MICROBIAL ECOLOGY

Microb Ecol (2003) 45:11–19
DOI: 10.1007/s00248-002-1032-1

Effect of Inorganic Nutrients on Relative Contributions of Fungi and Bacteria to Carbon Flow from Submerged Decomposing Leaf Litter

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Received: 10 June 2002; Accepted: 1 August 2002; Online publication: 27 November 2002

ABSTRACT

The relative contributions of fungi and bacteria to carbon flow from submerged decaying plant litter at different levels of inorganic nutrients (N and P) were studied. We estimated leaf mass loss, fungal and bacterial biomass and production, and microbial respiration and constructed partial carbon budgets for red maple leaf disks precolonized in a stream and then incubated in laboratory microcosms at two levels of nutrients. Patterns of carbon flow for leaf disks colonized with the full microbial assemblage were compared with those colonized by bacteria but in which fungi were greatly reduced by placing leaf disks in colonization chambers sealed with membrane filters to exclude aquatic hyphomycete conidia but not bacterial cells. On leaves colonized by the full microbial assemblage, elevated nutrient concentrations stimulated fungi and bacteria to a similar degree. Peak fungal and bacterial biomass increased by factors of 3.9 and 4.0; cumulative production was 3.9 and 5.1 times higher in the high nutrient in comparison with the low nutrient treatment, respectively. Fungi dominated the total microbial biomass (98.4 to 99.8%) and cumulative production (97.3 and 96.5%), and the fungal yield coefficient exceeded that of bacteria by a factor of 36 and 27 in low- and high-nutrient treatments, respectively. Consequently, the dominant role of fungi in leaf decomposition did not change as a result of nutrient manipulation. Carbon budgets indicated that 8% of leaf carbon loss in the low-nutrient treatment and 17% in the high-nutrient treatment were channeled to microbial (essentially fungal) production. Nutrient enrichment had a positive effect on rate of leaf decomposition only in microcosms with full microbial assemblages. In treatments where fungal colonization was reduced, cumulative bacterial production did not change significantly at either nutrient level and leaf decomposition rate was negatively affected (high nutrients), suggesting that bacterial participation in carbon flow from decaying leaf litter is low regardless of the presence of fungi and nutrient availability. Moreover, 1.5 and 2.3 times higher yield coefficients of bacteria in the reduced fungal treatments at low and high nutrients, respectively (percentage of leaf carbon loss channeled to bacterial

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production), suggest that bacteria are subjected to strong competition with fungi for resources available in leaf litter.

Introduction

Headwater woodland streams are primarily heterotrophic (detritus-based) since they receive substantial inputs of organic matter from the riparian vegetation while primary production is generally low [39]. Carbon and energy flow from submerged coarse particulate organic matter (CPOM, basically leaf litter and woody debris) to higher trophic levels is mediated by microorganisms. Fungi and bacteria convert a portion of detrital organic matter into microbial biomass and render leaf material a more palatable and nutritious food source for shredding invertebrates [4, 34]. In addition, aquatic hyphomycetes macerate leaf tissues with pectinolytic enzymes [8, 9, 37] and facilitate release of fine particulate organic matter which is an important resource for filtering invertebrates.

Recent advances in stream ecology point to the dominance of fungi in microbial communities associated with submerged decaying leaf litter. Fungi contribute 63–100% to total microbial (fungi plus bacteria) biomass [1–3, 14, 17, 41]. Bacteria may exhibit higher turnover rates but fungal production is equal to [2] or exceeds bacterial production associated with CPOM in lotic ecosystems by a factor of 0.9–627 [1, 31, 38, 41].

Elevated concentrations of N and P have been shown to accelerate leaf decomposition via enhanced microbial activity. Fungal biomass accrual and sporulation are stimulated by nutrient additions both in stream studies [16] and in laboratory microcosms [32, 36]. Positive effects of elevated inorganic nutrients (N and/or P) on bacterial growth rate and abundance have been demonstrated for planktonic bacteria in lentic ecosystems [6, 25]. However, only one study has addressed effects of nutrient enrichment on both fungi and bacteria associated with benthic CPOM in a headwater stream [17]. Both fungal and bacterial biomass, microbial respiration, and decomposition of two leaf species were higher in the nutrient-enriched reach of this stream. It is not clear, however, how elevated nutrient concentrations will affect the relative contributions of fungi and bacteria to carbon flow through these microbial compartments.

We recently examined the interactions that can occur between single species of a fungus and a bacterium on leaves in microcosms at two levels of nutrients [18]. The aim of the present study was to assess the relative contributions of the two types of microorganisms when natural communities of each participate in leaf decomposition at different levels of inorganic nutrients. To estimate microbial performances, we compared patterns of carbon flow from leaf disks colonized in a stream by the full microbial assemblage with those from leaves that were colonized by a natural bacterial community but in which fungal colonization was limited. The purpose of the fungal-reduction treatment was to examine the magnitude of bacterial activity in the transformation of leaf carbon. Both colonization treatments were examined at two levels of nutrient availability. We determined leaf mass loss, microbial production, and respiration and constructed partial decomposition budgets.

Methods

Field procedures

Leaf material for laboratory experiments was colonized in a headwater stream draining catchment 54 in the Coweeta Hydrologic Laboratory, Macon County, North Carolina, USA. This stream is small (avg. discharge about 1.5 L s^{-1}), circumneutral, and softwater and contains low nutrient concentrations (NO_3-N + NH_4-N <10 μg L^{-1}, soluble reactive phosphorus 2 μg L^{-1} at the time of leaf material introduction). The stream drains mixed deciduous forest in the southern Appalachians at an elevation of ca. 850 m a.s.l. A more detailed description of the stream is given by Cuffney et al. [11]. Water temperature during colonization period varied between 10.7 and 12.4°C as measured with an Optic StowAway temperature probe (Onset Computer Corp.) that recorded temperature every 30 min.

Red maple (*Acer rubrum* L.) leaf disks (11.8 mm diam.) cut from naturally abscised leaves were leached for several days at 5°C, dried at 60°C, weighed in sets of 15, and sterilized with gamma irradiation (2–3 megarads) [33]. Leaf disks were soaked for 1 day in deionized water at 5°C and enclosed in 10 × 10 cm fiberglass mesh bags (1 mm mesh size) or chambers designed to prevent fungal colonization. The design of these chambers is based on that described by McFeters and Stuart [22]. Chambers were constructed from three 6.5-mm thick 7 × 12 cm pieces of Plexiglas having two 38-mm diam. holes. Four filters (Millipore Isopore TMTP, 47 mm diam., 5 μm pore size) were sandwiched between the three pieces of Plexiglas held together with bolts forming two 6.5-mm high cylindrical chambers connected to the environment through the membrane filters. The pore size of the filters allowed access of bacteria into the chambers but excluded

conidia of aquatic hyphomycetes greater than 5 μm. Each chamber unit was enclosed in a 1 mm mesh size fiberglass bag to protect filters from mechanical damage in the stream. On November 5, 2001, leaf bags and leaf disks for colonization chambers were transported to the stream in a cooler and disks were placed in the chambers that were filled with filtered stream water (5 μm, TMTP filters). Leaf bags and chambers were tied to a line and secured to the stream bottom with nails in the upper reach of stream 54. After an 8-day colonization period they were retrieved, brought back to the laboratory, and stored overnight in stream water at 15°C. Leaf disks from each bag or chamber were removed, rinsed with filtered stream water (5 μm, TMTP filters), and placed into laboratory microcosms [33] that simulated stream conditions.

Laboratory Microcosms

Each microcosm was autoclaved and filled with 40 mL nutrient solution (see below), and then received 15 preweighed and precolonized leaf disks. Microcosms were incubated at 15°C and aerated aseptically with 80–100 mL air per minute using a pump supplying air to flow meters, each connected to a microcosm air inlet plugged with cotton. Two solutions with contrasting nutrient concentrations were used [18]. The low nutrient solution (control) contained 20 μg L^{-1} N (from (NH_4NO_3) and 2 μg L^{-1} P (from KH_2PO_4), whereas the high nutrient solution had 2000 μg L^{-1} N and 200 μg L^{-1} P, thus spanning nearly the entire range of nutrient variability occurring in lotic ecosystems. Solutions were buffered with MOPS (0.25 g L^{-1}), adjusted to pH 7.0 with KOH, and autoclaved. The fluid in the laboratory microcosms was replaced every 2 days to keep nutrient concentrations relatively constant and to determine sporulation by aquatic hyphomycetes.

On each sampling date, 12 microcosms were sacrificed (three replicates for each colonization type-nutrient level combination). Four mL of fluid and 5 disks (separately) were sampled from each microcosm for bacteria; 10 disks and the remaining fluid were used to determine respiration rates. After completion of respiration measurements, 5 disks were preserved in 5 mL methanol for determination of fungal biomass and 5 were used to determine leaf mass loss. The same scheme (except fluid subsampling) was used to sample leaf disks from leaf bags or colonization chambers at day 9 (respiration was measured in stream water in this case).

Details of microcosms subsampling and determination of leaf mass loss, fungal biomass, conidia production, and respiration rate are given in [18]. Briefly, ash-free dry mass (AFDM) remaining of leaf disks was estimated by weighing samples after drying at 100°C and taking into account the average ash content of 4.1% determined from combustion of sets of leaf disks at 500°C at the beginning and the end of the experiment. To determine fungal biomass, ergosterol was extracted and quantified by comparing absorbance at 282 nm after separation by HPLC (Shimadzu) with standard concentrations of ergosterol (Fluka) ([24], as modified in [38]). A conversion factor of 5.5 μg ergosterol per mg of mycelial dry mass was used [15]. Conidial suspensions were filtered through membrane filters (Millipore, 5 μm pore size), stained with trypan blue in lactic acid (0.1%), and counted. Respiration rates were determined from oxygen uptake at 15°C by recording dissolved oxygen concentration with YSI 5100 Dissolved Oxygen Meter (Yellow Springs, OH) for 30 min. Bacteria were enumerated with epifluorescence microscopy following staining with DAPI [40]. Samples were prepared and bacteria counted and sized according to [17]. Bacterial biovolumes were converted to bacterial carbon using formula $C = 89 \cdot V^{0.59}$, where the conversion factor has units of fg C μm^{-3}, to take into account for differences in carbon-to-volume ratios of different bacterial classes [30]. We picked five classes with biovolumes ranging from 0.018 to 0.81 μm^3 (0.14 and 0.23 μm^3 for the most abundant cocci and small rods) that translated into carbon contents from 463 to 97 fg C μm^{-3}, respectively (for the sixth class — filamentous bacteria — we used 97 fg C μm^{-3}). To calculate bacterial C, total bacterial counts and contributions of each class, biovolume data and specific conversion factors were used. Bacterial carbon estimates from leaf disks were corrected for ultrasonication dislodgment efficiency according to [18].

Statistical Analyses and Carbon Budgets

Decomposition rates, k, were estimated by linear regression of ln transformed AFDM remaining over the entire study (exponential model). Differences in k were determined with analysis of covariance (ANCOVA) followed by Tukey's test to compare slopes among treatments. Effects of nutrient concentration and composition of microbial community associated with leaf material on fungal biomass, sporulation rate of aquatic hyphomycetes, bacterial biomass, and microbial respiration were tested by repeated measures ANOVA [42]. Data derived from conidia and bacteria counts were $\ln(x + 1)$ transformed. All analyses were carried out with SYSTAT 10. Carbon budgets were constructed assuming 50% carbon content of leaf AFDM and fungal dry mass and a respiratory quotient of 1. Total bacterial production from fluid, total conidia production, and carbon losses due to respiration were calculated by summing over the entire experiment after linear interpolation for missing data for bacteria and respiration. Individual biovolumes or dry masses of aquatic hyphomycete conidia were taken from [5, 10] or estimated from microscopic measurements. Conidial biovolumes were converted to carbon using 150 fg C μm^{-3}.

Results

Decomposition rate was significantly faster (ANCOVA, $p < 0.001$) for leaf material colonized by the natural microbial assemblage and incubated at the high nutrient concentration in comparison with the other treatments (Fig. 1, Table 1). Reduced fungal colonization and/or incubation in low nutrient solution resulted in the decomposition coefficient, k, being roughly 2.5 times lower.

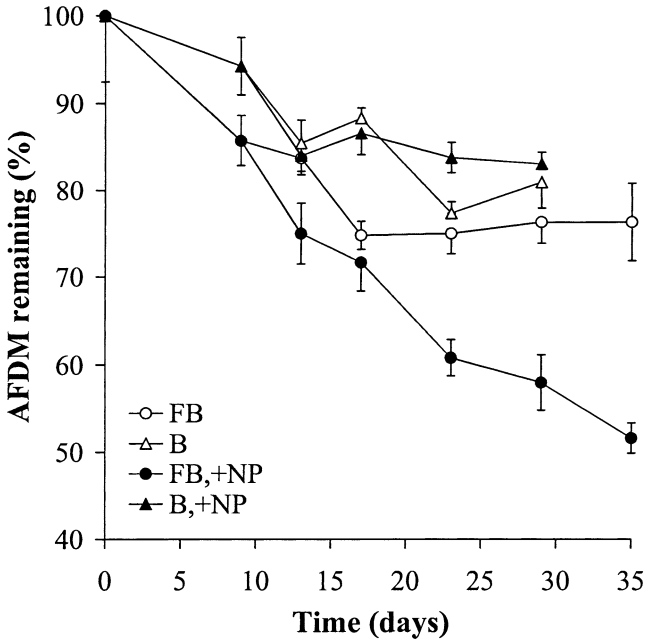

Fig. 1. Ash-free dry mass (AFDM) loss of maple leaf disks colonized in stream by natural microbial assemblage (i.e., fungi + bacteria) (FB) and in chambers designed to reduce fungal colonization (B) and then incubated in laboratory microcosms in control and nutrient enrichment (+NP) treatments. Symbols are means ± 1 SE.

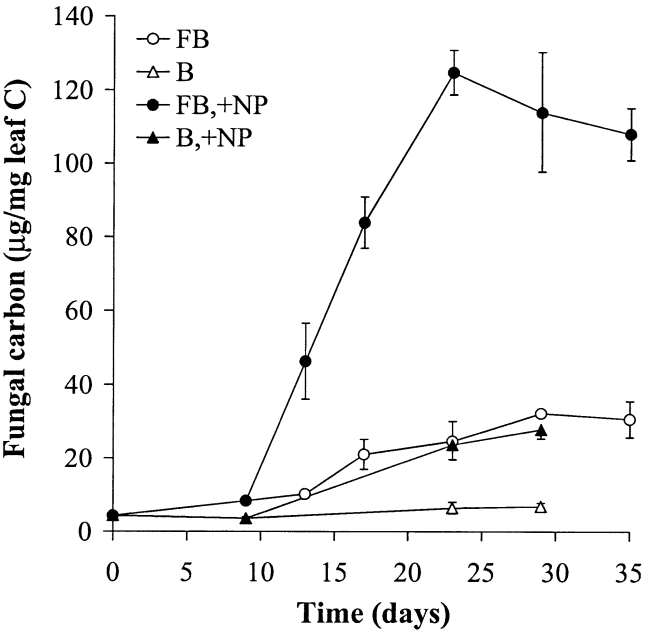

Fig. 2. Fungal biomass associated with leaf disks. For abbreviations, see Fig. 1. Symbols are means ± 1 SE.

The differences in fungal biomass associated with leaf material in low and high nutrient solutions for both the natural microbial assemblage and fungal-reduction treatments were significant (ANOVA, $p < 0.001$, Fig. 2). Leaf disks colonized by the full microbial assemblage in the stream started significant accumulation of fungal biomass very soon after being placed in the high-nutrient microcosms. Fungal biomass in this treatment reached a maximum after 23 d with fungal biomass corresponding to 12.5% of total detrital mass at that time in comparison to a maximum of 3.2% in the full assemblage–low nutrient treatment. Our attempt to exclude fungi was only partially successful as some gain in fungal biomass was observed in the fungal-reduction treatment at high nutrient concentration. However, fungal biomass of the full microbial assemblage exceeded that in fungal-reduction treatment (ANOVA, $p < 0.001$) by at least a factor of 4 irrespective of nutrient availability (Fig. 2).

Sporulation rates of aquatic hyphomycetes from leaf disks colonized by the full microbial assemblage were significantly higher in the high-nutrient than in the low-nutrient treatment (ANOVA, $p < 0.001$, Fig. 3). Sporulation was also detected in the fungal-reduction treatment as some fungal colonization occurred. However, total conidia output was more than an order of magnitude lower at both nutrient levels in comparison to that from leaf disks inoculated with the full microbial assemblage.

A total of 15 species of aquatic hyphomycetes were identified from the full microbial assemblage–high nutrient treatment in comparison to only 9 species in the low nutrient treatment (Table 2). One species (*Articulospora tetracladia*) dominated in the fungal-reduction treatment. We found a clear successional pattern in sporulation of the dominant species of aquatic hyphomycetes from the full microbial assemblage–high nutrient treatment. The relative abundances during the study period are illustrated in Fig. 4. *Tetrachaetum elegans* started conidia production early but gradually declined both in terms of percent

Table 1. Decomposition rates (k) of maple leaf disks colonized in stream by natural microbial assemblage (i.e., fungi + bacteria) (FB) and in chambers designed to reduce fungal colonization (B) and then incubated in laboratory microcosms in control and nutrient enrichment (+NP) treatments

Treatment	k (d^{-1}) ± 95% CL	r^2
FB	0.0068 ± 0.0033	0.46 a
B	0.0085 ± 0.0030	0.64 a
FB,+NP	0.0192 ± 0.0026	0.91 b
B,+NP	0.0069 ± 0.0029	0.56 a

95% confidence limits are included. Different letters indicate significant differences ($p < 0.001$) among slopes using Tukey's test

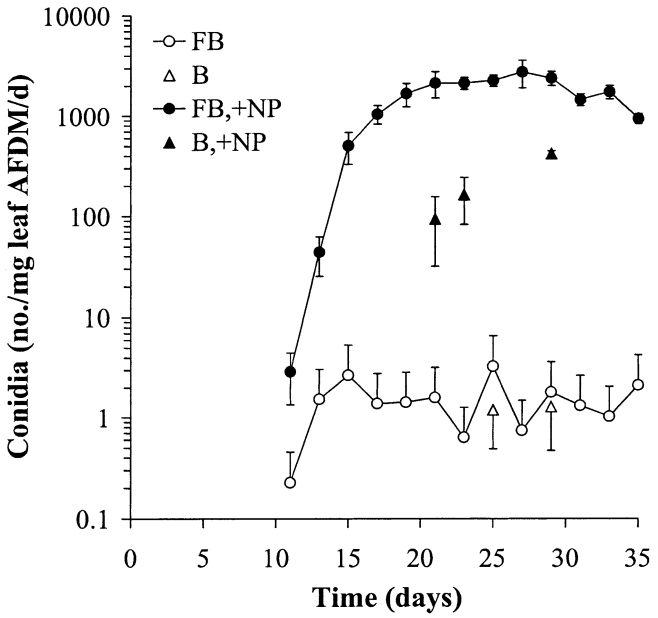

Fig. 3. Sporulation rate of aquatic hyphomycetes on leaf disks in laboratory microcosms. For abbreviations, see Fig. 1. Symbols are means ± 1 SE.

contribution to conidia pool and absolute numbers after its maximum at day 19 and was replaced by *Lunulospora curvula*. The contribution of *Articulospora tetracladia* remained relatively constant throughout the experiment. Other treatments did not show apparent changes in species abundances with time.

Bacterial biomass in laboratory microcosms was stimulated by higher nutrient concentrations in both natural microbial assemblage and fungal-reduction treatment (ANOVA, $p = 0.004$ and $p < 0.001$, respectively). However, at each nutrient concentration, bacterial biomass was not significantly affected by the inoculum the leaf disks received (ANOVA, $p = 0.23$, low nutrient concentration; $p = 0.12$, high nutrient concentration, Fig. 5).

Respiration rates of microorganisms from high-nutrient treatments exceeded those from low-nutrient microcosms for both natural microbial assemblage and fungal-reduction treatment (ANOVA, $p = 0.002$ and $p = 0.02$, respectively). Reduction of fungal colonization had a negative effect on respiration rates at both low and high levels of nutrients (ANOVA, $p = 0.03$ and $p < 0.001$, respectively, Fig. 6).

Carbon flow from leaf litter to the microbial compartments is illustrated in Table 3. Cumulative fungal production (and hence yield coefficient) in the full microbial assemblage exceeded bacterial production by a factor of 36 and 27 in the low and high nutrient treatments, respectively. Bacterial production was not stimulated in the fungal-reduction treatment. Respiration (mineralization) accounted for a significant portion (18–26%) of the leaf

Table 2. Aquatic hyphomycete conidia from laboratory microcosms (mean of percent contributions at each sampling date)

	Treatment			
Species	FB	B	FB,+NP	B,+NP
Alatospora acuminata Ingold	0.2		0.4	0.6
Anguillospora filiformis Greathead	3.4		0.9	
Articulospora tetracladia Ingold	34.5	100.0	23.4	83.3
**Casaresia sphagnorum* Gonz. Frag.	0.5		1.5	
Clavariopsis aquatica De Wild.			+	
Dimorphospora foliicola Tubaki			0.6	6.2
Goniopila monticola (Dyko) Marvanová & Descals			+	
Heliscella stellata (Ingold & V.J.Cox) Marvanová			0.1	
Heliscus lugdunensis Sacc. & Thérry			+	0.1
Lemonniera filiformis Dyko			0.1	0.8
Lemonniera pseudofloscula Dyko			0.2	0.6
Lunulospora curvula Ingold	0.8		22.0	
Tetrachaetum elegans Ingold	57.6		45.2	5.9
Tricladium chaetocladium Ingold	0.2		2.2	
Triscelophorus monosporus Ingold	1.0			
[#]Sigmoid (<60 μm)	1.8		3.5	2.5
Sum	100.0	100.0	100.0	100.0
Total no. of species	9	1	15	8

+, Mean percent contribution less than 0.1%

*, Observed only at first sampling date (day 11) and presumably did not sporulate on leaf disks

[#] Sigmoid spores may represent unbranched conidia of *A. acuminata*

For abbreviations, see Table 1

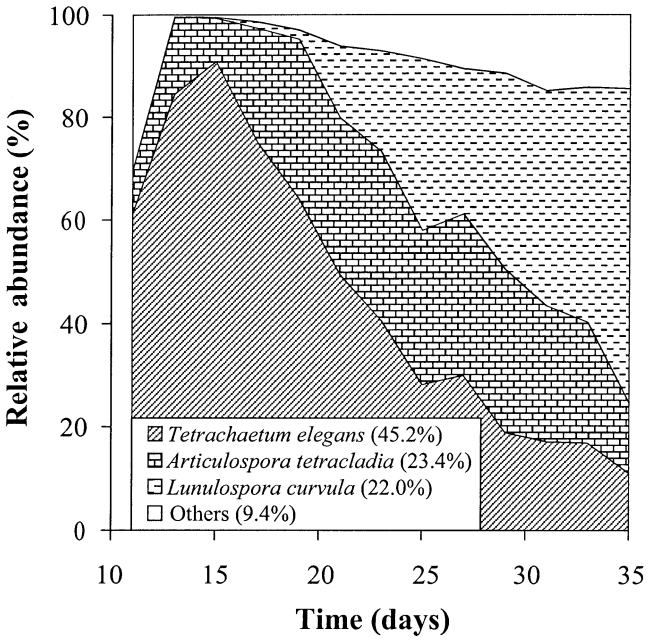

Fig. 4. Relative abundance of aquatic hyphomycete conidia released from leaf disks in laboratory microcosms in treatment FB, +NP. Means of percent contributions over the study period are given in parentheses.

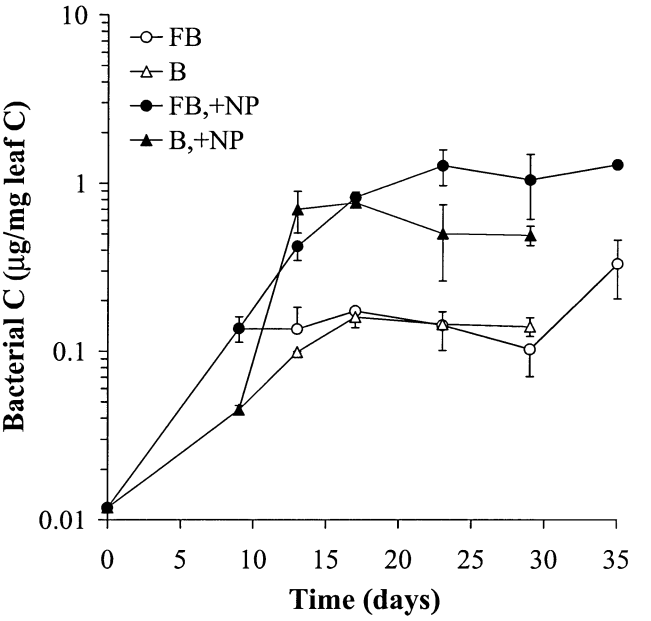

Fig. 5. Bacteria in laboratory microcosms (pooled data for bacteria associated with leaf disks and suspended in fluids). For abbreviations, see Fig. 1. Symbols are means ± 1 SE.

carbon loss. The leaf mass loss that was explained by the partial carbon budget ranged from 20 to 40% suggesting that significant leaf carbon was channeled to DOC and FPOC (not measured).

Discussion

Increased nutrient concentrations in experimental microcosms stimulated fungi and bacteria to a similar degree. When leaves were inoculated with the full microbial assemblage, the maximum fungal and bacterial biomass associated with leaf material was 3.9 and 4.0 times higher (7.4 times if bacteria in fluids are included) and cumulative productions were also 3.9 and 5.1 times higher in the high-nutrient than in the low-nutrient treatment. As a result the dominance of fungi did not change. During the course of this study the fungal contribution to the total microbial biomass associated with leaf material (or total microbial biomass of the microcosm including bacteria and conidia in fluid) varied from 98.4 to 99.8% in the low- and high-nutrient microcosms. These results are similar to those from other studies where simultaneous estimates of fungal and bacterial biomass have been made. The fungal contribution to total microbial biomass associated with decaying leaf litter in lotic ecosystems ranging from first-order streams to seventh-order river with contrasting nutrient concentrations varied from 88 to 100% [1–3, 14, 17, 41]. If we consider cumulative microbial production instead of biomass, the fungal contribution decreases to 97.3 and 96.5% in the low- and high-nutrient microcosms, respectively. Similar shifts toward higher bacterial contributions have also been found when bacterial and fungal production are compared using radiotracer techniques [1, 2, 41]. Fungal yield coefficients exceeded those of bacteria by factor of 36 and 27 in low and high nutrient treatments, respectively (Table 3).

Nutrient enrichment increased leaf decomposition rate only in the microcosms with the full microbial assemblages (Table 1). Leaf material in the fungal-reduction treatment amended with nutrients exhibited a decomposition rate similar to those in low-nutrient treatments, and it was not significantly different from that found for abiotic leaching/fragmentation of maple leaf disks under the same conditions in laboratory microcosms [18]. Positive effects of inorganic nitrogen and/or phosphorus on leaf decomposition have been demonstrated both in laboratory studies [20, 21, 27, 36] and in stream experiments [12, 16, 17]. Such a response is observed when either of these nutrients is limiting before amendment, but not when nutrients are in sufficient supply [23, 28]. Recently, accelerated leaf decomposition due to nutrient enrichment

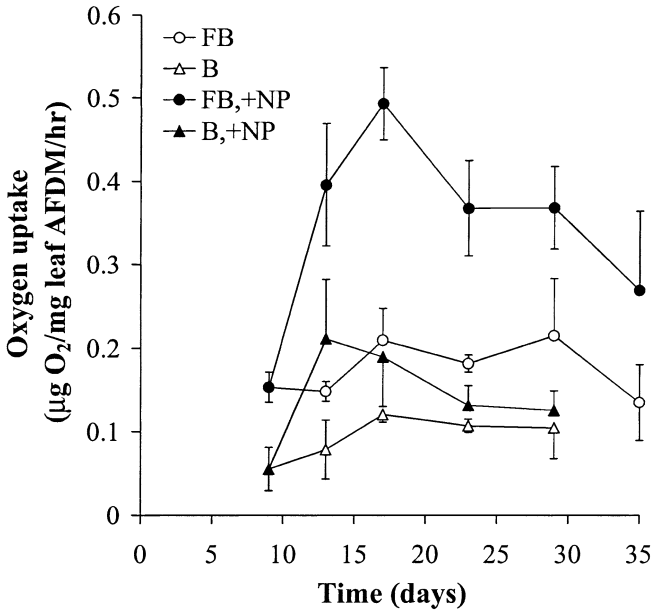

Fig. 6. Respiration of microorganisms from laboratory microcosms. For abbreviations, see Fig. 1. Symbols are means ± 1 SE.

has been linked to fungal activity [16, 32, 36, but see 26]; however, bacteria were not measured in those studies. Results of our study point to fungal and not bacterial activity as the major cause of the increased leaf decomposition rate after nutrient amendment. In field experiments, invertebrates have also been implicated in increased leaf breakdown following nutrient enrichment [26], presumably through enhanced fungal activity and conditioning of leaf detritus that constitutes an important food resource for shredders.

Analysis of the partial carbon budgets for decomposing leaf material colonized by the full microbial assemblage (Table 3) shows that 8% of leaf carbon loss in the low-nutrient treatment and 17% in the high-nutrient treatment was channeled to microbial (essentially fungal) production. Fungal yield coefficients have been reported to vary from 1 to 23% in a laboratory study depending on N and P availability [32]. Similar variability has been reported from streams of different chemistry (11-15%[35], 1.5-7.5%[41], 31%[32]). Respiration of the microbial community increased by 70% at the high nutrient concentration, but because cumulative production increased even more (see above), microbial production efficiency also increased by 72%. Of the leaf carbon lost in our laboratory microcosms, 24 and 22% was mineralized at low and high nutrient levels, respectively. However, the fate of a sufficient portion of leaf carbon lost in our experiments remained unexplained by these partial budgets. Losses of DOC (perhaps partially consumed by bacteria in fluids of microcosms) and FPOC (produced mainly because of macerating activity of aquatic hyphomycetes) jointly accounted for 68 and 60% of the total leaf carbon loss in low- and high-nutrient treatment, respectively (calculated by difference).

In comparison to a few studies where partial organic matter budgets have been constructed our estimates appear to be in reasonable agreement. Baldy et al. [1, 3] reported that

Table 3. Carbon budget for microorganisms associated with decomposing maple leaf disks by day 29 in laboratory microcosms

	Treatment			
Parameter	FB	B	FB,+NP	B,+NP
Initial leaf C, mg/chamber	43.3 ± 0.3	41.5 ± 0.4	41.7 ± 0.6	41.2 ± 0.5
Leaf mass loss, %	26.2 ± 2.3	19.7 ± 3.0	48.6 ± 3.7	20.1 ± 1.7
Final fungal biomass associated with leaf disks, µg C/mg leaf C	32.2 ± 1.0	6.8 ± 0.9	113.7 ± 16.2	27.8 ± 2.5
Net mycelial production, µg C/mg initial leaf C	20.3 ± 1.5	1.2 ± 0.6	60.5 ± 5.3	18.5 ± 1.7
Total conidia production, µg C/mg initial leaf C	0.11 ± 0.02	0.01 ± 0.00	19.3 ± 1.4	1.6 ± 0.2
Cumulative fungal production, µg C/mg initial leaf C	20.4 ± 1.5	1.2 ± 0.6	79.9 ± 6.5	20.0 ± 1.8
Final bacterial biomass associated with leaf disks, µg C/mg leaf C	0.06 ± 0.03	0.06 ± 0.02	0.92 ± 0.41	0.30 ± 0.06
Net bacterial production on leaf disks, µg C/mg initial leaf C	0.04 ± 0.02	0.04 ± 0.01	0.51 ± 0.24	0.24 ± 0.05
Total bacterial production from fluid, µg C/mg initial leaf C	0.53 ± 0.04	0.62 ± 0.05	2.40 ± 0.39	2.53 ± 0.36
Cumulative bacterial production, µg C/mg initial leaf C	0.57 ± 0.02	0.65 ± 0.06	2.91 ± 0.46	2.77 ± 0.31
Yield coecient—fungi, %	8.0 ± 1.3	0.6 ± 0.3	16.4 ± 0.1	10.0 ± 0.5
Yield coecient—bacteria, %	0.22 ± 0.02	0.34 ± 0.02	0.61 ± 0.12	1.39 ± 0.13
Respiration, µg C/mg initial leaf C	63.0 ± 6.8	34.6 ± 3.9	106.9 ± 6.5	52.3 ± 2.6
Microbial production eciency, %	25.4 ± 3.4	5.0 ± 1.7	43.6 ± 3.3	30.4 ± 2.7
Explained mass loss, %	32.2 ± 1.9	19.8 ± 4.9	39.5 ± 2.9	38.1 ± 4.1

[a] For abbreviations see Table 1. Data are means ± 1 SE. Leaf mass loss was corrected for final microbial biomass associated with leaf disks. Yield coefficient = cumulative production/leaf mass loss, production efficiency = cumulative production/(cumulative production + respiration), explained mass loss = (cumulative production + respiration)/leaf mass loss

41–65% of the leaf carbon lost in decomposition studies in the Garonne river was converted to fungal biomass and CO_2, somewhat higher than was explained by both fungal and bacterial activity of the full microbial assemblage in low (32%) and high (40%) nutrient treatments in our experiments (Table 3). Hieber and Gessner [19] gave more conservative estimates of fungal (15–18%) and bacterial (9–12%) contributions to total leaf mass loss (note, however, that shredder feeding accounted for 51–64% of leaf mass loss in that study). On the other hand, in another study by Baldy et al. [2] only 28% of the leaf mass loss was explained by all organic fractions measured (microbial production, respiration, DOC and FPOC release). This was attributed to the use of green instead of autumn-shed leaf material and extensive invertebrate feeding [2].

Our attempt to exclude fungal colonization by means of propagule size fractionation in colonization chambers in the stream was not completely successful. We believe that some cylindrical conidia branches of mainly *Articulospora tetracladia* (Table 2) passed through 5 μm membrane pores resulting in minor contamination. Nevertheless, we achieved a significant decrease in mycelial biomass (4.1–4.7 times) and conidia production (12 times) in comparison to chambers with the full microbial assemblage that led to significant changes in total microbial activity and decomposition (Tables 1, 3). This treatment did not significantly change cumulative bacterial production at either nutrient level, and leaf mass loss was negatively affected, suggesting that bacterial participation in carbon flow from decaying leaf litter is low regardless of presence of fungi and nutrient availability.

The higher yield coefficients (1.5 and 2.3 times) of bacteria in fungal-reduction treatments at low and high nutrients, respectively (percentage of leaf carbon loss channeled to bacterial production), suggest that bacteria are subjected to strong competition with fungi for resources available in leaf litter. Aquatic hyphomycetes are well equipped with hydrolytic enzymes capable of degradation of virtually all components of plant tissue [7, 29 and references therein]. In addition, the penetration of fungal hyphae into leaf tissue, in contrast to the growth of bacteria which is mainly restricted to the surface, allows fungi to achieve better acquisition of available resources. On the other hand, the observation that the final bacterial biomass in the full microbial assemblage was three times higher (but yet not statistically significant because of high variability, ANOVA, $p = 0.21$) than bacterial biomass in the fungal-reduction treatment at high nutrient concentrations suggests that bacteria associated with leaf litter may depend on fungal activity. This could be due to maceration of leaf tissue by fungi [37] that increase available colonization area, growing as epiphytes on fungal hyphae, obtaining organic nutrients from plant polymers partially digested by fungal extracellular enzymes or lysis of senescent fungal hyphae.

Results of the present study suggest that the relative contributions of fungi and bacteria to microbial decomposition of submerged leaf litter is unlikely to be significantly affected by the availability of inorganic nutrients in water. Fungi exhibit a dominant role among the leaf-associated microbiota at relatively high levels of nutrient enrichment through higher biomass, production, and activity and are major agents of decomposition of submerged leaf litter in streams.

Acknowledgments

This work was supported in part by the North Atlantic Treaty Organization under NSF-NATO Postdoctoral Fellowship in Science and Engineering awarded to VG and by NSF grant DEB 9806610.

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