AN ABSTRACT OF THE THESIS OF

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Abstract appro[redacted] Redacted for Privacy

Kermit Cromack, Jr.

In this study, biological and chemical characteristics were determined for two high-elevation meadow-to-forest transitions located in the Central Oregon Cascades. The chloroform fumigation incubation method (CFIM) was used to determine microbial biomass C (MBC) and the N flush due to fumigation (NF), and meadow values were compared to forest values for each. Meadow and forest MBC values were also compared for estimates of MBC determined with microscopy and these values were compared to CFIM estimates. Net N mineralization and C mineralization were determined for an 85-d incubation period and used as a measure of labile C and N. Microbial biomass C and NF were then compared to these labile pools in order to investigate the relationship between the amount of each nutrient stored in biomass and the magnitude of the respective labile nutrient

pool for each. Long-term and short-term net N mineralization rates and C/N ratios were also compared for meadow and forest soils, and the relationship between these two characteristics was examined.

In general, microbial biomass estimates made with the CFIM method did not show any significant differences between meadow and forest soils. Mean MBC for both sites as determined by CFIM was estimated to be 369 and 406 μg C g^{-1} soil in meadow and forest soils, respectively. Mean NF was estimated to be 37 and 56 μg N g^{-1} soil in meadow and forest soils, respectively. MBC estimates made using microscopy showed biomass C to be greater in the forest than in the meadow. Mean MBC as determined by microscopy was estimated to be 529 and 1846 μg C g^{-1} soil in meadow and forest soils, respectively. The NF measured as a percentage of the net N mineralized over 85 d was significantly greater in the forest than in the meadow soils, but was a substantial percentage in both. The means of these values were 30 and 166 % in meadow and forest soils, respectively. This led to the conclusion that biomass N may be a very important pool of stored labile N in this ecosystem. Net N mineralization rates were almost always greater in the meadow than in the forest soils. Net N mineralization for the 10-d incubations averaged 21 μg N g^{-1} soil in the meadow and 8 μg N g^{-1} soil in the forest Rates for long-term N mineralization averaged 126 μg N g^{-1} soil in the meadow and 52 μg N g^{-1} soil in the forest. Net N mineralization rates were correlated with C/N ratios for both short-term and long-term incubations.

Biology and Chemistry of a Meadow-to-Forest Transition in the Central Oregon Cascades

by

Rachel S. Heichen

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Rachel S. Heichen, Author

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Biology and Chemistry of a Meadow-to-Forest Transition in the Central Oregon Cascades

CHAPTER 1

GENERAL INTRODUCTION

During the past ten years, the development of molecular techniques for amplification and sequencing of genetic material has allowed microbial ecologists to examine the connection between genetic diversity and ecosystem function. One group of scientists at Oregon State University has begun a Microbial Observatory with the objective of studying this connection as it pertains to the nitrogen (N) cycle. The group is interested in investigating the question of whether or not diversities of specific N-cycling genes are related to the rates of processes for which those genes are associated. Previous studies have shown that vegetation can have an effect on N- cycling processes. For this reason, the group selected two meadow-to-forest transitions as study sites, with the expectation that differences in N-cycling characteristics between the two vegetation types will warrant further research regarding differences in the genes associated with N-cycling processes. Because no basic information existed on the biogeochemistry of these sites, some preliminary studies were performed in order to provide the group with baseline soil characteristics and to determine if differences in N- cycle process rates do indeed exist. My thesis begins as part of this preliminary investigation.

The first part of my study involved the use of the chloroform fumigation incubation method (CFIM) to determine microbial biomass carbon (MBC) and the nitrogen flush due to fumigation (NF) for meadow and forest soils at the two different sites. I was interested in whether I would find differences between meadow and forest soils using this method. I then compared measurements of MBC made with CFIM to those made with direct microscopic counts of bacteria

and fungi in order to investigate differences between the two methods. I also performed an 85-d long incubation of soils and measured mineralizable C and N. Microbial biomass C and NF were then compared to their respective mineralizable pools in order to determine how quantities of these pools related to C and N stored in the microbial biomass. Finally, I collated MBC data from different ecosystems around the world, all determined using the CFIM, in order to see where my numbers might fit into the global picture.

The second part of this study focused more on N dynamics. Both short-term (10 d) and long-term (77 d and 85 d) incubations were performed in order to study net N mineralization dynamics. I was interested in how net N mineralization rates might differ and how pool sizes of NH_4^+ and NO_3^- might shift over time for forest and meadow soil. I also investigated soil C/N ratios. I looked at how these ratios differed for meadow and forest soil, and how they were related to rates of net N mineralization.

Microbial Biomass Carbon

Under favorable environmental conditions, the microbial biomass functions as the major regulator of nutrient cycling. Although the biomass itself makes up only a small portion of the total organic matter in the soil, it transforms all organic matter into a form that can be used by other organisms. Thus, in the soil, as in all environments, microbial biomass recycles the nutrients that make life sustainable on this planet. In addition, the microbial biomass is a significant portion of the labile fraction of soil organic matter. It acts as a major nutrient sink during growth and a source during mineralization. For both of these reasons, the microbial biomass is considered to be an important indicator of soil quality and fertility (Voroney and Paul, 1984). Many methods have been developed through the years to measure MBC. These include direct microscopic counts, activity measures such as the substrate-induced respiration methods (SIR), and biochemical methods

including the CFIM and the chloroform fumigation extraction method (CFEM). The CFIM is the one that has been most commonly used since its introduction by Jenkinson and Powlson (1976). It was first noticed in the early 1900's that microorganisms were susceptible to fumigants, and that fumigant exposure would cause a flush of microbial growth to occur (Martens, 1995). The microorganisms that survived the fumigation were able to utilize the released labile nutrients. Jenkinson and Powlson (1976) related the increased respiration to the quantity of biomass that existed prior to the fumigation. The basic procedure has not changed much since its inception. After exposure of microorganisms to chloroform for a period sufficient to kill nearly all soil organisms, soils are allowed to incubate for a 10-d period, after which time carbon dioxide (CO_2) accumulation is measured in both a fumigated sample and an unfumigated control. Carbon dioxide resulting from the flush is converted into MBC using a conversion factor (k_c), which may vary in different soils (Voroney and Paul, 1984).

Jenkinson and Powlson (1976) recognized some limitations of CFIM even as the method was being developed. Mineralization of organic matter not associated with the living microorganisms results in a background respiration. Subtraction of an unfumigated control is used to correct for this extra respiration. This assumes, however, that the rate of mineralization of non-living substances is equal in both fumigated and unfumigated samples. This is not always true for samples that contain large amounts of easily degradable C sources. Low, or even negative biomass values may result in such cases (Adams and Laughlin, 1981). Thus, it is not recommended that the method be used to determine biomass C for soils that have had amendments added. Acidic soils with a pH of less than 5 can also be problematic when using CFIM. In soils with a pH of less than 4.5, MBC estimates were relatively low compared to other methods (Jenkinson, 1976; Williams and Sparling, 1984). One explanation offered by Jenkinson (1976) was that the microbes recolonizing the acidic soil after fumigation were less efficient at decomposing the dead biomass in the soil. The method also makes a few basic

assumptions: 1) fumigation kills "nearly" all of the soil microorganisms (however, there are enough organisms left in many soils that inoculation with fresh soil is unnecessary); 2) soil fumigation does not affect mineralization of the dead biomass by the recolonizing organisms; and 3) the amount of C mineralized with respect to the original quantity of biomass will be similar for different soils. When its limitations are observed, CFIM measurements correlated well with other methods used for determining MBC (Jenkinson, 1976).

The data listed in Table 1.1 have been collected from several different studies that used the CFIM to determine MBC. The studies represent a variety of ecosystems from geographic locations around the world. Along with biomass values, I have listed a few soil characteristics that are considered associated with the biomass values.

Net Nitrogen Mineralization

All life requires N. One of the critical functions performed by microorganisms is the transformation of organic N from decaying matter and waste products into its inorganic forms of ammonium (NH_4^+) and nitrate (NO_3^-). In this process, known as N mineralization, complex forms of N are broken down into forms that can be utilized by plants. Because N is often the limiting nutrient for plant growth, the quantity of available N is a major determinant of the amount of primary production that can take place, and thus affects growth of all organisms in the ecosystem's food web.

Attempts at quantification of N availability have been made since the early 1900's (Bundy and Meisinger (1994). Net N mineralization has been used as an index of N availability since the 1960's (Waring and Bremner, 1964). Net N mineralization (aerobic) is measured as the increase or decrease in the amount of NH_4^+ and NO_3^- produced per quantity of soil over a given incubation

Table 1.1 Microbial biomass C and soil characteristics for 26 soils from different ecosytems around the world.

Soil	Location	Vegetation	MBC (µg C g-1)	% C	% N	pH	Depth (cm)	Kc	Reference
1	U.K.	Arable soils	340	2.16	0.20	7.3	5-15	0.5	Jenkinson and Powlson, 1976
2	Oregon Cascades	Old growth conifer	985	5.11	0.22	5.8	0-15	0.41	Hart and Sollins, 1998
3	U.K.	Deciduous forest	414	4.09	0.35	5.5	5-15	0.45	Shan-Min et al., 1987
4	U.K.	Cut grassland	847	3.32	0.32	6.8	5-15	0.45	Shan-Min et al., 1987
5	New Zealand	Grassland	693	4.48	0.55	6.9	0-5	0.45	Sparling and Williams, 1986
6	West Africa	Moist savanna	70	0.61	0.05	5.5	0-10	0.45	Vanlauwe et al., 1999
7	West Africa	Forest	146	1.72	0.18	5.4	0-10	0.45	Vanlauwe et al., 1999
8	Kansas	Tallgrass prairie	844	2.73	0.25	6.7	5-15	0.41	Williams et al., 2000
9	U.K.	Forest	1354	6.40	0.52	5.8	0-10	varied	Vance et al., 1987
10	Bavaria	Forest	623	5.68	0.48	6.2	5-15	0.45	Beck et al., 1997
11	Bavaria	Arable soils	303	1.24	0.13	6.0	0-20	0.45	Beck et al., 1997
12	Spain	Forest	657	8.00	0.46	4.6	0-15	0.45	Diaz-Ravina et al., 1989
13	Negev, Israel	Desert, shrub	140	*	*	*	0-10	*	Sarig et al., 1994
14	Negev, Israel	Desert, open	95	*	*	*	0-10	*	Sarig et al., 1994
15	Amazon	Tropical rain forest	420	1.41	0.13	5.8	0-10	*	Feigl et al., 1995
16	Oregon	Old growth conifer	2976	18.60	0.64	5.1	0-10	0.41	Zak et al., 1994
17	New Mexico	Pinyon-juniper	80	2.00	0.15	8.3	0-10	0.41	Zak et al., 1995
18	New Mexico	Dessert-grassland	26	0.40	0.02	7.5	0-10	0.41	Zak et al., 1996
19	Colorado	Alpine tundra	813	10.20	0.71	5.1	0-10	0.41	Zak et al., 1997
20	Colorado	Shortgrass steppe	23	1.20	0.11	6.2	0-10	0.41	Zak et al., 1998
21	Minnesota	Tallgrass prairie	1017	1.60	0.09	5.6	0-10	0.41	Zak et al., 1999
22	Michigan	Hardwood forest	1526	6.10	0.41	6.3	0-10	0.41	Zak et al., 2000
23	New Hampshire	Hardwood forest	1787	25.50	1.20	4.0	0-10	0.41	Zak et al., 2001
24	North Carolina	Hardwood forest	1308	4.00	0.26	5.2	0-10	0.41	Zak et al., 2002
25	Oregon Cascades	High elevation forest	369	14.00	0.74	5.8	5-15	0.41	Heichen, present study
26	Oregon Cascades	High elevation meadow	407	10.10	0.78	5.9	5-15	0.41	Heichen, present study

* data not given

period. Net N mineralization can be broken down into several productive and consumptive processes occurring simultaneously. Productive processes include organic N transformation into NH_4^+ (ammonification) and NH_4^+ transformation into NO_3^- (nitrification). Both NH_4^+ and NO_3^- can be removed from the inorganic N pool via immobilization, the consumptive process that converts these inorganic forms into microbial tissues. Ammonium may also be fixed abiotically and NO_3^- may be lost from the soil via denitrification.

Net N mineralization can be quantified either aerobically or anaerobically. The anaerobic method usually consists of a 7 d waterlogged incubation (Bundy and Meisinger, 1994). The aerobic method may be 7-d, but is usually performed for 30 d (Bundy and Meisinger, 1994). To quantify net N mineralization, an initial concentration of inorganic N in the soil is first determined. At some later time point, the concentration of inorganic N is measured again, and the initial concentration is subtracted from the latter, giving a net amount of N mineralized over a period of time.

The main objectives of this study were: 1) to determine if meadow soils differ from forest soils in the quantity of MBC as measured by both CFIM and light microscopy; 2) to examine the relationship between labile C and N stored in biomass in relation to mineralizable C and N, respectively; 3) to determine how net N mineralization rates differ between meadow and forest soils; and 4) to examine the relationship between soil C/N ratio and net N mineralization rates.

CHAPTER 2

THE MICROBIAL BIOMASS

Abstract

Microbial biomass carbon (MBC) and the nitrogen flush (NF) due to fumigation were compared for high elevation meadow and forest soils at two sites: Carpenter Mountain and Lookout Mountain, located in the Central Oregon Cascades. Vegetation transitioned abruptly from grassy meadow to conifer forest at both sites. Mineral soils were sampled from a depth of 0-10 cm beneath the litter and organic layers during three different seasons, June, September, and November of 2000. The chloroform fumigation incubation method (CFIM) was used to determine MBC and NF. Mean MBC at Lookout, averaged over all three seasons, was estimated to be 446 and 437 μg C g^{-1} soil in the meadow and forest respectively. Mean MBC at Carpenter was estimated to be 292 and 377 μg C g^{-1} soil in the meadow and forest, respectively. June 2000 estimates of MBC were compared to estimates made by microscopy on the same soils. In the meadow, no significant differences were found between estimates made using the two methods. In the forest, microscopic estimates were approximately two and a half times as large as CFIM estimates at Lookout and five times as large as at Carpenter. At Lookout, the mean NF over two seasons (June and September of 2000) was estimated to be 36 and 63 μg N g^{-1} soil in meadow and forest soils, respectively. The NF at Carpenter was estimated to be 40 and 35 μg N g^{-1} soil in meadow and forest soils. With the exception of the September sampling date at Carpenter, no significant differences between meadow and forest NF were found. Both MBC and the NF were also examined in relation to the C and N mineralized during an 85-d incubation period. Microbial biomass C averaged between 12% and 16% of the C mineralized in the forest and meadow soils at both sites. At Lookout, NF averaged

approximately 36% of the N mineralized for the meadow and 111% of the N mineralized for the forest. In Carpenter soils, NF averaged approximately 23% in the meadow and 221% in the forest.

Introduction

By breaking down complex organic substances into simple mineral forms, the microbial biomass facilitates the constant exchange of C and N between the soil and its resident life forms. Nutrients that are not immobilized by microorganisms during the process of decomposition will be released back into the soil solution, where they have the possibility of meeting a variety of fates, including plant uptake, adsorption or fixation by various soil components, or re-assimilation by the microbial biomass (Myrold, 1999). The microbial biomass, which may turn over several times during a year (Waring and Schlesinger, 1985), represents a labile source of C and N. Microorganisms have typically been considered C limited (Smith and Paul, 1990). As such, the availability of C resulting from the quantity and quality of the vegetation will influence the size of the microbial biomass. However, recent studies have shown that the microbial biomass may be N-limited in some forest soils (Hart and Stark, 1997). If so, the availability of N and the C/N ratio may be a much larger controlling factor. There is also evidence that both C and N may be simultaneously limiting in different soil microsites (Chen and Stark, 2000). Thus, both the quality of C, and the quantity of C and N may be important determinants of the size of the microbial biomass.

Microbial biomass C and microbial biomass N (MBN) both represent portions of the total C and N pools. Microbial biomass N serves as an N reservoir, potentially available to plants during turnover of microbial biomass. Although heterotrophic microorganisms are considered superior to plants as competitors for inorganic N (Hart and Firestone, 1991), frequent microbial biomass turnover increases the events during which plants and their mycorrhizal symbionts may have

access to this inorganic pool, thus increasing the plant's chances of accumulating N in this way (Kaye and Hart, 1997). This could be an extremely important mechanism for plants to obtain N in ecosystems that have low decomposition rates, or systems in which microorganisms are N limited. In some systems, MBN may be mineralized in the largest quantities during the maximum growth season for plants. In an alpine dry meadow, Lipson et al. (1999) showed a sharp drop in biomass during July, August, and September, making N available to plants during the months of greatest plant activity in this system. Thus, biomass turnover could strongly affect N availability for plant growth.

As in many ecological associations, the relationship between microbial biomass and vegetation has evolved in such a way that each group of organisms influences the other. Thus, not only are plants affected by the size and turnover of the microbial biomass, but the microbial biomass also is likely influenced by vegetation characteristics. Various studies have shown that soils with different vegetation will differ in various aspects of their soil biology and biochemistry including N-cycling (Ross et al., 1996), decomposition rates (Hunt et al., 1988), and microbial community composition (Ingham et al., 1989). Comparing systems with contrasting vegetation, such as grassland and forest, could provide useful information about the influences of vegetation on characteristics of the biomass. Such studies have shown differences in: N immobilization rates and MBN turnover (Hart et al., 1993); the quantity of MBC (Ross et al., 1996); and time course profiles and points of peak release for N mineralization after chloroform fumigation (Davidson et al., 1989).

Numerous studies have measured MBC and MBN for meadow and forest ecosystems (Davidson et al., 1989; Zak et al., 1994), but the data for these two systems is usually obtained from different studies and/or sites. Comparisons are less equivocal if made using data from the same site, and using similar methodologies and treatments for all soils being compared. It is also important to consider that choosing a different methodology might produce a different result.

The CFIM has been the most commonly used since its introduction by Jenkinson and Powlson (1976). As with all methods used to quantify microbial biomass, it has limitations. Direct extraction is another biochemical method similar to CFIM that is also commonly used. Since there is no incubation period during which N may be immobilized by the microbial biomass, direct extraction may avoid some of the problems associated with CFIM. Activity measures such as substrate-induced respiration and direct microscopic counts are also used, and have been compared in some studies. In their original series of papers, Jenkinson and Powlson compare CFIM estimates to direct count estimates of bacterial biomass. Both methods showed similar results. More recent studies in different soil types have shown that this may not always be true (Ingham et al., 1991).

In this study, estimates of MBC (three seasons) and estimates of NF (two seasons) were made for adjacent meadow and forest soils at two locations. I was interested in whether or not differences or trends existed between the meadow and forest soils at each location. Estimates of MBC were also made using microscopy and compared to those made with CFIM. In addition, an 85-d incubation was performed, during which C and net N mineralization were determined as a measure of the readily mineralizable C and N pools respectively. Biomass C and N pools were then compared to C and net N mineralized.

Materials and Methods

Site description

June 2000

Two different locations at the H.J. Andrews Experimental Forest (HJA) in the Cascade Mountains were selected as study sites: Carpenter Mountain and Lookout Mountain (hereafter referred to as Carpenter and Lookout). Site selection was based on the availability at the HJA of sites containing a meadow adjacent to a

forest. Both sites are at an elevation of approximately 5000 ft. Lookout faces southwest and Carpenter faces due south. I have chosen to analyze each site as a separate case study, so that the information obtained from this study can be used by others to help answer questions pertaining to these specific sites In this observational study, three parallel, meadow-to-forest transects were placed at each site (see appendix). The treatments were defined as meadow (M), meadow/forest (MF), forest/meadow (FM), and forest (F). Transects were positioned to maximize the contrast between meadow and forest treatments. Along each transect, eight equally spaced circular plots (1 m diameter) were established, for a total of 24 plots. Transects were centered between MF and FM, with three meadow plots (M1, M2, and M3) and three forest plots (F1, F2, and F3) at either end. At Lookout, plots were spaced at 10 m intervals with a distance of approximately 20 m between transects. Since the ecotone was wider at Carpenter than at Lookout, plots were spaced 20 m, apart rather than 10 m apart. Transects 2 and 3 at Carpenter were separated by 20 m, while Transect 1 was located approximately 100 m upslope from the other transects. The uneven spacing between transects was necessary to work around areas where the meadow-forest transition was less clear and to avoid a hiking trail. A coring device consisting of 4.7-cm diameter PVC pipe was used to extract five soil cores from each plot. The litter and humus layers were removed from the cores (approximately 5 cm in forest and varying from 0 to 5 cm in meadow), and 10 cm of mineral soil were sampled from beneath these layers. Cores taken from the same plot were made into a single composite. Selected soil characteristics are given in Tables 2.1- 2.3.

Table 2.1 Textural analysis of soils from Lookout and Carpenter.

Site	Vegetation	Coarse	Sand	Silt	Clay
Lookout	meadow	0.0	68.7	19.4	2.1
Lookout	forest	0.2	66.6	18.6	5.4
Carpenter	meadow	0.0	66.4	18.6	5.6
Carpenter	forest	0.0	76.0	13.0	4.1

Table 2.2 Percent C and N and cation exchange capacity of soils from Lookout and Carpenter.

Site	Vegetation	% C	%N	CEC meq/100g
Lookout	meadow	11.24	0.89	39.5
Lookout	forest	14.98	0.94	45.1
Carpenter	meadow	9.46	0.69	39.0
Carpenter	forest	13.27	0.54	55.6

* Percent C and N are averages from soils collected along the three transects in June 2000

Table 2.3 Selected nutrient quantities of soils from Lookout and Carpenter

Site	Vegetation	P ppm	K ppm	Ca meq/100g	Mg meq/100g	Na meq/100g
Lookout	meadow	7	228	2.8	0.6	0.13
Lookout	forest	13	68	2.0	0.2	0.17
Carpenter	meadow	23	146	1.7	0.4	0.14
Carpenter	forest	19	67	0.9	0.3	0.11

September 2000

The locations that were utilized in June were sampled again in September, but because a related experiment was being set up simultaneously, core samples were not extracted from the original transects. Instead, a square grid was established in both the forest and the meadow at each site. Each grid contained 64 plots with 3 m between plots. There were only two treatments in this study, forest and meadow. Six cores were collected from randomly selected plots contained within the meadow grid and six from the forest grid at each location. Two random cores from each treatment and location were combined into a single composite, giving a total of three composite soil samples from meadow and forest respectively.

November 2000

The original transects that were sampled in June 2000 were utilized once again in November. Four plots were sampled along each transect (M2, MF, FM, and F2). Two cores were extracted from each plot and made into a single composite.

Treatment of soils prior to assay

Soils were transported in coolers from the HJA to OSU laboratories and stored at 4°C. The following day, all cores were sieved through a 4-mm sieve. All microbial biomass assays began within 48 h of soil collection.

Chloroform fumigation incubation estimates of microbial biomass C

Microbial biomass C was determined using the CFIM introduced and described by Jenkinson and Powlson, (1976). Replicate 25 g samples of field-moist soil were placed into 50-ml beakers, and soils were adjusted to 67% moisture content. Soils were fumigated with chloroform and left sealed in glass desiccators

for a period of 24 h, after which time the desiccators were repeatedly evacuated to remove the chloroform from the soil. Soil samples were left under a vented hood for approximately 2 h to allow any remaining chloroform to evaporate, and soil moisture was readjusted to 67% moisture content.

Soil samples were then transferred to specimen cups and prepared for incubation. Each specimen cup was placed in a mason jar and the lips of jars were coated with silicon grease. Water (1 ml) was added to the bottom of each mason jar to ensure that soil moisture would be retained during the incubation. A vial containing 5 ml of 1 M KOH was placed inside each mason jar, after which each jar was immediately sealed. An unfumigated control set of soil samples was also prepared for incubation. Both fumigated and unfumigated samples were incubated at 25°C for 10 d. At that time, the KOH vials were removed from the mason jars and 0.5 ml of 3.3 M $BaCl_2$ was added to each in order to precipitate CO_2. Carbon dioxide captured by each base trap was determined via titration with 0.26 M HCl. The amount of CO_2 in the unfumigated sample was regarded as background respiration and was subtracted from respiration in the fumigated sample. A K_c of 0.41 was used to convert C respired into MBC (Anderson and Domsch, 1978).

The following formula was used to convert the amount of KOH neutralized by the acid into microbial biomass C:

$MBC = (F-U) * 12 * (M_a) / [2 * Kc * Wt]$

MBC : microbial biomass C in mg g^{-1} dry weight of soil

F-U: acid titrated to neutralize KOH in the fumigated sample (ml) minus the amount titrated in the unfumigated sample

M_a: molarity of acid

12: mg C $mmole^{-1}$ of CO_2

2: $2H^+/CO_2$ evolved (For every CO_2 captured, KOH neutralizes 2 H+ ions.)

Kc = 0.41

Wt: g dry weight of soil

Nitrogen flush due to fumigation

The soil samples described above for the MBC portion of the study were utilized once again to determine the NF. After the 10-d incubation period, inorganic N was determined for both fumigated and unfumigated soils using a KCl extraction. Soil subsamples (10 g) were removed from the incubated soils, placed in specimen cups, and extracted with 40 ml of 2M KCl. Samples were shaken on a mechanical shaker for 45 min. Funnels lined with Whatman No. 1 filter paper were soaked with 0.5 M HCl in order to remove any contaminating inorganic N and then pre-leached with 2 M KCl. The contents of the shaken specimen cups were emptied into the funnels and the filtrates collected for analysis of NH_4^+ and NO_3^-. An Astoria Pacific series 300 autoanalyzer was used to colorimetrically determine NH_4^+ and NO_3^- concentrations. The NF was calculated as $NH_4^+ + NO_3^-$ in fumigated samples minus $NH_4^+ + NO_3^-$ in unfumigated samples. Because the K_n correction factor can be difficult to determine and may be quite different for meadow and forest soils, a correction factor was not used to convert numbers to microbial biomass N. Instead measurements are reported as the NF.

Microscopic estimates of microbial biomass C

Microscopy was used to determine MBC for bacteria and fungi. Fresh soil (1 g) was placed in 9 ml of 0.2 M phosphate buffer at pH 7.2. An agar film was prepared using 0.5 ml of the 1:10 soil suspension and 1 ml of liquefied 1.5% (w/v) agar (Ingham and Klein 1984). Differential interference contrast microscopy (200X) was used to count hyphal lengths. To quantify bacteria, a 1:100 soil suspension was made. Cells were stained with fluorescein isothiocyanate and filtered onto a 0.4-μm polycarbonate filter. Epifluorescent microscopy at 1000X with oil immersion was used to examine filters (Babiuk and Paul 1970). Bacterial and fungal biomass were calculated from the volume of bacterial cells or fungal hyphae in 1 g of dry soil using the visual estimates. It was assumed that bacterial

cell density averages 330 mg cm^3, and fungal tissue density averages 410 mg cm^3 (Ingham et al., 1991). Total biomass was converted to MBC by assuming that C averaged 50% of cellar mass (Ingham et al., 1991). All raw data for microscopic estimates of bacterial and fungal biomass C were provided by Kirk Waterstripe, Department of Forest Science, Oregon State University.

Respiration

Soil samples (50 g) were placed into specimen cups. The moisture level of each sample was brought to 67 % moisture content. The samples were placed in mason jars along with 5 ml of 1.0 M KOH as described above for the CFIM procedure. Two control mason jars containing KOH base traps, but no soil, were also prepared. Sealed mason jars were incubated at 25° C for a period of 85 d. Potassium hydroxide (KOH) vials were periodically removed and titrated with standard HCl in order to determine the amount of CO_2 captured by the KOH.

Respiration was determined by the following formula:

$R = [(A_c - A_r) * M_a * 12]/2 * soil$

R = respiration in µg C/ g dry weight soil

A_c = amount of HCl (ml) required to neutralize the control vial of KOH

A_r = the amount HCl (ml) required to neutralize non-control KOH

M_a = molarity of the HCl used for titration

12 = g C/ g CO_2

2 = 2 moles of H^+ ions for every mole of CO_2

Long-term N mineralization

Inorganic N was determined at approximately 1-week intervals for a period of 85 d. Soils were incubated in specimen cups, initially containing 50 g of soil. During each sampling period, 5 g of soil was removed and placed in new specimen cups. Inorganic N was extracted and measured using the method described above.

Net N mineralization was calculated as inorganic N (NH_4^+ and NO_3^-) at each sampling point less the initial inorganic N concentration.

Statistical analysis

All analyses were performed using SAS version 8 programming language. One-way analysis of variance (ANOVA) was used to determine all differences between meadow and forest soils. The categorical variable for all ANOVAs was vegetation with four levels (M, MF, FM, and F). The number of data points for each level varied with sampling season. Degrees of freedom (df) are given for all significant comparisons. Differences between sites and other comparisons that involved only two variables were done with t-tests. Non-constant variance revealed the need for a log-transformation of most of the response variables. Since back transformation of the logged averages results in medians rather than means, differences between meadow and forest soils were expressed in terms of their medians. Also, back-transformation results in multiplicative rather than additive differences. Thus a difference of two between treatments, for example, does not indicate that the treatments are two units apart, but rather indicates that the quantity measured for one treatment is twice as large as that of the other. All values reported as means are actual means, rather than back-transformed means. All P values equal to or less than 0.05 were considered significant and reported. The P values equal to or less than 0.10 were also reported for some comparisons. Least-squared linear regression was used to test for a relationship for all of the two-variable comparisons. Confidence intervals (CI) of 95% are given for all significant comparisons.

Results

Chloroform fumigation incubation estimates of MBC

No significant differences were found in CFIM MBC estimates between meadow and forest for any of the three sampling seasons at either Lookout or Carpenter. Figures 2.1, 2.2, and 2.3 (June, September, and November) show CFIM means and standard errors for each of the vegetation types sampled. Although not significant, differences in September were the greatest of the three sampling periods. The median MBC value at Carpenter was estimated to be 119% greater for the meadow than the forest (df = 3, 17, P = 0.1611, 95% CI: 0.65, 7.39). At Lookout in September, the median MBC value was estimated to be 54% greater in the meadow than in the forest. (df = 3, 16, P = 0.3902, 95% CI: 0.51, 4.62). In June and November, MBC values were similar for meadow and forest.

Trends along the individual transects were also examined (see appendix). No consistent trends were observed. Along some transects, MBC appears to be increasing from meadow to forest, while it appears to be decreasing along others. In general, variation among samples was large. Values of MBC ranged from 291 to 1287 μg C g^{-1} soil at Lookout and 61 to 645 μg C g^{-1} soil at Carpenter. The overall median MBC at Lookout was approximately 60% greater than that at Carpenter (t-test, 2-sided P = 0.0132, CI: 1.09, 2.03).

Nitrogen flush due to fumigation

All NF values were analyzed and graphed in a manner similar to MBC values (Figures 2.4 and 2.5). However, there were only two sampling periods, June and September, for NF. No significant differences were found for the June sampling period at either of the two sites. In September, however, the median meadow NF at Carpenter was significantly greater (54%) than the forest NF (df = 3, 20, P = 0.002, 95% CI: 1.82, 3.54). The NF values for meadow and forest were similar for Lookout in September.

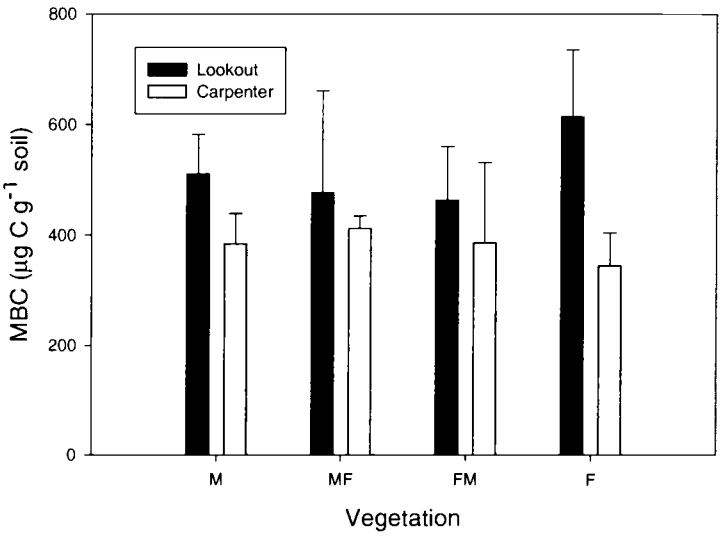

Figure 2.1. Microbial biomass C (CFIM) at Lookout and Carpenter for soil collected June 2000.

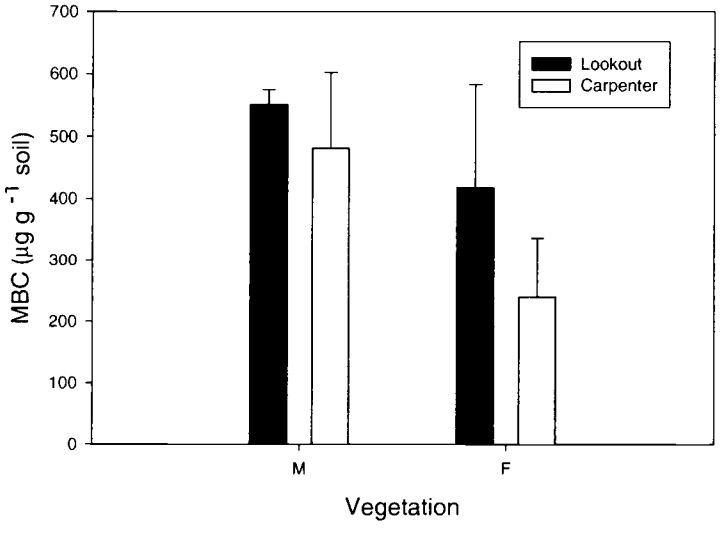

Figure 2.2. Microbial biomass C (CFIM) at Lookout and Carpenter for soil collected September 2000

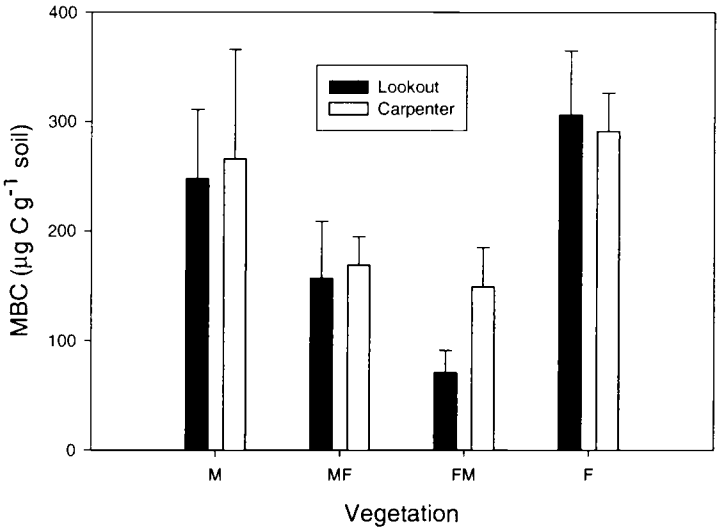

Figure 2.3 . Microbial biomass C (CFIM) at Lookout and Carpenter for soil collected November 2000

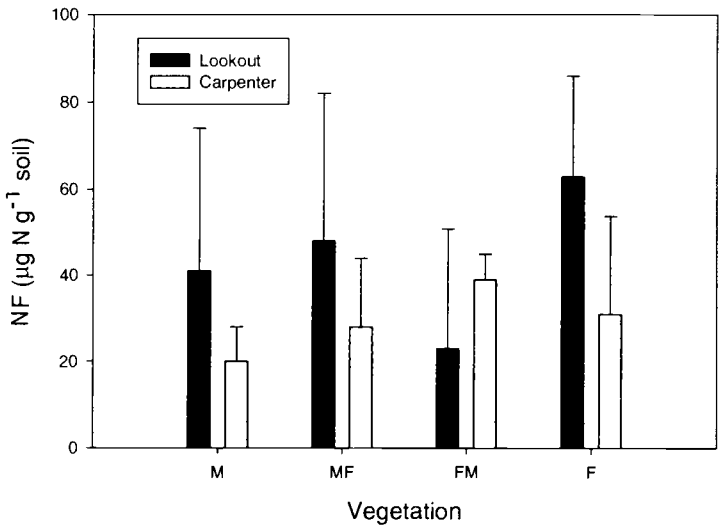

Figure 2.4 . Nitrogen flush at Lookout and Carpenter for soil samples collected in June 2000.

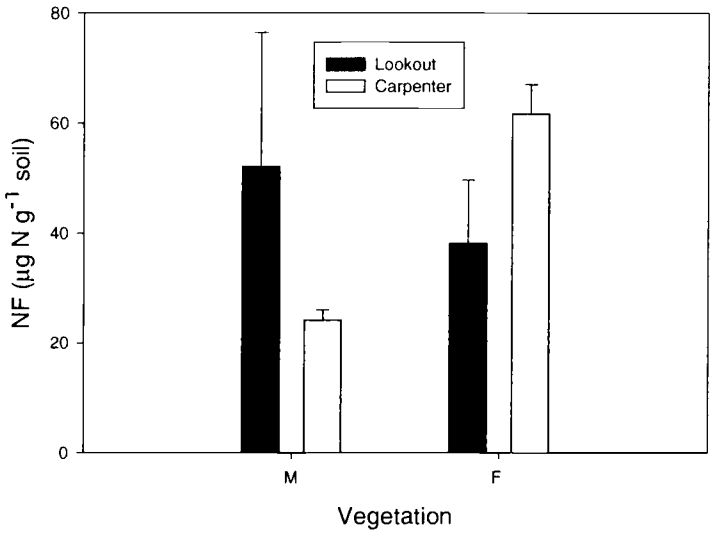

Figure 2.5. Nitrogen flush at Lookout and Carpenter for soil collected September 2000.

As with MBC, trends along transects (see appendix.) were inconsistent, and the variation was large. Values of NF ranged from –4.0 µg to 132.6 µg N g^{-1} soil at Lookout and from 3.5 µg to 74.0 µg N g^{-1} soil at Carpenter. As with MBC, NF was significantly greater at Lookout than at Carpenter by about 24% (t-test, 2-sided P = 0.0013, CI: 1.30, 2.75).

Microscopic estimates of fungal and bacterial biomass C

Microscopic estimates for MBC were significantly greater for the forest than the meadow for both Lookout and Carpenter (Fig 2.6). Microbial biomass C for the forest was approximately 215% greater than MBC for the meadow at both sites (Lookout df = 3, 20, P = 0.005, CI: 1.49, 6.67; Carpenter df = 3, 20, P = 0.0008, CI: 1.75, 5.71). In general, fungal biomass made up a far greater proportion of the total biomass than bacteria in both the meadow and the forest, and fungal biomass patterns mirrored the total microscopic estimates for MBC (Figures

2.6 and 2.7). Microscopic estimates for bacteria were similar for meadow and forest at Lookout. At Carpenter, microscopic estimates of bacterial biomass were 36% greater in the meadow than in the forest (df = 3, 20 P = 0.01, CI: 1.08, 1.72).

Chloroform fumigation incubation method vs. microscopic counts

Figures 2.9 (Lookout) and 2.10 (Carpenter) show comparisons between CFIM estimates and microscopic estimates of MBC. There was no significant difference between estimates made with two methods for meadow plots at either of the two sites. In the forest, however, MBC values were significantly greater using microscopy than CFIM at both Lookout and Carpenter. At Lookout Forest, the median value of MBC using microscopic methods was 145% greater than MBC values determined using CFIM (2 sample t-test, 2-sided P = 0.0263, CI: 1.13, 3.37). At Carpenter Forest, the median value of MBC using microscopic methods was 387% greater than that determined using CFIM (t-test, 2-sided P < 0.0001, CI: 2.69, 8.80).

Microbial biomass C and the N flush as percents of labile C and N

Figures 2.11 (Lookout) and 2.12 (Carpenter) show MBC as % C mineralized over an 85-d incubation period. All three transects are shown for each type of vegetation. There was no significant difference for meadow and forest values. However, the same trend that was observed for MBC (CFIM) can be seen once again in these graphs; there is a decrease from M to FM along all transects, and then an increase for F.

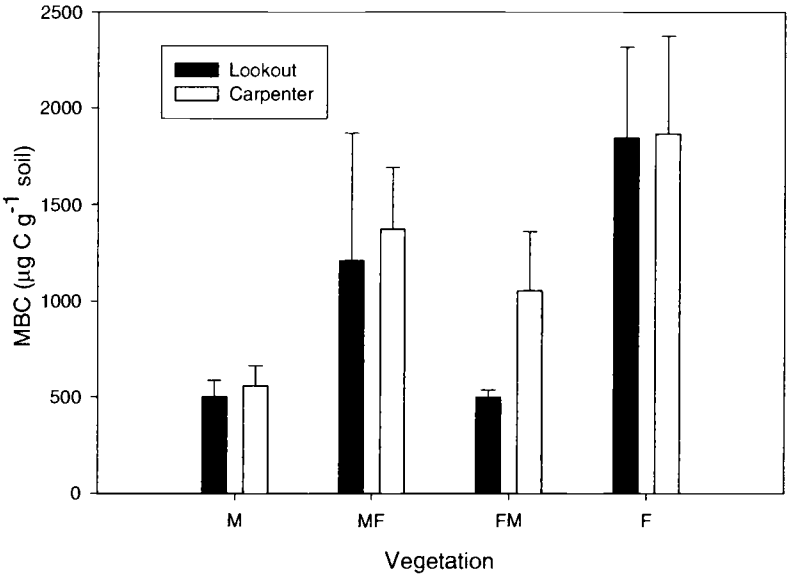

Figure 2.6. Microscopy estimates of MBC at Lookout and Carpenter.

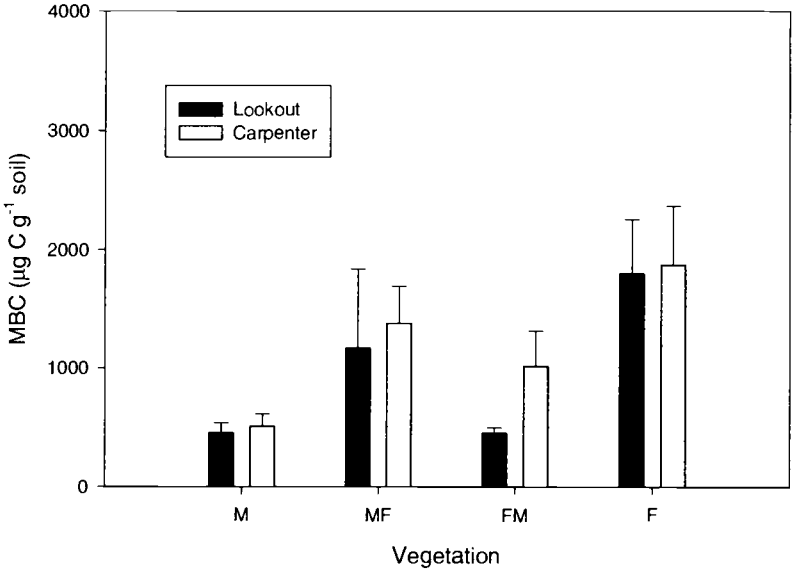

Figure 2.7. Microscopy estimates of fungal biomass C at Lookout and Carpenter.

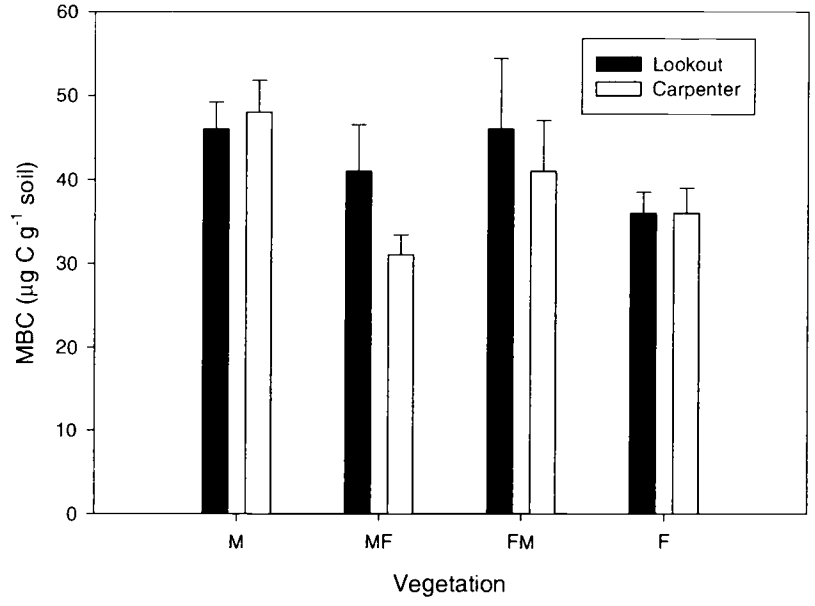

Figure 2.8. Microscopy estimates of bacterial biomass C at Lookout and Carpenter.

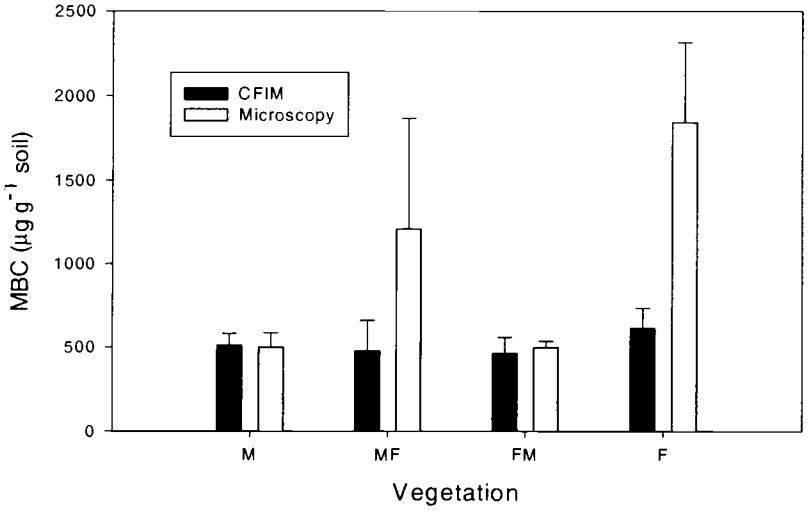

Figure 2.9. Microbial biomass C from both CFIM and microscopy at Lookout.

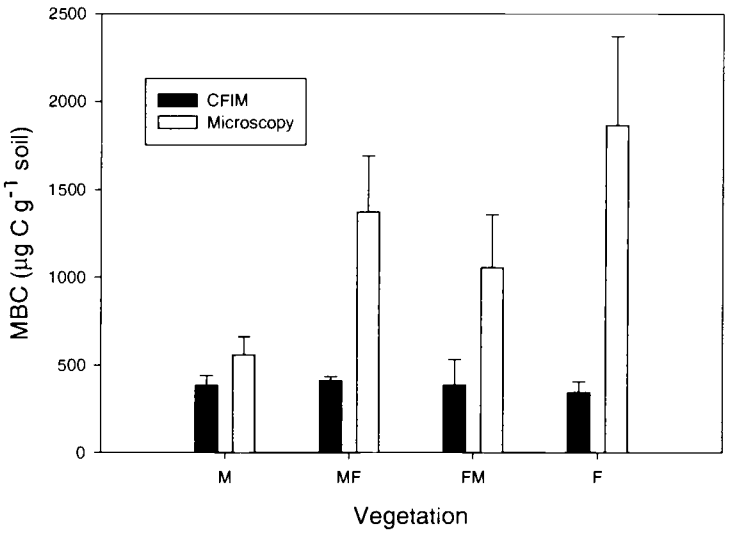

Figure 2.10. Microbial biomass C from both CFIM and microscopy at Carpenter.

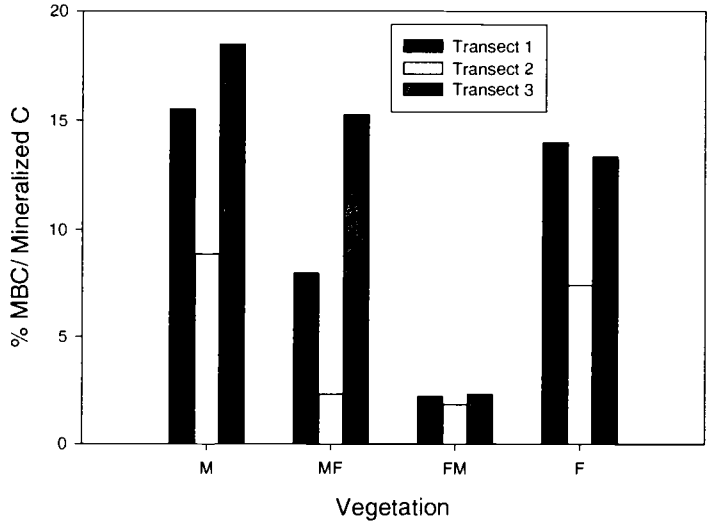

Figure 2.11. Microbial biomass C (CFIM) as a % C mineralized over 85 d at Lookout. Each bar represents the estimate of a single transect.

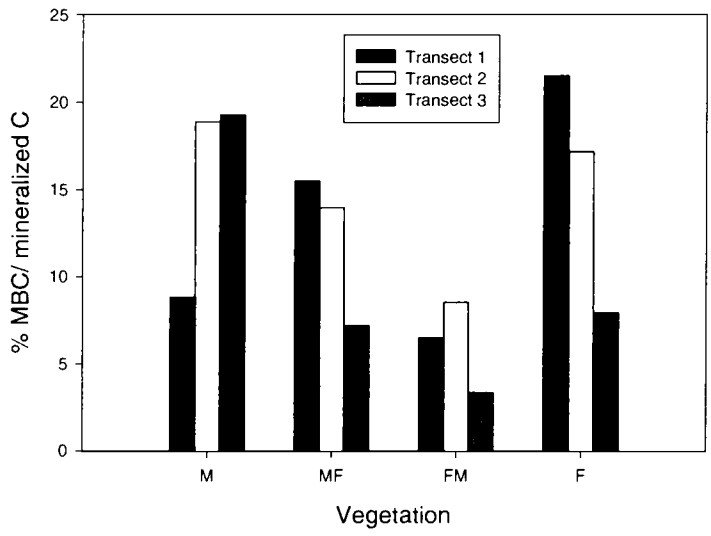

Figure 2.12. Microbial biomass C (CFIM) as % C mineralized over 85 d at Carpenter. Each bar represents the estimate of a single transect.

Figures 2.13 (Lookout) and 2.14 (Carpenter) show NF as % N mineralized over an 85-d incubation period. Because there was no NF data for November, the period during which the 85-d incubation took place, NF values from June were used instead. At both Carpenter and Lookout, NF as % N mineralized was significantly greater in the forest than in the meadow. At Carpenter, the forest value was estimated to be 14.5 times a large as that of the meadow (df = 3, 8, p = 0.0030, CI: 3.34, 63.32). At Lookout, the forest value was estimated to be 2.9 times a large as that of the meadow (df = 3, 8, p = 0.0477, CI: 1.01, 8.54).

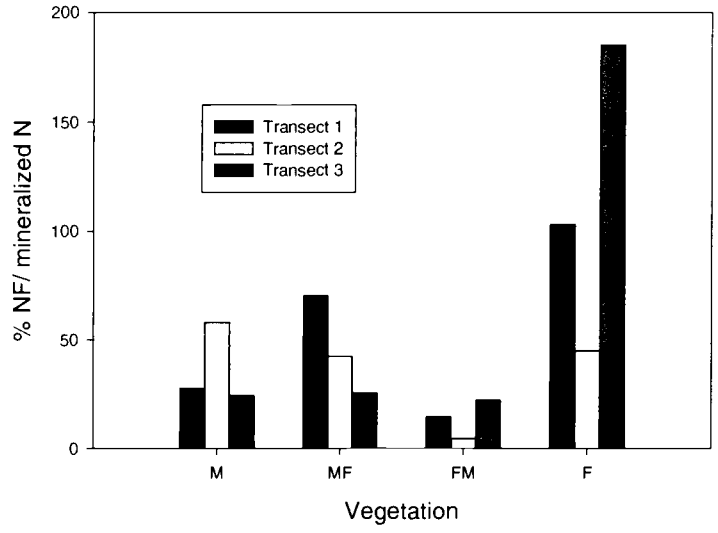

Figure 2.13. Nitrogen flush as % N mineralized over 85 d at Lookout.

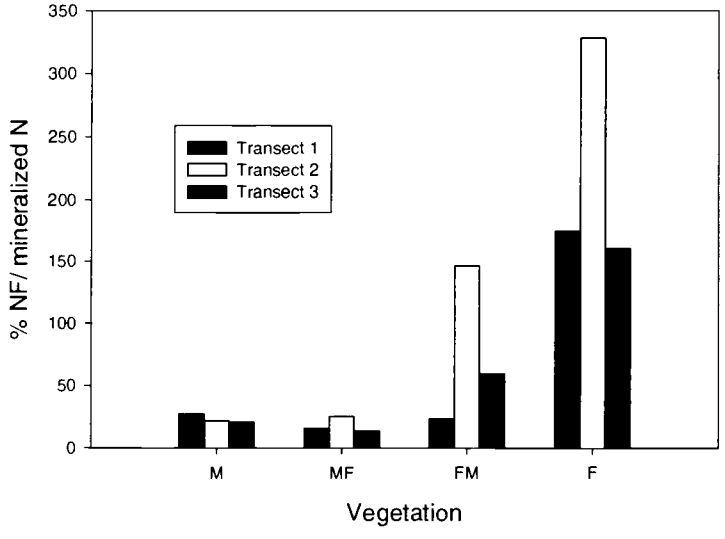

Figure 2.14. Nitrogen flush as % N mineralized over 85 d at Carpenter.

Discussion

Microbial biomass C averaged over all three sampling seasons was 407 μg C g^{-1} soil for the meadow soils and 369 μg C g^{-1} soil for the forest soils. In the interest of determining where these values fit into the global picture, I collected MBC values determined using CFIM from different studies in a variety of biomes (see Table 1.1). Forest values ranged from 160 μg in a West African forest soil to 2976 μg in an old-growth forest in Oregon. Grassland values ranged from 77 μg in a West African moist savanna to 1017μg in a tallgrass prairie in Minnesota. These values were extremely variable for grassland and forest ecosystems and did not show any particular trends for the two. I also compared the MBC values from my study with those from a study by Hart and Sollins (1998) using the CFIM method on soils from the HJA Experimental Forest in Oregon. Their median value for MBC from a 490 m elevation old-growth forest in the HJA was approximately 980 μg C g^{-1} soil, whereas the overall median value from the present study was 405 μg C g^{-1} soil. However, Hart and Sollins (1998) did not subtract an unfumigated control from their biomass values. Without subtracting a control, the mean MBC from the present study is approximately 600 μg C g^{-1} soil, still much lower than the values determined by Hart and Sollins (1998).

Microbial biomass N values are even more difficult to compare among studies than biomass C values because of the different methods and conversion factors that are used to calculate biomass N from NF. Part of the problem with determining a fixed conversion factor is that the N released from cells as a result of fumigation will be reimmobilized to varying degrees depending on the contents of the pre- and post- fumigation biomass (Voroney and Paul, 1983). Although I chose not to convert NF to biomass N for the purposes of comparing different treatments within this study, I looked at two classic papers that propose different K_n values,

and I used their methods to calculate microbial biomass C/N ratios for the June 2000 data set. In the first paper, Voroney and Paul (1984) account for the problem of reimmobilization by using a variable K_n factor. The value of K_n is dependent upon the ratio of the C flush (C_f) to the N flush (N_f) where C_f is determined without subtracting an unfumigated control and N_f is the quantity of NH_4^+ in the fumigated soil less the initial quantity of NH_4^+. Biomass N is caluculated as -0.014 (C_f/N_f) + 0.39. Because nitrification was considered insignificant, only NH_4^+ was used in their calculations for K_n and biomass N. In the present study, nitrification is significant in meadow soils and was included for both calculations, however, including NO_3^- had very little influence on K_n values. Using this method, K_n values for the present study ranged from –1.93 to 0.31 and biomass C/N ranged from 0.4 to 8.2. When the three negative K_n values were excluded , the average K_n was 0.22 and the average microbial biomass C/N ratio was 3.1. Since we have microscopic evidence that both meadow and forest soils were dominated by fungi, a microbial biomass C/N ratio of 3.1 seems unrealistically low. Shen et al. (1984) determined a much higher K_n value. Based on the relationship between the mineral N flush and the quantity of N in the biomass killed by fumigation, they established a fixed K_n of 0.68 . Using this value, biomass C/N values for my soils ranged from 2.2 to 27.1, and averaged 9.4. This average biomass C/N is much closer to what we might expect to find in a fungal dominated system.

Few microbial biomass comparisons have been made of meadow and forest vegetation paired plots. In one such study, a monoculture mountain beech forest in New Zealand was compared to an adjacent tussock grassland located above the beech treeline (Ross et al., 1996). Higher values of MBC were found for grassland soils than for forest soils at all sampling depths (0-10, 10-20, and 20-50 cm). In another comparison of grassy ecosystems with forested ecosystems, Hart et al. (1993) also found that the grassland soils had greater MBC than the mixed conifer forest soils in California. On the other hand, Billmore et al. (1995) found forest values of NF to be approximately three times the NF for grassland soils in Japan.

All of the above studies measured biomass using either CFIM or fumigation-extraction. Using a variety of different methods may result in different biomass numbers for some ecosystems. In a study that compared CFIM to microscopic MBC estimates in mycorrhizal mat and non-mat soils, Ingham et al. (1991) showed that microscopic biomass measurements ranged between 10 and 300 times greater than CFIM measurements. In the present study, microscopy estimates of MBC were significantly greater in the forest than in the meadow, whereas CFIM showed no difference between the two. However, the differences between microscopy and CFIM estimates were not nearly as striking as those found in the Ingham et al. (1991) study. For the meadow soils, CFIM estimates were similar to microscopy estimates at Lookout, and microscopy totals were approximately 67% greater than CFIM estimates at Carpenter. In the forest soils, microscopy estimates of biomass C were approximately three times greater than CFIM estimates at Lookout, and five times greater at Carpenter. Estimates from both Ingham et al. (1991) and the present study do not account for the efficiency of bacterial extraction. Extraction efficiency has been shown to be between 20 and 60% (Bottomley, 1994). However, even when bacteria are quadrupled, most of the results do not change substantially. Microscopic estimates for MBC were significantly greater for the forest than the meadow for both Lookout and Carpenter. Microscopic estimates of bacteria were similar for meadow and forest at Lookout and slightly greater for meadow at Carpenter. Only meadow comparisons between CFIM and microscopy were slightly different from the analysis that did not take efficiency extraction into account. Microscopy estimates were 24% greater than CFIM estimates at Lookout meadow, but this difference was not significant. At Carpenter meadow, microscopy estimates were 94% greater (2 sample t-test, $P = 0.03$, CI: 1.06, 3.55) than CFIM estimates.

It is thought that the discrepancy between the two methods may result from the resistance of some fungi to decomposition after fumigation; the CFIM only measures labile elements of the microbial biomass. In both forest and meadow

soils, CFIM estimates of MBC were 8-12 times greater than microscopic estimates of bacteria alone when extraction efficiency was assumed to be 100%, and CFIM estimates were 2-3 times greater when extraction efficiency was assumed to be 25%. Thus, it seems that at least some fungi are measured by this method.

In addition to looking at MBC, I also measured biomass N using the CFIM. However, I decided not to convert N flush measurements to microbial biomass because K_N values tend to vary more than K_c values, and are likely to be different for meadow and forest. Thus, microbial N is presented as the flush due to fumigation. There are no significant differences between meadow and forest for NF at Lookout during either of the two sampling periods, or at Carpenter during the June sampling period.

In September of 2000, NF at Carpenter was approximately 50% greater in the forest than in the meadow. Trends along transects were similar to those observed for MBC. It makes sense that these trends would be similar, because MBC correlated significantly with NF (see appendix). This correlation has been found in other studies (Ayanaba et al., 1976). One problem with measuring NF by the CFIM method is that the microbial community that grows during the incubation period may have a different C/N ratio than the community that was killed by fumigation. Fungi have much wider C/N than bacteria (Paul and Clark, 1989), but fast-growing bacteria probably comprise the majority of the community that grows back during the 10-d incubation period (Lynch and Panting, 1980). We might expect N to be immobilized more heavily in the forest soils than in the meadow soils during the incubation period, because the fungal:bacterial ratio was much greater in the forest than in the meadow. In the Billmore et al. (1995) research, CFIM was compared with the fumigation-extraction method. They estimated that fumigation extraction biomass N was approximately 2.6 times greater than that estimated by CFIM in the grassland soils. They concluded that immobilization was taking place in the grassland, but not in the forest soils.

Both MBC and NF were examined as a percentage of the total C and N mineralized, respectively. Long-term mineralization is one measure of labile C or N in the system, and the microbial biomass is one source of these labile nutrients. The relationship between microbial biomass and long-term mineralization provides information about the portion of the soil's labile C and N that is contained in microbial biomass. The November 2000 data show an interesting trend for MBC calculated as a percentage of C mineralized over an 85-d incubation period. Values ranged from 2% to 22% for both meadow and forest at both sites. There is a trend that shows a decrease along transects from the meadow to the edge of the forest boundary (FM). But once under the canopy of the forest, the values increase again and are similar to those observed for the meadow. This is the same trend that was observed for MBC (CFIM). The cause for this pattern is not known, it may be related to microclimate factors. Since tree roots will likely reach out into this region that we define as the forest meadow transition, soil properties are probably influenced by the trees. However, soils and soil organisms at this boundary are probably less protected from the environment than those that exist more deeply nested within the forest vegetation. The soils used for this analysis were collected in November, after some precipitation had fallen in the region. All soils were covered in a layer of snow, except for those located beneath the canopy.

The NF as a percent of N mineralized over an 85-d period showed a very different trend from C. The NF tends to represent a much larger proportion of labile N. This proportion would be even greater if NF had been converted to MBN as the C flush was converted to MBC. At both sites, values were an average of 3 to 5-fold greater in the forest than in the meadow. Meadow values averaged approximately 40% at Lookout and 25% at Carpenter, whereas, forest values averaged approximately 120 % at Lookout and 200% at Carpenter. This implies that microbial N may be the major pool of labile N in forest soil, and a significant pool of labile N in meadow soils as well.

Conclusions

In general, MBC and NF estimates made using CFIM did not result in differences for meadow and forest vegetation. Such differences observed in other studies have usually utilized grassy and forest plots from different sites. This study also showed significant differences in both MBC and NF between the two sites, but not between different vegetation types within a site. Microscopic estimates, on the other hand, did show differences in MBC between the forest and meadow soils. These differences were due to much higher fungal counts in the forest than in the meadow. When microscopic estimates were compared to CFIM, estimates for meadow soils were found to be similar for the two methods. However, microscopic estimates were much higher than CFIM estimates for the forest soils at both sites.

The relationship between biomass C and N and mineralizable C and N, provid some interesting information about the relative proportion that biomass represents of these labile nutrient pools. For the ecosystems examined, it seems clear that the N stored in biomass is a much larger portion of the total labile N pool than is MBC for the labile C pool. However, it is important to remember that mineralized C is a gross rate while mineralized N is a net rate. Mineralized C would be more accurately paired with gross N mineralization for this type of comparison. However, it may be more interesting to know how biomass N compares to available N, since this is the pool that will affect plant growth. It seems that the N pool may serve as the major form of labile N storage in the soil. Because vegetation is N limited in the majority of ecosystems, an understanding of the size and dynamics of the microbial N pool are critical to understanding plant accessibility to this key nutrient.

CHAPTER 3

NET N MINERALIZATION AND C/N RATIOS

Abstract

Net N mineralization rates were compared for meadow and forest soils at two sites, Carpenter Mountain and Lookout Mountain, located in the Central Cascades of Oregon. Vegetation occurred along a meadow to forest transition at both of these sites. Soils were sampled from a depth of 0-10 cm beneath the litter and organic layers. In June, September, and November 2000, net N mineralization was measured for a 10-d aerobic laboratory incubation. In November 2000 and June 2001, mineralization rates were followed over incubation periods of 85 and 77 d respectively. The rates in the meadow were greater than those in the forest at both sites and during all sampling periods except for the 10-d incubation at Lookout in September. Net N mineralization for the 10-d incubations averaged 21 $\mu g\ N\ g^{-1}$ soil in the meadow and 8 $\mu g\ N\ g^{-1}$ soil in the forest Rates for long-term N mineralization averaged 126 $\mu g\ N\ g^{-1}$ soil in the meadow and 52 $\mu g\ N\ g^{-1}$ soil in the forest. Soil C/N ratios were also examined in relation to net N mineralization. Meadow soils were compared to forest soils at each site and similar treatments (either meadow or forest) were compared for the two different sites. Forest C/N ratios were greater than meadow values at both sites, corresponding to lower mineralization rates in the forest than in the meadow. Carbon to N ratios were similar for Lookout meadow and Carpenter meadow, as were net N mineralization rates during most sampling periods. However, Carpenter forest had a significantly greater C/N ratio than Lookout forest, corresponding to greater net N mineralization rates for Lookout forest than for Carpenter forest. An experiment to determine gross rates of N mineralization and nitrification was also performed as

part of this study. However, production and consumption rates could not be determined from the measurements.

Introduction

In terrestrial environments, N is the nutrient usually limiting to plant growth. As such, the transformations of this nutrient that make it available to plants have received considerable attention. Most of the N is made available through the recycling of dead organisms during decomposition. As carbonaceous substances are broken down for use by heterotrophic microorganisms, N is usually released in the process. The conversion of N from an organic form to its more accessible, inorganic form of NH_4^+ is known as N mineralization. Net N mineralization, often used as a measure of available N, refers to the change in the size of the inorganic N pool (both NH_4^+ and NO_3^-) resulting from decompositon and the subsequent release of N into the soil as well as the immobilization of inorganic N by microorganisms. Net N mineralization rates, however do not reveal how much NH_4^+ or NO_3^- is actually produced since they are a measure of several concurrent productive and consumptive processes. Gross process rates (i.e., production of either NH_4^+ or NO_3^-) can be measured using isotope dilution.

In an ecological sense, the understanding of the effects of N cycling processes on vegetation are broadened by studies that focus on the other side of the relationship as well, the effects of vegetation on N dynamics. Many studies have shown differences in net N mineralization rates among soils associated with different plant species (Wedin and Tilman, 1990; Binkley and Giardina, 1998; and Thomas and Prescott, 2000). Hart et al., (1997) examined the effects of red alder (*Alnus rubra*), a N_2-fixing species, on soil N transformations. The inclusion of red alder in coniferous forest stands increased net N mineralization rates as well as other N-cycling processes. Comparisons have also been made between systems

containing contrasting vegetation such as grassland and forest ecosystems. In separate published papers, N-cycling processes for grasslands and forests in northern California from the same soil great group were measured (Jackson et al., 1988; Hart et al., 1992). Nitrogen cycling rates were found to be much smaller in the forest than in the grassland (Hart et al., 1993). This may be due in part to differences in the C/N ratios of the vegetation in these two systems. Net N mineralization is positive in most soils, but may be negative if microorganisms are N-limited. In theory, N limitation occurs when the C/N ratio of the detrital material passes some critical point, beyond which the N supply of the substrate is not enough to allow the microorganisms to utilize all of the available C. The critical C/N ratio is often reported to be between 20 and 30. Under perfect environmental conditions, whether N is mineralized or immobilized depends upon the C/N ratios of the materials that are breaking down; the C/N ratios of the decomposer microorganisms; and the efficiency with which the microorganisms are able to break down the given materials (Myrold, 1999).

Several studies have shown a relationship between C/N ratio and net mineralization (Wedin and Tillman, 1990; Pare and Bergeron, 1996; and Prescott et al., 2000). Pare and Bergeron (1996) showed N mineralization rates to be significantly correlated with both C/N and total N in the mineral soil beneath two out of three of the tree species examined. Prescott et al. (2000) showed a significant relationship between the net N mineralization rate of the forest floor and C/N ratio of the forest floor. Thomas and Prescott (2000) also showed a significant relationship between net N mineralization and C/N of the forest floor, although forest floor N concentration was even more highly correlated with net N mineralization in their study.

Since the soil is a heterogeneous material containing substances in many different stages of decomposition, factors such as C availability and substrate use efficiency may be extremely variable throughout, complicating the use of C/N ratio as a predictor of net N mineralization. As exemplified by Hart (1999), the C/N

ratio does not always correlate with net N mineralization. In a study that compared well-decayed bole material having a high C/N ratio to mineral soil, Hart (1999) found that the bole material had a greater rate of net N mineralization per total N than the mineral soil. This was a surprising result, given that higher C/N is usually associated with a lower net N mineralization. He concluded that the C in the bole material was more recalcitrant than that of the mineral soil. Thus, N was mineralized during decomposition of the available C pool, but the C/N ratio was determined using the much larger, total C pool.

In the present study, changes in net N mineralization along a meadow-to-forest transition are examined. Since soil samples are all extracted from the same site, the transition offers an opportunity to isolate more thoroughly vegetation's direct and indirect effects upon soil properties. The objectives are 1) to determine if differences in both short and long-term net N mineralization exist between meadow and forest vegetation at two different meadow-to-forest transitions and 2) to examine C/N ratios for meadow and forest at each site and determine if a relationship exists between C/N ratios and net N mineralization rates.

Materials and Methods

Site description

June 2000

Two different locations at the H.J. Andrews Experimental Forest (HJA) in the Cascade Mountains were selected as study sites: Carpenter Mountain and Lookout Mountain (hereafter referred to as Carpenter and Lookout). Site selection was based on availability at HJA of sites containing a meadow adjacent to a forest. I have chosen to analyze each site as a separate case study, so that the information obtained from my research can be used by others to help answer questions pertaining to these specific sites. In this observational study, three parallel,

meadow-to-forest transects were placed at each site (Figure A 1.). The treatments were defined as meadow (M), meadow/forest (MF), forest/meadow (FM), and forest (F). Transects were positioned to maximize the contrast between meadow and forest treatments. Along each transect, eight equally spaced circular plots (1 m diameter) were established, for a total of 24 plots. Transects were centered in-between MF and FM, with three meadow plots (M1, M2, and M3) and three forest plots (F1, F2, and F3) at either end. At Lookout, plots were spaced at 10-m intervals, with a distance of approximately 20 m between transects. Since the ecotone was wider at Carpenter than at Lookout, plots were spaced 20 m apart, rather than 10 m apart. Transects 2 and 3 at Carpenter were separated by approximately 20 m, while Transect 1 was located approximately 100 m upslope from the other transects. The uneven spacing between transects was necessary to work around areas where the meadow-forest transition was less clear and to avoid a hiking trail. A coring device consisting of 4.7-cm diameter PVC pipe was used to extract five soil cores from each plot. The litter and humus layers were removed from the cores, and the mineral soil was sampled to a depth of 10 cm. Cores taken from the same plot were made into a single composite.

September 2000

The locations that were utilized in June were sampled again in September, but because a related experiment was being set up simultaneously, core samples were not extracted from the original transects. Instead, a square grid was established in both the forest and meadow at each site. Each grid contained 64 plots with 3 m between plots. There were only two treatments in this study, forest and meadow. Six cores were collected from randomly selected plots contained within the meadow grid and six from the forest grid at each location. Two random cores from each treatment and location were combined into a single composite, leaving a total of six composite soil samples.

November 2000

The original transects that were sampled in June 2000 were utilized once again in November. Four plots were sampled along each transect (M2, MF, FM, and F2).

June 2001

The original transects were sampled. Three soil cores were removed from each plot, and all soil samples were composited into a single sample for each treatment (M, MF, FM, and F).

Treatment of soils prior to assay

Soils were transported in coolers from the HJA to OSU laboratories and stored at 4°C. The following day, all cores were sieved through a 4-mm sieve. All microbial biomass assays began within 48 h of soil collection.

Net N mineralization (10 d)

Net N mineralization was calculated as inorganic N (NH_3^+ and NO_3^-) concentration after a 10 d aerobic incubation less the initial (time zero) inorganic N concentration. Inorganic N was separated from soils using KCl extraction and subsequently measured on an Astoria Pacific series 300 autoanalyzer. Soil samples (25 g) were placed in specimen cups, brought to 67% water content, and incubated at 25° C. After a period of 10 d, replicate 10 g sub-samples were removed from the incubating soils and placed in specimen cups. Forty milliliters of 2M KCl were then added to each specimen cup, and samples were shaken on a mechanical shaker for 45 min. Funnels lined with Whatman No. 1 filter paper were soaked with 0.5 M HCl in order to remove any contaminating N and then pre-leached with 2M KCl. The contents of the shaken specimen cups were emptied into the funnels and the filtrates collected for analysis of NH_3^+ and NO_3^-.

Long-term net N mineralziation

Inorganic N was determined at approximately 1-week intervals for a period of 85 d in November 2000 and 77-d in June 2001. In November 2000, soils were incubated in specimen cups, initially containing 50 g of soil. In June 2001, soils were incubated in mason jars, containing approximately 300 g of soil. During each sampling period, 5 g of soil were removed from containers and placed in specimen cups. Inorganic N was extracted and measured using the method described above. Laboratory replicates were not used for the 77-d net N mineralization.

C/N ratios

C and N were determined on a Roboprep C/N analyzer linked to a Tracer Mass Isotope Ratio Mass Spectrometer.

Gross rates of N mineralization and nitrification

Gross rates of N mineralization and nitrification were measured for the June 2001 soils during three different sampling periods using the $^{15}NH_4^+$ and $^{15}NO_3^-$ isotope dilution methods described by Hart et al. (1994). The first sampling period was initiated two weeks after soils had been sieved and following a 1-week room temperature incubation period. The next sampling period was initiated 17 d after the first one. The final sampling period was initiated 24 d after the second one.

Labeling soils with ^{15}N

At each sampling period, one mason jar for each treatment was harvested. Two hundred g dry weight soil was removed from each mason jar and divided into two, 100-g sections. Each portion was gently spread out over a separate sheet of wax paper. Ten ml of KNO_3 (for NO_3^- addition) containing 100 μg of 99 atom% ^{15}N was added to one portion using a syringe. Ten ml of $(NH_4)_2SO_4$ (for NH_4^+

addition) containing 100 μg of 99 atom% ^{15}N was added to the other section of soil. The rate of N addition for both $^{15}NH_4^+$ and $^{15}NO_3^-$ was 1 μg N g^{-1} dry soil. The soils were gently mixed by folding over the wax paper from corner to corner several times. Soil sections were each divided into10-g subsamples and placed into specimen cups. Soil sections were sealed in polyethylene plastic bags and allowed to incubate at room temperature for a period of 12 hr. After this time, half of the $^{15}NH_4^+$ samples and half of the $^{15}NO_3$ samples were extracted with 50 ml of 2M KCl. The other samples were incubated in the specimen cups (with lids on) for 24 hr, at which point they were extracted with 50 ml of 2M KCl. Inorganic N concentrations were measured as described in the net N mineralization section. Approximately 20 ml of extract were stored frozen for later diffusion and ^{15}N analysis.

NH_4^+ diffusion

Frozen extracts from labeled soils were thawed and brought to approximately 40 ml of solution by adding 20 ml of KCl. New lids for the specimen cups were prepared earlier by gluing a small casing (lid from a plastic vial) onto the specimen cup lid in order to hold the Whatman #2 filter paper. Ten μl of $KHSO_4$ was pipetted onto the filter to serve as an acid trap for NH_4^+. The filter was immediately covered with a piece of Teflon tape and caped with the open half of the cut plastic vial. A marble was placed in each cup to enhance mixing during diffusion. One scoop of MgO buffer was then added to the specimen cup in order to volatilize the NH_4^+, allowing it to be trapped on an acidified filter paper. The lid was immediately placed onto the cup and securely tightened.

Specimen cups were incubated at approximately 35° C on an Orbit Shaker at 150 RPMs for 7 d. After that time, lids were removed and placed in a glass desiccator containing a beaker of concentrated $H_2SO_4^-$ for 2-3 days, until filter papers were completely dry. The cups were then placed back on the shaker in the

incubator for 1 d in order to volatilize any remaining NH_4^+ that was not diffused, and cups were set up for NO_3^- diffusion.

NO_3^- diffusion

The procedure is the same as described for NH_4^+ diffusion with the following changes:

1) One scoop of Devardas alloy was added to the solution in the specimen cup to convert NO_3^- into NH_4^+.
2) More MgO was not added since the solution contained enough of the buffering agent to continue the conversion of new NH_4^+ to NH_3.
3) Since H_2 gas is a byproduct of the reactions occurring during this diffusion, increased pressure in the specimen cup resulted in bulging of the cup lid. Teflon tape was threaded around the specimen cup in order to prevent the possibility of NH_3 loss with the increase in pressure.

After 2-3 days in the desiccator, filters were removed from lids and wrapped in small tin capsules. The ^{15}N atom% abundance was analyzed on a Tracer Mass Isotope Ratio Mass Spectrometer, utilizing a Dumas combustion/reduction apparatus. Gross N transformation rates were determined from changes in NH_4^+ and NO_3^- pool sizes and atom% ^{15}N excess between the two time points using the Kirkham and Bartholomew (1955) equations referenced from Hart et al. (1994).

Statistical analysis

All analyses were performed using SAS version 8 programming language. One-way analysis of variance (ANOVA) was used to determine all differences between meadow and forest soils. The categorical for all ANOVAs was vegetation with four levels (M, MF, FM, and F). The number of data points for each level varied with sampling season. Degrees of freedom (df) are given for significant comparisons. Differences between sites and other comparisons that involved only two variables were done with t-tests. Non-constant variance revealed the need for a

log-transformation of most of the response variables. Differences between meadow and forest soils were thus expressed as multiplicative differences between medians. All values reported as means are actual means, rather than back-transformed means. All P values equal to or less than 0.05 were considered significant and reported. The P values equal to or less than 0.10 were also reported for some comparisons. Least-squared linear regression was used to test for a relationship for all of the two variable comparisons. Confidence intervals (CI) of 95% were reported for all significant differences.

Results

Net N mineralization rates

For most of the sampling periods, the net N mineralization rate for a 10-d incubation was greater for meadow soils than for forest soils. Figures 3.1 (June) and 3.3 (November), representing mean net N mineralization across transects, show clear differences for forest and meadow soils. An exception to this trend was observed in September at Lookout, when net N mineralization was greater in the forest soils than in the meadow soils. Statistics for all differences with $P < 0.10$ are given below.

During June 2000, the median value for the net N mineralization rate over a 10-d period at Lookout was estimated to be 86% greater in the meadow than in the forest (df = 3, 19, P = 0.0956, 95% CI: 0.89, 3.88). The median net N mineralization rate for Carpenter was estimated to be 542% greater in the meadow than in the forest (df = 3, 20, $P < 0.0001$, 95% CI: 3.71, 11.32).

During November 2000, the median value for the net N mineralization rate over a 10-d period at Lookout Mountain was estimated to be 409% greater in the meadow than in the forest (df = 3, 8, P = 0.0596, CI: .91, 28.37). The median net N mineralization rate for Carpenter was estimated to be 511% greater in the meadow than in the forest. (df = 3,8, P = 0.0282, CI: 1.33, 28.01).

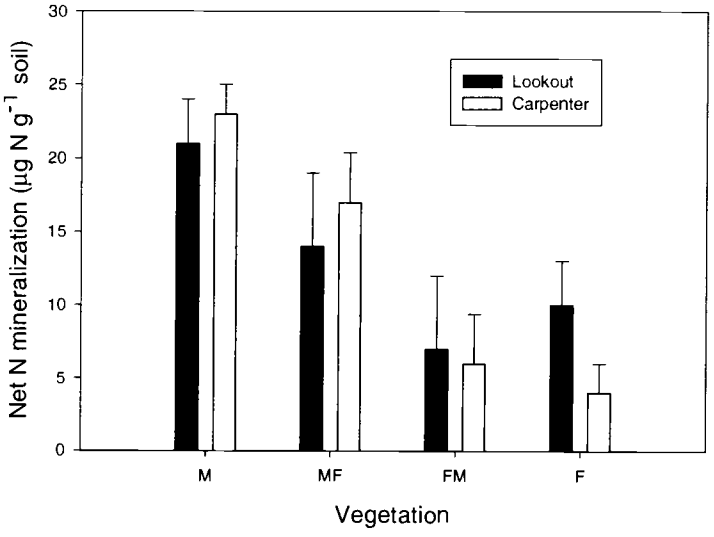

Figure 3.1. Net N mineralization rate during a 10-d aerobic incubation for June 2000.

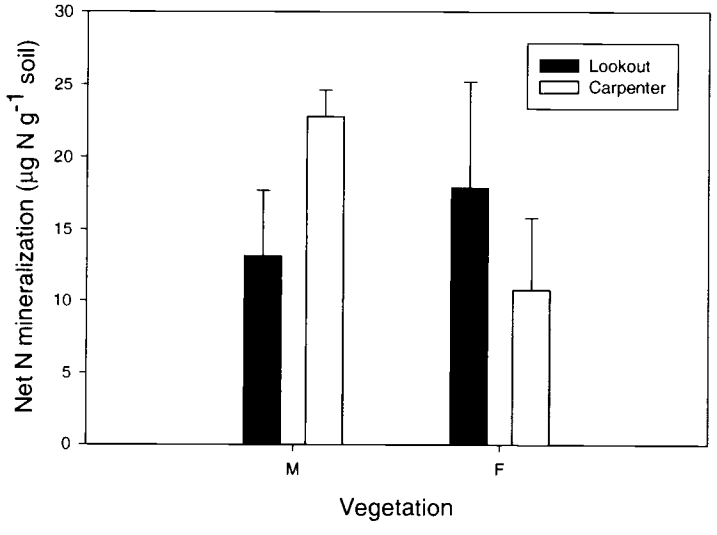

Figure 3.2. Net N mineralization rate during a 10-d aerobic incubation period for September 2000.

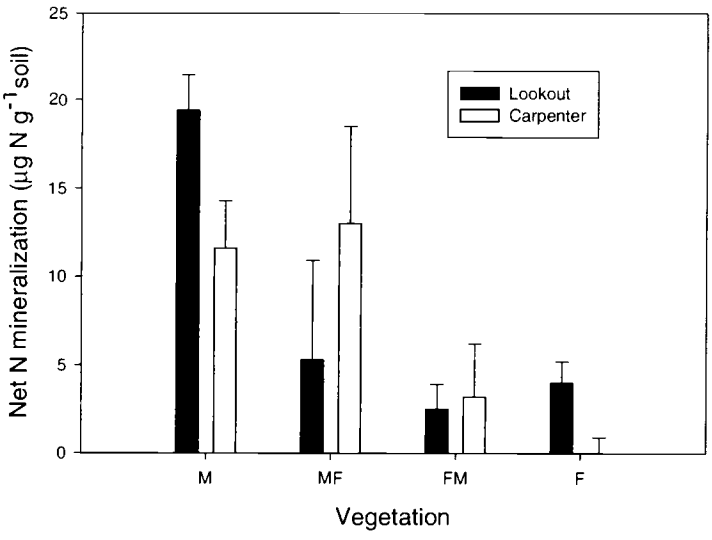

Figure 3.3. Net N mineralization rate during a 10-d aerobic incubation for November 2000.

During November of 2000, the median value for the net N mineralization rate over an 85-d period at Lookout was estimated to be 109% greater in the meadow than in the forest (df = 3, 8, P = 0.107, 95% CI: 0.72, 6.04). The median net rate of N mineralization for Carpenter was estimated to be 747% greater in the meadow than in forest. (DF = 3, 8 P = 0.0038, 95% CI: 2.50, 28.69).

During June of 2001, the net N mineralization rate over a 77-d period at Lookout Mountain was 24% greater in the meadow than in the forest. The net N mineralization rate for Carpenter was 839% greater in the meadow than in the forest. Because there was no replication, these numbers are not statistically significant.

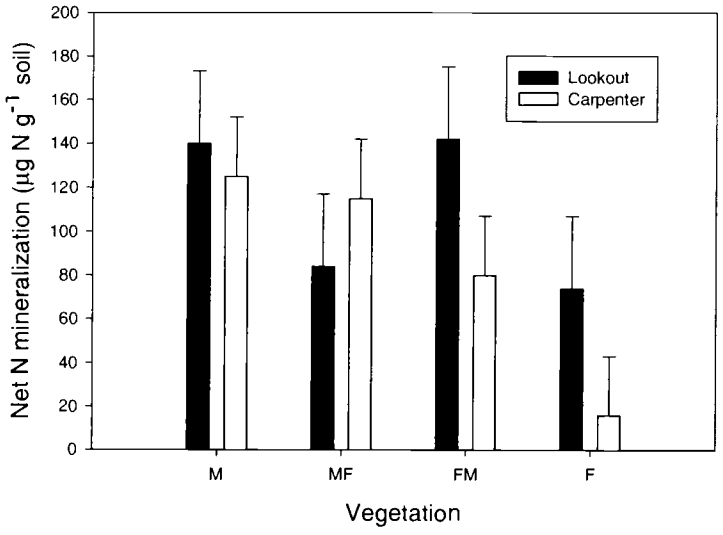

Figure 3.4. Net N mineralization rate during an 85-d incubation for Novermber 2000.

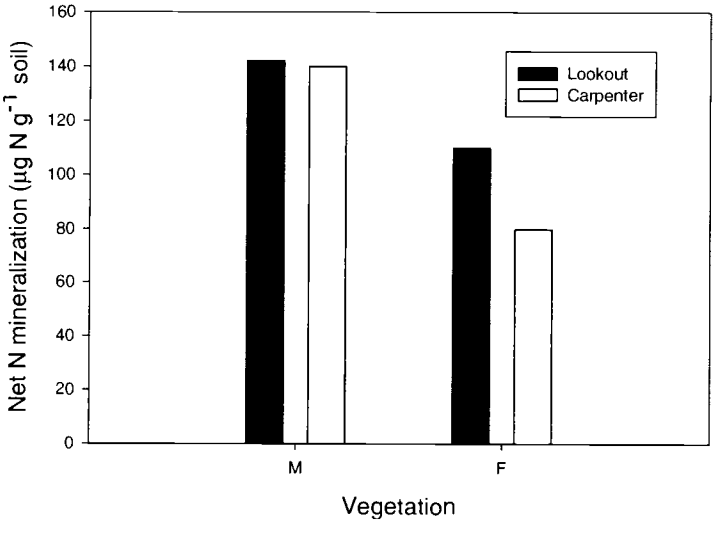

Figure 3.5. Net N mineralization rate during a 77-d incubation for June 2001.

Nitrogen mineralization time course data

Figures 3.6 – 3.13 show how NH_4^+ and NO_3^- concentrations change over time for both the November 2000 and the June 2001 incubation periods.

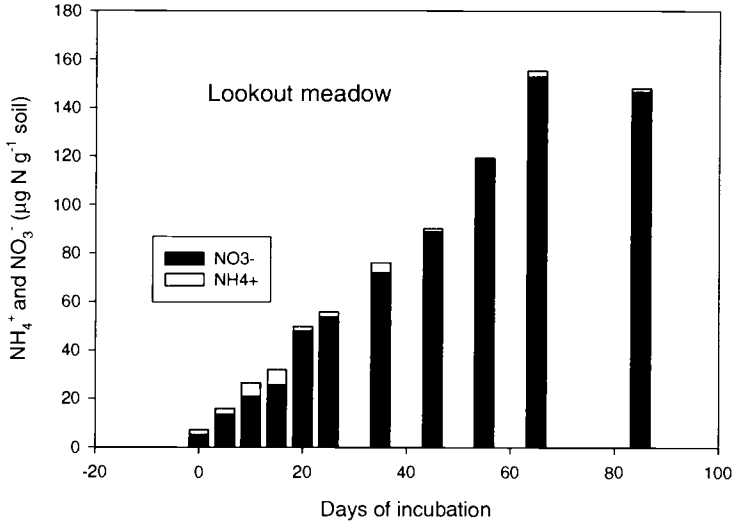

Figure 3.6. Lookout meadow NH_4^+ and NO_3^- pool sizes throughout an 85 d incubation beginning in November 2000.

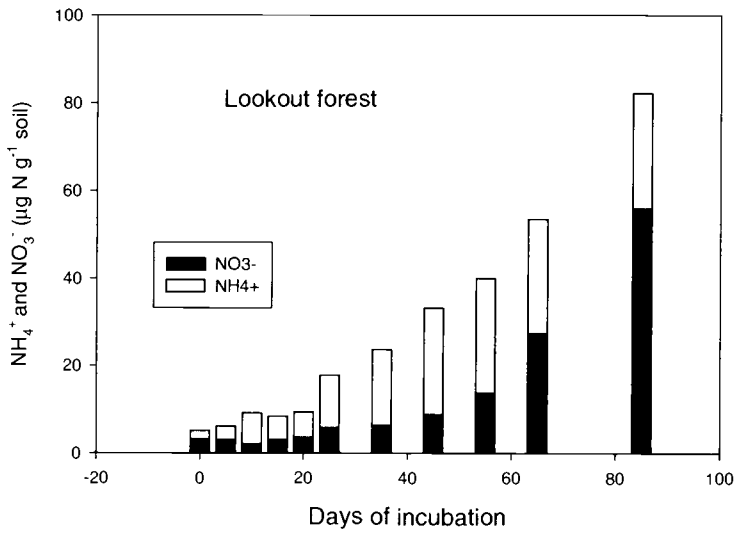

Figure 3.7. Lookout forest NH_4^+ and NO_3^- pool sizes throughout an 85 d incubation beginning in November 2000. Day 65 and 85 NH_4^+ concentrations are actually greater than those shown, but the

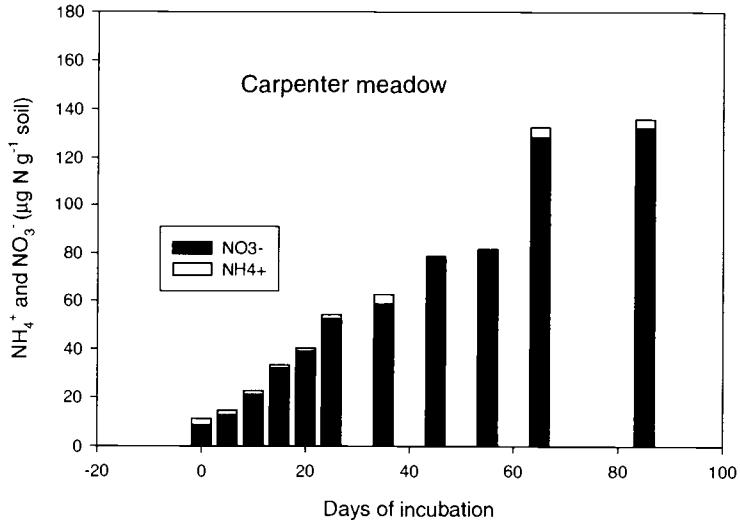

Figure 3.8. Carpenter meadow NH_4^+ and NO_3^- pool sizes throughout an 85 d incubation beginning in November 2000.

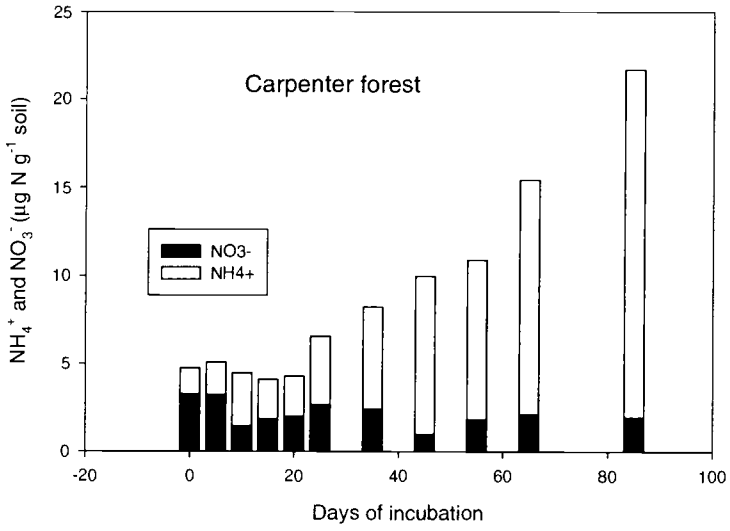

Figure 3.9. Lookout meadow NH_4^+ and NO_3^- pool sizes throughout an 85 d incubation beginning in November 2000.

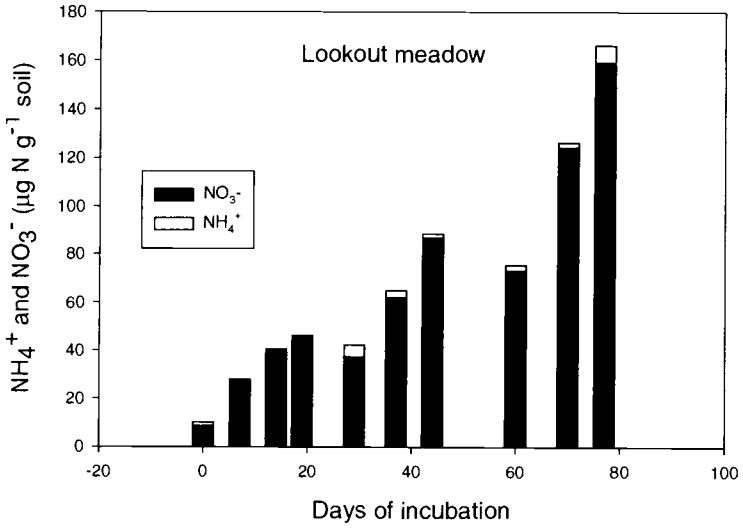

Figure 3.10. Lookout meadow NH_4^+ and NO_3^- pool sizes throughout a 77 d incubation beginning in June 2001.

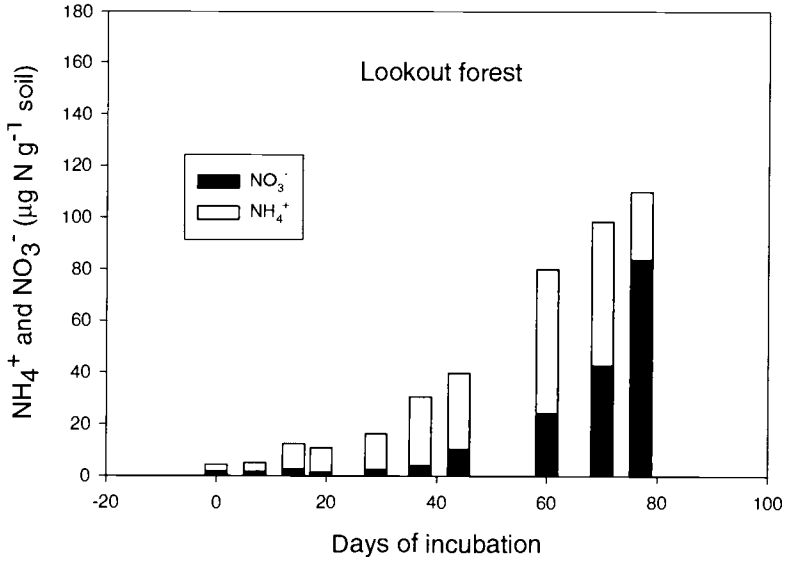

Figure 3.11. Lookout forest NH_4^+ and NO_3^- pool sizes throughout a 77 d incubation beginning in June 2001.

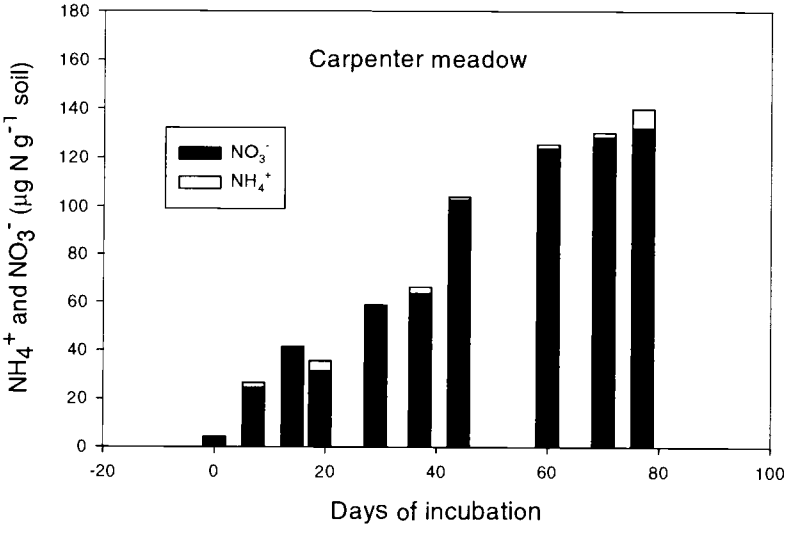

Figure 3.12. Carpenter meadow NH_4^+ and NO_3^- pool sizes throughout a 77 d incubation beginning in June 2001.

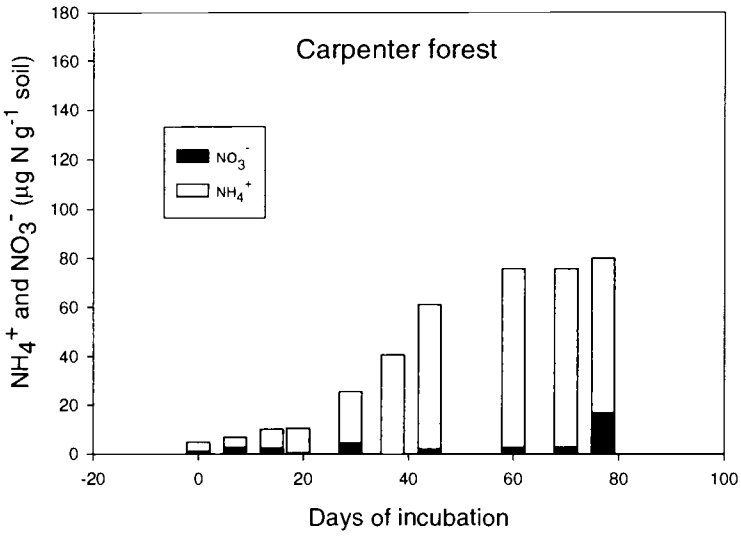

Figure 3.13. Carpenter forest NH_4^+ and NO_3^- pool sizes throughout a 77 d incubation beginning in June 2001.

C/N ratios

Figures 3.14 (Lookout) and 3.15 (Carpenter) show values for four vegetation types at each of the three transects. The C/N ratios were significantly greater in the forest than in the meadow at both Lookout and Carpenter. At Lookout, the C/N ratio of the forest was 25% greater than the C/N ratio of the meadow (df = 3, 20,
P = 0.0023, CI: 1.10, 1.43). At Carpenter, the C/N ratio of the forest was 81% greater that of the meadow (df = 3, 20, P < 0.0001, CI: 1.61, 2.03).

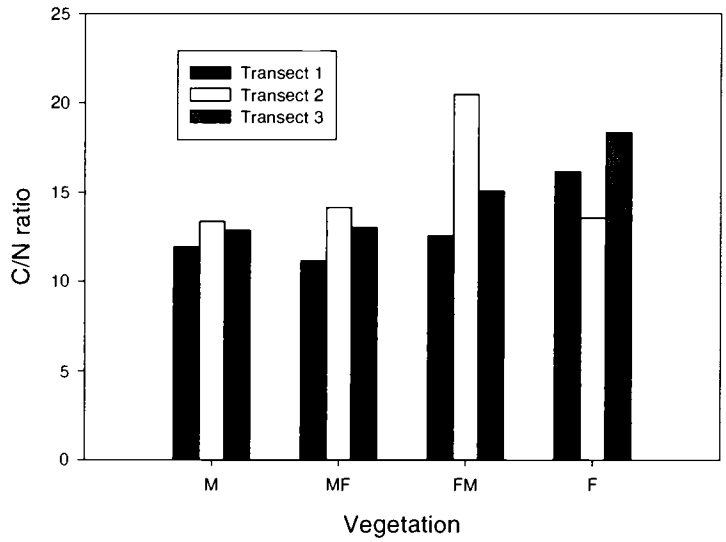

Figure 3.14. Soil C/N ratios at Lookout for June 2000. Each bar represents the value for a single transect.

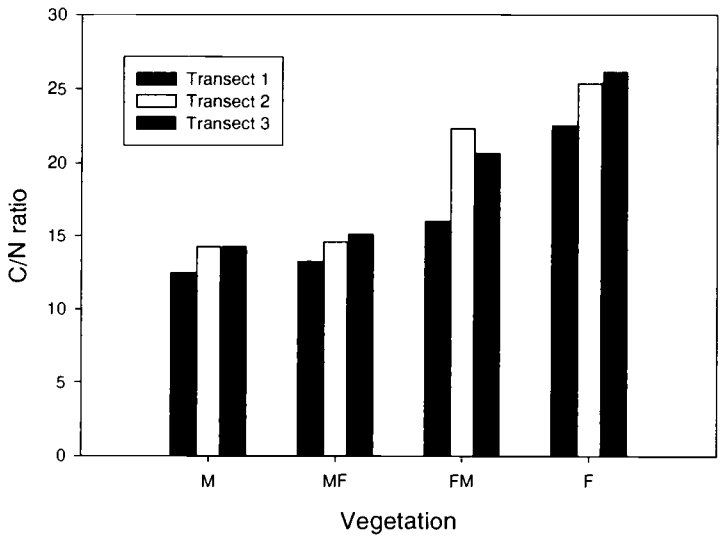

Figure 3.15. Soil C/N ratios at Carpenter for June 2000. Each bar represents the value of a single transect.

A comparison between the two sites showed similar C/N ratios at Lookout meadow and Carpenter meadow; however, the C/N ratio at Carpenter forest was significantly greater (53%) than the C/N ratio at Lookout forest (t-test, 2-sided $P < 0.0001$, CI: 1.33, 1.76)

Correlations between net N mineralization and C/N ratios were determined for 10-d mineralizations in June 2000 and for both 10-d (Fig. 3.16 and 3.17) and 85-d mineralizations in November 2000. Since total C and N were determined only for June soils, these values were used for both June and November correlations. Because fewer plots were sampled along each transect in November (only one for each treatment), C/N ratios for individual treatments were averaged along each transect in order to compare them to November net N mineralization rates.

Uneven distributions of the residuals for graphs of net N mineralization versus C/N ratio indicated the need for a log transformation of the net N mineralization variable. Thus a P value and r^2 (coefficient of determination) is given for each linear regression of C/N ratio on log-transformed net N mineralization. In June 2000, a significant correlation was found between C/N and log-transformed net N mineralization at both Carpenter ($n = 24$, $P< 0.0001$, $r^2 = 0.5359$) and Lookout ($n = 23$, $P = 0.0108$, $r^2 = 0.2717$). A significant correlation was not found between C/N and net N mineralization for the 10-d incubation in November at either site. However, there was a significant correlation between the two variables for the 85-d mineralization in November at both Carpenter ($n = 12$, $P = 0.0016$, $r^2 = 0.6418$) and Lookout ($n = 12$, $P = 0.0282$, $r^2 = 0.4291$) Scatterplots of untransformed net N mineralization versus C/N ratio are also displayed below (Fig. 3.18 and 3.19) in order to show the true relationship between these two variables.

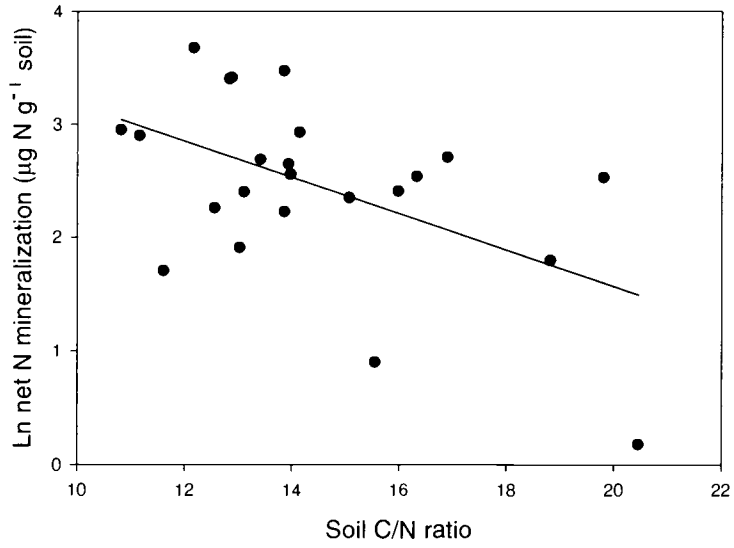

Figure 3.16. Log-transformed net N mineralization vs. soil C/N ratio at Lookout for10-d mineralization in June 2000

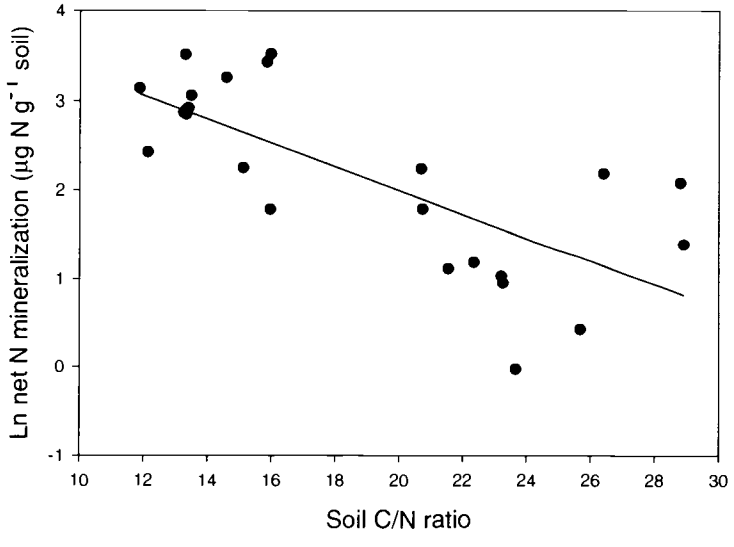

Figure 3.17 Log-transformed net N mineralization vs. soil C/N ratio at Carpenter for a 10-d mineralization in June 2000.

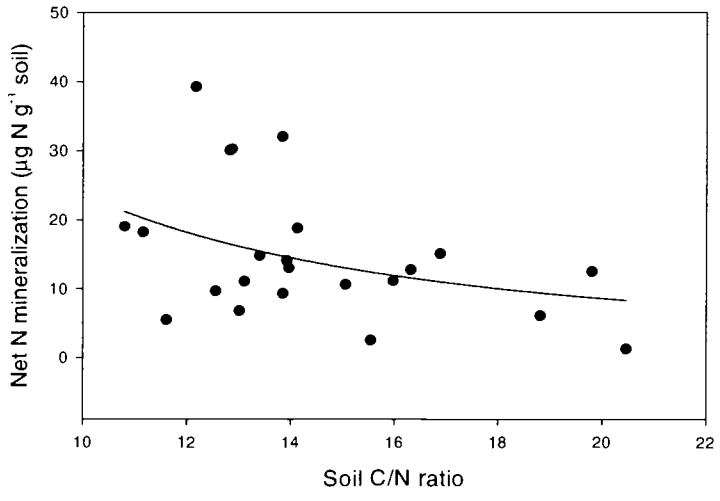

Figure 3.18. Net N mineralization vs. soil C/N ratio at Lookout for a 10 d mineralization in June 2000

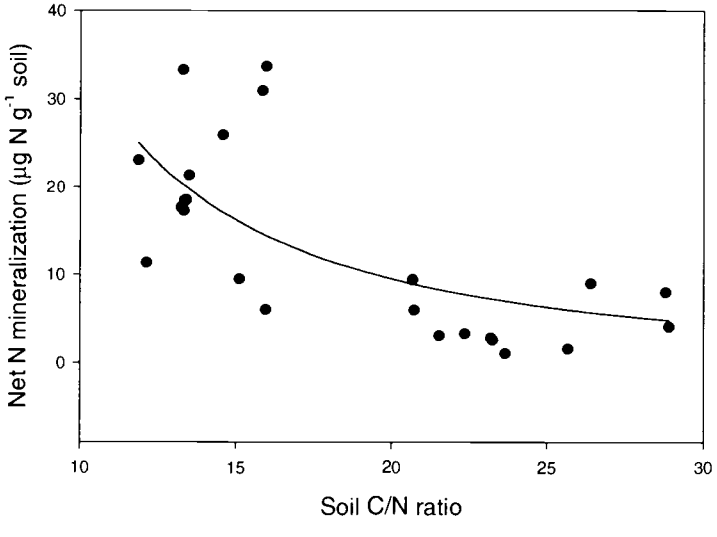

Figure 3.19. Net N mineralization vs. soil C/N ratio at Carpenter for 10 d mineralization in June 2000.

Gross rates of N mineralization and nitrification

The results of the ^{15}N isotope dilution experiment are shown in Tables 3.1-3.3. Percent N recovery and ^{15}N atom% as measured by the mass spectrometer are both reported. The calculated ^{15}N atom% excess (APE) is also reported, along with calculations for gross rates of production and consumption. Some of the calculations for gross rates of production and/or consumption produced negative results, given the measured N pool sizes and ^{15}N atom percent values, which may indicate methodology problems or that unknown processes were occurring.

Table 3.1 Gross rates of production and consumption for sampling period June 13th.

N form	Time (hrs)	Vegetation	% Recovery	AP	APE	Production	Consumption
						(μg N g^{-1} soil hr^{-1})	
NH_4^+	12	LM	78	0.44	0.64	0.21	0.21
		LF	76	1.01	0.99	0.35	0.40
		CM	78	0.51	0.74	0.22	0.23
		CF	81	0.84	1.00	0.41	0.41
	36	LM	85	0.37	0.13		
		LF	79	0.46	0.78		
		CM	86	0.37	0.10		
		CF	79	0.44	0.53		
NO_3^-	12	LM	67	3.17	2.81	0.10	-0.01
		LF	89	1.84	13.11	-0.06	0.08
		CM	69	3.52	3.15	0.13	0.06
		CF	103	1.73	12.30	-0.02	0.10
	36	LM	64	2.96	2.59		
		LF	99	0.98	25.60		
		CM	74	3.11	2.75		
		CF	100	0.91	15.40		

[a] N addition for both forms of N is at a rate of 1μg g^{-1} soil.
[b] Time is hours after labeling the soils.
[c] Vegetation is Lookout meadow (LM), Lookout forest (LF), Carpenter meadow (CM), and Carpenter forest (CF).
[d] % Recovery is the percent N measured by the mass spectrometer per the amount known to be in the diffused sample.
[e] AP is the atom percent measured by the mass spectrometer.
[f] APE is the calculated atom % excess (adjusted for natural abundance spike).

Table 3.2 Gross rates of production and consumption for sampling period June 30th.

N form	Time (hrs)	Vegetation	% Recovery	AP	APE	Production	Consumption
						($\mu g\ N\ g^{-1}$ soil hr^{-1})	
NH_4^+	12	LM	47	0.41	0.69	0.12	0.16
		LF	82	2.84	2.48	0.08	-0.11
		CM	62	0.39	0.55	0.07	0.09
		CF	83	2.33	1.96	0.13	0.26
	36	LM	83	0.37	0.11		
		LF	73	2.67	2.33		
		CM	69	0.37	0.10		
		CF	73	2.19	1.83		
NO_3^-	12	LM	67	2.68	2.31	0.06	0.00
		LF	107	1.94	19.66	0.04	-0.03
		CM	59	1.24	1.27	-0.19	-0.27
		CF	155	11.93	11.57	-0.05	-0.12
	36	LM	72	2.59	2.22		
		LF	101	2.22	14.75		
		CM	61	1.75	1.39		
		CF	95	2.18	16.35		

Table 3.3 Gross rates of production and consumption for sampling period July 24th.

N form	Time (hrs)	Vegetation	% Recovery	AP	APE	Production	Consumption
						($\mu g\ N\ g^{-1}$ soil hr^{-1})	
NH_4^+	12	LM	47	0.41	0.69	0.12	0.16
		LF	82	2.84	2.48	0.08	-0.11
		CM	62	0.39	0.55	0.07	0.09
		CF	83	2.33	1.96	0.13	0.26
	36	LM	83	0.37	0.11		
		LF	73	2.67	2.33		
		CM	69	0.37	0.10		
		CF	73	2.19	1.83		
NO_3^-	12	LM	67	2.68	2.31	0.06	0.00
		LF	107	1.94	19.66	0.04	-0.03
		CM	59	1.24	1.27	-0.19	-0.27
		CF	155	11.93	11.57	-0.05	-0.12
	36	LM	72	2.59	2.22		
		LF	101	2.22	14.75		
		CM	61	1.75	1.39		
		CF	95	2.18	16.35		

Discussion

In general, both the short- and long-term mineralization assays showed greater net N mineralization for meadow than for forest soils at both Lookout and Carpenter. Initially, almost all the N in the meadow was in the form of NO_3^-, whereas most of the N in the forest was in the form of NH_4^+. Over the course of the longer incubations, the NH_4^+ pool size for the meadow soils ranged from 0 to 8 $\mu g\ N\ g^{-1}$ soil, and NH_4^+ for the forest soils ranged from 0.9 to 73 $\mu g\ N\ g^{-1}$ soil. The NO_3^- pool size for the meadow soils ranged from 5 to 170 $\mu g\ N\ g^{-1}$ soil, and NO_3^- for the forest soils ranged from 0 to 115 $\mu g\ N\ g^{-1}$ soil. During all of the incubations, Carpenter forest soils produced very little net NO_3^- compared to Lookout forest soils. In general, NO_3^- levels for Carpenter forest soils stayed below 5 $\mu g\ N\ g^{-1}$ soil during both incubation periods. However, on the last day of the June soil incubation, the mean NO_3^- concentration for Carpenter forest soils increased to 17 $\mu g\ N\ g^{-1}$ soil. Lookout forest soils showed an increase in NO_3^- concentrations much earlier and to a much greater degree during both incubations. The average concentration of NO_3^- in Lookout forest soils was approximately 22 $\mu g\ N\ g^{-1}$ soil by the 60^{th} day and greater than 70 $\mu g\ N\ g^{-1}$ by the final day of the incubations.

In a study performed by Hart et al. (1994) on old-growth forest soils, also from HJA, significant increases in net NO_3^- production did not occur until after 140 d of incubation. Concentrations of NO_3^- remained below 5 $\mu g\ N\ g^{-1}$ soil for the first 140 d of the incubation, at which point levels increased to 40 $\mu g\ N\ g^{-1}$ soil. During the November incubation of the present study, net nitrification in Lookout forest soils averaged above 5 $\mu g\ N\ g^{-1}$ soil by the 25^{th} day of the incubation and had reached an average of 56 $\mu g\ N\ g^{-1}$ soil by the 85^{th} day. Carpenter forest soils didn't show any significant increase in net nitrification throughout the course of the incubation, however, it is possible that given time, NO_3^- concentrations would have increased in Carpenter soils as well. In the Hart et al. (1994) study, NH_4^+ levels increased steadily, reaching a maximum of approximately 25 $\mu g\ N\ g^{-1}$ soil at

140 days, immediately after which NH_4^+ levels dropped to near zero, and NO_3^- levels rapidly increased to approximately 40 μg N g^{-1} soil. The NH_4^+ concentrations in Lookout soils appeared to be reaching a peak at between 65 and 85 d with approximately 40 μg N g^{-1} soil. The NH_4^+ levels at Carpenter had reached an average of 20 μg N g^{-1} soil by the 85th day of the incubation. By day 100, NH_4^+ levels in Hart et al. (1994) soils were still under 15 μg N g^{-1} soil.

The C/N ratios tended to be higher in soils with a lower net N mineralization (Fig. 3.18 and 3.19). This was true for both the 10-d and 85-d mineralization assays. A curvilinear relationship was found for C/N ratio and net N mineralization for the 10-d incubation in June and the 85-d incubation in November. Prescott et al. (2000) also found a curvilinear relationship between net N mineralization and C/N of the forest floor of coastal Douglas-firs stands. In the Prescott et al. (2000) study, significant net mineralization only occurred where C/N ratios were less than 35. The C/N ratios in the present study ranged from 11.9 to 28.9 at Carpenter and 10.8 to 20.4 at Lookout. Net N mineralization was significant for all vegetation types during all of the incubations.

At both Lookout and Carpenter, forest soils had greater C/N ratios than meadow soils. The C/N ratio was 25 % and 81% greater in forest than meadow soils at Lookout and Carpenter, respectively. The C/N ratio for Carpenter meadow was similar to Lookout meadow and net N mineralization tended to be similar for the two meadow sites during most of the sampling periods. Carpenter forest soils, on the other hand, had a significantly higher C/N ratio than Lookout forest soils, and net N mineralization tended to be higher in Lookout soils than in Carpenter soils.

During the course of the present study, an attempt was also was made to quantify gross rates of N mineralization and nitrification with the intention of comparing those rates to net N mineralization rates. Some of the calculations produced impossible results for rates of production and consumption. Either the N pool sizes and/or ^{15}N APE values used in the calculations were not correct, or the

Kirkham and Bartholomew (1955) model used to determine rates of production and consumption did not reflect the N-cycling processes for the Lookout and Carpenter soils. The results (reported in Tables 3.1-3.3) will not be used for purposes of comparison to net N mineralization rates as originally intended.

Conclusions

The patterns observed in this study agree with evidence that shows a connection between net N mineralization and C/N ratios. However, the underlying causes are not always clear. In the Thomas and Prescott (2000) study, although there was a correlation between C/N and net N mineralization, there was a better correlation between net N mineralization and total N. In other studies, the lignin/N ratio has been shown to be more significant than C/N (Thomas and Prescott, 2000). In this study, we did not test for qualitative differences between meadow and forest or between sites, but this could be a useful point of further investigation.

CHAPTER 4

FINAL CONCLUSIONS

Many studies have examined differences in the biology and chemistry of soils associated with vegetation differences. In this study, I had the opportunity to look at some of these properties for meadow soils located adjacent to forest soils at two different locations in the HJA. This study was different from most other studies in that both types of vegetation existed on the same site, lending stronger evidence to the effect of vegetation as opposed to other site characteristics.

At both Carpenter and Lookout, differences in some soil properties are clearly associated with differences in vegetation. Net N mineralization rates provided the best evidence for these differences. Rates were almost always higher in the meadow than in the forest for both short-term and long-term incubations. My findings were consistent with the idea that most of the inorganic N in soils beneath grassy vegetation is in the form of NO_3^-, while most of the N in forest soils is in the form of NH_4^+. However, NO_3^- did begin to accumulate after a short incubation period at Lookout, and was beginning to accumulate after a comparatively longer time in some of the Carpenter soils as well.

The soils also differed in their C/ N ratios, with forest soils having a significantly higher C/N ratio than meadow soils. Differences were primarily driven by total N, which was greater in meadow soils, rather than total C, which was similar in the two soils. The C/N ratios were significantly correlated with net N mineralization, and wider C/N ratios in forest soils corresponded to smaller net N mineralization rates in these soils. This pattern was also true for site differences. Carpenter forest had wider C/N ratios than Lookout forest, corresponding to lower net N mineralization rates at Carpenter forest, while meadow soils at the two sites had both similar C/N ratios and similar net N mineralization rates.

Microbial biomass C and NF as measured by CFIM did not display the same meadow/forest divergence that was observed for net N mineralization. However, measurements of MBC determined by epifluorescent microscopy showed that MBC was significantly greater in the forest than in the meadow. This was due primarily to differences in the number of fungi; estimates for bacteria alone were similar at Lookout and only slightly greater at Carpenter. The discordance between the two methods brings into question what it is that each method actually measures. Although it appears that CFIM may not measure fungal biomass quantities, it may be very useful if the interest is in determining the quantity of labile nutrients stored within the biomass. A comparison of the amount of C and N contained in biomass with the quantities of C and N that can be mineralized over an 85-d incubation period should tell us something about how these two pools are related. In the case of N, it appears that the microbial biomass may be the major pool of labile N. Thus, an understanding of this microbial biomass and the dynamics of turnover may be extremely important to a fuller understanding of primary production in N limited soils.

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APPENDIX

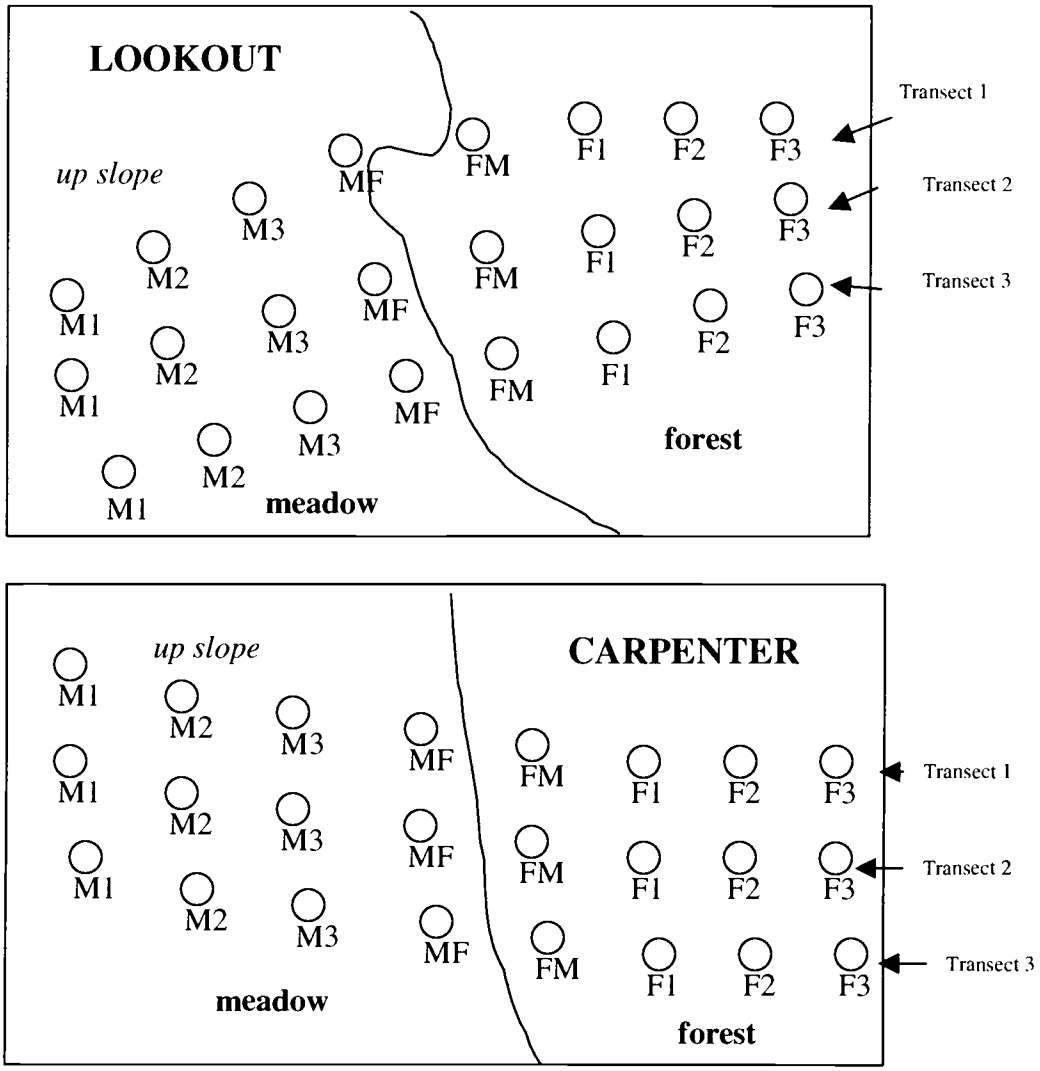

Figure A 1. Map of plots and transects at Lookout and Carpenter.

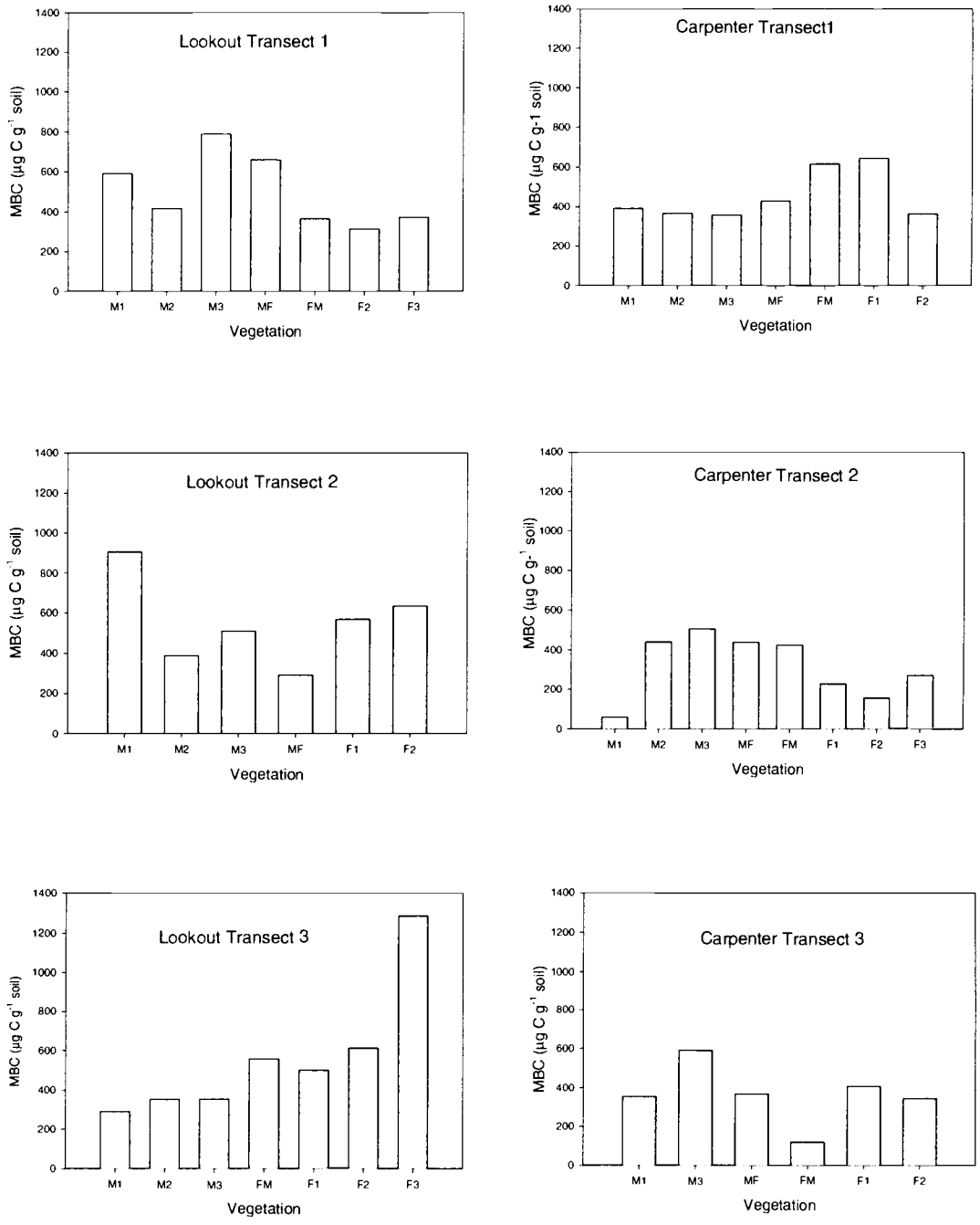

Figure A 2. Microbial biomass C vs. vegetation along each transect for soils collected in June 2000.

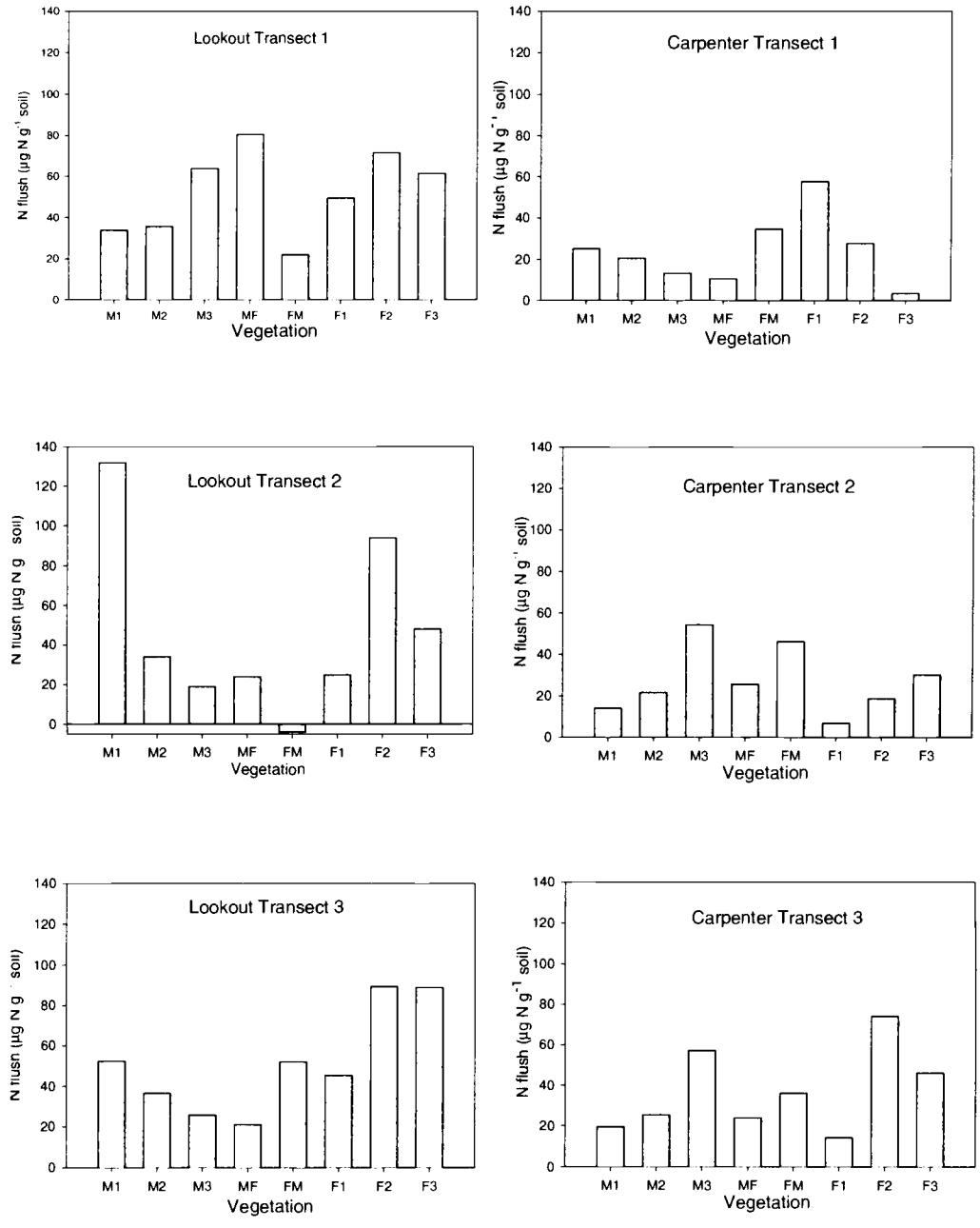

Figure A 3. Nitrogen flush vs. vegetation along each transect for soils collected in June 2000.

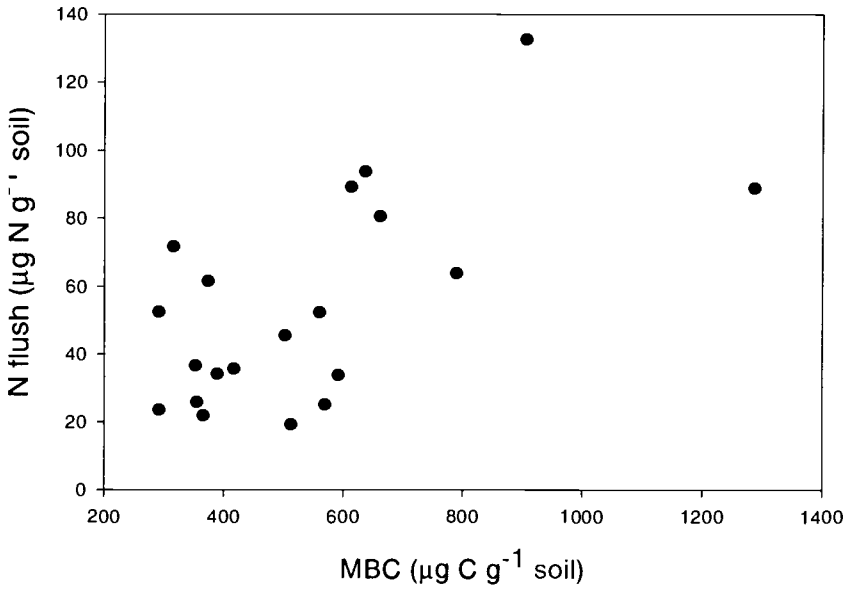

Figure A 4. Nitrogen flush vs. MBC for June 2000 Lookout soils.

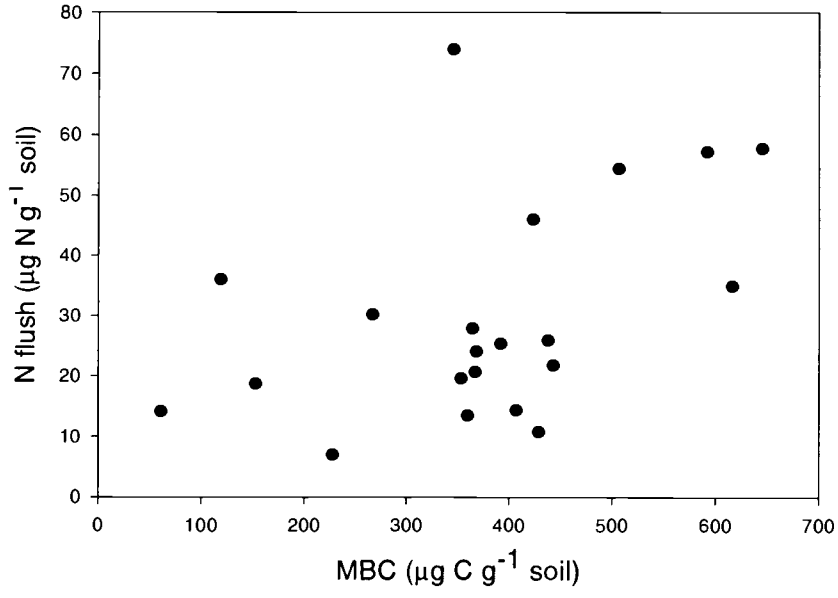

Figure A 5. Nitrogen flush vs. MBC for June 2000 Carpenter soils.

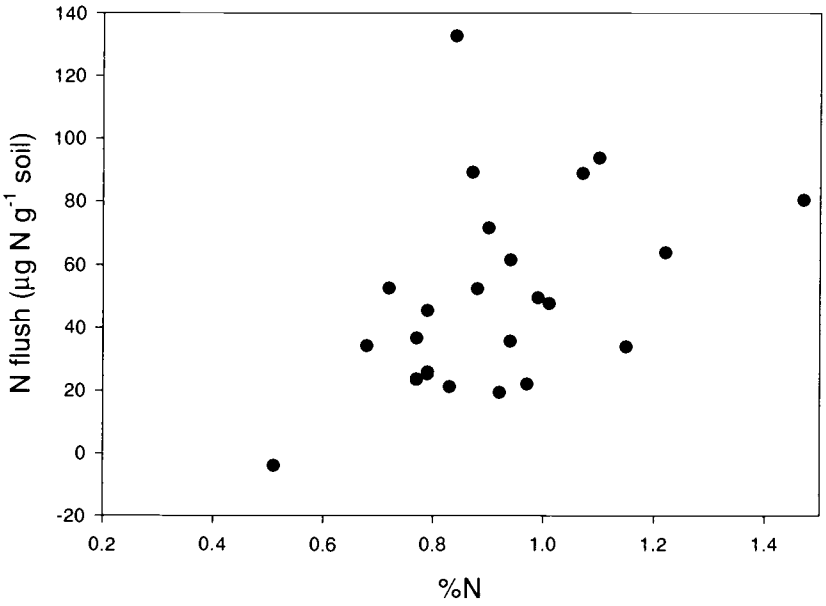

Figure A 6. Nitrogen flush vs. % N for June 2000 Lookout soils.

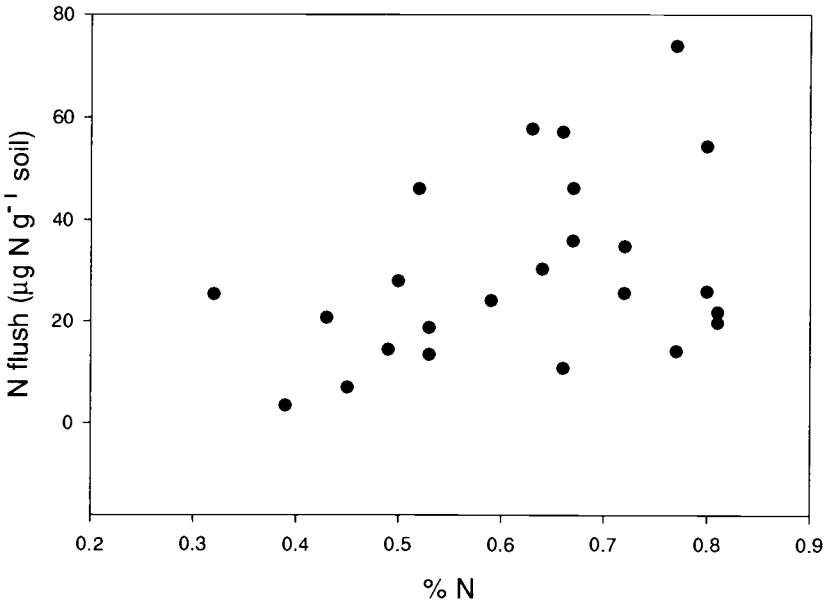

Figure A 7. Nitrogen flush vs. % N for June 2000 Carpenter soils.

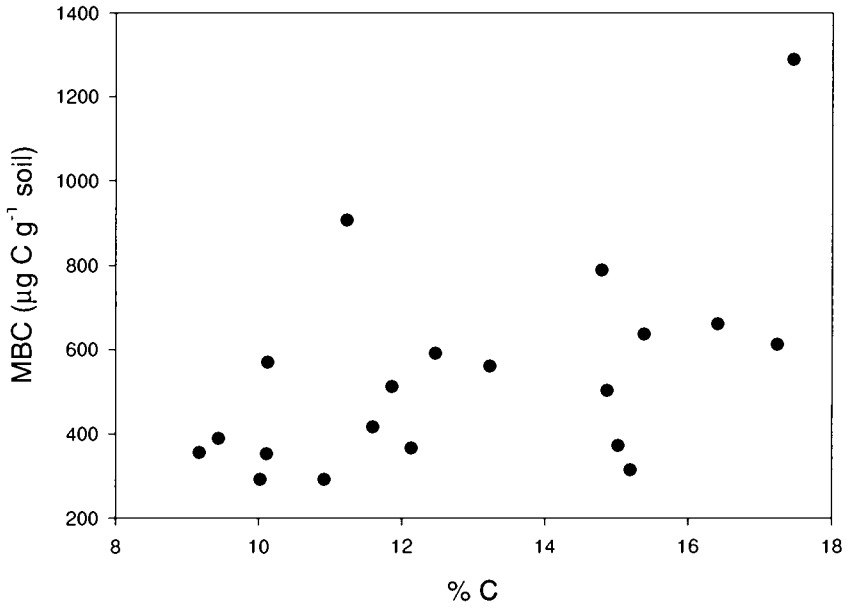

Figure A 8. Microbial biomass C vs. % C for June 2000 Lookout soils.

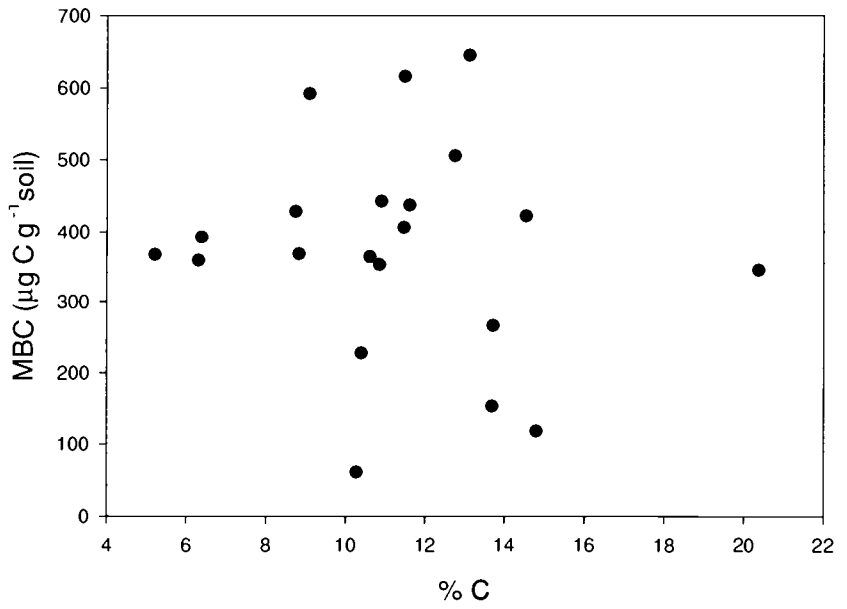

Figure A 9. Microbial biomass C vs. % C for June 2000 Carpenter soils.

Table A 10. Selected characteristics of Lookout mineral soil (5-15 cm) at each of 8 plots along 3 transects in June 2000.

Transect	Vegetation	pH	%C	%N	MBC	NF	$C:N_{soil}$
1	M	5.60	12.47	1.15	591	33.8	10.81
1	M	5.80	12.09	0.94	417	35.6	12.83
1	M	5.60	14.80	1.22	789	63.9	12.17
1	M/F	5.40	16.41	1.47	661	80.5	11.16
1	F/M	5.50	12.14	0.97	366	22.0	12.56
1	F	5.50	15.40	0.99	*	49.5	15.55
1	F	5.70	15.20	0.9	315	71.6	16.89
1	F	5.80	15.02	0.94	373	61.6	15.98
2	M	6.20	11.23	0.84	906	132.6	13.41
2	M	6.00	9.44	0.68	389	34.2	13.84
2	M	5.70	11.87	0.92	512	19.3	12.87
2	M/F	6.00	10.92	0.77	292	23.6	14.14
2	F/M	5.90	10.37	0.51	*	-4.0	20.46
2	F	5.50	10.13	0.79	569	25.2	12.82
2	F	5.80	15.39	1.1	636	93.8	13.97
2	F	5.60	14.10	1.01	*	47.7	13.93
3	M	5.90	10.02	0.72	291	52.5	13.85
3	M	5.90	10.11	0.77	353	36.6	13.11
3	M	5.70	9.17	0.79	355	25.8	11.61
3	M/F	5.80	10.79	0.83	*	21.1	13.02
3	F/M	5.90	13.23	0.88	560	52.4	15.07
3	F	5.60	14.87	0.79	502	45.5	18.82
3	F	5.60	17.24	0.87	613	89.3	19.81
3	F	5.90	17.46	1.07	1287	88.9	16.32

* No data.

Table A 11. Selected characteristics of Carpenter mineral soil (5-15 cm) at each of 8 plots along 3 transects in June 2000.

Transect	Vegetation	pH	%C	%N	MBC	NF	$C:N_{soil}$
1	M	6.10	6.40	0.48	392	25.3	13.33
1	M	6.10	5.21	0.43	367	20.7	12.13
1	M	5.80	6.32	0.53	359	13.5	11.87
1	MF	5.60	8.75	0.66	429	10.7	13.25
1	FM	5.90	11.50	0.72	616	34.9	15.96
1	F	5.80	13.11	0.63	645	57.8	20.74
1	F	6.10	11.61	0.5	364	27.9	23.26
1	F	5.90	10.18	0.43	*	3.5	23.66
2	M	5.50	10.28	0.77	61	14.2	13.35
2	M	5.80	10.91	0.81	443	21.8	13.49
2	M	5.90	12.76	0.8	506	54.4	15.85
2	MF	5.80	11.62	0.8	438	25.8	14.58
2	FM	5.70	15.05	0.67	423	46.2	22.36
2	F	5.80	11.56	0.4	228	7.0	28.90
2	F	6.00	13.55	0.53	153	18.7	25.68
2	F	6.00	13.72	0.64	267	30.2	21.54
3	M	5.70	10.86	0.81	353	19.6	13.40
3	M	5.90	11.05	0.83	*	25.5	13.31
3	M	6.30	11.34	0.71	592	57.2	15.97
3	MF	6.30	8.84	0.59	368	24.0	15.11
3	FM	6.10	13.87	0.67	119	36.0	20.68
3	F	5.60	11.48	0.49	407	14.4	23.21
3	F	5.40	20.45	0.71	345	74.0	28.80
3	F	5.60	13.80	0.52	*	46.1	26.41

* No data.

Table A 12. Lookout June 2000 data.

Transect	Vegetation	Net N min 10 d	CFIM		Microscopy	
			MBC	NF	Fungi	Bacteria
1	M1	19.06	591	33.84	349.8	88.6
1	M2	30.11	417	35.64	1129.9	96.8
1	M3	39.28	789	63.91	1155.4	109.4
1	MF	18.20	661	80.52	722.1	125.9
1	FM	9.63	366	21.95	632.2	103.0
1	F1	2.46	*	49.54	821.0	101.0
1	F2	15.02	315	71.57	1737.9	96.4
1	F3	11.12	373	61.55	6828.3	76.8
2	M1	14.77	906	132.59	200.3	91.6
2	M2	32.02	389	34.20	1060.2	128.1
2	M3	30.31	512	19.29	1490.7	57.7
2	MF	18.76	292	23.55	977.7	70.1
2	FM	1.19	*	-4.00	1411.8	72.3
2	F1	*	569	25.20	4295.6	79.7
2	F2	13.00	636	93.79	809.4	113.0
2	F3	14.11	*	47.72	3493.3	101.4
3	M1	9.27	291	52.53	531.1	86.2
3	M2	11.07	353	36.56	603.7	92.3
3	M3	5.51	355	25.81	1667.6	81.2
3	MF	6.73	*	21.11	1011.4	83.0
3	FM	10.54	560	52.35	4958.4	68.3
3	F1	6.07	502	45.53	4533.6	90.5
3	F2	12.51	613	89.25	1120.4	117.9
3	F3	12.74	1287	88.89	8706.5	116.7

* No data

Table A 13. Carpenter June 2000 data.

Transect	Vegetation	Net N min	CFIM		Microscopy	
		10 d	MBC	NF	Fungi	Bacteria
1	M1	17.32	17.32	392	25.32	1463.4
1	M2	11.41	11.41	367	20.66	701.5
1	M3	23.16	23.16	359	13.50	1064.4
1	MF	17.70	17.70	429	10.75	1246.5
1	FM	5.99	5.99	616	34.88	3811.9
1	F1	6.01	6.01	645	57.75	1580.8
1	F2	2.58	2.58	364	27.88	4655.7
1	F3	0.98	0.98	*	3.46	1943.5
2	M1	18.43	18.43	61	14.15	477.9
2	M2	21.43	21.43	443	21.76	638.5
2	M3	30.96	30.96	506	54.43	261.7
2	MF	25.93	25.93	438	25.81	3199.3
2	FM	3.30	3.30	423	46.19	1608.5
2	F1	4.06	4.06	228	6.97	2105.4
2	F2	1.54	1.54	153	18.74	4118.0
2	F3	3.03	3.03	267	30.19	2372.5
3	M1	18.52	18.52	353	19.61	1347.7
3	M2	33.33	33.33	*	25.54	886.6
3	M3	33.71	33.71	592	57.19	2327.9
3	MF	9.49	9.49	368	24.05	1642.0
3	FM	9.40	9.40	119	36.00	2628.2
3	F1	2.79	2.79	407	14.40	1824.3
3	F2	7.98	7.98	345	73.97	11229.5
3	F3	8.98	8.98	*	46.08	3125.1

* No data

Table A 14. Lookout September 2000 data.

Vegetation	CFIM MBC	CFIM NF	Net N min 10 d
M	592.54	55.23	16.11
M	553.57	92.62	19.24
M	508.91	8.70	4.41
F	738.64	15.19	28.43
F	190.57	49.13	21.21
F	325.36	50.42	3.97

Table A 15. Carpenter September 2000 data.

Vegetation	CFIM MBC	CFIM NF	Net N min 10 d
M	345.75	20.78	25.65
M	375.22	24.63	19.38
M	722.22	27.11	23.32
F	144.28	51.00	7.33
F	142.70	66.15	4.44
F	434.30	67.71	20.75

Table A 16. Lookout November 2000 data.

Transect	Vegetation	CFIM MBC	Net N min 10 d	Net N min 85 d	Respiration 10 d	Respiration 85 d
1	M	343	21.19	159.88	605.50	2212.11
1	MF	128	11.47	114.44	692.67	2330.69
1	FM	272	-0.34	150.14	855.78	3649.17
1	F	185	3.36	59.06	697.89	2528.34
2	M	57	25.82	106.70	475.35	1447.98
2	MF	229	-1.92	55.48	765.22	2486.96
2	FM	81	0.53	43.11	464.38	1751.47
2	F	32	7.45	123.53	724.01	2547.29
3	M	101	25.89	156.28	446.59	1470.18
3	MF	354	21.18	82.39	471.15	1499.12
3	FM	188	8.55	234.02	1077.55	4400.00
3	F	375	4.30	40.26	695.39	2806.56

Table A 17. Carpenter November 2000 data.

Transect	Vegetation	CFIM MBC	Net N min 10 d	Net N min 85 d	Respiration 10 d	Respiration 85 d
1	M	70	10.41	73.03	268.89	792.70
1	MF	331	10.66	67.81	304.62	789.74
1	FM	397	8.21	148.49	680.00	2675.00
1	F	122	-1.25	17.04	406.94	1590.16
2	M	211	22.65	137.57	471.72	1754.48
2	MF	174	26.91	102.09	498.93	1512.50
2	FM	173	6.96	31.57	587.96	2281.77
2	F	195	-1.66	5.67	376.18	1296.49
3	M	79	34.43	163.71	543.04	2059.37
3	MF	342	26.57	175.36	675.09	2425.03
3	FM	223	-3.71	60.60	620.96	2346.25
3	F	308	3.29	28.04	984.95	3875.23

Table A 18. Lookout June 2000 ANOVA tables.

	Source	Sum of squares	d.f.	Mean square	F-stat	p-value
	Model	65124	3	21708	0.33	0.8061
CFIM MBC	Error	1063490	16	66468		
	Corrected Total	1128614	19			

	Source	Sum of squares	d.f.	Mean square	F-stat	p-value
	Model	4031	3	1344	1.51	0.2425
CFIM NF	Error	17799	20	890		
	Corrected Total	21830	23			

Microscopy	Source	Sum of squares	d.f.	Mean square	F-stat	p-value
MBC for Bacteria	Model	762	3	254	0.7	0.5605
	Error	7215	20	361		
	Corrected Total	7977	23			

	Source	Sum of squares	d.f.	Mean square	F-stat	p-value
Microscopy	Model	37292663	3	12430888	3.28	0.0422
MBC for Fungi	Error	75771929	20	3788597		
	Corrected Total	113064592	23			

Net N min 10 d	Source	Sum of squares	d.f.	Mean square	F-stat	p-value
	Error	1421	19	611	6.01	0.0238
	Corrected Total	2097	22	75		

	Source	Sum of squares	d.f.	Mean square	F-stat	p-value
	Model	99	3	33	2.02	0.1454
C/N soil	Error	311	19	16		
	Corrected Total	411	22			

* Class variable = vegetation (M, MF, FM, F)

Table A 19. Carpenter June 2000 ANOVA tables.

	Source	Sum of squares	d.f.	Mean square	F-stat	p-value
	Model	11728	3	3909	0.15	0.9292
CFIM MBC	Error	447192	17	26305		
	Corrected Total	458920	20			

	Source	Sum of squares	d.f.	Mean square	F-stat	p-value
	Model	573	3	191	0.55	0.6518
CFIM NF	Error	6904	20	345		
	Corrected Total	7477	23			

Microscopy	Source	Sum of squares	d.f.	Mean square	F-stat	p-value
MBC for Bacteria	Model	4069	3	1356	3.55	0.0329
	Error	7639	20	382		
	Corrected Total	11709	23			

	Source	Sum of squares	d.f.	Mean square	F-stat	p-value
Microscopy	Model	32071599	3	10690533	2.63	0.078
MBC for Fungi	Error	81212082	20	4060604		
	Corrected Total	1.13E+08	23			

	Source	Sum of squares	d.f.	Mean square	F-stat	p-value
Net N min 10 d	Model	1822	3	607	17.05	< 0.0001
	Error	713	20	36		
	Corrected Total	2535	23			

	Source	Sum of squares	d.f.	Mean square	F-stat	p-value
C/N soil	Model	480	3	160	26.18	< 0.0001
	Error	122	20	6		
	Corrected Total	602	23			

* Class variable = vegetation (M, MF, FM, F)

Table A 20. Lookout September 2000 ANOVA tables.

	Source	Sum of squares	d.f.	Mean square	F-statistic	p-value
CFIM MBC	Model	26726	1	26726	0.64	0.468
	Error	166619	4	41655		
	Corrected Total	193346	5			

	Source	Sum of squares	d.f.	Mean square	F-statistic	p-value
CFIM NF	Model	291	1	291	0.27	0.6315
	Error	43334	4	1083		
	Corrected Total	4625	5			

	Source	Sum of squares	d.f.	Mean square	F-statistic	p-value
Net N min 10 d	Model	32	1	32	0.29	0.6178
	Error	438	4	110		
	Corrected Total	470	5			

Table A 21. Carpenter September 2000 ANOVA tables

	Source	Sum of squares	d.f.	Mean square	F-statistic	p-value
CFIM MBC	Model	86859	1	86859	2.41	0.19514
	Error	144051	4	36013		
	Corrected Total	230910	5			

	Source	Sum of squares	d.f.	Mean square	F-statistic	p-value
CFIM NF	Model	2103	1	2103	44.08	0.0027
	Error	190	4	48		
	Corrected Total	2293	5			

	Source	Sum of squares	d.f.	Mean square	F-statistic	p-value
Net N min 10 d	Model	214	1	214	4.99	0.0892
	Error	172	4	43		
	Corrected Total	386	5			

* Class variable = vegetation (M and F)

Table A 22. Lookout November ANOVA tables.

	Source	Sum of squares	d.f.	Mean square	F-stat	p-value
CFIM MBC	Model	95273	3	31758	4	0.0518
	Error	63478	8	7935		
	Corrected Total	158751	11			

	Source	Sum of squares	d.f.	Mean square	F-stat	p-value
Net N min 10 d	Model	834	3	278	6.53	0.0153
	Error	341	8	43		
	Corrected Total	1175	11			

	Source	Sum of squares	d.f.	Mean square	F-stat	p-value
Net N min 85 d	Model	11865	3	3955	1.23	0.3592
	Error	25636	8	3205		
	Corrected Total	37501	11			

	Source	Sum of squares	d.f.	Mean square	F-stat	p-value
Respiration 10 d	Model	133359	3	44453	1.4	0.3127
	Error	254568	8	31821		
	Corrected Total	387926	11			

	Source	Sum of squares	d.f.	Mean square	F-stat	p-value
Respiration 85 d	Model	4088473	3	1362824	2.31	0.1528
	Error	4717120	8	589640		
	Corrected Total	8805593	11			

* Class variable = vegetation (M, MF, FM, F)

Table A 23. Carpenter November 2000 ANOVA tables.

	Source	Sum of squares	d.f.	Mean square	F-stat	p-value
CFIM MBC	Model	44378	3	14792	1.5	0.2869
	Error	78906	8	9863		
	Corrected Total	123284	11			

	Source	Sum of squares	d.f.	Mean square	F-stat	p-value
Net N min 10 d	Model	1218	3	406	5.78	0.0211
	Error	562	8	70		
	Corrected Total	1780	11			

	Source	Sum of squares	d.f.	Mean square	F-stat	p-value
Net N min 85 d	Model	21428	3	7143	3.16	0.0856
	Error	18058	8	2257		
	Corrected Total	39485	11			

	Source	Sum of squares	d.f.	Mean square	F-stat	p-value
Respiration 10 d	Model	75481	3	25160	0.58	0.6461
	Error	348704	8	43588		
	Corrected Total	424185	11			

	Source	Sum of squares	d.f.	Mean square	F-stat	p-value
Respiration 85 d	Model	1916493	3	638831	0.81	0.522
	Error	6292081	8	786510		
	Corrected Total	8208574	11			

* Class variable = vegetation (M, MF, FM, F)