# BLACK SPRUCE (*Picea mariana* [Mill.] B.S.P.) BOREAL ECOSYSTEMS: HOW TREE-LENGTH AND FULL-TREE HARVESTING AFFECTS SOIL MICROBIAL POPULATIONS

by

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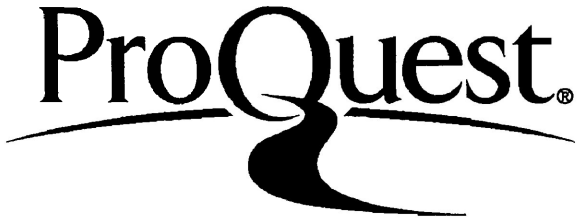

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# ABSTRACT

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Key Words: microbial biomass, soil organic matter, soil respiration, API 20E, substrate utilization, chloroform fumigation extraction.

To evaluate the hypothesis that microbial populations are affected by organic biomass removals, a study was designed to complement ongoing work in a black spruce (*Picea mariana* [Mill.] B.S.P.) Boreal ecosystem. The treatments included a control (uncut) and two harvest levels, tree-length (TL) and full-tree (FT). Soil samples from the organic and mineral horizons were taken from nine plots, representing three each of the treatments. Samples were taken once a month for four consecutive months; May through August during 1998. Soil respiration on two dates in September was estimated using the soda-lime technique. Bacterial cultures were prepared from the soil samples and pure strains identified using morphological and substrate utilization characteristics (specifically API 20E). Soil descriptors, including pH, total nitrogen, total phosphorus, organic matter content, and moisture content, were measured to investigate relationships with microbial biomass. Microbial biomass carbon ($MB_C$) and nitrogen ($MB_N$) were estimated using chloroform fumigation extraction. The data were statistically analyzed, using ANOVA, Pearson and Spearman correlations and, in the case of the $MB_C$ and $MB_N$, ANCOVA, to determine if there were any treatment or seasonal effects. Soil respiration demonstrated a significant treatment effect where the efflux was significantly greater on the control treatment compared to the harvest treatments. Five bacterial cultures were identified from the soil samples, *Chryseomonas luteola, Aeoromonas salmonicida, Serratia marcescens, Syntrophomonas multifilia,* and *Pseudomonas fluorescens*. $MB_C$ and $MB_N$ values measured were in agreement with other published values for boreal coniferous soils. The $MB_C$ in the organic horizon was significantly affected by the interaction of the treatment and month factors. There was a significant treatment effect on the $MB_C$ in the mineral horizon, with the control mean significantly higher than those of the harvest treatments. The $MB_N$ revealed no significant effects in either the organic or the mineral horizons. The author concludes that soil moisture and temperature did affect the values for microbial biomass and that these environmental conditions were likely impacted by the level of harvest.

# CONTENTS

# TABLES

# FIGURES

# INTRODUCTION

The microbial biomass, defined as the functional component of the micro-biota primarily responsible for decomposition, organic matter turnover, and nutrient transformation, is an important component of soil (Haron *et al.* 1998). Microbial biomass includes organisms such as bacteria, fungi, actinomycetes, microfauna and algae (Killham 1994). Acting as both a source and a sink of nutrients in soils, the microbial biomass, which depends mainly on organic matter for energy, influences the amount of available nutrients to plants via the mineralization-immobilization process (Gallardo and Schlesinger 1994). Microorganisms, therefore, play a major role in determining the relative quality of forest soil within the limitations of climate and topography (Entry *et al.* 1986).

Microbial biomass has been used to indicate and predict changes in various ecosystems caused by natural and anthropogenic disturbances (Weber 1990). Microbial biomass has been used as an indicator of global warming (Anderson and Joergensen 1997), sustainable development (Cairns and Meganck 1994), and biodiversity (Staddon *et al.* 1996). Microbial biomass has served many research applications in forestry. For example, it has been used in monitoring the effects of: municipal solid waste leachate on forest soils (Gordon *et al.* 1988); xenobiotic substances in soil (Dictor *et al.* 1998); fire (Diaz-Ravina *et al.* 1992); tillage induced changes (Carter 1986); pesticide application (Wardle and Parkinson 1990); response to thinning (Thibodeau *et al.* 2000) and microbial stress (Anderson and Joergensen 1997).

In addition, some components of the soil microbial biomass may act as natural biological control agents (Staddon *et al.* 1996). For example, the bacteria *Pseudomonas fluorescens* is antagonistic to soil-borne root pathogens that inhabit the soil rhizosphere (Killham 1994). This bacterial strain restricts a wide range of pathogens including: *Erwinia carotovora*, black leg in potatoes; *Pythium* spp., damping-off fungi; *Rhizoctonia solani*, cereal bare patch; and *Thielaviopsis basicola*, tobacco black root rot (Killham 1994). Continued study of these microorganisms and analysis of the molecular genetics of these antagonistic responses may lead to new biocontrols for other plant pathogens through genetic manipulation (Killham 1994).

Since little is known about the diversity of Boreal (northern) forest soil microorganisms, species loss or modification may deprive the ecosystem of processes, products, or genetic material provided by undescribed microbes (Staddon *et al.* 1996). Many studies have reported that the microbial biomass is affected by various timber harvesting treatments (Hendrickson *et al.* 1985; Entry *et al.* 1986; Foster and Morrison 1987; Weber 1990; Smolander *et al.* 1998). For example, timber harvesting may indirectly alter microbial biomass and activity by changing the amount and type of organic matter inputs, soil pH, soil temperature, and soil moisture (Entry *et al.* 1986).

The author was fortunate to have access to an established trial investigating the effects of various levels of biomass removal on a black spruce (*Picea mariana* [Mill.] B.S.P.) Boreal ecosystem. This study will attempt to augment the existing trial by asking the following questions:

1. Do increasing levels of biomass removal result in quantitative differences in soil microbial populations?

2. Do increasing levels of biomass removal result in differences in the bacterial species isolated from treated plots?

3. Do soil microbial populations exhibit seasonal responses and are these responses influenced by biomass removal?

4. Are there correlations between observed/measured fluctuations in soil microbial populations and soil parameters, such as pH, organic matter, total nitrogen, total phosphorous, and moisture content?

These questions will be addressed as follows:

1. Using the fumigation-extraction technique (Voroney *et al.* 1993), estimate the microbial biomass C and N found in representative samples from the study area,

2. Following the traditional taxonomic approach, isolate, culture and describe (Gram stain, cell and colony appearance, *etc.*) representative and unique bacteria,

3. Using the soda-lime respiration technique (Edwards 1982), estimate the soil respiration,

4. Using a functional or substrate utilization approach, specifically API 20E (Anonymous 1996), attempt to identify/characterize the isolated bacteria, and,

5. Using appropriate statistics, attempt to identify relationships between the microbial populations, treatment regimes and certain environmental parameters.

# LITERATURE REVIEW

The microbial biomass, which acts as both a source and a sink for nutrients, influences the availability of nutrients in the soil through the complementary processes of mineralization and immobilization. Mineralization is the process of releasing organically bound nutrients into mineral forms, which are then available for plant uptake, while immobilization refers to the process of microbial uptake of mineral nutrients, thus rendering them unavailable to plants (Killham 1994). The rates of these two processes depend, in part, upon the size of the microbial biomass, the composition of the microbial community (*e.g.*, fungi versus bacteria, nitrifiers versus denitrifiers), the quality of organic matter inputs, physical and chemical characteristics of the soil (*e.g.*, temperature, water content, % clay, pH) as well as the vegetation and climate above the ground. Each component will influence the others ultimately affecting the condition of the soil. Using forest canopy as an example, the presence of closely spaced spruce trees may cause the cycling of nutrients to slow down due to lower soil temperatures and acidic foliage inputs, whereas the presence of alder, with less acidic foliage and nitrogen fixing root nodules, may improve soil fertility (Bradley and Fyles 1995). Since microorganisms influence decay processes, mineral conversions, and plant root activity in the soil environment, plant growth conditions are therefore affected (Entry *et al.* 1986).

The elements affecting nutrient cycling and availability are numerous, complicated and interconnected. A change in any one may lead to subsequent changes in some or all of the other elements. The concept of "stability" or steady state in the microbial biomass

must, therefore, be used advisedly under such dynamic conditions. Nonetheless, some trends have been reported in the literature and will be summarized here.

In the literature review, the author will attempt to cover the major factors affecting microbial biomass quantity and composition: organic matter (OM) inputs, soil pH, soil temperature, soil moisture content, and seasonal conditions. Soil microbial activity measurements, specifically respiration, are also included due to their potential as ecosystem monitors following disturbance events. A discussion on microorganism culturing techniques follows, providing insights to the advantages and disadvantages of the various methodological approaches.

## ORGANIC MATTER INPUTS AND DECOMPOSITION

Approximately half of OM is carbon (C) (Paul and Clark 1989). Carbon compounds incorporated in the organic matter of soil are required primarily by the soil micro-biota for energy and nutrition (Paul and Clark 1989). Carbon consumption by microbes generates energy required to synthesize and break down other chemical compounds, such as organically bound nitrogen (N) and phosphorus (P), which are used for nutrition (Killham 1994).

Carbon and nitrogen are utilized by the micro-biota for cell wall formation and maintenance (Paul and Clark 1989). The cell walls of bacteria are made up of sugar derivatives, N-acteylglucosamine and N-acetylmuramic acid, that are linked by amino acids via peptide bonds (Paul and Clark 1989). Organic matter containing both these elements is therefore necessary for microbial biomass growth.

Carbon is present in the soil organic matter (SOM) in a variety of inorganic and organic forms. The latter, however, predominate and may include other elements like N and P. These organic compounds could occur as biomass C, root exudates, cellulose, lignin, chitin, or humus (Killham 1994). The biomass C is made up of soil microbes and animals (Killham 1994). This pool of C represents only 1-2% of the total organic C in soil. However, it is the backbone of the soil C-cycle and all other nutrient cycles (Killham 1994). Root exudates, a highly decomposable form of organic C (Paul and Clark 1989) represent less than 1% of total soil organic carbon, but provide an immediate substrate for many species of soil microbes (Killham 1994).

Cellulose and lignin (remnants of plant residues), chitin (remnants of soil animals and fungal residues), and soil humus (previously decomposed material) comprise 90% of the total soil organic C (Killham 1994). These compounds are highly resistant to decomposition due to their complex chemical nature. Decomposition follows a process whereby each compound is slowly broken down into smaller units, and then eventually into a form available for assimilation by the microbial biomass. Cellulose, for example, is broken down into glucose by specialized cellulytic saprophytes, such as the fungal species *Fusarium* and *Aspergillus*, or the bacterial species *Bacillus* and *Pseudomonas* (Killham 1994).

Lignins, having random structures and strong linkages, are even more resistant to decomposition than cellulose (Killham 1994). White rot fungi, such as *Coriolus versicolor* and *Phanerochaete chrysosporium* mediate the degradation of lignin into $CO_2$ and $H_2O$ (Paul and Clark 1989). Humus, the product of decomposition of fresher

material, has three constituents, humic acid, fulvic acid, and humin (Anderson and Schoenau 1993). Because each constituent is recalcitrant, humus is constantly present in the SOM and is continually being formed at the same time that it is being degraded (Alexander 1961).

The quality and availability of the substrate, as defined by the chemical composition of decomposing material, influences the microbial growth rate (Bosatta and Agren 1994). Thus, properties of the original litter together with soil physical factors are important in determining the amount of microbial biomass in the soil (Bosatta and Agren 1994). Bauhus *et al.* (1998) state that microbial biomass amounts are sensitive to changes in soil physical and chemical composition. For example, soils high in lignin residues can only support a low level of microbial activity. This is because lignin is relatively high in N and is a poor quality resource for soil microbes that require C (Brady and Weil 1996). Furthermore, Killham (1994) states that resource quality is described by C/N ratios; low C/N ratios indicate high litter quality and rapid rates of decomposition; high C/N ratios indicate poor litter quality and slow decomposition rates.

Litter inputs are basically from two sources - plant and animal residues. Plant litter may include any part of the plant from seed to root, and the decomposition rates of the various tissues vary according to the chemical structure involved (Haynes 1986). For example, the leaves and stems of a plant could contain up to 60% of N occurring as enzymes or proteins and 40% as free amino acid-N. The former require much time and energy for degradation, while the latter are water soluble and easily broken down (Haynes 1986).

Animal residues vary in components and turnover rates, just as plants do. Some important differences between animal and plant residues do exist, such as the presence of chitin in animal residues (Haynes 1986). Chitin, a contribution from the exoskeletons of arthropods and eggs of nematodes, is stable in soil (Haynes 1986). Furthermore, animals contribute urine and feces to the soil. Urine, containing 50-80% of N in the form of urea, is easily degraded following the soil process of hydrolysis to form ammonium ($NH_4^+$) which is readily immobilized by microbes and plants (Haynes 1986). Feces, in comparison, has a slower turnover rate due to its composition of highly resistant organic forms of N (Haynes 1986).

In addition, the type of organic matter in the soil is also dependent on the quality of foliage found in the ecosystem. For example, the soils of mixed-wood stands more often contain simple sugars, starches and cellulose (Rowell 1994) which are easily broken down and assimilated by microbes (Bradley and Fyles 1995). In contrast, the soil of coniferous stands includes more complex organic residues exhibiting slower turnover rates which in turn restricts microbial numbers (because of the poor quality and acidic soil conditions) thus slowing decomposition (Bradley and Fyles 1995). Furthermore, Bradley and Fyles (1995) state that previous studies have found that environmental factors, such as extremely low pH and reduced organic matter (OM) residues in coniferous stands, negatively influenced microbial activity and litter turnover rates. Also, as stand age increases the quality of organic matter inputs decreases, and this was reflected in declines in the microbial biomass carbon-to-soil organic carbon ratio and the microbial biomass nitrogen-to-soil organic nitrogen ratio found by Bauhus *et al.* (1998).

Assuming that sufficient C is available in the OM, it is appropriate to consider the presence and availability of macronutrients such as N and P. It is generally accepted that soil microbial populations under steady state conditions maintain an average C/N ratio of 8:1 (Rowell 1994) with the range being from 15:1 for some fungi to 3:1 for some bacteria (Paul and Clark 1989). Since only one-third of the carbon metabolized by microorganisms is assimilated (the rest is lost to respiration), substrates with a 24:1 C/N ratio could be considered ideal. Paul and Clark (1989) noted that OM inputs at 25:1 ratios result in no net mineralization or immobilization. Organic matter inputs in forest systems usually exceed this relatively low ratio. For example, leaves of oak trees have ratios of 65:1 while pine needles have ratios of 225:1 (Haynes 1986). When high C/N ratio residues are added to the soil, competition among microorganisms for scarce N resources restricts the rate of decomposition and limits the amount of inorganic N available for plant uptake. Complex chemical structures, such as lignins found in conifer needles, decompose very slowly as evidenced by low C oxidation and N mineralization rates (Brady and Weil 1996). Therefore, N may be limiting to microbial activity and numbers even when adequate C supplies are present (Brady and Weil 1996).

Gallardo and Schlesinger (1994) reported that P also has a limiting effect on microbial biomass. They found that P was less frequently immobilized than N by soil microorganisms in litter, causing an increase of N/P ratio in the litter. This ratio increase suggests that P and not N may be limiting in the F-H horizon. Phosphorous availability could also become limiting in the lower horizons through adsorption by Al and Fe sesquioxides (Gallardo and Schlesinger 1994).

The rate of OM decomposition is also dependent upon the various species of microbes present in the soil, and the timing of the OM inputs. Rapid turnover of biotic residues, for example, is mediated by zymogenous (bacilli and spore forming bacteria) soil microbes, which do not occur as a numerically significant component of the soil community under "normal" conditions, but proliferate when large amounts of organic residues are added into the soil (Killham 1994). The on-going, low level cycling of organic C is a function of autochthonous (coccoid bacteria) soil microbes (Killham 1994). These bacteria are most competitive at low substrate concentrations and persist actively in soil for long periods of time (Killham 1994). However, while bacteria alone may "stall" on complex substrates such as lignins, net mineralization has been noted when fungi are present (Paul and Clark 1989).

Timber harvesting can affect above- and below-ground aspects of the forest ecosystem. For example, the removal of a forest canopy will immediately affect annual organic matter inputs in the form of foliage and fine root mortality. One-time additions of coarse woody debris, such as large roots and branches, will drastically change the organic matter pools in the soil and at the forest floor (Chatarpaul *et al.* 1984; Hendrickson *et al.* 1985; Entry *et al.* 1986; Foster and Morrison 1987). Measured characteristics which support reported reductions in fungal and bacterial biomass include slowed rates of leaf and woody litter decay, excessive drying and high temperatures at the forest floor surface, increased $CO_2$ efflux and changes in soil N levels (Hendrickson *et al.* 1985). While the act of harvesting itself results in significant changes in the soil environment, there is evidence that harvesting intensity further influences the magnitude

of the change (Chatarpaul *et al.* 1984; Hendrickson *et al.* 1985; Entry *et al.* 1986; Foster and Morrison 1987). Thibodeau *et al.* (2000) investigated the effect of pre-commercial thinning on microbial populations in balsam fir stands in Quebec. They hypothesized that both the change in the soil temperatures and the input of foliage and branches would positively affect the microbial biomass immediately following treatment. In fact, no significant changes were noted in either $MB_C$ nor $MB_N$ in the mineral layer but a strong relationship was found between $MB_N$ and soil temperature.

In the Boreal forest of Canada, clear-cutting predominates. Most commonly, either a stems-only (also known as conventional) or full-tree technique is employed. In the former, the tree is processed at the stump leaving foliage and branches scattered throughout the site. In the latter, the tree is processed at roadside with foliage and branches being removed from the site. The chief difference in the two techniques lies in the amount and type of organic residue left in the stand. As of 1987, 65% of harvesting in Ontario's boreal forest was full-tree (Wiensczyk 1992).

Many authors have considered the question of nutrient capital depletion due to the removal of relatively nutrient rich foliage and small branches (for a summary see Wiensczyk 1992). The project upon which this thesis is based was established in response to concerns expressed about full-tree harvesting in the Class Environmental Assessment (Ontario Ministry of Environment 1994). In addition to the "balance-sheet" approach employed by many researchers, it is also necessary to consider potential changes in the microbiological system responsible for nutrient transformations.

Entry *et al.* (1986) observed that the bacterial biomass was significantly higher in a cutover where the organic residue was left on-site (*i.e.* stems-only) compared to treatments where organic residues were removed. Hendrickson *et al.* (1985) found that whole-tree harvesting resulted in decreased microbial biomass when compared to conventional harvest methods. The difference was attributed to a loss of residual OM in the former. Hendrickson *et al.* (1985) also reported that due to greater on-site traffic during the whole tree harvest, increased mixing of the forest floor with mineral soil resulted in a reduction of water-holding capacity and OM content of the soil, which implied a reduction in microbial activity.

After harvest, silvicultural treatments intended to promote the growth of crop tree species may also affect microbial populations. Ohtonen *et al.* (1992) found that intensive silvicultural activities (*e.g.*, scarification, fertilization and herbicide application) generally reduced microbial biomass on coniferous sites found in Central Ontario (Petawawa Research Forest). The modified scarification treatment alone, which removed the humus layer, caused a nutrient limitation as evidenced by a widening of the C/N ratio in the mineral soil (Ohtonen *et al.* 1992).

Bauhus *et al.* (1998) suggested that microbial biomass was influenced by soil texture and SOM quality. Microbial populations were found to be sensitive to changes in the soil chemistry and physical environment, and were negatively influenced by forest management practices. Management practices, such as harvesting and silviculture, could cause the microbial population to decline, and ultimately have a negative affect on OM turnover rates. Ohtonen *et al.* (1992) noted that the reduction of microbial biomass per

unit OM and the reduction of microbial biomass C in surface organic and mineral layers was indicative of a reduced capacity of the ecosystem to maintain its nutrient reservoir. This reduced nutrient reservoir results in slower decay rates because substrates containing readily mineralizable N were less available (Hendrickson *et al.* 1985).

## SOIL MOISTURE AND TEMPERATURE

The general consensus throughout the literature seems to be that moisture and temperature levels are key components in determining the nature and size of soil microbial populations. Soil bacteria require a water film for movement and can only remain active if there is suitable moisture in the soil, so that nutrients and waste products can diffuse in and out of the bacterial cell (Wong and Griffen 1976a & b). Thus, bacterial activity may increase or decrease as the water potential rises and falls, respectively. Changes in microbial activity can be estimated by soil respiration rates. According to Schlentner and Van Cleve (1985), soil respiration fluctuates as moisture or temperature changes, depending on which of the two parameters is most limiting at the time of measurement. At moisture contents less than 75%, by weight of soil, temperature increases had little effect on respiration, whereas at moisture contents of 100-250%, respiration increased with temperature. Alternatively, moisture levels had little influence on respiration when temperatures were below 5°C; however, at temperatures of 10-20°C, respiration increased with moisture changes.

Lundgren and Soderstom (1983) reported that in podzolic soils, seasonal changes in precipitation and moisture content exerted strong influences on soil bacteria numbers. Precipitation provides moisture and available nutrients to the soil, thus, immediately altering the soil environment. This increases the soil moisture content and the soil microbial biomass (Lundgren and Soderstom 1983). Evaporation, which is dependent on air temperature and humidity, may rapidly decrease this moisture content so that no detectable changes in soil bacteria populations can be found a few days after rainfall.

Alternately, Weber (1990) reported that rainfall events did not result in higher water contents or altered soil respiration rates of cut and burned aspen ecosystems at the Petawawa Research Forest. Furthermore, he indicated that temperature is more closely correlated to soil respiration, with a strong temperature control exerted over respiration patterns in both cut and burned treatments of aspen. Howard and Howard (1979) found that in hardwood stands, microbial numbers were not significantly correlated with moisture content. Moisture and temperature accounted for 5% and 64%, respectively, of the variation in soil respiration. Orchard and Cook's (1983) results correspond with those of Lundgren and Soderstom (1983), where they found a correlation between soil respiration (an indicator of microbial activity) with soil moisture content; as the soil moisture content decreased, respiration decreased, reflecting an estimated 10% reduction in microbial activity. Rapid increases in respiration rate immediately following rewetting of the soil indicated 1) the death of some organisms, and 2) that many microorganisms are capable of surviving water stress and resuming activity quickly in response to favourable changes in their environment. Orchard and Cook (1983) also suggested that it

was likely that an increase in activity, rather than in biomass, was responsible for increased respiration rates.

Berg *et al.* (1998) suggested that seasonal conditions might have a direct influence on the microbial biomass by inducing specific microbial community responses to soil moisture and temperature. For example, microbial biomass declines during periods with extreme climate conditions. They further speculated that seasonal effects on plant productivity and organic matter release also indirectly influence densities of soil fauna populations, and interactions between grazers and microflora.

Salonius (1983b) suggested that air drying of soils may lower species diversity, resulting in a significant reduction of the metabolic activity of the population (using a soil suspension method), as compared to that of an undried soil. Damage due to drying was found to be less in the H horizon than in the L and F horizons. Salonius (1983b) further recommended that if a soil is not to be studied immediately after sampling then it should be stored moist to maximize the amount of living microbial biomass.

Mixing of humus with mineral soil, as Salonius (1983a) reported, may also lead to increased soil temperatures, and enhanced organic matter decomposition. Clay colloids may act to buffer the soil environment against toxic accumulation of metabolic end products of the developing microbial population, thus allowing activity levels to be enhanced. However, this buffering effect may simply be more obvious in populations at temperatures of 20-40°C, as compared to less active populations at 10°C (Salonius 1983a). Anderson (1978) suggested that enhanced decomposition may be attributed to

increased microbial activity and species diversity resulting from the mixing of soil layers, which has subsequently created a greater diversity of micro-habitats.

**SOIL pH**

Any removal of forest vegetation may result in a change of soil pH, with subsequent consequences for the integrity of microbial functional groups, as well as for microbial processes (Gallardo and Schlesinger 1994). Gallardo and Schlesinger (1994) speculated that P limitations in soils with highly basic or acidic pH levels will affect the activity and nature of microorganisms present. Baath *et al.* (1995) suggested that pH may have the ability to alter other soil properties, such as the C/N ratio, which indirectly affect microbial community composition by restricting available nutrients. Staddon *et al.* (1996) reported that a change in soil pH results in a loss of species in microbial functional groups. They stated that cellulose decomposition, for example, is predominately mediated by filamentous fungi at conditions below pH 5.5, whereas other species of fungi and bacteria dominate at neutral to alkaline pH. The change in microbial functional groups, as environmental habitats change in soil pH and chemistry, may also limit the activity of the remaining members. Hendrickson *et al.* (1985) reported a significant pH increase (4.7 to 5.2) in the forest floor in a mixed-wood stand, after whole-tree harvesting. Foster and Morrison (1987) showed that forest removal using the full-tree method resulted in an acidifying effect of the forest soil and suggested that the incorporation of forest phytomass into the soil would have a neutralizing effect, thereby reducing the limiting effect of pH on microbial organisms.

Fungi tend to dominate the microbial community in acid forest soils (Anderson and Domsch 1975; Bewley and Parkinson 1985; Scheu and Parkinson 1994; Matthies *et al.* 1997). Matthies *et al.* (1997) reported that culturable fungi predominated over bacteria at a pH range of 2.2-6.5. Not surprisingly, they found that the bacterial populations in acidic forest soils were more tolerant of the ambient conditions than were bacteria from less acidic forest soils. It has also been reported that bacteria have predominated over fungi in acidic Boreal forest soils (Frostegard *et al.* 1993; Baath *et al.* 1995; Berg *et al.* 1998). After many pH-raising treatments, Baath *et al.* (1995) concluded that the fungal-to-bacterial biomass ratio remained fairly constant across a range of pH, suggesting that fungi and bacteria may not have varying pH optima. Further, Berg *et al.* (1998) noted that high N levels in soil may influence the shift of dominance from fungi to bacteria. They also hypothesized that high atmospheric N deposition may eventually lead to N saturation of the soil, which would impose a stress on fungal communities, and cause a decrease in their abundance and activity.

## METHODS OF DETERMINING MICROBIAL BIOMASS

Many methods have been developed and applied in the estimation of microbial biomass and activity. These approaches are: 1) direct and 2) indirect. Direct methods involve assays and measurements of the actual microbial biomass (Hartmann *et al.* 1997). Indirect methods estimate the size of the microbial biomass by measuring the metabolic activities of microbes (Hartmann *et al.* 1997). Direct techniques include: 1) chloroform fumigation incubation (CFI) (Jenkinson and Powlson 1976), and 2) chloroform

fumigation extraction (CFE) (Voroney *et al.* 1993). Indirect techniques include: 1) substrate induced respiration (SIR) (Sparling 1985), and 2) soil $CO_2$ evolution methods (Edwards 1982). The soil fumigation methods, as described by Jenkinson and Powlson (1976), assume that:

1. Carbon in dead organisms is more rapidly mineralized than that in living organisms.
2. Fumigation leads to a complete kill.
3. Death of organisms in the unfumigated soil is negligible compared with that in fumigated soil.
4. The only effect of soil fumigation is to kill the microbial biomass.
5. The fraction of dead biomass C mineralized over a given time period does not differ in different soils.

This method was originally developed for soils with a water holding capacity of 50-55% (Jenkinson and Powlson 1976), which may be limiting and problematic for soils outside

West 1988). Furthermore, the question of a correct "control" has plagued the methodology (Voroney 1985).

The CFE method, on the other hand, is reported to provide stable estimates of soil microbial biomass (Tate *et al.* 1988; Sparling and West 1988; Merckx *et al.* 1988; Vance *et al.* 1987). Chloroform fumigation extraction may be used on soils with low pH, high organic matter, and excessive water content (Inubushi *et al.* 1991). Further, CFE is not dependent on the physiological state of the soil microflora, suggesting that the dormant population may also be captured (Martens 1995). Feigl *et al.* (1995) concluded that the CFE method is more convenient and suitable for estimating both microbial C and N in the same extract, as compared to the CFI and SIR methods. Beck *et al.* (1997) also recommended CFE over CFI and SIR techniques with respect to forest soils. Martikainen and Palojarvi (1990) compared CFE to microscopic counting in ten forest soils with a range of pH (3.6-6.8) and organic C (2.6-36%), and concluded that CFE was better suited for all ten soils.

The SIR technique utilizes the physiological respiration response of soil organisms to substrate amendment to provide an estimate of soil microbial biomass C (Sparling 1985). Like the soil fumigation techniques, the SIR method also follows assumptions implicit in the estimation of microbial biomass. The SIR assumes that (Sparling 1985):

1. The response of different organisms to the method is reasonably constant.
2. The majority of the soil micro-biota will respond during the period of measurement.

3. Glucose is a suitable substrate to induce the maximal response of respiration.
4. The contribution to microbial C from non-glucose metabolizing organisms is insignificant or consistently low.

Unlike CFI, SIR can be applied to soils of low pH, and to leaf and forest floor materials (Sparling 1985). Also, very small soil samples can be analyzed and the relative contributions by bacteria and fungi to rhizosphere populations can be distinguished through the incorporation of inhibitors (Sparling 1985). A limitation is that SIR, like most other methods, requires calibration using another estimate of the microbial biomass (Feigl *et al.* 1995). Furthermore, SIR relies on the active soil population showing a respiratory response within a few hours after the addition of substrate. Therefore, the dormant population will not be captured by the assay (Feigl *et al.* 1995). Feigl *et al.* (1995) found SIR to be inappropriate for acidic soils with high clay content, in comparison to CFI and CFE methods. Ocio and Brookes (1990) suggested that the SIR response per unit of microbial biomass C may not be constant throughout the whole biomass range. It has also been reported that if the microorganisms are actively growing, SIR will overestimate the microbial biomass C (Sparling 1985).

Soil respiration, an indirect method, is defined as the sum total of all soil metabolic functions in which carbon dioxide is produced (Singh and Gupta 1977). It includes microbial, microfaunal, mycorrhizal, rhizospheral, and root respiration (Weber 1985). These components may be measured using dynamic or static procedures, both of which are forms of indirect sampling methods. Dynamic methods use an infrared gas analyzer (IRGA), whereby a sample of air of known composition is drawn over a known

area and the increase in $CO_2$ concentration is measured (Schlentner and Van Cleve 1985). Static techniques use alkali absorbents, like soda-lime, whereby an air-tight chamber covered with aluminum foil is inverted over an open tin can containing previously dried and weighed absorbent. After a measured period of time, the absorbent is removed and the amount of $CO_2$ absorbed is calculated (Edwards 1982). Pongracic *et al.* (1997) reported that estimates of soil $CO_2$ efflux using an IRGA were consistently greater than those calculated using soda-lime. However, explanations for the discrepancy between the two methods were not given.

The use and interpretation of soil respiration has historically been complicated by the difficulty of separating root respiration from microbial respiration. Attempts by various researchers, such as Singh and Gupta (1977) and Schlentner and Van Cleve (1985), have not resolved this problem. However, soil respiration remains a widely used method for assessing biological and metabolic activity (Reiners 1968; Schlentner and Van Cleve 1985; Weber 1985; Gordon *et al.* 1987; Weber 1990; Pongracic *et al.* 1997). As Weber (1990) stated, soil respiration measurements help to assess the metabolic activity of a site in relation to forest practices, thereby determining the degree of impact imposed by these practices on site productivity and recovery rates of ecosystem processes.

The usefulness of respiration measurements may be limited because of the occurrence of soil atmosphere alterations during sampling. This may affect the level of microbial activity (Prosser 1997) in that the gaseous phase in which the soil normally exists may be altered if there is a passage of gas over or through the soil. This could increase the mixing of the gases and ultimately change the concentration of the $O_2$ and

$CO_2$ , thereby resulting in a false measurement of microbial activity (Prosser 1997). Prosser (1997) suggested that since the microbial biomass is quite sensitive to the chemical and physical environment, and because the forest soil environment is considerably heterogeneous, it is difficult to measure microbial activity without error or bias.

Reiners (1968) speculated that soil respiration measurements did not capture the transfer of carbon compounds other than $CO_2$. While these losses may cause an under-estimation of the rate of energy release, the inclusion of tree root respiration may cause an over-estimation of activity rates. Schlentner and Van Cleve (1985), however, reported that static methods, such as soda-lime, are feasible approaches for estimating total soil respiration at remote field locations, such as forest sites.

## MICROORGANISM CULTURE AND IDENTIFICATION

Even though the fungal biomass exceeds that of bacteria, the latter will be the focus of this aspect of the study because bacteria act as excellent indicators of physicochemical conditions in the soil (Killham 1994) exhibiting higher sensitivity, over fungi, to changes in environmental parameters. Berg *et al.* (1998) reported that fungi were less susceptible to drying than bacteria, suggesting that spatial distribution patterns of bacteria may be predicted or determined by soil moisture. In addition, bacterial growth and activity are strictly limited by substrate quality (Killham 1994), and as such are indicators of how organic matter biomass removal influences the microbial biomass as a whole.

Microbial diversity encompasses a large array of taxonomic, physiological, and genetic characteristics, as well as the diversity of functional groups (Staddon *et al.* 1996). Difficulties occur in describing these attributes because current cultural methods for bacteria isolate no more than a small fraction of species (Staddon *et al.* 1996). This limitation is due to a variety of factors. For example, Staddon *et al.* (1996) described the effect of the association of *r*-strategist microbes (which grow well *in vitro*) with the K-strategist microbes (which are non-culturable) occurring in the same habitat. Drawbacks may also lie with the type of species of bacteria being cultured. For example, nitrifying bacteria are quite sensitive to techniques such as direct plating because organic materials introduced with the inoculum permit growth of heterotrophic contaminants (Schmidt and Belser 1982). Initial isolation of nitrifiers is difficult and, once isolated, these bacteria are slow growing in culture, sparse in yield, and susceptible to contamination (Schmidt and Belser 1982). Furthermore, bacteria occur in patches, which may be only a few cubic micrometres in volume, throughout soil (Coleman and Crossley 1996). Because bacteria are passive, they depend on episodic events such as rainfall or root growth for movement. Thus, bacterial distribution and abundance are difficult to estimate without a high variance about the mean (Coleman and Crossley 1996).

In order to isolate highly oxygen-sensitive anaerobic populations, specific cultivation techniques are required to avoid exposing the microbes to oxygen (Casida 1968). Bacteria which are parasitic on other bacteria may be present in the soil, but may not be demonstrated in isolations due to the lack of the presence of a suitable host (Casida 1968). Casida (1968) also suggested that much of the soil bacterial population

may exist in such a manner that antibiotics or other inhibitors in the soil stabilize their growth. Alexander (1961) advised that since the soil atmosphere contains such high levels of $CO_2$, the $CO_2$ level in the laboratory should be adjusted to reflect that found in the soil so that growth of soil microorganisms may occur under more natural conditions.

For these reasons, cultural methods tend to be biased in that only a minor fraction of the bacterial population may be available for characterization in pure cultures (Bakken 1997). Thus, the cultured isolates may or may not be representative of the bacterial species inhabiting the soil. Due to the diversity and variability of microbial communities found in the soil, classic taxonomic methodology does not always yield a clear identification (Bakken 1997). Sorheim *et al.* (1989) compared cultures of microbes growing on various nutrient media, and found only a partial overlap between populations growing on similar media. The Gram staining technique is used to identify between gram positive and gram negative bacteria based on cell wall characteristics. Gram negative bacteria belong to the family Enterobacteriaceae, which includes most bacteria that occur in the soil (Holt *et al.* 1994). The traditional method used in the identification of Gram negative bacteria is C substrate utilization (Palmieri *et al.* 1988). Such C sources may include glucose, xylose, mannitol, lactose, sucrose, maltose, fructose, galactose, mannose, rhamose, lysine, ornithine, arginine, phenylalanine, esculin, and gelatin (Palmieri *et al.* 1988). These bacteria will yield either a positive or a negative result in a pattern characteristic of a particular bacterial species. However, this approach may be impractical for assessments on diverse populations due to it being very labour intensive, time consuming, and expensive.

Modern automated systems, which are much less labour intensive and less expensive, were introduced originally and developed primarily for the identification of clinical isolates (Palmieri *et al.* 1988). Many of these commercial test systems have been modified to suit other situations, such as the identification of microorganisms isolated from soil. For example, the Biolog system has been used to determine activity patterns for assessing functional diversity of soil microorganisms (Zak *et al.* 1994). But, this system has limitations in that reactions are sensitive to inoculum densities, the selection of C sources is biased to clinical isolates, and it is unable to determine fungal activity (Zak *et al.* 1994).

Washington *et al.* (1971) evaluated the accuracy of another multitest micromethod system, Analytab, which is also used for the identification of soil Enterobacteriaceae. They found this system to be about 93% accurate after repeat testing with a heavier inoculum of those strains failing to ferment glucose initially (Washington *et al.* 1971). At the time, this system was the most complete commercially available test series for Enterobacteriaceae identification that provided an initial testing accuracy of 90%.

A study conducted by Robertson *et al.* (1976), revealed that identification kits, such as API 20E, have generally demonstrated satisfactory performance identifying clinical isolates when compared with traditional culturing methods. The authors concluded that the identification kits offered savings in both time and material costs, while allowing about the same rate of accuracy (exact percentage not stated). Furthermore, the study concluded that the kits offered, 1) improved quality control; 2) a

standardization of methods, which allowed the use of interpretative pattern directories; and 3) the application of these tests by less sophisticated operators (Robertson *et al.* 1976).

Palmieri *et al.* (1988), however, concluded that the API 20E system had limitations in the identification of various bacterial species in clinical trials. They stated that the API 20E system has deficient characterization of some test organisms, especially at the species level. This identification system was unable to distinguish, for example, between various *Pseudomonas* spp., and simply grouped them under the category of other *Pseudomonas* species (Palmieri *et al.* 1988). It was suggested that the API 20E system may be useful as a rapid screening method for preliminary characterization of various bacteria groups. The API 20E system has been employed successfully in the characterization and identification of bacteria isolates in other forest ecology studies, such as Mireku (1981) and Roy (1984). Nevertheless, whether traditional technique, or kit systems are used, there is no one technique ensures an accurate representation of all soil bacteria.

More recent techniques for assessing soil microbial biomass include phospholipid fatty acid (PLFA) profiles (Baath *et al.* 1995), fatty acid methyl ester (FAME) assays (Bailey *et al.* 1997), and deoxyribonucleic acid (DNA) extractions (Liesack *et al.* 1997). These methods are often used to determine the microbial population and community structure and activity via chemical means. The PLFA profiles use phospholipids, found in membranes of all living cells, as biomarkers (Morgan and Winstanley 1997). Phospholipids, comprising a constant proportion of the bacterial biomass, emit different

patterns for different subsets of microbial communities (Baath *et al.* 1995). This method is applicable to the study of mixed populations of varying degrees and complexity (Morgan and Winstanley 1997). The PLFA profiles can also be used to detect environmental changes, stress responses or periods of activity by tracking differences in lipid profiles of microbes (Morgan and Winstanley 1997).

The FAME assays are similar to PLFA's in that fatty acids are used to identify bacteria species rather than to estimate biomass (Bailey *et al.* 1997). The profiles are generated using extracted cellular fatty acid methyl esters that are assayed via gas/liquid chromatography (Bailey *et al.* 1997). A third technique uses DNA extraction. This entails direct cell lysis once separated from the soil matrix. The method retrieves up to 35% of the microbial biomass from the soil (Liesack *et al.* 1997). This technique, however, only selects for those microbes that have less affinity for soil particles (Liesack *et al.* 1997). Direct cell lysis, where the cells are first lysed in the soil then the DNA is extracted and purified, detects the overall genetic potential of the sample (Liesack *et al.* 1997). However, the method of lysis chosen is critical since this may influence the microbial biomass potential detected in the soil (Liesack *et al.* 1997).

Microbial diversity is not only demonstrated in the number of species contained within a community and in the number of functions they provide, but also in the number of rare habitats that they occupy (Staddon *et al.* 1996). Therefore, as Staddon *et al.* (1996) proposed, there may be a vast number of habitats yet unexplored, containing new species still unknown. Unless current culturing techniques are modified to include

methods that allow for these unknown species to be sampled, a clear understanding of microbial biodiversity may not be possible.

# METHODOLOGY

## SAMPLE SITE

The study site for the microbial biomass investigation is located about 80 km north of Thunder Bay, ON, to the west of highway 527 (Figure 1). The sample site consists of a 110 year old black spruce (*Picea mariana* [Mill.] B.S.P.) stand. The topography consists of gentle rolling hills, based on bedrock formed by morainal parent material. The site represents an ES20, spruce/pine feathermoss ecosite (Racey *et al.* 1996). The soil is classified as gleyed dystric brunisol with a very fresh moisture regime (3) (Sims *et al.* 1989). A description of the soil profile is provided in Table 1.

Harvested in the winter of 1994, four different biomass removal treatments were applied (Gordon *et al.* 1993), which included:

1. Tree-length harvesting system, where the slash is left near the stump.
2. Full-tree harvesting system, where the slash is removed to the roadside.
3. Whole-tree harvesting system, where the slash, duff, and stumps are removed to roadside.
4. Full-tree chipping system, where slash is removed to roadside, chipped and redistributed on cut blocks
5. Control, where the block remains uncut.

Only the tree-length, full-tree, and control treatments were considered for the purposes of the study reported here.

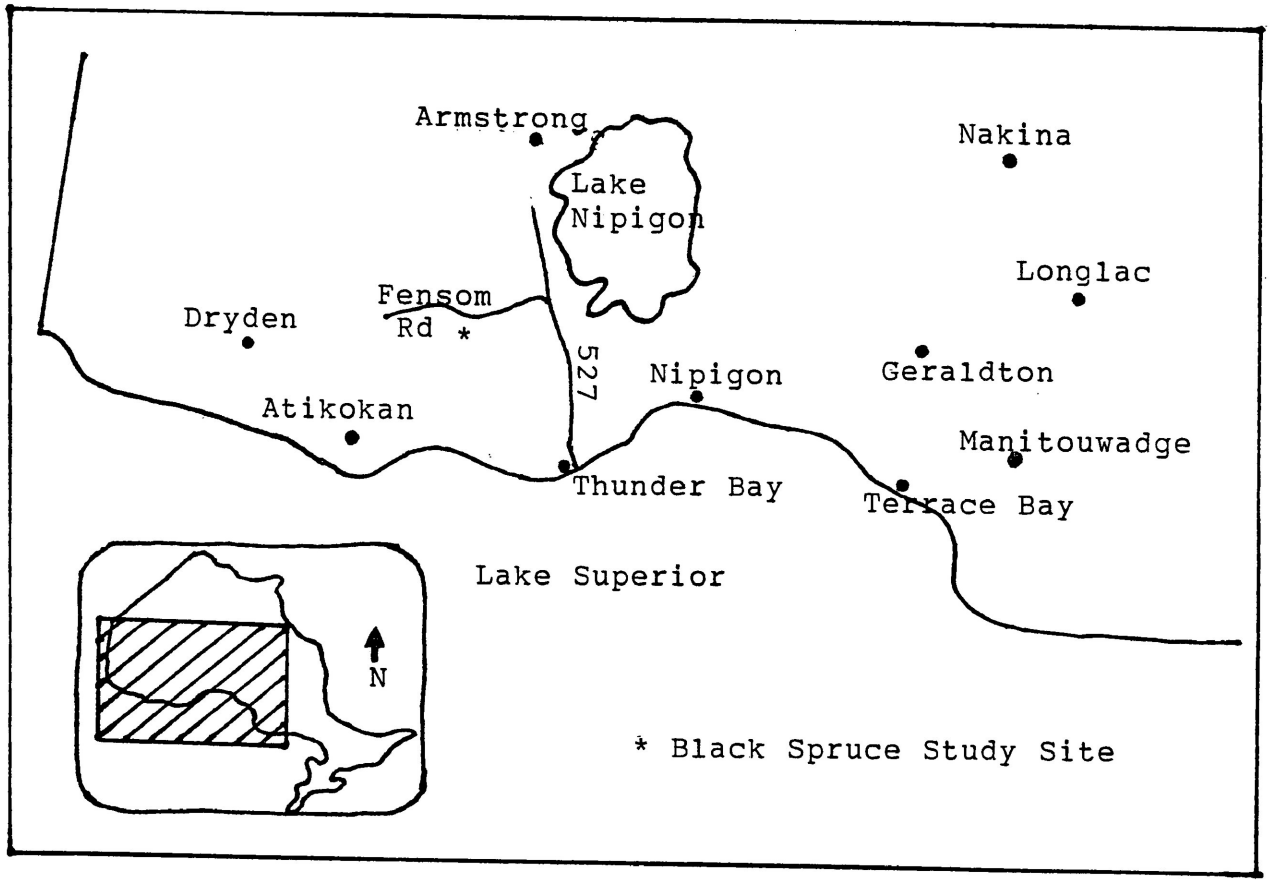


Figure 1. Map of northern Ontario identifying the black spruce study site location. (Not drawn to scale)

Table 1. Physical soil characteristics and classification of soil profiles associated with the black spruce study site.

| Horizon | Thickness (cm) | Soil Texture | Particle Distribution (%) | | | Soil Moisture | Soil Classification |
|---|---|---|---|---|---|---|---|
| | | | sand | silt | clay | | |
| F | 7 | | n/a | n/a | n/a | | |
| H | Discontinuous (<1 cm) | | | | | Very Fresh (3) | Gleyed Dystric Brunisol |
| Bm | 15 | Silty loam | 42 | 52 | 6 | | |
| Bcgj | 1 | Sandy loam | 46 | 49 | 5 | | |

Note: Coarse fragment content represented slightly over 32%, by volume, of the mineral soil profile.

## FIELD SAMPLING TECHNIQUES

Figure 2 demonstrates the layout of the treatment plots found on the study site. Originally, each plot ( 30 m x 30 m) was allocated a number from 1 to 12, and each plot was randomly allocated 1 of 4 different harvesting treatments, as specified earlier. The control plots (50 m x 50 m) were left untreated. Sampling for this study occurred once a month (at the end of the month), for four consecutive months, May, June, July, and August, in 1998.

Figure 3 presents the soil temperature and precipitation values for May through September of 1998. The temperature is averaged weekly, and the precipitation is event-based. Soil temperature, at a depth of 15 cm, was measured in the treatment plots. Precipitation was measured at a centrally located (on site) weather station. During the measurement period, the full-tree treatment displays consistently higher soil temperatures than do the tree-length or control treatments, with the control having the lowest temperatures. During the month of May, only 30 mm of rainfall was recorded. However, soil moisture content at this time was augmented by spring thaw and snow melt. June and August were relatively dry with only 42 mm and 55 mm of rain, respectively. July experienced the most amount of rain during the year of sampling (103 mm), with September following closely behind at 90 mm.

Samples were taken from nine plots, representing three each of the tree-length, full-tree and control treatments. These two harvest treatments were chosen because they are the most commonly used in the forest industry and represent a range of biomass removal options.

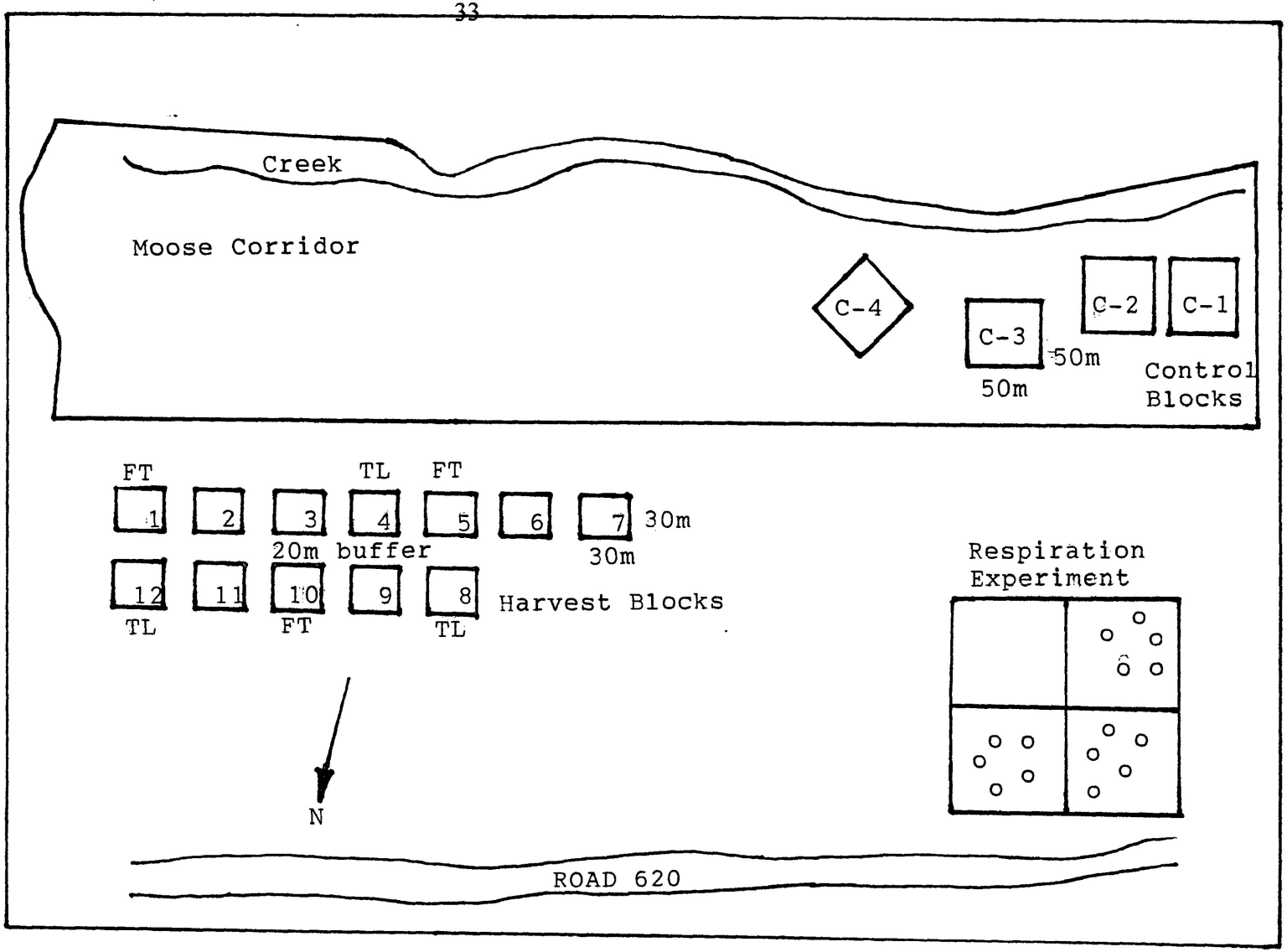


Figure 2. Map of the black spruce study site and treatment plot location. Plots sampled are marked as C (control, only 1 to 3), TL (tree-length, 4, 8, 12), and FT (full-tree, 1, 5, 10). The respiration experiment demonstrates the random allocation of the soda-lime tins on the plots.

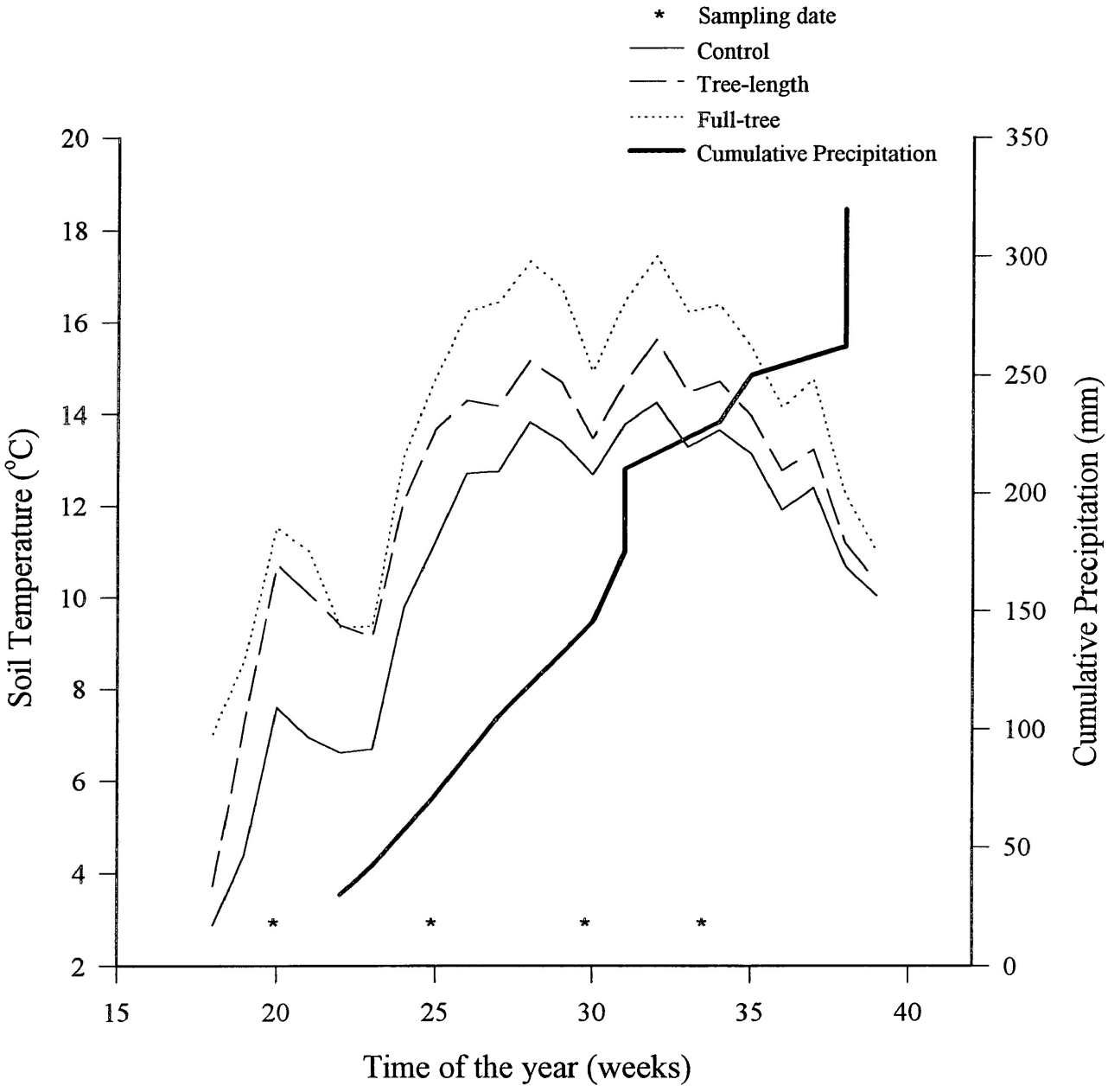


Figure 3. Average temperature and cumulative precipitation values associated with the black spruce study site for 1998.

Three sample points were obtained at random locations, in each plot. At each sample point, the litter (feather moss) layer was peeled back and two soil samples were taken using a trowel. One sample was taken from the upper organic layer, the F/H horizon, and the second sample was taken from the upper mineral layer, the $B_m$ horizon. Samples from each plot were bulked for each of the organic and mineral layers, so that there were nine organic and nine mineral samples taken each month. The samples were bulked in order to minimize the variability present within each plot. High spatial heterogeneity of chemical and physical properties of both the forest floor and soils has been recognized (Arp and Krause 1984) and bulking of samples is an attempt to reduce this variability. The samples were sealed in plastic bags and placed in frozen storage (-15°C) pending analysis.

## LABORATORY ANALYSIS

### Soil pH

The pH of each soil sample was measured following procedures outlined by Forster (1995a). The distilled water method was used in order to conserve soil which was dried and reused in a different test. Ten grams of air-dried and sieved (2mm) mineral soil was weighed into a glass beaker with 25 mL of distilled water (1:2.5 v/v). Five grams of organic soil was weighed into a glass beaker with 50 mL of distilled water added to the beaker (1:10 v/v). Each sample was stirred immediately for one minute and then allowed to stand for 30 minutes. After a second short stirring, the pH was measured with a pH meter (Orion 520A) and a glass electrode (Orion 91-57).

**Organic Matter Content**

The loss-on-ignition method described by Meyer and Vanson (1997) was used to determine the organic matter content of the soil sampled. The organic matter % of each sample was calculated as follows (Meyer and Vanson 1997): 1) In a crucible of known weight oven dried at 110°C, a 10 g sample of 2mm sieved soil was placed; 2) the sample was placed into a muffle furnace and the temperature was gradually raised to 600°C with the sample ignited for three hours; 3) samples were cooled in a desiccator, and reweighed to determine weight loss; 4) the organic matter content was calculated using the following formula:

organic matter content (%) = [(soil weight before - soil weight after) / soil weight before] * 100 Eq.1

**Water Content**

The gravimetric water content was measured following Forster (1995b). The procedure included weighing out 10 g of field-moist soil for each sample into an aluminium weighing tin, and the weight was recorded to the nearest 0.001g. The tins of soil were placed into a drying oven at 105°C for 24 hours. Upon removal, the tins were cooled in a desiccator, then reweighed. The per cent water content was calculated using the following formula:

% water content = [(moist weight - dry weight) / moist weight] x 100 Eq.2

**Total Nitrogen and Phosphorous**

A modified Micro-Kjedahl method was used to measure the total N and P in the soil samples. Original methodology follows steps outlined by Bremner and Mulvaney (1982). Soil was air dried at 65°C for 48 hours, ground by grinder, and sieved through a 2mm screen. The soil was placed in a container with an air-tight lid and labelled. For digestion, 1.0 g of soil was weighed and placed into a digestion tube. Two replications per sample were weighed out. One blank was included at the beginning and at the end of the digestion rack. Two or three boiling chips were added to each tube including the blanks and controls. Four mL of digestion solution (mixture of $H_2SO_4$ and salicylic acid) was added, and the tube was turned to wash down sides, then shaken gently. A rubber stopper was placed on all digestion tubes which were then left over night.

Stoppers were removed under the fumehood and one level scoop of crushed sodium thiosulphate was added to each tube. The digestion tubes were placed on a rack in the digester and placed along the sides were side plates. The digester was set to 350°C. After the sample tubes finished frothing, they were removed from the digester to a wooden stand and were allowed to cool for 5 minutes. One scoop of catalyst mixture (potassium sulfate, cupric sulfate, and selenium) was added to each tube and the tubes were returned to the digester at 400°C for 1 hour. Tubes were then removed from the digester and cooled for 10 minutes.

When cooled, 10 mL of distilled water was added. The samples were placed into the sonicator for several hours until the digest was dissolved. The rubber stoppers were removed from the tubes and distilled water was added to each sample until about 1 cm

from the top of the tube. The tubes were rubber stoppered again and inverted gently once or twice to mix the digest with the distilled water. An Autoanalyzer was used to measure the total nitrogen and phosphorus. Standards were used to establish a linear relationship for each element. Actual readings for each sample were then transformed using the line generated for the standards. This value, in ppm, could be calculated as a per cent using the following formula:

x = (y - a) / b, where a and b are calculated from a regression based on a series of standards;
y = reading in ppm from the Autoanalyzer;
x = ppm of nitrogen.
% N or P = [(ppm N (or P) x 0.075 L ) / weight of sample (mg)] x 100 Eq.3

Values were multiplied through by 1000 to reduce the number of decimal places, allowing for easier reporting and reading of figures.

**Culturing**

Culturing of bacteria followed methods outlined in Phillips *et al.* (1986). Twenty-five grams of fresh soil was placed in a 1 L graduated cylinder and distilled water was added so that the total volume was 250 mL. The suspension was then stirred and poured into a 1 L Erlenmeyr flask and shaken for 30 minutes. Using a sterilized pipette, 10 mL of the shaken suspension was drawn and transferred into a 90 mL sterile water blank. One mL of this diluted suspension was immediately transferred through successive 9 mL sterile water blanks, totalling six separate suspensions, each at various dilutions starting at 1:10 and ending with 1:1 000 000. Each dilution was shaken for a few seconds and was kept in motion while a sample was drawn into the pipette. Using the Eppendorf tip-ejector pipette 4700, 0.5 mL of each dilution was transferred aseptically to previously prepared

plates of Tryptic Soy agar medium (Anonymous 1996). Each plate was sealed with paraffin wax strips and incubated at 24°C for 48 hours. The bacteria colonies were then counted and described. Representative cultures were transferred to a separate plate of Tryptic Soy agar using the streak-plate method. The inoculated plates were incubated again at 24°C for an additional 36-48 hours.

**Identification**

Pure cultures were tested for Gram positive and Gram negative properties following the staining procedures outlined in Anonymous (1992). The identification of the isolated bacteria was done using a carbon substrate utilization system called API 20E (bioMerieux Vitek 1996). This system involved the following steps: 1) preparation of the inoculum, where a sterile loop of culture was mixed into a 5% saline solution; 2) preparation of the strips, where the holder was filled with sterile water and labelled; 3) inoculation of the strips, where each well was filled with the inoculation using a 5 mL pipette, and anaerobic tests were sealed with mineral oil; 4) incubation of the strips, where the strips were put into a dark chamber at 36 °C for 24 to 48 hours; 5) reading of the strips, where each well was identified by colour and designated a set number for that colour, leading to a nine digit number code; and 6) identification of microorganisms, where the number code is registered in a profile index, and each code is linked with a bacterial species (*i.e.* 0040024-10 *Pasteurella multocida*) and identified on a scale of poor to excellent probability.

**Soil Respiration**

Two respiration measurements were taken approximately one week apart, in September, using the soda-lime technique as described by Edwards (1982). One plot for each treatment was randomly selected. Each plot was further sub-divided into three sections (Figure 2); five tins of soda-lime were randomly placed on each sub-division (15 tins of soda-lime on each plot), plus one blank per plot. Within each pre-weighed tin, 35-40 grams of soda-lime was placed. The tins each have a diameter of 6.5 cm and a surface area of 33.18 $cm^2$, thus meeting the requirement for a minimum surface area (Edwards 1982). The tins of soda-lime were dried in an oven for 24 hours at 100°C and weighed before being placed on the sample sites. The tins were placed on the sites uncovered, and then a plastic tub (158.36 $cm^2$) covered in aluminium foil was placed over each tin. The foil helped to reflect the sunlight so that photosynthetic respiration was not falsely elevated. Also, the tins were placed such that a minimal amount of green material was in the area covered by the tub. The tins were allowed to stand for 24 hours, retrieved and capped. The blanks of soda-lime remained covered for this entire period. The soda-lime was again oven-dried for 24 hours at 100°C and reweighed to measure the amount of carbon dioxide evolved. Calculations followed the formula:

$CO_2$ evolved = (weight of sl. at time 2 - weight of sl. at time 1)/(# hours * area $m^2$) Eq.4

**Chloroform Fumigation-Extraction**

The method for determining microbial biomass carbon and nitrogen is described by Voroney *et al.* (1993). Ten grams of wet soil were oven-dried at 100°C for 24 hours to

calculate the moisture content. Twenty-five grams of fresh soil was weighed into a glass jar; four replicates per soil sample. Two replicates were fumigated with chloroform ($CHCl_3$) for 24 hours. The other two replicates of each set of four were immediately saturated with either 60 mL (organic samples) or 40 mL (mineral samples) of 0.5 M $K_2SO_4$. The replicates were shaken for one hour to allow for complete mixing of soil and $K_2SO_4$. The mixture was filtered through VWR glass fibre filter papers (grade.696), and the extracts were frozen at -10°C in plastic vials for later analysis. The fumigated samples, after repeated evacuation of the chloroform, were subject to the same treatment.

**Biomass N and Biomass C**

The extracts were analyzed for total N and C, using a colorimetric method (Anonymous 1978; 1984). The N containing compounds in the soil extracts were oxidized to nitrate by digestion in acidic and basic conditions with ultraviolet light. In order to avoid a suppression of N by C in the sample, the concentration of the potassium persulfate solution was increased from 1 to 3% (Luckai *et al.* in prep.). The nitrate was further reduced to nitrite by copper-hydrazine solution. The nitrite ion then reacted with sulfanilamide under acidic conditions to form a diazo compound, which then was coupled with N-(1-naphthyl)-ethylenediamine to form a reddish-purple colour. The intensity of the colour was measured at 520 nm.

In order to measure the total organic C, inorganic C (carbonate) was removed by entraining the acidified stream with a high velocity stream of N or C free air. The sample was transformed into a thin turbulent liquid film that was transported rapidly through a

large bore coil providing the necessary surface area for efficient $CO_2$ removal. At a purge rate of 500 mL per minute, up to 500 mg of inorganic C can be removed with minimal loss of volatiles. An aliquot of the carbonate free sample was air segmented, mixed with a stream of acid and potassium persulfate (4%) and subjected to UV radiation. The resultant $CO_2$ was dialyzed through a silicone rubber membrane and reacted with a weakly buffered phenolphthalein indicator. The decrease in colour of the indicator was proportional to the original C concentration. Calculations for biomass C and N followed the formulae outlined in Voroney *et al.* (1993):

1. Soil water content:

WS (%) = [ soil wet weight (g) - soil oven-dry weight (g) / soil oven-dry weight (g)]*100 Eq.5

2. Weight of soil sample (oven-dry weight equivalent) taken for microbial biomass measurements (MS):

MS (g) = [soil wet weight (g) * 100] / [100 + WS (%)] Eq.6

3. Total volume of solution in the extracted soil (VS):

VS (mL) = soil wet weight (g) - soil oven-dry weight (g) + extractant volume (mL) Eq.7

4. Total weight of extractable C and N in fumigated ($O_F$) and unfumigated ($O_{UF}$) soil samples:

$OC_F$, $OC_{UF}$ (g/ g soil) = extractable C (g/ mL) * [VS (mL) / MS (g)] Eq.8

$ON_F$, $ON_{UF}$ (g/ g soil) = extractable N (g/ mL) * [VS (mL) / MS (g)] Eq.9

5. Microbial biomass C and N in the soil (MB-C, MB-N):

a) MB-C (g/ g soil) = ( $OC_F$ - $OC_{UF}$) / $k_{EC}$ Eq.10

where: $k_{EC} = 0.25 \pm 0.05$ and represents the efficiency of extraction of microbial biomass C.

b) MB-N (g/ g soil) = ( $ON_F$ - $ON_{UF}$) / $k_{EN}$ Eq.11

where: $k_{EN} = 0.18 \pm 0.04$ and represents the efficiency of extraction of microbial biomass N.

## STATISTICAL ANALYSIS

### Overview

The variables of interest in this study which are subject to statistical analysis are microbial biomass carbon ($MB_C$) and nitrogen ($MB_N$), soil organic matter (OM), soil total nitrogen (N), soil total phosphorus (P), soil pH (pH), soil water content (WC), and soil respiration. The terminology and approach to the statistical analysis follows that of Zar (1996). Full data-sets can be found in Appendices I and II. The objective of the statistical analysis was to determine if the independent or fixed variables (*i.e.* harvesting treatment and time of sampling) affected the dependant or response variables. In all cases "α", or the probability of committing a Type I error, was set at 0.05.

The data were investigated carefully for outliers. Side-by-side dot plots were constructed to aid in the identification of any outliers. However, after a thorough search all datum points were left in the analysis because there was no just cause for elimination. For example, the pH value of one of the control plots in the organic soil horizon was measured at 3.12, which was much lower than all the other points measured. However, a pH of 3.12 is a reasonable value on a control plot that has a high input of acidic foliage. Therefore, this datum, as well as others like it, remain in the data sets.

In the case of the respiration data, an analysis of variance (ANOVA) was based on a two-way factorial, completely randomized design (see subsequent section for details). One-way analysis of variance (ANOVA) was employed to determine treatment effects, if any, on the soil descriptors (OM, N, P, pH, and WC). . When appropriate, the LSD Post

Hoc (Zar 1996) test for differences between treatment means was applied where the main effect (biomass removal) was found to be significant, and bar charts were constructed to demonstrate any differences. In an initial survey for variable response, $MB_C$ and $MB_N$ were also subject to two-way ANOVA; line graphs of $MB_C$ and $MB_N$ over time were used to identify trends and shed light on interaction effects.

As stated in the Literature Review, soil characteristics, such as pH, OM content and nutrient levels, are reported to 1) change in response to disturbance events and 2) appear to affect the structure and function of soil microbial communities. The investigative approach taken in this study therefore included a number of these variables in anticipation that the application of covariance analysis might assist in the interpretation of results through either 1) adjusting for sources of bias on the response variable or 2) throwing light on the nature of treatment effects in randomized experiments (Snedecor and Cochran 1967).

In order to explore and identify relationships between $MB_C$ and $MB_N$, and the soil descriptors, scatter plots were constructed and Pearson and Spearman correlation coefficients were computed. Both correlation tests compute a value between +1 and -1. The closer the value is to either extreme, the more highly correlated are the two parameters. The Spearman correlation differs from the Pearson in that it uses ranked data rather than absolute numbers. This reduces the effect of extreme data points (Zar 1996). Given that some soil descriptors exhibited correlation with either or both $MB_C$ and $MB_N$, the investigation of the data was then conducted using analysis of covariance (ANCOVA). Microbial biomass data are treated as a split-plot design with covariates, with the mineral

and organic layers separate. One difficulty associated with this approach and this data set is the resultant limited number of degrees of freedom, particularly when cases with unfilled cells were excluded from the ANCOVA.

All data were analyzed using DataDesk 6.0 software (Velleman 1997). Tests of normal variance were run on the data and they met a normal variance of distribution of means, as assumed by the Central Limit Theorem (Zar 1996). Expected mean square (EMS) tables were constructed to determine the appropriate tests for the null hypotheses, as well as to confirm the significance of the various response variables measured by the analysis of variance. Bar charts present treatment means and illustrate the results of the LSD Post Hoc tests.

**Soil Respiration**

As stated previously, the soil respiration data were subjected to a two-way analysis of variance with a completely randomized design (CRD). This design may be represented by the following equation:

$$Y_{ijkl} = \mu + T_i + D_j + TD_{ij} + \varepsilon_{(ij)k} \qquad \text{Eq 12.}$$

$$i = 1,2,3; \qquad j = 1,2; \qquad k = 1,2,\ldots,15$$

where

$Y_{ijk}$ = the carbon evolution of the $k^{th}$ tin of the $j^{th}$ sample date within the $i^{th}$ treatment.

$\mu$ = the overall mean.

$T_i$ = the fixed effect of the $i^{th}$ treatment.

$D_j$ = the fixed effect of the $j^{th}$ sample date.

$TD_{ij}$ = the fixed interaction effect of the $i^{th}$ treatment with the $j^{th}$ sample date.

$\varepsilon_{(ij)k}$ = the random effect of the $k^{th}$ tin in the $ij^{th}$ treatment combination, assumed IID $N(0, \sigma^2)$.

The treatment (T) factor represents three levels of biomass removal: control, tree-length, and full-tree. The sample date (D) is represented by two different sample dates on which the respired carbon was measured at different locations within the selected plots.

The expected means square (EMS) table (Table 2) will help to determine the test statistic for the following null hypotheses:

i. $H_o$: (T) = 0;

ii. $H_o$: (D) = 0;

iii. $H_o$: (TD) = 0.

Table 2. EMS table associated with Eq 12.

| Source | 3 F i | 2 F j | 15 R k | df | EMS |
|---|---|---|---|---|---|
| $T_i$ | 0 | 2 | 15 | 2 | $\sigma^2 + 30$ (T) |
| $D_j$ | 3 | 0 | 15 | 1 | σ2 + 45 (D) |
| $TD_{ij}$ | 0 | 0 | 15 | 2 | σ2 + 15 (TD) |
| $\varepsilon_{(ij)k}$ | 1 | 1 | 1 | 84 | σ2 |

**Microbial Biomass**

The analyses of the microbial biomass carbon and nitrogen in the organic and mineral soil horizons are represented by a two-factor analysis of covariance (ANCOVA) with the possible inclusion of up to five covariate variables (note: covariates were included only if the Pearson correlations were significant at $\alpha = 0.05$). This ANCOVA followed a split-plot design, whereby the harvest treatments were allocated to the main plots with 3 replicates and the month factor was allocated to the subplots. This experiment may be represented by the following linear model:

$$Y_{ijkl} = \mu + T_i + \omega_{(i)j} + \delta_{(ij)} + M_k + TM_{ik} + \omega M_{(i)jk} + \varepsilon_{(ijk)} \qquad \text{Eq 13.}$$

$$i = 1,2,3; \quad j = 1,2,3; \quad k = 1,2,3,4;$$

where

$Y_{ijk}$ = the microbial biomass of the soil sample for the $k^{th}$ month in the $j^{th}$ plot within the $i^{th}$ treatment.

$\mu$ = the overall mean.

$T_i$ = the fixed effect of the $i^{th}$ harvest treatment.

$\omega_{(i)}$ = the random effect of the $j^{th}$ plot within the $i^{th}$ harvest treatment. The $\omega_{(i)j}$'s are assumed to be IID N $(0, \sigma^2)$.

??$\delta_{(ij)}$ = the restriction error due to the restriction on the randomization of the 4 months within the $j^{th}$ plot within the $i^{th}$ harvest treatment. The $\delta_{(ij)}$'s are assumed to be IID N $(0, \sigma^2)$.

$M_k$ = the fixed effect of the $k^{th}$ month.

$TM_{ik}$ = the interaction effect of the $i^{th}$ harvest treatment with the $k^{th}$ month.

$\omega M_{(i)jk}$ = the interaction effect of the $k^{th}$ month with the $j^{th}$ plot within the $i^{th}$ harvest treatment.

$\varepsilon_{(ijk)}$ = due to bulking, there is no between sample variability of the $k^{th}$ month in the $j^{th}$ plot within the $i^{th}$ treatment.

The month (M) factor represents four points in time: May, June, July, and August. The treatment (T) factor is described by three levels of biomass removal: control, tree-length, and full-tree. Also, theoretically up to five covariates (pH, N, P, OM, and WC) could be included depending on the strength of their relationship to the dependent variable (measured with the Pearson Correlation test). The expected means square (EMS) table (Table 3) is used to determine the test statistic for the following null hypotheses:

i. $H_o$: (T) = 0;

ii. $H_o$: (M) = 0;

iii. $H_o$: (TM) = 0.

The table would include the covariate factors where appropriate.

Table 3. EMS table associated with Eq. 13.

| Source | 1 F i | 3 F j | 3 R k | 4 F l | df | EMS |
|---|---|---|---|---|---|---|
| $T_i$ | 1 | 0 | 3 | 4 | 2 | $\sigma^2 + 4\,\delta^2 + 4\,\omega^2 + 12\,\varphi$ (T) |
| $\omega_{(i)j}$ | 1 | 1 | 3 | 4 | 6 | $\sigma^2 + 4\,\delta^2 + 4\,\omega^2$ |
| $\delta_{(ij)}$ | 1 | 1 | 1 | 4 | 0 | $\sigma^2 + 4\,\delta^2$ |
| $M_k$ | 1 | 3 | 3 | 0 | 3 | $\sigma^2 + \omega_M^2 + 9\,\varphi$ (M) |
| $TM_{ik}$ | 1 | 0 | 3 | 0 | 6 | $\sigma^2 + \omega_M^2 + 3\,\varphi$ (TM) |
| $\omega M_{(i)jk}$ | 1 | 1 | 1 | 0 | 18* | $\sigma^2 + \omega_M^2$ |
| $\varepsilon_{(ijk)}$ | 1 | 1 | 1 | 1 | 0 | $\sigma^2$ |

*The degrees of freedom will vary depending on the number of covariate factors included in the analysis.

# RESULTS

## BACTERIOLOGY

The objective of the bacterial culturing was to determine if increasing levels of biomass removal would result in differences in the bacterial species isolated from the treated plots. Throughout the sampling season, the amount of cultures produced on the plates of solid media varied. Quantitatively speaking, in May a very high number of bacterial cultures were produced in each of the six dilutions up to 1:1 000 000. As the season of study progressed, the number of cultures gradually declined, and in August only a few cultures were produced in each of the first three dilutions, up to 1:1000.

Five different bacterial species were isolated from the soil collected from the sample sites: *Chryseomonas luteola, Pseudomonas fluorescens, Aeromonas salmonicida, Serratia marcescens,* and *Syntrophomonas multifilia* (Table 4). These species each occurred on all three treatment plots (control, tree-length, and full-tree), except for *P. fluorescens* which only occurred on the harvested plots. The number of colonies counted varied, however, a greater amount was generally observed from samples of the organic horizon compared to the mineral layer. Furthermore, species occurrence varied from month to month. For example, *S. multifilia* was found during June and July in the organic horizon, and during August in the mineral horizon. However, *C. luteola* was present throughout the entire season in both the organic and mineral horizons.

Table 4. A description and summary of the bacteria species isolated from organic and mineral soil samples, Summer 1998.

| SPECIES | DESCRIPTION | MONTH | RAW COLONY COUNT | | TREATMENT | API 20E READING |
|---|---|---|---|---|---|---|
| | | | ORGANIC SOIL | MINERAL SOIL | | |
| *Chryseomonas luteola* | Gram negative rods, 1µm long, 0.5µm wide; bright yellow, shiny, smooth, circular. | May<br>June<br>July<br>August | 652<br>417<br>114<br>103 | 379<br>206<br>209<br>109 | Control; Tree-length; Full-tree. | Good; Very Good |
| *Aeromonas salmonicida* | Gram negative rods, 1.5µm long, 0.5µm wide; pink, globular, smooth, shiny, circular. | May<br>June<br>July<br>August | 214<br>161<br>0<br>53 | 62<br>9<br>32<br>23 | Control; Tree-length; Full-tree. | Low Discrimination |
| *Serratia marcescens* | Gram negative rods, 1µm long, 0.25µm wide; white, globular, smooth, shiny, round. | May<br>June<br>July<br>August | 315<br>280<br>91<br>200 | 178<br>60<br>187<br>105 | Control; Tree-length; Full-tree. | Good; Very Good |
| *Syntrophomonas multifilia* | Gram negative rods, 1.25µm long, 0.5µm wide; pink, globular, shiny, scaley edges. | May<br>June<br>July<br>August | 0<br>245<br>200<br>0 | 0<br>0<br>0<br>209 | Control; Tree-length; Full-tree. | Good |
| *Pseudomonas fluorescens* | Gram negative rods, 3µm long, 0.75µm wide; beige, shiny, concentric circle pattern. | May<br>June<br>July<br>August | 0<br>0<br>138<br>0 | 0<br>0<br>0<br>0 | Tree-length; Full-tree. | Low Discrimination |

## SOIL RESPIRATION

Mean values for $CO_2$ respired (g $hr^{-1}$ $m^{-2}$) at locations in the control, tree-length and full-tree plots are summarized in Table 5. The values recorded range from 0.0069 to 0.0165 g $hr^{-1}$ $m^{-2}$. The control treatment had the highest mean for both sampling dates. Table 6 presents the results of the ANOVA for the respiration data.

Table 5. Summary of the amount of carbon respired (g $hr^{-1}$ $m^{-2}$) from the three treatments associated with the black spruce study site, Summer 1998.

| | C Efflux (Date 1) | | | C Efflux (Date 2) | | |
|---|---|---|---|---|---|---|
| Treatment | mean | max | min | mean | max | min |
| Control | 0.0123 | 0.0159 | 0.0094 | 0.0131 | 0.0165 | 0.0103 |
| Tree-Length | 0.0109 | 0.0139 | 0.0069 | 0.0114 | 0.0137 | 0.0074 |
| Full-Tree | 0.0101 | 0.0126 | 0.0069 | 0.0107 | 0.0133 | 0.0078 |

Date 1= September 5, 1998; Date 2= September 12, 1998.

Table 6. ANOVA table associated with $CO_2$ evolved (g $hr^{-1}$ $m^{-2}$).

| Source | df | Sums of Squares | F-ratio | Prob |
|---|---|---|---|---|
| Treatment (T) | 2 | 8.5 E - 05 | 12.867 | 0.000* |
| Sample Date (D) | 1 | 9.3 E - 06 | 2.8188 | 0.097 |
| TD | 2 | 2.7 E - 07 | 0.0412 | 0.959 |
| Error | 84 | 2.8 E - 04 | | |

* Significant at $\alpha = 0.05$.

The fixed effect of treatment (T) significantly affected the amount of carbon evolved. The raw data revealed a normal distribution. The LSD Post Hoc test revealed that the microbial activity (indexed by soil respiration) of plots in the uncut, mature stand differed significantly from those of the harvest treatments, even though soil temperatures were lower on the uncut areas (Figure 4). Neither the fixed effect of the sample date (D) nor the interaction (T x D) appeared to significantly affect the amount of carbon evolved.

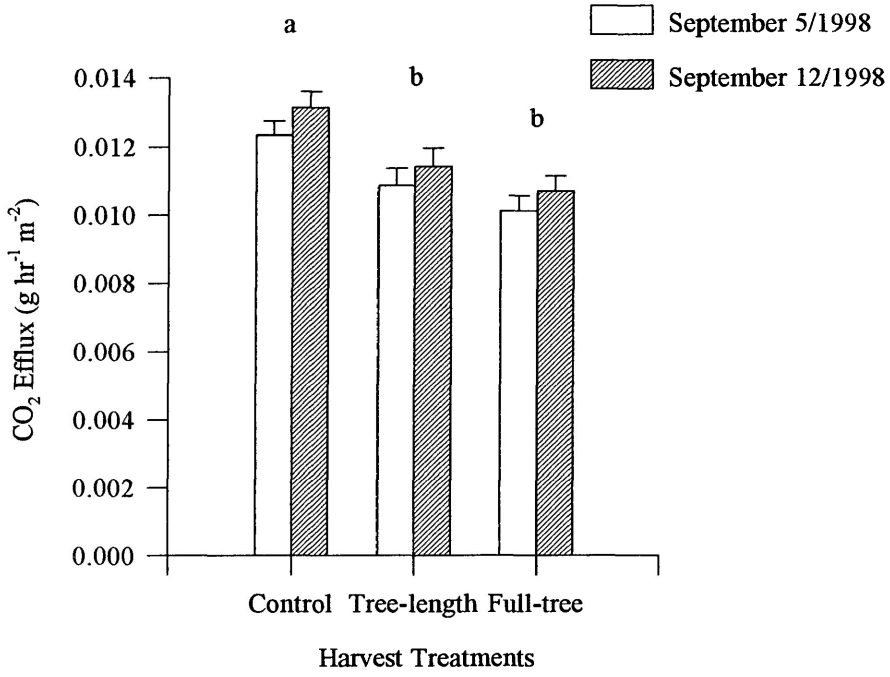


Figure 4. Results of the LSD Post Hoc test demonstrating similar and dissimilar harvest treatments, using mean $CO_2$ efflux (g $hr^{-1}$ $m^{-2}$) as the response variable. The letters on the bars represent which groups are similar to or different from each other.

## SOIL DESCRIPTORS

The soil descriptors measured in this experiment were pH, organic matter (OM), moisture content (WC), total P and total N. These parameters were measured in order to provide further insight into possible relationships with the microbial biomass. Mean treatment values within either the organic or mineral layers were similar (Table 7) however, one pattern did arise. Within the organic horizon, means for all descriptors except pH were highest for the tree-length treatment. With respect to pH, the range was from 3.12 (in a control sample) to 4.18 (in a full-tree sample). Ranges in the other parameters were relatively consistent from one treatment group to another with the exception of total N, where that of the full-tree treatment exceeded that of tree-length by a factor of 0.5 and that of the control by a factor of nearly three.

Within the mineral layer, no single treatment group exhibited consistently high or low values and ranges were, once again, very comparable with the exception of total N in the control group. Interestingly, with regard to pH, the lowest and highest values mirrored those found in the organic layer.

Table 7. Summary of the soil parameters measured in the organic and mineral soil horizons, summer 1998.

| Soil Parameter | Control | | | Tree-Length | | | Full-Tree | | |
|---|---|---|---|---|---|---|---|---|---|
| | mean | max | min | mean | max | min | mean | max | min |
| **Organic Horizon** | | | | | | | | | |
| pH | 3.47 | 3.93 | 3.12 | 3.92 | 4.02 | 3.84 | 4.09 | 4.18 | 3.98 |
| Organic Matter (%) | 82 | 95 | 69 | 84 | 91 | 77 | 78 | 93 | 59 |
| Moisture Content (%) | 65 | 70 | 55 | 74 | 77 | 71 | 71 | 76 | 61 |
| Total Phosphorus (mg/kg) | 151 | 161 | 144 | 159 | 181 | 150 | 149 | 161 | 133 |
| Total Nitrogen (mg/kg) | 1111 | 1138 | 1072 | 1178 | 1268 | 1139 | 1152 | 1232 | 1057 |
| **Mineral Horizon** | | | | | | | | | |
| pH | 3.73 | 4.34 | 3.38 | 3.83 | 4.18 | 3.7 | 4.04 | 4.26 | 3.8 |
| Organic Matter (%) | 13 | 20 | 8 | 11 | 13 | 9 | 12 | 16 | 8 |
| Moisture Content (%) | 31 | 42 | 20 | 36 | 38 | 34 | 34 | 40 | 28 |
| Total Phosphorus (mg/kg) | 32 | 36 | 30 | 32 | 38 | 26 | 33 | 36 | 28 |
| Total Nitrogen (mg/kg) | 167 | 225 | 118 | 135 | 141 | 121 | 136 | 142 | 121 |

One-way analysis of variance, where the treatment factor was tested against each of the five soil descriptors (pH, P, N, WC, and OM), was employed to determine if there were any effects due to the increasing levels of biomass removal. Table 8 presents a summary of the results. Only pH in the organic layer was significantly affected. The LSD test revealed that the control treatment mean was significantly lower than those of the harvest treatments (Figure 5). The one-way ANOVA employed on the mineral data (Table 8) revealed no significant effects of the treatment factor on any of the soil descriptors.

Table 8. One-way ANOVA table associated with the harvest treatment factor tested against the environmental parameters in the organic and mineral soil horizons.

| | Source | df | Sums of Squares | F-ratio | Prob. |
|---|---|---|---|---|---|
| **Organic Horizon** | | | | | |
| pH | Treatment | 2 | 1.3 E-7 | 4.110 | 0.029* |
| | Error | 25 | 3.9 E-7 | | |
| Nitrogen | Treatment | 2 | 54520 | 1.843 | 0.175 |
| | Error | 32 | 473222 | | |
| Phosphorus | Treatment | 2 | 788 | 0.662 | 0.523 |
| | Error | 31 | 18462 | | |
| Moisture Content | Treatment | 2 | 509 | 2.908 | 0.069 |
| | Error | 33 | 2886 | | |
| Organic Matter | Treatment | 2 | 119 | 0.387 | 0.683 |
| | Error | 24 | 3685 | | |
| **Mineral Horizon** | | | | | |
| pH | Treatment | 2 | 6.2 E-9 | 1.115 | 0.340 |
| | Error | 33 | 9.2 E-8 | | |
| Nitrogen | Treatment | 2 | 6058 | 1.016 | 0.373 |
| | Error | 33 | 98399 | | |
| Phosphorus | Treatment | 2 | 11 | 0.112 | 0.895 |
| | Error | 33 | 1649 | | |
| Moisture Content | Treatment | 2 | 129 | 0.232 | 0.794 |
| | Error | 33 | 9146 | | |
| Organic Matter | Treatment | 2 | 34 | 0.606 | 0.552 |
| | Error | 33 | 919 | | |

* Significant at $\alpha = 0.05$.

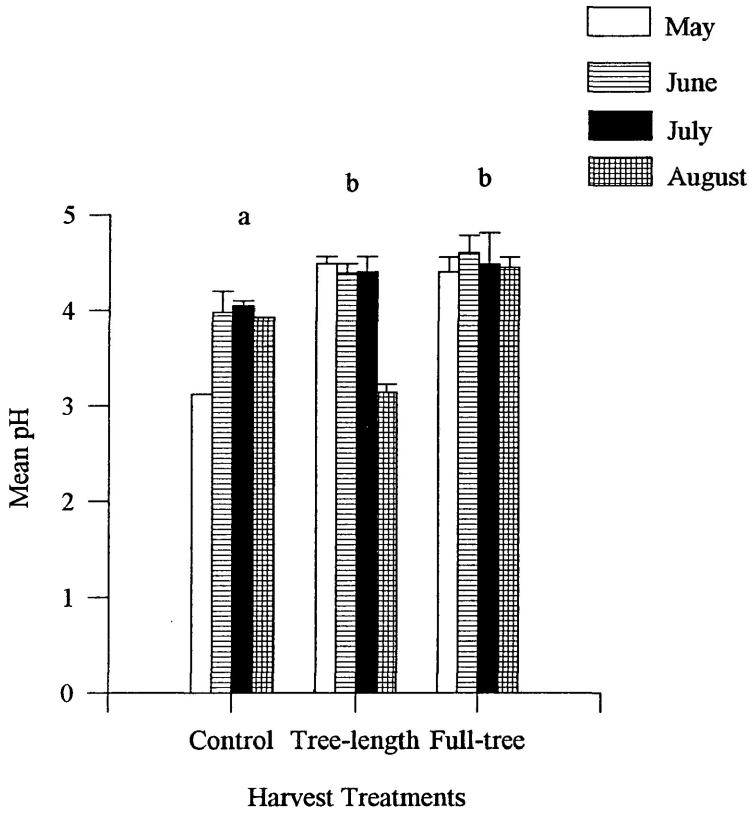


Figure 5. LSD Post Hoc test demonstrating similar and dissimilar harvest treatments in the organic soil horizon, using mean pH as the response variable. The letters on the bars represent which groups are similar to or different from each other.

## MICROBIAL BIOMASS

### Variability in Original Observations

Table 9 displays values for soil microbial biomass carbon and nitrogen (μg/g soil) in the organic and mineral soil horizons, respectively. The full data set can be found in Appendix I. A considerable numeric difference was observed between the microbial biomass carbon ($MB_C$) values of the organic and mineral soil layers regardless of the month. Biomass values for the organic layer range in the thousands (μg/g soil), whereas those of the mineral layer range in the hundreds (μg/g soil). Microbial biomass nitrogen ($MB_N$) follows a similar pattern, where the organic layer samples had much higher values than did those of the mineral layer. The C:N ratios (Table 9) are all generally within normal ranges for microbial biomass, that is 15:1 to 3:1 depending on the relative amount of fungi and bacteria (Paul and Clark 1989).

Results of the analyses of variance for the microbial biomass C are presented in Tables 10 and 11 (organic and mineral horizons, respectively). The ANOVA for the $MB_C$ in the organic layer revealed a significant treatment (T) factor, as well as the Treatment x Month interaction factor. In the mineral layer, the month and treatment main effects were found to significantly affect microbial biomass C.

Table 9: Summary of Microbial Biomass C and N for the Organic and Mineral Soil Horizon, summer 1998.

| Treatment | | May | | | June | | | July | | | August | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | $MB_C$ | $MB_N$ | C:N | $MB_C$ | $MB_N$ | C:N | $MB_C$ | $MB_N$ | C:N | $MB_C$ | $MB_N$ | C:N |
| **Organic Horizon** | | | | | | | | | | | | | |
| Control | Mean | 11120 | 1165 | 10.3 | 7785 | 1471 | 5.6 | 6821 | 985 | 7.1 | 5645 | 1181 | 5.6 |
| | SD | 3135 | 509 | | 629 | 435 | | 2007 | 269 | | 392 | 444 | |
| Tree-length | Mean | 5989 | 1381 | 4.6 | 5620 | 945 | 6.2 | 7599 | 1330 | 5.8 | 6323 | 1097 | 5.9 |
| | SD | 913 | 379 | | 1001 | 274 | | 624 | 269 | | 263 | 216 | |
| Full-tree | Mean | 7771 | 1458 | 5.4 | 5741 | 941 | 6.3 | 5665 | 1264 | 4.8 | 6112 | 1643 | 4.4 |
| | SD | 1112 | 334 | | 734 | 235 | | 1434 | 294 | | 833 | 775 | |
| **Mineral Horizon** | | | | | | | | | | | | | |
| Control | Mean | 1159 | 164 | 6.8 | 1337 | 158 | 8.6 | 611 | 54 | 11.5 | 320 | 53 | 6.8 |
| | SD | 588 | 70 | | 410 | 39 | | 119 | 17 | | 135 | 26 | |
| Tree-length | Mean | 441 | 84 | 5.4 | 355 | 78 | 4.9 | 600 | 96 | 6.6 | 336 | 42 | 7.8 |
| | SD | 79 | 11 | | 102 | 25 | | 303 | 30 | | 156 | 12 | |
| Full-tree | Mean | 861 | 147 | 9 | 404 | 141 | 3.1 | 410 | 83 | 5.5 | 240 | 55 | 4.6 |
| | SD | 219 | 118 | | 174 | 37 | | 65 | 29 | | 83 | 24 | |

Table 10. ANOVA table associated with the microbial biomass carbon in the organic soil horizon.

| Source | df | Sums of Squares | F-ratio | Prob. |
|---|---|---|---|---|
| Treatment (T) | 2 | 1.8 E + 7 | 6.0625 | 0.036* |
| Whole Plot Error (ω) | 6 | 9.1 E + 6 | no test | |
| Restriction Error (δ) | 0 | no est. | no test | |
| Month (M) | 3 | 1.3 E + 7 | 1.9697 | 0.156 |
| TM (TM) | 6 | 3.8 E + 7 | 2.9315 | 0.037* |
| ωM (ωM) | 17 | 3.7 E + 7 | no test | |
| Error | 0 | 0 | | |

* Significant at α = 0.05.

Table 11. ANOVA table associated with the microbial biomass carbon in the mineral soil horizon.

| Source | df | Sums of Squares | F-ratio | Prob. |
|---|---|---|---|---|
| Treatment (T) | 2 | 1.3 E + 6 | 20.011 | 0.002* |
| Whole Plot Error (ω) | 6 | 194645 | no test | |
| Restriction Error (δ) | 0 | no est. | no test | |
| Month (M) | 3 | 1.3 E + 6 | 5.0855 | 0.010* |
| TM (TM) | 6 | 1.4 E + 6 | 2.6236 | 0.053 |
| ωM (ωM) | 15 | 1.6 E + 6 | no test | |
| Error | 0 | 0 | | |

* Significant at α = 0.05.

The ANOVA for the $MB_N$ in the organic layer (Table 12) found no factors to be significant. In the mineral horizon (Table 13), however, the month factor was found to have a significant effect on the $MB_N$.

Table 12. ANOVA table associated with the microbial biomass nitrogen in the organic soil horizon.

| Source | df | Sums of Squares | F-ratio | Prob |
|---|---|---|---|---|
| Treatment (T) | 2 | 35454 | 0.0986 | 0.907 |
| Whole Plot Error (ω) | 6 | 1.1 E + 6 | no test | |
| Restriction Error (δ) | 0 | no est. | no test | |
| Month (M) | 3 | 175221 | 0.3441 | 0.793 |
| TM (TM) | 6 | 1.3 E + 6 | 1.2561 | 0.327 |
| ωM (ωM) | 15 | 2.9 E + 6 | no test | |
| Error | 0 | 0 | | |

Table 13. ANOVA table associated with the microbial biomass nitrogen in the mineral soil horizon.

| Source | df | Sums of Squares | F-ratio | Prob |
|---|---|---|---|---|
| Treatment (T) | 2 | 8093 | 3.5687 | 0.095 |
| Whole Plot Error (ω) | 6 | 6803 | no test | |
| Restriction Error (δ) | 0 | no est. | no test | |
| Month (M) | 3 | 41727 | 4.6240 | 0.014* |
| TM (TM) | 6 | 16248 | 0.9002 | 0.516 |
| ωM (ωM) | 15 | 54144 | no test | |
| Error | 0 | 0 | | |

* Significant at $\alpha = 0.05$.

Line graphs of the $MB_C$ (Figure 6) and $MB_N$ (Figure 7) were used to identify trends in the treatment means over time. These figures represent the unadjusted treatment means and any pattern illustrated is indicative of the sum of all environmental influences on the dependant variable. In Figure 6, it appears that biomass (as estimated by $MB_C$) declines from May through August and that these declines are most evident in the control samples. In both layers, control samples are clearly separated from harvest treatment samples in May and June, but achieve similar values in July and August. The pattern of increases and declines for tree-length is exactly the same in both the organic and mineral layers, while that for full-tree differs only in August.

In Figure 7, the $MB_N$ estimate of biomass varies widely in the organic layer as the season progresses. In the mineral layer, however, a general pattern of decline over the study period is evident. Initially, the full-tree treatment means are lower than the others, however, by July all three are displaying very similar amounts.

With the exception of a general decline from May to June, there is little similarity between the two estimators of microbial biomass.

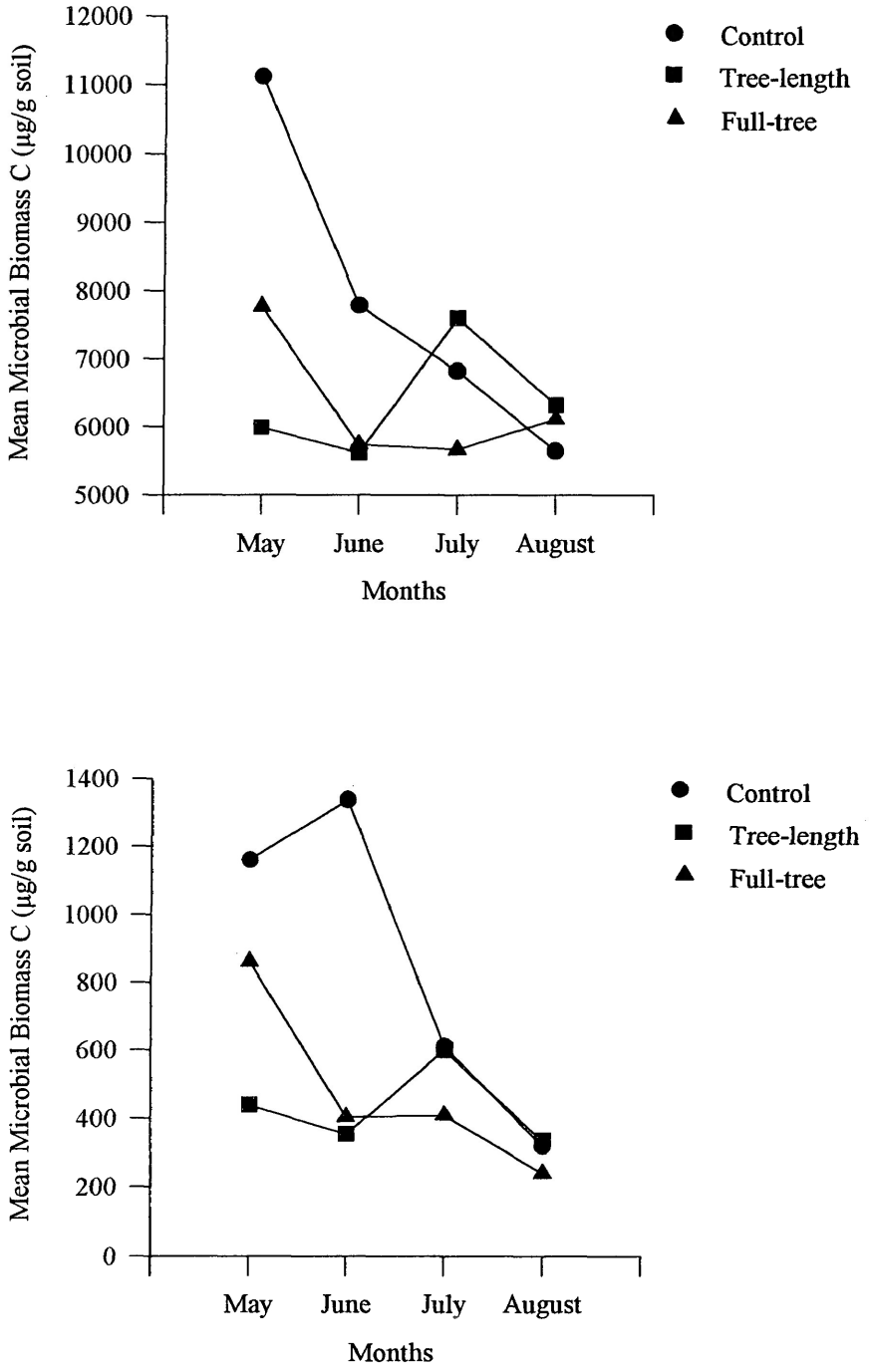


Figure 6. Mean $MB_C$ (µg/ g soil) throughout the sampling season, demonstrating interactions between harvest treatments influenced by soil descriptors: a) organic soil layer; b) mineral soil layer.

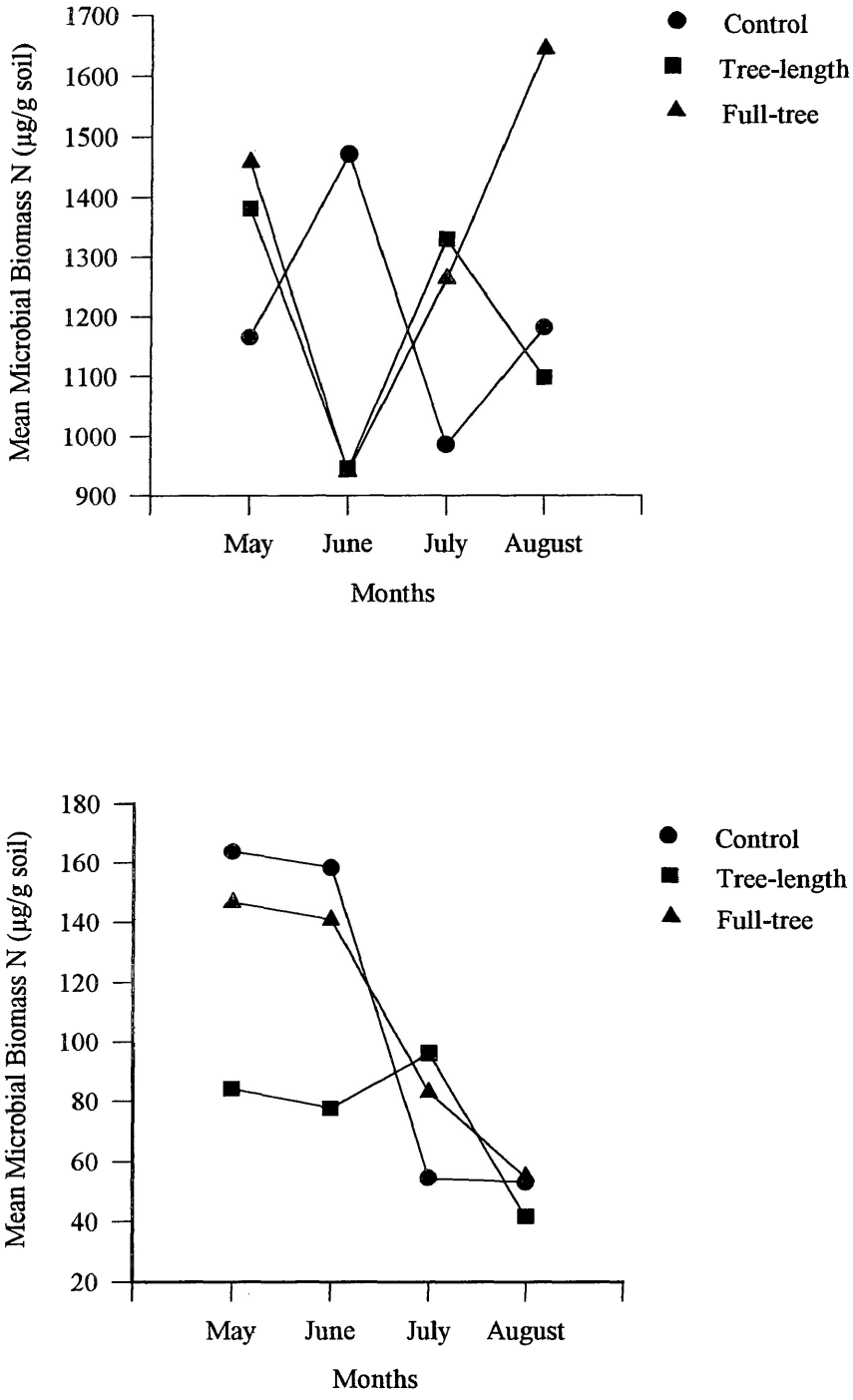


Figure 7. Mean $MB_N$ (µg/ g soil) throughout the sampling season, demonstrating interactions between harvest treatments influenced by soil descriptors: a) organic soil layer; b) mineral soil layer.

**Relationship Between Soil Descriptors and Microbial Biomass**

Scattergrams of the $MB_C$ and $MB_N$ against the soil descriptors demonstrate the association with each parameter (Figures 8 through 11). Comparisons between the organic and mineral layers must take scale into account. With the exception of pH, positive correlations between $MB_C$ and $MB_N$ and the soil descriptors are more apparent in the mineral layer.

Pearson and Spearman correlations were done to discover statistical relationships between the measured variables (Table 14). The pH and moisture content factors were found, by the Pearson correlation and not the Spearman, to have a significant association with the $MB_C$ in the organic soil layer. Total N, moisture content, and the OM content factors had a significant association with both the $MB_C$ and the $MB_N$ in the mineral soil horizon, as found by both the Pearson and Spearman correlations. Total N was found to be correlated with the $MB_N$ in the organic soil horizon. The Spearman correlation also found total P to be significant with $MB_N$ in the organic horizon.

Interestingly, and rather unexpectedly, the Pearson analysis revealed no correlation between $MB_C$ and $MB_N$ in the organic layer. However, Spearman's, which uses ranked, rather than absolute, values did identify this relationship as significant. The anticipated $MB_C$ to $MB_N$ relationship was identified by both tests in the mineral layer.

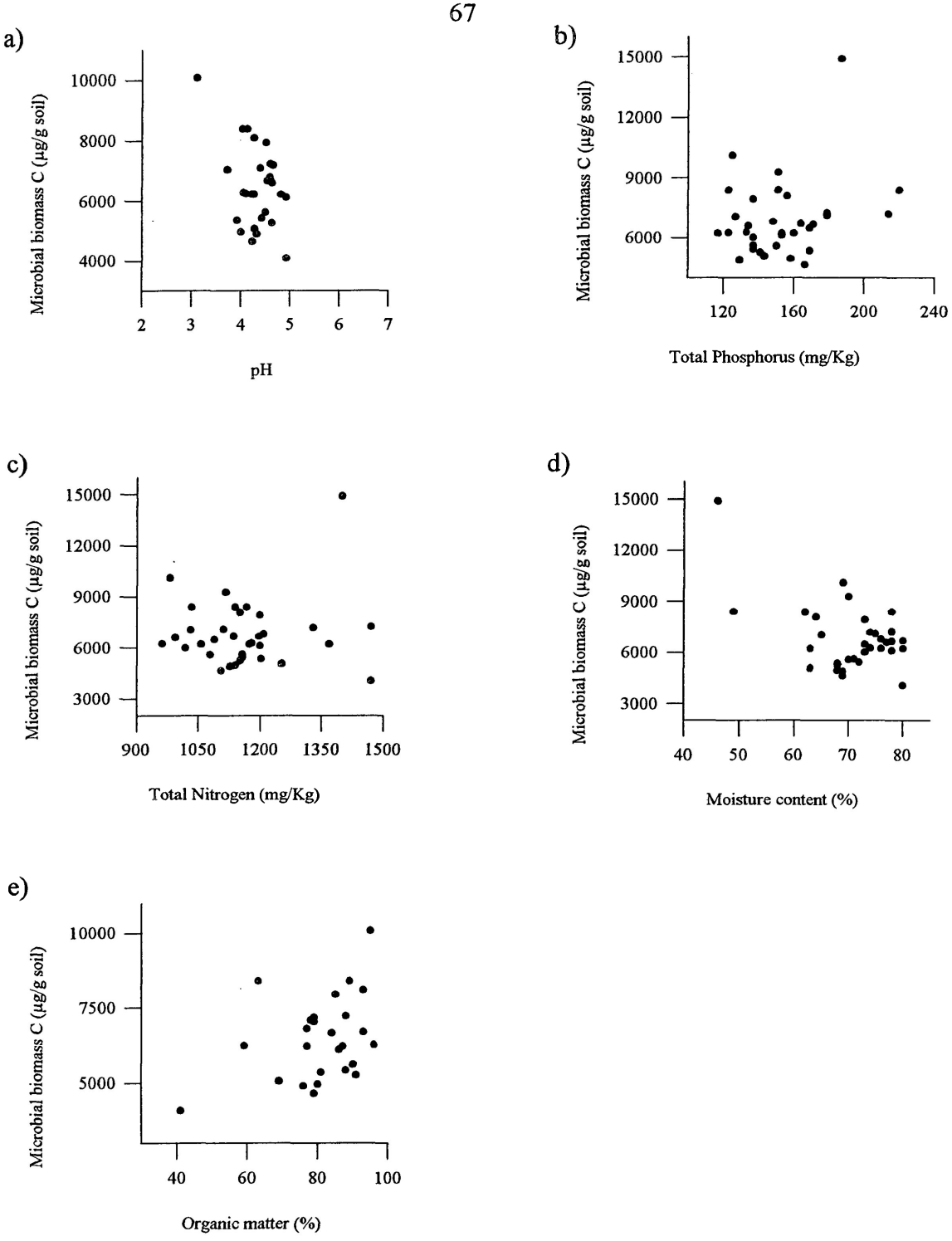


Figure 8. Scattergrams of the microbial biomass carbon with the soil description parameters in the organic soil horizon: a) pH; b) total P; c) total N; d) moisture content; and e) OM content.

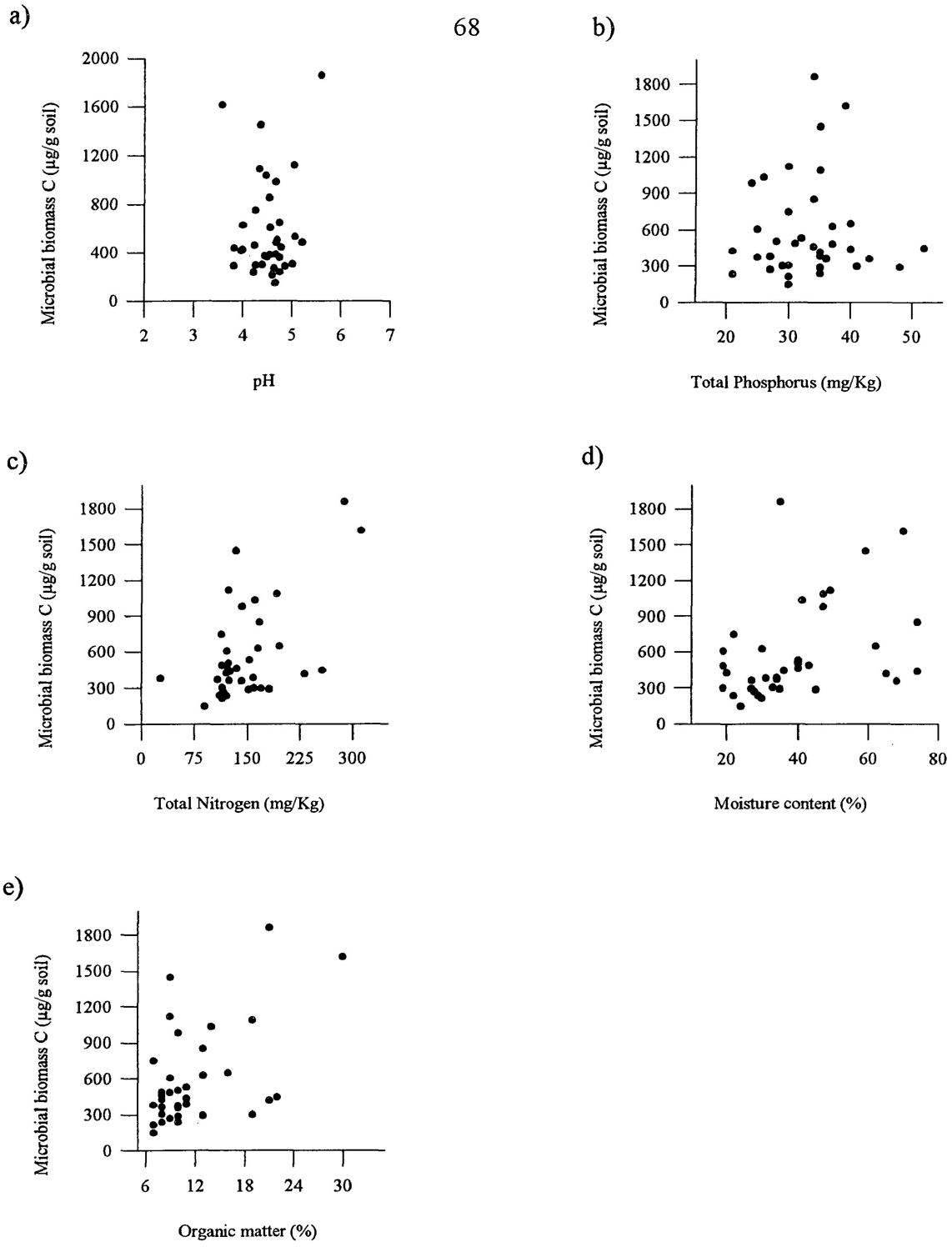


Figure 9. Scattergrams of the microbial biomass carbon with the soil description parameters in the mineral soil horizon: a) pH; b) total P; c) total N; d) moisture content; and e) OM content.

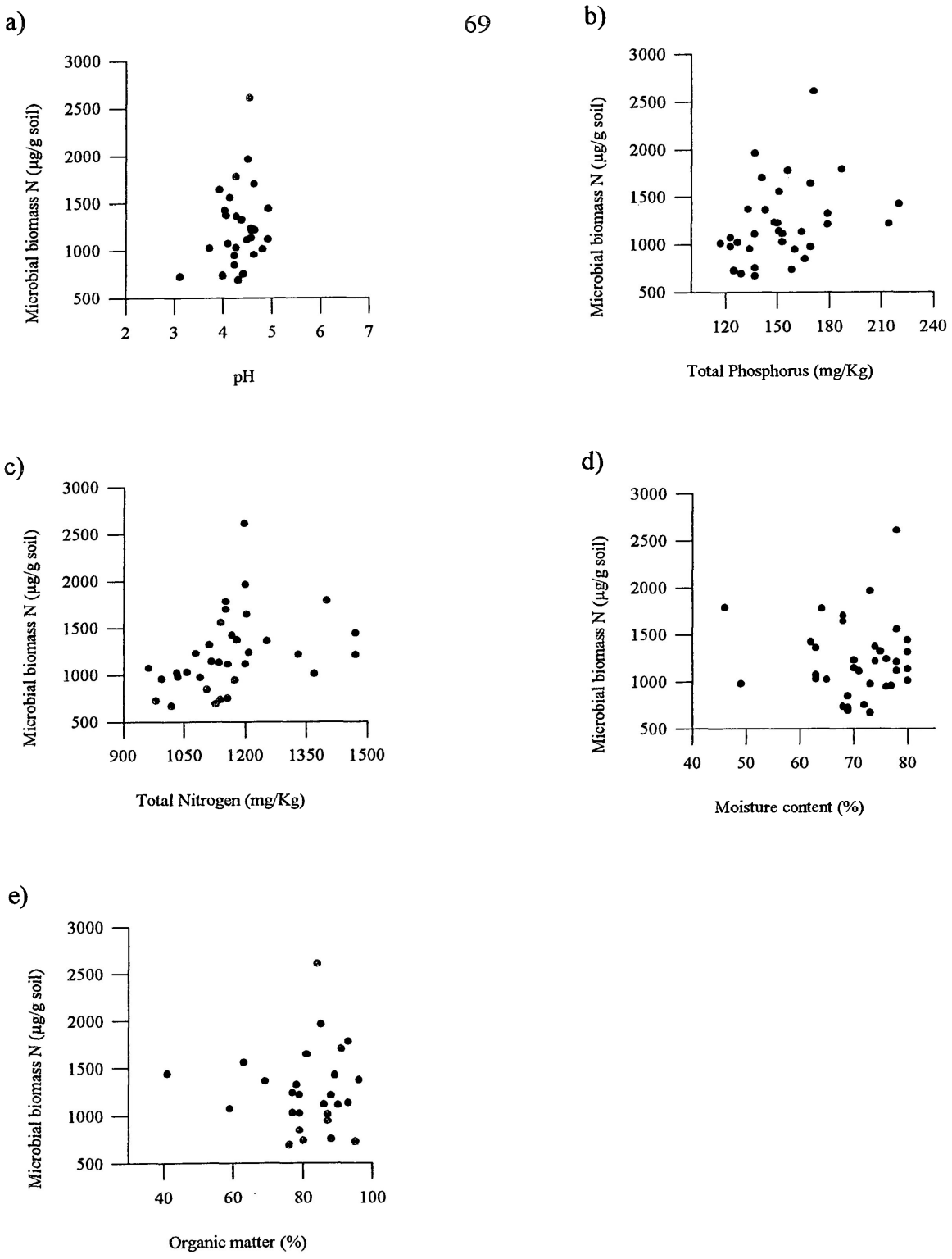


Figure 10. Scattergrams of the microbial biomass nitrogen with the soil description parameters in the Organic Soil Horizon: a) pH; b) total P; c) total N; d) moisture content; and e) OM content.

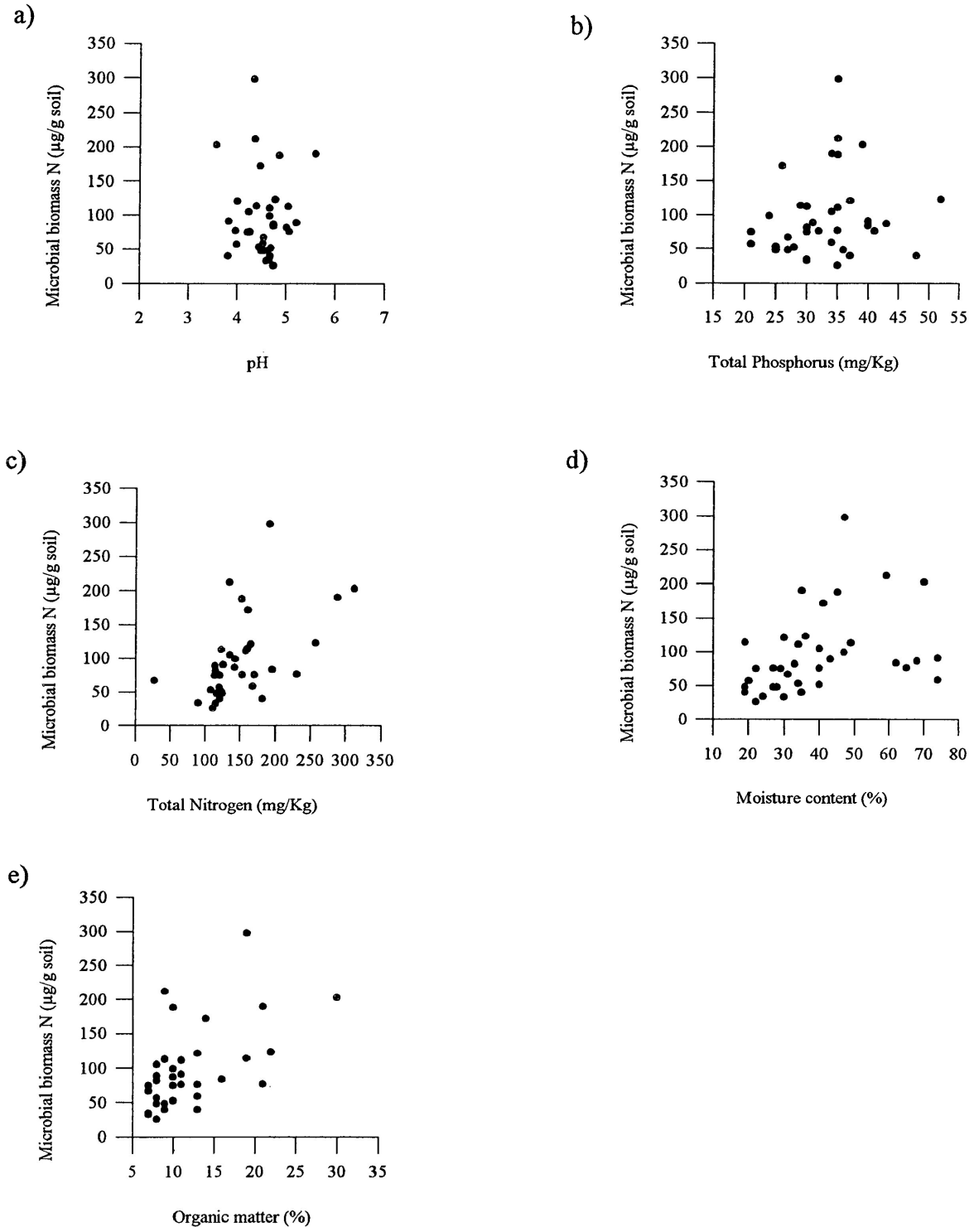


Figure 11. Scattergrams of the microbial biomass nitrogen with the soil description parameters in the Mineral Soil Horizon: a) pH; b) total P; c) total N; d) moisture content; and e) OM content.

Table 14. Results of the Pearson (lower triangle) and Spearman (upper triangle) Correlation analyses for the organic and mineral soil horizons.

| | $MB_C$ | $MB_N$ | WC | pH** | P | N | OM |
|---|---|---|---|---|---|---|---|
| **Organic Horizon** | | | | | | | |
| $MB_C$ | 1.000 | **.353*** | -.044 | .165 | .157 | -.065 | .288 |
| $MB_N$ | .258 | 1.000 | .193 | -.023 | **.349*** | **.407*** | -.067 |
| WC | **-.481*** | -.017 | 1.000 | **-.641*** | .040 | .243 | .055 |
| pH** | **.536*** | -.263 | -.250 | 1.000 | -.079 | **-.501*** | .031 |
| P | .247 | .323 | -.073 | -.225 | 1.000 | **.411*** | .004 |
| N | .078 | **.367*** | .101 | **-.403*** | **.448*** | 1.000 | .022 |
| OM | .367 | -.018 | -.032 | .223 | .074 | -.178 | 1.000 |
| **Mineral Horizon** | | | | | | | |
| $MB_C$ | 1.000 | **.584*** | **.468*** | -.017 | .037 | **.424*** | **.367*** |
| $MB_N$ | **.686*** | 1.000 | **.524*** | -.021 | .227 | **.598*** | **.582*** |
| WC | **.396*** | **.362*** | 1.000 | -.019 | .323 | **.476*** | **.470*** |
| pH** | .209 | .150 | .329 | 1.000 | .098 | .114 | .088 |
| P | .022 | .163 | **.348*** | .258 | 1.000 | **.557*** | **.427*** |
| N | **.554*** | **.525*** | **.389*** | **.415*** | **.519*** | 1.000 | **.913*** |
| OM | **.494*** | **.553*** | **.400*** | **.484*** | **.428*** | **.915*** | 1.000 |

* Correlation is significant at the 0.05 level (2-tailed).

**pH was calculated as hydronium ion concentration (M).

Note: Some data in the Organic Horizon was missing.

Total N was significantly correlated with many soil descriptors in both the organic and mineral soil horizons. In the case of total N and % OM, the relationship is so strong as to suggest the inclusion of only one or the other as a covariate.

**Variability in Microbial Biomass Adjusted for Covariates**

Summarized in Table 15 is the ANCOVA for the $MB_C$ in the organic soil horizon, revealing that the interaction of the treatment and month significantly affected the response variable. The ANCOVA results for the $MB_C$ in the mineral horizon are summarized in Table 16. This analysis showed that the treatment (T) significantly affected $MB_C$. The LSD Post Hoc test found the control treatment to be significantly different from both of the harvest treatments, tree-length and full-tree (Figure 12). Because total N was independently correlated with OM content, an ANCOVA was run without the total nitrogen covariate factor. This did not result in any significant differences from the original ANCOVA, therefore, only the original analysis is presented.

Table 15. ANCOVA table associated with the microbial biomass carbon in the organic soil horizon.

| Source | df | Sums of Squares | F-ratio | Prob. |
|---|---|---|---|---|
| pH (pH) | 1 | 3924 | no test | |
| Moisture Content (WC) | 1 | 155827 | no test | |
| Treatment (T) | 2 | 76222 | 0.0405 | 0.961 |
| Whole Plot Error (ω) | 6 | 5.6 E + 6 | no test | |
| Restriction Error (δ) | 0 | no est. | no test | |
| Month (M) | 3 | 207285 | 0.1303 | 0.939 |
| TM (TM) | 6 | 1.1 E + 7 | 3.6322 | 0.048* |
| ωM (ωM) | 8 | 4.2 E + 6 | no test | |
| Error | 0 | 0 | | |

* Significant at $\alpha = 0.05$.

Table 16. ANCOVA table associated with the microbial biomass carbon in the mineral soil horizon.

| Source | df | Sums of Squares | F-ratio | Prob. |
|---|---|---|---|---|
| Total Nitrogen (N) | 1 | 89050 | no test | |
| Moisture Content (WC) | 1 | 33172 | no test | |
| Organic Matter (OM) | 1 | 96716 | no test | |
| Treatment (T) | 2 | 527640 | 5.5325 | 0.043* |
| Whole Plot Error (ω) | 6 | 286111 | no test | |
| Restriction Error (δ) | 0 | no est. | no test | |
| Month (M) | 3 | 16141 | 0.0608 | 0.979 |
| TM (TM) | 6 | 606084 | 1.1416 | 0.386 |
| ωM (ωM) | 15 | 1.3 E + 6 | no test | |
| Error | 0 | 0 | | |

* Significant at $\alpha = 0.05$.

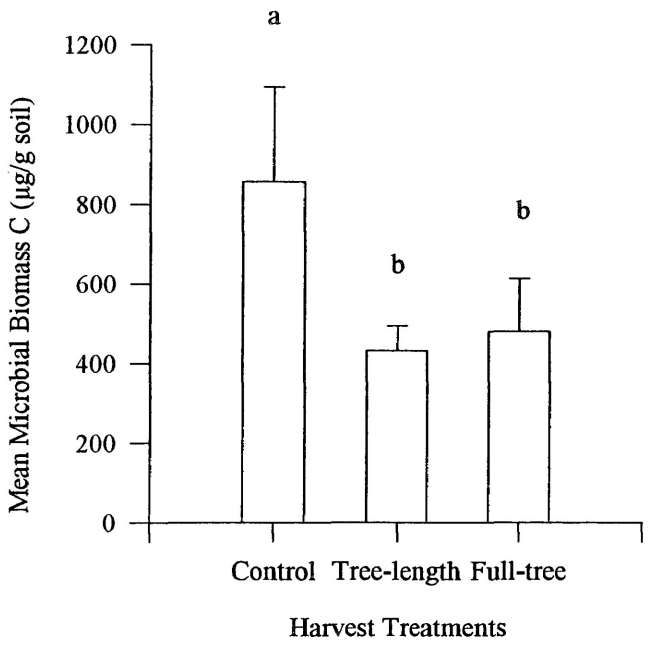


Figure 12. LSD Post Hoc tests demonstrating similar and dissimilar harvest treatments, using mean $MB_C$ (µg/ g soil) as the response variable in the mineral soil horizon. The letters on the bars represent which groups are similar to or different from each other.

The ANCOVA results for microbial biomass N in the organic soil horizon, summarized in Table 17, show that neither the factors nor their interaction influence the response. The ANCOVA was run with only the total nitrogen covariate factor in attempt to increase the degrees of freedom. This was done because the total N and the organic matter were highly correlated with one another according to the Pearson and Spearman correlation tests. The ANCOVA results for $MB_N$ in the mineral layer, summarized in Table 18, again revealed that no factors were found to be significant.

Table 17. ANCOVA table associated with the microbial biomass nitrogen in the organic soil horizon.

| Source | df | Sums of Squares | F-ratio | Prob |
|---|---|---|---|---|
| Total Nitrogen (N) | 1 | 39762 | no test | |
| Treatment (T) | 2 | 112421 | 0.6634 | 0.549 |
| Whole Plot Error (ω) | 6 | 508400 | no test | |
| Restriction Error (δ) | 0 | no est. | no test | |
| Month (M) | 3 | 100170 | 0.2351 | 0.870 |
| TM (TM) | 6 | 1.3 E + 6 | 1.4930 | 0.246 |
| ωM (ωM) | 15 | 2.1 E + 6 | no test | |
| Error | 0 | 0 | | |

Table 18. ANCOVA table associated with the microbial biomass nitrogen in the mineral soil horizon.

| Source | df | Sums of Squares | F-ratio | Prob |
|---|---|---|---|---|
| Total Nitrogen (N) | 1 | 4133 | no test | |
| Moisture Content (WC) | 1 | 2470 | no test | |
| Organic Matter (OM) | 1 | 4496 | no test | |
| Treatment (T) | 2 | 1326 | 0.5267 | 0.615 |
| Whole Plot Error (ω) | 6 | 7556 | no test | |
| Restriction Error (δ) | 0 | no est. | no test | |
| Month (M) | 3 | 1932 | 0.1988 | 0.895 |
| TM (TM) | 6 | 5348 | 0.2751 | 0.940 |
| ωM (ωM) | 15 | 48604 | no test | |
| Error | 0 | 0 | | |

# DISCUSSION

## BACTERIOLOGY

The culturing, isolation and identification of bacteria was undertaken to determine if different levels of biomass removal affected the number and type of bacterial species found in the treatment plots. In this investigation, the number of bacteria cultured declined as the sampling season progressed. This observation may be attributed to the soil temperature and moisture differences measured throughout the season. In the spring, warm temperatures and high amounts of moisture due to snow-melt (see Figure 3), appeared to stimulate microbial activity. Lundgren and Soderstom (1983) and Schlentner and VanCleve (1985) also attributed increased microbial activity to increased temperatures and moisture. As the climate continued to become warmer and drier, the number of cultures declined. A summary and description for each of the five bacterial species identified follows below.

*Chryseomonas luteola* was isolated on a number of occasions from the organic and mineral soil collected from all three treatment groups in this study, therefore, occurring in the general environment. *Chryseomonas luteola* is described in Bergey's Manual (Holt *et al.* 1994) as not being known to be present in the general environment but are saprophytes or commensals of humans. This discrepancy may be due to the limitations of the API 20E system to identify genetically similar strains (Palmieri *et al.* 1988). In a study by Anzai *et al.* (1997), it was found that *C. luteola* has a 93.9% sequence homology of 16S rRNA with *Pseudomonas* spp. Thus, Anzai *et al.* (1997) concluded that *Chryseomonas* is a junior subjective synonym of *Pseudomonas spp.* which are widely distributed in nature

(Holt *et al.* 1994). It is possible that what API 20E identified as *C. luteola,* is a subspecies of *Pseudomonas* thus explaining its high occurrence of in our soil.

Identified with a rating of "low discrimination", meaning that it may have been misidentified, *P. fluorescens* was cultured from only the harvested plots during the month of July. *P. fluorescens* is an aerobic microorganism with a respiratory type of metabolism (Holt *et al.* 1994) that functions in the synthesis of urease and in the process of denitrification (Killham 1994). Furthermore, *P. fluorescens* decomposes pure proteins with the formation of end products such as ammonia (Holt *et al.* 1994). *P. fluorescens* also functions as an antagonist, providing effective biocontrol of pathogens, such as *Gaeeumannomyces graminis* (take-all root disease) and *Pythium ultimum* (damping-off fungi) (Whipps 1997). Since it is commonly found in a number of niches, the low frequency of *P. fluorescens* observed here may be due to the limitations of culturing techniques as well as the possibility for misidentification by the API 20E system (Palmieri *et al.* 1988).

*Aeromonas salmonicida* was also identified with a "low discrimination" rating. Occurring on all three treatment plots throughout the entire sampling season, *A. salmonicida* is a facultative anaerobe that reduces nitrates and nitrites in the environment (Holt *et al.* 1994). This bacteria is chemolithotrophic (has both respiratory and fermentative types of metabolism); grows well at 22-28°C; occurs in fresh water and sewage; and may be pathogenic to frogs, fish, and humans (Holt *et al.* 1994).

Isolated throughout the entire sampling season from all three treatment plots, *Serratia marcescens* was identified with a confidence rating of "good to very good". *Serratia marcescens*, like *A. salmonicida*, is facultatively anaerobic, chemolithotrophic, and reduces nitrates (Holt *et al.* 1994). This bacteria is capable of growth at 30-37 °C (Holt *et al.* 1994). *Serratia marcescens* usually appears in soils enriched with chitin, and decomposes these proteins into various end products, such as ammonia (Holt *et al.* 1994). Further, this species functions as an antagonist, providing effective biocontrol of pathogens for plants (Whipps 1997).

*Syntrophomonas multifilia* was identified with a rating of "good", and was cultured only a few times during the sampling season; however, it did occur on all three treatment plots. Capable of growing at temperatures of 30-37 °C, *S. multifilia* functions as a fermenter (Holt *et al.* 1994). Mostly occurring in anoxic (without $O_2$) mud, this microbe obtains energy via β-oxidation of fatty acids, degrading the acids primarily to acetate and $H_2$ (Holt *et al.* 1994). This species was extracted from only those samples that happened to be from very wet pockets of soil. Having such a restricted niche, the low frequency of *S. multifilia* throughout the sampling season is therefore understandable.

The occurrence of the various bacterial isolates seems to respond to moisture and temperature levels rather than levels of organic residue. However, residues may, over time, influence the moisture and temperature of the soil (Hendrickson *et al.* 1985; Entry *et al.* 1986), which will eventually influence the type and amount of bacteria inhabiting the soil. Further, considering that the bacterial species identified were primarily nitrifying and

denitrifying species, there is the potential for short and long-term effects on nitrogen presence and availability on this site.

**SOIL RESPIRATION**

The objective of measuring the soil respiration was to determine if increasing levels of biomass removal would result in measurable differences. The ANOVA and subsequent LSD test revealed that $CO_2$ evolved in the control (uncut) plots was significantly higher than that evolved in the harvested plots. Since soil respiration consists of both root and microbial contributions, it is difficult to separate the two. Killham (1994) states that roots may contribute up to 30% of total soil respiration. In the harvest plots, the measuring apparati were intentionally placed so as to minimize the effect of living plant roots. The difference in $CO_2$ evolved (approximately 18%) could therefore be attributed to the presence or absence of roots. However, soil temperatures in the control plots were much lower than in the cut plots and, in general, respiration rates are reported to decline with temperature (Entry *et al.* 1986). For example, the $Q_{10}$ for root respiration has been measured at 2.5 (Singh and Gupta 1977) and at 2.4 (Uchida *et al.* 1998). Weber (1990) also reported a decline in $CO_2$ evolution when aspen stands were cut and burned. He attributed the difference to the loss of vegetation and biomass on site, contributing to lower metabolic activity. Furthermore, pH may be a factor affecting microbial respiration rates. Anderson and Domsch (1993) found that microbial communities released more $CO_2$-C (per unit MB) under acidic soil conditions than those with a more neutral pH.

Since the control plots had a more acidic pH than the harvested plots, the difference in $CO_2$ evolved may be related to this characteristic.

Given the opposing influences of root presence, soil pH and soil temperature, it seems reasonable to also consider that some of the difference was due to a change in either the quantity or activity level of the microbial component of the soil. From the statistical analyses of the estimates of microbial biomass (Table 11) it appears that treatment alone did not contribute significantly to quantitative differences in the organic layer although ANCOVA did reveal a statistically significant effect in the mineral horizon (Table 12). However, looking at the treatment means for the month of August, just before the respiration trials, it is apparent that differences between treatments at this time were minimal. It could therefore be surmised that quantity is not driving the differences in respiration. The level of microbial activity, on the other hand, may be a factor.

Orchard and Cook (1983) stated that an increase in respiration of a silt loam used for pastoral and crop farming, was most likely due to an increase in microbial activity. Weber (1990) attributed the decrease in soil respiration from uncut to harvested plots in his study to a decline in microbial activity. Metabolic activity of microorganisms is thought to depend on a number of factors including temperature, moisture and substrate (Orchard and Cook 1983; Lundgren and Soderstom 1983; Schlentner and Van Cleve 1985; Bosatta and Agren 1994; Berg *et al.* 1998; Bauhus *et al.* 1998). It is possible that the soil environment of the control plots is more conducive to higher rates of activity than is that of the cutover. Exposed areas may experience lower moisture levels, higher soil temperatures and lower quality organic matter inputs (*i.e.* essentially no root exudates or

foliar litterfall) all of which could depress microbial activity. Interestingly, chloroform-fumigation-extraction has as one of its advantages, the inclusion of all microbial organisms regardless of their metabolic state. Dormant organisms are therefore just as likely to be included in the quantitative estimate as are active organisms (Martens 1995).

Although this one time measurement must be considered cautiously, however, it appears to be an indicator of a change in state, if not quantity, of the microbial biomass after a forest removal event. Given that microbial populations may respond to crop removal events, there are implications for nutrient cycling, nutrient availability and, ultimately, the success and growth of vegetative regeneration. This change in state implies a reduction of microbial activity, which in turn may lead to a reduction in nutrient cycling and availability. Further investigation into the nature of change in microbial communities must be conducted before we can be sure of the direction of the trajectory.

## VARIABILITY IN SOIL DESCRIPTORS

One-way ANOVA for the soil descriptors revealed no significant treatment effects except for pH in the organic layer. It has been four years since the harvest treatments were imposed and it is interesting that, despite the obvious and dramatic change in the immediate environment, key soil characteristics such as % OM, moisture content, total P, total N and pH in the mineral layer do not differ significantly from one another. This observation must be made with caution however because other factors may have prevented the assignation of statistical significance. For example, there may be so much variability in the samples that trends due to treatment effect were obscured. Spatial

heterogeneity of the forest floor has been noted by other authors (Arp and Krause 1984). It is possible that random sampling may have captured a wide variety of combinations of mineral and organic elements. In this study, bulking of the samples was intended to reduce variation but may not have been sufficient given the number of samples actually incorporated. One means of determining the minimum sample size required to identify treatment differences is by calculating the required number of samples based on observed variance. Thus, the approximate minimum sample size required would be 1143. This is such a large number of samples that it is not practical to undertake. As always, scientific method must find a compromise between the resources available and the level of certainty associated with results.

In the case of pH in the organic layer, ANOVA and subsequent application of the LSD test for differences between treatment means revealed that control treatment means were significantly lower than those of the harvested areas. This maintenance of low acidity in the uncut, mature forest plots may be attributed to the continual inputs of acidic foliage (Brady and Weil 1986) and to higher cation uptake by roots. Hendrickson *et al.* (1985) and others (Baath *et al.* 1995; Smolander *et al.* 1998) have reported increases in soil pH after forest cover removal. However, this change may be temporary. The removal of forest vegetation has been shown to accelerate leaching of nitrates and weathering of pedogenic minerals, which release hydronium ions, thus promoting a return to the soil's original acidic level

(Rowell 1994).

## RELATIONSHIP BETWEEN SOIL DESCRIPTORS AND MICROBIAL BIOMASS

Correlation analysis was used to identify relationships between two independent variables; it is not expected that change in one variable is dependant upon change in the other (*i.e.* regression) but rather that the magnitudes of change are somewhat similar (Zar 1996). As stated previously, some of the results of the Pearson correlation test confirmed hypotheses about the samples while other results called our understanding of the system into question. A discussion of key findings follows.

In the organic soil layer, $MB_C$ was found not to be correlated with $MB_N$. This was somewhat unexpected considering that throughout the literature, the two are often linked with C/N ratios being used to confirm or estimate quantities of one or the other (Marumoto *et al.* 1982; Marumoto 1984; Ohtonen *et al.* 1992; Raubuch and Beese 1998; Haron *et al.* 1998). However, the result obtained here may be explained using two theories. For example, Alef (1993) suggested that fumigation of soil with chloroform ($CHCl_3$) liberates C and N that is chemically bound in humic fractions. These liberated minerals may inflate the results of the $MB_C$ and $MB_N$ calculations and lead to false conclusions from the correlation tests.

Another theory suggests that even a "reasonable" range of soil C:N ratios (*i.e.* 15:1 to 3:1) might not reveal a correlation. Specifically, the $MB_C$:$MB_N$ ratios for the full data set of the organic soil (Appendix I) ranges from 2.6:1 to 13.9:1. Even though these ratios are reasonable according to standards listed in the literature (Paul and Clark 1989),

the actual range of $MB_C$ and $MB_N$ values is so large that statistical relationships between the two may be difficult to determine.

The result that $MB_C$ and $MB_N$ were correlated in the mineral horizon adds weight to Alef's (1993) proposed argument that fumigation with $CHCl_3$ liberates C and N from humic fractions in addition to that from the MB. In samples from the mineral layer, mean % organic matter varied from 11 to 13 for the three treatments with a maximum of 30% measured in a control sample (high values are due to inexperienced sampling). These values are on the order of a fifth to a tenth that of the organic layer samples and are somewhat higher than others found in the literature. For example, Hendrickson *et al.* (1985) reported mean % OM values of 4.11% to 4.99% in the mineral soil and 57 to 76% in the organic layer of a mixed-wood forest in Central Ontario treated with conventional and whole-tree harvest methods. Chatarpaul (1987) studied conventional and whole-tree harvest methods on a mixed-wood forest of Central Ontario, where mean % OM values of the mineral soil ranged from 3.13% to 7.59%, with mean values of 54% to 69% in the organic soil.

The $MB_C$ and $MB_N$ were significantly correlated to % OM in the mineral layer but not in the organic layer. At first glance, these results may seem contradictory. However, two possible explanations for this apparent discrepancy present themselves. First, recall that samples from the organic layer averaged 80% (range 59 to 96%) organic matter while those of the mineral layer averaged 12% (range of 7 to 30%). Furthermore, estimates of $MB_C$ in the organic layer were at least an order of magnitude greater than those of the mineral layer and there were no apparent differences between treatment means for either

OM or $MB_C$ in the organic layer. It may be that variability in the measures of the organic layer was too great to reveal either similarities or differences in patterns of change. Second, the microbial biomass requires substrates. One might conclude that more OM in the soil matrix would be related to larger, or perhaps more active, populations of microorganisms (Hendrickson *et al.* 1985; Entry *et al.* 1986; Ohtonen *et al.* 1992; Bauhus *et al.* 1998). The range of OM in the mineral samples might have been sufficient to reveal a similar range in the microbial biomass while the much higher OM presence in the organic samples would be associated with a "saturation" effect. Treatment effects, either due directly to biomass removal or indirectly to changes in micro-environment, might be more easily detected in the mineral layer where numbers are naturally lower and populations are more likely to respond to moisture, temperature or substrate gradients.

Total N was significantly correlated with many factors in both the organic and mineral soil horizons. Specifically, with $MB_N$, pH and total P in the organic samples and with $MB_C$, WC, OM, pH and total P in the mineral layer. The correlation to OM in the mineral samples is understandable as this material, particularly after some decomposition, may be nitrogen rich (Haynes 1986; Killham 1994), while that of the more surficial layer may be less decomposed and therefore contain materials with wider C/N ratios. For example, compounds such as chitin, cellulose and lignin represent highly resistant pools of organic C (Killham 1994). These compounds, which are relatively N poor, may persist in the soil for long periods of time, requiring much time, energy, and import of N for degradation to occur (Killham 1994).

The correlation of total N to $MB_N$ in both layers may provide further evidence of the liberation of N due to CFE. With respect to the mineral samples, it may also be explainable in terms of larger microbial populations found in association with more nitrogen rich material. Nitrogen levels in the soil may be influenced by factors such as P levels, pH, and moisture content. For example, Gallardo and Schlesinger (1994) reported that P is less frequently immobilized than N by soil microbes, causing an increase of N/P ratios in the litter, and subsequently causing a limiting effect on the MB. They further speculated that P limitations in soils with extreme pH, where pH levels are highly acidic or highly basic, will affect the activity and nature of microorganisms present. In addition, Baath *et al.* (1995) suggest that pH may have the ability to alter other soil properties, such as C/N ratios, which indirectly affect microbial biomass by restricting available nutrients.

The level of moisture content may also affect the nitrogen levels, and subsequently C/N/P ratios. Soil bacteria, require a water film for movement and can only remain active if there is suitable moisture in the soil, so that nutrients and waste products can diffuse in and out of the cell (Wong and Griffen 1976a & b). Bacterial activity will increase or decrease as the water potential rises and falls, respectively. If moisture levels are inadequate, the mineralization-immobilization of nutrients may not occur, thus limiting the growth and activity of the microbial biomass.

In the organic layer samples, pH was correlated positively to $MB_C$ and negatively to WC and total N. Bosatta and Agren (1994) speculated that properties of the original litter and soil physical factors partly determine the amount of MB in the soil. Bauhus *et al.* (1998) stated that MB amounts are sensitive to changes in soil physical and chemical

composition. Therefore, in the organic samples, the pH was positively correlated to the $MB_C$ possibly because the organic residues remaining were less acidic, as compared to the continual inputs of more acidic spruce needles in the control plots. The less acidic sites (cut areas) did not have a continual input of acidic residues, making the environment tolerable for a greater variety of soil microbes. Although the site experienced a large deposit of needles during the harvest, this input may have been quickly assimilated, creating a surge in MB activity and growth, followed by a drop in biomass due to the lack of continual inputs.

Moisture content and total N were negatively correlated with pH, suggesting that the moisture content may be implicated in changes to pH. On the harvested sites, where the soil is less acidic, the moisture will run off, evaporate or drain through the soil because there is little vegetation uptake. In addition, the total N decreases on these harvested sites because there is little vegetation to take up and retain the nutrients. As water moves down through the soil, N as $NO_3^-$ ( as suggested by the bacteriology results) may be leached out and deposited into the water table (Haynes 1986). Also, the level of N-containing organic residues is decreased on the harvested sites due to the lack of continual inputs by vegetation.

However, in the mineral samples, pH is positively correlated with OM and total N because more nutrients and OM may be translocated into the mineral horizon from the organic layer. Since mineral turnover rates and chemical processes are much slower in mineral soil compared to organic soil it stands to reason that as the pH increases, the level of OM and N increases in the mineral layer, due to the lack of assimilation by plants.

## VARIABILITY IN MICROBIAL BIOMASS

With respect to actual measurements of biomass reported elsewhere for boreal forest soils, the values obtained here are reasonable. For example, Thibodeau *et al.* (2000) reported very similar values for both $MB_C$ and $MB_N$ in the soil of thinned and unthinned balsam fir stands. Furthermore, the values reported here account for 1-3% of the total soil organic C which is the standard accepted by most soil ecologists (Killham 1994). For these reasons, as well as the C:N ratios, it appears that the actual values measured are credible. This is an important point as the estimates were arrived at using CFE without calibration against another method (usually CFI). The latter is used to determine a suitable conversion factor however, it appears that the conversion factors applied in this study were appropriate.

### Microbial Biomass Carbon

Statistical analysis for the $MB_C$ in the organic horizon (ANCOVA, Table 15) found the interaction of the treatment and month factors to be significant. This suggests that a combination of seasonal conditions along with the amount of the organic residues present influences the amount of $MB_C$ found in the organic soil horizon. One way to decipher these influences is to consider the pattern of treatment means as presented in Figure 6a with the climatic information presented in Figure 3. As temperatures increased and moisture levels declined, the microbial biomass generally decreased. Specific evidence for the interplay between soil temperature and soil moisture is found in the values for the

control, tree-length and full tree treatments in July. Conditions on the full-tree plots, where all above-ground portions were removed, resulted in the lowest MBc value (approximately 5600 ug/g). It can be expected that the lack of coarse woody debris on the surface and/ vegetative regeneration led to high soil temperatures and evaporation. At this same time, values for the control and tree-length plots lie at 7000 ug/g 7800 ug/g, respectively. In the uncut plots, soil temperature is indeed lower and it could be predicted from a general knowledge of forested systems that soil water contents were reduced due to interception and uptake by the forest canopy. The latter effect would be exacerbated by a relative lack of precipitation over the growing season. In the tree-length harvested plots, soil temperatures would be higher but mitigated by surface debris while bulk precipitation would not be intercepted by standing vegetation. Thus, as indicated in the literature, microbial biomass is quite sensitive to unfavourable changes in temperature (Howard and Howard 1979; Weber 1990) and moisture content (Orchard and Cook 1983; Lundgren and Soderstom 1983). In this study, while microbial biomass numbers generally declined it is not clear which of treatment or environmental conditions were responsible.

The ANCOVA for the $MB_C$ in the mineral layer found the treatment factor to have a significant effect on the $MB_C$, suggesting direct influences of the harvest treatments. Figure 6b presents the treatment means by month. As noted earlier, the LSD test (based on ANOVA) confirms that mean $MB_C$ in the control plots is greater than that of the two harvest treatments. There are several possible reasons for this significant (at $\alpha$ = 0.05) difference: 1) the removal of standing biomass and annual inputs has been documented to result in quantitative differences in the soil microbial populations

(Hendrickson *et al.* 1985; Entry *et al.* 1986; Chatarpaul 1987; Foster and Morrison 1987); 2) a change in the microclimate to one less favourable for indigenous populations/communities may have contributed to declines in microbial numbers (Howard and Howard 1979; Orchard and Cook 1983; Lundgren and Soderstom 1983; Weber 1990; Berg *et al.* 1998); 3) a change in the soil chemistry to one less favourable; and, 4) loss of root exudates. For example, less organic matter inputs and change in pH due to loss of acidic foliar inputs from the previous forest stand causes the soil properties to change (Brady and Weil 1996).

However, it is important to note that qualitative changes in the microbial biomass are not captured by this method of measurement. It has been indicated in the literature that the removal of forest vegetation results in a change in the integrity of microbial functional groups (Gallardo and Schlesinger 1994; Baath *et al.* 1995; Staddon *et al.* 1996). A possible shift in microbial populations is further substantiated by the results for the soil respiration data, where higher levels of $CO_2$ efflux were found on the control treatment, indicating a higher level of microbial biomass/activity.

**Microbial Biomass Nitrogen**

The ANCOVA results for the $MB_N$ in both the organic and mineral horizons showed that the treatment and month factors, as well as their interaction, had no significant effects. Difficulties with the estimation of microbial biomass using nitrogen have been discussed elsewhere in this paper. Thibodeau *et al.* (2000) also reported a lack of change in the microbial biomass using both nitrogen and carbon as reference elements.

In general, Wardle and Ghani (1995) suggest that microbial biomass may not be reliably estimated when calibrating methods (CFE, CFI, and/or SIR) are employed on a spatially heterogeneous soil. Furthermore, they found that only comparatively large relative differences in the MB can be estimated reliably, suggesting that MB as a bioindicator of soil quality is limited. Therefore, as is the case with all experimental techniques, caution should be exercised when interpreting and attributing changes in soil microbial biomass to treatment or seasonal effects (Wardle and Ghani 1995).

# CONCLUSIONS

With respect to the isolations, only five bacteria that were cultured were in fact identified - two with low discrimination and three with good to very good discrimination. The species fulfilled a variety of roles in the soil ecosystem including N transformations. The API 20E system performed adequately on the organisms isolated. The author suspects that many more organisms were present than were cultured.

With respect to soil respiration measurements, it was evident that $CO_2$ evolved on control plots in the uncut, mature forest was significantly greater than that of the harvested plots. The author suggests that activity levels, and in turn, available C as root exudates, rather than biomass quantities, were responsible for the difference.

With respect to the soil descriptors, one-way ANOVA revealed no differences among the treatments for %OM, WC, total N and total P. Soil pH did, however, vary significantly with the mean of the control plots lower than those of the harvested plots. Despite obvious and dramatic changes in the environment above the soil, the soil itself does not exhibit statistically significant modification four years after the treatments were imposed.

Several of the soil descriptors exhibited correlation to each other as well as to the measures of microbial biomass. In the organic layer, it was noted that $MB_C$ and $MB_N$ did not in fact exhibit correlation with one another. A correlation between the two was determined in the mineral layer. The author presents several reasons for this apparent contradiction. Use of the chloroform fumigation extraction method without calibration by a second method gave reliable estimates of microbial biomass for boreal coniferous soils.

Application of ANCOVA to the measures of $MB_C$ and $MB_N$ produced different results for the two soil layers. Neither main effects nor the interaction term significantly affected $MB_N$ in either layer. The interaction of treatment and month was significant for $MB_C$ in the organic layer. Treatment alone significantly affected $MB_C$ in the mineral layer. In the latter, mean $MB_C$ in the control plots was nearly double that of the full-tree and tree-length plots. Throughout the data set, $MB_N$ appeared to fluctuate more so than $MB_C$; some reasons are suggested. The most sensitive indicator of change in the system appears to be microbial biomass C in the mineral layer. This may be because gradients in temperature, moisture and substrate quality are more readily apparent and indigenous microbial populations more likely to respond when changes do occur.

In general, descriptors of soil characteristics thought to reflect nutritional quality and quantity do not demonstrate statistically significant responses to harvest treatment although microbial response to soil moisture and temperature is evident. This particular black spruce system, four years after harvest, does not appear to have been adversely affected by the treatments imposed. It would be interesting to repeat this study in the future to determine if it is moving away from or closer to its pre-harvest set of conditions.

# RECOMMENDATIONS

Having had the opportunity to consider alternative approaches, the author would recommend the following as options for future work in this area:

1. Increase the number of samples analyzed in order to better capture and apply statistically the effect of spatial heterogeneity,
2. Avoid missing data by ensuring that generously sized soil samples are collected,
3. Consider soil texture and modify soil characteristics, such as moisture content, which may have affected the results of chloroform fumigation extraction (CFE),
4. Take respiration measurements much more frequently to account for rapid shifts in environmental conditions.
5. Use chloroform fumigation incubation or substrate induced respiration as a means to calibrate a site specific conversion factor for CFE,
6. Compare the impacts of anthropogenic disturbances to natural disturbances, such as wildfire, on soil microbial populations,
7. Using a chronosequence, assess microbial changes through successional stages of development.
8. Artificially change some of the soil descriptors in a controlled-factorial experiment to better isolate their effects.

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# APPENDIX I

# FULL DATA SET FOR THE ORGANIC AND MINERAL SOIL HORIZONS

# Appendix Ia. Complete data set for the Organic soil horizon.

| Harvest Treatment | Month of Sampling | $MB_C$ ($\mu$g/ g soil) | $MB_N$ ($\mu$g/ g soil) | C/N Ratio | $H^+$ Concentration (M) | Total Phosphorus (mg/Kg) | Total Nitrogen (mg/Kg) | Water Content (%) | Organic Matter (%) |
|---|---|---|---|---|---|---|---|---|---|
| Control | May | 14889 | 1790 | 8.3 | | 187 | 1399 | 46 | |
| Control | May | 8385 | 981 | 8.5 | | 123 | 1034 | 49 | |
| Control | May | 10087 | 724 | 13.9 | 0.0007585 | 125 | 981 | 69 | 95 |
| Tree-length | May | 5256 | 1702 | 3.1 | 0.0000229 | 141 | 1151 | 68 | 91 |
| Tree-length | May | 5618 | 1115 | 5.0 | 0.0000316 | 137 | 1157 | 71 | 90 |
| Tree-length | May | 7094 | 1326 | 5.3 | 0.0000407 | 179 | 1111 | 75 | 78 |
| Full-tree | May | 8086 | 1782 | 4.5 | 0.0000537 | 156 | 1151 | 64 | 93 |
| Full-tree | May | | | | | 164 | 1224 | 35 | |
| Full-tree | May | 6693 | 1136 | 5.9 | 0.0000257 | 164 | 1136 | 80 | 93 |
| Control | June | 7041 | 1025 | 6.9 | 0.0001862 | 127 | 1032 | 65 | 79 |
| Control | June | 8383 | 1425 | 5.9 | 0.0000912 | 220 | 1168 | 62 | 89 |
| Control | June | 7932 | 1963 | 4.0 | 0.0000309 | 137 | 1200 | 73 | 85 |
| Tree-length | June | 5416 | 753 | 7.2 | 0.0000380 | 137 | 1157 | 72 | 88 |
| Tree-length | June | 4647 | 845 | 5.5 | 0.0000575 | 166 | 1106 | 69 | 79 |
| Tree-length | June | 6797 | 1239 | 5.5 | 0.0000257 | 148 | 1209 | 76 | 77 |
| Full-tree | June | 6211 | 1013 | 6.1 | 0.0000157 | 117 | 1369 | 80 | 87 |
| Full-tree | June | 4896 | 691 | 7.1 | 0.0000478 | 129 | 1128 | 69 | 76 |
| Full-tree | June | 6114 | 1118 | 5.5 | 0.0000117 | 153 | 1200 | 78 | 86 |
| Control | July | 4956 | 734 | 6.8 | 0.0001000 | 158 | 1139 | 68 | 80 |
| Control | July | 6240 | 1075 | 5.8 | 0.0000776 | 123 | 962 | 63 | 59 |
| Control | July | 9268 | 1148 | 8.1 | | 151 | 1117 | 70 | |
| Tree-length | July | 7229 | 1213 | 6.0 | 0.0000251 | 179 | 1471 | 78 | 88 |
| Tree-length | July | 7179 | 1218 | 5.9 | 0.0000218 | 214 | 1330 | 74 | 79 |
| Tree-length | July | 8389 | 1559 | 5.4 | 0.0000724 | 151 | 1139 | 78 | 63 |
| Full-tree | July | 6696 | 1321 | 5.1 | | | | 80 | |
| Full-tree | July | 6217 | 1029 | 6.0 | 0.0000537 | 153 | 1057 | 63 | 77 |
| Full-tree | July | 4081 | 1441 | 2.8 | 0.0000117 | | 1471 | 80 | 41 |
| Control | August | 5347 | 1644 | 3.3 | 0.0001174 | 169 | 1202 | 68 | 81 |
| Control | August | 6004 | 667 | 9.0 | | 137 | 1018 | 73 | |
| Control | August | 5582 | 1233 | 4.5 | | 150 | 1078 | 70 | |
| Tree-length | August | 6270 | 1373 | 4.6 | 0.0000870 | 133 | 1180 | 74 | 96 |
| Tree-length | August | 6218 | 945 | 6.6 | 0.000575 | 160 | 1174 | 76 | 87 |
| Tree-length | August | 6483 | 974 | 6.7 | | 169 | 1089 | 73 | |
| Full-tree | August | 6666 | 2610 | 2.6 | 0.0000288 | 171 | 1197 | 78 | 84 |
| Full-tree | August | 5066 | 1364 | 3.7 | 0.0000524 | 143 | 1253 | 63 | 69 |
| Full-tree | August | 6603 | 955 | 6.9 | 0.0000229 | 134 | 994 | 77 | |

# Appendix Ib. Complete data set for the Mineral soil horizon.

| Harvest Treatment | Month of Sampling | $MB_C$ ($\mu$g/ g soil) | $MB_N$ ($\mu$g/ g soil) | C/N Ratio | $H^+$ Concentration (M) | Total Phosphorus (mg/Kg) | Total Nitrogen (mg/Kg) | Water Content (%) | Organic Matter (%) |
|---|---|---|---|---|---|---|---|---|---|
| Control | May | 1449 | 212 | 6.8 | 0.0000436 | 35 | 134 | 59 | 9 |
| Control | May | 1616 | 203 | 7.9 | 0.0002691 | 39 | 312 | 70 | 30 |
| Control | May | 414 | 77 | 5.4 | 0.0001071 | 35 | 231 | 65 | 21 |
| Tree-length | May | 528 | 76 | 7.0 | 0.0000085 | 32 | 153 | 40 | 11 |
| Tree-length | May | 358 | 87 | 4.1 | 0.0000177 | 43 | 142 | 68 | 10 |
| Tree-length | May | 437 | 91 | 4.8 | 0.0001479 | 40 | 126 | 74 | 11 |
| Full-tree | May | 851 | 59 | 14.5 | 0.0000288 | 34 | 168 | 74 | 13 |
| Full-tree | May | 1087 | 298 | 3.6 | 0.0000457 | 35 | 192 | 47 | 19 |
| Full-tree | May | 647 | 84 | 7.6 | 0.0000177 | 40 | 196 | 62 | 16 |
| Control | June | 1118 | 113 | 9.9 | 0.0000338 | 30 | 123 | 49 | 9 |
| Control | June | 1033 | 172 | 6.0 | 0.0000025 | 26 | 161 | 41 | 14 |
| Control | June | 1861 | 190 | 9.8 | 0.0000089 | 34 | 288 | 35 | 21 |
| Tree-length | June | 371 | 53 | 6.9 | 0.0000354 | 25 | 108 | 34 | 10 |
| Tree-length | June | 458 | 105 | 4.3 | 0.0000575 | 34 | 135 | 40 | 8 |
| Tree-length | June | 237 | 75 | 3.2 | 0.0000602 | 21 | 121 | 29 | 10 |
| Full-tree | June | 287 | 188 | 1.5 | 0.0000138 | 35 | 152 | 45 | 10 |
| Full-tree | June | 300 | 114 | 2.6 | 0.0000407 | 29 | 160 | 19 | 19 |
| Full-tree | June | 626 | 121 | 5.2 | 0.0000977 | 37 | 165 | 30 | 13 |
| Control | July | 482 | 40 | 11.9 | 0.0000208 | 37 | 121 | 19 | 9 |
| Control | July | 748 | 75 | 10.0 | 0.0000549 | 30 | 113 | 22 | 7 |
| Control | July | 605 | 48 | 12.5 | 0.0000275 | 25 | 121 | 19 | 9 |
| Tree-length | July | 981 | 99 | 9.9 | 0.0000213 | 24 | 143 | 47 | 10 |
| Tree-length | July | 443 | 123 | 3.6 | 0.0000165 | 52 | 257 | 36 | 22 |
| Tree-length | July | 378 | 67 | 5.6 | 0.0000281 | 27 | 27 | 31 | 7 |
| Full-tree | July | 486 | 89 | 5.5 | 0.0000060 | 31 | 114 | 43 | 8 |
| Full-tree | July | 362 | 48 | 7.5 | 0.0000323 | 36 | 124 | 27 | 8 |
| Full-tree | July | 383 | 111 | 3.4 | 0.0000213 | 35 | 158 | 34 | 11 |
| Control | August | 239 | 26 | 9.2 | 0.0000177 | 35 | 111 | 22 | 8 |
| Control | August | 296 | 76 | 3.9 | 0.0000549 | 41 | 170 | 27 | 13 |
| Control | August | 424 | 57 | 7.4 | 0.0001023 | 21 | 120 | 20 | 8 |
| Tree-length | August | 502 | 52 | 9.6 | 0.0000199 | 28 | 123 | 40 | 10 |
| Tree-length | August | 291 | 40 | 7.4 | 0.0001513 | 48 | 182 | 35 | 13 |
| Tree-length | August | 215 | 33 | 6.5 | 0.0000251 | 30 | 115 | 30 | 7 |
| Full-tree | August | 304 | 82 | 3.7 | 0.0000097 | 30 | 115 | 33 | 8 |
| Full-tree | August | 148 | 34 | 4.3 | 0.0000218 | 30 | 90 | 24 | 7 |
| Full-tree | August | 270 | 48 | 5.6 | 0.0000229 | 27 | 116 | 28 | 9 |

# APPENDIX II

# COMPLETE DATA SET FOR THE SOIL RESPIRATION STUDY

Appendix II: Complete data set for the Soil Respiration study.

| Treatment | C Efflux (09/05/98) ($gCO_2/hr/m^2$) | C Efflux (09/12/98) ($gCO_2/hr/m^2$) |
|---|---|---|
| Control | 0.0118 | 0.0121 |
| | 0.0159 | 0.0165 |
| | 0.0139 | 0.0141 |
| | 0.0138 | 0.0143 |
| | 0.0138 | 0.0141 |
| | 0.0127 | 0.0131 |
| | 0.0128 | 0.0136 |
| | 0.0094 | 0.0103 |
| | 0.0111 | 0.0116 |
| | 0.0117 | 0.0126 |
| | 0.0118 | 0.0123 |
| | 0.0107 | 0.0112 |
| | 0.0122 | 0.0165 |
| | 0.0121 | 0.0132 |
| | 0.0112 | 0.0114 |
| Mean | 0.0123 | 0.0131 |
| Tree-length | 0.0128 | 0.0133 |
| | 0.0100 | 0.0137 |
| | 0.0117 | 0.0128 |
| | 0.0126 | 0.0133 |
| | 0.0109 | 0.0152 |
| | 0.0139 | 0.0116 |
| | 0.0128 | 0.0084 |
| | 0.0129 | 0.0117 |
| | 0.0090 | 0.0099 |
| | 0.0095 | 0.0113 |
| | 0.0095 | 0.0105 |
| | 0.0088 | 0.0094 |
| | 0.0108 | 0.0109 |
| | 0.0069 | 0.0074 |
| | 0.0107 | 0.0118 |
| Mean | 0.0109 | 0.0114 |
| Full-tree | 0.0103 | 0.0103 |
| | 0.0119 | 0.0117 |
| | 0.0126 | 0.0133 |
| | 0.0094 | 0.0100 |
| | 0.0112 | 0.0111 |
| | 0.0114 | 0.0121 |
| | 0.0082 | 0.0078 |
| | 0.0069 | 0.0108 |
| | 0.0105 | 0.0116 |
| | 0.0092 | 0.0093 |
| | 0.0117 | 0.0123 |
| | 0.0107 | 0.0121 |
| | 0.0117 | 0.0117 |
| | 0.0084 | 0.0082 |
| | 0.0075 | 0.0079 |
| Mean | 0.0101 | 0.0107 |