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Warming mediates the resistance of aquatic bacteria to invasion during community coalescence

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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 invasion success of late-arriving ones through priority effects. This can be particularly important when early-arriving communities coalesce with another community during dispersal (mixing) events. However, the outcome of such community coalescence is unknown as we lack knowledge on how different factors influence the persistence of early-arriving communities and the invasion success of late-arriving taxa. Therefore, we implemented a full-factorial experiment with aquatic bacteria where temperature and dispersal rate of a better adapted community were manipulated to test their joint effects on the resistance of early-arriving communities to invasion, both at community and population level. Our 16S rRNA gene sequencing-based results showed that invasion success of better adapted late-arriving bacteria equaled or even exceeded what we

expected based on the dispersal ratios of the recipient and invading communities suggesting limited priority effects on the community level. Patterns detected at the population level, however, showed that resistance of aquatic bacteria to invasion might be strengthened by warming as higher temperatures (a) increased the sum of relative abundances of persistent bacteria in the recipient communities, and (b) restricted the total relative abundance of successfully established late-arriving bacteria. Warming-enhanced resistance, however, was not always found and its strengths differed between recipient communities and dispersal rates. Nevertheless, our findings highlight the potential role of warming in mitigating the effects of invasion at the population level.

Introduction

Variation in the composition of ecological communities can be the product of historical processes such as immigration, extinction and speciation (Fukami et al. 2007). The timing of immigrants' arrival (immigration history) can profoundly affect community structures and maintain diversity of communities in a landscape via a process known as priority effects (Fukami 2015). Priority effects imply that early-arriving species, through niche modification and/or resource depletion, gain advantage and become resistant to invasion of late-arriving ones, and therefore maintain high relative abundances over time (Lockwood et al. 1997, Rillig et al. 2015). Successful local adaptation by early-arriving species initiates priority effects that can reduce the establishment success of late-arriving species that are otherwise well-adapted to the local environment (Loeuille and Leibold 2008). On the other hand, priority effects can be absent or weak when late-arriving species generate species replacements. In the latter case, dispersal initiates species sorting processes even at very low rates of dispersal (Declerck et al. 2013), which eventually selects species that are better adapted to the given environment. All these processes can play an important role whenever mixing of communities (known as 'community coalescence') occurs (Rocca et al. 2020). Such community interchange events vary in the extent to which the environments of the coalescing communities are involved in the exchange, the mixing ratio of the communities, as well as temporal aspects of the event, which then all influence the resulting species establishments, exchanges and extinctions (Rillig et al. 2015). In cases where coalescence does not result in substantial environmental alteration and the mixing ratio is skewed towards the early arriving community (e.g., inflow of a stream into a lake or the movement of propagules on sea splash by wind) the outcome of the community coalescence is expected to be influenced by priority effects.

Previous studies have demonstrated that organisms with high growth rates have the capability to facilitate strong priority effects (De Meester et al. 2002, Peay et al. 2012, Lee et al. 2013, Tucker and Fukami 2014, Ruiz-González et al. 2015). Hence, any possible environmental factor that increases the abundance and growth of early-arriving species could possibly enhance priority effects, and thus increase community persistence and make the recipient communities resistant to further immigration (Chase 2010, Rudolf and Singh 2013). Therefore, priority effects are expected to be more important in species with short generation time (e.g., bacteria) (De

Meester et al. 2016). Aquatic bacteria are most likely continuously subject to priority effects due to mixing of waterbodies, for instance at river–lake interfaces. Two recent studies aimed to identify distinct roles of bacterial groups in priority effects during community succession in a biofilm (Brislawn et al. 2019) and experimental freshwater bacterial communities (Rummens et al. 2018). Brislawn at el. (2019) found that the majority of taxa had been replaced after a 56-day period of succession, thus were not persistent over time despite some indication of priority effects. In the study of Rummens et al. (2018), time lags in inoculation history resulted in priority effects but the responses of individual bacteria to immigration was diverse, meaning that the initial relative abundance of either the early-arriving or late-arriving bacteria could not predict the outcome of invasion, highlighting the unpredictability of a multispecies system. Focusing on macroorganisms, a recent study by Grainger et al. (2018) showed that warming increased the competitive exclusion of a late-arriving competitor and increased the importance of priority effects by aphids that arrived early.

The impact of ongoing climate change leads to increased mean water surface temperatures (IPCC 2014) that can increase the growth rates of aquatic organisms. However, microorganisms are generally understudied in climate change-context studies (Cavicchioli et al. 2019) and there is, to our knowledge, not a single study investigating the effects of warming on the outcome of community coalescence in aquatic bacteria. Although several studies suggest priority effects occur in a variety of aquatic bacterial communities (Tan et al. 2012, Lee et al. 2013, Andersson et al. 2014, Rummens et al. 2018, Svoboda et al. 2018), it is currently unclear how the joint effect of warming and mixing ratio influences priority effects and the outcome of coalescence of aquatic bacterial communities. Furthermore, we lack knowledge about the identity of key bacteria that can persist or successfully invade a local community following coalescence.

Therefore, we aimed to investigate whether the fates and roles of distinct aquatic bacteria during community coalescence differ in response to warming and if aquatic bacteria can resist immigration, for example due to priority effects. 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 priori adapted. After initial growth and establishment these communities became the 'recipient communities' that were exposed to invasion by Baltic Sea bacteria ('dispersal source community'). These late-arriving communities were mixed into the recipient communities at three different mixing (dispersal) rates.

Here, we wanted to focus on how potential priority effects by recipient communities can diminish the advantage of late-arriving Baltic Sea bacteria that are historically adapted to the environmental conditions (i.e., Baltic sea medium). The original recipient communities differed in their proximity to the Baltic Sea, in order to assess whether the geographic distance to the origin of the invading bacteria might affect the strength of community resistance. We expected 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 Sea. This could have resulted in a larger shared species pool, including larger numbers of bacteria of Baltic Sea origin in local lake seed banks (Comte et al. 2014). At the end of the experiment 16S rRNA-based bacterial community composition was analyzed, and the persistence of recipient communities and the immigration success of late-arriving bacteria were investigated both at community and population level.

We hypothesized that warming should increase resistance of aquatic bacterial communities (e.g., due to potential priority effects). In other words, late-arriving species from the Baltic Sea should be less successful in colonizing the recipient communities at higher temperature as a consequence of niche modification and/or resource depletion by the early-arriving bacteria in the recipient communities. Moreover, we assumed that recipient communities with lake inocula that are geographically closer to the Baltic Sea should contain larger numbers of species adapted to the applied Baltic Sea medium, and thus negatively impact the invasion success of their late-arriving peers.

Material and methods

Experimental design

In total, our experimental design resulted in 132 communities, three sets of recipient communities, exposed to three levels of dispersal and three temperatures, each with four replicates. 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 the 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 the 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; the concentrations of total carbon (TOC) and PO_4^{3-} was higher, while NO_3^- was lower in lakes 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 on day 0. 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 at each incubation temperature. The incubation of recipient cultures was started with one day delay (day 1) so that cell abundance would most likely be lower compared to the dispersal sources, in order to avoid strong dilution of the medium during the coalescence process.

Coalescence event

On day 7, after the successful establishment of recipient communities, measured as bacterial abundances (Fig. S2), community coalescence was performed by adding the dispersal source communities to the recipient communities. The coalescence event consisted of one

dispersal event at three different rates: no, low and high dispersal, wherein 0 %, 5 % and 20 % of the cells were exchanged with cells from the respective dispersal source (Fig. 1). For this, each replicate 'A' of the three recipient communities at the different incubation temperatures received cells from replicate 'A' of the dispersal source at the respective temperature level. Likewise, each replicate 'B' of the recipient communities received cells from replicate 'B' of the dispersal source and so on. The volume that needed to be exchanged in order to apply the 5 % or the 20 % dispersal rates was calculated based on the measured bacterial abundances in all cultures on day 7. To reach an equal final volume (564 mL) in all cultures the differences were compensated by adding additional cell-free incubation medium that was kept at the same conditions throughout the entire experiment. One 'additional medium' bottle (kept at 20 °C), broke during the experiment, hence, a mixture of the two other medium bottles (kept at 15 and 25 °C) were used after the dispersal treatments to reach equal volume in each incubation bottle. Both the cell exchange and the supply of additional medium were carried out under sterile conditions using sterile disposable pipettes.

Sample analyses

Throughout the experiment bacterial abundance was monitored (from day 2 till day 22) (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) as in Vass et al. (2020).

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) using the molybdenum-blue method (Menzel 1965) and by catalytic thermal decomposition method (Shimadzu TNM-L, Kyoto, Japan), respectively according to standard procedures. Further, ion chromatography was used to measure the concentrations of NH_4^+, NO_3^-, PO_4^{3-} as described previously (Attermeyer et al. 2019).

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 briefly in the Supplementary Material, and in detail in the protocol deposited to the protocols.io repository (dx.doi.org/10.17504/protocols.io.6jmhck6). Briefly, bacterial 16S rRNA gene was amplified with 341F (5'-CCTACGGGNGGCWGCAG-3') and 805R (5'- GACTACNVGGGTATCTAATCC-3') primers with Illumina adapters. 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 (Callahan et al. 2016) 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 (Quast et al. 2013).

Data analyses

All statistical analyses and visualizations were conducted in R version 3.3.2 (R Core Team 2015). The ASV table was analyzed using the packages 'phyloseq' (McMurdie and Holmes 2013) and 'vegan' (Oksanen et al. 2016). 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 (Oksanen et al. 2016) 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 coalesced communities would converge completely towards the dispersal source. When the coalesced community maintained a significant ($p < 0.05$) community distance from the dispersal source, we assumed that the early-arriving bacteria had enough community resistance (i.e., through priority effects by niche modification and/or resource depletion) to avoid complete replacement by the better adapted late-arriving bacteria.

To quantify community resistance, we performed a conservative mixing model following Székely & Langenheder (2017) in order to investigate whether the observed invasion success of the late-arriving bacteria is lower or higher than expected based on the applied cell exchange (dispersal rates). For this, we calculated the expected proportion of ASVs in the coalesced communities based on the ASV proportions in the recipient communities and in the corresponding dispersal source, and the applied cell exchange rates (i.e., 5 or 20 %). Thereafter, we calculated the Bray-Curtis dissimilarities between the coalesced communities and the corresponding dispersal sources for the measured and expected data matrices, respectively. The deviation of the measured Bray-Curtis dissimilarity from the calculated expected dissimilarity was multiplied by –1 to express 'invasion success' and tested using paired t-tests. A significant positive deviation ($p < 0.05$) indicates that the invasion success of the late-arriving bacteria from the dispersal source was higher than expected. In contrast, a significant negative deviation indicates that the late-arriving bacteria established less successfully than expected, which could be a consequence of priority effects.

Resistance at population level was investigated by determining the relative abundance of early-arriving lake ASVs of the recipient communities that persisted after the coalescence event. We further investigated the invasion success of late-arriving Baltic Sea ASVs of the dispersal source that were mixed into the recipient communities. For this, we first identified ASVs that were unique in either the recipient or the dispersal source communities, and fell in the above-mentioned categories by performing differential abundance analyses at each temperature level using the 'DESeq2' package (Love et al. 2014). First, we selected the unique abundant ASVs (> 0.5 %

relative abundance) in each recipient community and the dispersal source. Then, we determined separately for each recipient community whether the relative abundances (as a proxy for population size) of the abundant early-arriving ASVs changed after the effective dispersal treatments (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 $p < 0.05$; Benjamini and Hochberg method) negative differences in relative abundances as a sign of persistence, and grouped the corresponding ASVs 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 Baltic Sea ASVs in the dispersal source, we performed the conservative mixing model. For this, we calculated the expected relative abundances of the abundant late-arriving ASVs' (> 0.5 % in the dispersal source) in the effective dispersal treatments based on their relative abundances in the corresponding dispersal source, and the applied cell exchange rates (i.e., 5 or 20 %). For example, at 100 % efficacy, all dispersed late-arriving ASVs should have either 5 or 20 % of their relative abundances in the expected (coalesced) communities. Thereafter we assessed the difference between the measured abundances (i.e., in the coalesced communities) and the calculated expected values by performing differential abundance analyses. A non-significant difference 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 with subsequent Tukey's HSD test to assess if temperature and the origin of the recipient communities (inoculum origin) induced any differences on the relative abundance of persistent early-arriving ASVs and successfully established late-arriving ASVs.

Results

Community-level patterns

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 compared to the 15 °C treatment (two-way ANOVA, $F_{Temperature}$ = 76.09, $p < 0.001$; *post-hoc* Tukey's HSD test: $p_{adjusted} < 0.05$, Table S2), further, there were significant differences based on the origin of the inoculum ($F_{Inoculum\ origin}$ = 79.01, $p < 0.001$) and the interaction between inoculum origin and temperature was also significant ($F_{Temperature \times Inoculum\ origin}$ = 7.86, $p < 0.001$) on day 7. 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). Total phosphorus (TP) showed a slight decrease in the dispersal sources after day 7, while the TP concentrations of recipient samples showed high variation after day 1. Phosphate concentrations increased in all cultures after day 1. Ammonium concentrations showed the highest variance between replicates and some of them reached near zero values during the course of the experiment, but there was no clear trend in ammonium concentrations because the initial replicates (day 1) also had high variation (from ~0 to ~60 µg/L) (Fig. S4-S9).

The NMDS of the bacterial communities (Fig. 2) and PERMANOVA 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). The recipient communities exposed to dispersal (i.e., 5 and 20 % dispersal rate, brown and black dots on the NMDS plot) became more similar to the dispersal source (Fig 2; blue dots). However, 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 resistance or priority effects) did not occur in any of the communities.

The community dissimilarities measured between the coalesced communities and the corresponding dispersal sources were compared to the community dissimilarities calculated for the theoretical coalesced and dispersal communities estimated based on conservative mixing models where the resulting coalesced community is expected to follow the mixing ratio of the early- and late-arriving communities. The deviation between the measured and theoretically expected dissimilarities was used as an indicator for the invasion success of the late-arriving Baltic Sea bacteria. There was no case when the deviation was significantly negative, meaning that the

invasion success by the late-arriving bacteria was either as high as expected in case of conservative mixing, or even greater (Fig. 3). At 5 % dispersal, we found that the invasion success was in most cases higher than expected (positive deviation values), while at 20 % dispersal, there was no significant deviation ($p > 0.05$), indicating that the measured Bray-Curtis dissimilarity between coalesced and dispersal source communities did not differ from those expected.

Population-level patterns

We further examined changes in the dynamics of early- and late-arriving ASVs in response to warming. On a broad taxonomical level, we found that the abundant (> 0.5 % relative abundance) bacterial ASVs in the early-arriving communities belonged to the class Alphaproteobacteria, Gammaproteobacteria and Bacteroidia (Fig. S10). The most abundant genera (top three) were *Brevundimonas*, *Pseudomonas*, *Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium* (hereafter *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. S10). The 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. S10, Baltic Sea).

Differential abundance analyses revealed numerous ASVs of the abundant genera (> 0.5 %) that could be classified as either 'persistent' or 'forfeited' early-arriving ASVs or 'successful' or 'unsuccessful' late-arriving ASVs (see Methods). We identified several persistent early-arriving ASVs (belonging to the genera of *A-N-P-R*, *Brevundimonas*, *Flavobacterium*, *Limnobacter*, *Novosphingobium*, *Perlucidibaca*, *Pseudomonas*, *Rheinheimera* and *Sphingorhabdus*) that occurred in all three recipient communities. In addition, ASVs of *Fluviicola* and *Hydrogenophaga* were also persistent (i.e., did not show significant ($p_{adjusted} < 0.05$) changes in relative abundance after the mixing event) in Erken and Grytsjön recipient communities, but not in Lötsjön (Fig. 4, S11). Several ASVs within the above-mentioned genera were forfeited by the dispersal of late-arriving Baltic Sea bacteria, and in Lötsjön *Sphingobium* represented the only genus containing merely forfeited ASVs. There were also inconsistences because ASVs from the same genera (e.g. *Brevundimonas, Pseudomonas* and *Flavobacterium*) 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, *A-N-P-R*, *Brevundimonas* (especially in the case of Erken), *Fluviicola* and non-abundant groups (i.e. *Sediminibacterium, Chryseolinea and* unknown Bacteroidia grouped as 'other_Bacteroidia'; *Comamonas* and *Herbaspirillum* grouped as 'other_Gammaproteobacteria'; *Ferrovibrio* grouped as 'other_Alphaproteobacteria'in Fig. 4) tended to be more abundant and persistent at higher temperatures. Temperature had, however, 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: $F_{temperature} = 2.38$, $p = 0.109$; 20 % dispersal: $F_{temperature} = 0.74$, $p = 0.488$; no significant interactions in either case) (Fig. 4).

Among late-arriving Baltic Sea 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. 5, Fig. S12). The composition of successfully established late-arriving bacteria was similar regardless of the recipient communities into which they were dispersed. Their total relative abundances differed across temperature levels in both the 5 % and 20 % dispersal treatments (two-way ANOVA at 5 % dispersal: $F_{temperature} = 7.98$, $p = 0.002$; at 20 % dispersal: $F_{temperature} = 7.34$, $p = 0.002$; no significant interactions in either case) (Fig. 5). Significant differences in total relative abundances (based on post-hoc Tukey tests at $p < 0.05$) were found between 15 vs. 25 °C and 20 vs. 25 °C, respectively. Among the populations most impacted by warming were *Loktanella*, *Hydrogenophaga* and *Pseudomonas* which showed decrease in relative abundance at higher temperature levels (20 and 25 °C) (Fig. 5, Fig. S12).

Linking the population-level patterns (Fig. 5) to the community-level patterns (Fig. 3) clearly shows that, when 5 % dispersal was applied, the late-arriving bacteria were much more successful and established greater populations (higher sum of relative abundances) at the lower temperatures. This phenomenon was particularly pronounced in case of Lötsjön, i.e. the lake situated the furthest away from the Baltic Sea.

Effect of proximity to dispersal source

At 5 % dispersal, warming seemed to restrict the invasion success of late-arriving bacteria from lake inocula (e.g., Lötsjön and Erken) that are geographically further away from the

dispersal source (i.e., Baltic Sea) (Fig. 3). However, we found no effect of proximity on invasion success at 20 % dispersal.

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: $F_{\text{inoculum origin}} = 1.95$, $p = 0.159$; 20 % dispersal: $F_{\text{inoculum origin}} = 0.59$, $p = 0.562$, no significant interactions in either case) (Fig. 4). Nevertheless, it is worth mentioning that most of the forfeited ASVs occurred in Lötsjön (n = 27) and Grytsjön (n = 51) recipient communities, while only two forfeited ASVs (*Sediminibacterium* grouped as 'other_Bacteroidia') occurred in Erken recipient communities.

The total relative abundances of successfully established late-arriving bacteria differed between recipient communities (two-way ANOVA at 5 % dispersal: $F_{\text{inoculum origin}} = 3.83$, $p = 0.033$; at 20 % dispersal: $F_{\text{inoculum origin}} = 4.79$, $p = 0.015$; with no significant interactions in either case) (Fig. 5). Significant differences in total relative abundances (based on post-hoc Tukey tests at $p < 0.05$) were found between Erken and Grytsjön at 5 % dispersal and Lötsjön and Grytsjön at 20 % dispersal. Note that Grytsjön is the closest lake to the dispersal source.

Discussion

This study investigated how warming, dispersal rates and geographic proximity to the dispersal source influence the invasiveness of aquatic bacteria during community coalescence. At the community level, we found evidence for community persistence because immigration by presumably better adapted bacteria from the dispersal source did not cause complete convergence (community turnover) towards the dispersal source community. However, invasion success always approached or even exceeded the theoretical invasion success of late-arriving bacteria estimated based on our conservative mixing model, suggesting only minor role of priority effects. By investigating population-level patterns, however, it appeared that warming had the potential to restrict the establishment of late-arriving bacteria to some extent. This was in particular the case at low dispersal rate (i.e., 5 %) and in recipient communities that received inocula from lakes geographically further apart from the dispersal source (i.e., Baltic Sea). The detected patterns at both community and population level can, thus, vary and depend (i) on the dispersal rate of late-

arriving better adapted communities into recipient communities and (ii) on the composition of the recipient community.

Temperature-dependency of invasion success

In our study we tried to mimic natural dispersal events of bacterial communities which are often complex and involve mixing or coalescence of entire communities (Rillig et al. 2015, Rocca et al. 2020). This study provides experimental evidence that temperature-dependency of invasion (immigration) success can occur in complex pelagic bacterial communities wherein different bacterial groups are involved in different ways. The warming effect could be seen at both community and population levels. The analyses of patterns at the community level showed that the late-arriving bacteria successfully established populations and increased well above their expected relative abundances at lower temperatures and when dispersal rate was low. However, when more cells (20 %) were dispersed, further establishment (exceeding the theoretical relative abundances) was not found, indicating that invasion was restricted. One possible explanation for the lower establishment success of late-arriving bacteria and stronger persistence of early-arriving species at higher temperatures is that the resistance of recipient communities to invasion (dispersal) by late-arriving bacteria increased because higher temperature stimulated growth of the early-arriving bacteria (see Fig. S2 and Table S2). This, in turn, may have resulted in that recipient communities were closer to their carrying capacity when the dispersal (community coalescence) was performed, hence offering less opportunities for establishment. This resonated with our findings at population level which showed that the total relative abundance of successfully established (late-arriving) ASVs generally decreased with increasing temperature, whereas the sum of relative abundance of persistent early-arriving ASVs tended to show the opposite trends, even though this was not significant in any case.

Our experiment did not aim to directly test the presence of priority effects, but still allowed us to make assumptions about the consequences of the applied experimental treatments on the extent of priority effects. Priority effects can be due to two distinct mechanisms: niche-modification and niche preemption (Fukami 2015), but providing insights into the mechanisms underlying priority effects is difficult. For example, niche modification-driven priority effects of the different lake inocula could have influenced the identities of the successfully established late-arriving bacteria (Fukami 2015), which was, however, not the case because their identities were similar in each coalesced community (Fig. 5). Niche preemption might have influenced

communities grown at increased temperatures (20 and 25 °C) as communities had potentially reached their carrying capacity (their cell abundances started to level off) by the time when coalescence was performed. Since the establishment success of late-arriving ASVs decreased with increasing temperatures (see Fig. 5), this may indicate that the availability of resources in 20 and 25 °C treatments limited the possibility of late-arriving bacteria to increase in abundance. 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, without a clear sign of resource depletion (Fig. S4-S9), such effects appeared to be generally stronger at 5 % dispersal rate when warming occurred. This highlights that both dispersal rates and temperature are important mediators of community resistance, and that this resistance in general is likely to be greater if dispersal rates of late arrivals are relatively low (Loeuille and Leibold 2008) and at higher temperatures. However, this was not always the case because in cases where the sources of two mixing communities are geographically located close to each other (i.e., Grytsjön – Baltic Sea) we found no clear evidence that warming influenced the invasion success of the late-arriving bacteria.

Does distance matter?

The lake inocula included for the preparation of the recipient communities in this study differed in their geographic distance to the dispersal source (the late-arriving bacteria from the Baltic Sea). We therefore presumed that the potentially higher numbers of Baltic Sea bacteria in recipient communities closer to the Baltic Sea (i.e., Grytsjön) would lead to more resistance against invasion. Our results do, however, not clearly support this idea because the dissimilarities between the coalesced and dispersal source communities were similarly high in all cases (see Fig. 2), suggesting that the potential shared species pools with the Baltic Sea, including local seed banks of Baltic Sea taxa, was equal 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, and the total relative abundance of persistent early-arriving ASVs and successfully established late-arriving ASVs. For example, it seemed that warming did not influence the invasibility of recipient communities with inoculum from Grytsjön, the lake located closest to the Baltic Sea. This difference among coalesced communities might also be the consequence of the differences of chemical characteristic of the three lakes from which

their inocula originated (Table S1), or the compositional differences of the initial recipient communities (Figure S10). Moreover, intrinsic differences in traits (e.g. temperature optima) of ASVs that contribute to the invasibility of the different recipient communities may also be the explanation of the observed differences that, however, we cannot disentangle in our study.

Inconsistencies in population-level dynamics

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 their populations when invasion challenged them. Moreover, 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. Since similar results have been obtained in a number of studies (Fukami et al. 2007, Tucker and Fukami 2014, Rummens et al. 2018, Brislawn et al. 2019) of different complexity, our results suggest that species' responses to invasion at the population level are difficult to predict. Nevertheless, our finding corroborates results of other recent studies (Needham et al. 2017, García-García et al. 2019) 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 persist 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. Nevertheless, we cannot rule out the possibility that some ASVs detected through sequencing were dead or in dormant stages and thereby still be detected through sequencing, and therefore wrongly identified as persistent early-arriving or successfully established late-arriving ASVs.

Organisms across multiple kingdoms might be negatively affected by global warming, altering their ecosystem functions (Altermatt et al. 2008, Hall et al. 2008, Rudolf and Singh 2013, Dong et al. 2018). Despite the differences between micro- and macroorganisms (i.e., in their dispersal and dormancy capabilities), it seems that warming can influence community resistance to invasion to different extent. Nevertheless, we lack a comprehensive knowledge of these processes

in complex natural microbial communities where thermal conditions are not constant. In addition to warming, dispersal and the geographic proximity to dispersal source there might be other factors that can influence the invasibility of natural bacterial communities. For instance, it remains unclear what would happen in the presence of predation (e.g., bacterial grazers or phages) or multi-level trophic interactions. A few previous studies on zooplankton communities suggested that predation can be an important factor and can either reduce community resistance (Louette and De Meester 2007, Berga et al. 2015), or, in contrast, indirectly promote them (Ryberg et al. 2012). However, we lack a comprehensive knowledge on how predation could affect the invasibility of microbial communities and potential priority effects in those communities. Another aspect that need to be considered are temperature fluctuations that can promote the invasion success of dispersed species and maintain multiple species coexistence, thus, reducing historical contingency (Litchman 2010, Tucker and Fukami 2014, Toju et al. 2018).

Conclusions

Temperature has been shown to stimulate microbial invasions (e.g., spread of vibrios; Vezzulli *et al.* 2012) and influence the biogeographical patterns of microbes (Amalfitano et al. 2014). Our experimental study shows the potential of warming to mitigate the effects of invasion during events when communities are mixing. More precisely, higher temperatures increased the sum of relative abundances of persistent bacteria in the recipient communities and restricted the total relative abundance of successfully established late-arriving bacteria. It is, however, important to note that warming-enhanced resistance does not necessarily challenge invasion in any case as its strength depends on several other factors such as the dispersal (mixing) ratios of communities and their initial compositions. Nevertheless, our findings highlight the potential role of warming in mitigating the effects of invasion at the population level which can impact the biogeographical patterns of microbial communities as a consequence of ongoing climate change.

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Conflict of interest

The authors declare that they have no conflict of interest.

Data accessibility

The sequencing data supporting the results are archived in the public repository European Nucleotide Archive with accession numbers PRJEB34383. Additional data (e.g. ASV tables, sample and chemical data) are available on the openly available repository of Uppsala University (DiVA; http://urn.kb.se/resolve?urn=urn:nbn:se:uu:diva-409821) under the following ID: diva2:1427510.

Author contributions

S.L., M.V., A.J.S. and E.S.L. designed the study. M.V. performed the experiment. O.A.O. processed the samples. M.V. performed data processing, analyses and drafted the manuscript. All others contributed substantially to writing and revisions.

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Figure 1. Experimental design of the study. The recipient communities were comprised of three different lake inocula (Erken, Lötsjön or Grytsjön, indicated by the different cell symbols) inoculated separately into 'foreign' Baltic Sea incubation medium. The three lake inocula differed in their geographical distance from the Baltic Sea, with Grytsjön (in blue) being closest and Lötsjön (red) farthest away. The dispersal source constituted of the Baltic Sea community (dark blue cells) inoculated into cell-free incubation medium. Both the recipient (early-arriving lake bacteria) and the dispersal source (late-arriving Baltic Sea bacteria) communities were incubated at three different temperatures (15, 20 and 25 °C). Three different coalescence events (dispersals) were applied by replacing 0, 5 and 20 % cells in the recipient communities with cells from the dispersal source (indicated by the different colors of the recipient community symbols). Black and grey arrows represent the direction of the coalescence. The experiment was replicated four times. The recipient communities (n = 4, for each lake inocula) were always mixed with the corresponding dispersal source replicate (n = 4) at the respective temperature level.

Figure 2. Non-metric multidimensional scaling (NMDS) plot derived from abundance-based Bray-Curtis dissimilarities of bacterial community composition by the end of the experiment and facetted at the three temperature levels. Note that cultures with Baltic Sea inoculum were used as the dispersal source (n = 12), while cultures with lake inocula (Grytsjön, Erken and Lötsjön) were used as recipient communities (n = 108). All cultures were grown in Baltic Sea medium. Each symbol represents one replicate, and is shaped and colored by inoculum origin and dispersal rate, respectively. Goodness of fit (stress value): 0.105.

Figure 3. Deviation values representing the differences between the measured and expected Bray-Curtis dissimilarities of the coalesced communities and dispersal source communities were used as a metric of invasion success. The expected Bray-Curtis dissimilarity was determined by conservative mixing model based on the applied dispersal rate (5 % or 20 % cells exchange). Positive values indicate lower observed 'coalesced community–dispersal source' dissimilarity than expected, thus, greater invasion success by late-arriving bacteria. Black bars refer to significant deviations ($p < 0.05$). Error bars represent standard deviations.

Figure 4. Relative abundances of abundant persistent early-arriving bacteria (ASVs > 0.5 %

relative abundance) in the different dispersal (5 % or 20 %) and temperature treatments (15, 20 and 25 °C). ASVs are grouped by bacterial genus and were identified by differential abundance analysis (see Methods for the assessment procedure and Figure S6 for further results). *A-N-P-R* refers to the genus *Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium*.

Figure 5. Relative abundances of successfully established late-arriving species (ASVs, > 0.5 % relative abundance) in the different dispersal (5 % or 20 %) and temperature treatments (15, 20 and 25 °C). ASVs are grouped by bacterial genus and were identified by differential abundance analysis (see Methods for the assessment procedure and Figure S7 for further results).

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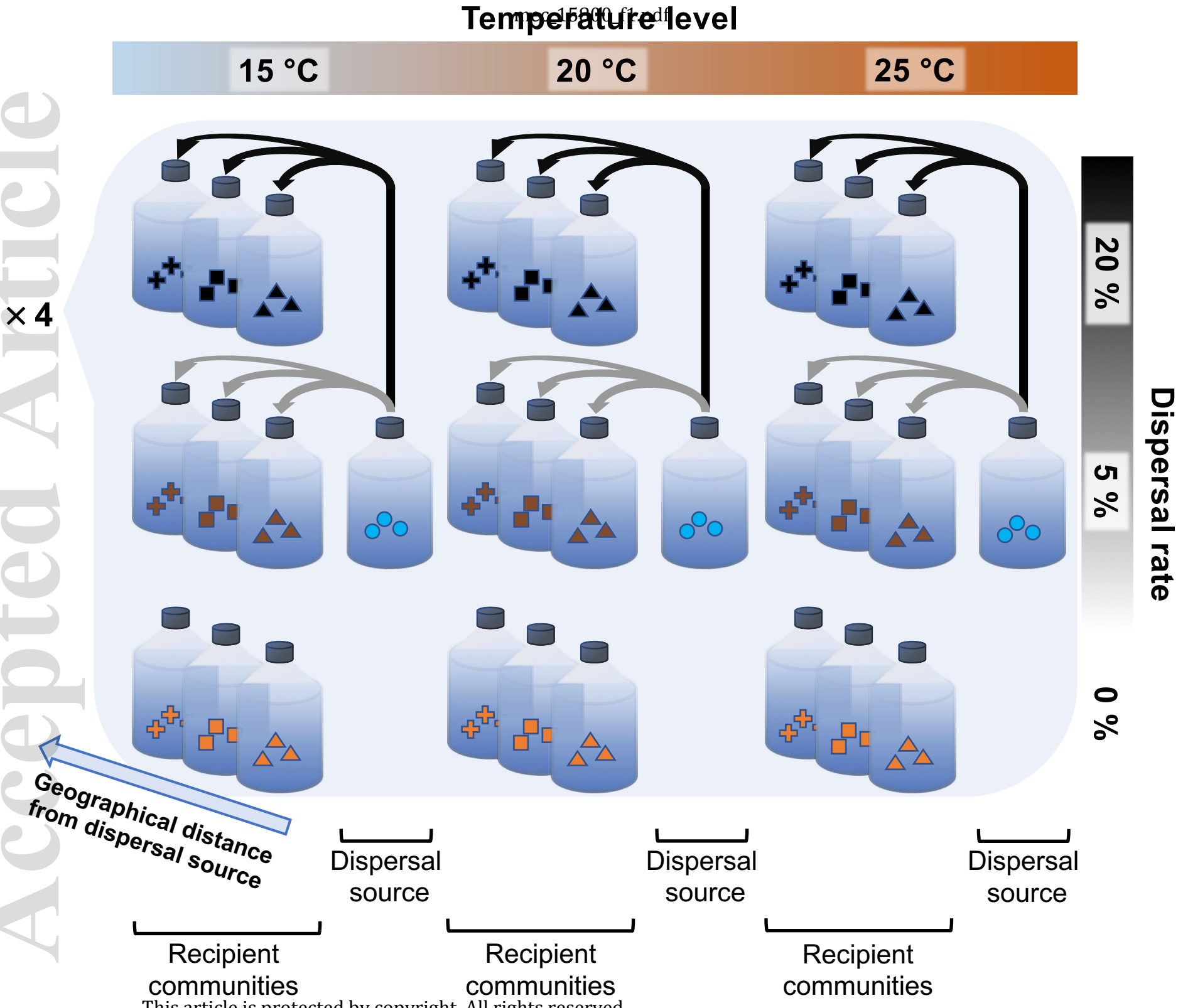

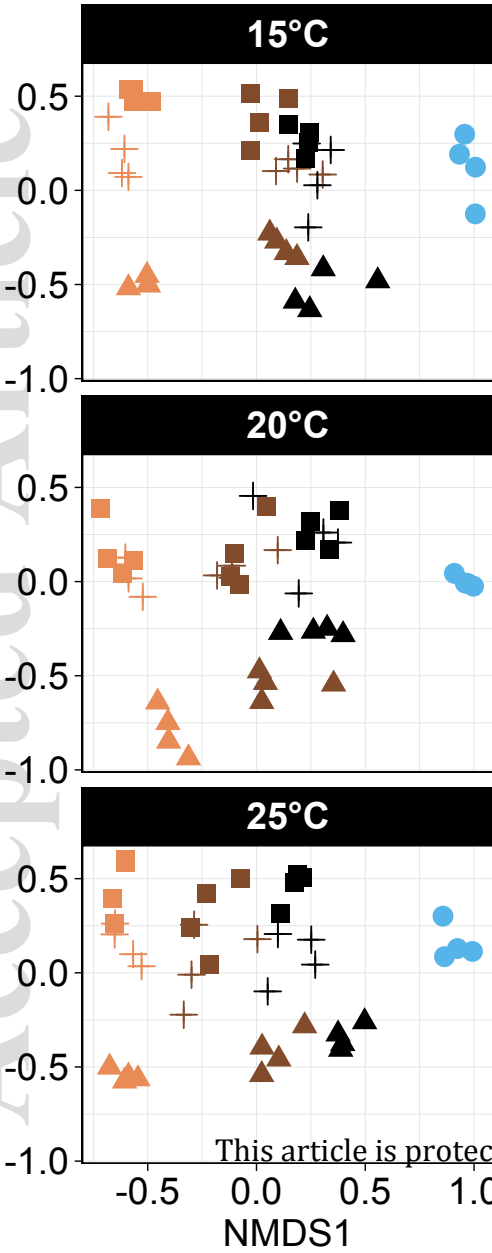

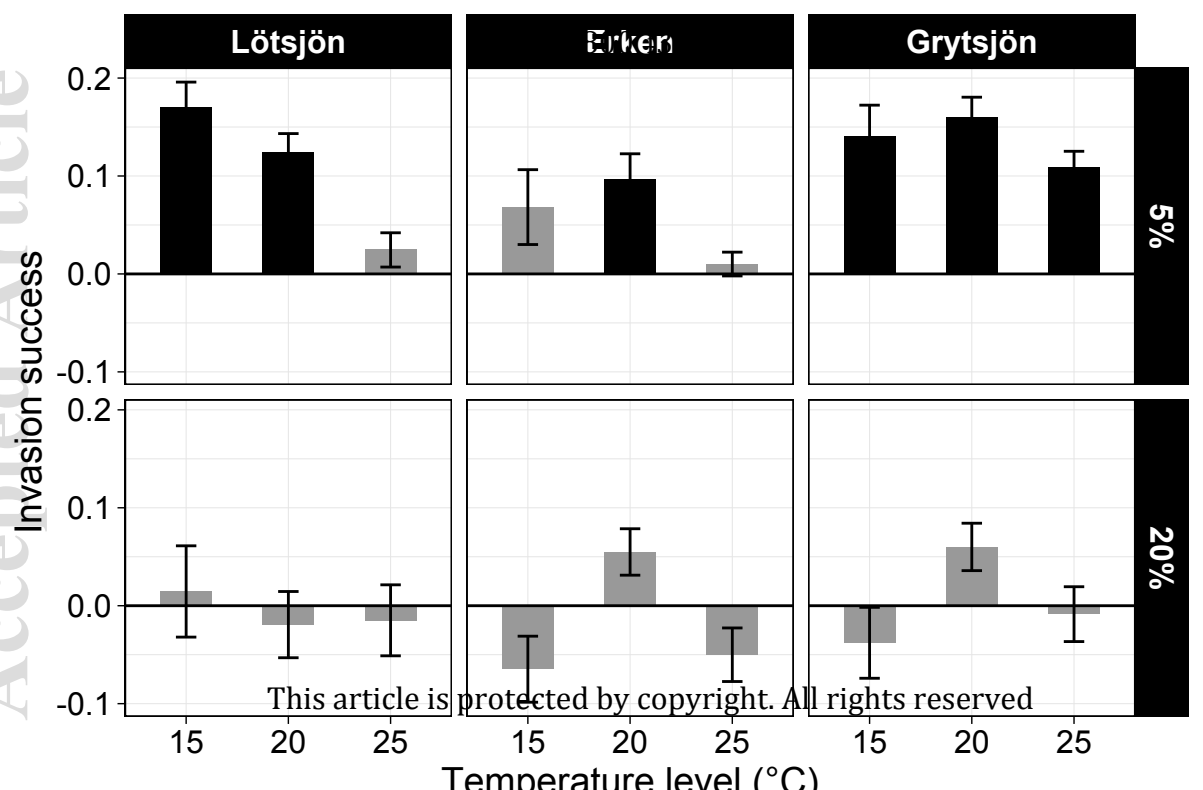

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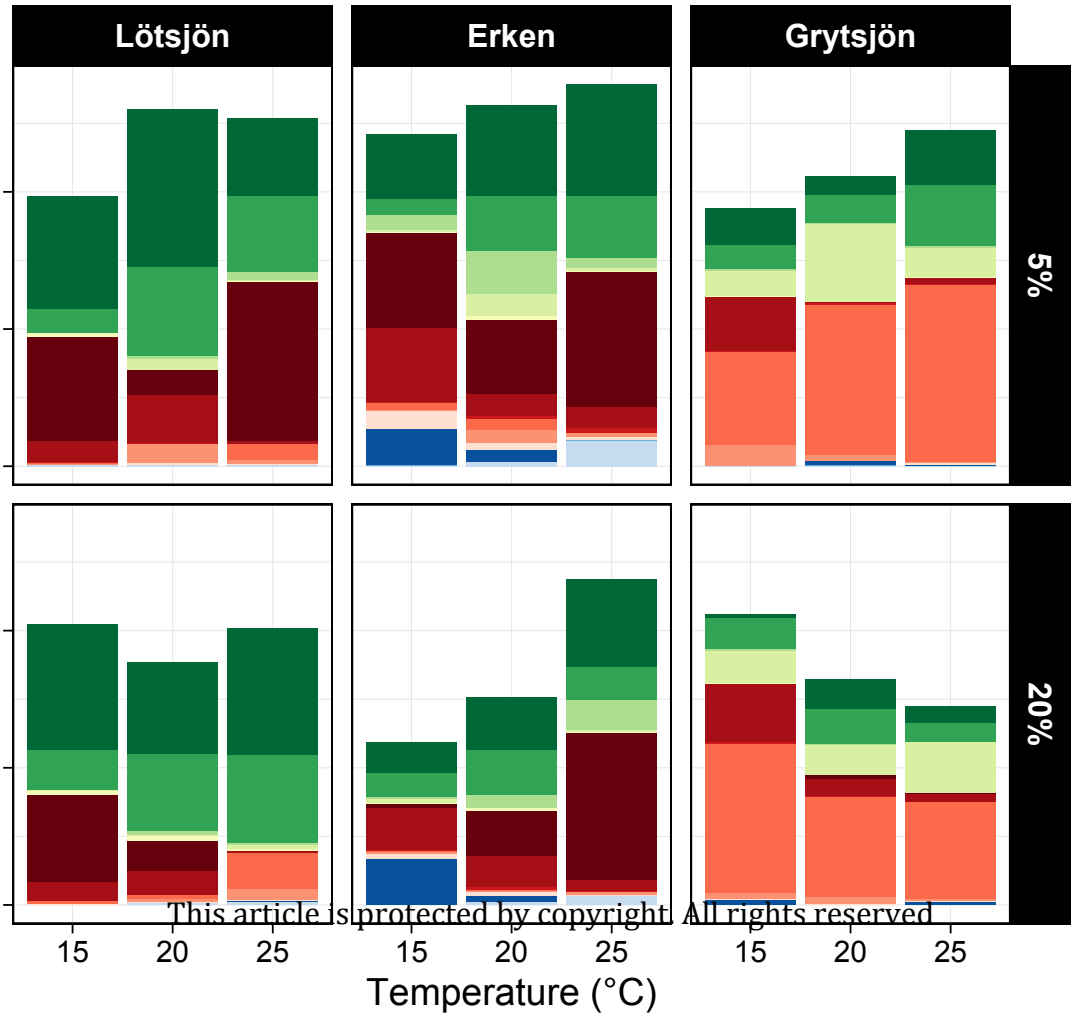

Taxonomy

- *Brevundimonas*
- *A-N-P-R*
- *Novosphingobium*
- *Sphingorhabdus*
- *other_Alphaproteobacteria*
- *Pseudomonas*
- *Rheinheimera*
- *Hydrogenophaga*
- *Limnobacter*
- *Perlucidibaca*
- *other_Gammaproteobacteria*
- *Flavobacterium*
- *Fluviicola*
- *other_Bacteroidia*