Fine Benthic Organic Matter (FBOM) Dynamics in Low-Order Mountain Streams: (1) Methods Evaluation and (2) the Effects of Stand Age, Season, and Elevation on FBOM Nutrient Availability and Microbiological Characteristics

by

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Robert Griffiths

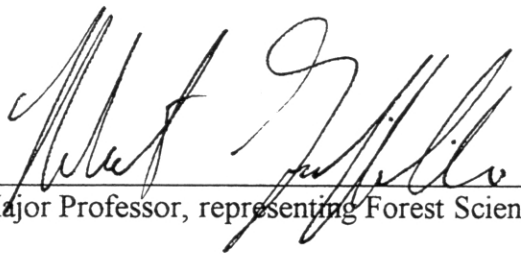
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David Hibbs

Allochthonous litter inputs are a primary source of organic matter in low-order forested streams. A major component of this litter moves through small streams as fine particulate organic matter (FPOM). Litter decomposition has been well studied, but few studies have examined benthic FPOM (FBOM) dynamics. The purpose of this study was to investigate, (1) how FBOM is controlled by headwater vegetation, elevation and seasons, (2) the links between organic matter inputs and FBOM substrate quality and (3) the relationships between FBOM substrate quality and microdecomposer activity. In preparation for this study, the stability of various microbial and chemical characteristics of FBOM during storage and analysis was determined. Denitrification potential (DNIT), phosphatase activity (PHOS), and extractable ammonium (EA) remained stable over a minimum of 11 hours of storage at 5°C. Mineralizable N (NMIN), respiration (RESP) rates, and ß-glucosidase (BGLC) activity all decreased within 12 hours of collection. Results varied for nitrogen fixation (NFIX). In response to these results, our laboratory protocol was altered to accomodate analyses with varying levels of stability. Following this protocol, FBOM was sampled and analyzed over a 10 month period from 14 first-

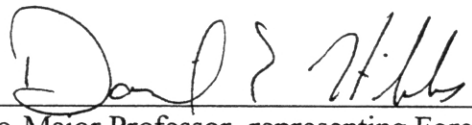
order streams in the Cascade Mountains of western Oregon. Streams ran through forests in three successional age classes: old-growth forest (OG) dominated by Douglas-fir (*Pseudotsuga menziesii*) and western hemlock (*Tsuga heterophylla*), and young regenerating stands (YS) either 10 years old, with a large riparian herbaceous component, or 30 years old, surrounded by deciduous trees such as red alder (*Alnus rubra*). Decreases in C:N, CTOT, NTOT and organic P (PORG) were mirrored by reciprocal increases in FBOM RESP, BGLC, PHOS and NFIX, all relative indicators of microbial activity. The lower C:N and higher DNIT, RESP, BGLC, PHOS, and NMIN observed in YS FBOM compared with OG suggests higher quality FBOM and faster decomposition rates in YS FBOM. Seasonal trends showed a major autumn deflection in FBOM C:N ratios and microbial activities, a likely result of increased leaf inputs following an early fall storm. Significantly lower C:N at high elevation (1220-1280 m) suggested the presence of more herbaceous vegetation and alder in high elevation riparian zones. Lower NTOT and CTOT and elevated DNIT, NFIX, RESP, PHOS at low elevation (580-800 m) suggested greater decomposition rates at low elevations. PORG was 2.2 and 3.6 mg P gOM^{-1} at high and low elevations respectively, a difference ($p \leq 0.05$) probably due to the young geologic age of parent material at high elevation. The data from this study suggest a potential link between headwater forest management and stream food web dynamics as mediated by shifts in FBOM.

Master of Science thesis of Heather Bonin presented on October 15, 1997

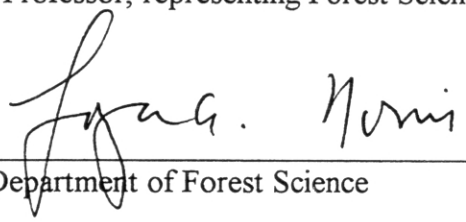
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Acknowledgments

I would like to thank all those that helped me in ways big and small to complete this study. My parents, Pierre and Laurie Bonin, deserve special thanks for their steadfast encouragement and support through the years. As my major adviser, Dr. Robert Griffiths provided financial support, invaluable insights and editing expertise, as well as the heartiest laugh I have ever heard. Drs. Gordie Reeves, Jim Sedell, and Fred Swanson all gave financial assistant for which I am very grateful. Carol Glassman performed many of the nutrient analyses and generously lent lab equipment. And finally, a resounding thank you to my friend and colleague Bruce Caldwell, without whom this project would never have succeeded--may the chanterelles always be bountiful.

Contribution of Authors

Dr. Robert Griffiths provided funding for this research and was involved in the interpretation of data and writing of each manuscript. Bruce Caldwell assisted in the experimental design, laboratory analyses and the editing of each manuscript.

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Dedication

This thesis is dedicated to the memory of my grandmother, Octavia Baptiste Bonin (1904-1990) -- the ecologist I would like to be.

Fine Benthic Organic Matter (FBOM) Dynamics in Low-Order Mountain Streams: (1) Methods Evaluation and (2) the Effects of Stand Age, Season, and Elevation on FBOM Nutrient Availability and Microbiological Characteristics

Chapter 1
Introduction

Dramatic, and often obvious, impacts to small streams occur when the surrounding forest is clear cut. After clear cutting, headwaters may simply dry up, or water temperature and sedimentation increase while channel complexity and particle retention decline due to a lack of woody debris.

As forest succession reinitiates, the quantity, quality, and timing of allochthonous inputs available for decomposition by stream invertebrates and microorganisms change. Woody debris and leaf litter (needles, leaves and herbaceous plants) are major components of organic inputs to small forested streams, and their decomposition dynamics have been well studied (Petersen and Cummins 1974, Suberkropp, Godshalk and Klug 1976, Findlay and Arsuffi 1989). However, most organic material moves through streams as fine particulate organic matter (FPOM) (Sinsabaugh et al. 1992), about which suprisingly little is known. Debate exists whether qualitative differences found in vegetation persist at the small size of FPOM (< 1mm); several studies have found few differences in FPOM microbial activity among streams running through different forest types, suggesting that headwater and riparian vegetation may have little influence in the chemical composition of FPOM (Hargrave 1972, Ward 1986, Sinsabaugh and Linkins, 1990, Sinsabaugh et al. 1992).

We examine benthic FPOM (FBOM), particles that have settled out of the water column to the stream bed. As depicted in the general model used as a framework for this study (Figure 1.1), FBOM is a potentially important link between terrestrial and aquatic environments and may help researchers understand and monitor how forest management affects stream productivity. This study focuses on how changes to forest vegetation via

clear cutting alter FBOM quality. The purpose was to examine the fundamental relationship between FBOM nutrient availability, or substrate quality, and microdecomposer activity and to investigate the link between organic matter inputs and FBOM substrate quality.

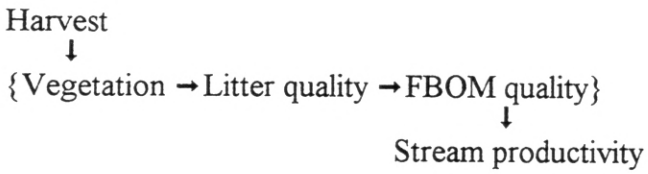


Figure 1.1. General model used to frame study. Research conducted on bracketed area.

The methods used to analyze FBOM quality were mostly adapted from those used for soil analyses, and thus were largely untested on sediments. Using FBOM collected from a subsample of the streams studied in chapter 3, I conducted time series analyses on the proposed methods and subsequently altered the experimental design to accomodate analyses with varying degrees of stability. Chapter 2 describes this study; the laboratory protocol is detailed in the Appendix.

Using these amended techniques, I then compared the qualitative characteristics of FBOM from streams flowing through forest in three successional age classes: old-growth forest dominated by Douglas-fir *(Pseudotsuga menziesii)* and western hemlock *(Tsuga heterophylla)* and young regenerating Douglas-fir stands either 10 years old, with a large herbaceous riparian components, or 30 years old, surrounded by deciduous trees. These results allowed me to examine the links between organic matter litter inputs, FBOM substrate quality, and microbial activity. Chapter 3 addresses this study.

Much research on FBOM remains to be done. Chapter 4 highlights conclusions drawn from the studies described in chapters 2 and 3 and suggests directions for future research.

Finally, a thought from my favorite philosopher, Lao Tzu.

"Nothing in the world

is as soft and yielding as water.

Yet for dissolving the hard and inflexible,

nothing can surpass it."[1]

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[1] Lao Tzu. Tao Te Ching: A New English Version. 1988. S. Mitchell (translator). Harper and Row, New York.

Chapter 2

Biochemical Analysis of Stream Sediment Fine Benthic Organic Matter: Methods Evaluation

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Abstract

Sample storage can significantly influence apparent microbial activities in stream fine benthic organic matter (FBOM), possibly confounding sample variability and perceived short-term changes in activity. Denitrification potential (DNIT), acetylene reduction (NFIX) and respiration (RESP) rates, mineralizable nitrogen (NMIN) and extractable ammonium (EA) concentrations, ß-glucosidase (BGLC) and phosphatase (PHOS) enzyme activities and C:N ratios of FBOM from first-order mountain streams in the Western Oregon Cascade Mountains were assayed at various times to determine potential storage effects. Denitrification potential, PHOS and EA remained stable over a minimum of 11 hours of storage at 5°C. Mineralizable nitrogen concentrations, RESP, and BGLC all decreased within 12 hours of collection. Results varied for NFIX. Once assay conditions were established, DNIT and RESP were linear with incubation time. At a one week interval, measures of NFIX, DNIT, RESP, BGLC, PHOS, and C:N ratios were generally repeatable within the same stream reaches. Correlations (r) between results from two sample dates 8 days apart were NFIX=0.30, DNIT=0.96, RESP=0.60, BGLC=0.42, PHOS=0.66 and C:N=0.89.

Introduction

Experimental design can quantitatively and qualitatively influence apparent biogeochemical transformations. Changes in microbial populations occurring with storage time have been well documented in water samples from marine (Ferguson et al. 1984, Marrase et al. 1992) and freshwater environments (Reinke and de Noyelles Jr. 1985, Amy and Hiatt 1989) and in aquifers (Hirsch and Rades-Rohkohl 1988). In soils, storage time has been shown to influence apparent microbial C, N, P, and ATP concentrations and soil enzyme activities (Pancholy and Rice 1972, Speir and Ross 1975, West et al. 1986, Tabatabai 1994). Sample perturbation has also been found to increase microbial activity

rates in aquatic samples (McIntire 1966, Logan and Kirchman 1991) and mineralizable N in soils (Cabrera and Kissel 1988).

As a major component of organic matter in streams, fine benthic organic matter (FBOM) is nutritionally important to stream microorganisms and invertebrates (Vannote et al. 1980, Benke et al. 1988, Cummins et al. 1989, Schlosser 1991). Few studies have documented the effects of sample storage or disturbance on stream sediment microbial or biochemical variables. Oxygen uptake by microbial populations in mud, sand and detritus in aerated water was not affected by sample size but was affected by stirring (Hargrave 1972). Other studies have found increases in microbial activity following disturbance of stream sediments (Christian and Wiebe 1979, Findlay et al. 1985) and marine or intertidal sediments (Oremland et al. 1984, Meyer-Reil 1987).

As a practical matter, it is frequently not possible to conduct a wide range of microbial activity measurements at the same time. The objective of our study was to determine the stability of various microbial and chemical characteristics of FBOM during storage and analysis. This study was designed to assess the relative stability of different microbial activities responsible for major biogeochemical transformations so that satisfactory sampling schemes could be designed.

Methods

Site Description

Fine benthic organic matter (FBOM) samples for time series analyses were collected from two first-order streams selected to represent a range of possibly influential environmental conditions on the H.J. Andrews Experimental Forest, in the Western Cascade Mountains, OR. One stream ran through an old-growth forest (OG) dominated by Douglas-fir *(Pseudotsuga menziesii)* and western hemlock *(Tsuga heterophylla)* and the other through a young Douglas-fir stand regenerating from a harvest in the late 1980s (CUT). Stream temperatures were 14°C and 11°C in OG and CUT respectively.

To examine temporal variability, FBOM was collected from 14 first-order streams running through OG or young Douglas-fir stands approximately 10 or 30 years old on the H.J. Andrews.

Experimental Design

To determine the stability of microbial populations in FBOM after collection, a time series was conducted on FBOM samples from two first-order streams on two separate occasions. To determine sampling variability, each analysis was repeated in duplicate on 3 or 4 subsets of samples collected at the same time and location. To determine the effects of storage time, analyses were performed from 3 to 15 hours post collection. Linearity of N_2O and CO_2 production was monitored repeatedly during the 15 hours following collection.

Temporal variability in denitrification potential, respiration, and acetylene reduction rates, and ß-glucosidase and phosphatase activity were measured twice separated by 8 days in the same 14 streams. C:N ratios were calculated for 9 of these streams on each date. This study was conducted under low-flow summer conditions to limit environmental variability.

Sample collection, preparation and storage

FBOM was collected from crevasses between rocks and the beds of first-order streams with a handheld vacuum pump connected to a 2 L collecting jar. The vacuum line was attached to a nozzle fitted with a 1 mm stainless steel screen, allowing the benthic material to be automatically wet sieved into the collecting jar, excluding particles > 1 mm. Samples were transferred to polystyrene jars (500 ml) which were filled with FBOM and stream water. These were stored in the field in an insulated chest filled with stream water and ice. In the laboratory, a slurry was prepared by decanting excess stream water from

the jars and mixing, keeping the FBOM suspended while subsampling. Subsamples were dispensed using plastic syringes with enlarged openings to facilitate transfer. Care was taken to limit sample exposure to ambient temperature by removing samples from 5°C storage only to dispense subsamples immediately prior to each analysis.

Laboratory analyses

Denitrification Potential Rates

Denitrification potential (DNIT) was determined as N_20 emission by anaerobically incubating FBOM supplemented with glucose and $NaNO_3$ (Martin et al. 1988). Duplicate 5 ml slurry samples in 25 ml Erlenmeyer flasks were capped with rubber stoppers and purged for 3 minutes with argon at a rate of at least 120 cc/min. In the middle of the purge, the flasks were gently shaken to ensure removal of air bubbles. The flasks incubated for one hour at 24°C and then 2 ml of sterile solution of 1 mM glucose and 1 mM $NaNO_3$ were injected through the stopper, with 2 ml of headspace subsequently removed. Flasks were incubated for another hour at 24°C. At zero and 2 h time, 0.5 ml headspace gas was removed from samples and assayed for N_2O in a gas chromatograph (GC) equipped with an electron capture detector. All GCs had stainless steel columns packed with Poropak-Q, either 50/80 or 80/100 mesh (Water Associates, Inc. Melford, MA).

Respiration Rates

To determine respiration rates (RESP), duplicate 5 ml samples in 25 ml Erlenmeyer flasks were capped with rubber stoppers. After incubating for one hour at 24°C, 0.5 ml of headspace was analyzed for CO_2 on a GC fitted with a thermal

conductivity conductor. After an additional two hours incubation under the same conditions, a second headspace sample was collected and analysed.

Acetylene Reduction Rates

Putative nitrogen fixation rates (NFIX) were determined using the acetylene reduction method (Weaver and Danso 1994). Samples were prepared in the same way as for DNIT except the headspace contained 1.5% O_2, 12.5% acetylene and 86% argon. After 24 hours of incubation, 0.5 ml of headspace gas was removed and analyzed for ethylene on a GC fitted with a flame ionization detector. A control was analyzed for ethylene production in the absence of acetylene.

Mineralizable Nitrogen and Extractable Ammonium Concentrations

For mineralizable nitrogen (NMIN) measurements, microbial conversion of organic N to inorganic N in the form of NH_4-N was measured by anaerobic incubation (Keeney 1982). Duplicate 10 ml sediment samples were added to 50 ml screw-topped test tubes which were then completely filled with deionized water (53ml), capped and incubated at 40°C for 7 days. After incubation an equal amount of 4 M KCL was added, shaken for 1 hour in the presence of 0.4 ml 10M NaOH and analyzed using a selective ion electrode (Corning ammonium electrode, Medford, MA). Extractable ammonium (EA) concentrations were measured by adding 50 ml of 2 M KCL to duplicate 10 ml samples in 250 ml Erlenmeyer flasks. The flasks were capped, shaken while incubating for 1 hour in the presence of 0.4 ml 10M NaOH and analyzed using a selective ion electrode to determine KCL-extractable ammonium concentration. To calculate net mineralization, EA concentrations were subtracted from NMIN concentrations to account for ammonium present prior to incubation.

Enzyme Activities

Phosphatase activity (PHOS) was determined using the spectrophotometric assay of Tabatabai and Bremner (1969), as modified by Zou et al. (1992). One ml of 50 mM *p*-nitrophenyl phosphate substrate was added to duplicate 1 ml subsamples containing suspended FBOM. The tubes were shaken and then placed in a 30°C waterbath for 1 hour, along with duplicate controls with no phosphatase substrate addition. After incubation, 1 ml of 50 mM Sigma 104 phosphatase substrate was added to the controls and reactions immediately stopped with the addition of two ml of 0.5 M NaOH and 0.5 ml of 0.5M $CaCl_2$. The assay mixtures were centrifuged for 5 minutes at 500 x g. From the supernatant, 0.2 ml of solution was diluted with 1.8 ml deionized water and the optical density measured at 410 nm. A standard curve was prepared from 0.02-1.0 umol ml^{-1} *p*-nitrophenol. Enzyme activity was expressed as μmol *p*-nitrophenol released gdw^{-1} h^{-1}.

The ß-glucosidase (BGLC) assay followed the same general procedure as for PHOS, except the substrate used was 1 ml of 10 mM p-nitrophenyl ß-D glucopyranoside, incubation period was 2 hours, and 2 ml of 0.1 M tris[hydroxymethyl]aminomethane at pH 12.0, instead of 0.5 M NaOH, were added to terminate the reaction.

Total Carbon and Nitrogen

Total carbon and nitrogen were determined by dry combustion with a Carlo-Erba NA 1500 Series II high temperature combustion furnace on oven-dried subsamples ground to pass 250 um.

Statistics

Pearson correlations were used to test linearity of DNIT and RESP rates and the similarity of assay results repeated in the same stream reaches at a one week interval.

Results and Discussion

The effect of storage time on FBOM varied for each analysis. Potential denitrification rates remained stable during 11-12 h of storage (Figure 2.1) and appeared to be constant during incubation periods once the assay conditions were established. Denitrification was linear from 2.0 to 9.5 hours in FBOM collected from OG and CUT stream reaches: $r^2 = 0.99$ for both ($p \leq 0.0001$, n=16), thus gas production rates could be calculated even if the time between headspace gas analyses was delayed. Similar results were found in sediments from a small forested stream hyporheic zone where DNIT was linear up to 3.3 hours (Duff and Triska 1990) and in pond sediment where rates were linear for at least 40 minutes following nitrate amendment (Murray et al. 1989). In intertidal estuarine sediments, DNIT was linear over 8 hours (Oremland et al. 1984). Similar results were found by Madsen (1979) and Oren and Blackburn (1979).

For NFIX, conclusions about the effects of storage time were more difficult to draw due to high subsample variability (Figure 2.1). The FBOM samples taken from OG and CUT showed significant increases after the initial storage period. Increased storage time may have allowed either the development of anaerobic microsites prior to the establishment of anoxic assay conditions or the growth of aerobic N-fixing microbial populations (Sprent 1969, Turner and Gibson 1980). Stored samples were stirred every 2-3 h while subsampling which likely aerated the samples prior to preparation for the nitrogen fixation assay. During preparation, sediments were exposed to an atmosphere with a very low O_2 concentration. However it is possible that optimum conditions for nitrogen fixation or the growth of nitrogen fixers were not achieved until the resident heterotrophs had further reduced O_2 concentrations with extended incubation.

Although EA remained stable during 11-12 hours of storage, NMIN declined severely within the first few hours of storage (Figure 2.2). From 5.3 hours to 7.8 hours post-collection, there were apparent 34% and 25% decreases in OG and CUT FBOM mineralizable N concentrations respectively. Given the lengthy incubation period for the NMIN assay (7 days), the reduction in NMIN with storage time is puzzling. Particle flocculation may have occurred after the first 2 hours of storage, resulting in a decline in

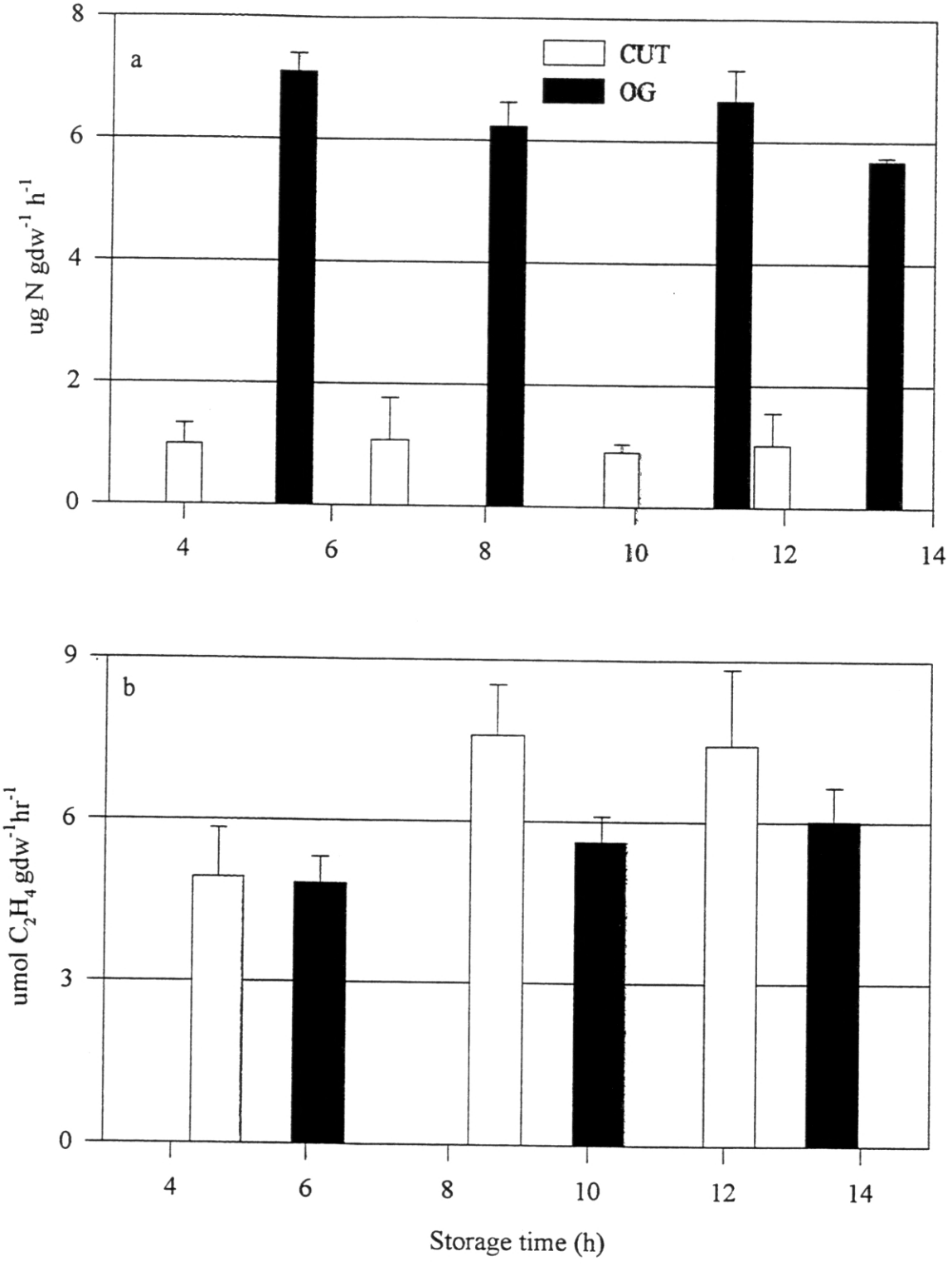


Figure 2.1. Time series results for (a) denitrification potential and (b) acetylene reduction rates. Storage time = time lapse from stream collection to start of assay incubation. Error bars = 1 standard deviation.

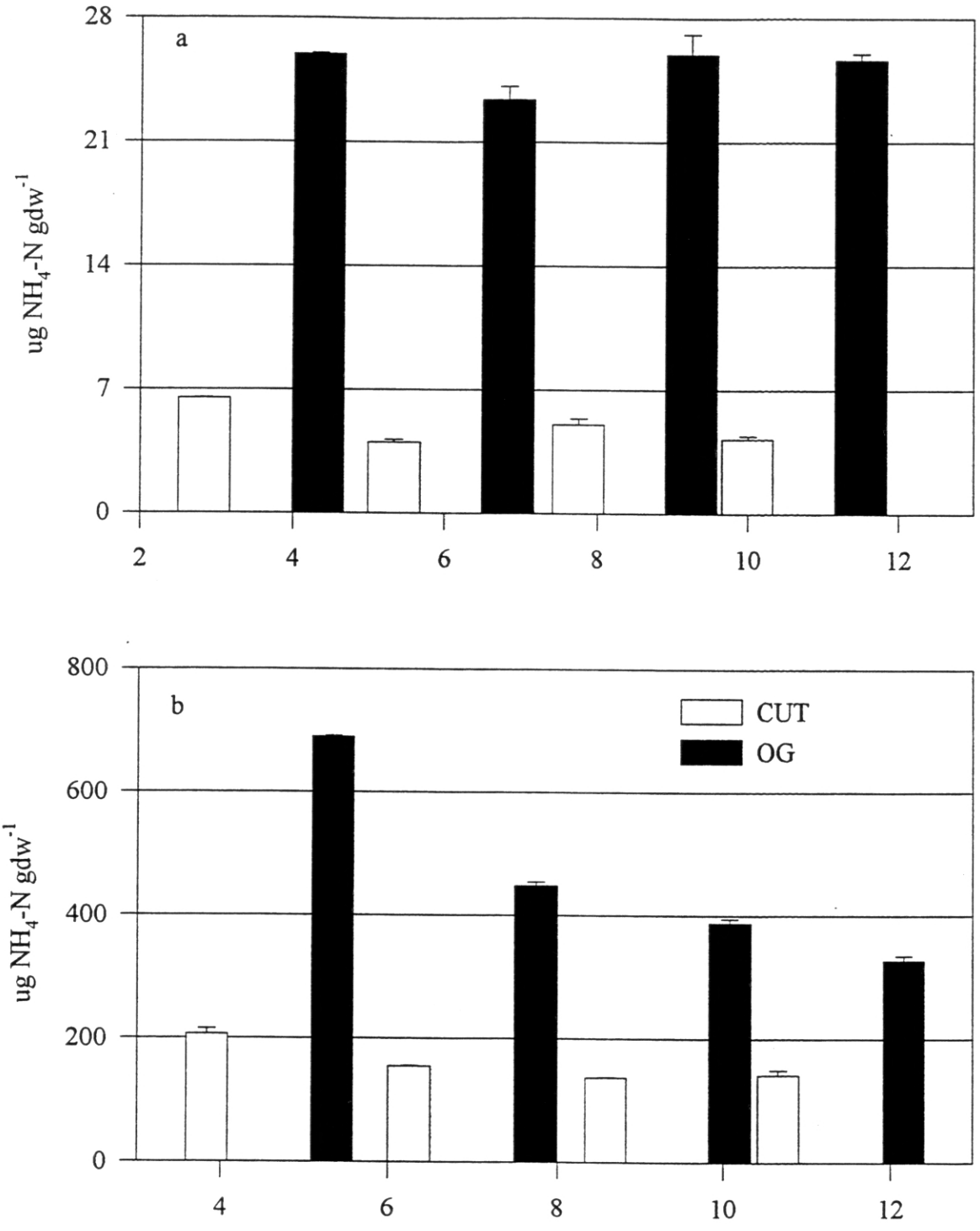


Figure 2.2. Time series results for (a) extractable ammonium and (b) mineralizable nitrogen (1 wk anaerobic incubation) concentrations. Storage time = time lapse from stream collection to start of assay incubation. Error bars = 1 standard deviation.

available N concentration as increased particle size decreased the active surface area of particles, reducing sites available for mineralization. Numerous processes can cause flocculation. The inorganic fraction of FBOM samples may have responded to increased ion concentrations in the thick slurry. In a study of microbial response to inorganic and organic particles in marine water, flocculation of inorganic particles occurred within 1 hour (Muschenheim et al. 1989). By using a blender to create turbulence for 2 minutes, particle flocculation increased approximately 6-fold within 10 hours (Lush and Hynes 1973). Flocculation may also have influenced the reductions in RESP and BGLC seen with incubation time (Figures 2.3-2.4).

Another possible explanation for the decline in NMIN was that there was rapid decomposition of a highly labile organic N fraction within the first 2.4 h after initial sampling. After this initial reduction in NMIN, the rate of loss decreased with time, a trend typical of decomposition curves.

For RESP and BGLC, sample stability was markedly compromised as post-collection time increased (Figures 2.3-2.4). Respiration rates in OG FBOM increased slightly between 5.0 and 10.0 hours of storage at 5°C. The most dramatic change occurred 8.5 and 10.0 hours following sample collection, when RESP decreased 87% and 48% for CUT and OG respectively. Note that rates remained steady for the same time period in samples incubating at 24°C (not shown); RESP was linear from 3.0 to 9.0 hours in FBOM from OG and CUT stream reaches: $r^2 = 0.99$ for both ($p \leq 0.0001$, n=8).

Respiration measurements were not amended and thus represent both microbial activity and substrate availability. A reduction in substrate availability as a limiting factor to respiration is suggested by the decline in BGLC activity. Beta-glucosidase activity decreased 80% and 46% between 4-6 hours of storage for CUT and OG respectively, approximately 2 hours prior to the decline in RESP (Figure 2.4). Beta-glucosidase hydrolyzes high molecular weight polysaccharides, generated by cellobiohydrolases, to low molecular weight glucose monomers which are directly available for bacterial uptake (Münster 1991). Evidence exists that enzymatic hydrolysis of polysaccharides and aquatic microbial uptake of sugars, such as glucose, are linked (Chróst and Rai 1993, Chróst

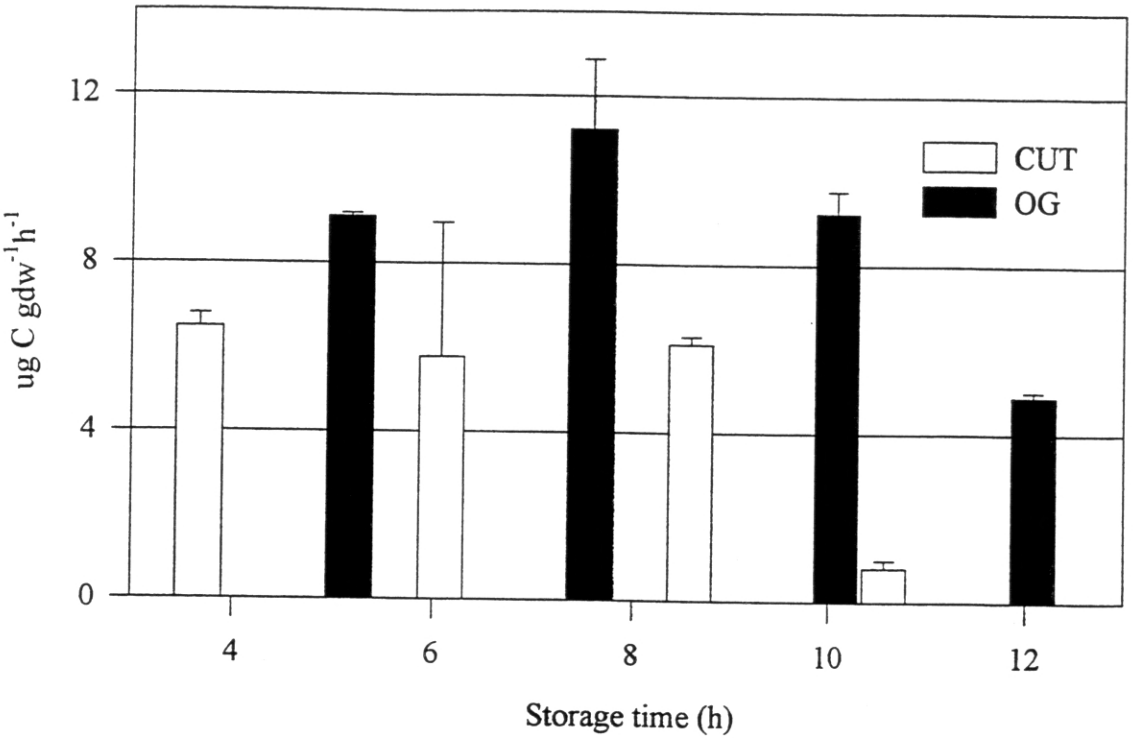


Figure 2.3. Time series results for respiration rates. Storage time = time lapse from stream collection to start of incubation. Error bars = 1 standard deviation.

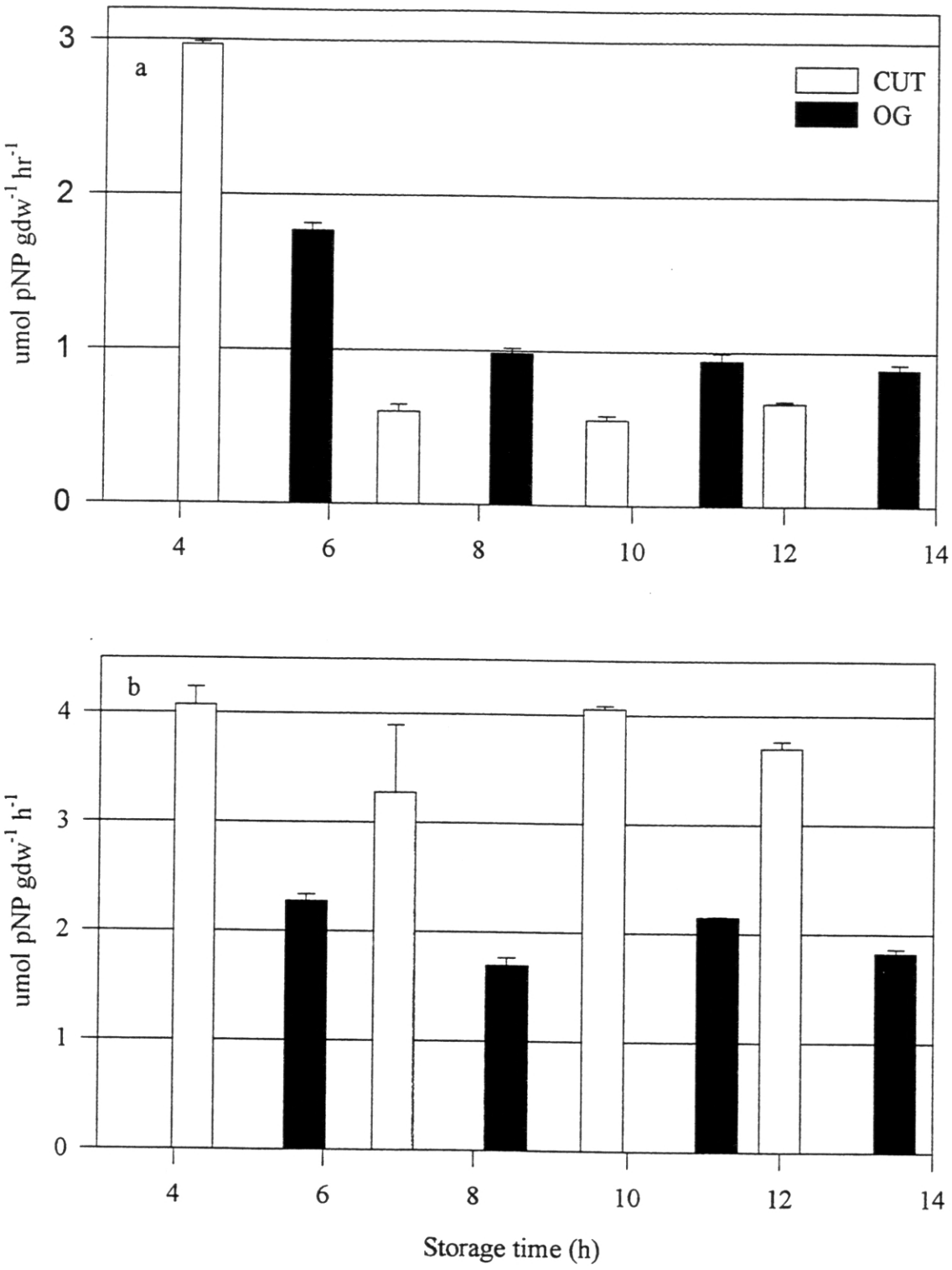


Figure 2.4. Time series results for (a) ß-glucosidase and (b) phosphatase activity rates. Storage time = time lapse from stream collection to start of assay incubation. Error bars = 1 standard deviation.

1990, Chróst 1989). A decline in BGLC activity and subsequent decrease in available substrate for uptake could reduce microbial growth and respiration.

Apparent decreases in activity could also be an artifact of substrate competition. Disturbance due to stirring the slurry may have increased the natural substrate available. Since the assay measured only synthetic substrate, preferential utilization of natural substrate by ß-glucosidase prior to use of synthetic substrate (*p*-nitrophenyl) added during assay would lead to an apparent decrease in activity rates due to direct competition between the natural and added artificial substrates. Prolonged exposure to low temperature (5°C storage versus 10°-14°C *in situ*) may also have lead to decreases in BGLC activity. In lake water with an *in situ* temperature of 7.5°C, low incubation temperatures (2°-15°C) significantly decreased BGLC activity and rates increased linearly up to the optimal temperature of 28°C (Chróst 1989). Note that in this assay, BGLC did incubate for 2 h at 30°C following storage.

Phosphatase activity remained stable for at least 12 h storage following collection (Figure 2.4). In stream bed sediments, PHOS was stable for 5 days at *in situ* temperatures (Marxsen and Schmidt 1993). Similar results have been found in soils (Speir and Ross 1975, Pettit et al. 1977, West et al. 1988). Phosphatase may be more stable when stored and more resistant to flocculation than ß-glucosidase, as a greater percentage of ß-glucosidase is associated with the cell-surface of heterotrophic bacteria than phosphatase. Dissolved free ß-glucosidase and phosphatase, unassociated with microorganisms, comprised 0-30% and 18-100% of total extracellular enzyme activity, respectively (Chróst 1989 and 1990, Wetzel 1991).

After assessing the stability of various microbial and chemical characteristics of FBOM during storage and analysis, we used these methods to examine within stream variability over time. Microbial activities were measured in 14 streams at two sample dates separated by 8 days. Despite the inherent heterogeneity of the stream environment, measures of microbial activity and C:N ratios from a given stream were generally repeatable during a one week interval, allowing in-stream comparisons through time and between-stream comparisons of FBOM biochemical properties. Correlations (r) between results from two sample dates 8 days apart were significant ($p \leq 0.05$, n=14 except C:N,

n=9) for DNIT=0.96, RESP=0.60, BGLC=0.66, and C:N=0.87. Those variables not showing significant correlations between the two sample dates were NFIX and PHOS, r=0.42 and r=0.30 respectively, n=14. With two exceptions, acetylene reduction rates ranged from 0.1 to 30.1 μmol C_2H_4 gdw^{-1} h^{-1}. When these two outliers were removed from analysis, the correlation for NFIX rates increased from r=0.30 to r=0.97 ($p \leq 0.05$, n=12).

A major concern was that FBOM sample heterogeneity within a given stream would be so high that consistent patterns of microbial activity among streams would not be reproducible at different sample times. It can be concluded from this study that FBOM microbial activities and chemical characteristics are relatively constant during the summer when low flows and small allocthonous inputs result in relatively stable conditions. This may not, however, be the case during periods of high flow when these variables may change rapidly over a 1 week period (Bonin, unpublished data). Evidence exists that there is a large FBOM pool with a long residence time supplemented by FBOM from seasonal events such as autumn inputs of fresh litter or spring algal blooms (Cushing et al. 1993) which could greatly influence microbial activities over relatively short time periods during periods of higher flow.

Conclusions

In summary, the application of methods of soil and water analyses to sediments requires reevaluation of the effects of handling and storage. It was found that FBOM analyses potentially varied in their robustness and reaction to different factors such as storage time and disturbance. Extractable ammonium, DNIT and PHOS remained stable for 12 hours of storage following collection. Acetylene reduction rates, RESP, BGLC, and NMIN varied with storage time. Results from biochemical analyses of FBOM from stream reaches were generally repeatable within one week's time, supporting the validity of within-stream comparisons with time and between-stream comparisons of FBOM characteristics.

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Chapter 3

Effects of Stand Age, Season, and Elevation on the Nutrients and Microbiological Characteristics of Low-Order Mountain Stream Fine Benthic Organic Matter

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Abstract

Allochthonous litter inputs are a primary source of organic matter in low-order forested streams. A major component of this litter moves through small streams as fine particulate organic matter (FPOM). Litter decomposition has been well studied, but few studies have examined benthic FPOM (FBOM) dynamics. From 14 first-order streams in the Cascade Mountains of western Oregon, FBOM was sampled over a 10 month period to investigate, (1) how FBOM is controlled by headwater vegetation, elevation and seasons, (2) the links between organic matter inputs and FBOM substrate quality and (3) the relationships between FBOM substrate quality and microdecomposer activity. Streams ran through forests in three age classes: old-growth forest (OG) dominated by Douglas-fir (*Pseudotsuga menziesii*) and western hemlock (*Tsuga heterophylla*), and young regenerating stands (YS) either 10 years old, with a large riparian herbaceous component, or 30 years old, with many stream side deciduous trees such as red alder (*Alnus rubra*). Carbon:nitrogen ratios ranged from 13-33 and were significantly correlated with total N (NTOT) ($r = -0.31$, $p \leq 0.05$) but not total C (CTOT). Decreases in C:N, CTOT, NTOT and organic P (PORG) were mirrored by reciprocal increases in respiration (RESP), ß-glucosidase (BGLC) and phosphatase (PHOS) activities and acetylene reduction (NFIX), all relative indicators of microbial activity. The lower C:N and higher DNIT, RESP, BGLC, PHOS, and mineralizable N (NMIN) observed in YS FBOM compared with OG suggests higher quality FBOM and faster decomposition rates in YS FBOM. One exception to this trend was NFIX which was greater in OG. Seasonal trends showed a major autumn deflection in C:N ratios and microbial activities, a likely result of increased leaf inputs following an early fall storm. Significantly lower C:N at high elevation (1220-1280 m) suggested the presence of more herbaceous vegetation and alder in high elevation riparian zones. Lower NTOT and CTOT and elevated DNIT, NFIX, RESP, PHOS at low elevation (580-800 m) suggested greater decomposition rates at low elevations. PORG was 2.2 and 3.6 mg P gOM^{-1} at high and low elevations respectively, a difference ($p \leq 0.05$) probably due to the young geologic age of parent material at high

elevation. The data from this study suggest a potential link between headwater forest management and stream food web dynamics as mediated by shifts in FBOM.

Introduction

Numerous studies have examined the qualitative shifts in leaf litter composition in the early to middle stages of decomposition (Petersen and Cummins 1974, Suberkropp, Godshalk and Klug 1976, Findlay and Arsuffi 1989). Changes in leaf species and composition lead to marked differences in microbial processing rates (Suberkropp and Klug 1976) and leaf and woody debris decomposition rates have been related to both litter C:N and extracellular enzyme activities (Taylor et al. 1989, Sinsabaugh et al. 1992, Sinsabaugh and Linkins 1993).

Since most organic matter moves through streams as fine particulate organic matter (FPOM) (Sinsabaugh et al. 1992), it is a potentially important link between terrestrial and aquatic environments. Although there is increasing interest in understanding FPOM dynamics, there have been few studies of factors influencing stream sediment benthic FPOM chemical or microbial characteristics. Fine benthic organic matter can vary in chemical composition by source and decay state (Ward et al. 1994) which may be reflected in changes in FBOM C:N ratios (Figure 3.1). Several studies have found few differences in FBOM microbial activity among streams running through different forest types, suggesting that headwater and riparian vegetation may have little influence in the chemical composition of FBOM (Hargrave 1972, Ward 1986, Sinsabaugh and Linkins 1990, Sinsabaugh et al. 1992). It is possible that in these studies, chemical differences in FBOM attributable to differences in stream-side vegetation may be swamped by FBOM generated from readily degradable stream algae, herbaceous plants or alder leaves during litter processing (Suberkropp and Klug 1980, Ward 1984).

The purpose of this study was to examine the fundamental relationship between FBOM nutrient availability, or substrate quality, and microdecomposer activity and to investigate the link between organic matter inputs and FBOM substrate quality. To this

end, we compared the qualitative characteristics of FBOM from streams flowing through stands with riparian zones dominated by coniferous, deciduous or herbaceous vegetation at two elevations for one year.

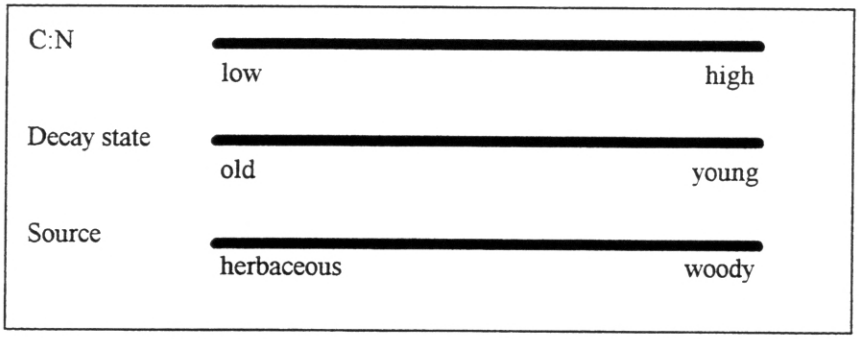


Figure 3.1 Relationship between FBOM C:N ratios and the decay state and vegetative source of particles, viewed as a continuum.

Methods

Site Description

FBOM samples were collected from 14 first-order streams flowing through forest in three successional age classes: three Douglas-fir *(Pseudotsuga menziesii)* stands approximately 10 years old (10YS), five 30 year old Douglas-fir stands (30YS) and six old-growth forest (OG) dominated by Douglas-fir and western hemlock *(Tsuga heterophylla)* in the H.J. Andrews Experimental Forest, in the Western Cascade Mountains of Oregon. Riparian vegetation of the eight young stands was dominated by herbaceous plants (10YS) or deciduous trees (30YS), primarily alder (*Alnus rubra*) and maple (*Acer macrophyllum*, *A. circinatum*). No riparian vegetation buffers were left on harvested stands and all stands had been replanted with Douglas-fir seedlings. Streams at high (1220-1280 m) and low elevations (580-800 m) were selected.

Sample collection, preparation and storage

Streams were sampled for FBOM at the same locations on 8 occasions: August 9, October 14, October 28, November 4, November 18 and December 9, 1995, and April 6 and May 12, 1996. FBOM was collected from stream beds with a hand vacuum pump into a 2 L collecting jar. The intake line was fitted with a 1 mm stainless steel screen, allowing benthic material to be wet-sieved during sampling. Samples were transferred to polystyrene jars (500 ml) and stored during field sampling in an insulated chest with stream water and ice. In the laboratory, a slurry was prepared by decanting excess stream water from the jars and mixing, keeping FBOM suspended while subsampling. Subsamples were dispensed using 1, 3, or 5 ml plastic syringes with enlarged openings. All laboratory analyses involving slurry began immediately upon return from FBOM collection which took 8-12 hours.

Laboratory analyses

Gas Analyses

Denitrification potential (DNIT) was determined as N_2O emission while anaerobically incubating FBOM supplemented with glucose and $NaNO_3$ (Martin et al. 1988). Duplicate 5 ml FBOM slurry samples in 25 ml Erlenmeyer flasks were capped with rubber stoppers and purged for 3 minutes with argon at 120 cc/min. During the purging, flasks were gently shaken to ensure removal of entrapped air bubbles. The flasks were incubated for one hour at 24°C and then 2 ml of sterile solution of 1 mM glucose and 1 mM $NaNO_3$ were injected through the stopper, with 2 ml of headspace subsequently removed. Flasks were incubated for another hour at 24°C. At one and three hours, 0.5 ml headspace gas was assayed for N_2O using a gas chromatograph (GC) equipped with an electron capture detector. Results were expressed as μg N gOM^{-1} h^{-1}.

Respiration (RESP) was measured on duplicate 5 ml FBOM slurry samples in 25 ml Erlenmeyer flasks capped with rubber stoppers. Following one hour pre-incubation at 24°C, 0.5 ml headspace samples after one and three hours were analyzed for CO_2 on a GC fitted with a thermal conductivity conductor. Results were calculated as μg C gOM^{-1} h^{-1}.

Putative nitrogen fixation rates (NFIX) were determined using the acetylene reduction method (Weaver and Danso 1994) with samples prepared as for DNIT except the headspace was replaced with 1.5% O_2, 12.5% acetylene and 86% argon. After 24 hours of incubation, 0.5 ml of headspace gas was removed and analyzed for ethylene on a GC fitted with a flame ionization detector. A control without acetylene was analyzed for endogenous ethylene production. Results were expressed as μmol C_2H_4 gOM^{-1} h^{-1}.

Nitrogen Chemistry

Mineralizable nitrogen (NMIN) measurements were determined using the 7 day anaerobic incubation method (Keeney 1982). Duplicate 10 ml FBOM samples in 50 ml screw-topped test tubes were filled with deionized water (53ml), capped and incubated at 40°C for 7 days. After incubation an equal amount of 4 M KCL was added, shaken for 1 hour in the presence of 0.4 ml 10M NaOH and analyzed for NH_4-N using a selective ion electrode (Corning ammonium electrode, Medford, MA).

Ammonium (EA) was extracted by adding 50 ml of 2 M KCL to duplicate 10 ml samples in 250 ml Erlenmeyer flasks. Flasks were capped, shaken while incubating for 1 hour in the presence of 0.4 ml 10M NaOH and analyzed using a selective ion electrode to determine KCL-extractable ammonium concentration. Net mineralization was calculated as NMIN - EA and NMIN and EA were expressed as mg NH_4-N gOM^{-1}.

Labile Polysaccharide Concentrations

Non-cellulose polysaccharide content (PSAC) of air-dried FBOM was estimated by colorimetric determination of total sugars (Lowe 1993) and reported as mg glucose equivalent gOM^{-1}.

Enzyme Activities

Phosphatase activity (PHOS) was determined using the spectrophotometric assay of Tabatabai and Bremner (1969), as modified by Zou et al. (1992). One ml of 50 mM *p*-nitrophenyl phosphate substrate was added to duplicate 1 ml subsamples containing suspended FBOM. The tubes were shaken and then placed in a 30°C waterbath for 1 hour, along with duplicate controls with no phosphatase substrate addition. After incubating, 1 ml of 50 mM *p*-nitrophenyl phosphate was added to the controls and all reactions were immediately stopped with the addition of 0.5 ml of 0.5M $CaCl_2$ and 2 ml of 0.5 M NaOH. The mixtures were centrifuged for 5 minutes at 500 x g. From the supernatant, 0.2 ml of solution was diluted with 2.0 ml deionized water and the optical density measured at 410 nm. A standard curve was prepared from 0.02-1.0 μmol ml^{-1} *p*-nitrophenol. Enzyme activity was expressed as μmol *p*-nitrophenol released gOM^{-1} h^{-1}.

ß-glucosidase (BGLC) followed the same general procedure as for PHOS, except the substrate used was 1 ml of 10 mM p-nitrophenyl ß-D glucopyranoside, incubation period was 2 hours, and 2 ml of 0.1 M tris[hydroxymethyl]aminomethane at pH 12.0, instead of 0.5 M NaOH, were added to terminate the reaction.

Total Carbon and Nitrogen

Total carbon and nitrogen (CTOT and NTOT) were determined by dry combustion with a Carlo-Erba NA 1500 Series II high temperature combustion furnace on oven-dried subsamples ground to pass 250 μm.

Total, Inorganic, and Organic Phosphorus

Total and inorganic phosphorus (PTOT and PINORG) was estimated colorimetrically in 1N H_2SO_4 extracts from dry samples ignited at 550°C and unignited samples respectively (Olsen and Sommers 1982). Organic P (PORG) was calculated by the difference PTOT-PINORG as mg P gOM^{-1}.

Statistical Analyses

Analysis of Variance

The computer program SAS 6.10 for Windows was used for all analyses of variance (ANOVA) to test for differences between treatment means and elevation means for each date. Non-parametric Spearman Rank correlations were generated between all microbial activity and nutrient availability variables.

Multivariate ANOVAs (MANOVA) were used in conjunction with univariate analyses as an additional explanatory tool to detect overall patterns over time; e.g. whether treatment or elevation differences in FBOM were significant over the entire sampling period and whether these differences were influenced by season. Repeated measures MANOVAs used SAS procedure "proc mixed" and the variance/covariance matrix resulting in the largest value for Akaike's Information Criterion for each variable (SAS Institute Inc. 1996). Repeated measures were conducted where sampling date

(TIME) and either treatment (TRT) or elevation (ELEV) were explanatory variables. There were 3 TRT levels: OG, 30YS and 10YS and two ELEV levels: HIGH (1220-1280 m) and LOW (580-800 m). Only OG and 30YS were included in repeated measures analyses for elevation effects; LOW 10YS were excluded because they had no high elevation counterpart. For all MANOVA, only samples from August-November were included. Results from December 9, April 6 and May 12 were excluded because the assumptions of repeated measures are violated by nonrandom missing data.

Principal Components Analysis

A matrix of 82 sample units (streams x dates) by 10 biochemical/activity variables and five environmental variables and site characteristics was analyzed by principal components analyses (PCA), using Euclidean distance measures and correlation on PC-ORD software (McCune and Mefford 1995). Missing data were replaced using recommendations by Tabachnik and Fidell (1989). Incomplete data led to deletion of 25 samples and three variables: DNIT, EA, and PTOT. The same variables were log-transformed for use in PCA as for (M)ANOVA. Five outliers were deleted. One had an unusually high rate of N-fixation for both duplicates on August 9; field notes remarked that this site had deep, slow moving pools, suggesting reduced oxygen concentrations could have stimulated N-fixation. Four outliers were deleted from the same 10YS which had consistently higher CTOT and NTOT than other 10YS and was the only site to have abundant devil's club (*Oplopanax horridum*).

Results

Seasonal Effects

All variables had either a significant TIME or TIME x TRT effect (MANOVA), indicating overall seasonal changes (Table 3.1) with most variables following one of two general seasonal patterns. A significant TIME main effect indicated that each treatment followed a similar seasonal pattern. A significant TIME x TRT interaction suggested that treatments changed differently over time. However, for variables with a significant interaction, treatments typically continued to follow the same seasonal pattern, suggesting that deviations from the general pattern were large enough to be statistically significant but minor when considering seasonal trends.

One pattern, typified by C:N (Figure 3.2), showed high ratios in August and early October, with a rapid, significant drop on October 28, and a recovery in early November. Similar patterns were observed for CTOT, NTOT, DNIT and PORG (Figures 3.2-3.4).

The second pattern (Figures 3.3-3.5), a reciprocal to the first, was found for NFIX, PTOT, RESP, BGLC, PHOS, and PSAC (Figures 3.3-3.5). Instead of decreases on October 28 and November 18, these variables pulsed upward, then returned to pre-pulse levels and generally remained steady until spring, when there was an upswing.

Extractable ammonium and NMIN did not follow the above trends, although missing data made patterns more difficult to interpret. EA and NMIN appeared to follow similar trends from November-May, reaching some of their lowest values in April and then increasing in May (Figure 3.3). EA and NMIN most differed in early October when concentrations increased for EA and decreased for NMIN. When EA and NMIN were normalized to total N instead of OM, seasonal patterns were similar.

Table 3.1. Repeated measures MANOVA with sampling date (TIME) and treatment (TRT) as main effects. Values represent least squared means or medians for log-transformed (†) variables. * $p \leq 0.1$, ** $p \leq 0.05$, *** $p \leq 0.01$. n=number of observations.

Variable	Units	Abbreviation	OG	30YS	10YS	Significance			n
						TIME x TRT	TRT	TIME	
C:N	no units	C:N	26	22	22	0.4388	0.2689	0.0001***	62
total carbon	%	CTOT	12.0	13.1	12.7	0.1871	0.8887	0.0001***	62
total nitrogen	%	NTOT	0.48	0.64	.60	0.0344**	0.5532	0.0001***	62
denitrification potential†	$\mu g\ N\ gOM^{-1}\ h^{-1}$	DNIT	30	50	67	0.0110**	0.1325	0.1231	49
mineralizable N†	$mg\ NH_4\text{-}N\ gOM^{-1}$	NMIN	2.1	2.4	2.3	0.0660*	0.7453	0.0001***	48
extractable ammonium	$mg\ NH_4\text{-}N\ gOM^{-1}$	EA	0.18	0.20	0.21	0.7611	0.2437	0.0001***	50
acetylene reduction†	$\mu mol\ C_2H_4\ gOM^{-1}\ h^{-1}$	NFIX	38	27	22	0.0001***	0.5650	0.0001***	52
respiration†	$\mu g\ C\ gOM^{-1}\ h^{-1}$	RESP	77	80	95	0.1423	0.1118	0.0001***	62
ß-glucosidase†	$\mu mol\ pNP\ gOM^{-1}\ h^{-1}$	BGLC	35	71	53	0.9889	0.0201**	0.0001***	62
phosphatase†	$\mu mol\ pNP\ gOM^{-1}\ h^{-1}$	PHOS	176	203	219	0.3309	0.0182**	0.0001***	62
labile polysaccharides	$mg\ glucose\ equivalent\ gOM^{-1}$	PSAC	261	225	274	0.0001***	0.6277	0.0001***	61
total P	$mg\ P\ gOM^{-1}$	PTOT	8.4	9.4	10.4	0.0389**	0.4484	0.0001***	51
organic P	$mg\ P\ gOM^{-1}$	PORG	2.4	2.4	2.5	0.2290	0.9910	0.0001***	51

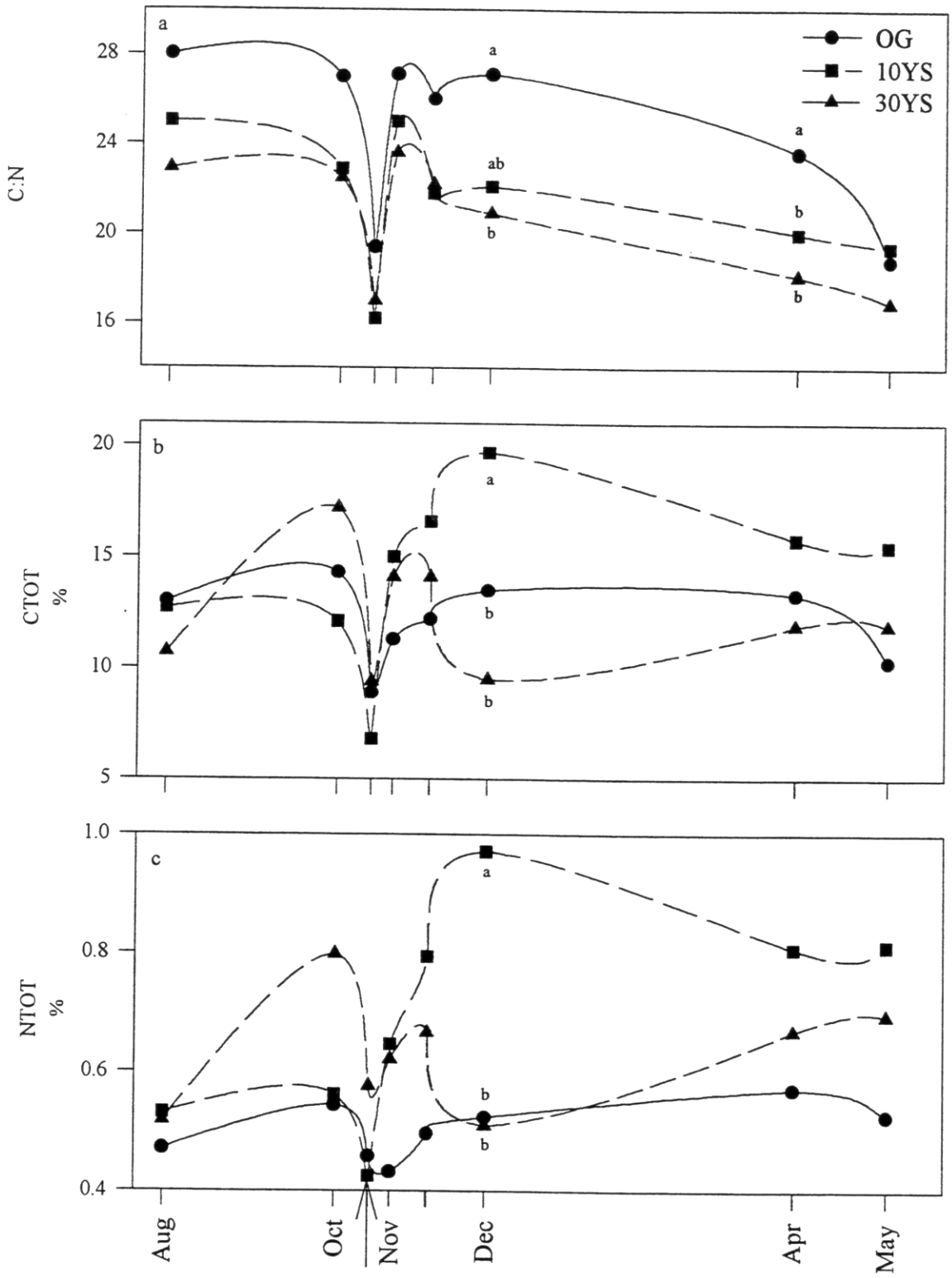


Figure 3.2. Mean values by treatment for FBOM a) C:N; b) total C; and c) total N. Different letter for same date indicates significant difference among treatments by ANOVA ($p <= 0.05$). Arrow points to October 28, 1995 sampling.

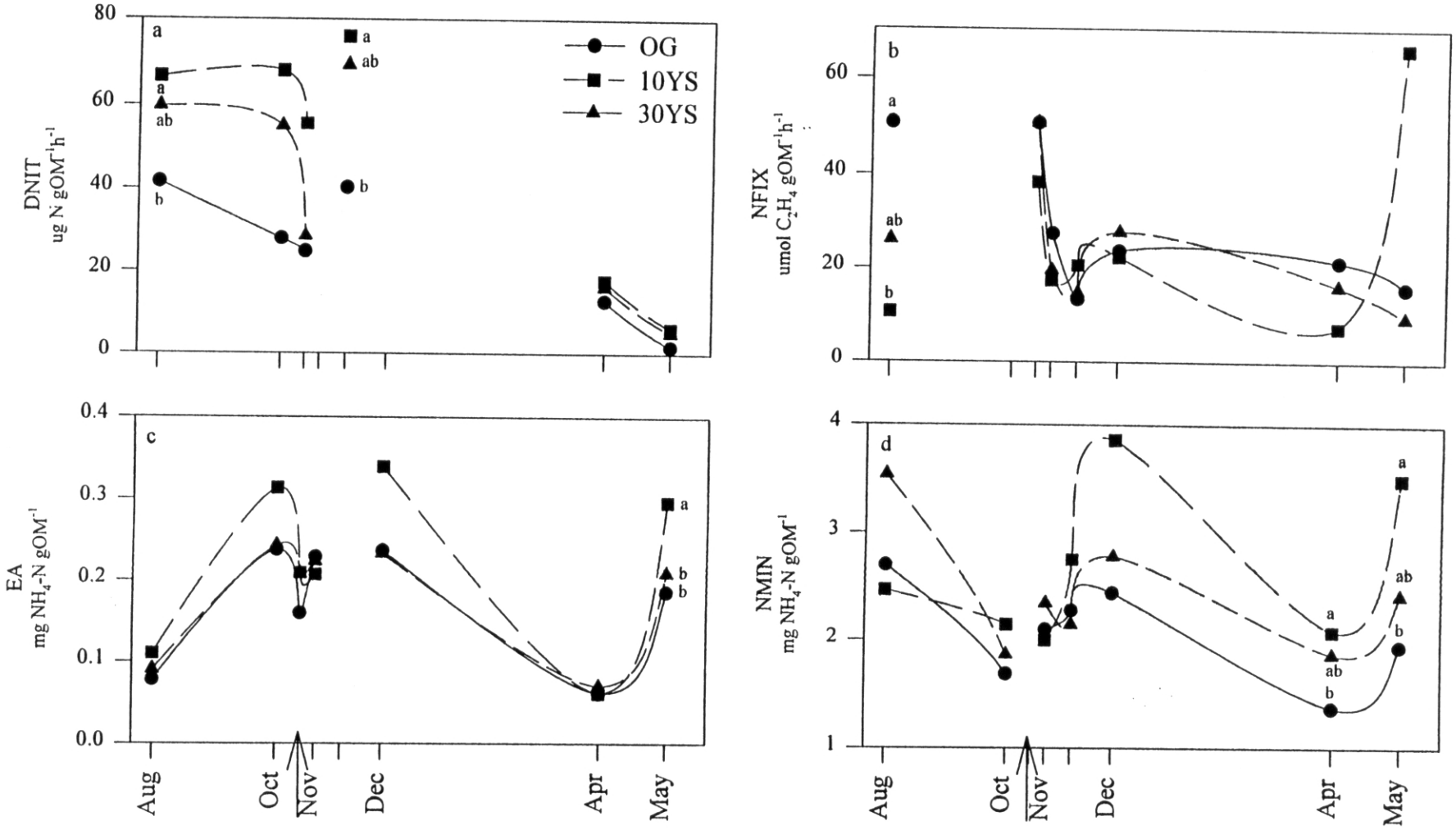


Figure 3.3. Mean values by treatment for FBOM a) denitrification potential; b) acetylene reduction; c) extractable ammonium; and d) mineralizable N. Different letter for same date indicates significant difference among treatments by ANOVA ($p<=0.05$). Arrows point to October 28, 1995 sampling.

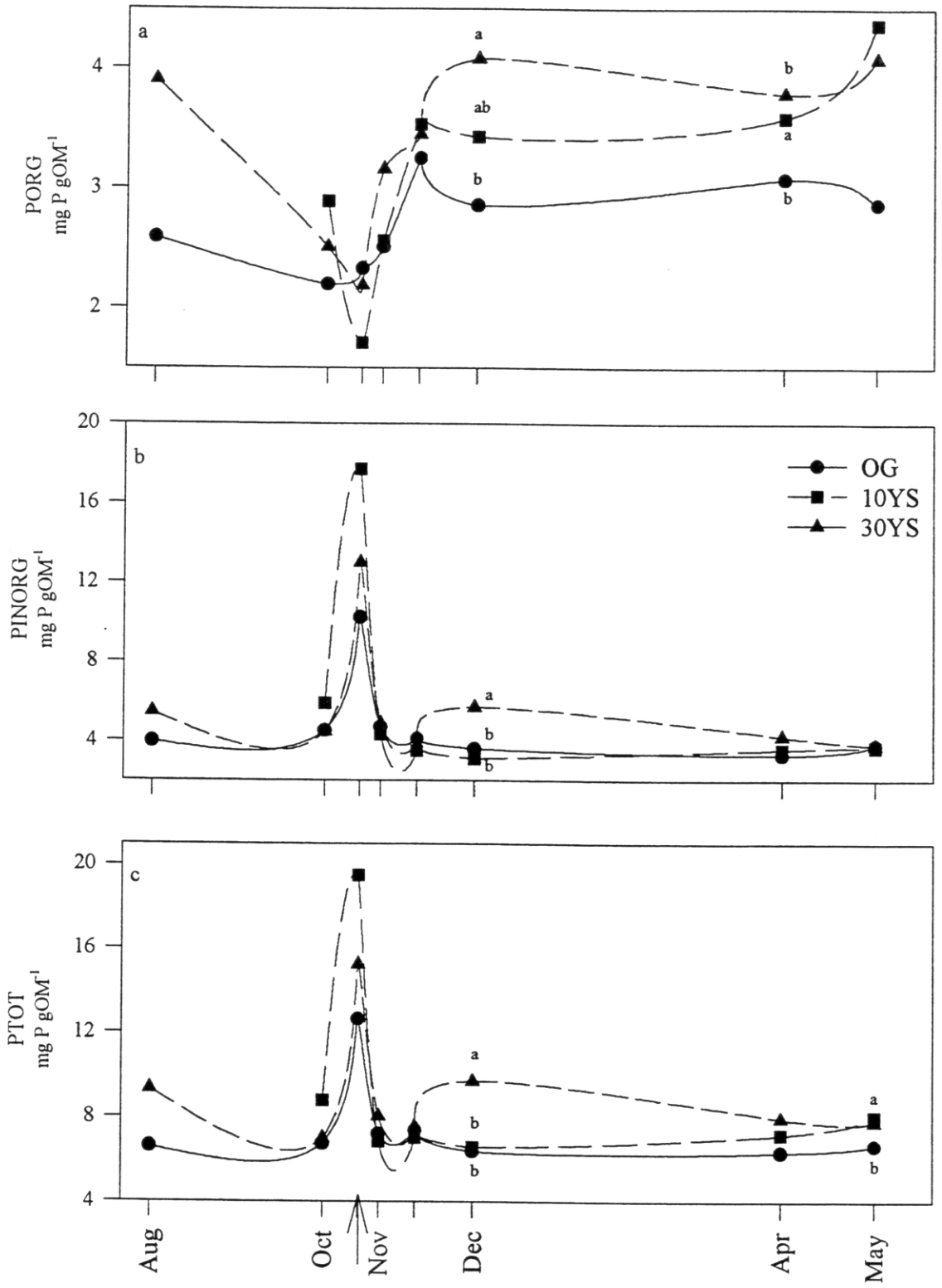


Figure 3.4. Mean values by treatment for FBOM a) organic P; b) inorganic P; and c) total P. Different letter for same date indicates significant difference among treatments by ANOVA ($p <= 0.05$). Arrow points to October 28, 1995 sampling.

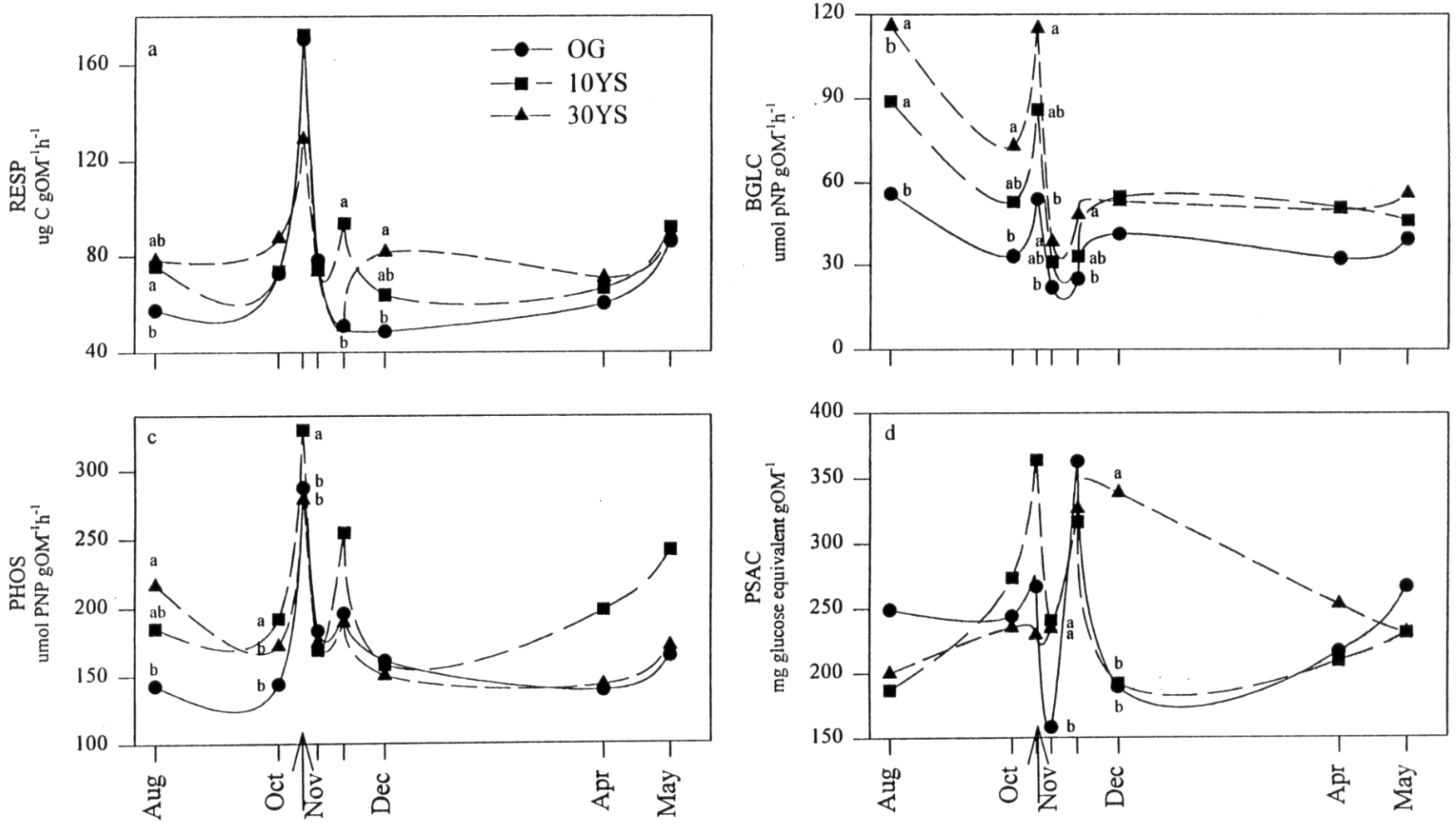


Figure 3.5. Mean values by treatment for FBOM a) respiration rates; b) ß-glucosidase activity; c) phosphatase activity; and d) labile polysaccharide concentrations. Arrows point to October 28, 1995 sampling.

Treatment Effects

The two enzyme activities showed significant TRT effects and six other variables showed significant TIME x TRT interactions (Table 3.1). In most cases, averages for 30YS fell between those observed for OG and 10YS.

Total Carbon and Nitrogen

Total C varied from 4.2-27.8%, although average values were very similar: 12.0, 13.1, and 12.7% for OG, 30YS and 10YS respectively. Total N was typically lowest in OG and, averaged over time, was 0.48, 0.64 and 0.60% for OG, 30YS and 10YS. Old-growth and 30YS showed significantly lower CTOT and NTOT values than 10YS during December (ANOVA, $p \leq 0.05$).

Overall, no significant treatment effect for FBOM C:N were found, however, mean ratios were highest in OG: a pattern repeated for 7 of the 8 sampling dates (Figure 3.2). Ratios averaged 26, 22, and 22 for OG, 30YS and 10YS respectively and varied from 13-33 (Table 3.1). When divided into 5 categories (Table 3.2), HIGH OG, LOW OG, HIGH 30YS, LOW 30YS, and LOW 10YS, to remove elevation influences, C:N was higher in HIGH and LOW OG than HIGH 30YS and LOW 30YS (MANOVA, $p \leq 0.1$, results not show).

Nitrogen assays

Denitrification potential was consistently lowest in OG FBOM and highest in 10YS (Figure 3.3). Differences between OG and 10YS were large at the beginning of the study and minimal by spring. Rates varied from 1 to 144 μg N gOM^{-1} h^{-1} and averaged 30, 50 and 67 μg N gOM^{-1} h^{-1} in OG, 30YS and 10YS respectively (Table 3.1).

Table 3.2. Mean or median (†) values ($\bar{x}$) and range for each variable, separated by both TRT and ELEV.

Variable	$\bar{x}$ Range				
	OG		30YS		10YS
	Low	High	Low	High	Low
stream temperature (°C)	7.7 5.0-11.5	5.3 4.0-8.0	7.8 6.0-11.0	5.6 4.0-9.5	8.3 5.0-11.0
C:N	26 19-33	22 15-27	24 16-30	20 13-26	21 13-32
CTOT	10.0 6.4-15.3	16.2 7.4-24.2	10.7 5.2-19.3	14.2 8.3-22.2	14.0 4.2-27.8
NTOT	0.86 0.25-0.53	0.73 0.49-0.96	0.45 0.30-0.72	0.75 0.46-1.40	0.69 0.29-1.39
DNIT†	35 1-108	19 5-49	60 4-114	41 7-93	54 6-144
NMIN†	2.3 1.2-3.2	1.7 1.1-2.3	2.3 1.5-4.1	2.1 1.3-3.6	2.6 1.5-5.0
EA	0.18 0.04-0.31	0.17 0.09-0.26	0.18 0.06-0.36	0.20 0.05-0.32	0.22 0.05-0.50
NFIX†	33 1-126	18 8-67	28 11-64	20 9-58	20 7-165
RESP†	71 38-177	69 38-360	80 42-159	72 40-186	81 43-219
BGLC†	39 11-71	42 21-62	55 41-136	62 21-202	55 17-150
PHOS†	183 121-288	140 93-180	213 134-397	185 146-357	181 140-423
PSAC	260 141-417	201 117-305	273 145-377	244 101-349	254 120-560
PTOT	6.6 4.3-10.9	8.6 5.8-17.4	6.6 4.2-9.8	10.6 5.6-20.2	8.3 5.2-19.0
PORG	2.3 1.4-3.3	3.4 2.0-4.7	2.3 1.0-3.2	3.8 1.6-5.7	3.3 2.1-5.1

Like DNIT, EA and NMIN were lowest in FBOM from streams flowing through old-growth (Figure 3.3). Averaged over time, NMIN was 2.1, 2.4, and 2.3 mg NH_4-N gOM^{-1} and EA was 0.18, 0.20 and 0.21 mg NH_4-N gOM^{-1} in OG, 30YS and 10YS respectively (Table 3.1). In December, April and May, NMIN in 10YS was significantly greater than in OG (ANOVA, $p = 0.06$, 0.07 and 0.06 respectively). These dates were excluded from MANOVA due to nonrandom missing data.

Acetylene reduction followed a seasonal trend opposite of other N assays and averaged 38, 27 and 22 μmol C_2H_4 gOM^{-1} h^{-1} in OG, 30YS and 10YS respectively.

Enzyme Activity

Old-growth FBOM had lower activities than FBOM from younger stands (Figure 3.5). Averaged over time, FBOM respiration rates were 77, 80 and 95 μg C gOM^{-1} h^{-1} for OG, 30YS and 10YS respectively and varied from 38 to 360 μg C gOM^{-1} h^{-1} (Table 3.2). ß-glucosidase and PHOS were the only variables to have an overall treatment effect (MANOVA, $p < 0.05$) (Table 3.1). Averaged over time, PHOS was 176, 203, and 219 μmol pNP gOM^{-1} h^{-1} and BGLC was 35, 71, and 53 μmol pNP gOM^{-1} h^{-1} in OG, 30YS and 10YS respectively (Table 3.1).

Labile polysaccharides

On average, labile polysaccharides were highest in 10YS (Table 3.1), but no obvious treatment effects were apparent (Figure 3.5).

Total and Organic Phosphorus

Total and organic P were typically lowest in OG (Figure 3.4). Averaged over time, PTOT was 8.4, 9.4 and 10.4 mg P gOM^{-1} and PORG was 2.4, 2.4 and 2.5 mg P gOM^{-1} in OG, 30YS and 10YS respectively (Table 3.1). By date PORG in 10YS was sometimes significantly higher than in OG and 30YS. On December 10, concentrations were 3.4 and 2.5 mg P gOM^{-1} in 10YS and OG respectively (ANOVA, $p = 0.02$), and similar results were seen on April 6 and May 12. These three dates were not included in MANOVA. When divided into 5 categories (Table 3.2), PORG was higher in high and low OG sites than in their 30YS counterparts (MANOVA, $p \leq 0.01$, results not shown).

Elevation Effects

There was an overall significant TIME x ELEV or singular elevation effect for 7 of the 13 variables listed in Table 3.3 (MANOVA, $p \leq 0.05$). Variables were generally higher at low elevation. Typically, the seasonal trend evident in the figures was similar at both elevations.

Total Carbon and Nitrogen

On average, FBOM C:N was higher and both CTOT and NTOT were lower at low elevation (Table 3.3). Mean C:N was 25 and 20 in LOW and HIGH respectively and was consistently lowest at high elevation. Total C and N were 10.0 and 0.45 % at low elevation and 15.1 and 0.75 % at high elevation and showed elevation effects (MANOVA, $p < 0.05$) (Figure 3.6). Aside from a decrease in values on October 28, average values remained relatively constant at both elevations.

Table 3.3. Repeated measures MANOVA with sampling date (TIME) and elevation (ELEV) as main effects. Values represent least squared means or medians for log-transformed (†) variables over all sampling dates. * $p \leq 0.1$, ** $p \leq 0.05$, *** $p \leq 0.01$. n=number of observations.

Variable	LOW	HIGH	Significance			n
			TIME x ELEV	ELEV	TIME	
C:N	26	20	0.0027***	0.0451**	0.0001***	62
CTOT	10.9	15.1	0.8825	0.0345**	0.0005***	62
NTOT	0.45	0.75	0.7435	0.0163**	0.0113**	62
DNIT†	53	29	0.0510*	0.0908*	0.0075***	49
NMIN†	2.4	2.0	0.5157	0.2344	0.0001***	48
EA	0.19	0.19	0.0809	0.9235	0.0036***	51
NFIX†	26	21	0.6090	0.5016	0.0036***	49
RESP†	84	78	0.3117	0.4038	0.0001***	62
BGLC†	46	56	0.3519	0.4561	0.0001***	62
PHOS†	214	166	0.8095	0.0236**	0.0001***	62
PSAC	269	225	0.0078***	0.1554	0.0001***	61
PTOT	8.8	10.0	0.6600	0.3138	0.0001***	44
PORG	2.2	3.6	0.6901	0.0074***	0.0013***	44

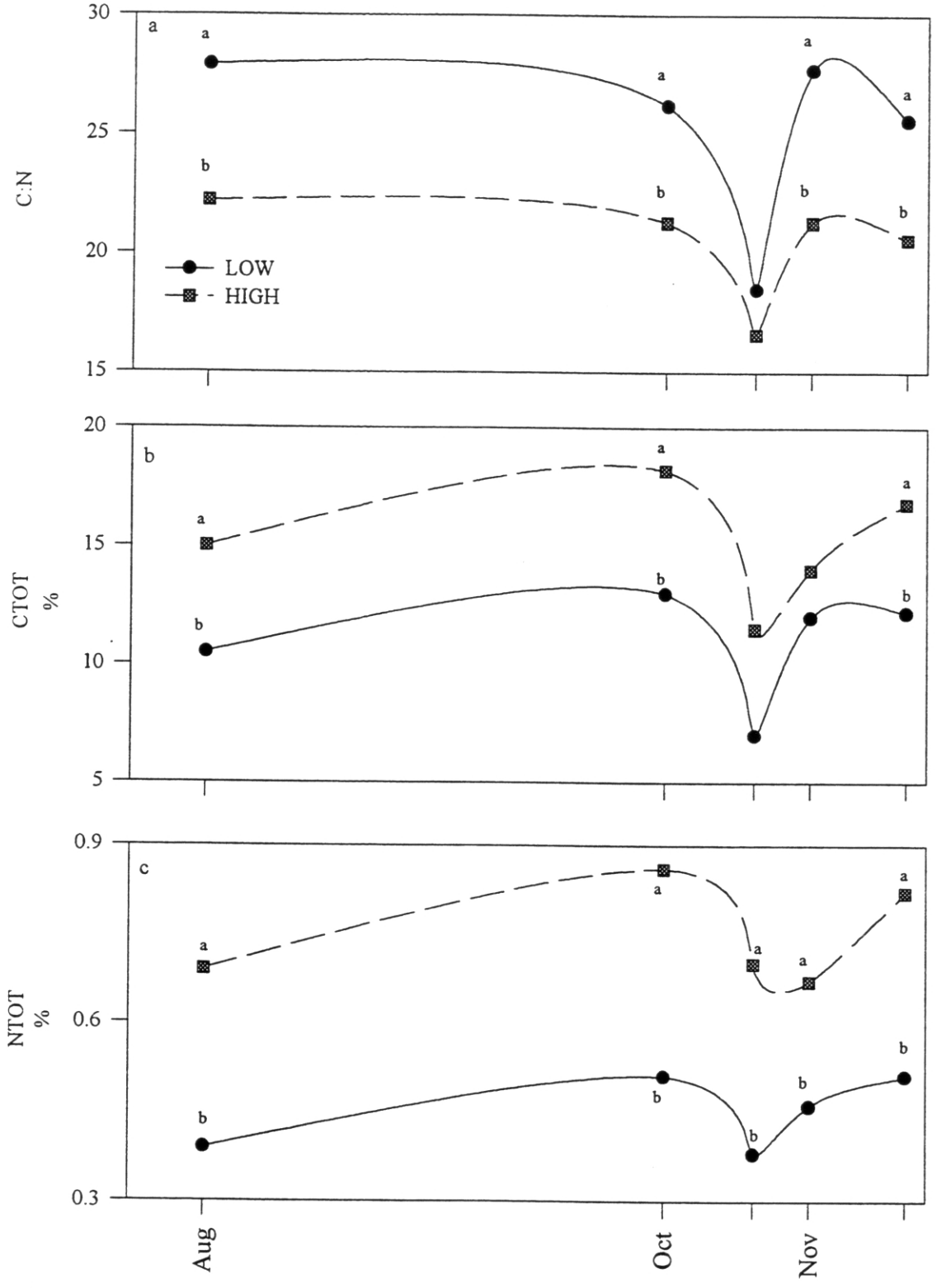


Figure 3.6. Mean values at low and high elevation for FBOM a) C:N; b) total C; and c) total N. Different letter for same date indicates significant difference among elevations by ANOVA ($p <= 0.05$).

Nitrogen assays

Like C:N, labile N was higher in FBOM at low elevations. Averaged over time, NMIN was 2.4 and 2.0 mg NH_4-N gOM^{-1} at LOW and HIGH respectively (Table 3.3). Although not significant, mean NMIN values for high elevation sites were consistently lower than those at low elevations (Figure 3.7). When NMIN was normalized to total N (%) instead of to gOM, there was an elevation effect (MANOVA, $p < 0.01$) as well as for August 9, October 14, and October 28 (ANOVA, $p \leq 0.05$, results not shown). EA had no consistent elevational trends, however stronger elevation effects were picked up when normalized to gN.

Average rates were greater in LOW versus HIGH for DNIT (53 and 29 mg N $gOM^{-1}\ h^{-1}$) and NFIX (26 and 21 μmol C_2H_4 $gOM^{-1}\ h^{-1}$) (Figure 3.7). Acetylene reduction followed the trend of the majority of variables with elevational effects observed on August 9 and November 18 (ANOVA, $p = 0.05$ and 0.09 respectively). DNIT was significantly higher in FBOM at low elevations on August 9 and October 14 and 28 (ANOVA, $p = 0.09$, 0.08, and 0.06 respectively). With the exception of the last sample date, all high elevation rates were lower than those at low elevations.

Enzyme Activity

Respiration rates and PHOS were also higher in FBOM from lower elevation streams. Although not significant, RESP rates averaged 84 and 78 μg C $gOM^{-1}\ h^{-1}$ at LOW and HIGH respectively (Table 3.3, Figure 3.8). PHOS was consistently higher at low elevations (MANOVA, $p = 0.02$, Table 3.3) and averaged 53 and 41 μmol pNP $gOM^{-1}\ h^{-1}$ at LOW and HIGH respectively. Although not significant, BGLC was typically greater at high elevation sites, unlike PHOS and RESP.

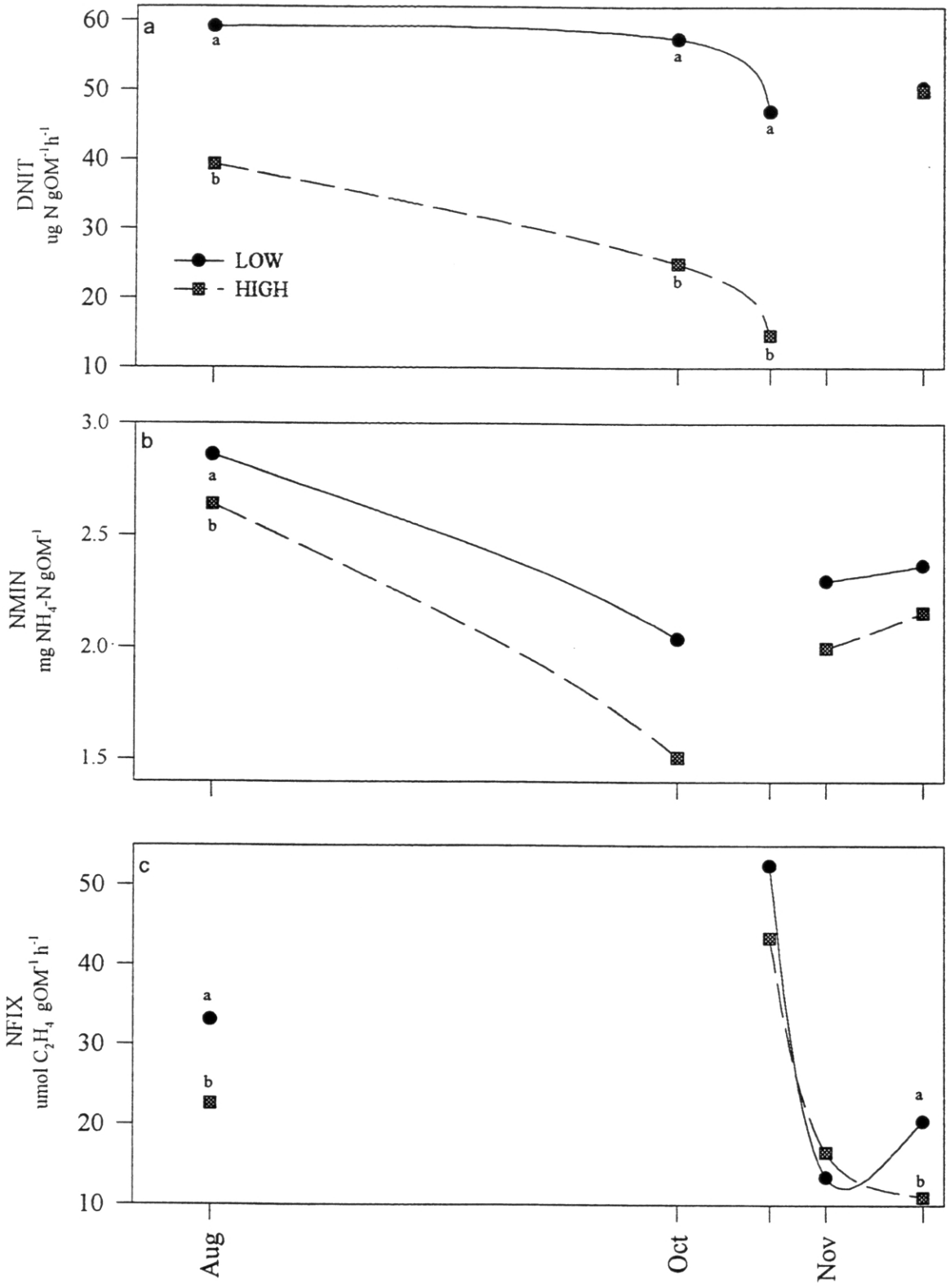


Figure 3.7. Mean values at low and high elevation for FBOM a) denitrification potential; b) mineralizable N; and c) acetylene reduction rates. Different letter for same date indicates significant difference among elevations by ANOVA ($p <= 0.05$).

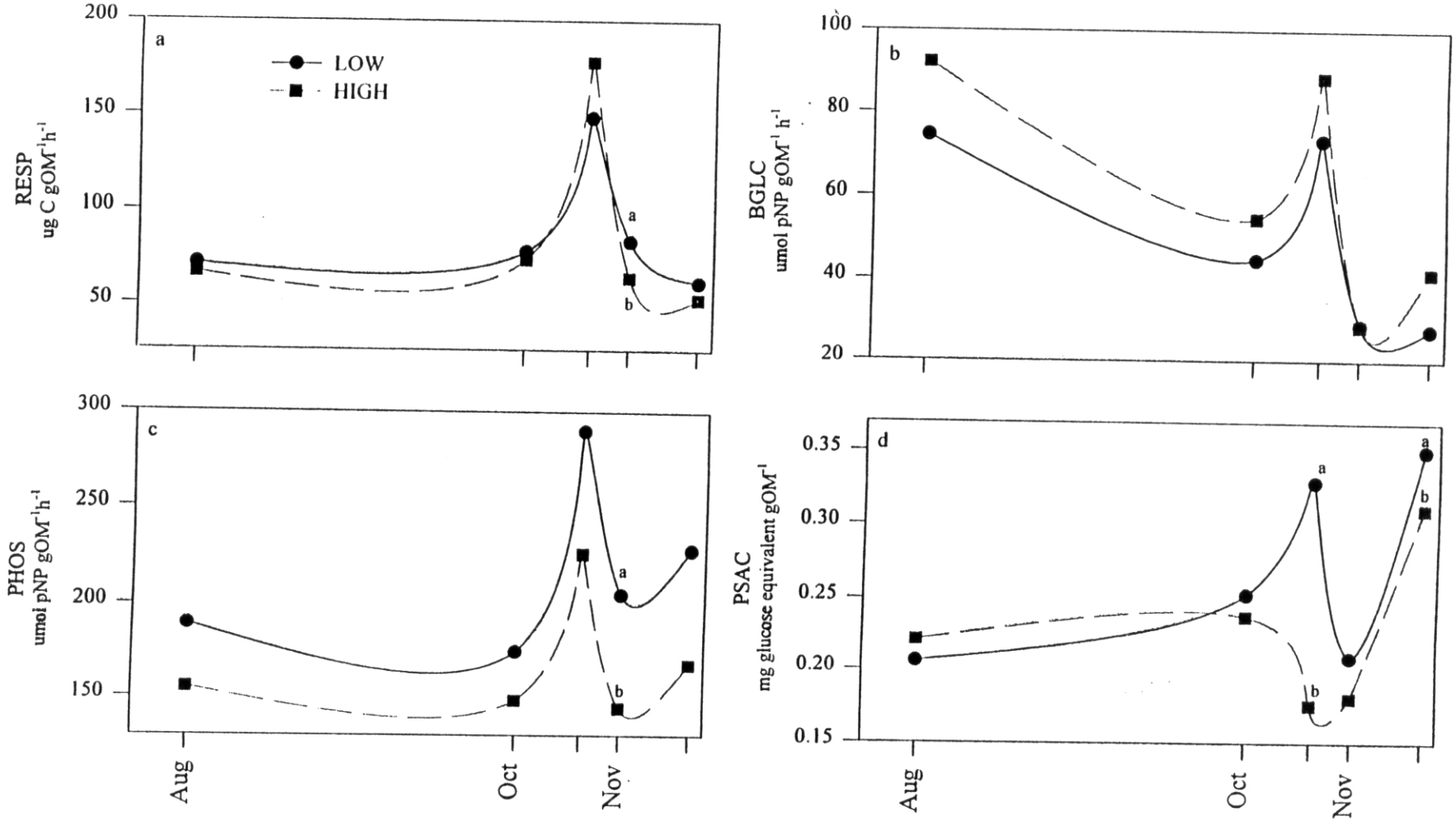


Figure 3.8. Mean values at low and high elevation for FBOM a) respiration; b) ß-glucosidase activity; c) phosphatase activity; and d) labile polysaccharide concentration. Different letter for same date indicates significant difference among elevations by ANOVA ($p <= 0.05$).

Labile polysaccharides

Labile polysaccharides averaged 269 and 225 mg glucose equivalent gOM^{-1} for LOW and HIGH respectively. Concentrations were greater (ANOVA, $p < 0.05$) at low elevation on October 28 and November 18, following the same general seasonal trend as RESP, PHOS, BGLC and PTOT (Figure 3.8).

Total and Organic Phosphorus

Total P followed the same trend as the majority of variables. Organic P appeared to follow the opposite seasonal trend, with a peak in PTOT and the lowest values for PORG on October 28 (Figure 3.9). Although means were unavailable for some sample dates, on average, PTOT and PORG were present in higher concentrations in FBOM at HIGH sites: 8.8 versus 10.0 mg P gOM^{-1} and 2.2 versus 3.6 mg P gOM^{-1} respectively (Table 3.3). For PORG, there were elevational differences (MANOVA, $p < 0.01$) but PTOT exhibited no siginificant elevation effects.

Principle Components Analysis

The first three dimensions explained 66.6 % of the variation in the data set. A comparison of broken-stick eigenvalues to observed eigenvalues indicated that the first three axes explained more variance than expected by chance alone. Table 3.4 shows loadings for each variable for axes 1-3. Axis 1 explained 31.2 % of the variation and was driven primarily by microbial activity, especially PHOS, BGLC and RESP as well as TOTC. Axis 2 explained 23.9 % and was driven by substrate quality, particularly C:N, CTOT, NTOT, and PORG. The 11.5 % of variation explained by axis 3 was driven by N availability, as shown by NMIN and NFIX.

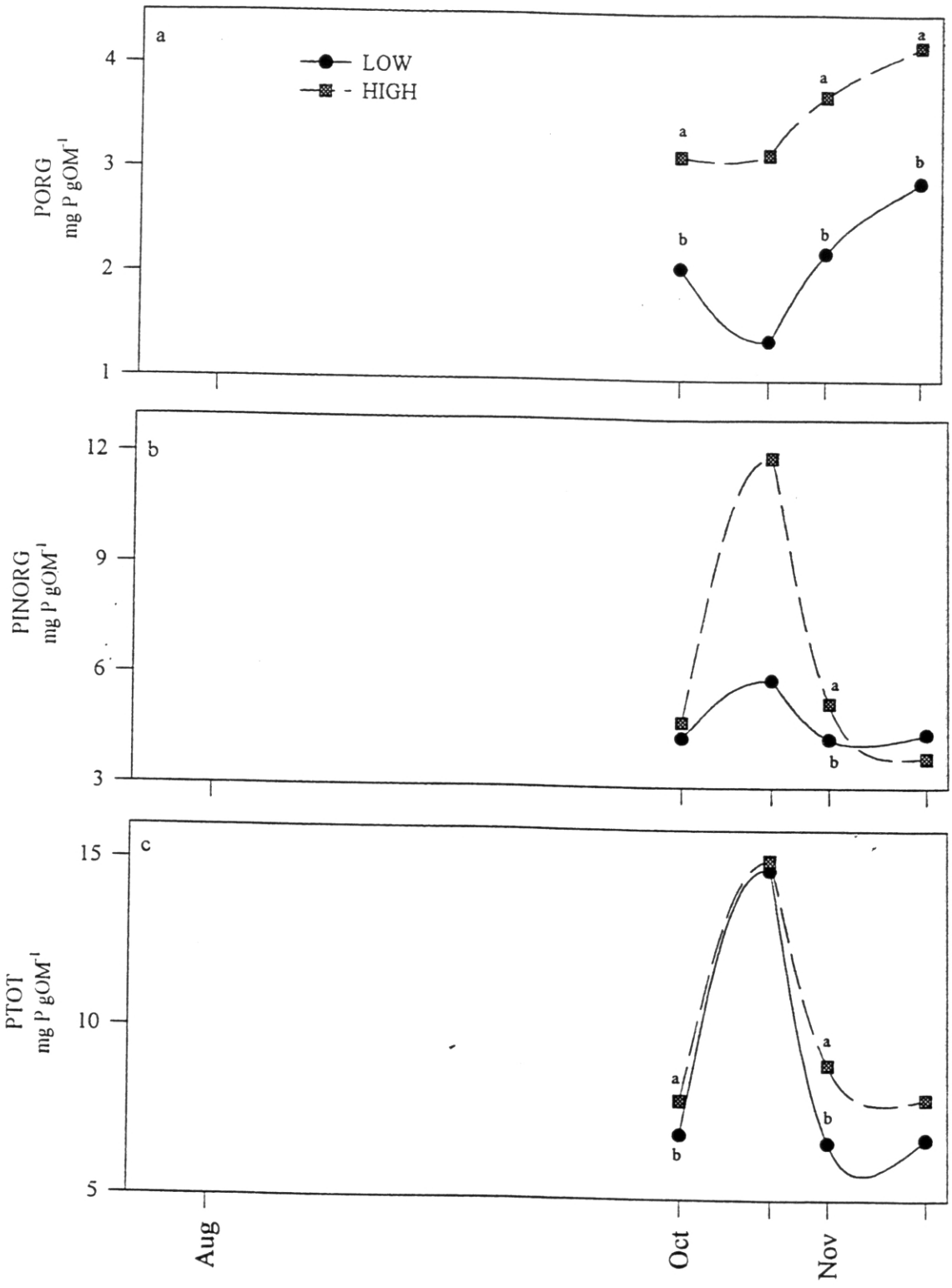


Figure 3.9. Mean values at low and high elevation for FBOM a) organic P; b) inorganic P; and c) total P concentrations. Different letter for same date indicates significant difference among elevations by ANOVA ($p <= 0.05$).

Table 3.4. Pearson Kendall correlations (r) of biochemical variables with ordination axes for PCA, n=82.

Variable	Axis 1	Axis 2	Axis 3
C:N	0.545	-0.643	0.180
CTOT	0.697	0.555	-0.060
NTOT	0.333	0.895	-0.048
NMIN	-0.499	0.229	0.608
NFIX	-0.376	-0.088	0.558
RESP	-0.653	0.170	-0.526
BGLC	-0.642	0.518	-0.105
PHOS	-0.864	0.062	0.004
PSAC	-0.482	-0.341	-0.039
PORG	0.126	0.625	0.372

Examination of TRT, TIME and ELEV in biochemical space elicit an interesting relationship between C, N, and microbial activity (Figure 3.10). The majority of FBOM samples fell into an area that had low total N and high total C (high C:N), and low activity rates, PORG and NMIN (Area I, Figure 3.10). This area included all OG (except one), low elevation sites and the majority of sample dates. In contrast, area II had low total C and N (low C:N), high activity rates and NMIN. It contained low elevation 10YS and 30YS sampled predominantly on the October 28 "pulse". This date represented a change from high C:N and low activity (date 2), to low C:N ratios and high activity rates (date 3, Figure 3.10c). Area III, which contained a small portion of samples, had low C:N as well, but total C and N and NMIN were high and activity rates were generally low, except BGLC. Most notably, area III had high ORGP. All high elevation sites had high concentrations of organic P. This area contained 30YS, 10YS and mostly high elevation sites.

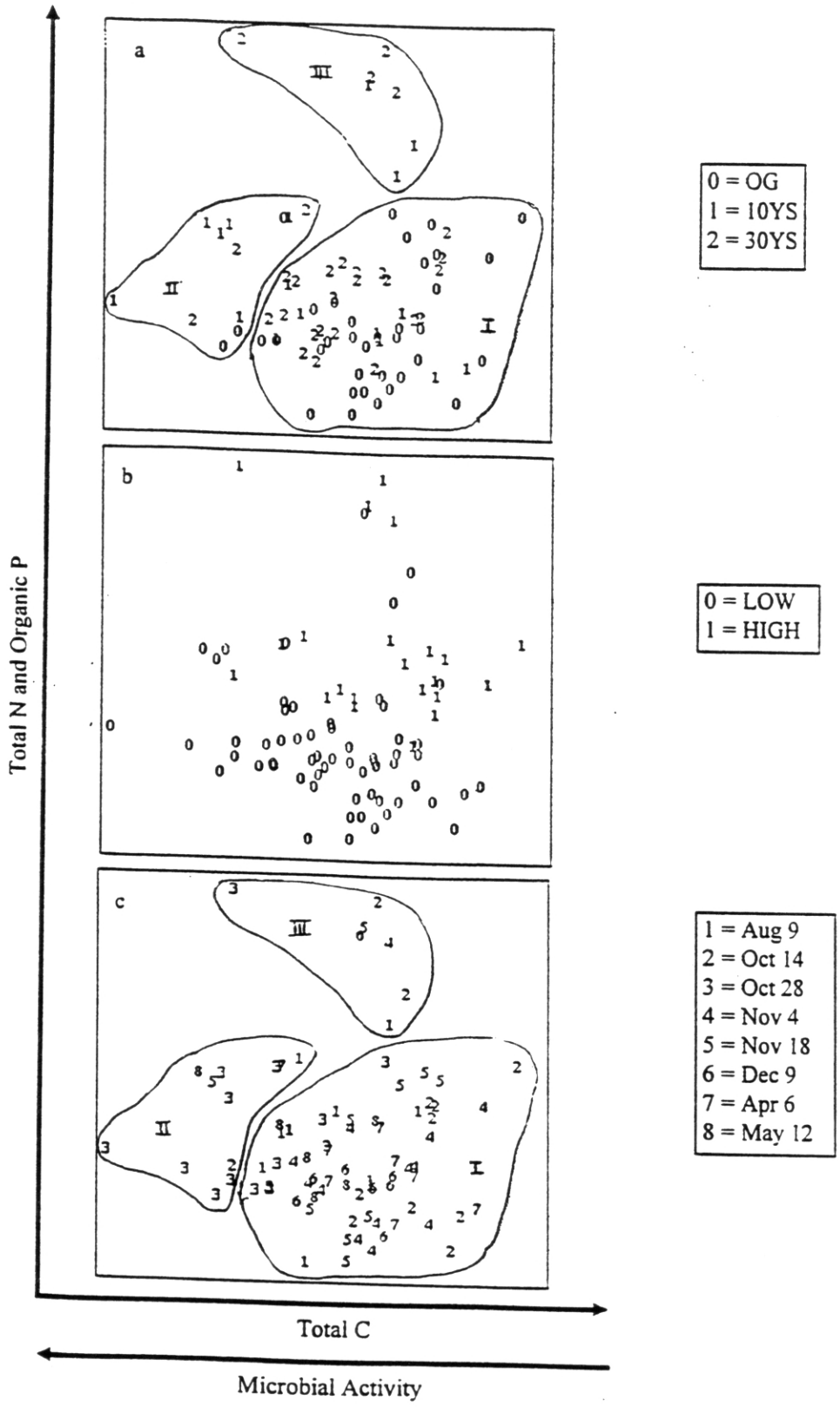


Figure 3.10 Overlays on PCA ordination of 82 sample units in chemical data space by a) treatment; b) elevation; and c) sampling date.

Discussion

FBOM Dynamics

If C:N is driven primarily by riparian vegetation detrital quality, we would expect negative correlations between C:N and measures of microbial activity and FBOM degradability. Readily decomposable litter with high N concentrations relative to C would be characterized by low C:N and high microbial activity. If C:N is influenced predominantly by the decay state of FBOM, these relationships should be positive. Low available C as well as N sequestered into humic materials would also result in FBOM with low C:N but with low microbial activity. All variables, except DNIT and NFIX ($r=0.02$ and 0.03 respectively) were inversely correlated with C:N (Table 3.5). Of these, all except EA and PSAC were significant ($p \leq 0.05$). These correlations suggest that the principle factor influencing FBOM substrate quality was not the decay state or degree of decomposition but the vegetative source (i.e., quality) of stream inputs.

Nitrogen is a known important regulator of decomposition (Taylor et al. 1989, Sinsabaugh et al. 1993) but its role in FBOM is relatively unknown. In this study, total N appears to drive C:N. Ratios were significantly correlated with NTOT, $r=-0.31$ but not with CTOT, $r=0.05$. Among treatments, variations in NTOT corresponded with lower microbial activity in OG; total N was 20-25% lower in OG (0.48%) than YS (0.60-0.64%) while average CTOT varied little among treatments. However, although NTOT and CTOT were highly correlated with each other $r=0.89$, neither was well correlated with microbial activity variables, suggesting the importance of examining both N and C availability as regulators of decomposer activity.

As a dynamic system with multiple inputs and outputs, FBOM does not appear to behave in a manner one would expect from simple decomposition dynamics. During litter decomposition, as organic mass is lost, total P increases over time, following a pattern similar to total N (Sinsabaugh et al. 1993). In laboratory streams, total P and N both

Table 3.5 Correlation coefficients between microbial and nutrient variables. Shaded variables normalized to gram dry weight (gDW). Without shade, to gram organic matter (gOM). * = significant at $p \leq 0.05$. (n) = number of observations. For any two variables, n is the same for both gDW and gOM correlations.

	C:N	CTOT	NTOT	DNIT	NMIN	EA	NFIX	RESP	BGLC	PHOS	PTOT	PORG	PSAC
C:N		0.05	-0.38*	0.10	-0.31*	-0.03	0.17	-0.31*	-0.40*	-0.22	-0.55*	-0.32*	-0.18
CTOT	0.05 (87)		0.89*	0.37*	0.73*	0.68*	0.26*	0.57*	0.70*	0.71*	0.66*	0.84*	0.70*
NTOT	-0.31* (87)	0.89* (87)		0.33*	0.80*	0.65*	0.18	0.65*	0.66*	0.80*	0.87*	0.91*	0.57*
DNIT	0.02 (65)	0.01 (65)	0.09 (65)		0.45*	0.18	0.11	0.17	0.48*	0.54*	0.33*	0.30*	0.40*
NMIN	-0.25* (73)	-0.09 (73)	0.09 (73)	0.25 (51)		0.68*	0.32*	0.67*	0.65*	0.75*	0.72*	0.74*	0.40*
EA	-0.20 (76)	0.21 (76)	0.26* (76)	0.07 (54)	0.20 (62)		0.17	0.48*	0.34*	0.49*	0.50*	0.60*	0.42*
NFIX	0.03 (74)	-0.12 (74)	-0.13 (74)	0.08 (74)	0.24 (60)	-0.09 (63)		0.28*	0.24*	0.22	0.19	0.12	0.17
RESP	-0.46* (87)	-0.29* (87)	-0.09 (87)	0.03 (65)	0.30* (73)	0.16 (76)	0.24* (74)		0.56*	0.60*	0.71*	0.48*	0.30*
BGLC	-0.55* (87)	-0.09 (87)	0.16 (87)	0.36* (65)	0.49* (73)	0.02 (76)	0.22 (74)	0.46* (87)		0.67*	0.65*	0.53*	0.31*
PHOS	-0.41* (86)	-0.48* (86)	-0.23* (86)	0.32* (64)	0.56* (72)	0.10 (75)	0.19 (73)	0.52* (86)	0.52* (86)		0.70*	0.67*	0.52*
PTOT	-0.67* (77)	-0.25* (77)	0.11 (77)	0.13 (55)	0.31* (70)	0.13 (66)	0.09 (64)	0.60* (77)	0.48* (77)	0.46* (76)		0.79*	0.34*
PORG	-0.51* (77)	0.31* (77)	0.54* (77)	-0.04 (55)	0.17 (70)	0.04 (66)	-0.23 (64)	-0.11 (77)	0.13 (77)	-0.06 (76)	0.36* (77)		0.61*
PSAC	-0.10 (85)	-0.43* (85)	-0.39* (85)	0.19 (63)	0.02 (71)	-0.01 (74)	-0.40* (85)	0.13 (85)	0.08 (85)	0.38* (84)	-0.08 (75)	-0.12 (75)	

increased over time during leaf decay (Howarth and Fisher 1976). In this study, total N and P were not significantly correlated (r=0.11). This finding is contrary to leaf litter studies, underscoring the importance of understanding FBOM dynamics.

In this study, all variables were normalized to OM, under the basic assumption that the organic fraction drives biological activity. When (M)ANOVAs and correlations were rerun with variables normalized to gram dry weight (gDW), notable changes occurred only among correlations (Table 3.5). Significant correlations ($p \leq 0.05$) were found in 62 versus 33 cases for gDW and gOM respectively. Normalizing to gDW improved correlations in 68 cases; for example, PTOT v NTOT changed from r=0.11 to 0.87, elucidating the positive relationship we would expect between these variables during decomposition. Correlations worsened when normalized to gDW in 8 cases, 6 of which where correlations with C:N. Nineteen cases changed sign from negative to positive when correlated to gOM and gDW respectively and most of these were associated with the substrate quality variables CTOT, NTOT, PORG and PSAC. Correlations for CTOT and NTOT with microbial activities were highly significant when normalized to gDW. For example, PHOS v CTOT changed from r = -0.48 to 0.71, suggesting caution is necessary when using correlations to aid understanding processes such as nutrient cycling. Overall, improvements in correlations suggest that the inorganic fraction of fine sediments affects microdecomposer activity.

Treatment effects

The range of C:N (13-33) was similar to previously reported ratios for FBOM from Cascade streams: 20-36 (Naiman and Sedell 1979). As predicted, C:N in OG FBOM tended to be higher than in YS. Wood is a major component of detrital inputs in low-order streams running through old-growth forests (Triska and Cromack 1980), resulting in higher C:N whereas YS should have more N-rich alder, herbaceous and algal inputs, driving down FBOM C:N. However, average C:N treatment differences were small (26 and 22 in OG and YS respectively). Ward (1986) found that despite qualitative

differences in allochthonous inputs to Oregon Cascade streams, FBOM lignin and cellulose concentrations were similar. One explanation for the similarity in FBOM composition was that particles were small enough to have reached a base level of decomposition. In this study, although C:N differences were not large between treatments, significant treatment differences in FBOM decomposition and nutrient availability persisted.

Detrital inputs in YS should be of higher quality and thus more readily degradable than OG woody inputs. This principle has been demonstrated in a study comparing decomposition rates of ground heartwood from red alder and Douglas-fir (Baker et al. 1983), as well as numerous litter bag studies (Suberkropp, Godshalk, and Klug 1976, Gregory 1978, Findlay and Arsuffi 1989). We expected that more readily degradable detritus with lower C:N would generate FBOM with higher microbial activities; this is what was observed. Respiration, DNIT, BGLC and PHOS were typically lowest in OG FBOM (Figures 3.3-3.5). Measuring extracellular enzymes such as BGLC and PHOS that mediate decomposition can provide more specific information about decomposition than general microbial activity measures such as RESP. ß-glucosidase, which is generally associated with fungi and bacteria, hydrolyzes disaccharides (cellobiose) generated by cellobiohydrolases from cellulose to low molecular weight glucose monomers which are directly available for microbial uptake (Münster 1991). Phosphatase hydrolyzes organic P esters to inorganic phosphate and is produced by a wide variety of biota, including bacteria, algae and fungi (Overbeck 1991). These two enzymes were the only variables in this study to have overall treatment effects (MANOVA) and harbor potential uses as means of evaluating stream recovery following harvest. Sinsabaugh et al. (1994) have successfully used enzyme activities to estimate mass loss rates of FBOM in a 4th order stream running through a mixed conifer/hardwood forest.

Assuming that detrital inputs in YS are higher quality and thus more readily degradable than OG woody inputs, we predicted that PSAC would also be lower in OG stream FBOM (Figure 3.5), however, this was not observed. As one of the first labile components to be degraded, labile polysaccharides are possibly mineralized before

reaching FBOM pools. Alternatively, many labile polysaccharides are totally or partially soluable and may have been significantly leached from particulate detritus.

We also anticipated that OG FBOM would have lower levels of biologically active N. This was observed in lower NMIN in FBOM collected from OG streams. Reduced N levels may also be directly related to lower total N also found in OG FBOM. When normalized to total N instead of to organic matter, there were no treatment differences for NMIN; EA remained lowest in OG. Greater N availability in YS was also observed as increased DNIT in YS FBOM. The relative activity of denitrifier populations may be regulated to a large degree by sediment inorganic N levels, thus sediments with elevated NMIN should also have elevated DNIT, which was observed. Correlations for DNIT-NMIN were $r = 0.25$ (ns) (normalized to gOM) and 0.45 ($p \leq 0.05$) when normalized to gDW.

Increased acetylene reduction rates in OG versus YS stream FBOM (Table 3.1) suggest that FBOM quality or conditions within OG streams enhances N-fixation. Young stands had more NTOT than OG, thus N limitation in OG may have stimulated N-fixation. Since microbial activity was also lower in OG FBOM, suggesting lower rates of decomposition, the energy source driving this increased fixation is unlikely to come from organic matter. Large gaps associated with OG Douglas-fir forests may provide the solar radiation needed for blue-green bacteria to fix N.

We hypothesized that following large disturbances such as clearcutting, stream sediment would display greater variability than in streams flowing through undisturbed old-growth forest. Indeed higher levels of variability were observed in FBOM collected from YS streams than those flowing through OG. Based on the range of each variable, variation in 10YS was apparently highest in 71% of the variables listed in Table 3.2 respectively.

Elevational Differences

With the exception of DNIT, NFIX and RESP, the differences between OG and 30YS FBOM microbial and chemical characteristics were greater in high elevation streams than in lower streams, suggesting increased elevation retards stream recovery following disturbance. In a study of the effects of clearcutting hardwood forest on soil processes and stream water chemistry, shallower soils and slower recovery of the vegetation increased disturbance effects and delayed recovery of higher elevation sites (Dahlgren and Driscoll 1994).

Specific elevation effects on litter and FBOM quality were not easily elucidated. Significant decreases in C:N found at higher elevation probably resulted from more herbaceous vegetation and alder in high elevation riparian zones (Figure 3.6). At higher elevations, the growing season is shortened and streams tend to be more open, with less riparian cover, which could lead to differences in algal production and streamside vegetation. Total C and N were significantly higher at high elevation suggesting that organic matter may take longer to break down due to slower decomposition rates. The lower microbial activities as shown by DNIT, NFIX, RESP and PHOS rates at higher elevations suggest slower decomposition rates. These reduced microbial activities and associated reductions in decomposition may be controlled by the lower stream temperatures found at higher elevations. Temperature decreases of 5°C in small streams flowing through deciduous or herbaceous stands in the southern Appalachian Mountains significantly decreased FBOM microbial activity (Peters et al. 1987). Both NMIN and PSAC (Figures 3.7-3.8) were higher at low elevation, which was unexpected. If decomposition rates were faster at lower elevation, these components should have been consumed first. It is possible that not only decomposition, but stream primary production rates were also elevated at low elevations, resulting in higher labile nutrient concentrations.

One of the most surprising aspects of this study was the strong influence of elevation on organic P concentrations, which were nearly double at high elevations (Figure 3.9). High organic P concentrations are a likely result of geologic age differences of

parent material. All the streams flow over andesitic and basaltic laval flows; however high elevation flows are as young as 4 million years, whereas at low elevation they are from the upper Oligocene and lower Miocene eras (Swanson and James 1975). The younger volcanic soils may contain higher concentrations of P.

Seasonal Effects

High sampling frequency in autumn captured a "pulse" on October 28 when C:N in FBOM dramatically decreased and then returned to pre-pulse values by November 4 (Figure 3.2). Daily precipitation from a nearby rain gauge (Figure 3.11) showed a storm event immediately prior to October 28 that may have loaded streams with litter, especially deciduous leaves and herbaceous riparian plants. Ratios as low as 13 on October 28 suggest an FBOM source with C:N lower than herbs or alder leaves, such as senescing algal detritus scoured by the rising flows (Table 3.6).

Table 3.6. Carbon:nitrogen ratios of vegetation and organisms common to streams in the western Cascades, Oregon.

Source	C:N	Reference
Woody material	220-1340	Cummins and Klug 1979
Douglas-fir needles	99	Yana Valachovic, per comm.
Big leaf maple leaves	59	Yana Valachovic, per comm.
Vine maple leaves	48	Yana Valachovic, per comm.
Red alder leaves	24	Yana Valachovic, per comm.
Macrophytes (moss, flowering plants)	13-69	Cummins and Klug 1979
Fungi	12	Staaf and Berg 1982
Periphyton	4-10	Cummins and Klug 1979
Bacteria	3-4	Paul and Clark 1989

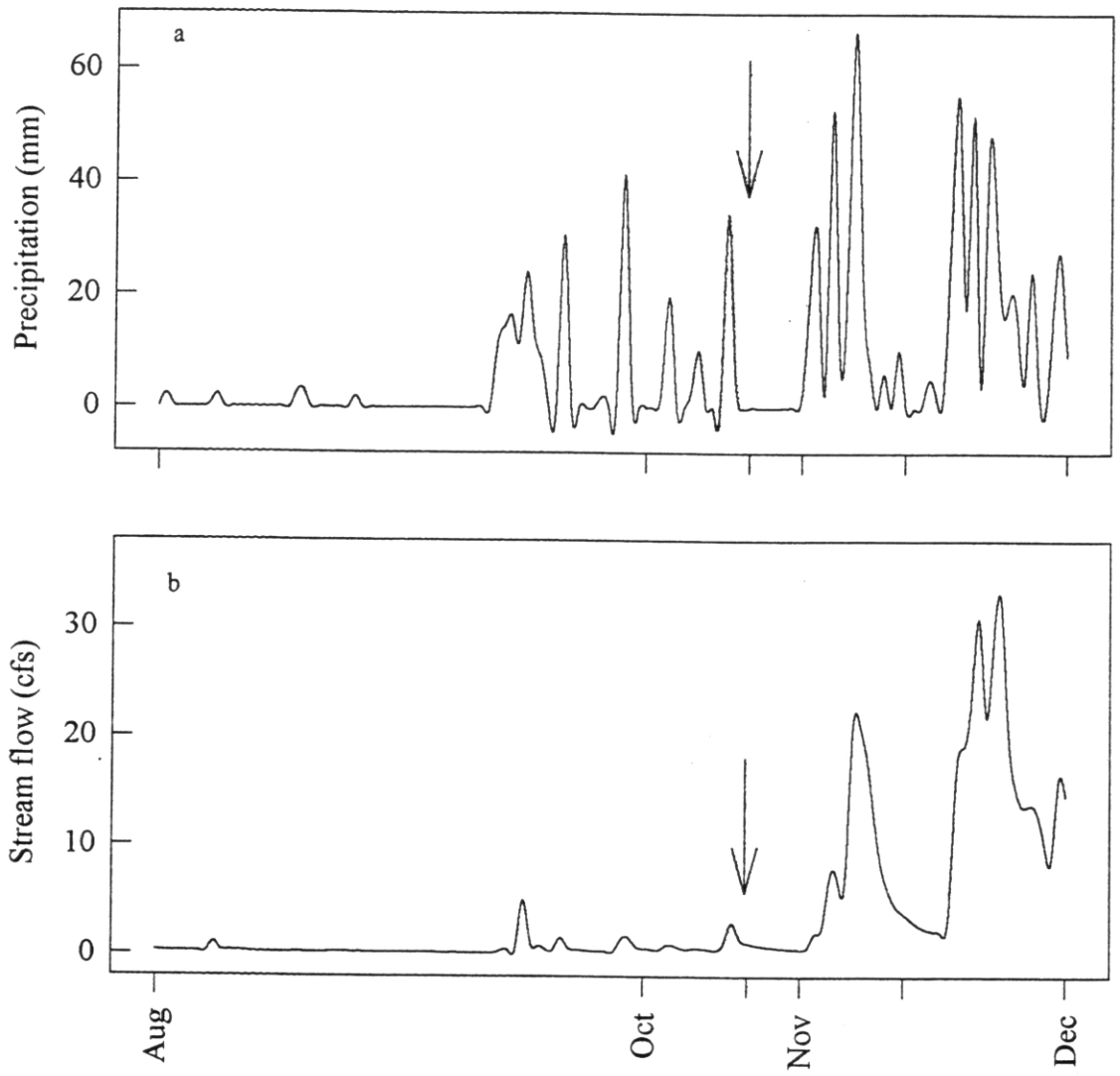


Figure 3.11. Daily rates of a) precipitation and b) total stream flow from a nearby meteorological station and stream gauge. Arrows point to October 28, 1995 sampling.

Concomitant with the lower C:N ratios observed during the October 28 pulse, increases in enzyme activities and respiration rates suggest a microbial response to the presence of readily degradable C associated with high quality nutrient inputs. The pulse of polysaccharide concentrations in YS streams may reflect this readily degradable component. Subsequent increased stream flow (Figure 3.11) may have transported these newer particles downstream, returning net FBOM C:N ratios to their higher, pre-pulse values. This result is supported by Cushing et al. (1993) who found that low-order stream FBOM contained a small pool of recently generated material that had extremely high biological activity, short turnover times and was transported from a reach within 24 hours following its generation. This contrasted the larger FBOM pool that had a longer turnover time and residence time within a stream reach. Studies of a large river system have shown that particles transported during low and high flows are compositionally different (Ittekkot et al. 1985). In forested, headwater streams in the southern Appalachian Mountains, maximum stream discharge and fine particulate matter export were high correlated ($r = 0.70$, Cuffney and Wallace 1989).

Similarly, while FBOM C:N were low in spring, microbial rates were typically high, again suggesting the presence of readily degradable C (Figure 3.5). Spring declines in C:N were accompanied by upswings in NMIN and EA (Figure 3.3) probably due to autochthonous algal production increasing available organic N. Consumption of large quantities of inorganic N by spring algal communities should lead to low denitrification potential rates, as seen in Figure 3.3.

From Figure 3.4, it is clear that a second pulse in FBOM labile polysaccharide concentrations occurred on November 18; this pulse was evident throughout the data set. November 18 occurred during another period of low flow after a storm that marked the first major increase in stream flows (Figure 3.11), suggesting a second pulse of leaf litter into streams drove down C:N and increased microbial activity rates and nutrient concentrations. After this date, rates of precipitation and stream flow were consistently higher and collection of sufficient FBOM proved difficult. The fall upswing in N-fixation (Figure 3.3) may be driven by the apparent increase in labile C which could provide energy

for this high-energy requiring transformation. Laboratory studies of N-fixation in stream sediments demonstrate a positive link between availability of energy-producing organics and N-fixation rates (Tam et al. 1981).

Results from PCA underscore the effects of treatment, elevation and season on litter quality, FBOM quality and microbial activity (Figure 3.10). Most FBOM showed high C:N, which suggests a source derived from wood which results in low microbial activity rates. Most OG sites fell in this area. Two other areas existed that had low FBOM C:N. These low ratios were associated with high activity rates and were comprised mostly of FBOM from 10YS and 30YS and FBOM from the main pulse date, October 28. Both these treatments and the heavy inputs in fall were sources of high quality litter and thus high nutrient availability. High organic P also greatly influenced FBOM from high elevations. Thus we see that vegetation, elevation and season influence FBOM substrate quality, particularly total C and N, C:N and organic P, which influence microbial activity associated with FBOM.

Since FBOM provides a key link between detrital inputs into streams and the aquatic detrital food chain, the changes in FBOM characteristics that we have documented suggest a strong causal link between forest management practices and stream productivity. In forested headwater streams, often less than one percent of total solar radiation reaches the stream, severely limiting primary production (Gregory et al. 1987), thus as the major source of organic matter in streams, FBOM is an important nutritional source for microorganisms and invertebrates (Vannote et al. 1980, Cummins et al. 1989, Schlosser 1991). Variation in invertebrate abundance has been linked to autumn leaf inputs, winter and spring leaf processing, summer reduced organic inputs, and summer transport of FBOM (Schlosser 1991, Wallace et al. 1991 and 1993). Since invertebrates serve as food for vertebrate predators such as fish and salamanders, forestry management practices that influence the type and amount of FBOM coming into streams will influence productivity at higher trophic levels.

Vannote et al. (1980) proposed the River Continuum Concept of longitudinal linkages, whereby organic matter from headwater streams can be transported large distances for later use by downstream communities. Management of headwater areas may

be as important to higher-order streams along the watershed as riparian management along those streams.

Conclusions

In small streams, changes in FBOM substrate quality affects microbial activity. FBOM quality, in turn, is linked to the quality of allochthonous litter inputs. Seasonal trends in FBOM suggest that more recalcitrant FBOM from poorer quality sources such as wood forms the bulk of FBOM that continuously resides in stream. This pool is seasonally supplemented by pulses of readily degradable FBOM from either autochthonous production or higher quality allochthonous inputs. Ward (1986) suggested that more recalcitrant FBOM from sources such as wood provided a stable and continuous energy source for headwater and downstream reaches that is punctuated by energy from readily degradable carbon sources processed in the headwaters and transported downstream. Stand vegetation effects and elevation differences on FBOM chemistry and activity persisted year round and were evident more than thirty years after clear-cutting. Clear-cutting increased the amount of variation in FBOM microbial and chemical factors as well.

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Chapter 4
Summary

The changes in FBOM characteristics that we have documented suggest a strong causal link between forest management practices and stream productivity. FBOM microbial activity and nutrient availability remained elevated more than 30 years after clear cutting. Disturbance effects were magnified at higher elevations as well.

Monitoring shifts in FBOM quality may be a promising method for assessing stream recovery following forest management activities. A reduced set of FBOM characteristics could be measured; enzyme assays are relatively quick and inexpensive to perform and also provide more specific information about decomposition than general measures of microbial activity such as respiration rates. Despite the apparent instability of ß-glucosidase activity found during time series analysis (Chapter 2), ß-glucosidase and phosphatase activities showed the strongest treatment effects. Knowledge of different enzyme activity rates could also elucidate the functional diversity of the bacterial community and the specific structures utilized during FBOM decomposition.

Although we found clear treatment differences in FBOM quality, we do not know whether these differences alter food web dynamics. Future research must examine how FBOM quality alters the quantity, growth rate, and size of members of the stream food web such as collectors, gatherers, and filter feeding invertebrates. Likewise, it is uncertain what share of the treatment, seasonal and elevational differences in FBOM quality evident in this study were due to allochthonous inputs, autochthonous algal production, or some other source. A more precise understanding of the sources of FBOM is necessary to understand how clear cutting and forest succession alter FBOM quality.

Understanding the effects of differences in FBOM quality on stream productivity is especially important in Oregon. Under the Oregon Department of Forestry's Water Protection rules, in the Coast range or western Cascades, small (< 2 cfs) streams without fish or domestic water use (classified as type N) require no retention of understory vegetation or conifers. That is, clear cutting may proceed to the stream edge, leaving no

riparian buffer and inviting a host of problems. Immediate impacts on these small streams are obvious -- increased sedimentation and water temperature, as well as changes to riparian vegetation. This study has shown that there may also be more subtle shifts in stream productivity mediated by the influence of riparian vegetation on the biology and chemistry of FBOM.

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Appendix

The data collected from during the methods evaluations (Chapter 2) were used to generate the laboratory analyses protocol followed during the study of the effects of stand age, season, and elevation on FBOM characteristics described in Chapter 3. In designing the laboratory protocol, my primary concern was to complete 7 different microbial and chemical analyses as quickly as possible after field collection and in the most appropriate sequence, so that results most accurately reflected conditions in the stream environment.

Field collection took 8-12 hours for myself and an assistant to complete. In actuality, the samples were collected during a more condensed time period (4.5-7 hours) due to lengthy travel time to the first site and collection of 5 unrelated samples toward the day's end.

During time series analyses, respiration (RESP), ß-glucosidase activity (BGLC), and mineralizable nitrogen (NMIN) were found to decrease in rate or concentration with storage time; in contrast, nitrogen fixation (NFIX) rates appeared to increase. Of these, RESP was the most stable, with apparent rate decreases occurring after 8.5-10.0 hours of storage. By starting this analysis first, I could analyze each sample before these storage effects occurred. After initiating the respiration analysis, I performed the three most stable analyses: denitrification potential (DNIT), extractable ammonium (EA) and phosphatase activity (PHOS). These were followed by the three least stable analyses, BGLC, NMIN and NFIX, in which storage effects appeared rapidly, within 4-7.8 hours of collection. The delay between collection of the final sample for this study and return from the field and the delay of initiation of these less stable analyses permitted the apparent storage effects and associated biological transformations to occur in all the samples before beginning these analyses. Thus, assays were done in the following order: RESP, PHOS, EA, DNIT, BGLC, NFIX and NMIN.