

Linking disturbance and resources to the invasion resistance and diversity of
microbial communities

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Abstract

Successful colonisations by invasive organisms are causing catastrophic changes to communities: altering their dynamics, reducing biodiversity and impeding ecosystem services. These ecological costs are only surpassed by habitat loss, and when combined with the huge associated economic costs, makes understanding how these events occur of growing importance. This study will focus on how disturbances, which change the availability of resources and habitat, may facilitate the establishment of novel species. First, we factorially separate resource influxes and habitat opening to test the mechanism by which disturbance increases invader success; using diversified populations of *Pseudomonas fluorescens*. We homogenised communities to open habitat and added nutrients to increase resources. Resource influxes were key in successful establishment, habitat opening had little effect and no interaction was found. Secondly, we expanded upon this by testing if resource abundance interacts with disturbance frequency; hypothesising when more resources are available disturbance-induced influxes, and thus invader success, would be greater. To do this communities of *P. fluorescens* were disturbed at different frequencies in three resource concentrations and invaded multiple times. We found disturbance and resources to interact: manipulating the mortality-growth rate balance, and thus success, of the invader. Resources also interacted with evolved biodiversity to effect invasion resistance. We finish by testing disturbance and resource effects on a stably coexisting 5-species bacterial community, using a 5x5 factorial design. Disturbance and resource both manipulated the variation in fitness between species: impacting biodiversity. Interactions were only found at high-disturbance-high-resources. This highlights the suitability of this system for future disturbance-resource studies on stably coexisting systems, including future invasion work.

In conclusion, we show disturbance, through adding resources, to be a key factor in invasion success: the extent to which being strongly affected by resource abundance. We also find disturbance and resource changes are likely to impact the stability of communities.

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Introduction

The successful establishment of novel species into communities is now a major global crisis (Vitousek *et al.* 1997). The rate at which these invasions can cause extinctions is second only to habitat loss, meaning they can have severe consequences to community biodiversity and stability (Vitousek *et al.* 1997; Davis *et al.* 2000; Fausch *et al.* 2001; O'Dowd *et al.* 2003; Lake and Leishman, 2004; Hooper *et al.* 2005). Not only are they ecologically very costly, but economically so, with invasive insects alone costing over 77 billion USD a year (Bradshaw *et al.* 2016). This has led to a growing interest in trying to understand traits that allow some novel organisms to become invasive, and what community traits influence their resistance to invasions in order to create a more conclusive picture of how invasions occur (Sher & Hyatt, 1999; Davis *et al.* 2000; Lake & Leishman, 2004; Altman & Whitlatch, 2007).

Despite substantial research into invasive species' traits and their receptive communities, invasion theory is still unclear and inconclusive. For example, in regard to the life-history of invaders, a classical theory has been fast reproduction is crucial for successful establishment (Rejmánek & Richardson, 1996; Allen *et al.* 2017); however, some now argue traits associated with future rather than current reproduction are more beneficial due to the decreased chance of demographic stochastic extinction (Jeppsson & Forslund, 2012; Sol *et al.* 2012). It is possible that differences in the success of traits reflects differences in propagule pressure, the number of invaders being added in each introduction event combined with the frequency of these events, a fact supported by invaders usually only establishing after numerous unsuccessful attempts (Rejmánek & Richardson, 1996; Sax & Brown, 2000; Tilman, 2004; Sol, 2007). This highlights when using observations to cognise traits that increase an invader's success, the lack of variable control may muddy conclusions. Studies looking into non-life-history associated traits include the similarity between the native and new predator, prey, host and parasite species, with increased similarity benefitting invading parasites and predators but disadvantaging invading hosts and prey (Lonsdale, 1999; Sax & Brown, 2000). Likewise, studies on community invasion resistance frequently focus on the similarity between the invader's new and native habitats (Lonsdale, 1999). The

role of biodiversity on community resistance has also been well studied yet remains debated, with both positive (Levine & D'Antonio, 1999; Hodgson *et al.* 2002) and negative impacts being found (Palmer & Maurer, 1997; Simberloff & Von Holle, 1999). These differences appear to depend on the level of niche saturation, with saturation and resistance being positively related (Tilman, 2004).

The importance of two community properties, niche and more notably resource availability, for invasion resistance are of increasing interest, especially as they are expected to change with an altering climate (Aplet *et al.* 1991; Davis *et al.* 2000; Tilman, 2004; Lembrechts *et al.* 2016). For example, Aplet *et al.* (1991) showed foraging by feral pigs in Hawaii opened niches and resulted in the increased establishment of some weed species; Huenneke *et al.* (1990) found fertilising (resource addition) serpentine grasslands caused an increase in non-native plant species. Lake and Leishman (2004) conducted an observational study looking at areas of an urban environment where exotic plant species had established; they found nutrient input to be a key factor and amplified by physical niche opening. A similar result was found in an experimental plant study by Lembrechts *et al.* (2016). Here, they aimed to experimentally disentangle the effects of increased resource and niche availability on invasion resistance. However, by removing plants (biomass) to open niches, resource abundance will have also increased through resident consumption reduction and the factors not effectively separated; this would also have occurred when the pigs opened niches in Aplet *et al.*'s (1991) study (Davis *et al.* 2000).

The following chapters explore aspects of a community that may increase their vulnerability to invasions. Principally, we focus on the role of disturbances: events that destroy biomass and lead to changes in resource abundances and the opening of habitat within a community (Roxburgh *et al.* 2004).

Although these destructive events are often integral parts of ecosystems, helping colonisers and competitors to coexist, they are also shown to facilitate biological invasions (Aplet *et al.* 1991; Sher & Hyatt, 1999; Buckling *et al.* 2000). Both experimental and observational studies have previously been used to try to decipher how this may be so, with changes to ecosystem composition, niche

or habitat opening and increased resource availability amongst the key hypotheses (Aplet *et al.* 1991; Sher & Hyatt, 1999; Davis *et al.* 2000; Tilman, 2004; Lembrechts *et al.* 2016).

Although the semi-natural setup of experimental plant studies could potentially give us a realistic picture of disturbance variable effects, variables are hard to control. This means the true effect of what is being tested may be missed: as for example, in the aforementioned study by Lembrechts *et al.* (2016). Another consideration is the number of manipulated generations being low, meaning many evolutionary processes are likely to be missed. For these reasons the use of microorganisms to test ecological questions has increased: their short generation times mean evolutionary processes can be observed over short time periods, the strict control of variables is possible, and many repeats can be undertaken. The experimental studies carried out in the following chapters all use bacteria grown in broth media under controlled conditions.

Previous experimental microbial work has shown that intermittent levels of disturbance can cause a peak in diversity due to a balance of niche-specialists forming (Buckling *et al.* 2000). A study utilising the same *Pseudomonas fluorescens* system later found increasing both diversity and productivity of communities increased their resistance to invaders (Hodgson *et al.* 2002); however, evidence of disturbance altering invasion success per se is lacking. Combining this previous work spawns the hypothesis that a quadratic relationship will occur whereby invasion success troughs at intermittent disturbance. Although, as disturbances alter variables other than just community composition, notably changes to niche and resource availabilities, it is likely its effect on invasion success will be more complex than a direct relationship with biodiversity (Tilman, 1994; Sher & Hyatt, 1999; Davis *et al.* 2000; Lembrechts *et al.* 2016).

In chapter one, we disentangle the mechanism by which disturbance (biomass removal) events may facilitate the establishment of novel species by factorially separating resource and habitat opening in communities of diversified *Pseudomonas fluorescens*. The use of microorganisms here allows us to test

these two effects of biomass removal without actually removing biomass: by homogenising to open habitat and adding more resource-rich growth media.

These two key-components of disturbance have not been effectively separated previously; habitat opening is frequently overlooked or indirectly leads to resource addition through consumption reduction by the residents. For this reason, resource addition is commonly thought to be the cause of invasions after disturbance. Separating these factors will help identify which disturbance types may reduce community invasion resistance the most as they are likely to differ between types.

In chapter two, we explore how community resource abundance influences the affect disturbance frequency has on invasion success. This builds upon the findings in chapter 1 that disturbances increase invasion success through adding resources, hypothesising disturbances in resource-abundant communities will have more of an effect than in resource-poor, due to greater resource addition per disturbance. As the multiple disturbance and resource treatments alter the way *P. fluorescens* diversifies, the impact of evolved biodiversity on invasion resistance is also tested (Buckling *et al.* 2000; Hodgson *et al.* 2002; Kassen *et al.* 2004). This is a factor previously shown to have mixed effects on invader success; here we explore if this is to do with interactions with other aspects of the community (Levine & D'Antonio, 1999; Simberloff & Von Holle, 1999; Hodgson *et al.* 2002; Green *et al.* 2011).

These two projects were carried out using *P. fluorescens*, relying on its fast and predictable rate of diversification into niche specialists to create diverse communities (Rainey & Travisano, 1998; Buckling *et al.* 2000; Kassen *et al.* 2004). In chapter three however, we test an ecologically stably coexisting community of five bacterial species in a disturbance frequency-resource abundance experiment, with the aim to gain an understanding of how diversity in this system is manipulated by these treatments. This data is essential for any future experimental invasion studies using this system, which may do so as its stable coexistence makes it potentially more realistic of natural communities. It also provides unique experimental data on how disturbance and resources affect biodiversity in stably coexisting multi-species communities; this will be

useful for predicting how stable communities may react to the current changes in disturbance regimes and resource abundances (Davis *et al.* 2000).

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Chapter 1: Disentangling the mechanisms underpinning disturbance-mediated invasion

Disturbances can play a major role in biological invasions: through destroying biomass they alter habitat and resource abundances. Previous field studies suggest that disturbance-mediated invader success is a consequence of resource influxes, but the importance of other covarying causes, notably the opening up of habitats, have yet to be directly tested. Using experimental populations of the bacterium *Pseudomonas fluorescens* we determined the relative importance of disturbance-mediated habitat opening and resource influxes, plus any interaction between them, for invader success. Two ecologically different morphotypes of the genetically marked *Pseudomonas fluorescens lacZ* strain were used as our invader. We found resource addition increased invasibility, and habitat opening to have little impact or interaction with resources: affects were equal on both invaders. Our results provide experimental support for the observation that resource input is a key mechanism through which disturbance increases invasibility.

Introduction

Biological invasions are a major global issue and widely accepted as the second biggest cause of extinctions after habitat loss (Vitousek *et al.* 1997). They reduce biodiversity, change ecosystem dynamics and cause huge financial costs (Vitousek *et al.* 1997; Sher & Hyatt, 1999; Shea & Chesson, 2002; Levine *et al.* 2003; Didham *et al.* 2005; Leishman *et al.* 2007). For these reasons it is essential to understand how some exotic species can become invasive and what makes an ecosystem vulnerable to invasion (Holway & Suarez, 1999; Sher & Hyatt, 1999; Lake & Leishman, 2004). A factor frequently shown to facilitate invasions is disturbances: events that, through destroying biomass, lead to changes in resource and habitat availability (Shumway & Bertness, 1994; Roxburgh *et al.* 2004; Lembrechts *et al.* 2016).

Biomass destruction can potentially alter invasion success in a number of inter-related ways; three key examples of these are: by increasing resource availability (defined as required substances such as light, soil nitrogen or water (Sher & Hyatt, 1999; Davis *et al.* 2000; Lembrechts *et al.* 2016), opening up

habitats (Tilman, 1994) and through the resulting community composition alterations (Buckling *et al.* 2000). The extent of these factors may vary between disturbance types and themselves differ in their impact on community invasibility; disentangling these differences is fundamental for understanding disturbance-mediated invader success. Disturbances can increase resource availability through associated resource inputs and resident mortality (Baldwin & Mitchell 2000; Davis *et al.* 2000; Gordon *et al.* 2008); this can allow invading populations to establish by reducing competition with residents (Tilman, 2004; Catford *et al.* 2009). Consequently, increased resource input is likely to particularly benefit fast growing, generalist invaders (Tilman, 1994; Rejmánek & Richardson, 1996; Lake & Leishman, 2004; Viollea *et al.* 2010; Fukami, 2015). The opening up of habitats reduces any advantage established residents have over invaders due to priority effects (larger population sizes and local adaptation) (Fargione *et al.* 2003; Urban & De Meester, 2009; Vannette & Fukami, 2014); if specific habitats are opened, it is most likely specialists will benefit over generalist invaders (Tilman, 1994; Rejmánek & Richardson, 1996; Lake & Leishman, 2004; Viollea *et al.* 2010; Fukami, 2015). The temporary loss of resident functional diversity associated with some disturbances (Connell, 1978; Buckling *et al.* 2000) can result in less efficient resource use and vacant habitats, again promoting invasions (Hobbs & Huenneke, 1992; Hodgson *et al.* 2002; Seabloom *et al.* 2003; Emery & Gross, 2007; Catford *et al.* 2009; Viollea *et al.* 2010; Fukami, 2015).

A large proportion of previous disturbance-invasion work concentrates on resource influxes as the cause of increased invasibility, with little consideration for habitat opening (e.g. Davis *et al.* 2000; Gross *et al.* 2005, *but see* Aplet *et al.* 1991; Lembrechts *et al.* 2016). Studies that do take other factors into account are either observational and do not actively disturb or invade communities (e.g. Lake & Leishman, 2004), ignore the opening of habitats entirely (e.g. Gross *et al.* 2005), or indirectly alter resource availability in habitat opening treatments through consumption reduction (e.g. Aplet *et al.* 1991; Davis *et al.* 2000; Lembrechts *et al.* 2016). Here, we experimentally investigate the relative importance of two aspects of disturbance-induced biomass destruction: resource availability and habitat opening, as well as their consequences for the resident community, in experimental populations of bacteria. In order to

independently manipulate these variables biomass destruction per se had to be avoided.

We used the bacterium *Pseudomonas fluorescens*, which has previously been used as a model for the causes and consequences of diversity (Rainey & Travisano, 1998; Gómez & Buckling, 2013) and invasion biology (Hodgson *et al.* 2002; Zhang & Buckling, 2016). When introduced into a spatially structured microcosm *P. fluorescens* diversifies into three distinct morphotypes: an air-broth interface growing wrinkly spreader (WS), a broth inhabiting smooth (SM) and the rarer bottom-dwelling fuzzy spreader (Rainey & Travisano, 1998; Kassen *et al.* 2000; Spiers *et al.* 2002; Koza *et al.* 2011; Hall *et al.* 2012). We independently manipulated two key consequences of disturbance in a full factorial design (habitat opening by homogenisation to open the surface niche, resources through directly adding nutrients), and then determined the change in resident community composition and the success of genetically marked and visually distinguishable *P. fluorescens lacZ* invaders. By using two different invading morphotypes: the faster growing SM and the more spatial niche-specific WS, it was possible to test whether different invader characteristics are predictably affected by resource input and habitat opening.

Methods

Strains

Ancestral *Pseudomonas fluorescens* SBW25 was grown overnight to carrying capacity in shaken glass vials (microcosms) containing 6ml of the growth medium King's medium B (KB), at 28°C with loose lids to allow oxygen transfer. This was inoculated into static microcosms and left to diversify for seven days, before being disturbed and invaded according to treatment group and left for a further two days (Fig. 1). On day nine all microcosms were thoroughly homogenised and a 900µl sample immediately frozen at -80°C in 900µl of 50% glycerol solution. Samples were plated at 10⁻⁵ and 10⁻⁶ dilutions on agar plates containing X-gal. For the invader, *P. fluorescens lacZ* was grown in static KB and left to diversify for six days before being plated; this strain is visually distinguishable from the wildtype in the presence of X-gal due to a colour change (Zhang & Rainey, 2007). A single SM and WS morph was selected, grown overnight then both frozen in glycerol stock and plated to check

morphotype purity. To stop any additional resources being added to the treatments, invaders were removed from their growth medium by centrifuging and re-diluted in m9 solution before addition.

Competition experiment

To test if the wildtype and invader had equal fitness, ancestral crystals of each were grown and shaken overnight before 3ml of each was mixed together in a fresh microcosm. This was plated to determine starting densities and 60 μ l used to inoculate 8 fresh microcosms that were then left to grow overnight before being replated. Relative fitness was calculated by wildtype growth rate/lacZ growth rate, with growth being $\log(\text{end density}/\text{start density})$.

Experimental design

Four treatment groups were used: homogenised + buffer (opened habitat), static + KB (added resources), homogenised + KB (full disturbance), and static + buffer (no disturbance/control, Fig. 1). Buffer was added to control for the increase in broth volume in the KB addition treatments. 2000 μ l of KB or buffer was added; homogenisation lasted for 30 seconds. All microcosms were then immediately invaded with 60 μ l of either the SM or WS invader. Both invader and resources were pipetted slowly down the side of the microcosm in order to minimise disruption to any biofilm. All treatments were replicated six times with both invaders, resulting in 48 microcosms.

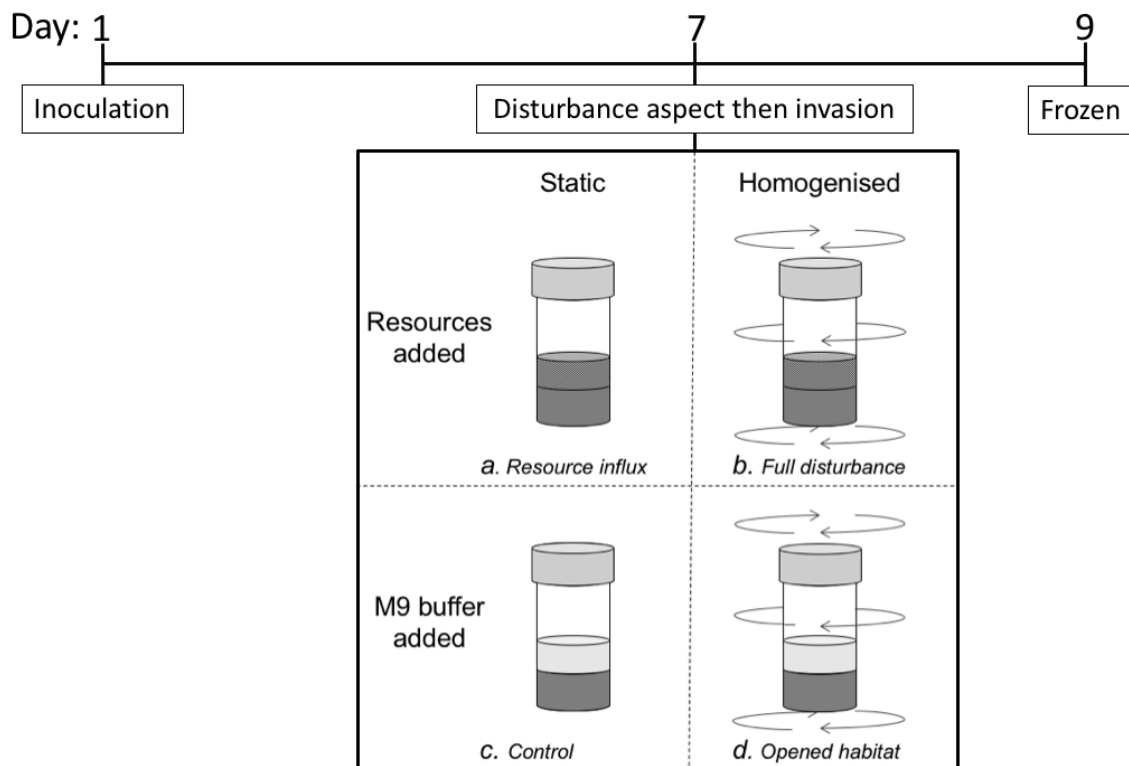


Figure 1 Timeline of methods. The four treatments: (a) static + KB (added resources), (b) homogenised + KB (full disturbance), (c) static + buffer (no disturbance/control) and (d) homogenised + buffer (opened habitat) were carried out on day seven. All treatments were invaded post-disturbance with either a Wrinkly Spreader or Smooth invader and replicated six times. Treatments ended two days later on day nine. Homogenisation lasted 30 seconds; 2ml of KB or buffer was added.

Statistical analysis

Invasion success was calculated as the proportion of invader (*lacZ* strain) on the plate (*lacZ* cfu/total cfu). ANOVAs were carried out in R (R Core Team, 2015) to test the affects and interactions of habitat opening, resource addition, invader morphotype and the resident WS and SM densities on invasion success; non-significant interactions ($p > 0.05$) were removed from the model. The effect of treatments and invader morphotypes on resident densities was tested with an ANOVA.

The relative fitness of the wildtype was compared to 1 with a t. test and found to be significantly less, showing *lacZ* to be fitter ($t=5.3$, $df=7$, $p=0.001$).

Results

Invasion success

We factorially manipulated resource availability and habitat opening to determine how they impacted the success of different invaders. Habitat opening did not significantly affect invasion success ($F_{1,43}=2.2$, $p=0.15$, Fig. 2), or interact with resources ($F_{1,42}=0.93$, $p=0.17$), but resource addition increased invasibility ($F_{1,43}=9.5$, $p=0.0036$). Invader morphotypes differed in their success, with mean SM invasion success (0.21) higher than WS (0.10) across the four treatments ($F_{1,43}=11.6$, $p=0.002$). There were no significant interactions between invader type and the resource and habitat manipulations in terms of invasion success ($p>0.2$ for all interaction terms).

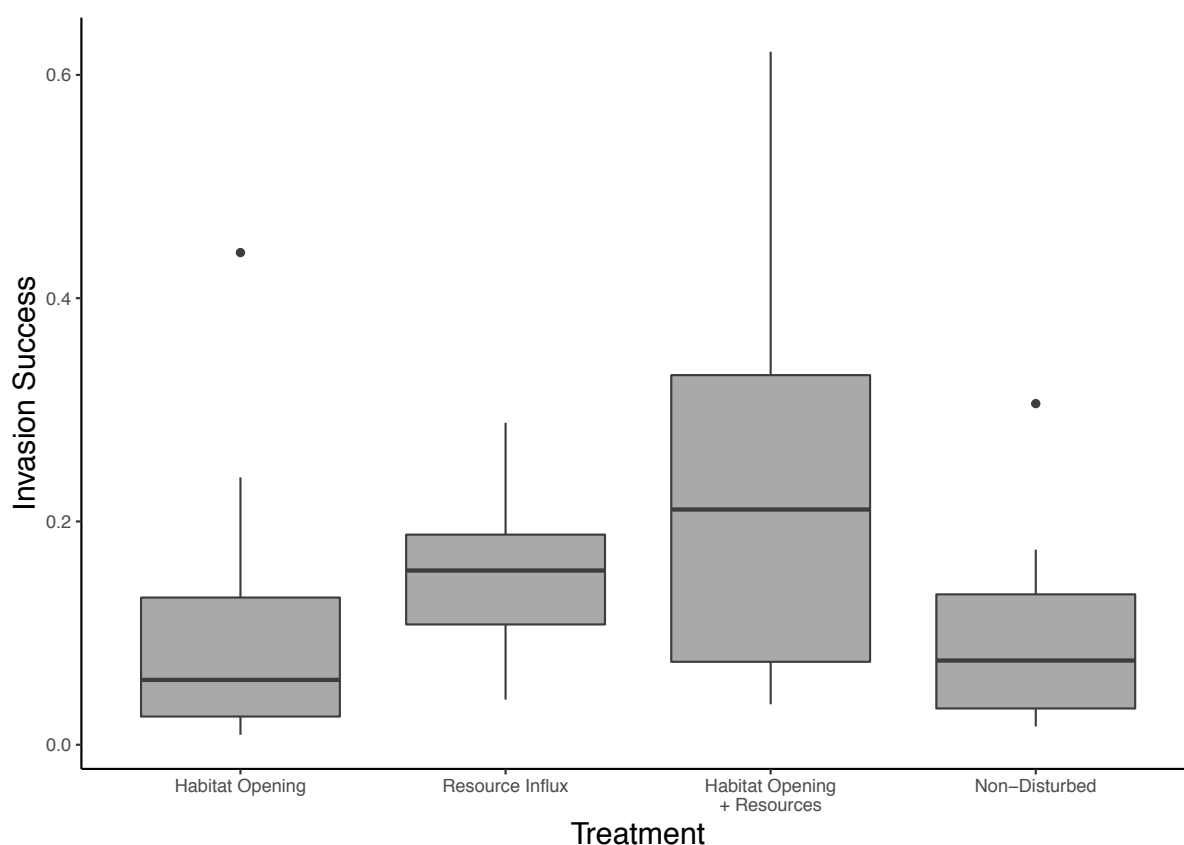


Figure 2 Invasion success (invader cfu/total cfu) in four treatments representing different aspects of disturbance: opened habitat, added resources, combined and no disturbance. Results are a combination of two invader morphotypes.

Resident morph density and its effect on invasion success

Total resident density did not significantly differ between treatments ($F_{3,44}=2.06$, $p=0.12$). However, both WS and SM resident density were affected in complex

ways, with significant three-way interactions between invader morph, resource input and habitat opening ($F_{3,40}=3.22$, $p=0.033$; $F_{3,40}=5.36$, $p=0.009$, respectively). To determine the relative importance of these changes in resident populations to the direct effects of the manipulations we included final SM and WS densities as terms in the statistical model for invasion success. There were no significant interactions between any combinations of density, resource, habitat or invader. As before, resource addition was the only manipulated variable that influenced invasion success ($F_{1,38}=10.0$, $p=0.003$), demonstrating that treatments had a direct effect on invasion, and not just an indirect effect through changes in resident populations. However, unlike WS density ($F_{1,38}=0.002$, $p=0.97$), resident SM density had a significant negative effect on invasion success ($F_{1,38}=5.5$, $p=0.024$, Fig. 3).

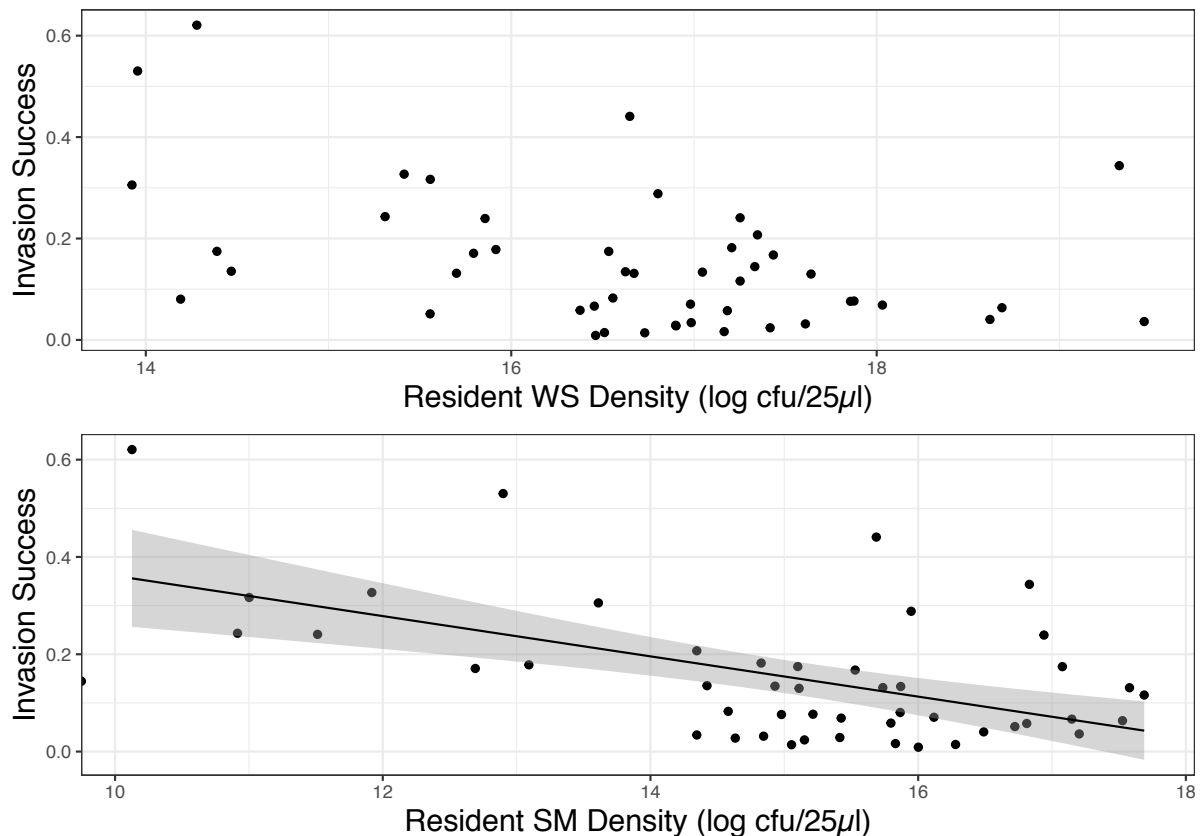


Figure 3 Invasion success (invader cfu/total cfu) against the log density (cfu/25 µl) of the resident wrinkly spreaders (WS: top panel) and smooths (SM: bottom panel). Regression lines are significant to $p<0.05$ with the shaded area showing the 95% confidence interval.

Discussion

Here, we experimentally determined, using a microbial system, the contributions of two distinct disturbance-induced processes predicted to enhance the success of invaders: resource influx and habitat opening. We found that resource influx provided a fitness advantage to the faster growing invaders over the residents, while habitat opening had no impact. These results support previous observational work that attributes post-disturbance invader success primarily to resource influxes (Lake & Leishman, 2004).

Invaders benefiting from resource influxes can be explained by reduced competition, and hence higher growth rate, allowing the invading genotypes to become established (Davis *et al.* 2000; Tilman, 2004). That the relative benefit of resource influx was independent of the life history of the invader (faster growing SM or mat-forming WS), adds to the generality of this finding. The opening up of the WS niche not affecting invasion success can be readily explained by priority effects (Fukami 2015); despite temporary destruction of the niche, the resident WS would still have had a numerical advantage, constraining the opportunities for invaders to become over-represented in the WS niche.

Resource influx had an indirect effect on invasion success through changes in resident community composition, in addition to the direct effect described above. While we do not offer (have) explanations for the complex three-way interaction between habitat opening, resource influx and invader type on the densities of both resident WS and SM, invader success decreased with resident SM density. By contrast, WS resident density did not affect invader success. This difference between the effect of resident SM compared with resident WS is possibly because SM are faster growing, and hence provide greater resistance to invaders, whose success is increased under conditions where fast growth is beneficial (i.e. resource influx). Their faster growth may mean they 'soak up' resource influxes by quickly turning them into biomass; this hypothesis needs further testing to clarify the mechanism of invasion resistance seen here. More generally, the interactions between invader type and disturbances demonstrates how invaders can modify their new environment and potentially create invasion feedback loops (Hobbs & Huenneke, 1992; Mack & D'Antonio, 1998). Biological

invasions themselves can act as disturbances; the establishment of invaders that alter resource abundances could have a major future impact on the community's invasibility (Levine & D'Antonio, 1999; Sher & Hyatt, 1999; Seabloom *et al.* 2003; Tilman, 2004; Fukami, 2015). An example of this is the invasion of volcanic rock by the nitrogen fixing *Myrica faya* tree, which escalates colonisation rates by increasing nitrogen availability (Vitousek *et al.* 1987)

While our results suggest resource influxes are a key driver of invasion success post-disturbance, the relative importance of resources and habitat opening is likely to be dependent on the community being disturbed (Hobbs & Huenneke, 1992; Sher & Hyatt, 1999; Lake & Leishman 2004). For example, habitat opening is expected to have a bigger affect when resident species are maladapted to the post-disturbance environment; this could be due to the disturbance itself or from niche modification by established invaders (Urban & De Meester, 2009; Fukami, 2015). Maladaptation will erode both priority (fitness advantage of being the first to occupy a niche) and dominance (disproportionally large influence by one species on invasion resistance, usually through competitive dominance over limiting resources) effects, ultimately reducing invasion resistance by weakening the residents' competitive dominance (Hobbs & Huenneke, 1992; Emery & Gross, 2006). Similarly, resource influxes are shown to have a bigger effect on invasibility in communities that were resource poor beforehand (Lake & Leishman, 2004). How communities respond to disturbance is frequently shown to depend on their previous disturbance regime (Hobbs & Huenneke, 1992; Sher & Hyatt, 1999; Lake & Leishman 2004), with deviations from this explaining twice the variation in invasion risk than disturbance per se (Moles *et al.* 2012). Moreover, invaders only being able to establish due to resource influxes does raise the question of whether their populations will be stable when resource abundances return to pre-disturbance levels. For example, Petryna *et al.* (2002) found that although disturbance facilitated invasions of grassland, invader populations reduced with time after disturbance. In summary, by using a microbial system we have provided experimental support for the proposed key role of resource influx in driving post-disturbance invasion success. However, more studies are needed to determine how disturbance history and other ecological variables will affect the generality of this conclusion.

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Chapter 2: Understanding disturbance-resource interactions and their influence on invasion success

Abstract

The introduction of novel species into communities is both ecologically and economically very costly. Disturbances have previously been found to facilitate these invasions, with the associated changes in resource availability proposed as a key factor. This study aims to see if community resource abundance interacts with a disturbance regime to alter its effect on invasion success. We invaded experimental populations of the bacterium *Pseudomonas fluorescens* in a factorial design containing multiple disturbance frequencies and resource abundances. As *P. fluorescens* populations rapidly diversify we were also able to test how evolved diversity affects invader success, both directly and through interactions with treatments. Disturbance regime and resource abundance effected invasion success both independently and through an interaction: resource abundance altered the quadratic relationship between disturbance frequency and invasion success. Biodiversity peaked at intermittent disturbance and was lowest in the low resource treatment, but only effected invasion success when resource abundance was high. We show the way disturbance affects invasion success is dependent on community resource ability, and this interaction is made more complex by interactions with diversity.

Introduction

Biological invasions are a global issue with severe consequences for native communities (Davis *et al.* 2000; Fausch *et al.* 2001; O'Dowd *et al.* 2003; Lake & Leishman, 2004). Successful invader colonisations can reduce biodiversity, alter community dynamics and cause large financial costs on a scale only surpassed by habitat loss (Vitousek *et al.* 1997; Sher & Hyatt, 1999; Fausch *et al.* 2001; Shea & Chesson, 2002; Levine *et al.* 2003; Didham *et al.* 2005; Altman & Whitlatch, 2007; Leishman *et al.* 2007). It is therefore essential to gain an understanding of how these events occur by studying the success of species entering novel communities and the way those communities react, in order to

create a predictive theory of invasibility (Holway & Suarez, 1999; Sher & Hyatt, 1999; Davis *et al.* 2000; Lake & Leishman, 2004).

A factor frequently shown to increase invasion success is disturbance: events that destroy biomass leading to the opening up of habitat and changes in the abundance of resources (Shumway & Bertness, 1994; Roxburgh *et al.* 2004; Altman & Whitlatch, 2007). These small or large-scale physical phenomena can facilitate invasions in a number of key ways, in particular by increasing resource availability, which reduces invader-native competition (Hobbs & Huenneke, 1992; Baldwin & Mitchell 2000; Davis *et al.* 2000; Tilman, 2004). They can also clear space for invaders to colonise, remove any priority effects and cause resident maladaptation (Davis *et al.* 2000; Stachowicz *et al.* 2002; Fargione *et al.* 2003; Altman & Whitlatch, 2007; Fukami, 2015).

Despite a large body of work showing disturbance to increase community vulnerability to invasion (Lake & Leishman, 2004; Roxburgh *et al.* 2004; Altman & Whitlatch, 2007; Lembrechts *et al.* 2016), support is equivocal, with studies also showing no or even a negative effect (Fausch *et al.* 2001). This may be due to disturbance interacting, or covarying with, other factors such as pre-disturbance resources which are known to affect invasion success both directly and potentially via changes in community composition (Huston, 2004; Hooper *et al.* 2005). We hypothesise that in communities with low resource abundances disturbance should not significantly alter their abundance (unless they are associated e.g. agricultural runoff, Gordon *et al.* 2008), as mechanisms such as consumption reduction will have little effect due to little biomass being removed (Davis *et al.* 2000). It is also possible that in these communities resident and invader populations will equally absorb any resources added through disturbance, resulting in invader and resident growth rates increasing evenly (Hobbs & Atkins, 1988; Davis *et al.* 2000). As resources become more abundant however, we predict disturbance is likely to play a bigger role in promoting invader establishment as the predicted increase in productivity will see more resources held up in biomass and depleted by consumption: disturbance will release these (Davis *et al.* 2000).

More productive communities are also likely to have stronger biotic resistance, through mechanisms such as dominant species and priority effects (Hodgson *et al.* 2002; Fargione *et al.* 2003; Fukami, 2015), which will be weakened by decreased native population sizes. Communities with greater biodiversity are often found to be more resistant to invasions (Levine & D'Antonio, 1999; Hodgson *et al.* 2002; Tilman, 2004; Brockhurst *et al.* 2006). This is thought to be due to multiple factors, with more diverse communities more likely to: contain species that disproportionately decrease invader establishment, have less available niches, and have positive species interactions that increase productivity (Hodgson *et al.* 2002; Fukami, 2015). These factors decrease invasion success by reducing access to resources (Hodgson *et al.* 2002; Seabloom *et al.* 2003; Tilman, 2004; Emery & Gross, 2007). There is growing argument however, that this relationship may not be the case and diversity may actually facilitate invasions through increased niche dimensionality (Simberloff & Von Holle, 1999; Ricciardi, 2001; Green *et al.* 2011); leading to the invasion paradox (Fridley *et al.* 2007).

This study will test this resource-disturbance interaction hypothesis by invading experimental populations of the bacterium *Pseudomonas fluorescens* at different disturbance frequencies and resource abundances in a fully factorial design. *P. fluorescens* has successfully been used in the past to test invasion theories (Hodgson *et al.* 2002; Zhang & Buckling, 2016) as well as the causes and consequences of diversity (Rainey & Travisano, 1998; Gómez & Buckling, 2013). Biodiversity arises due to *P. fluorescens* diversifying into three genetically distinct morphotypes when introduced into spatially structured microcosms: an air-broth interface growing wrinkly spreader (WS), a broth inhabiting smooth (SM) and the rarer bottom-dwelling fuzzy spreader (Rainey & Travisano, 1998; Kassen *et al.* 2000; Spiers *et al.* 2002; Koza *et al.* 2011; Hall *et al.* 2012). This rapid evolution of diversity will allow us to determine any additional effect of evolved biodiversity mediated by the disturbance-productivity regime on invasion success; a factor commonly stated to influence invasion resistance (Naeem *et al.* 2000; Tilman, 2004). As disturbance and productivity have both previously been found to be unimodally related to diversity, and diversity has been shown to influence the success of invaders, we hypothesise quadratic relationships between them and invasion success.

Methods

Strains

Ancestral *Pseudomonas fluorescens* SBW25 was grown overnight in shaken microcosms containing 6ml of the growth medium King's medium B (KB) at 28°C.

60µl of this was then transferred into the microcosms used in the treatments; these contained KB diluted 100, 10 or 0-fold with M9 buffer (to create different resource abundances). Microcosms were disturbed every 1, 2, 4, 8 or 16 days (Fig. 1); this involved a 1% transfer of homogenised broth into fresh media (99% mortality). *Pseudomonas fluorescens lacZ* was used as the invader: which is visually distinguishable from the wildtype when X-gal is present due to a colour change (Zhang & Rainey, 2007). This was left to diversify for five days in KB before being plated and a single WS and SM morphotype selected, grown and frozen; these genotypes were used for each invasion. Invasions took place in all microcosms every four days post-disturbance with 60µl of either 100-fold diluted smooth or wrinkly invader added; a non-invader control was also used. During treatments all microcosms were kept static at 28°C with loose lids to allow oxygen transfer.

Experiments finished on day 16 when all microcosms were homogenised and a 900µl sample frozen in 900µl of 50% glycerol. Once plated on KB agar containing X-gal, wildtype and invader SM, WS and fuzzy spreaders morphotypes colonies were counted.

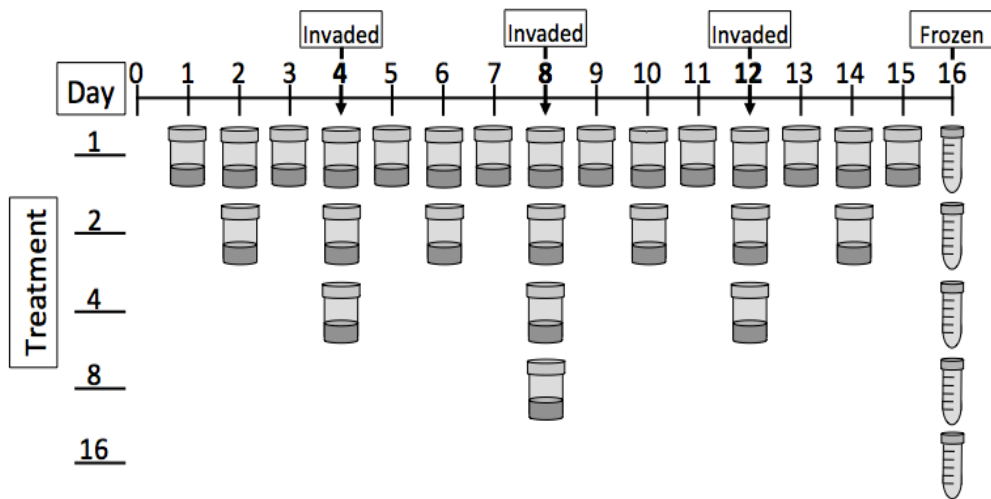


Figure 1 Treatments used: 1, 2, 4, 8 and 16-day disturbances at either 100, 10 or 0-fold resource dilution. All microcosms were invaded every four days and repeated six times.

Statistical Analysis

All counts were first standardised to per ml. Relative invader fitness (invasion success) was calculated as percentage change, v , of the proportion of invader to resident. V was used instead of direct proportion to control for different resident densities across the three resource treatments: as invader inoculation densities were consistent across all treatments it would be a lower proportion of the community when resident populations are more dense, for example when resources are abundant, compared to when resident populations are smaller. V was given by $v = x_2(1 - x_1)/x_1(1 - x_2)$, where x_1 is the initial invader proportion and x_2 the final (Ross-Gillespie *et al.* 2007). One was added to invader density (post volume standardisation) in order to eliminate zero inflation; v was transformed to $\log(v+1)$ to normalise the data. To analyse the effect disturbance plays on v at different resource abundances a model was fitted with interactions between resource abundance and disturbance, and disturbance as a quadratic term; a pairwise comparison was used to test if these terms differed between treatments.

An ANOVA was used to test treatment effects on evolved biodiversity: calculated using Simpsons index.

To test if resident populations influenced invader fitness an ANOVA was used with v as the response variable and disturbance, resource concentration and

biodiversity as explanatory variables; a linear regression was used to test the direction of any biodiversity interactions.

All statistical analyses were carried out in R (R Core Team, 2015).

Results

Invasion success (invader proportional change)

Disturbance and resource abundance significantly affected invasion success both as main effects ($F_{4,160}=14.6$, $p<0.0001$ & $F_{2,160}=22.6$, $p<0.0001$, respectively; Fig. 2), and through an interaction ($F_{8,160}=4.56$, $p<0.0001$). The quadratic relationships between disturbance and invasion success was significant ($F_{8,166}=15.5$, $p<0.0001$) in all resource treatments (Fig. 2); the relationship in the resource dense treatment significantly differed to the medium and low to $p<0.001$. Overall, the WS invader was more successful, having a greater invasion success ($F_{1,142}=4.14$, $p=0.044$; Fig. 2); invader morphotype significantly interacted with resource abundance ($F_{2,142}=7.23$, $p=0.001$) and disturbance ($F_{4,142}=9.00$, $p<0.0001$). however, the quadratic relationship between disturbance and invasion success did not differ between the two invader morphotypes ($F_{1,160}=0.031$, $p=0.86$): implying treatments had the same effect on each invader but were more pronounced in WS.

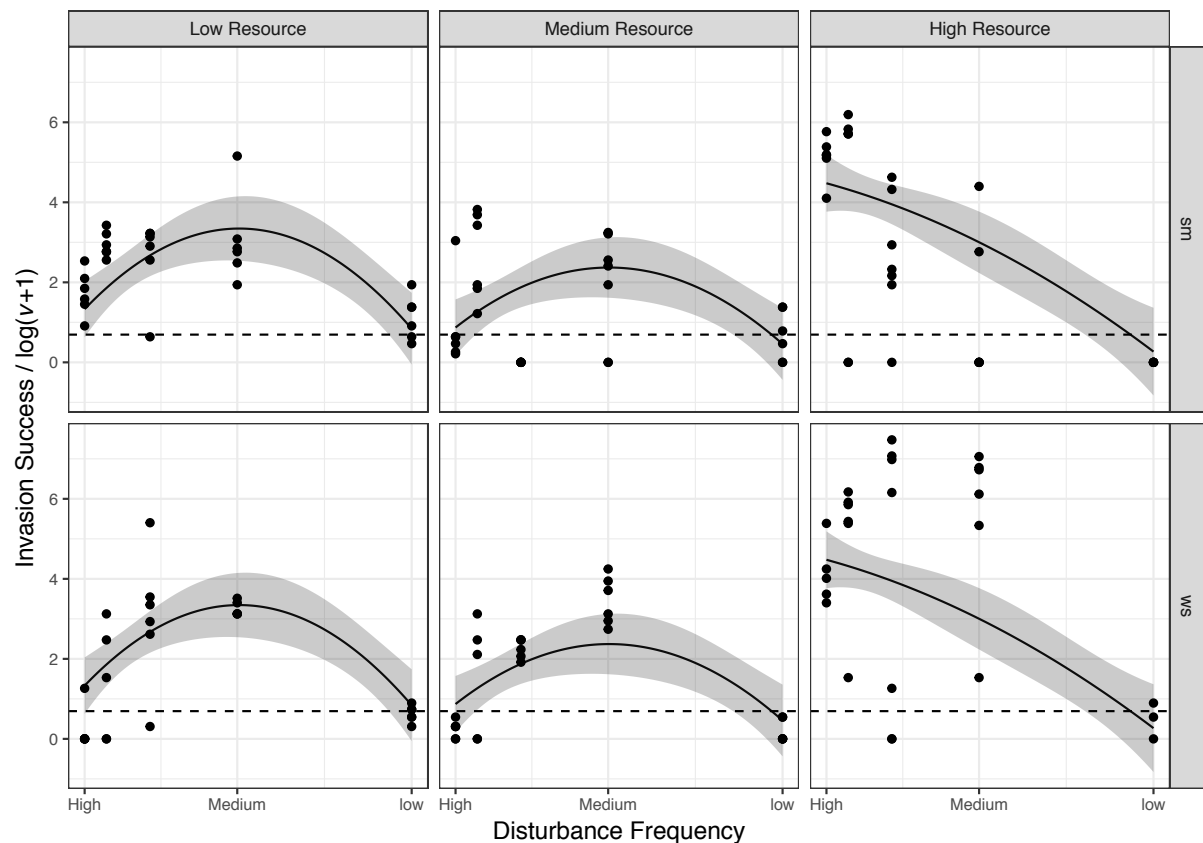


Figure 2 Invasion success, $\log(v+1)$, in response to different disturbance frequencies and resource abundances (abundances increase from left to right, frequency decreases left to right). V is the proportional change in invader density compared to the residents; the dashed line shows the value of equal population growth rate between residents and invaders after transformation (0.69). Top panels show the Smooth (SM) invader, bottom panels the Wrinkly Spreader (WS). Shaded areas show the 95% confidence interval.

Resident populations

Resident biodiversity differed between resource treatments ($F_{2,172}=22.2$, $p<0.0001$; Fig. 3), with high and medium being significantly more diverse than low ($p<0.0001$ for both), but not differing themselves ($p=0.42$). A significant unimodal relationship was found between disturbance and biodiversity ($F_{1,166}=18.0$, $p<0.0001$); this did not significantly interact with resource abundance ($F_{2,166}=0.60$, $p=0.55$).

Resident density significantly differed between resource treatments ($F_{2,172}=16.4$, $p<0.0001$; Fig. 4); this was due to the high resource treatment having

significantly more dense resident populations than the medium and low
($p < 0.0001$ for both).

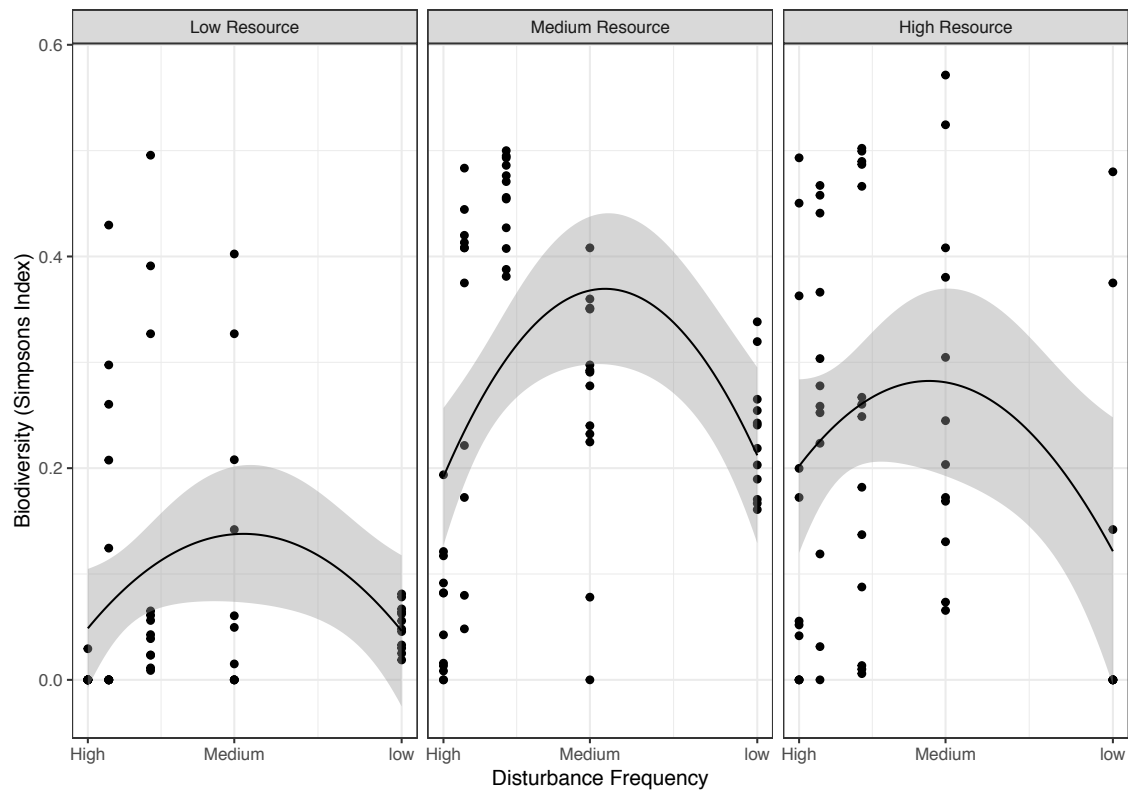


Figure 3 Evolved biodiversity in treatments of different resource abundances (increasing from left to right panels) and disturbance frequency (decreasing from left to right within panels). Biodiversity is calculated using the Simpsons index. Shaded areas show the 95% confidence interval.

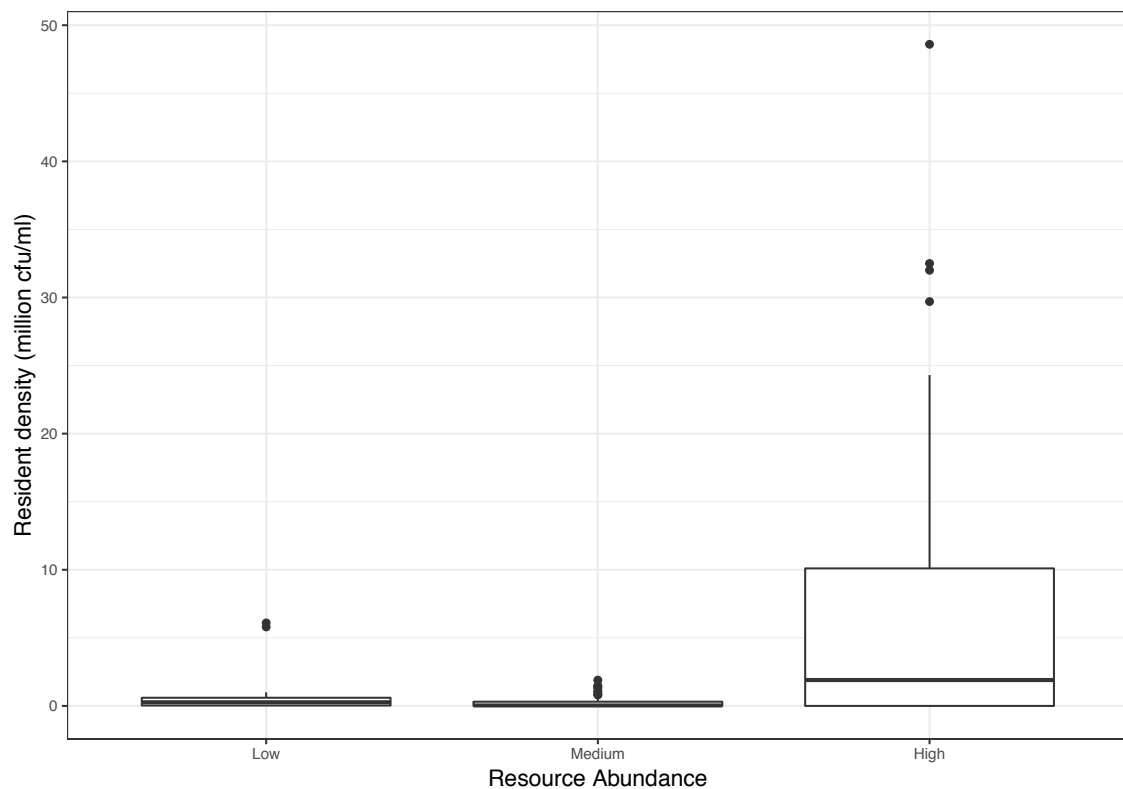


Figure 4 Density of resident populations after nine days in treatments of different resource abundances and disturbance frequencies; disturbance frequency data pooled to show densities per resource abundance. Density in the high treatment was significantly greater than the low and medium ($p < 0.0001$).

Linking Biodiversity and Invasion Success

Biodiversity had no significant main effect on invasion success ($F_{1,145}=1.45$, $p=0.24$). It did however, through a significant interaction with resource abundance ($F_{2,145}=3.10$, $p=0.048$, Fig. 5) show a negative relationship with invasion success in the high resource treatment ($F_{3,171}=5.45$, $R^2=0.071$, $p=0.009$). Biodiversity did not interact with invader morphotype ($F_{1,141}=3.50$, $p=0.064$).

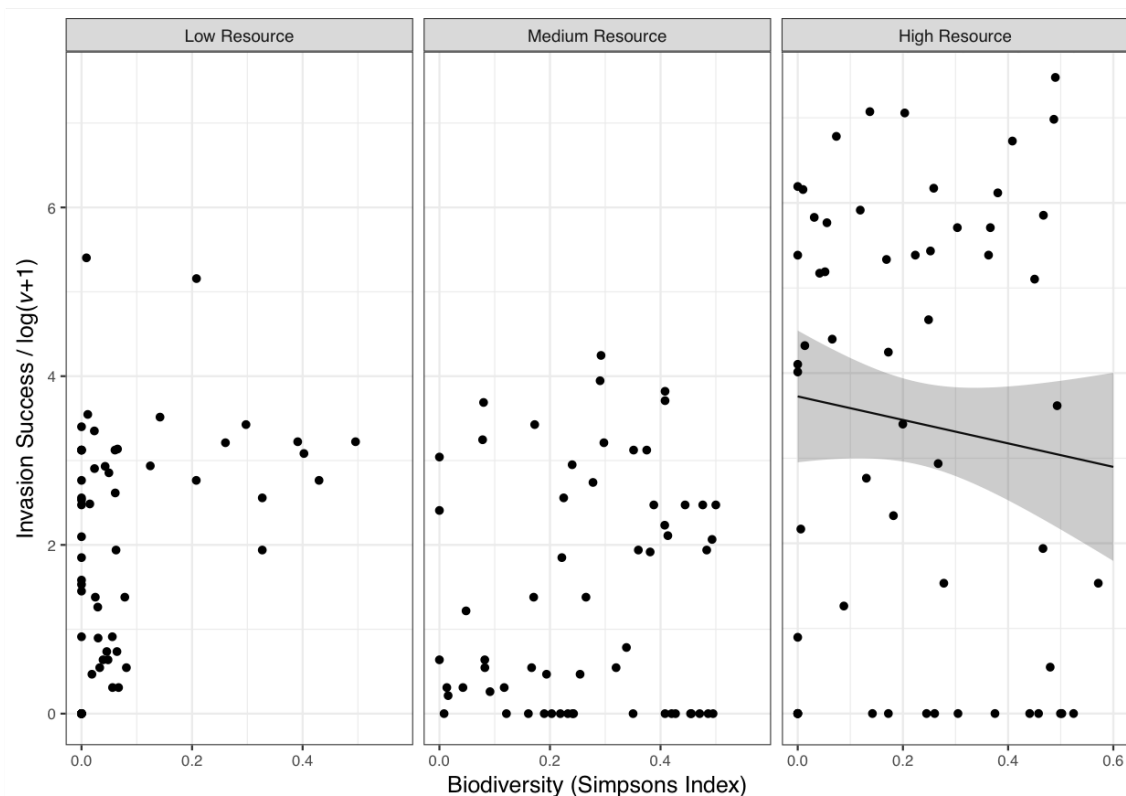


Figure 5 The effect of evolved biodiversity on invasion success in communities of varying resource abundance. Biodiversity is represented by the Simpsons index, invasion success by the proportional change, v , of both the SM and WS invader morphotypes combined; resources abundance increases from left to right panels. Regression lines are significant with shaded areas showing the 95% confidence interval.

Discussion

This paper aimed to determine how resource abundance affects the disturbance-invasion relationship, both directly and indirectly through evolved resident diversity. We found disturbance and resources to have direct effects both individually and through an interaction, as well as via manipulating resident biodiversity. Resource abundance was found to change the way disturbance affected invasion success.

The positive interaction between disturbance frequency and invasion success in the resource rich treatment shows the more 'windows of opportunity' an invader has to access resources, the more likely it is to succeed: each disturbance

opens up resources for the invader and reduces biotic resistance (Hodgson *et al.* 2002; Fargione *et al.* 2003; Fukami, 2015). The larger resource availability will allow high growth rates and populations to rapidly increase between disturbances, reducing the chance of small invader populations being stochastically removed by disturbance: an event likely to explain the low invasion success at high frequencies in the two lower resource treatments (Haddad *et al.* 2008). In this sense invader populations suffer allee effects, a fact shown by their negative proportional change in the majority of these treatments. This suggests the balance between disturbance-induced mortality and growth rate is an important factor deciding the outcome of the interaction, with resource abundance dictating growth rate and disturbance mortality. Invasion success dropping with decreasing disturbance frequency is most likely due to increasing biotic resistance from the growing resident populations and eventually the escalating broth toxicity. Although disturbance and resource availability affected the two invading morphotypes to different extents their overall impact was the same, showing these interactions occur in ecologically very different invading morphotypes: a fact likely explained by the mortality-growth reasoning being an ecologically fundamental process.

Disturbance timing and intensity have previously both been shown to affect community invasion resistance (Fasuch *et al.* 2001); here we show frequency to also matter, and the extent to which being dependent on community resource availability. It is probable intensity and resource availability will also interact, as greater intensities will open up resources analogously to increased frequencies (Hall *et al.* 2012). Compounding these findings show disturbances could be very effective conservation tools to reduce invasion risk if all factors are considered, and equally powerful invasion facilitators if wrong. We suggest frequencies and intensities should be calculated by considering the growth rates of the residents, with the highest frequency/greatest intensity disturbance that allows populations to recover to numbers capable of avoiding extirpation in the next disturbance being optimal.

The unimodal relationship between biodiversity and disturbance in our treatments supports the intermittent disturbance hypothesis (Connell, 1978; Wilkinson, 1999; Buckling *et al.* 2000; Cardinale *et al.* 2006; Benmayor *et al.*

2008; Viollea *et al.* 2010). This proposes diversity is lost at high disturbance due to species being unable to recover between events, lost at low through competitive exclusion and highest when disturbance facilitates a balance between tolerant and competitive species (Huston, 1979). In a similar way, diversity is low in resource poor environments due to few species being able to grow, medium abundances allow species to grow and coexist whereas high will once more lead to competitive exclusion (Huston, 1979). These patterns have previously been found in *P. fluorescens* (Kassen *et al.* 2004). Medium and high resource treatments' diversity not differing suggests the morphotype tolerant-exclusion balance was equal.

Biodiversity only having an effect when resource abundance was high could explain why some previous studies (e.g. Levine & D'Antonio, 1999; Hodgson *et al.* 2002; Tilman, 2004) found significant positive effects of diversity on invasion resistance whilst others (e.g. Simberloff & Von Holle, 1999; Ricciardi, 2001; Green *et al.* 2011) found negative. A possible explanation for this is that biodiversity in our study was a product of treatments rather than manipulation, suggesting the formation of diversity in the high resource treatment differed to the others. Biodiversity only having a significant effect when resources are easily obtainable could also be from biotic resistance increasing with resource availability: most likely due to their positive affect on resident density amplifying mechanisms associated with biotic resistance (Hodgson *et al.* 2002; Fargione *et al.* 2003; Fukami, 2015). These interactions are likely to sway from positive to negative depending on niche saturation, with saturation positively correlating with invasion resistance (Tilman, 2004). More diverse communities may contain more niche dimensionality (e.g. are less saturated) (Palmer & Maurer, 1997), which will allow an invader to obtain an empty niche and access resources (Simberloff & Von Holle, 1999); equally, more diverse communities may have less available niches due to residents occupying them (Shea & Chesson, 2002). As niche dimensionality decreases and saturation increases invaders will struggle to obtain enough resources to have a positive population growth rate and invasion resistance will increase (Shea & Chesson, 2002).

In conclusion, we find the effect disturbance has on invasion success to be highly dependent on community resource abundance, with interactions between

resources and evolved diversity further complicating outcomes. As our results imply these effects are due to ecologically fundamental processes, growth rate and mortality, and occur in the same way across two invader types, we suggest they are applicable outside of this microbial system. These complex interactions may go some way to explain the discrepancies found in previous disturbance-invasion literature. Finally, by understanding these interactions it may be possible, through ecological manipulations, to reduce the effect disturbances have on invasion resistance.

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Chapter 3: Effects of disturbance and resource abundance on the diversity of a stably coexisting microbial community

Abstract

Disturbance and resource abundance have both been shown to impact the formation and maintenance of biodiversity, yet if and how they interact remains uncertain. Stably coexisting communities exist when trade-offs and limited resource overlap occur between species for resources: allowing intra-specific competition to limit populations before inter-specific. It is unclear how disturbance and resources will affect these two competition types, either directly or through an interaction. Here, we test their effects using a stably coexisting community of five bacterial species in a 5x5 factorial design of disturbance and resource treatments. We find diversity to decrease with disturbance and to differ between resource treatments. Variables only interacted when resource abundance was in quadratic form, with the effect of resources differing between two disturbance treatments. We find disturbance to increase the variation in fitness amongst species: decreasing diversity through increasing inter-species competition relative to intra-species, and resources to both increase and decrease relative competition types. These results provide crucial evidence that stably coexisting communities may be under threat from the current global changes in disturbance regimes and resource abundances.

Introduction

The evolution and maintenance of biodiversity has long intrigued ecologists and evolutionary biologists alike, leading to it being the focus of many studies (Connell, 1978; Huston, 1979; Wilkinson, 1999; Buckling *et al.* 2000; Chesson, 2000; Kassen *et al.* 2000; Mackey & Currie, 2001; Fukami & Morin, 2003; Roxburgh *et al.* 2004). As biodiversity has been shown to play a fundamental part in stabilising communities: helping to aid invasion resistance, increase ecosystem services and maintain community dynamics, studying it could also have positive ecological and economic benefits (Hooper *et al.* 2005; Gamfeldt *et al.* 2013). Disturbance and resource abundance have both been shown

individually to strongly influence biodiversity (Srivastava & Lawton 1998; Kassen *et al.* 2000; Mackey & Currie, 2001; Fukami & Morin, 2003; Roxburgh *et al.* 2004; Hall *et al.* 2012), yet how these two factors interact remains uncertain (Huston, 1979; Kondoh, 2001; Haddad *et al.* 2008; Tonkin & Death, 2012).

Disturbances, defined as events that destroy biomass and consequently alter resource and niche abundances, are ubiquitous amongst communities and fundamental to structuring resident populations (Mackey & Currie, 2001; Roxburgh *et al.* 2004). These natural phenomena follow a regime described by their frequency - time period between events, their intensity - the degree of mortality and their extent - the size of the area effected (Hall *et al.* 2012; Miller *et al.* 2011). Disturbance frequency and intensity are key in shaping the disturbance-diversity relationship (DDR) of a community (Miller *et al.* 2011; Hall *et al.* 2012). These are often hump-shaped, but wide ranges of patterns have been observed (Mackey & Currie, 2001; Hodgson *et al.* 2002; Wilson & Tilman, 2002; Miller *et al.* 2011). The intermediate disturbance hypothesis (IDH) explains these unimodal relationships by hypothesising high disturbance frequencies to favour colonising species with high growth rates, low frequencies to favour competitively dominate species with efficient resource use and a balance of these types to occur at intermittent levels (Connell, 1978; Wilkinson, 1999; Buckling *et al.* 2000; Cardinale *et al.* 2006; Benmayor *et al.* 2008; Viollea *et al.* 2010).

The abundance of resources, which we define as any substance required by a species for positive population growth, will affect both the overall growth rate of a community and its productivity (assimilation of biomass) (Huston, 1979; Srivastava & Lawton 1998; Worm *et al.* 2002). Diversity has been shown to peak at intermittent levels of productivity in some studies (Kassen *et al.* 2000; Cardinale *et al.* 2006), not in others (Srivastava & Lawton 1998; Wilson & Tilman, 2002) and only under certain circumstances (Fukami & Morin, 2003). Resources are likely to be heavily competed for, with more adept competitors excluding others to the point of extinction. Niches arise when there is heterogeneity in the way resources can be accessed, with diversity usually being a function of niche abundance (Wilkinson, 1999; Wilson & Tilman, 2002).

Although the above factors have both been shown independently to influence biodiversity, it is becoming increasingly realised that single causal factors of biodiversity offer inadequate descriptions, and a multi-factor approach is more likely to offer an accurate representation (Fukami & Morin, 2003; Cardinale *et al.* 2006; Tonkin & Death, 2012). Many previous studies looking at the interaction have focused on communities that may not stably coexist (Agard *et al.* 1996; Chesson, 2000; Worm *et al.* 2002; Scholes *et al.* 2005; Cardinale *et al.* 2006; Tonkin & Death, 2012). A principal model for this is Huston's dynamic equilibrium model (DEM) (Huston, 1979; Agard *et al.* 1996). This expands upon the IDH by bringing in resource abundance: hypothesising how they may interact with disturbance regimes. It predicts communities are all heading towards a competitive equilibrium with low diversity: the rate of approach depending on the community's growth rate (productivity). Low growth rates allow diversity to be maintained for longer periods of time due to slower exclusion rates and an increasing likelihood of fluctuating selection. However, at extremely low growth rates diversity is again predicted to be low due to only a few species being able to survive. This hypothesis thus predicts a right-skewed, asymmetric 'hump' relationship between diversity and resource abundance. Disturbance is predicted to shift this peak: more severe to the right; less severe to the left, by stopping competitive exclusion occurring (Huston, 1979; Agard *et al.* 1996; Cardinale *et al.* 2006). Evidence for this model is mixed with both supportive (Agard *et al.* 1996; Cardinale *et al.* 2006) and unsupportive studies (Scholes *et al.* 2005; Tonkin & Death, 2012).

Though many of these predictions can theoretically be applied to stably coexisting communities there is little data confirming if this is so (Chesson, 2000; Kondoh, 2001). Coexistence is said to be stable when there are no long-term trends in species densities, with reductions in species' population numbers recovering from troughs (Chesson, 2000). A defining test of them is to invade communities with one species from rare: if it fully re-establishes the invasibility criterion is fulfilled and the community is said to be stable (Chesson, 2000). Stable coexistence can occur when intra-specific competition outweighs inter-specific. Inter-specific competition is based on R^* values: the lowest amount of a resource a species needs to persist, including to grow, reproduce and recover from biomass loss (Tilman, 1982; Chesson, 2000). The species with the lowest

R^* will win when the resource in question is limiting. However, if trade-offs mean that species' R^* values differ between resources, with one species having a lower R^* for one resource but a different species having a lower R^* for another, relative intra-specific competition can increase (Chesson, 2000). If there is limited resource overlap between species and trade-offs in the way each resource is utilised exist, then competition may become greater within species (intra-specific) than between (inter-specific). Meaning, as a species' population grows it becomes limited, and reduced, by its own density before it can competitively exclude other species. When resources are unevenly spread niches are created: such that a species' fitness is high (low R^*) in one niche and low (high R^*) in another; resulting in little niche overlap. An important addition to this is that each niche provides approximately equal proportions to the population (Kassen *et al.* 2000). Factors that decrease the variance of fitness between species (i.e. reduce inter-specific competition) can be referred to as equalising mechanisms and factors that increase relative intra-specific as stabilising mechanisms: increasing either of these leads to a higher chance of coexistence (Chesson, 2000). How disturbance and resource abundance affect these equalising and stabilising mechanisms has yet to be fully understood, despite much theoretical modelling (Chesson, 2000; Kondoh, 2001). If we apply concepts from non-equilibrium theory, we can make the following predictions:

Disturbances should make R^* values bigger as resources are needed to recover from biomass loss. However, if a species is more disturbance tolerant than other community members, for example by being more invested in generating offspring than growing large, it's R^* value may increase less and thus would become relatively lower: decreasing equalising terms and allowing it to contribute a larger proportion to the overall population whilst less-tolerant species recover. However, increasing resource abundance may counter this if its positive affect on colonisation rates mean less-tolerant species' populations recover quicker post-disturbance. This would make higher productivity an equalising mechanism for increasing disturbance: as predicted in the DEM.

Very high resource abundance is likely to decrease equalising mechanisms as trade-offs in resource acquisition will be less apparent: one niche will provide a higher proportion of individuals to the community resulting in a few large

populations taking over through competitive exclusion (Kassen *et al.* 2000, 2004; Worm *et al.* 2002). Frequent disturbance here will negate the change in equalising mechanisms by reducing interactions through lowering population densities, stopping competitive exclusion happening.

At intermittent productivity levels there is likely to be resource partitioning, creating a heterogenous environment in which niche numbers will increase: resulting in selection for specialists. This will increase the likelihood of coexistence by decreasing resource-overlap and amplifying trade-offs. Frequent disturbances here are predicted to restrict niche formation: lowering speciation and stopping the equalising term increasing. Low disturbance may allow one niche to eventually contribute disproportionately to the population: intermediate disturbance is favoured.

When resource abundance is low biodiversity is predicted to decline if resources drop below a species' R^* value for it. When this occurs generalists may begin to take over from niche-specialists due to their lower overall R^* from accessing multiple resource sources with limited trade-offs. Selection for a generalist would reduce coexistence by lowering both equalising and stabilising mechanisms. Disturbances will exaggerate this fact by destroying any remaining niches: low disturbance is favoured.

This study will test these predictions using a stably coexisting community of five bacterial species: *Pseudomonas lini*, *Stenotrophomonas rhizophilia*, *Achromobacter agilis*, *Variovorax guangxiensis* and *Ochrobactrum daejonense*. This has been shown to have stable coexistence, with each species being able to successfully invade from rare (fulfilling the invasion criterion) and the community coexisting for the full twelve weeks previously tested (Chesson, 2000; Pennycook *et al.* in press). We can manipulate energy input easily by diluting the growth medium. These facts distinguish it from previous microcosm studies of this interaction which only looked at genotypic responses (Kassen *et al.* 2004) or indirectly manipulated resource abundance (Scholes *et al.* 2005); both of which had coexistence through negative frequency-dependent selection. This system allows global, non-specific disturbances; multiple species, all in the same trophic level and no influence of metapopulation dynamics. It also allows

the use of very large populations in highly controllable and therefore repeatable conditions, which combined with asexual reproduction means genetic drift and hybridisation can be eliminated as factors effecting diversity (Kassen *et al.* 2004). We will test this system in a fully factorial design containing five disturbance frequencies ranging from 2 to 32 days, and five resource abundancies: 1/16-1/256 diluted growth medium; the intermittent value of both, 8-day disturbance in 1/64 growth medium, being based on the regime previously shown to be ecologically stable (Pennycook *et al.* in press).

Methods

Experimental communities

The bacterial species used in our community were: *Pseudomonas lini*, a gram-negative species occupying the rhizosphere (Delorme *et al.* 2002); *Stenotrophomonas rhizophilia*, a gram-negative species associated with both the rhizosphere (Hagemann *et al.* 2008) and plant tissue (Wolf *et al.* 2002), *Achromobacter agilis*, a nitrogen-fixing species from rhizospheric soil (Vandamme *et al.* 2016; Latt *et al.* 2018); *Variovorax guangxiensis*, a bacterium first isolated from the rhizosphere associated with banana plants (Gao *et al.* 2015) and *Ochrobactrum daejonense*, a gram-negative species first isolated from leachate treatment sludge (Woo *et al.* 2011).

Experimental design

Each of the five species was grown in monoculture in microcosms containing 6ml of the growth medium tryptic soy broth (TSB) for three days. After this period, optical density measurements were used to calculate the number of cfu per ml, based on previous measurements. A mastermix was then created with equal cfu numbers of each species. This was plated on King's medium B (KB) agar to confirm densities were equal and used to inoculate the microcosms used in the treatments. 25 treatments were used: five different resource abundances decreasing by a factor of two from 1/16th to 1/256th TSB and five disturbance frequencies doubling from every 2 to 32 days, with the 32-day treatment not being disturbed. All treatments were repeated six times and finished on day 32. Disturbances involved a 1% transfer of homogenised broth into fresh media, giving 99% mortality. Microcosms had loose lids to allow

oxygen transfer, were static and kept at 28°C throughout. On day 32 all treatments were thoroughly homogenised and frozen in 50% glycerol, defrosted and plated on KB agar.

Statistical Analysis

Cfu of each species were counted and standardised to per 25µl. Biodiversity was calculated using the Simpsons index. Resource and disturbance effects were tested using an analysis of variance with biodiversity as the response variable and resource and disturbance the explanatories. A similar model was used to test the effects on both total and individual species densities. All statistical tests were carried out in R (R Core Team, 2015).

Results

Community properties

Disturbance frequency and resource abundance significantly affected resident density both as main effects ($f_{4,124}=24.7$, $p<0.0001$ and $f_{4,124}=33.5$, $p<0.0001$, respectively; Fig. 1) and through an interaction ($f_{16,124}=5.76$, $p<0.0001$).

Biodiversity was also affected by both disturbance and resources ($f_{4,124}=7.4$, $p<0.0001$ and $f_{3,124}=6.6$, $p=0.0004$, respectively). There was no interaction between the linear term for resource abundance and disturbance ($f_{12,124}=1.1$, $p=0.39$), but was for the quadratic term ($f_{4,124}=3.3$, $p=0.012$): due to them differing between the 2 and 4-day disturbance treatments ($df=136$, $t.ratio=3.3$, $p=0.001$). This is despite the quadratic terms themselves being non-significant ($f_{1,124}=0.10$, $p=0.76$). Diversity increased linearly with decreasing disturbance frequency ($f_{4,144}=6.7$, $p<0.0001$).

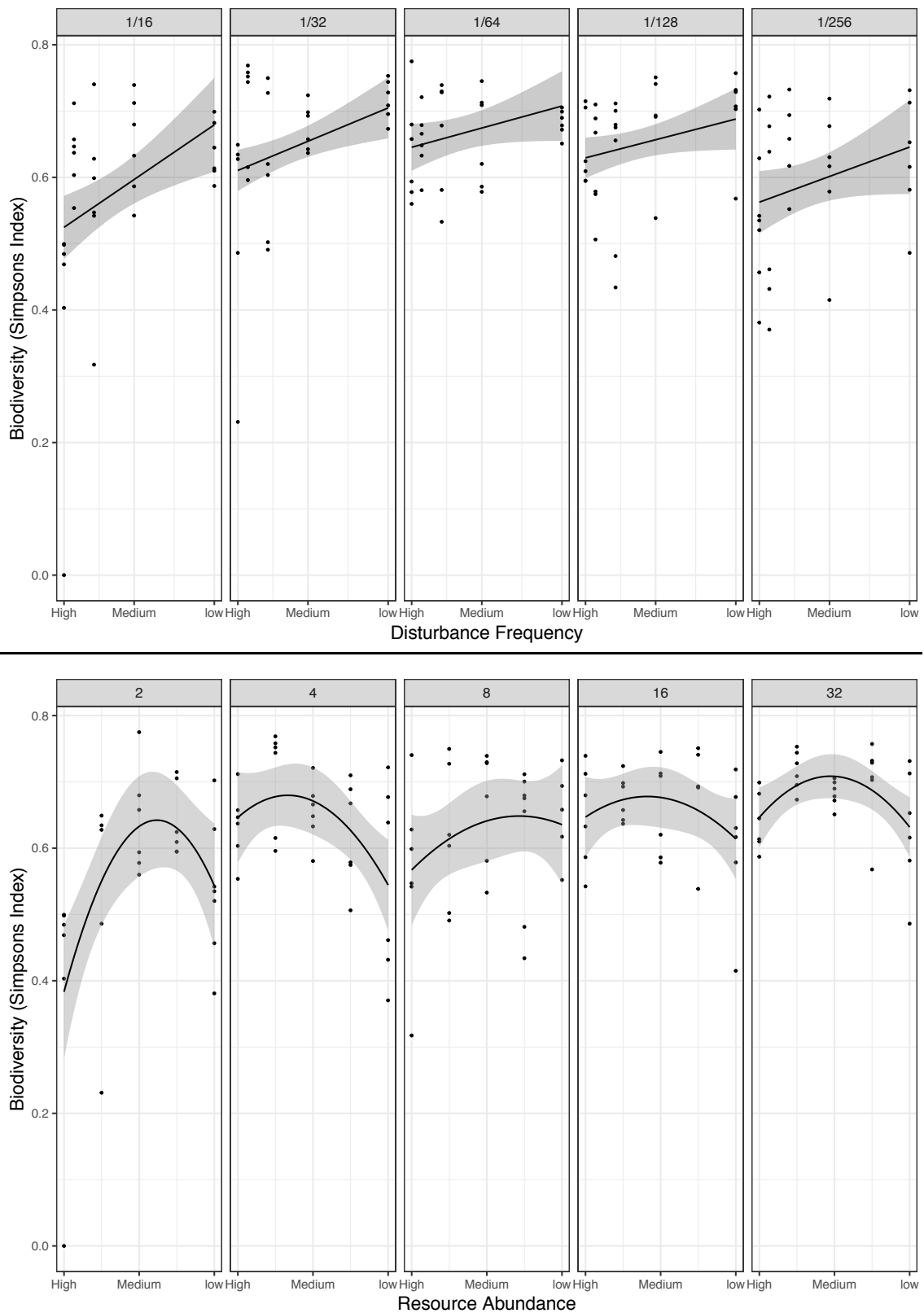


Figure 1 Biodiversity of five species in response to different disturbance regimes and resource concentrations. Top panels show the effect of disturbance within each resource treatment (resource abundance given as fraction diluted); bottom panels the effect of resource concentration within each

disturbance treatment. Disturbance frequency and resource abundance decreases from left to right. Biodiversity is measured using the Simpsons index. Regression lines in the top panel are significant to $p < 0.0001$; in the bottom panel the quadratic term in the 2-day disturbance treatment significantly differs to 4-day, but these do not differ to any others. Shaded areas show the 95% confidence interval.

Population properties

As treatments manipulate diversity by altering the populations of each species we looked at how these were affected. Disturbance, resources and their interaction affected all species except *V. guangxiensis*, which was only affected by resources; effects differed between species (table 1; Fig. 2). All species became extinct during some treatments: *O. daejonense* showed no trend, *V. guangxiensis* and *P. lini* showed opposing effects disappearing in the high and low resource treatments respectively; *S. rhizophilia* and *A. agilis* suffered in the high-high and low-low extremes. Effects were seen greatest in *P. lini*, which became extinct in $\sim 1/3^{\text{rd}}$ of treatments: the majority in low resources. Peak *S. rhizophilia* density shifted from low to high disturbance along a decreasing resource gradient. A decline in species density saw a decline in overall community density in all species except *V. guangxiensis* (table 1); this suggests different species are best suited to certain combinations of treatments.

Table 1 Significance of disturbance regime, resource abundance and their interaction on the densities of five bacterial species in a community; **bold** show non-significant effects. Effect on total density describes whether their density affected total density: if non-significant it suggests they were replaced by other species.

Genus	Disturbance affect	Resource affect	Disturbance* Resource interaction	Effect on total density
<i>Pseudomonas</i>	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$
<i>Ochrobactrum</i>	$p = 0.0004$	$p < 0.0001$	$p = 0.022$	$p < 0.0001$
<i>Stenotrophomonas</i>	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$
<i>Achromobacter</i>	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$
<i>Variovorax</i>	$p = 0.072$	$p = 0.0002$	$p = 0.071$	$p = 0.26$

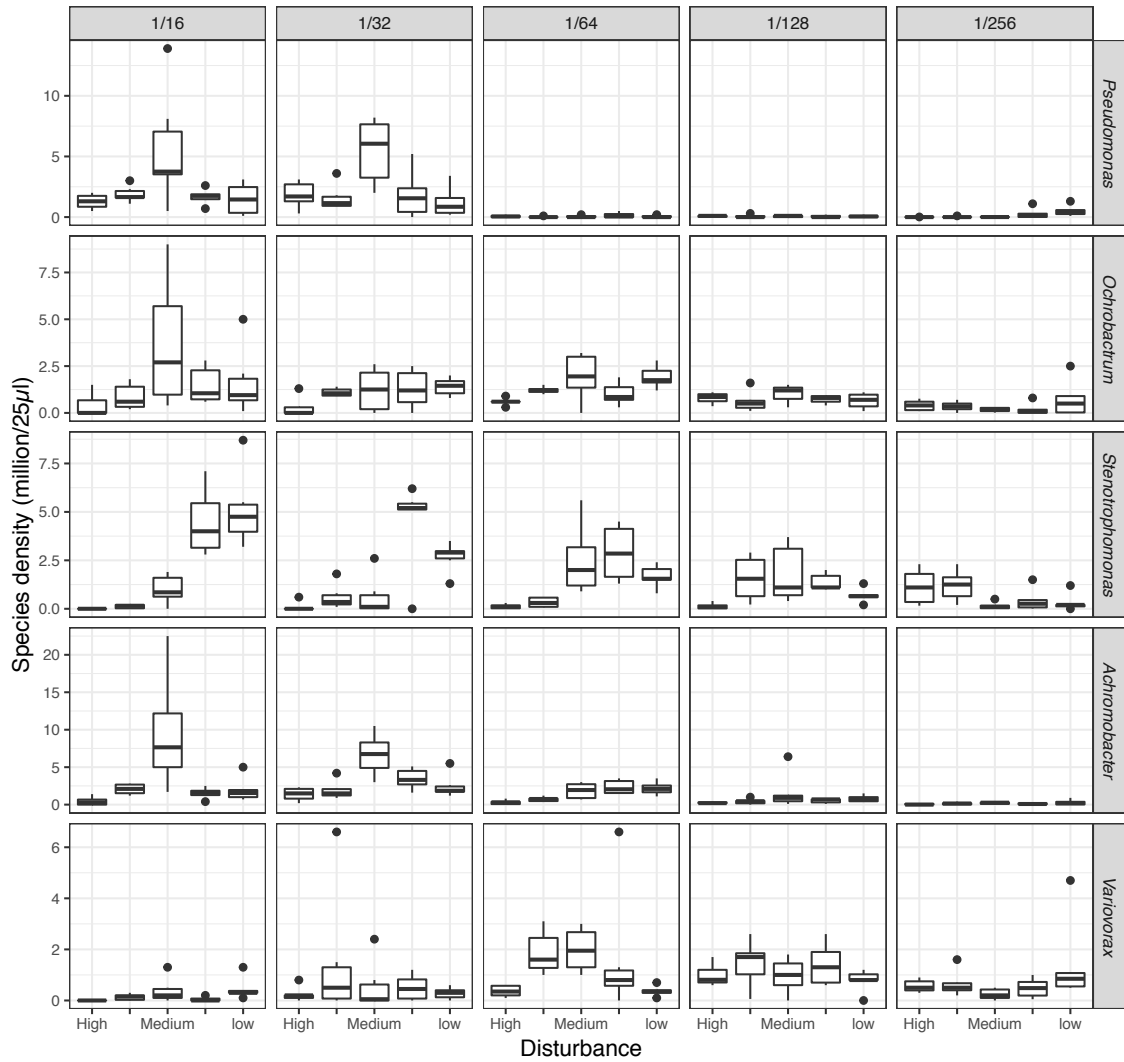


Figure 2 species densities (million cfu per 25µl) after exposure to different disturbance regimes and resource abundances. Resource abundance decreases from left to right panels by a factor of two each time (fraction diluted shown); disturbance frequency decreases from left to right within each panel.

Discussion

We found disturbance frequency and resource abundance to significantly alter the diversity of a stable bacterial community, showing both alter the variation in fitness between species. The linear terms did not interact suggesting the changes in equalising mechanisms by one variable are neither amplified nor offset by changes in the other; a surprising result. We did find however, an interaction between the quadratic term for resource abundance and disturbance

due to differences between two treatments: 2-day disturbance had considerably less diversity than 4-day at high resource abundance. Finding each variable to play major roles but not to interact along the whole disturbance and resource gradient is consistent with some previous work in non-equilibrium communities (Death, 2002; Haddad *et al.* 2008; Tonkin & Death, 2012), but not with others (Wilson & Tilman, 2002) or the DEM (Huston, 1979; Agard *et al.* 1996; Cardinale *et al.* 2006).

Disturbance frequency having a significant negative linear effect on biodiversity is consistent with previous non-equilibrium work (Death, 2002; Haddad *et al.* 2008) and implies disturbance is reducing equalising mechanisms by allowing more tolerant species to contribute greater proportions to the overall population. This may also be due to resource partitioning taking time to occur after disturbance, resulting in a delay in the formation of niches and their subsequent positive effects on coexistence. Niches arise when oxygen gradients, which naturally form in liquids but are amplified by the metabolic activities of bacteria, occur in the broth causing it to become spatially structured (Kassen *et al.* 2004). It could be by removing these gradients through re-oxygenising the broth that disturbance is manipulating equalising affects; a fact shown to be the case in populations of *P. fluorescens* (Kassen *et al.* 2004). Biodiversity is lost at high frequencies when species with slower growth rates become proportionally less represented after each disturbance. This causes a positive feedback loop to occur whereby smaller populations are stochastically more likely to have proportionally larger population reductions and thus become extinct (Haddad *et al.* 2008); this is seen clearest here in *S. rhizophilia* during the resource-dense treatments. This means a slight drop in equalising mechanisms will be amplified after each disturbance, as fitter species will become more and more represented. Species face an inherent trade-off between competitive ability in low-disturbance communities and colonisation rate in high, so those that suffer at high disturbance (e.g. here *S. rhizophilia*) are predicted to dominate when disturbance is low compared to biomass gain (Pianka, 1970; Cardinale *et al.* 2006; Haddad *et al.* 2008). This is due to sufficient time to colonise niches even with their slow growth or dispersal rate (Cardinale *et al.* 2006). The trade-offs here will decrease both stabilising and equalising mechanism as smaller populations will have less intra-specific competition and the better adapted

species with have a fitness advantage, further reducing community stability. The disturbance-diversity result not fitting with the unimodal prediction of the IDH is not rare amongst the literature (Mackay & Currie, 2001; Death, 2002; Haddad *et al.* 2008), and indicates disturbance usually reduces equalising mechanisms. One of our study species, *V. guangxiensis*, was not affected by disturbance. This could be due to visually different morphotypes being seen (unrecorded observations), leading to the prediction it adapts to disturbance regimes in a similar way to *Pseudomonas fluorescens* (Buckling *et al.* 2000; Kassen *et al.* 2004).

Resource abundance significantly affecting biodiversity is in line with previous studies (Wilson & Tilman, 2002; Kassen *et al.* 2004; Cardinale *et al.* 2006; Tonkin *et al.* 2012). Diversity is manipulated by resources regulating the strength of species interactions, including inter-species competition (Kassen *et al.* 2000; Cardinale *et al.* 2006). At higher resource abundances growth rates increase and potentially result in one niche providing more individuals to the overall population than a niche of previously equal fitness; rapidly increasing the variation in species' fitness (Chesson, 2000; Kassen *et al.* 2000; Haddad *et al.* 2008). This leads to an increased chance of species extirpation which, when coupled with less colonisation events, results in fewer species being present (Wilson & Tilman, 2002). When resources are low diversity is reduced due to species with larger R^* values, usually those able to dominate under high resource abundance, being unable to acquire enough for a positive population growth rate (Pianka, 1970; Chesson, 2000; Haddad *et al.* 2008): this was seen in *P. lini* which frequently became extinct in low resource treatments but dominated high.

Although we find the way resource abundance affects diversity to differ between 2 and 4-day disturbance treatments, we do not find an interaction between disturbance and resources during any other treatment, implying only when these variables are very high do they begin to interact in stable communities. This lack of an interaction between variables across all treatments is surprising as they are predicted to offset or amplify each other across their gradients. A likely explanation for this is that effects differed between each species, with different species doing better in high, medium and low treatments of both

variables. This means that disturbance and resource abundance are not acting as equalising mechanisms as they increase the variation in fitness between each species, with no overall peak in diversity due to each individual species' peak density being in a different place along each variable axis. A way to test this hypothesis would be to expand either the time period of the experiment or the treatment extremes (higher and lower resource abundances and disturbances frequencies) in order to increase the chance of extinctions occurring and cause diversity to decrease.

In conclusion, we find disturbance and resource abundance to alter equalising mechanisms in stable communities, resulting in changes in diversity. We find an interaction to only occur when both variables are at their highest extreme and that this reduces diversity. These results suggest this system's diversity is manipulated in two phases: first disturbance acts as an equalising mechanism, sorting by disturbance resilience; then resource abundance acts as a second equalising mechanism on the remaining populations to decide final diversity. At high disturbance frequency there is not enough time between disturbances for these processes to be separated, resulting in them interacting. We provide useful data on this new study system and demonstrate its potential for further disturbance, resource and biodiversity manipulation studies. Finally, we demonstrate how stably coexisting communities may react to changes in disturbance and resource abundances, which is crucial in a time where increasingly occurring global changes in resource availability and disturbance regimes are likely to bring changes to community diversity (Davis *et al.* 2000).

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Discussion

The aim of this thesis was to further our understanding of how disturbances may affect community invasion resistance, and how other factors may interact to alter these effects. In the first chapter we used bacterial populations to disentangle two key components of disturbance: resource and habitat opening. We find evidence that after biomass is destroyed by a disturbance it is the subsequent increase in resource availability that allows novel species to establish, rather than the opening of habitat. The separation of these variables is a useful step in creating a better picture of how invasion events occur, as although both variables will inevitably change during a disturbance, the relative amount is likely to differ between disturbance types. For example, communities exposed to a flood disturbance will have different resource changes if the flood-water contained nutrient-runoff from agricultural land compared to non-polluted water; similarly, static and flowing flood-water can have different effects on the abundance of resources such as nitrates (Unger *et al.* 2009). These two flood-types can also have different effects on habitat opening, with flowing flood water likely to remove the majority of dead biomass and leave soil bare: whereas static water will usually leave behind a more complex habitat containing debris. The ability to fully separate these closely intertwined variables whilst also being able to observe the impacts both on and from resident populations highlights the benefits of using microbes in these ecological studies. As this is the first study to factorial separate these factors of disturbance it naturally raises more questions for future study: is it the change in resource abundance that matters, or the relative change? If post-disturbance habitat differs to pre and residents are left maladapted, does habitat opening have more of an effect? Showing the diversity of the five-species community used in chapter three responds to disturbance and resource treatments, we recommend its use to answer these questions as stably coexisting multi-species communities are potentially more representative of natural communities than morphotypic variation within populations.

Chapter two expands upon the first by exploring how the abundance of resources within a community alters the effect of disturbance frequency. We found them to interact, with resource abundance altering the growth rate-

mortality balance of the invaders. Faster growth in higher resources enabled them to quickly 'bounce back' from disturbances and achieve success even at the highest disturbance frequency. These findings are supportive of those in the first chapter, as the influx of resources per disturbance would be greater in higher resource treatments. This also supports Davis *et al*'s (2000) fluctuating resource theory of invasion biology and shows reduced competition for resources can allow an invader to establish itself by gaining enough resources to grow and reproduce (Tilman, 2004).

Resource abundance and evolved biodiversity were found to interact, with biodiversity only having an effect in high-resource treatments. This suggests that the mixed results found in previous biodiversity-invasion literature can be explained by the lack of control over other interacting factors such as resource abundance. Further, it suggests when a biotic disturbance, such as invader establishment, causes extirpations of species the consequential loss of biodiversity may result in positive feedback loops to occur whereby future invasions are more successful (Levine & D'Antonio, 1999; Sher & Hyatt, 1999; Fukami, 2015). As mid and high resource treatments have the same diversity, but only in high does this have an effect, further study is needed into why this is so. It is probable resident density plays a role: we could not test this in this study due to resident density being included in our term for invasion success, v . This finding also shows us that even communities statistically as diverse as one another can have different biodiversity-induced invasion resistances, suggesting biodiversity may not be a suitable measure of biotic invasion resistance, and studies solely focusing on its role are potentially overlooking covarying factors. These results do also suggest the mechanisms determining how diversity evolves, such as disturbance and resources, are more important deciders of invasion resistance than diversity per se. Thus, testing hypothesis on factors interacting with diversity would be better taking place in the stable community used in chapter three, as diversity can be equal across treatments at the start; meaning it is only manipulated not created by treatments: a more representative study when looking at how changes in disturbance regime will affect invasion success.

Chapter three at first may appear unrelated to the first and second, but the use of a stable multi-species community will be a great advantage for future disturbance-resource-diversity studies, including invasion work. These findings also raise concerns that the current global changes to disturbance regimes and resource abundances may alter the stability of coexisting communities. In conclusion, we find the effect of disturbances on the establishment of novel species to be highly dependent on resources. We raise further questions regarding their interactive effects and provide evidence that a stable, coexisting community may offer a better way to test these.

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