

# Larger Area Facilitates Richness-Function Effects in Experimental Microcosms

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**ABSTRACT:** Species-area relationships (SAR) and biodiversity-ecosystem function (BEF) relationships are central patterns in community ecology. Although research on both patterns often invokes mechanisms of community assembly, both SARs and BEFs are generally treated as separate phenomena. Here we link the two by creating an experimental SAR in microcosm communities and show that greater species richness in larger areas is accompanied by greater ecosystem function. We then explore mechanisms of community assembly by determining whether rare, large, or high-biomass species are more likely to persist in the larger microcosms. Our results indicate that larger areas harbor more rare species of a wider range of body sizes and have higher functional diversity, implying that the addition of niche space that supports rare species underlies the effect of area on species richness and function. Our results suggest that the preservation of large areas is a potentially useful way of maximizing the provisioning of ecosystem services through the maintenance of biodiversity.

**Keywords:** species-area relationship, biodiversity ecosystem function, macroecology, size structure, reserve size, complementarity.

## Introduction

Multiple ecological forces, including competition, facilitation, and consumption, are involved in the generation and maintenance of biodiversity in ecological communities (Hutchinson 1959; Rohde 1992; Chesson 2000; Bascompte et al. 2006; Condit et al. 2006; Chase 2010). These forces fall into general categories of factors that minimize fitness differences among species (equalizing effects) or factors that limit interspecific competition (stabilizing; Chesson 2000). Although a consequence of these forces, species richness and/or functional diversity also can be causal factors driving community- and ecosystem-level properties, such as the aggregate func-

tioning of the system in terms of biomass production, nutrient cycling, or respiration and photosynthesis (i.e., ecosystem function; Tilman et al. 1997, 2014; Loreau et al. 2001; Hooper et al. 2005; Cardinale et al. 2006a). Moreover, the mechanisms by which species coexist within communities may influence the biodiversity-ecosystem function (BEF) relationship (Tilman 2000).

Although not a mechanism per se, one factor related to biodiversity is the area—*island size, habitat or patch size, or sampling area*—that is sampled for determining species richness (MacArthur and Wilson 1967; Preston 1973; Rosenzweig 1995; Scheiner 2003). Power-law-like and other positive relationships linking species richness to area (species-area relationships [SARs]) abound across nearly all data sets (Connor and McCoy 1979; Tjørve 2003; Horner-Devine et al. 2004; Bell et al. 2005; Drakare et al. 2006; Storch et al. 2012; Wilber et al. 2015). Multiple hypotheses have been proposed to explain SARs, including mechanistic hypotheses invoking niche and population dynamic processes as well as hypotheses invoking the probability of occurrence of certain species as a function of area (MacArthur and Wilson 1967; Connor and McCoy 1979; McGuinness 1984; Harte et al. 2009; McGill 2010; Storch et al. 2012). Within the hypotheses invoking niche processes, there are subhypotheses, such as that larger species may be successively added as area increases because of their larger home ranges (an allometric constraint; Post et al. 2000) or that rarer species would be added as area increases because they may have niches that tend not to occur in smaller patches (MacArthur and Wilson 1967; McGuinness 1984). It is unknown, however, whether an increase in ecosystem function accompanies the increase in species richness with area.

Species richness is often positively related to ecosystem function, either through the complementary effects of resource use or the increased likelihood of including high-function species (i.e., species with high biomass or high productivity) within experimental replicates as diversity increases (Huston 1997; Tilman et al. 1997, 2014; Loreau et al. 2001; Hooper et al. 2005; Cardinale et al. 2006a). Both of these possible mechanisms (a complementarity effect and a selec-

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tion effect, respectively) invoke species interactions that influence which and how many species can persist within a community, either through the partitioning of resources (e.g., through divergent body sizes or differential resource use) or the dominance of high-functioning species. Alternatively, neutral coexistence of species can foster diversity without altering ecosystem functioning, because the mechanism of coexistence is neither niche partitioning nor dominance but is rather turnover of species that have similar functions (Hubbell 2001; DeLong and Vasseur 2012; Tilman et al. 2014). Therefore, the increase in species richness with area should be accompanied by increases in ecosystem function if coexistence is supported by species interactions but not if it is supported by neutral processes.

Here we use experimental microcosms of different areas to generate an island SAR, and we test for an increase in ecosystem function with area. We measured community respiration and total carbon and nitrogen content as metrics of ecosystem function. We inoculated our microcosms with a diverse array of protists, after which the microcosms lost species through time and eventually came to an approximate equilibrium. Thus, in our experiment, the resulting SAR is the opposite of an extinction-area curve (Kitzes and Harte 2014).

We then evaluated features of the communities to determine whether there is evidence that complementarity or selection effects could be generating both the SAR and the effect of diversity on function. By definition, our microcosms are not subject to standard selection effects because all species were included in all dish sizes at the beginning of the experiment. Nonetheless, if the mechanism allowing higher richness and higher function in larger patches was the greater persistence of high-functioning species in larger patches, then high-functioning species (e.g., those that achieve higher abundance) would be the ones persisting in larger areas rather than being present across most areas. Thus, we would infer that selection effects are operating if we see high-functioning species being lost in the smaller dishes but present in the larger ones. In contrast, if there is evidence that species with different niches persist differentially across dish sizes, this would suggest that complementarity effects are operating (Cardinale et al. 2006a). For example, cell volume is a major niche dimension in protists because cell volume is linked to particle-size consumption (Fenchel 1980; Finlay and Fenchel 2001; DeLong and Vasseur 2012; DeLong et al. 2015) and influences swimming speed and the grain of the habitat that can be used. Other niche dimensions include trophic position or type (e.g., grazer or predator) and the use of space within the microcosms, such as swimming in the water column or staying at the bottom of dishes. If niche partitioning generated higher species richness and higher functioning in larger dishes, we would expect to see in larger dishes the addition

of species across multiple cell volumes (as opposed to similar cell volumes), differential space use, or increased functional diversity. Finally, if larger areas are required for larger species to persist (an allometric space constraint), we would expect to see the largest species persist to a greater extent in larger dishes, suggesting that niches specific to large species are the ones added to larger islands.

## Methods

We isolated 16 morphospecies of protist and algae from a pond at the Spring Creek Prairie Audubon Center near Denton, Nebraska, and a small pond on the campus of the University of Nebraska–Lincoln (Novich et al. 2014), along with a strain of *Tetrahymena thermophila* acquired from the *Tetrahymena* resource center (table 1). These species were extracted manually by pipette from pond samples and placed in single-species cultures until ready for use. Although some of the larger species (i.e., *Stentor* sp. and *Paramecium caudatum*) can feed on small flagellates, the pool of species overall rely on a range of bacteria and organic compounds in the medium for food. The species were all ciliates, flagellates, and algae. Our species pool, therefore, included taxa that are somewhat related (Violle et al. 2010), even though ciliates, flagellates, and algae are less related to one another than are all metazoans (Baldauf 1999). Nonetheless, we expected the pairwise interactions among species to be mostly independent of relatedness, as shown by Fritschie et al. (2014). We fed cells with locally collected and autoclaved pond water combined with protozoan medium (Carolina Biological Supply, Burlington, NC) mixed at a 1:9 ratio with filtered and autoclaved pond water and subsequently inoculated with a mixture of locally derived pond bacteria. We estimated the cell volume for each protist species from the literature and for the specific cell lines used where possible.

We used petri dishes with respective diameters of 35, 60, 100, and 150 mm (or 9.6, 28.3, 78.5, and 176.7 cm<sup>2</sup> in area) as microcosms. All microcosms contained protozoan medium to a constant depth (2.48 mm) across all dish sizes to standardize the vertical structure of the environment and the amount of light (~27,000 lux) that penetrated the dishes. We intentionally kept microcosms shallow so that the entire dish could be searched for the presence of each species on each sampling day without disrupting the microcosms. Each dish contained a number of brown rice grains to serve as slow-release sources of carbon, scaled to the microcosm volume so as not to alter the resource levels across dish sizes (table 2). We replenished each dish with filtered water on each sampling day to account for evaporation given measured evaporation at each dish size (table 2).

We inoculated each microcosm with the same 16 species. For the smallest dishes (35 mm diameter), we added three

**Table 1:** Species used in the microcosms

Rank	Species	Approximate avg. cell volume ( $\mu\text{m}^3$ )	Size rank	Density rank	Biovolume rank	Main food <sup>a</sup>
1	<i>Stentor</i> sp.	$5 \times 10^6$	1	13	14	B, A, P
2	<i>Spirostomum</i> sp.	$2.7 \times 10^6$	2	10	6	B
3	<i>Frontonia</i> sp.	$6 \times 10^5$	3	13	14	B, A
4	<i>Paramecium caudatum</i>	$5.8 \times 10^5$	4	8	4	B, A
5	<i>Euplotes</i> sp. 1	$7 \times 10^5$	5	4	1	B, A, P
6	<i>Paramecium bursaria</i>	$3.5 \times 10^5$	6	2	2	B, A, L
7	<i>Urostyla</i> sp.	$2 \times 10^5$	7	1	3	B, A, P
8	<i>Paramecium aurelia</i>	$1.1 \times 10^5$	8	5	5	B, A
9	<i>Euplotes</i> sp. 2	$1 \times 10^5$	9	13	13	B, A, L
10	<i>Closterium</i> sp.	$1 \times 10^5$	10	9	8	L
11	<i>Coleps hirtus</i>	$9 \times 10^4$	11	11	12	B, A, P
12	<i>Tetrahymena thermophila</i>	$4 \times 10^4$	12	12	11	B, O
13	<i>Vorticella</i> sp.	$2.5 \times 10^4$	13	3	7	B, A
14	<i>Peranema</i> sp.	$1 \times 10^3$	14	13	14	B, O
15	<i>Chilomonas paramecium</i>	$1 \times 10^3$	15	6	10	B, O
16	Unidentified flagellate	$1 \times 10^3$	16	7	9	B, O

Note: Also listed are cell volumes, ranked from largest to smallest, along with abundance (cells  $\text{mL}^{-1}$ ) rank, biovolume ( $\mu\text{m}^3 \text{mL}^{-1}$ ) rank, and main food, as indicated in Foissner and Berger (1996) or other observations.

<sup>a</sup> Diet types: bacterivores (B), algivores (A), predators (P), osmotrophs (O), photosynthetic (L).

individual cells for most species but added two cells for species that were less abundant in our source cultures (i.e., *Frontonia* sp. and *Coleps hirtus*). We added small unidentified flagellates in 1- $\mu\text{L}$  samples (roughly dozens of cells). For *Vorticella*, we added a variable number of individuals (1–5), as this organism typically forms clumps of individuals and collecting cells individually was difficult. Except for the flagellates, we transferred cells in a 50- $\mu\text{L}$  sample from the source population. Additional small flagellates appeared in some microcosms during the course of the experiment, although it is unclear whether they were already present and grew in density over the course of the experiment or whether they appeared from cysts that were inadvertently introduced in our microcosms. These species were unidentified, similar in size, and low in abundance and so were grouped together as “unidentified flagellates.”

We kept microcosms in incubators at 23°C and, during the 11-day experiment, searched all microcosms for 15 min each Monday, Wednesday, and Friday to find individuals of all added species. To be considered present, only one individual needed to be seen. We recorded common species

within a couple of minutes, and we systematically scanned the entire dish until the 15 min were up or we found all possible species, whichever came first.

At the end of the experiment, we estimated the density of each species in each microcosm by either counting all individuals in the dish or by counting individuals in a 0.2-mL sample taken from the microcosm. Also, we measured the oxygen consumption of a 0.75-mL sample using a fluorescent oxygen probe (OXY 10 micro, PreSens, Regensburg, Germany) and determined total carbon and nitrogen content for a 1-mL sample passed through a preburned 25-mm glass fiber filter and combusted in a Costech ECS 4010 (Valencia, CA) analyzer. We estimated total protist biovolume as the sum of the product of estimated density and cell volume for each species in each replicate. We then fit power-law functions with each community-level replicate measurement as dependent variables (species richness, oxygen consumption, carbon content, nitrogen content, and total biovolume) and microcosm area as the independent variable, with both dependent and independent variables log transformed, using linear models in Matlab. We also tested for a positive effect of species

**Table 2:** Characteristics of microcosms of different sizes used to build a species-area curve with protist communities

Diameter (mm)	Volume (mL)	Increase factor	No. seeds	Replenishment rate (mL per day)
35	2.38	1	1	.07
60	7.00	2.9	3	.2
100	19.48	8.12	8	.56
150	43.88	18.44	18	1.28

Note: All microcosms were filled to a depth of 2.48 mm to standardize light penetration through the water.

richness on oxygen consumption controlling for dish area with linear models using untransformed data.

Because protozoans show considerable variation in cell volume and niche shape and size (Foissner and Berger 1996; Caron et al. 2008), we tested for variation in functional diversity of the communities across dish area. We did this by assigning each species a vector of binary scores for not being (0) or being (1) bacterivores, algivores, predators, osmotrophs, or photosynthetic, using information from Foissner and Berger (1996), the presence of chloroplasts, or known ability for species to grow in axenic media (table 1). We then made a functional trait matrix with  $z$ -scores of these binary trophic traits and cell volume and calculated Euclidean pairwise differences among all traits. We constructed a dendrogram to assess clustering of species by function and used the sum of the branch lengths as a measure of functional diversity within dishes (Petchey and Gaston 2006). Finally, we assessed whether dish size influenced functional diversity and therefore was associated with differences in ecosystem function across dishes using the same power-law analysis as above.

We evaluated whether the population density of the six most persistent species varied across dish area on the final day using MANOVA, with densities as the dependent variables and dish area as a factor, followed up by ANOVAs for each species. We also tested whether variation in the density of these six species played a role in driving the overall function of the system by building linear models with the log of oxygen consumption, total carbon, and total nitrogen as dependent variables; the log of species richness as a predictor;

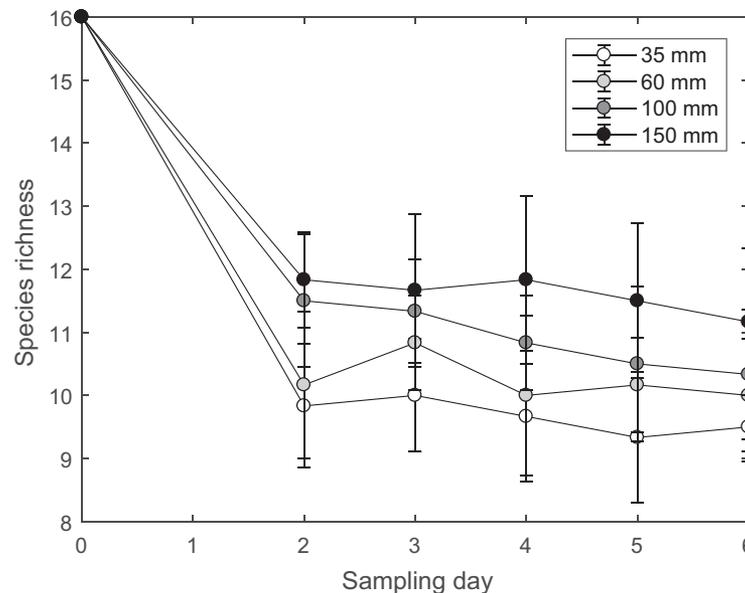
and the untransformed densities of each of the six species. We used backward elimination of insignificant terms to determine whether any particular species played a role in driving function in addition to the effect of species richness.

All data and code used to produce our analyses and figures are deposited in the Dryad Digital Repository (<https://dx.doi.org/10.5061/dryad.48cn500>; DeLong and Gibert 2019).

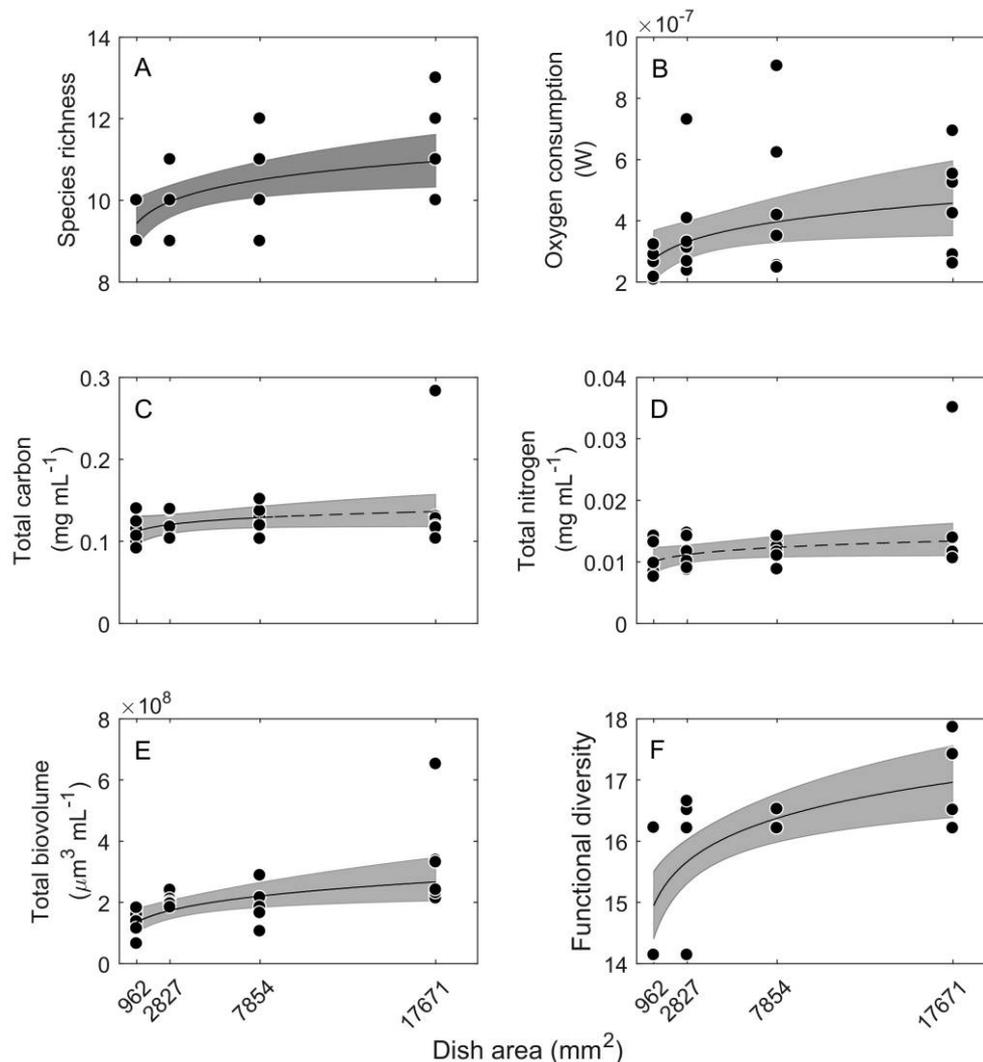
## Results

After seeding each microcosm with 16 species, diversity declined through time at first and then stabilized (fig. 1), which resulted in a positive relation between dish size and species richness (SAR scaling exponent  $z = 0.05 \pm 0.016$  standard error [SE];  $F = 10.2$ ;  $R^2 = 0.29$ ;  $P = .004$ ; fig. 2A). Concurrent with the increase in species number with dish area, community-level respiration per unit microcosm volume increased with dish area ( $z = 0.18 \pm 0.075$  SE;  $F = 5.57$ ;  $R^2 = 0.17$ ;  $P = .028$ ; fig. 2B). Total carbon content ( $F = 2.88$ ;  $R^2 = 0.08$ ;  $P = .1$ ; fig. 2C) and total nitrogen content ( $F = 3.4$ ;  $R^2 = 0.09$ ;  $P = .08$ ; fig. 2D) did not increase with dish area, but total biovolume per unit volume did ( $F = 10.7$ ;  $R^2 = 0.30$ ;  $P = .003$ ; fig. 2E).

Oxygen consumption was positively related to species richness ( $F = 2.84$ ;  $R^2 = 0.24$ ;  $P = .009$ ; fig. 3). To assess whether species richness influenced oxygen consumption independently from area or protist biovolume, we also conducted a linear model with species richness, dish area, and total biovolume as predictors of oxygen consumption. This analysis showed that species richness—and not dish



**Figure 1:** Realized species richness (mean  $\pm$  SD) through time at each dish size. Sampling occurred every 2–3 days over an 11-day period. Points are offset slightly on the X-axis for clarity.



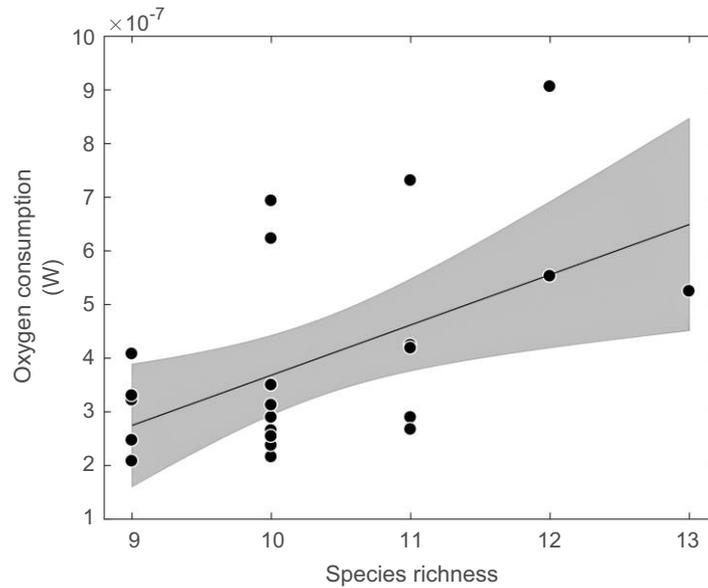
**Figure 2:** Relationship between species richness (A), whole-community oxygen consumption (B), total carbon per milliliter (C), total nitrogen per milliliter (D), estimated protist biovolume density (E), and functional diversity (F) for each replicate at each dish size. All regressions are shown with the fitted curve in solid black if significant and dashed black if not significant, with 95% confidence limits on the curve in gray. Data points from all replicates have been used, but there is some overlap, which may look like some data points have been omitted when they have not.

area or total protist biovolume—remained a significant predictor of oxygen consumption (species richness:  $t = 2.08$ ,  $P = .051$ ; area:  $t = 0.077$ ,  $P = .94$ ; biovolume:  $t = 0.22$ ,  $P = .83$ ).

There was no clear pattern about how species average cell volume was related to the likelihood of being present in dishes (fig. 4A). Both abundant species and those that went completely missing from all microcosms occurred across a range of cell volumes. However, a set of intermediate- to large-sized species were present in all dishes of all sizes. Average cell volume was not related to abundance ( $r = -0.11$ ,  $P = .68$ ), indicating the lack of a size scaling to the community structure. In contrast, the species that were progressively

more likely to persist in the larger dishes were species with low overall biovolume (fig. 4B). This indicates that the higher functioning occurring in the larger dishes was not a result of the addition of high biomass species but rather the maintenance of rarer species. However, there was a clear increase in functional diversity with dish area ( $F = 14.9$ ;  $R^2 = 0.38$ ;  $P = .0009$ ; fig. 2F).

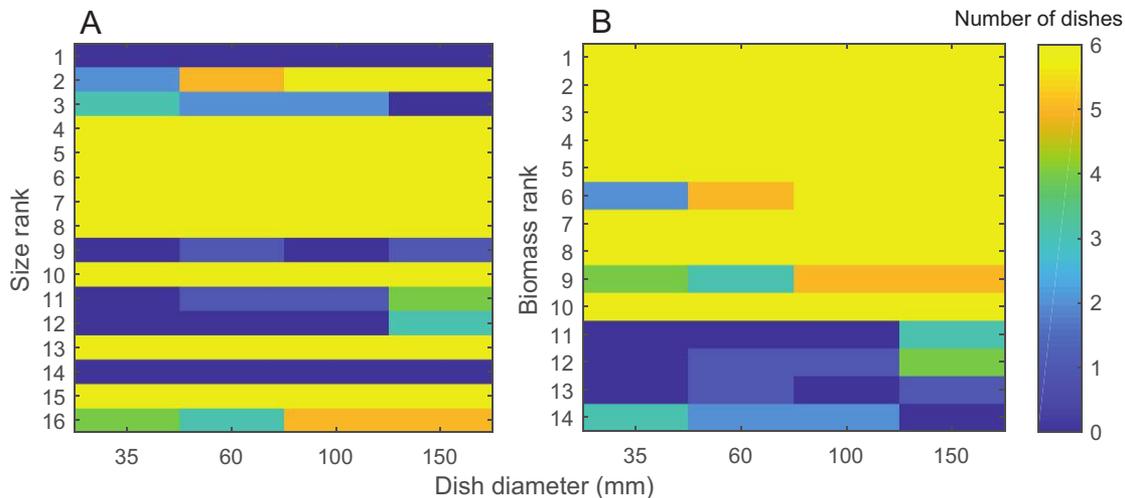
Six species of medium-to-large ciliates persisted across all dish sizes and replicates at relatively high biovolumes. The communities varied significantly in abundance of these species along two linear combinations of species ( $P = .009$ ; fig. 5A). Combination 1 was positively correlated with *Urostyla* ( $r = 0.6$ ,  $P = .002$ ) and *Euplotes* sp. 1 ( $r = 0.9$ ,



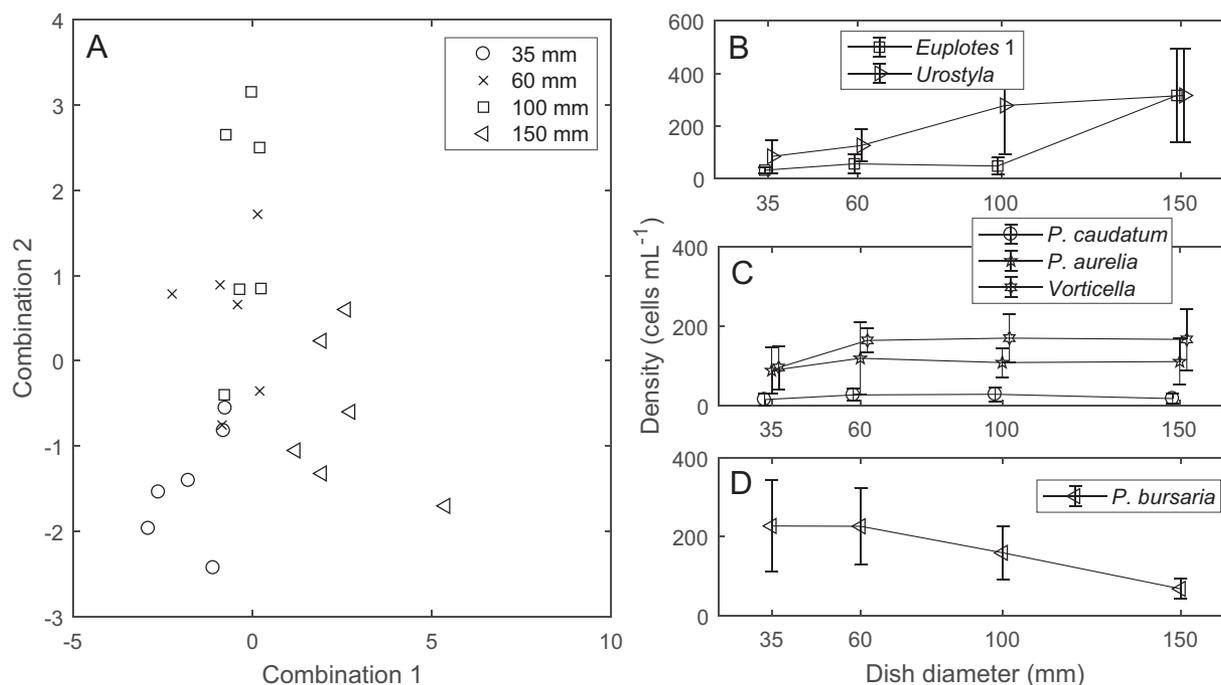
**Figure 3:** Relationship between whole-community oxygen consumption and species richness. Each circle is a replicate microcosm, and overall fit is shown with 95% confidence limits on the curve in gray.

$P < .001$ ) densities and negatively correlated with *Paramecium bursaria* ( $r = -0.72, P < .001$ ) density, and thus the largest dishes had more *Urostyla* and *Euplotes* and fewer *P. bursaria*. Combination 2 was positively correlated with *Paramecium caudatum* ( $r = 0.48, P = .018$ ) and *Vorticella*

( $r = 0.48, P = .018$ ) densities, and thus the smallest dishes tended to have fewer of these species. These patterns generally held up with univariate analyses: two species increased in density with increasing dish area (*Euplotes* sp. 1 [ $F = 13.23, P < .001$ ] and *Urostyla* [ $F = 4.17, P = .019$ ]; fig. 5B),



**Figure 4:** A, Number of replicate dishes of each size where each species is present, ordered by cell volume rank, where 1 is the largest species. Species could occupy from zero dishes (dark blue; i.e., extinct in all replicates of that size dish) to six dishes (bright yellow; i.e., present in all replicates at the size dish). Thus, horizontal bands of bright yellow indicate a species that was present in all replicates at all dish sizes. In contrast, horizontal bands that grade from blue to yellow are those species that showed up more frequently in larger dishes. The figure shows that some small, medium, and large species persisted in microcosms of all sizes, suggesting that species with different size-based niches may be able to coexist. B, Number of replicate dishes of each size where each species is present, ordered by rank of average abundance across all dishes, where 1 again is the most abundant species. Plot layout is as in A. The figure shows that high-abundance species are consistently present in all dish sizes, whereas low-abundance species tended to disappear in the smaller dishes.



**Figure 5:** Population density of six medium-to-large ciliates that persisted across all dish sizes and replicates. *A*, Two linear combinations of species density were associated with differences in dish size, as determined by MANOVA. Combination 1 was correlated with *Urostyla*, *Euplotes* sp. 1, and *Paramecium bursaria*, and combination 2 was correlated with *Paramecium caudatum* and *Vorticella*. These differences also can be seen species by species. Some species increased in density with dish area (*B*), others did not change in density across dish area (*C*), and one species declined in density across area (*D*). Points are offset slightly on the X-axis for clarity.

three species did not change in density with dish area (*P. caudatum* [ $F = 1.19, P = .34$ ], *Paramecium aurelia* [ $F = 0.25, P = .86$ ], and *Vorticella* [ $F = 2.22, P = .12$ ]; fig. 5C), and one species decreased in density with increasing dish area (*P. bursaria* [ $F = 4.82, P = .011$ ]; fig. 5D). Two of these species had positive effects on both the total amount of carbon and the total amount of nitrogen in the microcosm (*P. bursaria* [ $t = 2.67, P = .014$  for carbon and  $t = 3.14, P = .005$  for nitrogen] and *Euplotes* sp. 1 [ $t = 5.23, P < .001$  for carbon and  $t = 5.68, P < .001$  for nitrogen]). In contrast, none of these six species had independent effects on oxygen consumption.

### Discussion

Emergent properties of ecological communities by necessity arise from the processes linking species to each other and determining which species can persist in the community. Thus, the mechanisms allowing potentially competing species to coexist, at least temporarily, should play some role in driving emergent properties like total species richness and the overall functioning of the system (Tilman 2000). Our experiment links two classic community and ecosystem ecology patterns—the SAR and the BEF—to reveal that both

types of emergent property can operate simultaneously. Furthermore, because the additional physical space allows more species and higher functioning to occur in larger dishes, space itself may be an important factor driving ecosystem functioning in nature. Given the ubiquity of species-area curves and the importance of maintaining high ecosystem functioning for socioeconomic stability, conserving species-rich communities in large spatial extents may be an efficient way of fostering the positive effect of species richness on ecosystem functioning (Diamond 1975; Simberloff and Abele 1976).

A range of explanations have been proposed to explain species-area curves (MacArthur and Wilson 1967; Simberloff 1976; Connell 1983; McGuinness 1984; Post et al. 2000; Harte et al. 2009; McGill 2010; Storch et al. 2012). Some of these mechanisms invoke sampling processes in generating patterns, whereas other ideas invoke the likelihood of adding new niche space or specific species to the community as physical space is increased. In our experiment, larger area was not accompanied by increased resource concentration, as the depth of the microcosms (and thus light resources) was constant, and the release of carbon from rice grains was controlled and scaled to the microcosm volume itself (table 2). Thus, our results do not emerge from a species-energy effect, even though higher energy inputs can cause an in-

crease in species number through an overall increase in the number of individuals (Wright 1983; Schuler et al. 2015). In addition, because larger species were not more likely to be retained in larger dishes, there was not an allometric constraint operating to limit richness to small species in the smaller dishes. In fact, species across a range of cell volumes—including the smallest, some intermediate, and one of the larger species—were more likely to occur in the large dishes. Finally, since all species were added at the beginning of the experiment, there should have been no assembly history effects altering biotic interactions across dish size and thus influencing species richness (e.g., Fukami 2004).

Similar to other broadscale species-area relationships with microbes (Green et al. 2004; Bell et al. 2005), our microcosms showed a significant but relatively shallow species-area curve (fig. 2). It seems possible that the SAR relationship we observed was linked to enhanced niche space in the larger microcosms. Although difficult to quantify, that additional niche space may have arisen through the additional physical space or by increased spatial heterogeneity that could be exploited by the rarer species (e.g., Bell et al. 2005). Heterogeneity in microcosms, especially shallow, wide microcosms, can arise through the structure generated by biofilms, colony-forming bacteria, the build-up of detritus, and the spatial structure created by the rice grains, creating considerable scope for increased niche space. This type of heterogeneity is analogous to what could be observed by increasing the diversity of microclimates, soil types, or vegetative structures in terrestrial systems. Alternatively, an SAR could arise artificially if by virtue of sorting through more cells in the larger dishes we would detect more species (Bell et al. 2005). The finite number of species that were introduced in the dishes initially and our exhaustive searches, however, precluded this type of effect.

The presence of the BEF relationship accompanying the SAR gives clues as to what mechanisms may have structured our microcosm communities. In our experiment, all species were included in all dishes, and the species-area curve emerged through species loss. Thus, it would have been impossible to randomly include higher-functioning species only in large dishes to create a selection effect, although it would have been possible for high-functioning species to be lost more frequently in the smaller dishes. Our data on rank biovolume indicate, however, that high-functioning species persisted in all dish sizes and that high species richness and functioning resulted from the addition of rarer species across a wide range of cell volumes (fig. 4). Furthermore, no particular species played a strong role in driving oxygen consumption. This pattern suggests that rare species may be adding a detectable level of function to the system, similar to what has been seen in some grassland communities (Smith and Knapp 2003; Hooper et al. 2005). In short, there was no evidence that overall functioning in

the system was linked to high-functioning species, suggesting that selection effects were not operating.

By eliminating selection effects and observing an increase in functional diversity with dish area (fig. 2F), complementarity effects stand as the most likely mechanism generating the BEF in our system. Multiple traits influence the utilization of niches and subsequent coexistence of species (Cardinale 2011; Kraft et al. 2015). Potential niche dimensions that could enable complementary resource use in our microcosms include cell volume and space use (e.g., Cardinale 2011). Cell volume in protists is linked to particle size selection (Fenchel 1980; Finlay and Fenchel 2001; DeLong and Vasseur 2012; DeLong et al. 2015), suggesting that in the larger dishes, the addition of species from across a spectrum of cell volumes would foster greater complementary use of resources (i.e., the consumption of a greater diversity of particle sizes). Space use appears to have become more complementary in larger dishes as well. Behavioral differences were detectable in our communities, with *Paramecium caudatum* and *Paramecium aurelia*, for example, utilizing the full depth of the microcosm, while other species, such as *Urostylea*, *Euplotes*, and *Paramecium bursaria*, often settled on the bottom of the dish. Both *Urostylea* and *Euplotes* increased in relative abundance in the larger dishes (fig. 5), suggesting increased parsing of space by benthic and pelagic species. Thus, although niches remain exceedingly difficult to quantify, two fundamental aspects of niche space—what species eat and where they live—appear to have been more thoroughly exploited in larger dishes. This pattern is consistent with the idea that complementarity was driving the increased flux of energy in the systems.

Alternatively, the increased richness in the larger dishes may have influenced function in the system by increasing the abundance or richness of bacteria in the microcosms. Bacterial communities undoubtedly play a substantial role in the overall flux of energy in microcosm communities, especially where detritivores form much of the base of the food web. Protists may influence bacterial communities through their grazing action, their waste products, or the release of organic compounds (Fenchel and Harrison 1976; McGrady-Steed et al. 1997; Naeem and Li 1997; Krumins et al. 2006; DeLong and Vasseur 2012). As such, this effect could be seen as an indirect way in which the richness of one guild in a community positively influences the functioning of another (Worm and Duffy 2003; Cardinale et al. 2006b). This would be consistent with an observed link between protist species richness and decomposition in protist microcosms and plant communities (McGrady-Steed et al. 1997; Cardinale et al. 2011). In addition, some protists may act not as competitors with each other but as facilitators that allow other species to function at a higher level. Such facilitative effects are common in protist microcosm communities (DeLong and Vasseur 2012), so there may have been indirect

facilitative effects even among the protists. Our finding that total protist biovolume was not linked to oxygen consumption also suggests that increased functioning of bacterial decomposers played a role in generating the BEF pattern in our microcosms.

Despite the relationship between richness and oxygen consumption in this study, neither area nor diversity influenced the standing stock of carbon and nitrogen. Thus, not all aspects of functioning were related to richness. Variation in the standing stocks of carbon and nitrogen were positively associated with two mostly bottom-dwelling species: *P. bursaria* and *Euplotes* sp. 1. Thus, species with particular sets of behavioral or morphological traits may influence some aspects of function (i.e., standing stocks) while not influencing the overall productivity of the system. In larger, more speciose dishes, it appears that *Euplotes* sp. 1 became more abundant, possibly at the expense of *P. bursaria*, tilting the system toward less autotrophy, a pattern that has been seen in other protists microcosms where richness varied (Naeem and Li 1997; Petchey et al. 1999). The lack of an area or richness effect on standing stocks of carbon and nitrogen also could be related to our control of resource supply. That energy flux could increase given the same stocks of carbon and nitrogen suggests an increase in nutrient cycling, further suggesting an important role for bacterial activity in our systems.

In conclusion, our results point to links between emergent community patterns that may arise from the mechanisms of coexistence. We suggest that although there may be great utility in exploring emergent community ecology patterns on their own, exploring their intersection in a simple, experimental context can reveal processes that may be more challenging to detect in a natural system. Indeed, microcosm experiments using communities of protists have been used to test many important hypotheses, including some related to BEF and SARs (Naeem and Li 1997; Petchey et al. 1999; Fukami 2004; Bell et al. 2005; Long and Morin 2005; Violle et al. 2010; Altermatt et al. 2015). The difficulty of measuring ecosystem function at the whole-island level, for example, may be one reason that it was previously not clear that the species-area curve might be accompanied by a BEF relationship. The possibility that it is, however, implies that conservation of large areas could improve the provisioning of ecosystem services (Daily 1997).

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