

Testing the keystone community concept: effects of landscape, patch removal, and environment on metacommunity structure

EMLYN J. RESETARITS,^{1,3} SARA E. CATHEY,² AND MATHEW A. LEIBOLD¹

¹*Department of Integrative Biology, University of Texas at Austin, Austin, Texas 78712 USA*

²*Department of Biological Sciences, Virginia Polytechnic Institute and State University, Blacksburg, Virginia 24061 USA*

Abstract. Although the influence of regional processes on local patches is well studied, the influence of local patches and their spatial arrangement on regional processes is likely to be complex. One interesting idea is the keystone community concept (KCC); this posits that there may be some patches that have a disproportionately large effect on the metacommunity compared to other patches. We experimentally test the KCC by using replicate protist microcosm metacommunities with single-patch removals. Removing single patches had no effect on average community richness, evenness and biomass of our metacommunities, but did cause metacommunities to be assembled significantly less by local environmental conditions and more by spatial effects related to stochastic factors. Overall our results show that local patch removal can have large regional effects on structural processes, but indicate that more experiments are needed to find evidence of keystone communities.

Key words: conservation; dispersal; landscape ecology; microcosms; protists; variation partitioning.

INTRODUCTION

Communities have historically been studied at the local scale from a “niche-based” perspective, where processes driving community assembly are the abiotic environment and interspecific interactions (Hutchinson 1957, 1959, MacArthur 1969, Tilman 1982, Chase and Leibold 2003). From this perspective, communities are predicted to be explained primarily by local environmental factors; a prediction known as “species sorting” (Chase and Leibold 2003). At larger scales, regional processes cause stochastic extinctions of local communities and connect these local communities through processes of dispersal, structuring communities less by local environmental conditions and more by spatial connectivity (Hanski and Gyllenberg 1997, Hubbell 2001, Leibold and Loeuille 2015, Resetarits and Silberbush 2015). Although most studies focus on a single scale, the interaction of regional processes (dispersal, demography, extinction) and local “niche-based” processes for explaining community assembly is becoming increasingly apparent (Fagan 2002, Leibold et al. 2004, Cottenie 2005, Gravel et al. 2006, Leibold and McPeck 2006, Adler et al. 2007). Approaches to studying this interaction include metacommunity ecology (Leibold et al. 2004, Holyoak et al. 2005, Leibold 2011, Logue et al. 2011), and landscape ecology (Troll 1939, Naveh and Lieberman 1984, Urban et al. 1987, Turner et al. 2001, Urban and Keitt 2001). Most work in metacommunity

ecology focuses on this interaction between the local and regional scale in general terms and often ignores the spatial details of the metacommunity. In contrast, landscape ecology is focused on spatial context but often ignores more general community context such as patterns of biodiversity and the mechanisms of species interactions.

How local patches or communities contribute to regional patterns is an important, and primarily unaddressed, question regarding the interaction of local and regional processes (Urban and Keitt 2001). At the community level, this question requires the integration of the spatially-explicit aspects of landscape ecology with metacommunity ecology. Mouquet et al. (2012) proposed the keystone community concept (KCC)—an extension of Robert Paine’s (1966, 1969) keystone predator concept—which posits that the removal of some local patches may have disproportionate effects on the metacommunity. Mouquet et al.’s (2012) metacommunity-based models illustrate that particular local features of patches such as their size, productivity, or environmental distinctiveness can affect important features of the metacommunity such as its diversity or productivity. Economo (2011) and Gascuel et al. (2016) used neutral theory and landscape modeling to show that the particular spatial location of patches in a dispersal network could also affect overall diversity in complex ways even if there were no environmental differences among patches (see also Carrara et al. 2012). Thus both environmental features and position in a landscape could alter the contribution of individual patches to regional features of the metacommunity (Altermatt and Holyoak 2012, Carrara et al. 2014). Although the removal of a large or high complementary patch may have a strong impact on a metacommunity, this could be primarily due to direct effects, such as the

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³E-mail: eresetarits@utexas.edu

loss of species in that single patch. In this study, however, we are primarily interested in finding evidence of patches whose removal have significant indirect effects on the metacommunity resulting from downstream changes in neighboring patches.

The interaction of local and regional processes in shaping metacommunities is important not just for furthering ecological theory but also for environmental management. Habitat loss and fragmentation alter patterns of biodiversity and ecosystem attributes by changing the size and connectivity of the metacommunity, while habitat modification does so through changing the environmental conditions of patches. Such anthropogenic changes to both spatial and environmental context could interact to alter biodiversity and ecosystem services. The management of reserves and reserve networks seeks to counter these anthropogenic effects. Current approaches to evaluate and conduct triage, and reserve selection are primarily focused on combining landscape ecology with metapopulation theory (e.g., Moilanen et al. 2005, Leroux et al. 2016, Albert et al. 2017). They often look to preserve low connectivity, rare patches that have high endemism or complementarity (unique species not found in the other patches; Economo 2011) or to try to maximize connectivity to promote long-term persistence (Margules and Pressey 2000). However, these approaches may be ineffective if the species involved have strong interactions that also depend on environmental and spatial context, making it imperative that a metacommunity perspective be used when possible. Identifying certain patch attributes that predict “keystoneness” would be a critical tool for conservationists.

Protist microcosms are an ideal system for addressing conservation-related questions because they are well-studied, easily manipulated to mimic a variety of landscape types, and provide a needed bridge between ecological theory and on-the-ground conservation efforts. In this study, we used protist microcosms to experimentally test how the attributes of individual patches, including both location within the spatial network (high vs. low connectivity), and environmental distinctiveness (rare vs. common), altered properties of the metacommunity. We did this by comparing properties in a “N patch” control metacommunity and in different “N-1 patch” experimental metacommunities where we removed one patch only. Removal was done for all four combinations of high-vs.-low connectivity and rare-vs.-common environmental distinctiveness. We chose connectivity and environmental distinctiveness because they are local attributes dependent on regional context and because previous models suggest that these attributes should have indirect effects on the metacommunity (Mouquet et al. 2012, Fournier et al. 2016, Gascuel et al. 2016). Our metacommunity design combined the common elements of species sorting (habitat heterogeneity, niche partitioning between habitats, community assembly) with aspects of patch dynamics (background extinctions due to stochastic events and dispersal)

and neutral theory (demographic stochasticity in small local populations). We focused on two main metacommunity properties. First, we examined the effect of patch removal on diversity and ecosystem function metrics (average community richness, evenness, and biomass), and second, we used variation partitioning techniques (Borcard and Legendre 2002, Cottenie 2005, Peres-Neto et al. 2006, Logue et al. 2011) to assess the relative role of environmental conditions and spatial connectivity on community composition (referred to here as structuring processes). Additionally, we examined our results for evidence of “keystone communities” by looking for patches that had a disproportionate effect on the metacommunity.

We hypothesized that highly connected, rare patches should have the largest effect on both diversity/ecosystem function measures and on structuring processes at the metacommunity level. The removal of highly connected patches should maximally disrupt dispersal/recolonization from neighboring patches and rare patches should harbor unique species found in a smaller proportion of communities and thus be more susceptible to regional extinction and stochasticity in recolonization (Wilson and MacArthur 1967, Levins 1969, Hanski 1999).

Our study found no evidence for the presence of “keystone communities” and suggests that diversity measures (richness, evenness, biomass) of the metacommunity are robust to removal of single patches. However, we found that the removal of any single patch from a metacommunity caused community assembly to be driven less by environmental processes and more by spatial processes. Surprisingly, we found that landscape structure (i.e., the distribution of habitat types and stochastic extinctions across the metacommunity) had the largest effect on both diversity measures and structural processes. This study demonstrates the importance of assessing underlying regional processes in addition to local diversity measures in order to assess the impact of habitat loss at the regional scale.

MATERIALS AND METHODS

Experimental design

Experimental microcosms were optimal for testing metacommunity theory, because they allowed us to directly compare the effects of removing different patches on identical metacommunities. Our communities consisted of six protist species: *Colpidium striatum*, *Blepharisma* sp., *Euplotes* sp., *Philodina* sp., and *Vorticella* sp., which we ordered from Carolina Biological Supply (Burlington, NC), and *Paramecium* sp., which was obtained from Dr. Jin Liang at Georgia Tech. These species were used because they differ in characteristics that could affect their competitive ability, such as swimming speed, mean cell volume, and ontogenetic life stages, and because they vary in their preferred food source (bacteria vs. algae), thus allowing us to have two different patch types that contained distinct communities.

Metacommunities were composed of a patchwork of two different habitat types, algal-based (autochthonous) and bacterial-based (allochthonous) as described by Fukumori et al. (2015). Each patch was a Qorpak 60 mL jar containing 30 mL of COMBO culture media (Kilham et al. 1998). The two habitat types differed in their source of organic matter (algal production or bacterial decomposition of organic matter) for the protists to subsist on. Algal patches were inoculated with 25,000 cells (1 mL) of *Chlamydomonas*. Algal patches were made of clear polystyrene plastic to allow for optimal light penetration. Bacterial patches had a degraded wheat seed added for rapid bacterial colonization from the environment. Bacterial patches were made of a white polypropylene plastic and were cultured under dark conditions to minimize light penetration and prevent algal

growth. Both types of patches were cultured in the same culture chamber in order to hold all environmental parameters, except light conditions, constant.

All replicate metacommunities had an identical 6×6 virtual dendritic structure and were each composed of 36 patches (Control) or 35 patches (Treatments; see Fig. 1A). Metacommunities could either be algal-dominated (2/3 of the patches were algal-based) or bacterial-dominated (2/3 bacterial-based). In order to test the effect of patch removal on the metacommunity, each treatment consisted of the removal of a different “type” of patch. The patches we removed varied in both connectivity (high, low) and environmental distinctiveness (rare, common). Common patches had a habitat type that made up 2/3 of the metacommunity, and rare patches had habitat types that made up 1/3 of the

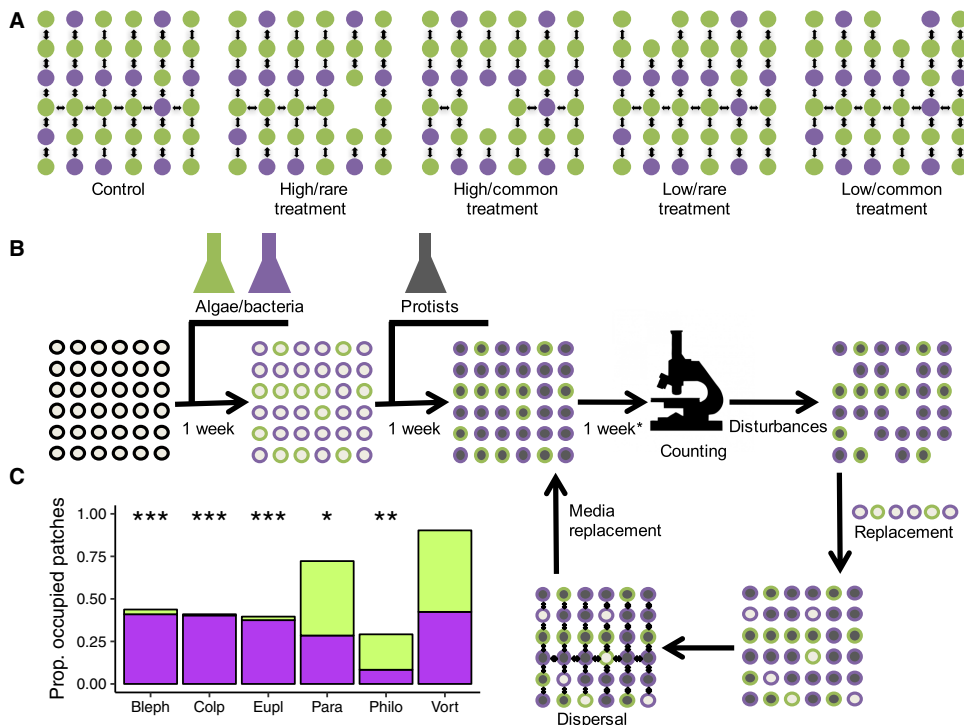


FIG. 1. (A) An example of an algal-dominated landscape, consisting of 5 replicate metacommunities (a control metacommunity and four treatment metacommunities) with identical extinctions and distributions of algal and bacterial patches. Arrows represent patches that are connected weekly by manual dispersal. Green circles represent algal patches and purple circles represent bacterial patches. The distribution of algal and bacterial patches varied between landscapes but were identical within a landscape. Our experiment contained 10 landscapes, half which were algal-dominated and half that were bacterial-dominated for a total of 1,760 patches and 50 metacommunities. (B) Experimental design: a single metacommunity consisting of 36 uncolonized patches (black open circles) were inoculated with algae or bacteria and allowed to grow for 1 week. Colored circles represent algal-based patches (green open circles) or bacterial-based patches (purple open circles). Six species of protist were then added to each patch (filled circles) and allowed to grow for 1 week. After 1 week, counts were taken (for control metacommunities only). Six patches from each metacommunity were then removed as disturbances and replaced with patches that had either algae or bacteria, but no protists (green and purple open circles). Dispersal of protists between connected patches (arrows connect patches) then occurred, causing new, empty patches to be colonized (closed circles). 10% of the media in each patch was then replaced to allow communities to persist. Protists were then allowed to grow for a week before the process was repeated for 9–10 weeks. *Starts after first disturbance-replacement-dispersal cycle. (C) The proportion of patches occupied by each of six protists (*Blepharisma* [Bleph], *Colpidium* [Colp], *Euplotes* [Eupl], *Paramecium* [Para], *Philodina* [Philo], *Vorticella* [Vort]) across two habitat types based on week 1 data (pre-dispersal) for landscapes 7–10, representing species sorting for each species. 72 bacterial-based patches (dark purple) and 72 algal-based patches (light green) were inoculated with all six protists and allowed to grow for 1 week. Significant species sorting (differential survival between the two habitats) is indicated as follows: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

metacommunity. The removal of high connectivity patches broke up the metacommunity into multiple unconnected sub-metacommunities, whereas the low connectivity patches did not. We therefore had four treatments, where a different type of patch was removed: high/common, high/rare, low/common, low/rare. These patches stayed empty for the entirety of the experiment, and we held their place with a container of ethanol (Fig. 1A).

The distribution of algal and bacterial patches within the 6×6 metacommunity was randomly assigned but was identical within a landscape. A landscape was applied as a block treatment and consisted of five replicate metacommunities (a control metacommunity and four treatment metacommunities) with identical habitat distributions and extinctions (see below; Fig. 1A). Our experiment contained 10 landscapes, half which were algal-dominated and half that were bacteria-dominated. We altered which habitat was dominant and the distribution of algal and bacteria-based habitat patches in order to maintain generality and to prevent our findings from being specific to one scenario. Our experiment used a total of 1,760 patches and 50 metacommunities.

Experimental protocol (Weekly)

Bacteria or algae were grown in patches for 1 week prior to the start of the experiment to ensure sufficient resources were available for protists (Fig. 1B). We then inoculated each patch in the metacommunities with approximately 20 individuals of each protist species and left them to grow undisturbed for 1 week. Each week, we replaced 3 mL (10%) from every patch with fresh COMBO media. We used the removed media to count protist densities (cells/mL) under a $10\times$ light microscope for each patch in the control treatment. We used these weekly densities to monitor the progress of all the metacommunities within each landscape.

Disturbances, dispersal and media replacement occurred weekly in all metacommunities. Each week, to introduce stochastic disturbances into the system, we replaced six patches (four common, two rare) from each metacommunity with fresh patches that contained only COMBO media and a source of organic matter (algae or bacteria) that had been growing for a week. These six patches were determined randomly, varied weekly, and were identical within a landscape. Dispersal was conducted after random patch disturbances to allow neighboring individuals to colonize the unoccupied patches. Dispersal was symmetrical and reciprocal between adjacent patches and was done manually by transferring 100 μ L aliquots between virtually connected microcosms (see Fig. 1B). One hundred microliters was chosen based on a pilot experiment because it produced moderate community richness, variation across the metacommunity (all species were not in all habitats), and allowed for spatial connectivity to be important without causing a large number of uncolonized (empty) patches (see Appendix S1: Fig. S1).

Final counts

We performed experiments from January 2015–August 2015. We ran landscapes 1–6 starting in January (experiment 1) and ran landscapes 7–10 (experiment 2) starting in May. Each experiment ran for 9–10 weeks, representing over 60 generations for most of the protist species. Algal-dominated landscapes were counted at 9 weeks and bacterial-dominated habitats were counted at 10 weeks due to time constraints and sampling intensity. Final counts were recorded as density (cells/mL) per species for each patch.

Diversity measures

In order to assess how removing certain patches from a metacommunity might affect diversity and ecosystem functioning, we calculated three broad community level measures: average community richness, evenness, and biomass. We used average community level measures in order to control for the direct effects of removing a patch on the metacommunity (35–36 patches) and to focus on the indirect effects. Richness was calculated as the average number of species found in each patch. Richness was not rarified because we scanned a larger volume of media for low density patches (<10 individuals) in order to detect rare taxa. Furthermore, because maximum richness of a patch is known (six species), rarefaction would potentially give us unrealistically high richness (>6 species). Community evenness was assessed by calculating Shannon's diversity (H') using the R package *vegan* and dividing it by the natural log of the richness.

Biomass was calculated by multiplying the density (cells/mL) of each species by an average volume/cell for that species. We calculated average volume for each species by photographing 10 specimens of each species (of varying size) from our original cultures, measuring length and width using ImageJ, and then applying the equation for the volume of an ellipsoid. *Paramecium* and *Vorticella* cultures used for biomass calculations were primarily grown on *Chlamydomonas* while *Blepharisma*, *Euplotes*, *Philodina*, and *Colpidium* were grown on bacteria. Size did vary between environmental conditions for *Blepharisma*, *Euplotes*, and *Colpidium*, but this was not taken into account since these species were uncommon in algal patches and only in low densities. Volume estimations were then averaged across the ten replicates to get an average volume/cell of each species. Biomass is expressed as the volume of protists per mL of media. All analyses, except variation partitioning, were done in R version 3.3.1 (Bug in Your Hair).

Variation partitioning

In order to determine the relative importance of spatial and environmental processes for structuring communities, we used variation partitioning techniques (Borcard and Legendre 2002, Cottenie 2005, Peres-Neto et al. 2006).

The environmental parameters used were habitat type (algal or bacterial) and age since most recent disturbance. In order to detect complex spatial patterning, we used Moran's Eigenvector Maps, which uses a weighted connectivity matrix to extract eigenvectors that maximize Moran's index of autocorrelation (Dray et al. 2006). We used a binary weights matrix and only included significant eigenvectors with positive autocorrelation.

Variation partitioning was performed for all 50 metacommunities at the end of the experiment to assess how well space (significant positive eigenvectors) and the environment (habitat type, age) explained community composition across the metacommunity (Borcard and Legendre 2002, Cottenie 2005). Raw community data was Hellinger transformed using the vegan package for all subsequent analyses (Legendre and Gallagher 2001). Redundancy Analyses (RDA) were done to test the significance of each component. Non-significant components for a metacommunity were given a value of zero in the data table used for subsequent analyses. However, including these non-significant components in the analyses did not qualitatively change the results, because non-significant components had very low values. R version 3.0.2 (Frisbee Sailing) and packages spacemakeR and packfor were used for variation partitioning analyses.

Statistical analyses

In order to assess habitat preferences for our six species, we calculated the number of algal and bacterial patches that possessed a given species at week 1 (after 1 week of protist growth). This measurement was only done for landscapes 7–10 due to misidentification of *Vorticella/Philodina* species for landscapes 1–6 during the first weeks of counting. We used a Pearson's chi-squared test to assess if species were found more often in one habitat than would be expected (50:50), given all species were inoculated into all patches.

We used a nested ANOVA to determine the effect of removing any patch (treatments vs. control) on each of our metacommunity properties (e.g., richness or environmental component) where landscape is nested within dominant habitat (Model: metacommunity property ~ treatment + experiment + landscape(dominant habitat) + treatment × dominant habitat). For metacommunity properties that were significantly affected by removing any patch, we looked to see if there was variation between treatments. In order to compare metacommunity properties between treatments, we standardized measurements for each treatment by the corresponding control in that landscape. This gave us an effect size for a given treatment (i.e., of a given patch) and allowed us to test for "keystone communities." One-way ANOVAs were done using this standardized dataset to see if there were differences between treatments (Model: effect size of metacommunity property ~ treatment). A significant difference in effect size between a single treatment and all other treatments signified a "disproportionate effect"—i.e., a "keystone community".

Additionally, in order to investigate what aspects of our landscapes significantly altered metacommunity properties (see *Results*), we assessed spatial autocorrelation and Weighted Recovery Time post-hoc. We assessed spatial autocorrelation (i.e., how well the two habitat types were dispersed throughout the landscape) using Moran's I. We calculated Weighted Recovery Time since disturbance using the equation,

$$\frac{\sum_{i=1}^n a_i \times c_i}{n} \quad (1)$$

where a_i is the age of a patch (i.e., time since last disturbance), c_i is the connectivity of the patch (0–4), and n is the number of patches in the metacommunity (35 or 36). In this equation, older or more connected patches contribute more to Weighted Recovery Time than newly disturbed or less connected patches. One-way ANOVAs were used to see if there were differences in Moran's I and Weighted Recovery Time between landscapes using all 50 metacommunities (Model: e.g., Moran's I ~ landscape). Pearson product-moment correlations were used to look for relationships between each metacommunity property (e.g., richness, environmental component) and Moran's I or Weighted Recovery Time.

RESULTS

Species environmental preferences

We found that protist taxa had different preferred habitats. *Blepharisma* sp. ($\chi^2 = 48.016$, $df = 1$, P -value < 0.0001), *Colpidium striatum* ($\chi^2 = 55.068$, $df = 1$, P -value < 0.0001), and *Euplotes* sp. ($\chi^2 = 45.632$, $df = 1$, P -value < 0.0001) were primarily found in bacterial patches, whereas *Paramecium* sp. ($\chi^2 = 4.654$, $df = 1$, P -value = 0.031) and *Philodina* sp. ($\chi^2 = 7.714$, $df = 1$, P -value = 0.005) were primarily found in algal patches (Fig. 1C). The only species that did not appear to have a habitat preference was *Vorticella* sp., which was the most common species across both habitats ($\chi^2 = 0.492$, $df = 1$, P -value = 0.483).

Landscape effects

There was considerable variation among our controls in terms of diversity measures and variation partitioning results (Table 1; Fig. 2A). Some of the variation in our diversity measures was due to differences between our two experiments (landscapes 1–6 vs. landscapes 7–10; Table 2). Another reason for this variation was that bacterial-dominated metacommunities exhibited significantly higher average community evenness ($F = 6.532$, $P < 0.05$), average community biomass ($F = 9.546$, $P < 0.01$), and were more spatially structured than algal-dominated metacommunities ($F = 9.157$, $P < 0.01$; Table 2; Fig. 2A). Our landscapes (i.e., block treatments), which each had a different distribution of algal and

TABLE 1. Data summary for diversity, ecosystem function measurements and variation partitioning for control and treatment metacommunities.

Metacommunity Property	Type	<i>n</i>	Mean	SE	Min	Max
Diversity and ecosystem function						
Biomass	Control	10	5.358E-05	5.580E-06	2.294E-05	7.710E-05
	Treatment	40	4.886E-05	3.076E-06	2.086E-05	9.648E-05
Evenness	Control	10	0.437	0.017	0.375	0.545
	Treatment	40	0.429	0.011	0.249	0.625
Richness	Control	10	2.772	0.143	2.306	3.556
	Treatment	40	2.723	0.060	1.857	3.400
Variation partitioning (%)						
Environmental (E S)	Control	10	26.668	2.584	12.316	38.281
	Treatment	40	20.303	1.604	0.000	42.761
Spatial (S E)	Control	10	11.906	3.504	0.000	28.413
	Treatment	40	19.061	1.769	0.000	40.183
Joint (EwS)	Control	10	10.864	3.323	0.000	29.354
	Treatment	40	11.818	1.077	0.000	23.538

Notes: Evenness was calculated by dividing Shannon's diversity (H') by the natural log of the richness. Biomass is measured in mL protist per mL media.

bacterial patches and had different weekly extinctions, significantly differed in average community richness ($F = 8.739$, $P < 0.0001$), average community evenness ($F = 2.990$, $P < 0.05$), the purely environmental (E|S) component ($F = 7.840$, $P < 0.0001$), and the joint environmental-spatial (EwS) component ($F = 6.463$, $P < 0.0001$). However, landscapes did not significantly vary in the purely spatial (S|E) component ($F = 1.864$, $P = 0.103$) or in average community biomass ($F = 1.421$, $P = 0.225$). Although our study was not designed to tease apart how different aspects of spatial arrangement may influence diversity measures and underlying processes, we found that Moran's I (spatial autocorrelation) and Weighted Recovery Time were significantly different between landscapes ($F = 33.080$, $P < 0.0001$; $F = 7.601$, $P < 0.0001$). Additionally, Moran's I was negatively correlated with average community biomass ($t = -4.687$, $df = 48$, $P < 0.0001$) and richness ($t = -2.584$, $df = 48$, $P < 0.05$; Fig. 2B), and Weighted Recovery Time was positively correlated with average community biomass ($t = 4.287$, $df = 48$, $P < 0.0001$), richness ($t = 2.372$, $df = 48$, $P < 0.05$), and evenness ($t = 2.594$, $df = 48$, $P < 0.05$; Fig. 2C). No significant correlations were found between these landscape metrics and variation partitioning results (Appendix S1: Table S2).

What is the effect of removing any patch?

There was no significant effect of removing a patch on average community richness ($F = 0.373$, $P = 0.545$), evenness ($F = 0.130$, $P = 0.721$), or biomass ($F = 1.536$, $P = 0.223$) (Table 2). However, we did find that removing any patch significantly decreased the purely environmental (E|S) component ($F = 7.741$, $P < 0.01$), which is how much local environmental factors determined community composition, and nearly significantly increased the purely spatial (S|E) component, which is how much stochastic

extinctions and dispersal between connected patches determined community composition ($F = 3.841$, $P = 0.057$; Table 2; Fig. 3A). On average, removing a patch decreased the variation explained by the purely environmental component from 26% to 20%, and increased the variation explained by the spatial component from 12% to 19% (Table 1). The joint environmental-spatial (EwS) component was, however, not impacted by removing any patch ($F = 0.220$, $P = 0.642$). There was no significant interaction between treatment and dominant habitat for any of our measurements, meaning that we did not find differing effects of treatment for algal and bacterial dominated metacommunities.

Are there keystone communities?

There were no significant differences in effect size (control – treatment) between treatments for either the environmental (E|S) component (F -value = 0.275, $df = 3$, P -value = 0.843), the spatial (S|E) component (F -value = 0.185, $df = 3$, P -value = 0.906), or the joint environmental-spatial (EwS) component ($F = 0.244$, $P = 0.865$; Fig. 3B). Thus, we found no patch type, based on connectivity and commonality, that exhibited keystone for any of our metacommunity attributes.

DISCUSSION

Most work on metacommunities has focused on how regional processes affect local patch properties (e.g. diversity or productivity; Mouquet and Loreau 2003, Economo and Keitt 2008). However, understanding how individual patches affect metacommunity-wide processes is key for predicting changes in biodiversity measures over long time scales and has been relatively understudied (Mouquet et al. 2012, Gascuel et al. 2016). In this study, we focused on how removing certain patches

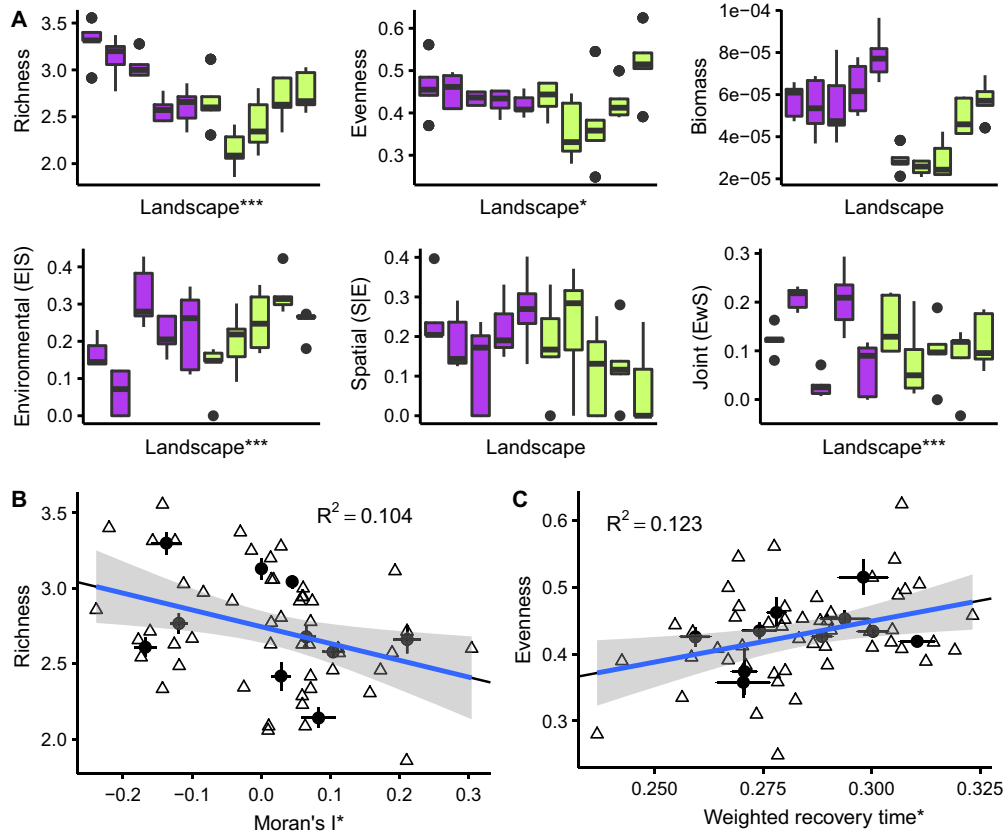


FIG. 2. (A) Boxplots showing the variation of diversity measures (Top: richness, evenness, and biomass) and variation partitioning results (Bottom: environmental component, spatial component, and joint) across our ten landscapes. Dark purple boxplots represent bacterial-dominated landscapes and light green boxplots represent algal-dominated landscapes. (B) The effect of spatial autocorrelation (Moran's I) on average community richness and (C) Weighted Recovery Time on average community evenness. Filled circles with error bars represent means and standard errors for each landscape for both parameters, showing that variation within landscapes is small. Open triangles represent individual metacommunities. Correlation coefficients and significance are based on individual metacommunity measurements. Shaded areas represents the 95% confidence interval. Moran's I varies between -1 (overdispersion) and 1 (positive autocorrelation). Significance effects are described as follows: period, $P < 0.1$; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

affected metacommunity-level diversity patterns and structural processes. We found that the metacommunity, in general, was highly resistant to the removal of a community, in terms of biodiversity and ecosystem function measures. On the other hand, we found that removing a patch did have a strong effect on underlying structural processes as identified by variation partitioning. Removing any patch decreased the importance of environmental filtering and increased the importance of spatial structure and dispersal for driving community assembly across the metacommunity.

Unexpectedly, we found that the strongest driver of our communities was our landscape (i.e., block) treatments. Landscapes differed in their distribution of algal and bacterial-based patches and the location of weekly stochastic patch disturbances. These effects were substantially larger than the removal of any single patch (and the concomitant dispersal linkages) and were not due to differences in metacommunity size, connectivity, or average disturbance

regime (all controlled or randomized in our experimental design) and presumably reflect particular details of the landscape arrangement of patches. Although our cursory investigation suggests that spatial autocorrelation of habitat types (Moran's I) and Weighted Recovery Time since disturbance may be important for community diversity and ecosystem functioning, the relative importance of each cannot be determined from this study. Additionally, much of the variation between landscapes, especially in environmental and spatial structuring, was not explained by either parameter. More nuanced metrics than those used here will likely be required to tease apart these landscape effects further.

One of the goals of this study was to identify keystone communities—patches that have a disproportionate effect on the metacommunity when removed (Mouquet et al. 2012). Although removing a patch did have a significant effect on the metacommunity (increase in spatial component, decrease in environmental component),

TABLE 2. ANOVA results for diversity, ecosystem function and variation partitioning measurements (Model: factor ~ treatment + experiment + landscape(dominant habitat) + treatment × dominant habitat).

Factor	Sum Sq	df	F value	P
Richness				
Treatment	0.019	1	0.373	0.545
Experiment	1.191	1	22.802	<0.0001
Dominant habitat	0.063	1	1.197	0.281
Landscape	3.195	7	8.739	<0.0001
Treatment × Dominant habitat	0.056	1	1.076	0.306
Evenness				
Treatment	4.59E-04	1	0.130	0.721
Experiment	4.68E-03	1	1.321	0.258
Dominant habitat	2.31E-02	1	6.532	0.015
Landscape	7.42E-02	7	2.990	0.013
Treatment × Dominant habitat	9.47E-03	1	2.674	0.110
Biomass				
Treatment	1.790E-10	1	1.536	0.223
Experiment	1.117E-09	1	9.609	0.004
Dominant habitat	1.110E-09	1	9.546	0.004
Landscape	1.156E-09	7	1.421	0.225
Treatment × Dominant habitat	2.200E-12	1	0.019	0.892
Environmental (E S)				
Treatment	0.032	1	7.741	0.008
Experiment	0.010	1	2.375	0.132
Dominant habitat	0.098	1	0.219	0.642
Landscape	0.139	7	7.840	<0.0001
Treatment × Dominant habitat	0.001	1	1.185	0.283
Spatial (S E)				
Treatment	0.041	1	3.841	0.057
Experiment	0.001	1	0.096	0.758
Dominant habitat	0.098	1	9.157	0.004
Landscape	0.139	7	1.864	0.103
Treatment × Dominant habitat	0.001	1	0.070	0.793
Combo (EwS)				
Treatment	7.29E-04	1	0.220	0.642
Experiment	8.57E-03	1	2.589	0.116
Dominant habitat	7.88E-03	1	2.379	0.131
Landscape	0.150	7	6.463	<0.0001
Treatment × Dominant habitat	1.93E-04	1	0.058	0.811

Note: Bolded values represent significant factors at a cutoff of $P < 0.05$.

there was no difference in the effect size based on what type of patch was removed (high/rare, high/common, low/rare, low/common). This is surprising, since removing a high connectivity patch broke up our metacommunities into smaller, disconnected metacommunities, and a large body of theory stemming from island biogeography and metapopulation ecology suggests that this should have a negative impact on species diversity and richness (Wilson and MacArthur 1967, Levins 1969, Urban and Keitt 2001). Not finding evidence for keystone

communities in this case certainly does not mean that they do not exist in other contexts. One reason there may not have been keystone communities in our system is that our metacommunities did not have enough habitat variability to produce patches with either unique attributes (e.g. super high nutrients, etc.; Mouquet et al. 2012) or high enough diversity to produce patches with unique species (Economo 2011). Additionally, the low species diversity of our system, combined with the large metacommunity size, may have dampened effects that would have been more pronounced given a smaller metacommunity and/or more diverse community. However, our results suggest that keystone communities, if found, may still have rather subtle effects.

Although in our experiment we omitted patches from a given metacommunity, what we were really interested in were the effects of patch removal. We do not believe that our results would have qualitatively changed if we had allowed full metacommunities to establish and then removed a patch rather than simply omitting a patch from the beginning. The reason being that omission and removal experiments should only differ if the starting conditions (i.e., species distributions across the metacommunity) differ. If, for example, we had inoculated only a few patches to begin with, or had some spatial community structure from the beginning, one could imagine removing a patch from the metacommunity could have different downstream (so to speak) effects than the omission of a patch. However, since we inoculated every patch with the same “cocktail” of species, we have not induced any spatial structure into our metacommunities that should cause omission of a patch to differ from the removal of a patch.

Our results support previous work showing that higher patch extinctions within a metacommunity cause a decrease in environmental structuring and an increase in spatial structuring (Fukumori et al. 2015, Leibold and Loeuille 2015). Additionally, the variation explained by both environment and space in our experiment mirror the results from low and medium dispersal treatments of Fukumori et al. (2015) which also had two distinct habitat types but had a grid-like spatial layout. We found that the loss of a single patch, which constituted less than a 3% (1/36) change in the size of the metacommunity, could significantly alter the fundamental processes structuring that metacommunity by decreasing the environmental component by roughly 20% and increasing the spatial component by roughly 50%. A decrease in the environmental component signifies a decrease in ‘species sorting’ (Leibold et al. 2004), which can alter ecosystem functioning (Leibold et al. 2017). Taken together with previous work these findings suggest that as anthropogenic forces disconnect or chip away at suitable wildlife habitat, the processes structuring these communities may be quickly changing in large, important ways, even if diversity and ecosystem properties appear unchanged.

Many conservation efforts focus on preserving habitat patches with high complementarity (i.e., that have unique

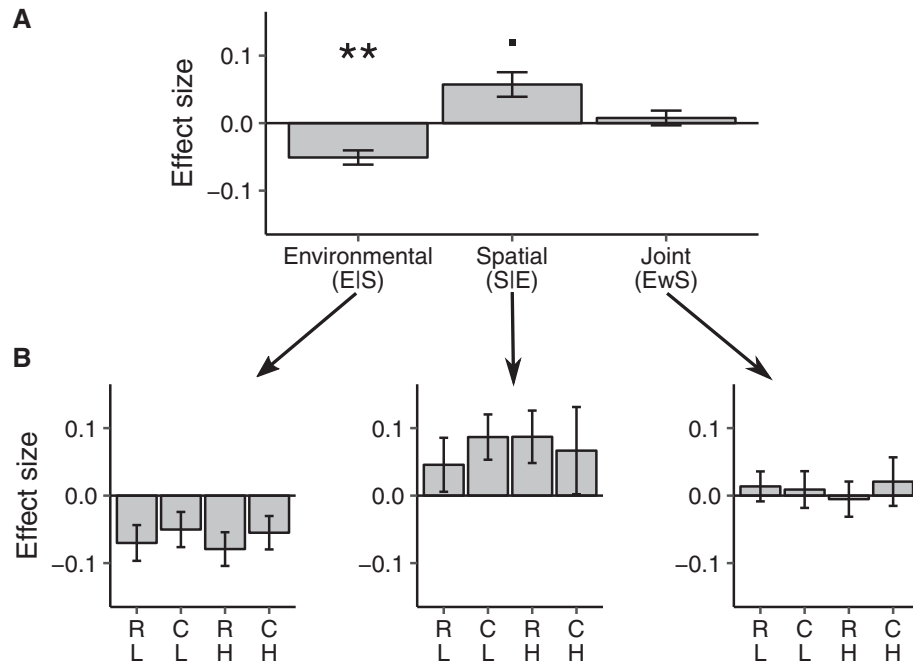


FIG. 3. (A) Bars represent the mean effect size (Treatment – Control) on the environmental, spatial, and joint environmental-spatial components when removing any community from the metacommunity. Removing a community increased the spatial component and decreased the environmental component. (B) Bars represent the effect sizes of individual treatments for the environmental, spatial, and joint environmental-spatial component. The top row of the x-axis represents the commonality of each treatment: Rare (R) or Common (C). The bottom row of the x-axis represents the connectivity of each treatment: Low (L) and High (H). Error bars represent standard error. Significance levels for the effect size of removing a community are as follows: period, $P < 0.1$; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

species), and these patches are often those that are environmentally distinct and poorly connected to other patches (e.g., islands; Margules and Pressey 2000). Using a neutral metacommunity (i.e., solely structured by space), Economo found that in the long term, both highly connected (redundant) and poorly connected (complementary) patches had the same long-term effects on diversity. In other words, Economo found that when metacommunities were driven solely by spatial structure (neutral), metacommunity diversity was directly related to metacommunity size, suggesting that conservation strategies to maintain species diversity may be ineffective if habitat loss is unavoidable. In non-neutral metacommunities (structured by both the environment and space), we found that the loss of any patch caused the metacommunity to become more structured by space. If species in metacommunities that are more structured by space are harder to conserve because of a strong causal relationship between metacommunity size and diversity, and a decrease in metacommunity size also causes an increase in the importance of spatial structure, this suggests that conservation efforts may become increasingly ineffective as habitat quantity decreases. Of course, more work across scales and different systems is needed in order to confirm this relationship between metacommunity size and spatial structuring before we can assess how applicable these results are to conservation.

As our questions and concerns become more global or large-scale, rigorous experiments are becoming less and less tractable. Especially for metacommunities, we have come to rely more and more on modeling, data mining, and observational studies rather than empirical testing. However, without rigorous experimentation, we lack the ability to identify intrinsic mechanisms and assess causality (Benton et al. 2007, Paine 2010). Fortunately, microcosm and mesocosm experiments using model organisms can allow us to directly address seemingly-intractable global questions (Altermatt and Holyoak 2012, Carrara et al. 2012, 2014, Fukumori et al. 2015).

As this study demonstrates, there is an ever-increasing need to include the role of spatial context and scale for understanding community and metacommunity dynamics (Bengtsson 2009, Leibold and Chase, *in press*). Although many studies focus on the impacts of disturbances on diversity, and the effects of diversity on community and ecosystem processes, fewer have focused on how disturbances effect underlying processes structuring communities. Our study clearly shows that even without changes in more traditional community measurements (diversity, etc.), there can still be strong structural changes occurring in the metacommunity. Future work will need to focus on understanding the role of these changes to underlying processes on long-term community diversity and stability.

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