

The influence of dissolved nutrients and particulate organic matter quality on microbial respiration and biomass in a forest stream

ROBERT S. STELZER*, JAMES HEFFERNAN[†] AND GENE E. LIKENS

Institute of Ecosystem Studies, Millbrook, NY, U.S.A.

**Present address: Department of Biology and Microbiology, University of Wisconsin Oshkosh, Oshkosh, WI, U.S.A.*

†Present address: Department of Biology, Arizona State University, Tempe, AZ, U.S.A.

SUMMARY

1. Although dissolved nutrients and the quality of particulate organic matter (POM) influence microbial processes in aquatic systems, these factors have rarely been considered simultaneously. We manipulated dissolved nutrient concentrations and POM type in three contiguous reaches (reference, nitrogen, nitrogen + phosphorus) of a low nutrient, third-order stream at Hubbard Brook Experimental Forest (U.S.A.). In each reach we placed species of leaves (mean C : N of 68 and C : P of 2284) and wood (mean C : N of 721 and C : P of 60 654) that differed in elemental composition. We measured the respiration and biomass of microbes associated with this POM before and after nutrient addition.
2. Before nutrient addition, microbial respiration rates and biomass were higher for leaves than for wood. Respiration rates of microbes associated with wood showed a larger response to increased dissolved nutrient concentrations than respiration rates of microbes associated with leaves, suggesting that the response of microbes to increased dissolved nutrients was influenced by the quality of their substrate.
3. Overall, dissolved nutrients had strong positive effects on microbial respiration and fungal, but not bacterial, biomass, indicating that microbial respiration and fungi were nutrient limited. The concentration of nitrate in the enriched reaches was within the range of natural variation in forest streams, suggesting that natural variation in nitrate among forest streams influences carbon mineralisation and fungal biomass.

Keywords: fungi, microbial respiration, nitrogen, organic matter, phosphorus

Introduction

Heterotrophic microbes associated with particulate organic matter (POM) in aquatic systems can obtain carbon and other nutrients from dissolved sources in the water column (Caraco *et al.*, 1998) and from the POM (Melillo *et al.*, 1984). The most abundant types of POM in freshwater benthic systems are dead leaves, wood and other plant material. This material typically has low concentrations of nitrogen and phosphorus

relative to carbon. Leaves entering aquatic systems generally have an initial molar C : N ratio of 20–80 and a C : P ratio of 1000–4000 (Gosz, Likens & Bormann, 1972; Melillo, Aber & Muratore, 1982; Gessner, Robinson & Ward, 1998). Wood generally has a C : N ratio of 150–1300 and a C : P ratio of 13 000–130 000 (Likens & Bormann, 1970; Melillo *et al.*, 1984). The elemental composition of heterotrophic microbes such as bacteria and fungi is constrained by their biomolecular composition (Elser *et al.*, 1996), although some plasticity in bacterial elemental ratios, particularly C : P, has been observed (Kirchman, 2000). Bacteria typically have a C : P ratio ranging from 30 to 100 (Jürgens & Güde, 1990) and a C : N ratio ranging from

Correspondence: Robert S. Stelzer, Department of Biology and Microbiology, University of Wisconsin Oshkosh, Oshkosh, WI 54901-8640, U.S.A. E-mail: stelzer@uwosh.edu

5 to 15 (Kirchman, 2000), and fungi have a C : N ratio ranging from 7 to 25 (Swift, Heal & Anderson, 1979), much lower than those of the main types of POM with which bacteria and fungi are associated.

The relatively high N and P content of microbes suggests that microbial activity will be sensitive to both dissolved nutrient concentrations and the chemical composition of POM. Experimental additions of dissolved nutrients to low-nutrient streams, lakes and mesocosms have often resulted in increases in microbial biomass (Pace & Cole, 1996; Suberkropp, 1998; Tank & Webster, 1998; Sridhar & Bärlocher, 2000; Grattan & Suberkropp, 2001), respiration (Howarth & Fisher, 1976; Elwood *et al.*, 1981; Tank & Webster, 1998) or the rate of organic matter decomposition (Howarth & Fisher, 1976; Elwood *et al.*, 1981; Robinson & Gessner, 2000). Other studies found no effect of added nutrients on microbial respiration or organic matter decomposition in aquatic systems (Triska & Sedell, 1976; Newbold *et al.*, 1983). Positive relationships between microbial activity and dissolved nutrient concentration have also been found in comparative studies (Suberkropp, 1995; Suberkropp & Chauvet, 1995; Rosemond *et al.*, 2002). In addition, POM chemical quality (e.g. C : N, C : P, lignin : N ratios) influences POM decomposition rate in aquatic systems (Melillo *et al.*, 1982; Enríquez, Duarte & Sand-Jensen, 1993; Gessner & Chauvet, 1994), suggesting that C mineralisation by microbes associated with the POM increases with POM quality.

Microbes associated with POM of low nutrient content would be expected to rely more on external (dissolved) nutrients for growth and reproduction whereas microbes colonising POM of high nutrient content may have a lesser need for external nutrients (Melillo *et al.*, 1984; Gessner & Chauvet, 1993). A previous study examined the simultaneous effects of dissolved nutrients and POM quality on microbial processes in the benthos (Royer & Minshall, 2001), although high ambient concentrations of dissolved nutrients may have confounded the authors' ability to determine the importance of dissolved nutrients. The objectives of our study were to determine the simultaneous effects of dissolved nutrient concentration and POM quality on microbial respiration and bacterial and fungal biomass in a low-nutrient forest stream. We predicted that: (i) microbial respiration and biomass would be higher on organic matter of higher quality at ambient (low) concentrations of

dissolved nutrients, and (ii) microbes associated with organic matter of lower quality would exhibit a stronger response to dissolved nutrients.

Methods

Site description

Norris Brook is in the eastern edge of Hubbard Brook Experimental Forest (HBEF), in the White Mountain National Forest (NH, U.S.A). The catchment is south facing and drains 87.2 ha (Hall *et al.*, 1980). The vegetation at Norris Brook is typical of that of a northern hardwood ecosystem. Dominant trees are American beech (*Fagus grandifolia*, Ehrh.), sugar maple (*Acer saccharum*, Marsh.) and yellow birch (*Betula alleghaniensis*, Michx. f.) (Likens & Bormann, 1995). Red spruce (*Picea glauca*, Sarg.) and Eastern hemlock (*Tsuga canadensis*, Carr.) are also abundant near the stream banks. The reference, nitrogen and nitrogen + phosphorus reaches (see 'Experimental Design') were heavily shaded, and had 93, 87 and 85% canopy cover, respectively, determined by digital analysis of scanned photographs taken in summer with a camera equipped with a fish-eye lens. The third-order stream is 1–2 m wide, depth ranges from 5 to 50 cm and discharge from 2 to 9 L s⁻¹ during the study. The 185-m long study reach consisted of riffles, runs and pools. The substratum was mainly boulder, cobble and gravel, but silt and sand were also abundant in pools. There was a substantial amount of dead wood in the stream channel and dead leaves and other plant material in less abundance. Norris Brook is weakly acidic (pH ranges from 6.2 to 6.7) and surface water temperature ranged from 8 to 19 °C during the study. Norris Brook was chosen for its low concentrations of dissolved inorganic P (<2 µg PO₄-P L⁻¹) and N (3–14 µg NO₃-N L⁻¹).

Experimental design

Nutrients and POM quality were simultaneously manipulated in three adjacent reaches of Norris Brook using a before-after control-impact (BACI) design (*sensu* Stewart-Oaten, Murdoch & Parker, 1986). Dissolved nutrients were added to the stream during the After period but not in the Before period (throughout the paper, Before and After will refer to the periods before and after the commencement of nutrient addition). The respiration and biomass of microbes

associated with five types of wood and two types of leaves placed in each reach were measured in the Before and After periods.

Dissolved concentrations of N and P were manipulated in the three reaches as follows, nutrients being delivered by peristaltic pumps. The most upstream reach served as the reference reach and received no added nutrients. Concentrations of PO_4^{-3} and NO_3^- were very low in this reach throughout the study (Table 1). The middle (nitrogen) reach, received continuous input of NO_3^- as NaNO_3 at the head of the reach, and the stream water NO_3^- concentration was approximately 20-fold greater than in the reference reach (Table 1). The downstream nitrogen + phosphorus reach received added PO_4^{-3} as $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$ and its PO_4^{-3} concentration was about 30-fold greater than in the reference reach. The N + P reach had increased NO_3^- because it was downstream of the N reach. Before nutrient addition, nutrient concentrations were uniformly low in all three reaches (Table 1).

Sugar maple and American beech leaves were collected from trees in the Hubbard Brook Valley after abscission during October 1999. Single-species leaf packs of 8–10 intact air-dried leaves were enclosed in pouches made of black Vexar material (1.3 cm mesh; DuPont, Wilmington, DE, U.S.A.). We used untreated wood veneer of sugar maple, American beech, northern red oak (*Quercus rubra*, Michx. f.), black cherry (*Prunus serotina*, Ehrh.) and yellow birch. These species were chosen based on their variation in elemental composition and abundance at HBEF (Table 2). The veneer was cut into rectangular pieces (85 × 35 cm; thickness ranged from 0.5 to 0.7 mm among species) and each type was fastened in a 3 × 6 matrix to Vexar mesh material. Thus, each veneer rack contained 18 veneer pieces of a single wood species. Veneer racks and leaf packs were attached to the stream bottom with metal spikes. For the Before period, three to four leaf packs of each species, and two veneer racks of each

species, were deployed in each reach during May 2000. For the After period, five to six leaf packs of each species, and three veneer racks of each species, were deployed in each reach in July 2000. The POM was positioned so that water depth (5–50 cm range) and velocity (5–30 cm s⁻¹ range) were similar among POM types. Nutrient addition to the reaches began 4 days after the July deployment.

We recognise that, because of seasonal changes in water temperature and potential changes in organic matter composition and other factors that may have affected microbial respiration and biomass, the environmental conditions were somewhat different for the Before and After periods. However, there was no evidence that the reaches were differentially affected by these natural factors in either the Before or After periods. Thus, we think that the experimental design was appropriate for addressing the objectives of our study.

Respiration measurements and routine sampling

Respiration of microbes associated with wood veneers and leaves was measured 5–8 weeks after substrate deployment during the Before period, and 6–9 weeks after deployment during the After period. Respiration for each leaf and wood species was measured on separate days (1 and 2 days per POM type for the Before and After period, respectively). The measurement dates were arranged in the After period so that, on average, each POM species had 53–55 days of colonisation since substrate deployment.

Respiration rates of POM were measured as oxygen change in cylindrical, acrylic chambers (2.1 L, 17-cm radius) equipped with lids that could be tightly sealed. Water was circulated in each chamber with a stir bar driven by a magnetic stir plate positioned under each chamber. Dissolved oxygen was measured with an YSI 52 dissolved oxygen meter equipped with a stirring

Table 1 Chemical and physical data for Norris Brook (means ± SD, *n* is given in parentheses). Before and After indicate periods before and after the commencement of nutrient addition

Period	Reach	Length (m)	$\text{NO}_3\text{-N}$ ($\mu\text{g L}^{-1}$)	$\text{PO}_4\text{-P}$ ($\mu\text{g L}^{-1}$)	DOC (mg L^{-1})*
Before	Reference	85	5.7 ± 4.6 (8)	0.5 ± 0.2 (7)	1.2 ± 0.2 (5)
	N	55	5.3 ± 3.6 (8)	0.9 ± 0.7 (8)	1.2 ± 0.1 (5)
	N + P	45	6.6 ± 3.4 (8)	0.8 ± 0.4 (8)	1.3 ± 0.1 (5)
After	Reference	85	9.9 ± 3.5 (17)	0.8 ± 0.3 (11)	1.4 ± 0.4 (7)
	N	55	196.1 ± 89.7 (18)	1.1 ± 0.6 (13)	1.4 ± 0.4 (7)
	N + P	45	174.0 ± 76.2 (17)	29.9 ± 13.1 (13)	1.5 ± 0.4 (7)

*Dissolved organic carbon.

POM type	%C	%N	%P	C : N	C : P
Red oak V*	48.1 ± 0.40	0.068 ± 0.01	0.001 ± 0.0003	833 ± 113	155610
Black cherry V	48.9 ± 0.29	0.072 ± 0.01	0.001 ± 0.0006	792 ± 63	88622
Sugar maple V	47.8 ± 0.27	0.077 ± 0.01	0.007 ± 0.0001	736 ± 120	18346
Yellow birch V	48.0 ± 0.31	0.086 ± 0.01	0.005 ± 0.0004	654 ± 34	26286
American beech V	48.0 ± 0.39	0.094 ± 0.01	0.009 ± 0.0004	593 ± 34	14406
Sugar maple Lt	46.2 ± 0.45	0.790 ± 0.04	0.049 ± 0.0042	68.2 ± 3	2412
American beech L	47.4 ± 0.40	0.817 ± 0.04	0.057 ± 0.0002	67.6 ± 3	2157

*Veneer, †Leaves.

probe inserted through a port in the lid of each chamber. Chambers were placed in water baths near the stream through which stream water was circulated by electric pumps to maintain respiration incubations at the ambient temperature of stream water. In each of three water baths, three chambers (one for each reach) received POM and filtered stream water (5- μ m mesh filter) and a fourth chamber received filtered stream water only. Each POM chamber contained four veneer pieces of the same species or a single leaf pack. Veneer pieces or leaf packs were randomly collected from the reaches and visible invertebrates and large detritus were removed before transfer to chambers. The POM was incubated with ambient stream water in the Before period while, in the After period, POM was incubated with stream water from its reach of origin (reference, N or N + P). The POM was acclimated for 30 min in the dark prior to the incubations and water baths were covered with black plastic to reduce photosynthesis throughout the incubations. Oxygen decline was linear throughout the 4-h incubations. Changes in oxygen in chambers containing only filtered stream water were attributed to probe drift and planktonic respiration and were subtracted from the oxygen change in the POM chambers. Respiration rates were scaled to POM biomass [as μ g O₂ per gram ash-free dry mass (AFDM) per hour] rather than surface area because fungi, which dominated the microbial community, can exploit nutrients in POM in three-dimensions by hyphal penetration (see brushed versus unbrushed comparison below). Prior to each daily run, metabolism chambers were washed with a 1% HCl solution and rinsed thoroughly to minimise microbial growth on the chambers walls. All chambers were tested for leaks and dissolved oxygen concentration did not change during 24-h incubations with tap water partially stripped of oxygen (<40% saturation).

The POM from the chambers was sub-sampled for bacterial and fungal biomass immediately after

Table 2 Initial chemical composition of wood and leaves as percentages of particulate organic matter dry mass or molar ratios (means \pm SD, $n = 3$ for %C and %N, and $n = 2$ for %P)

respiration incubations were completed. The POM for the analysis of ergosterol, used to estimate fungal abundance (Gessner & Chauvet, 1993), was placed in high-performance liquid chromatography (HPLC)-grade methanol and stored at 4 °C. The POM for bacterial abundance was preserved in 5% formalin and stored at 4 °C. The remaining POM from the chambers was placed at -20 °C for later determinations of AFDM.

To determine where bacteria and fungi were located in the biofilm-wood complexes we randomly chose eight yellow birch veneer pieces from each reach 7 weeks after nutrient addition began. Half of the pieces were brushed to remove the bulk of the biofilm and fine POM on the surfaces. The other wood pieces were not altered. Respiration rates were measured and samples for bacterial and fungal biomass were collected as described before.

From mid-May to early September water samples were collected weekly for phosphate, nitrate and dissolved organic carbon (DOC) at three primary sampling stations at the lower end of each reach and occasionally from three secondary sampling stations at the upper end of each reach. During periods of rapidly changing discharge, water samples for dissolved nutrients were collected daily. Drip rates or concentrations of nutrient solutions were adjusted accordingly when stream water nutrient concentrations deviated from our target concentrations (Table 1). Water samples were syringe-filtered through glass fiber filters (GF/F) in the field. Samples that were not analysed within 24 h of collection were stored at -20 °C prior to analysis. Ammonium was not measured. Ammonium concentration is typically very low in stream water at HBEF and is a very small fraction of dissolved inorganic N (Likens & Bormann, 1995; Bernhardt, Hall & Likens, 2002). Stream temperature was measured daily in the After period using a min/max thermometer and the water temperature

in the metabolism chambers was measured during each run with a sensor on the dissolved oxygen probe.

Microbial biomass determination

Ergosterol was extracted and measured according to Newell, Arsuffi & Fallon (1988). The POM samples in methanol were incubated in a water bath at 65 °C for 2 h. After addition of a saponification solution (4% KOH in 95% EtOH) samples were placed at 65 °C for 30 min. Ergosterol in samples was partitioned three times with *n*-pentane. The pentane was then evaporated under N₂. Ergosterol was quantified by absorbance at 282 nm using a reverse phase Waters HPLC (Milford, MA, U.S.A.) with a solvent flow rate of 2 ml methanol per minute through a 4.6 mm × 25 cm octadecyl reverse phase column. Ergosterol concentration of the samples was calculated from regressions of peak area (from the HPLC chromatograms) on ergosterol concentrations of standards. Ergosterol was converted to fungal C by assuming 1 mg fungal C per 11 µg ergosterol (Gessner & Chauvet, 1993).

The POM for bacterial counts was sonicated with a probe sonicator. Diluted sub-samples were stained with 0.1% acridine orange and filtered onto polycarbonate membrane filters (0.2-µm pore size) (Sinsabaugh & Findlay, 1995). Duplicate slides were made for each sample and 400 bacterial cells were counted per slide at 1250× magnification under epifluorescence. When the coefficient of variation of cell abundance among duplicate slides exceeded 20%, slides were remade. We measured cell volume on about 800 bacterial cells and mean cell volume (±SD) was $0.265 \pm 0.352 \mu\text{m}^3$, which we used to convert bacterial cell abundance to bacterial biovolume. Bacterial biovolume was converted to bacterial C assuming $100 \text{ fg C } \mu\text{m}^{-3}$ (Ducklow, 2000). For the After period fungal and bacterial biomass are reported only from the first of the two respiration runs for each POM type.

Chemical analysis of water and POM samples

The NO₃⁻ and PO₄⁻³ concentrations were measured with a Dionex (Sunnyvale, CA, U.S.A.) Ion Chromatograph with a AS4A anion column. Dissolved organic carbon was measured on a Shimadzu TOC analyser (Kyoto, Japan). The C and N content of wood veneer and leaves was measured on a Carlo-Erba CN analyser (CE Elantech, Lakewood, NJ, U.S.A.). The

total P content of POM was determined by combustion at 500 °C for 1 h and then exposure to 1 N HCl for 30 min. Phosphate in the leachate was analysed colorimetrically using the ascorbic acid method (APHA, 1992). The AFDM of veneers and leaves was determined as mass loss after ignition of dried samples at 500 °C for 2 h. The AFDM of POM sub-samples for ergosterol or bacteria cell counts was not measured directly because of the unknown mass of substances leached from the sub-samples by methanol and formaldehyde. The AFDM of this POM was estimated by multiplying its surface area by the AFDM : surface area ratio of the POM not sampled for microbial biomass from each metabolism chamber.

Statistical analysis

In studies conducted in whole stream reaches it is difficult, if not impossible, to replicate stream reaches. In experimental studies where reaches are not replicated, observed changes in response variables may be due to inherent differences (location effects) among the reaches instead of the treatments. By measuring microbial respiration and biomass associated with wood veneer and leaves before nutrient addition we could evaluate whether the contiguous reaches were inherently similar. If we could show that the reaches were similar before nutrient addition, difference in response variables among reaches after nutrient addition would be attributable to the nutrient manipulations.

We measured respiration and microbial biomass of each POM type on separate days so we could replicate POM type (three chambers for each POM type per reach) for our comparisons among the three reaches. In the After period respiration and microbial biomass were measured on two separate days (two runs) for each type of POM. Although the average colonisation time of each POM type was similar, there is the potential for the effects of our treatments to be confounded by variation in colonisation time among POM types. We addressed the potential effects of colonisation time on our results statistically. Our statistical model for determination of how reach, POM type and time affected microbial respiration rate and microbial biomass in the After period was a nested ANOVA model (after Sokal & Rohlf, 1995). In the model, reach ($n = 3$) was a fixed effect, POM type ($n = 7$) was nested within reach, time (i.e. POM run,

$n = 2$) was nested within POM type, and chamber ($n = 3$) was nested within time. In the Before period, we also used a nested ANOVA to determine how reach and POM type affected microbial activity. Reach was a fixed factor, POM type was nested within reach, and chamber was nested within POM type.

Results

Microbial activity and biomass before nutrient addition

There was no overall difference in the respiration rate of POM substrates among reaches prior to nutrient addition ($F_{2,18} = 0.115$, $P > 0.05$; Fig. 1a), suggesting that the three reaches were inherently similar (i.e. the null hypothesis that mean respiration was equal in the three reaches could not be rejected). Respiration rates differed among POM types ($F_{18,40} = 3.12$, $P < 0.01$) before nutrient addition and were higher for the leaves than the wood when expressed per unit of POM AFDM (Fig. 1a). The respiration rates on wood and leaves prior to nutrient addition were within the collective range of rates determined for wood and leaves under ambient stream conditions by other investigators (Tank, Webster & Benfield, 1993; Fuss & Smock, 1996; Royer & Minshall, 2001).

Bacterial C associated with POM, considered collectively, did not differ among reaches ($F_{2,18} = 0.022$, $P > 0.05$; Fig. 2a) prior to nutrient addition. Bacterial C differed among POM types ($F_{18,36} = 27.0$, $P < 0.001$) and was higher for the leaves than for the wood (Fig. 2a).

Fungal C associated with POM, considered collectively, was not different among reaches before nutrient addition ($F_{2,18} = 0.109$, $P > 0.05$; Fig. 3a). For individual POM types, such as yellow birch and beech veneer, there was more variation in fungal C among reaches than there was for bacterial C. Fungal C differed among POM types ($F_{18,40} = 9.03$, $P < 0.001$) and was higher for the leaves than the wood before nutrient addition (Fig. 3a).

Microbial activity and biomass after nutrient addition

After nutrient addition, there were large differences in microbial respiration among reaches ($F_{2,18} = 4.62$, $P < 0.05$; Fig. 1b). Respiration rates were generally highest in the N + P reach, intermediate in the N reach, and lowest in the reference reach. As in the

Before period, there were differences in respiration rates among POM types after nutrient addition ($F_{18,20} = 61.6$, $P < 0.001$; Fig. 1b). Respiration rate was highest for sugar maple leaves. There was no effect of colonisation time on respiration rates for the two separate days (runs) on which respiration was measured for each POM type after nutrient addition ($F_{20,73} = 0.491$, $P > 0.05$). We examined the response of each POM type to increased dissolved nutrients by calculating the relative change in respiration rate between the N and reference reaches and between the N + P and reference reaches. Respiration of wood, which had higher C : N and C : P ratios, responded

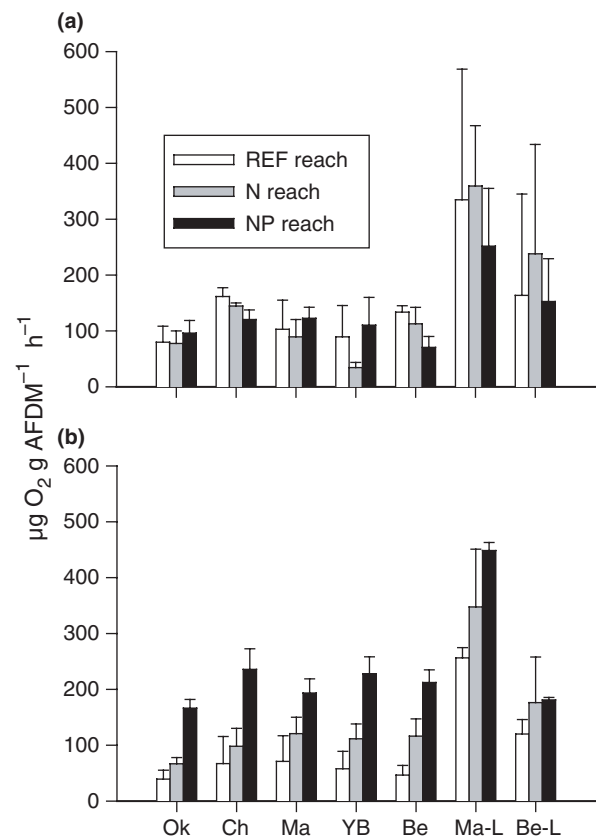


Fig. 1 Microbial respiration rates (mean + 1 SD) before (a) and after (b) nutrient addition. Before nutrient addition $n = 3$. After nutrient addition measurements from two separate days are combined for each particulate organic matter (POM) type ($n = 6$). POM types are arranged by decreasing C : N ratio. POM abbreviations are as follows: Ok (red oak wood), Ch (black cherry wood), Ma (sugar maple wood), YB (yellow birch wood), Be (American beech wood), Ma-L (sugar maple leaves), Be-L (American beech leaves). Bar abbreviations indicate reach as follows: REF (reference), N (nitrogen) and NP (nitrogen + phosphorus).

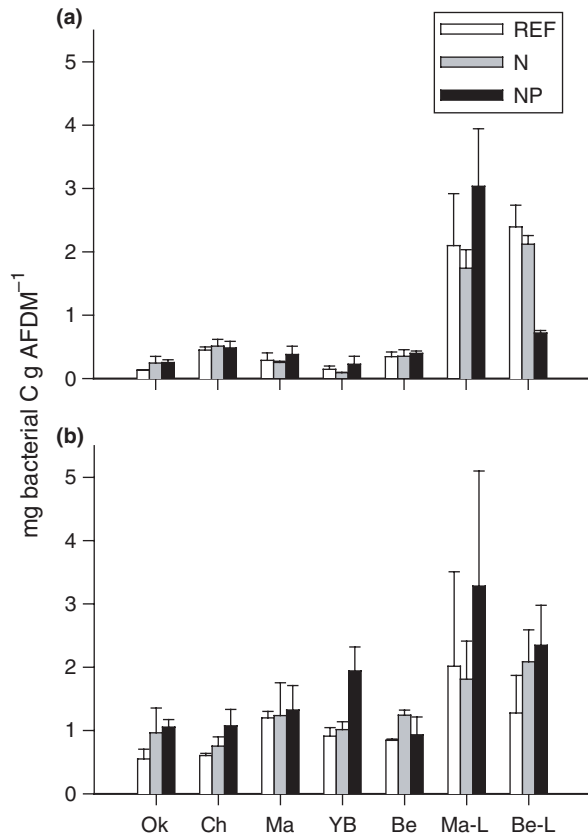


Fig. 2 Bacterial carbon associated with particulate organic matter (POM) (mean + 1 SD, $n = 3$) before (a) and after (b) nutrient addition. POM and reach abbreviations are as in Fig. 1.

more strongly to increased dissolved nutrients than leaves, which had lower C : N and C : P ratios (Fig. 4a, b).

Bacterial C associated with POM, considered collectively, did not differ among reaches after nutrient addition ($F_{2,18} = 1.78$, $P > 0.05$; Fig. 2b). Bacterial C was higher for leaves than wood after nutrient addition ($F_{18,37} = 3.03$, $P < 0.01$).

There were large differences in fungal C among reaches after nutrient addition ($F_{2,18} = 18.2$, $P < 0.001$; Fig. 3b). Fungal C was much higher in the N + P reach than the other reaches, particularly for the wood. There was no difference in fungal C among POM types after nutrient addition ($F_{18,39} = 1.64$, $P > 0.05$; Fig. 3b).

Comparison of fungi and bacteria

Bacterial C associated with POM was higher overall (averaged across three reaches) in the After period

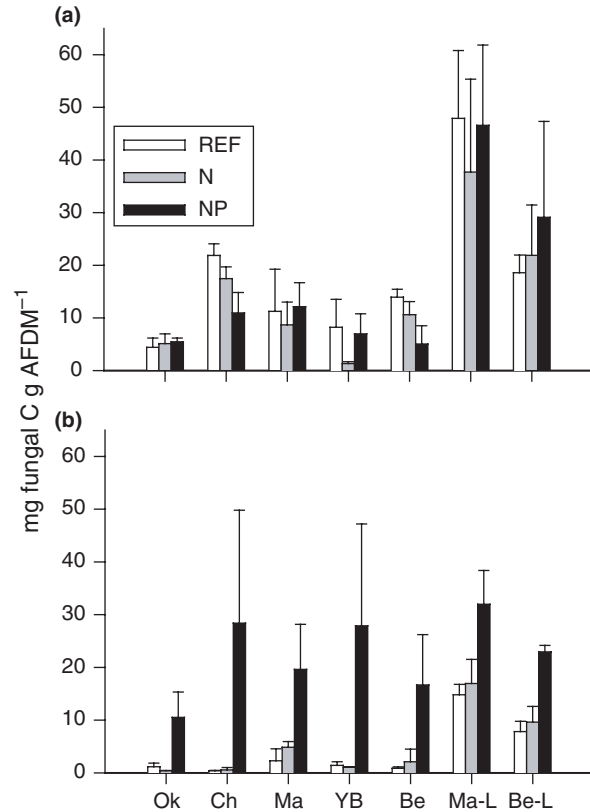


Fig. 3 Fungal carbon associated with particulate organic matter (POM) (mean + 1 SD, $n = 3$) before (a) and after (b) nutrient addition. POM and reach abbreviations are as in Fig. 1.

(1.37 mg C g AFDM⁻¹) than in the Before period (0.77 mg C g AFDM⁻¹). In contrast, fungal C associated with POM was lower overall for the After period (10.2 mg C g AFDM⁻¹) than for the Before period (16.4 mg C g AFDM⁻¹). For the reference reach alone, fungal C associated with POM was lower in the After period (4.11 mg C g AFDM⁻¹) than in the Before period (18.2 mg C g AFDM⁻¹). Microbial biomass was dominated by fungi prior to nutrient addition as $95.7 \pm 3.2\%$ (mean \pm SD) and $93.3 \pm 3.9\%$ of the microbial C associated with the wood and leaves, respectively, was fungal. The overall increase in bacterial C and decrease in fungal C from the Before to After period meant that bacterial C represented a larger percentage of the microbial C after nutrient addition. After nutrient addition $66.6 \pm 23.5\%$ and $88.3 \pm 5.4\%$ of the microbial C associated with the wood and leaves respectively, was fungal.

Bacterial C was much higher for unbrushed yellow birch wood than for brushed (Table 3) wood, whereas fungal C was as high or higher for the brushed wood

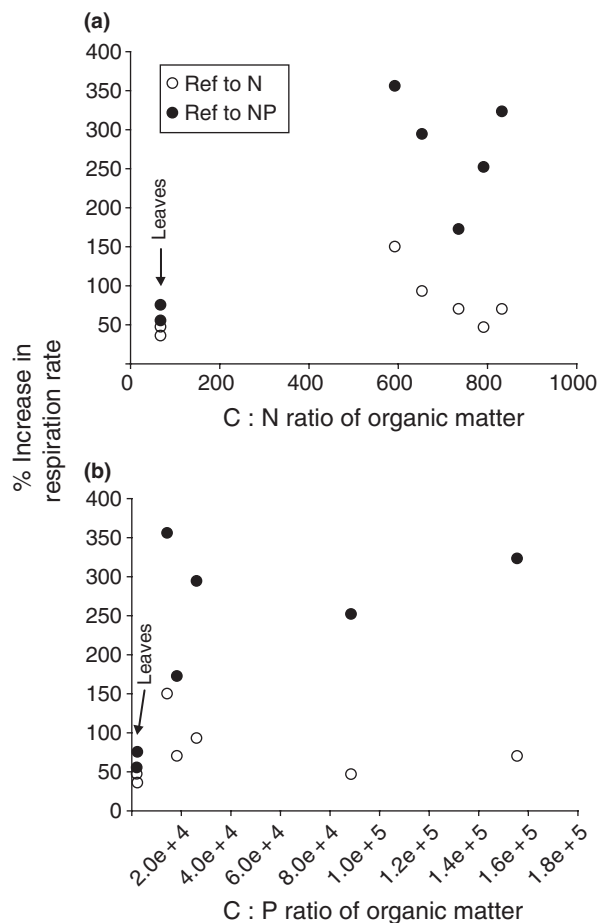


Fig. 4 The relative increase in microbial respiration rate between reference and nitrogen reaches (REF to N) and between reference and nitrogen + phosphorus reaches (REF to NP) after nutrient addition versus C : N ratio (a) and C : P ratio (b) of particulate organic matter (POM). POM abbreviations are as in Fig. 1.

in all three reaches after nutrient addition. Respiration rate was similar for unbrushed and brushed yellow birch wood from the enriched reaches and higher for the unbrushed wood than the brushed wood from the reference reach.

Physical/chemical attributes before and after nutrient addition

The mean daily minimum and maximum stream water temperatures were 13.3 ± 1.2 °C and 16.0 ± 0.9 °C, respectively, in the period after nutrient addition. During the 2 months prior to nutrient addition, water temperatures were more variable and lower, ranging from 8–15 °C. There was no

difference in water temperature among reaches in the Before and After periods based on measurements taken with a handheld thermometer during collection of water samples from each reach. Water temperature in chambers during metabolism runs was generally between 14.5 and 17.5 °C throughout the study. There was no difference in water temperature among chambers assigned to the three different reaches. Mean water temperature in chambers for sugar maple leaves and red oak veneer was 17 and 16.5 °C, respectively. For all other POM types mean water temperature in chambers was 15–15.5 °C. Although water temperature is known to influence respiration rate (e.g. Sinsabaugh, 1997), it is unlikely that the small differences in water temperature among POM types contributed to the main patterns in microbial respiration rate and biomass that we observed.

Discussion

Our two predictions were largely supported. Consistent with our first prediction, microbial respiration and biomass were higher for the POM of higher quality (i.e. leaves) when dissolved nutrient concentrations were low, as they were in the period before nutrient addition. When dissolved nutrient availability is low, microbes would be expected to increase their reliance on N and P in POM (Sinsabaugh *et al.*, 1993) and the higher microbial activity on leaves than on wood may have been due to the higher N and P content of leaves. Other studies have shown that the nutrient content or carbon quality (e.g. lignin content) of POM influences decomposition or respiration rates in aquatic systems (Melillo *et al.*, 1982; Enríquez *et al.*, 1993; Fuss & Smock, 1996). Microbial respiration rates were higher for leaves than wood when expressed as oxygen change per substrate volume or AFDM in other studies of microbial respiration in streams (Tank *et al.*, 1993; Fuss & Smock, 1996). The higher respiration rates and fungal C for sugar maple leaves compared with American beech leaves before nutrient addition (Fig. 1a), and after nutrient addition in the reference reach (Fig. 1b), suggests that sugar maple leaves were of higher quality. Nitrogen mineralisation and nitrification were higher in stands of sugar maple than American beech in a study along a nitrogen deposition gradient (Lovett & Rueth, 1999), consistent with our findings that sugar maple litter may be of higher quality than American beech. Variation in

Table 3 Bacteria and fungal carbon on unbrushed and brushed yellow birch veneer after nutrient addition (means \pm SD, $n = 2$)

Reach	Bacterial carbon (mg C g AFDM ⁻¹)*		Fungal carbon (mg C g AFDM ⁻¹)		Microbial respiration (μ g O ₂ g AFDM ⁻¹ h ⁻¹)	
	Unbrushed	Brushed	Unbrushed	Brushed	Unbrushed	Brushed
Reference	1.9 \pm 0.1	0.01 \pm 0.0002	2.0 \pm 0.6	1.8 \pm 0.3	77.2 \pm 30.5	46.3 \pm 0.1
N	1.4 \pm 0.1	0.02 \pm 0.01	0.4 \pm 0.3	4.1 \pm 0.9	125.2 \pm 5.2	115.8 \pm 2.3
N + P	1.8 \pm 0.3	0.33 \pm 0.03	38.1 \pm 16.6	39.4 \pm 7.8	255.4 \pm 47.2	283.1 \pm 13.7

*Ash-free dry mass.

C : N and C : P ratios among wood types did not affect respiration rates and microbial biomass in our study. The moderate range of variation among wood types in elemental composition, 10-fold for P but less than twofold for N, may have been insufficient to detect a potential effect on microbial processes.

In agreement with our second prediction, the quality of POM appeared to influence the response of microbes to increased concentrations of dissolved nutrients. Microbes associated with wood exhibited a larger relative increase in respiration rates with increased dissolved nutrients than microbes associated with leaves (Fig. 4a, b). This pattern was also apparent for the response of fungal biomass to N and P but was not found for bacteria, as no response to dissolved nutrients occurred (Figs 2b and 3b). Our results suggest that the response of microbes to increased dissolved nutrients was influenced by the quality of their substrate. Microbes associated with the leaves may have been less reliant on dissolved nutrients than microbes associated with wood. In another study, fungi associated with leaf disks were able to grow and sporulate in water with no dissolved N or P, suggesting that the fungi were able to obtain nutrients from the leaf material (Suberkropp, 1998). Addition of dissolved N and P resulted in higher growth and sporulation rates in Suberkropp's (1998) study, indicating that fungi can readily use dissolved nutrients from the overlying water.

In the Before period, statistical power was probably low because we had three replicates per treatment. If we had more replicates in the Before period we may have been able to show statistically significant differences among reaches. However, based on the data collected, the magnitude of any differences among reaches in respiration rate and microbial C would probably have been very small. In addition, in the Before period the reference reach tended to have relatively high microbial respiration rates and microbial C for the various POM types (Figs 1a, 2a and 3a).

Thus, our conclusions about the effects of nutrient addition on these parameters tended to be conservative.

Because we chose to study a single stream intensively we were constrained to having only three different nutrient treatments (reference, N, N + P) that were largely independent of one another. The addition of a 'high P' reach up-stream of the 'high N' reach, for example, would have affected the P availability in the 'high N' reach. We chose an N treatment as the uppermost treatment reach rather than P because large variation of dissolved N because of natural and anthropogenic causes is probably more common among northern hardwood forest streams than large variation in P. Thus, we could not independently test the effects of dissolved N and P on microbial processes. There was some evidence that microbes were co-limited by N and P as microbial respiration and fungal biomass were higher when both dissolved N and P were increased compared with dissolved N alone. However, without knowing how microbes would respond to raised P alone, we cannot determine if microbial communities were simultaneously limited by N and P. Our results strongly suggest that respiration and fungal biomass were limited by both N and P, but not necessarily simultaneously. Co-limitation of microbes associated with POM by nitrogen and phosphorus has been demonstrated in other studies (Tank & Webster, 1998; Sridhar & Bärlocher, 2000; Grattan & Suberkropp, 2001). In a meta-analysis of nutrient amendment experiments in streams, Francoeur (2001) found that co-limitation of algal communities was widespread.

The nitrate concentration in the N and N + P reaches in Norris Brook was well within the range of ambient nitrate concentration among other streams at HBEF (Likens & Bormann, 1995; Bernhardt *et al.*, 2002) and in many rivers (Caraco *et al.*, 1998). This result suggests that natural variation in nitrate concentration may have a widespread, important

influence on microbial respiration, and thus C mineralisation, in forest streams. The phosphate concentration in the N + P reach was considerably higher than that found in many forest streams (Likens & Bormann, 1995) and it is unclear to what extent natural variation in dissolved P among such streams will affect C mineralisation and other microbial processes.

Much of the evidence for the role of dissolved nutrients in regulating microbial respiration, biomass and organic matter decomposition in aquatic systems has been correlative (Rosset, Bärlocher & Oertli, 1982; Meyer & Johnson, 1983; Suberkropp, 1995; Suberkropp & Chauvet, 1995; Weyers & Suberkropp, 1996). Relatively few studies (Elwood *et al.*, 1981; Newbold *et al.*, 1983; Peterson *et al.*, 1993; Rosemond *et al.*, 2001, 2002; Gulis & Suberkropp, 2003) have examined how microbial processes such as respiration or decomposition respond to experimental enrichment of dissolved nutrients in whole stream reaches. Newbold *et al.* (1983) found no effect of continuous enrichment with ammonium on leaf decomposition or oxygen uptake in Walker Branch, Tennessee. It is possible that the moderate concentration of N in their enriched reach, $100 \mu\text{g NH}_4\text{-N L}^{-1}$, about half of our mean N concentration in the enriched reaches, was not sufficiently high to cause a stimulatory effect. Of the prior whole-stream enrichment studies only Elwood *et al.* (1981) and Gulis & Suberkropp (2003) found consistent effects of dissolved nutrients on heterotrophic microbial processes (microbial respiration and leaf decomposition). The average phosphate concentrations in the enriched reaches in Elwood *et al.* (1981) were at least twofold to 15-fold greater than our average phosphate concentration in the N + P reach. Our results suggest that microbes associated with POM may respond to considerably lower concentrations of dissolved P.

Fungal abundance responded positively to increased dissolved nutrients, particularly to N and P together, but bacterial abundance was not affected by nutrient enrichment. Why was the response of fungi and bacteria to increased nutrients uncoupled? The results of the brushed versus unbrushed comparison suggest that most of the fungal mycelia were in the interstices of the wood fibres whereas most of the bacteria associated with POM were in a superficial biofilm. Bacteria and fungi produce extracellular

enzymes (e.g. Zare-Maivan & Shearer, 1988) that break down large organic molecules and produce monomers that can be taken up by the microbes. Because of the ability of fungi to penetrate the wood, and presumably the leaves, enzymes produced by fungi may have had greater access to the POM carbon than did bacteria, enabling the fungi to exploit the increased supply of dissolved N and P in the enriched stream reaches. Dissolved organic carbon concentration was low (average of 1.4 mg L^{-1}) in Norris Brook, suggesting that microbes obtained at least a portion of their C from POM. In addition, the respiration of epilithic microbial communities in Norris Brook was not affected by increases in stream water nutrients (R.S. Stelzer, unpublished data), presumably because they were carbon limited, providing further evidence of the importance of POM C to fungi and bacteria in Norris Brook. In a study in Bear Brook at HBEF, bacterial abundance on inorganic surfaces (glass slides) did not respond to experimentally increased nitrate (Haack, Burton & Ulrich, 1988). Gulis and Suberkropp (2003) also compared the response of fungi and bacteria on leaves to nutrient enrichment in a forest stream. Both fungi and bacteria increased with nutrient enrichment. However, in their study, as in ours, fungal biomass was much higher than bacterial biomass on leaves.

The response of microbial respiration to increased dissolved nutrients was not proportional to the response of fungal C to nutrient enrichment. Although respiration rates of wood were three to four times higher from the N + P reach than the reference reach, for example, fungal C was 10–30 times higher from the N + P reach than the reference. This discrepancy may be due to uncertainty in the conversion factor used to convert ergosterol to fungal C (Gessner & Chauvet, 1993). The ratio of ergosterol to mycelial biomass can vary up to five-fold among different species of aquatic fungi (Gessner & Chauvet, 1993). Although the conversion factor used is an average for several different species, shifts in fungal species composition may lead to under- or overestimations of mycelial biomass when a single conversion factor is used. Given this potential uncertainty however, about half of the variation in microbial respiration could be explained by microbial C based on pooled data from all three reaches for before and after nutrient addition (Fig. 5). It is not surprising that respiration rates and heterotrophic microbial biomass would be related

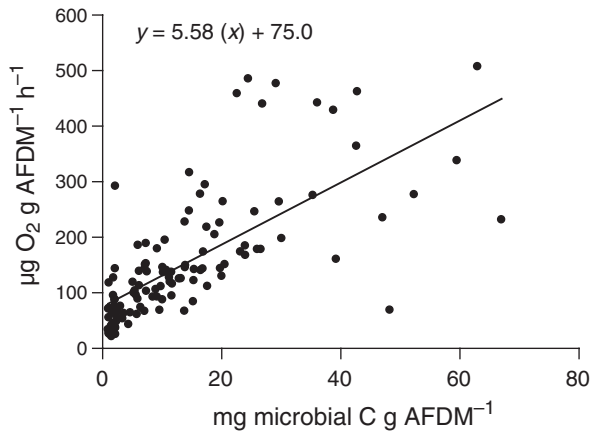


Fig. 5 Microbial respiration rate versus microbial carbon associated with particulate organic matter before and after nutrient addition ($r^2 = 0.49$, $F_{1,112} = 108.3$, $P < 0.001$).

because macroinvertebrates were removed prior to the incubations and autotrophic production was very low on the POM substrates in Norris Brook (R.S. Stelzer, unpublished data).

We do not know why bacterial C associated with the POM substrates was higher overall in the After than in the Before period, although bacteria were not affected by the nutrient enrichments. This change may have been related to accumulation of fine POM (FPOM). Stream flow was lower and there tended to be more FPOM on the wood and leaves during the period of nutrient addition. The relative abundance of bacteria and fungi increases and decreases, respectively, as average particle size decreases, as would occur when FPOM deposition is high (Sinsabaugh & Findlay, 1995). Although fungi responded strongly to the nutrient enrichments, especially to N + P, fungal C overall was higher in the Before than in the After period. This change in fungal C may have been related to seasonal changes in growth and reproduction of individual fungal species (Suberkropp & Klug, 1976). Despite these temporal changes in the abundance of bacterial and fungal C associated with the POM substrates, fungi were consistently more abundant than bacteria on the POM. Many other investigators have found that fungi are more abundant than bacteria on coarse POM such as wood and leaves (e.g. Findlay *et al.*, 2002).

We have demonstrated that microbial respiration and fungal biomass are limited by dissolved nutrients in a low-nutrient forest stream. Prior to nutrient

addition, leaves had higher microbial respiration rates and microbial biomass than wood which suggests that leaves, because of their higher N and P content, were superior substrates for microbes. The greater relative response of microbial respiration of wood to increased dissolved nutrients also suggests that the response of microbes to increased dissolved nutrients was influenced by POM quality. There are limitations in using simple ratios of elements or compounds to predict microbial processes in aquatic systems (Sinsabaugh *et al.*, 1993). In many cases, more detailed information about carbon quality may be necessary (e.g. Joffre *et al.*, 2001). Like all living organisms, heterotrophic microbes are fundamentally constrained by the supply of C, N, P and other essential elements available for growth and reproduction. Bulk measures of elements can be useful in describing the nutrient availability to microbes in single currencies with respect to these constraints. Future studies of microbial processes in streams may need to consider both the role of particulate and dissolved pools in providing essential elements for microbes. It is particularly important to understand how nutrient availability influences microbial processes, such as carbon mineralisation, in an era of global increases in anthropogenically-derived nutrients to ecosystems (e.g. Vitousek *et al.*, 1997).

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