

EFFECTS OF ALGAL RICHNESS ON COMMUNITY BIOMASS AND STABILITY
DEPEND ON HERBIVORY: AN AQUATIC MICROCOSM EXPERIMENT

by

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Abstract

Hundreds of studies exploring how changing biodiversity alters ecosystem functioning have led to a consensus that higher diversity tends to both increase and stabilize community biomass through time. However, the majority of this work has focused on a single trophic level, even though trophic interactions also influence community biomass and stability. The relatively few studies investigating the effects of changing diversity on trophic interactions such as herbivory have produced mixed results; whether such effects are important for community biomass and stability is unclear. We performed an experiment using freshwater laboratory microcosms to test for effects of algal diversity (one or four species) on community biomass and temporal variability in the presence of two different herbivores (cladocerans *Ceriodaphnia dubia* and *Daphnia pulex*). We measured algal biomass, herbivore density, and the variability of both measurements over four weeks. We also tested for effects of algal diversity on the strength of herbivory by comparing to a control treatment with no herbivores.

The effects of algal diversity differed qualitatively between herbivore treatments. With no herbivores, algal biomass was greater and less variable in the high diversity treatment. Total herbivory by *C. dubia* did not differ between diversity treatments, preserving the qualitative effects of diversity on algal biomass and variability, and leading to only weak effects of diversity on the herbivore population. In contrast, total herbivory by *D. pulex* was twice as great in polycultures, leading to a larger and less variable population of *D. pulex* but lower and more variable algal biomass in polycultures versus monocultures. Thus a differential effect of algal diversity on herbivory led to opposite effects of diversity on algal biomass and variability. Our

results suggest that trophic interactions lead to a richer array of diversity-function relationships than previously documented by studies that have focused on a single trophic level.

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Introduction

As biodiversity has declined globally (Newbold et al. 2015), an increasing amount of research has been dedicated to understanding how changes in biodiversity alter the functioning of ecosystems. Ecologists have paid particular attention to how species richness influences community biomass and the temporal variability of biomass, as these two metrics are widely used to describe ecosystem functioning (Hooper et al. 2005). A general consensus has emerged that species richness tends to increase the production of biomass and stabilize biomass through time (Hooper et al. 2005, Cardinale et al. 2012). However, this consensus largely stems from experiments that have manipulated the richness of terrestrial plants or aquatic algae and then measured the response without considering trophic interactions (Cardinale et al. 2009), even though trophic interactions also influence the biomass and variability of communities (McCann et al. 1998, Borer et al. 2005, O’Gorman and Emmerson 2009, Estes et al. 2011).

As ecologists have begun to merge food web research with biodiversity and ecosystem functioning research, studies have shown that trophic interactions like herbivory can have varying effects on diversity-biomass and diversity-stability relationships that exist for single trophic levels (Duffy et al. 2005, Thebault and Loreau 2006, Jiang and Pu 2009). The reason that trophic interactions have varying effects on diversity-function relationships seems to be that diversity can affect the strength of trophic interactions in various ways. For example, increasing producer diversity has been associated with reduced herbivory (Hillebrand and Cardinale 2004, Fox 2004), increased herbivory (Mulder et al. 1999, Pfisterer et al. 2003, Loranger et al. 2013), or no change in herbivory (DeMott 1998, Scherber et al. 2006) (see Cardinale et al. 2011 for a

meta-analysis). Theory and empirical evidence suggest that variation in herbivore traits such as body size and selectivity may explain differences among results (Duffy 2002, Thebault and Loreau 2005, Narwani and Mazumder 2010). For example, more selective herbivores may encounter their preferred food less often or spend more time handling food in a diverse community, decreasing herbivory rates (DeMott and Kerfoot 1982, Kratina et al. 2007, Vos et al. 2001). On the other hand, relative generalists may benefit from diverse diets that complement their nutritional needs or dilute toxins, thereby increasing herbivory (Pfisterer et al. 2003). However, few studies have measured herbivory of individual species to show how herbivore selectivity influences the effect of producer diversity on food webs, or have separated the effects of diversity on producers from the effects on herbivory. Both of these measurements are important for developing better models of how biodiversity affects ecosystem functions.

The way herbivores affect the relationship between producer diversity and community stability remains particularly poorly studied. Experiments have shown that producer diversity can stabilize producer community biomass in the presence of herbivores (Narwani and Mazumder 2012, Corcoran and Boeing 2012) and in more complex or natural food webs (Steiner et al. 2005, Tilman et al. 2006). In fact, a meta-analysis by Jiang and Pu (2009) suggests that trophic complexity may generally accentuate the positive impact of diversity on community stability. However, these experiments may not have evaluated scenarios in which producer diversity increases herbivory. A substantial increase in herbivory could de-stabilize producer community biomass, since strong top-down control tends to synchronize the temporal dynamics of prey species (Vandermeer 2004, Bauer et al. 2014). Furthermore, the bottom-up effects of diversity on the variability of higher trophic levels are poorly understood, with studies to date producing mixed results (Petchey 2000, Gonzalez and Descamps-Julien 2004, Narwani and

Mazumder 2012). In model systems with two trophic levels, species diversity (of both trophic levels) stabilizes both the producer community and the herbivores when diversity does not increase herbivory. But when diversity induces strong herbivory, producer biomass is de-stabilized while the herbivores are either stabilized or de-stabilized (Thebault and Loreau 2005). Few if any experiments have simultaneously measured the variability (and biomass) of two trophic levels and the strength of their trophic interaction to find whether these metrics interactively respond to varying producer diversity as suggested by theory.

Here we use experimental planktonic microcosms with two trophic levels to test for effects of producer diversity on the biomass and variability of the producer community, the density and variability of the herbivores, and the strength of the trophic interaction, herbivory. We tested these relationships with two different herbivores, only one of which (*Daphnia pulex*) we expected might increase its feeding in more diverse producer communities based on a study by Narwani and Mazumder (2010). In addition, unlike analogous experiments (e.g. Steiner et al. 2005, Corcoran and Boeing 2012, Narwani and Mazumder 2012), we included a control treatment with no herbivores that allowed us to measure herbivore selectivity, and to separate the effect of producer diversity on herbivory from the effect on producers. In accordance with past experiments and Thebault and Loreau (2005), we hypothesized that 1) without herbivores, producer species richness would increase and stabilize producer biomass; 2) if producer richness had weak or negative effects on net herbivory, then producer richness would similarly increase and stabilize both producer biomass and the herbivore population; and 3) if producer richness increased net herbivory, then producer richness would no longer increase producer biomass, and would de-stabilize producer biomass while increasing the herbivore population. Producer richness had weak effects on the strength of herbivory by one herbivore but strongly increased

herbivory by the other (*Daphnia pulex*), and all three hypotheses were largely met. Our results indicate that in line with theory, trophic interactions lead to a richer array of diversity-function relationships than previously documented by studies that have focused on a single trophic level.

Materials and Methods

Herbivores

We used cladocerans of the family Daphniidae (Crustacea: Cladocera) as model herbivores. Cladocerans are ubiquitous freshwater filter feeding zooplankton that form an important food chain link between phytoplankton and fish (Carpenter et al. 1987). As cyclical parthenogens with short generation times (3-10 days), cladocerans are able to quickly exploit resources. *Ceriodaphnia dubia* inhabits lakes and ponds worldwide (USEPA 2002). Adult *C. dubia* are <1 mm and their small (<35 μm) gape prevents them from easily consuming larger algal cells (Burns 1968). A previous study (Narwani and Mazumder 2010) suggested that *C. dubia* might feed at a similar or slower rate in polycultures versus monocultures of algae, based on results for *Ceriodaphnia reticulata*. The *Daphnia pulex* complex is also common and globally distributed (Crease 2012). *D. pulex* adults are larger and have a larger gape (45 μm) than *C. dubia*, allowing them to more easily consume larger particles (Burns 1968). In the same study by Narwani and Mazumder (2010), *D. pulex* demonstrated accelerated feeding in polycultures versus monocultures of algae.

We acquired both cladocerans from Sachs Systems Aquaculture, FL. Upon arrival, we inspected zooplankton cultures and removed all contaminating species. We then housed the cladocerans in 1-L borosilicate glass bottles in COMBO growth medium (Kilham et al. 1998) that was refreshed with media every 5 days. We incubated the bottles under a 16:8 hour light:dark cycle and fed the cladocerans a mixture of green algae (*Selenastrum capricornutum* and *Chlorella sorokiniana*) until the start of the experiment. In order to ensure that none of these

algae would contaminate the experiment, we rinsed and starved the cladocerans prior to inoculation by placing individuals into fresh sterile COMBO medium three times over eight hours.

Focal algae

We used five species of green algae (division Chlorophyta) for the experiment: *Chlorella sorokiniana*, *Scenedesmus acuminatus*, *Pediastrum duplex*, *Monoraphidium minutum*, and *Monoraphidium arcuatum*. These genera are found in 9-53% of North American freshwater lakes and range from the 67th to the 5th most commonly sampled taxa out of 262 genera identified in the Environmental Protection Agency's 2007 National Lakes Assessment (USEPA 2007). All of these species are small enough to be edible for both herbivores, but they represent a range of sizes (74.6 to 7250 μm^3 per cell), morphologies, and tendencies to clump or form colonies, which we presumed would lead to varying degrees of edibility by herbivores (Table 1). We obtained all five algal species from either the University of Texas Culture Collection (UTEX) or the Culture Collection of Algae at Goettingen University (SAG).

Experimental units and design

We grew experimental communities in 1-L borosilicate glass bottles with screw caps that were filled with 750-mL COMBO medium with animal trace elements, which is a standard freshwater medium for culturing phytoplankton and zooplankton. The screw caps were fitted with ports attached to rubber tubes, allowing sterile media exchanges to be performed without removing the caps. We stored the bottles horizontally on a stationary BellCo Digital Top Drive

Roller rack in an environmental walk-in chamber maintained at $20 \pm 0.5^{\circ}\text{C}$, with Phillips 32W coolwhite T8 fluorescent lights set to 18:6 hour light:dark cycles 6-cm from each row of bottles.

We manipulated algal species diversity as well as the presence/absence of each herbivore species. We grew each of the five algal species in monoculture, as well as all possible four-species combinations. There were three herbivore treatments: an herbivore-free control plus each of the two herbivore species inoculated separately. We replicated all ten algal compositions (five monocultures and five polycultures) five times within all three herbivore treatments, for a total of 150 microcosms.

Procedure

After autoclave sterilizing the media in the capped bottles, we inoculated each bottle with either 400,000 cells (533 cells/mL) of one algal species to create a monoculture, or 100,000 cells of each of four algal species to create polycultures with the same total cell count. We allowed the algae to grow for 12 days to increase the probability that the algae would sustain an herbivore population large enough not to undergo stochastic extinction. Then we began performing 8% (60 mL) medium exchanges every two days using 60 mL syringes. We used every third extraction for analysis (every 6 days), beginning with the first exchange. The day of this first exchange and sample was considered day 0. On day 3 we introduced 15 adult *D. pulex* or 25 adult *C. dubia* into the appropriate microcosms, so that the first algal sample represented a baseline prior to addition of herbivores. Our aim was to inoculate the microcosms with as many herbivores as possible with available supplies in order to minimize the probability of stochastic extinction; since we were comparing algal diversity treatments for each herbivore and not comparing the overall effects of the two herbivores, we did not standardize for equivalent biomass or density of the two

species. We continued the sampling schedule until day 24, yielding five samples. We re-suspended the algae daily by swirling each bottle for ten seconds.

Upon extracting the samples, we preserved 3-mL of each sample in formalin for estimation of the biovolume of each algal species. We counted up to 400 cells of each species per sample, or 1.8- μ L of medium, whichever came first. We did not observe any contamination. We then multiplied the resulting cell densities by the average cell biovolume for each species as measured on >70 cells with a Benchtop FlowCam using the area by diameter method (ABD). To estimate algal community biomass, we passed 50-mL of each sample through a Whatman glass fiber filter with 2.5- μ m pores. We placed each filter in a capped test tube with 10-mL anhydrous ethanol, covered it with aluminum foil and stored it in a freezer. After nine days, we analyzed the samples for fluorescence of chlorophyll-*a* as a proxy for algal community biomass following the method of Nusch (1980). We counted the herbivore population of each bottle by looking through the side of the bottle, beginning on day 10 and continuing every six days until day 28 to yield four population estimates per bottle. We recorded the exact population size if there were ≤ 5 individuals, and estimated to the nearest multiple of 5 if there were >5 .

Zooplankton populations went extinct in nine microcosms, which were excluded from analyses so that we only compared those with a consistent presence or absence of herbivory. We also excluded one microcosm from analyses that was a statistical outlier with orders of magnitude lower algal biomass than others. Additionally, two bottles were lost due to breakage. Therefore, a total of 138 experimental units remained in our final analyses, with at least three replicates per treatment-species combination.

Data analysis

We used linear mixed effects models to test for effects of algal diversity on mean algal biomass (measured as fluorescence of chlorophyll-*a*) and mean herbivore density. We natural log-transformed all data to improve normality, and added 1 to each measurement of herbivore density before transforming the data due to the presence of zeroes. We initially analyzed each response variable using the model

$$y = \beta_0 + \beta_1 S + \beta_2 H + \beta_3 t + \beta_4 S^*H + \beta_5 S^*t + \beta_6 H^*t + \beta_7 S^*H^*t + \varepsilon \quad \text{eqn 1}$$

where y is mean algal biomass or herbivore density, S is algal diversity (1 for polycultures, 0 for monocultures), H is the herbivore treatment (control, *C. dubia*, or *D. pulex*), t is time (day after start of the experiment), and ε indicates residual error. Because the 3-way interaction was significant for both response variables, indicating that time series varied between treatments, each herbivore treatment was then analyzed separately with the following model:

$$y = \beta_0 + \beta_1 S + \beta_2 t + \beta_3 S^*t + \varepsilon \quad \text{eqn 2}$$

We measured the variability of algal biomass (chlorophyll-*a*) and of herbivore density for each microcosm as the coefficient of variation (CV), which is the ratio of the standard deviation to the mean. Then we used two-way ANOVA to compare the CV of algal biomass (chlorophyll-*a*) and of herbivore density in monocultures versus polycultures. If there was a significant diversity by herbivore treatment interaction, then we used t-tests to analyze each herbivore treatment separately.

In order to represent the amount of herbivory affecting each experimental community, we calculated an ‘herbivory index’ as the ratio of each algal species’ biovolume in the presence of an herbivore to its biovolume in herbivore-free controls. This metric estimates the proportion of each species’ potential biovolume that is achieved in the presence of an herbivore. We estimated

95% confidence intervals for the herbivory indices by bootstrapping with 5,000 iterations. We performed all statistical tests using R version 3.1.1 (R Development Core Team 2014).

Results

Our results show that algal richness influenced both the mean and variability of algal biomass, but did so in qualitatively different ways depending on whether an herbivore was present, and which one was present. A repeated measures analysis of chlorophyll-*a* (a proxy for algal biomass) showed a significant 3-way interaction between the algal diversity treatment x herbivore treatment x time ($F = 24.26$, $P < 0.01$), indicating that time series varied among treatment combinations. Given this, we analyzed each herbivore treatment separately. A repeated measures of algal biomass in the control treatment (no herbivore) showed a significant treatment x time interaction (Table 2a), with polycultures having greater biomass than monocultures early in the time-series but both treatments converging by later dates (Fig. 1a). Despite differing time-trends, mean levels of algal biomass in the control treatment were generally higher in the algal polycultures (Fig. 1a, Table 2a), and the temporal variability of algal biomass was lower in polycultures (Fig. 2a, $t = 2.05$, $P = 0.05$).

In cultures containing the herbivore *C. dubia*, we found no difference in how algal richness affected algal biomass through time (see algal diversity x time, Table 2b); algal biomass declined through time equally for both richness treatments, but biomass was consistently higher in the algal polycultures (Fig. 1b, Table 2b). Similar to the control treatment with no herbivores, the variation of algal biomass through time was lower in algal polycultures compared to monocultures (Fig. 2b, $t = 2.6$, $P = 0.02$). Thus, cultures containing the herbivore *C. dubia* were

similar to controls in that algal biomass was greater, and variability lower, in cultures that had more algal species.

The relationships between algal diversity and algal biomass and variability in the presence of *D. pulex* stand in contrast to the other two treatments. After *D. pulex* were added to the experimental bottles, the biomass of algae declined rapidly, with these declines being greater for algal polycultures than monocultures (Fig. 1c, Table 2c, note algal diversity x time interaction). In addition, algal polycultures containing *D. pulex* had significantly higher variability of biomass than did algal monocultures (Fig. 2c, $t = -7.24$, $P < 0.01$). Thus, in the presence of *D. pulex*, algal species richness was associated with lower (not higher) biomass of algae, and higher (not lower) variation in algal biomass through time.

The contrasting effects of algal richness in the two herbivore treatments (*C. dubia* and *D. pulex*) is potentially explained by how algal richness differentially influenced grazing by the herbivores. Net grazing intensity by *C. dubia* was the same irrespective of algal richness treatment (see Total in Fig. 3a; error bars represent 95% confidence intervals). *C. dubia* appeared to consume all of the algal species with possible exception of *P. duplex* (note 95% confidence intervals in Fig. 3a overlap zero). *C. dubia* also appeared to graze nearly all species equally regardless of whether they were grown alone or in polyculture (note 95% CIs overlap algal richness treatments). The one exception was *Monoraphidium minutum*, which *C. dubia* grazed more when in a polyculture. However, *M. minutum* only contributed a small amount of biovolume to polycultures (Fig. 3c), which is why a greater grazing intensity on this species did not cause a change in total algal biovolume among treatments of algal richness.

In contrast, grazing by *D. pulex* in polycultures was more than twice as intense as grazing in monocultures. Algal biovolumes in monocultures were suppressed to 59% of the biovolume

achieved in the controls, whereas biovolumes in polycultures were suppressed to 26% (Fig. 3b). Like *C. dubia*, *D. pulex* grazed *M. minutum* more heavily in polyculture than in monoculture. However, unlike *C. dubia*, *D. pulex* also intensified its grazing of *Scenedesmus acuminatus* when the species was in polyculture, suppressing the species to 29% of the control biomass in monoculture and 6% in polyculture. The latter was the most intensely that any algal species was grazed by either herbivore, and is important because *S. acuminatus* was the dominant species in control polycultures, composing 67% of the mean algal biovolume (Fig. 3c). Therefore, the increase in herbivory by *D. pulex* in algal polycultures was largely caused by intensification of grazing on the dominant algal species, *S. acuminatus*.

Algal richness also influenced the population density and temporal variability of the two herbivore species differentially. A repeated measures analysis showed that *C. dubia* population densities increased through time more rapidly in algal polycultures compared to monocultures (Fig. 4a, Table 2d, note algal diversity x time interaction). *D. pulex* densities, however, were consistently higher in polycultures versus monocultures (Fig. 4b, Table 2e). While the temporal variability of *C. dubia* populations was no different between algal diversity treatments (Fig. 5a, $t = 0.80$, $P = 0.43$), the variability of *D. pulex* populations was lower in algal polycultures (Fig. 5b, $t = 3.93$, $P < 0.01$).

Discussion

The results of our study help reconcile the literature by showing that depending how different herbivores alter their consumption in response to changing producer diversity, diversity has qualitatively different effects on both community biomass and temporal variability. In agreement with the majority of biodiversity and ecosystem functioning studies to date, algal diversity tended to increase algal biomass while reducing its variability when no herbivores were present. Though the effects of algal diversity on algal biomass were positive, they weakened through time in the control treatments, contrasting with studies that have suggested biodiversity effects tend to grow stronger with time (Tilman et al. 2006, Cardinale et al. 2007). When the herbivore *C. dubia* was present in cultures, herbivory altered the temporal dynamics (eliminated an algal richness x time interaction), but was not affected by algal diversity, so diversity still increased and stabilized algal biomass in a way that was qualitatively similar to the control treatment. However, in cultures containing the herbivore *D. pulex*, increasing algal richness led to decreased (not increased) and less stable (not more stable) algal biomass. Similarly, algal diversity reduced the variability of the *D. pulex* population, but not of the *C. dubia* population, contrary to our hypothesis. Clearly, the effects of algal diversity on both algal biomass and the variability of both trophic levels was a function of the trophic interaction with herbivores; but, the two herbivores had differing effects.

Ours is one of only a few studies that have included trophic interactions in experiments looking at how biodiversity affects the functioning of ecosystems. The overly simplistic nature of biodiversity experiments to date has led several authors to call for the inclusion of more

‘vertical’ interactions (i.e. interactions among trophic levels) in studies that have historically focused solely on ‘horizontal’ interactions (i.e. interactions within a trophic level) (Thebault and Loreau 2006, Ives et al. 2005, Duffy et al. 2007, Cardinale et al. 2009). Of the few experiments that have included vertical interactions between producers and herbivores, results have been decidedly mixed, with no consistency in how producer diversity alters the strength of herbivory (Cardinale et al. 2011). Our study is useful because it points to certain characteristics of herbivores that might influence the relationship between producer diversity and herbivory, and in turn the diversity-biomass and diversity-stability relationships. The key factors responsible for contrasting results among herbivores in this study were the feeding rate and degree of selective feeding by herbivores when exposed to algal monocultures versus polycultures. The herbivore *C. dubia* consumed the same total amount of algae regardless of whether it was exposed to one or four algal species, and it did not change its feeding selectivity when algae were grown in polyculture. Because algal diversity did little to change the herbivore-producer trophic interaction, the qualitative effects of algal diversity on biomass and variability were very similar to controls where no herbivores were present. Perhaps as a result, we also found limited evidence that algal diversity influenced the population density or variability of *C. dubia*. In contrast, when *D. pulex* was exposed to greater algal richness, it not only consumed a greater total amount of algae, the herbivore shifted its feeding and selectively increased its consumption of the dominant algal species, *Scenedesmus acuminatus*. As a result, the biomass of algae grown in polyculture was substantially reduced by herbivory compared to algal monocultures, and this led to a reversal of algal diversity-biomass and diversity-stability relationships. Greater overall herbivory also caused the *D. pulex* population to be larger and less variable in the high algal richness treatment.

We do not know why *D. pulex* increased its total consumption, or fed more selectively on certain algal species when in algal polycultures. However, several hypotheses have been proposed to explain why herbivores can increase feeding in more diverse producer communities. For example, the “balanced diet” hypothesis proposes that diverse plant diets allow herbivore populations to proliferate if different plants offer complimentary nutritional needs for herbivores (e.g., different nutrients), or if plant diversity helps dilute potential toxins in herbivore diets (DeMott 1998, Pfisterer et al. 2003). The “diet quality” hypothesis proposes that monocultures (at least, some of them) tend to be a lower quality resource for herbivores than polycultures, and that herbivores compensate by passing lower quality food through their guts more slowly to maximize nutrient uptake (Sternern 1993). The “diet quality” hypothesis may explain why the per capita feeding rate of *D. pulex* declined in monocultures of algae compared to polycultures in an experiment by Narwani and Mazumder (2010). We do not have the data required to test these hypotheses, or to do anything more than speculate about why *D. pulex* increased its feeding rate and selectivity in algal polycultures. Nevertheless, it is worth noting that our results are consistent with models of how producer diversity impacts the functioning of two trophic-level systems (Thebault and Loreau 2005, Ives et al. 2005), which predict that herbivore feeding rates and selectivity dictate how producer diversity impacts biomass and variability.

Trophic interactions between producers and herbivores may be particularly important for understanding the functional role of biodiversity in aquatic ecosystems. Herbivores consume on average 50% or more of primary production in aquatic systems, which is significantly more than in terrestrial systems (Cyr and Pace 1993, Cebrian 1999). Despite this, only a few aquatic studies have explored how producer diversity and herbivory interact to affect community biomass and variability. One of these studies (Narwani and Mazumder 2012), which was similar

to ours, produced contrasting results showing that algal diversity tends to increase and stabilize algal biomass in the presence of both *C. dubia* and *D. pulex*. That experiment used different species of algae than we used in the current study, and included algal species that are generally considered to be less edible by *D. pulex* than the species we used. Reduced edibility may have prevented *D. pulex* from increasing its consumption rate in polycultures as occurred in our study; note, however, that Narwani and Mazumder (2012) did not include controls without herbivores in their study that would allow us to estimate changes in herbivory. Another laboratory experiment similarly showed that algal diversity increased and stabilized algal biomass in the face of herbivory by rotifers (Corcoran and Boeing 2012). The rotifers in this study apparently consumed all but one of the algal species, but seemed to reduce their grazing in polycultures despite the edibility of the polycultures. However there were no controls without herbivores in this experiment either, so the role of the trophic interaction between producers and herbivores can again only be speculated.

As results of this study are interpreted, it is important to keep several caveats in mind. First, our study was performed in an overly simplified laboratory environment that was in no way meant to represent the complexity of real lakes. For example, herbivore assemblages in real lakes rarely consist of a single species. Considering interactions among functionally diverse suites of grazers would allow a more realistic view of herbivory in nature, especially since it seems that different herbivores tend to respond differently to producer diversity (Narwani and Mazumder 2010). Experiments have also demonstrated that more diverse consumer assemblages cause stronger top-down control (Griffin et al. 2013), while predators could reduce herbivore impacts, so more work is needed to explore diversity-function relationships in more complex food webs. It should also be noted that the microcosms used for our study were a relatively

static environment compared to natural lakes where many abiotic factors that influence plankton fluctuate over time scales ranging from hours to decades (Levin 1992). One of the most important mechanisms by which biodiversity affects community variability is via asynchronous responses of different species to environmental fluctuations (Gonzalez and Loreau 2009, Loreau and de Mazancourt 2013), and the static environment used in our study may not have allowed this mechanism to be fully expressed.

Regardless of whether or not our study accurately portrays nature, the results are important because they suggest a richer variety of diversity-function relationships is possible when we begin to consider trophic interactions between producers and herbivores. The presence of one herbivore versus another closely related and functionally similar herbivore had the potential to completely reverse the relationship between producer diversity and both biomass and variability of biomass. This possibility had to date only been suggested by theory (Thebault and Loreau 2005, 2006), as few biodiversity experiments have explicitly manipulated trophic interactions. Our study implies that to predict the effects of changing biodiversity on ecosystem functions like biomass production and community stability, ecologists will need to adopt more of a food web approach. We need to understand which herbivores respond to decreasing (or increasing) producer diversity by increasing their consumption, which respond by decreasing their consumption, and which do not alter consumption at all. With this information in hand, we may begin to see generalities among different types of herbivores or food webs, and we can begin to develop models that more realistically predict the impacts of changing biodiversity on ecosystems.

Tables and Figures

Table 1. Source and relevant properties of algal species used in the experiment. UTEX = University of Texas Culture Collection; SAG = The Culture Collection of Algae at Goettingen University. Biovolumes were measured on >70 cells with a Benchtop FlowCam using the area by diameter method (ABD).

Genus and species	Source	Shape	Cell biovolume (μm^3)	Clumping/colonies
<i>Chlorella sorokiniana</i>	UTEX	sphere	74.6	solitary
<i>Monoraphidium minutum</i>	UTEX	compact prolate spheroid	113.42	solitary
<i>Scenedesmus acuminatus</i>	SAG	cylinder + two cones	400.13	solitary, rarely colonies of 2-6 cells
<i>Monoraphidium arcuatum</i>	SAG	elongate prolate spheroid	172.62	solitary with some large clumps
<i>Pediastrum duplex</i>	UTEX	rectangular prism	7250	colonies of 8-32 cells, rarely solitary

Table 2. Results of linear mixed effects models testing for effects of algal diversity and time on a) algal biomass without herbivores, b) algal biomass with *C. dubia*, c) algal biomass with *D. pulex*, d) *C. dubia* density and e) *D. pulex* density. a), c), and d) were modeled with equation 1 in the text, and b) and e) were modeled with equation 2 in the text.

Parameter	Estimate	SE	df	t value	Pr(> t)
a) Algal biomass (ln-transformed), no herbivores					
Intercept	8.322	0.075	192	110.824	<0.001
Algal diversity	0.405	0.105	47	3.860	<0.001
Time	0.038	0.004	192	9.787	<0.001
Algal diversity x Time	-0.011	0.005	192	-1.967	0.051
b) Algal biomass (ln-transformed), <i>C. dubia</i>					
Intercept	8.592	0.109	159	79.024	<0.001
Algal diversity	0.265	0.127	39	2.081	0.044
Time	-0.004	0.004	159	-0.909	0.365
c) Algal biomass (ln-transformed), <i>D. pulex</i>					
Intercept	8.462	0.132	189	64.178	<0.001
Algal diversity	0.161	0.186	46	0.866	0.391
Time	-0.014	0.007	189	-1.900	0.059
Algal diversity x Time	-0.074	0.010	189	-7.357	<0.001
d) <i>C. dubia</i> density (+1 then ln-transformed)					
Intercept	3.327	0.159	162	20.935	<0.001
Algal diversity	-0.232	0.212	39	-1.093	0.281
Time	0.007	0.007	162	0.999	0.319
Algal diversity x Time	0.027	0.010	162	2.771	0.006
e) <i>D. pulex</i> density (+1 then ln-transformed)					
Intercept	2.189	0.115	143	19.018	<0.001
Algal diversity	0.736	0.120	46	6.161	<0.001
Time	0.039	0.004	143	9.371	<0.001

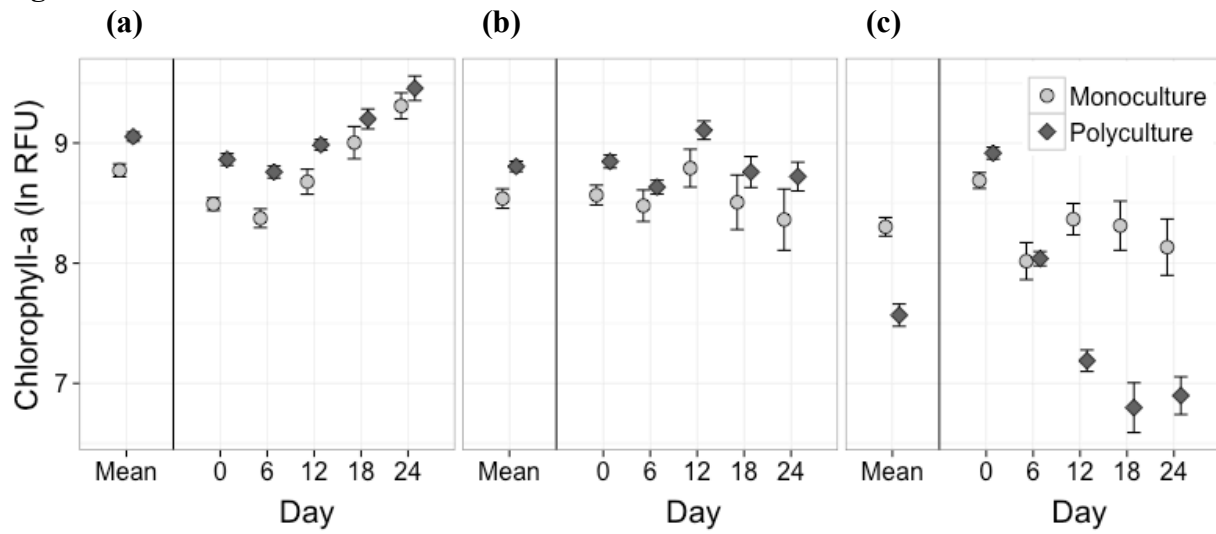
Fig. 1

Fig. 1. Time-averaged mean chlorophyll-*a* fluorescence, and chlorophyll-*a* fluorescence over time, by algal diversity and herbivore treatment (means \pm 1SE). **a**, Control (no herbivores), **b**, *Ceriodaphnia dubia*, **c**, *Daphnia pulex*. Herbivores were introduced on day 3.

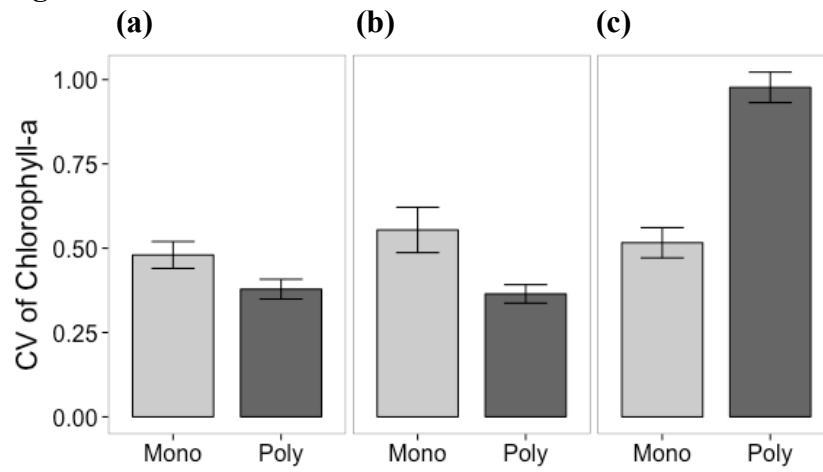
Fig. 2

Fig. 2. CV of chlorophyll-*a* fluorescence by algal diversity and herbivore treatment (means \pm 1SE). **a**, Control (no herbivores), **b**, *Ceriodaphnia dubia*, **c**, *Daphnia pulex*. Mono=algal monoculture, Poly=algal polyculture.

Fig. 3. a, b, Algal biovolume in the grazer treatments ($BV_{\text{herbivore}}$) relative to the control (BV_{control}), by algal species and diversity treatment. **a,** *Ceriodaphnia dubia*; **b,** *Daphnia pulex*. Error bars represent 95% confidence intervals. **c,** The proportion of total algal biovolume represented by each species in control polycultures, for reference. Total=all species; Ped=*Pediastrum duplex*; Chl=*Chlorella sorokiniana*; arc=*Monoraphidium arcuatum*; min=*Monoraphidium minutum*; Sce=*Scenedesmus acuminatus*.

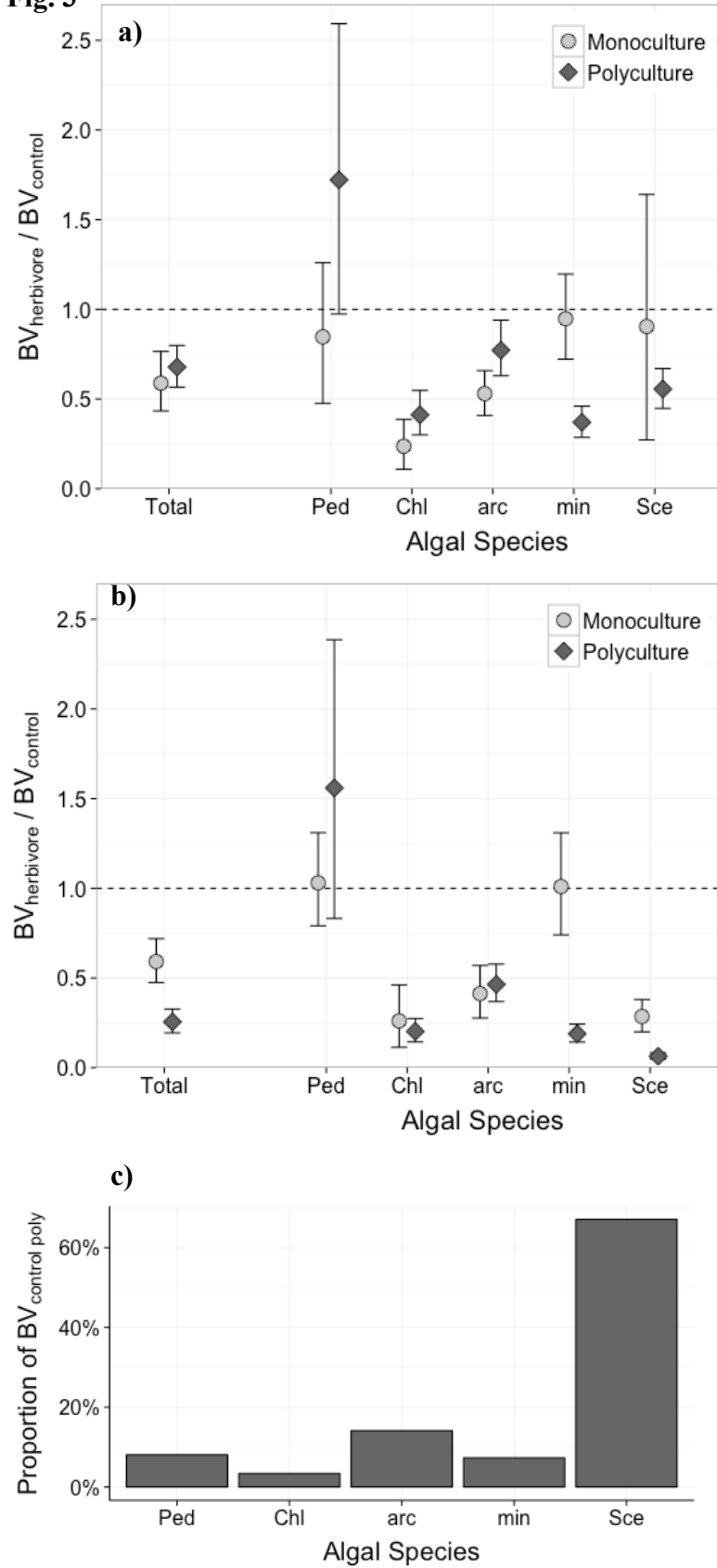
Fig. 3

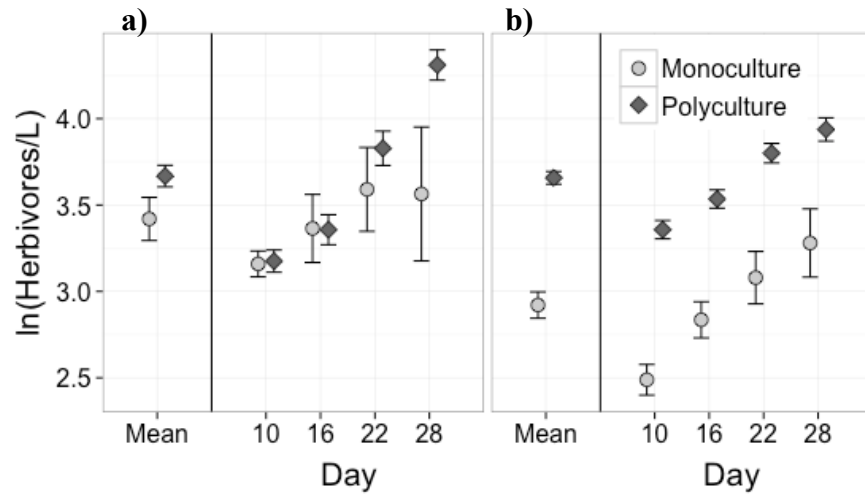
Fig. 4

Fig. 4. Time-averaged herbivores/L, and herbivores/L over time, by algal diversity and herbivore treatment (means \pm 1SE). **a**, *Ceriodaphnia dubia*, **b**, *Daphnia pulex*.

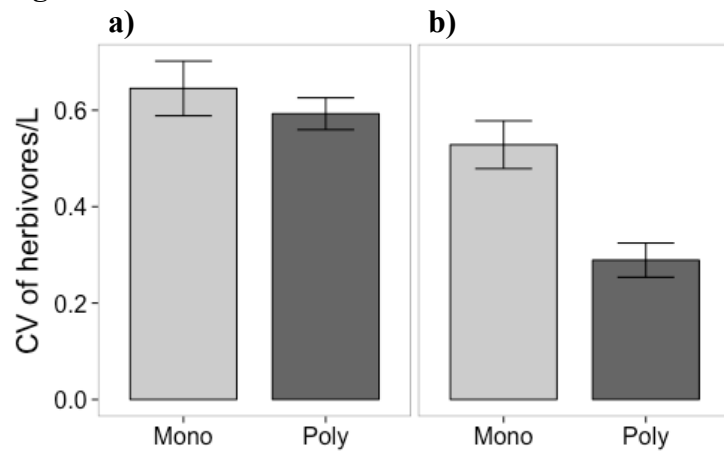
Fig. 5

Fig. 5. CV of herbivores/L by algal diversity and herbivore treatment (means \pm 1SE). **a**, *Ceriodaphnia dubia*, **b**, *Daphnia pulex*. Mono=algal monoculture, Poly=algal polyculture.

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