

GENERAL MECHANISMS FOR
NON-ADDITIVITY BETWEEN MULTIPLE STRESSORS
AT HIGHER TEMPORAL AND BIOLOGICAL SCALES

BY

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DECLARATION

I declare that this thesis has not been submitted as an exercise for a degree at this or any other university and it is, unless otherwise referenced, entirely my own work. I agree to deposit this thesis in the University's open access institutional repository or allow the Library to do so on my behalf, subject to Irish Copyright Legislation and Trinity College Library conditions of use and acknowledgement.

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SUMMARY

Multiple anthropogenic stressors threaten the diversity, stability and functioning of ecosystems worldwide. The combined effects of these stressors can, however, be surprisingly difficult to predict due to complex interactions across different levels of organisation. Ecosystem managers are particularly concerned by synergistic stressor interactions, which cause the combined effects of stressors to be greater than expected based on their individual effects alone. Multiple-stressor researchers have primarily used phenomenological approaches to search for generalities in the frequencies of stressor interactions, but have so far encountered an overwhelming amount of context-dependence. Consequently, there is growing interest in a mechanistic understanding of the interactions between stressors (Chapter 2). In this thesis, I integrate evolutionary and ecological theory into the empirical field of multiple-stressor research to describe mechanisms of non-additivity between stressors, that are not specific to particular species, ecosystems or stressors. I focus on general sources of non-additivity at higher temporal scales (Chapter 3) and at higher levels of biological organisation (Chapters 4 and 5). An overarching theme of this thesis is that non-additivity between stressors can not only be caused by natural effects, but can also arise due to observational effects. Natural sources of non-additivity can be physico-chemical interactions between the stressors themselves, or biological effects occurring at multiple levels of organisation from individuals to ecosystems. Conversely, observational sources of non-additivity stem from statistical effects during the measurement and prediction of responses to stressors. These effects can obscure the natural sources of non-additivity that researchers are actually interested in. Recognising these contrasting sources of non-additivity is an important step towards the accurate prediction of the ecological impacts of multiple stressors. A mechanistic understanding of stressor interactions will enhance the management and conservation of ecosystems in the Anthropocene.

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CHAPTER 1 | GENERAL INTRODUCTION

1.1 MULTIPLE-STRESSOR RESEARCH

An array of anthropogenic stressors are driving the rapid loss of global biodiversity (Ceballos et al., 2017; Sala et al., 2000). These stressors, including land-use change, invasive species, emerging contaminants, overexploitation and climate change, co-occur in time and space (Bowler et al., 2020; Geary et al., 2019; Halpern et al., 2015; Halpern et al., 2008). A major challenge for ecosystem managers is that the combined effects of stressors can be deceptively difficult to predict due to complex interactions across different scales. Consequently, multiple-stressor research, an interdisciplinary and predominantly empirical field of research, has emerged in the past two decades with the overall aim of understanding and predicting the interactions between stressors (Côté et al., 2016; Orr et al., 2020). There is now extensive evidence that the combined effects of stressors are often less than expected due to antagonistic interactions, or more than expected due to synergistic interactions (Crain et al., 2008; Jackson et al., 2016; Piggott et al., 2015). In some cases the combined effect of stressors can be explained by the individual effect of a single dominant stressor (Birk et al., 2020; Brennan & Collins, 2015; Garnier et al., 2017). In other cases, even if the individual effects of stressors are inconsequential, synergistic interactions can cause dramatic ecological impacts (Brook et al., 2008; Rillig et al., 2019). Stressors can even have positive individual effects but negative combined effects due to “reversal” stressor interactions (Breitburg et al., 1999; Sargian et al., 2007). Thousands of laboratory and field experiments, as well as a growing number of observational studies, have generated a diverse body of research on the ecological impacts of multiple stressors and their interactions. However, an overwhelming amount of context-dependence and a generally poor mechanistic understanding of stressor interactions, particularly at higher levels of organisation, prevents accurate prediction of the combined effects of stressors (De Laender, 2018; Jackson et al., 2021; Kroeker et al., 2017; Schäfer & Piggott, 2018)

Meta-analyses and phenomenological, vote-counting approaches have been the predominant tools in the search for generalities in the occurrences of stressor interactions. Syntheses of multiple-stressor research have focused on differences between classification systems (Piggott et al., 2015; Tekin et al., 2020), spatial scales (Birk et al., 2020) and life stages (Lange et al., 2018; Przeslawski et al., 2015), and on specific ecosystem types (Crain et al., 2008; Jackson et al., 2016; Leuzinger et al., 2011), combinations of stressors (Elser et al., 2007; Harvey et al., 2013; Holmstrup et al., 2010; Mantyka-Pringle et al., 2012) and biological responses (Darling & Côté, 2008; Yue et al., 2017). While these studies have provided valuable information on the interactions between specific stressors in specific settings, and have highlighted the prevalence of antagonism and synergism, they have not enhanced our abilities to predict interactions between stressors (Griffen et al., 2016; Schäfer & Piggott, 2018). Unfortunately, inferences based on the frequencies of different types of stressor interactions are flawed as the identity of stressor interactions depends on the classification system (Piggott et al., 2015) or null model used (Folt et al., 1999; Liess et al., 2016; Schäfer & Piggott, 2018; Tekin et al., 2020; Thompson et al., 2018a). Furthermore, classification of interactions is strongly impacted by the power of studies (e.g. via sample sizes) to statistically detect interactive effects (Schäfer & Piggott, 2018; Tekin et al., 2020) and is complicated when stressors have opposing effects (Galic et al., 2018; Piggott et al., 2015). There is even evidence of a publication bias towards synergism; the proportion of papers from the multiple-stressor literature mentioning synergism in their titles or abstracts is far higher than the proportion of synergism reported in meta-analyses (Côté et al., 2016). Gaining a mechanistic understanding of stressor interactions is therefore critical for accurate predictions of global change impacts and is particularly important when ecosystem managers use the identity of stressor interactions to prioritise which stressors to remove (Brown et al., 2014; Falkenberg et al., 2013; Fong et al., 2018).

1.2 MECHANISTIC UNDERSTANDING OF STRESSOR INTERACTIONS

Stressor interactions are defined as deviations from null models constructed from the individual effects of stressors. The additive null model, where the combined effect of stressors is predicted to be equal to the sum of their individual effects ($A + B$), is by far the most common in ecology (Folt et al., 1999; Schäfer & Piggott, 2018). Other models, mainly developed in toxicology, have been proposed as superior alternatives in specific settings (Bliss, 1939; Liess et al., 2016; Schäfer & Piggott, 2018; Tekin et al., 2020). For instance, the additive null model can produce unrealistic predictions when response variables are bounded. In such cases, the multiplicative null model, where the combined effect of stressors is predicted to be the probabilistic sum of their individual effects ($A + B - (A \times B)$), can be used to at least achieve predictions that are not beyond the realms of possibility (Folt et al., 1999; Schäfer & Piggott, 2018; Thompson et al., 2018a). However, by logarithmically transforming data to meet normality assumptions of statistical tests (e.g. ANOVA), researchers will change the underlying null model to the multiplicative null model even if they intended to use an additive null model (Griffen et al., 2016; Sih et al., 1998; Tekin et al., 2020). A recent framework outlined a mechanistic basis for null model selection where the stressors' modes of action, the sensitivities of organisms to stressors and the stressor-response relationship are used to inform the choice of null models (Schäfer & Piggott, 2018). The goal of this approach is to use null models that accurately predict the combined effect of stressors. Another approach to increase the predictive power of multiple-stressor research is to use the additive null model, the most intuitive null model that requires no prior knowledge, and gain a mechanistic understanding of the sources of non-additivity (De Laender, 2018; Thompson et al., 2018a, 2018b). The term “ecological surprise” originally described situations where the combined effect of stressors is not explained by their individual effects alone (Paine et al., 1998). However, considering the prevalence of antagonistic and synergistic interactions, it is more appropriate for the term to refer to situations where the mechanisms driving these stressor interactions are unknown (De Laender, 2018).

An important first step in gaining a mechanistic understanding of stressor interactions is recognising that non-additivity can be caused by natural effects or by observational effects. Natural sources of non-additivity can be the physical and chemical interactions between the stressors themselves, or they can be biological effects that occur at multiple levels of organisation from individuals to ecosystems (Boyd & Brown, 2015; Didham et al., 2007; Kroeker et al., 2017). For instance, non-additivity can arise from the physico-chemical interplay between warming and ocean acidification (Humphreys, 2017), or from the chemical interactions between multiple pesticides (Hernández et al., 2017). Similarly, biological mechanisms from individual physiology to community dynamics may introduce non-linear responses to stressors, and therefore non-additive effects (Brook et al., 2008; Griffen et al., 2016; Thompson et al., 2018b). Alternatively, other sources of non-additivity stem from the way that natural systems are observed (Schäfer & Piggott, 2018; Thompson et al., 2018a). This phenomenon is exemplified by how the choice of null model can dictate the statistical interaction detected between stressors. Disentangling the natural and observational sources of non-additivity is a key focus of this thesis. Integrating ecological and evolutionary theory into the primarily empirical field of multiple-stressor research may greatly enhance our understanding of non-additivity between stressors. If the sources of non-additivity can be properly understood, prediction of the combined effects of stressors will become a far less daunting task.

1.3 THESIS OUTLINE

The aim of this thesis is to identify general sources of non-additivity between multiple stressors. First, I provide a deeper introduction to multiple-stressor research and to the main themes in this thesis (chapter 2). I then use evolutionary and ecological theory to explore the sources and patterns of non-additivity at higher temporal scales (chapter 3) and at higher levels of biological organisation (chapters 4 and 5).

1.3.1 CHAPTER 2

This chapter serves as a detailed introduction to the research questions addressed in this thesis. I first carry out a quantitative bibliometric analysis of the multiple-stressor literature to highlight the divisions between freshwater, marine, and terrestrial ecologists, and ecotoxicologists, in their studies of multiple stressors. I show that disciplines use different terminology for predictor variables (“stressors”, “drivers”, “global change factors”) and for interactions (“antagonism” and “synergism”, “dampening” and “amplifying”). This leads to incomplete literature searches and meta-analyses, which slows the development of overarching theoretical concepts. I then establish a framework indicating the future of multiple-stressor research by summarising the research goals shared between researchers across disciplines. This conceptual framework lays the foundations for subsequent chapters.

1.3.2 CHAPTER 3

Although some meta-analyses have highlighted that interactions between stressors can change over time, the drivers of these temporal patterns have been unclear. Scaling up in time, I use evolutionary theory and empirical data to show that rapid evolution to multiple stressors generates a bias towards synergism. I develop a theoretical framework integrating performance trade-offs with synergistic interactions between stressors. Using data from an evolution experiment with the rotifer *Brachionus calyciflorus*, I empirically test this theory and illustrate that trade-offs in the adaptation to multiple stressors can introduce a bias towards synergism. Due to the generality of the underlying mechanism, these results suggest that adaptation should be accounted for to accurately predict the combined effects of multiple stressors.

1.3.3 CHAPTER 4

In this chapter I focus on the consequences of scaling up predictions to higher levels of organisation. Due to a geometric observation that “*in high dimensions there are more*

ways to be more different, than ways to be more similar”, scaling up predictions tends to systematically underestimate change. I explain the statistical mechanisms behind this effect, which is relevant to the study of any multi-dimensional system. I then use ecological simulations to explain the behaviour of various aggregate properties when community-level predictions are built from species-level predictions. As a practical case study, I relate this theory to multiple-stressor research where scaling up multiple-stressor predictions from one level (e.g. individuals, populations) to a higher level (e.g. communities, ecosystems) tends to introduce a bias towards synergism. This observational effect can influence the non-additivity between stressors at higher levels of organisation when a reductionist approach is employed.

1.3.4 CHAPTER 5

The aim of this chapter is to explain how non-additive effects at the community level are not intrinsic features of stressors, as they depend on how the community is observed. I use a simple model to explore how the non-additivity created by extinctions and invasions at the population level is observed by different metrics at community level. Critically, I find that community metrics often observe qualitatively different stressor interactions (antagonism, synergism) for the same stressors and the same biological system. Focusing on metrics that describe the composition, function and diversity of communities, I reveal the mechanisms that cause their observations of non-additivity to diverge. This work highlights the important effect of observation on the non-additivity at the community level and is a step towards the accurate prediction of the ecological impacts of multiple stressors.

1.3.5 CHAPTER 6

Finally, by summarising the work in previous chapters I explore the distinction between observational and natural sources of non-additivity. I explain that separating the two types of non-additivity is critical for a mechanistic understanding of stressor

interactions. I then suggest how future research could build on the work presented in this thesis to improve our understanding of non-additivity, and thus increase the predictive power of multiple-stressor research.

CHAPTER 2 | TOWARDS A UNIFIED STUDY OF MULTIPLE STRESSORS: DIVISIONS AND COMMON GOALS ACROSS RESEARCH DISCIPLINES

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AUTHOR CONTRIBUTION:

I wrote the first draft of the manuscript, conducted the bibliometric analysis, created the figures and organised contributions from co-authors with support from Jeremy Piggott. Ralf Schäfer, Michelle Jackson and Jeremy Piggott organised a cross-disciplinary workshop, *StressNet*, which inspired the writing of this manuscript. All authors contributed to the final manuscript.

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2.1 ABSTRACT

Anthropogenic environmental changes, or ‘stressors’, increasingly threaten biodiversity and ecosystem functioning worldwide. Multiple-stressor research is a rapidly expanding field of science that seeks to understand and ultimately predict the interactions between stressors. Reviews and meta-analyses of the primary scientific literature have largely been specific to either freshwater, marine or terrestrial ecology, or to ecotoxicology. In this cross-disciplinary study, we review the state of knowledge and use quantitative bibliometric analysis to identify a division between disciplines. Towards a unified research framework, we discuss the shared goals of increased temporal and ecological realism, with the overarching aim of improving predictive power. In a rapidly changing world, advancing understanding of the cumulative ecological impacts of multiple stressors is critical for biodiversity conservation and ecosystem management. Identifying and overcoming the barriers to interdisciplinary knowledge exchange is necessary in rising to this challenge. Division between ecosystem types and disciplines is largely a human creation. Species and stressors cross these borders and so should the scientists who study them.

2.2 INTRODUCTION

The most severe threats to global biodiversity and ecosystem functioning are anthropogenic environmental changes, or “stressors,” such as habitat loss, climate change, pollution and invasive species (Dirzo et al., 2014; Urban, 2015). These stressors often interact in complex and unexpected ways (Crain et al., 2008; Dieleman et al., 2012; Holmstrup et al., 2010; Jackson et al., 2016). Multiple-stressor research seeks to understand and predict interactions between stressors. Importantly, due to these interactions the combined effect of two or more stressors is frequently more than (synergistic) or less than (antagonistic) expected based on their individual effects (Folt et al., 1999; Piggott et al., 2015). The study of multiple stressors is not a novel pursuit

in science; toxicologists, and later ecotoxicologists, have been identifying the combined impact of multiple chemical stressors on individual organisms or populations for almost a century (Bliss, 1939; Loewe & Muischnek, 1926). Multiple-stressor research has now expanded to more diverse stressor combinations and has become a prominent feature of global change biology. Consequently, some of the concepts and terms used in the multiple-stressor literature have become common in mainstream ecology.

Aquatic, terrestrial, and ecotoxicological investigations into multiple stressors differ greatly in their approach. In the freshwater and marine ecology literature, numerous studies have measured biological responses to specific stressor combinations (Crain et al., 2008; Jackson et al., 2016). Such work has been conducted across the globe, from the Arctic (Andersen et al., 2017) to the Antarctic (Lenihan et al., 2018), and has focused on virtually all taxonomic groups, including bacteria (Salis et al., 2017), algae (Strain et al., 2015), invertebrates (Kaunisto et al., 2016), amphibians (Boone et al., 2007), and fish (Lange et al., 2018). Parallel to this research, and with almost no lateral exchange, the effects of multiple stressors on ecosystems have been the focus of many terrestrial experiments (Larsen et al., 2011; Rillig et al., 2019; Yue et al., 2017). Contrary to the freshwater and marine literature, the response variables of interest in terrestrial studies are mostly the fluxes and pools of matter such as carbon, nitrogen or other nutrients. Another discipline that has dealt with impacts of multiple stressors is ecotoxicology, which focuses on the effects of chemical pollutants and their interactions with other stressors (Holmstrup et al., 2010; Laskowski et al., 2010; Moe et al., 2013). Although freshwater, marine and terrestrial subdisciplines exist within ecotoxicology, they share a basic scientific foundation (e.g., methods, journals and conferences), which merits their aggregation as one discipline in this review.

Regardless of differing approaches, the underpinning concepts of multiple-stressor research are similar across the different disciplines. Despite this, exchange and cross-fertilization of ideas and conceptual models has been limited. For example, the co-tolerance concept (Vinebrooke et al., 2004), a number of stressor interaction classification systems (e.g., Piggott et al., 2015), and various null models predicting the

combined effect of stressors (e.g., Liess et al., 2016; Thompson et al., 2018a) have virtually escaped the terrestrial ecology community (Dieleman et al., 2012; Leuzinger et al., 2011; Yue et al., 2017). Moreover, models and methods developed in the context of ecotoxicology have largely been ignored in aquatic and terrestrial ecology (Schäfer & Piggott, 2018). Even reviews and meta-analyses of the multiple-stressor literature have primarily been specific to either freshwater (Jackson et al., 2016), marine (Crain et al., 2008) or terrestrial systems (Yue et al., 2017), or to ecotoxicology (Holmstrup et al., 2010) (but see: Côté et al., 2016; Darling & Côté, 2008).

Differences in terminology attest to the disconnection of freshwater, marine and terrestrial ecologists, as well as ecotoxicologists, from each other. For example, while the terms “stressors”, “antagonism” and “synergism” are common within the freshwater, marine and ecotoxicology literature (Gunderson et al., 2016; Jackson et al., 2016; Liess et al., 2016), many terrestrial and some marine ecologists often use the terms “drivers/factors”, “dampening” and “amplification”, respectively (Boyd & Brown, 2015; Leuzinger et al., 2011; Sirami et al., 2017; Yue et al., 2017). Other terms such as “cumulative effects”, “combined effects”, “net effects” or “interactive effects” are used across all disciplines (Crain et al., 2008; Harvey et al., 2013; Zhou et al., 2016). The pre-existing separation among scientific disciplines, exemplified by how ecologists tend not to cite work carried out in systems different from their own (Knapp et al., 2017; Menge et al., 2009), further contributes to the division in multiple-stressor research.

A better exchange between the different disciplines studying multiple stressors would be highly desirable. The separation of disciplines, including inconsistency in terminology, hampers progress as scarce resources are wasted due to the parallel development of similar methods and tools in different disciplines. Equally, incomplete literature searches and meta-analyses create an ignorance of the complete evidence, which can mislead research directions, impede the spread of ideas and slow down development of overarching theoretical concepts. In this cross-disciplinary review we use quantitative bibliometric analysis to identify the division between multiple-stressor researchers from different disciplines, we discuss qualitative differences in methods and

terminology between the disciplines, and we provide a common glossary to harmonise concepts and terminology. Subsequently, as a step towards a unified research framework we identify and discuss three common research goals that all multiple-stressor researchers share, specifically: (i) increased ecological complexity, (ii) increased temporal scale and realism, with the overarching aim of (iii) improving predictive power.

2.3 BIBLIOMETRIC ANALYSIS

2.3.1 METHODS

Using terms identified during our cross-discipline review we performed a search of the *ISI Web of Knowledge* database (<https://apps.webofknowledge.com>) to collect publications from the multiple-stressor literature (Appendix A.1). Next, we constructed citation networks where nodes represent specific publications and links indicate a citation between connected publications. Clustering algorithms and citation analysis were used to group publications that cite each other more than they cite other publications in the same network. The size of the nodes was based on the number of citations normalized by age of publication. To enhance visibility, only the 300 most highly cited publications were used to construct the citation networks. Given that this was biased towards marine and freshwater publications, the 25 next most highly cited terrestrial and ecotoxicological publications were added to ensure a similar number of publications across disciplines. The largest connected network (150 publications) from this pool of 350 publications was selected, ignoring publications outside the multiple-stressor literature. Supplementing our networks with additional publications reduced a bias in terms of nodes but may not have reduced a bias in terms of links (citations); on average the freshwater and marine publications had more citations than publications from the other disciplines. We therefore also constructed larger networks using a lower common threshold of citations resulting in networks based on the 500 and 1000 most

highly cited publications. For the main citation network, comprised of 150 publications, each publication was manually assigned to one of the four disciplines or as a “general” publication that was not specific to any discipline. A heat map was then produced to quantify the spread of the disciplines across the clusters of the networks. Finally, we created term networks, based on the titles and abstracts of the 150 multiple-stressor publications, using text-mining and clustering algorithms. For visibility, only terms that occurred at least 10 times were included, resulting in a network comprised of 161 terms. Citation and term networks were constructed using *VOSviewer 1.6.11* (Van Eck & Waltman, 2009).

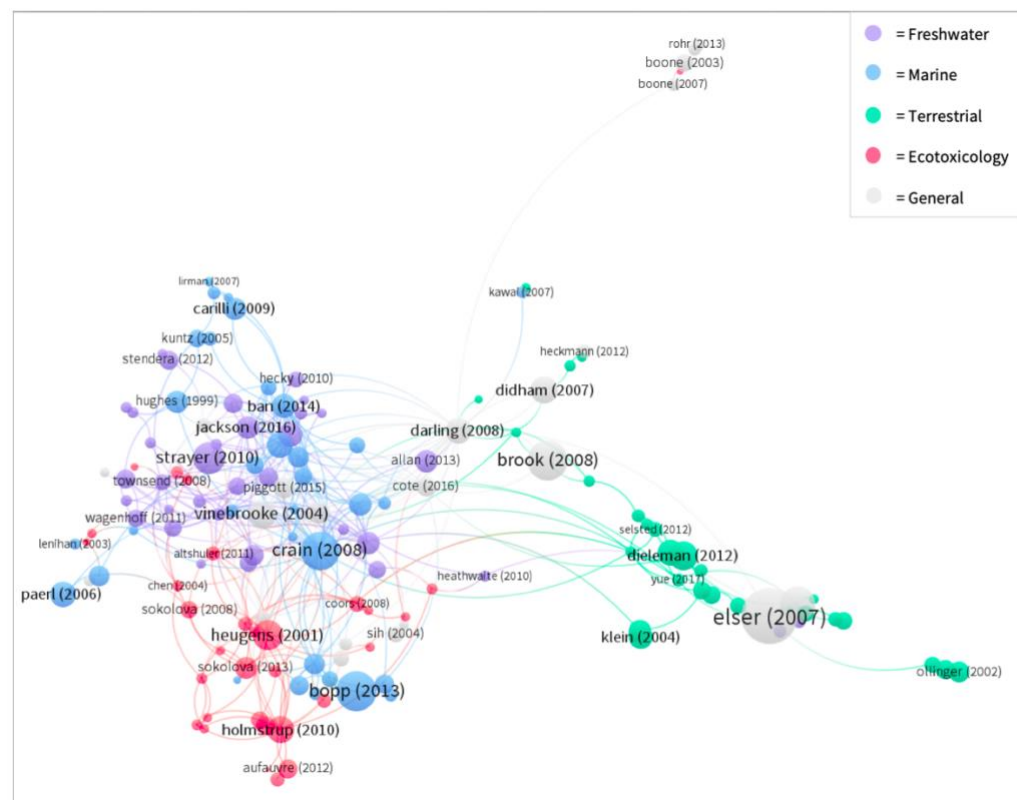


Figure 2.1: Citation network of the multiple-stressor literature. Nodes represent publications and links indicate the presence of a citation. The size of the nodes represents a publication’s number of citations normalized by age. The distance between nodes is calculated based on the number of times the connected publications cite each other. The colours of the nodes and their links represent the disciplines they belong to.

2.3.1 RESULTS

The citation network of 150 publications from the multiple-stressor literature (Appendix A.2) and the larger networks based on the 500 and 1000 most highly cited publications (Appendix A.3) had very similar clustering patterns and structures. Customizing the colours of the nodes to represent the different disciplines reveals the division between disciplines (Figure 2.1). Some of the most cited papers in the multiple-stressor literature are not specific to one discipline and are found towards the center of the networks (Côté et al., 2016; Darling & Côté, 2008; Folt et al., 1999; Piggott et al., 2015; Vinebrooke et al., 2004). Although the freshwater, marine and ecotoxicology literature clearly have their own clusters, there is substantial overlap between these disciplines (particularly freshwater and marine). In contrast, the terrestrial publications form a distinct cluster that is only connected to the rest of the network via five nodes, which are mostly meta-analyses or reviews (Christensen et al., 2006; Côté et al., 2016; Darling & Côté, 2008; Yue et al., 2017; Zhou et al., 2016). Based on the heat map (Appendix A.4), terrestrial publications are found almost exclusively in cluster 1 (82.8%) of the citation network (Appendix A.2). Ecotoxicological publications are found primarily in cluster 4 (54.8%). Freshwater publications are found primarily in clusters 2 (44.1%) and 6 (23.5%). Marine publications are well represented in all clusters in the network except for clusters 1 and 4.

In the term network, nodes towards the center of the network (e.g., effect, interaction, response) are used by all multiple-stressor researchers, whereas some nodes at the edges of the network are discipline-specific (Figure 2.2). The coloured nodes have been assigned to specific disciplines to outline the approximate location of disciplines in the network. These coloured terms act as markers against which the location of general terms of interest can be compared. For example, the term “multiple stressor” is found towards the edge of the network near freshwater, marine and ecotoxicological terms; it is on the opposite side of the network from where the terrestrial terms are. Similarly, the term “global change driver” is found among the terrestrial terms and away from the terms specific to the other disciplines.

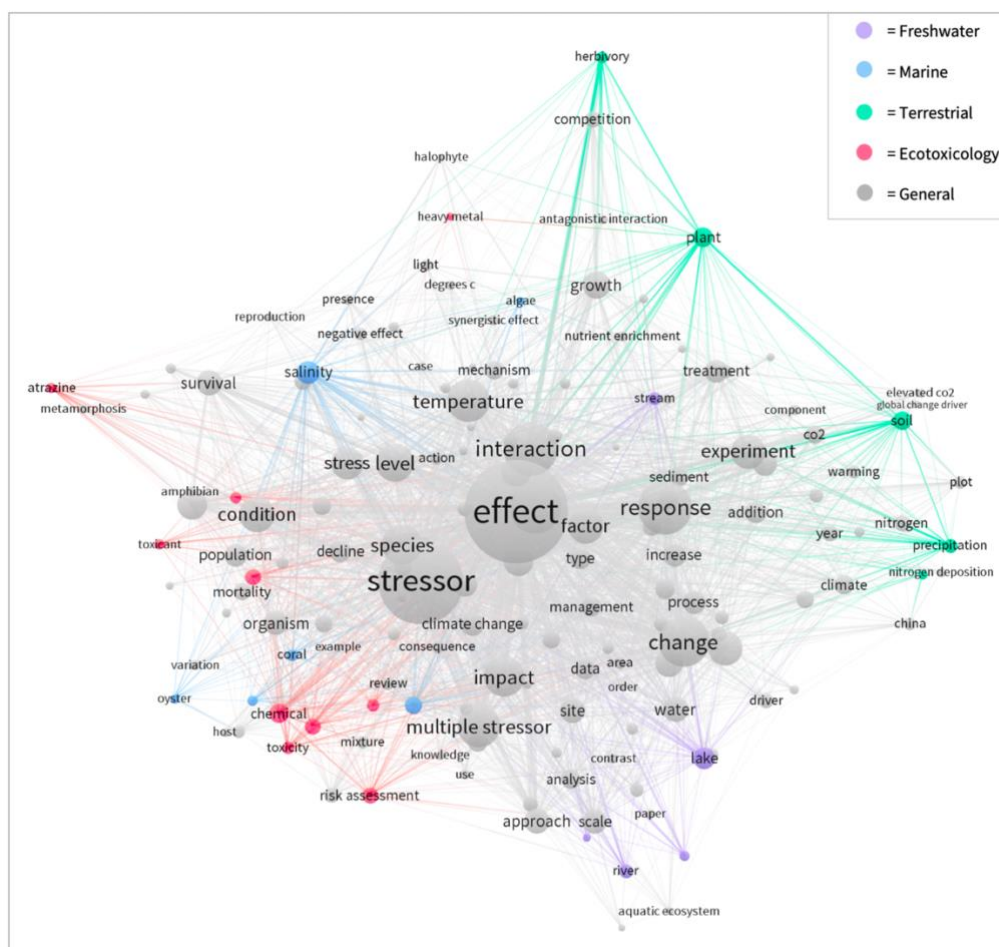


Figure 2.2: Network of terms from the multiple-stressor literature. Network constructed based on the titles and abstracts of the publications in the citation network (Figure 2.1). The size of the nodes represents the frequency of a term and the links represent co-occurrence. The colours of the nodes, which were assigned manually, represent the disciplines they are associated with.

2.4 SYNTHESIS

Our aim was to compare the terminology, predictor variables, response variables and methods across disciplines. We found that multiple-stressor researchers from different disciplines, despite studying fundamentally the same phenomena, are using different terminology for predictor variables and interactions and study different predictor and response variables (Table 2.1).

Another difference between disciplines is how researchers define a stressor. Many researchers associate stress with a negative biological response (Boyd & Hutchins, 2012; Vinebrooke et al., 2004) but others argue that the effect of stressors is context dependent and can be positive or negative (Côté et al., 2016; Piggott et al., 2015; Thompson et al., 2018b). For example, all common stressors (predictor variables) listed in Table 2.1 can cause positive or negative effects depending on the study species or the response variable. Another aspect to consider is whether a stressor can be natural, or only anthropogenic. Some researchers keep the definition as broad as possible (Côté et al., 2016; Kroeker et al., 2017) whereas others state that what separates a stressor from a “driver”, “factor” or “disturbance” is that it is anthropogenic (Piggott et al., 2015; Townsend et al., 2008). For the latter definition, it is important to note that natural factors such as predation or herbivory can become stressors under human modification.

There is a clear division between terrestrial researchers, who tend not to use the term “stressor”, and the rest of the multiple-stressor community. Terrestrial ecology has provided crucial evidence of the combined effect of stressors, but the language used leads to multiple-stressor meta-analyses missing these studies. That is because rather than using the common terminology of multiple-stressor research (e.g., stressor, antagonism or synergism), some studies only refer to the specific factors examined and describe effects as “dampening”, “amplifying” or “counteracting forces” (Borer et al., 2014; Gruner et al., 2008; Leuzinger et al., 2011). For example, in a meta-analysis of experiments examining the effects of multiple stressors on animal mortality in freshwater, marine and terrestrial communities the keywords used in the search included “synergy”, “antagonism” and “stress” but lacked “amplifying”, “dampening” or “factor/driver” (Darling & Côté, 2008). Potentially as a result of this, only four of the 112 experiments in the meta-analysis were conducted with terrestrial organisms (Darling & Côté, 2008). Hence, meta-analyses are useful in that they can identify knowledge gaps and pose new questions, but they reinforce division between disciplines when restricted to certain search terms.

As a result of the division between these research communities, certain ideas or approaches can become confined to different disciplines. For example, the terminology and distinction between global and local stressors is popular in the marine literature (Brown et al., 2013; Russell & Connell, 2012; Strain et al., 2015) but is rarely discussed elsewhere. Similarly, it seems that only freshwater ecologists use the term “reversals” when one stressor reverses the effect of another (Jackson et al., 2016). For instance, Christensen *et al.* (2006) found that a positive effect of acidification on phytoplankton became negative when warming was introduced. Ecotoxicologists have developed considerable theory on null model selection (Backhaus & Faust, 2012; Liess et al., 2016), which is only now being introduced to other communities of multiple-stressor research (Schäfer & Piggott, 2018). Novel concepts and approaches do not need to be (re-)discovered multiple times and all disciplines would benefit from a mutual exchange of ideas. We provide a glossary of terms (Table 2.2), with synonyms grouped, as a step towards the unification of multiple-stressor research.

Table 2.1: Comparison of multiple-stressor research across disciplines

Discipline	Terminology for predictors	Terminology for interactions	Common predictor variables	Common response variables	Key references
Freshwater	Stressor	Additive Synergistic Antagonistic Reversal	Temperature Altered flow Nutrients Toxicants Habitat modification Invasive species	Population metrics, Functional traits, Biodiversity	(Hering et al., 2015; Jackson et al., 2016; Ormerod et al., 2010)
Marine	Stressor Driver	Additive Synergistic Antagonistic	Temperature Acidification Nutrients Toxicants High/low salinity Hypoxia	Physiology, Population metrics, Functional traits, Biodiversity	(Crain et al., 2008; Gunderson et al., 2016; Przeslawski et al., 2015)
Terrestrial	Factor Driver	Additive Synergistic Antagonistic Dampening Amplifying Counteracting	Temperature Increased CO ₂ Land use change Nutrient modification Altered precipitation Invasive species	Fluxes and pools of elements, compounds and nutrients, Productivity, Biodiversity	(Borer et al., 2014; Yue et al., 2017; Zhou et al., 2016)
Ecotoxicology	Stressor Toxicant Toxic chemical	Additive Synergistic Antagonistic	Toxicants Increased temperature Salinization Drought Pathogens/predators	Physiology, Population metrics, Biodiversity	(Holmstrup et al., 2010; Liess et al., 2016; Moe et al., 2013)

Table 2.2: Glossary of terms from multiple-stressor research

Terms/Concepts	Definition	Source
Stressor Factor Driver	Any natural or anthropogenic variable that causes a quantifiable change, irrespective of its direction (increase or decrease), in a biological response. However, many researchers associate the term “stressor” with an anthropogenic variable that has a negative impact.	(Côté et al., 2016)
Multiple Stressors	Two or more co-occurring or sequential stressors.	n/a
Stressor Interaction	Modification of a stressor’s intensity or the sensitivity of an organism or ecosystem towards this stressor by another stressor or multiple other stressors. Thus, the term refers to the interaction between stressors in the real world. By contrast, concepts such as the multiplicative null model rely on mathematical interactions that do not necessarily imply interactions in the real world.	(Schäfer & Piggott, 2018)
Additive	When the combined effect of multiple stressors is equal to the sum of their individual effects, i.e. no interaction effect.	(Folt et al., 1999)
Antagonistic Dampening	Interactions between stressors that result in a lesser combined effect than that predicted by a null model (i.e. an interaction between stressors making their observed net effect less than expected).	(Schäfer & Piggott, 2018)
Synergistic Amplifying	Interactions between stressors that result in a greater combined effect than that predicted by a null model (i.e. an interaction between stressors making their observed net effect more than expected).	(Schäfer & Piggott, 2018)
Reversal	Interactions that result in the combined effect of two stressors being opposite in direction (negative or positive) from that of the sum of their single effects.	(Jackson et al., 2016)
Null Model	A model that predicts the combined effect of multiple stressors assuming the absence of interactions among stressors as defined above. However, some null models contain mathematical interactions to capture stochastic aspects in the action of two stressors, for example the multiplicative null model.	(Schäfer & Piggott, 2018)
Ecological Surprises	Scenarios where the mechanisms of stressor interactions are not understood and predictions based on null models fail.	(De Laender, 2018)

2.5 TOWARDS A UNIFIED RESEARCH FRAMEWORK

Despite the division between disciplines described above, all multiple-stressor researchers share the same goals. Elements of these common goals have been identified before but are scattered across the literature in both primary research and reviews. Here we integrate and develop on these shared research goals of (i) increased ecological complexity, (ii) increased temporal scale and realism, and (iii) increased prediction. Our conceptual framework offers a future direction for multiple-stressor research (Figure 2.3).

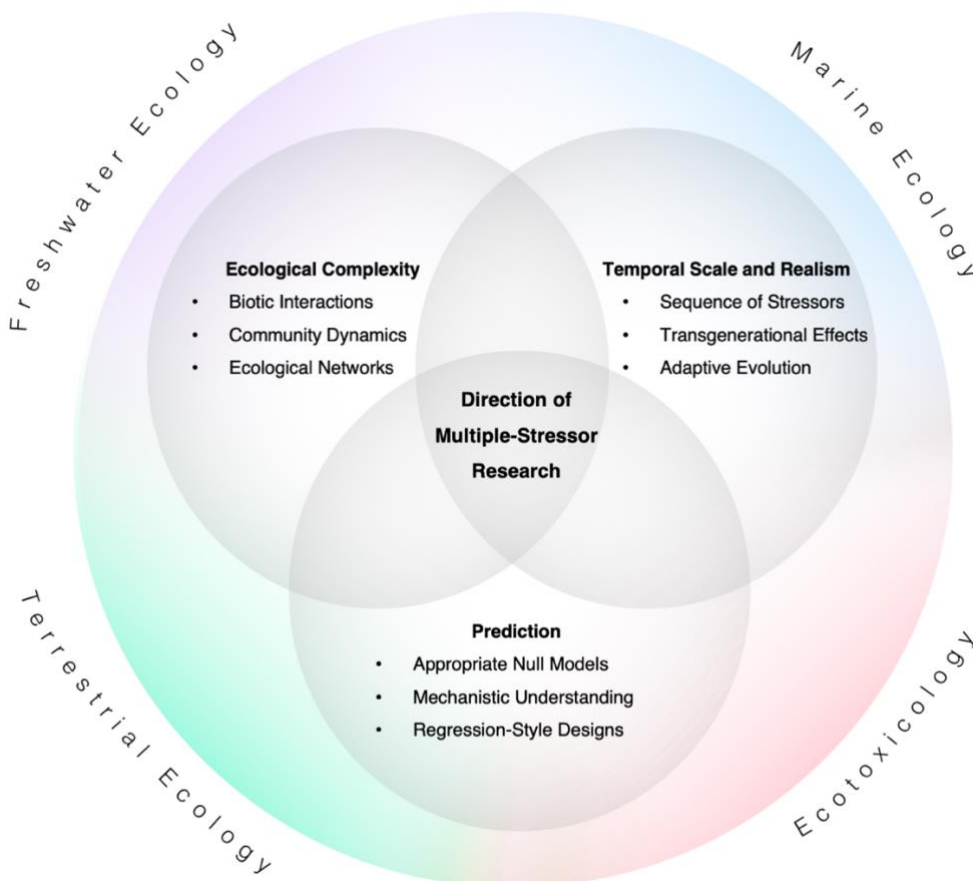


Figure 2.3: Towards a unified research framework. Conceptual framework integrating research goals shared by all disciplines to highlight the potential future direction of multiple-stressor research.

2.5.1 ECOLOGICAL COMPLEXITY

There is a need for multiple-stressor research to shift its focus towards the higher levels of biological organization to increase the field's relevance for conservation and ecosystem management (Crain et al., 2008; De Laender, 2018; Thompson et al., 2018a). Researchers have called for this increase in ecological complexity in freshwater (Bray et al., 2018; Schuwirth et al., 2016), marine (Boyd & Brown, 2015; Griffen et al., 2016) and terrestrial (Leuzinger et al., 2011) ecology as well as in ecotoxicology (Van den Brink et al., 2018). For instance, it is unclear if results obtained at lower levels of organisation scale up to the community and ecosystem level. Similarly, there is a poor understanding of the role of species interactions in introducing and modifying the interactions between stressors.

Different approaches have been taken to study how non-additivity between stressors is impacted by ecological complexity. Firstly, many meta-analyses have attempted to study how stressor interactions vary across levels of organisation. For example, in their review of 171 multiple-stressor studies in marine and coastal ecosystems, Crain *et al.* (2008) found that synergism was most common in population-level studies, but antagonism was most common in community-level studies. Similarly, Côté *et al.* (2016) found that synergism became less common as biological scale increased in their quantitative review across disciplines. However, Jackson *et al.* (2016) found no significant difference in the frequencies of interaction types at the different biological levels in their review of freshwater studies. Moving beyond this “vote-counting” approach, researchers have conducted specific experimental (Bruder et al., 2017; O’Gorman et al., 2012) and modelling (Galic et al., 2018; Griffith et al., 2019) research on this topic. Some theory has even been developed to predict the impacts of multiple stressors at higher levels of organisation (De Laender, 2018; Thompson et al., 2018a). De Laender (2018) showed how competition for common resources can lead to both synergistic and antagonistic effects of multiple stressors on species richness.

There is great interest in the connection between species interactions and stressor interactions. In general, it is thought that the combined effect of multiple

stressors can be amplified at the community level when stressors act on influential groups such as keystone species or ecosystem engineers (Gooding et al., 2009; Kroeker et al., 2017). Furthermore, species interactions may themselves change after exposure to stressors. For example, stressors may influence resource competition (Kroeker et al., 2013) and may change the susceptibility of hosts to pathogens and parasites (Lafferty & Holt, 2003; Lenihan et al., 1999). Equally, stressors can alter the trophic relationships of species (Arnold et al., 2012; Bruder et al., 2017). Schrama *et al.* (2017) applied multiple pesticides to pond mesocosms and used stable isotope analysis to show that these stressors and their interactions modified the flow of energy through the food web by inducing shifts in trophic links. Biotic interactions can themselves act as stressors and consequently interact with other stressors. For instance, the interactions between climate change and ungulate herbivory modulate effects on forest ecosystems (Didion et al., 2011). The importance of biotic interactions in understanding the effects of stressors highlights the need for an ecological network approach towards multiple-stressor studies (Bruder et al., 2019). Developments in DNA metabarcoding and stable isotope analysis are improving our ability to detect and quantify biotic interactions (Layman et al., 2012; Roslin & Majaneva, 2016). These empirical tools, as well as a solid theoretical foundation, will be required for multiple-stressor researchers to properly understand the role of species interactions and community dynamics in shaping the non-additivity between multiple stressors.

2.5.2 TEMPORAL SCALE AND REALISM

The combined effect of stressors depends on various, largely overlooked, factors related to different time scales (Côté et al., 2016; Gunderson et al., 2016). At the time scale within one generation, several temporal factors have been identified that may determine responses to multiple stressors. First, the sequence of exposure to stressors may be crucial. If species' responses to stressors are negatively correlated, sequence of exposure may be more important than if their responses are positively correlated (Vinebrooke et al., 2004). Specifically, if stressors exert different effect on species,

order of exposure may be less important than if their effects are redundant. Second, the time interval between stressors may influence their combined impact. Gunderson *et al.* (2016) developed a conceptual framework for physiological responses to stressors that predicts the interaction type between sequential exposure to two stressors to be additive when the time interval between exposure is long, but synergistic when time interval is short. Interactions between stressors can also depend on the developmental stage of an organism. Indeed, interactive effects may change, and even reverse, throughout ontogeny. Przeslawski *et al.* (2015) showed in a meta-analysis of marine organisms that the combination of thermal and salinity stress was more likely to be synergistic for embryonic than for larval life stages, yet the opposite pattern occurred between thermal and pH stress. Few studies, however, have tested variation in interactions across developmental stages within the same species (but see: Fitzgerald *et al.*, 2017).

At the time scale of a few generations, little is known about how the interaction type between stressors in offspring depends on the exposure of the parents to those stressors. As a rare example, a synergistic interaction between warming and a pollutant was detected in the mosquito *Culex pipiens* both in the parents and in the offspring of parents exposed to none or a single stressor. By contrast, an additive effect was present in the offspring of parents exposed to both stressors simultaneously, because in this condition the pesticide was already more lethal at the lower temperature (Tran *et al.*, 2018). At the time scale of tens of generations, the evolutionary adaptation to a stressor may shape tolerance to subsequent stressors due to pleiotropic effects where the same set of genes contributes to tolerance against different stressors. This may cause co-tolerance where the acquisition of genetic adaptation to one stressor increases tolerance to another (Bubliy & Loeschcke, 2005), which is likely as genetic mechanisms of tolerance to stressors are often conserved (Sikkink *et al.*, 2015). Yet, pleiotropic effects may also be antagonistic resulting in adaptation to one stressor actually reducing tolerance to a second (Hua *et al.*, 2017). Experiments should attempt to use realistic timing of stressors over meaningful timescales (e.g., Cheng *et al.*, 2015), but as this can be impractical, observational studies may be required (Hättenschwiler *et al.*, 1997).

Furthermore, certain stressors, for example nitrogen deposition (Payne et al., 2019), accumulate over time, which can delay ecological effects and further complicate multiple-stressor predictions. Understanding if and how interactions between stressors can change over time is a goal shared by all disciplines.

2.5.3 PREDICTION

The ultimate goal of multiple-stressor research is prediction of the combined effect of stressors. Over the past twenty years a vast amount of research has tested the effects of specific combinations of stressors on specific biological responses. However, very few, if any, general patterns have emerged from meta-analyses (Crain et al., 2008; Dieleman et al., 2012; Holmstrup et al., 2010; Jackson et al., 2016; Lange et al., 2018; Yue et al., 2017), except perhaps that non-additivity between stressors is ubiquitous. This approach to studying multiple stressors, calculating proportions of interaction types across different environments, conditions and responses, does not improve our predictive capacity of multiple stressors for a variety of reasons, including the existence of a publication bias towards synergism (Côté et al., 2016) and an overwhelming amount of context-dependency (Kroeker et al., 2017). There is a need to move beyond comparing proportions of antagonism and synergism and shift focus towards improving our mechanistic understanding of non-additivity. For example, using regression-style experimental designs, rather than typical factorial designs, would enhance our understanding of stressor-response relationships, thus increasing our ability to predict threshold responses (Boyd et al., 2018; Kreyling et al., 2018). When predicting the combined effects of multiple stressors, it is important to consider both the modes of action of stressors and their interactions. For example, the similarity or dissimilarity of stressors' modes of action may reveal important information about how they may interact (Folt et al., 1999; Vinebrooke et al., 2004). Equally, according to Boyd and Brown (2015), there are multiple modes of interaction between stressors at the physico-chemical, organismal, and ecosystem levels. This concept, of statistical interactions

between stressors occurring as a result of interactions between stressors at different scales, is gaining more attention (e.g., Griffen et al., 2016; Kroeker et al., 2017).

A major issue that needs to be resolved is the use of null models. The additive null model has been widely used, but also widely criticized for being inappropriate in many scenarios (Côté et al., 2016). For example, it is biased towards antagonism when metrics with a fixed boundary, such as mortality, are used as response variables (Folt et al., 1999; Lange et al., 2018). Many null models can be useful for multiple-stressor researchers, including both established models from the ecotoxicological literature and new developments such as the *Stress Addition Model* (Liess et al., 2016) and the *Compositional Null Model* (Thompson et al., 2018a). Researchers need to be aware of the different null models available and their association with statistical tests (Griffen et al., 2016). A recent framework for a mechanistic basis to null model selection aims to facilitate a shift towards a more predictive approach (Schäfer & Piggott, 2018). The objective here is to use null models that accurately predict the combined effects of stressors, or at least help us understand the source of non-additivity. “*Ecological Surprises*” arise when our null models are wrong, and researchers are unable to explain why. Debate over null models and the emerging publications have almost entirely bypassed the terrestrial global change research community, even though such considerations could influence the interpretation of some of their findings considerably. Predicting the impacts of multiple stressors is a common goal shared by all disciplines, and achieving this goal is vital for the sustainable management of resources and for the conservation of biodiversity and ecosystem services.

2.6 CONCLUSIONS

Multiple-stressor researchers from different disciplines are clearly separated. This was identified during our cross-disciplinary review and was confirmed using bibliometric analysis. The use of different terminology for predictor variables and for interactions

between those variables has reinforced this separation. Common terminology, or at least awareness of the different terms in online searches and meta-analyses, would greatly enhance cross-disciplinary collaboration and would encourage the integration of multiple-stressor research into mainstream ecology.

In future work, researchers should consider multiple-stressor literature from other disciplines for guidance on methods and analyses. Authors of primary research should include multiple terms in their keyword section to enhance the visibility of their research. Meta-analyses of the multiple-stressor literature should consider the broader range of terminology identified in this review (see common glossary: Table 2.2) and, where possible, be repeated to include relevant but previously missed studies. Multiple-stressor researchers from all disciplines are converging towards the same common goals, and the time is ripe for a unified approach. Division between ecosystem types and disciplines is largely a human creation. Species and stressors cross these borders, and so should the scientists who study them.

CHAPTER 3 | RAPID EVOLUTION GENERATES SYNERGISM BETWEEN MULTIPLE STRESSORS: LINKING THEORY AND AN EVOLUTION EXPERIMENT

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AUTHOR CONTRIBUTION:

I carried out the data analysis, created the figures and wrote the first draft of the manuscript. Pepijn Luijckx conducted the evolution experiment and provided the data. Myself and Jean-François Arnoldi developed the framework integrating multiple-stressor interactions and evolutionary trade-offs. Jeremy Piggott and Pepijn Luijckx initiated the project. All authors contributed to the final manuscript.

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3.1 ABSTRACT

Global change encompasses many co-occurring anthropogenic stressors. Understanding the interactions between these multiple stressors, whether they be additive, antagonistic or synergistic, is critical for ecosystem managers when prioritising which stressors to mitigate in the face of global change. While such interactions between stressors appear prevalent, it remains unclear if and how these interactions change over time, as the majority of multiple-stressor studies rarely span multiple generations of study organisms. Although meta-analyses have reported some intriguing temporal trends in stressor interactions, for example that synergism may take time to emerge, the mechanistic basis for such observations is unknown. In this study, by analysing data from an evolution experiment with the rotifer *Brachionus calyciflorus* (~35 generations, 31,320 observations), we show that adaptation to multiple stressors shifts stressor interactions towards synergism. We show that trade-offs, where populations cannot optimally perform multiple tasks (i.e. adapting to multiple stressors), generate this bias towards synergism. We also show that removal of stressors from evolved populations does not necessarily increase fitness and that there is variation in the evolutionary trajectories of populations that experienced the same stressor regimes. Our results highlight outstanding questions at the interface between evolution and global change biology, and illustrate the importance of considering rapid adaptation when managing or restoring ecosystems subjected to multiple stressors under global change.

3.2 INTRODUCTION

Ecosystems experiencing global change are threatened by many different anthropogenic stressors. Predicting the combined effects of these multiple stressors is a deceptively challenging goal. A diverse body of research has shown that the combined effect of stressors, or global change factors, is often more than or less than expected based on individual effects due to synergistic and antagonistic interactions, respectively (Crain et

al., 2008; Dieleman et al., 2012; Holmstrup et al., 2010; Jackson et al., 2016). Knowledge regarding the nature of stressor interactions can be used by ecosystem managers to prioritise which stressors to mitigate (Brown et al., 2013; Côté et al., 2016). Indeed, removing a stressor that interacts synergistically with other stressors will have a greater impact than removing a stressor that interacts antagonistically with other stressors (Brown et al., 2013; Falkenberg et al., 2013). There is, however, an overwhelming amount of context-dependence in multiple-stressor research with seemingly few, if any, general patterns in the occurrence of antagonism or synergism (Côté et al., 2016; Kroeker et al., 2017; Orr et al., 2020). Moreover, interactions between stressors can change over time (Darling & Côté, 2008; Debecker et al., 2017; Lange et al., 2018), which further complicates efforts to understand and predict global change impacts.

Meta-analyses that investigated temporal patterns in stressor interactions have yielded conflicting results. In an analysis of 29 multiple-stressor studies on freshwater fish the frequency of synergistic interactions increased with experimental duration (Lange et al., 2018). Conversely, a meta-analysis of 112 experiments that tested multiple-stressor effects on animal mortality found the opposite temporal trend, that antagonism became more common as experimental duration increased (Darling & Côté, 2008). To reconcile these apparently opposing results, it is critical to understand the mechanisms that change stressor interactions over time. Indeed, recent progress has identified that stressor interactions can vary across different life stages (Fitzgerald et al., 2017; Przeslawski et al., 2015; Sniegula et al., 2017) and between parents and offspring due to transgenerational effects (Tran et al., 2018, 2019). Furthermore, adaptive evolution to one stressor can change its interactions with another stressor (Cambronero et al., 2018; Debecker et al., 2017; Zhang et al., 2018). Despite these advances, general expectations of how stressor interactions change over time remain elusive.

Here, we focus on rapid evolution, seeking a mechanistic understanding of its impacts on stressor interactions. Evolution can take place over ecological timescales and is therefore relevant to global change biology, restoration ecology, and multiple-

stressor research (Stockwell et al., 2003; Thompson, 1998; Zhang et al., 2019). Rapid evolution, or contemporary evolution, can occur within a month for organisms with fast lifecycles (Baym et al., 2016; Luijckx et al., 2017) and there is evidence of adaptation within seven generations for multiple species, including guppies (*Poecilia reticulata*), water fleas (*Daphnia pulex*) and flour beetles (*Tribolium castaneum*) (Gorokhova et al., 2002; Reznick et al., 1997; Szűcs et al., 2017). Adaptation to global change can result in the “evolutionary rescue” of populations and communities that would have otherwise gone extinct (Bell, 2017; Fugère et al., 2020; Hendry et al., 2017; Strauss et al., 2008). However, given that global change is comprised of many interacting stressors, it is crucial to understand if, and how, rapid evolution to multiple stressors influences how those stressors interact.

Evolutionary theory can provide valuable insights into how adaptation to global change may alter stressor interactions. Performance trade-offs, where organisms cannot optimally perform multiple tasks, affect populations when adapting to multiple stressors (Agrawal et al., 2010; Hiltunen et al., 2018; Sheftel et al., 2018; Shoval et al., 2012; Tikhonov et al., 2020). Several evolutionary mechanisms can produce a trade-off, making it difficult for organisms to adapt to multiple stressors at once. Antagonistic pleiotropy, where one gene controls multiple and opposing phenotypes (Williams, 1957), may cause the adaptation to one stressor to decrease an organism’s tolerance to another (Anderson et al., 2013). For example, amphibian populations adapted to pesticides can have lower tolerance to certain parasites (Hua et al., 2017). Linkage disequilibrium (the non-random association of alleles) and epistasis (interactions between genes) are further potential causes of evolutionary trade-offs in multiple-stressor environments (Roff & Fairbairn, 2007; Østman et al., 2012). Finally, genetic bottlenecks associated with a reduction in population size due to intense stress may reduce genetic variation and impede adaptation to further stressors (Pedrosa et al., 2017; Ribeiro & Lopes, 2013). These trade-offs can be studied by plotting populations in performance space where each axis is defined by the adaptation of populations to a stressor. If evolved populations exhibit pareto optimality by falling on a convex hull of

optimum strategies, known as the pareto front (Shoval et al., 2012; Tikhonov et al., 2020), adaptation to multiple stressors will be less than the sum of the adaptation to individual stressors, resulting in stressor interactions changing over time.

Here, we study the role of rapid evolution to multiple stressors in creating temporal variation in stressor interactions by analysing a dataset from an evolutionary experiment with the rotifer *Brachionus calyciflorus*, conducted by Luijckx *et al.* (2017). Rotifers are ecologically important organisms in freshwater ecosystems world-wide (Arndt, 1993) and are commonly used as model organisms to address ecological and evolutionary questions (Becks & Agrawal, 2010; Declerck & Papakostas, 2017). In the experiment, rotifers were grown in a full factorial design of three stressors (eight environments in total) for ~35 generations (70 days), creating eight evolved lines of rotifers. Growth assays were conducted at the end of the experiment for each of the eight evolved lines of rotifers in each of the eight environments (64 combinations). We apply these data to a theoretical framework integrating the concepts of stressor interactions and pareto optimality in performance space to explore general mechanisms of temporal trends in stressor interactions. To this end, we asked three questions at the interface of evolutionary biology and global change research. First, do interactions between stressors change over time due to adaptive evolution? Second, do evolutionary trade-offs generate temporal variation in stressor interactions? Third, does removal of one or more stressors from populations evolved to those stressors increase or decrease their fitness?

3.3 METHODS

3.3.1 EVOLUTION EXPERIMENT AND TEMPORAL VARIATION IN STRESSOR INTERACTIONS

We analysed data from an evolution experiment where the rotifer *Brachionus calyciflorus* was allowed to adapt for 70 days (~35 generations) to individual and

multiple stressors in a full factorial design. For a detailed description of the methods we refer to Luijckx *et al.* (2017), which focused on the evolution of sexual reproduction opposed to adaptation to new environments. In short, rotifers were hatched from sediment collected from Lake Onondaga, New York, and grown under standard lab conditions (constant light at 22 °C) for six months (~100 generations) until the start of the experiment. Thirty-two rotifer populations were initiated (~6000 rotifers per population) from stock populations and exposed to eight different environments (four replicates per environment) where levels of salt (NaCl from 0 to 0.4g/L), copper (0 to 1.25µg of CuSO₄ per day) and temperature (reduced from 22 to 17.5 °C) were altered in all possible combinations (Figure 3.1a). To study evolutionary effects, these levels of environmental change were chosen as proxies for environmental and chemical stressors because they were known to have negative individual effects, but non-lethal combined effects. Indeed, adaptation occurred in six of the seven stressor environments (Luijckx *et al.*, 2017). Populations were maintained by providing 100 million algae per day and replacing 10% (50mL) of the culture media every second day. Every four days the size of all populations was standardized (to the third lowest population) to limit differences in evolutionary potential (i.e. the genetic variation available) between populations exposed to relatively benign environments and populations that experienced a large reduction in size due to high levels of stress (i.e. a genetic bottleneck). Adaptation during the experiment was monitored by measuring both density (five 1ml subsamples every four days) and lifetime reproductive success of individual rotifers (8-10 individuals per population for six timepoints). Growth assays were carried out at the end of the experiment where growth of 24 individuals from the 29 surviving populations (three populations from different treatments went extinct and were therefore not included in the analyses) were followed for five days to assess population growth rates in each of the eight environments (64 combinations of environment and evolved line with a total of 31,320 observations, Figure 3.1a). Data from growth assays were log transformed ($\ln(\text{data}+1)$) and a linear regression was fitted against time in order to obtain growth rates. Mean growth rates were then calculated for each population

(sample size of four, except for the evolved lines of copper, salt + copper and salt + temperature, where sample size was three). It should be noted that, while we have relatively few populations per treatment, high replication within each population ($n=24$) enhances our ability to detect differences within and between treatments.

We analysed the population growth rates in a multiple-stressor context to better understand the role of rapid evolution in creating temporal variation of stressor interactions. The effects of stressors on non-evolved populations were determined using the growth rates of the control populations in the stressor environments (blue points in Figure 3.1a). The effects of stressors on evolved populations were determined using the growth rates of these populations in the stressor environment that they evolved in (red points in Figure 3.1a). Stressor interactions were identified based on an additive null model using standardized effect sizes (*Hedges' g*) with 95% confidence intervals for all four combinations of multiple stressors in non-evolved and evolved populations (Gurevitch et al., 2000). When the 95% confidence intervals crossed zero, stressor interactions were classified as additive. Negative and positive interactive effects whose confidence intervals did not cross zero were classified as antagonistic and synergistic, respectively (Hale et al., 2017; Jackson et al., 2016). To identify temporal changes in stressor interactions, interactive effects were compared between non-evolved and evolved populations using a two-sided paired samples t-test. This comparison (blue vs red points in Figure 3.1a) represents a scenario where a factorial study design is used to determine stressor interactions before and after evolution. One could also consider an alternative scenario where the null model used to predict the combined effect of stressors remains fixed at a point prior to the occurrence of evolution. Under such a scenario one may expect combined effects to diminish due to evolution leading to a shift towards antagonism over time. Here we focus on the first scenario which represents full factorial designs, the bedrock of multiple-stressor research, but we refer to the Appendix B.1 and discussion for inferences and implications of the second scenario.

3.3.2 THEORETICAL FRAMEWORK: PERFORMANCE TRADE-OFFS AND SYNERGISM

To explore the role of performance trade-offs in creating temporal variation of stressor interactions we developed a theoretical framework integrating the concepts of multiple-stressor interactions and pareto optimality in performance space. First, we considered the effect of stressors individually and combined on separate axes to visualize additivity between stressors and to visualize adaptation to individual and multiple stressors (Figure 3.2a). By comparing the sum of adaptation to individual stressors and the adaptation to multiple stressors we define and quantify a shift in stressor interactions. We then considered performance space, where each axis is defined by the adaptation of populations to a single-stressor environment (Figure 3.2b). Here, if a performance trade-off exists, populations cannot optimally adapt to multiple environments and thus evolved states should approach the convex hull of optimum strategies, which is known as the pareto front (Shoval et al., 2012; Tikhonov et al., 2020). Under this scenario, for populations to improve fitness to one stressor, they will lose fitness to the other. Finally we compared the actual adaptation to multiple stressors (i.e. increase of fitness, ΔAB) and the taxi-cab distance ($\Delta^* AB$) in performance space between non-evolved populations and populations evolved to multiple stressors (Figure 3.2). If adaptation to multiple stressors can be decomposed into adaptation to one stressor and adaptation to the other stressor (additive adaptation, Appendix B.3) then these two values (ΔAB and $\Delta^* AB$) will be similar. We propose that if these two values are similar, and if there is evidence of a pareto front in performance space, then evolution and performance trade-offs will cause a shift towards synergism between stressors.

We then applied our theoretical framework to the population growth rate data. The growth rates of all populations in all environments are known (Figure 3.1a), allowing us to plot populations in performance space. To avoid artificially overestimating the strength of tradeoffs, however, we had to account for the effect that the removal of a stressor can have on population fitness. For example, populations evolved to salt + copper grown in the salt environment only will experience the removal

of the copper stressor, which they had started adapting to. When positioning these populations in performance space (the adaptation to salt alone on one axis, and the adaptation to copper alone on the other) we must account for the change in fitness caused by the absence of one of the stressors they had started to adapt to (copper and salt, respectively). Otherwise, the trade-off observed could simply be caused by the removal effect (see Appendix B.3 where we formalise this correction and clarify the underlying assumption of additive adaptation that it relies on). To showcase the existence of a pareto front, we thus plotted populations in performance space, with corrections for removal effects, for all combinations of stressors. We then compared the actual adaptation to multiple stressors (ΔAB) and the distance in performance space between non-evolved populations and populations evolved to multiple stressors ($\Delta^* AB$) to determine if performance trade-offs played a role in changing stressor interactions over time.

3.3.3 REMOVAL OF STRESSORS

Finally, to understand if rapid evolution to stressors influences restoration efforts we tested how removal of one or more stressors, after prolonged exposure, impacted population fitness. Specifically, we tested if growth rates of evolved populations in their own environment differed from their growth rates in the control environment (i.e. when stressors were removed) using a two-sided paired t-test, which controlled for differences between populations. Finally, to determine if the identity of the removed stressor was important, we compared the growth rates of the populations adapted to all three stressors when grown in the double stressor environments (e.g. lacking either temperature, salt or copper stressors) using a one-way ANOVA with Dunnett's "many-to-one" post hoc comparison (Dunnett, 1955). The assumptions of statistical tests (e.g., normality, homogeneity of variances, independence of data) were met and the data and code used for all statistical analyses and figures are available on *GitHub*: (<https://github.com/jamesaorr/evolution-multiple-stressors>).

3.4 RESULTS

Adaptive evolution to all individual and multiple-stressor environments occurred within one month (~20 generations, except potentially the multiple-stressor combination of salt + copper). For non-evolved populations, the combined effects of stressors were less than expected due to antagonistic interactions (blue points in Figure 3.1c). For evolved populations, however, the combined effects of stressors were more than expected due to synergistic interactions (red points in Figure 3.1c). Although not all stressor interactions were statistically detectable (1/4 non-evolved and 2/4 evolved), there was a clear shift towards synergism in the interactive effect sizes between non-evolved ($M=-1.46$, $SD=0.52$) and evolved ($M=2.11$, $SD=1.45$) populations ($t_3 = -5.03$, $p = 0.015$).

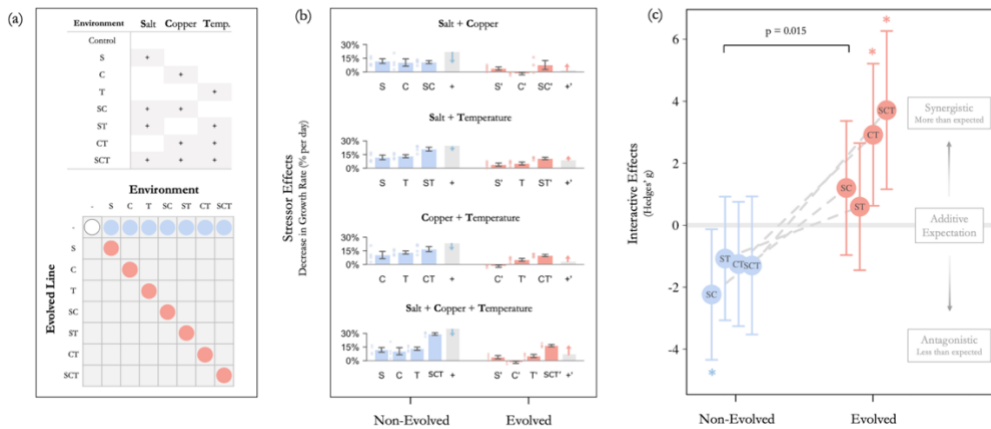


Figure 3.1: Bias towards synergism due to evolution. **(a)** A full factorial design of three stressors (salt, copper and temperature) created eight environments in which populations evolved. After evolution had occurred, growth rates of all populations in all environments were calculated. The white circle indicates how the baseline growth rate was determined. The blue and red circles represent non-evolved populations and evolved populations, respectively. **(b)** Individual and combined effects of stressors on the growth rates of non-evolved (blue) and evolved (red) populations for all combinations of stressors. The additive expectation for each combined effect is plotted in grey. Dots represent population replicates and error bars represent standard error. Arrows superimposed on the additive expectations represent interactive effects. Downward arrows indicate that the combined effect is less than expected (antagonism).

Upward arrows indicate that the combined effect is more than expected (synergism). **(c)** Interactive effects (*Hedges' g*) with 95% confidence intervals for the four combinations of multiple stressors for non-evolved (blue) and evolved (red) populations. Interactive effects below and above the grey line (at $y=0$) are antagonistic and synergistic, respectively. These effects are statistically significant (*) when 95% confidence intervals do not cross the grey line. Dashed lines connect evolved and non-evolved pairs of interactive effects. There is a clear shift from antagonism towards synergism due to evolution (paired t-test, $p\text{-value} = 0.015$).

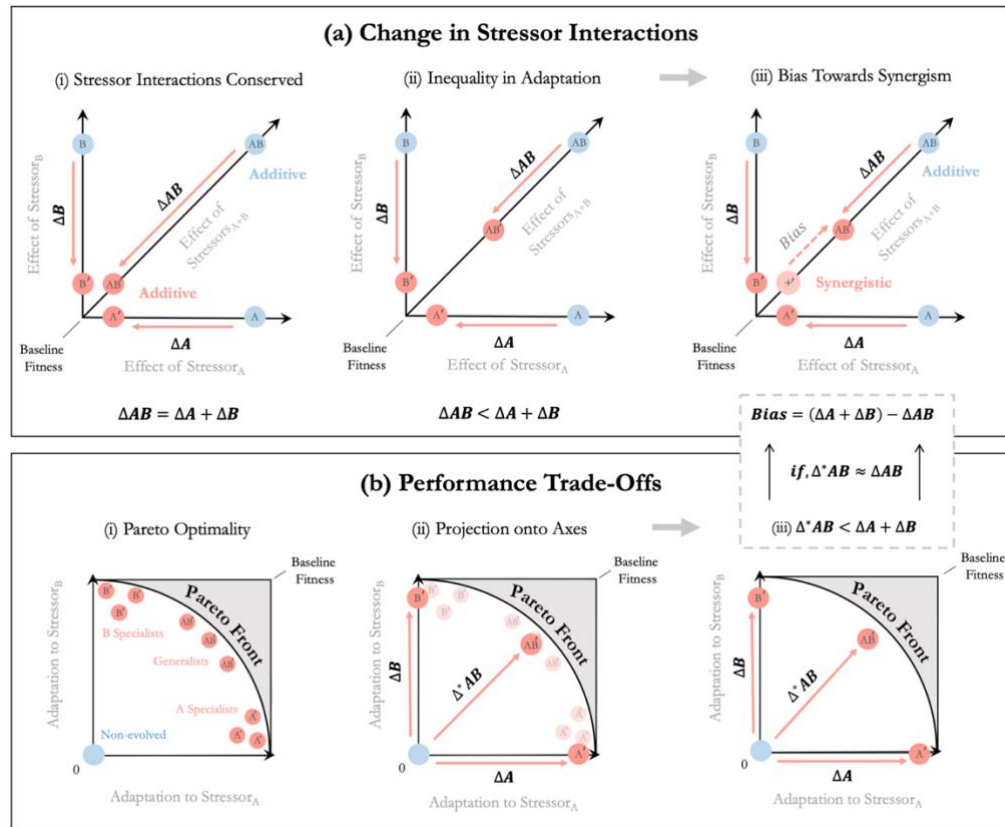


Figure 3.2: Performance trade-offs and a bias towards synergism. **(a)** The effects of two stressors, A and B, and their combined effect, AB, define three separate axes to visualize additivity between stressors. The effect of stressors on non-evolved (blue) and evolution (red) populations can only fall on these axes. Adaptation (i.e., reduction in effect of stressors) is represented as red arrows and is quantified by the difference (e.g., ΔA) between non-evolved (e.g., A) and evolved (e.g., A') populations. (i) If adaptation to multiple stressors is equal to the sum of the adaptation to individual stressors ($\Delta AB = \Delta A + \Delta B$) interactions between stressors (additive in this example) are conserved across evolution. (ii) However, if adaptation to multiple stressors is less than the sum of adaptation to individual stressors ($\Delta AB < \Delta A + \Delta B$), then (iii) interactions will shift towards synergism. The magnitude of this bias towards synergism is equal to the

difference between the adaptation to multiple stressors and the sum of the adaptation to individual stressors ($Bias = (\Delta A + \Delta B) - \Delta AB$). **(b)** Non-evolved populations and populations evolved to A, B or AB environments are plotted in performance space, where each axis is defined by the adaptation to an individual stressor. Populations can fall anywhere in this space. (i) If there is a performance trade-off, if populations cannot optimally adapt to multiple environments, evolved populations will be restricted to the pareto front, the convex hull of optimum strategies. (ii) Projecting the populations evolved to A or B onto their respective axes will recover the magnitude of adaptation to individual stressors ($\Delta A, \Delta B$). The taxi-cab distance in performance space between non-evolved populations and populations evolved to multiple stressors (Δ^*AB) is different to the actual adaptation to multiple stressors (ΔAB). (iii) A performance trade-off results in this distance being less than the sum of the adaptation to individual stressors ($\Delta^*AB < \Delta A + \Delta B$). If the distance in performance space between evolved and non-evolved states is roughly equal to the adaptation to multiple stressors ($\Delta^*AB \approx \Delta AB$) then a performance trade-off will generate a bias towards synergism.

Adaptation to multiple stressors (diagonal red arrows in Figure 3.3a) was always less than the sum of the adaptation to individual stressors (vertical and horizontal red arrows in Figure 3.3a). For example, populations adapted to copper or salt stressors regained all (C to C') or most (S to S') of the fitness lost due to the stressors, but fitness of population adapting simultaneously to both stressors (SC to SC') improved little (Figure 3.3a(i)). This inequality, between the magnitude of the diagonal red arrows and the sum of the magnitudes of the horizontal and vertical arrows in Figure 3.3a (e.g. $\Delta SC < \Delta S + \Delta C$), explains the shift towards synergism. In fact, as this inequality increases, the shift towards synergism also increases. The three-stressor combination had the greatest inequality between the adaptation to multiple stressors and the sum of the adaptation to individual stressors and therefore experienced the greatest shift towards synergism (steepest slope in Figure 3.1c). Plotting populations in performance space suggested the existence of pareto optimality for all multiple-stressor combinations except for salt + copper (Figure 3.3b). Furthermore, the actual adaptation to multiple stressors was similar to the distance between non-evolved and evolved populations in performance space (Figure 3.4).

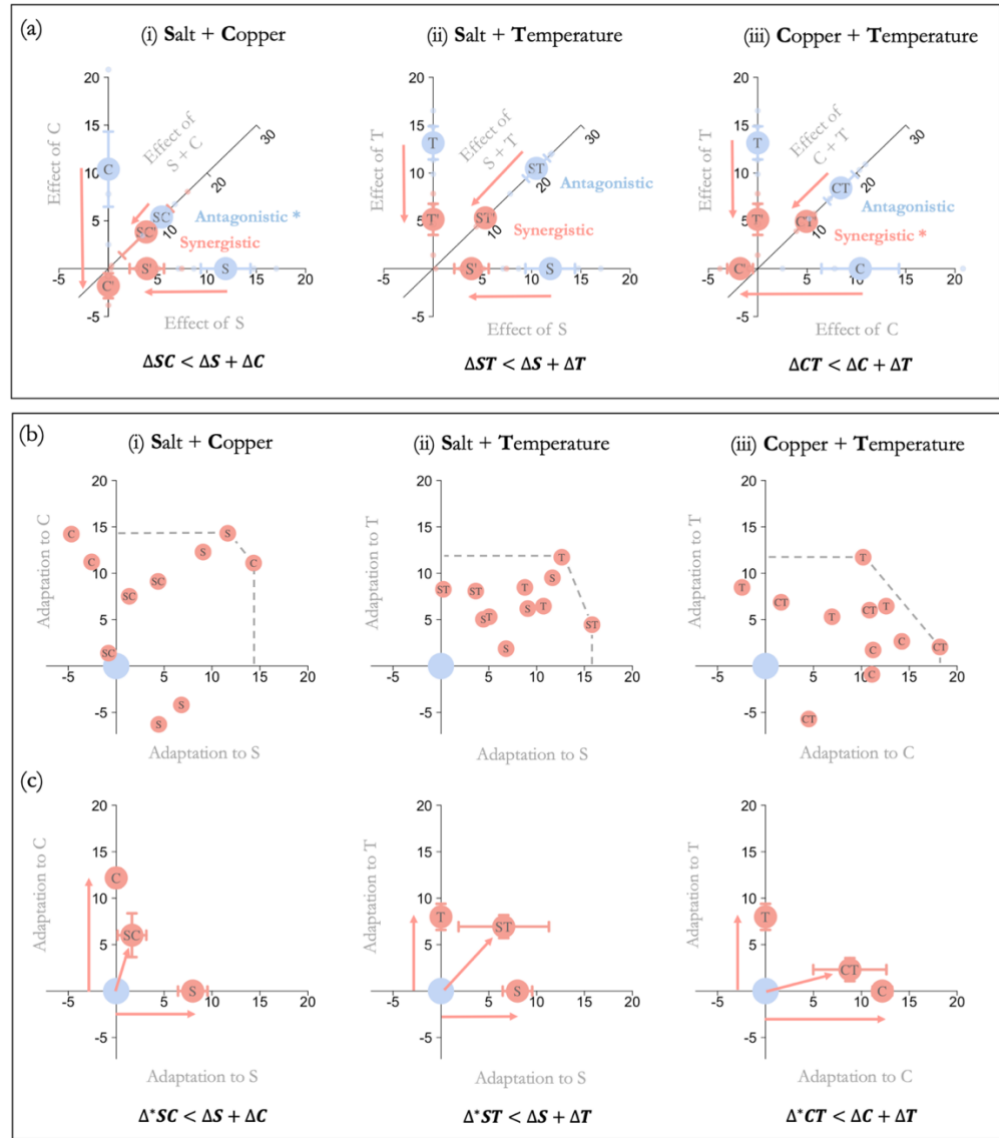


Figure 3.3: Empirical test of theoretical framework. **(a)** Population growth rate data for all pairs of stressors applied to the framework presented in Figure 3.2a. Effects of stressors are quantified by decrease in growth rates. Large points represent mean effects of stressors, error bars represent standard error and small points represent the effect of stressors on individual populations. For all pairs of stressors there is a bias towards synergism due to evolution (as in Figure 3.1c). **(b)** As per Figure 3.2b(i), populations that have evolved to one or both stressors are plotted in performance space, where axes are defined by the adaptation (change in growth rate) to individual stressor environments. Non-evolved populations (blue point) show no adaptation to stressors. Dashed lines indicate a potential Pareto front, where populations are expected to move towards if an evolutionary trade-off exists. **(c)** As in Figure 3.2b(ii), mean population growth rates with standard error are plotted and the populations evolved to individual stressors are projected onto their respective axis. For all pairs of stressors, the taxi-cab

distance in performance space between evolved and non-evolved states is less than the sum of the adaptation to individual stressors (e.g., $\Delta^*SC < \Delta S + \Delta C$).

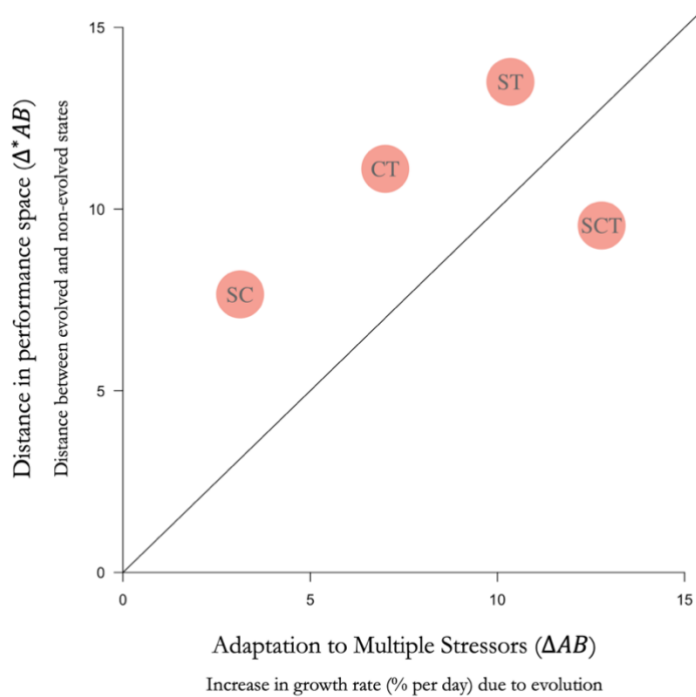


Figure 3.4: Test of additive adaptation. For each combination of multiple stressor (SC, ST, CT and SCT) the magnitude of adaptation (increase in growth rates) and the taxi-cab distance in performance space are plotted. The black diagonal line is the 1:1 line.

Removal of stressors from populations exposed to those stressors did not necessarily increase fitness (Figure 3.5). Growth rates of populations grown in absence of stressors were not significantly different from those where stressors were present ($t_{24} = 0.39$, $p = 0.699$). Moreover, when only considering populations that were exposed to one or two stressors, growth rates (% increase per day) of evolved rotifers decreased by 2.2% (95CI -0.2, +4.5) when stressors were removed, although this result was not statistically significant ($t_{20} = 1.88$, $p = 0.074$, note the contrasting pattern in Figure 3.5a for the triple stressor evolved line, which was excluded from this second analysis). Growth rates of the populations evolved to three stressors ($M = 5.4$, $SD = 1.9$) were not significantly impacted when the salt ($M = 7.2$, $SD = 3.3$, $p = 0.83$) or copper ($M = 2.7$, $SD = 2.9$, $p =$

0.59) stressors were removed, but their growth rates increased by $6.9 (\pm 2.5 \text{ SE})$ when the temperature stressor was removed ($M = 12.3$, $SD = 5.3$, $p = 0.046$, Figure 3.5b).

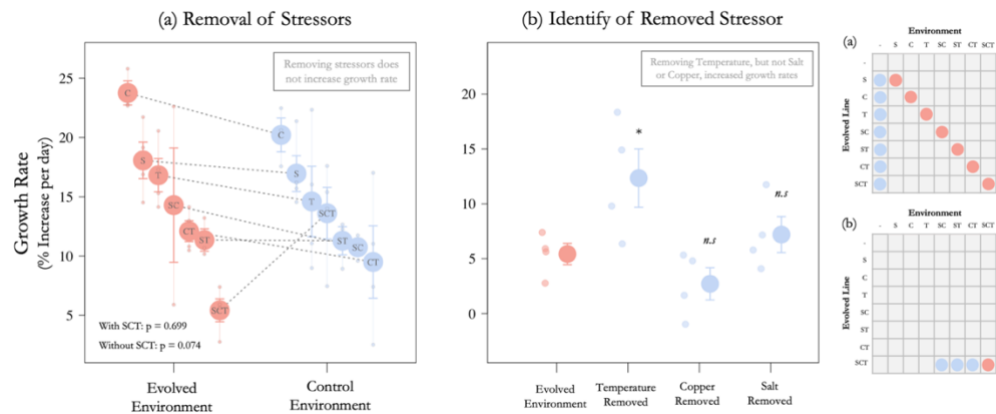


Figure 3.5: Removing stressors from evolved populations. **(a)** Mean growth rates of populations in their own environment (red) and in the control environment (blue) (i.e., when stressors have been removed). Small dots represent population replicates, error bars represent standard error and dashed lines connect each pair that is being compared. Removal of stressors does not increase growth rate. **(b)** Mean growth rates of populations evolved to all three stressors in their own environment and in all double stressor environments. Small dots represent population replicates and error bars represent standard error. Removal of temperature, but not copper or salt, increased growth rates (* = significant difference, *n.s.* = not significant). The comparisons being made in (a) and (b) are illustrated using the growth assay schema from Figure 3.1a.

3.5 DISCUSSION

In an evolution experiment with the rotifer *Brachionus calyciflorus* we found that rapid evolution to multiple stressors created an emergent bias towards synergistic stressor interactions. Because of the generality of the underlying mechanism, our results support the notion that contemporary evolution should be accounted for to accurately predict the impacts of global change. This synergism was independent of how the stressors themselves were interacting (e.g. via physico-chemical effects (Boyd & Brown, 2015)), but was instead, a consequence of an inequality between the adaptation to multiple

stressors and the sum of the adaptation to individual stressors. Crucially, this highlights the importance of having a mechanistic understanding of stressor interactions when making management decisions. This is particularly relevant when the management of local stressors is influenced by the identity of their interactions (antagonistic or synergistic) with global stressors such as climate change (Brown et al., 2013, 2014). Removal of a stressor should not be blindly prioritised based on the emergent synergism due to evolutionary trade-offs, but should rather be based on the magnitude of effects and on an understanding of the mechanisms of stressor interactions (Orr et al., 2020; Schäfer & Piggott, 2018). Furthermore, knowledge of how well adapted organisms are to existing stressors could inform decisions regarding the removal of stressors, as we found that removal of stressors from populations evolved to those stressors does not necessarily increase fitness. Evolution can change the optimum environmental conditions for populations meaning that removal of a stressor could potentially be a stressor itself. Our results reinforce the potential benefits of considering rapid evolution when studying how species and ecosystems are impacted by multiple stressors, and when predicting the ecological impacts of global change.

3.5.1 TEMPORAL VARIATION IN STRESSOR INTERACTIONS

Our main finding, that adaptation to multiple stressors generates synergism (question 1), is relevant to a scenario where factorial designs are employed, the standard approach in multiple-stressor research, where evolution to both multiple and individual stressors can be considered. In this context, our results suggest that as study duration increases, the probability of synergism also increases (until adaptation completely removes the effects of stressors). This corroborates the findings of a recent meta-analysis of the effects of multiple-stressors on fish (Lange et al., 2018). We also considered a second scenario, where evolution to multiple stressors, but not to individual stressors, is considered so the null models used to detect stressor interactions remain fixed over time (Appendix B.1). Although this is not how interactions between stressors are usually studied, it may be of general interest as it represents a situation where timeseries data is

collected in natural systems experiencing global change and may become relevant when comparing field and experimental studies of multiple stressors (e.g., Birk et al., 2020). Here, as the null models remain fixed (as the sum of the initial effects of stressors) but the combined effect of stressors is reduced over time due to adaptation, stressor interactions will be shifted in the opposite direction, towards antagonism. This may explain, in part, the results of a meta-analysis of terrestrial global change experiments, where effect sizes decreased with experimental duration (Leuzinger et al., 2011). In multiple-stressor research factorial designs are the rule, so the first scenario, where we generally predict a bias towards synergism over time, is more relevant. Nonetheless, we cannot rule out the possibility of adaptation to multiple stressors being greater than the sum of adaptation to individual stressors, which would bias stressor interactions towards antagonism (Appendix B.2). We reasonably expect, however, that such instances will be the exception rather than the rule (Agrawal et al., 2010; Barrett et al., 2005). It should be noted that these ideas are not specific to adaptation through evolution, but equally apply at smaller temporal scales when considering adaptation via phenotypic plasticity or epigenetic modifications (Fox et al., 2019; Rey et al., 2016; Scoville & Pfrender, 2010). Some meta-analyses have suggested that synergistic interactions take time to develop (Darling & Côté, 2008; Lange et al., 2018). Despite all of the context-dependence of multiple-stressor research, we have identified a general mechanism, adaptation to multiple stressors being less than the sum of the adaptation to individual stressors, that may explain this temporal trend.

3.5.2 PERFORMANCE TRADE-OFFS AND SYNERGISM

Our results support the theory that performance trade-offs created the inequality between the adaptation to multiple stressors and the sum of the adaptation to individual stressors (question 2). Performance trade-offs, visualized by pareto fronts, are seen for all combinations of stressors, except for salt + copper where some populations evolved to salt or copper have low adaptation in the other stressor environment but others have shown strong adaptation to both stressors (Figure 3.3b(i)). Given the high replication

within each population, this variation in adaptation is probably not related to sample size but instead, is caused by populations moving to different peaks within a fitness landscape (Wright, 1932). The similarity between adaptation to multiple stressors and the distance between evolved and non-evolved states in performance space (Figure 3.4), indicating that adaptation to multiple stressors can be approximately decomposed into the adaptation to one stressor and adaptation to the other stressor (additive adaptation), is why a link between performance trade-offs and emergent synergism can exist. In fact, performance trade-offs will not be relevant if the multiple-stressor environment is very different from a simple combination of the individual stressor environments, due to physico-chemical stressor interactions (Boyd & Brown, 2015). For example, consider two chemical stressors whose combination is effectively a third, novel chemical stressor (e.g. chemical interactions between multiple pesticides (Hernández et al., 2017)). This would typically result in adaptation to multiple stressors being greater than the distance between evolved and non-evolved states in performance space. If performance trade-offs do play a role in creating emergent synergism it is interesting to consider the consequences of increasing the number of stressors, and therefore increasing the dimensions of performance space and of pareto fronts themselves (Tikhonov et al., 2020). This is an idea that deserves further study, especially considering the recent interest in using the number of stressors, rather than their identity, to predict ecological and evolutionary responses to global change (Brennan et al., 2017; Komatsu et al., 2019; Rillig et al., 2019).

3.5.3 REMOVAL OF STRESSORS

Another key insight, potentially complicating ecosystem management, is that removal of stressors from evolved populations did not necessarily increase fitness (question 3). Evolution to stressors shifted populations' optimum environmental conditions leaving them maladapted to the original environment (Luijckx et al., 2017). A textbook example of adaptation to global change that mirrors our results is the rapid evolution of the peppered moth (*Biston betularia*) in response to the rise and fall of industrial pollution.

The frequency of melanistic peppered moths, which use their colouration as camouflage to avoid predation, rose with increasing levels of soot during the industrial revolution. As this stressor was removed during the 20th century the melanistic phenotype became rare as it was maladapted to the original environment (Cook et al., 2012; Kettlewell, 1958). Many studies have since explored the importance of considering adaptation to global change in the conservation and restoration of biodiversity (e.g., evolutionary rescue (Bell, 2017), adaptation to anthropogenic stressors (Rice & Emery, 2003; Stockwell et al., 2003), role of eco-evolutionary dynamics (Hendry, 2020)). Our study contributes to this interdisciplinary field by exploring the consequences of adaptation to co-occurring multiple stressors. Although multiple-stressor research primarily focuses on the effects of adding stressors, there have been some investigations into the effect of removing stressors (Brown et al., 2013; Falkenberg et al., 2013; Fong et al., 2018). However, to our knowledge none of these studies have considered the consequences of adaptation to stressors. In our study, we found that when populations had evolved to all three stressors the removal of different stressors had varying consequences. Fitness increased if the temperature stressor was removed (thus increasing temperature), which is unsurprising considering the relationship between temperature and metabolism (Brown et al., 2004). However, removal of the salt or copper stressors did not increase fitness (Figure 3.5b) as populations had successfully adapted to these new conditions. In this case, if ecosystem managers only had enough resources to remove one of the three stressors, having a mechanistic understanding of these stressors would be critical when prioritizing which stressor to remove. In addition, when removing stressors, (in particular temperature in our study) there was variation in fitness between populations that had evolved in the same environments (Figure 3.5b). This variation in the evolutionary trajectories of populations under the same conditions has been previously observed (Bennett & Lenski, 2007). The different paths evolution can take to reach peaks in fitness landscapes may add uncertainty to predictions of the long-term impacts of global change. Future multiple-stressor removal experiments, and

indeed management of multiple stressors, may be enhanced by taking adaptation to single and multiple stressors into account.

3.5.4 STUDY LIMITATIONS AND IMPLICATIONS FOR GLOBAL CHANGE

RESEARCH

Insights from evolutionary biology may be useful to global change ecologists, particularly considering the growing interest in the temporal dynamics of multiple stressors (Gunderson et al., 2016; Jackson et al., 2021; Orr et al., 2020; Ryo et al., 2019). From an evolutionary perspective, the ability of populations to adapt to stressors may be impacted by the timing of stressors. For instance, a recent mesocosm experiment showed that prior exposure to a stressor strongly predicted the evolutionary rescue of communities from that stressor at levels lethal to naïve communities (Fugère et al., 2020). In our study, stressors were applied simultaneously as constant press perturbations and the levels of environmental change were chosen to explore the role of evolution, rather than being based on specific global change scenarios. However, the rate of environmental change can have strong evolutionary and ecological consequences (Bell, 2017; Pinek et al., 2020). Indeed, it has been demonstrated theoretically and empirically that the probability of evolutionary rescue is higher when environmental change is gradual rather than abrupt as this allows time for advantageous mutations to arise (Bell, 2017; Lindsey et al., 2013). With mechanistic insights from evolutionary biology, future work could build on our proof of concept by investigating the extent of adaptation to more realistic stressor magnitudes and temporal regimes. Furthermore, our experimental design only allowed us to study the immediate effect of removing stressors. If given time to adapt, would these populations be able to evolve back to their original baseline fitness after the removal of stressors? This would depend in part on the available genetic variation. While stressors with large effects may impose greater selective pressures on populations and thus speed up evolution, large stressor impacts may also reduce population size and genetic variation (a genetic bottleneck) which may impede further adaptation. Moreover, available genetic variation would be higher if

adaptation to previous stressors occurred from standing genetic variation (soft selective sweep) than from a newly arisen mutation (hard selective sweep) (Burke, 2012; Payne & Wagner, 2019; Pedrosa et al., 2017). Similarly, genetic variation may be maintained at higher levels for local stressors or those that only occur periodically as genetic variation is maintained at a higher level in spatially or temporarily varying environments (Huang et al., 2016). Theories from evolutionary biology are clearly relevant to multiple-stressor research and may improve understanding and prediction of how populations will respond to future global change scenarios.

Our theoretical and empirical results, although based on a single-species evolution experiment, potentially have ramifications for the conservation of entire ecosystems facing global change. Rapid evolution will occur for species with short generations and fast turnover, such as bacteria, algae and the rotifers that we studied (Baym et al., 2016; Luijckx et al., 2017). These organisms are the foundations of food webs and their adaptation to stressors will have knock-on consequences for the larger, more charismatic species (Hiltunen et al., 2017; Jones & Ellner, 2007) that conservation and restoration projects are often focused on (Colléony et al., 2017). It is, however, unclear whether adaptive evolution to stressors would be as strong when organisms are entangled in a web of biotic interactions. Indeed, adaptation of bacteria to environmental change is restricted in complex communities (Scheuerl et al., 2020). Would the emergent synergism caused by adaptation to multiple stressors still exist when considering communities of species? Nonetheless, understanding the population-level consequences of rapid evolution to multiple stressors is required for accurate, mechanistic predictions of community-level responses to environmental change. This paper has raised a number of outstanding questions, that sit at the interface between ecology, evolution and global change biology, which we summarise in Table 3.1. Our results have revealed the importance of considering adaptation to global change when understanding and predicting the ecological impacts of multiple stressors.

Table 3.1: Outstanding questions at the interface between evolution and multiple-stressor research.

Q1	Does the emergent bias towards synergism created by evolutionary trade-offs, become stronger as the number of stressors increases?
Q2	Is removal of stressors based on their interaction types (antagonism/synergism) an effective strategy when adaptation to stressors has occurred?
Q3	If the stressors that populations have adapted to are removed, how long will it take for populations to adapt back to their original baseline fitness?
Q4	How much variation is there in the evolutionary trajectories of populations that have experienced the same stressor regimes?
Q5	Is adaptation to multiple stressors important when considering communities of species? How does adaptation to stressors scale up when considering ecosystem-level properties (diversity, function)?
Q6	How does environmental complexity influence evolvability? Is environmental complexity more closely approximated by the number of stressors or by some form of stressor diversity?

CHAPTER 4 | SCALING UP UNCERTAIN PREDICTIONS TO HIGHER LEVELS OF ORGANISATION TENDS TO UNDERESTIMATE CHANGE

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AUTHOR CONTRIBUTION:

I carried out the simulations, produced the figures and wrote the manuscript with Jean-François Arnoldi, who developed the theoretical framework. All authors contributed to the final manuscript.

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A version of this manuscript was published in *Methods in Ecology and Evolution* in May 2021 (Orr, Piggott, et al., 2021).

4.1 ABSTRACT

Uncertainty is an irreducible part of predictive science, causing us to over- or underestimate the magnitude of change that a system of interest will face. In a reductionist approach, we may use predictions at the level of individual system components (e.g. species biomass), and combine them to generate predictions for system-level properties (e.g. ecosystem function). Here we show that this process of scaling up uncertain predictions to higher levels of organization has a surprising consequence: it tends to systematically underestimate the magnitude of system-level change, an effect whose significance grows with the system's dimensionality. This stems from a geometrical observation: in high dimensions there are more ways to be more different, than ways to be more similar. We focus on ecosystem-level predictions generated from the combination of predictions at the species-level. In this setting, the ecosystem's relevant dimensionality is a measure of its diversity (and not simply the number of species). We explain why dimensional effects do not play out when predicting change of a single linear aggregate property (e.g. total biomass), yet are revealed when predicting change of non-linear properties (e.g. absolute biomass change, stability or diversity), and when several properties are considered at once to describe the ecosystem, as in multi-functional ecology. As an application we discuss the consequences of our theory for multiple-stressor research. This empirical field focuses on interactions between stressors, defined as the error made by a prediction based on their observed individual effects. Our geometric approach can be visualized and explored via the web application (<https://jamesaorr.shinyapps.io/scaling-up-predictions/>). Our findings highlight and describe the counter-intuitive effects of scaling up uncertain predictions, effects that can occur in any field of science where a reductionist approach is used to generate predictions.

4.2 INTRODUCTION

In natural sciences, uncertainty of any given prediction is ubiquitous (Dovers & Handmer, 1992). When considering predictions of change, uncertainty has directional consequences: uncertain predictions will lead to either over- or underestimation of actual change. The reductionist approach to complex systems is to gather and use knowledge about individual components before scaling up predictions to the system-level (Levins & Lewontin, 1985; Wu et al., 2006). Although scaling up to higher levels of organisation is general to the study of any complex systems, it is particularly well-defined in ecology. In this field, knowledge about the components at lower levels of organisation (individuals, populations) is commonly used to understand the systems at higher levels of organisation (communities, ecosystems) (Loreau, 2010; Woodward et al., 2010). An unbiased prediction of an individual component is one that makes no systematic bias towards over- or underestimation for that component (Table 4.1). But what happens when we scale up unbiased predictions to higher levels of organisation? If we do not systematically underestimate the change of individual components, will this still be true when considering many components at once? When addressing this question, one must be wary of basic intuitions as the problem is inherently multidimensional, thus hard to properly visualize.

As a thought experiment, consider two ecological communities, one species-poor (low dimension) and the other species-rich (high dimension). Both communities experience perturbations that change species biomass, and we assume that we have an unbiased prediction for this change, up to some level of uncertainty. We then scale up our predictions to the community-level, focusing on the change in Shannon's diversity index, caused by the perturbations. By comparing predicted and observed change we can quantify the degree of underestimation of our predictions, at the species and community-level. If we simulate this thought experiment (Figure 4.1a and Appendix C.3) we observe the following puzzling results, which motivate our subsequent analysis. Predictions of species biomass change may be unbiased (bottom row of Figure 4.1a), but when scaled up to the system level for the species-rich community, but not the

species-poor community, we see a clear bias towards underestimation of change (top right corner of Figure 4.1a). In fact, we can generalize this in silico experiment and see that for various ways of measuring change (Euclidean distance, difference in Shannon's diversity index, difference in invariability of total biomass), but not for others (difference in total biomass), this bias towards underestimation grows with species richness, i.e., with the dimension of the system (Figure 4.1b).

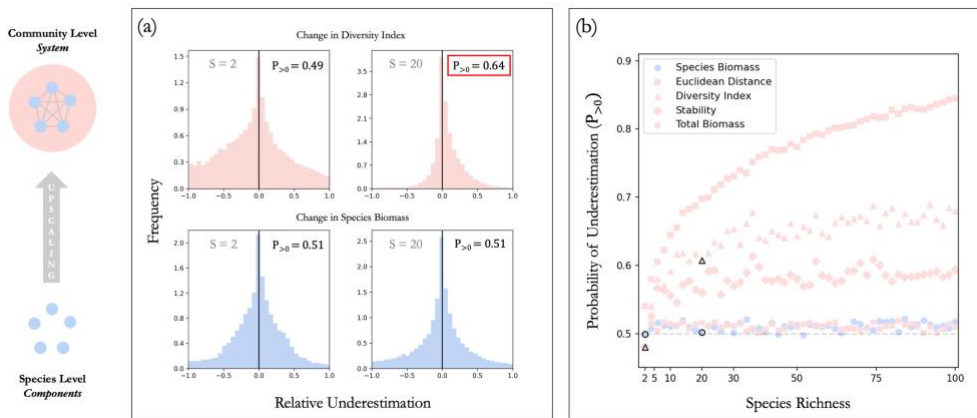


Figure 4.1: Scaling up predictions for two ecological communities. **(a)** Simulated communities of 2 species (left) and 20 species (right) experienced many simulated perturbations (change in species biomass), for which we assume unbiased predictions at the species-level. Uncertainty around those predictions is simulated as random terms of zero mean, independent across species. Histograms show the distribution of relative underestimation, defined as the difference between realized and predicted change expressed relatively to the predicted magnitude of change. The probability of underestimation, the probability of relative underestimation being positive, is shown in each plot. By construction, there is no bias towards underestimation at the species level (bottom row). We then scale up our predictions to the community level to generate predictions for Shannon's diversity index (top row). For the first, species poor community, this upscaling does not generate any bias. However, for the species rich community a bias emerges as 64% of realizations show an underestimated magnitude of change. **(b)** An extension of these simulations to many different communities ranging from 2 to 100 species shows that this bias towards underestimation grows with the dimensionality of the system for some aggregate properties (Euclidean distance, diversity index, stability measured as invariability of biomass) but not others (total biomass). The four points outlined in black correspond to the four histograms in part (a).

As we shall explain in depth, the reason for this emergent bias is that in high dimensions there are more ways to be more different, than ways to be more similar. Our goal is to make this statement quantitative and generally relevant to ecological problems. We start from a geometric approach showing that, in two dimensions, our claim can be visualized to reveal a positive relationship between magnitude of uncertainty and underestimation of change. Visualization is only possible in low dimensions, but a more abstract reasoning demonstrates that as dimensionality increases so does the bias towards underestimation, which is further strengthened by larger uncertainty. We note that dimensionality is not necessarily an integer value. We propose that the effective dimensionality most relevant to ecological upscaling of predictions is not the number of species, but instead is a specific diversity metric, the Inverse Participation Ratio (IPR) (Suweis et al., 2015; Wegner, 1980), comparable (but not equivalent) to Hill's diversity indices (Hill, 1973).

We then explain why the effect of dimensionality depends on how change is measured at the system level (Table 4.1). If a single linear function is used to aggregate components (e.g., total biomass), dimensionality has no effect. An unbiased prediction for individual components trivially scales up to produce an unbiased system-level prediction. But this is not true in general. Non-linear functions (e.g., Shannon's diversity index as in Figure 4.1), can remain sensitive to dimensional effects. Predictions of change of these properties, even if constructed from unbiased predictions of individual components can be systematically underestimated. The significance of this effect will depend on the relative significance of non-linearities in the function of interest. On simulated examples we will examine the behaviour of common ecosystem-level properties: diversity, stability and total biomass (the archetypal measure of ecosystem functioning (Loreau et al., 2001)). More generally, we emphasize that dimensional effects will occur as soon as system-level change is measured as a change in multiple properties at once (whether they are linear or not), as is the case in multi-functional descriptions of ecosystems (Manning et al., 2018).

As a seemingly different kind of ecological case-study, we then revisit core questions of multiple-stressor research in the light of our theory. In this field, there is a clear prediction (additivity of stressor effects), a high prevalence of uncertainty about the way stressors interact (resulting in non-additivity) and, ultimately, great interest in the ecosystem-level consequences of non-additive stressor interactions (synergism or antagonism) (Côté et al., 2016; Jackson et al., 2016; Piggott et al., 2015). Expressed in this context, our theory predicts the generation of bias towards synergism when multiple-stressor predictions are scaled up to higher levels of organisation.

Research has primarily focused on the causes of uncertainty, working hard to reduce it (Petchey et al., 2015). Here we take a complementary approach by investigating the generic consequences of uncertainty, regardless of the nature of the system studied or the underlying causes of uncertainty. Our theory becomes more relevant as the degree of uncertainty increases, which makes it particularly relevant for ecological problems. But, in fact, our findings could inform any field of science that takes a reductionist approach in the study of complex systems (e.g., economics, energy supply, demography, finance – see Appendix C.2), demonstrating how dimensional effects can play a critical role when scaling up predictions.

Table 4.1: Lexicon of concepts for Chapter 4

Reductionist view of complex systems

- *Components*: Individual variables B_i that together form a system (e.g. biomass of S species and abiotic compartments forming an ecosystem).
- *System state*: Point in *state space*, represented as a vector $B = (B_1, \dots, B_S)$ jointly describing all system components.
- *Difference (or magnitude of change) between states*: the Euclidean distance $\|B - B'\|$ between two joint states B and B' .

Scaling up uncertain predictions

- *Relative error*: Magnitude of error caused by uncertainty relative to the magnitude of predicted change.
- *Aggregate system-level property*: Scalar function of the joint state (e.g. total biomass or diversity index) – *Linear aggregate property*: Linear function of joint state variables (e.g. total biomass).
– *Non-linear property*: Non-linear function of joint state variables (e.g. diversity index).
- *Scaled up prediction*: A prediction made for the joint state, or a scalar property of the joint state, based on individual predictions for components.
- *Unbiased prediction*: A prediction that, despite uncertainties, does not systematically overestimate or underestimate the magnitude of change (of a joint state, a system component or an aggregate property).

Multi-functional view of complex systems

- Multivariate description of a complex system, based on multiple aggregate properties, or *functions* (production, diversity, respiration) instead of individual components (species biomass and abiotic compartments). The state of the system is the joint state $F = (F_1, \dots, F_{SF})$ of S_F functions. Difference between states is the distance between two joint functional states F and F' .

4.3 GEOMETRIC APPROACH

The central claim of this article is that *in high dimensions there are more ways to be more different, than ways to be more similar*. We propose an implication: a system-level prediction based on unbiased predictions for individual components, tends to underestimate the magnitude of system-level change.

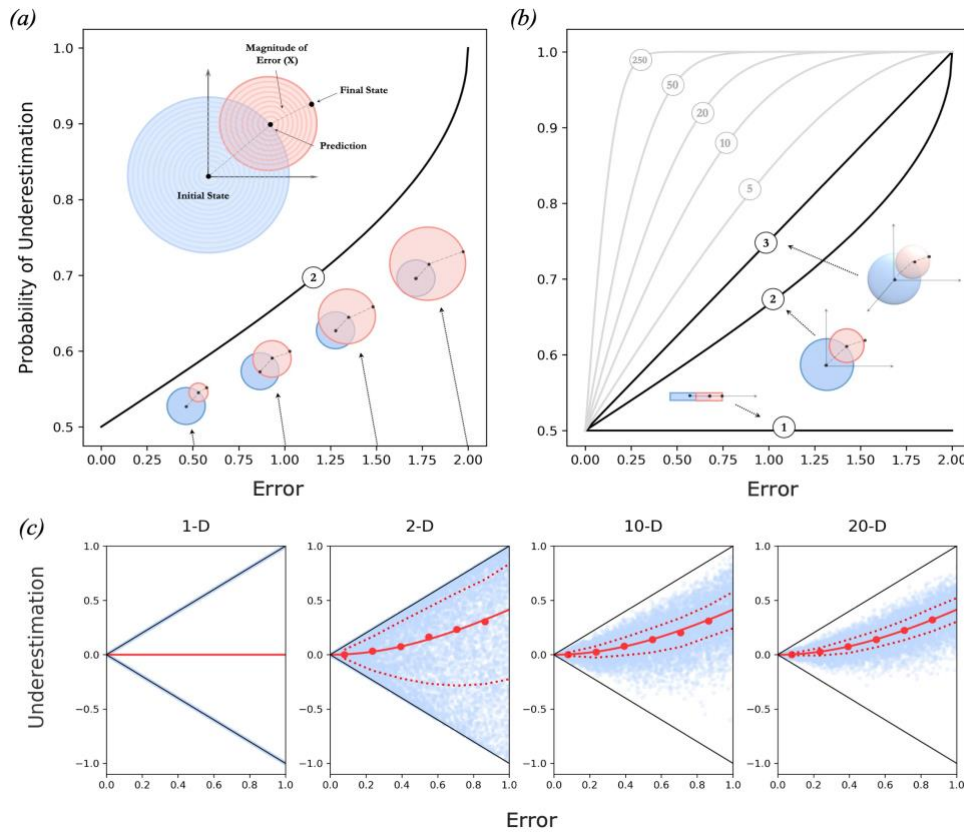


Figure 4.2: Geometric approach. **(a)** Already in two dimensions, the probability of underestimation increases as uncertainty increases. The centre of the blue circle is the initial state (its actual value is irrelevant) and its radius is defined by the predicted magnitude of change. The point at the centre of the red circle corresponds to the predicted state, while its radius represents the magnitude of error made by the prediction. By definition, final states thus fall on the edges of the red circle. If a final state falls inside the blue circle, then there has been an overestimation of change (it is closer to the initial state than what was predicted). If a final state falls outside the blue circle (as in the figure) then there has been an underestimation of change (it farther from the initial state than what was predicted). When uncertainty is small, error will be small thus the radius of the red circle is small, and the probability of underestimation is close to 0.5. As uncertainty (thus error) increases, however, there is increasing bias towards underestimation. Eventually when error is twice as large as the prediction only underestimation is possible. **(b)** This relationship between uncertainty and underestimation is strengthened by dimensionality. As dimensionality increases there becomes even more ways to be more different than ways to be more similar. Each curve corresponds to the probability of underestimation as a function of error for different dimensions labelled as circled numbers. For a fixed amount of error, the probability of underestimation will increase with dimension. **(c)** The relationship between the relative magnitude of error (x) and the relative magnitude of underestimation (y) based on uniform sampling of 1-D, 2-D, 10-D and 20-D intersecting hyper-spheres defined by

unbiased but uncertain predictions. The boundaries of this relationship are plotted in black and the median expectation $\tilde{y} = \sqrt{x^2 + 1} - 1$ as derived from Eq. 4.4 is plotted in red (except for 1-D where it does not apply). Blue points are simulated results, red points are the actual median values and dashed lines show the quantiles for vertical subsets of the simulated data. As dimensionality increases the width of the distribution decreases and converges towards its median, which effectively increases the probability of underestimation (b).

To understand these statements, it is useful to take a geometrical approach to represent the classic reductionist perspective, starting in two dimensions (Figure 4.2a). Picture two intersecting circles in a system's state-space (one blue, one red in Figure 4.2). The first, blue circle is centered on the system's initial state and its radius corresponds to the predicted magnitude of change. The second, red circle is centered at the predicted state (which lies on the blue circle) and its radius corresponds to the magnitude of realized error of the prediction, in other words, the realized outcome of the uncertainty associated with the prediction (red circle in Figure 4.2). The actual final state is thus somewhere on that red circle. If it falls inside the blue circle, the prediction has overestimated the magnitude of change. If, however, it falls outside the blue circle, the prediction has underestimated the magnitude of change. The proportion of the red circle lying outside of the blue circle measures the proportion of possible configurations that will lead to an underestimation of change. In other words, for a given magnitude of error caused by uncertainty, this portion of the circle represents the states that are more different from the initial state than predicted. As the relative magnitude of error increases (as the red circle's diameter becomes larger, relative to that of the blue circle) this proportion grows (Fig. 4.2a).

In three dimensions these two intersecting circles become two intersecting spheres. The proportion of interest is the surface of the spherical cap lying outside of the sphere centered on the initial state. Here, a non-intuitive phenomenon occurs: with the same radii as in the 2D case, in 3D there are now more configurations leading to underestimation. As dimensions increase this proportion increases, until the vast

majority of possible states now lie in the domain where change is underestimated (Figure 4.2b). This result can be made quantitative from known expressions for the surface of hyper-spherical caps. This gives us an analytical expression for the proportion of configurations leading to an underestimation of change, as a function of the relative magnitude of error (x) and dimension (S):

$$P_{>0}(x) = 1 - \frac{1}{2} I_{1-\frac{x^2}{2}} \left(\frac{S-1}{2}; \frac{1}{2} \right) ; \quad x = \frac{\|error\|}{\|prediction\|} \quad (4.1)$$

In the above equations $\| \cdot \|$ stands for the standard Euclidean norm of vectors, and $I_s(a, b)$ is the cumulative function of the β -distribution. This is what we mean by *in high dimensions there are more ways to be more different, than ways to be more similar*. To see how this relates to the scaling up of unbiased predictions of individual components (Table 4.1), we now take a statistical approach. Suppose we uniformly sample the intersecting circles, spheres and hyper-spheres defined above and drawn in Figure 4.2. The proportion (Eq. 4.1) becomes a probability, the probability of having underestimated change. This uniform sampling is precisely what happens if the uncertainty of individual variables are independent random normal variables with zero mean. This justifies our claim: a system-level prediction based on unbiased predictions for individual components, tends to underestimate the magnitude of change of the system state.

This reasoning is geometrical and relies on a computation of the surface of classic shapes such as hyper-spheres and spherical caps. But the core mechanism behind the behaviour of the probability of underestimation is more general and, in a sense, simpler. To see that, let us take a step back and analyse the relative magnitude of underestimation, defined as:

$$y = \frac{\|response\| - \|prediction\|}{\|prediction\|} \quad (4.2)$$

Given an angle θ between prediction and error vectors (resp. the vectors that point from initial to predicted state, and from predicted state to realized state) we can rearrange (Eq. 4.2) as:

$$y(x, \theta) = \sqrt{x^2 + 2x \cos(\theta) + 1} - 1 \quad (4.3)$$

the term $\cos(\theta)$ can take any values between -1 and $+1$. For the sake of simplicity, in what follows we will suppose that its mean and median are zero. This is the case if the errors associated with individual variables are drawn from independent symmetric distributions centered on zero (unbiased and unskewed predictions at the component level). In this case the median relationship between error (x) and underestimation (y) is:

$$\tilde{y} = \sqrt{x^2 + 1} - 1 \quad (4.4)$$

which is strictly positive as soon the error x is nonzero. This holds true in all dimensions greater than one, which can be seen in Figure 4.2c. The median underestimation $\tilde{y}$ does not depend on dimension, but the probability of underestimation, $\mathbb{P}(y \geq 0 : x)$, does. Indeed, $\mathbb{P}(y \geq 0 : x)$ is driven by the distribution of the random term $\cos(\theta)$ in (Eq. 4.3). If this distribution is narrow, realisations of y will fall close to $\tilde{y}$. Because the latter is positive and increases predictably with x , so will the probability of any realised y to be positive. Our framework assumes that the difference between two states, regardless of the system's dimensionality, is described by a single number. For multi-dimensional systems this allows for the counter-intuitive scenario where an uncertain prediction can nonetheless accurately predict the magnitude of system-level change.

A known fact from random geometry is that, given a random isotropic vector (i.e., a vector whose direction is uniformly distributed on the sphere), its angle θ with any other given vector satisfies:

$$\mathbb{E}(\cos \theta) = 0 ; \text{ and } \text{Var}(\cos \theta) = \frac{1}{S} \quad (4.5)$$

In other words, in high dimensions random vectors are approximately orthogonal, up to a variance inversely proportional to the dimension of state-space. In our context, this corresponds to normal i.i.d. distributions of errors, a particular case of independent unbiased and unskewed predictions. This explains why the probability of underestimation increases in Figure 4.2b with both dimension S and error x . In what follows we use the expression for the variance in (Eq. 4.5) as a definition of effective dimension. In doing so, we can free ourselves from the strict Euclidean representation of Figure 4.2 and generalize the theory beyond i.i.d. normal error distributions. This will be useful when applying our theory to ecological problems, where components are the biomass of species, are their contribution to ecosystem change are not equivalent, thus errors not i.i.d. Our geometric approach can be explored using the web application at <https://jamesaorr.shinyapps.io/scaling-up-predictions/>.

4.4 RELEVANCE TO ECOLOGY

4.4.1 EFFECTIVE DIMENSIONALITY

We now assume that the axes that define state-space represent the biomass of the species that form an ecological system. These species may have very different abundances, and thus will not all contribute equally to a given change. For instance, in response to environmental perturbations, biomass of species typically change in proportion to their unperturbed values (Arnoldi et al., 2018; Lande et al., 2003). The more abundant species (in the sense of higher biomass) will thus likely contribute more to the ecosystem-level change. Thus, if we use species richness as a measure of dimensionality, as the above section would suggest, we will surely exaggerate the importance of rare (i.e., low biomass) species. But using (Eq. 4.5) to define dimensionality, we can resolve that issue. In doing so we show that the relevant dimension when applying our ideas to ecological problems is really a measure of diversity of the community prior to the change, which may not be an integer, and will typically be smaller than the number of individual

components. In fact, if a species contribution to change is statistically proportional to its biomass B_i the effective dimensionality of a system is the Inverse Participation Ratio (IPR) of the biomass distribution, which reads:

$$IPR = \frac{(\sum_{i=1}^S B_i^2)^2}{\sum_{i=1}^S B_i^4} \quad (4.6)$$

This non-integer diversity metric was developed in quantum mechanics to study localisation of electronic states (Wegner, 1980). The IPR approaches 1 when a single species is much more abundant than the others, and approaches S when species have similar abundance – see Suweis *et al.* (2015) where this metric is used in an ecological context. Note that the IPR is closely related (but not equivalent) to Hill (1973)’s evenness measure ${}^2D = (\sum_i B_i)^2 / \sum B_i^2$. We can show that it is indeed the IPR that determines the variance (over a sampling of predictions and associated uncertainties of species biomasses) of the term $\cos \theta$ in (Eq. 4.3) so that:

$$Var(\cos \theta) = \frac{1}{IPR} \quad (4.7)$$

An uneven biomass distribution thus increases the width of the distribution of underestimation y therefore reducing the probability of a given realisation of change to have been underestimated. If species richness accurately predicted the width of the distribution of underestimation and therefore the probability of underestimation, the two simulated communities in Figure 4.3 would behave in the same way. However, the probability of underestimation is lower than expected based on richness, particularly for the community with a more uneven biomass distribution. Indeed, replacing richness S by the IPR in (Eq. 4.1) provides an excellent approximation of the behaviour of the probability of underestimation (Figure 4.3).

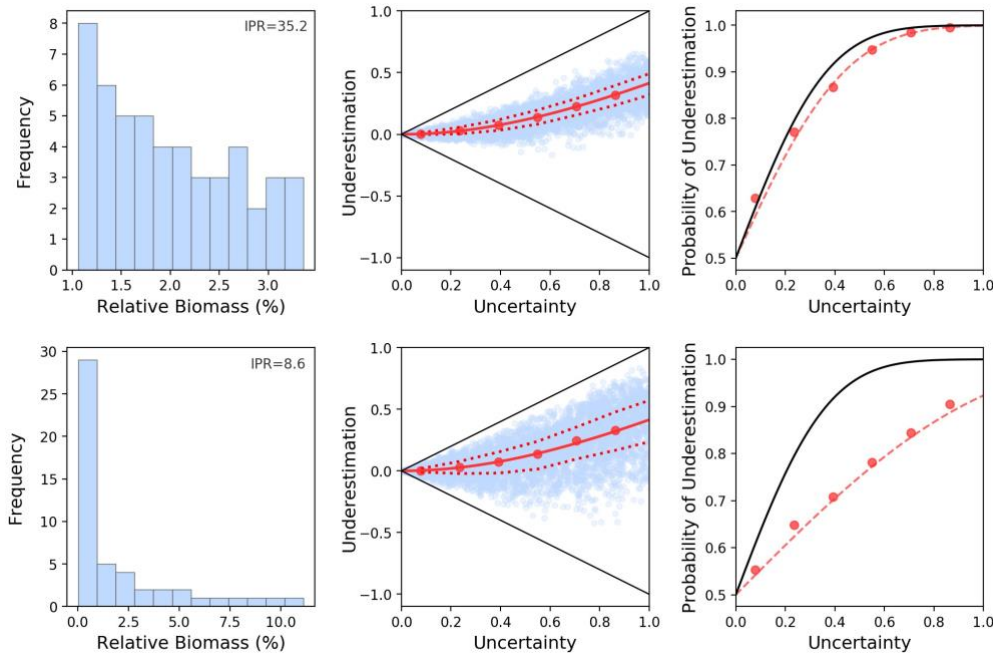


Figure 4.3: Effective dimensionality predicted by the IPR. Each row corresponds to simulations of 50 species communities with uneven biomass distributions that have experienced perturbations. The first column shows the biomass distributions of these communities. The two communities have IPR, and therefore effective dimensionality, of 35.2 and 8.6. The second column shows the relationship between error and underestimation of these two communities when unbiased predictions of biomass change are scaled up to change in state-space distance. As the biomass distribution becomes more uneven the variability around the median underestimation increases (dashed lines are quantiles), which effectively reduces the probability that a given change was underestimated. This can be seen in the third column where predictions using the dimension of state-space (50, black curves) are outperformed by predictions using the IPR (35.2 and 8.6, red curves). Red points show the actual probabilities of underestimation for vertical subsets of the simulated data and are accurately predicted using the IPR.

4.4.2 AGGREGATE PROPERTIES AND NON-LINEARITY

When scaling up predictions, there are different ways of measuring system-level change (Table 4.1). The classic reductionist approach is to quantify change via the Euclidean distance in state-space, thus keeping track of the motion of joint configurations. This is what we have done so far. Ecologically, this could correspond to measuring the absolute

biomass change of a community. Here, by construction, our theory is directly relevant. But other, non-reductionist, ways of quantifying change at the system-level are possible. In ecology, this could correspond to measuring changes in the diversity, stability or functioning of the ecosystem. Yet, if differences in these properties between two states correlate with the distance in the reductionist state-space, then our theory will remain relevant. As can be seen in Figure 4.4 this can be the case for diversity (Shannon's index) and stability (invariability of total biomass (Haegeman et al., 2016)). Our theory thus applies to those ecosystem-level properties. This leads us to the conclusion that their degree of change may be systematically underestimated by predictions built from species-level predictions.

On the other hand, changes of total biomass (ecosystem functioning) do not correlate well with changes in state-space Euclidean distance. This is due to the fact that total biomass is a linear function of species biomass (i.e., the sum). In fact, quantifying system-level change via a linear function acts as a projection from the state space onto a one-dimensional space defined by the function. Thus, despite the fact that the ecosystem might be constituted of many species (intrinsically high dimensional) the problem of scaling up predictions is essentially one dimensional. This is why bottom-up predictions of change of total biomass show no additional bias towards underestimation. More generally, when the linear part of the aggregate property of interest is dominant, dimensional effects are obscured. However, as soon as we consider changes of multiple properties at once, as in multi-functionality approaches in ecology (Table 4.1), dimensional effects will play out – even if all aggregate properties are essentially linear.

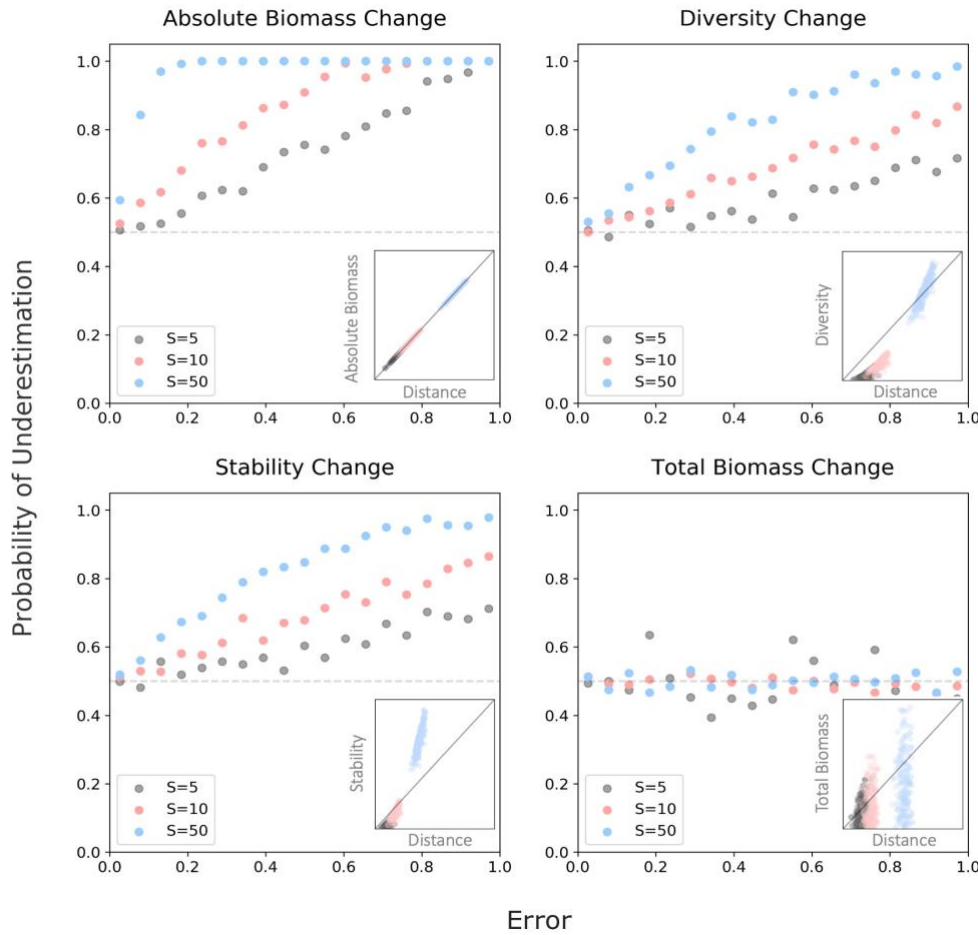


Figure 4.4: Behaviour of aggregate properties. Simulated communities of 5 (grey), 10 (red) and 50 (blue) species experienced some change in their biomass. Unbiased predictions of species' biomass change were scaled up to predictions of change in aggregate properties commonly used in ecological research. The relationship between uncertainty and the probability of underestimation is shown for changes in: (1) absolute biomass, (2) diversity, specifically the Shannon index, (3) stability, specifically invariability and (4) total biomass. Subplots show the relationship between changes in each aggregate property and changes in Euclidean distance. Absolute biomass change is analogous to Euclidean distance. Diversity and stability (non-linear functions) show some correlation with Euclidean distance and are therefore sensitive to dimensional effects. Total biomass (linear function) does not correlate with Euclidean distance so scaled up predictions of change of this aggregate property remain unbiased.

4.4.3 MULTI-FUNCTIONALITY

Scaling up predictions from individual components to an aggregate property can lead to a bias towards underestimation, due to dimensional effects. We explained that this occurs for non-linear aggregate properties, but not linear ones (such as total biomass). Is this to say that our theory is only relevant when predicting the change of non-linear system-level properties? Yes, but only in the restricted realm of one-dimensional approaches to complex systems.

Using a single function, for example a diversity index, to describe an ecosystem reduces the complexity of the system down to a single number and will potentially hide important details (Hurlbert, 1971). Consequently, there is a growing interest in multi-functionality approaches in ecology (Manning et al., 2018). These approaches are multivariate descriptions of ecosystems, an alternative to the reductionist perspective to account for the multidimensional nature of ecological systems (Table 4.1). By considering the change of multiple functions at once, even if these functions are essentially linear, dimensional effects will resurface.

To be clear, we still assume that we scale up predictions from the species to the ecosystem level. Only now we scale up predictions from species to several system-level properties at once, that describe the ecosystem's state from a multi-functional point of view. Let us suppose, for simplicity, that those aggregate properties (or functions) are linear. We have seen that considering a single linear function, in terms of upscaling of predictions, effectively reduces the problem to a single dimension. Likewise, considering multiple linear functions essentially reduces the effective dimensionality to the number of functions. Subtleties arise when the number of functions (S_f) and the dimensionality of the underlying system (e.g., IPR) are similar, and/or if the considered functions are colinear. For S_f independent functions measured on a community we find that the effective dimensionality (the one that determines the probability of underestimation of change) is:

$$S_{eff} \approx \frac{1}{\frac{1}{IPR} + \frac{1}{S_f}} \quad (4.8)$$

For example, if the change of an ecosystem with an IPR of 10 is measured using 10 linear functions at once, the effective dimensionality is ~ 5 (Appendix C.1). If functions are colinear the effective dimensionality will be even lower than S_f . This is to be expected, especially when thinking of an extreme case: if we measure the same function multiple times, we should see no dimensional effects. In summary, in a multivariate description of complex systems, dimensional effects will inevitably play out, in more or less intricate ways, whenever a prediction is scaled up from individual components to the system-level.

4.5 DISCUSSION

Our work demonstrates that a bias towards underestimation of change emerges when unbiased predictions of individual components (e.g., species biomass) are scaled up to the system-level (e.g., ecosystem function). Our geometric approach reveals a direct relationship between the probability of underestimation, the magnitude of error caused by uncertainty and a system's effective dimensionality. This effective dimensionality is not necessarily the number of individual components that form a system, but rather a measure of diversity *sensu* Hill (1973). In essence, these results come from the fact that in high dimensions there are more ways to be more different, than ways to be more similar (Figure 4.5). Importantly, it is non-linear aggregate properties (e.g., absolute biomass change, stability or diversity) that are sensitive to dimensional effects (Figure 4.5). For linear properties (e.g., total biomass), scaling up does not generate bias. Yet, even in this case, dimensional effects do play out when several functions are considered at once to describe the ecosystem, as in multi-functional approaches in ecology.

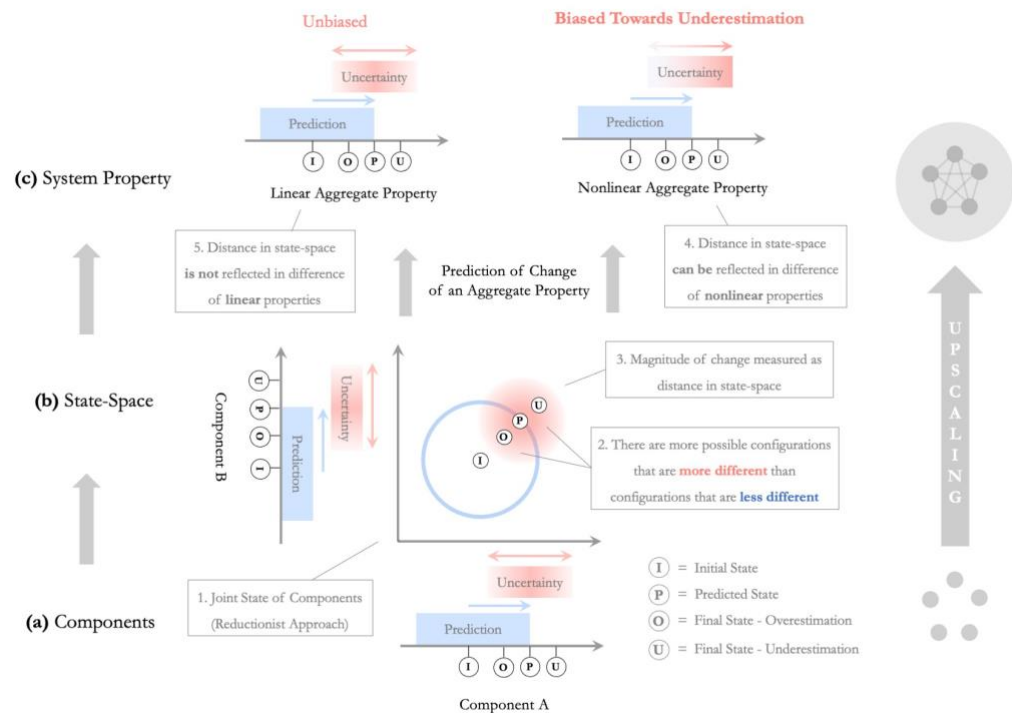


Figure 4.5: Overview of upscaling effects. **(a)** Two components, A and B are **(b)** considered at once to define a joint state (I). Suppose this state changes and falls near a predicted state (P). Then there are more ways for this state to be more different from (I), than ways to be more similar; more of the red disk is outside the blue circle than inside. Consequently, when predictions of change (blue) for individual components are scaled up to predictions of change of their joint state, unbiased uncertainties (red) become biased towards underestimation. In section Geometric Approach we quantified these surprising dimensional effects and investigate beyond the basic two-dimensional case shown here. **(c)** Magnitude of system-level change can be measured as distance in state space or by some other aggregate property. If an aggregate property is sensitive to changes in distance of the underlying state-space, dimensional effects, and therefore a bias towards underestimation, will be conserved. As we explained in section Aggregate Properties and Non-Linearity, it is the non-linear part of an aggregate property that controls its sensitivity to changes in state-space distance and thus the tendency of its degree of change to be underestimated by upscaled predictions.

Natural systems are intrinsically complex and the way that we describe them is necessarily multivariate (Loreau, 2010). It is generally accepted, in ecology, that there is a need for mechanistic predictive models, built from individual components and scaled up to the ecosystem-level (Mouquet et al., 2015; Poff, 1997; Woodward et al.,

2010). We have shown that dimensional effects will play out in this scaling-up, generating additional bias towards underestimation of any predicted system-level change. This is not to say that scaling up predictions is a faulty approach, but rather that one must keep track of dimensional effects when doing so.

Our theory provides a generic expectation for the consequences of uncertainty when predictions are scaled up from individual components to the system as a whole. By construction our results are based on “non-complex” behaviour, in the sense that we assume no correlations between unbiased prediction errors of individual components. Our theory can therefore be thought of as a baseline, a null model of what to expect if only dimensional effects are at play, against which we can test biological (or other) effects. Indeed, interactions between components (e.g., species interactions) may introduce systematic correlations between the prediction errors for each component, which we have assumed independent. This might in turn add further biases towards over- or underestimation, on top of the biases due to dimensional effects. Viewed this way, our research highlights a delineation between statistical and natural mechanisms controlling the behaviour of complex systems (Nekola & Brown, 2007).

We considered two levels of organisation: the level where predictions are made and the level where predictions are scaled up to. In principle, intermediate levels could be considered. For instance, given the increasing resolution of ecological data, predictions of change may originally be based at the level of individual organisms and could first be scaled up to species-level predictions and subsequently scaled up to ecosystem-level predictions. Here, if non-linear aggregate properties are used, dimensional effects will bias species-level predictions towards underestimation and will further increase this bias for ecosystem-level predictions. With an ever-increasing resolution of data, scaling predictions across multiple levels of organisation, and potentially introducing dimensional effects at multiple levels, may become more increasingly common.

Our work is theoretical and, in essence abstract. Yet it may be relevant for highly practical domains of ecology. To make this point, we now discuss some

implications of our theory to multiple-stressor ecological research, an essentially empirical field that explicitly deals with considerable uncertainty of predictions and holds great interest in its consequences.

4.5.1 MULTIPLE-STRESSOR RESEARCH

In the light of our theory, we propose to revisit a seemingly unrelated problem of wide ecological interest: what is the combined effect of multiple stressors on a given ecosystem? By translating our theory into the language of multiple-stressor research we aim to highlight some implications and to inspire further generalization. The combined effect of stressors on an ecological system is generally predicted based on the sum of their isolated effects, i.e., an additive null model (Folt et al., 1999; Schäfer & Piggott, 2018). Uncertainty around this additive prediction, which is ubiquitous in empirical studies (Crain et al., 2008; Holmstrup et al., 2010; Jackson et al., 2016), causes prediction errors called “non-additivity”. Uncertain predictions will either overestimate or underestimate the combined effect of stressors, respectively creating “antagonism” and “synergism” (Folt et al., 1999; Piggott et al., 2015). This translation of stressor interactions in terms of prediction uncertainty and under- or over-estimation leads us to the conclusion that scaling up uncertain multiple-stressor predictions generates bias towards synergism.

Here, scaling up predictions refers to multiple-stressor predictions (e.g., an additive model) at one level (e.g., individuals, populations) being used to build multiple-stressor predictions at higher levels of biological organisation (e.g., communities, ecosystems), an approach for which there is growing interest (Bracewell et al., 2019; Griffen et al., 2016; Kroeker et al., 2017; Orr et al., 2020; Thompson et al., 2018a). To be clear, scaling up predictions is not equivalent to simply scaling up investigations; our theory does not predict greater synergism at higher levels of organisation. In fact, we are not making predictions about how stressors will behave at higher levels of organization. What we claim instead is that, if we have a model for the combined effect of stressors at one level of organization and use that model to deduce their combined

effect at higher levels, the process of scaling up the model will introduce a bias towards an observed synergy between stressors, even if no systematic synergy was observed at the lower level.

Our theory has consequences for the interpretation of stressor interactions and is therefore relevant to the debate surrounding multiple-stressor null models (De Laender, 2018; Griffen et al., 2016; Liess et al., 2016; Schäfer & Piggott, 2018). Our findings are especially relevant to the *Compositional Null Model*, which employs a reductionist approach to the construction of multiple-stressor predictions (Thompson et al., 2018a). In such an approach, the baseline against which biological effects are tested must be shifted. Dimensional effects, quantified by the effective dimensionality of the underlying system and the non-linearity of aggregate properties, need to be accounted for to decipher a biological synergism from merely a statistical synergism.

4.5.2 CONCLUSIONS

In this paper we have addressed a subproblem of the reductionist program (Levins & Lewontin, 1985; Loreau, 2010). We investigated the consequences of uncertainty when unbiased predictions of individual components are scaled up to predictions of system-level change. Due to a geometric observation that in high dimensions there are more ways to be more different, than ways to be more similar, scaling up uncertain predictions tends to underestimate system-level change. Although we have primarily focused on ecology, and in particular on the response of ecosystems to perturbations; our general findings could inform any field of science where predictions about whole systems are constructed from joint predictions on their individual components, such as economics, finance, energy supply, and demography (Appendix C.2).

Given the generality of these results, why have these dimensional effects gone unnoticed until now? Firstly, we stress that these effects will only manifest when a reductionist approach is used to study complex systems and when distance in state-space or some other non-linear aggregate property is used to measure change at the system level. Secondly, even when these conditions are met, dimensional effects may be

obscured by the natural effects that also contribute to the overall probability of over or underestimation. Nonetheless, with increasing data quantity and resolution, scenarios where our theory is relevant could become increasingly common. If this is the case, understanding the generic dimensional effects that emerge when uncertain predictions are scaled up will be paramount to better predict the behaviour of complex systems.

CHAPTER 5 | NON-ADDITIVE INTERACTIONS AT THE COMMUNITY LEVEL ARE NOT INTRINSIC FEATURES OF STRESSORS: THE ROLE OF OBSERVATION

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AUTHOR CONTRIBUTION:

I carried out the simulations, produced the figures and wrote the first draft of the manuscript with support from Jean-François Arnoldi. All authors were involved in the conceptualisation of ideas.

STATUS:

This manuscript is in preparation.

5.1 ABSTRACT

Predicting the ecological impacts of global change requires a mechanistic understanding of antagonistic and synergistic interactions between multiple stressors. This non-additivity between stressors is widespread and can be caused by physical or chemical interactions between the stressors themselves, or by biological effects arising at different levels of organisation. Non-additivity, however, can also be caused by the way we choose to observe stressor impacts. Here, we explore observational sources of non-additivity at the community level, when the biological source of non-additivity occurs at the population-level, via extinctions and invasions following stressor exposure. Critically, we find community metrics often observe qualitatively different stressor interactions for the same combination of stressors and the same biological system. Focusing on descriptors of the composition, function and diversity of ecological communities, we reveal the drivers of the decorrelation between observations of different community metrics. We demonstrate that non-additive effects are not intrinsic features of stressors, but strongly depend on how we choose to observe the community. Our research is a step towards the understanding and prediction of the combined effects of multiple stressors on ecosystems.

5.2 INTRODUCTION

Predicting the combined effects of multiple stressors on ecological communities is a difficult task. The crux of the problem is that co-occurring stressors can interact in complex ways resulting in unexpected non-additive effects (Côté et al., 2016; Kroeker et al., 2017; Piggott et al., 2015). A growing number of studies have reported non-additive effects, either antagonism or synergism, of specific combinations of stressors on specific biological responses (Crain et al., 2008; Darling & Côté, 2008; Jackson et al., 2016; Yue et al., 2017). So far, however, the search for generalities in stressor interactions has primarily relied on phenomenological approaches, which have limited

predictive power (Griffen et al., 2016; Orr et al., 2020). This has resulted in a substantial amount of context-dependence in multiple-stressor research and has sparked interest in advancing a mechanistic understanding of stressor interactions (De Laender, 2018; Schäfer & Piggott, 2018).

Stressor interactions are defined as deviations from null models constructed from the individual effects of stressors (Folt et al., 1999). The additive null model –the most commonly used null model, in which the combined effect is the sum of individual effects– has been widely criticised as a predictive model (Schäfer & Piggott, 2018; Tekin et al., 2020), and a growing number of alternative null models, requiring varying degrees of prior knowledge, have thus been proposed (Liess et al., 2016; Tekin et al., 2020; Thompson et al., 2018a). These models attempt to accurately predict the combined effects of stressors, or at least fail in informative ways (Orr et al., 2020; Schäfer & Piggott, 2018). Another way of increasing predictive power and generalisability in multiple-stressor research, which is arguably a prerequisite for predictive models, is to build a thorough understanding of the sources and behaviour of non-additivity. Antagonism and synergism should only be called *Ecological Surprises* (Paine et al., 1998) when the sources of non-additivity are unknown (De Laender, 2018). If, however, non-additivity between stressors is understood and can be accounted for, combined effects of stressors may become more predictable.

To understand and predict stressor interactions it is first important to recognise that non-additivity between stressors can arise at multiple levels of organisation (Boyd & Brown, 2015; Kroeker et al., 2017). As an illustration, consider a scenario where an ecologist observes a synergistic combined effect of two stressors on the diversity of an ecosystem, and consequently asks: what caused this synergism? Perhaps it was caused by physical or chemical interactions between the stressors themselves (Boyd & Brown, 2015; Didham et al., 2007; Geary et al., 2019; Kroeker et al., 2017). Or, maybe the synergism was caused by non-linear responses at the individual level introduced by physiological or behavioural effects (Gunderson et al., 2016; Hale et al., 2017; Todgham & Stillman, 2013). Or, maybe non-linearity introduced by minimum viable population

or Allee effects at the population level caused the synergism (Brook et al., 2008). Perhaps the most obvious mechanisms of non-linear population-level responses to stressors, and therefore a potential source of this synergism, are extinctions and invasions following stressor exposure. Alternatively, the observed synergism could have been caused at the community-level by species interactions amplifying the effects of stressors throughout the community (Bruder et al., 2019; Kroeker et al., 2017; Thompson et al., 2018b). All of these biological sources of non-additivity are of great interest to ecologists and ecosystem managers who may use the identity of stressor interactions to inform conservation efforts (Brown et al., 2014; Côté et al., 2016). If, however, the synergistic effect on diversity was related to the *measure of diversity itself*, then that synergism would not transpose to other community properties as it will clearly not be an intrinsic feature of the stressors themselves. Understanding the potential sources of non-additivity, be they biological or observational, across different levels of organisation (Figure 5.1) is crucial for accurate predictions of the combined effects of stressors.

Here, we focus on observational effects of non-additivity observed at the community level, when the biological source of non-additivity is species turnover – extinctions and invasions caused by the stressors. Using a simple model for which extinctions and invasions are the only biological sources of non-linearity, we demonstrate that the choice of community metric used to quantify the effect of stressors will determine how non-linear responses at the population level scale up to an observed non-additive stressor interaction at the community level. Critically, we find a poor correlation between the non-additivity observed by different community metrics (e.g. species richness, diversity index, total biomass). There can even be qualitative differences in the stressor interactions (antagonism, synergism) depending on how the community is observed. We can, however, explain the mechanisms controlling the correlation between the non-additivity observed by different community metrics. As such, our work builds a mechanistic understanding of non-additivity at the community

level and is a step towards accurate predictions of the impacts of multiple stressors on ecosystems.

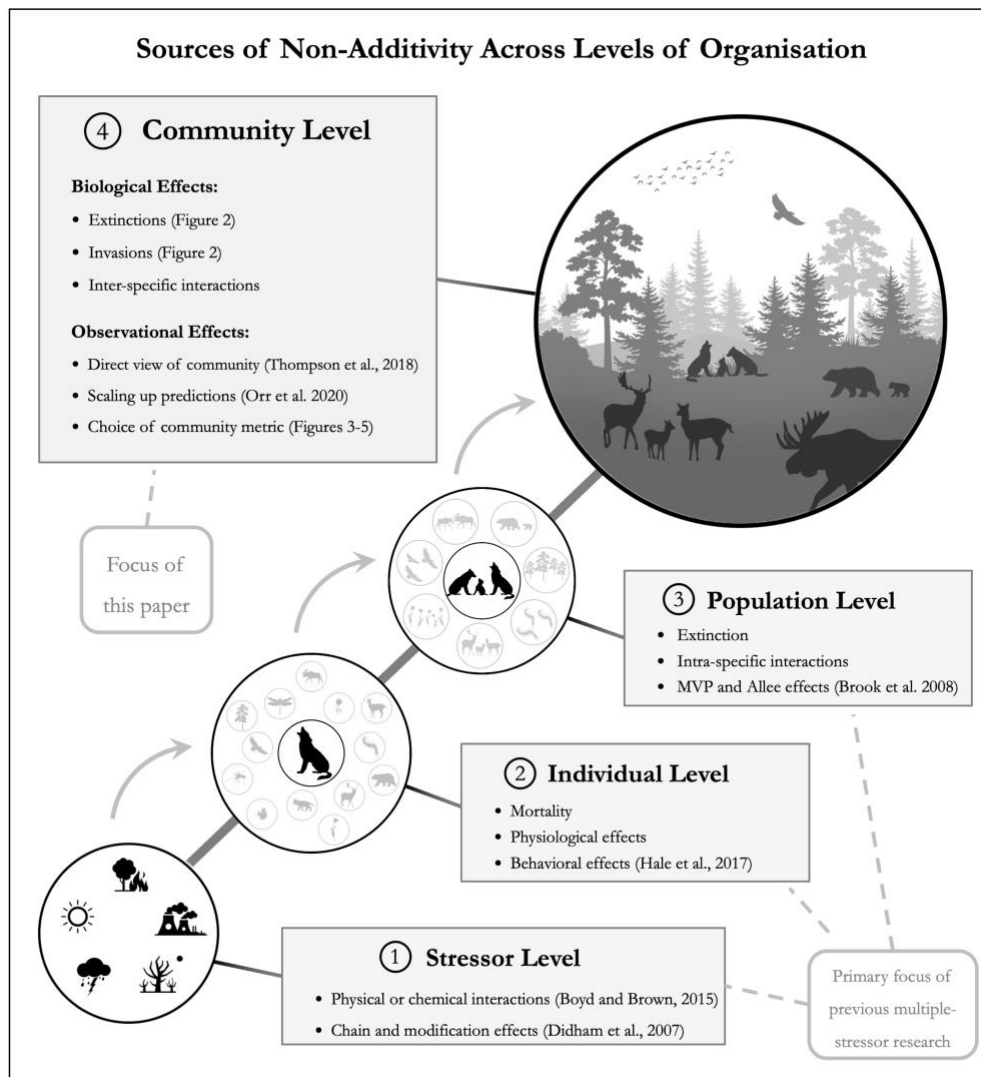


Figure 5.1: Sources of non-additivity across levels of organisation. Multiple-stressor researchers have primarily focused on sources of non-additivity arising at **(1)** the stressor level (e.g. physico-chemical effects), **(2)** the individual level (e.g. physiological or behavioural effects) and **(3)** the population level (e.g. intra-specific interactions, minimum viable populations, Allee effects). Our contribution, however, is a description of the non-additivity arising at **(4)** the community level with particular focus on the role of observation. We explain how biological effects (extinctions and invasions) are sources of non-additivity, which can be observed in different ways depending on the choice of community metric.

5.3 METHODS

We start from a simple, but non-trivial model: two non-interacting species. This model can be represented graphically and will provide valuable insight. We represent community states as points in a space where each axis is defined by the biomass of a single species. We define the impact of stressors at the community level as the Euclidean distance between initial and perturbed states, thus quantifying change of the community as a whole, in a classic mathematical way. The additive expectation for the combined effect of stressors follows simple tip-to-tail addition of the individual stressors (vector addition). The realized state of the community following exposure to multiple stressors, which may differ from the additive expectation, simply takes into account that species cannot have negative biomass. If the additive expectation overestimates or underestimates the magnitude of change then there is an antagonistic or synergistic stressor interaction respectively.

We then consider the high dimensional version of the above model: species-rich communities of non-interacting species. Here, species are characterized by their carrying capacities K_i . Positive carrying capacity is the abundance of the species, while species with negative carrying capacities are unable to establish (but we still consider their potential presence, as subsequent environmental conditions may permit them to invade). We model stressors as random relative perturbations of carrying capacities (Arnoldi et al., 2018), but our results are robust to other modelling choices. To control the direction of a stressor we draw a vector from a random normal distribution with center μ and standard deviation of 1, which we divide by its norm (so that it indeed defines a direction, i.e., a point on the hypersphere). If $\mu = 0$, the stressor can point in any direction. If $\mu > 0$, the stressor tends to have a negative effect, and if $\mu < 0$ the stressor tends to have a positive effect. The intensity of the entire stressor is drawn from a random uniform distribution between 0 and ϵ . A stressor's effect $\delta K = (\delta K_i)$ on all species of a community then reads:

$$\delta K = \text{intensity} \times \text{direction} \times |K| \quad (5.1)$$

Where $K = (K_i)$. Recall that K_i , if positive, is the species' abundance prior to stressor exposure. If negative, it can be thought of as the species' invasion growth rate. The final abundance of any species following exposure to a stressor is:

$$N_i = \max(K_i + \delta K_i, 0) \quad (5.2)$$

We quantify the effect of stressors using various community metrics describing the composition (Euclidean distance), diversity (species richness, Shannon's diversity index, Gini-Simpson index, Hill diversity) or function (total biomass) of the community (Hill, 1973; Roswell et al., 2021; Shannon & Weaver, 1963; Simpson, 1949). For each community metric we define absolute synergism as:

$$\Delta f(N_{12}) - (\Delta f(N_1) + \Delta f(N_2)) \quad (5.3)$$

where $\Delta f(N_j)$ refers to the change in a community metric caused by stressor j . Positive and negative values of absolute synergism reflect synergistic and antagonistic interactions respectively. We define non-additivity based on an additive expectation, but if a multiplicative null model was employed instead (Folt et al., 1999; Griffen et al., 2016), our results would still be relevant as long as a detection threshold was assumed. Using this framework, we can explore how non-additivity caused by the same mechanism is observed by different aggregate properties at the community level. By studying a general model with few parameters, the sources of non-additivity we describe will also be general and thus relevant when considering more complex models – or at least constitute a solid reference point. Code for all analyses is available on the *GitHub* repository: <https://github.com/jamesaorr/community-nonadditivity>.

5.4 TURNOVER AS A SOURCE OF NON-ADDITIVITY

Considering two non-interacting species already provides a useful conceptual model to understand how species turnover can determine community-level interactions between stressors (Figure 5.2). First, when stressors have negative effects on all species, extinctions are the only source of non-additivity and will cause antagonistic stressor interactions when distance in state-space is used to quantify change at the community-level (Figure 5.2a). This is not surprising. In fact, antagonism created when the additive null model surpasses the boundaries of response variables is well known to multiple-stressor researchers (Côté et al., 2016; Folt et al., 1999) and is often accounted for in predictive models (Schäfer & Piggott, 2018; Thompson et al., 2018a).

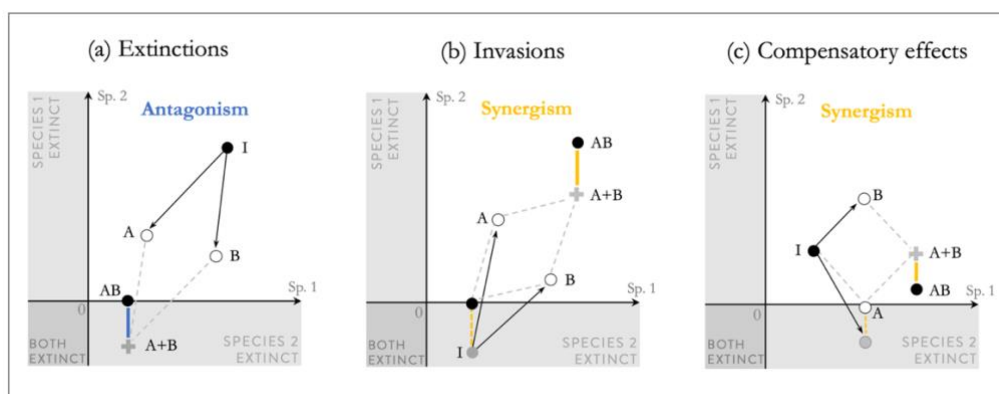


Figure 5.2: Species turnover as a source of non-additivity. Each axis describes the biomass of a single species. The black point, I, is the initial state of the community. The white points, A and B, are the state of the community following exposure to the individual stressors, which are represented as black arrows. The grey plus sign is the predicted state of the community based on an additive expectation constructed using the dashed grey lines. The black point, AB, represents the realised state of the community when exposed to both stressors. Antagonistic or synergistic interactions occur when the additive prediction overestimates or underestimates the combined effect of stressors. **(a)** When both stressors have negative effects, extinctions are the only source of non-additivity and they introduce antagonism. **(b)** When both stressors have positive effects, invasions are the only source of non-additivity and they introduce synergism. **(c)** When stressors have opposing or mixed effects, invasions and extinctions can occur. However, a third potential source of non-additivity is compensatory effects where one stressor independently causes an extinction but the other stressor prevents that extinction. An

overestimation of the effect of the positive stressor can cause the actual change to be underestimated by the additive expectation, resulting in a synergistic stressor interaction.

Conversely, when stressors have positive effects on all species, invasions are the only source of non-additivity and will cause synergistic stressor interactions at the community level. Species in the regional pool with negative growth rates are unable to join the assembled community but positive stressors can allow them to invade. The effect of a positive stressor can thus be split into two parts: (i) the increase in growth rate required for a species' to invade, and (ii) the increase in biomass thereafter. Importantly, the additive null model will not detect the former. When multiple positive stressors combine and an invasion occurs, there will be some redundant increases to the invading species' growth rate, which will be converted into additional biomass that was not predicted by the additive null model. Invasions will therefore systematically introduce synergism at the community-level when observed via state-space distance (Figure 5.2b).

Finally, when stressors are mixed (i.e. a single stressor has negative effects on some species but positive effects on others) or opposing (one stressor has purely negative effects and the other has purely positive effects), extinctions and invasions can both occur. Compensatory effects are, however, a third source of non-additivity for mixed or opposing stressors. Specifically, these effects occur when one stressor independently causes the extinction of a species but another stressor prevents that extinction when the stressors are combined. Similar to the case of invasions, here non-additivity stems from the fact that changes in growth rates below zero are undetected by the additive null model. The positive stressor will not be as strong as expected because it had to compensate for the decreases in growth rate below zero caused by the negative stressor. Depending on the specific directions of stressors, this can result in

the actual change being underestimated by the additive expectation, and therefore a synergistic interaction (Figure 5.2c).

Extinctions and invasions are perhaps the most obvious and trivial non-linear biological responses to stressors at the population level. However, when considering the effects of multiple stressors on species-rich communities, these mechanisms are likely ubiquitous, and generate rich patterns of non-additivity at the community level, depending on how the community itself is observed.

5.5 OBSERVATIONAL SOURCES OF NON-ADDITIVITY

So far we have quantified non-additivity using Euclidean distance in state-space, the definitive description of structural change of a community. Empiricists, however, typically quantify the effects of stressors on communities by measuring changes in their diversity or function (Jackson et al., 2016). We must therefore ask if the non-additivity observed by distance corresponds to the non-additivity observed by other community metrics. Unfortunately for multiple-stressor researchers: it depends.

Let us return to our two non-interacting species model, and consider how the non-additivity between two negative stressors is observed by distance and by species richness (the most simple diversity measure). Recall that extinctions are the only biological source of non-additivity between negative stressors, and always introduce antagonism in distance. Yet, when the impact of stressors are quantified using changes in species richness, the effect of extinctions can yield antagonistic or synergistic interactions, or can even be undetected entirely. This can be easily explained. For example, if the combined effect of stressors, but not the individual effects, cause extinctions, species richness will observe a synergistic stressor interaction (Figure 5.3a(i)). Alternatively, if both stressors independently cause the extinction of different species, their combined effect will be observed as additive by species richness (Figure 5.3a(ii)). Finally, if stressors individually cause the extinction of the same species, then the non-additivity observed by species richness will be antagonistic (Figure 5.3a(iii)).

For the same biological mechanism, species richness can observe all possible stressor interactions depending on minor details of this very simple model. This is not the first time that species richness has been shown to observe different stressor interactions for the same biological mechanism (De Laender, 2018). We refer to this introduction (or transformation) of non-additivity by community metrics as an observational effect.

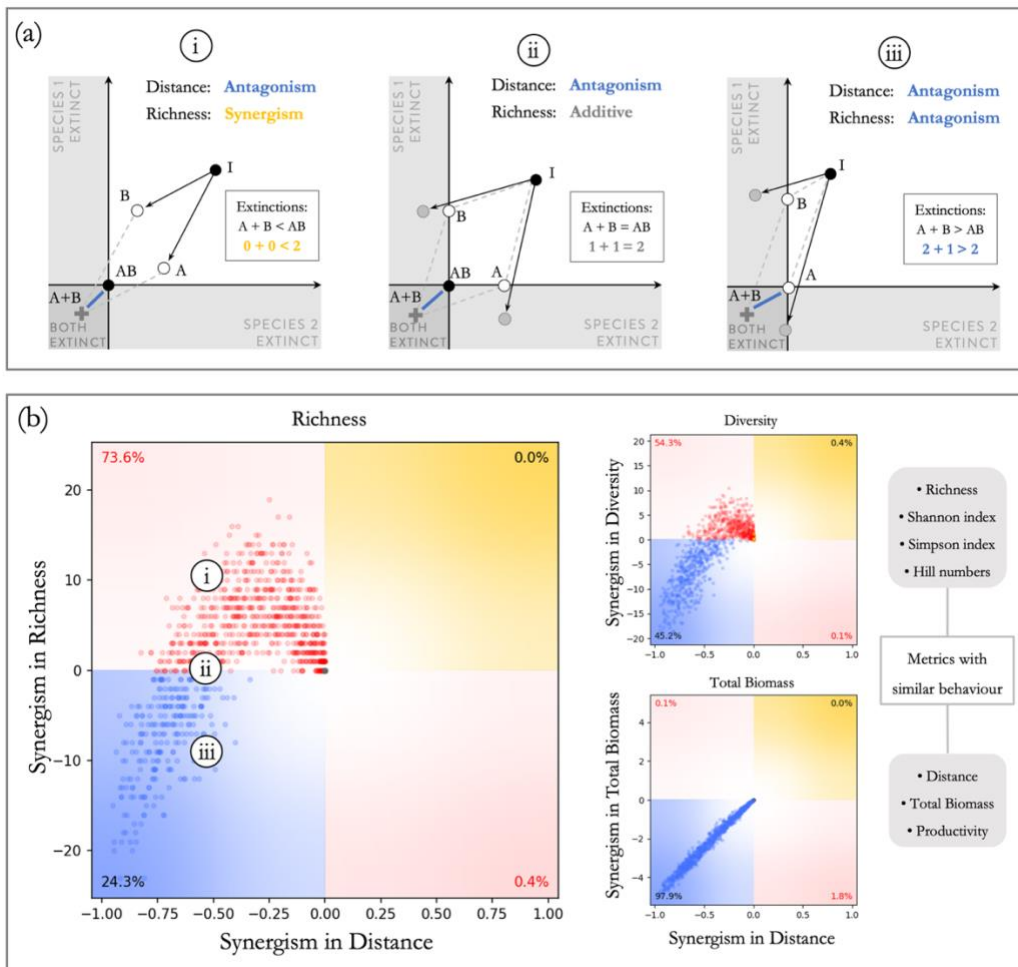


Figure 5.3: Observational sources of non-additivity. **(a)** Conceptual model of the effects of two stressors, A and B, on a community of two non-interacting species as in Figure 5.2a. For all three examples, the two stressors have negative effects on both species. Therefore, the only biological source of non-additivity is extinction, which will always lead to antagonistic stressor interactions when distance in state-space is used to quantify the effects of stressors. However, when species richness is used to quantify the effect of stressors, synergism (i), additivity (ii) or antagonism (iii) can be observed. **(b)** Synergism in distance plotted against synergism in richness (Eq. 5.3) for non-interacting communities exposed to multiple negative stressors, where extinction is the only

biological source of non-additivity. The blue and yellow quadrants contains pairs of stressors for which antagonism and synergism respectively were observed by both distance and richness. The red quadrants contain pairs of stressors for which distance and richness did not observe the same type of non-additivity. Synergism in Shannon diversity index correlates with synergism in richness, and synergism in total biomass correlates with synergism in distance.

We can generalise the results from the low dimensional conceptual model, to species rich communities. Plotting the synergism observed by distance and the synergism observed by richness (Eq. 5.3) for many pairs of stressors reveals a high frequency of mismatches between the observations of distance and richness (red points in Figure 5.3b). Even when extinctions are the only source of non-additivity, it is very common for synergistic stressor interactions to be observed by species richness, despite a systematic antagonistic effect on state-space distance. More generally, we can partition community metrics into two groups based on how they observe non-additivity between negative stressors: distance-like and diversity-like. In the first group we find aggregate properties describing the function of the community (i.e. total biomass, productivity), which observe non-additivity in a similar way to distance. The second group includes notions of diversity (richness, Shannon's index, Simpson's index, Hill diversity indices), whose observations of non-additivity are roughly comparable. The non-additivity observed by community metrics from different groups is typically incomparable. As we shall see, however, even community metrics in the same groups do not always observe non-additivity in the same way. Indeed, we find that the correlations between the non-additivity observed via community metrics is also strongly influenced by (i) the direction of stressors, (ii) the intensity of stressors and (iii) the fraction of potential invaders in the species pool.

5.5.1 DISTANCE AND FUNCTION

Based on Figure 5.3b, it appears that the non-additivity observed by distance (community-level property) and by total biomass (ecosystem-level property) are directly comparable. However, their actual relationship is complicated by properties of

the stressors (their direction and intensity) and by the composition of the species pool (the fraction of invaders). Firstly, it is true that distance and total biomass will observe the same non-additivity when stressors are purely negative or purely positive (Figure 5.4a). This is because the direction of non-additivity in state space (the vector of unaccounted change) will be approximately co-linear with the direction of total biomass (Appendix D.1a). However, when stressors have mixed or opposing effects the direction of non-additivity in state-space can be orthogonal to the direction of total biomass, resulting in the decorrelation between non-additivity in distance and non-additivity in total biomass (Figure 5.4a, Appendix D.1a).

The intensity of stressors also influences the correlation between non-additivity in distance and non-additivity in function, but only when stressors have mixed or opposing effects. Compared to purely negative stressors, mixed stressors that are orthogonal to the direction of total biomass have a larger distance to cover to cross extinction boundaries (and cause non-additivity). This geometric effect means that increasing intensity will allow mixed stressors that are orthogonal to the direction of total biomass to cause extinctions, and thus introduce non-additivity that will be observed differently by distance and by total biomass (Appendix D.1b). Consequently, increasing stressor intensity erodes the correlation between non-additivity in distance and non-additivity in total biomass (Figure 5.4b(ii)).

It is not just features of the stressors that are relevant here, but the fraction of invaders in the species pool also plays a role. The ratio between the number of species in the assembled community (i.e., species with positive carrying capacities) and the number species in the pool that are unable to invade the community (i.e., species with negative carrying capacities) will determine the relative potential of invasions and extinctions when stressors have mixed effects. This bias towards extinctions or invasions, determined by the fraction of invaders, shifts the effective direction of stressors and thus influences the correlation between the non-additivity in distance and the non-additivity in total biomass (Figure 5.4b(i)).

Total biomass is a *broad* ecosystem function influenced strongly by common species (Rivett & Bell, 2018). Other broad functions, such as productivity, will observe non-additivity in similar ways to total biomass. Alternatively, the average of many *narrow* functions, which describe specific tasks carried out by rarer species, will observe synergism in the same way as distance. Ultimately, when stressors have mixed or opposing effects, as is often the case in empirical data (Elmqvist et al., 2003; Frishkoff et al., 2018; Supp & Ernest, 2014), assuming that non-additivity observed at the community level (by distance) will be the same as the non-additivity observed at the ecosystem level (by function) is ill-advised.

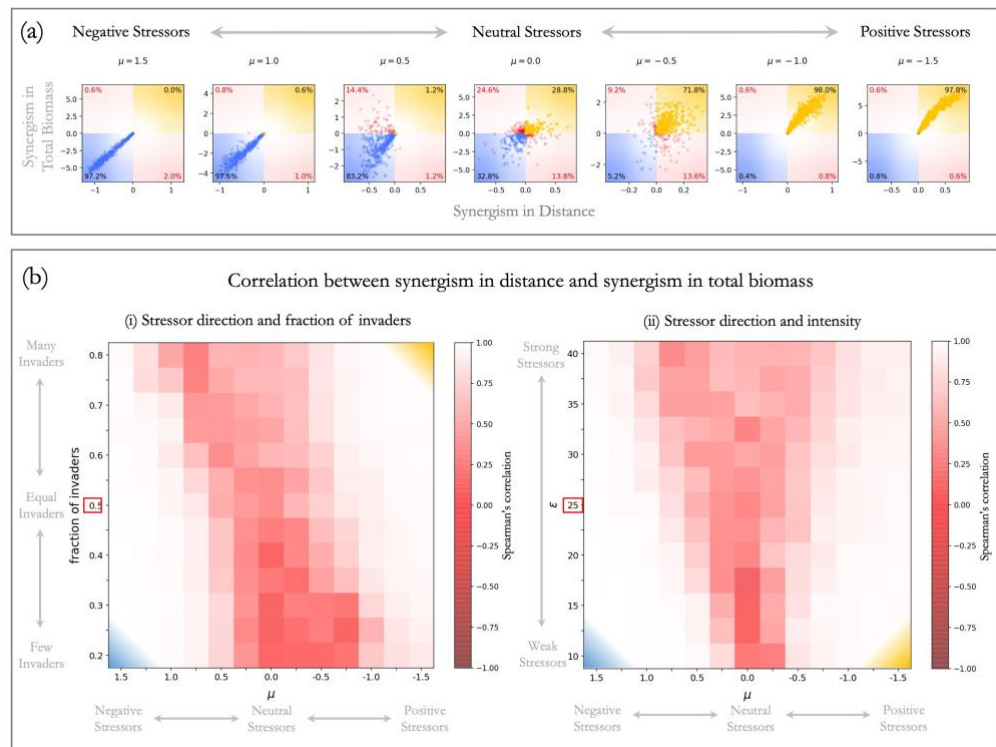


Figure 5.4: Correlation between synergism in distance and in total biomass. **(a)** Synergism observed by distance plotted against synergism observed by total biomass across a range of stressor directions from negative to neutral to positive stressors. **(b)** The correlation (Spearman's rank correlation coefficient) between synergism in distance and synergism in total biomass for different stressor directions and (i) different fractions of invaders and (ii) different stressor intensities. The colour of each square in the heatmap represents the correlation between synergism in total biomass and the synergism in distance for a set of 500 pairs of stressors. The seven boxes in the middle row of the heatmap (values highlighted in red) corresponds to the seven figures in part

(a). Shaded corners correspond to cases where distance and total biomass both observe the same stressor interactions, as either antagonistic (blue) or synergistic (yellow).

5.5.2 DIVERSITY

Empiricists often quantify the effects of stressors by measuring changes in the diversity of a community. This, however, can be an additional source of non-additivity between stressors (Figure 5.3). Indeed there is generally a very poor correlation between non-additivity observed by diversity and the non-additivity observed by distance. When stressors are weak and negative or strong and positive there is actually an inverse correlation between the observations of distance and diversity (Appendix D.2).

Despite this, we can still ask whether or not the non-additivity observed by different notions of diversity are at least comparable. Again, unfortunately for multiple-stressor researchers, even the non-additivity observed by different notions of diversity are not always comparable (Figure 5.5). To illustrate this we can consider the non-additivity observed by the family of Hill diversity indices, encompassing species richness ($q = 0$), the Shannon index ($q = 1$) and the Gini-Simpson index ($q = 2$) (Hill, 1973; Roswell et al., 2021). Species richness behaves quite differently to other notions of diversity, especially when stressors have mixed effects. The behaviour of the Gini-Simpson index (and indeed the original Simpson's index) is at least quite similar to that observed by the Shannon index. This is because hill indices range in their sensitivities to rare or abundant species, with species richness most sensitive to rare species and the Gini-Simpson index most sensitive to common species (Roswell et al., 2021). Critically, invading species will tend to be rare when stressors have mixed effects. Species richness will be most sensitive to this non-additive, caused by rare species, while the Gini-Simpson index will hardly observe it.

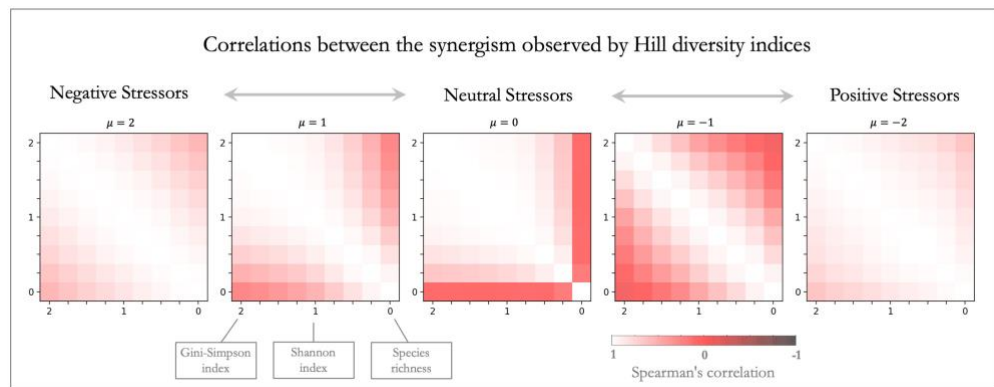


Figure 5.5: Correlation between the synergism observed by Hill's diversity indices. The family of Hill indices include the Gini-Simpson index ($q = 2$), Shannon index ($q = 1$) and species richness ($q = 0$). Correlations between these diversity indices are shown for five different values of stressor direction.

In general, community metrics often observe non-additivity between stressors in different ways, especially when stressors have mixed or opposing effects on species. It is concerning that commonly used response variables (e.g. total biomass, richness, diversity indices) may report different stressor interactions for the exact same stressors and the exact same biological system. Clearly, at the community level, stressor interactions are not only a feature of the stressors and the biological mechanisms, but are also features of our observations.

5.6 DISCUSSION

An understanding of the sources of non-additivity is required for accurate predictions of how ecosystems will respond to multiple stressors. Using a remarkably simple model we explored how non-additive stressor interactions caused by extinctions and invasions are perceived at the community level. At the population level, extinctions and invasions are perhaps the most obvious and ubiquitous sources of non-linearity. Yet, we found that they generate rich and potentially confusing patterns of observed stressor interactions at the community level.

Indeed, the measured non-additivity created by these biological mechanisms is strongly influenced by the community metric used to quantify change. Even metrics describing the same aspect of a community, for example its diversity, can observe qualitatively different stressor interactions. For the same combination of stressors and the same biological system, one community metric can observe antagonism while another community metric observes synergism. Our work reinforces the idea that changes in the composition of a community do not consistently scale up to changes in community properties (Supp & Ernest, 2014). Consequently, at the community level, stressor interactions are not just features of the stressors, or of biological systems, but are also features of our observations. It is well established in ecology that by reducing the complexity of an ecosystem down to a single number, community metrics can hide important information about the underlying system (Hurlbert, 1971; Tilman, 1996). Using multiple community metrics to quantify the ecological impacts of stressors, and indeed the interactions between stressors, is perhaps a more sensible approach (Manning et al., 2018).

By identifying the underlying mechanisms controlling the correlation between community metrics in their observations of non-additivity, we can anticipate when observations of different community metrics will and won't be comparable. Our work is a step towards the prediction of the effects of multiple stressors on ecosystems, and complements the literature surrounding null models in multiple-stressor research.

5.6.1 PREDICTION

The overarching goal of multiple-stressor research is accurate prediction of the combined effects of stressors (Côté et al., 2016; Orr et al., 2020). A focus on *predictive* null models that account for specific sources of non-additivity (e.g. extinctions) has emerged in recent years (Liess et al., 2016; Schäfer & Piggott, 2018). Most relevant to our work, is the *Compositional Null Model*, which scales up predictions from the species level to the community level (as we have done), and then accounts for non-additivity caused by extinctions (Thompson et al., 2018a). This null model, however, does not

consider invasions, or indeed any other biological mechanisms. Our approach is different; we do not try to account for non-additivity, but instead develop a mechanistic understanding of its sources. Indeed, a comprehensive overview of the many sources of non-additivity, particularly those related to observational effects, is essential for the development of predictive models in multiple-stressor research.

We have explained in detail that the choice of community metric can influence the type of non-additivity that is observed. However, this is not the only observational effect that controls the behaviour of non-additivity at the community level. Applying an additive null model to direct observations of changes in community metrics has also been highlighted as an observational source of non-additivity, which is accounted for by scaling up species-level predictions to community-level predictions (Thompson et al., 2018a). This reductionist approach to complex systems, however, can have an additional observational effect on non-additivity. Scaling up uncertain, but unbiased, population-level predictions tends to introduce a bias towards synergism at the community-level due to dimensional effects (Orr, Piggott, et al., 2021). Dimensional effects have not appeared in our models because invasions and extinctions are the only biological sources of non-additivity and these are strongly biased towards synergism and antagonism respectively. Nonetheless, having a clear understanding of the observational sources of non-additivity at the community level (Figure 5.1) is certainly a prerequisite for accurate prediction. When non-additivity between stressors is unknowingly influenced by observational effects, attempts to gain a mechanistic understanding of stressor interactions are compromised.

5.6.2 SPECIES INTERACTIONS

It is well-appreciated that species interactions are a source of non-additivity between multiple stressors at the community level (Bruder et al., 2019; Kroeker et al., 2017; Orr et al., 2020; Thompson et al., 2018b). As an example, consider two negative stressors whose combined effect, but not individual effects, cause the extinction of a keystone species. Although extinctions generally introduce antagonism in state-space distance,

the extinction of a keystone species will, by definition, disproportionately change the structure of an ecosystem via secondary extinctions and invasions (Eklöf & Ebenman, 2006; Paine, 1980). This will result in synergism, even in state-space distance. In an important first attempt to formalise the role of species interactions, Lotka-Volterra models have been used to show that different types of species interactions can introduce different types of non-additivity in species richness and total biomass (Thompson et al., 2018b). More generally however, the role of interaction strength (weak or strong) and distribution (connectance, symmetry) on the generation and transformation of non-additivity remains largely unclear.

Our general model offers a solid foundation to study the effects of more complex community dynamics on non-additivity between stressors. Species interactions are implicit in our model in that they may play a role in determining the effective direction and intensity of stressors, the initial state of the community and the fraction of invaders. We have, by construction, not considered other effects of species interactions. Indeed, species interactions may prevent or cause further extinctions and invasions. They may also introduce non-linearity in species' responses to stressors and may themselves be impacted by stressors (Barneche et al., 2021; Bruder et al., 2017; Woodward et al., 2010). Clearly, along with extinctions and invasions, species interactions are the primary biological mechanisms of non-additivity between stressors at the community level (Figure 5.1). Our results could be used as a reference against which the effects of species interactions could be quantified in future work.

5.6.3 CONCLUSIONS

Identifying the mechanisms of non-additivity is a key focus of multiple-stressor research. Using a general model we have revealed the importance of species turnover in introducing non-additivity between stressors in species-rich communities. Although these extinctions and invasions are not *Ecological Surprises*, they nonetheless introduce non-additivity at the community level. What is surprising, however, is how this non-

additivity will be observed differently depending on the choice of community metric used to quantify the effects of stressors.

Variation between community metrics in their observations of non-additivity shows how antagonism and synergism are not intrinsic features of stressors. Meta-analyses must therefore be careful not to lump together results obtained using different community metrics when trying to make conclusions about specific combinations of stressors. If ignored, observational sources of non-additivity may hinder the development of a mechanistic understanding of stressor interactions and may even mislead ecosystem management.

CHAPTER 6 | GENERAL DISCUSSION

6.1 NATURAL AND OBSERVATIONAL SOURCES OF NON-ADDITIVITY

Accurate predictions of global change impacts require a thorough understanding of multiple-stressor interactions (Côté et al., 2016; Schäfer & Piggott, 2018). Researchers across disciplines agree that improving prediction of the combined effects of stressors requires a shift from phenomenological to mechanistic approaches (Chapter 2). A recurring theme of this thesis has been that non-additivity between stressors is influenced by both natural effects and observational effects. In Chapter 3, I described a natural source of non-additivity related to adaptation and performance trade-offs. A bias towards synergism is introduced when adaptation to multiple stressors is less than the sum of the adaptation to single stressors, which is generally expected from the prevalence of performance trade-offs (Agrawal et al., 2010; Tikhonov et al., 2020). In Chapter 4, I described an observational effect generating a bias towards synergism when predictions are scaled up to higher levels of organisation. Unbiased non-additivity at the component level tends to be transformed into synergism at the system level when a reductionist approach is used to predict system-level change. In Chapter 5, I identified both natural and observational sources of non-additivity at the community level. Non-additivity between stressors can arise due to the biological effects of extinctions, invasions and species interactions. However, the type of non-additivity (synergism or antagonism) that is observed will vary depending on the community metric used to quantify the effect of stressors. Partitioning these two sources of non-additivity is essential as observational effects obscure the natural effects that researchers are interested in and may even mislead conservation efforts.

The focus on classifying interactions between specific stressors as either additive, antagonistic or synergistic is in part driven by the implications of stressor interactions for ecosystem management (Brown et al., 2013; Côté et al., 2016; Piggott

et al., 2015). Disrupting a synergistic interaction between stressors can be a cost-effective intervention, whereas mitigating a stressor that interacts antagonistically with other stressors may have limited impacts (Falkenberg et al., 2013; Fong et al., 2018). From the multiple-stressor literature, synergism is the concept that has gained the most traction in mainstream ecology and global change biology (Côté et al., 2016). Stereotypically, synergism represents the worst-case scenario for ecosystem managers as even if the individual effects of stressors are small, their combined effects can be severe. The research presented in this thesis describing general sources of synergism must therefore be interpreted with care. The emergent synergism due to performance trade-offs (Chapter 3), the bias towards synergism due to dimensional effects when scaling up predictions (Chapter 4), and the synergism introduced by community metrics (Chapter 5), are all very different to the classic, worst-case scenario notion of synergism (Brook et al., 2008; Folt et al., 1999; Paine et al., 1998; Sih et al., 2004). Although these mechanisms may lead to the statistical detection of synergistic interactions, they should not typically ring alarm bells. For instance, the synergism associated with trade-offs in the adaptation to multiple stressors should not be a major concern to ecosystem managers, as adaptation has already reduced the combined effect of stressors compared to the initial effect of stressors. Furthermore, synergistic interactions associated with observational effects from scaling up predictions or from the choice of community metric are not inherent features of the stressors. Indeed, just because a synergistic interaction is statistically detected does not mean that it is ecologically relevant. Observational sources of non-additivity must be understood and accounted for when studying the natural sources of non-additivity, and when managing ecosystems subjected to multiple stressors.

6.2 FUTURE RESEARCH

6.2.1 EXPERIMENTAL VALIDATION

The research in this thesis is some of the first to use ecological or evolutionary theory to understand multiple-stressor interactions (but see: De Laender, 2018; Thompson et al., 2018b; Vinebrooke et al., 2004). By taking a theoretical approach, I have demonstrated the existence of general, but previously unexplored, mechanisms of non-additivity. However, determining whether or not these mechanisms are important in natural systems is another question. As multiple-stressor research is primarily an applied field of research, the potentially relevant theories presented here require empirical testing. In Chapter 3, the theoretical framework integrating performance trade-offs and temporal variation in stressor interactions was already tested using empirical data from an evolution experiment with the rotifer *Brachionus calyciflorus* (Luijckx et al., 2017). However, single-species evolution experiments ignore the web of biotic interactions that populations in natural systems are entwined in. Indeed, the adaptation of a species to multiple stressors may be strongly influenced by its interactions with other species (Barracough, 2015; De Meester et al., 2019; Scheuerl et al., 2020). Mesocosm experiments are an ideal approach to study the adaptation of entire communities to multiple stressors due to their relatively high realism, control and replication (Fugère et al., 2020; Stewart et al., 2013). Microbial experimental systems may also provide useful platforms to unravel eco-evolutionary responses to multiple stressors (De Roy et al., 2014; Rillig et al., 2019). The theories about the sources and patterns of non-additivity at the community level (Chapters 4 and 5) also require empirical testing. Here, existing datasets that describe changes in species' biomass to multiple stressors could be used to test if the theories presented in this thesis are relevant to natural systems. For example, the behaviour of non-additivity when multiple-stressor predictions are scaled up from the species level to the community level could be examined for a range of communities and stressor combinations to see if the theoretical expectation of a bias towards synergism emerges. These data could also be used to compare the non-additivity

observed by different community metrics. For instance, how often do total biomass and diversity indices observe different magnitudes and directions of non-additivity for the same combination of stressors in empirical datasets? Investigating the role of species interactions in creating and transforming non-additivity (Chapter 5) is, however, a more daunting task due to the challenges of empirically accessing the community matrix (Delmas et al., 2017; Gray et al., 2014; Novak et al., 2016; Pringle & Hutchinson, 2020). However, with methodological developments (e.g., empirical dynamical models (Deyle et al., 2016), allometric diet breadth models (O’Gorman et al., 2019), DNA metabarcoding (Wirta et al., 2014)) quantifying the community matrix, so that theories linking stressor interactions and species interactions can be tested, is becoming more feasible.

6.2.2 REMOVAL OF STRESSORS

Multiple-stressor researchers primarily focus on the effects of adding stressors, rather than on the effects of removing stressors (but see: Falkenberg et al., 2013; Fong et al., 2018). Understanding the responses of ecosystems to the removal of stressors is important considering the highly applied focus of multiple-stressor research (Jackson et al., 2021). In Chapter 3, the effect of removing stressors that populations had already adapted to was explored. However, due to the experimental design I was unable to test if populations were able to adapt back to the original environment following the removal of stressors. Studying evolutionary responses to the removal of stressors, as well as studying the adaptation of entire communities to the addition and then removal of stressors, are worthwhile pursuits. More generally, although modelling studies have explored the efficacy of different strategies to manage ecosystems subject to multiple stressors, for example focusing restoration efforts on disrupting synergistic interactions (Brown et al., 2013), these ideas have not been empirically tested. Given the implications for conservation and ecological restoration, thorough testing is required to confirm that prioritising the removal of stressors based on their interactions is actually an effective management strategy. Designing field experiments where existing stressors

(e.g. invasive species, pollution) are removed and the recovery of the community is studied is a promising research avenue.

6.2.3 SIMULTANEOUS OR SEQUENTIAL STRESSORS

There is growing interest in the temporal dynamics of multiple stressors (Chapter 2, Cheng et al., 2015; Gunderson et al., 2016; Jackson et al., 2021; Ryo et al., 2019). Stressors do not always occur simultaneously at constant intensities, as was the case in Chapters 3 and 5, but may occur sequentially (Ashauer et al., 2017; Bruel et al., 2021) and have different rates of change (Pinek et al., 2020). An exciting research topic is exploring how the temporal dynamics of stressors is linked to their non-additivity. A conceptual framework has been developed to predict physiological responses of marine organisms to multiple sequential stressors (Gunderson et al., 2016), but a more general understanding of how the temporal overlap of stressors impacts non-additivity at the population, community and ecosystem levels is needed. From an evolutionary perspective, adaptation to multiple stressors may have different impacts if stressors occur simultaneously or sequentially. Indeed, if stressors occur in sequence, adaptation to one stressor may influence the effect of subsequent stressors. When the first stressor imposes a strong selective pressure, population bottlenecks or hard genetic sweeps may result in populations being more vulnerable to a second stressor (Pedrosa et al., 2017; Ribeiro & Lopes, 2013; Samani & Bell, 2016). Alternatively, species that survived the first stressor may be more tolerant to subsequent stressors, especially if the stressors have similar modes of action (Feckler et al., 2018; Fugère et al., 2020; Tlili et al., 2016). Adding further complication is the fact that different species will perceive the temporal overlap of stressors differently depending on their generation time (Jackson et al., 2021). From an ecological perspective, theories from the ecological stability literature may become useful when stressors occur in sequence. Non-additivity between simultaneous stressors and non-additivity between sequential stressors may be associated with different aspects of the notoriously multidimensional concept of ecological stability (Arnoldi et al., 2016, 2019; Donohue et al., 2016). For instance, non-

additivity between multiple sequential stressors may be controlled by the community's resistance to and recovery from stressors. Ecological and evolutionary theory will be required to establish generic expectations of how the temporal dynamics of stressors influences their interactions. Understanding ecological responses to more realistic stressor regimes is becoming a key focus of multiple-stressor research.

6.3 CONCLUSIONS

Multiple-stressor researchers have attempted to make generalisations regarding the frequency of stressor interactions using meta-analyses and vote-counting studies. Unfortunately, these phenomenological approaches have been plagued with high levels of context-dependence (Chapter 2, Kroeker et al., 2017; Schäfer & Piggott, 2018). Developing a mechanistic understanding of stressor interactions is thus essential for the transferability of knowledge from one system to another and will increase predictive power (Griffen et al., 2016; Mouquet et al., 2015; Yates et al., 2018). This thesis has demonstrated that using theory to explore general sources of non-additivity is a powerful way to unravel the mechanisms of stressor interactions. A key concept emerging from this work is that non-additivity can originate from both natural and observational sources, and delineating between these is required for accurate prediction of the combined effects of stressors. Ecosystems are increasingly threatened by unfamiliar pathogens, exotic invaders, newly-engineered nanomaterials and emerging contaminants (de Souza Machado et al., 2018; Reid et al., 2018). Accurate predictions of how these novel stressors will interact with existing stressors is essential for the management, conservation and restoration of ecosystems during the Anthropocene.

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APPENDIX A | SUPPLEMENTARY MATERIAL FOR CHAPTER

2

Title = ("multiple stress*" OR "stressors" OR "global change factors" OR "environmental factors" OR "global change drivers" OR "multiple drivers" OR "synerg*" OR "amplify*" OR "antagon*" OR "dampen*" OR "additive" OR "interactive effects" OR "multifactor" OR "nitrogen and phosphorus limitation" OR "multiple limiting resources" OR "nutrient co-limitation" OR "global change experiments")

AND

Topic = ("multiple stress*" OR "stressors" OR "global change factors" OR "global change drivers" OR "multifactor" OR "cumulative effect*" OR "net effect*" OR "combined effect*" OR "interacting" OR "nitrogen and phosphorus limitation" OR "multiple limiting resources" OR "nutrient co-limitation" OR "global change experiments")

AND

Web of Science Category = ("Ecology" OR "Toxicology" OR "Environmental Sciences" OR "Plant Sciences" OR "Zoology" OR "Marine Freshwater Biology" OR "Limnology" OR "Oceanography" OR "Multidisciplinary Sciences")

AND

Year Published = (1999-2019)

Figure A.1 Search of the *ISI Web of Knowledge* database

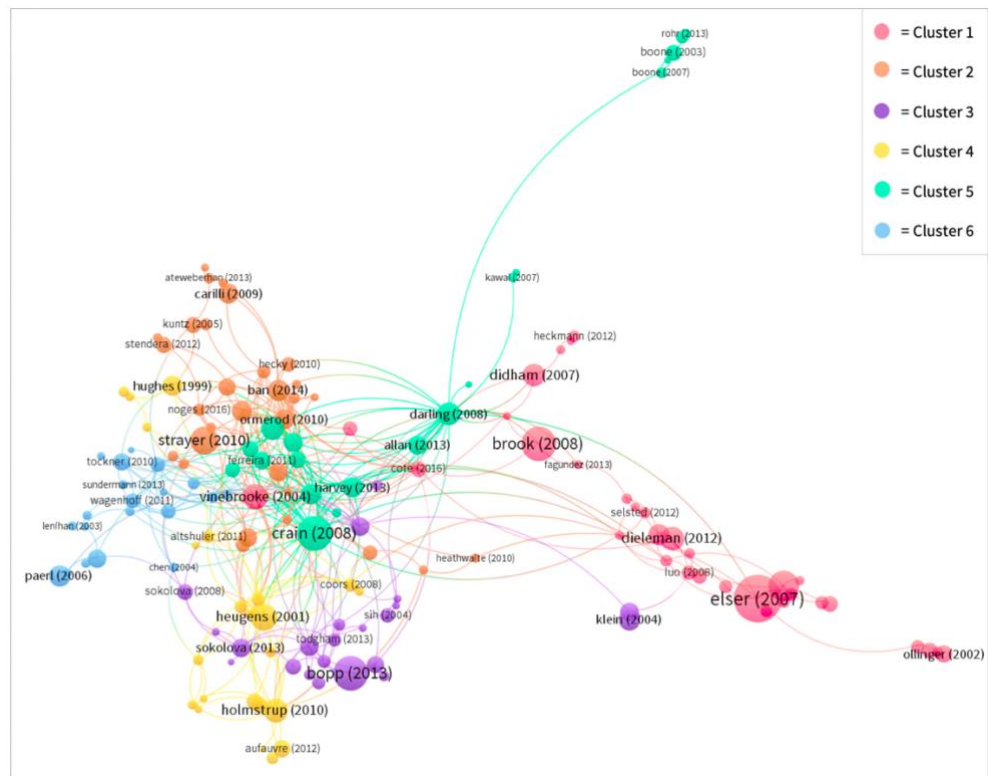


Figure A.2: Citation network with clusters highlighted. Nodes represent publications and links indicate the presence of a citation between connected publications. The size of the nodes represents the number of citations normalized by age. The distance between nodes is calculated using a citation analysis algorithm which determines the relatedness of items based on the number of times they cite each other. The colours of the nodes and their links represent the different clusters of publications.

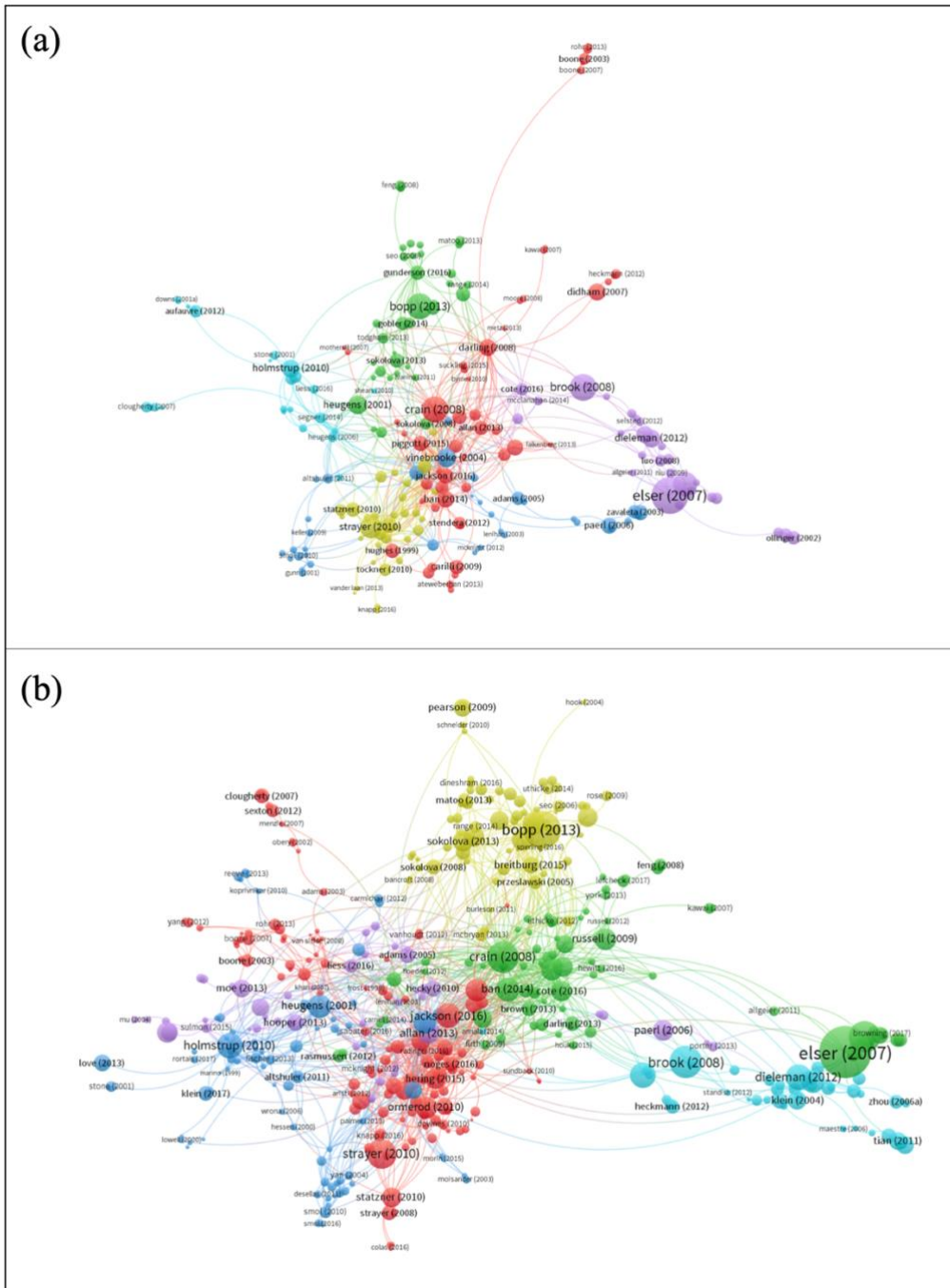


Figure A.3: Larger citation networks. Citation networks constructed using the top (a) 500 and (b) 1000 publications based on citations. Minimum cluster size was modified so that there was always the same number of clusters in each network to allow for comparisons to be made. The size of the nodes represents the number of citations normalized by age.

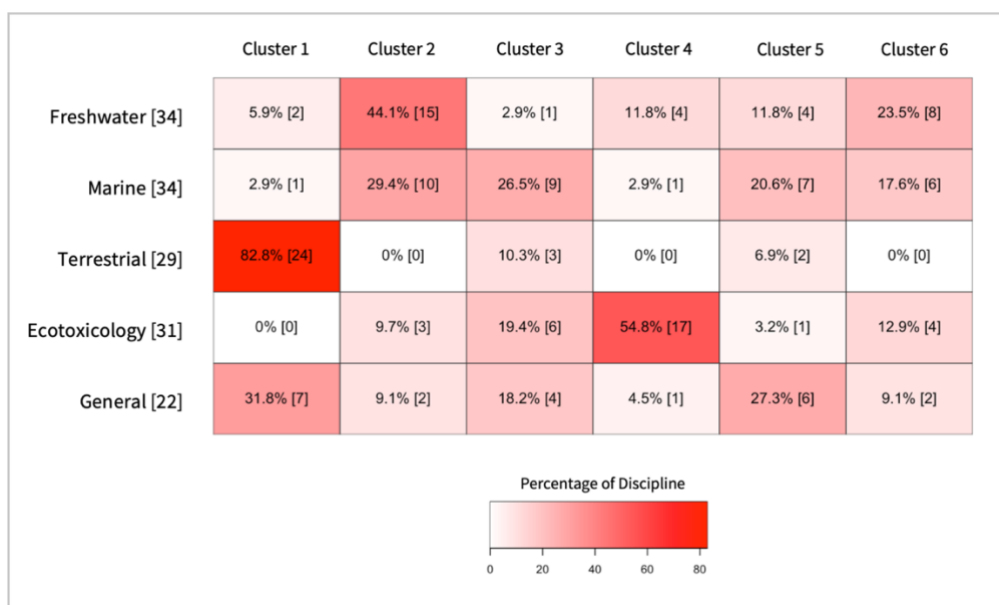


Figure A.4: Distribution of disciplines across clusters in the citation network. Cells in the heat map contain the percentage (and actual number in square brackets) of publications from each discipline found in each of the six clusters depicted in Figure A.2.

APPENDIX B | SUPPLEMENTARY MATERIAL FOR CHAPTER 3

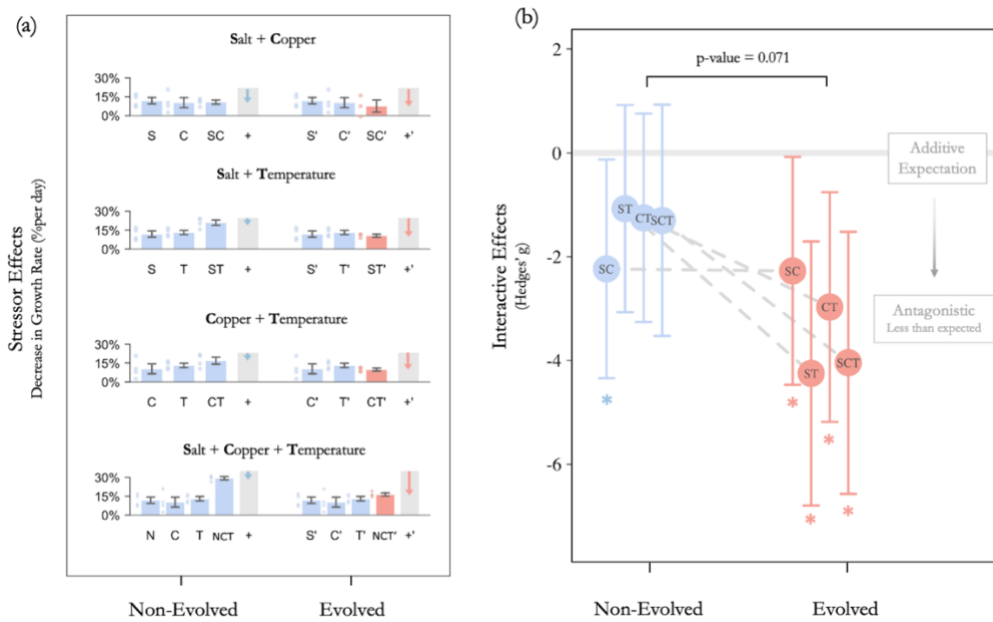


Figure B.1: Changes in stressor interactions for scenario two. Here the additive null model remains fixed, but the combined effect of stressors is reduced over time due to adaptation. This represents a scenario where timeseries data is collected in natural systems before and after application of stressors. **(a)** Individual and combined effects of stressors on the growth rates of non-evolved (blue) and evolved (red) populations for all combinations of stressors. The additive expectations, always constructed from the initial individual effects of stressors, are plotted in grey. Dots represent population replicates and error bars represent standard error. Arrows superimposed on the additive expectation represent the direction and magnitude of the interactive effects. **(b)** Interactive effects (*Hedges' g*) with 95% confidence intervals for the four combinations of multiple stressors for non-evolved (blue) and evolved (red) populations. Interactive effects below and above the grey line (at $y=0$) are antagonistic and synergistic, respectively. These effects are statistically significant (*) when 95% confidence intervals do not cross the grey line. Dashed lines connect evolved and non-evolved pairs of interactive effects. In this scenario, interactive effects become more antagonistic over time due to evolution (paired t-test, $p\text{-value} = 0.071$).

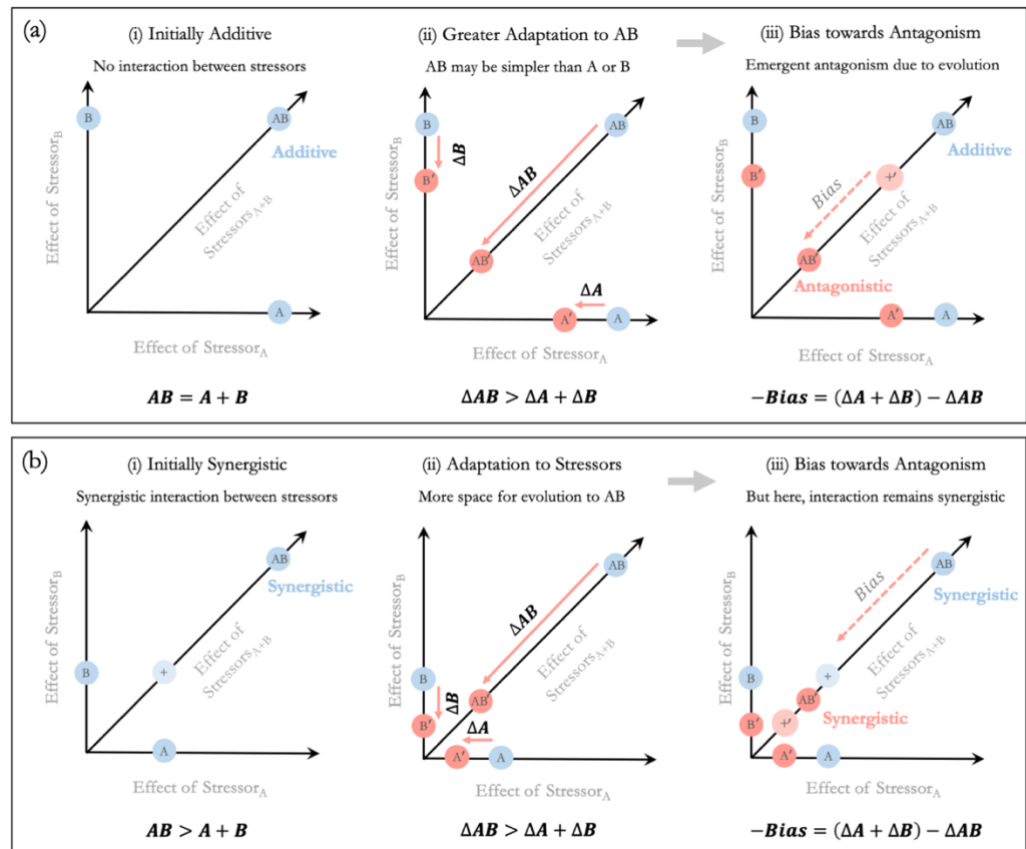


Figure B.2: How adaptation can shift stressor interactions towards antagonism. Two hypothetical examples of how adaptation to multiple stressors can be greater than the sum of the adaptation to single stressors (i.e., $\Delta AB > \Delta A + \Delta B$). **(a)** First, it is possible that a combination of stressors may be a simpler environment than any of its constituent parts (e.g., a mixture of chemicals). In this case, even if there was no interaction between stressors at first, after evolution had occurred there would be an emergent antagonism due to evolution. **(b)** Second, if the initial combined effect of stressors was greater than expected effect based on their sum (synergistic), then there would be more space for populations to evolve into. This inequality between the adaptation to multiple stressors and the sum of the adaptation to individual stressors would bias stressor interactions towards antagonism.

B.3 SUPPLEMENTARY NOTE ON ADDITIVE ADAPTATION AND REMOVAL EFFECT

The assumption of additive adaptation is that a population's fitness gain after adaptation to changes of multiple environmental parameters (each change of parameter representing an individual stressor) can be decomposed as the sum of adaptation to those individual environmental parameters:

$$\Delta W_{ab}(a'b') = A_{ab}(a') + B_{ab}(b') \quad (\text{B.3.1})$$

where subscript refers to the environment in which the population has evolved (ab) and in parenthesis is the environment where it is grown ($a'b'$), where the notation stresses that a' and b' need not, in principle, be equal to a and b . The fitness change after evolution is thus decomposed into two terms, one that only depends on the first environmental axis (taking value a' for instance) and the other that only depends on the second environmental axis (taking value b'). Here adaptation is the difference in fitness (in $a'b'$) between evolved (adapted to ab) and non-evolved populations (adapted to 00 , the control environment):

$$\Delta W_{ab}(a'b') = W_{ab}(a'b') - W_{00}(a'b') \quad (\text{B.3.2})$$

Note that we do not assume that the adaptation process itself can be decomposed as a sum of adaptation processes to single stressors, in fact we aim to demonstrate the occurrence of evolutionary trade-offs when populations adapt to multiple stressors, in which case,

$$A_{ab}(a) < A_{a0}(a) \text{ and } B_{ab}(b) < B_{0b}(b) \quad (\text{B.3.3})$$

To demonstrate evolutionary trade-offs we need to estimate the adaptation of populations evolved to ab , to a and to b separately, and verify that their sum is equal to $\Delta W_{ab}(ab)$ (Eq. B.3.1). It will then make sense to compare those adaptations to those

attained in the presence of a single stressor (Eq. B.3.3). However there is a subtlety, even under perfect adaptive additivity. If we grow a population that has evolved to ab in $a0$, its fitness difference with non-evolved populations will not be the value $A_{ab}(a)$ that we are after. Indeed,

$$\Delta W_{ab}(a0) = A_{ab}(a) + B_{ab}(0) \quad (\text{B.3.4})$$

where $B_{ab}(0)$ could even be negative if adapting to b implied maladaptation to the control environment. This information is not directly available in our data. However, we do have data from populations evolved to single stressors where, for example we have measurements for:

$$A_{a0}(0) = \Delta W_{a0}(00) \text{ and } B_{0b}(0) = \Delta W_{0b}(00) \quad (\text{B.3.5})$$

We can use this information to account for a removal effect by simply proposing that:

$$A_{ab}(0) \approx A_{a0}(0) \text{ and } B_{ab}(0) \approx B_{0b}(0) \quad (\text{B.3.6})$$

Thus the two identities

$$\begin{aligned} \Delta W_{ab}(a0) &= A_{ab}(a) + B_{ab}(0) \\ \Delta W_{ab}(0b) &= A_{ab}(0) + B_{ab}(b) \end{aligned} \quad (\text{B.3.7})$$

become, approximately

$$\begin{aligned} A_{ab}(a) &\approx \Delta W_{ab}(a0) - \Delta W_{0b}(00) \\ B_{ab}(b) &\approx \Delta W_{ab}(0b) - \Delta W_{a0}(00) \end{aligned} \quad (\text{B.3.8})$$

Which gives an approximation for the strict adaptation of populations to each stressor (assuming additivity). We can make a similar assumption for the three-stressor case:

$$\begin{aligned} B_{abc}(0) + C_{abc}(0) &\approx \Delta W_{0bc}(000) \\ A_{abc}(0) + C_{abc}(0) &\approx \Delta W_{a0c}(000) \end{aligned} \quad (\text{B.3.9})$$

$$A_{abc}(0) + B_{abc}(0) \approx \Delta W_{ab0}(000)$$

In this case we have that:

$$\begin{aligned} A_{abc}(a) &\approx \Delta W_{abc}(a00) - \Delta W_{0bc}(000) \\ B_{abc}(b) &\approx \Delta W_{abc}(0b0) - \Delta W_{a0c}(000) \\ C_{abc}(c) &\approx \Delta W_{abc}(00c) - \Delta W_{ab0}(000) \end{aligned} \quad (\text{B.3.10})$$

But another choice is possible, which uses information that is more closely related to the three stressor populations; the measurements of adaptation to two stressors out of three by the populations evolved to all three. If we make the approximation:

$$\begin{aligned} C_{abc}(0) &\approx \Delta W_{00c}(000) \\ B_{abc}(0) &\approx \Delta W_{0b0}(000) \\ A_{abc}(0) &\approx \Delta W_{a00}(000) \end{aligned} \quad (\text{B.3.11})$$

Then we have that:

$$\begin{aligned} \Delta W_{abc}(ab0) - \Delta W_{00c}(000) &\approx A_{abc}(a) + B_{abc}(b) \\ \Delta W_{abc}(a0c) - \Delta W_{0b0}(000) &\approx A_{abc}(a) + C_{abc}(c) \\ \Delta W_{abc}(0bc) - \Delta W_{a00}(000) &\approx B_{abc}(b) + C_{abc}(c) \end{aligned} \quad (\text{B.3.12})$$

which, as system of three equations for three unknowns that is invertible, gives:

$$\begin{pmatrix} A_{abc}(a) \\ B_{abc}(b) \\ C_{abc}(c) \end{pmatrix} \approx \frac{1}{2} \begin{pmatrix} 1 & 1 & -1 \\ 1 & -1 & 1 \\ -1 & 1 & 1 \end{pmatrix} \begin{pmatrix} \Delta W_{abc}(ab0) - \Delta W_{00c}(000) \\ \Delta W_{abc}(a0c) - \Delta W_{0b0}(000) \\ \Delta W_{abc}(0bc) - \Delta W_{a00}(000) \end{pmatrix} \quad (\text{B.3.13})$$

APPENDIX C | SUPPLEMENTARY MATERIAL FOR CHAPTER 4

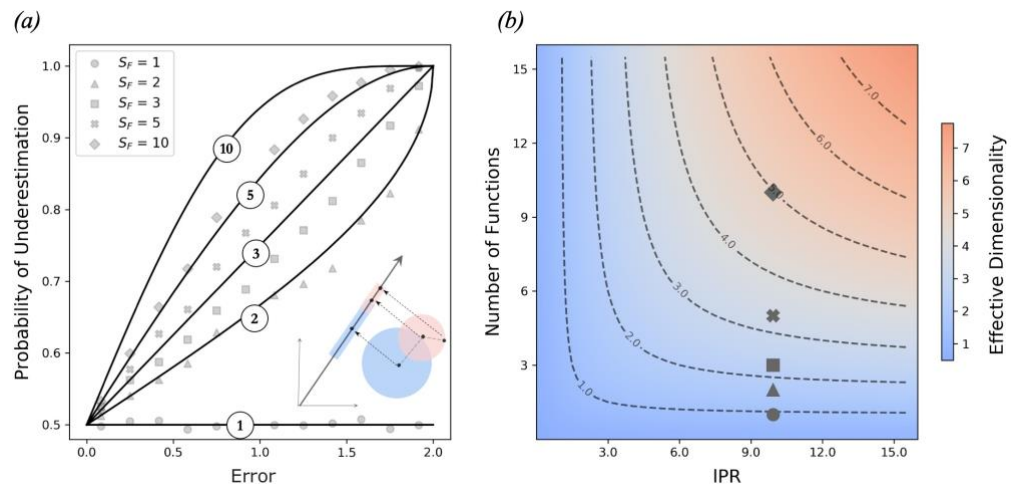


Figure C.1: Multifunctional approach. **(a)** The relationship between prediction error caused by uncertainty and the probability of underestimation for five simulations each scaling up predictions to a different number of aggregate properties (S_F). A community of 20 species, with IPR of 9.9, experienced change in biomass over 50,000 simulations. Unbiased predictions at the species level were scaled up to the community level using 1, 2, 3, 5 and 10 randomly drawn aggregate properties. Simulated results fall short of theoretical expectations for the probability of underestimation when the effective dimensionality is presumed to be the number of functions. The blue and red circles being projected onto a blue and red line represents a 2-D system being projected into 1-D functional space. **(b)** There is an interaction between the number of functions and the underlying dimensionality (IPR), which is illustrated by the heat-map. Usually the effective dimensionality is determined by the lower value of S_F and IPR. However, when these values are similar (e.g. diamond: 10 functions and IPR of 9.9) the effective dimensionality (~ 5) is much lower than either value.

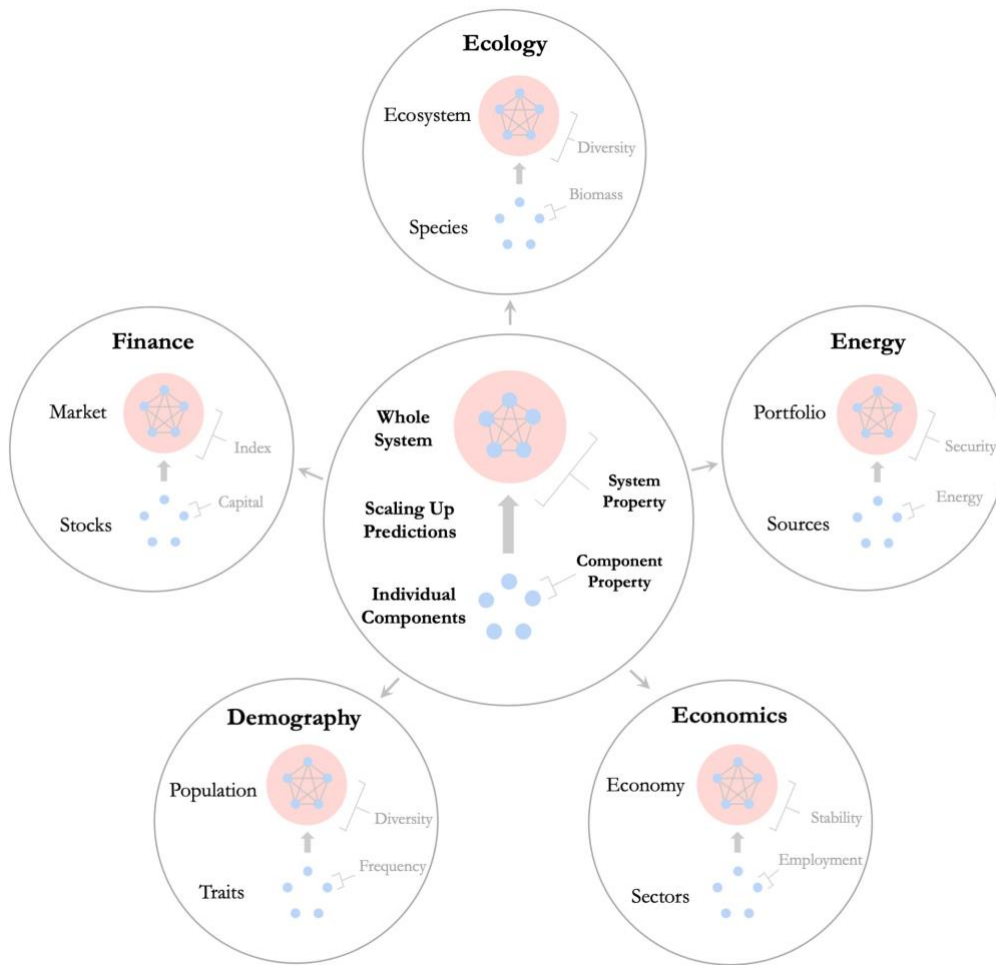


Figure C.2: Generalisation to other fields of science. Our findings could be relevant to other fields of science where: (i) there is interest in predicting change of complex systems based on knowledge about their individual components, and (ii) systems are described using multivariate coordinates and/or using non-linear properties of individual components. In **economics**, a region's economy can be viewed as a complex system comprised of individual sectors (e.g. agriculture, tourism, technology). Predictions of how employment numbers will change in individual sectors due to some perturbation could be scaled up to predictions of change of economy-level properties of interest such as stability, measured as, for example, the evenness of employment across sectors (Dissart, 2003; Halpern et al., 2012; Malizia & Ke, 1993). In the study of **energy supply**, different fuel or energy sources of a country (e.g. solar, wind, oil) can be considered together in a country's energy portfolio. Predictions of change of energy generation in each individual source could be scaled up to predictions of change of portfolio-level properties. Energy security is a system-level property of great interest that is quantified using diversity metrics (Chalvatzis & Ioannidis, 2017; Stirling, 1994) or variance-based approaches (Roques et al., 2008) based on *Mean-Variance Portfolio Theory*, which was originally developed to study risk or volatility of investment portfolios (Markowitz & Todd, 2000). In **demography**, populations can be thought of

as systems comprised of multiple different groups that are defined by traits (e.g. gender, age, ethnicity). Again, diversity is a system-level property of great interest in the study of populations that is quantified using non-linear aggregate functions (Reardon & Firebaugh, 2002; White, 1986). Changes in diversity of human populations is pertinent to many social sciences including sociology, economics and politics. In **finance**, markets are complex systems whose individual components are stocks. Predictions of how the capital of individual stocks will change could be scaled up to predictions of how stock market indices will change. Certain stock market indices, for example diversity-weighted indices, are non-linear aggregate properties that will be sensitive to dimensional effects (Chow et al., 2011; Fernholz et al., 1998). At a different financial scale, our theory may also be relevant to the study of investment portfolios. Here, analogous to energy security, portfolios are systems comprised of individual assets and the volatility or risk tolerance of a portfolio (measured using non-linear aggregate properties) is of great interest to investors (Bera & Park, 2008; Markowitz & Todd, 2000).

C.3 DETAILS OF SIMULATIONS

Initially, the theoretical relationship between error, underestimation and dimensionality was tested using numerical simulations. These simulations uniformly sampled the intersecting circles, spheres and hyper-spheres defined by a prediction of change and relative error (Figure. 4.2). This was done for 1-D, 2-D, 10-D and 20-D systems over 20,000 simulations. Specifically:

- a prediction of change and was randomly generated from a normal distribution of mean 0 and standard deviation 1 (defining the blue circle in Figure 4.2a).
- a direction of error was randomly generated from a normal distribution of mean 0 and standard deviation 1, and a magnitude of error was randomly generated from a uniform distribution between 0 and 2 (defining the red circle in Figure 4.2a).
- From these values, error (x) and underestimation (y) were calculated based on Euclidean distance and subsequently plotted in Figure 4.2c.
- The probability of underestimation $\mathbb{P}(y > 0; x)$ was calculated from the simulated results of error and underestimation.

As a next step, these simulations were modified to fit ecological problems. In Figure 4.1 and Figure 4.4 the intersecting shapes that are uniformly sampled had dimensions determined by the number of species in a simulated community. However, the dimensions of state space were given unequal weighting of how they respond to change in the form of uneven biomass distributions randomly generated from a log normal distribution of mean 0 and standard deviation 0.05.

In Figure 4.3 communities of 50 species were given unequal biomass distributions by drawing species' biomass from a log scale of varying range; the wider the range of the log scale the more uneven the biomass distribution. Underestimation (y) was calculated using Euclidean distance and a number of ecological relevant aggregate properties: the Shannon index (diversity), invariability (stability) and total biomass (functioning).

For Figure C1 our simulations were modified to illustrate that additional dimensional effects come into play when changes in multiple functions are considered at once. Over 50,000 simulations 20-D hyper-spheres (community of 20 species) with unequal weighting (IPR of 9.9) were uniformly sampled and the results were projected into functional space. Specifically, underestimation was measured for 1, 2, 3, 5 and 10 aggregate functions. Linear aggregate functions of the form:

$$F(B) = \sum_{i=1}^S F_i B_i$$

were defined via the coefficients F_i , i.e. their sensitivity to the change in the biomass of species i . The sensitivity of an aggregate function to each species was randomly drawn from a normal distribution of mean 0 and standard deviation 1. This corresponds to the case of statistically independent functions. State space was then defined by the number of functions. Simulations were conducted in Python with the Matplotlib, NumPy and SciPy libraries. Code is available in a *Jupyter* Notebook on GitHub: <https://github.com/jamesaorr/scaling-up-uncertain-predictions>.

APPENDIX D | SUPPLEMENTARY MATERIAL FOR CHAPTER 5

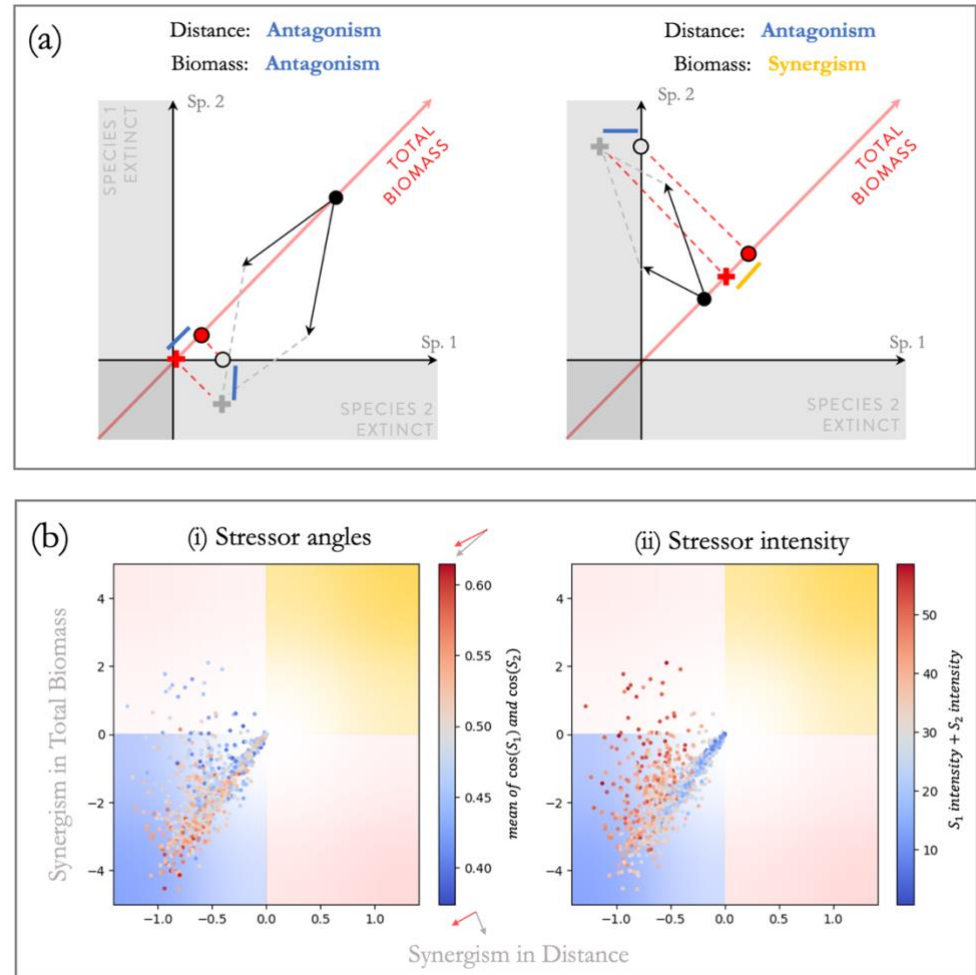


Figure D.1: The effect of stressor intensity. **(a)** Conceptual model of the effect of stressors on a community of two non-interacting species as in (Figure 5.2a). Here, the aggregate function total biomass (i.e. the sum of biomass), is represented by the red line. To quantify change using total biomass points in state-space are projected onto this 1D linear function. When the stressors, and therefore the non-additivity in distance, is roughly co-linear with the function of total biomass, the correlation between synergism in distance and synergism in total biomass will be strong. When, however, the stressors are orthogonal to total biomass (as can be the case for opposing stressors), there can be a mismatch between the synergism observed by the two metrics. **(b)** Synergism in distance plotted against synergism in total biomass for 1000 combinations of mixed stressors with strong negative skew in direction and a high intensity. Here, the system is closed to invasions so extinctions are the only source of non-additivity. Nonetheless,

total biomass can observe synergism when the stressors are orthogonal to total biomass (blue points in (i)) and have high intensity (red points in (ii)). Increasing intensity thus erodes the correlation between the synergism observed by these metrics by allowing the more positive stressors, that are less co-linear to total biomass, to reach the extinction threshold and cause non-additivity.

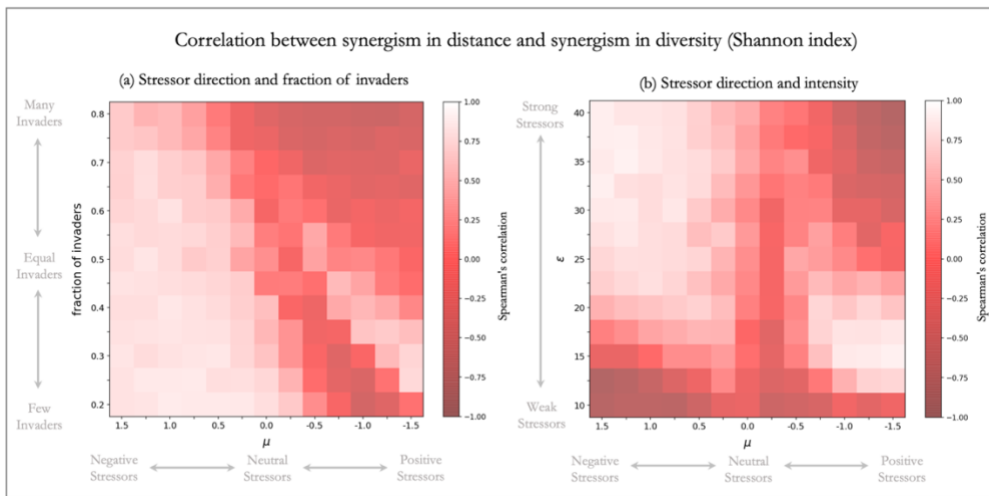


Figure D.2: Correlation between synergism in distance and in diversity, specifically Shannon index, for different stressor directions and **(a)** different fractions of invaders and **(b)** different stressor intensities. The colour of each square in the heatmap represents the Spearman's rank correlation between synergism in distance and synergism in diversity for a set of 500 pairs of stressors.