

EFFECTS OF SPECIES DIVERSITY ON COMMUNITY BIOMASS PRODUCTION CHANGE OVER THE COURSE OF SUCCESSION

JEROME J. WEIS,^{1,2} BRADLEY J. CARDINALE,^{1,2,3} KENNETH J. FORSHAY,² AND ANTHONY R. IVES²

¹*Department of Ecology, Evolution & Marine Biology, University of California-Santa Barbara, Santa Barbara, California 93106 USA*

²*Department of Zoology, University of Wisconsin, Madison, Wisconsin 53706 USA*

Abstract. Over the past decade an increasing number of studies have experimentally manipulated the number of species in a community and examined how this alters the aggregate production of species biomass. Many of these studies have shown that the effects of richness on biomass change through time, but we have limited understanding of the mechanisms that produce these dynamic trends. Here we report the results of an experiment in which we manipulated the richness of freshwater algae in laboratory microcosms. We used two experimental designs (additive and substitutive) that make different assumptions about how patches are initially colonized, and then tracked the development of community biomass from the point of initial colonization through a period of 6–12 generations of the focal species. We found that the effect of initial species richness on biomass production qualitatively shifted twice over the course of the experiment. The first shift occurred as species transitioned from density-independent to dependent phases of population growth. At this time, intraspecific competition caused monocultures to approach their respective carrying capacities more slowly than polycultures. As a consequence, species tended to over-yield for a brief time, generating a positive, but transient effect of diversity on community biomass. The second shift occurred as communities approached carrying capacity. At this time, strong interspecific interactions caused biomass to be dominated by the competitively superior species in polycultures. As this species had the lowest carrying capacity, a negative effect of diversity on biomass resulted in late succession. Although these two shifts produced dynamics that appeared complex, we show that the patterns can be fit to a simple Lotka-Volterra model of competition. Our results suggest that the effects of algal diversity on primary production change in a predictable sequence through successional time.

Key words: algal communities; biodiversity; productivity; species richness; succession.

INTRODUCTION

Over the past decade a leading challenge in ecology has been to understand the ecosystem-level consequences of species extinction (Tilman 1999, Chapin et al. 2000, Naeem 2002). A growing number of field and laboratory studies have manipulated the richness of species in a trophic group and found that diversity has a positive, but decelerating affect on the conversion of resources into biomass (reviewed by Schwartz et al. 2000, Schmid et al. 2001, Covich et al. 2004, Hooper et al. 2005, Srivastava and Vellend 2005, Balvanera et al. 2006, Cardinale et al. 2006a). But these effects often change in form and magnitude through time. For example, some studies have reported that effects of diversity grow stronger through time (Caldeira et al. 2001, Tilman et al. 2001, Jonsson 2006), others have reported they grow weaker (Cardinale and Palmer 2002, Bell et al. 2005, Cardinale et al. 2006b), and still others have reported transient positive effects of diversity that ultimately

become weak or non-significant as communities progress towards an equilibrium state (Fox 2004, Hooper and Dukes 2004). Despite clear evidence that biodiversity–ecosystem function relationships are temporally dynamic, there have been few attempts to generate a conceptual framework that might help interpret or predict these trends.

One framework that is potentially useful for understanding temporally dynamic effects of diversity is the successional patch mosaic. Successional dynamics in a patchy environment is one of the fundamental mechanisms of species coexistence (Pacala and Rees 1998, Amarasekare 2003, Leibold et al. 2004, Chase 2005), and occur when some fraction of patches in a landscape experience intermittent disturbance. After patches are colonized by initially low densities of organisms, recruiting populations undergo a period of density-independent growth and, given sufficient time between disturbances, they begin to experience intra- and inter-specific interactions that can ultimately limit community membership. Recent mathematical models suggest that species richness can have a qualitatively different effect on the production of biomass in patches at different stages of this successional sequence (Holt and Loreau 2001, Kinzig and Pacala 2001, Cardinale et al. 2004, Fox

Manuscript received 5 June 2006; revised 14 September 2006; accepted 18 September 2006. Corresponding Editor: N. J. Gotelli.

³ Corresponding author. E-mail: cardinale@lifesci.ucsb.edu

2004). For example, Cardinale et al. (2004) used Lotka-Volterra theory to argue that species richness can have no effect on community biomass during early stages of succession because species neither over- nor under-yield in polyculture during density-independent phases of growth. However, in later stages of succession when competition limits populations, several mechanisms can generate qualitatively similar effects of diversity on community biomass. Unfortunately, few experiments have tested this range of predictions. Instead, most have initialized species populations at high starting densities to ensure successful establishment, thus avoiding the density-independent phases of population growth (Symstad and Tilman 2001). And with few noteworthy exceptions, studies tend to be run for too short a time to achieve a late successional state.

Here we report the results of a laboratory study in which we used a simplified, but mechanistically tractable system of freshwater algae to explore how algal species richness affects the production of biomass from the point of initial colonization, through density-independent phases of population growth, into late stages of succession where a system is dominated by interspecific competition. We tested two hypotheses: First, we hypothesized that algal species richness has no impact on production of community biomass during early stages of succession when populations are in density independent phases of growth. If true, an important implication is that diversity–biomass relationships during early stages of succession depend solely on how a patch is initially colonized, and are not influenced by the traits of highly productive species. Second, we hypothesized that in late stages of succession when growth is reduced by intra- and interspecific interactions, aggregate biomass of a community is an increasing function of initial algal species richness. This could either be due to sampling effects where diversity increases the probability that a species with a high carrying capacity will be included in and ultimately dominate a community, or the partitioning of resources that cause a more diverse community to capture a greater fraction of available resources.

To test these hypotheses, we performed our experiment using two parallel designs that make different assumptions about how a patch is initially colonized. The first was the widely used substitutive design (also called the replacement-series) in which the initial number of recruits to a patch (individuals, seeds, biomass, etc.) is independent of the number of species. The second was the additive design in which the number of propagules of any given species is independent of initial richness. Running these designs together gives complementary information about the mechanisms operating to produce diversity effects over the course of succession—mechanisms that are not always visible in either design individually.

As we will show, our second hypothesis was not entirely supported. Although a positive effect of richness

on biomass developed during the intermediate phase of succession, this effect was ephemeral and transitioned into a negative effect of richness on biomass in late succession. This unexpected trend led us to fit a mathematical model to the data to investigate the mechanisms underlying the observed richness–biomass relationships. Collectively, our data and model show that differences in population growth rates and strong competitive asymmetries among species can alter the relationship between species diversity and community biomass from positive to negative over the course of succession. Although changes in the qualitative form of the richness–biomass relationship might suggest complicated dynamics, we show that they can, in fact, be intuitively predicted from the simple changes in species interactions that are captured by Lotka-Volterra equations.

METHODS

Focal species

Our experiment used three common Chlorophycean algal species: *Chlamydomonas debaryana* (Ch), *Scenedesmus quadricauda* (Sc), and *Selenastrum minutum* (Se). All three species have broad geographic distributions and are abundant in freshwater phytoplankton communities (Graham and Wilcox 2000). We chose these particular taxa for three reasons: (1) they differ in cell size and shape, which makes them easy to distinguish from one another; (2) they are available from commercial cultures (Culture Collection of Algae at the University of Texas at Austin); and (3) they all grow well under laboratory conditions using common growth media. Our cultures were grown in 1-L Erlenmeyer flasks filled with 500 mL of filtered COMBO growth media for two weeks prior to the start of the experiment (Kilham et al. 1998).

Experimental design

The experimental design was a factorial manipulation of two independent variables: initial algal species richness \times initial form of colonization. For initial richness we ran all species monocultures, each two-species polyculture, and the three-species polyculture. All seven species combinations were crossed with two forms of initial colonization, additive vs. substitutive. To standardize biomass, we first measured *in vivo* fluorescence of chlorophyll *a* in our stock cultures (Turner Aquafluor handheld fluorometer; Turner Designs, Sunnyvale, California, USA) and estimated algal biomass assuming chl *a* represents 1.5% of algal dry mass (Wetzel and Likens 2000). For the additive form of colonization, we held the biomass of each species constant across all levels of richness such that total initial dry biomass increased from 0.05 $\mu\text{g/L}$ in species monocultures to 0.10 and 0.15 $\mu\text{g/L}$ in the two- and three-species polycultures. In the substitutive design, we held the total biomass of the initial community constant at 0.05 $\mu\text{g/L}$ across all levels of richness. Thus, the biomass of each species was

cut in half and by one-third in the two- and three-species polycultures. Each experimental combination was replicated seven times. As the monocultures are the same for both forms of colonization, there were 11 rather than 14 treatment combinations (77 experimental units).

The experimental units were 140-mL mason jars filled with 110 mL of autoclaved COMBO growth media. Media was not replenished during the experiment as we specifically wanted to simulate a situation in which resource availability was fixed, potentially leading to strong competition among species. This might, for example, mimic conditions in a temperate lake where spring turnover generates a single pulse of nutrients before formation of an epilimnion, after which resources are depleted over a summer growing season. Jars were placed in randomized positions in a growth chamber fitted with six cool-light fluorescent bulbs. The chamber was set to 20°C and light was regulated on a 20 h : 4 h light : dark cycle. The experimental jars were loosely covered with plastic lids to prevent cross-contamination of algal species while still allowing gas exchange. Each jar was swirled by hand once per day to maintain cells in suspension.

Dependent variables

Jars were sampled on days 2, 5, 7, 9, 11, 15, 19, 23, and 33 of the experiment to determine the biomass of each species, as well as the total biomass in the polycultures. On each date we determined species population sizes by pipetting 2-mL samples from each jar, preserving samples in diluted Lugol's solution, and later counting cells on a hemacytometer under 200× magnification. To estimate the biomass of each population, we multiplied cell densities by the mean cell biovolume, determined by measuring the dimensions of cells for each species from photographs of the original stock cultures. Cellular dimensions were converted to biovolume (as in Hillebrand et al. 1999), and biovolume converted to biomass assuming a specific gravity of 1.0. It should be noted that this calculation of biomass (cell density × biovolume) differs from the method used to standardize initial colonization in the experimental units (in vivo fluorescence of chl *a*). While the two measures produce the same qualitative trends throughout the experiment, we have chosen to present estimates based on biovolume because these allow us to differentiate among the various species in polyculture. Thus, they also allow us to distinguish the growth of species when alone vs. when in polyculture.

Data analyses

To assess how the effect of species richness on biomass production changed through successional time, we used a repeated-measures ANOVA to model the log of algal biomass as a function of time (day of experiment), species richness, and the two-way interaction for each form of initial colonization (additive vs. substitutive). Algal biomass was weighted by (variance)⁻¹ to account for heterogeneity of variances across

levels of species richness. To help identify underlying mechanisms, we quantified the relative performance of species in poly- vs. monoculture using the proportional deviation in the observed biomass of species *i* in polyculture $O_{p,i}$ from its expected biomass $E_{p,i}$:

$$D_i = \frac{O_{p,i} - E_{p,i}}{E_{p,i}} \quad (1)$$

where

$$E_{p,i} = \frac{b_{p,i}(0)}{b_{m,i}(0)} \times O_{m,i}(t).$$

$E_{p,i}$ was calculated as the initial proportion of species *i* in poly- vs. monoculture, where $b_{p,i}(0)$ and $b_{m,i}(0)$ are the initial biomasses of species *i* in polyculture and monoculture, respectively, multiplied by the observed biomass in monoculture $O_{m,i}(t)$ at time *t*. A positive D_i is evidence of “over-yielding” in polyculture while a negative value indicates “under-yielding” (Hector 1998, Loreau 1998b). We then calculated the total proportional deviation of the three-species polycultures from expected values D_T as

$$D_T = \frac{\sum O_{p,i} - \sum E_{p,i}}{\sum E_{p,i}}. \quad (2)$$

Standard *t* tests were used to test for deviations in each metric from a null mean of zero.

RESULTS

General successional sequence

The mean population doubling times for the species monocultures were 6.06 ± 1.24 , 2.73 ± 0.06 , and 3.10 ± 0.06 d for *Chlamydomonas*, *Scenedesmus*, and *Selenastrum*, respectively (mean ± SE). This indicates that the 33-d time span of our experiment was sufficient to encompass between 6 and 12 generations of the focal species. The duration of our experiment was also sufficient to capture a number of key aspects of a successional sequence (Fig. 1). Initially, species populations grew exponentially with growth rates that were independent of the presence of other taxa. This is evident from Fig. 1, which shows a linear increase (on a log scale) in population density through approximately day 5 for all three species. The impacts of both intra- and interspecific competition on population growth rates became evident between days 9 and 15 of the experiment, depending on the species. This is clear from the decelerating growth curves of the monocultures and from the reduction in species population sizes in polycultures relative to their monoculture densities (Fig. 1). Although the population size of each species continued to increase through the final day of the study, growth rates between day 23 and 33 were near zero (0.09, 0.08, and 0.06 for *Chlamydomonas*, *Scenedesmus*, and *Selenastrum*, respectively, in monoculture), and densities in the additive three-species polycultures were reduced by 44–84% relative to monoculture values.

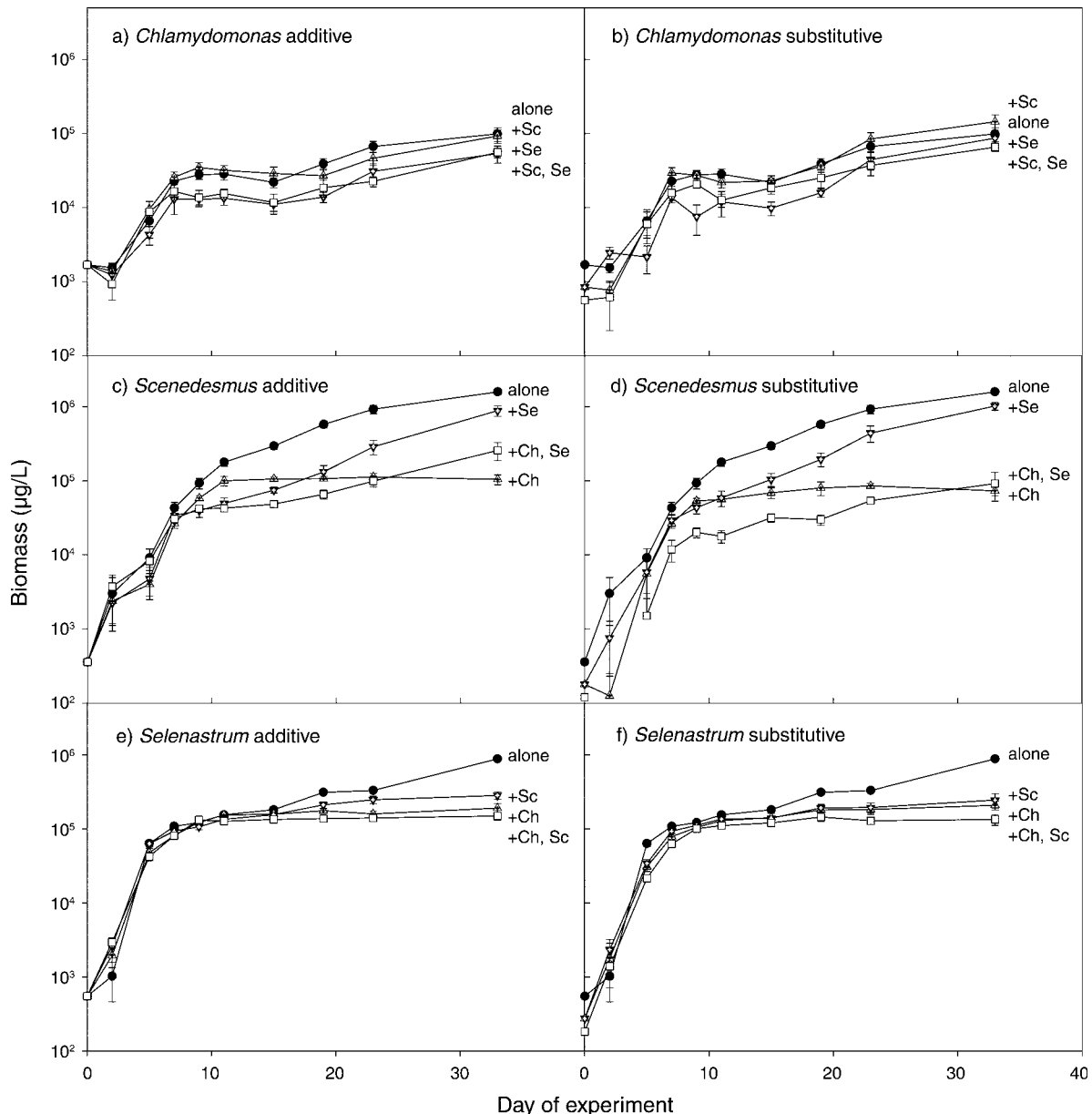


FIG. 1. Biomass of each of three focal species through time. (a, c, e) Standing biomass of each species monoculture, two-species polyculture, and three-species polyculture for the additive design. (b, d, f) The same data from the substitutive design. Each data point is the mean \pm SE of $N = 7$ replicates. Abbreviations: Sc, *Scenedesmus*; Se, *Selenastrum*; Ch, *Chlamydomonas*.

Thus, our study spanned the early stages of succession where species were in density-independent phases of growth, and later stages of succession where intra- and inter-specific interactions strongly limited population growth. Hereafter, we assume from Fig. 1 that the duration of our study was sufficient for the systems to approach equilibrium.

Effects of species richness on biomass production

Fig. 2a, b shows how community biomass changed through time as a function of algal species richness for

the additive and substitutive forms of colonization. Fig. 2c, d illustrates the same data in a complementary way, showing the slope \pm 95% confidence intervals for the relationship between species richness and community biomass on different dates of the study (i.e., the solution for fixed effects from the richness \times date interaction in a repeated-measures ANOVA). Note that between days 0 and 5 of the study when all species were experiencing exponential growth (representing one or two generations), the initial richness–biomass relationships remained qualitatively unchanged from their initial

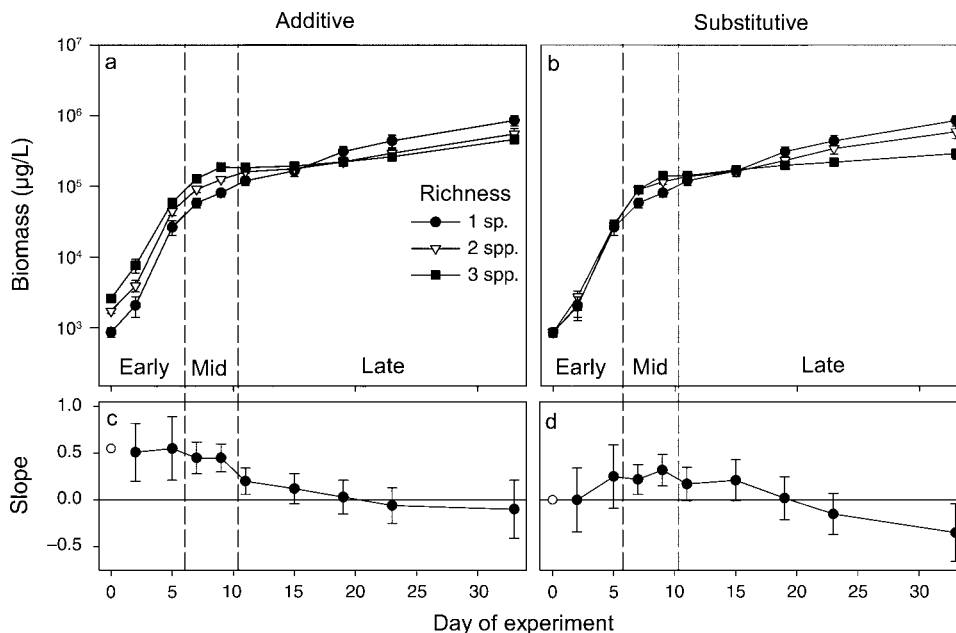


FIG. 2. Effects of algal species richness on production of community biomass. (a, b) Biomass (mean \pm SE) of species monocultures, as well as two- and three-species polycultures on each sampling date. Data are shown separately for the (a) additive and (b) substitutive forms of initial colonization. (c, d) The slope (mean with 95% CI) relating community biomass to algal species richness on each date (from a repeated-measures ANOVA). Vertical dashed lines are used to illustrate the different time periods referred to in the *Results* (roughly early, mid, and late succession); open circles are starting conditions.

conditions (compare open to solid circles in Fig. 2c, d). Between days 5 and 11 as population growth began to slow (representing 2–4 generations), the diversity–biomass relationship remained unaltered for the additive form of colonization, but a positive relationship emerged for the first time in the substitutive form of colonization. After day 11, biomass became an increasingly negative function of diversity in both designs, ultimately becoming significantly so in the substitutive design by day 33 (representing 6–12 generations).

To understand why the effect of diversity on community biomass changed in form and magnitude over the course of the experiment, it is informative to examine three representative dates in greater detail. On day 5 when species were still in density-independent phases of growth, the biomass of each species in the three-species polyculture was no different than what would be expected from the biomass they achieved in monoculture (Fig. 3a, d). Thus, species neither over- nor under-yielded, confirming that growth from day 0 to 5 was not influenced by the presence of other species. As a consequence, biomass increased proportionally across all levels of species richness such that the original relationships between richness and community biomass were simply maintained. It is interesting to note that this was true despite the fact that species differed substantially in their productivity. Between days 0 and 5, *Selenastrum* had an intrinsic rate of increase of 0.94 ± 0.06 , while growth rates of *Chlamydomonas* and *Scenedesmus* were far lower (0.18 ± 0.21 and $0.64 \pm$

0.14 , respectively). As a result, *Selenastrum* attained higher biomass in monoculture than the other two species (Fig. 3b, c), and dominated the polyculture (71–74% of total biomass).

On day 9, a positive relationship between species richness and community biomass emerged for the first time in the substitutive form of colonization (compare Fig. 3c–g). This positive relationship developed because two of the three species, *Chlamydomonas* and *Selenastrum*, began to “over-yield” in polyculture (Fig. 3h), meaning they produced more biomass than expected from their monoculture value. But this positive effect of diversity on community biomass was transient. By day 23, the slope relating community biomass to algal richness was significantly negative for the substitutive design (Fig. 3j, k), which occurred because of under-yielding by *Scenedesmus* (Fig. 3i). The average biomass of the three-species polyculture was roughly half that of the monocultures (462 vs. 854 mg/L) for additive design, but this was not sufficient to produce a significant negative slope (Fig. 3j).

A mathematical model

Results of the experiment generally support our first hypothesis that the relationship between algal diversity and community biomass during density-independent phases of growth is determined by the form of initial colonization and not influenced by the presence/absence of highly productive species. Indeed, we found that the initial richness–biomass relationships were maintained

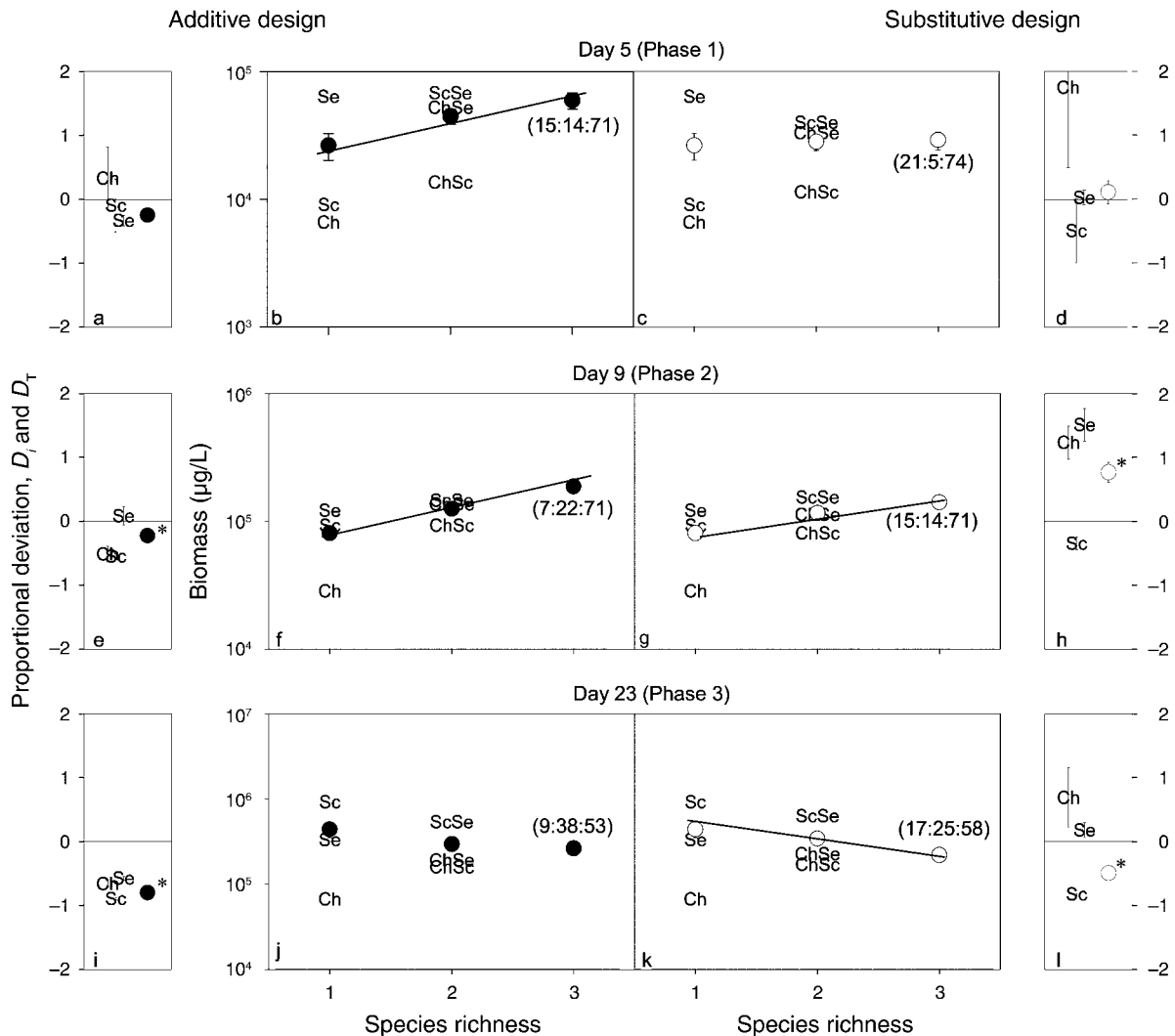


FIG. 3. The effect of algal species richness on community biomass for three dates chosen to represent early, mid, and late stages of succession. Center plots show the biomass (circles, mean \pm SE) for each treatment of species richness, as well as the biomass of each species–species combination (Ch, *Chlamydomonas*; Sc, *Scenedesmus*; Se, *Selenastrum*). Statistically significant relationships ($P < 0.05$) are noted by regression lines, and the percentage of biomass attributable to each species in polyculture is given in parentheses (Ch:Sc:Se). Plots on either side of the main graphs give the proportional deviation \pm SE of species biomasses from their initial values in monoculture. D_i 's (species labels) and D_T 's (circles) >0 indicate that a species "over-yielded," meaning it produced more biomass in polyculture for the interval from $t=0$ to t than it produced in monoculture. By contrast, values <0 indicate under-yielding. An asterisk denotes D_T values that are significantly different from zero ($P < 0.05$). Trends for the additive form of colonization are given in the left-hand panels, while trends for the substitutive form are given in the right-hand panels.

throughout density-independent growth despite the fact that a species with a high growth rate came to dominate the biomass of polycultures. However, our second prediction that the biomass of a community in late succession increases as a function of initial algal species richness was not supported. Instead, we found that diversity had a positive, but transient effect on community biomass that ultimately became negative or nonsignificant. To further explore why this might be so, we fit our data to a modified version of the Lotka-Volterra model of competition (see Appendix), and calculated the maximum likelihood parameter estimates

for species population growth rates and competitive abilities (see Appendix: Table A1). The maximum-likelihood parameter estimates suggest two conditions were true of the species used in our experiment. First, the species exhibited a trade-off between their growth rates and competitive abilities, with slow-growing species (e.g., *Chlamydomonas*) being competitively superior to fast-growing species (e.g., *Selenastrum*). Second, the late-successional competitive dominant (*Chlamydomonas*) had the lowest carrying capacity.

To determine if these two conditions are sufficient to reproduce the time trends observed in our experiment

(i.e., those in Figs. 2 and 3), we simulated them using a simple two-species Lotka-Volterra model:

$$b_i(t + 1) = b_i(t) \exp \left\{ r_i \left[1 - \frac{b_i(t)}{K_i} - \frac{\alpha_{ij} b_j(t)}{K_i} \right] \right\} \quad (3)$$

where populations grow with intrinsic growth rate r_i to carrying capacity K_i , and coefficients α_{ij} give the competitive effect of species j on species i . Note that the terms describing the impacts of intraspecific and interspecific competition, respectively, have been separated for clarity:

$$\frac{b_i(t)}{K_i} \text{ and } \frac{\alpha_{ij} b_j(t)}{K_i}.$$

To mimic the characteristics of our experimental species pool, we let $r_1 > r_2$ and $\alpha_{12} > \alpha_{21}$ (condition 1), and $K_1 > K_2$ (condition 2).

Fig. 4a, b illustrates the temporal dynamics for the average monoculture and the two-species polyculture (shown only for the substitutive form of initial colonization). Note that species richness has no effect on aggregate biomass during early succession (generations 1–10), a positive effect in intermediate succession (generations 10–20), and a negative effect in late succession (generations >20). The mechanisms that underlie these trends involve simple changes in the relative impacts of intra- and interspecific interactions in limiting population growth. In early periods of growth when $b_i(t) \ll K_i$, intra- and interspecific competition are both negligible, and Eq. 3 approximates exponential growth for both species. As a result, species achieve the same biomass whether growing alone or in the two-species system, and the biomass of the average monoculture increases by the same proportion per time step as does total biomass in the polyculture. Because a substitutive design assumes that initial diversity and biomass are independent, this independence is retained throughout all phases of density-independent growth. It is important to note that this is true despite the fact that species 1 is far more productive than species 2 ($r_1 > r_2$) and dominates biomass in polyculture (Fig. 4e). One thing this suggests is that a simple correlation between species richness and the probability that a productive species will come to dominate a community is not, by itself, sufficient to generate any relationship between diversity and biomass (Fig. 4b).

During intermediate succession (generations 10–20), a positive relationship between species richness and community biomass emerges as over-yielding by species 1 outweighs slight under-yielding by species 2 (Fig. 4a–c). Both trends occur because the high growth rate of species 1 allows it to achieve densities that not only affect species 2, but that begin to limit the growth of conspecifics. Intraspecific competition is key to understanding this phase because the substitutive form of colonization assumes that initial biomass of colonists is constant across all levels of richness. In turn, the initial

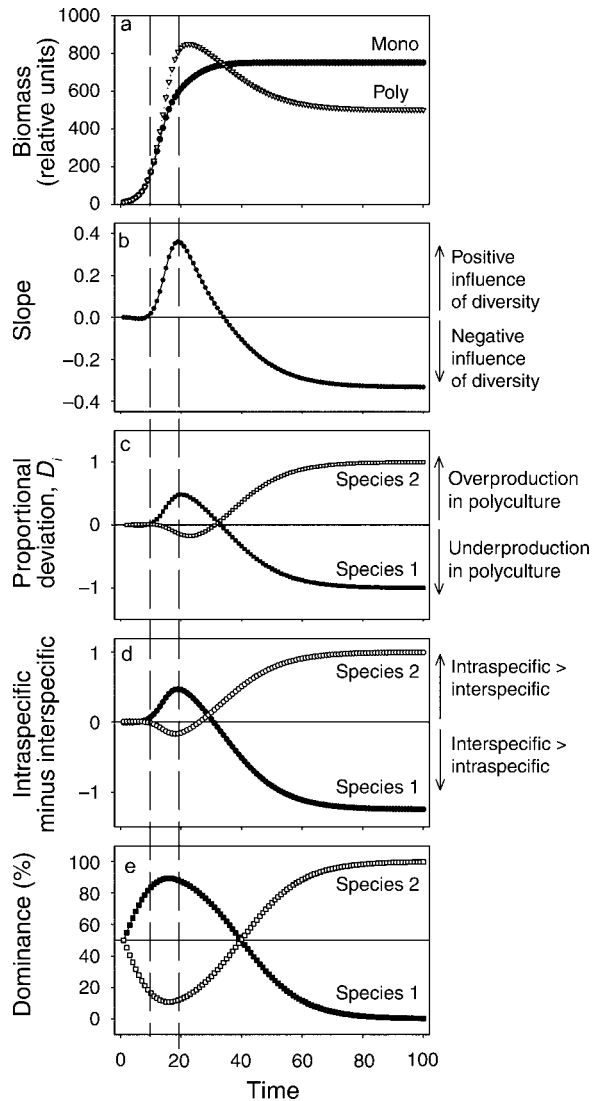


FIG. 4. Numerical simulations of Eq. 3 showing how species richness affects biomass production at different stages of succession for the substitutive form of colonization. (a) Change through time of standing biomass of the mean monoculture and the two-species polyculture. (b) Slope of the relationship between richness and biomass on a proportional scale (compare to Fig. 2d). (c) Proportional deviation D_i of biomass of each species in polyculture from values expected due to their growth in monoculture over the interval from $t = 0$ to t . (d) Net effect of intra- and interspecific competition on species in polyculture. A positive value indicates a stronger influence of intraspecific competition on population growth rates. (e) Percentage of total biomass (dominance) represented by each species in the polyculture. Dashed vertical lines separate what we refer to in the text as early, mid, and late succession. Parameter values for species 1 were chosen to depict a fast-growing early-successional species that is competitively inferior ($r_1 = 0.4$, $K_1 = 1000$, $\alpha_{12} = 2.5$), and values for species 2 depict a late-successional dominant with a lower carrying capacity ($r_2 = 0.2$, $K_2 = 500$, $\alpha_{21} = 0.25$).

density of conspecifics in a polyculture is one-half that of the monoculture, and reduced intraspecific competition in the polyculture causes species 1 to over-yield. But the resulting positive effect of diversity on biomass is transient and ultimately becomes a negative effect in late succession as the slower growing, but competitively superior species 2 comes to dominate the system (generations >20, Fig. 4e). This occurs simply because species 2 has the lower carrying capacity, causing biomass of the polyculture to be lower than that of the average monoculture.

DISCUSSION

We have shown that the effects of species diversity on the production of community biomass can change in both form and magnitude over the course of succession. Using a simple experimental system of freshwater algae, we documented two qualitative shifts in the relationship between species richness and biomass production. By fitting a model to the data, we showed that these shifts can be explained by simple Lotka-Volterra dynamics in which species have different carrying capacities and there are asymmetries in interspecific competition. These differences alter the relative impacts of intra- and interspecific competition on population growth rates that ultimately control the richness–biomass relationship. Results of our study support a growing body of evidence that the effect of species diversity on biomass production in any given community is temporally dynamic (Caldeira et al. 2001, Tilman et al. 2001, Cardinale and Palmer 2002, Fox 2004, Hooper and Dukes 2004, Bell et al. 2005, Cardinale et al. 2006b, Jonsson 2006), and they begin to outline a conceptual framework that can help interpret these trends.

Our results are consistent with the predictions of Cardinale et al. (2004), who used Lotka-Volterra theory to argue that species diversity should not affect the production of biomass for any community in density-independent phases of growth. This is because species achieve the same biomass whether growing alone or in polyculture, and diversity can have no effect on biomass when species neither over- nor under-yield. There are at least two important corollaries of this prediction, the first of which relates to how we interpret the most widely cited mechanism for diversity effects. The “sampling effect” (also called the selection-probability effect; Huston 1997, Loreau 1998b) has been invoked by numerous authors to explain how species diversity affects community biomass (see reviews by Schwartz et al. [2000], Schmid et al. [2001], Covich et al. [2004], Hooper et al. [2005], Srivastava and Vellend [2005], Balvanera et al. [2006], and Cardinale et al. [2006a]). Even so, there is considerable variation in, and perhaps some confusion about how this mechanism actually operates. Some have argued that the sampling effect is nothing more than a statistical inevitability that occurs when increasing diversity increases the probability that the most productive species will be included in, and ultimately dominate the biomass of, a community

(Huston 1997, Wardle 1999, Leps et al. 2001, Wojdak 2005). Results of our experiment do not support this interpretation. As evidence, take the results of our study during density-independent phases of population growth where (1) increasing diversity was correlated to the probability that the most productive species—*Selenastrum*, which had the highest rates of population growth—would be included in a given community, and (2) high growth rate allowed *Selenastrum* to dominate the biomass of polycultures. Yet, the probability of including this fast growing species in a diverse community was not sufficient to generate any effect of diversity, which is clear from the fact that richness and biomass remained independent in the substitutive design (Fig. 3c). Our model confirmed that the simple presence/absence of a highly productive species in a community does not, by itself, generate any relationship between species diversity and biomass production, which is true even when the traits of that species (e.g., high growth rate) allow it to dominate polyculture biomass. Rather, an effect of diversity can only manifest after species become sufficiently dense that local interactions alter the performance of one or more species in polyculture relative to their performance in monoculture. This finding is more consistent with alternative interpretations of the sampling effect that argue a correlation between species richness and the probability of a given species being included in a community must be coupled with distinct biological interactions (e.g., competition, facilitation) that cause the relative yields of species (polyculture:monoculture performance) to deviate from unity (Loreau 1998b, Cardinale et al. 2004, Ives et al. 2005).

If species richness cannot affect the biomass of a community while populations are in density-independent phases of growth, a second implication is that any relationship observed between species richness and biomass during early stages of succession should be solely determined by how a patch is initially colonized. This implication is potentially important because biodiversity experiments to date have, more often than not, assumed that initial colonization of a patch conforms to the substitutive design where initial biomass is independent of the richness of the regional species pool (see reviews by Schwartz et al. 2000, Schmid et al. 2002, Hooper et al. 2005). Balvanera et al. (2006) recently advocated this design as being superior to others, arguing that experiments which assume other forms of initial colonization are flawed. Aside from the fact that this argument ignores a long history of controversy over the merits of substitutive designs and their counterparts (Connolly 1988, Jolliffe 2000, Goldberg and Scheiner 2001), and aside from the fact that no single design is adequate for detecting the various mechanisms by which diversity might influence a process (Sih et al. 1998, Ives et al. 2005, Cardinale et al. 2006b), there is little evidence to suggest that a substitutive experimental design best mimics nature. In fact, some theory argues that it might not mimic nature much at all.

For example, meta-community theory has shown that when niche differences allow species to coexist in a "region" of patches, each species makes a unique contribution to biomass of the meta-community (Bond and Chase 2002, Mouquet et al. 2002, Loreau et al. 2003, Cardinale et al. 2004). If the number of propagules produced by a meta-community increases with regional biomass, the total number of propagules available to colonize any given patch is likely to be a monotonically increasing function of the number of species in the region. Uneven distributions in species abundance will likely prevent the relationship between regional richness and propagule abundance from being linear (i.e., purely additive), but few niche-based models allow the two to be completely independent (i.e., substitutive). Given this, we would argue that rather than advocating the superiority of one experimental design over another, it would be more useful for future research to detail how regional species richness influences propagule abundances. Only then will we better understand how richness and biomass are related in early successional communities (Cardinale et al. 2004).

One of the most interesting results of our experiment occurred in the substitutive experimental design where, during mid-succession, species richness had a positive, but transient effect on community biomass (Fig. 2d). This effect occurred because two of three species began to over-yield in polyculture (compare Fig. 3g–h). Importantly, those same species did not over-yield in the additive design during this period (Fig. 3e–f), which suggests that some feature of these two experimental designs influences the magnitude over-yielding. The difference in the two designs lies in how the initial density of conspecifics was standardized across levels of richness. For the additive design, the initial density of conspecifics was held constant, but for the substitutive design, conspecific density was decreased as richness increased. The decreasing density of conspecifics in the substitutive design appears to be key to interpreting the mechanism operating to produce a diversity effect in mid-succession. Using a model, we showed that lower conspecific density leads to reduced intraspecific competition in a polyculture for the substitutive design, and that this can generate over-yielding and a positive effect of diversity on biomass in mid-stages of succession. In our model, over-yielding results from differences in growth rates r_i among species. Fox (2004) has shown that similar trends are produced by varying K_i 's. In fact, all one needs to produce transient over-yielding is a time lag in the rate at which two species approach their respective K_i 's, which can be accomplished by modifying r , K , or the two in combination. Among other things, these results suggest that some caution is warranted when interpreting metrics of relative yield. Although over-yielding is commonly taken as evidence of niche partitioning among species and/or positive interspecific interactions, our model shows that reduced intraspecific interactions induced by the substitutive design can

produce over-yielding in a system of competitors that have no potential for resource partitioning (i.e., the system collapses to one species at equilibrium).

Whereas changes in the impacts of intraspecific interactions can explain the emergence of a positive relationship between species richness and community biomass in intermediate succession, the trends observed in late succession are best explained by interspecific interactions. The coupling of our empirical data with our model suggests that the species used in our experiment exhibited a trade-off between growth rates and competitive ability. As a result, the slowest growing species, *Chlamydomonas*, was also the late-successional dominant. Importantly, *Chlamydomonas* had the lowest carrying capacity of all three species such that its dominance in polyculture led to lower biomass than the average monoculture. It is unclear to us why *Chlamydomonas* had the lowest carrying capacity, but it may be related to the fact this species is flagellated and expends a substantial fraction of its ATP on mobility (Mitchell et al. 2005). Regardless, this condition produced what has been called a negative "sampling effect" (Loreau 1998a, Duker 2001), where increasing diversity increases the probability of dominance by a species with low productivity. A recent meta-analysis of biodiversity–ecosystem functioning studies suggests that negative sampling effects of diversity may not be widespread (Cardinale et al. 2006a). Nevertheless, the negative sampling effect documented in our study was somewhat fortuitous because the transitive positive effects of diversity in mid-succession may not have been detectable had the dominant species had the highest carrying capacity.

As is true for all "model" systems, the inferences from our study are inherently limited. Not only were the laboratory microcosms small and homogeneous, we specifically chose to work with a simplified system of three closely related species to maximize our ability to identify and interpret underlying mechanisms. Thus, it should be clear that our study in no way mimics the heterogeneous nature of real systems of freshwater algae that often contain hundreds of species. Still, when the results of our experiment are interpreted alongside those of other recent empirical and theoretical studies, the evidence collectively argues that any impact of species diversity on biomass production is likely to depend on the successional age of a community. To the extent this conclusion holds broadly true, the ecological consequences of species loss will be defined by the frequencies of disturbance and the rates of recolonization of disturbed sites that jointly define the successional state of a community.

ACKNOWLEDGMENTS

We thank Linda Graham for her expert advice on the algal species, Stanley Dodson for providing laboratory space and materials, and Adrienne Tandberg for assisting with sample processing. We also thank Jonathan Levine, Janneke Hille Ris Lambers, and two anonymous reviewers for comments on earlier versions of this manuscript. Funding was provided by

NSF grants to A. R. Ives (DEB 0108300) and B. J. Cardinale (DEB 0614428).

LITERATURE CITED

- Amarasekare, P. 2003. Competitive coexistence in spatially structured environments: a synthesis. *Ecology Letters* 6: 1109–1122.
- Balvanera, P., A. B. Pfisterer, N. Buchmann, J.-S. He, T. Nakashizuka, D. Raffaelli, and B. Schmid. 2006. Quantifying the evidence for biodiversity effects on ecosystem functioning and services. *Ecology Letters* 9:1146–1156.
- Bell, T., J. A. Newman, B. W. Silverman, S. L. Turner, and A. K. Lilley. 2005. The contribution of species richness and composition to bacterial services. *Nature* 436:1157–1160.
- Bond, E. M., and J. M. Chase. 2002. Biodiversity and ecosystem functioning at local and regional spatial scales. *Ecology Letters* 5:467–470.
- Caldeira, M. C., R. J. Ryel, J. H. Lawton, and J. S. Pereira. 2001. Mechanisms of positive biodiversity–production relationships: insights provided by delta C-13 analysis in experimental Mediterranean grassland plots. *Ecology Letters* 4:439–443.
- Cardinale, B. J., A. R. Ives, and P. Inchausti. 2004. Effects of species diversity on the primary productivity of ecosystems: Extending our spatial and temporal scales of inference. *Oikos* 104:437–450.
- Cardinale, B. J., and M. A. Palmer. 2002. Disturbance moderates biodiversity–ecosystem function relationships: experimental evidence from caddisflies in stream mesocosms. *Ecology* 83:1915–1927.
- Cardinale, B. J., D. S. Srivastava, J. E. Duffy, J. P. Wright, A. L. Downing, M. Sankaran, and C. Jouseau. 2006a. Effects of biodiversity on the functioning of trophic groups and ecosystems. *Nature* 443:989–992.
- Cardinale, B. J., J. J. Weis, A. E. Forbes, K. J. Tilmon, and A. R. Ives. 2006b. Biodiversity as both a cause and consequence of resource availability: a study of reciprocal causality in a predator–prey system. *Journal of Animal Ecology* 75:497–505.
- Chapin, F. S. I., E. S. Zavaleta, V. T. Eviners, R. L. Naylor, P. M. Vitousek, H. L. Reynolds, D. U. Hooper, S. Lavorel, O. E. Sala, S. E. Hobbie, M. C. Mack, and S. Diaz. 2000. Consequences of changing biodiversity. *Nature* 405:234–242.
- Chase, J. M. 2005. Towards a really unified theory for metacommunities. *Functional Ecology* 19:182–186.
- Connolly, J. 1988. What is wrong with the replacement series. *Trends in Ecology and Evolution* 3:24–26.
- Covich, A. P., M. C. Austen, F. Barlocher, E. Chauvet, B. J. Cardinale, C. L. Biles, P. Inchausti, O. Dangles, M. Solan, M. O. Gessner, B. Statzner, and B. Moss. 2004. The role of biodiversity in the functioning of freshwater and marine benthic ecosystems. *BioScience* 54:767–775.
- Dukes, J. S. 2001. Productivity and complementarity in grassland microcosms of varying diversity. *Oikos* 94:468–480.
- Fox, J. W. 2004. Effects of algal and herbivore diversity on the partitioning of biomass within and among trophic levels. *Ecology* 85:549–559.
- Goldberg, D. E., and S. M. Scheiner. 2001. ANOVA and ANCOVA: field competition experiments. Pages 69–93 in S. M. Scheiner and J. Gurevitch, editors. *Design and analysis of ecological experiments*. Oxford University Press, Oxford, UK.
- Graham, L. E., and L. W. Wilcox. 2000. *Algae*. Prentice Hall, Upper Saddle River, New Jersey, USA.
- Hector, A. 1998. The effect of diversity on productivity: detecting the role of species complementarity. *Oikos* 82: 597–599.
- Hillebrand, H., C. D. Durselen, D. Kirschtel, U. Pollinger, and T. Zohary. 1999. Biovolume calculation for pelagic and benthic microalgae. *Journal of Phycology* 35:403–424.
- Holt, R. D., and M. Loreau. 2001. Biodiversity and ecosystem functioning: the role of trophic interactions and the importance of system openness. Pages 246–262 in A. P. Kinzig, S. W. Pacala, and D. Tilman, editors. *The functional consequences of biodiversity: empirical progress and theoretical extensions*. Princeton University Press, Princeton, New Jersey, USA.
- Hooper, D. U., et al. 2005. Effects of biodiversity on ecosystem functioning: a consensus of current knowledge. *Ecological Monographs* 75:3–35.
- Hooper, D. U., and J. S. Dukes. 2004. Overyielding among plant functional groups in a long-term experiment. *Ecology Letters* 7:95–105.
- Huston, M. A. 1997. Hidden treatments in ecological experiments: re-evaluating the ecosystem function of biodiversity. *Oecologia* 110:449–460.
- Ives, A. R., B. J. Cardinale, and W. E. Snyder. 2005. A synthesis of subdisciplines: predator–prey interactions, and biodiversity and ecosystem functioning. *Ecology Letters* 8: 102–116.
- Jolliffe, P. A. 2000. The replacement series. *Journal of Ecology* 88:371–385.
- Jonsson, M. 2006. Species richness effects on ecosystem functioning increase with time in an ephemeral resource system. *Acta Oecologica-International Journal of Ecology* 29:72–77.
- Kilham, S. S., D. A. Kreeger, S. G. Lynn, C. E. Goulden, and L. Herrera. 1998. COMBO: a defined freshwater culture medium for algae and zooplankton. *Hydrobiologia* 377: 147–159.
- Kinzig, A. P., and S. Pacala. 2001. Successional biodiversity and ecosystem functioning. Pages 175–212 in A. P. Kinzig, S. W. Pacala, and D. Tilman, editors. *The functional consequences of biodiversity: empirical progress and theoretical extensions*. Princeton University Press, Princeton, New Jersey, USA.
- Leibold, M. A., M. Holyoak, N. Mouquet, P. Amarasekare, J. M. Chase, M. F. Hoopes, R. D. Holt, J. B. Shurin, R. Law, D. Tilman, M. Loreau, and A. Gonzalez. 2004. The metacommunity concept: a framework for multi-scale community ecology. *Ecology Letters* 7:601–613.
- Leps, J., et al. 2001. Separating the chance effect from other diversity effects in the functioning of plant communities. *Oikos* 92:123–134.
- Loreau, M. 1998a. Biodiversity and ecosystem functioning: a mechanistic model. *Proceedings of the National Academy of Sciences (USA)* 95:5632–5636.
- Loreau, M. 1998b. Separating sampling and other effects in biodiversity experiments. *Oikos* 82:600–602.
- Loreau, M., N. Mouquet, and A. Gonzalez. 2003. Biodiversity as spatial insurance in heterogeneous landscapes. *Proceedings of the National Academy of Sciences (USA)* 100: 12765–12770.
- Mitchell, B. F., L. B. Pedersen, M. Feely, J. L. Rosenbaum, and D. R. Mitchell. 2005. ATP production in *Chlamydomonas reinhardtii* flagella by glycolytic enzymes. *Molecular Biology of the Cell* 16:4509–4518.
- Mouquet, N., J. L. Moore, and M. Loreau. 2002. Plant species richness and community productivity: why the mechanism that promotes coexistence matters. *Ecology Letters* 5:56–65.
- Naeem, S. 2002. Ecosystem consequences of biodiversity loss: the evolution of a paradigm. *Ecology* 83:1537–1552.
- Pacala, S. W., and M. Rees. 1998. Models suggesting field experiments to test two hypotheses explaining successional diversity. *American Naturalist* 152:729–737.
- Schmid, B., A. Hector, M. A. Huston, P. Inchausti, I. Nijs, P. W. Leadley, and D. Tilman. 2002. The design and analysis of biodiversity experiments. Pages xii, 294 in M. Loreau, S. Naeem, and P. Inchausti, editors. *Biodiversity and ecosystem functioning: synthesis and perspectives*. Oxford University Press, Oxford, UK.

- Schmid, B., J. Joshi, and F. Schlapfer. 2001. Empirical evidence for biodiversity–ecosystem functioning relationships. Pages 120–150 in A. P. Kinzig, S. W. Pacala, and D. Tilman, editors. *The functional consequences of biodiversity: empirical progress and theoretical extensions*. Monographs in population biology 33. Princeton University Press, Princeton, New Jersey, USA.
- Schwartz, M. W., C. A. Brigham, J. D. Hoeksema, K. G. Lyons, M. H. Mills, and P. J. van Mantgem. 2000. Linking biodiversity to ecosystem function: implications for conservation ecology. *Oecologia* 122:297–305.
- Sih, A., G. Englund, and D. Wooster. 1998. Emergent impacts of multiple predators on prey. *Trends in Ecology and Evolution* 13:350–355.
- Srivastava, D. S., and M. Vellend. 2005. Biodiversity–ecosystem function research: Is it relevant to conservation? *Annual Review of Ecology, Evolution, and Systematics* 36:267–294.
- Symstad, A. J., and D. Tilman. 2001. Diversity loss, recruitment limitation, and ecosystem functioning: lessons learned from a removal experiment. *Oikos* 92:424–435.
- Tilman, D. 1999. The ecological consequences of changes in biodiversity: a search for general principles. *Ecology* 80:1455–1474.
- Tilman, D., P. B. Reich, J. Knops, D. Wedin, T. Mielke, and C. Lehman. 2001. Diversity and productivity in a long-term grassland experiment. *Science* 294:843–845.
- Wardle, D. A. 1999. Is “sampling effect” a problem for experiments investigating biodiversity–ecosystem function relationships? *Oikos* 87:403–410.
- Wetzel, R. G., and G. E. Likens. 2000. *Limnological analyses*. Third edition. Springer, New York, New York, USA.
- Wojdak, J. M. 2005. Relative strength of top-down, bottom-up, and consumer species richness effects on pond ecosystems. *Ecological Monographs* 75:489–504.

APPENDIX

Two-staged Lotka-Volterra model for the additive and substitutive experiments (*Ecological Archives* E088-058-A1).