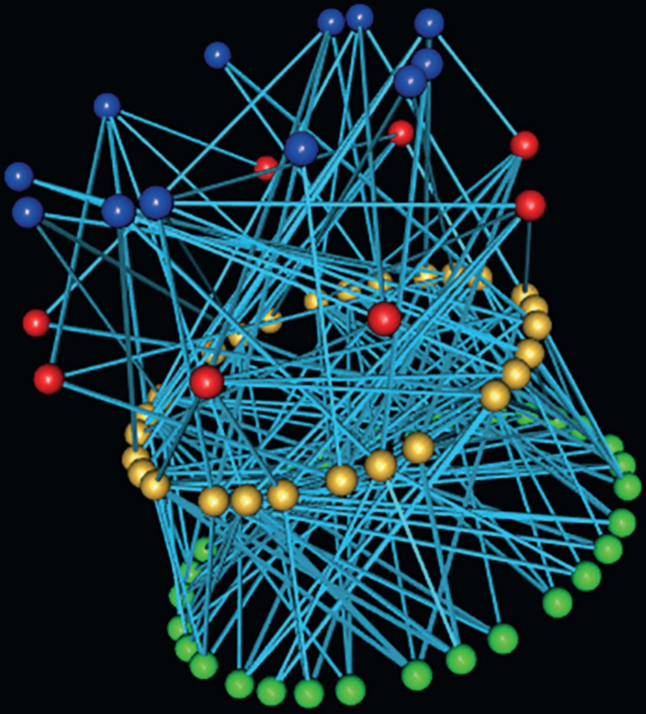
The influence of horizontal and vertical biodiversity on the effects of stressors on aquatic ecosystems

Qinghua Zhao



Propositions

1. Dividing diversity into horizontal and vertical dimensions is the key to understand "diversity-stability" relationships.

   (this thesis)

2. For ecological risk assessment of chemicals, protecting the most sensitive species is not sufficient to protect the whole ecosystem.

   (this thesis)

3. A good researcher knows when to stop probing the data.
4. The success of a PhD project is positively correlated with how busy your supervisors are.
5. Discussion face to face is much more efficient than the one online
6. The peak of working proficiency appears at the night before a deadline

Propositions belonging to the thesis, entitled

The influence of horizontal and vertical biodiversity on the effects of stressors on aquatic ecosystems

Qinghua Zhao

Wageningen, 27 January 2021

The influence of horizontal and vertical biodiversity on the effects of stressors on aquatic ecosystems

Qinghua Zhao

Thesis committee

Promotor

Prof. Dr Paul J. van den Brink

Personal chair, Chemical Stress Ecology

Wageningen University & Research

Co-promotor

Prof. Dr Frederik De Laender

Laboratory of Environmental Ecosystems Ecology

Research Unit in Environmental and Evolutionary Biology

University of Namur, Belgium

Other members

Prof. Dr Frank van Langevelde, Wageningen University & Research

Prof. Dr Lourens Poorter, Wageningen University & Research

Prof. Dr Peter C. de Ruiter, University of Amsterdam

Prof. Dr Johan van de Koppel, Royal Netherlands Institute for Sea Research (NIOZ), Yerseke

This research was conducted under the auspices of the Graduate School for Socio-Economic and Natural Sciences of the Environment (SENSE)

The influence of horizontal and vertical biodiversity on the effects of stressors on aquatic ecosystems

Qinghua Zhao

Thesis
Submitted in fulfilment of the requirements for the degree of doctor
at Wageningen University
by the authority of the Rector Magnificus
Prof. Dr A.P.J. de Mol,
in the presence of the
Thesis Committee appointed by the Academic Board
to be defended in public
on Wednesday 27 January 2021
at 16:00 in the Aula

Qinghua Zhao

The influence of horizontal and vertical biodiversity on the effects of stressors on aquatic ecosystems

160 pages

PhD thesis, Wageningen University, Wageningen, the Netherlands (2021)

With references, with summary in English

ISBN: 978-94-6395-602-4

DOI: https://doi.org/10.18174/533791

Contents

Chapter 1

General introduction

1.1 Why food webs

Food webs, *who eats whom*, exist almost everywhere on earth, from a tiny water-holding tree crotch to huge lakes. Robust food webs are essential for sustaining ecosystem structure, function, service and stability (Montoya *et al.* 2003; Thompson *et al.* 2012; Britten *et al.* 2014). For example, stable food webs in corals and lakes can ensure provision of commercial fisheries for humans (Rogers *et al.* 2014, 2017; Nõges *et al.* 2018). However, human activities are dramatically eroding diversity within food webs that have resulted in shifts in biomass production, energy flow, nutrient uptake and interaction strength between predator and prey (Worm & Duffy 2003; Srivastava & Vellend 2005). The declining diversity can further erode food webs stability (definition of stability see below) and make food webs more vulnerable to disturbances.

1.2 What is stability?

Stability describes system's ability to defy changes (Pimm 1984; Grimm *et al.* 1992). A system is deemed as stable if a system can return into its original state after disturbances. The illustration of stability intuitionally brings us two questions. First, how long will the return to the original state take? Second, if there is no return, how large is the maximum strength of disturbances that still permit the ecosystem to return to its original state? How long the return will take is coined as *engineering resilience* (Holling 1996). The maximum strength of disturbances that a system permits is called *ecological resilience* (Holling 1996). In this thesis, I study the *engineering resilience* and I will simply refer to *engineering resilience* as stability throughout thesis. How to quantify stability mathematically is listed at Box 1.

1.3 The relationship between diversity and stability

Whether higher diversity is good or bad for the stability of an ecosystem has been one of central questions in ecology (McCann 2000). Before the 1970s, empirical observations showed that higher diversity stabilizes communities. For example, (Elton 1958) showed that diversity can enhance stability against invasions in competitive systems (i.e. forests). However these early ideas were challenged by theoretical estimations such as Robert May's classic study published in 1973. May randomly constructed communities with randomly assigned interactive links between the species of the communities and he found that biodiversity tended to destabilize community dynamics (May 1973). His results seemed to counter reality, as real ecosystems were undoubtedly diverse and stable. (Pimm & Lawton 1977) separated species interaction types within communities into competition and predation. They constructed communities with varying number of competition and predation interactions and they found a contrasting pattern in their modelling simulations. A community having a higher number of competition interactions is more stable than a community only having predation interactions (i.e. a pure food chain). In later years, growing evidence showed that adding competitors into communities often stabilizes communities (McCann *et al.* 1998; Coyte *et al.* 2015; Yang *et al.* 2019), while adding predators often destabilizes them (Post 2002; Yang *et al.* 2019; Karakoç *et al.* 2020).

These contrasting relationships in diversity-stability relationships as described above provide the recommendation that distinguishing between competition and predation may be necessary to study the diversity-stability relationship. Competition operates among species that exist within each trophic levels. The number of species within trophic levels is called horizontal diversity (Figure 1.1). However, predation operates among species that exist across trophic levels. The number of trophic levels is called vertical diversity (Figure 1.1). If one characterizes biodiversity as horizontal and vertical diversity as done by Duff *et al.* (2007), competition and predation are easily distinguished, i.e. competition and predation operating in horizontal and vertical diversity, respectively. As a result, horizontal and vertical diversity may bring us a new insight to study diversity-stability debate.

1.4 Influence of diversity on effects of stress on ecosystems

Chemical used on agricultural fields to control pests provide considerable benefits to society (Cooper & Dobson 2007; Aktar *et al.* 2009). Chemicals can, however, enter adjacent aquatic ecosystems and therefore result in undesirable side effects on aquatic organisms, which have already been regarded as the second greatest threat to aquatic system behind habitat loss (Wilcove & Master 2005). To prevent undesirable side effects on the aquatic environment, authorities require an environmental risk assessment (ERA) of chemicals before marketing. Standardized single-species toxicity tests performed in the laboratory are the general data input for the effect assessment part of the ERA of chemicals (Daam & Van den Brink 2007; Artigas *et al.* 2012). However, these results bring a lot of uncertainties into the risk assessment, among others the unknown between species variation in sensitivity and the absence of ecological interactions (Daam & Van den Brink 2007; Artigas *et al.* 2012). This makes it difficult to extrapolate results from single-species toxicity tests to a larger scale of field conditions.

In the higher-tiers of the ecological risk assessment, multispecies experiments, using indoor or outdoor microcosms and mesocosms, are included as methods for ERA of chemicals (Brock *et al.* 2006; Artigas *et al.* 2012). They can provide a bridge between laboratory and field, providing the opportunity to study the combined effects of chemicals and ecological interactions on aquatic ecosystems. However, how complex the experimental setup and design should be has been widely recognized as a critical in the ERA of chemicals. Larger and more complex experiments (i.e. mesocosms) are more realistic than smaller scale and simpler experiments. In contrast, simple experiments (i.e. microcosms) are easier to conduct and manipulate. To date, the results from simple and complex experiments sometimes do not match (Fleeger *et al.* 2003; Daam *et al.* 2008b). For example, exposure to the same dose of the insecticide chlorpyrifos (1 ug*L^{-1}), sensitive herbivores (Cladocera) needed nearly 3 times longer to recover in complex experiments than in simple experiments (Fleeger *et al.* 2003; Daam *et al.* 2008b).

The complexity of ecosystems can be characterized by horizontal (diversity within trophic levels) and vertical (number of trophic levels) diversity. The division of diversity into two dimensions may help us understanding how complexity modifies the direct and indirect effect of chemicals on ecosystems.

The direct toxic effects of chemicals reduces sensitive species' abundance (Brock *et al.* 2000; Fleeger *et al.* 2003). It is noted that the intensity of direct effects may vary with biodiversity. For example, the direct negative effects of herbicide linuron on producer populations can be smaller when horizontal diversity of producer species is increased (Baert *et al.* 2016). A more diverse producers' community can include both sensitive and tolerant producers (Gonzalez & Loreau 2009; Baert *et al.* 2016). Reductions in populations of sensitive species can be compensated by an increase of tolerant species (Gonzalez & Loreau 2009; Baert *et al.* 2016).

Indirect effects of chemicals may lead to increased or decreased abundance of tolerant species (Brock *et al.* 2000; Fleeger *et al.* 2003). For example, insecticide exposure may increase producers' abundance though the decrease in sensitive herbivores but this can also result in a decrease in predators' abundance (Fleeger *et al.* 2003). The intensity of indirect effects may also vary with biodiversity. The presence of a predator (i.e. increasing vertical diversity) can result in a larger insecticide-induced increase in producers' abundance, as the presence of a predator serves as an extra top-down control on sensitive herbivores (Relyea & Mills 2001; Beketov & Liess 2006; Trekels *et al.* 2013).

1.5 Objectives

The main objective of this thesis is to study how food web stability is affected by diversity, anthropogenic impact (i.e. chemicals), climate change, and their interactions. This may provide a practical recommendation of how to conserve biodiversity to ensure stability and also of how to avoid anthropogenic impact. I first focus on dividing diversity into two dimensions (horizontal and vertical diversity) and on evaluating how changes of the two-dimensional diversity affect the food web stability. I then focus on how changes of the two dimensional diversity alter the effect of anthropogenic stressors, i.e. chemical exposure, on food webs. I will finally focus on the long-term effect of warming temperatures on food web stability.

To accomplish the objectives above, I combined experiments, long term monitoring datasets and modelling approaches. Combing these methods can bring us a better mechanistic understanding of how diversity changes, chemical exposure and warming temperatures alter the food web structure, function and stability.

1.6 Thesis outline

In **Chapter 2**, I aim to test that horizontal and vertical diversity may have a contrasting effect on food web stability. I first employed a widely used generic model to analyse the effect of the both kinds of diversity on stability. We use the model to ask 1) whether horizontal and vertical diversity may have a contrasting effect on stability; 2) whether horizontal diversity of producers and herbivores synergistically increase stability. I then conducted experiments with an empirical plankton food web to confirm whether the modelling estimations match with empirical evidence.

In **Chapter 3**, I aim to study how changes of species richness within trophic levels and number of trophic levels modify the effect of stressors (i.e. chemical exposure) on food webs. We conducted microcosm experiments mimicking planktonic food webs, which were exposed to either the herbicide linuron or the insecticide chlorpyrifos. For environmental risk assessment, the effects of pesticides on aquatic ecosystems are often assessed based on single species tests, disregarding the potential influence of species richness within trophic levels and number of trophic levels. Our study provides new insights about whether single species sensitivity will always represent a worst case estimate of ecological effects, whether protecting the most sensitive species ensures the protection of ecosystems.

In **Chapter 4**, I further study whether the initial horizontal and vertical diversity at the start of the experiments should be considered for the ecological risk assessment of chemicals. Microcosms and mesocosm experiments are often conducted to assess the ecological risks of chemicals. These experiments often vary in their horizontal and vertical diversity at the start of the experiment. I reanalysed experimental datasets spanning from microcosms to mesocosms and used structural equation modelling in order to evaluate the influence of biodiversity on the effect paths found. I evaluated whether differences in diversity at the start of the experiments altered the effects of chemicals on aquatic ecosystems.

In **Chapter 5**, I aim to study the long-term effect of warming on food web stability in natural systems. I will further study which factor (warming versus diversity) plays a larger role in the stability of natural food webs. I first employ empirical dynamic modelling to analyse long term field monitoring data sets of aquatic systems and to calculate time-varying stability of these food webs. I then quantify the direction and strength of the effect of warming and diversity on stability. By doing so, I am able to compare the relative importance of the effect of warming and diversity on stability.

Finally, **chapter 6** presents a synthesis, discussing results of all previous chapters in a broader context and extrapolating the results across scales.

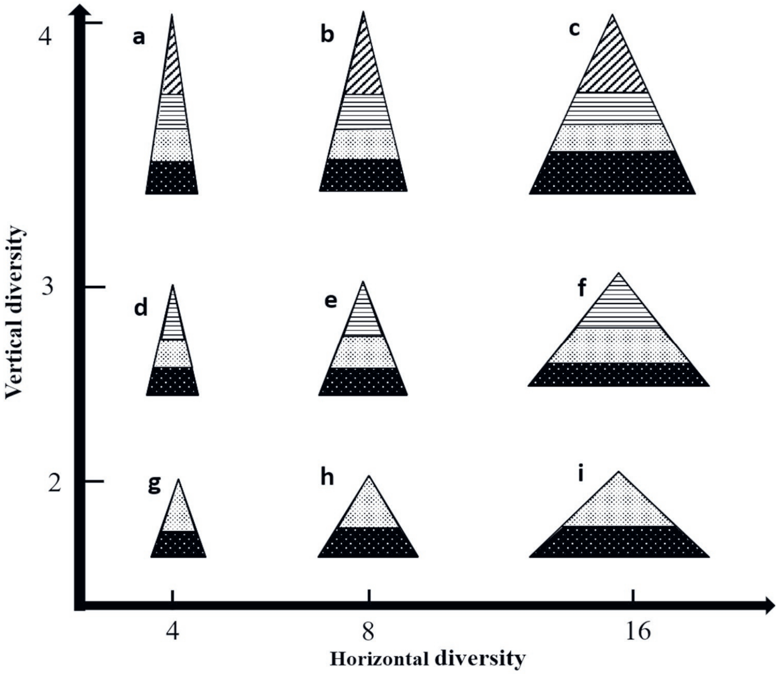


Figure 1.1 The schematic description of horizontal and vertical diversity within communities. The height of the pyramid here is number of trophic levels (vertical diversity) which varies from 2 to 4. The width of each hierarchy is the number of species within this trophic level (horizontal diversity). Specifically, basal width is the number of species in the first trophic level.

Box 1 Measurements of stability

Consider a community comprising m species ($m > 1$), whose community dynamics are described by differential equation 1.1,

$$\frac{dn_i(t)}{dt} = f_i(n_1(t), n_2(t), n_i(t) \dots, n_m(t)) \tag{1.1}$$

The rate changes of any species i, $\frac{dn_i(t)}{dt}$, is function of population size at time t, f_i. We can easily find the equilibrium population size for species i, $\hat{n}_i$, by solving m solutions below,

$$0 = f_i(\hat{n}_1, \hat{n}_2, \dots, \hat{n}_m) \tag{1.2}$$

If a small disturbance perturbs the population of i a distance away from the equilibrium, ε_i is written as,

$$\varepsilon_i(t) = n_i(t) - \hat{n}_i \tag{1.3}$$

The rate of changes of the distance, $\frac{d\varepsilon_i(t)}{dt}$, is finally obtained by equation 1.4, after Taylor expansion of equation 1.1 based on equilibrium $\hat{n}_i$ and ignoring high order terms.

$$\frac{d\varepsilon_i(t)}{dt} = \sum_m \left(\frac{\partial f_i}{\partial n_i}\Big|_{n_1(t)=\hat{n}_1,\, n_2(t)=\hat{n}_2, \dots\dots, n_m(t)=\hat{n}_m}\right) \varepsilon_i(t) \tag{1.4}$$

We write all m equations together as,

$$\begin{pmatrix} \frac{d\varepsilon_1(t)}{dt} \\ \frac{d\varepsilon_2(t)}{dt} \\ \vdots \\ \frac{d\varepsilon_m(t)}{dt} \end{pmatrix} = \begin{pmatrix} \overbrace{\begin{matrix} \frac{\partial f_1}{\partial n_1} & \frac{\partial f_1}{\partial n_2} & \cdots & \frac{\partial f_1}{\partial n_m} \\ \frac{\partial f_2}{\partial n_1} & \frac{\partial f_2}{\partial n_2} & \cdots & \frac{\partial f_2}{\partial n_m} \\ \vdots & \vdots & \ddots & \vdots \\ \frac{\partial f_m}{\partial n_1} & \frac{\partial f_m}{\partial n_2} & \cdots & \frac{\partial f_m}{\partial n_m} \end{matrix}}^{\text{Jacobian matrix}} \end{pmatrix} \begin{pmatrix} \varepsilon_1(t) \\ \varepsilon_2(t) \\ \vdots \\ \varepsilon_m(t) \end{pmatrix} \tag{1.5}$$

To simplify, we write equation 1.5 in matrix notation,

$$\frac{d\boldsymbol{\varepsilon}(t)}{dt} = \boldsymbol{A}\, \boldsymbol{\varepsilon}(t) \tag{1.6}$$

A community is stable only when all eigenvalues in matrix $\boldsymbol{A}$ are negative. The dominant one among all eigenvalues governs the long-term population dynamics. We quantify stability using the recovery time, defined as the negative reciprocal of the real part of the dominant eigenvalue ($-1/real(\lambda_{max})$). A larger recovery time indicates a lower stability.

As shown, the Jacobian matrix $\boldsymbol{A}$ is the key to measure stability. Transforming empirical data to $\boldsymbol{A}$, its diagonal elements that corresponded to the strength of the effect of population i on population i is often represented as function of minimum S, because it is hard to measure (more details see de Ruiter *et al.*, 1995; Neutel *et al.*, 2002; Schwarz *et al.*, 2017). For the computation of sequential Jacobian matrices and time-varying stability, I refer to Ushio *et al.* (2018) and Chapter 5.

Chapter 2

Horizontal and vertical diversity jointly shape food web stability against small and large perturbations

Zhao, Q., Van den Brink, P.J., Carpentier, C., Wang, Y.X., Rodríguez-Sánchez, P., Xu, C., Vollbrecht, S., Gillissen, F., Vollebregt, M., Wang, S. and De Laender, F., 2019. Horizontal and vertical diversity jointly shape food web stability against small and large perturbations. *Ecology letters*, 22, 1152-1162.

Abstract

The biodiversity of food webs is composed of horizontal (i.e. within trophic levels) and vertical diversity (i.e. the number of trophic levels). Understanding their joint effect on stability is a key challenge. Theory mostly considers their individual effects and focuses on small perturbations near equilibrium in hypothetical food webs. Here, we study the joint effects of horizontal and vertical diversity on the stability of hypothetical (modelled) and empirical food webs. In modelled food webs, horizontal and vertical diversity increased and decreased stability, respectively, with a stronger positive effect of producer diversity on stability at higher consumer diversity. Experiments with an empirical plankton food-web, where we manipulated horizontal and vertical diversity and measured stability from species interactions and from resilience against large perturbations, confirmed these predictions. Taken together, our findings highlight the need to conserve horizontal biodiversity at different trophic levels to ensure stability.

2.1 Introduction

Diversity (i.e., species richness) within food webs is important for sustaining ecosystem functions such as biomass production, energy flow and nutrient uptake (Otto *et al.* 2007; Rooney & McCann 2012; Soliveres *et al.* 2016; Barnes *et al.* 2018; Wang & Brose 2018). Diversity can be characterized in two dimensions (Duffy *et al.* 2007; Srivastava & Bell 2009; Wang & Brose 2018): the number of species within trophic levels (i.e., horizontal diversity) and the number of trophic levels (i.e., vertical diversity). Horizontal and vertical diversity both affect the functioning and stability of food webs, via different mechanisms (Duffy *et al.* 2007). Effects of horizontal diversity are driven by competitive interactions, while effects of vertical diversity are mediated by predation. Horizontal and vertical diversity may interact with each other (Duffy *et al.* 2007). For instance, producer coexistence can be indirectly mediated by consumer diversity (Brose 2008).

Until now, the effects of horizontal and vertical diversity on food web stability (i.e., via local stability analysis) have been mostly treated separately (Pimm & Lawton 1977; Duffy *et al.* 2007), and mainly using small trophic modules (Pimm & Lawton 1977; McCann *et al.* 1998; Thébault & Loreau 2005). No information is available on their joint effect in multitrophic food webs. Horizontal diversity of consumers is expected to increase stability (McCann *et al.* 1998), because a higher number of consumer species decreases the per capita energy flux in consumer-resource interactions by decreasing the per capita consumption rate (Crowder *et al.* 1997; Perna *et al.* 2004; Finke & Denno 2005), hence stabilizing the consumer-resource links (Rip & Mccann 2011; Gilbert *et al.* 2014). Producer diversity can increase stability (McCann 2000) by increasing the potential for niche differentiation among consumers (Novotny *et al.* 2006; Jetz *et al.* 2009; Poisot *et al.* 2013), or again weaken consumer-resource interactions (Berlow 1999; Hillebrand & Cardinale 2004; Edwards *et al.* 2010; Moore & Ruiter. 2012). In contrast, vertical diversity is expected to decrease stability in simple food chains via increasing recovery times (Pimm & Lawton 1977; Morin & Lawler 1995; Post 2002). This negative vertical diversity effect has been evoked as an explanation for the limited number of trophic levels in natural food webs (Pimm & Lawton 1977; Morin & Lawler 1995; McHugh *et al.* 2010; Sabo *et al.* 2010).

In natural systems, horizontal and vertical diversity will vary jointly. For example, the decrease of vertical diversity (e.g., the extinction of top predators) could cause cascades that lead to species extinction, lowering horizontal diversity (Crooks & Soulé 1999; Borrvall & Ebenman 2006; Srivastava & Bell 2009). In addition, ecosystem succession and degradation often change both horizontal and vertical diversity (Ferris & Matute 2003; Maharning *et al.* 2009; Yang *et al.* 2018). Hence, it is critical to understand how horizontal (both producer and consumer) and vertical diversity interact and shape food web stability.

The individual effects of horizontal and vertical diversity on local stability are often examined by analysing the Jacobian matrix (hereafter 'Jacobian'). This approach assumes that systems are near equilibrium and exposed to small perturbations (May 1973; Allesina & Tang 2012, 2015). However, ecosystems are often far away from equilibrium (Allesina & Tang 2015) and face large perturbations (De Laender *et al.* 2016). This makes it uncertain if stability analyses based on the Jacobian provide useful information for real-world perturbations (May 1973). Alternative stability measures have therefore been proposed (Grimm & Wissel 1997; Arnoldi *et al.* 2016; Donohue *et al.* 2016). Examples include population recovery and resistance following severe perturbations (Isbell *et al.* 2015; Baert *et al.* 2016; Hillebrand *et al.* 2018) and the coefficient of temporal variation of population dynamics (McCann 2000; Pennekamp *et al.* 2018). Recent work indicates that these alternative stability measures may correlate poorly (Ives & Carpenter 2007; Montoya *et al.* 2013; Hillebrand *et al.* 2018; Radchuk *et al.* 2019). For example, temporal stability is positively associated with diversity, while the latter is negatively correlated with resistance (Pennekamp *et al.* 2018).

In this paper, we combine models and experiments to examine the joint effect of horizontal and vertical diversity on food web stability. We define stability using two kinds of metric: either based on the assumption of small near equilibrium perturbations, or on biomass and compositional recovery following large perturbations away from equilibrium. To this end, we first analysed the joint effect of horizontal (the number of producer/consumer species) and vertical diversity (the number of trophic levels) on the Jacobian-based stability of randomly created food webs. Second, we manipulated horizontal and vertical diversity in an experiment with a planktonic food web and quantified their joint effect on stability, measured using empirically established Jacobian matrices. Finally, we quantified the effect of horizontal and vertical diversity on the stability of the same food web, but now measured as resilience following large perturbations caused by two types of chemicals.

Overall, our results show for the first time that the positive effect of producer diversity on stability increases with consumer diversity, regardless of vertical diversity. In contrast, vertical diversity always decreased stability. This trend emerged from all analyses and suggests that conserving diversity within multiple trophic levels is key to promote food web stability.

2.2 Materials and methods

2.2.1 Model and simulations

We conducted a full factorial design with 24 food web configurations: four levels of horizontal diversity at the first trophic level (producer diversity equalled 6, 7, 8, or 9), three levels of horizontal diversity at the second trophic level (consumer diversity equalled 3, 4, or 5), and two levels of vertical diversity (2 or 3 trophic levels). This design reflects the empirically observed triangularity of food webs (Woodward *et al.* 2005; Turney & Buddle 2016). We deliberately omitted omnivores (species consuming at multiple trophic levels), because omnivores have already been proven to stabilize food webs by creating weak predator-prey interactions (Neutel *et al.* 2002, 2007). Food web connectance (i.e. the number of links divided by the square of the number of species) was set to 0.10 (Dunne *et al.* 2002a, b; Williams *et al.* 2002). The links were randomly distributed between adjacent trophic levels.

We described community dynamics with generalised Lotka–Volterra equations (Eq. 2.1) (Emmerson & Yearsley 2004; Gibbs *et al.* 2018; Maynard *et al.* 2018):

$$\frac{dN_i}{dt} = N_i\left(b_i + \sum_j a_{ij}N_j\right) \tag{2.1}$$

where $N_\mathbf{i}$ and $N_\mathbf{j}$ are the population density of species i and j, respectively; b_i is the intrinsic per capita growth rate of species i. The b_i is positive for producers, where it represents the density independent growth rate, while b_i is negative for consumers and predators, where it represents a death rate. The a_{ij} is the per capita effect of species j on the growth rate of species i.

The growth rate b_i for all producers was equal to 1, which guaranteed that emergent food web patterns were a direct effect of horizontal/vertical diversity, rather than fitness differences among species (Maynard *et al.* 2018). For consumers and predators, we randomly drew b_i from a uniform distribution U(−0.001, 0) while b_i for predators was generated from U(-0.0001,0) (Eklöf & Ebenman 2006). We ensured that the b_i of predators were less negative than the b_i of consumers, because species at higher trophic levels often have larger body sizes, and therefore lower mortality rates (Borrvall *et al.* 2000). We ensured that intraspecific competition a_{ii} (i=j) was stronger for primary producers (-1) than for consumers and predators (-0.1) (Berg *et al.* 2011; Kadoya *et al.* 2018). Interspecific competitions a_{ij} (i≠j) among producers were sampled from U(-0.5, 0) and set symmetrically to avoid cycling or chaos (Eklöf & Ebenman 2006; Maynard *et al.* 2018). Consumers competed indirectly by sharing producers, and direct interspecific interactions among consumers were thus set to zero (Eklöf & Ebenman 2006).

Finally, the a_{ij} (i≠j), the per capita effect of consumers (or predators) species j on the per capita growth rate of producers (or prey) species i, were sampled from U(−0.5, 0) when a consumer (or predator) only consumed one producer (or prey) (Eklöf & Ebenman 2006). Considering that interaction strengths in natural systems communities often have skewed distributions with mostly weak and only few strong interactions (Borrvall *et al.* 2000), one strong a_{ij} was sampled from U(-0.4, 0) and assigned randomly (Eklöf & Ebenman 2006), if the number of producers (or prey) was larger than one. The weak a_{ij} were sampled from U(-0.1,0) divided by the number of prey species minus one (Borrvall *et al.* 2000; Borrvall & Ebenman 2006). Hence, the total effect of a consumer (or predator) on all its producers (or prey) a_{ij} always varied between -0.5 and 0, but the average per capita effect of a consumer (or predator) on its producers (or prey) decreased with the number of producers (or prey) (McCann *et al.* 1998; Borrvall *et al.* 2000). A rationale for this approach and more details can be found in the *supplementary information 2.1*. The effect of producers (or prey) on consumers (or predators) is given by a_{ji}, which is positive: $a_{ji} = -\mathrm{k} * a_{ij}$, with k representing the efficiency of the resources being converted into consumers, which was set at 0.2 (Borrvall & Ebenman 2006; Eklöf & Ebenman 2006).

Per food web configuration, we created 10,000 food webs, yielding 240,000 food webs. For each food web, we calculated stability as follows. First, we calculated equilibrium population density (directly solving the equations $0 = b_i + \sum_j a_{ij}\,\widehat{N}_j$ on Eq. 2.1) and verified if all equilibrium densities were positive. If this was the case, we retained the particular food web, otherwise we discarded it. For each food web configuration, more than 95% of the generated food webs were feasible with positive equilibrium densities (Table S2.1). Next, we used these equilibria to compute the Jacobian for this food web. Finally, we quantify stability using the recovery time, defined as the negative reciprocal of the real part of the dominant eigenvalue of the Jacobian, i.e. ($-1/\mathrm{real}(\lambda_{\max})$) (Pimm & Lawton 1977; Emmerson & Yearsley 2004; Moore & de Ruiter 2012). A larger recovery time indicates a lower stability. Finally, we conducted two sensitivity analyses to inspect how our results changed with the selected parameter ranges (Fig S2.1-2.3).

2.2.2 Experiments: general conditions

We experimentally tested the effect of horizontal and vertical diversity on the stability of a freshwater plankton food web representative of Dutch ditches. These two experiments, each lasted for 21 days, were performed in 900 mL glass jars, filled with 500 ml WC medium (Guillard & Lorenzen 1972; Frenken *et al.* 2018) and contained in a water bath at constant temperature (19.9 °C ± 0.8 °C) and a light regime of 12h: 12h (light: dark). The light intensity at the surface (measured with a LI-COR LI-250A, LI-COR Biosciences, Lincoln, USA) was 120 µmol m^{-2} s^{-1}, and was created using Ceramalux® Phillips 430 Watt

High Pressure Sodium Non-Cycling Lamps. We worked with field-collected organisms (details are in the *supplementary information 2.2*). The total initial bio-volume of producers (algae) and consumers (invertebrate grazers) was always 25 mm^3 and 0.2 mm^3, respectively, regardless of producer and consumer diversity (richness). For the systems with three trophic levels, we added one individual of predator *Chaoborus* to each system. The predators used in the experiments had mean individual body length 11.21 ± 0.04 mm. In both experiments, we worked with 4 replicates.

2.2.3 Experiment 1: empirical Jacobian matrices

The aim of the experiment was to examine how stability, based on empirically constructed Jacobian matrices varied with horizontal and vertical diversity. We manipulated horizontal diversity, at the first (producers; 1 or 5 species) and second trophic level (consumers; 1 or 4 species), and vertical diversity (2 or 3 trophic levels) in a full factorial design (Table S2.2). At all combinations, we estimated interactions (within and between tropic levels) to characterize the Jacobian on day 21 after the start of the experiment. The off-diagonal elements of this matrix are per capita interactions, which we estimated as the per capita material fluxes between consumers (or predators) and producers (or consumers) (Ruiter *et al.* 1995; Neutel *et al.* 2007; Schwarz *et al.* 2017). The effect of consumers (or predators) on producers (or consumers) is given by $J_{ji} = -\frac{F_{ij}}{M_j}$, and the effect of producers (or consumers) on consumers (or predators) is given by $J_{ij} = e_j \frac{F_{ij}}{M_i}$, where F_{ij} is the energy flux from i to j (e.g. from producers to consumers), e_j is the assimilation efficiency of j, and M_i and M_j (g m^{-2}) are the biomass of i and j, respectively (Schwarz *et al.* 2017). The diagonal elements of the Jacobian are $J_{ii} = -s\frac{X_i}{M_i}$, where X_i is the metabolism of trophic level i,and s is a free parameter between 0 and 1 (Schwarz *et al.* 2017). Because s cannot be determined empirically in complex food webs, we determined the smallest s leading to all eigenvalues of the Jacobian having negative real parts. The value of s represents the stability of the community against small perturbations, assessed based on estimated interactions (Schwarz *et al.* 2017). It is therefore conceptually similar to recovery time (smaller values indicate more stable food webs) obtained with the model and is referred to as the degree of self-damping. Details on the calculation of F_{ij} , X_i, and M are provided in the *supplementary information 2.3.*

2.2.4 Experiment 2: large perturbations

The objective of this experiment was to examine how horizontal and vertical diversity affected the stability against large perturbations. Here, we applied functional and compositional resilience as stability metrics. We manipulated the same experimental factors as in experiment 1, and added one additional factor: pesticide exposure (absent or present). We performed this experiment twice, once using the insecticide chlorpyrifos (1 µg l^{-1}), and once using the herbicide linuron (100 µg l^{-1}), selectively targeting consumers and producers, respectively (Wijngaarden *et al.* 1996; Daam *et al.* 2009). Experimental procedures were identical to the experiment 1. Information on chemical administration is provided in *supplementary information* 2.4. We measured community biomass, community composition (using the same methods as for experiment 1 and on days 6 and 21 day) and stability. To measure stability we first measured functional resilience (the recovery rate of total biomass) as (Isbell *et al.* 2015; Baert *et al.* 2016):

$$\text{functional resilience } = \frac{|B_{control,6} - B_{stress,6}|}{|B_{control,21} - B_{stress,21}|} \tag{2.2}$$

where $B_{\text{control},6}$, $B_{\text{control},21}$, $B_{\text{stress},6}$ and $B_{\text{stress},21}$ represent the total biomass in the control (no pesticide) and exposure (pesticide present) on days 6 and 21. Functional resilience is >1 if biomass differences between the control and stress treatment decrease between day 6 and 21, and <1 otherwise. Larger values mean faster recovery.

Next, we measured compositional resilience (compositional recovery) (Baert *et al.* 2016; Hillebrand *et al.* 2018):

$$\text{compositional resilience} = \left(\overbrace{1 - \frac{\sum_i |N_{i_{control,21}} - N_{i_{stress,21}}|}{\sum_i N_{i_{control,21}} + \sum_i N_{i_{stress,21}}}}^{BC_{21}}\right) - \left(\overbrace{1 - \frac{\sum_i |N_{i_{control,6}} - N_{i_{stress,6}}|}{\sum_i N_{i_{control,6}} + \sum_i N_{i_{stress,6}}}}^{BC_{6}}\right) \tag{2.3}$$

Compositional resilience can be considered an abundance-based change of Bray-Curtis similarity between day 6 (BC_6) and day 21 (BC_{21}) (Baert *et al.*

2016; Hillebrand *et al.* 2018), where N_i is abundance of species i. Positive values reflect that compositions of the control and disturbed communities converge between day 6 and day 21, while negative values imply compositional divergence. Again, larger values mean faster recovery.

2.2.5 Analysis of simulated and empirical data

To the simulated data, we applied linear regression to estimate the effect of producer, consumer, and vertical diversity, and their pairwise interactions, on the recovery time. To interpret potential effects on recovery time, we also tested for diversity effects on average interaction strengths, defined as the square root of the average of all the off-diagonal elements in the interaction matrix $J_{ij}(i \neq j)$ with total species T i.e., $(\sqrt{\frac{\sum_{i\neq j} J_{ij}^2}{T(T-1)}})$ (May 1973; Moore & Ruiter. 2012), again using linear regression.

To the data from experiment 1, we applied linear mixed models to test for the effect of producer, consumer, and vertical diversity, and their pairwise interactions, on the degree of self-damping, as calculated from the estimated interactions. We used species identity as a random effect to exclude the potential confounding effect of species identity.

To understand possible effects of diversity on the degree of self-damping, we examined diversity effects on three variables underlying the degree of self-damping: consumer biomass, the energy flux into consumers, and interaction strengths. We did so by first applied the mixed model to test for the effect of producer, consumer, and vertical diversity, and their pairwise interactions (again with species identity as a random effect) on these three variables. Next, we constructed linear regression models to examine the relationship between (1) consumer biomass and energy flux into consumers, (2) energy flux into consumers and the absolute value of interaction strength of consumers to producers, and finally (3) the absolute value of interaction strength of consumers to producers and degree of self-damping (minimum s). Again, we used mixed models with species identity as a random effect, and included interactions between horizontal and vertical diversity. We adopted the same approach for predator biomass, energy flux into predator, and absolute value of interaction strength of predator to consumer. However, note that by definition, vertical diversity here was always three, so we could only analyse the effects of horizontal diversity.

To the data from experiment 2, we again used linear mixed-effects models (species identity was again a random effect) to test for the effect of producer, consumer, and vertical diversity and their pairwise interactions on the two measures of recovery (Eq. 2.2 and 2.3). Because these measures depend on how total biomass changed with time, we also included sampling time and chemical concentrations into the analysis of total biomass. All models were fitted with the lme4 package in R (Bates *et al.* 2014).

2.3 Results

2.3.1 Model simulations

Producer and consumer diversity both promoted stability, i.e., decreased recovery time (Fig. 2.1). The positive effect of producer diversity on stability increased with increasing consumer diversity, and this trend was not qualitatively changed by vertical diversity. Vertical diversity on itself always decreased stability. Stability was highest at high horizontal (producer and consumer) diversity and low vertical diversity, and lowest at low horizontal diversity and high vertical diversity (Fig. 2.1a-b), indicating that high horizontal diversity can compensate the stability loss caused by vertical diversity. These results were robust to changing all parameters simultaneously from their reference value by -20% and +20% (Fig. S2.1). Outside of this range, the model results were sensitive to the conversion efficiency k (Fig. S2.2), where larger k destabilized the food webs and switched the diversity-stability relationship, as expected (Rip & Mccann 2011; Barbier & Loreau 2019). When fixing the conversion efficiency k to its reference value, the model results were robust to changes of up to -60% and +60% of all parameters except k (Fig. S2.3).

2.3.2 Experiment 1: empirical Jacobian matrices

Producer, consumer, and vertical diversity all affected food web stability. In line with the model predictions, both producer and consumer diversity increased food web stability (i.e., decreasing the degree of self-damping) and the impact of producer diversity on stability increased with increasing consumer diversity. Also in line with the model results, vertical diversity on itself decreased stability (Fig. 2.2a-b). Stability was highest at high horizontal (both producer and consumer) diversity and low vertical diversity, and was lowest at low horizontal diversity (producer and consumer) and high vertical diversity (Fig. 2.2a-b).

The effects of horizontal and vertical diversity on stability were associated with effects on consumer biomass, energy fluxes, and interaction strengths between trophic levels. Consumer biomass increased with producer and consumer diversity but decreased with vertical diversity (Fig. 2.2c-d). Diversity did not affect predator biomass (Table S2.3).

Interactions of producer, consumer, and vertical diversity affected the energy flux into consumers (Fig. 2.2e,f). At high vertical diversity (i.e., 3), horizontal diversity of either producers or consumers increased the energy flux into consumers (Fig. 2.2f). This higher energy flux was associated with higher consumer biomass (Fig. 2.3a). Under low vertical diversity (i.e., 2), however, horizontal diversity decreased the energy flux (Fig. 2.2e), while increasing consumer biomass (Fig. 2.3a). We found no effect of diversity on the energy flux into predators (Table S2.3).

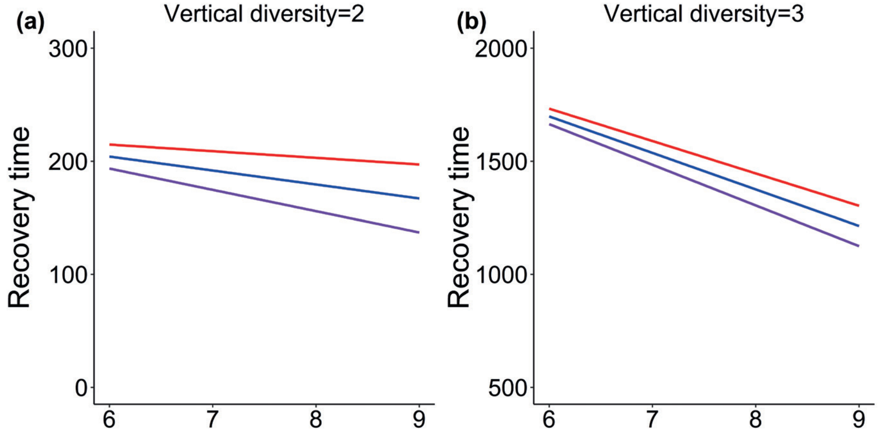


Figure 2.1 Model simulations illustrating the interactive effects of horizontal (producer and consumer) and vertical diversity on recovery time (a lower recovery time indicates a greater stability).

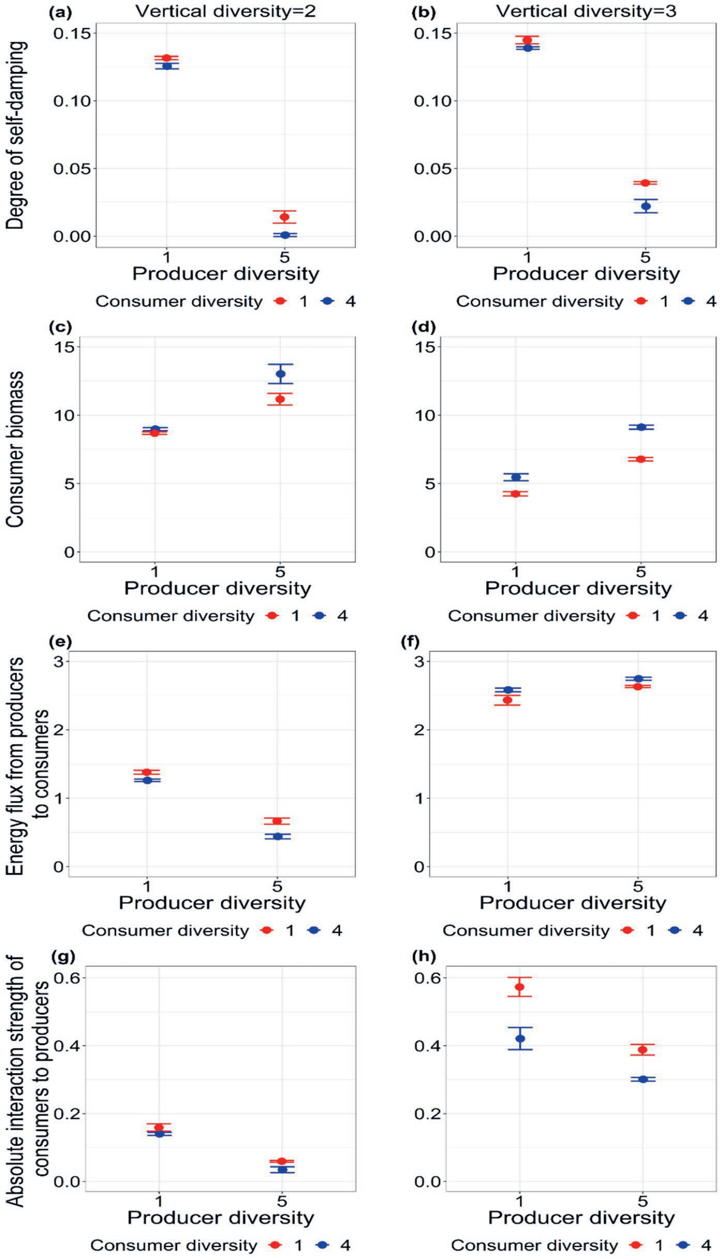


Figure 2.2 The interactive effects of horizontal (producer and consumer) and vertical diversity on stability (the degree of self-damping) (a, b), on consumer biomass (c, d), on energy flux from producers to consumers (e, f), and on the absolute value of interaction strength of consumers to producers (g, h). Plotted are sample mean ± one s.d. Detailed statistical results are listed in Table S2.4.

The interaction strength of consumers to producers was influenced by interactions of producer, consumer, and vertical diversity. Horizontal diversity decreased the interaction strength, whereas vertical diversity increased it (Fig. 2.2g,h). The interaction strength was lowest at high horizontal and low vertical diversity, but highest at low horizontal and high vertical diversity (Fig. 2.2g,h), where the interaction strength was positively correlated with the energy flux into consumers (Fig. 2.3b). No significant diversity effects were detected on the interaction strength of predators to consumers (Table S2.3). Finally, the interaction strength of consumers to producers was positively correlated with the degree of self-damping (Fig. 2.3c), indicating that strong interactions decreased food web stability.

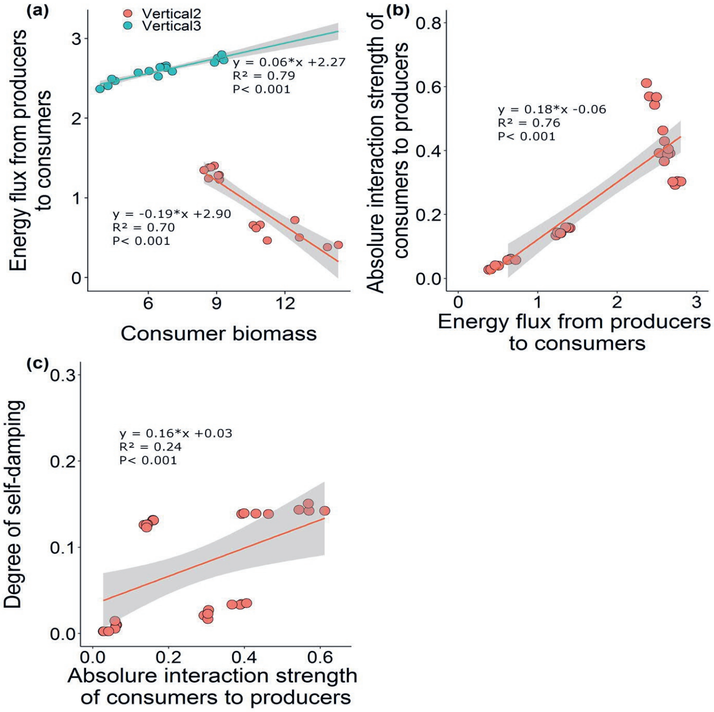


Figure 2.3 Relationships between consumer biomass (g m^{-2}) and energy flux from producers to consumers (g c m^{-2} h^{-1}) (a), between the energy flux from producers to consumers (g c m^{-2} h^{-1}) and the absolute value of interaction strength of consumers to producers (b), and between the absolute value of interaction strength of consumers to producers and the degree of self-damping (c).

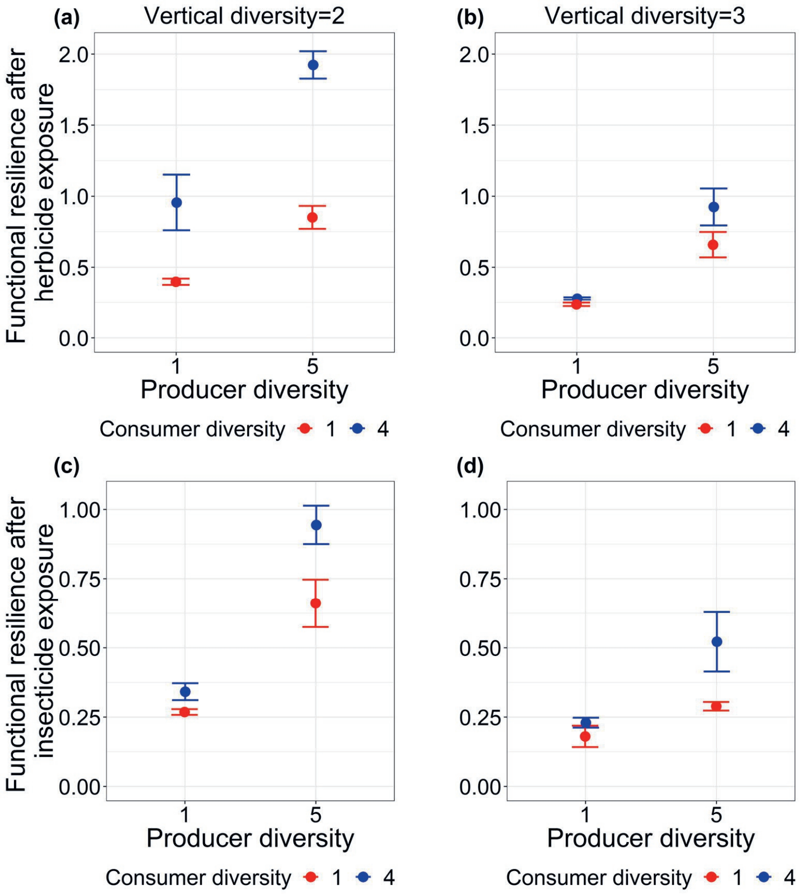


Figure 2.4 The interactive effects of horizontal (producer and consumer) and vertical diversity on the functional resilience after herbicide (a, b) and insecticide (c, d) exposure. Plotted are sample mean ± one s.d. Detailed statistical results are listed in Table S2.5.

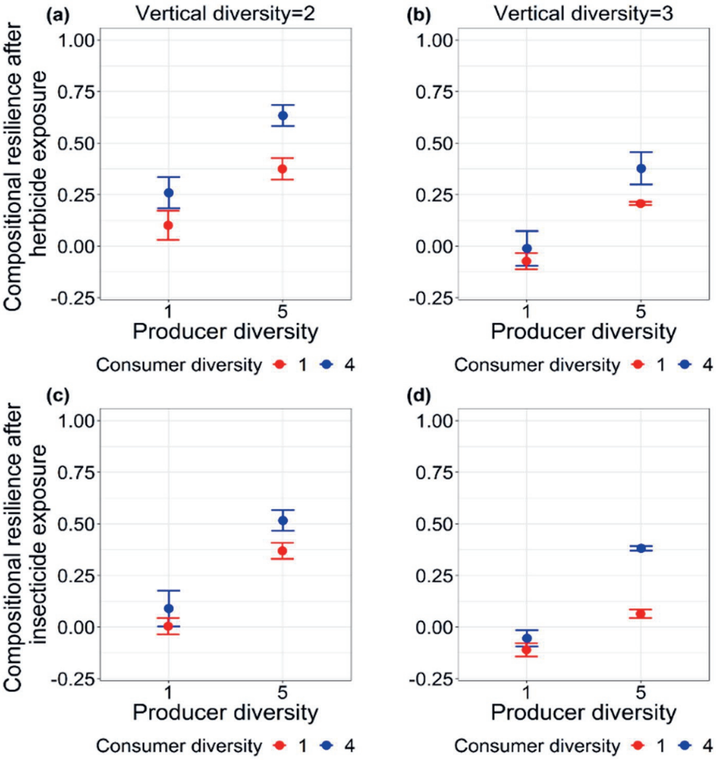


Figure 2.5 The interactive effects of horizontal (producer and consumer) and vertical diversity on the compositional resilience after herbicide (a, b) and insecticide (c, d) exposure. Plotted are sample mean ± one s.d. Detailed statistical results are listed in Table S2.5.

2.3.3 Experiment 2: large perturbations

In line with the results obtained with the Jacobian method for simulated and empirical food web data, producer and consumer diversity both increased stability (i.e. functional resilience) against severe perturbations and the positive effect of producer diversity was stronger when consumer diversity was high (Fig. 2.4a-d). Again, vertical diversity decreased stability (Fig. 2.4a-d). Therefore, functional resilience was highest at high horizontal diversity and low vertical diversity, and it was lowest when horizontal diversity was low and vertical diversity was high (Fig. 2.4a-d). We found qualitatively identical results for stability measured by the compositional resilience (Fig. 2.5a-d), even though the interactive effect of producer and consumer diversity was weaker for the case of herbicide exposure.

The effects of horizontal and vertical diversity on the functional and compositional resilience were associated with effects on total biomass (sum across all trophic levels) and composition, respectively. Total biomass showed signs of recovery after exposure to the herbicide and insecticide, but horizontal diversity increased the biomass recovery rate while vertical diversity decreased it. This result can be understood from the smaller effect the pesticides had on the horizontally more diverse communities (Fig. S2.4a-d and Fig. S2.5a-d). Indeed, this smaller effect translates to the numerator and especially denominator of Eq.2.2 being smaller at higher horizontal diversity, making their ratio (i.e. functional resilience) inevitably larger. The opposite occurred for vertical diversity, which increased biomass differences (Fig. S2.4e,f and Fig. S2.5e,f) and therefore decreased the recovery rate.

On average, the composition of the exposed and control communities was more similar on day 21 than on day 6, indicating compositional recovery. Horizontal and vertical diversity had also opposite effects on compositional recovery. Because producer abundance accounted for more than 97% of the whole community, the effects of horizontal and vertical diversity on compositional recovery can be understood by focusing on the producer community.

The herbicide directly decreased the abundance of sensitive producers (*Desmodesmus pannonicum, Chlorella vulgaris* and *Selenastrum capricornutum,* Fig. S2.6a) on day 6, but did not change consumer composition (Fig. S2.6c,d). A greater producer diversity caused an insurance effect as tolerant producers (e.g., *Scenedesmus obliquus* in Fig. S2.6a) became dominant, which caused compositional differences between the control and the herbicide-treated systems. This difference translates to the last term of Eq.2.3 (BC_6) being smaller at higher producer diversity (no composition changes on day 21), making the difference between BC_{21} and BC_6 (i.e., compositional resilience) inevitably greater. We also found that the magnitude of this insurance effect was increased by consumer diversity, but decreased by vertical diversity, which respectively increased and decreased compositional recovery (Fig. S2.6a-d).

The insecticide directly decreased the abundance of sensitive consumers (i.e., *Daphnia pulex*, and *Moina macrocopa* in Fig. S2.7a), and tolerant species (e.g., *Daphnia lumholtzi* in Fig. S2.7a) became dominant. The dominance of tolerant species had indirect, top-down, effects on its preferred algae (*Scendesmus acutus, C. vulgaris* and *S. capricornutum*), which increased the abundance of non-preferred algae (*D. pannonicum*), compensating the loss of the preferred algae (Fig. S2.7c). Again, this represents an insurance effect, but this time driven by consumer diversity. This mechanism caused composition to be more different between control and insecticide-exposed systems on day 6 (no composition discrepancy on day 21), which again translated to the last term of Eq.2.3 (BC_6) being smaller at higher consumer diversity, making the difference between BC_{21} and BC_6 (i.e. compositional resilience) inevitably greater. This insurance effect was again increased by producer diversity, but decreased by vertical diversity, which increase and decrease compositional recovery, respectively (Fig. S2.7a-d).

2.4 Discussion

Our model and empirical results show for the first time that horizontal diversity and vertical diversity jointly affect stability. Specifically, the effect of producer diversity was stronger when consumer diversity was higher, regardless of vertical diversity. Vertical diversity consistently decreased stability. Taken together, these results suggest that food webs that are horizontally diverse at various trophic levels, but contain relatively few trophic levels will be more stable. These conclusions are broadly supported. First, both model simulations and two independent experiments with natural food webs yield consistent results. Second, we applied both Jacobian-based stability assessments that assume small perturbations and population equilibrium, but also alternative stability measures following large perturbations.

The results from the simulations and empirical food webs (experiment 1) indicate that, under the assumption of small perturbations and population at equilibrium, horizontal and vertical diversity affect food web stability by changing (average) interaction strength. The individual and joint effects of producer and consumer diversity as well as the effect of vertical diversity, as found through modelling, can be understood from changing average interaction strengths (Fig. S2.8). The results from experiment 1 can be explained by biomass changes and energy flows between trophic levels, which finally change interaction strengths between trophic levels. We show that the well-known positive (and negative) effects of horizontal (and vertical) diversity on consumer biomass (Duffy 2002; Cardinale *et al.* 2003) underpin these proposed effects. The positive interactive effects of producer and consumer diversity on consumer biomass reflects a greater niche differentiation among producers and consumers, optimising consumer biomass (Cardinale *et al.* 2006; Tilman *et al.* 2014; Barnes *et al.* 2018). The negative effect of vertical diversity on biomass reflects predation on consumers. It should be noted that, in this study, we only added a single predator individual. Given that natural systems are controlled by predator populations (Cardinale *et al.* 2003; Snyder *et al.* 2008; Griffin *et al.* 2013), biomass depression by vertical diversity can be higher than reported here.

Increasing the biomass of a focal trophic group generally increases the energy flux into this group (Otto *et al.* 2007; Ehnes *et al.* 2011; Barnes *et al.* 2014). At high vertical diversity (i.e., 3), we found a positive interactive effect of producer and consumer diversity on consumer biomass, which was indeed positively associated with energy fluxes into consumers. However, the positive association between biomass and energy flux can be overruled by other factors such as body size structure (Barnes *et al.* 2014, 2018). Under low vertical diversity (i.e., 2), we detected that high consumer biomass was negatively correlated with the energy fluxes to consumers. We found some support that individual body mass distributions could explain this result (Fig. S2.9). The treatments with high consumer biomass had a higher proportion of large individuals, which have slower metabolic rates, and thus generate lower energy fluxes, than small organisms.

High energy flux between trophic levels can increase interaction strength (McCann 2000; Rip & Mccann 2011; Schwarz *et al.* 2017; Kadoya *et al.* 2018), which in turn decreases food web stability (McCann 2000; Rip & Mccann 2011; Ushio *et al.* 2018). We found that the large energy flux into consumers indeed increased the interaction strength between consumers and producers, which led to lower stability. More specifically, producer and consumer diversity positively interacted to decrease interaction strength, which increased food web stability. Vertical diversity increased the interaction strength and decreased stability.

Taken together, interactive effects of producer and consumer diversity can change consumer biomass and the energy flux into consumers, leading to weak interactions and increased stability. Vertical diversity, in contrast, makes for strong links which will decrease stability.

Pesticide effects on community biomass were a direct result of effects on community composition, and were buffered by horizontal diversity. This buffering effect has been shown before for competitive systems (Gonzalez & Loreau 2009; Isbell *et al.* 2015; Baert *et al.* 2016). Our findings suggest that this effect also holds for food webs. Importantly, we found that – in our system where producers were the largest community – this effect occurs both when the pesticide directly affects producers and when it affects producers indirectly by depressing consumers.

We are cognizant of our study's limitations. First, in our experiments, we only considered two levels per horizontal and vertical diversity treatment. Previous studies have shown that food webs with higher horizontal (producer or consumer) diversity have larger niche differentiation and lower consumption rate (Duffy *et al.* 2007; Edwards *et al.* 2010). We therefore expect the positive effect of producer diversity on stability to be stronger than reported here. Second, natural systems often vary not only in species richness but also in how species biomasses are distributed. Our results may therefore change when considering alternative diversity indices (e.g., Shannon's index in Kato *et al.* (2018)). However, a combination of Shannon's index and species richness may provide a deeper insight in future work. Third, our model assumed pairwise interactions and neglected potential higher-order interactions, i.e. pairwise interactions being modulated by a third species, which have been found to stabilize communities (Bairey *et al.* 2016; Grilli *et al.* 2017; Mayfield & Stouffer 2017; Letten & Stouffer 2019). We expect that adding high-order interactions will reinforce the positive effect of horizontal diversity we found here, but weaken the negative effect of vertical diversity on stability. Finally, our results cannot be extrapolated to food webs that include omnivores. Previous studies indeed showed that complex food webs with omnivores potentially hold many stabilizing weak links (Neutel *et al.* 2002, 2007), making the destabilizing effect of vertical diversity we report here possibly weaker. Recent studies demonstrated that the presence of omnivores can alter the relationship between vertical diversity and primary productivity in complex food webs (Wang *et al.* 2019).

Our results show that different aspects of biodiversity may affect stability in different ways, through effects on biomass, energy fluxes, and eventually interaction strengths. How our results scale up to more complex food webs is an outstanding question, but our findings suggest that the benefits of horizontal diversity can in theory overcompensate the negative effects of vertical diversity. Our results show that conserving horizontal diversity across trophic levels (multiple horizontal biodiversity) can offer a solution to maintain both functioning and stability of natural ecosystems with high vertical diversity.

Acknowledgment

We acknowledge feedback and advice from the editor and three anonymous referees. We thank Carlos Melian and Jurg W. Spaak for valuable suggestions and comments.

Supporting Information

Supporting Information to this article are available free at wiley online library (https://onlinelibrary.wiley.com/doi/full/10.1111/ele.13282).

Supplementary information

Supplementary information 2.1

We increased horizontal (both producer and consumer) diversity, starting from an initial food web configuration that contained 6 producer and 3 consumer species. Considering that interaction strengths in natural communities often have skewed distributions with mostly weak and only few strong interactions (Borrvall *et al.* 2000), the a_{ij} (i≠j), the per capita effect of consumer (or predator) species j on the per capita growth rate of producer (or prey) species i, was randomly drawn from U(−0.5, 0) when a consumer (or predator) only consumed one producer (or prey) (Eklöf & Ebenman 2006). If the number of producers (or prey) was larger than one, one strong a_{ij} was drawn from U(-0.4, 0) and assigned randomly (Eklöf & Ebenman 2006). Next, weak a_{ij} were set as random samples from U(-0.1,0) divided by the number of producers (or prey) minus one (Borrvall *et al.* 2000; Borrvall & Ebenman 2006). Hence, the total effect of a consumer (or predator) on all its producers (or prey) a_{ij} is always distributed as U(−0.5, 0). This makes the average per capita effect of a consumer (or predator) on its producers (or prey) decrease with increasing numbers of producers (or prey) (McCann *et al.* 1998; Borrvall *et al.* 2000). In what follows, we explain the rationale for this procedure.

Increasing consumer diversity.

Increasing consumer diversity can lead to multiple consumers consuming the same producer. With C the number of consumers, the proportion of a producer consumed by consumer 1, 2, 3……. to N alone is P_1, P_2, P_3……… to P_C, respectively. The proportion of a producer consumed by C consumers will not be the sum of the C proportions (Sih *et al.* 1998; Casula *et al.* 2006), for obvious reasons (proportions cannot exceed 100%) (Sih *et al.* 1998; Casula *et al.* 2006). Instead, we adopted the widely accepted idea that the proportion of producer individuals consumed by multiple consumers will follow a ‘multiplicative risk’ model (Sih *et al.* 1998; Casula *et al.* 2006). The expected proportion of producer individuals surviving the presence of C consumers is given by the product $(1-P_1)(1-P_2)(1-P_3)\ldots\ldots(1-P_C)=\prod(1-P_i)$. The amount of producer individuals surviving until the next time interval can be computed as $N_t = N_{t-1} \prod(1 - P_i)$, where P_i is the *per capita* consumption rate of consumer i. Hence, the average across all C consumer species of the per capita rate at which a producer is consumed is $\frac{1-\prod(1-P_i)}{C}$, which shows that increasing consumer diversity C decreases the average across all C consumer species of the per capita consumption rate of a consumer on a producer. In the generalized Lotka–Volterra equations, the a_{ij} are the consumption rates mentioned above explaining why we decreased the a_{ij} as C increases.

Increasing producer diversity.

Increasing producer diversity can lead to multiple producers being consumed by one consumer. Two meta-analyses have shown that the proportion of producer biomass consumed decreases as producer diversity increases, at invariant consumer richness (Hillebrand & Cardinale 2004; Edwards *et al.* 2010). In our model, interspecific competition between producers was weaker than intraspecific competition and was symmetric, leading to greater producer biomass at higher producer diversity. Hence, our choice to constrain the consumption rate a_{ij} always between -0.5 and 0 fixed the absolute consumption while producer biomass increased, as such decreasing the proportion of consumed producer biomass.

Supplementary information 2.2

Experimental organisms

We obtained all algae and consumers from cultures present at the Aquatic Ecology and Water Quality Management group of Wageningen University and Research. Five green alga (*Scendesmus acutus, Chlorella vulgaris, Desmodesmus pannonicum*, *Selenastrum capricornutum* and *Scenedesmus obliquus*) and four cladocerans (*Daphnia magna*, *Daphnia pulex*, *Daphnia lumholtzi* and *Moina macrocopa*) were randomly selected from this species pool for our experiments. These species were isolated in Dutch lakes or ditches, and then cultivated in the lab. These alga were cultured in WC medium in continuous light with a light intensity of 41.29 μmol m^{-2} s^{-1} created by OSRAM LUMILUX Cool Daylight lamps (18W/865) and cladocerans were cultured in RT medium according to (Tollrian 1993) in natural day/night rhythm, feeding them with the algae at a rate of 10^5 cells mL^{-1} per day. The predator (larva of *Chaoborus obscuripes*) was collected from Sinderhoeve Experimental Station in Renkum, The Netherlands. We kept all larva of *C. obscuripes* in a 5L plastic bucket with 1.5L pond water and 1.5L WC medium, stored them in a fridge (4-7 °C) to prevent moulting and fed them with cladocerans every three days. Before experiments, consumers and predators were separately moved into WC medium to starve for 24 h, so that their guts were cleared of pre-fed food.

Supplementary information 2.3

Calculation of energy fluxes (F_{ij}) and fresh biomass

To calculate energy fluxes from i to j, F_{ij}, we assumed the energy flux into each trophic level (producer, consumer, predator) was exactly balanced by losses through predation and metabolism (Schwarz *et al.* 2017). The energy flux F_{ij} was then $F_{ij} = \frac{1}{e_j}(X_j + L)$ with e_j the assimilation efficiency of j: 0.85 for *chaoborus* (Rall *et al.* 2010), and 0.35 for consumers (Kuiper *et al.* 2015). X_j (J h^{-1}) is the metabolic rate of all individuals of each trophic level j, and L (J h^{-1}) is the energy loss by predation. We first calculated energy flux to the predator *chaoborus* (since its energy loss to predation is zero), followed by the lower trophic levels. Energy flux was expressed as g C h^{-1}m^{-2} following a conversion factor 1g C=46 kJ (Salonen *et al.* 1976).

The metabolism X_j was calculated as the sum across all individuals within each trophic level. The individual metabolic rate I (J h^{-1}) was calculated as in as Ehnes *et al.* 2011, Barnes *et al.* 2014 and Schwarz *et al.* 2017: $InI = Ini_0 + a \times InM - E \times (\frac{1}{kT})$, where i_0 is the normalisation factor ($e^{21.97}$for *chaoborus* taken from Ehnes *et al.* 2011, $e^{19.75}$ and $e^{16.35}$ for consumers and alga from Brown *et al.* 2004), a is an allometric exponent factor (0.76 for *chaoborus* taken from Ehnes *et al.* 2011, 0.75 and 0.71 for consumers alga from Brown *et al.* 2004), E (eV) is the activation energy (0.66 for *chaoborus* taken from Ehnes *et al.* 2011, 0.69 and 0.63 for consumers alga from Brown *et al.* 2004), k is the Boltmann's constant (8.62×10^{-5} eV K^{-1}), T is the temperature in Kelvin, and M is the fresh biomass of each individual (mg).

To estimate the fresh cell weight of producers, we first estimated mean particle volume (mm^3 $cell^{-1}$) via dividing the total algal bio-volume (mm^3 ml^{-1}) by the number of counted particles (cell ml^{-1}) (Lampert et al. 1994; Lurling 2003). Algal bio-volume and density were measured with a cell counter (innovates AG CASY®- Technology, model TT). Dry cell weight was calculated from the mean particle volumes, multiplied by a conversion coefficient of 0.57 mg mm^{-3} (Boraas 1983), and by a conversion factor of 10 to convert to fresh weight (mg) (Havens 1995). To estimate the fresh body weight of consumers, we first counted individuals, and recorded their lengths L (mm) by microscopy. We then estimated their biovolume (mm^3) as $0.074 \times L^{2.92}$ (Horn 1991), and its fresh body weight by multiplying the biovolume with a conversion factor of 1 (mg mm^{-3}) (Havens 1995) and (Hwang & Heath 1999). To estimate fresh body weight of the predator, the dry biomass (mg) of one *Chaoborus* individual was taken as $0.05 \times L^{3.66}$ (L is length in mm) as done in (Sanful *et al.* 2012), and then transformed to fresh body weight by multiplying with a dry-wet weight conversion efficiency 10 (Havens 1995; Hwang & Heath 1999).

The total fresh biomass (mg) of each trophic level M_i was calculated by multiplying the fresh cell or abundance. Producer community composition was estimated from 900 µL subsamples from each replicate, stained with 100 µl lugol, and using an inverted light microscope (Nikon Corp., Tokyo, Japan) at 200x magnification (producers), and consumer composition was estimated using light microscopy Olympus szx10 (Olympus Corp, Tokyo, Japan) at 10x magnification, by sucking all individuals into a 50 ml culture dish filled with 20 ml WC medium.

Supplementary information 2.4

Pesticides applications

All stock solutions for both chemicals were created in such a way that 5 mL of stock solution with a desired concentration was achieved by dilution with

WC medium. For the stock solution of linuron, we diluted the commercial product Afalon® Flow with a linuron concentration (450 mg ml^{-1}) into 10 µg ml^{-1}. The stock solution of chlorpyrifos was achieved by diluting a commercial formulation Dursban® 4E with a chlorpyrifos concentration of 480 mg ml^{-1} in to 0.1 µg ml^{-1}. Then 5 mL of stock solution was hence added into the experimental system. Each system was filled WC medium up to 500 ml and stirred 16 seconds before the start of the experiment.

Supplementary Tables

Table S2.1 The feasibility for each food web configuration, indicating the percentages of communities with positive equilibrium density.

	Producer diversity	Consumer diversity	Vertical diversity	Feasible webs
1	6	3	2	98.99
2	6	4	2	98.26
3	6	5	2	99.01
4	6	3	3	96.13
5	6	4	3	96.13
6	6	5	3	97.27
7	7	3	2	98.97
8	7	4	2	99.23
9	7	5	2	98.97
10	7	3	3	96.05
11	7	4	3	96.44
12	7	5	3	96.05
13	8	3	2	98.97
14	8	4	2	98.93
15	8	5	2	98.97
16	8	3	3	95.99
17	8	4	3	95.91
18	8	5	3	95.98
19	9	3	2	98.96
20	9	4	2	98.96
21	9	5	2	98.96
22	9	3	3	95.98
23	9	4	3	96.35
24	9	5	3	96.00

Table S2.2 Details on the experimental design. Our experiment included three trophic levels: five algae species (*Scendesmus acutus, Chlorella vulgaris*, *Desmodesmus pannonicum*, *Selenastrum capricornutum* and *Scenedesmus obliquus*), four consumers (*Daphnia magna*, *Daphnia pulex*, *Daphnia lumholtzi* and *Moina macrocopa),* one predator (*Chaoborus obscuripes*). Experiments included eight food web configurations with two treatments of pure food chain (ss, single alga-single consumer; ssp, single alga-single consumer-present predator), two treatments of consumer adversity (sm, single alga-multiple consumers; smp, single alga-multiple consumers and present predator), two treatments of producer diversity (ms, multiple alga-single consumer; msp, multiple alga-single consumer-present predator), and two treatments including both producer and consumer diversity (mm, multiple alga-multiple consumer; mmp, multiple alga-multiple consumer-present predator).

Food webs	systems	Type	First trophic	Second trophic	Third trophic
1	*ss*	Pure food chain	*S. acutus*	*D. pulex*	0
2	*ssp*	Pure food chain	*S. acutus*	*D. pulex*	present predator
3	*sm*	consumer adversity	*S. acutus*	four consumers	0
4	*smp*	consumer adversity	*S. acutus*	four consumers	present predator
5	*ms*	producer diversity	five alga	*D. pulex*	0
6	*msp*	producer diversity	five alga	*D. pulex*	present predator
7	*mm*	producer + consumer adversity	five alga	four consumers	0
8	*mmp*	producer + consumer adversity	five alga	four consumers	present predator

Table S2.3 Results of linear mixed-effects models (LMMs) testing the effect of producer, consumer diversity, and their interactions on predator biomass, energy from consumers to the predator and absolute interaction strength of predator to consumers. (**P*< 0.05, ** *P* < 0.01, *** *P* < 0.001).

Parameters	Predator biomass	Energy flux into the predator	Absolute interaction strength of predator to consumers
Intercept	5.007***	0.810***	0.162
Producer diversity	0.079	0.010	-0.006
Consumer diversity	0.039	0.005	-0.003
Producer × Consumer diversity	-0.126	-0.008	0.001

Table S2.4 Results of linear mixed-effects models testing the effect of horizontal (producer and consumer) and vertical diversity and their pairwise interactions on degree of self-damping, consumer biomass, energy flux into consumers and absolute interaction strength of consumers to producers. (**P*< 0.05, ** *P* < 0.01, *** *P* < 0.001).

Parameters	Degree of self-damping	Consumer biomass	Energy flux into consumers	Absolute interaction strength of consumers to producers
Intercept	0.131***	8.546 ***	1.389 ***	0.168***
Producer	-0.116***	2.766 *	-0.732 ***	-0.117***
Consumer	-0.005**	0.577	-0.136 ***	-0.036*
Vertical	0.014***	-4.152 ***	1.036 ***	0.396***
Producer× Consumer	-0.009**	0.987 *	-0.071 *	0.029***
Consumer × Vertical	-0.002	1.053 *	0.304 ***	-0.098*
Producer × Vertical	0.009***	-0.516	0.947***	-0.050**

Table S2.5 Results of linear mixed-effects models testing the effect of horizontal (producer and consumer) and vertical diversity and their pairwise interactions on functional resilience after (herbicide or insecticide) exposure, and compositional resilience after (herbicide or insecticide) exposure. (*P< 0.05, ** P < 0.01, *** P < 0.001).

Parameters	Functional resilience after herbicide exposure	Functional resilience after insecticide exposure	Compositional resilience after herbicide exposure	Compositional resilience after insecticide exposure
Intercept	0.362	0.265	0.102	0.035
Producer	0.525*	0.399	0.272*	0.312*
Consumer	0.631***	0.079*	0.156***	0.0334
Vertical	-0.089	-0.081*	-0.176***	-0.157***
Producer× Consumer	0.369***	0.197***	0.105**	0.167***
Consumer × Vertical	-0.661***	-0.037	-0.093*	0.065
Producer × Vertical	-0.176*	-0.297**	0.010	-0.096*

Table S2.6. Results of linear mixed-effects models (LMMs) testing the effect of producer, consumer and vertical diversity, stress (herbicide, insecticide), day and the pairwise interactions on total biomass (sum all trophic levels). (**P*< 0.05, ** *P* < 0.01, *** *P* < 0.001).

Parameters	Total Biomass herbicide as stress	Total Biomass insecticide as stress
(Intercept)	25.061	-1.496
Producer	25.687***	-3.201
Consumer	3.578	-9.049
Vertical	-37.923**	12.418
stress	-0.929***	0.545*
day	-5.349**	-0.620
Producer× Consumer	-2.388**	0.683
Consumer × Vertical	4.900	1.274
Producer× Vertical	-3.903	0.344
Producer × stress	-0.099***	0.042*
Consumer × stress	-0.120***	0.0870**
Vertical × stress	0.613***	-0.456***
Producer × day	0.358*	0.905***
Consumer × day	-1.131***	-0.494*
Vertical × day	6.418***	3.276***
day × stress	0.052***	-0.026***

Supplementary Figures

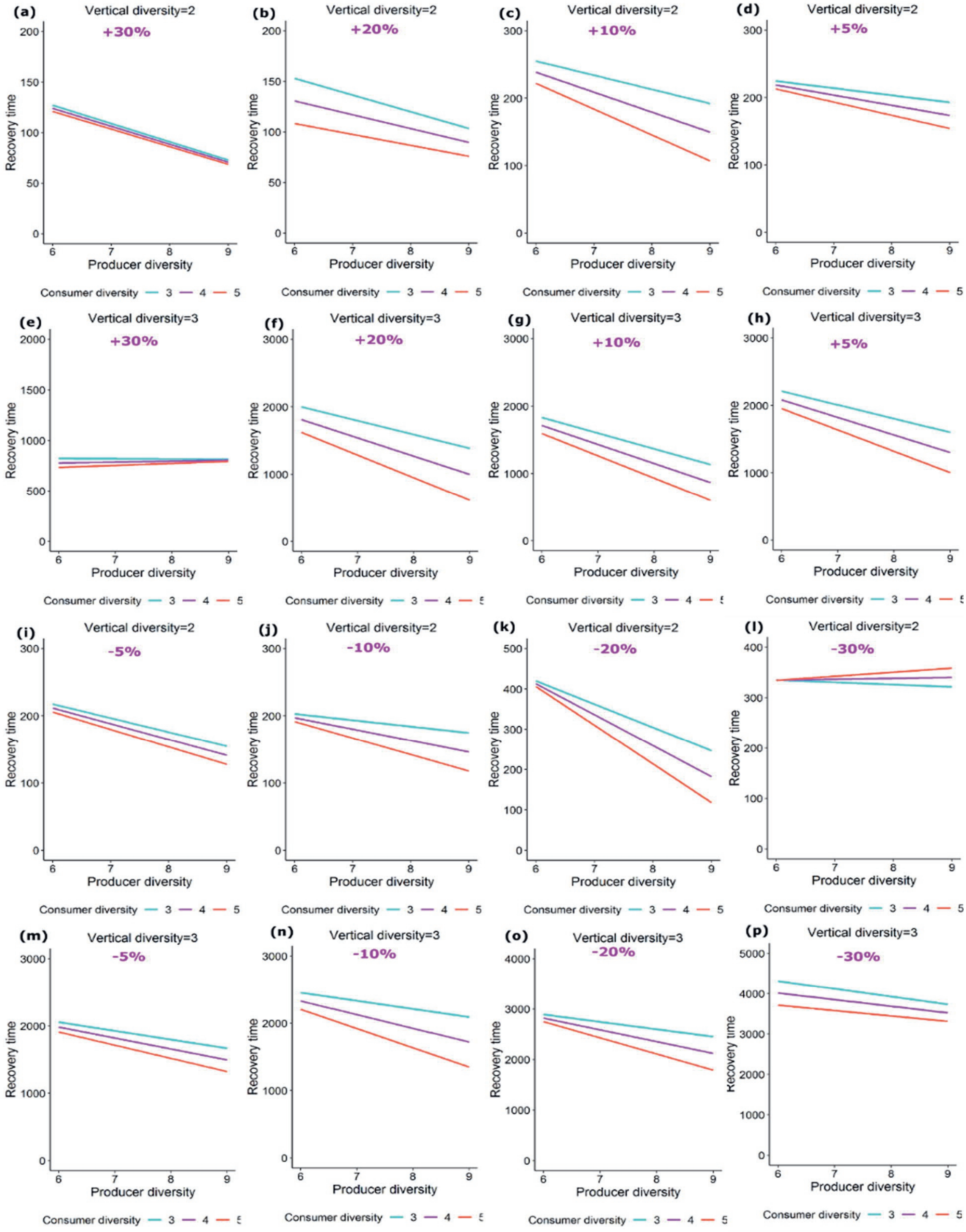


Figure S2.1 Model sensitivity analysis: The influence of varying all parameters simultaneously (global sensitivity analysis) from (-30%, -20%, -10%,-5%, +5%, +10%,+20%, to +30%) on the recovery time response to diversity.

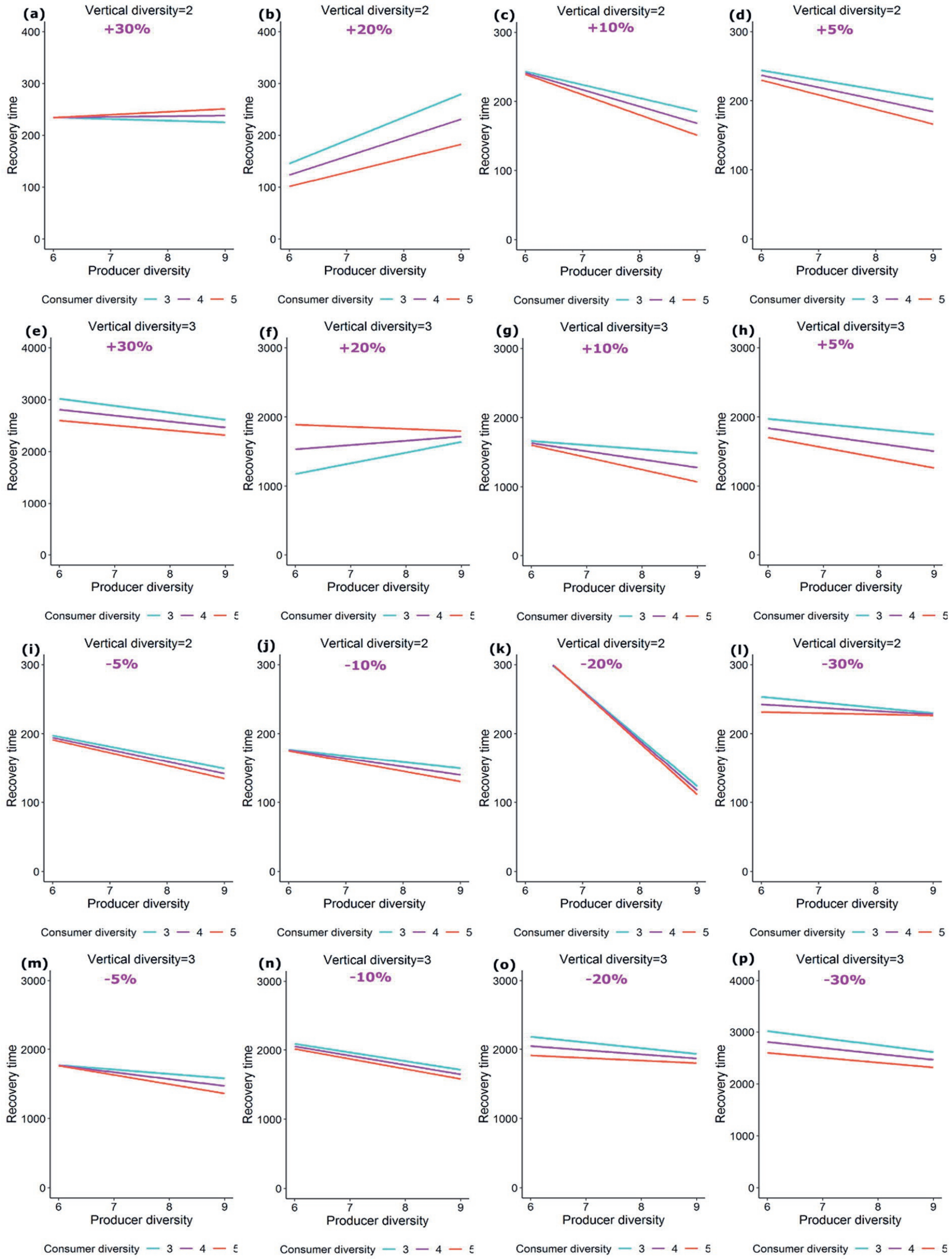


Figure S2.2 Model sensitivity analysis: The influence of varying only conversion efficiency k (-30%, -20%, -10%,-5%, +5%, +10%,+20%, to +30%), while keeping all other parameters fixed as in the main text (local sensitivity analysis), on the recovery time response to diversity.

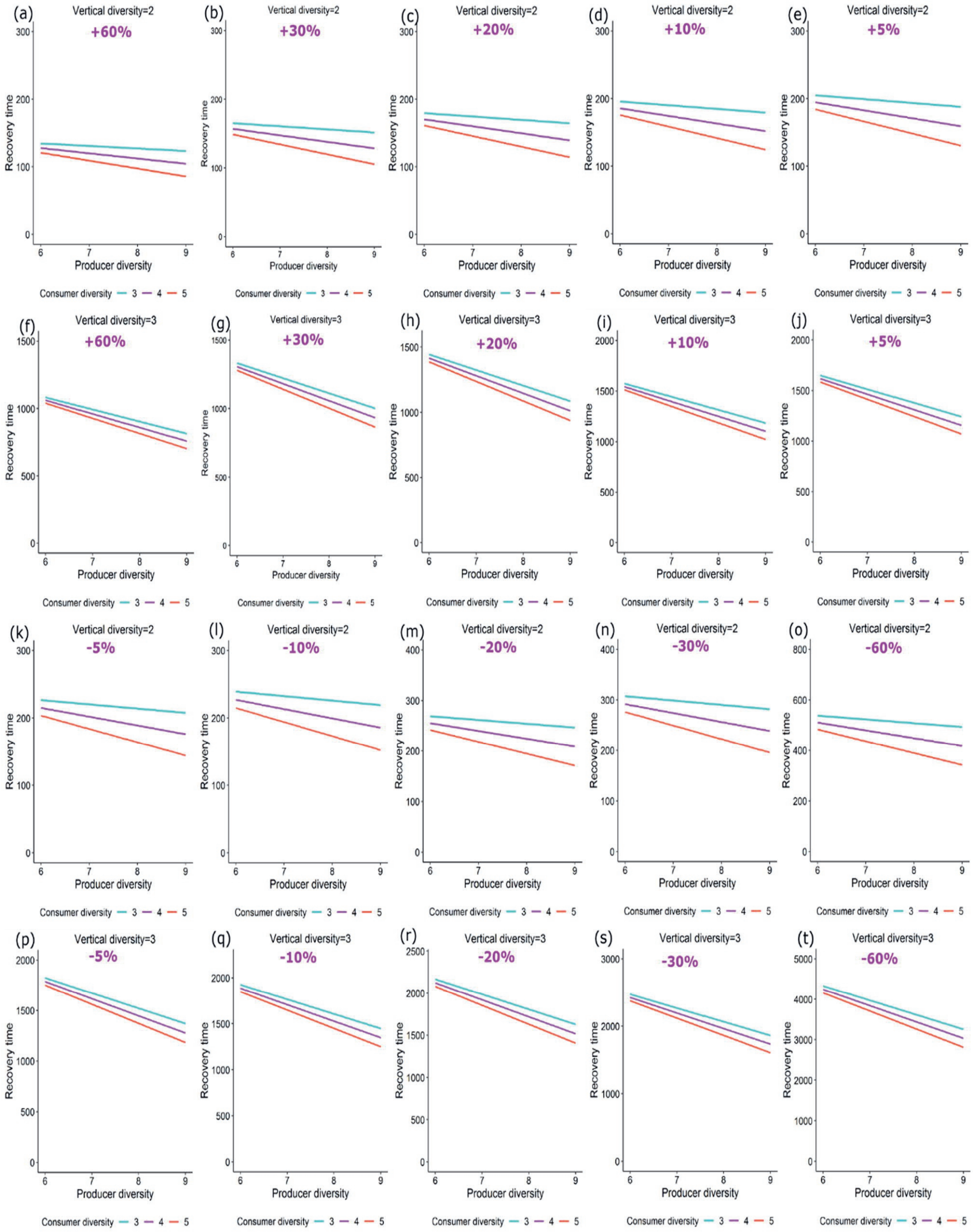


Figure S2.3 As Fig. S2.1, but keeping the conversion efficiency k fixed to the value specified in the main text. All other parameters were varied (-60%, -30%, -20%, -10%,-5%, +5%, +10%,+20%, +30% to +60%).

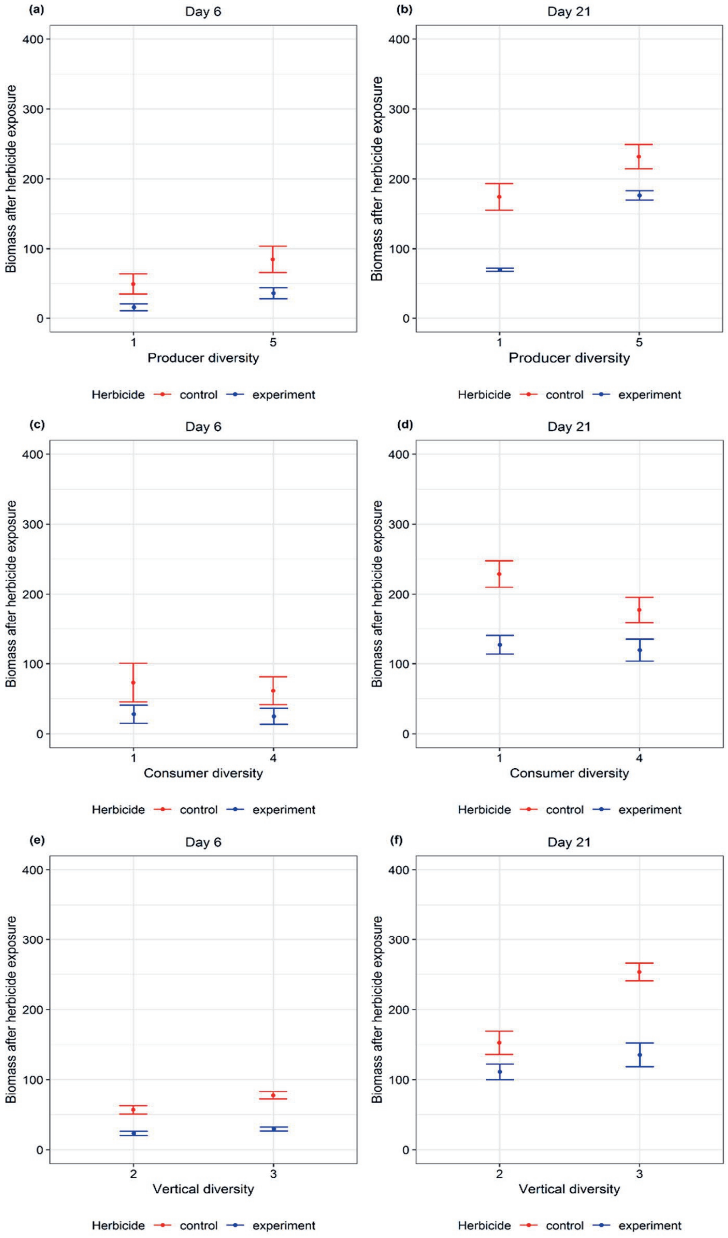


Figure S2.4 The effects of producer diversity (a, d), consumer diversity (b, e) and vertical diversity (c, f) on total biomass (sum all trophic levels) after herbicide exposure on day 6 and 21. Plotted are sample mean ± one s.d. Detailed statistical results are listed in Table S2.6.

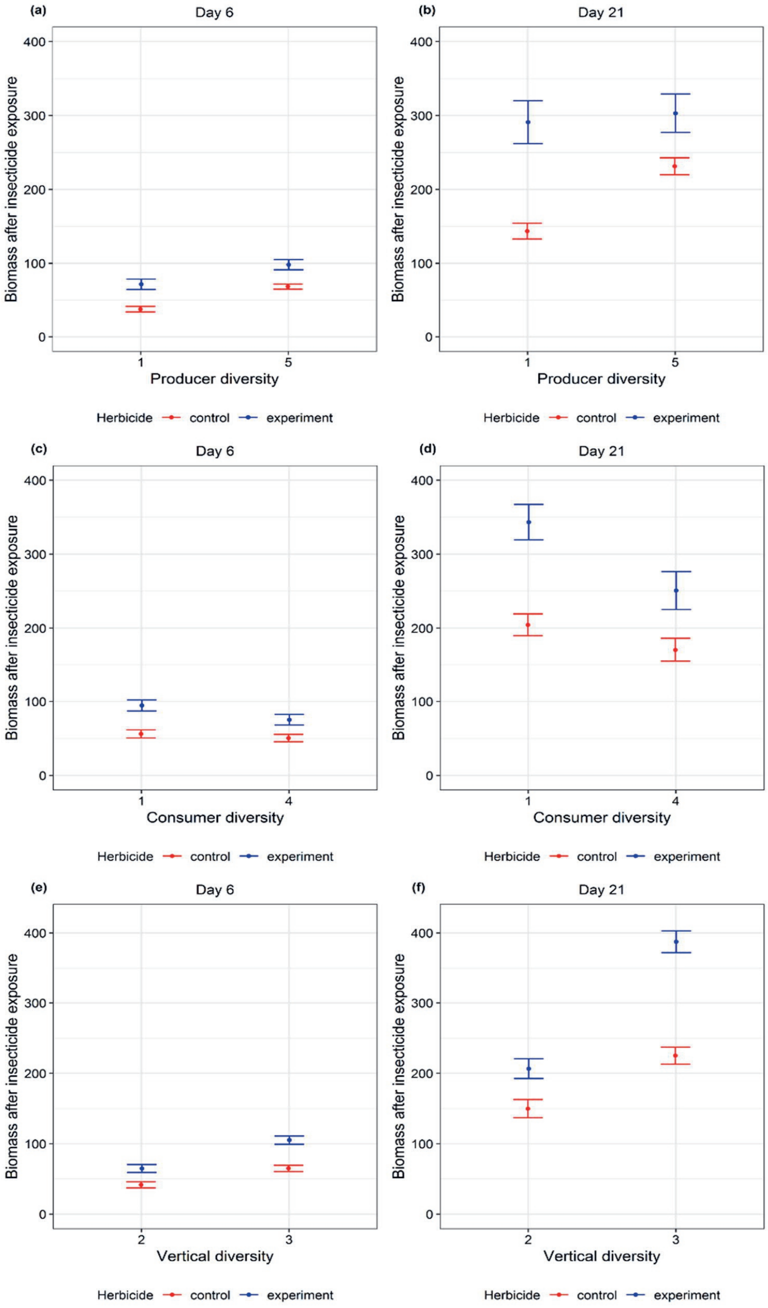


Figure S2.5 The effects of producer diversity (a, d), consumer diversity (b, e) and vertical diversity (c, f) on total biomass (sum all trophic levels) after insecticide exposure on day 6 and 21. Plotted are sample mean ± one s.d. Detailed statistical results are listed in Table S2.6.

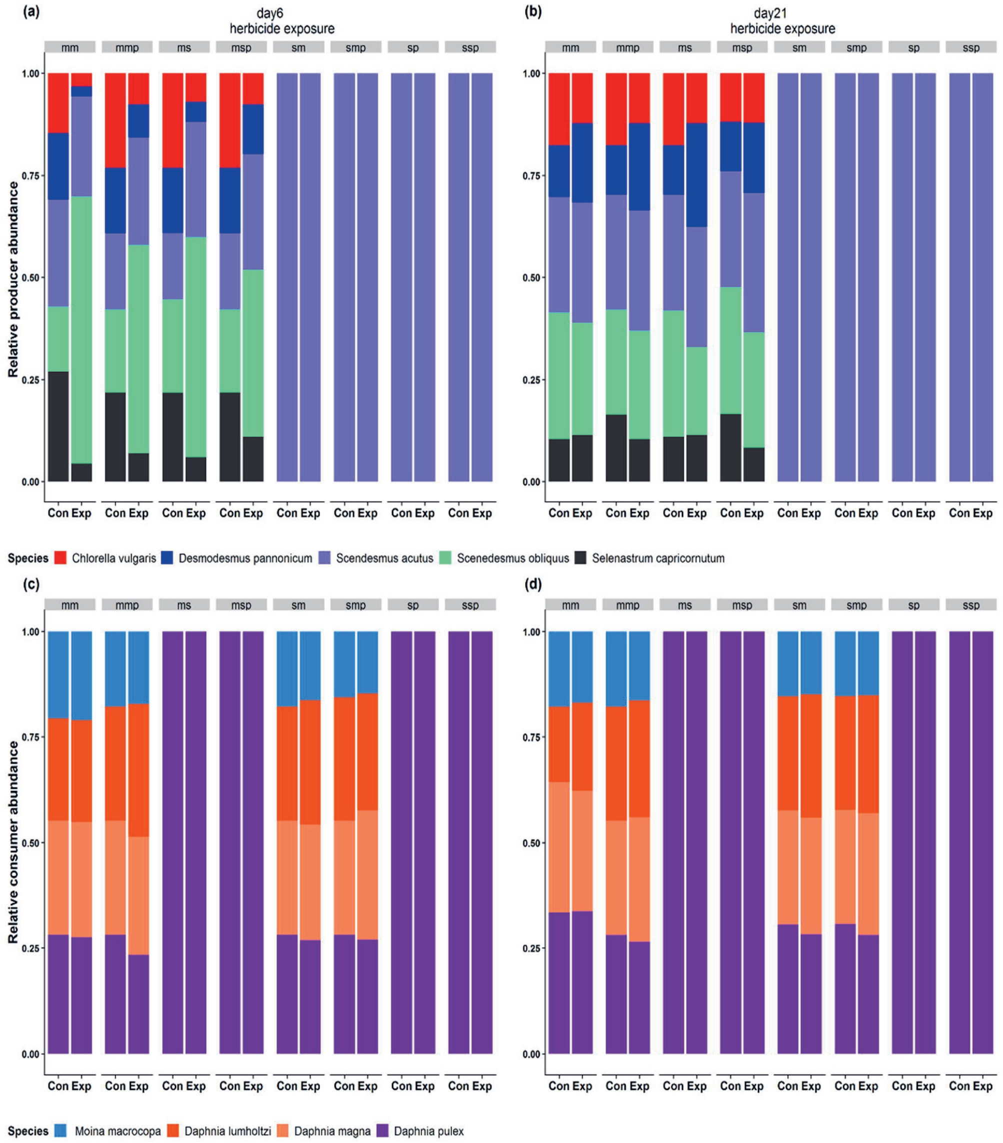


Figure S2.6 Relative producer (a and b) and consumer (c and d) abundance on the eight treatments after herbicide exposure on day 6 and 21. Con means control group, and Exp stands for exposure to chemicals. (Eight treatments include *ss*, single algae-single consumer; *ssp*, single algae-single consumer-present predator; *sm*, single algae-multiple consumers; *smp*, single alga-multiple consumers-present predator; *ms*, multiple algae-single consumer; *msp*, multiple algae-single consumer-present predator; *mm*, multiple algae-multiple consumer; *mmp*, multiple algae-multiple consumer-present predator).

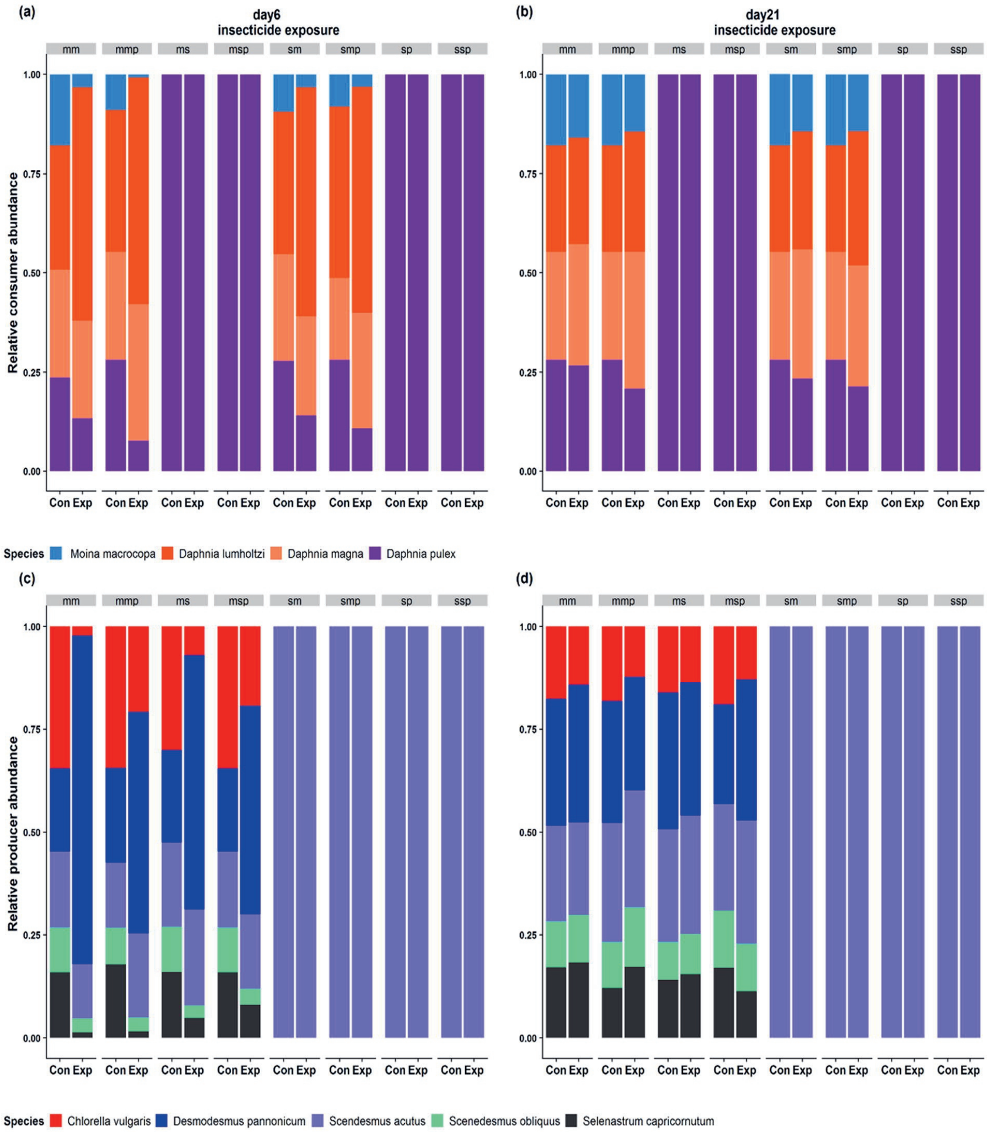


Figure S2.7 Relative producer (a and b) and consumer (c and d) abundance on the eight treatments after insecticide exposure on day 6 and 21. Con means control group, and Exp stands for exposure to chemicals. (Eight treatments include ss, single algae-single consumer; ssp, single algae-single consumer-present predator; sm, single algae-multiple consumers; smp, single alga-multiple consumers-present predator; ms, multiple algae-single consumer; msp, multiple algae-single consumer-present predator; mm, multiple algae-multiple consumer; mmp, multiple algae-multiple consumer-present predator).

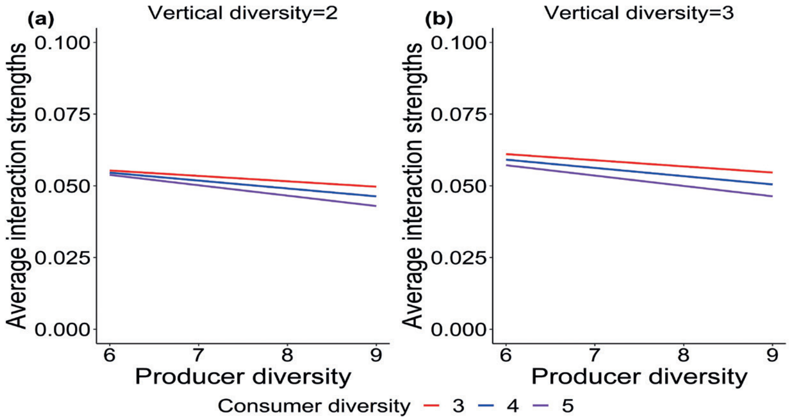


Figure S2.8 Model simulations illustrating the interactive effects of horizontal (producer and consumer) and vertical diversity on average interaction strengths.

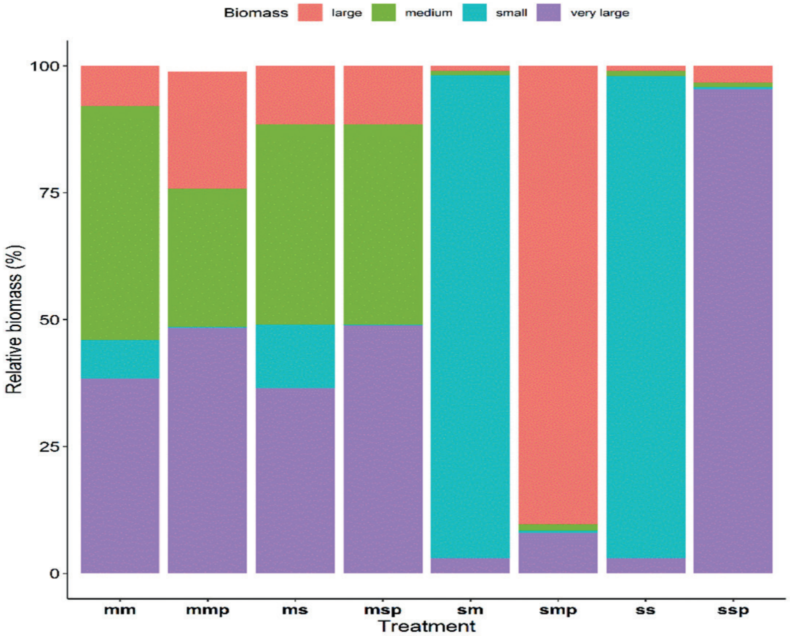


Figure S2.9 The proportion of fresh biomass composition for consumers across the eight treatments involving four order individual biomass (small, < 1.09×10^{-5} mg with body length range <0.10 mm; medium, 1.09×10^{-5} -0.43 mg with length range 0.10 -1.80 mm; large, 0.43-1.05 mg with length range 1.80 -2.30 mm; very large, >1.05 mg with length range >2.3 mm). (Eight treatments include ss, single algae-single consumer; ssp, single algae-single consumer-present predator; sm, single algae-multiple consumers; smp, single alga-multiple consumers-present predator; ms, multiple algae-single consumer; msp, multiple algae-single consumer-present predator; mm, multiple algae-multiple consumer; mmp, multiple algae-multiple consumer-present predator).

Chapter 3

Community composition modifies direct and indirect effects of pesticides in freshwater food webs

Zhao, Q., De Laender, F. and Van den Brink, P.J., 2020. Community composition modifies direct and indirect effects of pesticides in freshwater food webs. *Science of The Total Environment*, 739, 139531.

Abstract

For environmental risk assessment, the effects of pesticides on aquatic ecosystems are often assessed based on single species tests, disregarding the potential influence of community composition. We, therefore, studied the influence of changing the horizontal (the number of species within trophic levels) and vertical composition (number of trophic levels) on the ecological effects of the herbicide linuron and the insecticide chlorpyrifos, targeting producers and herbivores, respectively. We tested how adding, to a single primary producer, 4 selected competing producer species, 0-1-4 selected herbivore species, and one selected predator species resulting in 1, 2 and 3 trophic levels, changes the effects of the two pesticides.

Linuron decreased producer biovolume less (17%) when the 4 producers were added, because insensitive producers compensated for the loss of sensitive producers. However, linuron decreased producer biovolume 42% and 32% more as we increased the number of herbivore species from 0 to 4 and as we increased trophic levels from 1 to 3, respectively. The indirect negative effect of linuron on herbivore biovolume was 11% and 15% lower when more producer and herbivores were added, respectively. Adding a predator increased this indirect negative effect by 22%.

Chlorpyrifos decreased herbivore biovolume about 10% less when adding multiple herbivore or producer species. However, adding a predator magnified the direct negative impact on herbivores (13%). Increasing the number of producer, herbivore species and adding trophic levels increased the indirect positive impact on producer biovolume (between 10% and 35%).

Our study shows that changing horizontal composition can both increase and decrease the effects of the selected pesticides, while changing vertical composition by adding number of trophic levels always increased these effects. Therefore, single species sensitivity will not always represent a worst case estimate of ecological effects. Protecting the most sensitive species may not ensure protection of ecosystems.

3.1 Introduction

Ecological risk assessment of chemicals is mainly based on the results of single-species laboratory tests performed with algae, daphnia and fish, representing a limited set of standard test species (Brock *et al.* 2006; Artigas *et al.* 2012; Rohr *et al.* 2016). However, community composition in natural ecosystems often is more complex and how to address this difference in community composition is considered one of the most difficult challenges in ecotoxicology (De Laender & Janssen 2013; Rohr *et al.* 2016; Van den Brink *et al.* 2018). Community composition in natural systems can be characterised in two dimensions: the number of species within trophic levels (horizontal composition) and number of trophic levels (vertical composition). Both dimensions of composition could influence the effects of chemicals on aquatic communities (De Laender *et al.* 2015; Baert *et al.* 2016; Zhao *et al.* 2019). Recent work showed that the two dimensions had contrasting effects on the short-term stability of whole food webs (using total biomass as a proxy) after pesticide exposure (Zhao *et al.* 2019). However, how the two dimensions influence direct and indirect effects of chemicals after prolonged exposure is at present unknown.

The direct negative effects of herbicides on population size of primary producers (hereafter named ‘producers’) can be smaller when more producer species are added (Baert *et al.* 2016). A more diverse producers’ community can include both sensitive and tolerant producers (Baert *et al.* 2016). When environmental stressors reduce the population of sensitive producers, negative interactions among producers result in competitive release, so that reductions in populations of sensitive species can be compensated by an increase of tolerant species (Gonzalez & Loreau 2009; Baert *et al.* 2016; De Laender *et al.* 2016). In contrast, the direct negative effects of herbicides on producer populations can be larger as more herbivore species are added, because herbicides and herbivore grazing could interact to aggravate the herbicide effects (Rohr & Crumrine 2005; Rohr *et al.* 2006; Halstead *et al.* 2014). Conversely, the presence of a predator could suppress the herbivore population (Anderson *et al.* 1996; Pace *et al.* 1999), and the resulting decrease in grazing pressure could alleviate the direct negative effects of herbicides on producers.

The direct negative effects of insecticides on herbivores (population size) can be smaller as more herbivore species are added, again due to compensation. A more diverse herbivores' community can include both sensitive and tolerant herbivores, while more intolerant herbivores have larger probability to be included (Becker & Liess 2017). Insecticides decrease the populations of sensitive herbivores, resulting in its resource (producers) being released from grazing, which in turn can result in an increase of tolerant herbivores via an increase of food resources (Rohr & Crumrine 2005). The indirect benefit of insecticides on tolerant herbivores can thus compensate the decline of sensitive herbivores. The insecticide can also be hypothesized to affect herbivores less when more producer species are added, because of an increased probability that an edible producer would occur that promotes herbivore growth (Haddad *et al.* 2011). However, the insecticide could affect herbivore population size more severely when a predator is present, because of synergistic interactions between the insecticide and predation (Relyea & Mills 2001; Beketov & Liess 2006; Trekels *et al.* 2013). For example, Relyea and Mills (2001) reported that the pesticide carbaryl was 4 times more toxic to the prey (tadpoles) when a predator (*Ambystoma maculatum*) was present. Some studies, however, showed that interactions between insecticides and presence of a predator on herbivores can be additive or antagonistic (Campero *et al.* 2007; Trekels *et al.* 2011b; Janssens & Stoks 2013, 2017).

The indirect effects of pesticides are also expected to depend on horizontal and vertical composition. Herbicides could indirectly decrease herbivore population size, due to a decrease in edible producer biomass (Preston 2002; Fleeger *et al.* 2003; Bracewell *et al.* 2019). We expect that the herbicides could decrease herbivores even more when a predator is present, due to an increase of both bottom-up and top-down control (Rohr & Crumrine 2005; Rohr *et al.* 2006; Clements & Rohr 2009). In addition, insecticides could, indirectly, induce an increase of producer population size, because of the top-down induced release of producers (Rohr & Crumrine 2005; Rohr *et al.* 2006;

Clements & Rohr 2009; Halstead *et al.* 2014). It is thus expected that the release of producers could be stronger when a predator is present as this will serve as an extra top-down effect.

To test these hypotheses, we conducted microcosm experiments mimicking planktonic food webs in which we added 4 selected competing producer species to a single producer, 0, 1 or 4 selected herbivore species, and one selected predator species. By doing so we also changed vertical composition (1, 2 and 3 trophic levels). We then tested whether horizontal and vertical composition influences either the effects of the herbicide linuron or the effect of insecticide chlorpyrifos

3.2 Materials and Methods

3.2.1. Experimental conditions

We experimentally tested the effect of horizontal and vertical composition on simple food webs exposed to pesticides. The experiments, which lasted for 21 days, were performed in 900 mL glass jars, filled with 500 ml WC medium and contained in a water bath at constant temperature (19.9 °C ± 0.8 °C) and a light regime of 12h: 12h (light: dark). The light intensity at the surface (measured with a LI-COR LI-250A, LI-COR Biosciences, Lincoln, USA) was 120 µmol m^{-2} s^{-1}, and was created using Ceramalux® Phillips 430 Watt High Pressure Sodium Non-Cycling Lamps.

3.2.2. Organisms

We obtained all producers and herbivores from cultures present at the Aquatic Ecology and Water Quality Management group of Wageningen University. Five green alga (*Scenedesmus acutus, Chlorella vulgaris, Desmodesmus pannonicus, Raphidocelis subcapitata* and *Scenedesmus obliquus*) were randomly selected as producers while four cladoceran (*Daphnia magna*, *Daphnia pulex*, *Daphnia lumholtzi* and *Moina macrocopa*) species were randomly selected as herbivores. All these organisms were collected from Dutch lakes or ditches and then cultivated in the lab. The algae were cultured in WC medium under continuous light. The herbivores were cultured in RT medium using a natural day/night light rhythm (Tollrian 1993) and fed with algae *C. vulgaris* at 10^{-5} cell ml^{-1} day^{-1}. One individual of *Chaoborus obscuripes* was selected as a predator. *C. obscuripes* was collected from Sinderhoeve Experimental Station (www.sinderhoeve.org; Renkum, The Netherlands). Before addition, *C. obscuripes* was kept in a 5 L plastic bucket with 1.5 L pond water and 1.5 L WC medium, stored in a fridge (4-7 °C) to slower the moulting and fed with cladocerans every three days. Before the experiments started, herbivores and predators were separately moved into WC medium to starve for 24 h, so that their guts were cleared of pre-fed food.

3.2.3. Experimental setup

To a single randomly selected primary producer (*R. subcapitata*), we added 4 producers, 0, 1, or 4 selected herbivores, and one selected predator resulting in 1, 2 and 3 trophic levels (vertical composition). The other four producers were *S. acutus, C. vulgaris, D. pannonicus* and *S. obliquus*. The single herbivore was *M. macrocopa* (randomly assigned). The other three herbivores were *D. magna, D. pulex,* and *D. lumholtzi*. The predator was *C. obscuripes*.

We adopted a design where we manipulated horizontal and vertical composition, as well as the exposure to contaminants. To manipulate composition, we crossed horizontal composition of the producers (two levels; 1 or 5 species) and horizontal composition of the herbivores (three levels; 0, 1 or 4 species), resulting in 6 food-web structures. When consumers were present, we also manipulated the presence of a predator (absent or present), resulting in 4 more food-web structures. We therefore also manipulated vertical composition (1, 2 and 3 trophic levels). This gives a total of 10 different food-web structures. To manipulate exposure to contaminants, we either exposed these compositions to the insecticide chlorpyrifos (0 and 1 µg l^{-1}), or the herbicide linuron (0 and 100 µg l^{-1}). The 0 µg l^{-1} linuron and chlorpyrifos treatments served as controls. The nominal concentration of 100 µg l^{-1} linuron was chosen because it is higher than the 72d EC_{50} for relative growth inhibition of 6 µg l^{-1} for *Scenedesmus acutus* (Snel *et al.* 1998) and lower than the 21 days NOEC value (180 µg l^{-1}) for reproduction of *D. magna* (Crane *et al.* 2007). It was expected that the concentration had no direct toxic effect on herbivores but only on producers (Cuppen *et al.* 1997; Slijkerman *et al.* 2005).

The nominal chlorpyrifos concentration of 1 µg l^{-1} is the 48h LC_{50} value for *D. magna* (Kersting & van Wijngaarden 1992), so that treatment effects were supposed to not completely eliminate the herbivores and allow recovery (Van den Brink *et al.* 1996; Daam *et al.* 2008a).

We replicated each treatment four times, leading to 10 food web structures x 2 contaminants x 2 treatments (control and contaminant treatment) x 4 replicates = 160 vessels in total. The initial total biovolume of producer and herbivores was always 25 mm^3 and 0.2 mm^3, respectively, regardless of producer and herbivores richness. For the systems with all three trophic levels, we added one individual of the predator *C. obscuripes* to each system. We made sure the predators used in the experiments had a mean (±SD) individual body length of 10.46 ± 0.11 mm to avoid a bias introduced by body size-dependent feeding rates.

3.2.4. Chemical application and analysis

All stock solutions for linuron and chlorpyrifos were created in a same way that 5 mL of stock solution was diluted with WC medium to reach the desired concentration. For the stock solution of linuron, we diluted the commercial product Afalon® Flow with a linuron concentration of 450 mg ml^{-1} to 10 µg ml^{-1}. The stock solution of chlorpyrifos was achieved by diluting a commercial formulation Dursban® 4E, with a chlorpyrifos concentration of 480 mg ml^{-1} to 0.1 µg ml^{-1}. Then 5 mL of stock solution was added into the system. Each system was filled WC medium up to 500 ml and stirred 15 seconds immediately before the start of the experiment.

To monitor the chemical degradation during the experiment samples were taken after 1h, 2, 4, 6, 14 and 21 days of exposure. In order to analytically verify the linuron concentration of each experimental jar, 2 mL of water sample was added to 0.5 ml methanol. The chemical concentration was analysed according to Van den Brink et al. (1997), through Agilent Technologies LC-QQQ Mass spectrometer with a binary pump, Bin Pump, model G1312A, with MilliQ + 0.1% fatty acid as solvent A and methanol + 0.1 % fatty acid as solvent B with a ratio of 20:80. For the chlorpyrifos analysis, 8 mL samples were taken from each system and then 2 ml *n*-hexane was added, followed by vortex for 1 minute under 1000 revolution per second. A 1mL subsample was transferred to a GC vial, then followed by GC and electron capture detection to determine the exact concentration of chlorpyrifos (Rubach *et al.* 2011).

3.2.5. Ecological endpoints

We estimated the biovolume and composition of producers and herbivores, and biovolume of predators in each replicate on day 2, 4, 6, 14 and 21 day after the beginning of the experiment. At each sampling day, we first sampled the controls followed by the exposure jars to prevent cross contamination. Producer biovolume ($mm^3\ l^{-1}$) was measured with a CASY® Cell Counter model TT (innovates AG CASY®- Technology). In order to estimate algae composition, 900 µl algae samples were stained with 100 µl lugol preservative for microscopic enumeration of algal cells using an inverted light-microscope (Nikon Eclipse E100 microscope with a DS-2Mv-L2 camera; Nikon Corp., Tokyo, Japan) at 200 magnification. Herbivore biovolume ($mm^3\ l^{-1}$) in each replicate was calculated as abundance (individual l^{-1}) times individual biovolume (mm^3 individual^{-1}). The individual biovolume (mm^3 individual^{-1}) of herbivores and predators were measured by a formula $0.074 * L^{2.92}$ (L is length in mm) (Horn 1991), where the body length was estimated using light microscopy Olympus szx10 (Olympus Corp, Tokyo, Japan) at 10 magnification. Abundance and composition was recorded after sucking all individuals into an inverted 10 mL serological pipette to put into 50 ml culture dish that filled with 20 ml WC medium. Afterwards herbivores and predators were put back in their beakers for next sampling.

3.2.6. Data analyses

Biovolume or abundance were used to calculate the effect sizes for the producers, herbivores and the predator, while chlorophyll a was used to compute effect size for photosynthetic capacity. Effects sizes were calculated by dividing the value for the treatment by the mean of control so that an effect size smaller than 1 indicates a negative impact of the chemical on the producers, herbivores or the predator, a 1 no effect, while effect sizes larger than 1 indicates a positive impact. To each of chemicals, three-way ANOVA’s were used to estimate the effects of the horizontal composition of producers and herbivores, the vertical composition and with all combination of interactions on the effect sizes of producers (abundance, biovolume, chlorophyll *a*) and herbivores (abundance or biovolume) on sampling days 2, 4, 6, 14 and 21, respectively, yielding 50 three-way ANOVA's (5 response variables × 5 sample days × 2 pesticides). We adopted the same approach for the effect sizes of predator biovolume. However, note that by definition, in the case of the presence of a predator, the vertical composition was always three, so we could only analyse the effects of horizontal composition, yielding 10 two-way ANOVA's (1 response variables × 5 sample days × 2 pesticides). Normality of model residuals was verified by the QQ-plot.

The effects of herbicide (insecticide) on producers (herbivores) were the largest on day 6 (see results section). We used raw data (biovolume, density or chlorophyll *a*) on this day to understand the interactions between treatments. The raw data were natural log-transformed prior to analysis. For each pesticide data set, we applied four-way ANOVA's to estimate the effect of horizontal composition of producers and herbivores, vertical composition, pesticide and their pairwise interactions, on (1) producers (abundance, biovolume and chlorophyll *a*) and (2) herbivores (abundance and biovolume), respectively, yielding 10 four-way ANOVA's (5 response variables × 2 pesticides). Normality of model residuals was verified by the QQ-plot. We adopted the same statistical approach for the effect on predator biovolume. However, note that by definition, vertical composition is always three when a predator is present, so we could only analyse the effects of horizontal composition, yielding 2 three-way ANOVA's (1 response variable × 2 pesticides). Normality of model residuals was again verified by the quantile–quantile (QQ) plot. Finally, to evaluate the effects on community composition on day 6, the day with the maximum effects, we again used four-way ANOVA's to test the effect of horizontal composition of producers, herbivores and vertical composition, pesticide and their pairwise interactions, on ln(biovolume) of (1) the producer species (*R. subcapitata,* i.e. the single producer treatment) and (2) the herbivore species (*M. macrocopa,* i.e. the single herbivore treatment). For the other four producer species (*S. acutus, C.*

vulgaris, D. pannonicus and *S. obliquus*), a three-way ANOVA's was used to test the effect of horizontal composition of herbivores, vertical composition, pesticides and their pairwise interactions on ln(biovolume), because the horizontal composition of producers was always five. Similarly, we used three-way ANOVA's to test the effect of horizontal composition of producers, vertical composition, pesticide and their pairwise interactions on ln(biovolume) of each of the rest three herbivore species (*D. magna, D. pulex*, and *D. lumholtzi*), because the horizontal composition of herbivores was always four. The analysis of community composition yielded 18 ANOVA's (9 response variables × 2 pesticides). Normality of model residuals was again verified by the quantile–quantile (QQ) plot.

3.3 Results and discussion

3.3.1. Pesticide concentration

The mean start concentrations for linuron and chlorpyrifos in the experimental systems were 94.2 (± 8.4)% and 87.8 (± 9.4)% of the nominal concentrations, respectively (Fig 3.1). The dissipation half-life (DT50) for linuron could not be calculated (> 21 d; Fig 3.1), while the DT50 of chlorpyrifos was between 5-8 days. The observed persistence of linuron and chlorpyrifos were in line with those observed in other planktonic systems by Daam et al. (2008, 2009) and Daam and Van den Brink (2007) who reported DT50 values of > 21 days for linuron and 6-10 d for chlorpyrifos.

3.3.2. Effect of linuron

3.3.2.1 Influence of community composition on direct effects

Throughout experiments, the direct negative effect of the herbicide linuron on producer biovolume was, on average, 17% smaller when the 4 producer species were added (Fig. 3.2a). However, this direct negative effect was 42% larger when the number of herbivore species was increased from 0 to 4 and 32% larger when vertical composition was changed from 1 to 3 (Fig. 3.2b-c). On day 6, linuron had its maximum effect on producer biovolume (Fig. 3.2a-c). The negative effect of linuron on producer biovolume was larger when adding more herbivore species and when vertical composition was higher, regardless of the composition of the producer community (Fig. 3.3a-b). The negative effects were strongest when the number of producer species was lowest, the number of herbivore species highest, and vertical composition equal to 2 (Fig. 3.3a). These trends were similar when using chlorophyll *a* (photo- synthetic capacity) as an endpoint (Figs. S3.1a–c; S3.2a–b).

Adding producers decreased the direct negative effect on producer biovolume (Fig. 3.2a) and chlorophyll a (Fig. S3.1a), due to the decrease of sensitive chlorophytes biovolume (e.g. *R. subcapitata and C. vulgaris*) compensated by other tolerant chlorophytes (e.g. *S. obliquus*) (Fig. 3.4a). *R. subcapitata*, previously known as *Selenastrum capricornutum* and *Pseudokirchneriella subcapitata,* has a 5d EC_{50} of 67 μg l^{-1} based on abundance (USEPA, 2020), explaining its decrease in biovolume (Fig. 3.4a). *C. vulgaris* and to a lesser extent *S. acutus* also show a decrease in biovolume, which can be explained by their 7d EC_{50} of 50 μg l^{-1} also based on abundance and 3d EC_{50} of 8.9 μg l^{-1} based on population growth rate, respectively (Stephenson & Kane 1984; USEPA 2020). The other two species, *D. pannonicus* and *S. obliquus*, showed no response or an increase in biovolume (Fig. 3.4a) and, unfortunately, no sensitivity data is available, but *S. obliquus* became relatively abundant in small plankton dominated microcosms stressed by 150 μg l^{-1} linuron (Daam & Van den Brink 2007). Some semi-field experiments also showed that linuron had both positive and negative effects on Chlorophytes (Van den Brink *et al.* 1997; Slijkerman *et al.* 2005; Daam *et al.* 2009). For example, Daam et al. (2009) reported that some of chlorophytes (e.g., *Coelastrum cambricum* and *Pediastrum duplex*) decreased in population size, which was compensated by increases of other chlorophytes (e.g., *Ankistrodesmus falcatus*, *Oocystis pusilla* and *Oocystis lacustris*).

In contrast, the negative impact of linuron on producers was larger when more herbivores were added (Fig. 3.2b), due to a larger suppression of producer biovolume (e.g. *C. vulgaris*) (Fig. 3.4a) when multiple herbivores were present. Multiple herbivore species more effectively reduce producer population sizes than a single herbivore species because of larger consumption rates (Naeem & Li 1998; Duffy *et al.* 2003).

The presence of a predator (*C. obscuripes*) decreased the biovolume of the herbivores (Fig. 3.3c-d; Fig. 3.4b), as has also been reported by Black and Dodson (1990) and Hebert and Grewe (1985), due to predation. The presence of the predator hence alleviated the grazing pressure on producers, which made the direct negative effect of linuron on producers smaller than the treatments with producers and herbivores only, i.e. vertical composition=2, (Fig. 3.2c). However, the presence of the predator did not eliminate all herbivores. Thus, the herbivores still consumed producers (e.g., especially small sized *C. vulgaris*) (Fig. 3.4a). Hence, the presence of a predator and herbivores still made the negative effect of linuron on producers larger than the negative effect of linuron on producers in treatments where only producers were present, i.e. when vertical composition was equal to 1, (Fig. 3.2c).

3.3.2.2 Influence of community composition on indirect effects

The herbicide-induced decrease of producers led to indirect negative effects on herbivore biovolume (Fig. 3.2d-f). Some semi-field experiments found both negative and positive impacts of linuron on herbivores (Cuppen *et al.* 1997; Daam *et al.* 2009). For example, Cuppen et al. (1997) reported negative effects of linuron on Rotatoria but positive effects on Copepoda. They attributed these negative and positive linuron effects to the preferred resources of these herbivores, i.e. diatoms for Rotatoria and *Chlamydomonas* for Copepoda, respectively, the latter showing a large increase in the linuron stressed systems (Van den Brink *et al.* 1997). Here, we only found negative effects of linuron on herbivores, which can be attributed to herbivores consuming all producer species and the overall decrease in biovolume of the algae species (Fig. 3.4a) as no adaptation was found like as by Van den Brink et al. (1997).

In addition, the indirect negative effect of linuron on herbivore biovolume was 11% smaller when 4 producers were added and 15% smaller when the number of herbivore species was increased from 1 to 4 (Fig. 3.2d-e). This was because adding more producers and herbivores caused an increase of the absolute biovolume of herbivores on day 6 (Fig. 3.4b). However, the indirect negative effect of linuron on herbivores was 22% larger when vertical composition was changed from 2 to 3 (Fig. 3.2f), because predation decreased the absolute biovolume of each herbivore species (Fig. 3.4b). On day 6, linuron also had its maximum effect on herbivore biovolume (Fig. 3.2d-f). The negative effect of linuron on herbivore biovolume was smaller when more producers and herbivores were present, independent of vertical composition (Fig 3.3c-d). The negative effects were smallest when the number of producer species was highest, the number of herbivore species highest and the vertical composition equal to 2 (Fig 3.3c). We detected qualitatively identical results (single and interactive effects) using abundance as a proxy (Fig S3.1d–i; Fig. S3.2), even though the magnitude of decreases and increases was smaller. We did not detect significant effect of composition on the predator biovolume (Table S3.1).

3.3.3. Effect of chlorpyrifos

3.3.3.1 Influence of community composition on direct effects

As found for linuron, the direct effect of chlorpyrifos also depended on horizontal and vertical composition. The direct negative effect on herbivore biovolume was, on average, 7% smaller when the number of herbivore species was increased from 1 to 4 and 12% smaller when 4 producers were added, while the negative direct effect was 13% larger when vertical composition was changed by adding a predator (Fig. 3.5a-c). On day 6, chlorpyrifos also had its maximum effect on herbivore biovolume (Fig. 3.5a-c). The negative effect of chlorpyrifos on herbivore biovolume was smaller with adding more producers and more herbivores across any level of vertical composition (Fig 3.6a-b). The negative effects were smallest when the number of producer species was highest, the number of herbivore species highest, and the vertical composition equal to 2 (Fig 3.6a).

The negative direct effect of chlorpyrifos on herbivores was smaller when adding more herbivores (Fig. 3.5b), which was associated with the loss of sensitive herbivores (e.g. *M. macrocopa*, *D. magna* and *D. pulex*) being compensated by other more tolerant herbivores (e.g. *D. lumholtzi*) (Fig, 3.7a). Only for *D. magna*, *M. macrocopa* and *D. pulex*, single species toxicity values could be found with 2-6d EC_{50} values of 0.20, 0.27 and 0.21 $\mu g\, l^{-1}$, respectively (Na *et al.* 2012; USEPA 2020), explaining their decrease. The compensation of sensitive species by more tolerant ones has been shown previously (Van

Wijngaarden *et al.* 2005; Daam *et al.* 2008a). For example, Daam et al. (2008) showed that the decrease of Cladocera (e.g. *Streblocerus pygmaeus*) by chlorpyrifos was compensated by other tolerant Cladocera (e.g. *Dunhevedia crassa*).

In addition, we found that the direct negative effect of chlorpyrifos on the herbivore population was smaller when adding more of its resource (i.e. producer) (Fig. 3.5a), due to higher producers increasing the biovolumes of herbivores (e.g. *D. lumholtzi*) (Fig. 3.7a). However, the presence of a predator *C. obscuripes* made the negative effect of chlorpyrifos on herbivores larger (Fig. 3.5c; Fig. 3.6a-b), as expected (Relyea & Mills 2001; Van den Brink *et al.* 2017). For example, Relyea and Mills (2001) showed that, if a predator (*Ambystoma maculatum*) was present, the pesticide carbaryl was 4 times more toxic to the prey (tadpoles). Predation and chlorpyrifos has similar effects, and can produce synergistic effects when combined (Relyea & Mills 2001).

3.3.3.2 Influence of community composition on indirect effects

The chlorpyrifos-induced decrease of herbivores resulted in indirect positive effects on producer biovolume (Fig. 3.5d-f), as found by Daam and Van den Brink (2007). This indirect positive effect was 10% stronger when 4 producers were added (10% stronger), the number of herbivore species was increased from 1 to 4 (35% stronger) and vertical composition was changed from 1 to 3 (33% stronger) (Fig. 3.5d-f). On day 6, chlorpyrifos also had a maximum effect on producer biovolume (Fig. 3.5d-f). The positive effect of chlorpyrifos on producer biovolume was highest when the number of producer species was highest, more trophic levels were present and the number of herbivore species equal to 1 (Fig 3.6c-d). No significant effect of composition on predator biovolume was detected (Table S3.2). Again, we found qualitatively identical results (single and interactive effects) using abundance as proxy (Fig. S3.3d–i; Fig. S3.4c-f).

The positive effect on producer biovolume can be understood from the release of grazing. The decrease of herbivores especially promoted the growth of its producer food source (e.g. *D. pannonicus*) (Fig. 3.7b). The increase was reinforced by adding producers, herbivores and trophic levels (Fig. 3.7b), making the positive effect on producer biovolume larger (Fig. 3.5d-f). Previous studies only reported chlorpyrifos-induced increase of producers (Daam & Van den Brink 2007; Daam *et al.* 2008a). For example, Daam and Van den Brink (2007) showed that chlorpyrifos application decreased herbivore abundances (Cladocera) and consequently caused an increase in chlorophyll *a* levels. We further showed that the increase of producers could be reinforced by both horizontal and vertical composition, as explained above.

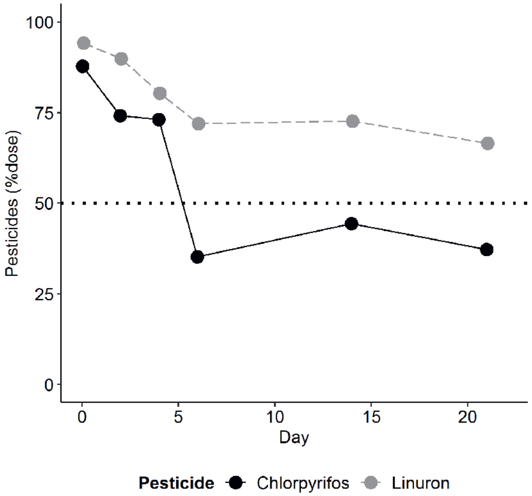


Figure 3.1 Concentration of linuron and chlorpyrifos in systems on sample 1h and day 2, 4, 6, 14 and 21.

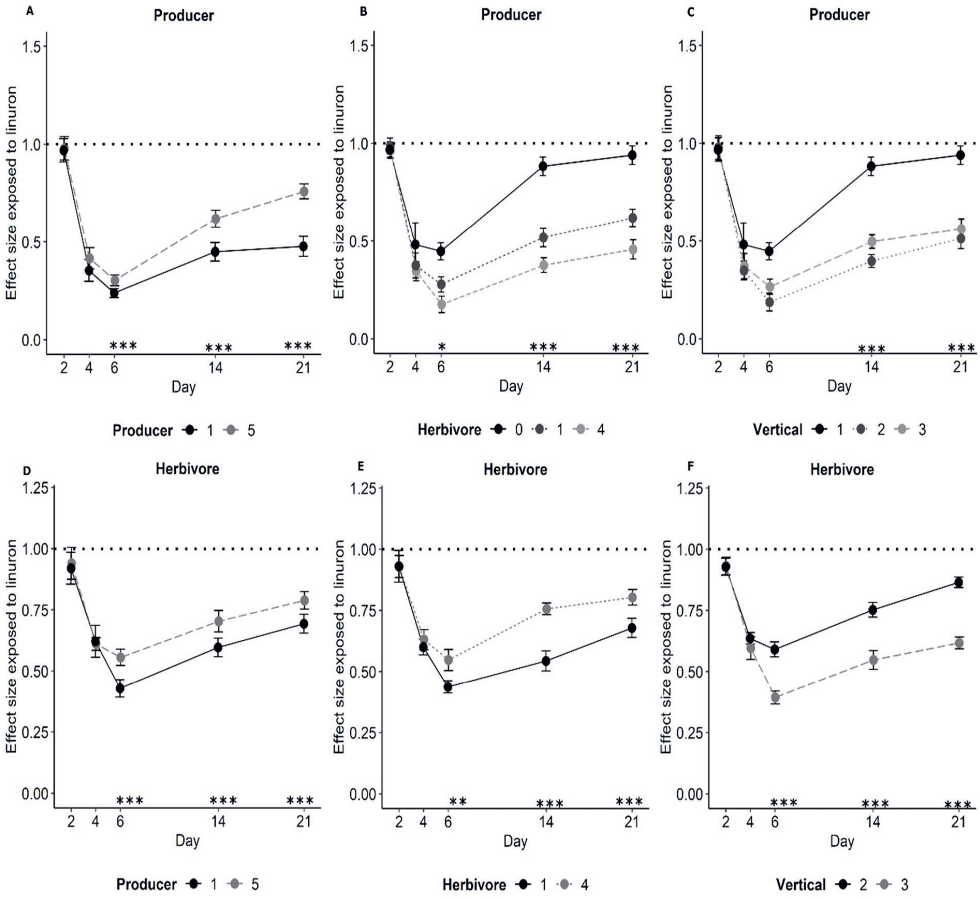


Figure 3.2 The effects of horizontal composition of producer and herbivore, and vertical composition on effect sizes (biovolume as proxy) of producers (A-C), herbivores (D-F) after exposure to linuron. Plotted are sample mean ± 1 SE. An effect size is 1 (treatment = control) indicating no effect of linuron, smaller than 1 (treatment < control) indicating a negative effect of linuron, and bigger than 1 (treatment > control) indicating a positive impact. The bigger deviation from effect size 1 (dash line) indicates larger effect of linuron. The effect size with 1 and 5 producers (A and D) was visualized by averaging effect sizes of all treatments with 1 and 5 producers, respectively, similar manipulation for the effect size under 0, 1 and 4 herbivores species (B and E) and for the effect size under 1, 2 and 3 vertical composition (C and F). Detailed statistical results are listed in Table S3.4.1-S3.4.2. (*$P< 0.05$, ** $P < 0.01$, *** $P < 0.001$).

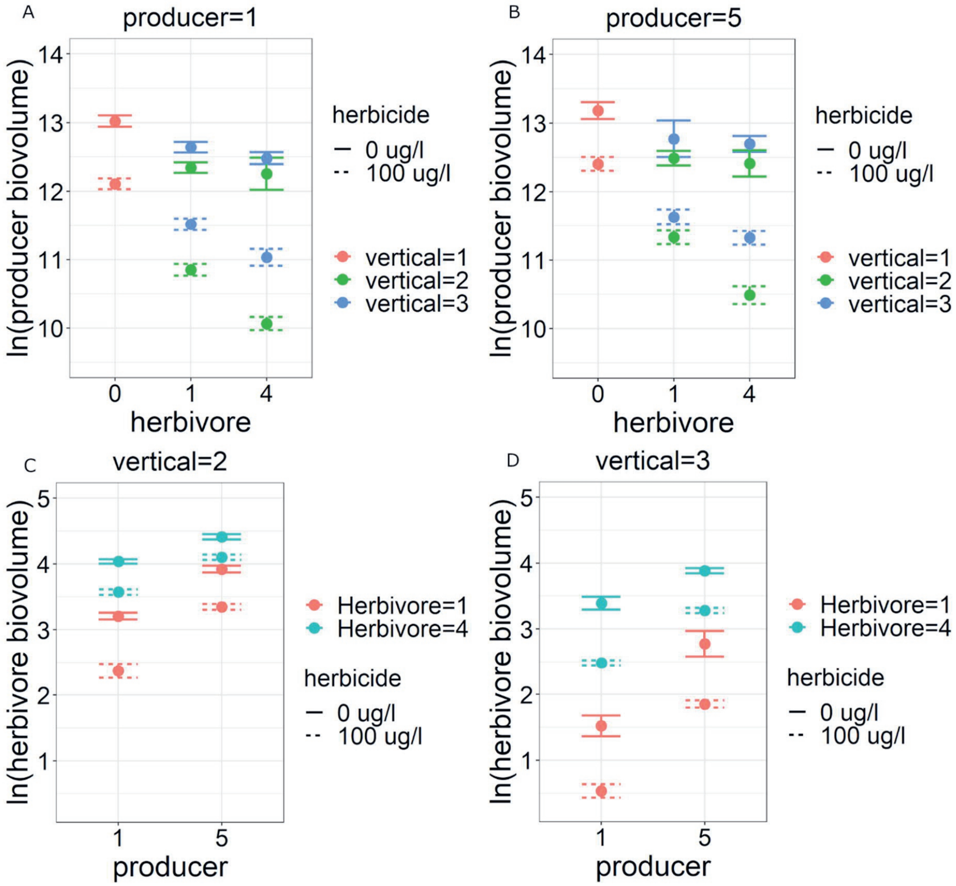


Figure 3.3 The interactive effects of horizontal (producers and herbivores) and vertical composition, herbicide linuron on ln(producer biovolume) (a, b) and on ln(herbivore biovolume) (c, d). Plotted are sample mean ± 1 SE. Solid error bars indicate linuron concentration of 0 µg l^{-1}, while dashed ones stand for linuron concentration of 100 µg l^{-1}. Detailed statistical results are listed in Table S3.5.

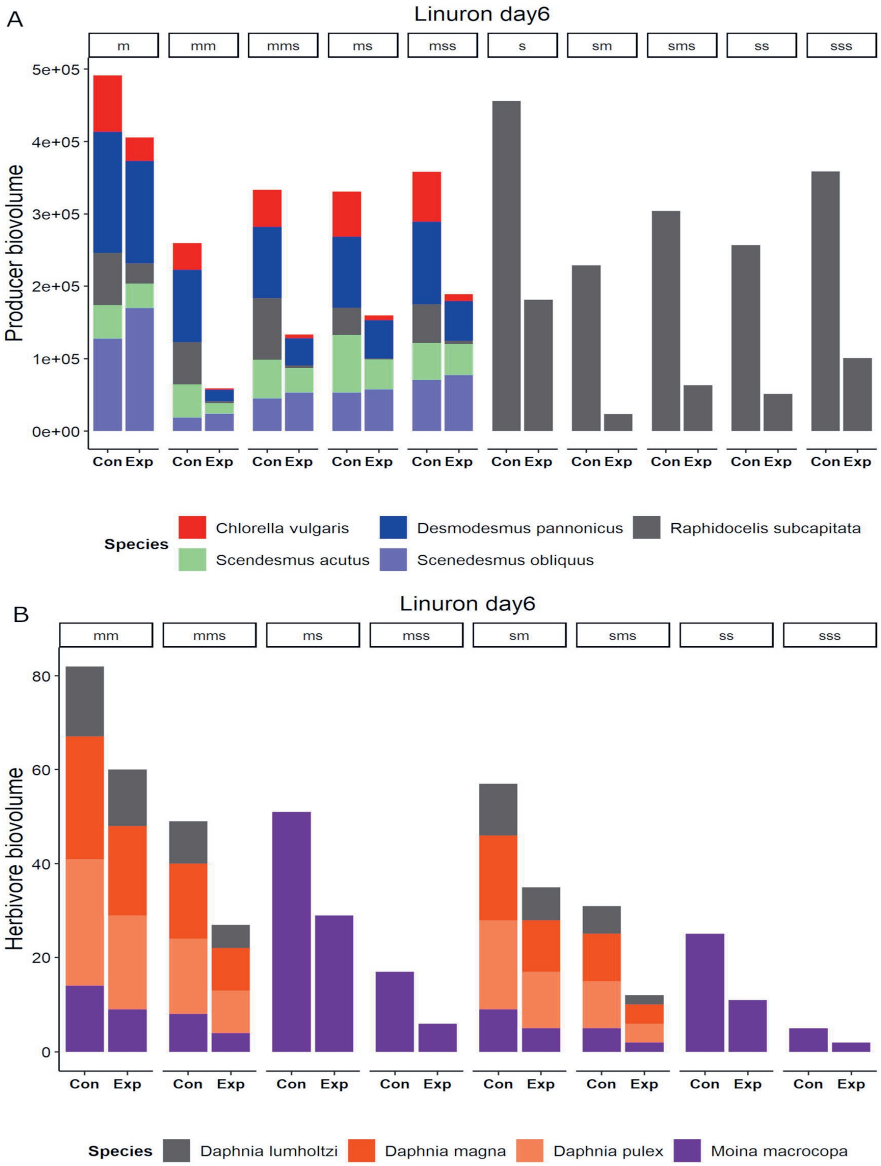


Figure 3.4 Species biovolume in the ten community types after linuron exposure on day 6. Con represents control group, and Exp stands for exposure. Ten treatments include: *s*, single algae; *ss*, single algae-single herbivore; *sss*, single algae-single herbivore-single predator; *sm*, single algae-multiple herbivores; *sms*, single algae-multiple herbivores-single predator; *m*, multiple algae; *ms*, multiple algae-single herbivore; *mss*, multiple algae-single herbivore-single predator; *mm*, multiple algae-multiple herbivores; *mms*, multiple algae-multiple herbivores-single predator). Detailed statistical results are listed in Table S3.6.1–3.6.2.

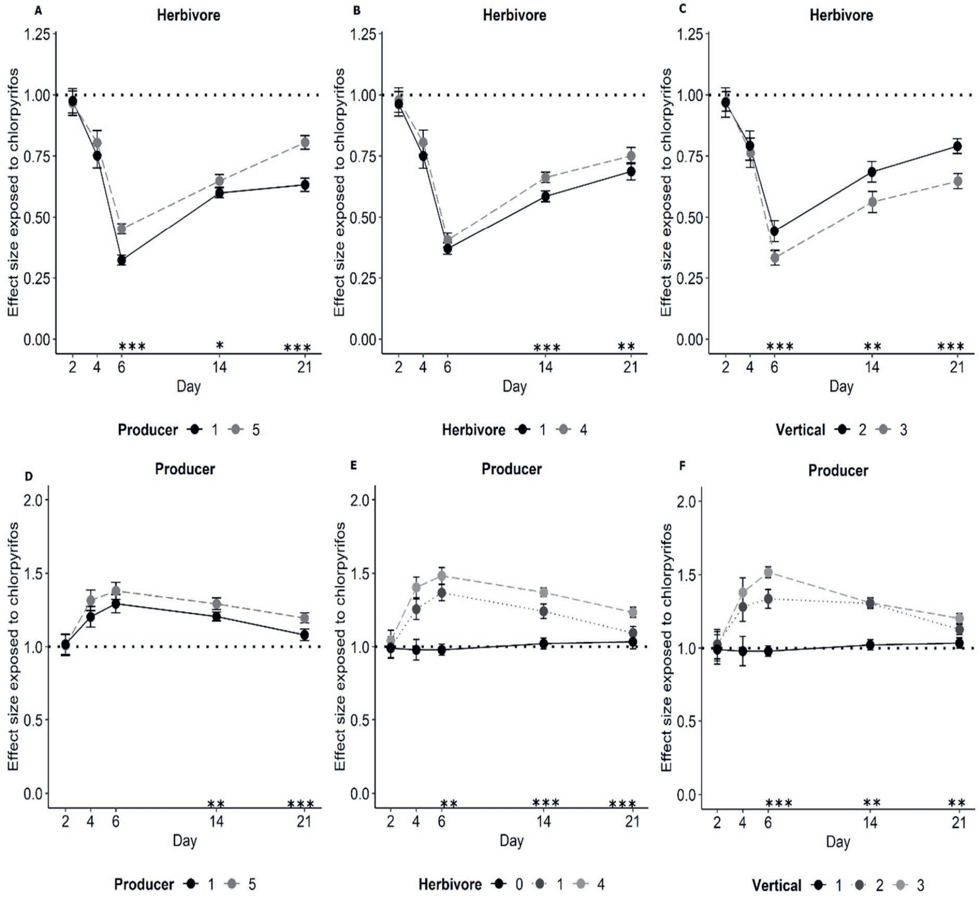


Figure 3.5 The effects of horizontal composition of producer and herbivore, and vertical composition on effect sizes (biovolume as proxy) for herbivores (A-C), producers (D-F) after exposure to chlorpyrifos. Plotted are sample mean ± 1 SE. An effect size is 1 (treatment = control) indicating no effect of chlorpyrifos, smaller than 1 (treatment < control) indicating a negative effect of chlorpyrifos, and bigger than 1 (treatment > control) indicating a positive impact. The effect sizes with 1 and 5 produces (A and D) was visualized by averaging effect size of all treatments with 1 and 5 producers, respectively, similar manipulation for the effect size under 0, 1 and 4 herbivores (B and E) and for the effect size under 1, 2 and 3 vertical composition (C and F). The bigger deviation from effect size 1 (dash line) indicates larger effect of chlorpyrifos. Detailed statistical results are listed in Table S3.7.1-S3.7.2. (*$P< 0.05$, ** $P < 0.01$, *** $P < 0.001$).

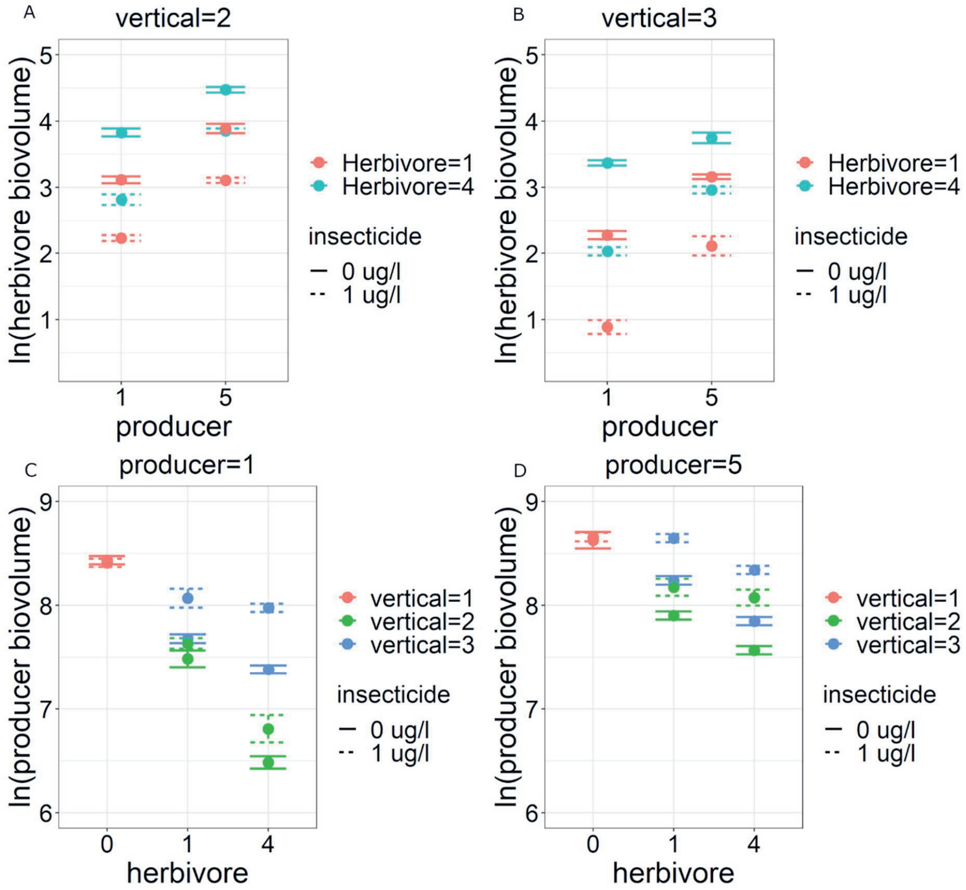


Figure 3.6 The interactive effects of horizontal (producer and herbivore) and vertical composition, insecticide chlorpyrifos on ln(herbivore biovolume) (a, b) and on ln(producer biovolume) (c, d). Plotted are sample mean ± 1 SE. Solid error bars indicate chlorpyrifos concentration of 0 μg l^{-1}, while dashed ones stand for chlorpyrifos concentration of 1 μg l^{-1}. Detailed statistical results are listed in Table S3.5.

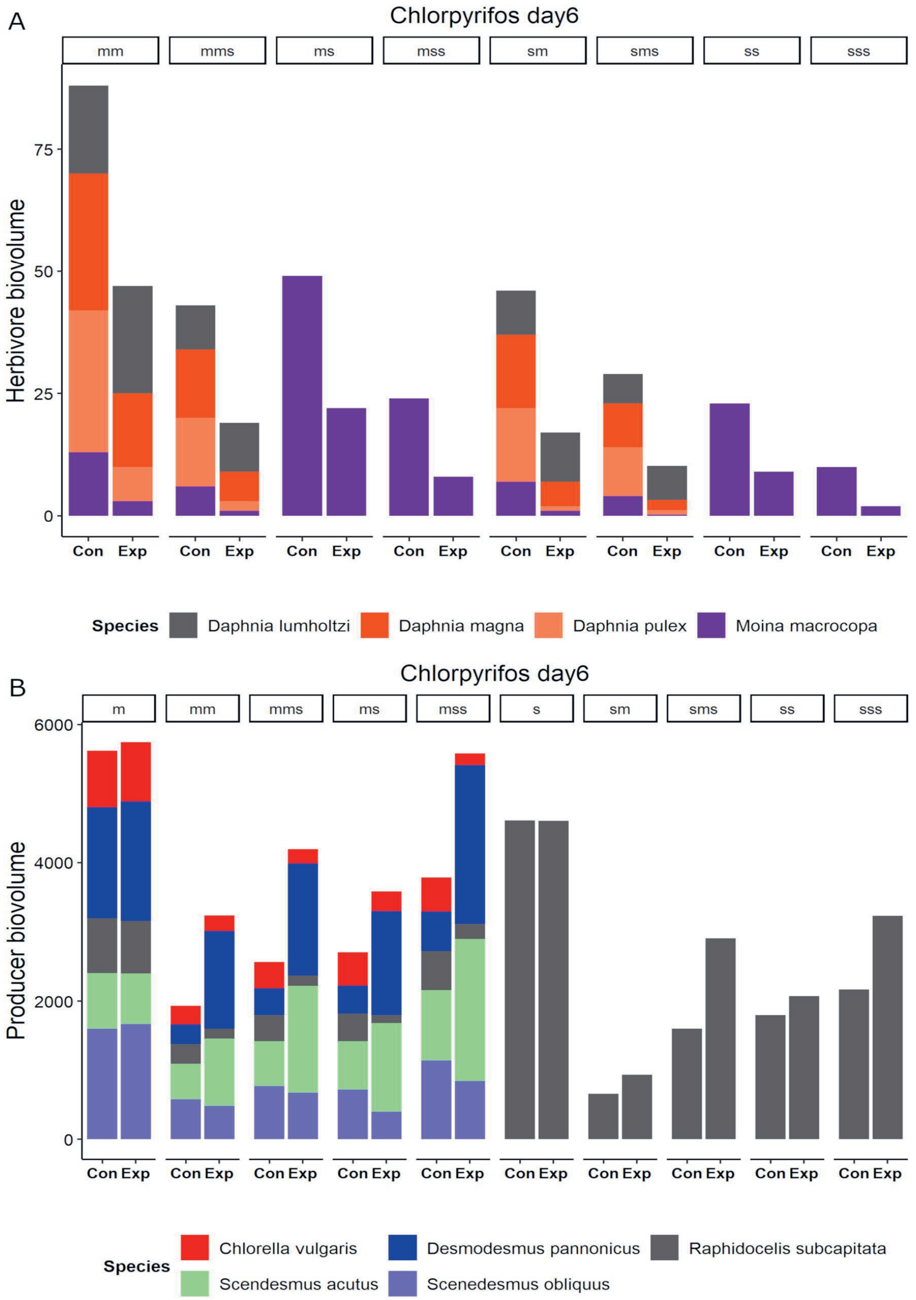


Figure 3.7 Species absolute biovolume in the ten community types after chlorpyrifos exposure on day 6. Con represents control group, and Exp stands for exposure. Ten treatments are same as Figure 3.4. Detailed statistical results are listed in Table S3.8.1–3.8.2.

3.5 Conclusions

Our experiment and analyses demonstrate that the direct and indirect effect of pesticides on aquatic ecosystems depends on horizontal and vertical composition. From these results, the following main conclusions can be drawn: (1) changing horizontal composition by adding species to our reference species increased or decreased the (in)direct effect of pesticides, depending on the type of pesticide used; (2) changing vertical composition by adding trophic levels always made (in)direct effects larger, regardless of the type of pesticide used. One important implication of our results is that the effects of pesticides on single species do not always correspond to worst-case scenarios and that protecting the most sensitive species does not protect the whole ecosystem. Given that community composition of natural systems widely varies between and within systems, we call for more research on how horizontal and vertical composition and diversity affect food-web resistance and resilience. Such studies will improve our understanding of the interaction between toxicological and ecological mechanisms, which is greatly needed to improve our understanding of the environmental impacts of chemicals and their risk assessment (Van den Brink *et al.* 2018).

Acknowledgement

We thank Silke Vollbrecht, Frits Gillissen and Marlies Vollebregt for their assistance during the experiments. We thank Sanne van den Berg, Anna Huang, Lara Schuijt, Markus Hermann and Annika Mangold-Döring for valuable suggestions. We also thank the cooperation of all other participants in this study.

Supporting Information

Supporting Information to this article can be found at ScienceDirect (https://www-sciencedirect-com.ezproxy.library.wur.nl/science/article/pii/S0048969720330485).

Supplementary tables

Table S3.1. Results of two way ANOVA's to assess the significant effect of horizontal composition of producers, herbivores and their interactions on the effect size of the predator (biovolume as proxy) after exposure to linuron over time. (*$P< 0.05$, ** $P < 0.01$, *** $P < 0.001$).

Parameters	Day2	Day4	Day6	Day14	Day21
	F	F	F	F	F
Producer	0.06	0.04	2.29	0.02	0.03
Herbivore	0.16	0.39	0.001	0.02	0.02
Producer: herbivore	0.16	0.04	0.57	0.15	0.03

Table S3.2. Results of two way ANOVA's to assess the significant effect of horizontal composition of producers, herbivores and their interactions on the effect size of the predator (biovolume as proxy) after exposure to chlorpyrifos over time. (*$P< 0.05$, ** $P < 0.01$, *** $P < 0.001$).

Parameters	Day2	Day4	Day6	Day14	Day21
	F	F	F	F	F
Producer	0.195	1.176	0.472	0.296	<0.001
Herbivore	0.049	0.131	0.472	<0.001	0.343
Producer: herbivore	<0.001	0.131	0.472	0.074	<0.001

Table S3.3 Species toxicity data using standard procedures after short-term exposed to pesticides (linuron/chlorpyrifos).

	Species	Pesticides	Exposure duration	Endpoint	EC_{50}	Reference
	Producers					
1	*R. subcapitata*	Linuron	5d	abundance	67 μg l^{-1}	USEPA, 2020
2	*C. vulgaris*	Linuron	7d	abundance	50 μg l^{-1}	Stephenson et al., 1984
3	*S. acutus*	Linuron	3d	growth rate	8.9 μg l^{-1}	USEPA, 2020
4	*D. pannonicus*	Linuron	Not reported	Not reported	Not reported	Not reported
5	*S. obliquus*	Linuron	Not reported	Not reported	Not reported	Not reported
	Herbivores					
1	*M. macrocopa*	chlorpyrifos	2d	abundance	0.20 μg l^{-1}	Na et al., 2012
2	*D. pulex*	chlorpyrifos	6d	abundance	0.21 μg l^{-1}	USEPA, 2020
3	*D. magna*	chlorpyrifos	3d	abundance	0.27 μg l^{-1}	USEPA, 2020
4	*D. lumholtzi*	chlorpyrifos	Not reported	Not reported	Not reported	Not reported

Table S3.4.1. Results of three way ANOVA's to assess the significant effect of horizontal composition of producers, herbivores, vertical composition, and their interactions on the effect size of the producers (biovolume as proxy) after exposure to linuron over time. (*$P< 0.05$, ** $P < 0.01$, *** $P < 0.001$).

Parameters	Day2	Day4	Day6	Day14	Day21
	F	F	F	F	F
Producer	0.002	0.57	24.01***	82.30***	190.31***
Herbivore	0.005	0.93	181.54*	271.73***	227.37***
Vertical	0.001	0.15	1.42	22.27***	21.65***
Producer: herbivore	0.002	0.06	2.92	3.79	6.33*
herbivore: vertical	0.001	0.001	0.70	3.98	12.36*
Producer: vertical	0.011	0.41	58.02***	107.15***	68.12**
Producer: herbivore: vertical	0.001	0.001	0.11	4.55*	1.20

Table S3.4.2. Results of three way ANOVA's to assess the significant effect of horizontal composition of producers, herbivores, vertical composition, and their interactions on the effect size of the herbivores (biovolume as proxy) after exposure to linuron over time. (*$P< 0.05$, ** $P < 0.01$, *** $P < 0.001$).

Parameters	Day2	Day4	Day6	Day14	Day21
	F	F	F	F	F
Producer	0.005	0.04	49.05***	65.75***	39.92***
Herbivore	0.001	0.28	36.78**	260.05***	68.35***
Vertical	0.001	0.48	116.84***	240.77***	268.82***
Producer: herbivore	0.001	0.01	9.65**	1.01	1.11
herbivore: vertical	0.005	0.79	0.45	5.22*	0.11
Producer: vertical	0.001	0.30	14.04***	20.11***	1.75
Producer: herbivore: vertical	0.001	0.01	13.23**	30.17***	0.18

Table S3.5 Results of four (or three) way ANOVA's to assess the significant effect of horizontal composition of producers, herbivores, vertical composition (only included into four way ANOVA), linuron (or chlorpyrifos) dose and their pairwise interactions on the ln(producer biovolume), ln(herbivore biovolume) and ln(predator biovolume) on day 6. (*P< 0.05, ** P < 0.01, *** P < 0.001).

Parameters	linuron			chlorpyrifos		
	ln(producer biovolume)	ln(herbivore biovolume)	ln(predator biovolume)	ln(producer biovolume)	ln(herbivore biovolume)	ln(predator biovolume)
	F	F		F	F	F
Producer	18.551***	386.277***	0.99	280.065***	514.219***	0.34
Herbivore	106.492***	861.153***	0.11	219.232***	447.342***	0.04
Vertical	56.748***	792.332***	---	175.667***	514.966***	---
Dose	691.919***	291.422***	0.30	84.331***	696.355***	0.93
Producer:Herbivore	0.096	39.609***	0.11	19.626***	6.622*	0.15
Producer:Vertical	6.139*	14.909***	---	19.103***	0.075	---
Herbivore:Vertical	8.898**	88.17***	---	14.796***	12.218**	---
Producer:Dose	6.804*	5.827*	0.11	0.658	21.791***	0.01
Herbivore:Dose	24.568***	9.814**	0.60	13.084***	1.323	1.34
Vertical:Dose	11.949***	14.087***	---	4.296*	17.863***	---

Table S3.6.1. Results of four (or three) way ANOVA's to assess the significant effect of horizontal composition of producers (i.e. only included in four way ANOVA's), herbivores, vertical composition, linuron dose and their pairwise interactions on the ln(biovolume) of each producer species on day 6. (*P< 0.05, ** P < 0.01, *** P < 0.001).

Parameters	*S. obliquus*	*S. acutus*	*C.vulgaris*	*D. pannonicum*	*S. capricornutum*
	F	F	F	F	F
Producer	---	---	---	---	57.62***
Herbivore	7.27*	5.54*	9.34**	0.61	4.00*
Vertical	5.65*	0.88	5.01*	0.76	0.20
Dose	7.56**	25.12***	77.73***	1.23	48.08***
Producer:Herbivore	---	---	---	---	3.78
Producer:Vertical	---	---	---	---	0.00
Herbivore:Vertical	2.86	0.00	2.60	3.72	2.42
Producer:Dose	---	---	---	---	22.88***
Herbivore:Dose	0.17	1.49	4.40*	2.53	0.29
Vertical:Dose	1.77	0.10	0.90	0.65	0.060

Table S3.6.2. Results of four (or three) way ANOVA's to assess the significant effect of horizontal composition of producers, herbivores (i.e. only included in four way ANOVA's), vertical composition, linuron dose and their pairwise interactions on the ln(biovolume) of each herbivore species on day 6. (*P< 0.05, ** P < 0.01, *** P < 0.001).

Parameters	*D.magna*	*D.lumholtzi*	*D.pulex*	*M.macrocopa*
	F	F	F	F
Producer	3.46	8.70**	0.99	14.61***
Herbivore	---	---	---	21.80***
Vertical	3.33	20.29***	6.56	69.40***
Dose	3.47	6.68*	2.75	31.61***
Producer:Herbivore	----	---	---	0.05
Producer:Vertical	1.08	0.00	0.00	0.65
Herbivore:Vertical	---	---	---	16.54***
Producer:Dose	0.26	0.54	0.67	0.55
Herbivore:Dose	---	---	---	1.24
Vertical:Dose	5.31*	0.19	0.41	13.99***

Table S3.7.1. Results of three way ANOVA's to assess the significant effect of horizontal composition of producers, herbivores, vertical composition and their interactions on the effect size of the herbivores (biovolume as proxy) after exposure to chlorpyrifos over time. (**P*< 0.05, ** *P* < 0.01, *** *P* < 0.001).

Parameters	Day2	Day4	Day6	Day14	Day21
	F	F	F	F	F
Producer	0.001	0.049	47.89***	6.04*	65.47***
Herbivore	0.003	0.056	3.47	15.21***	8.92**
Vertical	<0.001	0.016	34.88***	38.39**	44.68***
Producer: herbivore	<0.001	<0.001	8.22**	0.32	0.25
herbivore: vertical	0.001	0.022	1.18	1.29	0.01
Producer: vertical	0.003	0.002	0.84	0.03	0.001
Producer: herbivore: vertical	0.001	0.025	0.37	0.75	21.54***

Table S3.7.2. Results of three way ANOVA's to assess the significant effect of horizontal composition of producers, herbivores, vertical composition and their interactions on the effect size of the producers (biovolume as proxy) after exposure to chlorpyrifos over time. (**P*< 0.05, ** *P* < 0.01, *** *P* < 0.001).

Parameters	Day2	Day4	Day6	Day14	Day21
	F	F	F	F	F
Producer	0.001	0.09	2.22	9.83**	47.25***
Herbivore	0.026	0.55	25.67**	68.41***	93.00***
Vertical	0.002	0.20	22.18***	9.70**	9.74**
Producer: herbivore	0.004	0.06	0.31	3.46	32.35***
herbivore: vertical	0.002	0.001	0.08	0.09	0.38
Producer: vertical	<0.001	0.01	1.47	1.39	3.18
Producer: herbivore: vertical	<0.001	0.02	0.37	0.46	10.18**

Table S3.8.1. Results of four (or three) way ANOVA's to assess the significant effect of horizontal composition of producers (i.e. only included in four way ANOVA), herbivores, vertical composition, chlorpyrifos dose and their pairwise interactions on the ln(biovolume) of each producer species on day 6.

Parameters	*S. obliquus*	*S. acutus*	*C.vulgaris*	*D. pannonicum*	*S. capricornutum*
	F	F	F	F	F
Producer	---	---	---	---	9.17**
Herbivore	0.09	3.71	8.66**	5.56*	29.61***
Vertical	0.67	1.17	0.27	5.35*	12.82***
Dose	0.87	0.48	18.80***	5.34*	39.35***
Producer:Herbivore	---	---	---	---	0.19
Producer:Vertical	---	---	---	---	0.10
Herbivore:Vertical	1.15	0.05	0.11	1.08	0.66
Producer:Dose	---	---	---	---	0.20
Herbivore:Dose	0.78	1.62	0.60	0.35	7.78**
Vertical:Dose	0.05	0.43	0.02	0.09	0.04

Table S3.8.2. Results of four (or three) way ANOVA's to assess the significant effect of horizontal composition of producers, herbivores (i.e. only included in four way ANOVA), vertical composition, chlorpyrifos dose and their pairwise interactions on the ln(biovolume) of each herbivore species on day 6.

Parameters	*D.magna*	*D.lumholtzi*	*D.pulex*	*M.macrocopa*
	F	F	F	F
Producer	13.57*	5.68*	0.03	9.17**
Herbivore	---	---	---	29.61***
Vertical	6.48*	4.88*	4.38*	12.82***
Dose	16.68***	5.63*	5.72*	39.35***
Producer:Herbivore	----	---	---	0.19
Producer:Vertical	1.32	0.72	0.65	0.10
Herbivore:Vertical	---	---	---	0.66
Producer:Dose	5.84*	0.59	1.10	0.20
Herbivore:Dose	---	---	---	7.78**
Vertical:Dose	0.74	0.14	0.28	0.04

Supplementary figures

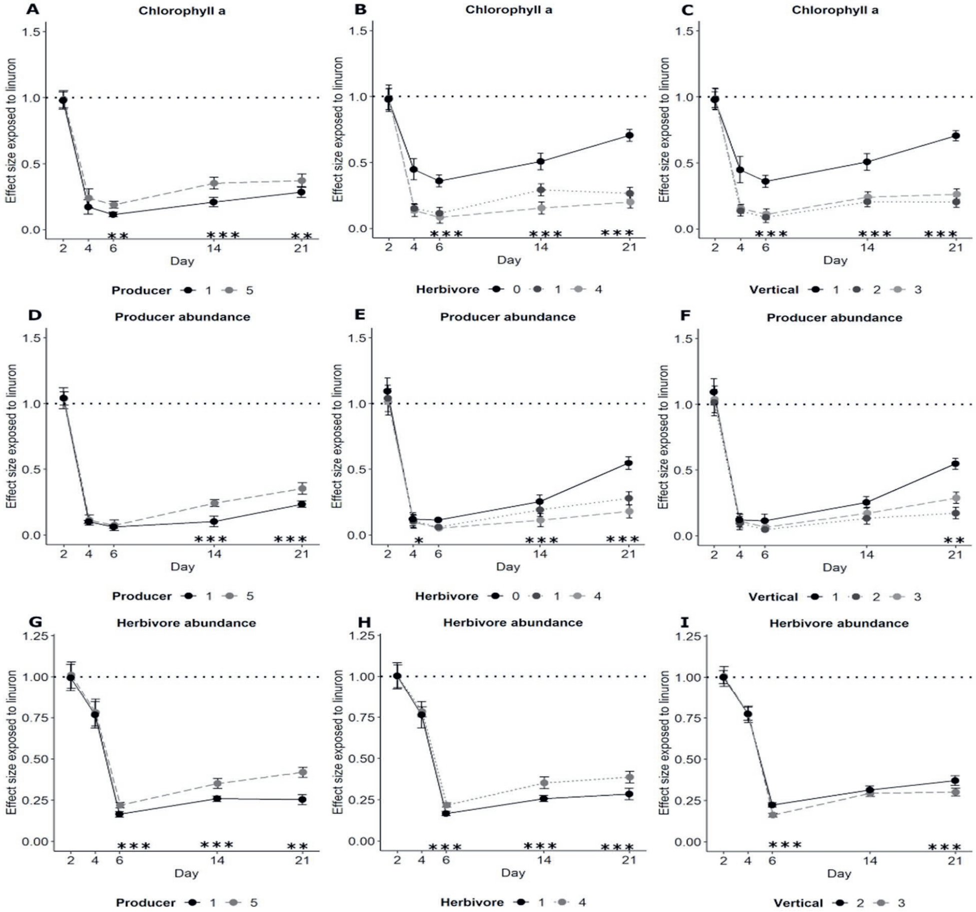


Figure S3.1 The effects of horizontal composition of producer and herbivore, and vertical composition on effect size (abundance as proxy) for chlorophyll *a* (A-C), producers (D-F) and herbivores (G-H) after exposure to linuron. Plotted are sample mean ± 1 SE. An effect size is 1 indicating no effect of linuron, smaller than 1 indicating a negative effect of linuron, and bigger than 1 indicating a positive impact. The bigger deviation from effect size 1 (dash line) indicates larger effect of linuron. The effect size under 1 and 5 producer species (A, D and G) was visualized by averaging effect size of all treatments with 1 and 5 producers, respectively, similar manipulation for the effect size under 0, 1 and 4 herbivores species (B, E and H) and for the effect size under 1, 2 and 3 vertical composition (C, F and I). (*$P< 0.05$, ** $P < 0.01$, *** $P < 0.001$).

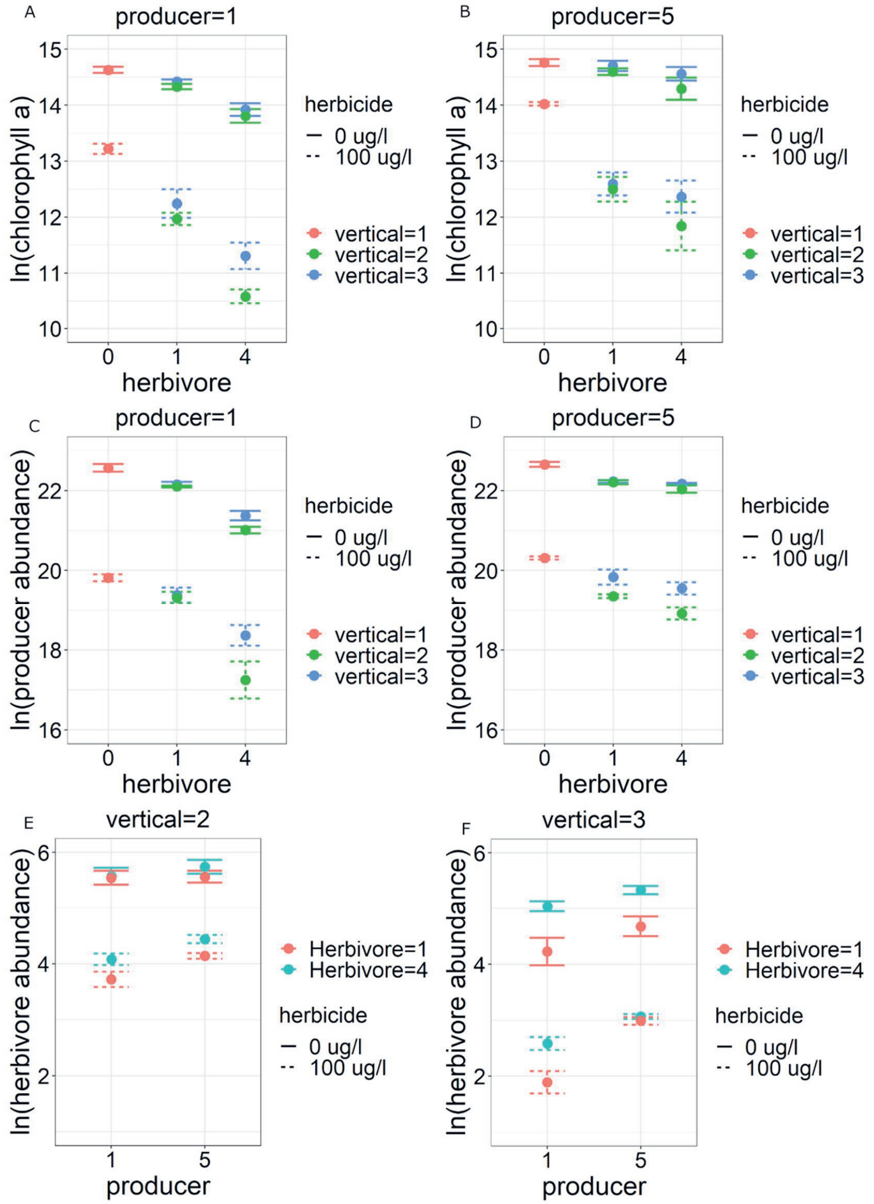


Figure S3.2 The interactive effects of horizontal (producer and consumer) and vertical composition, herbicide linuron on ln(chlorophyll *a*) (a, b), on ln(herbivore abundance) (c, d) and on ln(herbivore abundance) (e, f). Plotted are sample mean ± 1 SE. Solid error bars indicate linuron concentration of 0 μg l^{-1}, while dashed ones stand for linuron concentration of 100 μg l^{-1}.

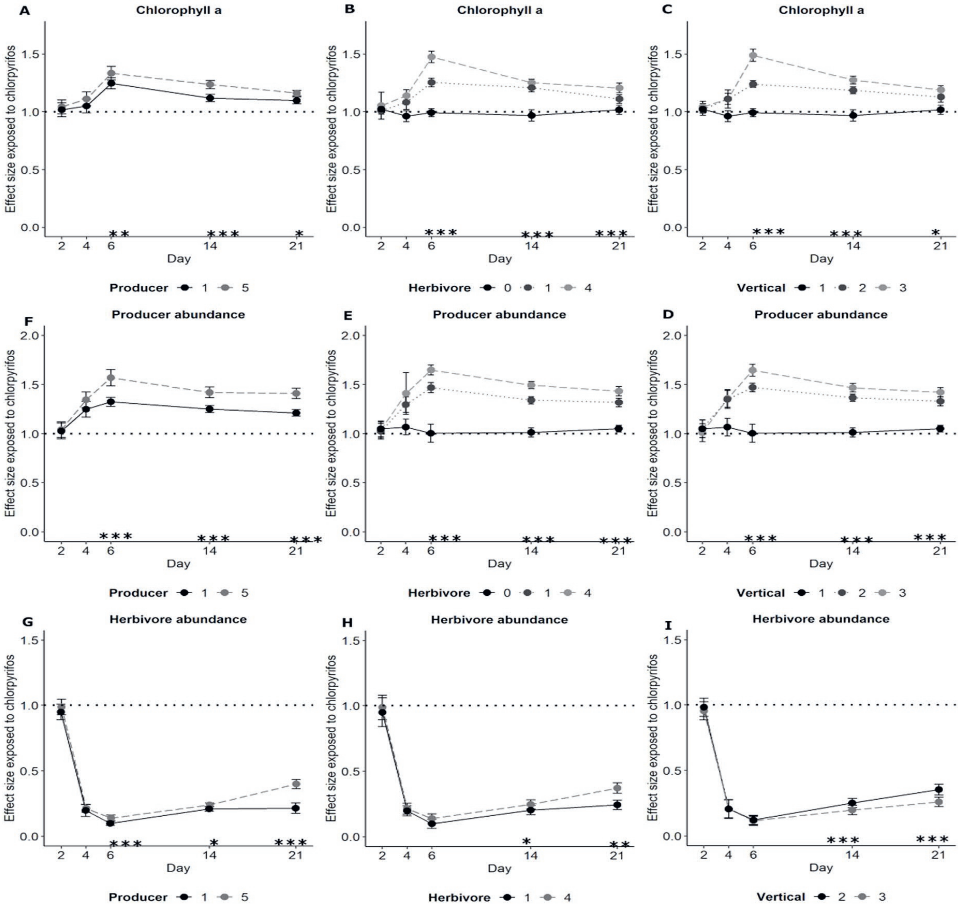


Figure S3.3 The effects of horizontal composition of producer and herbivore, and vertical composition on effect size (abundance as proxy) for chlorophyll *a* (A-C), producers (D-F) and herbivores (G-H) after exposure to chlorpyrifos. Plotted are sample mean ± 1 SE. An effect size is 1 indicating no effect of chlorpyrifos, smaller than 1 indicating a negative effect of chlorpyrifos, and bigger than 1 indicating a positive impact. The bigger deviation from effect size 1 (dash line) indicates larger effect of chlorpyrifos. The effect size under 1 and 5 producer species (A, D and G) was visualized by averaging effect size of all treatments with 1 and 5 producers, respectively, similar manipulation for the effect size under 0, 1 and 4 herbivores species (B, E and H) and for the effect size under 1, 2 and 3 vertical composition (C, F and I). (*P< 0.05, ** P < 0.01, *** P < 0.001).

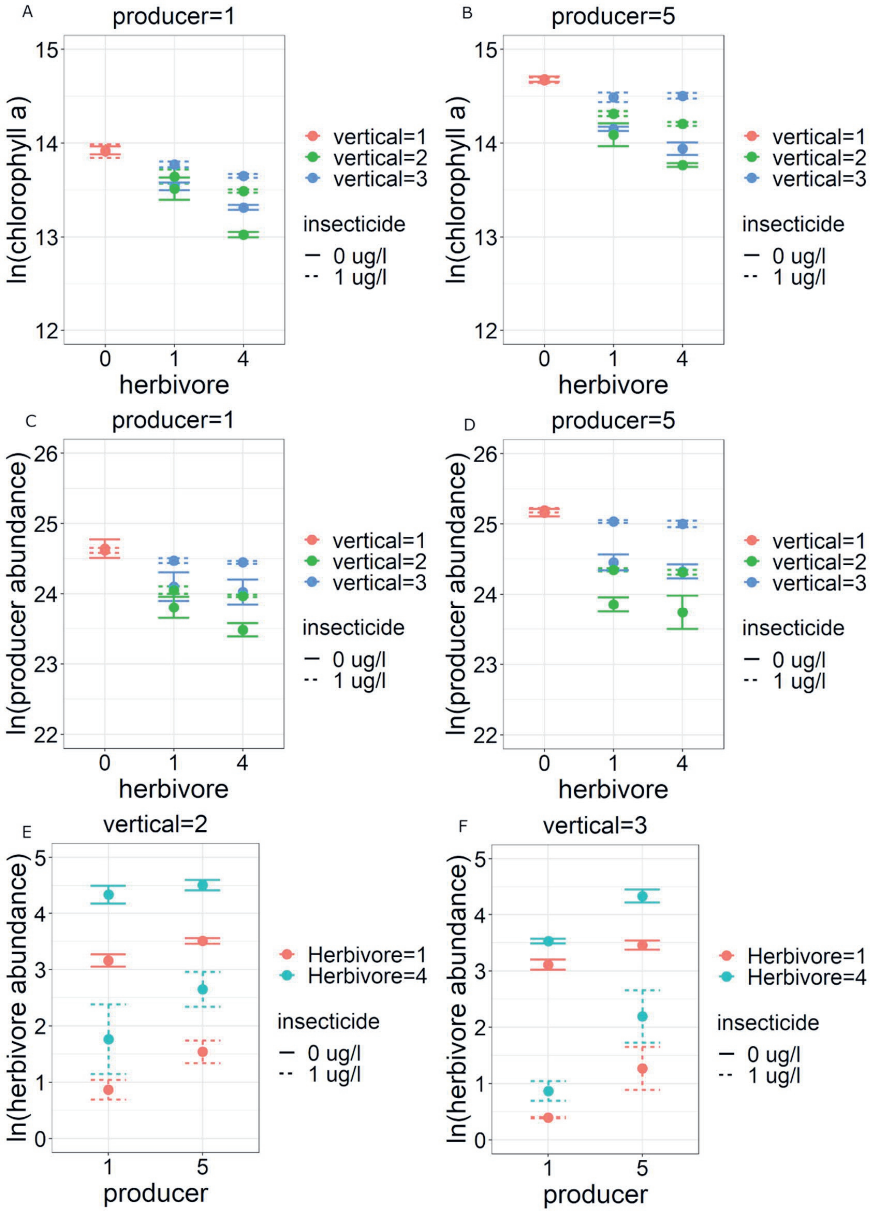


Figure S3.4. The interactive effects of horizontal (producer and consumer) and vertical composition, insecticide chlorpyrifos on ln(chlorophyll *a*) (a, b), on ln(herbivore abundance) (c, d) and on ln(herbivore abundance) (e, f). Plotted are sample mean ± 1 SE. Plotted are sample mean ± 1 SE. Solid error bars indicate linuron concentration of 0 μg l^{-1}, while dashed ones stand for chlorpyrifos concentration of 1 μg l^{-1}.

Chapter 4

The influence of initial experimental biodiversity on the effects of an insecticide on aquatic communities

Zhao, Q., Van den Brink, P.J. The influence of initial experimental biodiversity on the effects of an insecticide on aquatic communities

Abstract

Microcosm and mesocosm experiments are often conducted to assess the ecological risks of chemicals. These experiments vary in their horizontal (diversity within trophic levels) and vertical (number of trophic levels) biodiversity at the start of the experiment and differences in biodiversity may alter the effects of chemicals on aquatic communities. We reanalysed 7 experimental datasets spanning from microcosms to mesocosms and used structural equation modelling in order to evaluate the influence of biodiversity of effect sizes and paths. We found that the negative effects of chlorpyrifos on sensitive herbivore abundance was larger when dose, predator diversity and vertical diversity was higher, while the negative effects were smaller at higher herbivore diversity. The increases in chlorophyll *a* and changes in physicochemical parameters (electrical conductivity) induced by the decrease in grazing pressure were smaller at higher herbivore diversity. Our results suggest that ecological risk assessment of chemicals should consider the initial biodiversity at the start of the experiments.

4.1 Introduction

The results of single species laboratory tests are often used for the ecological risk assessment of chemicals (Van Wijngaarden *et al.* 2005). The use of these results introduces a lot of uncertainties into the risk assessment, among others the unknown between species variation in sensitivity and the absence of ecological interactions (Van Wijngaarden *et al.* 2005; Daam & Van den Brink 2007). Therefore, in the higher tiers of the ecological risk assessment multispecies experiments, using indoor or outdoor microcosms and mesocosms (cosms), are included as methods to assess the sensitivity and resilience of aquatic ecosystems to chemicals (Brock *et al.* 2006; Artigas *et al.* 2012). Although cosm experiments can provide an advantage over single species toxicity experiments of higher biodiversity and the inclusion of ecological interactions, their use raises the question how complex an experiment should be to provide realistic results for the field situation.

Cosm experiments often vary in their biodiversity at the start of the experiments, ranging from a relatively simple plankton community (Daam & Van den Brink 2007) till complex macrophyte dominated outdoor systems (Van den Brink *et al.* 1996). Until now it is unclear whether and to what extent the initial biodiversity difference could change the effect of chemicals on aquatic communities. Biodiversity in natural systems can be characterised in two dimensions: diversity within trophic levels (horizontal diversity) and number of trophic levels (vertical diversity) (Duffy *et al.* 2007). Both dimensions of diversity could modify the effects of chemicals on aquatic communities (Baert *et al.* 2016; Zhao *et al.* 2019, 2020). Recent studies used constructed food-webs in a simple indoor microcosm experiment to show that the two dimensions have contrasting impacts on the effect of the insecticide chlorpyrifos on herbivore population size (Zhao *et al.* 2019, 2020). The negative effect of chlorpyrifos on herbivores was smaller when food-webs had a higher number of herbivore species while the negative effects were larger in food-webs with a higher number of trophic levels (Zhao *et al.* 2020). However, whether these observed influences of horizontal and vertical diversity on the effects of chemicals on aquatic ecosystems are also observed under (semi-) field conditions, remains to be investigated.

In past experiments, the insecticide chlorpyrifos directly decreased sensitive herbivores' population size, which often resulted in an increase of chlorophyll *a*, an increase of ecosystem metabolisms (e.g. dissolved oxygen) and a decrease of predators' population size as indirect, cascading, effects (Brock *et al.* 2000; Fleeger *et al.* 2003). The interaction strength between these effects may depend on horizontal and vertical diversity.

The direct negative effect of chlorpyrifos on sensitive herbivores is expected to be larger at the presence of predators, because of the top-down control imposed to the sensitive herbivores by the predators (Beketov & Liess 2006; Trekels *et al.* 2013). The negative effects of chlorpyrifos on sensitive herbivores is, however, expected to be smaller when herbivore diversity is higher, because the increase in herbivore diversity increases the probability to include relatively insensitive herbivores (Zhao *et al.* 2020) , and the grazing pressure of the sensitive herbivore taxa is normally higher than that of the insensitive taxa.

The chlorpyrifos-induced decrease of herbivores may lead to an increase of chlorophyll *a* (Van Wijngaarden *et al.* 2005; Daam *et al.* 2008b). The increase of chlorophyll *a* is expected to be larger when predators are present, because of the top-down control by the predators on the sensitive herbivores. The increase of chlorophyll *a* is, however, expected to be smaller when herbivore diversity is increased, because of functional redundancy, i.e. that insensitive herbivores take over the function of the sensitive herbivores. Chlorpyrifos-induced increases in chlorophyll *a* may result in an increase of dissolved oxygen (Zafar *et al.* 2011; Halstead *et al.* 2014), because of the increase in photosynthesis (Halstead *et al.* 2014; Sumon *et al.* 2018).

In this study, we aim to study how and to what extent horizontal and vertical diversity at the start of a cosm experiment affects the direct and indirect effects of chlorpyrifos on the tested aquatic communities. We reanalysed published datasets from microcosm and mesocosm experiments evaluating the ecological effects of the insecticide chlorpyrifos of different complexity. For each dataset, we used a piece-wise structural equation model (SEM) to analyse the direct effect of the insecticide chlorpyrifos on the abundance of sensitive species and the indirect effects on chlorophyll *a*, ecosystem metabolisms and predators along the effect pathway. We finally used multiple linear models to correlate the resulting pathway coefficients from the SEMs with the initial horizontal and vertical biodiversity of the cosm experiments, hereby quantifying how horizontal and vertical diversity alter the pathway coefficients.

4.2 Materials and methods

We gathered 7 experimental datasets from microcosm and mesocosm experiments conducted with the insecticide chlorpyrifos by (Van den Brink *et al.* 1996; Van Wijngaarden *et al.* 2005; Daam & Van den Brink 2007; Daam *et al.* 2008a; Zafar *et al.* 2011), which had different levels of biodiversity at the start of the experiments (Table 4.1). We obtained the 7 datasets via a request to the data owner. We calculated the horizontal diversity of the herbivores and predators in each dataset using the Simpson index. The Simpson diversity rather than species richness alone was used as proxy of horizontal diversity, because both species richness and abundance were varying at the start of experiments. The vertical diversity in each dataset was computed as the number of trophic levels. Next, we divided the animal species (excluding detritivores) in each experiment into two trophic groups (i.e. herbivores and predators), based on their diet (Planque *et al.* 2014; Gray *et al.* 2015). Excluding detritivores was needed because the detritivores don't consume on producers, and, herewith are not included in the evaluated food-web (Fig. 4.1). The animal species in each trophic group were next divided into two subgroups (sensitive and incentive species), based on the results of the principal response curves (PRC) analysis or the LOEC and NOEC values provided in each paper (Table 4.1). For each dataset, we finally had data for seven measurement endpoints in total i.e. (in)sensitive predators' abundance, (in)sensitive herbivores' abundance, chlorophyll *a* and community metabolism (dissolved oxygen and electrical conductivity). The insensitive and sensitive predators' abundances were computed as the sum of abundance for all insensitive and sensitive predator species in each dataset, respectively. Followingly, (in)sensitive herbivores' abundances were computed as same way.

We employed a piecewise structural equation model (PSEM) to analyse the direct effects of chlorpyrifos on sensitive herbivores' and predators' abundance, and the resulting indirect effects on chlorophyll *a*, community metabolisms and insensitive herbivore and predator abundance along the effect pathway (Fig. 4.1). PSEM is a generalised path analysis that permits the assessment of causal relationships between variables even when sample size is low, making it an increasingly popular tool in ecology for the analysis of complex multivariate relationships (Lefcheck 2016). All PSEM calculations were done in the piecewiseSEM package version 3.5.2 in the R environment (Lefcheck 2016).

In PSEM, we made two assumptions on the effects of chlorpyrifos: 1) chlorpyrifos only directly affect the sensitive herbivores and sensitive predators and 2) all other possible effects of chlorpyrifos on the ecosystem are propagated through changes in the sensitive herbivores and sensitive predators, as depicted in Fig 4.1 (Brock *et al.* 2000; Fleeger *et al.* 2003). Each time the structural equation model was run, only two levels of chlorpyrifos dose was chosen to use, where 0 $\mu g*L^{-1}$ was always fixed as the control and another concentration (> 0 $\mu g*L^{-1}$), taken from the same experiment, was used to assess the effect of chlorpyrifos. For example, (Van den Brink *et al.* 1996) evaluated the effects of four dosages of chlorpyrifos concentration (0, 0.9, 6 and 44 $\mu g*L^{-1}$). Hence, three cases (0 versus 0.9; 0 versus 6 and 0 versus 44 $\mu g*L^{-1}$) were separately analysed in the structural equation model. Reanalysing the 7 experiments, resulted in 22 cases, so 22 runs with the same structural equation model (Table 4.1).

In order to quantitatively analyse the effect of biodiversity, all path coefficients from the 22 structural equation model results were extracted and used to further analysis. The coefficients in each of the pathways (e.g. the 1st pathway depicting the effect of chlorpyrifos on sensitive herbivores' abundance in Fig. 4.1) were treated as response variables, while horizontal and vertical biodiversity were treated as effect variables. We employed multiple linear regressions to analyse the effect of initial diversity at the start of experiment (horizontal diversity of herbivores and predators and vertical diversity), dose and their pairwise correlations with path coefficients.

Table 4.1 Micro- and mesocosm studies performed with chlorpyrifos included in the data analysis.

#	Species richness of herbivores	Species richness of predators	Number of trophic levels	Dose of chlorpyrifos (ug*L^{-1})	System	Study
1	101	28	3	0, 0.1, 0.9, 6, 44	Outdoor mesocosms	(Van den Brink *et al.* 1996)
2	46	20	3	0, 0.9	Outdoor microcosms	(Zafar *et al.* 2011)
3	21	0	2	0, 0.005, 0.05, 0.5, 5	Indoor microcosms	(Daam & Van den Brink 2007)
4	18	4	3	0, 0.1, 1, 10	Outdoor microcosms	(Daam *et al.* 2008b)
5	17	0	2	0, 0.01, 0.1, 1, 10	Indoor microcosms	(Van Wijngaarden *et al.* 2005)
6	33	0	2	0, 0.01, 0.1, 1	Indoor microcosms	(Van Wijngaarden *et al.* 2005)
7	34	0	2	0, 0.01, 0.1, 1	Indoor microcosms	(Van Wijngaarden *et al.* 2005)

4.3 Results and discussion

The negative effect of chlorpyrifos on sensitive herbivore abundance (the 1st pathway in Fig. 4.1) was significantly larger (more negative pathway coefficients) when predator and vertical diversity and dose were higher, while the negative effect was significantly smaller at higher herbivore diversity (Fig. 4.2a-4.2d).

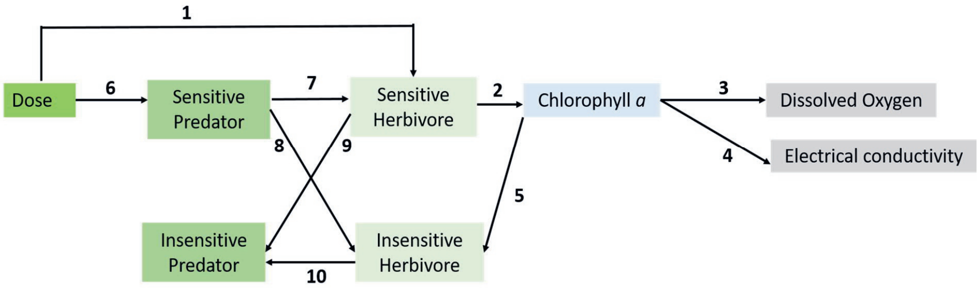


Figure 4.1 A structural equation model to analyse the influence of chlorpyrifos dose on (in)sensitive predator and herbivore abundance, chlorophyll *a* and metabolism (dissolve oxygen and electrical conductivity). The structural equation model considered all plausible pathways through chlorpyrifos dose influencing abundance, chlorophyll *a* and metabolism. Note that the (in)sensitive predator and pathways from 6 to 10 are removed from structural equation model when a dataset has no predator presence, otherwise, keep them when predators are present.

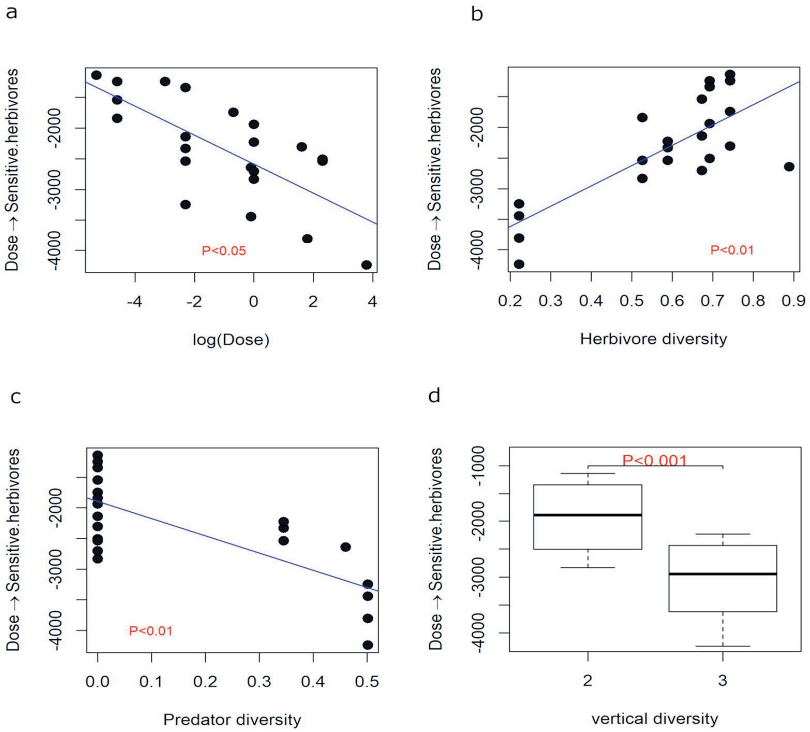


Figure 4.2 Multiple linear regressions show that chlorpyrifos dose (a), herbivore diversity (b), predator diversity (c) and vertical diversity (d) affect the negative effect of chlorpyrifos dose on sensitive herbivore abundance, expressed as the path coefficient corresponding with the 1st pathway in Fig. 4.1. A negative value indicates a negative effect, vice versa. Detailed statistical results are listed in Table S4.1.

Increasing vertical diversity from 2 to 3 (presence of multiple predators) made the negative effects of chlorpyrifos on sensitive herbivores larger in our study, due to addition of the top-down control by the predators (Fig. S4.1d). Here we found that increasing the predator diversity further increases the predation strength on sensitive herbivores' abundance (Fig. S4.1c). Predation and insecticides have similar effects, and can produce synergistic effects on sensitive herbivores when combined (Relyea & Mills 2001; Janssens & Stoks 2013; Trekels *et al.* 2013). For example, Relyea and Mills (2001) showed that the presence of a predator (*Ambystoma maculatum*) made the insecticide (carbaryl) 4 times more toxic to the prey (gray treefrog tadpoles, *Hyla versicolor*). Higher doses also significantly increased the negative effects of chlorpyrifos on sensitive herbivore abundance (Fig. S4.1a), which is a common phenomenon found in ecotoxicity experiments (Brock *et al.* 1992; Van den Brink & Ter Braak 1999). Here we found that higher herbivore diversity significantly decreased the negative effect of chlorpyrifos on sensitive herbivores. This is because the proportion of sensitive herbivores' abundance was smaller at higher herbivore diversity (Fig. S4.1b).

The positive effect of a decrease in sensitive herbivore abundance on chlorophyll *a* (the 2nd pathway in Fig. 4.1) was significantly larger at higher doses (Fig. 4.3a), because higher doses result in larger decreases of grazing pressure (Fig. S4.1a) leading to larger increases of chlorophyll *a* (Fig S4.2b). Previous studies also showed that higher doses induced larger increases of algae (Hughes *et al.* 1980; Brock *et al.* 1992; Fleeger *et al.* 2003). We found no significant effects of predator, herbivore and vertical diversity on chlorophyll *a* (Table S4.1).

The increase in chlorophyll *a* resulted in significant changes in community metabolism (dissolved oxygen and electrical conductivity) (Fig. 4.3b-4.3d). The positive correlation between chlorophyll *a* and dissolved oxygen (the 3rd pathway in Fig. 4.1) was significantly larger at higher doses (Fig. 4.3b), while the herbivore, predator and vertical diversity had no effect (Table S4.1). Higher doses led to larger positive correlations between chlorophyll *a* and dissolved oxygen, again because higher doses induced larger decreases of sensitive herbivores (fig. S4.1a) leading to larger increases of chlorophyll *a* (Fig. S4.2b) resulting in more dissolved oxygen production (Fig. S4.2c) through photosynthesises (Webber *et al.* 1992; Brock *et al.* 2000).

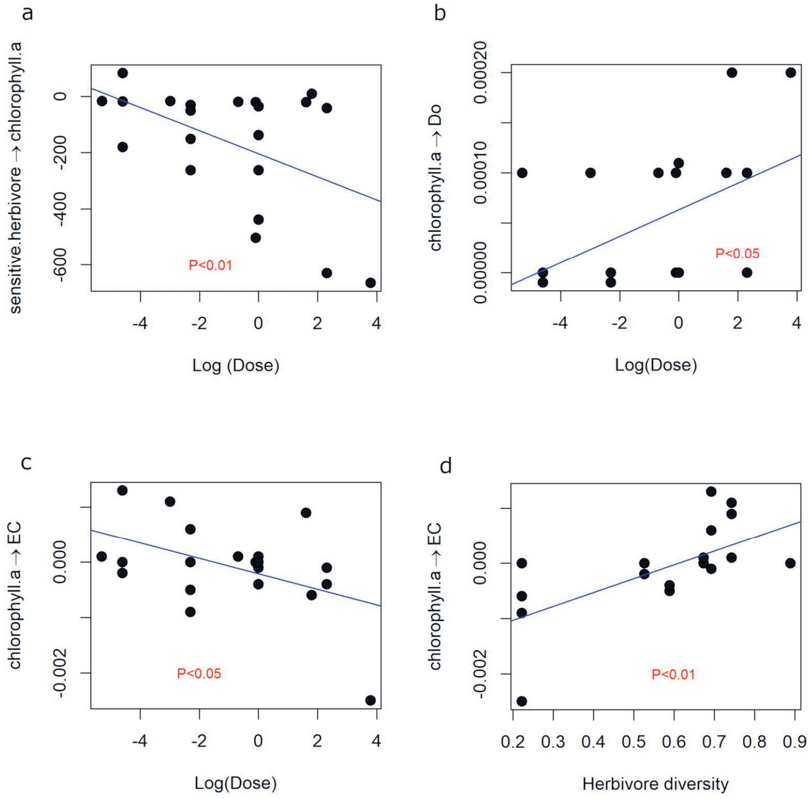


Figure 4.3 Multiple linear regressions show that chlorpyrifos dose, herbivore diversity, predator diversity and vertical diversity affect the negative correlation between sensitive herbivores and chlorophyll *a* (a), positive correlation between chlorophyll *a* and dissolved oxygen DO (b), negative correlation between chlorophyll *a* and electrical conductivity (c, d), expressed as the path coefficient corresponding with 2st, 3rd 4th pathway in Fig. 4.1, respectively. A negative value indicates a negative effect, vice versa. Detailed statistical results are listed in Table S4.1.

The negative correlation between chlorophyll *a* and electrical conductivity (the 4th pathway in Fig. 4.1) was significantly smaller at higher herbivore diversity and significantly larger at higher dose (Fig. 4.3c-4.3d). The predator and vertical diversity had no effect (Table S4.1). Higher herbivore diversity significantly decreased the negative correlation between chlorophyll *a* and electrical conductivity, because of a lower effect of chlorpyrifos on the grazing of the algae when herbivore diversity is high (Fig. S4.1b, S4.2b, S4.2d). Increasing herbivore diversity leads to increasing chance of the inclusion of insensitive herbivores (Fig. S4.1b), which maintain their grazing pressure under chlorpyrifos stress (Fig. S4.2b). Previous studies only reported that insecticides-induced algae increase led to decrease of electrical conductivity (Webber *et al.* 1992; Peither *et al.* 1996; Brock *et al.* 2000). For example, Webber *et al.* (1992) showed that insecticide (lindane) exposure decreased herbivores' abundance (cladocerans, adult copepods and nauplii), and consequently caused an increase in producers and finally decrease electricity conductivity. For the first time we showed that the negative correlation between chlorophyll *a* and electrical conductivity can be larger at higher herbivore diversity, as explained above.

The positive correlation between chlorophyll *a* and insensitive herbivores abundance (the 5th pathway in Fig. 4.1) was significantly larger at higher dose (Fig. 4.4a), while herbivore, predator and vertical diversity had no effect (Table S4.2). Higher chlorpyrifos dose significantly increased the positive correlation between chlorophyll *a* and insensitive herbivores, because higher dose indirectly reduced the competition over food for the insensitive herbivores with the sensitive herbivores (Fig. S4.1a, S4.2b, S4.2e). (Rohr & Crumrine 2005) reported similar results for the insecticide endosulfan, which directly decreased the populations of sensitive herbivores (chironomid larvae), resulting in its resource (producers) being released from grazing, which in turn resulted in an increase of tolerant herbivores (snails and tadpoles) via an increase of food resources.

The negative effect of chlorpyrifos on sensitive predator abundance (the 6th pathway in Fig. 4.1) was significantly larger when the dose was higher (Fig. 4.4b). We found no effect of predator, herbivore and vertical diversity on the sensitive predator abundance (Table S4.1). Higher dose increased the negative effects on sensitive predators, again due to higher acute effects at higher doses (Fig S4.2a), which is common in cosm experiments (Boyle *et al.* 1996; Peither *et al.* 1996; Fleeger *et al.* 2003). For example, Peither *et al.* (1996) reported that population size of predators (predatory zooplankton, such as *Chaoborus flavicans*) can be reduced to zero when insecticide (lindane) dose is larger than 12 ug*L^{-1}.

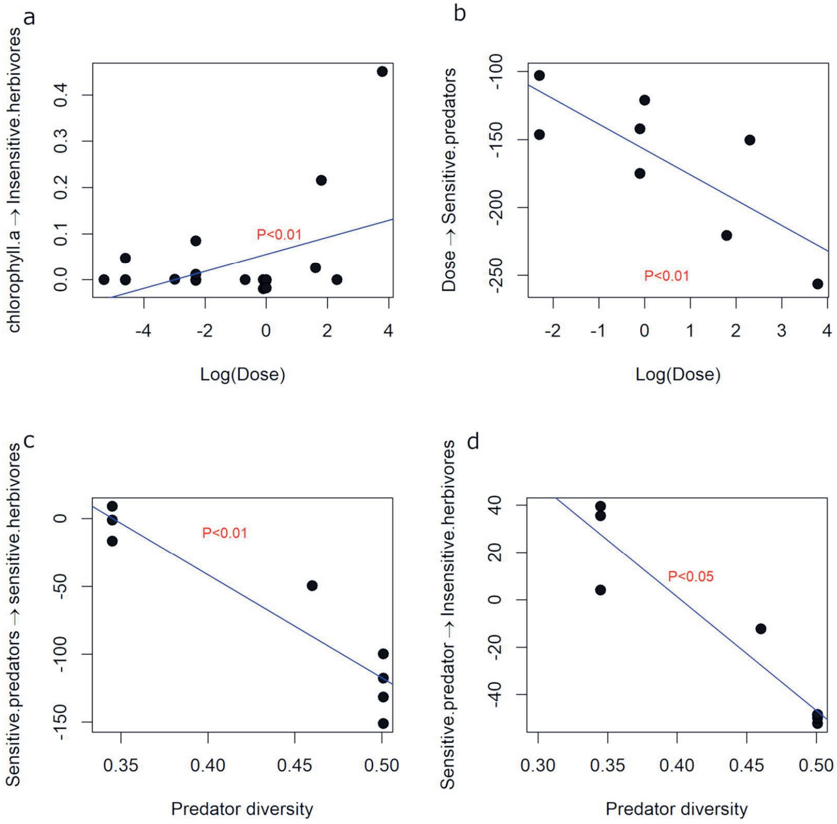


Figure 4.4 Multiple linear regressions show that chlorpyrifos dose, herbivore diversity, predator diversity and vertical diversity affect the positive correlation between chlorophyll *a* and insensitive herbivores (a), negative effect of dose on sensitive predators (b), negative correlation between sensitive predators and sensitive herbivores (c) and negative correlation between sensitive predators and insensitive herbivores (d), expressed as the path coefficient corresponding with 5st - 8th pathway in Fig. 4.1, respectively. A negative value indicates a negative effect, vice versa. Detailed statistical results are listed in Table S4.1-S4.2.

The negative correlation between sensitive predators and (in)sensitive herbivores' abundance (the 7th and 8th pathway in Fig. 4.1) are significantly larger at higher predator diversity (Fig. 4.4c-4.4d), while herbivore diversity, vertical diversity and dose have no effect on these negative effects (Table S4.2). Higher predator diversity increased the negative correlation between sensitive predators and (in)sensitive herbivores which can be attributed to higher predation pressure (Fig. S4.1c; S4.2f). Higher predator diversity maintained a larger predation pressure on herbivores than lower predator diversity (Sih & Wooster 1998; Schmitz 2007). Dose, herbivore and predator diversity have no effect on the effect of both sensitive and insensitive herbivores to insensitive predators (the 9th and 10th pathway in Fig. 4.1; Table S4.2).

4.4 Conclusions

Our results showed for the first time that the initial experimental biodiversity at the start of a cosm experiment modifies the effects of an insecticide on aquatic communities. From these results, the following main conclusions can be drawn: (1) the negative effects of chlorpyrifos on sensitive herbivores' abundance was larger when dose, predator diversity and vertical diversity was higher, while the negative effects were smaller at higher herbivore diversity; (2) the effects of the "herbivore decrease" induced increase in chlorophyll *a* on physicochemical parameters (electrical conductivity) was smaller at higher herbivore diversity. Given that indoor or outdoor cosm experiments are widely used for ecological risk assessment of chemicals, we call for more research on how horizontal and vertical diversity affect ecosystem function and resilience. Such studies will improve our understanding of the role of biodiversity in the interaction between toxicological and ecological mechanisms.

Acknowledgement

We thank Michiel Daam, René van Wijngaarden and Mazhar Zafar for providing the data sets.

Supplementary figures

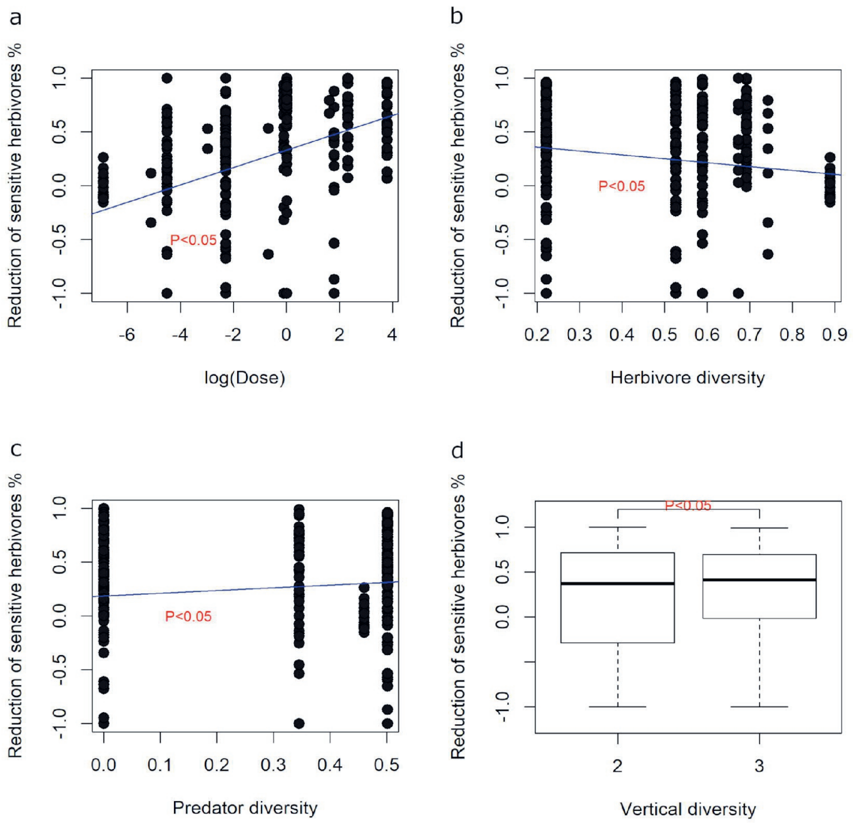


Figure S4.1 Multiple Linear regressions show that chlorpyrifos dose (a), herbivore diversity (b), predator diversity (c) and vertical diversity (d) affects the proportion of reduction of sensitive herbivore abundance. Detailed statistical results are listed in Table S4.3.

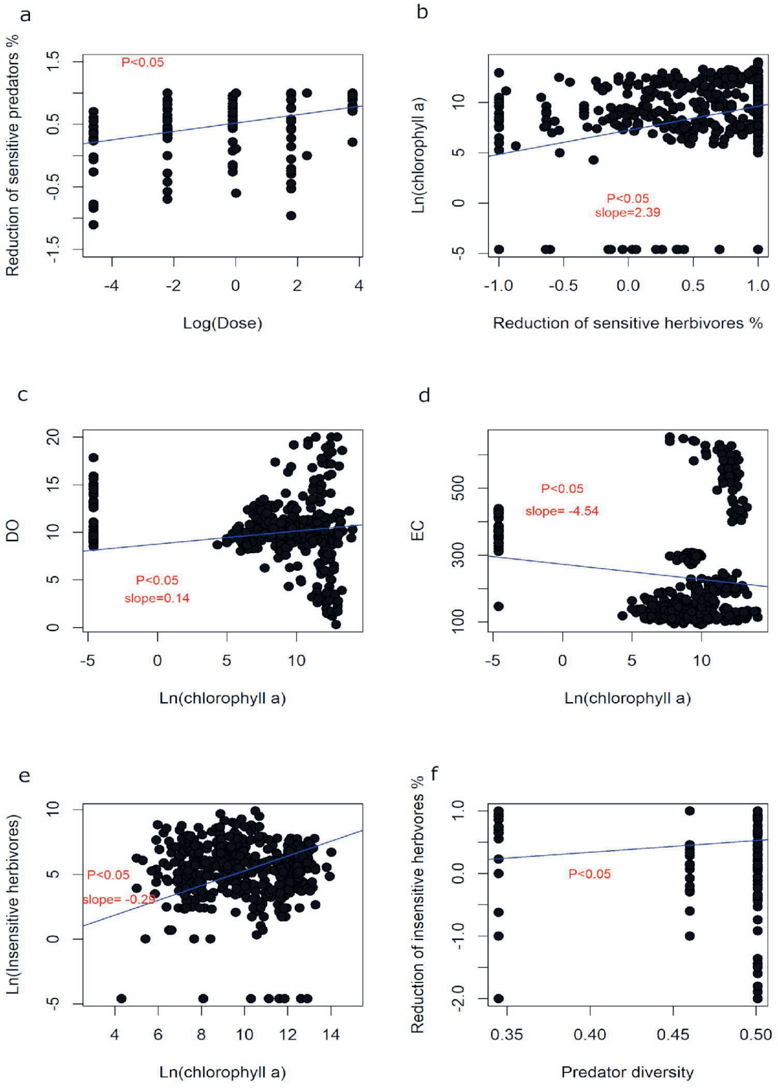


Figure S4.2 Multiple Linear regressions shows that chlorpyrifos dose, herbivore diversity, predator diversity and vertical diversity affects the proportion of reduction of sensitive predator abundance (a) and proportion of reduction of insensitive herbivore abundance (f). The simple linear regression showed the relationship between proportion of reduction of sensitive herbivore abundance and ln(chlorophyll *a*) (b), between ln(chlorophyll *a*) and dissolved oxygen (DO) (c), between ln(chlorophyll *a*) and electrical conductivity (DO) (d), ln(chlorophyll *a*) and insensitive herbivores (e). Detailed statistical results are listed in Table S4.3.

Supplementary tables

Table S4.1 Results of multiple linear regression to assess the significant effect of initial diversity at the start of experiment (horizontal diversity of herbivores, predators and vertical diversity), ln(dose) and their pairwise interactions on negative effect of dose on sensitive predators, on negative effect of dose on sensitive herbivores, on negative correlation between sensitive herbivores and chlorophyll *a*, on positive correlation between chlorophyll *a* and dissolved oxygen (DO), on negative correlation between chlorophyll *a* and electrical conductivity (EC) (*$P < 0.05$, ** $P < 0.01$, *** $P < 0.001$).

Parameters	Dose ↓ Sensitive predators	Dose ↓ Sensitive herbivores	Sensitive herbivores ↓ Chlorophyll *a*	Chlorophyll *a* ↓ DO	Chlorophyll *a* ↓ EC
	coefficient	Coefficient	coefficient	coefficient	coefficient
Intercept	-54.02	-9830.9***	-1426.9	-3.4×10^{-5}	-6.0×10^{-4}
ln(dose)	-53.1**	-235.9*	-387.5**	1.3×10^{-5}*	-1.4×10^{-4}*
herbivores	8.0	3304.1**	435.5	4.1×10^{-4}	2.5×10^{-3}**
predators	4.5	-2804.2**	-414.9	7.6×10^{-4}	4.3×10^{-4}
vertical	----	-1126.0***	443.0	-9.9×10^{-5}	-9.3×10^{-4}
ln(dose): herbivores	2.2	236.0	103.9	1.5×10^{-4}	-1.9×10^{-3}
ln(dose):predators	3.0	-210.6	-220.3	1.2×10^{-5}	-5.5×10^{-5}
ln(dose):vertical	----	234.2	134.7	-3.1×10^{-5}	7.2×10^{-4}
herbivores: predators		-7718.7**	-287.6	-6.7×10^{-5}	-3.3×10^{-4}
herbivores: vertical	----	383.5	53.1	1.2×10^{-5}	1.5×10^{-4}
Predators: vertical	----	374.0	94.6	1.4×10^{-5}	2.6×10^{-4}

Table S4.2 Results of multiple linear regression to assess the significant effect of initial diversity at the start of experiment (horizontal diversity of herbivores, predators and vertical diversity), ln(dose) and their pairwise interactions on positive correlation between chlorophyll *a* and insensitive herbivores, positive correlation between insensitive herbivores and insensitive predators, positive correlation between sensitive herbivores and insensitive predators, negative effect correlation between sensitive predators and insensitive herbivores and negative correlation between sensitive predators and sensitive herbivores (*P< 0.05, ** P < 0.01, *** P < 0.001).

Parameters	Chlorophyll *a* ↓ Insensitive herbivores	Sensitive predators ↓ Insensitive herbivores	Sensitive herbivores ↓ Insensitive predators	Insensitive herbivores ↓ Insensitive predators	Sensitive predators ↓ Sensitive herbivores
	coefficient	coefficient	coefficient	coefficient	coefficient
Intercept	0.21	-85.8	-0.31	0.31	-721.9
ln(dose)	0.31**	24.3	0.01	0.04	-18.8
herbivores	0.79	7.23	0.09	-0.03	1427.6
predators	0.12	-479.37**	0.60	-0.59	-759.5**
vertical	-0.15	----	----	----	----
ln(dose): herbivores	0.50	0.05	0.60	0.05	44.67
ln(dose):predators	0.04	-0.08	0.07	-0.09	40.68
ln(dose):vertical	-0.17	----	----	----	----
herbivores: predators	-0.58	-0.33	0.03	-0.48	51.84
herbivores: vertical	0.61	----	----	----	----
Predators: vertical	<0.01	----	----	----	----

Table S4.3 Results of multiple linear regression to assess the significant effect of initial diversity at the start of experiment (horizontal diversity of herbivores, predators and vertical diversity), ln(dose) and their pairwise interactions on negative effect of dose on proportion of sensitive predators' reduction, sensitive herbivores' reduction and insensitive predators' reduction.

Parameters	Reduction of sensitive herbivores %	Reduction of sensitive predators %	Reduction of insensitive herbivores %
	coefficient	coefficient	coefficient
Intercept	-1.24	2.39	-3.91
ln(dose)	0.08***	0.06***	-1.03*
herbivores	-0.37*	-3.68	7.91
predators	0.25*	0.52	1.44*
vertical	0.12*	----	2.01
ln(dose): herbivores	-0.92	0.37	1.74
ln(dose):predators	0.24*	0.32	0.39
ln(dose):vertical	0.31	----	3.34
herbivores: predators	-0.42	-0.42	7.26
herbivores: vertical	0.24	----	6.29
Predators: vertical	0.21	----	3.82

Chapter 5

Warming has a greater long-term effect on stability than biodiversity across natural aquatic food webs

Zhao, Q., Van den Brink, P.J., Wang, Y.X., Widdicombe, C., Atkinson, A., De Laender, F. Warming has a greater long-term effect on stability than biodiversity across natural aquatic food webs.

Abstract

Climate warming and biodiversity are pivotal to food web structure, function and stability. Knowledge of how food web stability responds to climate warming and biodiversity changes has been mostly done using short term experiments or using static food-web structure modelling estimates. We synthesized 12 long-term food web data sets (11 from freshwater lakes and 1 from a marine system) spanning between 10 to 30 years across three continents. We next employed empirical dynamic modelling to recover community dynamics from these time series and thus the dynamic effects of warming and biodiversity on stability. We showed that warming causally decreased food web stability while diversity (species richness and Simpson diversity) increased stability. On average, the absolute effect of the observed rise in temperature on stability was 2.1 times larger than the effect of biodiversity increase. Our findings suggest that global warming could impose a larger damage to ecosystem stability than biodiversity changes.

5.1 Introduction

Climate warming is dramatically modifying consumer–resource interactions (Rall *et al.* 2010; Gilbert *et al.* 2014; Uszko *et al.* 2017), and as a consequence alters food web structure and function (Gilbert *et al.* 2014; Schwarz *et al.* 2017). Warming can decrease the local stability (henceforth "stability") of food webs (Gilbert *et al.* 2014), when it increases per capita consumer rates (Gilbert *et al.* 2014; Schwarz *et al.* 2017; Uszko *et al.* 2017). However, some studies showed that warming can also cause no effect on stability, or even increase stability (Gilbert *et al.* 2014; Schwarz *et al.* 2017). In addition, biodiversity (species richness or Simpson diversity) can also affect food web stability (McCann 2000; Worm & Duffy 2003; Rooney & McCann 2012). For example, greater biodiversity can decrease consumption rates, and therefore weaken consumer–resource links, which acts to stabilize food webs (McCann *et al.* 1998; McCann 2000; Rooney & McCann 2012). Alternative food web structures may bring about different effects of biodiversity on stability (Rooney *et al.* 2006; Rooney & McCann 2012). Because warming and biodiversity changes are expected to go hand in hand, understanding their joint impact is pivotal.

Studies on the effect of warming or biodiversity on stability have mostly used short-term experiments or model simulations (Gilbert *et al.* 2014; Kéfi *et al.* 2019). These settings convey a number of important limitations for the translation of these results to natural ecosystems (Kéfi *et al.* 2019). First, there is a focus on a limited number of temperature shifts (e.g. ambient *versus* forecasted temperature) (Rineau *et al.* 2019). This setting does not reflect expected temperature dynamics, which include diurnal, seasonal, and annual fluctuations. Second, relationships among species and responses to temperature are often considered fixed. This precludes accounting for the adaptation of reaction norms (Norberg *et al.* 2012; Schaum *et al.* 2017; Rineau *et al.* 2019) and the waxing and waning of species interactions through time, which has been observed in face of environmental change (Ushio *et al.* 2018; Bartley *et al.* 2019). Importantly, population dynamics can fail to obey specific equations, which precludes application of standard modelling approaches

(Sugihara *et al.* 2012; Ye *et al.* 2015). Third, the effects of warming and biodiversity on food web stability are often analysed through local stability analysis (May 1973; Gilbert *et al.* 2014). This approach assumes that systems have at least one equilibrium (i.e., a point attractor), and that the asymptotic return rate to that equilibrium after a small perturbation is a proxy of stability (May 1973). However, ecosystems are often far away from equilibria and, more fundamentally, have nonpoint attractor(s) (e.g., strange attractors) (Ives & Carpenter, 2007).

In this study, we quantify the long-term effects of warming and biodiversity on the stability of natural food webs. In order to account for the three shortcomings listed above, we employed empirical dynamic modelling. We first synthesized 12 monthly-sampled food web datasets (11 from freshwater lakes and 1 from a marine system) which span between 10 and 30 years across Europe, Asia and North America. We then employ convergent cross mapping (CCM) to detect causal links among consumers and resources using the empirical time series. Next, the interaction strength among predator-prey (elements in Jacobian matrices) at each time point was computed by the multivariate S-map method. Food web stability at each time point was analysed by computing the real part of dominant eigenvalue of the time-varying Jacobian matrix. Finally we again employed CCM and S-map to detect the causal effect of temperature or biodiversity on stability, and to quantify the direction and strength of the effects.

5.2 Materials and methods

Obtaining time series datasets. We used twelve long-term time-series datasets representing eleven freshwater (lakes) and one marine (Western English Channel) ecosystems to test food web stability responses to biodiversity and temperature (Table S5.1). The 11 lake datasets were obtained by searching the freely available open dataset base from DataONE, North Temperate Lakes, UK Centre for Ecology & Hydrology, JaLTER and NERC (Table S5.1). We selected the datasets using four criteria: 1) data were collected for at least 4 years, 2) the number of trophic level was at least 2, 3) taxa were identified at the species level and 4) temperature was reported. The criterion that data were collected for at least four years was used to ensure that the length of the dataset was sufficient for CCM analysis. The minimum number of trophic levels was set at 2 to ensure food web interactions. Identification of taxa to the species level was required to build high resolution food webs and to uniformly define biodiversity. The temperature was required to ensure test of the effects of warming. The single marine dataset was obtained directly from the Plymouth Marine Laboratory. Overall, we obtained the 12 datasets which are monthly sampled, spanning from 10 to 30 years and originate across the continents of North America, Europe and Asia (Table S5.1). We divided all species into producers, herbivores, omnivores and predators by their diet (Planque *et al.* 2014; Gray *et al.* 2015). We only selected the species which were counted at least once per year for the analyses, otherwise the dataset contained too many zero values for CCM analysis. Finally, the time series of abundance and temperature were normalized to unit mean and variance.

Identifying causal interactions among species using CCM. From the data sets (previous section) we identified the causal links (i.e. competition and predation) within all species pairs in each dataset using convergent cross-mapping (CCM). CCM is based on Takens's theorem for nonlinear dynamical systems (Sugihara *et al.* 2012; Ushio *et al.* 2018). Takens's theorem states that the original attractor of a dynamical system can be reconstructed from the time series of a single observational variable and that same time series but delayed in time. If the cause variable X and effect variable Y belong to the same dynamical system, it is feasible to predict the current state of X using time lags of Y. If two variables (X and Y) are causally related, the accuracy of this prediction (cross-mapping skill) will rapidly increase as the time series of X and Y get longer, but needs to converge at some time series length. Convergence is thus a necessary condition for causality. In addition, when X and Y are driven by a common factor (e.g. seasonality) spurious causality can occur. This can be solved by developing a null test with surrogate time series, as shown by (Deyle *et al.* 2016). In order to generate surrogates for any cause variable X, we calculated year average of X, and also calculated seasonal anomaly as the difference between the observed value X and the year average

X. Then we random shuffled the seasonal anomaly, and added the shuffled anomalies back to the year average of X. As result, this new time series, termed surrogate time series, had same seasonal average as X, but with random anomalies. If X really causes Y, Y should be not only be sensitive to the seasonal components of X, but also to anomalies (Deyle *et al.* 2016). Thus, Y should be better to estimate real time series X than the surrogates of X. To test of this was indeed the case, we produced 100 seasonal surrogates for X.

In this study, an interaction link (e.g. between a prey and a predator) was regarded as significant if each of two criteria were satisfied: (1) the difference between the cross-mapping skill at the smallest and largest library sizes (Δρ) was larger than 0 (convergence) (Ushio *et al.* 2018); (2) cross-mapping skill (ρ) in the real time series was higher than the 95% confidence intervals of surrogates (Ushio *et al.* 2018). Furthermore, considering that the prey species often exhibit time-delayed effects on predator species (Rohner 1995), we carried out 0 to 6 month time-lagged CCM analyses (Ye *et al.* 2019), in which we retained the CCM with the highest ρ (Ye *et al.* 2019). The embedding dimension E (the number of dimensions we needed to recover dynamics) was examined from 1 to 24, which allows for the influence of previous populations up to two year prior, with the best E determined by mean absolute error (Ushio *et al.* 2018).

Quantifying time-varying interaction strength among species. To quantify the interaction matrix (Jacobian matrix) of the food webs at each time point, we used the multivariate S-map method (Ushio *et al.* 2018). For example, if producer species P_1 is causally influenced by a producer P_2 and a herbivore species H_1, and best E equals 6, the state space is reconstructed by $\{P_1(t), P_2(t), H_1(t), P_1(t-1), P_1(t-2), P_1(t-3)\}$. The number of variables used to reconstruct the attractor was equal to the best E. The coefficients of $P_2(t)$ from the multivariate S-map were the interspecific interaction strengths of $P_2(t)$ to $P_1(t)$, and the coefficients of $H_1(t)$ from multivariate S-map were the predation of $H_1(t)$ on $P_1(t)$, while the other coefficients ($P_1(t-1)$, $P_1(t-2)$, $P_1(t-3)$) were excluded because they either were not the interspecific or predatory ones.

Computing stability and quantifying the effects of temperature and biodiversity on the dynamic stability. The time varying stability of the food webs, i.e. dynamic stability, was calculated as described in *Supplement 5.1*. The real part of dominant eigenvalue in the Jacobian matrix D in *Supplement 5.1* was used as a proxy of dynamic stability. A smaller $Re(eigen)$ indicates higher dynamic stability of a food web. In this way, we were able to compute dynamic stability (stability per time point) for every system. To quantify how temperature and diversity (species richness and Simpson diversity) affected dynamic stability, we again employed convergent cross-mapping (CCM) to test their causal effects on stability. Once a causal effect was detected, we employed the multivariate S-map method to quantify the strength and direction of the effect of temperature and diversity on stability. Before analysis, all data were again normalized to unit mean and variance. In the multivariate S-map method, we used the partial derivatives as proxy of the direction of the effect, via reconstructing state space as Re(eigen)(t), species richness(t), Simpson diversity(t) and time-lag terms. If a factor, e.g. species richness, had a negative partial derivative coefficient, this indicated that species richness negatively affected $Re(eigen)$, so higher species richness had a smaller $Re(eigen)$. Hence, in this case, we concluded that higher species richness increased the dynamic stability, as expressed by a reduced $Re(eigen)$.

The absolute effect of temperature and diversity (species richness and Simpson diversity) on dynamic stability was directly calculated as their absolute net effects. The significance of the net and absolute effect of temperature and diversity on dynamic stability was tested by a Kruskal-Wallis test.

Quantifying the effects of temperature and biodiversity on the mean interaction strength in food webs, and quantifying the effects of mean interaction strength on dynamic stability. We tested to what extent the effect of temperature and biodiversity on stability can be understood from changes in mean interaction strength. Mean interaction strength over time was calculated as the geometric mean of the absolute of all the off-diagonal elements in the matrix J_0. J_0 is a part of the Jacobian Matrix D (*Supplement*

5.1) and contains the interaction strengths among species, while excluding all other submatrices in D.

Next, the causal effect of temperature and diversity on the mean interaction strength in each of the 12 datasets was quantified by CCM and multivariate S-mapping. Similarly, the causal effect of mean interaction strength on dynamic stability in each of the 12 datasets was quantified by CCM and multivariate S-mapping.

Sensitivity analysis. Determining the best E is a core step to perform CCM analysis. There are two criteria to determine the best E: 1) lowest mean absolute error and 2) maximal predictive skill. In this chapter, the lowest mean absolute error was used to choose the best E (Ushio *et al.* 2018). To test how robust our results were, we also used the maximal predictive skill as the criterion to determine the best E. These results are shown in Table S5.3.

In addition, 6 out of 11 lakes provided time series of fish species (Table S5.2.1-S5.2.6). These fish species, however, were not high resolute, i.e. only sampled annually rather than monthly. Given that fish species have much smaller death rates than phytoplankton, zooplankton, and macroinvertebrates, we used this annual abundance as monthly abundance. We aim to test whether our results are still robust after adding the fish species into food webs. The causal effect of temperature and diversity on stability in each of the 6 lakes was again quantified by CCM and multivariate S-mapping. These results are shown in Table S5.4.

5.3 Results and discussions

Overall, the number of causal links among species was different among data sets, varying from 9 to 192 links (Fig 5.1). Food web stability was time-varying and was either stable or unstable among the 12 datasets (Fig. 5.2), with the food web being stable if $Re(eigen)$ was smaller than 1 and vice versa. A smaller $Re(eigen)$ indicated a more stable food web, so lake Big Muskellunge and Sparling had the most stable food webs (Fig. 5.2).

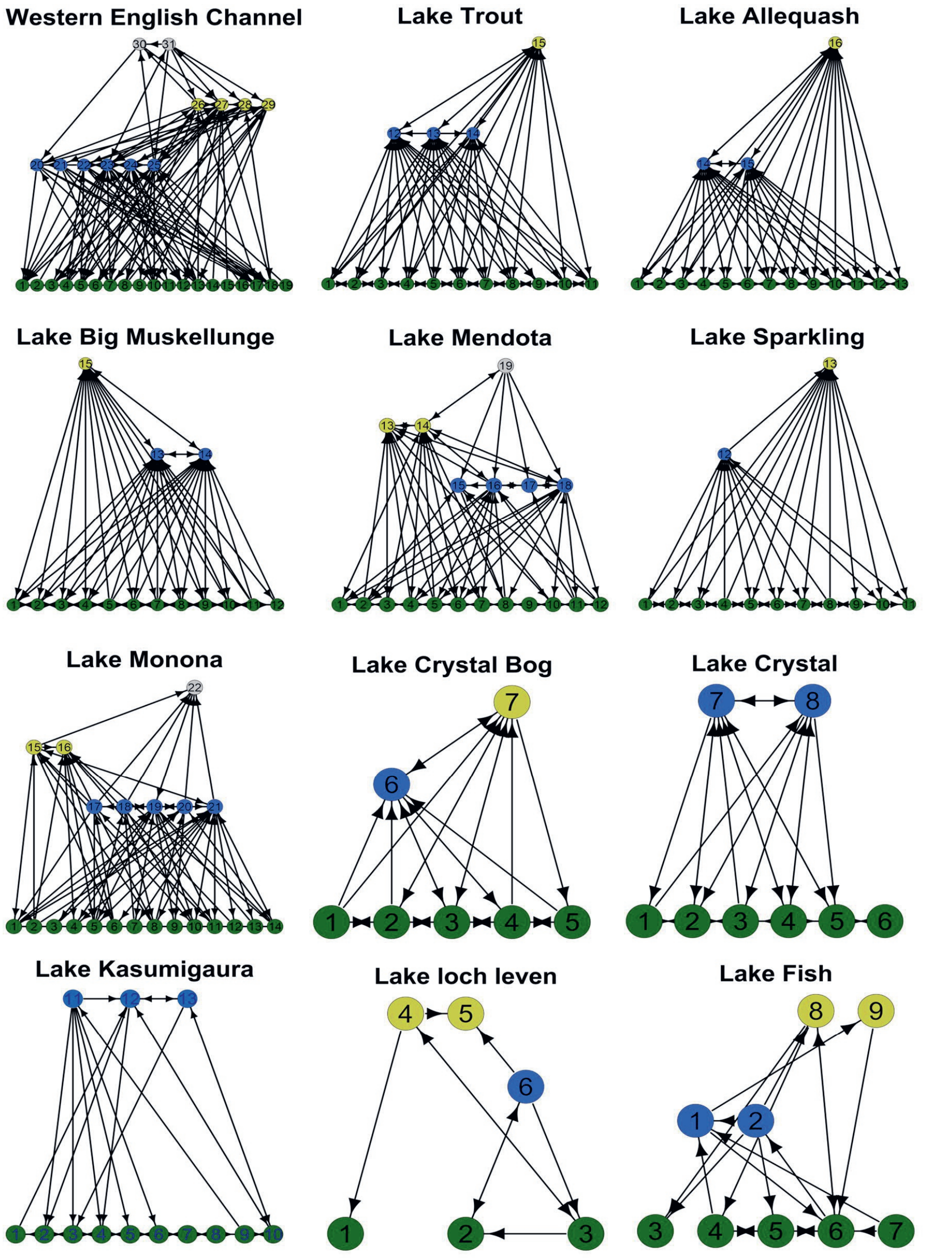


Figure 5.1 Interaction networks from 12 natural food webs. Arrows indicating causal links between each pair of species, as identified by convergent cross-mapping (CCM). The letter within each circle is species number. Circles with the same colour within a food web indicate the same trophic level.

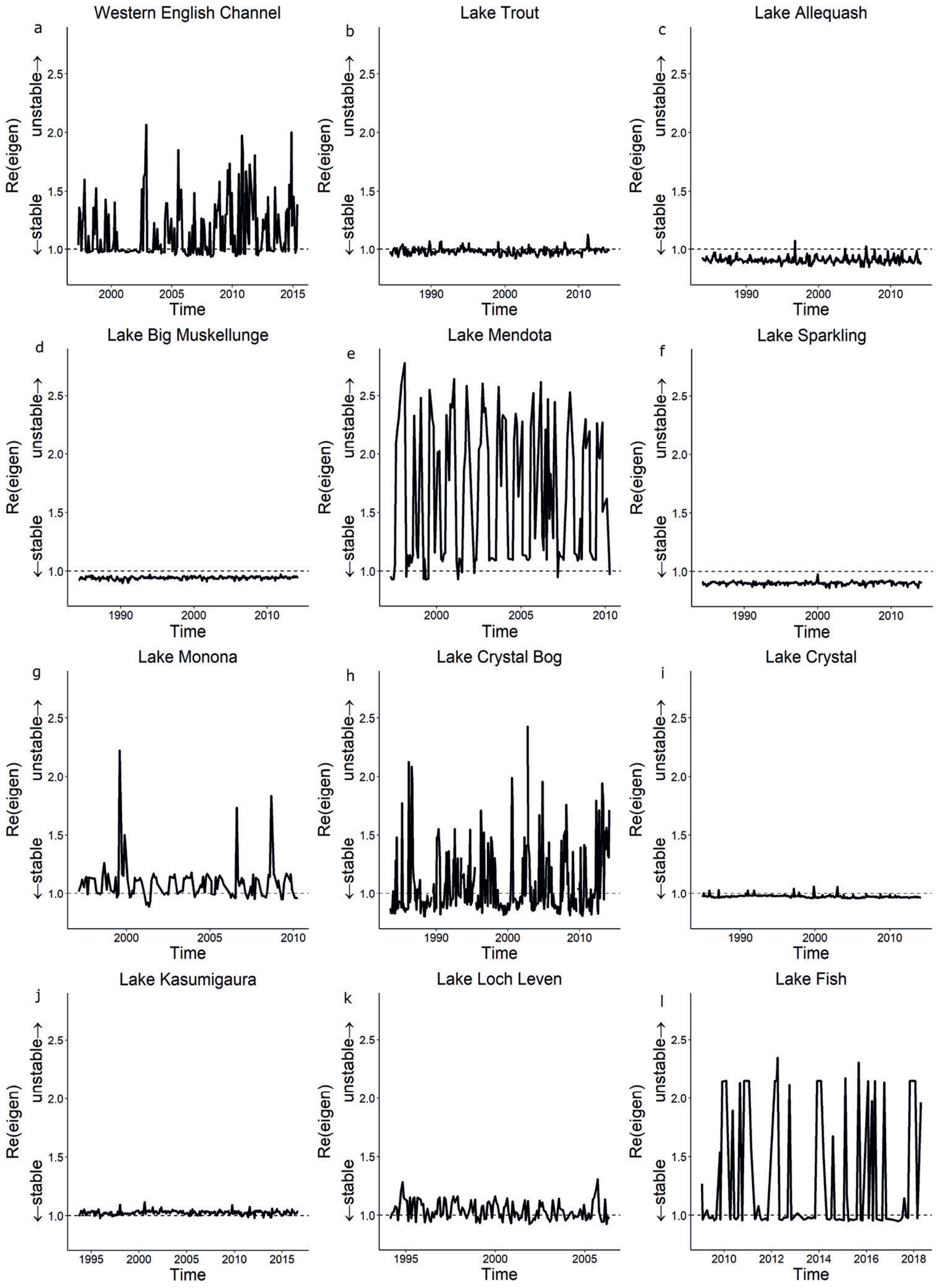


Figure 5.2 Time-varying real part of the dominant eigenvalue $Re(eigen)$ in all datasets.

Temperature positively affected $Re(eigen)$ across all datasets (Fig. 5.3). That is, higher temperatures made for larger $Re(eigen)$, which decreased stability. However, higher biodiversity (species richness and Simpson diversity) always increased stability via decreasing $Re(eigen)$ (Fig. 5.3). The absolute effect of the observed increase in temperature on stability was significantly larger than the effect of the observed increase in biodiversity across all datasets (Fig. S5.1). On average, temperature had 2.2 and 2.0 times larger absolute effects on stability than richness and Simpson, respectively. In addition, using maximal predictive skill as the criterion to determine the best E didn't change these general trends, while species richness and/or Simpson started to become insignificant in some of lakes (Table S5.3). These trends were similar when adding fish species to the food webs (Table S5.4).

In 10 out of the 12 datasets, warming decreased stability because it increased mean interaction strength among species (Fig. 5.4, Table S5.5). Given that the mean interaction strength and stability were calculated from the same Jacobian matrices, it was not surprising that mean interaction strength causally decreased stability in all datasets. Previous modelling studies have shown that warming can increase interaction strength between predators and prey, decreasing stability (Gilbert *et al.* 2014), yet empirical evidence of such effects on long time scales has been lacking. Empirical evidence from short-term experiments having limited number of warming treatments (ambient, +1.7°C, +3.4°C) has shown that warming had no effect on food web stability (Schwarz *et al.* 2017).

Higher biodiversity increased stability, which is again associated with the mean interaction strength (Table S5.5). In again 10 out of 12 datasets, higher species richness and Simpson diversity causally decreased the mean interaction strength (Fig. 5.4), which causally increased stability (Table S5.5). Previous studies have shown that species richness can increase food web stability by reducing the interaction strength between predators and prey, but evidence has again only been provided by modelling and short term experiments (McCann *et al.* 1998; Kéfi *et al.* 2019).

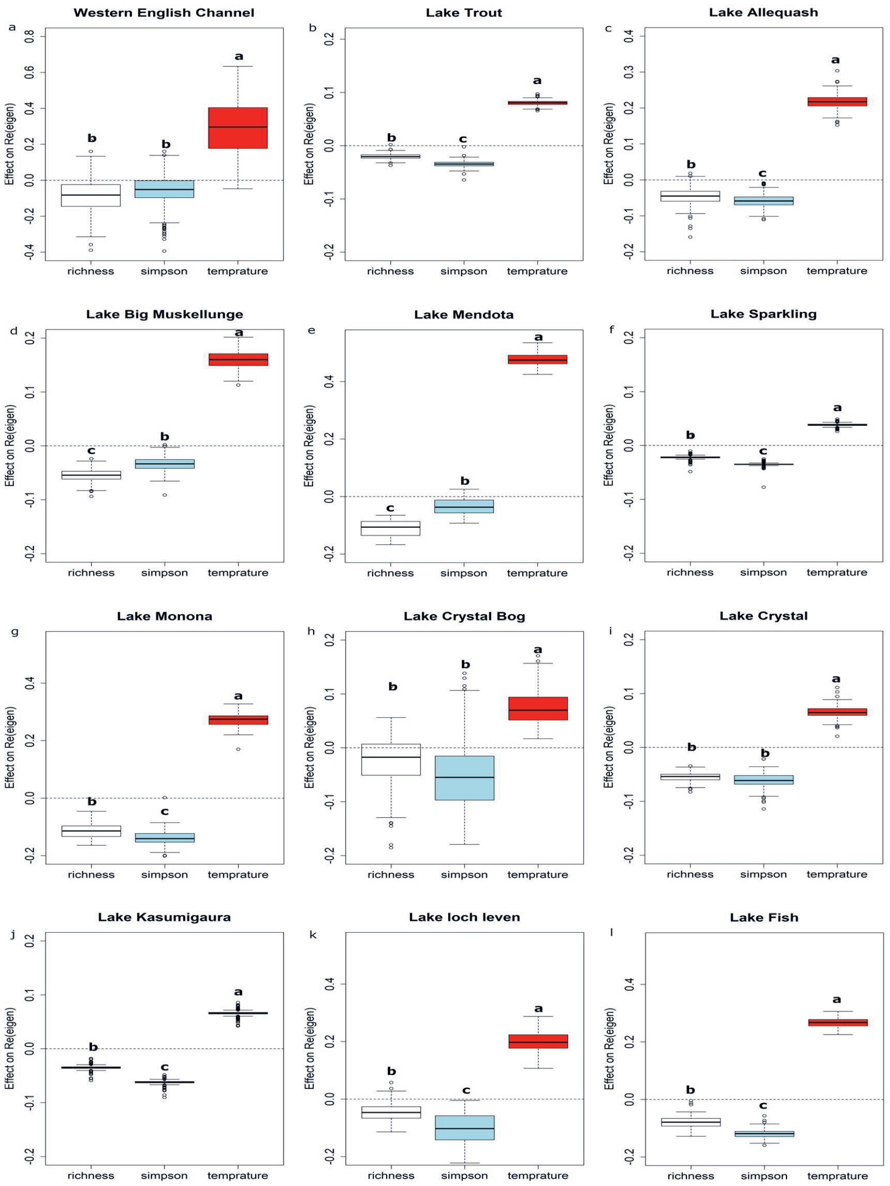


Figure 5.3 The direction and strength of the effect of temperature and biodiversity (species richness and Simpson diversity) on the real part of dominant eigenvalue $Re(eigen)$. Negative values indicate negative effects on $Re(eigen)$ while positive value indicated positive effects, vice versa. A smaller $Re(eigen)$ indicates higher stability of a food web. The significant causal effect of temperature and biodiversity on $Re(eigen)$ was given in Supplementary Figure 5.2.

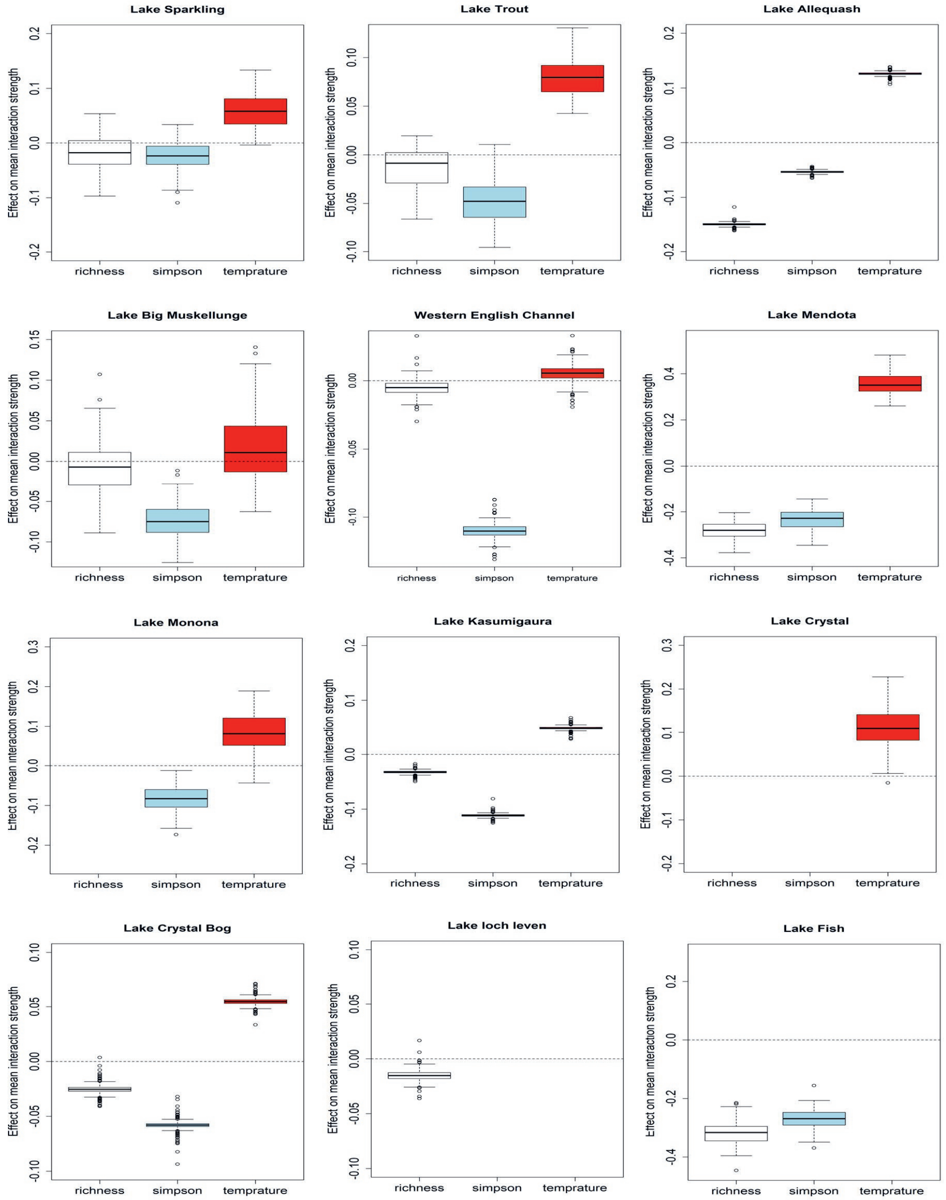


Figure 5.4 The direction and strength of the effect of temperature and biodiversity (species richness and Simpson diversity) on mean interaction strength. Negative values indicate negative effects while positive value indicated positive effects. The significant causal effect of temperature and biodiversity on mean interaction strength was given in Supplementary Figure 5.3.

5.4 Conclusions

Our results show for the first time that warming temperatures causally decreases the long term stability of twelve food webs in natural aquatic ecosystems, while biodiversity (Species richness and Simpson diversity) increased stability. On average, the absolute effect of the observed increases in temperature on stability is larger than the observed increases in biodiversity. Our findings suggest that current levels of global warming could impose a greater threat to ecosystem stability than observed levels of biodiversity change.

Data availability

All lake data used in this study are freely publicly available and data sources can be found in Table S5.1. Station L4 marine data are archived at the Western Channel Observatory (https://www.westernchannelobservatory.org.uk/) and are freely available upon request to Dr. Claire Widdicombe (clst@pml.ac.uk) and Angus Atkinson (aat@pml.ac.uk) at Plymouth Marine Laboratory.

Supplementary

Supplement 5.1 Iteration of dynamic stability. The interaction strength among species was the partial derivative for each time point, which was quantified by the multivariate S-map. Here we iterate the dynamic stability of a food web with 13 species in Lake Kasumigaura as following and other food webs in rest datasets could be iterated as same way. Population dynamics from species 1 to 13 including 6 time-lag effects in Lake Kasumigaura are described as

$$N_1(t+1) = \frac{\partial N_1(t+1)}{\partial N_1(t)} N_1(t) + \frac{\partial N_1(t+1)}{\partial N_2(t)} N_2(t) + \cdots\cdots + \frac{\partial N_1(t+1)}{\partial N_{13}(t)} N_{13}(t) + \frac{\partial N_1(t+1)}{\partial N_1(t-1)} N_1(t-1) + \frac{\partial N_1(t+1)}{\partial N_1(t-2)} N_1(t-2) + \cdots\cdots + \frac{\partial N_1(t+1)}{\partial N_1(t-6)} N_1(t-6) + C_1 \tag{5.1}$$

$$N_2(t+1) = \frac{\partial N_2(t+1)}{\partial N_1(t)} N_1(t) + \frac{\partial N_2(t+1)}{\partial N_2(t)} N_2(t) + \cdots\cdots + \frac{\partial N_2(t+1)}{\partial N_{13}(t)} N_{13}(t) + \frac{\partial N_2(t+1)}{\partial N_2(t-1)} N_2(t-1) + \frac{\partial N_2(t+1)}{\partial N_2(t-2)} N_2(t-2) + \cdots\cdots + \frac{\partial N_2(t+1)}{\partial N_2(t-6)} N_2(t-6) + C_2 \tag{5.2}$$

Population dynamics from species 3 to 13 was described similarly with equation (5.1) and (5.2). Population dynamics from species 1 to 13 can be written as matrix notation as

$$\boldsymbol{N}(t+1) = J_0\boldsymbol{N}(t) + J_1\boldsymbol{N}(t-1) + J_2\boldsymbol{N}(t-2) + J_3\boldsymbol{N}(t-3) + J_4\boldsymbol{N}(t-4) + J_5\boldsymbol{N}(t-5) + J_6\boldsymbol{N}(t-6) + \boldsymbol{C} \tag{5.3}$$

N is a 13-dimensional vector of abundance, **J** is 13×13-dimensional matrix of partial derivatives (interaction strength) and **C** is 13-dimensional vector of Intercept.

After combing with Identity and zero matrix I and O respectively, we can write equation (5.3) as

$$\underbrace{\begin{pmatrix} \boldsymbol{N}(t+1) \\ \boldsymbol{N}(t) \\ \boldsymbol{N}(t-1) \\ \boldsymbol{N}(t-2) \\ \boldsymbol{N}(t-3) \\ \boldsymbol{N}(t-4) \\ \boldsymbol{N}(t-5) \end{pmatrix}}_{\boldsymbol{Y}(t+1)} = \underbrace{\begin{pmatrix} J_0 & J_1 & J_2 & J_3 & J_4 & J_5 & J_6 \\ I & O & O & O & O & O & O \\ O & I & O & O & O & O & O \\ O & O & I & O & O & O & O \\ O & O & O & I & O & O & O \\ O & O & O & O & I & O & O \\ O & O & O & O & O & I & O \end{pmatrix}}_{\boldsymbol{D}} \underbrace{\begin{pmatrix} \boldsymbol{N}(t) \\ \boldsymbol{N}(t-1) \\ \boldsymbol{N}(t-2) \\ \boldsymbol{N}(t-3) \\ \boldsymbol{N}(t-4) \\ \boldsymbol{N}(t-5) \\ \boldsymbol{N}(t-6) \end{pmatrix}}_{\boldsymbol{Y}(t)} + \underbrace{\begin{pmatrix} C \\ O \\ O \\ O \\ O \\ O \\ O \end{pmatrix}}_{\boldsymbol{E}} \tag{5.4}$$

To simplify, we then write equation (5.4) as

$$\boldsymbol{Y}(t+1) = \boldsymbol{DY}(t) + \boldsymbol{E} \tag{5.5}$$

Then we assume Y is at steady state $\widehat{\boldsymbol{Y}}$, which is not a local stable equilibrium of the community. Note that, here, we did not assume the community has a local stable equilibrium (i.e. the community always returning this local stable equilibrium after small perturbations).

$$\widehat{\boldsymbol{Y}}(t+1) = \boldsymbol{D}\widehat{\boldsymbol{Y}}(t) + \boldsymbol{E} \tag{5.6}$$

Next, we combine equation (5.5) and (5.6),

$$\boldsymbol{Y}(t+1) - \widehat{\boldsymbol{Y}}(t+1) = \boldsymbol{D}(\boldsymbol{Y}(t) - \widehat{\boldsymbol{Y}}(t)) \tag{5.7}$$

We could finally write equation (5.7) as

$$\boldsymbol{\varepsilon}(t+1) = \boldsymbol{D}\boldsymbol{\varepsilon}(t) \tag{5.8}$$

In equation (5.8), the deviation ε from the steady state will shrink only when the absolute value of the real part of the dominant eigenvalue of the Jacobian matrix (**D**) <1. If the absolute value of the real part of the dominant eigenvalue i.e. dynamic stability, is less than 1, it indicates that a food web tends to recover faster from perturbations.

Supplementary Table

Table S5.1 Metadata for 12 time series used in analysis.

	lake Name	Country	continent	Latitude	Longitude	Year	Data Source
1	Crystal Lake	United States	North America	46.0018	-89.6136	1983-2014	https://lter.limnology.wisc.edu/data
2	Sparkling Lake	United States	North America	46.0091	-89.6995	1983-2014	https://lter.limnology.wisc.edu/data
3	Trout Lake	United States	North America	46.0461	-89.6751	1983-2014	https://lter.limnology.wisc.edu/data
4	Western English Channel	England	Europe	50° 15′	4° 13′	1992-2017	https://lter.limnology.wisc.edu/data
5	Lake Crystal Bog	United States	North America	46.0076	-89.6063	1983-2014	https://lter.limnology.wisc.edu/data
6	Lake Kasumigaura	Japan	Asia	36.0275	140.3963	1992-2016	http://db.cger.nies.go.jp/gem/moni-e/inter/GEMS/database/kasumi/index.html
7	Lake Loch Leven	Scotland	Europe	56.1940	-3.3754	1982-2012	data.gov.uk/
8	Lake Mendota	United States	North America	43.1113	-89.4255	1997-2008	https://lter.limnology.wisc.edu/
9	Lake Monona	United States	North America	43°4′9″	N89°21′34″	1997-2008	https://lter.limnology.wisc.edu/
10	Lake Fish	United States	North America	38.5502	111.7078	2008-2018	https://lter.limnology.wisc.edu/
11	Lake Allequash	United States	North America	46° 7' 20"	89° 48' 42"	1983-2014	https://search.dataone.org/
12	Lake Big Muskellunge	United States	North America	46° 7' 20"	89° 48' 42"	1983-2014	https://search.dataone.org/

Table S5.2.1 Zooplankton and fish species in lake Trout and their trophic level classification.

Lake Trout				
Herbivores		**Omnivores**	**Predators**	
Cladocera	Rotifer	Copepod	Copepod	Zooplanktivorous fish
1. Bosminidae sp	*2. Kellicottia longispina*	*12. Diacyclops thomasi*	*15. Leptodora sp*	*16. Pimephales notatus*
	3. Keratella cochlearis	*13. Leptodiaptomus minutus*		*17. Ambloplites rupestris*
	4. Keratella quadrata	*14. Mysis sp*		*18. Micropterus dolomieu*
	5. Conochilus sp			
	6. Asplanchna sp			
	7. Polyarthra dolichoptera			
	8. Keratella crassa			
	9. Polyarthra vulgaris			
	10. Keratella hiemalis			
	11. Polyarthra remata			

Table S5.2.2 Zooplankton and fish species in lake Sparkling and their trophic level classification

Lake Sparkling					
Herbivores		**Omnivores**	**Predators**		
Cladocera	Rotifer	Copepod	Copepod	Zooplanktivorous fish	Piscivorous fish
1. Bosminidae sp *11. Daphnia longiremis*	*2. Kellicottia longispina* *3. Keratella cochlearis* *4. Keratella earlinae* *5. Keratella quadrata* *6. Conochilus sp* *7. Asplanchna sp* *8. Polyarthra dolichoptera* *9. Polyarthra vulgaris* *10. Polyarthra remata*	*12. Tropocyclops prasinus mexicanus*	*13. Leptodora sp*	*14. Pimephales notatus* *15. Etheostoma nigrum* *16. Notropis volucellus* *17. Lepomis gibbosus* *18. Ambloplites rupestris* *19. Micropterus dolomieu*	*20. Sander vitreus*

Table S5.2.3. Zooplankton and fish species in lake Crystal and their trophic level classification.

Lake Crystal			
Herbivores		**Omnivores**	**Predators**
Cladocera	Rotifer	Copepod	Zooplanktivorous fish
4. Diaphanosoma birgei	*1. Kellicottia longispina* *2. Keratella cochlearis* *3. Conochilus sp* *5. Polyarthra vulgaris* *6. Polyarthra remata*	*7. Leptodiaptomus minutus* *8. Diacyclops thomasi*	*9. Perca flavescens*

Table S5.2.4 Zooplankton and fish species in lake Crystal Bog and their trophic level classification.

Lake Crystal Bog				
Herbivores		**Omnivores**	**predators**	
Cladocera	Rotifer	Copepod	Copepod	Zooplanktivorous fish
1. *Bosminidae sp* *2. Holopedium gibberum*	3. *Polyarthra vulgaris* 4. *Keratella taurocephala* 5. *Kellicottia bostoniensis*	6. *Tropocyclops prasinus mexicanus*	7. *Mesocyclops edax*	8. *Perca flavescens* 9. *Umbra limi*

Table S5.2.5 Zooplankton and fish species in lake Big Muskellunge and their trophic level classification.

Lake Big Muskellunge						
Herbivores			**Omnivores**	**Predators**		
Copepod	Cladocera	Rotifer	Copepod	Copepod	Zooplanktivorous fish	Piscivorous fish
1. Skistodiaptomus oregonensis	*2. Bosminidae sp* *12. Daphnia pulicaria*	*3. Kellicottia longispina* *4. Keratella cochlearis* *5. Keratella earlinae* *6. Keratella quadrata* *7. Conochilus sp* *8. Synchaeta sp* *9. Keratella crassa* *10. Polyarthra vulgaris* *11. Polyarthra remata*	*13. Diacyclops thomasi* *14. Tropocyclops prasinus mexicanus*	*15. Mesocyclops edax*	*16. Lepomis macrochirus* *17. Pimephales notatus* *18. Etheostoma nigrum* *19. Notropis volucellus* *20. Cottus bairdii* *21. Ambloplites rupestris* *22. Micropterus dolomieu* *23. Catostomus commersonii* *24. Perca flavescens* *25. cisco sp*	*26. Sander vitreus*

Table S5.2.6 Zooplankton and fish species in lake Allequash and their trophic level classification.

Lake Allequash						
Herbivores			**Omnivores**	**Predators**		
Copepod	Cladocera	Rotifer	Copepod	Copepod	Zooplanktivorous fish	Piscivorous fish
1. Skistodiaptomus oregonensis	*2. Bosminidae sp*	*3. Gastropus stylifer* *4. Kellicottia longispina* *5. Keratella cochlearis* *6. Keratella earlinae* *7. Conochilus sp* *8. Synchaeta sp* *9. Ascomorpha ovalis* *10. Keratella crassa* *11. Polyarthra vulgaris* *12. Keratella hiemalis* *13. Polyarthra remata*	*14. cyclopoid sp* *15. Tropocyclops prasinus mexicanus*	*16. Mesocyclops edax*	*17. Pomoxis nigromaculatus* *18. Lepomis macrochirus* *19. Pimephales notatus* *20. Ambloplites rupestris* *21. Catostomus commersonii* *22. Perca flavescens*	*23. Micropterus salmoides* *24. Sander vitreus*

Table S5.3 Selecting best E based on the maximal predictive skill. The causal effect of species richness, Simpson diversity, and temperature on $Re(eigen)$ in each food web. Only significant results were shown ($p < 0.05$, the test of significance see “Methods”), while insignificant results were remarked as *n.s*. Influential strengths were estimated by S-map at each time point (see “Methods”), and were averaged over time. Causal effects were determined by convergence cross-mapping (CCM; see “Methods”).

#	locations	Effect of species richness on Re(eigen)	Effect of Simpson on Re(eigen)	Effect of temperature on Re(eigen)
1	Western English Channel	-0.09	n.s	0.28
2	Lake Trout	-0.06	-0.03	0.08
3	Lake Allequash	-0.04	-0.06	0.21
4	Lake Big Muskellunge	-0.05	-0.03	0.16
5	Lake Mendota	-0.06	n.s	0.15
6	Lake Sparkling	-0.02	n.s	0.04
7	Lake Monona	n.s	-0.03	0.09
8	Lake Crystal Bog	n.s	-0.05	0.07
9	Lake Crystal	-0.07	n.s	0.12
10	Lake Kasumigaura	-0.05	-0.03	0.18
11	Lake Loch Leven	-0.03	n.s	0.12
12	Lake Fish	-0.08	n.s	0.26

Table S5.4 Adding fish species into food webs. The causal effect of species richness, Simpson diversity, and temperature on $Re(eigen)$ in each food web. Influential strengths were estimated by S-map at each time point (see “Methods”), and were averaged over time. Only significant results were shown ($p < 0.05$, the test of significance see “Methods”), while insignificant results were remarked as *n.s.* Causal effects were determined by convergence cross-mapping (CCM; see “Methods”). Values in brackets indicate the absolute effect species richness, Simpson diversity, and temperature on $Re(eigen)$, while values in outside brackets indicate net effects. Bold values indicate the effects when present of fishes, while un-bold values indicate the effects when absent of fishes. The species of each lake was shown at Table S5.2.1-5.2.6.

#	locations	Effect of species richness on Re(eigen)	Effect of Simpson on Re(eigen)	Effect of temperature on Re(eigen)
1	Lake Trout	**-0.05(0.05)**	**-0.04(0.04)**	**0.18(0.18)**
		-0.02(0.02)	-0.03)0.03)	0.08(0.08)
2	Lake Allequash	**-0.08(0.08)**	**-0.01(0.02)**	**0.11(0.11)**
		-0.04(0.04)	-0.05(0.05)	0.21(0.21)
3	Lake Big Muskellunge	**-0.08(0.08)**	**-0.05(0.05)**	**0.10(0.10)**
		-0.06(0.06)	-0.03(0.03)	-0.17 (0.17)
4	Lake Sparkling	**-0.02(0.02)**	**-0.01(0.01)**	**0.04(0.04)**
		-0.01(0.01)	-0.03(0.03)	-0.04(0.024)
5	Lake Crystal Bog	**-0.03(0.03)**	**-0.02(0.02)**	**0.13 (0.13)**
		-0.01(0.03)	-0.05(0.06)	0.07(0.07)
6	Lake Crystal	**-0.07(0.07)**	**-0.03(0.04)**	**0.12(0.12)**
		-0.05(0.05)	-0.07(0.06)	-0.07(0.07)

Table S5.5 The causal effect of temperature on species richness and on Simpson diversity in each food web, and the causal effect of mean interaction strength (mean IS) on $Re(eigen)$. Only significant results were shown ($p < 0.05$, the test of significance see "Methods"), while insignificant results were remarked as *n.s*. Influential strengths were estimated by S-map at each time point (see "Methods"), and were averaged over time. Causal effects were determined by convergence cross-mapping (CCM; see "Methods").

#	locations	Effect of temperatures on Species richness	Effect of temperature on Simpson	Effect mean IS on $Re(eigen)$
1	Western English Channel	n.s	-0.06	0.07
2	Lake Trout	n.s	-0.22	0.063
3	Lake Allequash	-0.16	-0.15	0.11
4	Lake Big Muskellunge	-0.08	-0.05	0.006
5	Lake Mendota	-0.20	-0.18	0.16
6	Lake Sparkling	-0.09	-0.22	0.05
7	Lake Monona	-0.27	-0.15	0.06
8	Lake Crystal Bog	-0.02	-0.17	0.03
9	Lake Crystal	-0.20	n.s	0.12
10	Lake Kasumigaura	-0.12	n.s	0.005
11	Lake Loch Leven	n.s	-0.13	0.02
12	Lake Fish	n.s	n.s	0.27

Supplementary figures

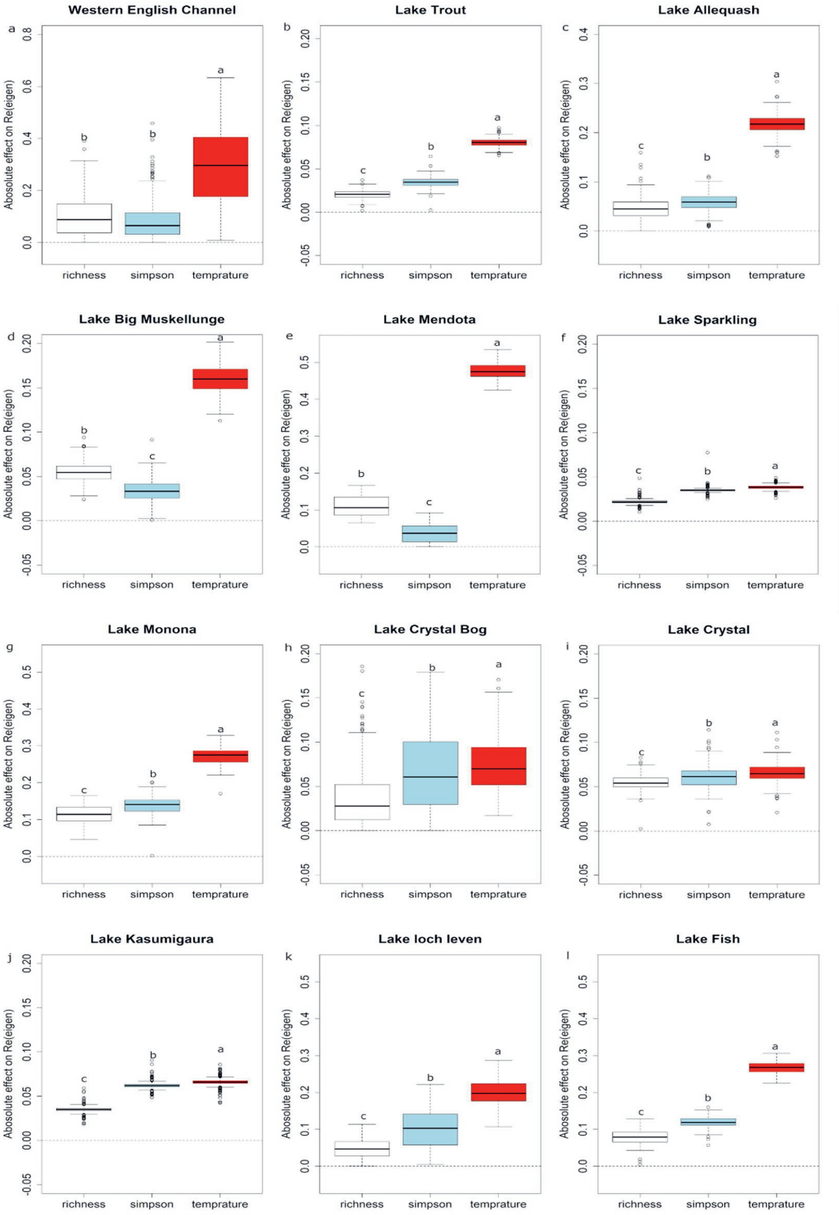


Figure S5.1. Absolute effect of temperature and biodiversity (species richness and Simpson diversity) on real part of dominant eigenvalue $Re(eigen)$. Negative values indicate negative effects on $Re(eigen)$ while positive value indicated positive effects, vice versa. A smaller $Re(eigen)$ indicates higher stability of a food web.

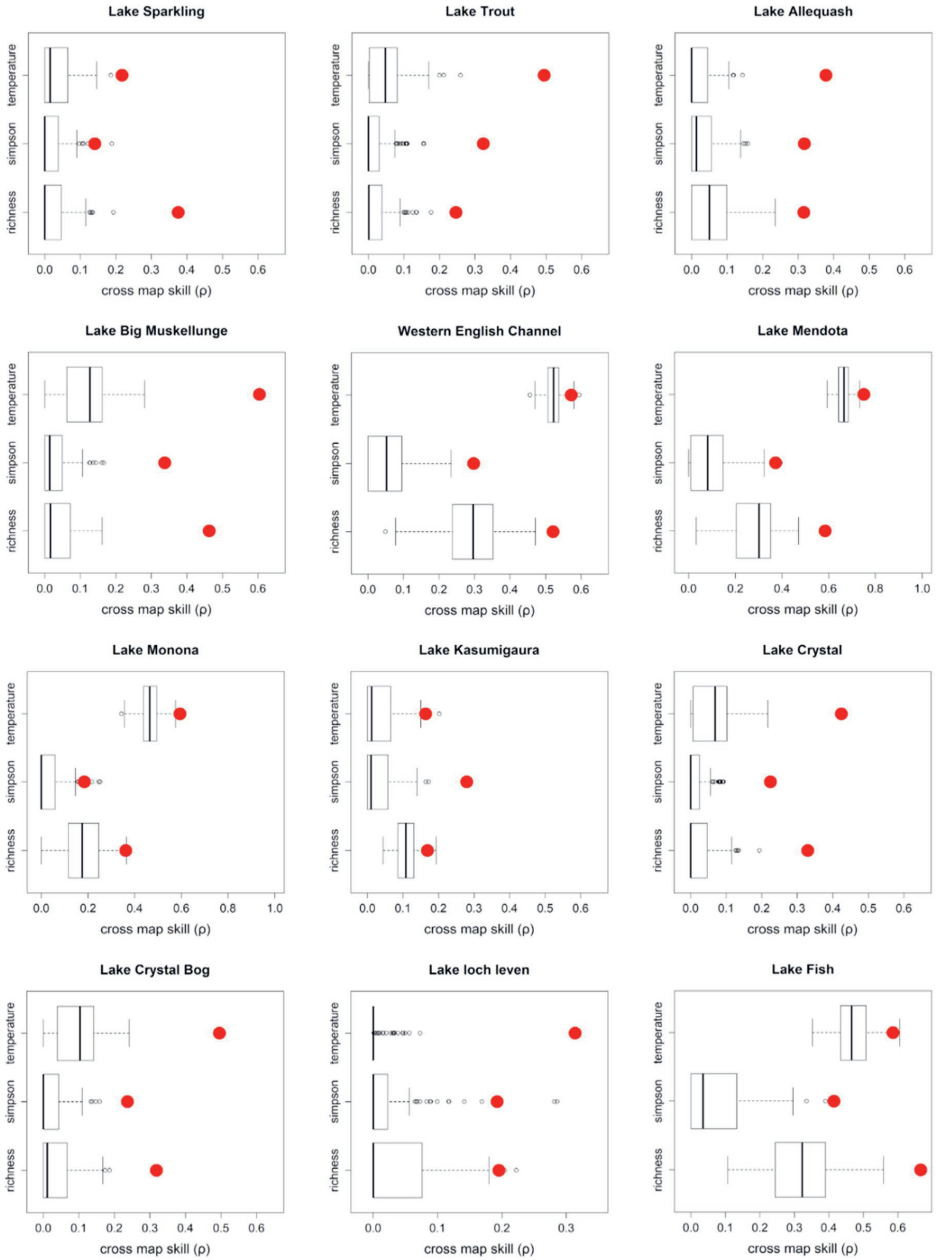
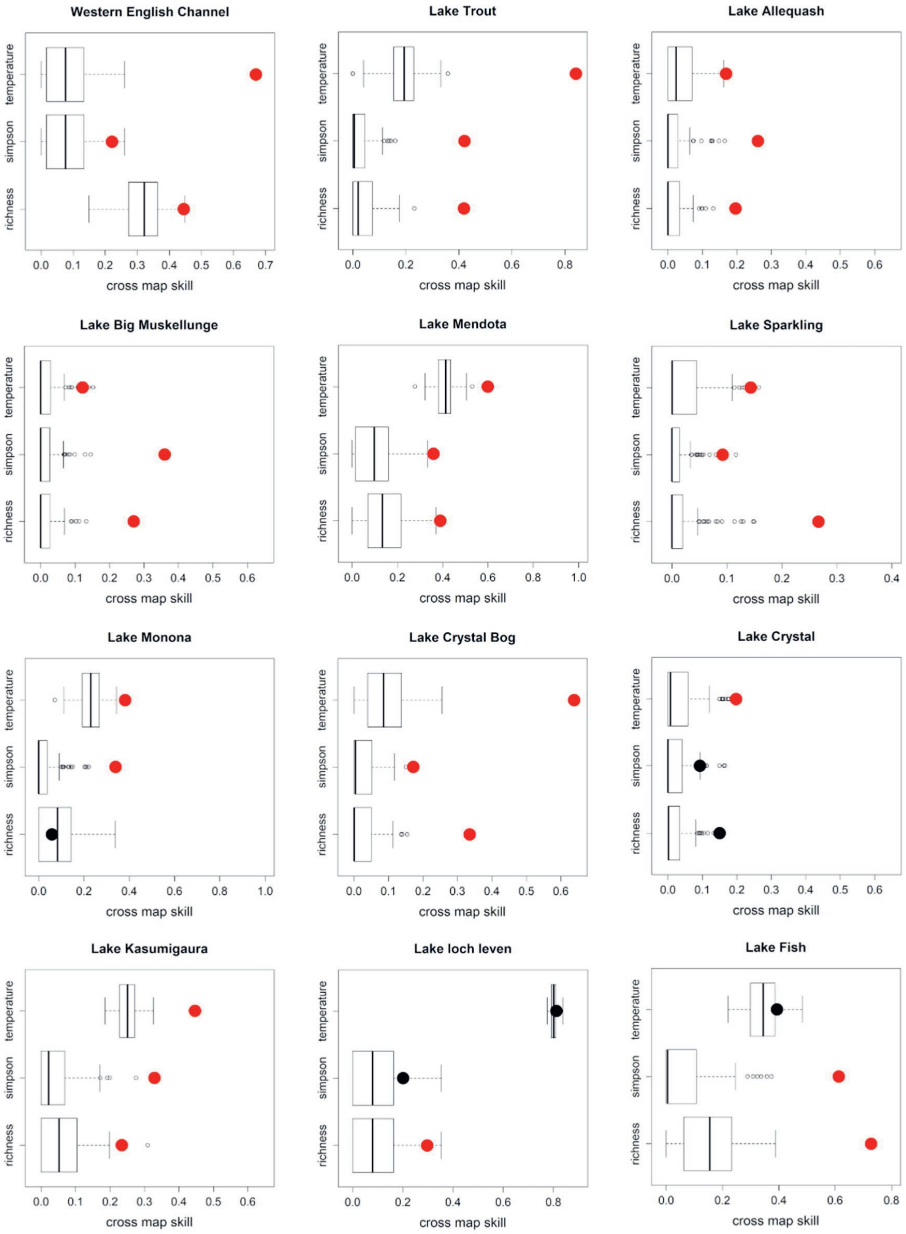


Figure S5.2. Convergent cross-mappings (CCM) showing significant causal effects of temperature and biodiversity (species richness and Simpson diversity) on real part of dominant eigenvalue $Re(eigen)$. Red circles show a significant effect on $Re(eigen)$. A black circle indicated no causal effect.


Western English Channel

Lake Trout

Lake Allequash

Lake Big Muskellunge

Lake Mendota

Lake Sparkling

Lake Monona

Lake Crystal Bog

Lake Crystal

Lake Kasumigaura

Lake loch leven

Lake Fish

Figure S5.3. Convergent cross-mappings (CCM) showing significant causal effects of temperature and biodiversity (species richness and Simpson diversity) on mean interaction strength. Red circles show a significant effect on mean interaction strength. A black circle indicated no causal effect.

Chapter 6

Synthesis

Characterising diversity into horizontal and vertical diversity can help us to understand the relationship between diversity and stability. Theoretical studies often show that diversity tends to decrease stability, while empirical evidence show that biodiversity tends to increase it. The empirical evidence results from experiments including either only the first trophic level (i.e., primary producer) or only simple trophic interactions. Hardly any information is available about the joint effect of vertical and horizontal diversity on stability (Table 6.1).

Moreover, horizontal and vertical diversity can help us to inform environmental risk assessment (ERA) of chemicals. Lower-tier laboratory single-species tests in ERA don't consider species interactions. In the higher-tiers of ERA, multiple species experiments performed using microcosm or mesocosm, often either address only one dimensional diversity (e.g. only vertical diversity), or don't characterise diversity into the two dimensions (i.e. horizontal and vertical diversity) (Table 6.2). The joint influence of horizontal and vertical diversity on the ecological effect of chemicals received little attention.

The objective of my thesis is to enhance the understanding of the importance of horizontal and vertical diversity on food web stability and ERA of chemicals. This study is among the first to consider the joint effects of the horizontal and vertical diversity on stability and ERA of chemicals. In this chapter, I synthesise the results of my work and discuss how they contribute to diversity-stability debate and ERA of chemicals.

Table 6.1 Review of the effect of diversity on the community stability (*engineering resilience*) as observed in empirical and theoretical studies. The table shows for each study whether diversity was divided into horizontal and vertical diversity (Yes, No), and whether the effects of horizontal and vertical diversity were considered or not (Yes, No). Null indicates that the perturbations are very small and unitless.

#	Type of study	Perturbation	Type of community	Division diversity into horizontal and vertical diversity	Horizontal diversity	Vertical diversity	Joint effect of horizontal and vertical diversity	Citation
1	empirical	drought	grassland	Yes	Yes	No	No	(Vogel *et al.* 2012)
2	empirical	chemicals	algae	Yes	Yes	No	No	(Baert *et al.* 2016)
3	empirical	null	Food webs	Yes	No	Yes	No	(Karakoç *et al.* 2020)
4	empirical	drought	grassland	Yes	Yes	No	No	(Isbell *et al.* 2015)
5	empirical	null	fish	Yes	Yes	No	No	(Ushio *et al.* 2018)
6	empirical	null	Food webs	No	No	No	No	(Neutel *et al.* 2007)
7	empirical	CO_2 increase N/P/K fertilization Temperature C amendments	Food webs	No	No	No	No	(Allison & Martiny 2008)
8	empirical	heat	algae	Yes	Yes	No	No	(Allison 2004)
9	empirical	heat	Food webs	No	No	No	No	(Wertz *et al.* 2007)
10	empirical	drought	grassland	Yes	Yes	No	No	(Van Ruijven & Berendse 2010)
11	empirical	sterilization	Food webs	No	No	No	No	(Meola *et al.* 2014)
12	empirical	Invasions	Food webs	No	No	No	No	(Resources & Brunswick 2006)

13	theoretical	null	Food webs	No	No	No	No	(May 1973)
14	theoretical	null	Food webs	No	No	No	No	(Thébault & Loreau 2005)
15	theoretical	null	Food webs	No	No	No	No	(Yang *et al.* 2019)
16	theoretical	null	Food webs	No	No	No	No	(Allesina & Tang 2012)
17	theoretical	null	Food webs	Yes	Yes	No	No	(McCann *et al.* 1998)
18	theoretical	null	Food webs	No	No	No	No	(Butler & O'Dwyer 2018)
19	theoretical	null	Food webs	No	No	No	No	(Leigh 1965)
20	theoretical	null	Food chain	Yes	No	Yes	No	(Pimm & Lawton 1977)
21	theoretical	null	Food chain	Yes	No	Yes	No	(Sterner *et al.* 1997)
22	theoretical	null	Food webs	Yes	No	Yes	No	(Emmerson & Yearsley 2004)
23	empirical	drought	grassland	Yes	Yes	No	No	(Tilman & Downing 1994)

Table 6.2 Review of the effects of chemicals (i.e. pesticides) on aquatic communities in experiments. The table shows for each study whether diversity was divided into horizontal and vertical diversity (Yes, No), and whether the effects of horizontal and vertical diversity were considered or not (Yes, No).

#	Type of experiments	Type of community	Division diversity into horizontal and vertical diversity	Horizontal diversity	Vertical diversity	Joint effect of horizontal and vertical diversity	Citation
1	microcosm	algae	Yes	Yes	No	No	(Baert *et al.* 2016)
2	mesocosm	Food webs	No	No	No	No	(Van den Brink *et al.* 1996)
3	microcosm	algae	Yes	Yes	No	No	(Mensens *et al.* 2017)
4	microcosm	Food webs	No	No	No	No	(Zafar *et al.* 2011)
5	microcosm	Food webs	No	No	No	No	(Daam *et al.* 2008b)
6	microcosm	Food webs	No	No	No	No	(Daam & Van den Brink 2007)
7	microcosm	Food chain	Yes	No	Yes	No	(Tran *et al.* 2019)
8	microcosm	Food webs	No	No	No	No	(Van Wijngaarden *et al.* 2005)
9	microcosm	Food chain	Yes	No	Yes	No	(Beketov & Liess 2006)
10	microcosm	Food chain	Yes	No	Yes	No	(Relyea & Mills 2001)
11	microcosm	Food webs	Yes	No	Yes	No	(Trekels *et al.* 2011b)
12	microcosm	Food chain	Yes	No	Yes	No	(Beketov & Liess 2006)
13	microcosm	Food webs	Yes	No	Yes	No	(Trekels *et al.* 2013)
14	microcosm	Food chain	Yes	No	Yes	No	(Campero *et al.* 2007)
15	microcosm	Food chain	Yes	No	Yes	No	(Janssens & Stoks 2017)
16	microcosm	Food chain	Yes	No	Yes	No	(Janssens & Stoks 2013)
17	microcosm	Food webs	No	No	No	No	(Daam *et al.* 2009)
18	microcosm	Food chain	Yes	No	Yes	No	(Dinh Van *et al.* 2014)
19	microcosm	Food webs	No	No	No	No	(Brock *et al.* 1992)
20	mesocosm	Food webs	No	No	No	No	(Van den Brink & Ter Braak 1999)
21	mesocosm	Food webs	No	No	No	No	(Hughes *et al.* 1980)

22	mesocosm	Food webs	No	No	No	No	(Cuppen *et al.* 1997)
23	microcosm	Food webs	No	No	No	No	(Sumon *et al.* 2018)
24	microcosm	Food webs	No	No	No	No	(Thompson *et al.* 2006)
25	microcosm	Food webs	No	No	No	No	(Kersting & van Wijngaarden 1992)
26	microcosm	Food webs	No	No	No	No	(Snel *et al.* 1998)
27	mesocosm	Food webs	No	No	No	No	(Stephenson & Kane 1984)
28	microcosm	Food webs	Yes	Yes	No	No	(Van den Brink *et al.* 1997)
29	mesocosm	Food webs	No	No	No	No	(Traunspurger *et al.* 1996)
30	mesocosm	Food webs	No	No	No	No	(Peither *et al.* 1996)
31	mesocosm	Food webs	No	No	No	No	(Boyle *et al.* 1996)
32	mesocosm	Food webs	No	No	No	No	(Rohr & Crumrine 2005)
33	mesocosm	algae	Yes	Yes	No	No	(Bretherton *et al.* 2019)
34	microcosm	Food webs	No	No	No	No	(Vonesh & Kraus 2009)
35	mesocosm	Food webs	No	No	No	No	(Relyea & A 2005)
36	microcosm	Food webs	No	No	No	No	(Muturi *et al.* 2017)
37	microcosm	Food webs	No	No	No	No	(Laabs *et al.* 2007)
38	mesocosm	Food webs	No	No	No	No	(Vischetti *et al.* 2008)
39	microcosm	Food chain	Yes	No	Yes	No	(Pestana *et al.* 2009)
40	mesocosm	Food webs	No	No	No	No	(Trekels *et al.* 2011a)
41	microcosm	Food webs	No	No	No	No	(Van Wijngaarden *et al.* 2004)
42	mesocosm	Food webs	No	No	No	No	(Rumschlag *et al.* 2019)
43	mesocosm	Food webs	No	No	No	No	(Groner & Relyea 2011)
44	microcosm	Food webs	No	No	No	No	(Fliedner *et al.* 1997)
45	mesocosm	Food webs	No	No	No	No	(Hasenbein *et al.* 2016)
46	microcosm	Food webs	Yes	No	Yes	No	(Barry & Davies 2004)
47	microcosm	Food webs	Yes	No	Yes	No	(Ridal *et al.* 2001)
48	microcosm	Food webs	No	No	No	No	(Nowell *et al.* 2014)

49	mesocosm	Food webs	No	No	No	No	(Bendis & Relyea 2016)
50	mesocosm	Food webs	No	No	No	No	(Hegde *et al.* 2014)
51	microcosm	Food webs	No	No	No	No	(Dellagreca *et al.* 2004)
52	mesocosm	Food webs	No	No	No	No	(Hayasaka *et al.* 2012)
53	microcosm	algae	Yes	Yes	No	No	(Tien & Chen 2012)
54	microcosm	Food webs	No	No	No	No	(Van den Brink *et al.* 1995)
55	microcosm	macrophytes	Yes	Yes	No	No	(Hand *et al.* 2001)
56	microcosm	Food webs	No	No	No	No	(Cedergreen *et al.* 2006)
57	microcosm	Food chain	Yes	No	Yes	No	(Lima-Fernandes *et al.* 2019)
58	microcosm	algae	Yes	Yes	No	No	(DeNoyelles *et al.* 2016)
59	microcosm	Food webs	No	No	No	No	(Xiao *et al.* 2017)
60	microcosm	Food webs	No	No	No	No	(Heimbach *et al.* 1992)
61	microcosm	Food webs	No	No	No	No	(Isensee & Jones 1975)
62	microcosm	algae	Yes	Yes	No	No	(Brockway *et al.* 1984)
63	mesocosm	Food webs	No	No	No	No	(Chang *et al.* 2005)

6.2 The effect of diversity on food web stability depends on horizontal and vertical diversity

In **chapter 2**, we first modelled the joint effects of horizontal and vertical diversity on food web stability. Stability is analysed by local stability analysis, which assumes that systems are exposed to small perturbations near equilibrium. In modelled food webs, we found that horizontal and vertical diversity increased and decreased stability, respectively, with a stronger positive effect of producer diversity on stability at higher consumer diversity. Microcosm experiments with a plankton food web, again analysed with local stability analysis, confirmed these modelling predictions. Given that ecosystems often face large perturbations, we secondly exposed the same food webs as used for local stability analysis to larger perturbations (chemicals). We again confirmed the modelling predictions. Taken together, our results indicate that high horizontal diversity can compensate the stability loss caused by a high vertical diversity.

In **chapter 2**, exposure to larger disturbances (chemicals) and differences in horizontal and vertical diversity had contrasting effects on the stability. Horizontal diversity decreased the negative effects of chemicals on total biomass of food webs which enhanced stability, while vertical diversity increased the negative effects, reducing stability. These results indicate that two dimensional diversity can modify the effect of chemicals on food webs and can provide an perspective on the use of the results of single and multiple species tests in the ERA of chemicals, which was thoroughly studied in **chapter 3**.

6.3 The effect of chemicals depends on horizontal and vertical diversity

In **chapter 3**, we thoroughly studied how changes in number of species within trophic levels and number of trophic levels modify the direct and indirect effects of chemicals (an insecticide and a herbicide). We found from the results of the microcosm experiments that the effect of the herbicide linuron on producer abundance was lower when the number of producers increased from 1 to 5. However, linuron decreased producer abundance to a higher extend when the number of herbivore species was increased from 0 to 4 and when the number of trophic levels was increased from 1 to 3. The indirect negative effect of linuron on herbivore abundance was lower when the number of producers or herbivores was higher. Adding a predator, however, increased the indirect negative effects of linuron on herbivore abundance.

The insecticide chlorpyrifos decreased herbivore abundance less when the number of herbivores increased from 1 to 4 and when number of producers increased from 1 to 5. However, when the number of trophic levels increased from 2 to 3, the direct negative impact on herbivores was higher. Increasing the number of producer and herbivore species and trophic levels always increased the indirect positive impact on producer abundance. The results in chapter 3 indicate that the effects of pesticides on single species do not always represent a worst case ERA. Protecting the most sensitive species may not protect the whole ecosystem.

It should be noted that results in chapter 3 are from a microcosm experiment, including a limited number of species (from 1 up to 10). We therefore scaled up our analysis to microcosm and mesocosm experiments which included higher numbers of species (from 17 up to 129 species) and reanalysed 7 experimental datasets (**chapter 4**). We aimed, in chapter 4, to evaluate how robust the observed interactions of horizontal and vertical diversity and the effects of insecticide chlorpyrifos as observed in **chapter 3**, are.

In **chapter 4**, we found similar results as in chapter 3. That is, increasing species diversity within trophic levels (horizontal diversity) and the number of trophic levels (vertical diversity) had contrasting influence on the effect of chlorpyrifos on herbivore abundance as observed in microcosm and mesocosm experiments. Chapter 4 additionally showed that the decrease in herbivore abundance induced an increase in chlorophyll *a* and subsequent changes in physicochemical parameters (electricity conductivity). These indirect effects were smaller at higher herbivore diversity. Given that the datasets used in **chapter 4** are synthesized from experiments with different scales of biodiversity, the results suggest that diversity at the start of the experiments should be considered for the use of their results in the ERA of chemicals. The results of chapter 3 and 4 bring us to the conclusion that horizontal and vertical diversity indeed modify the effects of chemicals on aquatic ecosystems in both simple systems like planktonic ecosystems, as well as more complex macrophyte dominated aquatic ecosystems.

6.4 The effect of climate change on food web stability

Warming temperatures could decrease, increase and even have no effect on food web stability (Binzer *et al.* 2012; Gilbert *et al.* 2014; Schwarz *et al.* 2017; O'Gorman *et al.* 2019). Previous studies about warming temperatures were either tested in short term empirical studies or estimated by modelling simulations. Short term empirical studies (e.g. field experiments) normally study a small changes in the dynamics of food webs and usually evaluate two levels of temperature (i.e. ambient *versus* future projections) (Schwarz *et al.* 2017). Although evaluating the effects of climate factors using two levels in experiments and modelling assessments are quite common, it may not reflect reality (Rineau *et al.* 2019). Temperature in real systems is not fixed but varies within the day and over seasons and years. Moreover, modelling estimations do not completely reflect the real situation in nature (Gauzens *et al.* 2020). The aim of **chapter 5** was to evaluate the effect of warming on food web stability based on long term monitoring data from natural systems using a model-free approach.

In order to evaluate the effects of temperature on food web stability in **chapter 5**, we synthesized data from 12 food webs, 11 from freshwater lakes and one from marine system, spanning between 10 to 30 years and three continents. We employed the empirical dynamic modelling, not assuming population equilibrium and not assuming any set of equations governing the systems, but instead recovering dynamics from time series data to quantify the effect of warming on the stability for all these food webs. We found that warming decreases stability via increasing the mean interaction strength between predator and prey. We additionally found that biodiversity (estimated as species richness and as the Simpson index) increased stability. Moreover, on average, the absolute effect of the observed increase in temperature on stability is 2.1 times larger than the observed increase in biodiversity. The findings in **chapter 5** suggest that current levels of global warming could impose a larger damage to ecosystem stability than current levels of biodiversity changes.

In general, this thesis provides new ways to study the relationship between diversity and stability, and to study the influence of chemicals on aquatic ecosystems, by dividing diversity into horizontal (number of species within trophic levels) and vertical diversity (number of trophic levels). The feasibility of this division is based on the fact that the two dimensional diversity mediate food webs via different mechanisms. The effect of horizontal diversity is mediated by competition, while the effect of vertical diversity is mediated by predation. We also provide a new perspective on how warming temperatures affect food web stability in the long term.

References

Aktar, W., Sengupta, D. & Chowdhury, A. (2009). Impact of pesticides use in agriculture : their benefits and hazards. *Interdiscip. Toxicol.*, 2, 1–12.

Allesina, S. & Tang, S. (2012). Stability criteria for complex ecosystems. *Nature*, 483, 205–208.

Allesina, S. & Tang, S. (2015). The stability–complexity relationship at age 40: a random matrix perspective. *Popul. Ecol.*, 57, 63–75.

Allison, G. (2004). The influence of species diversity and stress intensity on community resistance and resilience. *Ecol. Monogr.*, 74, 117–134.

Allison, S.D. & Martiny, J.B.H. (2008). Resistance, resilience, and redundancy in microbial communities. *Proc. Natl. Acad. Sci.*, 105, 11512–11519.

Anderson, J.S., Teutsch, M., Dong, Z., Delgado-lo, F., Nu, F., Vera, J.C., *et al.* (1996). A meta-analysis of the freshwater trophic cascade. *Proc. Natl. Acad. Sci.*, 93, 7723–7726.

Arnoldi, J., Loreau, M. & Haegeman, B. (2016). Resilience , reactivity and variability : a mathematical comparison of ecological stability measures. *J. Theor. Biol.*, 389, 47–59.

Artigas, J., Arts, G., Babut, M., Caracciolo, A.B., Charles, S., Chaumot, A., *et al.* (2012). Towards a renewed research agenda in ecotoxicology. *Environ. Pollut.*, 160, 201–206.

Baert, J.M., De Laender, F. & Janssen, C.R. (2017). The consequences of nonrandomness in species-sensitivity in relation to functional traits for ecosystem-level effects of chemicals. *Environ. Sci. Technol.*, 51, 7228–7235.

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

Bairey, E., Kelsic, E.D. & Kishony, R. (2016). High-order species interactions shape ecosystem diversity. *Nat. Commun.*, 7, 12285.

Barbier, M. & Loreau, M. (2019). Pyramids and cascades: a synthesis of food chain functioning and stability. *Ecol. Lett.*, 22, 405–419.

Barnes, A.D., Jochum, M., Lefcheck, J.S., Eisenhauer, N., Scherber, C., O'Connor, M.I., *et al.* (2018). Energy flux: the Link between multitrophic biodiversity and ecosystem functioning. *Trends Ecol. Evol.*, 33, 186–197.

Barnes, A.D., Jochum, M., Mumme, S., Haneda, N.F., Farajallah, A., Widarto, T.H., *et al.* (2014). Consequences of tropical land use for multitrophic biodiversity and ecosystem functioning. *Nat. Commun.*, 5, 1–7.

Barry, M.J. & Davies, W. (2004). Effects of invertebrate predators and a pesticide on temporary pond microcosms used for aquatic toxicity testing. *Environ. Pollut.*, 131, 25–34.

Bartley, T.J., McCann, K.S., Bieg, C., Cazelles, K., Granados, M., Guzzo, M.M., *et al.* (2019). Food Web Rewiring in a Changing World. *Nat. Ecol. Evol.*, 3, 345–354.

Bates, D., Mächler, M., Bolker, B. & Walker, S. (2014). Fitting linear mixed-effects models using lme4. *J. Stat. Softw.*, 67, 1–48.

Becker, J.M. & Liess, M. (2017). Species diversity hinders adaptation to toxicants. *Environ. Sci. Technol.*, 51, 10195–10202.

Beketov, M.A. & Liess, M. (2006). The influence of predation on the chronic response of *Artemia* sp. populations to a toxicant. *J. Appl. Ecol.*, 43, 1069–1074.

Bendis, R.J. & Relyea, R.A. (2016). Wetland defense: naturally occurring pesticide resistance in zooplankton populations protects the stability of aquatic communities. *Oecologia*, 181, 487–498.

Berg, S., Christianou, M., Jonsson, T. & Ebenman, B. (2011). Using sensitivity analysis to identify keystone species and keystone links in size-based food webs. *Oikos*, 120, 510–519.

Berlow, E.L. (1999). Strong effects of weak interactions in ecological communities. *Nature*, 398, 330–334.

Binzer, A., Guill, C., Brose, U. & Rall, B.C. (2012). The dynamics of food chains under climate change and nutrient enrichment. *Philos. Trans. R. Soc. B Biol. Sci.*, 367, 2935–2944.

Boraas, M.E. (1983). Population dynamics of food-limited rotifers in two-stage chemostat culture. *Limnol. Oceanogr,* 28, 546-563.

Borrvall, C. & Ebenman, B. (2006). Early onset of secondary extinctions in ecological communities following the loss of top predators. *Ecol. Lett.*, 9, 435–442.

Borrvall, C., Ebenman, B. & Jonsson, T. (2000). Biodiversity lessens the risk of cascading extinction in model food webs. *Ecol. Lett.*, 3, 131–136.

Boyle, T.P., Fairchild, J.F., Robinson-Wilson, E.F., Haverland, P.S. & Lebo, J.A. (1996). Ecological restructuring in experimental aquatic mesocosms due to the application of diflubenzuron. *Environ. Toxicol. Chem.*, 15, 1806–1814.

Bracewell, S., Verdonschot, R.C.M., Schäfer, R.B., Bush, A., Lapen, D.R. & Van den Brink, P.J. (2019). Qualifying the effects of single and multiple stressors on the food web structure of Dutch drainage ditches using a literature review and conceptual models. *Sci. Total Environ.*, 684, 727–740.

Bretherton, L., Kamalanathan, M., Genzer, J., Hillhouse, J., Setta, S., Liang, Y., *et al.* (2019). Response of natural phytoplankton communities exposed to crude oil and chemical dispersants during a mesocosm experiment. *Aquat. Toxicol.*, 43–53.

Britten, G.L., Dowd, M., Minto, C., Ferretti, F., Boero, F. & Lotze, H.K. (2014). Predator decline leads to decreased stability in a coastal fish community. *Ecol. Lett.*, 17, 1518–1525.

Brock, T.C., Arts, G.H., Maltby, L. & Van den Brink, P.J. (2006). Aquatic risks of pesticides, ecological protection goals, and common aims in European Union Legislation. *Integr. Environ. Assess. Manag.*, 2, e20–e46.

Brock, T.C.M., van den Bogaert, M., Bos, A.R., van Breukelen, S.W.F., Reiche, R., Terwoert, J., *et al.* (1992). Fate and effects of the insecticide Dursban® 4E in indoor Elodea-dominated and macrophyte-free freshwater model ecosystems: II. Secondary effects on community structure. *Arch. Environ. Contam. Toxicol.*, 23, 391–409.

Brock, T.C.M., Lahr, J. & Van den Brink, P.J. (2000). *Ecological risks of pesticides in freshwater ecosystems Part 1 : Herbicides. Alterra-Report 088*.

Brockway, D.L., Smith, P.D. & Stancil, F.E. (1984). Fate and effects of atrazine in small aquatic microcosms. *Bull. Environ. Contam. Toxicol.*, 32, 345–353.

Brose, U. (2008). Complex food webs prevent competitive exclusion among producer species. *Proc. R. Soc. B Biol. Sci.*, 275, 2507–2514.

Brown, J.H., Gillooly, J.F., Allen, A.P., Savage, V.M. & West, G.B. (2004). Toward a metabolic theory of ecology. *Ecology*, 85, 1771–1789.

Butler, S. & O'Dwyer, J.P. (2018). Stability criteria for complex microbial communities. *Nat. Commun.*, 9.

Campero, M., Slos, S., Ollevier, F. & Stoks, R. (2007). Sublethal pesticide concentrations and predation jointly shape life history: Behavioral and physiological mechanisms. *Ecol. Appl.*, 17, 2111–2122.

Cardinale, B.J., Harvey, C.T., Gross, K. & Ives, A.R. (2003). Biodiversity and biocontrol: emergent impacts of a multi-enemy assemblage on pest suppression and crop yield in an agroecosystem. *Ecol. Lett.*, 6, 857–865.

Cardinale, B.J., Srivastava, D.S., Duffy, J.E., Wright, J.P., Downing, A.L., Sankaran, M., *et al.* (2006). Effects of biodiversity on the functioning of trophic groups and ecosystems. *Nature*, 443, 989–992.

Casula, P., Wilby, A. & Thomas, M.B. (2006). Understanding biodiversity effects on prey in multi-enemy systems. *Ecol. Lett.*, 9, 995–1004.

Cedergreen, N., Kamper, A. & Streibig, J.C. (2006). Is prochloraz a potent synergist across aquatic species? A study on bacteria, daphnia, algae and higher plants. *Aquat. Toxicol.*, 78, 243–252.

Chang, K.H., Sakamoto, M. & Hanazato, T. (2005). Impact of pesticide application on zooplankton communities with different densities of invertebrate predators: An experimental analysis using small-scale mesocosms. *Aquat. Toxicol.*, 72, 373–382.

Clements, W.H. & Rohr, J.R. (2009). Community responses to contaminants: using basic ecological principles to predict ecotoxicological effects. *Environ. Toxicol. Chem.*, 28, 1789–1800.

Cohen, I. & Post, A.F. (1993). The heterotrophic connection in a photoautotrophic chlorella vulgaris dominant in wastewater oxidation ponds. *Water Sci. Technol.*, 27, 151–155.

Cohen, J.E., Jonsson, T. & Carpenter, S.R. (2003). Ecological community description using the food web, species abundance, and body size. *Proc. Natl. Acad. Sci. U. S. A.*, 100, 1781–6.

Cooper, J. & Dobson, H. (2007). The benefits of pesticides to mankind and the environment. *Crop Prot.*, 26, 1337–1348.

Coyte, K.Z., Schluter, J. & Foster, K.R. (2015). The ecology of the microbiome: networks, competition, and stability. *Science.*, 350, 663–666.

Crane, M., Maycock, D., Watts, C.D., Atkinson, C. & Johnson, I. (2007). *Proposed EQS for water framework directive annex VIII substances: 2,4- dichlorophenol.* Environment Agency, Almondsbury, Bristol, UK.

Crowder, L.B., Squires, D.D. & Rice, J.A. (1997). Nonadditive effects of terrestrial and aquatic predators on juvenile estuarine fish. *Ecology*, 78, 1796–1804.

Cuppen, J.G.M., Van den Brink, P.J., Hartgers, E.M., Fettweis, U., Crum, S.J.H., Van Donk, E., *et al.* (1997). Sensitivity of macrophyte-dominated freshwater microcosms to chronic levels of the herbicide linuron. *Ecotoxicol. Environ. Saf.*, 38, 13–24.

Daam, M.A. & Van den Brink, P.J. (2007). Effects of chlorpyrifos, carbendazim, and linuron on the ecology of a small indoor aquatic microcosm. *Arch. Environ. Contam. Toxicol.*, 53, 22–35.

Daam, M.A., Van den Brink, P.J. & Nogueira, A.J.A. (2008a). Impact of single and repeated applications of the insecticide chlorpyrifos on tropical freshwater plankton communities. *Ecotoxicology*, 17, 756–771.

Daam, M.A., Crum, S.J.H. & Nogueira, P.J.V. den B.A.J.A. (2008b). Fate and effects of the insecticide chlorpyrifos in outdoor plankton-dominated microcosms in Thailand. *Environ. Toxicol. Chem.*, 27, 2530–2538.

Daam, M.A., Rodrigues, A.M.F., Van den Brink, P.J. & Nogueira, A.J.A. (2009). Ecological effects of the herbicide linuron in tropical freshwater microcosms. *Ecotoxicol. Environ. Saf.*, 72, 410–423.

De Laender, F. & Janssen, C.R. (2013). Brief communication: the ecosystem perspective in ecotoxicology as a way forward for the ecological risk assessment of chemicals. *Integr. Environ. Assess. Manag.*, 9, e34–e38.

De Laender, F., Morselli, M., Baveco, H., Van den Brink, P.J. & Di Guardo, A. (2015). Theoretically exploring direct and indirect chemical effects across ecological and exposure scenarios using mechanistic fate and effects modelling. *Environ. Int.*, 74, 181–190.

De Laender, F., Rohr, J.R., Ashauer, R., Baird, D.J., Berger, U., Eisenhauer, N., *et al.* (2016). Reintroducing environmental change drivers in biodiversity–ecosystem functioning research. *Trends Ecol. Evol.*, 31, 905–915.

Dellagreca, M., Iesce, M.R., Cermola, F., Rubino, M. & Isidori, M. (2004). Phototransformation of carboxin in water. Toxicity of the pesticide and its sulfoxide to aquatic organisms. *J. Agric. Food Chem.*, 52, 6228–6232.

DeNoyelles, F., Kettle, W.D. & Sinn, D.E. (2016). The responses of plankton communities in experimental ponds to atrazine, the most heavily used pesticide in the United States. *Ecology.*, 63, 1285–1293.

Deyle, E.R., Maher, M.C., Hernandez, R.D., Basu, S. & Sugihara, G. (2016). Global environmental drivers of influenza. *Proc. Natl. Acad. Sci.*, 113, 13081–13086.

Dinh Van, K., Janssens, L., Debecker, S. & Stoks, R. (2014). Warming increases chlorpyrifos effects on predator but not anti-predator behaviours. *Aquat. Toxicol.*, 152, 215–221.

Donohue, I., Hillebrand, H., Montoya, J.M., Petchey, O.L., Pimm, S.L., Fowler, M.S., *et al.* (2016). Navigating the complexity of ecological stability. *Ecol. Lett.*, 19, 1172–1185.

Duffy, J.. (2002). Biodiversity and ecosystem function: the consumer connection. *Oikos*, 99, 201–219.

Duffy, J.E., Cardinale, B.J., France, K.E., McIntyre, P.B., Thébault, E. & Loreau, M. (2007). The functional role of biodiversity in ecosystems: incorporating trophic complexity. *Ecol. Lett.*, 10, 522–538.

Duffy, J.E., Paul, J. & Elizabeth, A. (2003). Grazer diversity effects on ecosystem functioning in seagrass beds. *Ecol. Lett.*, 6, 637–645.

Dunne, J.A., Williams, R.J. & Martinez, N.D. (2002a). Food-web structure and network theory: the role of connectance and size. *Proc. Natl. Acad. Sci.*, 99, 12917–12922.

Dunne, J.A., Williams, R.J. & Martinez, N.D. (2002b). Network structure and biodiversity loss in food webs: robustness increase with connectance. *Ecol. Lett.*, 5, 558–567.

Edwards, K.F., Aquilino, K.M., Best, R.J., Sellheim, K.L. & Stachowicz, J.J. (2010). Prey diversity is associated with weaker consumer effects in a meta-analysis of benthic marine experiments. *Ecol. Lett.*, 13, 194–201.

Ehnes, R.B., Rall, B.C. & Brose, U. (2011). Phylogenetic grouping, curvature and metabolic scaling in terrestrial invertebrates. *Ecol. Lett.*, 14, 993–1000.

Eklöf, A. & Ebenman, B. (2006). Species loss and secondary extinctions in simple and complex model communities. *J. Anim. Ecol.*, 75, 239–246.

Elton, C. (1958). *The ecology of invasions by animals and plants*. Springer Nature.

Emmerson, M. & Yearsley, J.M. (2004). Weak interactions, omnivory and emergent food-web properties. *Proc. R. Soc. B Biol. Sci.*, 271, 397–405.

Ferris, H. & Matute, M.M. (2003). Structural and functional succession in the nematode fauna of a soil food web. *Appl. Soil Ecol.*, 23, 93–110.

Finke, D.L. & Denno, R.F. (2005). Predator diversity and the functioning of ecosystems: the role of intraguild predation in dampening trophic cascades. *Ecol. Lett.*, 8, 1299–1306.

Fleeger, J.W., Carman, K.R. & Nisbet, R.M. (2003). Indirect effects of contaminants in aquatic ecosystems. *Sci. Total Environ.*, 317, 207–233.

Fliedner, A., Remde, A., Niemann, R., Schäfers, C. & Stein, B. (1997). Effects of the organotin pesticide azocyclotin in aquatic microcosms. *Chemosphere*, 35, 209–222.

Frenken, T., Wierenga, J., van Donk, E., Declerck, S.A.J., de Senerpont Domis, L.N., Rohrlack, T., *et al.* (2018). Fungal parasites of a toxic inedible cyanobacterium provide food to zooplankton. *Limnol. Oceanogr.*, 63, 2384–2393.

Gauzens, B., Rall, B.C., Mendonça, V., Vinagre, C. & Brose, U. (2020). Biodiversity of intertidal food webs in response to warming across latitudes. *Nat. Clim. Chang.*, 10, 264–269.

Gibbs, T., Grilli, J., Rogers, T. & Allesina, S. (2018). Effect of population abundances on the stability of large random ecosystems. *Phys. Rev. E*, 98, 1–16.

Gilbert, B., Tunney, T.D., Mccann, K.S., Delong, J.P., Vasseur, D.A., Savage, V., *et al.* (2014). A bioenergetic framework for the temperature dependence of trophic interactions. *Ecol. Lett.*, 17, 902–914.

Gonzalez, A. & Loreau, M. (2009). The causes and consequences of compensatory dynamics in ecological communities. *Annu. Rev. Ecol. Evol. Syst.*, 40, 393–414.

Gray, C., Figueroa, D.H., Hudson, L.N., Ma, A., Perkins, D. & Woodward, G. (2015). Joining the dots: an automated method for constructing food webs from compendia of published interactions. *Food Webs*, 5, 11–20.

Griffin, J.N., Byrnes, J.E.K. & Cardinale, B.J. (2013). Effects of predator richness on prey suppression: a meta-analysis. *Ecology*, 94, 2180–2187.

Grilli, J., Barabás, G., Michalska-Smith, M.J. & Allesina, S. (2017). Higher-order interactions stabilize dynamics in competitive network models. *Nature*, 548, 210–213.

Grimm, V., Schmidt, E. & Wissel, C. (1992). On the application of stability concepts in ecology. *Ecol. Modell.*, 63, 143–161.

Grimm, V. & Wissel, C. (1997). Babel, or the ecological stability discussions: an inventory and analysis of terminology and a guide for avoiding confusion. *Oecologia*, 109, 323–334.

Groner, M.L. & Relyea, R.A. (2011). A tale of two pesticides: How common insecticides affect aquatic communities. *Freshw. Biol.*, 56, 2391–2404.

Guillard, R.R. & Lorenzen, C.J. (1972). Yellow-green algae with chlorophyllide C12. *J. Phycol.*, 8, 10-14.

Haddad, N.M., Crutsinger, G.M., Gross, K., Haarstad, J. & Tilman, D. (2011). Plant diversity and the stability of foodwebs. *Ecol. Lett.*, 14, 42–46.

Halstead, N.T., Mcmahon, T.A., Johnson, S.A., Raffel, T.R., Romansic, J.M., Crumrine, P.W., *et al.* (2014). Community ecology theory predicts the effects of agrochemical mixtures on aquatic biodiversity and ecosystem properties. *Ecol. Lett.*, 17, 932–941.

Hand, L.H., Kuet, S.F., Lane, M.C.G., Maund, S.J., Warinton, J.S. & Hill, I.R. (2001). Influences of aquatic plants on the fate of the pyrethroid insecticide lambda-cyhalothrin in aquatic environments. *Environ. Toxicol. Chem.*, 20, 1740–1745.

Hasenbein, S., Lawler, S.P., Geist, J. & Connon, R.E. (2016). A long-term assessment of pesticide mixture effects on aquatic invertebrate communities. *Environ. Toxicol. Chem.*, 35, 218–232.

Hassan, F.M. & Alkam, F. (2008). Phytoplankton and related nutrients in sawa lake, iraq. *J. Dohuk Univ.*, 11, 67–76.

Havens, K.E. (1995). Insecticide (Carbaryl, 1-napthyl-N-methylcarbamate) effects on a freshwater plankton community: zooplankton size, biomass, and algal abundance. *Water, Air, Soil Pollut.*, 84, 1–10.

Hayasaka, D., Korenaga, T., Suzuki, K., Saito, F., Sánchez-Bayo, F. & Goka, K. (2012). Cumulative ecological impacts of two successive annual treatments of imidacloprid and fipronil on aquatic communities of paddy mesocosms. *Ecotoxicol. Environ. Saf.*, 80, 355–362.

Hebert, P.D.N. & Finston, T.L. (2001). Macrogeographic patterns of breeding system diversity in the *Daphnia pulex* group from the United States and Mexico. *Heredity.*, 87, 153–161.

Hegde, G., Mandya, M., Gokarnakar, S.S., Babu, V.N., Shivaramaiah, V.N. & Krishnamurthy, S. V. (2014). Influence of combinations of pesticides and fertilizers on aquatic productivity. *J. Environ. Prot.*, 05, 434–440.

Heimbach, F., Pflueger, W. & Ratte, H. T. (1992). Use of small artificial ponds for assessment of hazards to aquatic ecosystems. *Environ. Toxicol. Chem.*, 11, 27–34.

Hillebrand, H. & Cardinale, B.J. (2004). Consumer effects decline with prey diversity. *Ecol. Lett.*, 7, 192–201.

Hillebrand, H., Langenheder, S., Lebret, K., Lindström, E., Östman, Ö. & Striebel, M. (2018). Decomposing multiple dimensions of stability in global change experiments. *Ecol. Lett.*, 21, 21–30.

Holling, C.S. (1996). *Engineering resilience versus ecological resilience. Eng. within Ecol. constraints*. National Academy of Sciences, USA.

Horn, W. (1991). The influence of biomass and structure of the crustacean plankton on the water transparency in the Saidenbach storage reservoir. *Hydrobiologia*, 225, 115–120.

Hughes, D.N., Boyer, M.G., Papst, M.H., Fowle, C.D., Rees, G.A. V. & Baulu, P. (1980). Persistence of three organophosphorus insecticides in artificial ponds and some biological implications. *Arch. Environ. Contam. Toxicol.*, 279, 269–279.

Hwang, S.J. & Heath, R.T. (1999). Zooplankton bacterivory at coastal and offshore sites of Lake Erie. *J. Plankton Res.*, 21, 699–719.

Isbell, F., Craven, D., Connolly, J., Loreau, M., Schmid, B., Beierkuhnlein, C., *et al.* (2015). Biodiversity increases the resistance of ecosystem productivity to climate extremes. *Nature*, 526, 574–577.

Isensee, A.R. & Jones, G.E. (1975). Distribution of 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) in Aquatic Model Ecosystem. *Environ. Sci. Technol.*, 9, 668–672.

Janssens, L. & Stoks, R. (2013). Synergistic effects between pesticide stress and predator cues: conflicting results from life history and physiology in the damselfly Enallagma cyathigerum. *Aquat. Toxicol.*, 132–133, 92–99.

Janssens, L. & Stoks, R. (2017). Chlorpyrifos-induced oxidative damage is reduced under warming and predation risk: explaining antagonistic interactions with a pesticide. *Environ. Pollut.*, 226, 79–88.

Jetz, W., Kreft, H., Ceballos, G. & Mutke, J. (2009). Global associations between terrestrial producer and vertebrate consumer diversity. *Proc. R. Soc. London B Biol. Sci.*, 276, 269–278.

Kadoya, T., Gellner, G. & McCann, K.S. (2018). Potential oscillators and keystone modules in food webs. *Ecol. Lett.*, 21, 1330–1340.

Karakoç, C., Clark, A.T. & Chatzinotas, A. (2020). Diversity and coexistence are influenced by time-dependent species interactions in a predator–prey system. *Ecol. Lett.*, 23, 983–993.

Kéfi, S., Domínguez-García, V., Donohue, I., Fontaine, C., Thébault, E. & Dakos, V. (2019). Advancing our understanding of ecological stability. *Ecol. Lett.*, ele.13340.

Kersting, K. & van Wijngaarden, R. (1992). Effects of chlorpyrifos on a microecosystem. *Environ. Toxicol. Chem.*, 11, 365–372.

Kuiper, J.J., Van Altena, C., De Ruiter, P.C., Van Gerven, L.P.A., Janse, J.H. & Mooij, W.M. (2015). Food-web stability signals critical transitions in temperate shallow lakes. *Nat. Commun.*, 6, 1–7.

Laabs, V., Wehrhan, A., Pinto, A., Dores, E. & Amelung, W. (2007). Pesticide fate in tropical wetlands of Brazil: An aquatic microcosm study under semi-field conditions. *Chemosphere*, 67, 975–989.

Lampert, W., Rothhaupt, K.O. & von Elert, E. (1994). Chemical induction of colony formation in a green alga (*Scenedesmus acutus*) by grazers (*Daphnia*). *Limnol. Oceanogr.*, 39, 1543–1550.

Lefcheck, J.S. (2016). piecewiseSEM: piecewise structural equation modelling in r for ecology, evolution, and systematics. *Methods Ecol. Evol.*, 7, 573–579.

Leigh, E.G. (1965). On the relation between the productivity, biomass, diversity, and stability of a community. *Proc. Natl. Acad. Sci.*, 53, 777–783.

Letten, A.D. & Stouffer, D.B. (2019). The mechanistic basis for higher-order interactions and non-additivity in competitive communities. *Ecol. Lett.*, 22, 423–436.

Lima-Fernandes, E., Bundschuh, M., Bakanov, N., Englert, D., Schulz, R. & Schäfer, R.B. (2019). Effects of a systemic pesticide along an aquatic tri-trophic food Chain. *Bull. Environ. Contam. Toxicol.*, 103, 507–514.

Lurling, M. (2003). The effect of substances from different zooplankton species and fish on the introduction of defensive morphology in the green alga *Scenedesmus obliquus*. *J. Plankton Res.*, 25, 979–989.

Maharning, A.R., Mills, A.A.S. & Adl, S.M. (2009). Soil community changes during secondary succession to naturalized grasslands. *Appl. Soil Ecol.*, 41, 137–147.

May, R. (1973). *Stability and complexity in model ecosystems*. Princeton University Press,Princeton, NJ.

Mayfield, M.M. & Stouffer, D.B. (2017). Higher-order interactions capture unexplained complexity in diverse communities. *Nat. Ecol. Evol.*, 1, 0062.

Maynard, D.S., Serván, C.A. & Allesina, S. (2018). Network spandrels reflect ecological assembly. *Ecol. Lett.*, 21, 324–334.

McCann, K., Hastings, A. & Huxel, G.R. (1998). Weak trophic interactions and the balance of enriched metacommunities. *Nature*, 395, 794–798.

McCann, K.S. (2000). The diversity–stability debate. *Nature*, 405, 228–233.

McHugh, P.A., McIntosh, A.R. & Jellyman, P.G. (2010). Dual influences of ecosystem size and disturbance on food chain length in streams. *Ecol. Lett.*, 13, 881–890.

Mensens, C., De Laender, F., Janssen, C.R., Sabbe, K. & De Troch, M. (2017). Different response–effect trait relationships underlie contrasting responses to two chemical stressors. *J. Ecol.*, 105, 1598–1609.

Meola, M., Lazzaro, A. & Zeyer, J. (2014). Diversity, resistance and resilience of the bacterial communities at two alpine glacier forefields after a reciprocal soil transplantation. *Environ. Microbiol.*, 16, 1918–1934.

Montoya, J.M., Rodríguez, M.A. & Hawkins, B.A. (2003). Food web complexity and higher-level ecosystem services. *Ecol. Lett.*, 6, 587–593.

Moore, J.C. & Ruiter, P.C. de. (2012). *Energetic food webs: an analysis of real and model ecosystems*. 1st edn. Oxford University Press, Oxford, UK.

Morin, P.J. & Lawler, S.P. (1995). Food web architecture and population dynamics: theory and empirical evidence. *Annu. Rev. Ecol. Syst.*, 26, 505–529.

Muturi, E.J., Donthu, R.K., Fields, C.J., Moise, I.K. & Kim, C.H. (2017). Effect of pesticides on microbial communities in container aquatic habitats. *Sci. Rep.*, 7, 1–10.

Na, W., Lili, L., Kaifeng, S. & Shunshan, D. (2012). Analysis of structure-activity relationship and toxicity of organophosphorus pesticide to plankton. *Ecol. Environ. Sci.*, 21, 118–123.

Naeem, S. & Li, S. (1998). Consumer species richness and autotrophic biomass. *Ecology*, 79, 2603–2615.

Neutel, A.-M., Heesterbeek, J.A.P. & Ruiter, P.C. de. (2002). Stability in real food webs: weak links in long loops. *Science.*, 296, 1120–1124.

Neutel, A.M., Heesterbeek, J.A.P., Van De Koppel, J., Hoenderboom, G., Vos, A., Kaldeway, C., *et al.* (2007). Reconciling complexity with stability in naturally assembling food webs. *Nature*, 449, 599–602.

Nõges, T., Anneville, O., Guillard, J., Haberman, J., Järvalt, A., Manca, M., *et al.* (2018). Fisheries impacts on lake ecosystem structure in the context of a changing climate and trophic state. *J. Limnol.*, 77, 46–61.

Norberg, J., Urban, M.C., Vellend, M., Klausmeier, C.A. & Loeuille, N. (2012). Eco-evolutionary responses of biodiversity to climate change. *Nat. Clim. Chang.*, 2, 747–751.

Novotny, V., Drozd, P., Miller, S.E. & Kulfan, M. (2006). Why are there so many species of herbivorous insects in tropical rainforests? *Science.*, 738, 1115–1118.

Nowell, L.H., Norman, J.E., Moran, P.W., Martin, J.D. & Stone, W.W. (2014). Pesticide toxicity index-a tool for assessing potential toxicity of pesticide mixtures to freshwater aquatic organisms. *Sci. Total Environ.*, 476–477, 144–157.

O'Gorman, E.J., Petchey, O.L., Faulkner, K.J., Gallo, B., Gordon, T.A.C., Neto-Cerejeira, J., *et al.* (2019). A simple model predicts how warming simplifies wild food webs. *Nat. Clim. Chang.,* 9, 611-616.

Otto, S.B., Rall, B.C. & Brose, U. (2007). Allometric degree distributions facilitate food-web stability. *Nature*, 450, 1226–1229.

Pace, M.L., Cole, J.J., Carpenter, S.R. & Kitchell, J.F. (1999). Trophic cascades revealed in diverse ecosystems. *Trends Ecol. Evol.*, 14, 483–488.

Peither, A., Jüttner, I., Kettrup, A. & Lay, J.P. (1996). A pond mesocosm study to determine direct and indirect effects of lindane on a natural zooplankton community. *Environ. Pollut.*, 93, 49–56.

Pennekamp, F., Pontarp, M., Tabi, A., Altermatt, F., Alther, R., Choffat, Y., *et al.* (2018). Biodiversity increases and decreases ecosystem stability. *Nature*, 563, 109–112.

Perna, M., Pinto, M. Di & Roualec, J.M. (2004). Predator diversity dampens trophic cascades. *Nature*, 429, 407–410.

Pestana, J.L.T., Loureiro, S., Baird, D.J. & Soares, A.M.V.M. (2009). Fear and loathing in the benthos: responses of aquatic insect larvae to the pesticide imidacloprid in the presence of chemical signals of predation risk. *Aquat. Toxicol.*, 93, 138–149.

Pimm, S.L. (1984). The complexity and stability of ecosystems. *Nature*, 307, 321–326.

Pimm, S.L. & Lawton, J.H. (1977). Number of trophic levels in ecological communities. *Nature*, 268, 329–330.

Planque, B., Primicerio, R., Michalsen, K., Aschan, M., Certain, G., Dalpadado, P., *et al.* (2014). Who eats whom in the Barents Sea: a food web topology from plankton to whales. *Ecology*, 95, 1430–1430.

Poisot, T., Mouquet, N. & Gravel, D. (2013). Trophic complementarity drives the biodiversity –ecosystem functioning relationship in food webs. *Ecol. Lett.*, 16, 853–861.

Post, D.M. (2002). The long and short of food chain length. *Trends Ecol. Evol.*, 17, 269–277.

Preston, B.L. (2002). Indirect effects in aquatic ecotoxicology: implications for ecological risk assessment. *Environ. Manage.*, 29, 311–323.

Rall, B.Ö.C., Vucic-Pestic, O., Ehnes, R.B., EmmersoN, M. & Brose, U. (2010). Temperature, predator-prey interaction strength and population stability. *Glob. Chang. Biol.*, 16, 2145–2157.

Relyea & A, R. (2005). The impact of insecticides and herbicides on the biodiversity and productivity of aquatic communities. *Ecol. Appl.*, 15, 618–627.

Relyea, R.A. & Mills, N. (2001). Predator-induced stress makes the pesticide carbaryl more deadly to gray treefrog tadpoles (*Hyla versicolor*). *Proc. Natl. Acad. Sci. U. S. A.*, 98, 2491–2496.

Resources, N. & Brunswick, N. (2006). Population and community resilience in multitrophic communities. *Ecology*, 87, 996–1007.

Ridal, J.J., Mazumder, A. & Lean, D.R.S. (2001). Effects of nutrient loading and planktivory on the accumulation of organochlorine pesticides in aquatic food chains. *Environ. Toxicol. Chem.*, 20, 1312–1319.

Rineau, F., Malina, R., Beenaerts, N., Arnauts, N., Bardgett, R.D., Berg, M.P., *et al.* (2019). Towards more predictive and interdisciplinary climate change ecosystem experiments. *Nat. Clim. Chang.*, 9, 809–816.

Rip, J.M.K. & Mccann, K.S. (2011). Cross-ecosystem differences in stability and the principle of energy flux. *Ecol. Lett.*, 14, 733–740.

Rogers, A., Blanchard, J.L. & Mumby, P.J. (2014). Vulnerability of coral reef fisheries to a loss of structural complexity. *Curr. Biol.*, 24, 1000–1005.

Rogers, A., Blanchard, J.L. & Mumby, P.J. (2017). Fisheries productivity under progressive coral reef degradation. *J. Appl. Ecol.*, 55, 1041–1049.

Rohner, C. (1995). Great horned owls and snowshoe hares : what causes the time lag in the numerical response of predators to cyclic prey ? *Oikos.*, 74, 61–68.

Rohr, J.R. & Crumrine, P.W. (2005). Effects of an herbicide and an insecticide on pond community structure and processes. *Ecol. Appl.*, 15, 1135–1147.

Rohr, J.R., Kerby, J.L. & Sih, A. (2006). Community ecology as a framework for predicting contaminant effects. *Trends Ecol. Evol.*, 21, 606–613.

Rohr, J.R., Salice, C.J. & Nisbet, R.M. (2016). The pros and cons of ecological risk assessment based on data from different levels of biological organization. *Crit. Rev. Toxicol.*, 46, 756–784.

Rooney, N., McCann, K., Gellner, G. & Moore, J.C. (2006). Structural asymmetry and the stability of diverse food webs. *Nature*, 442, 265–269.

Rooney, N. & McCann, K.S. (2012). Integrating food web diversity, structure and stability. *Trends Ecol. Evol.*, 27, 40–45.

Rubach, M.N., Crum, S.J.H. & Van den Brink, P.J. (2011). Variability in the dynamics of mortality and immobility responses of freshwater arthropods exposed to chlorpyrifos. *Arch. Environ. Contam. Toxicol.*, 60, 708–721.

Ruiter, P.C. De, Neutel, A., Moore, J.C., Ruiter, P.C. De, Neutel, A. & Moore, J.C. (1995). Energetics, patterns of interaction strengths, and stability in real ecosystems. *Science.*, 269, 1257–1260.

Rumschlag, S.L., Halstead, N.T., Hoverman, J.T., Raffel, T.R., Carrick, H.J., Hudson, P.J., *et al.* (2019). Effects of pesticides on exposure and susceptibility to parasites can be generalised to pesticide class and type in aquatic communities. *Ecol. Lett.*, 22, 962–972.

Salonen, K., Sarvala, J., Hakala, I. & Viljanen, M. L. (1976). The relation of energy and organic carbon in aquatic invertebrates1. *Limnol. Oceanogr.*, 21, 724–730.

Sanful, P.O., Frempong, E., Aikins, S., Hall, R.I. & Hecky, R.E. (2012). Secondary production of *Chaoborus ceratopogones* (Diptera: Chaoboridae) in Lake Bosumtwi, Ghana. *Aquat. Insects*, 34, 115–130.

Schaum, C.E., Barton, S., Bestion, E., Buckling, A., Garcia-Carreras, B., Lopez, P., *et al.* (2017). Adaptation of phytoplankton to a decade of experimental warming linked to increased photosynthesis. *Nat. Ecol. Evol.*, 1, 1–7.

Schmitz, O.J. (2007). Predator diversity and trophic interactions. *Ecology*, 88, 2415–2426.

Schwarz, B., Barnes, A.D., Thakur, M.P., Brose, U., Ciobanu, M., Reich, P.B., *et al.* (2017). Warming alters energetic structure and function but not resilience of soil food webs.

Nat. Clim. Chang., 7, 895–900.

Sih, A., Englund, G. & Wooster, D. (1998). Emergent impacts of multiple predators on prey. *Trends Ecol. Evol.*, 13, 350–355.

Sih, A. & Wooster, D. (1998). Emergent impacts of multiple predators on prey. *Trends Ecol. Evol.*, 13, 350–355.

Slijkerman, D.M.E., Moreira-Santos, M., Jak, R.G., Ribeiro, R., Soares, A.M.V.M. & Van Straalen, N.M. (2005). Functional and structural impact of linuron on a freshwater community of primary producers: the use of immobilized algae. *Environ. Toxicol. Chem.*, 24, 2477–2485.

Snel, J.F.H., Vos, J.H., Gylstra, R. & Brock, T.C.M. (1998). Inhibition of photosystem II (PSII) electron transport as a convenient endpoint to assess stress of the herbicide linuron on freshwater plants. *Aquat. Ecol.*, 32, 113–123.

Snyder, G.B., Finke, D.L. & Snyder, W.E. (2008). Predator biodiversity strengthens aphid suppression across single- and multiple-species prey communities. *Biol. Control*, 44, 52–60.

Soliveres, S., Van Der Plas, F., Manning, P., Prati, D., Gossner, M.M., Renner, S.C., *et al.* (2016). Biodiversity at multiple trophic levels is needed for ecosystem multifunctionality. *Nature*, 536, 456–459.

Song, C., Altermatt, F., Pearse, I. & Saavedra, S. (2018). Structural changes within trophic levels are constrained by within-family assembly rules at lower trophic levels. *Ecol. Lett.*, 21, 1221–1228.

Srivastava, D.S. & Bell, T. (2009). Reducing horizontal and vertical diversity in a foodweb triggers extinctions and impacts functions. *Ecol. Lett.*, 12, 1016–1028.

Srivastava, D.S. & Vellend, M. (2005). Biodiversity-ecosystem function research: Is it relevant to conservation? *Annu. Rev. Ecol. Evol. Syst.*, 36, 267–294.

Steiner, C.F. (2002). Context-dependent effects of *Daphnia pulex* on pond ecosystem function: Observational and experimental evidence. *Oecologia*, 131, 549–558.

Stephenson, R.R. & Kane, D.F. (1984). Persistence and effects of chemicals in small enclosures in ponds R. *Arch. Environ. Contam. Toxicol.*, 13, 313–326.

Sterner, R.W., Bajpai, A. & Adams, T. (1997). The enigma of food chain length: abasence of theoretical evidence for dynamic contraints. *Ecology*, 78, 2258–2262.

Sugihara, G., May, R., Ye, H., Hsieh, C.H., Deyle, E., Fogarty, M., *et al.* (2012). Detecting causality in complex ecosystems. *Science.*, 338, 496–500.

Sumon, K.A., Ritika, A.K., Peeters, E.T.H.M., Rashid, H., Bosma, R.H., Rahman, M.S., *et al.* (2018). Effects of imidacloprid on the ecology of sub-tropical freshwater microcosms. *Environ. Pollut.*, 236, 432–441.

Sze, P. (1980). Seasonal succession of phytoplankton in Onondaga Lake, New York (USA). *Phycologia*, 19, 54–59.

Thébault, E. & Loreau, M. (2005). Trophic interactions and the relationship between species diversity and ecosystem stability. *Am. Nat.*, 166.

Thompson, D.G., Solomon, K.R., Wojtaszek, B.F., Edginton, A.N. & Stephenson, G.R. (2006). The impact of insecticides and herbicides on the biodiversity and productivity of aquatic communities. *Ecol. Appl.*, 16, 2022–2027.

Thompson, R.M., Brose, U., Dunne, J.A., Hall, R.O., Hladyz, S., Kitching, R.L., *et al.* (2012). Food webs: reconciling the structure and function of biodiversity. *Trends Ecol. Evol.*, 27, 689–697.

Tien, C.J. & Chen, C.S. (2012). Assessing the toxicity of organophosphorous pesticides to indigenous algae with implication for their ecotoxicological impact to aquatic ecosystems. *J. Environ. Sci. Heal. Part B.*, 47, 901–912.

Tilman, D. & Downing, J.A. (1994). Biodiversity and stability in grasslands. *Nature*, 367, 363–365.

Tilman, D., Isbell, F. & Cowles, J.M. (2014). Biodiversity and ecosystem functioning. *Annu. Rev. Ecol. Evol. Syst.*, 45, 471–493.

Tiwari, A. & Chauhan, S.V.S. (2006). Seasonal phytoplanktonic diversity of Kitham lake, Agra. *J. Environ. Biol.*, 27, 35–38.

Tollrian, R. (1993). Formation in *Daphnia pulex* as an example of continuous phenotypic plasticity: morphological effects of *Chaoborus* kairomone concentration and their quantification. *J. Plankton Res.*, 15, 1309–1318.

Tran, T.T., Janssens, L., Dinh, K. V. & Stoks, R. (2019). An adaptive transgenerational effect of warming but not of pesticide exposure determines how a pesticide and warming interact for antipredator behaviour. *Environ. Pollut.*, 245, 307–315.

Traunspurger, W., Schäfer, H. & Remde, A. (1996). Comparative investigation on the effect of a herbicide on aquatic organisms in single species tests and aquatic microcosms. *Chemosphere*, 33, 1129–1141.

Trekels, H., van de Meutter, F. & Stoks, R. (2011a). Habitat isolation shapes the recovery of aquatic insect communities from a pesticide pulse. *J. Appl. Ecol.*, 48, 1480–1489.

Trekels, H., Van de Meutter, F. & Stoks, R. (2011b). Effects of species-specific interactions with predation risk on the relative species sensitivities to a pesticide in water boatmen (*Corixidae*). *Oikos*, 120, 897–905.

Trekels, H., Van de Meutter, F. & Stoks, R. (2013). Predator cues magnify effects of the pesticide endosulfan in water bugs in a multi-species test in outdoor containers. *Aquat. Toxicol.*, 138–139, 116–122.

Turney, S. & Buddle, C.M. (2016). Pyramids of species richness : the determinants and distribution of species diversity across trophic levels. *Oikos*, 125, 1224–1232.

USEPA. (2020). *Sustainable Materials Management: the road ahead;U.S. Environ. Prot. Agency Washington, DC, June 2009.*

Ushio, M., Hsieh, C.H., Masuda, R., Deyle, E.R., Ye, H., Chang, C.W., *et al.* (2018). Fluctuating interaction network and time-varying stability of a natural fish community. *Nature*, 554, 360–363.

Uszko, W., Diehl, S., Englund, G. & Amarasekare, P. (2017). Effects of warming on predator–prey interactions–a resource-based approach and a theoretical synthesis. *Ecol. Lett.*, 20, 513–523.

Van den Brink, P.J., Boxall, A.B.A., Maltby, L., Brooks, B.W., Rudd, M.A., Backhaus, T., *et al.* (2018). Toward sustainable environmental quality: priority research questions for Europe. *Environ. Toxicol. Chem.*, 37, 2281–2295.

Van den Brink, P.J. & Ter Braak, C.J.F. (1999). Principal response curves: analysis of time-dependent multivariate responses of biological community to stress. *Environ. Toxicol. Chem.*, 18, 138–148.

Van den Brink, P.J., Hartgers, E.M., Fettweis, U., Crum, S.J.H., Van Donk, E. & Brock, T.C.M. (1997). Sensitivity of macrophyte-dominated freshwater microcosms to chronic levels of the herbicide linuron. *Ecotoxicol. Environ. Saf.*, 38, 13–24.

Van den Brink, P.J., Klein, S.L. & Rico, A. (2017). Interaction between stress induced by competition, predation, and an insecticide on the response of aquatic invertebrates. *Environ. Toxicol. Chem.*, 36, 2485–2492.

Van den Brink, P.J., Van Wijngaarden, R.P.A., Lucassen, W.G.H., Brock, T.C.M. & Leeuwangh, P. (1996). Effects of the insecticide Dursban® 4E (active ingredient chlorpyrifos) in outdoor experimental ditches: II. Invertebrate community responses and recovery. *Environ. Toxicol. Chem.*, 15, 1143–1153.

Van den Brink, P.J., Van Donk, E., Gylstra, R., Crum, S.J.H. & Brock, T.C.M. (1995).

Effects of chronic low concentrations of the pesticides chlorpyrifos and atrazine in indoor freshwater microcosms. *Chemosphere*, 31, 3181–3200.

Van Ruijven, J. & Berendse, F. (2010). Diversity enhances community recovery, but not resistance, after drought. *J. Ecol.*, 98, 81–86.

Van Wijngaarden, R.P.A., Brock, T.C.M. & Douglas, M.T. (2005). Effects of chlorpyrifos in freshwater model ecosystems: the influence of experimental conditions on ecotoxicological thresholds. *Pest Manag. Sci.*, 61, 923–935.

Van Wijngaarden, R.P.A., Cuppen, J.G.M., Arts, G.H.P., Crum, S.J.H., Van Den Hoorn, M.W., Van Den Brink, P.J., *et al.* (2004). Aquatic risk assessment of a realistic exposure to pesticides used in bulb crops: a microcosm study. *Environ. Toxicol. Chem.*, 23, 1479–1498.

Vischetti, C., Cardinali, A., Monaci, E., Nicelli, M., Ferrari, F., Trevisan, M., *et al.* (2008). Measures to reduce pesticide spray drift in a small aquatic ecosystem in vineyard estate. *Sci. Total Environ.*, 389, 497–502.

Vogel, A., Scherer-Lorenzen, M. & Weigelt, A. (2012). Grassland resistance and resilience after drought depends on management intensity and species richness. *PLoS One*, 7.

Vonesh, J.R. & Kraus, J.M. (2009). Pesticide alters habitat selection and aquatic community composition. *Oecologia*, 160, 379–385.

Wang, S. & Brose, U. (2018). Biodiversity and ecosystem functioning in food webs: the vertical diversity hypothesis. *Ecol. Lett.*, 21, 9–20.

Wang, S., Brose, U. & Gravel, D. (2019). Intraguild predation enhances biodiversity and functioning in complex food webs. *Ecology*, e02616.

Webber, E.C., Bayne, R. & Seesock, C. (1992). Ecosystem-level testing of a synthetic pyrethroid insecticide in aquatic mesocosms. *Environ. Toxrcology und Chemrstry*, 1, 87–105.

Wen, Z., Mian-Ping, Z., Xian-Zhong, X., Xi-Fang, L., Gan-Lin, G. & Zhi-Hui, H. (2005). Biological and ecological features of saline lakes in northern Tibet, China. *Hydrobiologia*, 541, 189–203.

Wertz, S., Degrange, V., Prosser, J.I., Poly, F., Commeaux, C., Guillaumaud, N., *et al.* (2007). Decline of soil microbial diversity does not influence the resistance and resilience of key soil microbial functional groups following a model disturbance. *Environ. Microbiol.*, 9, 2211–2219.

Wilcove, D.S. & Master, L.L. (2005). How many endangered species are there in the United States ? *Frontiers in Ecology and the Environment.*, 3, 414-420.

Williams, R.J., Berlow, E.L., Dunne, J.A., Barabasi, A.-L. & Martinez, N.D. (2002). Two degrees of separation in complex food webs. *Proc. Natl. Acad. Sci.*, 99, 12913–12916.

Woodward, G., Ebenman, B., Emmerson, M., Montoya, J.M., Olesen, J.M., Valido, A., *et al.* (2005). Body size in ecological networks. *Trends Ecol. Evol.*, 20, 402–409.

Worm, B. & Duffy, J.E. (2003). Biodiversity, productivity and stability in real food webs. *Trends Ecol. Evol.*, 18, 628–632.

Xiao, P., Liu, F., Liu, Y., Yao, S. & Zhu, G. (2017). Effects of pesticide mixtures on Zooplankton assemblages in aquatic microcosms simulating rice paddy fields. *Bull. Environ. Contam. Toxicol.*, 99, 27–32.

Yang, Q., Fowler, M.S., Jackson, A.L. & Donohue, I. (2019). The predictability of ecological stability in a noisy world. *Nat. Ecol. Evol.*, 3, 251–259.

Yang, X., Yan, C., Zhao, Q., Holyoak, M. & Fortuna, M.A. (2018). Forest ecology and management ecological succession drives the structural change of seed-rodent interaction networks in fragmented forests. *For. Ecol. Manage.*, 419, 42–50.

Ye, H., Beamish, R.J., Glaser, S.M., Grant, S.C.H., Hsieh, C.H., Richards, L.J., *et al.* (2015).

Equation-free mechanistic ecosystem forecasting using empirical dynamic modeling. *Proc. Natl. Acad. Sci. U. S. A.*, 112, E1569–E1576.

Ye, L., Chang, C.W., Matsuzaki, S. ichiro S., Takamura, N., Widdicombe, C.E. & Hsieh, C. hao. (2019). Functional diversity promotes phytoplankton resource use efficiency. *J. Ecol.*, 107, 2353–2363.

Zafar, M.I., Van Wijngaarden, R.P.A., Roessink, I. & Van den Brink, P.J. (2011). Effects of time-variable exposure regimes of the insecticide chlorpyrifos on freshwater invertebrate communities in microcosms. *Environ. Toxicol. Chem.*, 30, 1383–1394.

Zhao, Q., Brink, P.J. Van den, Carpentier, C., Wang, Y.X.G., Rodrıguez-Sanchez, P., Xu, C., *et al.* (2019). Horizontal and vertical diversity jointly shape food web stability against small and large perturbations. *Ecol. Lett.*, 22, 1152–1162.

Zhao, Q., Laender, F. De & Brink, P.J. Van Den. (2020). Community composition modifies direct and indirect effects of pesticides in freshwater food webs. *Sci. Total Environ.*, 739, 139531.

Summary

Summary

The aim of this thesis is to advance the understanding of how horizontal and vertical diversity affect food web stability, how the two dimensions of diversity modify the effect of anthropic stressors (i.e. pesticides) on food webs, and how warming temperatures affect stability of ecosystems in the long-term. I studied this interaction via synthesizing the results of mathematical modelling, conducting experiments, reanalysing multi-species experiments and analysing long term monitoring datasets (Chapter 1).

I first attempted to address a classical problem in ecology, i.e. the relationship between diversity and food web stability (Chapter 2). While theoretical studies indicated that diversity decreases food web stability, empirical evidence often indicated that diversity enhances it. After disentangling diversity into horizontal and vertical diversity, we combined the results of mathematical models and experiments, and showed that horizontal and vertical diversity increased and decreased stability, respectively. The positive effect of producer diversity on stability was higher at higher consumer diversity. The results indicate that high horizontal diversity can compensate the stability loss caused by vertical diversity.

The changes of species richness within trophic levels and number of trophic levels can both modify the (in)direct effect of pesticides (herbicide and insecticide) in food webs (chapter 3 and 4). In chapter 3, I show, using microcosm experiments, that the negative effect of the insecticide chlorpyrifos on herbivore abundance was smaller when number of herbivore species was increased, while the negative effects on herbivores was larger when number of trophic levels was increased. I further scaled up the results from simple indoor microcosms to more complex indoor and outdoor microcosm and mesocosm experiments, via reanalysing several existing datasets (chapter 4). I showed that the results of chapter 3 were still robust for more complex ecosystems. Given that the datasets in chapter 4 are synthesized from experiments at different scales of biodiversity, the results in chapter 4 suggest that diversity at the start of the experiments should be considered for the use of their results in the ecological risk assessment of chemicals. The results of

chapter 3 and 4 together bring us to the conclusion that horizontal and vertical diversity can indeed modify the effects of chemicals on aquatic communities across microcosms to mesocosms experiments.

In chapter 5, I focus on field monitoring datasets from ocean and lakes. The aim of this chapter was to assess the long term effect of warming temperatures on food web stability in natural systems, and also assess which factor (warming temperatures versus diversity) has the largest effect on food web stability. We found that warming temperatures decreased food web stability, while diversity increased it. The absolute effect of the observed increase in temperature on stability was 2.1 time larger than that of the observed decrease in diversity, indicating that warming could have a bigger negative effect on stability than diversity change.

From the results in this thesis, I conclude that 1) horizontal and vertical diversity can have a contrasting effect on food web stability; 2) horizontal and vertical diversity modify the effects of chemicals and 3) observed increases in temperature has a larger long term effect on food web stability than observed decreases in diversity.

SENSE

Netherlands Research School for the
Socio-Economic and Natural Sciences of the Environment

DIPLOMA

for specialised PhD training

The Netherlands research school for the
Socio-Economic and Natural Sciences of the Environment
(SENSE) declares that

Qinghua Zhao

born on 22 March 1988 in Sichuan, China

has successfully fulfilled all requirements of the
educational PhD programme of SENSE.

Wageningen, 27 January 2021

Chair of the SENSE board

Prof. dr. Martin Wassen

The SENSE Director

Prof. Philipp Pattberg

The SENSE Research School has been accredited by the Royal Netherlands Academy of Arts and Sciences (KNAW)

KONINKLIJKE NEDERLANDSE
AKADEMIE VAN WETENSCHAPPEN

The SENSE Research School declares that **Qinghua Zhao** has successfully fulfilled all requirements of the educational PhD programme of SENSE with a work load of 47.0 EC, including the following activities:

SENSE PhD Courses

- Environmental research in context (2016)
- Research in context activity: ‘Making a video on “The definition and measurements of ecosystems’ stability”’ (2020)
- Model training for scenario analysis: River export of nutrients from land to sea (2018)

Other PhD and Advanced MSc Courses

- Introduction to R, PE&RC and SENSE graduate schools (2016)
- Basic Statistics, PE&RC and SENSE graduate Schools (2016)
- Ecological Methods 1, Wageningen University (2017)
- Complexity in ecological systems , Wageningen University (2018)
- Structural equation modelling, PE&RC graduate school (2018)
- Modelling population dynamics with Physiologically Structured Population Models (PSPM), PE&RC graduate school (2018)
- Consumer-resource interactions PE&RC graduate school (2018)
- Dynamic Models in R: Programming, parameter estimation and model selection, PE&RC graduate school (2018)
- Mathematical Models in Ecology and Evolution, RSEE graduate school (2018)

Management and Didactic Skills Training

- Supervising MSc student with thesis entitled ‘Testing a methodology to assess the influence of horizontal and vertical diversity on ecosystem stability after a pesticide application’ (2017)
- Assisting in the MSc courses ‘Chemical Stress Ecology and Ecotoxicology’ (2019)

Oral Presentation

- *The effect of pesticides on target communities depends on multi- trophic diversity*, 8th Young Environmental Scientists Meeting, 5 - 10 February 2019, Ghent, Belgium

SENSE coordinator PhD education

Dr. ir. Peter Vermeulen

The research described in this thesis was financially supported by China Scholarship Council, under Grant Agreement Number 201606190229. Printing of this thesis was financially supported by the Aquatic Ecology and Water Quality Management Group of Wageningen University.

Cover design: Met Vriendelijke Groet.

Layout: Qinghua Zhao & Proefschriftmaken.

Printed by: Proefschriftmaken.