The effect of immigration on the adaptation of microbial communities to warming

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Abstract

Theory predicts that immigration can either enhance or impair the rate at which species and whole communities adapt to environmental change, depending on the traits of genotypes and species in the source pool relative to local conditions. These responses in turn will determine how well whole communities function in changing environments. We tested the effects of immigration and experimental warming on microbial communities during an 81 day field experiment. The effects of immigration depended on the warming treatment. In warmed communities immigration was detrimental to community growth whereas in ambient communities it was beneficial. This result is explained if colonists came from a local species pool pre-adapted to ambient conditions. Loss of metabolic diversity, however, was buffered by immigration in both environments. Communities showed increasing local adaptation to temperature conditions during the experiment and this was independent of whether or not they received immigration. Genotypes that comprised the communities were not locally adapted, however, indicating that community local adaptation can be independent of adaptation of component genotypes. Our results are consistent with a greater role for species interactions rather than adaptation of constituent species in determining local adaptation of whole communities, and confirm that immigration can either enhance or impair community responses to environmental change depending on the environmental context.

Introduction

The growth and functioning of communities arises from traits of their component species. Likewise, whether or not communities maintain ecological functioning in changing environments may depend on adaptation of component species to the new environment, as well as on changes in species abundances and interactions. In microbial communities, these processes can act over short-timescales and have large impacts on ecosystem services such as decomposition (Kardol et al. 2010) and water purification (Stottmeister et al. 2003). Yet, despite our reliance on the services that microbial communities provide, there is limited understanding of how these complex communities respond to environmental change. A key question is to what extent do communities respond to environmental change locally, for example by adaptation of constituent species or species sorting, rather than by the immigration of genotypes or species already adapted to new conditions?

Immigration has been shown to enhance adaptation of single species to environmental change in laboratory organisms, such as *Pseudomonas aeruginosa* to antibiotics (Perron et al. 2007) and yeast to increasing salinity (Bell & Gonzalez 2009). Immigration can even prevent local extinctions by providing genetic variation for natural selection to act upon (Holt & Gomulkiewicz 1999; Tallmon et al. 2004; Blanquart et al. 2012). However, intermediate rates of dispersal may be optimal for adaptation under some circumstances (Vogwill et al. 2008, Bell & Gonzalez 2011). This is because dispersal can hinder adaptation if the influx of maladapted genotypes swamps the process of natural selection (Mayr 1963; Moore & Hendry 2009). The effect of immigration on adaptation therefore depends critically on whether a population is locally adapted or not; i.e., on whether native genotypes have higher fitness in their environment than foreign genotypes (Kawecki & Ebert 2004). Reciprocal transplant experiments often show that species are locally adapted (e.g. Joshi et al. 2001; Belotte et al. 2003; Laine 2008), but this is not always the case (e.g. Fox et al. 2015).

The growth and functioning of a whole community, which arises from the traits of component species, should depend similarly on immigration. Whether or not ecological functions are maintained in diverse communities will depend on whether species can persist and adapt to new conditions (Gonzalez et al. 2013). For example, functions that are performed by rare species, such as the metabolism of recalcitrant compounds (McGuire & Treseder 2010) may be dependent on immigration to prevent local extinction of those species. Adaptation of component species could be enhanced or impaired by immigration as outlined above. In addition, immigration might result in colonisation by new species or genotypes that have tolerance for a wide-range of conditions and are therefore pre-adapted to the new environmental conditions (Ackerly 2003; de Mazancourt et al. 2008; de Meester et al. 2011), especially with long-range microbial dispersal (Nemergut et al. 2011). These colonists might restore, maintain or even enhance ecological functions (Székely et al. 2012). If whole-community adaptation strongly depends on species interactions, however, then the nature of interactions (negative, neutral or positive) might be as influential as immigration in shaping adaptation (Lawrence et al. 2012). If species are co-adapted to local environments, then local extinction might cause greater loss of ecological functioning than can be restored by immigration of new species in the short term. The complexity of natural communities makes it challenging to understand the interplay between adaptation, immigration and species interactions and this limits our ability to predict community-wide responses to environmental change.

Warming is of particular interest. Microbial communities are predicted to face warming over the coming decades due to climate change (Parry et al. 2007) and river and lake ecosystems are already being affected by urban heat pollution (Daufresne & Boët 2007). Temperatures change not only over decades but also seasonally and diurnally. Species that can survive a broad range of temperatures over short periods may not withstand long-term

changes and this could have implications for community composition and ecosystem functioning. How diverse communities respond to warming will depend on many processes. Warming can alter community composition, for example if sensitive species are lost and species tolerant of higher temperatures increase in abundance or invade (Wang & Kanehl 2003; Dang et al. 2009; Wang et al. 2011; Grigaltchik et al. 2012). Changes in composition can further impact species interactions; for example, if coevolved mutualisms are disturbed (Warren & Bradford 2014). In addition, species might cope with higher temperatures via phenotypic plasticity or may evolve tolerance through genetic changes. Together, these processes will determine the robustness of community functioning to the increase in temperature. These different mechanisms by which the composition, abundance and interactions between species may change could lead community functioning to be altered in response to environmental changes such as warming. In fact, community local adaptation may be different to - or even independent of - adaptation of component species.

Here we use experimental manipulations of decomposer microbial communities to investigate how immigration affects local adaptation at both the community and species levels in a changing environment. Many studies have experimentally warmed communities or observed natural variation to investigate the effects of warming. They find that respiration rates increase with warming (Demars et al. 2011) and that this is associated with changes in community composition (Zhang et al. 2005). Yet, this effect is often temporary (Eliasson et al. 2005); for example, over the long term there is little difference in microbial respiration rates in temperate and cool oceans (Rivkin et al. 1996) and ambient and warmed soils (Bradford et al. 2008). This might reflect either immigration of genotypes and species able to cope with higher temperatures or in situ sorting and evolution of the initial species. Distinguishing these alternatives requires direct comparison of open and closed communities.

We exposed naturally assembled diverse communities of microbes to experimental warming in the field and controlled immigration into them. Following a period of warming we assessed the effect of immigration and warming on community growth and metabolic functional diversity as a measure of ecological functioning. Our set-up allowed natural fluctuations in abiotic conditions and immigration of wild phenotypes but also retained some of the control and tractability of laboratory experiments. We also tested whether the local adaptation of communities was related to the local adaption of component species. If species in a community have similar responses to abiotic conditions we would expect local adaptation of individual species within a community to mirror local adaptation of the whole community. If instead species interactions predominate in determining the growth of the entire community, growth of species isolates in 'native' conditions compared to isolates from 'foreign' conditions might poorly predict the growth rate of the entire community, as we previously observed in a laboratory mesocosm of 4 species (Lawrence et al. 2012).

Material and Methods

Microcosms, media and treatments

Following Bell (2010), we used artificial aquatic microcosms that mimic tree-holes of beech trees (*Fagus sylvatica*) as a controlled system in which we could manipulate migration and temperature but which contained naturally co-occurring microbes in a diverse community of hundreds of species. Our factorial design exposed 5 replicated microcosms to each combination of two levels of warming: i) ambient temperature and ii) increasing temperature; and two levels of immigration i) no immigration and ii) immigration. Before the start of the experiment microbes were allowed to colonise open bottles containing sterile beech tea medium. Beech tea was prepared by autoclaving 50g of autumn-fall beech leaves in 500ml of water and diluting the filtrate 32-fold. In July 2011 six open bottles each containing 240ml of

sterile beech tea were placed under beech trees along a ~100m transect. After 22 days 2ml aliquots were frozen at -80^{0}C and the remainder was homogenised to create a single starter culture. Microcosms were 250ml Duran bottles filled with 220ml of sterile beech tea and 20ml of the starter culture. During the evolution experiment immigration was prevented using rubber stoppers (BugStoppers, GE Healthcare, USA), which were pierceable and contained a breathable membrane impenetrable to microbes. Bottles were placed under beech trees in five blocks (between 20-100m apart), each of which contained a single bottle for each treatment combination.

Controlling migration

To ensure that the microcosms differed only in whether or not they received immigration, all microcosms remained sealed throughout the experiment to control for confounding effects of different rates of evaporation or addition of organic material that could occur if vials from the immigration treatment were left open. Immigration was then simulated as follows. Each vial from the immigration treatment was paired with an open vial containing 220ml sterile beech tea (placed directly adjacent to and maintained at the same temperature as the experimental bottle). Every 9 days, 1ml of liquid from the open vial was transferred to the sealed bottle. The open vial was then replaced with one containing sterile beech tea. Bottles in the immigration treatment therefore received inocula exposed to the environment without altering physical conditions.

Sampling and temperature manipulation

Samples were taken every 9 days for 81 days. At each time-step a sterile syringe and needle were used to remove 30ml from each microcosm. The sample was replaced with 30ml of sterile beech tea (or 29ml in the immigration treatments plus 1ml from the paired open vial). Samples were returned to the laboratory and 2ml aliquots frozen at -80^{0}C in 80% glycerol.

These samples were used later for culturing isolates and T-RFLP. Growth assays and metabolic function assays were performed on fresh culture on the same day as samples were taken from the field. The warmed microcosms were maintained at a constant temperature above ambient (Figure A1) using heating coils (Parasene soil warming cable, PPW Services Ltd, Oxford, UK). Average temperatures were verified using temperature loggers (ibutton, Maxim, USA). Immediately following sampling, which took place every 9 days, the thermostats were adjusted so that the divergence in temperature between treatments gradually increased during the experiment. For warmed microcosms this resulted in an average increase above ambient of 0.5^{0}C every 9 days (Figure A2). During the experiment the mean seasonal change in temperature was a decrease of approximately 8^{0}C (from end of August to beginning of November 2011) and so the mean ambient temperature decreased during the experiment whereas the warmed treatment had a relatively constant temperature over time (Figure A1). Diurnal fluctuations in temperature reached a maximum of approximately 13^{0}C. The difference in mean temperature of the warmed and ambient treatments ranged between a minimum of 7°C (days 9 to18) and a maximum of 13^{0}C (days 63 to 72) (Figures A1&A2). This is extent of temperature change is representative of some cases of anthropogenic warming of aquatic ecosystems, for example, loss of riparian shading (Hester & Doyle 2011) and discharge from power plant cooling systems (Madden et al. 2013) have been shown to elevate temperatures to a similar degree. The maximum temperature reached in warmed communities was 38^{0}C compared to 28^{0}C in ambient communities. Therefore the maximum elevation in mean temperature caused by the warming treatment is equivalent to diurnal temperature fluctuations but caused the upper temperature extreme to be higher. The temperature elevation was sustained over 81 days and as a result the warming treatment was expected to cause a significant perturbation to the microbial communities.

The difference in mean temperature between ambient and warmed microcosms increased significantly over the course of the experiment (Pearson's correlation = 0.73, df = 6, t = 2.59, p = 0.041, Figure A2).

Community growth, metabolic function assays and t-RFLP profiles

Samples from each microcosm were reciprocally assayed for total growth in their 'native' environment (the average temperature that they were exposed to during the preceding 9 days) and in the 'foreign' environment (the average temperature of the other treatment during the preceding 9 days). At every time-step (every 9 days) growth was measured using optical densities at 600nm (OD_{600}) averaged across 3 replicates for each sample: 20μl of each sample was suspended in 180μl of beech tea in a 96 well plate and incubated for 96 hours. OD_{600} was measured every 24 hours and subtracted from negative controls of sterile media.

Metabolic functional diversity was measured throughout the experiment to determine how the treatments affected metabolic performance of the communities. At the start of the experiment and every third time-step (i.e. at days 27, 54 and 81) metabolic function of whole community samples was measured for replicates 1, 3 and 5 from each treatment using Biolog GN2 microplates, which assay growth rates of the community on 95 different carbon sources. Biolog GN2 plates were incubated at 25^{0}C for 24 hours. OD_{570} was measured at 0 hours and 24 hours. Of the 95 carbon substrates 11 were not metabolised by any community and were excluded from subsequent analyses.

We used terminal-restriction fragment length polymorphisms (t-RFLP) to profile the bacterial communities contained within the microcosms. T-RFLP provides a 'fingerprint' of bacterial genotypes within the community by cutting amplified 16S DNA using a restriction endonuclease. The range of fragment sizes that results gives an indication of the diversity of genotypes in the community. Profiles of the initial community and replicate communities 1, 3

and 5 from days 27, 54 and 81 were analysed (for t-RFLP protocol see Text A1). Only fragments that fell in the range 40-510nt were used for analysis. Relative abundances were calculated as the ratio between the fluorescence of each terminal restriction fragment (t-RF) and the total fluorescence of all t-RFs of that sample. Shannon diversity was calculated using the number of t-RFs per sample.

Local adaptation of isolates

We isolated separate genotypes by diluting 100µl of cryopreserved samples from day 81 (replicates 1, 3 and 5) in 100µl of beech tea. Samples were then spread on hard R2 agar (20 µl of sample). After 18 days of growth at 21°C 42 randomly-selected isolates from each community were picked and resuspended in 100µl of beech tea. After growth for 72 hours at 20°C, 5µl of the culture was plated on hard R2 agar and allowed to grow at 21°C for 7 days before randomly selecting 10 colonies per mesocosm that appeared to be monoculture to resuspend in 200µl of beech tea. These cultures were incubated at 24°C for 48 hours. Within the 10 colonies selected per mesocosm there were at least 3 or 4 colony phenotypes (with the exception of one case where there were 2). Based on past 16S sequencing of tree-hole bacteria these phenotypically different colonies are likely to be different species (Lawrence et al. 2012; Fiegna et al. 2015). Colony phenotypic diversity did not differ significantly between treatments (ANOVA, $F_{2,9} = 1.98$, $p=0.19$). For growth assays of the isolates, 10µl of the bacterial cultures were resuspended in 90µl of beech tea and the OD_{600} was measured immediately and every 24 hours for 96 hours. All cultures were assayed in their ‘native’ environment (the average temperature that they were exposed to during the preceding 9 days) and the ‘foreign’ environment (the average temperature of the other treatment during the preceding 9 days).

Statistical analyses

We used linear mixed effects models (lmer, lme4 library) with random error structures to account for temporal pseudoreplication caused by repeated sampling of the same microcosms over time and spatial autocorrelation caused by blocking. In all models the main explanatory variables were warming treatment, immigration treatment and time-step (as a factor). In some models, additional explanatory variables, such as species diversity, were used. To select the minimum adequate model we began with the maximal model and sequentially removed the least significant interaction terms. At every step we used analysis of variance (ANOVA) using maximum likelihood to ensure that the simplification did not significantly reduce explanatory power of the model. ANOVA were used to contrast particular treatments of interest. To report the direction and effect size of differences among treatments, we used the total change in optical density over 24h to 96h of growth assays as a measure of total growth. Total growth of the community during the assay was used as the measure of growth rather than maximum growth rate because the experimental evolution regime meant that resources were only replenished every 9 days and therefore rapid early growth would not necessarily be advantageous to the genotypes within the communities. To test the hypothesis that immigration would prevent the loss of metabolism of complex compounds, 43 of the carbon substrates present on Biolog plates were identified as either recalcitrant (23 substrates) or labile (20 substrates) according to their molecular structure (Treseder et al. 2011) and this was included as an additional explanatory variable in the minimum adequate model for change in metabolic function over time. T-RFLP data and metabolic profiles were analysed with multivariate statistics (Text A2).

The degree of local adaptation of communities defined as:

(growth of native community- growth of foreign community) / growth of native community

was compared to the mean local adaptation of isolates within each community, defined for each community as the mean of: (growth of native isolate - mean growth of foreign isolates) / growth of native isolate. Local adaptation of communities and mean local adaptation of isolates were combined into a single vector and used as a response variable in an lmer with the explanatory variables of temperature regime (ambient or warm), migration treatment (immigration or no immigration) and measure type (community or isolates) with microcosm as a random effect. A Tukey HSD test was used to compare local adaptation for each temperature regime. All statistical analyses were performed in R (R Development Core Team 2011).

Results

Effect of warming and immigration on total community growth over time

Experimental warming caused the total growth of communities to decrease between the initiation of the experiment and day 45. After this growth recovered marginally but remained depressed relative to the start of the experiment (Figure 1A). In contrast, communities that experienced ambient temperatures maintained high total growth until day 54 and then suffered a sudden decrease in total growth between days 54 and 63 followed by a marginal recovery towards the end of the experiment (Figure 1A). These differing effects of temperature regime on total community growth are reflected by the significant interaction between time-step and warming treatment that explains approximately 30% of the model deviance in total growth (ANOVA of lmer interaction between warming treatment and time-step; $F_{7,117} = 21.0$, MSE = 0.0006, $p < 0.001$, Table A1). The variance in total growth between replicate communities did not vary significantly during experimental evolution (lm of variance over time; $F_{1,30} = 0.32$, $p>0.05$).

We hypothesised that immigration would be beneficial to community growth regardless of the warming treatment, because immigration would introduce genetic diversity

for natural selection to act upon and novel species. Contrary to this, we found that the effect of immigration depended on the warming treatment. Immigration enhanced total community growth in the ambient communities but reduced total community growth in the warmed communities (ANOVA of lmer interaction between warming treatment and immigration; $F_{1,117} = 9.7$, MSE = 0.0003, $p < 0.01$; Figure 1A, Table A1). There was not a significant effect of immigration treatment on Shannon diversity (lmer, $F_{1,19}= 0.97$, MSE = 33.0, p=0.34).

The shape of the community growth curves varied considerably between warming treatments and over time (Figure A3). During the first half of the experiment both warmed and ambient communities had Monod-shaped growth curves with rapid early growth approaching an asymptote towards the end of the assay. As the temperature dropped, ambient communities did not maintain rapid doubling rates and whole-community growth curves were no longer significantly non-linear (ANOVA comparing models with time as a factor versus time as a continuous variable, likelihood ratio = 2.18, df = 158 and 156, p = 0.30). In contrast, microbes in warmed communities retained rapid early growth approaching an asymptote throughout the experiment (ANOVA comparing models with time as a factor versus time as a continuous variable, likelihood ratio = 3.09, df = 158 and 156, $p < 0.05$). Thus, warming (resulting in a relatively constant temperature over time) facilitated the maintenance of rapid doubling rates in these communities (Figure A4).

Effect of warming and immigration on metabolic functional diversity

Metabolic functional diversity declined rapidly in the warmed treatments before recovering slightly towards the end of the experiment (Figure 1B). Functional diversity declined in the ambient communities during the experiment, but at a slower rate than in the warmed communities throughout. The interaction between warming treatment and time-step on metabolic functioning was significant but explained only a small amount of model deviance

in metabolic functional diversity (ANOVA of lmer interaction between warming treatment and time-step, $F_{2, 911} = 15.8$, MES = 0.59, $p < 0.001$; Table A2).

Warmed and ambient communities diverged in their use of carbon substrates highlighting underlying changes in metabolism during warming (Figure 2, Text A3). For example, warmed communities improved in their ability to metabolise organic acids such as citric acid and amino acids such as L-alanine but became worse at metabolising the polymer tween and sugar alcohols such as xylitol. For ambient communities the opposite was true. The total change in metabolic profiles was greater for warmed communities than ambient ones (ANOVA of lmer of distance moved in multivariate space weighted according to the variance explained by the PCs, $F_{1, 18} = 7.7$, MSE = 10.21, $p < 0.05$; Table A3), indicating that warmed communities showed more variation through time in the resources they could metabolise than ambient communities.

If immigration buffered metabolic functional diversity we would expect that communities receiving immigration would be able to metabolise more carbon sources than communities that remained closed. In agreement with this, we find that metabolic functional diversity declined less in communities receiving immigration than in closed communities irrespective of the warming treatment (ANOVA of lmer interaction between immigration treatment and time-step, $F_{2, 911} = 5.1$, MES = 0.19, $p < 0.01$, Figure 1B, Table A2) and communities that received immigration had on average approximately 20% greater metabolic diversity than those that did not. We hypothesised that immigration may have allowed communities to retain the ability to metabolise complex compounds; in fact, we found no difference in the metabolism of recalcitrant and labile compounds between immigration treatments (lmer of interaction between immigration treatment and carbohydrate structure, $F_{1,453} = 0.54$, $p > 0.05$, Table A8, Figure A5). Shannon diversity was not significantly different between communities that received immigration and those that did not, however, the

higher metabolic diversity associated with immigration may have been related to Shannon diversity, in part, since in communities that received immigration Shannon diversity had a positive effect on total metabolic function (number of substrates that could be metabolised) (Figure A6). This association between Shannon diversity and metabolic function was weak and may be driven by one community that was particularly diverse in both metabolic function and species composition. In closed communities there was no relationship between Shannon diversity and total metabolic function (ANOVA of lmer of interaction between immigration and Shannon diversity, $F_{1, 14} = 16.8$, MSE = 1377, $p < 0.01$; Table A4, Figure A6). These results are consistent with the hypothesis that colonisation of species would buffer metabolic functional diversity but this effect is not entirely explained by immigration increasing species diversity.

Community local adaptation during environmental change

If communities are locally adapted to their temperature regime then they will have greater growth in their native conditions than a community from a foreign temperature regime and also poorer growth in foreign conditions compared to communities native to those conditions. Because we are comparing two temperature treatments (ambient and high), local adaptation is indicated by the crossing of community reaction norms on Figure 3 (local maladaptation would be indicated by crossing of reaction norms where growth of foreign communities is greater than local communities). We expected that community local adaptation should increase during the experiment because if communities do not exchange individuals then over time there should be greater divergence in species composition or relative abundances of species between communities and also more opportunity for selection to occur, which could affect overall community functioning and growth. For the same reasons, and also because the immigration of conspecifics may swamp species adaptation or limit species sorting, we also

predicted that local adaptation should be greatest in communities that did not receive immigration.

Contrary to our hypothesis, immigration did not have a significant impact on local adaptation of communities. A large proportion of the variation in total growth of communities during the experiment was attributable to the temperature regime, time-step and assay temperatures and only a small proportion to the immigration treatment. Accordingly, in the full model the interaction between warming treatment, migration treatment, assay conditions and time-step was not significant and was removed during model simplification (lmer, $F_{7,231} = 1.81$, p=0.09).

In the minimum adequate model the interaction between warming treatment, assay conditions and time-step was highly significant (lmer, $F_{7,261} = 3.77$, $p<0.001$, Table A5). This result indicates that the degree of community local adaptation to the temperature regime changed over time (Figure 3). To better interpret this outcome, we analysed local adaptation separately at each time-step. Growth assays performed on days 18, 27 and 45 show that foreign communities grew equally as well as local communities of both temperature treatments; therefore in general there was not local adaptation early in the experiment (Figure 3). The lack of interaction between temperature regime and assay conditions on these sampling days also suggests that there is not local adaptation at these sampling points (lmers of interaction between temperature regime and assay conditions; day 18: $F_{1,9} = 0.37$, p=0.56; day 27: $F_{1,9} = 4.75$, p=0.057; day 45: $F_{1,9} = 1.00$, p=0.34). In contrast, on day 36 and from day 54 onwards there were significant interactions between temperature regime and assay conditions (lmers of interaction between temperature regime and assay conditions; day 36: $F_{1,9} = 10.32$, p=0.01; day 54: $F_{1,9} = 11.98$, $p<0.01$; day 63: $F_{1,9} = 23.92$, $p<0.001$; day 72: $F_{1,9} = 27.81$, $p<0.001$; day 81: $F_{1,9} = 14.62$, $p<0.01$) and on days 63, 72, and 81 growth of communities in their local conditions exceeded that of foreign communities indicating that

communities were locally adapted (Figure 3). The degree of local adaptation, as indicated by the strength of the interaction between temperature regime and assay conditions, was positively correlated with time-step suggesting that community local adaptation increased over time. Together these data show that local adaptation to the temperature regime increased during the evolution experiment and that immigration did not influence community local adaptation.

Local adaptation of isolates

After 81 days in warmed or ambient conditions the microbial communities showed local adaptation to the temperature conditions (lmer of the subset of microcosms from which genotype isolates were taken at day 81; $F_{1,3}$=14.13, p=0.036, Table A6) and we expected that genotype isolates would also show local adaptation. We found that isolates from communities selected under ambient temperature had greater growth on average than those from the warmed treatment in both ambient and warmed assay conditions regardless of the immigration treatment (Figure 4 and A7). Neither the three-way interaction between immigration treatment, warming treatment and assay conditions was significant (lmer, $F_{1,241}$ = 2.55, p = 0.11) nor was the two-way interaction between warming treatment and assay conditions (lmer, $F_{1,241}$ = 0.022, p=0.88), indicating that isolates were not locally adapted and that immigration did not affect this. Therefore, we found no evidence of local adaptation of genotype isolates, rather isolates from ambient communities were fitter overall (Figure 4). A linear model confirmed that local adaptation of communities and isolates were significantly different (lmer of interaction between type of population (community or isolate) and temperature treatment on local adaptation; $F_{1,8}$ = 8.26, p= 0.021, Table A7), this is driven by the high growth of isolates from ambient communities in warm assay conditions. These data suggest that growth responses to a foreign environment differ between communities and the isolates embedded within them.

Discussion

Immigration had contrasting effects on community growth between warmed and ambient treatments. Total growth was impaired by immigration in warmed communities but enhanced by immigration in ambient communities. This finding is contrary to our initial hypothesis that immigration would be beneficial regardless of the warming treatments. We had expected that colonists would include genotypes adapted to a wide range of conditions thanks to long-range dispersal of bacterial propagules, which should improve adaptation by increasing genetic variation for selection to act upon (Tallmon et al. 2004) and by allowing colonisation of pre-adapted species or genotypes (de Meester et al. 2011). Instead, our finding is consistent with colonising microbes coming from a local species pool that experienced the same conditions as the ambient communities. Immigrants to ambient communities would then be better adapted to their conditions than those introduced to warmed communities. Maladapted immigrants to warmed communities would make a poor contribution to community growth leading to decreased average total growth. Although microbes have long been regarded not to exhibit dispersal limitation, distance-decay relationships and local dispersal that could explain our results have been found previously over short distances (Vos & Velicer 2008) and in experiments with artificial mesocosms facing only ambient conditions (Bell 2010).

There were declines in total community growth and in metabolic diversity during experimental evolution suggesting that experimental conditions caused declines in abundance of some species or even local extinctions. Immigration reduced the loss of metabolic diversity in warmed and ambient communities indicating that immigration acted as a buffer to the loss of functioning that may be associated with environmental change. A buffering effect of immigration has been reported in previous mesocosm experiments with zooplankton exposed to environmental change (Thompson & Shurin 2012). In theory, the species that should be most readily lost when growth rates decline are rare species (Gonzalez & Chaneton 2002) and

in decomposer communities these tend to be slow growing specialists that metabolise recalcitrant compounds (McGuire & Treseder 2010). We found no evidence that metabolism of recalcitrant compounds was preserved by immigration. Another possibility is that immigration prevents loss of metabolic functioning by maintaining high species diversity through a balance of immigration and extinction (Loreau & Mouquet 1999). Immigration did not affect Shannon diversity here, however. Communities that received immigration therefore either had more varied resource use across species or constituent species each used a wider range of metabolic resources. Our speculation that immigration caused an increase in generalists is consistent with the theory that generalists should be more successful colonists (Dall & Cuthill 1997) and agrees with the outcome of an experiment by Székely et al. (2012) which reported that when microbial communities were transplanted between environments of different salinity an overrepresentation of generalists was associated with enhanced functioning.

Local adaptation of communities became stronger during the experiment, consistent with our initial hypothesis. We argue that this may have occurred because the longer communities were exposed to the temperature regimes the greater the effect of processes such as species sorting or species adaptation to the abiotic conditions. Species-specific responses to temperature could have contributed to divergent community growth and metabolic functioning between temperature treatments. In support of this, there was a marked divergence in the metabolic profiles of warmed and ambient communities indicating that the metabolic functions lost at the start differed between the treatments. Therefore in the warmed communities different species were sensitive to the environmental change than those lost in the ambient communities. Species-specific responses to warming in fungal communities have also been found to cause shifts in community composition with consequences for ecosystem functioning (Dang et al. 2009). This is notable because the provision of ecosystem services

may rely on the balance between different taxa, for example the ratio of fungi to bacteria in soil can affect decomposition rates (Güsewell & Gessner 2009). An alternative explanation is that community growth and functioning may have been contingent on changes that occurred early in the experiment and therefore differences between communities would increase over time, even without ongoing species sorting or evolution. However, variance between replicate communities of the same treatment did not increase significantly over time and was small compared to variance between treatments, indicating that the responses to the temperature regimes were consistent between replicates. Therefore community local adaptation is unlikely to have been driven only by chance effects early in the experiment and was more likely due to the ongoing temperature regimes and ecological and evolutionary outcomes arising from them.

Contrary to our expectations, immigration did not decrease community local adaptation indicating that there was not a strong homogenising effect of immigration in this experiment. In our experiment we estimate that at least 10^8 cells were introduced in the immigration treatment every 9 days, but if colonisation depends on rare events that transfer large amounts of material, for example a dead animal falling into a tree-hole, then immigration might have larger impacts in real systems. Alternatively, the relatively small effect of immigration could reflect high diversity and functional redundancy of local bacterial communities, so that pre-adapted forms are present and can increase in abundance even in a closed local community.

In order to identify potential mechanisms behind community responses, we compared community-wide patterns with the local adaptation of samples of genotype isolates. Isolates from ambient communities grew better in warmed and ambient conditions compared to isolates that were sampled from warmed communities. Hence, in contrast to communities, genotype isolates did not show local adaptation. Genotypes were isolated at an intermediate

temperature, meaning that genotypes from both warmed and ambient treatments may have had some opportunity to acclimate to the foreign temperature conditions. We would not expect, however, that this should result in isolates from ambient communities growing better in both warmed and ambient conditions, as we found. As far as we are aware, the idea that community local adaptation can be very different to, or even independent of adaptation of component genotypes, has not been tested before. Studies have investigated how local adaptation of genotypes varies spatially and temporally (e.g. Belotte et al. 2003, Kraemer et al. 2015) while others have investigated the contribution of species sorting to changes in ecological functioning of the whole community (McClellen et al. 2008). Experiments linking local adaptation of species with that of their community have been lacking, despite theory that links species' physiological and evolutionary responses to ecological responses of the community (Collins & Gardner 2009).

Our results match other findings that the dynamics of single species do not always correlate predictably with their dynamics when in a group (Fridley 2002; Schmidtke et al. 2010). One reason could be that the dynamics of the isolates depends on whether other species are present; for example through quorum sensing bacteria can alter the chemicals that they produce (Miller & Bassler 2001) or alternatively species may rely on the presence of others to obtain resources (Lawrence et al. 2012). Therefore, a species that may grow well in isolation could be hindered when in the community (or vice versa). Our data show that genotype isolates from ambient communities performed better than expected when removed from the biotic environment that they had experienced during the evolution experiment. This suggests that interactions within these communities were antagonistic on average, whereas the growth of isolates taken from warmed communities followed a similar pattern to that of the community, suggesting that on average there was a balance between positive and negative interactions in warmed communities. Hence, the prevalence of antagonistic, neutral and

synergistic interactions in these communities depended on the environmental conditions during experimental evolution. Although we cannot elucidate the mechanisms behind the altered interactions reported here, previous work using simplified microbial communities has shown that eco-evolutionary feedbacks can influence the nature of species interactions with consequences for community functioning (Lawrence et al. 2012, Andrade-Dominguez et al. 2014).

In conclusion, immigration affected community growth and functioning but did not influence community local adaptation, which became stronger the longer the temperature regimes were imposed. Bacterial communities were generally locally adapted to the prevailing temperature, yet genotypes that comprise the communities were not locally adapted. We propose that this reflects the importance of species interactions in determining community growth and functioning, as indicated by experiments with fewer species in the laboratory. In line with prior theory, the effects of immigration depended on environmental context as well as on which aspect of community function was measured. Our results have implications for the robustness of community functioning to environmental change. For example, one might predict that ecosystem functioning would be degraded if component species do not adapt to environmental change; however, our results show that growth and functioning of microbial communities may be maintained, at least in the medium term, even if species are not locally adapted. More studies are now needed to track responses of natural bacterial communities in detail, to quantify the relative role of immigration, evolution and changes in species interactions in ecosystem responses to environmental change.

Acknowledgements

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University Research Fellowship to TB and a Grantham Institute for Climate Change BBSRC funded PhD studentship to DL.

Online Appendix A – supporting graphs, tables and text

Figure A1

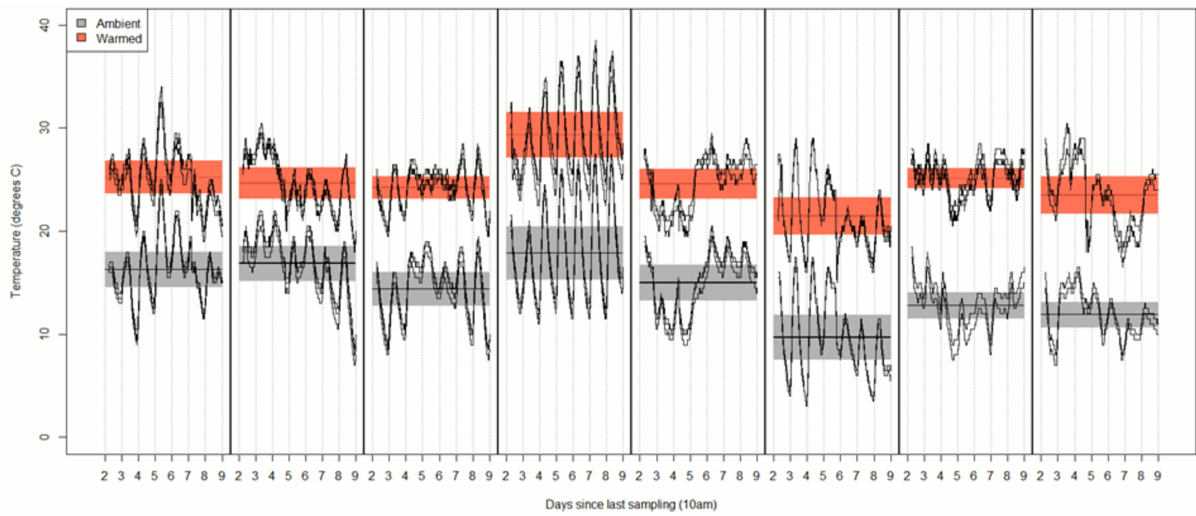

Figure A2

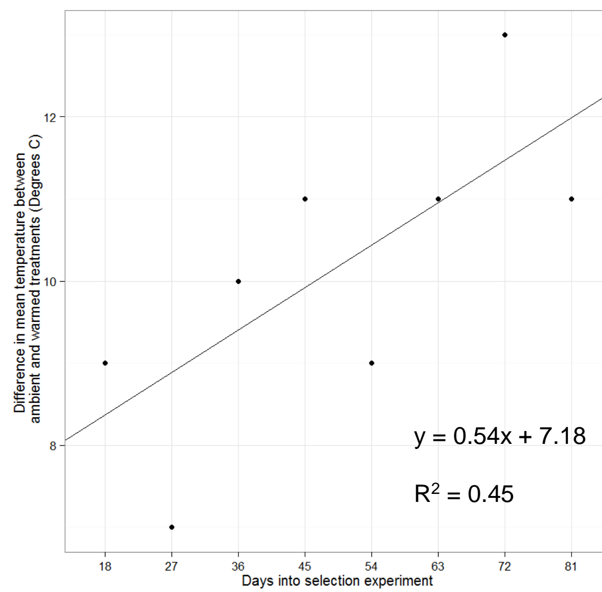

Figure A3

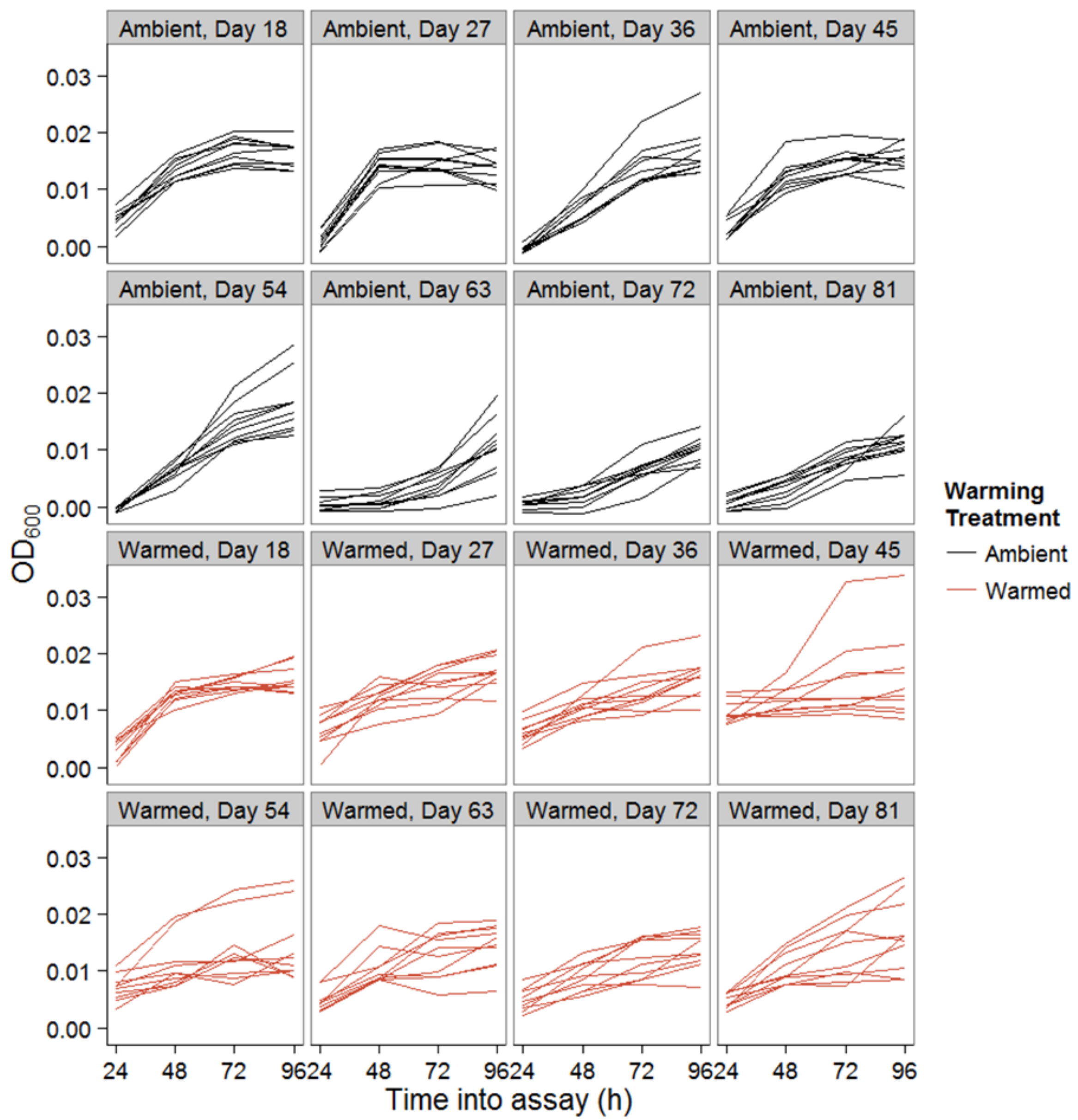

Figure A4

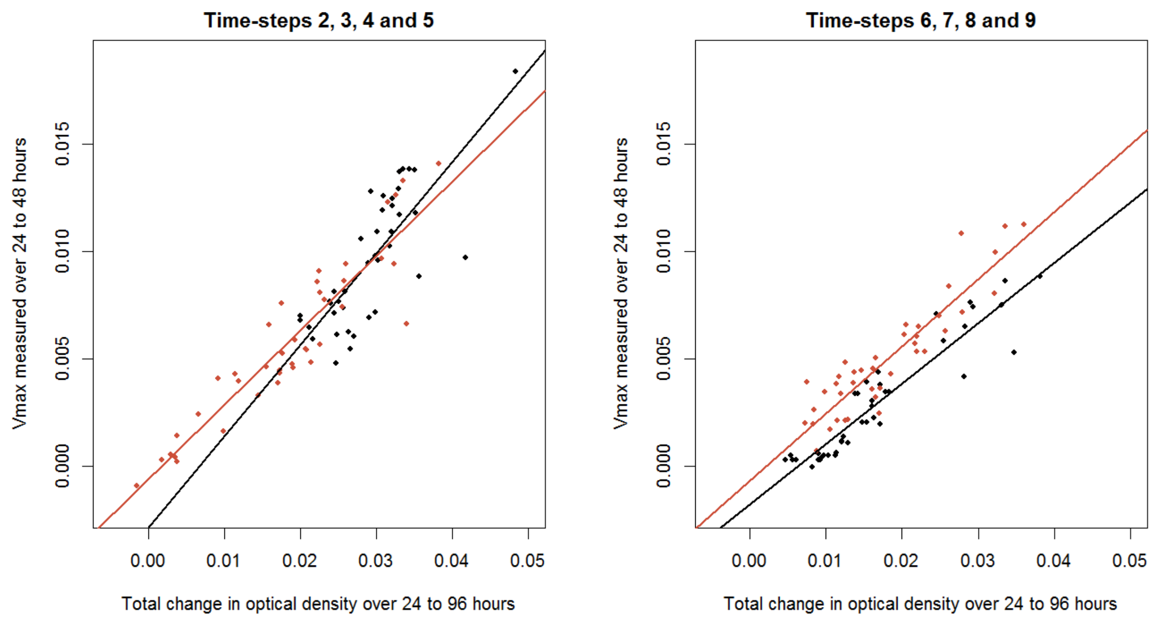

Figure A5

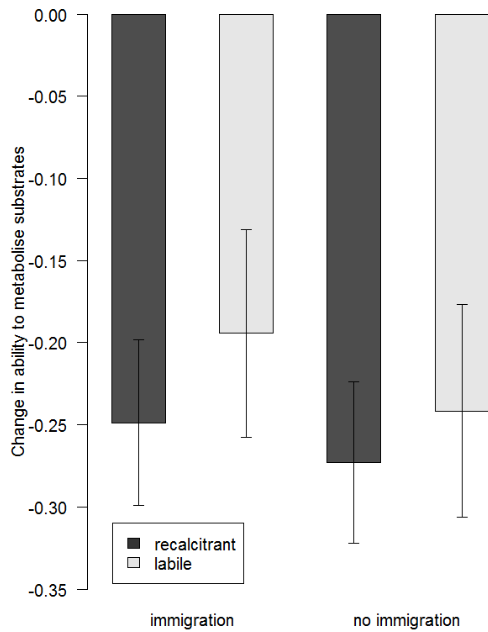

Figure A6

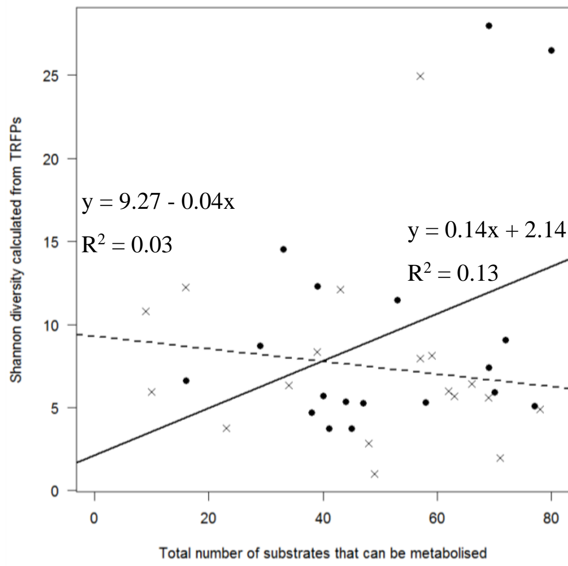

Figure A7

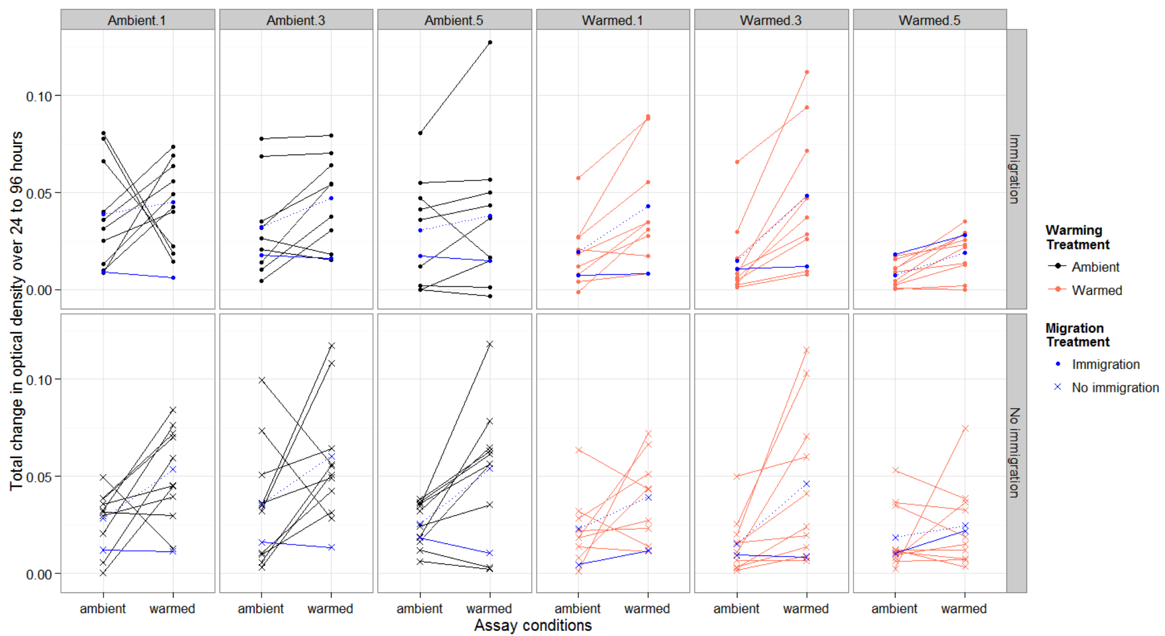

Table A1. Output of final lmer for total growth during environmental change.

Fixed effect / interaction	Mean square error	Numerator degrees of freedom	Denominator degrees of freedom	F value	p value	Deviance explained (%)
time-step	0.0005	7	117	16.03	<0.001	23.46
warming	0.0009	1	117	29.73	<0.001	6.21

treatment						
immigration treatment	1x10^{-5}	1	117	0.18	>0.05	0.04
warming X time-step	0.0006	7	117	21.01	<0.001	30.75
warming X immigration	0.0003	1	117	9.69	<0.01	2.03

The minimum adequate model was warming X immigration + warming X time-step with the random effects of microcosm and block.

Table A2. Output of final lmer for change in metabolic function during environmental change.

Fixed effect / interaction	Mean square error	Numerator degrees of freedom	Denominator degrees of freedom	F value	p value	Deviance explained (%)
time-step	0.79	2	911	21.00	<0.001	1.44
warming treatment	1.17	1	911	31.10	<0.001	1.06
immigration treatment	0.49	1	911	13.16	<0.001	0.45
warming X time-step	0.59	2	911	15.81	<0.001	1.08
immigration X time-step	0.19	2	911	5.15	<0.01	0.35

The minimum adequate model was warming X immigration + warming X time-step with the random effects of microcosm, block and substrate.

Table A3. Output of final lmer for change in community metabolic profile over the course of environmental change.

Fixed effect /	Mean	Numerator	Denominator	F	p value	Deviance

interaction	square error	degrees of freedom	degrees of freedom	value		explained (%)
warming treatment	10.21	1	18	7.65	<0.05	1.65
immigration treatment	1.16	1	18	0.87	>0.05	0.19

The minimum adequate model was warming + immigration with the random effects of microcosm and principle component.

Table A4. Output of final lmer for total metabolic function given community Shannon diversity during environmental change.

Fixed effect / interaction	Mean square error	Numerator degrees of freedom	Denominator degrees of freedom	F value	p value	Deviance explained (%)
Shannon diversity	71.34	1	14	0.87	>0.05	0.51
Immigration treatment	99.63	1	14	1.12	>0.05	0.71
Warming treatment	328.69	1	14	4.00	>0.05	2.35
Time-step	189.27	2	14	2.31	>0.05	2.71
Shannon diversity X immigration	1337.38	1	14	16.78	<0.01	9.83

The final model was diversity X immigration + warming + time-step with the random effects of microcosm and block.

Table A5. Output of final lmer for community local adaptation during environmental change.

Fixed effect / interaction	Mean square error	Numerator degrees of freedom	Denominator degrees of freedom	F value	p value	Deviance explained (%)
time-step	0.0013	7	261	54.66	<0.001	29.25
warming treatment	$1x10^{-5}$	1	261	1.60	0.21	0.12

immigration treatment	$1x10^{-5}$	1	261	0.23	0.63	0.02
Assay temperature	0.0025	1	261	105.6	<0.001	8.07
warming X assay temperature	0.001	1	261	41.38	<0.001	3.16
Warming X time-step	0.0008	7	261	4.80	<0.001	2.57
Assay temperature X time-step	0.0081	7	261	48.57	<0.001	25.99
Immigration treatment X assay temperature	0.0003	1	261	12.64	<0.001	0.97
warming X time-step X assay temperature	0.0001	7	261	3.77	<0.001	2.02

The minimum adequate model was warming X time-step X assay temperature + migration X assay temperature with the random effects of microcosm and block.

Table A6. Output of lmer for local adaptation of a subset of communities (replicates 1, 3 and 5) at day 81.

Fixed effect / interaction	Mean square error	Numerator degrees of freedom	Denominator degrees of freedom	F value	p value	Deviance explained (%)
Assay conditions	$5x10^{-6}$	1	3	0.77	0.45	0.82
Immigration treatment	$6x10^{-6}$	1	3	0.83	0.43	0.89
Warming treatment	$2x10^{-6}$	1	3	0.33	0.61	0.35
Assay conditions X warming treatment	$9x10^{-5}$	1	3	13.14	0.036	14.12
Assay conditions X immigration treatment	$4x10^{-8}$	1	3	0.006	0.94	0.01

The minimum adequate model was warming treatment X assay temperature + immigration treatment X assay temperature with the random effects of microcosm and block.

Table A7. Output of lmer of local adaptation of communities and isolates.

Fixed effect / interaction	Mean square error	Numerator degrees of freedom	Denominator degrees of freedom	F value	p value	Deviance explained (%)
Warming treatment	3.13	1	8	21.74	<0.01	29.96
Type of population	2.67	1	8	18.57	<0.01	25.58
Warming x type of population	1.19	1	8	8.26	0.021	11.39

The minimum adequate model was warming treatment X type of population (community or isolates) with microcosm as a random effect.

Table A8. Output of lmer of change in metabolic function using a subset of the full dataset for which carbon substrates are identified as recalcitrant or labile.

Fixed effect / interaction	Mean square error	Numerator degrees of freedom	Denominator degrees of freedom	F value	p value	Deviance explained (%)
time-step	1.20	2	453	38.13	<0.001	6.77
immigration treatment	0.14	1	453	4.28	<0.05	0.38
structure	0.02	1	453	0.70	>0.05	0.06
warming treatment	0.93	1	453	29.61	<0.001	2.63
immigration X time-step	0.14	2	453	4.32	<0.05	0.77
structure X time-step	0.21	2	453	6.51	<0.01	1.16
immigration X structure	0.02	1	453	0.54	>0.05	0.05
warming X time-step	0.56	2	453	17.82	<0.001	3.16
warming X structure	0.13	1	453	3.97	<0.05	0.35
warming X time-step X structure	0.15	2	453	4.79	<0.01	0.85

Structure was included as a two-factor explanatory variable in the model and the models were compared using ANOVA. The model including structure explained the data significantly better (p<0.001) indicating that structure should be included as an explanatory variable. The

output of the minimum adequate model is qualitatively the same as the lmer using the full data set (Table A2).

Text A1 T-RFLP protocol

Total nucleic acid was extracted from 1ml of homogenised sample using methods previously described by Griffiths et al. (2000). The 16S genes were then amplified using PCR. 2µl of extracted DNA was mixed with the forward primer 63F (5′-CAGGCCTAACACATGCAAGTC-3′) labelled at the 5′ end with 6FAM, the reverse primer 519R (5′- GWATTACCGCGGCKGCTG-3′) and molecular grade water and added to PCR beads (illustra PureTaq^{TM}Ready-To-Go^{TM}, GE Healthcare, USA). This mixture was then heated to 95°C for 2mins and cycled 34 times through 1min at 95°C, 1min at 55°C and 2mins at 72°C and then was maintained for 10mins at 72°C before finally being cooled to 4°C. Each sample was amplified three times using PCR and the resulting products were combined for PCR clean up. SureClean (Bioline, UK) was added at an equal volume to that of the PCR product, this was centrifuged at 4°C and 3000rpm for 30mins, the supernatant removed, 100µl cold 70% ethanol added, this was centrifuged again and the supernatant removed. Any ethanol remaining was removed by evaporation and the precipitated DNA was dissolved in 10µl of molecular grade water for 1h. The samples were then digested by adding 2µl of the sample to the restriction enzyme MSP1, BSA, restriction endonuclease buffer and molecular grade water and incubating at 37°C for 2 hours. Subsequent fragment analysis was carried out using an ABI 3130xl Genetic Analyzer (Applied Biosystems) and the resulting data were analysed using GeneMarker software.

Text A2 Analysis of T-RFLP and metabolic profile data using multivariate statistics

Metabolic data was close to linear so it was analysed using principal components analysis (PCA). The distances between samples in the PCA plots reflect their Euclidian distances in

multivariate space and retain information on the relative differences between samples. Therefore we used the response variable of distance moved in multivariate space to compare changes in metabolic profile during the environmental change experiment. Again, lmers were used to account for the repeated sampling of the same microcosm and experimental blocking. Data on community profiles obtained from T-RFLP analyses were non-linear and contained many zero values therefore nonmetric multidimensional scaling (NMDS) was used to visualise similarities/differences in community composition between treatments. NMDS preserves rank differences, not absolute differences, and therefore it is not appropriate to compare distances moved in multidimensional space between treatments (Ramette 2007). We used the function envfit in the Vegan package to analyse and display the influence of the explanatory variables on the position of samples in the ordination plot.

Text A3 Principal components calculated from Biolog assays

Principal component 1 represents the change in mean OD and therefore indicates how well communities could metabolise the carbon sources in general.

Principal component 2 represents change in the ability to metabolise a number of carbon sources, in particular it indicates a decrease in the ability to metabolise sugar alcohols (e.g. D-melibiose and methyl-D-glucoside) and some organic acids (e.g. D-galacturonic acid and D-glucosaminic acid) and an improvement in metabolism of polymers (e.g. tween), amino acids (e.g. L-alanine, L-leucine, L-ornithine and L-proline) and some organic acids (e.g. keto buteric acid, keto valeric acid, propionic acid and hydroxybuteric acid).

Similarly, principal component 3 represents a number of carbon sources and indicates a decrease in the ability to metabolise the polymer tween, some sugar alcohols (e.g. xylitol and mono-methyl-succinate) and some organic acids (e.g. keto valeric acid) and an improvement

in the metabolism of some organic acids (e.g. citric acid, quinic acid and D-saccharic acid) and some amino acids (e.g. hydroxyl-L-proline, L-alanine and amino butyric acid).

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Figure Legends

Figure 1. Growth and metabolic function of microbial communities throughout the warming experiment. A) Total community growth and **B)** metabolic functional diversity throughout the experiment. Error bars are ± standard errors of community growth of 5 replicate communities and of metabolic function of 3 replicate communities. Black lines represent communities exposed to ambient conditions and red line represent communities exposed to experimental warming. Solid lines denote communities that received immigration and dashed lines show those that did not receive immigration.

Figure 2. Change in principal components representing metabolic function during the environmental change experiment. The boxplots represent changes in PC1, PC2 and PC3 over time of 3 replicate communities in each treatment compared to the starter community. A value of zero indicates no change. PC1 represents a decrease in overall metabolism, PC2 represents change in the ability to metabolise a number of carbon sources, in particular a decrease in the ability to metabolise sugar alcohols (e.g. D-melibiose and methyl-D-glucoside) and some organic acids (e.g. D-galacturonic acid and D-glucosaminic acid) and an improvement in metabolism of polymers (e.g. tween), amino acids (e.g. L-alanine, L-leucine, L-ornithine and L-proline) and some organic acids (e.g. keto buteric acid, keto valeric acid, propionic acid and hydroxybuteric acid). PC3 represents a decrease in the ability to metabolise the polymer tween, some sugar alcohols (e.g. xylitol and mono-methyl-succinate) and some organic acids (e.g. keto valeric acid) and an improvement in the metabolism of some organic acids (e.g. citric acid, quinic acid and D-saccharic acid) and some amino acids (e.g. hydroxyl-L-proline, L-alanine and amino butyric acid).

Figure 3. Community growth in ambient and warmed conditions throughout experimental warming. Error bars display ± the standard error of the mean total growth of 5 communities. Black lines represent communities exposed to ambient conditions and red lines

represent communities exposed to experimental warming. Solid lines denote communities that received immigration and dashed lines show those that did not receive immigration. Each panel shows total growth of communities sampled on a given day into the evolution experiment and assayed in warmed and ambient assay conditions.

Figure 4. Mean growth of communities and isolates sampled at day 81 in different assay conditions. Error bars display ± the standard error of the mean total growth of 3 communities and of mean growth of isolates sampled from those 3 communities. **A)** Total growth of communities in warmed and ambient assay conditions, **B)** total growth of isolates in warmed and ambient assay conditions.

Figure A1. Fluctuations in temperature during the experiment. Shaded bars show ± standard error in mean temperature of 3 temperature loggers. Upper sets of lines show temperature of the warmed treatment recorded every 10 minutes and lower lines show the temperature of the ambient treatment. Days since last sampling are indicated on the x-axis, vertical lines correspond to 10am on a given day. The mean temperature of control communities decreases during the experiment and that of warmed communities remains close to initial temperatures, hence there was a divergence in mean temperatures over the course of the experiment.

Figure A2. Divergence in mean temperature for the warmed and ambient treatments. Points display the difference in mean temperatures of the ambient and warmed treatments at each sampling day.

Figure A3. Growth curves of whole communities measured at each time-step of the environmental change experiment. Black lines represent communities that experienced ambient conditions and red lines are those that were warmed, growth curves of immigration and non immigration treatments are combined in the plots, there are 5 replicates for each

treatment. During time-steps 2 to 6 (days 18 to 54) growth curves for both warming treatments are generally monod-shaped with rapid initial growth followed by an asymptote. In the warmed communities this non-linear growth pattern is maintained but in ambient communities growth curves become linear.

Figure A4. Relationship between V_{max} and total growth rates in the first and second half of the environmental change experiment. Red dots and lines represent warmed communities and black dots and lines ambient communities. A) V_{max} and total change in optical density on sampling days 18, 27, 36 and 45. B) V_{max} and total change in optical density on sampling days 54, 63, 72 and 81.

Figure A5. The effect of immigration on the metabolism of recalcitrant and labile compounds. Error bars show ± standard error of 3 replicate communities sampled at 27, 54 and 81 days into the evolution experiment. Dark bars show the change in metabolism of recalcitrant compounds and light bars represent that of labile compounds.

Figure A6. Relationship between total metabolic function and community composition. Circles represent communities that received immigration and crosses represent closed communities.

Figure A7. Growth of isolates and communities in ambient and warmed assay conditions. Each panel shows community and isolate total growth within a microcosm. Solid blue lines show the total growth of communities in ambient and warmed assay conditions and blue dotted lines show the mean growth of the isolates (9 or 10 isolates per community), which are denoted individually by the red and black solid lines.

Figure

A

Mean total growth of communities

0.00
0.01
0.02
0.03
0.04

18 27 36 45 54 63 72 81

Days into experimental evolution

Warming Treatment
Ambient
Warmed

Migration Treatment
Immigration
No immigration

B

Change in mean metabolic diversity compared to starter community

−0.1
−0.2
−0.3
−0.4

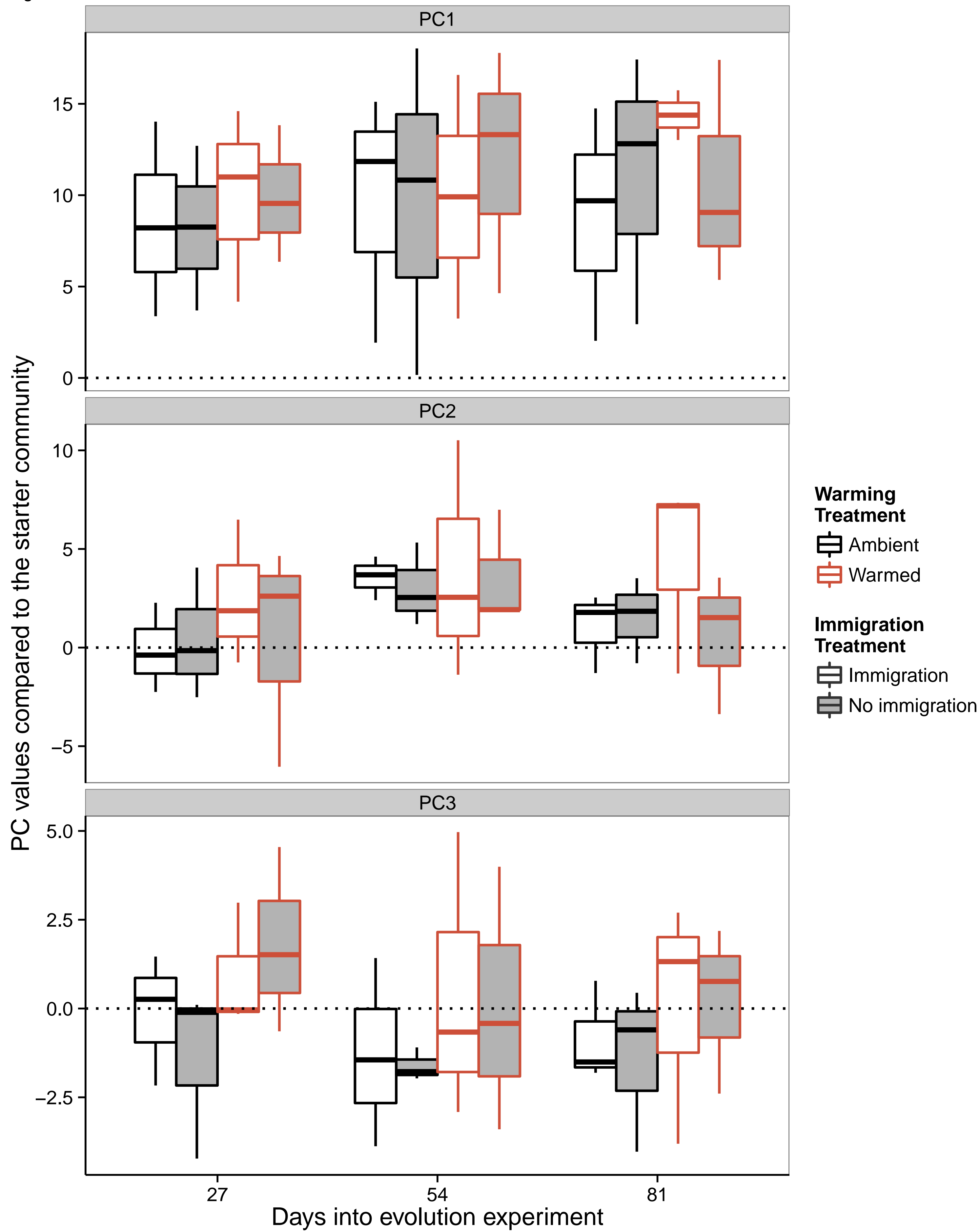
18 27 36 45 54 63 72 81

Days into experimental evolution

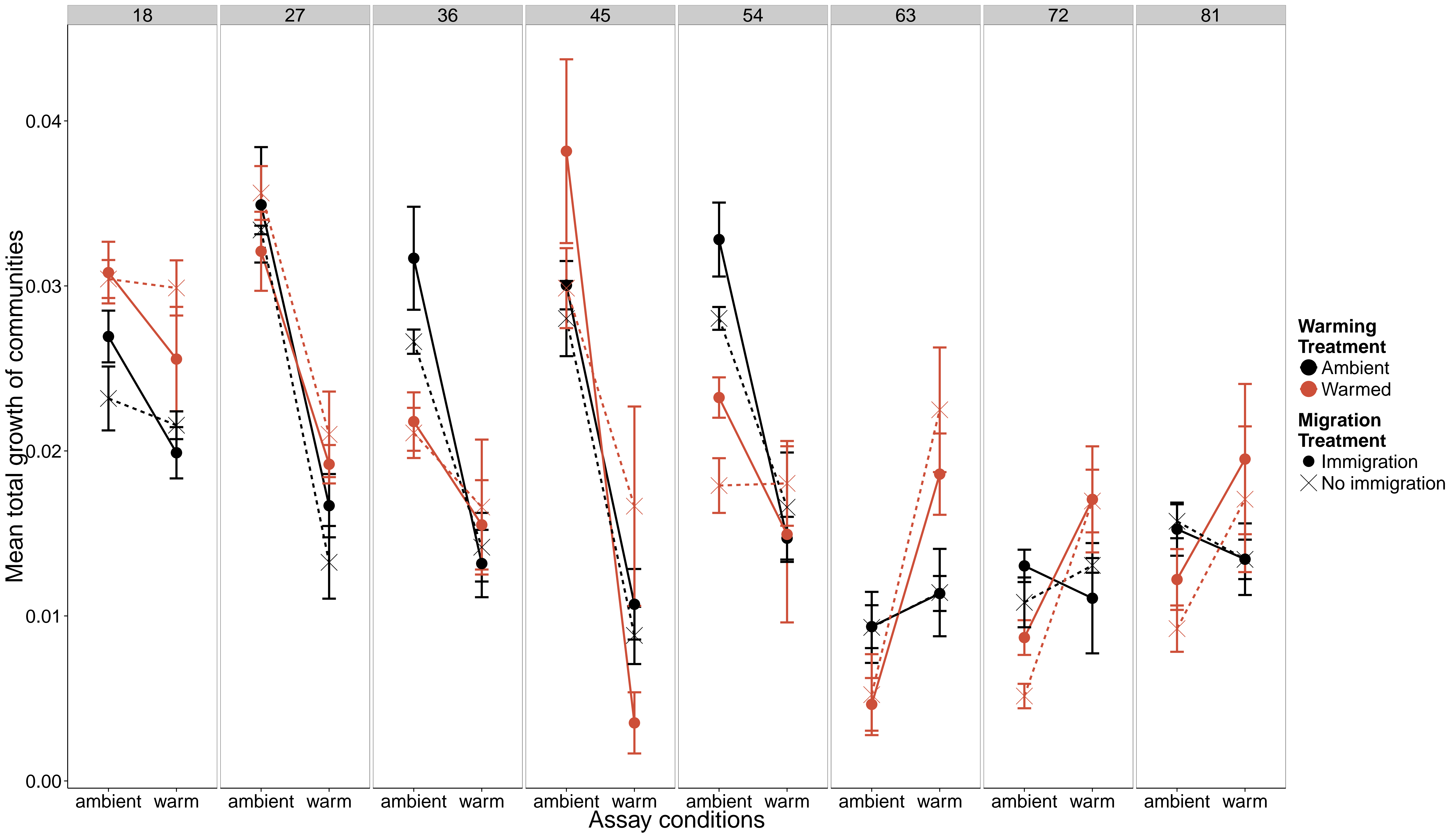
Warming Treatment
Ambient
Warmed

Migration Treatment
Immigration
No immigration

Figure



Figure



Figure