Relationships between microbial physiological status and

nitrogen availability in forest soils

by

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A thesis submitted to the Faculty of Graduate Studies and Research

in partial fulfilment of the requirements for the degree of

Master of Science

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I dedicate this work to my mother,

Sylvia Au (*A.D.* 1931-1990)

ABSTRACT

Although the physiological nitrogen demand of the soil microbial biomass is a major determinant of N mineralization in forest soils, the exact nature of the relationship is unclear. This study investigated the relationships between a respiration-based indicator of microbial physiological N demand (NIR) and N availability in forest soils. NIR was found to correlate significantly with net mineralized N in the field and annual foliar litterfall N fluxes. In a laboratory incubation, NIR was shown to be sensitive to changes in soil available C and N pools. These results demonstrated that microbial physiological N demand is determined by relative availabilities of labile C and N, and that it is significantly related to N cycling in forest soils. Results from a seasonal study of a forested watershed suggest that nutrient availability determined tree production and soil C availability, which in turn determined microbial physiological N demand and nitrogen dynamics in the forest.

RÉSUMÉ

La biomasse microbienne est un contrôle important de la minéralisation de l'azote (N) dans les sols des forêts, mais les effets de la demande physiologique microbienne pour N sur l'abondance de N dans le sol sont peu connus. Nous avons donc étudié les interactions entre un indicateur de la demande physiologique microbienne pour N (NIR) et l'abondance de N dans des sols forestiers. NIR était corrélé significativement avec la minéralisation nette de N et le flux annuel de N dans la litière foliaire. Durant une incubation de laboratoire, NIR était sensible aux changements d'abondance de C et N labile dans le sol. Ces résultats ont démontré que la demande physiologique microbienne pour N est déterminée par l'abondance relative de C et N labile, et que sa relation avec le cyclage de N est significative. Les résultats d'une étude saisonnière d'un écosystème forestier suggèrent que l'abondance des nutriments est un facteur associé à la productivité des arbres et l'abondance de C dans le sol, ces derniers ayant un effet sur la demande physiologique microbienne pour N et le flux de N dans le forêt.

Suggested short title-

Microbial physiological status and forest soil nitrogen cycling.

(W. R. Au)

ACKNOWLEDGEMENTS

Sonja Kosuta, Bernard Pelletier, Dan Simard, Stephen Yamasaki, and Claudia Zan- thank you for all the support you gave during hard times, and for all the fun we shared during good times. And my fondest thanks and love are extended to Mercy Peterson, whose help, companionship, and love have made the past two years of my life a most wonderful experience.

PREFACE

Unless otherwise referenced, the material presented in this thesis is an original contribution to science made by the candidate and his collaborators. The relationships between microbial physiological status and nitrogen availability in forest soils are examined in the following five chapters. Chapter I is a general introduction that provides the context of the research and the overall objectives of the study. Chapters II, III, and IV are in manuscript format and constitute the main body of the thesis. Chapter V is a brief conclusion that summarizes the findings of the study and directs the needs for future research.

The following five paragraphs are excerpted from *Guidelines for Thesis Preparation*, published by the Faculty of Graduate Research, and are included to inform the external examiner of Faculty regulations:

Candidates have the option of including, as part of the thesis, the text of one or more papers submitted or to be submitted for publication, or the clearly-duplicated text of one or more published papers. These texts must be bound as an integral part of the thesis.

If this option is chosen, **connecting texts that provide logical bridges between the different papers are mandatory**. The thesis must be written in such a way that it is more than a mere collection of manuscripts: in other words, results of a series of papers must be integrated.

The thesis must still conform to all other requirements of the "Guidelines for Thesis Preparation". **The thesis must include:** A Table of Contents, an abstract in English and French, an introduction which clearly states the rationale and objectives of the study, a comprehensive review of the literature, a final conclusion and summary, and a thorough bibliography or reference list.

Additional material must be provided where appropriate (e.g. in appendices) and in sufficient detail to allow a clear and precise judgment to be made of the importance and originality of the research reported in the thesis.

In the case of manuscripts co-authored by the candidate and others, **the candidate is required to make an explicit statement in the thesis as to who contributed to such work and to what extent**. Supervisors must attest to the accuracy of such statements at the doctoral oral defense. Since the task of the examiners is made more difficult in these cases, it is in the candidate's interest to make perfectly clear the responsibilities of all the authors of the co-authored papers.

Each of the three chapters that constitute the main body of the thesis is a research article that is either published or destined for publication in a refereed scientific journal. As such, each has been formatted to conform to the specifications of its respective journal. The first manuscript (Chapter II), entitled "Microbial biomass respiration response to added carbon and nitrogen as an indicator of nitrogen availability in forest soils", is a short communication that was accepted for publication in the Canadian Journal of Soil Science in August of 1998, and is currently *in press*. The second manuscript (Chapter III), entitled "Response of the microbial biomass and nitrogen induced respiration to differences in C and N availability in a forest soil", was submitted to the editorial staff of Soil Biology & Biochemistry in July of 1998, and is currently *in review*. The third manuscript (Chapter IV), entitled "Relationships between tree growth, nutrient fluxes, and soil microbial biomass dynamics in a northern hardwood forest", is in paper format suitable for submission to Ecology.

The candidate is first author on all of the manuscripts and his supervisor, Dr. James W. Fyles, is co-author for having provided invaluable inspiration, direction, and funding for the projects, as well as keen editorial remarks on the

manuscripts. Dr. Benoit Côté is included as co-author on the article for Chapter II for his editorial and funding contributions. The manuscript for Chapter IV draws upon the research from a larger inter-disciplinary project taking place in the Hermine watershed of the Station de Biologie des Laurentides, at St-Hippolyte, Québec. A great part of the funding and data that was obtained for this study was as a result of the contributions of many people involved in the project. The precipitation and throughfall data presented in the study were collected and analyzed by Dr. François Courchesne of the Université de Montréal. From within our department, Dr. Willie Hendershot and Mlle Hélène Lalande were responsible for the collection and analyses of the soil chemistry data. Finally, Dr. Benoit Côté was responsible for the collection of tree growth data, in addition to being a main editor of the manuscript. As such, these individuals are included as co-authors on the manuscript presented in Chapter IV.

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CHAPTER I

GENERAL INTRODUCTION

The availability of nitrogen is generally considered the most limiting factor on forest productivity (Gosz 1981, Vitousek et al. 1982, Mahendrappa et al. 1986). Nitrogen is used by plants as a primary constituent of proteins, nucleic acids, coenzymes, membrane constituents, and other secondary products (Marschner 1986). Consequently, its uptake in sufficient quantities is crucial for tree growth and survival. Although temperate and boreal forest soils typically possess a large capital of total nitrogen, over 90% of the nitrogen may be organically bound (Carlyle 1986), while less than 2% may be available at any one time in the soil mineral nitrogen pools (Scarsbrook 1965). As trees predominately take up nitrogen in its mineral forms of ammonium and nitrate (Kirkby 1981), they are dependent on the continual replenishment of the mineral nitrogen pools by the mineralization of organic nitrogen (Carlyle 1986).

The development of reliable methods to assess and predict the capacity of a soil to supply mineral nitrogen has been of long-standing interest, as this information would provide the basis on which increased efficiency and reduced environmental impacts associated with forest production could be achieved (Jarvis et al. 1996). The challenge has been formidable, however, as mineralization processes are governed by complex interactions within and between the biotic and abiotic components of the forest ecosystem (Gosz 1981). Thus, despite the scores of methods that have been developed to assess mineral nitrogen availability over the past decades (Keeney 1980, Binkley and Hart 1989), there is as yet no generally accepted method that consistently describes and predicts nitrogen mineralization in forest soils.

The role of the soil microbial biomass in nitrogen mineralization

In forest ecosystems, the major pathway for the return of nitrogen to the soil is through above- and belowground litter inputs (Staaf and Berg 1981). Consequently, the release of plant available nitrogen is intimately linked to heterotrophic decomposition processes. The primary agent responsible for the decomposition of soil organic matter, and the subsequent mineralization of

organic nitrogen to mineral nitrogen, is the soil microbial biomass (Smith and Paul 1990). The soil microbial biomass, defined as the living component of the soil organic matter, excluding plant roots and soil animals larger than about $5 \times 10^3 \mu m^3$ (Jenkinson and Ladd 1981), attacks and decomposes organic matter in soil and litter to meet its energy and nutrient requirements (Aber and Melillo 1991). When the amount of nitrogen released through the decomposition of organic matter exceeds their physiological demands for nitrogen, microorganisms will excrete the excess nitrogen as ammonium into the soil solution (Tisdale et al. 1985, Drury et al. 1991). In this way, organically-bound nitrogen in the soil is recycled and made once again plant available through microbial decomposition and mineralization activities.

The microbial biomass nitrogen pools can also impact mineral nitrogen availability by acting as a reservoir of labile nitrogen which is made plant available as the microbial population turns over (Jenkinson and Ladd 1981). Furthermore, when the soil microbial biomass itself is nitrogen limited, it can immobilize nitrogen from the soil mineral pools, and thus compete with plants for mineral nitrogen uptake (Paul and Clark 1989). The net amount of nitrogen that is available to plants, consequently, is the balance between microbial mineralization and release of excess nitrogen into the soil mineral pools, and immobilization from the soil mineral pools by the microbial biomass to meet its physiological demands (Paul and Clark 1989, Jarvis et al. 1996). Clearly, the soil microbial biomass is intimately linked to the fundamental processes of soil nitrogen mineralization, and its significance in determining the nitrogen supplying capacity of a soil is increasingly becoming recognized (Myrold 1987, Alef et al. 1988). Further research into the mechanisms of microbial control on mineral nitrogen availability, however, is still needed to improve our understanding and ability to predict nitrogen mineralization in soils (Smith and Paul 1990, Jarvis et al. 1996).

A microbial indicator of nitrogen availability

The development of rapid methods to quantitatively estimate the soil

microbial biomass, such as soil ATP measurement (Paul and Johnson 1977), fumigation-incubation (Jenkinson and Powlson 1976), and substrate-induced respiration (Anderson and Domsch 1978), has greatly facilitated the investigation of nitrogen cycling at the organism level (Smith et al. 1985). Moreover, the development of indicators based on microbial biomass measurements have been useful at comparing microbial activities across different soils (Franzluebbers et al. 1995). Indicators such as microbial biomass per unit of soil organic-C (Insam and Domsch 1988), the metabolic quotient for CO_2 (Anderson and Domsch 1993), and the kinetically derived labile carbon measure AC (Bradley and Fyles 1995a), have often been shown to be more sensitive and informative than chemical determinations of soil constituents (Anderson and Domsch 1989). The development of a similar indicator that could quantify the physiological nitrogen demand of the microbial biomass would provide insight into how microbial physiological demands control nitrogen availability in the soil.

The substrate-induced respiration method of Anderson and Domsch (1978) is a simple and rapid technique that estimates microbial biomass content in the soil by eliciting a physiological response from the soil microbial biomass. The method assumes that if a saturation pulse of a labile energy source (glucose) is added to the soil, the amount of CO_2 evolved by the microbes during the lag phase prior to biomass synthesis is proportional to the total microbial biomass carbon in the soil, as measured by the fumigation-incubation technique of Jenkinson and Powlson (1976). In concern over the effect of mineral nutrient depletion during incubation studies on the substrate-induced respiration response of the microbial biomass, Smith and co-workers (1985) modified the original method by supplementing their glucose amendments with nutrient broth. They found that the microbial respiratory response to the glucose + nutrient broth amendment was greater than the microbial respiratory response to the glucose only amendment.

In their study, Bradley and Fyles (1995b) similarly used the modified substrate-induced respiration technique described above, but with the objective of generating measures of the physiological status of the microbial biomass. They

proposed that the magnitude of the absolute respiratory response to the added glucose was proportional to the fraction of the microbial biomass that was *energy-limited*, whereas the magnitude of the absolute respiratory response to the added nutrient broth was proportional to the fraction of the microbial biomass that was *nutritionally-limited*. In their studies, they found that these microbial physiological indices reflected differences in belowground carbon allocation by different tree species (Bradley and Fyles 1995*b*), potential nitrogen mineralization (Bradley and Fyles 1995*b*), and mineral nitrogen availability in a fertile forest soil (Bradley et al. 199?). These results suggest that the microbial respiratory response to added labile carbon and nitrogen may be a useful indicator of the physiological status of the microbial biomass.

Elucidating the relationships between microbial physiological nitrogen demand and nitrogen mineralization would improve our understanding of the microbial controls on soil nitrogen availability. Furthermore, if consistent relationships between microbial physiological nitrogen demand and nitrogen mineralization were found, then microbial physiological nitrogen demand could also be used as a predictive index of nitrogen mineralization in the soil. In order to meet these objectives, however, a rapid and reliable method to quantify the physiological nitrogen demand of the microbial biomass must first be developed. The respiration-based method proposed by Bradley and Fyles (1995) to measure microbial physiological limitation appears theoretically sound and its practical application is simple. As such, further research to determine its usefulness as a measure of microbial physiological nitrogen demand is warranted. It was with these general goals in mind that this study was undertaken.

Objectives of the study

The specific objectives of this research were to:

1. develop a rapid respiration-based indicator that quantified the physiological nitrogen demand of the microbial biomass,

2. determine the relationships between microbial physiological nitrogen demand and carbon and nitrogen availability in the soil, and

3. determine if an indicator of microbial physiological nitrogen demand could be applied in field studies to elucidate the linkages between carbon and nitrogen dynamics in forests.

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CONNECTING PARAGRAPH I

The importance of the physiological demand for nitrogen of the soil microbial biomass in controlling nitrogen availability in forest soils was discussed in Chapter I. The need to develop a practical and reliable method to quantify this physiological demand, in order to further investigate the relationships between microbial physiological status and nitrogen mineralization, was also emphasized. The following chapter describes the application of a simple, respiration-based measure of the microbial physiological nitrogen demand, as an indicator of mineral nitrogen availability and historical nitrogen inputs in a forest soil.

CHAPTER II

MICROBIAL BIOMASS RESPIRATION RESPONSE
TO ADDED CARBON AND NITROGEN AS AN INDICATOR OF
NITROGEN AVAILABILITY IN FOREST SOILS

ABSTRACT

The relationship between a microbial biomass respiration response to added C and N (NIR) and N mineralized in buried-bags in the field was examined. Significant negative correlations were found between organic horizon NIR values and the amount of mineralized N measured at various times, however no significant correlations were found in the mineral horizon. These results suggest that NIR has potential for use as a physiological indicator of forest floor N availability.

Key words: microbial biomass, respiration, SIR, N mineralization.

INTRODUCTION

Nitrogen availability in forest soils is controlled by the mineralization of soil organic-N to mineral-N by the soil microbial biomass (SMB). The physiological N demand of the SMB determines to what extent N will be mineralized or immobilized (Paul and Clark 1989). The development of a method to measure the physiological N demand of the SMB would be useful in that it would provide a physiological measure of the capacity of a soil to mineralize N, as well as an indirect measure of soil N availability.

The substrate-induced respiration (SIR) method of Anderson and Domsch (1978) was originally developed as a physiological method for quantifying the microbial biomass in soils. The method has been used extensively to determine microbial biomass-C in soils because it is rapid and easy to use. The method assumes that if a saturation pulse of a readily available carbon/energy source (glucose) is added to the soil, the amount of CO_2 evolved by the SMB during the lag phase prior to biomass synthesis is a reflection of the actual size of the SMB.

Smith et al. (1985) modified the original SIR method, to avoid long-term nutrient deficiencies during incubation studies, by amending soils with both glucose and nutrient broth. They found that these "nutritionally complete" amendments resulted in a greater respiration response compared to the glucose-only amendments. Bradley and Fyles (1995) suggested that the absolute increase in respiration due to the glucose (SIR) represented the carbon/energy deficiency of the SMB, while the absolute increase in respiration due to the nutrient broth (NIR) represented the nutritional deficiency of the SMB. We suggest that the NIR response is a measure of the physiological N demand of the SMB, and that it may be used as a simple and rapid physiological indicator of a soil's capacity to mineralize N. The objective of this study was to examine the relationship between NIR responses of forest soils and the total N mineralized from the same soils over a growing season.

MATERIALS AND METHODS

Study site and soils

The study site was the Hermine watershed located at the Station de Biologie des Laurentides near St-Hippolyte, Québec, Canada (45°59' N, 74°01' W). The catchment has a surface area of ca. 5 ha with a drainage system consisting of an intermittent first-order stream. The soils have developed over anorthositic glacial till and are classified as Orthic Ferro-Humic Podzols (Humic Cryorthods) with a moder humus form. The forest floor has a pH (in H_2O) of 4.3, a total CEC of 29.5 cmol (+) / kg, and a total N content of 16.8 mg/g dry soil. The mineral Bfh horizon has a pH of 5.0, a total CEC of 5.4 cmol (+) / kg, a total N content of 3.3 mg/g dry soil, and an organic C content of 6.9%. The watershed is divided into three zones (Z1, Z2, Z3) according to canopy species composition, with each zone represented in three 300 m^2 replicate plots. Z1 is dominated by sugar maple (*Acer saccharum*), Z2 is dominated by sugar maple with some red maple (*A. rubrum*), and Z3 is equally dominated by sugar maple, red maple, birch (*Betula* spp.), and aspen (*Populus grandidendata*).

Four subsamples from both the forest floor organic (FH) and mineral (Bfh) horizons were taken from each plot in mid-June of 1995. The soils were passed through a 4.5 mm sieve to remove large roots and coarse woody debris, bulked to form a composite horizon sample for the plot, and sealed in polyethylene bags and placed over ice. The soils were returned to the laboratory where they were stored in the dark at 4° C until analyzed.

Respirometry measurements

Soil pre-conditioning and respirometry techniques followed the method of Bradley and Fyles (1995). Soils were removed from cold storage and pre-incubated in their polyethylene bags at room temperature for five days. Soils were

brought to field capacity moisture content by the addition of deionized-distilled water, spread on paper towels for three hours at room temperature to reduce moisture content to a level found in preliminary experiments to support optimal respiration, and replaced in their bags to incubate for another two days. Following the pre-incubation period, 30 and 70 g wet weight organic and mineral subsamples, respectively, were removed from the bulk samples from each plot and analyzed for basal respiration, SIR, and substrate and nutrient induced respiration (SNIR). The resulting SIR and SNIR values were used to calculate microbial biomass-C, metabolic quotient (qCO_2), and NIR values.

For the determination of basal respiration, subsamples were placed in 130 mL specimen jars, flushed with ambient air for five minutes using an aquarium pump, and sealed with lids equipped with rubber septa. After 2 h, a 3 cc air sample from the headspace of each jar was taken with a syringe and injected into a Hewlett Packard 5890-II gas chromatograph (Hewlett Packard, Avondale, PA) to determine the CO_2 concentration of the air sample. Soils were dried at 101°C to determine the dry weight of each subsample. Basal respiration was calculated as the mean of the duplicate subsamples and reported as micrograms of CO_2-C per gram of dry soil per hour. All respiration rates were corrected for changes in room temperature and pressure, assuming ideal gas laws and a $Q_{10} = 2$.

For the determination of SIR, each subsample was placed into a 500 mL specimen jar and amended with D-glucose at a rate of 667 µg/g and 143 µg/g wet weight organic and mineral soil, respectively, applied in the form of a 250 mg glucose-talc mixture. These concentrations were found in preliminary studies to be the minimum concentrations required to induce maximal respiration responses in each horizon. The talc mixtures were stirred into the soil using an electric hand blender, after which samples were transferred into 130 mL specimen jars and left uncovered at room temperature for 1.5 h. Samples were then flushed with ambient air for five minutes using an aquarium pump, and covered with lids equipped with septa. After 0.5 h, the CO_2 concentration of the air within the headspace of the jars was determined as described above for basal respiration. SIR values for each plot

were calculated as the mean of the duplicate subsamples and reported as micrograms of CO_2-C per gram of dry soil per hour. SIR values were converted to microbial biomass-C values using the regression equation milligrams of $C_{micr} = 40.04 \cdot SIR + 0.37$ developed by Anderson and Domsch (1978). qCO_2 was calculated as the ratio of basal respiration to microbial biomass-C.

The determination of SNIR was carried out in the same way as the determination of SIR, with the exception that soils were amended with a 250 mg talc mixture including both glucose and nutrient broth. The amendment contained D-glucose at concentrations of 667 µg/g and 143 µg/g wet weight organic and mineral soil, respectively, and Difco nutrient broth at concentrations of 833 µg/g and 357 µg/g wet weight organic and mineral soil, respectively. SNIR values were calculated as the mean of the duplicate subsamples and reported as micrograms of CO_2-C per gram of dry soil per hour. The NIR value for each plot was calculated as the difference between the mean SNIR and SIR values for the plot.

Net N mineralization

Net N mineralization was measured during the growing seasons of 1994 and 1995 (May to October) using the sequential buried-bag technique of Eno (1960). Sixteen samples of both organic (F) and mineral (Bfh) soil were collected from each plot and bulked into composite horizon samples. A subsample from the bulk composite samples of each horizon (ca. 10 g organic soil and 20 g mineral soil) was sealed individually in a polyethylene bag, and placed over ice. These samples were brought back to the laboratory where they were immediately extracted in 1 N KCl solution. Extracts were analyzed colorimetrically for NH_4^+-N (nitroprusside-salicylate) and NO_3-N (Cd reduction) concentrations using a Quickchem AE automated analysis system. A second subsample (ca. 10 g organic and 20 g mineral) from each of the original bulk samples was sealed in a polyethylene bag and buried in a hole (ca. 20 cm deep) in the centre of each plot and left to incubate *in situ* for a period of 4 weeks. After the 4 weeks, the samples

were removed, placed over ice, and returned to the laboratory where their NH_4^+-N and NO_3^--N concentrations were determined as described above. The entire procedure was repeated every four weeks until the end of October. Net N-mineralization for the 4 week period was calculated as the difference between post and pre-incubation concentrations of mineral N. Total mineralized-N for the year was calculated by summing the net mineralized-N of all the 4 week periods.

Statistical analyses

SMB respiration parameters and soil N mineralization data were compared across zones using the one-way ANOVA procedures of the Statistix statistical software (Analytical Software 1996). Comparison of zone means, following the significance of the ANOVA, was performed using an LSD (t) test, with values of $P < 0.05$ considered significant. Linear regression analyses were performed to test for significant correlations between NIR responses and N mineralization data.

RESULTS AND DISCUSSION

Microbial parameters

In the organic horizon, basal respiration and qCO_2 were not significantly different across the zones (Table 2.1). The similar basal respiration rates suggest that the SMB in the organic horizon of the three zones stabilize to a similar baseline metabolic level. Differences in qCO_2 values between microbial biomasses have been attributed to differences in microbial community structure (Insam and Haselwandter 1989) or to differences in environmental stress experienced by the SMB (Anderson and Domsch 1993). The similar metabolic quotients suggest that the SMBs in the three zones are similar in community structure, or are experiencing similar environmental stresses. Microbial biomass

Table 2.1. Microbial biomass and N mineralization parameters (means ± 1 SE) of the organic horizon.

Zone	Basal	SIR	NIR	Biomass-C	qCO_2	Mineralized N		
						June 1995	1994 Total	1995 Total
	(μg CO$_2$-C·g^{-1} dry soil·h^{-1})			(mg C$_{micr}$/g dry soil)	(μg CO$_2$-C·mg^{-1} C$_{micr}$·h^{-1})	(μg N/g dry soil)		
Z1	9.3 ± 0.7	12.0 ± 0.7	3.4 ± 0.4	0.9 ± 0.03	9.4 ± 0.3	219 ± 54	687 ± 136	731 ± 130
Z2	7.2 ± 1.1	9.2 ± 0.8	2.3 ± 0.2	0.7 ± 0.03	8.5 ± 0.9	221 ± 17	917 ± 170	1149 ± 86
Z3	9.4 ± 0.3	9.0 ± 0.2	4.7 ± 0.3	0.7 ± 0.01	10.1 ± 0.3	143 ± 19	407 ± 18	374 ± 44

(SIR) and NIR were found to differ significantly across the zones ($P = 0.02$ and 0.002 respectively; Table 2.1). Z1 was found to have a significantly larger SIR response than Z2 and Z3. This suggests that the SMB in Z1 had a greater physiological demand for carbon or energy relative to the biomasses in Z2 and Z3, presumably due to having a larger biomass. In contrast, Z3 was found to have the largest NIR response, with Z1 having an intermediate response and Z2 having the smallest NIR response. This suggests that the biomass in the forest floor of Z1, with a high SIR response and a relatively low NIR response, had a greater demand for carbon than for nitrogen. Conversely, the low SIR response and high NIR response of the forest floor biomass of Z3 suggests that it had a greater demand for nitrogen than for carbon. The smaller SIR and NIR responses of Z2 may reflect a smaller SMB that is experiencing less carbon and nitrogen limitations relative to the other two zones, or may reflect an SMB that is limited by other factors.

In the mineral horizon, none of the measured parameters were found to be significantly different from one another across the zones (Appendix A). This suggests that the three zones may all have qualitatively similar organic matter in the mineral horizon. As a result, the mineral horizons of the three zones may be supporting microbial biomasses that live in similar conditions, and thus do not differ significantly in their physiological responses. Because no significant differences were found in the mineral horizon, the remainder of the discussion will focus on the comparison between the organic horizon SMB parameters and the N mineralization data.

Net N mineralization

Mineralized N was not found to differ significantly across the zones at the time of sampling, nor over the 1994 growing season (Table 2.1). Differences were found, however, in the total mineralized N over the growing season of 1995 ($P = 0.003$) between zones. A similar pattern across the zones (Z2 > Z1 > Z3) was found in all mineralization measurements, and was opposite to the pattern

observed in the NIR data. This is consistent with our hypothesis, in that zones with greater mineralized-N support a microbial biomass that is less N-limited and consequently produce smaller NIR responses. Litterfall foliar N flux in 1994, determined as part of another study (unpublished data), was found to be significantly higher in Z2 (2.2 g N/m) than in Z1 and Z3 (1.5 and 2.0 g N/m, respectively). These results are consistent with the microbial and mineralization results: zones with greater litterfall N input mineralized more N over the growing season, and as a result supported microbial biomasses that were less N-limited and produced smaller NIR responses.

These results also suggest that forest floors developed under sugar and red maple tend to support a microbial biomass that has a greater demand for carbon, whereas forest floors under aspen and birch tend to support a microbial biomass that has a greater demand for nitrogen. Birch is known to be a soil ameliorating species that may increase the cycling of nutrients (Miles and Young 1980; Miller 1984). Our data seem to support the theory proposed by Bradley and Fyles (1995) that the greater below-ground C inputs of birch result in a microbial biomass that is relatively more N-limited.

Correlations between microbial and N mineralization measures

Significant correlations were observed between NIR and total mineralized-N over the 1994 ($r^2 = 0.49$, $P = 0.02$) (Fig. 2.1a) and 1995 growing seasons ($r^2 = 0.85$, $P = 0.0002$) (Fig. 2.1b), and the amount of mineralized-N at the time of sampling ($r^2 = 0.38$, $P = 0.05$). These relationships suggest that the physiological N demand of the forest floor SMB is strongly correlated to the amount of N that is mineralized in the field. NIR was not found to be correlated with any of the N mineralization data in the mineral horizon, suggesting that microbial controls on N mineralization in the mineral horizon are different from those in the organic horizon. It is not evident from this data whether the amount/rate of N mineralization controls the N demand of the forest floor SMB, or whether another
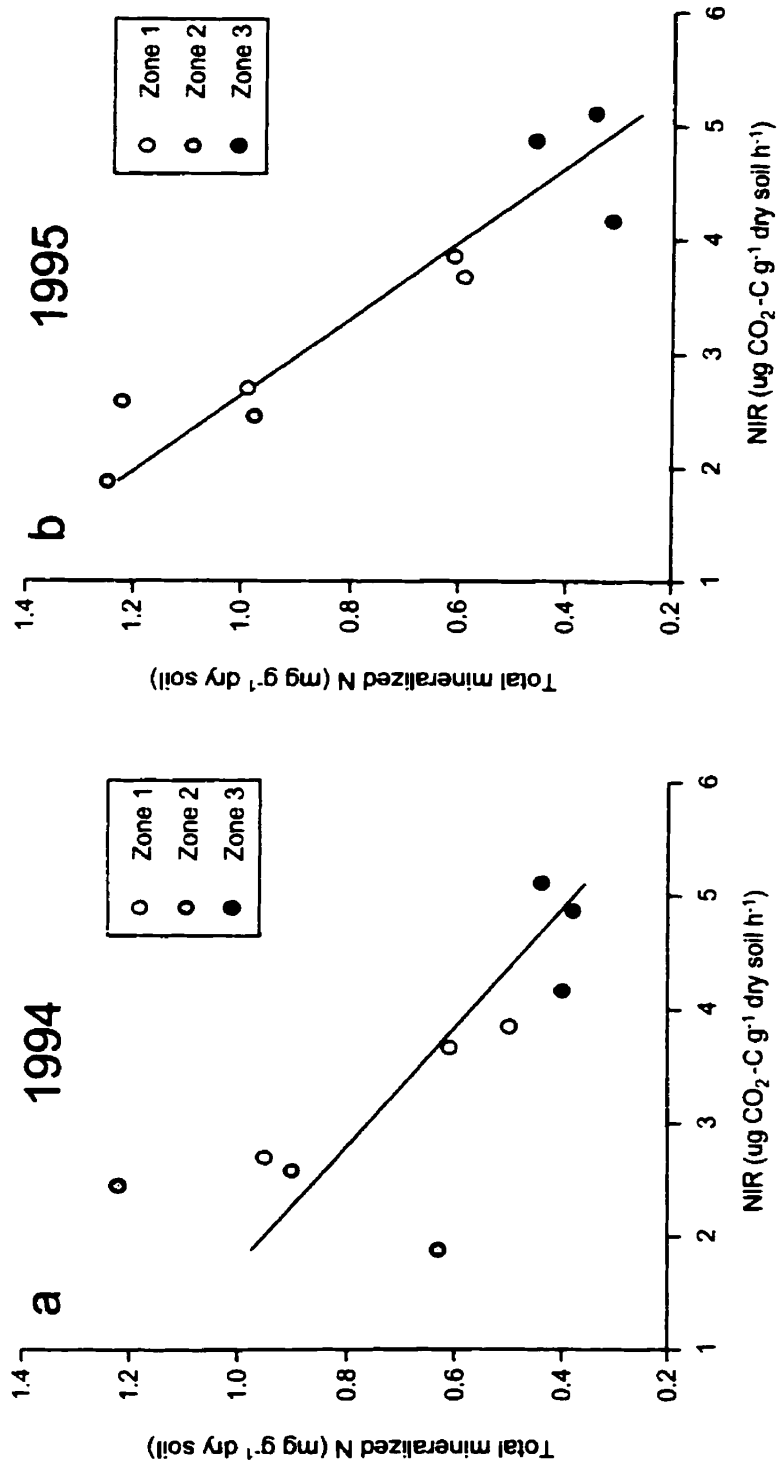
Fig. 2.1. Relationship between the forest floor NIR response and the total mineralized N in the organic horizon over the (a) 1994 and (b) 1995 growing seasons.

external factor controls both of these parameters together. Further work is needed to clarify the controls of this system.

The results of this study show that there is a strong relationship between the SMB physiological parameter NIR and the amount of N-mineralized in the organic horizon. Further work is required to examine how microbial N demands might change over time, and how they respond to differences in soil C availability. The results of this study, however, suggest that NIR may be a useful indicator of soil N-availability and that further research is justified.

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CONNECTING PARAGRAPH II

In Chapter II, NIR was shown to be negatively correlated with the total amount of nitrogen that was mineralized over the current and previous growing season in the forest floor, as well as with historical litter inputs of nitrogen to the soil. These results suggest that the microbial physiological nitrogen demand and nitrogen cycling in the forest floor are interrelated. The need for clarification of the controls on the NIR response were emphasized, in particular, how changes in the available carbon and nitrogen pools affected microbial physiological status. The next chapter describes a six week laboratory incubation that investigated the response of the microbial biomass and NIR to changes in soil carbon and nitrogen availability.

CHAPTER III

RESPONSE OF THE MICROBIAL BIOMASS AND
NITROGEN INDUCED RESPIRATION TO
DIFFERENCES IN C AND N AVAILABILITY IN A FOREST SOIL

SUMMARY

The response of the microbial biomass and the microbial physiological N-limitation indicator *nitrogen-induced respiration* (NIR) of a forest soil to relative differences in readily-available C and N were investigated over a six week laboratory incubation. In both the mineral and organic soil horizons, samples amended weekly with C (glucose) had significantly greater ($P \leq 0.05$) available C (AC), basal respiration, and microbial biomass-C than soils amended weekly with N (ammonium sulfate) or control soils. C amended mineral soils had significantly greater carbon availability index (CAI), metabolic quotient, and NIR than N amended or control soils, suggesting that the increased C availability in these soils supported a microbial community that was less C efficient and more N limited. CAI and metabolic quotient did not differ significantly among treatments in the organic horizon, suggesting that the chronic C inputs were readily metabolized by a biomass already adapted to high C conditions. N amended organic soils had significantly greater mineral N levels than both C amended and control soils, and had significantly lower NIR responses than C amended soils, suggesting that the increased N availability in these soils supported a microbial biomass that was less N limited. The significant positive correlations between NIR, microbial parameters, and soil C availability in the mineral horizon suggest that microbial N limitation in the mineral horizon is predominately determined by C availability. In the organic horizon, the weaker correlations between NIR and soil C availability in conjunction with the significant negative correlation between NIR and mineral-N availability in the organic horizon suggests that microbial N limitation is predominately determined by N availability. These results suggest that NIR is an effective indicator of microbial physiological N limitation, and that NIR is sensitive to changes in soil C and N availability.

INTRODUCTION

Forest productivity is often limited nutritionally by the availability of soil nitrogen (N). Trees in general take up N in its inorganic forms and are thus dependent on the processes that affect the soil mineral N pools (Carlyle 1986). Much research has been done over the past decades in an effort to understand the factors that control N mineralization and immobilization in the soil. Our knowledge of the biological controls on these processes is still incomplete however, and needs to be further developed to better understand their impacts on soil N availability (Jarvis et al. 1996).

One of the main biological components of the soil N cycle is the soil microbial biomass (SMB). The SMB is the primary agent responsible for the mineralization and release of mineral N during the decomposition of litter and soil organic matter (Jenkinson and Ladd 1981, Smith and Paul 1990). Furthermore, the SMB can draw upon the soil mineral N pool to meet its own N requirements. Net N mineralization, then, is the balance between the amount of N that is mineralized and the amount of mineral N that is immobilized to meet the physiological N demand of the SMB (Jarvis et al. 1996). Consequently, the microbial physiological N demand (ie. the degree of N-limitation experienced by the biomass) is a major control on net N mineralization in the soil (Paul and Clark 1989).

The development of a method to measure the SMB physiological N demand could provide a useful measure of readily-available N in the soil, as well as provide a predictive index of mineralization and/or immobilization. Soil analyses and measurements based on the physiological responses of the SMB have been suggested to be more sensitive and informative than chemical determinations of soil constituents (Anderson and Domsch 1989). The metabolic quotient for CO_2 (qCO_2- the ratio of basal respiration per unit microbial biomass-C) is a widely used microbial physiological index that has been linked to factors such as environmental stress (Killham 1985, Anderson and Domsch 1993) and

forest successional stage (Insam and Haselwandter 1989). The carbon availability index (CAI- the ratio of basal respiration to substrate-induced respiration; Parkinson and Coleman 1991) and the kinetic parameter available carbon (AC-derived from fitting microbial respiration responses to varying substrate concentrations to a modified Michaelis-Menten equation; Bradley and Fyles 1995) are both physiological indices that have been used to measure the abundance of readily-available carbon (C) in the soil.

In a previous study, we suggested that relative differences in the magnitude of the microbial respiration parameter nitrogen induced respiration (NIR- the absolute increase in microbial respiration due to an added N source) could be used as a physiological indicator of relative differences in soil-N availability (Au et al. 1998, Chapter II). In the study, a strong negative correlation was found between the NIR response of the SMB and the total amount of N that was mineralized in the field over the growing season. The purpose of this study was to further clarify the relationships between the NIR response and C and N availability in the soil. To this end, we undertook a six week laboratory incubation to observe how the microbial community and the NIR response of a forest soil responded to chronic inputs of readily-available C and N. The aims of this study were: (1) to measure the changes over time in the SMB community in response to relative differences in C and N availability, and (2) to determine whether the NIR response is sensitive to changes in soil available-C and mineral N levels.

MATERIALS AND METHODS

Soils and sampling

The soils used in this study were collected from a 100 year old mixed species stand in the Morgan Arboretum near Montréal, Québec, Canada (45°25'N, 73°57'W). The stand is ca. 2 ha in size and is dominated by beech, hemlock, and

red maple. The microtopography is moderately mounded and the drainage is good to moderate. The soils have developed over a fluvial sand deposit and are classified as humo-ferric podzols with a mor humus form.

Soil samples were collected in November 1996 from four 1 m^2 sampling points of similar slope position and microtopography. Forest floor material (FH) and the top 15 cm of the mineral Bfh horizon were taken from each point, with soils from each horizon bulked respectively, and immediately stored in the dark at 4°C until used.

Soil conditioning

Soils were passed through a 4.5 mm sieve to remove large roots and coarse fragments, and were mixed thoroughly. An initial aliquot from the bulk samples of each horizon was removed for preliminary testing to determine the soil moisture contents, amendment concentrations, and rate of treatment applications that were optimal for respirometry measurements. Soils were divided into nine equal samples of ca. 1.3 kg and 2.9 kg fresh weight organic and mineral soil respectively, and each sample was placed in a 10 L plastic container. Duplicate 10-20 g fresh weight soil subsamples were removed to determine the moisture contents of the soils in each container. Soils were subsequently brought up to 200% and 25% moisture content (w/w), for organic and mineral samples respectively, by the addition of distilled-deionized water, and covered with polyethylene sheets to maintain moisture levels. Soils were pre-incubated at room temperature for one week.

Chronic soil treatments

To contrast the effects of relative differences in soil C and N availability on the physiological status of the soil microbial biomass, soils were amended with chronic C and N treatments in the form of aqueous solutions added on a weekly basis for a period of six weeks. The nine soil samples from each horizon were

randomly divided into one of three treatments replicated three times: + C, + N, or no substrate (control). Soils receiving the + C treatment were amended with a solution containing D-glucose as the carbon substrate at a concentration of 2880 μg C/g and 240 μg C/g wet weight organic and mineral soil, respectively. Soils receiving the + N treatment were amended with a solution containing ammonium sulfate $((NH_4)_2SO_4)$ as the nitrogen source at a concentration of 240 μg N/g and 20 μg N/g wet weight organic and mineral soil, respectively. Soils receiving the control treatment were amended with distilled-deionized water. The selected glucose and ammonium sulfate concentrations were found, in preliminary tests, to be the minimum concentrations required to induce maximal respiratory responses in the soils.

On the day prior to the addition of treatment solutions to soils, the polyethylene sheets were removed from the containers and soils were left uncovered overnight. Treatment solutions were added to the soils the following morning. This method was found to maintain soils at the desired moisture levels, as the water that was added in the treatment solutions offset the moisture that was lost overnight to evaporation. For the first three weekly treatments, 96 mL and 78 mL of treatment solution were added to the organic and mineral samples, respectively. Because of the destructive removal of soil for analyses at the end of the third week, the volume of added treatment solution for the three remaining weekly treatments was reduced to 62 mL and 50 mL for the organic and mineral samples, respectively. Soils were mixed thoroughly and recovered with the polyethylene sheets. Soils were allowed to incubate undisturbed during the following six days until the next treatment. The first treatment was made on day 1, with subsequent treatments made every seven days thereafter.

Soil analyses

Soil analyses were performed on days 0, 21, and 42 of the incubation. To determine the effects of the treatments on the physiological status of the SMB, the

following microbial respiration-based parameters were measured: basal respiration, microbial biomass-C, qCO$_2$, and NIR. Furthermore, CAI and AC values (for day 42) were determined as measures of readily available-C in the soil. Soil mineral N levels were determined as a measure of readily available-N in the soil.

Respirometry

Six 30 g and 60 g subsamples of wet weight organic and mineral soil, respectively, were removed from each container and placed into 130 mL specimen jars. The subsamples were divided into three pairs of duplicate subsamples, with one pair of duplicates being used for each of the following measurements: basal respiration, substrate-induced respiration (SIR), and substrate and nitrogen-induced respiration (SNIR). The resulting SIR and SNIR values were used to calculate microbial biomass-C and NIR values, respectively.

For the determination of basal respiration, soil subsamples were flushed with ambient air for five minutes using an aquarium pump. Soils were subsequently amended with 2 mL of distilled-deionized water and covered with a lid equipped with a rubber septum. After 1 h. a 4 cc air sample from the headspace within the specimen jar was removed with a syringe and injected into a Hewlett Packard 5890-II gas chromatograph (Hewlett Packard, Avondale, PA) equipped with a Poropak Q column and thermal conductivity detector to determine the CO$_2$ concentration of the air sample. Soils were dried overnight at 101°C to determine the dry weight of each subsample. Basal respiration was calculated as the mean of the two subsamples and reported as micrograms of CO$_2$-C per gram of dry soil per hour. All respiration rates were corrected for changes in room temperature and pressure, assuming ideal gas laws and a Q$_{10}$ = 2.

For the determination of SIR, subsamples were flushed with ambient air and amended with a 2 mL D-glucose solution at concentrations of 2880 µg C/g and 240 µg C/g wet weight organic and mineral soil, respectively. Soils were

covered for 1 h and the CO_2 concentration of the air in the headspace of each specimen jar was determined as described above for basal respiration. SIR was calculated as the mean of the two subsamples and reported as micrograms of CO_2-C per gram of dry soil per hour. SIR values were converted to microbial biomass values using the Anderson and Domsch (1978) regression equation of milligrams of $C_{micr} = 40.04 \cdot SIR + 0.37$. qCO_2 values were calculated as the amount of basal respiration per unit of microbial biomass and was reported as micrograms of CO_2-C per milligram C_{micr} per hour. CAI was calculated as the ratio of basal respiration to SIR.

The determination of SNIR was carried out in the same way as the determination of SIR, with the exception that subsamples were amended with a 2 mL solution containing both D-glucose and L-glycine. Previously, we had generated SNIR responses by amending soils with a glucose solution containing nutrient broth as the nitrogen source. Nutrient broth, however, is not only a complex mixture of various nutrients, but also a potential organic energy source. As such, the microbial respiratory response generated by its addition may not be a strict measure of microbial-N demand, as it would confound the response of the SMB to the other nutrients and energy sources that occur in the broth. An inorganic N source would be ideal in isolating a pure measure of microbial-N demand, however previous work has shown that amendment of mineral N salts to the soil can result in reduced microbial respiration and activity (Salonius 1972, Nohrstedt et al. 1989). Therefore, assuming that the SMB would preferentially metabolize glucose for its C and energy requirements, we elected to use a simple amino acid in the SNIR solution rather than nutrient broth, so as to generate a respiration response that would be strictly due to the addition of a readily-available N source.

Solutions used for organic soil subsamples contained D-glucose and L-glycine at concentrations of 2880 μg C/g and 240 μg N/g wet weight soil, respectively. Solutions used for mineral soil subsamples contained D-glucose and L-glycine at concentrations of 240 μg C/g and 12 μg N/g wet weight soil,

respectively. CO_2 concentration of the air in the headspace of each specimen jar was determined as described above for basal respiration. SNIR was calculated as the mean of the two subsamples and reported as micrograms of CO_2-C per gram of dry soil per hour. NIR was calculated as the difference between the mean SNIR and SIR values for each soil sample.

AC determination

The kinetic parameter AC was determined on day 42 using the method described by Bradley and Fyles (1995). Briefly, the method is based on plotting the microbial respiration responses (v) to a range of glucose concentrations (S_A) ranging from 0 to saturation levels. The resulting curve is then fitted to a modified Michaelis-Menten curve: $v = (V_{max})(S_A + AC)(K_m + S_A + AC)^{-1}$, where V_{max} is the maximum respiratory response to glucose at saturation levels, K_m is the Michaelis constant, and AC is the extrapolated x-intercept value representing the glucose-equivalent of soil available C.

Seven 30 g and 60 g subsamples of wet weight organic and mineral soil, respectively, were removed from each container, placed into 130 mL specimen jars, and flushed with ambient air for five minutes. One of seven different 2 mL glucose solutions was then added to each subsample, with solutions ranging in concentrations from 0 to 2880 µg C/g wet weight soil for organic subsamples, and from 0 to 240 µg C/g wet weight soil for mineral subsamples. CO_2 concentration of the air in the headspace of each specimen jar was determined as described above for basal respiration. The parameters V_{max}, K_m, and AC were estimated by nonlinear regression analysis using the NONLIN procedures of the SYSTAT statistical software (Wilkinson 1990). The AC value estimated for each soil sample was reported as micrograms of glucose-equivalent C/g dry soil.

Mineral N availability

Mineral nitrogen availability in the soil was determined by extraction of soils in KCl solution. After determining the moisture content of the soils in each container, ca. 20 g and 30 g of wet weight organic and mineral soil, respectively, were removed from each container and sealed in individual polyethylene bags. Soils were kept frozen in their bags at -10°C until analyzed. Frozen samples were thawed overnight in a refrigerator at 4°C. Organic and mineral samples of 10 g and 15 g wet weight soil, respectively, were placed into 250 mL Erlenmeyer flasks and 100 mL of 1N KCl solution was added. Flasks were shaken on an overhead shaker at 120 rpm for 1 h, after which extracts were filtered through Whatman #5 filter papers. The filtrate was collected in 120 mL plastic bottles and immediately frozen at -10°C until analyzed. Filtrates were thawed overnight at room temperature, and analyzed for NH_4^+ (nitroprusside-salicylate) and NO_3^- (Cd reduction) using a Quickchem AE automated analysis system. Mineral N availability was reported as μg mineral N/g dry soil.

Statistical analyses

Statistical analyses for treatment, horizon, and treatment x horizon interaction effects on soil variables for each date were tested by two-way ANOVA using the PROC GLM procedures of the SAS system (SAS Institute 1989). In all statistical tests, F values with corresponding probabilities $\leq$ 0.05 were considered significant. Where significant treatment x horizon interactions were found, the simple effect of treatments in each of the soil horizons was tested using one-way ANOVA. Comparison of means between treatments, upon significance of the ANOVA, was performed using Tukey's (HSD) multiple-range test. Pearson's correlation coefficients between variables measured on day 42 were generated using the Linear Models procedures of the Statistix statistical software (Analytical Software 1996).

RESULTS

The two-way ANOVA of day 0 data showed no significant treatment or treatment x horizon interaction effects, but did show significant horizon effects in all measured variables ($P < 0.0004$), with the organic horizon generally producing values two to ten times greater than those of the mineral horizon. With the exception of CAI, observed values in the organic horizon remained significantly greater than those of the mineral horizon on both days 21 and 42. By day 21, significant treatment effects were observed in four of the six measured variables, and by day 42, significant treatment effects were observed in all seven of the measured variables. Although significant treatment x horizon interaction effects were observed in only some of the measured variables on both days 21 and 42, the data for each soil horizon will be presented separately in order to facilitate data presentation and discussion. As such, the results and discussion presented hereafter will treat each horizon separately and will focus on the results of days 21 and 42.

Soil C availability

Samples that were amended with C tended to have greater levels of soil available C, however the patterns of response between horizons as well as between available C measurements were not similar. On day 21, significant treatment and treatment x horizon interaction effects were observed in soil C availability as measured by CAI. In the mineral horizon, the CAI in C amended soils had increased and become significantly greater than the CAI in both N amended and control soils, which remained unchanged and decreased, respectively (Fig. 3.1A). In the organic horizon, however, CAI values were not found to differ significantly across treatments.

On day 42, the patterns observed in soil C availability as measured by CAI were different from those as measured by AC. On this date, no significant
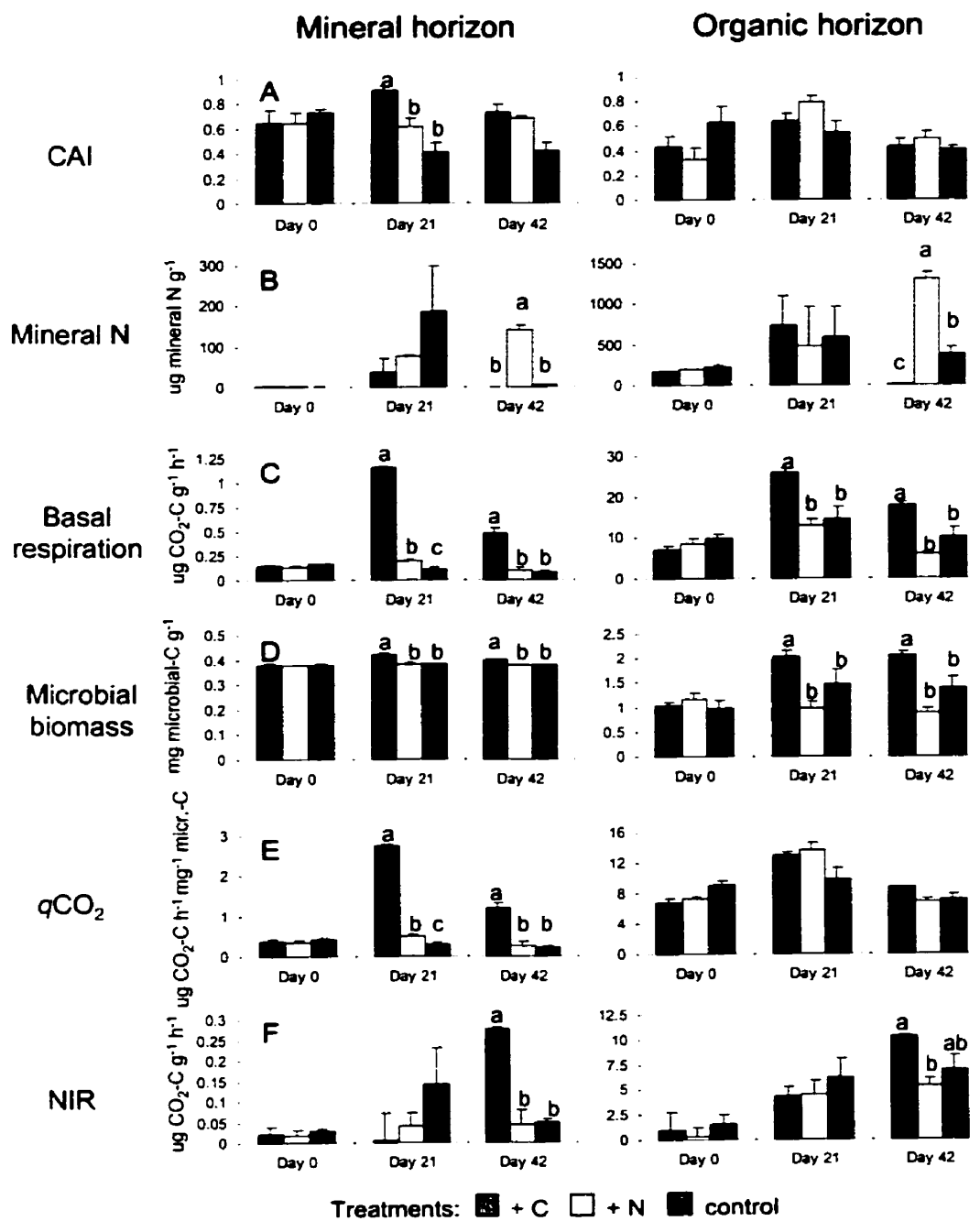
Fig. 3.1. Means (with standard error bars) of measured variables of each horizon for days 0, 21, and 42.

[a-c]Means on each date denoted with different letters were significantly different at $P < 0.05$.

Table 3.1. Estimated AC values (means ± 1 SE) for the mineral and organic horizons on day 42.

Treatment	AC (μg glucose equivalent-C/g)	
	Mineral horizon	Organic horizon
C	38.4 ± 3.78	68.3 ± 1.88
N	4.8 ± 3.95	0.1 ± 0.03
control	1.5 ± 0.73	40.8 ± 23.4

treatment effects on soil C availability were detected in CAI values. In the mineral soil, CAI values in the C amended soils had fallen, but still tended to be greater than the values of the control treatment which remained low. CAI values in the organic horizon were again not found to be significantly different across treatments. In contrast, significant treatment effects on soil C availability were detected in the AC data, although no significant treatment x horizon interaction was observed (Table 3.1). Soils that were amended with C were found to have significantly greater AC values than soils amended with N. The control soils in the organic horizon were highly variable in their AC values, and as a result were not found to be statistically different from either C or N amended soils.

Soil mineral N availability

Day 21 mineral N levels in both horizons were characterized by relatively high variation in all treatments. As a result, no significant treatment or treatment x horizon interaction effects were observed on this date. Stabilization in mineral N levels seemed to occur by day 42, as variation within treatments subsided and gave rise to strong treatment and treatment x horizon interaction effects. On this date, soils amended with N had significantly greater levels of mineral N than either C amended or control soils in both soil horizons (Fig. 3.1B). Furthermore, the mineral N levels in the control soils of the organic horizon were significantly greater than in the C amended soils, where mineral N levels fell to levels lower than those observed on day 0.

Microbial parameters

Treatment and treatment x horizon interaction effects were statistically significant for both basal respiration and microbial biomass on both dates and on day 42 for NIR, whereas only treatment effects were found to be significant for qCO_2 on both dates. In both soil horizons, basal respiration in C amended soils was significantly greater than in N amended or control soils for both days 21 and

42 (Fig. 3.1C). Basal respiration in N amended soils in the mineral horizon was greater than in the control soils, but only significantly so on day 21. The opposite trend was observed in the organic horizon, where basal respiration rates in N amended soils tended to be lower than in control soils, but differences were not significant. Actual increases in basal respiration rates in the mineral horizon were observed only in the C amended soils, where values peaked at 1.2 μg CO_2-C·g^{-1} dry soil·h^{-1} on day 21, representing an increase of ca. 800% relative to day 0 values. In contrast, basal respiration rates in the organic horizon increased in all treatments on day 21 with the maximum occurring in the C treatment at 26 μg CO_2-C·g^{-1} dry soil·h^{-1}, representing an increase of only ca. 250% relative to day 0 values. By day 42, basal respiration rates in both horizons tended to decrease slightly in all treatments.

Microbial biomass patterns among treatments were similar to those observed in basal respiration in both horizons. On days 21 and 42, C amended mineral and organic soils supported significantly greater microbial biomass levels than N amended and control soils. In the mineral soil, only C amended soils showed changes in biomass levels relative to day 0 values, peaking and stabilizing on day 21 (Fig. 3.1D). Overall, microbial biomass levels in the mineral soils remained within the range of 0.37 to 0.42 mg C_{micr}/g dry soil throughout the incubation. In the organic soils, biomass levels increased in the C amended and control soils on day 21, and remained at these levels on day 42. N amended soils, which did not generally fluctuate from day 0 values, supported significantly lower biomass levels than C amended soils on both dates, but were not significantly different from the control soils.

Only significant treatment effects were observed in SMB $q CO_2$ values, and these were primarily dominated by the changes that occurred in the mineral horizon, as $q CO_2$ values in the organic horizon tended not to differ across treatments. Soils amended with C were found to have significantly greater $q CO_2$ values than N amended or control soils on both days 21 and 42 (Fig. 3.1E). In the mineral horizon, $q CO_2$ values in C amended soils increased by ca. 600% on day

21 to 2.7 µg CO_2-C/g mg C_{micr}, but subsequently fell to 1.2 µg CO_2-C/g mg C_{micr} by day 42. These increases over day 0 values were largely due to the increases in basal respiration, as microbial biomass levels in the mineral horizon fluctuated very little over the course of the incubation. qCO_2 values remained relatively unchanged in the N amended soils and declined in the control soils of the mineral horizon. In the organic horizon, qCO_2 values for all treatments increased on day 21, and subsequently decreased slightly on day 42, however these changes were not significant.

The NIR responses of both horizons on day 21 were characterized by high variation, which resulted in no discernible treatment or treatment x horizon effects on this date (Fig. 3.1F). By day 42, variation was reduced and C amended soils were found to have significantly greater NIR responses than the N amended soils in the organic horizon, and greater responses than both the N amended and control soils in the mineral horizon. NIR in the mineral horizon showed inconsistent changes over time, but C amended soils showed a clear increase over the other treatments on day 42 to a high value of 0.28 CO_2-C·g^{-1} dry soil·h^{-1}. In the organic horizon, NIR responses in all the treatments were found to increase on day 21. The N and control treatments maintained these same levels on day 42, whereas the NIR response in the C amended soils increased further on day 42 to a value of 10.3 µg CO_2-C·g^{-1} dry soil·h^{-1}.

Correlations between soil variables on day 42

In the mineral horizon, basal respiration, microbial biomass, and qCO_2 were found to have strong positive correlations with one another, and all three had significant positive correlations with both measures of soil C availability (Table 3.2). NIR was also found to have significant positive correlations with the microbial parameters and with both measures of soil C availability. Mineral N availability was found to have negative but statistically insignificant correlations with all other measured variables.

Table 3.2. Correlation coefficients between microbial parameters and mineral N availability in the mineral horizon on day 42.

	Biomass	$q\mathrm{CO_2}$	CAI	AC	NIR	KCl-N[a]
Basal	**0.98	**0.99	*0.76	**0.98	*0.76	-0.48
Biomass		**0.98	*0.68	**0.96	*0.72	-0.53
$q\mathrm{CO_2}$			*0.77	**0.98	*0.76	-0.48
CAI				*0.73	*0.70	-0.22
AC					*0.70	-0.47
NIR						-0.50

[a] KCl-extractable mineral N

*,** Significant correlation at $P \leq 0.05$ and $P \leq 0.001$, respectively.

Table 3.3. Correlation coefficients between microbial parameters and mineral N availability in the organic horizon on day 42.

	Biomass	$q\mathrm{CO_2}$	CAI	AC	NIR	KCl-N^a
Basal	**0.99	*0.86	-0.30	*0.71	**0.92	*-0.79
Biomass		*0.77	-0.44	*0.75	**0.95	*-0.77
$q\mathrm{CO_2}$			0.19	0.54	0.64	-0.63
CAI				-0.36	-0.53	0.37
AC					0.65	-0.51
NIR						*-0.66

^{a} KCl-extractable mineral N
*,** Significant correlation at $P \leq 0.05$ and $P \leq 0.001$, respectively.

The strong correlations between microbial parameters and soil available-C that were found in the mineral horizon were not as evident in the organic horizon (Table 3.3). Where CAI was found to be significantly correlated with all other variables except mineral N availability in the mineral horizon, it was not found to be significantly correlated with any variable in the organic horizon. Basal respiration, microbial biomass, and qCO_2 in the organic horizon were once again strongly correlated with one another, however only basal respiration and microbial biomass were found to correlate with C availability as measured by AC. NIR was found to be strongly correlated once again with basal respiration and microbial biomass, as well as being significantly negatively correlated with mineral N availability and possibly with AC (P = 0.06). It was, however, not found to be significantly correlated with CAI or qCO_2. With the exception of CAI, mineral N availability was negatively correlated with all other variables, with significant correlations occurring with basal respiration, microbial biomass, and NIR.

DISCUSSION

Soil C availability

Both CAI and AC are measures of soil available-C, however they are subject to different interpretations. CAI values represent the ratio between the microbial activity levels that are sustained at the soil's "basal" C levels, and the maximum activity levels that the SMB can achieve when C is not limiting. Thus, CAI is a measure of the C limitation that prevents the SMB from realizing its maximum potential activity levels with respect to C availability. CAI values that approach 0 reflect a microbial community that is far from reaching its maximum potential activity level because of C limitation, whereas CAI values that approach 1 reflect a microbial community that is near its maximum potential activity level and not C limited (Cheng et al. 1996). In contrast, the curve-fitting method for

determining *AC* not only considers the microbial activity levels at basal and saturation C levels, but also incorporates the respiratory responses of the SMB between these two points. The slope of this curve dictates the value of *AC*, and also gives an indication of the affinity of the SMB for C. Thus, *AC* values also inherently incorporate a measure of the affinity of the SMB for C.

Although different patterns were observed in CAI and *AC* measurements, soils that were chronically amended with glucose were generally found to have greater soil available-C. In the mineral horizon, the consistently greater CAI values in C amended soils suggests that the SMB in these soils was less C limited than the SMB in the other two treatments, which in turn suggests that C amended soils had relatively greater C availability. The increase in biomass levels in C amended mineral soils was relatively small, however, suggesting that the amount of microbial biomass that can be supported in the mineral horizon may be determined more by other factors than soil C availability. As such, the rate of added glucose to these soils may have exceeded the capacity of the SMB to use C, resulting in a build-up of available C. The organic horizon could be generally characterized as having higher substrate quality than the mineral horizon, and thus likely supports a microbial community that is already adapted to higher labile-C levels. The lack of a similar increase in CAI values in the C amended organic soils suggests that the rate of C input did not exceed the metabolic C demands of the SMB, and as a result, microbial C-limitation in these soils remained high.

In addition to reflecting greater available-C levels in C amended soils on day 42, the *AC* data also suggests that the SMB in C amended soils had a lower affinity for C than the SMB in N amended soils. Either the presence of high levels of C in the C amended soils reduced the affinity of the SMB for C, or the presence of high levels of mineral N in the N amended soils stimulated the affinity of the SMB for C. The lack of effect that mineral N amendment had on CAI values of either horizon, however, suggests that the presence of readily available N in the soil did not promote additional metabolism of C from the soil available C pools.

The bulk of the treatment effects on basal respiration, microbial biomass, and qCO_2 occurred in the C amended soils, suggesting that the SMB is responsive to inputs of labile-C. Microbial activity and growth are first and foremost limited by C availability (Smith and Paul 1990). The greater basal respiration rates and microbial biomass levels in C amended soils suggests that the chronic input of glucose into these soils alleviated the C limitation of the SMB and allowed it to increase both its activity and size. Other incubation studies have also reported increases in microbial activity and numbers to added glucose over time (Sparling et al. 1981, Sparling and Williams 1986, Bradley and Fyles 1995). The controlling effect of available C on microbial activity and growth is further supported by the strong positive correlations observed between basal respiration and microbial biomass and both available carbon measurements in the mineral soil, and with AC in the organic soil.

In the mineral horizon, the proportionally large increases in basal respiration with relatively small concomitant increases in biomass levels in the C amended soils suggests that microbial activity in the mineral horizon is highly C limited, whereas microbial growth is limited by other factors besides C availability. This disproportionate increase in basal respiration with respect to biomass levels resulted in the high qCO_2 values of these soils. Low qCO_2 values are associated with a slow-growing but C efficient autochthonous microbial community, whereas high qCO_2 values are associated with a more opportunistic but C inefficient zymogenous microbial community (Insam and Haselwandter 1989). The relatively low substrate availability in the mineral horizon would normally support a relatively autochthonous microbial community. The steep increase in qCO_2 values in C amended soils on day 21 suggests that the chronic glucose treatments raised soil available-C levels, resulting in a shift in the SMB from an autochthonous to a zymogenous community structure. The subsequent reduction in qCO_2 values by day 42 was likely due to the adaptation of the SMB

to higher available-C levels, and its consequent stabilization to a more efficient metabolic state.

In contrast, the proportional increases in both basal respiration and microbial biomass in the C amended organic soils resulted in no significant changes in qCO_2 values of these soils over those of the other treatments. The forest floor is a C-rich environment relative to the mineral horizon, and likely supports a microbial community that is already zymogenous in structure and adapted to high C conditions. Thus, the pulses of labile C into these soils may have been simply mineralized by the existing SMB, without having shifted the microbial community structure in the forest floor to an even more zymogenous state. This is reflected in the lack of significant correlations between qCO_2 values and either C availability index.

Mineral N availability on microbial dynamics

In contrast to C availability and microbial dynamics where consistent patterns had established themselves by day 21, mineral N availability in the soil was still highly variable on day 21 and did not stabilize into discernible patterns until day 42. Although not reflected in the measured biomass values, there may have been a significant turnover of microbial populations occurring over the first four weeks of the incubation as the soils acclimatized to laboratory conditions, which may have resulted in extensive N mineralization. The variation early in the incubation suggests that mineral N availability may be more sensitive to changes in the soil environment than either soil C or SMB parameters, and may take a longer period of time to stabilize.

Although mineral N levels became significantly greater in N amended soils on day 42, this had relatively small observable effects on the measured microbial parameters. Despite slight increases in basal respiration and qCO_2 values relative to the control soils, N amended mineral soils did not differ significantly from the control soils in the other measured variables. This suggests

that SMB activity and dynamics in the mineral horizon are much more responsive to fluxes in soil C availability than to fluxes in soil N availability.

In the organic horizon, the observed negative effects that mineral N amendment had on basal respiration and biomass levels are consistent with findings from other studies that reported the inhibitory effects of mineral-N additions to the soil on microbial activity and biomass (Salonius 1972, Nohrstedt et al. 1989). In some of these studies, the depressing effect was attributed to decreases in soil pH due to the amendment of ammonium, however pH was not found to change over the course of this incubation (data not shown). The decrease in microbial respiration may have been due to increasing SMB exploitation of the mineral N source, which could be taken up without the concomitant mineralization of C that accompanies the metabolism of organic N sources.

Relationships between NIR, soil C, N, and microbial dynamics

NIR responses were similar to mineral N responses in that they were highly variable on day 21, but settled into discernible patterns by day 42. The high variation in the soil mineral N levels was likely itself a major cause of the variation in the microbial physiological N demand on this date. In this respect, the NIR response may be more sensitive to N dynamics in the soil than the microbial parameters or the indices of C availability, and thus may be more reflective of the N fluxes that occur in the soil. Its usefulness as an effective indicator of microbial N demand, however, may be limited when mineral N levels have not yet stabilized, as NIR values were not found to be significantly correlated with any of the other measured variables on this date (data not shown).

The stabilization of mineral N levels by day 42, however, resulted in the NIR data falling into the expected patterns of response. The significant increases in the NIR responses of C amended soils on this date suggest that the SMB in these soils had developed a strong physiological demand for N. The input of labile-C into these soils likely increased the microbial physiological N demand by: a) alleviating the C limitation, resulting in N becoming the next limiting nutrient,

and by b) allowing the SMB to proliferate, resulting in a larger microbial biomass competing for the same soil N pools. This relationship is further supported by the strong correlations between NIR values and microbial biomass values and soil AC values. Thus, increased C availability in the soil stimulated microbial growth and activity, both of which resulted in the increase of the SMB physiological demand for N, and consequently the magnitude of its NIR response.

NIR was clearly responsive to C availability in the soil, however its response to soil N availability was different in each horizon. In the mineral horizon, the significantly greater levels of mineral N in the N amended soils did not affect NIR values relative to the control. This, coupled with the significant positive correlations between NIR and both measures of C availability and the lack of significant correlations with mineral N availability, suggests that microbial N limitation in the mineral horizon is determined more by the abundance of available C than by the abundance of available N. Because microbial activity in the mineral horizon is much more C limited, the SMB may be capable of exploiting available N sources only when available C sources are at hand.

The N amended soils of the organic horizon, on the other hand, did have significantly lower NIR responses than the C amended soils. Furthermore, organic horizon NIR values were found to be significantly correlated with mineral N values, and not with either C availability index. This suggests that microbial N limitation in the organic horizon is determined more by the abundance of available N than by the abundance of available C. Again, the substrate rich environment of the forest floor likely supported a microbial community that was more limited by N, and as a result, the addition of a readily available N source would have reduced the physiological N limitation of the SMB.

In summary, these results suggest that after N dynamics in the soil have stabilized, the NIR response is a good indicator of the physiological N limitation of the microbial biomass. NIR was found to be more sensitive to changes in N availability than the other microbial measurements, and may be a more sensitive indicator of the physiological status of the SMB with respect to N demand than

qCO$_2$. The application of NIR measurements in field situations where N dynamics have likely already reached steady state could allow for useful comparisons of microbial N limitations between soils, as well as provide a predictive index as to which soils would mineralize or immobilize more nitrogen. Further work is required to determine the applicability of NIR to other soils and to test the strength of the relationships between NIR and other established measures of N availability.

Acknowledgements-

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CONNECTING PARAGRAPH III

In Chapter III, evidence supporting the usefulness of NIR as an indicator of microbial physiological nitrogen demand was presented. NIR was found to be more sensitive to changes in soil nitrogen availability than other microbial measures, and was also found to be sensitive to changes in soil carbon availability. Having established its usefulness allows the application of NIR in field studies to determine the impacts of microbial physiological nitrogen demand on nitrogen cycling in the forest. Chapter IV describes a long-term watershed study in which the relationships between nutrient availability, tree productivity, microbial physiological status, and nitrogen cycling were investigated.

CHAPTER IV

RELATIONSHIPS BETWEEN TREE GROWTH, NUTRIENT FLUXES,

AND SOIL MICROBIAL BIOMASS DYNAMICS

IN A NORTHERN HARDWOOD FOREST

ABSTRACT

In the forest ecosystem, the input of reduced C to the soil from trees is the driving force behind microbial growth and turnover of nutrients. As such, we reasoned that factors that affect tree productivity should, as a result, affect tree C allocation to the soil and ultimately microbial activity and growth. Furthermore, we reasoned that changes in microbial activity should impact nitrogen mineralization in the soil. To test these hypotheses, the relationships between nutrient availability, tree productivity, soil C and N availability, and microbial dynamics were investigated in a northern hardwood forest. Strong positive correlations between foliar litter K, Mg, and P fluxes and basal area growth suggested that the availability of these nutrients were limiting on growth. The development of K and Mg limitations may have been attributable to reductions in atmospheric inputs of these nutrients. Moreover, increased basal area growth and litterfall nutrient fluxes were associated with greater soil C availability. We hypothesized 1. that trees with greater nutritional status were able to fix more carbon and as a result allocate more C belowground, or 2. that trees with greater below ground C allocation stimulated microbial nutrient turnover, allowing for greater nutrient uptake, nutritional status, and growth in the trees. Soil C availability was thereafter found to be related with the activity, substrate-use efficiency, and physiological N demand of the soil microbial biomass. The physiological N demand of the microbial biomass, in turn, was related with the amount of N that was mineralized in the soil. In this way, tree production and C allocation impacted soil N dynamics by influencing the physiological status of the microbial biomass. Microbial physiological N demand was also correlated with foliar litterfall N flux, suggesting that the magnitude of N inputs from litter also influences the microbial physiological demand for N. Results indicate that plant productivity, microbial dynamics, and nitrogen mineralization were all closely linked, and that impacts in one component of the ecosystem will be expressed in the other components of the ecosystem.

INTRODUCTION

The flow of energy and nutrients through terrestrial ecosystems is dependent on the interactions between the autotrophic and heterotrophic components of the ecosystem (Aber and Melillo 1991). In forest ecosystems, trees are the main autotrophic source of C input to the system, however their productivity is limited by mineral nutrient availability (Mahendrappa et al. 1986). The heterotrophic microbial community, on the other hand, mediates the turnover and release of mineral nutrients in the soil, but its activities are limited by the availability of labile C sources in the soil (Smith and Paul 1990). In this way, the plant and microbial components of the ecosystem are inextricably linked to one another, as tree inputs of reduced carbon to the soil fuels microbial growth and activity, which in turn drives the turnover of soil nutrients upon which tree growth is dependent (Coleman et al. 1983, Pastor and Post 1986, Holmes and Zak 1994). As such, we should expect to see relationships between tree production, soil C availability, and microbial activity and nutrient cycling in forest ecosystems.

Numerous studies have examined the relationships between nutrient availability and tree productivity and nutrition, as well as the relationships between the soil microbial biomass and nutrient turnover in the soil. We know of few studies, however, that have integrated tree productivity with microbial biomass dynamics and nutrient turnover. The few studies that have investigated these relationships have found significant linkages between plant production and microbial activity and biomass on regional scales (Myrold et al. 1989, Zak et al. 1994). Clearly, further investigations into the linkages between autotrophic C cycling and microbial nutrient cycling are required to improve our understanding of ecosystem functioning.

Furthermore, most nutrient cycling studies are concerned with the cycling of C and N in the ecosystem, as it is often assumed that these are the most limiting factors on production in terrestrial ecosystems. The response of organisms in the ecosystem, however, integrates the cycling of all the nutrients in the system.

Consequently, this opens the opportunity for any single nutrient cycle to control the cycling of the other nutrients, and ultimately impact tree productivity. This was apparent in studies on maple decline, which showed that climatic stresses triggered deterioration in P and K nutrition in trees, which was subsequently followed by forest decline (Côté and Ouimet 1996). Further research on how perturbations in one nutrient cycle are expressed in other nutrient cycles and components of the ecosystem may give us insight into the potential impacts of management and other disturbances in forest ecosystems.

The long-term watershed monitoring project established at the Station de Biologie des Laurentides, in southern Québec, was developed with the aim of improving our understanding of carbon and nutrient cycling in forests, and how seasonal and interannual variation in different components of the ecosystem impact these cycles. In this paper, we report the findings of a three year study, undertaken as part of this project, that was directed at investigating tree-soil-nutrient linkages in the forest. The primary objectives of the study were to elucidate the relationships between 1. litterfall nutrient fluxes and aboveground tree production, 2. aboveground tree production and soil C availability, 3. soil C availability and microbial physiological status, and 4. microbial physiological status and nitrogen availability and cycling.

METHODS

Study site

The Hermine watershed is located at the Station de Biologie des Laurentides, near St-Hippolyte, Québec, Canada (45°59' N, 74°01' W). The watershed is situated in the Great-Lakes-St. Lawrence forest region of Rowe (1972). The regional climate is classified as cool continental, with long, cold winters and short, cool summers with a growing season that begins in mid-May and ends in mid-November. The area receives 1185 ± 111 mm (SD) of

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precipitation annually, with over half falling during the growing season in the form of rainfall. The mean air temperature in December and July are -10°C and 19°C, respectively.

The catchment has a surface area of ca. 5 ha with a drainage system consisting of an intermittent first-order stream. The soils have developed over anorthositic glacial till and are classified as Orthic Ferro-Humic Podzols (Humic Cryorthods) with a moder humus form. Some general soil properties are given in Table 4.1, and a detailed description of a typical soil profile can be found in Courchesne and Hendershot (1988). The canopy vegetation in the watershed is dominated by sugar maple (*Acer saccharum* Marsh.), with lesser amounts of red maple (*A. rubrum* L.), American beech (*Fagus grandifolia* Ehrh.), yellow birch (*Betula alleghaniensis* Britt.), paper birch (*B. papyrifera* Marsh.), and large-tooth aspen (*Populus grandidendata* Michx.). Nine 300 m^2 plots, representing the range of species and elevations found in the watershed, have been established from which vegetation and soil data were collected. Some site characteristics of the plots are given in Table 4.2.

Precipitation

Bulk precipitation in the years of 1994 to 1996 was sampled with two collectors made of 20 cm diameter plastic funnels attached to 2 L polyethylene bottles that were kept in an insulated box. The collectors were installed on top of a 15 m meteorological tower located on the watershed divide, ca. 100 m away from the plots. A nylon screen was placed in the funnels to avoid solution contamination by particulate matter. Solutions were sampled every two weeks during the growing season and at monthly intervals during the rest of the year. Samples were filtered through a 0.4 µm polycarbonate membrane, with total Ca and Mg concentrations of the filtrate determined by atomic absorption spectrophotometry (Varian) and total K and N determined by ion chromatography (Waters). Concentrations of P were not detected in precipitation samples, and therefore were not determined.

Table 4.1. Some general soil properties (means ± 1 SE) of the Hermine watershed in 1996.

Horizon	pH (1:10 soil:H$_2$O)	Total N (mg / g dry soil)	Exchangeable cations (mmol (+) / kg dry soil)			
			Ca	K	Mg	Total CEC
FH	4.3 ± 0.06	14.3 ± 0.5	215 ± 19	21.9 ± 1.9	7.2 ± 0.7	290 ± 20
Bfh	5.0 ± 0.02	3.0 ± 0.1	14 ± 1	1.4 ± 0.1	0.8 ± 0.1	44 ± 2

Table 4.2. Some site and vegetation characteristics of the plots in the Hermine watershed measured in 1996.

				% basal area by species					
				Acer		*Betula*		*Fagus*	*Populus*
Plot	Age	Slope position	Mean DBH (cm ± 1 SE)	*saccharum*	*rubrum*	*papyrifera*	*alleghaniensis*	*grandifolia*	*grandidendata*
1	180	lower	23.1 ± 2.1	99	-	-	-	1	-
2	180	lower	25.5 ± 3.6	98	-	-	-	2	-
3†	180	lower	29.2 ± 6.4	88	-	-	-	10	-
4	180	mid-upper	19.8 ± 1.8	76	24	-	-	-	-
5	180	mid-upper	19.8 ± 1.8	92	6	-	2	-	-
6	180	mid-upper	16.6 ± 2.8	81	10	-	9	-	-
7	90	upper	17.2 ± 1.4	26	20	-	27	6	21
8	90	upper	18.8 ± 1.8	20	10	16	12	2	40
9	90	upper	21.3 ± 2.2	26	11	3	-	3	57

† Striped maple (*Acer pennsylvaticum*) comprised ≈2% of the basal area in plot 3.

Basal area growth- In the fall of 1993, all trees with a stem diameter greater than 9 cm at 1.3 m aboveground (diameter at breast height- DBH) within each plot were measured for DBH. Trees were remeasured annually in the fall to determine their annual increase in DBH. Annual increases in tree diameter were converted to annual basal area increments, with total basal area increment per plot determined by summing the annual basal area increments of all trees in the plot. The basal area increment of each plot was extrapolated to a per hectare basis.

Litterfall- During the snow-free seasons of 1994 to 1996, litter was collected monthly from five 0.25 m^2 litter traps randomly distributed within each plot. Litter samples from each plot were bulked across all sampling dates in that year, and dried in a forced-air oven at 65°C for 48 h. Dried litter was weighed and sorted by species into foliage, seed, and miscellaneous fractions, with a subsample of each fraction ground in a cyclotec mill to pass through a 40-mesh screen. Ground litter samples were digested in a mixed H_2O_2/H_2SO_4 reagent (Allen 1989), with digests analyzed for their total N and P concentrations using a Quickchem AE autoanalyzer, and their total Ca, K, and Mg concentrations by atomic absorption spectrophotometry. Total annual litterfall mass and foliar litterfall nutrient fluxes for each plot were calculated and reported on a gram per square meter basis.

Soils

Collection- Soil collection occurred monthly from May to September of 1996. The FH layer of the forest floor and the top 15 cm of the mineral Bfh horizon were taken from four sampling points within each plot. Soils were passed through a 4.5 cm sieve to remove large roots and coarse woody debris. Soils from each horizon were bulked, respectively, to form composite forest floor and mineral horizon samples for each plot. Samples were thereafter placed into

polyethylene bags and brought back to the laboratory where they were processed within 48 h.

Extractable P- Extractable P in forest floor samples was determined for the months of May, July, and September. On each of these dates, a portion from the bulk forest floor sample of each plot was air-dried at room temperature. Extractable P was subsequently determined by extraction of the dried samples in Mehlich III solution (Mehlich 1984). Extracts were analyzed colorimetrically for their P concentrations using a Quickchem AE automated analysis system. Extractable P was reported on a microgram per gram of dry soil basis. Values across months were averaged to determine mean seasonal extractable P for each plot.

Net N mineralization- Net N mineralization was determined based on the sequential buried bag technique of Eno (1960). On each soil sampling date, duplicate subsamples (ca. 15 g and 20 g fresh weight forest floor material and mineral soil, respectively) were taken from each of the bulk horizon samples and sealed into their own respective polyethylene bags. One set of subsamples was returned immediately to the lab and kept frozen at -10°C until analyzed. The other set of subsamples was buried in a 15 cm deep hole at one of the sampling points in the plot and left to incubate *in situ* until the following sampling date. On the following sampling date, a new batch of *in situ* incubations were started, and buried subsamples from the previous month were removed from the ground and returned to the lab where they were frozen at -10°C until analyzed.

At the end of the growing season, all frozen subsamples were thawed overnight in a refrigerator at 4°C. After determining the moisture content of each subsample, aliquots of forest floor material and mineral soil of ca. 10 g and 15 g fresh weight soil, respectively, were extracted in 100 mL of $1N$ KCl solution. Extracts were filtered and frozen at -10°C until analyzed. Filtrates were thawed overnight at room temperature and then analyzed colorimetrically for NH_4^+-N (nitroprusside-salicylate) and NO_3^--N (Cd reduction) concentrations using a

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Quickchem AE automated analysis system. Net N mineralization for the monthly period was calculated as the difference between the post- and pre-incubation mineral N concentrations of that monthly period, and was reported as micrograms of mineral N per gram of dry soil. Total mineralized N for the 1996 growing season was calculated as the sum of all monthly net N mineralization values.

Microbial respiration measurements- The respirometry techniques used in this study to determine microbial respiration and calculate microbial physiological parameters were based on the methods described in Au et al. (1998, Chapter II). After determining the moisture content of the bulked horizon samples of each plot, soils were brought up to 70 % (w/w) field capacity moisture content by the addition of deionized-distilled water. Soils were mixed thoroughly and incubated in their polyethylene bags at room temperature for 7 d. Following the incubation period, 30 g and 70 g wet weight forest floor material and mineral subsamples, respectively, were removed from the bulk samples of each plot and analyzed for basal respiration, substrate-induced respiration (SIR), and substrate and nutrient-induced respiration (SNIR).

For the determination of basal respiration, subsamples were placed in 130 mL specimen jars, flushed with ambient air for five minutes using an aquarium pump, and sealed with lids equipped with a rubber septum. After 1 h, a 4 cc air sample from the headspace of each jar was removed with a syringe and injected into a Hewlett Packard 5890-II gas chromatograph equipped with a Poropak Q column and thermal conductivity detector (Hewlett Packard, Avondale, PA) to determine the CO_2 concentration of the air sample. Soils were dried at 70°C to determine the dry weight of each subsample. Basal respiration was calculated as the mean of the duplicate subsamples and reported as micrograms of CO_2-C per gram of dry soil per hour. All respiration rates were corrected for changes in room temperature and pressure, assuming ideal gas laws and a $Q_{10} = 2$.

For the determination of SIR, each subsample was placed into a 500 mL specimen jar and amended with *D*-glucose at a rate of 667 µg/g and 143 µg/g wet weight forest floor material and mineral soil, respectively, applied in the form of a

250 mg glucose-talc mixture. The talc mixtures were stirred into the soil using an electric hand blender, after which samples were transferred into 130 mL specimen jars and left uncovered at room temperature for 1.5 h. Samples were then flushed with ambient air for five minutes, and covered with lids equipped with septa. After 0.5 h, the CO_2 concentration of the air within the headspace of the jars was determined as described above for basal respiration. SIR values for each plot were calculated as the mean of the duplicate subsamples and reported as micrograms of CO_2-C per gram of dry soil per hour.

From the above data, it was possible to estimate the microbial biomass-C content of the soil, the specific respiration rate (qCO_2) of the microbial biomass, and soil carbon availability index (CAI). Microbial biomass-C content was calculated using the equation milligrams of microbial-C = 40.04·SIR + 0.37 developed by Anderson and Domsch (1978). qCO_2 is an ecophysiological index that has been used as an indicator of differences in microbial substrate-use efficiency (Anderson and Domsch 1989, Insam and Haselwandter 1989). qCO_2 values were calculated as the ratio of basal respiration to microbial biomass-C and reported as micrograms of CO_2-C per milligram of microbial-C. Soil CAI, the ratio of basal respiration to SIR, was used as a relative microbial measure of the abundance of readily available C in the soil (Parkinson and Coleman 1991). CAI values that approach 0 reflect a microbial biomass whose activity levels are highly C limited, whereas CAI values that approach 1 reflect a microbial biomass whose activity levels are not C limited (Cheng et al. 1996).

The determination of SNIR was carried out using the same method as described for the determination of SIR, except that soils were amended with a 250 mg talc mixture including both glucose and Difco nutrient broth. The mixture contained D-glucose at concentrations of 667 µg/g and 143 µg/g wet weight forest floor material and mineral soil, respectively, and Difco nutrient broth at concentrations of 833 µg/g and 357 µg/g wet weight forest floor material and mineral soil, respectively. SNIR values were calculated as the mean of the

duplicate subsamples and reported as micrograms of CO_2-C per gram of dry soil per hour.

By taking the difference between the SNIR and SIR values of each plot, it was possible to determine the respiration response of the microbial biomass to added nutrients (NIR) for each plot. NIR has been shown in previous studies to be a sensitive and accurate indicator of microbial physiological N demand in response to differences in soil C and N availability (Au and Fyles *in review*, Chapter III), and was shown to correlate negatively with net N mineralization and foliar litterfall N flux in the field (Au et al. 1998, Chapter II). The concept of the NIR measure assumes that the magnitude of the microbial respiration response to an added labile N source is directly proportional to the physiological N demand of the microbial biomass. Thus, a microbial biomass that is more N limited would have a relatively larger NIR response than a microbial biomass that is less N limited. NIR was calculated by subtracting the mean SIR value from the mean SNIR value for each plot, and was reported as micrograms of CO_2-C per gram dry soil per hour. Mean seasonal values of all respiration-based parameters for each plot were thereafter calculated by averaging values across all months.

Statistical analyses

Statistical analyses for differences across years of sampling in tree growth and litterfall data were tested by repeated measures ANOVA using the PROC GLM procedures of SAS (SAS Institute, 1989). Adjusted Greenhouse-Geisser F values with corresponding probabilities ≤ 0.05 were considered significant. Comparison of means, upon significance of the ANOVA, was performed using an LSD (t) test. Statistical analyses for differences between plots in soil and microbial data were tested by one way ANOVA using the PROC GLM procedures of SAS. Statistical analyses of soil measurements were performed on each horizon separately. F values with corresponding probabilities ≤ 0.05 were considered significant. Comparison of means, upon significance of the ANOVA, was performed using Bonferroni's multiple comparison test. Correlation analysis

between tree growth, litterfall, and mean seasonal soil variables, and between microbial and soil variables across the 1996 season, was performed using the Linear Models procedures of the Statistix statistical software (Analytical Software, 1996). Pearson's correlation coefficients with corresponding probabilities ≤ 0.10 were considered significant.

RESULTS

Precipitation chemistry

Among the three measured years, 1994 was characterized by average precipitation levels with relatively high atmospheric inputs of K^+ and Mg^{2+} (Table 4.3). 1995 was the driest of the three years, with ca. 8% less total precipitation than the three year mean. Furthermore, all nutrient inputs in 1995 fell to levels below their three year means, with marked decreases observed in K^+ and Mg^{2+} inputs. Precipitation levels in 1996 were the greatest of the three years, with most atmospheric inputs of nutrients generally approaching or exceeding 1994 levels. K levels, however, increased only slightly and remained below 1994 levels.

Tree growth and litterfall nutrients

Mean basal area growth was significantly greater in 1996 than in 1994, but was statistically equivalent to growth in 1995 (Table 4.4). Litterfall mass, however, was not found to differ significantly across the years (Table 4.4). Patterns in foliar litterfall nutrient fluxes varied across the years and between nutrients. Foliar litter Ca flux was significantly lower in 1996 than in both 1994 and 1995, whereas foliar litter P flux was significantly greater in 1996 than in 1994 (Table 4.4). Foliar litter N flux may have differed significantly across the years ($P = 0.07$), with the greatest levels measured in 1996 and the lowest levels in 1994. Neither foliar litter K or Mg fluxes ($P = 0.12$ and 0.10, respectively) were

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Table 4.3. Annual budget of aboveground nutrient inputs to the Hermine watershed for the years of 1994 - 1996.

Year	Source	H$_2$O ($\cdot 10^6$ L / ha)	Inputs (mg·m^{-2}·y^{-1})				
			Ca	K	Mg	N	P
1994	Precip.	12.3	501	340	95	2850	ND
	(+1993 litterfall)		3470	851	467	4690	95
1995	Precip.	11.3	469	59	61	2610	ND
	(+1994 litterfall)		3540	850	499	4535	78
1996	Precip.	13.4	721	86	78	2830	ND
	(+1995 litterfall)		4215	866	550	4982	89
Mean	Precip.	12.3	564	162	78	2763	ND
	(+litter-fall)		3898	855	505	4736	87

Table 4.4. Annual tree productivity and total foliar litterfall nutrient fluxes (means ± 1 SE) in the Hermine watershed from 1994 - 1996.

Year	Basal area growth $(cm^2 \cdot ha^{-1} \cdot y^{-1})$	Litterfall mass $(g \cdot m^{-2} \cdot y^{-1})$	Foliar litterfall nutrient flux $(mg \cdot m^{-2} \cdot y^{-1})$				
			Ca	K	Mg	N	P
1994	2630 ± 180	287 ± 12	3541 ± 64	791 ± 66	438 ± 31	1925 ± 116	78 ± 8
1995	5170 ± 230	285 ± 12	3494 ± 117	780 ± 92	472 ± 34	2153 ± 118	89 ± 8
1996	5070 ± 240	267 ± 12	3317 ± 115	725 ± 40	430 ± 24	2163 ± 174	94 ± 13

found to differ significantly across the years, however both fluxes tended to be lower in 1996 than in the two previous years.

Soil and microbial biomass dynamics

With the exception of CAI, net mineralized N and microbial biomass parameters in the forest floor were generally 0.5 to 1 order of magnitude greater than those in the mineral horizon. In the forest floor, none of the variables were found to differ significantly across the plots. Total mineralized N appeared to be greater in plots 3 to 6, whereas NIR tended to be lower in plots 3 to 6 (Fig. 4.1). In the mineral horizon, significant differences between plots were observed in microbial biomass, NIR, and soil moisture (Fig. 4.2). Microbial biomass was significantly greater in plot 2 than in plots 4 and 9. NIR was significantly greater in plot 1 than in plots 4, 6, and 9. Soil moisture was significantly greater in plot 1 than in plots 5 and 9.

Correlation analysis

Basal area growth- Basal area growth in 1994 was not found to correlate significantly with any foliar litterfall nutrient flux ($P \geq 0.44$). In 1995, however, strong positive correlations between basal area growth and foliar litter P ($P = 0.01$) and K fluxes ($P = 0.001$) were observed (Fig. 4.3). Similar relationships between basal area growth and foliar litter K, Mg, and P fluxes in 1996 were also observed (Fig. 4.4), although the strength of the correlations was slightly lower ($P \leq 0.05$) than in 1995. Neither foliar litter Ca flux ($P \geq 0.12$) nor N flux ($P \geq 0.23$) were found to correlate with basal area growth in any of the years. To determine if the above correlations were influenced by a species gradient across the plots, basal area growth was also plotted against sugar maple litterfall nutrient fluxes, as sugar maple occurs ubiquitously throughout the watershed. Basal area growth was found to significantly correlate with sugar maple foliar litter Mg flux ($P = 0.02$), and nearly correlated with foliar litter K ($P = 0.12$) and P fluxes ($P = 0.11$) in
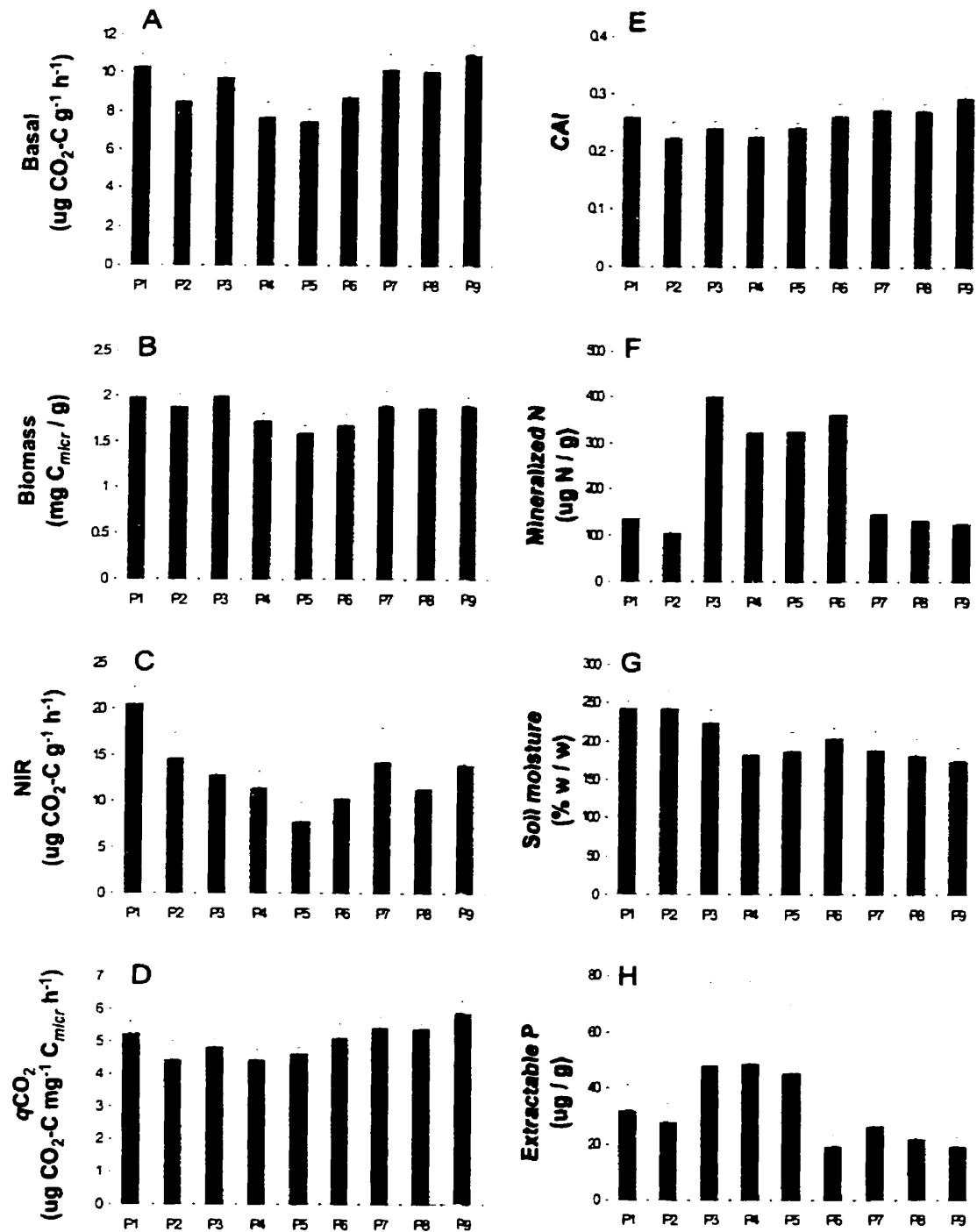
Fig. 4.1. Forest floor basal respiration (A), microbial biomass C (B), NIR (C), qCO$_2$ (D), CAI (E), total mineralized N (F), soil moisture content (G), and Mehlich extractable P (H) in 1996 for the nine plots of the Hermine watershed. Values = means ± 1 SE.
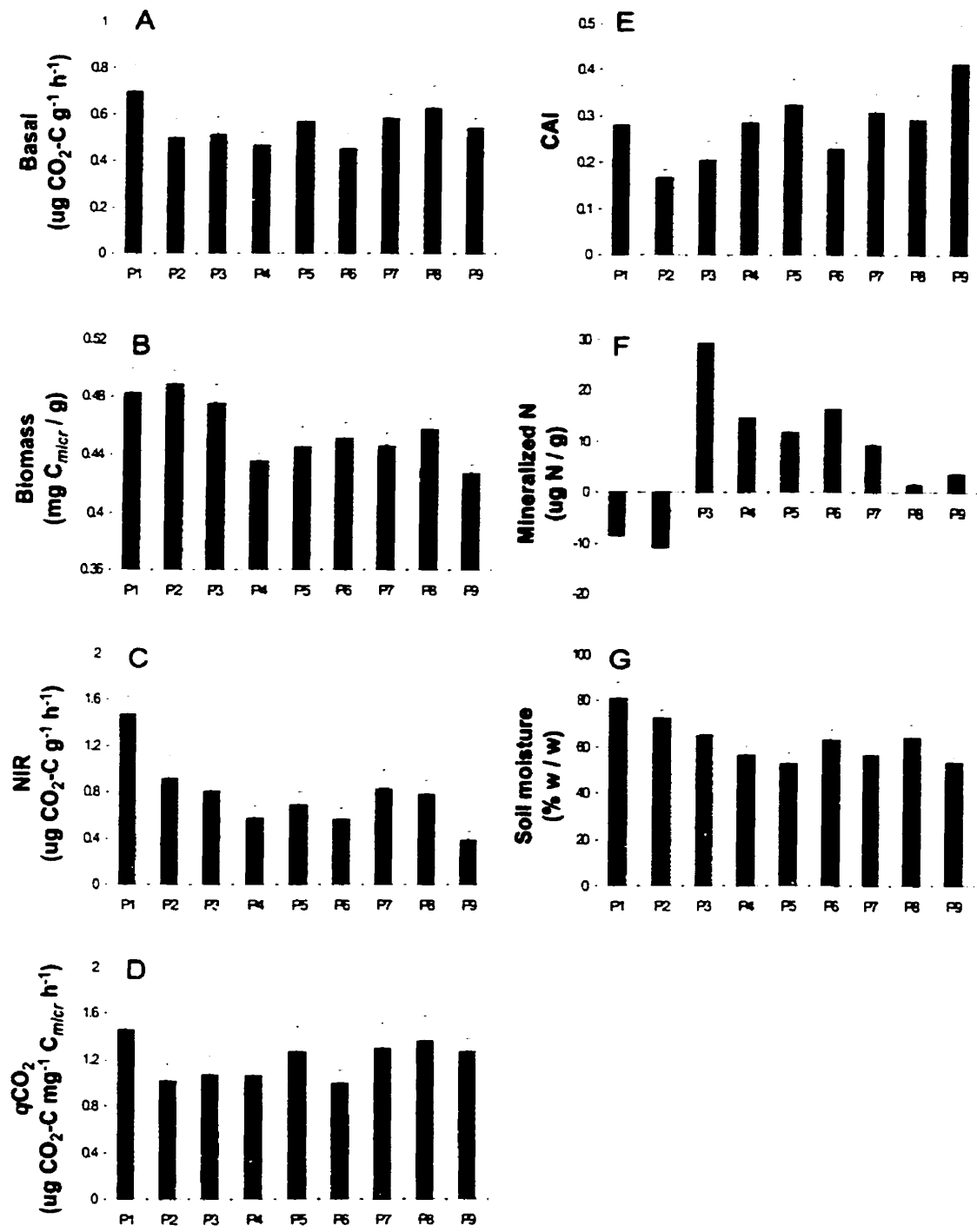
Fig. 4.2. Mineral horizon basal respiration (A), microbial biomass C (B), NIR (C), qCO_2 (D), CAI (E), total mineralized N (F), and soil moisture content (G) in 1996 for the nine plots of the Hermine watershed. Values = means ± 1 SE.
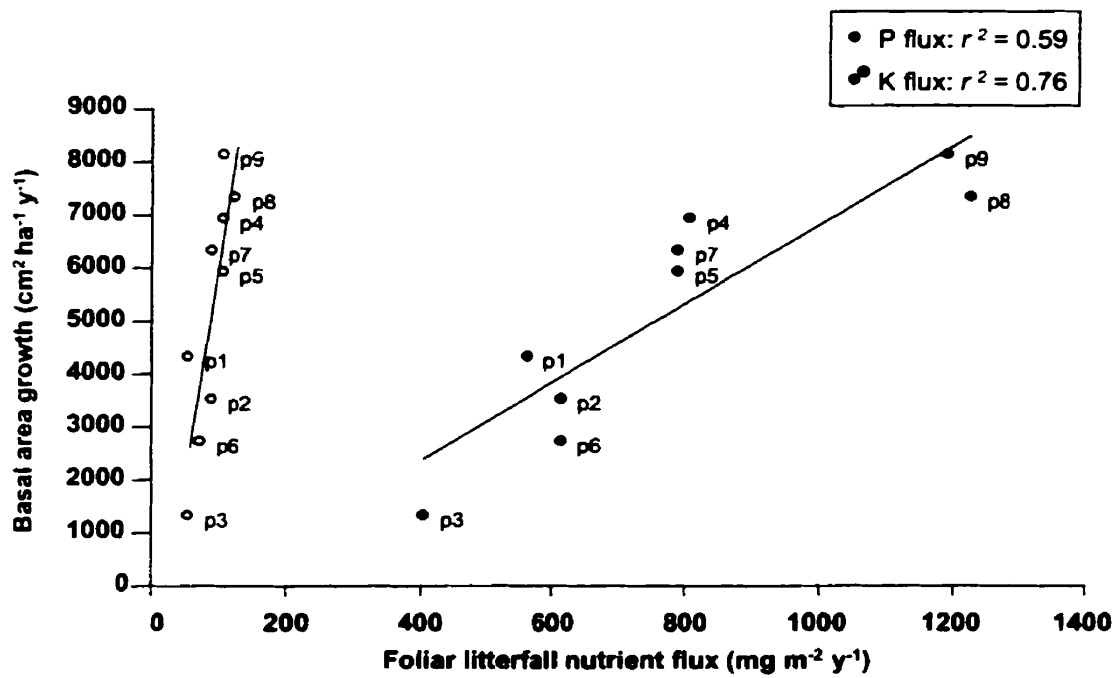
Fig. 4.3. Relationship between mean foliar litterfall P and K flux and basal area growth in 1995 in the 9 plots of the Hermine watershed.
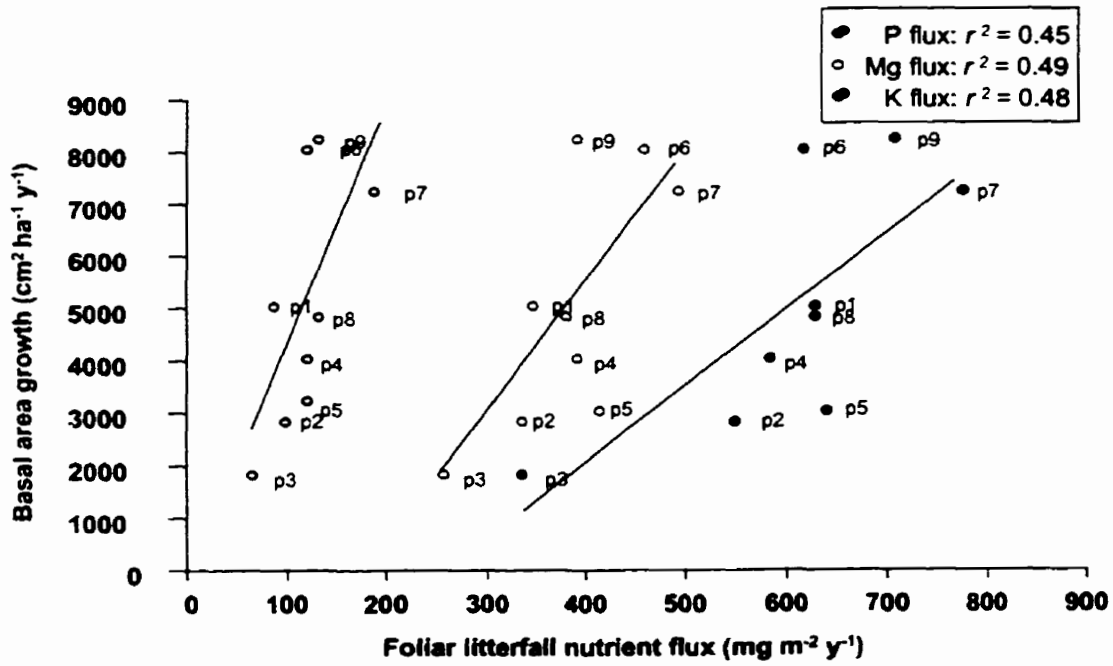
Fig. 4.4. Relationship between mean foliar litterfall P, Mg, and K flux and basal area growth in 1996 in the 9 plots of the Hermine watershed.

Table 4.5. Correlation coefficients between basal area growth, sugar maple foliar litterfall nutrient fluxes, and mean seasonal CAI values in 1996.

	Sugar maple foliar litterfall nutrient flux				
	Ca	K	Mg	P	N
CAI$_{FH}$†	-0.04	**0.76	*0.60	0.45	0.18
CAI$_{Bfh}$	0.10	***0.77	*0.59	*0.58	0.16
BAG‡	0.11	0.56	**0.76	0.57	0.32

† Carbon availability index (with horizon in subscript).
‡ Basal area growth.
*,**,*** Significant correlation at $P \le 0.1$, $P \le 0.05$, and $P \le 0.01$, respectively.

sugar maple (Table 4.5). With respect to soil variables, mean seasonal extractable P was found to negatively correlate with basal area growth in 1996 (P = 0.02), however no other soil variable was found to correlate significantly with basal area growth.

Mean seasonal soil CAI- Mean seasonal CAI values in each horizon were significantly correlated with one another (P = 0.03) (Table 4.6). CAI in the mineral horizon was found to be strongly correlated with basal area growth in 1996 (P = 0.008), however similar relationships between forest floor CAI and basal area growth were not observed (P = 0.14). CAI in both horizons were found to correlate only with the total foliar litter flux of K, although total foliar litter P flux was found to nearly correlate with CAI in the mineral horizon (P = 0.11). Significant correlations were found, however, when CAI was plotted against the foliar litterfall nutrient fluxes of sugar maple (Table 4.5). CAI in the forest floor was found to correlate with sugar maple foliar litter K (P = 0.02) and Mg (P = 0.09) fluxes, while CAI in the mineral horizon was found to correlate with sugar maple foliar litter K (P = 0.01), Mg (P = 0.10), and P fluxes (P = 0.10).

Mean seasonal nitrogen measures- Mean seasonal NIR in the forest floor was found to have a strong negative correlation (P = 0.005) with total foliar litter N flux in 1995 (Table 4.7). Correlations between mean seasonal NIR in the forest floor and total foliar litter N flux in 1996 and total mineralized N in the mineral soil were less strong but still significant (P ≤ 0.1). A nearly significant negative correlation (P = 0.12) between mean seasonal forest floor NIR and total mineralized N in the forest floor was also observed. Mean seasonal NIR values in the mineral horizon were significantly correlated with values in the forest floor (P = 0.02). Although mean seasonal NIR in the mineral soil showed negative relationships with foliar litter N flux in both years and total mineralized N in both horizons, a significant correlation was observed only with 1995 foliar litter N flux (P = 0.06). Total mineralized N in the forest floor was correlated with total mineralized N in the mineral soil (P = 0.002), however neither were significantly correlated with foliar litter N flux in either 1995 or 1996.

Table 4.6. Correlation coefficients between basal area growth, total foliar litterfall nutrient fluxes, and mean seasonal CAI values in 1996.

| | CAI_{Bfh} † | BAG‡ | Total foliar litterfall nutrient flux | | | | |
			Ca	K	Mg	P	N
CAI_{FH}	**0.71	0.53	-0.01	**0.66	0.40	0.48	0.06
CAI_{Bfh}		***0.81	-0.14	*0.61	0.45	0.57	0.05

† Carbon availability index (with horizon in subscript).
‡ Basal area growth.
*,**,*** Significant correlation at $P \leq 0.1$, $P \leq 0.05$, and $P \leq 0.01$, respectively.

Table 4.7. Correlation coefficients between mean seasonal NIR values, foliar litterfall N fluxes, and total mineralized N in 1996.

	NIR_{Bfh}	FLN flux 1995†	FLN flux 1996	Mineral-N_{FH}‡	Mineral-N_{Bfh}
NIR_{FH}	**0.74	***-0.83	*-0.63	*-0.58	-0.56
NIR_{Bfh}		*-0.64	-0.41	-0.34	-0.49
FLN flux 1995			0.40	0.40	0.43
FLN flux 1996				0.28	0.18
Mineral-N_{FH}					***0.88

† FLN = Foliar litterfall N.

‡ Total mineralized N (with horizon in subscript).

*,**,*** Significant correlation at $P \leq 0.1$, $P \leq 0.05$, and $P \leq 0.01$, respectively.

Seasonal data in 1996- Analysis of forest floor microbial data across the 1996 season showed that basal respiration, NIR, and CAI were all positively correlated with one another ($P \leq 0.01$) (Table 4.8). Furthermore, basal respiration, microbial biomass, and soil moisture were also positively correlated with one another ($P \leq 0.01$). Microbial biomass, however, was not found to correlate significantly with NIR, CAI, or net mineralized N. Net mineralized N and CAI were negatively correlated with each other ($P = 0.08$), however neither were significantly correlated with soil moisture ($P > 0.33$). NIR was strongly correlated with CAI ($P = 0.005$) and negatively with net mineralized N ($P = 0.003$), and with soil moisture to a lesser degree ($P = 0.08$).

In the mineral horizon, basal respiration and microbial biomass were both strongly correlated with NIR ($P \leq 0.002$), however the correlation between one another was less strong as it was in the forest floor ($P = 0.05$) (Table 9). Of the three, however, only NIR was found to correlate with soil moisture content ($P = 0.07$). CAI was found to be positively correlated with basal respiration and negatively correlated with microbial biomass ($P = 0.001$), but was not found to correlate significantly with NIR ($P = 0.95$). Net mineralized N was not found to correlate significantly with any variable except soil moisture.

DISCUSSION

Atmospheric inputs through precipitation can be an important source of mineral nutrients to the forest ecosystem (Likens and Bormann 1977, Bormann and Likens 1979). Grennfelt et al. (1985) reported that up to 19% of K and 10% of Mg inputs to a forested watershed in south-western Sweden originated from precipitation sources. Similar values for the Hubbard Brook Experimental Forest were reported, with 11% of the K inputs and 15% of the Mg inputs to this area originating from precipitation (Likens et al. 1977). Although these inputs of K (and to a lesser extent Mg) are relatively small compared to the nutrient cycling

Table 4.8. Correlation coefficients between microbial and soil variables across the 1996 season.

Horizon		Biomass	NIR	qCO_2	CAI	Min. N†	Moisture
FH	Basal	***0.69	***0.42	***0.79	***0.63	-0.18	***0.42
	Biomass		0.12	0.12	-0.10	0.01	***0.60
	NIR			***0.46	***0.41	***-0.52	*0.26
	qCO_2				***0.97	*-0.27	0.06
	CAI					*-0.26	-0.09
	Min. N						-0.15
Bfh	Basal	**0.29	***0.53	***0.98	***0.55	-0.18	0.07
	Biomass		***0.54	0.10	***-0.56	-0.20	*0.25
	NIR			***0.44	-0.01	-0.18	*0.27
	qCO_2				***0.69	-0.16	0.03
	CAI					-0.06	-0.14
	Min. N						*-0.25

† Net mineralized N

*, **, *** Significant correlation at $P \leq 0.1$, $P \leq 0.05$, and $P \leq 0.01$, respectively.

that occurs through litterfall and retranslocation within plant tissues (Miller 1986), they nevertheless represent fresh input of labile K and Mg into the system. As such, abrupt changes in the magnitude of these inputs would likely have an impact on the abundance of plant-available K and Mg in the system.

If we assume that the total annual aboveground input of a mineral nutrient to the soil is equal to the sum of precipitation inputs of that year and litter inputs from the previous year (throughfall was not calculated and will not be included), K and Mg inputs from precipitation in 1994 contributed 40% and 20% of the total annual aboveground K and Mg inputs, respectively, in 1994 (1993 litterfall data not shown). K and Mg inputs from precipitation, however, contributed only 7% and 12%, respectively, of the total aboveground K and Mg inputs in 1995, and 11% and 15%, respectively, of the total aboveground K and Mg inputs in 1996. Thus, not only did total inputs of K and Mg in 1994 exceed the total inputs in 1995 and 1996, but also a greater proportion of the K and Mg inputs in 1994 originated from precipitation sources, which unlike litterfall sources, do not have to undergo decomposition and/or leaching prior to becoming plant-available (Kaupenjohann and Zech 1992). These results suggest that the availability of labile K and Mg for tree uptake should have been greater in 1994 than in both 1995 and 1996.

Correlation analysis between basal area growth and foliar litterfall K and Mg flux support this observation, as no significant correlations were found in 1994, whereas basal area growth was strongly correlated with K flux in 1995, and with both K and Mg flux in 1996. This suggests that growth in 1995 was limited by the availability of K, and that growth in 1996 was limited by the availability of both K and Mg, whereas growth in 1994 was limited by other factors. Analysis of nutrient status in mature foliage would provide further insight into the relationships between tree nutritional status and growth, however this data was not available at the time of preparation of this manuscript.

The importance of P availability in controlling forest productivity is increasingly becoming recognized (Chapin et al. 1978, Pastor et al. 1984). The

positive correlations between foliar litterfall P flux and basal area growth in 1995 and 1996 suggests that P availability was also limiting on tree growth in these two years. Unlike K and Mg, however, P inputs to the watershed from precipitation were negligible, and as annual foliar litterfall P flux in 1996 was actually greater than in both of the previous years, the nature of the limitation may lie within differences in uptake from the soil available-P pools. The negative correlation between basal area growth and mean seasonal soil extractable-P suggests that plots where trees had greater P uptake at the expense of the soil available-P pools had greater growth in 1996.

Foliar litterfall K, Mg, and P fluxes and basal area growth were similarly correlated with CAI in the mineral horizon and nearly correlated with CAI in the forest floor. The nature of correlation analysis does not allow for causality to be established in the relationship between variables, but the results lead us to hypothesize on some of the processes that may be occurring independently or concurrently.

(*1*) *Trees with greater nutritional status, as reflected by greater litterfall nutrient fluxes, had less of a nutrient limitation on their productivity, and consequently had greater above- and belowground C allocation, as measured by basal area growth and soil CAI, respectively.* Increases in tree production in response to greater nutrient availability have been well documented, however there is as yet no clear consensus on the nature of the relationship between above- and belowground C allocation in forest ecosystems. Some studies have suggested that belowground C allocation is proportional to aboveground production (Nadelhoffer et al. 1985, Zak et al. 1994), whereas others have suggested that belowground production on infertile sites comes at the expense of aboveground production (Keyes and Grier 1981). Given the interpretation that CAI values are directly proportional to the amount of readily available C in the soil, the relationship between basal area growth and CAI suggests that above- and belowground C allocation can be proportional to one another. Basal area growth, however, is the last aboveground sink into which trees allocate C over the course

of the growing season (Kozlowski et al. 1991). As such, trees that have already met the C demands of root and shoot growth may be able to allocate "surplus" C to secondary sinks such as basal area growth and root exudates proportionally. We recognize that CAI is not an actual measure of the labile C pool in the soil, but that it provides a relative microbial physiological indicator of the abundance of readily-available C in the soil. There currently is no standard method to accurately measure soil labile C pools, however, and the use of the ratio of basal respiration to SIR as a measure of C limitation appears to be theoretically sound, as the only difference between the two respiration responses is the addition of available carbon (Cheng et al. 1996).

(2) *Trees with greater below ground C allocation, as measured by soil CAI, intensified microbial activity and nutrient turnover, resulting in greater nutrient availability to the trees and greater tree production.* Microbially-mediated transformations of nutrients in the soil are important processes in the cycling of most nutrients, particularly in the cycling of soil N and P (Chapin et al. 1978, Jenkinson and Ladd 1981, Paul and Clark 1989, Wardle 1992). It is suggested that microbial turnover of nutrients is not limited by the supply of nutrient sources in the soil, but by lack of available C or energy sources to mineralize these nutrients (Smith and Paul 1990). Consequently, plants that allocate greater amounts of C to the soil may stimulate microbial activity and increase turnover of soil nutrients (Aber and Melillo 1991). Berendse et al. (1991) proposed that the stimulation of microbial activity and mineralization of indigenous soil N on a site once dominated by the ericaceous shrub *Erica tretralix* L. was as a result of the greater root labile C inputs from the invading perennial grass *Molinia caerulea* (L.). Bradley and Fyles (1995) found that soils developed under birch seedlings had accelerated nutrient cycling compared to soils developed under other species, and attributed this to the superior rhizodeposition of birch roots which stimulated microbial nutrient acquisition. Of the three litterfall nutrients that were correlated with basal area growth, the availability of P is the most strongly influenced by microbial turnover (Yanai 1991). As such, trees

that allocated more C belowground may have stimulated greater microbial immobilization and subsequent turnover of P, and as a result their growth may have benefited from greater P uptake.

Closer inspection of the above correlations reveals that the upper slope plots tend to consistently occur near the top of the regressions between basal area growth and foliar litterfall fluxes of P, K, and Mg. Despite the lack of clear differences in microbial and soil characteristics between plots, a potential influence on these correlations may arise from the transition from shade tolerant climax species in the lower slope plots, to progressively more shade intolerant, pioneer species in the upper slope plots. Early successional species such as birch and aspen are known for their rapid growth and high nutrient uptake (Bormann and Likens 1979, Pastor and Bockheim 1984). As such, it is possible that the greater annual production and foliar litterfall nutrient fluxes observed in the upper slope plots are as a result of the greater occurrence of birch and aspen in these plots. Both basal area growth and CAI, however, showed similar relationships when correlated with sugar maple foliar litterfall nutrient fluxes alone. This suggests that sugar maples occurring in the upper slope plots also had greater basal area growth and nutritional status. Thus, it seems unlikely that the observed relationships are due only to a species gradient across the plots. Alternatively, the presence of birch and aspen in the upper slope plots may be stimulating greater nutrient turnover in the plot as a whole by virtue of greater belowground C allocation. In this way, the presence of birch and aspen could have a stimulatory effect on the nutrient uptake and growth of the other species occurring in the plot.

As microbial activity is driven by plant inputs of C (Coleman et al. 1983, Holmes and Zak 1994) we should expect to see relationships between microbial activity and soil C availability. As such, it is not surprising that significant positive correlations between soil CAI, basal respiration, qCO_2, and NIR were found in the forest floor. C availability in the forest floor was not only a major control on the activity levels of the microbial biomass, but also on the substrate-use efficiency and physiological N demand of the microbial biomass. Soils with

greater available C not only supported a relatively N limited microbial biomass, but would also supported a microbial community that was less efficient at substrate use (Insam and Haselwandter 1989, Bradley and Fyles 1995).

Whereas microbial activity was strongly correlated with soil CAI, microbial biomass content in the soil showed insignificant relationships with forest floor CAI, and even negative relationships with mineral horizon CAI. The latter seemed to be strongly influenced by decreases in microbial biomass and concurrent increases in CAI in September. Nevertheless, these results suggest that biomass levels are determined by other factors than labile-C availability. McGill et al. (1986) found that microbial biomass levels were predominately controlled by long term C availability and environmental fluctuations. Our results support this observation, as microbial biomass levels showed stronger positive relationships with soil moisture than with soil CAI. The negative effect of soil drying on the active component of the soil microbial biomass has been reported extensively (Bottner 1985, West et al. 1987, Wardle and Parkinson 1990), and has been attributed to death of the active component of the biomass due to desiccation (Bottner 1985) and/or the shift of part of the active component of the biomass to a dormant state (Wardle and Parkinson 1990). Soil moisture in both horizons, however, was poorly related to CAI and qCO$_2$, suggesting that fluctuations in moisture content had little impact on microbial carbon limitation and substrate-use efficiency.

Only weak relationships were observed between net mineralized N and microbial respiration, biomass, qCO$_2$, and CAI. This suggests that measures of C availability and microbial biomass parameters related to carbon are inadequate at predicting N dynamics in the soil. The strong negative correlation between forest floor NIR and net mineralized N, however, suggests that C availability in the forest floor affected nitrogen mineralization by controlling the physiological N demand of the microbial biomass, which in turn determined the amount of N that was mineralized. In this way, soil C availability, as determined by plant C allocation, influenced forest floor N availability by controlling the physiological N

demand of the microbial biomass. Foliar litterfall N flux was not found to correlate with total mineralized N in either horizon, however was found to be negatively correlated with NIR in both horizons. Thus, the physiological N demand of the microbial biomass appears to be also linked to the magnitude of historical litter N inputs to the soil. Similar linkages were not apparent in the mineral horizon, and this is consistent with the results reported in Au et al. (1998). In the mineral horizon, N mineralization seemed to be predominately controlled by environmental controls (soil moisture), whereas microbial physiological N demand seemed to be more directly related to microbial activity and size than to levels of available C or N.

In summary, our results suggest that linkages between atmospheric, tree, and soil level processes controlled the C and N cycling in the Hermine watershed in 1996. Relative decreases in atmospheric K and Mg inputs in 1995 and 1996, in contrast to 1994, may have resulted in K and Mg nutrient limitations on productivity in those years, as evidenced by strong correlations between basal area growth and foliar litterfall K and Mg fluxes. The availability of P also appeared to be limiting productivity, as significant correlations between foliar litterfall P fluxes and basal area growth were also observed. This was further supported by the negative correlation between basal area growth and extractable P in the soil, suggesting that trees with greater P uptake at the expense of the soil available P pools had increased growth.

Plots with greater basal area growth and foliar litterfall nutrient fluxes were also found to have greater soil C availability, leading us to put forth two hypotheses: 1. trees with greater nutritional status were able to fix more carbon and as a result allocate more C belowground, and/or 2. trees with greater below ground C allocation stimulated microbial nutrient turnover, allowing for greater nutrient uptake, nutritional status, and growth in the trees. Soil C availability was thereafter found to correlate with the activity and physiological N demand of the soil microbial biomass, with the latter found to be linked with the amount of N mineralized in the soil. In this way, tree production and C allocation impacted soil

N dynamics by influencing the physiological status of the microbial biomass. NIR was also correlated with foliar litterfall N flux, suggesting that NIR and nitrogen mineralization are also related to litter N inputs.

Our work suggests that ecosystem level variations in a particular energy or nutrient cycle can be expressed in the other nutrient and energy cycles by virtue of the multitude of linkages within the ecosystem. Further long-term studies that monitor seasonal and annual variations in the forest will be useful in determining the impacts of acute and chronic disturbances on forest ecosystems. Moreover, the interdependence between the plant and microbial components in determining the flows of C and nutrients in the ecosystem were emphasized in this study. Additional work to investigate the relationships between plant production and microbial activity and nutrient turnover will help us to understand how these components will respond to disturbances at different levels of the ecosystem.

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CHAPTER V

GENERAL SUMMARY AND CONCLUSIONS

The primary objective of this study was to examine the mechanisms of microbial control on soil N availability. As the microbial biomass impacts the soil mineral N pools through the processes of mineralization and immobilization, it was hypothesized that the physiological demand for N of the biomass should be a major determinant of whether N was being mineralized into or immobilized out of the soil mineral N pools. The development of the respiration-based parameter NIR, which quantified the physiological N demand of the microbial biomass, provided us with the opportunity to test this hypothesis.

The results from this study suggest that N mineralization is strongly governed by the physiological demands of the soil microbial biomass. The availability of labile C in the soil appears to be a main determinant of the microbial physiological N demand. Historical N availability and inputs also appears to affect the magnitude of N limitation experienced by the microbial biomass. Thus, microbial physiological status is the integrated response to both C and N availability in the soil. Results from this study emphasize the need for studies that integrate the flows of C and N in both the autotrophic and heterotrophic components of the forests ecosystem. Finally, the consistent relationships that were found between NIR and N mineralization in the forest floor suggest that NIR may be a potentially useful predictive index of forest floor N mineralization.

Although the application of NIR in studies of nitrogen cycling appears promising, at times the relationship between microbial physiological status and soil N availability are unclear. While the negative relationship between NIR and N mineralization in the forest floor was generally found to be consistent, it was often not observed in the mineral horizon. Furthermore, NIR and N mineralization were not found to be significantly related when soil N dynamics were highly variable. Although NIR is clearly related at times to N availability in the soil and could potentially be applied as a predictive index of N mineralization, further investigation to determine the controls on microbial physiological status when

NIR is not correlated with N availability are needed before NIR can be widely accepted.

This research has also been valuable in establishing a framework upon which further studies can be developed. The application of ^{15}N and ^{14}C tracer techniques in subsequent studies on the physiological status of the microbial biomass may provide additional insight into the nature and controls of the NIR response. Furthermore, the concept of NIR is based upon eliciting a physiological respiration response from the soil microbial biomass to an added N substrate. By changing the nature of the added substrate, this approach could be used to determine the microbial physiological demands for other soil nutrients. Finally, comparisons between NIR and other established indices of N availability will be required on a range of benchmark soils in order to determine the robustness of NIR as a measure of soil N availability.

Appendix I. Microbial biomass and N mineralization parameters (means ± 1 SE) of the mineral horizon.

Zone	Basal	SIR	NIR	Biomass-C	qCO_2	Mineralized N		
						June 1995	1994 Total	1995 Total
	($\mu g\ CO_2\text{-}C \cdot g^{-1}$ dry soil·h^{-1})			($\mu g\ C_{micr}$/g dry soil)	($\mu g\ CO_2\text{-}C \cdot mg^{-1}$ $C_{micr} \cdot h^{-1}$)	(μg N/g dry soil)		
Z1	0.57 ± 0.08	0.68 ± 0.04	0.32 ± 0.12	27.6 ± 1.8	1.37 ± 0.18	9.1 ± 2.2	N.D.	0.03 ± 0.01
Z2	0.79 ± 0.10	0.78 ± 0.06	0.31 ± 0.06	31.7 ± 2.4	1.90 ± 0.25	2.4 ± 0.2	N.D.	0.02 ± 0.01
Z3	0.74 ± 0.14	0.64 ± 0.11	0.31 ± 0.12	25.9 ± 7.9	1.80 ± 0.31	1.3 ± 1.2	N.D.	0.00 ± 0.00