The ecological and evolutionary responses of groups of species to environmental change

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DECLARATION OF ORIGINALITY:

This thesis is the result of my own work and includes no work done in collaboration with or by others, except where specifically stated.

Diane Lawrence, April 2013

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ABSTRACT

The natural environment is being altered by anthropogenic activity at an unprecedented rate. The effects of climate change and damage caused by other human activities show little sign of decelerating. The ubiquity of the forecast changes means that all species are likely to face alterations in their environment and consequently may have to migrate or adapt to avoid extinction. Yet, as species respond they may initiate additional ecological changes, creating further selection pressures on species they interact with. Research has shown that ecological and evolutionary dynamics such as these occur on the same time-scale and produce dynamics that cannot be explained if evolution is ignored. Thus, to understand and predict how species will respond to environmental change, it is important to consider both ecology and evolution and the feedback between them. In this thesis I investigate the ecological and evolutionary responses of groups of species to a number of environmental changes using experimental evolution with naturally co-occurring bacteria and mathematical modelling. After reviewing and introducing the field in Chapter 1, in Chapter 2 I investigate the effect of species diversity on evolution to a novel environment. In Chapters 3 and 4 I report on the results of a field experiment in which I manipulated immigration into diverse microbial communities while exposing them to experimental warming. Specifically, in Chapter 3 I investigate how warming and immigration affect local adaptation and also whether the local adaptation of the community is predictable from that of component species and in Chapter 4 I discuss the impact of warming on predictability. Finally, in Chapter 5 I use mathematical modelling to explore how species' evolutionary responses to stress affect their interspecific interactions. My research places particular emphasis on the importance of species interactions to evolution and, furthermore, how these changes in biotic interactions affect ecosystem functioning.

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Chapter 1:

THESIS INTRODUCTION

1.1 Background

Anthropogenic activities are expected to cause a suite of abiotic changes to the environment. Climate change is predicted to increase mean global temperature and to make extreme events such as drought and flooding more frequent (Parry et al. 2007). Furthermore, as atmospheric CO_2 concentration increases, more CO_2 is expected to become dissolved into the oceans causing acidification. Acidification of the Arabian Sea has already caused inhibition of calcification rates in planktic foraminifera resulting in shell thinning (de Moel et al. 2009) and the global consequences of ocean acidification are predicted to be widespread (Sinutok et al. 2012). In addition to the global effects of climate change, localised anthropogenic activities such as agricultural intensification and urban development are having impacts on terrestrial and freshwater ecosystems, for example, through changes in land use (Foley et al. 2005), nutrient enrichment and warming (Allan 2004; Woodward et al. 2012). The detrimental effects of eutrophication on lake and stream diversity and ecological functioning have been well studied (Smith et al. 1999) and eutrophication has even been demonstrated to be responsible for reversals in speciation of whitefish by removing ecological niches (Vonlanthen et al. 2012). Furthermore, there is evidence that warming is having direct impacts on food provision. For example, in Lake Tanganyika fish yields have been estimated to have been reduced by up to 30% due to climate warming and, in this case, the effect of climate change on ecosystem services has been greater than the impact of overfishing (O'Reilly et al. 2003). Environmental changes such as these mean that in the near future species may face novel conditions which they cannot tolerate. There is growing concern about how species will cope with environmental change (Walther 2010) and how best to protect ecosystems at risk (Montoya and Raffaelli 2010).

Broadly speaking, when faced with environmental change species can either respond in an ecological or evolutionary way. Ecological responses to environmental changes include altering abundance (e.g. Parmesan and Yohe 2003), phenology (Both et al. 2009) or range (Davis et al. 2005). Responses such as these can indirectly affect other species through both biotic and abiotic effects (Suttle et al. 2007; van der Putten et al. 2010). For example, in a study using freshwater mesocosm communities, warming was found to alter community composition resulting in an increase in the size structure of the community. This made the transfer of energy between trophic levels inefficient and caused a decrease in microbial decomposition rates (Dossena et al 2012). In addition to ecological responses, species may react to environmental change by evolutionary adaption (e.g. Franks and Weis 2008; Parmesan 2006). It is now appreciated that evolution can take place over short timescales which are relevant when thinking about the responses of groups of species to environmental change (Thompson 1999), however, there is concern about whether species will be able to adapt at a fast enough rate to keep pace with climate change (Berteaux et al. 2004). The ability of component species in a community to adapt to environmental change is likely to depend on many factors including the type, rate and extent of change and the influence of species interactions. Elucidating what factors make species more likely to be able adapt to survive environmental change is a key challenge for the future if we are to protect species and ecosystem services that we value.

In the past decade there has been growing appreciation that ecological and evolutionary dynamics occur on the same time-scale and that there is an on-going interplay between them (Yoshida et al. 2003). Because species face selection pressure from the abiotic environment and the other species they interact with, this makes predicting the effect that biotic interactions will have on adaptation challenging. Some experiments using two species have found that antagonistic coevolution can promote rapid adaptation through coevolutionary arms races (Pal et al. 2007), and alternatively, theoretical studies indicate that trade-offs or niche conservatism due to competition could limit adaptation in diverse communities (de Mazancourt et al. 2008; Johansson 2008). As almost all species interact with many others this is an issue that needs further research.

Our understanding of species' adaptation to has come in a large part from experimental evolution in which evolution can be monitored over very short time-scales. The most famous experimental evolution study, observing the adaptation of *Escherichia coli* to a novel environment, has been running continuously since it was initiated by Lenski in 1988 (e.g. Lenski 2004; review of experimental evolution Kawecki et al. 2012). To date the 12 replicate populations of bacteria have undergone over 57000 generations and have revealed numerous insights about the adaptive process including how the rate of mutation changes over time (Sniegowski et al. 1997), the importance of key innovations (Blount et al. 2008) and repeatability in evolution (Woods et al. 2006). This experiment inspired many others and experimental evolution has been used to investigate processes as diverse as adaptive radiation (Brockhurst et al. 2007a), the evolution of sex (Becks and Agrawal 2010) and the evolution of multicellularity (Ratcliff et al. 2012). The value of experimental evolution stems from the use of organisms with short generation times and large population sizes meaning that mutations may arise frequently and therefore evolution can be rapid. Furthermore, experimental evolution typically uses organisms which can be frozen so that the ancestral and evolved strains can be compared thereby providing a time-line of evolutionary changes.

The ability of a single species in isolation to adapt to a new or changing environment has been much investigated using an experimental evolution approach (e.g. Lenski 2004; Barrett et al. 2005; Bell and Gonzalez 2009). More complex scenarios have been studied less commonly no doubt because doing so contradicts one of the benefits of experimental evolution - that the highly simplified environment means that external influences can be discounted and the mechanisms behind adaptation fully explored. But if we wish to understand how species and ecosystem services may be affected by environmental change it would be beneficial to perform experimental evolution studies under more natural conditions. Accordingly, there is presently movement towards using experimental evolution to explore more complex scenarios. For example, Gómez and Buckling (2011) have investigated antagonistic coevolution of their well understood bacteria-phage system in soil and found that rapid coevolution occurred even in this spatially complex environment. Other studies have enhanced biological realism by increasing the genetic complexity of the interacting organisms. For example, in

an experiment with three genotypes of the algae *Chalamydomonas reinhardtii* Collins (2011) showed that the abiotic stress of rising CO_2 coupled with intergenotypic competition caused a trade-off between adaptation to the environment and adaptation to compete. Experimental evolution is also being performed in the field. For example, using dormant propagules of daphnia from lake soil cores Urban et al. (2012) are able to compare the adaptation of contemporary and historical daphnia to experimental warming. Despite the progress being made there are still gaps in our understanding of evolution in groups of species. For example, it is not clear how important processes that have been found to be influential in laboratory experiments, such as immigration and coevolution, are to the evolution of species and resulting community functioning in the wild. Therefore, experiments of these types, which incorporate further complexity, are valuable to help us establish whether findings from simple laboratory experiments can be extrapolated to explain evolutionary dynamics in the natural environment.

One of the most effective ways that ecologists have to protect species and ecosystems from the detrimental effects of climate change is to make predictions about their likely responses to environmental change so that management efforts can be focussed in the correct way (Millar et al. 2007). Unfortunately, predicting ecological and evolutionary responses to environmental change is not straightforward. For example, theory predicts that as environments become more stressful interactions between species may be altered (Bertness and Callaway 1994) and, furthermore, it has been found that environmental disturbances themselves may reduce the predictability of responses (Murphy and Romanuk 2012). Thus, theories developed by studying species and ecosystems under relatively benign conditions could be rendered inaccurate in the future as climate change causes environmental conditions to become increasingly stressful. So, to ensure effective management of ecosystems under environmental change it is important for research to be carried out into the consequences of abiotic stress on traits such as species interactions and response predictability.

Beyond understanding how groups of species may respond to environmental change it is important to recognize the implications these changes in community dynamics or composition may have for ecosystem function and ecosystem services. If environmental changes cause extinctions, for example,

this could have a negative impact on the productivity of a community. In the case of river algae communities, decreases in diversity can reduce the rate at which water is denitrified (Cardinale 2011). Or, if extinction is biased towards species with certain traits, such as slow growing specialist bacteria which decompose recalcitrant compounds, this may affect ecosystem function in ways that could not predicted without prior knowledge of the system (McGuire and Treseder 2010). For these reasons it is important to gain as much knowledge as possible of the ways groups of species might respond to environmental change before it is too late to mitigate the potential negative impacts on biodiversity and ecosystem services.

1.2 Aims and Thesis Outline

The broad aims of this thesis are to investigate how biotic interactions affect adaptation of species in diverse communities exposed to environmental change and the impacts this has on ecosystem functioning and, furthermore, to consider the effects that warming has on the predictability of ecological processes. My work addresses a number of gaps in the literature in a variety of distinct but related areas, below I briefly discuss the thesis chapters.

Chapter 2: investigates the effect that species diversity has on adaptation to a novel environment using experimental evolution with bacteria. I show that in diverse communities species interactions led to niche partitioning and facilitation through cross-feeding. This resulted in enhanced productivity in communities composed of coevolved species compared to communities composed of species that evolved in isolation.

Chapter 3 uses a field experiment with diverse microbial decomposer communities to test the hypotheses that immigration will enhance adaptation to experimental warming and that the local adaptation of communities is predictable from that of constituent isolates. I find that immigration is beneficial to community growth in ambient but not warmed communities and I suggest that this may be due to the immigration of maladapted individuals from the surrounding species pool swamping evolution in warmed communities. Furthermore, I find that the local adaptation of the community is

not predictable from that of the isolates that constitute it indicating the growth of isolated species may not accurately reflect how community function is impacted by environmental change.

Chapter 4 uses results from the same field experiment to test the hypothesis that warming decreases the predictability of responses. In agreement with the hypothesis, communities and isolates exposed to experimental warming had less replicable responses than those that experienced ambient conditions. My results indicate that warming itself may alter ecosystem properties and make responses to further environmental changes increasingly difficult to predict.

Chapter 5 uses mathematical models to investigate the stress-gradient hypothesis (SGH) which posits that as an environment becomes more stressful the importance of facilitative interactions increases relative to competitive interactions. Specifically, I aim to discover whether the SGH could be a more prevalent occurrence than is currently recognised by testing whether it can arise through evolution of fundamental interactions such as resource use. My results indicate that as abiotic stress increased species became more specialised in their resource use and this led to an increase in community-level facilitation. Furthermore, the mortality caused by allelopathy also declined as stress intensified. These results lend support to the idea that the SGH could be a widespread phenomenon.

Chapter 2:

SPECIES INTERACTIONS ALTER EVOLUTIONARY RESPONSES TO A NOVEL ENVIRONMENT

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2.1 Abstract

Studies of evolutionary responses to novel environments typically consider single species or perhaps pairs of interacting species. However, all organisms co-occur with many other species, resulting in evolutionary dynamics that might not match those predicted using single species approaches. Recent theories predict that species interactions in diverse systems can influence how component species evolve in response to environmental change. In turn, evolution might have consequences for ecosystem functioning. I used experimental communities of five bacterial species to show that species interactions have a major impact on adaptation to a novel environment in the laboratory. Species in communities diverged in their use of resources compared with the same species in monocultures and evolved to use waste products generated by other species. This generally led to a trade-off between adaptation to the abiotic and biotic components of the environment, such that species evolving in communities had lower growth rates when assayed in the absence of other species. Based on growth assays and on nuclear magnetic resonance (NMR) spectroscopy of resource use, all species evolved more in communities than they did in monocultures. The evolutionary changes had significant repercussions for the functioning of these experimental ecosystems: communities reassembled from

isolates that had evolved in polyculture were more productive than those reassembled from isolates that had evolved in monoculture. My results show that the way in which species adapt to new environments depends critically on the biotic environment of co-occurring species. Moreover, predicting how functioning of complex ecosystems will respond to an environmental change requires knowing how species interactions will evolve.

2.2 Introduction

Understanding how species adapt to novel environments is an important task both for understanding the dynamics of living systems and for predicting biotic responses to anthropogenic changes in the natural environment (Winder and Schindler 2004; Davis et al. 2005; Berg et al. 2010). However, most studies of evolutionary adaptation consider single species in isolation. Although this approach is useful for uncovering genetic mechanisms, virtually all species co-occur with many other species. Faced with a new abiotic environment, communities might respond by evolution of component species, but ecological changes in species' abundances and distributions can also occur. If ecological interactions such as competition affect evolutionary responses (de Mazancourt et al. 2008; Johansson 2008), then results from single species studies might not accurately predict evolutionary dynamics in diverse assemblages.

Although there has been growing interest in how evolution affects ecological dynamics (Yoshida et al. 2003; Fussmann et al. 2007; Ozgul et al. 2009; Gravel et al. 2011), most studies have still considered single species or pairs of interacting species. In addition, the question of how ecological interactions affect evolutionary responses to novel abiotic environments has received even less attention (Thompson 2005; Siepielski and Benkman 2007). If ecological interactions among species are weak, then evolutionary changes should be the same as those predicted in single species studies. However, if species use overlapping resources or otherwise interact, the extent and type of evolutionary responses might differ from those predicted if the same set of species each adapted to the new abiotic conditions in isolation (Schluter et al. 1985; Liow et al. 2011).

Several mechanisms might influence evolutionary dynamics in mixtures of species. First, species in diverse communities might have their resource use restricted by competitors, lowering effective population sizes and therefore reducing the rate at which beneficial mutations arise and the species adapts to a novel environment (Rich et al. 1979; Johansson 2008; Zhang and Buckling 2011). In this scenario, species in communities should adapt to the new environment as they would in isolation, but

the rate of adaptation would be reduced. Second, if trait variation among species exceeds variation within species, a new abiotic environment might act on the relative abundance of different species (ecological sorting) rather than on genetic variation within species (de Mazancourt et al. 2008; Johansson 2008). In models of this mechanism, pre-adapted species increase in abundance at the expense of less well-adapted species and the average amount of evolution in surviving species is typically reduced compared to responses of the same species in monoculture (although in rare scenarios the amount of evolution can increase de Mazancourt et al. 2008). Third, there might be a trade-off between adaptation to biotic and abiotic components of the environment (Collins 2011). Such trade-offs might result from the production of costly adaptations involved in species interactions such as defences (Agrawal et al. 1999; Fine et al. 2006) or from selective interference between adaptations to the biotic and abiotic environment (Ridenhour 2005). In this case, species that evolved in communities should be less well adapted to the abiotic environment than if they adapted in isolation and vice versa. In the most extreme case, species might adapt to use resources generated by other species (Benkman and Parchman 2009), in which case they will evolve entirely different resource use depending on whether other species are present.

These mechanisms could change both the magnitude and direction of evolutionary change in communities compared to predictions from single species studies. However, evidence for an effect of diversity is currently scarce. Experiments have shown that diversity can inhibit evolution; for example, Brockhurst et al. (2007) showed that niche occupation restricts adaptive radiation of a single bacterial strain. Similarly, Collins (2011) found that diversity limits adaptation to elevated CO_2 in algae and Perron et al. (2011) showed that diversity limits the evolution of multi-drug resistance (although this effect was alleviated by horizontal transfer of resistance mutations). However, these studies considered genetic diversity within species rather than species diversity within communities. There is abundant evidence that coevolution drives fast evolution between species with strong ecological interactions (Buckling and Rainey 2002; Brockhurst et al. 2003) and that pairwise coevolution can change the direction of evolution compared to adaptation in isolation (Schulter et al. 1985). Furthermore, studies of diffuse coevolution have shown that adaptation of a focal species to

particular interacting species, such as insect herbivores, is influenced by interactions with other species, such as vertebrate herbivores (Iwao and Rausher 1997; Stinchcombe and Rausher 2001; Strauss et al. 2005; Siepielski and Benkman 2010). For example, character displacement of limnetic and benthic species pairs of sticklebacks only occurred in lakes with low species diversity of other fish (Ormond et al. 2011). To the best of my knowledge, however, the evolution of interactions among multiple species in a community has not been investigated using an experimental evolution approach.

Evolutionary dynamics in diverse systems will have important consequences for ecosystem functioning in altered environments. Ecosystem functions such as decomposition and productivity emerge from the degree to which species are adapted to their biotic and abiotic environments (Cadotte et al. 2008; Harmon et al. 2009; Bassar et al. 2010). Following a change in the environment, ecosystem functioning might be disrupted either because the species abundances change or because component species fail to adapt to the new environmental optimum. Alternatively, coevolution among species might act to enhance ecosystem properties, for example if species evolve complementary resource use and thereby increase ecosystem productivity (Hillesland and Stahl 2010; Poltak and Cooper 2011). Understanding of these processes is needed to predict how ecosystem functioning will respond to environmental changes over evolutionary timescales.

Here, I test whether species diversity influences environmental adaptation and ecosystem functioning using naturally co-occurring decomposer bacteria from temporary pools around the roots of beech trees (*Fagus sylvatica*), which have previously been used successfully for experimental ecology (Bell et al. 2005; Bell 2010). I chose five species of bacteria differing in colony colour and shape so that each species could be isolated from species mixtures (Appendix 2.1 and 2.2). Sequencing of 16S rDNA showed that isolates belong to five different families (Appendix 2.1). I refer to them as species since they represent genetically and phenotypically distinct clusters that co-occurred naturally. Monocultures of each species and polycultures containing all five species were allowed to adapt to laboratory conditions by regular serial transfer on beech-leaf extract (Figure 2.1). Laboratory

conditions represent a new environment and differ from wild tree-holes in several ways: tree-holes receive a larger quantity and variety of resources, are spatially complex, and have an unpredictable input of water and leaves, whereas laboratory cultures experienced regular dilution with uniform medium in a shaken container. Growth assays were used to determine evolutionary responses. I predicted that species should adapt to laboratory conditions by evolving faster growth rates on the beech tea medium, but that the presence of other species might change the direction and extent of adaptation by one of the mechanisms outlined above.

To measure species interactions and changes in resource use, my approach was to grow one species on beech tea, then to filter-sterilize the medium and to assay the growth of a second species on the "used" beech tea. If the second species used similar resources to the first (i.e., if their niches overlapped), the second species should grow less well on "used" beech tea than on "unused" beech tea because its resources would have been consumed. If the two species were specialized on different resources (i.e., occupied different niches), the second species should grow equally well on "used" and "unused" tea. Finally, if the second species used resources produced by the first (called facilitation or cross-feeding (Turner et al. 1996)), the second species should grow better on "used" tea than on "unused" tea. While this method does not provide direct information on competitive interactions in mixtures, it provides a tractable and reproducible measure of changes in resource use of each species during evolution. Because other types of interaction, such as direct inhibition by bacteriocides (Riley and Wertz 2002), might also affect growth rates, I also used nuclear magnetic resonance (NMR) spectroscopic profiling of "used" and "unused" tea to investigate changes in resource use directly.

Finally, I tested whether adaptation to the presence of other species affected productivity (rate of production of CO_2) by reassembling communities with different evolutionary histories using isolates that either evolved in monoculture or co-evolved in the same polyculture. If adaptation increased community productivity, I expected communities reassembled with isolates that evolved in polycultures to be more productive than those reassembled with isolates that had evolved in monoculture.

2.3 Materials and Methods

2.3.1 Species and media

Bacteria were isolated and sequenced by Francesca Fiegna using the methods described below. Bacteria were isolated from single colonies from temporary pools formed by the roots of a beech tree at Silwood Park, Berkshire, United Kingdom, in November 2008 (Appendix 2.3). BLAST and Ribosomal Database Project (Altschul et al. 1990) matches and photographs of colonies of each species are provided in Appendix 2.1 and Appendix 2.2. Species A and E belong to families Sphingobacteriaceae and Flavobacteriaceae, respectively (both in the phylum Bacteroidetes); species B and C belong to families Enterobacteriaceae and Pseudomonadaceae, respectively (both in the class Gammaproteobacteria of the phylum Proteobacteria); and species D belongs to the family Sphingomonadaceae (in the class Alphaproteobacteria of the phylum Proteobacteria). Note that my isolation protocol means that all of the bacteria are expected to be aerobic heterotrophs. Isolates were grown on beech-leaf tea prepared by autoclaving 50 g of autumn fall beech leaves in 500 ml of water and diluting the filtrate 32-fold (Bell et al. 2005).

2.3.2 Evolution experiment

Fifteen replicates of each species in monoculture and of each five-species community were set up following the protocol in Figure 2.1 and Appendix 2.3. The tubes were incubated at 25°C and shaken at 100 rpm. Every 3 and 4 d, 100 µl from each microcosm was transferred to 2 ml of fresh media for a total of 15 serial dilutions over 8 wk. Cell densities prior to transfer were estimated by colony counts on R2A agar. Bacteria were isolated from final cultures by plating on R2A agar, selecting single colonies, and re-suspending them in 1 ml of 1/32× beech tea. Isolates were stored at −84°C for use in subsequent assays.

2.3.3 Growth assays on unused and used beech tea

Growth assays were performed in 1 ml of 32× beech tea in 24-well plates inoculated with 250 µl of bacteria from a liquid culture grown up for 4 d from stored frozen isolates. The plates were kept at 25°C for 4 d without shaking and growth measured daily using OD_{600}. Readings were subtracted from negative controls of sterile medium placed on each column of the plate. Nine replicates were used for each Species×Treatment combination. "Used" beech tea was prepared by inoculating 14 ml of beech tea with 200 µl of single bacterial species and allowing growth at 25°C for 14 d. The first and second isolate used for each assay always belonged to the same treatment—that is, both ancestral, both monoculture, or both polyculture isolates. Substrates were then filter sterilized using a 0.2 µm membrane to remove bacterial cells and leave any unused nutrients in the substrate. Sterility was confirmed by plating on agar. Growth was measured as described for growth assays on unused beech tea for nine replicates of each Species×Substrate×Treatment combination.

2.3.4 Nuclear Magnetic Resonance (NMR) analyses

Samples of unused beech tea, tea used previously by one isolate, and tea used previously by one isolate and then a second isolate (as described in the previous section) were initially analysed by Volker Behrends and Jake Bundy using proton NMR. Because of the low concentration of carbon substrates in the beech tea, 5 ml of each sample were lyophilized and resuspended in 550 µl 90% $^{2}H2O$ (superscript numbers are atomic weights; i.e., $^{1}H2O$ is "normal" water and $^{2}H2O$ is deuterated) containing 1 mmol l^{-1} 3-(trimethylsilyl)propane-1-sulfonic acid (DSS) and, 5 mmol l^{-1} sodium azide. The $^{2}H2O$ provided a field frequency lock for the spectrometer and the DSS served as an internal chemical shift reference. Spectra were acquired on a Bruker 800 US^{2} NMR spectrometer (Bruker BioSpin), with a magnetic field strength of 18.8 T and resulting ^{1}H resonance frequency of 800 MHz, equipped with a 5-mm cryogenic probe. Spectra were acquired following the approach given in (Beckonert et al. 2007). Briefly, a one-dimensional NOESY pulse sequence was used for water suppression; data were acquired into 64 k data points over a spectral width of 12 kHz, with eight dummy scans and 256 scans per sample. Spectra were phased in iNMR 3.6 (Mestrelab) and exported

to Matlab 2010b (Mathworks) for further analysis. Distinct peaks were integrated and baseline-corrected using in-house scripts and assigned where possible using in-house databases. One resonance with a singlet at chemical shift $\delta = 3.22$ ppm was assigned as choline; a COSY spectrum of the unused medium showed a cross-peak at δ 4.05/3.52 ppm, as would be expected for the methylene protons of choline (although the resonances were too low intensity to be visible in the 1D spectra). To measure resource use or production, I calculated the size of each peak in medium obtained after the growth of an isolate minus the size of the peak in the medium before the species had grown on it. Positive values indicate net production of a compound and negative values indicate net consumption. I used correlation tests to identify correlated peaks with $r>0.95$, which might indicate multiple peaks derived from the same compound. Contaminant peaks derived from methanol and acetonitrile were removed from the dataset. I explored variation in resource use and production across isolates using principal components analysis of unscaled variances implemented with the prcomp() function in R (R Development Core Team 2010): I used unscaled rather than scaled variances to focus on compounds showing larger changes in their absolute concentrations.

2.3.5 Productivity of assembled communities

MicroResp kits were used to measure community respiration. Respired CO_2 results in a change in colour of cresol red indicator dye suspended above each well of a 96-well plate. Ten replicates were used per treatment in a single plate and the experiment was repeated in triplicate. Each well contained 840 µl of 1/32× beech tea and 40 µl of each species from a stock culture of standard density. The plate was sealed and the change in optical density (OD) at 570 nm of the indicator gel measured after 6 h as recommended by the manufacturers (Campbell et al. 2003). The change in OD of blank wells (filled with 1 ml 1/32× beech tea) was used to account for the base level of CO_2 in the vials. The rate of CO_2 respiration per ml of culture medium was calculated using the formula provided in the MicroResp manual (Campbell et al. 2003).

2.3.6 Statistical analysis

To calibrate OD_{600} in terms of cell density per ml of culture medium (Daalgard et al. 1994), I performed serial dilution and colony counts of stock cultures of isolates of each species from each treatment. I fitted a linear model with log (colony count)/ml as the response variable and species, treatment, and OD_{600} as explanatory variables, including interaction terms. The model simplified to retain species and OD_{600}, but no interaction terms (i.e., different intercept for calibration line for each species, but same slopes, $F_{4,67} = 32.9$, $p<0.0001$, $r^2 = 0.64$, Appendix 2.4). The fitted lines were used to calibrate in units of log(number of cells) per ml. I used linear mixed effects models of repeated measures of cell density over time to compare growth of bacteria among treatments and species in the growth assays (Appendix 2.3). To report the direction and effect size of differences among treatments, I used the rate of change in density over the first 48 h as a simple measure of V_{MAX}—that is, the maximum rate of growth from low densities (Appendix 2.5). Analysis of variance (ANOVA) and Tukey's Honest Significant Difference tests were used to identify significant contrasts between particular treatments of interest. There was no evidence of different evolutionary trends in carrying capacity of isolates (i.e., using density at 96 h) as opposed to growth rate (Appendix 2.6 versus Appendix 2.7). To test for significant differences in NMR profiles between treatments, I used Monte Carlo simulation tests shuffling profiles randomly among species and treatments. The Euclidean distance between samples was recorded, and the mean distance between both evolved treatments in turn and ancestral isolates was used to measure the amount of evolution, and the mean distance between each species within a treatment was used to measure the amount of divergence in resource use among species. Observed values were compared to randomised values from 10,000 random permutations. Two-tailed tests were used.

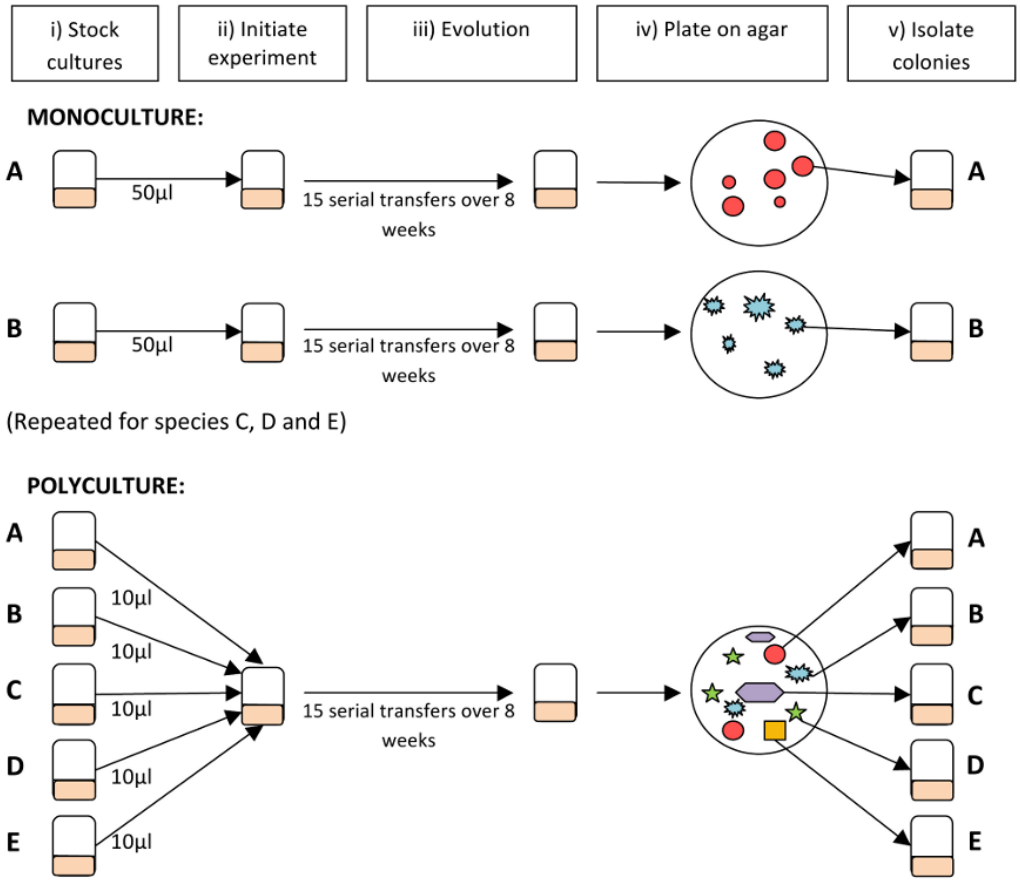


Figure 2.1. Experimental design for the evolution experiments. (i) Stocks of wild isolates were grown up, each comprising a single starting genotype of each species. **(ii)** Experiments were started with each species in monoculture or in polyculture (all five species mixed together). **(iii)** To stimulate active growth and promote adaptation to the laboratory conditions, each culture was diluted 20-fold in fresh medium twice weekly for 8 wk. Tubes were shaken to prevent the formation of biofilms and maintain spatial homogeneity. Numbers of generations ranged from 60.9 to 82.2 across cultures and effective population sizes ranged from 5.3×10^5 to 9.9×10^6 (Appendix 2.8). **(iv)** Final cultures were plated on agar. **(v)** Single colonies of each species were isolated for growth assays described in the main text.

2.4 Results

2.4.1 Growth rates on beech tea of monoculture isolates

Although able to grow on beech tea in the lab at the start of the experiment, one species (E) dwindled to low cell densities during the evolution experiment (Appendix 2.8) and was excluded from growth assays and subsequent experiments because it failed to re-grow from frozen cultures. Species A to D were recoverable in all treatments and were used for subsequent experiments. Across species, final isolates that evolved in monoculture grew on average faster than ancestral isolates of the same species on unused beech tea (dark bars, first and second rows, Figure 2.2), consistent with the prediction that they adapted to laboratory conditions of serial dilution in beech tea medium by increasing growth rates on this medium. The effect was significant in species B, C and D, which grew between 47% and 120% faster after evolving in monoculture compared to their ancestral isolates. Growth rates of evolved monoculture isolates of species A were not significantly different from its ancestral isolate. Note that phenotypic plasticity and parental effects can be discounted as explanations for differences among treatments. In all my assays, frozen isolates were first grown in beech tea medium for 4 d (= 4 to 6 generations, Appendix 2.8), and then an aliquot was taken from these cultures to start the assay cultures. Differences in phenotypes between treatments were therefore maintained after several generations of growth in identical environments and cannot be readily explained by phenotypically plastic responses.

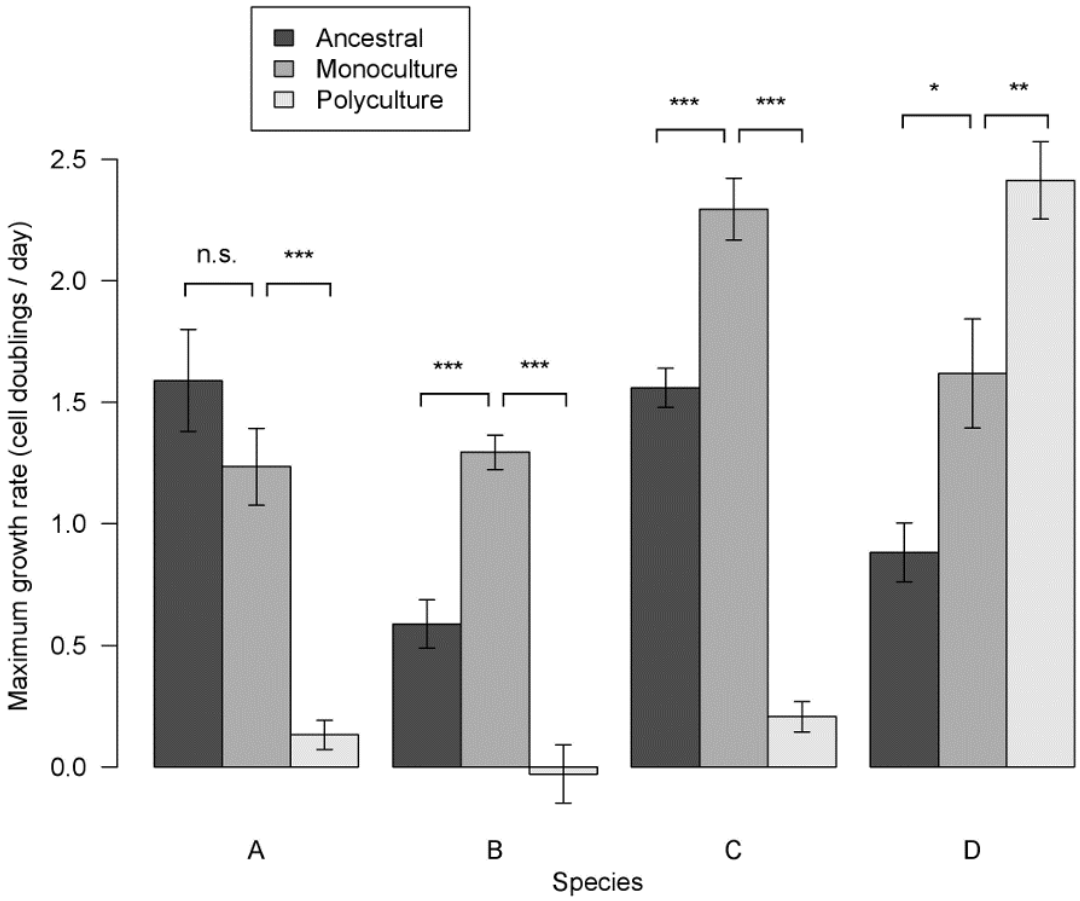


Figure 2.2. Maximum growth rates of isolates after evolution under each diversity treatment. Maximum rate of growth from low densities, V_{MAX}, of each species grown on unused beech tea under assay conditions. Dark bars, growth rates of ancestral isolates. Mid grey bars, growth rates of monoculture isolates. Pale bars, growth rates of polyculture isolates. Standard error bars are shown. Tukey Honest Significant Difference test contrasts between treatments: *** $p<0.001$, ** $p<0.01$, * $p<0.05$; n.s., not significant (see also Appendix 2.9). Species A evolved slower maximum growth rates in polycultures compared to its ancestral and monoculture isolates. Species B and C evolved faster maximum growth rates on unused beech tea in monocultures, but far slower maximum growth rates in polycultures compared to ancestral isolates. Species D evolved faster maximum growth rates in monocultures compared to its ancestral isolate and even faster maximum growth rates in polycultures.

2.4.2 Growth rates on beech tea of polyculture isolates

Isolates of species A, B, and C that evolved in polyculture grew significantly slower on unused beech tea than their corresponding ancestral isolates and than the isolates that evolved in monoculture (Figure 2.2). Growth rates were 87% to 100% slower after evolving in polyculture compared to the corresponding ancestral isolates. This is consistent with the existence of a trade-off between adaptation in the presence of other species and adaptation in the absence of other species; when evolving in the presence of other species, isolates of A, B, and C nearly lost the ability to grow on unused beech tea. In contrast, the polyculture isolate of species D grew significantly faster on unused beech tea than either ancestral or monoculture isolates. By adapting in the presence of the other species, species D evolved to grow at a faster rate on beech tea when assayed with other species absent. This result is not readily predicted by the general theories outlined in the introduction and is discussed further below.

2.4.3 Species interactions between ancestors and between isolates evolved in monoculture

Reduced growth of ancestral isolates on beech tea previously used by other ancestral isolates showed that species had generally negative interactions (Figure 2.3a, Appendix 2.6), as predicted if species used overlapping resources. The exception was species D, whose growth was not reduced on tea previously used by other species even though tea used by species D reduced the growth of other species (arrows towards species D on Figure 2.3a). This result might indicate that species D used a greater range of resources than the other species, but which included the resources used by the other species. Growth of monoculture isolates on tea previously used by monoculture isolates of other species showed that negative interactions among species were reinforced: now species D also grew significantly slower on tea previously used by other species (Figure 2.3b). These results would be expected if species in monoculture converged to use a more similar set of resources.

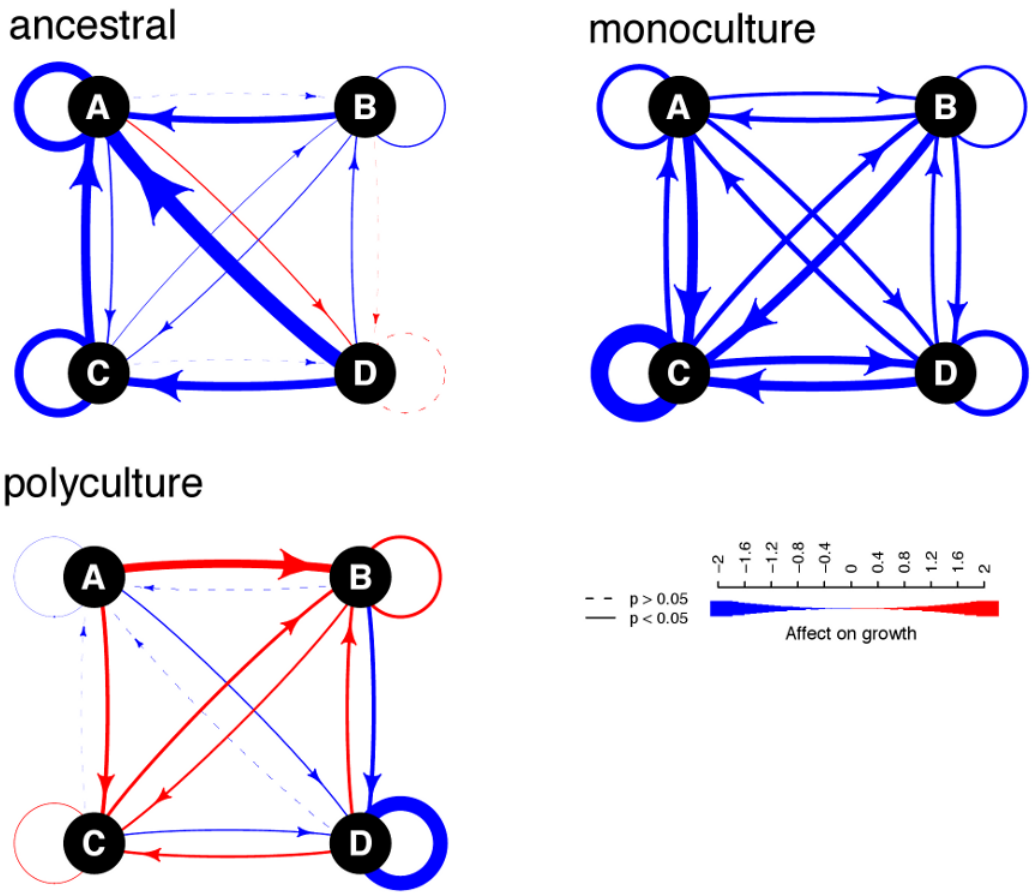


Figure 2.3. The interspecific impacts of resource use on relative growth. Interspecific effect on relative growth among species inferred from their ability to grow on sterile beech tea previously used by each other species, shown separately for each treatment. Blue arrows indicate negative effects on growth, and red arrows indicate positive effects on growth. The width of the arrow represents the maximum growth rate (V_{MAX}) on used tea minus the maximum growth rate on unused tea (underlying data in Appendix 2.6 and linear model in Appendix 2.9). Dashed lines indicate that growth on used tea was not significantly different from growth on unused tea.

2.4.4 Species interactions between isolates that evolved in polycultures

Species interactions evolved to be more positive between polyculture isolates than between ancestral or monoculture isolates (Figure 2.3c). Species B and C evolved in polyculture to grow significantly faster on tea previously used by other species than on unused beech tea (Figures 2.3c and Appendix 2.6). Thus, interactions shifted to facilitation as predicted if species adapted to use resources being produced by other species as waste products of metabolism. Polyculture isolates of D remained negatively affected on substrate used by other species, although less so than their monoculture isolates (Appendix 2.6, relative growth rate on used tea versus unused tea: in monoculture, 0.24±0.05, and in polyculture, 0.77±0.07), indicating that species D also adapted to the presence of other species. Polyculture isolates of species A grew poorly on all substrates (Appendix 2.6), but again the interactions were significantly less negative than between ancestral and between monoculture isolates.

2.4.5 Resource use of ancestral, monoculture, and polyculture isolates

Forty-three separate resonances (i.e., peaks) were distinguished and integrated from the NMR spectra (Appendix 2.10 and 2.11). Variation in the net use and production of peaks in the spectra across ancestral, monoculture, and polyculture isolates of each species confirmed that resource use evolved in each of the species in ways that matched findings from the growth assays (Figures 2.4, Appendix 2.10, and 2.11). Considering the multivariate space of resource use and production across all compounds, polyculture isolates displayed greater differences from ancestral isolates than did monoculture isolates (across species, mean and standard error of Euclidean distance between paired ancestral isolates and monoculture isolates = 1.20±0.26; mean and standard error of distance between ancestral isolates and polyculture isolates = 2.42±0.37, $p = 0.003$, Monte Carlo simulation). Moreover, although species evolved, if anything, to have marginally more similar resource use in monoculture (not significantly so, $p = 0.36$, Monte Carlo simulation), patterns of resource use and production diverged significantly between species in polycultures ($p = 0.010$, Monte Carlo simulation; mean and standard error of Euclidean distance between species: ancestral isolates = 2.27±0.01; monoculture isolates = 1.98±0.01; polyculture isolates = 3.41±0.01). Together these results show that

species' use of NMR-visible carbon substrates in the beech tea evolved more in polyculture treatments than in monoculture treatments and did so in a way to increase the differences in metabolism between species and thereby to reduce negative interactions between them.

Principal components analysis identified the main axes of variation in net use or production of these compounds across ancestral, monoculture, and polyculture isolates of each species (Figure 2.4). The first principal component distinguished isolates based on the degree to which they used glucose, choline, formate, and succinate to produce pyruvate (Appendix 2.12). The second principal component distinguished isolates based on whether they used up or produced acetate, formate, and lactate. Notable changes in polyculture isolates were as follows: species A evolved to produce 96% more acetate and to produce rather than use formate; species B evolved to use up to 84% more choline, formate, and lactate and to use rather than produce succinate; species C evolved to use rather than produce acetate; and species D evolved to produce rather than to use lactate and to use rather than produce acetate (Appendix 2.11).

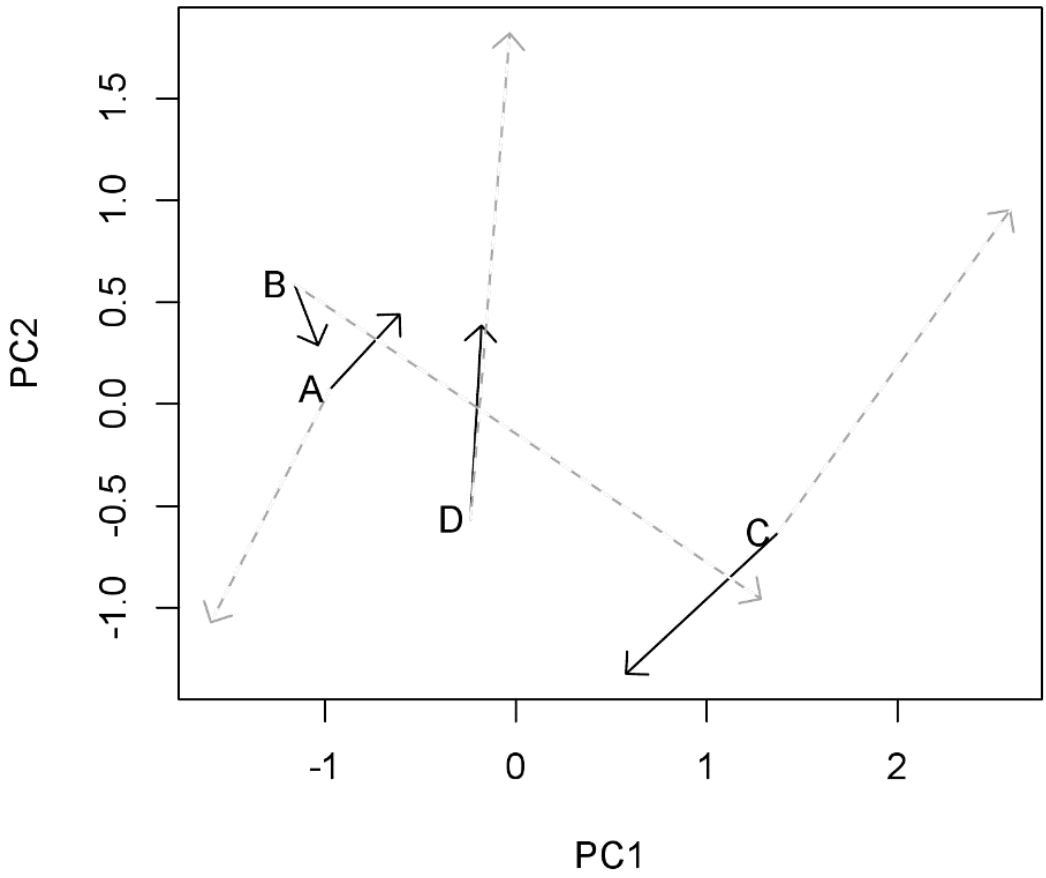


Figure 2.4. Evolution of resource use. Trajectories of evolution in monoculture (solid black arrows) and polyculture (grey dashed arrows) of each species with respect to the first two principal components summarizing variation in their ability to use and produce compounds identified by NMR. The start of each arrow indicates the position of the ancestral isolates along these axes. Increasing PC1 is correlated with using more glucose, choline, formate and succinate, and producing more pyruvate (Appendix 2.12). Increasing PC2 is correlated with using more acetate, formate, and producing more lactate. Species resource use evolved more in polyculture than in monoculture (dashed grey arrows are longer than solid black ones), and polyculture isolates display greater divergence in resource use and production than either ancestral or monoculture isolates (dashed grey arrows point towards the four corners of the plot).

These observed changes indicate possible cases of cross-feeding evolving in polycultures, which might explain the positive interactions observed in growth assays. For example, species D evolved to produce lactate in polycultures and species B to use it. To test whether species generally evolved increased use of other species' waste products in polycultures, I quantified the amounts of substrates produced by each species grown on beech tea and the amounts of the same substrates that were used by a subsequent species grown on the "used" beech tea (Appendix 2.13). On average across species, polyculture isolates displayed significantly increased use of substrates (i.e., a more negative change in the amounts of the substrate on the *y*-axis of Figure 2.5) that were produced in increased amounts by other species (a more positive change in the amount of substrates on the *x*-axis of Figure 2.5, Pearson's correlation, $r = -0.74$, $p<0.0001$), relative to ancestral isolates. Moreover, although monoculture isolates were also able to use waste products generated by other monoculture isolates, the correlation between increased production and increased use (relative to ancestral isolates) was significantly weaker (Pearson's correlation $r = -0.20$, $p = 0.03$; significant interaction between slope and treatment, linear model results in Figure 2.5). Polyculture isolates therefore appear to have evolved greater use of waste products generated by polyculture isolates of other species.

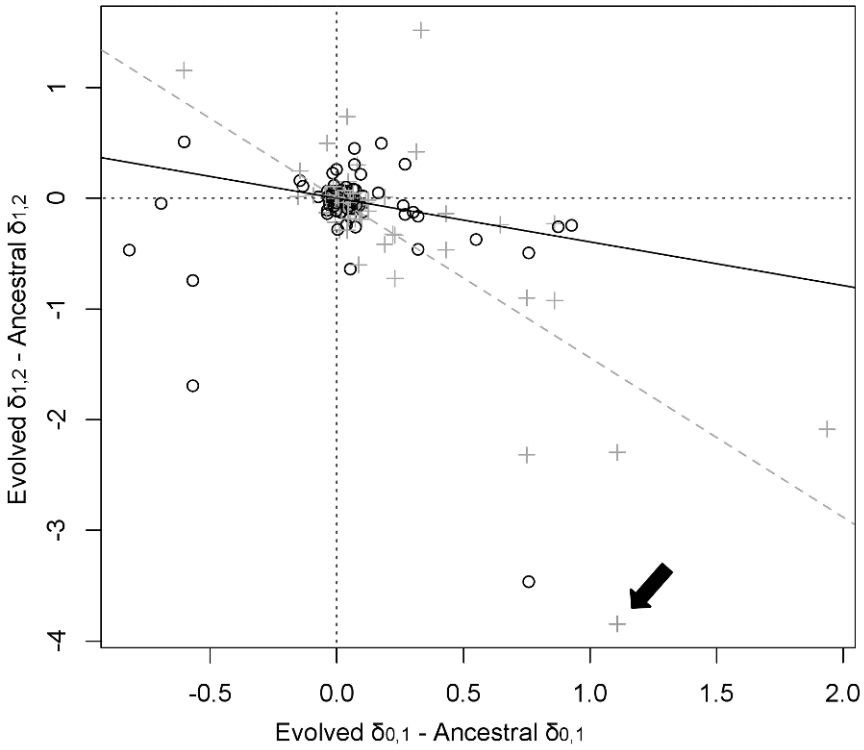


Figure 2.5. Correspondence between compounds being generated and compounds being used up by other species in polycultures. The data summarize results from assays growing one species on beech tea medium, filtering that medium, and then growing a second species on the used medium. I calculated two quantities: $\delta_{0,1}$ = the amount of compound in the filtrate from species 1 minus the amount of compound in beech tea (relative to the amount of the DSS standard); $\delta_{1,2}$ = the amount of compound in the filtrate from species 2 minus the amount of compound in filtrate from species 1. Positive δ indicates production of compounds during the assay and negative δ indicates consumption. I then compared δ between evolved and ancestral isolates for different species pairs: each point shows the comparison for a given species pair and either monoculture (black circles) or polyculture (grey crosses) treatments. The *x*-axis is $\delta_{0,1}$ of the evolved isolate minus $\delta_{0,1}$ of the corresponding ancestral isolate. More positive values indicate that the evolved isolate of the first species produced more of that compound than did its ancestral isolate. To focus on waste products as potential targets of cross-feeding, only compounds that were produced by the evolved isolate were included. The *y*-axis is $\delta_{1,2}$ for evolved isolate minus $\delta_{1,2}$ for the corresponding ancestral isolate. More negative values indicate that the evolved isolate of the second species used more of the compound than did its ancestral isolate. For example, the point indicated by the arrow represents increased production of acetate by species A in polyculture relative to ancestral isolates (*x*-axis) and its increased use by species D in polyculture relative to ancestral isolates (*y*-axis, all changes shown separately by species and compound in Appendix 2.13). There is a general negative trend: if the first species produces more of a compound, the second species is likely to use more of it. However, the effect is significantly stronger in polyculture isolates (grey dashed line) than in monocultures (black line): polyculture isolates have evolved increased consumption of compounds that have increased in production in polyculture isolates of other species. Linear model of y = x * treatment (monoculture or polyculture), interaction term coefficient = −1.13, t = −5.4, p<0.0001.

2.4.6 Ecosystem functioning

Communities were reassembled to contain one isolate of each of the four surviving species. Communities reassembled using isolates that evolved in polycultures displayed significantly higher productivity, measured as CO_2 production rate, than communities reassembled using isolates that evolved in monoculture (Figure 2.6). Adaptation to the biotic environment of co-occurring species therefore increased community productivity.

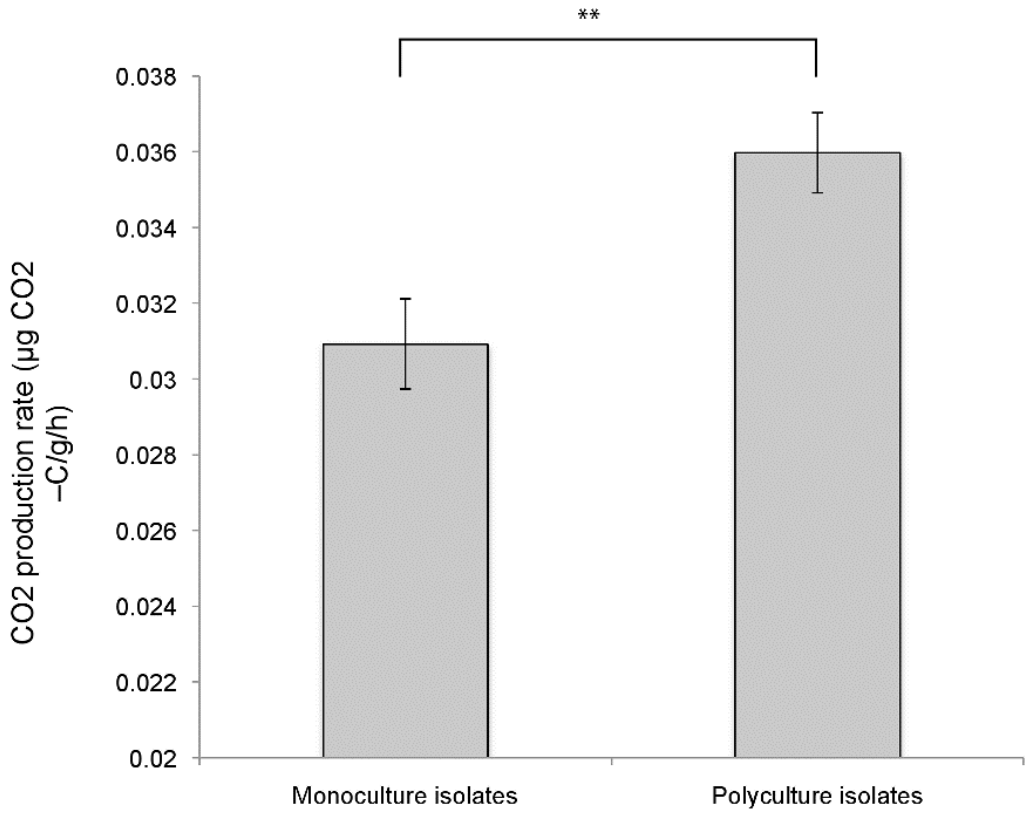


Figure 2.6. The effect of diversity during evolution on ecosystem function of reassembled communities. The mean rate of CO_2 release over a 6-h period by communities reassembled from isolates that evolved under the two diversity treatments. Standard errors are shown. Tukey Honest Significant Difference test contrasts between interaction types: ** $p<0.01$.

2.5 Discussion and Conclusions

My results show that species interactions had a major impact on how species adapted to the new environment in the laboratory. In all four surviving species, the magnitude of evolution in terms of changes in growth rate on beech tea medium and changes in use of NMR-visible resources was significantly greater in polycultures than in monocultures. Moreover, species diverged in resource use in polycultures compared to monocultures and ancestral isolates. This provides experimental evidence for a classic scenario of character displacement reducing the overlap of resources used by interacting species (Macarthur and Levins 1967). Furthermore, not only were negative interactions reduced, but species also adapted to use waste products of other species in polycultures, leading to positive interactions between some pairs of species. Together, these changes led to increased productivity of the entire community. By evolving to use different resources, and to metabolise waste products of other species, the species collectively decomposed substrates in the beech tea more effectively. Similar results have been observed for cross-feeding ecotypes evolving during monoculture experiments (Poltak and Cooper 2011; Treves et al. 1998); here I show that cross-feeding also evolves readily between distantly related species of bacteria.

The effect of species interactions on evolution varied among species. In three species, A, B, and C, there was a trade-off between adaptation to the laboratory environment in the presence of other species and adaptation in the absence of other species: polyculture isolates grew less well when assayed in isolation than did monoculture isolates. In species B and C this occurred because they adapted to use waste products generated by other species, which was demonstrated both by their increased growth on medium previously used by other species and by their increased use of waste products from other species. In species A it occurred because this species changed to use different carbon sources than the other species: its interactions became less negative in the polyculture treatment than between ancestral or monoculture isolates (but not positive) and it used more trehalose and less glucose and lactate (Appendix 2.11).

In contrast, species D displayed a positive effect of diversity on its adaptation to abiotic conditions: the polyculture isolate had enhanced growth rate when assayed on its own compared to either the ancestral or monoculture isolates. There is no evidence that species D polyculture isolates evolved to use any of the NMR-visible resources more effectively than any other isolates. I therefore hypothesize that polyculture isolates of species D evolved increased use of complex carbon sources that cannot be distinguished by NMR. One clue supporting this hypothesis is that polyculture D produced large amounts of lactate and was the only isolate to do so and without correlated negative change in any other compound. I suggest therefore that species D could be producing lactate from metabolism of compounds not distinguishable by NMR—for example, macromolecular structures such as mixtures of proteins. None of the general theories outlined in the introduction readily explain why species D should enhance its ability to grow on its own after evolving in polyculture. However, in rare circumstances in the niche simulation model by de Mazancourt et al. (2008), competition among species could "push" one species to evolve into a wider range of niches than it would do so when in the absence of competitors. The observation that species D has shifted away from its ancestral and monoculture isolates in resource use and away from the polyculture isolates of other species is consistent with this possibility (Figure 2.4).

Despite differences in response among the species, in all cases the effects of diversity arose because co-adaptation between species altered their ability to grow in an environment free of other species. The other mechanisms outlined in the introduction cannot explain my results. Effective population sizes were generally lower in polycultures (Appendix 2.11), but still exceeded 10^6 in all surviving species, and polyculture isolates did not adapt more slowly than monocultures. Instead, co-adaptation with other species rendered species A, B, and C even less well adapted to the abiotic environment in the absence of other species than their ancestors, and species D better adapted. Similarly, my results do not reflect the damping of evolutionary responses by ecological sorting, because species' use of NMR-visible compounds in fact evolved more in polycultures than in monocultures. Species E might have dwindled to low numbers in polycultures because of one of these two mechanisms (Appendix

2.11), but in any case it failed to sustain large populations during the experiment even in monocultures.

The NMR results show that changes in resource use can explain observed changes in interactions and productivity (see also Jasmin and Kassen 2007). It remains possible that other interactions could be operating among these species as well, but which remained undetected by my assays. Some of the metabolites generated by species could have had toxic effects on other isolates, and some of the observed metabolic changes could have been to reduce toxic effects rather than increase resource use. Also, bacteria are known to produce signalling molecules that can have inter-specific effects—for example, antimicrobial properties (Riley and Wertz 2002) or positive effects such as stimulating enzyme production (West et al. 2007). In principle, these could have caused some of the changes in growth rates I observed in interaction assays and they would be interesting traits to investigate in future studies. However, typical signalling molecules such as quorum sensing compounds are usually not produced at high enough concentrations for detection by NMR (Behrends et al. 2009), and therefore the changes observed here reflect changes in resource use rather than changes in signalling. Because the NMR results match inferences from the growth assay results, it is more parsimonious to conclude that changing resource use is the dominant mechanism explaining my findings.

My results provide among the first experimental evidence supporting recent theories that species interactions in diverse communities affect evolutionary responses to an environmental change. The way in which species adapted to new conditions in the laboratory when in monoculture—the setting assumed for many evolutionary theories and experiments—provided little information on the outcome of evolution in the diverse community. Co-occurring species modified the environment by generating new resources, and thereby altered the selection pressures on other species and how they used the available resources. Other experiments have reported that genetic diversity inhibited adaptation to the environment (Brockhurst et al. 2007; Collins 2011) but have not investigated whether adaptation to the biotic environment of co-occurring species changed how species adapt to a new abiotic environment. If the processes I observed here are common in other communities, including

multicellular eukaryotes over longer timescales, then attempts to understand evolutionary dynamics in the wild must take into account the biotic environment of co-occurring species (Gomez and Buckling 2011; Liow et al. 2011) .

As well as being important for understanding evolutionary dynamics, my experiments show that evolutionary interactions had important consequences for ecosystem-level functions. Co-adaptation for approximately 70 generations—not an unrealistic timescale for responses of annual eukaryotic organisms to predicted changes over the next hundred years—acted to enhance community productivity, through the evolution of complementary use of resources. Niche complementarity and facilitation are known to be important determinants of community productivity (Loreau and Hector 2001; Cardinale 2011), and my results add to growing evidence from microbial systems that niche evolution can exert a strong influence on productivity (Venail et al. 2008; Gravel et al. 2011). Recent work has shown that biofilms derived from a single clone of *Burkholderia cenocepacia* evolved cross-feeding morphotypes that together had enhanced productivity compared to the morphotypes grown alone (Poltak and Cooper 2011) and that coevolution between obligate mutualists can enhance community productivity (Hillesland and Stahl 2010): my study demonstrates similar processes operating between phylogenetically distinct species which were not obligate syntrophs. It remains to be determined whether adaptation generally acts to enhance ecosystem productivity (Hughes et al. 2008; Williams and Lenton 2010), but if so, it will be an important process to consider in predicting the impacts of current environmental changes on ecosystem services. Ecosystem functions such as decomposition rate might be reduced by local extinction of species providing important functions, but it is important to know whether evolution of surviving species will restore (as found here) or further disrupt those functions.

My communities were far less diverse and far simpler than natural communities. A single tree-hole likely contains thousands of bacterial species, including anaerobes and many other functional groups excluded by my isolation protocol. Even the comparatively depauperate community of multicellular eukaryotes in tree-holes would typically contain many more than four or five species (Srivastava

2005). A major goal for future research is to understand whether my findings scale to natural ecosystems and how other ecological mechanisms such as predation affect evolutionary outcomes in diverse communities. Strong interactions have been demonstrated between bacteria and their phages in natural settings (Vos et al. 2009), but reciprocal co-adaptation between bacterial species might be rare compared to adapting to the general biotic environment because of the large number of potential pairwise interactions among species (Futuyma and Agrawal 2009). Another important process in natural communities is immigration, which can add variants (new genotypes or species) that might swamp evolutionary responses (Perron et al. 2007). Understanding how natural assemblages respond to new environments, such as those caused by global warming, ocean acidification, or pollution, depends critically on understanding the balance between ecological and evolutionary responses of the kind I demonstrate here.

2.6 Acknowledgements

I thank Francesca Fiegna for performing isolation and 16S sequencing of the bacteria and Volker Behrends and Jake Bundy for performing the initial NMR analyses.

Chapter 3:

IMMIGRATION AND LOCAL ADAPTATION OF WARMED MICROBIAL COMMUNITIES

3.1 Abstract

Theory predicts that immigration should increase the rate at which species adapt to environmental change. Yet, species do not exist in isolation and biotic interactions may influence the outcome of immigration on adaptation. In theory, the rate of adaptation of species in a community may be increased by immigration, or alternatively, species diversity or the introduction of maladapted species may swamp adaptation. Furthermore, little is known about how local adaptation of constituent species affects the functioning of communities as a whole. I used naturally assembled microbial decomposer communities to investigate the effect of immigration and experimental warming on the growth of communities in their contemporary environmental conditions and in foreign conditions. Furthermore, I assayed representative species in isolation to investigate whether the local adaptation of constituent species predicted that of the community as a whole. The replicate microbial communities were exposed to experimental warming during an 81day field experiment and their growth and functioning measured in laboratory experiments. My results indicate that the effect of immigration was dependent on the warming treatment. In warmed communities immigration was detrimental to growth and local adaptation whereas in ambient communities it had a beneficial effect. I suggest this was due to the immigration of maladapted individuals into the warmed communities swamping local adaptation. I also found that the local adaptation of isolates was a poor predictor of the local adaptation of the community as a whole. My results suggest it would be valuable for further research to be carried out into the implications of environmental change on local adaptation of species and the consequences of this for the functioning of communities.

3.2 Introduction

Environmental change is being accelerated by anthropogenic activities (Parry et al. 2007). To protect species and ecosystems in the future we must understand and predict how they will respond to environmental changes. Many vital processes are performed by diverse microbial communities so understanding the impacts of environmental change on these communities should be of central importance (Ducklow 2008). Whether or not species can persist and ecological functions can be maintained in changing environments will depend on whether species can adapt to keep pace with change (Gonzalez et al. 2013). Here I use experimental manipulations of microbial decomposer communities to investigate the hypothesis that immigration will aid adaptation to environmental change and I investigate whether the degree of local adaptation of a community is predictable from that of representative constituent species.

Despite our reliance on diverse microbial communities for valuable ecosystem functions such as decomposition (Kardol et al. 2010) and water purification in both natural water bodies and in manmade systems such as sewage treatment (Stottmeister et al. 2003) we have only a limited understanding of how these communities are likely to respond to environmental change. In order to fully explore ecological and evolutionary theories researchers have commonly focused on explaining the mechanisms behind the dynamics of just one or a few interacting species (e.g. Lenski 2004; Brockhurst et al. 2007b). Natural communities, however, are clearly more complex than model systems that can be constructed and explored in the laboratory. Thus, it is not clear whether factors which have been shown to be influential to ecological and evolutionary dynamics in laboratory experiments - such as immigration and coevolution (e.g. Bell and Gonzalez 2011; Lawrence et al. 2012) - will be as important in natural ecosystems in which high species diversity may swamp their effects.

Over the coming decades mean global temperature is predicted to increase due to climate change (Parry et al. 2007) and additionally urban heat pollution caused, for example, by run-off from paved

urban areas is already warming rivers and lakes with implications for stream ecology (Daufresne and Boët 2007). For example, if species differ in their sensitivity this may alter community composition (Wang and Kanehl 2003), which may impact functioning as was found in stream denitrifying microbial communities exposed to watershed urbanization (Wang et al. 2011) and fungal and freshwater communities exposed to warming (Dang et al. 2009; Dossena et al. 2012). Accordingly, because reactions to warming can be species specific and the dynamics of single species do not always correlate predictably with their responses when in a group (Hall et al. 2008; Kordas et al. 2011), forecasting community level responses to increasing temperatures is not straightforward. This has been demonstrated in experiments examining how the differential responses of multiple species to warming can alter species interactions (Helland et al. 2011, Grigaltchik et al. 2012). Therefore warming, or indeed any stress for which species' responses differ, could have unexpected consequences for ecological communities.

Many studies that have experimentally warmed or observed natural differences in temperature of communities report that respiration rates increase with warming (Demars et al. 2011). Yet, this effect is often only temporary (e.g. Eliasson et al. 2005) and meta-analyses have indicated that over the long term there is little difference in respiration rates of microbes in temperate and cool oceans (Rivkin et al. 1996) and ambient and warmed soils (Bradford et al. 2008). Other experiments suggest that short-term increases in respiration concurrent with warming may be due to changes in community composition (Zhang et al. 2005) and it is common for microbial communities to shift populations seasonally (Hall et al. 2008) signifying that immigration may be highly influential to microbial community functioning.

The impacts of immigration on adaptation have been extensively modelled (e.g. Holt and Gomulkiewicz 1999; Blanquart et al. 2012). Theories predict that in a sink population, one that will decline to extinction without intervention, immigration can enhance the likelihood of successful rescue by providing genetic variation for natural selection to act upon (review of dispersal and genetic rescue Tallmon et al. 2004) or by increasing population sizes so that beneficial mutations are more likely to arise (Rich et al. 1979). These theories have been supported by evidence from experimental

studies. For example, Perron et al. (2007) found that immigration facilitated adaptation of *Pseudomonas aeruginosa* to increasing levels of antibiotic and also decreased the trade-offs associated with the evolution of resistance. Also, in an experiment with *Saccharomyces cerevisiae* Bell and Gonzalez (2009) found that local dispersal aided adaptation to increasingly saline environments. Yet the influence of immigration can be complicated; in a source population, one that does not require immigration to maintain it, dispersal may hinder adaptation because the introduction of maladapted genotypes can swamp the process of natural selection (Mayr 1963). There is partial evidence that dispersal of maladapted individuals has been swamping selection for evolution towards an optimal body shape in natural communities of threespine stickleback (Moore and Hendry 2009) and in some experimental evolution studies intermediate rates of dispersal produce the greatest adaptive potential (Vogwill et al. 2008, Bell and Gonzalez 2011). These theoretical models and empirical studies indicate that the outcome of immigration on adaptation is likely to depend on whether a population is a source or a sink and therefore whether it is under stress. In a population that is not under stress immigration may swamp adaptation but in a dwindling population immigration may be more likely to aid adaptation.

The picture is further complicated when we consider immigration of multiple species into diverse communities. If adaptation within species is independent of interactions between species the predictions are the same as in populations of single species; that immigration will be more beneficial to populations under stress than those in stable environments. On the other hand, if adaptation is influenced by species interactions then the nature of interactions (antagonistic/synergistic) may be as influential as immigration or the abiotic environment in shaping adaptation. In addition to this, in diverse communities enhanced functioning in local conditions compared to foreign conditions, or "community local adaptation", could occur as a result of species sorting (Ackerly 2003; de Mazancourt et al. 2008) or the immigration of preadapted species (de Meester et al. 2011) rather than by *de novo* mutation or micro-evolution. And, as they are not mutually exclusive, community local adaptation could also arise from a combination of these mechanisms. Thus, the complexity of natural communities makes it challenging to ascertain the mechanism by which they become locally adapted

or maladapted. To the best of my knowledge no other studies have experimentally tested how immigration affects adaptation to a changing environment using diverse naturally assembled communities.

It has often been found that microbial decomposer populations can have a "home field advantage" so that they are locally adapted to the soil or litter in which they are found (Ayres et al. 2009; Bishoff et al. 2006). Yet, it is unclear how the local adaptation of communities is determined by the local adaptation of species within those communities. In theory, community local adaptation could be the product of the average local adaptation of species that constitute the community so that the balance of locally maladapted and adapted species predicts that of the community. Or, alternatively, the average local adaptation of species may deviate from that of the community. This could arise because without the detrimental (or beneficial) effects of interspecific interactions species' growth in isolation may be uncorrelated with their growth when in a mixture (Lawrence et al. 2012). It is also possible that in diverse communities just a few species may contribute to the majority of community functioning and therefore if we only observe the local adaptation of a subset of species we may be unlikely to capture dominant (Smith and Knapp 2003; Wertz et al. 2006) or rare species and these may have the greatest influence on community functioning (Mouillot et al. 2013) and therefore on measures of local adaptation. Through mechanisms such as these isolated species could grow well in an environment that was detrimental to the growth of the community (or vice versa). It has been demonstrated in numerous experiments that the growth of a species in monoculture can differ from that in polyculture and that without prior knowledge of the system community function is not necessarily predictable from the function of component species (e.g. Fridley 2002; Schmidtke et al. 2010). Yet, I know of no attempts to assess the predictability of community local adaptation from the local adaptation of community members.

3.2.1 Predictions

Hypothesis I: Immigration will be more beneficial in a changing environment than a stable one.

For a population to adapt to keep pace with environmental change it is necessary for it to contain genetic variation for selection to act upon or for individuals with novel variation to be introduced. Both of these attributes are likely to be boosted by immigration therefore I predict that immigration will be generally beneficial to total community growth and local adaptation. However, as high levels of immigration may swamp adaptation, I predict that immigration will be more advantageous to a community under stress than one in relatively benign conditions.

Hypothesis II: The local adaptation of the communities will be predictable from the local adaptation of species that constitute those communities.

If species in a community have similar responses to abiotic stress I would expect that the degree of local adaptation of individual species within a community will be consistent. Furthermore, I predict that the average local adaptation of isolates will reflect the local adaptation of the community as a whole.

To test these hypotheses I exposed naturally assembled diverse communities of microbes to experimental warming in the field and manipulated immigration into them. Following this period of environmental change I determined what effect immigration and warming had on community growth, local adaptation and metabolic function and how the local adaptation of isolates related to that of the communities. This set-up permitted natural fluctuations in environmental conditions and immigration of wild phenotypes but also retained some of the control and tractability of laboratory experiments.

3.3 Materials and Methods

3.3.1 Microcosms, media and treatments

The microcosms used in this experiment were modelled on treeholes of beech trees (*Fagus sylvatica*), which are pools of water formed where the roots of the trees buttress together. These natural microcosms contain hundreds of species of microbes as well as protists and insect larvae. As treeholes act like meta-communities they are useful for testing ecological theories; for example, they have been used in studies of the species-area relationship (Bell et al. 2005a) and in testing how biodiversity affects ecosystem functioning (Bell et al. 2005b). I modified these natural microcosms to create a more controlled system in which I could manipulate migration and temperature but which contained naturally co-occurring microbes in a realistically diverse community.

The experiment was fully factorial and had two levels of warming: i) ambient temperature and ii) increasing temperature and two levels of immigration i) no immigration and ii) a limited influx of immigration. There were 5 replicates of each dispersal-stress combination.

3.3.2 Creating the start culture

For 22 days before the start of the experiment microbes were allowed to colonise 6 open bottles containing 240ml of sterile 32X beech tea. These bottles were placed under beech trees along a ~100m transect. Samples from each of these bottles were frozen at -80^{0}C and the remainder was homogenised to create the start culture.

3.3.3 Initiating the experiment

The microcosms were 250ml Duran bottles filled with 240ml of 32X beech tea. Beech tea was prepared by autoclaving 50g of autumn fall beech leaves in 500ml of water, and diluting the filtrate 32-fold. Immigration was prevented using rubber stoppers (Bugstoppers). The stoppers were piercable and contained a breathable membrane which was impenetrable to organisms.

To initiate the experiment sterile 250ml Duran bottles were filled with 220ml of sterile 32X beech tea and 20ml of the start culture was added to each. Sterile stoppers were fitted and the bottles were then taken to the field site and placed under beech trees along the transect.

3.3.4 Controlling migration

All microcosms remained sealed for the duration of the experiment to control for confounding effects that could occur if vials from the immigration treatment were left open (for example there may be different rates of evaporation or addition of organic material between treatments). To simulate immigration, each vial from the immigration treatment was paired with an open vial (open vials were maintained at the same temperature as their pair) and, after samples were taken at each time-step, 1ml of bacteria from the open vial was transferred to the sealed bottle. The open vial was then replaced with one containing sterile beech tea. At the next time-step 1ml from this was transferred to the immigration microcosm and so on.

3.3.5 Sampling

Samples were taken every nine days for nine time-steps. The microbes were therefore exposed to a changing environment for a total of 81d. At each time-step a hypodermic needle and sterile syringe were used to remove 30ml of beech tea from each microcosm. The sample was replaced with 30ml of sterile 32X beech tea (or 29ml in the case of immigration treatments where 1ml was also added from the open vial). Samples were returned to the laboratory to perform growth assays and measure metabolic function. Samples from each microcosm at each time-point were frozen in 80% glycerol thereby providing a timeline of community changes. Growth and metabolic function assays were performed on the same day that the samples were taken from the field.

3.3.6 Increasing temperature

The temperature of the heat-stressed microcosms was controlled with under-soil heating coils and maintained at a constant level above ambient (Appendix 3.1). At the beginning of each time-step the thermostat was increased by 2 notches. Six microcosms containing 240ml of beech tea and a

temperature logger (ibutton) were used to verify average temperatures achieved by ambient and heated microcosms. 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*) and warmed microcosms diverged from the controls by an average of 0.5^{0}C per time-step (Appendix 3.2).

3.3.7 Growth assays

Samples from each treatment were reciprocally assayed in their 'home' environment (the average temperature that they were exposed to during the previous time-step) and in the 'away' environment (the average temperature of the other treatment during the previous time-step). Assays were performed in triplicate for each sample and the optical densities at 600nm (OD_{600}) averaged for use in analyses.

To measure growth rates 20µl of each sample was suspended in 180µl of 32X beech tea in a 96 well plate and allowed to grow in an incubator for a total of 96 hours. OD_{600} was measured every 24 hours. Cell densities were subtracted from average negative controls of sterile media placed around the perimeter of each 96 well plate.

3.3.8 Metabolic function

Metabolic function was measured throughout the experiment to determine how the treatments affected ecosystem functioning of the communities. At the initiation of the experiment and every three time-steps the metabolic function of whole community samples were measured. Metabolic function assays were performed for replicates 1, 3 and 5 from each treatment. Biolog GN2 microplates containing 95 different carbon sources were used to assess metabolic function of the communities. If microbes in the sample could metabolise the carbon source the well would turn purple. Colour change of the wells was compared to a control well containing only water to determine the metabolic function of the bacterial community. The Biolog plates were set up as per the manufacturer's instructions and 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 therefore excluded from analyses of change in metabolic diversity.

3.3.9 Local adaptation of isolates

To generate the isolates 100µl of frozen samples from replicates 1, 3 and 5 of each treatment from the final time-step were resuspended in 100µl of 32X beech tea and 20 µl was spread on hard R2A agar. After 18 days of growth 42 random colonies were picked from each plate and resuspended in 100µl of 32X beech tea. Cultures were grown for 72 hours and 5µl of these cultures were plated on hard R2A agar and allowed to grow for 7 days. 10 isolates were randomly selected for use and cells were resuspended in 200µl of 32X beech tea. These cultures were incubated at 24°C for 48 hours and used to initiate growth assays.

To measure total growth of the isolates, 10µl of the bacterial cultures were resuspended in 90µl of 32X beech tea and the OD_{600} was measured immediately and every 24 hours for 96 hours. Cell densities were subtracted from average negative controls of wells containing sterile media. All cultures were assayed in 'home' and 'away' conditions.

3.3.10 T-RFLP analysis

Profiles of the initial community and replicate communities 1, 3 and 5 from every third time-step were analysed by comparing terminal-restriction fragment length polymorphisms (T-RFLP). Briefly, 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). 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 these were combined for PCR clean up. An equal volume of

SureClean (Bioline) to the volume of sample was added, 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 and the resulting data were analysed by using GeneMarker software. 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 and the total fluorescence of all terminal restriction fragments of that sample.

3.3.11 Statistical analyses

To statistically analyse community growth curves during assays and also total community growth and metabolic function over the course of the environmental change I used linear mixed effects models (lmer). In these models I used random error structures to account for temporal pseudoreplication that could be caused by repeated sampling of the same microcosms over time and spatial autocorrelation that could be caused by blocking. In all models the main explanatory variables were warming treatment, immigration treatment and time-step (as a factor) and in some models (as described in the Results section) additional explanatory variables, such as species diversity, were also used. To select the minimum adequate model (the model that best explained the data using the minimum number of explanatory variables) I began model simplification with the maximal model (model including interactions between all explanatory variables) and sequentially removed the least significant interaction terms. At every step of the simplification I used analysis of variance (ANOVA) to compare models fitted using the maximum likelihood criterion to ensure that the simplification did not significantly reduce the explanatory power of the model (detailed model simplifications are shown in the Appendix as indicated in the Results section). ANOVA and Tukey's Honest Significant Difference tests - calculated from lmers - were used to contrast particular treatments of interest. To report the direction and effect size of differences among treatments, I used the total change in optical

density over 24h to 96h of growth assays as a measure of total growth. Total growth was used as a proxy for fitness because it was considered to be a more important trait than maximum growth rate for these bacteria as their selection regime meant that resources were only replenished every 9 days and therefore rapid early growth would not necessarily be an advantage. Community and isolate local adaptation was calculated as the total growth over 24 to 96h in the foreign environment subtracted from total growth in their contemporary environment. Therefore a positive value indicates local adaptation and a negative value local maladaptation. Paired t-tests were used to compare the local adaptation of isolates and 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 used as an explanatory variable in an lmer.

T-RFLP data and metabolic profiles were analysed with 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 I used the response variable of distance moved in multivariate space to compare changes in metabolic profile during the environmental change experiment. 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). I 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. All statistical analyses were performed in R (R Development Core Team 2011).

3.4 Results

3.4.1 Effectiveness of the experimental warming

Over the course of the experiment mean warmed and ambient temperatures diverged significantly (Pearson's correlation = 0.73, df = 6, t = 2.59*) by an average of 0.5^0C per time-step (Appendix 3.2). The experiment was carried out in summer/autumn 2011 and so the ambient temperature declined as the experiment progressed whereas the temperature of the warmed microcosms was maintained at a similar level to at the initiation of the experiment (Appendix 3.1).

3.4.2 Community growth curves during environmental change

At every time-step whole communities were assayed in the laboratory under the experimental treatment conditions. During the course of the environmental change experiment there was considerable variation in the shape of the growth curves between warming treatments and also between sampling time-steps (Appendix 3.3). Throughout the first half of the experiment both warmed and ambient communities tended to show monod-shaped growth curves with rapid early growth approaching an asymptote towards the end of the assay. In the second half of the experiment, however, growth patterns differed between warming treatments. Over time-steps 6 to 9 warmed communities continued to display monod-shaped growth curves which were significantly non-linear (ANOVA comparing a model with time as a factor versus a model with time as a continuous variable, likelihood ratio = 3.09, df = 158 and 156, $p<0.05$) whereas the growth curves of ambient communities became linear (ANOVA comparing a model with time as a factor versus a model with time as a continuous variable, likelihood ratio = 2.18, df = 158 and 156, p = 0.30). Thus, the warming treatments affected the shape of the community growth curves. Specifically, early growth rate, V_{max} (change in OD_{600} from 24 to 48h), was maximised in warmed communities whereas ambient communities showed more consistent growth rates through time. This difference in growth patterns is emphasised when comparing total growth (total change in OD_{600} over 24 to 96h) with V_{max}. Although total growth and V_{max} are strongly correlated for both ambient (Pearson's correlation = 0.94, df = 38, t

= 16.7***) and warmed communities (Pearson's correlation = 0.91, df = 38, t = 13.9***) warmed communities have a significantly higher intercept (ANOVA of effect of warming on V_{max}, $F_{1,77} = 52.2$, MES = 0.00055, $p<0.001$) (Appendix 3.4) indicating that a greater proportion of their total growth was achieved over 24 to 48h compared to ambient communities. Taken together these data indicate that as the temperature became cooler species in ambient communities were unable to maintain rapid doubling rates (the "ancestral" pattern of community growth) and as a result their growth curves became linear. In contrast, microbes in warmed communities displayed rapid early growth approaching an asymptote throughout the experiment. Thus, warming may have facilitated the maintenance of rapid doubling rates in these communities.

3.4.3. The effect of warming on total growth varied over time

Due to the varying growth strategies in warmed and ambient communities and because rapid early growth was not necessarily expected to be an advantage (media was replenished only every 9 days therefore species were not expected to be constantly in exponential growth and therefore natural selection could result in a number of different growth strategies) total growth achieved by communities from 24 to 96h was used as the measure of fitness. The total growth rates of communities that experienced warming decreased rapidly from the initiation of the experiment to time-step 5 and then recovered marginally (Figure 3.1). This initial decline was particularly steep for warmed communities which received immigration. Total growth of the warmed communities recovered marginally towards the end of the experiment but remained lower than those at the start of the experiment. The control communities, which experienced ambient temperatures, responded differently and maintained relatively high total growth rates until time-step 6 under both immigration scenarios (Figure 3.1). These ambient communities then suffered a sudden decrease in total growth rates from time-step 6 to time-step 7 but recovered marginally towards the end of the experiment. This variable effect of the warming treatment over time is reflected by the significant interaction between these variables which explains approximately 30% of the model deviance (ANOVA of lmer interaction between warming treatment and time-step; $F_{7,117} = 21.0$, MSE = 0.0006, $p<0.001$, model simplification Appendix 3.5 and final model statistics Appendix 3.6).

3.4.4 The effect of immigration was dependent on the warming treatment

The effect of immigration on total community growth depended on the warming treatment. When the temperature was ambient immigration enhanced average community growth rates whereas in the warmed treatment immigration had a negative effect on total community growth (ANOVA of lmer interaction between warming treatment and immigration; $F_{1,117} = 9.7$, MSE = 0.0003, $p<0.01$, Figure 3.1). This is in contrast to the prediction that immigration would be generally beneficial to total growth rates.

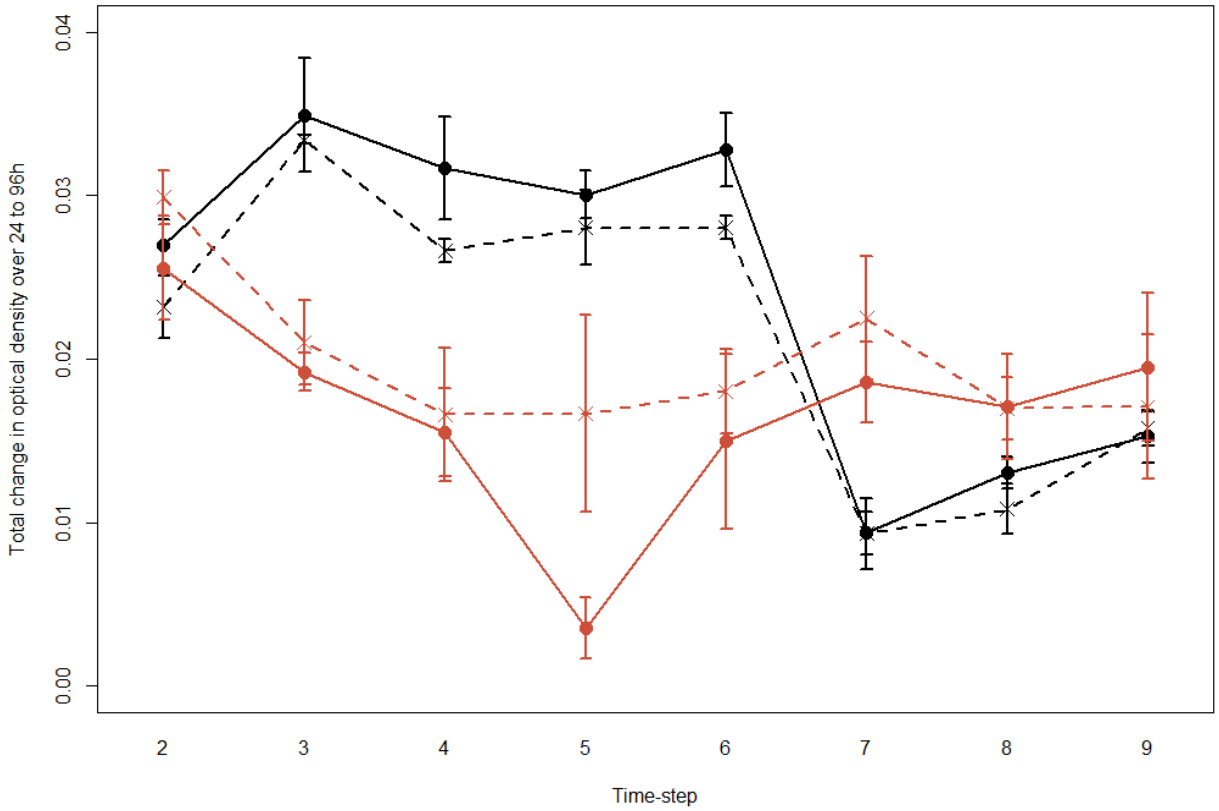


Figure 3.1. Total growth of microbial communities at each time-step of the warming experiment. Black lines indicate that samples came from control communities and red lines indicate that they were from warmed. Solid lines with circular points represent samples from communities with immigration and dashed lines with crosses represent those from communities that were prevented from receiving immigration. Error bars are standard errors of mean total change in optical density over 24h to 96h of 5 replicate communities. Control communities had initially high total growth but this declined significantly over time whereas warmed communities had lower total growth overall but their decline in total growth was less. There was a significant interaction between immigration and warming. In ambient communities immigration enhanced total growth whereas in warmed communities immigration reduced total growth (ANOVA of lmer interaction between warming treatment and immigration; $F_{1,117} = 9.7$, MSE = 0.0003, $p<0.01$).

3.4.5 Community local adaptation during environmental change

There is a large amount of variation in the degree of local adaptation of microbial communities over the course of the warming experiment (Figure 3.2). Communities sometimes grew better in a foreign environment than their home environment, nonetheless, on average communities were significantly locally adapted during the experiment (lmer, intercept = 0.005, SE = 0.002, t = 2.30*, model simplification Appendix 3.7 and final model statistics Appendix 3.8).

3.4.6 The effect of the warming treatment on local adaptation varied over time

During the course of the experiment the local adaptation of warmed communities increased (gradients of the red lines in Figure 3.2 and Appendix 3.9 change from positive to negative) whereas that of ambient communities fell (shallower negative gradients of the black lines in Figure 3.2 and Appendix 3.9) as indicated by the significant interaction between warming and time-step (ANOVA of lmer interaction between warming treatment and time-step; $F_{7, 117} = 45.3$, MES = 0.002, $p<0.001$). This interaction explained approximately half (51%) of the model deviance. At the conclusion of the experiment the mean degree of local adaptation of ambient communities had declined whereas warmed communities had improved from being locally maladapted on average to having local adaptation which was marginally greater but not significantly different to that of the ambient communities (Tukey contrasts from lmer at time-step 9, difference = 0.006, SE = 0.004, $z = 1.31^{n.s.}$).

3.4.7 Local adaptation was hindered by immigration in warmed but not ambient communities

Similarly to in the growth assays, the effect of immigration depended on the warming treatment. In warmed communities the introduction of individuals was detrimental to local adaptation whereas it was beneficial in ambient communities (ANOVA from lmer interaction between immigration and warming treatment, $F_{1, 117} = 11.8$, MES = 0.0006, $p<0.001$). Although this interaction was significant it explained only approximately 2% of the model deviance. These results are contrary to the prediction that immigration would be more beneficial to local adaptation in a changing environment compared to a stable one.

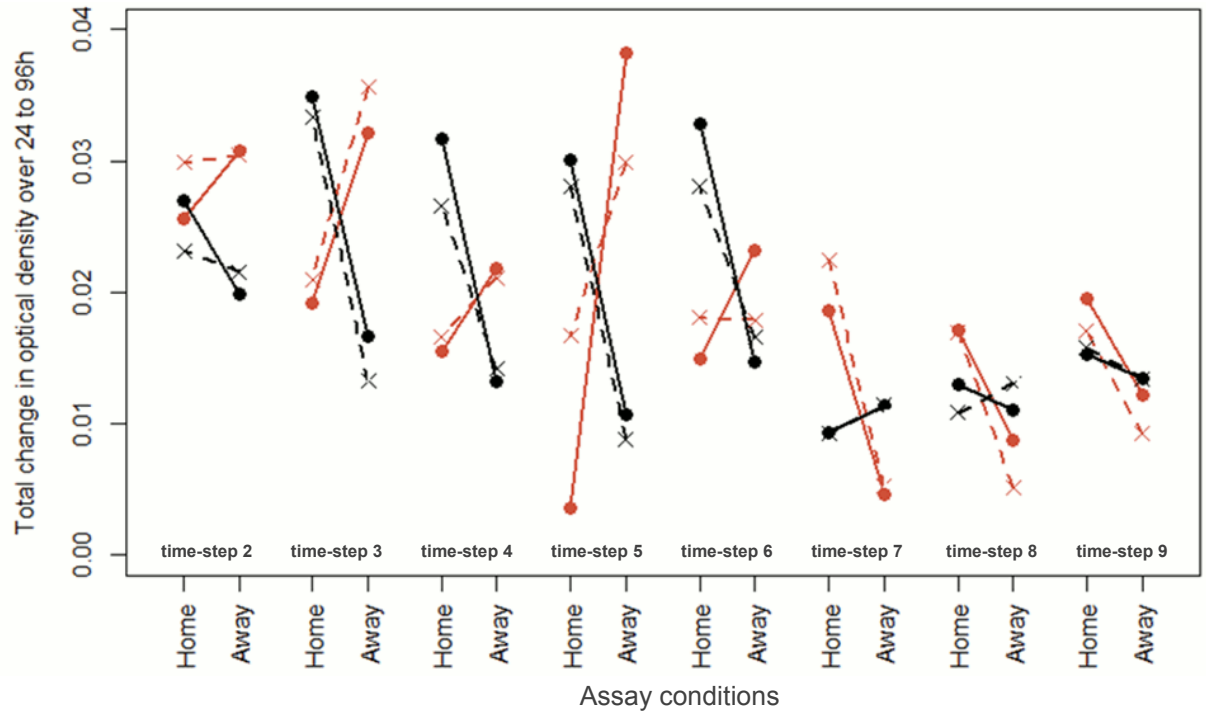


Figure 3.2. The difference between growth in 'home' and 'away' environments throughout the experimental warming. Black lines denote communities that remained at ambient temperature and red lines those that were warmed. Solid lines with circles represent communities that received immigration and dashed lines with crosses indicated that the communities were closed. The first point in the pair shows total growth in the contemporary environment for that community and the second point is total growth in the foreign conditions. Lines that have a negative slope indicate greater growth in 'home' environments than the away environments and therefore show local adaptation. During the first half of the experiment ambient communities consistently show local adaptation indicating that they were generally well locally adapted, however, they became less locally adapted during the experiment. Warmed communities, in contrast display poor local adaptation from time-steps 2 to 6 followed by a marked improvement during the final three time-steps. Contrary to expectations immigration was detrimental to local adaptation in warmed communities but beneficial in ambient communities (ANOVA from lmer interaction between immigration and warming treatment, $F_{1, 117} = 11.8$, MES = 0.0006, $p<0.001$).

3.4.8 Does the local adaptation of isolates predict that of the communities?

At the conclusion of the environmental change experiment communities were locally adapted on average, however, there was a large amount of variability in the local adaptation of isolates that constituted these microbial communities and most communities were composed of both locally adapted and maladapted isolates (Appendix 3.10). In warmed communities isolate local adaptation reflected that of the community reasonably well and there was not a significant difference in the degree of local adaptation between warmed isolates and communities (paired t-test, mean of the differences = 0.015, df = 5, t = $2.37^{n.s.}$). Isolates from ambient communities, in contrast, were poor predictors of community local adaptation and they consistently and significantly underestimated community local adaptation (paired t-test, mean of the differences = 0.021, df = 5, t = 4.69**) (Figure 3.3). This occurred because, in contrast to the communities as a whole, ambient isolates tended to prefer warmed conditions to the cool ones that they had experienced.

In the majority of warmed communities at least 85% of the isolates sampled were locally adapted to the increased temperatures indicating that on average species in warmed communities were better locally adapted than those from ambient communities (t-test on the proportion of locally adapted and maladapted isolates in each community, difference in means = 0.79, df = 5.98, t = 7.07***).

The immigration treatment did not have a significant effect on the proportion of locally adapted isolates sampled from the communities (t-test, difference in means = 0.16, df = 9.67, t = $0.86^{n.s.}$).

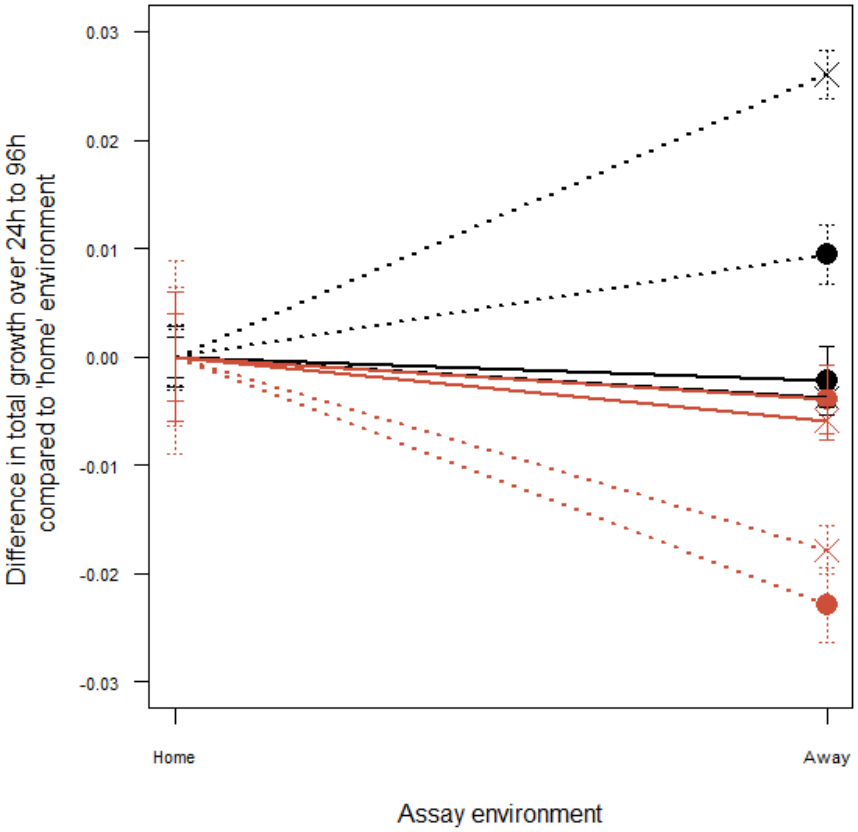


Figure 3.3. Comparison of mean local adaptation of isolates and communities. Solid lines show the difference in total growth of communities in their 'home' and 'away' environments and dotted lines show that of isolates. Circles represent communities with immigration and crosses are those that were prevented from having immigration. Black show microbes that were exposed to ambient conditions and red show those exposed to warmed conditions. Error bars represent the standard error of the mean for 3 replicate communities. Growth rates have been standardised according to total growth in 'home' environment to account for experimental blocks. Isolates from warmed communities had average local adaptation that was not significantly different to that of the whole community; however, ambient isolates were poor predictors of community local adaptation.

3.4.9 Metabolic function declined initially but recovered over time

All treatments showed an initial decrease in total metabolic function (Figures 3.4, 3.5a and 3.5c positive movement along PC1) followed by a small regain in total function between time-steps 6 and 9 (Figure 3.5a and 3.5c negative movement along PC1) with the exception of ambient closed communities which continued to decline throughout the experiment. This meant that overall the decrease in total metabolic function between the start of the experiment and the end was greater for warmed communities than ambient and the difference in the trajectories of the changing metabolic functioning between warming treatments is reflected by the significant interaction between warming and time-step (ANOVA of lmer interaction between warming treatment and time-step, $F_{2, 911} = 15.8$, MES = 0.59, $p<0.001$; model simplification Appendix 3.11 and final model statistics Appendix 3.12).

These changes in metabolic functioning mirror those found in the growth assays performed during the warming experiment showing that heated communities suffered a large decrease in functioning up to time-step 6 followed by a recovery to time-step 9 and that ambient communities suffered a shallower decline in functioning compared to warmed communities but continued to decline after time-step 6.

3.4.10 Immigration maintained metabolic diversity

Communities that received immigration had a shallower loss of functioning than closed communities and regained greater functioning as the experiment progressed (Figure 3.4) as shown by the significant interaction between immigration and time-step (ANOVA of lmer interaction between immigration treatment and time-step, $F_{2, 911} = 5.1$, MES = 0.19, $p<0.01$). There was, however, no effect of immigration on the metabolism of recalcitrant and labile compounds (ANOVA of lmer interaction between immigration and metabolism of cyclic and non-cyclic carbon compounds on change in metabolic diversity, $F_{1, 453} = 0.54$, MES = 0.002, $p>0.05$; final model statistics Appendix 3.13). Taken together these data indicate that immigration helped to preserve community metabolic functioning to some extent but this was not due to the immigration treatment promoting the colonisation or preservation of species that metabolised recalcitrant compounds.

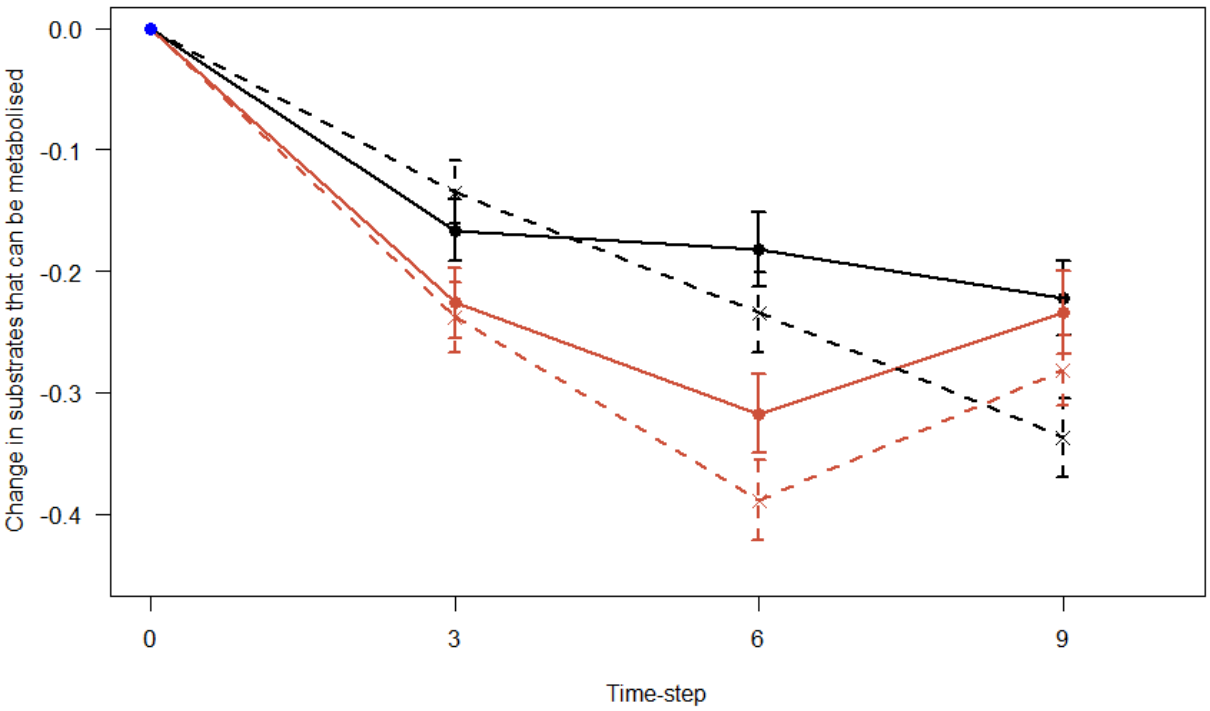


Figure 3.4. Change in metabolic diversity from the initiation of the experiment to the final time-step. Solid lines with circles indicate that the communities were allowed immigration and dotted lines with crosses indicate that the microcosms were closed. Red lines represent warmed communities and black lines ambient communities. Error bars are standard errors of 3 replicates. All immigration-stress combinations suffered a decrease in metabolic diversity over the first 6 time-steps. Warmed communities regained some function over the final 3 time-steps whereas ambient ones continued to decrease. Communities that were allowed immigration suffered less steep declines in function over the first 6 time-steps and regained more function on average over the final 3 time-steps than those that did not receive immigration. Therefore immigration helped to preserve community metabolic function during environmental change (ANOVA of lmer interaction between immigration treatment and time-step, $F_{2, 912} = 5.1$, MES = 0.19, $p<0.01$).

3.4.11 Communities from warmed microcosms had greatest variation in resource use over time

Warmed and ambient communities show a clear divergence in their use of some specific carbon substrates (Appendix 3.14) highlighting underlying changes in metabolism arising during warming (divergence of red and black lines along PC3 in Figures 3.5b and 3.5c). Divergence in PC3 indicates that warmed communities improved in their ability to metabolise some organic acids such as citric acid and amino acids such as L-alanine but became worse at metabolising the polymer tween and some sugar alcohols such as xylitol. For ambient communities the opposite was true. These differences in metabolic functioning of the communities exposed to ambient and warmed conditions decreased as the experimental warming progressed.

The total change in metabolic profiles calculated using principal components 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, $p<0.05$; model simplification Appendix 3.15 and final model statistics Appendix 3.16) indicating that during the experiment microbes from warmed communities showed more variation in the resources they could metabolise than microbes from ambient communities.

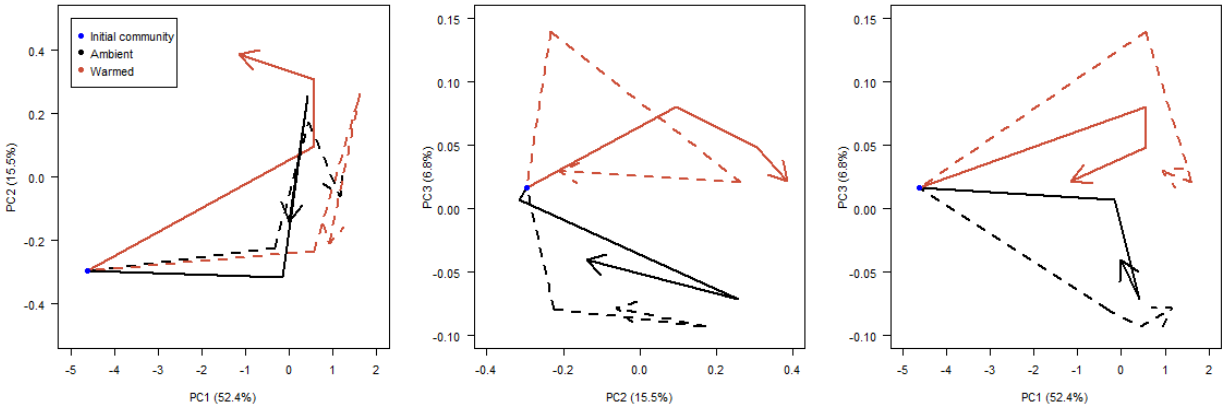


Figure 3.5. Change in principal components representing metabolic function during the environmental change experiment. Solid lines signify communities with immigration and dotted lines signify closed communities. Red lines represent warmed communities and black lines ambient communities. The lines represent average position of 3 replicate communities in multivariate space. PC1 represents a decrease in overall metabolism, PC2 and PC3 represent changes in the ability of the community to metabolise certain carbon substrates (see Appendix 3.14). **a)** Principal components 1 and 2. **b)** Principal components 2 and 3. **c)** Principal components 1 and 3. On average, warmed communities move a significantly greater distance in multivariate space during indicating that they have greater variability in their metabolic profiles during the environmental change experiment than ambient communities (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, $p<0.05$).

3.4.12 Community composition, diversity and evenness

Multivariate analyses of T-RFLPs indicate that samples were clustered by the time-step from which they were taken (metaMDS, $r^2 = 0.20$*) (Figure 3.6a). Therefore there were parallel successional changes in species composition that were independent of the immigration or warming treatments. Shannon diversity (calculated from the number of T-RFLP peaks in each sample), community evenness and time-step explained 71%, 35% and 20% of variation in community profiles respectively (Figure 3.6b).

Community evenness displayed a general decline over time (Appendix 3.17) but was significantly lower at time-step 6 than at time-steps 3 and 9 and was lower at time-step 9 than time-step 3 (Tukey contrasts from lmer of community evenness over time, $p<0.05$; model simplification Appendix 3.18 and final model stats Appendix 3.19). Conversely, Shannon diversity tended to increase through the experiment (Appendix 3.17) however, this effect was not significant (ANOVA of lmer of Shannon diversity over time, $F_{1, 17} = 0.46$, MES = 16.74, $p>0.05$; model simplification Appendix 3.20 and final model statistics Appendix 3.21) and there was no correlation between diversity and evenness (Pearson's correlation = 0.022, df = 35, $t = 0.13^{n.s.}$). In addition to this, the warming and immigration treatments had non-significant effects on Shannon diversity and community evenness (see Appendix 3.19 and 3.21 for full model statistics).

3.4.13 Metabolic diversity increased with species diversity in communities with immigration

Species diversity helped to explain the change in total metabolic function (the total number of carbohydrate substrates that could be metabolised) of communities over the course of environmental change. There was a significant interaction between immigration treatment and species diversity that explained approximately 10% of the model deviance (ANOVA of lmer of interaction between immigration and species diversity, $F_{1, 14} = 16.8$, MSE = 1377, $p<0.01$; model simplification Appendix 3.22 and final model stats Appendix 3.23). In communities that received immigration metabolic function increased as species diversity increased, however, in closed communities there was no relationship between species diversity and total metabolic function (Appendix 3.24).

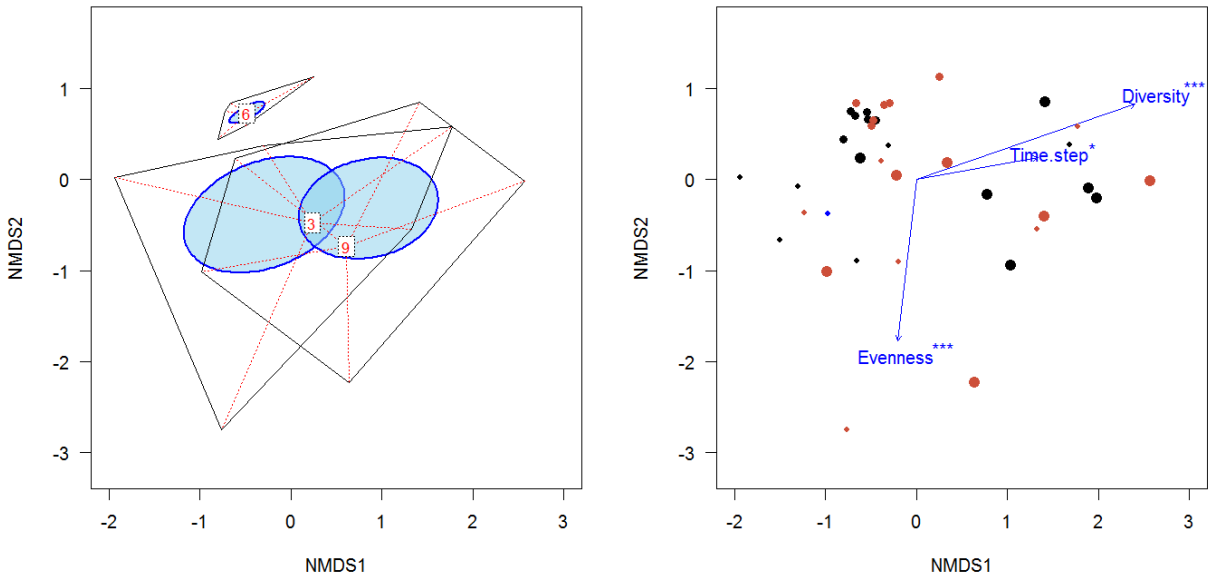


Figure 3.6. Multivariate analyses of community profiles from T-RFLPs. Ordination plots of community profiles from time-steps 3, 6 and 9. **a)** Clustering indicates that communities from time-steps 3 and 9 were relatively similar to each other whereas samples from time-step 6 were distinctly separated from other time-steps. **b)** Each circle represents a community sample. Black circles are samples from ambient communities, red circles are from warmed communities and the blue circle is the initial community. The size of the circle is relative to time-step so the smallest circles are samples from time-step 3 and the largest are from time-step 9. Positive movement along NMDS1 is correlated with increasing Shannon diversity (metaMSD, r^2 = 0.71***) and time-step (metaMDS, r^2 = 0.20*). Negative movement along NMDS2 is correlated with increasing community evenness (metaMSD, r^2 = 0.35***).

3.5 Discussion

Immigration was beneficial to total growth and local adaptation in the ambient communities. This is in agreement with theoretical models and laboratory experiments that predict that immigration should increase local adaptation through mechanisms such as increasing genetic variation for selection to act upon (Tallmon et al. 2004) and the introduction of preadapted species or genotypes (de Meester et al. 2011). However, contrary to expectations, immigration was detrimental to total growth and local adaptation in the warmed communities that were under the most stress during the initial weeks (in terms of total community growth rates).

The immigrating microbes were taken from open vials maintained at the same temperature as the experimental microcosms, however, all colonisers of these open vials came from the same surrounding (ambient) species pool. Therefore it is possible that immigrants to ambient communities were better preadapted to the conditions than those introduced to warmed communities. Therefore, one explanation for immigration being detrimental to warmed communities could be that locally maladapted immigrants to warmed communities had poor contribution to community growth and this could have decreased average total growth rates. Alternatively, the introduction of maladapted individuals into warmed treatments may have swamped natural selection. Maladapted individuals may have effectively been resetting the process of natural selection and thereby hindering local adaptation in warmed communities (Mayr 1963). This swamping process may be similar in outcome to that observed in some populations of stickleback in which individuals from lakes immigrate into rivers and prevent local adaptation towards the optimum body morphology for survival in the rivers (Moore and Hendry 2009). This is, to the best of my knowledge, the first time that the effect of immigration on local adaptation in diverse natural communities exposed to environmental change has been experimentally investigated.

The warming treatment was initially stressful and warmed communities suffered significantly greater decreases in total growth than communities that experienced ambient conditions. These decreases in

total growth occurred in paralleled with declines in metabolic diversity suggesting that the experimental conditions may have caused severe decreases in the abundance of some species or even local extinctions. Furthermore, there was a marked divergence in the metabolic profiles of warmed and ambient communities indicating that the metabolic functions lost at the initiation of the experiment differed between the treatments. Hence, 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). The provision of ecosystem services may rely on the balance between species in a community, for example the ratio of fungi to bacteria in soil can affect decomposition rates (Güsewell and Gessner 2009). Therefore, if warming commonly alters the species composition of communities, even without reducing total diversity, this could have negative impacts on ecosystem services in the future.

Immigration into communities reduced the extent of the loss of metabolic diversity at the beginning of the experiment and also helped to boost metabolic diversity as the experiment progressed 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 also been reported in mesocosm experiments with zooplankton exposed to environmental change, although the benefit of immigration depended on the nature of the stress (Thompson and Shurin 2012). In theory, the species that should be most readily lost when growth rates decline are rare species (Gonzalez and Chaneton 2002) and in decomposer communities these tend to be slow growing specialists that metabolise recalcitrant compounds (McGuire and Treseder 2010).Thus, one way in which immigration could improve functioning would be to prevent these rare species from becoming locally extinct or to reintroduce them. In this experiment, however, immigration did not affect the extent to which communities metabolised complex carbon compounds suggesting that immigration did not maintain metabolic diversity by promoting the colonisation of or survival of species that metabolise recalcitrant compounds. It might be anticipated that the main way in which immigration would prevent loss of metabolic functioning would be to increase or maintain species diversity (Loreau and Mouquet 1999).

My results, however, indicate that immigration did not affect species diversity. Therefore it follows that in communities that received immigration there was either more varied resource use between the species or that, on average, the constituent species had greater metabolic diversity. Immigration may have provided an opportunity for species with different metabolic profiles to members the resident community to (re)establish by exploiting vacant niches and this could produce a community in which component species have more complementary resource use. Niche partitioning of this kind has been found to increase productivity of algal communities (Cardinale 2012). Or alternatively, in closed communities as a consequence of tradeoffs (or due to a lack of selection pressure to retain metabolic diversity) species may have evolved to specialise on a narrower range of resources, whereas, in communities that received immigration this loss of function may have been prevented by gene flow. Unfortunately it is not within the scope of this study to differentiate between these two mechanisms but if further experiments were possible it would be interesting to investigate whether the beneficial effect of immigration on metabolic diversity could be explained by species becoming more specialised - and thereby enhancing niche partitioning - or by species retaining a broad metabolic profile - potentially allowing the community to metabolise a greater variety of resources.

Warming had a greater effect size than immigration on all of the aspects of community functioning and composition that I measured; indicating that in these communities the abiotic change in environment was more influential to ecological and evolutionary processes than the biotic change. However, if the sensitivity of species to the change in temperature differed between treatments (as the metabolic data suggest) this means that biotic factors such as species interactions may also have been altered by the warming. Thus, species would not only have to adapt to the physical change in environment but also to other direct and indirect pressures caused by biotic responses to warming, such as competition for resources and tolerance to allelopathic chemicals. Rather than a single environmental alteration causing directional selection many simultaneous changes may increase the number of traits under selection (Fisher 1930). Adaptation may then be hindered because, via mechanisms such as pleiotropy, adaptation of one trait may cause another trait to move further from the optimum (Orr 2000). Thus, I suggest that warming may have had a greater impact than

immigration on community functioning in this experiment because of the multiple secondary effects that warming could encompass.

In contrast to my hypothesis, the local adaptation of isolates was a poor predictor of the local adaptation of the communities from which they originated. This is not entirely unexpected as it has been shown previously that the dynamics of single species do not always correlate predictably with their dynamics when in a group (Fridley 2002; Schmidtke et al. 2010). However, to the best of my knowledge, the predictability of the local adaptation of a community based on the local adaptation of community members has not previously been assessed. One reason that the local adaptation of isolates was a poor predictor of community local adaptation could be that the dynamics of the isolates is dependent on whether other species are present; for example through quorum sensing bacteria can alter the chemicals that they produce depending on cell densities (Miller and 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). It is also acknowledged that the scale at which traits are measured can affect their interpretation (Noda 2004) and that at a broader scale (i.e. community) traits are less variable than at a narrower scale (i.e. species). This may occur, for example, through functional redundancy so that functions that would be lost by poor growth of a sensitive species are performed by other species in the community (Naeem 1998). If some species are able to maintain the functions that are lost by sensitive community members the local adaptation of isolates may have little bearing on that of the whole community.

Isolates from ambient communities were particularly poor predictors of community local adaptation and in fact average local adaptation of ambient isolates was significantly lower than that of the ambient communities. This occurred because the isolated species were maladapted; they grew better in warm assay conditions than in the ambient conditions which they evolved in. It is often the case that bacteria grow faster at warmer temperatures (Ratkowsky et al. 1982) so this itself is not surprising, however, the communities as a whole grew better at cooler (ambient) temperatures and therefore displayed local adaptation. A possible mechanism to account for this could be that there

were a small number of dominant species in these communities which were locally adapted and contributed the majority of community growth (Smith and Knapp 2003; Wertz et al. 2006) but were not included in the randomly selected subset that were assayed as isolates. I know of no reason, however, that this should have happened to a greater extent in ambient communities than warmed communities. Another potential explanation is that the selection regime may have unknowingly favoured species which grew well in warm conditions and therefore the maladaptation of isolates from ambient communities could be an artefact of the selection protocol (although there is no obvious reason that this would be the case).

Isolates from warmed communities had local adaptation that was not significantly different from that of the whole community and had a greater proportion of locally adapted isolates than ambient communities. This indicates that overall isolates from warmed communities were better locally adapted than those from ambient communities. This may have occurred if, due to the higher temperatures, generation times were faster on average in warmed communities over the course of the experiment as this could potentially allow beneficial mutations to arise more frequently and spread to fixation more quickly (Rich et al. 1979). This theory is supported to some extent by the finding that the shape of the growth curves differed between warmed and ambient communities during the second half of the environmental change experiment. Warmed communities displayed rapid initial growth rates and reached an asymptote during the assays whereas ambient communities displayed linear growth rates throughout the assays. Hence, if when growing in larger microcosms species in warmed communities maintained rapid doubling rates for longer, they could have undergone more generations within the same length of time compared to species in ambient communities. This would mean that species in warmed communities could have effectively have had more opportunity to adapt. This could be one explanation for warmed communities having a greater proportion of locally adapted species than ambient communities.

The warmed communities recovered towards the end of the environmental change in terms of total growth, local adaptation and metabolic function so that at the conclusion of the experiment the performance of warmed communities was not significantly different to that of ambient communities.

Yet, the evolutionary histories of species within the treatments differed. This meant that when species were isolated from their communities there were differences in total growth and local adaptation between those that evolved in warmed and ambient communities. So, although total community function was similar, the traits of the species within those communities differed. It has been demonstrated in laboratory experiments that adaptation and particularly the adaptation of key innovations may be contingent on a species evolutionary history (Blount et al. 2008) and that the amount that species are able to adapt in response to stress can depend on the rate of the environmental change (Mitchell and Hoffman 2010). Therefore, the future adaptation of species may depend on both their evolutionary history and the environmental change they have experienced. My findings suggest that ecosystem functioning of diverse microbial communities may be maintained or recovered despite environmental stress. Yet, if we only observe community level responses we may be obscuring damage done to the adaptive potential of constituent species which could have longer term consequences for ecosystem services.

In laboratory experiments with just a few species the evolution of focal species can be traced and the mechanisms behind it thoroughly examined but the relevance to natural diverse communities in changeable environmental conditions is debatable. On the other hand, field experiments which include environmental variability and diverse communities are undoubtedly preferable in terms of realism but they can lack general applicability. By carrying out the environmental change in the field but examining mechanisms in the laboratory I hoped to avoid some of these limitations but take advantage of the benefits. Below I discuss some of the limitations of my experiment.

Although my results do indeed show a convincing effect of warming and immigration on aspects of community functioning I acknowledge that in using these diverse communities my ability to rigorously explore the mechanisms behind the findings was constrained. For example, I was unable to determine whether the species in the initial community persisted and adapted to the environmental change or whether species sorting allowed the community to respond without adaptation of component species. One of the original aims of this experiment was to follow a few species throughout the experiment and observe how their local adaptation was altered by warming and

immigration and test whether these responses were consistent across species and replicate communities. Unfortunately, due to practical constraints and time limitations, this was found to be unfeasible but I still believe there would be value in this approach especially when considering how keystone or particular species of interest may respond to multiple simultaneous stressors as might be expected due to climate change (Parry et al. 2007). I suggest this would be a valuable direction for future research.

Another limitation of the experiment was that when species were assayed in isolation they were removed from the biotic conditions in which they evolved and so interspecific interactions were ignored. Therefore, it is possible that my measures of local adaptation of the isolates may not truly reflect their contribution to community local adaptation. Unfortunately, this was a necessary compromise because I was unable to measure the growth of individual species when embedded in a polyculture. In theory individual species' growth in microbial communities could have been tracked using real-time PCR or by artificially introducing fluorescently labelled genotypes or antibiotic resistant markers into the community (Gomez and Buckling 2011). This would, however, have compromised efforts to make the communities as natural as possible and, besides, transformed genotypes are commonly poor competitors (with the exception of *E. Coli* O157:H7 developed by Richie et al. 2003) so there would be no guarantee of them surviving the experiment. Having said this, there is no obvious reason to assume that species interactions would have been more influential to growth in one treatment than another. Therefore removing biotic interactions should have had the same effect in the 'home' and 'away' environments and if this is true my estimation of local adaptation should be valid. One on-going avenue of research is to study coevolution of microbial decomposers in their natural communities and this type of research should give us more understanding of the importance of species interactions in diverse communities and also whether environmental changes will affect the strength and nature of interactions.

3.6 Conclusions

My results demonstrate that warming and immigration had substantial impacts on the functioning and local adaptation of microbial communities. I found that the effect of immigration on community growth and local adaptation was dependent on the abiotic conditions; immigration was detrimental to communities that were under stress due to experimental warming but beneficial to ambient communities. I suggest this may be due to the immigration of maladapted individuals swamping evolution in warmed communities. The data indicate that the local adaptation of the community as a whole is not well predicted by the local adaptation of species within the communities and I suggest that it would be beneficial for other studies to address how exposure to stress affects the long term functioning of communities and the evolution of component species.

Chapter 4:

WARMING AND REPLICABILITY

4.1 Abstract

A major aim of ecological research is to predict how species and ecosystems will respond to environmental change, however, previous work has shown that environmental disturbances themselves can affect the predictability of responses. The effect of warming on predictability has rarely been studied, yet, warming is one of the principle outcomes forecast due to climate change. Therefore in this research I test the hypothesis that warming will decreases response predictability and, furthermore, I also investigate how immigration and scale affect predictability. Using data from an 81day field experiment in which I experimentally warmed microbial decomposer communities, described in Chapter 3, I find that warming increases the variability in growth and metabolic functioning between replicate communities. This is consistent with the hypothesis that warming would decrease response predictability. My results imply that this may be because faster growth rates in warmed communities allowed greater divergence in traits such as metabolic profile between replicate communities. In addition, total growth throughout the warming experiment was positively correlated with predictability, suggesting that high variability may warn of an ecosystem under stress. Thus, in conclusion, my results strongly indicate that warming can decrease the predictability of species and community growth and functioning. This could have important implications for our forecasts of the impacts of climate change in the future and for the management of ecosystems exposed to environmental change.

4.2 Introduction

One of the major aims of ecological research is to predict how species and ecosystems will respond to environmental disturbances. Yet we know that predictability may vary depending on factors such as the type of disturbance (Murphy and Romanuk 2012), the scale at which is it measured (Noda 2004) and the species richness of the community (Loreau 2000; Ptacnik et al. 2008). Therefore in order to accurately forecast ecosystem dynamics in response to environmental change there is a need to study predictability itself. Here I report on the results of an experiment in which I manipulated temperature and immigration into diverse communities of microbial decomposers. I examine how immigration and warming affect predictability of community traits and, furthermore, whether predictability is consistent across levels (i.e. individual species and community).

Research into the responses of species and communities to environmental change commonly focuses on changes in mean trait values such as abundance or productivity (as described for my warming experiment in Chapter 3). Yet, disturbances can also increase the variability of responses in ecological systems (Fraterrigo and Rusak 2008). Recent experimental and theoretical research has indicated that increases in variability can precede significant ecological regime shifts such as switches from oligotrophy to eutrophy in lakes (Carpenter and Brock 2006). Other studies have indicated that increased variability may be an indication of a species or community under stress (Warwick and Clarke 1993). This means that even if average trait values are not significantly affected by a disturbance increased variance may be indicative of a population or community at risk. Therefore the predictability of responses, defined as the among-replicate variation in a trait of interest (Carpenter and Brock 2006), is an important variable to consider when assessing the impacts and consequences of environmental change.

Anthropogenic activities are accelerating environmental change (Parry et al. 2007) and as a result we can expect greater disturbances in the future and potentially less predictable outcomes. This phenomenon may make it increasingly difficult to distinguish background variance from that caused

by environmental change and therefore it may take longer and require changes of a greater magnitude before we can statistically identify the impacts of climate change or other disturbances (Cottingham et al. 2000). This may impair effective management of ecosystems or species threatened by environmental change or overexploitation.

A major branch of ecological research has considered the impacts species extinctions may have on community functioning and ecosystem stability. It has been found that decreasing biodiversity commonly leads to reduced stability (Loreau 2000; Ptacnik et al. 2008). This can occur because in more diverse communities redundancy may allow functionally similar species to act as a buffer to maintain functioning despite local extinctions (Cottingham and Carpenter 1998). In addition to this, in less species rich communities there is a greater chance that species abundances will fluctuate in synchrony (Gonzalez and Descamps-Julien 2004), which would potentially produce more variable responses between replicates. It has also been shown that more variable ecosystems can be prone to losing rare species (Pimm 1988) and this may have a disproportionate impact on functioning in some ecosystems (McGuire and Treseder 2010). Therefore if disturbances caused by anthropogenic activities do increase variability this may increase the likelihood of extinctions or damage to ecosystem services beyond those expected to be caused by the environmental change alone.

One of the foremost disturbances anticipated due to climate change is an increase in mean global temperature (Parry et al. 2007) so it is crucial to investigate whether predictability is affected by warming. In theory, ecosystem and species traits may be more variable at higher temperatures because process rates are increased. At warmer temperatures traits such as generation times and species turnover may be faster (Brown et al. 2004; Hillebrand et al. 2010) and therefore discrete communities may diverge in their composition or dynamics over a shorter timescale than they might in ambient conditions. Additionally, predictability of responses in warmed conditions may be decreased because warming may cause local extinctions thereby decreasing biodiversity and potentially ecosystem stability (Loreau 2000). In a field experiment exposing an Arctic plant community to warming and herbivore removal Post (2013) reported that warming decreased community stability and suggested that this was primarily due to a decrease in species diversity. This research by Post (2013) is the only

other experiment to directly assess the impact of warming on response predictability that I am able to find in the literature. Therefore it is vital that more research be carried out into the effect of warming on predictability.

In a meta-analysis of the effects of disturbance on predictability Murphy and Romaiuk (2012) did not detect a significant effect of warming on variability. Unfortunately the study suffered from a lack of appropriate data on responses of aquatic systems to warming and therefore could not disentangle results from aquatic and terrestrial systems. Aquatic systems may be expected to be particularly prone to decreased predictability with disturbance because traits that may increase response variability such rapid rate of biomass turnover (Shurin et al. 2006) and strong species interactions (Shurin et al. 2002) may be elevated compared to terrestrial systems. Ocean surface temperatures have already increased due to anthropogenic activities (Barnett et al. 2005) meaning that some of the primary regulators of the global carbon cycle such as marine phytoplankton (Falkowski et al. 2000) are already experiencing warming. Thus, it is vital to understand how disturbance affects predictability in aquatic systems to ensure accuracy of climate change forecasting.

Another expected consequence of climate change is species migrations (Parmesan and Yohe 2003). Broadly speaking, if a species cannot tolerate environmental conditions it must adapt or migrate to avoid extinction. There have already been changes to species distributions (Walther 2010) and there are expected to be more in the future (Parmesan and Yohe 2003) so the impact of migration on ecosystem predictability is of particular interest. There are a number of mechanisms by which immigration may increase predictability. Firstly, immigration may boost or maintain species diversity (Jackson and Sax 2009) and, as discussed earlier, this should increase predictability of ecosystem responses (Naeem and Li 1997). Secondly, immigration may cause homogenisation (Rahel 2002) thereby reducing variation in species composition between communities, which may make community growth and functioning traits more consistent. Finally, mathematical models indicate that immigration should increase community stability by simplifying complex dynamics (Holt 1983) and, as stability may be considered analogous to a lack of variability (Steiner 2005), the more stable a system is the more predictable its responses should be. There is also evidence, however, that high

levels of immigration could destabilise population dynamics (Stone and Hart 1999). In a mesocosm experiment exposing zooplankton communities to differing nutrient addition and dispersal regimes Forrest and Arnott (2007) found that the effect of dispersal on predictability was dependent on both nutrient addition and the identity of the species introduced indicating that there may not be a consistent effect of immigration on predictability.

A large body of research has shown that the spatial scale at which ecosystem properties are measured has been shown to affect predictability with broader scales producing more replicable responses than narrower scales (Levin 1992). For example it has been found that even in patches with similar species composition local dynamics can vary and traits such as primary productivity and nutrient cycling are often contingent on the history of the patch, species interactions and local idiosyncrasies (Huston 1999; Cottenie et al. 2003). Therefore traits are often more reproducible when observing regions rather than patches. A similar hierarchical structure exists when considering the dynamics of individual species compared to the more broad dynamics of the community (Noda 2004). This may occur because component species rarely fluctuate in synchrony (Doak et al. 1998) and at the community-level functional redundancy may obscure the more variable species-specific responses to environmental change. As a result, responses may be more predictable between replicate communities than between their constituent species.

4.2.1 Predictions

Hypothesis I: Warming will increase the variability between replicate communities.

This may occur because in warmed communities there may be faster generation times and rates of species turnover and this could increase the rate of divergence in traits between replicate communities compared to in ambient conditions.

Hypothesis II: Immigration will increase predictability.

If immigration is sufficient to cause homogenisation of community composition this will make communities more similar and this could also constrain divergence of community traits.

Hypothesis III: Community responses will be more predictable than individual species' responses.

This is predicted to occur because processes such as functional redundancy may allow the community to maintain function despite idiosyncratic responses of component species.

Here I report on the results of an experiment in which I assess how the predictability of community growth, functioning and composition is affected by warming and immigration over an 81day field experiment using diverse communities of decomposer bacteria. I also investigate differences between the predictability of individual species and replicate communities. I discuss the implications my results may have for climate change forecasting and for the management of ecosystems exposed to environmental change.

4.3 Materials and Methods

The data presented here were collected during the field experiment and laboratory experiments described in Chapter 3 therefore please see Chapter 3 pages 46-50 for experimental Materials and Methods. Statistical analyses differ from Chapter 3 and are described below.

4.3.1 Statistical analyses

To measure variability between replicate communities or isolates from within a community I used the natural log of absolute deviates from the median total growth rates (change in OD_{600} over 24 to 96h) as response variables. This scaled the deviates as a function of the medians. I compared standardised deviates from different treatments using linear mixed effects models (lmer) with error structures to account for repeated sampling of the same microcosm over time and spatial blocking. For all lmers the main explanatory variables were warming treatment, immigration treatment and, where multiple sample were taken from the same microcosm over time, time-step (as a factor). To select the minimum adequate model I began model simplification with the maximal model and removed the least significant interaction terms one at a time. At every step of the simplification I used analysis of variance (ANOVA) to compare models fitted using the maximum likelihood criterion to ensure that the simplification did not significantly reduce the explanatory power of the model (detailed model simplifications and final model statistics are shown in the Appendix as indicated in the Results section). ANOVA and Tukey's Honest Significant Difference tests - calculated from lmers - were used to contrast particular treatments of interest. To measure the synchrony of isolate responses to a novel environment I calculated the difference in total growth over 24 to 96h in contemporary conditions ("home") compared to foreign conditions ("away"; the change in environment consisted of a change in temperature to match that experienced by samples from the other level of warming during the preceding time-step). I then calculated the natural log of the absolute deviation of each isolate from the median difference between growth in "home" and "away" conditions. This was used as a response variable in an lmer with a random effect to account for spatial blocking.

To compare the effect of immigration and warming on the variability in metabolic profile of the replicate communities a principal components analysis (PCA) was performed and the metabolic profiles were plotted on to ordination space. The difference between metabolic profiles of replicate communities at each time-step was used as a measure of variation between communities and was calculated as the perimeter of a triangle connecting the replicates in multivariate space. Principal components (PCs) 1, 2 and 3 were used therefore giving three measures of between-replicate variation. An lmer was used to account for these repeated measures and, again, to account for the spatial blocking and repeated measures of the same samples over time.

4.4 Results

4.4.1 The effect of warming and immigration on predictability of community growth rates

Comparison of between-replicate community variability (calculated from standardised deviates of total growth) indicate that ambient communities had approximately half as much variability in total growth as warmed communities. The magnitude of the effect warming on replicability varied over time but was greater on average in warmed communities during all time-steps except for time-step 7 (Figure 4.1). This change in the variability of warmed and ambient communities during the experiment is shown by the significant interaction between warming and time-step which explained approximately 10% of the model deviance (ANOVA of lmer of between replicate variability, $F_{7,118}$ = 3.48, MES = 0.22, $p<0.01$; model simplification Appendix 4.1 and final model statistics Appendix 4.2; Figure 4.1). This interaction was driven by the particularly high variability of warmed communities at time-step 5. Between-replicate variability of warmed communities was significantly greater at time-step 5 than at all other sampling points with the exception of time-step 9 (Tukey HSD of final lmer, $p<0.05$). In contrast to this, there were no time-steps at which variability of ambient communities was significantly elevated (Tukey HSD of lmer, $p>0.05$). These results are consistent with the prediction that warming would decrease replicability.

Contrary to predictions, there was no significant effect of immigration on predictability; communities that received immigration had slightly, but not significantly, more variability in total community growth rates than those remained closed (ANOVA of lmer of between replicate variability, $F_{1,118}$ = 0.003, MES = 0.0002, $p>0.05$).

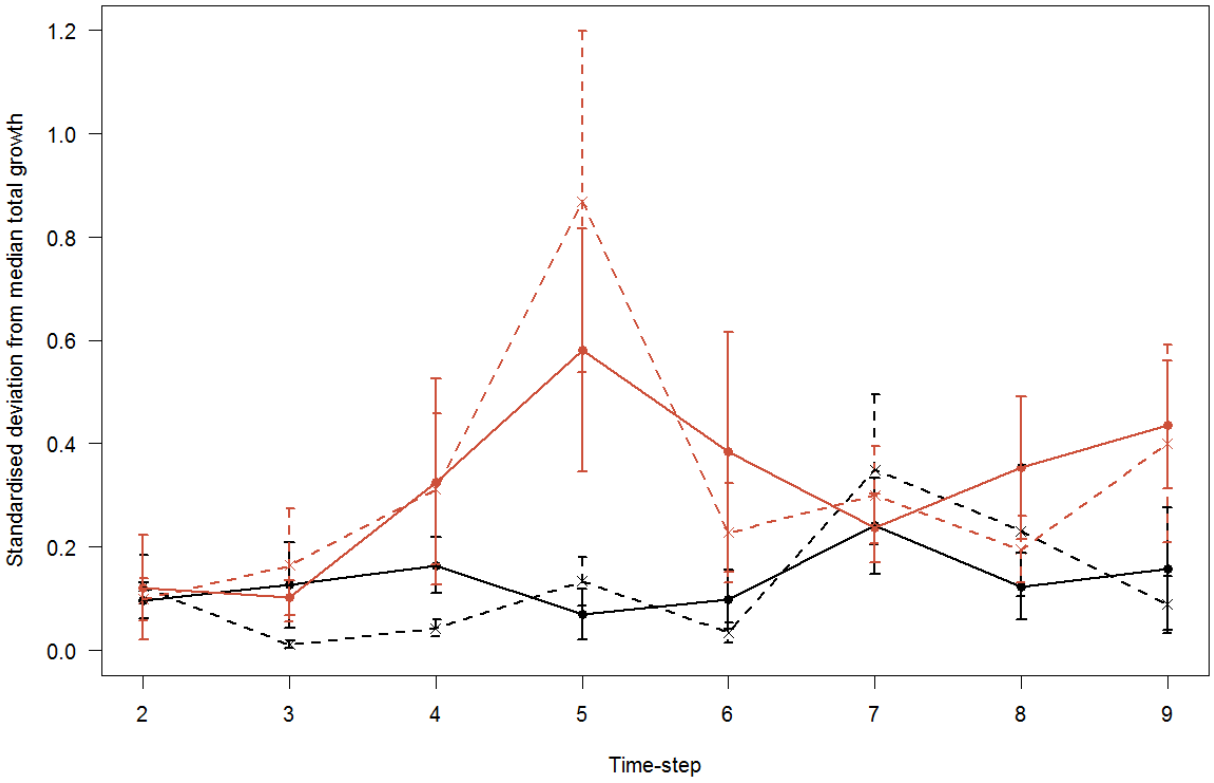


Figure 4.1. Standardised deviates from the median treatment total growth at each time-step of the environmental change experiment. Red lines represent warmed communities and black lines represent ambient. Solid lines with circles denote communities that received immigration and dashed lines with crosses are those that remained closed. Error bars show standard error of the mean for 5 replicate communities. The variability of total growth rates of warmed communities was significantly greater than that of ambient communities (ANOVA of lmer of interaction between time-step and warming treatment on between replicate variability, $F_{7,118} = 3.48$, MES = 0.22, $p<0.01$) indicating that warming may decrease predictability of community processes.

4.4.2 Variability correlated negatively with total growth

There was a negative correlation between the total growth of communities and the between-replicate variation in total growth (Pearson's correlation = -0.24, df = 158, t = -3.05**; Figure 4.2) indicating that, on average, when community growth rates are low there is a large amount of variability in performance of the replicates. This supports the hypothesis that variability itself may be a sign of poor community functioning or of a community under stress.

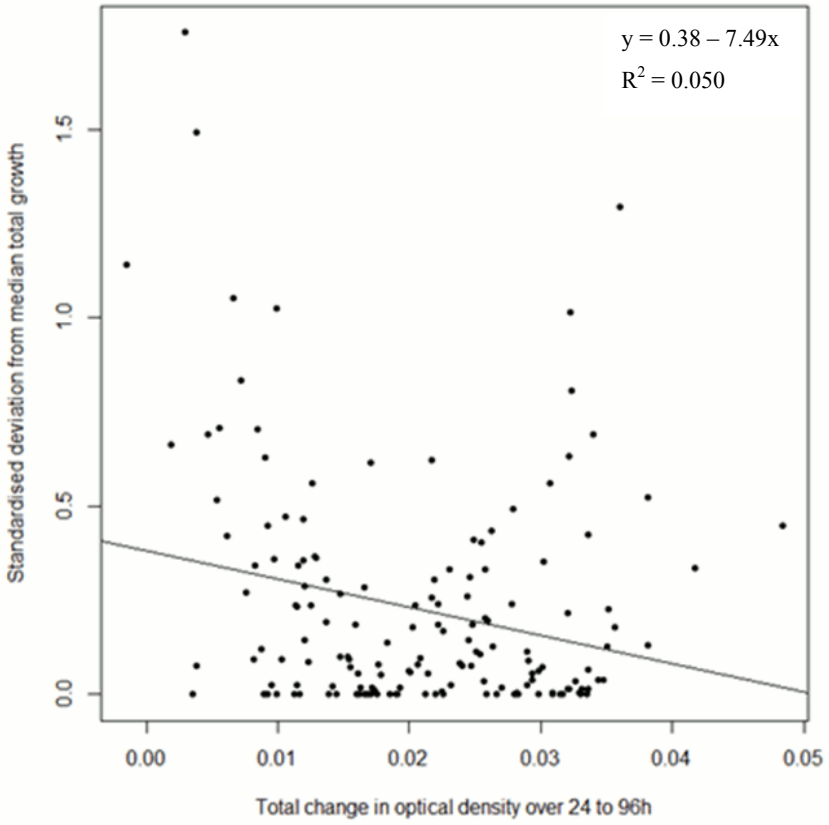


Figure 4.2. Relationship between total growth of communities and between-replicate variability in total growth. Standardised deviations from median total growth plotted against community total growth rates for each treatment at each time-step (n = 160). There is a negative correlation between variability and total growth (Pearson's correlation = -0.24, df = 158, t = -3.05**) indicating that when communities grew poorly variability between replicates was high.

4.4.3 Variability in metabolic function between replicate communities

The divergence in metabolic profiles between replicate communities (calculated by measuring distance between replicate communities in multivariate space of PCA plots) was significantly greater for warmed communities than ambient ones (ANOVA of lmer of variability in PCs of replicate communities, $F_{1,19} = 4.7$, MES = 380.8, $p<0.05$; model simplification Appendix 4.3 and final model statistics Appendix 4.4; Figure 4.3 and Appendix 4.5) and this explained approximately 10% of the model deviance. This indicates that the metabolic functioning of warmed communities was more variable and therefore less predictable than that of ambient communities.

Similarly to the growth assays there was no significant effect of immigration on between replicate variability in metabolic profile (ANOVA of lmer of divergence in metabolic profile, $F_{1, 19} = 0.69$, MES = 55.8, $p>0.05$).

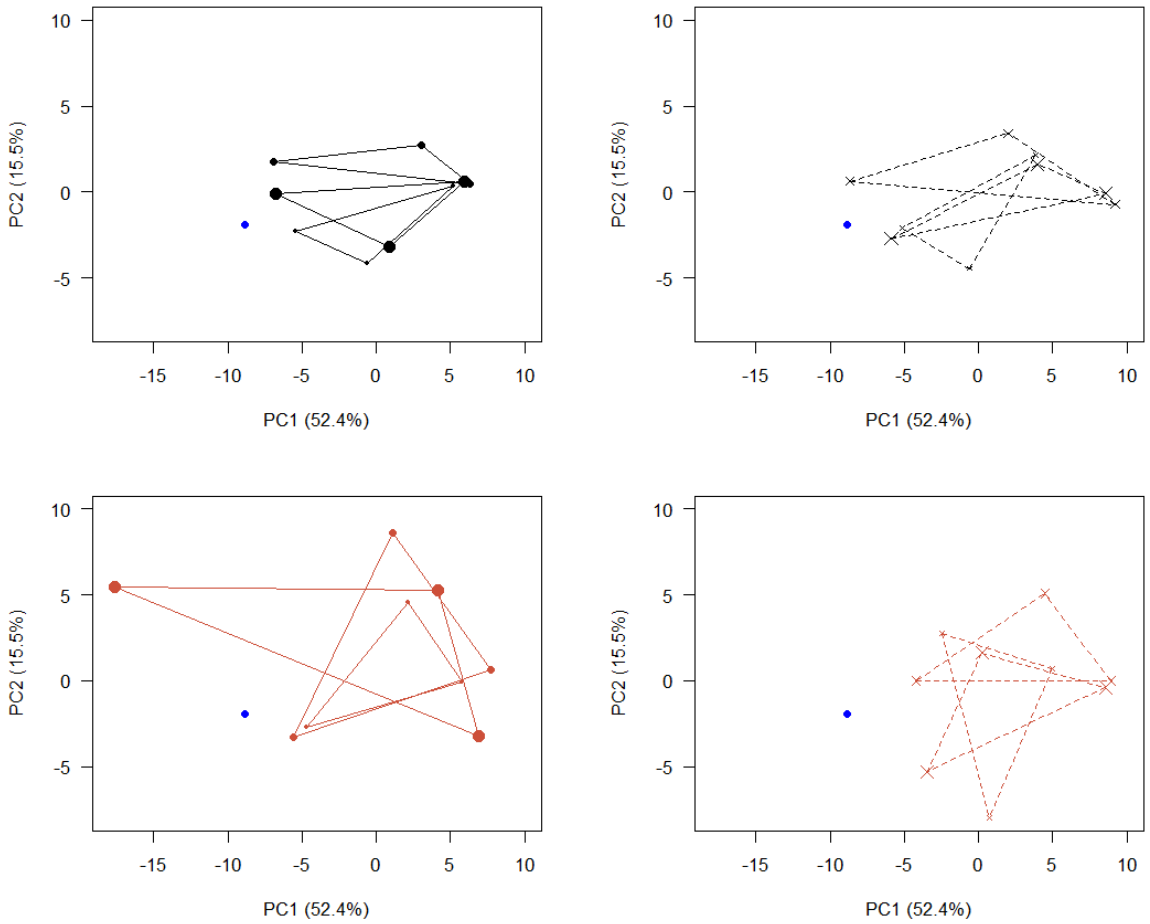


Figure 4.3. Variability in the metabolic profile of replicate communities during the environmental change experiment. Principal components analysis was used to represent ordination space (only PCs 1 and 2 are shown here, see Appendix 4.5 for PC3 graphs) and triangles are drawn to connect the metabolic profile of three replicate communities from each immigration-warming treatment at time-steps 3, 6 and 9. Black lines represent ambient communities and red lines warmed communities. Solid lines with circles denote communities that received immigration and dashed lines with crosses are those that remained closed. The size of the points represents the time-step from which the sample was taken so that smallest points are time-step 3 and largest are time-step 9. The blue point represents the metabolic function of the initial community. The perimeter of the triangles is used as a measure of the variability in metabolic profile between replicate communities. Warmed communities had significantly greater variation in metabolic profile compared to ambient communities (ANOVA of lmer of variability in PCs of replicate communities, $F_{1,19} = 4.7$, MES = 380.8, $p<0.05$).

4.4.4 Predictability of isolate and community growth

At the conclusion of the environmental change experiment the between-isolate variability in total growth was greater than that of communities (ANOVA of lmer of isolate and community standardised deviates, $F_{1, 130} = 5.9$, MES = 4.37, $p<0.05$; model simplification Appendix 4.6 and full model statistics Appendix 4.7), however, this only explained about 4% of the model deviance. These results indicate that variability was greater between species within a community than between replicate communities and therefore responses were more predictable at the community level than at the species level. This is consistent with the hypothesis that functioning should be most predictable at broad scales.

Isolates within warmed communities had less variability in their total growth rate than those from ambient communities although not significantly so (ANOVA of lmer of between-isolate variability, $F_{1, 113} = 2.62$, MES = 2.19, $p>0.05$; model simplification Appendix 4.8 and final model statistics Appendix 4.9). This is the opposite finding to community variability in which in between replicate variability was significant greater for warmed communities compared to ambient ones. Therefore the effect of warming on variability was not consistent across isolates and communities.

In agreement with whole community results, immigration did not have a significant effect on between-isolate variability in growth rates (ANOVA of lmer of between-isolate variability, $F_{1, 113} = 0.41$, MES = 0.34, $p>0.05$).

4.4.5 Synchrony of responses of isolates from the same community to a change in environment

There was a significant interaction between the warming and immigration treatments on the deviation in responses of isolates from the same community to a change in environment (ANOVA from lmer comparison of standardised deviates from median difference in total growth, $F_{1, 113} = 24.3$, MES = 10.85, $p<0.001$; model simplification Appendix 4.10 and final model Appendix 4.11, Figure 4.4) which explained approximately 16% of the model deviance. This interaction was driven by the high variability in the responses of isolates from warmed communities that did not receive immigration. In

these communities variability in isolate responses were significantly greater than that of all other immigration-warming treatments (Tukey HSD of lmer, comparisons of isolates from warmed closed communities with all other treatments $p<0.05$). Other differences between treatments did not reach significance (Tukey HSD of lmer, all comparisons $p>0.05$). These results indicate that species' responses to an environmental change were less synchronous in warmed communities than ambient communities.

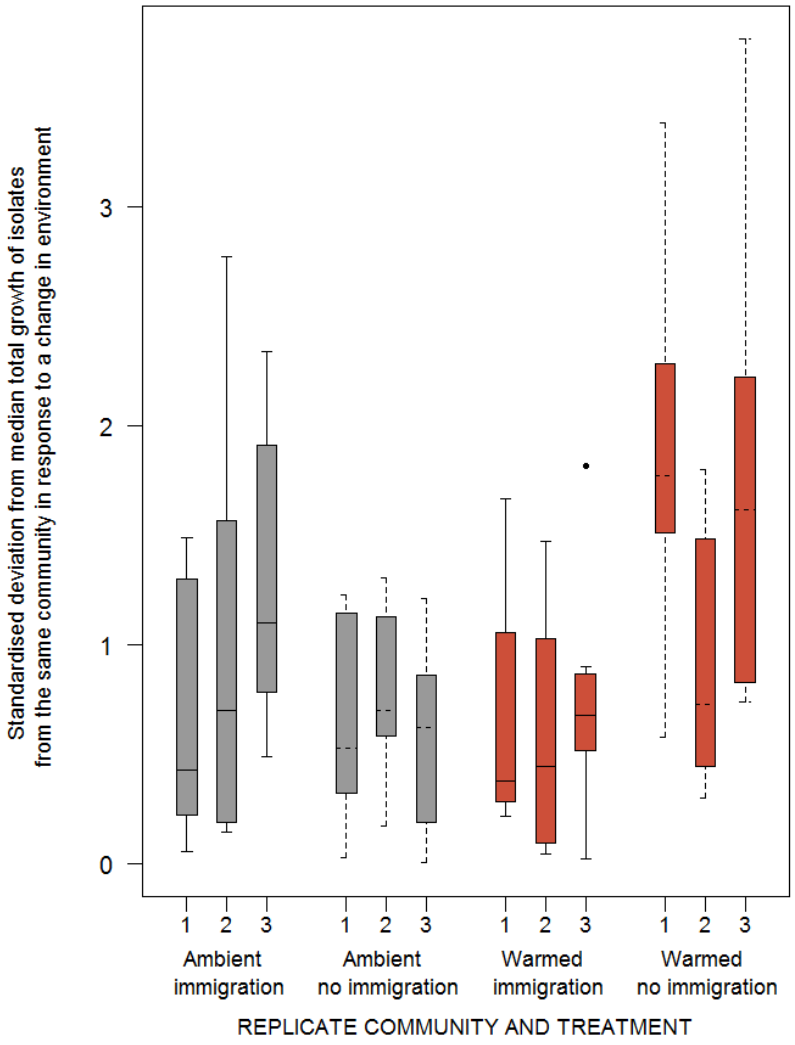


Figure 4.4. Deviation in the responses of isolates from the same community to a change in environment. Boxes display the variation in difference in total growth in a contemporary and foreign environment (local (mal)adaptation) for 10 isolates from the same community. Data were standardised according to the median within-community difference in total growth of the isolates in the two environments. Lower values of deviation indicate greater synchrony in isolate responses to the change in environment. There was a significant interaction between the immigration and warming treatments (ANOVA from lmer comparison of standardised deviates from median difference in total growth, $F_{1, 113} = 24.3$, MES = 10.85, $p<0.001$). Isolates from warmed communities that did not receive immigration had significantly less synchronous responses to warming than all of the other immigration-warming treatments (Tukey HSD from lmer, $p<0.05$).

4.5 Discussion

Experimental warming increased the between-replicate variability in the total growth and metabolic function of communities. This is consistent with the hypothesis that warming would decrease predictability. Below I discuss three non-mutually exclusive mechanisms that could explain this finding.

Firstly, in theory, if warming caused a decrease in species richness this could reduce community stability (Loreau 2000; Ptacnik et al. 2008). This mechanism was found to be responsible for decreases in stability caused by warming in a field experiment by Post (2013), however, as I found no significant difference between diversity of warmed and ambient communities (reported in Chapter 3 section 3.4.12 page 64 and Appendix 3.19 and 3.21) this mechanism cannot explain the reduced response predictability in this experiment.

Secondly, if species within the warmed communities had more synchronous responses than those in ambient communities this could make variability between communities greater. So rather than redundancy allowing community growth to be maintained despite some species declining in abundance, similar responses among species may have limited the benefit of functional redundancy. If these effects were of variable magnitudes between replicates of if replicates happened to be sampled at different stages in a fluctuating cycle (Thibaut and Connolly 2013) this would result in greater variability between replicates of warmed compared to ambient communities. This hypothesis is refuted by the finding that isolates within warmed communities that did not receive immigration had significantly greater variability in growth than isolates within communities from the other treatments. Hence, species in ambient communities responded to warming in a similar way to each other whereas those in warmed communities without immigration had more idiosyncratic and therefore asynchronous responses. Thus, in this experiment, impaired functional redundancy via synchronous responses between species within a community cannot explain the increased variability in warmed communities.

Thirdly, faster process rates in warmed communities (Brown et al. 2004; Hillebrand et al. 2010) may have increased the divergence in growth and functioning between replicate communities. This mechanism is supported by the finding that over the second half of the environmental change experiment warmed communities achieved a significantly greater proportion of their total growth during 24 to 48h of the growth assay than ambient communities (reported in Chapter 3 section 3.4.2 pages 52-53, Appendix 3.3 and 3.4). This occurred because warmed communities had rapid early growth rates and approached carrying capacity faster. Hence, the maintenance of faster doubling rates throughout the environmental change experiment in warmed communities could have allowed processes such as species sorting and evolution to occur more rapidly. This could explain the greater divergence in traits such as metabolic profile between replicate warmed communities compared to replicate ambient communities.

Thus the only mechanism supported by the data to account for the decreased replicability in warmed communities is that warming facilitated the maintenance of faster process rates thereby allowing greater divergence in traits between warmed communities. Due to the paucity of research on the effect of warming on predictability I am unable to say how common or important this mechanism may be in natural ecosystems in general but as warming is one of the principle outcomes anticipated due to climate change (Parry et al. 2007) it would certainly be beneficial for further research to be done into the effect of warming on predictability. This finding is relevant for forecasts of the impacts of environmental change in the future. For example, species distribution models incorporate variability into their forecasts but assume that environmental changes do not affect variability, however, in light of this research, I suggest that it would be beneficial to question whether this assumption provides us with the best estimates of the future distribution of species particularly when considering the impacts of warming (Elith and Leathwick 2009).

My results are in contrast to a meta-analysis performed by Murphy and Romaiuk (2012) who found no significant effect of warming on predictability. This difference in results could have arisen because my experiment was performed using aquatic communities whereas their analyses were more heavily weighted towards terrestrial systems. In aquatic communities mechanisms such as strong species

interactions (Shurin et al. 2002) and rapid rates of biomass turnover (Shurin et al. 2006) that could potentially increase variability may be more prevalent than in terrestrial communities. Consequently, predictability may be expected to be low in aquatic systems. Aquatic communities are of particular importance in terms of climate change forecasts. Unicellular marine phytoplankton are estimated to be responsible for up to half of global net primary productivity and are a major contributor to global carbon cycling (Falkowski et al. 2000) and as a result their responses to warming may have profound impacts on climate change in the future. Freshwater aquatic systems such as rivers and lakes are also influential to carbon cycling and their carbon emissions are thought to be on a par with those caused by land-use change (Cole et al. 2007). Moreover, freshwater ecosystems are also valuable because they provide other essential ecosystem services such as denitrification and water purification (Ducklow 2008). Both freshwater and marine ecosystems are already experiencing warming due to anthropogenic activities and this is having detectable effects on their ecology (e.g. Sinutok et al. 2012). Thus, the impact of warming on aquatic communities is a timely and potentially very important issue. For these reasons it is vital for us to understand the effect warming may have on predictability, particularly in aquatic communities, so that we can accurately forecast future climate change and protect ecosystem services that we value.

In contrast to the prediction that immigration would increase replicability of community responses I found that immigration did not have a significant effect on the predictability either community growth rates or metabolic functioning. In fact, the only significant effect of immigration that was detected in this experiment was that it made the responses of warmed isolates to a change in environment more synchronous. There is little consensus arising from previous research into the effect of immigration on predictability. For example, in a mesocosm experiment with seagrass communities France and Duffy (2006) found that the effect of dispersal on stability was dependent on diversity and in an experiment with zooplankton Forrest and Arnott (2007) found that dispersal had varying effects on predictability depending on nutrient enrichment and the identity of the species that immigrated. These studies and the data I present here suggest that immigration does not have a consistent effect on predictability in communities. It would be beneficial to explore highly simplified scenarios to determine whether this

attributable to the complexity of the communities that have been studied in the past or whether it is a general result. The effect of immigration on predictability of ecological functioning is likely to become increasingly important in the future as climate change causes increased species migrations (Huntley et al. 2006).

Isolates were found to have less predictable total growth than communities indicating that the level of complexity at which functioning is measured can influence interpretation of the effects of environmental change. Isolate variability could have been enhanced in this experiment because when species are grown in isolation species-specific responses are exposed but when in a community these may be averaged out or obscured entirely (due to functional redundancy). It has long been recognised that the spatial scale at which community and species traits are measured can be important (Levin 1992). This work provides further evidence that responses can be inconsistent across species and communities and, furthermore, that the level of complexity that traits are measured at can affect interpretation of ecological processes. Thus, in order to understand predictability of ecosystem processes under environmental change we should consider the complexity level at which we are measuring traits (Noda 2004).

Notably, my results indicate that predictability is positively correlated with total growth demonstrating that variability may correspond directly to stress. Therefore in this system between-community variability was a proxy for stress. This result is agreement with findings from some marine systems (Warwick and Clarke 1993). If this association between variability and stress is found to be a common trait in natural populations or communities it could be useful in monitoring and management as variability could be used as an indicator of a species or community at risk before mean trait values, such as population density, begin to decline.

4.6 Conclusions

Warming decreased the predictability of growth and functioning in these experimental communities. My results suggest that this was not due to increased synchrony of responses between species within

communities or decreased diversity in warmed communities but because faster processes rates led to more divergent traits. If warming commonly reduces predictability of community responses this could have significant implications for the accuracy of forecasts of climate change and for monitoring the impacts of warming. I also found that predictability was lower at the individual level compared to the community level highlighting that the level of complexity at which measurements are taken can influence interpretation of them. Finally, total growth throughout the warming experiment was positively correlated with predictability suggesting that high variability may warn of an ecosystem under stress. Together these results indicate that to improve our forecasts of ecological responses to warming, at both the community and species level, we should also consider the impact that warming can have on predictability.

Chapter 5:

TESTING THE STRESS-GRADIENT HYPOTHESIS

5.1 Abstract

The stress-gradient hypothesis (SGH) posits that as abiotic stress increases the importance of facilitative interactions relative to negative interactions will increase. The SGH was developed largely with plant communities in mind and as a result mathematical models designed to test it have tended to favour types of interaction specific to plants. Yet, in recent years the SGH has been found to accurately predict how interactions change in response to stress in species such as algae, mussels and moths implying that more generally applicable mechanisms may be responsible for the SGH. In this research I used a mathematical model to investigate whether patterns consistent with the SGH would arise when species could only interact in very fundamental ways i.e. through resource use and allelopathy. The species resource use trade-offs were defined so that one species would tend to use the primary resource and the second species would typically to use the waste product of the metabolism of the primary resource. In this way the species' evolution of resource use could alter the degree to which facilitation (cross-feeding) occurred. In addition to this, the species had varying sensitivities to the stress thereby increasing the realism of the model. My results were consistent with the SGH and species interactions became more facilitative as average stress intensified. This occurred because at greater stress the species evolved to become specialists on the two resources thereby decreasing overlap in resource use and increasing facilitation through cross-feeding. In addition to this allelopathy decreased as stress intensified due to density dependent effects. My results suggest that the SGH could arise through fundamental interactions that are common to almost all organisms and therefore that the SGH could be a more wide-spread phenomenon than previously recognised.

5.2 Introduction

The stress-gradient hypothesis (SGH) posits that community-wide facilitative interactions increase in importance relative to competitive interactions along gradients of increasing abiotic stress (reviewed by He et al. 2013). The SGH was first proposed by Bertness and Callaway (1994) in an article drawing attention to the importance of positive interactions in ecology. In the past two decades facilitation, defined as an improvement in the growth of a beneficiary species without detriment to the facilitator species, has become a more prevalent research topic. For example, facilitation is recognised to play an important role in biodiversity-ecosystem functioning (Cardinale et al. 2002) and community assembly (Bruno 2000) and positive interspecific interactions have also been shown to influence evolution (Lortie 2007; Kikvidze and Callaway 2009) and coevolution (Lawrence et al. 2012). The SGH, however, remains a somewhat contentious theory because empirical tests have both supported and contradicted it (e.g., Bertness and Ewanchuk 2002; Maestre and Cortina 2004), and because it was originally empirically-derived, there has been little formal theory to support it. Here I report on the results of a mechanistic model of the SGH that examines how facilitation through cross-feeding and competition through allelopathy in a pair of microbes changes along a gradient of increasing stress.

Ecosystems worldwide are in a state of rapid change caused by anthropogenic activities (Parry et al. 2007). Climate change is predicted to make extreme events such as droughts and floods more frequent and it is already causing gradual changes across terrestrial and aquatic ecosystems with significant consequences (Walther 2010; Sinutok et al. 2012). Furthermore, intensive agriculture and urbanisation are triggering eutrophication and warming in rivers and lakes (Ptacnik et al. 2008) and associated land-use changes are decimating natural habitats (Foley et al. 2005). Processes such as these will result in species increasingly experiencing environments for which they are not adapted and this may reduce individuals' survival, growth and reproduction. Therefore stress, defined as a reduction in the conversion of energy to biomass due to constraints imposed by the abiotic environment (Grime 1977), is likely to be exacerbated in future. For this reason it is important that we

consider the way that stress may affect community functioning so that we can prepare for or mitigate the negative consequences of environmental change.

One potentially important but often overlooked factor that can influence ecosystem properties is facilitation. Competition has commonly been the principal focus of ecological research and two of the major and perhaps best established ecological theories are based on competitive interactions. Niche theory (Hutchinson 1957) and competitive exclusion (Gause 1934) together have been used to help explain processes as diverse as speciation, range expansion, community assembly and adaptation (Colwell and Rangel 2009). These theories assume that facilitative interactions have a negligible effect on community processes. Yet for centuries ecologists have recognised that facilitative interactions are common in nature (e.g., Clements 1916) and recently facilitation has been shown to be as important as competition in influencing some aspects of community functioning (Stachowicz 2001). For example, by creating new habitats or producing resources which another species can use (Crooks 2002) or ameliorating stressful abiotic conditions (Bertness and Leonard 1997) facilitation can expand the realised niches of interacting species (Bruno et al. 2003). Consequently, in some situations facilitation can enhance the diversity or productivity assemblages of species (Stachowicz 2001; Mulder et al. 2001; Cardinale 2011). For these reasons there has been a drive to include facilitation and mutualism in ecological theories with the hope of enhancing their predictive power (Bruno et al. 2003; Michalet et al. 2006).

The imminent intensification of stress that is predicted to happen as a consequence of climate change makes it imperative that we research the SGH and the resulting effects changes in species interactions may have on community functioning. If, as the SGH predicts, positive interactions do become more important as stress increases this could, in theory, preserve some level of ecosystem functioning even when environmental change is severe. Or alternatively, increases in facilitation concurrent with stress may be insufficient to counteract the negative effects of stress on growth and survival. Therefore, to help guide conservation strategies we need to discover if the SGH is a ubiquitous phenomenon and, moreover, we need to determine how changes in species interactions along gradients of stress will affect ecosystem functioning.

The SGH has most commonly been investigated in vascular plant communities, for example, in salt marsh, alpine and arid ecosystems (e.g., Bertness and Leonard 1997; Callaway et al. 2002; Pablo Lopez et al. 2013). In plant communities such as these, facilitation can take the form of neighbouring plants ameliorating stressful conditions by preventing soil instability and wind damage or nurse plants providing increased water through shading and hydraulic lift (Brooker et al. 2008). These mechanisms are somewhat unique to vascular plants; yet results consistent with the SGH (or the "hump-shaped hypothesis" as discussed later) have been reported in non-vascular plants such as microalgae (Bulleri et al. 2011) and even in animals, for example, mussels (Rius and McQuaid 2009), detritivorous invertebrates (Fugère et al. 2012) and herbivorous moths (Dangles et al. 2012). In addition to this, a recent study has reported a decrease in parasitism of insects with increasing stress (Péré et al. 2013). Hence, this new research suggests that the SGH may be a widespread phenomenon. If the SGH is common to numerous communities containing species with diverse traits then it follows that more widely applicable mechanisms might be responsible for it. For this reason I believe it would be beneficial to test whether the SGH is found in very simple communities in which species can interact in only the most fundamental ways.

A number of general mechanisms have been suggested to account for the SGH. Firstly, in stressful environments the strength of negative interactions will be reduced due to density dependence. In other words, individuals are less likely to interact in harsh environments because their low population densities mean that the rate at which they encounter each other is reduced. Secondly, if facilitation acts to ameliorate the harsh environment and thereby make it more favourable for a beneficiary species this may allow the beneficiary to survive where it could not previously (Bertness and Callaway 1994). This niche expansion may mean that the species that are found in high stress environments will predominantly be those that display facilitative interactions. Therefore facilitative interactions will increase in prevalence compared to competitive ones. Niche expansion could also take the form of enhanced or novel cross-feeding (using the waste products of another species). Cross-feeding may allow a beneficiary species to sustain rates of reproduction in an environmental that might otherwise be too harsh for it to survive in. Thirdly, the SGH could occur as a result of there

being different constraints on growth in low and high stress environments which result in differing interactions along the stress-gradient. In low stress environments, when species' abundances are high, growth is likely to be limited by resource availability and therefore there will be strong interspecific competition for the resources, however, when stress is high, growth may instead be limited by the abiotic environment (Pugnaire and Luque 2001; Bertness and Ewanchuk 2002). This would thereby reduce the importance of competitive interactions in the harsh environment. Although these mechanisms have been found in experimental tests of the SGH, to the best of my knowledge, there has been little attempt to model the effect of stress on species interactions.

In past research the SGH has been tested both when growth is limited by a stressor that is also the resource (e.g., water or light) and when growth is limited by a non-resource stressor (e.g., temperature or salinity). Interestingly the characteristics of the stress investigated commonly affect whether evidence of the SGH is found. As Maestre et al. (2009) point out, when the stress factor is a non-resource, for example salinity in salt marsh ecosystems (Bertness and Ewanchuk 2002) or temperature in alpine plant communities (Callaway et al. 2002), the SGH is frequently found to apply yet when the stress is a resource, such as water in arid environments, the SGH is often invalidated (Tielbörger and Kadmon 2000). This indicates that the nature of the stress could be an important consideration when predicting the impacts of climate change on species interactions and the resulting impacts on ecosystem functioning.

The SGH predicts that the prevalence of positive interactions relative to negative interactions should increase monotonically as stress intensifies; however the shape of the relationship is inconsistent. The SGH has been found to be unimodal in several studies (e.g., Maestre and Cortina 2004; Holmgren and Scheffer 2010) and occasionally asymptotic (Kawai and Tokeshi 2007). These inconsistencies have led to revision of the SGH and the development of the theory that facilitation will be greatest at intermediate levels of stress (Maestre et al. 2006). This is sometimes referred to as the "hump-shaped hypothesis". The following mechanisms have been hypothesised to account for situations in which the relationship between facilitation and stress is unimodal and therefore facilitation peaks at intermediate stress. Firstly, the physiological responses of the component species to the stress may be non-linear

thereby leading to a non-linear change in interactions along the stress gradient (Malkinson and Tielbörger 2010). Secondly, if the stress has a greater detrimental effect on the facilitator than the beneficiary then at very high stress the ability of the facilitator to deliver benefits to the beneficiary may be impaired thereby decreasing community-level facilitation (Maestre and Cortina 2004). Finally, if the stress factor is a resource, competition for the limiting resource may be very strong in the harshest environments but facilitation may ameliorate this in environments with intermediate stress (Holmgren and Sheffer 2010). Asymptotic relationships between facilitation and stress, where facilitation increases with environmental severity but reaches an asymptote, have been studied less extensively but the mechanisms described above may also account for asymptotic curves if interactions become neutral rather than negative at high stress. Another explanation for asymptotic relationships between facilitation and stress is that these arise in experiments that fail to incorporate the whole spectrum of stress (Lortie and Callaway 2006). In these cases, interactions in the most stressful environments may not be sampled and this would prevent the shape of the relationship between stress and facilitation from being correctly identified. The predictions that these curves make about species interactions are most conflicting at the high-stress end of the gradient. It is in these particularly harsh environments that we are often most concerned about protecting species and ecosystem functioning. Therefore, elucidating the shape of the relationship between stress and facilitation will be important to ensure that ecosystems are managed effectively.

Although most evidence supporting and opposing the SGH has come from field experiments and observational studies it has also been investigated mathematically. A popular approach to modelling the SGH is with the dual-lattice model which has been useful in particular to investigate how species' positive and negative interactions along a gradient of stress limit their ranges (Travis et al. 2006). One drawback of this approach, however, is that species are assumed to be equivalent to one another with the same tolerances to stress and competitive abilities. This assumption is perhaps too simplistic as Gause's competitive exclusion principle (1934) indicates that species with exactly the same niches cannot coexist and, furthermore, in natural communities it is well recognised that species' frequently have different responses to stress (McCann 2000). Moreover, in some cases conditions that are

stressful for one species may be beneficial for another. Species' specific responses to environmental change have been shown to be important to ecosystem functioning (Mulder et al. 2001; Wang et al. 2011) and for the outcome of biotic interactions in natural communities (Liancourt et al. 2005). Therefore to improve biological realism and relevance it would be beneficial for models of the SGH to incorporate variable species' sensitivities to stress and also trade-offs between competitive ability and tolerance to stress. In their spatially explicit model to test the SGH Chen et al. (2009) address this by investigating the effect of trade-offs between a competitor and stress-tolerant species on the interplay of positive and negative interactions along a gradient of stress. In this model the interacting species can occupy patches in a lattice and facilitation occurs through aiding populations in neighbouring patches. This model represents a progression in the realism of SGH modelling and has the advantage of observing species interactions over long timescales, however, its spatial structure means that the results are still specific to communities in which constituent individuals are static. The SGH was first proposed with plant communities in mind, so it is unsurprising that mathematical models have been designed in a way that favours mechanisms specific to plant-plant interactions. Nonetheless, empirical studies are now shifting to investigate the SGH with species as diverse as mussels (Rius and McQuaid 2009), microalgae (Bulleri et al. 2011) and invertebrates (Fugère et al. 2012; Dangle et al. 2012) and I believe that the emphasis of the mathematical modelling should also shift in line with this.

In this research I use mathematical models of two interacting microbes to explore the possibility that the SGH could be found in more widespread ecological scenarios than vascular plant-plant interactions by investigating if the SGH could theoretically arise through one of the most basic mechanisms of interaction, resource use, which is common to all species. The stress factor in the model is a non-resource such as temperature or salinity that has a density independent impact on the mortality of the species. I run the model under different scenarios of species tolerance to stress and also investigate whether allelopathy between the microbial species is reduced along a gradient of increasing stress. Uniquely to SGH modelling, the microbial species are able to evolve during each model simulation and thereby alter their resource use in response to the stress imposed and the

resource use of the other species. In the simulations one species can facilitate the other by providing waste produces that the beneficiary can consume. The degree to which the species evolve to become specialists or generalists influences community-level facilitation along the gradient of increasing stress.

5.3 Methods

The model considers the evolution of two microbes growing on two resources. Firstly I describe the model for two species and two resources, assuming a single phenotype in each species. Secondly, I describe how abiotic stress is included in this model. Thirdly, I describe how both species can interact antagonistically through the production of inhibitory chemicals. Finally, I describe how I added evolution of resource use by modelling a spectrum of phenotypes in each species. In addition to this I describe the trade-offs associated with V_{max}, the phenotypic trait that is subject to selection.

The model is based on a chemostat model in which Resource 1 enters the system at a constant rate through influx and resources and microbial cells are removed from the system through efflux. Resource 2 is produced as a waste product of the metabolism of Resource 1. The model makes the following assumptions:

the rate of change of Resource 1 concentration = influx – consumption – efflux (1)

the rate of change of Resource 2 concentration =

metabolism of Resource 1 – consumption – efflux (2)

the rate of change of population density of each species = growth – efflux (3)

5.3.1 Resource consumption, recycling and microbial growth

The extracellular Resource 1 (concentration, S_1) is converted to ATP by both Species 1 and Species 2. Resource 2 (concentration, S_2) is created as a waste product of this reaction. Growth of bacteria on each resource is modelled as a Michaelis-Menton shaped curve so that growth is limited by the resource abundance. The rate of the pathway degrading Resource 1, denoted by J^{S1}, is given by:

$$J^{S_1} = \frac{V_{max_1} S_1}{K_m - S_1} \quad (4)$$

where V_{max1} denotes the maximal growth rate on Resource 1and K_m the Michaelis-Menton constant. An equivalent equation for the Resource 2 pathway, denoted by J^{S2}, is given by:

$$J^{S_2} = \frac{V_{max_2} S_2}{K_m - S_2} \tag{5}$$

where V_{max2} denotes the maximal growth rate on Resource 2. For both species there are trade-offs between V_{max} and K_m which are defined later.

The change in concentrations of Resource 1and Resource 2 and changes in population density of Species 1, N_1, and Species 2, N_2, at time t are modelled in the following way:

$$\frac{dS_1}{dt} = D(S_0 - S_1) - J^{S_1} N_1 - J^{S_1} N_2 \tag{6}$$

$$\frac{dS_2}{dt} = D(-S_2) + J^{S_1} N_1 + J^{S_1} N_2 - J^{S_2} N_1 - J^{S_2} N_2 \tag{7}$$

$$\frac{dN_1}{dt} = n_{ATP} J^{S_1} N_1 + n_{ATP} J^{S_2} N_1 - D N_1 \tag{8}$$

$$\frac{dN_2}{dt} = n_{ATP} J^{S_1} N_2 + n_{ATP} J^{S_2} N_2 - D N_2 \tag{9}$$

where D denotes the dilution rate of the chemostat and describes the rate at which resources are brought into the system and also the rate at which cells and unused resources are removed from the system. The concentration of Resource 1 in the input medium is S_0. The parameter n_{ATP} represents the number of ATP molecules produced by metabolism of one molecule of resource. For both species there is a trade-off between n_{ATP} and V_{max} and these trade-offs are defined later.

5.3.2 Abiotic stress

To test the hypothesis that interactions become more positive under greater abiotic stress, a stress was imposed on the species in the form of density independent mortality. This represents a non-resource stressor such as temperature or salinity which in nature would act independently of population density. Species' tolerance to the stress (γ) varied between 0.15 and 0.95 with higher values representing greater tolerance and therefore lower mortality. Species sensitivity to the stress is the inverse of their tolerance ($1/\gamma$) and the mortality (M) caused by the abiotic stress is the product of the density of the species and their sensitivity to stress so that $M = N/\gamma$. Stress induced mortality can be included in the model by modifying equations 8 and 9 as follows:

$$\frac{dN_1}{dt} = n_{ATP} J^{S_1} N_1 + n_{ATP} J^{S_2} N_1 - \frac{D N_1}{\gamma_1} \quad (10)$$

$$\frac{dN_2}{dt} = n_{ATP} J^{S_1} N_2 + n_{ATP} J^{S_2} N_2 - \frac{D N_2}{\gamma_2} \quad (11)$$

The model was run with a range of tolerance values for each species to investigate the effect of differing species sensitivities to stress on the relationship between increasing stress and facilitation.

5.3.3 Competition through the release of inhibitory chemicals

In addition to interspecific interactions that arise through competition for resources or cross-feeding, species were also able to interact through the production of allelopathic chemicals. Bacteria commonly excrete signalling molecules at a rate that is correlated with their population density; this allows members of their own species to assess their population density but also provides an opportunity for other species to detect their concentration. Some species of bacteria can alter their gene expression according to concentrations of signalling molecules excreted by other bacterial species (Federle and Bassler 2003) thereby allowing them to use quorum sensing to regulate their release of antimicrobials (Tashiro et al 2013). So in this model I assume that species detect the

density of the other species through quorum sensing and release allelopathic chemicals when their own abundance is exceeded by that of the other species. The concentration of allelopathic chemicals produced by Species 1, A_1, and Species 2, A_2, at time t resulting from excretion by the producer species and removal when used against the target species or lost through efflux is modelled in the following way:

$$\frac{dA_1}{dt} = eq_1N_1 - ec_1N_2A_1 - DA_1 \quad (12)$$

$$\frac{dA_2}{dt} = eq_2N_2 - ec_2N_1A_2 - DA_2 \quad (13)$$

where e denotes the efficiency of production of the chemical (i.e. how many A produced per individual) and q denotes the likelihood of producing the chemicals (ratio of target species to producer species with a maximum of 1). Production of the chemicals was defined in this way so that species would produce chemicals at their maximal rate only if their abundance was exceeded by that of the other species. This was thought to be a realistic assumption because in nature there are frequently trade-offs associated with defensive or antagonistic traits and therefore I assume that species have mechanisms to detect the abundance of their neighbours to avoid unnecessary expenditures of energy. The likelihood of producing allelopathic chemicals was limited to 1 so that the maximum rate of production was one molecule of chemical per individual per time-step. c denotes the probability of an individual of the target species meeting a chemical and is the product of the abundance of the target population and the concentration of the chemical. For Species 1 this can be written as $c_1 = N_1 A_2$ and for Species 2 as $c_2 = N_2 A_1$. The allelopathic chemicals are eliminated from the system when they interact with an individual of the target species or by efflux. Equations 10 and 11 can be modified in the following ways to include the cost of producing inhibitory chemicals and the mortality inhibitory chemicals impose on the target species:

$$\frac{dN_1}{dt} = n_{ATP} J^{s_1} N_1 + n_{ATP} J^{s_2} N_1 - \frac{D N_1}{\gamma_1} - \boldsymbol{eq_1 p N_1} - \boldsymbol{c_1 s_1 N_1 A_2} \quad (14)$$

$$\frac{dN_2}{dt} = n_{ATP} J^{s_1} N_2 + n_{ATP} J^{s_2} N_2 - \frac{D N_2}{\gamma_2} - \boldsymbol{eq_2 p N_2} - \boldsymbol{c_2 s_2 N_2 A_1} \quad (15)$$

where *p* denotes the penalty imposed on the producer species for synthesising the chemical and *s* denotes the susceptibility of the species to the chemical produced by the other species. There is a trade-off between a species' tolerance to stress (γ) and its resistance to the inhibitory chemicals produced by the other species (*s*) which can be written as $s_1 = 1 / \gamma_1$ for Species 1 and $s_2 = 1 / \gamma_2$ for Species 2. This trade-off means that when species are tolerant to stress they have a high susceptibility to damage by inhibitory chemicals.

5.3.4 Phenotypic mutations and the evolutionary model

The above model was expanded so that each species consists of *n* phenotypes each with a different V_{max} value residing in the range [0.1,0.9]. Maximal growth rate on Resource 1, V_{max1}, is traded-off against maximal growth rate on Resource 2, V_{max2}, and can be described by $V_{max1} = 1 - V_{max2}$. In addition to this, V_{max} is also affected by species-specific ATP yield trade-offs and also resource affinity trade-offs as described later. The combination of these trade-offs and their effect on V_{max} describe the species average growth rates on both resources. I refer to this trait as "phenotype *x*" and describe it in more detail later.

Each species' phenotype evolves based on a *no-flux* boundary model of evolution as described in Gudelj et al. (2007). Briefly, mutations occur during clonal reproduction at mutation rate ε. If the offspring is a mutant it will have equal probability of having a phenotypic trait value one step greater or less than the parent. In the cases where the parent cell is at the boundary of the phenotypic range, mutations can only give rise to offspring with a phenotype one step greater than the parent (at the lower boundary) or one step less (at the upper boundary). Equation 14 can be modified to show the evolutionary model for Species 1:

$$\frac{dN_{1_1}}{dt} = \boldsymbol{\varepsilon}\left(\boldsymbol{N_{1_2}} - \boldsymbol{N_{1_1}}\right) + n_{ATP_1} J^{S_{1_1}} N_{1_1} + n_{ATP_1} J^{S_{2_1}} - \frac{D N_{1_1}}{\gamma_1} - eq_1 p N_{1_1} - c_1 s_1 N_{1_1} A_2 \quad (16)$$

$$\frac{dN_{1_i}}{dt} = \boldsymbol{\varepsilon}\left(\frac{\mathbf{1}}{\mathbf{2}}\boldsymbol{N_{1_{i-1}}} + \frac{\mathbf{1}}{\mathbf{2}}\boldsymbol{N_{1_{i+1}}} - \boldsymbol{N_{1_i}}\right) + n_{ATP_i} J^{S_{1i}} N_{1_i} + n_{ATP_i} J^{S_{2i}} N_{1_i} - \frac{D N_{1_i}}{\gamma_1} - eq_1 p N_{1_i} - c_1 s_1 N_{1_i} A_2$$

for $i = 2$, ... $n - 1$ (17)

$$\frac{dN_{1_n}}{dt} = \boldsymbol{\varepsilon}\left(\boldsymbol{N_{1_{n-1}}} - \boldsymbol{N_{1_n}}\right) + n_{ATP_n} J^{S_{1n}} N_{1_n} + n_{ATP_n} J^{S_{2n}} N_{1_n} - \frac{D N_{1_n}}{\gamma_1} - eq_1 p N_{n_1} - c_1 s_1 N_{n_1} A_2 \quad (18)$$

where the phenotypic trait is split into n bins each with a different value of V_{max1} (or V_{max2}) and where N_i denotes the density of individuals of $V_{max1\ i}$ (or $V_{max2\ i}$) for $i = 1,..., n$. In this model $n = 9$. The equivalent equations for the evolution of Species 2 are written by modifying equation 15 in the same way.

5.3.5 Trade-offs

In this model phenotype x is defined by three trade-offs related to V_{max} i) the ability of individuals of a given phenotype to use the two resources, ii) affinity with the resource and iii) the number of ATP obtained by metabolism of the resource. Similar trade-offs to those described have been found to be important to the evolution of V_{max} in microbial systems (Pfeiffer and Bonhoeffer 2004).These three trade-offs are described below.

i) Firstly, I assume that there is a linear trade-off between growth using Resource 1 and growth using Resource 2. Meaning that for any given phenotype $V_{maxS1} = 1 - V_{maxS2}$.

ii) Secondly, I assume that V_{max1} and V_{max2} are constrained by the affinity (f) of a phenotype for the resource whereby an increase in V_{max1} or V_{max2} leads to a decrease in the resource affinity. f is a linear increasing function of V_{max} and can be written as $K_m = f(V_{max1})$ for growth on Resource 1 or $K_m = f(V_{max2})$ for growth on Resource 2. For simplicity, the affinity trade-off (f) was the same in both species.

iii) Thirdly, I assume that V_{max1} and V_{max2} are traded-off against the number of ATPs produced during metabolism (g) so that the greater the V_{max} the lower the ATP yield. The shape of this trade-off function is different for the species so that competitive exclusion is avoided. For Species 1 the yield trade-off function is g_1 and for Species 2 is g_2. Therefore for Species 1 the yield trade-off functions can be written as $n_{ATP} = g_1(V_{max1})$ when growing on Resource 1 and $n_{ATP} = g_1(V_{max2})$ on Resource 2. For Species 2 the yield trade-off functions are $n_{ATP} = g_2(V_{max2})$ for growth on Resource 1 and $n_{ATP} = g_2(V_{max2})$ on Resource 2. The functions are quadratic so that ATP yields are maximised at either end of the phenotypic distribution and therefore species tend to evolve to become specialists on either resource rather than generalists (Appendix 5.1). Specifically, the shape of the n_{ATP} yield trade-offs were defined so that Species 1 would tend to evolve to specialise on Resource 1 and Species 2 on Resource 2. Therefore Species 1 is a facilitator because it produces Resource 2 as a waste product of its metabolism of Resource 1 which Species 2, the beneficiary, is able to consume.

Low values of phenotype x correspond to enhanced growth on Resource 1 and high values to enhanced growth on Resource 2. Taking the affinity and yield trade-offs into account, equations 4, 5, 14 and 15 become:

$$\mathrm{J}^{S_1} = \frac{\mathbf{V}_{max_1} S_1}{f(\mathbf{V}_{max_1}) - S_1} \tag{19}$$

$$\mathrm{J}^{S_2} = \frac{\mathbf{V}_{max_2} S_2}{f(\mathbf{V}_{max_2}) - S_2} \tag{20}$$

$$\frac{dN_1}{dt} = g_1(\mathbf{V}_{max_1})\mathrm{J}^{S_1} N_1 + g_1(\mathbf{V}_{max_2})\mathrm{J}^{S_2} N_1 - \frac{DN_1}{\gamma_1} - eq_1 pN_1 - c_1 s_1 N_1 A_2 \tag{21}$$

$$\frac{dN_2}{dt} = g_2(\mathbf{V}_{max_1})\mathrm{J}^{S_1} N_2 + g_2(\mathbf{V}_{max_2})\mathrm{J}^{S_2} N_2 - \frac{DN_2}{\gamma_2} - eq_2 pN_2 - c_2 s_2 N_2 A_1 \tag{22}$$

where f denotes the affinity trade-off function and g_1 denotes the yield trade-off function for Species 1 and g_2 the yield trade-off function for Species 2.

The model was run with varying levels of species' sensitivity to stress to investigate how species interactions change along a gradient of stress. One set of simulations was run without the capacity for the species to produce allelopathic chemicals and one set of simulations allowed the production of chemicals. I started simulations with the populations of both species normally distributed around phenotype 0.5 and therefore the species were generalists.

5.4 Results

5.4.1 The effect of stress on abundance

Throughout the Results and Discussion sections when discussing "average stress" this is the average sensitivity to stress (mortality induced by the stress) of the two species in a model simulation.

The abiotic stress had the desired effect and caused a negative impact on community total abundance (Figure 5.1). When the difference in sensitivity to stress exceeded 25% extinction was common (Figure 5.1) therefore the data I report on here only comprise simulations in which the difference in species' sensitivity to stress was not greater than 25%.

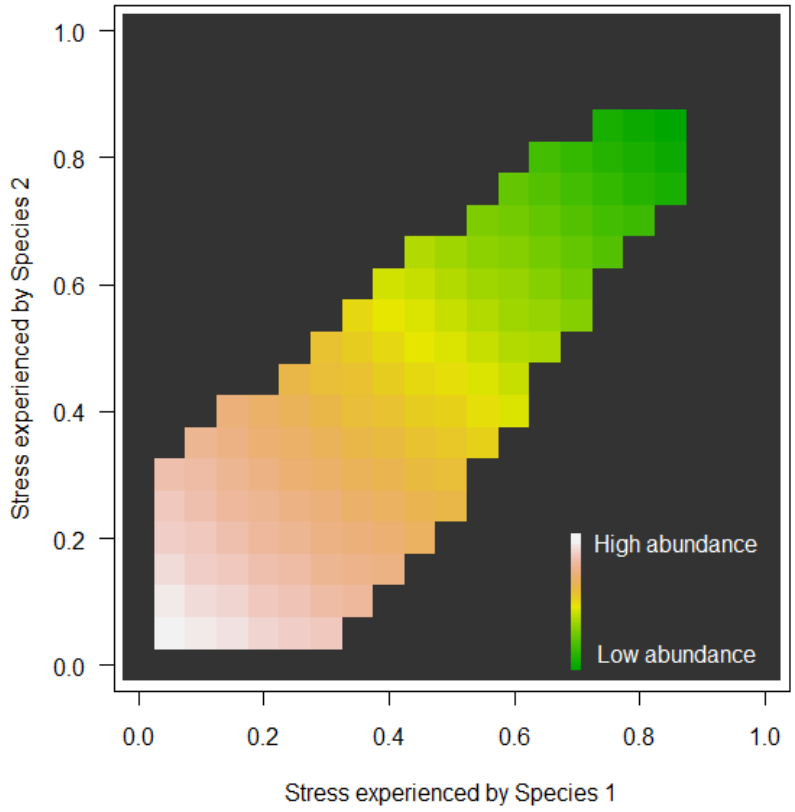


Figure 5.1. Stress decreases total abundance. Total abundance at equilibrium for all combinations of stress tolerance for Species 1 and 2. There is a clear gradient in total abundance; when stress is high total abundance is low. Dark grey blocks represents simulations in which one or both species declined to extinction or where equilibrium was not reached during the model iterations.

5.4.2 Stress leads to resource specialisation and increased cross-feeding

I investigated the SGH by testing how resource use of the two species changed as abiotic stress increased. There are two broad outcomes of the model. The two species can evolve overlapping phenotype distributions and thereby compete for the same resources. Alternatively, the species can evolve non-overlapping phenotype distributions and specialise to use different resources. Because Resource 2 is produced as waste product from metabolism of Resource 1, the latter is cross-feeding.

Competition for resources between the two species was measured as the overlap in phenotypic distributions. Overlap was calculated as the sum of the abundance of individuals that shared each phenotype divided by total community abundance (Appendix 5.2). The model outputs indicate that as abiotic stress increased the overlap in resource use decreased and species evolved to become more specialised - their phenotypic distributions moved towards the boundaries (Figure 5.2a).

The reason for increased resource partitioning when average stress was high is as follows. Due to the trade-offs defined in the model the relative growth rate of specialists was higher than that of generalists. Therefore, even when there was no stress, the intermediate genotypes always grew slower and had a lower steady state density than the specialists. This means that when stress was added, although all genotypes suffered the same relative penalty to their growth rate, the growth rate of the intermediate genotypes dropped lower than the dilution rate of chemostat. Hence, the density of intermediate genotypes dwindled and they were lost. As a result, more resources were available to the extreme genotypes. Thus, even though the extreme genotypes suffered the same relative penalty on growth caused by stress, this was overcompensated for by the greater availability of resources and therefore the extreme genotypes attained a higher density (blue lines in Figure 5.2a).

This niche partitioning at higher average stress led to enhanced facilitation at the community level. In more stressful conditions Species 1, the facilitator, specialised to use Resource 1 and lost the ability to use Resource 2. As a consequence Species 1 was unable to take advantage of its own waste (lower set of lines in Figure 5.3a). Species 2, in contrast, specialised to use Resource 2 which made its survival dependent on Species 1 producing the secondary metabolite. As a result, the facilitation of Species 2

was enhanced as average stress increased (upper set of lines in Figure 5.3a). Taken together these results indicate that when average stress increased - and specialisation was greater - Species 2 obtained the majority of its energy through cross-feeding ultimately leading to enhanced average use of Resource 2 at the community level (Figure 5.3b). Therefore, consistent with predictions of the SGH, community facilitation was enhanced along a gradient of increasing stress.

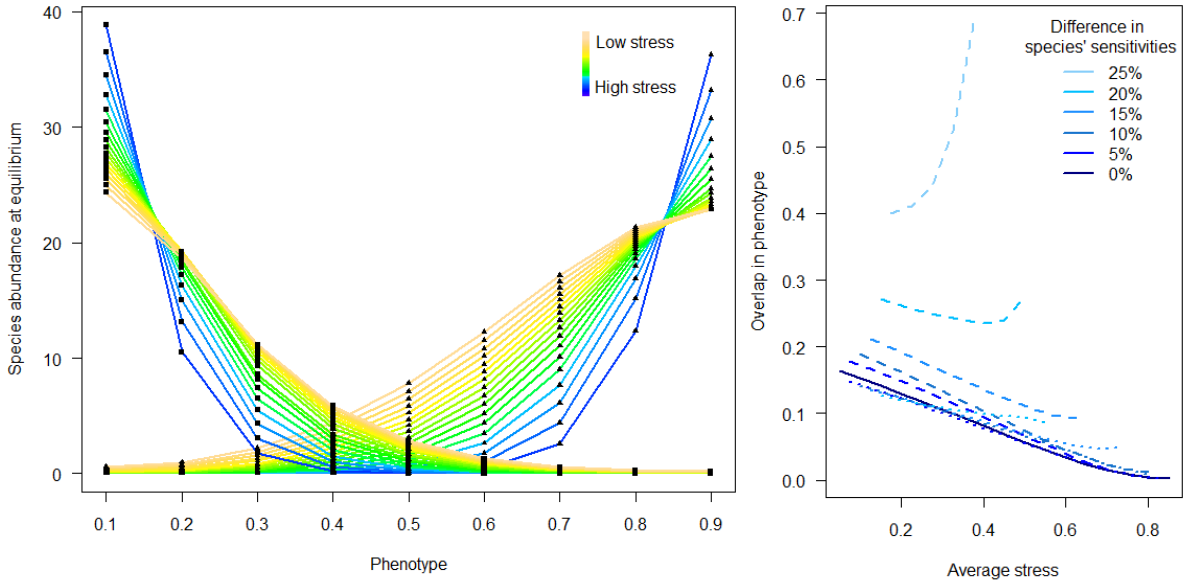


Figure 5.2. Evolution of phenotypic distributions led to increased specialization as abiotic stress increased. a) Lines of the same colour represent equilibrium phenotypic distributions of the species from the same simulation. High values = specialisation on Resource 2, low values specialisation on Resource 1, intermediate values = equal use of both resources. Species 1 is represented by triangular points and Species 2 by square points. Each set of lines show phenotypic distributions when species' sensitivities to stress were equal. As abiotic stress increased, species evolved to have less overlap in their phenotypic distributions. **b)** Overlap in phenotypic distribution controlled for community abundance as average stress increases. The solid line shows results when species sensitivities were equal, the dashed lines denote cases where Species 2 had greater tolerance to stress and the dotted lines are when Species 1 had greater tolerance to the stress. The gradient in colour from dark to light represents increasing divergent in species' tolerances to stress. When species' had very divergent tolerance to stress the relationship between stress and overlap in resource use was altered.

This trend of decreasing overlap in resource use and subsequent greater facilitation along a gradient of stress was independent of the difference in species' sensitivities to stress (overlapping lines on Figure 2b and almost parallel lines in Figure 5.3b) with the exception of communities in which Species 2 was considerably more tolerant to stress than Species 1 (dashed light lines on Figures 5.2b and 5.3b). When Species 2 was between 20 and 25% more tolerant to stress than Species 1 the overlap in resource use increased as average stress intensified and the relationship between stress and community facilitation was hump-shaped. In these rare cases Species 2 had evolved to become a generalist and Species 1 survived at low abundances consuming Resource 1 (Appendix 5.3). Hence, by evolving to use Resource 1 as well as Resource 2, Species 2 could cross-feed on its own waste. Yet, on average this led to lower levels of cross-feeding at the community level and produced the hump-shaped relationship between facilitation and stress. When the situation was reversed, and Species 1 was considerably more tolerant to the stress, Species 2 also evolved towards being a generalist (these data are not shown because there were too few simulations in which species could coexist to draw a trend line).

Therefore, in the majority of cases, increased stress led to resource specialization and enhanced community facilitation, however, in cases where one species was close to extinction the dominant species evolved to be a generalist and the facilitation curve became hump-shaped.

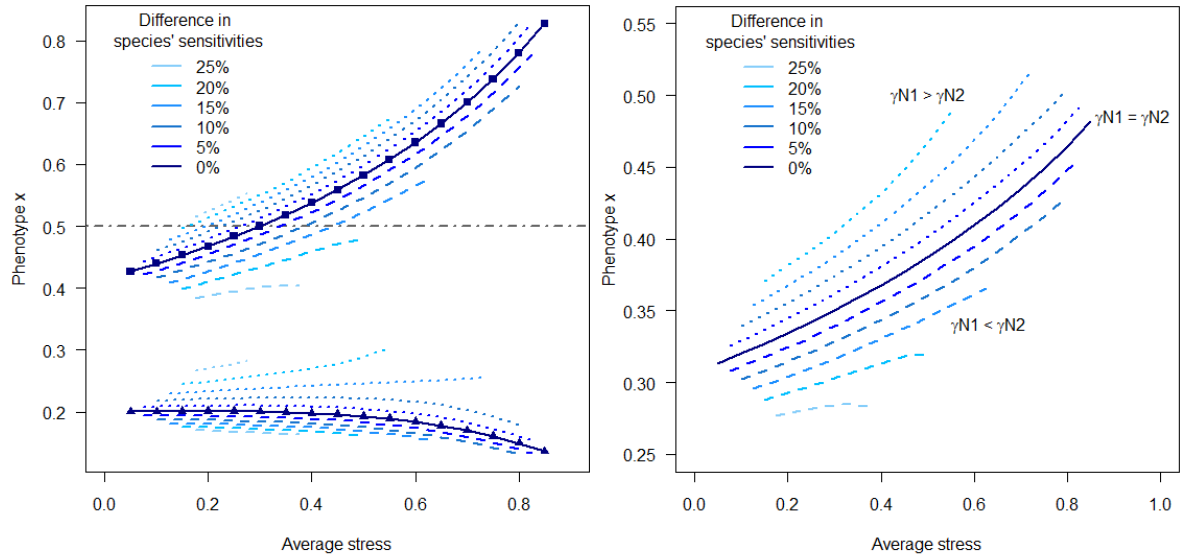


Figure 5.3. Species resource use along a gradient of increasing stress. The lines represent the value of phenotype x (or the average value for the entire community) along a gradient of stress when the difference in species' tolerances to stress are held constant. High values of x = specialisation on Resource 2, low values on Resource 1, a value of 0.5 = equal growth on both resources. As x increases, the maximum rate of growth on Resource 2 increases and the maximum rate of growth on Resource 1 decreases. The dashed grey line indicates the phenotype with equal growth rate on both resources. The solid lines show results when species sensitivities were equal, the dotted lines denote cases where Species 1 was more tolerant and the dashed lines are when Species 2 was more tolerant to the stress. The gradient in colour from dark to light represents increasing divergence in species' tolerances to stress. **a)** Change in phenotype x of the species along a gradient of stress. The triangular points represent Species 1, the facilitator, and square points represent Species 2, the beneficiary. As stress intensified specialisation of resource use led metabolism of Resource 2 by Species 1 to be decreased whilst metabolism of Resource 2 by Species 2 was enhanced. **b)** Average phenotype *x* of the two species along the gradient of stress. At the community level increasing stress had a positive effect on facilitation through cross-feeding.

5.4.3 Production of antagonistic chemicals peaked at intermediate stress

In the model simulations that allowed allelopathy, as well as interaction through sharing or competing for resources, species were able to compete directly through the production of extracellular chemicals that harm the other species. The resistance of individuals of the target species to the inhibitory chemicals of the producer species is traded-off against their tolerance to stress. Thus, when species are sensitive to stress (i.e. when "average stress" is high) they have a lower susceptibility to damage by chemicals. For this reason, when assessing how the gradient of stress affects species interactions, I had to measure not only the volume of chemicals produced but also the damage they caused to the target species.

In these analyses I used average inhibitory chemical production of the two species at equilibrium because there was a strong positive correlation between the production of chemicals by Species 1 and Species 2 during each model simulation (Pearson's correlation, correlation = 0.995, df = 110, t = 106.12***) . This correlation occurred because chemical production of both species was strongly associated with the population density of the least abundant species (because of the way q was defined in the model). Likewise, the average mortality caused by the inhibitory chemicals within each simulation was used in these analyses because the mortalities caused by inhibitory chemicals were highly correlated for the two species within a model simulation (Pearson's correlation, correlation = 0.929, df = 110, t = 26.29***). This correlation was less strong than that of chemical production, however, because the efficacy of the chemicals depended on the other species' trade-off in tolerance to stress versus tolerance to chemicals and on the rate of encounter between the target species and the inhibitory chemical.

The shape of the relationship between chemical production and average stress was dependent on which species was the most abundant in the community (Figures 5.4a and 5.4b). When Species 2 was the most abundant there was a linear decline in production of inhibitory chemicals along the gradient of increasing stress (Figure 5.4a). This was caused by two mechanisms: firstly, as stress increased community total abundance decreased and, as chemical production was limited to a maximum of 1

molecule per individual per time-step, this meant that the rate of excretion of chemicals was reduced. Secondly, the evenness of the community decreased as stress increased and therefore the volume of antimicrobials produced by the most abundant species (as described by parameter q in the model) was reduced (Appendix 5.4a). In contrast, when Species 2 was least abundant there was a unimodal relationship between increasing stress and the total production of allelopathic chemicals (Figure 5.4b). Again, the same two mechanisms determined the average production of allelopathic chemicals but the outcome differed. In this case, as stress intensified, the community became more even and this increased the average probability of individuals producing antimicrobials (parameter q) (Appendix 5.4b). However, when this increased antimicrobial production was balanced against the limitation on production caused by the concurrent decrease in total abundance, a hump-shaped relationship between stress and the production of inhibitory chemicals was produced (Figure 5.4b).

The relationship between average stress and the production of allelopathic chemicals was conserved when species sensitivities to stress differed (parallel lines on Figures 5.4a and 5.4b) but the volume of chemicals produced at a given intensity of stress was altered (different heights of lines in Figures 5.4a and 5.4b). For example, when Species 1 was increasingly more tolerant to stress than Species 2 (dotted lines in Figure 5.4b) the average volume of chemicals produced at a given stress was reduced. This pattern occurred because as the species' tolerances to the stress became more divergent the relative abundance of Species 2 decreased and therefore the amount of antimicrobial that Species 1 was induced to produce was decreased. The same was true when Species 2 was more tolerant to stress than Species 1 (Figure 5.4a).

5.4.4 Damage caused by inhibitory chemicals decreased along the stress gradient

Rates of chemical production are not the same as rates of mortality because the efficacy of the inhibitory chemicals depends on i) the target species' abundance - because rates of encounter are increased at high abundance and ii) the target species' resistance to the antimicrobial which is traded-off against their tolerance to stress.

When average stress was low to intermediate species generally produced relatively high volumes of allelopathic chemicals (Figures 5.4a and 5.4b). These chemicals had a large impact on mortality at low stress (Figures 5.4c and 5.4d). This occurred for two reasons, firstly, because when average stress was low there was a high abundance of the target species and this made it more likely that the inhibitory chemicals would be encountered. Secondly, at low average stress species were tolerant to stress but (due to the trade-off between tolerance to stress and tolerance to inhibitory chemicals) were sensitive to chemicals produced by the other species. Consequently, inhibitory chemicals had the greatest impact on mortality at low average stress.

In contrast, when average stress experienced by species was high little mortality was caused by inhibitory chemicals (Figures 5.4c and 5.4d). This occurred because at high average stress species' abundances were low and this meant that the rates at which chemicals were produced (Figures 4a and 4b) and encountered were also low. Furthermore, at high average stress when species were sensitive to abiotic stress they were resistant to the antimicrobials (due to the trade-off between tolerance to stress and tolerance to inhibitory chemicals). Hence, the impact of allelopathic chemicals on mortality was minimal when average stress was high. As a result, there was a negative relationship between average stress and mortality caused by inhibitory chemicals (Figures 5.4c and 5.4d).

This trend of decreasing mortality caused by antimicrobials was consistent across the sensitivity curves but the steepness of the relationship varied. At low average stress the simulations had divergent rates of mortality and these corresponded to the rate of production of inhibitory chemicals. Yet at high average stress, because encounter rates were low, the mortality induced by antimicrobials tended to converge towards a similar low value. This is decrease in the importance of antagonistic interactions in stressful conditions is consistent with predictions of the SGH.

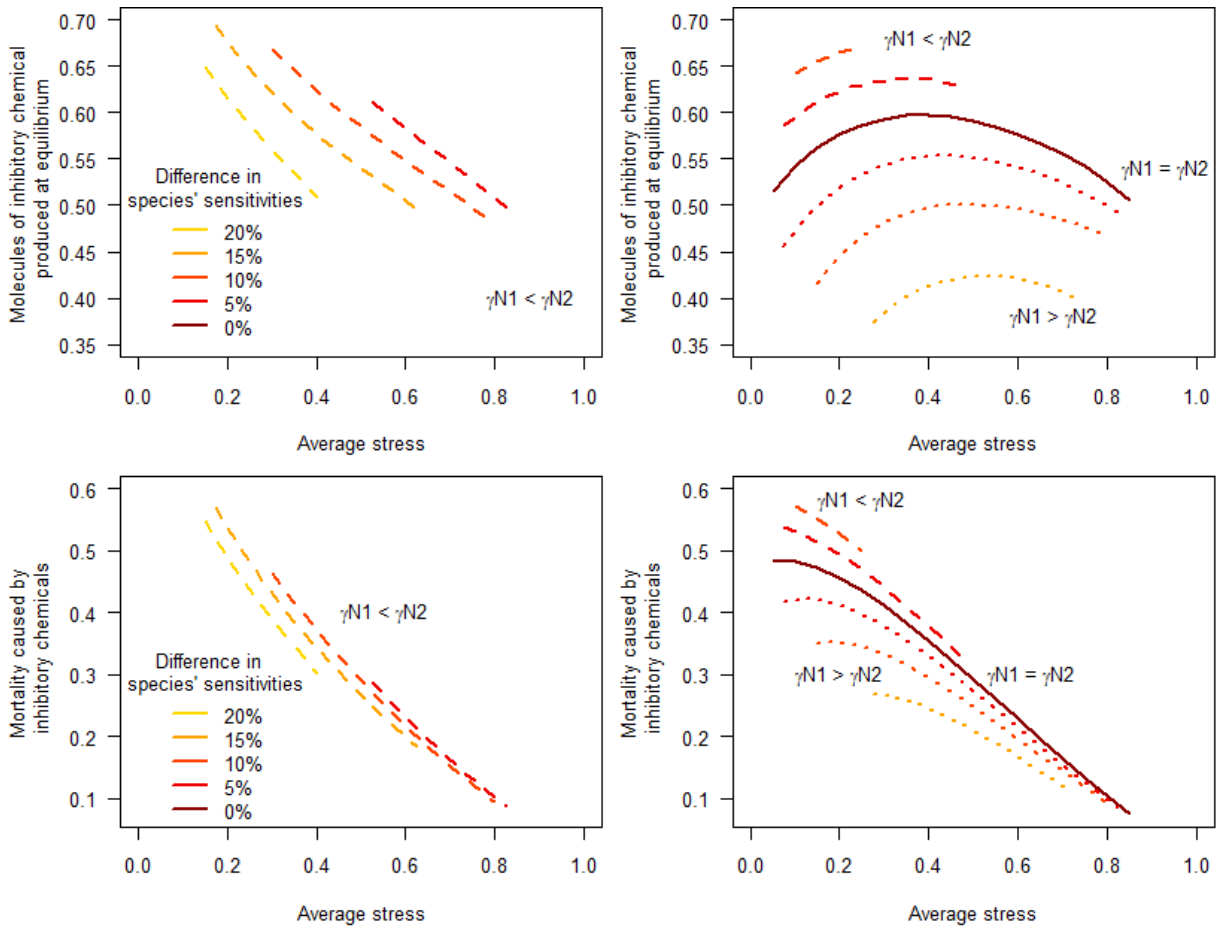


Figure 5.4. Average community level production of inhibitory chemicals and resulting mortality. Figures a) and b) show the production of inhibitory chemicals where Species 1 is the least abundant in the former and Species 2 in the latter. Figures c) and d) show the resulting mortality caused by the production of inhibitory chemicals in a) and b) respectively. For all graphs the solid line represents simulations in which species have an equal tolerance to stress, the dotted line when Species 1 is more tolerant to stress than Species 2 and the dashed line when Species 2 is more tolerant to stress than Species 1. The gradient in colour from dark to light represents an increasing difference in species' tolerances to stress. **a)** Production of inhibitory chemicals along a gradient of stress when Species 1 was less abundant than Species 2. As stress increased production of chemicals decreased. **b)** Production of inhibitory chemicals along a gradient of stress when Species 2 was less abundant than Species 1. There is a unimodal relationship between production of inhibitory chemicals and average stress. The hump-shape is due to a peak in species evenness and total abundance leading to greater production of allelopathic chemicals at intermediate stress. **c)** Mortality caused by inhibitory chemicals along a gradient of stress when Species 1 was less abundant than Species 2. Mortality decreased steeply as severity of the stress increased. **d)** Mortality caused by inhibitory chemicals when Species 2 was less abundant than Species 1. For figures c) and d) the mortality induced by inhibitory chemicals decreased as the gradient of stress increased because when stress was high, and therefore species' abundances were low, there was a decreased rate of encounter between inhibitory chemicals and the target species.

5.4.5 Comparison of the patterns of facilitation in the simulations with and without allelopathy

Including inhibitory chemicals in the models had an impact on the shape of the relationship between community facilitation and stress. In agreement with the simpler model, as the intensity of stress increased species had more facilitative interactions through increased cross-feeding (Figure 5.5a). Although, rather than the trend being linear, as in the majority of cases in the model without inhibitory chemicals, the trend was for a convex increase in facilitation with stress. At low average stress the rate of cross-feeding was greater in the model with allelopathy than in the model without allelopathy. This was due to selection for the evolution of greater specialisation of resource use in benign conditions when inhibitory chemicals were present (Figure 5.5b) and this resulted in greater use of Resource 2 overall. The damage caused by inhibitory chemicals declined as stress increased (Figures 5.4c and 5.4d) and therefore, taken together, these results indicate that as species' antagonism through inhibitory chemicals declined, their facilitation through cross-feeding increased. At the greatest levels of stress the curves from the two models converge and therefore when stress was severe facilitation was independent of whether species could interact through allelopathic chemicals. This occurred because the damage done by inhibitory chemicals was negligible under intense stress.

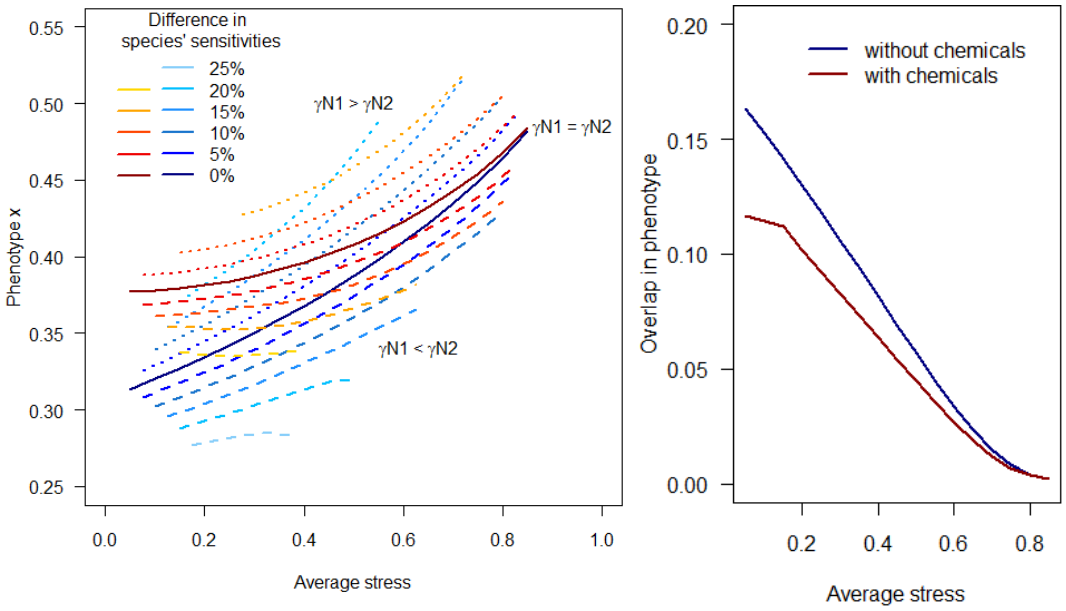


Figure 5.5. Comparison of the models including inhibitory chemicals and without inhibitory chemicals on average community phenotype x along a gradient of stress. a) Lines represent the average phenotype *x* of the communities along a gradient of stress when the difference in species' tolerances to stress were held constant. The solid lines show results when species tolerances were equal, the dotted lines denote cases where Species 1 was more tolerant than Species 2 and the dashed lines are when Species 2 is more tolerant to the stress than Species 1. The gradient in colour from dark to light represents increasing difference in species' tolerances to stress. Blue lines represent simulations in which allelopathic chemicals could not be produced (taken directly from Figure 3b) and red lines represent simulations in which species were capable of producing allelopathic chemicals. The general trend of increased phenotype *x* with stress was consistent in the models however the shape of the relationship changed from linear in the simulations without chemicals to convex in those with chemicals. **b)** Change in the overlap of species' resource use along a gradient of stress. For clarity, only the simulations in which species' had equal tolerances to stress are shown. There was less overlap in resource use in the model which includes inhibitory chemicals indicating that in relatively benign conditions antagonistic interactions led to the evolution of greater specialisation.

5.5 Discussion

Broadly speaking, my results are consistent with the SGH (Bertness and Callaway 1994); species interactions became increasingly facilitative along a gradient of increasing stress. In the model without allelopathy species evolved increasing levels of resource specialisation as the abiotic environment became more stressful and this led to enhanced cross-feeding and greater facilitation when measured at the community-level.

To the best of my knowledge, the effect of stress on resource specialisation has not been studied before and this is the first time that an increase in specialisation with abiotic stress has been reported. Resource partitioning like this has been shown to increase functioning in algal communities (Cardinale 2011) and coevolved bacterial communities (Lawrence et al. 2012). Therefore, if our results are consistent with dynamics in laboratory and mesocosm experiments, increasing stress may lead to resource partitioning and thereby create communities that have more complementary resource use than those in benign environments. In the face of rapid environmental change this may be good news for the safeguarding of ecosystem services. However, this idea will have to be thoroughly investigated empirically before we would know if specialisation is a common response to stress and, moreover, whether this would actually alleviate some of the negative effects of abiotic stressors associated with climate change.

I found that facilitation increased monotonically with average community stress when the species' sensitivities to stress were similar and this is in line with predictions of the SGH. Monotonic increases in facilitation with stress have also been reported in experimental studies of alpine plant communities (Callaway et al. 2002) and herbivorous moths (Dangle et al. 2012) among others. Although the relationship between facilitation and stress was consistently linear in my model (with the exception of simulations in which species' sensitivities to stress were very divergent as discussed in detail below), I cannot rule out the possibility that the complexity of species responses and interactions in wild communities could alter the shape of the relationship. For example, the necessary simplicity of my

model meant that I did not include possible traits such as non-linear physiological responses to stress which Malkinson and Tielbörger (2010) suggest could be important in shaping the SGH and, additionally, my model dealt only with a simple two-consumer model rather than with more complex food web structures. Future work should aim to build on the basic model presented here by including factors like this and testing their effect on the shape of the relationship between stress and facilitation.

When species' sensitivities to stress were considerably divergent their resource use was altered and the relationship between facilitation and stress became hump-shaped. Rather than specialising on each resource, the more stress-tolerant species became dominant and evolved to consume both resources whilst the stress-sensitive species survived at low abundance. In these rare cases overlap of resource use increased with stress and as a consequence cross-feeding declined. Maestre and Cortina (2004) also recognised that species-specific sensitivities to stress may cause facilitation to be impaired in the harshest environments however they suggest a different mechanism. Rather than the beneficiary being able to adapt to use the resources that were unexploited by the facilitator, as in my model, Maestre and Cortina (2004) suggest that at high stress the facilitator may become unable to provide benefits to the beneficiary and this will decrease community facilitation in the harshest environments. Both mechanisms have the same outcome; when the difference in species' sensitivities to abiotic stressors is large the relationship between stress and facilitation becomes hump-shaped. Therefore these results suggest that to gain a greater understanding of the SGH it would be beneficial put a greater emphasis on investigating the effects of species-specific responses to stress.

It is possible that the results of the model simulations were heavily influenced by the model parameters. For example, the ATP yield trade-off functions were defined so that one species would typically evolve to become the facilitator and the other the beneficiary. The trade-offs were defined in this way so that the species would be able to coexist in a wide range of simulations even with divergent species-specific sensitivities. Although I would expect to find species with different resource preferences commonly coexisting in nature I acknowledge that this is not representative of all species interactions. One way in which the model could be adapted in further research would be to

alter the trade-offs associated with V_{max} so that cross-feeding is not a predetermined trait and therefore interactions between species with varying preferences for the resources can be investigated.

Not only did my model outputs show that species interactions became more facilitative under increasing stress, I also found that mortality caused by allelopathy decreased as abiotic stress increased. This is consistent with competitive interactions becoming less important in harsh environments as predicted by the SGH. In my model antagonism via allelopathy decreased with increasing stress for a combination of reasons. Firstly, at high abiotic stress species' population densities were low and this limited the rate of production of inhibitory chemicals and also the rate at which individuals of the target species encountered the chemicals. Secondly, due to the trade-off between tolerance to stress and tolerance to antimicrobials, at high average stress (when species were sensitive to stress) species had high resistance to inhibitory chemicals. The combination of these effects resulted in inhibitory chemicals inducing low levels of mortality at high stress. Therefore, in the harshest environments the rate at which chemicals were produced and encountered and also their efficacy was reduced. Density dependent antagonism similar to that described here may alter the rates of predator-prey and host-parasite interactions in along gradients of increasing stress which could have consequences for community functioning (Holbrook and Schmitt 2002).

In my model the production of allelopathic chemicals was determined by the relative abundance of the species. When a species was the least abundant it would always produce inhibitory chemicals at its maximum rate whereas when a species was the most abundant it would produce inhibitory chemicals at a rate that was proportional to the density of the other species. The production of chemicals was defined in this way because it was the simplest mechanism that would allow species detect and respond to their competitors and is in line with the type of quorum sensing that microbes are known to be capable of (Millar and Bassler 2001; Tashiro et al 2013). In benign environments the results of the simulations may be particularly sensitive to the way in which this parameter was defined, however, at high stress it is unlikely that altering this parameter would have a substantial effect on mortality caused by inhibitory chemicals because the encounter rate between the target species and the chemicals would remain low. One interesting modification to the model would be to allow the species

to have different rates of chemical production or efficacy; this may change the shape of the relationship between species interactions and stress in a similar way as varying the species' sensitivities to stress. Future work could add complexities like these to increase the realism of antagonistic interactions included in SGH modelling.

Interestingly, including allelopthy in my model changed the shape of the relationship between increasing stress and facilitation. In the more basic model, facilitation increased almost linearly with stress, however, when species were able to interact antagonistically the curve became convex. This occurred because, in the model with allelopathy, strong competition via antimicrobials in the benign environment caused species' average resource use to diverge leading to greater average facilitation by cross-feeding. As the average stress increased and the mortality caused by antimicrobials decreased, the facilitation curves of the two models converged (producing the convex-shaped curve for the model with allelopathy), and in the harshest environments the effect of inhibitory chemicals was negligible. To the best of my knowledge, this convex relationship between facilitation and stress in the simulations with allelopathy is not an outcome predicted by the SGH or reported in empirical tests of the SGH. This inconsistency may have arisen because research into the SGH usually considers antagonism through resource competition (e.g., Fugère et al. 2012) or competition for space (e.g., Rius and McQuaid 2009; Bowker et al. 2010) rather than allelopathy which requires energy expenditure beyond that usually required for survival and reproduction. Therefore it would be interesting to investigate whether different modes of antagonism such as allelopathy, resource competition, parasitism or predation should be expected to be affected by stress intensification in different ways.

It is somewhat counterintuitive that the beneficiary species would produce antimicrobials to harm the facilitator species which provides the secondary metabolites from which it gains the majority of its energy. Yet the species have no foresight and, as allelopathy cannot evolve in this model, the species' production of inhibitory chemicals is defined by their relative abundances rather than by what may ultimately improve their fitness. Therefore an improvement to this model would be to allow species' production of allelopathic chemicals to evolve so that it is sensitive to selection pressure imposed by

the other species and the abiotic stress. The development of a model of allelopathy and facilitation via cross-feeding along a gradient of stress could be based on models of social evolution theory (e.g., Bucci et al. 2011).

In recent decades facilitation has been shown to be influential to numerous ecological and evolutionary processes (e.g., Bruno 2000; Cardinale et al. 2002; Kikvidze and Callaway 2009; Lawrence et al. 2012) and therefore understanding the balance between positive and negative interactions in communities is vital if we are to be able to predict how ecosystem functioning and ecosystem services will be affected by stress caused by climate change. Conservation management has already been influenced by research into the SGH, for example, shrubs have been planted to enhance ecosystem restoration in Mediterranean mountains (Gómez-Aparicio et al. 2004) and dominant grasses used to promote shrub establishment in semi-arid steppe ecosystems (Maestre et al. 2001). Results from this study may encourage further research into how generally applicable the SGH is and the possible mechanisms behind it and this future work may inform conservation management of a wide range of ecosystems under stress.

5.6 Conclusions

The model presented here is intended to explore whether two basic modes of species interaction could explain the SGH. I found that evolution of resource use alone was sufficient to produce patterns of facilitation consistent with those predicted by the SGH. Specifically, in harsh conditions species evolved specialisation of resource use and consequently community-level facilitation increased. Furthermore, when species were very divergent in their sensitivities to stress, a hump-shaped relationship between stress and facilitation was evident, and this finding builds upon previous hypotheses in the literature. I also found evidence that competition through allelopathy decreased as facilitation and abiotic stress increased due to density-dependent effects. These results are influenced to a degree by the specific parameters of the model, however, the basic model presented here can be developed further to investigate various types species interactions or trade-offs. It is somewhat

surprising that results consistent with the SGH can be produced when modelling such simple interactions because in the ecosystems in which the SGH is commonly studied facilitation takes the form of more complex interactions. This research lends support to the possibility of the SGH being a widely applicable phenomenon and therefore makes it even more important that research is carried out into species and community responses to stress before the effects of climate change become more severe.

Chapter 6:

THESIS CONCLUSIONS

The overarching aim of this thesis has been to investigate how species interactions affect ecological and evolutionary responses to environmental change and the consequences this has for ecosystem functioning. I have researched this broad subject through a variety of methods but my findings have been somewhat consistent between chapters. Here I discuss some of main conclusions of my research that link across chapters, how they fit into current scientific theory and the direction that I believe research in this area should progress in the future.

One interesting and somewhat unexpected result of my research was that species' adaptation to a novel (Chapter 2) or stressful environment (Chapter 5) led to niche partitioning by specialisation of resource use. In both cases this resulted in increased facilitation at the community-level through enhanced cross-feeding. To the best of my knowledge, no other studies have found that adaptation of species to a novel environment or evolution in increasingly stressful environments can promote resource use specialisation. In theory, resource specialisation could arise because abiotic stress coupled with interspecific competition may cause disruptive selection thereby selecting for niche partitioning. In addition to this, interspecific competition may select for a trade-off in diversity of resource against efficiency of resource use so that species have enhanced growth on a narrower range of resources. If specialisation is a common outcome of abiotic stress in diverse communities this could have implications for ecosystem functioning and therefore ecosystem services that are important to human wellbeing. Ecosystem services often arise through the growth and interactions of multiple species (Balvanera et al. 2006), for example, nutrient recycling, denitrification and decomposition are performed by highly diverse microbial communities (Ducklow et al. 2008). If facilitation is enhanced when conditions are stressful, this may counteract the detrimental effects of

climate change by sustaining ecosystem services to some degree. On the other hand, the epidemiological implications may be less welcome, for example, in a patient infected with multiple pathogens, abiotic stress caused by antibiotics could trigger a decrease in antagonism between pathogenic species - or even encourage facilitation - and thereby hinder treatment. Given the potential importance the outcomes of evolution could have for human wellbeing it is vital for further research to be done in this area.

In Chapter 2 I found that species interactions influenced adaptation in ways that could not be predicted by observing evolution of the same species in monoculture. This outcome was mirrored in Chapter 3 in which I found that the local adaptation of representative species in a community did not correspond to local adaptation at the community-level. Species-specific responses are not a novel idea (e.g. Dang et al. 2009) and it is well recognised that processes such as functional redundancy can obscure idiosyncratic species' growth when in a community (Naeem 1998). Yet, until now there has been little investigation of how evolution of component species affects functioning of the community. Together my findings suggest that experiments investigating the evolution of single species' in response to environmental change may not necessarily reflect evolutionary dynamics that occur in diverse communities. There has been a wealth of excellent evolution experiments involving single or pairs of species that have contributed greatly to our understanding of processes such as antibiotic resistance (Perron et al. 2007), antagonistic coevolution (Brockhurst et al. 2007b) and evolutionary rescue (Bell and Gonzalez 2011). However, without investigating whether similar dynamics can be expected in natural diverse communities the benefit of these studies may be limited. In the future research in experimental evolution could address this constraint by developing experiments that capture the complexities of the wild but can still fully explore dynamics of a few focal species. This will require innovative experimental design but the benefits associated with understanding how evolution of component species contributes to community functioning in natural ecosystems would be substantial.

A common theme through some of the chapters of my thesis has been predictability. In Chapter 4 my results indicated that warming decreased response predictability and in Chapter 5 I found evidence

that changes in species interactions are, to some extent, predictable from the abiotic environment. Predictability is currently a popular research theme in ecology and evolution because anthropogenic activities are causing ubiquitous changes to the environment which, in many cases, it is too late to reverse (Washington et al. 2009). Therefore, research that enhances our predictive accuracy may help to mitigate some of the negative effects of disturbances (Thuiller et al 2008). Through mathematical modelling I found evidence that the stress-gradient hypothesis - facilitative interactions increasing along gradients of stress - could be a common phenomenon. If this is conclusion is found to be justified then it will aid our understanding and predictions of species responses to stress. Likewise, my finding that that warming itself may decrease the predictability of ecosystem properties will hopefully be useful to modify predictions of responses to environmental change. My hope is that these results will encourage further research into the effects of disturbance on predictability of ecosystem responses and species interactions with the aim of understanding the mechanisms behind these phenomena so that we can predict how communities will respond to environmental change in the future and thereby understand how best to manage them.

To conclude, in this thesis I have investigated the ecological and evolutionary responses of groups of species to environmental change using mathematical modelling, experimental evolution and a field experiment. My research has produced novel insights into how species interactions influence evolution, how the local adaptation of communities compares to that of their constituent species and about the predictability of ecosystem processes and species interactions under environmental change. My findings build on hypotheses and on-going research in this field and I hope that they inspire further research that can broaden our understanding of the likely impacts of environmental change on the evolution of species and resulting ecosystem functioning.

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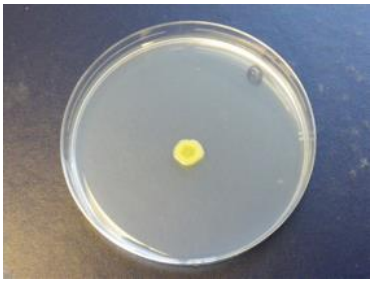
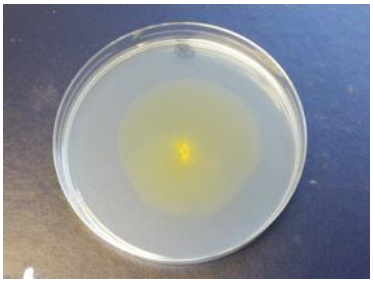
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2.1. Molecular identification of bacterial isolates (performed by F. Fiegna). All species are likely to be anerobic heterotrophs.

Species	16S rDNA sequence	Closest BLAST hits on NCBI	Closest match on Ribosomal Database Project II database
A	TACCGTACATTCAGCTTCTCA CACGTGAAAAGGTTTATTCCG GTACAAAAGCAGTTTACAACC CGTAGGGCCGTCTTCCTGCAC GCGGCATGGCTGGTTCAGGCT TGCGCCCATTGACCTAATATT CCTTACTGCTGCCTCCCGTAG GCCCCCCGTGCCCCCGCCCCG CCCGCCGCGCGCGGCGGGCG GGGC	10 uncultured bacteria clones (99% identity) Sphingobacteriaceae bacterium (99% identity) *Pedobacter sp.* (99% identity)	Unclassified Sphingobacteriaceae
B	ACGTCATGTTCAGTGCTATTA ACACTTAACCCTTCCTCCTCG CTGAAAGTGCTTTACAACCCG AAGGCCTTCTTCACACACGCG GCATGGCTGCATCAGGCTTGC GCCCATTGTGCAATATTCCCC ACTGCTGCCTCCCGTAGGCCC	*Yersinia ruckeri* (100% identity) 26 *Yersinia ruckeri* (99% identity) Uncultured proteobacterium (99% identity) *Serratia proteomaculans* (99% identity) *Yersinia ruckeri* (97% identity) *Rahnella* sp. (97% identity)	Unclassified Enterobacteriaceae
C	TACGTCAAACAGCAAAGTATT AATTTACTGCCCTTCCTCCCA ACTTAAAGTGCTTTACAATCC GAAGACCTTCTTCACACACGC GGCATGGCTGGATCAGGCTTT CGCCCATTGTCCAATATTCCC CACTGCTGCCTCCCGTAGGCC CCCCGTGCCCCCGCCCCGCC	10 *Pseudomonas* sp. (100% identity) 2 uncultured bacteria clones (100% identity) 15 *Pseudomonas* sp (99% identity)	Unclassified Pseudomonaceae
D	TACTGTCATTATCATCCCTGG TAAAAGAGCTTTACAACCCTA AGGCCTTCATCACTCACGCGG CATTGCTGGATCAGGGTTGCC CCCATTGTCCAATATTCCCCA CTGCTGCCTCCCGTAGGCCCC CCGTGCCCCCGCCCCGCCCGC CGCGCGCGGCGGG	4 uncultured bacteria clones (100% identity) *Sphingomonas sp.* (100% identity) *Novosphingobium* sp. (100% identity) 7 uncultured bacteria clones (100% identity)	Unclassified Alphaproteobacteria
E	GTACCGTCAGCTGATTCACGA ATCAGTGTTTCTTCCTGTGCA AAAGCAGTTTACAATCCATAG GACCGTCATCCTGCACGCGGC ATGGCTGGTTCAGGCTTGCGC CCATTGACCAATATTCCTCAC TGCTGCCTCCCGTAGGCCCCC CGTGCCCCCGCCCCG	Uncultured Bacteriodetes bacterium clone (100% identity) Uncultured bacterium clone (99% identity) 2 *Flavobacterium sp*. (99% identity) 4 uncultured bacteria clone (99% identity) 3 *Flavobacterium sp*. (97% identity)	Unclassified Flavobacteriales

2.2. Description and photographs of growth morphology of each species on agar plates. Initial bacterial isolation performed by F. Fiegna. Photographs courtesy of F. Fiegna.

Species A	**Species B**	**Species C**
		
Pink colonies with a glossy, smooth surface.	Opaque white colonies with an irregular edge.	Translucent white colonies with a smooth edge.
Species D	**Species E**	
		
Opaque yellow colonies with a glossy surface.	Yellow colonies, opaque centre, translucent at edge.	

2.3. Additional methods.

Isolating bacteria

To ensure that starting species comprised single genotypes, single colonies from R2A agar plates spread with tree-hole water were re-suspended in liquid medium and then re-plated on R2A agar, before picking off final single colony isolates for storage in glycerol at -84ºC. Isolates were identified from 16S rDNA sequence using primers 27F (Lane 1991) and 534R (Muyzer et al. 1993).

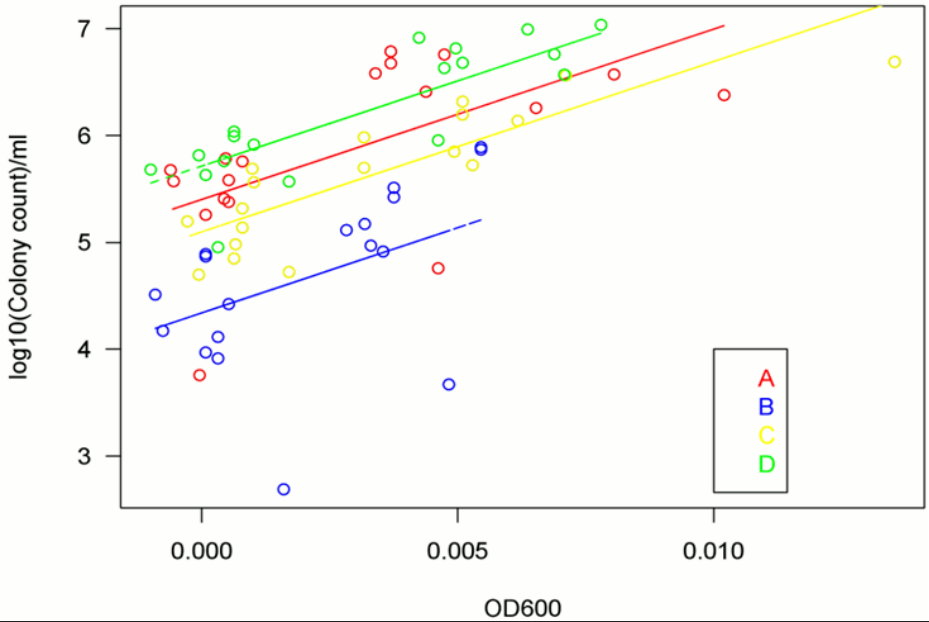
Evolution experiments

Cultures were established in 30ml universal tubes containing 2ml of beech tea and inoculated initially with 50µl from established cultures of bacteria in beech tea. In 'monoculture' treatments, cultures were started with 50µl of each species in turn cultured in 2ml of medium in isolation. In 'polyculture' treatments, inoculates of 10µl of each of the species was added to the same tube to create a five-species community of bacteria. The optical densities at 600nm (OD_{600}) were measured for all of the stock cultures before inoculation to ensure that starting densities were similar for all species.

Linear mixed effects models

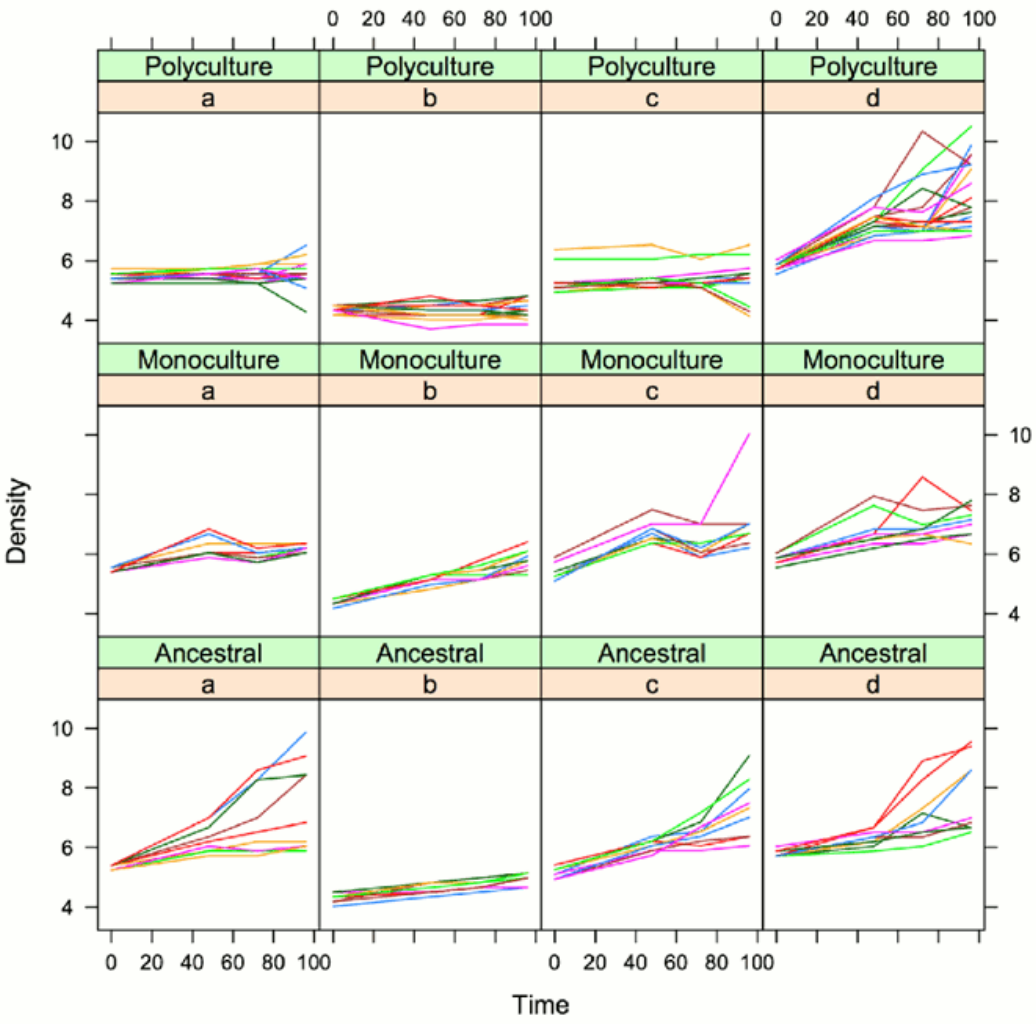
For each analysis, I first fitted the most complex model with all interactions among the explanatory variables as fixed effects, and time nested within a grouping factor specifying each assay well as random effects. Visual inspection of residuals against fitted values and of quantile normal plots were used to judge model assumptions (Crawley 2007). Following statistical comparison of alternative models of variance-covariance structure, density was included as a variance covariate in all models (Pinheiro and Bates 2000). To test for non-linear growth over time, I repeated the procedure both assuming time to be continuous and as a factor (four levels), and used ANOVA to compare models fitted using the maximum likelihood criterion. Finally, I attempted to simplify the maximum model by removing highest order interaction terms and using ANOVA to compare nested models fitted using maximum likelihood. The minimum adequate model was refitted using restricted maximum likelihood (REML) for reporting. For assays on used beech tea, I compared models with either 'interaction type' (unused, used by intraspecific isolate, or used by interspecific isolate) or 'substrate' (unused, used by species A, used by species B etc.) to test for significant differences in interaction strengths among species. I used growth over the first 48 hours to summarize changes in maximum growth rate, *Vmax*: in all assays, growth was either linear or fastest during the first 48 hours (Appendix 2.5). Similar conclusions about the evolutionary effects of species interactions were reached using density at 96 hours as a measure of carrying capacity instead of focusing on initial growth rate (Appendix 2.7).

2.4 Scatter plot showing the linear relationship between OD_{600} and log colony counts.



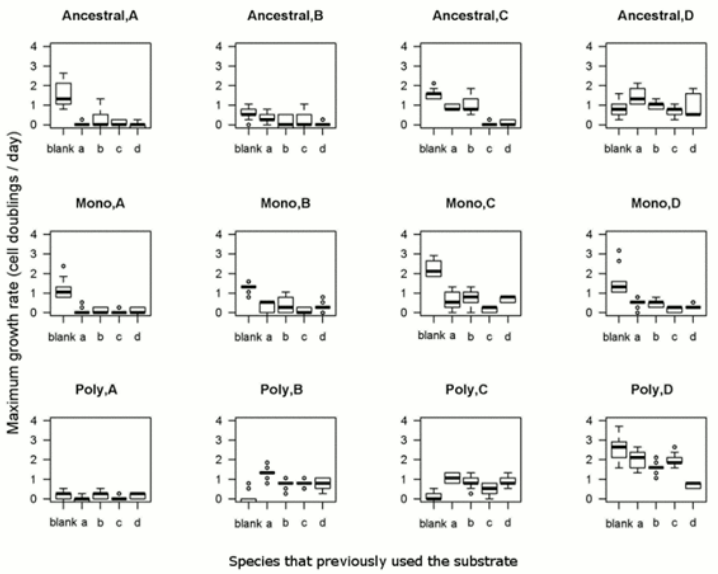
Appendix 2.4. Scatter plot showing the linear relationship between OD_{600} and log colony counts. The model simplified to retain species and OD_{600}, but no interaction terms (i.e., different intercept for calibration line for each species, but same slopes, $F_{4,67} = 32.9$, $p<0.0001$, $r^2 = 0.64$). The fitted lines were used to calibrate in units of log(number of cells) per ml.

2.5 Growth of replicates of each species on beech tea



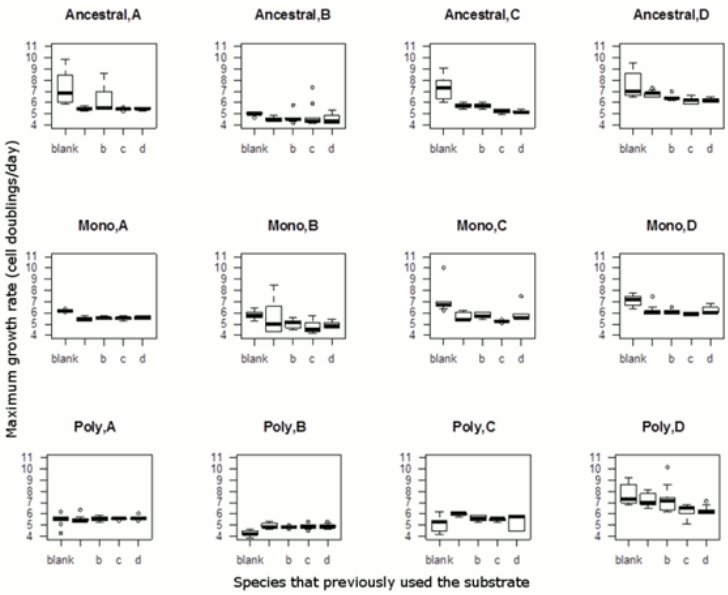
Appendix 2.5 Growth of replicates of each species in assays on unused beech tea across the three treatments. The *y*-axes are log(cell counts per ml), and *x*-axes are time since start in hours. Ancestral isolates of all four species grew linearly over the assay period on unused beech tea (ANOVA comparing a model with time as a factor versus a model with time as a continuous variable, likelihood ratio = 6.9, $df = 13$ and 21, $p = 0.55$). The monoculture isolates displayed significantly non-linear growth (ANOVA comparing models with time as a factor and as a continuous variable, L-ratio 39.3, $p<0.0001$). In species A, B, and C there was a reduction in growth rate between day 2 and 3 followed by recovery by day 4. In species D, there was a successive decline in growth rate. In each case, growth between day 0 and day 2 was faster than at any later period. Polyculture isolates grew linearly over the assay period (ANOVA comparing models with time as a factor and as a continuous variable, L-ratio 27.7, $p<0.001$).

2.6 Maximum growth rates for each species and treatment in "used" and "unused" beech tea



Appendix 2.6. Maximum growth rates for each species and evolution treatment when grown in "used" and "unused" substrate. Boxplots of maximum growth rates, V_{MAX}, in cell doublings per day across evolution treatments, species, and substrates. The dark line shows the median, the box limits show the inter-quartile range, and whiskers/points indicate extreme values.

2.7 Boxplots of the density after 4d across species and substrates



Appendix 2.7. Boxplots of the density after 4d (log10) across species and substrates. The dark line shows the median, the box limits show the inter-quartile range, and whiskers/points indicate extreme values. Key findings based on comparing Vmax remain the same when comparing amount of growth by day 4: species A grows well on unused tea in ancestral and monoculture treatments, but not when it has evolved in polyculture. Species B and C shift from having reduced growth on used tea in ancestral and monoculture isolates to having enhanced growth in polyculture treatments. Species D evolves to have stronger negative effects of used tea in monoculture than in ancestral isolates, but evolves even better growth on unused tea when it evolves in polycultures than in either ancestral or monoculture isolates.

2.8 Densities, doubling rates, and effective population sizes of each species during the evolution experiments.

Treatment	Species	N_0	N_t	No. cell doublings per day	Total number of generations	Effective population size
Monoculture	A	6.23 ± 0.04	7.51 ± 0.04	1.21 ± 0.07	67.7 ± 4	6.99 ± 0.02
	B	5.34 ± 0.21	6.64 ± 0.21	1.19 ± 0.34	66.6 ± 18.8	5.97 ± 0.17
	C	5.56 ± 0.09	6.71 ± 0.13	1.09 ± 0.09	60.9 ± 5	6.27 ± 0.1
	D	6.13 ± 0.03	7.45 ± 0.04	1.25 ± 0.08	70.2 ± 4.8	6.91 ± 0.03
	E	4.14 ± 0.35	5.21 ± 0.36	1.09 ± 0.3	61.2 ± 17	4.53 ± 0.33
Polyculture	A	4.94 ± 0.05	6.27 ± 0.07	1.27 ± 0.12	71.1 ± 6.9	5.72 ± 0.04
	B	5.04 ± 0.18	6.58 ± 0.2	1.47 ± 0.13	82.2 ± 7.3	5.89 ± 0.18
	C	5.53 ± 0.06	6.73 ± 0.09	1.14 ± 0.1	63.6 ± 5.4	6.26 ± 0.06
	D	5.35 ± 0.06	6.62 ± 0.06	1.2 ± 0.08	67 ± 4.5	6.11 ± 0.04
	E	4.34 ± 0.13	5.71 ± 0.12	1.37 ± 0.29	76.6 ± 16.1	5.08 ± 0.05

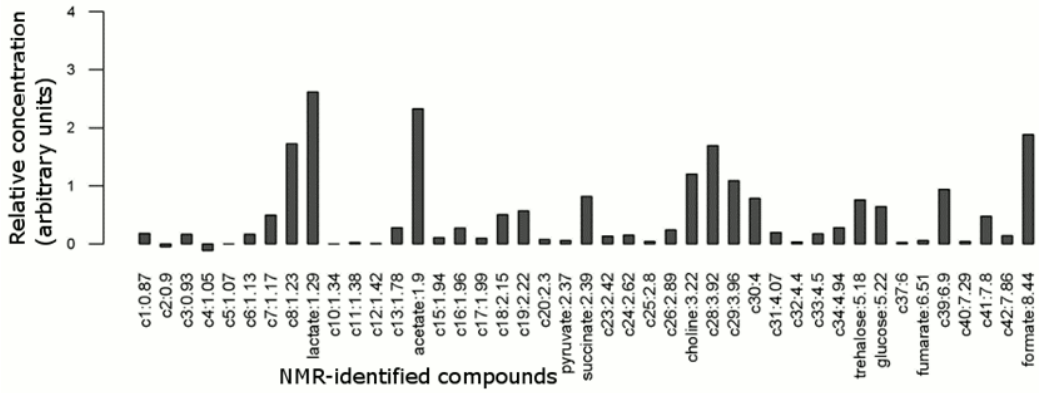
2.9. Linear mixed effects model comparisons.

Models compared	Likelihood ratio	Degrees of freedom	p
1) Model 1: species X treatment X substrate X time 2) Model 1 but removing the highest order interaction term*	585.9	217, 325	<0.0001
1) Model 1 2) Model 2: species X treatment X interaction X time #	449.9	197, 325	<0.0001

* Comparison shows that all explanatory variables remain in model including the four-way interaction term

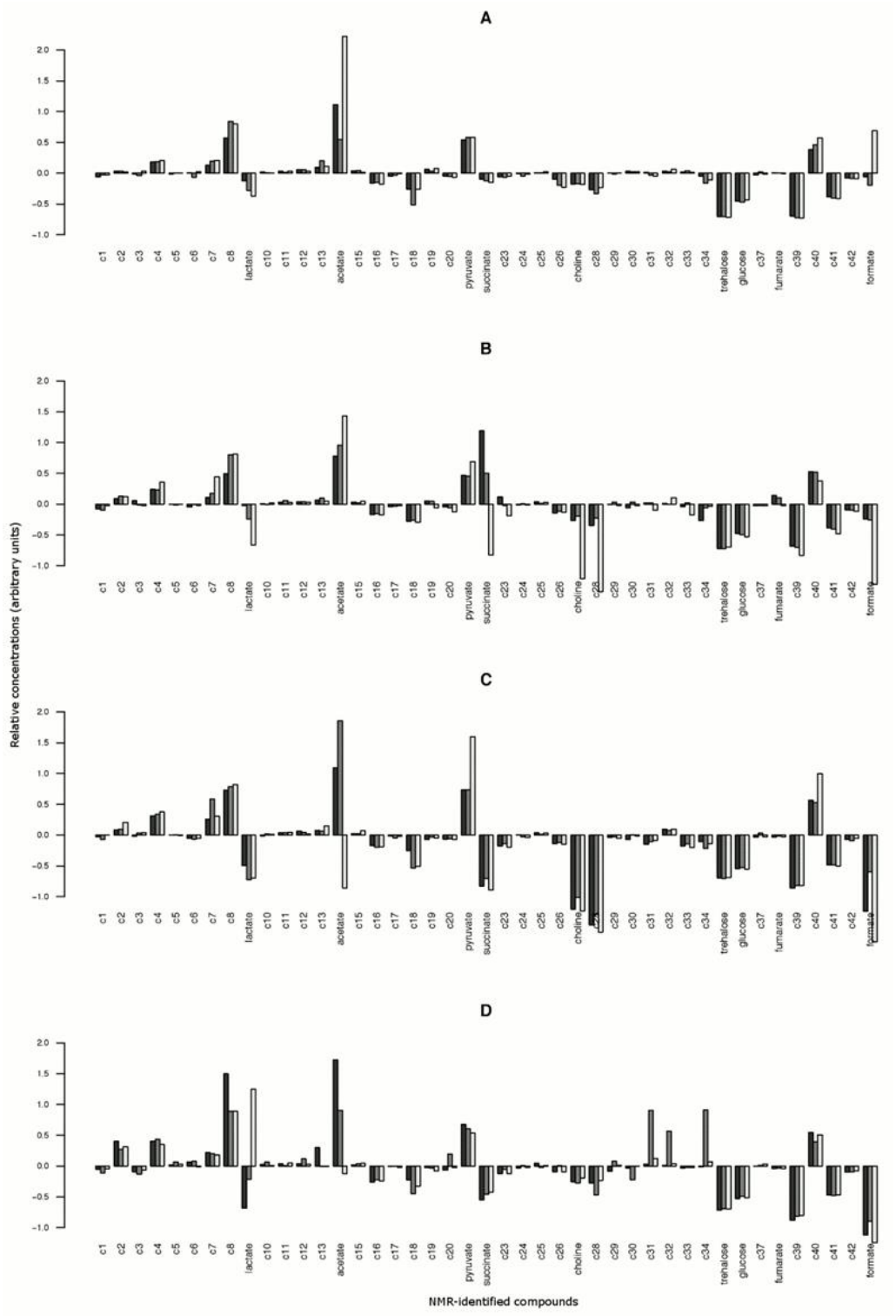
Comparison shows that there are differences in interaction strength between different combinations of species: substrate (= used by species a, b, c or d) is a better explanatory variable than interaction (=unused, used by own species, used by a different species).

2.10 Compounds identified in unused beech tea



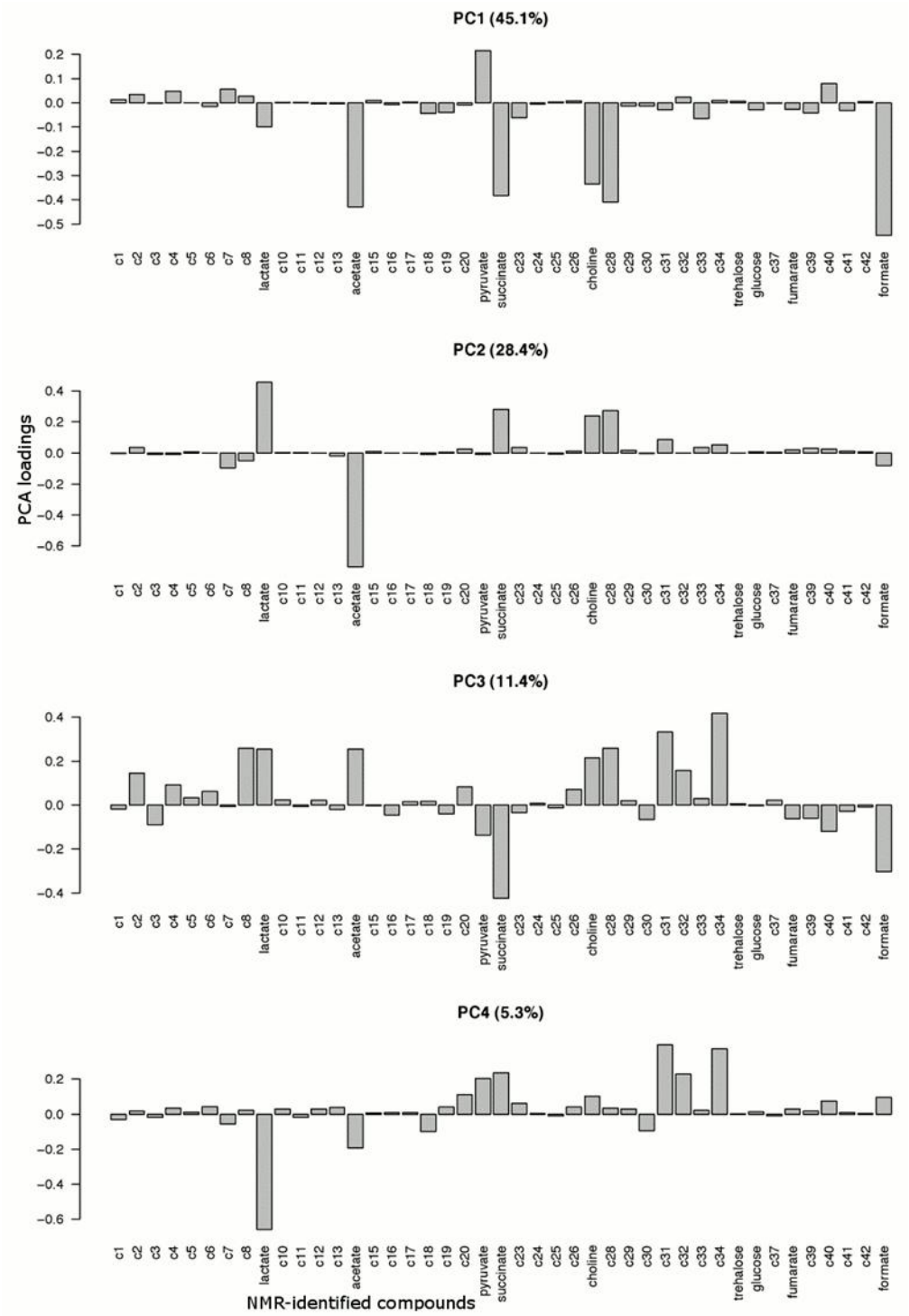
Appendix 2.10. Amounts of compounds identified from distinct peaks in the NMR spectrum of unused beech tea. Bars show the size of the major peak for each distinct compound relative to the size of the standard, DSS; hence peak heights are dimensionless. The location of each peak on the spectrum is shown after each name (peak shift in parts per million).

2.11 NMR peaks for each species and treatment



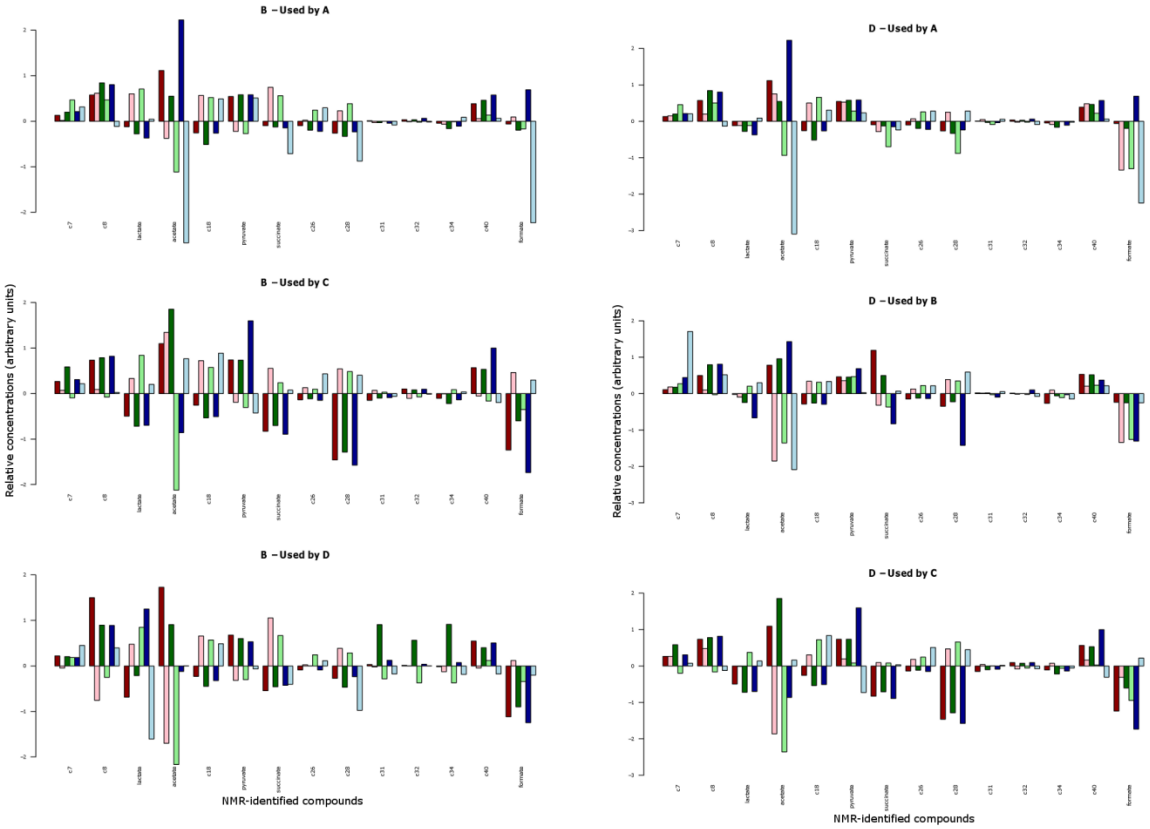
Appendix 2.11. NMR peaks for each species and treatment. The difference in the size of NMR peaks between tea used by ancestral (dark grey), monoculture (mid grey), and polyculture (light grey) in turn and the size of peaks in unused beech tea. Positive values indicate production of a compound, and negative values indicate consumption of a compound. Peak sizes are expressed relative to the size of the standard, DSS, and hence are dimensionless.

2.12 Contribution of each compound to variation between treatments



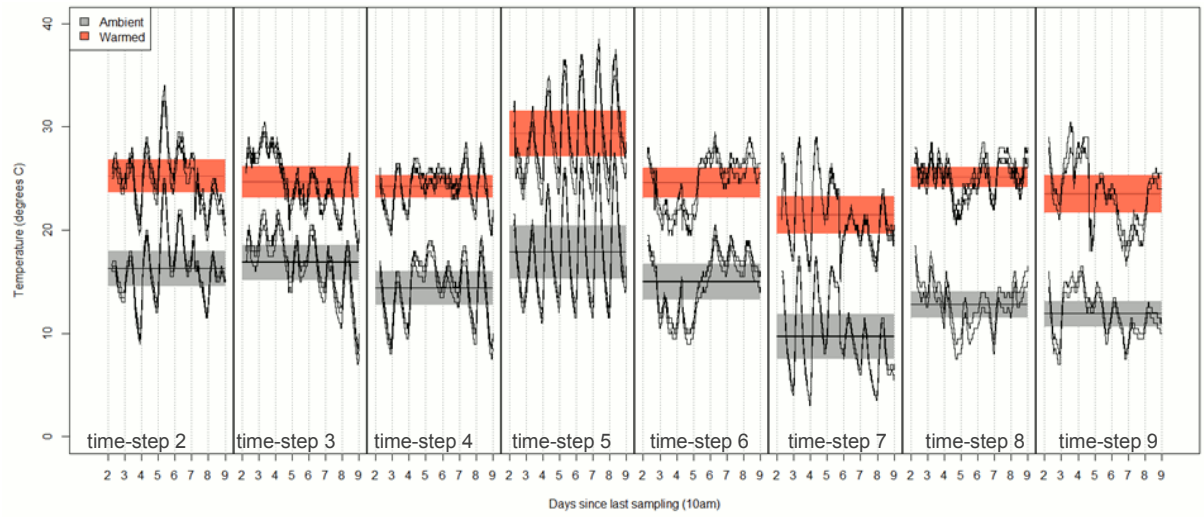
Appendix 2.12. Contribution of each compound to variation between treatments. Loadings of the first four principal components of resource use and production of the four surviving species across ancestral, monoculture, and polyculture treatments. The input data were the difference between the size of the peak in medium used by the isolate and the size of the peak in the beech tea (i.e., the data in Appendix 2.11). Bars indicate the correlation coefficient between variation in each compound and the relevant principal component. The percentage of total variation described by each principal component is shown above each plot; together they explain 90.1% of the total variation.

2.13 Changes in substrate composition after use by a first species then species B or D



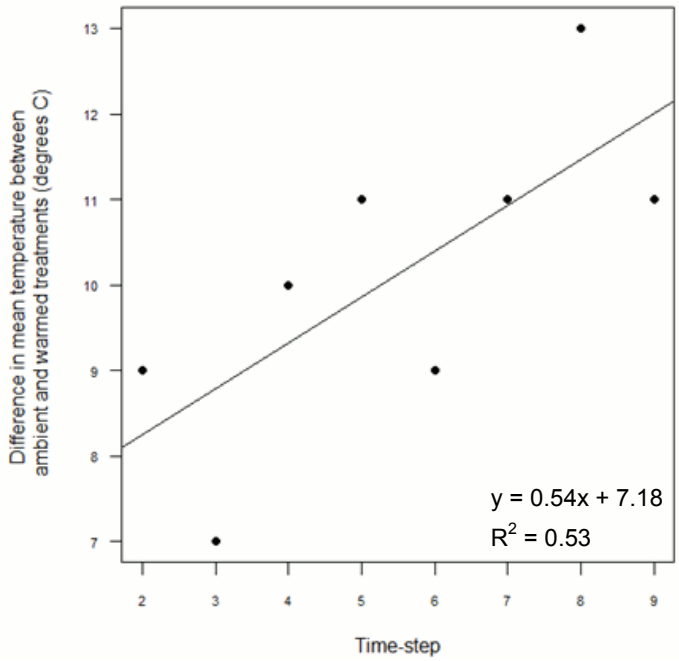
Appendix 2.13. Changes in substrate composition after use by a first species and then species B or D. The difference in the relative size of NMR peaks between tea used by a first species' ancestral (red), monoculture (green), and polyculture (blue) in turn and the relative size of peaks in unused beech tea; together with the change in the size of the peak after a second species grew on medium already used by the first species (then filter sterilised) for the same treatments (ancestral, pink; monoculture, light green; polyculture, light blue). The order of bars for each compound is first species ancestral, second species ancestral, first species monoculture, second species monoculture, first species polyculture, and second species polyculture. Positive values indicate production of a compound, and negative values indicate consumption of a compound relative to the starting medium. To improve clarity of the figure and focus on compounds of interest for cross-feeding, only compounds in which at least one isolate generated an increase in peak size of 0.5 are shown. Only species B and species D were used as the second species, chosen to represent two species showing different results in the growth assays. Evidence of evolved cross-feeding in polyculture is apparent when high blue peaks (generation of the compound by the first species) are associated with low purple peaks (use of the compound by the second species). For example, the species A polyculture isolate produces formate, which in turn is used up by both species B and D.

3.1 Fluctuations in temperature throughout environmental change experiment



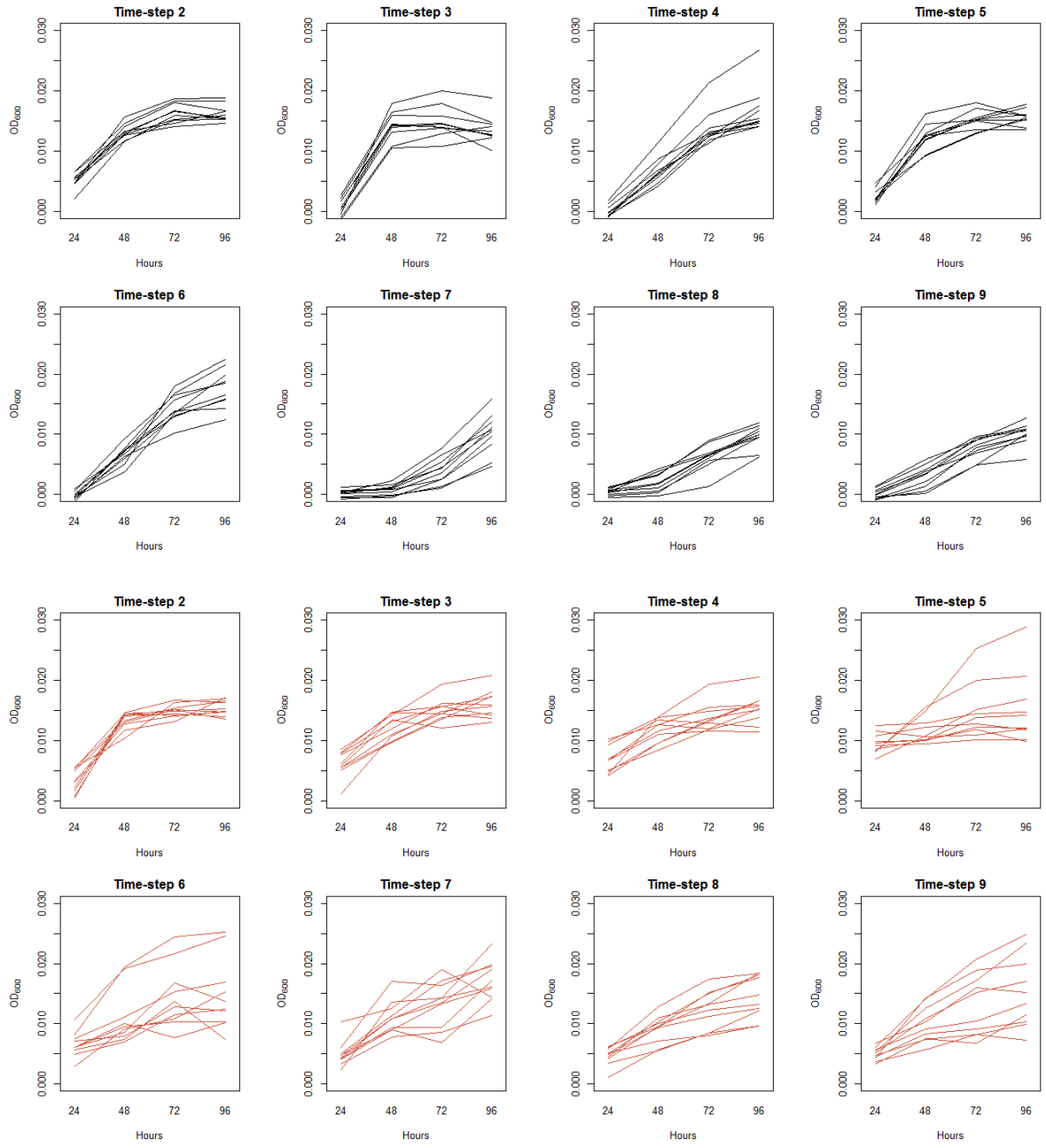
Appendix 3.1. Fluctuations in temperature during each time-step. Shaded bars show standard error in the temperature during the time-step. Upper lines show warmed temperature recorded every 10 minutes and lower lines show the ambient temperature. The mean temperature of control communities decreases during the experiment and that of warmed communities remains close to initial temperatures therefore there was a divergence in mean temperatures over the course of the experiment.

3.2 Divergence in mean temperature during the environmental change



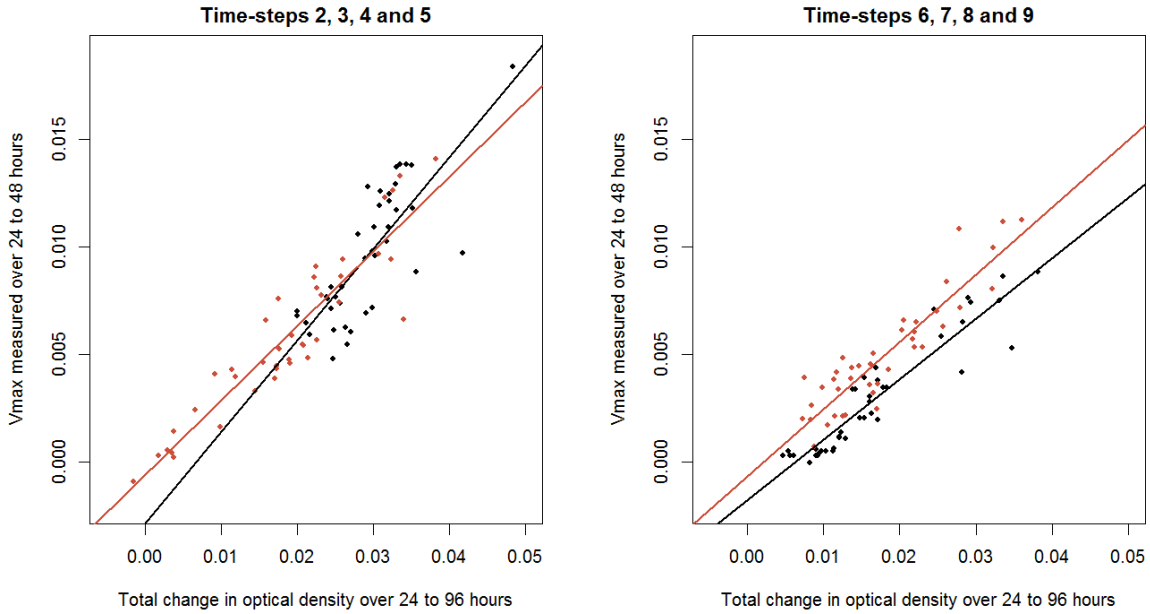
Appendix 3.2. Divergence in mean temperature for the warmed and ambient treatments. There is a significant positive increase in the difference between mean temperatures of warmed and ambient microcosms during the course of the experiment (Pearson's correlation = 0.73, df = 6, t = 2.59*).

3.3 Whole community growth curves during environmental change



Appendix 3.3. 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. For time-steps 2 to 6 growth curves 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.

3.4 Correlation between V_{max} and total growth rates in the first and second half of the environmental change experiment



Appendix 3.4. Correlation between total growth rate over 24 to 96h and V_{max} (change in OD_{600} over 24 to 48h). Throughout the experiment there is a strong correlation between total growth and Vmax, however, in the second half of the experiment the intercept of warmed communities is significantly higher than that of ambient communities. This indicates that later in the environmental change experiment a greater proportion of total growth was achieved over 24 to 48h in warmed communities compared to in ambient communities.

3.5 Simplification of lmer of total growth during environmental change. In all models compared the random effects were "microcosm" to account for the same microcosm being sampled at each time-step and "block" to account for spatial autocorrelation that may be caused by samples being in the same experimental block. ANOVAs were used ensure that simplification did not result in significant loss of explanatory power. Simplification indicated that model 3 is the minimum adequate model to explain total community growth during the environmental change experiment.

Models compared	**AIC**	**Degrees of freedom**	**p value**
Model 1 warming X immigration X time-step	-1146	35	
Model 2 warming X immigration + warming X time-step +immigration X time-step	-1152	28	Model 1 compared to Model 2: p>0.05
Model 3 warming X immigration + warming X time-step	-1159	21	Model 2 compared to model 3: p>0.05
Model 4 immigration + warming X time-step	-1152	20	Model 3 compared to model 4: p<0.01
Model 5 immigration X warming + time-step	-1069	14	Model 3 compared to model 5: p<0.001

3.6 Output of final lmer for total growth during environmental change. The minimum adequate model was warming X immigration + warming X time-step with the random effects of microcosm and block.

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 treatment	0.0009	1	117	29.73	<0.001	6.21
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

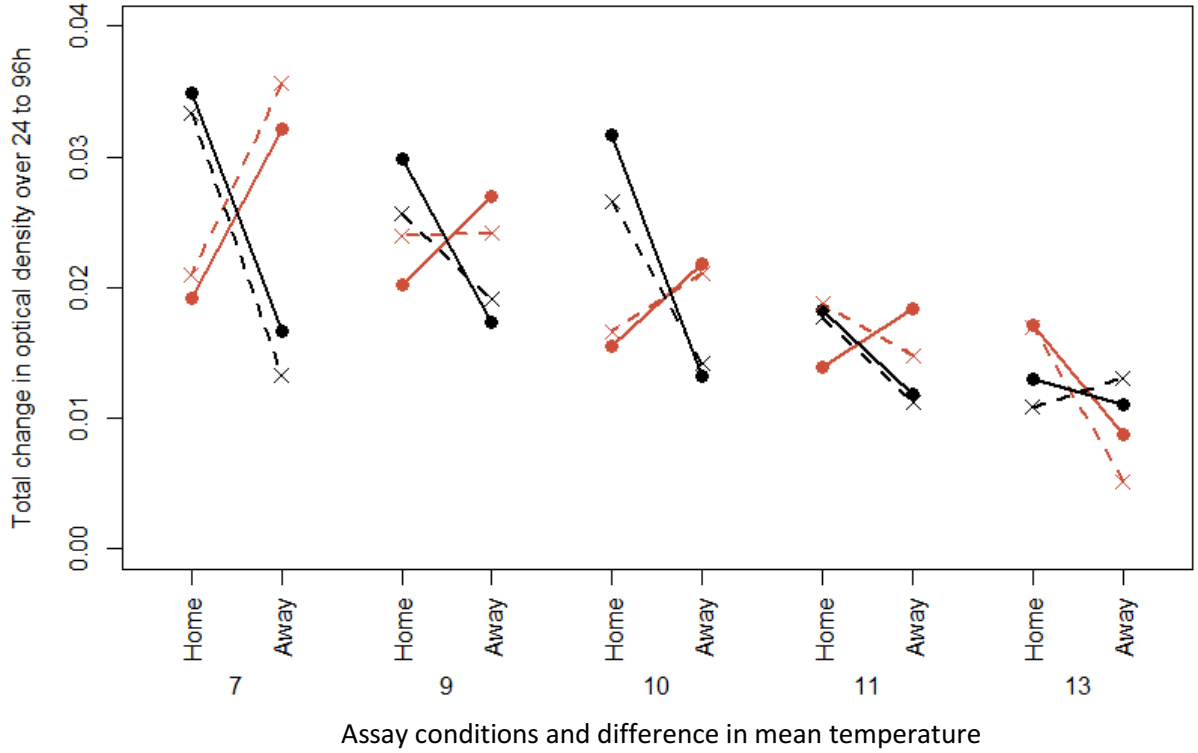
3.7 Simplification of lmer of community local adaptation during environmental change. In all models compared the random effects were "microcosm" to account for the same microcosm being sampled at each time-step and "block" to account for spatial autocorrelation that may be caused by samples being in the same experimental block. ANOVAs were used ensure that simplification did not result in significant loss of explanatory power. Simplification indicated that model 3 is the minimum adequate model to explain community local adaptation over the course of the environmental change experiment.

Models compared	AIC	Degrees of freedom	p value
Model 1 warming X immigration X time-step	-1078	35	
Model 2 warming X immigration + warming X time-step +immigration X time-step	-1080	28	Model 1 compared to Model 2: p>0.05
Model 3 warming X immigration + warming X time-step	-1083	21	Model 2 compared to model 3: p>0.05
Model 4 immigration + warming X time-step	-1074	20	Model 3 compared to model 4: p<0.001
Model 5 immigration X warming + time-step	-923	14	Model 3 compared to model 5: p<0.001

3.8 Output of final lmer for community local adaptation during environmental change. The minimum adequate model was warming X immigration + warming X time-step with the random effects of microcosm and block.

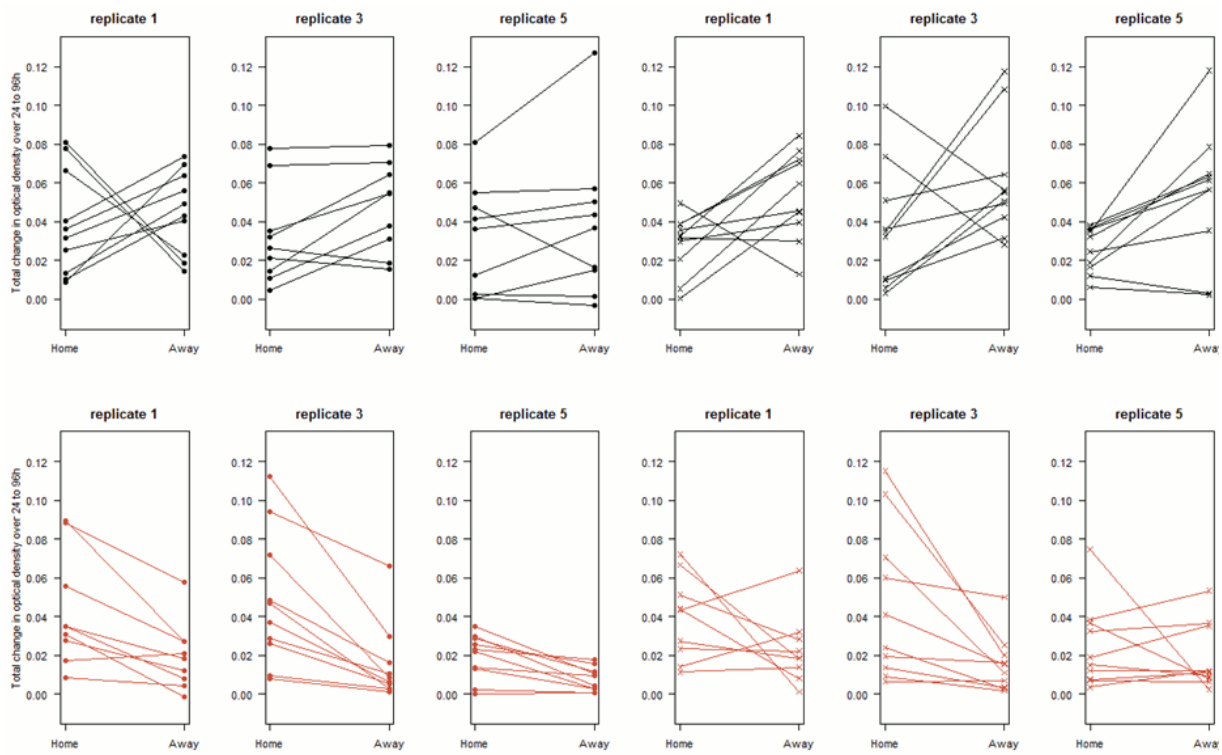
Fixed effect / interaction	Mean square error	Numerator degrees of freedom	Denominator degrees of freedom	F value	p value	Deviance explained (%)
time-step	0.0002	7	117	3.52	<0.01	3.97
warming treatment	0.0050	1	117	98.57	<0.001	15.88
immigration treatment	0.0001	1	117	1.45	>0.05	0.23
warming X time-step	0.0023	7	117	45.34	<0.001	51.13
warming X immigration	0.0006	1	117	11.80	<0.001	1.90

3.9 Local adaptation of communities as assay temperatures diverge during the environmental change experiment



Appendix 3.9. Difference between growth in 'home' and 'away' environments as assay temperatures diverge. The first point in the pair shows total growth in the in the same average conditions as that community experienced during the environmental change experiment and the second point shows total growth in the foreign conditions. Lines that have a negative slope indicate greater growth in 'home' environments and therefore show local adaptation. Ambient communities showed high local adaptation when difference in mean temperature was lower and their degree of local adaptation tended to decline as the conditions diverged; indicating that the local adaptation of ambient communities declined during the experiment. Conversely, warmed communities showed maladaptation when the experimental conditions were similar and their local adaptation tended to improve as the experimental conditions diverged.

3.10 Local adaptation of isolates at the end of the environmental change experiment



Appendix 3.10. Local adaptation of isolates. Each panel represents a community and each line represents a randomly isolated colony. Black lines are isolates from ambient communities and red lines are isolates from warmed communities. Dots represent isolates from communities with immigration and crosses represent isolates from communities without immigration. The left point shows total growth in the 'home' environment for that isolate and the right point is total growth in the 'away' environment. Negative slopes indicate local adaptation and positive slopes show local maladaptation. Isolates from ambient communities are predominantly locally maladapted and isolates from warmed communities are predominantly locally adapted.

3.11 Simplification of lmer of change in total metabolic function during environmental change. In all models compared the random effects were "microcosm" to account for the same microcosm being sampled at each time-step and "substrate" to account for growth rate being measured on the same substrates repeatedly ANOVAs were used ensure that simplification did not result in significant loss of explanatory power. Simplification indicated that model 3 is the minimum adequate model to explain the change in metabolic functioning over the course of the environmental change experiment.

Models compared	**AIC**	**Degrees of freedom**	**p value**
Model 1 warming X immigration X time-step	-160	15	
Model 2 warming X immigration + warming X time-step +immigration X time-step	-160	13	Model 1 compared to Model 2: $p>0.05$
Model 3 immigration X time-step + warming X time-step	-162	12	Model 2 compared to model 3: $p>0.05$
Model 4 immigration X time-step + warming	-135	10	Model 3 compared to model 4: $p<0.001$
Model 5 time-step X warming + immigration	-156	10	Model 3 compared to model 5: $p<0.01$

3.12 Output of final lmer for change in metabolic function during environmental change. The minimum adequate model was warming X immigration + warming X time-step with the random effects of microcosm, block and substrate.

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

3.13 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. 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 (Appendix 3.12).

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

3.14 Principle components calculated from Biolog assays

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

Principle 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, principle 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).

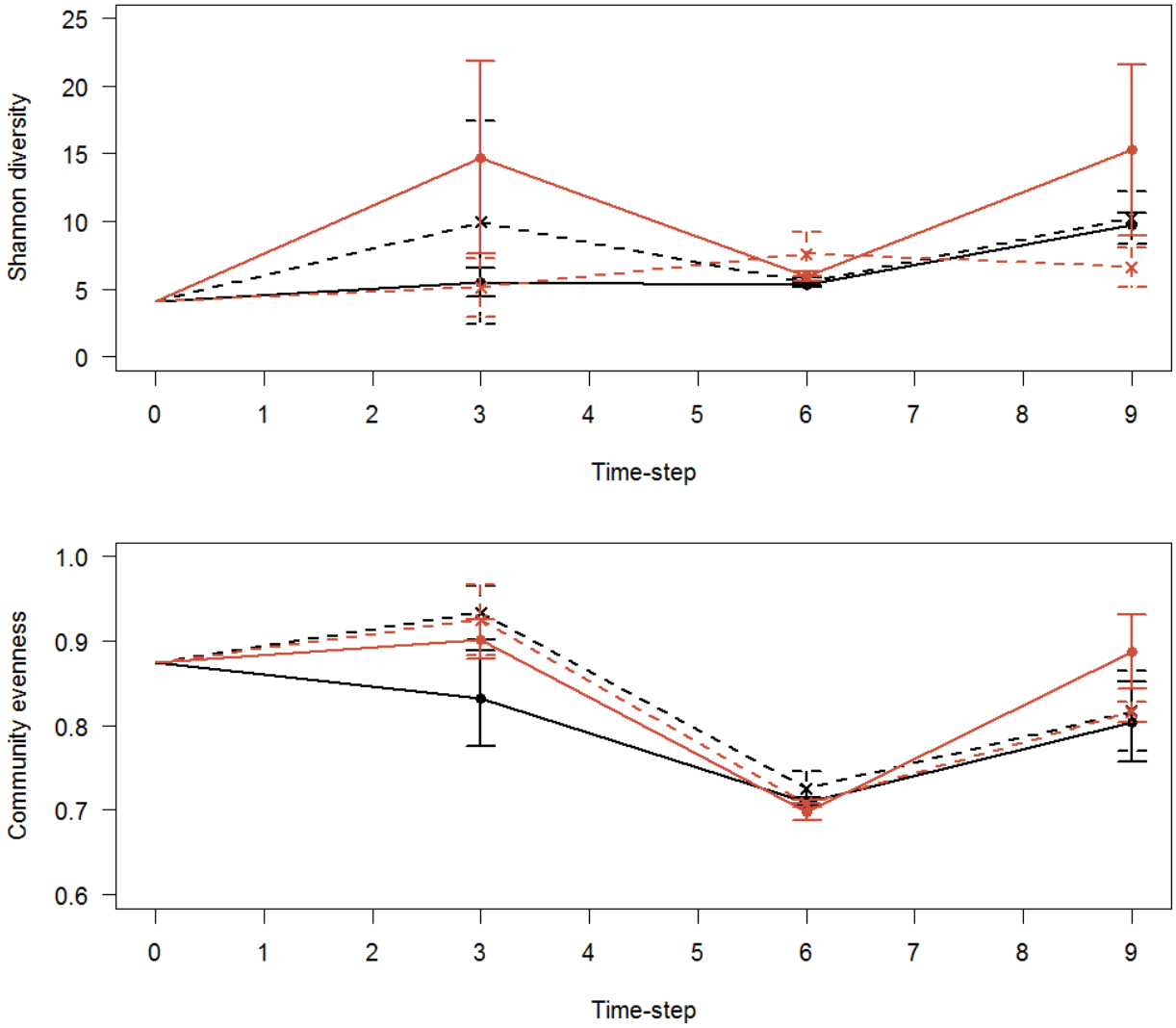
3.15 Simplification of lmer of movement of community metabolic profile in multivariate space over the course of environmental change. In all models compared the random effects were "microcosm" to account for repeated sampling of the same microcosm through time and "principal component" to account for the inflated variance within samples caused by the weighting of the distance travelled according to the variance explained by the principal components. ANOVAs were used ensure that simplification did not result in significant loss of explanatory power. Simplification indicated that model 3 best explained the change in community metabolic profile without significant loss of explanatory power, however, as I am interested in the main fixed effects of immigration and warming model 2 is used as the final model.

Models compared	**AIC**	**Degrees of freedom**	**p value**
Model 1 warming X immigration	142	7	
Model 2 warming + immigration	140	6	Model 1 compared to Model 2: $p>0.05$
Model 3 warming	139	5	Model 2 compared to model 3: $p>0.05$
Model 4 immigration	144	5	Model 2 compared to model 4: $p<0.05$
Model 5 no fixed effects	139	4	Model 3 compared to model 5: $p<0.05$

3.16 Output of final lmer for change in community metabolic profile over the course of environmental change. The minimum adequate model was warming + immigration with the random effects of microcosm and principal component.

Fixed effect / interaction	**Mean square error**	**Numerator degrees of freedom**	**Denominator degrees of freedom**	**F value**	**p value**	**Deviance 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

3.17 Change in community diversity and evenness over time



Appendix 3.17. Change in communities diversity and evenness calculated from T-RFLP profiles during environmental change. Black lines denote ambient communities and red warmed communities. Solid lines with circles indicate that the communities received immigration and dashed lines with crosses indicate that the communities were closed. There were 3 replicates per immigration warming condition. Analyses indicate that there were no significant effects of warming and immigration on diversity over time. There was, however, a general decrease in community evenness over time with communities at time-step 6 being significantly less even than those at time-steps 3 and 9 and communities at time-step 9 being less even than those at time-step 3.

3.18 Simplification of lmer of community evenness during environmental change. In all models compared the random effects were "microcosm" to account for repeated sampling of the same microcosm through time and "block" to account for the spatial blocking structure. ANOVAs were used ensure that simplification did not result in significant loss of explanatory power. Simplification indicated that model 7 best explained the change in community evenness without significant loss of explanatory power, however, as I am interested in the main fixed effects of immigration, warming and time-step model 5 is used as the final model.

Models compared	**AIC**	**Degrees of freedom**	**p value**
Model 1 warming X immigration X time-step	-87	15	
Model 2 warming X immigration + warming X time-step + immigration X time-step	-90	13	Model 1 compared to Model 2: $p>0.05$
Model 3 warming X immigration + immigration X time-step	-92	11	Model 2 compared to model 3: $p>0.05$
Model 4 time-step X immigration + warming	-91	10	Model 3 compared to model 4: $p>0.05$
Model 5 time-step + immigration + warming	-90	8	Model 4 compared to model 5: $p>0.05$
Model 6 time-step + immigration	-91	7	Model 5 compared to model 6: $p>0.05$
Model 7 time-step	-93	6	Model 6 compared to model 7: $p>0.05$
Model 8 intercept only	-59	4	Model 7 compared to model 8: $p<0.001$

3.19 Output of final lmer for community evenness during environmental change. The final model was warming + immigration + time-step with the random effects of microcosm and block.

Fixed effect / interaction	**Mean square error**	**Numerator degrees of freedom**	**Denominator degrees of freedom**	**F value**	**p value**	**Deviance explained (%)**
time-step	0.108	2	16	35.7	<0.001	63.56
warming treatment	0.003	1	16	1.07	>0.05	0.95
immigration treatment	0.002	1	16	0.71	>0.05	0.63

3.20 Simplification of lmer of Shannon diversity during environmental change. In all models compared the random effects were "microcosm" to account for repeated sampling of the same microcosm through time and "block" to account for the spatial blocking structure. ANOVAs were used ensure that simplification did not result in significant loss of explanatory power. Simplification indicated that model 8 best explained the change in diversity without significant loss of explanatory power, however, as I am interested in the main fixed effects of immigration, warming and time-step model 5 is used as the final model.

Models compared	**AIC**	**Degrees of freedom**	**p value**
Model 1 warming X immigration X time-step	251	15	
Model 2 warming X immigration + warming X time-step + immigration X time-step	250	13	Model 1 compared to Model 2: p>0.05
Model 3 warming X immigration + immigration X time-step	246	11	Model 2 compared to model 3: p>0.05
Model 4 time-step + immigration X warming	244	9	Model 3 compared to model 4: p>0.05
Model 5 time-step + immigration + warming	245	7	Model 4 compared to model 5: p>0.05
Model 6 time-step + immigration	244	7	Model 5 compared to model 6: p>0.05
Model 7 time-step	243	6	Model 6 compared to model 7: p>0.05
Model 8 intercept only	242	4	Model 7 compared to model 8: p>0.05

3.21 Output of final lmer for Shannon diversity during environmental change. The final model was warming + immigration + time-step with the random effects of microcosm and block.

Fixed effect / interaction	**Mean square error**	**Numerator degrees of freedom**	**Denominator degrees of freedom**	**F value**	**p value**	**Deviance explained (%)**
time-step	58.43	2	16	1.72	>0.05	8.14
warming treatment	20.01	1	16	0.59	>0.05	1.39
immigration treatment	33.02	1	16	0.97	>0.05	2.30

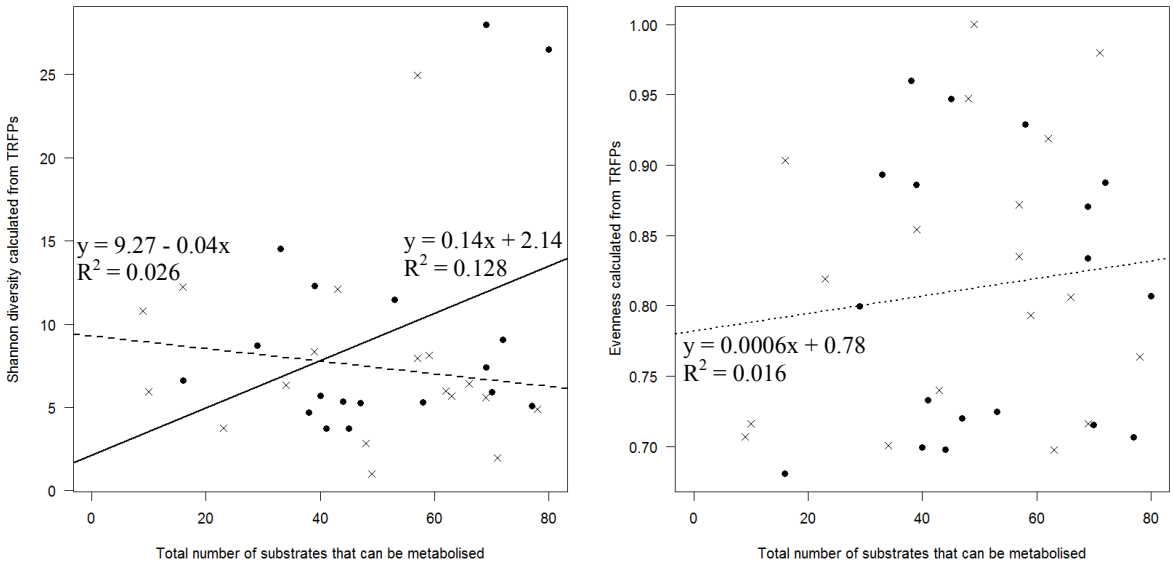
3.22 Simplification of lmer of community metabolic function during environmental change with Shannon diversity as an explanatory variable. In this model the response variable was total community metabolic function, measured as the total number of substrates that could be metabolised. In all models compared the random effects were "microcosm" to account for repeated sampling of the same microcosm through time and "block" to account for the spatial blocking structure. Simplification indicated that model 14 best explained the data without significant loss of explanatory power, however as time-step is an explanatory variable of interest it is retained in the model and therefore the final model is model 11.

Models compared	**AIC**	**Degrees of freedom**	**p value**
Model 1 warming X immigration X time-step X diversity	304	27	
Model 2 warming X immigration X time-step + warming X time-step X diversity + immigration X time-step X diversity + diversity X immigration X warming	302	25	Model 1 compared to Model 2: p>0.05
Model 3 warming X immigration X time-step + warming X time-step X diversity + diversity X immigration X warming	299	23	Model 2 compared to model 3: p>0.05
Model 4 warming X time-step X diversity + diversity X immigration X warming + immigration X time-step +immigration X warming	295	21	Model 3 compared to model 4: p>0.05
Model 5 diversity X immigration X warming + immigration X time-step +immigration X warming + time-step X diversity +warming X time-step	294	19	Model 4 compared to model 5: p>0.05
Model 6 immigration X time-step +immigration X warming + time-step X diversity + immigration X diversity + warming X diversity +warming X time-step	295	18	Model 5 compared to model 6: p>0.05
Model 7 immigration X time-step +immigration X warming + time-step X diversity + warming X diversity + diversity X immigration	293	16	Model 6 compared to model 7: p>0.05
Model 8 immigration X time-step + time-step X diversity + warming X diversity + diversity X immigration	292	15	Model 7 compared to model 8: p>0.05
Model 9 immigration X time-step + warming X diversity + diversity X immigration	292	13	Model 8 compared to model 9: p>0.05
Model 10 immigration X time-step + diversity X immigration + warming	292	12	Model 9 compared to model 10: p>0.05
Model 11 diversity X immigration + warming + time-step	292	10	Model 10 compared to model 11: p>0.05
Model 12 diversity + immigration + warming + time-step	303	9	Model 11 compared to model 12: p<0.001
Model 13 diversity X immigration + time-step	298	9	Model 11 compared to model 13: p<0.01
Model 14 diversity X immigration + warming	292	8	Model 11 compared to model 14: p>0.05

3.23 Output of final lmer for total metabolic function given community diversity during environmental change. The final model was diversity X immigration + warming + time-step with the random effects of microcosm and block.

Fixed effect / interaction	Mean square error	Numerator degrees of freedom	Denominator degrees of freedom	F value	p value	Deviance explained (%)
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
Diversity X immigration	1337.38	1	14	16.78	<0.01	9.83

3.24 Relationship between total metabolic function and community composition.



Appendix 3.24. Relationship between total metabolic function and community composition. Circles represent communities that received immigration and crosses represent closed communities. **a)** Correlation between total metabolism and species diversity for each immigration regime. There was a positive but not significant correlation between metabolism and species diversity in communities that received immigration (solid line). In contrast, in closed communities there was a slight negative correlation (dashed line). b) There was a slight positive correlation between community evenness and total metabolism and no interaction between immigration and evenness (dotted line).

4.1 Simplification of lmer of variability between replicate communities during environmental change. In all models compared the random effects were "microcosm" to account for the same microcosm being sampled at each time-step and "block" to account for spatial autocorrelation that may be caused by samples being in the same experimental block. ANOVAs were used ensure that simplification did not result in significant loss of explanatory power. Simplification indicated that model 6 is the minimum adequate model that best explains total community growth during the environmental change experiment, however, as I am interested in the main effect of immigration this is retained and therefore model 4 is used as the final model..

Models compared	**AIC**	**Degrees of freedom**	**p value**
Model 1 warming X immigration X time-step	77	35	
Model 2 warming X immigration + warming X time-step +immigration X time-step	67	28	Model 1 compared to Model 2: $p>0.05$
Model 3 time-step X immigration + warming X time-step	65	27	Model 2 compared to model 3: $p>0.05$
Model 4 immigration + warming X time-step	56	20	Model 3 compared to model 4: $p>0.05$
Model 5 immigration + warming + time-step	65	13	Model 4 compared to model 5: $p<0.01$
Model 6 warming X time-step	54	19	Model 4 compared to model 6: $p>0.05$
Model 7 warming + time-step	63	12	Model 6 compared to model 7: $p<0.01$

4.2 Output of final lmer for variability between replicate communities during environmental change. The minimum adequate model was warming X time-step + immigration with the random effects of microcosm and block.

Fixed effect / interaction	**Mean square error**	**Numerator degrees of freedom**	**Denominator degrees of freedom**	**F value**	**p value**	**Deviance explained (%)**
Warming treatment	1.20	1	118	18.93	<0.001	8.08
Time-step	0.20	7	118	3.20	<0.01	9.55
Immigration treatment	$2x10^{-4}$	1	118	0.003	>0.05	0.01
Warming X time-step	0.22	7	118	3.48	<0.01	10.40

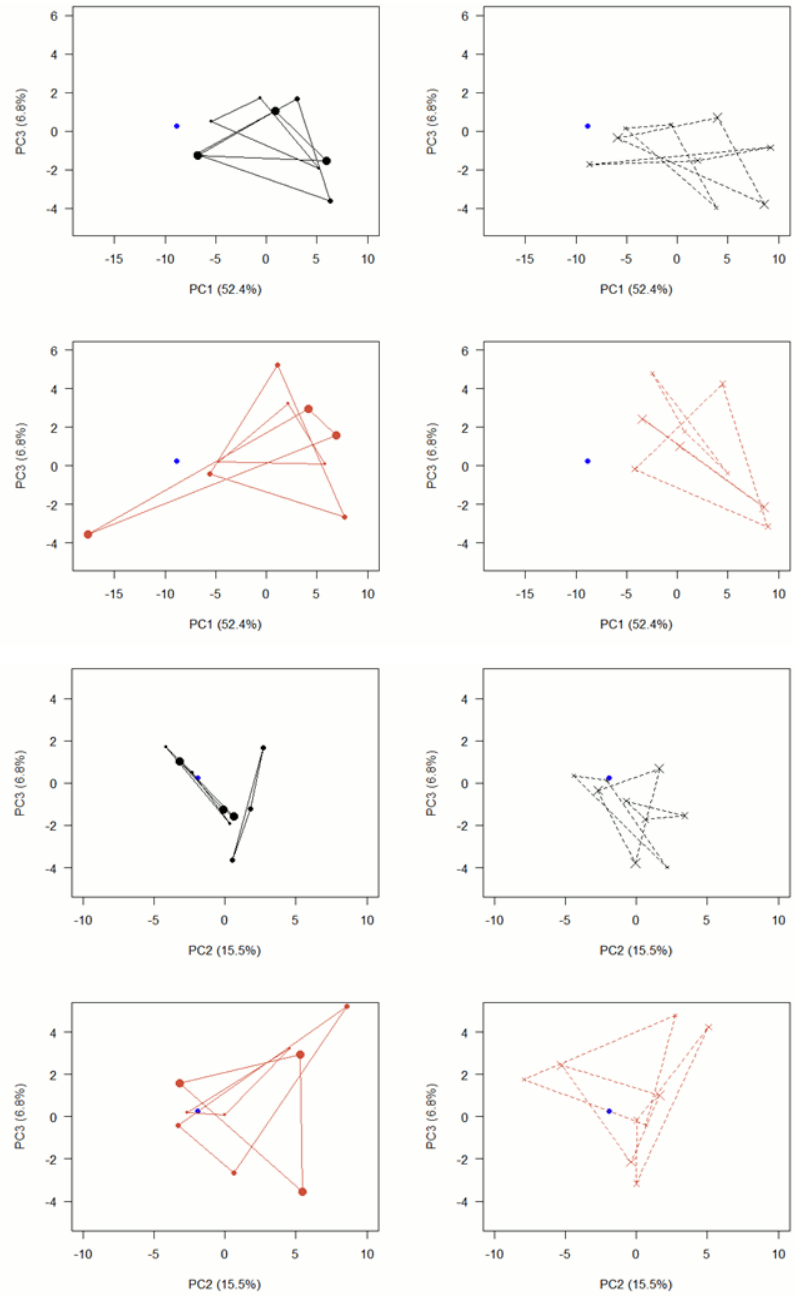
4.3 Simplification of lmer of divergence in community metabolic profiles during the environmental change experiment. In all models compared a random effect was used to account for the same sample having multiple data points because divergence between replicate microcosms was measured along principal components 1, 2 and 3. ANOVAs were used ensure that simplification did not result in significant loss of explanatory power. Simplification indicated that model 8 is the minimum adequate model that best explains the divergence in community metabolism during the environmental change experiment, however, as I am interested in all the main effect they were retained and therefore model 5 is used as the final model.

Models compared	**AIC**	**Degrees of freedom**	**p value**
Model 1 warming X immigration X time-step	277	14	
Model 2 warming X immigration + warming X time-step +immigration X time-step	277	12	Model 1 compared to Model 2: p>0.05
Model 3 time-step X immigration + warming X immigration	275	10	Model 2 compared to model 3: p>0.05
Model 4 time-step + warming X immigration	273	8	Model 3 compared to model 4: p>0.05
Model 5 immigration + warming + time-step	274	7	Model 4 compared to model 5: p>0.05
Model 6 warming + time-step	273	6	Model 5 compared to model 6: p>0.05
Model 7 warming	273	4	Model 6 compared to model 7: p>0.05
Model 8 intercept only	275	3	Model 7 compared to model 8: p>0.05

4.4 Output of final lmer for divergence in community metabolic profiles during environmental change. The minimum adequate model was warming + time-step + immigration with a random effect to account for pseudoreplication.

Fixed effect / interaction	**Mean square error**	**Numerator degrees of freedom**	**Denominator degrees of freedom**	**F value**	**p value**	**Deviance explained (%)**
Warming treatment	380.8	1	19	4.71	<0.05	9.94
Immigration treatment	55.8	1	19	0.69	>0.05	1.46
Time-step	188.5	2	19	2.33	>0.05	9.84

4.5 Between-replicate variation in community metabolic profiles during environmental change



Appendix 4.5 Between-replicate variation in community metabolic profiles during environmental change. Principal components analysis was used to represent ordination space (for PC1 plotted against PC2 see Figure 4.4) and triangles are drawn to connect the metabolic profile of three replicate communities from each immigration-warming treatment at time-steps 3, 6 and 9. Black lines represent ambient communities and red lines warmed communities. Solid lines with circles denote communities that received immigration and dashed lines with crosses are those that remained closed. The size of the points represents the time-step from which the sample was taken so that smallest points are time-step 3 and largest are time-step 9. The blue point represents the metabolic function of the initial community. The perimeter of the triangles is used as a measure of the variability in metabolic profile between replicate communities. Warmed communities had significantly greater variation in metabolic profile compared to ambient communities (ANOVA of lmer of variability in PCs of replicate communities, $F_{1,19} = 4.7$, MES = 380.8, $p<0.05$).

4.6 Simplification of lmer of variability of isolates and communities. In all models compared a random effect was used to account for spatial autocorrelation that could be caused by blocking. ANOVAs were used ensure that simplification did not result in significant loss of explanatory power. Simplification indicated that model 7 is the minimum adequate model that best explains the divergence in community metabolism during the environmental change experiment, however, as I am interested in all the main effect they were retained and therefore model 5 is used as the final model.

Models compared	**AIC**	**Degrees of freedom**	**p value**
Model 1 warming X immigration X complexity	367	10	
Model 2 warming X immigration + warming X complexity + immigration X complexity	365	9	Model 1 compared to Model 2: p>0.05
Model 3 complexity X warming + warming X immigration	363	8	Model 2 compared to model 3: p>0.05
Model 4 complexity + warming X immigration	363	7	Model 3 compared to model 4: p>0.05
Model 5 immigration + warming + complexity	364	6	Model 4 compared to model 5: p>0.05
Model 6 warming + complexity	362	5	Model 5 compared to model 6: p>0.05
Model 7 complexity	362	4	Model 6 compared to model 7: p>0.05
Model 8 intercept only	366	3	Model 7 compared to model 8: p<0.05

4.7 Output of final lmer for variability of isolates and communities. The final model was warming + complexity + immigration with a random effect to account for spatial pseudoreplication.

Fixed effect / interaction	**Mean square error**	**Numerator degrees of freedom**	**Denominator degrees of freedom**	**F value**	**p value**	**Deviance explained (%)**
Warming treatment	1.27	1	130	1.72	>0.05	1.16
Immigration treatment	0.35	1	130	0.48	>0.05	0.32
Complexity	4.37	1	130	5.93	<0.05	4.00

4.8 Simplification of lmer of isolate variability. In all models compared a random effect was used to account for spatial autocorrelation that could be caused by blocking. ANOVAs were used ensure that simplification did not result in significant loss of explanatory power. Simplification indicated that model 5 is the minimum adequate model that best explains the divergence in community metabolism during the environmental change experiment, however, as I am interested in all the main effect they were retained and therefore model 2 is used as the final model.

Models compared	**AIC**	**Degrees of freedom**	**p value**
Model 1 warming X immigration	326	6	
Model 2 warming + immigration	326	5	Model 1 compared to Model 2: p>0.05
Model 3 warming	323	4	Model 2 compared to model 3: p>0.05
Model 4 immigration	327	4	Model 2 compared to model 4: p>0.05
Model 5 intercept only	325	3	Model 3 compared to model 5: p>0.05

4.9 Output of final lmer for variability of isolates. The final model was warming + immigration with a random effect to account for spatial pseudoreplication.

Fixed effect / interaction	**Mean square error**	**Numerator degrees of freedom**	**Denominator degrees of freedom**	**F value**	**p value**	**Deviance explained (%)**
Immigration treatment	0.34	1	113	0.41	>0.05	0.33
Warming treatment	2.19	1	113	2.62	>0.05	2.13

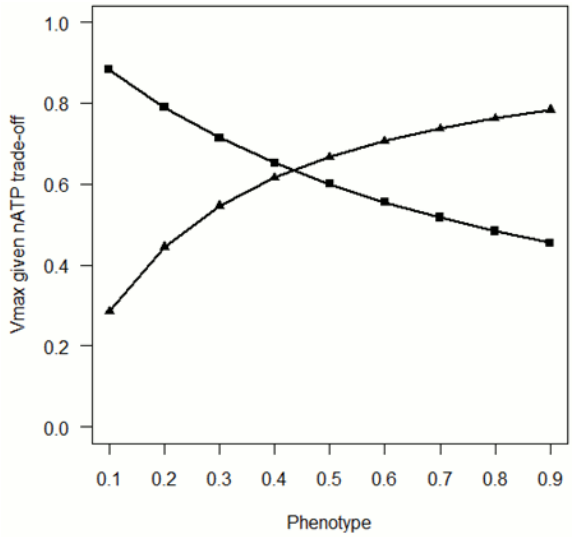
4.10 Simplification of lmer of synchrony of isolate responses to a change in environment. In all models compared a random effect was used to account for spatial autocorrelation that could be caused by blocking. ANOVAs were used ensure that simplification did not result in significant loss of explanatory power. Simplification indicated that model 1 is the minimum adequate model that best explains the deviation in responses of isolates from the same community to a change in environment.

Models compared	**AIC**	**Degrees of freedom**	**p value**
Model 1 warming X immigration	256	6	
Model 2 warming + immigration	276	5	Model 1 compared to Model 2: p>0.05
Model 3 warming	278	4	Model 2 compared to model 3: p>0.05
Model 4 immigration	278	4	Model 2 compared to model 4: p>0.05
Model 5 intercept only	279	3	Model 3 compared to model 5: p>0.05

4.11 Output of final lmer for synchrony of isolate responses to a change in environment. The minimum adequate model was warming X immigration with a random effect to account for spatial pseudoreplication.

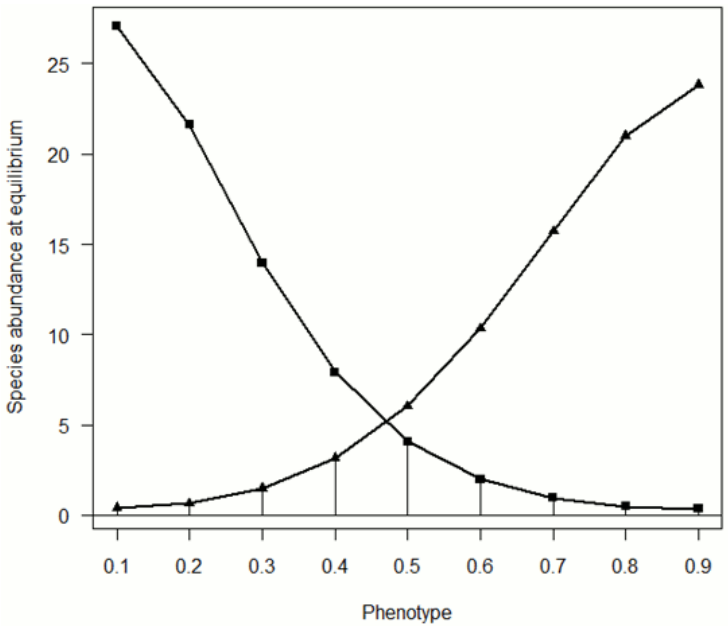
Fixed effect / interaction	**Mean square error**	**Numerator degrees of freedom**	**Denominator degrees of freedom**	**F value**	**p value**	**Deviance explained (%)**
Immigration treatment	1.92	1	113	4.29	<0.05	2.78
Warming treatment	1.96	1	113	4.39	<0.05	2.84
Immigration X warming	10.85	1	113	24.28	<0.001	15.74

5.1 ATP yield trade-off functions



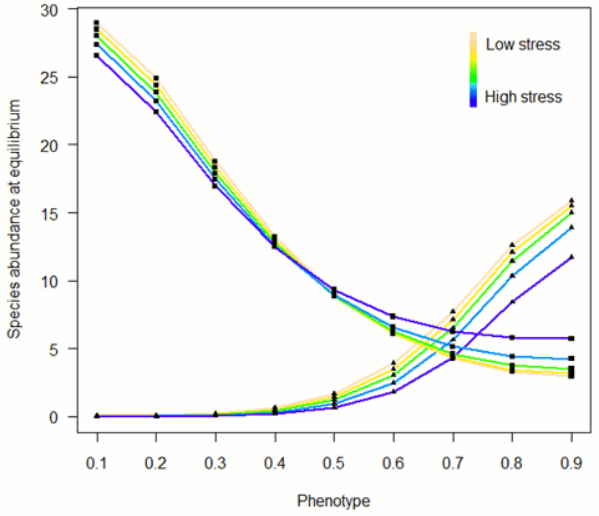
Appendix 5.1. ATP yield trade-off functions. The curves describe the V_{max1} of the phenotypes of each species. Triangular points represent Species 1 and square points Species 2. For example, individuals of Species 1 with a phenotype of 1 have a V_{max1} of approximately 0.3 and individuals with a phenotype of 9 have a V_{max1} of approximately 0.8. Hence, individuals of Species 1 with higher phenotypes gain greater energy from consumption of Resource 1. Due to these trade-off curves Species 1 typically evolves to specialise on Resource 1 and Species 2 on Resource 2.

5.2 Overlap in species' phenotypic distributions



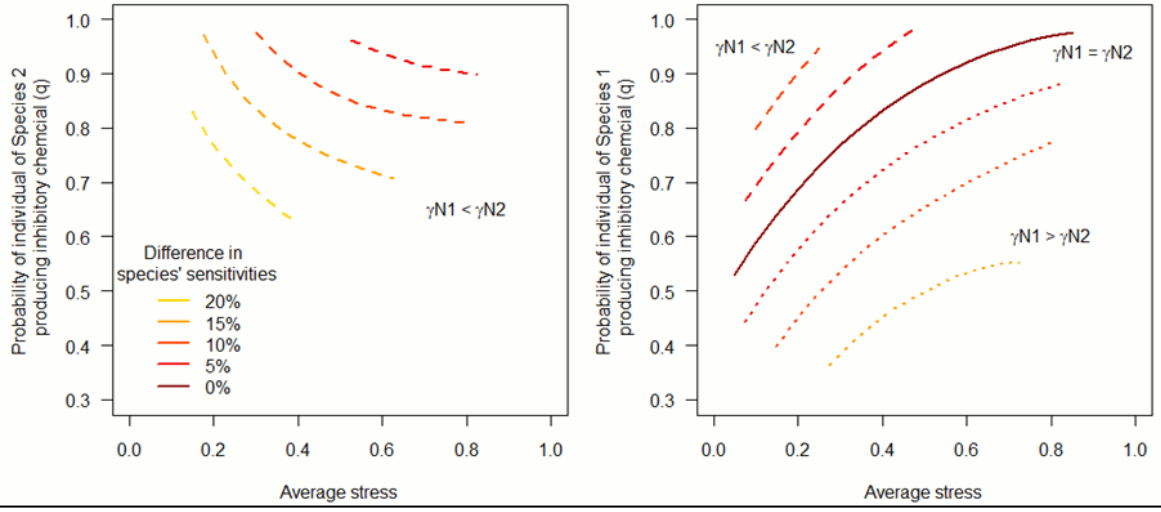
Appendix 5.2. Overlap in species' phenotypic distributions. Example of a typical distribution of phenotypes at equilibrium (in this case when both species have a tolerance to stress (γ) of 0.95). The vertical lines indicate the overlap in individuals of each phenotype and the sum of these was divided by the total abundance of the two species to produce the measure of overlap used in the analyses. Species 1 is represented by triangular points and Species 2 by square points.

5.3 Equilibrium phenotypic distributions when Species 2 is more stress-tolerant than Species 1



Appendix 5.3. Equilibrium phenotypic distributions when Species 2 is more stress-tolerant than Species 1. Each set of coloured lines represent the species' equilibrium phenotypic distributions in simulations in which Species 1 was 25% more sensitive to the stress than Species 2. Lines with triangular points represent Species 1 and those with square points Species 2. As abiotic stress increased Species 2 became more generalist and Species 2 declined in abundance leading to increased overlap of phenotypic distributions in more stressful scenarios.

5.4 Probability of production of allelopathic chemicals as stress intensifies



Appendix 5.4. The probability of the more abundant species in the community producing allelopathic chemicals as average stress intensifies. For both graphs the solid line represents simulations in which species have an equal tolerance to stress, the dotted lines when Species 1 is more tolerant to stress than Species 2 and the dashed lines when Species 2 is more tolerant to stress than Species 1. The gradient in colour from dark to light represents an increasing difference in species' tolerances to stress. **a)** The probability of an individual of Species 2 producing antimicrobials when it was more abundant than Species 1. The likelihood of production decreased as average stress increased due to communities becoming more uneven. **b)** The probability of an individual of Species 1 producing antimicrobials when it was more abundant than Species 2. As average stress intensified the probability of producing antimicrobials increased because the communities became more even.