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Multitrophic diversity effects depend on consumer specialization and species-specific growth and grazing rates

Joanna Filip, Barbara Bauer, Helmut Hillebrand, Anna Beniermann, Ursula Gaedke and Stefanie D. Moorthi

J. Filip, H. Hillebrand, A. Beniermann and S. D. Moorthi (stefanie.moorthi@uni-oldenburg.de), Inst. for Chemistry and Biology of the Marine Environment (ICBM), Carl-von-Ossietzky Univ. Oldenburg, Germany. AB also at: Inst. of Biological Didactics, Univ. of Gießen, Germany. – B. Bauer and U. Gaedke, Inst. of Biochemistry and Biology, Univ. of Potsdam, Germany. BB also at: Helmholtz Centre for Ocean Research Kiel (GEOMAR), Germany.

Ecosystem functioning is affected by horizontal (within trophic groups) and vertical (across trophic levels) biodiversity. Theory predicts that the effects of vertical biodiversity depend on consumer specialization. In a microcosm experiment, we investigated ciliate consumer diversity and specialization effects on algal prey biovolume, evenness and composition, and on ciliate biovolume production. The experimental data was complemented by a process-based model further analyzing the ecological mechanisms behind the observed diversity effects. Overall, increasing consumer diversity had no significant effect on prey biovolume or evenness. However, consumer specialization affected the prey community. Specialist consumers showed a stronger negative impact on prey biovolume and evenness than generalists. The model confirmed that this pattern was mainly driven by a single specialist with a high per capita grazing rate, consuming the two most productive prey species. When these were suppressed, the prey assemblage became dominated by a less productive species, consequently decreasing prey biovolume and evenness. Consumer diversity increased consumer biovolume, which was stronger for generalists than for specialists and highest in mixed combinations, indicating that consumer functional diversity, i.e. more diverse feeding strategies, increased resource use efficiency. Overall, our results indicate that consumer diversity effects on prey and consumers strongly depend on species-specific growth and grazing rates, which may be at least equally important as consumer specialization in driving consumer diversity effects across trophic levels.

Synthesis

In a microcosm experiment, we investigated multitrophic consumer diversity and specialization effects using ciliate consumers and microalgal prey. Consumer diversity increased consumer biovolume, which was highest in combinations containing both generalists and specialists. Specialist consumers showed a stronger negative effect on prey biovolume and evenness than generalists. These experimental data were supported by a process-based model, indicating that the large effect of the specialists was based on high per capita grazing rate on the two most productive prey species. Species-specific traits such as growth and grazing rates were equally important for multitrophic diversity effects than consumer specialization.

Natural food webs represent a complex array of diversely cross-linked multiple trophic levels, in which ecosystem functioning is affected by horizontal (within trophic groups) and vertical (across trophic levels) biodiversity (Duffy et al. 2007). Numerous ecological studies have addressed the consequences of species diversity on ecosystem functioning. After an initial phase of studies within trophic levels, more recently, diversity effects across multiple trophic levels have been addressed (see studies by Downing and Leibold 2002, Naeem and Li 1997, see reviews by Duffy et al. 2007, Srivastava et al. 2009, Griffin et al. 2013). These multitrophic studies, however, yielded inconsistent conclusions regarding the strength and the direction of consumer diversity (i.e. richness) effects on biomass and composition within and across trophic levels (Gamfeldt et al. 2005, Steiner et al. 2005, Dzialowski and Smith 2008), indicating that these effects are highly context-dependent. Further studies demonstrated that diversity effects may be strongly determined by food web configuration and/or consumer identity, involving relevant species-specific traits, such as consumer specialization and grazing rates (Steiner 2001, Straub and Snyder 2006, Finke and Snyder 2008). For instance, Filip et al. (2012) demonstrated that altering species composition (and thus the degree of trait variation) in a microbial food web with the same number of species led to significant quantitative differences in consumer performance. Likewise, Narwani and Mazumder (2012) demonstrated in a recent study that community composition

generally explained more of the variation in population, community and ecosystem properties than species diversity per se, when investigating the effects of prey diversity and composition on the density and stability of resource and consumer populations in planktonic food webs.

In a theoretical study, Thébault and Loreau (2003) analyzed the impact of consumer specialization (number of feeding links) on the relationship between diversity and ecosystem functioning in a model plant–herbivore system. Generalist consumers had a stronger negative effect on prey biomass than specialists, as the consumption rate on each prey species increased when generalists were added, while specialists did not ingest all prey species. Consumer biomass initially increased with increasing generalist diversity more than in the presence of only specialists, presumably due to a greater prey spectrum. However, generalist biomass decreased at high diversity levels due to strong dietary overlap and enhanced interspecific competition, while adding more specialists further increased consumer production at all diversity levels due to non-overlapping diet spectra and higher resource use efficiency.

The present study aimed at testing these predictions of the model of Thébault and Loreau (2003) by investigating the effects of ciliate consumer richness and specialization on algal prey and ciliate consumer biovolume in experimental microbial microcosms. Moreover, we investigated consumer richness and specialization effects on prey composition and evenness, as consumer identity and grazing preferences were shown to determine effects on prey community structure (Duffy et al. 2003, Burkepile and Hay 2008). We chose four algal species, which differed in their edibility by four different ciliate consumers and were thus able to study a relatively complex food web in a small experimental set-up (Fig. 1). We classified the ciliate consumers as generalists or specialists based on their feeding preferences (species with wider diet breadth were considered generalists and vice versa), and manipulated their richness and composition to test the following hypotheses on the basis of the model results formulated by Thébault and Loreau (2003): H1) Increasing consumer species richness decreases prey biovolume. H2) Generalist and specialist consumers have divergent effects on prey biovolume and evenness. In this context, we expect generalists to have a stronger impact on prey biovolume than specialists due to higher consumption rates on each prey species according to the model of Thébault and Loreau (2003). Conversely, we expect specialists to have a stronger impact on prey evenness due to enhanced grazing on only particular prey species, decreasing prey evenness due to unequal grazing. H3) Increasing consumer species richness increases consumer biovolume. H4) Generalist and specialist consumers have divergent effects on consumer biovolume. In this context, we expect higher biovolume for generalists than for specialists due to a greater prey spectrum and highest biovolume in mixed assemblages due to highest complementary resource use. At high consumer richness levels (> nine species), Thébault and Loreau (2003) predicted consumer biomass to decrease again after reaching a plateau due to increased competition among generalists with a strong dietary niche overlap; however this prediction could not be tested in our experimental setup which only included four consumer species.

Our experimental design matched the model design from Thébault and Loreau (2003) and their predictions regarding consumer biovolume were supported by our experiment. However, their predictions concerning consumer effects on prey were not supported, as in our system specialists decreased prey biovolume more efficiently than generalists. To deepen our understanding of the mechanisms behind this discrepancy, we used a mathematical model, which included species-specific growth (in the case of prey) and grazing rates (in the case of the consumers). Such potential species-specific differences were ignored by the model of Thébault and Loreau (2003), but have subsequently been suggested to be important for the identification of the relevant ecological mechanisms driving biodiversity effects (Cardinale et al. 2011). Our study presents one of the first multitrophic biodiversity experiments coupled with a process-based model that takes these potential species-specific differences and consumer specialization into account, thus advancing our mechanistic understanding of biodiversity–ecosystem functioning relationships across trophic levels.

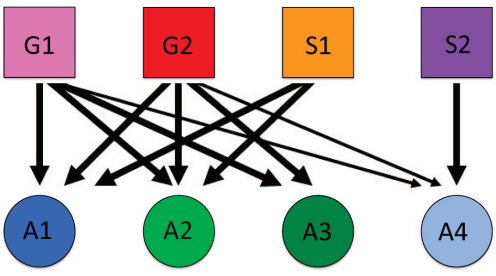

Figure 1. Food web configuration in our experiment and model based on prior feeding experiments. Squares represent consumers (S1: *Frontonia angusta*, S2: *Nassula sorex*, G1: *Stylonychia* sp., G2: *Coleps hirtus*) and circles prey (A1: *Chlamydomonas terricola*, A2: *Cryptomonas* sp., A3: *Fragilaria capucina*, A4: *Plectonema* sp.). Thick arrows represent strong feeding relations (with high (0.9, 1) preference values of consumers in the model) and thin arrows weak feeding relations (with low (0.4) preference values of consumers in the model, Table 2).

Material and methods

Organisms and culture conditions

For the experiment we used four different algae (prey, A1–A4) and four heterotrophic ciliate consumers (generalists G1 and G2 and specialists S1 and S2, Table 1), which potentially co-occur in freshwater habitats. As both phytoplankton and microzooplankton occur in nature with a wide range of different traits, occupying different niches, we chose four microalgae of different sizes, forms and taxonomic groups and four consumers differing in their growth and feeding characteristics with the aim of maximizing trait diversity at both trophic levels. All ciliate consumers chosen for the present study are mainly herbivorous and none of them is able to survive purely on bacteria. Therefore, we neglected potential ciliate bacterivory in our

Table 1. Species used in the experiment (A1 − 4 = algal prey; S1 + S2 = specialist ciliate consumers; G1 + G2 = generalist ciliate consumers).

Denotation	Taxonomic group	Species	Source
	Algae		
A1	Chlorophyceae	*Chlamydomonas terricola*	culture collection of
A2	Cryptophyceae	*Cryptomonas* sp.	algae, Botanical Inst.,
A3	Bacillariophyceae	*Fragilaria capucina*	Univ. of Cologne Germany (A1–A3)
A4	Cyanobacteria	*Plectonema* sp.	culture collection of algae and protozoa, Inst. of Freshwater Ecology in Cumbria, UK
	Ciliates		
S1	Oligohymenophorea	*Frontonia angusta*	provided by U.-G. Berninger, Univ. of Salzburg, Austria)
S2	Nassophorea	*Nassula sorex*	culture collection of algae and protozoa
G1	Spirotrichea	*Stylonychia* sp.	own isolation from a pond at the Univ. of Cologne, Germany
G2	Prostomatea	*Coleps hirtus*	provided by U.-G. Berninger

study and only investigated the grazing impact on the phytoplankton assemblage. For cultivation prior to the experiment we fed all ciliates with a cryptophyte (A2), except for the specialist S2, which was fed with a filamentous cyanobacterium (A4). We kept all cultures in a climate chamber at 18°C with a light/dark cycle of 12 : 12 h and a light intensity of 60 µmol m^{-2} s^{-1} in transparent culture flasks (volume 50 ml). Ciliates were kept in mineral water (Volvic), while algae were cultivated in WEES - culture medium (McFadden and Melkonian 1986) with the addition of silicate according to concentrations used in WC – culture medium (Guillard and Lorenzen 1972). We fed the ciliates weekly and transferred all cultures twice a month to new medium.

Feeding preferences and grazing rates

Prior to the main diversity experiment, we tested consumer feeding preferences and grazing rates in order to classify them as generalists or specialists. In these feeding experiments, we offered the four single algal species separately to the four different ciliates in monocultures. Each inoculum contained equal biovolume of ciliates (2×10^7 µm^3 ml^{-1}) and algae (3×10^6 µm^3 ml^{-1}), respectively. For that, we measured the cell length and width of 20 cells per species and calculated the individual biovolume of algae and ciliates sensu Hillebrand et al. (1999) and Moorthi et al. (2008). Organisms were measured alive before preservation, as they can change their shape after fixation (Stoecker and Gifford 1994). We conducted the feeding experiments in 12-well cell culture plates (volume 6 ml, area of growth: 3.8 cm^2) in WEES culture medium (+ silicate, McFadden and Melkonian 1986) and determined the number of ingested prey cells by counting the remaining algae after 1, 3 and 24 h, respectively, in preserved samples (1% Lugol's iodine solution) using an inverted microscope to determine whether algal cells were ingested at all. Only if the ciliates showed positive growth on particular algae after 72 h, though, the algae were assumed to be suitable prey for the ciliate consumer. Based on these results, ciliates were classified as generalists or specialists depending on the number of algal species they grew on.

Diversity experiment

In the main diversity experiment we manipulated ciliate consumer richness across three different diversity levels, comprising all consumer monocultures (four treatments), all possible two-species combinations (six treatments) and the four-species combination (one treatment), feeding on a constant mixture of all four algal species. These 11 ciliate species combinations were replicated four times. Additionally, we set up a control without consumers in four replicates, resulting in a total of 48 experimental units. At the beginning of the experiment we inoculated algal and ciliate species with equal biovolume in all diversity levels and species combinations, respectively, (algae: 6×10^5 µm^3 ml^{-1}, ciliates: 5×10^6 µm^3 ml^{-1}), i.e. for ciliate two-species and four-species combinations we inoculated half or a quarter of what was inoculated for individual consumer species in monocultures, respectively.

The experiment was conducted in 250 ml Erlenmeyer-flasks in WEES-culture medium (+ silicate) with a volume of 150 ml in a climate chamber at 18°C; a light intensity of 60 µmol $m^{-2}s^{-1}$and a 12 h : 12 h light/dark cycle for 15 days. This time frame was appropriate in order to observe trophic interactions and detect potential consumer diversity and specialization effects. Note that this experiment was not designed to investigate temporal dynamics of predator–prey interactions (i.e. potential oscillations), but to investigate the direct short-term effects of consumer diversity and specialization within and across trophic levels. All algal species were light limited, except for the diatom (A3), which was silicate limited due to unintentional low Si concentrations in the medium. Every third day, 10 ml subsamples were taken from each experimental unit and preserved with Lugol's iodine solution at 1% final concentration. Subsamples of 3 to 5 ml were counted in 12-well cell culture plates (volume: 6 ml, area of growth: 3.8 cm^2) using an inverted microscope at 100 × magnification for the ciliates and 400 × magnification for algae. The sampled volume was replaced by new culture medium at every sampling time point (7% media exchange every third day) to promote grazing effects on phytoplankton over nutrient effects.

Statistical analysis

For the main diversity experiment, data were analyzed from the last sampling day (day 15), as the strongest treatments effects were observed at that time point. Ciliate and algal cell abundances were converted to biovolume, and prey evenness was calculated according to the Pilou's evenness index (Pilou 1975). We tested the effects of consumer species combination on algal biovolume and evenness by conducting a one-way analysis of variance (ANOVA) with species

composition as independent factor. Thereby, we avoided unequal treatment level and nested designs of composition within our diversity treatments and were able to pursue a fully balanced analysis. In case of a significant treatment effect we conducted planned comparisons to compare selected consumer treatment groups as a direct test of our hypotheses: 1) algal mixture without consumers versus algal mixture with consumers, testing for an overall consumer effect on prey; 2) one-species consumer treatments versus all ciliate polyculture treatments and 3) two-species combinations versus four-species combinations, both (2 + 3) testing for a consumer richness effect on prey; 4) generalists (treatments containing generalists only) versus all mixed treatments (treatments containing both, specialists and generalists in combination), 5) specialists treatments (treatments containing specialists only) versus all mixed treatments, and 6) specialists versus generalists, the three latter comparisons (4 − 6) testing for differences in effects on prey among differently specialized consumers. To correct the type I error occurring due to repeated testing, we used sequential Bonferroni adjustment of significance levels (Rice 1989).

To test the effects of consumer species combination on consumer biovolume we also conducted a one-way ANOVA. In case of significant treatment effects we performed planned comparisons (see above, planned comparisons 2–6), testing for consumer richness and specialization effects on consumer biovolume.

All analyses were conducted with R ver. 2.15.1.

Modeling approach

To further advance our understanding of the ecological mechanisms behind the consumer richness and specialization effects observed in our study, we used a process-based model as suggested by Cardinale et al. (2011). We represented the four algal and four ciliate species used in the diversity experiment and their interactions with state variables in an ODE (ordinary differential equations) model. We ran 12 simulations corresponding to the 12 consumer treatments of the experiment by varying the number and identity of consumers in the model. We conducted these simulations with one parameter set including all the information about species-specific variables (growth and grazing rates) that was available to us from the main diversity experiment (for details see Supplementary Material Appendix 1). In addition, we ran model scenarios where we ignored the measured differences among species in growth and/or grazing rates and omitted small differences in other parameters. These scenarios intended 1) to investigate the importance of species-specific growth and grazing rates for consumer richness effects by comparing simulations ignoring these to simulations including them and 2) to avoid the danger of drawing conclusions based on results which might only be true for the specific parameterization used, given the inevitable insecurity in model parameterization.

We used a modified version of the multispecies Rosenzweig–MacArthur ODE model (Rosenzweig and MacArthur 1963). We included a prey preference parameter (q_{ij}) which was 0 if consumer j did not graze on prey i and nonzero (≤ 1) otherwise, reflecting relative species-specific prey preferences (Table 2). We simulated the biovolume dynamics of the prey (P_i) and consumer (C_j) species according to the following equations:

Table 2. Prey preference values for the consumer–prey pairs used in the simulation model. We inferred the existence and strength of feeding links from direct observation of the feeding behavior of consumers during the feeding experiments. For existing feeding links we then used values of either 1 (strong preference) or 0.4 (weak preference). These values were obtained by fitting.

Prey	A1	A2	A3	A4
Consumers				
G1	1	1	1	0.4
G2	1	1	1	0.4
S1	1	1	0	0
S2	0	0	0	1

$$\frac{dP_i}{dt} = r_i \times P_i - \sum_j g_{ij} \times C_j \quad (1)$$

$$\frac{dC_j}{dt} = \left(\varepsilon \sum_j g_{ij} - d \right) C_j \quad (2)$$

where r_i is the growth rate of species i, g_{ij} is the maximum grazing rate of consumer j on prey i, while ε and d are the growth efficiency and the mortality rate of the consumers, respectively.

We assumed density-dependent growth for all prey species with a maximum growth rate of r'_i. The diatom (A3) was unintentionally silicate-limited in the experiment. The lack of silicate was the main limiting factor of the growth of A3 and constrained its biomass to a very low level. Meanwhile, the other prey species were only limited by light. Therefore, A1, A2 and A4 had a common carrying capacity K (Eq. 3) while A3 had its own capacity (K_{A3}) in the model (Eq. 4).

$$r_i = r'_i \left(1 - \frac{\sum_i P_i}{K} \right) \quad (3)$$

$$r_{A3} = r'_{A3} \left(1 - P_3 / K_{A3} \right) \quad (4)$$

The consumers had a Holling type II functional response where g'_j was the maximum grazing rate of consumer j while M was the half-saturation constant of the consumers (Eq. 5). Prey preference values (q_{ij}) were either 0, 0.4 or 1 (Table 2).

$$g_{ij} = g'_j \frac{g_{ij} \times P_i}{(food_j + M)} \quad (5)$$

$$food_j = \sum_i q_{ij} \times P_i \quad (6)$$

We defined a parameter set (in the following called the 'standard parameterization') to represent the experiment as

Table 3. The standard parameter set used in the model.

Parameter description	Symbol	Value	Unit
Growth rate			
A1	r'_i	0.7	day^{-1}
A2		0.9	
A3		0.3	
A4		0.2	
Max. grazing rate			
S1	g'_j	5	day^{-1}
S2		1	
G1		0.6	
G2		1	
Growth efficiency	ε	0.35	day^{-1}
Mortality	d	0.05	day^{-1}
Carrying capacity			
A1, A2, A4	K	5×10^7	$\mu m^3\ ml^{-1}$
Carrying capacity			
A3	K_{A3}	10^6	$\mu m^3\ ml^{-1}$
Half-saturation constant	M	6.5×10^6	µm

closely as possible. For this aim, we determined the growth rates of the prey and the carrying capacity from the treatment without consumers. We fitted the grazing, efficiency and mortality parameters based on the diversity experiment and literature values (Tirok and Gaedke 2010, for details see Supplementary material Appendix 1). All parameter values are provided in Table 3. The mean biovolume of the four replicates measured on the third day of the experiment were used as initial values, as ciliate abundances decreased during the first few days of the experiment as a result of stress during the inoculation procedure, before they started growing (Supplementary Material Appendix 1 Fig. A1.2). The biovolume dynamics were simulated until day 15. We simulated the dynamics of the four prey species 1) without consumers, 2) with each of the consumers, 3) with all possible two-species combinations of consumers, and 4) with all four consumers, in complete analogy with the diversity experiment (Fig. 2, 3).

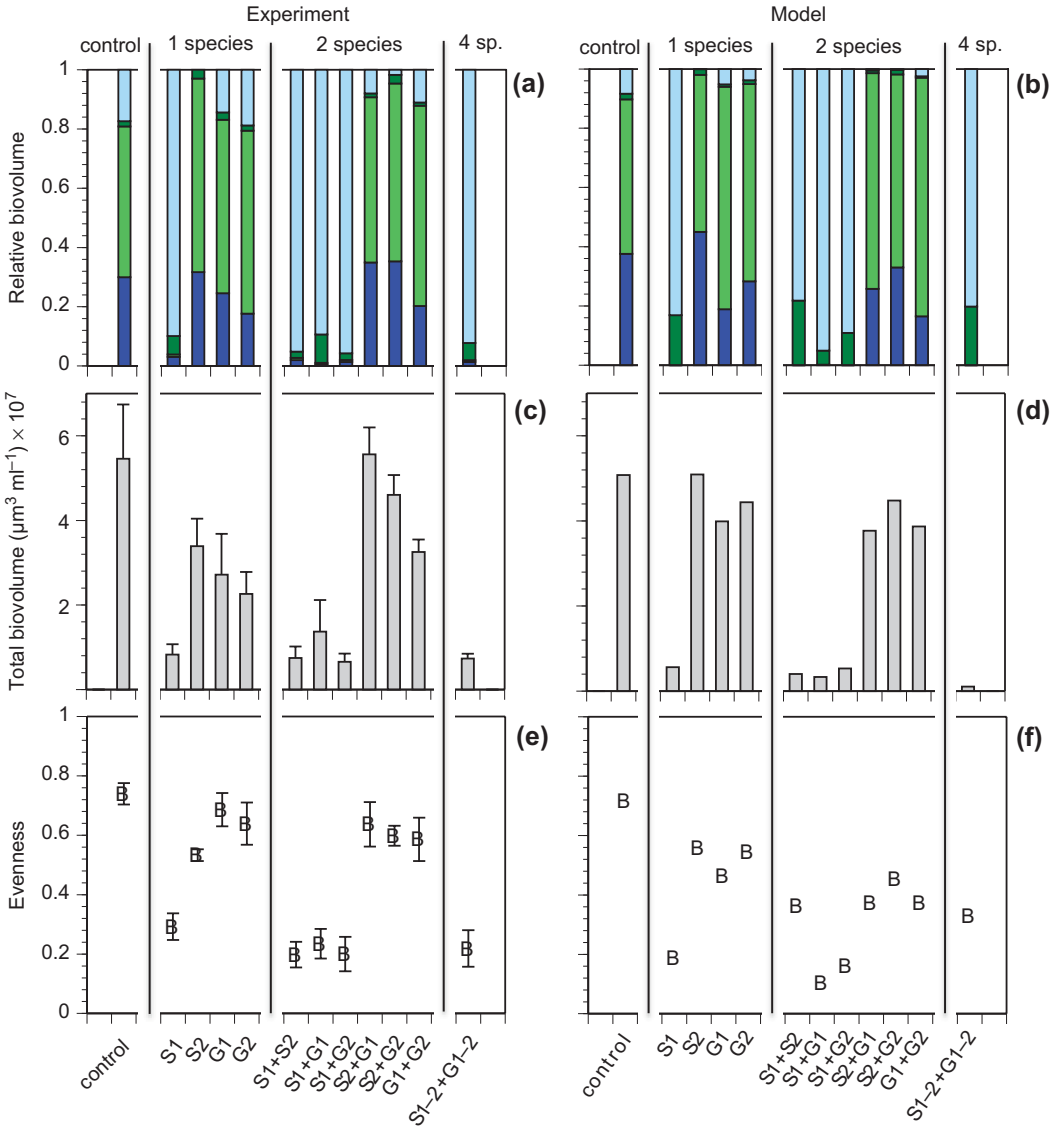

Figure 2. Average relative biovolume of algal prey species (a, b; A1 – dark blue, A2 – light green, A3 – dark green, A4 – light blue), total biovolume (c, d) and evenness (e, f) in the experiment (left panel: a, c, e; mean ± SE) and in the simulation model (right panel: b, d, f) in all consumer species combinations.

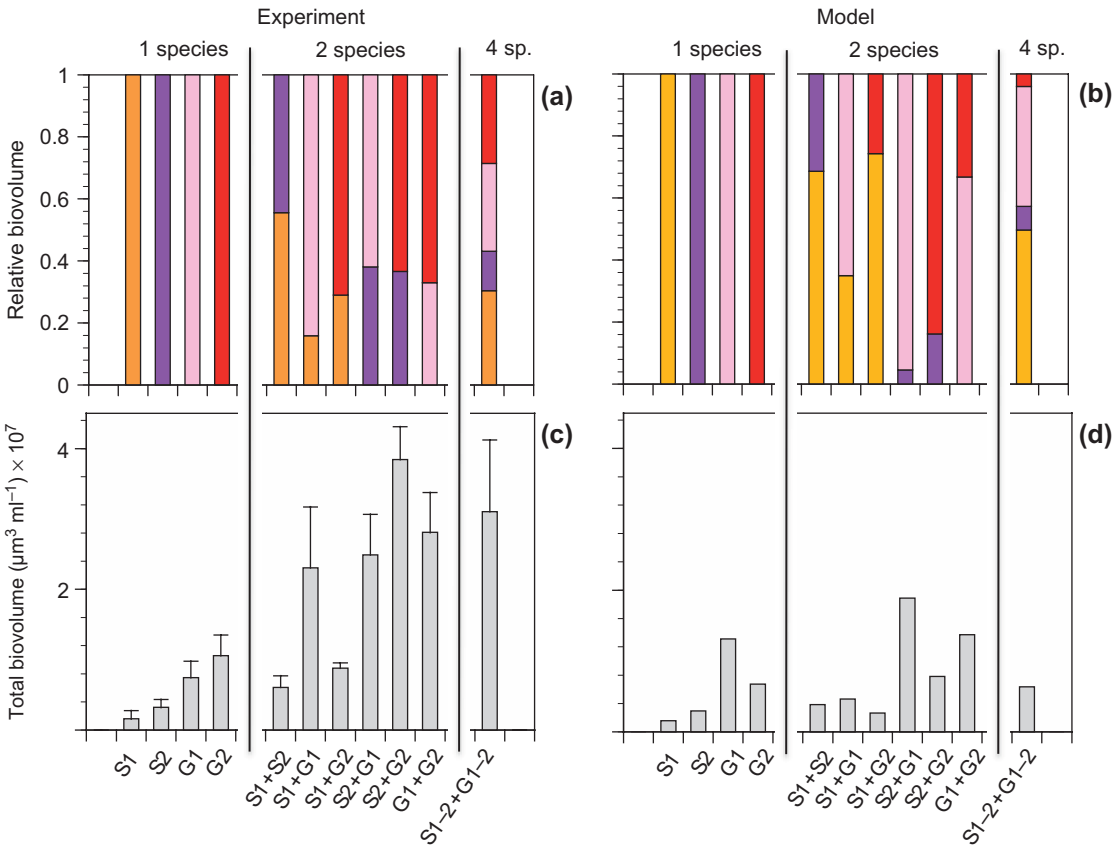

Figure 3. Average relative biovolume of ciliate consumer species (a, b; S1 – orange, S2 – purple, G1 – pink, G2 – red) and total biovolume (c, d) in the experiment (left panel: a, c; mean ± SE) and in the simulation model (right panel: b, d) in all consumer species combinations.

In addition to simulations including the standard parameterization we ran four scenarios with a different parameterization. Within these simulations, we altered the prey growth rates and the relative grazing characteristics of the consumers to assess their importance in driving the observed patterns of the experiment. The four scenarios were the combinations of two types of prey growth scenarios (1P, 2P) and two types of consumer grazing scenarios (1C, 2C). (1P) In the 'species-specific prey growth rates' scenario, prey growth rates were set as in Table 3 (i.e. as in the 'standard model'). (2P) In the 'equal prey growth rates' scenarios, all prey growth rates were set to 0.5, which is close to the mean value of the growth rates used in the standard parameterization and A3 also competed for the common carrying capacity. (1C) Similarly, in the 'equal grazing rates' scenarios, all grazing rates were set to 3, close to the mean value of the species-specific grazing rates in the 'standard model' (Table 3). (2C) In the 'strong S1' scenario the grazing rates of S2, G1 and G2 were set to 0.8 and that of S1 to 5, as S1 was the most effective consumer in reducing prey biovolume in the diversity experiment. For all scenarios, the initial conditions were set equal (each prey biovolume: 6×10^5 µm^3 ml^{-1}, total consumer biovolume: 5×10^6 µm^3 ml^{-1}, divided equally among the number of species present in the treatment), and all nonzero prey preference values (q_{ij}) were set to 1, which means that we ignored species-specific differences in prey preferences and only included the information on the presence or absence of trophic links in the model. Thus, in all scenarios, G1 and G2 were identical as they differed neither in their grazing rates nor in their preferences. Hence, in the scenarios we included only the following combinations of species: four prey species with 1) S1 and S2, 2) S1 and a generalist (as G1 and G2 were identical), 3) S2 and a generalist, 4) two identical generalists, or 5) four consumers (Fig. 4). This way, the effect of the growth and grazing rates and the food web structure could be observed without potentially confounding effects of the small differences in realized initial conditions (we started the standard model with biovolumes measured on day 3), the prey preferences of the consumers and the lower carrying capacity of A3. Other parameters were set according to the standard parameterization.

Results

Feeding preferences and ingestion rates

Based on the feeding experiments prior to the main diversity experiment, we classified the ciliates according to their feeding preferences as generalists or specialists (Fig. 1). The generalists G1 and G2 fed and grew on all four offered prey species. The specialist S1 only grew on A1 and A2, the two fastest growing prey species, while the specialist S2 exclusively grew on the cyanobacterium A4, one of the slower growing prey species in addition to A3. Thus, the two

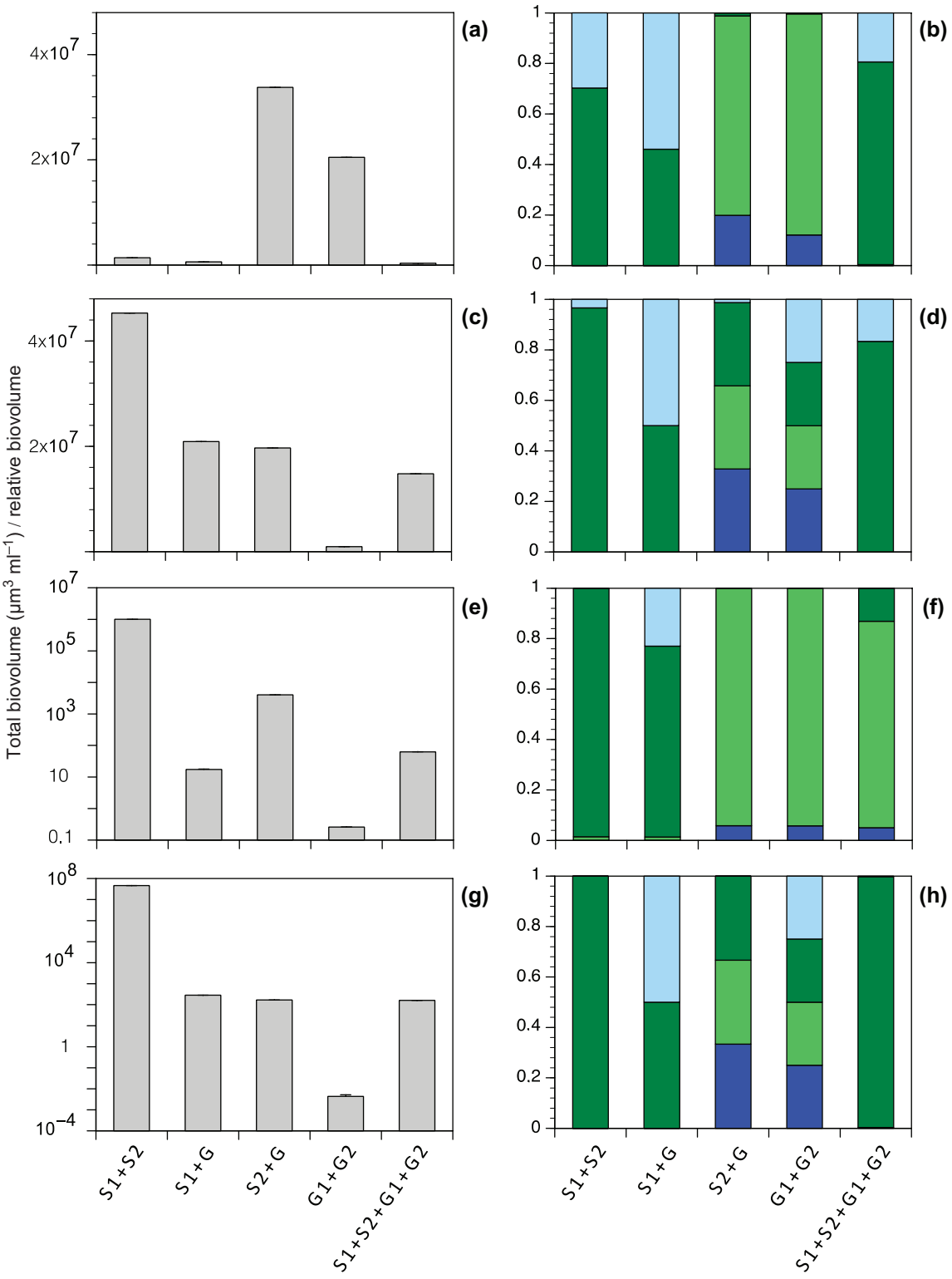

Figure 4. Total (a, c, e, g) and relative (b, d, f, h) biovolume of algal prey species in four simulated scenarios with (a–b) strong specialist S1 and species-specific prey growth rates, (c–d) strong specialist S1 and equal prey growth rates, (e–f) all consumer grazing rates equal and species-specific prey growth rates and (g–h) all consumer grazing rates equal and equal prey growth rates. Relative biovolume: A1 – dark blue, A2 – light green, A3 – dark green, A4 – light blue. These model scenarios represent simplified versions of the standard model, with the degree of simplification increasing from the upper to the lower panels. Corresponding results of the standard model are shown on Fig. 2. See Methods for a more detailed description of the scenarios. Note the different scalings on the y-axes (a + c: linear, e + g: log-scale) and the different range of the y-axes.

Table 4. Results of the one-way ANOVA and summary of the planned comparisons testing the effects of consumer species combination on prey biovolume, prey evenness and consumer biovolume by comparing selected consumer treatment groups ('all mixed treatments' contain both, specialist and generalist consumers in mixture; 'generalist' treatments contain generalists only, 'specialist' treatments contain specialists only).

	Prey biovolume			Prey evenness		Consumer biovolume		
	DF	F	p	F	p	DF	F	p
One way ANOVA	11,36	9.30	$<0.001^{**}$	14.51	$<0.001^{**}$	10,33	11.59	$<0.001^{**}$
No./planned comparison								
1. consumer present - consumer absent	1,46	4.93	0.031	5.6	0.022			
2. one species treatments – all polycultures	1,42	0.37	0.547	6.19	0.017	1,42	30.03	$<0.001^{**}$
3. two species – four species	1,26	2.41	0.133	1.46	0.237	1,26	1.24	0.275
4. generalist – all mixed treatments	1,30	1.96	0.172	9.76	0.004^{**}	1,30	4.9	0.035
5. specialist – all mixed treatments	1,30	0.72	0.402	0.06	0.806	1,30	46.48	$<0.001^{**}$
6. specialist – generalist	1,22	6.92	0.015	11.09	0.003^{**}	1,22	14.48	$<0.001^{**}$

**significant after Bonferroni adjustment.

specialists had complementary diet spectra, while those of the generalists overlapped with each other and with the two specialists, especially with S1 (Fig. 1).

Diversity experiment and model

Consumer composition had significant effects on all response variables, i. e. prey biovolume, prey evenness and consumer biovolume (Table 4). In the following, we refer to the planned comparisons (PC) conducted within the one-way ANOVA as listed in Table 4, comparing effects of different consumer species combinations on prey biovolume, prey evenness and consumer biovolume to test for consumer presence (PC 1), consumer richness (PC 2 – 3) and consumer specialization (PC 4 – 6) effects.

Hypotheses H1 and H2: consumer effects on prey biovolume and evenness

Consumer specialization but not consumer richness strongly influenced the prey assemblage. Prey evenness was significantly lower in treatments containing only specialists compared to treatments containing only generalists (Table 4, Fig. 2). This effect was not significant for prey biovolume (Table 4, Fig. 2). Prey evenness in mixed treatments (containing both generalists and specialists) was also significantly lower compared to pure generalist treatments (Table 4, Fig. 2). In contrast, neither consumer presence nor consumer diversity significantly affected prey biovolume or prey evenness (Table 4, Fig. 2).

Consumer specialization effects on prey were mainly driven by the specialist consumer S1, as prey biovolume and evenness were lower in all treatments containing this particular ciliate (Fig. 2a, c, e), even though its biovolume was low compared to the generalists (Fig. 3a, c). S1 strongly grazed on the two most productive microalgae A1 and A2, indirectly promoting A4, which became dominant and suppressed prey evenness. A3 biovolume was low in all of the treatments due to the unintentional silicate limitation. Using the standard model (that is, implementing the observed species-specific growth and grazing rates) we ran simulations with species combinations reflecting all consumer treatments of the experiment. Similarly to the experiment, the presence of S1 had a substantial negative effect on prey evenness and biovolume at the end of the simulations in this model version (Fig. 2b, d, f). Other model versions only reproduced this effect if they included a higher grazing rate of S1 and species-specific prey growth.

Hypotheses H3 and H4: consumer effects on consumer biovolume

Ciliate biovolume significantly increased with increasing ciliate richness in the experiment (Table 4, Fig. 3). Furthermore, ciliate specialization affected ciliate biovolume in both, the experiment (Table 4, Fig. 3c) and the model (Fig 3d). In the experiment, consumer biovolume was significantly higher for generalists compared to specialists, and highest in mixed consumer combinations (generalists and specialists together, Table 4, Fig. 3c). The model predicted that both generalists produce more biovolume when growing together with S2 compared to S1 (Fig. 3d). In the model, S1 in combination with the generalists increased until it overexploited the two most productive prey species, A1 and A2, subsequently hampering its own growth and that of the generalists, resulting in low total consumer biovolume. This model prediction was only supported for G2 in the experiment. S2 did not have such a negative effect on the generalists as S1. In the experiment it even promoted them, probably as it was grazing on the less productive filamentous cyanobacteria A4, thereby releasing the two more productive phytoplankton species, A1 and A2, from competition. In the model, the magnitude of this effect on consumer biovolume was smaller than observed in the experiment, as A4 had a low biovolume, and thus, small competitive effects on A1 and A2.

Scenarios with alternative growth and grazing characteristics

We performed simulations with the same consumer specialization and standard parameterization, but altered growth and grazing characteristics of prey and consumers to test their influence on the observed consumer effects on prey biovolume and evenness. In the 'strong S1' scenario, that is, implementing a high grazing rate for S1, and lower, equal grazing rates for the other consumers (Fig. 4a–d), the strong effect of S1 on prey community composition was reproduced regardless of the prey growth rates being species-specific (Fig. 4b) or equal (Fig. 4d). Regarding effects on prey biovolume, however, the strong biovolume-reducing effect of S1 could only be reproduced

in the scenario with species-specific prey growth rates (Fig. 4a). For equal prey growth rates, consumer effects on prey biovolume differed from the experiment, as the modeled prey biovolume was lower in the generalist treatments (Fig. 4c) since the generalist consumers built up higher biovolume when feeding on four equally productive prey. Thus, the scenario with species-specific prey growth rates and consumer-specific grazing rates was sufficient to reproduce the main qualitative patterns seen in the diversity experiment in terms of prey biovolume and community composition. Including additionally species-specific feeding preferences of consumers (as opposed to only including the information on the presence or absence of trophic links) and taking the biovolume values from the measurements of the third day of the experiments as initial values (instead of setting them equal) was only important for reproducing the observed patterns quantitatively (compare Fig. 4a to Fig. 2c and d, and Fig. 4b to Fig. 2a and b).

Without differences in grazing rates ('equal grazing rates' scenarios, Fig. 4e–h) all combinations of consumers except for the '2 specialists' combination resulted in unrealistically low prey biovolume with or without species-specific prey growth rates.

In general, equal prey growth rates (Fig. 4c–d, g–h) resulted in higher evenness of the prey communities in most treatments compared to species-specific growth rates. In all scenarios, the negative effects of the four – consumer treatment on prey biovolume and evenness did not substantially exceed the effects of the most potent two – consumer treatment. Therefore, the effect of consumers on prey did not directly depend on richness but on the presence of particular consumers (those with the strongest effects on prey biovolume), either S1 (in the 'strong S1' scenario) or the generalists ('equal grazing rates' scenario).

Discussion

In our study consumer composition strongly affected prey biovolume and evenness, as well as consumer biovolume. Increasing consumer richness had no significant effect on prey biovolume, refuting hypothesis H1. Hypothesis H2 on divergent effects of generalist and specialist consumers on prey biovolume and evenness was partly supported and partly refuted. Consumer specialists negatively affected prey evenness (supporting H2), while in contrast to H2, specialists also tended to have a stronger negative impact on prey biovolume than the generalists. This effect was mainly induced by a single specialist consumer with high per capita grazing rates on the two most productive prey species, emphasizing the importance of species-specific traits in determining consumer effects on prey. Both, consumer richness and consumer specialization had major effects on consumer biovolume. Corroborating hypothesis H3, consumer richness increased consumer biovolume. Hypothesis H4 on divergent effects of generalist and specialist consumers on consumer biovolume was also supported. As expected, consumer biovolume was higher for generalists compared to specialists, and highest in mixed consumer combinations (generalists and specialists together). In the following, we will discuss the effects of consumer richness and specialization on prey and consumer dynamics in more detail.

Hypotheses H1 and H2: effects of consumer richness and specialization on prey biovolume and evenness

Consumer richness per se had no significant effect on prey biovolume and prey evenness, contrasting negative consumer richness effects on prey biomass reported in several model (Holt and Loreau 2001, Thébault and Loreau 2003) and experimental studies (Gamfeldt et al. 2005, Jaschinski et al. 2009, Matthiessen et al. 2007). Other studies, however, also revealed no or only weak consumer richness effects on prey biovolume and diversity (Bruno and O'Connor 2005, Steiner et al. 2005), indicating that consumer effects on prey are not always determined by consumer diversity, but may be context-dependent and driven by species-specific traits.

The model by Thébault and Loreau (2003) provides a mechanism explaining divergent effects of consumer richness on prey biomass depending on consumer specialization. Our study was explicitly designed to test these predictions and in fact, consumer specialization was an important factor influencing consumer richness effects in our experiment. However, the experimental data did not match the model prediction of Thébault and Loreau (2003) with regard to sign and magnitude of the effects. We found a stronger decrease in prey biovolume with consumer specialists (more precisely, S1) rather than consumer generalists, whereas Thébault and Loreau (2003) predicted stronger prey suppression with generalists, as these increased consumption of all prey species. The difference between the model predictions and our experimental outcome is explained by species-specific differences in grazing rates. Our model reproduced the finding of Thébault and Loreau (2003) when we assumed equal grazing rates for all consumers, whereas our empirical findings were reproduced when we included different grazing rates and particularly a high grazing rate for S1 in our model.

Combining our experiment and the model including specific consumer grazing rates thus allows the conclusion that specialist consumers may have stronger assemblage-wide grazing effects than generalists if they exhibit high per-capita grazing rates. Specialist consumers were shown to be more effective in either locating, capturing or consuming prey in case of some insects (Egan and Funk 2006, Wang and Keller 2002), marine invertebrates (Behrens Yamada and Boulding 1998) and in zooplankton (Norberg 2004). Thus, the pattern that a specialist reduces prey biovolume more than the relatively 'inefficient' generalists might be common in nature. This motivates further research on the role of tradeoffs between species-specific traits in the biodiversity–ecosystem function relationship.

S1 specialized on the two most productive algae A1 and A2, which means that besides effectively reducing prey biovolume, it released the less productive algal species A4 from competition, thereby substantially changing prey community composition and reducing prey evenness. Similar effects on prey composition were shown before, as consumer richness reduced prey richness by shifting prey community composition from a diverse assemblage of

edible autotrophs towards few grazing resistant invertebrates in a seagrass community (Duffy et al. 2003), and/or altered prey community structure (Bruno and O'Connor 2005, Burkepile and Hay 2008). Burkepile and Hay (2008) demonstrated in a coral reef community that herbivore richness reduced algal biomass and diversity, suppressing upright macroalgae and turf algae and facilitating crustose coralline algae, thus also facilitating coral survivorship and growth. A mesocosm study revealed that predator richness effects on a trophic cascade strongly depended on predator specialization and grazing rate (Bruno and O'Connor 2005). Generalist carnivores substantially limited herbivore abundance and grazing, resulting in doubled macroalgal biomass, while this trophic cascade was much weaker in presence of a less effective carnivore and completely short-circuited in presence of an omnivore, which fed on both herbivores and macroalgae.

Consumer specialization and species-specific traits of consumers and their prey such as grazing and growth rates determined consumer diversity effects in our study within and across trophic levels. In a recent study, Narwani and Mazumder (2010) demonstrated that consumer feeding selectivity and prey community composition were also the key factors in determining the direction and magnitude of prey diversity effects on consumption rates. Most likely, these findings are also relevant for natural communities. Feeding specialization, for instance, is an important feature that determines species' niche partitioning in nature. In order to estimate the effects of consumer species loss, it is therefore crucial to carefully consider specific traits of the species lost and of the other interacting food web components.

Hypothesis H3 and H4: consumer richness effects on consumer biovolume production

Increasing consumer richness increased total consumer biovolume, corroborating previous empirical studies (Gamfeldt et al. 2005, Moorthi et al. 2008, Steiner et al. 2005). We successfully investigated the tentative mechanisms behind the increased production. Consumer biovolume was higher for generalists compared to specialists, which is in accordance with Thébault and Loreau (2003), and may be explained by the broader diet spectrum of the generalists. Consumer biovolume was highest in mixed combinations, including generalists and specialists, indicating that consumer functional diversity (i.e. more diverse feeding strategies) increases complementary resource use.

Using the process-based model, we disentangled how diversity effects on consumer biovolume were driven by interactions between consumer specialization and species-specific growth and grazing rates. The specialist S1 did not reach substantially higher biovolume or abundances compared to other consumer species at any time of the experiment, with a few exceptions (Supplementary material Appendix 1 and 2 Fig. A1.2, A2.1, A2.2). However, it consistently suppressed prey biovolume much more efficiently than the other consumers in all treatments (Fig. 2). Thus its strong effect on the prey assemblage was not due to higher abundances/biovolume but due to its unique traits. The model suggested that these traits were 1) its specialization on the two most productive algal species and 2) its higher grazing rate compared to other consumers.

S1 is the prototype of a keystone predator (Menge 1995, Paine 1966), a species strongly affecting trophic interactions while not being important in terms of relative biomass. Keystone effects have also been observed in other microcosm studies such as ours. Worsfold et al. (2009), for example, demonstrated that a specialist predator, though rare, substantially altered the effects of a generalist predator on a prey community in an experiment with three trophic levels.

Generalist consumers produced higher biovolume than specialists and dominated in mixtures with specialists (two species treatments) despite attaining low monoculture yields in our experiment. Here, discrepancies between the model and the experiment occurred regarding consumer biovolume in the combination of S1 and G1 and the treatment with four consumers. In the model, these treatments resulted in low total consumer biovolume, while they were high in the experiment. In the experiment, S1 might have promoted the growth of G1 by preventing grazing inhibition of G1 induced by too high prey concentrations. Reduced grazing and consumer growth have been observed when culturing the generalist ciliate G1 with prey concentrations comparable to the high concentrations used in our experiment (Moorthi unpubl.). Similarly, Montagnes and Lessard (1999) demonstrated grazing inhibition at high food concentrations exceeding 10^4 prey cells per ml for the planktonic marine ciliate *Strombidinopsis multiauris* in laboratory experiments. In our experiment, ciliates were inoculated with high prey densities even exceeding 10^4 prey cells per ml, which might have been high enough to hamper consumer grazing and, thus, secondary production. As the model does not consider facilitative effects by reducing prey density, the potential positive effect of S1 on G1 was not reproduced. In the model, S1 increased in combination with G1 until it overexploited the two most productive prey species, A1 and A2, hampering its own growth and that of G1. Thus, in the model the combination of S1 and G1 resulted in a low total consumer biovolume.

The second specialist S2 indirectly promoted the generalists by a different mechanism, i.e. by grazing on the filamentous cyanobacteria A4 that was not much preferred by any of the other consumers, releasing the more productive prey A1 and A2 from competition. Thereby, the generalist consumers encountered higher absolute and relative abundances of their preferred prey. These results are supported by a study of Kratina et al. (2007), who demonstrated in experimental microcosms that both, the density and diversity of non-prey species (not preferred species) can decrease predation rates and thus significantly weaken the strength of predator–prey interactions.

Overall, the results of our study indicate that with respect to consumer diversity effects on prey and consumers, species-specific growth and grazing rates may be at least equally important as consumer richness and specialization in driving consumer diversity effects within and across trophic levels. The mechanistic explanation behind consumer richness effects in our study was a selection effect, i.e. the inclusion of a very effective specialist consumer,

which emphasizes that diversity effects on ecosystem functioning depend on multiple species traits and their potential tradeoffs.

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Supplementary material (available online as Appendix oik-01219 at <www.oikosjournal.org/readers/appendix>). Appendix 1, 2.