EFFECTS OF ALGAL AND HERBIVORE DIVERSITY ON THE PARTITIONING OF BIOMASS WITHIN AND AMONG TROPHIC LEVELS

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Abstract. Factorial manipulations of biodiversity on different trophic levels can reveal the mechanisms that govern how biomass is partitioned within and among trophic levels. Here I report results of a laboratory microcosm experiment crossing eight algal compositions (seven monocultures and a polyculture of all seven species) with five ciliate herbivore compositions (no herbivores, three monocultures, and a polyculture of all three species). Microcosms allowed comparison of short-, medium-, and long-term (dozens of generations) results. Niche differentiation allowed competing algae to coexist in the absence of herbivores. Surprisingly, algal polycultures produced more biomass than the most productive monocultures only in the medium term. A temporally variable relationship between the productivity of diverse and depauperate algal assemblages might reflect changes in competitive mechanisms over time, and/or transient dynamics, and contrasts with the predictions of simple competition models.

Herbivores had little effect on total algal biomass or algal composition, in part because of the lack of a strong trade-off between algal competitive ability and edibility. Increasing algal diversity significantly reduced the biomass of one herbivore, apparently because competition from inedible algae reduced the productivity of edible algae. A negative effect of inedible algae on herbivore biomass accords with simple food web theory and indicates that increasing diversity on one trophic level can have negative consequences for other trophic levels.

Herbivores varied little in their abilities to graze different algae. Total algal biomass, algal composition, and total herbivore biomass were independent of herbivore diversity and composition, and algal composition had only minor effects the outcome of competition among herbivores. However, the outcome of herbivore competition was predictably asymmetrical, indicating that herbivores varied in other ways, such as ability to consume alternative food sources (bacteria). Further factorial manipulations of diversity and trophic structure are needed to improve understanding of the likely consequences of species loss in food webs. These experiments should last as long as possible, since the relationship between biodiversity and ecosystem properties may depend as much on when the relationship is measured as on species identity and the degree of niche differentiation among species.

Key words: algae; biodiversity; ecosystem function; food webs; microcosms; protists; trophic structure.

INTRODUCTION

Two of the most fundamental properties of an ecological community are the number of species it contains and the total biomass of those species. Many theories treat questions about diversity as questions about the rules that govern how the total biomass of a community or assemblage is partitioned among species (e.g., Tilman 1982, Holt et al. 1994). These theories emphasize the role of competition in determining how biomass is partitioned among species within trophic levels (Tilman 1982), and the role of trophic structure in determining how biomass is partitioned among trophic levels (Abrams 1993). Diversity and biomass are typically linked through the assumption that both total biomass, and the rules that govern how that biomass is partitioned, reflect the availability of limiting resources (Holt et al. 1994).

Other studies treat diversity as an independent rather than a dependent variable, and emphasize the importance of species diversity in determining the total biomass and productivity of a system (Loreau et al. 2001, Hector and Hooper 2002). In this view, resource availability may determine the potential biomass a system can support, but the extent to which that potential is realized will depend strongly on species diversity and composition. In particular, plants are a dominant component of biomass in most ecosystems, directly controlling primary productivity and nutrient retention. Experimentally increasing plant diversity often increases total plant biomass and productivity (Bell 1991, Naeem et al. 1996, Hector et al. 1999, Tilman et al. 2001, Fridley 2002; but see Bell 1990, Hooper and Vitousek 1997).

However, whether increasing plant diversity increases total plant biomass may depend on the composition
of the community in which plants are embedded (Mulder et al. 1999, Naem et al. 2000). Changes in plant biomass due to the removal or altered composition of higher trophic levels often exceed the changes in plant biomass produced by experimental manipulations of plant diversity (Hector et al. 1999, Shurin et al. 2002). A complete understanding of the relationship between diversity and biomass, and therefore of the consequences of biodiversity loss, will require an understanding of how species interact within food webs (Duffy 2002).

Here, I describe a microcosm experiment in which I manipulated algal and herbivore (ciliate protist) diversities in a factorial fashion. By conducting factorial manipulations, I can separate the effects of changes in diversity on each trophic level. The only previous factorial manipulation of diversity on multiple trophic levels focused on interactions between algae and bacterial decomposers (Naem et al. 2000). Protist microcosms trade off realism to allow collection of long-term population dynamic data from replicated communities of known trophic structure under controlled conditions (Lawler and Morin 1993, Petchey et al. 2002). Long-term data from mechanistically tractable model systems like protist microcosms complement the short term data typically provided by field experiments on larger organisms (Petchey et al. 2002, but see Tilman et al. 2001). Long-term data are likely to be particularly important in studies of biodiversity and trophic structure, since feeding relations connect species via chains of indirect effects that may not be revealed in short experiments.

The experiment asked the following questions: (1) What is the relationship between algal diversity and total algal biomass in the absence of herbivores? (2) What are the joint effects of algal and herbivore diversity on total algal biomass and composition? (3) What are the joint effects of algal and herbivore diversity on total herbivore biomass and composition? (4) To what extent do algal responses and effects reflect niche differentiation (e.g., differences in resource use and/or vulnerability to herbivores), and to what extent do they reflect selection effects (e.g., eventual dominance of initially-diverse communities by more productive algal species)? (5) To what extent do herbivore responses and effects reflect niche differentiation (e.g., preferences for different algae) vs. selection effects?

Materials and Methods

Microcosm assembly, maintenance, and sampling

I selected seven algal species (Asterionella formosa, Chlamydomonas reinhardtii mating type +, Chlorella vulgaris, Cryptomonas ovata, Haematococcus lacustris, Nitzchia sp., and Synechococcus leopoliensis) for the experiment. I chose algae from a wide taxonomic, morphological, and physiological range to ensure some possibility of niche differentiation, and to facilitate identification. I selected three species of algivorous (“herbivorous”) ciliate protists (Euplotes patella, Paramecium caudatum, and Paramecium aurelia). Feeding trials indicated that all three herbivores readily consume and grow on two of the algae (C. reinhardtii and C. ovata). Two other algae (C. vulgaris and S. leopoliensis) are an appropriate size for consumption by herbivores, and probably are consumed at least occasionally, but do not support herbivore growth on their own.

The other three algae are too large for the herbivores to consume. For brevity, I will refer to C. reinhardtii and C. ovata as “edible” algae, and the other five species as “inedible” algae. Feeding trials also indicated that P. caudatum can grow on bacteria alone. The other two herbivores cannot grow on a bacteria-only diet, although other strains of P. aurelia can do so (personal observation). I obtained all organisms from Scienco (Manchester, UK). The algae and herbivores commonly co-occur in nature (J. Fox, personal observation; Fenchel 1987), but were not chosen to mimic any particular natural system.

Microcosm assembly, maintenance, and sampling followed standard protocols (Lawler and Morin 1993). Microcosms were 240-ml glass bottles containing 100 mL of growth medium (0.56 g Protozoan Pellets [Carolina Biological Supply, Burlington, North Carolina, USA], plus 0.05 g sodium metasilicate per liter of distilled water) and two wheat seeds. These materials were steam autoclaved before use. Bottles were loosely capped to prevent contamination while allowing gas exchange. I inoculated the medium with the bacterium Bacillus subtilis 24 h before adding algae, to provide an initially uniform decomposer fauna (introduction of algae and protists unavoidably introduced other bacterial taxa).

I randomly assigned treatments to bottles. I added algae as 0.3 mL inocula drawn from high-density stock monocultures. Initial densities of each species in monoculture and polyculture were thus ~0.3% of monoculture carrying capacity. I added five to seven individuals of each herbivore species via micropipet after 30 d of algal growth. Herbivores grew on C. reinhardtii before the experiment. Adding herbivores individually via micropipet avoided introducing any C. reinhardtii along with the herbivores.

I placed the bottles in a randomized rectangular array on a laboratory bench in a climate-controlled room (20°C, 16:8 light:dark cycle). Light came from two sources, the fluorescent room lights, and a 60W incandescent bulb ~50 cm above the center of the array. I randomly repositioned the bottles daily. I renewed the nutrient medium once per week by briefly agitating the bottles to homogenize their contents, and then replacing 10 mL of medium from each bottle with fresh, sterile medium.

I sampled bottles approximately once per week, briefly agitating the bottles before sampling. All organisms were counted live to facilitate identification. Sampling of herbivores followed the procedure of Lawler and
Morin (1993). I counted smaller algae (C. reinhardtii, C. vulgaris, S. leopoliensis) using a haemacytometer, counting as large a volume as necessary (up to a maximum of 0.0018 mL) to count at least 20 cells. I sampled larger algae using a Sedwick-Rafter cell, examining as large a volume as necessary (up to a maximum of 0.3 mL) to count at least 20 cells. On the rare occasions when a smaller algal species was entirely absent from a haemacytometer sample (<1% of samples), I confirmed its absence by inspecting the larger Sedwick-Rafter sample. Analyses of results suggested that this sampling effort was adequate for the highly controlled system used here. I converted all counts to number per milliliter, and converted all densities to biovolumes (an index of biomass) using the mean volume of 10–20 cells. I estimated cell volumes from standard geometrical shapes (Wetzel and Likens 1999).

**Experimental design, predictions, and statistical tests**

The experiment was a full factorial design crossing eight algal compositions (monocultures of each species, and a polyculture of all seven species) and five herbivore compositions (no herbivores, monocultures of each species, and a polyculture of all three species). There were two replicates/treatment for a total of 80 bottles. Logistical constraints required the experiment to run in two temporal blocks, the second starting 7 d after completion of the first. It was not always possible to sample both blocks the same number of days after algal addition. The species used here have generation times of ~24 h, so sampling points only a few days apart may not be comparable. I therefore provide time series plots of species’ biovolumes, but restrict statistical analyses to samples taken 7, 30, or 60 d after algal addition. These three sampling points are the only ones at which I sampled both blocks. Below I describe the ecological hypotheses and the corresponding statistical tests. I conducted all statistical analyses using R 1.7.0 for Windows (Ihaka and Gentleman 1996), except power analyses (G*Power 2.0 for Windows; Erdfelder et al. 1996).

Factorial manipulations of species composition on two trophic levels raise challenging statistical issues. Standard multivariate approaches (e.g., MANOVA) are inapplicable, since not all species are added to all experimental units. I employed separate univariate analyses, correcting for multiple comparisons where necessary using the sequential Bonferroni procedure (Rice 1989). For certain hypotheses, I was able to use planned orthogonal contrasts rather than post-hoc tests that would require correction for multiple comparisons (Crawley 2002).

**Algae.—**I tested the effect of algal composition on total algal biovolume after 7 and 30 d (i.e., before herbivore addition), and after 60 d in bottles to which no herbivores were added. Analyzing the data for each sampling date separately reveals differences between short-term (7 d), medium-term (30 d), and long-term (60 d) results. A repeated measures analysis would have been complicated by the fact that only herbivore-free bottles were sampled on all three sampling points. I modeled the data using generalized linear models (GLMs), with F tests for hypothesis testing. I fit the models using quasilikelihood with a log link function (Crawley 2002). Inspection of residuals verified their approximate normality and homoscedasticity. Each GLM included a block effect, but no interactions between block and other effects (Crawley 2002). I used sequential Bonferroni correction (Rice 1989) to ensure that the overall probability of detecting a significant effect of algal composition on total algal biovolume in the absence of herbivores on any of the three sampling dates was ≤0.05. Data on algal biovolume after 60 d from one herbivore-free monoculture of S. leopoliensis were inadvertently lost and therefore excluded from the analyses.

When GLMs revealed a significant effect of algal composition on total algal biovolume, I used the fitted model coefficients to conduct planned orthogonal contrasts between total biovolume in polyculture vs. each of the monocultures (Crawley 2002). Significantly greater biovolume in polyculture than in any monoculture (transgressive overyielding, Trenbath 1974) provides unambiguous evidence for an important role for niche differentiation and/or positive interactions in determining total algal biovolume. Theory predicts that transgressive overyielding is likely to develop only after many generations of algal growth (Pacala and Tilman 2002). However, even in the absence of transgressive overyielding, long-term algal coexistence in polyculture indicates that algae occupy different niches, so that niche differentiation contributes to the relationship between algal diversity and total algal biovolume.

Even in the absence of niche differentiation, theory predicts that algal polycultures should outperform monocultures on average because all polycultures, but only some monocultures, contain the most productive alga (a “sampling” or “selection” effect; Tilman et al. 1997, Loreau and Hector 2001). Simple exploitative competition theory predicts that the algal species with the highest monoculture biovolume should attain the same biovolume in polyculture, while other species should attain greatly reduced biovolume in polyculture compared to monoculture (Tilman et al. 1997). I used a GLM to test the effects of algal species identity, culture type (mono- or polyculture), and their interaction, on algal biovolume, handling block effect, and model fitting as described above. I conducted separate GLMs on days 7 and 30 and judged statistical significance after sequential Bonferroni correction. I used GLM coefficients to conduct planned orthogonal contrasts comparing the biovolume of each algal species in mono- vs. polyculture. Note that the performance of individual algal species in mono- vs. polyculture is independent of the relationship between total algal bio-
volume in mono- vs. polyculture. The analysis of individual species is not redundant with the analysis of total biovolume described above, even though both analyses use some of the same data. On day 60, I used a GLM to test the effects of algal species identity, culture type, herbivore presence/absence, and their interactions, on algal biovolume. Planned orthogonal contrasts compared the biovolume of each species in the presence vs. absence of herbivores, in mono- vs. polyculture in the absence of herbivores, and in mono- vs. polyculture in the presence of herbivores. This analysis reveals whether the selection effect favors different algae in the presence vs. absence of herbivores, and whether herbivores release inedible algae from competition by suppressing edible algae. The planned contrasts can also reveal the effects of processes besides exploitative competition. For instance, algae that attain significantly higher biovolume in polyculture benefit from the presence of other algae.

Even in the absence of the monodominance predicted by simple exploitative competition models, a positive association between monoculture and polyculture biovolume would be broadly consistent with a selection effect driven by exploitative competition (Fox 2002). I tested for an association between the mean biovolume of each algal species in monoculture and polyculture using correlation coefficients. I calculated correlations for bottles without herbivores (after 7, 30, and 60 d), and for bottles with herbivores (after 60 d), and applied sequential Bonferroni correction to determine the statistical significance of these four correlation coefficients.

I tested for effects of algal species richness, herbivore species richness, and their interaction, on total algal biovolume after 60 d using a GLM, treating both algal and herbivore richness as discrete factors. This analysis is not redundant with those described above, since herbivores might alter total algal biovolume without detectable effects on the biovolumes of individual algal species, and since the effects of herbivores might vary with herbivore diversity. Increasing herbivore diversity should cause increasingly large reductions in algal biovolume, because of a selection effect for more effective herbivores, and/or because of complementary patterns of resource use among herbivores (Norberg 2000, Sommer et al. 2001). The interactive effects of algal and herbivore diversity on total algal biovolume are challenging to predict, as no published theory is available. Increasing algal diversity might reduce the effects of herbivores on algal biovolume, because in algal polycultures inedible algae should compensate for herbivory on edible algae (Grover 1994). However, the extent to which inedible algae will compensate for grazing on edible algae will depend on the intensity of competition among algae. If algae differ greatly in resource use, competition will be weak, and inedible algae will hardly compensate for grazing. Alternatively, increasing algal diversity might increase the effects of herbivores, by promoting herbivore coexistence and increasing the scope for differential resource use by herbivores. If there is a strong trade-off between algal competitive ability and herbivore resistance, herbivory should prevent edible algae from competitively excluding inedible algae (Grover 1994). The results of the GLM for effects of algal species identity, culture type (mono- or polyculture), and herbivore presence/absence can identify compensatory shifts in algal composition between edible and inedible algae.

I examined all possible sequences of model terms in analyses testing for effects of algal and herbivore richness, and herbivore presence/absence, as these analyses are unbalanced (Schmid et al. 2002). The order of model terms never affected the results.

Herbivores—I tested the effects of algal composition (C. reinhardtii monoculture, C. ovata monoculture, or polyculture), herbivore composition (E. patella, P. aurelia, P. caudatum, or a polyculture of all three), and their interaction, on total herbivore biovolume after 60 d using a GLM. Herbivores always went extinct from monocultures of inedible algae, so these treatments were excluded from analyses of herbivore biovolume. Theory predicts that algal polycultures will reduce herbivore biovolume because inedible algae compete with edible algae, thereby reducing the productivity of the herbivores’ food supply (Grover 1994). However, this effect may be small if algae differ in their resource use and therefore compete only weakly. Increasing herbivore diversity should increase mean herbivore biovolume because of a selection effect for more effective herbivores (Holt and Loreau 2002), and/or because herbivores may have different, complementary patterns of resource use (Norberg 2000, Sommer et al. 2001). Interactive effects of algal and herbivore composition might arise if, e.g., only algal polycultures allow niche complementarity among herbivores.

I tested for selection effects among herbivores with a GLM analyzing the effects of herbivore species identity, culture type (mono- or polyculture of herbivores), algal composition (C. reinhardtii monoculture, C. ovata monoculture, or polyculture), and their interactions, on herbivore biovolume. Planned orthogonal contrasts compared the performance of each herbivore species in mono- vs. polyculture, and compared herbivore performance on different algae. Comparisons of herbivore performance on different algae were conducted as separate comparisons for each combination of herbivore species and culture type.

Responses of total herbivore biovolume to changes in algal composition, and effects of herbivores on algae, will depend on how herbivores vary in grazing effectiveness. The most effective grazer might be expected to dominate herbivore polycultures. Competition theory suggests that a useful way to quantify grazing effectiveness is to measure $R^*$, the level to which a consumer is able to reduce a resource in the absence
of other species (Tilman 1982). To identify variation in grazing effectiveness among herbivores, I tested the effect of herbivore composition, algal composition (C. reinhardtii or C. ovata in monoculture), and their interaction, on total algal biovolume after 60 d using a GLM.

**RESULTS**

**Algae**

Inspection of population dynamic time series indicated that rates of change in algal biovolume generally slowed over the course of the experiment, generally approaching a quasi-steady state after 60 d (Fig. 1a, b).

Mean total algal biovolume varied significantly among algal compositions after 7, 30, and 60 d in the absence of herbivores (day 7, \( F_{6,71} = 136.0, P < 0.001 \); day 30, \( F_{6,71} = 36.1, P < 0.001 \); day 60, \( F_{6,6} = 23.3, P < 0.001 \); Fig. 2). Block effects were nonsignificant (\( P > 0.2 \) in this and all other analyses). Total biovolume in each of the three most productive monocultures significantly exceeded that in polyculture on day 7 (Fig. 2). Total biovolume in polyculture significantly exceeded that in any monoculture on day 30 (Fig. 2). Total biovolume in each of the two most productive monocultures significantly exceeded that in polyculture on day 60 (Fig. 2).

Mean algal biovolume in monoculture was significantly correlated with mean biovolume in polyculture on day 7 (Fig. 3a). The correlations on days 30 and 60 in the absence of herbivores were marginally significant after sequential Bonferroni correction (\( P = 0.023 \) and \( P = 0.032 \), respectively) (Fig. 3b, c).

After 7 d, algal biovolume varied significantly among algal species (\( F_{6,125} = 268.4, P < 0.001 \); Fig. 3a). Day 7 biovolumes were significantly lower in polyculture than monoculture (\( F_{6,125} = 781.9, P < 0.001 \); Fig. 3a), although the effect of mono- vs. polyculture growth varied significantly among algal species (interaction term \( F_{6,125} = 6.5, P < 0.001 \); Fig. 3a). Planned contrasts (\( F \) tests) indicated that every species except A. formosa attained significantly lower biovolume in polyculture than monoculture (\( P < 0.045 \) for all species except A. formosa \( P = 0.068 \); Fig. 3a).

After 30 d, algal biovolume varied significantly among algal species (\( F_{6,125} = 56.5, P < 0.001 \); Fig. 3b). The effect of mono- vs. polyculture growth varied significantly among algal species (\( F_{6,125} = 7.5, P < 0.001 \); Fig. 3b), with a marginally significant general trend toward reduced biovolume in polyculture (\( F_{1,125} = 3.18, P = 0.073 \); Fig. 3b). Planned contrasts indicated that C. reinhardtii attained significantly lower biovolume in polyculture than monoculture (\( P = 0.001 \)), while C. vulgaris attained significantly higher biovolume in polyculture (\( P = 0.002 \); Fig. 3b).

After 60 d, biovolume varied significantly across algal species (\( F_{6,130} = 41.4, P < 0.001 \)). Polyculture growth significantly reduced algal biovolume compared to monoculture (\( F_{1,129} = 183.7, P < 0.001 \); Fig. 3c, d), but the effect of mono- vs. polyculture growth varied among algal species (\( F_{6,116} = 20.4, P < 0.001 \); Fig. 3c, d). Algae varied significantly in their response to herbivores (algal species × herbivore presence–absence interaction, \( F_{6,125} = 3.5, P = 0.003 \); Fig. 3c, d). Planned contrasts indicated that this effect was due to significantly reduced biovolume of C. reinhardtii in the presence of herbivores (\( P = 0.031 \)). Planned contrasts also indicated that C. reinhardtii and C. vulgaris attained significantly lower biovolume in polyculture than monoculture in the absence of herbivores (\( P = 0.001 \); Fig. 3c, d).

**Fig. 1.** Time series of total algal and herbivore biovolumes (originally measured in units of mm\(^3\)/mL). Each time series shows data from one representative replicate. (a) Total algal biovolumes in herbivore-free replicates. Algal monocultures are abbreviated with the first two letters of their genera, except for C. reinhardtii (Ca) and C. vulgaris (Co); "7" denotes a polyculture of all seven algae. (b) Total algal biovolumes in herbivore polycultures. Only algal compositions containing edible algae are shown. (c) Total herbivore biovolumes from the same replicates shown in panel (b).
Fig. 2. Contrasts (model coefficients from GLMs, +1 se) comparing mean total biovolume in each algal monoculture to mean total biovolume in polyculture, in the absence of herbivores. Abbreviations of algal monocultures are as in Fig. 1. Positive contrasts indicate greater algal biovolume in monoculture. Key: open bars, day 7; hatched bars, day 30; stippled bars, day 60. Asterisks indicate significant contrasts ($P < 0.05$); filled circles indicate marginally significant contrasts ($0.05 < P < 0.06$).

0.021 and $P = 0.001$, respectively). In the presence of herbivores three algae ($A. formosa$, $C. vulgaris$, and $S. leopoliensis$) attained significantly lower biovolume in polyculture than monoculture ($P = 0.008$, $P < 0.001$, and $P = 0.029$, respectively), while $H. lacustris$ attained significantly higher biovolume in polyculture ($P = 0.002$).

After 60 d, algal richness, herbivore richness, and their interaction had no significant effect on total algal biovolume (all $P > 0.2$; Fig. 4). Failure to detect significant effects may reflect lack of statistical power. To examine this possibility I repeated the analysis as a two-way ANOVA on log-transformed total algal biovolume, and conducted a post-hoc power analysis on the ANOVA results. The power of the ANOVA should provide a good approximation to the power of the GLM as long as the ANOVA residuals are reasonably well behaved. The probability of detecting effects of algal and herbivore richness the size of those observed was $\sim 0.8$. The probability of detecting effects $2.5 \times$ the size of those observed was $\sim 0.5$.

One large inedible alga, $H. lacustris$, attained extremely high biovolume in two polycultures with herbivores (Fig. 3d). Repeating all analyses of the 60-d algal data without these two polycultures did not materially affect the results.

**Herbivores**

Most herbivore populations approached a quasi-steady state by the end of the experiment (Fig. 1c). Total herbivore biovolume on day 60 varied significantly with algal composition ($F_{2,11} = 10.1$, $P = 0.003$; Fig. 5), but not with herbivore composition or the interaction of algal and herbivore composition (all $P > 0.17$; Fig. 5). Monocultures of $C. ovata$ supported the fewest herbivores, while monocultures of $C. reinhardtii$ supported the most. Failure to detect significant effects of herbivore composition, or a significant interaction term, may reflect a lack of power. The probability of detecting effects the size of those observed was $\sim 0.5$.

Analysis of the performance of individual herbivore species indicated significant differences among herbivores ($F_{2,17} = 4.12$, $P = 0.035$; Fig. 5). Since the analysis of total herbivore biovolume did not detect significant differences among herbivore monocultures, these differences must largely reflect differences in polyculture performance. Planned contrasts indicated that both $E. patella$ and $P. aurelia$ attained lower biovolume in polyculture than monoculture, although the significance of both contrasts was marginal ($P = 0.088$ and 0.085, respectively; Fig. 5). These two contrasts also explain the significant effect of culture type (mono- or polyculture) in the GLM ($F_{1,17} = 17.0$, $P < 0.001$; Fig. 5). A GLM also indicated that herbivore species’ biovolumes varied significantly with algal composition ($F_{2,17} = 10.5$, $P = 0.001$; Fig. 5). This variation in individual herbivore species’ biovolumes is similar to the effects of algal composition on total herbivore bio-
FIG. 3. Mean algal biovolume in monoculture and polyculture. Algal abbreviations are as in Fig. 1. Dashed lines give 1:1 relationships. Asterisks indicate points that deviate significantly ($P<0.05$) from the 1:1 line as indicated by planned orthogonal contrasts, and statistically significant correlation coefficients. (a) Algal biovolume after 7 d (no herbivores). (b) Algal biovolume after 30 d (no herbivores). (c) Algal biovolume after 60 d (no herbivores). (d) Algal biovolume after 60 d (+herbivores). The filled symbol Ha, in (d) indicates mean biovolume of *H. lacustris* calculated without two outliers (both polycultures) in which *H. lacustris* attained extremely high biovolume.

FIG. 4. Effects of algal diversity, herbivore diversity, and their interaction on total algal biovolume after 60 d. Bars show fitted values from GLM (+1 SE), controlling for block. Key: open bars, no herbivores; hatched bars, one herbivore species; filled bars, three herbivore species.

**Discussion**

**Algae**

Many experimental biodiversity manipulations last only one to three generations of the study organisms (e.g., Hooper and Vitousek 1997, Hector et al. 1999, Fridley 2002). Both theory (Pacala and Tilman 2002) and experiments (Tilman et al. 2001) suggest that short- and long-term effects of biodiversity on ecosystem properties may differ. My results support this suggestion. In the absence of herbivores, algal polycultures exhibited transgressive overyielding in the medium term (after 30 d), but not in the short (7 d) or long term (60 d).

In the short term, most algae attained significantly lower biovolume in polyculture than in monoculture, indicating competition. Algae attaining high biovolume in monoculture dominated the polycultures, consistent with exploitative competition (Tilman 1982, Tilman et al. 1997). In the medium term, algae attaining high monoculture biovolumes continued to dominate competition in polyculture, again consistent with exploitative competition. *C. vulgaris* attained the highest bio-

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Herbivores did not vary significantly in grazing effectiveness (all $P > 0.81$; Fig. 6). The probability of detecting effects as small as those observed was $0.4–0.5$.

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Planned comparisons testing the effects of algal composition on different combinations of herbivore species and culture type, indicated that monocultures of *E. patella* (i.e., *E. patella* without other herbivores), and *P. aurelia* in herbivore polycultures, produced significantly higher biovolume when growing on monocultures of *C. reinhardtii* than when growing on algal polycultures ($P = 0.010$ and $0.033$, respectively; Fig. 5). A similar trend for monocultures of *P. caudatum* was only marginally significant ($P = 0.072$; Fig. 5).

Herbivores did not vary significantly in grazing effectiveness (all $P > 0.81$; Fig. 6). The probability of detecting effects as small as those observed was $0.4–0.5$. 
Fig. 5. Effects of algal composition, herbivore composition, and their interaction on total herbivore biovolume after 60 d. Bars show fitted values from GLM (+1 SE), controlling for block. Algal compositions are C. reinhardtii monocultures (Ca), C. ovata monocultures (Cr), and all seven species (7). Herbivore compositions are E. patella (Eu; open bars), P. aurelia (Pa; hatched bars), P. caudatum (Pc; filled bars), and all three species (3; stacked bars illustrating proportional contribution of each herbivore species to total herbivore biovolume).

Fig. 6. Effect of herbivore composition on the monoculture biovolume of each edible algal species. Bars show fitted values from GLM (+1 SE), controlling for block. Key: open bars, C. reinhardtii monocultures; hatched bars, C. ovata monocultures. Herbivore compositions are E. patella (Eu), P. aurelia (Pa), P. caudatum (Pc), and all three species together (3).

volume in polyculture, and is known to be highly competitive for both nutrients and light (Grover 1991, Huisman et al. 1999).

However, lack of medium-term competitive exclusion after ~30 generations of algal growth suggests the algae were not all competing for the same resource. Even the most productive species in monoculture (C. reinhardtii) exhibited reduced biovolume in polyculture, also consistent with the hypothesis that algae differed in their resource use (Tilman et al. 1997). If algae all competed for the same resource, the dominant competitor eventually would be unaffected by competition from other algae (Tilman et al. 1997). Although algae attaining high biovolume in monoculture tended to dominate polycultures in the medium term, polyculture biovolume was not strongly determined by a selection effect for any one alga. Three algae each produced between 10% and 54% of the mean polyculture biovolume after 30 d. The medium-term results suggest that variation in algal resource use allowed both coexistence and transgressive overyielding in the medium term.

In the long term, transgressive overyielding in the absence of herbivores disappeared because the most productive algal monocultures increased in biovolume throughout the experiment, while polycultures did not. Lack of a significant correlation between long-term monoculture and polyculture biovolume in the absence of herbivores may indicate that exploitative competition was not the only mechanism of interaction among algae over the second half of the experiment. Possibly, chemically mediated interference competition became important as the bottles aged. Bell (1990) found reduced growth of C. reinhardtii in filtered media preconditioned by previous algal growth. Growth inhibition increased in media subjected to more lengthy preconditioning, suggesting that growth-inhibiting compounds accumulated over time (Bell 1990). C. vulgaris, which dominated the polycultures, is known to inhibit the growth of some of the other algae used in this experiment (Inderjit 1994). Any growth inhibition that did occur was apparently more intense among than within species, since monocultures of several algae increased in biovolume throughout the experiment.

Interference competition could generate priority effects. This might explain why two algal polycultures with herbivores developed extremely high biovolume of H. lacustris, although herbivory could also generate priority effects (Grover 1994). Repeating the experiment with a higher rate of medium replacement could test whether accumulation of growth-inhibiting compounds contributed to the results. Growth inhibition may affect algal composition in natural communities as well, although its importance is unclear (Inderjit 1994). Autolysis and/or viral lysis of algal cells, followed by recycling of nutrients from decomposing cells, might also have affected long-term algal composition.

Any interference competition or other mechanisms generally did not lead to strong selection effects. Four algae each contributed between 8% and 42% of the mean polyculture biovolume in the absence of herbivores after 60 d, indicating that selection effects for particular species remained weak. Whatever the long-term mechanisms of competition, coexistence of several species for ~60 generations indicates niche differentiation among the algae, although this differentiation did not translate into long-term transgressive overyielding.
Long-term loss of transgressive overyielding might also reflect the transient dynamics of the system, rather than (or in addition to) a shift in competitive mechanisms over time. Pacala and Tilman (2002) used simple Lotka-Volterra models to argue that plant biodiversity experiments will exhibit transgressive overyielding, if at all, only in the long term. In the short term, total polyculture biovolume should approximately equal the monoculture biovolume of the species with the fastest exponential growth rate. A grassland experiment conducted by Tilman et al. (2001) supports these predictions, but my results do not. In the short term, the most productive algal monocultures significantly outperformed the polycultures, indicating that polyculture biovolume did not simply reflect that of the fastest-growing species. However, my short-term data represent approximately seven generations of algal growth, probably sufficient time for competition to become important. Simulations of simple Lotka-Volterra competition models indicate that many combinations of parameter values and initial conditions can produce medium-term transgressive overyielding that disappears in the long term (e.g., Fig. 7). Possibly, an even longer experiment might have revealed further shifts in the results. However, increasingly slow rates of change in algal and herbivore biovolumes over the course of the experiment argue against this possibility.

**Herbivores**

Coexistence of algae and lack of a strong selection effect in the absence of herbivores likely limited the scope for herbivores to produce large shifts in algal composition. Herbivores strongly reduced the biovolume of only one edible algal species (C. reinhardtii), and only in C. reinhardtii monocultures. While C. reinhardtii was one of the most productive algae in both monoculture and polyculture, it did not exclude the other algae, and was reduced in biovolume by competition from them. Lack of a strongly dominant competitor indicates the absence of a strong trade-off between algal competitive ability and herbivore resistance. Herbivory would not be expected to cause major shifts in algal composition without such a trade-off. Mulder et al. (1999) found modest effects of insect herbivores on the relationship between plant diversity and total plant biomass, a similar result to mine. Experiments in systems where producers exhibited a strong competitive ability/herbivore resistance trade-off likely would reveal larger effects of herbivores than observed here.

Herbivores did not vary in grazing effectiveness as measured by $R^*$ values, despite differences in body size, feeding morphology, and use of space (Fenchel 1987; personal observation). Although failure to detect significant differences may well reflect lack of statistical power, the observed differences in $R^*$ values may well be too small to translate into large differences among herbivores in their biovolume production or effects on algae. Working in a similar system, Fox (2002) found that $R^*$ values reliably predicted competitive outcomes only when one species had an $R^*$ value $<\sim50$–60% of that of its nearest competitor. The effects of smaller differences in mean grazing effectiveness were apparently obscured by other factors such as intraspecific variation in grazing ability (Clark et al. 2003). Large, consistent differences among herbivores in grazing effectiveness likely would have been detected.

Consistent with this relative lack of differentiation, total herbivore biovolume, total algal biovolume, and algal composition did not vary significantly with herbivore composition, and algal composition did not significantly affect the outcome of competition among herbivores. Sommer et al. (2001) also reported that assemblages of similar herbivores (either cladocerans or copepods) had little effect on total algal biomass. While a more highly replicated experiment likely would have detected significant effects of herbivores on total algal biovolume, the observed (nonsignificant) effects seem small compared to the large effects of consumers on their resources in other protist microcosm experiments (e.g., Lawler and Morin 1993, Naeem and Li 1998, Fox 2002).

When growing without other herbivores, E. patella (and, to a lesser extent, P. caudatum) attained greatly reduced biovolume when grown in algal polycultures, compared to monocultures of C. reinhardtii. C. reinhardtii was the only edible alga present at appreciable biovolume in algal polycultures, and competition from inedible algae reduced C. reinhardtii biovolume in the absence of herbivores. Reduced E. patella biovolume in algal polycultures compared to C. reinhardtii monocultures therefore indicates that competition from inedible algae reduced the biovolume of E. patella that C. reinhardtii could support. Simple models of algal-herbivore dynamics predict that competition between
edible and inedible algae will reduce herbivore biomass (Grover 1994), but few experiments demonstrate this effect (Steiner 2001). Reduced *E. patella* biovolume in algal polycultures indicates that increasing diversity on one trophic level can have negative as well as positive consequences for other trophic levels.

Surprisingly, competition between edible and inedible algae had no significant effect on biovolume of *P. aurelia*, which, when grown without other herbivores, attained similar densities in algal polycultures and *C. reinhardtii* monocultures. Consumption of bacteria and/or less edible algae may explain the lack of effect of inedible algae on *P. aurelia* in algal polycultures. Many species of micro- and mesoplankton (including the keystone herbivore *Daphnia* spp.) consume both bacteria and eukaryotic algae (Jürgens 1994). Bacteria may affect the outcome of herbivore–alga interactions in natural systems.

Interestingly, *P. caudatum* failed to persist in monocultures of inedible algae, even though this strain of *P. caudatum* attains densities >100 individuals/mL when growing on bacteria alone (J. Fox, personal observation). The reason for this failure to persist is unclear. Possibly, competition from inedible algae reduced bacterial density. However, this seems unlikely, as Protozoan Pellet medium has a high carbon:nutrient ratio, which should favor bacteria in exploitative competition with algae (Rothaupt 1992, Hulot et al. 2001). Using the same medium, Hulot et al. (2001) found that two species of algae (*C. vulgaris* and *Scenedesmus* sp.) each had no affect on bacterial density. Alternatively, inedible algae might release compounds that inhibit ciliate growth (Hulot et al. 2001). However, *P. caudatum* attained similar densities in algal polycultures and monocultures of edible *C. reinhardtii*, arguing against the hypothesis that inedible algae inhibit ciliate growth.

**Conclusions and future directions**

This experiment shows that short-term effects of biodiversity on ecosystem properties may not reflect long-term effects. Further, theory suggests that the effects of changes in producer diversity likely will depend on consumer diversity (Grover 1994, Holt et al. 1994, Duffy 2002). Understanding the implications of loss of biodiversity (as opposed to loss of plant diversity) will require long-term manipulations of diversity within multiple trophic groups, crossed with more traditional manipulations of the number/composition of groups (e.g., removal of entire trophic levels). Although such factorial experiments require many treatments, this problem may not be as serious as has been argued (Naeem 2002) because biologically infeasible trophic structures (e.g., predators with no prey) can be ignored. A combination of theory, observations, and factorial experiments (including those conducted in model systems) should help ecologists develop an understanding of the consequences of species loss in a multtrophic context.

**Acknowledgments**

Thanks to Marcel Holyoak, Charles Godfray, Jim Grover, Claire de Mazancourt, Owen Petchey, and an anonymous referee for helpful discussion and comments, and to Tim Benton for statistical advice.

**Literature Cited**


