Microbial
Ecology

Relative Importance of Bacteria and Fungi in a Tropical Headwater Stream: Leaf Decomposition and Invertebrate Feeding Preference

M.S. Wright and A.P. Covich

Institute of Ecology, University of Georgia, Athens, GA 30602, USA

Received: 6 March 2004 / Accepted: 23 June 2004 / Online publication: 28 July 2005

Abstract

Bacteria and fungi provide critical links between leaf detritus and higher trophic levels in forested headwater food webs, but these links in tropical streams are not well understood. We compared the roles of bacteria and fungi in the leaf decomposition process and determining feeding preference for two species of freshwater shrimp found in the Luquillo Experimental Forest, Puerto Rico, using experimental microcosms. We first tested the effects of four treatments on decomposition rates for leaves from two common riparian species, *Cecropia scheberiana* (Moraceae) and *Dacryodes excelsa* (Burseraceae), in laboratory microcosms. Treatments were designed to alter the microbial community by minimizing the presence of bacteria or fungi. The fastest decay rate was the control treatment for *D. excelsa* where both bacteria and fungi were present ($k = -0.0073$ day^{-1}) compared to the next fastest rate of $k = -0.0063$ day^{-1} for the bacterial-conditioned *D. excelsa* leaves. The fastest decay rate for *C. scheberiana* was also the control treatment ($k = -0.0035$ day^{-1}), while the next fastest rate was for fungal-conditioned leaves ($k = -0.0029$ day^{-1}). The nonadditive effect for leaf decomposition rates observed in the control treatments where both fungi and bacteria were present indicate that bacteria and fungi perform different functions in processing leaf litter. Additionally, leaf types differed in microbial colonization patterns. We next tested feeding preference for leaf type and microbe treatment in microcosms using two species of freshwater shrimp: *Xiphocaris elongata*, a shredder, and *Atya lanipes*, a scraper/filterer. To estimate feeding preferences of individual shrimp, we measured change in leaf surface area and the amount of particles generated during 5-day trials in 16 different two-choice combinations. *X. elongata* preferred *D. excelsa* over *C. scheberiana*, and leaves with microbial conditioning over leaves without conditioning. There was no clear preference for fungal-conditioned leaves over bacterial-conditioned leaves. This lack of preference for which microbes were responsible for the conditioning demonstrates the importance of both bacterial and fungal resources in these tropical stream food web studies.

Introduction

One tenet in stream ecology maintains that forested headwater streams are detritus-based ecosystems where allochthonous inputs comprise a significant source of energy and nutrients for food webs [2, 17, 48]. Because allochthonous inputs are a critical component of headwater food webs, leaf litter breakdown remains an important target of research. Although it is well documented that microbial conditioning is often a necessary phase before macroinvertebrate use [3, 15, 18], little is known about how fungi and bacteria interact to process leaves in stream food webs [30].

Microbial conditioning of leaf matter aids in the transfer of energy to higher trophic levels by increasing the nutritional value and palatability of leaf matter [3, 11, 23] and by metabolizing recalcitrant leaf components such as lignin and hemicellulose [1, 35, 42]. Microbes also contribute to the physical degradation of leaf material and particle production for invertebrate use [37]. The dominant view currently is that fungi perform a critical role in conditioning leaf material before subsequent invertebrate use while bacteria are thought to play a secondary role [13]. This perspective is the result of several studies showing that fungal biomass and production are significantly higher than that of bacteria on decomposing leaf matter [5, 19, 21]. These studies, however, do not directly address whether production and biomass are the best predictors for assessing the role of fungi and bacteria in leaf decomposition. Some authors

---

Correspondence to: M.S. Wright; E-mail: mswright@uga.edu

DOI: 10.1007/s00248-004-0052-4 • Volume 49, 536–546 (2005) •

suggest that the role of bacteria in leaf decomposition has been underestimated, and that bacteria alter leaf structure and composition in a fundamentally different manner from that of fungi [20, 30, 35]. Findlay et al. [12] suggest that while bacteria may not be quantitatively significant in invertebrates' diets, they may be qualitatively important. The difference between fungal and bacterial importance in transferring energy and nutrients to higher trophic levels may be less distinct than previously thought if measures other than microbial biomass and production are tested (e.g., leaf breakdown rates and invertebrate feeding preference).

The relative importance of bacteria and fungi in leaf decomposition and invertebrate feeding may be influenced by the chemical composition of leaves. Studies have examined leaf chemical attributes such as lignin content and secondary compound concentrations as possible predictors for substrate preferences among microbes and detritivores [32, 36, 43]. Studies examining the effects of secondary compounds on decomposition rates and microbial colonization in aquatic ecosystems are equivocal, often finding that other factors are better predictors of leaf quality [6, 32, 33]. The one leaf characteristic that consistently predicts microbial colonization and leaf decomposition rates is lignin content. The chemical structure of lignin renders it a particularly recalcitrant leaf component, and while some microbes possess enzymes capable of hydrolyzing it, most invertebrates do not [35]. Higher lignin concentrations are associated with slower microbial colonization rates and have a corresponding effect on reducing invertebrate feeding [4, 14].

The vast majority of leaf decomposition studies are conducted in temperate streams, and less is known about the importance of microbial processing in tropical systems [but see 13, 18, 22, 29, 38]. Some researchers speculate that microbes in the tropics play a more significant role in leaf decomposition [22, 26, 38, 43]. Warm water temperatures and the relatively continuous input of leaf litter may influence the microbial community on leaf substrates in tropical streams [22, 43, 48]. Moreover, the relatively high species richness of riparian forests in tropical catchments results in a greater complexity of leaf chemistry compared to most temperate riparian forests [7]. These factors may lead to differences in microbial functioning compared to temperate streams.

A critical assessment of the relative importance of both fungi and bacteria in lotic food webs is ongoing but incomplete [4, 13, 19, 27, 30, 45]. In this study we manipulated the microbial assemblage on two leaf species to assess the role of fungi and bacteria in transferring energy to higher trophic levels in tropical headwater streams. This energy transfer can be via the consumption of both microbial biomass or microbial byproducts such as carbon-rich exopolysaccharides. We used two species of freshwater shrimp that have different modes of feeding to elucidate how this difference affects the relative importance of fungi and bacteria in stream food webs. We tested the effect of leaf attributes on the relative importance of bacteria and fungi using two leaf types that represent different secondary compound content and lignin composition. We anticipated that shrimp would prefer leaves colonized by both fungi and bacteria when given the choice, but preference between fungi- and bacteria-conditioned leaves would be less distinct, and that shrimp species would differ in their preference due to differing modes of feeding. We expected shrimp to prefer lower-lignin leaves and that the lower-lignin leaves would decompose faster.

Methods

Study Site and Organisms. This study took place in the Luquillo Experimental Forest (LEF) in northeastern Puerto Rico, latitude 18°18' N, longitude 65°47' W. This secondary mature tabonuco forest is dominated by *Dacryodes excelsa* (Burseraceae), *Cecropia scheberiana* (Moraceae), and *Prestoea acuminate* (Palmae) in riparian zones. Mean annual precipitation is 3600 mm, and stream temperatures typically range between 20°C and 26°C with little seasonal change. Headwater streams are heavily shaded and have low nitrogen and phosphorus levels resulting in limited primary production [8].

Atyidae shrimp are abundant in headwater streams and are important components of food webs in upper elevations of larger rivers in the LEF [10, 28]. *Atya lanipes* is a collector/scraper under low-flow conditions and opportunistically filter feeds when flow is >5 cm s^{-1} [8]. The freshwater shrimp, *Xiphocaris elongata*, is an omnivorous feeder on coarse particulate organic matter (CPOM) and aquatic insect larvae. In headwater streams, this species is considered to be a member of the shredder functional group. These two species dominate the shrimp assemblage in LEF first- and second-order streams numerically and in biomass and therefore play a significant role in Puerto Rican stream food webs [9, 10].

Leaf Types and Preparations. We used freshly senesced leaves from *D. excelsa* (tabonuco) and *C. scheberiana* (cecropia) collected from the forest floor for these experiments. Tabonuco is high in secondary compound content (hydrolyzable tannins) but low in lignin, whereas cecropia is low in secondary compound content but has a high percentage of lignin [16, 25, 46]. Tabonuco and cecropia have roughly the same initial nitrogen content, but cecropia leaves are typically 10 times larger. To standardize leaf area, we cut intact leaves into 3 cm × 3 cm squares using square templates as guides. We minimized differences in initial leaf mass by standardiz-

Table 1. Sixteen different two-choice test combinations used for short-term feeding trials and *X. elongata* feeding preference results over all test dates

Test	*Choice 1*		*Choice 2*		*Trials where choice 1 was preferred*	*Trials where shrimp fed*	*Possible trials*
1	Tabonuco	Control	Cecropia	Control	12	16	22
2		Fungi		Fungi	16	19	22
3		Bacteria		Bacteria	16	19	22
4		Neither		Neither	9	15	22
5	Cecropia	Control	Cecropia	Neither	14	15	22
6		Control		Bacteria	5	8	22
7		Control		Fungi	7	12	22
8		Fungi		Neither	9	14	22
9		Fungi		Bacteria	7	13	22
10		Bacteria		Neither	8	14	22
11	Tabonuco	Control	Tabonuco	Neither	13	19	22
12		Control		Bacteria	15	21	22
13		Control		Fungi	8	18	22
14		Fungi		Neither	16	19	22
15		Fungi		Bacteria	10	19	22
16		Bacteria		Neither	14	18	22

Tests 1–4 examine leaf type effect, tests 5–10 examine microbe effect within the cecropia leaf type, and tests 11–16 examine microbe effect within the tabonuco leaf type. Choice 1 represents the predicted preferred choice of the two-choice combination. Control: fungi and bacteria present; fungi: fungi present, bacteria minimized; bacteria: bacteria present, fungi minimized; neither: fungi and bacteria minimized.

ing leaf thickness and avoiding holes and midvein sections.

Leaf Decomposition Rate in Laboratory Experiments. For the leaf decomposition experiment, we set up 24 microcosms (59 cm × 43 cm × 15 cm) in a completely randomized order. Each microcosm was aerated and covered with shade cloth to minimize light variability, algal growth, and water temperature, and each contained 5 L of stream water collected from Quebrada Prieta, a second-order stream in the LEF. There were four treatments (“fungi”: fungi present, bacteria minimized; “bacteria”: bacteria present, fungi minimized; “neither”: both bacteria and bacteria minimized; “control”: both groups present) replicated six times. For “bacteria” microscosms, we initially placed 250 mg of the fungicide nystatin (Sigma Aldrich) into each container. To minimize bacteria from “fungi” microcosms, we used 250 mg of both tetracycline and chloramphenicol (Sigma Aldrich). For the “neither” microscosms, we added all three chemicals as described above. These target concentrations of 50 mg L^{-1} were suggested by Sigma Aldrich [40] as effective for cell culture applications, and nystatin was used by Kaushik and Hynes [23]. We added no chemicals to the “control”containers where both bacteria and fungi were present. To determine the effectiveness of the antimicrobial treatments, we plated approximately seven leaf fragments removed from random leaf disks on nutrient broth agar, and monitored the development of microbe colonies growing out from these fragments. Plates for evaluating bacterial growth contained nystatin. After 72 h of incubation, digital photographs were taken and the images were later scored for bacterial and fungal growth. The number of fragments demonstrating bacteria or fungal growth was recorded, and a percentage was calculated for each sampling date. Though many microbes are not culturable, this technique served as a rough estimate of what was present.

Once per week, we removed 1 L of water from each microcosm and added 1 L of fresh water from Quebrada Prieta and the appropriate chemicals based on a 50 mg L^{-1} concentration to maintain a supply of microbe colonizers and nutrients. Once we established that water temperature varied little over the course of the day, we measured water temperature once per week for every aquarium. We randomly collected one leaf disk of both cecropia and tabonuco from each container once per week for 12 weeks to estimate leaf decomposition. We also collected disks on day 2 to determine leaf mass lost due to leaching. Disks were immediately dried at 50°C for 48 h and then weighed.

Shrimp Feeding Trials. We used 16 different two-choice test combinations (Table 1), replicated four times to create 64 tests per feeding trial. Feeding aquaria were 10 cm × 20 cm × 34 cm and contained 1.5 L of water collected from Quebrada Prieta. Each aquarium was aerated using one airstone attached to an aquarium pump and covered with shade cloth to minimize algal growth. Feeding trials for both *X. elongata* and *A. lanipes* were performed after 2 days of leaf incubation, and then at 2-, 4-, 6-, 8-, and 10-week leaf incubation intervals.

We obtained shrimp from baited traps placed in Quebrada Sonadora, a third-order stream in the LEF, for use in feeding trials. We used shrimp of the appropriate species and a size range between 10 and 17 mm in carapace length, which constitutes a median size class for shrimp found in this stream. Freshly collected shrimp were used in each trial. Prior to use in the feeding trials, shrimp were kept in the lab for a 2-day period in aerated aquaria without food to allow for acclimatization and the passage of fecal matter.

Experiments with *X. elongata* during week 8 were exceptional in that only 32 tests were performed (two replications of the 16 tests) because of a combination of lost traps and shrimp mortality.

We randomly assigned a test combination to each container, and one shrimp was then placed in each container with the two leaf disks collected from the leaf decomposition microcosms. After 5 days, the shrimp was removed, while leaf disks were photographed, dried for 48 h at 50°C, and weighed. Particles from the water were collected by filtration (250 μm pore size) and then air dried and photographed for digital image analysis to quantify the amount of particles produced.

Digital Image Analysis. Images were obtained using an Olympus Camedia C-2040 Zoom camera with a 3× optical zoom, 2.5× digital zoom, and 2.1 megapixel resolution. We used the ImageJ 1.29 program [31] to analyze particle filters for the percentage of the image covered by particles. We used Image Tool Version 3.0 software [47] to analyze leaf surface area for the leaf disks.

Leaf Toughness. To determine the effect of each microbe treatment on leaf toughness, we used a penetrometer and recorded what weight was necessary to penetrate the leaf surface. We performed this test on three leaves of both cecropia and tabonuco from all 24 aquaria at the end of the experiment (t = 84 days). As an initial reference, we tested the toughness of 18 disks of each species after leaching them for 2 days in stream water.

Statistical Analysis. For leaf decomposition in aquarium microcosms, we fit a linear regression line using SAS to the natural log of the average percent remaining using microbe treatment and leaf type as covariates [41]. We compared decay coefficients using the Student-Newman-Keuls (SNK) multiple comparison method with $\alpha = 0.05$.

In order to establish feeding preference we first calculated the change in leaf area by subtracting the final leaf area from the initial reference area of 9 cm^2 for each leaf disk. No leaf disks in *A. lanipes* trials exhibited evidence of feeding, so these leaves were not analyzed for change in leaf surface area. To quantify preference in *X. elongata* trials, we next subtracted the change in area for the first leaf disk from the change in area for the second disk in the paired combination from each trial. The first leaf disk in the equation was always the hypothesized "preferred" leaf of the two-choice combination. Therefore, the difference between the two changes in leaf area would be positive according to the null hypothesis. Positive differences received a "1" and negative values received a "0," and we used logistic regression to analyze whether the proportion of trials receiving a "1" was statistically greater than 0.5 for each of the 16 test combinations. This point represents the proportion that would arise by chance if no preference existed. No test had a significant date effect or date and test interaction, so we dropped these terms from the model and used the intercept as an estimate of the proportion of shrimp that showed a preference.

We censored those shrimp that did not eat anything for analysis of feeding preference. However, we used this information to examine whether shrimp did not eat because of laboratory conditions, or because they chose not to feed on either of the offered choices, by comparing the percent of trials where shrimp fed across tests and over time. We analyzed the effect of time on the amount of leaf material consumed by running an ANOVA with date, microbe/leaf treatment combination, and the interaction term as factors to calculate slope estimates on data from trials where shrimp fed. We used ANOVA to examine shrimp species, test, and date effects on the percentage of filter area covered by particles and particle count. We then compared tests using the SNK method. We log-transformed the data because plots of residuals versus predicted values indicated increasing variance. We also examined leaf mass change as another potential response for establishing feeding preference, but this measure was too variable to provide accurate results. This likely is due to differences in initial leaf mass and microbial biomass, rendering it difficult to separate the shrimp feeding effect on mass from this variability.

To evaluate the effects of microbe treatment and leaf type on leaf toughness, we used ANOVA with microbe treatment and leaf type as covariates [41]. Means for each microbe treatment/leaf type combination were compared using the SNK multiple comparison method with $\alpha = 0.05$.

Results

Microbial Leaf Decomposition. Water temperature in microcosms varied little over time and ranged from 25 to 29°C. This temperature is not above the range found in LEF streams. Culture plates revealed that the microbial community was significantly altered among treatments and that treatments generally were effective at reducing the appropriate microbe group (Table 2). On average, approximately 25% of the leaf fragments in "fungi" and "neither" leaf disks had bacterial growth compared to 100% of fragments from the "bacteria" and "control" leaf disks. In all, 14.5% of leaf fragments from "bacteria" and 22.7% of fragments from "neither" leaf disks had

Table 2. Mean percentage (± 1 s.e.) of cultured leaf fragments exhibiting bacterial or fungal growth from each microbe treatment over the course of the 10-week microcosm leaf decomposition

Microbe treatment	*Bacterial growth*	*Fungal growth*
Bacteria	100 ± 0.0	14.5 ± 7.5
Fungi	25.9 ± 5.9	98.6 ± 1.4
Neither	24.3 ± 4.4	22.7 ± 8.9
Control	100 ± 0.0	83.2 ± 6.4

fungal growth, compared to 98.6% and 83.2% of leaf fragments from "fungi" and "control" leaf disks, respectively.

Leaves on average lost 40–48% of their initial weight over the first 2 days, and 43–50% within in the first week (Fig. 1). Between day 7 and day 14, leaves across treatments increased in mass, with the 'fungi' and 'control' leaves increasing the most. After day 14, mass generally declined, but some periods experienced increasing mass, particularly in the 'fungi' treatment (Fig. 1). On day 35, there was an increase in mass of leaves from the 'neither' treatment suggesting fungal contamination due to methodological issue related to the nystatin supply, but on subsequent days this effect diminished once the contamination was eradicated when a new supply of nystatin was used.

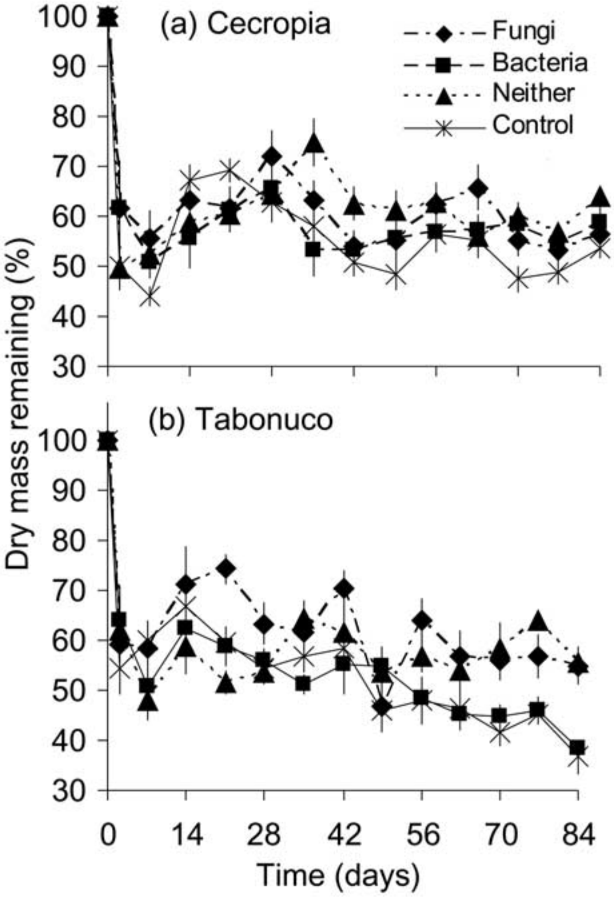

Figure 1. Mean percent mass remaining for leaves in aquarium microcosms by microbe treatment and leaf type. Error bars represent one standard error ($n = 6$).

Table 3. Leaf decomposition coefficients (*k*) from aquarium microcosms

Leaf type	*Microbe treatment*	*k*	*SNK grouping*
Cecropia	Neither	−0.00083	a
	Bacteria	−0.00021	a
	Fungi	−0.0029	a
	Control	−0.0035	ab
Tabonuco	Neither	−0.0014	a
	Bacteria	−0.0063	bc
	Fungi	−0.0037	ab
	Control	−0.0073	c

Cecropia patterns in the "fungi" treatment where bacteria were minimized indicated that mass followed a cyclical pattern increasing and decreasing, while mass in the "control" declined (Fig. 1). No cecropia treatment reached a mass below that of mass on day 7, the mass before microbial buildup. Tabonuco leaves exhibited a general decline in remaining mass over time. This decline is especially evident for "control" and "bacteria" treatments.

Cecropia leaves had significantly slower decay coefficients compared to tabonuco in the "control" and "bacteria" treatments but not in the "neither" and "fungi" treatments (Table 3). Within cecropia, none of the microbe treatment *k*-values differed significantly from each other, but the "control" *k*-value was grouped with the tabonuco "bacteria" and "fungi" treatment values, indicating that decomposition was faster than the other treatments. Within tabonuco, the "neither" treatment was not different from the "fungi" treatment, but it was significantly slower than the "bacteria" and "control" treatments. The "fungi" treatment *k*-value was grouped with the "bacteria" treatment, but was significantly slower than the "control" treatment. The "bacteria" and "control" treatments did not differ significantly. In summary, differences in decomposition were most pronounced between leaf types in terms of the relative importance of fungi and bacteria. Bacteria were effective in increasing decomposition of tabonuco but not cecropia.

Penetrometer Results. There was a significant interaction between leaf species and microbe treatment in determining leaf toughness ($p < 0.0001$, $df = 4$). Initial leaf toughness did not differ significantly between cecropia and tabonuco (Fig. 2). However, after 84 days of incubation, cecropia was significantly softer than tabonuco regardless of microbial exposure. Microbe treatments also differed with "control" and "fungi"

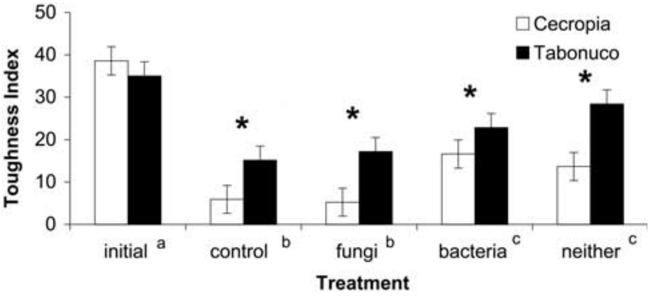

Figure 2. Leaf toughness after initial 2 and 84 days of incubation as measured by number of washers needed to penetrate leaf surface (± 1 s.e.). Significant differences based on the SNK multiple comparison method ($\alpha = 0.05$, $df = 170$, SE = 2.347) between tabonuco and cecropia are marked with asterisks. Significant differences between microbe treatments based on SNK groupings are marked by different letters ($n = 18$).

treatments being significantly softer than "bacteria" and "neither" treatments for both cecropia and tabonuco based on SNK multiple comparisons. All were grouped as significantly softer than initial leaves.

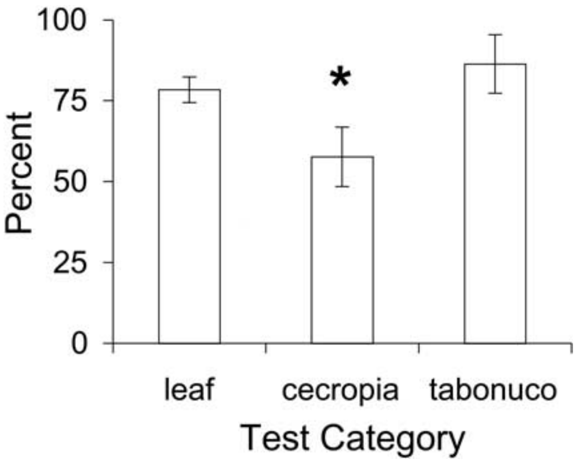

Figure 3. Percent of trials in which *X. elongata* fed based on change in leaf surface area. "Leaf" represents the mean of tests 1–4 (possible trials = 88). "Cecropia" represents the mean of tests 5–10 (possible trials = 132), while "tabonuco" represents the mean of tests 11–16 (possible trials = 132). Error bars represent 95% confidence intervals.

Shrimp Feeding Trials. Temperature in feeding aquaria ranged between 25 and 28°C. *X. elongata* did not eat any leaf material in 93 of a possible 352 feeding trials, but there was no trend over time in the percent of shrimp feeding. Percent feeding on a given date ranged between 72% for day 2 tests and 88% for day 28 tests. The exception to this day 14, when shrimp ate in 55% of the trials. Because there was no time trend in the percent of shrimp eating, data on the percent of shrimp feeding were pooled over sampling dates. *X. elongata* in tests 1–4 (leaf preference test) and tests 11–16 (tabonuco tests) fed in significantly more trials compared to shrimp in tests 5–10 (cecropia tests), while there was no difference between tabonuco and leaf preference tests (Fig. 3).

The tests of leaf type preference for each microbe treatment indicate that *X. elongata* preferred tabonuco over cecropia (Fig. 4a). The proportion of trials where

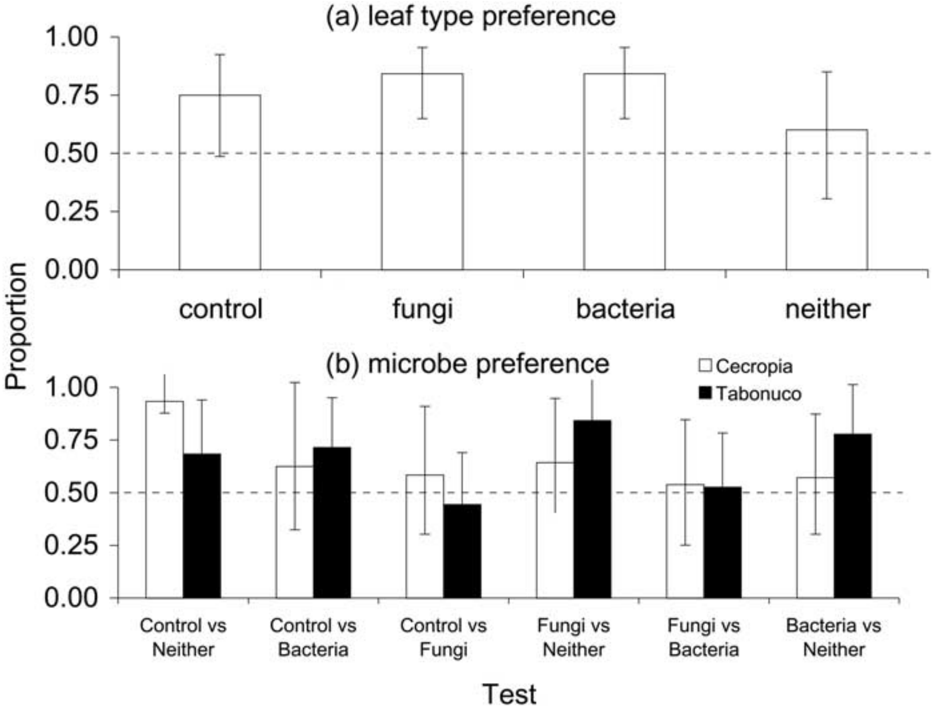

Figure 4. Proportion of tests where *X. elongata* preferred the hypothesized treatment based on change in leaf surface area. (a) Proportion of trials where tabonuco was preferred over cecropia for each microbe treatments. (b) Proportion of trials where the first listed microbe treatment was preferred over the second treatment for each leaf type (see Table 1). Preference is indicated by a proportion significantly greater than 0.5. Error bars represent 95% profile likelihood confidence intervals.

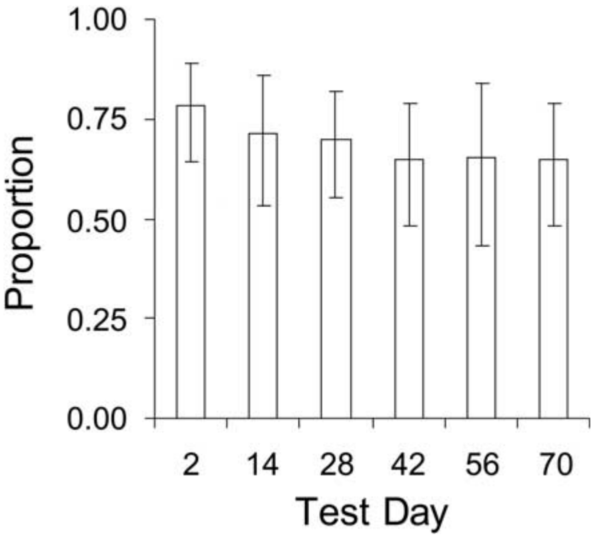

Figure 5. Feeding preference results by date averaged over the 16 tests for *X. elongata* demonstrating declining preference over time. Error bars represent 95% confidence intervals with $n = 64$ for all dates except day 56, where $n = 82$.

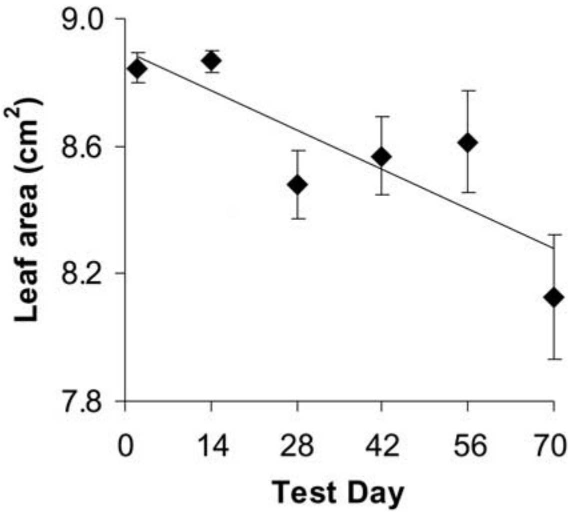

Figure 6. Average leaf area remaining at the end of feeding trials for *X. elongata* demonstrating an increase in the amount of leaf material consumed. Error bars represent one standard error.

tabonuco was preferred is not significantly different from 0.5 for the neither treatment, but is for the other three microbe treatments. Tests 1–4 in Table 1 list the outcomes for these tests. Results from microbe preference trials indicate that preference was generally as predicted (Fig. 4b and tests 5–16 in Table 1). When the "neither" treatment was one choice in the tabonuco trials, the other three microbe treatments were significantly preferred, although the lower confidence interval for the tabonuco "control" test extends to 0.488. Tabonuco leaves in the "control" treatment were marginally preferred over bacteria leaves, but not for cecropia tests. The only statistically significant preference in the cecropia trials was for the "control" versus 'neither' test, in which 14 out of 15 *X. elongata* preferred the "control" leaf. None of the other tests were statistically significant, but estimates indicate there is some evidence for preference for the predicted choice except in the "fungi" versus "bacteria" trials where estimates were close to 0.5.

Time Effect. The percent of *X. elongata* eating did not change over time, but within those shrimp that ate, feeding preference for the predicted preferred leaf declined over time when data were pooled over all tests (Fig. 5). The quantity of leaf material remaining at the end of the trial significantly decreased across test dates (Fig. 6). However, there was no significant treatment effect or date and treatment interaction on the amount of material remaining.

Particles. *X. elongata* sad *A. lanipes* differed in particle production. *A. lanipes* had higher production than *X. elongata* in the first feeding trial (0.38% and 0.20% average cover in original scale respectively, data not shown), but the trend was opposite by the day 70 test (0.54% and 0.65%). Particle production increased over time for both *X. elongata* and *A. lanipes*. There was no significant test effect as the SNK multiple comparison method grouped all tests within a shrimp species together.

Discussion

Microbial Leaf Decomposition. Percent mass remaining in microcosms reveal that decomposition patterns differed between tabonuco compared to cecropia depending on which microbe groups were present. Tabonuco "control" and "bacteria" leaves and, to a lesser extent, cecropia "control" lost mass over time. This trend could be due to a decline in leaf mass, a decline in microbial biomass over time, or most likely, a combination of both. Possible explanations for a decline in microbial biomass include a reduction in the amount of easily usable substrates, the accumulation of fungal and bacterial inhibitory compounds, or nutrient limitation. The presence of inhibitory compounds and nutrient limitations are less likely to explain the observed differences between cecropia and tabonuco because the two leaves were kept in the same microcosm containers. Also, microcosms were refreshed weekly with new stream water, thereby replenishing nutrients and diluting inhibitory compounds. This suggests that the two leaf species differ in carbon usability. Suberkropp et al. [45] noted changes in leaf chemistry through time, as simple leaf components were consumed earlier leaving recalcitrant materials such as lignin, cellulose, and hemicellulose. They further suggested that bacteria are limited in their ability to degrade these recalcitrant components, but that some fungi have enzymes capable of digesting these. Cecropia contains more lignin, and this likely limited bacterial growth. Tabonuco is high in tannin content, but many of these compounds leached out within the first week as indicated by the darkening of microcosm water color (personal observation). This potentially reduced the inhibitory effect for microbes associated with leaf material.

In a similar study, Gulis and Suberkropp [19] found comparable decay coefficient patterns for *Acer rubrum* leaves with both bacteria and fungi (k = 0.0068 day^{-1}) and leaves with bacteria but reduced fungi (k = 0.0085 day^{-1}). They suggest that fungi possibly limit bacterial activity, perhaps because of competition for nutrients. However, they do not refute the alternative that bacteria are partially dependent on fungi for increasing colonization area and substrate availability. They propose that bacteria are limited in what leaf components they can utilize because bacteria are unable to access interior sections of leaves, but that these sections become available after fungal hyphae penetrate leaf tissue. The seemingly synergistic effect of fungi and bacteria, which led to the fastest tabonuco decomposition rate when both groups were present in this study, may in part be explained by this possible interaction between bacteria and fungi. It should be noted that the use of leaf disks increased microbial access to interior leaf sections compared to whole leaves, which may therefore diminish bacterial reliance on fungi for access to interior leaf components. Contrastingly, Mille-Lindblom and Tranvik [30] report an antagonistic relationship between bacteria and fungi, as both bacterial and fungal biomass were depressed when grown together. An antagonistic effect may be seen in the difference between the "fungi" treatment and "control" patterns in this study. Leaves in the "fungi" treatment had higher mass increases than "control" treatments, indicating that fungi can attain higher biomass without the presence of bacteria. Thus, fungi and bacteria may act antagonistically toward one another, but act synergistically in decomposing leaf material. This antagonistic–synergistic effect may be less pronounced on cecropia in part because its lignin content may have limited bacterial growth. "Bacteria" leaves had faster decomposition rates than "fungi" treatments, and this could be due to the higher fungal biomass on leaves masking reductions in leaf mass.. Studies show that fungi account for a larger percentage of microbial biomass than bacteria [13, 19, 27], and by just quantifying the percent mass remaining, this measure includes changes in microbial biomass as well. To fully assess mechanisms for these observed patterns, additional studies quantifying enzyme activities, inhibitory compound concentrations, bacterial and fungal biomass and activities, and changes in leaf chemistry are necessary.

Leaf toughness results further support the idea that bacteria and fungi process leaf material in fundamentally different manners. Cecropia leaves have higher lignin content than tabonuco, but tabonuco leaves have a thick, waxy cuticle, and this may explain why cecropia leaves were significantly softer after 84 days of incubation. Fungi are likely responsible for softening leaf tissue through hyphal penetration as evidenced by differences between treatments where fungi were not minimized ("control" and "fungi") and those where they were ("bacteria" and "neither").

The "control" leaf decay values for both tabonuco (-0.0073 day^{-1}) and cecropia (-0.0035 day^{-1}) can be classified as a medium and a slow rate, respectively, according to a system developed by Peterson and Cummins [34] that is based on the combined effect of physical, microbial, and invertebrate processing. Leaf decay values from a companion decomposition study using the same leaf disk sizes in the LEF stream, Quebrada Prieta, were -0.016 day^{-1} (± 0.0027 SE) for cecropia and -0.027 day^{-1} (± 0.0028 SE) for tabonuco [49]. Leaves in this study were kept in 1 mm mesh bags designed to prevent macroinvertebrate access; thus the observed processing can be mainly attributed to microbial and physical decomposition. Bobeldyk and Ramirez (personal communication) found a rate ($k = -0.0034$ $\text{day}^{-1} \pm 0.0003$) very similar to the cecropia "control" in this study when using cecropia leaves in Quebrada Prieta in 90 μm mesh bags meant to exclude all invertebrates. This small mesh size probably also limited physical processing leaving microbes as the main processing mechanism. By comparing decay coefficients from the microcosms in this study and the stream study observed in Wright [49], we estimate that microbes may account for ~27% and 22% of the macroinvertebrate-independent processing of tabonuco and cecropia, respectively. We predict that this percentage is higher in tropical streams because of warmer water temperatures and a year-round supply of leaf substrates. Additional studies separating microbial from invertebrate processing should thus be undertaken to fully address differences between tropical and temperate streams in regard to the role of microbes in leaf decomposition.

Feeding Preference Trials. Results indicate that *X. elongata* preferred tabonuco over cecropia leaves except for the comparison within the "neither" treatment, possibly because the lack of microbes left tabonuco and cecropia essentially equal in terms of feeding preference. Similarly, the less apparent preference in the "control" may be a result of microbial conditioning that rendered differences between the two leaf types less critical. Fungi and bacteria have differing enzyme capabilities for breaking down compounds such as tannins, lignin, and cellulose, and their combined presence may have acted to nullify the differences between the two leaf types more than in treatments with only bacteria or only fungi. The reduction in preference over time also supports the idea that microbial conditioning reduces differences between leaf types. The overall preference for tabonuco suggests that lignin is a stronger negative influence than initial secondary compound content in predicting invertebrate feeding preference. Other studies have also concluded that lignin is the dominant factor controlling decomposition rates for both invertebrates and microbes [e.g., 14]. Preference for tabonuco does not support the hypothesis that leaf toughness is also a factor determining feeding preference, as cecropia was significantly softer than tabonuco for all microbe treatments.

The clear preference for leaves other than the "neither" treatment indicates the importance of having some microbial conditioning. This conditioning effect is well supported by other studies [e.g., 23, 24]. However, the lack of significant preference in tests other than "neither" suggests that shrimp are less discerning at the level of which microbes are responsible for the conditioning. Alternatively, it could be that preference does exist, but that not enough shrimp fed to establish a significant preference. Figure 6 indicates that trends do exist for some tests. However, the estimates for tests involving "fungi" versus 'bacteria' treatments and "fungi" versus "control" treatments are at ~0.5, suggesting that *X. elongata* did not distinguish between the two choices. Kostalos and Seymour [24] found that *Gammarus minus* (Amphipoda) preferred leaves with fungal conditioning over leaves with bacterial conditioning in a similar study for a temperate system. Shredding invertebrates may vary in their reliance on microbial conditioning depending on factors such as gut morphology and the endosymbiotic microflora colonizing their digestive systems. For example, Rong et al. [39] revealed that *Tipula caloptera* (Diptera) was less reliant on microbial conditioning because of its basic gut pH and active microbiota community, while *Gammarus tigrinis* (Amphipoda) was more reliant on conditioning because of its gut physiology. We hypothesize that *X. elongata* is less selective in making food choices, in part because its omnivorous feeding habits allow it to utilize numerous substrates and because their morphology does not require organic matter to be softened before they are able to tear it. Alternatively, *X. elongata* may be more selective because of its pronounced mobility and, therefore, ability to sample multiple food resources. That *X. elongata* does not require leaf material to be conditioned by microbes is supported by the observation that the number of shrimp feeding did not vary over time. Shrimp fed even after only 2 days of leaf conditioning. However, the fact that more leaf material was consumed during later trials suggests that more microbial conditioning results in more consumption.

The only significant effect in the analysis of particle production was time, as more microbial conditioning led to more particle production. This increased particle production is not surprising because of the related increase in the amount of leaf material consumed. Particles consisted of both fecal material and leaf fragments in the case of *X. elongata*, with the majority being fecal particles (personal observation). Fecal production is a function of many factors beyond the amount of material ingested, including the nutritional content of the material and the individual's metabolism. These sources of variability are likely to explain why no tests were significantly different. *A. lanipes* produced more particles than *X. elongata*, indicating that *A. lanipes* were feeding during the trials. However, the methods used in this study were not able to separate differences between microbe and leaf treatments and thus, any preference by *A. lanipes* could not be determined.

Although *X. elongata* did not feed in ~25% of the trials, differences among tests in the percent of shrimp feeding indicate that shrimp did not feed solely because they were stressed by laboratory conditions. Cecropia tests had a significantly lower percentage of trials where shrimp fed, suggesting that it is of lower quality. Direct preference tests between cecropia and tabonuco affirm this.

Possible Methodological Issues. Because methods employed in this study for manipulating microbe assemblages relied on the use of antibiotics, one of its potential limitations is complications related to treatment efficacy. This includes incomplete eradication of fungi or bacteria due to resistant microbes. The goal of the treatments in this study was thus to minimize the presence of bacteria or fungi. Several lines of evidence suggest these treatments were effective in doing so, including culture plates revealing abundant fungal growth in fungi-containing treatments and the minimization of such growth in "bacteria" and "neither" treatments. Also, qualitative evidence includes abundant biofilm growth on leaves in bacteria-containing microcosms that was absent in "fungi" and "neither" treatments over the course of the study. Additional interesting evidence supporting treatment efficacy is that the water in microcosms containing bacteria was noticeably lighter in color compared to "fungi" and "neither" microcosms by the end of the study. We believe this to be due to the breakdown of the leached tannins by bacteria over the course of the study that initially had rendered the water in all microcosms the same dark tea color. Another potential concern is the effect of antibiotic treatments on shrimp feeding, but this is not likely a major issue because of high percentages of shrimp feeding throughout the different trials and because shrimp feeding trials were conducted in separate containers from that of the leaf incubations. Additionally, the volume of water in shrimp feeding containers was large relative to the leaf disks, and this likely would dilute any antibiotic residues. These lines of evidence all suggest that treatments were effective at manipulating the microbe assemblages in the designed manner.

Summary. *X. elongata* is selective at the level of leaf type, and secondarily, prefers leaves with some microbial conditioning. Whether fungi or bacteria are responsible for the conditioning is apparently of less importance to these species of detritivores. Leaf decomposition results imply that fungi and bacteria may have different functions in the leaf decomposition process through such possible mechanisms as exoenzyme production and differing metabolic capabilities, and thus that both are critical for maximum leaf processing rates. Differences between leaf types have implications for energy flow and nutrient cycling in these food webs. For example, stream reaches receiving primarily tabonuco leaf inputs may process leaf material faster compared to reaches receiving mostly cecropia inputs. Information concerning the effects of different leaf types on microbial diversity and the diversity of enzymes produced is likely to provide an increased understanding of the effects of leaf chemistry and subsequent differences in patterns of microbial processing of leaf litter.

Acknowledgments

We are indebted to our research assistant, Anthony Cappa, and thank D. Jean Lodge and Stephanie Rohan for their assistance. We are also grateful to Alonso Ramirez and Angela Bobeldyk for providing useful data. We thank William H. Clements, F. Brent Reeves, and anonymous reviewers for helpful comments on earlier manuscripts. This research was supported by grant DEB-9411973 from the National Science Foundation to the University of Puerto Rico and the International Institute of Tropical Forestry, USDA, as part of the Long-Term Ecological Research Program in the Luquillo Experimental Forest. Additional support was provided by the Forest Service (USDA), the University of Puerto Rico, and Colorado State University.

References

1. Abdullah, SK, Taj-Aldeen, SJ (1989) Extracellular enzymatic activity of aquatic and aero-aquatic conidial fungi. Hydrobiologia 174: 217–223
2. Abelho, M (2001) From literfall to breakdown in streams: a review. Sci World 1: 656–680
3. Arsuffi, TL, Suberkropp, K (1989) Selective feeding by shredders on leaf-colonizing stream fungi: comparison of macroinvertebrate taxa. Oecologia 79: 30–37
4. Baldy, V, Gessner, MQ, Chauvet, E (1995) Bacteria, fungi and the breakdown of leaf litter in a large river. Oikos 74: 93–102
5. Boulton, AJ, Boon, PI (1991) A review of methodology used to measure leaf litter decomposition in lotic environments: time to turn over a new leaf? Aust J Mar Freshwat Res 42: 1–43
6. Campbell, IC, Fuchshuber, L (1995) Polyphenols, condensed tannins, and processing rates of tropical and temperate leaves in an Australian streams. J North Am Benthol Soc 14: 174–182
7. Covich, AP (1988) Geographical and historical comparisons of neotropical streams: biotic diversity and detrital processing in highly variable habitats. J North Am Benthol Soc 7: 361–386
8. Covich, AP, McDowell, WH (1996) The stream community. In: Reagan, DP, Waide, RB (Eds.) The Food Web of a Tropical Rain Forest, University of Chicago Press, Chicago
9. Covich, AP, Crowl, TA, Johnson, SL, Pyron, M (1996) Distribution and abundance of tropical freshwater shrimp along a stream corridor: response to disturbance. Biotropica 28: 484–492
10. Crowl, TA, McDowell, WH, Covich, AP, Johnson, SL (2001) Freshwater shrimp effects or detrital processing and nutrients in a tropical headwater stream. Ecology 82: 775–783
11. Cummins, KW (1973) Trophic relations of aquatic insects. Ann Rev Entomol 18: 183–206
12. Findlay, S, Meyer, JL, Smith, PJ (1984) Significance of bacterial biomass in the nutrition of a freshwater isopod (*Liceus* sp.). Oecologia 63: 38–42
13. Findlay, S, Tank, J, Dye, S, Valett, HM, Mulholland, PJ, McDowell, WH, Johnson, SL, Hamilton, SK, Edmonds, J, Dodds, WK, Bowden, WB (2000) A cross-system comparison of bacterial and fungal biomass in detritus pools of headwater streams. Microb Ecol 43: 55–66
14. Gessner, MO, Chauvet, E (1994) Importance of stream microfungi in controlling breakdown rates of leaf litter. Ecology 75: 1807–1817
15. Gessner, MO, Chauvet, E, Dobson, M (1999) A perspective on leaf litter breakdown in streams. Oikos 85: 377–384
16. Gonzalez, G, Seastedt, TR (2001) Soil fauna and plant litter decomposition in tropical and subalpine forests. Ecology 82: 955–964
17. Graca, MAS (2001) The role of invertebrates on leaf litter decomposition in streams—a review. Int Rev Hydrobiol 86: 383–393
18. Graca, MAS, Cressa, C, Gessner, MO, Feio, MJ, Callies, KA, Barrios, C (2001) Food quality, feeding preferences, survival and growth of shredders from temperate and tropical streams. Freshwat Biol 46: 947–957
19. Gulis, V, Suberkropp, K (2003) Effect of inorganic nutrients on relative contributions of fungi and bacteria to carbon flow from submerged decomposing leaf litter. Microb Ecol 45: 11–19
20. Hall Jr, RO, Meyer, JL (1998) The trophic significance of bacteria in a detritus-based stream food web. Ecology 79: 1995–2012
21. Hieber, M, Gessner, MO (2002) Contribution of stream detritivores, fungi, and bacteria to leaf breakdown based on biomass estimates. Ecology 83: 1026–1038
22. Irons III, JG, Oswood, MW, Stout, RJ, Pringle, CM (1994) Latitudinal patterns in leaf litter breakdown: is temperature really important? Freshwat Biol 32: 401–411
23. Kaushik, NK, Hynes, HBN (1971) The fate of dead leaves that fall into streams. Arch Hydrobiol 68: 465–515
24. Kostalos, M, Seymour, RL (1976) Role of microbial enriched detritus in the nutrition of *Gammarus minus* (Amphipoda). Oikos 27: 512–516
25. Le Caro, F, Rudd, RL (1985) Leaf litter disappearance rates in Puerto Rican montane rain forest. Biotropica 17: 269–276
26. Lodge, DJ (1996) Microorganisms. In: Reagon, DP, Waide, RB (Eds.) The Food Web of a Tropical Rain Forest, University of Chicago Press, Chicago

27. Maarmi, A, Barlocher, F, Pattee, E, Chergui, H (2001) Fungal and bacterial colonization of *Salix pedicellata* leaves decaying in permanent and intermittent streams in Eastern Morocco. Int Rev Hydrobiol 86: 337–348
28. March, JG, Pringle, CM, Townsend, MI, Wilson, AI (2002) Effect of freshwater shrimp assemblages on benthic communities along an altitudinal gradient of a tropical island. Freshwat Biol 47: 377–390
29. Mathuriau, C, Chauvet, E (2002) Breakdown of leaf litter in a neotropical stream. J North Am Benthor Soc 21: 384–396
30. Mille-Lindblom, C, Tranvik, LJ (2003) Antagonism between bacteria and fungi on decomposing aquatic plant litter. Microb Ecol 45: 173–182
31. National Institute of Health (2003) Image J: image processing and analyzing in Java. http://rsb.info.nih.gov/ij/. Date of download: 3, January 2003
32. Ostrofsky, ML (1993) Effects of tannins on leaf processing and conditioning rates in aquatic ecosystems: an empirical approach. Can J Fish Aquat Sci 50: 1176–1180
33. Ostrofsky, ML (1997) Relationship between chemical characteristics of autumn-shed leaves and aquatic processing rates. J North Am Benthol Soc 16: 750–759
34. Peterson, RC, Cummins, KW (1974) Leaf processing in a woodland stream. Freshwat Biol 4: 343–368
35. Pusch, M, Fiebig, D, Brettar, I, Eisenmann, H, Ellis, BK, Kaplan, LA, Lock, MA, Naegeli, MW, Traunspurger, W (1998) The role of micro-organisms in the ecological connectivity of waters. Freshwat Biol 40: 453–495
36. Quinn, JM, Smith, BJ, Burrell, GP, Parkyn, SM (2000) Leaf litter characteristics affect colonisation by stream invertebrates and growth of *Oltinga feredayi* (Trichoptera: Conoesucidae). N Z J Mar Freshwat Res 34: 272–287
37. Rader, RB, McArthur, JV (1994) Relative importance of mechanisms determining decomposition in a southeastern blackwater stream. Am Midl Nat 132: 19–31
38. Ramirez, A, Pringle, CM, Molina, L (2003) Effects of stream phosphorous levels on microbial respiration. Freshwat Biol 48: 88–97
39. Rong, Q, Sridhar, KR, Barlocher, F (1995) Food selection in three leaf-shredding stream invertebrates. Hydrobiologia 316: 173–181
40. Sigma Aldrich (2002–2003) Biochemicals and Reagents for Life Science Research. Sigma-Aldrich, St. Louis, MO
41. The SAS Institute (2002) The SAS System for Windows V8. SAS Institute, Cary, NC
42. Sinsabaugh, RL, Linkins, AE, Benfield, EF (1985) Cellulose digestion and assimilation by three leaf-shredding aquatic insects. Ecology 66: 1464–1471
43. Stout, J (1989) Effects of condensed tannins on leaf processing in mid-latitude and tropical streams: a theoretical approach. Can J Fish Aquat Sci 46: 1097–1106
44. Suberkroopp, K, Klug, MJ (1976) Fungi and bacteria associated with leaves during processing in a woodland stream. Ecology 57: 707–719
45. Suberkropp, K, Godshalk, GL, Klug, MJ (1976) Changes in the chemical composition of leaves during processing in a woodland stream. Ecology 57: 720–727
46. Sullivan, NH, Bowden, WB, McDowell, WH (1999) Short-term disappearance of foliar litter in three species before and after a hurricane. Biotropica 31: 382–393
47. The University of Texas Health Science Center at San Antonio Dental School (2003) Image Tool Version 3.0. http://ddsdx.uthscsa.edu/dig/itdesc.html. Date of download: 3 January 2003
48. Wallace, JB, Eggert, SL, Meyer, JL, Webster, JR (1997) Multiple trophic levels of a forest linked to terrestial litter inputs. Science 277: 102–104
49. Wright, MS (2003) Microbial and invertebrate processing of leaf litter in a Puerto Rican headwater stream. M.S. Thesis, Colorado State University, Fort Collins, CO