AN EXPERIMENTAL TEST OF HOW MICROBIAL COMMUNITIES RESPOND TO WARMING IN SYSTEMS CONNECTED BY DISPERSAL

by

Evgeniya Yangel

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The following individuals certify that they have read, and recommend to the Faculty of Graduate and Postdoctoral Studies for acceptance, a thesis entitled:

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Examining Committee:

Mary O'Connor, Zoology, UBC
Co-supervisor

Laura Wegener Parfrey, Zoology and Botany, UBC
Co-supervisor

Michelle Tseng, Zoology and Botany, UBC
Supervisory Committee Member

Diane Srivastava, Zoology, UBC
Additional Examiner

Abstract

Community dynamics and structure are greatly affected by climate change through warming. Temperature directly affects the rates of biochemical reactions that in return affect growth, resource use, organismal abundance, species interactions, and, therefore, communities and biodiversity. In addition, low connectivity can limit dispersal between communities, reducing the potential for demographic rescue effects. Therefore, the effects of temperature on diversity and community structure in patchy landscapes can depend on the degree of connectivity among landscapes. We tested whether the effects of temperature on communities contingent on the degree of connectivity using experimental pond metacommunities, each comprised of four-1000L mesocosms spanning a 4.5°C spatial temperature gradient and connected by one of three dispersal rates. This spatial temperature gradient was maintained, while also allowing the mesocosm temperatures to fluctuate temporally with seasonal weather variation. Bacterial communities in the mesocosms were sampled in the summer to evaluate whether dispersal rate at the metacommunity level affects local and regional community response to seasonal fluctuations in temperature. We predicted that higher levels of dispersal would raise local (alpha) diversity and decrease species turnover among ecosystems (beta diversity) and metacommunity-level (gamma) diversity. However, we found no effect of dispersal on local and regional diversity metrics. We also predicted that dispersal rates would differently affect species compositional differences along the thermal gradient. At low dispersal rates among communities, we observed differences in species composition associated with temperature. At higher dispersal rates, communities were not structured by temperature and composition was similar within a metacommunity, which was not observed in any other dispersal treatment. This emphasizes the

homogenizing effect high dispersal has on bacterial community structure. Our findings demonstrate that bacterial diversity metrics do not follow metacommunity predictions about dispersal effects on diversity. However, we found support for the hypothesis of high dispersal homogenizing communities. This suggests there are other processes that influence bacterial community diversity patterns, but dispersal can erode the effect of the environment on bacterial community structure.

Lay Summary

Climate change and loss of habitat connectivity are two of the major stressors affecting organisms and their interactions. However, we do not truly know how these interacting forces affect biodiversity. Furthermore, most of the empirical studies focus on larger organisms, overlooking microbes that are ubiquitous and are essential to ecosystem health. Therefore, one of my main objectives was to understand what drives bacterial communities in environments experiencing variable temperatures with a different degree of connectivity. In addition, my other objective was to examine whether degree of connectivity by itself affects local and regional biodiversity patterns. I found that temperature determined bacterial community structure, but its effects were lost in highly connected habitats. At the same time, different levels of connectivity did not influence bacterial biodiversity patterns at local or regional scales. These results contribute to understanding of how bacterial communities will respond to climate change in habitats with variable degrees of connectivity.

Preface

Mary O'Connor and Patrick Thompson designed the overall experiment and further collaborated with Laura W. Parfrey and Bianca Segovia Trevizan on the project idea. Evgeniya Yangel and Bianca Segovia Trevizan did the majority of microbial sampling at UBC Experimental Ponds Facility. Evgeniya Yangel did all the molecular work, sequencing and statistical analyses with input from Laura W. Parfrey and Bianca Segovia Trevizan. Evgeniya Yangel wrote the manuscript and Mary O'Connor and Laura W. Parfrey provided feedback. Mary O'Connor, Laura Parfrey and Michelle Tseng provided feedback on writing, analyses and discussion. Chapter 2 will be edited and prepared as manuscript which will be submitted for publication in a peer-review journal.

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List of Abbreviations

ANOVA: Analysis of Variance

DF: Degrees of Freedom

NMDS: Non-metric Multidimensional Scaling

PCR: Polymerase Chain Reaction

PERMANOVA: Permutational multivariate analysis of Variance

iNEXT: Interpolation and Extrapolation for Species Diversity

IQR: Interquartile Range

N: sample size at a metacommunity level

n: sample size at a tank level

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Dedication

I dedicate my thesis to my grandmother, Lubov Fedotovna Zaytseva, who to me is the embodiment of *"Per aspera ad astra"*. To the person, who lived during unimaginably difficult times, but who maintained a positive mindset and always proved that impossible is possible if you really have a goal and work hard for it. I love you very much. I am always thinking of you and hope you are in the most fantastic place surrounded by the loved ones.

Chapter 1: Introduction

1.1 How warming and habitat fragmentation affect community dynamics and structure

Climate change has been manifesting through global warming and more frequent extreme weather events as a result of anthropogenic activities (IPCC 2014). Consequently, these changes in environmental conditions are in turn affecting natural ecosystems and their communities (IPCC 2014, Cavicchioli et al. 2019). Among the effects of rapid global change are species loss, species range distribution shifts, and also declines in species abundance and biomass with an increasing number of threatened species being at the risk of extinction (IUCN Red List; Hoffmann & Sgrò 2011). The issue of losing biodiversity is quite profound, as not only are we losing charismatic megafauna that have social and cultural values, but we are also losing other organisms like trees (García-Valdés et al. 2018) and invertebrates (Harley 2011), though less charismatic, that greatly contribute to ecosystem function and ecosystem services. The environments experiencing warming require a greater number of species to maintain ecosystem functioning for two reasons: 1) more diverse communities are more likely to include species whose thermal optima is within their optimum range and can maintain their function, 2) higher diversity provides higher variability in the functional traits that in turn promote ecosystem functioning through complementarity in resource use (García et al. 2018). Higher community diversity can therefore mediate the effects of warming on biodiversity-ecosystem functioning relationships (García et al. 2018).

In addition to warming, there are other disturbances occurring that affect ecosystems and their inhabitants, including habitat loss and fragmentation due to human activities that can lead to

species loss (Tilman et al. 2001), community compositional change (Tilman et al. 2001) and time-delayed extinctions (Krauss et al. 2010). Habitat loss and fragmentation restrict species movement, but also increase competition for resources, including nutrients and space. Additionally, reduction in habitat size also directly leads to population decline and consequent species losses. Such habitat modifications could drive some species to relocate and colonize the neighbouring habitats if available. In turn, successful dispersal and colonization depends on the degree of fragmentation and whether there are matrix and/or habitat corridors available for species to disperse through and successfully reach a new habitat. Meanwhile populations with higher spatial isolation have restricted movement and are at risk of extinction.

Warming and fragmentation do not occur in isolation and it is important to understand and estimate what the combined effects of two or more forces occurring simultaneously are, in order to execute efficient conservation management practices. Species responses to climate change vary due to differences in thermal traits and there are a variety of species interactions within the communities that can be impacted by the change. Habitat loss and fragmentation are considered to affect species more than warming (Sala et al. 2000; Jetz et al. 2007) but there is a lot of evidence suggesting the synergistic negative interaction of climate change with fragmentation cause even more biodiversity losses (Opdam & Wascher, 2004; Brook et al., 2008). For example, deforestation and habitat fragmentation lead to localized drying and regional rainfall shifts that in turn increase risk of fire and reduce species ability to move in response to abiotic changes (Brook et al. 2008). Biodiversity loss reduces ability of communities to provide ecosystem functions such as biomass production, decomposition, nutrient cycling and more (Cardinale et al. 2012).

More diverse systems increase temporal stability of ecosystem functions such as biomass production and total resource capture (Allan et al. 2011; Cardinale et al. 2012). These communities have more genes, species and functional groups of organisms which influence a productivity to a greater extent through greater efficiency of capturing biologically essential resources and, consequently, their conversion into biomass (Cardinale et al. 2012). Additionally, such communities are more likely to contain species that are functionally redundant making them more resilient to environmental disturbance (Aronson et al. 2004; McLean et al. 2019), even though in some cases it may not sufficiently compensate for a loss of certain species (Aronson et al. 2004).

Many ecological studies and conservation efforts focus on larger organisms, but it is essential to recognize that microscopic organisms have a profound direct and indirect contribution to ecosystem functioning and services (Juburg & Salles 2015). Microbes and bacteria drive biogeochemical cycles (Field et al 1998, Behrenfeld 2014), they control decomposition and mineralization processes (Cavicchioli et al. 2019), but also promote organismal growth and productivity (van der Heijden et al. 2008) as well as the health (Bourne et al. 2016, Engel et al. 2016; Guo & Narisawa 2018). Being ubiquitous and so important to ecosystem functioning and ecosystem health, it is crucial to investigate how changing environments and habitat connectivity modifications affect microbial communities. In particular, there is a major concern associated with how the change in microbial diversity and functioning will translate into ability of other organisms to withstand and respond to the environmental change (Cavicchioli et al. 2019).

There are different trends that have been observed in response to warming in microbial systems. In marine environments, warming has been shown to increase bacterial respiration, bacterial losses to their grazers and resulting bacterial-grazer biomass flux within the microbial food webs, and higher bacterial production when sufficient resources are available (Sarmento et al. 2010). The diversity trends are less clear; warming has been shown to increase (Sheik et al. 2011, Zhou, Wang and Luo 2020), decrease (Sheik et al. 2011) and have no effect (Zhou, Wang and Luo 2020, Song et al. 2020) on different diversity metrics. For example, warming has not been found to alter alpha diversity in soil microbes but to increase beta diversity (Zhou, Wang and Luo 2020). At the same time, warming has a different effect on richness when coupled with other factors; for example, with precipitation: it decreases diversity under normal precipitation, but increases species richness during the drought conditions (Sheik et al. 2011). In addition, in the light of accelerating habitat alteration, there are multiple studies that examined how habitat connectivity via dispersal affects microbial communities: dispersal has been shown to increase local richness (Shen et al. 2018) and weaken the importance of local environment on microbial community structure (Lindström & Östman, 2011). The difference in community response to each abiotic factor emphasizes the importance of studying the influence of a couple or multiple abiotic factors interaction on the community structure. In the real world, none of the abiotic factors occur in isolation and it is important to understand the combined effects of factors occurring simultaneously. Currently, we are lacking empirical studies which test how environmental change and fragmentation interactively influence bacterial communities in heterogeneous landscapes.

1.2 Main objectives of the thesis

The main objective of my thesis is to understand how bacterial communities respond to warming across environments with different degrees of connectivity. To do so, I relied on metacommunity theory to draw predictions about how habitats with variable temperature regimes and different degrees of dispersal affect bacterial communities. The metacommunity framework explains patterns of diversity in sets of local communities linked by dispersal, where a community is a set of populations of different species occupying a particular habitat patch and dispersal refers to the movement of individuals from site to site (Wilson 1992; Leibold et al. 2004; Holyoak et al. 2005). The benefit of the metacommunity framework is that not only it can unify local and regional scales and relevant theories, but it also allows us to understand biodiversity as a function of abiotic spatial variation among habitat patches and dispersal together (Thompson et al. 2020).

Dispersal is thought to be a key process that allows biodiversity and functioning to be maintained in changing environments (Loreau et al. 2003, Gonzalez et al 2009). Since we live in the world of changing climate, it is essential we incorporate ecological theory and its predictions of community response to changes in environmental conditions. Metacommunity theory predicts that if local communities are connected by dispersal, species in metacommunities can offset the effects of warming on local diversity by tracking their environment through movement in space and time (Loreau et al. 2003, Thompson & Gonzalez 2017, Thompson & Fronhofer 2019). This theory emphasizes the importance of dispersal as one of the processes that can preserve community biodiversity during stressful abiotic conditions and it predicts resultant community structure and diversity patterns.

In order to address the main objective, we have designed an experimental metacommunity of freshwater mesocosms to answer the following questions: 1) How do bacterial communities respond to warming when dispersal is introduced into the system? Does the degree of dispersal rate (intermediate versus high) have a different effect on community structure? 2) Does dispersal promote microbial diversity in the communities at local scale (alpha diversity)? How does dispersal affect bacterial diversity among-localities (beta diversity) and regionally (gamma diversity)? To answer these questions, we draw on predictions from theory that are described in detail in Chapter 2.

Chapter 2: An experimental test of how microbial communities respond to warming in systems connected by dispersal

2.1 Introduction

Biodiversity is an essential feature of ecological systems and is undergoing rapid change in many ecosystems as anthropogenic climate change and habitat alteration accelerate. Environmental warming associated with climate change affects population growth rates and fitness and drive changes in diversity at the community level (Dell et al. 2011; Yvon-Durocher et al. 2015; Barneche et al. 2019). Biodiversity in a community depends not only on local conditions and population dynamics, but also on regional processes, primarily immigration from other communities in the region (Holyoak et al. 2005; Loreau et al. 2003, Thompson et al. 2020). Therefore, the effects of temperature on local diversity may depend on regional diversity and how much connectivity there is among communities within the region. To date, our understanding of how warming affects diversity and function is largely derived from experimental studies that lacked connectivity and regional variation in diversity.

Metacommunity theory explains how connectivity among communities occupying patches in heterogeneous landscapes can maintain diversity at local and regional scales (Mouquet & Loreau 2003, Loreau et al. 2003, Thompson et al. 2020). The main concept of this theory is that dispersal, the occasional movement of individuals among patches to colonize new habitats, allows species to track their optimal environments as the environmental conditions change in space or time. “Regions” are often represented in metacommunity theory by different habitats

with different abiotic conditions, while different species in the region have different responses to habitat and environmental variation. This process is referred to in the metacommunity literature as 'species sorting' (Leibold et al 2004). Species sorting allows for niche-partitioning and dominance of certain species at a given space and time. Regionally, there can be a lot of heterogeneity in habitat quality among discrete habitat patches. Therefore, species abundance and presence vary from habitat to habitat reflecting the biotic and abiotic environment. If there is no connectivity between habitat patches, species are unable to colonize new habitat patches when historically favorable patches become no-longer habitable, which can lead to changes in species' abundance and even diversity loss. Dispersal between different patches in the region, however, allows species to track their environment and escape stressful abiotic conditions (Mouquet & Loreau 2003, Loreau et al. 2003, Thompson et al. 2020). This idea in metacommunity theory is known as a spatial insurance hypothesis, which emphasizes the importance of dispersal as one of the processes that can preserve community biodiversity during stressful abiotic conditions (Loreau et al. 2003). As dispersal increases, local richness increases and communities have higher temporal turnover (temporal β), regardless of abiotic niche breadths or competition, while spatial and regional diversity erode (Thompson et al. 2020). Both spatial and temporal turnover are promoted by strong competition or narrow abiotic niche breadth, but in case of high dispersal rates both are eroded (Thompson et al. 2020). Spatial insurance dynamics allow dispersal from a regional species pool to increase overall species richness by introducing heat tolerant taxa that partially compensate for the loss of local heat sensitive species (Thompson & Shurin, 2012). Dispersal also has a buffering capacity against acidification, but only for certain trophic groups

(Limberger et al. 2019). Therefore, in the light of climate change, increasing connectivity can offset the biodiversity loss in response to stressful abiotic conditions.

In environments in which local patches differ in temperature, species composition and diversity may differ in ways that reflect physiological and ecological effects of temperature (consistent with the species sorting idea). Warming has been shown to reduce diversity and organismal functioning in communities (Petchey et al. 1999; Fussmann et al. 2014; O'Gorman et al. 2019). For example, warmer temperatures may be outside a species' thermal niche, leading to the loss of that species from a habitat that has warmed. Also, an increase in the metabolic needs of organisms can reduce biodiversity if the community's metabolic demand cannot be met by available energy or nutrient supplies, supporting less individuals and leading to eventual species loss (Brown et al. 2004). Moreover, with ongoing climate change and increasing magnitude of warming there are projections of more abrupt community disruptions, leading to more severe biodiversity loses than predicted before (Trisos et al. 2020). At the same time, at the community level, warming affects the biodiversity-ecosystem functioning relationship and more species may be needed to maintain ecosystem functioning under thermal stress (García et al. 2018). However, if local communities are connected by dispersal, populations of species in metacommunities can offset the negative effects of warming on local diversity by tracking their environment through movement that allows them to colonize habitats that may have previously been unsuitable (Loreau et al. 2003, Thompson & Gonzalez 2017, Thompson & Fronhofer 2019).

The evidence to date in support of the spatial insurance hypothesis operating as a mode of resilience to warming comes predominantly from zooplankton in aquatic systems (Thompson & Shurin 2012, Symons & Arnott 2013). In the same aquatic systems, microbes play critical roles in community dynamics and ecosystem processes. For example, nutrient cycling (e.g. carbon and nitrogen), food webs, plant and animal health - all depend on microbial dynamics, so it is crucial to understand how aquatic microbial communities are impacted by abiotic conditions and habitat connectivity under global change. As in larger-bodied taxa, bacterial diversity can increase (Sheik et al. 2011, Barton et al. 2016), remain unchanged (Song et al. 2020) and decrease (Sheik et al. 2011, Barton et al. 2016) following changes in their thermal environment. To our knowledge, there is limited understanding of whether microbial community responses to warming are affected by dispersal rates, as would be predicted by metacommunity theory and as is observed for other planktonic communities. There is evidence, however, that dispersal does modify community responses to environmental conditions: following diversity loss resulting from a salinity stress, alpha diversity of marine bacteria increased with dispersal and declined in the absence of dispersal among habitats (Shen et al. 2018). Local environmental variables, such as lake water quality, also explain a great proportion of variation in community composition, but the magnitude of effect decreases with higher dispersal (Lindström & Östman, 2011). In particular, communities with low dispersal are the most compositionally dissimilar to each other, while high dispersal has a homogenizing effect, decreasing the importance of species sorting (Lindström & Östman, 2011). However, under the stress of acidification, dispersal does not affect bacterial alpha diversity (Limberger et al. 2019). Overall, these different findings indicate that bacterial response is variable.

In addition to dispersal, community composition could also be driven by demographic stochasticity (Shoemaker et al. 2019) and by order and timing of species arrival during community assembly (priority effects) depending on the order in which species arrive to the community and whether they can coexist with the present species (Fukami et al. 2016). If stochasticity and priority effects are dominant and species composition is not driven by environmental conditions, we would expect considerable site-to-site variation in otherwise similar environments. We expect stochasticity is less important to bacteria because bacterial communities are strongly shaped by abiotic factors and fast generation times coupled with large population sizes (Louca et al. 2016).

According to metacommunity theory, colonization can also influence community composition via competition-colonization tradeoff in which subdominant species can only persist by colonizing newly disturbed patches before dominant species arrive (Holyoak et al. 2005). Therefore, we may observe community composition differences based on the timing of species' arrival timing in addition to species sorting according to environmental factors.

However, it is likely that the competition-colonization trade-off is less important for structuring microbial communities, than for the plant and animal communities this theory has so far been tested in, because dispersal is more efficient for microbes due to their small size compared to invertebrates. Microbes have large population sizes per milliliter of water and higher population growth rates due to shorter generation times and therefore can quickly track environmental

changes (Korhonen et al. 2010 as cited in De Bie et al, 2012). Because of their small size, microbes can move passively (Finlay 2002; Nemergut et al. 2013) through vectors like air, water or host organisms that travel between different habitats (Vanschoenwinkel et al. 2008). Also, this passive movement does not depend on traits that might trade-off with competitive dominance as observed in plants and other organisms (Pellissier 2015). In addition, many microbes have dormant forms that persist in the environment in unfavourable conditions (Locey 2010). Consequently, various microbes are initially present in the species pool in dormant stages and they can bloom in response to a favourable change in abiotic conditions (Locey 2010, Jones & Lenon 2010; Lennon & Jones 2011). Hence, microbes can move passively and survive this movement because of their dormant stages that allow microbes to persist in the environment. Thus, microbes that will become dominant in different conditions are more likely to be a part of the species pool due to higher background levels of dispersal and dormancy, in comparison to macro-organisms which are thought to rely more heavily on active dispersal, which brings new species that thrive in changing conditions.

To test the hypothesis, derived from metacommunity theory, that effects of habitat temperature on bacterial community composition caused by species sorting can be overwhelmed by dispersal in bacterial metacommunities (**hypothesis 1, H1**), I manipulated temperature and dispersal rate of organisms in experimental aquatic ecosystems. I measured the diversity and compositional patterns of bacteria to test the following specific predictions:

- **Prediction 1.1:** Local communities differing in temperature regime will be compositionally different in metacommunities, consistent with species sorting to environmental temperature regime
- **Prediction 1.2:** Differences in community composition due to species sorting disappear at high dispersal rates because species present and abundant in local communities are being moved among communities in a metacommunity. This pattern happens despite the species immigration from more to less favourable areas (also known as mass effects).
- **Prediction 1.3**: I predict that increasing dispersal rates among local habitats that differ in their temperatures raises alpha diversity and reduces spatial beta and gamma diversity within a metacommunity.

Hypothesis 2: Temporal beta diversity (species turnover within a local community over time) will be the highest at low dispersal rates.

- If true, **prediction 2.1**: Communities that differ due to temperature as a result of species sorting (Hypothesis 1) and are isolated from other communities will change more over time than communities connected to a larger (regional) species pool via dispersal.

For the background dispersal treatment, I predicted two possible scenarios depending on which processes dominated: 1) if abiotic factors structured the communities (species sorting), we expected more similar community composition under the same environmental conditions, or 2) if demographic stochasticity and priority effects through initial inequalities in the compositional differences of the seeded communities were dominant and composition was not driven by

environment-related traits, we expected considerable site-to-site variation in otherwise similar environments.

2.2 Methods

2.2.1 Experimental design and sample collection

To test my hypotheses, I estimated bacterial diversity and composition in an experiment designed to test the metacommunity theory predictions for freshwater ecosystems including zooplankton, phytoplankton and microbiota. The experiment consisted of twelve metacommunities that each contained four 'local communities' (mesocosms), for a total of 48 mesocosms (Figure 1). To test our hypotheses, we needed to establish an environmental gradient and varying dispersal treatments within metacommunities. To achieve the former, we established a constant thermal gradient using heaters within each metacommunity. Subsequently, we introduced different levels of dispersal within metacommunities through reciprocal exchange of water and corresponding biota.

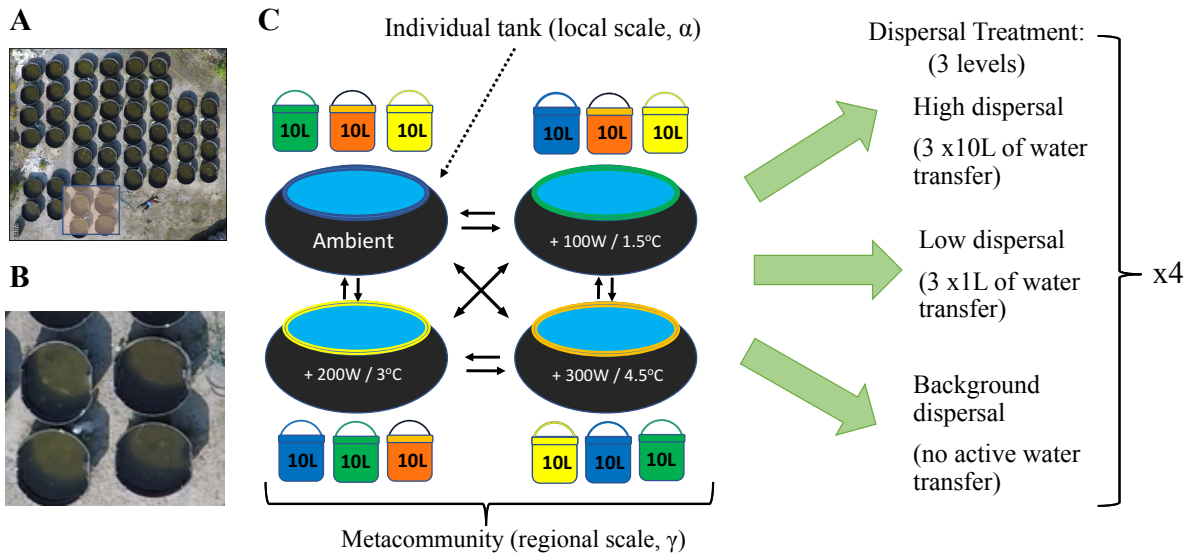

Figure 1. Experimental design. A) All 48 mesocosms (each mesocosm contains one 'local community'), and B) one experimental unit, a metacommunity, comprised of four mesocosms (four local communities) linked by manual experimental dispersal events. C) A schematic of experimental set up. Metacommunities included four local communities differing in their temperature regimes. Metacommunities (N=12) were assigned one of the three dispersal rate treatments, indicated by green arrows: background, low or high. Buckets indicate that 3 X 10L was drawn from each tank regardless of the treatment and buckets are colour-coded by the local habitat's temperature treatment where the samples were drawn from. Note that the final amount dispersed by the bucket depended on the assigned dispersal rate. Metacommunities were sampled at a tank level (alpha diversity), and data was analyzed at all local (alpha), between-local (beta) and regional or metacommunity (gamma) scales.

2.2.1.1 Establishing experimental conditions

Each mesocosm was a 300 US Gallon (~1136L) Rubbermaid Stock Tank (Rubbermaid USA), hereafter referred to as a mesocosm. The mesocosms were filled with water from the artificial ponds from UBC Ponds facility on May 24th, 2018. To establish a thermal gradient within metacommunities, we used aquarium heaters. In each metacommunity, we placed submersible

aquarium heaters (JÄGER TruTemp, EHEIM GmbH & Co KG, Germany) of different wattages into each of the four mesocosms (100W, 200W or, 300W and control of 0W that was represented by a plastic rod of a similar size). Heaters were randomized within each metacommunity, and were turned on May 25th. The thermostats were set at maximum to ensure delivery of the constant amount of heat throughout the experiment. The mesocosms that were supplied with heaters were underlaid with styrofoam sheets (Coe Lumber) to reduce heat loss. We used HOBO temperature loggers with ±0.5°C measurement accuracy to measure water temperature in each experimental ecosystem. On June 12th, we added nitrogen and phosphorus in forms of nitrate (109 ug /L) and hydrogen phosphate (15 ug/L) to each mesocosm to boost productivity.

Dispersal treatments were both assigned and implemented at a metacommunity-level as background dispersal (no active dispersal), low/intermediate (3L water transfer in total per tank) or high (30L water transfer per tank). Each treatment was replicated four times. Dispersal was performed on a weekly basis within each metacommunity. To implement dispersal treatments, we collected 3 X 10L of water from each mesocosm (Figure 1). We used a 3L integrated Van Dorn sampler to collect water and biota, and we poured the samples into three 19L buckets for each mesocosm regardless of the treatment, as procedural control. Based on the assigned treatment, the appropriate water quantity was dispersed between tanks of the same metacommunity such that each mesocosm received sourced water from the other 3 local communities. For example, in case of high dispersal treatment, 30L of water was collected from each of the four local communities and each mesocosm received 30L combined from the other three local communities. In the case of background and low dispersal treatments, the remaining

water (30L or 27L) was returned back to the original tanks accounting for any disturbance effect of dispersal. The buckets were rinsed with water after every dispersal event, before moving to the next metacommunity, to minimize background dispersal. Since the buckets were not sterilized between each dispersal event, we did not have a true zero dispersal treatment, so we refer to this as background dispersal. The first dispersal treatment marked the start of the experiment on June 14th, 2018; the experiment lasted 12 weeks, terminating on September 6th, 2018.

The mesocosms were located outdoors and open to the air, meaning this was not a closed system and dispersal may have occurred outside our experimental water movement via airflow or animals (birds and insects were present). We have no reason to think this type of background dispersal would have been influenced by experimental treatments, and thus applies equally to all communities.

2.2.1.2 Collecting experimental organisms and establishing experimental communities

The mesocosms were filled with freshwater from the artificial ponds from UBC Ponds. The sourced pond water contains a natural assemblage of bacteria, phytoplankton and zooplankton (Arnegard et al. 2014). Following that, tank communities were additionally seeded from the same regional pool of species collected from nine water bodies in Metro Vancouver region, May 2018. We collected sediment and water samples primarily to get zooplankton, but the samples also included the passively sampled microbial communities from nine water bodies (three lakes from the Malcolm Knapp Research Forest, Rice, Burnaby, Deer, Shirley and Whyte lakes and

the experimental pond at UBC) in Metro Vancouver Regional district to get a better representation of the regional species pool. Sediment samples were collected using Ekman grab and kicknet, with benthic invertebrates sorted by hand. Water samples were collected using a 64 um plankton tow (with a 30cm diameter) that was repeatedly drawn through the water column till a dense population of zooplankton was visible in a 19L bucket of lake water. Water samples from all lakes were combined into 300L buckets, mixed by stirring, then 1L of the mixture was added to each mesocosm on May 28th. Benthic invertebrate samples from each lake were combined in 100L buckets and were divided equally by hand and added to the mesocosm the same day. Consequently, the mesocosms were seeded from the regional species pool with equivalent starting communities.

After the mesocosms were seeded with biota on the 24th and 28th of May, heaters were turned on May 25th and the mesocosms were left untouched allowing communities to establish and acclimate to the temperature gradient before the first dispersal event on June 14th.

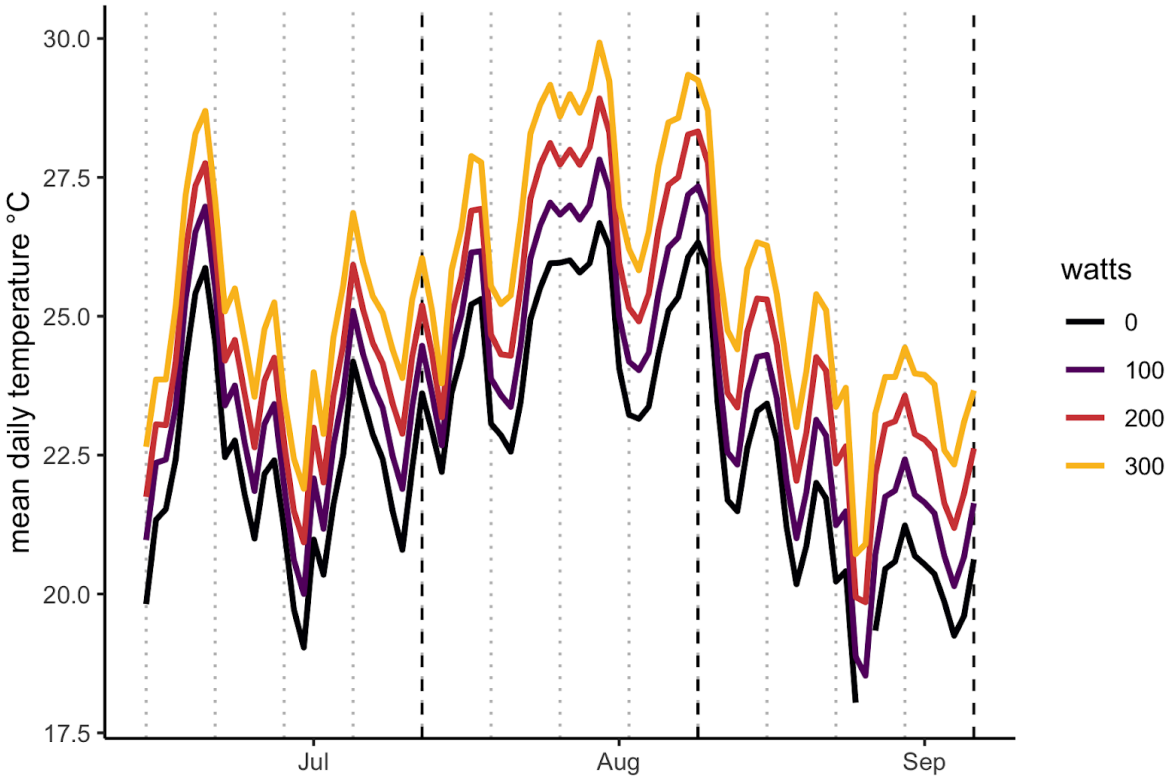

Figure 2 (Reprinted with permission from Thompson et al. in prep). Time series of mean daily water temperature over the course of the experiment. The coloured lines represent mean values across all replicate mesocosms with heaters of a given wattage level. Thick dashed vertical lines indicate the three sampling dates for zooplankton (Jul 12th, Aug 9th, Sep 6th). Thin dashed vertical lines indicate the dates on which the dispersal treatment was applied (in July: 5th, 12th, 19th and 26th; in August: 2nd, 9th, 16th, 23rd and 30th, 2018). Bacteria were sampled on May 30th, July 6th and August 15th, 2018.

2.2.1.3 Bacterial Sampling

To estimate bacterial community diversity and composition, we sampled each mesocosm at the start of the experiment (May 30th), and at two times during the experiment: July 6th (Day 42) and August 15th (Day 82), 2018. For each sample, the water was pushed through the Sterivex filters

(Sterivex GP 0.22 um) via 60mL syringe (BD Luer-Lok Tip, REF 309653). We filtered 500mL in May (12 tanks sampled) and 200ml in July and August (all 48 tanks sampled) due to filter clogging as a result of development of plankton populations and in some cases sediment particle resuspension because of dispersal treatment days prior. The May tanks were sampled on the 6th day of heating before dispersal treatment was introduced (June 14th) to describe the community before the start of the experimental manipulations. Filters were placed in individual pre-labelled Whirl-Pak® bags and stored on ice until they were transported to the lab and stored at -70^{o}C until further processing.

2.2.2 Molecular methods:

To get a taxonomic profile of bacterial communities from which we could estimate diversity metrics and composition, DNA was extracted from Sterivex filters using the Qiagen PowerSoil® HTP 96 Well DNA and Qiagen PowerSoil Pro® Extraction Kits according to manufacturer's protocol. To prepare the samples for extraction, each Sterivex™ filter was opened in sterile conditions and separated from its casing similar to Cruaud et al. (2017). Each Sterivex™ cartridge was cracked open near an open flame burner by a sterilized hammer and cut in half with a sterile scalpel blade. Subsequently, a half of the filter was placed into a well within a 96 well extraction plate from the kit and the other half was placed in a sterile Cryovial and frozen at -70^{o}C for backup. This was repeated for all the samples until the plate was filled and then it was left overnight at -20^{o}C. The tools were sterilized with 80% ethanol, flame treated and subsequently sprayed with an RNAse AWAY^{TM} (Thermo Scientific^{TM}) between each sample. Samples from different time points and treatments were randomized on two extraction plates.

The V4 region of 16S rRNA gene was targeted for amplification using primers 515F: GTGYCAGCMGCCGCGGTAA and 806R: GGACTACHVGGGTWTCTAAT with Illumina adapters and a 12nt barcode on the 515F primer. The PCR cycle protocol was Phusion Flash PCR with 30 cycles (the master mix: BSA 10mg/ml 1ul, H20 6 ul, Phusion ul, Forward primer 10mM 1ul, Reverse primer 10mM 1 ul, DNA 1ul). Amplified DNA was imaged on a 1% agarose gel (2.5g agarose + 250 ml TAE). Amplified samples were further cleaned up of residual PCR reagents. Samples extracted with the Powersoil HTP 99 well kit were purified the using Qiagen Ultra Clean 96 PCR kit, and samples extracted with Qiagen PowerSoil Pro using QIAquick PCR purification kit (Qiagen). Purified DNA amplicons were quantified using PicoGreen (company). All samples were then pooled in equal concentration (17 ng). The final pool was submitted for Illumina MiSeq amplicon sequencing to Integrated Microbiome Resource facility at the Centre for Comparative Genomics and Evolutionary Bioinformatics at Dalhousie University (Halifax, Canada) according to standard protocols (Comeau et al. 2017).

2.2.3 Sequence data analysis:

Raw sequencing reads were demultiplexed using idemp (Wu, 2014). Demultiplexed reads were then processed into amplicon sequence variants (ASVs) using DADA2 16s pipeline, including trimming to a minimum sequence length was 150bp, removing chimeras (Morien, 2020). ASVs were assigned taxonomy using the SILVA v 132 (Callahan, 2018) database clustered at 97% similarity. ASVs with fewer than 100 reads in the dataset were filtered out, as were ASVs unassigned at the domain level. Following this, data were loaded into phyloseq using R (version

3.6.3) and chloroplasts and mitochondria were removed. Relative abundance taxa plots used non-rarefied data. For all other analyses, samples were rarefied to 20,000 reads. Samples with fewer than 20,000 reads were removed from the analysis, including all negative controls (5 extraction negatives and 2 PCR negatives) and three samples (29a, 3m, 4a). The total number of unique ASVs detected in the study was 1229.

2.2.3.1 Removal of contaminated samples:

All samples were initially randomized on the extraction plate to enable detection of cross-contaminated samples. We detected two samples that were nearly identical despite coming from different tanks in different months (38a and 37j) but were adjacent on the extraction plate. We conservatively removed both samples from the analysis. No other potential cross contamination was detected.

2.2.4 Statistical analyses and hypothesis testing:

One premise of our hypotheses is that dispersal rates influence microbial community composition. To verify that dispersal rates in our experiment were sufficient to move microbial taxa among communities, we compared community dissimilarity, by ASV relative abundance, for local communities within each metacommunity and across metacommunities separately for each dispersal rate using one-way ANOVA with dispersal as a fixed effect to understand if dispersal had any effect on diversity within the metacommunity.

To test whether effects of habitat temperature on bacterial community composition caused by species sorting can be overwhelmed by dispersal in bacterial metacommunities (**hypothesis #1**), I estimated similarity in bacterial composition among local communities within each metacommunity, and then compared these estimates among metacommunities in the background, low and high dispersal treatments to address predictions 1.1 and 1.2. I only used the July diversity estimates to test this hypothesis, because this allowed enough time since the start of the experiment to allow communities to establish. In addition to the beta diversity estimates, I used non-metric multidimensional Scaling (NMDS) to ordinate samples based on Bray-Curtis (relative abundance) and Jaccard (presence-absence) dissimilarity indexes to visualize similarity of communities between different temperature treatments in metacommunities with different dispersal rates. PERMANOVA was used to test for the differences in community structure between treatments (a factorial combination of temperature and dispersal treatments) using the "Adonis" function in the vegan package for R (Oksanen et al 2005). To determine which treatments within each independent variable were distinct, I ran pairwise PERMANOVA with the wrapper function "pairwise.adonis" for vegan package (Martinez Arbizu, 2020). P-values were adjusted using Bonferroni method. PERMANOVA is robust to differences in dispersion, but only with balanced sampling designs (Anderson and Walsh 2013) so I tested for the presence of heterogeneity of dispersion for July data. The assumption of homogeneity was met, so no additional data manipulation was needed. All permutational test statistics were generated using 9,999 permutations. Note here that all analyses were run on all 46 tanks in July, but NMDS was plotted with 45 tanks (n=45) due to tank 44 community being the most different and skewing the plot (see Appendix Figures 4A and 5A for plots with tank 44 included in the analyses). However,

removal of that sample did not affect the statistical outcomes for both hypothesis 1 and 2 (Table 1A, Table 2A), so we justify presenting NMDS without tank 44.

To test if increasing dispersal rates among local habitats that differ in their temperatures raises alpha diversity and reduces spatial beta and gamma diversity within a metacommunity (**prediction 1.3**), we estimated the relationship between dispersal and three biodiversity metrics for communities in July:

a) **Alpha diversity**: We estimated local diversity as the extrapolated Chao richness using iNEXT package in R (Hsieh et al. 2016). We performed a linear regression to test whether alpha diversity increased with dispersal rates and metacommunity as a random effect.

b) **Gamma diversity:** At the regional (metacommunity) scale, we estimated asymptotic taxonomic richness using the iNEXT package in R (Hsieh et al. 2016). We used a linear regression model to test the hypothesis that gamma diversity of metacommunities differed across dispersal treatments, predicting that it would decrease with higher dispersal.

c) **Beta diversity (spatial):** To test the effect of dispersal on beta diversity, we calculated both Bray-Curtis and Jaccard distances between all possible pairings of local communities within each metacommunity, then obtained an average distance for each metacommunity. Following this, we ran a linear regression to test whether beta diversity (dissimilarity) decreased with increasing dispersal. This test is different from the above comparison of dissimilarity across versus within the metacommunities because this test

examines dissimilarity across dispersal treatments, but not within versus across metacommunities within a dispersal treatment.

To test **hypothesis #2,** that compositional change over time within a local community depends on dispersal rate within the metacommunity, for each community we compared composition in July with composition in August. We calculated temporal beta diversity for each local community by calculating dissimilarity distances (both Bray-Curtis and Jaccard) between two months. We used linear regression with dispersal as a fixed effect and metacommunity as a random effect and community dissimilarity as the response variable.

All statistical analyses were performed using R version 3.6.3. Linear regressions were fit using nlme (version 3.1.152). Estimates of alpha diversity were made using iNEXT (version 2.0.20). PERMANOVA analyses were carried using vegan package (version 2.5.6).

2.3 Results

Overall, we found that high dispersal homogenized bacterial communities within metacommunities (Figure 3). Bacterial communities were more similar within metacommunities than among metacommunities for the high dispersal treatments (ANOVA: $F1=22.81$, $p=0.000002$), but not in the lower ($F=1.22$, $p=0.27$) or background ($F=0.14$, $p= 0.71$) dispersal treatments. We therefore verified our hypothesis premise as high dispersal rates were sufficient to move microbial taxa among communities

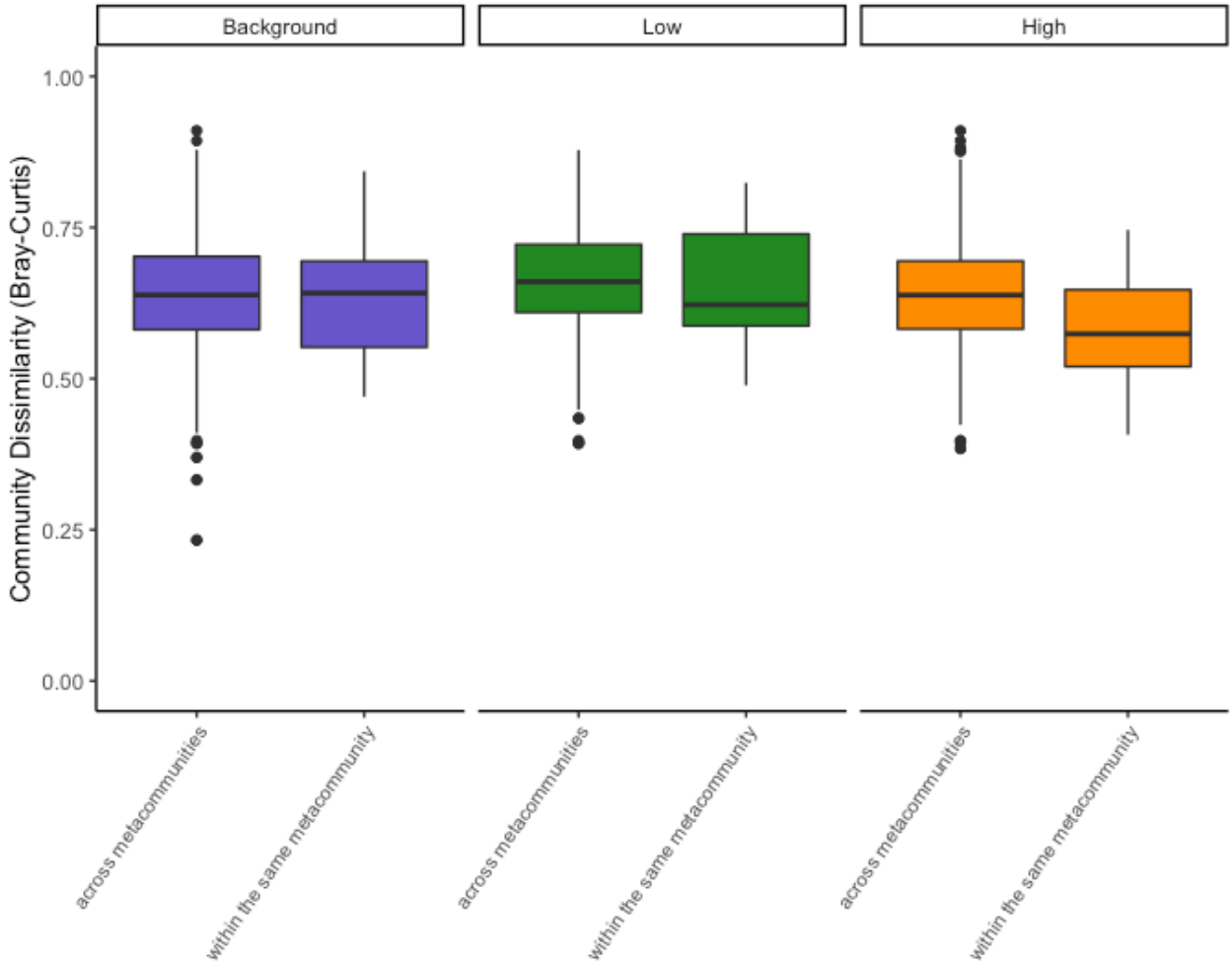

Figure 3. Bacterial community dissimilarity (Bray-Curtis, spatial beta diversity) for all possible pairs of local communities (n = 46 mesocosms) across background (lowest), low and high dispersal rate treatments. Boxes define the interquartile range (IQR) between the first and third quartiles (25th and 75th percentiles, respectively), and the horizontal line inside the box represents the median. Whiskers represent the lowest and the highest values within 1.5 times of the IQR from the first and third quartiles. Boxes are colour-coded by dispersal treatment with purple, green and orange denoting background, low and high dispersal treatments respectively.

Hypothesis 1: Effects of habitat temperature on bacterial community composition caused by species sorting can be overwhelmed by dispersal in bacterial metacommunities

In July, at low dispersal rates microbial community structure differed significantly among local communities differing in their temperature treatment (PERMANOVA: $F_{3,15}$=1.53, R2=0.25, p=0.01) within metacommunities, and among local communities from different metacommunities (PERMANOVA on Bray Curtis dissimilarity: $F_{3,15}$=1.65, R2=0.27, p= 0.004) (Figure 4). This pattern also holds for species composition when compared using only presence/absence data (the Jaccard index), for which community structure depended on local temperature regime and metacommunity identity (Table 1). Furthermore, we found composition of local communities was more similar within metacommunities than among metacommunities, suggesting communities were more compositionally similar within than among metacommunities (PERMANOVA Bray-Curtis dissimilarity: $F_{3,14}$=2.673, R2=0.39898, p=0.0001), but temperature did not structure the communities ($F_{3,14}$=1.360, R2=0.20299, p=0.0721); in other words, at high dispersal, there were no detectable effects of temperature treatment on bacterial community composition, but at low dispersal rates, local community composition differed between temperature groups. Similar results were found for an analysis of community similarity based on the Jaccard index (Table 1). As for metacommunities experiencing background-level dispersal rates, we found that composition differences among local communities were explained by experimental temperature only (PERMANOVA on Bray-Curtis dissimilarity $F_{3,14}$=1.685, R2=0.30418, p=0.0017) and not metacommunity identity (PERMANOVA: $F_{3,14}$=1.1875, R2=0.21439, p=0.1584), suggesting community composition was more similar in local communities exposed to the same temperature and was not structured by

their ‘region’ identity (in this case, metacommunity). Further, we ran pairwise PERMANOVA analyses on the significant variables of PERMANOVA for each dispersal rate (specifically: background dispersal - temperature, low dispersal - temperature and metacommunity, high dispersal - metacommunity), but none of these pairwise comparisons were significant (p adjusted >0.05).

Consequently, we do not reject our predictions that bacterial composition differences are explained by temperature when dispersal rates are relatively low (observed in both background and low dispersal treatments), consistent with species sorting (prediction 1.1). In contrast, temperature did not explain variation in community composition in metacommunities at higher dispersal rates, consistent with our prediction 1.2. However, even though the failure to find a temperature effect at high dispersal is consistent with prediction 1.2, I cannot exclude the possibility that the analysis did not have the statistical power to detect a real effect. This could be a result of using data subset by dispersal and running analysis at that level, which resulted in smaller sample size affecting the statistical power. As for background dispersal (the lowest dispersal treatment level), we found communities to be more similar under same temperature regimes, indicative of species sorting, and corroborating one of the predicted scenarios suggesting that abiotic factors are more important in our experiment as opposed to demographic stochasticity and priority effects. Overall, our findings support our predictions 1.1 and 1.2 (see Table 1). We do not reject our hypothesis that in the absence of dispersal species sorting drives the differences in bacterial community compositions, while high dispersal overrides its effects

and causes communities to compositionally converge irrespective of the abiotic environments the species came from.

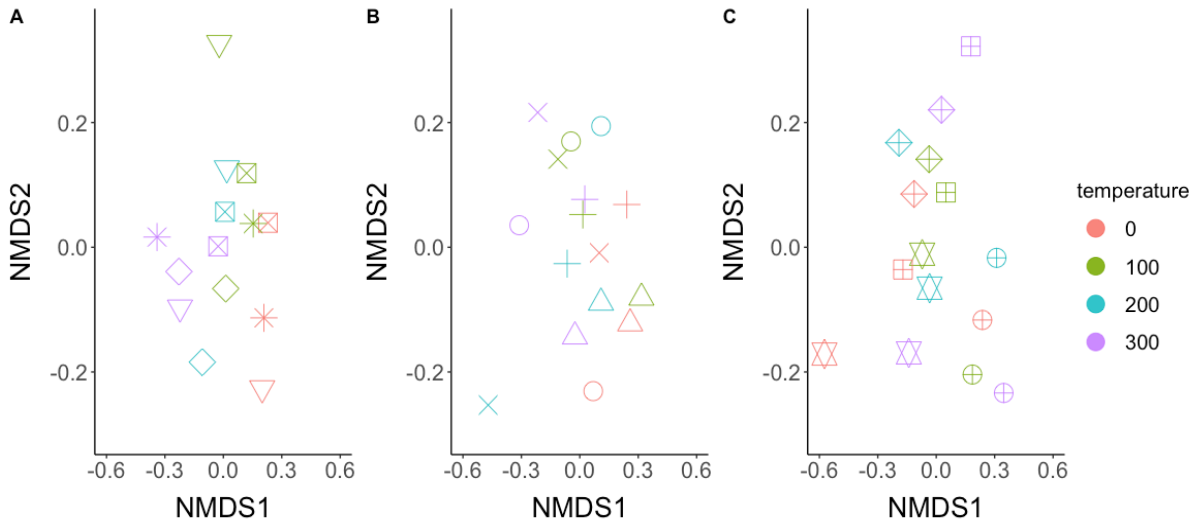

Figure 4. Non-metric Multidimensional Scaling (NMDS) plots based on Bray-Curtis dissimilarities of bacterial communities at the lowest (background) (panel A, n=14*), low (panel B, n=16) and high (panel C, n=15) dispersal treatments in July. Each point represents an ordination value for the tank which identity is indicated by shape for metacommunity, and by colour for temperature treatment. *Note here for panel A: n=14, as we removed the data point for tank 44 for this graph. However, the statistical analyses were run with and without this tank, and results were unaffected (see Appendix: Figure 4A with the tank 44).

Table 1. Summary statistics and hypothesis 1 testing the effect of dispersal rate (background, low and high) on community structure. Dissimilarity indexes used for analyses are Bray-Curtis (BC) – relative abundance, and Jaccard – presence absence. P<0.05 are bolded to indicate the significance. PERMANOVA was run on a model of bacterial dissimilarity ~ temperature + metacommunity.

Dispersal rate	**PERMANOVA**				
	Dissimilarity Index	**Variable**	**F**	**R2**	**P**
Background (Figure 4, Panel A)	Bray-Curtis	Temperature	$F_{3,14}$=1.69	0.3	**0.002**
		Metacommunity	$F_{3,14}$=1.19	0.21	0.16
	Jaccard	Temperature	$F_{3,14}$=1.54	0.29	**0.002**
		Metacommunity	$F_{3,14}$=1.14	0.21	0.16
Low (Figure 4, Panel B)	Bray-Curtis	Temperature	$F_{3,15}$=1.53	0.25	**0.01**
		Metacommunity	$F_{3,15}$=1.65	0.27	**0.004**
	Jaccard	Temperature	$F_{3,15}$=1.32	0.23	**0.01**
		Metacommunity	$F_{3,15}$=1.41	0.25	**0.005**
High (Figure 4, Panel C)	Bray-Curtis	Temperature	$F_{3,14}$=1.36	0.2	0.07
		Metacommunity	$F_{3,14}$=2.67	0.4	**0.0001**
	Jaccard	Temperature	$F_{3,14}$=1.20	0.2	0.11
		Metacommunity	$F_{3,14}$=2.08	0.35	**0.0001**

In addition, our other goal was to understand how different dispersal rates affected bacterial communities and alpha, spatial beta and gamma diversity. We predicted that increasing dispersal rates among local habitats that differ in their temperatures raises alpha diversity and reduces spatial beta and gamma diversity within a metacommunity (**prediction 1.3**). We found:

Alpha diversity: Local estimated taxonomic richness varied among metacommunities, but not with dispersal (nested ANOVA: $F_{2,9}$=0.02, p=0.98; Figure 5). Therefore, we reject our prediction that alpha diversity would increase with dispersal.

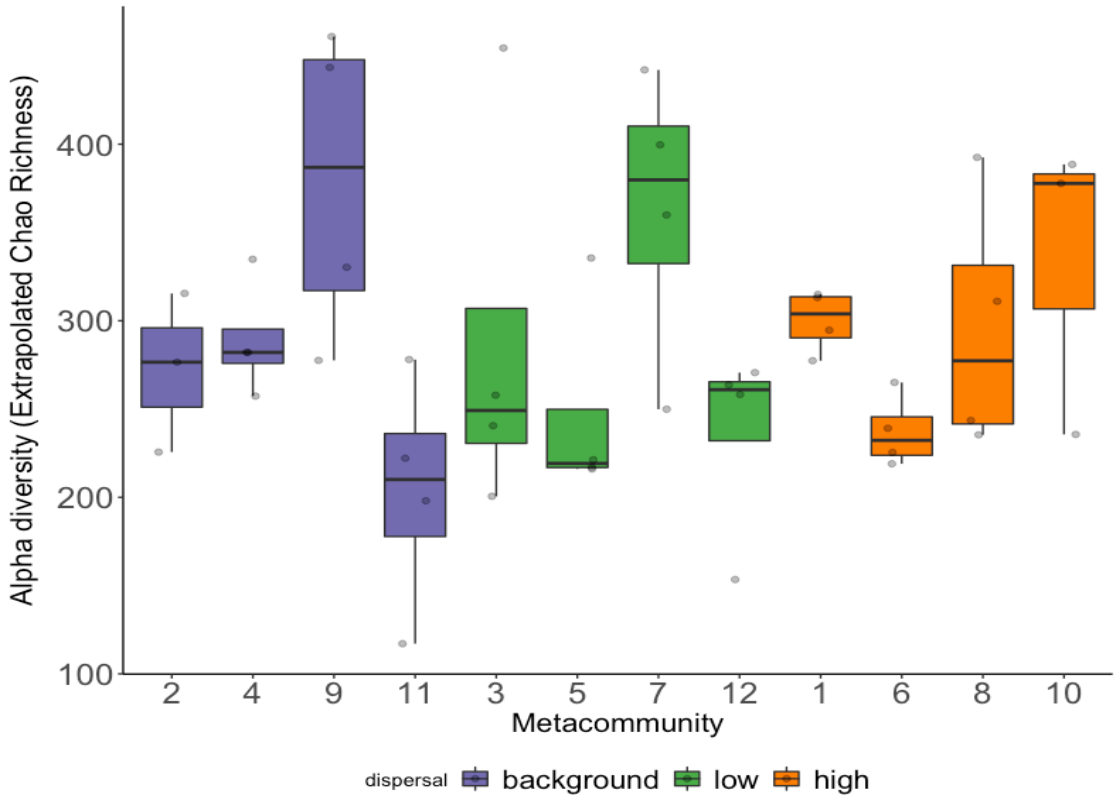

Figure 5. Alpha diversity of bacterial communities estimated as richness across metacommunities (N=12) in July. Each point (n=46) represents a richness estimate for a given mesocosm. Boxes define the interquartile range (IQR) between the first and third quartiles (25th and 75th percentiles, respectively), and the horizontal line inside the box represents the median. Whiskers represent the lowest and the highest values within 1.5 times of the IQR from the first and third quartiles. Boxes are colour-coded by dispersal treatment with purple, green and orange denoting background, low and high dispersal treatments, respectively.

Table 2. Linear mixed effects models of alpha diversity estimated as extrapolated Chao richness with metacommunity as a random effect in July (n=46). Abbreviations: numDF – numerator degrees of freedom, denDF – denominator degrees of freedom.

Model	Fixed effect				
	effect	numDF	denDF	F value	p value
Extrapolated Chao Richness ~ dispersal + 1|metacommunity	intercept	1	34	357.87	<0.0001
	dispersal	2	9	0.02	0.98
Extrapolated Chao Richness ~ temperature x dispersal + 1|metacommunity	intercept	1	25	291.62	<0.0001
	temperature	3	25	2.35	0.1
	dispersal	2	9	0.01	0.97
	temperature x dispersal	6	25	0.57	0.75

Gamma diversity:

Overall, observed regional (metacommunity) diversity ranged between 413 and 611 ASVs, and extrapolated - between 415 and 647 unique ASVs (Figure 6). However, we found no significant effect of dispersal rate (F_2=1.237, p= 0.335; Figure 6), and we reject our prediction that higher dispersal rates reduce gamma diversity.

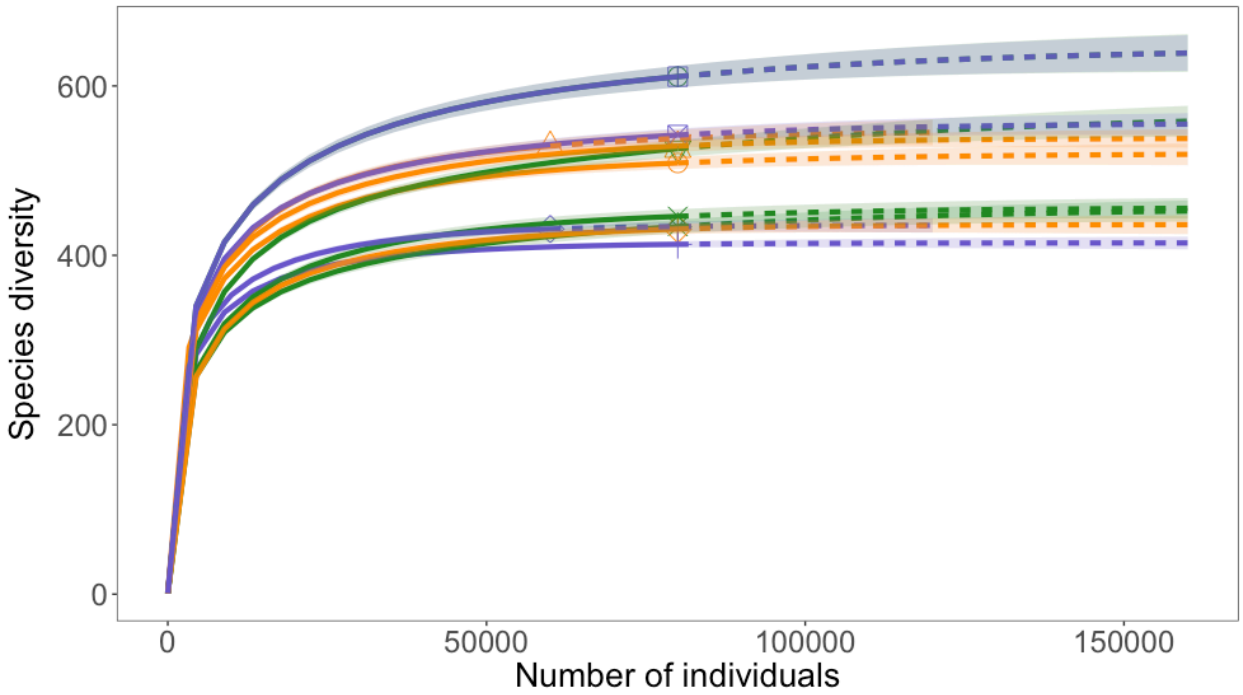

Figure 6. Estimated Gamma Diversity at the metacommunity scale, based on sample-size-based rarefaction (solid line segment) and extrapolation (dashed line segments) sampling curves with 95% confidence intervals (shaded areas) for bacterial richness data across three dispersal treatments (N = 12 metacommunities) in July. Each curve represents the estimates for a single metacommunity by pooling observations from the four constituent local communities within each metacommunity. Each metacommunity's species accumulation curve is colour-coded by dispersal, with violet, green and orange denoting background, low and high dispersal treatments respectively. Each shape represents a cumulative observed species diversity value for each metacommunity.

Beta diversity (spatial):

Measures of beta diversity, both relative abundance and presence-absence of ASVs, were variable across all metacommunities and across dispersal treatments. Mean bacterial community dissimilarity at metacommunity level appeared to be higher in low dispersal treatment. This is partially consistent with our prediction that local communities within metacommunities would

appear more different with dispersal treatment specifically in case of low dispersal (prediction 1.1). There was a lot of variation across metacommunities, but there was no significant effect of dispersal on community dissimilarity for relative abundance (Bray-Curtis index: $F_{2,9}$=2.308, p=0.155, see Figure 8A in the Appendix) or presence-absence (Jaccard index: $F_{2,9=}$2.163, p= 0.171). However, the statistical power of this analysis was reduced by smaller sample size due to estimating dissimilarity at a metacommunity level. Therefore, we cannot exclude that the lack of effect of dispersal on spatial beta diversity was likely due to insufficient statistical power.

Hypothesis 2: temporal beta (β) diversity (species turnover within a patch over time) is the highest at low dispersal rates.

Overall, we found that local community composition differed substantially between July and August (Figure 7). Dissimilarity values were above 0.75 when comparing observations from each community in August with the same community in July, suggesting turnover was quite high between the two months. When looking into the most abundant taxa in both months, we saw a complete turnover in the dominant ASVs between July and August (see Appendix: Figures 9A & 10A). Dispersal did not explain patterns in temporal turnover within local communities (Bray-Curtis, ANOVA: $F_{2,26}$=1.11, p = 0.34; Jaccard, ANOVA: $F_{2,26}$=1.12, p = 0.34; Figure 7), so we rejected this hypothesis.

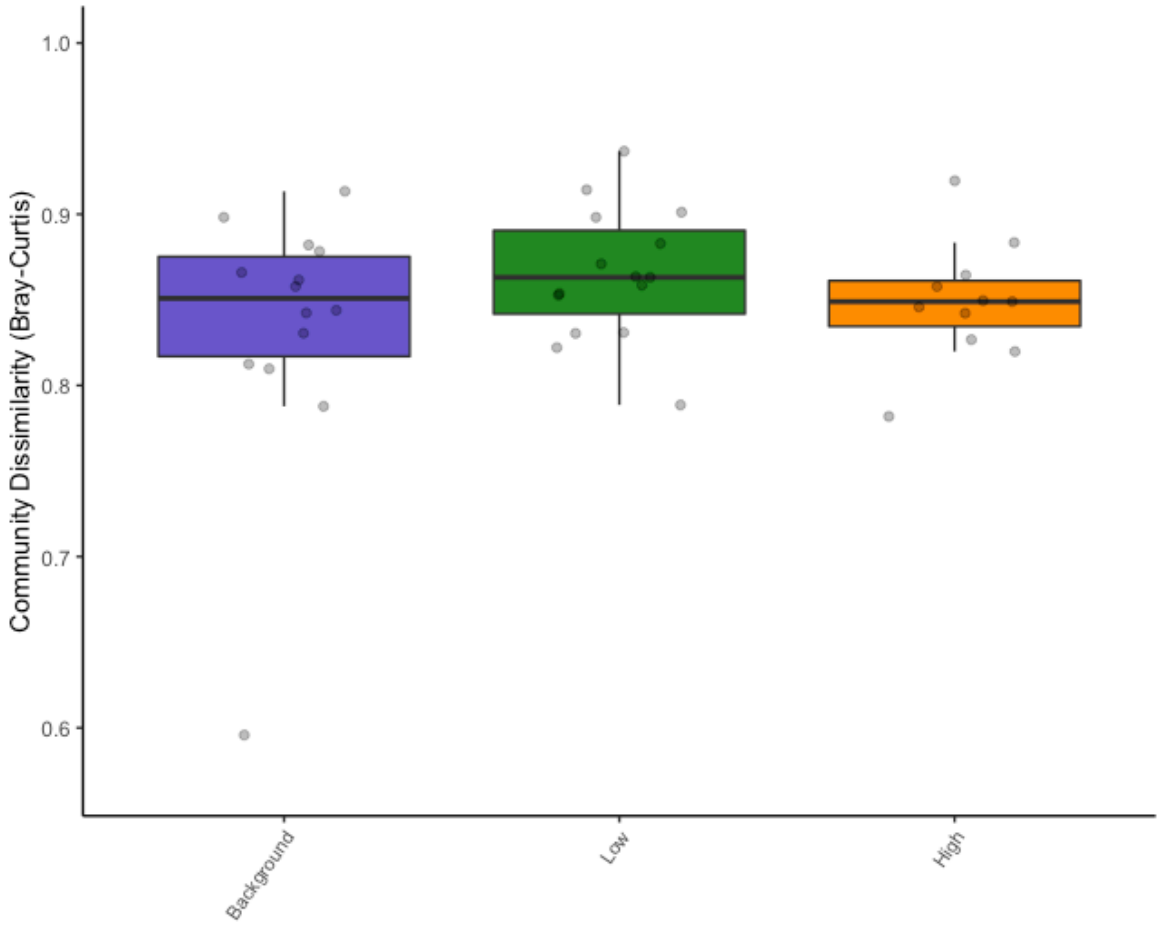

Figure 7. Temporal beta diversity does not differ across dispersal treatments. Each point is the Bray-Curtis dissimilarity for one tank between July and August (n=40).

2.4 Discussion

The main objective of this study was to test whether warming affects microbial communities differently in habitats with different degrees of connectivity. Overall, we found that variation in bacterial community composition was explained by temperature regime, but the effect of temperature was much less pronounced and non-significant in high dispersal environments where dispersal homogenized the communities. Thus, dispersal eroded the effect of temperature

on local community composition, in agreement with prediction 1.2. Contrary to prediction 1.3 that was drawn from metacommunity theory, we found no support for dispersal rates affecting local or regional taxonomic richness of bacterial communities. We did observe that low dispersal rates yielded communities with very different spatial community structures in terms of both ASV relative abundance and presence-absence (Figure 4, Table 1 and Appendix: Figure 4B). In addition, community dissimilarity was high in between the months, but temporal diversity trends were unaffected by dispersal rates at the metacommunity level. In summary, our study suggests that a high degree of connectivity by dispersal leads to homogenization of bacterial communities, weakening the effect of environment on community structure, even when there are no measurable effects of dispersal rates on local and regional richness.

We did find support for a homogenizing role of dispersal, but why did we not find any effect of dispersal on diversity? The Baas-Becking hypothesis in microbial biogeography suggests that "everything is everywhere, but environment selects", implying that dispersal limitation is unimportant for free-living microbes with highly resilient life stages (Baas-Becking, 1934; de Wit & Bouvier, 2006 as cited in Locey 2010). However, the results of this study disprove this as we observe community homogenization at high dispersal rates causing spatial beta diversity to become more similar (Figure 4, 4A, 4B and 8A). As for no effect of dispersal on alpha or gamma diversity, a possible explanation for no significant change in both metrics may have to deal with dormancy contributing to the maintenance of microbial diversity. Dormancy can be either physical through spores and cysts or physiological through reduced metabolic activity and suppressed reproduction, and these dormant populations can be present at almost undetectable

densities (Locey 2010). Many microbes experience dormancy and less than 10% of microbial community members within soil and aquatic environments are active at a time (Gasol et al., 1995; del Giorgio et al.,1997; Luna et al., 2002 in Locey 2010). Importantly, dormancy can be driven by stressful abiotic conditions, but it can be reversed under optimal conditions (Jones & Lenon 2010; Lennon & Jones 2011). In this study, we did not directly measure real-time biological activity, but our comparison of the most abundant taxa does not reject the idea of dormancy and "microbial seed bank." We observed a large turnover of the dominant ASVs across months (Figures 9A, 10A and 11A). Moreover, we found some ASVs were specific to a certain month (Figure 12A**)**. Considering our metacommunities were seeded from the same species pool, it is possible that the unique ASVs in July were present in May, but were dormant and present at extremely low densities, so were not detected until abiotic conditions became favourable in July and they became "active". Thus, the dormant bacteria can potentially represent a vast reservoir of bacterial diversity in metacommunities, and dormancy can explain the lack of effect of dispersal on local and regional diversity.

Our results showed that temporal beta diversity was unaffected by dispersal rates, but beta community dissimilarity between the months was high regardless of dispersal (Figure 7). This pattern can be explained by environmental selection being a more deterministic factor of community composition than priority effects. The local environment explains some compositional variation even in the presence of dispersal (Lindström & Östman, 2011). Our metacommunities spanned a range of temperatures within a metacommunity but were also open to the environment and experienced thermal fluctuations on a daily and weekly basis (Figure 2).

Consequently, the same tank within a metacommunity experienced variation in thermal conditions, and because the initial metacommunity assembly was similar and without systematic variation among metacommunities, it is likely that there was an initial selection for microbes with suitable thermal optima. Therefore, when the temperature was changing, microbes with different thermal niches outperformed others. Under the likely scenario of a dormant microbial seed bank contributing to high diversity, turnover may have happened through selection of species that were already present in the species pool suitable to the new temperature. Because communities experienced so much thermal variation within a month, it is possible that there was enough bacterial variation present within the metacommunity for the environment to select suitable microbes. Consequently, dispersal would not contribute to temporal turnover by bringing new colonizers as the environment already has the "dormant" microbial pool to select from.

We conducted a novel test of metacommunity theory predictions for bacterial communities in environments with a spatio-thermal gradient and different connectivity levels. Our research was part of a larger collaborative study (Thompson et al. in prep), allowing us to compare bacterial community responses to those of phytoplankton and zooplankton in the same experimental system. Overall, the results differed among trophic levels in terms of which experimental treatments best explained variation among the communities but all trophic levels had a similar alpha diversity patterns. We hypothesized community structure to vary with temperature and dispersal. It appeared that zooplankton communities were clearly structured by temperature, but not by dispersal (Thompson et al., in prep). Interestingly, for microbes, we found communities to

be structured by temperature in the background and low dispersal treatments but not in high dispersal (Figure 4, panel C). Contrary to the predictions from metacommunity theory, we found no effect of dispersal on bacterial richness at the local and regional level and our results were matched in zooplankton communities where alpha diversity also did not vary with dispersal.

It previously has been suggested that the potential for dispersal to mitigate effects of environmental change may vary with trophic level. Complementary to our results, freshwater bacteria and zooplankton richness were unaffected by dispersal under different pH conditions (Limberger et al. 2019), though no other study has examined the synergistic effect of warming and dispersal on bacterial communities. One study tested metacommunity theory predictions of how temperature and dispersal affects protist and rotifer communities associated with a carnivorous plant at two successional stages in the laboratory (Parain, Gray and Berier 2019). A hump-shaped relationship was observed between diversity and dispersal in late-successional communities, but higher temperatures decreased alpha- and gamma-diversity (Parain, Gray and Berier). These findings suggested climate change will drive extinctions at both local- and global scales but emphasized the buffering capacity of dispersal at intermediate rates (Parain, Gray and Berier 2019). These findings are in line with metacommunity predictions, but the study design differs greatly from ours which may explain the differences in our conclusions. One major difference in the experimental design is that our metacommunities had a thermo-spatial gradient and therefore species were dispersed from communities with different thermal environments while Parrain et al. (2019) metacommunities were exposed to one temperature regime per treatment, therefore precluding species sorting. This is important, as the Parrain et al. (2019)

design implied homogeneity of the patches, while in the real world there is a lot of heterogeneity between habitat patches, especially on the regional scale, and theory suggests dispersal may buffer biodiversity against the effects of changing environment under low/intermediate dispersal and high regional heterogeneity (Mouquet and Loreau 2003, Thompson et al. 2020).

From a community composition perspective, there is also evidence that bacterial communities are more similar under high dispersal as a result of homogenization, and the most compositionally variable under low dispersal where species sorting plays a deterministic role (Lindström & Östman, 2011). However, there is evidence that dispersal can affect communities in a different manner across different trophic levels, but 1) it primarily comes from research solely focused on the effect of dispersal on diversity, and 2) the effect of dispersal on diversity depends on whether the environments examined are homogenous or heterogenous. For example, Gilbert et al. (1998) found terrestrial arthropod immigration was boosted by introduction of corridors and both local and regional richness was higher than in disconnected systems and there was no evidence of reduced diversity caused by homogenization. Matthiessen and Hillebrand (2006) observed a humped-shaped relationship between dispersal and both the species richness and species biomass of benthic macroalgae (with the highest values at intermediate dispersal). Forbes and Chase (2002) found quite a different pattern in freshwater zooplankton: increased connectivity had no effect on local diversity but it decreased regional diversity and caused higher community similarity. The authors suggest that habitat quality/homogeneity may play a role; Gilbert et al. (1998) allowed dispersal between relatively homogenous patches, while in their

study fragments were heterogeneous. Therefore, connectivity between homogenous patches can lead to different diversity patterns when compared to heterogeneous sites.

Do these results suggest bacterial communities will be affected in light of climate change?

Climate is changing and habitat fragmentation is ongoing, so it is crucial to think about the contribution of science to forecasting how these forces will shape biological communities. Bacteria are among the most abundant organisms on the planet **(**Curtis et al. 2002) and more importantly - are key contributors to biogeochemical and metabolic processes on which other organisms rely. Consequently, it is crucial to understand how they are impacted by global change. Based on our findings, bacterial community structure in habitats with low connectivity is structured by the local environment (in our case, temperature), while the habitats with high degree of dispersal do not vary with environmental temperature. Therefore, bacterial community compositions in the light of climate change are more likely to shift in habitats with low connectivity. In addition to community structure, there is a lot of concern associated with diversity trends in the light of preservation of species diversity. While it has been proven effective to increase habitat connectivity for larger organisms to maintain biodiversity (Bailey 2007; Tambosi et al., 2013), neither bacterial richness nor regional diversity metrics were affected by dispersal. This finding is in line with the "everything is everywhere, but environment selects" hypothesis and dormancy driving bacterial diversity patterns. Passive dispersal plays a role in broad bacterial distribution, while high diversity, dormancy, fast generation time and large population sizes make bacteria less susceptible to the negative effects of fragmentation and climate change.

Chapter 3: Conclusion

Our experiment tested the predictions derived from metacommunity theory in regard to how microbial communities are affected by warming in metacommunities with different degrees of connectivity. Overall, we find support for the homogenizing role of dispersal, but found no effect of dispersal on bacterial diversity.

Our first hypothesis states that dispersal would homogenize communities, leading to more compositionally similar communities across thermal environments with increasing dispersal, while lower dispersal metacommunities would be most compositionally different by the treatment as it would allow for species to track their environment. Our data support this hypothesis as we observed background and low dispersal metacommunities to be structured by temperature, while under high dispersal the effect of temperature weakened, and communities appeared more compositionally similar. Furthermore, when comparing how dissimilar bacterial communities were when compared by dispersal treatment across different metacommunities versus within the same metacommunity, we found high dispersal to significantly homogenize communities within the same metacommunity appearing statistically compositionally different from other metacommunities. This supports our hypothesis that high dispersal has a homogenizing effect on bacterial community composition.

Further, our second hypothesis stated that dispersal will lead to higher alpha diversity, but lower beta and gamma diversity. However, we found no evidence of dispersal having any effect on alpha, gamma and temporal beta-diversity (third hypothesis) metrics. Spatial beta diversity

trends appeared to be lower for background and high dispersal treatments and increased for low dispersal metacommunities (more dissimilarity between low dispersal metacommunities, though there was a lot of variation), but the trend was not strong or statistically significant. We speculate that one of the plausible explanations for the lack of observed effect of dispersal on richness or diversity lies in communities having a dormant microbial seed bank that represents the regional diversity. Since only around 10% of bacteria are active at a time in the community, it is highly possible that all possible ASVs were present initially in the regional species pool with which we seeded our mesocosms. However, since different abiotic conditions favour different bacteria, we saw a turnover in the most dominant taxa over time. Additionally, we saw some ASVs that were unique to a data point sampled, but it does not exclude the possibility that those ASVs were present throughout the experiment but were either dormant or were present at undetectable densities and were not detected in sequencing.

This study was originally designed for zooplankton, which imposed certain limitations to studying the effects of treatment on the bacterial communities. The dispersal events were not carried out in sterile conditions (even though there was the effort to minimize passive dispersal) and the mesocosms were open to the environment, making it impossible to have a control with absolutely zero dispersal. As was discovered by Louca et al. (2020), if initial microbial community assembly and dispersal were controlled, community dynamics are very similar under the same abiotic conditions over time. Since we did not control background dispersal throughout the experiment, we acknowledge our experimental limitations and assigned the passive dispersal as a background treatment. We assume that a much greater quantity of organisms was transferred

by the movement of 3 and 30L of water in the experimental treatments. Thus, this experiment tests the influence of background, low, and high levels of dispersal. Something that this study could have benefited from was having more frequent taxonomy profile sampling events to track community composition changes on a finer scale, especially around the extreme weather events, like the heatwave in August. Because we only took bacterial profiles at three time points, we can only speculate about the importance of seasonality or extreme weather events on community structure, but it would be interesting to see a higher resolution of community change before, during and after the heatwave.

This study is of great importance, as it is the first one to assess how bacterial communities respond to warming in habitats with different levels of connectivity. We recognize that abiotic environments structure bacterial communities, but high dispersal rates are homogenizing and can erase the filtering effects of the abiotic environment. However, since dispersal did not have an effect on diversity, there is no argument from the conservation point-of-view regarding higher habitat connectivity being beneficial for preservation of bacterial diversity. This is the opposite that has been found for larger organisms, but bacteria are smaller sized, have faster generation times and larger population sizes, making them great passive dispersers, compared to many macroorganisms. Also, it is important to note that this was a short-term study, and we cannot predict how diversity would change over a longer period of time, especially if more extreme climatic events were to occur. We recognize the importance of long-term monitoring to forecast diversity patterns in the future, but this was outside the scope of this study. Therefore, future studies would benefit from a longer study duration and record of bacterial community dynamics

and structure during different times of the year to examine the importance of seasonality and extreme weather events.

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Appendix

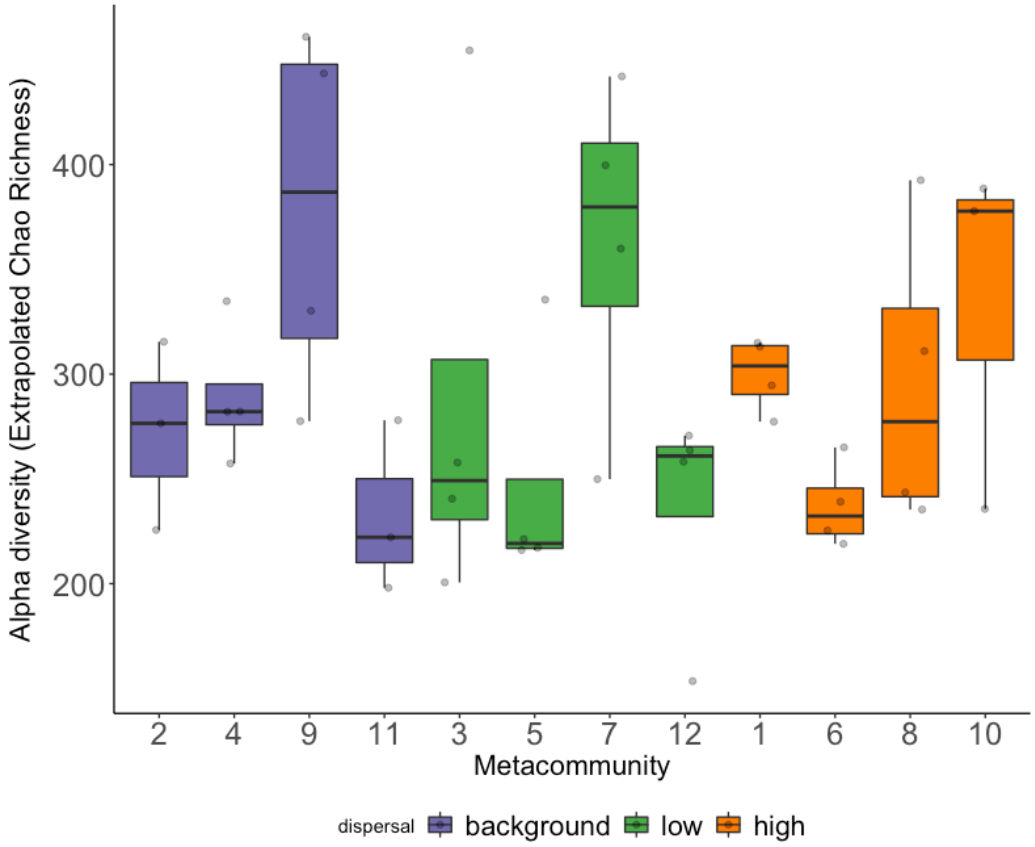

Figure 5A. Alpha diversity of bacterial communities estimated as richness across metacommunities (N=12) in July with tank 44 excluded from the analysis. Each point (n=45) represents a richness estimate for a given mesocosm. Boxes define the interquartile range (IQR) between the first and third quartiles (25th and 75th percentiles, respectively), and the horizontal line inside the box represents the median. Whiskers represent the lowest and the highest values within 1.5 times of the IQR from the first and third quartiles. Boxes are colour-coded by dispersal treatment with purple, green and orange denoting background, low and high dispersal treatments, respectively.

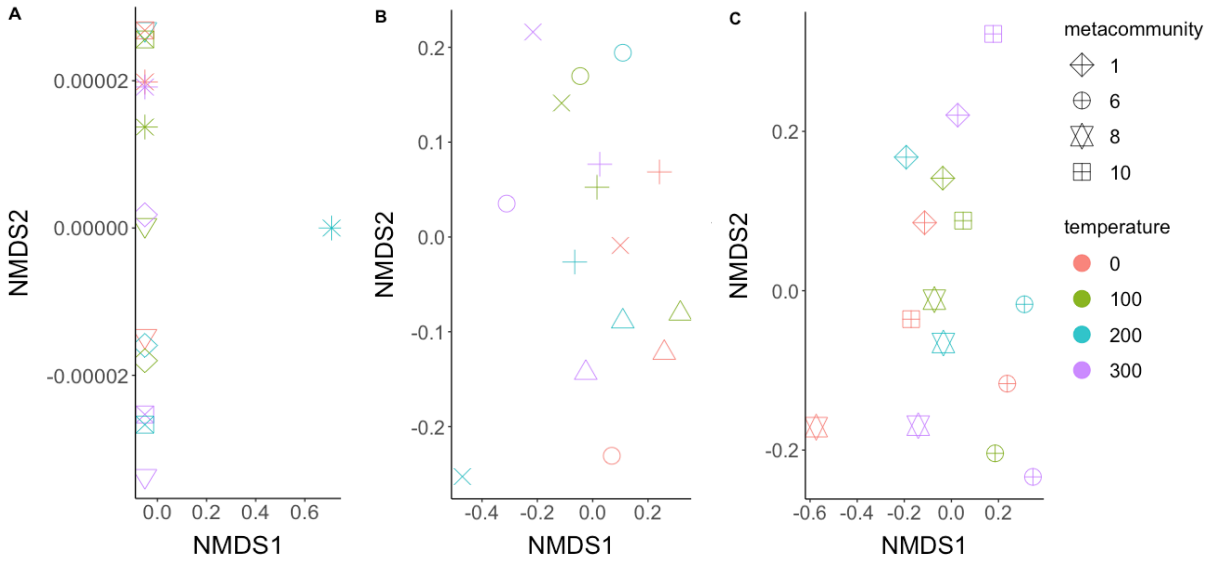

Figure 4A. Non-metric Multidimensional Scaling (NMDS) plots based on Bray-Curtis dissimilarities of bacterial communities at the lowest (background) (panel A, n=15) low (panel B, n=16) and high (panel C, n=15) dispersal treatments in July. Each point represents an ordination value for the tank which identity is indicated by shape for metacommunity, and by colour for temperature treatment.

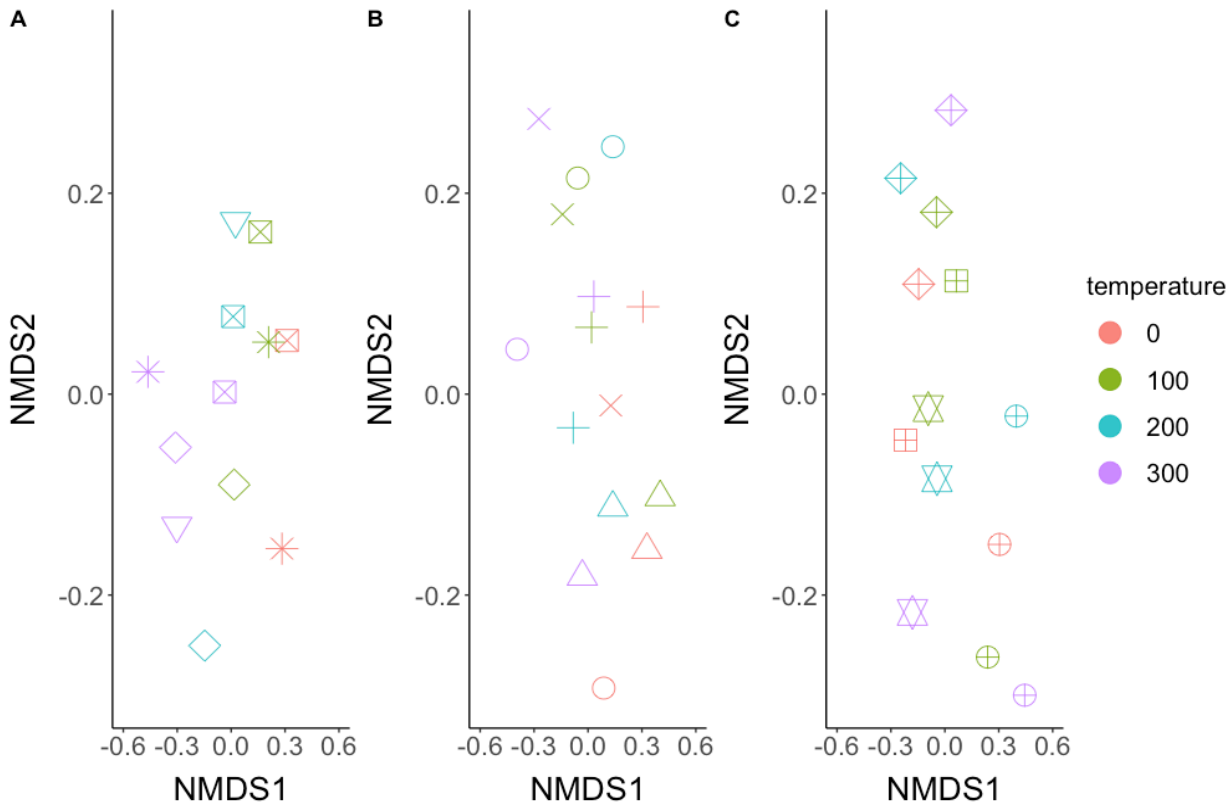

Figure 4B. Non-metric Multidimensional Scaling (NMDS) plots based on Jaccard dissimilarities of bacterial communities at the lowest (background) (panel A, n=14*), low (panel B, n=16) and high (panel C, n=15) dispersal treatments in July. Each point represents an ordination value for the tank which identity is indicated by shape for metacommunity, and by colour for temperature treatment. *Note here for panel A: n=14, as we removed the data point for tank 44 for this graph. However, the statistical analyses were run with and without this tank, and results were unaffected (see Table 1 and Appendix: Table 1A).

Table 1A. Summary statistics and hypothesis 1 testing the effect of background dispersal rate on community structure on July data without tank 44. Dissimilarity indexes used for analyses are Bray-Curtis (BC) – relative abundance, and Jaccard – presence absence. P<0.05 are bolded to indicate the significance. The model on which I ran PERMANOVA was done on a subset of data by dispersal with distance matrix for each dissimilarity index explained by two independent factors: temperature and metacommunity (dissimilarity ~ temperature + metacommunity).

Dispersal rate	**PERMANOVA**				
	Dissimilarity Index	**Variable**	**F**	**R2**	**P**
Background (Figure 4, Panel A)	Bray-Curtis	Temperature	$F_{3,13}$=1.92	0.35	**0.001**
		Metacommunity	$F_{3,13}$=1.21	0.22	0.19
	Jaccard	Temperature	$F_{3,13}$=1.65	0.32	**0.002**
		Metacommunity	$F_{3,13}$=1.14	0.22	0.21

Table 2A. Linear mixed effects models of alpha diversity estimated as extrapolated Chao richness with metacommunity as a random effect in July with tank 44 excluded from the analysis (n=45). Abbreviations: numDF – numerator degrees of freedom, denDF – denominator degrees of freedom.

Model	Fixed effect				
	effect	numDF	denDF	F value	p value
Extrapolated Chao Richness ~ dispersal + 1|metacommunity	intercept	1	33	432.08	<0.0001
	dispersal	2	9	0.07	0.94
Extrapolated Chao Richness ~ temperature x dispersal + 1|metacommunity	intercept	1	24	344.16	<0.0001
	temperature	3	24	1.69	0.2
	dispersal	2	9	0.03	0.97
	temperature*dispersal	6	24	0.67	0.68

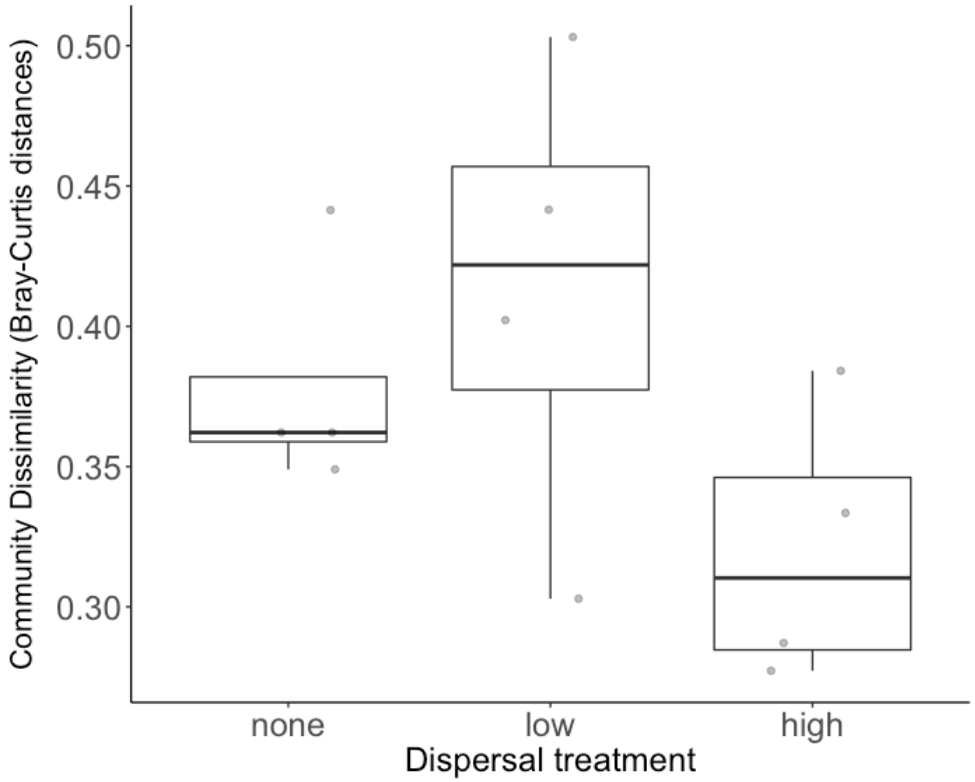

Figure 8A. Community dissimilarity (Bray-Curtis beta diversity) for bacteria across three levels of dispersal treatment. Each point represents a value for an average Bray-Curtis distance value for each metacommunity (n=12 metacommunities). Boxes define the interquartile range (IQR) between the first and third quartiles (25th and 75th percentiles, respectively), and the horizontal line inside the box represents the median. Whiskers represent the lowest and the highest values within 1.5 times of the IQR from the first and third quartiles.

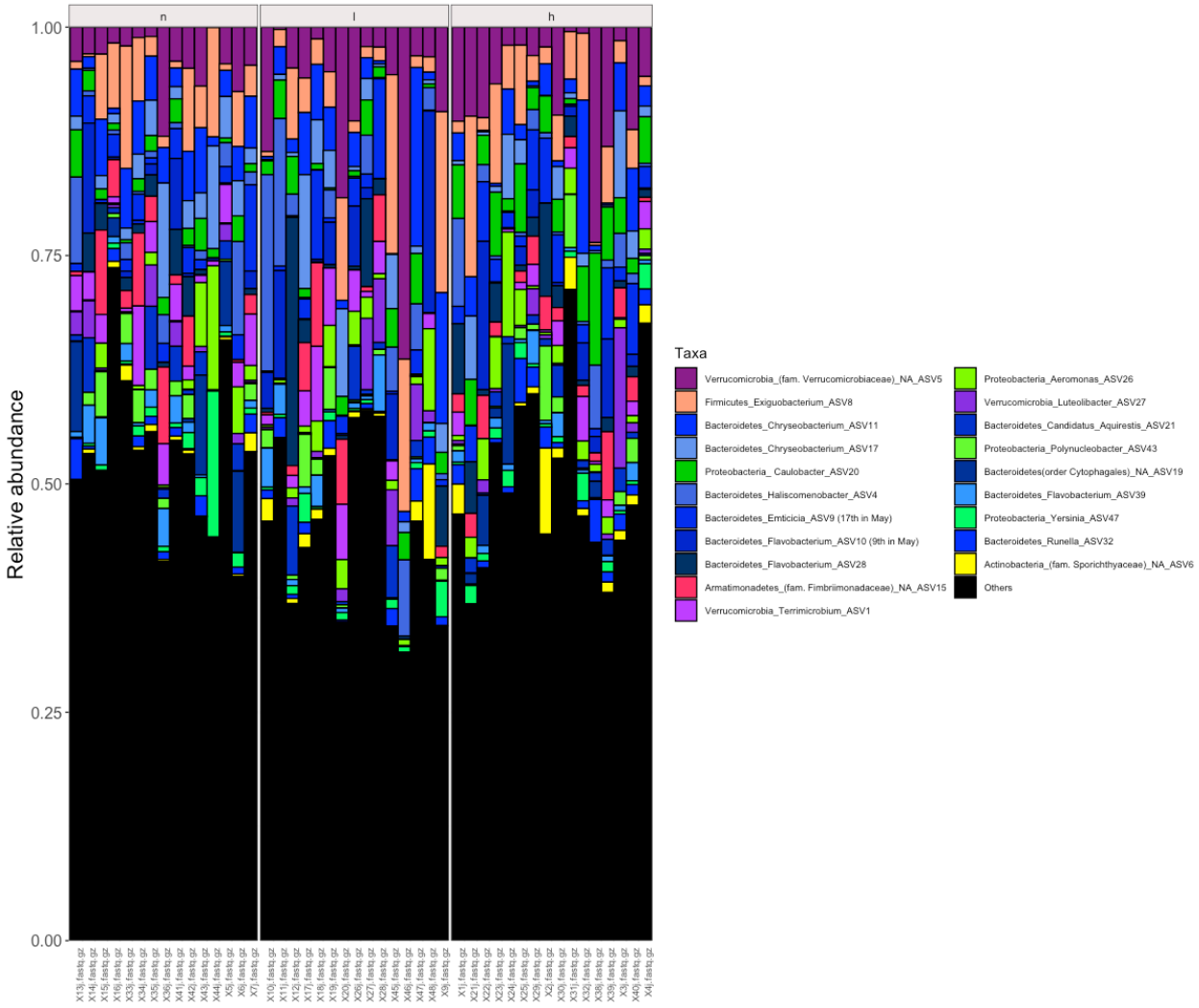

Figure 9A. Relative abundance of the top 20 most abundant bacterial ASVs in July across tanks (n=46) with different dispersal levels represented by the panels. Panels abbreviations stand for: n – background (no active dispersal), l – low, and h - high. Each colour is unique to an individual ASV.

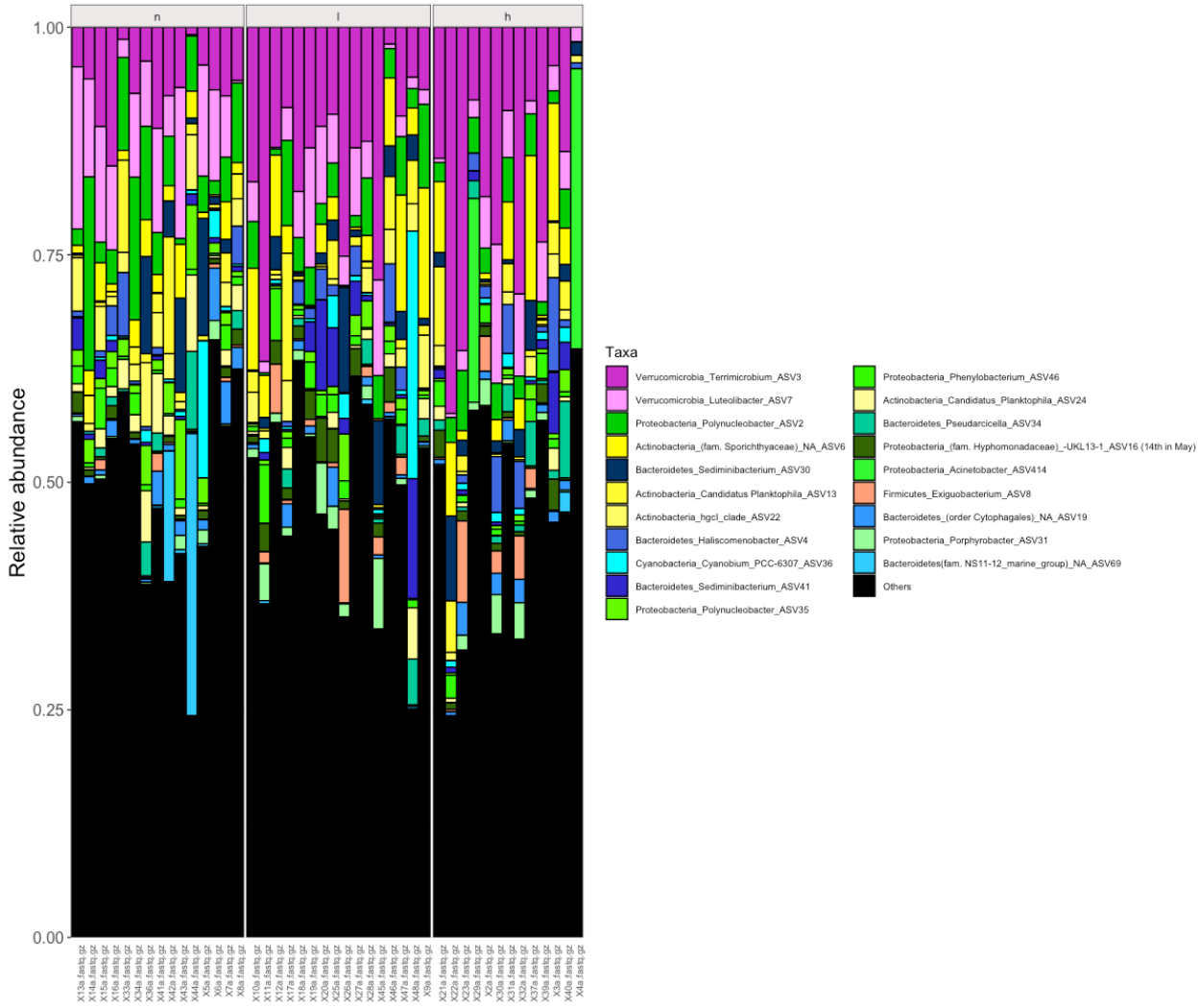

Figure 10A. Relative abundance of the top 20 most abundant bacterial ASVs in August across tanks (n=44) with different dispersal levels represented by the panels. Panels abbreviations stand for: n – background (no active dispersal), l – low, and h - high. Each colour is unique to an individual ASV.

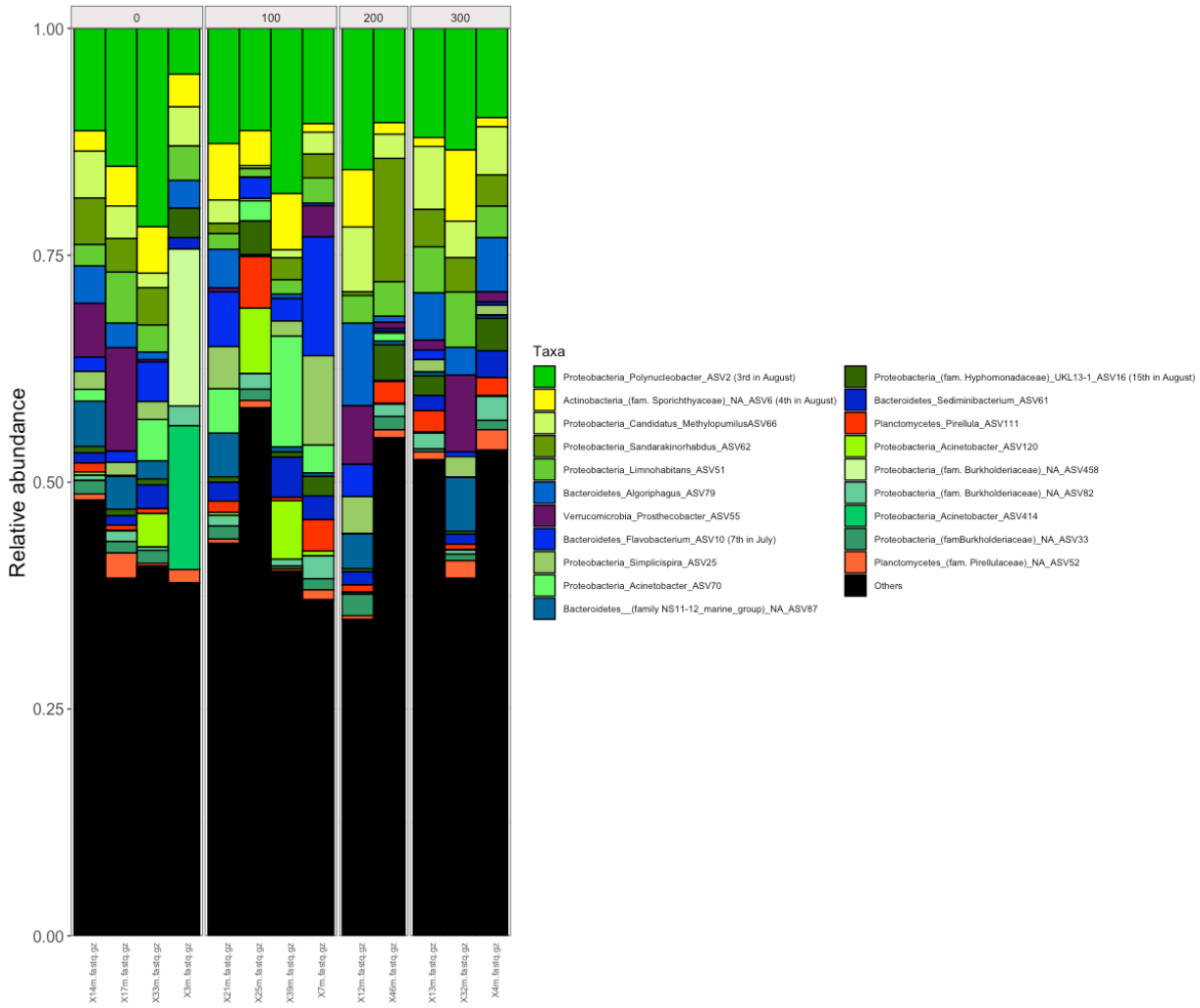

Figure 11A. Relative abundance of the top 20 most abundant bacterial ASVs in May across tanks (n=13) with different dispersal levels represented by the panels. Panels abbreviations stand for: n – background (no active dispersal), l – low, and h - high. Each colour is unique to an individual ASV.

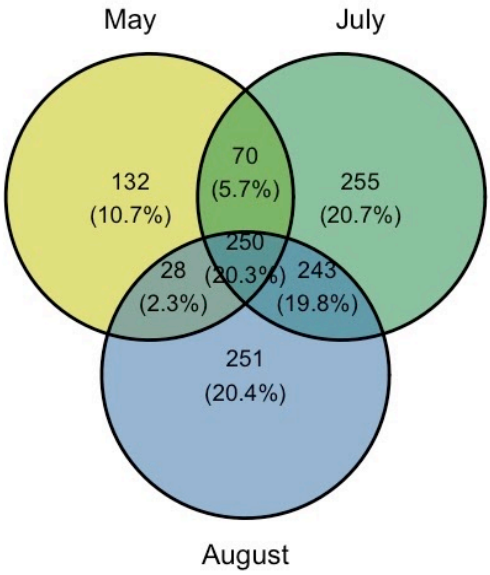

Figure 12A. Venn Diagram showing proportion of variation in bacterial community structure explained by sampling time (month).