

Ecosystem and physiological scales of microbial responses to nutrients in a detritus-based stream: Results of a 5-year continuous enrichment

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Abstract

Our study examined the response of leaf detritus-associated microorganisms (both bacteria and fungi) to a 5-yr continuous nutrient enrichment of a forested headwater stream. Leaf litter dominates detritus inputs to such streams and, on a system-wide scale, serves as the key substrate for microbial colonization. We determined physiological responses as microbial biomass and activity expressed per unit mass of leaves and system-level responses by quantifying leaf litter standing crop monthly and expressing responses per unit area of streambed. Physiological (mass-specific) trends differed from system-level (area-specific) trends. Physiological responses to enrichment were generally positive. With the exception of bacterial biomass, nutrients increased all metrics expressed per unit mass leaf litter in the treatment stream relative to the reference (fungal biomass and production, bacterial production, microbial respiration). This positive physiological response to nutrient enrichment was associated with lower leaf litter standing crop in the treatment stream, resulting in less substrate for microbial colonization. Consequently, during most years on a system-level scale, only fungal production and microbial respiration were positively affected by nutrients, whereas fungal biomass was negatively affected. Thus, from a whole-stream perspective, nutrients led to a lower quantity of leaf detritus with greater variation, resulting in net reductions of associated fungal biomass and greater intra-annual variability in both fungal biomass and respiration. Our results demonstrate profound effects of nutrients on heterotrophic pathways that mediate detritus processing in stream ecosystems. Similar effects on heterotrophic microbes and detrital resources may be a widespread consequence of anthropogenic nutrient enrichment.

Heterotrophic microorganisms have been shown to be stimulated by increases in inorganic nutrient concentrations. However, long-term effects of nutrients on their biomass and activity, consequences for the detrital substrates they colonize, and effects on microbial production at system-level scales are unknown. Previous studies have shown that nutrient enrichment stimulates leaf decomposition, fungal biomass, and spore production associated with decomposing leaves in streams, particularly in systems characterized by relatively low nutrient concentrations (Howarth and Fisher 1976; Grattan and Suberkropp 2001; Ferreira et al. 2006; but see Newbold et al. 1983; Royer and Minshall 2001). However, these studies have typically followed the short-term decomposition dynamics of a group of leaves or leaf pieces placed in a stream. Much less is known about the long-term fate of leaf litter standing crop and the activity of leaf-decomposing microorganisms in the common situation of a stream being enriched with nutrients over several annual cycles. In contrast to lotic ecosystems that depend on autochthonous primary production, in which nutrient enrichment has been shown to increase biomass at the base of the food web (Peterson et al.

1993; Slavik et al. 2004), nutrient enrichment of detritus-based systems may increase the rate of leaf litter decomposition and therefore decrease detrital mass. Consequently, even though the leaf detritus in a nutrient-enriched stream may initially have higher microbial biomass and be a better food source for detritivores, it will disappear more rapidly and may ultimately limit food web production. Accelerated decomposition may also have serious ecosystem-level consequences related to shifts in carbon (C) pathways that are mediated by microorganisms (e.g., increased respiration and downstream transport of C; Benstead et al. 2009).

Microorganisms associated with detrital substrates can be examined on the basis of their biomass or activity per unit mass of detritus, providing a measurement of their physiological responses, or, alternatively, these metrics can be expressed on an areal basis (i.e., per unit habitat area) and provide an indication of system-level responses. The former approach has been the most commonly used procedure (Gessner and Chauvet 1994; Gulis and Suberkropp 2003a). However, if the amount of detritus per unit area is known and the appropriate parameters are determined for microorganisms associated with naturally occurring detritus, calculation of biomass or activity on an areal basis can provide a system-scale estimate (Suberkropp 1997). The first method provides an indication of biomass and physiological activity associated with a given amount of detritus and, thus, the food quality for detritivores feeding on this material. The second approach provides the amount of microbial biomass or its activity associated with detritus available to higher trophic levels on

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a reach or ecosystem scale. As such, areal metrics are particularly useful for assessing effects of environmental change such as nutrient enrichment, because they can be used in comparisons to other ecosystem-scale measurements of energy supply or demand, including organic matter budgets or estimates of production by other consumer groups.

In this study, we conducted a long-term (5-yr) nutrient (nitrogen and phosphorus) enrichment of a detritus-based headwater stream. Because leaf litter is the primary energy source for consumers in these streams (Wallace et al. 1997, 1999; Hall et al. 2000), our primary objectives focused on the biomass and production of fungi and bacteria, as well as the microbial respiration associated with naturally occurring leaf detritus. Microbial metrics (expressed on both a physiological basis, i.e., per gram of leaf litter, and a system-level basis, i.e., per square meter of stream) in the treatment stream were compared with those in an adjacent, unaltered reference stream. After the pretreatment year in which no manipulation occurred, one of the streams was continuously enriched with both inorganic nitrogen and phosphorus for the remainder of the study. Leaf litter standing crop, fungal biomass, and respiration were determined at approximate monthly intervals during the 6-yr period, whereas fungal and bacterial production were determined at monthly intervals during the first 3 yr and during the sixth year of the study.

Methods

Study sites and sampling—The study was conducted from July 1999 to July 2005 in two headwater streams at Coweeta Hydrologic Laboratory (a Long Term Ecological Research site), Macon County, North Carolina, at an altitude of ca. 850 m a.s.l. The streams were surrounded by mixed hardwoods with a dense understory of *Rhododendron maximum* L., which results in year-round shading. Consequently, these streams are primarily heterotrophic, i.e., they rely on allochthonous organic matter as their primary source of C and energy (Hall et al. 2000). The streams are ca. 200 m apart on the same south-facing slope and are similar with respect to physicochemical characteristics. They are small (average discharge about 1 L s⁻¹), circumneutral, and softwater, and have low ambient nutrient concentrations (additional stream information in Greenwood and Rosemond 2005). One of the streams was designated as a reference (catchment 53) while the other (catchment 54) received continuous nutrient enrichment initiated in July 2000 that continued throughout the rest of the study. The treatment stream received a solution of ammonium nitrate and potassium phosphate. A metering pump upstream of a 145-m experimental reach was connected to a flow meter and delivered a nutrient solution (proportional to instantaneous discharge) to a pipe with multiple outlets fed with stream water and laid along the streambed (Gulis et al. 2004; Greenwood and Rosemond 2005). Dissolved nutrient concentrations in both streams were relatively low prior to enrichment. Dissolved inorganic nitrogen (DIN; NH₄-N + NO₃-N) was $26.2 \pm 9.8 \mu\text{g L}^{-1}$, $n = 17$, and soluble reactive phosphorus

(SRP) was $8.1 \pm 3.8 \mu\text{g L}^{-1}$, $n = 17$ (July 1999–July 2000). Dissolved nutrient concentrations were 10–20× higher in the treatment stream compared to the reference during the study period (July 2000–August 2005) (DIN: $506.2 \pm 36.3 \mu\text{g L}^{-1}$, $n = 116$; SRP: $80.0 \pm 5.6 \mu\text{g L}^{-1}$, $n = 116$). Details of water sampling and analysis are presented in Gulis et al. (2004) and Greenwood and Rosemond (2005). Water temperature was continuously monitored with Optic StowAway temperature probes (Onset Computer) and was similar between the two streams (annual means of 12.0–12.5°C, range 1–19°C) (Gulis and Suberkropp 2004; Gulis et al. 2008). The streams had similar discharge, which was much lower in the first 3 yr of the study (range 0.20–0.62 L s⁻¹ average annual discharge in both streams during a period of drought; Benstead et al. 2009) compared to higher discharge (range 0.95–1.54 L s⁻¹ average annual discharge in both streams) during the last 3 yr of the study.

Standing crop of leaf litter was determined monthly by collecting leaf material from ten 0.15-m-wide transects spanning the wetted stream width. Oak (*Quercus* spp.) and rhododendron (*R. maximum*) leaves were the major leaves in the streams throughout the study. Shortly after leaf fall (November and December), leaves that decomposed more rapidly (e.g., *Acer rubrum* L., *Cornus florida* L., *Liriodendron tulipifera* L.) were present, but these leaves were rare at other times. Transects were randomly selected (one per 10-m reach) on each sampling date in both streams. Leaves were weighed, subsampled unless standing crop was low, taken to the laboratory, oven-dried (100°C), weighed, combusted (500°C), and reweighed to determine ash-free dry mass (AFDM) per square meter. At streamside, 5 disks (6.5-mm diameter), 15 disks (11.6-mm diameter), and 10 disks (15.6-mm diameter) were cut from leaves collected from each of five transects in each stream and used for microbial analyses (see below).

Fungal biomass, growth rate, production—Fungal biomass was estimated from ergosterol content of plant detritus and fungal growth rates were estimated from rates of [¹⁴C]acetate incorporation into ergosterol (Newell and Fallon 1991; Suberkropp and Weyers 1996) as described in Gulis and Suberkropp (2006). Briefly, five sets of five leaf disks (11.6-mm diameter, cut from leaves collected at every other transect) from each stream were placed in glass tubes filled with 3.95 mL filtered stream water (0.45-μm pore size, Millipore). Sodium [¹⁴C]acetate (MP Biomedicals) was added (final acetate concentration 5 mmol L⁻¹, activity 1 MBq per sample) and tubes were incubated for 3 h at stream temperature with aeration. Tubes were placed in an ice bath and samples were filtered (934AH glass fiber, Whatman), rinsed, preserved in 5 mL methanol, transported to the laboratory, and stored at -20°C. Lipids from leaf disks were extracted with alcoholic KOH and partitioned into pentane. Extracts were evaporated to dryness, reconstituted in methanol, and filtered (0.2-μm pore size, Fisher Scientific). Ergosterol was quantified with a high-pressure liquid chromatograph (Shimadzu) equipped with a Whatman Partisphere C18 column and ultraviolet detector set at 282 nm, and compared with external ergosterol

standards (Acros). Ergosterol fractions were collected with a fraction collector (Advantec) and mixed with Ecolume scintillation cocktail (ICN Biomedicals). Radioactivity in the ergosterol fraction was measured in a scintillation counter (Beckman) that corrected for quenching. Corresponding sets of five leaf disks cut from the same leaves were dried at 100°C, weighed, ashed at 500°C, and reweighed to estimate AFDM of the extracted set. Fungal biomass of plant litter was calculated using the conversion factor of 5.5 mg ergosterol g⁻¹ fungal dry mass (Gessner and Chauvet 1993). Empirical conversion factors of 19.3 µg fungal biomass nmol⁻¹ acetate incorporated for leaf litter (Suberkropp and Weyers 1996) were used to calculate fungal growth rates. Daily fungal production was calculated based on fungal growth rate and biomass estimates (Suberkropp 1997).

Bacterial biomass and production—Bacterial biomass was estimated from abundances and biovolume determinations. Bacteria were enumerated using direct counting with epifluorescence microscopy following staining with 4',6-diamidino-2-phenylindole (DAPI; Velji and Albright 1993). Five sets of five leaf disks (6.5-mm diameter) from each stream were preserved in 5 mL of 2% buffered formalin solution (in saline) at streamside and stored until analysis. One milliliter of tetrasodium pyrophosphate solution (NaPPi, 60 mmol L⁻¹; Tween 80, 60 mg L⁻¹; formalin, 1%) was added and bacteria were dislodged by vortexing, incubating on a shaker for 20 min, sonicating for 20 min (Branson 1200 sonication bath), and re-vortexing. This preparation was diluted, stained with DAPI, filtered through black polycarbonate filters (0.22-µm, Osmonics), and examined with a Leitz Diaplan microscope equipped for epifluorescence observation. At least 10 grids (approx. 20–30 cells per grid) per filter were counted. Photographs (Kodak Elite Chrome 400 slide film) were taken for biovolume estimates. These were scanned and four bacteria classes were picked and sized using Scion Image software (Scion). Bacterial biovolumes were converted to bacterial C using the following empirical formula (Simon and Azam 1989) to take into account differences in C: volume (V) ratios of different bacteria classes: $C = 89V^{0.59}$, where the conversion factor has units of fg C µm⁻³. For our bacteria classes with biovolumes ranging from 0.14 to 0.81 µm³, it yielded values from 197 to 97 fg C µm⁻³, respectively. To calculate bacterial C associated with leaf samples, total bacterial counts, contributions of each class, biovolume data, and specific conversion factors were used for each sampling date. An additional conversion factor was applied to account for dislodgment efficiency as described in Gulis and Suberkropp (2003a).

Bacterial production was estimated from rates of [³H]leucine incorporation into bacterial protein (Thomaz and Wetzel 1995; Suberkropp and Weyers 1996). Five sets of five leaf disks (11.6-mm diameter) from each stream were incubated at streamside in tubes containing 3.9 mL of filtered stream water to which 0.1 mL of [4,5-³H]leucine (final concentration, 400 nmol L⁻¹, 142 GBq mmol⁻¹) was added and incubated for 30 min. Incorporation was stopped by adding trichloroacetic acid (5% final concentration).

Corresponding sets (five from each stream) of leaf disks to which trichloroacetic acid was added before the radioactive leucine served as killed controls. In the laboratory, all samples were heated at 80°C for 20 min and cooled in an ice bath. Samples were then filtered through polycarbonate filters (0.22-µm pore size) and leaf disks were washed 3× with ice-cold trichloroacetic acid and 2× with 80% ethanol. Leaf disks and filters were digested with 1 mol L⁻¹ NaOH for 1 h at 80°C and incubated for an additional 24 h at room temperature. An aliquot was removed and decolorized with 30% H₂O₂ overnight, scintillation fluid (Ecolite, ICN Biomedicals) was added, and the radioactivity of each sample was determined with a scintillation counter (Beckman) that corrected for quenching. Bacterial production was calculated following Ward (2006).

Microbial respiration—Microbial respiration was measured as oxygen uptake of plant litter with YSI 5100 dissolved oxygen meters at streamside (Gulis and Suberkropp 2003a). On each collection date, five sets of 10 leaf disks (15.6-mm diameter) from each stream were placed separately in stream water in respiration chambers (26 mL) and incubated at stream temperature. Oxygen concentrations were recorded periodically for 30 min in the dark. Oxygen consumption was determined from the slope of a regression of oxygen concentration vs. time minus the control slope determined from stream water alone in chambers without leaf disks. AFDM of leaf disks was determined as described above to calculate respiration rate per unit mass of plant litter.

Calculations and statistical analyses—The parameters expressed on an areal basis were calculated as the product of that parameter expressed per gram and the leaf litter standing crop. Consequently, variances for these products were calculated as described by Goodman (1960). Annual fungal and bacterial production and microbial respiration (per square meter of stream) were estimated by interpolating between sampling dates and summing up daily production or respiration estimates over the year. We used bootstrapping to estimate mean leaf standing crop, mean fungal and bacterial biomass, annual fungal and bacterial production, and annual microbial respiration and corresponding 95% confidence limits (CLs). Each raw data set was resampled with replacement and data recombined to produce 1000 bootstrap sets, from which means and 95% CLs were calculated. To test for differences between estimates for the two streams, we used a bootstrap test of significance (Manly 1991) testing whether the mean difference between estimates was different from zero. To convert our data to C units, 50% C content of fungal biomass and a respiratory quotient of 0.95 were assumed. We also made an approximate estimate of the proportion of leaf litter standing crop mass loss that could be attributed to microbial respiration by dividing mean areal respiration rates by the decline in leaf litter standing crop that occurred in each year (from maximum to minimum standing crops). For these calculations, we used only respiration values from the same time periods over which losses occurred.

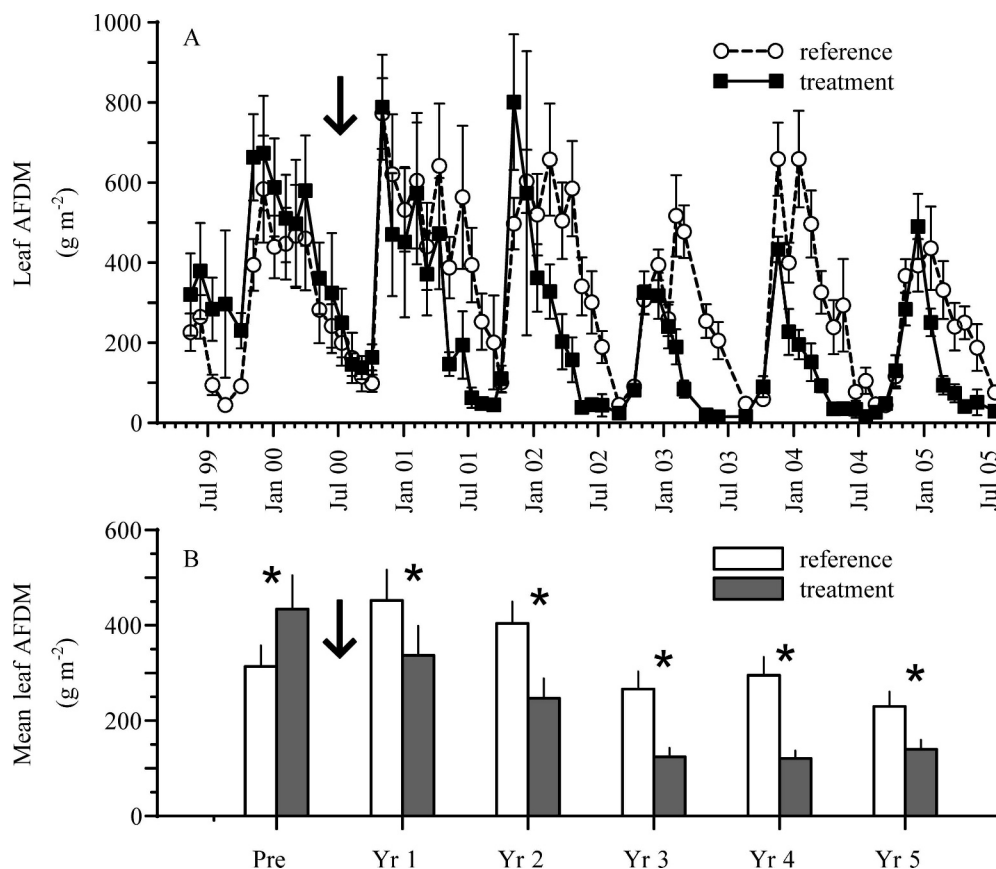


Fig. 1. Leaf litter standing crop in reference and treatment streams. (A) Leaf litter standing crop. Streams differed after treatment began (RIA, $p < 0.001$). Error bars indicate $\pm$ SE, $n = 10$. (B) Mean leaf litter standing crop in each year. Years in which streams differed are indicated with an asterisk (bootstrap test of significance, $p < 0.05$). Error bars equal $\pm 95\%$ CL. Nutrient addition began in July 2000, as indicated by the arrows.

Because streams were not replicated, we used randomized intervention analysis (RIA) (Carpenter et al. 1989) to test the null hypothesis of no change in parameters in the treatment stream relative to the reference stream following initiation of nutrient addition. For all variables, a year of pretreatment data was obtained for both streams. Some variables could be collected for the whole 5-yr enrichment period that followed, whereas some variables were only measured in the first, second, and fifth years of enrichment. RIA tests were run on the full study period when such data were available and for the first 2 yr and the fifth year of enrichment for the other variables. We also determined the intra-annual coefficient of variation (CV) of variables for which we had data for all years to assess treatment effects on within-year variation in response variables.

Results

We observed two microbially mediated responses to nutrient enrichment that affected seasonal patterns in microbial activity and biomass associated with leaf detritus in the treatment stream. The first response was the positive effect of nutrients on the biomass and production of microorganisms, particularly fungi, on a physiological

basis, i.e., per unit mass of leaf detritus. The second response was the subsequent loss of organic matter from the treatment stream that was presumably due to increased microbial activity, as well as macroinvertebrate feeding. Thus, on an ecosystem basis, microbial activity and biomass were elevated in the treatment stream in the autumn, when leaf litter inputs entered the stream. In the late spring and summer, however, reduced organic matter in the treatment stream meant lower whole-system microbial activity and biomass relative to the reference.

Leaf litter—Leaf litter standing crop exhibited predictable peaks in both streams during the autumn (November–December), as leaves were shed from the riparian vegetation, and declined during the rest of the year (Fig. 1A). Patterns of the annual decline in leaf standing crop were similar during the pretreatment year in both streams, but leaf standing crop in the treatment stream declined more rapidly than in the reference stream after nutrient enrichment began (Fig. 1A). During the pretreatment year, the mean leaf standing crop in the treatment stream was greater than in the reference, and it declined relative to the reference following nutrient enrichment (Fig. 1B). In addition, the mean standing crop of leaf litter in both

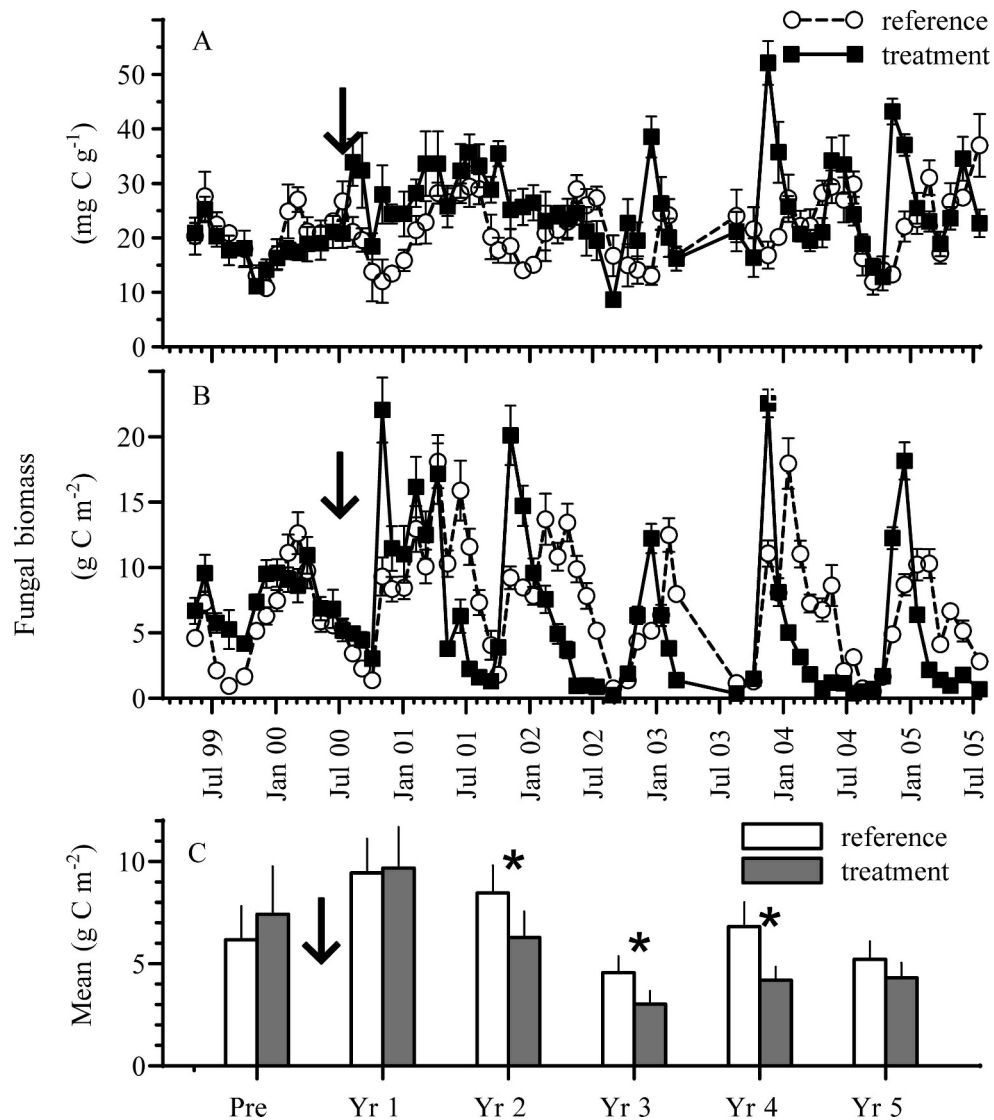


Fig. 2. Fungal biomass carbon associated with naturally colonized leaves in the reference and treatment streams. (A) Concentration of fungal biomass carbon g^{-1} leaf litter AFDM. Streams differed after treatment began (RIA, $p = 0.017$). Error bars indicate $\pm \text{SE}$, $n = 5$. (B) Fungal biomass carbon associated with leaves m^{-2} of stream. Error bars indicate $\pm \text{SE}$. (C) Mean fungal biomass carbon m^{-2} in each year. Years in which streams differed are indicated with an asterisk (bootstrap test of significance, $p < 0.05$). Error bars equal $\pm 95\%$ CL. Arrows as in Fig. 1.

streams was lower during the last 3 yr of the study in comparison to the first 3 yr (Fig. 1B), but always declined more rapidly in the treatment stream (Fig. 1A).

Fungal biomass and production—The concentration of fungal biomass associated with leaves was similar in both streams during the pretreatment year, but was greater in the treatment stream than in the reference stream once enrichment began (Fig. 2A). In the reference stream, the average concentration of fungal biomass during the enrichment period was 4.3% (1.6–7.4%) whereas in the treatment stream, fungal biomass averaged 5.3% (1.7–10.4%) of total leaf mass. Once nutrient enrichment started, the treatment stream exhibited higher fungal biomass m^{-2} following leaf fall but it declined rapidly thereafter

(Fig. 2B) as the leaf standing crop declined. Consequently, from the second through the fourth year of enrichment, the mean fungal biomass m^{-2} was actually greater in the reference than in the treatment stream (Fig. 2C), because of the lower amount of leaf litter in the treatment stream (Fig. 1B), whereas no differences were found for other years.

Daily fungal production g^{-1} was similar in both streams during the pretreatment year, and was stimulated by nutrient enrichment in the treatment stream (Fig. 3A). Fungal production m^{-2} was also higher in the treatment than in the reference stream when leaf litter was abundant in the treatment stream, but declined below values in the reference stream (Fig. 3B) when the standing crop of leaf declined to low levels (Fig. 1A). However, overall, fungal

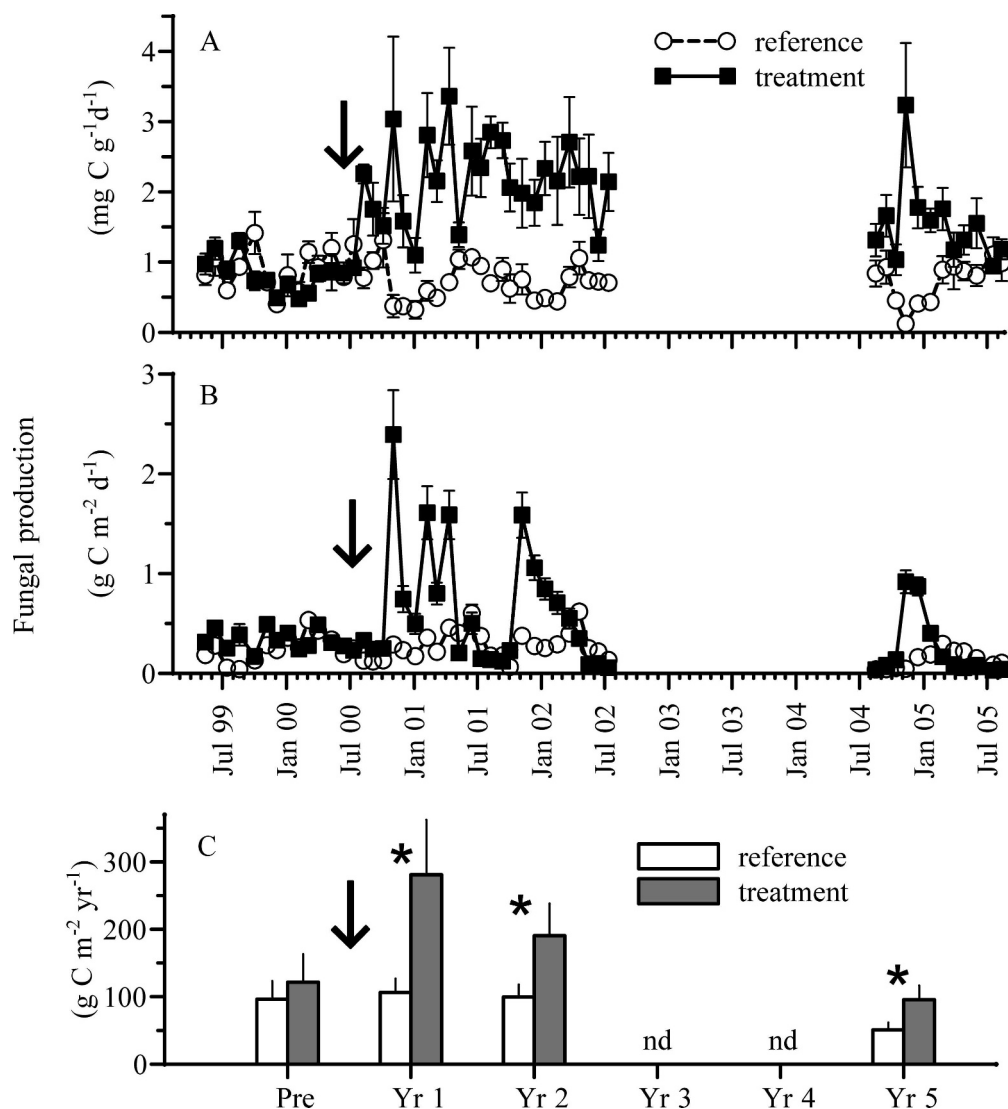


Fig. 3. Fungal carbon production associated with naturally colonized leaves in the reference and treatment streams. (A) Fungal carbon production g⁻¹ leaf litter AFDM. Streams differed after treatment began (RIA, $p < 0.001$). Error bars indicate $\pm$ SE, $n = 5$. (B) Fungal carbon production associated with leaves m⁻² of stream. Error bars indicate $\pm$ SE. (C) Annual fungal carbon production m⁻² in each year. Years in which streams differed are indicated with an asterisk (bootstrap test of significance, $p < 0.05$). Error bars equal $\pm 95\%$ CL. nd, fungal production was not measured. Arrows as in Fig. 1.

production g⁻¹ leaf litter was ca. $3\times$ higher in the treatment (mean = $2.2 \text{ mg C g}^{-1} \text{ d}^{-1}$) than in the reference stream (mean = $0.7 \text{ mg C g}^{-1} \text{ d}^{-1}$) during the first 2 yr of enrichment and again in the fifth year of enrichment. Consequently, annual fungal production m⁻² remained higher in the treatment than in the reference stream once nutrient enrichment began (Fig. 3C).

Bacterial biomass and production—Concentrations of bacterial biomass associated with leaf litter did not differ between the streams during pretreatment or the first 2 yr of nutrient addition (Fig. 4A), and were not measured during the remainder of the study. Likewise, the bacterial biomass m⁻² in the streams was similar in both streams during this period on an areal basis and was not affected by nutrient addition (Fig. 4B,C). In contrast, bacterial production g⁻¹

leaf litter was higher in the treatment than in the reference stream during the enrichment period (Fig. 5A). However, bacterial production m⁻² was only greater in the treatment stream when there were high leaf standing crops and declined below that in the reference stream when the leaf standing crop declined (Fig. 5B). Because bacterial production g⁻¹ was only slightly higher in the enriched than in the reference stream and bacterial production m⁻² fluctuated depending on leaf litter standing crop, annual bacterial production m⁻² was similar in the two streams (Fig. 5C).

Annual production to biomass (P : B) ratios—Annual P : B ratios and turnover times for fungi were similar in both streams during the pretreatment year. Annual P : B increased and turnover times decreased in the treatment stream after nutrient enrichment began (Table 1). Annual

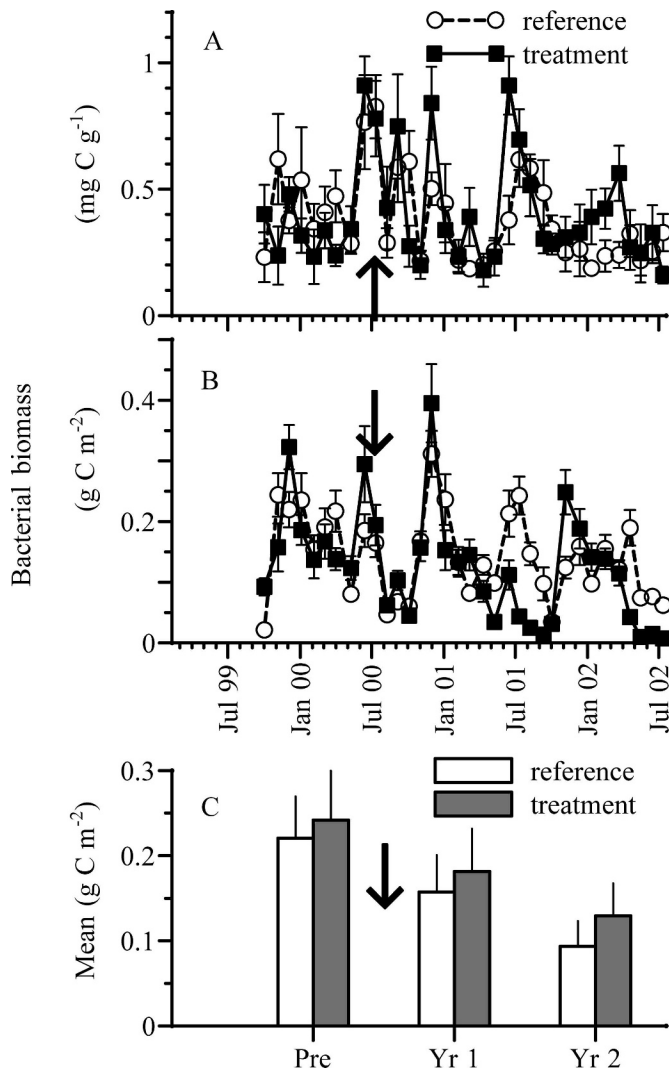


Fig. 4. Bacterial biomass carbon associated with naturally colonized leaves in the reference and treatment streams. (A) Concentration of bacterial biomass carbon g⁻¹ leaf litter AFDM. Streams did not differ after treatment began (RIA, $p = 0.09$). Error bars indicate $\pm$ SE, $n = 5$. (B) Bacterial biomass carbon associated with leaves m⁻² of stream. Error bars indicate $\pm$ SE. (C) Mean bacterial biomass carbon m⁻² in each year. Years in which streams differed are indicated with an asterisk (bootstrap test of significance, $p < 0.05$). Error bars equal $\pm 95\%$ CL. Arrows as in Fig. 1.

P: B ratios and turnover times for bacteria were similar in both streams and there were no detectable trends due to enrichment. Bacterial turnover times were shorter than those of fungi.

Microbial respiration—Microbial respiration g⁻¹ leaf litter was similar in both streams during the pretreatment year but was stimulated by nutrient enrichment (Fig. 6A). Although respiration rates on a whole-stream basis can vary considerably from day to day (Roberts et al. 2007), we believe that the multiple daily “snapshots” of respiration that we measured over 6 yr reflect robust trends in regard to nutrient effects. In a pattern similar to that of microbial

production, respiration m⁻² was greater in the treatment than in the reference stream during the period after leaf fall and declined to lower levels later in the year as leaf litter disappeared from the treatment stream (Fig. 6B). Annual total respiration m⁻² was greater in the treatment than in the reference stream in the pretreatment year and in the first 2 yr and the last year of enrichment, but was similar in the two streams in years 3 and 4 of enrichment (Fig. 6C). The percentage of litter loss attributed to microbial respiration was estimated to be 25% (interannual range 15–41%) in the reference and 34% (interannual range 18–49%) in the treatment stream.

Temporal variation in response—Prior to nutrient enrichment, fungal biomass m⁻² (Fig. 2B) and respiration m⁻² (Fig. 6B) were largely driven by similar trends in litter standing crop (Fig. 1A) and were generally higher in the treatment stream compared to the reference. Differences between treatment and reference streams were not widely variable temporally. Following nutrient enrichment, there was greater seasonal variation in detrital resources and in fungal biomass m⁻² and respiration m⁻² in the treatment stream relative to the reference, as demonstrated by higher intra-annual CVs based on monthly data (Fig. 7).

Discussion

Our study showed that chronic input of nutrients to a detritus-based system dramatically altered microbial activity, the standing crop of microorganisms and detritus, and their intra-annual variability. Although previous studies have shown that detritus-associated heterotrophic microbes respond positively to increased supply of inorganic nutrients (Grattan and Suberkropp 2001; Rosemond et al. 2002; Ferreira et al. 2006), no previous study to our knowledge has determined how these effects play out over a long time period on a whole-system scale by incorporating the consequent effects on detrital standing crops.

In this donor-controlled system, C inputs in the form of leaf litter are delivered in a pulse in the autumn. Typically, this organic matter is processed through subsequent months, supporting a diverse array of consumers in forested headwater streams with life histories timed to this resource pulse (Cuffney et al. 1990). Nutrient enrichment disrupted the typical timing of microbial and detrital resources in the treatment stream, which became highly variable temporally. Coefficients of variation for litter standing crop, fungal biomass, and microbial respiration all shifted from below to above 100% in the treatment stream following nutrient enrichment. Such increased variability may be an excellent indicator of ecosystem-level disturbance, as suggested by Fraterrigo and Rusak (2008).

System- and physiological-scale responses in microbial biomass, activity, and production—In the treatment stream, fungal biomass g⁻¹ often declined to lower levels than found in the reference stream during the summer, leading to large fluctuations between the maxima (autumn–winter) and minima (summer) in fungal biomass concentrations in the treatment stream (Fig. 2A). During the summer, leaves

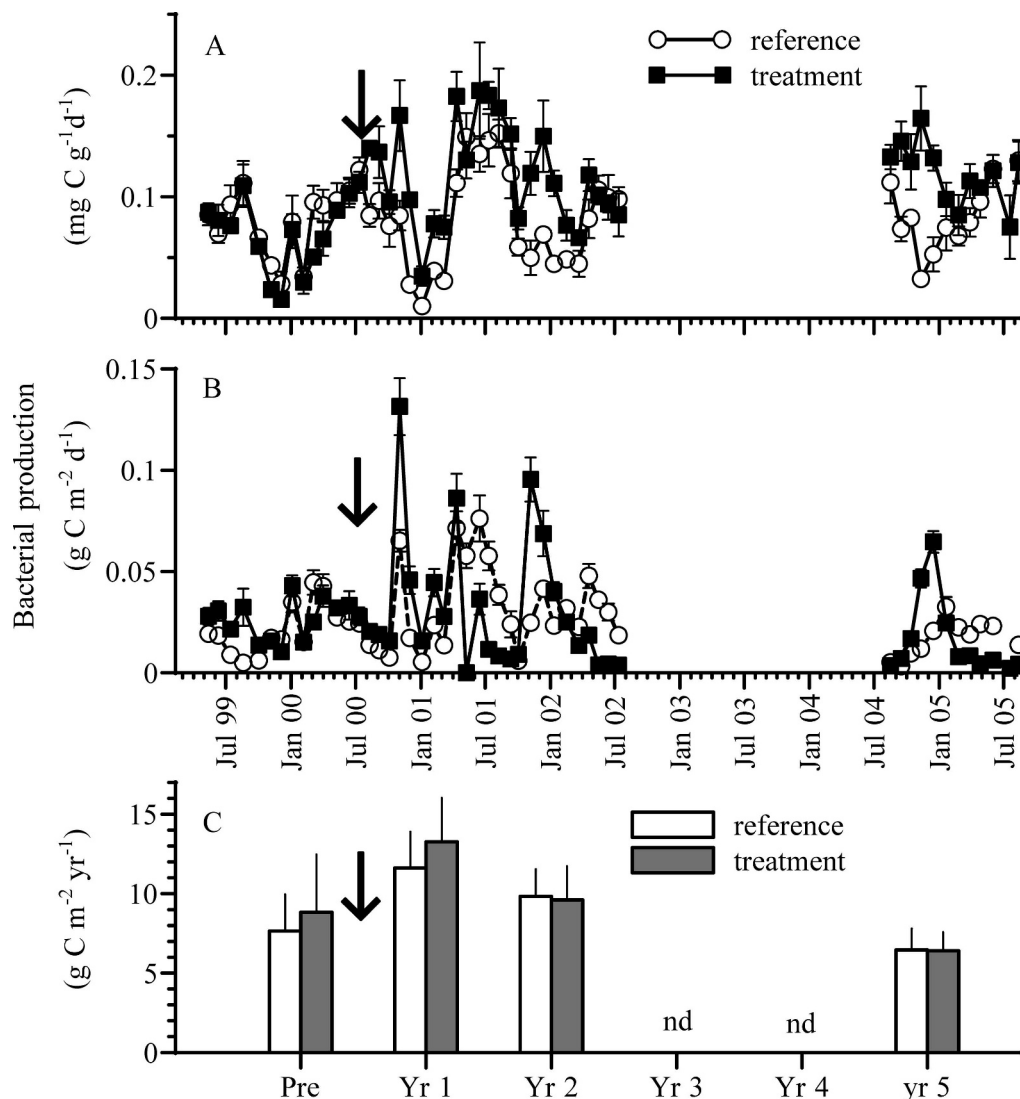


Fig. 5. Bacterial carbon production associated with naturally colonized leaves in the reference and treatment streams. (A) Bacterial carbon production g^{-1} leaf litter AFDM. Streams differed after treatment began (RIA, $p < 0.001$). Error bars indicate $\pm \text{SE}$, $n = 5$. (B) Bacterial carbon production associated with leaves m^{-2} of stream bottom. Error bars indicate $\pm \text{SE}$. (C) Annual bacterial carbon production m^{-2} in each year. Years in which streams differed are indicated with an asterisk (bootstrap test of significance, $p < 0.05$). Error bars equal $\pm 95\% \text{ CL}$. nd, bacterial production was not measured. Arrows as in Fig. 1.

were scarce in the treatment stream because of rapid decomposition and consumption of leaves by detritivores. Consequently, most of the leaves sampled had only recently entered the stream at the time of sampling (K. Suberkropp pers. obs.) and were not yet extensively colonized by fungi. Fluctuations in fungal biomass on a system-wide basis (m^{-2} ; Fig. 2B) were consequently caused by a combination of shifts in fungal biomass g^{-1} (Fig. 2A), leaf litter standing crop (Fig. 1A), and possibly aspects of litter conditioning time.

The difference in daily fungal production g^{-1} (Fig. 3A) between the two streams was greater than the differences we observed in fungal biomass g^{-1} (Fig. 2A). One reason for this is that a portion of fungal production goes to production of conidia that are transported downstream. Conidia production can account for up to 60–80% of total

fungal production (Suberkropp 1991; Gulis and Suberkropp 2003b). Once nutrient enrichment began, the conidia concentration in the water of the treatment stream increased to 4.5–6.9 $\times$ that of the reference (Gulis and Suberkropp 2004), indicating greatly increased conidia production. Other studies have also noted larger increases in conidia production than in biomass after nutrient enrichment (Suberkropp 1998; Grattan and Suberkropp 2001; Gulis and Suberkropp 2003a), suggesting that a considerable proportion of the increase in fungal production resulting from nutrient enrichment was shunted to reproduction. The second reason for the relatively small, yet significant, differences in fungal biomass between the nutrient-enriched and the reference stream was potentially higher invertebrate feeding associated with higher macroinvertebrate shredder biomass and production in the

Table 1. Fungal and bacterial annual production to biomass (P:B) ratios and turnover times calculated from annual mean biomass (g m^{-2}) and annual production ($\text{g m}^{-2} \text{yr}^{-1}$). nd, not determined.

	Stream	Pre	Yr 1	Yr 2	Yr 5
Fungal					
P:B (yr^{-1})	Reference	16	11	12	10
	Treatment	16	29	30	22
Turnover time (d)	Reference	23	32	31	37
	Treatment	22	13	12	16
Bacterial					
P:B (yr^{-1})	Reference	35	74	105	nd
	Treatment	52	88	91	nd
Turnover time (d)	Reference	10	5	3	nd
	Treatment	7	4	4	nd

treatment stream (Cross et al. 2006). Some shredders can preferentially feed on leaf patches colonized by fungi and even prefer pure fungal biomass in laboratory trials (Suberkropp 1992). Feeding on fungal hyphae by invertebrates in the treatment stream increased from $2.3 \text{ g AFDM m}^{-2} \text{yr}^{-1}$ during the pretreatment year to $9.2 \text{ g AFDM m}^{-2} \text{yr}^{-1}$ during the first 2 yr of nutrient addition (Cross et al. 2007). Invertebrate feeding consequently exerted stronger top-down control on fungal biomass in the nutrient-enriched stream.

Annual fungal production associated with leaf detritus on an areal basis was higher in both streams ($51\text{--}280 \text{ g C m}^{-2}$) than has been found previously for other streams (18 g C m^{-2} or less; Suberkropp 1997; Gulis et al. 2006). However, the range of fungal production g^{-1} organic matter in Coweeta streams was similar or lower than found in those streams. The major reason for the high annual production in Coweeta streams is that they are very retentive of leaf litter and maintain mean leaf standing crops of $120\text{--}450 \text{ g AFDM m}^{-2}$. In other streams that have been examined, leaves are typically washed out shortly after leaf fall, and mean annual leaf standing crops are less than $100 \text{ g AFDM m}^{-2}$ (Gulis et al. 2006). This suggests that organic matter quantity is critical to the maintenance of the microbial resource base in headwater stream food webs, as suggested by both donor-control theory (Huxel and McCann 1998) and manipulative experiments (Wallace et al. 1997).

We found both fungal biomass and production to be much higher ($51\text{--}75\times$ and $10\text{--}20\times$, respectively) than bacterial biomass and production on naturally colonized leaves. Such dominance of fungi has previously been reported from stream decomposition studies in litter bags (Baldy et al. 1995; Weyers and Suberkropp 1996). We also found that fungal production:biomass (P:B) and turnover times were enhanced more by nutrient addition than bacterial P:B and turnover times (Table 1). The stimulation in fungal production was similar to the stimulation in microbial respiration associated with leaves, suggesting that a major portion of the respiration we measured was due to fungal activity. Indeed, fungal (but not bacterial) production correlated well with microbial respiration over 4 yr in both streams ($r = 0.62$, $p < 0.0001$, $n = 96$).

Implications for alterations in quantity, quality, and variability of leaf detritus—Leaf litter is the primary energy source for consumers in forested headwater streams (Wallace et al. 1997) and dominates C inputs to these and other forested streams (Benstead et al. 2009). Despite standing crops of wood that are $2\text{--}3\times$ greater than leaf standing crops, fungal production was an order of magnitude higher per unit mass and was $4\times$ higher per unit stream area for leaf vs. wood substrates (Gulis et al. 2008). Energy flow from autotrophic microbes in these streams is minimal (Hall et al. 2000) and their response to nutrient enrichment was limited (Greenwood and Rosemond 2005). Thus, leaf-associated bacteria and fungi quantitatively dominate microbial activity in this system and appear to be key in determining declines in detrital C resources.

A C budget of this stream based on the first 2 yr of enrichment showed altered storage and fluxes of C due to nutrient enrichment, largely because of increased downstream transport of fine particulate organic matter (FPOM), and secondarily by increased losses due to respiration (Benstead et al. 2009). The results shown here corroborate and extend the trends found in the C budget regarding the reduction in the standing crop of leaf detritus and increased losses due to respiration. Enrichment stimulated respiration in most years (Fig. 6C), and the proportion of leaf litter loss due to respiration was slightly higher in the treatment (34%) than in the reference (25%) stream (although large interannual variation occurred in both streams). In addition, detritus standing crop in the budget analysis was determined by benthic cores (Benstead et al. 2009), whereas our dataset quantified leaf detritus from separate data collections (monthly transects). Not only do these independent data show that leaf detritus continued to decline in the treatment stream through years 3, 4, and 5 of enrichment, but our analysis of leaf standing crop, fungal biomass, and respiration also revealed increased intra-annual variation of these parameters. Consequently, nutrient enrichment resulted in two temporal patterns in organic matter loss: greater intra-annual variability because of faster loss of annual litter inputs, and gradual year-to-year attrition of total organic matter storage (Benstead et al. 2009). Both of these patterns were driven by increased biological activity, resulting in loss of C from the system through both elevated transport of FPOM and increased conversion to CO_2 .

Greater proportions of losses due to microbial respiration (above) as well as macroinvertebrate consumption (Cross et al. 2007) altered fates of organic matter inputs and resulted in declines in standing crop of leaf detritus in the treatment stream. These losses are consistent from a budget perspective with reduced storage and standing crop of organic matter in the treatment stream (Benstead et al. 2009; Fig. 1A). Litterbag studies conducted in these streams indicate that leaves decomposed faster (Greenwood et al. 2007) and had higher fungal biomass and activity with nutrient enrichment (Gulis and Suberkropp 2003a), and that faster decay rates can be attributed to microbial and macroinvertebrate pathways (Cross et al. 2007; C. J. Tant and A. D. Rosemond unpubl.).

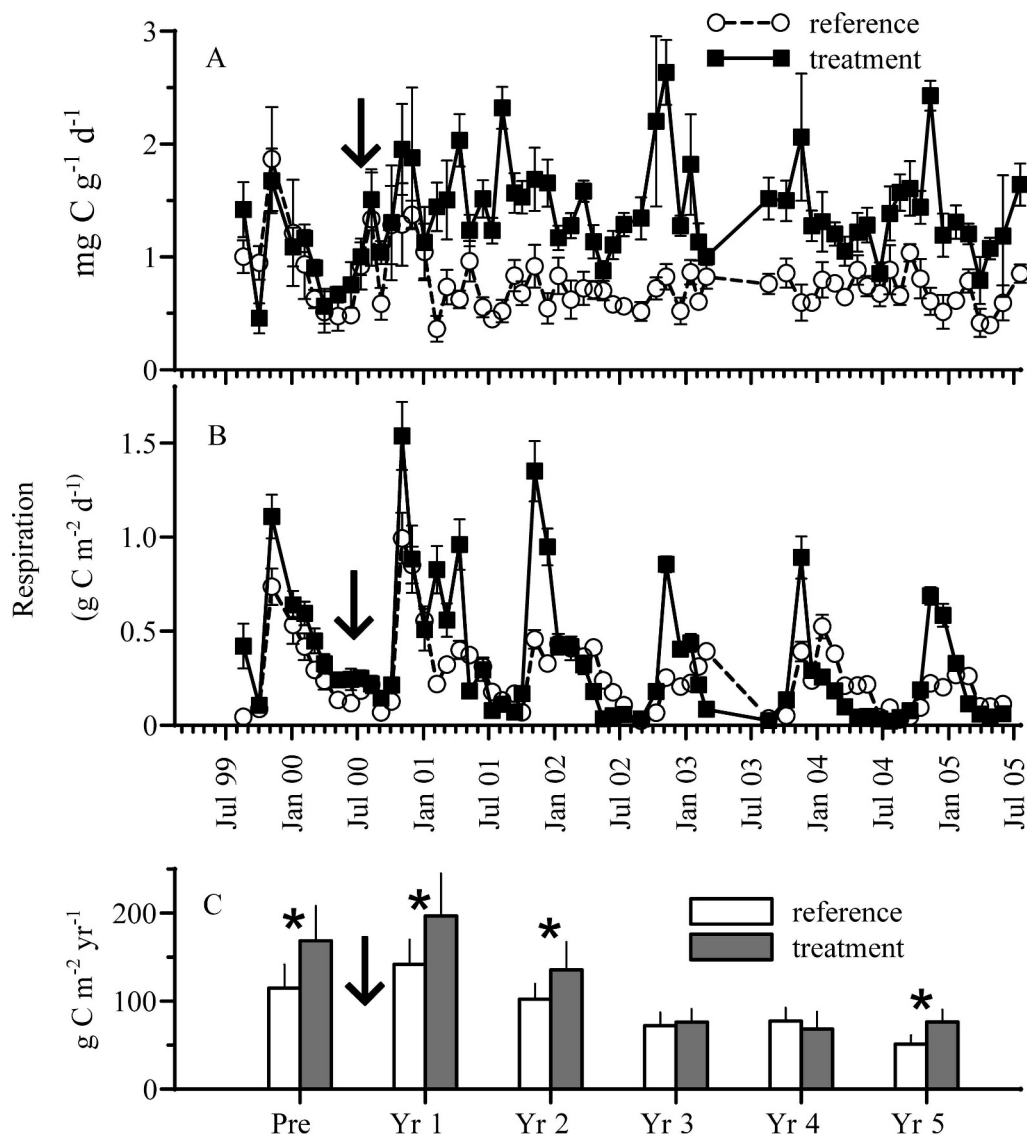


Fig. 6. Microbial respiration calculated as carbon respired from naturally colonized leaves in the reference and treatment streams. (A) Respiration g^{-1} leaf litter AFDM. Streams differed after treatment began (RIA, $p < 0.001$). Error bars indicate $\pm \text{SE}$, $n = 5$. (B) Respiration associated with leaves m^{-2} of stream. Error bars indicate $\pm \text{SE}$. (C) Annual total respiration associated with leaves m^{-2} in each year. Years in which streams differed are indicated with an asterisk (bootstrap test of significance, $p < 0.05$). Error bars equal $+95\%$ CL. Arrows as in Fig. 1.

The rapid disappearance of leaf detritus in the treatment stream led to low standing crops during the summer, which had implications for leaf-associated microbial biomass and activity, and likely also affected other aspects of stream microbial activity and higher trophic levels with respect to habitat availability and production. During the first 2 yr of enrichment, secondary production of invertebrates was stimulated $1.2\text{--}3.3\times$ over that predicted from prior data from these streams (Cross et al. 2006). In years 4 and 5 of enrichment, total secondary production of invertebrates was even higher in the treatment stream, but production of some groups of consumers was ultimately negatively affected (J. M. Davis and A. D. Rosemond unpubl.). Initial increases in total macroinvertebrate production suggest that the greater amount of microbial production

associated with leaves led to higher food quality that offset the timing of the availability of leaf detritus. In headwater streams that exhibit summer minima in leaf standing crop, the availability of leaf detritus throughout the year may be critical for some consumers. Eggert and Wallace (2003) identified a critical low threshold of benthic leaf litter ($< 25\text{--}50 \text{ g AFDM m}^{-2}$) that was associated with maintenance of secondary production of certain consumers. Leaf litter standing crop was below 50 g AFDM m^{-2} on only four sampling dates in the reference stream, but fell below these values in the treatment stream on 17 sampling dates during the enrichment period, and dropped below 25 g AFDM m^{-2} in the treatment stream on five of those dates. Therefore, some populations of macroinvertebrates may have been limited by periods of severely low detritus

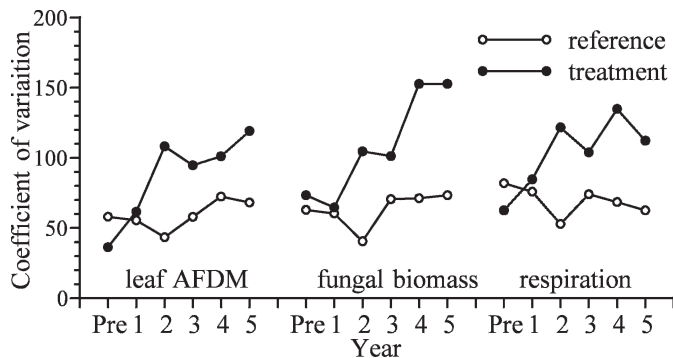


Fig. 7. Coefficients of variation for annual means of leaf litter standing crop m^{-2} , fungal biomass m^{-2} , and respiration m^{-2} .

standing crops, via habitat or food availability, that occurred during the summer months due to nutrient enrichment.

Both streams exhibited a decline in leaf litter standing crop during the last 3 yr of the study (Fig. 1A,B), apparently due to increased precipitation and discharge that increased organic matter export from the study reaches (A. D. Rosemond unpubl.). Thus, hydrologic variability apparently combined with nutrient enrichment to determine retention and temporal availability of detritus. Notably, even in years of very high flows associated with tropical depressions (e.g., two tropical depressions tracked over the study area in 2004; <http://maps.csc.noaa.gov/hurricanes/viewer.html>), biotically driven patterns of lower leaf litter standing crop in the treatment stream remained robust.

As a result of nutrient enrichment that stimulated fungal production, the concentration of fungal biomass associated with leaf detritus increased, making it a better food source. However, this led to an increased rate of disappearance of leaf detritus in the stream, lower C standing crops, and a decline in the overall amount of fungal biomass in the system (per unit area) on an annual basis. Such changes stimulated overall macroinvertebrate secondary production during the course of this study but may eventually lead to lower secondary production or curtailment of life cycles of some groups of macroinvertebrates in affected stream reaches. Our results also suggest that downstream export of microbially derived C may also have been stimulated, because a significant portion of fungal production was shunted into reproduction and was likely transported out of the stream reach as conidia. The microbially driven pathways promoted by nutrient enrichment changed the quantity and timing of availability of organic matter and associated microbial resources in this headwater stream. Detrital pathways in other systems exposed to chronic nutrient enrichment may be similarly affected.

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