Productivity gradients cause positive diversity–invasibility relationships in microbial communities

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Abstract
Models predict that community invasibility generally declines with species diversity, a prediction confirmed by small-scale experiments. Large-scale observations and experiments, however, find that diverse communities tend to be more heavily invaded than simple communities. One hypothesis states that large-scale environmental heterogeneity, which similarly influences native and invasive species, can cause a positive correlation between diversity and invasibility, overriding the local negative effects of diversity on invasibility. We tested this hypothesis using aquatic microbial communities consisting of protists and rotifers consuming bacteria and nanoflagellates. We constructed a productivity gradient to simulate large-scale environmental heterogeneity, started communities with the same number of species along this gradient, and subjected equilibrail communities to invasion by non-resident consumer species. Both invaders and most resident species increased their abundances with resource enrichment, resulting in a positive correlation between diversity and invasibility. Intraspecific interference competition within resident species and the positive effect of enrichment on the number of available resources probably accounted for the higher invasibility with enrichment. Our results provide direct experimental evidence that environmental heterogeneity in productivity can cause a positive diversity–invasibility relationship.

Keywords
Biological invasions, community invasibility, environmental heterogeneity, invasive species, species diversity.

INTRODUCTION
Biological invasions of non-native species pose a serious threat to native species and ecosystems (D’Antonio & Vitousek 1992; Vitousek et al. 1996; Mack et al. 2000; Sakai et al. 2001; Grosholz 2002). Invasive species are ranked as the second leading cause of global biodiversity loss (Wilcove et al. 1998), and have caused and will continue to cause tremendous economic losses (Pimentel et al. 2000). It is, therefore, of considerable interest among ecologists and conservation biologists to understand ecological mechanisms underlying biological invasions, particularly those regulating the success and failure of invading species.

Many factors can potentially influence the susceptibility of a community to biological invasions (invasibility), including evolutionary history, habitat suitability and disturbance, community structure, and propagule supply. Local species diversity, as part of community structure, has long been thought to play an important role in determining community invasibility (Elton 1958). The classic view is that species diversity should enhance community resistance to biological invasions, as apparently first proposed by Elton (1958). Although largely based on anecdotal evidence, Elton’s proposition is strongly supported by subsequent models (Robinson & Valentine 1979; Post & Pimm 1983; Case 1990; Drake 1990; Law & Morton 1996; but see Moore et al. 2001) and by small-scale experimental manipulations of local species diversity (McGrady-Steed et al. 1997; Knops et al. 1999; Stachowicz et al. 1999, 2002; Levine 2000, 2001; Naeem et al. 2000; Symstad 2000; Kennedy et al. 2002). In contrast, large-scale observational studies and experiments conducted along natural diversity gradients often found that diverse communities tend to be more heavily invaded than simple communities (Planty-Tabacchi et al. 1996; Wiser et al. 1998; Lonsdale 1999; Stohlgren et al. 1999, 2003; Levine 2000, 2001; reviewed in Levine & D’Antonio 1999; Levine...
et al. 2002). These seemingly contradictory findings between studies conducted at local and larger regional scales call for an understanding of underlying mechanisms behind the observed diversity–invasibility patterns.

Two biological mechanisms, the sampling effect (Huston 1997; Tilman et al. 1997; Wardle 2001) and the resource use complementarity effect (Tilman et al. 1997; Tilman 1999), can account for the negative relationship between diversity and invasibility found in small-scale experiments. What causes the positive diversity–invasibility relationship in large-scale studies is less understood. One hypothesis emphasizes the importance of large-scale heterogeneity in environmental factors (henceforth the environmental heterogeneity hypothesis), and suggests that environmental factors influencing native species diversity, such as nutrient availability, propagate supply, and disturbance, often have a similar effect on invasive species, provided that native and invasive species are not strikingly different in key features of their ecology. Positive diversity–invasibility trends emerge because similar responses of native and invasive species to environmental heterogeneity over large scales often overwhelm the negative effect of diversity on invasibility at small scales (Huston 1994; Levine & D’Antonio 1999; Stohlgren et al. 1999; Naeem et al. 2000; Wardle 2001; Levine et al. 2002; Shea & Chesson 2002). This environmental heterogeneity hypothesis is supported by a recent model demonstrating that variation in the number of available resources could turn the negative diversity–invasibility relationship at small scales into a positive relationship at large scales (Byers & Noonburg 2003).

Although intuitively appealing, the environmental heterogeneity hypothesis has not been directly tested by experiments. Here, we take the first step in the experimental exploration of whether large-scale environmental heterogeneity could generate a positive relationship between species diversity and community invasibility. As in many other studies (e.g. Stachowicz et al. 1999; Levine 2000), we operationally define invasibility as the ability of invaders to increase when rare and become established, which can be operationally measured by the numerical abundance of invaders after a standard interval of time. We conducted the experiment in aquatic microcosms, where we could readily manipulate productivity levels and monitor responses of consumers (protozoa and rotifers). We established a productivity gradient to simulate large-scale among-community environmental heterogeneity, started resident communities with the same number of species along the gradient, and then invaded equilibrial resident communities with low densities of a ‘novel’ consumer/invader. We show that after small-scale within-community species interactions, both resident species richness and invader abundances still responded positively to increased productivity, resulting in a positive relationship between diversity and invasibility.

MATERIALS AND METHODS

A total of 18 protozoan and rotifer species were used in the experiment, including 15 ciliates: Coleps sp., Colpidium striatum, Colpoda cucullus, Colpoda sp., Halteria sp., Loxocephalus sp., Paramecium bursaria, Paramecium caudatum, Paramecium tetraurelia, Spirostomum sp. (large), Spirostomum sp. (small), Tetrahymena pyriformis, Tetrahymena thermophila, Urotricha sp. and one unidentified hypotrich, two rotifers: Lepadella sp. and Rotaria sp., and one flagellate: Chilomonas sp. All species use bacteria and nanoflagellates as food resources, under the conditions of the experiment. The generation times of these species are generally short, ranging from hours to days. Most of the species were isolated from natural ponds, while a few were obtained from the Carolina Biological Supply Company (henceforth CBS; Burlington, NC, USA) or the American Type Culture Collection (Rockville, MD, USA). Each species had been maintained in stock cultures separately for many generations before the experiment.

Two species, Coleps and P. bursaria, were randomly selected as model invaders to challenge resident communities. Resident communities, termed community A and community B for convenience, were initially assembled from the species pool without each invader. Community A contained each species in the species pool except for the small Spirostomum, the unidentified hypotrich, and the invader Coleps, totalling 14 species. Community B contained every species in the species pool except for Halteria, and the invader P. bursaria, totalling 15 species. Communities A and B were assembled at different times, and the difference in their composition reflects the availability of species in stock at the time of assembly, though they still had many species in common.

The experimental microcosms were 250 mL Erlenmeyer flasks, each containing 100 mL of nutrient medium. We made the medium by dissolving CBS protozoan pellets in filtered well water, and used different concentrations of the medium to manipulate productivity levels (see below). We sterilized the flasks and medium before the experiment. The medium was inoculated with three bacterial species: Serratia marcescens, Bacillus cereus and Bacillus subtilis, all obtained from CBS, and with an assemblage of nanoflagellates collected from the Rutgers University Display Garden Pond (New Brunswick, NJ, USA). Resident consumers were introduced into the microcosms 24 h after the inoculation of bacteria and nanoflagellates. A minimum of 20 individuals was introduced for each consumer species to enhance the probability of their initial establishment.

Previous work has shown that abundance of bacterial resources is positively correlated with the concentration of the nutrient medium (Kaunzinger & Morin 1998; Fox et al. 2002). We created a productivity gradient consisting of
Repetitive-measures ANOVAs were used to analyze invasion (10 mL).
The abundance of rare species in a larger sample volume with fresh medium every week to replenish nutrients and Japan. We replaced 10% of the medium in each microcosm number of individuals of each species in the sample with a medium from each well-mixed microcosm and counted the richness and abundance. We withdrew for community A and 8 weeks for community B.

Each microcosm was sampled weekly to monitor species richness and abundance. We withdrew 0.35 mL of the medium from each well-mixed microcosm and counted the number of individuals of each species in the sample with a Nikon SMZ-U microscope (Nikon Corporation, Tokyo, Japan). We replaced 10% of the medium in each microcosm with fresh medium every week to replenish nutrients and reduce accumulation of metabolic wastes. The medium removed was also inspected to obtain a better estimate of the abundance of rare species in a larger sample volume (10 mL).

Invader abundances (density, measured as number per millilitre) were used as the measure of invasion success. Repeated-measures ANOVAs were used to analyze invasion success over time. Nonparametric Spearman correlation coefficients (r) were calculated to relate invasion success to resident species richness, as the data violated assumptions of parametric analyses. We used pre-invasion resident species richness (week 4) in all analyses because post-invasion resident species richness is likely to be affected by the invading species (Levine & D’Antonio 1999). Using post-invasion resident species richness in analyses does not change the results. All the species abundance data were log transformed [log_{10} (abundance ml^{-1} + 1)] to reduce heteroscedasticity.

RESULTS

Patterns in community A

After 4 weeks of community development, four resident species (Colpoda sp., Halteria sp., P. caudatum and T. pyriformis) went extinct in all microcosms, regardless of productivity levels. Of the 11 resident species present in week 4, six increased in abundance with enrichment, one (Chilomonas) was detected at the two highest productivity levels, two (Lacooephalus and large Spirostomum) were only present at one productivity level, and two (C. eucullus and Rotaria) were present at most productivity levels but did not change their abundances along the gradient (Fig. 1). Only four species were present at the lowest nutrient level, in contrast with eight species at the three highest nutrient levels (Fig. 2a). ANOVA confirmed that resident species richness increased with productivity (Fig. 2a; one-way ANOVA, productivity: F_{9,20} = 9.99, P < 0.0001). Note that for the six species that showed significant positive responses to enrichment, abundances reached a plateau at higher levels of productivity (Fig. 1).

The invader Colpods attained higher abundance at higher nutrient levels (Fig. 2b, c; one-way repeated-measures ANOVA, nutrient: F_{9,20} = 17.43, P < 0.0001). Repeated-measures ANOVA also revealed a significant time effect (one-way repeated-measures ANOVA, time: F_{2,40} = 7.28, P = 0.002), primarily because of the general decline in Colpods abundance from week 6 to week 7 (Fig. 2b). Although Colpods abundance increased from week 6 to week 7 for some productivity levels (Fig. 2b), the interaction between time and productivity was not significant (one-way repeated-measures ANOVA, time × nutrient: F_{18,40} = 1.67, P = 0.0893).

Because both invader abundance and resident species richness increased with productivity, there was a strong positive correlation between pre-invasion species richness and invasibility (measured by the abundance of the invader) at the end of experiment (Fig. 2d; Spearman rank coefficient r_s = 0.83, P < 0.0001). This relationship disappeared after the effect of productivity was taken into account using a partial correlation analysis (partial Spearman rank coefficient r_s = 0.26, P = 0.1679).

Patterns in community B

After 4 weeks of community development, four of the resident species (Colpoda sp., T. pyriformis, T. thermophila and the unidentified hypotrich) became extinct in all microcosms, regardless of nutrient levels. The specific extinction patterns differed from those seen in community A, where P. caudatum became extinct, but T. thermophila did not. Of the 12 resident species present in week 4, eight increased in abundance with productivity, Lacocephalus was present at three highest nutrient levels, small Spirostomum was present only at two productivity levels, Rotaria showed a positive, albeit nonsignificant response to enrichment, and C. eucullus was unaffected by productivity (Fig. 3). Consequently, as in community A, resident species richness increased significantly with increasing productivity in community B (Fig. 4a; one-way ANOVA, productivity: P < 0.0001).
Figure 1 Effects of nutrient enrichment (increasing productivity) on the abundances of 11 resident species present in week 4 in community A. Values are mean ± 1 SE. The $P$-value reported in each panel is from ANOVA for the effect of nutrient enrichment. For species significantly affected by enrichment, mean abundances at sufficiently high productivities under the horizontal bar do not differ significantly ($P > 0.05$, Tukey’s Studentized Range test). Species are listed in the increasing order according to their body size, i.e. Uronema < Chilomonas < Tetrahymena < Loxocephalus and so on.
$F_{9,20} = 4.70, P = 0.0019$). Note that for the eight species that showed significantly positive responses to enrichment, abundances reached a plateau at higher levels of productivity (Fig. 3).

Abundance of the invader, *P. bursaria*, increased at higher productivity levels (Fig. 4b,c; one-way repeated-measures ANOVA, productivity: $F_{9,20} = 6.72, P = 0.0002$). *P. bursaria* abundance increased continuously during the post-invasion period (Fig. 4b), as shown by the significant time effect in the repeated-measures ANOVA (one-way repeated-measures ANOVA, time: $F_{2,40} = 685.01, P < 0.0001$). There was also a significant interaction of time and productivity (one-way repeated-measure ANOVA, time · productivity: $F_{18,40} = 2.26, P = 0.0161$).

As in community A, a significant positive correlation emerged between pre-invasion resident species richness and invasibility at the end of experiment (Fig. 4d), Spearman rank coefficient $r_s = 0.37, P = 0.0458$), because of their similar responses to enrichment. This apparent relationship, however, vanished after the effect of productivity was taken into account (partial Spearman rank coefficient $r_s = -0.25, P = 0.1865$).

**DISCUSSION**

Our experiment clearly demonstrated that large-scale environmental heterogeneity in the form of variation in productivity across communities could lead to a positive relationship between diversity and invasibility. A positive diversity–invasibility correlation emerged in two different communities, where both resident species diversity and invader abundance responded positively to nutrient enrichments that manipulated productivity. These findings provide the first direct experimental evidence for the environmental heterogeneity hypothesis proposed to account for the positive diversity–invasibility relationship observed in some natural communities (Huston 1994; Levine & D’Antonio 1999; Stohlgren et al. 1999; Naeem et al. 2000; Wardle 2001; Levine et al. 2002; Shea & Chesson 2002).

One important question is why the abundance of the invaders continues to increase with enrichment, given that resident communities established long before invasions took place (in terms of generation times) had the potential to reduce resources to uniformly low levels, regardless of the initial level of enrichment. Our results suggest two possible explanations. First, many resident species appeared to be regulated by intraspecific interference competition, because their similar responses to enrichment. This apparent relationship, however, vanished after the effect of productivity was taken into account (partial Spearman rank coefficient $r_s = -0.25, P = 0.1865$).
high nutrient levels, where abundances of many resident species failed to increase with further enrichment (Figs 1 and 3). In our system, intraspecific interference may be caused by production of growth inhibitors or waste products from conspecifics. Intraspecific interference competition was also reported for several bacterivorous ciliates in another microcosm study (Fox 2002). Intraspecific interference competition can also occur in terrestrial plant communities, where most invasion studies have been conducted. For example, plants may affect the survival and growth of their conspecific neighbours by releasing toxins or growth inhibitors. Interference competition may play a role in positive diversity–invasibility relationships found in natural plant communities, but direct evidence for it is scant.

Second, enrichment may have promoted invader abundances by increasing the number of different resources available to consumers, which may have been limited at low productivity. Models show that both resident species diversity and invasion success can respond positively to increases in the number of resources, which can create a positive relationship between diversity and invasibility (Byers & Noonburg 2003). Because we did not directly monitor resources in our experiment, we were unable to directly evaluate the relevance of this mechanism. Future work examining how productivity affects resource diversity should address this question.

The assumption that native and invading species will respond similarly to nutrient enrichment is essential to generate a positive diversity–invasibility relationship. In the present study, the invaders as well as most resident species showed similar positive responses to increasing resource supply. However, a few resident species appeared to be unaffected by resource availability. For example, C. cucullus, a resident species in both communities A and B, showed virtually no response to productivity (Figs 1 and 3). One characteristic that separated C. cucullus from other species in

Figure 2 Patterns of resident species richness and invader (Coleps) abundance in community A. (a) Resident species richness along the nutrient (productivity) gradient just before Coleps invasion; (b) Responses of Coleps abundance over time at different nutrient levels; different symbols represent different nutrient levels; (c) Coleps abundance along the nutrient gradient on the final sampling date (week 7); (d) Coleps abundance vs. resident species residence. Values are means ± 1 SE in panels (a)–(c).
Figure 3 Effects of nutrient enrichment (increasing productivity) on the abundance of 12 resident species present in week 4 in community B. Values are mean ± 1 SE. The $P$-value reported in each panel is from ANOVA for the effect of nutrient enrichment. For species significantly affected by enrichment, mean abundances at sufficiently high productivities under the horizontal bar do not differ significantly ($P > 0.05$, Tukey's Studentized Range test). Species are listed in the increasing order according to their body size, i.e. Uronema < Chilomonas < Loxocephalus < Colpidium and so on.
the experiment is its life cycle. *C. cucullus* can quickly form and emerge from dormant cysts (Fenchel 1987), which can uncouple abundance from productivity. Had we used *C. cucullus* as invaders, we probably would observe that neither productivity nor resident species richness affected its invasion success. The idea that invading species may possess unique physiology or life history characteristics and show different responses to environmental change is not new. For example, Huenneke et al. (1990) reported that native and exotic plant species in California serpentine grassland, which have different resource use strategies, responded differently to nutrient enrichment.

One potential caveat in this study is that the two resident communities were not substantially different in composition. This compositional similarity reflected our decision to arbitrarily choose one species as the invader and use the rest species in the species pool as resident species for each community, in an effort to create a large diversity gradient. Nonetheless, our general findings should not change if communities with little species overlap were used, because most of the members in the species pool showed similar positive responses to increases in resource supply.

Reviews indicate that species diversity can show various responses (positive, negative, hump-shaped or no pattern) to increased productivity in natural communities (Waide et al. 1999; Mittelbach et al. 2001). Numerous explanations have been proposed to account for the observed productivity–diversity patterns (Rosenzweig & Abramsky 1993; Tilman & Pacala 1993; Fukami and Morin 2003). In the context of biological invasions, the key question remains how the productivity–diversity relationship affects community invasibility. It can be argued, though, that a positive diversity–invasibility relationship could be common along productivity gradients, independently of the emergent productivity–diversity patterns. This is because the same ecological processes regulating native species abundance and diversity should have similar effects on exotic species abundance and diversity, provided native and exotic species are not markedly different (Huston 1994; Wardle 2001).

Previous studies have either focused on how species diversity changes with productivity or how diversity affects community invasibility. Our finding suggests that studies of how community invasibility and species diversity both change along a productivity gradient may be particularly illuminating.

In a recent synthesis, Davis et al. (2000) emphasized the fundamental importance of resource availability for invading species and argued for a productivity-based framework for invasion. They suggest that increasing availability of limiting resources, either by direct fertilization or by reduced resource consumption through disturbance, would increase community invasibility. This idea is supported by the results.
of our experiment and several field experiments where resource availability was manipulated (Huenneke et al. 1990; Burke & Grime 1996; Davis & Pelsor 2001). There is also some evidence that nutrient-rich natural communities are more likely to be invaded by non-native species as compared to their nutrient-poor counterparts (Wiser et al. 1998; Stohlgren et al. 1999). This resource-based approach may also help reconcile the apparent contradiction between the negative diversity–invasibility relationship in diversity-manipulation experiments and the positive diversity–invasibility relationship in observational studies as well as natural experiments. We suggest that a common positive resource–invasibility relationship should be behind both types of studies, because high diversity generally reduces resource availability in diversity-manipulation experiments under initially homogenous environmental conditions, and high resource availability generally promotes diversity in natural communities under initially heterogeneous environmental conditions. Finally, along with other authors (e.g. Leibold et al. 2004), we suggest that additional progress in understanding the causes of invasions and other questions in community ecology will come from careful synthesis of processes operating at local (e.g. richness within communities) and regional (e.g. productivity levels among communities) scales.

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