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It's complicated:

The role of timing in microbial community coalescence

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Abstract
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In recent years, the importance of historical contingency has been increasingly recognized in microbial communities. During community coalescence, immigration history, and dispersal history can become decisive for the developing community. For example, an early arriving pioneer can inhibit the immigration success of a late invader by resource consumption/alteration, also known as priority effects. Alternatively, the signal of past dispersal in the resident community can be long-lasting and contribute more to the communities' composition than contemporary dispersal. The overall aim of this thesis was to investigate the potential importance of arrival timing and dispersal timing in complex natural lake bacterial communities. This was done by examining the role of priority effects in experiments as well as the role of dispersal, including past dispersal, in natural lakes. Priority effects were difficult to detect on a whole community level but were found in high nutrient levels and in the absence of grazing. In the lakes, the internal production or internal dispersal was the most important assembly mechanism. However, external sources, including dispersal from the groundwater and the main inlet, were also important. Past dispersal, at times, contributed more to the lake bacterial community composition (BCC) than contemporary dispersal. Further, the results showed that past dispersal can leave a long-lasting signal in lake BCC, which mainly resulted from the dispersal of inactive cells. In conclusion, this thesis highlights the potential importance of temporal dynamics in complex freshwater bacterial communities and emphasizes the need to incorporate arrival and dispersal timing in future community coalescence studies.

Keywords: community coalescence, arrival timing, dispersal timing, microbial communities, community composition, lake bacteria

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Denn nur Arbeit kann dir sagen ob's Ideen wirklich bringen
Because only work can show you if ideas are worth pursuing
– Der Nino aus Wien

List of Papers

This thesis is based on the following papers, which are referred to in the text by their Roman numerals.

I. Lumpi, T., Langenheder, S., Lindström, E. S. (2023) The challenges of detecting underlying mechanisms for priority effects in complex bacterial lake communities. *Manuscript*

II. Lumpi, T., Guo, X., Lindström, E. S. (2023) Nutrient availability and grazing influence the strength of priority effects in bacterial freshwater communities. *Submitted*

III. Logue, J. B., Lumpi, T., Lindström, E. S. (2023) Sources of dispersal and timing matter for lake bacterial community assembly. *Manuscript*

IV. Lumpi, T., Langenheder, S., Lindström, E. S. (2023) Present and past dispersal effects on a lake bacterial community. *Manuscript*

Contents

Abbreviations

ALW	Artificial Lake Water
ASV	Amplicon Sequence Variant
BCC	Bacterioplankton Community Composition
DNA	Deoxyribonucleic Acid
HNF	Heterotrophic Nanoflagellate
MST	Microbial Source Tracking
NMDS	Non-metric Multidimensional Scaling
OTU	Operational Taxonomic Unit
PCoA	Principal Coordinates Analysis
PCR	Polymerase Chain Reaction
RO	Reverse Osmosis
RNA	Ribonucleic Acid
rRNA	Ribosomal Ribonucleic Acid
ST	Source Tacker
TP	Total Phosphorus
TN	Total Nitrogen
TFF	Tangential Flow Filtration
TOC	Total Organic Carbon

Introduction

Community composition – how do natural communities assemble?

One overarching goal in the field of community ecology is to disentangle the complicated web of species and to find general mechanisms behind the diversity and structure of communities. The task at hand is daunting as species interact among themselves and with their environment along a continuous time axis where the conditions change constantly.

Community assembly describes the process of communities forming under certain environmental conditions and within a given species pool (Kraft & Ackerly, 2014).

Historically, competition-predation models (Lotka, 1925; Volterra, 1928) and the concept of ecological niches (Hutchinson, 1957) led to a strong emphasis on local habitat conditions as the major driving forces of the community assembly. This bore the risk of ignoring essential mechanisms shaping communities on a wider spatial and temporal scale (Lawton, 1999; Ricklefs, 1987). An integrative concept of both local as well as regional processes shaping community composition (= metacommunity concept) can be understood as a major achievement made in the past two decades (Chase & Leibold, 2002; Hanski, 1998; Holyoak et al., 2005; Leibold et al., 2004). A metacommunity describes a set of local communities that are connected by the dispersal of multiple potentially interacting species (Leibold et al., 2004). As such, an interplay of regional processes (i.e., dispersal from other habitats) and local dynamics (i.e., local interactions) drive local community assembly. Similarly, another conceptual framework was brought forward by Vellend (2010) after which community assembly can be explained by an interplay of four major processes: selection (the fitness difference between species), drift (stochastic changes in population abundances), speciation (the creation of new species) and dispersal (the migration of species across space).

The above-described frameworks have helped to conceptualize a myriad of ecological processes into a few major drivers and to integrate larger spatial scales into local community patterns. Importantly, regional and local

processes can affect communities in a hierarchical manner; for instance, when potential colonizers are drawn from the regional species pool and the local environment eventually selects for newly establishing species in the habitat.

Community assembly in microorganisms

The above-mentioned theoretical concepts have been studied rigorously in bacterial and other microbial communities in various ecosystems.

One of the most important findings is the recognition of local environmental selection as the predominant assembly mechanism (Langenheder & Lindström, 2019). Local environmental selection pressures for microbial communities include, among others, the chemical habitat conditions (e.g., pH, salinity, oxic/anoxic), bottom-up (e.g., resource availability) and top-down (e.g., predation and other mortality) forces as well as other interspecific interactions (e.g., mutualism). Nevertheless, numerous field studies have repeatedly identified spatial distance effects s (Martiny et al., 2006), therefore, indicating the relevance of dispersal for the assembly of microbial communities. The rate of dispersal can affect communities in opposing ways. If the dispersal rate is high, it can exceed a species' extinction rate thus leading to a mismatch between community composition and local habitat conditions (=mass effects). If the dispersal rate is limited, on the other hand, the local community can become species depleted as a result of local extinction events and the inability of suitable taxa to reach the habitat (Leboucher et al., 2020).

Null model approaches have further shown that stochastic assembly processes (i.e., random colonization and drift), as opposed to deterministic processes (i.e., environmental selection), can have a large influence on community composition and diversity (Stegen et al., 2013; Zhou & Ning, 2017). Legacy effects, which describe the influence of biotic and abiotic events that occurred in the past (e.g., past environmental conditions, past dispersal events) can also be seen as stochastic assembly mechanisms (Zhou & Ning, 2017). One example is the occurrence of priority effects where the (stochastic) arrival timing of species or communities to a new habitat impacts the development of the newly forming community (Fukami, 2015).

In recent years, several studies have found arrival history (e.g., priority effects) to be important during community assembly (Debray et al., 2022; Fukami, 2015). This has led to the recognition of historical contingency potentially being important in the field of microbial community ecology (Langenheder & Lindström, 2019).

Priority effects in microbial communities

The immigration history (i.e., the arrival order and timing) of a species or community to a new habitat can have a long-lasting effect on community structure through priority effects (Fukami, 2015). They occur when the first colonist exhausts (niche-preemption) or alters (niche-modification) resources or habitat conditions, therefore, affecting the establishment success of the later immigrant (Fukami, 2015). Spatially dissimilar communities can therefore theoretically be found in identical habitats as a result of historical contingency (Chase, 2003; Fukami, 2009).

Priority effects can be benefitting (facilitative priority effect) or inhibiting (inhibitory priority effect) the immigration success of the second arriver (Debray et al., 2022). In this thesis, I focus on inhibitory priority effects, which arise when the early arriver has a numerical advantage over the late arriver (De Meester et al., 2016). Priority effects can therefore be understood as density-dependent processes in which the population size of the first pioneer impacts the later community composition as a result of positive frequency-dependent selection. Positive frequency-dependent selection occurs when the fitness of a species increases with the increasing number of individuals (Vellend, 2016). Consequently, priority effects are expected to be stronger in environmental conditions that favor the growth of the first colonist (Chase, 2003).

Factors that promote the occurrence of priority effects

The strength of priority effects can vary with the environmental context.
Local factors that facilitate fast population dynamics fostering rapid growth include high productivity (Chase, 2010; Vannette & Fukami, 2014) and small habitat patches since carrying capacity is generally reached sooner (Fukami, 2004a). Also, the absence of predation can increase the likelihood of priority effects since very successful first colonizers who can take advantage of freely available resources are expected to be preferably preyed upon (Chase et al., 2009). Finally, the absence of temporal variability and environmental fluctuations is also expected to influence priority effects (Tucker & Fukami, 2014). However, since the growth rate of the first colonizer only matters in comparison to the immigration rate of the second immigrant, the dispersal rate of later arrivers equally matters.

Although this thesis aims to primarily highlight ecological dynamics, evolutionary dynamics can similarly influence the importance of priority effects. Local adaptation could promote priority effects via niche pre-emption whereas theoretical work suggests genetic variation as the main driver (Urban & De Meester, 2009). Likewise, characteristics of the regional species pool can have

an effect on the strength of priority effects, such as its richness (Fukami, 2004b) and species trait composition (Vannette & Fukami, 2014).

Most of the above-described work on priority effects has been done on relatively simple model communities, such as well-studied yeast microbial communities and aquatic invertebrate and amphibian communities. While priority effects have been investigated in complex bacterial communities in recent years, most studies have focused on well-characterized microbe-host systems (Debray et al., 2022). Nevertheless, little is still known about priority effects during the mixing of whole complex natural communities (= community coalescence). Knowledge of the role of historical contingency in natural communities is thus lacking and needs to be investigated further to identify the potential role of priority effects in nature.

Moreover, small and passive dispersers with the ability to grow and adapt rapidly are hypothesized to be prone to priority effects (De Meester et al., 2016). Microbial communities are therefore well suited to study priority effects experimentally and in nature, however, very few studies exist (Rummens et al., 2018; Svoboda et al., 2018; Vass et al., 2021)

Community coalescence and the role of timing

Microbial communities meet and mix regularly. Prominent examples feature the mixing of soil and stream communities in the river bed (Mansour et al., 2018), leaf and soil communities after litterfall (Rillig et al., 2016), and oral communities, when two romantic partners kiss (Kort et al., 2014). Community coalescence describes the process of previously separate communities mixing thus forming a new entity (Rillig et al., 2015). Predictions on microbial community coalescence outcomes are extremely difficult, if not impossible, to make as this would require a thorough understanding of between-community and within-community interspecific interactions (Castledine et al., 2020), including higher trophic levels. The role of arrival timing is equally unexplored, however, intuitively should become important as soon as communities mix into a new environment.

In natural communities, the most likely scenario of community coalescence is the immigration of a new community into an already existing resident community. The resident community is expected to have an advantage through higher population numbers and the monopolization of available resources, much like niche pre-emption sensu Fukami (2015). Local adaptation to the prevailing selection pressure (e.g., predation) could give the resident community an additional advantage (Castledine et al., 2020). However, during coalescence also the different habitats mix, and the resulting environmental shift

could undermine the expected advantages of the resident community (Castledine et al., 2020). Another important consideration is a possible shift in activity in microbial communities depending on the timing of profitable habitat conditions (Aanderud et al., 2015). Microorganisms have the capacity to enter reversible states of reduced metabolic activity (=dormancy) if environmental conditions become unfavorable (Lennon & Jones, 2011). This adds another layer of complexity to community coalescence since the effect of the dispersed community on resident community composition can occur with a time delay. Past dispersal events could therefore have a longer impact on e.g., a lake community if dispersed cells become active at a later time in the lake. Yet, very little is known about the importance of past dispersal in microbial communities.

Likewise, the duration of priority effects in free-living microbial communities is relatively unexplored. Theory predicts that priority effects can be permanent or transient (Fukami, 2015) and microbe-host studies have found priority effects to shape microbial succession across several generations (Debray et al., 2022). Still, more long-term studies and a higher sampling resolution are needed to further explore the longevity of priority effects.

Why study microbial communities?

Microbial communities make exceptionally good models to study broad ecological and evolutionary questions. Their microscopic sizes allow for small experimental units and with that experimental designs that cover a wide range of environmental variability with sufficient replication and thus a high degree of experimental control (Jessup et al., 2004). Further, microorganisms are accessible for genetic manipulation (e.g., to study diversification as in Knope et al. (2012)) and can be stored easily over long periods of time before their resurrection and continued use. Alternatively, microbial communities can be kept in culture easily and thanks to their short generation times are unparalleled to study temporal and evolutionary dynamics (Jessup et al., 2004).

Lastly, bacterial and microbial communities are the backbone of our ecosystems' biogeochemical cycles and perform fundamental functions such as the transformation and recycling of organic matter and nutrients (Falkowski et al., 2008). Their metabolic diversity and short generation times make them susceptible to rapid changes in community composition as environmental conditions vary. A deeper knowledge of the different assembly mechanisms that govern microbial communities is therefore needed to understand important factors that influence microbial community composition and ultimately function (Martiny et al., 2006).

Aims of this thesis

Arrival timing and dispersal timing can be crucial to the community assembly outcome of coalescing communities (Castledine et al., 2020; Rummens et al., 2018; Svoboda et al., 2018). The overall aim of this thesis is therefore to investigate the potential importance of timing in complex lake bacterial communities.

More specifically, the first part of this thesis focuses on results from two laboratory experiments. In the experiments, I investigated the importance of arrival timing of two naïve communities to a new environment (study I). Further, I examined the importance of arrival timing of a mal-adapted community in comparison to a pre-adapted community to a new environment (study II).

The second part of this thesis examines the importance of dispersal, including past dispersal, in natural lake communities based on two field studies. Here, I focused on the importance of external dispersal sources, including past dispersal, to a lake bacterial community (study III). Lastly, the duration of a dispersal event in a lake bacterial community over several weeks was investigated (study IV).

The main research questions of each study can be summarized as:

Study I: aimed to investigate whether the effect of arrival timing of two naïve lake bacterial communities would be stronger at higher nutrient levels and with low immigration rates of the late invader community.

Study II: aimed to investigate if the effect of arrival timing of a maladapted pioneer community into a pre-adapted invader community depended on nutrient availability and the presence/absence of grazing.

Study III: estimated present-day as well as delayed effects of external dispersal sources such as inlets, atmospheric deposition, and groundwater on lake bacterial community composition (BCC).

Study IV: investigated the persistence of a dispersal signal of a strong dispersal event into a lake in present-day epilimnion communities. Further, the study aimed to identify important persistent taxa and determine the main assembly mechanism behind successful persistent amplicon sequence variants (ASVs) in the epilimnion.

Methods – Studies I and II

Batch culture experimental designs

For **study I**, lake water of two dissimilar oligotrophic lakes was sampled and their bacterial communities were used as inocula to test the importance of arrival timing between the first "pioneer" and the second "invader" community. The communities were thereafter introduced a) simultaneously and b) sequentially with a time delay of 96 hours (= 4 days) to five different nutrient levels (10 μgL^{-1}, 50 μgL^{-1}, 100 μgL^{-1}, 200 μgL^{-1} and 400 μgL^{-1} total phosphorus (TP)). The second invader community was introduced at a 1%, 10%, and 50% dispersal rate in comparison to the cell count of the first pioneer communities at the time of inoculation. This was done for five nutrient levels and three dispersal rates resulting in 15 treatments. Single-community controls for "pioneer" and "invader" communities were incubated separately and in the same manner as simultaneous and sequential communities, with four replicates each (Figure 1). After a successful inoculation, the communities were left to grow for 12 days, meanwhile, bacterial cell abundances were monitored by flow cytometry at least every other day. On day 16, the experiment was stopped by filtration onto a 0.2 µm membrane, and the BCC was analyzed by sequencing of 16S rRNA gene amplicons.

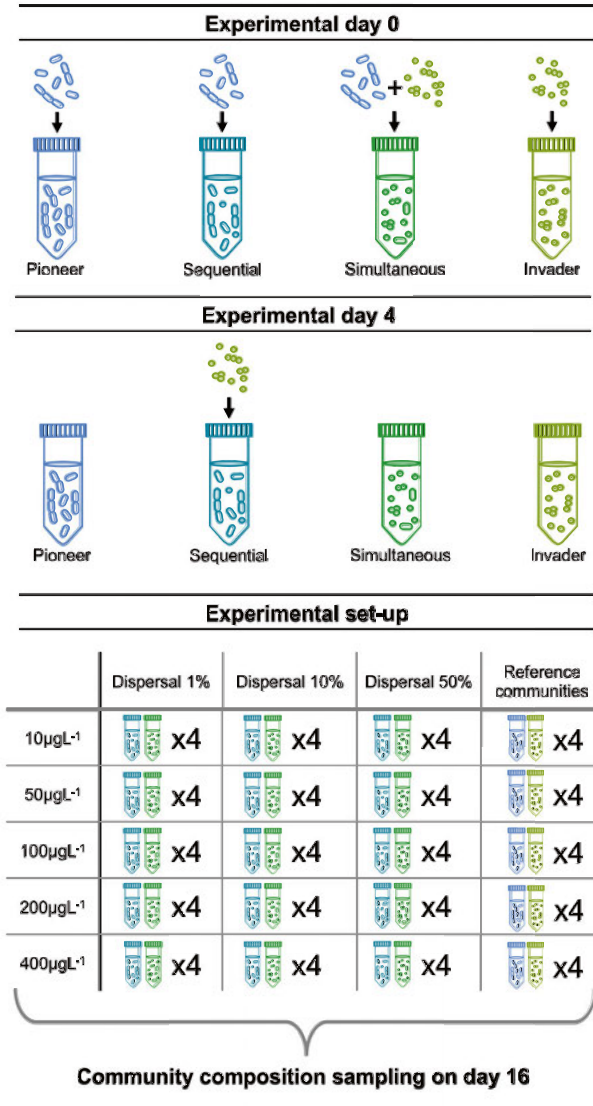


Figure 1: Conceptual overview of the experimental design in study I. Blue rod-shaped cells represent the pioneer communities, light green coccoid cells represent the invader communities. On experimental day0, simultaneous communities were incubated with pioneer and invader communities at the same time whereas sequential communities were incubated with pioneer communities only. Additionally, pioneer and invader communities were inoculated separately without the other competing community (= reference communities). On experimental day4 (96 hours in the experiment), invader communities were added to sequential communities. All experimental communities were introduced into 5 different nutrient levels ($10\mu gL^{-1}$, $50\mu gL^{-1}$, $100\mu gL^{-1}$, $200\mu gL^{-1}$,$400\mu gL^{-1}$) and invaders were introduced at dispersal rates of 1%, 10% and 50% of the cell count of the pioneer communities.

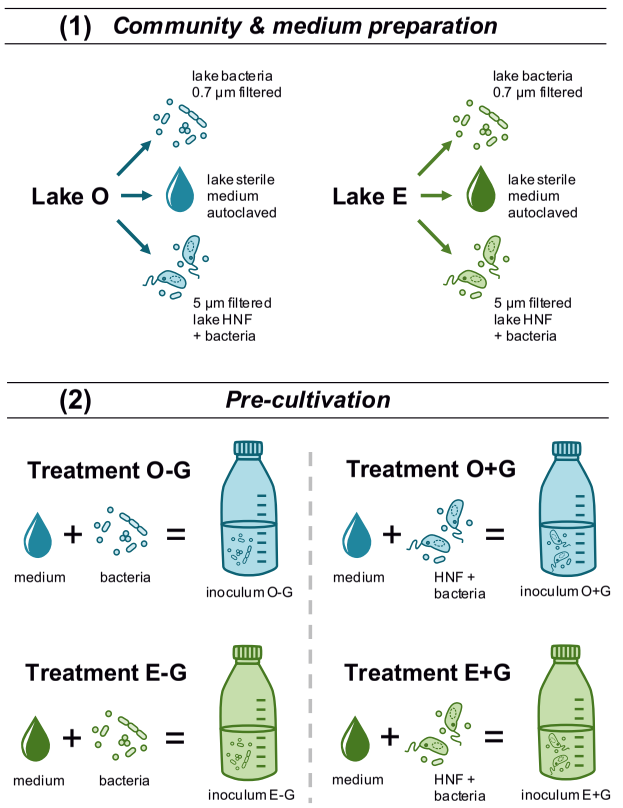
For **study II**, lake water of one oligotrophic and one eutrophic lake were sampled and bacterial communities were used as inocula to test the importance of arrival timing between the first maladapted community and the second pre-adapted community. Prior to the start of the experiment, the communities were pre-cultivated in their respective lake water for 12 days. This was done in the presence and absence of heterotrophic nanoflagellates (HNF) resulting in four experimental treatments: eutrophic with grazing, eutrophic without grazing, oligotrophic with grazing, and oligotrophic without grazing. The pre-cultivated communities were thereafter introduced a) simultaneously and b) sequentially with a time delay of 38 hours where the maladapted community C1 was introduced first followed by the pre-adapted community C2. Here, maladapted and pre-adapted were related to the differences in nutrient levels between home and alien environments. This was done both in the presence and in absence of HNF grazing resulting in the four treatments. Again, single-community controls for all maladapted and pre-adapted communities were incubated separately and in the same manner as simultaneous and sequential communities, with 5 replicates each (Figure 2). After the start of the experiment, the batch cultures were left to grow for 15 days. During the experiment, bacterial cell abundances were monitored by flow cytometry, and HNF abundances were monitored by counting DAPI-stained cells microscopically. Samples for BCC were taken on experimental days 10 and 15 by filtering 200µl of each experimental unit onto a 0.1µm membrane. On day 15, the experiment was stopped and BCC was analyzed by sequencing the 16S rRNA gene.

Bacterial inoculum and experimental sterile medium

To grow complex bacterial communities under laboratory conditions, bacterial lake communities in **study I** were pre-filtered through a 0.7 µm membrane to remove bacterial grazers. The grazer-free communities were thereafter concentrated using tangential flow filtration (TFF) with 0.2 µm membrane pore size, which resulted in concentration factors of 10x the original cell concentration. This step served to achieve small (< 1 mL) inoculation volumes. Consequently, the processed communities were introduced into their respective experimental sterile medium. In **study I**, artificial lake water (ALW) was prepared according to Bastviken et al. (2004) where differences in nutrient levels were achieved by varying concentrations of Na_2HPO_4, NH_4Cl and Reverse Osmosis (RO) concentrate as the carbon source. We strived for achieving a C:N:P ratio of 106:16:1 according to Redfield Ratio (Redfield, 1934).

Bacterial lake communities in **study II**, were partitioned into communities without (0.7 µm filtered communities), and with grazers (HNF) (5 µm filtered communities). The communities were thereafter pre-cultivated in their home lake environment (0.7 µm-filtered and autoclaved lake water) and left to grow

for 12 days in laboratory conditions. The resulting pre-cultivated communities served as inocula for their respective experimental sterile medium. The experimental sterile medium was again composed of the 0.7 μm filtered and subsequently autoclaved lake water of the two lakes. The original pH of the lake water was restored in the medium by HCl addition after autoclavation.


(1) Community & medium preparation
lake bacteria
0.7 µm filtered
Lake O
lake sterile
medium
autoclaved
5 µm filtered
lake HNF
+ bacteria
lake bacteria
0.7 µm filtered
Lake E
lake sterile
medium
autoclaved
5 µm filtered
lake HNF
+ bacteria
(2) Pre-cultivation
Treatment O-G
medium
bacteria
inoculum O-G
Treatment O+G
medium
HNF +
bacteria
inoculum O+G
Treatment E-G
medium
bacteria
inoculum E-G
Treatment E+G
medium
HNF +
bacteria
inoculum E+G

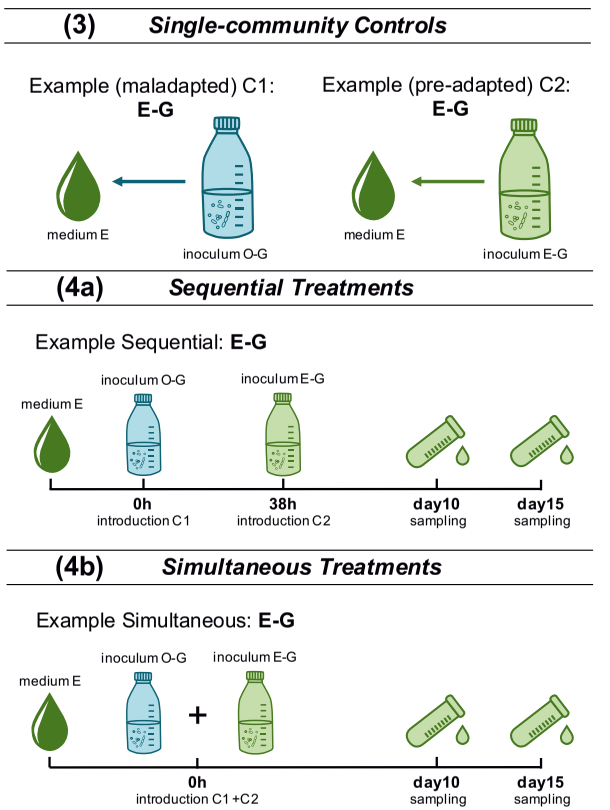

(3) Single-community Controls
Example (maladapted) C1:
E-G
medium E
inoculum O-G
Example (pre-adapted) C2:
E-G
medium E
inoculum E-G
(4a) Sequential Treatments
Example Sequential: E-G
medium E
inoculum O-G
inoculum E-G
0h
introduction C1
38h
introduction C2
day10
sampling
day15
sampling
(4b) Simultaneous Treatments
Example Simultaneous: E-G
medium E
inoculum O-G
inoculum E-G
0h
introduction C1 +C2
day10
sampling
day15
sampling

Figure 2 (page 21): Overview of (1) community and media preparation, (2) pre-cultivation, (3) single-community controls, (4) sequential and simultaneous treatments in study II. (1) Lake water O (=oligotrophic) was used to provide sterile medium O, oligotrophic communities with bacteria only and oligotrophic communities with HNF present were used as inocula. Lake water E (=eutrophic) was used for sterile medium E, eutrophic bacterial communities and eutrophic communities with HNF present were used as inocula. (2) The resulting media and communities were used to prepare 4 pre-cultivated communities: O-G, O+G, E-G and E+G which were left to develop for 12 days in the dark before the start of the experiment. (3) We introduced single-community controls at the start of the experiment by introducing C1 and C2 separately without the competition of another community. (4a) sequential treatments were inoculated with C1 on day0 and C2 38 hours later. (4b) Simultaneous treatments were inoculated with C1 and C2 concurrently on day0. Single-community controls, sequential treatments and simultaneous treatments were sampled on experimental days 10 and 15. Note that panels 3 and 4 only show examples for treatment E-G.

Cell abundances and bacterial secondary production

In all studies, bacterial abundances were quantified by flow cytometric determination by volumetric counting of SYTO 13-stained cells as described in del Giorgio et al. (1996) .

HNF cell abundances in study II were determined by epifluorescence microscopy using DAPI-stained cells under UV excitation.

Microbial community composition

In studies I and II, total nucleic acids were extracted from the bacterial communities. Following, the variable V3-V4 region of the 16S rRNA gene was amplified with polymerase chain reactions, and the resulting amplicons were sequenced by Illumina MiSeq sequencing.

The raw sequencing data were processed using the Divisive Amplicon Denoising Algorithm (DADA2). This included quality filtering and trimming, amplicon sequence variant (ASV) inference, and chimeria removal. Taxonomic assignment was done using the SILVA reference alignment according to the DADA2 workflow (Callahan et al., 2016).

Statistical analyses

A number of statistical analyses were used in this thesis, here key methods are highlighted as they were recurring throughout all studies or consequential for individual studies.

Differences in the bacterial communities in studies I and II were visualized and tested using principal coordinates analysis (PCoA) and permutational multivariate analysis of variance (PERMANOVA) of Aitchison distances. Aitchison distances were chosen to accommodate the compositional nature of amplicon sequence data and were computed using Euclidean distances of centered log-ratio (clr) transformed data (Gloor et al., 2017).

To assess priority effects on a population level, differential abundance analysis (DeSeq2) was used to identify taxa that contributed to the observed differences in sequential and simultaneous communities in studies I and II. For the analysis, we selected the 20 most abundant ASVs in the pooled replicates of the first pioneer and second invader communities, respectively. Following, the selected dominant taxa from each reference community in sequential and simultaneous communities were tested, using DESeq2 analysis (Love et al., 2014).

Main results – Studies I and II

The importance of priority effects has been shown to vary with the ecological context in simple model communities (Leopold et al., 2017). Environmental conditions which favor population growth of the first arriver are expected to promote the occurrence of priority effects, in cases of niche pre-emption and niche modification. Such environmental conditions can be for instance, higher nutrient availability (Vannette & Fukami, 2014), higher temperatures (Vass et al., 2021), or the absence of predation (Chase et al., 2009) or low viral-induced mortality. Many of these conditions are still unexplored for the strength of priority effects in complex bacterial communities.

Can nutrient availability and dispersal rate of the invader influence the importance of priority effects? (Study I)

In **study I** we investigated the importance of nutrient availability for the occurrence of priority effects in two dissimilar lake communities. We hypothesized stronger priority effects at higher nutrient levels due to the higher growth rates of the first pioneer. The growth rate of the first arriver, however, is only relevant if compared to the immigration rate of the second invader (Fukami, 2015). Therefore, we further hypothesized to detect stronger priority effects with lower dispersal rates of the invader.

If priority effects were strong, we expected sequential communities to more closely group to the reference pioneer treatments and consequently expected simultaneous communities to group closer to reference invaders (as visualized in Figure 3).

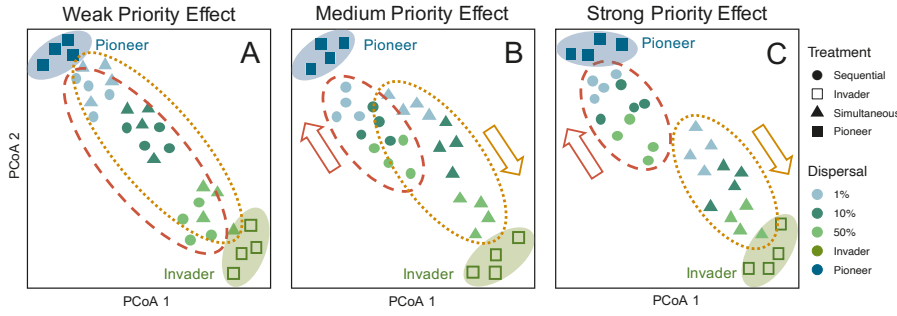


Figure 3: Conceptual figure for study I and II of the expected development of simultaneous and sequential communities in relation to their respective reference invader and reference pioneer communities in cases of (A) weak, (B) medium and (C) strong priority effects. (A) In cases of weak priority effects, we expected no significant differences between simultaneous and sequential communities and that all treatments aligned between reference pioneer and invader communities, depending on the dispersal rate of the invader. (B) For medium priority effects, we expected a separation between simultaneous and sequential communities as well as a lower distance of sequential communities to reference pioneers. Consequently, we expected a lower distance of simultaneous treatments to reference invaders. (C) For strong priority effects, we expected an even stronger separation between simultaneous and sequential communities and even lower distances between sequential-pioneer and simultaneous-invader communities.

The PERMANOVA results showed that simultaneous treatments differed significantly from sequential communities, which indicated that arrival timing mattered for the development of the coalesced communities. However, we could not detect that sequential communities more closely resembled reference pioneer communities as would have been expected in cases of priority effects. Also, similarly, simultaneous communities did not more closely resemble invader communities (Figure 4). On the ASV level, we could only find a small number of taxa in the pioneer communities which contributed to the observed differences in simultaneous and sequential communities. Instead, the biggest differences in BCC could be explained by differences in nutrient availability, thus highlighting the superior role of species sorting compared to arrival timing (see Table 1 in **study I).**

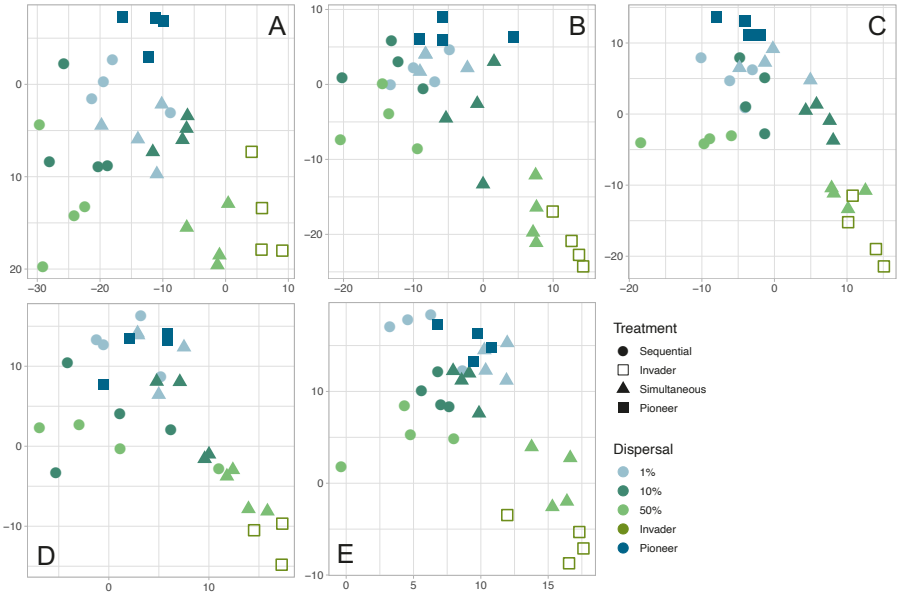


Figure 4: Principal coordinate analysis (PCoA) derived from Aitchison distance of BCC at the five nutrient levels in study I. The plots represent the different nutrient treatments: 10µg L^{-1} (A), 50µg L^{-1} (B), 100µg L^{-1} (C), 200 µg L^{-1} (D), 400µg L^{-1} (E). Axis 1 has a relative eigenvalue of 0.094, axis 2 has a relative eigenvalue of 0.081. Single-community reference invader and pioneer communities shown in each plot were grown for each nutrient separately. Evidently, the experimental communities did not show the expected development in cases of priority effects, thus making it impossible to attribute the difference between simultaneous and sequential communities to priority effects.

To summarize, we were unable to reliably detect priority effects on the community level in **study I**. Since we could not attribute the differences between simultaneous and sequential communities to priority effects, I refrain from discussing the importance of nutrient availability and the dispersal rate of the invader for arrival timing. The differences between simultaneous and sequential communities were likely caused by other factors which will be discussed in the discussion section.

Can nutrient availability and the presence or absence of grazers influence the importance of priority effects? (Study II)

Study II explored the importance of nutrient availability and predation for the strength of priority effects. We hypothesized stronger priority effects in eutrophic treatments, again, due to the expected higher growth rates of the first arriving community C1. Additionally, we hypothesized stronger priority

effects in the absence of grazing as a likely negative influence of grazing on the population numbers of the early C1.

The result showed the expected development of simultaneous and sequential communities in cases of priority effects, that is, sequential communities more closely resembling C1 and simultaneous communities more closely resembling C2 (see conceptual Figure 3). We therefore concluded that arrival history mattered for the coalesced communities in **study II**. The expected BCC outcome, however, according to our hypotheses, could only be found in the high nutrient treatment and in the absence of HNF grazing. Higher nutrient availability and the connected higher bacterial abundances of C1, thus, mattered for the detection of priority effects (Figures 5 & 6).

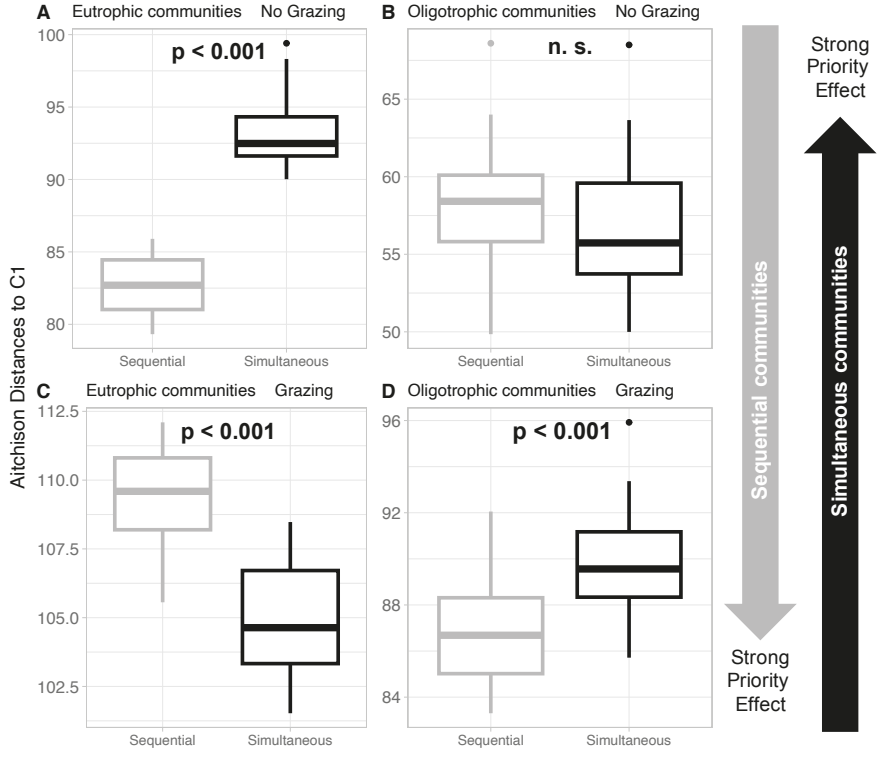


Figure 5: Study II - Aitchison distances of sequential and simultaneous communities to C1 sampled on experimental day 10. Differences in the distances were tested using paired Wilcoxon signed rank tests. Arrows display the expected higher/lower similarity of sequential and simultaneous communities to C1 in case of strong priority effects.

The presence of grazers in eutrophic treatments clearly changed community composition when compared to eutrophic treatments without grazing. Importantly, opportunistic taxa which were important for the detection of priority effects in the eutrophic, non-grazing treatments seemed to be vulnerable to

predation. Increased HNF grazing pressure likely reduced the net growth rates and thus advantage due to the early introduction of C1.

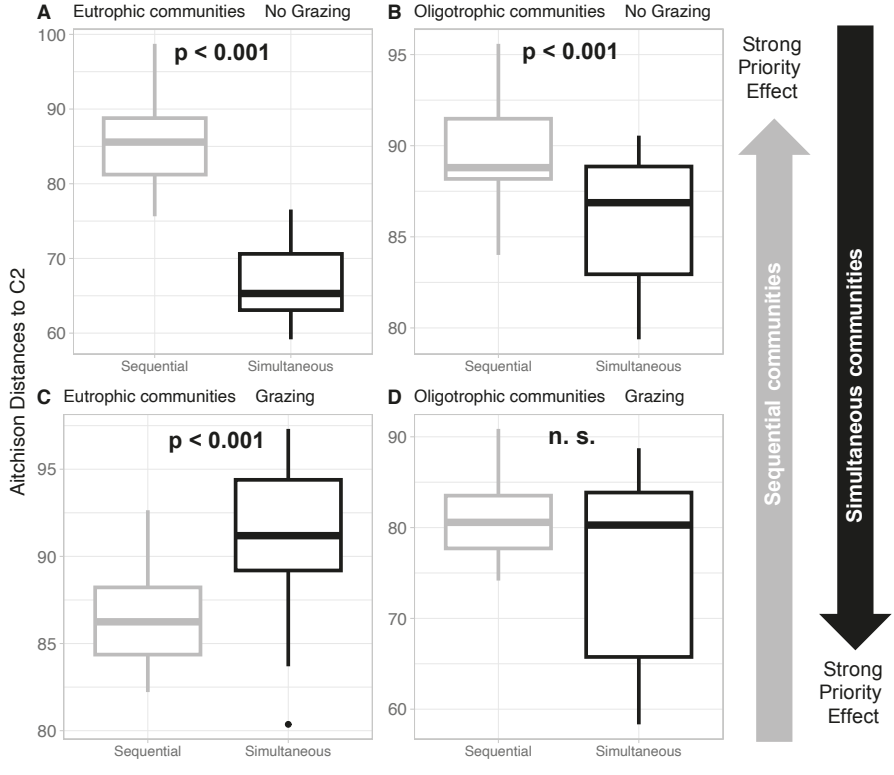


Figure 6: Study II - Aitchison distances of sequential and simultaneous communities to C2 sampled on experimental day 10. Differences in the distances were tested using paired Wilcoxon signed rank tests. Arrows display the expected higher/lower similarity of sequential and simultaneous communities to C2 in case of strong priority effects.

However, differences between communities explained by arrival timing were smaller than the differences explained by nutrient availability and grazing, illustrating the superior role of environmental selection in **study II** (see Table 1a in **study II**).

In summary, in **study II** I found that higher nutrient availability and the absence of grazing promoted the occurrence of priority effects in our experiment. When comparing the importance of arrival timing to the importance of species sorting as predictors for BCC, arrival timing seems to be of minor importance.

Methods – Studies III and IV

Lake studies

The oligotrophic forest lake Digernästjärnen in Jämtland County was sampled for **study III**. To identify the most important dispersal sources to the lake water and the lake sediment sinks, cell transport rates from three different inlets into the lake, atmospheric deposition, and groundwater inflow were determined during a seven-day sampling period. To account for spatial variability, the dispersal sources and lake water samples were taken at four different sampling locations in the lake (Ra-Rd in Figure 7). Samples for BCC were taken *in situ* by filtering 0.15 L onto a 0.2 μm filter membrane and immediately preserving the sample in liquid nitrogen. Similarly, 1 g sediment was weighed and immediately stored in liquid nitrogen until further processing and sequencing of the 16S rRNA gene and transcript. Bacterial cell abundances were quantified using flow cytometry and the internal bacterial production of the lake bacterial community was measured by thymidine incorporation into DNA.

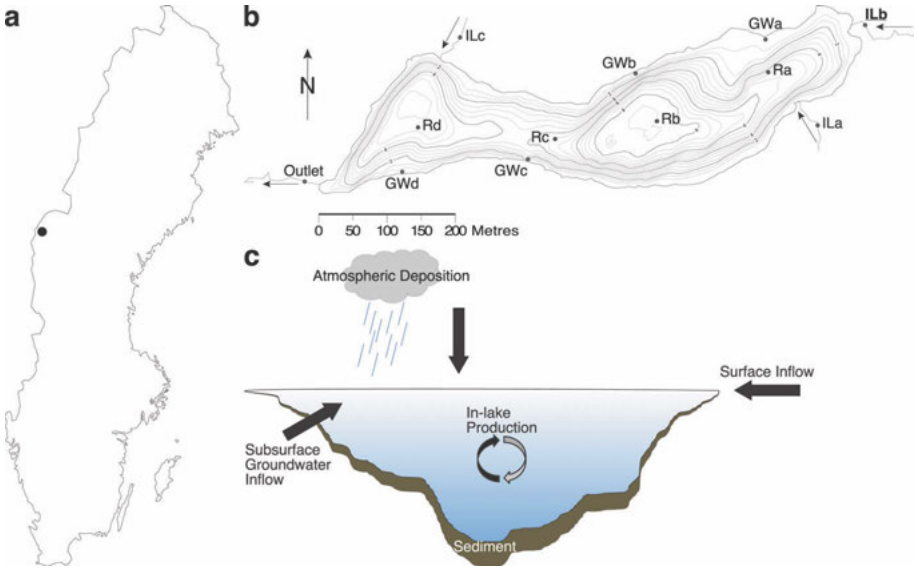


Figure 7: Overview of the sampled lake in study III. (a) Map pointing to the study lake, Lake Digernästjärnen. Panel (b) depicts Lake Digernästjärnen's bathymetry as well as all sampling stations. Panel (c) illustrates a lake's hypothetical cross-section, highlighting the various possible routes of bacterial immigration (i.e., dry and wet atmospheric deposition, subsurface groundwater, and surface inflow). Abbreviations are: GW, subsurface groundwater inflow; IL, surface inflow (i.e., inlets); R, raft (i.e., in-lake station, where atmospheric deposition, epi- and hypolimnion, and sediment sampling was carried out).

For **study IV**, the oligotrophic forest lake Siggeforasjön in Uppsala County was sampled. The sampling campaign was conducted between February and June and aimed to capture a large dispersal event (spring flood) from the main inlet into the lake. Inlet discharge measurements were taken weekly along with inlet and lake water samples to estimate bacterial abundances, physico-chemical parameters, and BCC. The lake samples were taken at the deepest point of the lake and divided into epilimnion and hypolimnion samples. To monitor mixis and stratification of the lake water body, a temperature and oxygen profile was taken, again, at the deepest point of the lake. Bacterial abundances were quantified using flow cytometry and BCC was analyzed by sequencing the 16S rRNA gene and transcript.

Cell abundances and bacterial secondary production

In all studies, bacterial abundances were quantified by flow cytometric determination of SYTO 13-stained cells as described in del Giorgio et al. (1996). Bacterial secondary production in **study III** was measured as tritiated thymidine incorporation into DNA according to Bell (1993).

Microbial community composition

In **studies III and IV**, DNA and RNA were co-extracted from collected bacterial cells. The consideration of the RNA fraction served as a proxy for metabolic activity in the community as RNA is needed for protein synthesis in the cell (Laursen et al., 2005). Subsequently, RNA samples from **studies III and IV** were DNase treated and reverse transcribed to generate complementary DNA (cDNA) for downstream processing. The variable V3-V4 region of the 16S rRNA gene was amplified according to the methods described for **studies I and II** (see above). The resulting amplicons were sequenced by either 454-pyrosequencing (**study III**) or Illumina MiSeq sequencing (**study IV**).

The raw sequencing data were processed using different pipelines. For study **III**, raw sequencing data were processed using AmpliconNoise, Perseus as well as an in-house Perl script. Further, 454-pyrosequences were classified using RDP naïve Bayesian Classifier, operational taxonomic units (OTUs) were clustered at 97% sequence identity and aligned per SILVA reference alignment. For **study IV**, quality filtering and trimming, amplicon sequence variant (ASV) inference, and chimeria removal were done using the Divisive Amplicon Denoising Algorithm (DADA2). Taxonomic assignment was done using the SILVA reference alignment according to the DADA2 workflow (see **studies I and II**).

Statistical analyses

Differences in bacterial communities in study III were visualized and tested using non-metric multidimensional scaling (NMDS) ordination and PERMANOVA based on Morisita-Horn distances. Beta-diversities between bacterial communities in study IV were estimated using NMDS ordination based on Bray-Curtis dissimilarities.

SourceTracker (ST) analyses in studies III and IV were used to determine the most important past and present dispersal sources to the lake sink communities. SourceTracker is a machine learning algorithm and uses a Bayesian approach to estimate the relative contribution of multiple source communities to a sink community, including unknown sources (Knights et al., 2011).

In study III, the studied external sources of the lake DNA (LD) sink consisted of three inlets (ILa-ILc), atmospheric deposition (ADa-ADd) collected in four sampling stations in the lake (Ra-Rd), and groundwater discharge measured in four different sampling locations in the lake (GWa-GWd) (see Figure 7). Additionally, we included the lake RNA fraction (LR) as a source (Figure 8, B) as we relied on the assumption that rRNA is proportional to the growth of

the lake community. This step served to compare the lake's internal production to the external dispersal. Past dispersal sources were sources from previous sampling occasions that contributed substantially (> 10%) to the lake DNA sink at the time.

In study IV, we investigated the contribution of the present-day lake hypolimnion communities, present-day inlet dispersal communities and communities from a past strong dispersal event ("spring flood samples") to the present-day epilimnion BCC as the sink. To identify ASVs stemming from the spring flood event in study IV, we selected taxa with a probability of ≥ 60% to originate in one of the spring flood sources, according to the ST model outputs. Subsequently, the relative read abundances as well as RNA:DNA ratios for the selected ASVs were calculated to investigate potential shifts in relative abundance and activity between the inlet and lake environment.

Main results - Studies III and IV

The way natural communities assemble has been conceptualized and extensively studied in the last two decades (Langenheder & Lindström, 2019; Leibold et al., 2004; Vellend, 2010). In freshwater bacterial communities, the common consensus is that local environmental conditions and dispersal from the regional species pool best explain the natural communities' diversity and composition patterns (e.g. Van der Gucht et al., 2007; Crump, 2012; Adams et al., 2014; Barberán and Casamayor, 2014; Souffreau et al., 2018). Understanding the quantitative contribution of the different dispersal sources is critically important, but has been understudied in the past (Langenheder & Lindström, 2019). Moreover, depending on the environmental context, stochastic community assembly mechanisms (e.g., dispersal timing) can become important (Aguilar & Sommaruga, 2020; Stegen et al., 2013) in the lake BCC. Regardless, so far temporal dynamics have rarely been considered for microbial community assembly studies.

What is the importance of external dispersal sources to a model lake and does past dispersal matter? (Study III)

Study III explored the relative contribution of the main bacterial dispersal sources to a model lake and their potential impact on BCC. The studied sources were composed of three inlets, atmospheric deposition, and groundwater discharge, to the lake water and lake sediment sinks. The aim of **study III** was to relate the observed dispersal rates with the internal growth-related inputs within the lake. Additionally, we aimed to investigate the role of present-day dispersal versus past dispersal.

The most influential factor for community assembly was the lake's internal production according to the bacterial production results as well as the lake RNA fraction in the SourceTracker (ST) models. On all three sampling occasions, the ST models also identified the main inlet (ILb) as well as different groundwater sources (mainly GWa and GWc) as important dispersal sources for the lake BCC (Figure 8).

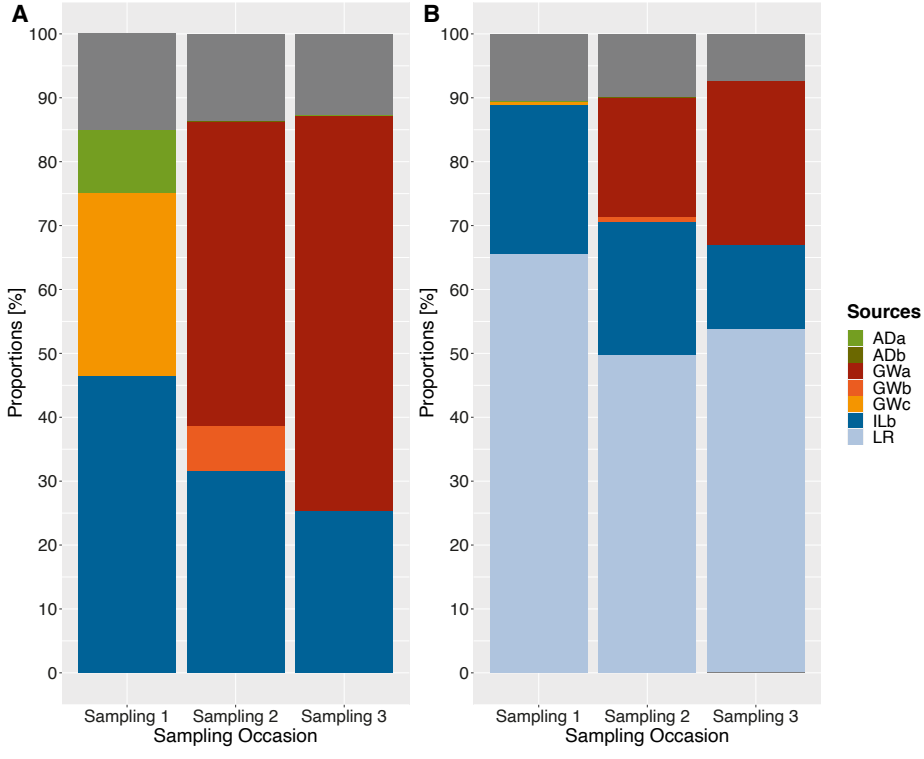


Figure 8: Results from SourceTracker analyses in study III with lake bacterial DNA (LD) as the sink. The graphs visualize the estimated proportions of the most important sources derived from ST models including a) ADa, ADb, GWa-c, ILb as sources, and b) ADa, ADb, GWa-c, ILb, LR as sources. Abbreviations are as follows: AD, atmospheric deposition; GW, subsurface groundwater inflow; IL, surface inflow (i.e., inlets); LR, lake bacterial RNA; a-d, sampling stations a-d; Sampling 1-3, sampling occasion 1-3.

As expected, we identified differences in the ST models depending on the presence or absence of the lake RNA fraction (LR) as a source, since the results of ST models depend on the number of included sources (Figure 8). However, the major inlet (ILb) and one of the groundwater sources were important sources also when LR was included. When excluding LR in the ST models, both ILb and groundwater sources increased in relative contribution while atmospheric deposition always was of low importance. Surprisingly, we found no relationship between the estimated dispersed cells and the ST model results.

Past external dispersal sources, especially dispersal from groundwater as well as ILb, also mattered for present-day lake BCC. The final ST model which included dispersal sources from sampling occasion 1 and 2, showed that past dispersal contribution even outnumbered present-day dispersal at times (Figure 9).

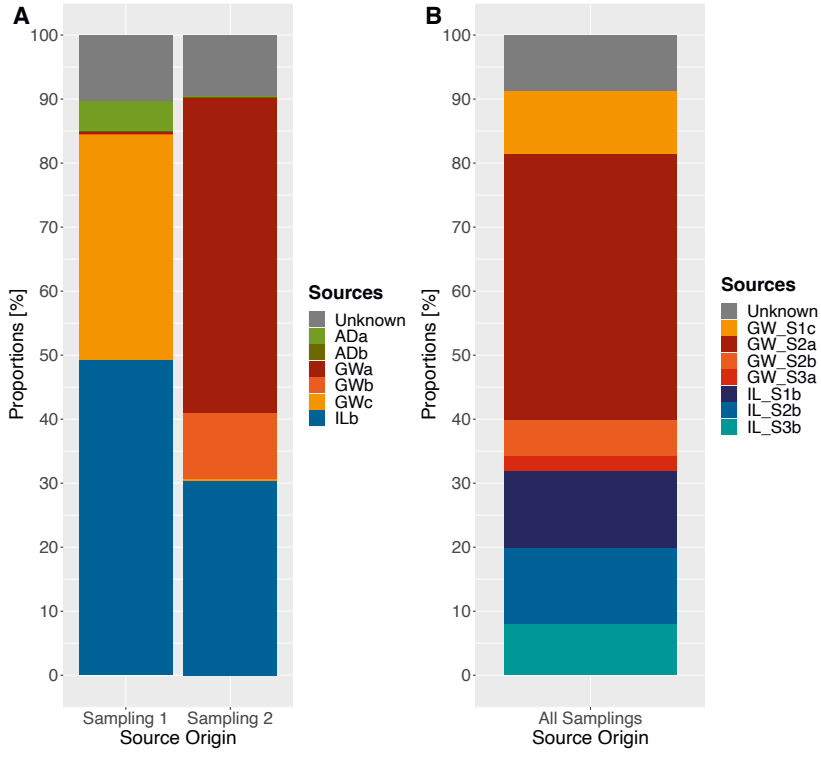


Figure 9: Results from SourceTracker models in study III with lake bacterial DNA (LD) from Sampling 3 were used as the sink. The graphs show the estimated proportions of the most important sources derived from earlier ST models. A) Sources from exclusively sampling 1 (AD_S1a, AD_S1b, GW_S1a, GW_S1b, GW_S1c, IL_S1b) or exclusively sampling 2 (AD_S2a, AD_S2b, GW_S2a, GW_S2b, GW_S2c, IL_S2b) are considered. B) Most important sources from sampling 1 and sampling 2 are included (GW_S1c, GW_S2a, GW_S2b, GW_S3a, IL_S1b, IL_S2b, IL_S3b). Abbreviations are as follows: AD, atmospheric deposition; GW, subsurface groundwater inflow; IL, surface inflow (i.e., inlets); a-d, sampling stations a-d; Sampling 1-3, sampling occasion 1-3.

In summary, **study III** found species sorting the predominant assembly process in the studied lake bacterial community. Nevertheless, groundwater and the main inlet clearly were relevant for the lake BCC, with or without including the RNA fraction in the ST models. Finally, we were able to detect the importance of past dispersal sources according to our ST models, which is interesting to consider for future community assembly studies.

How long can the signal of past dispersal be detected in lake BCC? (Study IV)

Study IV monitored a model lake and the water discharge from its main inlet during the time of a strong dispersal event (= spring flood) and up to two months after this event. The aim was to measure the duration of the spring flood signal in the present-day epilimnion community. Further, **study IV** aimed to identify important dispersed inlet taxa that were persistent in the epilimnion community. To differentiate between potentially actively growing cells in the lake environment versus inactive downstream cell transport, we compared relative abundances and RNA:DNA ratios of pre-selected ASVs. This step was included to determine the main assembly mechanism behind successful persistent taxa in the lake epilimnion.

SourceTracker model results illustrated that the signal of the past spring flood event was detectable until four weeks after the pre-defined spring flood. The relative contribution of past dispersal to the BCC of the epilimnion was always minor in comparison to the hypolimnion source or present-day inlet dispersal (Figure 10).

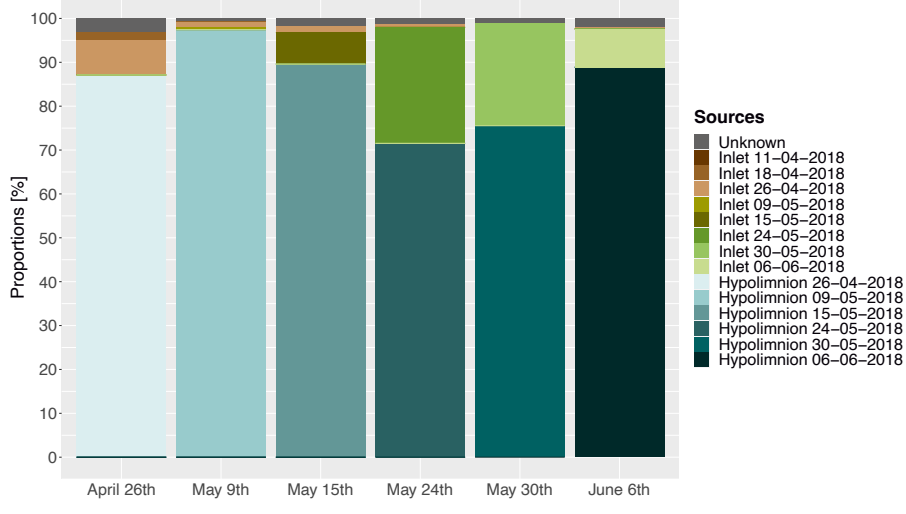


Figure 10: Results of SourceTracker (ST) models in study IV. Each bar represents the contribution of the sources (inlet spring flood samples: Inlet 11-04-2018, Inlet 18-04-2018 & Inlet 26-04-2018, present-day inlet and present-day hypolimnion) to the sink (present-day epilimnion) for each sampling occasion.

ASVs that stemmed from the spring flood and persisted in the lake epilimnion were overwhelmingly classified as inactive in both inlet and epilimnion thus suggesting a higher importance of the dispersal of inactive cells along the aquatic continuum. Several opportunistic taxa that were active in the inlet

switched to becoming inactive in the lake or were not detectable in the RNA fraction of the epilimnion. Only a small number of opportunistic ASVs were active in both inlet and epilimnion which suggests an active recruitment into the new environment. Finally, only one ASV was identified that switched from inactive in the inlet to active in the lake environment thus showing an insignificant role of the inlet acting as a "seed bank" for the lake BCC.

In summary, **study IV** showed that lake epilimnion appeared largely influenced by the dispersal from the present-day hypolimnion and present-day inlet dispersal and only minorly by past inlet dispersal. Yet, we could show a long-lasting signal of past inlet dispersal which primarily resulted from the dispersal of rare and inactive cells from the upstream source.

Overall discussion

Are priority effects detectable in uncultured and complex bacterial communities? (Studies I & II)

Insight into historical assembly processes in complex microbial communities has long been restricted due to the methodological challenges when characterizing microbial community members and their interactions (Debray et al., 2022). The growing evidence of priority effects in microbial communities in recent years mostly focuses on well-described microbe-host systems or simpler culturable communities (Furman et al., 2020; Lee et al., 2013; Martínez et al., 2018; Vannette & Fukami, 2014). Yet, little is still known about priority effects during the coalescence of complex natural communities. Pioneering studies are scarce and not always conclusive (Rummens et al., 2018; Svoboda et al., 2018; Vass et al., 2021).

In **studies I and II** we aimed to measure priority effects of whole communities as the difference between simultaneous communities and sequential communities. Further, we compared the coalesced communities to their single-community references as a way to measure the direction of community development in simultaneous and sequential communities (see conceptual Figure 3). In **study I**, we found significant differences between simultaneous and sequential communities which would have indicated the relevance of arrival history. However, the results did not agree with what would have been expected in cases of priority effects, as discussed above.

While higher nutrient levels promoted the growth of the first pioneer, the second invader continuously showed higher bacterial abundances in single-community controls thus possibly overwriting any signs of positive frequency-dependent selection from the pioneer community. However, even if invader communities were the better competitors, we would have expected convergence of both simultaneous and sequential communities towards the reference invaders for each nutrient treatment. Surprisingly, this was not the case.

One methodological concern was the risk of changes in the composition and diversity of the invader community between the introduction into simultaneous communities and sequential communities. While we identified a change

in richness, we could not verify that a change in the invader community from day 0 to day 4 affected the outcome of our experiment substantially. A previous study has used cryopreserved stock communities as the second invader (Rummens et al., 2018), thus avoiding this methodological bias.

In **study II**, on the contrary, we were able to observe priority effects according to our expectations. The reason behind this could have been a methodological one. Similar to previous studies investigating priority effects in coalescing aquatic communities (Rummens et al., 2018; Svoboda et al., 2018), **study II** measured the strength of priority effects as the invasion resistance to the better-adapted second community. By introducing maladapted and pre-adapted communities to their respective sterile alien and home environment, one can follow the pre-determined development of the experimental communities better, which, in turn, makes the interpretation of the results easier. The additional pre-cultivation step before the start of the experiment helped to adjust the natural lake communities to laboratory conditions, thus, resulting in less community turnover at the onset of the experiment. The individual experimental replicates in **study II**, therefore, showed less variability compared to the replicates of **study I** which ultimately influenced the interpretation of the results and the statistical power of each experiment.

What are the underlying factors influencing the strength of priority effects? (Study II)

Previous studies on simple model communities found a positive effect of nutrient availability on the occurrence of priority effects (Vannette & Fukami, 2014). In contrast, predation is hypothesized to temper the occurrence of priority effects (Chase et al., 2009). To our knowledge, no study has so far investigated the importance of grazing on the occurrence of priority effects in bacterial communities.

In **study II** we could identify priority effects according to our assumptions, however, the effects of arrival timing were only observed in high-nutrient treatments and in the absence of grazing. Eutrophic treatments also depicted the highest bacterial abundances of C1 recipient communities at the time of C2 community invasion (= 38 hours in the experiment). The importance of higher population numbers of the first arriver C1 for the occurrence of priority effects is in line with previous literature (Chase, 2010) as it promotes positive frequency-dependent selection (Vellend, 2016). Moreover, a higher bacterial abundance of C1 in eutrophic treatments increases the likelihood of resource pre-emption and/or modification, which are the underlying mechanisms of priority effects (Fukami, 2015). Likewise, a previous study found the effects

of invasion resistance to be stronger in treatments with higher bacterial abundances in the resident community (Vass et al., 2021).

Comparing grazing to non-grazing treatments under eutrophic conditions, we found big compositional differences between the communities. Importantly, we were not able to identify priority effects in eutrophic grazing treatments. While bacterial abundances of recipient C1 communities at the time of C2 arrival were also high, the presence of grazers prevented strong exponential net growth (as in the non-grazing treatments), thus likely reducing the advantage of an early introduction of C1. To our knowledge, no other study has investigated the effects of grazing on the occurrence of priority effects in complex bacterial communities. However, it has been shown that zooplankton predation can enhance the importance of deterministic community assembly processes in bacterial communities (Berga et al., 2015). This in turn would counteract the degree of stochastic community assembly processes such as priority effects.

In summary, we found that environmental factors that promote the growth of an early arriver have potential to promote priority effects. Contrarily, conditions that counteract the development of the early-arriving community likely decreased the chances of priority effects, which is in agreement with previous literature (Chase et al., 2009; Vannette & Fukami, 2014; Vass et al., 2021).

Several methodological choices likely led to an underestimation of arrival timing in both **studies I and II.** For example, the introduction of the second invading community while the first pioneering community was still in exponential growth phase. Our chosen time delay between pioneer and invader mimicked previous study designs which successfully detected priority effects (Rummens et al., 2018; Svoboda et al., 2018). However, stronger effects of arrival timing were found with longer time delays and when recipient pioneer communities were in stationary growth phase (Rummens et al., 2018).

What is the importance of external dispersal sources to a model lake and does past dispersal matter? (Study III)

The results in **study III** showed an overwhelming importance of the lake's internal production compared to external dispersal sources, thus highlighting the importance of species sorting for lake BCC. This conclusion was supported by bacterial secondary production data, a decline in alpha diversity from inlet streams to the lake as well as SourceTracker (ST) models including the RNA fraction as a proxy for the actively growing part in the bacterial community. The use of RNA data as a proxy for growth rates has been criticized

because the rRNA content of different taxa has been shown to vary at a given metabolic rate (Steven et al., 2017). Further, ribosome accumulation has been observed in some dormant bacteria (Sukenik et al., 2012). However, the use of RNA data is in general considered a robust measure of microbial activity when compared with other community-wide metrics of cellular activity (Bowsher et al., 2019; Loeppmann et al., 2018). Additionally, a great number of studies have also found a superior role of species sorting when compared to external sources for the BCC in lakes (e.g. Van der Gucht et al., 2007; Barberán and Casamayor, 2010; Logue and Lindström, 2010; Adams et al., 2014; Barberán and Casamayor, 2014; Souffreau et al., 2015; Souffreau et al., 2018), thus supporting our findings.

Regarding the external dispersal sources, the observed contribution of the main inlet is not surprising as several previous studies have found an influence of surface water flow from, for instance, inlets on lake BCC (Adams et al., 2014; Comte et al., 2017; Crevecoeur et al., 2022; Crump et al., 2012). However, we found no relationship between the estimated dispersed cells and ST model results. Since past effects of inlet dispersal seemed at times more important than present-day dispersal into the lake, a delay in dispersal effects on lake BCC could be an explanation for the incoherent results.

The dispersal from the groundwater was even more complex with different groundwater sites contributing at different sampling occasions. Generally, we found a high contribution of GWa both as present-day dispersal source as well as past dispersal source. With a few exceptions (e.g., see Ortiz-Álvarez et al., 2020) the role of groundwater in lake BCC has so far not been sufficiently explored. We, therefore, encourage future studies to incorporate groundwater as a viable dispersal source in aquatic ecosystem assembly studies.

How long can the signal of past dispersal be detected in lake BCC and who are the major contributors? (Study IV)

SourceTracker models in **study IV** could identify the signal of past dispersal from the inlet spring flood in the epilimnion BCC up until four weeks later. This result is remarkable, especially since the lake mixed directly after the end of the defined spring flood, thus, resulting in severe environmental changes and cell dispersal from the hypolimnion.

The majority of ASVs stemming from the spring flood were either inactive along the aquatic continuum or shifted from active in the inlet to inactive in the epilimnion (= ”filtered”). Ubiquitous generalist taxa can have attenuated

growth rates even in changing environmental conditions such as along the aquatic continuum in **study IV** (Crevecoeur et al., 2022; Klappenbach et al., 2000). High resource efficiency allows for low activity and could be the key to wide ecological tolerances in these taxa (Crevecoeur et al., 2022). On the other hand, if strong environmental pressure from the inlet to the lake environment acts on the dispersed cells, they can become dormant or die, thus, leaving only their DNA traces behind.

Few metabolically active ASVs from the inlet remained active in the epilimnion, indicating metabolic activity and cell growth in the new environment. However, all taxa originating from the inlet shifted from sub-dominant or rare to even lower relative abundances in the epilimnion. This suggests a strong environmental barrier between the inlet and the lake. Although we could not detect a strong environmental gradient, other unmeasured determinants for instance the available substrate (e.g. DOM) as well as grazing pressure and viral load (Adams et al., 2014) could have served as environmental filters. Nevertheless, rare taxa in bacterial communities can exhibit high growth rates, despite low abundances. Strong grazing pressure can limit their numerical dominance even though they substantially contribute to the system's food chain and microbial processes (Neuenschwander et al., 2015; Šimek et al., 2014). This further highlights the need to combine RNA and DNA tools in future microbial studies.

Generally, however, the observed contribution of past dispersal of cells into the present-day epilimnion BCC was relatively small. The most important dispersal source was the lake's hypolimnion followed by the present-day dispersal from the inlet.

In conclusion, **study III** and **study IV** showed that past signals from external dispersal sources mattered for the lake's BCC. While species sorting was the most important assembly mechanism detected in **study III**, external dispersal from the main inlet as well as groundwater discharge further represented important sources to the lake's bacterial community. Here, past dispersal, at times, even exceeded the signal from present-day dispersal, thus, making the interpretation of our results more complex. **Study IV**, showed that the lake's hypolimnion largely influenced community composition of the epilimnion followed by present-day inlet dispersal. Past spring flood dispersal only contributed to a small proportion, but showed a long-lasting signal in the epilimnion BCC, which could be due to dispersal of rare and inactive cells from the upstream source.

Combined, **studies III** and **IV** highlight the need to incorporate temporal dynamics in large-scale microbial field studies in the future (Aguilar & Sommaruga, 2020; Stadler & del Giorgio, 2022). The two study designs had

very different sampling intervals ranging from 48 hours to 7 days which makes direct comparisons of the results difficult. Generally, microbial communities have been described to react to environmental changes quickly owing to their fast generation times and population numbers (Judd et al., 2006; Van der Gucht et al., 2007). I, therefore, recommend a shorter sampling interval to equally capture small-scale changes, especially at the RNA level. Additionally, **studies III** and **IV** emphasize the significance of RNA analyses in future community assembly studies to help differentiate between active recruitment and transport of inactive cells. This will ultimately help to better resolve the major assembly mechanisms in large-scale microbial field studies (Crevecoeur et al., 2022).

General Conclusions and Outlook

The overall aim of this thesis was to investigate the potential importance of arrival timing and dispersal timing in complex natural lake bacterial communities.

The first part of the thesis focused on the possible detection of priority effects in two separate experiments. Theory predicts that priority effects should become more important in communities with functional plasticity upon arrival to a new environment (De Meester et al., 2016). Further, short generation times and contemporary genetic variation are expected to facilitate the monopolization of resources upon early arrival (De Meester et al., 2016). All these criteria are met by natural lake bacterial communities, however, I generally found priority effects difficult to detect in experimental studies I and II. Also, if priority effects were successfully detected (study II), the importance of arrival timing compared to e.g., the environmental selection for BCC was of minor importance.

The undertaking of measuring priority effects in bacterial communities was challenging both from a methodological and conceptual point of view. The various methodological difficulties included among others the storage of the invader community as well as the question of invasion timing and have largely been discussed in the discussion section above. Aside from the methodological concerns, another likely explanation for the undetected priority effects in **study I** and the minor importance in **study II** is the inherent complexity of bacterial communities. While the experimental conditions for simultaneous and sequential communities were kept constant, we introduced the invader communities at different growth phases of the early establishing pioneer community. These differences in the relative abundance of single populations within the pioneer community can lead to strong differences in interspecific interactions between the invading ASVs and the already growing pioneer community. Priority effects can act individually on each ASV in the community. The number of possible assembly outcomes in highly diverse bacterial communities, therefore, increases super-exponentially with the growing complexity of the community (Figure 11) (Song et al., 2021). Consequently, the outcome of priority effects in complex communities is very hard to predict.

Further, since priority effects can act on a species level, I was uncertain about the expected outcome in whole communities. Preceding studies (Rummens et al., 2018; Svoboda et al., 2018) have managed to successfully identify priority effects in complex bacterial communities, however, on a whole community level a thorough investigation of the interspecific mechanisms leading to these patterns is nearly impossible. This inherent problem of unknown mechanisms driving the patterns we detect on a community level is difficult to solve, yet, future studies should try to consider this caveat.

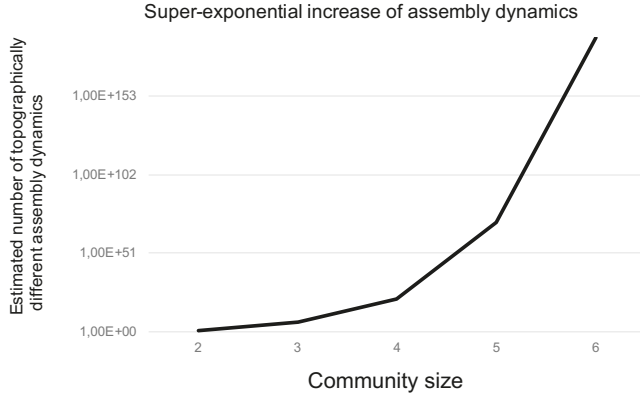


Figure 11: With growing community size, the estimated number of different assembly dynamics increases super-exponentially. Adapted from Song et al., 2021.

Nevertheless, I acknowledge that our experimental design could have led to an underestimation of the importance of priority effects. One main source of variation and uncertainty in study I was the direct use of natural lake communities in a laboratory setting. While a shift in BCC upon the introduction of natural bacterial communities into the experimental medium was expected, we could observe a high degree of variation among experimental replicates. A combination of selection and ecological drift (Gilbert & Levine, 2017) could be an explanation for the high variability we observed in study I. The high community turnover could be the result of selected and stochastic changes in the population abundances in the communities. In study II, I aimed to counteract selection and drift by pre-cultivating the experimental communities under laboratory conditions. The pre-cultivation step helped to minimize selected and random extinction events upon the start of the experiment and generally shaped the community composition to facilitate a prediction of the likely development in the course of the experiment. Future experiments should consider including this step as a way to minimize a shift in BCC and general variability in their experimental communities.

Despite the considerable number of challenges, I managed to detect priority effects in high nutrient levels and in the absence of grazing in study II. The results were interesting also on a population level and ASVs associated with priority effects were generally labeled “opportunistic taxa". With these taxa, I suspected a broad ecological tolerance and competitiveness upon arrival to favorable habitats. Here, more detailed information on the competitive ability, potential functional cohorts, and growth rates of such successful competitors under high nutrient levels and in the absence of grazing would be desirable in future work. Likewise, reasons for the absence of priority effects in high-nutrient treatments but in presence of grazing should be studied in more detail. Competition-predation tradeoffs describe the dilemma of taxa having to either invest in the strategy of being a good competitor (e.g., small cell sizes allow for efficient phosphorus uptake) or grazing resistant (e.g., by investing in grazing defenses) (Winter et al., 2010). Such “killing the winner” mechanisms likely hampered the detection of priority effects in eutrophic no-grazing treatment but at what level of competitive success of the first arriver do these killing the winner mechanisms act? On the contrary, predation has in theory the potential to also promote priority effects by decreasing the establishment success of the second invader but much more work needs to be done on these open questions. The work on simpler microbial communities could for instance help to clarify some unresolved questions, especially regarding the role of grazing for priority effects.

In the second part of the thesis, I examined the importance of dispersal and dispersal timing (including past dispersal) in natural lake communities. This was done in two lake studies where the detection of past dispersal effects in the residence lake bacterial communities relied heavily on the use of SourceTracker models. In studies III and IV we used microbial source tracking (MST) to estimate the contribution of different source microbial communities to a specific sink microbial community. SourceTracker (ST) (Knights et al., 2011), is a machine learning algorithm that uses a combination of Bayes’ Theorem and Gibbs sampling to model the sink community as a convex mixture of sources. The model has performed well in the past using synthetic datasets and real datasets under controlled conditions (Raza et al., 2021). A benefit of using SourceTracker over other MST methods, is the algorithm’s feature to assign sources as “unknown” in cases of low compatibility with the assigned sources.

Using ST models to estimate the proportion of contemporary and delayed dispersal, I was able to detect a relevance of past dispersal sources in both study III and study IV. Study IV showed interesting results as the signal of past dispersal lasted longer than expected. Here, it would be interesting to investigate the duration of even stronger dispersal events (e.g., including potential mass effects) into a lake and without an immediate disturbance event (e.g., lake

mixis) following the spring flood. Additionally, the combined use of RNA and DNA data has great potential to further decipher community assembly mechanisms in natural communities and should be considered in future studies.

However, SourceTracker can be sensitive to biases such as the variability of low contribution sources between ST model runs (Henry et al., 2016). In study III, I have seen the higher variability of low contribution sources in atmospheric deposition and some groundwater sites and should be cautious when interpreting the results. It has been suggested that multiple ST model runs should be considered to establish a confidence interval (Li et al., 2020), which could still be done in study III to get a measure of variability in these low contribution sources. Study IV shows more robust results which could stem from a greater sequencing depth as this has been shown to increase ST model consistency (Li et al., 2020).

Further, the ST algorithm can have difficulties in differentiating sources of similar composition (Brown et al., 2017). Regarding this, source samples in study III showed high variability in BCC between the sampling stations and sampling occasions and should have been able to differentiate by the model. In study IV, the epilimnion and hypolimnion samples are very similar in their BCC which could have led to an overestimation of hypolimnion-contribution in the models. This bias should also be considered when investigating past dispersal sources since the temporal community turnover might not be strong enough for the algorithm to reliably detect differences in BCC over time. Future studies could investigate the degree of species overlap in source samples and the resulting biases in SourceTracker results. However, as said above, study III showed high variability between the different sampling occasions and the ST models should have been able to differentiate between them. In study IV, we used the spring flood inlet samples compared to later low discharge inlet samples in the same ST models which also showed distinct differences in BCC between each other so I am, again, confident in our results.

A conceptual criticism of the use of ST models in ecological studies was brought forward by Wang et al. (2023). According to the authors, ever-changing microbial interactions and the occurrence of priority effects upon community coalescence render it impossible to model dynamic microbial communities. While I agree that it is important to acknowledge the dynamic nature of microbial communities, I nevertheless see no better alternative than the use of "static" models to try and explain BCC at the time of sampling. To the best of my knowledge, there are no reliable statistical tools that capture the inherent properties of microbial communities such as interspecific interactions and stochastic assembly mechanisms.

Taking into account several of the above-described limitations, SourceTracker can be a powerful tool also in ecological studies. Since the machine-learning algorithm uses source libraries as training datasets to identify unique patterns between the sources, one has to be careful to ensure representative high-quality source samples to increase accuracy in the models (Li et al., 2020).

In conclusion, the thesis provides evidence that arrival timing and dispersal timing can be important for complex bacterial communities. Although priority effects and dispersal timing can be difficult to measure, microbial communities seem to be affected by historical contingency. Future studies should therefore incorporate temporal dynamics into their study designs to help improve our understanding of how microbial communities assemble.

Summaries

Summary (English)

Bacterial communities are hard to observe, harder to understand, and impossible to live without. We humans are dependent on microbial communities in almost every aspect of our lives: our immune and digestion systems, the soil and food production, our drinking water, and the degradation of pollutants in the environment. The list goes on since these are only a few of the major services these tiny, yet abundant organisms, provide for our daily well-being.

In the collective quest to gather knowledge about this highly interesting and diverse domain of life, different fields in the natural sciences go about collecting information in various ways. Microbial ecologists try to find answers to simple, yet fundamental questions such as: why do we find a certain species in one location when it is absent in another? Or, on a bigger scale (since one microorganism seldom comes alone): how do microbial communities form? In the field of community ecology, four major factors of the presence or absence of a species at a certain place in time have been identified:

the selection of the "fittest" by the environment (=selection),
random birth or death (= drift),
the creation of species (= speciation),
the migration of species from one place to another (= dispersal).

Interestingly, these four mechanisms cannot explain all of the patterns typically observed in microbial communities. Instead, in recent years, other factors including the legacy of an environment or the community have been explored to help explain the unaccounted variation.

One example of the legacy of a community is the importance of arrival timing to a new and "empty" habitat. A habitat can become new or "empty" by for example a strong disturbance event during which the environmental conditions change drastically, thus, leaving little chance for the locally adapted species to survive. Prominent examples include wildfires, floods, the drying of a pond in the summer, etc. After such a disturbance event, the habitat will be colonized anew and the (random) order and timing of the species' arrival can

leave a long-lasting impression on the developing community, a phenomenon called priority effects.

The influence of the first colonizer on the second arriver can be both beneficial or inhibitory, but in the thesis, I focused on the scenario where a first colonizer hinders the successful establishment of the second arriver. This is done on a "first come, first served" basis where the first colonizer exhausts or modifies the available habitat resources so that the later arriver cannot establish equally successful on account of being too late. However, this might not be the case if the second arriver comes in high population numbers (= mass effects) or is better adapted to the new habitat conditions. Then the advantage of the first colonizer might not be enough to stop the second arriver from successfully invading and can, therefore, be replaced. In the last years, a growing number of studies indicated that priority effects might be important for microbial communities, however, much remains still unknown.

In the first part of my thesis, I investigated the occurrence of priority effects in bacterial lake communities using experiments. Previously, the advantage of the first colonizer has been shown to be more pronounced under environmental conditions that favor its growth. On the other hand, if the establishment of the first colonizer is hindered by e.g., low nutrients or predation, priority effects are expected to play a small or no role. In my experiments, I found that priority effects were measurable at higher nutrient levels and in the absence of predation, as previously hypothesized. However, I found the detection of priority effects in complex bacterial communities to be difficult and future studies should consider their experimental design carefully. Further, priority effects can act differently on the myriad of species in bacterial communities, making the prediction of the results more complicated.

In nature, priority effects in microbial communities are difficult to measure since disturbance events are rarely strong enough to extinguish the majority of the residing microbial community, thereby creating "new" habitats. However, a common phenomenon is the mixing of entire microbial communities, a process called community coalescence. Community coalescence can happen on various scales and in various environments, for example when a river leads into a lake, in agriculture during tillage or fertilization of the soil, or during colonization of the gut microbiome. During community coalescence in nature, the most common scenario is a new community immigrating into an already existing resident community. The resident community is expected to have an advantage due to the higher population number and the pre-adaptation to the habitat's selection pressure. However, a great number of studies have shown that the immigration of new species (= dispersal) can have a long-lasting influence on the resident community.

In the second part of my thesis, I, therefore, focused on the importance of dispersal and dispersal timing in natural lake bacterial communities. In the first lake study, I compared the importance of several dispersal sources (precipitation, groundwater, inlets) to the internal bacterial growth in the lake. While external sources mattered for the lake BCC, I also found that past dispersal explained a considerable amount of variation in my data. In the second lake study, I observed a delayed effect of a strong dispersal event into the lake, which lasted for up to four weeks. Additionally, I investigated if the delayed signal came from the dispersal of inactive cells or actively growing cells upon arrival to the lake environment. The results showed that the signal of past dispersal was mostly caused by the dispersal of inactive cells which remained inactive in the lake.

Taken together, all of my studies showed that, although environmental conditions were more important for community composition, dispersal, and dispersal timing played a role during community assembly. Thus, temporal dynamics should receive more attention in future studies as they could help explain some of the observed unexplained variation in microbial community composition.

Finally, why do we study the importance of dispersal, timing and stochastic processes in microbial communities? As I clarified in the beginning, we depend on microbial communities in almost every aspect of our lives. Being able to predict how communities assemble and by extension understand their functional capabilities is therefore critical. This is especially the case considering our rapidly changing world in the context of climate change, increased land use, and environmental pollution. Further, we humans actively manipulate microbial communities for our health and industrial benefit which requires in-depth knowledge of community composition and stability. Prominent examples are for instance the colonization of the gut microbiome in newborn babies and the use of probiotics and fecal transplant therapy in patients with chronic illnesses. In agriculture, microorganisms can positively enhance plant growth and crop yield and arrival timing can decidedly change the communities' structure. Many more examples of the use of controlled microbial cultures exist in research, medicine, and industry. The consideration of arrival and dispersal timing could, therefore, help improve our understanding and use of microbial communities in the future.

Sammanfattning (Svenska)

Bakteriesamhällen är svåra att observera, svårare att förstå och omöjliga att leva utan. Vi människor är beroende av mikrobiella samhällen i nästan alla aspekter av våra liv; för vårt immunförsvar och matsmältningssystem, för jorden och livsmedelsproduktionen, för vårt dricksvatten och för nedbrytningen av föroreningar i miljön. Detta är dock bara några av de viktiga ekosystemtjänsterna som dessa små men talrika organismer tillhandahåller för vårt dagliga välbefinnande, listan kan göras mycket längre.

Mikrobiella ekologer försöker hitta svar på enkla men grundläggande frågor som: varför hittar vi en viss art på en plats när den saknas på en annan? Eller, i större skala, eftersom en mikroorganism sällan kommer ensam, hur formas mikrobiella samhällen? Inom området samhällsekologi har fyra huvudfaktorer, som påverkar närvaron eller frånvaron av en art vid en viss tidpunkt, identifierats:

naturligt urval,
slumpmässig födelse eller död (= drift),
artbildning,
spridning.

Intressant nog kan dessa fyra mekanismer dock inte förklara alla mönster i mikrobiella samhällens diversitet. Istället har andra faktorer, som till exempel historiska förändringar i miljön, undersökts med syfte att förklara den outredda variationen. Ett annat exempel på betydelsen av historiska händelser är vikten av tidpunkten vid vilken en art anländer till en ny och "tom" livsmiljö. En livsmiljö kan bli ny eller "tom" genom till exempel en kraftig störning under vilken miljöförhållandena förändras drastiskt, vilket ger låga chanser för de lokalt anpassade arterna att överleva. Exempel på detta är skogsbränder, översvämningar, dammar som torkar ut på sommaren etc. Efter en sådan störning kommer livsmiljön att koloniseras på nytt och den (slumpmässiga) ordningen och tidpunkten för arters ankomst kan lämna ett långvarigt avtryck i sammansättningen av samhället, ett fenomen som kallas priority effects, vilket skulle kunna översättas till ”prioritetseffekter” på svenska.

Den första invandrarens påverkan på senare inflyttare kan vara både positiv och negativ, men i avhandlingen fokuserade jag på scenariot där en första invandrare hindrar senare invandrare att etablera sig. Det är alltså "först till kvarn"-principen som gäller; där den första invandraren tömmer eller modifierar de tillgängliga resurserna i habitatet så att den som anländer senare inte kan etablera sig lika framgångsrikt. Detta är dock kanske inte fallet om den senare inflyttaren anländer i höga antal (så kallade ”masseffekter”) eller är bättre anpassad till miljöförhållandena på platsen. I sådana fall kanske

fördelarna den första invandraren har inte räcker för att stoppa den senare inflyttaren från att framgångsrikt invadera och blir därför ersatt. Under de senaste åren har ett växande antal studier visat att prioritetseffekter kan vara viktiga för mikrobiella samhällen, men mycket är fortfarande okänt.

I den första delen av min avhandling undersökte jag förekomsten av prioritetseffekter i bakteriella sjösamhällen med hjälp av experiment. Tidigare har det visats att fördelen den första invandrare har är mer uttalad under miljöförhållanden som gynnar dess tillväxt. Å andra sidan, om etableringen av den första invandrare hindras av t.ex. på grund av låga koncentrationer av näringsämnen eller predation, förväntas prioritetseffekter spela en liten eller ingen roll. I mina experiment var prioritetseffekter mätbara vid högre näringsnivåer och i frånvaro av predation, som förväntat. Jag fann dock att det var svårt att fastställa förekomsten av prioritetseffekter i komplexa bakteriesamhällen vilket ställer stora krav på designen i framtida experiment. Vidare kan prioritetseffekter agera olika för de otaliga olika arter som finns i bakteriesamhällen, vilket gör det komplicerat att förutsäga vilka de mätbara resultaten blir.

I naturen är prioritetseffekter i mikrobiella samhällen också svåra att mäta eftersom störningar sällan är tillräckligt starka för att utradera majoriteten i det levande mikrobiella samhället, och därmed skapa en "ny" livsmiljö. Ett vanligt fenomen är dock att hela mikrobiella samhällen blandas, en process som kallas community coalescence. Community coalescence kan ske i olika skalor och i olika miljöer, till exempel när en flod mynnar i en sjö, i jordbruket under jordbearbetning eller gödsling av marken eller under kolonisering av tarmmikrobiomet. Vid dessa tillfällen förväntas det redan etablerade samhället ha en fördel på grund av stora populationer och att det redan är anpassat till miljön. Ett stort antal studier har dock visat att invandring av nya arter kan ha ett långvarigt inflytande på samhällets sammansättning.

I den andra delen av min avhandling fokuserade jag därför på betydelsen av spridning och tidpunkten för spridning i naturliga sjöbakteriesamhällen. I den första sjöstudien jämförde jag betydelsen av flera spridningskällor (nederbörd, grundvatten, inlopp) med bakterietillväxten i sjön. Även om de externa källorna var viktiga för sjöbakteriesamhällena, fann jag också att spridning förklarade en stor del av variationen i mina data, även flera dagar efter att själva spridningen hände. I den andra sjöstudien observerade jag en liknande effekt av en kraftig spridning till sjön, som var mätbar i upp till fyra veckor senare. Dessutom undersökte jag om den kvardröjande effekten kom från spridningen av celler som var inaktiva eller aktivt växande vid ankomsten till sjön. Resultaten visade att signalen från tidigare spridning mestadels orsakades av spridningen av inaktiva celler som förblev inaktiva i sjön.

Sammantaget visade alla mina studier att även om miljöförhållandena var viktigare för samhällets sammansättning, spelade spridning och tidpunkten för spridning en roll för samhällets sammansättning. Således bör tidsdynamiken få mer uppmärksamhet i framtida studier eftersom det kan hjälpa till att förklara en del av den observerade oförklarade variationen i sammansättningen av mikrobiella samhällen.

Slutligen, varför studerar vi betydelsen av spridning, timing och slumpmässiga processer i mikrobiella samhällen? Som nämnts tidigare är vi beroende av mikrobiella samhällen i nästan varje aspekt av våra liv. Att kunna förutsäga hur samhällen bildas och förstå deras funktionella kapacitet är därför avgörande. Detta är särskilt viktigt med tanke på vår snabbt föränderliga värld där klimatförändringar, ökad markanvändning och miljöföroreningar är viktiga inslag. Vidare manipulerar vi människor mikrobiella samhällen för att uppnå fördelar för vår hälsa och i industriella sammanhang, vilket kräver djupgående kunskap om samhällets sammansättning och stabilitet. Viktiga exempel är till exempel koloniseringen av tarmmikrobiomet hos nyfödda barn och användningen av probiotika och fekal transplantationsterapi hos patienter med kroniska sjukdomar. Inom jordbruket kan mikroorganismer positivt förbättra växternas tillväxt och skördar och spridningstidpunkten kan förändra samhällenas struktur. Många fler exempel på användning av kontrollerade mikrobiella kulturer finns inom forskning, medicin och industri. Överväganden över tidpunkten för ankomst och spridning kan därför bidra till att förbättra vår förståelse och användning av mikrobiella samhällen i framtiden.

Zusammenfassung (Deutsch)

Bakterielle Gemeinschaften sind schwierig zu beobachten, noch schwieriger zu verstehen und unverzichtbar für unser tägliches Leben. Mikroben bestimmen menschliches Leben in fast allen Bereichen: unser Immun- und Verdauungssystem, unsere Böden und damit die Nahrungsproduktion, unser Trinkwasser und der Abbau von Umweltschadstoffen sind von ihnen bestimmt. Die Liste an wesentlichen Leistungen, die uns diese winzigen, aber allgegenwärtigen Lebewesen zur Verfügung stellen und somit unser tägliches Leben und Wohlbefinden ermöglichen, ist lang. Im kollektiven Streben der Naturwissenschaften, mehr Wissen über diese hochinteressante und vielfältige Domäne des Lebens herauszufinden, haben sich verschiede Forschungsfelder etabliert.

Mikrobiologen im Bereich der Ökologie erstreben Antworten zu einfachen, aber grundlegenden Fragen wie zum Beispiel: warum kommt eine bestimmte Art an einem bestimmten Ort vor, während sie an einem anderen Ort fehlt? Oder aber, im größeren Zusammenhang (da eine Art selten alleine vorkommt): wie entstehen mikrobielle Artgemeinschaften? Im Fachbereich der Ökologie von Lebensgemeinschaften werden vier Faktoren, die besonders wichtig für die An- oder Abwesenheit von einer Art an einem bestimmten Ort der Betrachtung sind, beschrieben.

Die natürliche Selektion des am besten Angepassten durch Umweltfaktoren (=Selektion);
Zufallsereignisse der Geburt und des Sterbens (=ökologische Drift);
Die Entstehung neuer Arten (= Artenbildung);
Artenwanderung von einem zu einem anderen Ort (= Ausbreitung).

Interessanterweise können diese vier Faktoren nicht alle Mechanismen, die man häufig in mikrobiellen Artgemeinschaften beobachtet, erklären. Stattdessen, sind in den vergangenen Jahren andere Faktoren, wie beispielsweise historische Veränderungen in den Umweltbedingungen und der Artgemeinschaft, in den Fokus gerückt um diese Abweichung zu erklären.

Ein Beispiel für solche historischen Veränderungen ist die Bedeutung des Besiedelungszeitpunkt einer Art in einem neuen, unbesiedelten Habitat. Ein Habitat kann durch das Auftreten eines starken Störungsereignisses, welches die Umweltbedingungen drastisch verändert, unbewohnt und somit unbesiedelt sein. Die an die alten Lebensbedingungen angepasste Arten haben infolgedessen geringere Überlebenschancen. Bekannte Beispiele hierfür sind Waldbrände, Überschwemmungen, Austrocknung von kleinen Gewässern während der Sommermonate, usw. Nach solchen Störungsereignisse werden veränderte Habitate durch Arten neu besiedelt. Hierbei könne die (willkürliche)

Reihenfolge und der Zeitpunkt der Neubesiedelung einer Art einen teilweise langanhaltenden Einfluss auf die sich entwickelnde, neue Artengemeinschaft haben. Dieses Phänomen wird in der Wissenschaft als „Priority Effect“, also die Vorrangfolge der Artenzusammensetzung bezeichnet.

Der Einfluss des Erstbesiedlers kann sowohl positive als auch negative Auswirkungen auf die nachfolgende Art haben. In dieser Arbeit liegt der Fokus auf letzteren, also die hemmenden Wirkungen der ersten auf die folgende Art. Dies beruht auf dem Prinzip „wer zuerst kommt, mahlt zuerst“, indem der Erstankömmling das besiedelte Habitat zu seinen Gunsten verändert, so dass die nachfolgende Art sich weniger erfolgreich etablieren kann. Jedoch kann dieser Vorteil des Ersten auch durch die nachfolgende Art, beispielsweise durch hohe Anzahl an Individuen der zweiten Art (= Massenwirkung) oder der Besiedlung einer besser angepassten Art im Vergleich zur ersten Art, aufgewogen werden. In diesen Fällen könnte der Vorteil der früheren Ankunft des Erstbesiedlers nicht ausreichen, um eine Kolonisierung der zweiten Art zu verhindern und es kann zu einer Verdrängung der ersten Art kommen. In den letzten Jahren hat eine wachsende Anzahl von wissenschaftlichen Studien darauf hingewiesen, dass die Vorrangfolge der Artzusammensetzung, also der „Priority Effect“ möglicherweise eine wichtige Rolle für mikrobielle Artgemeinschaften spielt, vieles bleibt aber nach wie vor unklar.

Der erste Teil dieser Arbeit befasst sich mit der Erfassung von „Priority Effects“ in bakteriellen Artengemeinschaften von Seen, welche mit Hilfe von Experimenten durchgeführt wurde. Zuvor hat sich gezeigt, dass wachstumsbegünstigende Umweltfaktoren des Erstbesiedlers eine groβe Rolle spielen. Andererseits wird angenommen, dass sich ein geringer oder kein Effekt der Vorrangfolge ergibt, wenn der Erstbesiedler durch Faktoren, wie zum Beispiel geringe Nahrungsverfügbarkeit oder Prädation behindert wurde. Meine Experimente zeigten, wie bereits angenommen, dass die Vorrangfolge durch höhere Nahrungsverfügbarkeit und geringere Prädation positive beeinflusst wird. Dennoch wurde durch diese Experimente deutlich, dass die Nachweisbarkeit der Vorrangfolge schwierig ist und zukünftige Studien sollten daher dem Versuchsaufbau besondere Aufmerksamkeit schenken.

In der Natur sind Vorrangfolgen der Artenzusammensetzung schwierig zu messen, da natürliche Störungsereignisse selten stark genug ausgeprägt sind um angestammte mikrobielle Arten mehrheitlich auszulöschen und somit ein komplett neues Habitat hervorzubringen. Allerdings kommt es oft zu einer Vermischung von mikrobiellen Artengemeinschaften in der Natur; dieser Prozess wird Verschmelzung (”community coalescence”) von Artgemeinschaften genannt. Diese Verschmelzung kann in unterschiedlichem Ausmaß und Ökosystemen vorkommen, beispielsweise im Bereich eines Seezuflusses, in der

Landwirtschaft bei der Düngung von Feldern, oder während der Besiedlung der Darmflora durch Bakterien.

In der Natur kommt eine Vermischung der Artengemeinschaften häufig dadurch zustande, dass eine neue Artgemeinschaft eine bestehende, etablierte Gemeinschaft besiedelt. Es wird erwartet, dass die etablierte Gemeinschaft aufgrund der höheren Individuenzahl und Voranpassung an das Habitat und den damit verbundenen Selektionsdruck einen Vorteil genießt. Dennoch haben viele Studien aufgezeigt, dass die Einwanderung von neuen Arten (= Ausbreitung) langwierigen Einfluss auf die etablierte Artgemeinschaft haben kann.

Demnach befasste ich mich im zweiten Teil dieser Arbeit mit der Bedeutung von Artenausbreitung und dem Zeitpunkt der Ausbreitung oder Immigration in natürliche Artengemeinschaften von Seen. In der ersten See-Studie wurde untersucht wie sich die Bedeutung mehrere Ausbreitungsquellen (Niederschlag, Grundwasser, Zufluss) auf das Bakterienwachstum im See auswirkt. Während externe Einflüsse für die bakterielle Artgemeinschaft des Sees wichtig sind, konnte die Studie auch aufzeigen, dass vergangene Immigrationsereignisse einen Einfluss hatten.

In der zweiten See-Studie konnte ein verzögerter Effekt eines starken Immigrationsereignisses in den untersuchten See beobachtet werden, welcher bis zu vier Wochen anhielt. Zusätzlich wurde untersucht, ob das Signal des Verzögerungseffekts durch inaktive oder aktive bakterielle Zellen während der Ankunft in den See bedingt wurde. Die Ergebnisse haben gezeigt, dass das Signal von vorangegangenen Immigrationsereignissen hauptsächlich durch inaktive Zellen begünstigt wurde, die inaktiv im See zurückgeblieben sind.

Zusammenfassend zeigten alle Studien, dass die Artausbreitung und der Zeitpunkt der Ausbreitung zu dem Prozess der Bildung der Artengemeinschaft beitragen, obwohl die Umweltbedingungen eine größere Rolle für die Artzusammensetzung spielten. Deshalb sollte in zukünftigen Studien mehr Aufmerksamkeit auf den Faktor der zeitlichen Dynamik gelenkt werden. Eine Berücksichtigung der zeitlichen Dynamik könnte dazu beitragen, einige der beobachteten, unerklärten Variationen in der Zusammensetzung der mikrobiellen Artgemeinschaft besser zu erklären.

Abschließend die Frage: Warum untersuchen wir die Bedeutung von Ausbreitung/Immigration, Besiedelungszeitpunkt und willkürliche Prozesse in mikrobiellen Artgemeinschaften? Wie ich eingangs verdeutlicht habe, sind wir in fast allen Aspekten unseres Lebens von mikrobiellen Gemeinschaften abhängig. Es ist daher von entscheidender Bedeutung, vorhersagen zu können, wie sich Gemeinschaften zusammensetzen, und im weiteren Sinne ihre

funktionalen Fähigkeiten zu verstehen. Dies gilt insbesondere in Anbetracht einer sich schnell ändernden Umwelt im Zusammenhang mit globalen Klimawandel, Intensivierung der Landnutzung und zunehmender Umweltverschmutzung. Darüber hinaus manipuliert der Mensch mikrobielle Gemeinschaften aktiv für medizinische und industrielle Zwecke; dies erfordert ein tiefgreifendes Wissen über die Zusammensetzung und Stabilität von Gemeinschaften. Bekannte Beispiele sind etwa die Besiedlung der Darmflora von Neugeborenen und der Einsatz von Probiotika und Spenderstuhltransplantationen für Patienten mit chronischen Darmerkrankungen. In der Landwirtschaft können Mikroorganismen das Pflanzenwachstum und den Ernteertrag verbessern, wobei der Besiedelungszeitpunkt die Struktur der Artgemeinschaft entscheidend beeinflussen kann. Viele weitere Beispiele für den Einsatz von mikrobiellen Kulturen existieren in der Forschung, Medizin und Industrie. Die Berücksichtigung des Besiedelungszeitpunkts und der Moment der Ausbreitung/Immigration könnte daher dazu beitragen, unser Verständnis und die Nutzung mikrobieller Artgemeinschaften in Zukunft zu verbessern.

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"*Limno is special*" – Alina Mostovaya

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