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Effects of nutrient enrichment on the decomposition of wood and associated microbial activity in streams

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SUMMARY

1. We determined the effects of nutrient enrichment on wood decomposition rates and microbial activity during a 3-year study in two headwater streams at Coweeta Hydrologic Laboratory, NC, U.S.A. After a 1-year pretreatment period, one of the streams was continuously enriched with inorganic nutrients (nitrogen and phosphorus) for 2 years while the other stream served as a reference. We determined the effects of enrichment on both wood veneers and sticks, which have similar carbon quality but differ in physical characteristics (e.g. surface area to volume ratios, presence of bark) that potentially affect microbial colonisation and activity.

2. Oak wood veneers (0.5 mm thick) were placed in streams monthly and allowed to decompose for approximately 90 days. Nutrient addition stimulated ash-free dry mass loss and increased mean nitrogen content, fungal biomass and microbial respiration on veneers in the treatment stream compared with the reference. The magnitude of the response to enrichment was great, with mass loss 6.1 times, and per cent N, fungal biomass and microbial respiration approximately four times greater in the treatment versus reference stream.

3. Decomposition rate and nitrogen content of maple sticks (ca. 1–2 cm diameter) also increased; however, the effect was less pronounced than for veneers. Wood response overall was greater than that determined for leaves in a comparable study, supporting the hypothesis that response to enrichment may be greater for lower quality organic matter (high C : N) than for higher quality (low C : N) substrates.

4. Our results show that moderate nutrient enrichment can profoundly affect decomposition rate and microbial activity on wood in streams. Thus, the timing and availability of wood that provides retention, structure, attachment sites and food in stream ecosystems may be affected by nutrient concentrations raised by human activities.

Keywords: breakdown, fungal biomass, nitrogen, phosphorus, respiration

Introduction

Wood is an important resource in streams. Large woody debris provides structure and habitat variability through the creation of pools and retention of other forms of organic matter (Bilby & Ward, 1991). Wood snags are the most productive substrata in sandy-bottom rivers and serve as important attachment sites and habitat for many stream invertebrates (Benke *et al.*, 1984). Wood provides physical and

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biological stability in streams in part because it is so slow to decompose. The slow decomposition is because of several characteristics of wood, both chemical and physical, including higher concentrations of lignin and lower concentrations of nitrogen than in other forms of organic matter such as leaves (Melillo *et al.*, 1983).

Changes in the quality or quantity of wood affect physical, chemical and biological dynamics of streams. The quantity of woody debris has been significantly altered by humans in terms of wholesale removal (Sedell & Froggatt, 1984), but more subtle processes, such as loss through nutrient-accelerated microbial breakdown, have been addressed very little. In streams where food webs rely on dead organic matter, heterotrophic microorganisms play critical roles in colonising and decomposing organic matter and thus controlling quality and availability of food resources to higher order consumers. Several previous field studies have shown that experimental increases or natural gradients in dissolved nutrients (N and/or P) positively affect leaf breakdown (e.g. Elwood *et al.*, 1981; Pearson & Connolly, 2000; Robinson & Gessner, 2000) and associated microorganisms (Suberkropp & Chauvet, 1995; Grattan & Suberkropp, 2001; Rosemond *et al.*, 2002; Gulis & Suberkropp, 2003) (but see Triska & Sedell, 1976; Newbold *et al.*, 1983; Rosemond *et al.*, 2001; Royer & Minshall, 2001; Huryn *et al.*, 2002). Fewer comparable studies have been conducted to determine nutrient effects on wood, but where effects have been tested, positive responses of wood-associated microorganisms (Tank & Webster, 1998; Stelzer, Heffernan & Likens, 2003; Tank & Dodds, 2003) have been found.

The effect of nutrients on organic matter may depend on the latter's composition and quality. Particulate organic matter entering headwater streams can be comprised of labile to refractory carbon, have variable lignin and nutrient contents and differ in physical characteristics such as surface area to volume ratio and barriers to microbial colonisation (e.g. bark on wood, waxy cuticles on leaves). Because microorganisms can obtain nutrients from both the substrate and the overlying water (Melillo *et al.*, 1984; Mulholland *et al.*, 1984), their response to dissolved nutrients may depend on the nutrient content of the organic matter they colonise. Recent work showed that microbial response to nutrient enrichment was greater on high C : N wood than on lower C : N leaves (Stelzer *et al.*, 2003). Wood is not only lower in nutrient content than leaves, but has higher lignin, lower surface area to volume ratio and a physical barrier to microbial colonisation (i.e. bark). Microbial activity on wood may become limited by the low diffusion of oxygen below the surface layers (Aumen *et al.*, 1983; Harmon *et al.*, 1986). This slows the rate of decomposition, particularly of lignin, as its degradation requires oxygen (Kirk & Farrell, 1987). Consequently, one could also predict a limited response of wood to changes in dissolved nutrients.

The objective of this study was to determine the effects of whole-stream nutrient enrichment on wood decomposition and associated microbial activity. Our long-term experiment allowed us to assess the effects on standardised substrates (wood veneers) and also determine realistic decomposition rates and effects of enrichment on sticks. Comparisons of veneers and sticks allowed us to examine the magnitude of nutrient enrichment effects on organic matter that was similar in lignin and nutrient content, but differed in physical characteristics (bark, surface area to volume ratio). In addition, we compared the magnitude of the response observed on veneers and sticks to the effect of nutrients on leaves measured in a concurrent study. Our experiment was conducted in two streams over 3 years, one pretreatment year and 2 years in which the treatment stream received continuous enrichment with inorganic nitrogen and phosphorus. We placed oak veneers in both streams monthly and sampled them after approximately 90 days. To characterise the microbial communities associated with the veneers, we measured microbial respiration, fungal biomass and N content. We also placed maple sticks in both streams at the beginning of the study and sampled them periodically over 3 years to determine decomposition rate and associated N concentration.

Methods

Study sites

The study was conducted in two headwater streams draining catchments 53 (reference) and 54 (treatment) in Coweeta Hydrologic Laboratory, Macon County, NC, U.S.A. The streams drain south-facing slopes covered by mixed deciduous forest in the southern Appalachian Mountains at an altitude of ca. 850 m

a.s.l. Because the dense understory of *Rhododendron maximum* L. results in year-round shading, the streams are primarily heterotrophic, i.e. they rely on allochthonous organic matter and energy (Hall, Wallace & Eggert, 2000 and references therein). These streams are small (average discharge about 1 L s^{-1}), circumneutral, softwater and have low ambient nutrient concentrations. Streams are located about 200 m apart and their physical and hydrochemical characteristics are very similar (Cuffney, Wallace & Lugthart, 1990; Wallace *et al.*, 1999). Water temperature was continuously monitored with Optic StowAway temperature probes (Onset Computer Corp.) and was similar between the two streams over the 3-year period, ranging annually from 1–19 °C (mean 12.1 °C for both streams) (Gulis & Suberkropp, 2004).

Pretreatment observations on both streams began in June 1999 and nutrient addition was started in the treatment stream on 11 July 2000 while stream 53 continued to serve as a reference. The treatment stream was enriched continuously with N and P in a flow-dependent manner. A metering pump situated 145 m above the flume delivered a concentrated solution of ammonium nitrate and potassium phosphate to a pipe fed with stream water, which then dripped out at multiple points along the study reach. Delivery of the nutrient solution was controlled electronically, via a signal from an ISCO flow meter that a given flow volume had passed the flume. The pump was powered by a deep cycle marine battery, which was kept charged by a solar panel (AMJ Equipment Corp., Lakeland, FL, U.S.A.).

Water samples were taken twice a month from one point in the reference stream, five points along the enriched reach at approximately 30 m intervals and one point upstream of the nutrient delivery system. Samples were filtered (Millipore HA filters, 0.45 μm) into acid-washed bottles, which were kept on ice and then frozen until analysis. All samples were analysed for ammonium, nitrate and soluble reactive phosphorus (SRP) with an Alpkem Rapid Flow Analyzer 300 by the Stable Isotope/Soil Biology Laboratory in the Institute of Ecology, University of Georgia, GA, U.S.A.

Wood veneers

Five oak (*Quercus alba* L.) veneers (150 × 25 × 0.5 mm) were fastened to sections of plastic gutter mesh, labelled and three sets were placed in each stream monthly. After approximately 90 days, the three sets of veneers were removed from each stream. Preweighed veneers (two per set) were taken back to the laboratory, dried at 60 °C, weighed and cut in half. Subsamples were weighed again, combusted at 500 °C, and reweighed to determine ash-free dry mass (AFDM). The other half of each veneer was ground in a Wiley Mill and subsamples were analysed for C and N content (Carlo Erba NA 1500 Elemental Analyzer, Stable Isotope/Soil Biology Laboratory, Institute of Ecology). The veneers that had not been preweighed were cut into 1 × 1 cm pieces on site. Five wood squares per replicate set of veneers were placed in methanol, stored at −20 °C, and later analysed for ergosterol. The other set of five pieces was placed in respiration chambers situated in the stream, and the rate of oxygen uptake was measured. After respiration measurements, these pieces were taken to the laboratory and their AFDM determined as described above.

Ergosterol was extracted from pieces of veneer by refluxing them in alcoholic KOH (30 mL) at 80 °C for 30 min (Newell, Arsuffi & Fallon, 1988; Suberkropp & Weyers, 1996). After 10 mL of water was added, ergosterol was partitioned into pentane (three times) and evaporated to dryness under a stream of nitrogen gas. The residue was dissolved in methanol and filtered (0.45 μm Acrodisc PTFE, Pall, Gelman Laboratory, Ann Arbor, MI, U.S.A.). Ergosterol was separated with a Whatman Partisphere C-18 column in a high performance liquid chromatograph (Shimadzu, Scientific Instruments, Columbia, MD, U.S.A.) using methanol as the mobile phase (1 mL min^{-1}) and detected by measuring absorbance at 282 nm and comparing peak area to ergosterol standards (Fluka, Buchs, Switzerland). A standard conversion factor of 5.5 μg ergosterol per milligram fungal biomass was used to convert ergosterol concentrations to fungal biomass (Gessner & Chauvet, 1993).

Respiration was measured using YSI 5100 dissolved oxygen meters equipped with a stirrer. Respiration chambers (26 mL) contained a stainless steel screen to separate wood pieces from the stirrer but allowed mixing of the water in the chamber. Once wood pieces were placed in the chambers, the chambers were positioned in the stream and covered with black plastic to inhibit potential photosynthesis. Oxygen concentrations were recorded at intervals over 25–40 min. Rates of respiration were determined from

slopes of oxygen concentrations versus time and the AFDM of the veneer pieces. Rates of oxygen consumption with only water in the chambers were subtracted from rates exhibited by veneers to correct for drift, if any.

Maple sticks

Branches of red maple (*Acer rubrum* L., 1.1–1.9 cm in diameter) were collected from living trees, returned to the laboratory, cut into 12.5 cm long lengths and air-dried for 40 days. They were then weighed, tagged and fastened in sets of 11 to plastic gutter mesh. Six replicate sets of sticks were placed in each stream on 4 August 1999, and sticks were retrieved periodically over the next 3 years. When sampled, sticks were cut in half, dried at 100 °C, and both halves weighed. One half was then combusted at 500 °C and reweighed to determine AFDM. The other half was ground in a Wiley Mill and subsamples were analysed for C and N content as above.

Calculations and statistical analyses

As not all veneers were sampled exactly 90 days after they were placed in the stream, AFDM remaining of the veneers was corrected to reflect a 90-day decomposition period. Data on AFDM remaining were fitted to an exponential decay model for the number of days veneers were actually in the stream (73–115 days, most were 84–104 days) and then AFDM remaining after 90 days was calculated using decomposition rates (k). As true replication in whole ecosystem experiments is difficult or impossible, we used a before–after control–impact experimental design that involves parallel observations of reference and treatment systems before and after manipulation. To find out if the changes in the parameters measured occurred following nutrient enrichment, we performed randomised intervention analysis (RIA, Carpenter *et al.*, 1989). Despite recent criticism (Murtaugh, 2002), we believe that this analysis was appropriate in our case as the three half-series mean values (pretreatment mean values in both streams and post-treatment mean value in the reference stream) were very similar and temporal differences between streams were not pronounced. Mean values of the parameters associated with veneers in the two streams were also compared for both before and after treatment periods with paired t-tests. Decomposition rates of maple sticks were compared with ANCOVA followed by Tukey's test and differences in their N contents were analysed with ANOVA.

Results

Dissolved inorganic nitrogen was less than 30 μg L^{-1} in the reference stream and in the treatment stream prior to enrichment, and increased to an average of about 400 μg L^{-1} in the treatment stream during the enrichment. SRP was less than 10 μg L^{-1} prior to enrichment and in the reference stream and was increased to ca. 50 μg L^{-1} in the treatment stream. These represented 11–14 times increases in inorganic nitrogen and six to nine times increases in SRP in the treatment stream (Table 1).

Table 1 Mean and range of inorganic nutrient concentrations in the experimental streams. For the pretreatment period (30 June 1999 to 6 July 2000), sampling date values are based on one to four samples from the reference and treatment streams. For the enrichment period (11 July 2000 to 24 July 2002), one sample was taken from the reference stream and five samples were taken from the treatment stream on each sampling date to confirm that nutrients were increased evenly along the study reach.

		NO_3-N + NO_2-N (μg L^{-1})		NH_4-N (μg L^{-1})		SRP (μg L^{-1})	
Period/stream	n	Mean	Range	Mean	Range	Mean	Range
Pretreatment (1999–2000)							
Reference	5	15	9–26	9	0*–30	8	0*–20
Treatment	12	19	4–40	10	0*–25	9	0*–22
Enrichment (2000–2002)							
Reference	33	17	0*–151	10	0*–76	4	0*–17
Treatment	44	309	11–1711	105	6–566	51	0*–268
Treatment – above the pump	31	22	0*–251	14	0*–70	6	0*–40

n, number of sampling dates; SRP, soluble reactive phosphorus.
0*, concentration below detection limit.

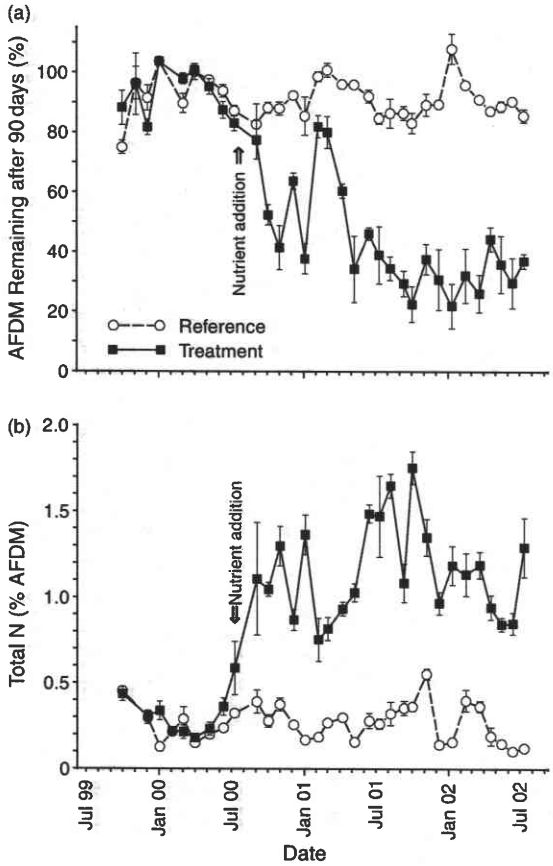

Fig. 1 (a) The percent ash-free dry mass (AFDM) remaining of oak veneers after 90 days incubation in the streams, $n = 4$–6. (b) Total nitrogen as percent of the AFDM of oak veneers after ca. 90 days incubation in the streams, $n = 3$. Vertical bars = ±SEM.

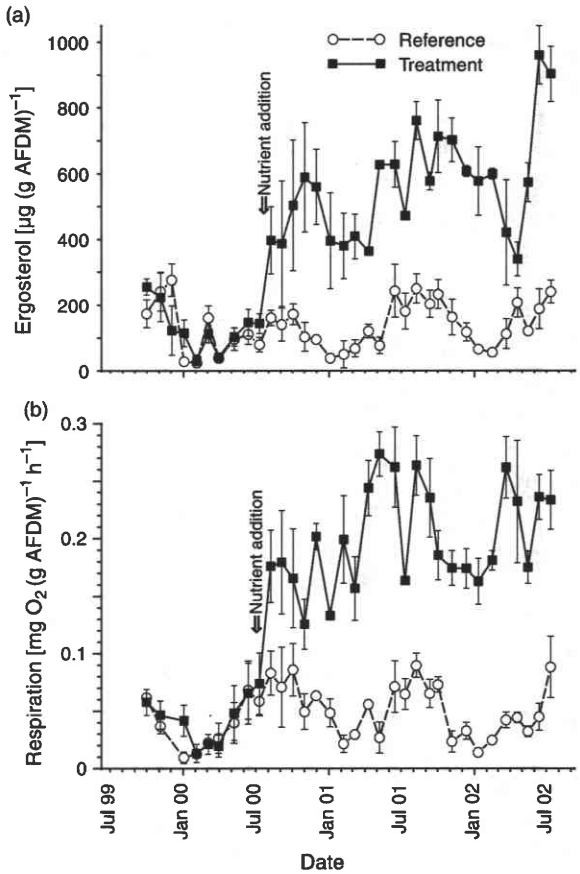

Fig. 2 Ergosterol concentration (a) and respiration as rate of oxygen uptake (b) associated with oak veneers after ca. 90 days incubation in the streams, $n = 3$. Vertical bars = ±SEM.

During the pretreatment year, the mean AFDM losses by wood veneers after 90 days (Fig. 1a) were similar in both streams (paired *t*-test, $P = 0.95$) at 7.2 and 7.3%. After the nutrient enrichment began, the mean AFDM loss after 90 days in the treatment stream (56.6%) differed (paired *t*-test, $P < 0.0001$) from that found in the reference stream (9.3%) (6.1 times difference). On the basis of the comparison of pretreatment and treatment data for both streams, RIA indicated that breakdown of wood veneers increased following nutrient enrichment ($P < 0.0001$).

Mean nitrogen concentration (Fig. 1b) of the veneers in the two streams was also similar in the pretreatment year (paired *t*-test, $P = 0.12$), with veneers containing 0.26% N in the reference stream and 0.32% N in the treatment stream. During the 2-year nutrient enrichment, the mean N content of veneers in the treatment stream increased to 1.15% N and was higher (paired *t*-test, $P < 0.0001$) than the mean found in the reference stream (0.27% N) (4.3 times difference). RIA indicates that significant changes in nitrogen content occurred after nutrient addition started ($P < 0.0001$).

Ergosterol concentration associated with the veneers exhibited a similar pattern (Fig. 2a). During the pretreatment year, veneers in both streams had similar mean ergosterol concentrations (paired *t*-test, $P = 0.78$) corresponding to 22 (reference stream) and 24 (treatment stream) mg fungal biomass g AFDM^{-1}. During the nutrient enrichment, the mean concentration of fungal biomass was higher in the treatment stream (102 mg g AFDM^{-1}) than in the reference stream (26 mg g AFDM^{-1}) (paired *t*-test, $P < 0.0001$). Fungal biomass associated with the veneers increased 3.9 times following nutrient enrichment (RIA, $P < 0.0001$).

Microbial respiration associated with the veneers also increased after nutrient enrichment (Fig. 2b, RIA,

$P < 0.0001$). Before the enrichment began, the mean respiration rate associated with veneers was similar in the two streams (paired *t*-test, $P = 0.21$) averaging 0.037 mg O_2 g $AFDM^{-1}$ h^{-1} in the reference stream and 0.043 mg O_2 g $AFDM^{-1}$ h^{-1} in the treatment stream. During the 2-year enrichment period, the mean respiration rate associated with veneers in the treatment stream (0.200 mg O_2 g $AFDM^{-1}$ h^{-1}) was 3.9 times higher (paired *t*-test, $P < 0.001$) than that in the reference stream (0.052 mg O_2 g $AFDM^{-1}$ h^{-1}).

Seasonal maxima and minima were evident in both fungal biomass and respiration rate associated with veneers, particularly in the reference stream (Fig. 2), and appear to be because of seasonal temperature fluctuations. Similar fluctuations occurred in the amount of AFDM loss in the reference stream (Fig. 1a, as AFDM remaining) but appeared to lag somewhat behind fungal biomass and respiration rate. This may be due to the fact that veneers were colonised for approximately 90 days in the streams before measurements were made and weight loss represents an integration of activity over that time.

Decomposition rate (k) of maple sticks in the two streams was similar during the pretreatment year (ANCOVA, Tukey's test) with k in the reference stream of 0.00062 day^{-1} and k in the treatment stream of 0.00051 day^{-1} (Fig. 3a). During the nutrient addition, decomposition rate in the two streams differed (ANCOVA, Tukey's test, $P < 0.001$), increasing in the treatment stream (0.00138 day^{-1}) while decomposition in the reference stream remained slow (0.00051 day^{-1}). The N content of the maple sticks also followed the same pattern (Fig. 3b). Before enrichment, sticks in both streams exhibited similar N concentration (ANOVA, $P = 0.71$). During enrichment, N concentration in the sticks in the nutrient enriched stream was higher than that in the reference stream (ANOVA, $P < 0.001$).

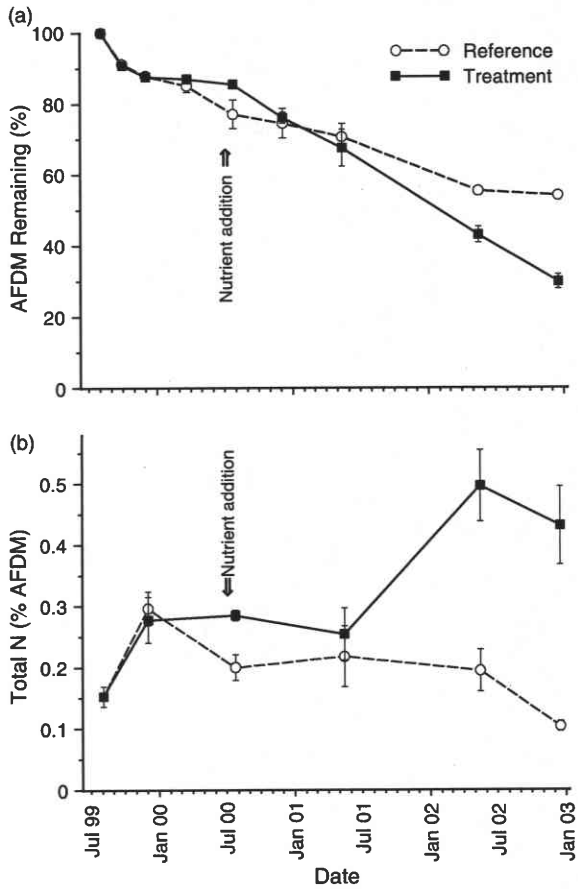

Fig. 3 (a) The percent AFDM remaining of maple sticks during decomposition over the 3-year study period, $n = 3$–6. (b) Total nitrogen as percent of the AFDM of maple sticks, $n = 3$. Vertical bars = ±SEM.

Discussion

Effects of nutrients on wood decomposition

Our results showed profound effects of experimental nutrient enrichment on the decomposition rate of wood. Experiments in laboratory microcosms (Aumen *et al.*, 1983; Melillo *et al.*, 1984; Aumen, Bottomley & Gregory, 1985) and correlative field studies (Golladay & Webster, 1988; Golladay & Sinsabaugh, 1991; Díez *et al.*, 2002; but see Wold & Hershey, 1999) have similarly shown effects of nutrients on woody substrates. Our study is the longest continuous running experimental enrichment of a heterotrophic system that we are aware of. Thus, the effects observed here may reveal more efficiently than previous studies the effects of nutrients on wood in streams, isolated from other confounding factors. Normally, an increase in nutrient concentration does not occur in isolation from other environmental changes (e.g. sedimentation), but this experiment illustrates the potential role of raised nutrients alone on wood-associated microbes and breakdown rate.

The breakdown rate of oak veneers in the absence of enrichment (mean 0.0011 day^{-1}) was similar to that (0.0015 day^{-1}) reported for a nearby stream at Coweeta by Tank & Webster (1998). In the present

study, the breakdown rate of veneers following nutrient enrichment increased to a mean of 0.0126 day^{-1}. Simon & Benfield (2001) also reported relatively high values for oak veneers (0.0040–0.0065 day^{-1}) under high nutrient conditions. We also showed that nutrient enrichment increased the decomposition rate of sticks, which allows for a more realistic extrapolation and prediction of nutrient effects on wood resources in streams. In the present study, maple sticks decomposed slower than veneers in the reference stream, as expected given the lower surface area to volume ratio of sticks in comparison to veneers and the presence of bark, which is thought to retard fungal colonisation (at least by aquatic hyphomycetes, Shearer & Webster, 1991; Gönczöl & Reváy, 1993). Breakdown rates of sticks (ca. 1–2 cm diameter) in the absence of enrichment (0.00051–0.00062 day^{-1}) were similar to previously reported values for sticks of 0.5–3 cm diameter decomposing in Coweeta streams (0.00031–0.00066 day^{-1}; Golladay & Webster, 1988; Webster *et al.*, 1999; Eggert & Wallace, 2003). Faster decay has been observed for smaller sticks (0.5 cm diameter) (0.00100–0.00115 day^{-1}, calculated from graph; Chergui & Pattee, 1991) and slower for slightly larger sticks (3 cm diameter) than used in our study (0.00015–0.00048 day^{-1}, Díez *et al.*, 2002). Obviously, the species of wood tested and nutrient concentrations in stream water will contribute to variation in decomposition rate, but the k value of 0.00138 day^{-1} observed here in response to enrichment is faster than any previous values reported for sticks of this general size. In our study, after 2 years of enrichment, bark was mostly lost from the maple sticks in the treatment stream but was still attached in the reference stream. Following loss of bark, we predict that breakdown rates would increase in response to nutrient addition, such that rates would be ultimately greater than we have found following 2 years of enrichment.

The response we observed was probably due primarily to microbial processes, as the effect on decomposition was consistent with effects on measures of microbial biomass and activity (Fig. 1a versus Fig. 1b and 2a,b). However, the increase in mass loss was 1.5 times greater than the increase in microbial responses to nutrient enrichment, suggesting that invertebrate feeding also contributed to mass loss. Invertebrate biomass and production increased in the treatment stream relative to the reference following nutrient addition (W. Cross, unpublished data) and greater invertebrate biomass may have contributed to greater mass loss of wood. However, we did not quantify invertebrate biomass or density on the wood surfaces in this study.

Effects of nutrients on microbial activity

The effect of nutrients was probably largely driven by fungal, rather than bacterial, response to nutrient enrichment. We have found a much greater response of fungi than bacteria to nutrient addition in our treatment stream based on monthly measurements of leaf-associated fungal and bacterial biomass and production (K. Suberkropp, unpublished data). These results are consistent with work showing that increased nutrients significantly increased microbial biomass and activity in other streams (Tank & Webster, 1998; Stelzer *et al.*, 2003; Tank & Dodds, 2003) and are also consistent with a presumed greater relative contribution of fungi versus bacteria to driving the response to enrichment (Stelzer *et al.*, 2003).

Fungi are considered to be the main decomposers of submerged wood. Golladay & Sinsabaugh (1991) and Sinsabaugh *et al.* (1992) have shown that activities of lignocellulose degrading enzymes extracted from submerged wood correlated with both fungal biomass and wood mass loss. Many aquatic hyphomycetes and ascomycetes have been reported to grow, reproduce, cause mass loss and possess a wide array of wood degrading enzymes (see reviews by Shearer, 1992, 1993; Wong *et al.*, 1998). Although we did not quantitatively examine the composition of the fungal communities in this study, we observed *Casaresia sphagnorum* Gonz. Frag., *Tricladium chaetocladium* Ingold, *Clavariopsis aquatica* De Wild., *Heliscina campanulata* Marvanová and an unidentified discomycete (Helotiales) to be common on wood veneers in this study.

Although fungal biomass and/or microbial respiration associated with decomposing wood has been estimated in a few studies (e.g. Golladay & Sinsabaugh, 1991; Tank, Webster & Benfield, 1993; Maharning & Bärlocher, 1996; Hendel & Marxsen, 2000; Díez *et al.*, 2002; Stelzer *et al.*, 2003), direct comparisons are often impossible because of different sizes of wood used and duration of experiments. Our estimates of fungal biomass from veneers in the absence of enrichment are similar to previous estimates from

similar oak veneers in Coweeta streams (Tank *et al.*, 1998; Tank & Webster, 1998). Fungal biomass associated with oak veneers in several cave streams, as reported by Simon & Benfield (2001), was as high as we found in the nutrient enriched stream, i.e. up to 170–180 mg g AFDM^{-1}. Nutrient enrichment (N + P) by means of nutrient-releasing substrata had no effect on fungal biomass associated with veneers in streams with relatively high ambient nutrient concentrations (Simon & Benfield, 2001) but stimulated fungal biomass accrual in low nutrient streams in Coweeta (Tank & Webster, 1998) and in six of 10 streams throughout North America (Tank & Dodds, 2003), which agrees with our results. Similarly, microbial respiration rates previously reported from veneers (Tank & Webster, 1998; Stelzer *et al.*, 2003) are similar to those found in our study and were stimulated by experimental nutrient enrichment. Microbial respiration reported by Simon & Benfield (2001) was two to three times higher than observed in our study, but both incubation temperature and nutrient concentrations were relatively high in their study; not surprisingly, nutrient addition did not result in increased respiration rates in six out of the seven streams they studied. Mean nitrogen content of veneers after about 90 days decomposition in our reference stream (0.27%) was comparable with values reported for woody substrates by other researchers (0.15–0.6%) (Sinsabaugh *et al.*, 1993; Díez *et al.*, 2002) and increased because of nutrient enrichment (1.15%). Mean C : N ratios of veneers after 90 days incubation and sticks by the end of the study were more than four times lower in the treatment versus reference stream. Such microbially mediated increases in N can change both biogeochemical cycling in the stream and food quality for invertebrates (Cross *et al.*, 2003).

The magnitude of the response of wood to nutrient addition

We compared nutrient enrichment effects on veneers and sticks (this study) to effects observed in a concurrent study on leaf breakdown (J. Greenwood, unpublished data) (Table 2). The effect of nutrient addition on wood processing and associated microbial activity in our study was greater than that for leaves over the same 2-year period (Table 2) and for leaf data reported previously from a single season (Gulis & Suberkropp, 2003). For veneers, the breakdown rate in the treatment stream during nutrient enrichment was on average 11 times higher than in the reference stream (note that mass loss after 90 days was 6.1 times higher; the discrepancy is because of the nonlinear nature of the k estimate). In contrast, breakdown rates of red maple leaves were 2.2 times greater in the treatment versus reference stream (Table 2) and were similarly affected in the study by Gulis & Suberkropp (2003) (1.8 and 3.7 times for red maple and rhododendron, respectively). Nitrogen immobilisation and changes in C : N ratio were also higher for wood than leaves following nutrient enrichment (Table 2). Fungal biomass associated with veneers in the nutrient enriched stream was four times greater than in the reference stream, whereas the maximum fungal biomass associated with leaves was 2.3–2.4 times that in the reference reach (Gulis & Suberkropp, 2003). Notably, even sticks were relatively more affected by nutrient enrichment than leaves (Table 2), despite a lower surface to volume

Table 2 Comparison of response of wood veneers, sticks and leaves to nutrient enrichment. Values are averaged over 2 years of enrichment. For veneers, decomposition rate k, nitrogen content and C : N ratio mean values are based on 23 samples per stream at the end of about 90 days incubation. For sticks, k based on one decomposition experiment per stream, nitrogen contents and C : N ratios are mean values for the final sampling date. For leaves (*Acer rubrum*) mean k based on two experiments (year 1 and 2 of enrichment) for each stream, nitrogen contents and C : N ratios are mean values on a comparable date in each year that corresponded to 70% leaf mass loss in the treatment stream (day 88 in year 1 and day 56 in year 2, $n = 5$ samples per stream per date) (J. Greenwood, unpublished data)

Parameter	Veneers	Sticks	Leaves
Carbon quality	low	low	high
Surface area to volume ratio	high	low	high
Initial nitrogen content (%)	0.13	0.15	0.48
Initial C : N ratio	373	321	104
Decomposition rate, reference stream (day^{-1})	0.0011	0.0005	0.0088
Decomposition rate, treatment stream (day^{-1})	0.0126	0.0014	0.0192
Magnitude of change (no. of times)	11	2.7	2.2
Nitrogen content, reference stream (%)	0.27	0.10	0.66
Nitrogen content, treatment stream (%)	1.15	0.43	1.08
Magnitude of change (no. of times)	4.3	4.2	1.6
C : N ratio, reference stream	228	452	74
C : N ratio, treatment stream	48	108	44
Magnitude of change (no. of times)	4.8	4.2	1.7

ratio and the presence of bark, which are barriers to microbial colonisation. Our results suggest that the magnitude of the response to nutrient enrichment may be driven in part by the initial nutrient content of the substrate, and not hampered by increased lignin content of wood versus leaves. In a study that was designed specifically to contrast effects of nutrients on leaves versus wood, Stelzer *et al.* (2003) also found that nutrient addition had the greatest effect on microbial respiration on substrates of the highest C : N.

The magnitude of the response of microbial assemblages and breakdown rates of veneers to nutrient enrichment was dramatic and surpasses most previous measures of biotic response, including autotrophs, grazers and microbial assemblages, to experimental nutrient additions in freshwater ecosystems [see meta-analyses by Brett & Goldman (1997); Francoeur (2001); Hillebrand (2002), and other studies on veneers by Stelzer *et al.* (2003) and Tank & Dodds (2003)]. We attribute this large response to several factors, some of which are particular characteristics of the substrate tested. As previously mentioned, veneers have a high C : N and surface to volume ratio and, thus, associated microbes have a great potential response to water column nutrients. The long-term nature of our study also probably contributed to the large response observed. Note that breakdown rates in year 2 were much greater than in year 1 of the study, indicating that maximum response was not observed until after at least 1 year of continuous enrichment of the whole stream reach.

Gessner & Chauvet (2002) advocate the use of leaf litter breakdown as an ecosystem-level process that provides insight into stream health. Similarly, wood breakdown might also be used to assess stream function, as suggested by Spänhoff & Meyer (2004). The consistent values reported for breakdown and the relatively large response to nutrient enrichment in our study suggest that veneers, as a standardised wood substrate, may be useful for determining heterotrophic response to increased nutrient concentrations and can serve as a functional assay tool.

Ecosystem-level consequences of nutrient effects on wood

Wood plays a critical role in ecosystem processes as both structure and a source of organic carbon. Accelerated breakdown of wood because of increased nutrient concentrations is also likely to have complex effects on stream function. These effects include positive effects of food quality for consumers via increased N content. Although we have not observed an increased dependence on wood by consumers in the first 2 years of nutrient enrichment, overall invertebrate production was much higher in the treatment stream and can be attributed to increased consumption of leaves, fungi and detritus of unknown origin (which may be partially derived from wood) (W. Cross, unpublished data). Leaf litter standing crop has declined in the treatment stream (K. Suberkropp, unpublished data), and previous studies have shown that, when leaf litter was reduced in a nearby stream, invertebrates consumed greater quantities of wood (S. Eggert, unpublished data). This may result in a greater use of wood by consumers as enrichment proceeds. Indirectly, faster disappearance and a lower standing crop of wood can, in turn, affect habitat complexity, retention and, consequently, decomposition rates of other forms of particulate organic matter and so alter food resources available to consumers. Our results indicate that increased nutrient mobilisation because of human activities may contribute to such effects.

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