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Shaped by the past

Resilience and ecological memory in microbial communities

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Shaped by the past: Resilience and ecological memory in microbial communities

Abstract

Microorganisms play a fundamental role in global biogeochemical cycles, which are increasingly being altered by the current rapid changes in the environment, threatening biodiversity and the provisioning of key ecosystem services. This thesis addresses key uncertainties surrounding the assessment of ecological resilience and the role of previous disturbances in shaping the stability especially of microbial communities to future disturbances. Firstly, a quantitative framework was developed for the assessment of ecological resilience, which combines current knowledge into a reiterative approach that gradually reduces uncertainty about the level of resilience in a system. Following this, I conducted laboratory experiments in which bacterioplankton communities were exposed to repeated disturbances, to investigate the role of disturbance history in shaping functional and structural stability under subsequent disturbances. I found that disturbance history can affect functional stability in response to both new and familiar disturbances, providing evidence of an "ecological memory" effect which then declined over time. In contrast, microbial community composition appeared to be dominated by a stochastic process, i.e. drift. This suggests the more deterministic responses of the microbial functions arose from sub-lethal effects of the disturbance on microbial activities rather than changes in community composition. These results highlight potential uncertainties associated with application of resilience frameworks developed for multicellular communities to microbial organisms, especially where non-deterministic drift dominates microbial community assembly, and variation in community structure is not strongly connected with variation in functioning.

Keywords: Ecological resilience, stability, bacterioplankton, community assembly, ecological memory, disturbance history, drift

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Historiens avtryck: Motståndskraft och ekologiskt minne i mikrobiella samhällen

Sammanfattning

Pågående miljöförändringar hotar ekosystemens förmåga att tillförlitligt tillhandahålla ekosystemtjänster, nu och i framtiden. Mikroorganismer spelar grundläggande roller i biogeokemiska kretslopp, vilka i sin tur är väsentliga för andra ekosystemtjänster. Syftet med den här avhandlingen är att besvara några av de viktiga frågorna kring bedömningen av ekologisk motståndskraft och hur tidigare störningar påverkar (mikrobiella) samhällens stabilitet vid framtida störningar. Ett kvantitativt ramverk utvecklades för bedömning av ekologisk motståndskraft. Metoden reducerar osäkerheten i uppskattningen av graden av motståndskraft i ett system genom upprepade mätningar av stabiliteten i systemet. För att undersöka vikten av störningshistorik för hur ekosystem påverkas av efterföljande störningar, utsattes bakterieplanktonsamhällen för upprepade störningar i laboratorieförsök. Dessa studier visar att störningshistorik kan leda till ökad funktionell stabilitet hos mikrobiella samhällen som utsätts för samma sorts störning på nytt eller för en ny sorts störning. Försöken visade också att denna skyddande effekt minskar över tid, och att den skiljer sig mellan olika ekosystemprocesser. Dessutom tycktes ekologisk drift spela en viktig roll för bakteriesamhällens sammansättning vid störningar, medan funktioner påverkades på ett mer deterministiskt sätt. Detta tyder på att sub-letala effekter av störningen har större betydelse för mikrobiella processer än förändringar i samhällens sammansättning. Slutligen visar mina resultat att det kan vara mer komplicerat att uppskatta graden av ekologisk motståndskraft hos mikrobiella jämfört med multicellulära organismsamhällen, särskilt om icke-deterministisk drift påverkar samhällssammansättningen, som i mina experiment.

Nyckelord: Ekologisk motståndskraft, stabilitet, bakterieplankton, samhällsammansättning, ekologiskt minne, störningshistorik, drift

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Gevormd door het verleden: Veerkracht en ecologisch geheugen bij microbiële gemeenschappen

Abstract

Toenemende milieuveranderingen bedreigen het vermogen van ecosystemen om ecosysteemdiensten te leveren. Micro-organismen spelen een fundamentele rol in mondiale biogeochemische cycli die ten grondslag liggen aan de ecosysteemdiensten. Het doel van dit proefschrift is om de kennis over ecologische veerkracht en de rol van eerdere verstoring by het vormen van de stabiliteit van ecosystemen, inclusief microbiële gemeenschappen, bij blootstelling aan verdere verstoringen, te vergroten. Hiervoor is een methode ontwikkeld, die door de huidige kennis te combineren en het herhaaldelijk verrichten van metingen het begrip van de ecologische veerkracht van een ecosysteem geleidelijk verbetert. Daarnaast werden bacterieplanktongemeenschappen in het laboratorium blootgesteld aan herhaalde verstoringen, om te onderzoeken hoe eerdere verstoringen de stabiliteit beïnvloeden bij verdere verstoringen. Deze studies tonen aan dat de eerdere verstoringen de stabiliteit van sommige ecosysteemprocessen kan verhogen bij blootstelling aan zowel nieuwe als bekende verstoringen, en dat dit effect in na verloop van tijd afneemt. Daarentegen lijkt de soortensamenstelling van microbiële gemeenschappen vooral gevormd te worden door een stochastisch process, d.w.z., drift. Dit wijst er op dat de meer deterministische reacties van ecosysteemprocessen op de verstoringen eerder het gevolg zijn van sub-letale effecten dan van veranderingen in de soortensamenstelling. Als gevolg kan het meten van ecologische veerkracht bij mico-organismen ingewikkelder zijn dan bij meercellige organisment, met name wanneer de soortensamenstelling sterk wordt gestuurd door stochastische processen.

Trefwoorden: ecologische veerkracht, stabiliteit, bacterieplankton, gemeenschapsvormende processen, ecologisch geheugen, verstoringen, drift

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Dedication

To my family ~ the one I was given and the ones I chose

“I think I’m going to need a bigger flowchart.”

The Doctor

Contents

List of publications

This thesis is based on the work contained in the following Papers, referred to by Roman numerals in the text:

D. L. Baho, C. R. Allen, A. S. Garmestani, H. B. Fried-Petersen, **S. E. Renes**, L. Gunderson, and D. G. Angeler (2017). A quantitative framework for assessing ecological resilience. Ecology and Society, 22 (3):17

S.E. Renes*, J. Sjostedt*, I. Fetzer and S. Langenheder (2020). Disturbance history can increase functional stability in the face of both repeated disturbances of the same type and novel disturbances. Scientific Reports, 10 (1), pp. 11333-11333

S. E. Renes, E. S. Lindström, H. B. Fried-Petersen, S. Langenheder, C.R. Allen, D. G. Angeler, B. G. McKie (Manuscript). Ecological memory and the functional responses of microbial communities to disturbance

S. E. Renes, B. G. McKie, H. B. Fried-Petersen, S. Langenheder, D. G. Angeler, E. S. Lindström (Manuscript). Microbial community composition under disturbance: deterministic or stochastic responses?

Papers I-II are reproduced with the permission of the publishers.

*Shared first authorship

The contribution of Sophia Renes to the Papers included in this thesis was as follows:

I. SR contributed to idea development and writing.

II. SR and Johanna Sjöstedt contributed equally to this Paper, and had an equal responsibility for acquisition and analysis of the data and drafting the manuscript. SR also contributed to conception/design of the study, interpretation of the data and manuscript revision.

III. SR had shared responsibility for the study design and main responsibility for planning and performing the experiment, data analysis and interpretation as well as drafting the manuscript and incorporating comments and revisions from coauthors.

IV. SR had shared responsibility for the study design and main responsibility for planning and performing the experiment, data analysis and interpretation as well as drafting the manuscript and incorporating comments and revisions from coauthors.

1. Introduction

We face multiple, interlinked ecological and societal challenges today, arising from environmental change caused by human activities. Among these, climate change is expected to lead to more extremes in basic environmental variables, including temperature and pH (IPCC, 2014). Environmental change endangers our ecosystems, and poses a threat to the ecosystem processes and functions underpinning ecosystem services, many of which are performed or mediated by microbial organisms (Rousk & Bengtson, 2014; Gessner *et al.*, 2010; Millennium Ecosystem Assessment, 2005; Folke *et al.*, 2004). Environmental impacts arising from climate change, and the resulting loss of species can trigger sudden catastrophic changes, causing an ecosystem to shift to another regime in which other processes and species dominate the ecosystem (Folke *et al.*, 2004; Scheffer *et al.*, 2001). This often comes at a high cost to society, and can be very difficult, if not impossible, to reverse.

In our attempts to understand and predict the effects of environmental change on ecosystems, many different perspectives have arisen as part of different research fields within ecology (Grimm & Wissel, 1997), which are now increasingly being drawn together (Donohue *et al*., 2016; Truchy *et al*., 2015). Two main lines of thinking regarding ecosystems response to environmental change are discussed in this thesis. One view has the main focus on the stability of ecosystems, where a key view during initial development of the theory was that ecosystems fluctuate around an equilibrium, and measures stability as particular aspects of the deviations from this equilibrium (Donohue *et al.*, 2016; Gunderson, 2000). The other view is based on the observation of the occurrence of so-called regime shifts. Regime shifts are seen as evidence that the above assumption of ecosystem

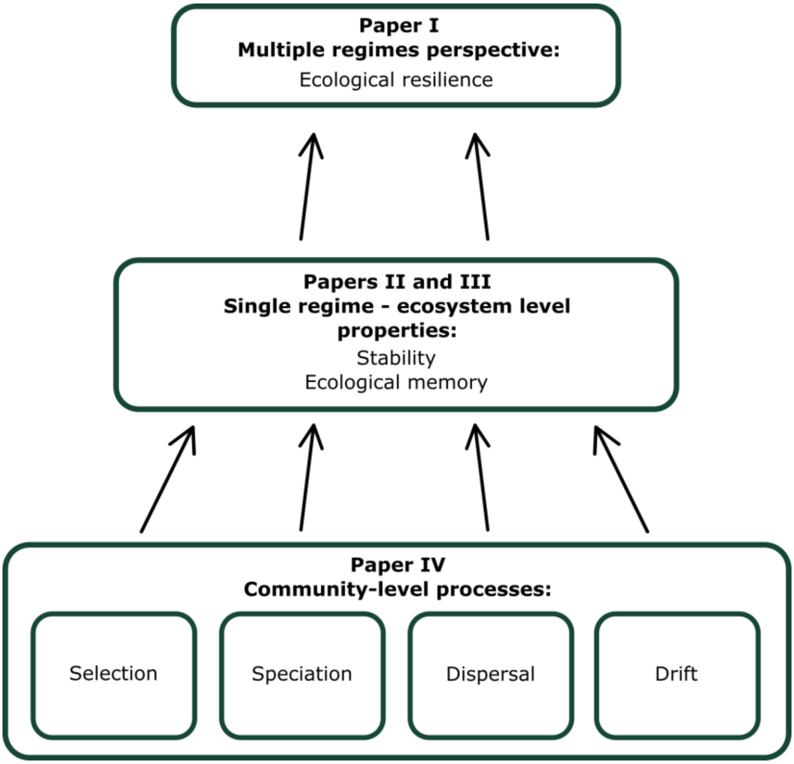


Figure 1. Schematic of the theoretical perspective of the papers in this thesis and the relationship between them.

equilibrium in a single regime does not hold. Instead the second framework uses the concept of ecological resilience, which is the strength of a disturbance an ecosystem can withstand before undergoing a regime shift (Gunderson, 2000). Both perspectives have in common that innate characteristics of ecosystems determine how well they respond to environmental change. As such, ecosystem responses can be affected by past events that shape the structure and processes in ecosystems, and as a result, determine the response to future conditions (Johnstone *et al.*, 2016; Bengtsson *et al.*, 2003). This concept, ecological memory, will be another major focus of this thesis. Further, underpinning any kinds of changes in biological communities are the community assembly processes selection, dispersal, drift and speciation (Vellend, 2010).

This thesis will move from the broadest to the narrowest perspective through these levels of theory on how ecosystems are shaped by and respond to the environment (Fig. 1). **Paper I** takes the view of ecological resilience as an emergent ecosystem property that takes into account the possibility of multiple regimes, while **Papers II and III** focus on the single regimes perspective, addressing ecological stability and ecological memory. Finally, **Paper IV** touches on the underlying mechanisms of community assembly. While **Paper I** is general and conceptual in scope, **Papers II, III and IV** are based on experimental work with microbial communities. Because of their small size and short generation times, microbial organisms are very suitable model organisms for ecological research, allowing for experimental manipulation and measurement of large numbers of organisms over a large number of generations in a controlled environment (Jessup *et al.*, 2004). However, they are worth studying in their own right, because of their key importance for biogeochemical cycles, such as the carbon and nitrogen cycles. They contribute both directly and indirectly to degradation of organic matter, primary production and nitrogen fixation (Rousk & Bengtson, 2014; Nazaries *et al.*, 2013; Offre *et al.*, 2013; Gessner *et al.*, 2010).

1.1 Disturbance, ecological resilience and stability

Research on ecosystem response to environmental change often uses some kind of term describing a change that might impact an ecosystem. Terms such as perturbations, pressures, stressors and disturbances are used in various ways to indicate events or conditions in the environment that impact ecosystems either via long-lasting or short-term exposure (Borics *et al.*, 2013). The definition of *disturbance* used in this thesis is "[…] a discrete, punctuated killing, displacement, or damaging of one or more individuals (or colonies) that directly or indirectly creates an opportunity for new individuals (or colonies) to become established" (Sousa, 1984). In reality, ecosystems often face various types of environmental disturbances occurring simultaneously and/or in sequence, which can cause non-additive effects (Herren *et al.*, 2016; Odum, 1981). The experiments in this thesis focus on sequential disturbances, primarily repeated disturbances of the same type, but sometimes followed by a second type.

In the stability point of view, based on the idea that ecosystems and ecosystem processes fluctuate around a single equilibrium, the response of an ecosystem to disturbances is often divided into a number of measurable components. *Resistance* (or insensitivity) describes the level of change directly after exposure to a disturbance, *resilience* (also called engineering resilience), is the rate at which the ecosystem parameter returns to its original or baseline value, *recovery* is the degree to which the system returns to its original state (often measured at the end of an experiment), and *temporal variability* is the variation over time (Hillebrand *et al.*, 2018; Donohue *et al.*, 2016; Shade *et al.*, 2012).

In contrast with this view on stability, the concept *ecological resilience* acknowledges the possibility that ecosystems do not return to a single equilibrium (within a "single regime") after a disturbance, but rather might undergo shifts between different regimes. Such a regime shift moves the ecosystem towards a new equilibrium that is fundamentally different than the first (Gunderson, 2000). Ecological resilience is defined as "[…] a measure of the amount of change needed to change an ecosystem from one set of processes and structures to a different set of processes and structures" (Angeler & Allen, 2016). A common way to illustrate the concept of ecological resilience, is the ball-in-cup metaphor (Scheffer *et al.*, 1993). The ball represents the ecosystem state, and the cup is the regime, or domain of attraction. Smaller disturbances push the ball up the sides of the cup, after which it returns back to the center, but large disturbances can push the ball over the edge of the cup, after which is falls into the next cup. This represents a shift to a different regime, which might be hard to return from if the cup is deeper than the first (i.e. it is more resilient). Besides very strong disturbances, long-term environmental change can erode the resilience of the system, making the cup shallower, so a small disturbance is enough to cause a regime shift (Scheffer *et al.*, 1993). The two views are by no means mutually exclusive, and are often used in combination (e.g. Shade *et al.*, 2012; Ives & Carpenter, 2007).

There are still many uncertainties involved in quantifying ecological resilience in a way that can meaningfully inform management of ecosystems (Cumming *et al.*, 2005), therefore one aim of this thesis was to set up a framework for quantifying ecological resilience (**Paper I**).

1.2 Ecological memory

Various definitions of *ecological memory* include the species, individual organisms, interactions, adaptations as well as the materials and abiotic structures that are present in an ecosystem after a disturbance, and that determine the response of an ecosystem to future disturbances (Johnstone *et al.*, 2016; Bengtsson *et al.*, 2003; Nystrom & Folke, 2001). Or more simply put, ecological memory is “the ability of the past to influence the present trajectory of ecosystems” (Hughes *et al.*, 2019). An understanding of the influence of past disturbances on current ecological responses will help in managing ecosystems.

Different mechanisms have been proposed for how past disturbances can affect ecosystem response to subsequent disturbances. *Acquired stress resistance* in microbes, also called stress priming, is a phenomenon where exposure to a disturbance triggers a physiological response in the cell that increases the organism’s chance of surviving a subsequent, possibly stronger, disturbance, even of a different type (Andrade-Linares *et al.*, 2016). A recent meta-analysis on acquired stress resistance in microbes found that previous exposure to a disturbance increased survival in the face of a subsequent disturbance by a factor ten, irrespective of the combination of disturbance type (Andrade-Linares *et al.*, 2016). This type of research has typically been performed in pure culture, but acquired stress resistance is likely to have long-term consequences for communities, since differences in the ability to gain this type of resistance, as well as the cost involved, can lead to fitness differences between organisms (Rillig *et al.*, 2015).

In environmental risk assessment the concept *pollution-induced community tolerance* is used as a method to determine how severely a biological community is affected by a contaminant. The theory is that tolerant species survive exposure, so when a community is experimentally exposed to a known contaminant, high tolerance in the community indicates that the community has been degraded, i.e. the sensitive species were already gone due to previous exposure (Tlili *et al.*, 2016; Blanck, 2002). This concept has been expanded to disturbance research with the term *stress-induced community tolerance*. Vinebrooke *et al*. (2004) described a theoretical framework where the degree and sign of the correlation between the tolerances of species in a community to separate disturbances determines the effect of one disturbance on the tolerance of the resulting community to the second disturbance. This correlation is called *co-tolerance*. In the case of positive co-tolerance, exposure to one disturbance results in a community that is more tolerant to a subsequent disturbance, while negative co-tolerance will result in a community that is more sensitive (Vinebrooke *et al.*, 2004; Blanck, 2002).

Positive co-tolerance can possibly be explained by differences in niche width between organisms (Sjostedt *et al.*, 2018). Niche width is a measure of habitat specialization, where the niche width of generalists is high, as they are able to live in a wider variety of habitats. Pandit *et al.* (2009) found that generalists are less affected by local environmental factors than specialists, suggesting that disturbances should select for generalists. This is supported by several disturbance studies in microbial systems that have shown increased tolerance and substrate use after exposure to disturbances, suggesting that generalists became more dominant in the communities (Atlas *et al.*, 1991; Mills & Mallory, 1987).

For some of these mechanisms, the duration of this effect is explicitly considered (Andrade-Linares *et al.*, 2016; Hilker *et al.*, 2016). Others have also proposed that the effects of past environmental conditions on communities are likely to be transient (Vass & Langenheder, 2017). There have been calls for long time-series studies to help disentangle the effects of past environmental conditions on current ecosystems (Langenheder & Lindstrom, 2019). This thesis addresses the effects of repeated disturbances on the response to a subsequent disturbance of the same type (**Papers II, III and IV**) and of a different type (**Paper II**), as well as the role of time between these disturbances (**Paper III and IV**).

1.3 Community assembly processes

The field of community ecology has produced a very large number of theories to describe the processes regulating community assembly (Vellend, 2016). In recent years, efforts have been made to simplify these into a general theory of community ecology (Vellend, 2010). Vellend (2010) proposes that all community assembly processes can be summarized into the four *high-level processes*: 1. Selection; 2. Dispersal; 3. Drift; and 4. Speciation, analogous to the four major processes in population genetics. All other processes are considered *low-level processes* and are special cases of one or more of the high level processes (Vellend, 2016; Vellend, 2010). *Selection* describes a deterministic force where community changes are caused by differences in fitness between individuals. *Dispersal* is the movement of individuals into and out of a habitat, where the number and identity of these individuals determines how the taxonomic composition of the community is changed. *Drift* is a process where stochastic birth and death events change a community in non-deterministic ways. *Speciation*, finally, is the process by which new species come into existence (Vellend, 2016; Vellend, 2010). This framework has been readily accepted in the field of microbial ecology (Zhou & Ning, 2017; Hanson *et al.*, 2012). However, some modifications of the framework have been proposed for use in microbial ecology. For example, the term *diversification* is sometimes used instead of speciation, due to the problematic species concept in asexual microbes (Zhou & Ning, 2017). In addition, some have argued that no distinction should be made between ecological and evolutionary processes, because selection can work on multiple levels (e.g. genes, individuals and taxa) for many microbial organisms, (Hanson *et al.*, 2012). For the sake of simplicity, this thesis will use the terms as defined by (Vellend, 2010).

While there is inconsistent evidence regarding the importance of the various community assembly processes in microbial ecosystems, selection is still thought to be the dominant processes in most cases (Langenheder & Lindstrom, 2019; Lindstrom & Langenheder, 2012; Martiny *et al.*, 2006). One aim of this thesis was to investigate the role of stochastic (e.g. drift) versus deterministic (e.g. selection) community assembly processes in microbial communities undergoing disturbances (**Paper IV**).

2. Objectives

The aim of this thesis was to address key uncertainties surrounding the assessment of ecological resilience and the role of previous disturbances in shaping the stability of (microbial) communities in response to future disturbance. First, I present a framework of methods for assessing and managing for general resilience in ecosystems (**Paper 1**). This is followed by experimental studies using microbial communities as model ecosystems, allowing us to investigate the functional and structural responses of microbial communities to disturbances. This includes a focus on the role of ecological memory effects arising from exposure to previous disturbances, the types of disturbances the communities are exposed to, community assembly processes, and the degree of concordance of structural and functional responses (**Papers II-IV**).

Specifically, I aimed to:

- create a quantitative framework for assessing ecological resilience (**Paper I**)
- investigate the effects of disturbance history on functional and compositional stability of microbial communities in response to a familiar or novel disturbance (**Paper II, III and IV**)
- assess the importance of the time since last disturbance for the response to a subsequent disturbance (**Papers III and IV**)
- explore the role of stochastic versus deterministic community assembly processes in microbial communities undergoing disturbances (**Paper IV**)

3. Methods

Here I present an overview of the key methods used in this thesis. Note that **Paper I** is not listed here as it was primarily a conceptual article that did not involve any specific experimental or sampling methodology. For more details on the methods described below, please see **Papers II-IV**.

3.1 Study design

In **Paper II**, bacterioplankton communities were grown in semi-continuous culture and exposed to weekly temperature disturbances of either 25°C or 35°C for 15 h, or a control treatment without temperature change, for four weeks (Fig. 2). After this the content of each of the four replicate communities per temperature treatment group was split into two, and one community from each pair was then exposed to an acidification event where the pH was lowered from pH 8 to 6. The communities were then allowed to recover for one more week at background laboratory conditions (in the dark, at 13 °C; Fig. 2). Samples for analysis of bacterial abundance and bacterial production were taken daily during the first four weeks of the experiment and four times during the last week (after the pH disturbance). Samples for community analysis and β-glucosidase and cellobiohydrolase activities were taken once per week during the whole experiment.

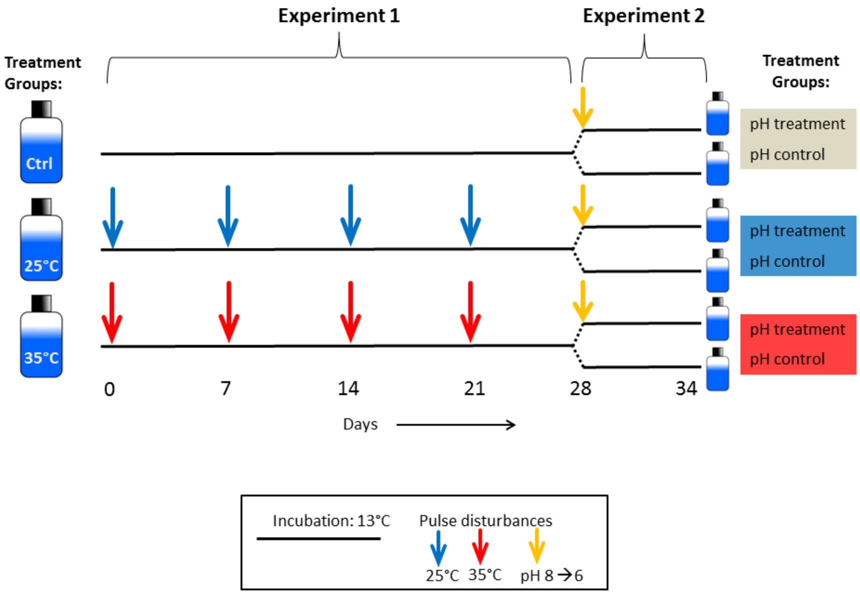


Figure 2. Experimental design for the experiment in **Paper II**. Horizontal lines represent the timeline for each treatment group. After four weekly temperature pulse disturbances (heating to 25°C, 35°C, or control, no heating), each of four replicate communities in a treatment group was split in two. One half of each pair was then exposed to an acidification event (pH 8→6) and allowed to recover for one week. Figure from **Paper II**.

Papers III and IV are based on a single experiment, performed in continuous culture, with 4 replicates each in 2x3 levels of experimental treatments. Bacterioplankton communities were exposed to one of three exposure history treatments during the first three weeks of the experiment: a *background* treatment, which was held at background laboratory conditions (in the dark, at 20°C), and the *long* and *short recovery treatments*, which were both exposed to three mild salinity pulse disturbances (5 ppt NaCl) over the timespan of a week (Fig. 3). The *long recovery treatment* was then allowed a two-week recovery time and the *short recovery treatment* was allowed one week of recovery before four of the eight replicate communities from each exposure history treatment was exposed to the ‘main pulse’ disturbance (15 ppt NaCl, *pulse* replicates), while the other four were left at background laboratory conditions (*no pulse* replicates, Fig. Y). After the main pulse, the communities were allowed to grow at background

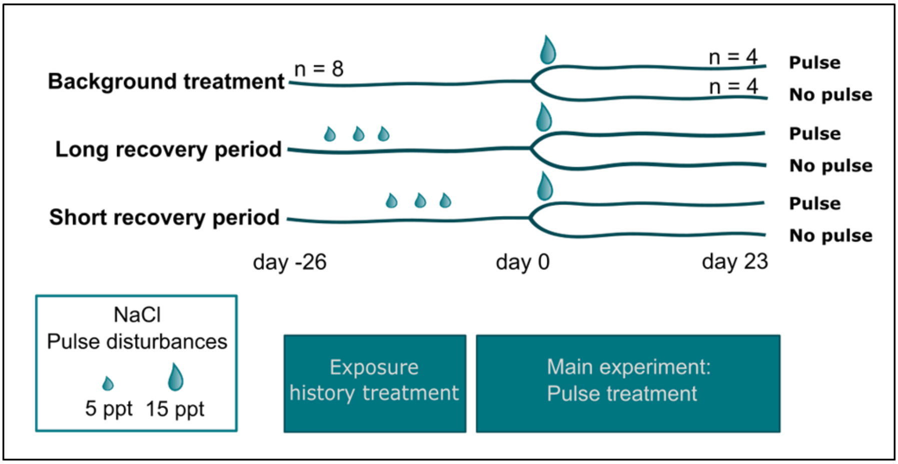


Figure 3. Experimental design used in **Papers III and IV**. Horizontal lines represent the timelines for the six treatment groups (four replicates each). Small drops represent a series of three 5ppt salinity pulses (‘Exposure history treatment’) over a timespan of one week during the exposure history period. The large drop represents a single 15 ppt salinity pulse, counted as day 0 in **Paper III** (‘Pulse treatment’). Assessment of community functioning for **Paper III** took place every second day during the ‘main experiment’, which started 5 days before the pulse treatment. Samples for analysis of community composition for **Paper IV** took place every second day throughout the whole experiment, including the period during the exposure history treatment. Positive and negative numbers indicate days after and before the main pulse disturbance, respectively. Figure from **Paper III**.

laboratory conditions for another 26 days (Fig. 3).

Paper III focuses on measurements of ecosystem functions taken every second day during the ‘main experiment’ (Fig. 3), starting 5 days before the main pulse (15 time points). **Paper IV** follows the change in community composition, also sampled every second day, from the start of the exposure history period until the end of the experiment (26 time points).

3.2 Seeding communities and growth medium

The seeding community and lake water used as growth medium for **Paper II** came from Lake Ekoln near Uppsala, Sweden. The growth medium was prepared by sterile-filtering the lake water (0.2 µm filters) and autoclaving it twice before use. The inoculum was filtered through a 0.7 µm filter and allowed to acclimate at 13°C for two days before starting the experiment.

Figure 4. The source of the seeding community and carbon amendment for the growth medium. Water is being pumped out of the stream, through a series of filters, to the reverse osmosis machine (not shown).

The seeding community for **Papers III and IV** came from a forest stream upstream of Fiby Lake in the Uppland region of Sweden (Fig. 4). The bacterial communities were filtered through 0.7 µm filters to remove large particles and potentially predatory organisms. An artificial lake water medium was prepared according to Bastviken (2004), with dissolved organic matter from the sampling location of the seeding communities as a carbon source. The carbon was collected using a portable reverse osmosis machine (Fig. 5) and autoclaved before use. Before starting the experiment, we allowed the communities to acclimate to laboratory conditions, first 4 days in batch culture (without addition of medium), after which the inflow of sterile medium was started in the continuous culture setup, and they were allowed to acclimate in continuous culture for an additional two and a half weeks. After the acclimation period, the content of the 24 continuous culture vessels was homogenized and redistributed over the vessels just prior to starting the exposure history treatments.

Figure 5. Portable reverse osmosis machine in action. This kind of machine is usually used for water purification. However, we discarded the ‘pure’ water permeate and collected the retentate (dissolved matter from the stream) into a bucket in order to concentrate it many times in order to facilitate transportation and storage of large quantities of natural dissolved organic matter for reproducible use in experiments.

3.3 Culture methods, sampling and disturbances

The experiment in **Paper II** was performed in semi-continuous culture with a daily 20% medium exchange rate. The first part of the experiment was performed in 500 µL volumes in 1 L bottles, and the second part of the experiment was performed in half volumes, after splitting each replicate community in half. Samples for analysis of functional and compositional parameters were taken from the medium that was removed during medium exchange. The temperature disturbances were applied to the cultures by moving them into incubators set to the desired temperature for 15 hours. The pH disturbance was administered by pipetting a sterile HCl solution into the bottles under sterile conditions.

The experiment in **Paper III and IV** was performed using a continuous culture setup in 200 mL volumes and a dilution rate of 0.02 h^{-1} (Fig. 6). Every second day, 20 mL samples were taken from the water column using a mounted syringe. For the disturbances, an autoclaved NaCl solution was administered to the vessels through the same mounted syringe.

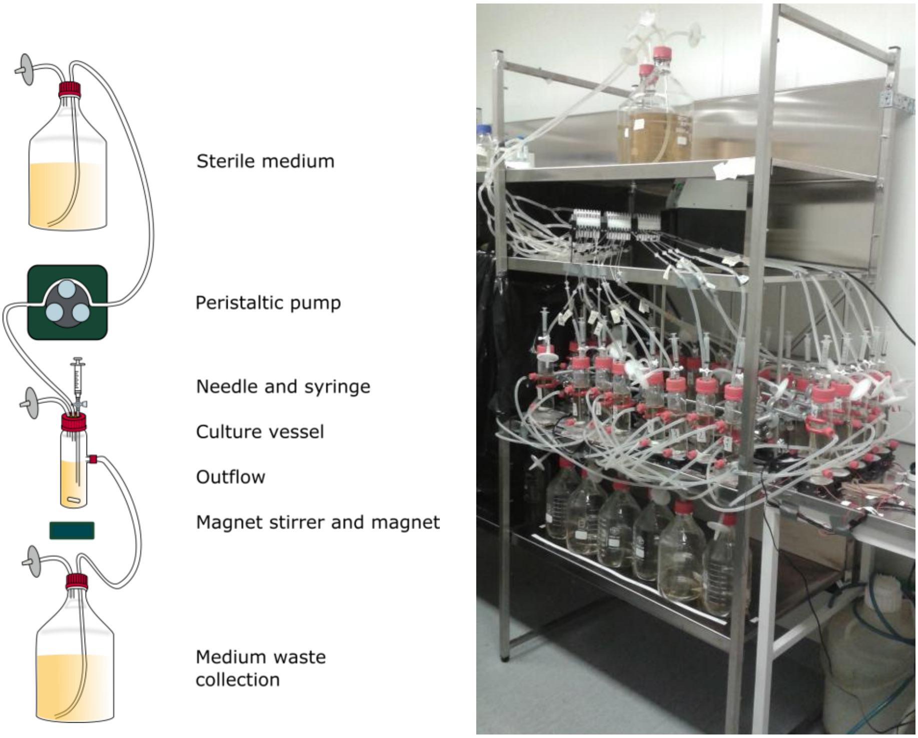


Figure 6. Continuous culture set-up. Left, schematic: Sterile medium was pumped from large medium bottles into culture vessels using a peristaltic pump. The medium entered the culture vessel from the top, and excess medium flowed out on the side. The cultures were stirred using magnet stirrers. Samples were taken and salinity disturbances were applied through a mounted needle and detachable syringe. Medium outflow was collected in waste bottles. All bottles had an air filter, allowing exchange of sterile air to prevent pressure build-up. Right, reality: 24 culture vessels in a climate control room.

3.4 Measuring abundance and community functioning

In both **Papers II and III** we measured bacterial abundance, bacterial production, and the activities of the extracellular enzymes β-glucosidase and cellobiohydrolase. In addition, in **Paper III** we measured the activity of the extracellular enzyme leucine-aminopeptidase. We used the following methods:

- *Bacterial abundance:* flow cytometry (del Giorgio *et al.*, 1996);
- *Bacterial production:* leucine incorporation method (Smith & Azam, 1992);

- *Extracellular enzyme activities:* incubation with 4-methyllumbelliferone- and 7-amino-4-methylcoumarin- linked substrates (Ylla *et al.*, 2013).

3.5 Community composition and community metrics

Both experiments assessed community composition though MiSeq sequencing of the 16S rRNA gene. In **Paper II** the resulting sequences were clustered into operational taxonomic units (OTUs), while in **Paper IV** amplicon sequences variants (ASVs) were used for downstream analysis.

The following community and diversity metrics were calculated:

- Presence-absence and abundance-weighted *niche width* (Pandit *et al.*, 2009, **Paper II**);
- *Richness* and evenness based on OTUs (**Paper II**);
- *Richness*: mean number of ASVs of 100 rounds of subsampling the ASV sequence data down to 7137 reads per sample (**Paper IV**);
- *Aitchison distances*: a distance metric used by us to quantify the variation between replicate communities, as a measure of β-diversity (Gloor *et al.*, 2017). We calculated mean pairwise Aitchison distances for all possible pairs of replicates within each treatment group and time point (**Paper IV**).

3.6 Stability assessment and statistical analysis

Various statistical methods were used in **papers II- IV**. Here I only briefly present a few key methods, as well as methods used to assess functional stability.

In **Paper II** response ratios, the ratio between functional values in the disturbed treatment and those in the controls at the same time point, were used to determine functional resistance and recovery (Griffiths & Philippot, 2013). In a time point directly after a disturbance, the response ratio was interpreted as a measure of resistance, at later time points the response ratio was interpreted as a measure of recovery.

In **Paper III**, a mixed-model ANOVA was used to test the effects of *exposure history, pulse* and *time* on the functional response variables. The three-way interaction between these terms was the main term of interest. If significant, it would mean that exposure history affected the functional response to the pulse. The form of that functional response, based on four component of functional stability adapted from Donohue *et al.* (2016), were evaluated as follows:

- *Resistance*: the difference in level of functioning between the pulse and no pulse replicates from the same exposure history treatment on the day after the main pulse;
- *Recovery*: a treatment group that was sensitive on the day after the main pulse was considered to have recovered when the pulse replicates returned to at least 95% of the levels of functioning in the no pulse replicates on the same day;
- *Recovery time*: the time point at which the pulse replicates in an exposure history treatment group recovered to at least 95% of the no pulse replicates;
- *Long-term trajectories*: longer term divergence in levels of functioning between the pulse and no pulse replicates in the same exposure history treatment group in combination with a three-way interaction between exposure history, pulse and time indicates a difference in long-term trajectories between the different exposure history treatments.

In **paper IV** a linear mixed-effects model was performed using Aitchison distance as a response variable, in order to test whether the replicate communities within a treatment group became more dissimilar over time and whether the dissimilarity was less during exposure to disturbances. Exposure history, pulse and time were modelled as fixed effects and a new term ‘disturbance’ was added to describe the time points at which the communities were exposed to heightened salinity. A separate term, ‘pair ID’ was included as a random factor to account for differences in background variation between replicate pairs.

4. Results and discussion

4.1 A quantitative framework for assessing ecological resilience

The word “resilience” has been used in at least two ways in ecological theory: as engineering resilience, which is the rate of recovery to an equilibrium after a disturbance, and as ecological resilience, which is the level of disturbance an ecosystem can withstand before it reorganizes into an alternative regime, governed by different processes and structures (Gunderson, 2000; Holling, 1973). Unlike engineering resilience, ecological resilience takes into account the existence of alternative regimes. However, while the engineering resilience definition can be measured fairly easily, ecological resilience is difficult to quantify due to the complexity of the system behind it (Cumming *et al.,* 2005).

The aim of **Paper I** was to set up a framework to operationalize the ecological resilience concept for use in management. This framework first defines four attributes of ecological resilience, which are then used to formulate hypotheses about the measurable aspects of resilience in each of four possible scenarios (“premises”) of the level of resilience in the system. This framework uses a reiterative approach where repeated assessments of monitoring data, or multiple snap-shot measurements in the ecosystem are used as a basis for gradual improvement of knowledge about the resilience of the ecosystem and the effects of management interventions.

The four attributes of ecological resilience used in this framework are: scale, adaptive capacity, thresholds, and alternative regimes. The attribute *scale* describes the hierarchical organization by which ecosystem structures and dynamics are divided over particular scales in time and space (Allen *et* *al.*, 2014), which can be assed objectively using discontinuity analysis (Angeler & Allen, 2016). This is important to resilience assessments, since functional redundancy (e.g. Allen & Holling, 2008), effects of disturbances (Nash *et al.*, 2014) and response diversity (e.g. Tomimatsu *et al.*, 2013; Elmqvist *et al.*, 2003) can vary across scales and, as a result, contribute to ecological resilience.

Adaptive capacity is the ability of an ecosystem to remain in a particular regime by adjusting to the changing 'stability landscape' (Gunderson, 2000). In this framework, the focus of adaptive capacity is on patterns of species rarity and dominance. Abundant taxa have been found to correlate with particular temporal scales, while rare taxa are often stochastic and can take over after a disturbance and help stabilize ecosystem functioning, allowing the system to recover within the same basin of attraction. (Angeler *et al.*, 2014; Baho *et al.*, 2014; Lyons *et al.*, 2005; Walker *et al.*, 1999). The attribute *thresholds* is implied by the existence of multiple regimes, and emphasizes the precise point at which an ecosystem reorganizes into an alternative regime. Experiments to detect thresholds are not usually feasible, therefore observational studies are often used to identify regime shifts after the fact, for example, using early warning indicators (Scheffer *et al.*, 2012) or multivariate regime shift indicators (Sundstrom *et al.*, 2017; Eason *et al.*, 2016). The existence of *alternative regimes*, finally, is very important for management, since regimes are often held in place by self-reinforcing feedbacks (i.e. negative feedbacks) that make it very difficult to return a system to its previous state after a regime shift (e.g. Scheffer *et al.*, 2001).

These four attributes of resilience are then linked to the following four scenarios regarding the level of adaptive capacity and ecological resilience present in the system: 1. resilience and adaptive capacity are high and the system stays within its basin of attraction; 2. adaptive capacity and resilience are reduced; 3. the adaptive capacity of the system is gone and the system is at a threshold, actively undergoing a regime shift; 4. the system has reorganized into an alternative regime, where it has gained new adaptive capacity and resilience to stay within that regime. Each scenario implies a number of hypotheses:

- *Scenario 1:* stability metrics (resistance, engineering resilience, persistence, temporal variability) as well as response diversity and within and cross-scale redundancy are high, the presence of stochastic species indicates high adaptive capacity.

- *Scenario 2:* The same indicators are used as for scenario 1, but all these levels are reduced compared to before. In addition, early warning signs, such as changes in dynamic system order (i.e. Spanbauer *et al.*, 2016) and critical slowing down (Dakos *et al.*, 2015) can be observed.
- *Scenario 3:* All dynamics are unstable; community composition, abundances, food web structures and species interactions as well as species dynamics are stochastic and unsynchronized.
- *Scenario 4:* The same indicators are used as for scenario 1, with the addition that the within-and cross-scale structures are not the same as before (in the old regime).

As it is unclear which scenario holds when the assessment is started, this procedure is always reiterative, with the results being compared to previous rounds of assessment in order to gradually gain information on the general resilience of the ecosystem. Depending on which premise appears to hold, management can be aimed retaining the current regime or scenario planning can be used in order to prepare for the potential outcomes of an impending regime shift (Chaffin *et al.*, 2016). Alternatively, if a regime shift has occurred, management can be aimed at retaining the new regime, if it has desirable outcomes, or, if the new regime is undesirable, aimed at trying to design management interventions to reduce the resilience of this regime in order to push it past a threshold into a different regime.

This framework uses a reiterative approach where (ideally) time series based on regular monitoring, or multiple snap-shot inventories in the ecosystem make it possible to compare the changes over time and gradually reduce the uncertainty surrounding the resilience of the ecosystem and the effects of management strategies. This approach is aimed to facilitate the assessment of general resilience, as opposed to specific resilience, which is the resilience in response to one particular type of disturbance or stressor.

4.2 The effects of disturbance history on functional and compositional stability of microbial communities in response to a familiar or novel disturbance

The three experimental papers all addressed the question of how disturbance history affects stability of microbial communities in response to a subsequent disturbance. **Paper II** addressed this question by exposing freshwater bacterioplankton communities to a series of temperature pulses followed by an acidification event, measuring both functional and community responses. **Papers III and IV** focused separately on the functional and compositional aspects of this question by exposing freshwater bacterioplankton communities to a series of three mild salinity pulses, followed by a stronger salinity pulse disturbance.

4.2.1 Changes in community composition happened early in both experiments

In **Paper II** the largest changes in community composition, richness, evenness and niche width (both presence-absence and abundance-weighted) took place during the first week in all treatment groups. At this point, one week after the first disturbance, the community composition of communities that were exposed to weekly temperature disturbances of 35°C clearly separated from the controls and the communities exposed to 25°C disturbances treatment. Community composition in the 35°C treatment did not recover or change much more after this (Table 1). After the first week, richness and the two niche width measures decreased with each subsequent exposure to the 35°C treatment, while evenness remained constant, but lower than in the other treatment groups (Table 1). The controls and 25°C treatment group did not differ in any community and diversity parameters during the course of the experiment. The acidification event, in the second part of the experiment in **Paper II**, did not affect community composition in any treatment group (Table 1).

Table 1. (*Next page):* Effect of disturbance history on functional or compositional stability in response to a subsequent disturbance. Summary of results from **Papers II-IV**. The 25°C treatment from **Paper II** is excluded, as it was largely similar to the controls. Niche width measures were either presence-absence (p.a.) or abundance weighted (a.w.). Non-significant results are indicated with ‘n.s.’ Functional and compositional responses that were not assessed in a particular paper are indicated with

‘NA’.

	Paper II Temperature (35°C)	**Paper II pH***	**Paper III Salinity**	**Paper IV Salinity**
Abundance	Decreased resistance /recovery	n.s.	Low persistence /different long-term trajectory	NA
Bacterial production	Decreased resistance /recovery	Increased resistance /recovery	Low persistence /different long-term trajectory	NA
B-glucosidase activity	Increased Resistance /recovery	n.s.	n.s.	NA
Cellobihydrolase activity	Increased resistance /recovery	n.s.	n.s.	NA
Leucine aminopeptidase activity	NA	NA	n.s.	NA
Community composition	Increased resistance	Inconclusive	NA	n.s.
Richness	sensitive	NA	NA	
Evenness	resistant	n.a	NA	NA
Niche width (p.a.)	sensitive	NA	NA	NA
Niche width (a.w.)	sensitive	NA	NA	NA

* The effect of a history of repeated heating pulses on the response to pH disturbance. All other columns show results where the disturbance history and the subsequent disturbance were of the same type.

Similarly to **Paper II**, most of the changes in community composition and richness that took place in **Paper IV** happened during the first week of the experiment, although this was before the first disturbances were applied to all but the long recovery treatment. We found no significant effect of exposure history on the compositional response to the main pulse disturbance, and any statistically significant effects of the treatments by themselves had very small effect sizes (Table 1). At the same time, the replicate communities in all treatment groups diverged over time, and most of this divergence also took place during the first week of the experiment.

4.2.2 Responses of different functions to different disturbances

After the first disturbance, each consecutive disturbance was followed by higher recovery in both β-glucosidase and cellobiohydrolase activities in **Paper II** (Table 1). Bacterial abundance and production, in contrast, rather showed a slight reduction in resistance and recovery over time. In the second part of the experiment in **Paper II**, the bacterial communities that had previously been exposed to four temperature disturbances of 35°C showed increased resistance and/or recovery of bacterial production rates in response to the pH disturbance. The stability of bacterial abundance and β-glucosidase and cellobiohydrolase activities, on the other hand, were not significantly affected (Table 1). In **Paper III** we similarly found that the responses differed between functions (Table 1). Bacterial abundance and production had different long-term trajectories depending on whether the bacterial communities had been exposed to salinity before the main pulse disturbance and how long ago this was. However, the response of the extracellular enzyme activities, β-glucosidase and cellobiohydrolase and leucine aminopeptidase did not differ significantly between exposure history treatments, although there were signs of possible differences in resistance and recovery (**Paper III**, Table 1).

4.2.3 Mechanisms behind compositional and functional responses

The lack of a difference between the 25°C treatment group and the controls in community composition, diversity metrics and functioning in **Paper II** indicates that the communities were both functionally and compositionally resistant to being heated to 25°C (Table 1). They were, in contrast, sensitive to heating to 35°C. The fact that no large changes in community composition happened after the first disturbance, suggests that many sensitive taxa were lost from the community after this first disturbance, resulting in a community that was more tolerant to the three subsequent temperature disturbances. This is consistent with stress-induced community tolerance (Vinebrooke *et al.*, 2004). However, richness continued to decrease with each subsequent exposure to the 35°C temperature disturbances. Although this is consistent with findings in the literature that richness can decrease as a result of disturbances (Downing & Leibold, 2010; Allison, 2004), it also indicates a continued sensitivity to the disturbance after the first week that was not very apparent in community composition. The two niche width measures also continued to decrease with each subsequent temperature disturbance of 35°C, indicating that the temperature pulses gradually selected for a community more dominated by specialist taxa (Pandit *et al.*, 2009). The stability patterns of the different community functions in response to repeated temperature disturbances varied between functions (Table 1). This is similar to the results in **Paper III**, where previous exposure to disturbances affected the response to a subsequent disturbance of the same type in different ways for different functions (Table 1). Other studies have also found that the effect of previous disturbances in on the response to a subsequent disturbance varies between functions (Sjostedt *et al.*, 2018; Berga *et al.*, 2017; Berga *et al.*, 2012), suggesting that this might be a general phenomenon.

The fact that the acidification event in **Paper II** increased resistance and/or recovery of bacterial production rates in response to the pH disturbance in the 35°C treatment (Table 1) is consistent with the findings of earlier studies that previous disturbances can increase resistance to additional disturbances (Sjostedt *et al.*, 2018; Philippot *et al.*, 2008). Since there was no additional community change (Table 1), the increased functional resistance might be due to acquired stress-resistance, where exposure to a disturbance triggers cellular changes that increase resistance to a subsequent disturbance (Rillig *et al.*, 2015). The level of protection that exposure to one type of disturbance causes in response to a different, subsequent disturbance, likely depends on the similarities in the stress-response mechanisms associated with the different disturbance types. Temperature stress has been found to have the ability to trigger this kind of acquired stress-resistance towards other types of disturbances, presumably because temperature stress triggers a host of general stress-responses in the cell (Andrade-Linares *et al.*, 2016).

The lack of compositional response to both the pH disturbance in **Paper II** and salinity disturbance **Paper IV** raises the question whether these disturbances were strong enough to constitute a true disturbance to the communities. Nonetheless, both disturbances led to clear changes in community functioning, indicating that both disturbances had sub-lethal effects on cell activities, which altered microbially mediated ecosystem processes (Table 1). In contrast, various other studies have found the levels of salinity used in **Papers III and IV** to have definite structuring effects on community composition (Berga *et al.*, 2017; Baho *et al.*, 2012; Berga *et al.*, 2012). It is possible that the divergence between replicates in Paper IV that largely took place in the first week of the experiment, before most of the communities had been exposed to any disturbances, drowned out any systematic effects of the disturbance on community composition.

The results of these Papers show that previous exposure to disturbances can increase the functional stability of bacterioplankton communities in response to a subsequent disturbance. This is the case both when the disturbance history and the subsequent disturbances are of the same type and when they are different types of disturbances. However, this depends on the function that is measured, since various functions show very different stability responses.

4.3 The importance of the time since last disturbance for the response to a subsequent disturbance

The previous section describes how previous exposure to disturbances can affect the stability of bacterioplankton communities in response to subsequent disturbances based on **Papers II-IV**. In addition, **Papers III and IV** address the question of what the importance is of the amount of time between the disturbance history and a following disturbance. **Paper III** focuses on the functional responses and **Paper IV** addresses the compositional response.

For community function, the response to the main pulse was affected by the previous disturbances (**Paper III**). However, this was only the case for bacterial abundance and production, that both had different long-term trajectories in the different exposure history treatments. The responses we observed in bacterial abundances, in particular, suggest that ecological memory can diminish over time. The long-term trajectories of communities without a history of disturbance were most similar to those of communities that had previously been exposed to salinity disturbances followed by a long recovery period (Fig 7).

One possible explanation for the change in functional response to the main pulse as a results of previous exposure to mild salinity pulses is stressor-induced community tolerance (Vinebrooke *et al.*, 2004; Blanck, 2002). This would mean that the mild salinity disturbances during the exposure history period selected for a subset of the community that was tolerant to the salinity disturbances, so that the resulting community was more tolerant to the main pulse disturbance, and as a result, have higher functional stability. However, there was no evidence for consistent community change as a result of the mild salinity disturbances in **Paper IV**. In contrast, the effect of the treatment on community composition was very minor, and the compositional response to the main salinity pulse was not affected by the previous exposure to salinity. This was irrespective of the time interval between the series of minor salinity pulses and the main pulse disturbance.

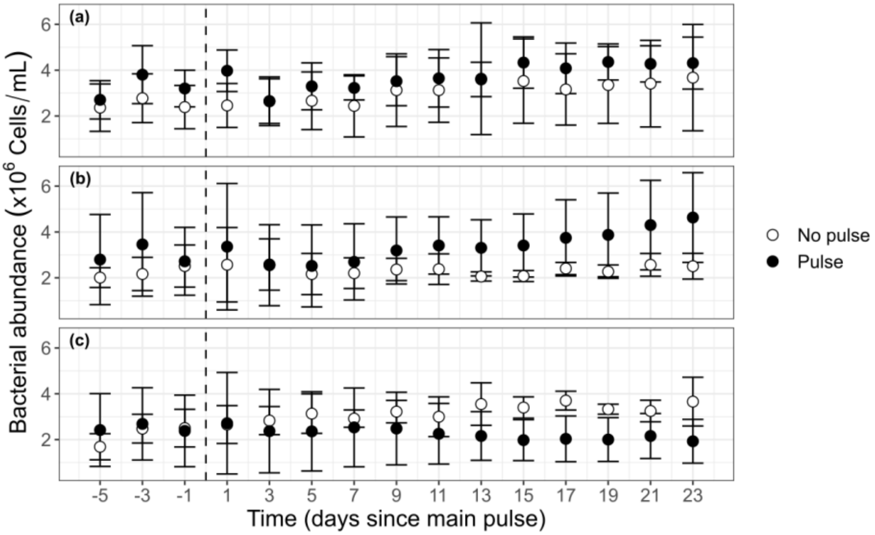


Figure 7. An example of a response that indicates that the effects of previous disturbances on the response to subsequent disturbance is reduced over time. Long-term trajectories in bacterial abundance in the background treatment (a) more closely resembles that in the long recovery treatment (b) than the short recovery treatment (c). The vertical dashed line indicates the timing of the main pulse disturbance. Figure from **Paper III**.

An alternative explanation for the change in functional stability as a result of previous exposure to disturbances is acquired stress-resistance, where changes at a cellular level caused by the initial disturbances have a protective effect to future disturbances (Andrade-Linares *et al.*, 2016). This kind of effect is mainly studied over short time-scales, while the time between the last mild salinity pulse and the main pulse in my experiment reported in **Papers III and IV** would have spanned 7 or 14 generations, depending on the treatment group. However, aquired stress-resistance is thought to have long-term effects in mixed communities, as the fitness differences incurred by the cost of acquiring stress-resistance as well as the costs of sensitivity to disturbance can lead to changes in succession patterns (Rillig *et al.*, 2015). It is reasonable to believe that such changes would be temporary, because a long period without disturbances (such as the 14 days between the mild disturbances and the main pulse in the long recovery treatment) could be enough for sensitive taxa to become more dominant again. Again, we do not see a consistent pattern of change in community composition to support this.

The inconsistency between the functional and compositional results might have been due to the compositional variation between replicates within treatment groups that increased over time (**Paper IV**). Most of this divergence occurred during the first week of the experiment, before most of the disturbances were applied. Therefore, it is hardly surprising that we were only able to detect very small differences between treatments, as effects of the disturbances could easily have been drowned out by the high variation between replicates. At the same time, the divergence between replicates over time suggest that drift had an important role in shaping the community composition in **Paper IV** (Vellend, 2010, see section 4.4 below).

In conclusion, we found that the effects of previous disturbances on the response to a subsequent disturbance is modulated by the amount of time between the previous and subsequent disturbances. However, this effect differs between functions and we did not observe this effect on community composition in either of the two experiments. Is has been observed that functional and compositional stability are not necessarily linked, or at least not linked in a straightforward way (Hillebrand & Kunze, 2020), although it appears that community composition is often more sensitive and less likely to recover than function (Hillebrand & Kunze, 2020; Shade *et al.*, 2012). However, these studies are often based on a single function (Hillebrand &

Kunze, 2020), and we are likely to find more complex patterns when we take multifunctionality into account.

4.4 The role of stochastic versus deterministic community assembly processes in microbial communities undergoing disturbances

Together the four processes selection, dispersal, drift and speciation (Vellend, 2010) shape communities. Although there is support for the roles of the other processes, selection (a deterministic process) is thought to be the most important community assembly mechanism in microbial communities (Langenheder & Lindstrom, 2019; Lindstrom & Langenheder, 2012; Martiny *et al.*, 2006). However, the roles of ecological memory and exposure to repeated disturbances in shaping the mechanisms governing community composition and diversity have been little considered in previous research. We did not observe any strong effect of the disturbances on community composition in **Paper IV**, despite the clear effects on ecosystem functioning in **Paper III**. In addition, we observed that variation between replicates appeared to increase with time (Fig. 8), leading us to suspect that drift, a stochastic process, was playing a bigger role than expected (**Paper IV**). So in the remainder of **Paper IV** we addressed the role of drift by testing whether the rate of divergence between replicate communities, exposed to the same conditions, was affected by exposure to salinity disturbances.

We hypothesized that variation between replicates would increase over time (due to drift) and that this divergence would be halted or reduced during exposure to the disturbances, because of selection/species sorting overcoming the effects of drift (Vellend, 2010). We found that the replicates within a treatment group became more dissimilar over time (Fig. 8). Most of this change occurred during the first week, which coincided with a decrease in richness during the same period. Furthermore, and contrary to expectations, the variation between replicates was on average higher during the disturbances than in the communities that had not been exposed to any disturbances (Fig. 8). This is, however, consistent with a modelling study that investigated how community assembly is affected by dispersal and selection, and found higher stochasticity in communities exposed to strong selection (Evans *et al.*, 2017).

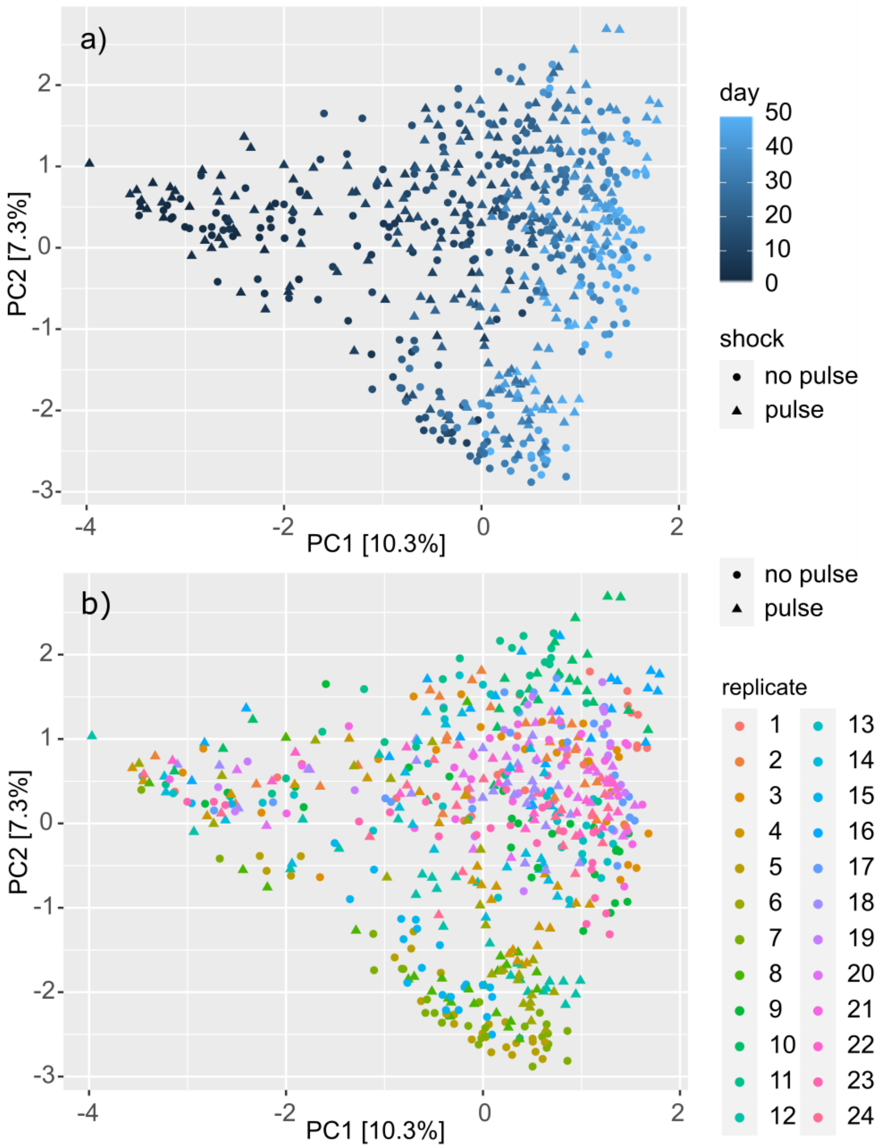


Figure 8. Principal component analysis showing deviation of community composition between replicates. Data for all communities throughout the whole experiment. a) Colored by time point (n = 26). The darker colors represent samples from the beginning of the experiment, lighter colors show samples from later in the experiment. b) Colored by replicate (n = 24). Each color shows a single replicate across all time points. Figure from **Paper IV**.

Besides drift, which is purely stochastic, speciation and dispersal can have stochastic elements to them, which makes it difficult to fully disentangle these processes (Evans *et al.*, 2017; Zhou & Ning, 2017). We argue that it is very unlikely that speciation events occurred during this experiment (**Paper IV**), but the role of dispersal might be important. On the whole, the experimental set-up did not allow for dispersal, but the initial division of the starting cultures over the culture vessels, as well as the homogenization step just prior to the start of the experiment can be seen as massive dispersal events. Both dispersal limitation and massive dispersal may have contributed to stochasticity in the communities (Evans *et al.*, 2017; Zhou & Ning, 2017).

In conjunction with this, selection might still have played a part in shaping the communities in different ways. Small initial differences in composition as a result of dispersal and drift might have led to larger divergence through different species interactions in the replicate communities.

In conclusion, our results show that drift might have an increased importance during disturbances, although dispersal limitation and species interactions might also play key roles, which we were unable to explicitly elucidate. In addition, this study has also pointed out some methodological implications: experiments without dispersal can have very high variation over time and increasing compositional variation between replicates, so it is important to have high replication and many measurement points over time in order to capture community dynamics.

4.5 Synthesis

This thesis addresses questions of ecological resilience, ecosystem stability and ecological memory using both a more conceptual (**Paper I**) as well as experimental (**Papers II-IV**) perspectives. Based partly on my work and on my reading of the literature I argue that these perspectives can be seen as complementary approaches towards better understanding how ecosystems are shaped by, and respond to, changes in the environment. The approaches differ in the level of organization on which they focus. The ecological resilience view focuses on the emergent phenomenon that ecosystems operate in regimes, determined by the underlying ecosystem processes and structures that keep it in place (Gunderson, 2000, Fig. 9). It also acknowledges the possibility of multiple regimes, so that if an ecosystem is disturbed enough, or if there is an erosion of resilience (e.g. through losses of response diversity), the system can reorganize and enter a new regime, which is regulated by a different set of structures and processes (Angeler & Allen, 2016). The stability view can be thought of as nested within this, where the focus is on patterns of change and recovery in measures of ecosystem functions and structure in response to disturbances (Donohue *et al.*, 2016; Gunderson, 2000). Ecological memory, then, can be seen as a phenomenon that is relevant in both frameworks, in that it connects the underlying materials, organisms, information and structures in an ecosystem to the emergent stability response of the ecosystem within a regime (Fig. 9).

Various definitions of ecological memory exist, as well as of legacies of past disturbances, which can be considered to be part of the same concept. Ecological memory is divided into internal and external legacies (Bengtsson *et al.*, 2003), information legacies (species traits in the community, adaptation to past disturbances) and material legacies (propagules, individuals, biotic & abiotic residues; Johnstone *et al.*, 2016), or biological and structural legacies, active and passive mobile links, and support areas that can act as a source for recolonization of species (Nystrom & Folke, 2001). In addition, legacies can be permanent or transient, depending on time lags in species sorting and priority effects, and the duration of the effect might depend on selection, growth rates, dispersal and colonization rates as well as changes in environmental conditions (Vass & Langenheder, 2017). Although different research groups emphasize different elements that may contribute to ecological memory and legacy effects, together, they form a cohesive view (Fig. 9). Finally, the different components of ecological memory clearly point out the role of the community assembly processes that underlie all changes in community structure (Vellend, 2010, Fig. 9). The division of internal and external memory clearly points at the role of dispersal, where connectivity between habitats in a meta-ecosystem allows for the movement of individual organisms that can facilitate recovery of a community after a disturbance (Nystrom & Folke, 2001). Selection determines which organisms survived after the last disturbance, depending on which organisms were present and what kind of adaptations they had. Information legacies such as stressor-induced community tolerance (SICT) can also result from selection. Furthermore, abiotic or structural material legacies can be a source of selection within an ecosystem. Speciation is a process that can occur within the species pool inside an ecosystem. The same

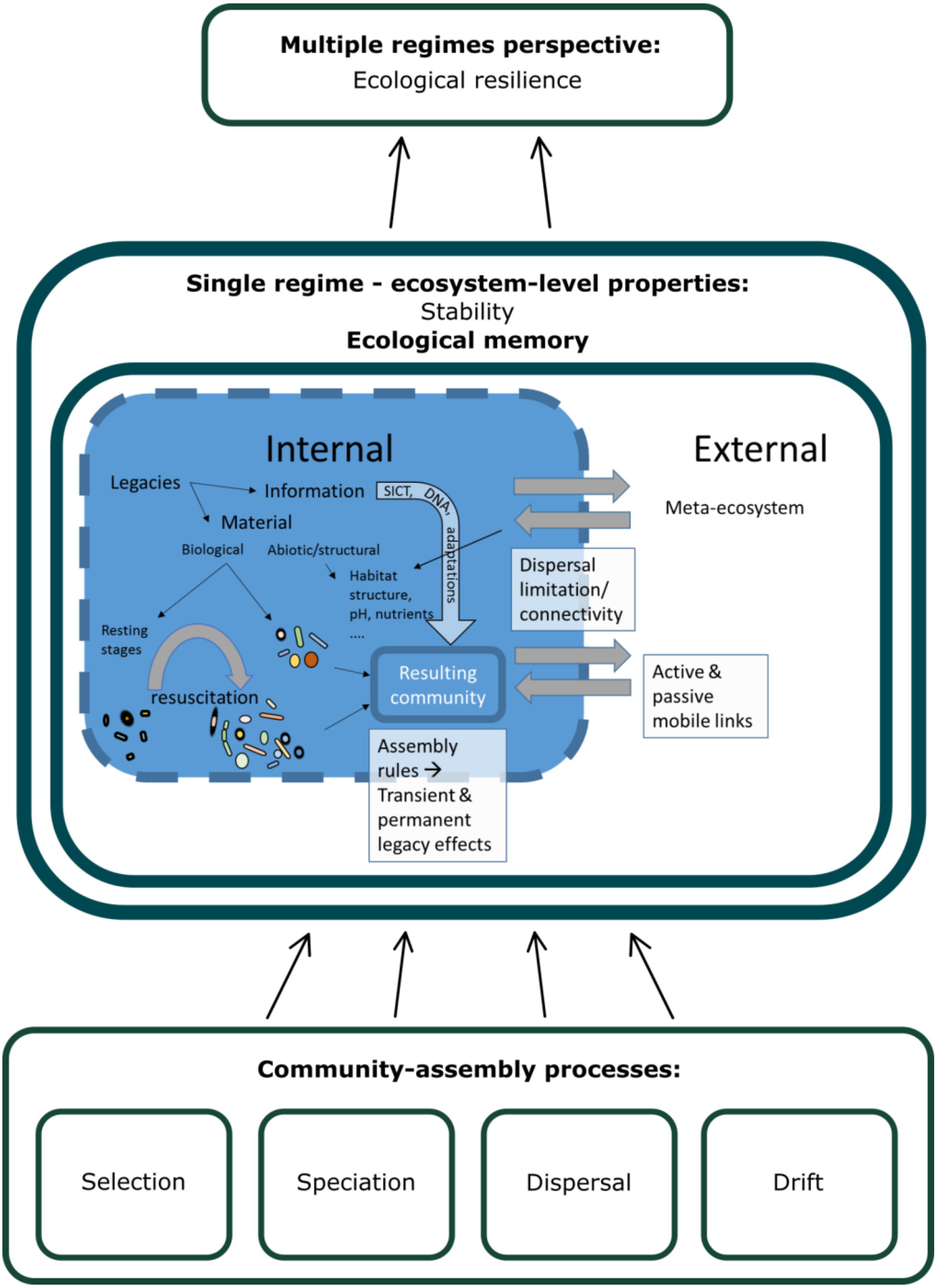

Multiple regimes perspective:
Ecological resilience
Single regime - ecosystem-level properties:
Stability
Ecological memory
Internal
External
Legacies
Information
SICT, DNA, adaptations
Material
Biological
Abiotic/structural
Habitat structure, pH, nutrients ....
Resting stages
resuscitation
Resulting community
Meta-ecosystem
Dispersal limitation/ connectivity
Active & passive mobile links
Assembly rules → Transient & permanent legacy effects
Community-assembly processes:
Selection
Speciation
Dispersal
Drift

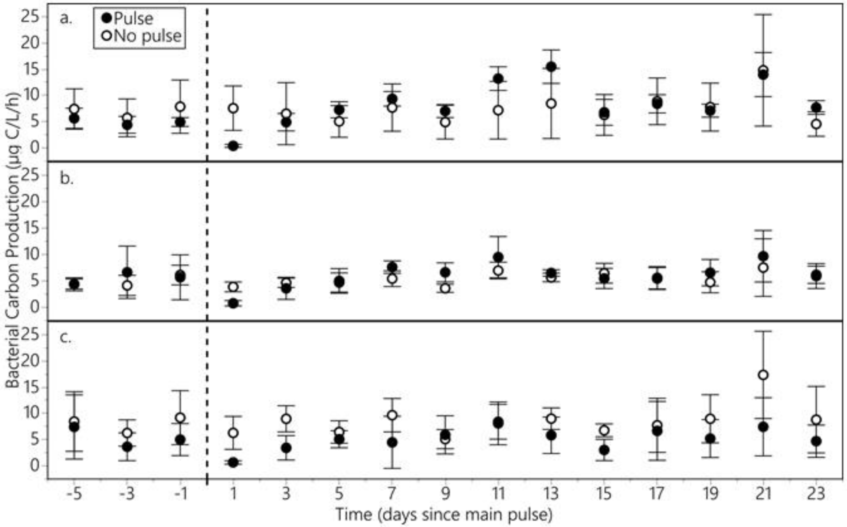
Figure 9. (*Previous page):* Conceptual figure showing the relationship between the different theoretical perspectives addressed in this thesis. Ecological resilience is an emergent property of an ecosystem, from a multiple-regimes perspective. Stability and ecological memory are ecosystem properties in the single-regime perspective. The various components of ecological memory are illustrated inside the inner box. The components of ecological memory are largely driven by or affected by the underlying community assembly processes.

goes for drift, although the relative importance of drift is affected by the roles of the other community assembly processes and species abundance (Fig. 9).

This experimental work in **Papers II-IV** of this thesis only addressed a subset of the elements of ecological memory presented in Fig. 9. These elements were primarily internal memory, including the active biological community and the information legacies they contained. Any memory effects I observed in these studies would have arisen form changes in either the identity of organisms present in the system (i.e. community composition) and/or the information legacies they contained as a result of past disturbances. Stress-induced community tolerance and acquired stress resistance can be seen as examples of such changes (Fig. 9).

In **Paper II** we observed the largest change in community composition and other community parameters such as richness and niche width in response to the first temperature disturbance. The changes in response to the 35°C treatment separated it from the other treatment groups, indicating that the disturbance selected for tolerant members of the community. This can be seen as a biological legacy containing the information legacy of tolerance to the disturbance. The community composition in the controls and 25°C treatment groups also changed most in this first week. This is consistent with our observations from the salinity experiment (**Paper IV**), where the largest changes in community composition and richness also took place during the first week, even before any disturbances were applied. The changes in community composition found in **Paper IV** did not lead to communities separating from each other by treatment as in **Paper II**. Nonetheless, the

A.


a.
b.
c.
Pulse
No pulse
Bacterial Carbon Production (µg C/L/h)
25
20
15
10
5
0
-5
-3
-1
1
3
5
7
9
11
13
15
17
19
21
23
Time (days since main pulse)

B.

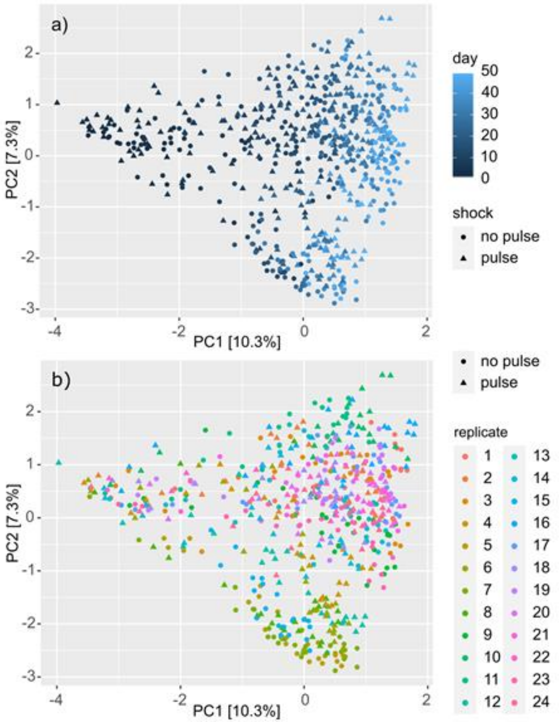

a)
b)
PC1 [10.3%]
PC2 [7.3%]
-4
-2
0
2
-3
-2
-1
0
1
2
day
50
40
30
20
10
0
shock
no pulse
pulse
replicate
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Figure 10. (*Previous page*): Deterministic effects of the salinity pulse on bacterial production immediately after the disturbance (A.) occurred while the communities were drifting stochastically in different directions (B). This indicates that the more deterministic functional responses reflect sub-lethal effects of the disturbance on, e.g. microbial activity rates rather than systematic changes in community composition. Figure A. from Paper III and Figure B. from Paper IV.

quick divergence between replicates (or in **Paper II**, between the 35°C treatment group and the rest of the communities) followed by a relative stabilization and lack of recovery of community composition might indicate the existence of multiple regimes (Faust *et al.*, 2015). In terms of Fig. 9, this would indicate a break-down of the processes and structures present in the regime, including any elements of ecological memory, followed by a reorganization into a new regime. Further analysis, using one of many possible approaches to assess the existence of multiple regimes (e.g. Faust *et al.*, 2015, or **Paper I**), would be necessary in order to establish which of these two scenarios hold (ecological memory through biological and information legacies or multiple regimes).

Paper I aims to facilitate management of ecosystems for general resilience, which is something of a holy grail for ecological management, because it would allow for development of a general best practice for particular ecosystem types. However, **Paper IV** shows that communities have the potential to respond very differently to the same environmental conditions, in this case attributable to initial tiny differences in composition that were enhanced over time due to drift and possibly species interactions. In Fig. 9 this would be a legacy effect caused by the community assembly process drift leading to different outcomes of selection in initially very similar communities. This shows that care is needed in which assumptions are made, as even apparently similar communities might not respond in a highly deterministic fashion to the same disturbance or management intervention (Fig. 10).

Interestingly, though, in my experiments, community functioning showed more evidence of systematic responses to the imposed disturbances (Fig. 10). Ecosystem managers are more likely to be interested in the stability or resilience of functions mediated by microbes, since these are crucial to key ecosystem services and cultural/aesthetic aspects of ecosystems (e.g. whether lake water is clear and suitable for bathing and fishing or murky, smelly, and thus repellent for human recreational activities) than in the particular composition of the (microbial) communities. In contrast with this view, **Paper I** focuses mainly on patterns of community structure and only address functioning in terms of functional trait diversity, rather than realized or potential functional process rates, or changes in ecosystem service provisioning. The results of **Papers II-IV** highlight the importance of incorporating quantification of functioning and services in ecological resilience assessment, in particular when the stability of functions is the management goal.

When stochastic processes such as drift dominate community assembly it is important to be careful with assumptions that similar ecosystems will respond similarly to disturbances. When, on the other hand, more deterministic processes dominate community assembly, a framework such as in **Paper I** is more likely to be of help in informing management. A big open question is then, how often drift is a dominant process for microbes (or other types of organisms) in nature.

Just as the results of the experiments in this thesis show that it is important to measure ecosystem function in order to understand the stability and resilience of the system, it is also important to measure multiple functions in parallel. In accordance with the literature, **Papers II-IV** show that measures of functional and compositional stability can tell very different tales about the stability of an ecosystem, complicating the assessment of ecological resilience as proposed in **Paper I** (Hillebrand & Kunze, 2020; Sjostedt *et al.*, 2018; Berga *et al.*, 2017; Berga *et al.*, 2012; Shade *et al.*, 2012). It has often been shown that high biodiversity is needed in order to support multiple functions in an ecosystem (Lefcheck *et al.*, 2015). Nonetheless, a recent simulation study showed that individual functions are affected in non-additive ways by biodiversity, but when multifunctionality of an ecosystem is taken as a whole, the effects of biodiversity are evened out (Gamfeldt & Roger, 2017). Similarly, multiple stressors were found to affect individual functions in contrasting ways, but the level of multifunctionality of the system was found to be unaffected by the stressors (Alsterberg *et al.*, 2014). This further emphasizes the importance of considering multiple ecosystem measures in order to assess stability and ecological resilience.

5. Conclusions & outlook

In order to improve our understanding of ecosystem stability in the face of environmental change, a quantitative framework was developed for the assessment of ecological resilience. This framework combines the current knowledge about ecological resilience and stability assessment into a reiterative approach that gradually reduces uncertainty about the level of resilience in a system. In addition, several experimental studies were performed using aquatic microbial communities in order to address open questions about the role of disturbance history in shaping resilience and stability of ecosystems in response to future disturbances.

The main conclusions of this thesis are:

- Previous exposure to disturbances can increase functional stability in response to a subsequent disturbance of both the same and a different type, providing evidence of an effect of ecological memory on microbial responses to disturbances
- The effect of disturbance history on stability in response to a subsequent disturbance is reduced with increasing time between the previous disturbance and subsequent disturbance.
- The effect of disturbances and disturbance history on stability differs between functions and between function and composition
- Variation between replicate communities in a closed system can diverge over time, and this effect is stronger during disturbances, indicating that stochastic processes dominate community assembly, and that these processes can be increased by disturbances

The results of the experiments in this thesis indicate that it may be risky to apply the framework from **Paper 1** to ecosystems dominated by stochastic community assembly. In such cases, community response to disturbances and management interventions might vary widely among very similar systems. Future studies should aim to discover to what extent stochastic community assembly occurs in natural microbial communities. In addition, this thesis shows that microbial community functioning can respond deterministically to disturbances, even while the underlying community composition is subject to drift. This suggests that the microbial community functions I quantified might be more predictable than community composition, and hence more tractable to management. The implicit aim of ecosystem management is to preserve ecosystem service provisioning, but assessment of ecological resilience is largely aimed at aspects of community structure rather than function. Frameworks for assessing ecological resilience should therefore be modified to more explicitly consider ecosystem process quantification. Furthermore, acknowledging multifunctionality of ecosystems is essential for correct assessment of ecosystem stability, since individual functions vary in their response to disturbances.

This thesis addressed two main components of ecological memory: internal biological and information legacies, which consist of the active organisms in the ecosystem as well as the information contained in them in the form of adaptations, acquired stress resistance and stress-induced community tolerance. Other components of ecological memory remained unaddressed in this thesis. In particular, the role of dispersal and dispersal limitation in modulating the relative importance of stochastic and deterministic community assembly processes deserves more attention.

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Popular science summary

It is impossible to miss all the news about climate change. Changing temperatures, rising sea levels, hurricanes, droughts, the list goes on. All of these changes threaten ecosystems, risk killing species and changing how ecosystems function. Humans are dependent on the things that nature gives us – food, water, clean air, materials for clothes and buildings and so on. Microscopic organisms (microbes), such as bacteria, help make this possible. Among other things, they break down organic matter, process nitrogen so that plants can use it for growth, and affect the rate of global warming by regulating green-house gas emissions. In short, it is important to understand how our ecosystems, and in particular microbial ecosystems, are affected by environmental change and how this affects all the things they do for us. This thesis is meant as a step towards reaching that goal.

My coauthors and I set up a framework for measuring ecological resilience in ecosystems. Ecological resilience is the strength of a disturbance an ecosystem can handle before it breaks down. It is a very difficult thing to measure, especially if we consider different types of disturbances. There is still a lot we do not know. But by measuring the stability of an ecosystem and looking at how this changes over time, we can gradually get a better idea of how close it is to breaking down.

In addition, I performed laboratory experiments on freshwater microbes from a lake and a stream in Sweden, using these microbes as miniature ecosystems. I exposed them to repeated disturbances in the form of heat waves, acid, or salt, to see how the disturbances I created affected how the ecosystems coped with disturbances I exposed them to later on. For each type of disturbance in these experiments, I had four separate bottles that were filled with the same microbes and received the same treatment. Some of these treatments only involved one type of disturbance. For others, I first disturbed the ecosystems with one kind of disturbance, and then with another kind. I found that the microbial ecosystems developed a kind of ‘memory’ of the disturbances that helped them function better in some ways, but not others, when they faced another disturbance. This happened, no matter if the last disturbance was of the same type they had experienced before, or a new type. This ‘memory’ also seemed to become weaker over time. Our results show that one needs to be very careful about what one measures, to avoid drawing the wrong conclusions. If one only measures one aspect of an ecosystem one might believe that it is more stable than it really is.

Next, I looked at what kinds of microbes were present in my experimental ecosystems at different times during the experiments. I found out that the groups of microbial ecosystems that were identical at the start of the experiment, and were disturbed in the same way (in separate bottles) became dissimilar over time. Different types of microbes died out, while others became dominant, even though they were scarce at the beginning. These changes had to be happening by chance, because if one type of microbe is easily killed by a disturbance, it should be killed in all the ecosystems that are exposed to that disturbance. This means that the group of microbes surviving the disturbances should similar in the ecosystems exposed to the same disturbance. I compared the speed of change of these ecosystems to groups of ecosystems that were not disturbed, expecting the disturbances to slow down the speed at which the ecosystems became more dissimilar. I found the opposite of what I expected! The ecosystems were more dissimilar when they were disturbed than when they were not. This indicates that different ecosystems that look the same can respond very differently to the same type of disturbance.

In summary, my thesis shows that disturbances can affect ecosystems in complex ways. Ecosystems can ‘remember’ but also ‘forget’ the past. It matters what you measure, so if you only measure one aspect of an ecosystem this can fool you! And just because two ecosystems look the same, it doesn’t mean that they will behave the same way.

Populärvetenskaplig sammanfattning

Det är omöjligt att undgå alla nyheter om klimatförändringarna. Ökande temperaturer, stigande havsnivåer, orkaner och torka. Listan bara fortsätter. Alla dessa förändringar hotar världens ekosystem, riskerar att utrota arter och förändra hur ekosystem fungerar. Människan är beroende av det som naturen ger oss - mat, vatten, ren luft, material till kläder och byggnader, och så vidare. Mikroskopiska organismer (mikrober), såsom bakterier, är en viktig del av detta. Bland annat bryter de ner organiskt material, omvandlar kväve så att växter kan använda det för tillväxt, och de påverkar den globala uppvärmningshastigheten genom att reglera utsläppen av växthusgaser. Kort sagt är det viktigt att förstå hur våra ekosystem, och i synnerhet mikrobiella ekosystem, påverkas av miljöförändringar och hur detta påverkar alla saker de gör för oss. Den här avhandling är tänkt som ett steg på vägen mot att nå det målet.

Mina medförfattare och jag utvecklade ett ramverk för att mäta ekologisk motståndskraft i ekosystem. Ekologisk motståndskraft kan beskrivas som styrkan i en störning som ett ekosystem kan hantera innan det kollapsar. Det är mycket svårt att mäta, och särskilt om vi tittar på olika typer av störningar. Det finns fortfarande mycket vi inte vet, men genom att mäta stabiliteten i ett ekosystem och titta på hur den förändras över tid kan vi gradvis få en bättre uppfattning om hur nära systemet är att kollapsa.

Som en del i undersökningen utförde jag ett laboratorieexperiment på sötvattensmikrober, från en sjö och en bäck i Sverige, där jag använde dessa mikrober som miniatyrekosystem. Jag utsatte dem för upprepade störningar i form av värmeböljor, försurning eller salt, för att se hur de störningar jag skapade påverkade hur ekosystemen hanterade störningar jag utsatte dem för senare. För varje typ av störning hade jag fyra separata flaskor som fylldes med samma mikrober – och som fick samma behandling. Några av dessa behandlingar bestod bara av en typ av störning, medan jag i andra fall störde ekosystemen med först en typ av störning och sedan en annan. Jag upptäckte att de mikrobiella ekosystemen utvecklade ett slags ‘minne’ av störningarna som hjälpte dem att fungera bättre på vissa, men inte på andra, sätt när de senare mötte en annan störning. Detta hände, oavsett om den senaste störningen var av samma typ som de hade upplevt tidigare eller en ny typ. Detta ‘minne’ tycktes också bli svagare med tiden. Mina resultat visar att man måste vara mycket försiktig när man väljer vad man ska mäta, för det är lätt att dra fel slutsatser. Om man bara mäter en aspekt av ett ekosystem kan man komma att tro att det är mer stabilt än det egentligen är.

Efter det här tittade jag på vilka typer av mikrober som fanns i mina experimentella ekosystem vid olika tidpunkter under experimenten. Jag upptäckte att de mikrobiella ekosystem som hade varit identiska i början av experimentet och som hade störts på samma sätt (i separata flaskor) hade blivit olika med tiden. Olika typer av mikrober hade dött ut, medan andra blev dominerande, även om de var sällsynta i början. De här förändringarna måste ha skett av en slump, eftersom alla mina ekosystem hade behandlats på samma sätt. I nästa steg undersökte jag då om störningarna saktat ner förändringshastigheten. Det jag förväntade mig var att om en typ av mikrober lätt dödas av en störning, bör detta hända i alla ekosystem som utsätts för den störningen. Det innebär att gruppen av mikrober som överlever störningarna borde vara mer lika varandra. För att undersöka det här jämförde jag förändringshastigheten för dessa ekosystem med grupper av ekosystem som inte stördes och hittade motsatsen till vad jag förväntade mig! Ekosystemen var mer olika när de stördes än när de inte gjorde det. Detta visar att ekosystem som liknar varandra kan reagera på väldigt olika sätt på samma typ av störningar.

Sammanfattningsvis visar min avhandling att störningar kan påverka ekosystem på komplexa sätt. Ekosystem kan ‘komma ihåg’ men också ‘glömma’ det förflutna. Det spelar roll vad man mäter, så om man bara mäter en aspekt av ett ekosystem kan man bli lurad! Och bara för att två ekosystem ser likadana ut, betyder det inte att de kommer att bete sig på samma sätt.

Populairwetenschappelijke samenvatting

We horen steeds meer over de gevolgen van klimaatverandering. Stijgende temperaturen, stijgende zeespiegel, orkanen, droogtes, de lijst gaat maar door. Al deze veranderingen bedreigen soorten met uitsterven en veranderen de manier waarop ecosystemen functioneren. Mensen zijn afhankelijk van de producten en middelen die de natuur ons geeft: voedsel, water, schone lucht, grondstoffen voor kleding en gebouwen, en ga zo maar door. Micro-organismen (microben), zoals bacteriën, zijn daar een essentieel onderdeel van. Zo breken ze bijvoorbeeld organisch materiaal af, ze zetten stikstof om in andere vormen zodat planten het kunnen gebruiken voor groei, en ze beïnvloeden de snelheid van de opwarming van de aarde door de uitstoot van broeikasgassen te beïnvloeden. Kortom, het is belangrijk dat we begrijpen hoe onze ecosystemen, en in het bijzonder microbiële ecosystemen, worden beïnvloed door veranderingen in het milieu en welke invloed dit heeft op alle dingen die ze voor ons doen. Dit proefschrift draagt bij aan onze kennis over hoe ecosystemen worden beinvloed door milieuveranderingen.

Mijn coauteurs en ik hebben een methode ontwikkeld voor het meten van de ecologische veerkracht in ecosystemen. De ecologische veerkracht is de tegenkracht die een ecosysteem kan leveren tegen een verstoring van buiten. Als de kracht van de verstoring te groot wordt, wordt een kantelpunt bereikt, wat grote negatieve ecologische gevolgen heeft. Dit is heel moeilijk te meten, vooral als we kijken naar verschillende soorten verstoringen. Er is nog veel dat we niet weten. Maar door de stabiliteit van een ecosysteem te meten en te kijken hoe dit in de loop van de tijd verandert, kunnen we geleidelijk aan een beter beeld krijgen van hoe dichtbij zo’n kantelpunt is.

Daarnaast heb ik laboratoriumexperimenten uitgevoerd waarbij ik zoetwatermicroben uit een meer en een beekje in Zweden gebruikte als miniatuurecosystemen. Ik stelde hen bloot aan herhaalde verstoringen in de vorm van hittegolven, verzuring of verzilting, om te zien hoe deze verstoringen de response van de ecosystemen op latere verstoringen beïnvloedden. Voor elk type verstoring in deze experimenten waren er vier afzonderlijke flessen, met dezelfde microben, die dezelfde behandeling kregen. Bij sommige van deze behandelingen ging het om één soort verstoring. Bij andere ondergingen de experimentele ecosystemen éérst de ene, en daarna de andere soort verstoring. Ik ontdekte dat de microbiële ecosystemen zich de verstoringen als het ware konden 'herinneren', wat hen hielp om op bepaalde manieren, maar niet op alle manieren, beter te functioneren nadat ze met een andere verstoring werden geconfronteerd. Dit gebeurde ongeacht of het ging om dezelfde soort verstoring of een verstoring waaraan het ecosysteem nog niet eerder was blootgesteld. Wel leek deze 'herinnering' in de loop van de tijd zwakker te worden. Onze resultaten laten ook zien dat we goed moeten opletten met wat we meten, omdat we makkelijk de verkeerde conclusies kunnen trekken. Als we maar één aspect van een ecosysteem meten, concluderen we misschien dat het stabieler is dan het in werkelijkheid is.

Vervolgens keek ik naar welke soorten microben op verschillende tijdstippen in mijn experimentele ecosystemen aanwezig waren. Ik ontdekte dat de groepen microbiële ecosystemen die aan het begin van het experiment zeer sterk op elkaar leken en daarna aan precies dezelfde verstoringen werden blootgesteld (in afzonderlijke flessen), in de loop van de tijd van elkaar gingen verschillen. Sommige soorten microben stierven uit, terwijl andere overheersend werden, hoewel ze eerst misschien weinig voorkwamen. Deze veranderingen moesten door toeval komen, aangezien mijn ecosystemen allemaal dezelfde behandeling hadden ondergaan. Ik wilde weten of de snelheid van de veranderingen in de samenstelling afnam als gevolg van de verstoringen die ik had toegediend. Als een type microbe gemakkelijk wordt gedood door een verstoring, zou in alle ecosystemen moeten gebeuren die aan die verstoring zijn blootgesteld. Dit betekent dat de groepen microben die de verstoringen overleven, hetzelfde zouden moeten zijn in alle flessen. De snelheid waarmee deze ecosystemen veranderden heb ik vergeleken met groepen ecosystemen die niet verstoord waren. Het resultaat was het tegenovergestelde van wat ik had verwacht! De ecosystemen verschilden meer van elkaar wanneer ze verstoord werden dan wanneer dat niet het geval was. Dit laat zien dat ecosystemen met dezelfde soortensamenstelling, heel verschillend kunnen reageren op dezelfde soort verstoring.

Samenvattend laat mijn proefschrift zien dat verstoringen een complex effect kunnen hebben op ecosystemen. Ecosystemen kunnen zich het verleden ‘herinneren’ maar het ook weer ‘vergeten’. Het maakt uit wat je meet, dus als je maar één aspect van een ecosysteem meet, kun je jezelf voor de gek houden! En dat twee ecosystemen dezelfde soortensamenstelling hebben, wil nog niet zeggen dat ze op dezelfde manier reageren op verstoringen.

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It takes a village to raise a child, they say. Well, it takes a department to raise a PhD! Nobody can do a PhD on their own, and I have had the luck and privilege of calling two departments home.

Now I will not attempt to break any records on longest acknowledgements section ever, so I will keep it simple.

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Microorganisms play a fundamental role in global biogeochemical cycles, which are increasingly being altered by environmental change. Here, a quantitative framework was developed for the assessment of ecological resilience to facilitate ecosystem management, and in experiments with microbial communities the role was investigated of past disturbances in shaping the stability of microbial communities to future disturbances. “Ecological memory” effects in community functioning were found to decline over time, while microbial community composition appeared to be dominated by stochastic assembly processes.

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