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The importance of bacterivorous protists in the decomposition of stream leaf litter

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SUMMARY

1. Allochthonous organic matter, in the form of senesced leaves, is a major source of carbon supporting detrital food webs. While studies have documented the role of bacteria and fungi in the decomposition of leaf litter, little information is available regarding the role of protists in the decomposition process.
2. We tested the hypothesis that the presence of stream-dwelling bacterivorous protists leads to an increased rate of leaf decomposition through grazing pressure on bacteria. We isolated live protists from decomposing leaves collected in a stream in Northern Virginia, U.S.A. (Goose Creek) and established laboratory cultures of common bacterivorous protists.
3. Recently senesced leaves from the field were used in laboratory microcosm experiments to determine if the rate of litter decomposition differed between four treatments: bacteria only, bacteria + flagellates, bacteria + flagellates + ciliates, autoclaved stream water (control). We determined the dry weight of leaf remaining, bacterial abundance, flagellate abundance and ciliate abundance for each replicate on days 0, 7, 14, 30, 60 and 120.
4. The rate of leaf decomposition was significantly higher in treatments with protists than without and bacterial abundance declined in protist treatments compared with bacteria only treatment. Weight loss in the presence of flagellates was three to four times higher when protists were present compared with treatments with bacteria alone. These results provide experimental evidence that protists could play a significant role in the detrital processes of streams.

Keywords: bacteria, leaf decomposition, litter, protist, stream

Introduction

The important energetic role of detritus has long been recognised in terrestrial and aquatic ecosystems. In many streams, inputs of leaf litter comprise all or a significant part of the food base (Cummins *et al.*, 1989). The widely accepted representation of litter decomposition involves early leaching of soluble compounds, followed by microbial colonisation and growth, culminating in fragmentation by mechanical means and invertebrate shredders. Factors known to influence this process in streams include temperature, flow, nutrients, leaf chemistry and macroinvertebrate consumers (Bärlocher & Kendrick, 1975; Findlay *et al.*, 1996; Ostrofsky, 1997; Wetzel, 2001a). Macroinvertebrates have a large effect both through the mechanical shredding of litter and their microbial grazing (Webster & Benfield, 1986). The microbes (bacteria and fungi) are also responsible for a significant fraction of total litter decomposition in streams (Heiber & Gessner, 2002) and much of their biomass is transferred to higher trophic levels (Bott & Borchardt, 1999; Gessner & Chauvet, 2002). However, how protists (the smallest consumers of microbes) affect *stream* leaf matter processing is largely unknown. This is despite the fact that several freshwater protists rely primarily

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on bacteria for their food source (Finlay & Esteban, 1998) and decomposing litter is a rich habitat for bacteria (Suberkropp & Klug, 1976; Findlay, Howe & Austin, 1990). One would thus expect protists to be abundant in litter and to have the potential to influence its decomposition through their effect on the microbes.

Grazing by protists on water column and sediment bacteria has been documented in other freshwater and marine environments, and some researchers have suggested that protists may act as top-down controls of bacterial abundance and carbon flow (Stuart, Lucas & Newell, 1981; Fenchel, 1986; Berninger, Finlay & Kuuppo-Leinikki, 1991; Finlay & Esteban, 1998). Microbial stimulation by consumers has been suggested for systems as diverse as soils and pelagic food webs (Sherr & Sherr, 1994; Mikola & Setälä, 1998). If protistan consumers keep bacteria in log phase growth by actively grazing on cells, or if protists produce waste products that are readily metabolised by bacteria and fungi (Johannes, 1965; Fenchel & Harrison, 1976; Fenchel & Jørgensen, 1977; Jansson *et al*., 1999), then protists could have a negative effect on detrital biomass in streams.

We designed experiments to test the hypothesis that the presence of bacterivorous protists influences bacterial biomass and decomposition of stream litter. We isolated stream-dwelling bacterivorous ciliates and flagellates from submerged leaf litter collected from a warm-water, low gradient stream, cultured the protists in the laboratory and then conducted controlled microcosm experiments. Using recently senesced leaves from the dominant deciduous tree box elder (*Acer negundo* L.) along our study stream, and protist and bacterial densities comparable with that on leaf litter in the stream, we measured the rate of litter decomposition in the presence of bacteria alone, with both bacteria and flagellates present, and with bacteria, flagellates and ciliates present. We tracked litter mass loss, as well as protist and bacterial abundances at regular intervals over a 120-day period.

Methods

Study site

Leaves for our experiments and protists for cultures were collected from a fourth-order Piedmont stream (Goose Creek) in Virginia, U.S.A. The study site, representative fauna, and composition of the riparian vegetation are described extensively elsewhere (e.g. Palmer, 1990; Poff *et al*., 1993; Hakenkamp *et al*., 2001). The stream is perennial but flow is typically near zero for 3–5 months of the year (summer into autumn; Palmer *et al*., 2000). The streambed has abundant leaf litter throughout the year and >50% of the leaf 'packs' on the streambed consist of single leaves most of which are box elder (Silver *et al*., 2002). The rest of the leaves are typically found in large accumulations in stagnant areas near woody debris (Palmer *et al*., 1995). To determine which protists to use in our experiments, we collected decomposing box elder leaves from the streambed in the autumn of 1999. Bacterivorous protists were the most common trophic group on the leaves (using Foissner, Berger & Schaumburg, 1999 and references there-in to assign trophic groups), outnumbering predaceous and algivorous ciliates by a factor of almost 10 (Ribblett, 2002). Thus, we focused on bacterial microbes as the decomposers and isolated and cultured bacterivorous ciliates and flagellates for our experiments.

Cultures

Box elder leaves freshly collected from the streambed were rinsed with filtered stream water (Whatman GF/F filter) and about 600 mLs of the resulting fluid were placed in a sterile 1-L culture flask containing 2–3 g of crushed, dry, box elder leaves. The infusion was maintained at 10 °C under a 14 : 10 light : dark cycle, with a light flux of 80 μE m^{-2} s^{-1} supplied by cool-white fluorescent bulbs. The infusion was incubated for 3 weeks, gently mixed, subsampled and filtered to produce two fractions: a < 5 μm (Whatman Nucleopore® filter, Whatman Inc., Clifton, NJ, U.S.A.) for isolating protists and a < 0.45 μm (Acrodisc® filter, Gelman Sciences, East Hills, NY, U.S.A.) size-fraction for bacterial cultures. A 50-mL flask of sterile Cerophyll™ medium (Lee & Soldo, 1992) was inoculated with 1 mL of the <0.45 μm size-fraction to establish a mixed bacteria culture. The bacterial culture was maintained in the dark at 10 °C, and propagated by weekly transfer into sterile medium.

The <5 μm size-fraction was serially diluted into 3-mL tubes to increase the likelihood of obtaining a single flagellate species. Stock bacterial culture was added to each tube, and then tubes were incubated in

the dark at 10 °C. One, 3-mL tube containing an assemblage of bacteria and a single flagellate, *Spumella* sp., was propagated by weekly transfer into 50-mL flasks of sterile Cerophyll™ medium – thus, the flagellate + bacteria culture was established. To establish the ciliate + flagellate + bacteria culture, a clonal culture of the ciliate *Dexiostoma campyla* (Stokes, 1886 *sensu* Jankowski, 1967; Ganner & Foissner, 1989) was obtained by single cell isolation of specimens from the infusion of box elder leaves. Ciliates were grown on stock flagellate + bacteria Cerophyll™ culture, maintained in the dark at 10 °C, and propagated by weekly transfers. Samples from cultures were visually checked for contamination using phase contrast microscopy before each weekly transfer.

Experiment

Recently senesced box elder leaves collected from the riparian zone were soaked briefly in distilled water until pliable, cut into 12-mm disks, air-dried and stored. Forty leaf disks were placed in separate preweighed aluminium dishes, dried, cooled and weighed to determine their initial dry weight. The number of leaf disks was chosen based on the ability to get accurate weight change estimates determined during preliminary experiments. Each dish of leaf disks was placed into a clean experimental container, wrapped with aluminium foil, and sterilised in an autoclave (45 min at 121 °C), then immediately taken to a sterile transfer room (UV irradiated) and stored for ≤2 day before initiating the experiment.

Three of the 147 containers were randomly selected for use in determining if any change in leaf weight had occurred up to this point. Fifty-eight millilitre of filtered (Whatman™ GF/F) and autoclaved stream water were added to the remaining 144 containers, which were then placed in an incubator at 10 °C without light. Leaves were left for 14 day in sterile water prior to start of the experiment to allow for leaching, so that our measures of weight loss (decomposition rate) would exclude losses because of leaching. After the 14-day period, the 144 containers were separated into four sets of 36. For the treatments, target densities of bacteria, flagellates and ciliates were chosen based on field densities for our site and other streams (Table 1). The amount of inoculate needed to establish target densities was determined by cell counts, and then we inoculated containers with the appropriate stock cultures (sterile stream water for the control) to establish four 'microbial' treatment groups ($n = 36$ containers per group): bacteria alone, bacteria-flagellate, bacteria-flagellate-ciliate, and sterile controls. We ensured that each container had the same final volume of fluid (60 mL).

Table 1 Densities of bacteria and protists on stream leaf litter at our study site and in published literature compared with densities established in microcosms at the onset of our experiments (day 0)

Reported field densities	Experimental densities
Bacteria	
10^8–10^9 cm^{-2}*	1.65×10^6–3.30×10^6 cm^{-2}†
3.8×10^7 cm^{-2}‡	2.46×10^6 cm^{-2}§
	1.63×10^6 cm^{-2}¶
Flagellates	
8.52×10^4–2.27×10^5 cm^{-2}**	9.16×10^2 cm^{-2}§–
	1.17×10^3 cm^{-2}¶
Ciliates	
7.95×10^2–1.59×10^3 cm^{-2}**	1.0×10^1 cm^{-2}¶
3.00×10^1–3.90×10^2 mL^{-1}††	
1.23×10^2–2.76×10^2 cm^{-2}‡‡	

*Suberkropp & Klug, 1976; †present paper – for bacteria alone treatments (see Methods); ‡Heiber & Gessner, 2002; §present paper – for bacteria-flagellate treatments (see Methods); ¶present paper – for bacteria-flagellate-ciliate treatments (see Methods); **Bott & Kaplan, 1989; ††Franco *et al.* 1998; ‡‡Ribblett 2002.
Note: some studies report abundances per millilitre while others report abundances per leaf area (cm^2); where possible we have converted values to per area for ease of comparison.

Containers were randomly assigned to one of two incubation conditions, stationary or gently swirled (on a shaker table, 4 rpm). For each incubation condition, there were 18 control containers, 18 bacteria containers, 18 bacteria-flagellate containers, and 18 bacteria-flagellate- ciliate containers. The incubator was kept at 10 °C without illumination throughout the duration of the experiment. Based on preliminary data, we chose to conduct a 120-day experiment to ensure that decomposition rate would be measurable. Thus, three containers from each 'microbial treatment × incubation condition' were randomly sampled without replacement 0, 7, 14, 30, 60 and 120 days after inoculation.

For determination of bacterial and protistan abundance in each container, sampling involved agitating the container then collecting two aliquots: a 5-mL aliquot was preserved in glutaraldehyde (1% final concentration) and a 10-mL aliquot fixed using acid Lugol's solution (1% final concentration). After

aliquot removal, leaf disks selected at random were examined microscopically to verify that protists had been detached from leaves. The agitation process was successful; however, as with all methods for detaching bacteria from the substratum, the process was not 100% efficient (Buesing & Gessner, 2002) and so some cells remained attached to leaves. Harsher methods (e.g. grinding, sonication) would destroy the fragile decomposing leaf tissue that was required for leaf weight loss determinations. As our methods and substrata (leaf discs) were constant across treatments, we made the usual assumption that bacterial numbers in our aliquots may be underestimates but any bias would be comparable across treatments (Epstein & Rossel, 1995).

After aliquots of water were taken, leaf disks were removed from each container using forceps and their dry weight determined. For estimates of bacterial abundance, aliquots of water from the glutaraldehyde-fixed samples were diluted by two to three orders of magnitude using 0.2-μm filtered (Whatman Nucleopore®), autoclaved distilled water. Two separate, 1-mL aliquots of each diluted sample were stained with 4′-6-Diamidino-2-phenylindole (DAPI) following the procedures of Porter & Feig (1980). Stained preparations were examined by epifluorescence microscopy (Zeiss Axioskop; 1000×; 390 nm excitation filter; 430 nm barrier filter) and bacteria counted until 100 fields of view had been scanned. Typically, 500–2000 bacteria were counted per stained preparation, with mean number of bacteria per field used to determine bacterial abundance for each sample.

To determine flagellate abundance, Palmer-Maloney (0.1-mL) chambers were filled with Lugol's-fixed sample and examined using inverted microscopy (Zeiss phase-contrast; 400×, Carl Zeiss International, Göttingen, Germany). Flagellates present in individual fields of view were enumerated until 100 cells were counted, with two chambers examined per sample. Mean number of cells per field was used to determine flagellate abundance for each sample. For ciliate counts, three 1-mL Sedgewick-Rafter™ chambers (Wildlife Supply Co., Buffalo, NY, U.S.A.) were filled with a Lugol's-fixed sample and examined using phase contrast microscopy (Zeiss Axioskop; 200×). Ciliates present in random longitudinal transects were enumerated until ≥100 ciliates were counted, except when cell numbers were low the entire chamber was counted. Mean number of ciliates per transect was used to calculate ciliate abundance for each sample.

Statistics

For each sample, the fraction of leaf mass remaining was obtained by dividing final dry weight of the leaf disks by their initial dry weight. Data for leaf fraction remaining were arcsin, square-root transformed and analysed using linear model regression (PROC MIXED, SAS Institute, 2001) to determine if there were differences in leaf weight loss between control, bacteria, bacteria-flagellate, and bacteria-flagellate-ciliate treatments. Assumptions of homogeneity of variance and normality were met; Tukey pairwise comparisons were used to compare means.

Microbial abundances in all treatments increased to an asymptote without a sigmoidal inflection point and therefore bacteria data were modelled by nonlinear regression (PROC NLIN, SAS Institute, 2001). Bacterial abundance for each treatment was $\log_{10}$ transformed and fit to Mitscherlich's equation (Ratkowsky, 1983) as the following model:

$$Y_{ij} = \beta_{0,i} \times \left[1 - e^{-\beta 1,i(\text{day}-\beta 2,i)}\right] + \in_{ij}$$

where parameters $Y_{ij} = y$-axis (log cells mL^{-1}), $\beta_0 =$ asymptote (log cells mL^{-1}), $\beta_1 =$ slope (Δ cells mL^{-1} day^{-1}), and $\beta_2 = x$ intercept (time in days). A Wald's chi-square test (Rao, 1973) was used to determine if estimated parameters indicating asymptote height or rate of bacterial growth, differed between treatments. Flagellate and ciliate abundance were $\log_{10}$ transformed, and fit to the same nonlinear model. Flagellate abundance was compared for differences between treatments.

Results

Incubation condition (i.e. stationary or swirled) had no significant effect on the abundance of bacteria, flagellates, ciliates or leaf weight during the 120 day experiment ($P > 0.05$), and thus data for the two conditions were pooled by treatment for subsequent analysis. Regressions between time and fraction of leaf weight remaining were highly significant (equations and P-values in Fig. 1) with steeper slopes in the protist treatments (slope comparisons in Fig. 1). At the end of the 120-day experiment, the percent of leaf

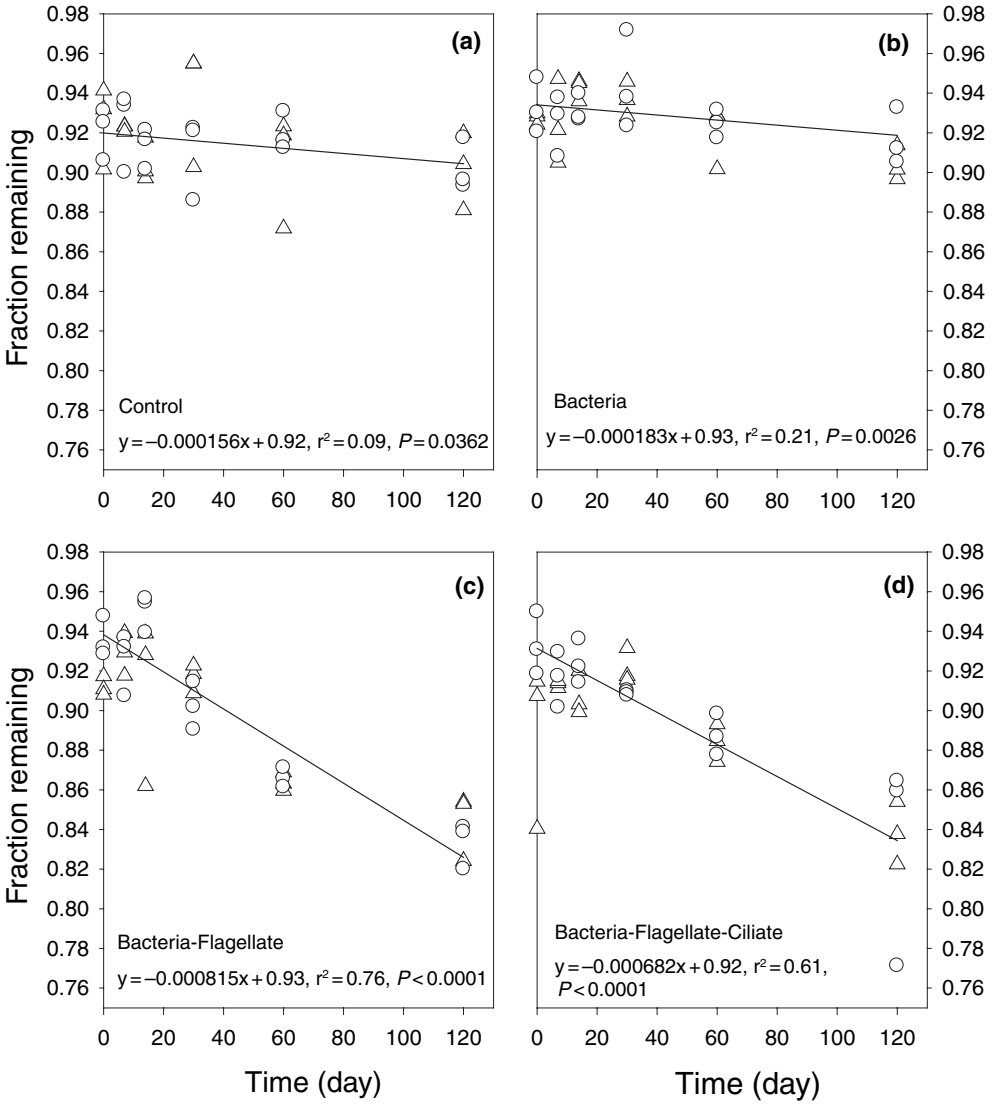

Fig. 1 Dry weight fraction remaining for data pooled between (Δ) stationary and (○) shaken incubation conditions. In all cases (a–d), slopes were determined from arcsin square-root back transformed data to fraction remaining; *P*-values indicate slopes were significantly different from zero. Statistical contrasts of slopes with Tukey multiple comparisons test ($\alpha = 0.05$, experiment wise error rate) between control (a) versus bacteria (b), bacteria-flagellate (c), bacteria-flagellate-ciliate (d) had adjusted *P*-values of 0.0360, 0.0015 and 0.0001, respectively. Contrasts of slopes (as above) between bacteria (b) versus bacteria-flagellate (c) and versus bacteria-flagellate-ciliate (d) both had adjusted *P*-values of <0.0001. Contrast of slopes between bacteria-flagellate (c) and bacteria-flagellate-ciliate (d) had an adjusted *P*-value of 0.9140.

weight remaining in the control and bacteria-only containers ranged from 87 to 92 and 90–93%, respectively (Fig. 1a,b); whereas, the percent of leaf weight remaining in the bacteria-flagellate or bacteria-flagellate-ciliate treatments ranged from 82 to 86 and 77–87%, respectively (Fig. 1c,d). Average weight loss was 8.5% in the presence of bacteria and flagellates and 7.5% in the bacteria + flagellate + ciliate treatment, compared with 1.9 and 2.0% for bacteria alone and controls; thus, the rate of decomposition was 3.5–4 times higher in the presence of protists. The rate of leaf decomposition (slope of linear regression of weight loss over time) was significantly higher in all treatments relative to the control (Fig. 1; $P < 0.05$, PROC MIXED with Tukey pairwise comparisons).

The presence of bacterivorous protists enhanced the decay of leaf material, with the rate of decomposition being significantly higher in the bacteria-flagellate and

bacteria-flagellate-ciliate treatments than in the bacteria alone treatment [$P < 0.05$ PROC MIXED; equations with rate (slope values) in Fig. 1]. However, 'complexity' of the bacteriovore community did not influence the loss of leaf mass – decomposition rates for bacteria-flagellate and bacteria-flagellate-ciliate treatments were not statistically different ($P > 0.05$, PROC MIXED).

Bacterial abundance at the start of the experiment ranged from $4.96 \pm 0.41 \times 10^6$ to $9.88 \pm 0.22 \times 10^6$ cells mL^{-1} in the treatments (Fig. 2b–d, note – figures show log abundance per volume). Per total leaf area in each dish, this corresponds to 1.65–3.29×10^6 bacteria cm^{-2}. Bacterial abundance increased to an asymptote, reaching maximum densities by day 14 in the control and by day 7 in all other treatments (Fig. 2a–d). Bacteria were also present in the control, thus representing a *lower* bacterial abundance treatment rather than a complete bacteria-free control. Bacterial abundances for asymptotes were 2.31×10^7 (control), 9.12×10^7 (bacteria only), 6.59×10^7 (bacteria-flagellate) and 4.27×10^7 cells mL^{-1} (bacteria-flagellate-ciliate). Maximum bacterial abundance in the bacteria only treatment

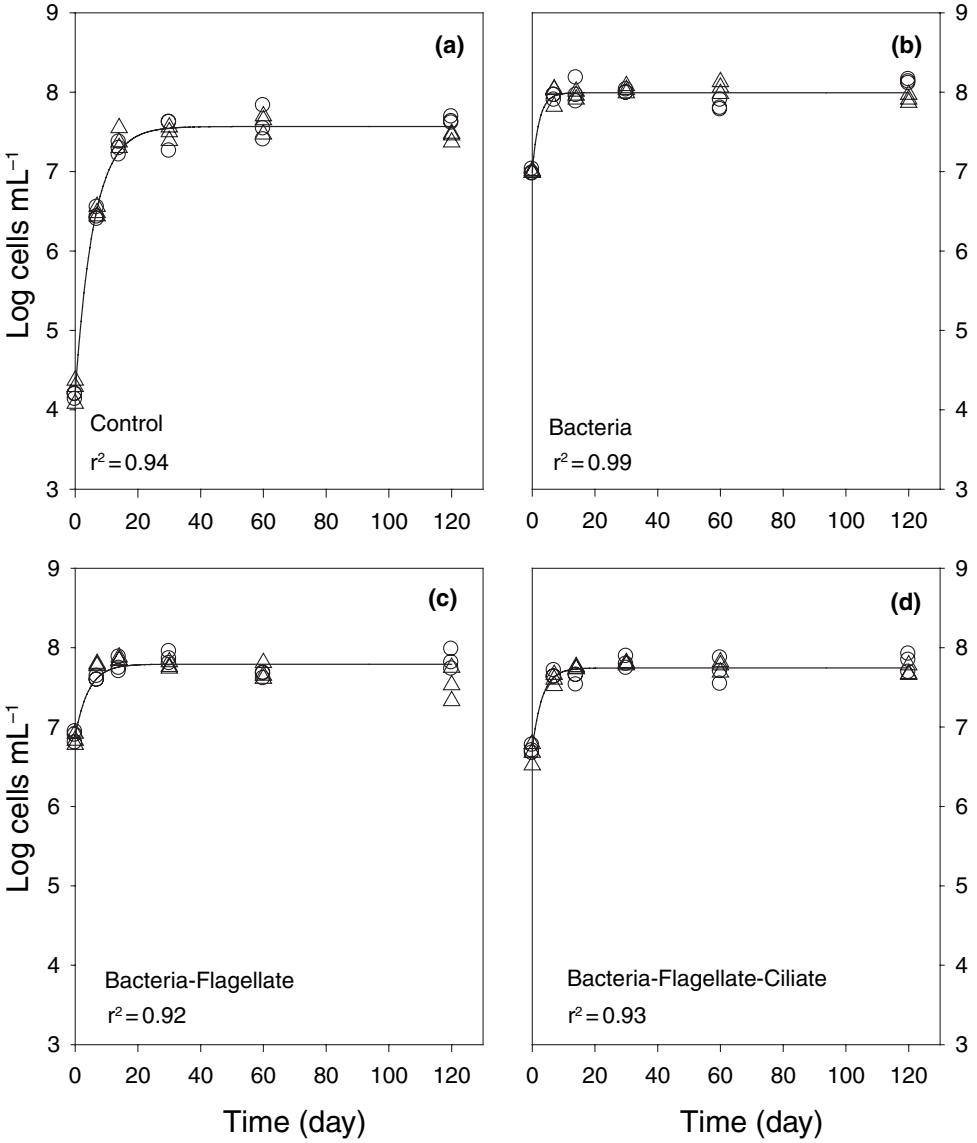

Fig. 2 Log bacterial density for control (a), bacteria (b), bacteria-flagellate (c), bacteria- flagellate-ciliate (d), ($n = 6$ for each sampling date); regression coefficients from the nonlinear analysis are given for each treatment. From the nonlinear analysis, estimates of the asymptote were the only significant parameter, where $P < 0.05$.

was significantly higher than in all other treatments (Fig. 2b; $P < 0.05$, PROC NLIN, SAS Institute, 2001) but there were no differences in bacterial abundances between the control, bacteria-flagellate, and bacteria-flagellate-ciliate treatments.

Flagellate abundance immediately following inoculation of containers averaged $2.75 \pm 0.20 \times 10^3$ and $3.51 \pm 0.31 \times 10^3$ cells mL^{-1} in the bacteria-flagellate and bacteria-flagellate-ciliate treatment, respectively (Fig. 3a,b; note – figures show log abundance per volume). Per total leaf area in each dish, this corresponds to 9.16×10^2 flagellates cm^{-2} of leaves for the bacteria-flagellate treatment and 1.17×10^3 flagellates cm^{-2} of leaves for the bacteria-flagellate-ciliate treatment. Flagellate density increased to an asymptote, with maximum abundance reached by day 7 (Fig. 3a,b) when they were higher in the bacteria-flagellate treatment than in the bacteria-flagellate-ciliate treatment ($P < 0.05$ PROC NLIN, SAS Institute, 2001). Mean ciliate abundance was $3.1 \pm 0.53 \times 10^1$ cells mL^{-1} at the start of the experiment and increased to an asymptote of $2.0 \pm 0.09 \times 10^3$ cells mL^{-1} by day 7 (Fig. 3c). This initial abundance corresponds to 1.03×10^1 cells cm^{-2} of leaves and 6.67×10^2 cells cm^{-2} by day 7.

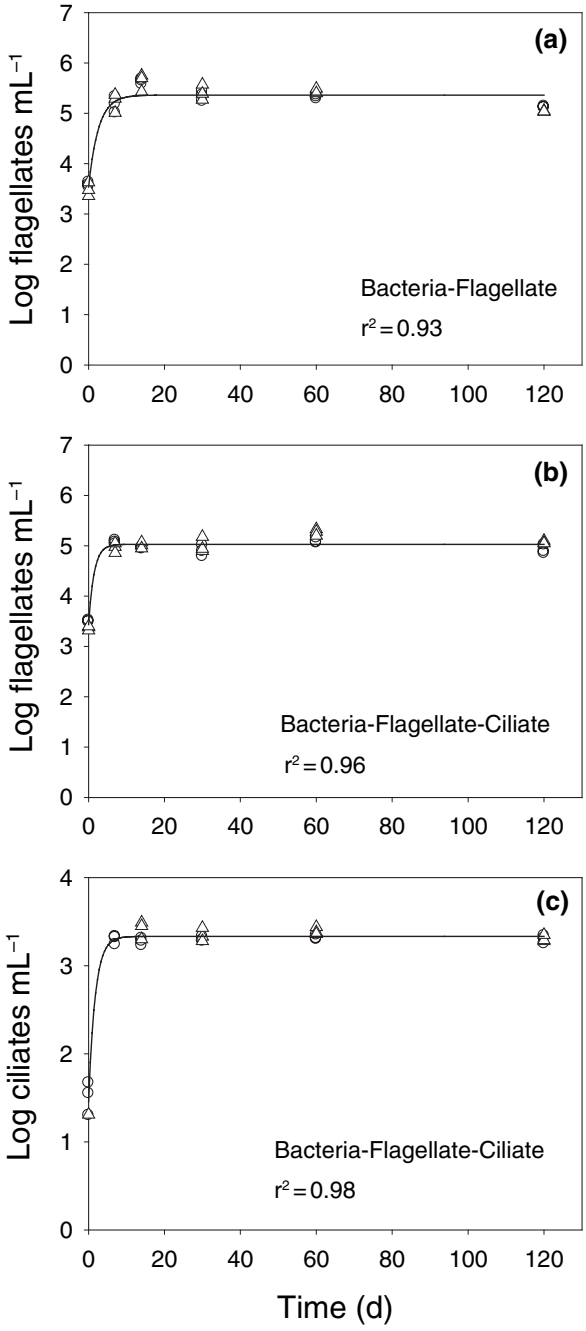

Fig. 3 Log flagellate abundance for bacteria-flagellate (a) and bacteria-flagellate-ciliate (b) and ciliate abundance for bacteria-flagellate-ciliate (c), ($n = 6$ for each sampling date); regression coefficients from the nonlinear analysis are given for each treatment. From the nonlinear analysis, estimates of the asymptote were the only significant parameter, where $P < 0.05$.

Discussion

Protists are among the most speciose forms of life and play an important role in essential ecological processes in soil and water (Fenchel, 1986; Finlay & Esteban, 1998). Despite this, they remain extremely understudied. Indeed, Wetzel (2001b) made an urgent plea for work on protists suggesting, that most organic carbon mineralisation is influenced by them yet a miniscule amount of research has focused on them. There has also been a plea for a mechanistic understanding of what regulates detrital decomposition and its availability to higher trophic levels (Wegener, Oswood & Schimel, 1998; Gessner & Chauvet, 2002). Here we report on work targeting both issues. Using laboratory microcosms, we show that in the presence of stream-dwelling bacterivorous protists, the rate of litter decomposition was enhanced, thereby reducing the standing stock of detritus.

Laboratory studies on quite different systems (detrital hay, seagrasses, macrophyte leaves and dinoflagellate theca) have also found positive effects of protists on decomposition rate (Harrison & Mann, 1975; Fenchel & Harrison, 1976; Fenchel, 1977; Sherr, Sherr & Berman, 1982) but this is apparently the first study for stream protists. Given the wealth of evidence from laboratory studies that protists are

functionally important, the next step is to take such experiments to the field to verify that the effects are measurable *in situ*, given the added complexity of the natural systems. Many factors are well known to influence the breakdown of leaves in running-waters (e.g. macroinvertebrates, litter chemistry, DOC, pH, O_2; Rounick & Winterbourn, 1983; Ostrofsky, 1997; Wallace *et al.*, 1997) and thus the shift from the laboratory to the field is not trivial. Because our experiment was designed to determine *if* the presence of protists could alter decomposition rates, we chose to use a controlled laboratory setting. Only in such a setting could we track the response of individual protist populations to the type of controlled manipulations required to answer this question. While microcosm studies, as described here, cannot exactly mimic natural systems, they have been widely used as experimental systems to test complex ecological questions while controlling potentially confounding factors [e.g. the relationship between ecosystem function and biodiversity (Morin, 1999; McGrady-Steed & Morin, 2000)]. We used protists isolated from our field site, recently senesced, field-collected leaves from the dominant riparian tree species at this site, and set our target densities of bacteria and protists based on field estimates. Further, as >50% of the leaves in Goose Creek are found in stagnant areas of the stream (Palmer *et al.*, 1995) and flow is typically at or near zero for 3–5 months per year (Palmer *et al.*, 2000), the no-flow or intermittently swirled microcosms were not unrealistic compared with field conditions. These aspects of our experiment design, and the fact that bacterivorous protists are among the earliest colonists of decomposing material (Pratt & Cairns, 1985; Kusano, Kusano & Watanabe, 1987) and may even be the dominate protistan functional feeding group on leaf litter in streams (Franco, Esteban & Téllez, 1998; Ribblett, 2002), lead us to speculate that stream protists have the potential to influence the availability of allochthonous matter to higher trophic levels in the field. Furthermore, our results should be comparable with other studies conducted in systems like the backwaters of large rivers and streams, ponds, or pools in which leaf litter accumulates and water flow is slight.

As the protists used in our experiments were strict bacteriovores (i.e. no evidence exists that they graze directly on leaf material or on fungi (Ganner & Foissner, 1989), we hypothesise that the reduced litter biomass in our protists treatments was related to enhanced mineralisation of detritus. Enhanced mineralisation would presumably be due to high rates of bacterial turnover in response to grazing pressure by consumers (Sherr & Sherr, 1994; Snyder & Hoch, 1996; Mikola & Setälä, 1998). We did find statistically lower abundances of bacteria in our two protists treatments compared with our bacteria-only treatment, suggesting a grazing effect. Because we only measured bacterial abundance in our experiments, and not productivity, a lower bacterial abundance but a higher bacterial turnover rate in the presence of protists remains a hypothesis at this point. Although work by Snyder & Hoch (1996) shows that bacterial production on protist excretion products can reach maxima of two- to threefold higher in with protist treatments than in protist-free controls.

Our study was designed to test the hypothesis that protists influence the rate of stream leaf litter decomposition, not to produce an accurate estimate of decomposition that is realistic for in-stream (field) conditions. Because the effects of both bacteria and protists on decomposition were measurable and significant (Fig. 1), we were able to test this hypothesis. The fact that the rates we report are less than those reported from studies measuring decomposition *in situ* is not surprising because we (a) purposely excluded weight loss because of leaf leaching, (b) we excluded all macroinvertebrates from our study [note, Ostrofsky (1997) excluded those >1.5 cm], and (c) there was no mechanical breakdown of leaves by turbulent flow. It is also possible that our rates are low because we autoclaved the leaf disks prior to the experiment, and this could have altered their chemical composition. There is presently no evidence to support or refute this and, in fact, autoclaving is a technique that has been used by others, (e.g. Bengtsson, 1992).

Implications

Allochthonous leaf litter plays a pivotal role in many aquatic ecosystems and for some streams represents all or most of the basal resource that supports the food web. Litter decomposition (which governs the availability of this resource for consumers) is considered so important in many streams that it has been identified as a putative indicator of ecosystem integrity (Gessner & Chauvet, 2002). Most estimates of leaf litter decomposition rates in streams are from studies in

which leaves are exposed to invertebrate consumers or are enclosed in mesh bags that exclude invertebrates (Petersen & Cummins, 1974; Chauvet, 1987; Baldy, Gessner & Chauvet, 1995). There have been several criticisms of using mesh bags (e.g. flow artefacts), but one aspect that has not been fully investigated is that the mesh does not exclude the smallest consumers, the protists, and thus decomposition rates reflect not only bacterial and fungal activity but also their interactive effects with protists. In food web diagrams for stream systems, the primary emphasis with respect to protists has focused on their association with dissolved organic matter and with processes within the microbial loop. Our results suggest a link between protists and leaf organic matter and would entail a more complex conceptual model of food web dynamics.

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