Microbial Respiration on Decaying Leaves and Sticks

Along an Elevational Gradient

of a Southern Appalachian Stream.

by

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(ABSTRACT)

Microbial respiration on sticks and leaves, measured as oxygen uptake rate, was compared among four (2nd-4th order) sites along an elevational gradient at Coweeta Hydrologic Laboratory, North Carolina. Rhododendron and birch leaves were placed in mesh bags in the stream at each site on 21 Oct 90. Oxygen uptake rates were measured for both leaf species beginning 15 Dec 90 and continuing monthly until leaves were no longer intact (birch-2 months, rhododendron-7 months). Microbial oxygen uptake rates were also measured monthly for one year on qualitative collections of sticks (1-3 cm diameter) found in the stream at each site. Oxygen uptake rates (mg O_2/hr) were calculated on both a surface area and AFDM basis. Overall mean respiration rate per unit surface area was highest for sticks, followed by rhododendron, and birch. When expressed on an AFDM basis, respiration rates were highest for birch, followed by rhododendron, and sticks. Based on continuous measurements, mean monthly stream temperatures were significantly different among sites, but mean incubation temperatures were not significantly different among sites, Respiration rates were significantly correlated with temperature for both rhododendron leaves and sticks on both an AFDM and surface area basis. Respiration rates on rhododendron also increased with exposure time. Results indicate that differences in respiration rates among sites, for both

rhododendron leaves and sticks, can be explained primarily by changes in temperature. Additionally, the high respiration rates per unit surface area on sticks indicate that small woody debris may play a significant role as a substrate for microbial metabolism.

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Finally, thanks go to my family. Without you all behind me, this would have been impossible. Thanks Dad for your interest and encouragement, and for loving biology.

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Introduction

In this study I measured microbial respiration on leaves and sticks along an elevational and stream-size gradient. Microbial respiration in streams involves the conversion of organic carbon to carbon dioxide (mineralization) by bacteria and fungi colonizing organic matter. Because carbon is lost from the system and organic substrates are decomposed, microbial respiration is an important component in the energy dynamics of forested headwater streams. The magnitude of this process in streams of various sizes is not well known.

In streams, organic matter standing stocks are determined by upstream inputs, local riparian contributions, and instream production. Forested headwater streams are heavily shaded and depend primarily on riparian organic matter inputs to fuel stream processes (e.g., Minshall et al. 1983). Riparian inputs of leaves and wood are the major energy inputs into headwater streams and are broken down by both abiotic and biotic processes. Leaching (within the first 24- 48 hours) and physical fragmentation make up the abiotic components of organic matter breakdown. Decreases in standing stocks of organic matter are also due to microbial respiration, macroinvertebrate assimilation, and ultimately, downstream transport (Petersen et al. 1989). Microorganisms mediate the

biotic component of organic matter processing through metabolism, and their presence increases the palatability and nutritional quality of the substrate as a food source for macroinvertebrates (Cummins 1974). Microorganisms and macroinvertebrates represent the biotic component involved in organic matter breakdown. This study focuses on the role of microbial respiration in the breakdown of allochthonous organic matter in small mountain streams.

Initial stages of microbial colonization of organic matter are dominated by fungi, primarily aquatic hyphomycetes (Barlocher and Kendrick 1974, Suberkropp and Klug 1976). As fragmentation occurs and planar surfaces of material decrease, fungal biomass declines and bacterial abundances on decomposing leaves becomes more important (Suberkropp and Klug 1981). Increases in bacterial numbers during the latter stages of decay reflect increased surface area available for colonization as a results of fungal activity (Suberkropp and Klug 1976). Findlay and Arsuffi (1989) determined that fungi and bacteria play approximately equal roles in leaf decomposition in terms of carbon assimilation (mineralization). Despite lower standing stocks of bacteria in comparison to fungal standing stocks, bacterial production is similar to that of fungi because of high bacterial turnover times.

Various methods have been used to measure benthic respiration in streams. Respiration can be measured *in situ* using either open system or chamber methods. Open system gas change methods, measuring either O_2 or CO_2, are complicated by the difficulty in accurately estimating reaeration coefficients, especially in small, turbulent streams (Bott et al. 1985). However, gas change measured in chambers can be used in any stream system. Additionally, detailed analyses of chamber contents can be determined (i.e., surface area, AFDM, etc.) and metabolic variability within a reach (or substrate) can be analyzed. Finally, using a multiple chamber system allows for replication of measurements and can be used to determine heterogeneity within the stream

system (Bott et al. 1978). Bott et al. (1985) found no advantage (i.e., accuracy) of measuring changes in O_2 vs. CO_2 in chamber methods. A more direct method of measuring microbial activity may be to measure microbial biomass on organic matter, but no clear relationship between microbial biomass and metabolic rate has been determined. Dynamic population numbers make estimates in population useless as determinants of microbial activity, therefore oxygen consumption is a more useful way to compare microbial activity on different organic matter types (Hargrave 1972).

The four sites chosen for this study are located along an elevational gradient, and as elevation decreases, stream size, stream temperature and discharge increase, while retention of organic matter decreases (Vannote et al. 1980). Previous studies have demonstrated that both temperature and discharge affect microbial respiration rates. Also, microbial respiration varies with the type of organic matter.

The effect of temperature on respiration has been examined in several previous studies. Higher temperatures increase rates of organic matter breakdown by increasing microbial processes (Suberkropp and Klug 1974, Petersen and Cummins 1974). Respiration rate varies with season, and peaks in respiration occur at times of highest temperatures (summer) (McIntyre and Phinney 1965, Cuffney et al. 1990). Naiman (1983) determined that course particulate organic matter respiration in first through third order streams was best predicted by temperature. Conversely, organic matter content, and not temperature, was the best predictor for the respiration rates of sediments (Hedin 1990).

Benthic respiration along a stream-size gradient were studied within the framework of the River Continuum (Vannote et al. 1980). Bott et al. (1985) found that respiration differences in Michigan and Pennsylvania streams over a stream-size gradient were best explained by temperature. Other studies have shown that respiration per gram of organic matter does not change with increasing stream size (1st-5th order)(Naiman and Sedell 1980, Naiman 1983, Minshall et al. 1983). The magnitude of microbial respiration

on organic matter in streams has been studied primarily as a function of total bottom (benthic) respiration per m^2 of streambed (e.g. Naiman 1983). If estimates of microbial respiration on discrete substrates (leaves and wood) are applied to standing stocks of each organic matter type at each site, the relative contribution of organic matter type to total metabolism of the streambed can be estimated. Overall decreases in standing stocks of organic matter in a downstream direction result in overall decreases in microbial respiration rates per m^2 of streambed (Naiman 1983, Bott et al. 1985).

Microbial colonization and metabolism vary with organic matter quality, but studies on respiration in streams have primarily focused on relatively labile organic matter types (FPOM and leaves). Woody debris is also an important source of organic matter in forested headwater streams (e.g., Triska and Cromack 1980), and its abundance decreases in a downstream direction (Naiman and Sedell 1979). Wood breakdown rates are generally very slow as a result of high lignin and cellulose content and high carbon to nitrogen ratios (Harmon et al. 1986). Wood size also affects breakdown rates. Small woody debris (i.e., sticks) has a higher surface area to volume ratio and therefore breaks down more quickly than larger pieces (Golladay and Webster 1988). Because wood decomposes more slowly than leaves, studies of energy dynamics in streams have generally discounted the importance of wood decomposition (Triska and Cromack 1980). Slow breakdown rates of wood may mask the extent of microbial colonization of wood in streams. Recent studies have shown that the microbial colonization of wood is extensive (Aumen et al. 1983). Substrate stability has been emphasized as a major factor contributing to extensive biofilm development on wood when comparisons were made between leaves and wood (Golladay and Sinsabaugh 1991).

Microbial respiration and decomposition have both been shown to vary with organic matter type. Petersen and Cummins (1974) and others have shown a wide range of decomposition rates with various leaf types. Hedin (1991) found that overall, forested

streams have lower rates of benthic respiration than do lakes and marine systems because detritus in streams is primarily of terrestrial origin and is more refractory than the aquatic vascular plant and algal material of lake and marine systems. The birch leaves chosen for this study are quick decomposers, whereas broadleaf evergreens, like rhododendron, are slow decomposers possibly as a result of a thick waxy cuticle that is thought to slow microbial colonization. Birch leaves placed in the stream provide the opportunity to measure microbial respiratory activity on a labile substrate. Rhododendron provides a relatively stable substrate for colonization thereby offering the opportunity to measure microbial activity over a longer period of time, reflecting seasonal temperature and flow changes.

In this study I investigated microbial respiration of three substrates: labile leaves (birch); refractory leaves (rhododendron); and sticks. I then extrapolated respiration rate estimates to standing stocks of leaves and sticks in Coweeta streams. Finally, the effect of stream size, temperature, and flow differences were examined by measuring respiration over an elevational and stream-size gradient.

Methods

Site description

The stream system used in this study is located at Coweeta Hydrologic Laboratory, Macon County, North Carolina, USA. This Forest Service facility is a 2270-ha experimental forest located in the Nahantahala National Forest and is part of the Long-Term Ecological Research Program supported by National Science Foundation. The climate at Coweeta is characterized by high moisture and mild temperatures. Precipitation occurs primarily as frequent rains of low-intensity with little snowfall (Swank and Crossley 1988). Coweeta is located in the eastern part of the southern Appalachian Blue Ridge. The metasedimentary rocks of Coweeta consist of the Tallulah Falls Formation overlain by the Otto Formation, and the Coweeta Group. The Tallulah Falls Formation is dominated by feldspar and biotite rich metasandstones, as well as aluminous schists and mafic volcanic rocks. The Otto Formation is made up of quartz-rich two-mica feldspathic metasandstones with interlain aluminous schists. The Coweeta Group consists of quartz diorite gneiss, metasandstone, and pelitic schist (*in* Swank and Crossley

1988). The forest is dominated by oaks (*Quercus* spp.), red maple (*Acer rubrum*), tulip poplar (*Liriodendron tulipifera*), dogwood (*Cornus florida*), and rhododendron (*Rhododendron maximum*) (Swank and Crossley 1988).

Four 100m reaches were chosen along an elevational gradient from upper Ball Creek at the base of Watershed 27 (WS27), a second-order stream, and continuing down to Coweeta Creek (CC), a fourth-order stream formed by the confluence of Ball Creek and Shope Fork (Fig. 1). Upper Ball Creek (UBC) and Lower Ball Creek (LBC)(Fig. 1) constitute the middle elevational sites, respectively. Elevation ranged from 1070 m at the high elevation site (WS 27) to 675m at the low elevation site (CC). Stream gradient ranged from 0.16 m/m at Watershed 27 to 0.02 m/m at Coweeta Creek. Width, canopy, substrate, and discharge are described in Table 1.

Stream nutrients ($O\text{-}PO_4$, $NO_3\text{-}N$, $NH_4\text{-}N$) generally show no trend as one moves from high to low elevation sites. $O\text{-}PO_4$ remains at the minimum level of detection year round (0.001 mg/L). $NO_3\text{-}N$ levels are usually less than 0.05 mg/L. While $NO_3\text{-}N$ is generally lower at WS27 (high elevation site) than CC, in the autumn $NO_3\text{-}N$ drops at CC (low elevation site)(data from CHL). $NH_4\text{-}N$ is generally less than 0.005 mg/L throughout the year.

Organic matter inputs are primarily terrestrial in these heavily shaded mountain streams, and there is very little instream primary production (Webster et al. 1982). Huryn and Wallace (1987) found that mean standing crop of total benthic organic matter (BOM) at WS27 ranged from 401.9 gAFDM/m^2 in riffles to 1419.2 gAFDM/m^2 in pools. Wood (> 1 cm^2) comprised 52% of total BOM in riffles and 57% of total BOM in pools, while leaves were 8% of total BOM in riffles and 6% of total BOM in pools. Recent studies of BOM standing stocks in WS27 (E.F. Benfield and J.R. Webster, unpublished data) indicate that wood composes about 50% of total CBOM, but standing stocks of wood are much lower (85.94 gAFDM/m^2) than the previous estimate. Wood

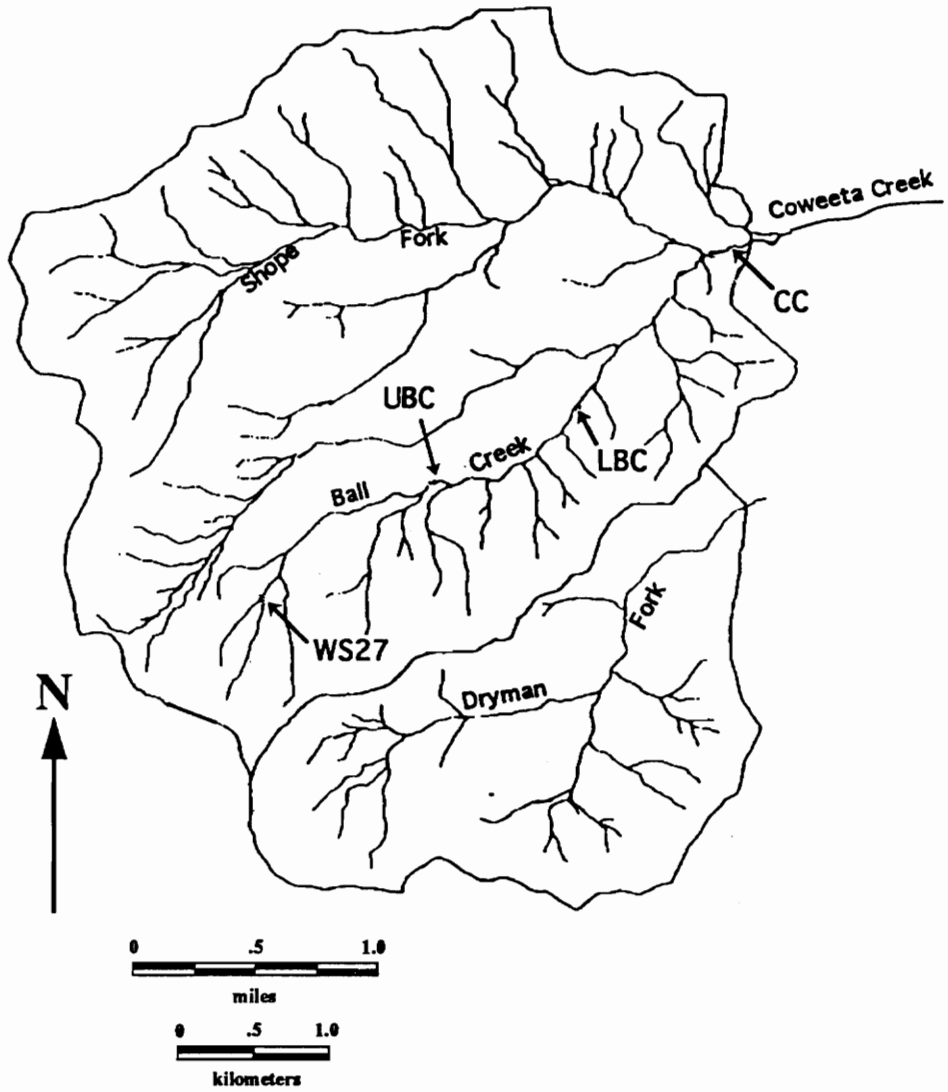

Figure 1. Map of Coweeta Basin: Study sites were located at Coweeta Hydrologic Laboratory, Macon Co., North Carolina. Four sites were located on Ball Creek- Coweeta Creek and are marked by arrows.

Table 1. Characteristics of study sites along Ball Creek- Coweeta Creek.

	WS27	UBC	LBC	CC
Stream Order[1]	2	3	3	4
Elevation (m)[1]	1070	922	721	675
Distance from source (m)[2]	950	2600	3850	5550
Gradient (m/m)[2]	0.16	0.17	0.04	0.02
Width (m)[2]	2-5	3-5	4-5	7-8
Canopy	heavy shade	heavy shade	medium shade	partially open canopy
Substrate	cobble, boulder	variable bedrock to sand	mostly large cobble	mostly large cobble
Mean Annual Discharge (L/s)[3]	20.5	96.8	148.8	590.4
Drainage Area (ha)[1]	38.8	206.8	380.5	1561.6

[1]From Coweeta 1: 7200 map or Coweeta Hydologic Laboratory (CHL) Forest Service files.

[2]From J. Webster et al., unpublished.

[3]From CHL Forest Service data. WS27 based on mean annual discharge in Shope Fork (1935-1991) and regression of WS27 data (1945-1991). UBC and LBC based on regression of data at wiers 20 and 15 (1938-1942) versus Shope Fork. CC based on drainage area and regression of discharge versus drainage area for sites along Ball Creek (Wiers 9, 15, 20, and 27). J. Webster, personal communication.

decreased at the downstream sites to a low of 1.26 gAFDM/m^2 at CC. Leaf standing stocks also decreased in a downstream direction, ranging from a high of 63.94 gAFDM/m^2 at WS27 to a low of 15.22 gAFDM/m^2 at CC.

Field Studies

Respiration rates, indicated by microbial oxygen uptake, were measured monthly at each of the four sites. Respiration rates were determined for two leaf types of known age, sweet birch (*Betula lenta*) and rhododendron (*Rhododendron maximum*), and a qualitative collection of sticks found in the stream. Rhododendron and birch leaves were collected just prior to abscission in Autumn 1990, air dried, and placed by species into litter bags (5mm mesh size). Bags were tied to string and placed in the stream at each of the four sites on 27 October 90. Microbial respiration on leaves was measured monthly at each site beginning 15 December 90 and continuing until leaf material was no longer intact (2 months for birch, 7 months for rhododendron). Small woody debris (sticks 1-3 cm diameter) was also collected from the stream at each site, and microbial respiration rates were determined monthly for 1 year. In December, January, and February, measurements were made at UBC because diel temperature differences at any one site were greater than differences among sites.

After removing invertebrates and excess silt, either leaves or sticks were placed in respiration chambers and oxygen change was measured over a 3-5 hour incubation. Respiration chambers were constructed from opaque PVC pipe (23 cm diameter, 30.5 cm length, 1.6 liter volume) equipped with recirculating pumps and YSI Model 5739 polarigraphic oxygen probes. Each dissolved oxygen probe was connected to a multi-

channel signal conditioner that determined oxygen concentration (Fig. 2) (see appendix for detailed description of method). Six respiration chambers were run in parallel for each incubation using 2-3 replicates per organic matter type. An empty chamber was included with each incubation to determine if the probe consumed a significant amount of oxygen or if respiration by fine particulate organic matter in the water column was significant. Dissolved oxygen concentrations for each of the six chambers were determined every 10 seconds, averaged over 2 minute periods, and recorded on tape by a Campbell 21x datalogger.

Continuous stream temperature was recorded during incubations using a Radio Shack thermistor connected to the datalogger. Additionally, stream temperature was recorded continuously (every 2 hours) from December 1990 to December 1991 at the two middle elevation sites, Upper Ball Creek (UBC) and Lower Ball Creek (LBC), using Ryan recording thermographs (J.B. Wallace, UGA, unpublished data). Watershed 27 (WS27) and Coweeta Creek (CC) had thermographs for the period June 1990 to December 1991. Missing temperature data was extrapolated by regressing available temperature data for WS27 to UBC data, and CC to LBC data ($r^2 = \simeq 1.0$ for both.)

Laboratory Analyses

Dissolved oxygen concentrations recorded for each chamber during the incubation were retrieved from the tape and exported to the computer. Oxygen decreased linearly over time, and the rate of oxygen consumption was determined as the change in oxygen concentration (mg/l) over time (minutes).

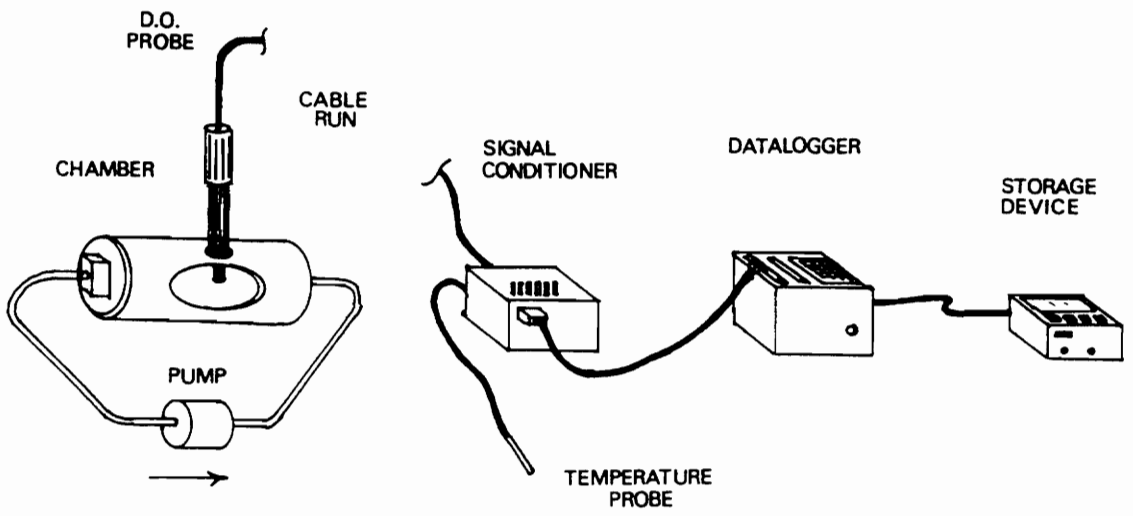

Figure 2. Field apparatus for measuring respiration rates on organic matter in streams: The chamber, dissolved oxygen probe, and temperature probe were placed in the stream. The signal conditioner, datalogger, and storage device were located on the bank.

After each incubation, leaves were returned to the lab, and a penetrometer was used to estimate leaf conditioning or softness (Feeney 1970, Suberkropp and Klug 1981). Penetrance was determined as the weight needed to push a 5 mm diameter rod through the leaf matrix. Eight measurements of penetrance were taken for each chamber of leaves incubated. A digitizer was used to measure total leaf surface area (m^2) per chamber using xerox copies of leaves. Stick surface area was estimated from length and diameter measurements. Organic matter from each chamber was oven dried (48 hrs for leaves, 1 week for sticks) at 50 °C and weighed. Leaf and stick samples were ground using a Wiley mill and subsamples were ashed for 35 minutes at 550 °C, re-wetted to restore the weight of hydration, and re-weighed to determine total ash free dry mass (AFDM) for each chamber. Respiration rates per chamber were expressed on an AFDM or surface area basis.

Additional incubations were run on yellow poplar (*Liriodendron tulipifera*) and white pine (*Pinus strobus*) sticks placed in streams draining a hardwood and a white pine forest 37 months and 33 months previously. Respiration rates for these sticks of known age and species were compared to the random qualitative collections of sticks made monthly at each site. In addition, at each site, selective collections of qualitatively determined "old" and "new" sticks were separated for incubations. I separated "old" and "new" sticks by appearance of decay (i.e. bark cover, grooving, softness etc.). I then compared these respiration rates with the random collections of sticks used in the monthly determinations of respiration rate.

Statistical Analyses

Respiration data for leaves was analyzed using a multiple factor ANOVA with site, species, conditioning time (in days), temperature, and penetrance values (g) as independent variables. Stick respiration data was analyzed using a 2 factor ANOVA with site and temperature as independent variables. Regressions for respiration versus temperature were determined for birch, rhododendron, and sticks. A multiple regression was also run for leaf respiration versus days and temperature. Q_{10}'s were determined for each leaf species and sticks over the entire year using the slope of the regressions of log respiration rate vs. temperature. Residuals of the regression of respiration rate vs. temperature were used to determine site differences independent of temperature differences at each site. All statistical analyses were run using SAS (SAS 1991).

Results

Differences in microbial respiration among species

Birch leaves lasted about 77 days in the stream before becoming skeletonized, and I was able to complete only two monthly respiration runs. The highest and lowest respiration rates for birch were found in December (day 49 of incubation). When respiration rate was expressed per unit surface area, the highest rate observed for a chamber was 1.83 mg $O_2/m^2/h$ and the lowest was 0.95 mg $O_2/m^2/h$. The overall substrate mean for all chambers and sites for the two months of incubations was 1.23 mg $O_2/m^2/h$ (Table 2). Individual site means ranged from 1.01 to 1.48 mg $O_2/m^2/h$. When respiration rate was expressed on the basis of total gAFDM per chamber, the high and low rates were 0.17 and 0.08 mg O_2/gAFDM/h, both observed during December runs. The overall substrate mean was 0.12 mg O_2/gAFDM/h, and overall site means ranged from 0.10 to 0.14 mgO_2/gAFDM/h.

Table 2. Mean respiration rates for each organic matter type in streams at Coweeta.

		mg $O_2/m^2/h$	mg O_2/gAFDM/h
Birch (2 mo)[1]	WS27	1.48	0.14
	UBC	1.01	0.10
	LBC	1.31	0.13
	CC	1.04	0.10
	Mean[4]	1.23	0.12
Rhododendron (7 mo)[2]	WS27	4.44	0.08
	UBC	3.58	0.07
	LBC	5.06	0.10
	CC	4.64	0.10
	Mean[4]	4.72	0.09
Sticks (12 mo)[3]	WS27	11.84	0.01
	UBC	11.47	0.01
	LBC	11.09	0.01
	CC	15.58	0.02
	Mean[4]	12.52	0.01

Respiration is expressed per unit surface area and per gAFDM.
[1]Incubation temperature range= 6.6-9.9 °C.
[2]Incubation temperature range= 4.6-16.7 °C.
[3]Incubation temperature range= 4.6-18.4 °C.
[4]Mean is based on all samples for that substrate type.

Rhododendron leaves were incubated in the stream for a total of 243 days (7 monthly runs) before advanced decomposition made it impossible to measure respiration. The highest respiration rate for rhododendron was measured in June, the last month of incubation (day 243), while lowest respiration was measured in March. When respiration rate was expressed per unit surface area, the highest rate observed for a chamber was 6.84 mg $O_2/m^2/h$ and the lowest was 1.87 mg $O_2/m^2/h$. The overall substrate mean for all chambers and sites for the seven months of incubations was 4.42 mg $O_2/m^2/h$ (Table 2). Individual site means ranged from 3.58 to 5.06 mg $O_2/m^2/h$. When respiration rate was expressed on the basis of total gAFDM per chamber the high and low rates were 0.16 and 0.03 mg O_2/gAFDM/h in June and March, respectively. The overall substrate mean was 0.09 mg O_2/gAFDM/h, and overall site means ranged from 0.07 to 0.10 mgO_2/gAFDM/h.

Stick respiration was measured monthly for 12 months using qualitative collections of small woody debris (sticks) found in the stream at each site. For sticks, the highest respiration rate was measured in August for surface area (28.91 mg $O_2/m^2/h$), while the lowest respiration rate was measured in January (2.93 mg O_2/gAFDM/h). The overall substrate mean for all chambers and sites for the 12 months of incubations was 12.52 mg $O_2/m^2/h$ (Table 2). Individual site means ranged from 11.09 to 15.58 mg $O_2/m^2/h$. When respiration rate is expressed on the basis of total gAFDM per chamber, the high and low rates were 0.03 and 0.01 mg O_2/gAFDM/h in May and January, respectively. The overall substrate mean was 0.01 mg O_2/gAFDM/h, and overall site means ranged from 0.01 to 0.02 mgO_2/gAFDM/h.

When microbial respiration was expressed on the basis of AFDM/chamber, both leaf types showed respiration rates in the same range-- close to 0.1 mg O_2/gAFDM/h. When expressed on a surface area basis, microbial respiration rates for rhododendron were two to five times higher than those for birch leaves. Overall, respiration expressed

per unit surface area varied over a larger range for all organic matter types than did respiration per AFDM. Microbial respiration rates for sticks per unit surface area were ten times greater than those for birch leaves and three times greater than rates for rhododendron (Fig. 3). When expressed on a AFDM basis, the trend in respiration reversed: birch leaves had the highest rate of respiration, followed by rhododendron and then sticks (Fig. 4). Means of respiration rates may indicate differences between birch, rhododendron, and sticks but it is important to note that overall incubation temperature means were different for each substrate type. Birch leaves were run only in midwinter (temperature $\bar{x}$= 9.2 °C); rhododendron leaves were run in winter, spring, and early summer (temperature $\bar{x}$= 10.9 °C); and sticks were run monthly for a year (temperature $\bar{x}$= 12.5 °C). Analysis of variance (ANOVA) followed by least squares means (LSM) comparisons indicated that for respiration per AFDM and per unit surface area, the means for rhododendron, birch, and sticks were significantly different, when adjusted for temperature ($p < .05$).

Relationship of microbial respiration to temperature

Analysis of the continuous temperature data indicated that mean daily stream temperatures varied with site and month, and that month accounted for most of the variability (ANOVA, $p < .0001$). Duncan's multiple range test indicated that mean daily stream temperatures were significantly different among sites, ranging from a low at WS27 (10.85 °C) and a high at CC (11.88 °C)(Table 3). Monthly high temperatures occurred in July, while low temperatures occurred in February (Fig. 5).

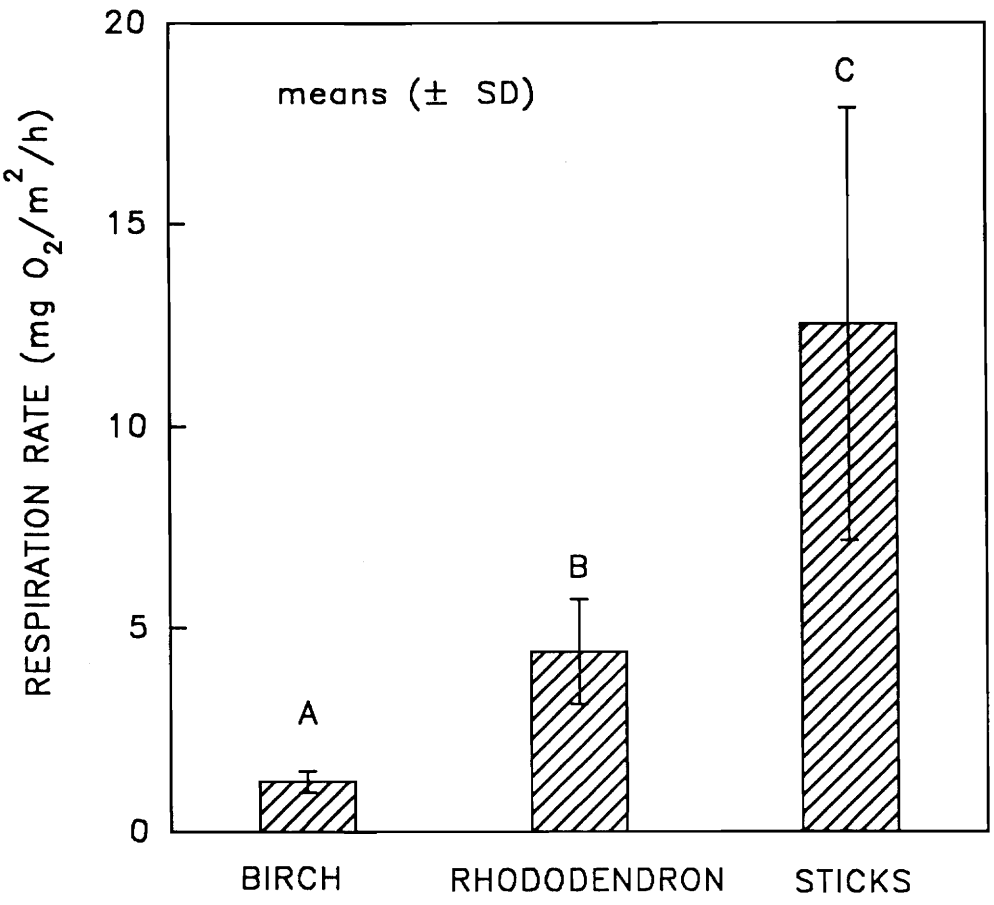

Figure 3. Respiration rate per unit surface area plotted by substrate type: Mean respiration rates are expressed in mg $O_2/m^2/h$. Significant differences between substrates were determined by ANOVA, LSM ($p < .0001$) accounting for temperature and site as factors. Substrate types with the same letter were not significantly different.

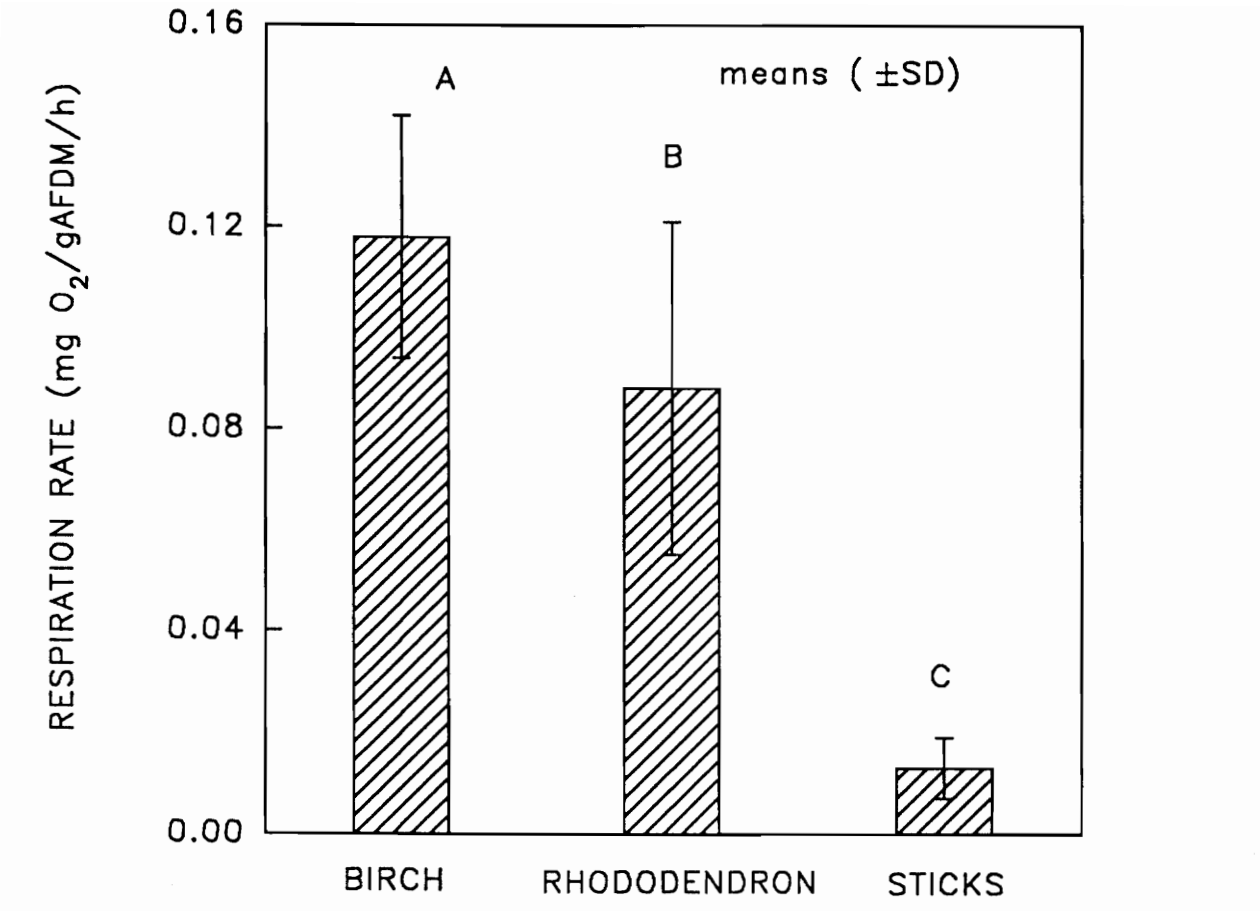

Figure 4. Respiration rate per gAFDM plotted by substrate type: Mean respiration rates are expressed in mg O_2/gAFDM/h. Significant differences between substrates were determined by ANOVA, LSM ($p < .05$) accounting for temperature and site as factors. Substrate types with the same letter were not significantly different.

Table 3. Summary of continuous temperature data

Site	Mean Annual Temperature (°C)	
WS27	10.85	A
UBC	11.19	B
LBC	11.50	C
CC	11.88	D

Data are expressed as mean annual stream temperature for each site. Means with different letters were significantly different.

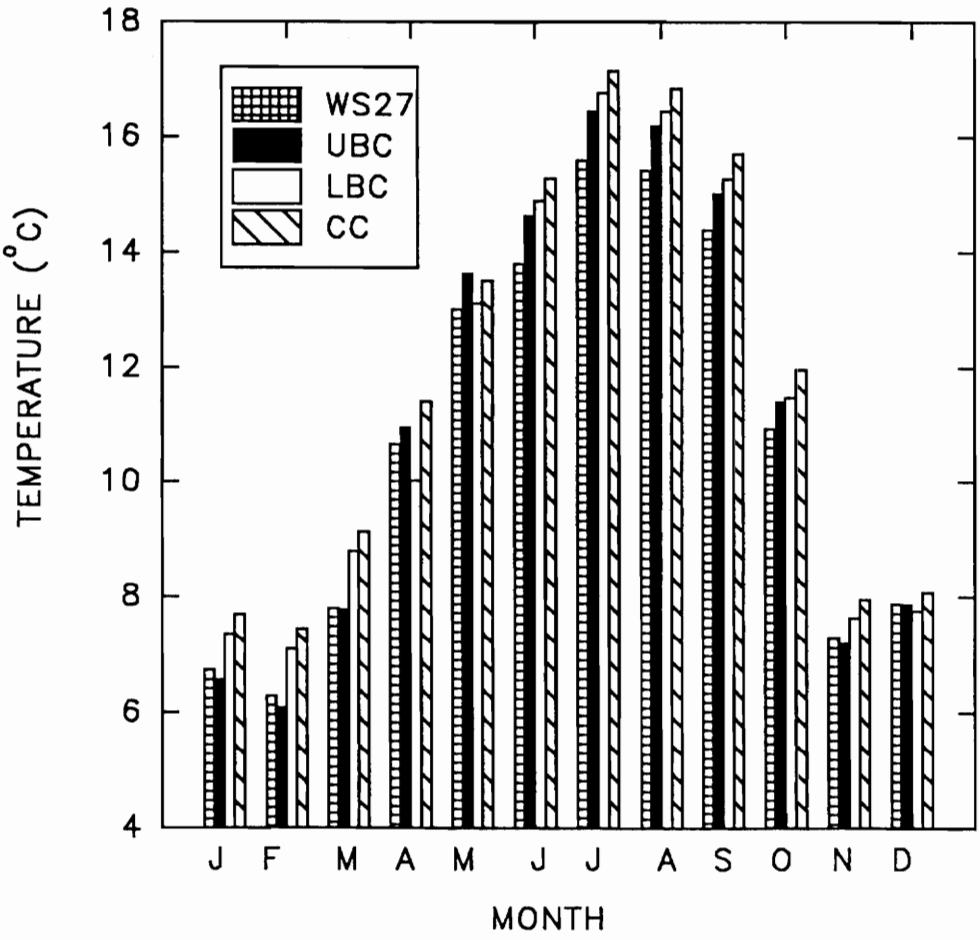

Figure 5. Monthly stream temperatures plotted by site: Mean monthly stream temperatures were calculated from continuous temperature data from November 1990- October 1991 (J.B. Wallace, UGA, unpublished data).

Stream temperature data collected during respiration incubations varied significantly with both site and date of incubation (ANOVA, $p < .0001$). Date was responsible for most of the incubation temperature variability. WS 27, at the highest elevation, had the lowest mean incubation temperature for the 12 months of data collection (11.7 °C), and the other sites were clustered around a mean of 12.5 °C (Fig. 6). The mean incubation temperature at WS 27 was significantly different from all the other sites (ANOVA, LSM, $p < .0001$).

Microbial respiration increased with increasing incubation temperature for both birch and rhododendron leaves, as well as for sticks. The regressions of respiration per unit surface area (m^2) for sticks ($r^2 = .62$, $N = 98$, $p < .01$) and rhododendron ($r^2 = .43$, $N = 63$, $p < .01$) were significantly different from zero (Fig. 7). The regression for birch leaves was not significant, but the temperature range over which birch leaves were incubated was very small. The regressions of microbial respiration per AFDM for rhododendron ($r^2 = .59$, $N = 63$, $p < .01$) and sticks ($r^2 = .59$, $N = 98$, $p < .01$) were significant, but the regression for birch was not (Fig. 8). Slopes for the relationships between respiration rate and temperature were different between rhododendron leaves and sticks for respiration expressed both on a surface area and AFDM basis (ANCOVA, $p < .0001$)(Fig. 8).

The relationship of metabolism (as represented by respiration rate) to temperature is often represented by a Q_{10} function:

$$R = R_0 \, Q_{10}^{(T - T_0)/10}$$

where R is respiration rate and T is temperature (°C). The equation can be modified to solve for Q using a linear regression of ln(respiration) vs. temperature:

$$lnR = \ln(R_0 \, Q_{10}^{T_0/10}) + T/10 \, lnQ$$

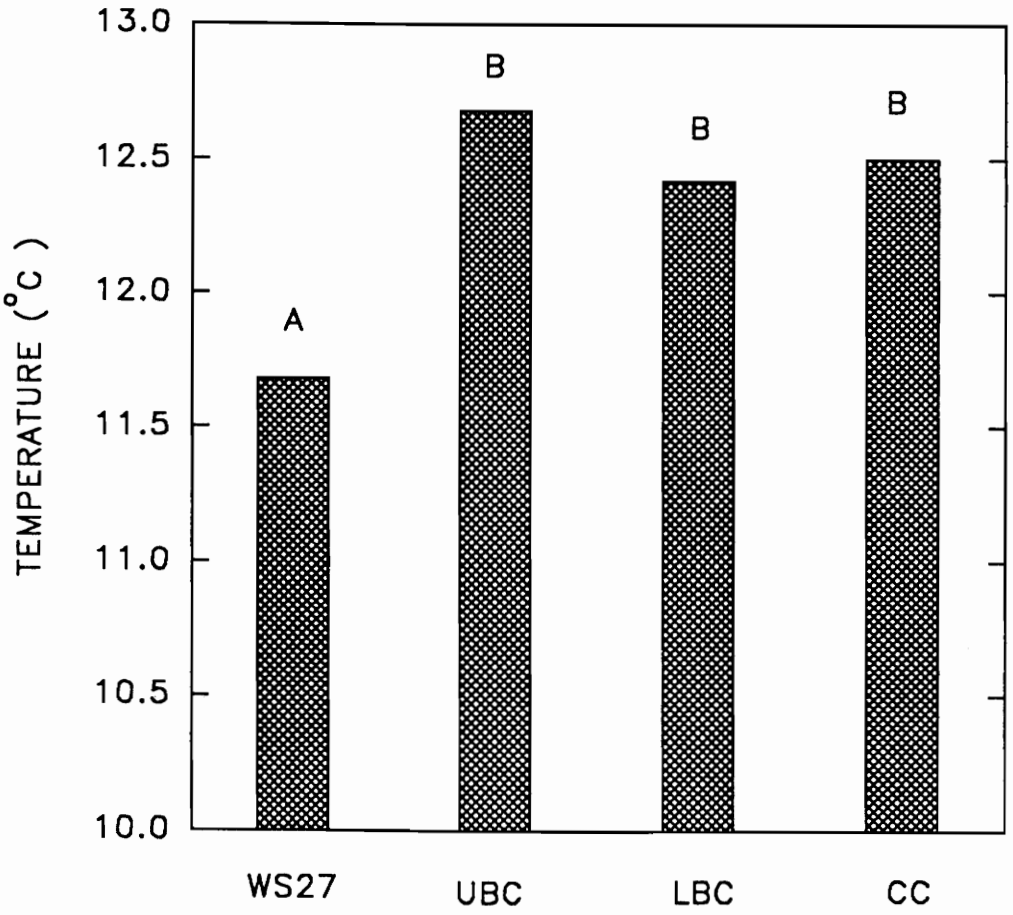

Figure 6. Mean annual incubation temperature for each site: Mean incubation temperatures were determined for 12 months of temperature data recorded at the time of incubation. Differences in sites were determined by ANOVA, LSM ($p < .0001$). Sites with the same letter were not significantly different.

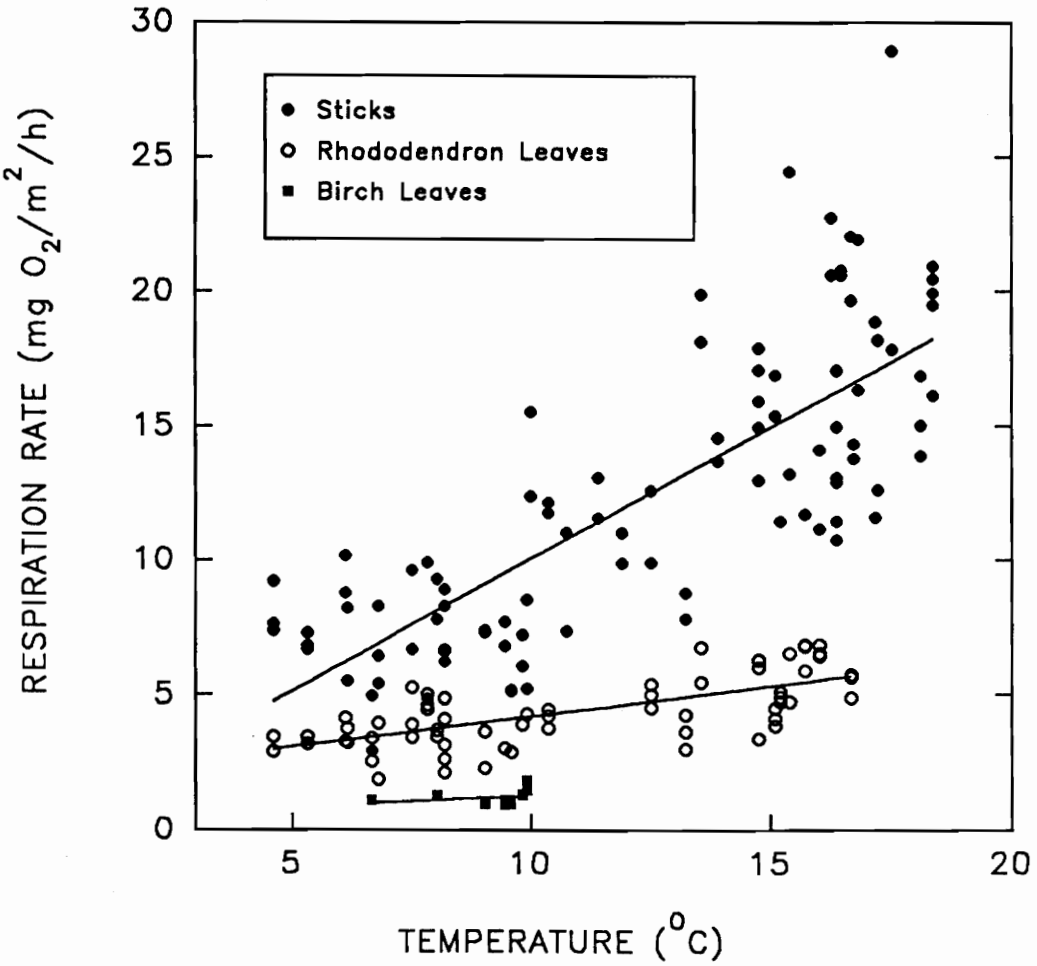

Figure 7. Respiration per unit surface area vs. incubation temperature plotted by substrate type: The regressions of respiration rate vs. temperature for sticks ($r^2 = .62$, $N = 98$, $p < .01$) and rhododendron ($r^2 = .43$, $N = 63$, $p < .01$) were significantly different from zero, but the regression for birch was not.

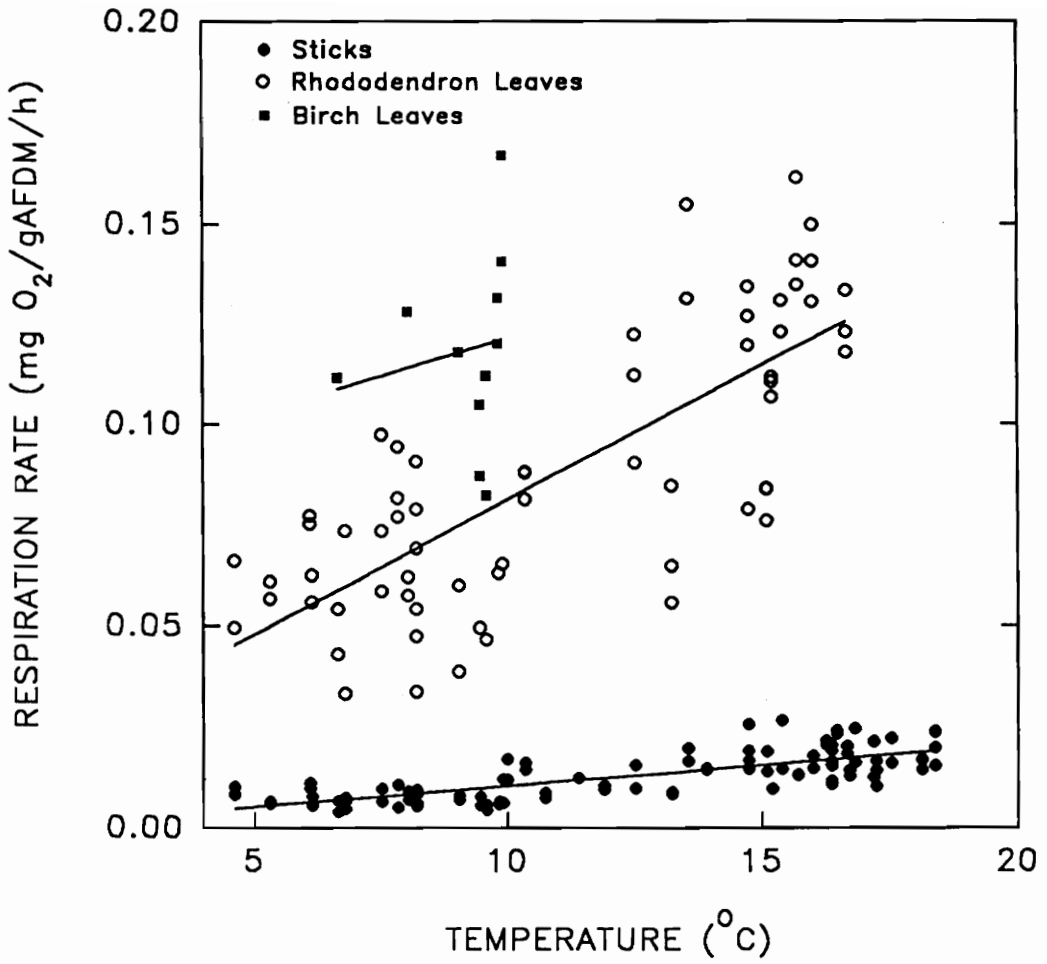

Figure 8. Respiration per gAFDM vs. incubation temperature plotted by substrate type: The regressions of respiration rate vs. temperature for sticks ($r^2 = .59$, $N = 98$, $p < .01$) and rhododendron ($r^2 = .59$, $N = 63$, $p < .01$) were significantly different from zero, but the regression for birch was not.

where lnR is the y intercept and lnQ/10 is the slope of the function lnR = f(T). We can then determine the Q_{10} value using the equation

$$Q_{10} = e^{(10 \times slope)}$$

Q_{10}'s for both rhododendron leaves and sticks were determined in this way. Regressions using birch leaves were not significant. When expressed in terms of surface area, the Q_{10} was 1.7 for rhododendron the Q_{10} was 2.4 for sticks. On an AFDM basis, rhododendron had a Q_{10} of 2.2, and sticks had a Q_{10} of 2.4. For Q_{10}'s determined using respiration data expressed per unit surface area, the value for rhododendron was significantly different than the Q_{10} for sticks (ANCOVA, $p = .001$). Using respiration data expressed per AFDM, Q_{10}'s for rhododendron and sticks were not significantly different (ANCOVA).

Microbial respiration on rhododendron leaves related to exposure time in streams

Microbial respiration rates on leaves increased with stream exposure time for respiration expressed per unit surface area and per unit AFDM (Fig. 9, Fig. 10). Regressions for rhododendron respiration rate per unit surface area ($r^2 = .39$, $N = 63$, $p < .01$) and per AFDM ($r^2 = .58$, $N = 63$, $p < .01$) were significant from zero. Regressions for birch were not significant.

From Figures 9 and 10, it is evident that temperature changed during the time leaves were being conditioned. Depending on time of year, microbial respiration rates

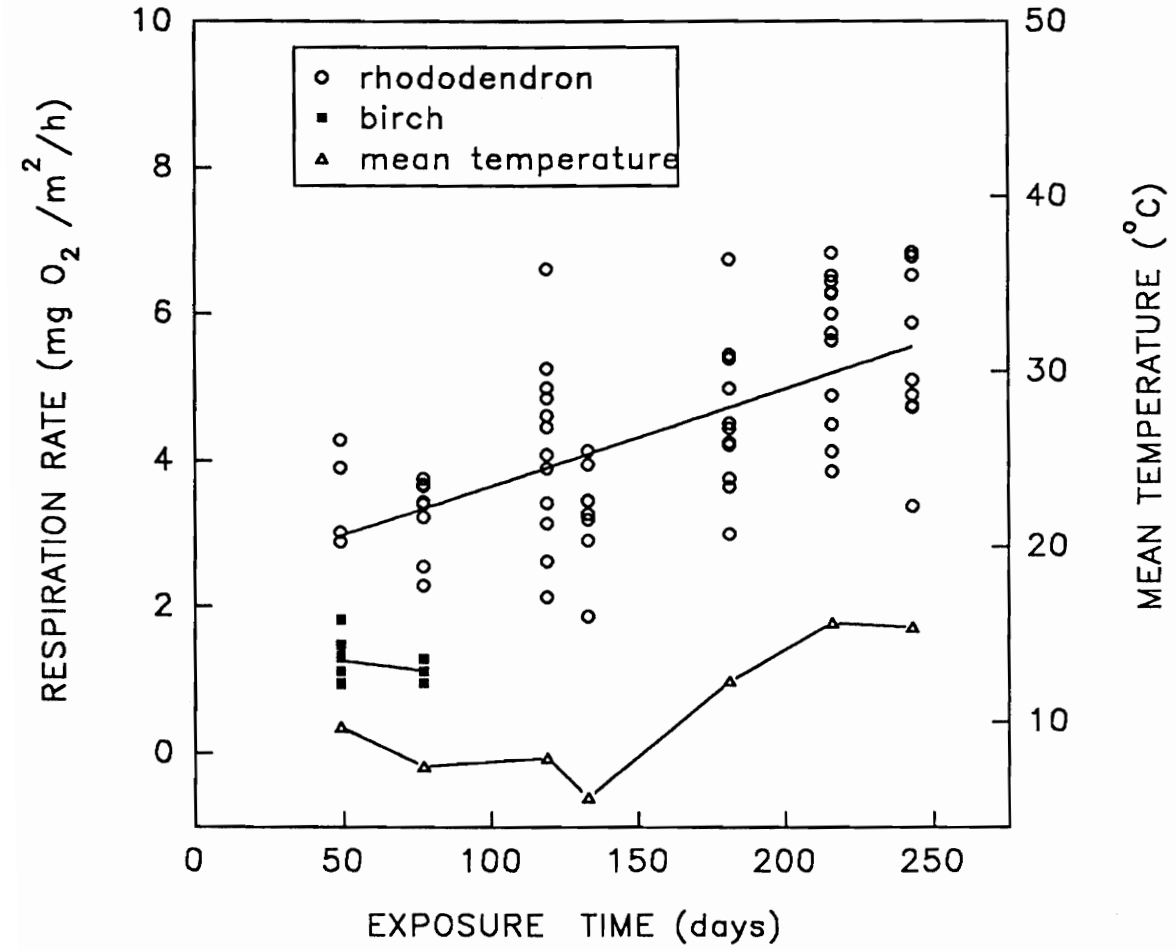

Figure 9. **Rhododendron respiration rate per unit surface area as a function of exposure time:** Incubation temperature is also plotted on the Y axis. The regression of respiration rate vs. exposure time was significantly different from zero ($r^2 = .39$, $N = 63$, $p < .01$). The birch regression was not significant.

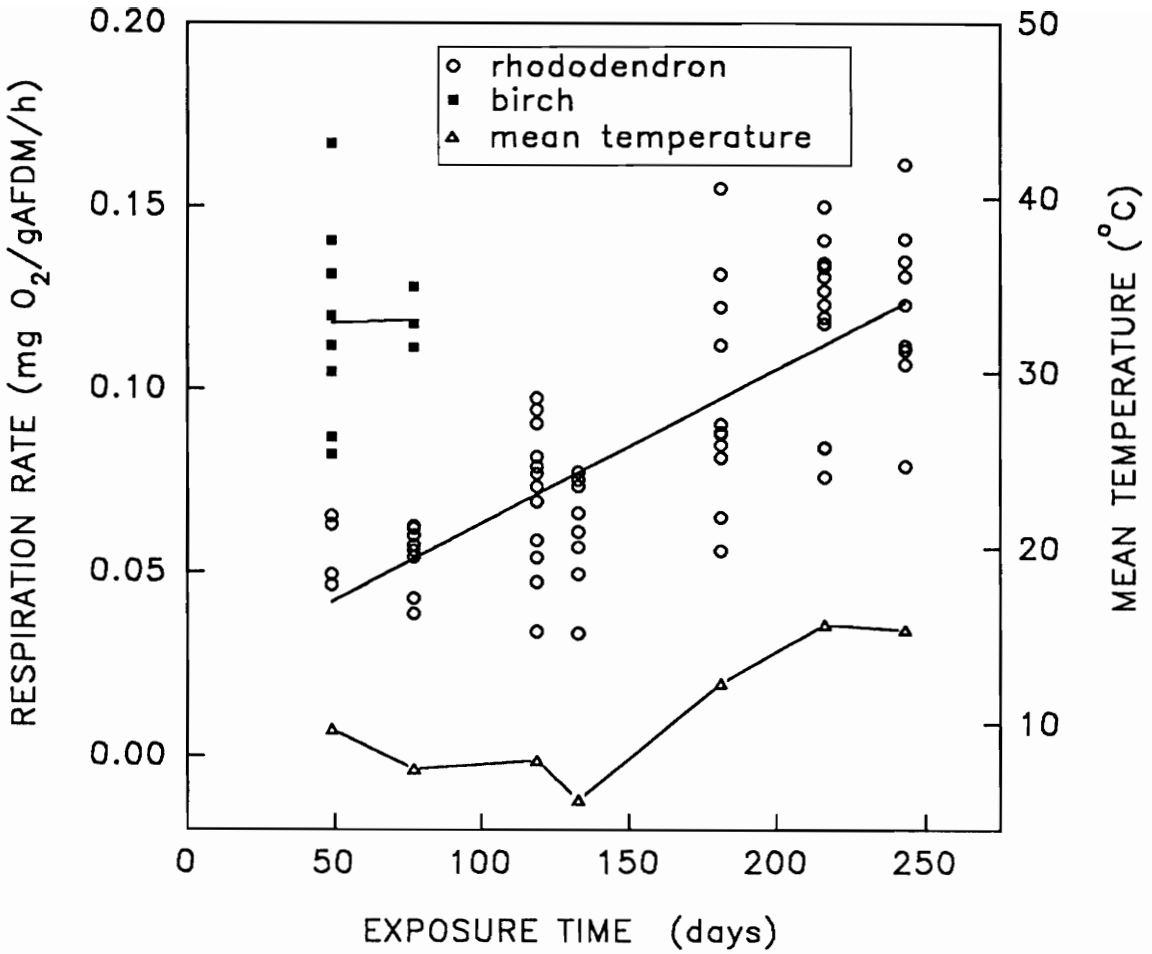

Figure 10. Rhododendron respiration rate per gAFDM as a function of exposure time: Incubation temperature is also plotted on the Y axis. The regression of respiration rate vs. exposure time was significantly different from zero ($r^2 = .58$, $N = 63$, $p < .01$). The birch regression was not significant.

on rhododendron responded differently to the combined effects of temperature and exposure time. For exposure time less than day 134 (December through March), incubation time was increasing but stream temperature was still decreasing, reaching a low in March. When I included only data from day 0-134, the regressions of rhododendron respiration expressed per unit surface area vs. exposure time and temperature, alone or combined were not significant. Conversly, for respiration expressed per AFDM, before day 134, the regression of rhododendron respiration vs. exposure time was significant ($r^2 = .20$, $N = 31$). The multiple regression of respiration vs. exposure time and temperature ($r^2 = .20$, $N = 31$) was also significant, while the regression of rhododendron respiration vs. temperature alone was not significant. For the multiple regression, exposure time explained most of the variability in rhododendron respiration rate.

Both exposure time and stream temperature were increasing for exposure time after day 134 (April through June). For rhododendron respiration expressed per unit surface area, the linear regression of respiration rate vs. temperature was significant ($r^2 = .23$, $N = 31$), as was the multiple regression of respiration vs. exposure time and temperature ($r^2 = .23$, $N = 31$). The regression of rhododendron respiration rate vs. exposure time alone was not significant. For the multiple regression, temperature explained most of the variability in rhododendron respiration. For respiration expressed per AFDM, linear regressions of respiration vs. exposure time and respiration vs. temperature were significant ($r^2 = .14$, $r^2 = .25$, $N = 31$, respectively). Additionally, the multiple regression of rhododendron respiration vs. exposure time and temperature was significant ($r^2 = .25$. $N = 31$). For the multiple regression of respiration/AFDM, temperature explained a significant amount of the variability in respiration. To summarize, before day 134, respiration rate increased with incubation time, despite declining temperatures, and variability in respiration rates was best explained by incubation time. After day 134, variability in respiration rates was best explained by stream temperature.

Leaf softness was measured as an indicator of microbial conditioning. Log of the mean penetrance per chamber (8 replicate leaves) for rhododendron decreased with exposure time ($r^2 = .77$, $N = 63$, $p < .0001$)(Fig. 11). Softness increased with rhododendron exposure time and therefore weight needed for penetrance decreased. Additionally, the coefficient of variation increased with exposure time in the stream, illustrating the patchy nature of decomposition (Fig. 12). The regression for coefficient of variation (CV) of penetrance for rhododendron vs. exposure time was significant ($r^2 = .43$, $N = 63$, $p < .01$).

Regressions and correlations of variables affecting leaf decomposition

Regressions of temperature, leaf exposure time, and penetrance vs. rhododendron respiration rates (both per AFDM, per m^2) were all significant (Table 4). Microbial respiration on rhododendron leaves expressed per unit surface area or AFDM, was related most strongly to temperature ($r^2 = .44$, $r^2 = .59$, respectively), but the regressions were not significantly different from the relationships to exposure time, and days. For respiration/AFDM, exposure time was equally related to respiration rate as temperature ($r^2 = .58$)(Table 4). It is not suprising that all regressions were significantly different from zero, and not significantly different from each other, because Pearson correlation coefficients for temperature, incubation time, and penetrance demonstrated that all three variables were correlated (Table 5). Temperature and incubation time were most strongly correlated despite the fact that temperature was decreasing during the first four months of incubations (Dec-Mar) ($R = .77$, $N = 75$).

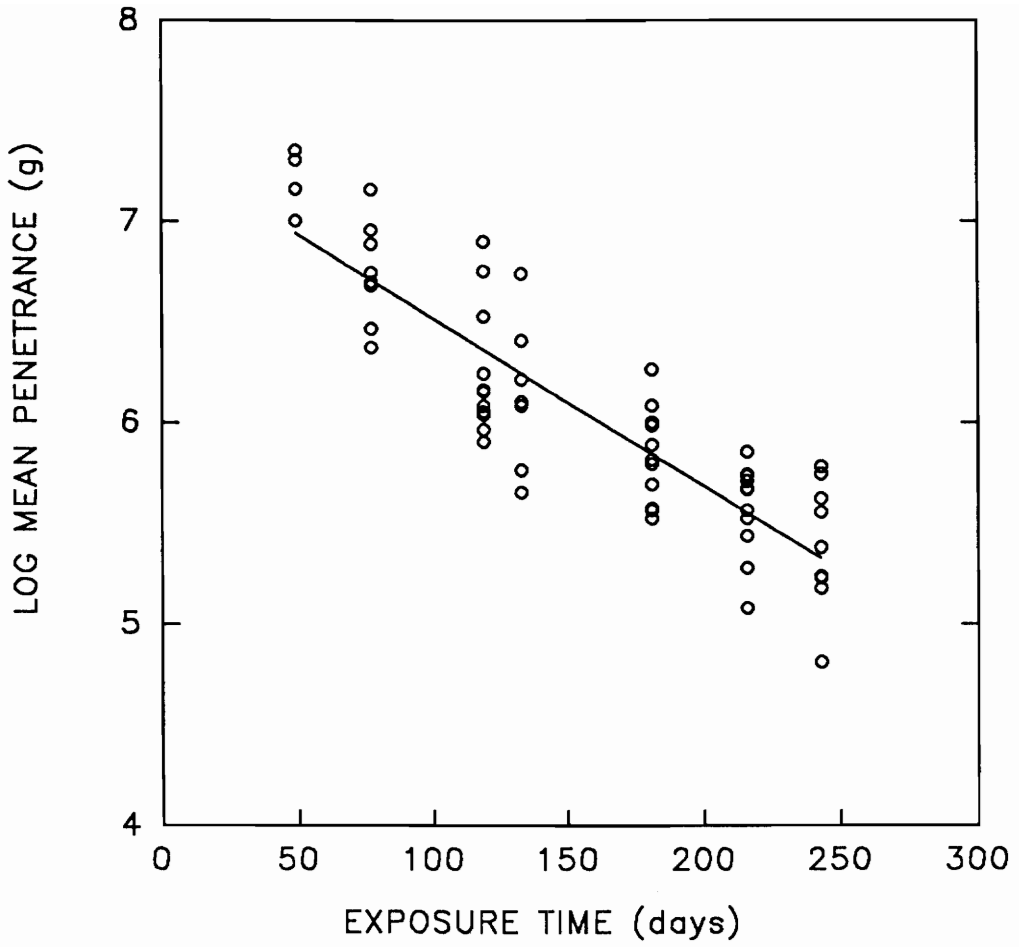

Figure 11. Penetrance for rhododendron leaves as a function of exposure time: Penetrance expressed as g weight to penetrate the leaves. Log of mean penetrance (from 8 leaves per chamber) was plotted against leaf exposure time. The regression of log mean penetrance vs. exposure time was significantly different from zero ($r^2 = .77$, $N = 63$, $p < .0001$).

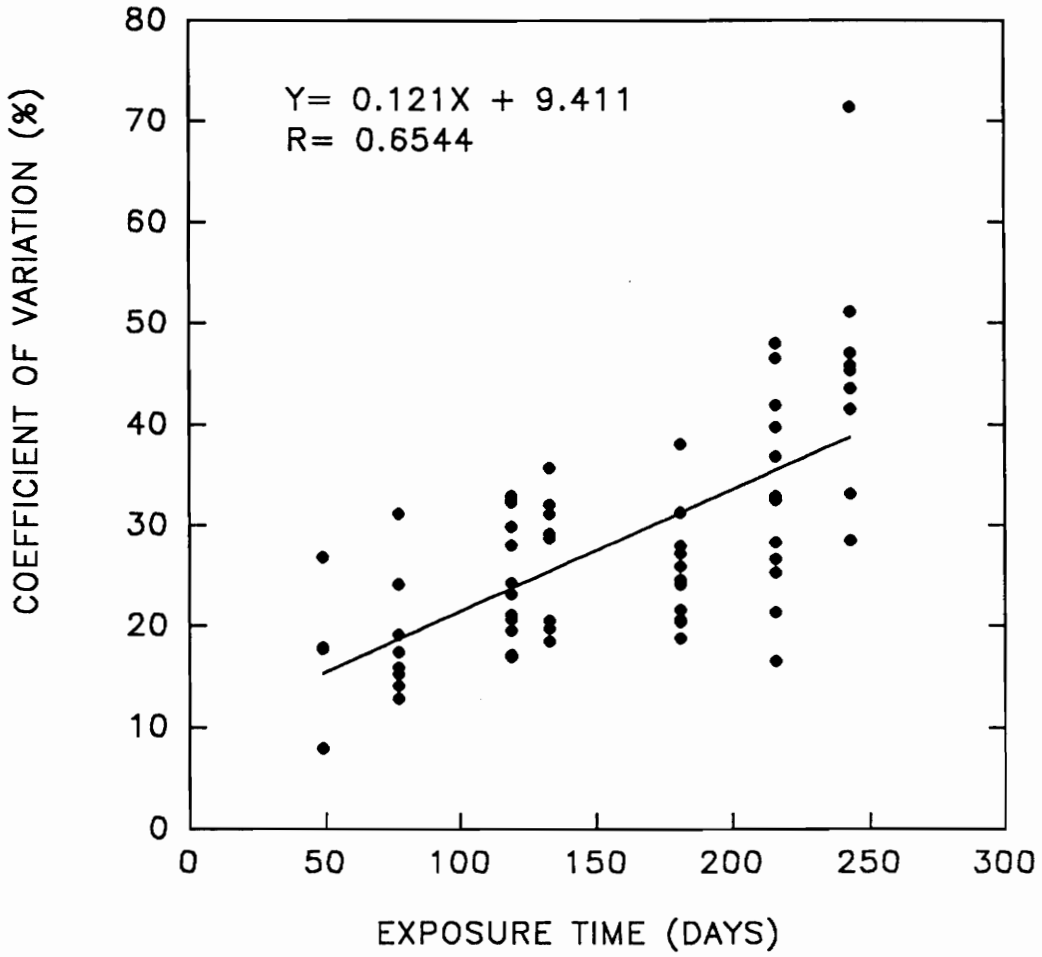

Figure 12. Variability in penetrance as a function of exposure time: The regression of the coefficient of variation for penetrance vs. exposure time was significantly different from zero ($r^2 = .43$, $N = 63$, $p < .01$).

Table 4. Coefficients of variation for regression analysis of rhododendron leaf respiration

	mg $O_2/m^2/h$	mg O_2/gAFDM/h
Independent Variable	r^2	r^2
Exposure time (days)	0.39	0.58
Temperature (°C)	0.43	0.59
Penetrance (g)	0.33	0.45

Respiration is expressed per unit surface area and per gAFDM. All regression slopes were significantly different from zero ($N = 63$, $p = .0001$).

Table 5. Pearson correlation coefficients (r) among variables affecting rhododendron respiration rates

	Exposure Time	Temperature	Penetrance
Exposure Time (days)	—	0.767 (0.0001)[1]	-0.449 (0.0001)
Temperature (°C)	0.767 (0.0001)	—	-0.386 (0.0006)
Penetrance (g lead shot)	-0.449 (0.0001)	-0.386 (0.0006)	—

[1]Numbers in parenthses indicate levels of statistical significance for the correlation coefficients.

Site differences in microbial respiration rates

Initially, mean microbial respiration rates were determined for each site, and no trends were apparent for respiration/unit surface area or respiration/AFDM for leaves or sticks (Figs. 13 and 14). I analyzed rhododendron respiration rate as a function of site alone, and as a function of site combined with combinations of leaf exposure time, temperature, or penetrance using Analysis of Variance (SAS 1991). Additionally, I analyzed stick respiration rates as a function of site alone, and as a function of site and temperature, combined, using Analysis of Variance (SAS 1991).

The ANOVA using site as the independent variable indicated that respiration rates were significantly affected by site and rhododendron respiration at UBC was lower than all other sites when expressed on a surface area basis ($p = .0064$)(Fig. 13). When temperature or incubation time was included in the analysis along with site, the model was significant (ANOVA, $p < .0001$) and mean respiration for UBC was again lower than all other sites (LSM). When penetrance was included in the analysis of variance with site, respiration rates at UBC were different only from LBC (ANOVA, LSM, $p < .001$).

When expressed per AFDM, analysis of respiration rates on rhododendron as a function of site was significant and showed that UBC was lower than LBC and CC, but not from WS27 (ANOVA, LSM, $p < .01$)(Fig. 14). With temperature or incubation time added to the analysis including site, respiration rates at UBC were lower than all other sites (ANOVA, LSM $p < .001$). In addition, when exposure time was added to the analysis of variance including site, WS27 respiration rates were also different from all other sites, while LBC and CC were not distinct from each other (ANOVA, LSM, $p < .001$). The ANOVA of respiration rate using site and penetrance as factors indicated

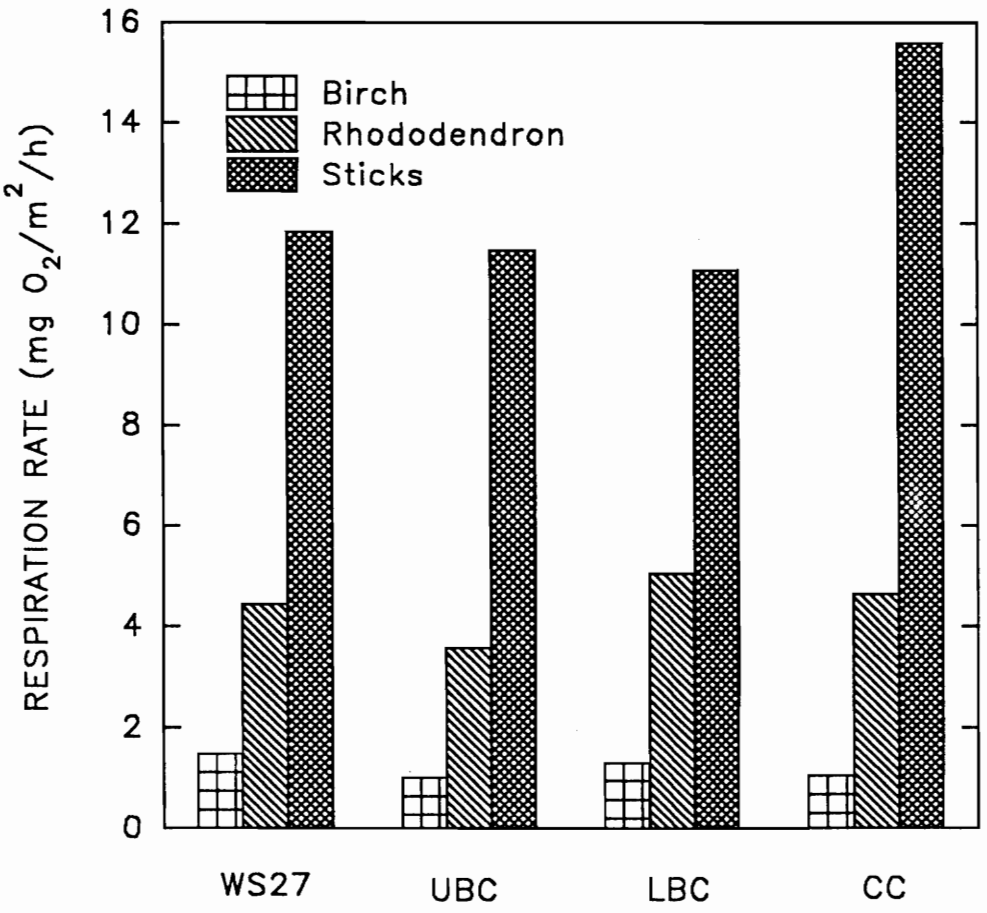

Figure 13. Mean respiration rates per unit surface area plotted by site for each substrate type: Significant differences among sites for each substrate type are described in the text.

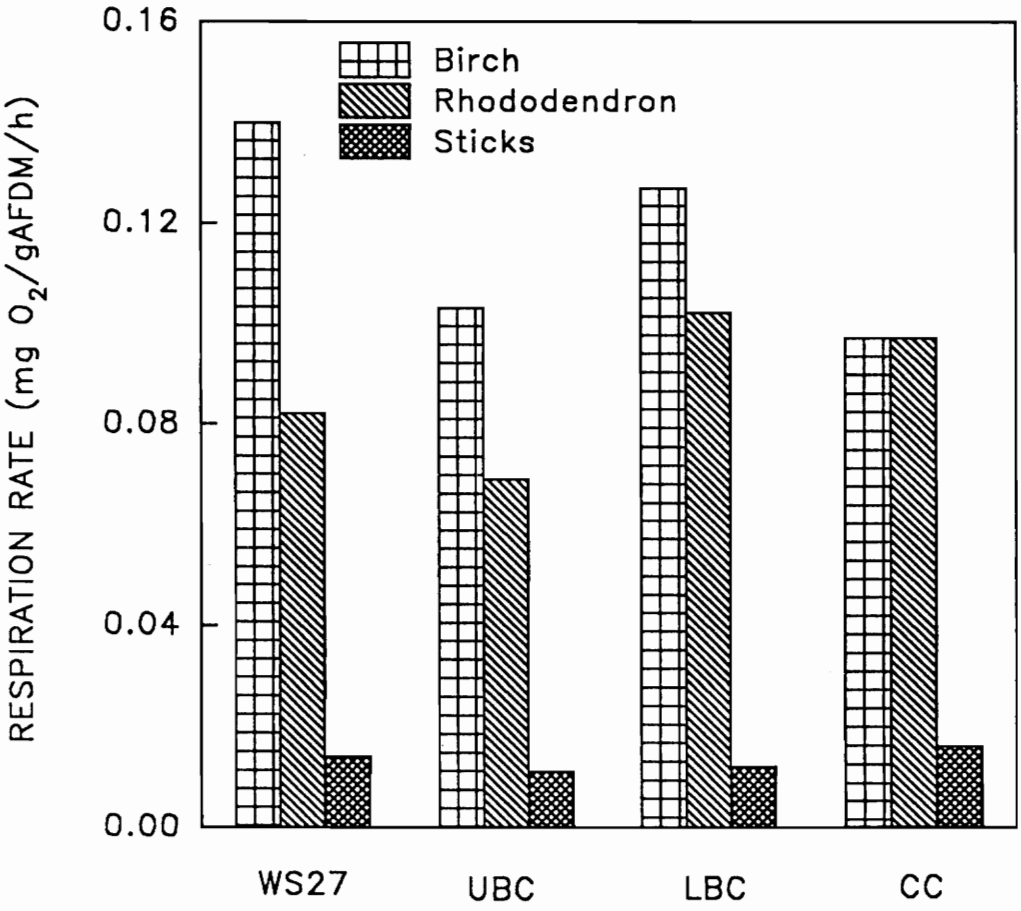

Figure 14. **Mean respiration rates per gAFDM plotted by site for each substrate type:** Significant differences among sites for each substrate type are described in the text.

that respiration rates for UBC and LBC are different, similar to the results found for the analysis of respiration per unit surface area (ANOVA, LSM, $p < .001$).

For microbial respiration on sticks, there were no interactions for the general linear model using site and temperature as independent variables. The analysis using site alone was significant ($p = .009$) and least squares means analysis indicated that stick respiration rates at CC were higher than all other sites when expressed per unit surface area ($p < .01$)(Figure 13). When temperature was included in the analysis, both site and temperature were significant ($p = .0001$, $p = .0001$) and mean respiration for CC was again higher than all other sites (LSM, $p < .01$).

When stick respiration was expressed per AFDM, ANOVA using site alone was significant ($p < .01$) and respiration rates at WS27 were different from UBC and LBC (LSM, $p < .05$)(Figure 14). When temperature was included in the analysis, both site and temperature were significant ($p = .0001$, $p = .0001$), and CC respiration was different from LBC, and WS27 is different from UBC and LBC (ANOVA, LSM, $p < .01$).

Another analysis was also used to determine if there were differences in respiration rates among sites for the same substrate type. The residuals from the linear regression of respiration vs. temperature for both sticks and rhododendron leaves were used as the dependent variable in an analysis of variance to determine if there was an effect of site on respiration rates, independent of temperature differences. The analysis of variance was based on the assumption that the relationship between respiration rate and temperature for either sticks or rhododendron leaves was the same at each site (i.e., the slopes were the same for each site). This assumption was true (ANCOVA, $p < .05$) (Figs. 15 and 16). Analysis of variance on the residuals of the regression between respiration rate and incubation temperature indicated that for respiration per unit surface area and per AFDM, site significantly explained the variability in residuals and that the mean residual for UBC was lower than all other sites ($p = .0001$). These results corroborate the

results from the analysis of variance for rhododendron respiration rates described previously.

For sticks, the slopes of the regressions of respiration vs. temperature were the same for respiration expressed per AFDM, and three of four were similar for respiration expressed per unit surface area (LBC was different from CC and UBC, the sites immediately above and below)(Figure 17 and 18) (ANCOVA, $p < .05$). The slope of the regression of respiration vs. temperature for LBC may have been different as a result of some high data points. Analysis of variance on the residuals from the regression of respiration rate vs. temperature indicated that for respiration per unit surface area site significantly explained the variability in residuals and that the CC mean residual was higher than all other sites ($p = .0007$). These results corroborate the results from the analysis of variance of stick respiration rates described above. When expressed as respiration per AFDM, site again explained the variability in residuals and residuals from CC were higher than LBC and UBC, and LBC residuals were different from WS27 ($p = .0002$).

By analyzing the residuals of the regressions of respiration rate vs. temperature temperature differences at incubation are being accounted for. When temperature differences were taken into account, the analysis of residuals, for both sticks and leaves, were in agreement with the analysis of individual respiration rates: generally, there were no differences in respiration rates that were not accounted for by temperature.

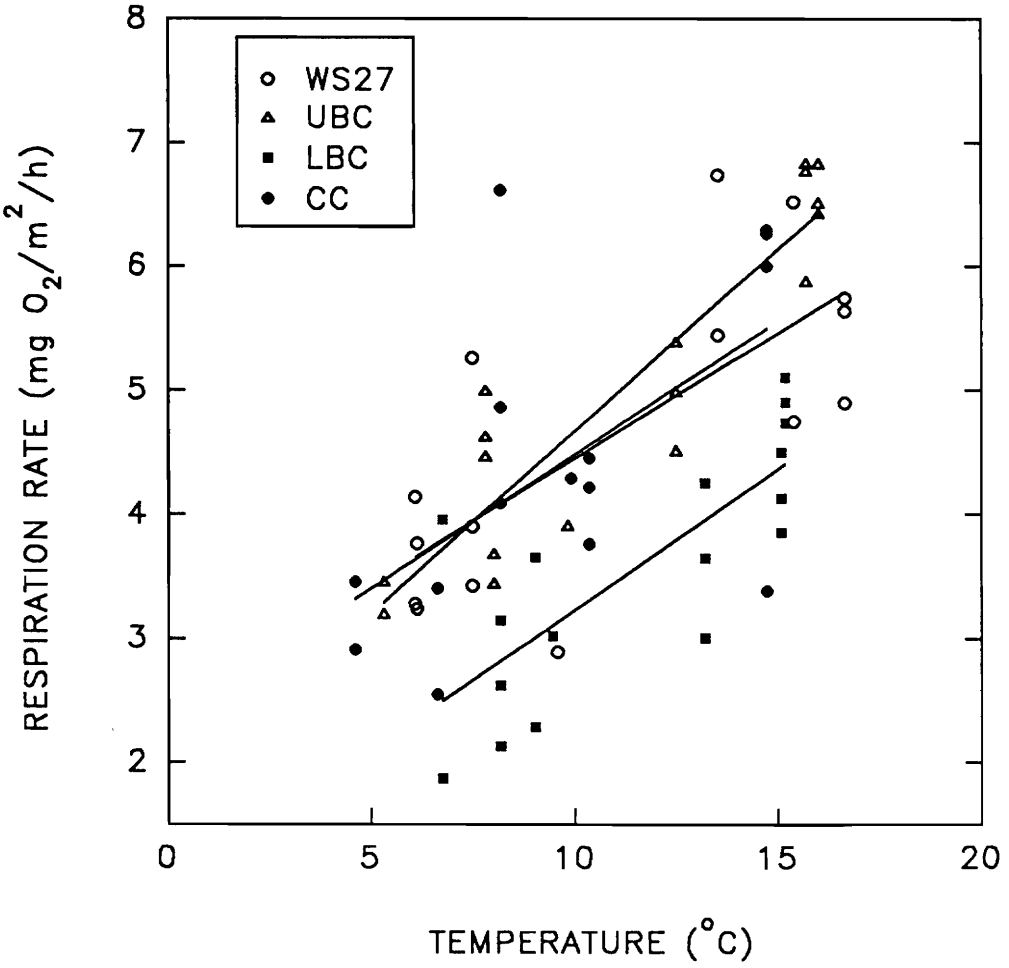

Figure 15. **Rhododendron respiration rate per unit surface area vs. temperature plotted by site:** The slopes for each regression were not significantly different among sites (ANCOVA, $p < .05$).

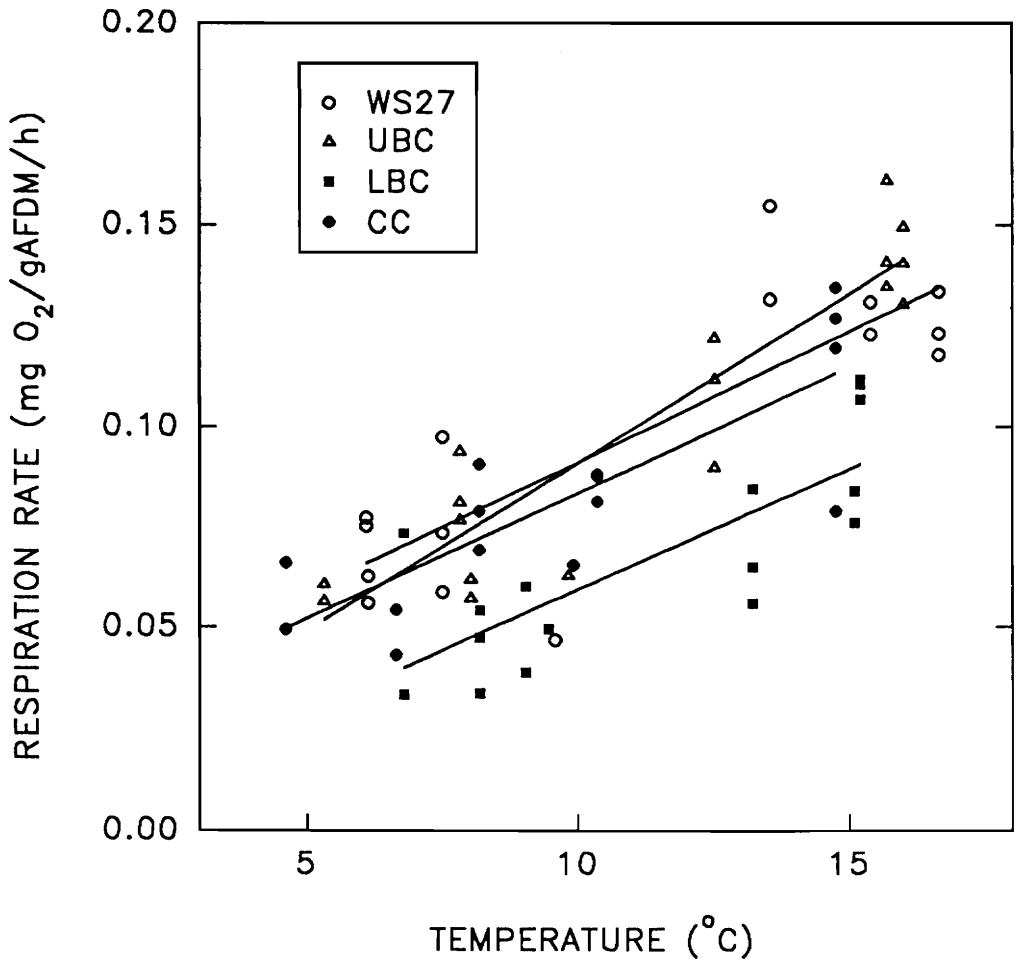

Figure 16. Rhododendron respiration rate per gAFDM vs. temperature plotted by site: The slopes for each regression were not significantly different among sites (ANCOVA, $p < .05$).

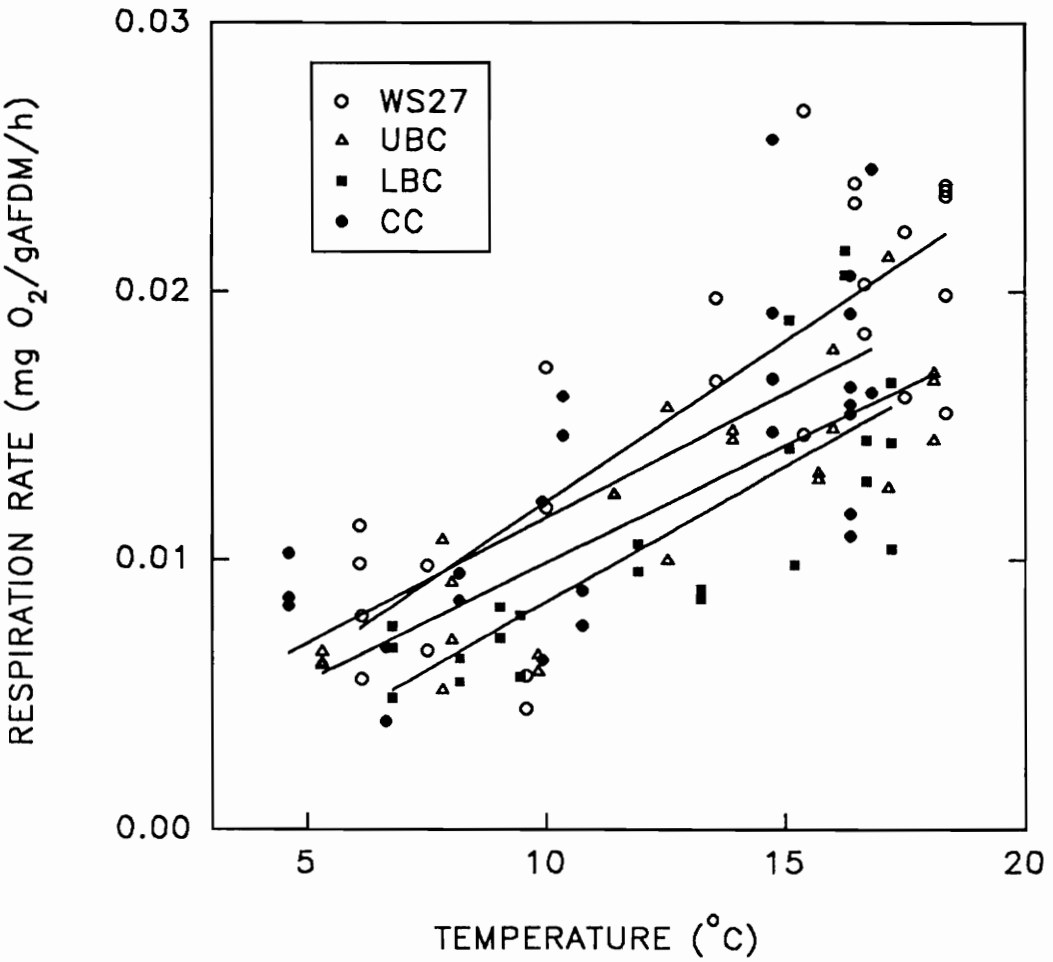

Figure 17. Stick respiration rate per gAFDM vs. temperature plotted by site: The slopes for each regression were not significantly different among sites (ANCOVA, $p < .05$).

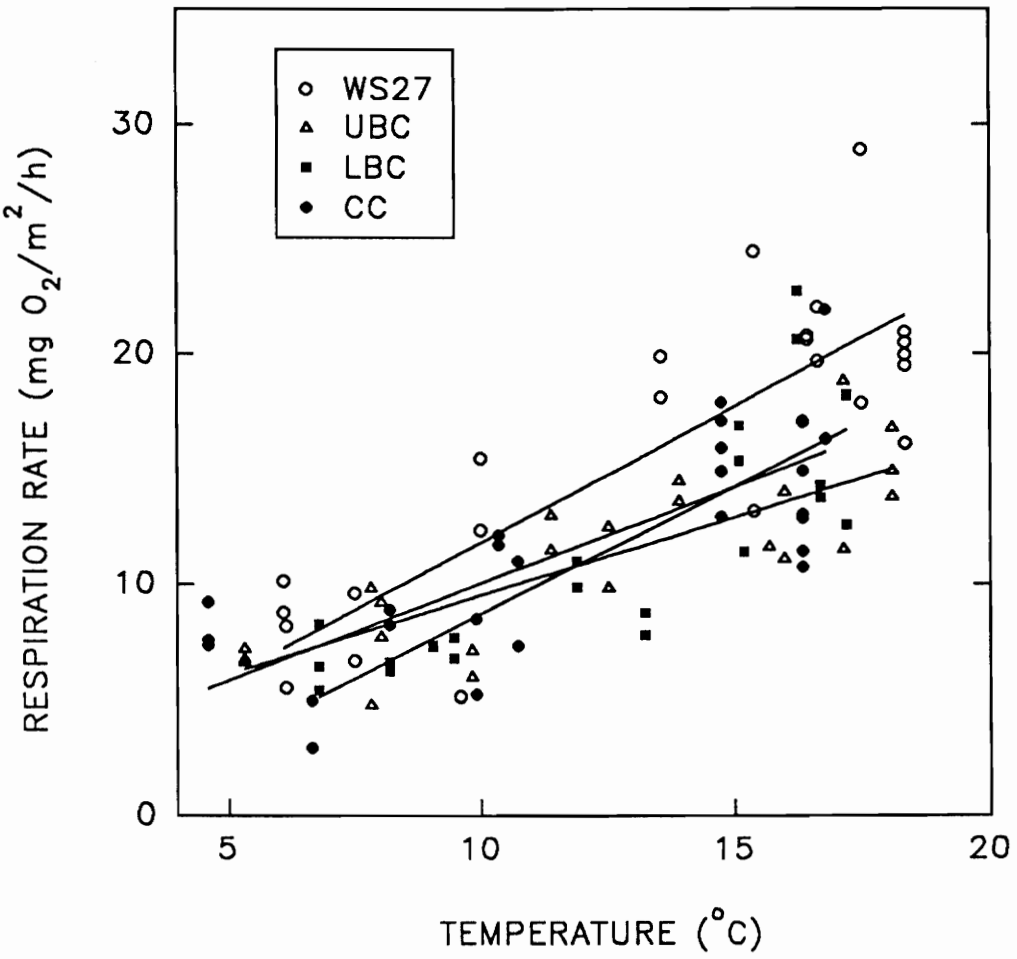

Figure 18. Stick respiration rate per unit surface area vs. temperature plotted by site: The slopes for the regressions for WS27, UBC, and CC were not significantly different from each other, but LBC was significantly different from UBC and CC (ANCOVA, $p < .05$).

Discussion

General trends in respiration at all sites

Comparison of leaf respiration rates at Coweeta to other studies

Mean daily respiration rates expressed per unit surface area from this study ranged from 0.02-0.04 g $O_2/m^2/d$ for birch leaves, 0.09-0.12 g $O_2/m^2/d$ for rhododendron leaves, and 0.28-0.37 g $O_2/m^2/d$ for sticks (calculated from hourly rates from Table 2). Respiration rate estimates from past studies have not been expressed per m^2 of substrate.

Mean daily respiration rates from this study, expressed on an AFDM basis, ranged from 2.40-3.36 mg O_2/gAFDM/d for birch leaves, 1.68-2.40 mg O_2/gAFDM/d for rhododendron leaves, and 0.24-0.48 mg O_2/gAFDM/d for sticks (calculated from hourly rates from Table 2). With the exception of the study by Bott et al. (1978), respiration estimates from this study fall at the high end of the range of values from past studies measured on an AFDM basis (Table 6). The respiration rates for White Clay Creek,

Pennsylvania (Bott et al. 1978), were estimated at high summer temperatures in an open canopy reach of stream, thereby explaining their high respiration rates. Cuffney et al. (1990) reported very similar estimates of microbial respiration on wood and leaves for two headwater streams at Coweeta.

Rates of decomposition differ across leaf types (Findlay and Arsuffi 1989), and decomposition depends in part on microbial colonization as well as the chemical quality of the leaf type. Petersen and Cummins (1974) described a processing continuum, which included a hierarchy of leaf species ranked in order of decomposition rates. Therefore, we would expect respiration rates to vary with leaf type, for example, Findlay et al. (1986) found that a labile leaf type (alligatorweed) stimulated greater rates of respiration than did the more refractory leaf type (oak). As would be predicted from relative decomposition rates (Petersen and Cummins 1974), birch leaves decomposed much more quickly than rhododendron leaves during my study. After 77 days in the stream, birch leaves were almost completely skeletonized while rhododendron leaves were in the stream 243 days before advanced decomposition prevented measurement of microbial respiration. Leaves of different decay rates should harbor peak microbial biomass at different times (Findlay and Arsuffi 1989), a trend supported by my respiration rates. Mean respiration rate per gAFDM was higher for birch leaves (0.12 mg O_2/gAFDM/h) than for rhododendron leaves (0.09 mg O_2/gAFDM/hr)(ANOVA, $p < .05$). The maximum respiration rate for birch (0.17 mg O_2/gAFDM/hr) was very close to the rhododendron maximum respiration rate (0.16 mg O_2/gAFDM/hr), but the peak occurred at a much lower temperature for birch (Fig. 8). Expressing respiration per gAFDM underestimates biological activity on organic matter with different surface area: volume ratios, including different leaf types. The resulting values show rhododendron and birch leaves having very similar activity. In contrast, mean respiration rates expressed per square meter of substrate were higher for rhododendron (4.42 mg $O_2/m^2/h$)

Table 6. Benthic respiration in streams in eastern United States. All measurements were made by oxygen uptake in chambers. Respiration rates are esxpressed per gAFDM.

Stream	Order	Respiration Rate (mg O_2 $gAFDM^{-1}$ d^{-1})	Notes	Reference
McKenzie River System, Oregon	1-7	0.17	CPOM metabolism, same for all sites	Naiman and Sedell 1980
Beaver Creek, Quebec, Canada	1-2	0.05-0.35	CPOM < 10cm diameter, per g dry weight	Naiman 1983
White Clay Creek, Pennsylvania	3	10-17	trays of natural substrate, measured 1 day in August	Bott et al. 1978
Beaver Creek, Quebec, Canada	2 2	0.11 0.11	wood < 10 cm CPOM	Naiman et al. 1986
WS53, WS54, WS55 North Carolina	1 1	0.03-0.44 0.59-2.65	wood CPOM	Cuffney et al. 1990
Ball Creek/ Coweeta Creek North Carolina	1-4 1-4 1-4	2.40-3.36 1.68-2.40 0.24-0.48	birch leaves rhododendron leaves sticks < 5cm diameter	This study

than for birch (1.23 mg $O_2/m^2/h$), although not significantly different (ANOVA, $p < .05$). The difference between the maximum respiration per surface area for rhododendron (6.84 mg $O_2/m^2/h$) and for birch (1.83 mg $O_2/m^2/h$) was greater than when respiration was expressed per AFDM. Broadleaf evergreens like rhododendron breakdown slowly (Webster and Waide 1982), but once colonized, support an active microbial community, as demonstrated by high rates of respiration/ unit surface area comparable to birch, the more labile leaf type (Fig. 7). High microbial activity on wood has been attributed to stability of substrate, and perhaps this can be applied to the more stable and slow decomposing leaf species such as rhododendron (Golladay and Sinsabaugh 1991).

Comparison of stick respiration at Coweeta to other studies

Stick respiration per g AFDM (mean= 0.01 mg O_2/gAFDM/h) was much lower than respiration rates for the two leaf types. Reporting respiration on a mass basis under-represents the high microbial activity which is often restricted to the outer surface of wood, regardless of volume (Petersen et al. 1989). When stick respiration was expressed per unit surface area, respiration rates were ten times higher than birch and three times higher than rhododendron leaves (Table 2). Petersen et al. (1989) measured wood respiration using a Gilson respirometer and expressed their results on a substrate surface area basis. My estimates of stick respiration were lower than those reported by Petersen et al. (1989) who estimated stick respiration to be 91 mg $O_2/m^2/h$. The maximum respiration measured for sticks in my study was 28.9 mg $O_2/m^2/h$. Overall, mean respiration rates (per unit surface) may be highest on sticks because microbial respiration is dependent, in part, on biofilm development, which is directly dependent on the amount of surface area for colonization. Sticks are often grooved and therefore provide a com-

plex substrate for colonization different from the relatively smooth surface of leaves. Therefore you underestimate real surface area resulting in an overestimate of respiration rate.

In the past, the importance of wood in streams has been studied primarily as a structural component in streams. Woody debris provides physical stability within the stream channel (Bilby and Likens 1980, Triska and Cromack 1980), habitat for aquatic organisms, and retention of organic matter through debris dams (Bilby 1981, Benke and Wallace 1990). Although turnover rates for wood are estimated in terms of tens to hundreds of years, wood generates significant amounts of FPOM as a result of physical abrasion and microbial activity (Ward and Aumen 1986). They estimated that FPOM generated through wood processing could be greater than FPOM generation associated with leaf and needle fall in 3rd order Mack Creek, Oregon. Estimates of FPOM production by wood included FPOM lost to mineralization. The authors assumed that mineralization rates for wood were very low compared to physical fragmentation. This assumption may be inaccurate in light of the high respiration rates per unit surface area I measured on small woody debris in Coweeta streams.

When expressed on an AFDM basis, respiration rates on birch leaves were 12 times greater than sticks, and rhododendron respiration rates were 9 times greater than sticks (Table 2). This does not necessarily mean that wood respiration is insignificant in streams. Rates of mineralization of wood carbon by microbes varies with wood size. Large woody debris (< 10cm) has commonly been discounted as a source of microbial respiration because of its low decomposition rates resulting from low surface area to volume ratio and assumed low rates of respiration due to limited oxygen diffusion for fungal and bacterial communities (eg. Naiman et al. 1986). On the other hand, fine woody debris (< 10 cm) decomposes more quickly and contributes substantially to energy flow in woodland stream systems (Triska and Cromack 1980). As a microbial

substrate, fine woody debris can be thought of as an intermediate between large woody debris and leaf litter (Triska and Cromack 1980).

High rates of microbial respiration (per m^2) found on woody debris in Coweeta streams are assumed to be associated with high microbial colonization. Organic surfaces are unique in that they are not just a surface for colonization (as in epilithic biofilms) but also provide a metabolic substrate (Golladay and Sinsabaugh 1991). Although few studies have measured respiration rates on wood separately from other organic matter types, a number of recent studies have found that woody debris supports an active microbial biofilm. Aumen et al. (1983) examined the distribution of the biofilm on wood, and measured lignocellulose mineralization using ^{14}C techniques. Through wood corings, plate counts, evolution of $^{14}CO_2$, and SEM, they determined that microbial colonization and degradation was limited to the surface of wood. Sinsabaugh et al. (1991) showed that ATP (estimate of biomass), ergosterol (estimate of fungal activity), and various exoenzymes were all higher for biofilms found on wood than on glass, as a result of increased surface area, better attachment sites, and the supplemental carbon source provided by the wood substrate.

The first direct comparisons of biofilm development on wood vs. leaf substrates were those of Golladay and Sinsabaugh (1991). Their results support the high respiration rates (per surface area) that I found on sticks in Coweeta streams. Biofilm biomass was greater on white birch ice cream sticks than on leaves, which was attributed to the increased physical stability of wood as compared to leaves. Leaves did decay faster than sticks, because of higher lignin and cellulose content and high C: N ratio in wood (Webster and Benfield 1986), but ATP standing stocks on wood were 4-7 times greater than on leaves (Golladay and Sinsabaugh 1991). Ergosterol standing stocks on wood also increased over the course of the study, indicating that fungi were important in biofilm development. As new layers of wood of the same chemical composition are ex-

posed, fresh substrate becomes available for microbial colonization. They concluded that the extensive biofilm development on wood could be attributed to wood's physical stability (Golladay and Sinsabaugh 1991). I believe that the high respiration rates on wood (per m^2) in my study were similarly due to a more highly developed biofilm resulting from wood stability.

Microbial respiration on rhododendron leaves and stream exposure time

Leaf breakdown occurs in three phases: an initial weight loss due to leaching; microbial conditioning and decomposition as a result of mineralization; and fragmentation (both physical and invertebrate)(Webster and Benfield 1986). The relationship between respiration rate and exposure time emphasizes the temporal component of the microbial conditioning phase of leaf breakdown. For rhododendron leaves, there was a positive linear relationship between microbial respiration rate and exposure time of leaves in the stream (Figs. 9 and 10). Because birch leaves decomposed very quickly (77 days), the relationship between respiration and exposure time was not significant.

Birch leaves in this study reached maximum respiration rates by day 50, but declined by day 77 (Fig. 9), as a result of tissue loss (skeletonization). Birch leaves decompose quickly (Petersen and Cummins 1974), and the decrease in birch respiration over time is supported by past studies examining fungal and bacterial activity on other fast-decomposing leaves. Findlay and Arsuffi (1989) found that fungal hyphal biomass (82-96% of total microbial biomass) and spore production peaked before 30 days and that respiration rates for fast decomposing sycamore leaves tripled over a two week period, before declining in the third week of decomposition. Golladay and Sinsabaugh (1991) also found that indicators of biofilm structural development on leaves (ATP,

ergosterol, and chlorophyll) were not consistently correlated with exposure time. This is understandable, as fungal populations peak and fall during decomposition, and chlorophyll *a* concentrations have high variability over time. In contrast, rhododendron leaves from this study showed no decline in respiration rates, but rhododendron, as mentioned before, is a slow decomposer and maintains its structural stability for a long time (Figs. 9 and 10).

Softening of leaf material (an indication of microbial conditioning) increased over stream exposure time (Fig. 11). Suberkropp and Klug (1981) also found a decrease in penetrance of leaf disks over time, with a maximum decrease corresponding to peak ATP levels, FPOM production, and fungal spore production. They concluded that skeletonization of leaves ("loss of original integrity") was a result of both microbial mineralization as well as production of smaller size classes of particulate organic matter. Additionally, my results show that the coefficient of variability of penetrance increased with stream incubation time (Fig. 12), demonstrating the patchy nature of leaf decomposition as a result of the "complex nature of plant material" (Webster and Benfield 1986).

Relationship of microbial respiration to temperature

Stream temperature was measured during monthly incubations from December 1990 to December 1991 and continuously for the same period with recording thermistors. Stream temperature, measured continuously, varied from a monthly low of 6.1 in February 1991 at UBC to a high of 17.2 in July 1991 at CC. Mean monthly stream temperatures from continuous data were significantly different between all sites (Table 3), but mean monthly stream temperatures measured during incubations were

generally similar (only WS27 was significantly different from the other 3 incubation sites)(Fig. 6). Despite the overall similarity in temperature during incubations respiration rates for rhododendron leaves and sticks were measured year round and therefore measured under a variety of temperature regimes. The relationship between respiration rates and temperature for each substrate was examined both as a direct relationship between respiration and stream temperature and by estimating the Q_{10} value. There was a positive linear relationship between respiration rate (both per unit surface area and per AFDM) and incubation temperature for both rhododendron leaves and sticks. Hargrave (1969) noted that by using 12 months of respiration data, seasonal effects of temperature (ie. size of microbial community) would be reflected in the slope of the relationship between respiration rate and temperature. The slopes for this relationship were significantly different between rhododendron leaves and sticks on both a surface area and AFDM basis (Figs. 7 and 8)(ANCOVA, $p < .05$) Additionally, Q_{10}'s were determined using linear regression of the ln(respiration rates) vs. temperature. When respiration was expressed on an AFDM basis, there was no difference between the Q_{10} for rhododendron ($Q_{10} = 2.2$) and sticks ($Q_{10} = 2.4$). However, when the Q_{10}'s were determined using respiration per unit surface area, rhododendron ($Q_{10} = 1.7$) was significantly different from sticks ($Q_{10} = 2.4$)(ANCOVA, $p = .001$). My Q_{10} estimates for sticks and leaves in Coweeta streams were similar to Q_{10}'s reported in various studies. For example, Hargrave (1969) reported a Q_{10} of 2.2 for the benthic microbial community in Marion Lake, British Columbia at temperatures from 10 °- 20°C. White et al.(1991) examined the relationship between specific growth rates and temperature for bacterial communities in a variety of marine and freshwater habitats and expressed the relationship as Q_{10} values. Over the entire temperature range (6°- 27°C), Q_{10} values were 2.1-3.9.

However, the literature does not support the differences I found in the response of respiration rates to changes in temperature between leaves and sticks. The microbial community has been hypothesized to be the same on all substrate types and therefore affected by temperature in the same way. Chamier and Dixon (1982) found that the same "fungal consortia" colonizes all litter types for a particular stream, though the taxonomic composition may change over time (Suberkropp and Klug 1976). Microbial oxygen consumption is a result of chemical oxidation of reducing agents and the respiration of organisms, and temperature affects both of these processes, in addition to the diffusion rate of oxygen and reducing substances across the water-substrate interface (Hargrave 1969). Although the rate of colonization on a particular organic matter type may vary as a result of differences in organic matter chemistry, the relationship between respiration and temperature should be the same. In other words, although the y-intercept of the regression may be different, indicating a different level of activity, the slope (or Q_{10}) should be the same (e.g., Fig. 7)

The slope of the relationship between respiration and temperature was higher for sticks on a surface area basis and higher for rhododendron on an weight basis but overall, the diffrences were not great. I believe that the difference between the Q_{10}s of rhododendron and sticks using respiration per unit surface area resulted from overestimating rhododendron surface area during the later months of decomposition. The overestimate occurred because I was unable to account for areas of leaf that may have been devoid of mesophyll due to invertebrate feeding. I assumed, during digitizing, that all leaf material present was available for microbial colonization. Overestimating leaf surface area in the later months of decomposition results in lower respiration rates, and therefore lower Q_{10} than sticks.

Another possible explanation for a difference in Q_{10}'s between sticks and rhododendron leaves may be an actual change in microbial physiology at higher tem-

peratures. Oxygen diffusion through the biofilm is decreased during summer high temperatures, and therefore microbial respiration may be lower.

Conflicting views on the importance of temperature in determining respiration rates have been described in the literature. Cuffney et al. (1990) found that respiration rates for wood and leaves at Coweeta varied with season and that respiration lows occurred in winter (January) and peak respiration rates occurred in late May (early summer). Similarly, my results show peaks of respiration rates for rhododendron in June and in May (per AFDM) and August (per m^2) for sticks. Low respiration rates for my study occurred in March for rhododendron leaves and January for sticks. On the other hand, Hedin (1990), in an extensive review of studies estimating sediment community respiration, determined that respiration correlated best with sediment organic matter, but not temperature. When he added temperature as an independent variable, it only slightly improved the regression with sediment organic matter (increased r^2 from .84 to .88). Hedin noted that temperature alone did not correlate with respiration rate as a result of low variability in stream temperature across the data set and that temperature would have a stronger effect on respiration on an annual basis. Baker (1986) found that stream sediment respiration rates per gram organic matter, but not per gram dry weight, were correlated with sediment and stream temperature. Early research by McIntyre and Phinney (1965) showed a decrease in community respiration in laboratory stream systems associated with seasonal decreases in temperature.

Despite conflicting studies of the relationship between respiration rate and temperature, it is well documented in the literature that temperature affects decomposition in streams (reviewed by Webster and Benfield 1986). Higher temperatures increase breakdown rates by affecting microbial processes (Suberkropp and Klug 1974, Petersen and Cummins 1974). Simply, increased stream temperatures increases microbial development (eg. Findlay and Arsuffi 1989). My results show that the microbial community

colonizing organic matter is active even at very low temperatures (e.g., Fig. 8). Early studies of leaf decomposition showed that aquatic hyphomycetes were the prevailing fungi at stream temperatures close to 0 °C and that they are the "main agents of decay" during the winter, following leaf fall (Barlocher and Kendrick 1974). Tam et al. (1963) found that leaf weight loss associated with decomposition at 0 °C was 40% of the weight loss at 20 °C. Similarly, Sinsabaugh et al. (1981) demonstrated that cellulose activity of microbes at 0°C is about 30% of activity measured at 25 °C. Additionally, fungal growth and microbial respiration was measurable at 0°C in various streams (Buttimore et al. 1984). Although past studies show that biofilms are active at low stream temperatures, it has been suggested that stream microbial communities may be maintained in a state of reduced growth. Suberkropp and Klug (1976) demonstrated that bacterial isolates from a 3rd order woodland stream exhibit maximum growth in the lab at temperatures 5-10 degrees above stream temperatures. Under natural conditions populations were "optimally adapted" to temperatures 5-20 °C higher than stream temperatures (Bott 1975). Golladay and Sinsabaugh (1991) found very high estimates of respiration on wood and leaves as a result of high stream temperatures during their study (20-25 °C)

Development of the river continuum concept (RCC) initiated a number of stream studies that examined the factors affecting stream processes along a gradient from headwater streams to larger rivers (Vannote et al. 1980). The role of changing temperature was a major focus of these studies. Naiman (1983) found that CPOM (< 10cm) respiration does change with season and peaks in the warmest months, and that temperature was most important in predicting rates of community respiration of benthic detritus in small streams (1st-3rd order), similar in size to the streams used in my study. In Pennsylvania and Michigan streams, variation in benthic community respiration (measured in trays) was primarily due to temperature (Minshall et al. 1983, Bott et al. 1985). Because of high standing crop of detritus, Bott et al. (1985) estimated that respi-

ration was highest in the headwater regions, but that respiration increased in a downstream direction as a result of higher temperatures. Minshall et al. (1983) also predicted that respiration would be highest in headwater streams but found that respiration often increased in a downstream direction as a result of either particle size or the addition of periphyton respiration.

Interaction of temperature and exposure time on rhododendron respiration rates

Hanson et al. (1984) developed a negative exponential model for leaf decomposition in streams and found that exposure time, in conjunction with accumulated temperature, worked best as independent variables to explain leaf decomposition rates. My results show that there is some interaction between rhododendron respiration rates and the effects of exposure time and temperature. As noted above, both respiration per unit surface area and respiration per unit weight increased with incubation time (Figs. 9 and 10). Also, for the entire incubation period (243 days), a multiple regression of respiration rate vs. incubation time and temperature indicated that temperature explained most of the variability in respiration (ANOVA, $p < .05$). But temperature did not increase consistently over the incubation period. From day 0 to day 134, stream temperature decreased (Dec-Mar), and yet respiration rate continued to increase (e.g. Fig. 9). After day 134, temperature did increase (April-June) as would be expected from changing seasons. Results from regression analysis for data from day 0-134 indicated that exposure time for rhododendron leaves explained most of the variability in respiration rates (per AFDM). After day 134, regression analysis indicated that temperature explained most

of the variability in respiration rates (per AFDM, surface area). But during this time, temperature and exposure time were positively correlated.

Data from the literature support the apparently conflicting result of increasing respiration rates despite decreasing temperature. Aquatic hyphomycetes depend on substrate availability for colonization, and a pulse of substrate becomes available during autumn leaffall. Therefore, despite decreasing fall-winter temperatures, productivity of aquatic hyphomycetes increases. Suberkropp and Klug (1981) compared fungal activity at room vs. stream temperatures, and the density of aquatic hyphomycetes was highest for leaves incubated at colder stream temperatures. The authors concluded that these fungi are adapted for high growth at a time when substrate becomes available (i.e., autumn) thereby outcompeting other fungal species (Suberkropp and Klug 1981). I conclude that increased respiration rates before day 134 were a result of increasing aquatic hyphomycete productivity despite decreasing temperatures.

Site trends in respiration rates at Coweeta

The four sites used in this study were located along an elevational gradient. Both stream discharge and temperature increased with increasing stream order (Tables 1 and 3). One would expect that temperature and discharge differences would affect microbial colonization of organic matter and ultimately respiration rates.

As described earlier, mean daily temperature measured continuously with a recording thermister, was significantly different among sites (Table 3), and temperature increased in a downstream direction (ANOVA, $p < .0001$). But temperature data from incubation periods showed that only WS27 was significantly lower in mean annual in-

cubation temperature than the other sites (ANOVA, $p < .0001$)(Fig. 6). Overall mean respiration rates calculated for each site revealed no apparent trend in respiration rates on leaves or sticks other than could be accounted for by temperature (Figs. 13 and 14). Generally, the analysis of variance corrected for temperature indicated two site differences repeatedly (one for rhododendron, one for sticks).

Analysis of respiration rates on rhododendron indicate that UBC (2nd order) had significantly lower mean rates of respiration than the other three sites, both on an AFDM and surface area basis (ANOVA, $p < .05$). I believe this may be a result of leaf pack placement in the stream. UBC has fast-flowing riffle down the center of the stream, and pools or slow backwater areas along the sides. To insure that leafpacks remained secure, I placed them in pool areas along the sides and sedimentation may have been a problem. I believe that the occasional burying of leaf packs retarded biofilm development, and hence, microbial respiration was lower.

Analysis of respiration rates on sticks indicated that CC, the most downstream site, had significantly higher rates of respiration than the other three sites (ANOVA, $p < .05$). Higher respiration rates at this site could be a result of two possibilities. The higher respiration rates at CC could be a function of stick retention time. Discharge is notably higher at this site (Table 1), and the stream is wider with fewer backwater areas. Overall, this site is the least retentive of the four sites (Covich and Webster, unpublished data). Wood found at this site was usually located in one or two large debris dams, and sticks appeared to be well decomposed (punky and grooved). Because the sticks found at CC were lodged in one of only a couple of large, long-term debris dams, the sticks collected may be preferentially older than those found at sites with more transient debris dams. Older wood may have a more developed biofilm and higher rates of respiration. Another possibility may be that the tree species at this site are different than the other sites (J.R. Webster, personal communication), and as discussed earlier, may have resulted in sig-

nificantly different rates of respiration. The tree species may be more labile than the ones found upstream, and therefore have higher rates of respiration. Other differences in respiration rates among sites seen only for stick respiration per AFDM may be a result of variability in the density of sticks found at each site. Sticks supporting an equally developed biofilm may vary in density and therefore have very different respiration rates (per gAFDM) that would not be demonstrated if respiration was expressed per unit surface area.

Respiration rates at various sites for each substrate were primarily a function of temperature. Other differences among sites (e.g. discharge, canopy openess, etc.) apparently did not have significant effects on respiration rate as I found no site trends once temperature was corrected for in the statistical model. The relationship between respiration rate and stream temperature at each site was generally the same (i.e., same slope) for both rhododendron (Figs. 15 and 16) and for sticks (expressed per AFDM)(Fig. 17)(ANCOVA, $p < .05$). For stick respiration expressed per unit surface area, the slope for the relationship between respiration and temperature for LBC is significantly different than the other three sites due to several high data points (Fig. 18).

Vannote et al. (1980) predicted that community respiration will be high but similar in 1st through 5th order streams, decreasing through 10th order, then increasing 11-12th order. Studies on benthic metabolism have generally found that respiration rates, expressed per unit AFDM, do not change with increasing stream order (Naiman and Sedell 1980, Naiman 1983), thereby supporting the results from my study. Webster et al. (1992) summarized respiration rates for streams of the Eastern United States, and the analysis of pooled data showed no significant correlation between respiration rate and stream order. Comparison of intermittent and perennial prairie streams of differing order revealed that respiration rates on leaf litter were similar for all streams (Hill et al. 1992).

While temperature affects respiration on an immediate time scale (i.e., at time of incubation), flow/ discharge differences at sites may affect microbial biofilm development and ultimately respiration on a more long-term scale. My results showed no significant differences in respiration rates among sites despite the large differences in annual discharge between sites, ranging from 20.5 L/sec to 590.4 L/sec. Golladay and Sinsabaugh (1991) found that flow regimes do not affect biofilm structure and function for sticks and leaves. Additionally, Naiman et al. (1986) found that respiration rates per AFDM of fine woody debris (< 10cm) were not different between riffle and pool sites.

Benthic respiration rates applied to Coweeta BOM standing stocks

Although respiration rates per unit weight, or surface area of substrate, may not change with stream order, standing stocks of leaves or sticks do change with increasing stream order. For example, Naiman (1983) found that because CPOM standing stocks decreased in a downstream direction, there was a decrease in metabolism per m^2 of stream bottom. When I extrapolated respiration rates of sticks and rhododendron leaves to annual standing stocks of sticks and leaves, I found similar results for Coweeta streams.

Standing stocks of benthic CPOM (leaves) and wood (small sticks > $1cm^2$ < $3cm^2$) were estimated for each of the four sites, five times, from April 1991 to February 1992 (E.F. Benfield, VPI, unpublished data). Three habitat types were sampled at each site; cobble-riffle, sandy reach, and rockface-boulder. Mean annual standing stock (g/m^2) at

each site was calculated using means weighted by habitat type, thereby estimating standing stocks while proportions of each habitat type were accounted for at the four stream sites. As stream size increased, the proportion of boulder-rockface habitat decreased, while cobble-riffle habitat increased (Table 7). Annual standing stocks, calculated from weighted means from each habitat type, of both leaves and sticks decreased with increasing stream size (except for sticks at LBC)(Table 8). Leaf biomass was generally higher than corresponding stick biomass, except at WS27 where wood dominates annually (Table 8).

Mean annual respiration rates for each site (in mg O_2/gAFDM/h) for sticks and rhododendron were applied to annual standing stocks for both leaves and sticks to obtain estimates of respiration rates per m^2 of streambed at each site (Table 9). Respiration rates per m^2 of streambed for sticks and leaves generally decreased with increasing stream size. Of course all of the standing stocks of leaves were not rhododendron, but for illustrative purposes, I will assume this is the case. In a study examining benthic metabolism in various biomes, Bott et al. (1985) also found that respiration was highest in the headwater site as a result of a larger standing stock of detritus. As stream size increases, reductions in respiration rate are linked to reduced organic matter standing stocks (Naiman 1983).

In my study, annual community respiration rates for leaves were 6- 61 times greater than for sticks. The relatively higher standing stocks of leaves and higher respiration rates per gAFDM for rhododendron resulted in the respiration rates (per m^2 of streambed) of leaves contributing more to the total CBOM respiration than did sticks (Table 9). However, although not as important at the lower sites (UBC, LBC, and CC), stick respiration at WS27 were 14% of total CBOM respiration (Table 9).

Respiration rates for the Ball Creek- Coweeta Creek continuum are low compared to respiration in other streams (in g O_2/m^2 streambed/d)(Table 10). These differences

Table 7. Habitat composition of study sites along Ball Creek- Coweeta Creek

	WS27	UBC	LBC	CC
Cobble/riffle	45%	53%	69%	78%
Sandy reach	17%	22%	9%	13%
Rockface/boulder	38%	25%	22%	9%

Data from J.B. Wallace, UGA, unpublished data.

Table 8. Annual standing stocks of sticks and leaves at study sites along Ball Creek- Coweeta Creek

	WS27	UBC	LBC	CC
STICKS	85.94 (57%)	2.95 (10%)	13.16 (42%)	1.26 (8%)
LEAVES	63.94 (43%)	26.24 (90%)	18.13 (58%)	15.22 (92%)

Data are expressed in g AFDM/m^2 streambed. Mean standing stocks are calculated as weighted means based on the area of each habitat type at each site. Habitat types are given in Table 6. Percentages of total standing stock of CPOM at each site are given in parenthesis. Data from E.F. Benfield, VPI&SU, unpublished data.

Table 9. CBOM respiration for along Ball Creek- Coweeta Creek expressed in mg O_2/m^2 streambed/h

	WS27	UBC	LBC	CC
STICKS	0.86 (14%)	0.03 (2%)	0.13 (7%)	0.03 (2%)
LEAVES	5.12 (86%)	1.84 (98%)	1.81 (93%)	1.52 (98%)

Values are calculated from standing stocks (gAFDM substrate/m^2 streambed) and mean annual rhododendron respiration rates (mg O_2/gAFDM substrate/h) at each site (Table 2). Percentage of total CPOM respiration for each site are given in parentheses.

may be a function of several factors. I based my leaf respiration/ m^2 streambed on rhododendron respiration rates/ AFDM. This is definitely an underestimate of total leaf respiration in the stream because other faster decomposing leaf types, with higher rates of respiration (e.g., birch), have not been included in the estimates. Birch and rhododendron had similar peak respiration rates, but the birch peak occurs more quickly than rhododendron. Additionally, I used respiration estimates for two organic matter types, leaves (rhododendron really) and sticks, in the CPOM pool and no fine particulate organic matter or algal respiration is included in the estimates. Coweeta streams are cold mountain streams, whereas many of the studies reported in Table 10 were in large warm-water systems. Respiration rate estimates from most studies in Table 10 were measured using trays of benthic substrate (leaves, sticks. FPOM, and rock of various sizes) and the amount of organic matter in trays is probably underestimated, thereby inflating overall respiration rates (J. R. Webster, personal communication).

Wood standing stocks are highest at WS27 (Table 9) and therefore wood respiration contributes the most to total benthic respiration at this headwater site. Annually, sticks accounted for 57% of the standing stock, and contributed 23% to total CBOM respiration. These data indicate that wood does play a significant role in total CBOM respiration at the headwater site, despite low respiration rates per gAFDM of substrate, because of the high standing stock of wood. Hedin (1990) described wood as a "focal site of metabolic activity in headwater streams". He primarily referred to the role of debris dams in retention of fine particulate organic matter. I believe his definition may also apply to the metabolic activity of the microbial community colonizing wood itself, thereby expanding the importance of wood beyond its structural characteristics. Generally, there was no trend in respiration rates with increasing stream size, and other variables associated with stream size (e.g., discharge, quality of organic matter) did not

Table 10. Benthic respiration estimates in streams in eastern United States. All measurements were made by oxygen uptake in chambers (except where noted). Respiration rates are expressed per m^2 of streambed.

Stream	Order	Respiration Rate ($g\ O_2\ m^{-2}\ d^{-1}$)	Notes	Reference
McKenzie River System, Oregon	1-7	0.03-0.35	highest in 1-2 order streams	Naiman and Sedell 1980
Augusta Creek, Site 1,	1	0.6	wooded	King and Cummins, 1989a, b
Site 2,	1	0.5	wooded	
Site 3,	2	1.8	meadow	
Site 4,	3	1.9	cleared	
Site 5, Michigan	3	0.8	wooded	
Chippewa River, Michigan	3	0.3	measured over sediments	Brown and King, 1987
Beaver Creek, Quebec, Canada	1-2	0.02-0.23	CPOM < 10cm diameter	Naiman 1983
White Clay Cr., Pennsylvania	1-4	1.11-2.17	FPOM and cladophora	Bott et al. 1985
Augusta Cr., Michigan	1-4	0.71-2.88	primarily potomageton	
Hubbard Brook, New Hampshire	1-2	0.03-0.4	CO_2 production in chambers, RQ = 0.85	Hedin, 1990
Artificial Streams, Oregon	NA	0.3-2.5	laboratory streams	McIntyre and Phinney 1965
Beaver Creek,	2	0.03	wood < 10 cm	Naiman et al. 1986
Quebec, Canada	2	0.01	benthic CPOM	
White Clay Creek, Pennsylvania	3	2.5-3.4	trays natural substrate	Bott et al. 1978
Stillhouse Br., WS54	1	2.2	benthic bottom trays	Cuffney and Wallace, unpublished data
Satellite Br., WS55 North Carolina	1	1.9		
Ball Creek/			estimated from BOM standing stocks	This study
Coweeta Creek	1-4	0.04-0.12	rhododendron	
North Carolina		0.001-0.02	sticks < 5cm diameter	

significantly affect respiration rates. Overall, the most important factor affecting respiration on leaves and sticks in streams is temperature.

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Appendix A. A modified field apparatus for measuring benthic metabolism in streams

Introduction

Changes in dissolved oxygen concentration are widely used to measure metabolism in recirculating chambers equipped with YSI dissolved oxygen probes (Bott et al. 1978, Cuffney et al. 1990). To use a multi-chamber, replicated system, we needed a multi-channel dissolved oxygen meter. Low cost systems are not commercially available. The signal conditioner described below can be built at relatively low cost and allows the user to compare oxygen changes in each of six chambers incubated at the same temperature regime. The apparatus we used included the signal conditioner, six recirculating chambers with YSI Inc. dissolved oxygen probes, and a Campbell 21x micrologger (Figure 1). The signal conditioner is battery operated for field use, and data output is measured in mg/L or % saturation.

Materials

The signal conditioner was comprised of two circuits, a dissolved oxygen circuit and a temperature circuit. The circuit shown in Figure 2 measured dissolved oxygen. It is a current to voltage converting amplifier that accepts input from a YSI Model 5739 dissolved oxygen probe and puts out a proportional voltage to a Campbell micrologger (YSI Inc. Technical Disclosure 1968). The circuit required two forms of temperature compensation, one for probe characteristics and the other to account for the variable solubility of oxygen in water due to temperature changes (YSI Inc. Technical Disclosure 1968). The YSI probe has two onboard thermistors, which were easily incorporated into the circuit. They were located in the feedback loop to independently regulate these effects. The feedback thermistor was responsible for membrane temperature compensation, and the output voltage divider thermistor adjusted for variability in oxygen solubility as a function of temperature to determine percent saturation. This eliminated the need to manually look up the saturation of oxygen in water at a given temperature. Atmospheric pressure compensation was handled numerically in the datalogger program by multiplying by a correction factor given altitude (see manual for YSI Model 57 dissolved oxygen meter). The probes required an 800 mv excitation voltage, which was provided by the datalogger. A voltage source must be provided if the datalogger is not used. Since the circuit used is an inverting amplifier, the output voltage is negative. Another inverting amplifier can be cascaded if needed.

Because both probe thermistors were dedicated in the circuit, a separate thermistor was used for direct measurement of temperature (Figure 3). A Radio Shack thermistor was selected because of widespread availability, low cost, linearity over usable range, and supplied calibration data. The temperature circuit consisted of a Wheatstone Bridge probe configuration and a voltage amplifier, which pro-

vided gain, offset, and common mode rejection to eliminate interference (Horowitz and Hill 1987). The probe was connected to the amplifier using four conductor twisted pair wire (e.g. telephone wire).

Operational amplifiers require a split voltage supply, so a battery and charger system was incorporated to provide the positive and negative voltages. This avoided the complexity of a AC-DC converter, while also eliminating power supply noise. Nickel-Cadmium batteries were well suited for this application. A constant current source was used to charge the batteries (Figure 4).

A probe simulator was constructed to provide a known signal for trouble-shooting and alignment. This was accomplished by selecting a temperature in the operating range and substituting fixed resistors for both of the thermistors. A third resistor was substituted for the probe electrodes at a value that provided a current equal to a probe in oxygen saturated water at the selected temperature. The simulator was constructed in an Amphenol connector identical to those used with the YSI dissolved oxygen probes (Figure 5).

Good quality operational amplifiers (e.g. FET input) insured reliable results over a wide temperature range. The signal changes resulting from normal temperature change over a 12 hour period were minimal when compared to the dissolved oxygen changes measured.

The circuit diagram shown in figure outputs a voltage proportional to percent saturation and is optimized for 10 °C. A voltage proportional to mg/L dissolved oxygen is achieved by shorting thermistor R4 and substituting R2 with a 900 ohm resistor.

Procedure

The datalogger was programmed to convert the six independent signals (in millivolts) from the signal conditioner to corresponding dissolved oxygen concentrations (in mg/L). We designated 15 mg/L as full scale dissolved oxygen value. The datalogger full scale input voltage used was -50 mv. Therefore, scaling the voltage from the signal conditioner by -.02 results in a reading of a % of full scale mg/L dissolved oxygen. This was then multiplied by the full scale value. Additionally, the signal for temperature from the signal conditioner (in volts) was converted to degrees Celsius by dividing by 10.

Calibration of the system was accomplished by either of 2 ways. The most precise method was calibration against a Winkler sample of dissolved oxygen in the stream (Standard Methods 1989). The calibration potentiometers for all six probes were set such that the datalogger value for each channel equaled the Winkler value. Second, calibrating the probes against saturated air was done by using the temperature probe to record ambient air temperature. The recorded temperature value was then used to calculate saturated dissolved oxygen, such that:

$$\text{mg oxygen/liter} = 468/(31.6 + t)$$

where t = temperature in °C at sea level (Cole 1979). A conversion factor was needed for altitudes above sea level (Standard Methods 1989). This value became the new full scale value (instead of 15 mg/L used above). The probes were left in air and the potentiometers for each channel were adjusted to obtain this value.

Conclusions

The multi-channel signal conditioner was an affordable method to simultaneously measure dissolved oxygen change in stream incubated chambers. The results show a strong similarity to theoretical estimates of dissolved oxygen over a tem-

perature range, as well as to an individual dissolved oxygen meter's readings. This method permits low cost construction of a multi-channel dissolved oxygen system using easily obtained components.

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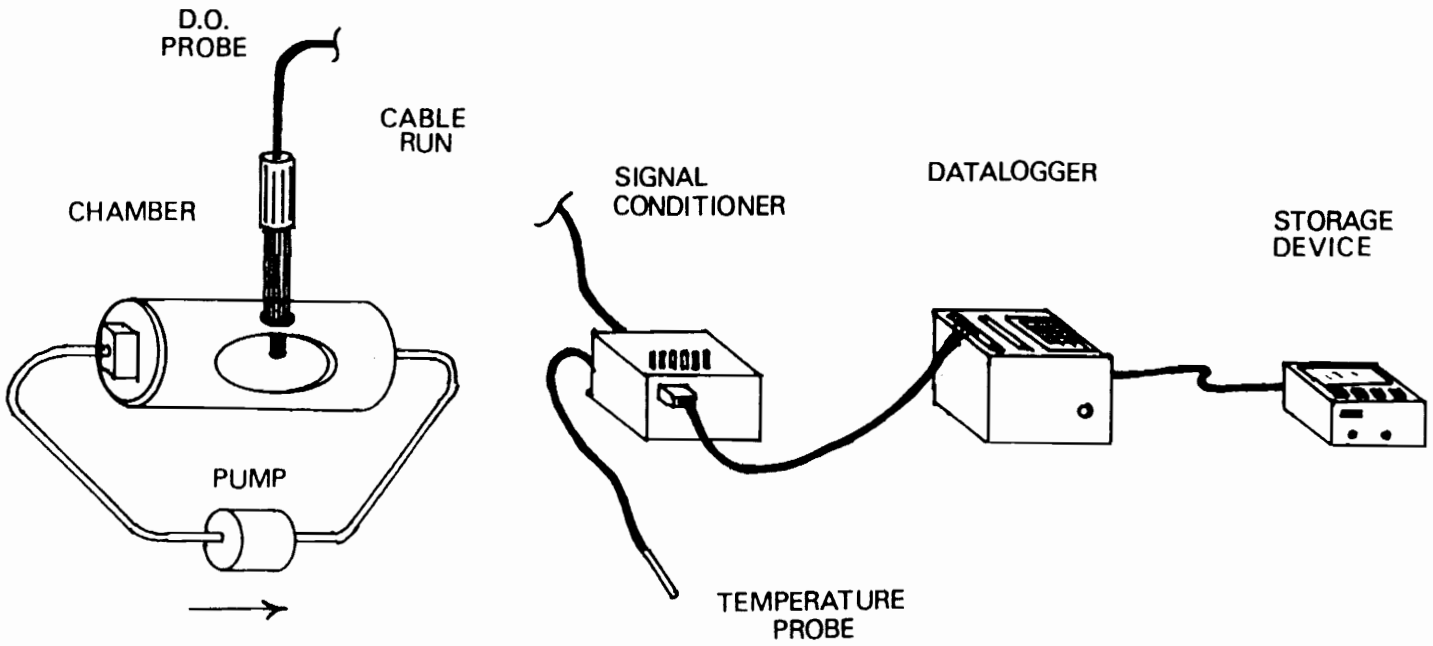
We would like to thank Tom Cuffney and UGA for assistance during early development of the method. Additionally, Raye Heggeman at YSI, Inc., gave technical advice regarding the circuit designs.

Figure 1

FIELD APPARATUS



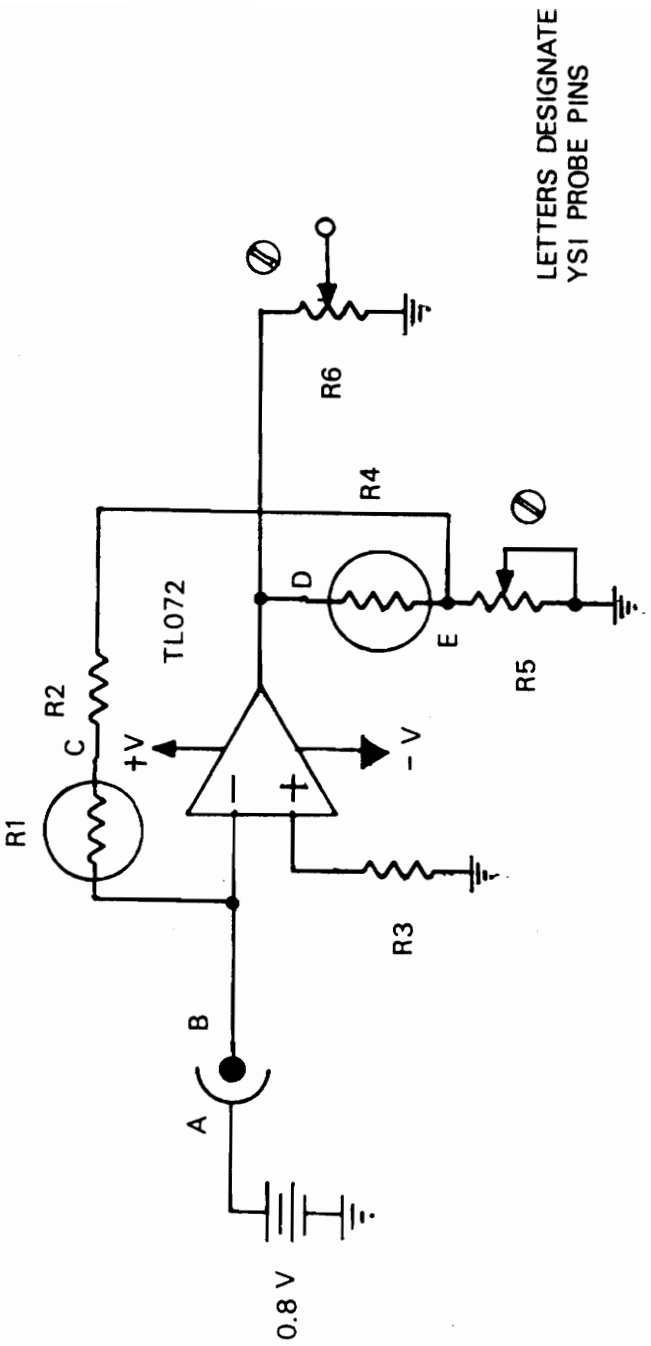


Figure 2

D.O. AMPLIFIER

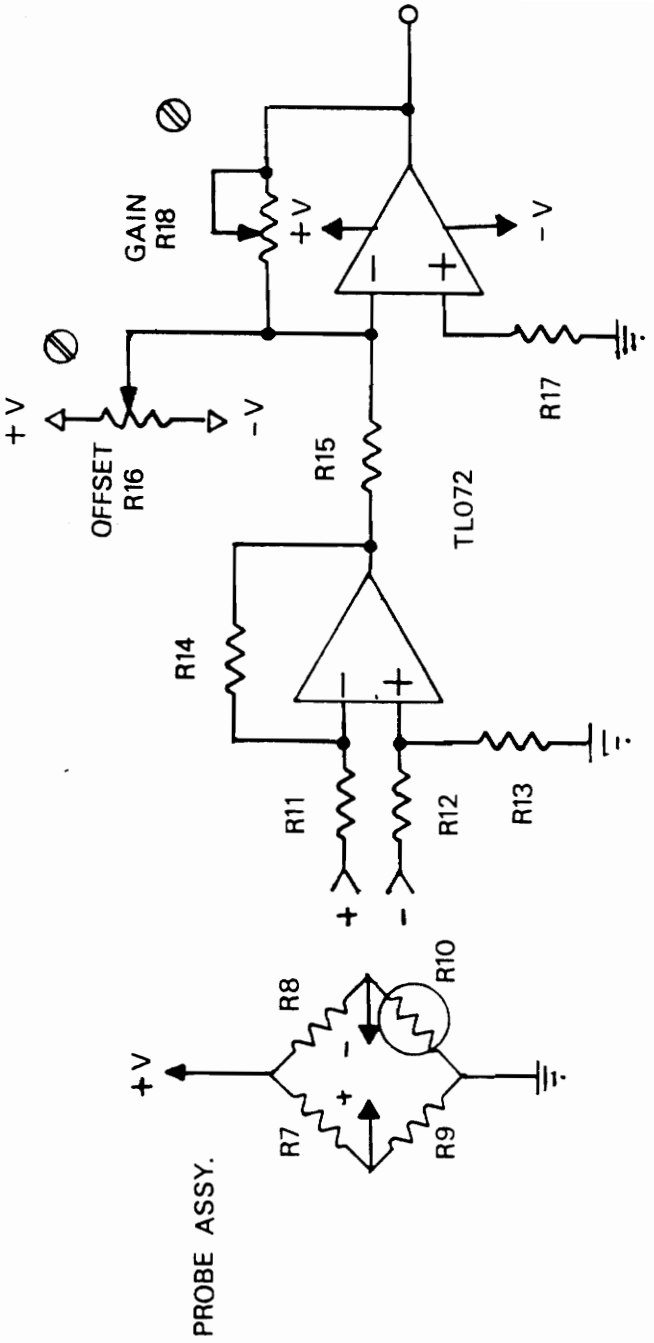


Figure 3

TEMPERATURE AMPLIFIER

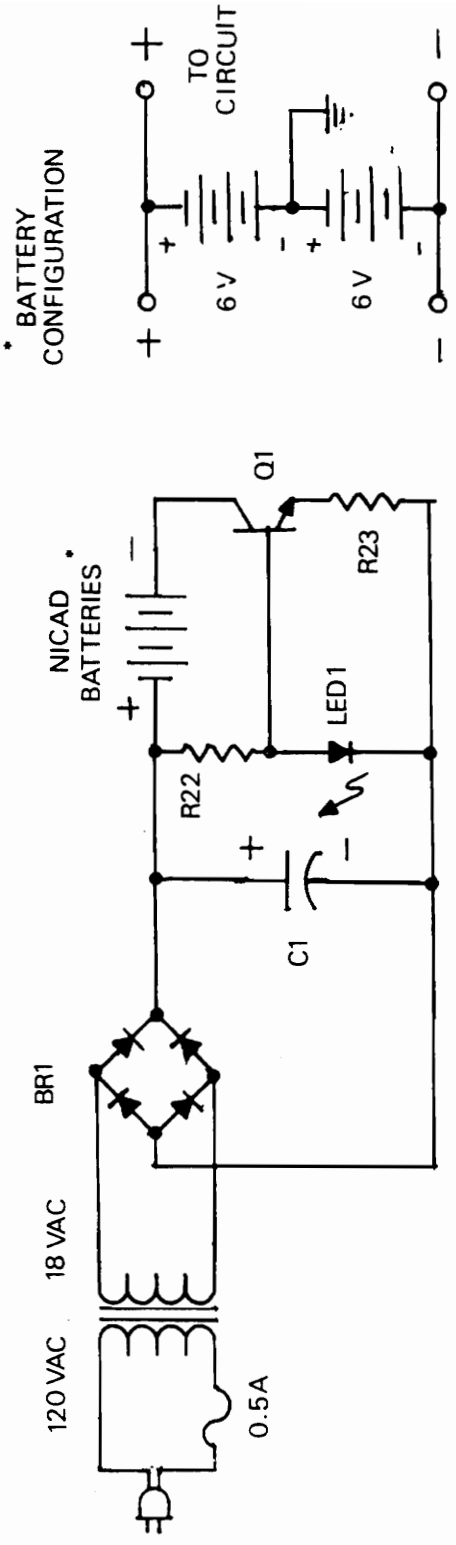
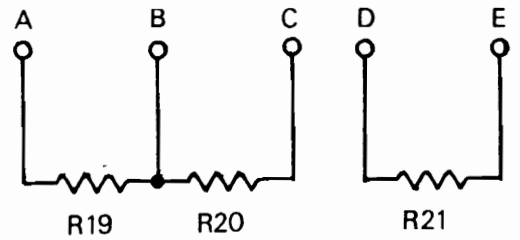


Figure 4

BATTERY CHARGER

Figure 5

PROBE SIMULATOR



LETTERS DESIGNATE
CONNECTOR PINS

COMPONENT LIST

1. D.O. Amplifier:

R1	Thermistor Onboard YSI Probe
R2	22K 1/4w 5% Resistor
R3	3.3K 1/4w 5% Resistor
R4	Thermistor Onboard YSI Probe
R5	2K 1/4w 5% Resistor
R6	10K 10-turn Precision Potentiometer
IC1	TL072 Dual FET Operational Amplifier

2. Temperature Amplifier:

R7-R9	18K 1/4w 5% Resistors
R10	Thermistor 10K @ 25° C (Radio Shack)
R11-R14	10K 1/4w 1% Precision Resistors
R15	1K 1/4w 5% Resistor
R16	5K 10-turn PC Potentiometer
R17	900 Ohm 1/4w 5% Resistor
R18	5K 10-turn PC Potentiometer
IC1,IC2	TL072 Dual FET Operational Amplifier

3. Probe Simulator:

R19	59K 1/4w 5% Resistor
R20,R21	2.7K 1/4w 5% Resistor

4. Battery Charger:

R22	2K 1/4w 5% Resistor
R23	5 Ohm 5w Power Resistor
C1	220 uF 35v Electrolytic Capacitor
BR1	5 amp Bridge Rectifier
LED1	Light Emitting Diode
Q1	2N3055 NPN Power Transistor
MISC.	120VAC-18VAC 3 amp Transformer; 1500 mAhr NiCd Batteries Associated Hardware

Vita

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1985-86	University of Michigan
1987-88	Michigan State University, B.S. in Zoology
1989-present	Virginia Polytechnic Inst. and State University, M.S. Thesis topic: Microbial respiration on decaying leaves and sticks along an elevational gradient of a Southern Appalachian stream. Major Advisor: Dr. J.R. Webster.

Professional Experience:

1985-86	Research Technician, Behavioral Training in Primates, Kresge Hearing Research Institute, University of Michigan, Ann Arbor, MI.
1988	Research Technician, Dept. of Physiology, Michigan State University, Lansing, MI.

1988	Internship, Surface Water Quality, Dept. of Natural Resources, State of Michigan, Lansing, MI.
1989	Research Technician, Stream Ecology, Dept. of Zoology, Michigan State University, Lansing, MI.
1989-present	Teaching Assistant, General Biology, Principles of Biology, Freshwater Ecology, Field & Lab Ecology, VPI & SU, Blacksburg, VA.
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Webster, J.R. and J.L. Tank. Book review of The biology of particles in aquatic systems. R.S. Wotten (editor). Journal of the North American Benthological Society 10(3):339.

Manuscripts in Preparation:

Tank, J.L. and J.C. Musson. A modified field method for measuring benthic metabolism in streams (in prep).

Abstracts and Presentations:

Tank, J.L., J.R. Webster, and E.F. Benfield. Microbial respiration on decaying leaves and sticks along an elevational gradient of a southern Appalachian stream. Bulletin of the North American Benthological Society 9:139. Presented at the annual North American Benthological Society meeting, Louisville, May 1992.

Tank, J.L. and J.C. Musson. A modified field apparatus for measuring benthic respiration in streams. Bulletin of the North American Benthological Society 8:136. Presented at the annual North American Benthological Society meeting, New Mexico, May 1991. Awarded an honorable mention for the Hydrolab Award for best poster presentation.

Webster, J.R., J.L. Tank, and T.V. Crockett. Retention of large particulate organic matter in streams at Coweeta Hydrologic Laboratory. Bulletin of the North American Benthological Society 8:75. Presented at the North American Benthological Society meeting, New Mexico, May 1991.

Tank, J.L. The effect of elevational change on benthic microbial respiration rates in Ball Creek, Macon Co., NC. Presented at the All-Coweeta LTER meeting. June 1991

Grant Proposals/Fellowships Funded:

Tank, J.L. Awarded Cunninham Fellowship for August 1992- May 1995 for study in the Biology Department at Virginia Tech. Advisor: Dr. J.R. Webster

Tank, J.L. Awarded Rotary Foundation Fellowship for February 1993- January 1994 to study at the University of Canterbury, Christchurch, New Zealand. Advisor: Dr. Mike Winterbourn

Tank, J.L. Effects of elevational change on benthic microbial respiration rates in streams. Funded by Sigma Xi for $400.00 May 1988. Matched by Department of Biology VPI&SU for $400.00.

Tank, J.L. GSA Travel Fund for travel to NABS 1991, Santa Fe, NM. Funded for $300.00 and matched by department for $100.00.

Professional Activities:

Nominated by the Department of Biology for the Graduate Student Service Award, February 1992.
Selected to participate in the national teleconference "What is Graduate School?" in Washington DC , 3 Oct 91, sponsored in part by NSF and GEM.
Elected as the GSA representative to the University Commission on Research, 1991-92
Elected Representative to the Biology Graduate Student Advisory Committee, 1990-91, 1991-92.
Graduate Student Assembly Delegate, 1989-90, 1990-91, 1991-92.
Assisted Program Committee at NABS meeting, May 1990, hosted at VPI & SU.
Graduate Student Member of the Graduate Recruitment Committee, Fall 1990

References:

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