The Dissertation Committee for George F. Livingston Certifies that this is the approved version of the following dissertation:

Using Microcosms to Bridge Metacommunity Theory with Natural Patterns

Committee:

Mathew Leibold, Supervisor

Lawrence Gilbert, Co-Supervisor

Tim Keitt

Christine Hawkes

Jeremy Fox

Using Microcosms to Bridge Metacommunity Theory with Natural Patterns

by

George F. Livingston, B.S.

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Dedication

This work is dedicated to my family, Yuexin, Artie, Sue, Francis, Melba, Junjia, Meilan, and Yuzhen and, in memory, to Xingshu and Kay.

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Using Microcosms to Bridge Metacommunity Theory with Natural Patterns

George F. Livingston, Ph.D.

The University of Texas at Austin, 2014

Supervisors: Mathew Leibold and Lawrence Gilbert

Metacommunties are sets of interacting species embedded in landscapes and interconnected via dispersal. The development of metacommunity theory has greatly outpaced its experimental testing. This situation restricts the feedbacks between theory and natural systems, hindering the development of useful theory and limiting application of theory to natural patterns. My dissertation aims to accelerate the testing of metacommunity theory using three microcosm experiments ranging from highly to more loosely constrained. The first experiment implemented a competition-colonization tradeoff between two strains of bacteria and tested if the tradeoff produced the expected patterns of coexistence and ecosystem function. Generally, the results conformed closely to theoretical expectations, though high stochasticity limited coexistence. The second experiment utilized multi-trophic protist communities to test if assembly history followed by complete mixing can produce situations where one

community replaces another. Results indicate that community replacement can occur under mixing, though it may be buffered by trophic structure. The third experiment tested the ability of variance partitioning to attribute landscape patterns to process in a one-predator two-prey system. Results indicate that both predators and dispersal can generate similar spatial patterns. Distinguishing between the two requires explicitly incorporating the predator into the partition. In summary, each of these three experiments reinforces aspects of existing theory while illuminating new paths for future theoretical and empirical exploration.

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Chapter 1: Competition-colonization dynamics in experimental bacterial metacommunities

Abstract: One of the simplest hypotheses used to explain species coexistence is the competition–colonization trade-off, that is, species can stably coexist in a landscape if they show a trade-off between competitive and colonization abilities. Despite extensive theory, the dynamics predicted to result from competition–colonization trade-offs are largely untested. Landscape change, such as habitat destruction, is thought to greatly influence coexistence under competition–colonization dynamics, although there is no formal test of this prediction. Here we present the first illustration of competition–colonization dynamics that fully transposes theory into a controlled experimental metacommunity of two *Pseudomonas* bacterial strains. The competition–colonization dynamics were achieved by directly manipulating trade-off strength and colonization rates to generate the full range of coexistence conditions and responses to habitat destruction. Our study successfully generates competition–colonization dynamics matching theoretical predictions, and our results further reveal a negative relationship between diversity and productivity when scaling up to entire metacommunities.

Introduction

Competition–colonization (CC) trade-off models predict that species can coexist in landscapes with patch turnover by means of spatial niche partitioning[1, 2, 3, 4]. Under CC dynamics, species can occupy a 'colonization niche' by efficiently colonizing empty habitat patches (that is, 'fugitive species') or a 'competition niche' by outcompeting other species within sites[5]. Depending on allocation trade-offs, such as those determined by life history, these alternative strategies (that is, 'colonizers' versus 'competitors') reduce the ratio of interspecific to intraspecific competition and allow coexistence to occur without environmental heterogeneity among patches[6]. Although early CC models[1, 2] somewhat unrealistically assumed strict trade-offs, including habitat homogeneity and the absence of both patch pre-emption and stochasticity, CC model predictions have been shown to be applicable to situations of greater complexity[4, 7].

CC models predict that changes to landscape structure impact coexistence even in the absence of changes in environmental conditions at the local scale[8]. A key prediction of such models is that habitat destruction (or any other environmental change that reduces overall colonization rates) should preferentially suppress superior competitors or drive them extinct, whereas increasing the regional abundance of inferior competitors[8]. If these landscape-level processes are resolved on long time scales, then extinction debts (that is, species committed to extinction) are expected to develop[9]. It is also likely that changes in the prevalence of competitively dominant species could alter ecosystem functioning if such species are more productive than inferior competitors[5].

Despite a general acceptance of the theoretical framework, to our knowledge there is no direct experimental treatment of the general predictions of the CC theory; in fact, indirect tests that attempted to document the existence of the CC trade-off have produced contradictory results in experimental[10, 11, 12, 13] and observational studies of natural species assemblages[14, 15, 16, 17, 18]. Moreover, because the existence of a CC trade-off in an assemblage does not necessarily indicate that CC dynamics are determinant, indirect approaches have limited power to evaluate the theory.

In this study, we present the first experimental illustration of a CC dynamic by manipulating the trade-off strength and colonization rate in an experimental system of *Pseudomonas fluorescens* bacteria in 96-well plate metacommunities. *Pseudomonas* has emerged as a model for experimental ecology and evolution[19, 20, 21]. We first tailored the general CC model[2, 3] to our experimental system, which allowed us to make quantitative predictions regarding the conditions under which different patterns of coexistence and dominance are predicted in the metacommunity. We constructed and manipulated the two key mechanisms of CC dynamics in the model (trade-off strength and colonization rate) within a controlled setting to impose an experimental CC dynamic. We subsequently tested the predicted consequences of this dynamic for coexistence, landscape change and ecosystem function by monitoring strain persistence, local dominance, patch occupancy and metacommunity productivity. Our experiment is thus a targeted test of CC dynamics; however, it is an implementation rather than a test of underlying mechanisms.

Results

Experimental system

We used two *Pseudomonas* bacterial strains (analogous to ecological species[22]), a strong competitor ('competitor') and a poor competitor ('colonizer'), to generate a strict asymmetry of competition in local patches (that is, the colonizer is completely excluded from local patches). The rationale underlying the use of a two-strain system is consistent with other existing experimental and empirical studies of the CC (for example, [13, 15, 18]) and a variety of other studies that use microbes to investigate ecological phenomenon (for example, [23, 24, 25]). We manipulated the strength of the CC trade-off by controlling the colonization of the two strains independently; both strains could have the same colonization rate in the experiment (no trade-off treatment) or the colonization rate of the colonizer could be strongly increased relative to the competitor (strong trade-off treatment). The manipulation of colonization rate was achieved by diluting each strain when building the 'colonizers pool' (when inoculating a new microplate between two time steps of our experiment); greater dilution reduced the number of cells that entered the colonizers pool and consequently reduced the probability that a strain would successfully colonize each inoculated well (see Methods and Supplementary Methods 1).

In contrast to most CC models that assume continuous colonization processes, we aimed to conduct our colonization treatments at discrete-time intervals for logistical feasibility. To achieve this goal, we needed to derive a discrete version of the CC

competition model that we could parameterize for our experimental system (see Methods and Supplementary Methods 1). The following experiments and model conditions were implemented. An initial metacommunity was inoculated with each of the two strains in half of the wells of a 96-well microplate. Every 24 h, the regional abundances (that is, microplate scale) of the two strains were estimated and used to build a 'colonizer pool,' which was subsequently used to inoculate a new microplate containing fresh medium. The relative contribution of the two strains in the colonizer pool and the dilution of this pool were adjusted before each transfer (Fig. 1.1), to manipulate the absolute and relative colonization rates (and thus the strength of the CC trade-off) of the two strains in the metacommunity. This procedure ensured that our experimental manipulations remained constant throughout the experiment, but that potential deviations from the predicted trajectories in the preceding transfers would be taken into account in the computations of the colonization pool. The overall dilution ensured that colonization rates were low enough to approximate limited or near-limited dispersal and generate an exponential growth phase. To prevent any possible confounding of evolutionary changes in each strain from affecting the dynamics, at each new transfer, the bacterial colonization pool was reconstituted from initial frozen stock and not from the bacteria of the previous transfer. Using this experimental setting and our model (Fig. 1.2, see Methods), we predicted the outcome of metacommunity dynamics under different trade-off and colonization scenarios (Fig. 1.3). We subsequently assigned our primary experimental treatments along this parameter space to generate all predicted outcomes, which consisted of the exclusion of the colonizer or

competitor, coexistence and the extinction of both strains (Supplementary Table 1.1). In general, our experimental results closely corresponded to the predictions of our model.

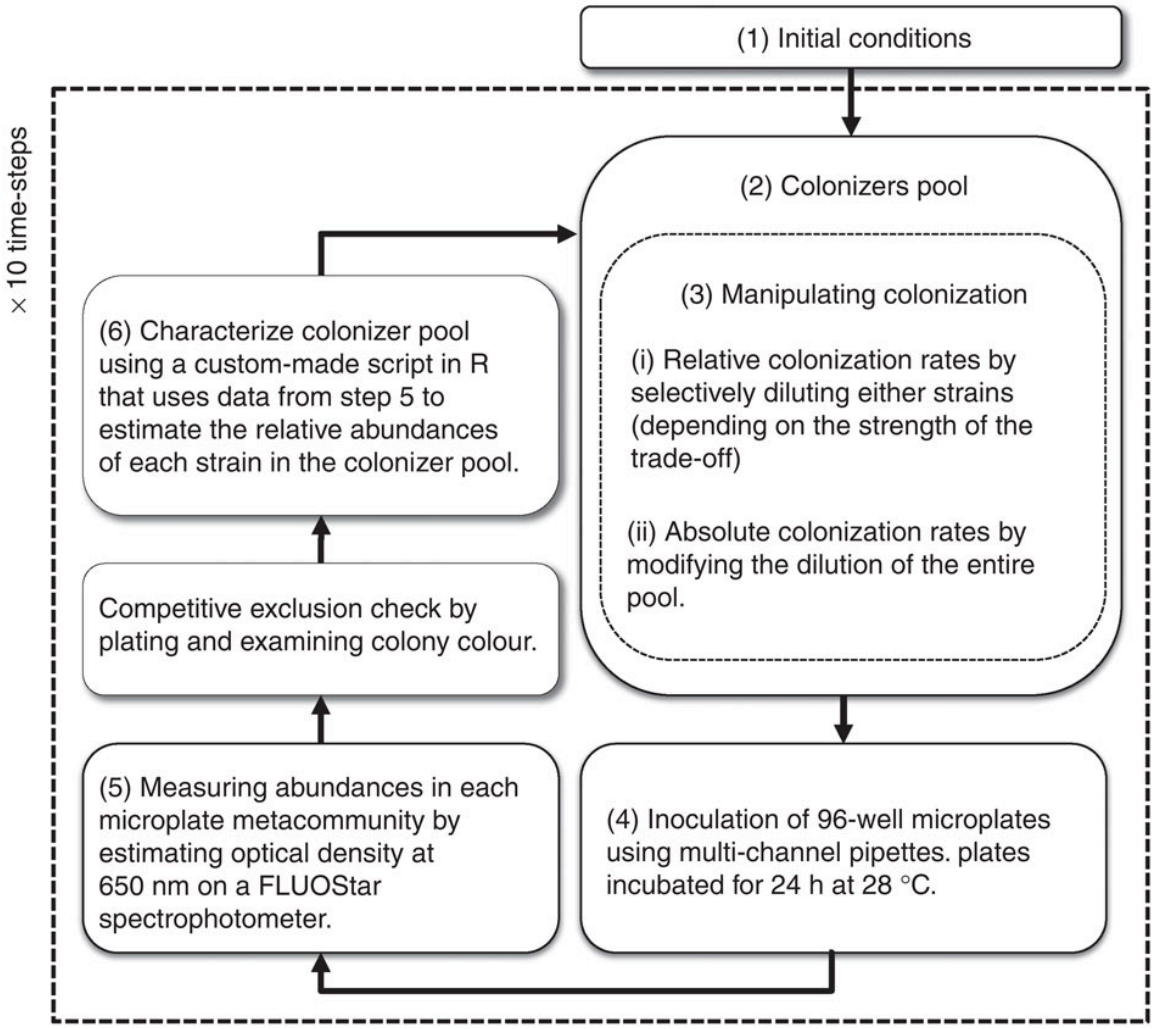

Figure 1.1: **Implementing competition–colonization trade-offs.** The experiment consisted of six basic steps: (1) set up an initial metacommunity with equal proportions of both strains in all wells (48 wells at random occupied by each strain); (2) build a new 'colonizer pool' based on the regional abundances of the two strains after every 24 h; (3) modify the relative contribution of the two strains in the colonization pool and the dilution of this pool according to each experimental treatment (see Supplementary Table 1.1) to manipulate the absolute and relative colonization rates (and thus the strength of the CC trade-off) of the two strains in the metacommunity; and finally (4) inoculate new metacommunities (that is, new microplates containing fresh media); (5) measuring abundances in each microplate metacommunity by estimating optical density; presence/absence results were confirmed via plating. Finally, (6) characterize colonizer

pool using data from step 5 to estimate the relative abundances of each strain in the colonizer pool. This experimental procedure was run for ten transfers.

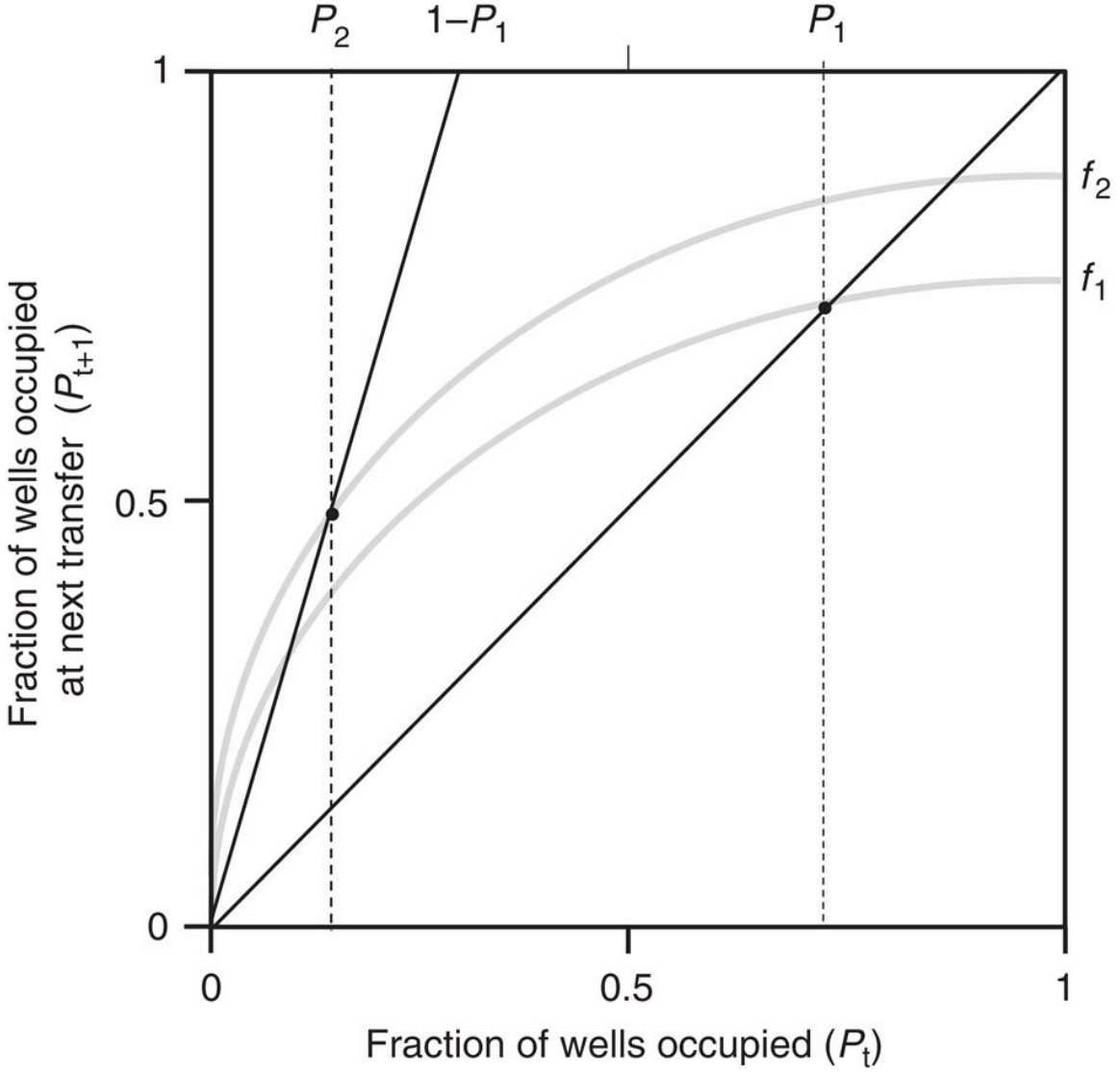

Figure 1.2: Cobweb plot for the patch occupancy model. Patch occupancies after one transfer (P_1 for strain 1 and P_2 for strain 2 at $t+1$) are functions of their current values through the probability of successful colonization following dispersal (f_1 and f_2). The equilibrium is stable provided that f is concave ($f'' < 0$), and under this condition, the persistence conditions are $f'_1(0) > 1$ for strain 1 and $f_2'(0) > 1/(1-P_1)$ for strain 2. One can determine the equilibrium occupancy of the competitor (P_1) and, subsequently, the equilibrium occupancy of the colonizer (P_2), given P_1.

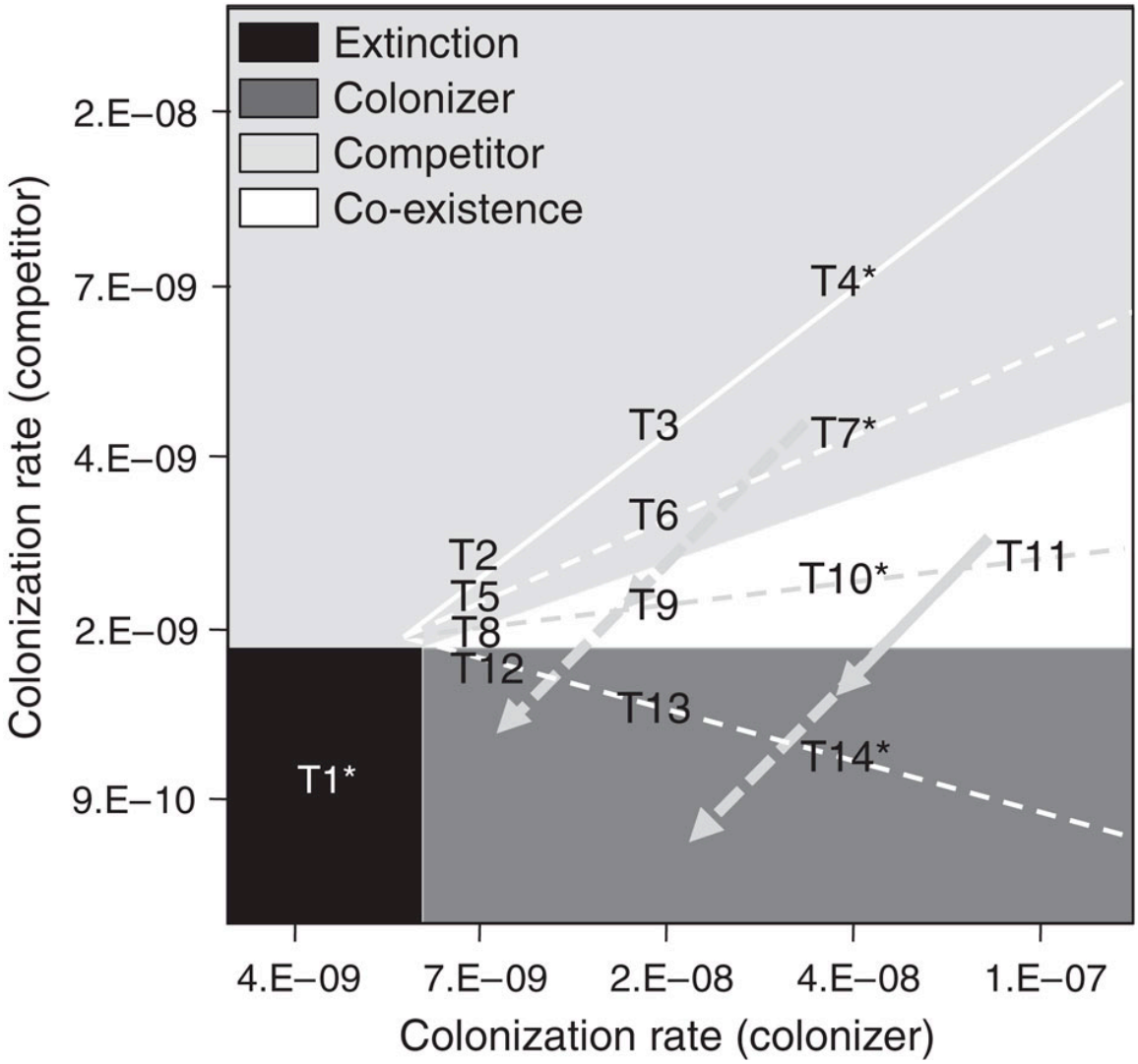

Figure 1.3: Coexistence predictions. These predictions take into account the colonization rates (that is, dilution rates) of strong competitor (competitor) and poor competitor (colonizer) strains. Numbers indicate the treatment corresponding to each combination of strain colonization rates (obtained from the model presented in Fig. 2). Axes indicate the colonization rates of the competitor and colonizer strains as the concentrations used before inoculation at each transfer; smaller values (that is, greater dilutions) indicate that fewer cells colonize each well (that is, are inoculated); greater values indicate more cells being dispersed. Light gray, colonizer excluded by the competitor; white, coexistence; dark gray, competitor excluded by the colonizer; black, extinction of both strains. Lines correspond to the four trade-off strengths: no trade-off (solid white line), weak, medium and strong trade-offs (all indicated by white dashed lines). Thicker arrows indicate the predicted trajectories of isolation treatments (H7 and H11) subjected to reductions in colonization rate (70 or 90%). *Treatments whose observed dynamics are displayed in Fig 1.4. Dynamics for the remaining treatments are shown in Supplementary Fig. 1.1.

Coexistence and patch occupancy

The persistence of both strains was most prevalent and resulted in the most even relative patch occupancies at moderate trade-off strength (Figs 1.4 and Fig. 1.5, and also see Supplementary Fig. 1.1). Treatments with no or weak trade-off strength resulted in the rapid exclusion of the colonizer, whereas strong trade-offs caused extinction of the competitor. Low absolute colonization resulted in the extinction of both strains. There was a highly significant interaction between strain identity and trade-off strength in predicting equilibrium patch occupancy (Fig. 1.5; generalized-linear model, $P<0.0001$, $df=74$, $\chi^2=32.81$, Supplementary Table 1.2), which occurs because the two strains are affected in opposite ways by trade-off strength. Our experimental results thus showed that CC dynamics may regulate the outcome of species competition in a landscape that conforms to theoretical assumptions of strictly asymmetric competition, patch homogeneity and discrete global dispersal. Interestingly, the most important deviation between our predictions and the experimental results occurred under conditions for which our model predicted coexistence (Supplementary Fig. 1.2). In half of such cases, either the competitor or the colonizer went extinct (four colonizer and two competitor extinctions). This evidence suggests that stochasticity may be important in CC dynamics, because it can lead to apparently random extinctions of either species, even under conditions that are favourable to the persistence of both strains. Our simulations indicate that these effects are not solely due to the finite size of the metacommunity[13] or to demographic stochasticity (Supplementary Fig. 1.2), and that they are influenced by temporal variation (among transfers) in the overall colonization

rates of the two species. In our experiments, this situation occurs because the actual colonization rates that we impose are highly sensitive to (1) density estimates in our metacommunities and stock cultures, (2) unavoidable procedural imprecision during dilution and (3) variability in the number of colonizing cells per well. This effect is likely to be comparable to the temporal stochasticity in overall dispersal that may occur in natural metacommunities.

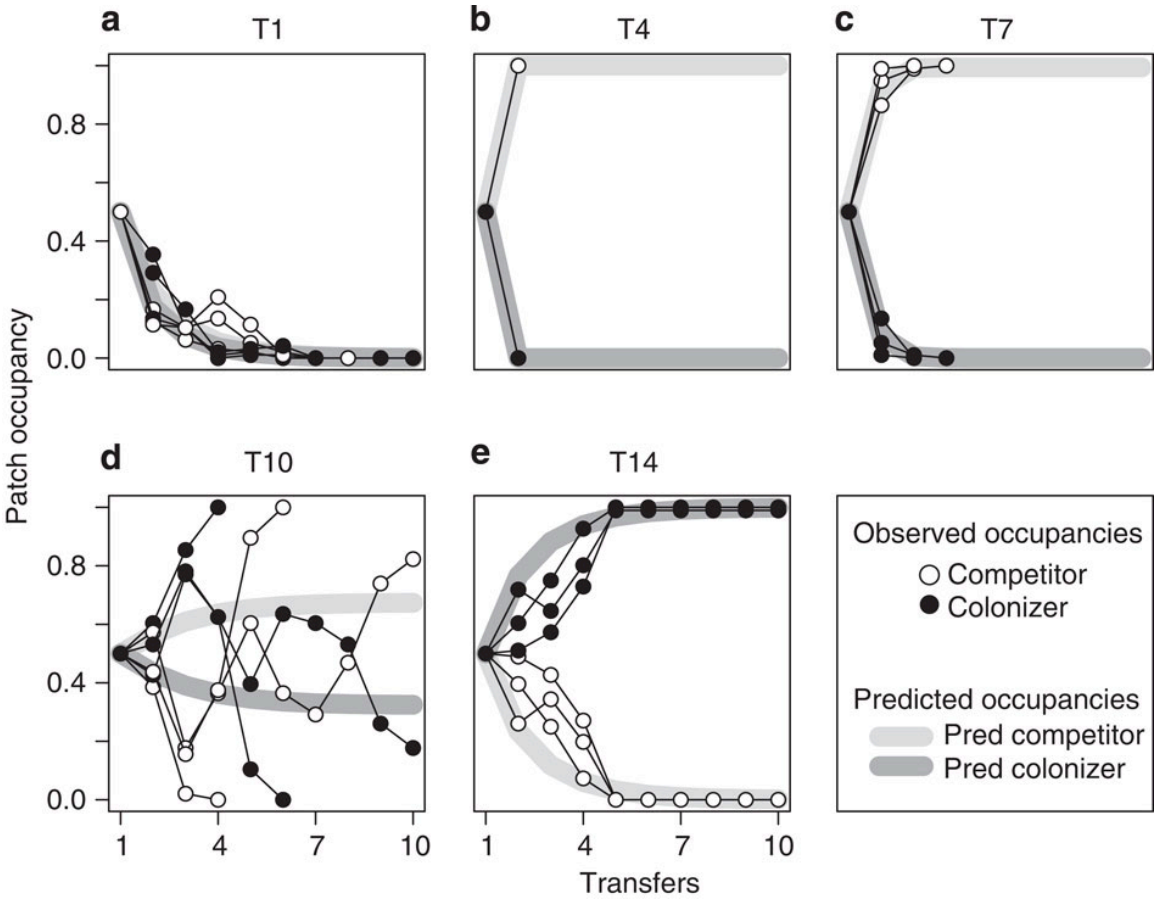

Figure 1.4: Time series for all replicates from select treatments. Treatments T1, T4, T7, T10 and T14 are included. Panels **a**–**e** show observed occupancies (points) and model predictions for each strain (thick lines). Each treatment included three replicates and was run for a maximum of ten transfers. Replicates were stopped after the extinction of one or both strains occurred. Aside from treatment T10, which showed strong variability, all other treatments were consistent with the model predictions. Dynamics for the remaining treatments from the main experiment are shown in the Supplementary Fig. 1.1.

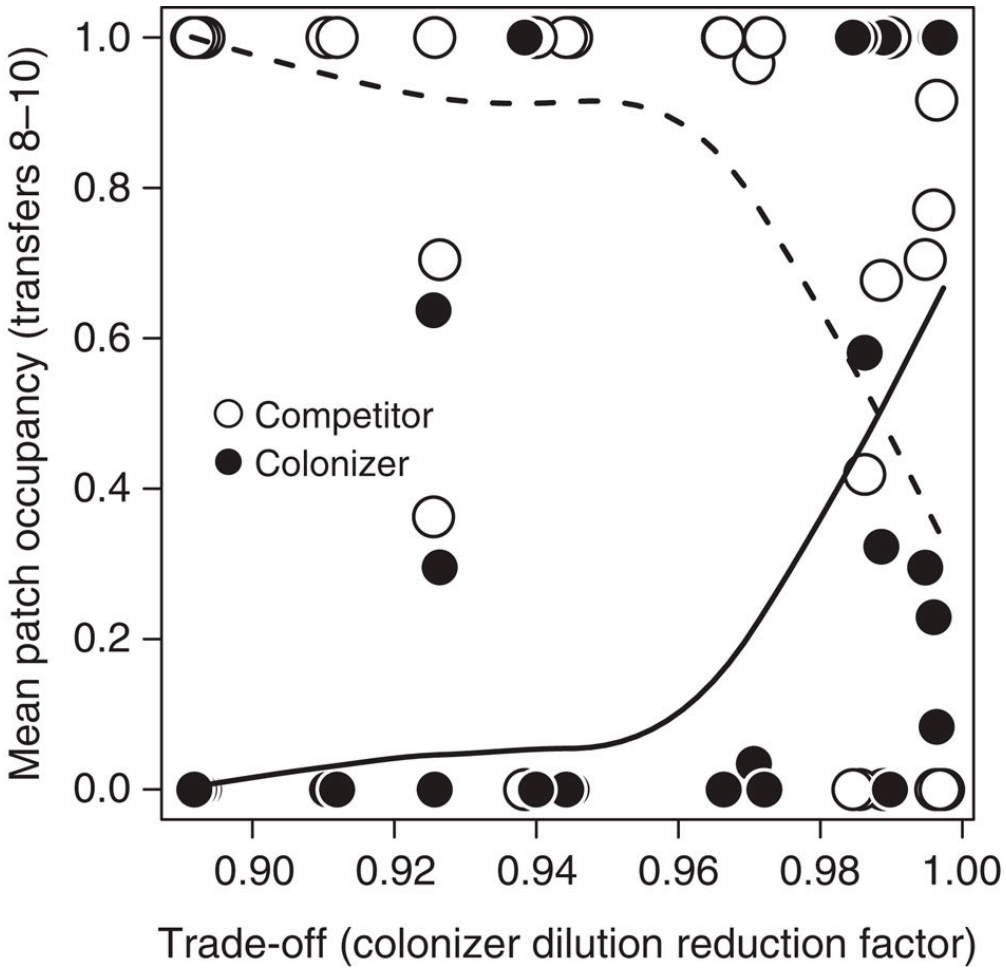

Figure 1.5: Effect of trade-off strength on equilibrium patch occupancy by strain. Each point indicates the average proportion of occupied patches by each strain in each replicate (averages of transfers eight to ten in our experiment). The 'competitor dilution reduction factor' represents the proportional reduction in the overnight culture volume of the competitor before dilution (the strength of the trade-off in terms of reduced colonization rate). Treatment T1 was not included in this analysis because the extinction of both strains was predicted. A smoothing loess line is fitted to points from each strain to highlight the colonization pattern. All points were jittered to avoid overlap and improve the clarity of the figure.

Metacommunity productivity

We found evidence for functional consequences of CC dynamics in a negative relationship between regional diversity and metacommunity productivity (Fig. 1.6). Productivity was measured as the sum of cell densities (estimated by optical density) in

all of the wells in each microplate averaged over the last three transfers. Taking in account that there were only two strains in this system, we calculated the Shannon index (or Shannon entropy) to measure diversity using the relative density of each strain within each microplate (that is, metacommunity). If both species were equally common, the Shannon index would be maximized. The more unequal the abundances of each strain are in the metacommunity, the smaller the corresponding Shannon index. If only one strain is contributing to the overall biomass and the other strain is very rare (or absent), the Shannon index approaches zero[26]. These scenarios are analogous to those in communities in which there are substantial changes in the relative proportions of abundant species, although not in numbers of species (for example, [27, 28]). In our experimental system, the competitor was much more productive (that is greater cell densities) than the colonizer (Supplementary Fig. 1.3). Taking into account that these strains cannot coexist locally, the asymmetry in productivity implies that metacommunities dominated by the colonizer have lower productivity than those dominated by the competitor do. In fact, the low-diversity metacommunities often consisted of monocultures of the competitor with high productivity, whereas high-diversity metacommunities resulted in mixtures of productive (occupied by the competitor) and unproductive patches (occupied by the colonizer) that lead to a negative relationship between regional diversity and productivity (Fig. 1.6). Theoretically, the low-diversity communities could also have consisted of monocultures of the colonizers, which would lead to a unimodal relationship between productivity and diversity[29], but this scenario was not observed because there were far more treatments

in which the competitor was expected to be more likely to persist than the colonizer (Fig 1.3).

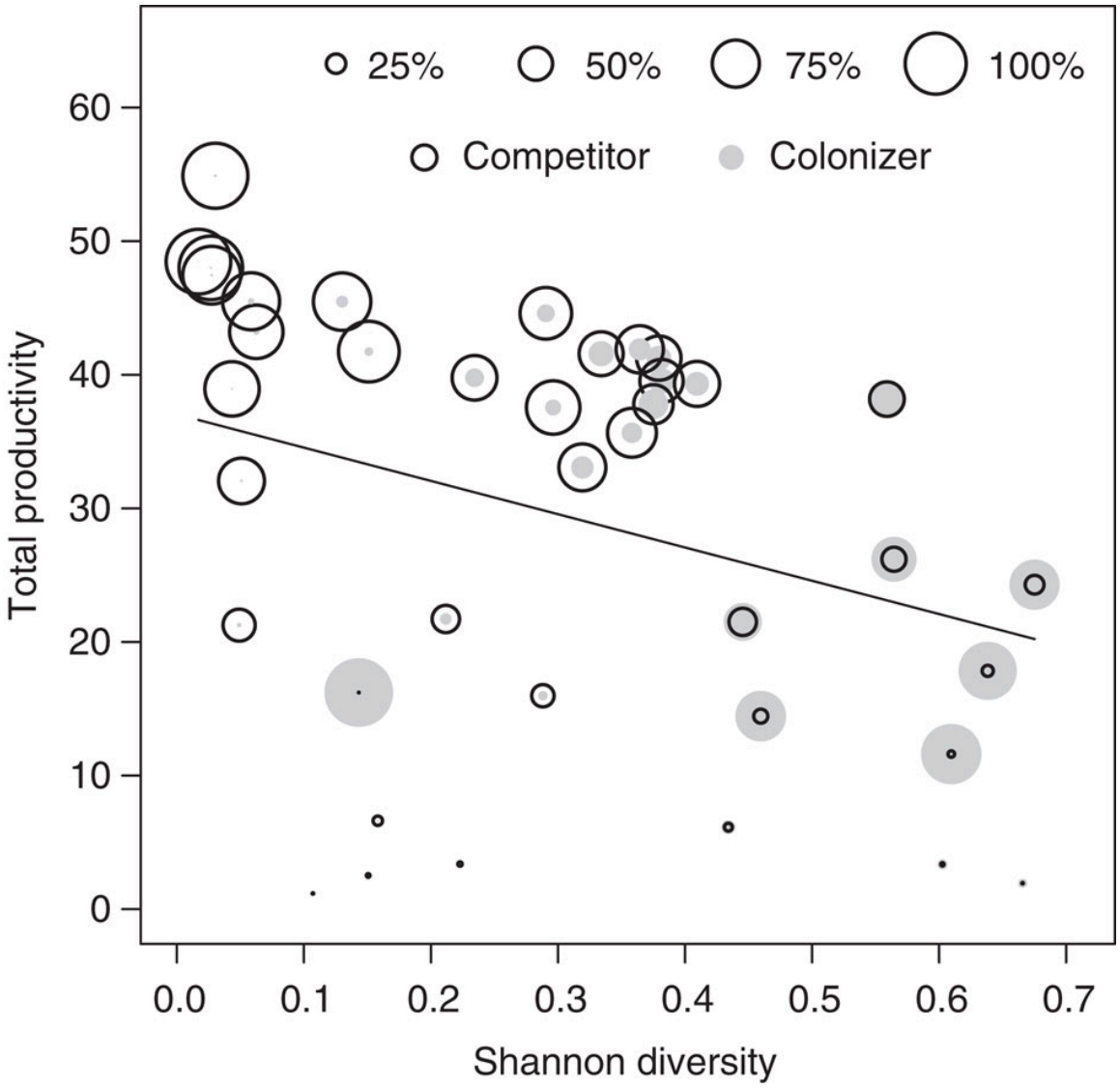

Figure 1.6: Total metacommunity productivity and Shannon diversity. Points are averages from each replicate in the 14 main experimental treatments over transfers 8–10. Circle sizes are proportional to the mean patch occupancy of each strain (see scale in the figure). Notably, diversity was calculated based on the productivity and not the occupancy data, so plates with uneven occupancy can have high diversity (P<0.036, R^2=0.108, F=0.57, n=42).

As opposed to other regional mechanisms of species coexistence that have found a positive biodiversity-ecosystem function (BEF; for example, source-sink dynamics[30, 31]), our experiment showed how CC dynamics may produce a rarely observed negative BEF relationship[32, 33], which is likely explained by a combination of patch dynamics and the regional dominance of a productive and competitive species (a 'negative selection effect', *sensu*[34]). These results suggest that the definition of complementarity and selection effects, as the main drivers underlying the BEF relationship, may need to be revisited to accommodate broader coexistence mechanisms. Complementarity is based on niche differentiation and/or facilitation[35, 36] and leads to a positive BEF, whereas the selection effect assumes that the dominant species are driving the BEF pattern and predicts either a positive or a negative relationship, depending on whether the traits of dominance are positively or negatively correlated to ecosystem functioning[34]. Although complementarity is clearly linked to the mechanism of species coexistence, the selection effect does not integrate any information on what drives species coexistence or abundances. In our experiment, dominance is linked to the proportion of patches occupied in the metacommunity and this is driven by an interaction between species traits (CC trade-off strength) and an external driver (perturbation rate) that 'selects' for dominance of either the competitor or colonizer. As dominance here is decoupled from species productivity (that is, the dominant species can be either the most or the least productive species), it leads to a negative selection effect where the most diverse metacommunities are less productive because they are dominated by the less productive species. Besides illustrating one of the first experimental negative BEF

relationships (see also [32, 37]) our results stress the need for redefining the drivers of the BEF based on the relationship between the traits driving coexistence (for example, CC trade-offs versus local niche differentiation) and traits related to ecosystem functioning *per se*. Future research will need to disentangle the mechanisms driving species coexistence at local and regional scales, and reveal the manner in which niche traits at multiple scales are related to ecosystem functioning to advance a mechanistic understanding of the BEF relationship[29, 38, 39].

Increasing patch isolation

In a second experiment, we investigated whether increased patch isolation could modify the outcome of metacommunity dynamics[8, 9]. We reproduced two experimental treatments from the first experiment that revealed (1) the strong persistence of both strains and (2) rapid exclusion of the colonizer (treatments H11 and H7, respectively, in Fig. 1.7). We subsequently generated new predictions from the model, which showed that decreased overall colonization by 70 and 90%(corresponding to highly isolated patches) in each treatment (Fig. 1.3) should qualitatively affect coexistence outcomes (eliminating the competitor from the H11 treatment, inducing coexistence in the H7 treatment). We ran these treatments in a second experiment with identical conditions for ten transfers. As predicted, we found that the persistence of both strains was enhanced for the 70%reduction and that the extinction of the best competitor (referred to as PI) was observed for the 90% reduction (Fig. 1.7). Extinctions and reversals in persistence scenarios required multiple transfers to resolve relative to controls (especially in H7, Fig. 1.7), suggesting the existence of an 'extinction debt'[9]. This situation illustrates the

susceptibility of communities structured by the CC trade-off to the changes in absolute colonization rate that are predicted under human impacts on landscapes, including habitat destruction[9, 40], fragmentation[8] or changes in inter-habitat matrix quality[41]. Previous experimental studies have been limited to binary or coarse-scale manipulations of colonization rate to simulate reductions in landscape connectivity[42, 43]. By coupling our model predictions to a tailored experimental system, we were able to identify and test critical thresholds in the effects of decreased connectivity on metacommunities. Future developments will include measuring how these thresholds are affected by other scenarios of competitive interaction (for example, [4]) or landscape topology and dispersal patterns (for example, [44]).

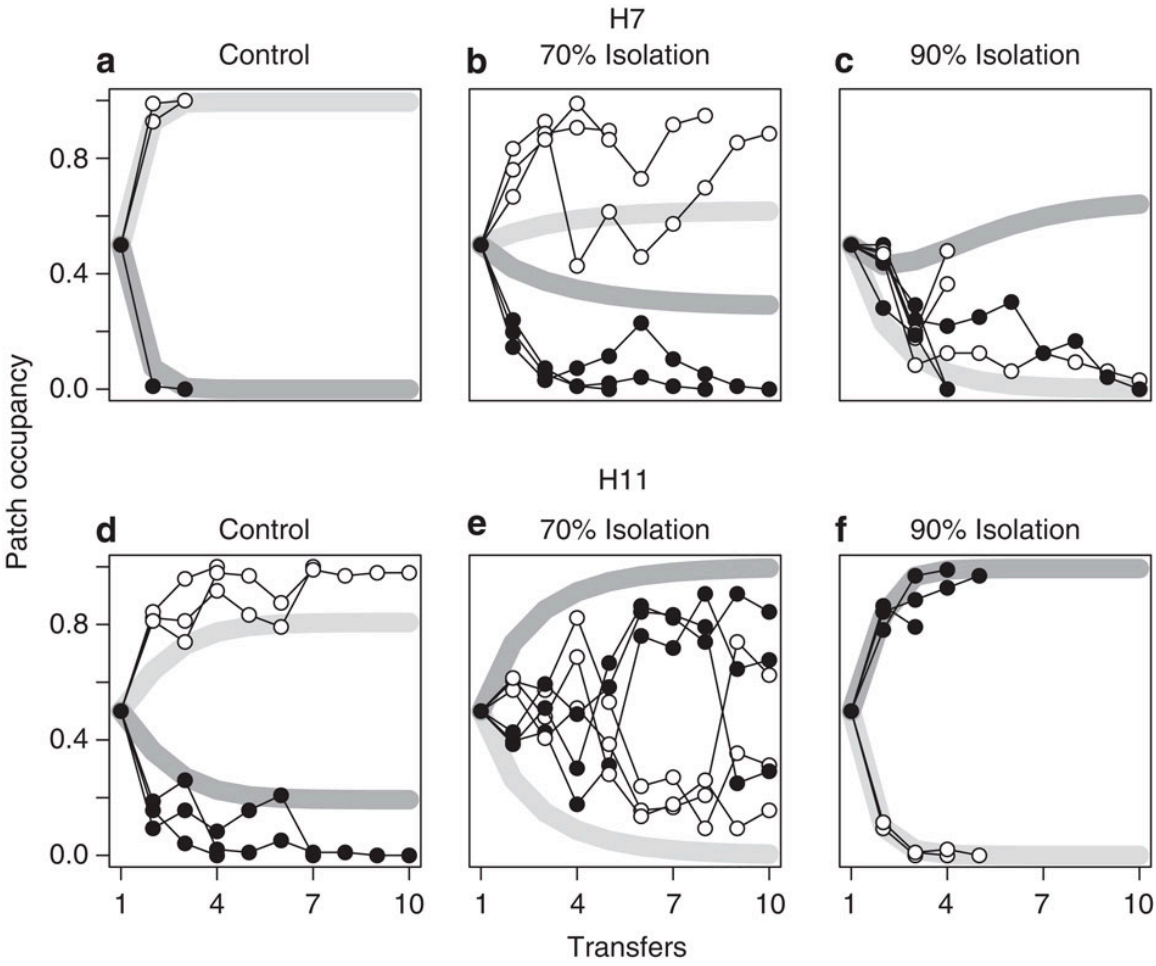

Figure 1.7: Time series for all replicates from all treatments in the habitat isolation experiment. Panels **a**–**f** show observed occupancies (points) and model predictions for each strain (thick lines). Each treatment included three replicates and was run for a maximum of ten transfers. Replicates were stopped after the extinction of one or both strains occurred.

Discussion

In the context of increasing global change, ecologists are being urged to shorten the loop between theory and application[45]. Although ecologists have now adapted to include larger-scale mechanisms through the use of metapopulation, metacommunity and meta-ecosystem concepts and models[46, 47, 48, 49], experimental work targeting biodiversity and

ecosystem effects at larger spatial scales is still in the initial stages[50]. The reality is that many theoretical models, often used to understand the consequences of global change, are difficult to test in natural systems, especially without first evaluating their performance in highly controlled experimental settings. Our study was done using a unitrophic community in an aquatic microcosm system, which is a common feature of many experimental tests of metacommunity paradigms[50]. These systems closely approximate theoretical assumptions and have a long history in ecology serving as bridges between theory and natural systems[51, 52], making them ideal for theory that is challenging to test like CC dynamics. Here we demonstrated that CC dynamics can indeed be implemented in a controlled microcosm system, facilitating the comprehensive and quantitative evaluation of these theoretical models in more robust situations.

Our combined theoretical–experimental framework opens the way for further investigation of different aspects of CC dynamics (1) as a coexistence mechanism, (2) as a determinant of patterns of biodiversity loss under anthropogenic landscape impacts and (3) as a driver of regional BEF relationships. A prime example of the potential applications of our framework is the demonstration that the effects on landscape-level coexistence could be closely linked to those on regional level BEF relationships, suggesting that the effects of fragmentation on extinction debts may also cascade to ecosystem attributes. Our findings illustrate that BEF relationships may depend on the spatial scale over which they occur and the mechanisms generating the gradient of diversity[29, 30,38].

We believe that our experimental framework is likely to prompt further investigations into the mechanisms underlying the evolution of CC dynamics in natural communities by exploring the particular ecological scenarios that trigger the evolution of CC trade-offs. As the changes in colonization rates in our experiment have analogous effects to reductions in the number of susceptible hosts or reduced transmissibility in epidemiological models[53, 54], our experimental setting has strong potential for use beyond community and landscape ecology questions; for example, to investigate questions related to spatial epidemiology.

Our ability to bridge advances in theory with natural systems partially relies on using formal tests of predictions derived from theoretical models[51, 52]. Although the highly controlled nature of our system and related experiments (for example, [55, 56]) does not allow direct extrapolation of our primary results to natural landscapes, they are a key step toward the understanding of natural metacommunity dynamics and properties. These approaches provide critical mechanistic explanations that will ultimately facilitate the application of theory towards the conservation and management of biodiversity and ecosystems on large scales.

Methods

A model for CC dynamics in microplate metacommunities

We used a patch occupancy model to describe how the proportion of wells (patches) in a microplate that are occupied by a particular bacterial strain changes through successive transfers from one plate to the next. This model can be seen as the discrete-

time equivalent of seminal community ecology models[2, 3] or the limiting case of a haystack metapopulation model when local competitive exclusion is rapid (see [57]). With P_1 and P_2 as the proportion of wells occupied by strain 1 (competitor, equation 1) and strain 2 (colonizer, equation 2), respectively, the dynamic equations are as follows:

$$P_1(t+1) = f_1(P_1(t)) = f(P_1(t)n_1d_1) \qquad (1)$$

$$P_2(t+1) = (1 - P_1(t+1))f_2(P_2(t)) = (1 - P_1(t+1))f(P_2(t)n_2d_2) \qquad (2)$$

where n_1 and n_2 are the local densities of each strain and d_1 and d_2 are their colonization rates; fs any function, including a Poisson zero-term function $f=a(1-e(-bx))$, such as the one used in this study. Important assumptions are that (1) strain 1 is completely dominant so that strain 2 disappears from any well where strain 1 is found; and (2) at the end of a transfer, both strains reach a given density in any colonized well, irrespective of initial conditions.

The equilibrium occupancies can be determined graphically for any function f (see Fig. 1.2). Coexistence occurs if the proportion of successfully colonized wells exceeds zero for both strains and strain 2 has colonization rate sufficiently greater than strain 1 (Fig. 1.3). We took dispersal to follow a Poisson distribution, which was appropriate for our method of diluting cell cultures[58], and we assumed that at least one cell was needed for successful colonization, yielding $f(x)=1-e(-x)$. This function was validated in preliminary trials (Supplementary Fig. 1.4).

Bacterial strains

We used two isogenic strains for both experiments; *Pseudomonas* SBW25-LacZ (LacZ, 'colonizer') and an unknown strain of *Pseudomonas* were isolated on Gould S1 medium (sucrose, glycerol, casamino acids, $NaHCO_3$, $MgSO_4$ $7H_2O$, K_2HPO_4 $3H_2O$, N lauroyl sarcosine sodium, trimethoprim) from soil samples collected in Montpellier, France[59]. We refer to the second strain as 'PI' ('competitor'). LacZ was chosen because it can be distinguished under plating with X-gal (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside) and IPTG (isopropyl-β-D-thio-galactoside). PI was chosen because it clearly outcompeted LacZ (see Supplementary Methods 1, Competitive assay section). In all trials and the main experiment, bacteria were grown for 24 h (overnight) at 28 °C on KB medium (glycerol 10 µl l^{-1}+20 g l^{-1} protease peptone H3+1.5 g l^{-1} K2HPO_4+1.5 g l^{-1} $MgSO_4$; autoclaved 20 min at 121 °C) under constant agitation[21].

Model parameterization

Preliminary trials showed that increasing the dilution rates generated a Poisson distribution of growth success in microplate wells (Supplementary Fig. 1.4) and that this distribution was similar between the two strains. This result confirmed that we could manipulate colonization using pre-inoculation dilution and allowed us to use a specific distribution (Poisson) in our model. We parameterized our model with the average strain-specific growth in a single well after 24 h of 1.027×10^8 for LacZ and 9.517×10^8 cells for PI and the number of wells per microplate, which was 96. Colonization (dilution) rates were selected based on an exploration of model coexistence state outputs. Cell number was assayed following the droplet procedure described above.

This model was implemented in Mathematica 8 (Wolfram). Model results are shown in Figs 3, 4 and 7.

Basic experimental design

The main experiment consisted of manipulating trade-off strength (that is, weak, moderate, strong and no trade-off) across 14 treatments (Fig. 1.3), with 3 independent replicates for a starting total of 42 microplates (metacommunities), or 4,032 wells (patches). Our experiment consisted of six basic steps as follows: (1) set up an initial metacommunity with equal proportions of both strains in all wells (48 wells at random occupied by each strain); (2) build a new 'colonizer pool' based on the regional abundances of the two strains after every 24 h; (3) modify the relative contribution of the two strains in the colonization pool and the dilution of this pool according to each experimental treatment (see Fig. 1.1) to manipulate the absolute and relative colonization rates (and thus the strength of the CC trade-off) of the two strains in the metacommunity; and finally (4) inoculate new metacommunities (that is, new microplates containing fresh media); (5) measuring abundances in each microplate metacommunity by estimating optical density at 650 nm on a FLUOStar spectrophotometer; and (6) characterize colonizer pool using a custom-made script in R that uses data from step 5 to estimate the relative abundances of each strain in the colonizer pool (for complete description of these steps see Supplementary Methods 1, Detailed experimental protocol, and Supplementary Note 1 for R script). This experimental procedure was run for 10 transfers, which we considered appropriate following model simulations (Figs 1.4 and 1.7). The treatment- and strain-specific pre-

inoculation dilution, concentrations of each strain and isolation levels can be found in Supplementary Table 1.1.

Similar to the main experiment, the isolation experiment had six basic steps as follows: (1) set up an initial metacommunity; (2) build a 'colonizer pool'; (3) modify the relative contribution of the two strains in the colonizers pool and the dilution of this pool according to each experimental treatment and (4) inoculate new metacommunities. To mimic isolation, we imposed an additional reduction in overall colonization by reducing the inoculation volumes of the two strains by 0 (control), 70 or 90%in replicate microplates. The two coexistence scenarios that we implemented were the same as treatment T7 and T11 in the main experiment. Following the implementation of the additional reduction we proceeded with steps 5 and 6 as in the previous experiment. This experiment consisted of a starting total 18 microplates or 1,728 patches and was run for 10 transfers.

Stock cultures and experimental duration

A key issue in this experiment was to ensure that no substantial evolution occurred between transfers so as not to interfere with the ecological CC dynamics predicted by our model. For this purpose, we generated a uniform stock library of bacteria by freezing (–80 °C) 300 µl aliquots from overnight cultures in KB medium and glycerol (60/40 bacteria to glycerol, 80% v/v ratio). Before each transfer, we defrosted (for 5 min) and vortexed (for 10 s) new frozen aliquots to remove the glycerol from the aliquot before inoculation. Separate overnight tubes with 6 ml of KB were inoculated with 125 µl of

LacZ and 30 µl of PI from the defrosted and vortexed aliquots. Previous pilot experiments have shown that these inoculation volumes ensured relative congruence between overnight growth and growth in the microplates. This detail meant that our procedure relating optical density-estimated cell counts in overnight cultures to microplate estimates (see Supplementary Methods 1, Measuring abundances and Detailed experimental protocol) was not correcting for great differences in cell counts. Overnight cultures were grown overnight at 28 °C under constant orbital shaking.

Statistical analysis

Productivity was measured as the sum of bacterial growth in all wells of each microplate. Diversity was calculated as the Shannon diversity index that takes into account the relative contribution to productivity of each strain. The Shannon index used was calculated as $H=-\Sigma p_i \log(b) p_i$, where p_i is the proportional abundance of species i, and b is the base of the logarithm. This index is also known as Shannon entropy [26]. If both species are equally common, the Shannon index is maximized. The more unequal the abundances of each strain are in the metacommunity, the smaller the corresponding Shannon index. If only one strain is contributing to the overall biomass and the other strain is very rare (or absent), the Shannon index approaches zero[26]. These calculations were performed using the vegan package in R[60]. We tested for an interaction between strain identity and trade-off strength in predicting equilibrium patch occupancy using generalized-linear model (Supplementary Table 1.2).

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Chapter 2: The dynamics of community assembly under sudden mixing in experimental microcosms

Abstract: Landscape connectivity has been shown to alter community assembly and its consequences. Here we examine how strong, sudden changes in connectivity may affect community assembly by conducting experiments on the effects of 'community mixing', situations where previously-isolated communities become completely connected with consequent community re-organization. Previous theory indicates that assembly history dictates the outcome of mixing: mixing randomly-assembled communities leads to a final community with random representation from the original communities, while mixing communities that were assembled via a long history of colonizations and extinctions leads to strong asymmetry, with one community dominating the other. It also predicts that asymmetry should be stronger in the presence of predators in the system. We experimentally tested and explored this theory by mixing aquatic microcosms inhabited by a complex food web of heterotrophic protists, and algae. Our results confirm the prediction that long assembly history can produce asymmetry under mixing and suggest these dynamics could be important in natural systems. However, in contrast to previous theory we also found asymmetry weaker under mixing of communities with more complex trophic structure.

Introduction

Spatial effects are increasingly recognized to be important to community assembly at local and regional scales (Leibold et al. 2004, Holyoak et al. 2005). A central finding is that the degree of connectivity among local communities in a metacommunity can alter patterns of diversity, composition, and food web and ecosystem attributes (Massol et al. 2011, Logue et al. 2011). To date, most of this work has focused on cases where connectivity is fixed in time. However connectivity can change due to both natural and anthropogenic factors. Although some attention has been given to altered fragmentation corresponding to rapidly decreased connectivity (Didham et al. 2012), a largely unexplored and interesting possibility is the reverse: a sudden increase in connectivity through 'community mixing'.

Community mixing occurs when previously isolated communities merge into one. The phenomenon is likely widespread in nature, but it is difficult to identify cases of mixing because field data is not typically collected with a delineation of past community isolation or of changes in connectivity across time. However, community mixing does occur. For example, over long geologic time scales macroecological events such as the collision of landmasses or the merging of ocean or river basins (Vermeij 1991, Wilkinson et al. 2006) provide opportunities for community mixing. On decadal time scales, defragmentation processes may also represent contemporary mixing (e.g. reforestation, Sitzia et al. 2010, Didham et al. 2012). Seasonally, recurrent rainfall events induce rivulets to form among rock pool communities and this dispersal mode accounted for the majority of dispersal volume of invertebrates in one system

(Vanschoenwinkel et al. 2008). Even on daily time scales, tidal action floods and re-isolates coastal water bodies (Larkin et al. 2008) and the mixing of some microbial communities may occur at even higher frequency (Gonzalez et al. 2012).

What is the outcome of such events? Tilman (2011) reviewed cases of the historical merging of "biogeographic realms." In general, coexistence was the predominant outcome and wholesale extinctions of taxa from either of the two source realms did not occur, nor did the overall extinction rate among both groups of taxa increase. A notable exception to this pattern comes from the extinction of 12 of 20 genera of South American ungulates after the collision of the North and South American landmasses possibly due to direct or apparent competition with Northern taxa (Webb 1976). Although these examples provide patterns, it is extremely difficult to determine the processes responsible. Possible processes involve pre-contact evolutionary community assembly like character displacement, coevolution, phylogenetics, species range evolution (Urban et al. 2008) and trade-off surfaces (Tilman 2011), or post-contact ecological community assembly involving rapid re-assembly through species interactions and environmental heterogeneity among the previously isolated realms.

Simple assembly models based on competition can aid interpretation of the general pattern of coexistence observed in biogeographic contexts. These models reveal the expected consequences of community mixing under simple, widely applicable ecological scenarios. Gilpin (1994) used Lotka-Volterra models to show that after mixing, one community could mostly or entirely replace the other, much as in the partial replacement of the South American mammal fauna by the Northern. He contrasted

situations where the initial communities were ‘random’ vs. ‘non-random.’ The ‘random’ ones were created by drawing species at random from a species pool and finding sets of species that could stably coexist but were subject to no other mechanisms of community assembly. The ‘non-random’ communities were those that initially contained an unstable set of species, but collapsed under deterministic extinctions to a smaller subset of species that could stably coexist. He found that mixing two ‘random’ communities usually resulted in a final community that comprised roughly equal numbers of species from the two initial communities. In contrast, he found that mixing of two ‘non-random’ communities resulted in final communities that were often completely or partially biased to contain species only from one or the other of the initial ones (asymmetry). The results illustrate that ‘non-random’ communities are likely to have a set of species with a more globally stable species composition (across many possible species combinations) and can thus “trap” mixed communities more than do ‘random’ ones with local stability (across few possible species combinations). However, only 29% of mixes among ‘non-random’ communities produced asymmetry, indicating that the occurrence of asymmetry is sensitive to the composition of mixed communities. Subsequent theoretical work also showed that communities with few species tend to overtake more species-rich ones (Toquenaga 1997).

Incorporating trophic structure by adding a trophic level to communities enhances asymmetry due to the increased global stability imposed by the predator relative to competitors (Wright 2008). This increased global stability occurred because the effects imposed by predators on their prey tended to be larger than the interaction coefficients

among competitors. Under the mixing of non-random communities with predators, asymmetry in the final community is further enhanced by the need for the prey from each initial community to coexist with a novel predator and for those predators to coexist with one another (Wright 2008). Although unexplored theoretically, mixing a single trophic level community with a two level community could produce similarly enhanced asymmetry.

Although intriguing, this theory is poorly developed, being represented by only the three related simulation studies summarized above. Conversely, examples from natural systems suffer from a lack of control that hinders interpretation of patterns. An experimental approach can complement theory and observations from nature, reveal if asymmetric outcomes of mixing are possible in communities comprised of real (as opposed to theoretical) organisms, and allow causation to be attributed to the mixing event (as opposed to, e.g., pre-mixing evolutionary history).

We use multi-trophic aquatic microbial communities to explore the dynamics resulting from assembly in isolation followed by sudden mixing. Microbial microcosms are a powerful model system for bridging the gap between theory and nature (Lawler and Morin 1993, Morin 1999, Cadotte et al. 2005, Fox et al. 2010). We used a well-characterized food web involving producers, herbivores, omnivores, and predators. To address the effect of trophic structure, communities included interaction networks whose species composition was manipulated to produce with either mostly competitive (C) or trophic (predator-prey) (T) interactions among species. We mixed two competitive

communities (CC), two trophic communities (TT), and one of each (TC) in replicated pairs.

We tested two hypotheses about the mixing of two isolated communities: H1) asymmetric outcomes (one community largely or entirely replaces the other) occur more often than expected by chance; and H2) asymmetry is more frequent in TT and TC than in CC mixes. We explored potential biological mechanisms underlying the mixing process using an analysis of asymmetry within each of six trophic guilds used in our experiment: bacterivores, inedible algae, edible algae, herbivores, predators, and omnivores.

Materials and methods

Experimental design

The 52 species in the experiment included edible algae, inedible algae, bacterivores, omnivores, herbivores, and predators. We generated 132 eight-species communities divided equally into two types: communities that were dominated by trophic interactions (T); and communities that were dominated by competitive interactions (C). Like Gilpin (1994) and Wright (2008), we choose to initiate our communities with equal numbers of species. Unlike Wright's (2008) model, both C and T communities include trophic and competitive interactions, however, we varied the ratio of trophic to competitive species in an analogous way to Wright's method of substituting one prey for a predator.

We then paired communities in three ways: 22 pairs of competitive communities (CC), 22 pairs where one community was a competitive community and the second was a trophic community (TC), and 22 pairs in which both were trophic (TT) (66 total pairs, Fig. 2.1). Each community in the experiment contained a unique species composition and there was no overlap in composition between paired communities. We mixed community pairs 45 days after establishment and allowed the mixed communities to reassemble for an additional 45 days. These periods are similar to previous microcosm assembly experiments (Fox 2008) and represent approximately 90 generations by the end of the experiment for mid-trophic-level species (other groups have longer or shorter generation times).

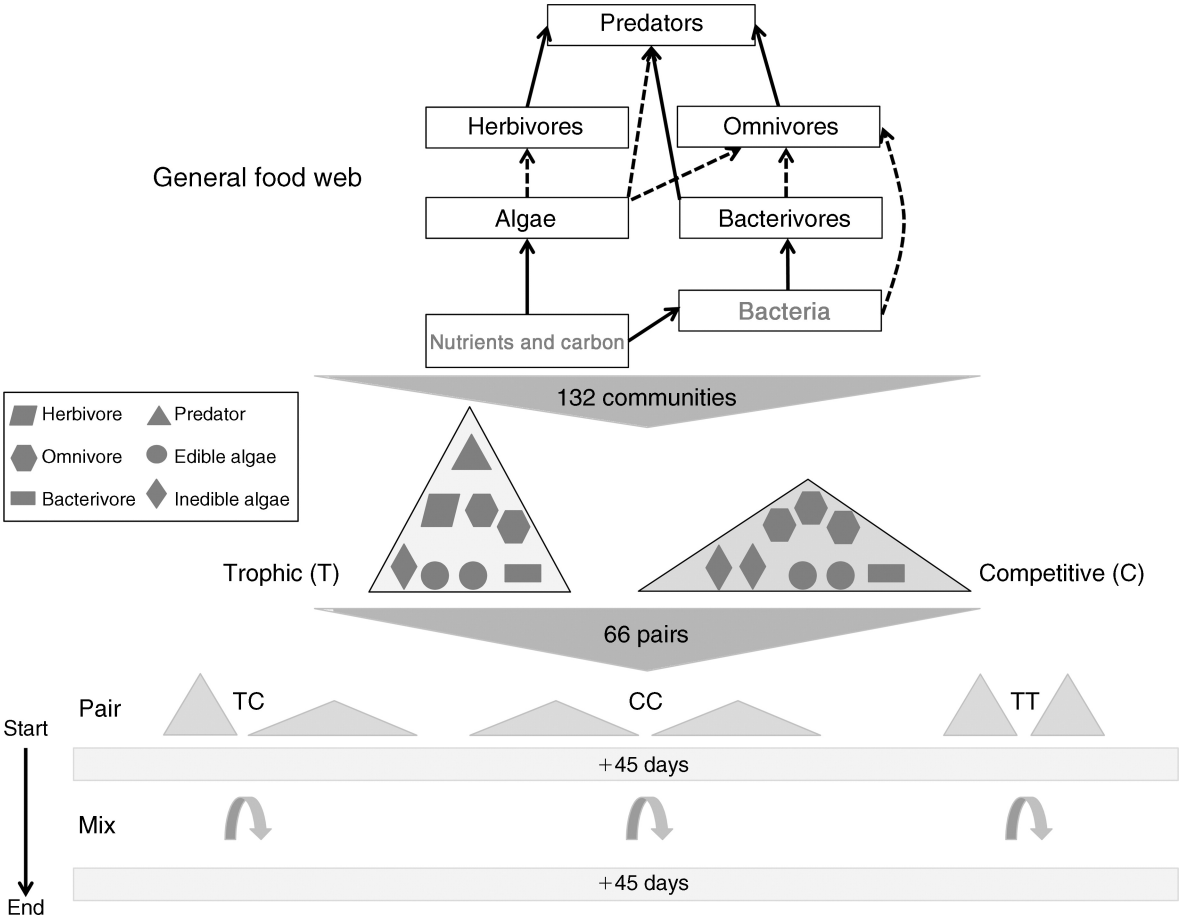

Figure 2.1: Schematic of experimental design. The general food web was adapted from the feeding trial (Appendix B) and Petchey et al. (1999). Dashed lines indicate context-dependent feeding links. 132 trophic (T) and competitive (C) communities each with 8 species were randomly generated and paired in TC, CC, and TT combinations (66 total pairs and 22 of each type, see Methods). These communities were assembled for 45 days, mixed, and assembled for another 45 days until the end of the experiment.

Trophic and Competitive Community Assembly

Trophic communities were inoculated with one species each of bacterivore, inedible algae, edible globular algae, edible non-globular algae, herbivore, predator, and two omnivore species. In competitive communities, we increased the frequency of competitive interactions by removing the herbivore and predator and adding an additional omnivore and inedible algae species. Competitive communities were

inoculated with one each of bacterivore, edible globular algae, edible non-globular algae, two inedible algae, and three omnivore species (Fig. 2.1). Edibility of algae was determined by size (Appendix A2 and methods in Appendix B2). We classified the majority of heterotrophic species into trophic categories based on a combination of feeding trials (Appendix B2 and C2), mouthpart size, and published observations (see refs in Appendix A2). Although precise feeding relationships were context dependent, all herbivores, predators and omnivores used in the experiment are considered to be generalists.

Stock cultures were obtained from biological supply companies and contributions from other microbiological labs (see Appendix A2). We inoculated communities from high-density stock cultures grown under similar conditions for 10 days prior to the start of the experiment. Bacterivores, omnivores in pure culture, and algae were added as 250 ul innocula. *Spirogyra* sp., a filamentous algae, was added as a single strand. For omnivores, herbivores, and predators, we isolated 5 individuals for innoculation by serial dilution. Food webs were assembled from the base up, with bacterivores and algae added at 7 d, and omnivores, herbivores, and predators added at 14 d.

Microcosm conditions

We used deep 100 x 25 mm petri dishes as microcosms filled with 39 ml of COMBO culture medium (Kilham et al. 1998), on which all species exhibited vigorous growth. Lids were vented allowing gas exchange. To initiate bacterial populations we inoculated autoclaved wheat seeds (carbon source) with a mixture of three unknown

bacterial strains that were isolated from cultures of bacterivorous protists. We then added two of these wheat seeds to each microcosm. Our cultures were not axenic, and other unknown bacterial species and possibly nanoflagellates were unavoidably introduced. However, additions of these unknown organisms were consistent across replicates and are unlikely to confound our results (Fox 2008).

Microcosms were placed under a single fluorescent tube light in a growth chamber with a 16:8 light dark cycle at 20 °C. Each week we replaced 11% of culture volume with fresh sterile medium to prevent the accumulation of toxic metabolites. Trays of 18 petri dishes were rotated weekly within the growth chamber to ensure uniform lighting conditions, but all community pairs were placed adjacent to each other to assure that each pair was subject to identical conditions.

Mixing and sampling procedures

Mixing involved pouring the contents (including wheat seeds) of both petri dishes simultaneously into a single new sterile dish. A sterile cell scraper was used to transfer attached biofilms. Mixing doubled the volume in each microcosm to 78ml.

We sampled communities at mixing and at the end of the experiment. Communities were placed under a stereomicroscope and scanned until all species were accounted for or a maximum of 10 minutes. Preliminary trials and other studies (Fox 2008) demonstrate that this length of time is sufficient to find all species present. Because of logistical constraints we only sampled species abundances for 24 community pairs (distributed evenly across treatments). If supplemented with

abundance data, presence-absence provides sufficient information to address our main hypotheses. Thus, for the remaining 42 community pairs we scanned communities for presence/absence only. To check if extinctions were still occurring at mixing, we scanned 13 communities selected at random for extinctions at 28 d.

For those communities where abundance was estimated, 300 ul was extracted using a micropipette after swirling the dish and counted under a stereoscope (Fox 2002). Algae that were either too abundant or too small to count using this method were estimated by transferring approximately 30 ul to a haemocytometer for counting under a compound scope. High-density samples were diluted until they reached countable densities.

Statistical analyses

We calculated asymmetry in composition as the absolute value of the difference in post-mixing extinction rates from each initial community within a mixed pair. Let A represent asymmetry and e_1 and e_2 represent the proportion of species present at the time of mixing that went extinct after mixing in communities 1 and 2. The equation for asymmetry is thus: $A = |e_1 - e_2|$. This method controls for variation in species richness between each community in a pair prior to mixing and allowed us to look at both the randomness of the overall distribution of asymmetry outcomes as well as the outcome for any particular pair of communities.

Our core results involve comparing the mean, variance and skew of our observed asymmetry in composition with that predicted by a null model (described below). We

make comparisons overall, within treatments, and to aid interpretation of community-level results, within guilds. Skew is only reported for the overall distribution because among treatments and guilds it never significantly deviated from the null model. For our guild-level analysis, we calculated asymmetry separately for each guild within paired communities. We calculated asymmetry using the same general equation presented above, but only for those cases where both communities contained at least one species in a given guild.

We neither expected nor observed all species to persist in all microcosms because of species interactions and demographic stochasticity. This allowed us to analyze our results using a null model. We used each species' pre-mixing persistence probabilities to simulate extinctions in observed communities from the time of mixing until the end of the experiment. The pre-mixing persistence probability for each species was calculated as the number of communities with that species present after 45 d divided by the total number of communities inoculated with that species (probabilities are reported in Appendix A2). This model assumes that species persistence probabilities are unaffected by mixing, i.e. that persistence probabilities are the same during the first and last 45 d of the experiment. We test this assumption by correlating pre and post-mixing persistence probabilities. After 1,000 simulation runs, we inferred the likelihood of observing symmetric versus asymmetric outcomes by generating confidence intervals (mean, variance, skew of distributions, and deviance of asymmetry values from expected for each mixed pair of communities).

For the 48 communities with abundance data, we calculated asymmetry in Raup-Crick dissimilarity to address the possibility that focusing on presence/absence data missed important patterns using the vegan package in R (Oksanen et al. 2013). Asymmetry was calculated as the difference in dissimilarity of the communities in each pair relative to the final mixed community. This asks if, in terms of Raup-Crick dissimilarity, the final community is more similar to one of the two pre-mixing communities. The Raup-Crick index was used to avoid confounding effects of differing species richness among communities (Chase et al. 2011).

We explored two mechanisms that could have determined the identity of dominant communities. For the first, we asked if the presence of particular species was associated with communities showing significantly higher or lower asymmetry than expected under the null model. For the second, we asked if the pre-mixing extinction rate in each community correlated with the post-mixing extinction rate or with asymmetry using a GLM with a logit link function. The simulations and all statistical analyses were implemented in R (v2.14.1; R Development Core Team 2012, Supplement 2).

Results

Pre-mixing assembly

Species persistence probabilities prior to mixing ranged widely from 0% to 100% (Appendix A2). Trophic communities had significantly higher pre-mixing extinction rates

than competitive communities (Fig. 2.2a). Persistence probabilities differed significantly among guilds, being highest for inedible algae and lowest for predators and herbivores (Fig. 2.2b). The average number of extinctions among pre-mixing communities ranged from zero to six with an average of 2.3 (+/- St. Dev. 1.2). The weekly extinction rate during the first 28 d of assembly was 5.5% and between 28 and 45 d of assembly it was 5%. This indicates the extinction rate was not increasing and may have attained levels reflecting background stochasticity prior to mixing.

Post-mixing assembly

After mixing, the extinction rate among species from the 13 communities during the first 28 d was 3.5%, but dropped to 0.01% between 28 and 45 d. This indicates that extinctions had stabilized by the final sample. Species persistence probabilities again ranged from 0% to 100% and were significantly correlated with pre-mixing probabilities (Fig. 2c, Appendix A2). Overall the extinction rate was significantly lower post-mixing and CC, TC and TT mixes had similar post-mixing extinction rates (Fig. 2a). Among guilds, three (bacterivores, herbivores and predators) exhibited significantly lower extinction rates after mixing (Fig. 2b). These reduced extinction rates occurred because two bacterivore species, three herbivore species, and two predators had extremely low or zero pre-mixing persistence probability (Appendix A2) and were thus eliminated before mixing. The number of post-mixing extinctions per community again ranged from zero to six with an average of 2.5 (+/- St. Dev. 1.4).

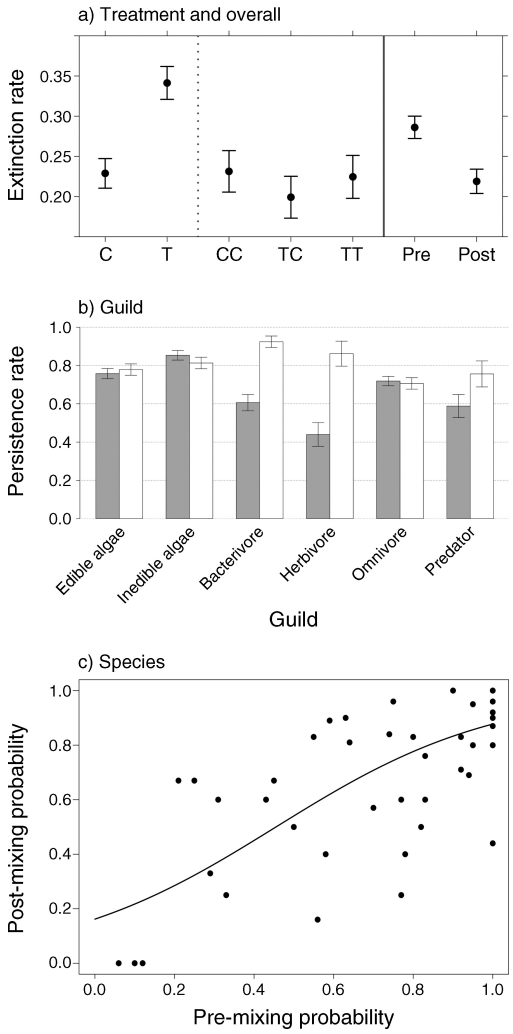

Figure 2.2: Pre and post-mixing effects on extinction/persistence rates. Error bars show standard error. (a) Mean extinction by treatment. C=competitive; T=trophic; CC, TC, TT are mixes. Pre and post are overall rates before and after mixing, respectively. N=1056 in pre and N= 748 in post. The difference between C and T and pre and post is significant (GLM, $P<0.001$, df=1055, $X^2=885.29$, and $P<0.01$, df=1806, $X^2=1629.34$, respectively), but not among mixing types. (b) Mean persistence by guild. There is a significant effect of guild on persistence rate before (GLM, $P<0.00001$, df=1055, $X^2=782.69$) and after mixing (GLM, $P<0.001$, df=743, $X^2=625.64$). (c) Mean pre and post-mixing persistence for all species present at both times (N=48). Fitted with a binomial GLM ($P<0.01$, df =47, $X^2=23.32$).

Asymmetry

We observed significantly greater overall mean asymmetry and also greater variance relative to the null model (Table 2.1), supporting H1. Comparing the frequency distributions shows a fatter tail of high asymmetry communities in the observed data (Fig. 2.3). By analyzing individual pairs of pre-mixing communities, we found 10 pairs of communities deviated from predicted with significantly greater asymmetry (Fig. 2.4). Asymmetry was significantly greater than expected in CC mixes, but not in TC or TT, failing to support H2 (Table 2.1, Fig. 2.4). Disregarding the null model, observed asymmetry among CC, TC, and TT treatments did not differ significantly. Among guilds, we found significantly greater asymmetry than expected under the null model for all groups except bacterivores and omnivores (Table 2.1). Asymmetry in Raup-Crick dissimilarity is correlated with asymmetry in extinction rate (Appendix 2D).

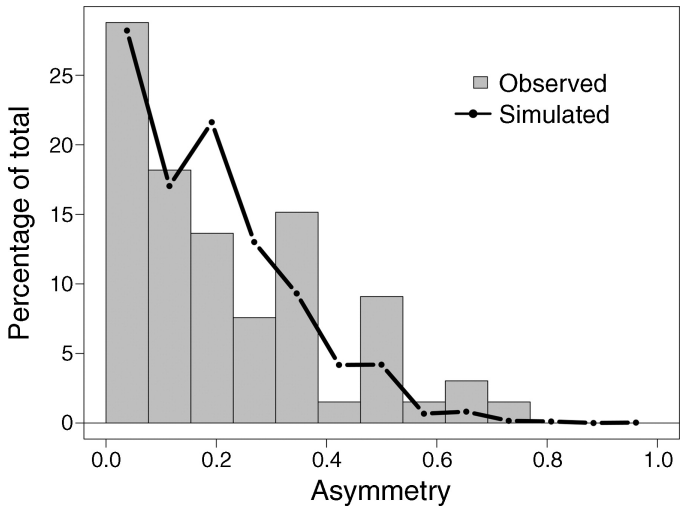

Figure 2.3: Histogram of asymmetry in composition. N=66 for observed data and N=66,000 for simulation results. Shows observed and simulated composition asymmetry.

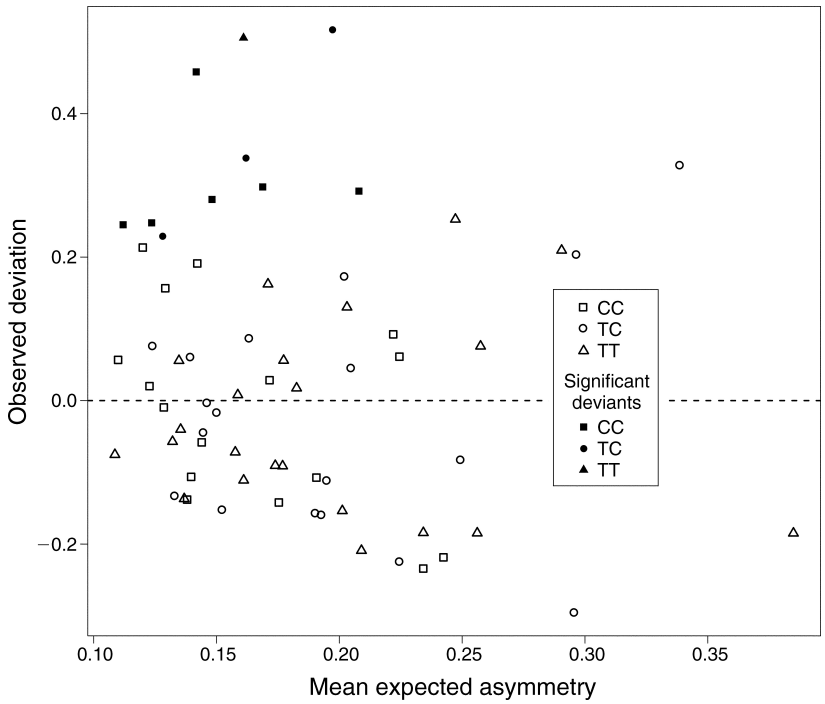

Figure 2.4: Scatterplot of observed and expected asymmetry by community. The "Observed Deviation" represents the difference between the mean expected asymmetry (simulated) and the observed value for each of the 66 post-mixing communities. Significant deviants are calculated at the $P<0.05$ level.

Table 2.1: Summary statistics by treatment and guild for asymmetry in composition.

Treatment and statistic	Observed	Simulated	SD	95% CI
Overall				
Mean	**0.215**†	0.182	0.017	0.149–0.216
Variance	**0.036**	0.023	0.005	0.014–0.032
Skew	0.838	0.838	0.321	0.210–1.467
CC				
Mean	**0.235**	0.167	0.026	0.116–0.218
Variance	**0.032**	0.018	0.006	0.006–0.030
TC				
Mean	0.224	0.193	0.034	0.128–0.259
Variance	**0.048**	0.024	0.008	0.009–0.040
TT				
Mean	0.188	0.196	0.032	0.134–0.258
Variance	0.032	0.025	0.009	0.008–0.042
Bacterivores				
Mean	0.105	0.212	0.062	0.090–0.334
Variance	0.099	0.165	0.037	0.093–0.238
Edible algae				
Mean	**0.367**	0.200	0.035	0.131–0.269
Variance	0.143	0.108	0.018	0.072–0.144
Herbivores				
Mean	**0.333**	0.025	0.015	−0.004–0.055
Variance	0.333	0.026	0.015	−0.003–0.054
Inedible algae				
Mean	**0.282**	0.079	0.026	0.028–0.130
Variance	**0.137**	0.061	0.020	0.021–0.101
Omnivores				
Mean	0.372	0.322	0.043	0.238–0.406
Variance	0.097	0.104	0.016	0.073–0.136
Predators				
Mean	**0.857**	0.084	0.027	0.031–0.138
Variance	**0.143**	0.077	0.023	0.032–0.122

Notes: $N = 66$ pairs of communities overall, $N = 22$ pairs for each mix type, and $N = 19$, 60, 55, 3, 52, and 7 pairs for bacterivores, edible algae, inedible algae, herbivores, omnivores, and predators, respectively. C stands for competitive; T, trophic; CC, TC, TT are mixes. Boldface type highlights those observed statistics that are significantly higher ($P < 0.05$) than expected under the null model. Simulations were run for 1000 times.

† $P = 0.05$.

Mechanisms

We observed that the presence of four species, *Anabaena* sp., *Campylomonas reflexa*, *Loxocephalus* sp., and *Tetrahymena pyriformis*, before or after mixing was associated with asymmetry in composition ($P<0.05$, none after Bonferroni correction), but there was no tendency for these species to be associated with the dominant final community. More extinctions prior to mixing did not affect the chances a community would dominate another after mixing, or the likelihood of extinctions post-mixing.

Discussion

Community mixing results in asymmetrical outcomes more often than expected under our null model, supporting H1. Asymmetry occurs despite the fact that pre-mixing communities did not differ in their evolutionary history or environment, were assembled and paired at random, and that "wining" and "losing" communities were not consistently associated with the presence of particular species. Although our experiment does not include unmixed control communities, similar overall and per species extinction rates pre and post-mixing suggest that the microcosm environment was largely unaffected by mixing and our null model is a reasonable approximation. Significant asymmetry in composition occurred in 18% of 66 mixes. By comparison, Gilpin (1994) observed asymmetry in 29% of simulated mixes among communities structured only by competition and Wright (2008) observed this number to be 38% when including

predation. However, these percentages are sensitive to arbitrary choices of model parameters, so that no particular value is necessarily expected.

Previous models of community mixing found both an effect of assembly history and a combinatorial effect (Gilpin 1994, Toquenaga 1997, Wright 2008). This is because even mixing among 'random' communities could sometimes produce asymmetry due to the rarity of multi-species attractors in phase space just as with the 'non-random' communities. Our experiment does not include 'random' communities. This is because in an experimental setting it is not possible to generate randomly assembled communities since the effect of deterministic assembly history cannot be eliminated. This is not a shortcoming of our approach, but rather it is an indication of the probable rarity of completely randomly assembled communities in nature. Furthermore, several lines of evidence suggest that asymmetry at the community and guild-level were influenced by assembly history (H1) and were not random. First, although cases of asymmetry occurred in TT and TC, we found significant overall asymmetry only among CC mixes which included guilds with more species per guild than in TC or especially TT mixes. This is concordant with Gilpin's simulations, in which competition-driven extinctions during assembly enhanced the likelihood of asymmetry under mixing. Second, extinctions were quite frequent during the assembly process. We did not observe any consistent effects of extinction rate during assembly on the outcome of mixing, but the timing, order, and species identity of extinctions and density changes likely had strong effects (Drake 1990, Lundberg et al. 2000, Fukami 2004) analogous to the assembly method in Gilpin's model.

The significant trend towards asymmetry is a robust result because our experimental system both maintained key features of theoretical models and incorporated substantial novel complexity. Consequently, we also find evidence for effects that have not been considered by theory. In contrast to our results that found lower asymmetry in TT and TC than CC mixes, Wright (2008) found substantially more asymmetry when simulating mixing of simple trophic communities than mixing competitive communities (H2). Our failure to support H2 may be explained by three complexities that are absent from Wright's model relating to the guild structure of our trophic communities. First, Wright's model did not include unsaturated communities. Our trophic communities have fewer species in the omnivore and algae guilds, making it more likely that under mixing a 'niche-filling' process occurs on resource heterogeneity (Davies et al. 2009). This could reduce post-mixing competition and enhance persistence. A role for niche-filling is suggested by non-significant asymmetry for bacterivores (a guild likely not to be saturated at two species, Davies et al. 2009). Second, the traits of species in Wright's model were fixed. However, species may show adaptive feeding responses (behavior or plasticity) to mixing in trophic communities that reduce resource competition and enhance persistence (Petchey 2000). Supporting this possibility, we found that omnivores (generally most flexible in diet) show non-significant asymmetry despite containing relatively more species than other guilds. Third, Wright's model including only a predator and prey guild whereas our experiment included up to six guilds. Trophic and competitive communities differ in their capacity to "swap" guild modules rather than individual component species. Upon mixing, TT or TC mixes can,

for example, maintain the omnivores from one community but the herbivores and predators from another. Mean asymmetry is often high within guilds (higher than the overall community-level asymmetry in four of five guilds, Table 1) suggesting that between guild-interaction is more intense under sudden mixing than interactions between whole communities.

Although asymmetry may seem only marginally more common than under our null model, it is important to emphasize that even the occasional occurrence of a highly asymmetric outcome of mixing can be a major event that produces the complete replacement of local community composition. Our experiment has only considered the mixing of environmentally identical patches, however in nature synergistic or antagonistic interactions between communities and environmental heterogeneity among patches could affect asymmetry or the composition of dominant communities. Further, the frequency of asymmetry might be higher under other assembly scenarios, such as those involving concurrent and directional environmental change (deterministically favoring one community over another, Croft 2001) or interactions involving direct and obligate facilitative or parasitic interactions (producing linked extinctions, (Goodnight 2011)). Lastly, study of anthropogenic interaction with ecosystems has focused on the effects of lowered connectivity while the role of increased connectivity is less well understood in contexts like species introductions producing invasive communities and invasional meltdown (Simberloff 2006), canal and corridor construction, or restoration projects requiring enhanced connectivity (Gilpin 1994, Lockwood et al. 2005).

Theoretical frameworks like sudden mixing theory are urgently needed in the context of rapid anthropogenic change to ecosystem connectivity.

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Chapter 3: Predators and disturbance interact to regulate spatial pattern in multi-trophic microcosms

Abstract: Variation partitioning methods are a key tool for the application of metacommunity theory to natural landscapes. However, the interpretation of environmental, spatial and residual variation is challenging because multiple ecological processes involving dispersal, disturbance, heterogeneity in niche breadth, and predation can produce similar patterns. Consequently, the application of variance partitioning to situations where underlying ecological processes are well characterized can help define appropriate interpretations in more challenging natural settings. We applied variance partitioning methods to experimental multi-trophic protist microcosm landscapes with one predator, two competing prey, two patch types, and localized dispersal. To test the ability of variance partitioning to identify underlying processes, we applied three disturbance regimes to our landscapes with the expectation that the impact of the predator would be reduced by disturbance. We find that the environmental and spatial components are indistinguishable among treatments and that the predator was the most important explanatory factor in no and intermediate disturbance, while in high disturbance spatial effects were predominant due to the increased extinction rate of the predator. Spatial segregation developed in the no disturbance treatment between the predator and its preferred prey, while this pattern weakened with disturbance. Overall, our results indicate that spatial patterns like those produced by trophic interactions should be explicitly incorporated into variance partitioning analyses.

Introduction

Metacommunity theory currently centers on the interplay of the local environment with spatial effects generated by dispersal (Leibold 2011, Logue et al. 2011, Winegardner et al. 2012). Variation partitioning methods are the primary quantitative approach linking this theory to natural patterns in community composition across space (Gilbert and Bennett 2010). Variance partitioning addresses multicolinearity (Lawler and Edwards 2006) and attributes unique variances explained by local environmental variables versus spatial variables (Borcard and Legendre 1994, Legendre and Legendre 1998). Strong environmental signal is thought to indicate species sorting, strong spatial signal is hypothesized to indicate either patch or neutral dynamics, and a combination is hypothesized to indicate mass effects (Cottenie 2005). A meta-analysis (Cottenie 2005) and subsequent studies using variance partitioning on natural communities most frequently report either environmental or a mixture of environmental and spatial effects (Smith and Lundholm 2010, Logue et al. 2011).

Despite its widespread application, the diagnostic use of variance partitioning to test among metacommunity models has been questioned for both statistical and ecological reasons. The statistical issues associated with variance partitioning are related to the mischaracterization of explanatory environmental and spatial matrices and sampling design (Gilbert and Bennett 2010). This results in over or underestimation of the variances attributed (Gilbert and Bennett 2010). Fortunately, improved statistical techniques exist (Yee 2006) or are under development (Diniz-Filho *et al.* 2012) and

variance partitioning can be supplemented with other independent statistical analyses, such as those describing spatial pattern formation (Kefi et al. 2010, Biswas and Wagner 2012).

The ecologically based problems center on the fact that variance partitioning is correlative while multiple ecological processes can produce the same patterns (Guichard and Steenweg 2008). In addition to the frequently hypothesized role of dispersal rate (e.g. (Beisner *et al.* 2006)), three other factors have been shown to mediate the relative importance of environmental, spatial and residual components: 1) it has been theoretically and experimentally demonstrated that increasing disturbance rates can increase residual and spatial effects by forcing recolonization with species from neighboring patches that are not optimally adapted to local conditions (Leibold and Loeuille *in review*, Fukumori et al. *in review*, (Ohashi and Hoshino 2014)), 2) heterogeneity in the niche breadth of species within communities can produce divergent patterns for specialists versus generalists (Pandit et al. 2009, Coyle et al. 2013), and 3) species interactions such as predation can produce spatial patterns in the absence of underlying environmental heterogeneity (Borcard and Legendre 1994), such as cascading effects when higher trophic levels are dispersal limited (Verreydt *et al.* 2012).

Although the effects of disturbance, niche breadth, and predation on variance partitions have been studied in isolation, these factors occur simultaneously in natural metacommunities and they likely interact. This makes disentangling their effects with natural datasets difficult. However, the application of variance partitioning to situations where underlying ecological processes are well characterized can help define

appropriate interpretations in more challenging settings (Gilbert and Bennett 2010). We implemented these three factors in experimental protist microcosm metacommunities. Protist microcosms are a powerful tool to bridge theory with natural patterns (Cadotte et al. 2005, Benton et al. 2007), including among metacommunities (Carrara et al. 2012, Livingston et al. 2013). Specifically, we applied variance partitioning methods to landscapes with multi-trophic communities including one predator and two competing prey each with a different niche breadth, two patch types, and localized dispersal. Predators are susceptible to increasing rates of patch disturbance and face metapopulation extinction beyond critical thresholds (Swihart et al. 2001, Staddon et al. 2010). This means that community variance explained by predation should weaken with increasing disturbance. To explore this, our landscapes were replicated across three levels of disturbance from none to 30 percent of patches defaunated weekly. We test how well variance partitioning techniques can distinguish among the combined effects of disturbance, variable niche breadth, and the predator. We do this by 1) partitioning prey community variance using separate environmental, predator, and spatial explanatory matrices. We then 2) crosscheck and supplement the variance partitioning using a spatially explicit analysis of species pairwise clustering patterns.

Methods

Overview

Our experimental system is based on that used in Fukumori et al. (*in review*), however the experimental design used here differs in several important aspects. Each landscape consisted of a lattice with an equal mixture of illuminated patches that support algal production and herbivory and patches with a wheat seed to support decomposition and both herbivory and bacterivory. Landscapes were inoculated with two competing omnivorous (mixed herbivore and bacterivore) protists, one (*Paramecium* sp.) a strong herbivore, and the other (*Colpidium striatum*) a strong bacterivore. A voracious shared predator (*Didinium nasutum*) was added to this two-prey system. Disturbance was manipulated at three levels by replacing patches weekly with sterilized medium to achieve "defaunation." Dispersal was implemented weekly by transferring a small amount of medium from one to the next patch from four directions. We monitored the number of individuals weekly for six-weeks and performed statistical analyses on the observed two-species prey metacommunities.

Microcosms and study species

Microcosms consisted of 60 mm × 15 mm vented petri dishes. Landscapes consisted of a 10 × 10 lattice of patches with half of the patches with wheat seeds and half without. Seedless patches consisted of 11 ml of sterilized medium with 0.55 g/L of protozoa pellets (Carolina Biological Supply, Burlington, North Carolina, USA) in deionized water and 800 ul of stock algae (*Chlamydomonas reinhardtii*, NIES-2239, NIES Culture Collection). Seed patches consisted of 12 ml of pellet medium and one

sterilized wheat seed as a source of slowly released carbon. Once a week, 10% of medium was removed from each patch and replaced with fresh medium to reduce the build-up of toxic metabolites. We generated three 100-patch landscapes at each disturbance level for a total of nine landscapes or 900 communities.

We used three protozoan species: *Colpidium striatum*, *Paramecium* sp. and *Didinium nasutum*. *Colpidium* was obtained from Carolina Biological Supply. *Paramecium* and *Didinium* were obtained from other laboratories. Both prey species are omnivores. Paramecium grows equally well on a mixed or pure algae diet, while *Colpidium* grows best on bacteria alone and supports extremely low growth when cultured on algae alone (Fukumori et al. *in review*, Figure 3.1). Both species are competitors, although they can coexist (Fukumori et al. *in review*, Figure 3.1). The niche breadth of *Paramecium* includes both patch types while *Colpidium* is effectively restricted to patches with wheat seeds (Figure 3.1). *Didinium nasutum* is a voracious predator on *Paramecium* and upon colonization typically drives a *Paramecium* population to extinction within one week (Luckinbill 1973). Although *Didinium* fed on *Colpidium* in our experimental system, it was not observed to reduce the population size of *Colpidium* and in fact *Colpidium* benefits from the presence of *Didnium* due to the associated removal of its competitor (Figure 3.1). After exhausting its prey, *Didinium* formed resting cysts that hatch following the recolonization of *Paramecium* and that once again eliminate the founding *Paramecium* population (Beers 1937). *Colpidium* populations did not induce the formation or hatching of cysts. Given our weekly

sampling window, we conceptualize patches with cysts as permanent predator populations.

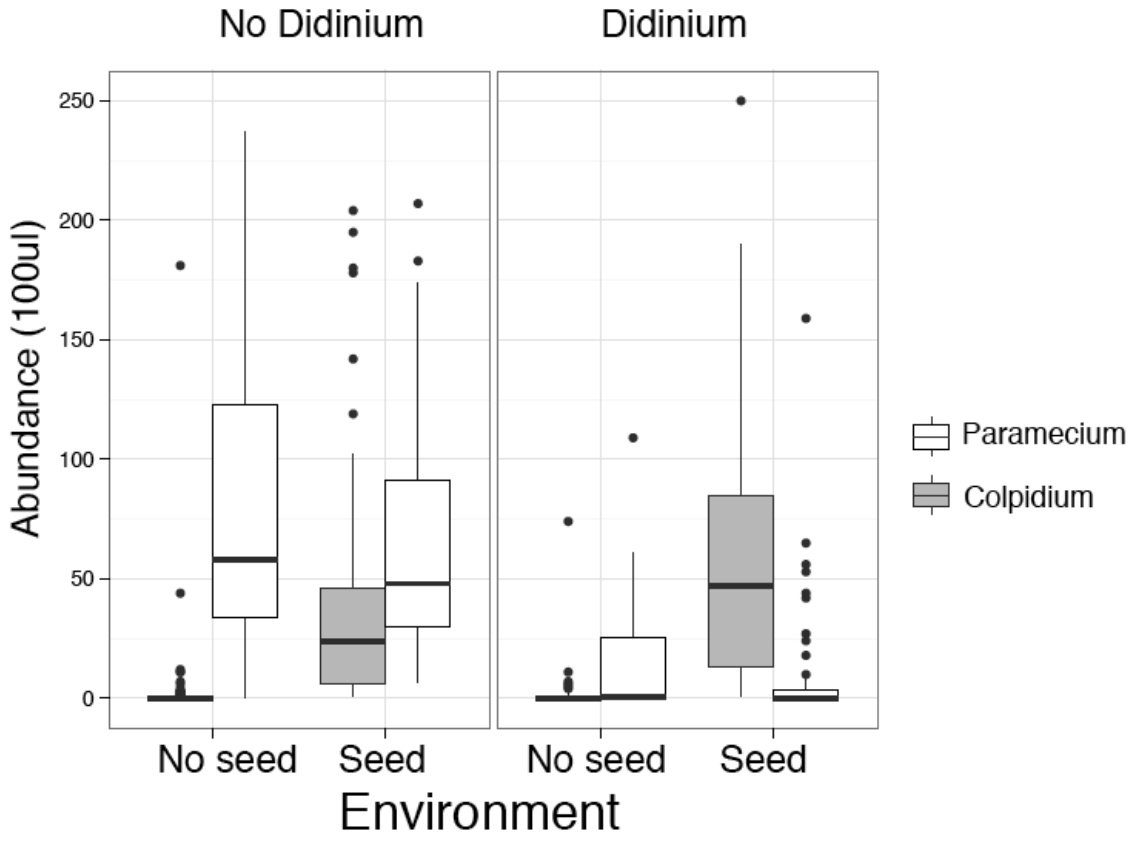

Figure 3.1: Abundance of prey species in patches with and without wheat seeds and with and without Didinium. Data are from all replicates combined from week six. Didinium significantly decreases the abundance of Paramecium ($P<0.0001$, $df=298$, $F=108$) and marginally significantly increases the abundance of Colpidium ($P=0.068$, $df=250$, $F=3.372$).

Initial conditions and dispersal

All patch types were assigned at random in each replicate. All landscapes were started with 1/3 of the patches inoculated at random with one or the other of the two prey species and 1/3 of the landscape being empty. We inoculated with 200ul of the two

prey protists from high-density stock cultures. One individual of *Didinium* was added at random to 1/3 of the patches at week 2. Experimental cultures were kept at 20 C and a 14:10 h light:dark cycle.

Dispersal was implemented by transferring a small amount of medium (≈3µl) from one to the next patch from four directions of the lattice design using a dental toothpick (Fukumori et al. *in review*). We estimate that each dispersal event between two adjacent patches typically transferred 0-9 individuals from established prey populations (Fukumori et al. *in review*). Dispersal was started one week after inoculation.

Disturbance treatments

The exact same local patch type alignment was used in each of three replicate landscapes across the disturbance treatments (disturbance treatment was blocked by landscape alignment). Disturbance was manipulated by varying the frequency of patches replaced at random with a new patch containing sterilized fresh medium (no disturbance =0% (0 patches), intermediate disturbance =15% (15 patches/week), and high disturbance =30% (30 patches/week)). Preliminary trials had shown that higher extinction rates led to rapid global extinctions of some or all species in the metacommunity (Fukumori et al. *in review*). The disturbance treatments were performed weekly beginning the second week to allow protists to attain multiple large populations

before disturbance. Throughout the experiment, disturbances occurred immediately before a dispersal treatment.

Sampling

We counted all individuals of the three species (including *Didnium* cysts) in a 100-*μ*L sample. Due to logistical constraints, it was not feasible to count all landscapes every week. One replicate was counted only during weeks two, three and six, while the other two replicates were counted every week beginning on week two for a total of 36 landscape counts. In addition to population size, we sampled several ecosystem properties to determine if our results had possible consequences for ecosystem function. The only known impact of the protist species used in this experiment on ecosystem properties is the herbivory of *Paramecium* on *Chlamydomonas*. By reducing *Chlamydomonas* abundance, this has the potential to regulate concentrations of nutrients used by *Chlamydomonas*. We measured bulk chlorophyll fluorescence during week three to six, and NO4 and NH4 concentrations during week five in two replicates. Chlorophyll fluorescence (ex440 em680 (Sher *et al.* 2011)) and colorimetric assays for NO4/NH4 (Sims et al. 1995, Doane and Horwáth 2003) were measured in 96 well microplates using a plate reader (CS-9300 microplate reader (Shimadzu, Tokyo, Japan)). Results of chlorophyll and nutrient sampling are reported in the supplement.

Statistical analyses

Our core set of statistical analyses use partial canonical analyses (Legendre and Legendre 2012) to partition community variation into environmental and spatial components. In some analyses, we also partition variation explained by *Didinium* and by fine and broad scale spatial variables. In total, we utilized five explanatory matrices: environment (no seed/seed), *Didinium* (presence/absence), total space, fine scale space, and broad scale space. Variation partitioning works by subtracting the variation explained individually by each explanatory matrix (conditional variation) from the variation explained by all explanatory matrices combined (total variation) to estimate independent and covariance fractions (Gilbert and Bennett 2010). A full description of this technique can be found in (Peres-Neto et al. 2006).

All analyses were performed using R (version 3.0.6). Variance partitioning was performed using the varpart function in Vegan (Oksanen *et al.* 2013). The adjusted canonical R-squared produced by this function are analogous to the adjusted R-squared in multiple regression (Peres-Neto *et al.* 2006). Species abundances were transformed using the Hellinger transformation (Legendre and Legendre 2012). Environmental and Didinium variables were not transformed. We characterized space using PCNM, a technique that captures spatial dependencies at multiple spatial scales using a truncated distance matrix among sites (Dray *et al.* 2006). Following forward selection using the QuickPCNM function (PCNM library), we grouped the resulting positive and negative spatial axes into broad and fine spatial scales by selecting two sets of relatively more contiguous axes (Borcard *et al.* 2011). We did not detrend our data prior to using PCNM because linear gradients likely result from dispersal in our experimental

landscapes. Differences among disturbance treatments and the effect of time on variation components was analyzed using ANOVA.

To relate the spatial patterns in the PCNM to known species interactions among the protists, we used the Kcross summary statistic (Lotwick and Silverman 1982) implemented using the envelope function in Spatstat. Kcross is related to Ripley's K and measures the degree of clustering or segregation among pairwise combinations of species using point pattern analysis. For each landscape and each of the three possible pairwise combinations of species, we identified those that showed significant clustering at any sample radius (P=0.01, Monte Carlo test, runs=199, (Ripley 1981)) and summed the observed Kcross statistics at spatial scales where it fell outside the upper or lower critical envelope.

Results

Patch occupancy and metacommunity abundance

From the initial 30% of patches inoculated, *Paramecium* rapidly increased in occupancy to a mean of 77% by week two, while *Colpidium* increased more slowly (Figure 3.2). Across treatments, both species continued to increase significantly or marginally significantly from weeks two to six (F=3.55-7.35, df=36, P=0.02-0.08), except for *Colpidium* in the high treatment (F=3.1, df=36, P=0.11). The dip in *Paramecium* occupancy observed from week two to three is due to the addition of *Didinium*.

Throughout the experiment *Paramecium* occupied more patches (>60%) than *Colpidium* (<60%), although the two species sometimes overlapped in metacommunity abundance. There was no significant effect of disturbance treatment on *Paramecium* or *Colpidium* occupancy ($F=0.12$, $df=36$, $P=0.88$; $F=1.54$, $df=36$, $P=0.23$, respectively) or abundance ($F=0.38$, $df=36$, $P=0.68$; $F=1.03$, $df=36$, $P=0.37$, respectively).

Increasing disturbance had a significantly negative effect on *Didinium* occupancy ($F=13.84$, $df=27$, $P<0.0001$) and cyst abundance ($F=4.02$, $df=27$, $P<0.05$, Figure 3.2). *Didinium* occupancy significantly increased over time in the no disturbance treatment to a mean of 34% by week six ($F=12.02$, $df=8$, $P<0.01$), while there was a nonsignificant decline at high disturbance to 13% ($F=0.63$, $df=8$, $P=0.45$).

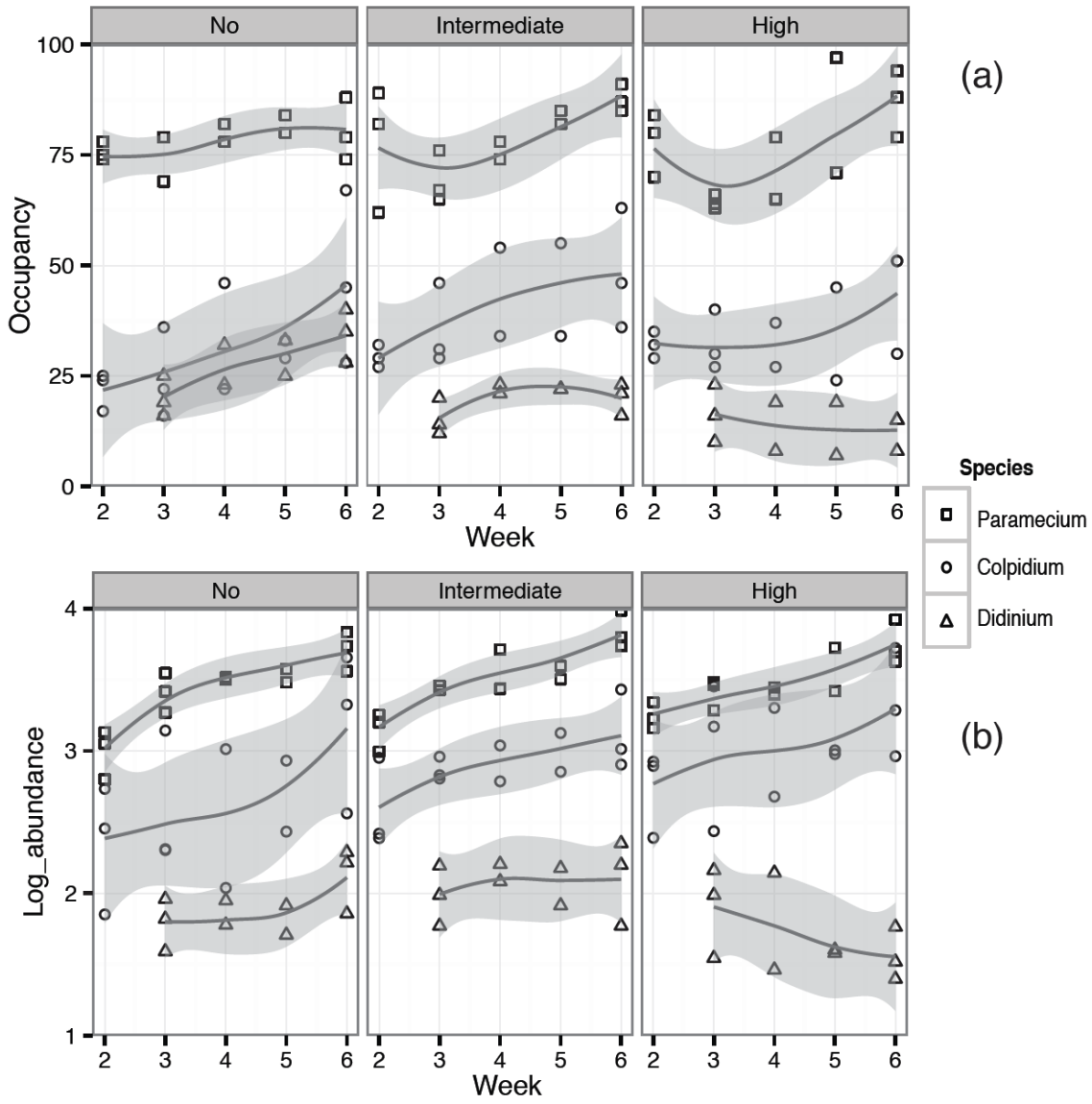

Figure 3.2: Patch occupancy (a) and metacommunity abundance (b) patterns for each of the three species from week two to six in three disturbance treatments. Didinium was added on week two and first counted on week three. Didinium abundance represents the total number of cysts observed. Points are fit with a Loess smoother.

Variance partitions

From initially random conditions, the variation partitions for each landscape changed over time across the treatments (Figure 3.3). In all treatments, the environmental component increased as *Colpidium* spread to patches with seeds (No: $F=7.12$, $df=11$, $P<0.05$; Intermediate: $F=27.36$, $df=11$, $P<0.01$; High: $F=7.55$, $df=11$, $P<0.05$). None of the other components showed a significant trend with time (Figure 3.3), likely due to the relatively small number of landscapes per week by treatment combination. Qualitatively, in the no disturbance treatment spatial and residual components decreased, while *Didinium* showed a hump-shaped trend with time. This pattern became progressively weaker with disturbance, with little temporal pattern apparent in the high disturbance treatment.

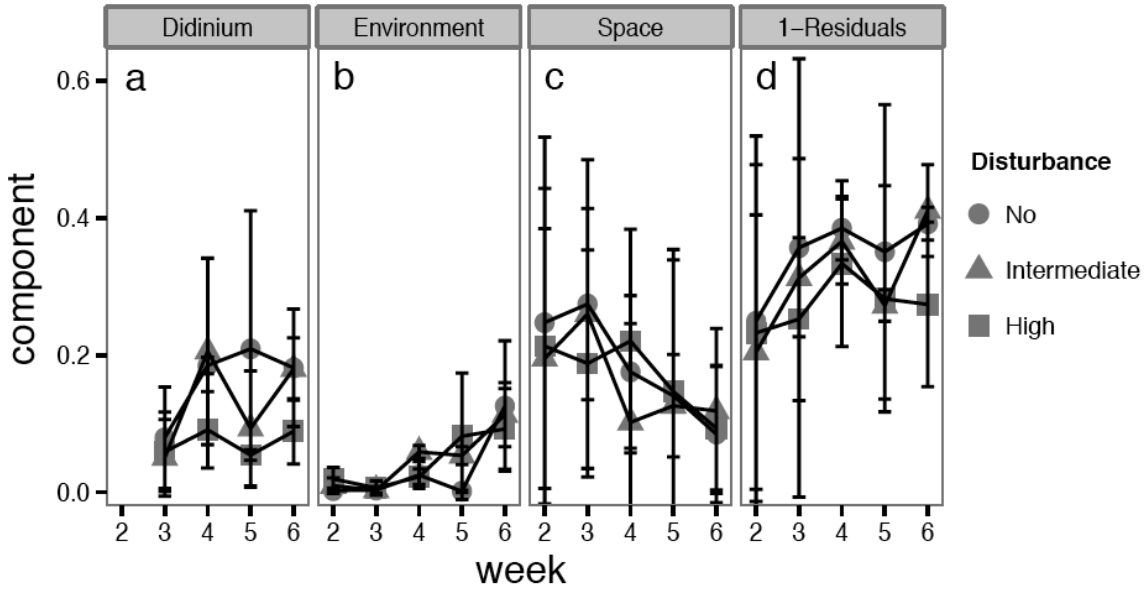

Figure 3.3: Variation partitioning through time by explanatory factor: (a) Didinium, (b) environment, (c) space, and (d) 1-residuals. Error bars show standard deviation. Component is the unique proportion of variance explained.

To examine absolute differences in variation components among treatments, we combined the values for the last three weeks after the landscapes had time to depart from initial conditions. We also further decomposed space and *Didinium* with space into broad and fine spatial scales (Figure 4). Overall, residuals were the largest component in all treatments (greater than 50%). In the no disturbance treatment residuals were significantly lower than under high disturbance ($F=4.11$, $df=18$, $P<0.05$, TukeyHSD). The variance explained by *Didinium* was significantly higher in the no versus high disturbance ($F=3.08$, $df=18$, $P<0.05$, TukeyHSD) and *Didinium* with space was marginally significantly higher ($F=3.25$, $df=13$, $P=0.07$, TukeyHSD). Fine scale space was highest in the high disturbance treatment, although this was only significant for the intermediate to high comparison ($F=4.14$, $df=13$, $P<0.05$). There were no significant effects of disturbance on environment, broad scale space, or *Didinium* with fine scale space.

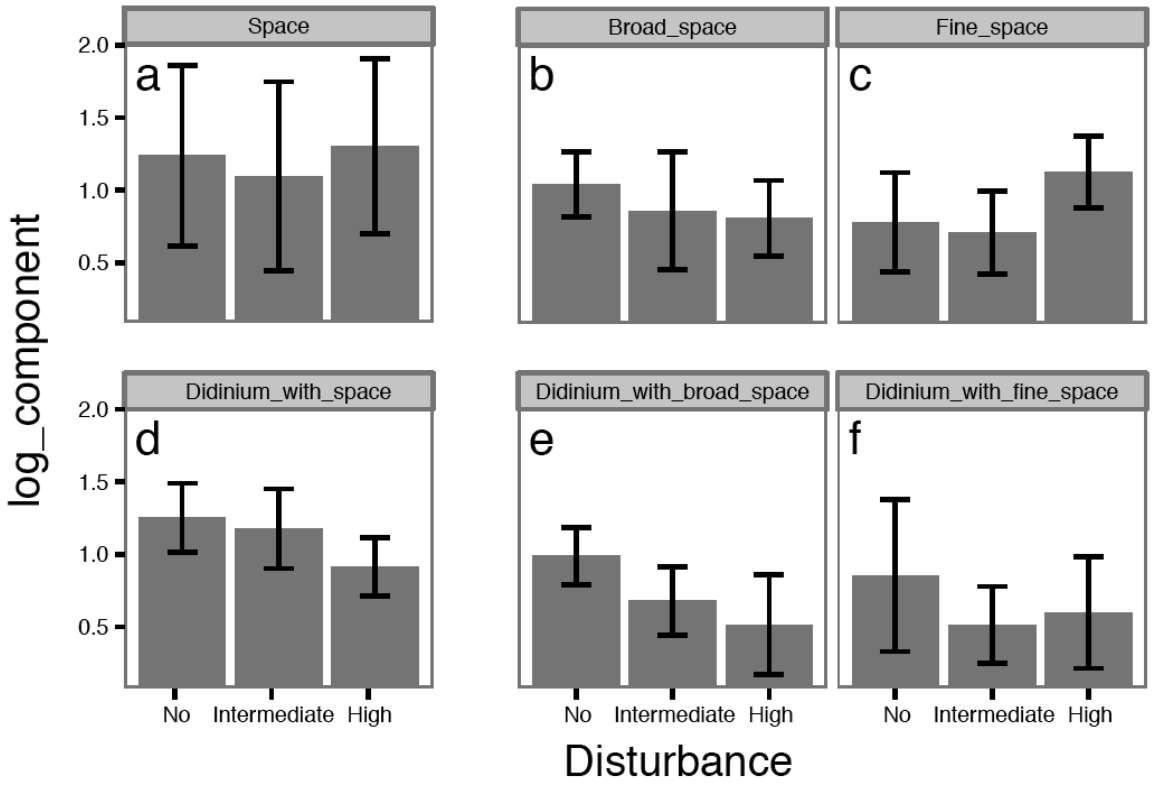

Figure 3.4: The variance partition by spatial scales for weeks 4 to 6. Space (a) is divided into broad (b) and fine (c) scales. Didinium with space (d) is the shared component between Didinium and space and is also split into broad (e) and fine (f). Broad and fine space sum to total space or total Didinium with space. Error bars show standard deviation.

Spatial co-clustering

Among the three pairwise combinations of species (*Paramecium-Didinium*, *Colpidium-Didinium*, and *Paramecium-Colpidium*), in all weeks we observed 26 significant deviations (P=0.01) from the expected value in no disturbance, 25 in intermediate, and 18 in high disturbance. A majority of deviations were below the expected value (62%), indicating segregation was more common than clustering. The number of significant pairs increased with time in the no disturbance treatment (weeks

3-6 only to account for the addition of *Didinium*; $F=13.98$, $df=8$, $P<0.01$), while there was no significant effect of time at intermediate or high disturbance ($F=1.96$, $df=8$, $P=0.20$; $F=0.02$, $df=8$, $P=0.9$). Including both clustering and segregation, 49% of deviations under no disturbance were either *Paramecium*-*Didinium* (37%) or *Colpidium*-*Didinium* (11%) pairs (Figure 5). This percentage decreased to 38% at intermediate and to only 19% at high disturbance. Thus under high disturbance, 81% of deviations were *Paramecium*-*Colpidium* pairs.

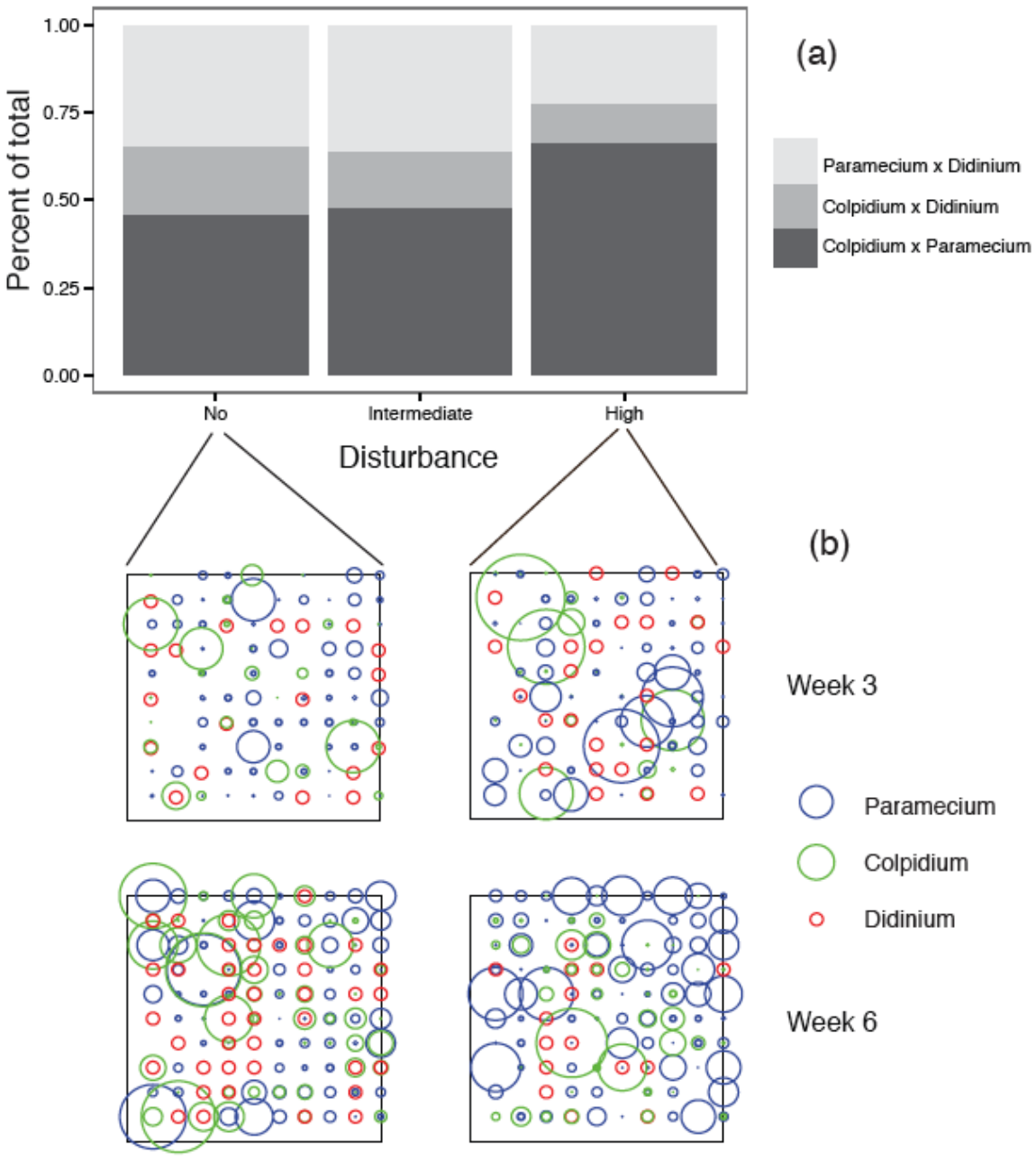

Figure 3.5: Significant spatial clustering or segregation among pairs of species. Clustering was measured using the Kcross statistic. (a) Bars show percent of the total number of significant associations detected in all replicates and weeks of each treatment combined. (b) Landscape maps from a representative replicate at week three when Didinium was added and at the end of the experiment (week 6). The size of circles is proportional to the population size in each patch, except for Didinium which is shown as presence/absence only.

Herbivory, chlorophyll and nutrients

We found that the correlation between *Paramecium* abundance and Chlorophyll across patches was negative in no and intermediate disturbance landscapes and that there was no correlation under high disturbance (Supplementary Figure 1, F=3.18, df=16, p=0.07, TukeyHSD). NO4 was at undetectable levels in our landscapes (<0.05ppm). NH4 showed the same pattern with *Paramecium* as Chlorophyll, however this result was not significant (Supplementary Figure 3.1).

Discussion

Summary

We find that in our microcosm landscapes variation partitioning successfully distinguishes among the effects of 1) disturbance, 2) heterogeneous niche breadth and 3) the predator. The effect of disturbance is detectable in the decreased variance explained by *Didinium* and *Didinium* with space as disturbance increased. As we hypothesized, this is due to the reduced occupancy and abundance of *Didinium*. The effect of heterogeneous niche breadth is detectable in the environmental component that can be attributed entirely to the spread of *Colpidium*. Lastly, the variance explained by the predator is the largest non-residual component in the no and intermediate disturbance treatments, demonstrating the strong control it exhibits on landscape

pattern. However, the pure spatial component is similar across treatments and cannot readily be attributed to dispersal limitation versus species interactions. We discuss the implications of these results for variance partitioning of natural landscapes.

Scaling to natural systems

Although we controlled and manipulated key aspects of our microcosms, our experiment reproduces several features common in natural systems including randomness, unmeasured or unknown environmental variables, and long transient effects. All of our initial conditions were completely random and this caused variation among replicates. We characterized the environment coarsely as seed or no seed, while in reality the uncharacterized bacterial community in both patch types likely contributed to both spatial and temporal variation in protist abundance (Simek *et al.* 1997). Both initial randomness and unmeasured environment likely contributed to the high residuals in the variance partition even under no disturbance. Such high residual variation is commonly observed in variance partitions (Cottenie 2005). Lastly, the increasing prey abundances and decreasing predator abundance under disturbance indicate our system was not at steady state after six weeks. Steady state under no disturbance would likely involve all patches being occupied by *Didinium* cysts, whereas under high disturbance *Didinium* is likely to have gone extinct. However, our intention was not to investigate steady state behavior, but rather to explore the transient dynamics occurring after a reasonable number of protist generations had passed (~45-135 generations in

continuously occupied patches by week six). Transient dynamics are commonly observed in natural systems, particularly those under disturbance regimes (Fukami and Nakajima 2011).

Importance of trophic structure

Our results underscore the importance of including explanatory factors that characterize trophic structure into variance partitioning because they can substantially improve the total variance explained. Several other studies have recognized the cascading effects of limited predator dispersal on prey (Verreydt *et al.* 2012) or utilized entire predator or parasite communities as factors in variance partitions (Halpern et al. 2006, Kurek et al. 2011, Rzanny et al. 2012). However, the number of studies that have included predators as explanatory matrices is small relative to the availability of data on predator abundance. Our study is the first to explicitly compare variation components produced by trophic versus environmental and spatial factors in controlled and highly replicated landscapes. The strong control of trophic structure in our system is a function of the strength of predation of *Didinium* on *Paramecium* and the lack of environmental effects on *Didinium*. Our results suggest the impact of predators on the focal metacommunity is likely to be strongest in systems with low levels of disturbance where those interactors are themselves spatially structured by dispersal and unaffected by environmental gradients within the study area. For example, in aquatic systems, the fish versus fishless community context is a strong effect of a dispersal-limited predator

(Reissig *et al.* 2006) that is also susceptible to high rates of disturbance (WILSON *et al.* 2008). Even in systems where predators are not dispersal limited, fluctuating or cyclical predator prey-dynamics make trophic structure a key aspect of the local environment (Gouhier *et al.* 2010).

Interpreting space

We find that attribution of the pure spatial component to a single causal process is particularly challenging. Fundamentally, this is because environmental variables are identified before they are measured, whereas spatial variables are produced ad hoc using statistical procedures and then used as indirect signatures of process. Spatial signal is most frequently considered a signature of dispersal limitation or of mass effects if environmental signal is also observed (Cottenie 2005). In our experiment, the Kcross statistics indicate that the predominate cause of spatial pattern under no disturbance is *Didinium*, whereas under disturbance the predominant cause is random clustering resulting from frequent re-colonization and dispersal limitation. In addition, we find that fine scale spatial patterns increase under high disturbance. This supports the hypothesis that spatial effects should be more common at fine scales that are smaller than the scale of environmental drivers (Dray *et al.* 2012), in our case *Didinium*. Together, these results suggest that spatial components of variance partitioning can be better understood if decomposed with scale and supplemented with independent tests of spatial pattern.

Conclusions

Our results highlight three important considerations for variance partitioning:

1) Attributing variance to multiple causal processes may be facilitated by splitting sampled sites into separate groups using estimates of disturbance rate.

2) Predators should be included as separate explanatory matrices. Future analyses including predators may help reduce residual variation and including them as separate matrices may provide deeper insights into assembly processes.

3) Spatial components should be interpreted with caution and supplemented with independent analyses investigating the possible role of predation or other species interactions in generating spatial signal.

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Appendix: Supplemental Information

Chapter 1 Supplemental Information

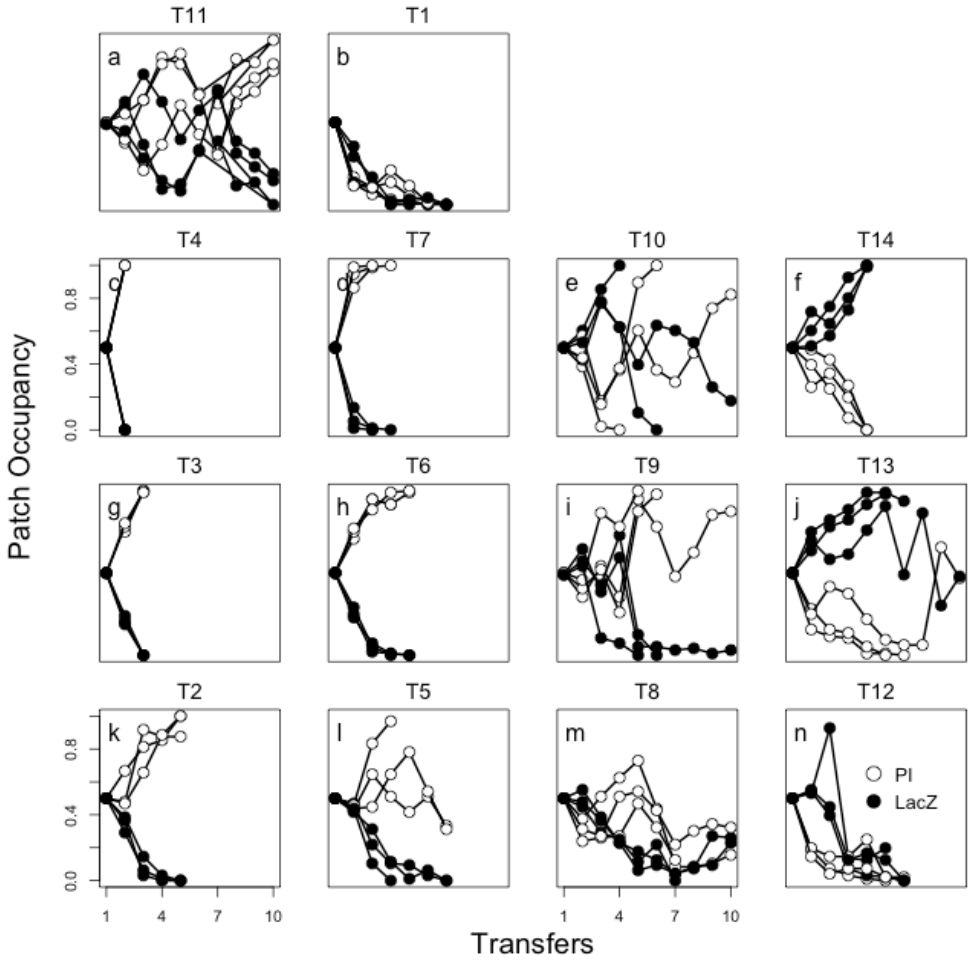
Supplementary Table 1.1: Treatment parameters. The “Treatment” column shows the treatment numbers (corresponding to those in Fig. 2 for treatments T1-T14). The isolation experiments are H7 and H11 and correspond to the initial parameters used in treatment T7 and T11. Metacommunity “isolation” refers to the proportion by which absolute colonization rates were reduced in the isolation experiment. “Dilutions LacZ” (the weak competitor) and “PI” (the strong competitor) are the negative exponent (base 10) of the “Concentration LacZ” and “PI” after dilution for each treatment. The “PI dilution reduction factor” represents a proportional reduction in the volume of PI before serial dilution was implemented (the strength of the trade-off).

Treatment	Isolation	Dilutions LacZ (10^{-X})	Dilutions PI (10^{-X})	Concentration LacZ	Concentration PI	PI dilution reduction factor (trade-off)
T1	-	7	7.968	1.00E-07	1.08E-08	0.892
T2	-	6.5	7.468	3.16E-07	3.40E-08	0.892
T3	-	6	6.968	1.00E-06	1.08E-07	0.892
T4	-	5.5	6.468	3.16E-06	3.40E-07	0.892
T5	-	6.5	7.55	3.16E-07	2.82E-08	0.911
T6	-	6	7.256	1.00E-06	5.54E-08	0.945
T7	-	5.5	6.962	3.16E-06	1.09E-07	0.965
T8	-	6.5	7.633	3.16E-07	2.33E-08	0.926
T9	-	6	7.545	1.00E-06	2.85E-08	0.972
T10	-	5.5	7.456	3.16E-06	3.50E-08	0.989
T11	-	5	7.368	1.00E-05	4.29E-08	0.996
T12	-	6.5	7.715	3.16E-07	1.93E-08	0.939
T13	-	6	7.833	1.00E-06	1.47E-08	0.985
T14	-	5.5	7.95	3.16E-06	1.12E-08	0.997
H7	0.7	5.5	6.962	3.16E-06	1.09E-07	0.966
H7	0	5.5	6.962	3.16E-06	1.09E-07	0.966
H7	0.9	5.5	6.962	3.16E-06	1.09E-07	0.966
H11	0.7	5	7.368	1.00E-05	4.29E-08	0.996
H11	0	5	7.368	1.00E-05	4.29E-08	0.996
H11	0.9	5	7.368	1.00E-05	4.29E-08	0.996

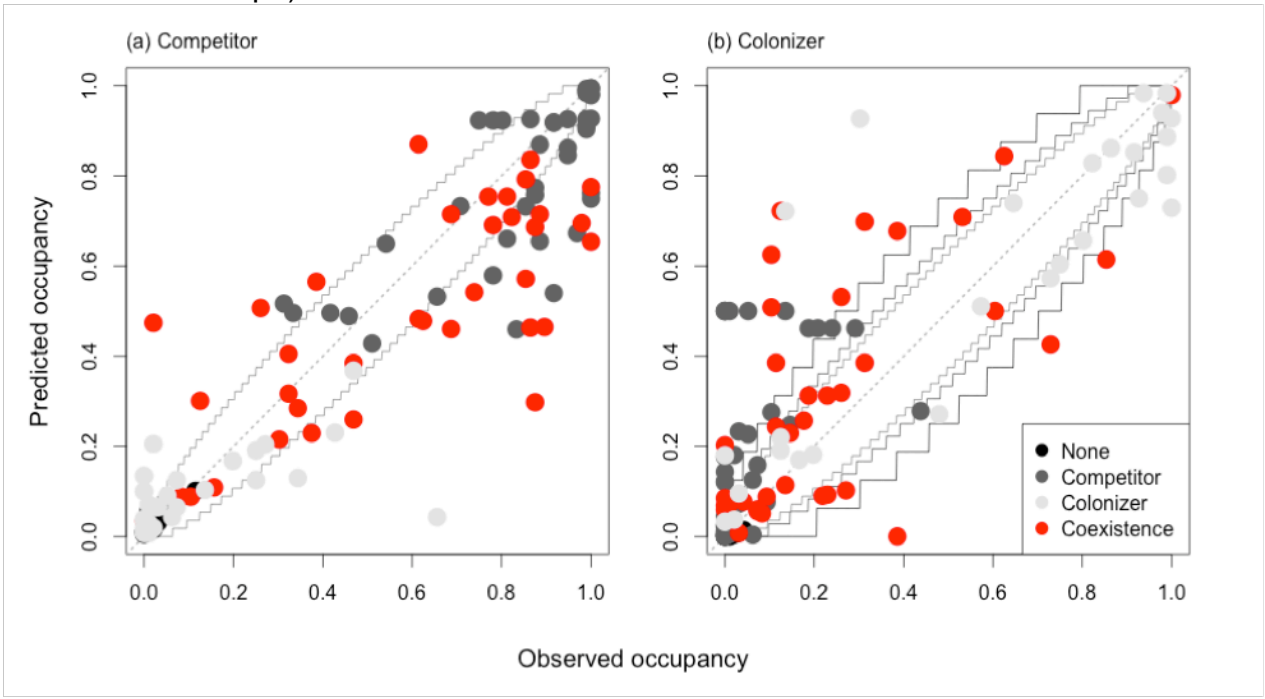
Supplementary Table 1.2. Generalized linear model (GLM) fitting with a binomial error distribution and a logit link function (GLM function in R [61]). Levels of significance: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1. The response variable is the proportion of occupied patches by each strain in each replicate (averages of transfers 8-10). The trade-off predictor is the 'Competitor dilution reduction factor' (see Table S2) or the proportional reduction in the overnight culture volume of the competitor prior to dilution (the reduction in colonization rate). Strain is the strain identity; colonizer (LacZ) or competitor (PI). Treatment T1 was not included in this analysis because the extinction of both strains was predicted. Strain details $n = 78$.

Coefficients:	Estimate	Std. Error	Z value	Pr(>|z|)
(Intercept)	33.86	13.29	2.547	0.010864*
Trade-off	-34.31	13.73	2.499	0.012444*
Strain	-74.75	20.82	3.589	0.000331***
Trade-off x Strain	75.67	21.44	3.530	0.000416***

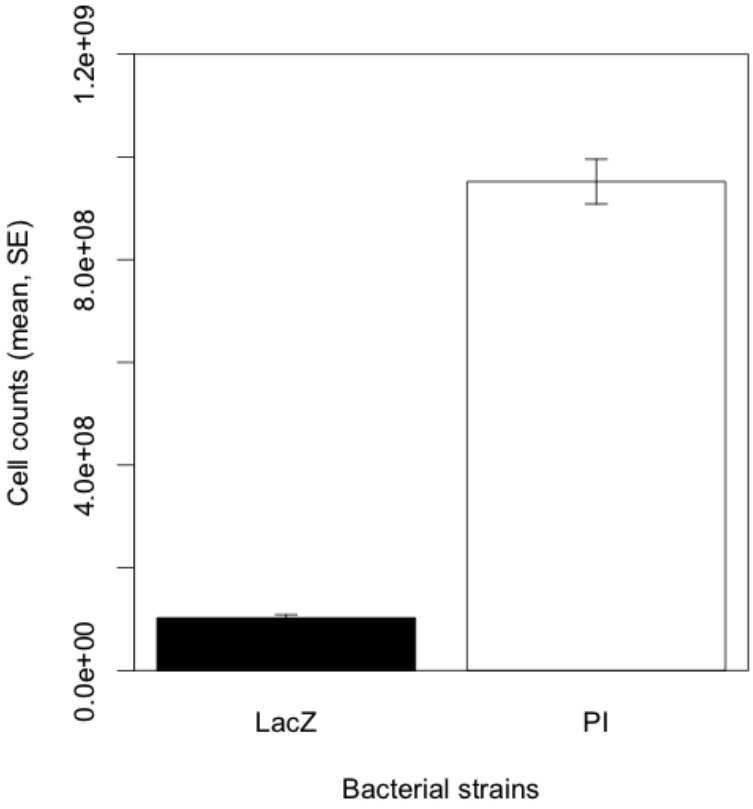
Supplementary Figure 1.1: **Time series for all treatments (T1-T14) from the main experiment.** Panel a shows treatments T11 and T1, which are outside the three main absolute dilution levels (see Fig. 2). Panels c-f, g-j and k-m each cross the coexistence zone along a range of absolute dilution rates (see Fig. 2). Each treatment included three replicates and was run for a maximum of 10 transfers. Replicates were stopped after the extinction of one or both strains occurred. Different circle colors indicate different strains: LacZ, black circles; PI, white circles.



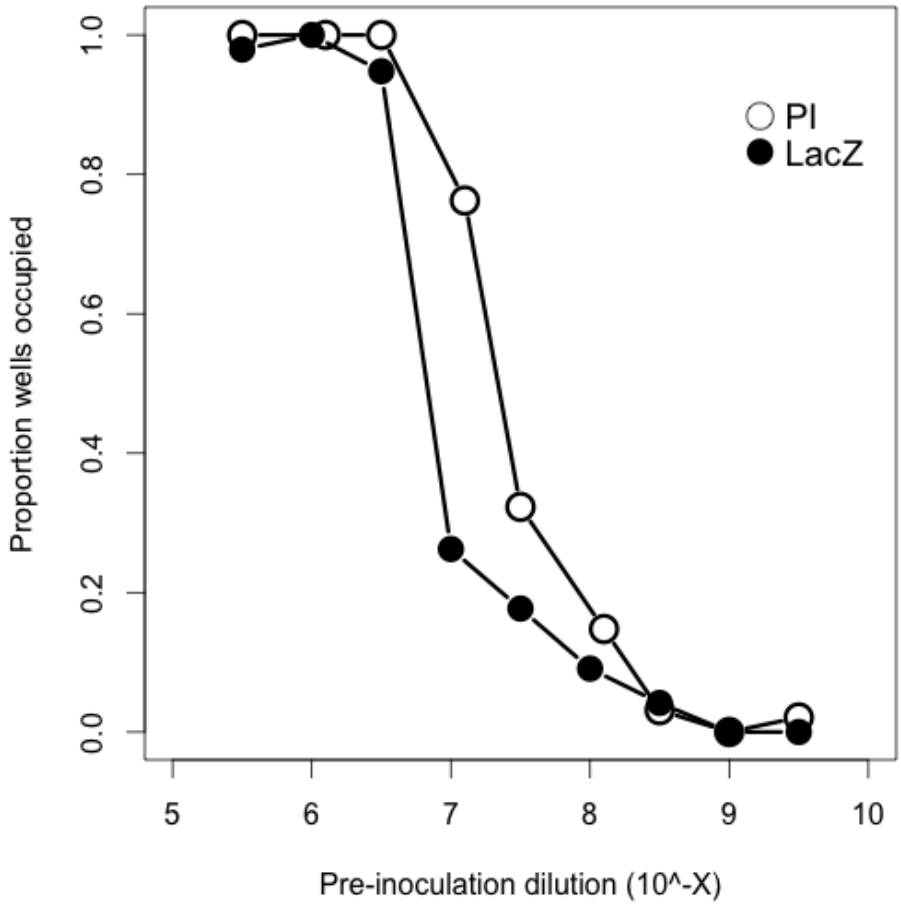
Supplementary Figure 1.2: **Observed and predicted occupancy for each strain after each transfer.** Colors indicate predicted persistence scenario. Four different scenarios are indicated by colored circles: the persistence of the two strains (red); the competitor persists (dark gray); the colonizer persists (light gray) and none of the strains persist (black). The dashed line indicates the 1:1 (perfect) match between predicted and observed values. Points above and below this line indicate that observed occupancies were greater or less than those predicted by the model, respectively. Full lines indicate 95% confidence envelopes from the stochastic binomial distribution of colonization events. Predicted and observed occupancy for the colonizer are scaled to available patches (i.e. those unoccupied by the competitor), and the different envelopes correspond to increasing proportions of available patches (from 1 – inner envelope to 0.2 – outer envelope).



Supplementary Figure 1.3: **Growth of LacZ (weak competitor) and PI (strong competitor).** Cells were counted after 24 hours of growth in wells filled with 200 μl KB in a 96-well microplate using droplet plating (15 μl droplets diluted to a 10^-6 concentration, replicated 3 times) on a selection of 5 wells with equal inoculation proportions of each strain. Bars represent means values for the total estimated number of cells in a single well and error bars represent standard error.



Supplementary Figure 1.4: **Proportion of wells containing each strain in 96-well plates as a function of the pre-inoculation dilution.** Different colors of circles indicate different strains: LacZ, black circles; PI, white circles. Overnight cultures of each strain were serially diluted equal amounts before inoculation onto separate 96-well microplates and grown for 24 h in 200 μl of KB. Each dilution level per strain was not replicated. Notably, the initial cell density was not equalized among strains prior to this trial, generating variation among the curves for each strain.



Supplementary Methods 1

This methods section describes two experiments that manipulated competition-colonization trade-offs of bacterial metacommunities in experimental

microcosms. In the first experiment – the main experiment – we quantitatively manipulated two key parameters: trade-off strength and absolute colonization rate. In the second experiment – the isolation experiment – we manipulated the isolation of each community from the species pool by imposing reductions in overall colonization rate. These experiments were conducted with marked *Pseudomonas fluorescens* strains of known competitive ability that can be made to approximate a two-species system. The supplementary methods detail three aspects of the design: 1) competition assays; 2) measuring abundances; and 3) the experimental protocol.

Competition assays. We assayed the competitive ability of both strains after 24 and 48 h in KB medium at 28°C in 96-well (240-μl) microplates (Falcon USA #353072). We used the range of relative inoculation densities of the two strains that would be used in our experiment by diluting LacZ and PI from overnights (incubated together) at rates corresponding to the same 14 treatments implemented in the main experiment (Supplementary Table S1; three replicates per treatment). This method produced a range from equal initial proportions of each strain to strongly biased toward LacZ. After 24 h of growth (conditions described in Methods *Bacterial strains*), we used a 96 pin replicator (Boekel well model 140500) to check for the presence/absence of each strain from one microplate per treatment (selected at random). One microliter of culture was filed onto petri dishes that contained a mixture of LB agar (Luria-Bertani medium with

agar; 5 $g.l^{-1}$ yeast extract + 10 $g.l^{-1}$ tryptone + 5 $g.l^{-1}$ NaCl, autoclaved 20 min at 121°C), X-gal and IPTG. PI colonies (yellow) can easily be distinguished from the LacZ (blue) on this medium. We further confirmed the pin replicator results using droplet plating (15-μl droplets diluted to a 10^-6 concentration, replicated 3 times) on a selection of 5 wells from the microplate with equal inoculation proportions of each strain (Supplementary Fig. S3).

We observed complete exclusion of LacZ after 24 h in all equal proportion wells and in 99% of the 1344 wells in the 14 microplates. However, when the initial inoculation proportion was biased toward LacZ by one order of magnitude or greater, complete exclusion in the remaining 1% of wells was not observed until after 48 h with plating using a pin replicator. Importantly, PI dominated these wells so that our measurement procedure for microplates (see Methods *Basic experimental design*) recorded these wells as occupied by PI even though LacZ was not excluded until 48 h. From this, we considered the competitive exclusion of LacZ by PI in patches as effectively instantaneous after 24 h.

Measuring abundances. Preliminary trials showed that optical density (OD) measurements (650 nm) of growth were sufficiently accurate for our high cell density microplates and that a simple linear relationship exists between OD values and bacterial cell counts ($R^2 = 0.943$, $P < 0.0001$, $F = 133$, cells = 1.247×10^8 * OD + 0.971). We developed this equation by droplet plating overnight cultures ($n = 10$, 5 per strain) with cell counts (replicated 3x at 10^{-6} dilution for

plating) ranging from 3.33 x 10^5 cells/15 μl to 7.14 x 10^7 cells/15 μl and regressing the counts against OD values (n = 10) for the same cultures. All OD measurements were performed with a FLUOstar Optima spectrophotometer (BMG LABTECH).

We developed a procedure to determine the presence of a strain in a well by calculating maximum growth thresholds for each strain. This calculation could be achieved because the mean OD-estimated growth at 10^{-5} initial dilution of LacZ (0.606, +/- 0.026) is substantially lower than PI (1.092, +/- 0.037). After 24 hours, these differences hold across all dilutions used in the experiment, with the maximum LacZ value of 0.666 never exceeding the minimum PI value of 0.893. In addition, the minimum growth of LacZ is always greater than OD values for wells with blank medium. Given these values, we considered that wells with OD values < 0.750 could be classified as LacZ, whereas those > 0.750 could be classified as PI. We are confident that this method is accurate; although an average of 4% of wells identified as PI at 24 h had not yet reached exclusion of LacZ in high dilution treatments, 100 % of wells classified as PI at 24 hours showed exclusion at 48 h. Wells with values less than the maximum blank value +0.10 were identified as no-growth wells. Due to the large volume of microplates, some minimal error in OD measurement is expected over the course of the experiment. To ensure the accuracy of the identification of LacZ and PI, a randomly selected replicate from each treatment was pin-replicated each transfer using a 96-pin replicator. Following 48 h incubations, the correlation between OD

strain/no-growth classifications and plated values was strong and highly significant (PI: $R^2 = 0.996$, $F = 18477$; LacZ: $R^2 = 0.969$, $F = 2285$, $P < 0.0001$; $n = 75$ for both strains).

Detailed experimental protocol. Here, we provide a detailed account of our transfer procedure, which involved setting up initial conditions, determining relative abundances of each strain, and generating the colonizer pool for the subsequent transfers. This procedure was supported by an R script (see Supplementary Note) that compiles and analyzes information about observed growth and occupancy by taking into account experimental treatments (i.e. trade-off strength and colonization rate). The script outputs the inoculation volumes for the subsequent transfer independently for each replicate. This procedure allowed us to follow the dynamics of each replicate separately and control for potential daily variation in overnight growth from our frozen stocks. For clarity, we divided this protocol into six steps 1) initial conditions, 2) building pool of colonizers, 3) implementing trade-offs, 4) inoculation, 5) measuring abundances, and 6) characterizing colonizers pool.

1. **Initial conditions**. To achieve an initial dilution approaching the limit of <100% successful colony growth across the microplate, we diluted 4000 μl of LacZ and 2344 μl of PI (OD corrected, higher OD value corrected to lower) in culture volume separately for each strain 5x serially in 18 ml of M9 minimal salts medium (NH_4Cl, 1 g l^{-1}; Na_2HPO_4, 6 g l^{-1}; KH_2PO_4, 3 gl^{-1};

NaCl, 0.5 g l^{-1}, autoclaved 20 min at 121°C). Using a multi-channel pipette, 10 μl of each strain was added with 190 μl of KB in two separate halves of a 96-well microplate as arbitrary non-equilibrium starting conditions. All microplates were placed in the incubator at the same time at 28°C for 24 hours, after which OD was measured for each microplate. All transfers included three microplates designated as "blanks." Blank microplates received 10 μl of M9 minimal salts media without bacteria, but were otherwise subjected to the same procedures as the treatment microplates. A second set of overnight tubes was grown with these microplates to initiate the second transfer.

2. **Building a pool of colonizers:** The first step to generate the colonizer pool was to determine how much of each strain is present in the metacommunity. This task was achieved by categorizing the growth value for each well as either LacZ or PI (see the *Measuring abundances* section) to calculate regional abundances of each strain per replicate. Because the dilution amount was computed using cell densities, all OD values were converted to estimated cell counts using the equations previously described in the *Measuring abundances* section.
3. **Implementing trade-offs:** The estimated cell counts were subsequently multiplied by the proportion of each microplate occupied by each strain and the strength of the trade-off (applied as a proportional reduction to PI only and produced using predicted dilution rates from our model; see

Supplementary Table S1 for values) and corrected against the overnight OD values to determine the volume of each strain (adjusted from 200 μl) to add. The equation implemented for PI was Volume = cells * trade-off * (cells/cells_overnight) * 200, and for LacZ, it was Volume = cells * (cells/cells_overnight) * 200. Note that for "no trade-off" scenarios, the dilution rates were not equal between the two strains because the final growth of PI is always greater than LacZ. To equalize the number of cells inoculated, the dilution rate was higher for PI (1.08^{-08}, and 1.0^{-07} for LacZ) under "no trade-off" scenarios.

4. **Inoculation:** The inoculation volume generated by the previous step was subsequently diluted serially in wells with 1600 μl (the initial volume was adjusted up or down to accommodate the exact volumes added for each strain).

5. **Measuring abundances**: Abundances were estimated in each microplate metacommunity by estimating optical density at 650nm on a FLUOStar spectrophotometer. See details on the abundances estimates in the "Measuring abundances" above.

6. **Characterize colonizer pool**: A custom made script in R was used to estimate the relative abundances of each strain in the colonizer pool based on the data from step 5). This script takes in account the bacterial in each individual well and calculates the exact contribution of each strain

for the colonizers pool of the subsequent step. These calculations were done separately for each replicate of each treatment.

Ending the experiments: We stopped transferring a replicate if one or two strains reached zero occupancy. After each transfer, all microplates were discarded, and new microplates were inoculated using data from the previous transfer. All replicates were processed independently at each step.

Supplementary Note: R script describing the calculations involved in each transfer, including serial dilution and routines for correcting variation within 96-well microplates due to edge effects.

```
#########################################################################
#
# This R script describes the calculations involved in the experiments described in the paper:
#
# Competition-colonization dynamics in experimental bacterial metacommunities
#
# 1. George Livingston*^, 2. Miguel Matias*, 3. Vincent Calcagno, 4. Claire Barbera, 5. Marine
 Combe, 6. Mathew A. Leibold and 7. Nicolas Mouquet
#
# *These authors contributed equally to this work
# ^Current address
# (1,2,4,5,7) Institut des Sciences de l'Evolution – CNRS UMR 5554 - Université de Montpellier II
 - CC 065 34095 MONTPELLIER Cedex 05
# (3) INRA Sophia-Antipolis 400 Route des Chappes - BP 167 06903 Sophia-Antipolis FRANCE
# (6,^) Section of Integrative Biology University of Texas at Austin 1 University Station C0930
# Austin, TX 78712, USA
#
# For additional information please contact the corresponding at at glivingston@utexas.edu
#
#########################################################################
# libraries

library(SCiAn)

#########################################################################
# set working directory

path <- c("/your path/")
```

```
#########################################################################
# set parameters

# upload table with parameters
FINALtable <- read.csv(paste(path,"/treatment_tables/treatments_table.csv",sep=""))

# set number of transfers transfer
transf=10

# set the overnight values measured using optical density
piOV=c(0.9132)
lOV=c(0.6316)
blankOV=c(0.466)

# remove the background signal from the growth medium
piOV=piOV-blankOV
lOV=lOV-blankOV

# calculate cells concebntrations based on previously established relationship
logCellOVI <- 3.316*piOV+.899
logCellOVL <- 3.316*lOV+.899

# determine the actual cell densities for each strain
CellOVI <- 10^logCellOVI
CellOVL <- 10^logCellOVL

# set treatments (this example refers to the second habitat destruction experiment)
treatments=c("L5","L8","5","8","H5","H8")

# set number of replicates
replicates=c(1:3)

#########################################################################
# Calculate mean and max blanks

# Load blank plates and using Scian functions. These functions are available at CRAN and
 facilitate the upload from Spectrophotometer's output files.

data.blank=formatDF(read.cinetic(paste(path,"/data/D_", transf,"_B_", 1, ".csv", sep="")))
blank1 <- data.frame(position=NA,measure=NA)
for (i in 1:length(data.blank)) {blank1[i,2]<-data.blank[i]}

data.blank=formatDF(read.cinetic(paste(path,"/data/D_", transf,"_B_", 2, ".csv", sep="")))
blank2 <- data.frame(position=NA,measure=NA)
for (i in 1:length(data.blank)) {blank2[i,2]<-data.blank[i]}

data.blank=formatDF(read.cinetic(paste(path,"/data/D_", transf,"_B_", 3, ".csv", sep="")))
blank3 <- data.frame(position=NA,measure=NA)
for (i in 1:length(data.blank)) {blank3[i,2]<-data.blank[i]}

# determine the max blank value
max(blank1[,2])
```

```
max(blank2[,2])
max(blank3[,2])

# determine the mean blank value per rows and columns to adjust for potential edge effects
firstrowmaxblank <- max(cbind(blank1[1:12,2],blank2[1:12,2],blank3[1:12,2]))
firstrowmeanblank <- mean(cbind(blank1[1:12,2],blank2[1:12,2],blank3[1:12,2]))
siderowmaxblank <-
 max(cbind(blank1[c(24,36,48,60,72,84),2],blank2[c(24,36,48,60,72,84),2],blank3[c(24,36,48,60,
 72,84),2]))
siderowmeanblank <-
 mean(cbind(blank1[c(24,36,48,60,72,84),2],blank2[c(24,36,48,60,72,84),2],blank3[c(24,36,48,60
 ,72,84),2]))
bottomrowmaxblank <- max(cbind(blank1[85:96,2],blank2[85:96,2],blank3[85:96,2]))
bottomrowmeanblank <- mean(cbind(blank1[85:96,2],blank2[85:96,2],blank3[85:96,2]))
interiormeanblank <- mean(cbind(blank1[-c(1:12,24,36,48,60,72,84,85:96),2],blank2[-
 c(1:12,24,36,48,60,72,84,85:96),2],blank3[-c(1:12,24,36,48,60,72,84,85:96),2]))

# determine the max blank values for the entire set of plates using boxplot.stats function. These
 adjustments are essential to improve the accuracy of the method of determining the type of
 strain based on their growth

allblank <- rbind(as.matrix(blank1[1:12,2]),as.matrix(blank2[1:12,2]),as.matrix(blank3[1:12,2]))
blankstats <- boxplot.stats(allblank)
firstrowmaxblank <- blankstats$stats[5]

allblank <-
 rbind(as.matrix(blank1[c(24,36,48,60,72,84),2]),as.matrix(blank2[c(24,36,48,60,72,84),2]),as.mat
 rix(blank3[c(24,36,48,60,72,84),2]))
blankstats <- boxplot.stats(allblank)
siderowmaxblank <- blankstats$stats[5]

allblank <- rbind(as.matrix(blank1[85:96,2]),as.matrix(blank2[85:96,2]),as.matrix(blank3[85:96,2]))
blankstats <- boxplot.stats(allblank)
bottomrowmaxblank <- blankstats$stats[5]

allblank <- rbind(as.matrix(blank1[-c(1:12,24,36,48,60,72,84,85:96),2]), as.matrix(blank2[-
 c(1:12,24,36,48,60,72,84,85:96),2]), as.matrix(blank3[-c(1:12,24,36,48,60,72,84,85:96),2]))
blankstats <- boxplot.stats(allblank)
interiormaxblank <- blankstats$stats[5]

#########################################################################
# Main loop

counter=1
for (treat in treatments) {

 for (rep in replicates) {

       #Read DO measure file
       data.plaque=formatDF(read.cinetic(paste(path,"/data/D_", transf,"_", treat,"_", rep, ".csv" ,
 sep="")))

       mat <- data.frame(strain=NA,measure=NA)
```

```
      for (i in 1:length(data.plaque)) {mat[i,2]<-data.plaque[i]}


  # Determine the type of strain based on optical density with adjustments for edge effects. The
cut-offs are adjusted based on daily overnights and blanks.

      # Interior
      for (i in 1:96)        {
   if (mat[i,2] < interiormaxblank+0.010) {mat[i,1]="NG"} # No growth
            if (mat[i,2] >= interiormaxblank+0.010) {if (mat[i,2] < 0.800) {mat[i,1]="L"}} # Lac
strain
            if (mat[i,2] >= 0.800) {mat[i,1]="PI"} # Pi strain
            }

      # Cut-offs top row!!
      for (i in 1:12)        {
            if (mat[i,2] < firstrowmaxblank +0.010) {mat[i,1]="NG"} # No growth
            if (mat[i,2] > firstrowmaxblank +0.010) {if (mat[i,2] < 0.870) {mat[i,1]="L"}} # Lac strain
            if (mat[i,2] > 0.870) {mat[i,1]="PI"} # Pi strain
            }

      # Cut-offs bottom row!!
      for (i in 85:96)       {
            if (mat[i,2] < bottomrowmaxblank +0.010) {mat[i,1]="NG"} # No growth
            if (mat[i,2] > bottomrowmaxblank +0.010) {if (mat[i,2] < 0.870) {mat[i,1]="L"}} # Lac
strain
            if (mat[i,2] > 0.870) {mat[i,1]="PI"} # Pi strain
            }

      # Cut-offs side row!!
      for (i in c(24,36,48,60,72,84))    {
            if (mat[i,2] < siderowmaxblank+0.010) {mat[i,1]="NG"} # No growth
            if (mat[i,2] > siderowmaxblank+0.010) {if (mat[i,2] < 0.870) {mat[i,1]="L"}} # Lac strain
            if (mat[i,2] > 0.870) {mat[i,1]="PI"} # Pi strain
            }

      #### Edge effect corrections ####

  # Calculate means for each position
      mfirstrow <- mean(mat[1:12,2])
      innertop <- mat[13:48,2]
      minnertop <- mean(innertop[-c(12,24,36)])
      sidetop <- mean(innertop[c(12,24,36)])
      mbottomrow <- mean(mat[85:96,2])
      innerbottom <- mat[49:84,2]
      minnerbottom <- mean(innerbottom[-c(12,24,36)])
      sidebottom <- mean(innerbottom[c(12,24,36)])
      # correct top
      for (i in 1:12) {mat[i,2] <- mat[i,2]*(minnertop/mfirstrow)}
      # correct bottom
      for (i in 85:96) {mat[i,2] <- mat[i,2]*(minnerbottom/mbottomrow)}
      # correct last column top
      for (i in c(24,36,48)) {mat[i,2] <- mat[i,2]*(minnertop/sidetop)}
```

```
    # correct last column bottom
    for (i in c(60,72,84)) {mat[i,2] <- mat[i,2]*(minnerbottom/sidebottom)}


    # Blank correction
    mat[1:96,2]=mat[1:96,2]-interiormeanblank # remove interior blank


#### Daily transfer routine ####

#### 1. Take the mean DO values and divide them by overnight DO values for each strain.
# Multiply these proportions by 400ul for each strain to caculate DO corrected volumes (VL,VI).

    #Calculate the mean measure by strain and counts
    matPI <- subset(mat,mat$strain=="PI",select="measure")
    countsPI <- nrow(matPI)
    meanPI <- mean(matPI)
    if (countsPI==0) meanPI=0

    logCellI <- 3.316*meanPI+.899
    CellI <- 10^logCellI# actual values 10^6 cells

    matL <- subset(mat,mat$strain=="L",select="measure")
    countsL <- nrow(matL)
    meanL <- mean(matL)
    if (countsL==0) meanL=0

    logCellL <- 3.316*meanL+.899
    CellL <- 10^logCellL

    # Daily overnight adjustment

    piadj <- CellI/CellOVI
    ladj <- CellL/CellOVL

    # Dilution volumes adjustment

    VI1 <- piadj*400
    VL1 <- ladj*400

    #### 2. Calculate from the layout the number of wells (out of 96) occupied for each strain.
Multiply VL and VI from #1 by these proportions.

    propI <- countsPI/96
    propL <- countsL/96

    # Volume by occupancy adjustment
    VI2 <- VI1*propI
    VL2 <- VL1*propL

    #### 3. Mutiply volume by occupancy (VI) by the treatment specific reduction factor.

    VI3 <- VI2*FINALtable[counter,"IDF"] # The table with reduction factors can be found in the
```

```
supplementary material provide with the paper
      VL3 <- VL2

      #### 4. Mutiply VI3 by the destrction factor (This step is only for the second experiment)
  # This factor reduces the volume added to the dispersal pool by a proportion equivalent to the
global reduction in dispersal rate

      if (treat=="L5") {
            VI4 <- VI3*FINALtable[counter,"Destruction"]
            VL4 <- VL3*FINALtable[counter,"Destruction"]
            }
      if (treat=="L8") {
            VI4 <- VI3*FINALtable[counter,"Destruction"]
            VL4 <- VL3*FINALtable[counter,"Destruction"]
            }
      if (treat=="H5") {
            VI4 <- VI3*FINALtable[counter,"Destruction"]
            VL4 <- VL3*FINALtable[counter,"Destruction"]
            }
      if (treat=="H8") {
            VI4 <- VI3*FINALtable[counter,"Destruction"]
            VL4 <- VL3*FINALtable[counter,"Destruction"]
            }
      if (treat=="5") {
            VI4 <- VI3
            VL4 <- VL3
            }
      if (treat=="8") {
            VI4 <- VI3
            VL4 <- VL3
            }

  # Initial dilution. The corrections below are implemented to ensure all innocualtion volumes are
greater than 10ul.
  # Dilutions are implemented in 2ml x 4 x 6 microplates by changing tips after each ejection and
with 200ul of transfer volume except for some final volumes which are 632ul if it is a half dilution
step

      dilI=NA
      if (VI4<10) { # correction in case the volume of VI$ is <10
   dilI <- VI4
            VI4=VI4*10
            IniDil <- 2000-VI4-VL4
   }
  if (VI4<10) { # correction in case the volume of VI4 is still <10
   VI4=VI4*10
   IniDil <- 2000-VI4-VL4
   }
  if (VI4<10) { # correction in case the volume of VI4 is still <10
   VI4=VI4*10
   IniDil <- 2000-VI4-VL4
   }
  dilL=NA
```

```
  if (VL4<10) { # correction in case the volume of VL4 is <10
    dilL <-VL4
    VL4=VL4*10
    IniDil <- 2000-VI4-VL4
    }

       if (VI4>=10) {IniDil <- 2000-VI4-VL4}
       if (VL4>=10) {IniDil <- 2000-VI4-VL4}

       # Output table

       FINALtable[counter,"Transfer"] <- transf
       FINALtable[counter,"Max_blank"] <- interiormaxblank
       FINALtable[counter,"Mean_blank"] <- interiormeanblank
       FINALtable[counter,"OV_Lac"] <- lOV
       FINALtable[counter,"OV_PI"] <- piOV
       FINALtable[counter,"CellOVI"] <-CellOVI
       FINALtable[counter,"CellOVL"] <-CellOVL
       FINALtable[counter,"CountsI"] <- countsPI
       FINALtable[counter,"CountsL"] <- countsL
       FINALtable[counter,"MeansI"] <- meanPI
       FINALtable[counter,"MeansL"] <- meanL
       FINALtable[counter,"CellI"] <-CellI
       FINALtable[counter,"CellL"] <-CellL
       FINALtable[counter,"piadj_"] <- piadj
       FINALtable[counter,"ladj_"] <- ladj
       FINALtable[counter,"VI1_"] <- VI1
       FINALtable[counter,"VL1_"] <- VL1
       FINALtable[counter,"propI_"] <- propI
       FINALtable[counter,"propL_"] <- propL
       FINALtable[counter,"VI2_"] <- VI2
       FINALtable[counter,"VL2_"] <- VL2
       FINALtable[counter,"VI3_"] <- VI3
       FINALtable[counter,"VL3_"] <- VL3
       FINALtable[counter,"VI4_"] <- VI4
       FINALtable[counter,"VL4_"] <- VL4
       FINALtable[counter,"Initial.dilution"] <- IniDil
       FINALtable[counter,"correctionI"] <- dilI
       FINALtable[counter,"correctionL"] <- dilL


 counter=counter+1
 }# for (rep in replicates)

} #for (t in treat)

write.table(FINALtable[-c(2,7,8,9,11,14,15,16,17,18),],paste(path,
 transf,".txt",sep=""),row.names=FALSE)

#########################################################################
# Plot dynamics

graphtable <- FINALtable[,c("Treatment","Replicate","propL_","propI_")]
```

```
graphL <- tapply(graphtable$propL_,graphtable$Treatment,mean)
graphI <- tapply(graphtable$propI_,graphtable$Treatment,mean)
labels <- unique(FINALtable[,"Treatment"])

graphbind <- cbind(labels, graphL, graphI, graphL+ graphI)

barplot(t(graphbind[,-1]),beside=TRUE,legend.text=c("Lac","Pi","Total"))

##############################################################################
```

Supplementary References

61. R Development Core Team (2011). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL: http://www.R-project.org/.

Chapter 2 Supplemental Information

APPENDIX A. Summary table of species used in the experiment.

SUPPLEMENTARY TABLE 2.1. Summary of species used in the experiment. Trial prey refer to those species used as prey during the feeding trial. Per = *Peranema sp.*; Tetra = *Tetrahymena pyriformis*; Col = *Colpidium striata*; Chl = *Chlorella vulgaris*; Crypto = *Cryptomonas erosa;* Chloro = *Chloromonas clathrata*. (-) signifies not applicable.

Species	Type	Trophic Guild	Pre-mix Pers. Prob. (mean, N, SE)	Post-mix Pers. Prob. (mean, N, SE)	Trial Prey	Cell Biovolume	Supporting Guild/Biovolume Citations	Culture Source
Colpoda sp.	Protist	Bacterivore	0.00, 17, 0.000	-	Chl	-	Violle et al. 2010 (1)	Jiang Lab
Euplotes diadaleos	Protist	Bacterivore	0.63, 16, 0.125	0.90, 10, 0.100	Chl	-	Kusch et al. 1995 (2)	Jiang Lab
Halteria grandinella	Protist	Bacterivore	0.75, 32, 0.078	0.96, 25, 0.040	Chl	-	Jiang et al. 2011 (3)	Jiang Lab
Spirostomum sp.	Protist	Bacterivore	0.21, 14, 0.114	0.67, 3, 0.333	Per/Chloro	-		Carolina Biological Supply
Unk. Ciliate	Protist	Bacterivore	0.64, 28, 0.092	0.81, 16, 0.101	Chl	-		Jiang Lab
Uronema sp.	Protist	Bacterivore	1.00, 25, 0.000	1.00, 25, 0.000	Chl	-		Jiang Lab

Brachionus calyciflorus	Rotifer	Herbivore	0.00, 12, 0.000	-	-	-	Mohr and Adrian 2002 (4)	Fussman Lab
Brachionus havanensis	Rotifer	Herbivore	0.00, 8, 0.000	-	-	-	Mohr and Adrian 2002 (4)	Fussman Lab
Brachionus rubens	Rotifer	Herbivore	0.10, 10, 0.100	0.00, 1	-	-	Aránguiz-Acuña et al. 2011 (5)	Fussman Lab
Parameciu m caudatum	Protist	Herbivore	0.55, 11, 0.157	0.83, 6, 0.167	Per/Chloro	-		Carolina Biological Supply
Parameciu m sp. 1	Protist	Herbivore	0.92, 13, 0.077	0.83, 12, 0.112	Per/Chloro	-		Jiang Lab
Unk. Rotifer	Rotifer	Herbivore	0.83, 12, 0.112	1.00, 10, 0.000	Per/Chloro	-		Carolina Biological Supply
Glaucoma scintellans	Protist	Omnivore (b/h)	0.94, 34, 0.041	0.69, 32, 0.083	Per/Chloro	-		Jiang Lab
Loxocepha lus sp.	Protist	Omnivore (b/h)	0.77, 40, 0.067	0.60, 30, 0.091	Per/Chloro	-	Jiang et al. 2011 (3)	Jiang Lab
Parameciu m bursaria	Protist	Omnivore (b/h)	0.83, 35, 0.065	0.76, 29, 0.081	Per/Chloro	-		Carolina Biological Supply
Tetrahyme na pyriformis	Protist	Omnivore (b/h)	0.56, 34, 0.086	0.16, 19, 0.086	Per/Chloro	-		Carolina Biological Supply

Tetrahymena thermophila	Protist	Omnivore (b/h)	0.58, 26, 0.099	0.40, 15, 0.131	Per/Chloro	-		Jiang Lab
Colpidium kleini	Protist	Omnivore (b/h/p)	0.74, 34, 0.077	0.84, 25, 0.075	Per/Chloro	-		Jiang Lab
Colpidium striatum	Protist	Omnivore (b/h/p)	0.45, 33, 0.088	0.67, 15, 0.126	Per/Chloro	-		Carolina Biological Supply
Euplotes patella	Protist	Omnivore (b/h/p)	0.59, 34, 0.086	0.89, 19, 0.072	Per/Chloro	-	Holyoak and Sachdev 1998 (6), Fox 2004 (7)	Carolina Biological Supply
Tetrahymena vorax	Protist	Omnivore (b/h/p)	0.80, 30, 0.074	0.83, 23, 0.081	Col/Chloro	-		Jiang Lab
Parameciu m sp. 2	Protist	Omnovore (p/h)	0.90, 31, 0.054	1.00, 28, 0.000	Per/Chloro	-		Jiang Lab
Amoeba proteus	Protist	Predator	0.25, 12, 0.131	0.67, 3, 0.333	Col/Chloro	-		Carolina Biological Supply
Asplanchna brightwelli	Rotifer	Predator	0.31, 16, 0.120	0.60, 5, 0.245	Col/Crypto	-		Fussman Lab
Blepharisma americanum	Protist	Predator	1.00, 12, 0.000	0.92, 12, 0.083	Col/Chloro	-		Carolina Biological

								Supply
Dileptus monilatus	Protist	Predator	0.50, 8, 0.189	0.50, 4, 0.289	Tetra/Chloro	-		Carolina Biological Supply
Stentor coeruleus	Protist	Predator	0.70, 10, 0.153	0.57, 7, 0.202	Col/Chloro	-		Carolina Biological Supply
Unk. Protist sp.	Protist	Predator	0.90, 10, 0.100	1.00, 9, 0.000	Tetra/Chloro	-		Unknown
Campylomonas reflexa	Globular alga	Edible to protists	1.00, 24, 0.000	0.96, 24, 0.042	-	1507	Elena Litchman	UTEX
Chlamydomonas reinhardtii	Globular alga	Edible to protists	0.92, 24, 0.058	0.71, 21, 0.101	-	45	Elena Litchman	Carolina Biological Supply
Chloromonas clathrata	Globular alga	Edible to protists	1.00, 20, 0.000	0.90, 20, 0.069	-	179.5	UTEX photos	UTEX
Chroomonas pochmanii	Globular alga	Edible to protists	0.12, 25, 0.066	0.00, 3, 0.000	-	151	Elena Litchman	UTEX
Cryptomonas erosa	Globular alga	Edible to protists	0.77, 26, 0.084	0.25, 20, 0.099	-	1507	Elena Litchman	UTEX
Vischeria helvetica	Globular alga	Edible to proti	1.00, 16, 0.000	1.00, 16, 0.000	-	381.51	Algaebase (8)	UTEX

		sts						
Ankistrodesmus falcatus var. aci	Non-globular alga	Edible to rotifers	1.00, 21, 0.000	1.00, 21, 0.000	-	151	Elena Litchman	UTEX
Chlorokybus atmophyticus	Non-globular alga	Edible to rotifers	0.82, 17, 0.095	0.50, 14, 0.139	-	267.95	UTEX photos	UTEX
Colacium vesiculosum	Non-globular alga	Edible to rotifers	0.29, 24, 0.095	0.33, 6, 0.211	-	492	Elena Litchman	UTEX
Pediastrum sp.	Non-globular alga	Edible to rotifers	0.95, 21, 0.048	0.95, 21, 0.048	-	3200	Elena Litchman	UTEX
Scenedesmus gladiosum	Non-globular alga	Edible to rotifers	1.00, 23, 0.000	0.96, 23, 0.043	-	300	Elena Litchman	UTEX
Sellaphora pupula var. rectangularis	Non-globular alga	Edible to rotifers	0.43, 23, 0.106	0.60, 10, 0.163	-	266.24	UTEX photos	UTEX
Anabaena sp.	Filamentous alga	Inedible	1.00, 26, 0.000	0.96, 26, 0.038	-	-		UTEX
Atractomorpha echinata	Filamentous alga	Inedible	1.00, 16, 0.000	0.44, 16, 0.128	-	-		UTEX
Boldia erythrosiphon	Filamentous alga	Inedible	0.06, 17, 0.059	0.00, 1	-	-		UTEX
Closterium	Glob	Inedi	1.00,	0.92,	-	175840	Elena	UTEX

acerosum	ular alga	ble	13, 0.000	13, 0.077			Litchman	
Eudorina unicocca var. periphe	Globular alga	Inedible	1.00, 15, 0.000	0.80, 15, 0.107	-	268, 8 cells per colony	Olenina et al. 2006 (9)	UTEX
Haematococcus lacustris	Globular alga	Inedible	1.00, 11, 0.000	1.00, 11, 0.000	-	6000	Weger et al. 2002 (10)	UTEX
Oscillatoria amoena	Filamentous alga	Inedible	1.00, 23, 0.000	1.00, 23, 0.000	-	-		UTEX
Pleodorina californica	Globular alga	Inedible	0.78, 9, 0.147	0.40, 5, 0.245	-	33493.33	Prescott 1962 (11)	UTEX
Spirogyra occidentalis	Filamentous alga	Inedible	1.00, 23, 0.000	0.87, 23, 0.072	-	-		UTEX
Staurastrum gladiosum	Globular alga	Inedible	0.83, 12, 0.112	0.60, 10, 0.163	-	1616	Elena Litchman	UTEX
Volvox rousseletti	Globular alga	Inedible	0.33, 12, 0.142	0.25, 4, 0.250	-	8000–17000, cell size 45	Elena Litchman	UTEX
Zygnema circumcarinatum	Filamentous alga	Inedible	0.95, 21, 0.048	0.80, 20, 0.092	-	-		UTEX

Supporting citations:

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5. Aránguiz-Acuña A., R. Ramos-Jiliberto, and R. O. Bustamante. 2011. Experimental evidence that induced defenses promote coexistence of zooplanktonic populations. Journal of Plankton Research 33:469–477.

6. Holyoak, M., and S. Sachdev. 1998. Omnivory and the stability of simple food webs. Oecologia117:413–419.

7. Fox, J. W. 2004. Effects of algal and herbivore diversity on the partitioning of biomass within and among trophic levels. Ecology85:549–559.

8. www.algaebase.org/search/?genus=Vischeria

9. Olenina, I., S. Hajdu, L. Edler, A. Andersson, N. Wasmund, S. Busch, et al. 2006. Biovolumes and size-classes of phytoplankton in the Baltic Sea.

10. Weger H. G., J. K. Middlemiss and C. D. Petterson. 2002. Ferric chelate reductase activity as affected by the iron-limited growth rate in four species of unicellular green algae (Chlorophyta). Journal of Phycology 38:513–519.

11. Prescott, G. W. 1962. Algae of the Western Great Lakes Area. Revised ed. Brown, Dubuque, Iowa, USA.

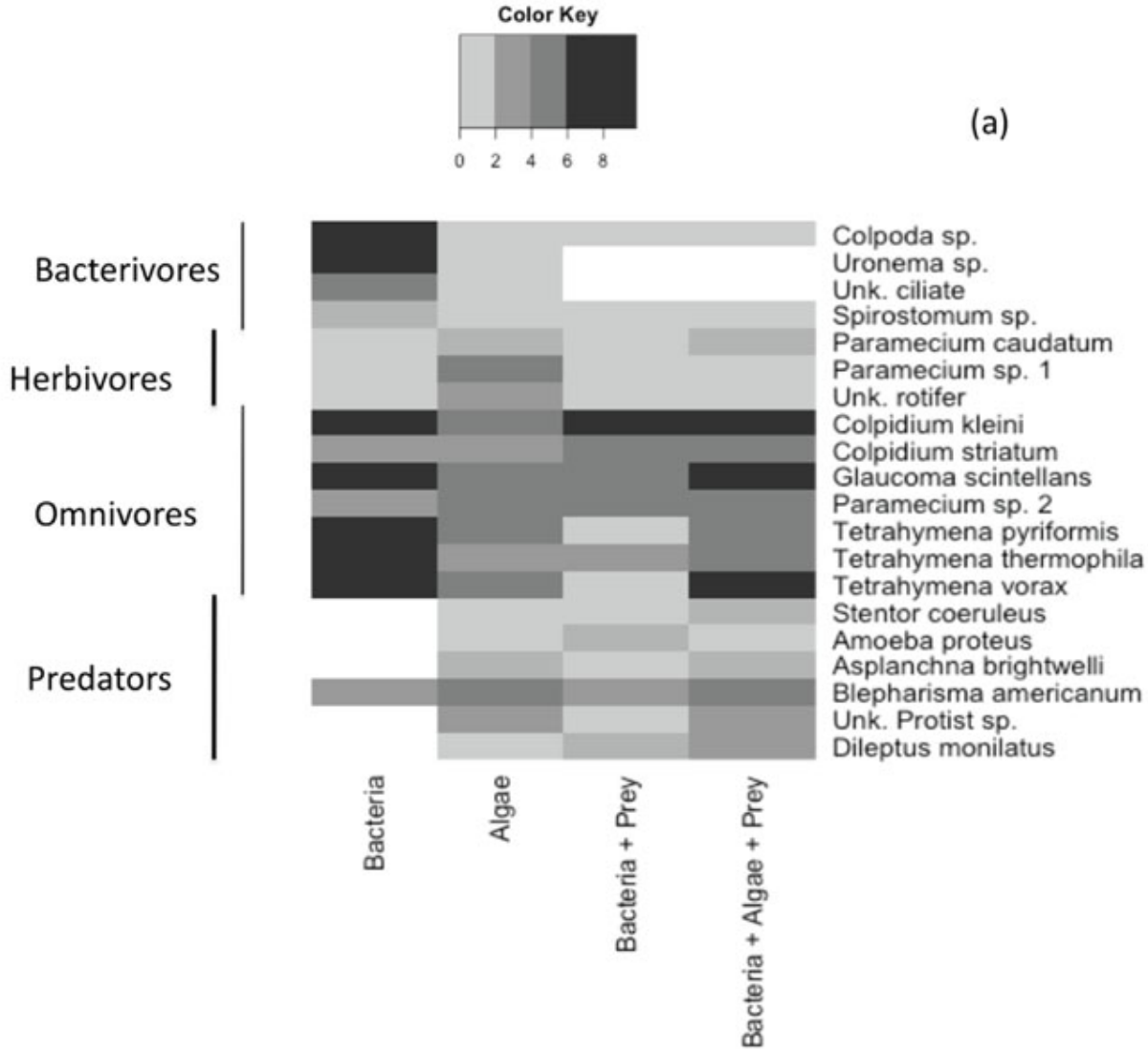
Appendix B. Methods for feeding trials and characterization of trophic structure.

We classified the majority of heterotrophic species into trophic categories based on a combination of feeding trials, the literature, and mouthpart size. We inoculated five individuals of each species into each of four culture conditions: (1) wheat seed only, (2) wheat seed and protist prey, (3) algae only, and (4) algae and protist prey. Based on culture experience and the literature, suitable potential prey were selected for each species (Appendix A). Petri dishes were filled with 30mL of COMBO and algae/prey were grown for one week prior to inoculation of focal species. Trials were not replicated and density was sampled using the same methods as in the main experiment after one or two weeks depending on when the species attained a countable density. Bacteria, prey protists, and algae were given one week to grow before species were inoculated. Species with growth on wheat seeds only were classified as bacterivores, those with growth on protist prey were classified as predators, those with growth on algae were classified as herbivores, and those with growth on some combination of bacteria, algae, and protists were classified as various types of omnivores (Fig. 2a, Appendix A).

Eight of the twenty-eight species in the trial failed to grow on at least one resource due either to strong Allee effects or low establishment success in mono-inoculation (i.e., co-inoculation with many other species increases establishment success). For these species, we inferred their trophic position from the literature, culturing experience, and cases in which they grew successfully in the feeding trial. All of these eight species are well described in the literature and assigning them to guilds was not ambiguous (supporting citations for each species in Appendix A).

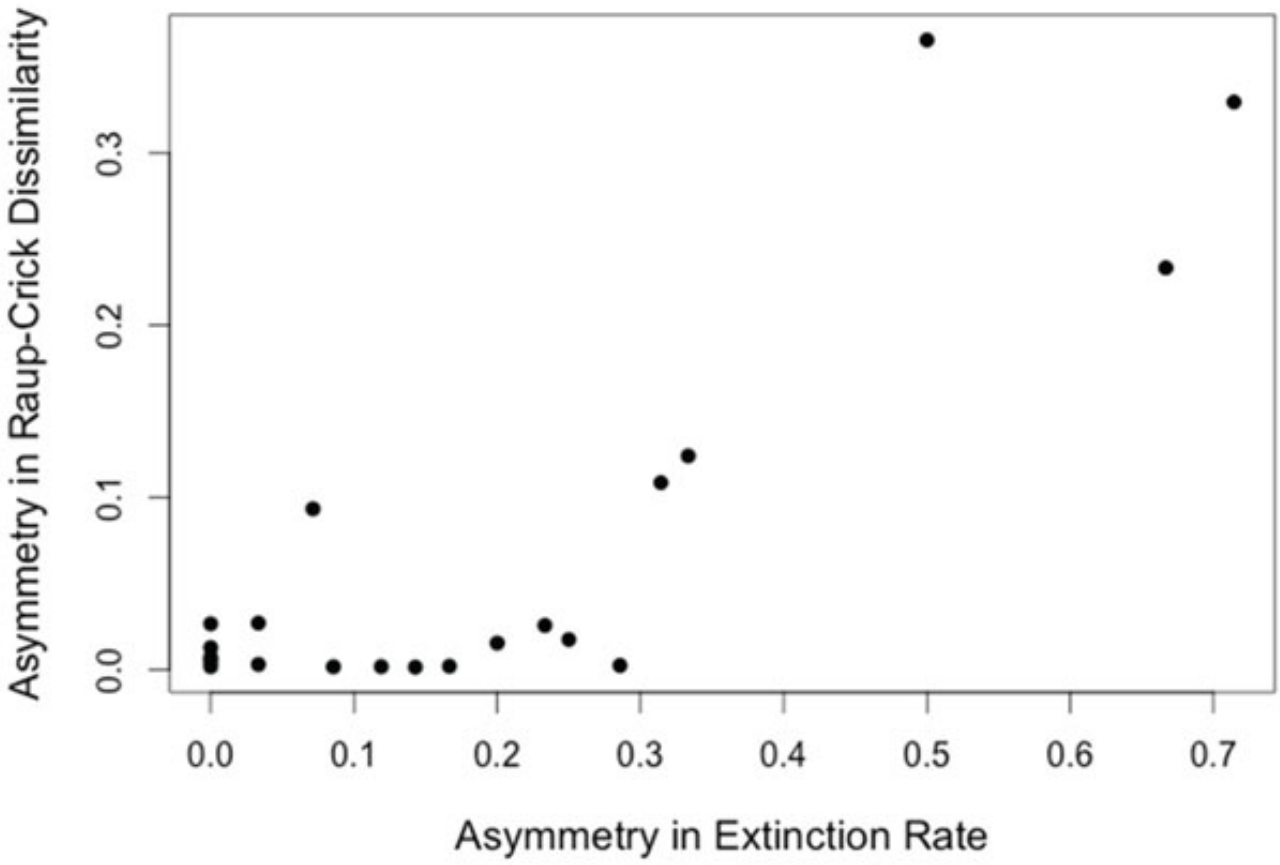
Algae were classified as edible or inedible based on size. Edible species were less than 20μm in both width and length (generally 15μm maximum) and inedible species were greater than 20μm in at least one dimension.

Appendix C. Heat map of feeding trial growth rates and the general experimental food web.



Supplementary Fig. 2.1. Heat map of feeding trial growth rates and the general experimental food web. (a) Growth rates of all species with complete information from the feeding trial. White squares represent NA values were growth was not assayed because protists were either too small to potentially feed on candidate prey (in the case of bacterivores) or known not to grow on bacteria alone (in the case of predators). Methods for the feeding trial are described in Appendices A and B.

Appendix D. Scatterplot of asymmetry in species composition.



Supplementary Fig. 2.2. Scatterplot of asymmetry in species composition (based on extinctions) and asymmetry based on Raup-Crick dissimilarity ($N = 20$). The correlation is non significant (GLM, $P = 0.07$, df = 19, $X^2 = 29$), but note the positive trend.

Supplemental R Script

The supplement is a script (R_script_simulation.r) demonstrating the implementation of our simulations and subsequent analysis of asymmetry. The simulation method we utilized is nonparametric and user-defined. For this reason we have included the script. As described in the methods section of our associated manuscript, the simulation involves imposing species extinctions in our post-mixing communities and using the output as a null model against which we compare our observed results. The script includes annotations describing the function of each section of code.

```
##Nov. 27, 2012
#R code for Livingston et al.##
################ Load Packages ###############
library(moments)
library(gdata)
library(lattice)
############################## Read in Data ############################
setwd("~/Desktop/R input files")  # Set working directory

# Read in data
mydata<-read.csv("~/Desktop/R input files/Emerge/data_07062011.csv",header=TRUE) #
"data_07062011.csv" is the raw file of each species' occurrence per community pre- and post-
mixing
mydata.pre.ori<-mydata[mydata$sample=="Pre",] #initial communities (subset of mydata)
mydata.post.ori<-mydata[mydata$sample=="Post",] # species composition at the time of mixing
(subset of mydata)
mix.com.name<-names(summary(mydata$mixcommunity))[-1] # # Retrieve the names of all
mixed communities

persistance_pr<-read.csv("~/Desktop/R input files/Emerge/persistance_prob.csv",header=TRUE)
# "persistance_prob" is species' persistence probability


connect_deviant_data<-read.csv("~/Desktop/R input files/Emerge/composition
asymmetry.csv",header=TRUE) # Observed asymmetries

simmulation.no<-10 # The number of simulation runs

#############################################################################
############### Asymmetry in Species Composition#############

################################## Define Functions ##################
# 1) Function: Asym.Comp()
# Function Asym.Comp() takes in two datasets, "mydata.mix" (communities present after
simulated extinctions) and "mydata.post.ori" (communities present at the time of mixing). This
function is used to calculate the overall asymmetry in species composition and asymmetry in
species composition in each community by guild.

Asym.Comp<-function (mydata.mix,mydata.post.ori) {
  # Create null vectors
```

```
  Diff<-c()
  exC1<-c()
  exC2<-c()
  S1<-c()
  S2<-c()

  #Retrieve the name of the two mixed communities in each "mix" communities pairs
  for (i in 1:length(mix.com.name)){
    mix1.com<-mydata.mix[mydata.mix$mixcommunity==mix.com.name[i],]$mix1[1]  # Retrieve
the name of the first pre-mix community
    mix2.com<-mydata.mix[mydata.mix$mixcommunity==mix.com.name[i],]$mix2[1] # Retrieve the
name of the second pre-mix community

    # Asymmetry Calculation
    exC1<-length(mydata.post.ori[mydata.post.ori$community==mix1.com,]$sppID) -
length(mydata.mix[mydata.mix$community==mix1.com,]$sppID) # exC1=simulated number of
species that went extinct post-assembly in the first premixing community
    exC2<-length(mydata.post.ori[mydata.post.ori$community==mix2.com,]$sppID) -
length(mydata.mix[mydata.mix$community==mix2.com,]$sppID) # exC2=simulated number of
species that went extinct post-assembly in the second premixing community
    S1<-length(mydata.post.ori[mydata.post.ori$community==mix1.com,]$sppID)  #S1= the
number of species present at the time of mixing in the first premixing community
    S2<-length(mydata.post.ori[mydata.post.ori$community==mix2.com,]$sppID)  #S2= the
number of species present at the time of mixing in the second premixing community

    Diff.temp<-abs((exC1/S1)-(exC2/S2))  # Asymmetry: the absolute difference in extinction rates
    Diff<-cbind(Diff, as.numeric(Diff.temp)) # Diff contains the asymmetry values of each mixed
community in each simulation run
  }
  return(Diff)
}
##### the end of function Asym.Comp() #########

# 2) Function: Asym.Comp.Treat()

# Function Asym.Comp.Treat() takes in three datasets, "mydata.mix" (simulated data:
communities present after mixing), "mydata.post.ori" (experimental data: communities present at
the time of mixing) and "mix.com.name" (a vector of names of all mixed communities). this
function calculates the asymmetry in species composition in each community by treatment.

Asym.Comp.Treat<-function (mydata.mix,mydata.post.ori,mix.com.name)
{
  # Create null vectors
  Diff<-c()
  exC1<-c()
  exC2<-c()
  S1<-c()
  S2<-c()

  #Retrieve the name of the two mixed communities in each "mix" pair of communities
  for (i in 1:length(mix.com.name))
  {
    mix1.com<-mydata.mix[mydata.mix$mixcommunity==mix.com.name[i],]$mix1[1]  # Retrieve
```

```
the name of the first pre-mix community
    mix2.com<-mydata.mix[mydata.mix$mixcommunity==mix.com.name[i],]$mix2[1]  # Retrieve
the name of the second pre-mix community

    # Asymmetry Calculation:
    # exC1/exC2=simulated number of species went extinct post-assembly in each of the
premixing communities; S1/S2= the number of species actually present at the time of mixing in
each of the premixing communities
    exC1 <- length(mydata.post.ori[mydata.post.ori$community==mix1.com,]$sppID) -
length(mydata.mix[mydata.mix$community==mix1.com,]$sppID)
    exC2 <- length(mydata.post.ori[mydata.post.ori$community==mix2.com,]$sppID) -
length(mydata.mix[mydata.mix$community==mix2.com,]$sppID)
    S1<-length(mydata.post.ori[mydata.post.ori$community==mix1.com,]$sppID)
    S2<-length(mydata.post.ori[mydata.post.ori$community==mix2.com,]$sppID)
    Diff.temp<-abs((exC1/S1)-(exC2/S2)) # Asymmetry: the absolute difference in extinction rates
    Diff<-cbind(Diff, as.numeric(Diff.temp)) # Diff contains the asymmetries of each mixed
community in each simulated dataset
  }
  return(Diff)
}
##### the end of function Asym.Comp.Treat() #########

# 3) Function: Asym.Comp.Stat()

# Function Asym.Comp.Stat() takes in a dataset "Asymmetry.t" (each row of Asymmetry.t
contains simulated asymmetries in species compostition in each mixed communites in one
simulation run) and calculate an overall mean asymmetry across all communities across all
simulation runs, with standard deviation and 95% confidence interval.

Asym.Comp.Stat <- function (Asymmetry.t) {
  # transpose dataset
  Asymmetry<-t(Asymmetry.t)

  # Create null vectors
  asymmetry_mean_total<-c()
  asymmetry_mean_total_temp<-c()
  asymmetry_sd_total<-c()
  asymmetry_low_total<-c()
  asymmetry_high_total<-c()
  Difference_total<-c()

  # Extract mean asymmetry of each communities (a total of 66 mean asymmetries) across all
simulation runs for following calculations
  for (i in 1:simmulation.no) {
    asymmetry_mean_total_temp <-cbind(asymmetry_mean_total_temp, mean(Asymmetry[,i],
na.rm=TRUE))
  }

  asymmetry_mean_total<-mean(as.numeric(asymmetry_mean_total_temp),na.rm=TRUE)
#calculate the overall mean asymmetry
  asymmetry_sd_total<-sd(as.numeric(asymmetry_mean_total_temp),na.rm=TRUE) #calculate
SD
  asymmetry_low_total<-qnorm(0.025,mean= asymmetry_mean_total,sd=asymmetry_sd_total)
```

```
#calculate the lower boundary of 95% CI
  asymmetry_high_total<-qnorm(0.975,mean= asymmetry_mean_total,sd=asymmetry_sd_total)
#calculate the upper boundary of 95% CI
  #Export data
  Difference_total <- as.data.frame(cbind(t(asymmetry_mean_total), t(asymmetry_sd_total),
t(asymmetry_low_total), t(asymmetry_high_total)))
  colnames(Difference_total)<-c("mean","SD","95% CI","95% CI")
  return(Difference_total)
}
###### Function "Asym.Comp.Stat.Var" Ends ######
.

# 4) Function: Asym.Comp.Stat.Var()
# Function Asym.Comp.Stat.Var() takes in a dataset "Asymmetry.t" (which contains all simulated
asymmetries in species compostition in each mixed communites in each simulation run) and
calculate an overall mean variance of asymmetry across all communities, with standard deviation
and 95% confidence interval.

Asym.Comp.Stat.Var <- function (Asymmetry.t) {
  # transpose dataset
  Asymmetry<-t(Asymmetry.t)

  # Create null vectors
  asymmetry_var_total<-c()
  asymmetry_var_total_temp<-c()
  asymmetry_sd_var_total<-c()
  asymmetry_low_var_total<-c()
  asymmetry_high_var_total<-c()
  Difference_var_total<-c()

  # Extract the variance in asymmetry of all communities in each simulation run for following
calculations
  for (i in 1:simmulation.no) {
    asymmetry_var_total_temp<-
cbind(asymmetry_var_total_temp,var(Asymmetry[,i],na.rm=TRUE))
  }

  asymmetry_var_total<-mean(as.numeric(asymmetry_var_total_temp),na.rm=TRUE) #calculate
the overall mean variance of asymmetry
  asymmetry_sd_var_total<-sd(as.numeric(asymmetry_var_total_temp),na.rm=TRUE) #calculate
SD
  asymmetry_low_var_total<- qnorm(0.025,mean=asymmetry_var_total,
sd=asymmetry_sd_var_total)  #calculate the lower boundary of 95% CI
  asymmetry_high_var_total<-qnorm(0.975,mean=
asymmetry_var_total,sd=asymmetry_sd_var_total)  #calculate the upper boundary of 95% CI

  #Export data
  Difference_var_total<-as.data.frame (cbind(t(asymmetry_var_total), t(asymmetry_sd_var_total),
t(asymmetry_low_var_total), t(asymmetry_high_var_total)))
  colnames(Difference_var_total)<-c("mean","SD","95% CI","95% CI")
  return(Difference_var_total)
}
```

```
######### Function "Asym.Comp.Stat.Var" Ends ########



##############################Simulations###################################

# Create null vectors for overall asymmetry in species composition
mydata.left<-c()
Asymmetry.t<-c()

# Create null vectors for asymmetry in species compostition by guild
mydata.mix.Bac<-c()
Asymmetry.t.Bac<-c()
mydata.mix.Edible<-c()
Asymmetry.t.Edible<-c()
mydata.mix.Herb<-c()
Asymmetry.t.Herb<-c()
mydata.mix.Inedible<-c()
Asymmetry.t.Inedible<-c()
mydata.mix.Omni<-c()
Asymmetry.t.Omni<-c()
mydata.mix.Predator<-c()
Asymmetry.t.Predator<-c()

# Create null vectors for asymmetry in species compostition by treatment
mydata.mix.1_2<-c()
Asymmetry.t.1_2<-c()
mydata.mix.3<-c()
Asymmetry.t.3<-c()
mydata.mix.CC<-c()
Asymmetry.t.CC<-c()
mydata.mix.TC<-c()
Asymmetry.t.TC<-c()
mydata.mix.TT<-c()
Asymmetry.t.TT<-c()

for (i in 1: simmulation.no) {
  cat("simulation",i,"\n") #Print current simulation number
  persist<-c()
  mydata.left<-c()
  #The following "for loop"" finds the persistence prob of each species in "mydata", and simulates
whether it will persist or not and return the result as TRUE/FALSE
  for (i in 1:length(mydata$species)) {
    p<-c()
    p<-c(p,persistance_pr[persistance_pr[1]==as.character(mydata$species[i]),2]) # For each
species in "mydata", find its persistance probability in "persistance_pr" and save it as p
    persist.temp<-sample(c(TRUE,FALSE),size=1,replace=TRUE,prob=c(p,1-p))   #based on
persistance probability, estimate whether each sample from each species would persist or not
    persist<-c(persist,persist.temp) #save the result above as "persist"
  }
  #After simulating every species in "mydata", now "persist" is a vector with the same length as
the number of rows in "mydata", and filled with TURE or FALSE
  mydata.left<-mydata[persist,] #species that persist after simulation will now be stored in a new
```

```
vector "mydata.left"

  mydata.mix<-mydata.left[!is.na(mydata.left$mix1),]  #Extract all mix communities from
"mydata.left"

  ######### Asymmetry in Species Composition Overall #########
  Diff<-Asym.Comp(mydata.mix,mydata.post.ori)
  Asymmetry.t<-rbind(Asymmetry.t,Diff) # Asymmetry.t contains all simulated asymmetries in
species compostition in each mixed communites in each simulation run

  ######## Asymmetry in Species Composition by Guild#########
  # Extracting asymmetry results by guild
  #Bacteria
  mydata.mix.Bac<-mydata.mix[mydata.mix$guild=="Bac",]
  mydata.post.ori.Bac<-mydata.post.ori[mydata.post.ori$guild=="Bac",]
  Diff.Bac<-Asym.Comp(mydata.mix.Bac,mydata.post.ori.Bac)
  Asymmetry.t.Bac<-rbind(Asymmetry.t.Bac,Diff.Bac)
  #Edible
  mydata.mix.Edible<-mydata.mix[mydata.mix$guild=="Edible",]
  mydata.post.ori.Edible<-mydata.post.ori[mydata.post.ori$guild=="Edible",]
  Diff.Edible<-Asym.Comp(mydata.mix.Edible,mydata.post.ori.Edible)
  Asymmetry.t.Edible<-rbind(Asymmetry.t.Edible,Diff.Edible)
  #Herbivore
  mydata.mix.Herb<-mydata.mix[mydata.mix$guild=="Herb",]
  mydata.post.ori.Herb<-mydata.post.ori[mydata.post.ori$guild=="Herb",]
  Diff.Herb<-Asym.Comp(mydata.mix.Herb,mydata.post.ori.Herb)
  Asymmetry.t.Herb<-rbind(Asymmetry.t.Herb,Diff.Herb)
  #Inedible
  mydata.mix.Inedible<-mydata.mix[mydata.mix$guild=="Inedible ",] #"Inedible+blank"
  mydata.post.ori.Inedible<-mydata.post.ori[mydata.post.ori$guild=="Inedible ",]
  Diff.Inedible<-Asym.Comp(mydata.mix.Inedible,mydata.post.ori.Inedible)
  Asymmetry.t.Inedible<-rbind(Asymmetry.t.Inedible,Diff.Inedible)
  #Omnivore
  mydata.mix.Omni<-mydata.mix[mydata.mix$guild=="Omni",]
  mydata.post.ori.Omni<-mydata.post.ori[mydata.post.ori$guild=="Omni",]
  Diff.Omni<-Asym.Comp(mydata.mix.Omni,mydata.post.ori.Omni)
  Asymmetry.t.Omni<-rbind(Asymmetry.t.Omni,Diff.Omni)
  #Predator
  mydata.mix.Predator<-mydata.mix[mydata.mix$guild=="Predator",]
  mydata.post.ori.Predator<-mydata.post.ori[mydata.post.ori$guild=="Predator",]
  Diff.Predator<-Asym.Comp(mydata.mix.Predator,mydata.post.ori.Predator)
  Asymmetry.t.Predator<-rbind(Asymmetry.t.Predator,Diff.Predator)



  ######## Asymmetry in Species Composition by treatment#########
  # Extracting asymmetry resuls by treatment
  # CC
  mydata.mix.CC<-mydata.mix[mydata.mix$mixtype=="CC",]
  mydata.post.ori.CC<-mydata.post.ori[mydata.post.ori$mixtype=="CC",]
  mix.com.name.CC<-names(summary(drop.levels(mydata.post.ori.CC$mixcommunity)))
  Diff.CC<-Asym.Comp.Treat(mydata.mix.CC,mydata.post.ori.CC,mix.com.name.CC)
  Asymmetry.t.CC<-rbind(Asymmetry.t.CC,Diff.CC)
```

```
  # TC
  mydata.mix.TC<-mydata.mix[mydata.mix$mixtype=="TC",]
  mydata.post.ori.TC<-mydata.post.ori[mydata.post.ori$mixtype=="TC",]
  mix.com.name.TC<-names(summary(drop.levels(mydata.post.ori.TC$mixcommunity)))
  Diff.TC<-Asym.Comp.Treat(mydata.mix.TC,mydata.post.ori.TC,mix.com.name.TC)
  Asymmetry.t.TC<-rbind(Asymmetry.t.TC,Diff.TC)

  # TT
  mydata.mix.TT<-mydata.mix[mydata.mix$mixtype=="TT",]
  mydata.post.ori.TT<-mydata.post.ori[mydata.post.ori$mixtype=="TT",]
  mix.com.name.TT<-names(summary(drop.levels(mydata.post.ori.TT$mixcommunity)))
  Diff.TT<-Asym.Comp.Treat(mydata.mix.TT,mydata.post.ori.TT,mix.com.name.TT)
  Asymmetry.t.TT<-rbind(Asymmetry.t.TT,Diff.TT)
}

# SIMULATION ENDS


#### Statistical Analysis of Asymmetry in Species Composition#####

# Mean asymmetry with SD and 95% CI
Asym.Comp.Stat(Asymmetry.t)

# Asymmetry by guild with SD and 95% CI
Asym.Comp.Stat(Asymmetry.t.Bac)
Asym.Comp.Stat(Asymmetry.t.Edible)
Asym.Comp.Stat(Asymmetry.t.Herb)
Asym.Comp.Stat(Asymmetry.t.Inedible)
Asym.Comp.Stat(Asymmetry.t.Omni)
Asym.Comp.Stat(Asymmetry.t.Predator)

# Asymmetry by treatment with SD and 95% CI
Asym.Comp.Stat(Asymmetry.t.CC)
Asym.Comp.Stat(Asymmetry.t.TC)
Asym.Comp.Stat(Asymmetry.t.TT)

# Overall mean variance in asymmetry with SD and 95% CI
Asym.Comp.Stat.Var(Asymmetry.t)

# Mean variance in asymmetry with SD and 95% CI by guild
Asym.Comp.Stat.Var(Asymmetry.t.Bac)
Asym.Comp.Stat.Var(Asymmetry.t.Edible)
Asym.Comp.Stat.Var(Asymmetry.t.Herb)
Asym.Comp.Stat.Var(Asymmetry.t.Inedible)
Asym.Comp.Stat.Var(Asymmetry.t.Omni)
Asym.Comp.Stat.Var(Asymmetry.t.Predator)

# Mean variance in asymmetry with SD and 95% CI by treatment
Asym.Comp.Stat.Var(Asymmetry.t.CC)
Asym.Comp.Stat.Var(Asymmetry.t.TC)
Asym.Comp.Stat.Var(Asymmetry.t.TT)
```

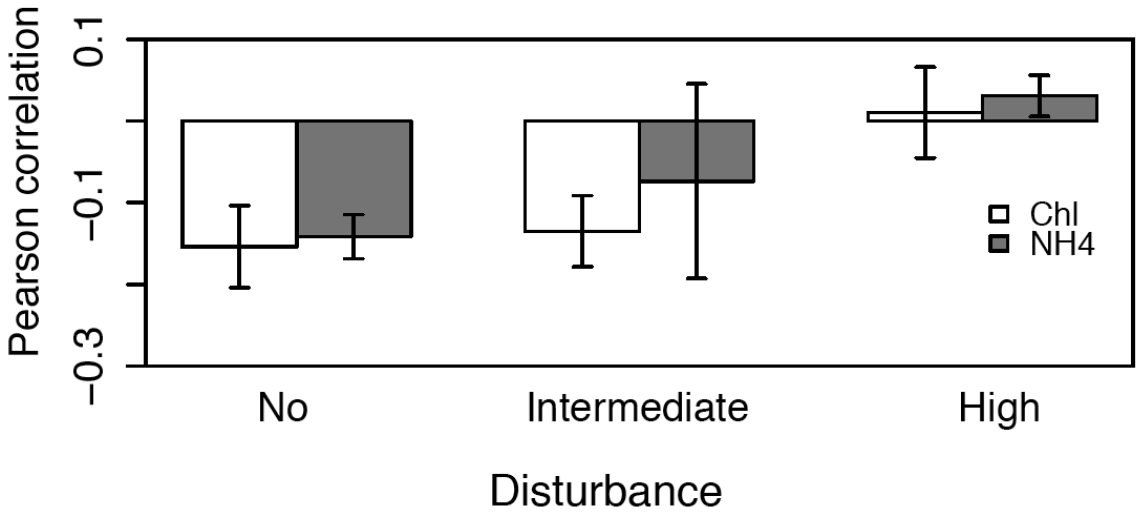
```
#Idividual community level deviants from 95% CI in asymmetry
# Transpose dataset
asym.comp.temp<-t(Asymmetry.t)
rownames(asym.comp.temp)<-names(summary(mydata.mix$mixcommunity))[-1]

# Create null vectors
mix_mean_indiv.comp<-c()
mix_sd_indiv.comp<-c()
mix_low_indiv.comp<-c()
mix_high_indiv.comp<-c()
connect_asym_indiv.comp<-c()

# Calculate the mean asymmetry in species composition for each mix community across all
simulation runs and store the result in connect_asym_indiv.comp
for (i in 1:66) {
  mix_mean_indiv.comp<-cbind(mix_mean_indiv.comp,mean(asym.comp.temp[i,],na.rm=TRUE))
  mix_sd_indiv.comp<-cbind(mix_sd_indiv.comp,sd(asym.comp.temp[i,],na.rm=TRUE))
  mix_low_indiv.comp<-cbind(mix_low_indiv.comp,qnorm(0.00038,mean=
mean(asym.comp.temp[i,],na.rm=TRUE),sd= sd(asym.comp.temp[i,],na.rm=TRUE)))
  mix_high_indiv.comp<-cbind(mix_high_indiv.comp,qnorm(0.99962,mean=
mean(asym.comp.temp[i,],na.rm=TRUE),sd=sd(asym.comp.temp[i,],na.rm=TRUE)))
}
connect_asym_indiv.comp <- as.data.frame(cbind(t(mix_mean_indiv.comp),
t(mix_sd_indiv.comp), t(mix_low_indiv.comp), t(mix_high_indiv.comp)))
names(connect_asym_indiv.comp)<-c("mean","SD","95%_CI.low","95%_CI.hi")
rownames(connect_asym_indiv.comp)<-mix.com.name

#Identfy Deviants
sum(connect_deviant_data$comp_diff < connect_asym_indiv.comp[3]) #The number of deviants
below CI
sum(connect_deviant_data$comp_diff > connect_asym_indiv.comp[4]) #The number of deviants
above CI
```

Chapter 3 Supplemental Information



Supplementary Figure 3.1: Pearson correlation coefficients between abundance of Paramecium and chla and NH4. NH4 data was only collected for landscapes in week 5 from two replicates. Chlorophyll (chl) data was collected for weeks 3-6. Error bars show standard deviation.

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