Warming-enhanced priority effects at population and community levels in aquatic bacteria

Máté Vass*, Anna J. Székely, Eva S. Lindström, Omneya A. Osman, Silke Langenheder

Department of Ecology and Genetics/Limnology, Uppsala University, Norbyvägen 18 D, 75236 Uppsala, Sweden

*Corresponding author contact information: mate.vass@ebc.uu.se

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Abstract

The immigration history of communities can profoundly affect community composition. For instance, early-arriving species can have a lasting effect on community structure by reducing the immigration success of late-arriving ones through priority effects. Warming could possibly enhance priority effects by increasing growth rates of early-arriving species. Here we implemented a full-factorial experiment with aquatic bacteria where both temperature and dispersal rate of a better adapted community were manipulated to test their effects on the importance of priority effects. Our results suggest that priority effects might be strengthened by increasing temperature as warming increased the resistance of recipient communities against dispersal, and decreased the relative abundance of successfully established late-arriving species. However, warming-enhanced priority effects were not always found and their strengths differed between recipient communities and dispersal rates. These findings highlight the importance of context dependent studies of priority effects.

Introduction

Variation in the composition of ecological communities can be the product of historical processes such as immigration, extinction and speciation [1]. The sequence and timing in which species or their propagules reach an ecological community (immigration history) can profoundly affect community structures and maintain diversity of communities via a process known as priority effects [2]. Priority effects imply that early-arriving species gain advantage and become resistant to invasion of late-arriving ones, and therefore maintain high relative abundances over time [3]. Priority effects are enforced by mechanisms that increase the ecological opportunity of the early-arriving species. For instance, factors such as time lag between arrivals, high growth and evolutionary rates of the early-arriving species (i.e., rapid local adaptation), are processes that reduce the establishment success of late-arriving species [4, 5]. Successful local adaptation by early-arriving species initiates strong priority effects that can even reduce the establishment success of late-arriving species that are otherwise well-adapted to the local environment [6]. On the other hand, priority effects can be absent or weak when the better adapted late-arriving species generate species replacements. In the latter case, dispersal initiates species sorting processes, which has been shown to occur even at very low rates of dispersal [7]. In general, strong priority effects are expected if growth rates and/or the adaptive potential of local communities are high in relation to the time it takes for better adapted species and/or dormant resident species to arrive or resuscitate and grow to become abundant community members [8].

The most likely reason for priority effects is that early-arriving species induce rapid niche-modification or preempt resources so that late-arriving species will not be able to successfully establish in a local community [9, 10]. Hence, any possible environmental factor that increases growth rates of early-arriving species could possibly enhance priority effects

[11, 12]. This includes the impact of ongoing climate change that leads to increased mean water surface temperatures [13]. For instance, a recent study by Grainger *et al.* (2018) has shown that priority effects can be temperature-dependent in aphid species under the projected IPCC temperature scenarios. In addition, results suggesting that warming might promote priority effects have been obtained from yeast [15] and zooplankton communities [16], but, in general, the number of studies on this topic is low.

Previous studies have demonstrated that organisms with high growth rates have the capability to facilitate strong priority effects [4, 9, 17], and therefore priority effects are expected to be more important in bacteria compared to other organism groups [5]. Results from several studies suggest that priority effects occur in a variety of aquatic bacterial communities [18–20], but their relationship to temperature remain unexplored. Furthermore, we lack knowledge about the identity of bacteria that are key players in either causing priority effects or being hindered in their establishment by them. Even though two recent studies aimed to identify distinct roles of a bacterial groups in priority effects during community succession in biofilm [21] and in experimental freshwater bacterial communities [19], it remains unclear whether the fates and roles of distinct bacteria in priority effects differ in response to warming.

Therefore, we performed a full-factorial experiment, where bacteria from three Swedish lakes were inoculated and grown in cell-free Baltic Sea medium at three temperature levels. These lake communities represented the early arrivals that were allowed to colonize the ‘foreign’ (Baltic Sea) medium to which they were not a priori adapted. After initial growth and establishment these communities became the ‘recipient communities’ that were exposed to invasion by Baltic Sea bacteria that were well-adapted to the incubation medium (i.e., Baltic Sea medium). These late-arriving communities were dispersed into the recipient communities at three different rates. We generated the recipient communities using three lake

inocula that differed in their proximity to the Baltic Sea, as we expected that the distance of the origin of the early- and late-arriving communities might alter priority effects. At the end of the experiment, bacterial community composition was analyzed and the potential priority effects were investigated both at the community and population level (see Methods).

We hypothesized that warming results in stronger priority effects in bacterial communities, namely, that recipient communities grown at high temperature will be more resistant to dispersal of late-arriving species from the Baltic Sea. Moreover, recipient communities that are geographically closer to the Baltic Sea might harbor larger internal seed banks containing Baltic Sea bacteria and share the regional species pool with Baltic Sea to a greater extent than lakes further apart. Hence, we assumed that lakes geographically closer to the Baltic Sea would be more prone to maintain priority effects than more distant lakes due to their higher ratio of species adapted to the applied Baltic Sea medium.

Material and methods

Experimental design

In total, our experimental design resulted in 132 communities, three sets of recipient communities, each with four replicates exposed to three levels of dispersal and three temperatures. For each temperature there was a dispersal source with four replicates and a control with four replicates consisting of cell-free medium (Fig. 1).

For the preparation of the Baltic Sea incubation medium used in this experiment, 120 L seawater was collected at the Swedish Baltic Sea coast on 19 June, 2018, at Barnens Ö (N 59°55'11.9", E 18°54'52.2"). The water was filtered through a 20 µm net *in situ* to remove zooplankton and kept in dark at 4 °C overnight. Then, the medium was autoclaved (121 °C

for 40 mins) and its pH was adjusted to its original level (pH = 8.18) by HCl addition. Afterwards, the medium was filtered through sterile 0.2 μm 47 mm membrane filters (Supor-200, Pall Corporation, Port Washington, NY, USA), and distributed into sterile 1,000 mL glass bottles, and autoclaved once more at 121 °C for 20 minutes in order to achieve a sterile cell-free incubation medium. Until inoculation, the bottles containing the sterile medium were kept in dark at 4 °C.

For the preparation of the inoculum communities, water samples were collected from three Swedish lakes (Lötsjön – N 59°51'44.0", E 17°56'37.6"; Erken – N 59°50'09.2", E 18°37'57.9"; and Grytsjön – N 59°52'21.1", E 18°52'53.6") and from the Baltic Sea (same location as above) on 4 July 2018 (Fig. S1). The distances of the three lakes from the Baltic Sea sampling location were 54.5 km (Lötsjön), 18.3 km (Erken) and 5.6 km (Grytsjön). The chemical characteristics of these lakes were slightly different, and altogether the concentrations of total carbon (TOC) and PO_4^{3-} increased, while NO_3^- decreased as lakes were located closer to the sea coast (Table S1). All samples were sequentially filtered to remove bacterial grazers, first through a 20 μm net *in situ* to remove zooplankton and then through GF/F filters (0.7 μm, Whatman, UK) prior inoculation to remove protozoans.

The dispersal source communities were established by inoculating 100 mL of GF/F filtered Baltic seawater into bottles containing 900 mL of cell-free Baltic Sea medium. The batch cultures were incubated at three different temperature levels (15, 20 and 25 °C) in the dark with four replicates at each incubation temperature. The established dispersal source communities were used in the dispersal treatments and represented the late-arriving species arriving at different rates (Fig. 1).

To create the recipient communities of early-arriving species 50 mL of GF/F filtered lake water inocula was added to bottles containing 450 mL cell-free Baltic Sea medium, and incubated at three different temperature levels (15, 20 and 25 °C) in dark with four replicates

126 at each incubation temperature. The incubation of recipient cultures was started with one day
127 delay so that cell abundance would most likely be lower compared to the dispersal sources, in
128 order to avoid strong dilution of the medium during the dispersal process (see below).
129
130 *Dispersal*
131 On day 7, after the successful establishment of recipient communities, measured as
132 bacterial growth (Fig. S2), bacteria from the dispersal source communities were added to the
133 recipient communities. The dispersal treatment consisted of one dispersal event at three
134 different rates: no, low and high, wherein 0 %, 5 % and 20 % of the cells were exchanged
135 with cells from the respective dispersal source (Fig. 1). For this, each replicate 'A' of the
136 three recipient communities at the different incubation temperatures received cells from
137 replicate 'A' of the dispersal source at the respective temperature level. Likewise, each
138 replicate 'B' of the recipient communities received cells from replicate 'B' of the dispersal
139 source and so on (Fig. 1). For this, we measured the bacterial abundances (for details see
140 'Sample analyses' below) in all cultures and calculated the volume that needed to be replaced.
141 To reach an equal final volume (564 mL) in all cultures the differences were compensated by
142 adding additional cell-free incubation medium that was kept at the same conditions
143 throughout the entire experiment. One 'additional medium' bottle (kept at 20 °C), broke
144 during the experiment, hence, a mixture of the two other medium bottles (kept at 15 and 25
145 °C) were used after the dispersal treatments to reach equal volume in each incubation bottle.
146 Both the cell exchange and the supply of additional medium were carried out under sterile
147 conditions.
148
149 *Sample analyses*

Throughout the experiment, bacterial abundance was monitored (Fig. S2) using a CytoFLEX flow cytometer (Beckman Coulter, Indianapolis, IN, USA) with 2.27 μM of SYTO 13 fluorescent nucleic acid stain (Invitrogen, Eugene, Oregon, USA).

To follow changes in environmental conditions in the cultures, samples for chemical analyses were collected three times: on day 1 after lake inocula were distributed into the medium, after the dispersal treatment (day 7), and on the last day of the experiment (day 22). Total phosphorus (TP), total nitrogen (TN) and total carbon (TOC) were measured spectrophotometrically (Perkin Elmer, Lambda 40, UV/VIS Spectrometer, Massachusetts, USA) and by catalytic thermal decomposition method (Shimadzu TNM-L, Kyoto, Japan), respectively according to standard procedures. Further, ion chromatograph was used to measure the concentrations of NH_4^+, NO_3^-, PO_4^{3-} as described previously [22].

Bacterial community composition

At the end of the experiment (day 22), the cultures (564 mL) were filtered by vacuum filtration onto 0.2 μm 47 mm membrane filters (Supor-200, Pall Corporation, Port Washington, NY, USA). DNA extraction from the membrane filters was performed using the DNeasy PowerSoil Kit (Qiagen, Venlo, Netherlands). The 16S rRNA gene amplicons were prepared using a two-step PCR protocol described in detail in the protocol deposited to the protocols.io repository (dx.doi.org/10.17504/protocols.io.6jmhck6). Amplicon paired-end sequencing was performed on Illumina MiSeq platform at the SciLifeLab SNP&SEQ Technology Platform hosted by Uppsala University, using Illumina MiSeq v3 sequencing chemistry. Raw sequences have been deposited to the European Nucleotide Archive with the accession number PRJEB34383.

Sequences were processed using DADA2 pipeline [23] in R on the server of Uppsala Multidisciplinary Center for Advanced Computational Science (UPPMAX). First, forward

and reverse sequences were trimmed to 280 and 220 bp long, respectively, after quality filtering (truncQ = 2) with maximum expected errors set to 2 and 5 for forward and reverse sequences, respectively. Secondly, sequences were dereplicated and sequence variants were inferred. Finally, chimeric sequences were removed and the final amplicon sequence variants (ASVs) were assigned against SILVA 132 core reference alignment [24].

Data analyses

All statistical analyses and visualizations were conducted in R version 3.3.2 [25]. The ASV table was analyzed using the packages 'phyloseq' [26] and 'vegan' [27]. Chloroplast ASVs and unassigned ASVs were discarded. Samples were rarified to an even depth of 6,366 reads per sample that eventually resulted in an ASV matrix with 5,598 ASVs in 120 samples. The taxonomic distribution of reads was visualized with Krona (http://sourceforge.net/projects/krona).

Principal component analysis was applied to assess if dispersal treatments induced any differences in nutrient concentrations during the experiment, including original, unfiltered lake and Baltic Sea water samples as references. Differences in bacterial abundance in dependence of temperature and the origin of the recipient community was tested using a two-way ANOVA and a subsequent Tukey's HSD test.

Differences in community composition among samples were tested with permutational multivariate analysis of variance (PERMANOVA, permutations: 999) using the *adonis* function in 'vegan' package [27] and visualized using non-metric multidimensional scaling (NMDS), both based on the abundance-based Bray-Curtis dissimilarities. In the absence of priority effects, the well-adapted late-arriving bacteria from the Baltic Sea dispersal source should outcompete the originally maladapted early-arriving lake bacteria of the recipient community. Hence, the composition of the recipient communities would converge completely

towards the dispersal source. On the opposite, we assumed the presence of priority effects when recipient community maintained a significant dissimilarity compared to the dispersal source. To assess to what extent the recipient community shifted towards the dispersal source as a measure of the strength of priority effects, we first calculated the Bray-Curtis dissimilarity between each recipient community and its respective dispersal source. Our assumption was that priority effects are the stronger the higher the dissimilarity between recipient communities and the dispersal source. In case of complete priority effects at the level of the entire community, the recipient community should completely maintain its dissimilarity from the dispersal source. Finally, to be able to specifically address how dispersal influenced the strength of potential priority effects, we subtracted the Bray-Curtis dissimilarities between the recipient and dispersal source in the dispersal treatments with that of the respective 0 % dispersal treatment. This was done to correct for shifts in community composition that occurred in the recipient communities in the absence of dispersal.

Priority effects at the population level were investigated by determining the relative abundance of early-arriving ASVs of the recipient communities that persisted after exposure to dispersal, and late-arriving ASVs of the dispersal source that established successfully in the recipient communities. For this, we identified ASVs that fall in the above-mentioned categories by performing differential abundance analyses at each temperature level using the 'DESeq2' package [28]. First, we selected the most abundant ASVs (> 0.5 % relative abundance) in each recipient community and the dispersal source. Then, we determined separately for each recipient community if the relative abundances (as a proxy for population size) of all abundant early-arriving ASVs changed after the effective dispersal treatment (i.e., 5 % and 20 % dispersal rate treatments) compared to their relative abundances in the no dispersal (0 %) communities. Here, we interpreted the lack of significant (adjusted $p < 0.05$) negative differences in relative abundances as a sign of priority effects and grouped them as

'persistent early-arriving ASVs'. On the other hand, if their relative abundances were significantly lower (adjusted $p < 0.05$) in treatments receiving dispersal from the Baltic Sea, we categorized them as 'forfeited early-arriving ASVs'. Second, for the late-arriving ASVs from the Baltic Sea dispersal source, we performed a conservative mixing analysis following Székely & Langenheder (2017). For this, we calculated for the most abundant ASVs' (> 0.5 % in the dispersal source) their expected relative abundances in the dispersal rate treatments based on their relative abundances in the 0 % dispersal rate treatment and the dispersal source, and the applied cell exchange rates (i.e., 5 or 20 %). Thereafter we assessed the deviation of the measured abundances from the expected values. A non-significant deviation or a significantly (adjusted $p < 0.05$) higher relative abundance of a late-arriving ASV compared to the expected one provides a sign of successful establishment, while a significantly (adjusted $p < 0.05$) lower abundance indicates unsuccessful establishment of the late-arriving ASVs.

Finally, we used two-way ANOVAs to test how temperature and the origin of the recipient communities (inoculum origin) affected the community shift induced by dispersal and the relative abundance of persistent early-arriving ASVs and successfully established late-arriving ASVs., respectively.

Results

After the initial inoculation of early-arriving bacteria in the Baltic sea medium all recipient communities showed typical growth patterns of dilution cultures and increased in abundance at least until day 7 (Fig. S2). The temperature increase (i.e., 20 and 25 °C) resulted in significantly higher abundances on day 7 (two-way ANOVA, $F_{Temperature} = 76.09$, $p < 0.001$;

post-hoc Tukey's HSD test: $p_{adjusted} < 0.05$, Table S2), further, significantly differences by inoculum origin ($F_{Inoculum\ origin} = 79.01$, $p < 0.001$; $F_{Temperature \times Inoculum\ origin} = 7.86$, $p < 0.001$). Bacterial abundances remained stable throughout the experiment in all treatments. Despite some initial variation, the chemical conditions of the cultures inoculated with different recipient communities did not experience any pronounced shift or showed clustering patterns in response to the dispersal treatments (Fig. S3A) or to the different incubation temperatures (Fig. S3B).

The NMDS of the bacterial communities (Fig. 2) shows that without dispersal (i.e., 0 % dispersal rate) all three recipient communities (Lötsjön, Erken and Grytsjön) were compositionally different (Fig. 2 orange dots; PERMANOVA: $F_{Inoculum\ origin} = 9.96$, $R^2 = 0.35$, $p = 0.001$), and were affected by the temperature manipulation ($F_{Temperature} = 3.02$, $R^2 = 0.11$, $p = 0.001$). Meanwhile, the recipient communities exposed to dispersal (i.e., 5 and 20 % dispersal rate, brown and black dots) became more similar to the dispersal source (Fig 2; blue dots).

PERMANOVA results showed that the recipient communities exposed to dispersal were significantly dissimilar from the dispersal source in all cases (Table S3), thus, complete convergence to the dispersal source (i.e., complete absence of priority effects) did not occur in any of the communities. We found a similar pattern when assessing the degree of dissimilarity of recipient communities from the dispersal source, namely, that recipient communities receiving either 5 % or 20 % dispersal shifted towards the dispersal source, without a complete convergence (Bray-Curtis dissimilarity = 0), compared to the 0 % dispersal (Fig. 3). Recipient communities with the highest dispersal (i.e. 20 %) shifted the most towards the dispersal source (Fig. 3). When Bray-Curtis dissimilarities were calculated in relation to the 0 % dispersal treatment to assess the degree of community shift induced by dispersal (as a

proxy for the strength of the priority effects), the shift was greater in the 20 % dispersal compared to 5 % dispersal rate treatment (Fig. 4). Both temperature and the inoculum origin of the recipient community had a significant effect on this relationship at 5 % dispersal (two-way ANOVA, $F_{Temperature}$= 5.97, p = 0.006, $F_{Inoculum\ origin}$= 5.76, p = 0.007), but not at 20 % dispersal (two-way ANOVA, $F_{Temperature}$ = 1.99, p = 0.153, $F_{Inoculum\ origin}$ = 0.33, p = 0.724) (Fig. 4). This indicates that the strength of priority effects was affected by temperature and recipient community origin at 5 % dispersal but not at 20 %. There were no significant interactions between temperature and inoculum origin in any of the dispersal rates tested.

We further examined changes in the dynamics of early- and late-arriving ASVs in response to temperature changes. On a broad taxonomical level, we found that the most abundant (> 0.5 % relative abundance) bacterial ASVs in the early-arriving communities belonged to the class Alphaproteobacteria, Gammaproteobacteria and Bacteroidia (Fig. S4). The most abundant genera (top three) were *Brevundimonas*, *Pseudomonas*, *Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium* (thereafter *A-N-P-R*) in the Lötsjön and Erken recipient communities and *Limnobacter*, *Algoriphagus* and *A-N-P-R* in the Grytsjön recipient communities (Fig. S4). The most abundant (> 0.5% relative abundance) members of the dispersal source communities (i.e., late-arriving bacteria) were ASVs belonging to Alphaproteobacteria (mainly *Loktanella*, *A-N-P-R* and *Roseibacterium*), Gammaproteobacteria (mainly *Hydrogenophaga*, *Pseudomonas*, *Rheinheimera*) and Bacteroidia (mainly *Algoriphagus*) (Fig. S4, Baltic Sea).

Differential abundance analyses revealed numerous ASVs of the most abundant genera (> 0.5 %) that could be classified as either ‘persistent’ or ‘forfeited’ early-arriving ASVs or did undergo an (un)successful establishment as late-arriving ASVs (see Methods). We identified several persistent early-arriving ASVs that taxonomically differed between the

three recipient communities (Fig. 5, Fig. S5). Specifically, in the Lötsjön recipient communities, ASVs of *A-N-P-R*, *Brevundimonas*, *Pseudomonas* and *Rheinheimera*; in Erken recipient communities, ASVs of *Flavobacterium*, *A-N-P-R*, *Brevundimonas*, *Pseudomonas*, *Rheinheimera*, *Hydrogenophaga* and *Novosphingobium* were persistent, while in Grytsjön recipient communities, ASVs of *A-N-P-R*, *Sphingorhabdus*, *Limnobacter* and *Rheinheimera* showed persistence (i.e., did not show significant ($p_{adjusted} < 0.05$) changes in relative abundance in the dispersal treatments) (Fig. S5). However, there were also inconsistences because ASVs from the same genera (e.g. *Pseudomonas*, *Flavobacterium* and *Rheinheimera*) could be categorized both as forfeited and persistent early-arriving bacteria. Interestingly, changes in the composition of persistent early-arriving ASVs were found as the temperature level increased. For example, there was a general trend showing that *Flavobacterium* and *Rheinheimera* were more persistent at lower temperature (15 °C and 20 °C) than at the highest temperature level (25 °C). In contrast, *Brevundimonas* and not abundant members of Bacteroidia (i.e. *Comamonas*, *Curvibacter*; grouped as 'other_Bacteroidia' in Fig. 5) tended to be more abundant and persistent at higher temperatures. Temperature and inoculum origin had no effect on the total relative abundance of the persistent early-arriving ASVs in the recipient communities with different dispersal treatments (two-way ANOVA at 5 % dispersal: $F_{temperature} = 2.38$, $p = 0.109$, $F_{inoculum\ origin} = 1.95$, $p = 0.159$; 20 % dispersal: $F_{temperature} = 0.74$, $p = 0.488$, $F_{inoculum\ origin} = 0.59$, $p = 0.562$, no significant interactions in either case) (Fig. 5).

Among late-arriving bacteria, mainly ASVs of *Algoriphagus*, *Loktanella*, *Roseibacterium*, *Hydrogenophaga* were the ones that showed successful establishment, thus, maintained or significantly increased (adjusted $p < 0.05$) their relative abundances after being dispersed into recipient communities (Fig. 6, Fig. S6). The composition of successfully established late-arriving bacteria was similar regardless of the recipient communities into which they were dispersed. Their total relative abundances decreased with warming in both

the 5% and 20 % dispersal treatments and differed between recipient communities with different inoculum origin (two-way ANOVA at 5 % dispersal: $F_{temperature}$ = 7.80, p = 0.002, $F_{inoculum\ origin}$ = 3.83, p = 0.033; at 20 % dispersal: $F_{temperature}$ = 7.34, p = 0.002, $F_{inoculum\ origin}$ = 4.79, p = 0.015, no significant interactions in either case) (Fig. 6). Among the populations most impacted by warming were *Loktanella*, *Hydrogenophaga* and *Pseudomonas*, thus, showed decrease in relative abundance at higher temperature levels (20 and 25 °C) (Fig. 6, Fig. S6).

Discussion

Our study shows that warming has the potential to promote priority effects, but that it depends (i) on the rate of dispersal of late-arriving better adapted communities into recipient communities and (ii) on the composition of the recipient community. More specifically, and in agreement with previous studies that have used similar approaches to ours [18, 19], we found evidence of priority effects in aquatic bacterial communities. We also found that dispersal of the late-arriving bacteria induced some species replacement, i.e. decreased priority effects, because all recipient communities converged towards the dispersal source communities to some extent. However, warming could reduce such effects of dispersal, even though this effect varied among recipient communities (Fig. 4).

Dispersal events in bacterial communities from nature are complex and involve mixing or coalescence of entire communities [29], which we tried to mimic in our study. Therefore, it is difficult to relate our results to previous studies that have primarily focused on priority effects related to differences in the assembly history of individual species or strains [1]. Thus, in this study we aimed to upscale the number of studied species involved in priority

effects compared to previous studies by investigating the roles of the members of complex communities. Our findings represent, to our knowledge, the first experimental evidence that temperature-dependency of priority effects can occur in pelagic bacterial communities wherein different bacterial groups are involved in different ways. The warming effect could be seen at the population level since less successful establishment of late-arriving ASVs were found in the recipient communities in response to increasing temperature. Specifically, in the case of the recipient communities, the total relative abundance of successfully established ASVs generally decreased with increasing temperature, whereas the relative abundance of persistent early arriving ASVs tended to show the opposite trends, even though this was not significant in any case.

One possible explanation for the lower establishment success and stronger persistence of resident species at higher temperatures is that the resistance of recipient communities to invasion (dispersal) by late-arriving bacteria increased as a result of temperature-stimulated high growth rates of the early-arriving bacteria (see Fig. S2 and Table S2). Similarly, Grainger *et al.* (2018) demonstrated that increased temperature increased growth rates of aphid species, thus, allowing them to more rapidly change and deplete resources which altogether increased the competitive exclusions of competitor species that arrived late. In our experiment, such effects appeared to be generally stronger at 5 compared to 20 % dispersal rates. This highlights that dispersal rates are an important mediator of the strength of priority effects in natural communities, and that this strength in general is likely to be higher if dispersal rates are relatively low [6].

We identified several persistent early-arriving ASVs that taxonomically differed between the three sets of recipient communities. Hence, distinct sequence variants (ASVs) of early-arriving bacteria played a role in maintaining priority effects. However, we also found that there can be inconsistencies at the genus level in the response to dispersal of different

sequence variants as ASVs belonging to the very same genus (e.g., *Pseudomonas*, *Flavobacterium* and *Rheinheimera*) can be categorized both as persistent and forfeited ASVs. This corroborates results of other recent studies [30, 31] that emphasize the importance to evaluate population level dynamics at the deepest taxonomical resolution possible. Moreover, the composition of persistent early-arriving ASVs differed between the different temperature treatments, suggesting that, as temperature conditions change, the identity of bacterial populations that maintain priority effects changes as well. On the other hand, in the case of the dispersal source community, we found consistency in the identity of the successfully established late-arriving ASVs as, irrespective of the identity of the recipient community or the temperature treatment, they typically belonged to the same genera. In summary, our findings suggest that different species can be involved in the development of priority effects of aquatic bacterial communities under different circumstances. Since similar results have been obtained in a number of studies [1, 9, 19, 21] of different complexity, our results suggest that species' responses to invasion at the community level are difficult to predict.

The lake inocula included for the preparation of the recipient communities in this study differed in their geographical distance to the dispersal source (the late-arriving bacteria from the Baltic Sea). Therefore, we presumed that recipient communities closer to the Baltic Sea might have been exposed to dispersal from the Baltic Sea in their recent history to a greater extent than those farther from the Baltic. This could have resulted in a larger shared species pool, including larger numbers of bacteria of Baltic Sea origin in local lake seed banks [32]. We therefore presumed that the potentially higher numbers of species that are adapted to environmental conditions in the Baltic Sea in recipient communities closer to the Baltic would lead to stronger priority effects. Our results do, however, not support this idea because the dissimilarities between the recipient and dispersal source communities were similarly high in

all cases (see Fig. 3). Hence, the results do not support our hypothesis of stronger priority effects in lakes closer to the Baltic Sea. Further, it suggests that the shared species pools with the Baltic Sea, including local seed banks of Baltic Sea taxa, were equally low irrespective of the distance of the lake to the Baltic Sea. There were nevertheless differences among lakes regarding the pattern of how temperature affected dispersal-induced shifts in community composition at the community level and the total relative abundance of persistent early arriving ASVs and successfully established late ASVs. These differences might be the consequence of the differences of chemical characteristic of the three lakes, or the result of intrinsic differences in traits (e.g. temperature optima) of ASVs that contribute to priority effects in the different lakes that we cannot disentangle in our study.

Priority effects can be due to two distinct mechanisms: niche-modification and niche preemption [2], but providing insights into the mechanisms underlying priority effects is difficult. In our experiment niche modification-driven priority effects of the different lake inocula should have influenced the identities of the successfully established late-arriving bacteria, which was, however, not the case. On the contrary, niche preemption might have had an influence in communities grown at increased temperatures (20 and 25 °C) as communities had attained higher abundances at the time when the dispersal source was added, while the establishment success of late-arriving ASVs decreased with increasing temperatures (see Fig. 6). This indicates that the availability of resources was probably reduced to such an extent in 20 and 25 °C treatments that this limited the abundances of late-arriving bacteria.

In addition to warming and dispersal there might be other factors that can influence the importance of priority effects in natural bacterial communities. For instance, it remains unclear what would happen in the presence of predation (e.g., bacterial grazers) or multi-level

trophic interactions. A few previous studies on zooplankton communities suggested that predation can be an important factor and can either reduce priority effects [16, 33], or, in contrast, indirectly promote them [34]. However, we lack a comprehensive knowledge on how predation could affect priority effects in particular in microbial communities. Another aspect that need to be considered are temperature fluctuations that can promote the immigration success of dispersed species and maintain multiple species coexistence, thus, reducing historical contingency [9, 35, 36].

Organisms across multiple kingdoms might be negatively affected by global warming, altering their ecosystem functions [11, 37–39]. Temperature has been shown to stimulate microbial invasions (e.g., spread of vibrios; Vezzulli *et al.* 2012) and influence the biogeographical patterns of microbes [41]. However, priority effects could play an important role by dampening the establishment success of invasive bacteria [42]. Our experimental study shows that this can be the case also in aquatic bacterial communities where early-arriving species, through priority effects, may to some extent be more resistant to invasion of late-arriving bacteria from an external dispersal source at higher temperatures. However, our findings also highlight that the overall strength of warming-enhanced priority effects is context dependent and differs depending on the composition of early-arriving communities as well as dispersal rates of the late-arriving ones.

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Conflict of interest

The authors declare that they have no conflict of interest.

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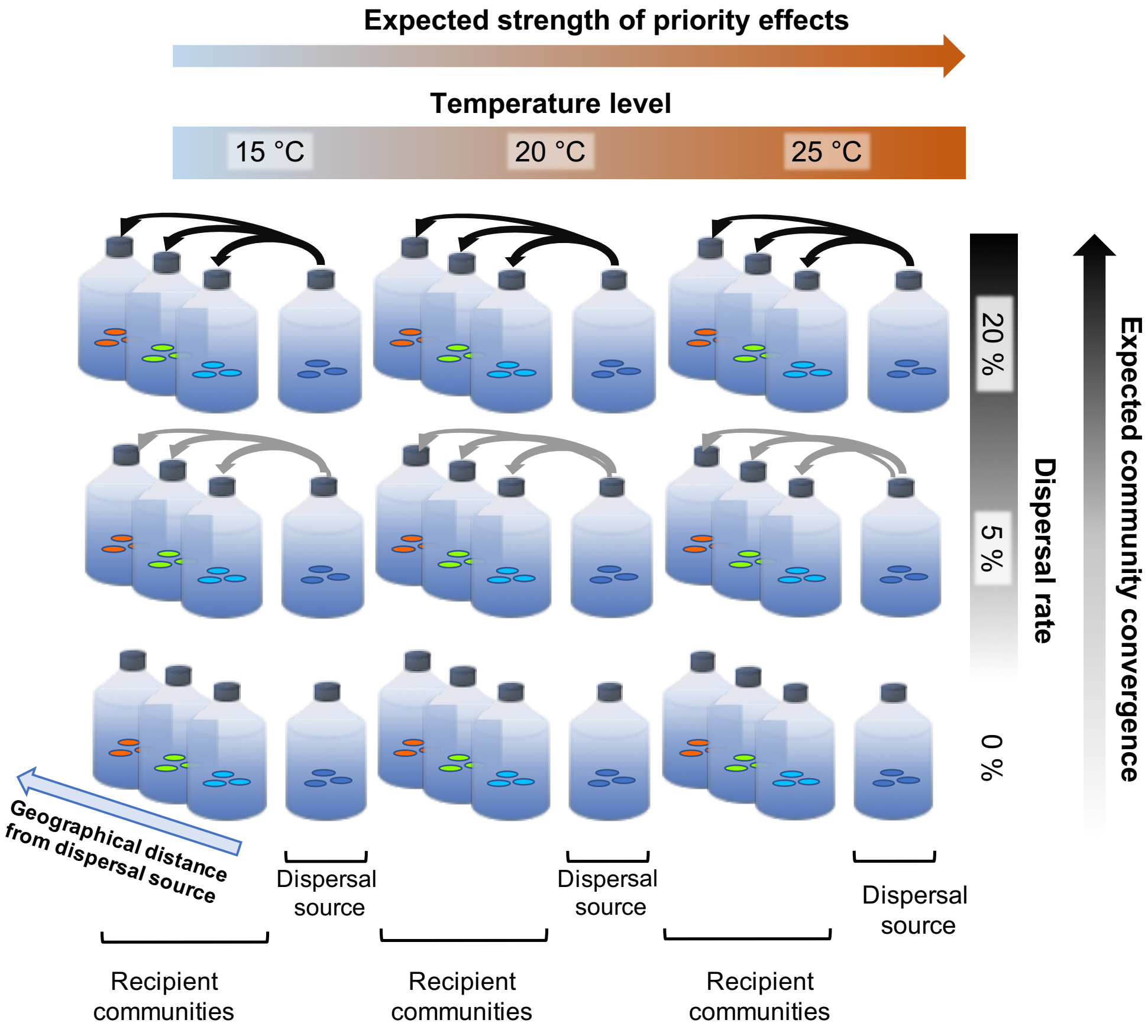
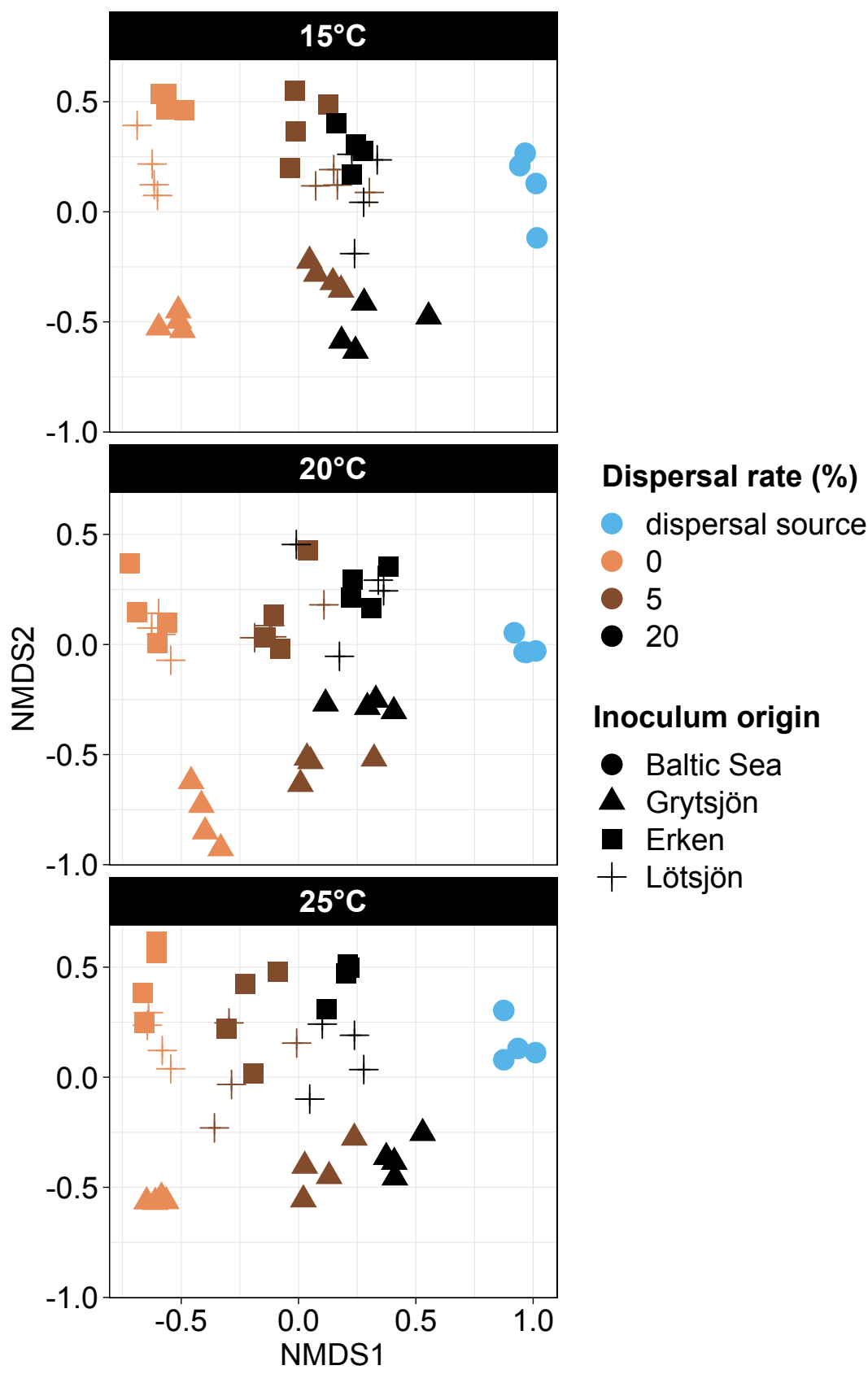
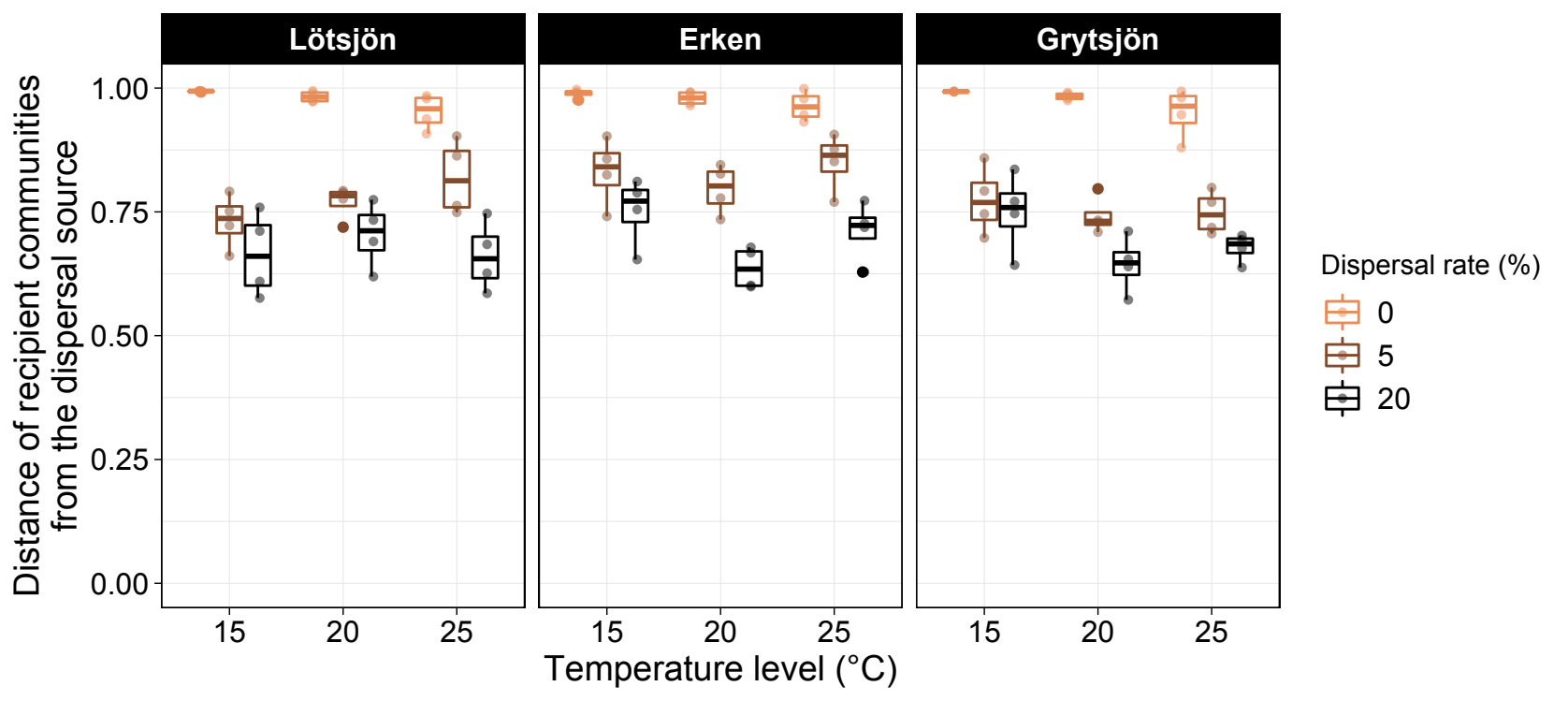
564

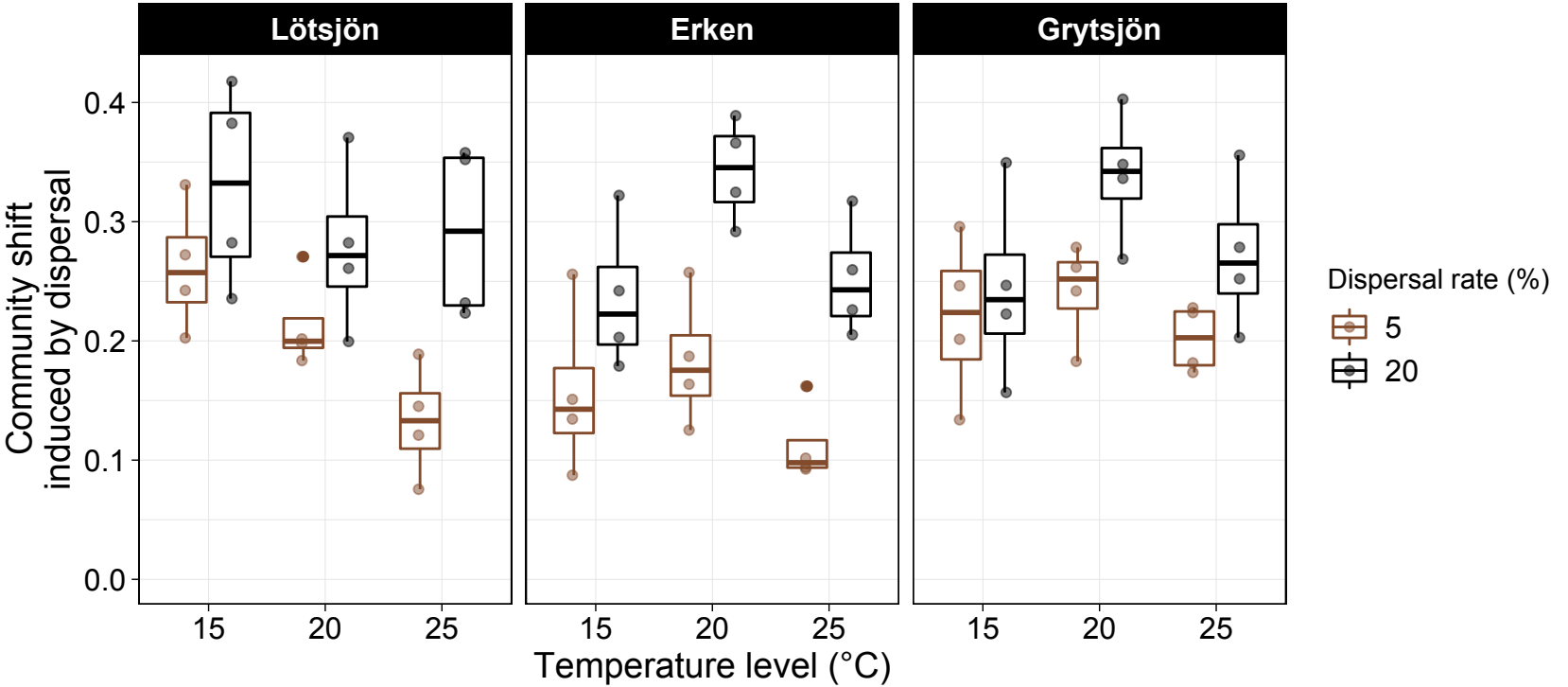
565 **Figure 1.** Experimental design of the study. The recipient communities were comprised of
566 three different lake inocula (Erken, Lötsjön or Grytsjön, indicated by the different cell colors)
567 inoculated separately into 'foreign' Baltic Sea incubation medium. The three lake inocula
568 differed in their geographical distance from the Baltic Sea, with Grytsjön (in blue) being
569 closest and Lötsjön (red) farthest away. The dispersal source constituted of the Baltic Sea
570 community (dark blue cells) inoculated into cell-free incubation medium. Both the recipient
571 (early-arriving species) and the dispersal source (late-arriving species) communities were
572 incubated at three different temperatures (15, 20 and 25 °C). Three different dispersal
573 treatments (cell exchange) were applied by replacing 0 %, 5 % and 20 % cells in the recipient
574 communities with cells from the dispersal source. Black and grey arrows represent the
575 direction of the dispersal treatments. The experiment was carried out in four replicates for all
576 treatments and for all recipient and dispersal communities. The recipient communities were
577 always dispersed with the corresponding dispersal source replicate at the respective
578 temperature level. Community convergence induced by dispersal was tested by measuring
579 Bray-Curtis community dissimilarities between recipient and dispersal source communities as
580 an indicator of the strength of priority effects: the less the communities converge toward the
581 dispersal source, i.e., the more resistant recipient communities are against dispersal, the
582 stronger are priority effects. The main hypothesis to be tested is that the strength of priority
583 effects increases with temperature.
584
585 **Figure 2.** Non-metric multidimensional scaling (NMDS) plots derived from abundance-based
586 Bray-Curtis dissimilarities of bacterial community composition at the three temperature levels
587 by the end of the experiment. Note that cultures with Baltic Sea inoculum were used as the
588 dispersal source, while cultures with lake inocula (Grytsjön, Erken and Lötsjön) were used as
589 recipient communities. All cultures were grown in Baltic Sea medium. Symbols are shaped

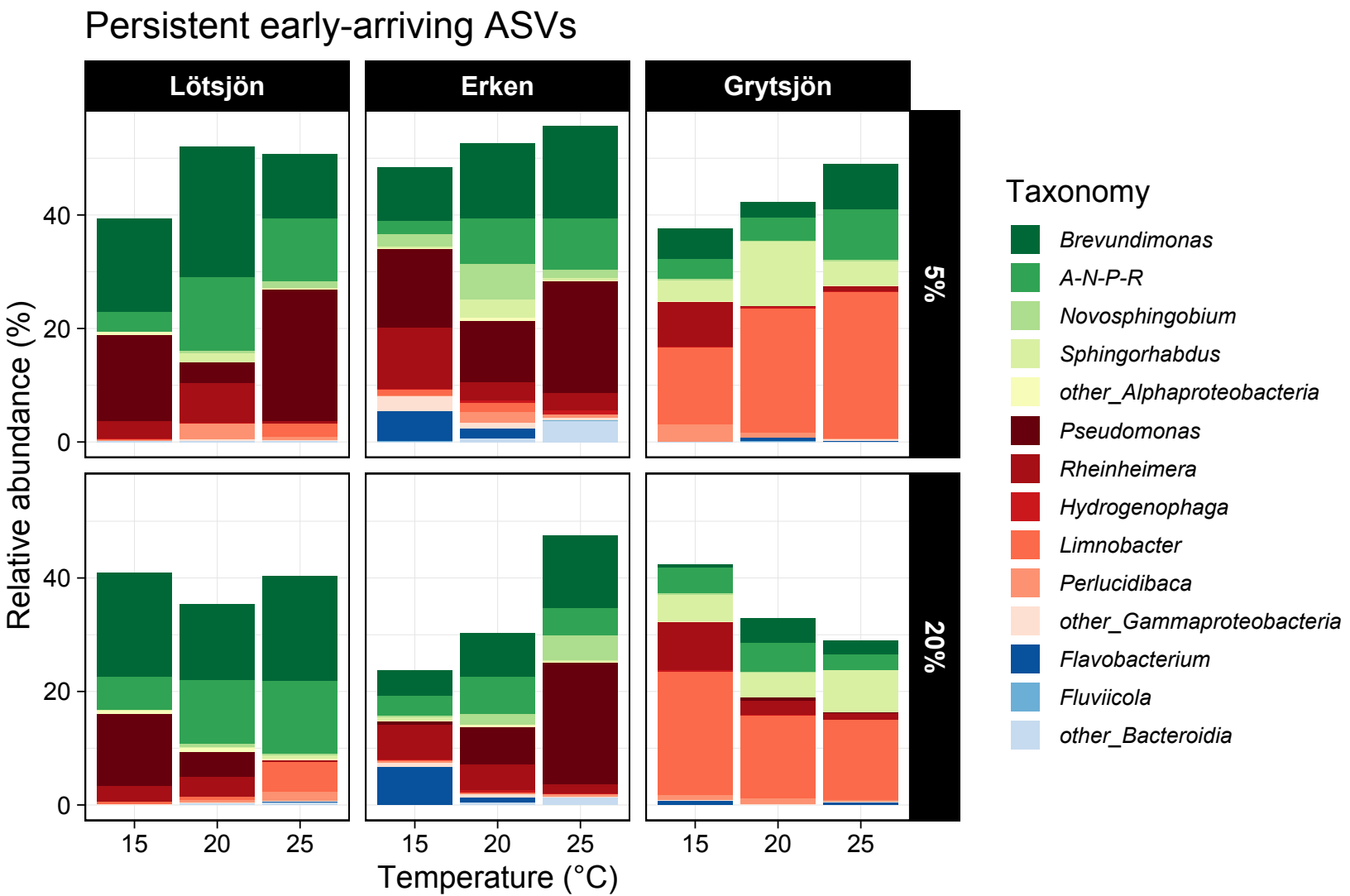
590 and colored by inoculum origin and dispersal treatment, respectively. Goodness of fit (stress
591 value): 0.105.
592
593 **Figure 3.** Distance of recipient communities from the dispersal source (late-arriving species)
594 communities (based on Bray-Curtis dissimilarity) at different temperature levels. Recipient
595 communities were exposed to either 0, 5 or 20 % dispersal (cell exchange) from the dispersal
596 source.
597
598 **Figure 4.** Dispersal-induced shifts in community composition in relation to temperature.
599 Differences in Bray-Curtis dissimilarities between recipient communities and the dispersal
600 source in the 5 and 20% dispersal treatments in relation to the 0 % dispersal treatment.
601
602 **Figure 5.** Changes in the relative abundances of persistent early-arriving species (ASVs > 0.5
603 % relative abundance) in the different dispersal (5 % or 20 %) and temperature treatments
604 (15, 20 and 25 °C). ASVs are grouped by bacterial genus and were identified by differential
605 abundance analysis (see Methods for the assessment procedure and Figure S6 for further
606 results). *A-N-P-R* refers to the genus *Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium.*
607
608 **Figure 6.** Changes in the relative abundances of successfully established late-arriving species
609 (ASVs, > 0.5 % relative abundance) in the different dispersal (5 % or 20 %) and temperature
610 treatments (15, 20 and 25 °C). ASVs are grouped by bacterial genus and were identified by
611 differential abundance analysis (see Methods for the assessment procedure and Figure S7 for
612 further results).
613

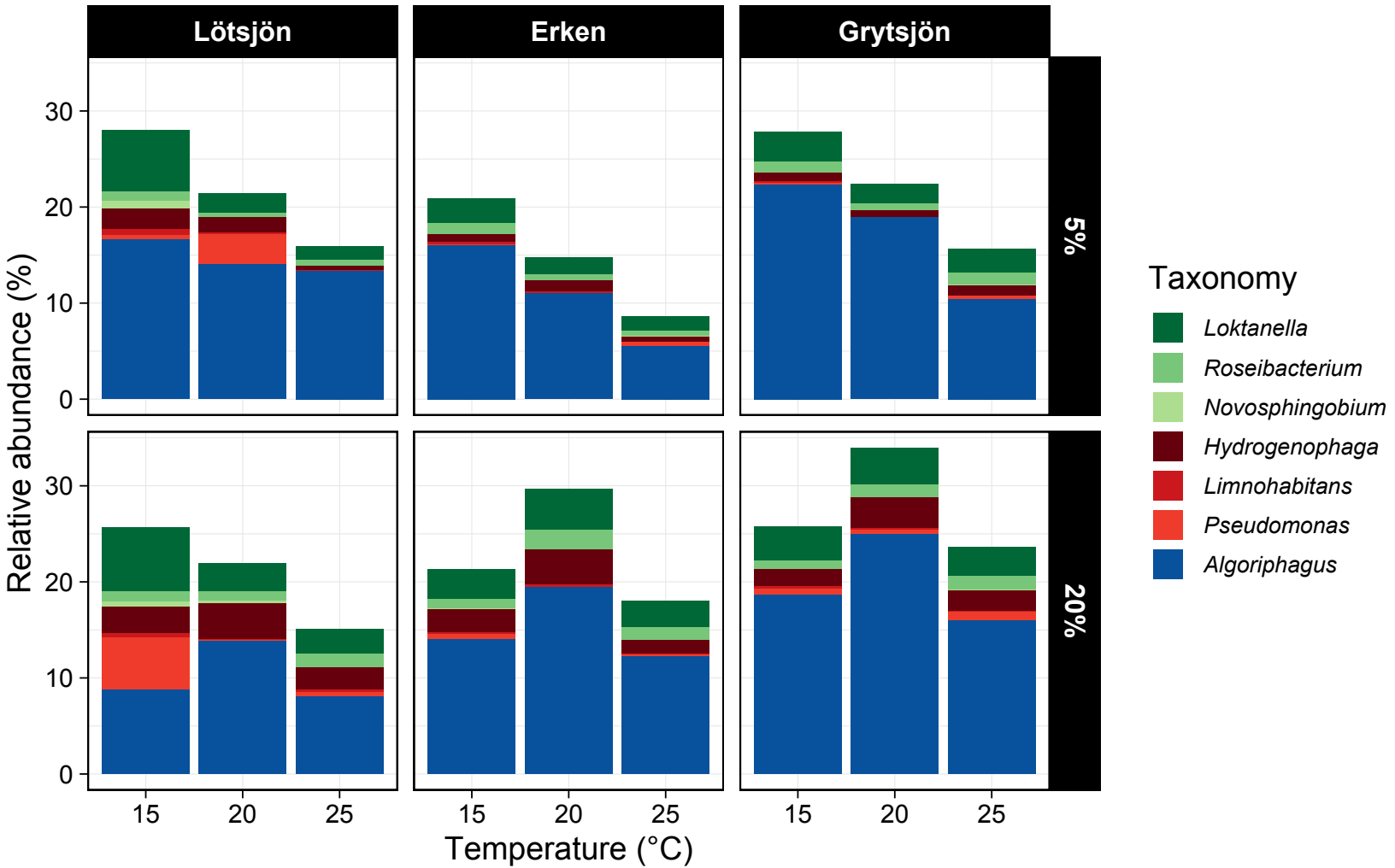

Expected strength of priority effects
Temperature level
15 °C
20 °C
25 °C
20 %
5 %
0 %
Dispersal rate
Expected community convergence
Geographical distance from dispersal source
Dispersal source
Dispersal source
Dispersal source
Recipient communities
Recipient communities
Recipient communities


15°C
20°C
25°C
0.5
0.0
-0.5
-1.0
NMDS2
-0.5
0.0
0.5
1.0
NMDS1
Dispersal rate (%)
dispersal source
0
5
20
Inoculum origin
Baltic Sea
Grytsjön
Erken
Lötsjön


Lötsjön
Erken
Grytsjön
Distance of recipient communities from the dispersal source
1.00
0.75
0.50
0.25
0.00
15
20
25
Temperature level (°C)
Dispersal rate (%)
0
5
20


Lötsjön
Erken
Grytsjön
Community shift induced by dispersal
0.0
0.1
0.2
0.3
0.4
15
20
25
Temperature level (°C)
Dispersal rate (%)
5
20


Persistent early-arriving ASVs
Lötsjön
Erken
Grytsjön
5%
20%
Relative abundance (%)
0
20
40
15
20
25
Temperature (°C)
Taxonomy
Brevundimonas
A-N-P-R
Novosphingobium
Sphingorhabdus
other_Alphaproteobacteria
Pseudomonas
Rheinheimera
Hydrogenophaga
Limnobacter
Perlucidibaca
other_Gammaproteobacteria
Flavobacterium
Fluviicola
other_Bacteroidia


Successfully established late-arriving ASVs
Lötsjön
Erken
Grytsjön
5%
20%
Relative abundance (%)
0
10
20
30
15
20
25
Temperature (°C)
Taxonomy
Loktanella
Roseibacterium
Novosphingobium
Hydrogenophaga
Limnohabitans
Pseudomonas
Algoriphagus