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Primary Research Paper

Nutrient limitation in detritus-based microcosms in *Sarracenia purpurea*

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Abstract

Most prior work on the role of top-down and bottom-up effects in aquatic communities has ignored the significant detrital component that occurs in natural systems. We investigated the effects of specific nutrients (carbon, phosphorus, and nitrogen), as well as a top predator (the mosquito *Wyeomyia smithii*), on the structure of the detritivore community found in the water-filled leaves of the pitcher plant *Sarracenia purpurea*. The concentrations of three nutrients and the presence of the predators were manipulated in a factorial design, while the response of the remaining community was quantified. Bacterial growth was found to be strongly carbon-limited and somewhat less limited by phosphorus and there was an interaction between the effects of the two nutrients. Neither carbon or phosphorus addition affected protozoan or rotifer abundance, and nitrogen had only a minor effect. The presence of the predator, however, significantly reduced the abundance of the four numerically dominant bacteriovores. There were no interactions between top-down and bottom-up effects; the strong direct reciprocal effects between adjacent trophic levels seem to be greatly attenuated as they are propagated farther up or down the food chain.

Introduction

Aquatic communities are structured by multiple forces within trophic webs: light and nutrient availability from lower trophic levels can determine potential growth whereas consumption from higher trophic levels can restrict population sizes which may affect competitive interactions and species diversity. Although these ''bottom-up'' and ''top-down'' forces have been well documented in many communities, ecologists now seek generalizations about their relative magnitudes and interactions. In typical aquatic communities, nutrients such as nitrogen and phosphorus are generally thought to have strong bottom-up effects through limitation of phytoplankton growth (see, e.g., Bell et al., 1993; Vrede et al., 1999), while top predators are thought to exert large effects that ''cascade'' down through trophic levels below, affecting abundance and diversity (see, e.g., Carpenter et al., 1985). Both resources and predators can affect intermediate species, but bottom-up and top-down effects generally diminish as they are passed through each trophic level (see, e.g., McQueen et al., 1986).

Many studies of aquatic communities, however, overlook their significant detrital component, although the great majority of primary production eventually must pass through detritivores (O'Neill & Reichle, 1980; Wetzel & Ward, 1992; Azam et al., 1994). In aquatic systems, bacteria are known to play a vital role in the mineralization of detritus,

potentially controlling nutrient flow to the remainder of the community (Sterner & Elser, 2002). Some freshwater studies have suggested that bacteria are often nutrient, especially phosphorus, limited (e.g., Jones, 1977; Wang et al., 1992; Vrede et al., 1999). Bacteria are thought to generally outcompete algae for these nutrients, but can in turn be dependent on algae, directly or indirectly, for dissolved organic carbon (Bratbak & Thingstad, 1985). These studies have generally been conducted under laboratory or artificial conditions in the absence of consumers and other higher trophic levels (however, see Vadstein, 2000). Studies that combine nutrient additions with top-predator manipulations are rare. Rosemond et al. (2001) found significant effects of nutrients and predators in stream detritus communities and suggested that effects of nutrients moving up through food webs may be greater in detrital than in autotrophic communities. Conversely, Kaufman et al. (2002) manipulated nutrients and predators in tree-hole communities and found that predators had a much larger effect than nutrients on the abundances of bacteriovores. The roles of nutrients and consumers in detrital communities remain unclear.

The communities found in the water-filled leaves of the pitcher plant *Sarracenia purpurea* L. are an ideal detritus-based system in which to study the relative roles of nutrients and predators in a natural community. Newly opened leaves fill with rainfall and attract a variety of insects that drown in the water. Nutrients from these insects form the energetic basis for a specialized aquatic community that colonizes the leaf. This inquiline community includes bacteria as primary decomposers and protozoans and rotifers (mainly *Habrotrocha* cf. *rosa* Donner) as bacteriovores. Larvae of the pitcher-plant mosquito *Wyeomyia smithii* are top predators feeding primarily on the bacteriovores (e.g., Addicott, 1974; Kneitel & Miller, 2002; Miller & Kneitel, 2005), although they may also consume bacteria. Primary producers, such as algae, are very rarely found in active pitchers in North America (Buckley et al., 2003). Although previous studies have quantified species interactions in this community (e.g., Kneitel & Miller, 2002; Miller et al., 2002), no study has used this community to investigate nutrient limitation.

This detrital community is novel in that the component species may also interact with the host plant through nutrient dynamics. Carnivorous plants are thought to be nitrogen or phosphorus limited, with the nutrients from decomposing prey ultimately being absorbed by the plant itself (Bradshaw & Creelman, 1984; Bledzki & Ellison, 1998). Species in the water-filled leaves may control the rate of flow of nutrients to the plant or actually compete with the plant for nutrients obtained through prey capture. However, nitrogen uptake by the plant is relatively slow (Bradshaw & Creelman, 1984), whereas bacteria uptake of nutrients is thought to be relatively rapid (Vadstein, 2000). Implementing daily additions of nutrients (i.e., a press experiment) over 7 days allows a significant amount of time for bacteria (generation times of 3–4 h) and protozoans (generation times of approximately 8–10 h) to respond, while minimizing significant interactions with the host plant.

We conducted a press experiment by adding all combinations of elemental carbon, nitrogen, and phosphorus, and larval mosquitoes into natural pitcher plant leaves in a factorial design. The responses of bacteria, protozoans, and rotifers to these top-down and bottom-up treatments were quantified. The first objective was to determine if carbon, nitrogen, or phosphorus (or some combination thereof) is limiting for the species within the inquiline community of the pitcher plant. While phosphorus is often limiting for communities in freshwater systems, very little is known about detritivorous communities where the lack of primary producers may limit the availability of dissolved organic carbon. The second objective was to quantify any interactions between bottom-up (nutrient limitation) and top-down (mosquito predation) forces in limiting the abundances of bacteria, protozoans or rotifers.

Materials and methods

Sarracenia purpurea is found in wetlands throughout much of eastern North America, from throughout Canada to northern Florida. The populations in north Florida, Mississippi and Alabama may represent a separate species, *Sarracenia rosea* (Naczi et al., 1999; Ellison et al., 2004). Our research was conducted in Crystal Bog in the Apalachicola National Forest, near Wilma,

Florida. The bog is found in a treeless area that follows the edge of a wood thicket near the Hostage River, with *Cyrilla racemiflora* and *Taxodium distichum* running along the river on one side of the bog and dense *Aristrida stricta* along the other side. The dominant herbaceous cover includes *Aristida stricta, Xyris fimbriata, Eriocaulon compressum*, and other carnivorous species such as *Sarracenia flava* and *S. psittacina*.

We added mosquito larvae and three different nutrients to inquiline communities in a factorial design. Glucose was added as an organic carbon (C) source, ammonium chloride (NH_4Cl) as an inorganic nitrogen (N) source, and sodium phosphate monobasic dihydrate ($NaH_2PO_4 \times 2H_2O$) as an inorganic phosphorus (P) source. The full design consisted of five replicates of 16 treatments (2 mosquito treatments $\times$ 2C $\times$ 2N $\times$ 2P).

We determined ambient concentrations of selected nutrients in randomly chosen leaves using cadmium reduction (N), calicylate (ammonium), and ascorbic acid (phosphate) spectrophotometry, all according to standard methods (APHA, 1985). Average ammonium level was 0.51 ppm (s.d. 0.73, $n = 157$ leaves), average nitrate was 0.16 ppm (s.d. 0.13, $n = 68$), and average orthophosphate level was 0.36 ppm (s.d. 0.61, $n = 120$). Ambient carbon levels were not determined.

Treatments were initiated in the first week of October of 2002. Plants were located along a 5×50 m area strip running north to south parallel to the river. While the plant community was similar along this entire strip, soil moisture was decreased and the density of *Aristida stricta* increased towards the northern end. One healthy water-filled (>10 ml) leaf was chosen on each of 80 different pitcher plants in Crystal Bog. Because previous work has demonstrated that soil moisture is correlated with small changes in the inquiline community (Buckley et al., 2004), the leaves were divided into five approximately 5×10 m blocks, and one treatment was assigned randomly to each leaf within a block. We homogenized the initial communities by collecting a total of 1500 ml of fluid from >80 plants throughout the field. This water was taken back to the laboratory, where mosquito larvae were removed and set aside, and any large matter (sticks, dead leaves, etc.) was removed by passage through a 500-μm filter. On the first day of the experiment, 10 ml of the filtered and mosquito-free water was placed into each of 80 macrocentrifuge tubes. Five 3rd instar mosquito larvae were added to each of 40 tubes; the other 40 received no mosquito larvae. The volumes and numbers of mosquitoes were chosen to approximate ambient levels (Miller et al., 1994; Kneitel & Miller, 2002).

In the field, the selected leaves were washed out thoroughly with deionized water and emptied before the contents of one tube were poured in. A 0.25-ml aliquot of fluid was then taken from each replicate and brought back to the lab for analysis. Mesh bags were placed over the leaves to prevent any new insects from entering.

Nutrient treatments were devised to increase ambient nutrient levels but to be similar to those used in previous manipulations of aquatic systems (e.g., Carlsson & Caron, 2001). A small amount (0.1 ml) of concentrated nutrient solution (1800 ppm glucose, 107 ppm ammonium chloride, or 31 ppm sodium phosphate) was added daily to each appropriate community during the experiment. These additions should increase ambient N and P levels by approximately 100% and 50%, respectively. The degree to which carbon levels were increased is unknown.

Fluid in the leaves was sampled every 24 h by gently mixing the fluid, then removing a 0.25 ml mosquito-free aliquot using a sterile pipette. The appropriate nutrient enrichments were then added, with sterile water being added to control leaves. The fluid in each leaf was mixed well with a sterile pipette, and the leaf was re-covered with its mesh bag. On the 7th day, the entire contents of each experimental leaf were removed with a sterile pipette and placed in a sterile 50-ml macrocentrifuge tube for transport to the laboratory.

The 0.25-ml aliquot of fluid taken daily from each pitcher was used to census bacteria, protozoans, and rotifers. Cell growth on agar plates was used to estimate the relative abundance of bacteria by counting colony forming units (CFUs). Dilution plates usually are only able to culture between 0.1 and 10% of the viable organisms in samples (Kirk et al., 2004) and so should be evaluated only as a measure of relative bacterial abundance. Prior research has demonstrated that CFUs from plate counts do increase proportionally as prey are introduced into pitchers (Kneitel & Miller, 2002; Miller et al., 2002) and that bacterial abundances

are significantly correlated with changes in the number of ants captured by pitchers through time (Miller & Kneitel, 2005). A 0.05-ml subsample was used to create a 10^{-4} dilution, then 0.1 ml of the dilution was spread on a half-strength Luria broth plate (Cochran-Stafira & von Ende, 1998; Kneitel & Miller, 2002). The plates were incubated at 28 °C for 72 h, after which the number of CFUs were determined by direct count.

A 0.l-ml subsample of the 0.25-ml aliquot was used for determination of protozoan and rotifer abundance and richness. Live samples were counted using a Palmer counting cell under a compound microscope at 100× (Kneitel & Miller, 2002). We find that it is very difficult to count some species using preserved samples because individuals can be amorphous and often occur inside detritus. If the concentrations of protozoa were over 3000 individuals/ml, a 0.05 ml subsample was counted with a hemacytometer. Unfortunately, use of Palmer cells gave a poor resolution of rotifer abundances, which are often on the order of 10/ml.

When the entire contents of the pitchers were collected on the last day, the volumes were recorded and the mosquito larvae were counted and photographed. Bacteria, rotifers, and protozoans were counted as above, and mosquito lengths were determined from the photographs using Sigma Scan 4.0 software (SYSTAT Software Inc., Richmond, CA, USA). Despite the mesh bags covering the leaves, new dead ants were found in some leaves. Ant numbers were initially used as a covariate in the analyses, but were never found to contribute significantly; the results shown here do not include ant numbers. Any large-insect debris in the water was also recorded. Nitrate, ammonium, and orthophosphate concentrations were also determined for two randomly chosen leaves from each treatment according to standard methods (APHA, 1985) as noted above.

After log transformation, the effects of nutrient additions on nutrient levels (log x) and mosquito lengths (log (x + 1)) at day 7 were determined by ANOVA. The treatments effects were quantified after waiting 3 days for any species responses to become established. Treatment effects were analyzed with a repeated-measures restricted maximum-likelihood model, with nutrients and mosquitoes as main fixed effects in a full factorial design, with day as the repeated factor (days 4–7), and with leaf as a nested random effect. As these analyses demonstrated no day-by-treatment interactions, we chose to average the abundances from days 4 to 7 for bacteria and protozoa, then to test for treatment effects using a full factorial ANOVA (2 N × 2 C × 2 P × 2 mosquito levels). Means are presented throughout with standard errors. All analyses were conducted with JMP 5.0 (SAS Institute, Cary, NC, USA).

Results

One leaf was lost to herbivory during the experiment. Little rain fell during the experiment and the average leaf content recovered was 8.2 ml (0.32) of the original 10 ml. The mean number of mosquitoes recovered from the mosquito-addition treatments was 3.2 (0.33) as some of the original 5 mosquitoes died or emerged as adults. Mean mosquito lengths were not significantly affected by the addition of any nutrient (Table 1). The mean mosquito number in the ''mosquito-free'' treatments was 0.6(0.18). These individuals were first instars that probably hatched from eggs that survived the initial washing of each pitcher.

Nutrient levels

The nutrient additions generally had small effects on nutrient availability in leaves. Nitrate levels were not significantly affected by the daily addition of ammonium chloride, but did significantly increase with the addition of glucose and decrease with the addition of mosquito larvae (Table 2, Fig. 1). There were also several significant

Table 1. Summary of the ANOVA results for the effects of nutrient (C, N, and P) treatments on average mosquito length

Source	df	*F* Ratio	Prob > *F*
C	1	1.22	0.279
N	1	0.30	0.587
P	1	1.64	0.211
C*N	1	0.86	0.363
C*P	1	0.19	0.665
N*P	1	1.51	0.229
C*N*P	1	3.77	0.062

interactions among treatments on nitrate levels that are difficult to interpret (Table 2). Ammonium levels also were not significantly affected by the addition of ammonium chloride, but marginally declined due to glucose addition. Finally, orthophosphate levels were not affected significantly by any treatments, including the addition of sodium phosphate, although there was a significant interaction between the additions of glucose and ammonium chloride (Table 2).

Bacterial abundance

Leaves varied widely in bacterial abundance (estimated by CFUs), ranging from undetectable (less than 10^4 cells per ml) to over 10^8 cells per ml. Addition of carbon had a strong effect, increasing CFUs by almost an order of magnitude (Table 3). The addition of phosphorus also increased the number of CFUs, but primarily in combination with carbon, resulting in positive interactions between carbon and phosphorus (Fig. 2). The addition of nitrogen had no effect on bacteria, nor did presence of mosquitoes.

Table 2. *F*-values from the ANOVA of the effects of nutrient (C, N, and P) addition treatments on water nutrient levels in the leaves of *Sarracenia purpurea*

Source	NO_3	NH_4	OPO_4
C	15.18**	4.17$^+$	0.04
N	1.69	1.35	0.88
P	0.23	0.07	0.05
Mosq	7.23*	1.35	0.28
C*N	0.88	1.03	13.39**
C*P	0.63	2.28	0.74
N*P	7.46*	1.22	0.01
N*Mosq	0.01	0.63	0.72
C*Mosq	1.45	0.02	0.59
P*Mosq	1.15	0.21	2.94
C*N*P	0.00	0.29	3.86
C*N*Mosq	6.49*	0.04	1.85
C*P*Mosq	3.32	0.70	2.59
N*P*Mosq	11.16**	0.02	0.89
C*N*P*Mosq	1.22	0.05	0.36
ANOVA	3.93**	0.89	2.14$^+$
R square	0.80	0.46	0.71

$^+p < 0.08$, $*p < 0.05$, $**p < 0.01$, df = 1 for each variable in the model.

Bacteriovore abundance

Three species constituted over 98% of the number of protist individuals observed: *Bodo* (possibly *B. menges*), *Poterioochromonas* (species undetermined), and *Colpoda* (species undetermined). A fourth bacteriovore, the pitcher-plant rotifer *Habrotrocha rosa*, was also a common inhabitant. Nitrogen addition significantly decreased the abundance of *Poterioochromonas*, but no other effects of nutrients on bacteriovores were detected (Table 3). Mosquitoes reduced abundances of all four bacteriovore species significantly (Table 3), with particularly strong effects on the protozoa species (Fig. 3). No significant interactions among nutrient additions or between nutrient additions and mosquito treatments were observed.

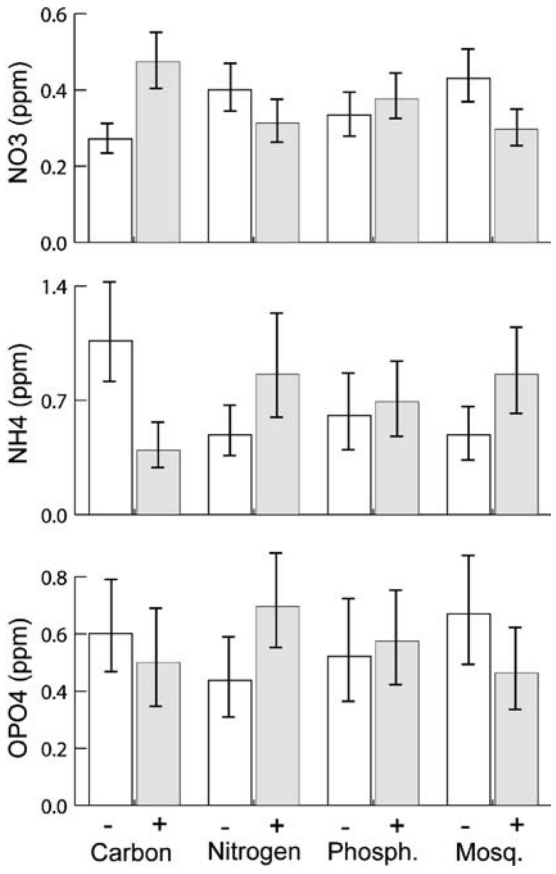

Figure 1. The effects of the addition of glucose (C) , ammonium chloride (N), sodium phosphate (P), and mosquitoes on the concentrations of nitrate, ammonium, and orthophosphate levels. Means and standard errors shown are back transformed from originally log-transformed data.

Table 3. Summary of the ANOVA results for the effects of nutrient (C, N, and P) and predator (mosquito) treatments on bacteria and bacteriovore abundances. The *F* value for the full model for each species and the variance explained are in the final two rows

Source	Poterioochromonas		Bodo		Colpoda		Rotifers		Bacteria	
	F Ratio	Prob > *F*	*F* Ratio	Prob > *F*	*F* Ratio	Prob > *F*	*F* Ratio	Prob > *F*	*F* Ratio	Prob > *F*
C	1.56	0.216	0.30	0.589	0.08	0.777	1.30	0.258	16.45	0.0001
N	7.25	0.009	1.39	0.243	0.37	0.544	0.16	0.687	1.10	0.298
P	0.15	0.703	0.09	0.763	0.36	0.553	0.07	0.797	5.45	0.023
Mosq	21.62	< 0.0001	4.66	0.035	19.05	< 0.0001	20.65	< 0.0001	0.5392	0.466
C*N	1.57	0.215	0.05	0.831	0.11	0.740	1.98	0.165	3.20	0.079
C*P	0.12	0.731	0.41	0.523	1.14	0.290	0.42	0.517	4.10	0.047
N*P	0.37	0.543	0.06	0.813	0.00	0.982	2.52	0.118	0.01	0.917
N*Mosq	0.99	0.323	0.38	0.541	0.35	0.558	2.63	0.110	1.63	0.206
C*Mosq	0.00	0.996	0.21	0.645	0.67	0.417	0.31	0.581	0.90	0.347
P*Mosq	0.20	0.657	1.40	0.240	2.30	0.135	2.37	0.129	0.72	0.399
C*N*P	1.00	0.321	2.95	0.091	0.05	0.816	1.04	0.312	4.28	0.043
C*N*Mosq	1.06	0.307	0.66	0.419	1.04	0.319	0.11	0.746	0.11	0.737
C*P*Mosq	0.12	0.733	0.43	0.514	0.00	0.963	0.78	0.382	0.56	0.456
N*P*Mosq	0.01	0.928	0.01	0.920	0.01	0.925	1.27	0.265	1.15	0.289
C*N*P*Mosq	0.25	0.620	0.45	0.504	0.11	0.740	0.41	0.523	0.17	0.682
ANOVA	2.45	0.007	0.90	0.566	1.71	0.073	2.41	0.008	2.65	0.004
R square		0.368		0.177		0.289		0.365		0.387

Discussion

Although the nutrient-addition treatments had some effects on the communities we studied, these effects did not move up the food chain to higher trophic levels, as no effects of carbon or phosphorus addition on bacteriovore abundances were observed. In fact, the only nutrient limitation observed was a significant effect of nitrogen addition on one protist species, *Poterioochromonas*.

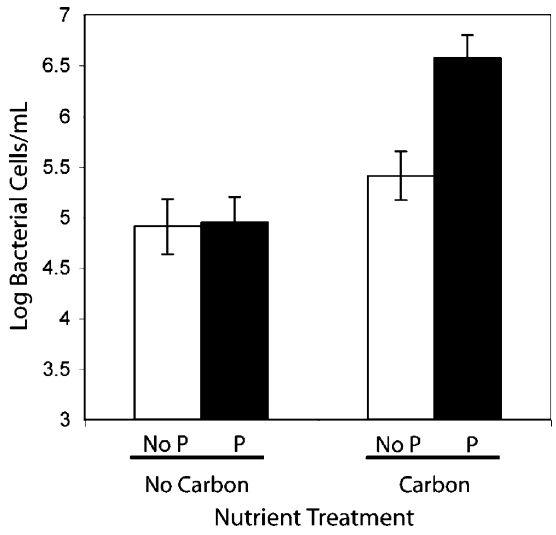

Figure 2. Interactions between the effects of carbon and phosphorus supplements on bacterial abundance in the water-filled leaves of *Sarracenia purpurea*. Error bars show standard errors.

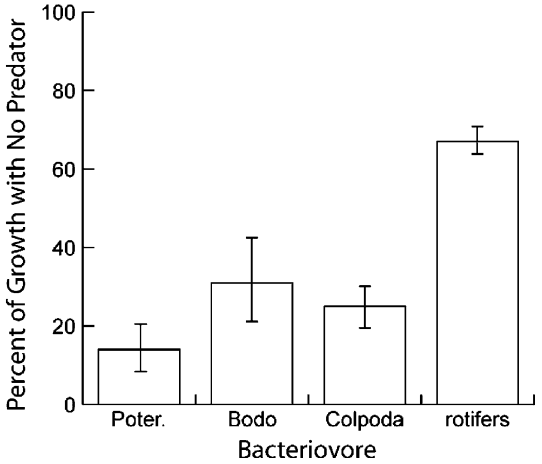

Figure 3. The effect of predation by larvae of the mosquito *Wyeomyia smithii* on the abundances of bacteriovores. Values are given as the mean abundance with the predator present as a proportion of the abundance of each species when the predator is absent. Means and standard errors shown are back transformed from originally log-transformed data.

Instead, the presence of the predator, *Wyeomyia smithii*, significantly reduced the abundance of all four bacteriovores we counted.

Top-down and bottom-up effects did not appear to interact, which is consistent with Kneitel and Miller (2002). Although treatments demonstrated significant direct effects of resources on bacteria and of mosquitoes on protozoa and rotifers, almost no indirect effects through intermediate trophic levels were apparent. For example, carbon addition increased bacterial abundance, but this increase in bacteria had no effect on the abundance of any of the bacteriovores. Similarly, mosquitoes had strong effects on bacteriovores, but this suppression of bacteriovore abundances did not cascade down to cause increases in bacteria.

The bacterial and bacteriovore responses to nutrient additions should be interpreted with some caution. The use of plate counts to enumerate relative changes in bacterial abundance assumes that the proportion of total number of bacterial cells that will grow on the media is independent of treatment. If for example, the addition of carbon disproportionately increases the abundance of culturable bacteria, then we may have overestimated the potential effects of carbon addition on the bacteriovores (however, see Bakken, 1997).

Species-specific responses of the bacteriovores must be understood in the context of the natural history of each species. The ciliate *Colpoda* is a filter-feeding bacteriovore (Lee et al., 2000). *Bodo* is a relatively small raptorial flagellate, known to be primarily a bacteriovore. The chrysophyte, *Poterioochromonas*, is also a small flagellate and may be a mixotroph (Lee et al., 2000). However, the *Poterioochromonas* in pitcher plants in N. Florida have never been observed to contain chloroplasts (Miller, personal observation) and appear to actively feed on bacteria. In our lab, all three species appear to go through several generations per day; we estimate that generation times of approximately 8 h. The bdelloid rotifer *H. rosa* is largely restricted to the leaves of *S. purpurea* (Bledzki & Ellison, 2003) and feeds on bacteria and particulate matter. It has a slower population growth rate than the protozoa, with intrinsic growth rates estimated at 0.1–0.3 and doubling times from 2 to 6 days (Bledzki & Ellison, 1998; Kneitel, 2002).

Species responses to nutrients or predation are the result of some combination of direct treatment effects and indirect effects occurring through the rest of the community (see Bender et al., 1984). It may be, for example, that effects of nutrients addition on *Bodo* were masked by competition with *Colpoda* (Cochran-Stafira & von Ende, 1998). However, such indirect interactions would also have generally resulted in significant interactions between top-down and bottom-up effects. The lack of interaction terms suggests that nutrient effects were consistent with and without the significant effects of predation, such that competition was probably not masking individual responses to nutrients.

Previous studies in pitcher-plant microcosms found similar, but not completely consistent, results. Manipulating mosquito abundance has been shown to affect bacteriovores and generally not bacteria, as found in the study reported here. Addition of insect detritus (dead fire ants) to pitchers produced an increase in bacteria (Kneitel & Miller, 2002; Miller et al., 2002), similar to the response we observed following the addition of carbon. However, these insect-addition treatments in earlier experiments have also led to increases in bacteriovore abundance (Kneitel & Miller, 2002; Miller et al., 2002), but we did not observe such increases following the addition of nutrients in this study. Previous studies were carried out over a longer time period (e.g., 23 days), allowing a longer time for bacteriovores to increase with bacteria in the absence of mosquito predators. In addition, the ant-addition treatments used in previous studies may either have added more nutrients overall or added a unique combination of nutrients.

We know of only two similar studies in detritus-based ecosystems. Rosemond et al. (2001) found that indirect bottom-up effects of phosphorus on chironomids were greater than the direct top-down effects of predatory fish and shrimp in a detritus-based food web in a tropical stream. Kaufman et al. (2002) controlled nutrients and predators in treehole communities of mosquitoes, protozoa, rotifers, and bacteria, which are natural microcosms similar to those used in this study (Srivastava et al., 2004). They found carbon to limit bacterial growth, whereas phosphorus and nitrogen had no significant effects. As in our study, they concluded that top-down effects of mosquito

predators were much greater than bottom-up effects of nutrients.

Taken together, these studies illustrate the difficulties of drawing conclusions about the relative strengths of and interactions between top-down and bottom-up (e.g., trophic-cascade) effects from experiments in natural communities. Generally both top-down and bottom-up effects have been found to be significant, but their relative importance depends on the degree to which each is manipulated. We suggest that the magnitude of the top-down and bottom-up treatments should be standardized by the natural variance of each to allow meaningful comparisons for any given community (as they were in our study). If treatments are standardized, however, the interactions between top-down and bottom-up effects may be relatively weak, if effect strengths dramatically decline as they pass through trophic levels. This generalization may prove a poor one if specific nutrient requirements of higher trophic levels occur (see, e.g., Rosemond et al., 2001). Overall, the damping of resource and predator effects as they move through trophic levels is a perplexing result that calls for further study.

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