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A Cross-System Comparison of Bacterial and Fungal Biomass in Detritus Pools of Headwater Streams

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ABSTRACT

The absolute amount of microbial biomass and relative contribution of fungi and bacteria are expected to vary among types of organic matter (OM) within a stream and will vary among streams because of differences in organic matter quality and quantity. Common types of benthic detritus [leaves, small wood, and fine benthic organic matter (FBOM)] were sampled in 9 small (1st–3rd order) streams selected to represent a range of important controlling factors such as surrounding vegetation, detritus standing stocks, and water chemistry. Direct counts of bacteria and measurements of ergosterol (a fungal sterol) were used to describe variation in bacterial and fungal biomass. There were significant differences in bacterial abundance among types of organic matter with higher densities per unit mass of organic matter on fine particles relative to either leaves or wood surfaces. In contrast, ergosterol concentrations were significantly greater on leaves and wood, confirming the predominance of fungal biomass in these larger size classes. In general, bacterial abundance per unit organic matter was less variable than fungal biomass, suggesting bacteria will be a more predictable component of stream microbial communities. For 7 of the 9 streams, the standing stock of fine benthic organic matter was large enough that habitat-weighted reach-scale bacterial biomass was equal to or greater than fungal biomass. The quantities of leaves and small wood varied among streams such that the relative contribution of reach-scale fungal biomass ranged from 10% to as much as 90% of microbial biomass. Ergosterol

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concentrations were positively associated with substrate C:N ratio while bacterial abundance was negatively correlated with C:N. Both these relationships are confounded by particle size, i.e., leaves and wood had higher C:N than fine benthic organic matter. There was a weak positive relationship between bacterial abundance and streamwater soluble reactive phosphorus concentration, but no apparent pattern between either bacteria or fungi and streamwater dissolved inorganic nitrogen. The variation in microbial biomass per unit organic matter and the relative abundance of different types of organic matter contributed equally to driving differences in total microbial biomass at the reach scale.

Introduction

Carbon, energy, and inorganic nutrient budgets of many headwater streams are dominated by processes associated with large pools of detrital organic matter (OM) [45], and these processes are presumed to be mediated by heterotrophic microbes, principally bacteria and fungi [21]. Microbial standing stocks and metabolic activities are expected to vary with the physical nature and biochemical composition of the organic matter (e.g., [44]). For instance, fungal biomass and production generally exceed bacterial biomass and production on intact leaf litter [10, 47], whereas bacteria dominate heterotrophic biofilms on fine particulate organic matter [7]. Microbial biomass will vary at larger spatial scales because of variation in the standing stocks of different types of detrital material. The surrounding vegetation, climate, and stream reach characteristics will control the quantity, timing, and characteristics of allochthonous loading, whereas stream geomorphology and hydrology will affect net standing stocks (see [20]). This combination of intrinsic and extrinsic controlling factors may act to magnify differences among streams (or stream reaches) in quantity of microbial biomass per unit area of stream bottom, or they may tend to cancel out differences.

Variability in the absolute standing stocks of microbes and the relative proportion of bacteria and fungi may result in heterogeneity in food quality or availability for a range of detritivorous animals. Although it is clear that microbial biomass is not the sole source of detritivore nutrition, it is more readily assimilated than the nonliving organic substrate [9]. It is also clear that invertebrate abundance often correlates with standing stocks of OM and its associated microbes [30, 36]. Moreover, the contribution of microbial organic matter as a food resource varies greatly among different groups of insects. There was a 10-fold variation in the contribution of bacterial carbon to a range of aquatic insects in a headwater stream food web, with collectors of fine benthic organic matter deriving the greatest proportion of their assimilated carbon from bacterial biomass [15]. At smaller scales, detritivore feeding preferences may vary depending on fungal taxa present [2]. Evidence for selective feeding on bacteria is less clear; certain taxa are particularly efficient in depressing bacterial numbers [27], while grazing pressure from other taxa appears to have a trivial impact on benthic bacteria [3]. At very small scales, model systems suggest differences in susceptibility to grazing by various protozoa [48].

Bacteria and fungi play significant roles in organic matter degradation, but their contributions will vary across detrital types and among habitats (e.g., pool, riffle, debris dam) in streams. Physical retention structures such as log dams lead to accumulation of coarse-particulate organic matter, and these represent "hot spots" of carbon degradation in stream ecosystems [17, 11]. It is likely that fungal metabolism dominates these habitats [13], so net carbon transformations will reflect the degradative ability of the fungal community. Concurrent with decomposition and assimilation of organic matter into microbial biomass, the low nutrient content of many types of allochthonous detritus suggests microbial growth may be affected by the supply of inorganic nutrients from the water column. Variation in dissolved inorganic nitrogen among streams is important in controlling microbial degradation of leaf litter [40] and small woody debris [42]. There is also evidence for immobilization of external phosphorus by leaf-degrading fungi [14] and stimulation of leaf decomposition [28]. Nutrient uptake in streams has been investigated extensively, most often from the perspective of periphyton nutrient limitation and nutrient spiraling (see [37]) but heterotrophic microbes may dominate nutrient retention in shaded streams with large allochthonous loadings and in the hyporheic zone [22, 23].

Although microbes are important components of stream ecosystems, there has not been an explicit con-

sideration of their relative distribution among organic matter types, nor has there been a larger scale study of variation among streams. To address both these scales, microbial biomass associated with several types of organic matter was determined for nine streams distributed across North America. These streams were selected to vary in a suite of potential controlling factors such as ambient nutrient concentrations and abundance of several detrital pools. By sampling across divergent stream types at different times of year we hoped to encompass a large proportion of total variability among streams. Our intent was not to describe any particular stream in detail but to span the range in potential differences among streams. The broad range of streams sampled and the consistent methodology for sampling and measurement provide a unique opportunity for cross-site comparisons of sources of variation in microbial biomass.

Methods

Study Sites

The 9 streams selected for study vary from arid- to humid-temperate and humid-tropical headwater streams and provide wide gradients in water chemistry and nutrient availability, metabolic rates, hydrology, food web complexity, and geomorphology. Nitrate (reported as NO_3-N) was measured using the cadmium reduction method [1] and ammonium (NH_4-N) and soluble reactive phosphorus (SRP) concentrations were analyzed spectrophotometrically [46].

Sampling

At each of the nine streams, four common types of organic matter were collected: leaves, biofilms on small woody debris, and FBOM from the streambed surface as well as FBOM from 2–5 cm below the surface. Additionally, at two of the sites (Sycamore Creek, AZ, and Kings Creek, KS) filamentous algae were common enough to be included as a type of OM and contributed to reach-scale estimates of microbial abundance. The reaches sampled at each of the nine streams ranged from 150 to 450 m and were selected to encompass the general geomorphological and ecological characteristics such as pools, riffles, and debris accumulations.

For each of the four primary organic matter types (leaves, small wood, surface and subsurface FBOM) we estimated both microbial biomass (bacterial and fungal) and OM standing stocks to yield reach-scale microbial biomass. To generate a representative estimate of microbial biomass on a particular type of OM, we sampled at approximately 10 points spread along the stream reach, making sure to collect samples from all the representative habitat types (i.e., backwater/pool, riffles, debris dams). Leaves and small woody debris for microbial abundance estimates were collected by hand and all collected material of each OM type was pooled and then prepared for subsampling. Leaves were cut up into small pieces using forceps and clean, but not sterilized, scissors; wood biofilm was collected by scraping wood surfaces with a razor blade. Subsamples (5 replicates each, 5–15 g wet mass) for leaves or wood biofilm were preserved in 20 mL of 5% buffered formalin (for bacterial abundance) or 20 mL of HPLC-grade MeOH (for extraction of ergosterol). Surface and subsurface FBOM slurries were collected using a suction device, and again, samples from approximately 10 different locations along the experimental reach (from varying habitat types) were collected and pooled to achieve representative FBOM samples for the reach. Subsurface FBOM was exposed for collection using a trowel to scrape away the top 2–5 cm of sediments. From each pooled slurry, five subsamples each were taken for bacterial and fungal biomass (approximately 10 mL of slurry per subsample) and fixed with buffered formalin or MeOH, respectively. For all four organic matter types, we determined a wet wt:ash wt conversion factor by taking additional subsamples from each pooled OM sample, determining wet weight of each replicate, drying, weighing, ashing at 550°C, and reweighing. We report variability among subsamples of microbial biomass to provide some measure of analytical variability but do not use these as error estimates for reach-scale observations.

Bacterial abundance was estimated from acridine orange direct counts following homogenization (2 min) and sonification (2 min at 10 W with Ultrasonics W-380 Microprobe) to release and disperse the cells. One filter was prepared from each of the five subsamples and fluorescing cells were counted for at least five different locations on each filter (minimum of 100 cells counted per filter), yielding coefficients of variation of 20% or less for a particular sample type. For consistency, the same person prepared and counted slides from all sites. Ergosterol, a sterol specific to fungi, was extracted in MeOH at 80°C for 2 h, then saponified, separated in pentane, and quantified on a Waters HPLC [24].

Standing stocks of small wood, leaves, and FBOM were determined for use in calculating reach-scale microbial biomass from estimates of microbial biomass per unit organic mass of each OM type. At each stream, we sampled at 10–12 locations along each experimental reach in a stratified random fashion, to obtain estimates for both riffle and pool habitats. We placed a metal cylinder (0.07 m^2) into the sediments and removed coarse benthic organic matter (CBOM) by hand, separating it into wood and leaves. Fine benthic organic matter was sampled by sealing a metal cylinder on the stream bottom, mixing the sediments to approximately 5 cm, pumping streamwater and particles into a bucket, measuring total volume, subsampling, and filtering onto a GF/F filter. Samples of each OM type were dried at 55°C, weighed, ashed at 550°C, and reweighed to determine standing stocks in g AFDM/m^2 streambed. Additionally, %C and %N were determined for subsamples of each OM type using a Carlo Erba NA 1500 CN Analyzer (University of Georgia). Wet wt:ash wt. conversion

factor, bacterial or fungal biomass estimates, and habitat-weighted standing stock estimates were used to determine reach-scale estimates of microbial biomass for each OM type.

For direct comparison of microbial biomass, bacterial cell abundance and ergosterol concentrations were converted to units of carbon. All bacterial cell numbers were converted to carbon units assuming a common carbon content of 2×10^{-14} g C/cell, which is equivalent to a mean cell biovolume of 0.2 μm^3/cell multiplied by 100 fg C/μm^3 (cf. [6]). Ergosterol was converted to fungal carbon assuming 1 mg fungal C/11 μg ergosterol [12].

To compare microbial abundance across types of organic matter, the mean value of microbial biomass on a particular type of OM for each stream was used as an independent observation in a one-way ANOVA. Since all streams yielded samples of FBOM, but Sycamore Creek had no samples of leaves or wood and Kings Creek had insufficient quantities of small wood to sample without causing depletion, the number of observations ranged from 9 to 7. Potential relationships between microbial biomass and controlling factors such as carbon:nitrogen ratio or external factors such as streamwater temperature or nutrient content were explored with regression analyses.

Results

Microbial Abundance

Bacterial and fungal biomass varied dramatically among types of OM and across sites with overall coefficients of variation (SD/mean for all sites, OM types combined) for bacteria of 0.83 and for fungi of 1.9. There were significant differences in standing stocks of both bacteria and fungi among types of organic matter (Fig. 1) with generally higher biomass of bacteria per unit mass on finer particles and higher fungal biomass on coarse particulate organic material (leaves and small wood). Bacterial biomass differed significantly among types of OM ($p = 0.03$) with both types of FBOM supporting significantly higher bacterial biomass than leaves or wood (based on least significant difference [LSD] at $p = 0.05$ [34]). The maximum difference in bacterial biomass among types of organic matter was somewhat greater than threefold and occurred in the comparison of leaves and surface FBOM. Fungal biomass also differed significantly among types of OM ($p = 0.015$) with values per unit organic mass of leaves and small wood significantly higher than either type of FBOM (LSD $p < 0.05$). The range in fungal biomass among types of organic matter (~40-fold) was greater than the range in bacteria among types of organic matter. Bacterial and fungal biomasses were negatively correlated ($r = -0.36$, $p < 0.05$) considering the common OM types from all sites.

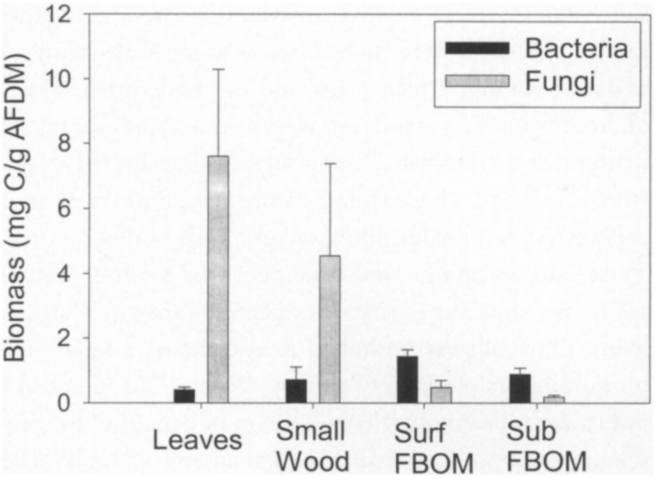

Fig. 1. Bacterial and fungal biomass for the four types of organic matter collected from all streams. Values are means of 7–9 streams ±1 SE.

Variation in microbial biomass among sites was compared for bacterial biomass on surface FBOM (OM with highest average bacterial biomass) and fungal biomass on leaves (OM with highest average ergosterol concentration). These data cannot be analyzed statistically to detect differences among streams, but they do show a larger range across streams for fungal biomass (~10-fold, Fig. 2B) than for bacterial biomass (3-fold, Fig. 2A). There was not a significant cross-site relationship between bacterial and fungal biomass on leaves ($p = 0.44$) or FBOM ($p = 0.47$).

Detritus Standing Stocks

Standing stocks of detrital material varied considerably among streams with surface FBOM, the major component in 6 of 9 cases, averaging 69% of total detrital standing stock (Table 1). Study streams were intentionally selected to span the range in abundance of the various types of OM so the relative size of the various pools ranges from almost 100% FBOM (Kings Creek, KS) to roughly a 50:50 mix of FBOM and small wood (Eagle Creek, MI). This variation in the standing stocks of different types of detritus combined with differences in microbial abundance among OM types resulted in substantial differences among streams in the relative and absolute biomass of bacteria and fungi and how that biomass was distributed among detritus pools. As was evident in the comparison of microbial biomass across types of OM, fungi show greater variation than bacteria in both their abundance per g OM and the resultant areal standing stock.

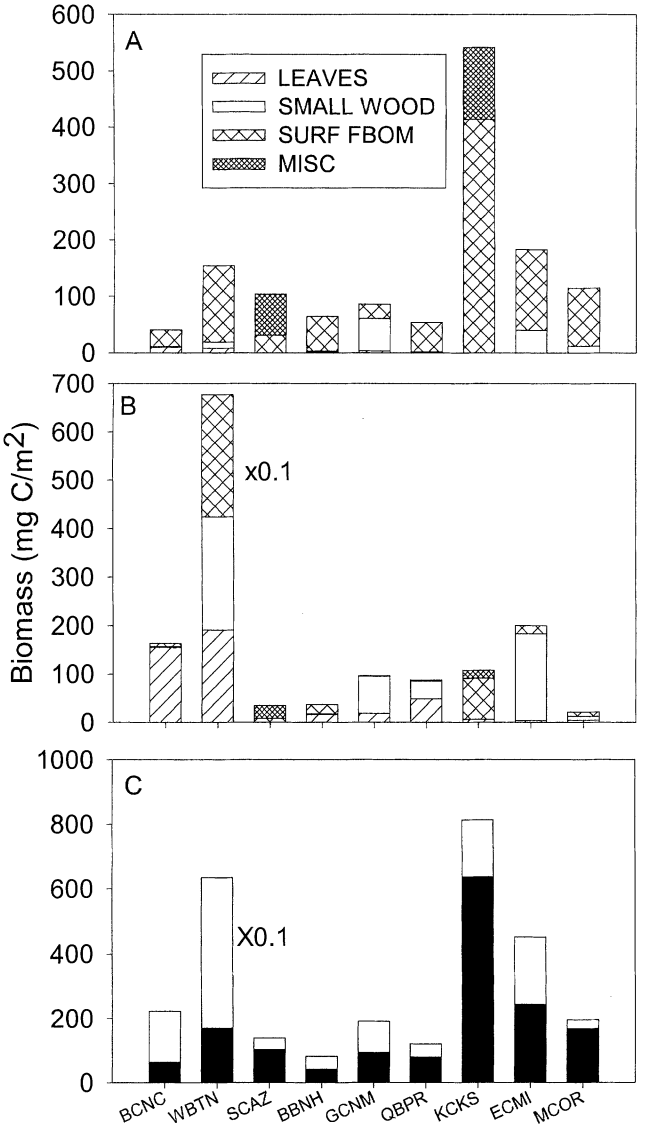

Fig. 2. Habitat-weighted reach-scale estimates of (A) bacterial biomass, (B) fungal biomass, and (C) total microbial biomass for the 9 stream reaches. Values for fungal biomass in Walker Branch, TN, have been multiplied by 0.1 to bring them onto a common scale. The miscellaneous category is filamentous algae in Sycamore Creek (SCAZ) and epilithic material in Kings Creek (KCKS). Site abbreviations are given in Table 1.

Variation in fungal biomass per unit mass of organic matter and mass of organic matter per unit area of stream bottom combine to generate large variations in fungal biomass per unit area. The CV for leaf fungal biomass per m^2 (2.4) was greater than the CV for fungal biomass per unit OM (1.0) or for the standing stock of leaves per m^2 (1.6) across streams. In contrast, the CV for FBOM bacterial biomass (0.4) was lower than the CV of the standing stock of FBOM per m^2 (0.8) across streams. The net result is that bacterial biomass is less variable than fungal biomass among stream ecosystems.

The majority of reach-scale bacterial biomass was associated with the FBOM component (Fig. 2A), whereas fungal biomass was more variable with the major contribution from leaves and small wood in varying proportions (Fig. 2B). Summing fungal and bacterial habitat-weighted biomass yields an estimate of total microbial biomass representative of the mix of OM pools found in these nine study reaches (Fig. 2C). Total microbial biomass ranged from about 40 mg C/m^2 (Sycamore Creek, AZ) to just over 4500 mg C/m^2 (Walker Branch, TN).

The contribution of microbial carbon to the detrital standing stocks of carbon was quite low (0.2–2%) and showed considerable variation among OM classes (Fig. 3) with the highest contribution of microbial carbon on leaves and small wood. The relative contribution of bacteria and fungi to the total microbial biomass varies dramatically among types of organic matter examined, ranging from over 90% fungal carbon on decomposing leaves and wood to 80% bacterial carbon on subsurface FBOM.

Controls on Microbial Biomass

There appeared to be qualitative effects of substrate composition on microbial biomass. There was a significant negative relationship between bacterial abundance and detritus C:N when data from all classes of OM were combined (excluding single datum for wood from Mack Creek) (Fig. 4A). There was a significant positive relationship between fungal biomass and detritus C:N (Fig. 4B).

The generally low nutrient content of detrital organic matter (particularly for wood and leaves) suggests the availability of dissolved inorganic nitrogen (DIN) or phosphorus in the water column might influence microbial communities. Despite the broad range in detritus C:N (~10–50) and a wide range in streamwater dissolved inorganic nitrogen (NH_4 + NO_3) concentrations (3–130 μg N/L; (Table 1) there was no significant relationship between either bacterial (r = 0.11) or fungal biomass (r = 0.07) and average DIN. There was a marginally significant (p = 0.06) and weak (r = 0.44) positive relationship between bacterial biomass and streamwater soluble reactive phosphorus, but no relationship between fungal biomass and SRP (r = −0.07). There was no significant effect of temperature on bacterial or fungal biomass when all data were combined or when each class of OM was analyzed separately (r = 0.05 for fungal biomass, all data; r = -0.01

Table 1. Site names and abbreviations, nutrient concentrations, streamwater temperature, and standing stocks of three types of organic matter [fine benthic organic matter (FBOM), leaves and wood surfaces] across the 9 streams[a]

Variable	Upper Ball, Creek, NC	Walker Branch, TN	Sycamore Creek, AZ	Bear Brook,[b] NH	Gallina Creek, NM	Quebrada Basley, PR	South Kings Creek, KS	Eagle Creek, MI	Mack Creek, OR
Site abbreviation	BCNC	WBTN	SCAZ	BBNH	GCNM	QBPR	KCKS	ECMI	MCOR
Collection date	25 Oct 96	31 Mar 97	12 May 97	20 Jun 97	27 Aug 97	20 Feb 98	23 Apr 98	28 Jun 98	1 Aug 98
TEMP (°C)	7.2	12.4	23	14.3	7.2	22	15.5	23	13.1
DISCHARGE (L/s)	129.6	17.5	43	9.1	4	20.2	15.8	202	56.6
NH4 (μg N/L)	3.3	4.1	6	4.3	4.7	3.3	3.0	16	2.2
NO3 (μg N/L)	2.3	18.7	9	54.4	4.2	167.2	2.3	17.5	59.2
SRP (μg P/L)	2.9	3.3	14	3.6	8	14.3	3.3	3.1	13
Epilithon (g AFDM/m^2)	1.3	3.8	18.3	2.7	3.5	3.5	76.0	5.8	2.9
Filamentous algae (g AFDM/m^2)	nc	nc	172.3	nc	nc	nc	2.502	nc	Nc
FBOM (g AFDM/m^2)	43.6	197.0	20.5	46.5	28.25	32.2	212.0	164.6	108.6
C:N (mass/mass)	19.65	18.6	9.0	18.0	18.3	9.9	17	14.1	27
Leaves (g AFDM/m^2)	62.5	76.0	nc	4.2	6.9	6	1.4	0.9	0.4
C:N	50.2	47.5	nc	21.0	23.2	32.2	29.4	18.2	46.0
Wood (g AFDM/m^2)	2.8	112.0	nc	2.7	84.7	6.0	1.4	197.7	3.9
C:N	45.5	48.5	nc	36.9	41.8	32.2	41.7	36.6	59.6

[a] Carbon-to-nitrogen mass ratios are shown for the three common types of organic matter. Temperature, discharge, and nutrient concentrations are average values determined over approximately a 6-week period. Organic matter standing stocks are means based on approximately 10 sampling points in the reach collected at the start of the experiment. nc, not collected due to low abundance.

[b] Bear Brook at the Hubbard Brook Experimental Forest, NH

for bacterial biomass, all data). The statistical power of these analyses is low (see [4] for general discussion and calculations) because of low values for the correlation coefficients rather than inadequate number of observations. For the relationships with temperature or DIN, correlation coefficients were 0.1 or smaller, so power for an analysis of n = 22 to 25 is approximately 0.1 while the power for the relationship between bacteria and SRP was moderate (0.4).

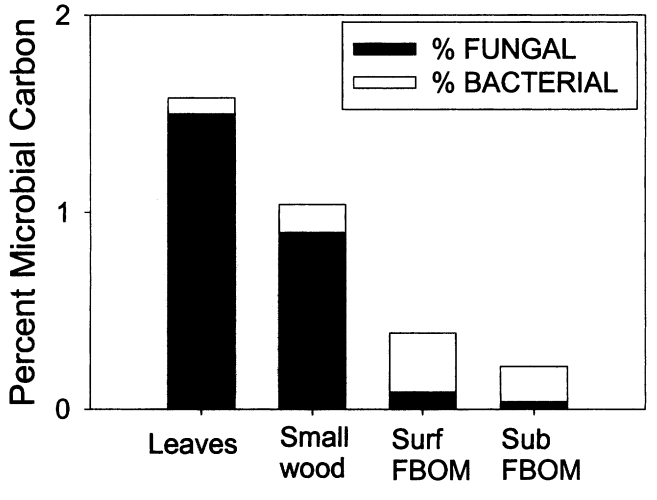

Fig. 3. Percentage of each detrital pool made up of microbial carbon calculated using average values across all sites for bacterial or fungal biomass on each type of organic matter and assuming detritus AFDM was 50% carbon.

Discussion

Controls on Microbial Biomass

Our comparison among these nine streams supports the general patterns that have emerged from individual studies, and the generality of these patterns is strengthened by our explicit attempt to include a diverse range of streams with very different characteristics. Also, our results show how variation in microbial biomass per unit OM interacts with the relative abundance of OM types to determine overall microbial biomass in diverse stream ecosystems. Furthermore, this study reveals the greater sensitivity of fungi to OM type relative to the more even distribution of bacterial biomass across categories of OM.

Fungi are clearly the predominant microbes on coarse particulate organic material (i.e., leaf litter and woody debris), presumably as a consequence of their broad degradative capacity and need for suitable physical substrate

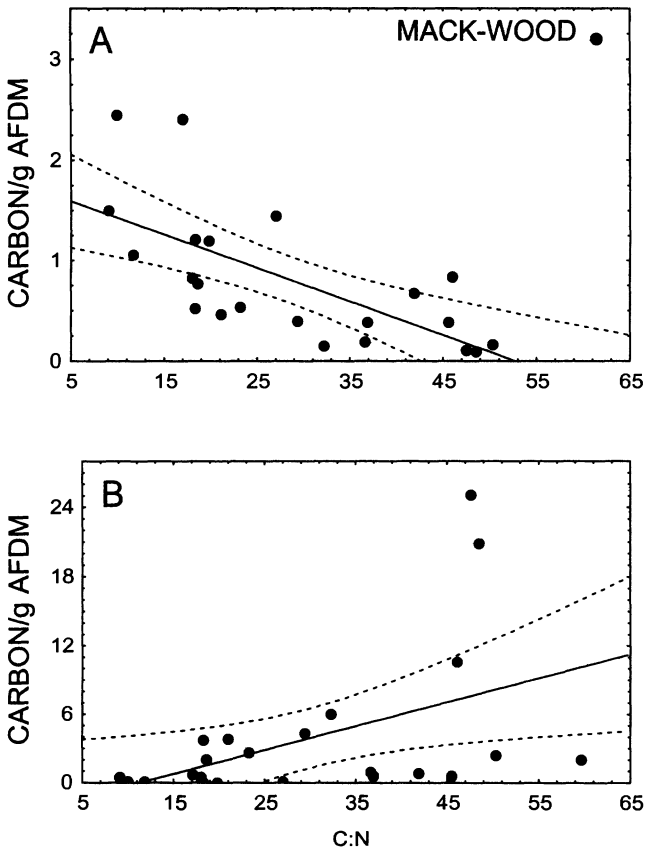

Fig. 4. Relationship between bacterial (A) and fungal (B) biomass and substrate C:N ratio. The correlation for bacteria calculated without the high point (Mack, Wood) was significant (p = 0.0006, r = −0.67). For fungal biomass the correlation is significant (p = 0.02) but weak (r = 0.47).

to support their mycelial mass. The ergosterol concentrations measured for leaf litter across the nine streams in this study (mean = 84 μg ergosterol/g leaf AFDM; range = 30–275) were at the low end of the range of previously reported values (maximum reported ergosterol concentrations = 50–900 μg/g AFDM; see Table 3 in [26]; 200–500 μg ergosterol/g leaf AFDM in [38]; 200–500 μg ergosterol/g leaf DM in [13]). Most previous studies have followed a time sequence of early fungal colonization and growth by measuring ergosterol concentrations on a cohort of leaves after they fell or were placed into a stream [38]. Dynamics of aquatic hyphomycete fungi on leaves have generally shown rapid increases in ergosterol up to the point where sporulation begins followed by stable or declining values. Leaf samples in our comparative study were *in situ* material collected from various sites in each stream during different seasons and so represent a more complete spectrum of leaf ages in a stream. Except for Upper Ball Creek, NC (studied during a period of peak litterfall), our samples of extant leaf litter included a greater representation of older leaves and slow-decaying species than most cohort studies.

There are fewer comparative data on bacterial abundances in small streams, but our estimates for bacteria on leaf litter and FBOM are within the previously reported range. In a study of decomposition of three leaf species (oak, sycamore, and elm) Findlay and Arsuffi [10] found bacterial abundances ranging from 1 to 5 × 10^{10} cells/g AFDM varying with length of decay and leaf species. The values for bacterial density from the present study range from 0.5 to 4 × 10^{10} cells/g AFDM of leaf with an average of 2 × 10^{10} cells/g AFDM leaf. In a comparison of five streams in Ontario, Hudson et al. [19] found bacterial abundances on FBOM ranged from 1 to 5 × 10^{10} cells/g AFDM, slightly lower than the mean of 7 × 10^{10} cells/g AFDM for our nine streams.

Our comparative study also confirms previous suggestions about the relative amounts of bacteria and fungi on various types of detritus in aquatic and terrestrial environments. Fungi dominate the larger size classes of particulate material that retain the original structural integrity of the plant tissues, and their penetrative hyphae can access the interior spaces of these particles. In contrast, bacteria dominate in finer particulate matter that offers a high surface area for colonization and uptake of dissolved organic matter [8]. Prior studies of bacteria and fungi on leaf litter have clearly demonstrated higher productivity and biomass of fungi than bacteria, particularly during the early stages of leaf decay (e.g., [47]). Scanning electron micrographs of biofilms colonizing wood in acid and circumneutral streams in New Zealand were also dominated by fungal hyphae [41]. Smaller size classes of particulate organic matter in wetlands had much higher bacterial than fungal biomass [31].

Detritus quality, often measured as C:N ratio, might also affect microbial abundance. Observed relationships (Figs. 4A, 4B) between microbial biomass and detritus C:N are confounded for two reasons. First, there is covariation between C:N and particle size with FBOM having a much lower mean C:N (16.6) than leaves (C:N = 33) or wood (C:N = 42) (Table 1). Second, microbial biomass contributes to the carbon and nitrogen content of the detrital complex. If this contribution is substantial, the correlation with bacterial biomass becomes circular. As microbial biomass increases, the C:N of the mixture will decline because microbial biomass has a lower C:N ratio than the detritus. To remove the effect of particle size we examined

potential relationships between microbial biomass and C:N within classes of organic matter, although this leaves at most 9 observations in the data set. There was no significant relationship ($p > 0.05$) between bacterial abundance and C:N considering just the FBOM classes, nor was there a relationship between ergosterol and C:N of leaves alone or leaves and wood combined.

The confounding effect of microbial contribution to substrate C and N is only a problem for the bacteria–C:N relationship. The positive association between ergosterol and C:N is actually opposite the pattern predicted if the contribution of nitrogen-rich fungal biomass (cf. [35]) was influencing substrate C:N ratios. High values of fungal biomass would lead to decreases in the C:N of the mixture and a circular correlation would be negative, not positive as observed. Therefore, increasing fungal biomass in the larger size classes was probably due simply to particle size, and higher fungal biomass actually occurs on lower quality (higher C:N) classes of organic matter. The effect of increasing bacterial biomass on the detrital C:N can be assessed by subtracting bacterial carbon and nitrogen from the detrital complex assuming a C:N of 5 for bacterial cells. Following this correction the pattern is still significant ($p<0.05$) with only a slightly lower correlation coefficient ($r = -0.625$ instead of $r = -0.67$) suggesting the contribution of bacterial nitrogen was not driving the observed pattern. Thus, higher bacterial biomass is associated with both smaller particle sizes and higher quality (as measured by C:N ratio) and the relative effects of these two factors cannot be isolated.

A wide array of factors influences microbial standing stocks and activities in aquatic ecosystems, and temperature is usually one of the first controlling factors to be considered. Other studies have reported strong effects of temperature on stream respiration [18, 33], although the strength of the correlation between temperature and metabolism can be highly variable [18]. The observed lack of temperature effects on microbial biomass suggests that while temperature may influence metabolic rates, biomass is perhaps limited by one or a combination of alternative factors including carbon and nutrient availability, grazing pressure, or disturbance frequency. External nutrient supplies are known to affect decomposition of leaf litter [39] and wood [42] in streams, yet we found little evidence that ambient DIN or SRP influenced microbial biomass in this cross-site comparison. For the streams sampled there was a reasonably broad range in mean DIN concentrations (the range is 3× the mean; Table 1) and many of the detritus substrates had high C:N ratios. One possible explanation for lack of dissolved nutrient effects relates to our sampling of a broader range of OM ages than is typically represented in studies of individual leaf species. Older organic matter may have a smaller proportion of available carbon and so dissolved nutrients do not have as strong an effect as for younger types of OM. If newly abscised leaves entering a stream have high C:N (or C:P) yet contain assimilable carbon compounds, then an external source of N or P may greatly stimulate the ability of microbes to grow on and decompose that leaf litter. Later in the decay sequence the recalcitrant nature of the residual macromolecules becomes the rate-limiting factor [32] and external nutrients may be a second-order control. Phosphorus addition has been shown [28] to stimulate leaf mass loss, perhaps because fertilized leaves attracted more macroinvertebrate shredders but fungal biomass (ergosterol) was not increased by fertilization. Alternatively, it is the turnover rate, not the standing concentrations of inorganic nutrients, that determines flux from the water column into biomass [5] and a correlation between microbial biomass and ambient inorganic nutrient concentration should not be expected. The conclusion based on our data is that external factors (nutrients and temperature) do not emerge as major controlling factors relative to variation among types of organic matter despite our attempt to include a broad range in these independent variables.

Use of standing stocks to assess the relative importance of bacteria and fungi underestimates both these communities because a significant quantity of fungal biomass is released as spores [10, 38] and the rapid turnover of bacteria will increase their contribution to metabolic processes. Also, estimates of conversion factors from bacterial cell numbers to carbon or from ergosterol to carbon can vary by roughly a factor of 2 (e.g., [12]). In the absence of detailed bacterial biovolume data or knowledge of the actual fungal taxa colonizing different substrates at the different sites, we have simply used consistent conversion factors across all samples and sites. It seems unlikely that site-to-site variation in these factors would be capable of homogenizing the patterns we have documented. For instance, the difference in numerical abundance of bacteria on leaves vs surficial FBOM is 3.5-fold (2 vs 7.1 $\times 10^{10}$ cells/g AFDM) so leaf bacteria would have to be on average 3.5× larger than FBOM bacteria to negate the difference. For comparison,

bacterial biovolumes on leaves in New York, were estimated to be 0.28 um^3/cell [10], and biovolumes on FBOM in Ontario, CANADA, were 0.21 um^3/cell [19]. Similar biovolumes were found on wood surfaces (0.28μm^3) and leaves (0.19 μm^3) in a different stream in the Hubbard Brook Experimental Forest (Norris Brook) [R. Stelzer, pers. comm]. However, relatively small differences in linear dimensions can yield a 3.5× volume difference (a 5 vs 3.3 μm diameter coccus, for instance), so we use these carbon-per-cell conversions to bring our data into common units while recognizing the potential inaccuracies.

Among-Stream Variation

Stream ecosystems are heterogeneous in distribution of both organic matter and organisms because of a complex interplay of catchment features, physical disturbance, in-stream retention, substrate stability, and biotic interactions. Examining scales of patchiness and potential controls on heterogeneity may lead to new insights into stream ecosystem function [25]. The patchy distribution of detritus pools and associated microbes will generate significant differences in availability of these resources to consumers over relatively small spatial scales. For instance, nonselective feeders in a leaf pack or debris dam will acquire much more fungal carbon than nonselective feeders in depositional areas rich in FBOM. FBOM collectors should be more tightly linked to bacteria than leaf shredders, and a tracer study in a small stream confirmed that the contribution of bacteria was strongly associated with the amount of amorphous fine particulate organic matter in an animal's gut [15]. Prior studies of leaf-shredders have shown that fungal carbon makes a much greater contribution to the consumers' nutrition than does bacterial carbon, although assimilated microbial carbon alone could not balance carbon lost to respiration [9].

The present study also confirms that the standing stock of microbial carbon is a relatively small proportion of the detrital–microbe complex, comprising less than 2% of bulk detrital carbon. The relative contribution of microbial carbon may be higher during early stages of decay [13, 26] and the contribution of microbial nitrogen will be greater than the carbon contribution due to generally lower C:N for microbial biomass. The chloroform fumigation technique was employed to estimate the microbial N content in several of these streams. In three of the streams, microbial nitrogen made up 2–20% of total N [29], and microbial N comprised 1–18% of total N in Eagle Creek [16]. Although not all of the N measured with the fumigation method is necessarily assimilable, these independent measures support the general pattern that microbial carbon is a very small proportion of the organic carbon in the detritus–microbe complex. Microbial nitrogen makes up a greater proportion but is still not the major fraction.

Differences among streams in absolute biomass and relative contribution of bacteria and fungi to reach scale microbial biomass can be driven differences in microbial biomass per unit OM as well as by differences in standing stocks of OM per unit area. We illustrate this combined effect with bivariate plots (Figs. 5A, 5B) showing the microbial biomass associated with one type of organic matter (FBOM for bacteria, leaves for fungi) per unit area of the various streams. The microbial carbon isopleths show the product of microbial biomass per unit OM and OM standing stocks per unit area. For reach-scale estimates of

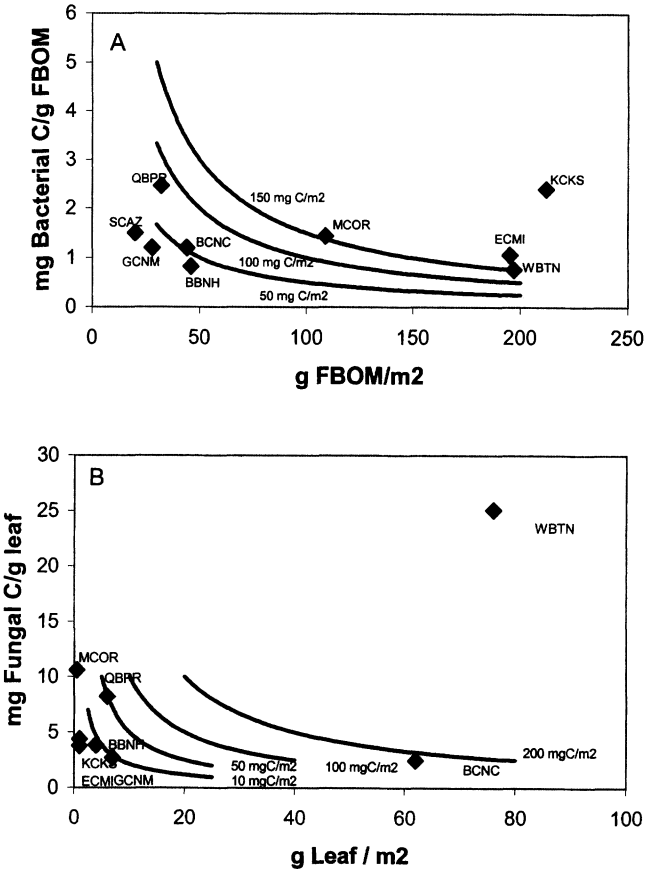

Fig. 5. Bacterial biomass per m^2 on FBOM (A) and fungal biomass per m^2 on leaves (B) showing variation among streams in detrital standing stocks (horizontal axis) and quantity of microbial biomass per unit detritus (vertical axis). Site abbreviations are given in Table 1.

bacterial carbon associated with FBOM there appear to be two groups (Fig. 5A): (1) streams where high bacterial biomass was primarily due to high standing stocks of FBOM (Eagle, Kings and Mack Creeks, and Walker Branch), and (2) streams where low bacterial biomass was primarily due to low standing stocks of FBOM (Sycamore, Gallina, and Upper Ball Creeks, and Bear Brook). The range in bacterial biomass per unit OM is relatively small (~ threefold) and most of the variability among streams was due to differences in FBOM standing stock. Factors affecting differences in annual means among streams in OM pools were synthesized recently [20] and include climate, riparian vegetation and in-stream retention devices. On shorter time scales, shredders and discharge clearly affect FBOM dynamics [43].

The pattern for reach-scale estimates of fungal biomass on leaves is less obvious (Fig. 5B), but there was clearly a group of streams with low fungal biomass per unit area of stream bottom (<10 mg C/m^2) due to low leaf standing stocks (Kings, Eagle, Mack, and Gallina Creeks, and Bear Brook). There was a tremendous range in fungal biomass per unit area for the other three streams spanning 50 to >1000 mg fungal C/m^2 associated with leaf litter (Quebrada Bisley, Upper Ball Creek, and Walker Branch). Seasonal patterns of allochthonous organic matter inputs and retention may greatly influence patterns in fungal biomass. In one of the few annual studies of microbial production, most of the temporal variability in areal fungal biomass and production was due to seasonal changes in abundance of leaf litter, rather than differences in biomass of fungi per g of litter [38].

As shown above, reach-scale standing stocks of fungal carbon were more variable among streams than standing stocks of bacterial carbon. This variation can be attributed to both variability in fungal biomass per unit OM and differences in standing stocks of OM per m^2. To further illustrate the differences in sensitivity of reach-scale fungal and bacterial biomass to changes in standing stocks of OM per unit area, we generated areal estimates of fungal and bacterial carbon in hypothetical streams having different proportions of leaves and FBOM. We used the grand mean ergosterol per g AFDM of leaf and grand mean bacterial abundance per g AFDM of FBOM to calculate their respective biomasses. Holding the total quantity of detritus constant at 50 g AFDM/m^2 but varying the proportion in leaves vs FBOM yields 100-fold differences in fungal carbon per m^2 among streams but less than fourfold difference in bacterial carbon per m^2 (Fig. 6).

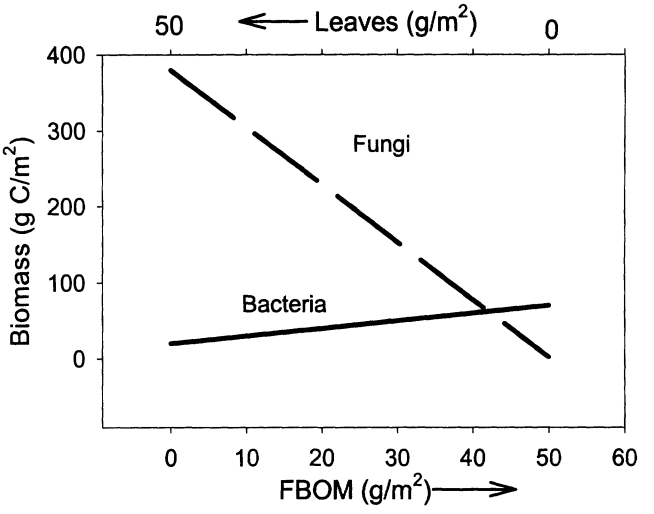

Fig. 6. Variation in bacterial and fungal biomass per m^2 in a hypothetical stream with varying proportions of leaf OM and FBOM holding the total organic matter pool at 50 g AFDM/m^2. Bacterial and fungal biomass were calculated using bacterial and fungal carbon per g AFDM averaged across all sites.

These differential "sensitivities" of microbes to the composition of the bulk detrital pool mean that even relatively small differences among streams in standing stock of leaf litter will result in large differences in quantities of fungal biomass per unit area. Also, even small quantities of leaf litter in a stream will allow fungal carbon to become the predominant component of reach-scale microbial biomass. The more even distribution of bacterial biomass across types of organic matter makes bacteria less sensitive to differences among streams in the size of these detrital pools and would presumably make bacterial biomass more consistent across seasons within individual streams. Therefore bacteria will be a more predictable food resource as the various pools of organic matter vary seasonally or among stream reaches. Similarly, the relative constancy in their biomass suggests the bacterial contribution to carbon flow and energy transfer may also be less variable over time and space. Although microbial biomass is not a perfect surrogate for other measures of microbial metabolism, a relatively stable pool of biomass will act to reduce spatial and temporal heterogeneity in the contribution of bacterial metabolism to stream ecosystem processes. In contrast, the large variability in fungal biomass suggests the contribution of fungi to metabolic processes will vary dramatically among streams or over time, particularly if the abundance of leaf litter is highly variable.

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