

**Microbial invasion ecology: community-level effects of biological invasions in bacterial microcosm model systems**

Volume 1 of 1

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## Chapter 1

# Invasion Ecology: A variety of factors influence the success of biological invasions

### Introduction

Here, a brief history of environmental factors affecting the dynamics of biological invasions into ecological systems, and some of the experimental techniques used to investigate these, will be described. The potential efficacy of using laboratory microcosms to predict real-world ecological processes will be discussed, with a view to identifying the strengths and weaknesses in creating predictive models of the real world using laboratory systems.

Ecological studies have traditionally been focussed on field work and large common garden experiments, which are both time-consuming and expensive. The use of laboratory microcosms for the study of ecological processes has in the past been criticized as having “limited relevance” to real-world ecology (Carpenter, 1996), however strong counter-arguments have been made that microcosm experiments do in fact have direct relevance to ecological questions on a larger scale (Drenner & Mazumda, 1999; Jessup *et al.*, 2004; Benton *et al.*, 2007). Lending further credibility to the idea of using microbial microcosm experiments to answer broad ecological questions are bacterial microcosm studies that report results paralleled by traditional large-scale field studies; for example, species richness-habitat size relationship (Kohn & Walsh, 1994; Ricklefs & Lovette, 1999; Delgado-Baquerizo *et al.*, 2018), the unimodal relationship between habitat resource availability and plant community diversity (Theodose & Bowman, 1997; Bedford *et al.*, 1999; Kassen *et al.*, 2000) and synergy between propagule pressure and disturbance events during biological invasions (Eschtruth & Battles, 2009; Jones and Ramoneda, 2017). Given that different field systems often report conflicting results (Jeschke *et al.*, 2012), it is unlikely that any single microcosm experiment will be predictive of processes in all ecosystems, and it would be unreasonable to expect such.

Bacterial microcosm experiments offer a rapid, high-throughput method for testing hypotheses, thereby providing the basis for well-informed and targeted large-scale and long-term field studies. In addition to their relevance as models for macro-ecological processes, the direct study of microbial ecological systems is also important due to the crucial role of microbiota within real-world systems; the interplay between macro-organisms and their local microbiome can have profound impacts on the structure and function of these macro-communities (Hale *et al.*, 2006; Van Der Heijden *et al.*, 2008).

As much of the literature about broad ecological theory is based on studies conducted in the field, it is important that current understanding of real-world ecological and evolutionary processes is used to inform the design of laboratory microcosm experiments. As more of the results of real-world studies are replicated in microcosm systems the power of these systems to make rapid predictions about, and inform management of, real-world processes will increase. Here the focus will be on the causes and consequences of biological invasions, and the broad ecological processes that may contribute to invasion success or ecosystem resilience to these invasions.

The successful invasion of macrofauna and macroflora into non-native habitats has had devastating impacts on native ecosystems and economies in numerous cases; examples include the European rabbit (*Oryctolagus cuniculus*) worldwide (Smith & Boyer, 2008), the western corn rootworm, (*Diabrotica virgifera*) in Europe (Gray *et al.*, 2009; Blandino *et al.*, 2017), and Himalayan balsam, (*Impatiens glandulifera*) in central and northern Europe (Kollmann & Bañuelos, 2015).

Invasion ecology is influenced by biotic and abiotic factors in any given environment, and concepts linked to these are important at all biological scales and across all groups of organisms (Dillon *et al.*, 2005; Holle & Simberloff, 2005; Catford *et al.*, 2009). The mechanisms underlying the successful invasion of new habitats by non-native species are likely to vary between invasions, however a combination of basic mechanisms may be key to invasion success (Holle & Simberloff, 2005). Understanding the mechanisms underlying community resilience and species invasiveness is vital to future success in mitigating the impact of detrimental invasions, in the contexts of biological conservation, food security and epidemiology.

Here, previous literature regarding the ecology of invasions is discussed, with particular focus on the application of laboratory studies to compliment real-world studies. Various aspects determinant to invasion success, and their impacts will be investigated, and the potential to mitigate these impacts with properly informed policy will be considered.

### **Invader Traits**

As some species seem to be more successful at invading new habitats than other very similar, often closely related, species, there are likely to be specific traits that increase the chances of invasion success (Stuart *et al.*, 2014; Čuda *et al.*, 2015). Direct competitive invasion-interactions between ecologically and morphologically similar species have been observed widely in invaded environments (Ricciardi *et al.*, 1998; Stuart *et al.*, 2014; Carboni *et al.*, 2016; Mazzamuto *et al.*, 2017).

A key feature of a number of invasive organisms is an r-selected life history, able to rapidly reproduce and take advantage of niche openings within an ecosystem (Sakai *et al.*, 2001; McMahon, 2002). Examples of such r-selected species are the European rabbit (Smith & Boyer, 2008) and the western corn rootworm (Blandino *et al.*, 2017), which are economically important crop pests. These type of rapidly reproducing species can rapidly expand from a small founder population, and may be particularly able to take advantage of the opportunities for genetic diversification offered by successive multiple introductions to an invaded environment (Malacrida *et al.*, 2007). The potential for a species to quickly expand from a small founder population, and to adapt to novel environmental conditions via rapid evolution, may provide an advantage over K-selected species that will take longer to fill a potential niche, and may potentially fill a smaller niche-space due to their inability to rapidly adapt to new local conditions.

Similarly to the potential for rapid evolutionary adaptation, phenotypic plasticity may increase an organism's chance of success when invading a novel environment. For example, *O. cuniculus* has successfully colonised habitats across a wide temperature gradient (Flux, 1994, cited in Lees & Bell, 2008), the species' success in this regard is likely to be closely linked to developmental plasticity in thermoregulatory morphology (Williams & Moore, 1989). Similarly, the invasive dandelion *Taraxacum officinale* successfully responds to differing environmental light availability through plastic developmental responses in leaf morphology (Brock *et al.*, 2005). A study of German-origin brown trout (*Salmo trutta*) populations in Patagonia found plastic life history traits, migration and

spawning time, to be more important than genetic adaptation for the local success of the species in this novel environment (Valiente *et al.*, 2010).

Comparisons between introduced populations of the South American alligator weed (*Alternanthera philoxeroides*) in the USA and China found that genetic diversity of the Chinese populations was comparatively extremely low (Geng *et al.*, 2016). However, despite the lack of genetic diversity in the Chinese populations, high phenotypic plasticity has allowed the species to colonise environments with equal success as the considerably more diverse populations in the USA. Plastic phenotypes may be a particularly important trait during the early stages on an invasion, providing invaders with a rapid adaptive response to novel conditions, prior to subsequent evolutionary adaptation to local conditions (Si *et al.*, 2014; Zenni *et al.*, 2014). As with rapid evolution potentially found in r-selected species, the ability of phenotypically plastic organisms to quickly respond to the novel conditions presented by a new environment may allow them to quickly maximise their utilisation of available niche-space. Early success of an invader within a novel ecosystem may then allow selection processes to act upon the population, further increasing its ability to exploit and reproduce within the new niche, and potentially to expand the occupied niche-space within the new environment.

Generalism may also contribute to invasion success, through greater opportunity to invade an unoccupied ecological niche, or take advantage of mutualist interactions with members of the resident community, as can be the case with invasive plant or pollinator species (Olesen *et al.*, 2002; Emer *et al.*, 2016). This may be of particular importance in perturbed environments, where niche space is opened up by resident mortality or niche creation (Xiankai *et al.*, 2008; Bobbink *et al.*, 2010). The timing of a biological invasion may often be key to its success, discussed in more detail below under “Disturbance”, niche openings due to resident mortality provide the opportunity for a competitive invader to re-colonise a disturbed site more rapidly than native populations. This is of particular importance to generalist species, as their potential to utilise varied niche-opportunities will inevitably allow them to successfully take advantage of a wider range of disturbance-types to colonise an environment. This potential to respond to a more varied range of opportunities may make the timing of arrival of propagules less important to these generalist species, as they are able to quickly take advantage of niche-openings offered by varied cyclic and one-off disturbances, rather than requiring exactly the right environmental conditions to establish an initial population.

Similarity to native species may, in many cases, be a common trait of successful invaders; for example, a study conducted on a wide variety of invaders in French grassland systems found the most successful invaders shared many similarities with native species (Carboni *et al.*, 2016). Close similarities with resident species is intuitively an important trait for an invader, indicating a preadapted suitability for the novel environment. These kind of similarities are particularly striking in the case of well-known competitive interactions as the result of invasion events, such as between native red (*Callosciurus erythraeus*) and invasive grey (*Sciurus vulgaris*) squirrels in Europe (Mazzamuto *et al.*, 2017). Competition between ecologically similar populations is easily investigated in a laboratory environment; previous studies testing the effects of the advantage of greater metabolic generalism in *Vibrio cholera* (Almagro-Moreno & Boyd, 2009), and fitness-lifespan relationships in *Caenorhabditis elegans* (Jenkins *et al.*, 2004), have revealed large fitness disparity between two populations of the same species with relatively small ecological or life-history trait differences. As with the progression of adaptation to an environment due to natural selection within a species, relatively small differences in fitness between species will drive an expansion of the population of the fitter population at the expense of the less fit population. Particularly in the case of a species under low selection pressure in an environment with abundant resources, the sudden

arrival of a similar, but more suitably adapted species, may lead to rapid displacement of the native population.

Propagule pressure, or number of reproductively viable individuals invading an environment, is another concept important to biological invasions. A potential invader with an extremely low propagule pressure may fail to reach population sizes necessary to maintain a viable population within a novel environment (Lockwood *et al.*, 2009), whereas an extremely high propagule pressure may permit invasion of a diverse and invasion-resilient ecosystem (Holle & Simberloff, 2005). A bacterial microcosm study (Jones & Ramoneda, 2017) to investigate the importance of propagule pressure over time found it to be an important factor in successful establishment of an invader, particularly when a member of the resident community is a strong competitor for resources. As noted regarding generalist species, the potential for a species to take advantage of a wide variety of ecological niches will effectively increase any propagule pressure exerted upon an environment, as the invading population will be able to take opportunistic advantage of diverse opportunities presented by a range of disturbances or changes in local resource use by native populations.

A high adaptive potential of a species may increase its invasiveness towards novel environments, and may arise through a number of mechanisms. High propagule pressure on an environment from diverse sources can provide the raw material for adaptation to a new environment through intrinsic genetic variation. A study in Florida, USA, on the invasive brown anole (*Anolis sagrei*) found that the invasive population was more genetically diverse than the populations in their native ranges, due to successive invasions from multiple locations. They further suggested that the associated increase in genetic diversity promoted the species' invasiveness as it adapted to its new habitat (Kolbe *et al.*, 2004). Similar conclusions were drawn from another study in the USA on the invasive European wetland grass, *Phalaris arundinacea*, finding that high genetic diversity within the invasive populations was due to repeat invasions from diverse sources (Lavergne, 2007). Low diversity or absence of native biota can drive diversification of an invading species to fill available ecological niches; a laboratory study on the adaptation of the soil bacteria *Pseudomonas fluorescens* to a soil substrate found significantly greater diversification and adaptation to available ecological opportunities in the absence of the natural soil community, highlighting the importance of ecological opportunity and niche incumbency during these diversification events (Gómez & Buckling, 2013).

Many of the mechanisms discussed above highlight the importance of adaptability and resource-use flexibility for the success of an invader within a novel environment. Generalist life-history and resource-use traits, phenotypic plasticity, and rapid potential for evolutionary adaptation all provide a potential invader with a wider range of opportunities, across both space and time, than a potential competitor lacking these traits. As previously discussed, many successful invasive species are successful in a range of novel environments, and their successful invasion of these diverse habitats may highlight the importance of generalism and rapid adaptation as key traits for successful invaders.

### **Community Traits**

Pre-existing biotic interactions play a key role in the resilience of a community to invasion. A study, conducted in Cambridgeshire, UK, found that grazing by livestock reduced the coverage of native competitive plant species compared to ungrazed control plots, and was linked to an associated increase in the abundance of invasive *C. helmsii* (Dean *et al.*, 2015). Similarly, mathematical modelling of invader success at varying levels of resident community diversity and interaction has

predicted greater community resilience to invasion at higher diversities and closer inter-specific interactions (Case, 1990; Diez *et al.*, 2012).

Natural communities are constructed in relation to their available resources, peak diversity being achieved by successional and evolutionary processes leading to the occupation of available niche-space within an environment (Pigot & Tobias, 2013). The formation of diverse communities within an environment inevitably leads to a state of low available resources, whereby further colonisation of the environment is limited by the presence of niche-incumbent residents (Tilman, 2004). Invasion success is reliant on the establishment of the novel population within an environment, meaning there must be available resources for propagule pressure to translate into an established population (Tilman, 2004). As well as net resource availability, this will also be affected by “niche incumbency”, whereby a resident species may competitively exclude ecologically similar species, as has been found amongst populations of Hispanic anole lizards (*Anolis spp.*) (Algar *et al.*, 2013). A microcosm study, using the bacterium *Pseudomonas fluorescens* as a focal-invader species, found that although an invader can rapidly diversify to take advantage of niche opportunities in a complex soil environment, the presence of a diverse resident community constrains this adaptive radiation (Gomez & Buckling, 2013). As discussed in relation to propagule pressure, it is necessary for propagules to find available niche-space within an environment in order to establish a successful invasive population. Where an existing community is making extensive use of available resources, and potentially adapted to local cyclic disturbances, any potential invader may find it difficult to establish a sustainable population within the ecosystem.

High invasion capability does not necessarily equate with high success in all suitable environments, and “enemy release” may play an important role in the success of many biological invasions, whereby invasion of a new habitat allows a species to escape natural predators or parasites through geographic separation. *O. cuniculus* is an extremely successful invasive species with high fecundity, allowing rapid colonisation of new habitats and re-colonisation of disturbed areas. Despite their success as an invasive species, *O. cuniculus* is in decline in its native range and listed as “near-threatened” by the IUCN (Smith & Boyer, 2008). Anthropogenic mortality is a great factor in the species’ native decline; however human persecution has not had a similar effect in colonised habitats. Biotic interactions may be a key factor in this context, while *O. cuniculus* is the primary prey species for over 40 large vertebrate predators in its native range, many colonised habitats present a much reduced threat from predation (Jasic *et al.*, 1979; Lees & Bell, 2008). Without the pressure on *O. cuniculus* populations from predators, their ability to recover numbers after anthropogenic culling may be greatly increased.

A number of other plant and animal species are similarly successful as invaders but at threat in their native ranges; including Monterey pine, (*Pinus radiata*) (Farjon, 2013; Calviño-Cancela & van Etten, 2018), the purple pitcher plant (*Sarracenia purpurea*) (Schnell *et al.*, 2000; Franklin *et al.*, 2017), and the Himalayan Tahr (*Hemitragus jemlahicus*) (Bhatnagar & Lovari, 2008; Cruz *et al.*, 2017). Whether due to predator density, diversity, or another unrelated factor, identification of the ecological pressures and mechanisms underlying this phenomenon may help inform future control of biological invasions. In regards to competitive fitness of these populations, it may not be surprising that populations suffering from threat and vulnerability within their natural range are often successful invasive species. Whilst conditions in the novel environment are an important factor during a potential invasion, these species may be pre-adapted by the high selection pressure present upon them in their native range. Extreme selection pressures leading to ecological vulnerability in the native environment of an invading species may be an important determinant in the species’ potential for success when invading a novel environment. This mechanism of pre-adaptation has

been investigated in real-world invasive species, using comparisons of invasive populations in their native and invasive ranges that indicate high selection pressure in their native environment, and associated adaptations, can prime a species for invasive potential. Pre-adaptation for invasion success has been identified in comparisons of 14 congeneric pairs of invasive European plants (Schlaepfer *et al.*, 2010), and by a single-species analysis of phenotypic plasticity reaction norms in *I. glandulifera* (Elt, *et al.*, 2016). Further investigation of pre-adaptation to novel environmental factors in more diverse phyla will be essential to identify the importance of these processes during biological invasions. As with other aspects of invasion biology, microcosm experiments offer an opportunity to rapidly build predictive data regarding these processes, however an increased availability of parallel real-world data will be required in order to identify suitable predictive laboratory experiments for varying real-world scenarios.

As is the case with predators, disease has the potential to limit the expansion and maintenance of an invasive population, assuming the population is vulnerable to the disease, and that the disease has a negative impact upon the fitness of the population. Introduced globally as a biocontrol method against populations of the European rabbit (*O. cuniculus*), the myxoma virus initially proved successful in eradicating up to 99% of the individuals in infected populations; as a lesson for future similar biocontrol projects, this strong selection pressure rapidly led to the emergence of large myxomatosis-resistant populations of *O. cuniculus* (Ross & Sanders, 1984; Kerr *et al.*, 2004). This example highlights how a rapidly-adaptive, r-selected population can overcome the obstacle presented by a deadly disease. In this instance, the rapid reproduction of the invasive species allowed it to recover from almost extinction-level mortality, to re-establish itself with a highly adaptive disease-resistance now fixed within the population.

Many biological invasions may be facilitated by new mutualistic, or positive, interactions with species present in the new ecosystem (Richardson *et al.*, 2000; Traveset & Richardson, 2014). Invasion of pacific islands by the giant African land snail (*Achatina fulica*) has been found to be facilitated indirectly by preferential predation of potential competitors by ant supercolonies (Green *et al.*, 2011), whereas direct interactions between seed-dispersing ant species and invasive plants has been shown to facilitate the spread of the plant species (Prior *et al.*, 2015). Novel positive interactions via a passive, for example the non-preferential predation of *A. fulica*, or active, seed dispersal by native species, mechanism opens new, unoccupied niche-space for the invading species in the form of a “mutualist-capture”, acting similarly to enemy-release by opening new competitive advantages within a new environment. Further investigation of positive invader-native interspecific interactions will be key to understanding the importance of these interactions in contemporary and future biological invasions.

## **Disturbance**

For clarity, “disturbance” will be defined here as “any relatively discrete event in time that disrupts ecosystem, community, or population structure and changes resources, substrate availability, or physical environment” (Pickett & White, 1985). These events vary in their nature, being driven by abiotic or biotic factors, but usually result in a change in resident community structure (Banks, *et al.*, 2013) and often a reduction of resident population size (Rykiel Jr., 1985). Environmental disturbance events play an important role in governing community structure and function, and ecosystem chemical cycles at all ecological (Sousa, 1984; Pickett *et al.*, 1989) and biological scales (Chauvat *et al.*, 2003; Bekker & Taylor, 2010).



Major, but infrequent disturbance events including the end-Permian (Erwin, 1994) and the Cretaceous-Paleogene boundary (Schulte et al., 2010) mass extinction events, have been historically crucial influences on community structure at a global scale. Subsequent to these, and similar events, novel groups of organisms rapidly adapted and evolved to fill the niche space left open by the previous occupants (Raup, 1994; Kauffman & Harries, 1996; Botha & Smith, 2006; Brayard *et al.*, 2009; Fawcett *et al.*, 2009). These global-scale events demonstrated the importance of niche incumbency and the potential for rapid evolution and invasion by novel species when these niches are evacuated due to mass-mortality disturbance events.

Regular, punctuated disturbances can have a similar impact on invasion by opening new niches either via resident mortality, or changes in the environment, into which an invader can establish a population; for example, in New Zealand, the invasive herb *Hieracium lepidulum* has been found to rapidly colonise forest areas following canopy disturbance events, and the resulting increase in light and water availability in these areas (Spence *et al.*, 2010).

Continuous environmental perturbations do not fall within Pickett and White's (1985) definition of disturbance as a discrete event, or necessarily have the high mortality and rapid changes in population structure often associated with environmental disturbance. The effects of continuous perturbation on the colonisation and dispersal of invasive species do, however, share characteristics with those of discrete disturbance events and therefore merits attention. Continuous local disturbance can promote the spread or establishment of invasive species; the abundance and dispersal rate of *H. lepidulum* is higher in continuously perturbed creek-margin habitats than adjacent areas (Miller *et al.*, 2015). Similarly, a study of 21 focal exotic species in the H.J. Andrews Experimental Forest (Oregon, USA) found the highest frequency of species along in-use roads, followed by abandoned roads and streams (Parendes & Jones, 2000). Streams and roads present continuous, or extremely high frequency, physical perturbation of the environment, with low intensity and good opportunities for physical dispersal.

Propagule pressure from source populations of an invader is likely to play a key role in disturbance-mediated invasion events. The previously mentioned bacterial microcosm study by Jones and Ramoneda (2017) found propagule pressure to be an important factor in successful invasions, they further reported that the timing of propagule arrival is important, success being higher when a community is actively growing, and niche incumbency effects weak as a result (i.e. immediately following a mortality-inducing disturbance event). These results reflect the findings of an earlier field study into the relationship between canopy disturbance effects and propagule pressure of three invasive species in forest systems in Pennsylvania and New Jersey, USA (Eschtruth & Battles, 2009). This field study identified an increase in light availability, following disturbance, associated with high propagule pressure from the three invasive species to be predictive of successful spread of the species.

While the immediate succession subsequent of disturbance events is likely to be the key focus of studies on disturbance-population dynamics, it is also important to take into account long term successional process that will take place after such events. It has been suggested that focus on rapid-growing early-succession invaders in forest ecosystems has left late-successional, and potentially ecologically damaging, species relatively ignored by invasion-disturbance literature (Martin *et al.*, 2009).

Disturbance plays an important role in structuring of natural communities, and the application of or release from environmental stress by disturbance events can have profound impacts on these community structures. Anthropogenic impacts are causing novel mechanical and chemical disturbances at local scales, and anthropogenic influence on a global scale is causing chemical

disturbance, and changing the frequency and intensity of natural disturbance events (Banks *et al.*, 2013) via pollution and climate change. As these anthropogenic influences increase in magnitude over time it will be important to understand how ecological systems may respond to these changes and how environmentally detrimental impacts may be mitigated. Identification of the roles of mortality, dispersal, resource influx and invasive species propagule pressure during these events may prove crucial to informing management decisions for conservation, health and agricultural industries.

### **Abiotic Conditions**

Abiotic interactions between a community and its environment play an important role in community structure and, therefore, its resilience to invasion. O<sub>2</sub>, moisture, light and nutrient availability, in addition to pH and temperature, play an important role in governing the suitability of any environment for the successful establishment of a species. The invasive Argentine ant, *Linepithema humile*, is a successful invasive species in seasonally dry areas of Southern California (Menke & Holway, 2006); associated with anthropogenic moisture enrichment, the ants were found to withdraw from areas where human-mediated water was experimentally cut off from the environment. Similarly, rapid range-expansion of invasive, or competitive local species, is often observed after increases in favourable abiotic factors, for example, invasion of grasses into heathland (Heil & Diemont, 1983) and similar low-nutrient environments (Bobbink *et al.*, 2010) following nutrient influx, or establishment of herbaceous invaders in woodland environments following canopy disturbance, and the resulting increase in resource availability (Spence *et al.*, 2010).

Changes in abiotic chemical conditions can have dramatic consequences on an ecosystem's abiotic and biotic interactions. Resource-poor habitats will tend to favour stress-tolerant species, while resource-rich environments tend to favour highly competitive species. For example, the floral assemblage in a given habitat will be highly dependent on nutrient availability. *Calluna spp.* are stress-tolerant but not highly competitive, the genus tends to inhabit low-nutrient heathland areas, where highly competitive grass species are unable to dominate, however chemical disturbance via enrichment of the environment with nitrogen and phosphorous can lead to the rapid invasion and dominance of grass species (Heil & Diemont, 1983). These invasions of heathland by grass species can be largely attributed to these simple changes in abiotic conditions. The grasses are not "new invaders", as they were already propagating locally, however increased nutrient availability alters the relationship between plant communities and their environment. Similarly, reviews by Xiankai *et al.* (2008) and Bobbink *et al.* (2010) identify nitrogen deposition as a leading potential cause of changes to plant community structures, whereby fast-growing, competitive species may capitalise on improved nutrient availability at the expense of slower growing stress-tolerant species.

The availability of resources within an environment is a key link between all previously discussed mechanisms and processes involved in invasion biology. Without available resources, there is no niche-space for an invader to utilise within the novel environment, therefore even under conditions of high propagule pressure the potential to establish an invasive population will be extremely limited. Particularly in the case of autotrophic species, access, whether opened up via abiotic processes, for example nutrient influx, or biotic processes, such as native population mortality, to abiotic resources is essential for growth and reproduction.

## Impacts on Resident Communities

Biological invasions have long been recognised as an important process in community ecology, potentially driving rapid and extreme changes in both the biotic and abiotic components of affected ecosystems. These changes can be driven by direct competition for resources, predation or grazing, the spread of disease, or habitat modification.

Direct competition for resources between an invader and resident species may be particularly intense where species' ecological niche is closely aligned. A study on a pair of closely related balsam species, *Impatiens spp.*, found the longer growing season of the invasive Himalayan balsam, *Impatiens glandulifera*, allowed it to invade novel environments and outgrow competitors, providing an advantage in competition for light resources (Čuda *et al.*, 2015). Despite the otherwise closely aligned ecological niches of the species involved, this competitive advantage allows *I. glandulifera* to make better use of local abiotic resources, displacing less-competitive native members of the genre.

As discussed earlier, generalist species may often have greater ecological opportunity than specialists, due to their ability fill a wider range of ecological niches; the impact of generalists is likely to be greater than that of specialists as they will tend to have direct interactions with a large portion of the resident community. Invasive pollinators are likely to be in direct competition for resources with native pollinator species (Emer *et al.*, 2016); specialist invaders will generally compete with a low number of native specialists for a particular resource, whereas generalist invaders will compete both with other local generalist and specialist species for resources, potentially leading to greater impacts on the resident community.

As vectors or causative agents of disease, invasive species-related disease has had dramatic impacts upon resident populations. In Europe, the spread of squirrelpox virus by the disease-carrying invasive gray squirrel, *Sciurus carolinensis*, into resident red squirrel, *Sciurus vulgaris*, populations has been linked to declines in abundance of the native species (Sainsbury *et al.*, 2008; Chantry *et al.*, 2014). Similar findings or concerns have followed investigations into disease-spread by non-native invasive species in populations of animals, including crayfish (Capinha, *et al.*, 2013) and racoons (Beltrán-Beck, *et al.*, 2012), and plants,

As ecosystem engineers, invasive species can dramatically alter their host environment. Invasion of exotic European earthworm species into North American hardwood forests has directly impacted local nutrient cycling regimes and soil structure (Gundale *et al.*, 2005; Resner *et al.*, 2015). By engineering changes in these important soil properties and processes these invasive earthworms have impacted the soil fungal communities, leading to reductions in understory mycorrhizal-associated plant species and domination of these environments by previously rare non-mycorrhizal species (Hale *et al.*, 2006). Similarly effected changes in community composition have been documented where other ecosystem engineers have invaded ecosystems, including the removal of native New England littoral algae canopies by the invasive snail, *Littorina littorea*, through grazing and sediment removal (Bertness 1984); salt marsh expansion at the expense of open sand and mudflat habitats via stabilisation and compaction of sediments by common cordgrass, *Spartina anglica* (Gray & Benham, 1990; Van Wesenbeeck *et al.*, 2007), can lead to significant reductions in the total abundance and diversity of native mollusc and crustacean species (Hedge & Kriwoken, 2000). These examples show how the mechanical and chemical effects of an invasive species on the abiotic conditions within an ecosystem can have profound effects on the structure of the resident communities.

Although many biological invasions have a direct and obviously negative impact on native ecosystems, this is not always the case. The highly successful invasive plant *Crassula helmsii* has colonised large areas of the UK, but has been reported to have no significant impact on the diversity of plant communities in affected habitats in south-east England, however changes in resident floral community structure were associated with invasions (Smith & Buckley, 2015).

The impacts of an invasive species upon the resident community can be varied, however almost always impact the native community structure. Identification of the species most likely to be affected by the introduction of a particular invader may often be difficult, as the effect upon some species may be indirect, but nevertheless significant. A broad understanding of species-species interactions within both natural and invaded ecosystems, and the effects of disruption to these interactions, will be important to future prediction of the impacts of invasions.

### **Evolution and Ecology During Invasion Events**

Adaptation of an invasive species to environmental conditions in a novel environment can be key to the species' establishment. Any new environment will present some variance upon conditions previously experienced by a species and, where developmental plasticity cannot provide means to cope with these changes, selection pressure is likely to drive adaptation during successful invasions. The rapid spread of an invasive species is often preceded by a lag phase, subsequent to its introduction, this may be explained in some cases by simple ecological mechanisms relating to population size and reproductive capacity of the introduced population, however in many cases this lag phase may represent a period of adaptation to the new environment (Ellstrand & Schierenbeck, 2000; Clements & Ditommaso, 2011). In a number of cases, introduced populations have only begun a rapid advance within a new environment after multiple introductions from a variety of source populations, intra- or interspecific hybridisation providing the raw genetic diversity for selective pressure to act upon (Abbott, 1992; Roy *et al.*, 2015).

The hybrid species bohemian knotweed, *Fallopia × bohémica*, a cross between japanese knotweed, *F. japonica*, and giant knotweed, *F. sachalinensis*, which displays high genetic diversity across its invasive range in North America (Gillies *et al.*, 2016), is able to spread faster than either of the parent species, and is capable of viable sexual reproduction (Clements *et al.*, 2016). Rapid evolution seen in one causative agent of Dutch elm disease, *Ophiostoma novo-ulmi*, has led to increased resistance of the fungus to viral pathogens, as a result of increased genetic diversity via outcrossing with populations of the closely related *O. ulmi*, a less competitive causative agent of Dutch elm disease (Brasier, 2001).

These above are examples of rapid evolution due to hybridisation between populations, however selection pressure differentials between an invader's native and new environments can drive adaptive evolution within a population. European-invasive populations of *Impatiens glandulifera* of differing age were investigated for the strength of defence against invertebrate herbivores (Gruntman *et al.*, 2017). Older populations were found to have diminished chemical defences in comparison to more recently introduced populations, supporting the hypothesis that enemy release allows adaptive reallocation of resources away from defence and into growth. Similar results were published by Friman *et al.* (2013), whereby *Pseudomonas aeruginosa* populations isolated from cystic-fibrosis patients suffering long-term infection were more susceptible to infection by bacteriophage enemies than those isolated after recent infection; here enemy-release again

provides an environment in which defence and resistance mechanisms are not selected for, allowing adaptive allocation of resources to alternative fitness-enhancing traits.

These rapid evolutionary events will not always affect only the invading species. A study of anole lizards on Florida islands found the native *A. carolinensis* species evolved larger toe-pads over 20 generations as a result of inter-specific spatial competition with the invasive *A. sagrei*. These adaptive changes are due to a vertical shift in population habitat use to higher tree-perches as a result of interspecific competition. This study shows the potential for not only rapid adaptation of an invader to a new environment, but for the rapid adaptation of native species in response to selection pressures exerted by an invader (Stuart *et al.*, 2014).

The adaptation of species during invasion events presents an opportunity to study species' responses to environmental change, not only does this provide an opportunity to investigate questions relating of immediate importance to biological conservation and agricultural concern, but may provide insights into how species will adapt to global environmental changes as a result of anthropogenic impacts; understanding the processes by which species adapt to novel conditions may provide key answers to how negative impacts on global biodiversity may be mitigated as global warming, pollution and land-use changes continue to impact global ecosystems. Investigating these changes in the context of microbial microcosms can provide a laboratory model for community resource-use changes in response to competition from a novel invader.

### **Bacterial Community Ecology**

On a microbiological scale, the invasion or fixation of drug resistant bacterial communities in hospitals (Bradley *et al.*, 2017) or agricultural soils (Economou & Gousia, 2015; Sundin & Wang, 2018), and invasion by novel pathogenic microbial species are of direct concern to human health and food security.

As available agriculturally productive land is rapidly diminishing, due to increasing demand and degradation of existing agricultural space, efficient usage of available productive soils will become ever more important in the future (Lal, 2009; Popp *et al.*, 2013; Rulli *et al.*, 2013). Aside from the concerns regarding invasion of plant pathogenic bacteria in agricultural systems, yield-enhancing associations between crops and soil microbiota are of further importance to future food security (Sturz & Nowak, 2000). Microbial soil community members, including bacteria promote, nutrient mineralisation, increasing the efficiency of agricultural land use (Bender & van der Heijden 2015). In these contexts, it is important to understand how focal pathogenic species may invade and fix within a soil environment, and equally, what the effects of a novel species can be on the resident soil community, in order to inform management strategies for the prevention of plant pathogen spread and the promotion of yield-enhancing communities.

Investigation of the effects of native community composition and resource availability upon invasion success in a microcosm experiment (Mallon *et al.*, 2015b) has found resource availability to be key to the success of an invading focal species. Where a diverse resident community is established and maximising use of available niche-space, invasion success was found to be low; a low diversity community may fail to exploit available niches, leaving niche-space open to exploitation by a potential invader. In the same study, it was found that not only is resident interaction with existing niche-space important during invasion events, but that environmental disturbance in the form of nutrient influx has a similar effect to reductions in resident community diversity. Influx of resources

that cannot be fully exploited by the resident community effectively creates a niche which an invader may take advantage of to establish itself within the environment

A soil microcosm study on the effects of resource utilisation and historical disturbance similarly found exploitation of available resources to be predictive of invasion success. By using four distinct bacterial strains, differential ability to utilise resources was shown to affect the success of each in a consistent environment. Further, heat disturbance of the media and resident community prior to inoculation of the focal species reduced resident diversity and promoted invasion by the focal species (Ma *et al.*, 2015).

Soil bacterial communities are an important, and often overlooked, functional component of ecosystems worldwide, and vital to the health of soils used to feed a growing world population. Understanding the impacts of disruption to these communities, and what biotic and abiotic factors may contribute to such disruption, is essential for the maintenance of the ecological and agricultural systems they help to support.

## **Conclusion**

There are many factors to consider when investigating the causes and effects of biological invasions. As discussed above, the various processes linked to the success, or failure, of any given biological invasion are often overlapping, and can be difficult to distinguish. For example, disturbance is a mechanism whereby new resources will usually become available within a system either by making a previously unavailable resource available, or through mortality within populations previously utilising the resource. Simply increasing the abundance of a single abiotic resource may have a range of outcomes, including encouraging new interspecific interactions, new biotic-abiotic interactions, and abiotic-abiotic chemical or physical interactions that may affect the ecosystem as a whole.

Selection pressure in both the native and novel environments of an invasive species may play an important part in its success. Whether via pre-adaptation to the new environment as a result of conditions within its natural range, or via selection pressure for in-situ adaptation within the invasive range, understanding how and why some species are able to rapidly adapt, or are pre-adapted, to a novel environment is likely to be key to understanding the long-term success of contemporary and future established invasive populations.

With such complex, interrelated systems, it is important that studies identify points of variability and control for them appropriately, in order to properly investigate the role of a focal process. The various ecological processes considered above can all be controlled for within a laboratory setting, although experimenters should be careful that manipulation of one factor does not have unintended effects upon another potentially important controlled factor (for example, when investigating population density of a bacterium in relation to environmental pH, the population density itself may have a direct influence upon the experimentally manipulated pH via production of metabolites). The study of ecological processes in a laboratory environment allows fine control, and accurate measurement of both biotic and abiotic environmental factors and, particularly in the case of microbial microcosm studies, allows experimenters to take snapshots in time during their experiment by freezing samples of the microcosm, providing an accessible living-fossil record of the stages of invasion and succession within the experimental system.

Designing model systems that prove to be predictive of, and analogous to, real-world ecological systems and processes have the potential to offer a rapid diagnostic method for predicting

outcomes during time-sensitive real world situations. An understanding of how biological organisms interact with their abiotic and biotic environment is important when taking management decisions, for example during environmentally detrimental biological invasions, crop pest outbreaks, or human disease outbreaks. Laboratory systems have been used, to varying extents, to investigate questions relevant to real-world ecology, and often find congruent results. Future work with laboratory model systems will rely on availability of real-world data to validate the predictive power of these controlled systems, however, as a deeper understanding is gained of both laboratory and real-world systems the power of laboratory models to independently predict real-world outcomes is likely to increase.

The subsequent chapters will describe three experiments conducted to investigate the effects of some of the previously discussed diverse mechanisms governing biological invasions and the structuring of communities within their environments. While the investigation of the entire range of mechanisms discussed was not undertaken, consideration of these various factors was taken into account either by experimentally manipulating or controlling for them.

The first experiment investigated the impacts of population diversity of an invading bacterium on the structure of a resident soil microbial community. The author undertook the laboratory work for this experiment, with support from Elze Hess. Experimental design was developed with Elze Hess and Angus Buckling, with input from other colleagues. Daniel Padfield and Elze Hess led the final analysis of the resulting complex whole-community dataset. The results of the experiment were published separately in Padfield *et al.* (2020).

The second experiment was conducted to test the effects of adaptation to local disturbance regimes upon the resident community's resilience to invasion. The third experiment aimed to lay the foundations for a novel microbial community for use in future microbial microcosm studies.

## Chapter 2

# Genetic diversification determines the impact of rapid evolution on microbial community structure

### Introduction

It is known that the introduction of an invading species has an impact upon the resident community of an ecosystem, and that the presence of a resident community constrains the evolution of the invading species to fill new metabolic niches (Gomez & Buckling, 2013).

The introduction of *P. fluorescens* clones preadapted to the abiotic and biotic conditions of an environment has been shown to have a greater impact upon the resident community structure, compared to introduction of clones not adapted to these conditions (Gómez *et al.*, 2016). The effects of the fitness and the diversity of the invader on the structure of the resident community has not so far been investigated. Here the effects of adaptation to local biotic and abiotic conditions, the diversity of invading population, and impacts of sympatric or allopatric evolution of the members of the invading population were investigated. This study aims to investigate the effects of diversity verses the effects of fitness of the focal invading species during these events.

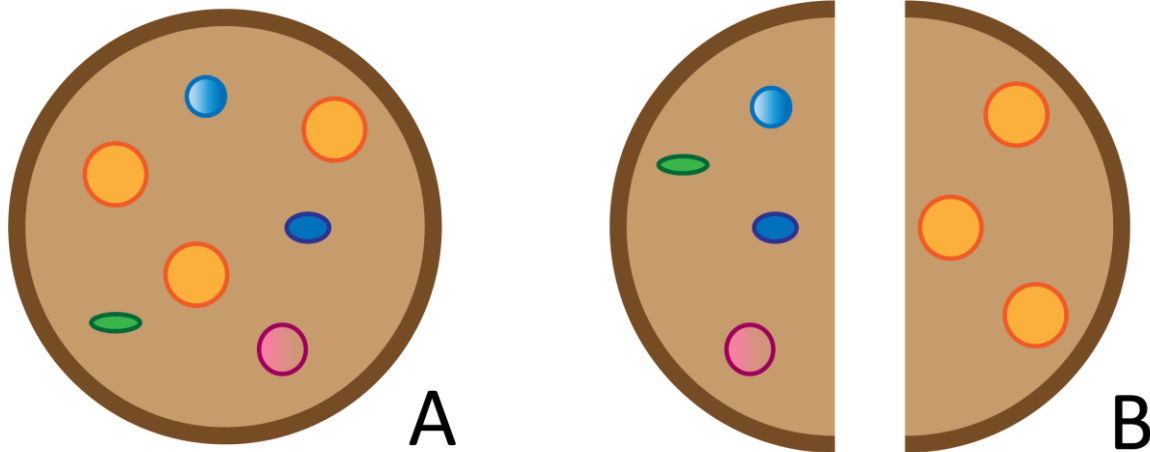
During previous studies on the diversification of bacterial populations conducted using *P. fluorescens* SWB25, which can rapidly diversify to adapt to available ecological niches (Rainey & Travisano, 1998), diversification of *P. fluorescens* was shown to be restricted by the presence of diverse niche-occupying communities (Gómez & Buckling, 2013). These results are in concordance with prior theory and studies on the adaptive radiation of species in relation to ecological opportunity (Van Valen, 1965; Gavrillets & Losos, 2009; Algar & Mahler, 2015; Stroud and Losos, 2016). Similarly, survival of the *Escherichia coli* as an invader in a soil microcosm experiment had an inverse relationship with the diversity of the resident community (van Elsas, 2012). In a pristine environment, lacking an existing community, the diversification of an invading species will be driven by intraspecific competition and niche availability, driving rapid diversification into open niche-space, particularly in a complex, niche-rich environment such as soil (Bolnick, 2001). Novel adaptations of this kind include the evolution of citric acid metabolism by *E. coli* in Lenski's "Long Term Evolutionary Experiment", whereby evolution of a novel metabolic trait occurred in response to ecological opportunity (Blount *et al.*, 2012).

The natural microbial community (NMC), here treated as the native, or "resident", community, and the physical and chemical environment (John Innes #2 compost) are consistent between treatments in this study, therefore maintaining a consistent community and ecosystem resilience to invasion. Population size, or propagule pressure, of the "invading species" (*P. fluorescens* SBW25, and experimentally evolved lines of the same) was also constant; therefore, propagule pressure was consistent between treatments.

As discussed in Chapter 1, diverse populations, and those pre-adapted to environmental conditions found in the host environment, are often successful as an invasive species. An "ancestral" *P. fluorescens* SBW25, and 24 experimentally evolved and phenotypically diverse *P. fluorescens* SBW25 clones (referred to here as "pre-adapted" populations) were selected as the focal species for this study. Preadapted populations had all been experimentally evolved in John Innes #2 compost prior to sampling, half of the populations sampled had undergone this pre-adaptation in isolation



(allopatric evolution), and half in the presence of a soil microbial community similar to the resident community used in this study (sympatric evolution) (see Figure 1 below). By introducing mixed populations of these clones, the diversity of the invading population was manipulated, whilst maintaining a constant “propagule pressure” between treatments.



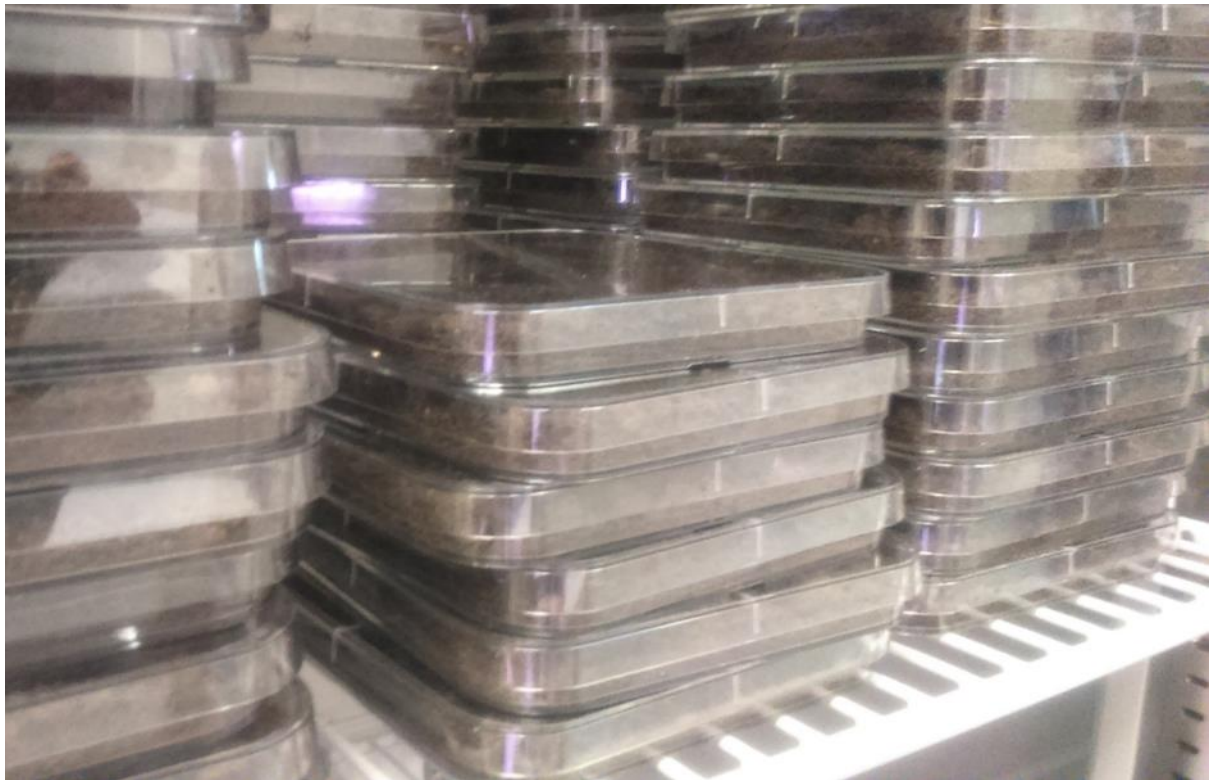
**Figure 1. Allopatric and Sympatric adaptation:** The concepts of allopatric and sympatric evolution are frequently referenced in regards to the reproductive isolation of populations, relevant here due to the potential for gene-flow between even distantly related bacteria. Here the pertinence of these differing types of evolutionary adaptation are in regards to the pre-adaptation (or lack of it) of the invading species for wider interspecific interactions with the resident community. A shows our focal species (solid orange circles) interacting and co-evolving in an environment with a diverse community (sympatric preadaptation); B shows our focal species in orange circles evolving in spatial isolation from a diverse community (allopatric preadaptation).

Starting at low population sizes, compared with the carrying capacity of the soil microcosm, this study is most analogous to a recently disturbed environment, with native and invasive populations competing to fill available niche space, and utilise abundant available resources. By manipulating only the community structure of invading populations, this study aims to identify the relative importance of invader diversity during invasion events, and the magnitude of impact of this diversity upon the resident community.

## Materials and Methods

### Microcosms (x102) – see Figure 2

- 10x10cm square petri dish
- 75g soil substrate (twice-autoclaved John Innes #2 compost)
- $1 \times 10^7$  Colony Forming Units (CFU) of invader (except NMC control microcosms)
- Resident NMC (50 $\mu$ l of soil wash in 1950 $\mu$ l M9(1x))
- Nystatin (prevention of fungal growth)
- 28°C humidified incubator
- 8ml sterile H<sub>2</sub>O (more as required to maintain soil moisture throughout experiment)



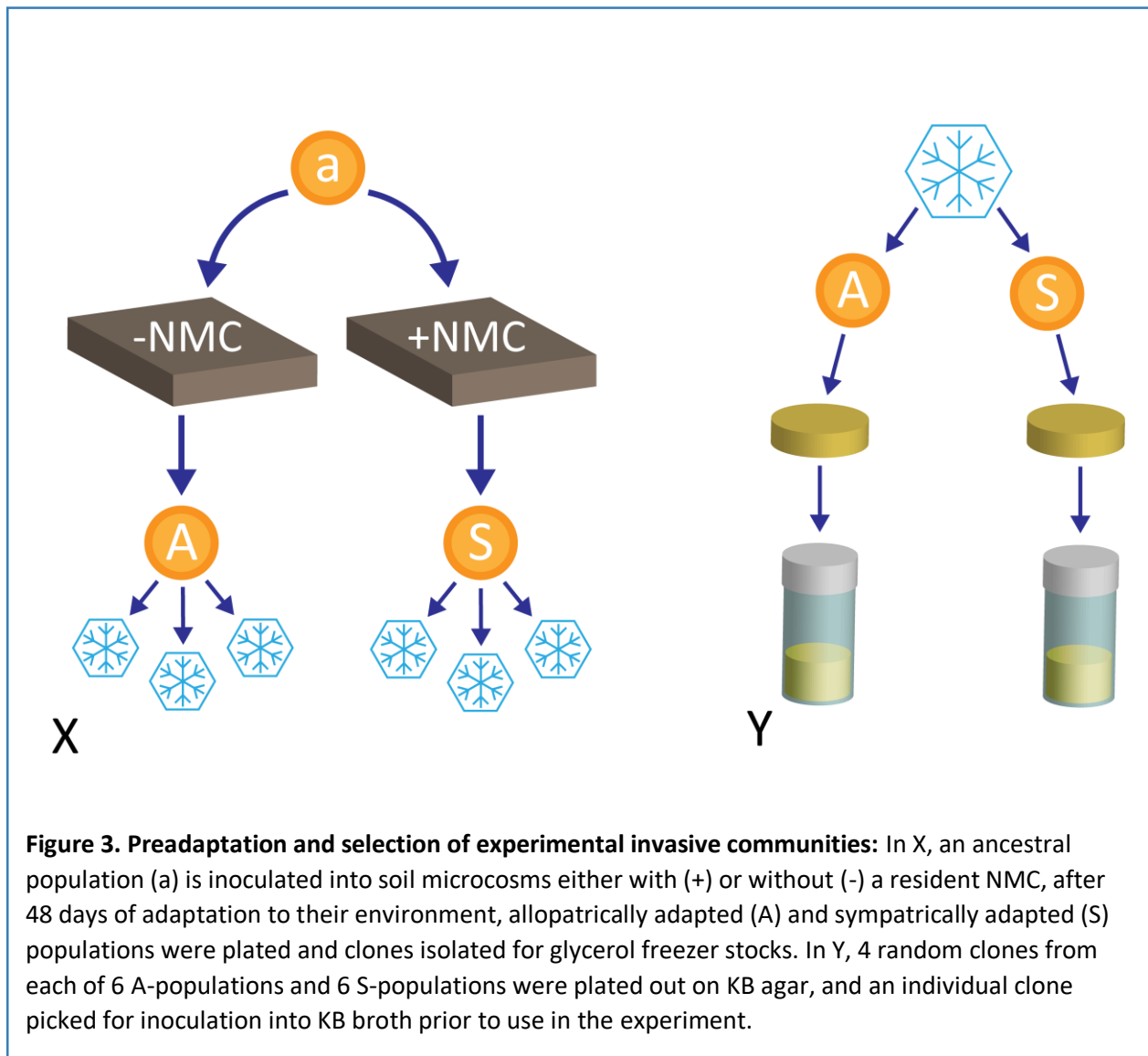
**Figure 2.** Experimental microcosms containing 75g of John Innes No.2 compost and microbial communities.

### NMC (resident community)

The soil NMC of John Innes #2 was sampled by soil-wash, a mix of 40g compost and 200ml M9(1x) in an Erlenmeyer flask. The soil-wash was shaken at 28°C for 24 hr prior to being sampled for immediate inoculation to the microcosms, and freezing with glycerol at -70°C.

### Invader Treatments (102)

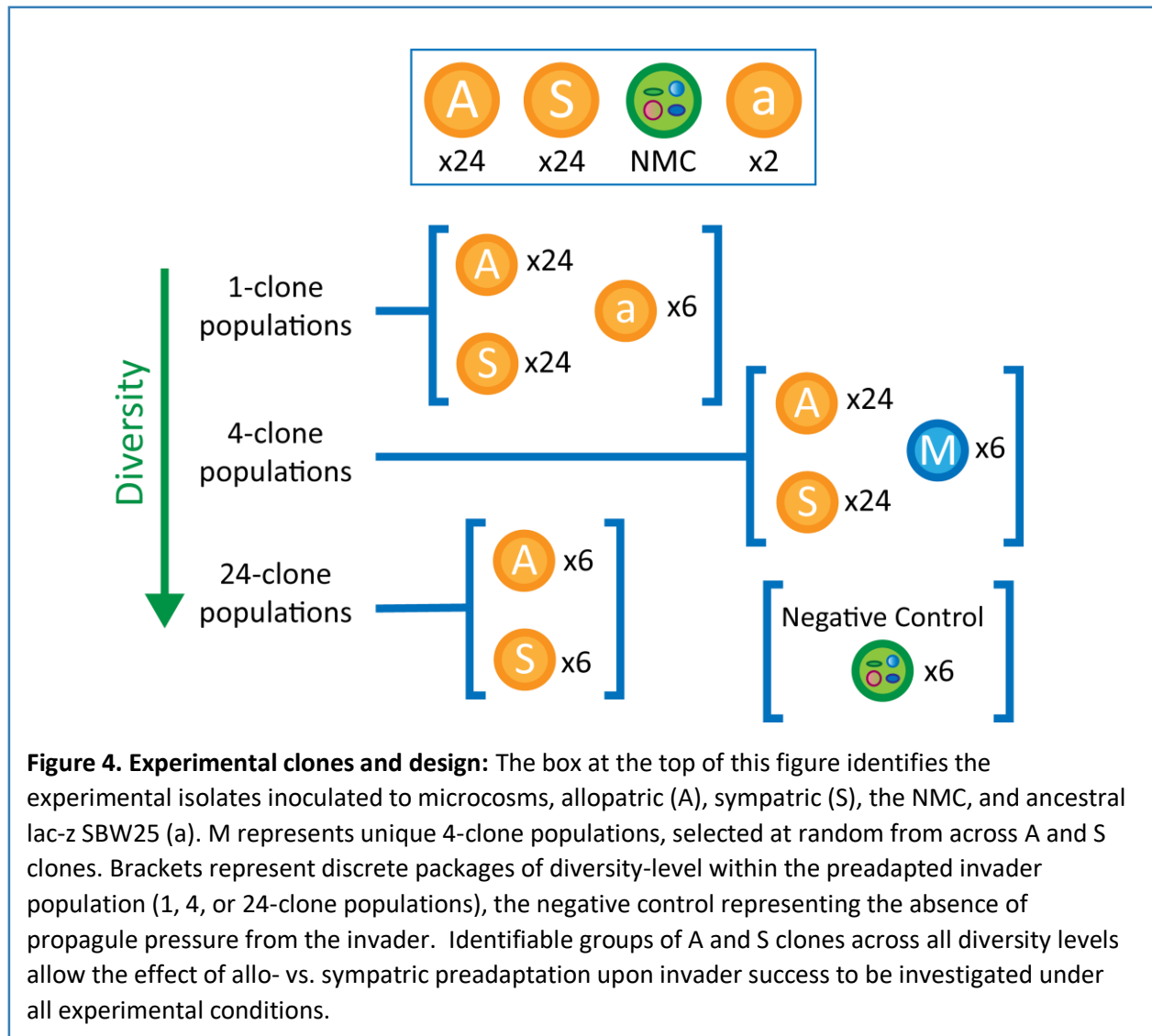
Invader populations were sampled as individual clones from existing stock of experimentally evolved *P. fluorescens* SBW25, and treatments built from these individual clones. 24 clones were sampled from allopathically evolved populations, 4 clones from each of 6 populations; clones were grown in standard LB broth from freezer stocks, then plated out and picked from a single colony to grow in LB broth at 28°C. 24 sympatrically evolved clones, and 2 ancestral *P. fluorescens* SBW25 clones were sampled using the same method (Figure 3).



Prior to inoculation to microcosms, all populations were plated out for density counting, cultures were maintained at 5°C for 24 hours during this process. Experimental populations were then constructed as below:

- Sympatric populations (6 populations of 4 co-evolved clones)
- Allopatric populations (6 populations of 4 co-evolved clones)
- NMC Control (NMC only, 6 replicates)
- Mixed (randomised) populations (12 populations of 4 clones, random selection of 1 clone from different preadapted populations)
- Mixed allopatric populations (2 (4-clone) populations, 6 replicates)
- Mixed sympatric populations (2 (4-clone) populations, 6 replicates)
- Individual clones (48 populations)
- Ancestral controls (SBW25, 2 clones (lac-z and non-lac-z), 6 replicates)

## Experimental Design



Experimental groups were formed from the 48 preadapted clones into populations of 1 (individual clones), 4 and 24 (Figure 4). The groups of 4 clones consisted of the sub-populations taken from freezer stocks, and of “mixed sub-populations” randomly selected to create artificial sub-populations that had all undergone allopatric preadaptation. 2 populations of 24 clones consisted of the entire experimental group of either allopatrically or sympatrically preadapted clones.

The individual clone treatments were used to test for the effects of fitness on the structure of the resident community, while the experimental groupings of different numbers of clones provide a gradient of diversity to test for the effects of invader diversity upon the resident community. Additionally, both lac-z marked and unmarked populations of the ancestral SBW25 clone were assayed for their effects on the resident biota. The negative control group consisted of microcosms containing only the resident community.

Assays were conducted in 90×90mm square petri-dishes containing 75g of compost. All media was treated with nystatin to limit fungal growth during the 6-week experimental period. Experimental microcosms were incubated at 28°C and 80% humidity. 8ml of sterile H<sub>2</sub>O was added to each microcosm prior to incubation, and as needed to maintain soil moisture during the experimental period. Each microcosm was inoculated with 1×10<sup>7</sup> CFU of *P. fluorescens*, and 50μl of soil wash supernatant, in M9 buffer to a total of 2ml.

### **Fitness Assaying**

All clones were assayed for fitness using a 6-day competitive fitness assay against the unmarked ancestral SBW25 *P. fluorescens* ancestral strain. Fitness assays were conducted in 30ml glass universals containing 3g of compost. 1.5 × 10<sup>6</sup> cells of each the preadapted and ancestral clones were added to the compost and were incubated at 28°C for 6 days. Clones were then recovered as a soil-wash and plated onto King's B (KB) agar plates containing x-gal in order to score densities of the populations at the end of the assay.

### **Metabolic Assaying**

Metabolic diversity of the preadapted and ancestral strains was conducted using GN2 MicroPlates (Biolog Inc., Hayward, CA), this system allows indirect measurement of metabolic activity on a range of substrates via optical-density (OD) measurements of tetrazolium violet dye response to redox reactions within the wells. The following protocol was followed for preparation of the GN2 Microplates:

Day 1 – Strains were inoculated and shake-incubated overnight in 6ml of KB broth at 28°C.

Day 2 – Populations were sampled and diluted 100x, then shake-incubated overnight at 28°C in a 96-well plate, 150μl per well.

Day 3 – A new 96-well plate was filled with 200 μl M9 (1x) per well and inoculated from the culture-containing plates using a 96-well plate comb. These plates were then static-incubated at 28°C for 2 hours, before transfer of 150μl of M9-culture mix to the GN2 MicroPlates. MicroPlates were then incubated statically at 28°C for 24 hours.

Day 4 – 24-hour experimental measurements were taken using a 96-well plate-reader at 590nm wavelength.

Day 5 – 48- hour experimental measurements were taken using a 96-well plate-reader at 590nm wavelength.

Of 50 total plates assayed (48 preadapted clones, 1 SBW25, and 1 control), 3 were discarded due to failure of metabolism. Otherwise, OD<sub>590</sub> readings were normalised by the OD<sub>590</sub> of the control well on each plate, and analysed in R (v3.5.1) (Team 2013).

In analysis, variation in resource use was identified as phenotypic variation ( $V_P$ ).  $V_P$  was divided into genetic variation ( $V_E$ ), environmental variation ( $V_E$ ), and genotype x environment variation ( $V_G$ ). Differences in  $V_P$ ,  $V_G$ , and  $V_{GE}$  between the preadapted and ancestral clones may then be used to infer successful diversification of resource use in the preadapted clones.  $V_P$  was calculated as the

average Euclidian distance between all clones after Hall & Colegrave (2007),  $V_G$  as the mean variance of OD<sub>590</sub> measurements between clones on an individual substrate after Venail, *et al.* (2008), and  $V_E$  as the mean variance of OD<sub>590</sub> measurements of individual clones across all substrates.  $V_{GE}$  was calculated and split into responsiveness (R), a measure of environmental variance indicating differences between generalist and specialist clones, and inconsistency (I), a measure of non-correlation between the metabolic activity of clones.

$$R = \frac{(i - j)^2}{2G(G-1)}$$

where  $G$  is the number of genotypes tested within a population and  $i$  and  $j$  are the standard deviations of each genotype tested.

The inconsistency component,  $I$ , indicates non-correlations between genotypes over environments:

$$I = \frac{\sum_{i,j} (1 - p_{ij})}{G(G-1)}$$

where  $p_{ij}$  is the correlation of performance across substrates between each pair of genotypes.

A lack of correlation implies that genotypes have adapted to utilise different resources, so that inconsistency is a measure of niche differentiation and the evolution of diversity within populations. As there was only a single replicate of the ancestral population, a Mann-Whitney test was used to test the null hypothesis that there is no difference in variation between preadapted and ancestral populations in regards to  $V_P$ ,  $V_G$ , and  $V_{GE}$ .

### Focal Species Density Analysis

The final density of *P. fluorescens* across treatments was analysed using linear models. The data was first log<sub>10</sub> transformed and one replicate was removed where no *P. fluorescens* was cultured (individual clone, allopatric preadaptation). The impact of preadaptation history upon final density of the focal species was investigated by splitting the data into levels of diversity (individual clones, 4 clones and 24 clones) where all clones were either pre-adapted with or without the natural community.

Separate linear models were run on each subset of the data, with log<sub>10</sub> abundance g<sup>-1</sup> soil as the response and pre-adaptation history as the predictor. Model selection was done using likelihood ratio tests and the *fdr* method was used to adjust p values.

The effect of diversity upon *P. fluorescens* density was investigated by pooling the different pre-adaptation history treatments at each level of diversity: LacZ ancestor (n=6), single clone (n=48), four clones (n=12), and 24 clones (n=12). Differences in abundance between treatments were tested for using a linear model. Model selection was done as above and comparisons were done between individual treatments using the R package 'emmeans' (Lenth 2018). Despite the unbalanced sample sizes between treatments, the variance was not higher in treatments with more samples, and the use of standard linear models is relevant to the data.

## Community DNA Sequencing and Analysis

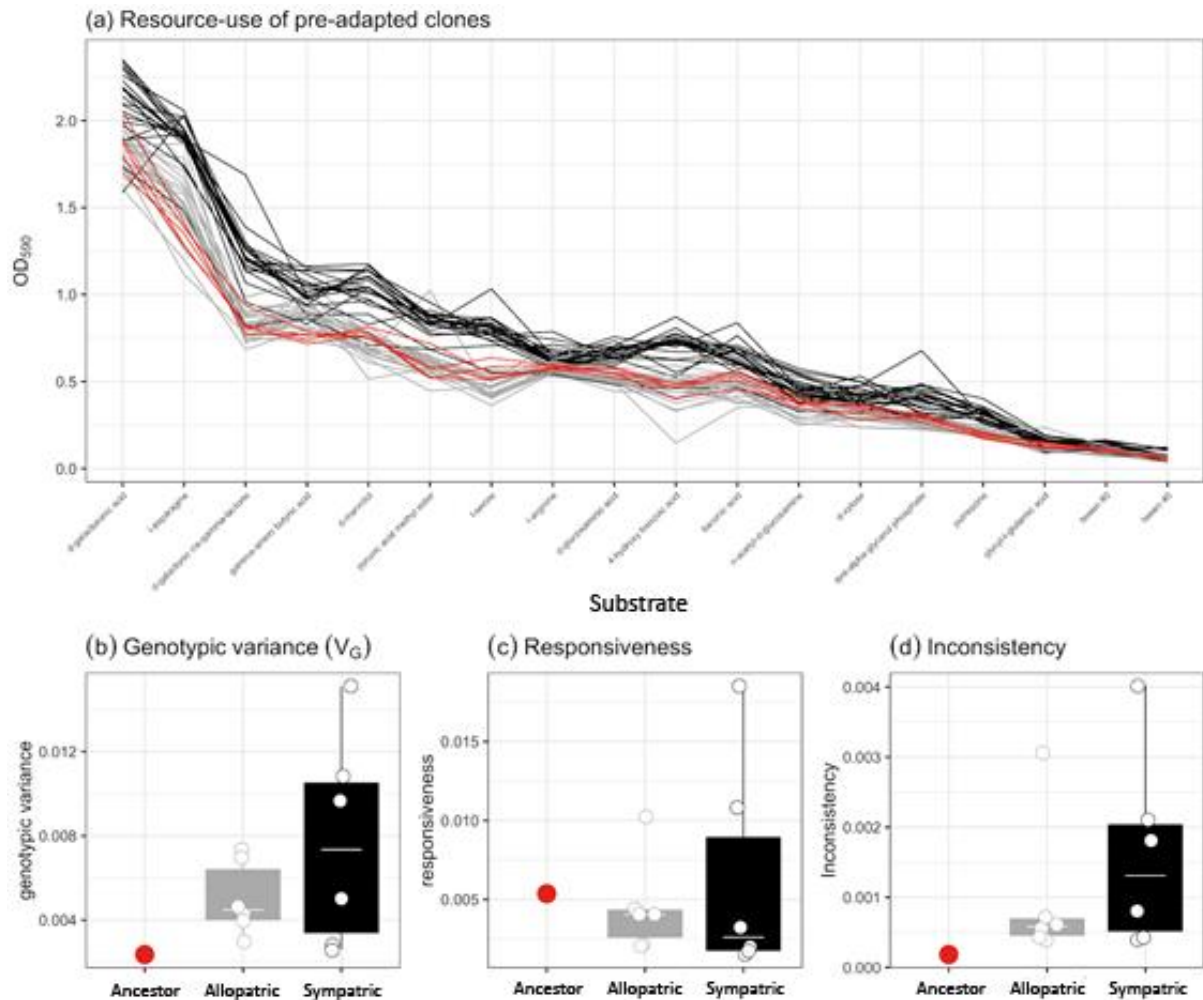
Analysis focussed upon dissimilarity indices to identify structural differences between resident communities. Phylogenetic analysis was undertaken on abundance of amplicon sequence variant (ASV) DNA markers, and results are reported as weighted Unifrac distance. Comparison of community structures was conducted using the “phyloseq” and “vegan” R packages. Permutational ANOVAS were run using vegan’s ‘adonis’ function.

## Results

### Metabolic Assay

Preadapted clones, both allopatrically and sympatrically evolved, were found to have a significantly greater metabolic diversity than the lac-z marked *P. fluorescens* SBW25 ancestral clone, as measured by  $V_P$  and  $V_G$  (Wilcoxon rank sum:  $p=0.0006$ ), however no significant differences in metabolic diversity were found between, or within, allopatric and sympatric preadapted populations.





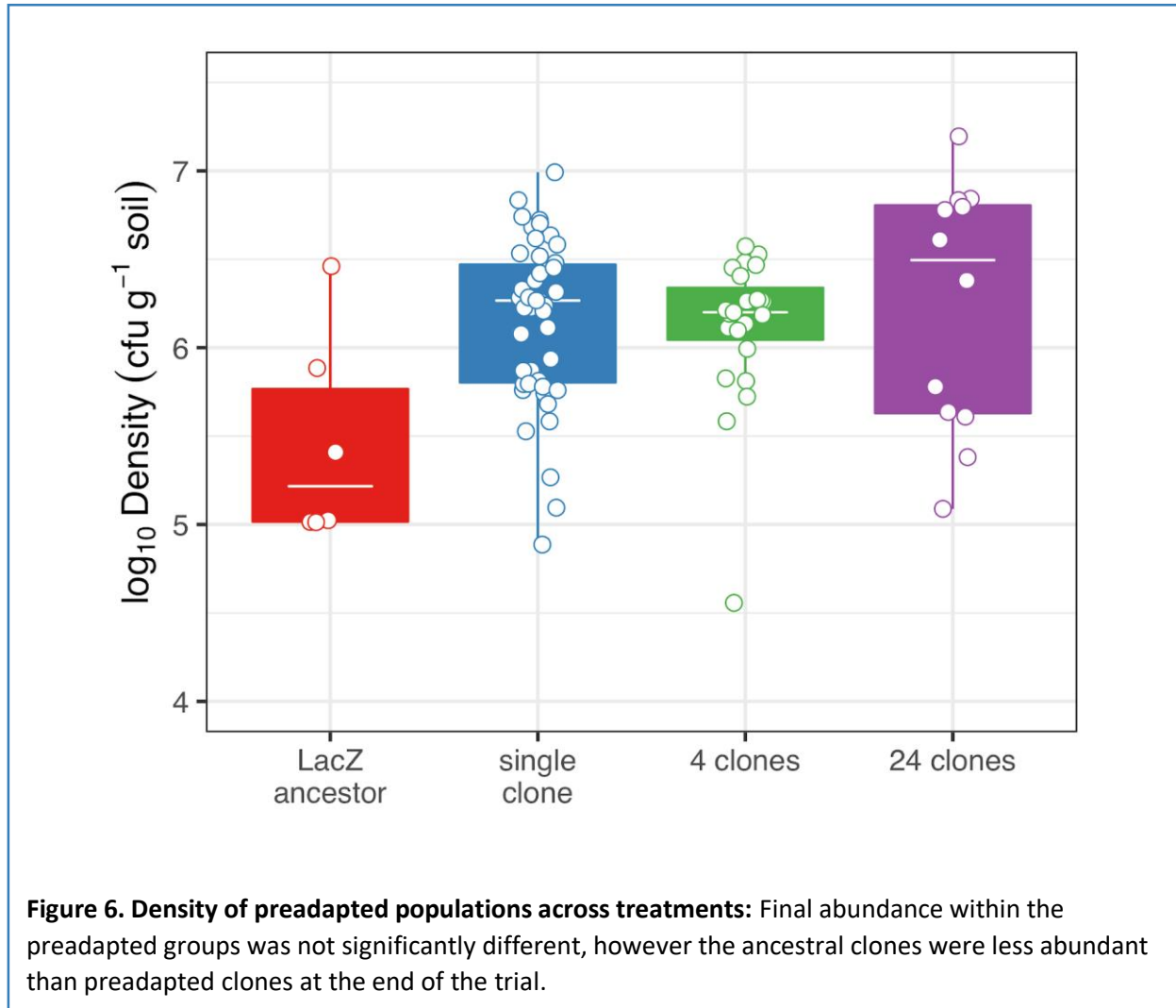
**Figure 5. Comparisons in resource-utilisation between ancestral, and allopatrically and sympatrically preadapted populations:** (a) shows the top 18 GN2 Microplate substrates ranked by mean utilisation (■ = ancestral clones, ■ = allopatric clones, ■ = sympatric clones). (b) allopatric clones displayed higher  $V_G$  than sympatric clones. (c) no differences in responsiveness ( $R$ ) were found between groups. (d) preadapted populations displayed higher resource specialisation, or inconsistency ( $I$ ), than ancestral populations, allopatrically preadapted populations displayed the highest  $I$ . In panels b-d, tops and bottoms of bars represent the 75<sup>th</sup> and 25<sup>th</sup> percentiles of the data, the white lines show the medians, and the whiskers extend from their respective hinge to the smallest or largest value, no further than 1.5x interquartile range, points represent the mean value per population.

Analysis of the  $V_{GE}$  component found no differences in  $R$  (generalism vs. specialism) between preadapted species, or between the preadapted populations and the ancestral population (Wilcoxon rank sum:  $p=0.677$ ), and while no significant difference was found between allo- and sympatrically preadapted populations, all preadapted populations had adapted to specialise on specific substrates when compared with the ancestral population (Wilcoxon rank sum:  $p=0.0006$ ) (Figure 5).

### Invader Population Density

Allopatric and sympatric preadaptation had no significant effect upon final abundance (at 48 days) of *P. fluorescens* across all levels of diversity ( $p>0.05$ ). Diversity, however, altered the final abundance

of the focal species in the microcosms (ANOVA comparing models with and without diversity:  $F_{3,84} = 3.78$ ,  $p = 0.013$ ), however this apparent variation was found to be driven by relatively low abundance of the ancestral strain and no significant differences were found when comparing preadapted populations (Figure 6).

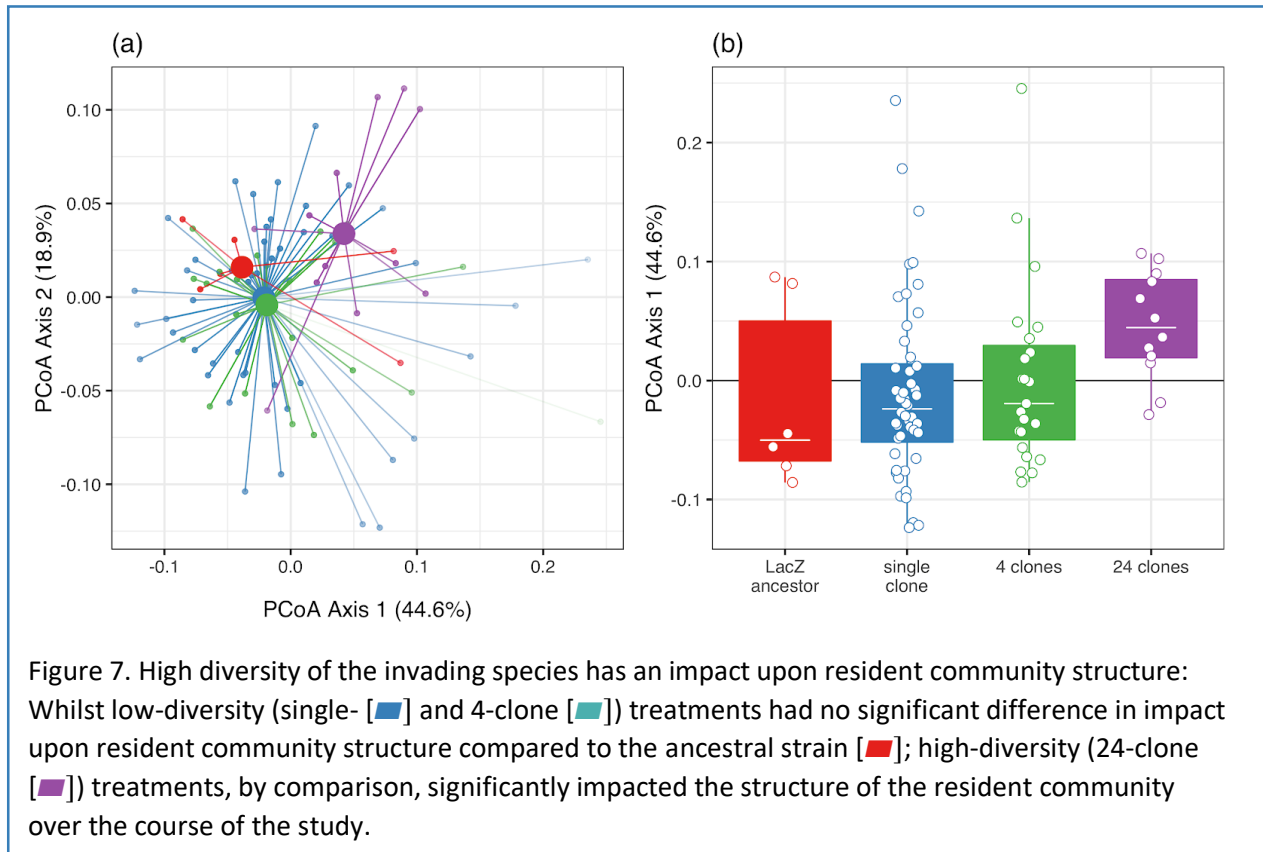


### Impacts of the Presence of Preadapted Populations Upon Resident Communities

There was no effect of pre-adaptation history (allo- or sympatrically evolved) on resident community composition (based on weighted Unifrac distances) across all levels of focal species diversity, from an individual to 24 clones. Investigation of the difference in impact between invader populations that co-adapted with those from different populations found no significant impact (PERMANOVA:  $F_{1,21} = 0.6$ ,  $R^2 = 0.03$ ,  $p = 0.63$ ).

Diversity significantly altered community composition (PERMANOVA,  $F_{3,85}=2.30$ .  $R^2=0.08$ ,  $p=0.01$ ). The first principal coordinate explained 44.6% of the total variation and partially separated the highest diversity treatment (24 clones) from the other levels of diversity; 83% of communities that

contained 24 clones of the focal species mapped to a positive axis 1 score, compared to an average of 31% of single clone samples, 44% of four clone samples and 33% of samples inoculated with the lac-z ancestor. To determine whether this separation was significant, we ran the multiple pairwise permutational ANOVAs. Resident communities that were inoculated with the highest diversity treatment of the invader species, 24 clones, had a different composition than communities grown with a single preadapted clone (PERMANOVA:  $R^2 = 0.08$ ,  $p=0.01$ ), four preadapted clones (PERMANOVA:  $R^2 = 0.12$ ,  $p_{adj} = 0.008$ ), and the LacZ ancestral population (PERMANOVA:  $R^2=0.17$ ,  $p=0.036$ ). None of the contrasts between any of the other diversity treatments were significant (PERMANOVAs: all  $p>0.05$ ) (Figure 7).



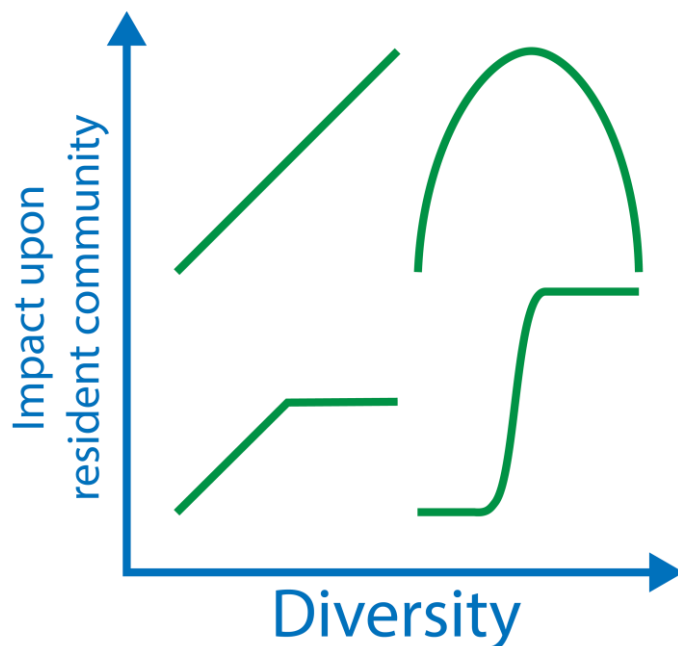
## Conclusion and Discussion

A group of *P. fluorescens* populations experimentally evolved in a soil substrate was found to have diversified significantly from the ancestral laboratory SBW25 strain in regards to resource-use. This finding confirms that the expected diversification of a species within a niche-rich environment holds true. Greater differences may have been expected between the allopatric and sympatric evolution treatments, however the confirmation of genetically fixed phenotypic traits as the result of a 48-day laboratory microcosm experiment is promising for the future study of evolutionary ecology on short timescales within a laboratory environment.

Diversity was found to be the only important factor in structuring the NMC during this study, with significant differences in NMC structure across invader diversity-levels. Diversity within the invading populations was found to be more important than individual adaptations to the environment.

These results corroborate previous field-work investigating the impact of diversity within invading populations discussed in Chapter 1. High levels of diversity have previously been associated with invasion success (Kolbe et al., 2004; Lavergne, 2007; Abbott, 1992; Roy *et al.*, 2015; Clements et al., 2016; Brasier, 2001), providing both the variation within a population to exploit niche-opportunity, and for natural selection to act upon. Whilst it is clear that diversity can affect the impact of a biological invasion in both real-world and laboratory microcosm contexts, the mechanisms involved remain unclear. Further study of the underlying mechanisms behind the impacts of invader diversity may reveal a benefit from raw diversity in terms of niche-acquisition potential, or the potential for a species to specialise subsequent to invasion as a result of selection for an optimal genotype from within the population. Regardless of future findings, it is clear that high diversity is a potentially important factor in the successful establishment and maintenance of invasive populations; when considering control strategies for the control of an outbreak, whether ecologically damaging invasive species or agricultural pathogen, restriction of mixing between populations is clearly important. In the case of an ecologically damaging invasive species, once introduced, measures should be put in place to limit the introduction of related populations. In the case of agriculturally important pathogens, measures including sanitisation and avoidance of movement of equipment between contaminated sites may prevent increases in diversity of populations.

In regards to the impact of increasing diversity upon the resident population in this study, the question is the open as to whether this relationship is linear. Future work may examine the relationship between invader diversity and community structure impact in light of a possible “peak diversity”, whereby increases in resident community impacts may potentially reach a maximum, or decline in relation to invasive population diversity. Whether there is a broadly applicable pattern to this relationship, e.g. analogous to the classic bacterial growth curve, will require future work focussed upon a broader range of invader diversity.



**Figure 8. Increasing diversity of an invading population may not have a linear impact upon resident community structure:** Diversity of an invader may linearly impact the structure of a resident community, however biological and ecological relationships are often not this simple. The relationship may prove to follow a number of models, and potentially more than one under differing conditions. The curves depicted against the axes above illustrate some of the possible relationships between invader diversity and the impact of the invading population upon resident community structure.

The easy manipulation of these environments allows the investigation of many important facets of invasion ecology, some of which have already been investigated, including resource availability and disturbance (Ma *et al.*, 2015, Mallon *et al.*, 2015b), and enemy-release effects using a bacteriophage as a predator (Gomez & Buckling, 2013). Simple manipulations to these small laboratory environments are analogous to large-scale real-world events, due to the large populations, relative size of the microcosm to individual bacterium, and long timescales as measured in generations (Jessup *et al.*, 2004).

Further work using similar systems may benefit from preliminary experiments using simplified media and communities, in order to identify fine-scale effects of the invasive species upon the resident community. For example, due to the complexity of the NMC used in this experiment, it is not possible to identify possible adaptive evolution of members of the NMC in response to the invasion of the focal species. Nor is it possible to properly quantify the level of resource use by the NMC in relation to available niche-space. Use of simplified soil media and synthetic communities for this type of experiment may yield answers to questions that investigations using natural media and communities may find difficult to untangle, with contemporary knowledge of these systems. That said, the soil microcosm system has the proven potential to rapidly answer important questions relating to evolutionary ecology in real-time.

While experimentation under standard laboratory conditions provides an environment for fast growth of species of interest, it may also have important impacts on natural communities that are not adapted to such high temperatures, conducting similar microcosm experiments across a gradient

of temperatures may reveal important effects of temperature and allow identification of effects imposed by laboratory conditions. Replication of these experiments using different focal species, NMCs and media will provide insight into the generalisability and consistency of the findings.

Laboratory studies, such as this one, have proved to be predictive of real-world ecological outcomes, and therefore have the potential to inform real-world policy. Systems capable of replicating decades of ecological process within a matter of weeks are a powerful tool for prediction of the trajectory of future invasion-related scenarios.

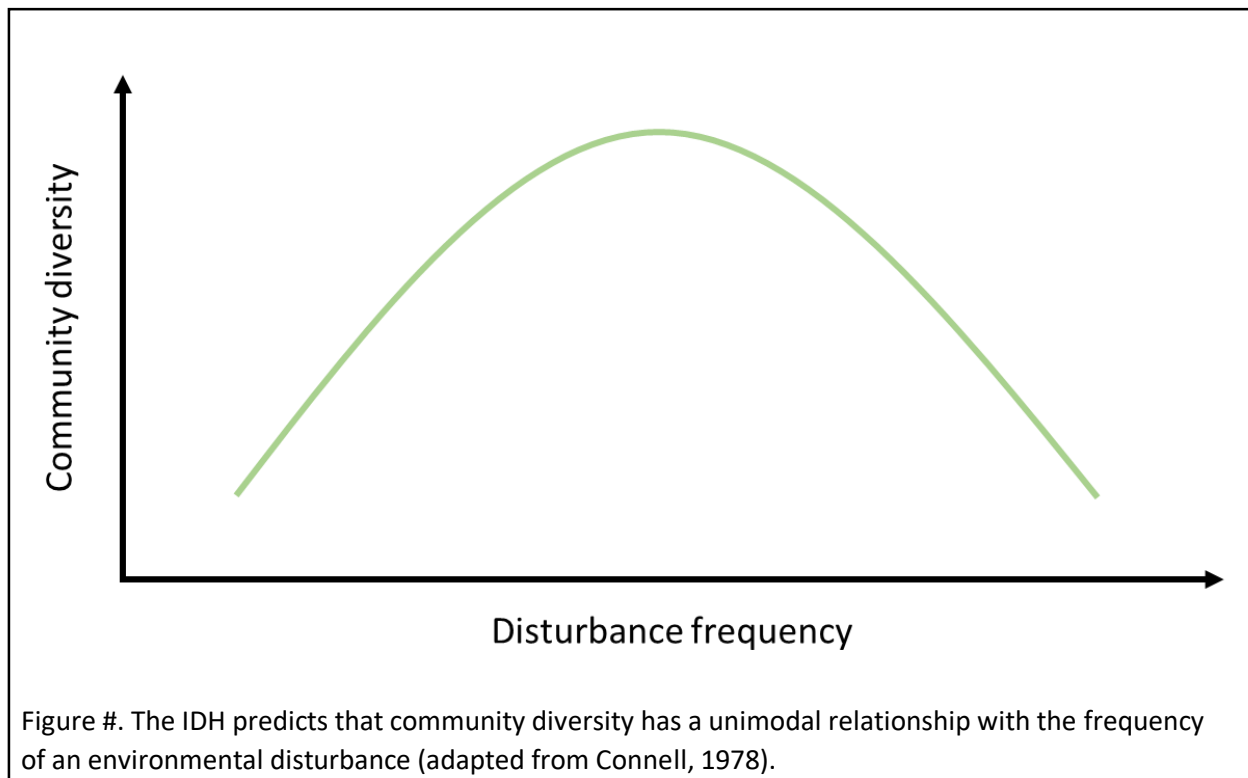
## Chapter 3

# Disturbance: intermediate disturbance effects and resilience to invasion

### Introduction

Disturbance events can have profound impacts on community assembly and structure, potentially driving the generation and maintenance of biodiversity or, conversely, driving homogeneity within communities. Disturbance events open up ecological niches both for recolonization by the resident community, and potentially for new invading species (Fawcett *et al.*, 2009, Ramoneda, 2017). A recent meta-analysis of disturbance studies identifies changes in disturbance regime to be an important predictor of invasion success in these conditions, as opposed to disturbance *per se* (Moles *et al.*, 2012). Here, the prediction is that adaptation to historical disturbances drives the resilience of resident communities to invasion, allowing them to resist influx of novel species under historical, but not novel, conditions.

Connell's (1978) intermediate disturbance hypothesis (IDH) predicts that disturbances of intermediate frequency will maintain high levels of diversity within an environment, compared with low- or high-frequency disturbance events (see fig.1); species will also be adapted to historically experienced disturbances, but vulnerable to the effects of novel disturbances. The IDH is now a long-established ecological principle, the idea was suggested even before Connell's (1978) paper (Wilkinson, 1999). Studies of plant communities invaded by alien species support the IDH, but show how fast-growing r-selected species can shift the peak of the disturbance-diversity curve towards more frequent disturbance (Catford *et al.*, 2012), while intuitive, this neatly illustrates the fact that what is defined as 'intermediate' is highly dependent upon the life-history traits of the organisms affected by the disturbance (Shea *et al.*, 2004).



During interspecific competitive interactions, disturbance frequency interacts with other disturbance properties to either enhance or suppress co-existence; spatial extent, intensity and duration of disturbance all have important interactions in this context (Shea *et al.*, 2004; Johst & Huth, 2005; Miller *et al.*, 2012). In this study, spatial extent, intensity and duration were all fixed parameters, testing only for the effect of disturbance frequency on preadaptation and invasion.

In a heterogeneous environment, *P. fluorescens* will rapidly diversify to fill available spatial niches; in liquid media the ancestral smooth (SM) morphotype inhabits the liquid-phase as a free-floating population, adapted populations of the wrinkly spreader (WS) and fuzzy spreader (FS) morphotypes inhabit the liquid-air interface and bottom of the microcosm respectively (Rainey & Travisano, 1998; Buckling *et al.*, 2000). These morphotypes are easily distinguished when plated to King's B agar for abundance scoring. The study by Buckling *et al.* (2000) found intermediate disturbance frequency to be an important factor in the maintenance of diversity of *P. fluorescens* populations. Moreover, diversity protects against invasion in this system (Hodgson *et al.*, 2004) and in some natural contexts (Hector *et al.*, 2001).

Here, between prior adaptation to disturbance regime contemporary disturbance to affect resilience to invasion using communities of *P. fluorescens* SBW25. Evolved diversity of resident populations was additionally measured to determine how this was affected by disturbance and in turn how this affected invasion success.



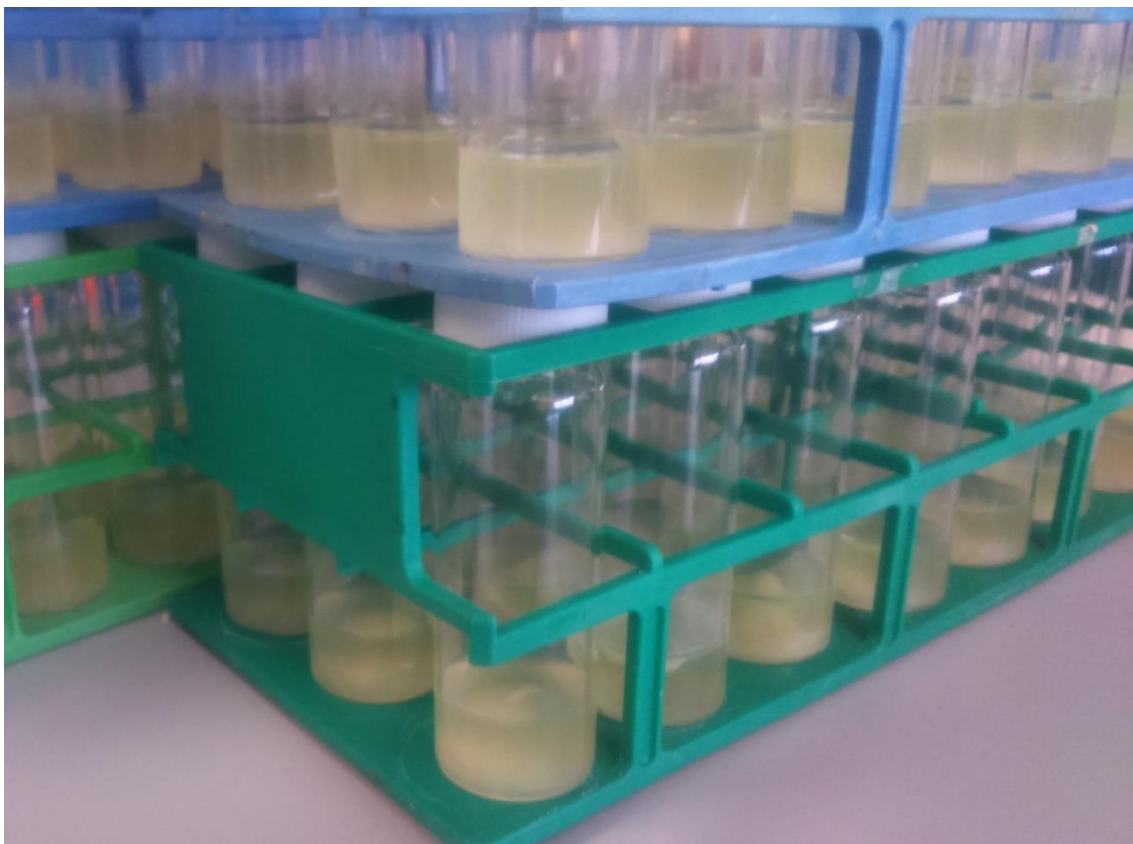
## Methods and Materials

### Preadaptation and diversity assaying

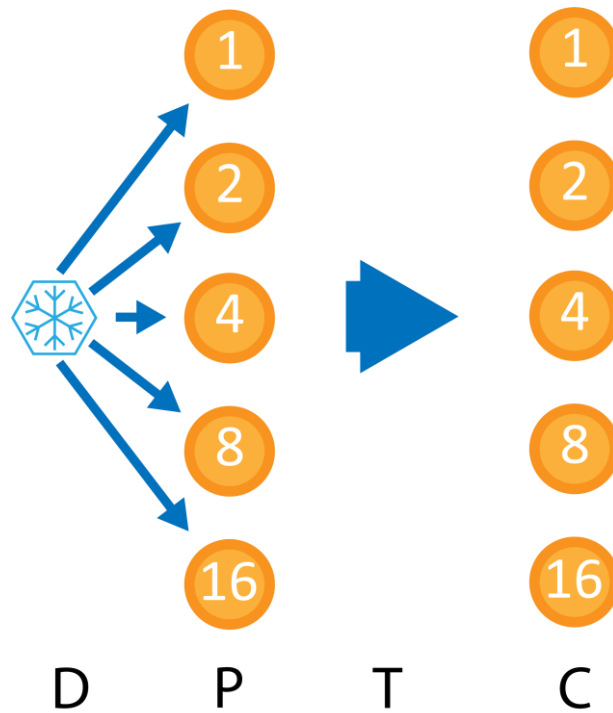
'Resident' populations of the *P. fluorescens* SBW25 strain were preadapted to disturbance regimes in 30ml glass universals (Figure 1) containing 6ml of KB broth, statically incubated at 28°C. Disturbance effects were applied by vortexing microcosms, and transferring 60µl of homogenised culture to fresh media. Disturbance took place at 1, 2, 4 and 8-day intervals during the 16-day preadaptation phase, a final non-disturbance control was not transferred during this period. 6 replicates were used for each treatment.

At the end of the preadaptation period all treatments, including the control, were transferred to new media using the same protocol as disturbance application. 6 replicate populations from each preadaptation treatment were then subjected to disturbance at all regime frequencies, in competition with a lac-z marked ancestral SBW25 clone 'invader' population (Figure 2). During set-up of the competition assays  $1 \times 10^6$  cells of the ancestral clone were inoculated to each microcosm.

All microcosms were sampled at the end of the preadaptation and competition phases of the experiment. Morphology and abundance were scored on KB agar plates, x-gal was added to the KB for scoring of the competition assays via blue-white colony differentiation. Diversity was measured by plating populations to KB agar and scoring colonies by eye.



**Figure 1.** Experimental microcosms were 30ml glass universals containing 6ml of King's B broth.

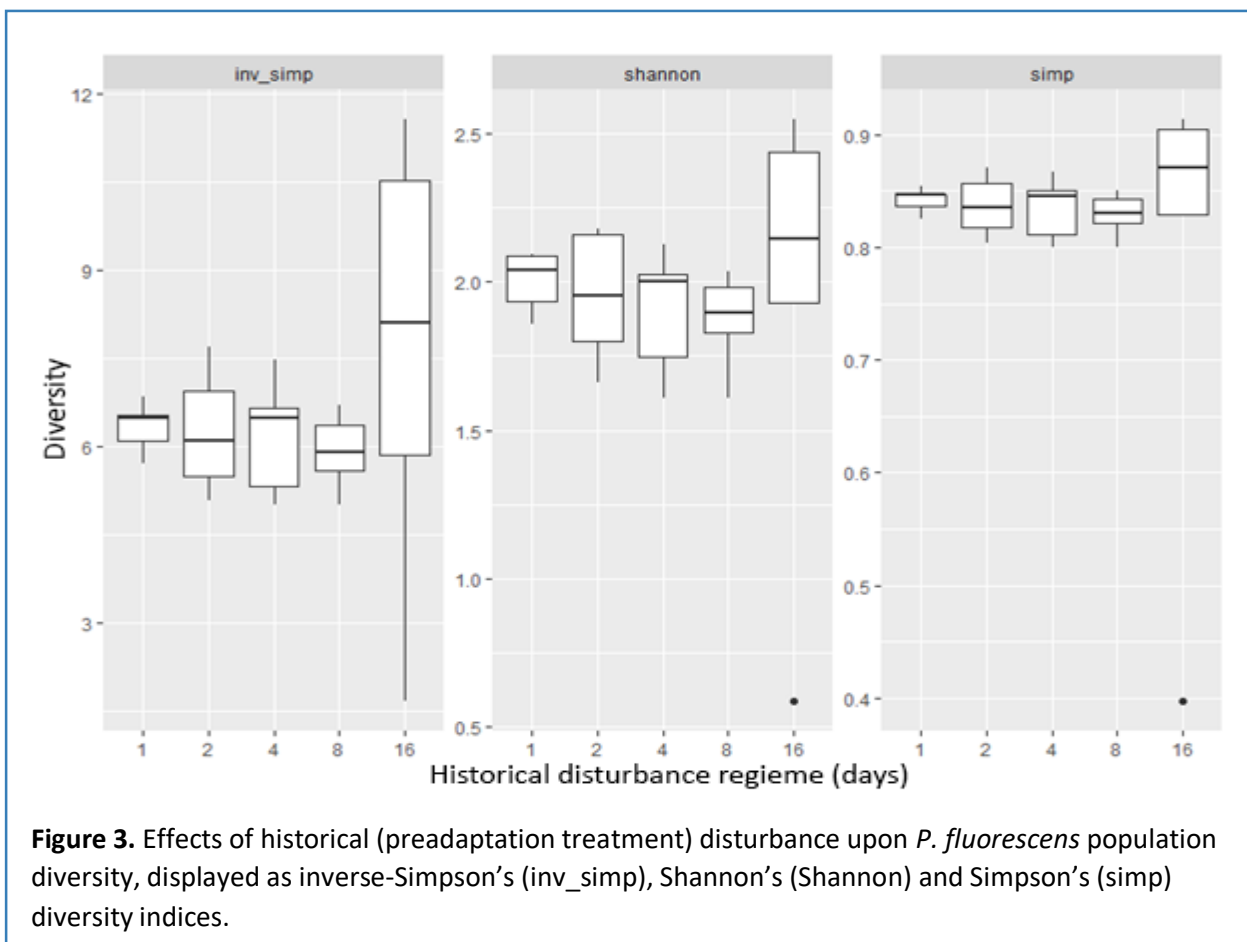


**Figure 2.** *P. fluorescens* SBW25 clones were defrosted, isolated and grown, before inoculation to disturbance preadaptation treatments (D). Clones were then incubated in static conditions for 16 days, experiencing vortexing (spatial disturbance) and 99% population mortality (via sampling and transfer to new media) with a frequency equal to the number of days indicated in circles (P). At the end of the 16-day preadaptation period, all preadapted populations were then sampled for inoculation into new microcosms (T). Competitive assays were performed with a marked ancestral strain, all preadapted populations competed against this ancestor under all disturbance regimes (C).

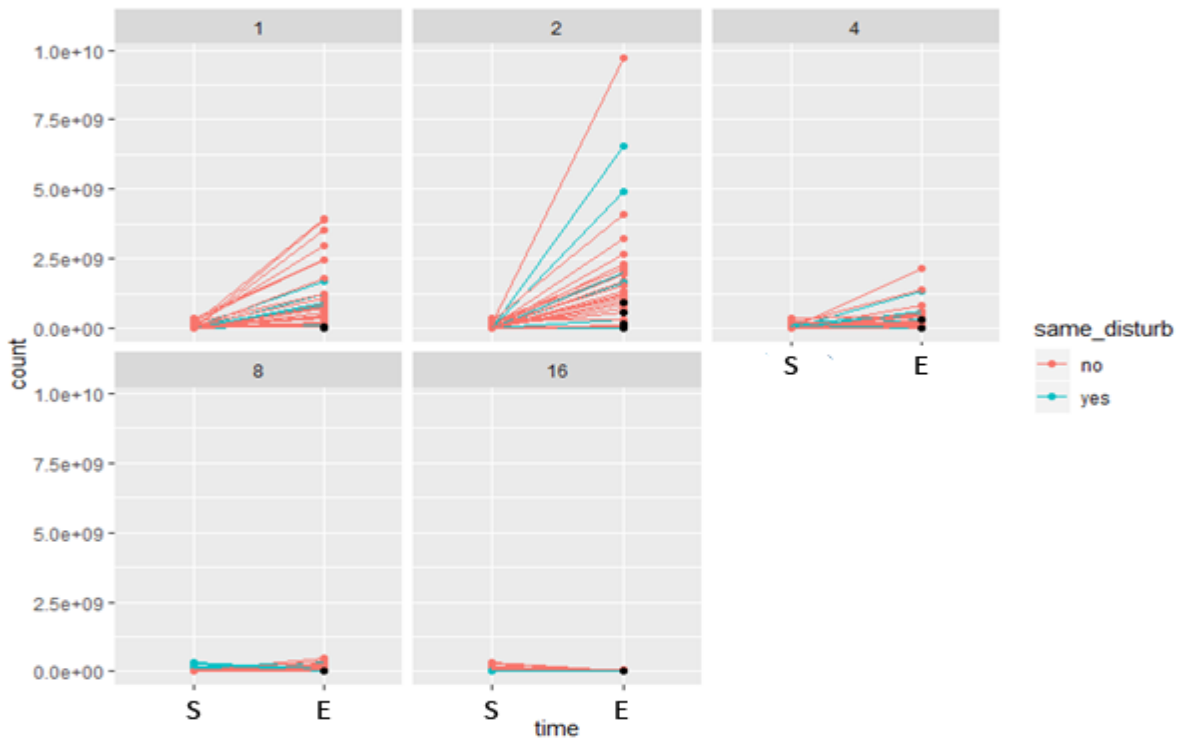
### Analysis

Analysis was conducted in R using a zero-inflated poisson model, due to low survivorship of invading populations. Diversity indices were calculated using both Simpson's and inverse Simpson's indices, and Shannon's diversity index.

## Results

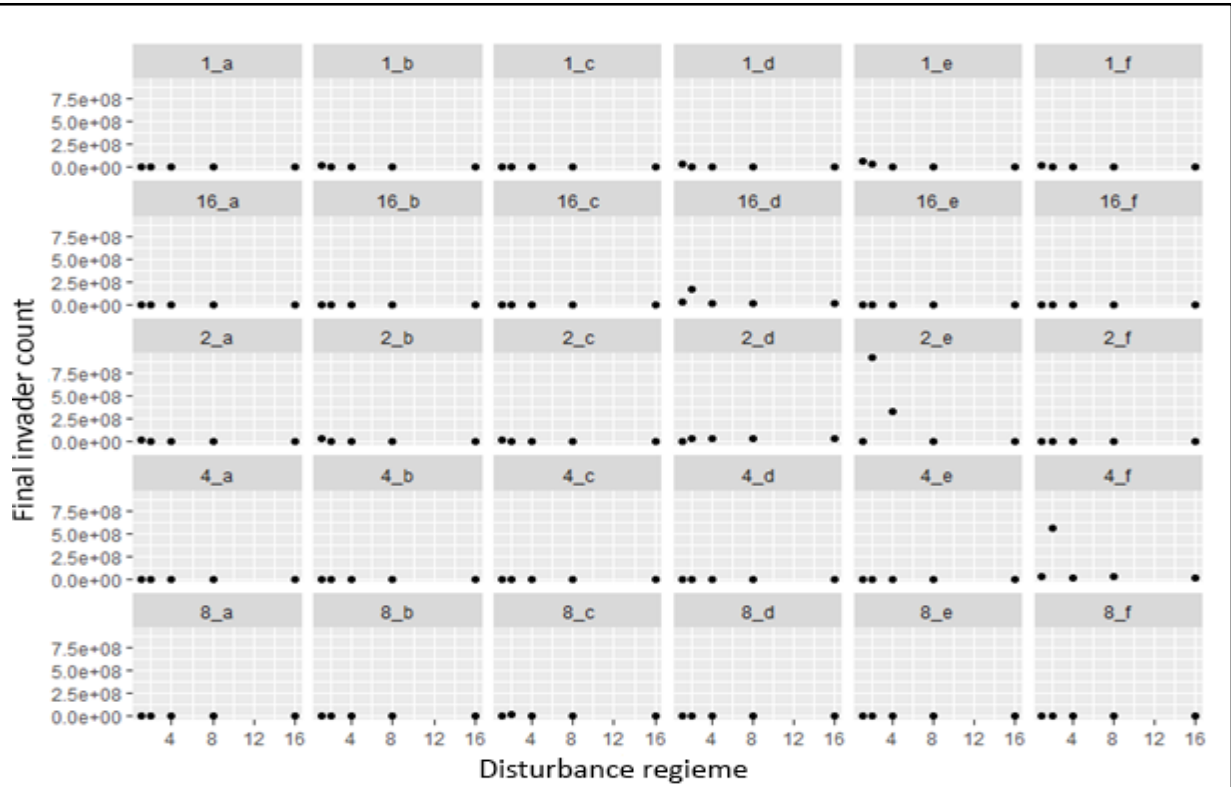


No significant effect of historical or current disturbance regime was found upon population diversity of populations into WS and FS morphotypes. Generalised linear modelling of the interactions found no significant effects on the proportion of the invader of historical ( $df=20$ , residual deviance=16.014,  $p=0.0589$ ) or current disturbance regime ( $df=16$ , residual deviance=10.720,  $p=0.2584$ ), and interactions between the two were not possible to compare due to the model having no residual degrees of freedom ( $n=1$ ,  $df=0$ ) (Figure 3). The highest variability in diversity was found in the undisturbed control treatment, and the abundance of CFU in this treatment was extremely low by day 16 (Figure 4).



**Figure 4.** Abundance (CFU/ml) of preadapted clones under historical (1,2,4,8, and 16-day) disturbance regimes (blue) and under novel disturbance regimes (red), and ancestral invader (black) before (S) and after (E) the competitive fitness assay across treatments.

Diversity metrics did not reveal a clear pattern in support of the intermediate disturbance hypothesis. Historical disturbance was not found to be predictive of abundance of *P. fluorescens* populations, or presence-absence scoring of the ancestral invader.



**Figure 5.** Abundance of ancestral invader across all treatments, each box represents an individual replicate from the preadaptation phase, disturbance during the competition phase indicated on the x-axis. The number (1/2/4/8/16) at the top of each box represents the historical disturbance regime under which the resident community was preadapted, the letter (a-f) corresponds to each of 6 replicates.

The invading ancestral clone performed poorly across almost all disturbance regimes and against all preadapted populations, as measured by survival compared to the resident populations. The invader was most competitive under 2-day disturbance regimes, but not consistently or significantly between preadaptation treatments or replicates (Figure 5).

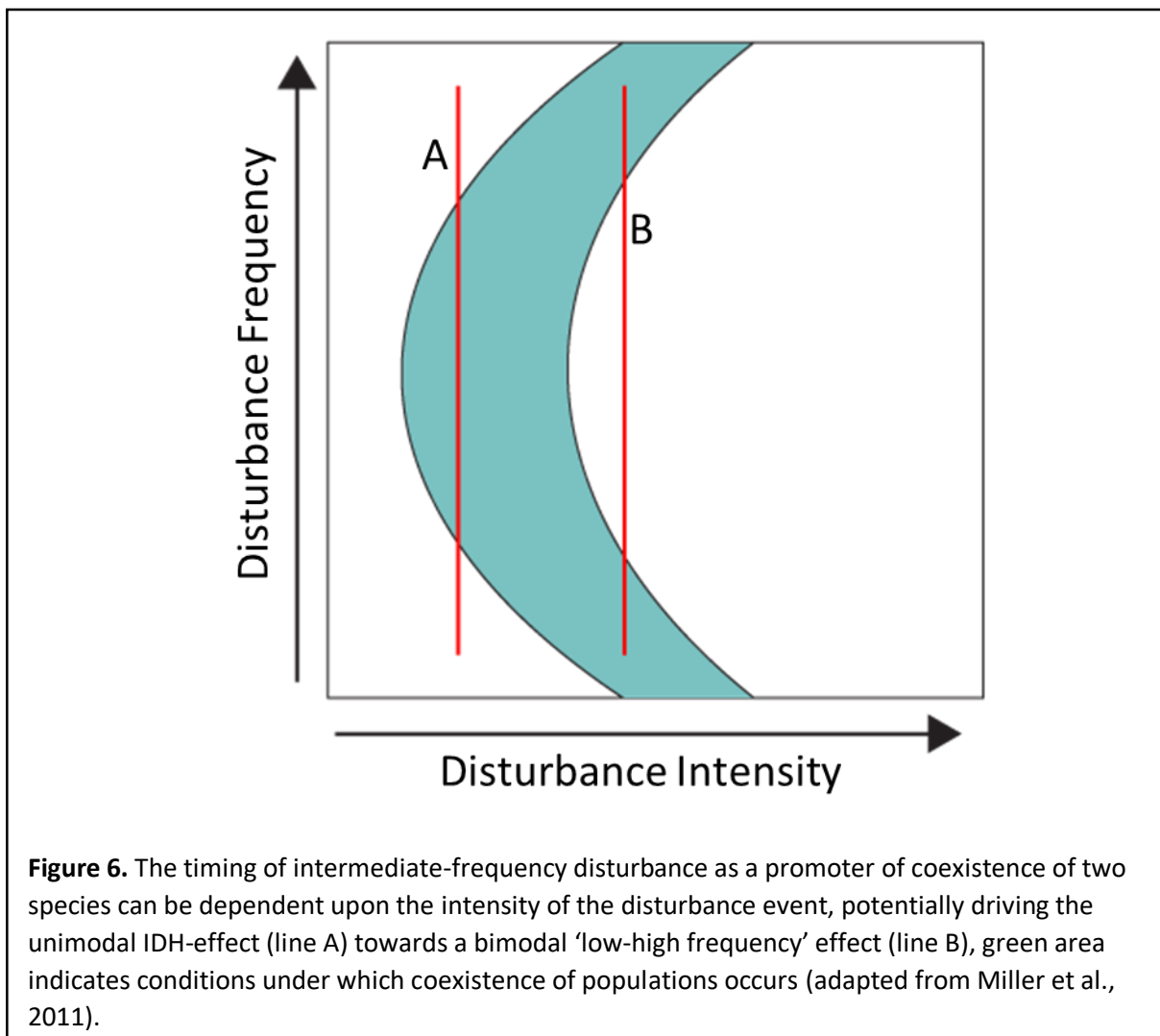
## Discussion

This experiment did not replicate the findings of Buckling *et al.* (2000), however due to an order of magnitude in the difference in the intensity of disturbance (99% mortality in this study, 99.9% in Buckling *et al.*) this may be accounted for. Here, past disturbance was found to have a non-significant impact upon invasion success, the current disturbance was found to have lesser impact, potentially pointing towards adaptation to historical regimes as an important factor in community stability. Whilst these results are non-conclusive, they suggest a potential relationship between resident adaptation to disturbance and resistance to invasion under the same conditions. Further investigation may consider a longer preadaptation period for the 'resident' community

Disturbance frequency-intensity interactions can dramatically shift the theoretical ecological 'space' under which stable coexistence of two species in an environment is promoted, higher intensity driving coexistence states towards diverging high- or low-frequency, see fig.6 on next page (Miller *et*

*al.*, 2011). Compared to the previous work by Buckling et al. (2000), the intensity of disturbance here was an order of magnitude lower, potentially causing a dramatic shift in the timing of an optimal intermediate disturbance pattern. Diversity was found to correlate non-significantly with disturbance frequency, potentially implying that peak diversity may be found at 1-day frequencies, or below.

As bacterial microcosm experiments represent a high number of generations (potentially hundreds of generations in 4 days) compared to many field experiments, it is possible that the 99.9% mortality imposed (high intensity) by Buckling et al. (2000) has pushed the coexistence region towards an extreme low-frequency disturbance optimum.



Although a peak of preadapted resident diversity was not found at 'intermediate' levels of disturbance here, the highest levels of coexistence of the resident and ancestral invader were found in 2-day disturbance treatments. This indicates the action of an intermediate disturbance effect on community diversity, and represents a shift in the coexistence region towards higher-frequency

disturbance, negatively correlating with disturbance intensity, as previously discussed. Line B on fig.6 may represent the pattern seen in diversity at the end of the preadaptation phase of the experiment, whereby coexistence is most likely at high and low frequencies. This bimodal diversity-disturbance relationship has been reported previously, also finding that the strength of this type of relationship is often weaker than when a unimodal relationship is identified (Johst & Huth, 2005).

Populations were found to be extremely low abundance in the 16-day treatments, and the invader driven extinct by the resident community where the resident had been preadapted to 8- or 16-day disturbance. This may represent adaptation towards a stress-tolerant state, rather than to the disturbance regime *per se*. Regular sampling of this treatment to study population dynamics through time may provide insight into successional patterns associated with r-k selected species. If the observed high relative abundance (although low total abundance) of resident preadapted clones in the 16-day treatment is due to stress tolerance, it can be expected that they will be relatively rare compared to the ancestral invader during the early stages of 'succession' following the disturbance and invasion event. As nutrients are depleted and toxic secondary metabolites accumulate, the relative abundance of the invader should be expected to fall, eventually leading to local extinction found in this study, as it is outcompeted by the locally adapted population.

As the competition assay was treated as a continuation of historical disturbance regimes from the perspective of the locally adapted populations, resident community abundance was not equal across treatments. Low population size did not have any effect on the resilience of the locally adapted populations, indicating that this is not as important for community resilience to invasion as local adaptation itself.

Future work in this system may be informed by investigation of the curve of the intensity-frequency dependency of the coexistence region of *P. fluorescens* in a heterogeneous broth environment. If low intensity disturbance does indeed take the intermediate disturbance optimum for coexistence below one day, it would require intensive work to conduct such an experiment, but due to the rapid evolution and generation times of the organism, such investigations would be much more feasible to undertake than similar field studies.

## Chapter 4

# Construction of a small synthetic community for the purposes of experimental manipulation

### Introduction

Microbial populations can grow and evolve at a rapid pace, which allows for the investigation of questions relating to evolutionary and ecological concepts to be tested under relatively short timescales. The laboratory model *P. fluorescens* has begun to be widely used in studies investigating the interplay between rapid evolution and resident communities, due to its rapid diversification into niche specialists in both broth media and soil substrates (Brockhurst et al., 2007; Fukami et al., 2007; Gómez & Buckling, 2013; Gómez et al., 2016). Previous research has shown that the presence of a NMC constrains the adaptive divergence of *P. fluorescens* (Gómez & Buckling, 2013), whilst the prior adaptation of *P. fluorescens* to the environment enhances the effects of its presence in an environment on the structure of the resident NMC (Gómez et al., 2016). These interactions have important implications in environments perturbed by human activities, such as medical (Blair et al., 2015) and agricultural environments (Soman et al., 2016), for management of antibiotic resistance and food security.

Further investigation of the transferability of effects of focal species traits to, and between, environments containing novel abiotic and biotic factors will further inform knowledge of the interactions between focal species fitness, diversity and the structure of resident communities. This work should build upon previous work in the field, identifying where previous results may be generalised to diverse environmental conditions, and further investigating the effects of these variable conditions where generalisations cannot be drawn.

Whilst the current literature provides important insights into microbial evolutionary ecology, studies have relied on either oversimplified “communities”, consisting of just one species (Brockhurst et al., 2007; Fukami *et al.*, 2007), or highly complex, but poorly defined, natural communities sampled from natural environments, containing many unculturable species (Eilers *et al.*, 2010; Gómez & Buckling, 2013; Gómez *et al.*, 2016).

Although experiments with extremely simplified single-species or extremely complex natural communities provide important insights into community ecological processes, they lack precision in regards to the identification of impacts upon individual species within a community. Synthetic communities have been suggested as an important tool for the study of microbial community interactions for the future (Großkopf & Soyer, 2014). A small and well-characterised community would provide the opportunity to identify individual interactions between community members and environmental changes, and allow a fine scale understanding of the impacts these changes have on community structure and functioning. While not providing the level of realism and complexity found in whole natural microbial communities, this approach offers a system much more amenable to experimental manipulation, and may provide a basis from which to make predictions about and design experiments for much more complex systems. A recent study of large-scale community assembly identified highly stochastic outcomes at species level, whilst finding higher taxonomic-level assembly to be predictable and likely linked to functional use of carbon resources (Goldford *et al.*, 2018). In the context of assemblage of small synthetic communities, the findings of Goldford *et al.*



(2018) are promising, as this higher-order predictability in community assemblage will translate to predictable assemblage at the species level when a low number of species representing diverse, functionally disparate taxa are present.

The preparation of defined soil media allows the manipulation of soil properties in a highly controlled manner (Pronk *et al.*, 2016). This type of very defined media has been utilized in only a small number of microbiological experiments (Ellis, 2004; Guenet *et al.*, 2011). The type of complex, yet highly defined, media described by Guenet *et al.* (2011) may help bridge the gap between oversimplified in-vitro and less well-characterised ecological methodologies. This specific artificial soil media has already been shown to be suitable for the culture of a variety of environmental bacteria, including *P. fluorescens* (Guenet *et al.*, 2011).

Further work using SCs and/or soils will lack some of the biological complexity of experimentation with 'natural' soil-compost microcosms, but will allow for a much finer control of experimental variables. Explicit control over the texture, structure, organic matter (OM) content, and other key soil variables will allow investigation of biological interactions in relation to variation of specific abiotic variables. The construction of a well-defined SC will also allow for specific control over the biotic aspect of an experimental environment, allowing the structure and relative abundances of the 'NMC' to be strictly controlled at the outset of an experiment. This type of manipulation is currently redundant in the case of single-species communities, and impractical in the case of whole natural microbial communities.

In the same way that well characterised model organisms are used to provide a highly controlled and replicable study species, a laboratory-constructed "model community" of defined species would provide an easily characterised system, highly amenable to experimental manipulation, for the investigation of microbial ecology.

Here, preliminary work on the construction of a synthetic community has focused on nutrient availability as a potential key driver of community diversity and structure. By picking characteristic morphotypes from a diverse population of laboratory-incubated bacteria, this short study aims to identify laboratory conditions under which a stable population of limited-diversity microbial species can form.

## Methods

An initial population (IP) of 27 bacterial populations (Table 1) was made by plating out freezer-stocks to KB agar, then picking clones and inoculating into 6ml of KB broth in 30ml glass universals. The IP was incubated overnight at 28°C, shaking at 180rpm.

<b>Family</b>	<b>Genus</b>
Alcaligenaceae	<i>Achromobacter</i>
Alcaligenaceae	<i>Bordatella</i>
Alcaligenaceae	<i>Candidimonas</i>
Alcaligenaceae	<i>Pigmentiphaga</i>
Alcaligenaceae	<i>Pusillimonas</i>
Bacillaceae	<i>Bacillus</i>
Bruvelliaceae	<i>Ochrobactrum</i>
Burkholderiaceae	<i>Cupriavidus</i>
Caluobacteraceae	<i>Brevundimonas</i>
Cellulomonadaceae	<i>Oerskovia</i>
Comamonadaceae	<i>Acidovorax</i>
Comamonadaceae	<i>Variovorax</i>
Flavobacteriaceae	<i>Flavobacterium</i>
Hyphomicrobiaceae	<i>Devosia</i>
Microbacteriaceae	<i>Agromyces</i>
Microbacteriaceae	<i>Microbacterium</i>
Micrococcaceae	<i>Arthrobacter</i>
Nocardiaceae	<i>Rhodococcus</i>
Paenibacillaceae	<i>Paenibacillus</i>
Planococcaceae	<i>Lysinibacillus</i>
Pseudomonadaceae	<i>Pseudomonas</i>
Rhizobiaceae	<i>Rhizobium</i>
Rhodobacterales	<i>Paracoccus</i>
Rhodocyclaceae	<i>Shinella</i>
Sphingobacteriaceae	<i>Pedobacter</i>
Staphylococcaceae	<i>Staphylococcus</i>
Xanthomonadaceae	<i>Stenotrophomonas</i>

**Table 1.** Bacterium inoculated to media as the IP.

The universal containing the IP was vortexed before inoculation to experimental media dilutions, 60µl of IP was inoculated into 6ml of media in 30ml glass universals. 6 replicates were made of each treatment. Media used were KB broth and tryptone-soya broth (TSB); concentrations of 1, ½, ¼, 1/8, 1/16, 1/32 and 1/64 diluted with H<sub>2</sub>O were used for all media, and all combinations were incubated at both 15°C and 28°C.

All treatments were statically incubated for 7 days, before being vortexed and plated on KB for scoring. Treatments were qualitatively scored for diversity via visual identification of colony morphs.

One colony each species was picked from a plate containing 5 easily distinguishable colony types (Table 2), and inoculated together in a single glass universal containing 6ml of KB for overnight

growth before transfer of 20µl of culture-media into experimental 30ml universals containing 6ml of 1/64 TSB. Weekly samples were taken for scoring and at the same time as 60ul of each population was transferred to fresh TSB media.

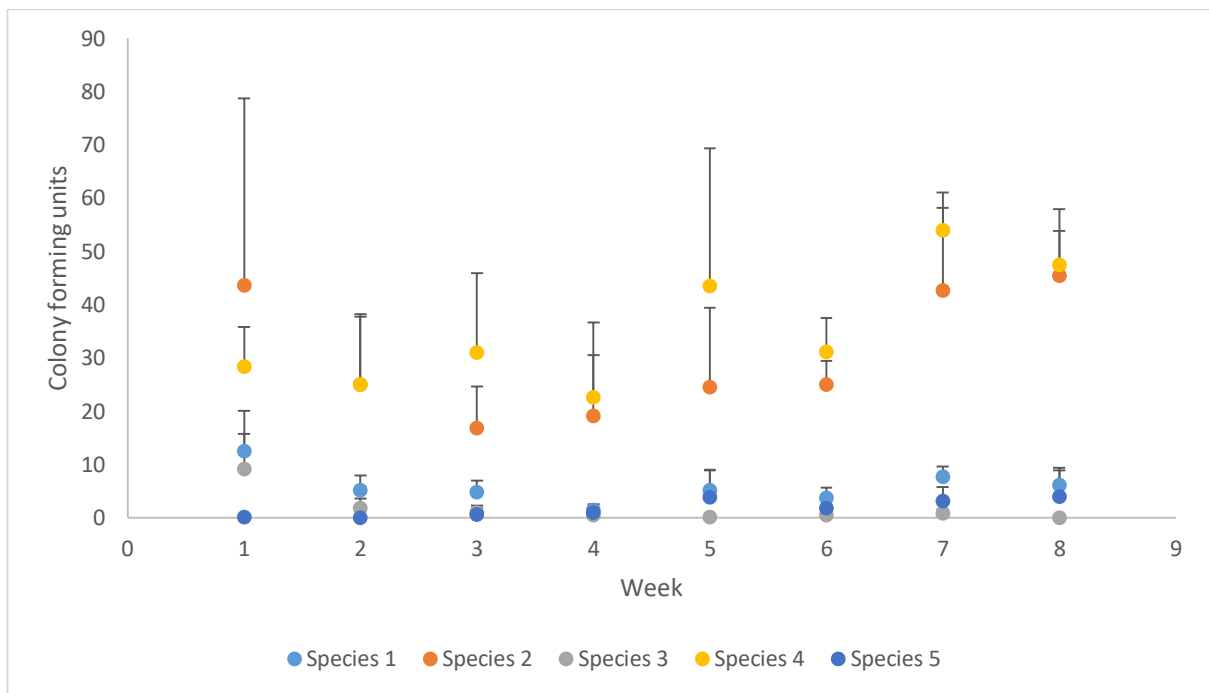
Name	Description
Species 1	Smooth, opaque yellow colonies
Species 2	Smooth, translucent brown-cream colonies
Species 3	Fuzzy, translucent yellow colonies
Species 4	Smooth, opaque white-cream colonies
Species 5	Fuzzy/wrinkly, translucent pale brown colonies

**Table 2.** Morphotype colonies were visually characterised and assigned a number for use in this experiment, pending future genus and species-level assignment via molecular identification.

## Results

Qualitative visual scoring of initial population structuring found that high-nutrient treatments led to complete dominance of communities by 1 or 2 species in every instance. The other 25 or 26 species were either extinct or present in undetectably low abundances. Even at high dilution, KB media encouraged a similar dominance by a single morphotype. Low concentrations of the complex TSB media were found to have the highest levels of diversity, morphotypes used for the longer co-existence assay (Figure 1) were from a single 1/64 concentration, 15°C-incubated TSB IP microcosm.

Community composition varied during the 8 weeks of study, showing a clear trend towards two dominant community members and three low-frequency members (Figure 1). Species 3 was not identified on any plates at week 8, but was confirmed as present in all treatments during subsequent counts after the end of this study (data not published). Between 8-12 weeks the local adaptation of populations caused morphological variation, making distinction of community members by eye increasingly difficult and unreliable.



**Figure 1.** Mean colony forming units as counted weekly (25 $\mu$ l of a  $1 \times 10^{-5}$  dilution of experimental media per plate). Positive error bars show the standard deviation of measurements of each morphotype at each time-point, across replicates.

## Discussion

This experiment shows the relative ease with which a small, stable SC can be constructed and maintained. Further study of this system would be required in order to truly understand the dynamics of this population through time, however the consistency of the hierarchy of abundant and rare species throughout the experimental period has promising potential for experimental manipulation. The potential for the use of this type of system to conduct meaningful ecological experiments in a laboratory setting is high, SC morphotypes were easily identifiable by eye beyond the 6-week period these experiments are often conducted over (Gómez & Buckling, 2013; Gómez et al., 2016), meaning that the use of time consuming or expensive molecular identification techniques will often be unnecessary. Molecular identification techniques would allow the confident identification and quantification of species abundances beyond 8-10 weeks where required.

The small number of morphotypes in the SC will allow detailed analysis of phenotypic and genetic changes in populations that would not be practical with a full natural community. This will allow study of the changes to individual populations within the community in response to environmental changes, and co-evolutionary dynamics between community members and focal invader species.

The small size of the SC constructed should allow for relatively easy analysis of the niche of each community member, via study of their metabolism and deconstruction of the community to study individual species-species interactions. Conducted in a low-nutrient complex media, this experiment identifies the potential for co-existence of species in this kind of environment in a laboratory setting. Similar to the floral-community dynamics of heathland habitats (Heil & Diemont, 1983), this low-nutrient environment prevents the dominance of highly competitive species, by limiting their

available resources, allowing stress-tolerant and K-selected species to co-exist with other, potentially more competitive, morphotypes.

Transfer of SC from a liquid medium to an artificial soil medium can be informed by the requirements for low-nutrients but diverse opportunities to maintain SC diversity found here. The increase in complexity of environment from broth to structured heterogeneous environment would be a further step towards modelling the natural world in a simplified, understandable, and most importantly, controllable way. Further work on the construction of this type of small community should focus on diversity of carbon utilisation within the community, by providing species with targeted niche-space the controlled and predictable assemblage of SCs in a laboratory environment should be easily attainable.

## **Acknowledgements**

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