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Sultana Sarvatara Shah

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**DECADAL RESPONSES IN SOIL N DYNAMICS AT A PAIRED WATERSHED**

**EXPERIMENT IN MAINE**

By

Sultana Sarvatara Shah

B.S. The Evergreen State College, 2000

A THESIS

Submitted in Partial Fulfillment of the

Requirements for the Degree of

Master of Science

(in Plant, Soil, and Environmental Sciences)

The Graduate School

The University of Maine

December, 2002

**Advisory Committee:**

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Thesis Advisor: Dr. Ivan J. Fernández

An Abstract of the Thesis Presented  
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Nitrogen is commonly thought of as the most limiting nutrient to plant growth, yet elevated N deposition can result in N accumulating in excess of biotic demand, a condition known as "N Saturation." Excess N can perturb soil microbial N transformations and may cause initial increases in net N mineralization rates followed by decreases in net N mineralization with concomitant increases in net nitrification. Along with increases in net nitrification and N loss, N saturation is often associated with a loss of forest productivity. Understanding nitrogen dynamics in soil under enhanced N deposition is key to predicting future forest health. We studied forest floor and mineral soils at the Bear Brook Watershed in Maine (BBWM), a paired watershed experiment with one watershed serving as a reference and another treated with  $(\text{NH}_4)_2\text{SO}_4$ . We used both lab incubations and *in situ* measurements to evaluate net N mineralization and net

nitrification in both watershed soils. Significantly higher net N mineralization and net nitrification rates were observed in the treated watershed by both methods. For example *in situ* net N mineralization was  $4.25 \text{ mg kg}^{-1} \text{ day}^{-1}$  in the treated watershed compared to  $3.00 \text{ mg kg}^{-1} \text{ day}^{-1}$  in the reference watershed. Soil under differing dominant forest types present in these watersheds resulted in different N cycling rates and different response to long-term N fertilization: hardwoods had higher N mineralization rates in the O horizons, however softwoods had higher rates in the mineral soils. Despite different N cycling rates, influenced by forest cover and treatment, input-output estimates suggested ~80% N retention in treated watershed, despite the long-term N amendments to this watershed, and ~96% N retention in the reference watershed.

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## CHAPTER 1: LITERATURE REVIEW

### INTRODUCTION

Nitrogen (N) cycling in terrestrial ecosystems is one of the most complex of the major nutrient cycles, which has led to extensive research on this topic in both agricultural and forestry settings over the past century. Nitrogen is also considered to be the most commonly limiting plant nutrient throughout the world (Vitousek and Howarth, 1991; Schlesinger, 1997). However, in the past 50-100 years atmospheric N deposition has increased significantly resulting in it being considered a potential pollutant in terrestrial ecosystems (Schlesinger & Hartley 1992; Galloway et al. 1995; Torseth and Semb, 1997). Increasing N deposition has been a prominent issue in the last decade and consequently more effort has been put into understanding its effects. Increasing N deposition has largely been due to the combustion of fossil fuels, resulting in the increase of N oxides in the atmosphere (Galloway et al. 1995; Lovett et al., 2000). Nitrogen oxides can be transported long distances and can create strong mineral acids in the atmosphere that are deposited back into ecosystems as acid deposition, otherwise known as acid rain (Fernandez and Adams, 2000). Acidic N deposition is problematic for many reasons, one of which is that many plant species in terrestrial ecosystems are adapted to, and function optimally in, soils with low N availability (Vitousek et al., 1997). Changes in N availability can result in changing dynamics of plant populations and their primary consumers and ultimately all species that depend on plants (Aerts and Berendse, 1988; Wedin and Tilman. 1996; Tilman, 1987; Vitousek et al., 1997). Increased N deposition has serious repercussions on all aspects of the nitrogen cycle and consequently on many other elemental biogeochemical cycles (Lovett et al., 2000; Aber et al., 1989; Vitousek et

al., 1997). One consequence of soil acidification is root damage, resulting in reduced ability of N uptake (Henriksen and Hessen, 1997), while other problems include: increasing emissions of  $\text{NO}_x$  which contributes to the formation of ozone (Seinfeld 1986), nitrate leaching resulting in acidification of streams, eutrophication of estuaries and coastal waters (Murdoch and Stoddard, 1992; Henriksen and Hessen, 1997; Smith et al., 1999), spruce forest decline (Johnson et al., 1994; McNulty et al., 1996), mercury accumulation in fish (Driscoll et al., 1994), increased Al mobility (Lawrence and David 1997) and increased contamination of  $\text{NO}_3\text{-N}$  in drinking water (Spalding and Exner, 1993).

Due to persistent perturbation of the N cycle resulting mainly from the activities of humans; it is critical to understand how N dynamics change over time, particularly as a result of increased N inputs to terrestrial and aquatic ecosystems. A specific way to quantify changes wrought in the soil N cycle by enhanced N availability is to measure rates of N mineralization and nitrification; two important microbial processes that govern the availability of N to plants and themselves. These processes, usually measured as N mineralization and nitrification can provide an accurate benchmark as to where the system is in terms of saturation; a condition where N availability exceeds biotic demand.

### **Objectives**

- To examine N mineralization and nitrification rates at the Bear Brook Watershed in Maine (BBWM), where one artificially N-enriched watershed is compared against a control watershed. This will be done against a backdrop of other potential influences including forest cover.

- To evaluate the relationship between potential net nitrification and nitrification potential.
- To determine the effects of a lab incubation period (7, 14, 28 days) on N mineralization; commonly used incubation periods found in the N cycling literature.

## **NITROGEN CYCLE**

In a forested ecosystem, N is a dynamic and essential nutrient. Nitrogen is also commonly thought of as the most limiting nutrient for plant growth. The main processes that affect N in soils includes: atmospheric deposition, N<sub>2</sub> fixation, mineralization, nitrification, immobilization, denitrification and leaching (Stoddard et al., 1994).

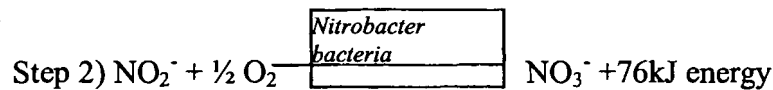
Almost all the N that enters a terrestrial ecosystem by natural processes is derived from biological N<sub>2</sub> fixation and atmospheric deposition (Stevenson and Cole, 1999). Nitrogen deposition occurs in a variety of forms, including NO<sub>3</sub><sup>-</sup>, NH<sub>4</sub><sup>+</sup>, gaseous forms, and organic N in wet and dry deposition. Concentrations of NO<sub>3</sub><sup>-</sup>, NH<sub>4</sub><sup>+</sup> and organic N in wet and dry deposition vary widely throughout the US (Stoddard et al., 1994).

Although a large pool of N exists in our atmosphere ( $3.9 \times 10^{18}$  kg or approximately 78% of the partial pressure of the atmosphere), it is present as an inert gas and as such cannot be used by most plants or animals. The N<sub>2</sub> molecule has a triple covalent bond that is difficult to break; yet some microorganisms and some free-living and plant-associated microorganisms, known as diazotrophs are capable of incorporating N into organic molecules. The N<sub>2</sub> molecule can then be utilized by microorganisms

within nodulated leguminous plants (e.g., *Rhizobium* sp.), non-leguminous root associated actinomycetes (e.g., *Frankia* sp.) and by free-living non-photosynthetic bacteria and cyanobacteria (Stevenson and Cole, 1999).

Mineralization is the process of microbial decomposition of this fixed-N within organic matter resulting in production of  $\text{NH}_4\text{-N}$  and  $\text{NO}_3\text{-N}$ . Mineralization is an important process in non-leguminous plant ecosystems such as forests, as it internally recycles N that would otherwise be tied up in organic matter and unavailable for plant use (Stoddard et al., 1994).

Nitrification (part of the mineralization process) is the microbially mediated oxidation of  $\text{NH}_4^+$  to  $\text{NO}_3^-$  carried out by autotrophic bacteria. Nitrification is a two-step process:



(Brady and Weils, 1999).

Winogradsky (1890) was the first to discover that nitrification was a two-step process as well as finding that *Nitrosomonas* and *Nitrobacter* were the organisms involved in this oxidative reaction (Stevenson and Cole 1999). Although *Nitrosomonas* and *Nitrobacter* are most commonly thought of as the dominant bacteria species that perform this oxidative process, there are a number of other microbes (bacteria such as *Nitrospira* sp., and



possibly heterotrophic fungi) capable of nitrifying  $\text{NH}_4\text{-N}$  and gaining energy from this process (Sylvia et al., 1998).

It has been assumed that little if any nitrification goes on in highly acidic forest soils (Brierley et al., 2001), mainly because isolated autotrophic nitrifying microorganisms rarely carry out nitrification below pH 6.0 in pure culture. However, Brierley et al. (2001) and Klein et al. (1983) both found that nitrification proceeded at pH ranges of 3-6. The authors hypothesized this could be due to the pH of microhabitats differing enough from the average stand pH to allow for autotrophic nitrification, that there are acid-tolerant autotrophic nitrifiers that have yet to be investigated, or that acid-tolerant heterotrophic nitrifiers are carrying out the nitrification (Brierley et al., 2001). Regardless of the mechanisms of nitrification by either autotrophic or heterotrophic organisms, it has been found by a number of researchers that nitrification does proceed in acidic forest soils (Brierley et al., 2001; De Boer and Kowalchuk, 2001; Pennington and Ellis, 1993; Tietema et al., 1992).

There are three main processes that make mineral N unavailable to plants: immobilization (either through biotic demand or abiotic sorption), denitrification, and leaching. Biotic immobilization is the conversion of inorganic N ( $\text{NH}_4\text{-N}$  and  $\text{NO}_3\text{-N}$ ) to organic forms, the reverse process of mineralization, although the two processes often occur simultaneously in soils. Denitrification is the process of  $\text{NO}_3^-$  being converted to gaseous forms of N such as  $\text{N}_2\text{O}$  and  $\text{N}_2$  by facultative and obligate anaerobic soil microorganisms (Brady and Weil, 1999). Although denitrification results in a loss of available N for plants,  $\text{N}_2\text{O}$  is also a greenhouse gas 230 times more effective than  $\text{CO}_2$  at trapping heat radiation and its atmospheric lifespan is 3-5 times that of  $\text{CO}_2$  (IPCC, 1995;

Powlson, 1993). The combination of  $N_2O$  and  $CO_2$  is not only the source of eighteen to fifty percent of current global warming, but these gasses are also involved in reactions that lead to ozone depletion in the stratosphere (Warneck et al., 1988). Leaching of  $NO_3^-$ -N is another way (besides denitrification)  $NO_3^-$ -N is lost from terrestrial ecosystems. Leaching of  $NO_3^-$ -N occurs because the negatively charged  $NO_3^-$ -N ions are not adsorbed by the predominately negatively charged colloids and surfaces that are found in most soils (Brady and Weil, 1999). This results in  $NO_3^-$ -N ions being easily leached by water percolating through soils. Nitrate leaching is a concern for a number of reasons including: a decrease in available N for plants, leaching of  $NO_3^-$ -N causes acidification of streams and eutrophication of estuaries and coastal waters (Murdoch and Stoddard, 1992; Henriksen and Hessen, 1997).

## **NITROGEN DEPOSITION AND SATURATION**

Acidic deposition first emerged in scientific literature in the 1960's in Sweden and around Scandinavia when researchers began seeing acidic precipitation and surface water acidification (Oden 1968). Hubbard Brook in New Hampshire was the first site in North America to report acidic deposition effects around this time (Likens et al., 1972). Acidic deposition has increased the concentration of  $H^+$ ,  $SO_4^{2-}$ , and  $NO_3^-$  ions in soils which has led to increased rates of leaching of base cations, increased  $Al^{3+}$  mobility and the resulting acidification of soils (Driscoll et al., 2001). Since the implementation of the Clean Air Act in 1963, 1970, and revised again in 1990, Stoddard et al. (1999) reports that lake and stream  $SO_4^{2-}$  concentrations in the northeastern United States have declined

significantly; a result of SO<sup>2</sup>- scrubbers in coal burning utilities and use of coal with lower S content. Conversely, NO<sub>3</sub>-N concentrations in streams have only slightly decreased due to the 1990 legislation probably owing to the required 10% reduction in N deposition (Stoddard et al. 1999; McNulty et al., 1996).

A consequence of chronically enhanced N deposition is that the ecosystem could become N saturated, a term used to describe a state where the availability of mineral N is in excess of plant and microbial demand. This is usually evident by significant amounts of NO<sub>3</sub>-N leaching from the catchment (Aber et al., 1989; Aber et al., 1998). Because increasing NO<sub>3</sub>-N concentrations in streams are indicative of a watershed reaching N saturation, it is important to understand the factors controlling all forms of N in soils and streams.

Aber et al., (1989) proposed four stages of N saturation that have since been experimentally tested and revised in Aber et al., (1998). Stage 0 is defined as characteristics of N cycling under N limiting conditions (ambient N loading); while during stage 1 there is a fertilization effect; e.g., an increase in plant growth and other N-limited biological processes. Since N is limiting in most ecosystems, this initial pulse of added N often increases N availability and N cycling rates such as mineralization and nitrification. Nitrogen saturation occurs at stage 2 resulting in negative effects although they may be relatively subtle and difficult to measure. These negative effects can include loss of frost-hardiness in conifer trees (Bobbink et al., 1992; Fangmeier et al., 1994, but see Taulavuori 1998; Wiemken et al., 1996), elevated amino acid content (Näsholm and Ericsson, 1990; Näsholm et al., 1994) and lowered phosphorus and lignin content in foliage, NO<sub>3</sub>-N leaching from soils, and increased emissions of N<sub>2</sub>O (Aber et al., 1998).

Stage 2 is also where Aber et al., (1998) found N mineralization to decrease in four sites in New England. This is contrary to the original hypothesis where N mineralization rates continued to increase over the course of the saturation continuum due to expected continued decrease in the soil C/N ratio due to the increased N deposition. Stage 3, the final stage of N saturation, is where some visible effects are observed such as disruption of both above and below ground forest structure resulting in tree death. Other indications of stage 3 include a reduction in total net photosynthesis and a reduction in overall forest productivity (Aber et al., 1989; Aber et al., 1998). However, some ecosystems are considered predisposed to N saturation due to factors such as chronic elevated N deposition, advanced stand age (Stoddard et al., 1994), artificial or natural disturbances (Goodale and Aber 2001) and large soil organic and inorganic N pools (Stoddard et al., 1994).

In N limited soils, the rate of nitrification is low compared to N enriched soils as autotrophic nitrifiers are poor competitors for available  $\text{NH}_4\text{-N}$ . In an N limited system,  $\text{NH}_4\text{-N}$  is retained by soils through adsorption to cation exchange sites and little is typically leached, however as N accumulates in ecosystems, changes may occur in the N cycle. Increased N availability in the soil results in faster and more complete decomposition of N-containing soil organic matter manifested as increased N mineralization and nitrification rates (Aber 1993; McNulty et al., 1996). Aber et al., (1995) found a consistent set of responses to chronic N additions in a study of five northern temperate forested ecosystems, including the Bear Brook Watershed in Maine. Some of the responses to chronic N additions included: initial increases in net N mineralization followed by decreases during stage 2 of N saturation, continual increases

in net nitrification and declining tree growth in coniferous stands. These findings demonstrate how soil processes such as N mineralization and nitrification can provide an accurate benchmark for determining the effects of increased N deposition and may be used to place an ecosystem on the N saturation continuum. These results are consistent with those found in alpine watersheds in the Front Range of the Colorado Rocky Mountains which reached N saturation from anthropogenically derived sources of N (Williams et al., 1996).

#### **NITRATE IN STREAMS**

Large amounts of  $\text{NO}_3\text{-N}$  ions leached to streams is an important and often deleterious result of increasing N deposition and consequently much effort has gone into understanding the factors that control terrestrial  $\text{NO}_3\text{-N}$  loss to streams. Some of these controlling factors have been found to include disturbances such as forest harvesting (Likens et al., 1970; Goodale and Aber 2001), insect damage/defoliation (Swank et al., 1981; Webb et al., 1995; Lovett et al., 2002), land use history, vegetation/tree species composition and decreasing tree growth rates (Lovett et al., 2000; Goodale and Aber 2001). Broader ecological or biogeochemical factors that control  $\text{NO}_3\text{-N}$  loss to streams include microbial or hydrological variables (Vitousek et al., 1979), atmospheric deposition of N-containing compounds (Lovett et al., 2000) and abiotic interactions in soil (Berntson and Aber, 2000; Dail et al., 2001). All of these factors influence  $\text{NO}_3\text{-N}$  loss in streams with most increasing the amount of  $\text{NO}_3\text{-N}$  that is leached to streams.

Peterson et al. (2001) reported that the most rapid uptake and transformation of inorganic N in streams occurred in the smallest streams included in their large in-stream N release studies. This phenomenon is likely the result of interactions between flowing waters (and the inorganic-N therein) with the streambeds and sediments as the volume of flow in contact with sediments is greater in smaller streams. These researchers also found that  $\text{NH}_4\text{-N}$  entering the streams was removed from the water within tens to hundreds of meters, (Mulholland et al., 1990) while  $\text{NO}_3\text{-N}$  was removed only after traveling 5-10 times the distance of  $\text{NH}_4\text{-N}$ . Even though low  $\text{NH}_4\text{-N}$  concentrations were found in most of the streams studied, the nitrification rates were high indicating that small streams may be important sources of  $\text{NO}_3\text{-N}$ , and hence  $\text{N}_2\text{O}$  to the atmosphere when  $\text{NO}_3\text{-N}$  laden waters encounter anaerobic sediments.

Lawrence (2002) investigated the hydrologic constraints on stream acidification and found that episodic as opposed to chronic acidification of streams, was more widespread. The term episodic acidification is used to indicate the increasing acidity of a stream due to a high flow event such as a storm. Although episodic acidification can occur naturally, the intensity of acidification (usually associated with significant declines in pH) in many streams during hydrologic episodes has been associated with increased acidic deposition (Wigington et al., 1996). This is likely due to high flow events moving water quickly through the soil and reducing the mitigating biotic and abiotic factors that retain acidifying-N species. The anion neutralization capacity (ANC) depends on cation exchange reactions that buffer decreases in the pH of soil solutions through the release of bases (mostly  $\text{Ca}^{2+}$ ) adsorbed to particle surfaces. Since exchangeable  $\text{Ca}^{2+}$  in the mineral soil can decrease due to acidic deposition, the result of persistent acid deposition storm

events can be a loss of neutralization of soil waters occurring in the mineral soils. (Lawrence 2002).

## **NITROGEN MINERALIZATION AND NITRIFICATION**

There is evidence that N mineralization and nitrification are controlled by a number of factors including soil depth, forest species composition, amount and quality of organic matter present, and varying seasons (Boone 1992; Federer et al., 1983; Finzi et al., 1998; Campbell et al., 2000). There is also evidence that the effects of tree species on surface soil N dynamics can occur over short time scales (Gower and Son 1992; Binkley 1995; Verchot et al., 2001). Pastor and Post (1988) demonstrated that changes in temperature and precipitation caused a northward migration of the hardwood forest borders in North America. The transition occurred from conifer- to hardwood-dominated forests and led to increases in N availability because hardwood species have high growth rates, high tissue N concentrations, and rapid rates of litter decomposition due to lower lignin and other polyphenolic concentrations (Finzi et al., 1998; Perry et al., 1987). The findings of Finzi et al. (1998) are consistent with Ferrari (1993) who found a two-fold difference in the annual rate of N mineralization in sugar maple and hemlock stands on the same soil type in the organic soil horizons. Cole and Rapp (1981) found organic matter turnover in the forest floor to take on average four times longer and N turnover more than three times longer in conifer stands as opposed to deciduous stands. In contrast, Mladenoff (1987) found no significant difference in the rate of N mineralization beneath sugar maple and hemlock trees in Wisconsin. Interspecific differences in soil

organic matter (SOM) quality appear to regulate the rate of net N mineralization in northeastern forests (Finzi et al., 1998). Low rates of nitrification are observed in many acid forest soils (Davidson et al., 1992; Stark and Hart 1997) and increases in the pH of soils can often increase the rate of net nitrification (Vitousek and Matson 1985; Persson et al., 1995).

Nitrogen mineralization has been found by a number of researchers to vary significantly with depth (Federer et al. 1983; Boone 1992; Binkley and Hart 1989; Persson et al. 1995; Friedland et al., 1991). Federer et al. (1983) demonstrated that N mineralization declined with increasing depths in New Hampshire and Connecticut, but that mineralization in Maine was independent of depth. Persson et al. (1995) found that N mineralization and potential nitrification were significantly different between organic and mineral soils in an acidic Norway spruce forest in southern Sweden and eastern Denmark, with 78% of the net N mineralization occurring above a 10cm depth. Nitrogen mineralization generally decreases with increasing depth because there is less organic matter for microbes to decompose (Persson et al., 1995). When the organic matter effect is removed by expressing mineralization on a mass per area basis, a general decrease with depth still occurs. This is hypothesized to be partly due to decreasing temperatures with increasing depths, yet that only explains part of the decrease. Federer et al. (1983) suggests that organic matter may become older and more difficult to break down as depth increases.

Nitrogen mineralization has been found to vary according to dominant vegetation and leaf litter composition. Some researchers have reported greater N mineralization rates in soils dominated by deciduous trees as opposed to coniferous stands (eg. Campbell



et al., 2000; Finzi et al., 1998; Nadelhoffer et al., 1984; Boone 1992; Aber et al., 1993), although this is dependant on substrate quality. Similar results have been found in deciduous versus coniferous leaf litter (Tietema and Wessel 1992). In an experiment conducted by Campbell et al. (2000), they found hardwood stands to have higher net N mineralization rates in the organic horizons of the soils sampled, but the mineral soils had equally low rates of N mineralization and nitrification in both stand types. Campbell et al., (2000) attributed the increased N mineralization in the organic horizons of the hardwoods to differences between stand types in soil moisture, temperature, pH, and biotic controls related to competition between plants and microbes. They also hypothesized that the lower N cycling rates found in the softwood stands could indicate that softwoods might be more sensitive to increased N deposition. Finzi et al., (1998) and Fernandez et al., (2000) both found results similar to Campbell et al., (2000) with hardwood stands demonstrating significantly higher net N mineralization rates compared with softwoods in the organic horizons. This was attributed to differences in SOM quality such as the C/N. Aber et al., (1993) found similar results at the Harvard Forest, MA after 3 years of chronic N fertilization. Immediately following treatment, Aber et al., (1993) found higher rates of net N mineralization and net nitrification in the softwood stands in the mineral soils and the opposite true for the organic horizons in the hardwood stands. Aber et al., (1993) also found the softwoods to show larger changes in extractable N, foliar N, nitrification and N leaching loss, lending support to the premise that softwoods may be more sensitive to increased N deposition (Campbell et al., 2000).

Net N mineralization rates have been found to vary seasonally in temperate forest soils with greater rates in the growing season, especially midsummer than the winter

(Garten et al., 1994; Boone 1992; Nadelhoffer et al., 1984; Vitousek and Matson 1985).

Temporal trends in soil  $\text{NH}_4\text{-N}$  and net N mineralization potential also suggest that maximum soil N availability occurs during the time of high nutrient demands by forests to initiate spring root growth (Garten et al., 1994). Vitousek and Matson (1985) demonstrated a 10-fold seasonal range for potential net N mineralization (PNNM) in the mineral soil and found that temperature and moisture were largely responsible for the large variation observed.

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## **CHAPTER 2 : DECADAL RESPONSES IN SOIL N DYNAMICS AT THE BEAR BROOK WATERSHED IN MAINE**

### **INTRODUCTION**

Over the past 50-100 years atmospheric N deposition has increased across both Europe (Gunderson 1995; Dise and Wright, 1995) and the northeastern U.S. (Galloway et al., 1995; Aber et al., 1993) due largely to human activities including fossil fuel combustion and changes in agricultural practices. Although N is considered to be limiting in most forest ecosystems throughout the world (Schlesinger 1997), it is also considered a potential pollutant in some ecosystems (Torseth and Semb, 1997). Furthermore, increasing emissions of  $\text{NO}_x$  contributes to the formation of ozone (Seinfeld 1986). N in excess of biological demand can lead to soil acidification, which in turn can cause root damage, thereby reducing the ability of plants to take up N (Henriksen and Hessen, 1997). Other consequences of increased N deposition include: spruce forest decline (McNulty et al., 1996), increased Al mobility (Lawrence and David 1997), mercury accumulation in fish (Driscoll et al., 1994), increased  $\text{NO}_3\text{-N}$  leaching resulting in acidification of streams, eutrophication of estuaries and coastal waters (Murdoch and Stoddard, 1992; Henriksen and Hessen, 1997), and increased contamination of  $\text{NO}_3\text{-N}$  in drinking water (Spalding and Exner, 1993).

In N-limited soils, the rates of ammonification and nitrification are low compared to N-enriched soils. This is because in an N-limited ecosystem,  $\text{NH}_4\text{-N}$  made available via mineralization is quickly utilized by both plants and soil microbiota or retained by

soils through adsorption to cation exchange sites. This can result in little  $\text{NH}_4\text{-N}$  being leached or available for nitrification. However, once assimilatory needs have been met, additional  $\text{NH}_4\text{-N}$  is available for nitrifier communities that oxidize  $\text{NH}_4\text{-N}$  to  $\text{NO}_3\text{-N}$ . This  $\text{NO}_3\text{-N}$  can then be leached owing to its low retention by most soils and under conditions of low oxygen availability denitrification can convert much of  $\text{NO}_3\text{-N}$  to  $\text{N}_2\text{O}$  or  $\text{N}_2$ . (Aber 1993; McNulty et al., 1996; Mohn et al., 2000).

Nitrification and N mineralization rates are one among many microbial activities that have been studied to assess effects of disturbance including fertilization (Gilliam et al., 1996; McNulty et al., 1993), harvesting (Fenn et al., 1998; Frazer et al., 1990), land use history and other disturbance (Goodale and Aber 2001; Goodale et al., 2000) and climate (Mitchell et al., 1996; Pastor and Post 1988; Rustad et al., 2000; Peterjohn et al., 1994). Nitrogen mineralization has also been used in forest ecology to evaluate forest succession (Gower and Son 1992) and the subsequent effects on forest types (Cole and Rapp 1981; Campbell et al., 2000). A number of researchers have found O horizons soils under hardwoods to have significantly higher rates of N mineralization compared to O horizon soils under softwoods (Campbell et al., 2000; Aber et al., 1993; Finzi et al., 1998; Ferrari 1993). This has been attributed to differences in soil moisture, pH, temperature, carbon to nitrogen (C/N) ratios and biotic mechanisms related to competition between plants and microbes (Campbell et al., 2000; Aber et al., 1993). Campbell et al., (2000) and Aber et al., (1993) have hypothesized that the lower N cycling rates observed in softwoods could indicate that softwoods might be more sensitive to deleterious effects of increased N deposition than hardwoods.

Long-term elevated N deposition can result in N saturation at a site where N availability is chronically in excess of microbial and plant demand (Aber et al., 1989; Ågren and Bosatta 1988). Nitrogen saturation is manifested in an ecosystem by initial increases in net N mineralization rates (thought to be a fertilization effect) followed by sharp declines. These declines in N mineralization parallel increases in net nitrification rates and are often followed by declining tree growth after saturation is reached (Aber et al., 1998).

Since many of Europe's ecosystems have progressed farther on the N saturation continuum than in North America, researchers there have provided valuable insights into the progression of N dynamics during the evolution of N saturation. The EXperimental MANipulation of Forest Ecosystems project in Europe (EXMAN) (Rasmussen et al., 1990) and the NITROgen Saturation EXperiments in Europe (NITREX) (Dise and Wright, 1992) addressed the effects of increased N deposition on biogeochemical cycling in European coniferous forests. In both Europe and North America the most commonly used indicator of the N status of a site is stream  $\text{NO}_3\text{-N}$  export (e.g. Aber et al., 1993, Adams et al., 1997, Andersson et al., 2002). However, stream chemistry is a function of internal watershed processes such as N mineralization and nitrification in soils. Therefore, this study was designed to evaluate N dynamics at the Bear Brook Watershed in Maine (BBWM) after more than a decade of experimental N enrichment. The specific objectives of this research were to determine the effects of increased N deposition on net N mineralization and net nitrification at BBWM and the influence of forest type on those processes.

## MATERIALS AND METHODS

### Site Description

The Bear Brook Watershed in Maine (BBWM) is located in eastern Maine at 44°52' north latitude and 68°06' west longitude, approximately 60 km from the coast of Maine (Figure 1.1) and is located on the upper 210 m of the southeast slope of Lead Mountain. BBWM is a paired watershed experiment that began in 1987. BBWM was established to evaluate a whole ecosystem response to elevated N and S deposition in a low alkalinity forested stream watershed in northern New England (Norton et al., 1999) utilizing the paired watershed approach (Likens et al., 1977). Both watersheds are topographically similar (Wang and Fernandez, 1999) and had similar patterns of output fluxes for elements prior to manipulation (Norton et al., 1999). The East Bear watershed is 10.95 ha while West Bear is 10.26 ha. A first order stream drains each watershed with an average slope from the top of the watershed to the weirs of 31% (Norton et al., 1999).

The vegetation at BBWM includes both hardwoods and softwoods, with hardwoods and mixed woods dominating the lower ~60% of the watersheds. Hardwoods include American beech (Fagus grandifolia Ehrh.), sugar maple (Acer saccharum Marsh.), red maple (Acer rubrum L.), with minor yellow birch (Betula alleghaniensis Britt.) and white birch (Betula papyrifera). The hardwood forest is successional following logging prior to 1945 (Wang and Fernandez, 1999). The upper areas of the watersheds are nearly pure softwood stands 80-120 years old including red spruce (Picea rubens Sarg.), balsam fir (Abies balsamea L.) and hemlock (Tsuga Canadensis L. Carr).  
Softwood, mixed wood,

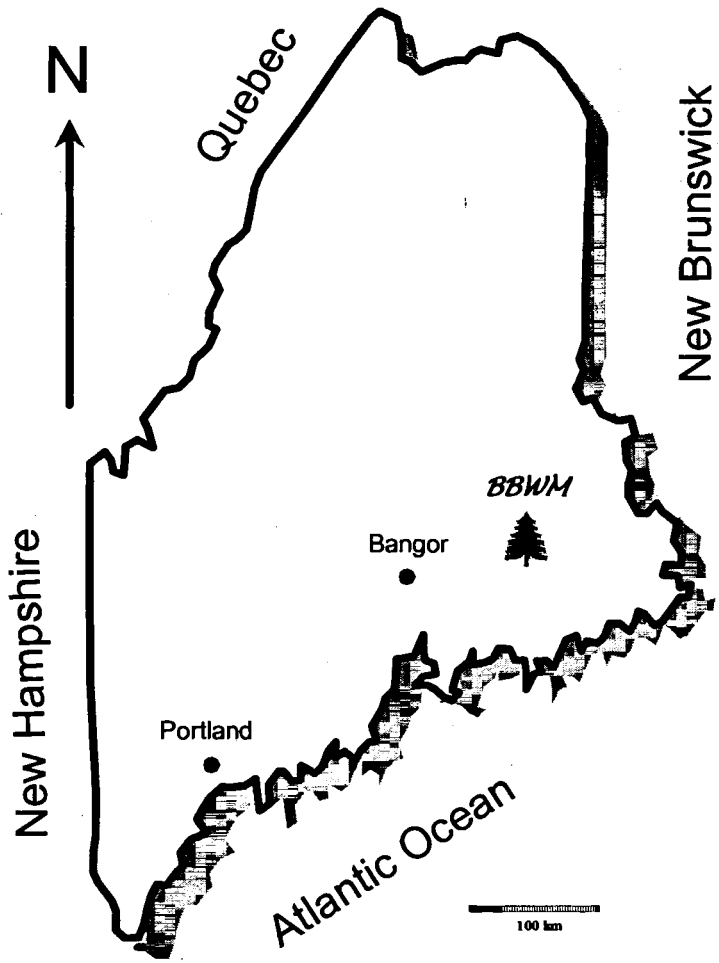


Fig. 1.1. Relative location of the Bear Brook Watershed in Maine.

and hardwoods cover approximately 25, 40, and 35% of the total watershed areas, respectively (Wang and Fernandez, 1999).

The soils are acidic, have low base saturation, cation exchange capacity, and sulfate adsorption capacity (Norton et al., 1999). Bedrock geology consists of metamorphosed quartzites and calc-silicate gneiss. Further details of the study site can be found in Norton et al. (1999) and Fernandez and Adams (2000).

Nitrogen additions to the West Bear watershed were initiated in 1989 and consisted of bimonthly additions of dry  $(\text{NH}_4)_2\text{SO}_4$  typically with two applications to the snowpack, two during the growing season, one in the spring and one in the fall. The West Bear watershed receives  $25.2 \text{ kg N ha}^{-1}\text{year}^{-1}$  of N treatments resulting in estimated total N inputs (wet + estimated dry + treatment) of  $33.6 \text{ kg N ha}^{-1}\text{year}^{-1}$ . The reference East Bear watershed receives  $8.4 \text{ kg N ha}^{-1}\text{year}^{-1}$  of ambient wet plus estimated dry deposition (Norton et al., 1999).

### **Experimental Design**

Within each watershed, four 10x10 m plots were established with two of the four plots in each watershed in hardwoods and two in softwoods. Plots were chosen to have comparable slopes, dominant tree species, and proximity to streams between watersheds. Four replicate soil samples were collected from each 10x10 m plot on five different dates (September 19, 2000, June 11 and 12, 2001, July 2, 2001, August 6 and 7, 2001 and September 17, 2001). *In-situ* incubations varied for logistical reasons by sampling period and were 35, 28, 21, 14, and 14 days, respectively. Soil sampling depth increments included the O horizon and the uppermost 15 cm of the B horizon using a 15x15 cm



frame for sampling, excluding the E horizon when present. In the lab, samples were sieved and each sample was then divided into three subsamples. Subsamples were numbered to indicate that they were (1) “time zero” or the initial NO<sub>3</sub>-N and NH<sub>4</sub>-N extractions prior to incubations, (2) subsamples for the 14 day laboratory incubations, or (3) subsamples that were placed in labeled polyethylene bags and re-buried for *in-situ* net N mineralization for 14 to 35 days according to Eno (1960).

Soils were transported on ice packs and stored at 4°C prior to extraction. Soil extractions were completed within 24 hours of collection. Extracts were frozen until they could be analyzed for NH<sub>4</sub>-N and NO<sub>3</sub>-N. Analyses were conducted by similar methods at both the Institute of Ecosystem Studies (IES) in Millbrook, NY and the University of Maine's Analytical Laboratory. A subset of samples were analyzed at both laboratories to assure data quality with excellent agreement between facilities.

### **Net N Mineralization and Net Nitrification**

Net N mineralization and net nitrification were assessed using both a 14-day laboratory incubation (Hart et al., 1994) and an *in situ* method (Eno 1960). Both methods were chosen to assess N mineralization and net nitrification in order to estimate actual rates in the field exposed to variable temperatures (*in situ* method) as well as measuring the potential for mineralization by the nitrifying communities present in the soil under more ideal laboratory conditions (14-day laboratory incubation method). While laboratory incubations do not quantitatively measure actual rates of these processes under field conditions, they are widely used for their value as an index of N dynamics and for some of their practical advantages in the context of field research. Net N mineralization

is defined as the difference between the sum of  $\text{NO}_3\text{-N}$  and  $\text{NH}_4\text{-N}$  before and after incubation, while net nitrification is the difference for  $\text{NO}_3\text{-N}$  alone.

Field moist O horizon soils were sieved through a 6 mm mesh sieve and mineral soils were sieved through a 2 mm mesh sieve. A  $15 \pm 0.05$  g subsample of field moist soil was placed in a plastic cup and incubated in the dark at  $\sim 22^\circ\text{C}$  for 14 days. A  $\sim 5$ g subsample of field moist soil was also used to determine oven-dry moisture content (O horizon soils were dried at  $65^\circ\text{C}$  and mineral soils were dried at  $105^\circ\text{C}$ ). At the initiation of the experiment, "time zero" subsamples were extracted immediately with 100 mL of 2M KCl to determine initial  $\text{NO}_3\text{-N}$  and  $\text{NH}_4\text{-N}$  concentrations. After 14 days of laboratory incubation or 14-35 days of *in-situ* incubation, soils were extracted as above. Net N mineralization was calculated as  $\text{NO}_3\text{-N}$  plus  $\text{NH}_4\text{-N}$  (at time 14 days for laboratory incubations or varying *in situ* times) minus  $\text{NO}_3\text{-N}$  plus  $\text{NH}_4\text{-N}$  (time zero for initials). Soil pH was determined in deionized water according to Hendershot et al. (1993) for samples collected in September 2000. Soil pH from this research was highly correlated with soil pH measured during a quantitative soil study in 1998 at BBWM (Fernandez, unpublished data) when evaluated at the plot level. Concentrations of  $\text{NO}_3\text{-N}$  and  $\text{NH}_4\text{-N}$  were determined on an OI Analytic Dual-Channel Automated Ion Analyzer at the University of Maine's Analytical Laboratory and on a Perstop Flow Solutions 3000 Injection Analyzer at IES.

### **N Budget Calculations**

Biomass N was calculated as the sum of above and belowground biomass N content, both of which were estimated using allometric equations from the literature.

Total soil N was calculated from a quantitative soil study conducted in 1998 at BBWM (Fernandez, unpublished data). Total N in the mineral soils was defined in this study as all soil below the O horizons, excluding the E horizon, but including all B and C horizons to a 1 m depth. Total pedon values for total N were the sum of the O horizon and all mineral soils. Extractable N data from the 1998 soil studies were only available for the O horizons and mineral soils to a depth of 5 cm. Annual *in situ* net N mineralization was taken as growing season net N mineralization and calculated for May 1, 2001 to October 31, 2001. Since soils were collected in June, July, August and September 2001, June and September 2001 were used to extrapolate estimates for both May and October.

### **Statistical Design**

The statistical design was a split-split plot between treatments, forest types and time. In this design, factor A was the East and West Bear watersheds, factor B was the hardwood and softwood forest types and factor C was time. Analyses were performed separately on O horizons and mineral soils given the dramatically different characteristics of each horizon. Data required rank transformations and were subsequently analyzed by ANOVA on the Statistical Analysis System (SAS System, 1999) with an alpha level of 0.05.

## RESULTS AND DISCUSSION (Net Nitrogen Mineralization and Net Nitrification)

### Watershed Effects

Organic horizons potential net N mineralization (NNM) was significantly higher in the treated West Bear watershed compared to the reference East Bear watershed (Table 1.1). Wang and Fernandez (1999) reported potential NNM after four years of treatment at BBWM and found no significant differences between the watersheds. They attributed their findings to either no treatment effect on potential NNM, or that differences were masked by high variability in the data. Furthermore, they pointed out that watershed level comparisons did not take into account the effects of forest type and suggested that watershed by dominant forest type contrasts were more appropriate. The occurrence of significantly higher potential NNM in West Bear O horizons in this study, after twelve years of continuous treatment, could reflect the evolution of N accumulation in the treatment watershed. All of the net N mineralization results showed a consistent trend towards higher numerical means in West Bear compared with East Bear. West Bear *in situ* net N mineralization means were nearly 40% higher in the O horizons of West Bear but nearly the same in the mineral soils although neither was significantly different between watersheds. It is logical that potential NNM, an indicator of incipient change carried out under more ideal laboratory conditions for microbial communities might best reveal a shift in N dynamics in the O horizon before *in situ* NNM, measured under variable field conditions that are often less than ideal.

Table 1.1. Net N mineralization and net nitrification ( $\text{mg N kg}^{-1} \text{ soil d}^{-1}$ ) for both watersheds and forest types (standard errors in parentheses)

	Soil Horizon	Net N mineralization		Net Nitrification	
		— $\text{mg N kg}^{-1} \text{ soil day}^{-1}$ —		— $\text{mg N kg}^{-1} \text{ soil day}^{-1}$ —	
		<i>In-situ</i>	14 day lab incubation	<i>In-situ</i>	14 day lab incubation
<u>Watershed</u>					
East Bear	O Horizon	3.00 (0.46)	4.82 (0.53) †	0.07 (0.03) †	0.14 (0.05) †
	Mineral	0.24 (0.09)	0.91 (0.09)	0.13 (0.02) †	0.21 (0.04) †
West Bear	O Horizon	4.25 (0.59)	10.30 (1.07)	0.83 (0.15)	2.68 (0.37)
	Mineral	0.21 (0.07)	1.01 (0.19)	0.19 (0.07)	0.71 (0.18)
<u>Forest Type</u>					
Softwood	O Horizon	2.17 (0.25) †	4.76 (0.47) †	0.19 (0.04)	0.55 (0.11)
	Mineral	0.32 (0.09)	1.28 (0.09) †	0.20 (0.02)	0.61 (0.07)
Hardwood	O Horizon	5.07 (0.66)	10.36 (1.09)	0.72 (0.16)	2.27 (0.40)
	Mineral	0.13 (0.07)	0.65 (0.18)	0.12 (0.07)	0.31 (0.17)

† indicates significance at the 0.05 level for contrasts within either watershed or forest type within horizons.

Increased N mineralization rates in northeastern forests soils in response to experimental N enrichment have been reported previously at the Harvard Forest, Massachusetts (Magill et al., 1997), Mt. Ascutney, Vermont (Aber et al., 1995), and the Fernow Experimental Forest, West Virginia (Gilliam et al., 2001). Increased N mineralization rates at the beginning of N additions are attributed to a fertilization effect (Aber et al., 1998). Aber et al., (1998) hypothesized that during stages 2 and 3 along a temporal N saturation continuum, N mineralization rates decrease due to one of two hypotheses: 1) increased N deposition results in the randomization of chemical bond structures in N containing soil organic matter thereby reducing efficiencies of extracellular catabolic enzymes resulting in decreasing decomposition rates or 2) the production of humus-degrading microbes is suppressed in the presence of elevated N.

Stream chemistry for the treated West Bear watershed indicated that BBWM is at stage 2 of N saturation (Aber et al., 1995; Fernandez and Adams 2000). Stage 2 in the N saturation continuum is consistent with higher mean net N mineralization rates found in West Bear compared to East Bear.

Rates of both potential and *in situ* net nitrification (NN) were significantly higher in the West Bear compared to the East Bear watershed in both the O horizon and mineral soils (Table 1.1). The relative difference was much greater for O horizons than mineral soils interpreted as a reflection of responsiveness to added N. Wang and Fernandez (1999) also found significantly higher potential NN rates in the forest floor of the treated West Bear watershed compared to the East Bear watershed early in the study.

Inorganic N in acidic forest soils tends to be dominated by  $\text{NH}_4\text{-N}$  rather than  $\text{NO}_3\text{-N}$ . This is due in part to the fact that  $\text{NH}_4\text{-N}$  is the initial form of inorganic N produced from mineralization with subsequent nitrification often limited in forest soils by organic matter quality and acidity, and that  $\text{NO}_3\text{-N}$  is easily leached. The dominance of  $\text{NH}_4\text{-N}$  was evident in the net N mineralization data from the O horizons in both watersheds (Table 1.2) and has been reported for other sites in the northeastern United States (McNulty et al. 1996; Aber and Melillo 1991). Although the majority of significant differences in this study were observed in potential and *in situ* NN (Table 1.1), differences in net nitrification did not generally translate into significant differences in N mineralization because in most cases mineralized N was almost entirely attributable to  $\text{NH}_4\text{-N}$  (Table 1.2). A notable exception to the  $\text{NH}_4\text{-N}$  dominated soils at BBWM is West Bear mineral soils that showed  $\text{NO}_3\text{-N}$  comprised the majority of net N

mineralization in both hardwood and softwood forest types (Table 1.2) as did the *in situ* East Bear hardwood mineral soils.

That the treated West Bear mineral soils demonstrated higher  $\text{NO}_3\text{-N}$  concentrations compared to  $\text{NH}_4\text{-N}$  is consistent with hypotheses regarding the progression of the stages of N saturation (Aber et al., 1998), where  $\text{NO}_3\text{-N}$  becomes an increasingly larger component of net N mineralization especially in the mineral soils. Fenn et al., (1998) hypothesized that elevated N additions may increase nitrification rates by supporting larger nitrifying microbial populations in soils due to the increase of substrate  $\text{NH}_4\text{-N}$ . Magill et al., (1997) found an increased percentage of net nitrification in relation to net N mineralization in N enriched plots at the Harvard Forest, Massachusetts. They showed that net nitrification, as a percentage of net N mineralization, increased from 17% at the beginning of N additions in 1988 to 51% in 1993, with most of this increase occurring in the mineral soil of a monoculture red pine stand. No clear explanation for the relative importance of net nitrification to net N mineralization in the East Bear hardwood mineral soils is evident but rates of N turnover were exceedingly low in this soil material.

Gilliam et al., (2001) reported that environmental factors such as soil temperature and soil moisture strongly influenced net nitrification rates in the untreated forested watersheds at the Fernow Experimental Forest (FEF) in West Virginia. However, they found that these environmental factors were not as strongly correlated with net nitrification rates in their experimental watershed between four and six years after the beginning of treatments with  $(\text{NH}_4)_2\text{SO}_4$ . They suggested that N additions might alter the relationship between microbes and their environment, causing microbes to become more

Table 1.2. Percentage of net N mineralization comprised of NH<sub>4</sub>-N and NO<sub>3</sub>-N averaged over the five collection periods.

Watershed	Forest Type	Horizon	Method	N min dominated by NH <sub>4</sub> -N (%)	N min dominated by NO <sub>3</sub> -N (%)
East Bear	Hardwood	O Horizon	14 day	98	2
			<i>in situ</i>	98	2
		Mineral	14 day	78	22
			<i>in situ</i>	0	100
East Bear	Softwood	O Horizon	14 day	98	2
			<i>in situ</i>	98	2
		Mineral	14 day	77	23
			<i>in situ</i>	60	40
West Bear	Hardwood	O Horizon	14 day	75	25
			<i>in situ</i>	79	21
		Mineral	14 day	21	79
			<i>in situ</i>	7	93
West Bear	Softwood	O Horizon	14 day	88	12
			<i>in situ</i>	84	16
		Mineral	14 day	28	72
			<i>in situ</i>	16	84



sensitive to N additions rather than environmental factors. Koopmans et al., (1995) found similar results in coniferous forests in the Netherlands where environmental factors were not as strongly correlated with net nitrification rates in the high N deposition plots compared to the ambient and low N deposition plots. No significant correlations between soil temperature or soil moisture and potential or *in situ* NNM were found in our data at BBWM. The disparity in results between this study and that of Gilliam et al., (2001) may be due to climatic or soil differences between the sites. The Fernow Experimental Forest has a mean annual temperature 5<sup>o</sup> C higher and receives on average 15 cm year<sup>-1</sup> more precipitation than BBWM leading to warmer soil temperatures and possibly higher moisture contents in the soil (Fernandez and Adams 2000).

### **Forest Type Effects**

Table 1.1 shows the potential and *in situ* NNM means by major forest type at the BBWM. Both potential and *in situ* methods showed significantly higher rates of net N mineralization in hardwood compared to softwood O horizons. In contrast, there was significantly lower potential NNM in hardwood compared to softwood mineral soils. Net nitrification results showed no significant differences between forest types although numerical trends paralleled the contrasting patterns seen in NNM between O and mineral soil horizons.

Opposing trends in net N mineralization between organic and mineral horizons in hardwoods versus softwoods have been shown in other studies in the northeastern United States (Aber et al., 1993; Fernandez et al., 2000; Campbell et al., 2000; Finzi et al., 1998).

Campbell et al., (2000) attributed the higher N cycling rates in hardwood O horizons to differences in soil moisture, pH, and biotic controls related to competition between plants and microbes. They found lower rates of N mineralization in the mineral soils although there were no significant differences in mineral soils between forest types.

Fernandez et al., (2000) looked at potential NNM, potential NN and potential net ammonification at 20 hardwood stands and 9 softwood stands across Maine including the reference watershed at BBWM. They found significantly higher O horizon potential NNM and potential NN in hardwoods compared to softwoods. They also found that both forest types had similar O horizon N concentrations, but total C concentrations were higher under softwoods leading to a higher C/N ratio under softwoods. Higher C/N ratios are often correlated with lower N mineralization rates in forest soils (Fernandez and Adams 2000; Vitousek et al., 1982; Blair et al., 1990). Quantitative soil excavations and analyses at BBWM (Fernandez, unpublished data) showed softwoods had significantly higher C/N ratios than hardwoods in both organic and mineral horizons (Table 1.3) which could explain why hardwoods had significantly higher NNM rates in the O horizons compared with softwoods.

Results from the European NITREX project showed C/N ratios could be an indicator of  $\text{NO}_3\text{-N}$  leaching (Gunderson et al., 1998; Dise et al., 1998). Gunderson et al., (1998) found that conifer stands in temperate forest ecosystems in Europe having a C/N ratio below 25 leached  $\text{NO}_3\text{-N}$  or had elevated  $\text{NO}_3\text{-N}$  concentrations. They suggested a C/N ratio continuum where the potential for  $\text{NO}_3\text{-N}$  leaching is low with O horizon C/N ratios above 30, moderate for sites with C/N ratios between 25-30 and high for those with C/N ratios below 25. Thus, in addition to soil moisture, pH, and competition, litter

Table 1.3. Soil pH, total N, total C and total C:N ratio by horizon and depth from the quantitative soil excavations in 1998 (standard errors given in parenthesis).

Watershed	Forest Type		pH <sub>w</sub>	Total N	Total C		Total C:N	Fine Earth kg ha <sup>-1</sup>
					-----mg kg <sup>-1</sup> -----			
East Bear	Hardwoods	O horizon	4.01 (0.06) †	1.56 (0.06)	34.3 (1.49) †	22 (0.99) †	82652 (9957) †	
		5-cm	4.28 (0.04) †	0.49 (0.06)	8.83 (0.84)	19.41 (0.81) †	146059 (20177)	
		5-25 cm	4.66 (0.03) †	0.32 (0.03)	6.14 (0.52)	20.24 (0.56) †	804855 (99647)	
East Bear	Softwoods	O horizon	3.54 (0.04) ††	1.41 (0.03)	40.64 (0.81) ††	29.20 (0.94) ††	159393 (27270) †	
		5-cm	4.07 (0.05) †	0.39 (0.04)	9.28 (0.82)	24.39 (0.62) ††	144733 (12750)	
		5-25 cm	4.36 (0.06) †	0.32 (0.03)	7.69 (0.70)	24.08 (0.41) ††	680275 (60916)	
West Bear	Hardwoods	O horizon	3.97 (0.06) †	1.44 (0.05)	33.63 (1.45)	23.31 (0.54)	86111 (19293) †	
		5-cm	4.18 (0.05) †	0.43 (0.03)	8.59 (0.64)	19.92 (0.37) †	120254 (11001)	
		5-25 cm	4.59 (0.05) †	0.33 (0.03)	6.65 (0.57)	20.60 (0.39) †	704367 (81687)	
West Bear	Softwoods	O horizon	3.69 (0.05) ††	1.49 (0.05)	37.54 (1.26) ‡	25.68 (1.25) ‡	139519 (11661) †	
		5-cm	4.09 (0.04) †	0.46 (0.05)	9.81 (0.95)	22.22 (0.67) &	143093 (12571)	
		5-25 cm	4.43 (0.04) †	0.30 (0.02)	6.47 (0.38)	22.28 (0.68) ††	768772 (50000)	

"†" indicates significance between vegetation types within a watershed at the 0.05 level.

"‡" indicates significance between watersheds within a vegetation type at the 0.05 level.

quality (e.g., C/N ratio) also is a determinate of NNM and NN. Table 1.3 shows softwood stands at BBWM have O horizon C/N ratios of 29 and 26 for East and West Bear watersheds, respectively (Table 1.3); indicating that the softwood stands at BBWM may be leaching moderate amounts of NO<sub>3</sub>-N according to the findings of Gunderson et al. (1998). During 1997 the West Bear watershed discharged ~5 kg N ha<sup>-1</sup> while East Bear discharged ~0.1 kg N ha<sup>-1</sup> (Kahl et al., 1999) paralleling the differences in the C/N ratios between watersheds. Similar to Gunderson et al., (1998), Dise et al. (1998) examined the hypothesis that C/N ratios of the O horizons could be used to estimate the

level of  $\text{NO}_3\text{-N}$  leaching from an ecosystem, but furthered Gunderson et al. (1998) work by examining a range of N deposition conditions and evaluated how N deposition affects both C/N ratios and  $\text{NO}_3\text{-N}$  leaching. Dise et al., (1998) found that at low levels of N deposition ( $<9 \text{ kg N ha}^{-1} \text{ year}^{-1}$ )  $\text{NO}_3\text{-N}$  leaching was minimal regardless of the O horizon C/N ratio. At intermediate ( $9\text{-}18 \text{ kg N ha}^{-1} \text{ year}^{-1}$ ), high ( $18\text{-}30 \text{ kg N ha}^{-1} \text{ year}^{-1}$ ), and very high ( $>30 \text{ kg N ha}^{-1} \text{ year}^{-1}$ ) levels of N deposition,  $\text{NO}_3\text{-N}$  leaching increased with increasing N deposition and decreasing C/N ratios. BBWM receives an estimated ambient total N deposition of  $8.4 \text{ kg N ha}^{-1} \text{ year}^{-1}$ , with the treated West Bear watershed receiving  $33.6 \text{ kg N ha}^{-1} \text{ year}^{-1}$  as both ambient atmospheric deposition plus treatment (Kahl et al., 1999). According to Dise et al., (1998) the very high N deposition ( $>30 \text{ kg N ha}^{-1} \text{ year}^{-1}$ ) to West Bear should induce higher  $\text{NO}_3\text{-N}$  leaching compared to East Bear. This is reflected in the higher stream  $\text{NO}_3\text{-N}$  export in West Bear compared with East Bear (Kahl et al., 1999). However, other parameters need to be taken into account when predicting  $\text{NO}_3\text{-N}$  leaching besides C/N ratios and amounts of N deposition such as site and land-use history (Goodale and Aber 2001; Ollinger et al., 2002; Gunderson et al., 1998; Dise et al., 1995, 1998).

Studies examining C/N ratios as a predictor of  $\text{NO}_3\text{-N}$  leaching, N mineralization, and nitrification have also been conducted in the U.S. (McNulty et al., 1991; Lovett and Reuth 1999; Goodale and Aber 2001; Ollinger et al., 2002). Typically negative correlations have been reported between O horizon soil C/N ratios and N mineralization and nitrification rates (Goodale and Aber 2001; McNulty et al., 1991; McNulty et al., 1996; Ollinger et al., 2002). The strongest correlations are usually between soil C/N ratios and nitrification rates. Lovett and Reuth (1999), Ollinger et al., (2002) and

McNulty et al., (1996) all reinforced the premise that a threshold C/N ratio of 20-25 in both softwoods and hardwoods exists where nitrification sharply increases at or below this range.

The discussion of O horizons above, where lower C/N ratios correlate with higher net N mineralization and NO<sub>3</sub>-N leaching, may explain why the O horizons in the hardwood stands have significantly higher net N mineralization rates compared to the softwood stands. It does not explain why the opposite trend exists in the softwood mineral soils. We hypothesize one of two possibilities for the softwood mineral soil results. The first hypothesis is that O horizons in hardwoods mineralize N at a faster rate than softwoods because of higher tissue N concentrations (Nadelhoffer et al., 1995) and more rapid rates of litter decomposition (Finzi et al., 1998). This leaves only the more recalcitrant humic materials to illuviate into the mineral soils below. In contrast, softwood litter is slower to decompose in the O horizons because of its higher lignin:N and C/N ratios (Ferrari 1993), resulting in less O horizon mineralization and humification with more mineralizable substrate illuviating into the mineral soils. Thus, more labile C is available in softwood mineral soils to respond to N enrichment. The second hypothesis is that softwood and hardwood mineral soils mineralize N at similar rates, but softwood mineral soils are more sensitive to increased N deposition causing an increase in net N mineralization to result in the softwoods with N enrichment. A higher sensitivity to N deposition in softwoods for N mineralization has also been hypothesized by others (Campbell et al., 2000; Aber et al., 1995). Campbell et al. (2000) hypothesized that softwood species may be more sensitive to N deposition if softwood sites have lower

rates of N assimilation into foliage and bolewood compared to hardwood sites (Nadelhoffer et al., 1995).

No significant differences were observed for potential or *in situ* NNM or NN between watersheds by dominant stand type (Figure 1.2). Similar numerical patterns were observed between watersheds and forest types: 1) O horizons had higher net N mineralization and net nitrification rates than mineral soils; 2) potential NNM and NN rates were higher than *in situ* NNM and NN rates; 3) hardwood soils, particularly in West Bear, had higher nitrification rates compared to softwoods. Wang and Fernandez (1999) also found no significant differences in potential NNM between the two watersheds by dominant stand type. In this study, West Bear hardwood O horizon soils had twice the rates of potential and *in situ* NNM than East Bear hardwood soils (Figure 1.2) after twelve years of continuous treatment. The higher rates of potential and *in situ* NNM in West Bear hardwoods compared to East Bear hardwoods could reflect the progressive accumulation of ecosystem N. The lack of significant differences in potential NNM between watersheds by dominant stand type in both this study and Wang and Fernandez (1999) could be attributed to: (a) high variability, (b) rapid immobilization of added N by soil microbes and plant roots, (c) adsorption of  $\text{NH}_4\text{-N}$ , (d) denitrification, or (e) nitrification and subsequent  $\text{NO}_3\text{-N}$  leaching. In response to these possibilities, (a) high variability in this study was evident by the large standard deviation of the mean (120% of the mean for the 14 day laboratory incubations and 160% of the mean for the *in situ* incubations), (b) immobilization by soil microbes and plant roots was not in the scope of this study, however, there is evidence for this immobilization mechanism in both soil

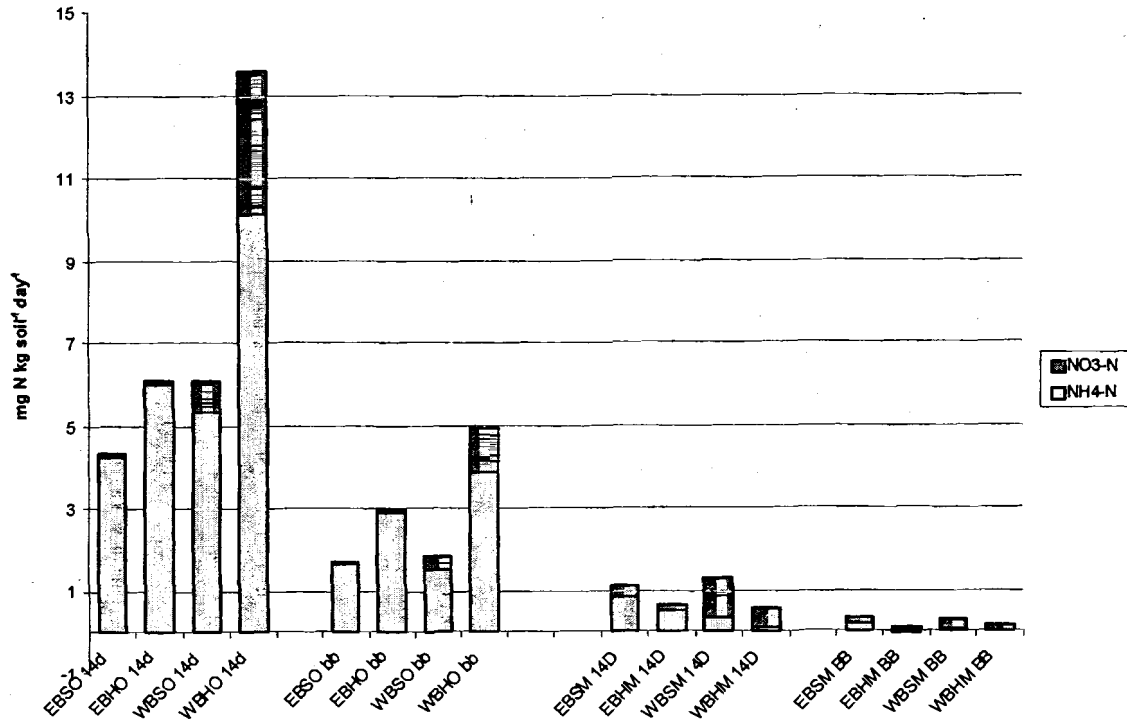


Fig. 1.2. Net N mineralization between watersheds, tree types and soil horizons in both the laboratory and *in situ* incubated samples. Note: WB – the West Bear watershed; EB – the East Bear watershed; S – softwood forest type; H – hardwood forest type; O – O horizons; M – mineral soils; 14D – 14 day laboratory incubations; IS – *in situ* incubations.

biota and trees from previous work at BBWM (White et al. 1999; Nadelhoffer et al., 1999), (c) given the high CEC of the soils at BBWM as well as the greater exchangeable  $\text{NH}_4\text{-N}$  in the West Bear watershed, it is probable that there was adsorption of  $\text{NH}_4\text{-N}$ , (d) there is no denitrification data from BBWM at this time, (e) there is evidence of increased nitrification and  $\text{NO}_3\text{-N}$  leaching in streams as noted above from the treated West Bear watershed at BBWM (Kahl et al. 1999).

### **Temporal Patterns**

There was a temporal trend for declining NNM and potential NN over time in this study (Figure 1.3). These trends appear to be attributable to declining moisture over the study period (Figure 1.4). Both temperature and moisture strongly influence microbial activity in soils and subsequently N mineralization and nitrification rates (Arnold et al., 1999; Sarathchandra et al., 1989). Although warmer soils can lead to increased N mineralization rates (Fenn et al., 1998), warmer soils can also lead to decreases in soil moisture that may have negative effects on microbial populations (Van Gestel et al., 1993). Arnold et al., (1999) reported on microbial biomass at different experimental soil temperatures and resultant moisture regimes in the O horizons at the Howland Integrated Forest Study site in Maine. They suggested that a moisture threshold may exist in the O horizons between 20-120% moisture content, above which soil temperature had a strong influence on microbial biomass, and below which moisture was the dominant factor limiting microbial activity. Results from this study support the hypothesis that a moisture threshold exists in the O horizons of these forest soils between approximately 75 to 130%



moisture content (Figure 1.3). Above this threshold, soil temperature had a greater influence on soil microbial processes compared to soil moisture and below this threshold the opposite was true. Since *in situ* NNM rates declined after the July 2001 collection (Figure 1.4) coincidental with an O horizon soil moisture decline to approximately 75% (Figure 1.3), we hypothesize that moisture was primarily responsible for the decline in *in situ* NNM rates and controlled the temporal pattern of *in situ* NNM over time in this study. This does not negate the possibility of substrate availability, substrate quality, phenology or other factors contributing to these results. The decline of *in situ* NNM rates were most clearly demonstrated by East and West Bear hardwoods and West Bear softwoods (Figure 1.4). Similar declines were evident in potential NNM and potential NN but the declines in potential NNM did not occur until after the August 2001 collection. Potential NNM may have demonstrated an effect of declining moisture later because soil mixing and laboratory incubations at  $\sim 22^{\circ}\text{C}$  made microbial communities less sensitive to moisture stress compared to *in situ* measurements subjected to variable field conditions. It should be noted that this trend was not demonstrated in the mineral soils where relative moisture declines were less marked over the duration of the study.

### **Soil N Content and Watershed N Budgets**

Similar trends in soil N dynamics were observed between watersheds and forest types when data were expressed on a mass of N per unit surface area basis, as was reported above for soil concentration data. Notable exceptions that differed from the concentration results for the main effects of watershed and forest type were: (1) *in situ*

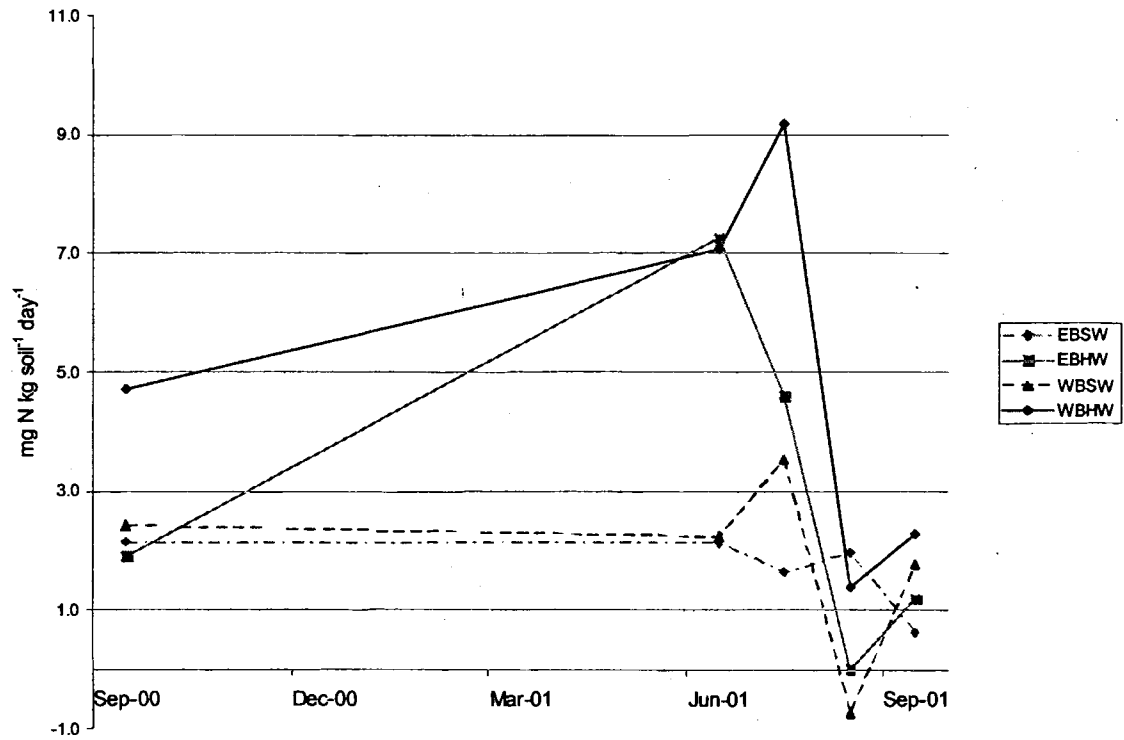


Fig. 1.3. *In situ* net N mineralization compartmental rates over time. EBSW – East Bear watershed, softwood forest type; EBHW – East Bear watershed, hardwood forest type; WBSW – West Bear watershed, softwood forest type; WBHW – West Bear watershed, hardwood forest type.

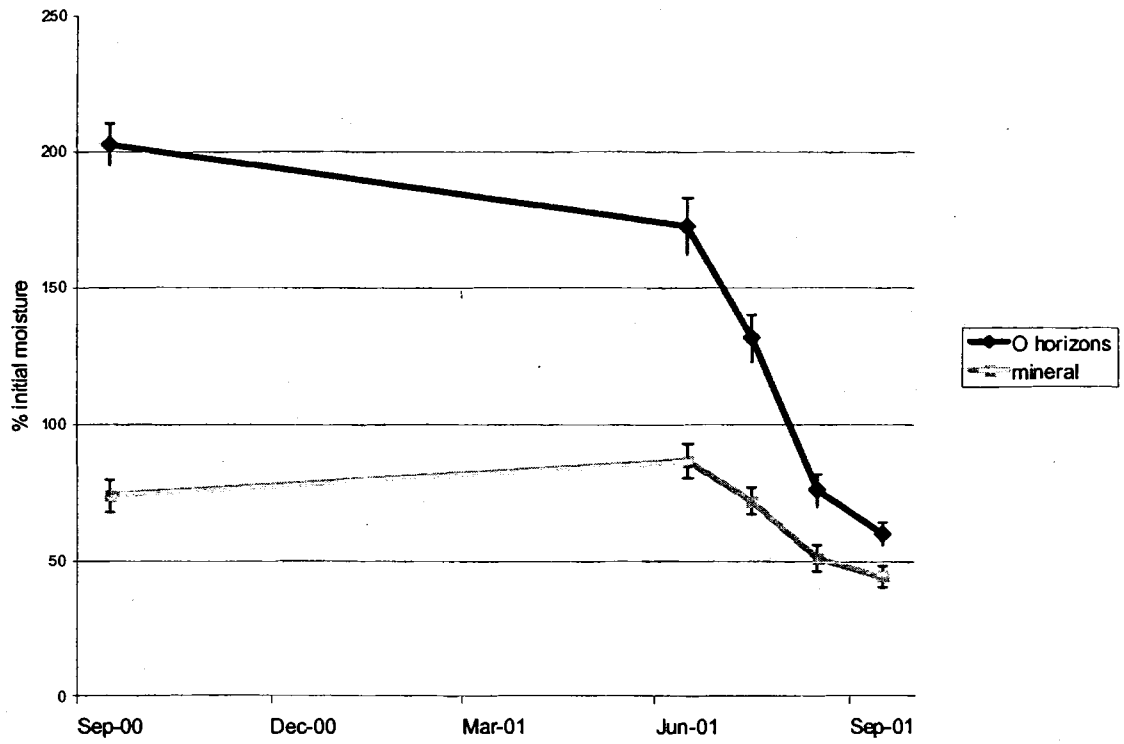


Fig. 1.4. O horizon and mineral soil percent initial moisture over time (standard errors in parentheses).

NNM had significantly higher means in West Bear compared with East Bear in the O horizons, and (2) potential NNM was no longer significantly higher in hardwood O horizons compared to softwood O horizons or softwood mineral soils compared to hardwood mineral soils. The loss of a statistical significance was the result of the slightly different soil masses between watersheds although numerical trends remained the same. Table 1.4 shows mass per unit area data for the interaction between watershed and forest type for both net N mineralization and net nitrification. West Bear NNM was significantly higher than East Bear for both softwoods and hardwoods. It is noteworthy

Table 1.4. In situ net N mineralization and nitrification ( $\text{kg N ha}^{-1} \text{ soil day}^{-1}$ ) for watersheds by forest type (standard errors in parentheses).

	West Bear		East Bear	
	----- $\text{kg N ha}^{-1} \text{ soil day}^{-1}$ -----		----- $\text{kg N ha}^{-1} \text{ soil day}^{-1}$ -----	
	Softwoods	Hardwoods	Softwoods	Hardwoods
	<u>net N mineralization</u>			
O Horizon	0.476 (0.064) †	0.464 (0.079) †	0.100 (0.029)	0.386 (0.075)
Mineral	0.076 (0.012)	0.071 (0.069)	0.139 (0.059)	0.065 (0.021)
	<u>net nitrification</u>			
O Horizon	0.066 (0.015)	0.103 (0.020)	0.004 (0.002)	0.009 (0.004)
Mineral	0.071 (0.008)	0.063 (0.067)	0.055 (0.011)	0.063 (0.015)

† indicates significance between watersheds by forest type at the 0.05 level.

that the increase in West Bear over East Bear in the O horizons for NNM is ~5X for softwoods but only ~1.2X for hardwoods. The higher softwood NNM in West Bear compared to the reference East Bear watershed suggests that this forest type may be more responsive to changes in N dynamics in the forest floor after twelve years of treatment, in contrast to results from earlier in the BBWM experiment when it was suggested that hardwoods had a higher sensitivity to treatments (Wang and Fernandez 1999). The data reported here show that both forest types have a higher and now roughly equal rate, of NNM in the O horizons presumably as a result of the different relative increases they showed in response to treatments.

Annual net N mineralization and net nitrification were estimated using data extrapolated from May 1 to October 31 and assuming no N turnover during the dormant season. These data were then used to construct a simple N budget for BBWM to provide a whole ecosystem context for data from this study. Estimated biomass N was small compared to total soil N, and represented only 6-7% of total soil N in both watersheds while estimated annual NNM was ~0.6% of total soil N in East Bear and only ~1% in West Bear. Although the majority of total soil N was found in the mineral soils, the majority of NNM occurred in the O horizons in both the treated and reference watersheds (Figures 1.5 and 1.6). A lower rate of decomposition and N turnover in the mineral soils presumably reflects the fact that soil organic matter is older and progressively more recalcitrant with depth (Federer et al., 1983; Persson et al., 1995). The O horizons also contained more extractable N than the mineral soils, a logical byproduct of higher rates of N cycling. Extractable inorganic N was 0.2-0.6% of total N in both watersheds for the O horizons and upper mineral soil. The most notable difference between watersheds was

that total NNM was approximately 60% greater in West Bear compared to East Bear due largely to the higher rates of NNM in West Bear O horizons compared to East Bear O horizons. In contrast to the higher NNM found in the O horizons compared to mineral soils, higher NN was found in the mineral soils compared to O horizons. Net nitrification under N treatment in the mineral soils was also proportionally greater and comprised up to 100% of the NNM in the mineral soils as evidenced by the West Bear mineral soil data (Figures 1.5 and 1.6). In this estimate of the BBWM watershed N budget it should be noted that total soil N includes soil depth increments to the bottom of a one meter pedon. We confined our study of net N mineralization and net nitrification to the O horizon and upper mineral soil increments. This was due to both practical limitations and an expectation that the majority of the N mineralization in these soils occurs in these upper increments as reported in literature (Federer et al., 1983; Persson et al., 1995). Therefore, estimates of whole soil net N mineralization and net nitrification were not possible. Also, since actual measurements were limited to growing season data and winter rates were assumed to be negligible, these annual estimates are likely conservative.

These data for N dynamics at BBWM are comparable to those reported in the literature for other low elevation forests in the region. Literature values for total soil N appear to range from 1034 and 2275 kg N ha<sup>-1</sup> in the O horizon and mineral soil, respectively, for a low elevation spruce-fir forest in Howland, Maine (Fernandez et al. 1993), to 1932 kg N ha<sup>-1</sup> and 877 kg N ha<sup>-1</sup> in the O horizon and mineral soil, respectively, for the Adirondack Region of New York (Mitchell et al. 2001). Federer et al. (1983) reported the total N in a softwood stand in Maine was 1802 and 3224 kg N ha<sup>-1</sup>, and net N mineralization was 15 and 27 kg N ha<sup>-1</sup> year<sup>-1</sup>, for O horizons and mineral soils,

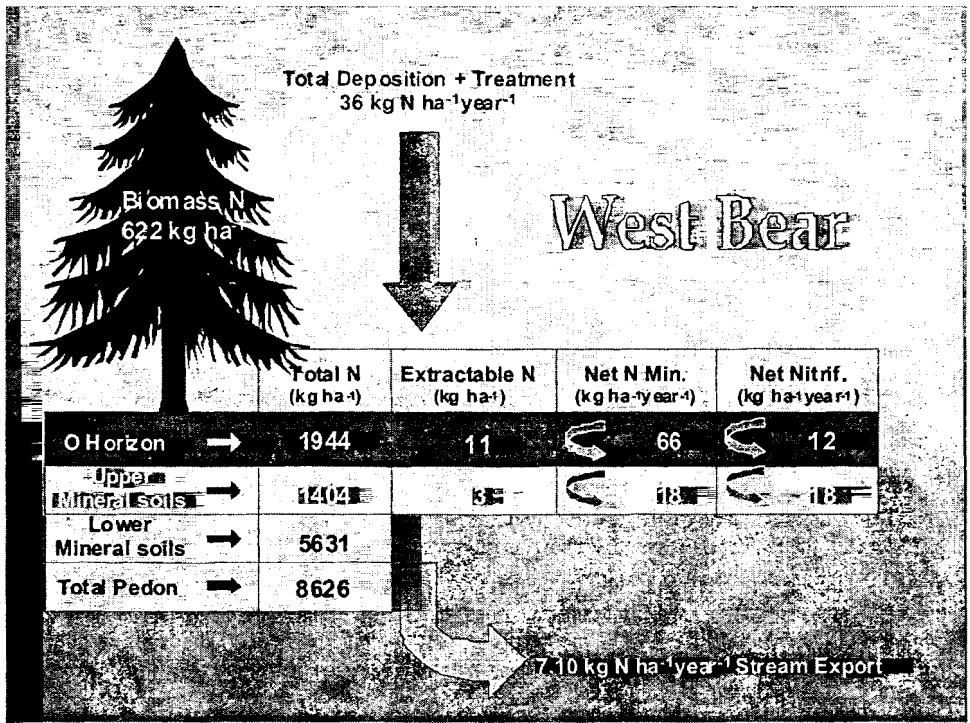


Fig. 1.5. Watershed budget for the West Bear watershed.

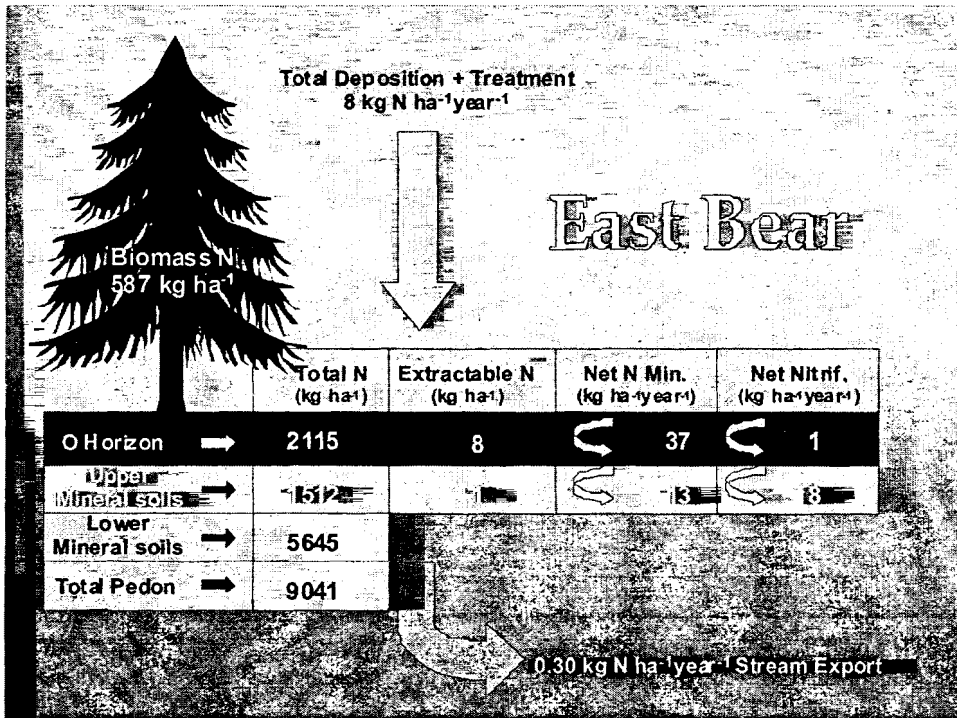


Fig. 1.6. Watershed budget for the East Bear watershed.



respectively. Cole and Rapp (1981) analyzed data from 14 sites included in the International Biological Program (IBP) from around the world and found temperate coniferous forest soils contained on average  $6821 \text{ kg N ha}^{-1}$  and temperate deciduous forest soils contained on average  $5177 \text{ kg N ha}^{-1}$ . Devito et al. (1999) reported net N mineralization values for O horizons in Canadian soils ranged from  $114 \text{ kg N ha}^{-1}\text{year}^{-1}$  for deciduous forest types to  $140 \text{ kg N ha}^{-1}\text{year}^{-1}$  for mixed conifer forest types. They also reported  $52$  and  $46 \text{ kg N ha}^{-1}\text{year}^{-1}$  for deciduous and mixed forest types, respectively, in the upper 10 cm of the mineral soils, which is  $\sim 4$  times that at BBWM. In their measurements of annual net N mineralization, they included the winter months for which they reported high rates of net N mineralization (49 to 92% of annual net N mineralization). It will be important in future research to better define dormant season forest soil N dynamics across a range of climatic regimes for northern forest types. Other researchers have also found similar increases in net nitrification in the mineral soils compared to the O horizons at N treated sites (Andersson et al 2002; Magill et al. 1997). The results from this study suggest higher proportions of mineralized N being nitrified, consistent with the concepts of the evolution of N saturation in forested ecosystems (Aber et al., 1998).

Input-output estimates showed that  $\sim 20\%$  and  $\sim 4\%$  of the total N inputs were exported annually in West and East Bear streams, respectively. Therefore  $\sim 80\%$  of the input N was still retained in West Bear, despite the long-term N amendments to this watershed. Other investigators in both Europe and in the United States have reported high retention of inorganic N even after experimental N additions in forested ecosystems. Bergholm and Majdi (2001) reported 93% retention of N inputs in a Norway spruce stand

in Sweden treated with  $(\text{NH}_4)_2\text{SO}_4$ , and 96% retention for their reference watershed. They suggested that the spruce stand had a relatively high capacity to accumulate N due to high aboveground production. They reported  $326 \text{ kg ha}^{-1}$  in aboveground biomass. Mitchell et al., (1996b) reported that an untreated watershed in the Adirondack State Park of New York retained 74% of wet inputs of N. Similarly, Magill et al., (1997) observed that 85-99% of N additions ( $50$  and  $150 \text{ kg N ha}^{-1}\text{year}^{-1}$  of  $\text{NH}_4\text{NO}_3$ ) were retained at the Harvard Forest in Massachusetts. It must be noted that none of these studies measured gaseous loss, ostensibly by way of denitrification, which may temper the high estimates of N retention. Even so, it is likely that even under ambient deposition and treatment N amendments these forest soils have at once a large potential for further accumulation of N in soil N pools, and yet a high susceptibility for increased N amendments to induce accelerated N mineralization, nitrification, and  $\text{NO}_3\text{-N}$  export in soil solutions and streams.

## **Conclusions**

Our results indicate that after twelve years of whole-watershed experimental N enrichment, the West Bear watershed demonstrated higher rates of net N mineralization and net nitrification consistent with stages in the evolution of N enrichment or “N Saturation”. However, watershed retention of N inputs was nearly 80% in the treated West Bear watershed and over 95% in the reference East Bear watershed, indicating a significant potential in both watersheds for continued N accumulation. Of greater importance are the changes in N dynamics within the ecosystem, which respond to increased N inputs rapidly despite high overall N retention in the watershed. Forest soil

nitrification plays an increasing role in net N mineralization in the treated watersheds that are otherwise dominated by  $\text{NH}_4\text{-N}$ . Increased stream  $\text{NO}_3\text{-N}$  in the treated West Bear watershed may best reflect the notably higher rates of N mineralization in hardwood O horizons compared to softwoods, although higher mineral soil N mineralization was evident in softwoods compared to hardwoods. In addition, earlier studies of N dynamics at BBWM showed little or no response in softwoods to the treatments. This suggests that forest type is important in understanding watershed response to N deposition. The results also indicate that changes in N dynamics not evident earlier in this whole watershed N enrichment experiment are emerging after over a decade of treatments and are providing insights into long-term processes often undetected in research of limited duration.

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## CHAPTER 3. METHODOLOGICAL APPROACHES FOR ASSESSING NET N MINERALIZATION AND NET NITRIFICATION

### INTRODUCTION

A common goal of both researchers and managers concerned with N cycling in forested ecosystems is the evaluation of both the amount of nitrogen (N) in forest soils, N turnover, and its availability to plants. Generally speaking measuring total N in soils has become routine, but this measurement belies the complexity of N forms and their availability. Measuring labile N is much more complex, and methods range from attempts to estimate actual rates of N turnover and availability in the field, to indices of these same N dynamics by laboratory methods wherein more variables can be controlled. Considerable interest in N dynamics in forest soils has also resulted from concerns for the effects of long term atmospheric N deposition on forest ecosystems, and the phenomenon called N saturation, an ecosystem condition where inorganic N exceeds plant and microbial demand (Aber et al., 1989). The most common techniques seek to measure the rates of oxidation of organic N to inorganic N (N mineralization), or the transfer of ammonium ( $\text{NH}_4$ ) to nitrate ( $\text{NO}_3$ ). Measurements of N mineralization can be either measurements of net N mineralization or gross N mineralization (Verchot et al., 2001) with net measurements being much more common due to the ease and relative low cost of the technique. Common approaches to measuring net N mineralization include incubating soils in a laboratory or *in situ* incubations for one or more weeks (Hart et al. 1994; Raison et al. 1987). Researchers in tropical ecosystems often use a 7-day incubation since N mineralization proceeds more rapidly under increased temperature and moisture regimes (Veldkamp et al., 1999; Ellingson et al., 2000). Researchers in

temperate ecosystems have more often used both 14-day and 28-day incubations, both *in situ* and in the laboratory (Knoepp and Swank, 1995, 1996; McNulty et al., 1996; Eno 1960; Wang and Fernandez, 1999; Fernandez et al., 2000; Verchot et al., 2001; Kraske and Fernandez, 1990). Therefore, methodology has been inconsistent in the literature regarding the length of the incubation period when measuring potential net N mineralization using laboratory or *in situ* incubation techniques. Moreover, comparison of laboratory rates to those obtained in the field has brought the former into question (Knoepp and Swank, 1995). We chose to examine the difference between these incubation times and the subsequent effects on net N mineralization to provide insight into the relationship between 7-, 14-, and 28-day incubations. Since a measurement of net nitrification can also be made from N mineralization assays, we also sought to compare NO<sub>3</sub>-N production in our temporal N mineralization assays. We compared *in situ* potential net nitrification (Eno 1960), 14-day laboratory potential net nitrification (Hart et al. 1994) and nitrification potential (Hart et al. 1994) to assess the degree of correlation among methods. We also considered these data in the context of a watershed N manipulation experiment to determine which method might be more sensitive for detecting differences in forest type or N status as a result of long term N amendments which have altered the availability of mineral N. The nitrification potential assay (Hart et al., 1994) measures the activity of the autotrophic nitrifying soil community by providing ideal conditions for nitrification where the limiting factor is no longer NH<sub>4</sub>-N but the nitrifying population itself. Acidic forest soils are thought to be dominated by autotrophic nitrifying populations as opposed to heterotrophic nitrifying populations, the latter for whom assimilable organic-C could also limit growth (Zhu and Carreiro 1999).

## MATERIALS AND METHODS

### Soils Used in this Study

Soils from the Bear Brook Watershed in Maine (BBWM) were used in these methodological comparisons. The BBWM is located in eastern Maine at 44°52' north latitude and 68°06' west longitude, approximately 60 km from the coast of Maine and is located on the upper 210 m of the southeast slope of Lead Mountain. BBWM is a paired watershed experiment that began in 1987 and was established to evaluate a whole ecosystem response to elevated N and S deposition in a low alkalinity forested stream watershed in northern New England (Norton et al., 1999).

The vegetation at BBWM includes both hardwoods and softwoods, with hardwoods and a mix of the two dominating the lower ~60% of the watersheds. Hardwoods include American beech (Fagus grandifolia Ehrh.), sugar maple (Acer saccharum Marsh.), red maple (Acer rubrum L.), with minor yellow birch (Betula alleghaniensis Britt.) and white birch (Betula papyrifera). The higher elevations are nearly pure softwood stands 80-120 years old including red spruce (Picea rubens Sarg.), balsam fir (Abies balsamea L.) and hemlock (Tsuga canadensis L. Carr). Softwood, hardwoods and a hardwood-softwood mix cover approximately 25, 35, and 40% of the total watershed areas, respectively (Wang and Fernandez, 1999).

The soils are acidic, have low base saturation, cation exchange capacity, and sulfate adsorption capacity (Norton et al., 1999). Bedrock geology consists of metamorphosed quartzite and calc-silicate gneiss. Further details of site characteristics can be found in Norton et al. (1999) and Fernandez and Adams (2000).



## **The Nitrogen Amendments**

Nitrogen additions to the West Bear watershed were initiated in 1989 and consisted of bimonthly additions of dry  $(\text{NH}_4)_2\text{SO}_4$  typically with two applications to the snowpack, two during the growing season, one in the spring and one in the fall. The West Bear watershed receives  $25.2 \text{ kg N ha}^{-1}\text{year}^{-1}$  of N treatments resulting in estimated total N inputs (wet + estimated dry + treatment) of  $33.6 \text{ kg N ha}^{-1}\text{year}^{-1}$ . The reference East Bear watershed receives  $8.4 \text{ kg N ha}^{-1}\text{year}^{-1}$  of ambient wet plus estimated dry deposition (Norton et al., 1999). To the experimental design of the plot layout use for sampling is described in Shah et al., (2002).

## **In Situ Net N mineralization and Net Nitrification**

*In situ* net N mineralization and net nitrification were assessed using the buried bag method of Eno (1960). Soils were incubated in the field for 35 and 28 days in September 2000 and June 2001, respectively. Net N mineralization was defined as the difference between the sum of  $\text{NO}_3\text{-N}$  and  $\text{NH}_4\text{-N}$  at initiation and after incubation, while net nitrification was the difference for  $\text{NO}_3\text{-N}$  alone. Soil samples were kept in plastic bags in a cooler for transport from the field to the laboratory. Field moist O horizon soils were sieved through a 6 mm mesh sieve and mineral soils were sieved through a 2 mm mesh sieve. Replicate subsamples (~5g) of field moist soil were used to measure oven-dry moisture content (O horizon soils were dried at  $65^\circ\text{C}$  and mineral soils were dried at  $105^\circ\text{C}$ ). At the initiation of the experiment, "time zero" subsamples were immediately extracted with 100 mL of 2M KCl (solution to soil ratio m/v) to determine initial  $\text{NO}_3\text{-N}$  and  $\text{NH}_4\text{-N}$  concentrations. After incubating in the field, buried soil bags were collected

and extracted as above. Concentrations of  $\text{NO}_3\text{-N}$  and  $\text{NH}_4\text{-N}$  were determined on an OI Analytic Dual-Channel Automated Ion Analyzer at the University of Maine's Analytical Laboratory and on a Perstop Flow Solutions 3000 Injection Analyzer at The Institute of Ecosystem Studies.

### **Laboratory Net N Mineralization and Net Nitrification Incubations**

Net N mineralization and net nitrification were assessed using a 14-day laboratory incubation (Hart et al., 1994). Soils were returned to the laboratory and sieved as described above for the *in situ* incubation. A  $15 \pm 0.05$  g subsample of field moist soil was placed in a plastic cup covered with parafilm perforated for ventilation and then incubated in the dark at  $\sim 22^\circ\text{C}$  for 14 days. A  $\sim 5$ g subsample of field moist soil was also used to determine oven-dry moisture content and correct for the dry mass equivalent of the incubating soils (O horizon soils were dried at  $65^\circ\text{C}$  and mineral soils were dried at  $105^\circ\text{C}$ ). Soils were then extracted and analyzed as described above.

### **Nitrification Potential Assay**

The nitrification potential assay of Hart et al., (1994) was chosen because it measures the rate of nitrification in a soil by creating conditions where  $\text{NH}_4\text{-N}$  is no longer the limiting factor, with the assumption that rates of nitrification are then limited by the capacity of the nitrifying community itself. Samples were handled according to the shaken slurry method described by Hart et al. (1994). A  $15 \pm 0.05$  subsample of soil was taken from each sample and mixed with 100ml of a solution containing 1.5 mM  $\text{NH}_4^+$  and 1 mM  $\text{PO}_4^{3-}$  in a 250-ml flask. Slurries were shaken on a wrist-action shaker for 24

hours. Approximately one-quarter of the slurry was removed at 2, 4, 22, and 24 hours. These subsamples were then filtered through Whatman #40 filter papers that had been previously leached with deionized water to remove any inorganic-N contamination. Samples were analyzed for NO<sub>3</sub>-N on a Perstop Flow Solutions 3000 Injection Analyzer at IES.

### **Statistical Analyses**

The nitrification potential data was analyzed using a repeated measures design on the Statistical Analysis System (SAS System, 1999) with an alpha level of 0.05. The statistical design for the laboratory and *in situ* incubations was a split-split plot among treatments, forest types and time. In this design, factor **A** was the reference East and treated West Bear watershed soils, factor **B** was the hardwood and softwood forest types and factor **C** was time. Analyses were performed separately on O horizons and mineral soils given the clearly different characteristics of each horizon. All data except the nitrification potential required rank transformations and were subsequently analyzed by ANOVA on the Statistical Analysis System (SAS System, 1999) with an alpha level of 0.05.

## RESULTS AND DISCUSSION

### **The Importance of Incubation Time in Measuring Potential Net N Mineralization**

Figures 2.1 and 2.2 show net N mineralization grand means for O horizon and mineral soils respectively, across watersheds and forest types for 7-, 14- and 28-day incubations. Data are expressed on a per day basis to normalize for the obvious differences that would occur from cumulative results over different incubation times. These data suggest that the 7-day incubation results in a mineralization rate distinctly less per unit time with an asymptotic decrease in slope to the 14- and 28-day incubation periods for the O horizon soils (Fig 2.1). The results suggest that using the 7-day incubation in these northern US forest soils would likely underestimate potential net N mineralization and given the slope of the curve at 7 days, perhaps small differences in substrate quality or environmental conditions such as temperature or moisture could have disproportionate influences on the results. Although the 28-day results are higher than the 14-day results in the O horizon soils, the difference between these two longer incubation periods is relatively small and the results from both are highly correlated ( $r^2$  of 0.94) (Figure 2.3). However, the mineral soils suggest that a 14-day incubation would also likely underestimate the potential of these soils to mineralize N. These results of the O horizon soils suggest that either of these longer incubation periods might adequately assess N mineralization potential of a soil, or that scientific and practical considerations could govern the final choice of technique. However, in the mineral soils these data suggest that a 28-day incubation would be needed to adequately assess N mineralization potential of a soil.

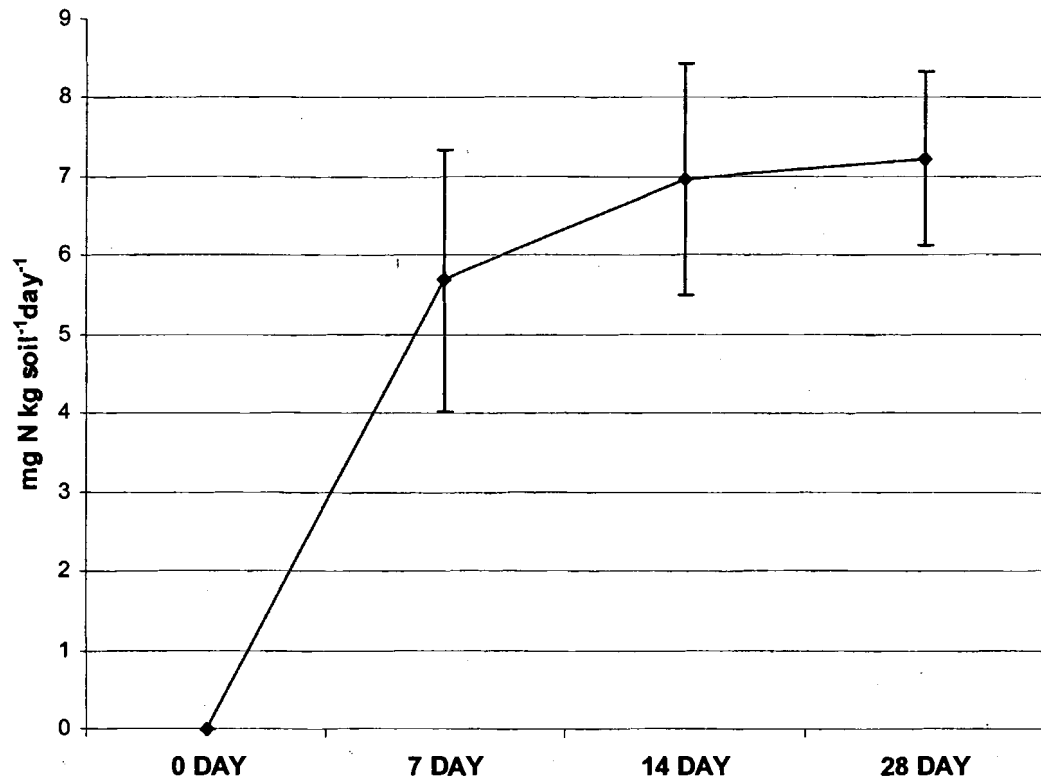


Fig 2.1. Organic horizon net N mineralization compartmental means over 7, 14, 28 day laboratory incubations. Error bars represent standard error of the mean.

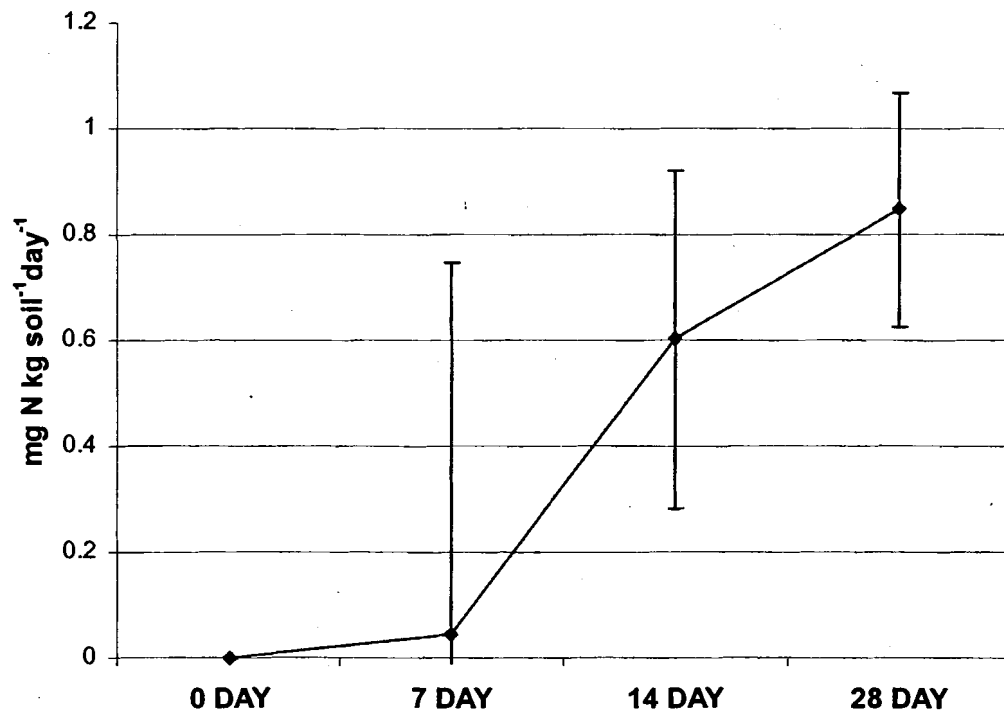


Fig 2.2. Mineral soil compartmental net N mineralization means over 7, 14, 28 day laboratory incubations. Error bars represent standard error of the mean.

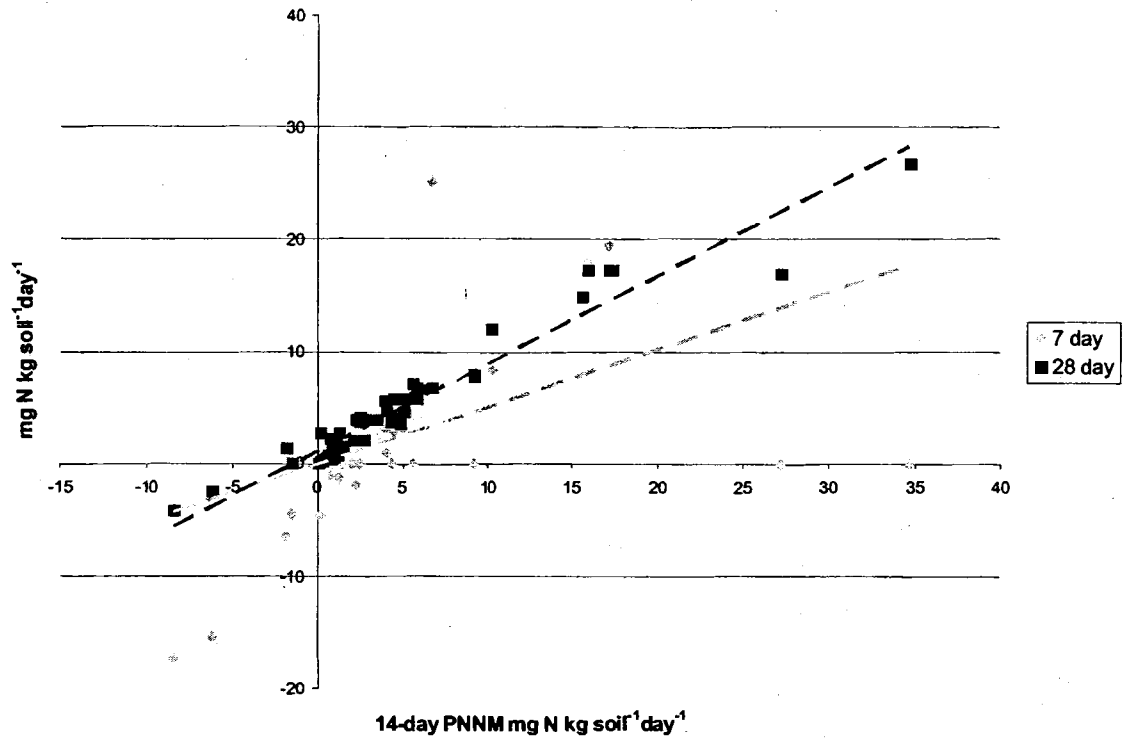


Fig. 2.3. Fourteen day potential net N mineralization vs. 7 day and 28 day potential net N mineralization for both watersheds, forest types and soil horizons in September 2000. The  $r^2$  of the 14 day vs. 7 day is 0.30; the  $r^2$  of the 14 day vs. 28 day is 0.94.

Overall, we would suggest that a 14-day incubation be used to minimize the time involved in obtaining this type of data, while still assuring adequate incubation times for the soil to more fully express its potential for N mineralization by this type of index method. Figure 2.3 also suggests that conclusions drawn on 14- and 28-day ( $r^2$  of 0.94) results might be nearly interchangeable whereas the correlation to 7-day incubation results is much weaker ( $r^2$  of 0.29 between the 7- and 14-day incubations). It is important to note that these results are for a single intensive research site, and similar comparisons should be conducted across a range of forest and soil types in northern climate forest ecosystems to draw conclusions on the best methods to use.

#### **Potential Net Nitrification, *In Situ* Net Nitrification, and Nitrification Potential: A Methodological Comparison**

All three methods were applied to O horizons material and mineral soils from the BBWM and Table 2.1 shows the means and associated standard error of the mean for each method, presented by both watershed and forest type. All three methods showed similar significant differences when N-mineralization rates were compared between watersheds for both O horizon and mineral soils. Each method revealed that N-treatments to the West Bear watershed have resulted in higher potential rates of nitrification. Differences between forest types were less consistent among the methods we used. The *in situ* method resulted in significant differences in O horizons between forest types but not mineral soils, while the nitrification potential assay demonstrated significant differences in the mineral soils between forest types but not O horizons (Table 2.1). Interestingly, the nitrification potential assay was the only method that detected



Table 2.1. Potential net nitrification and nitrification potential for both watersheds and forest types by soil horizon (standard errors in parentheses) † indicates significance at the 0.05 level for contrasts within either watershed or forest type within horizons

Nitrification Potential (mg N kg soil <sup>-1</sup> )				
<u>Horizon</u>	<u>Watershed</u>		<u>Forest Type</u>	
	<u>East</u>	<u>West</u>	<u>Hardwood</u>	<u>Softwood</u>
<u>Organic</u>	5.68 (2.12)†	86.26 (18.05)	77.74 (19.33)	14.2 (4.59)
<u>Mineral</u>	9.31 (1.94)†	28.92 (3.31)	12.44 (2.02)†	26.66 (4.07)

14 day potential net nitrification (mg N kg soil <sup>-1</sup> d <sup>-1</sup> )				
<u>Horizon</u>	<u>Watershed</u>		<u>Forest Type</u>	
	<u>East</u>	<u>West</u>	<u>Hardwood</u>	<u>Softwood</u>
<u>Organic</u>	0.18 (0.09)†	3.21 (0.56)	2.80 (0.61)	0.60 (0.16)
<u>Mineral</u>	0.21 (0.05)†	0.64 (0.31)	0.26 (0.30)	0.60 (0.09)

<i>In situ</i> potential net nitrification (mg N kg soil <sup>-1</sup> d <sup>-1</sup> )				
<u>Horizon</u>	<u>Watershed</u>		<u>Forest Type</u>	
	<u>East</u>	<u>West</u>	<u>Hardwood</u>	<u>Softwood</u>
<u>Organic</u>	0.06 (0.02)†	0.87 (0.20)	0.75 (0.20)†	0.19 (0.05)
<u>Mineral</u>	0.11 (0.02)†	0.16 (0.12)	0.08 (0.12)	0.19 (0.03)

numerically higher means in the mineral soils of the softwoods compared to the hardwoods (Table 2.1). These differences between horizons by forest types has been demonstrated for both *in situ* and 14-day laboratory incubations in a study conducted by Shah et al., (2002) spanning a period of two years, and is consistent with the findings of others for northeastern U.S. forest soils (Aber et al., 1993; Fernandez et al., 2000; Campbell et al., 2000; Finzi et al., 1998). This suggests that the nitrification potential assay may be more sensitive for detecting differences, such as between softwood and hardwood forest types, which are not readily expressed under less than ideal conditions for the relevant microbial communities. It should be noted that all three methods proved to be significantly correlated (Table 2.2).

Table 2.2. Pearson correlation coefficients for 14 day PNN, *in situ* PNN, nitrification potential spanning both collection months (Sept. 00 and June 01). N=40. † indicates significance at the 0.05 level.

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<u>O Horizons</u>		
	<u><i>In situ</i></u>	<u>Nitrification Potential</u>
14 day	+0.81†	+0.62†
<i>In situ</i>		+0.64†

<u>Mineral Soils</u>		
	<u><i>In situ</i></u>	<u>Nitrification Potential</u>
14 day	+0.44†	+0.76†
<i>In situ</i>		+0.42†

Verchot et al. (2001) examined the differences between gross and net mineralization and nitrification and also compared the sensitivity of lab incubations, *in situ* incubations and the nitrification potential assay in determining functional differences in net nitrification between hardwood forest stands dominated by different canopy tree species in Millbrook, NY and the Catskill Mountains of NY. They found that gross rates of N mineralization and nitrification were not good indicators of differences in forest types. Furthermore, they reported that net N mineralization and net nitrification, particularly the laboratory incubation, proved to be better indicators of differences between forest types by using a 14-day incubation technique both in the laboratory and *in situ*. Knoepp and Swank (1995) compared 28-day laboratory incubations to both *in situ* buried bags and *in situ* incubated cores and found the *in situ* incubated cores to be preferable because they found them to best incorporate site-specific changes in moisture and soil temperature. The findings of Verchot et al. (2001) concur with this study in that nitrification potential appeared to be more sensitive to differences in forest types than net nitrification in laboratory incubations or *in situ* incubations.

Although the three methods proved to be significantly correlated, the magnitude of the correlation differed between methods and between soil horizons. In the O horizons, the 14-day laboratory incubation and the *in situ* incubation proved to be best correlated, however in the mineral soils, the 14-day laboratory incubation and the nitrification potential assay proved to be best correlated out of the three methods used (Table 2.2). The differences between the correlation coefficients are likely due to sample size and variability between the three methods.

A clear distinction among the three methods is the mean rate of nitrification as shown in Table 1.1. The nitrification potential assay had the highest rates, nearly an order of magnitude greater than either the 14-day laboratory incubation or the *in situ* incubation. For the latter two methods, the 14-day laboratory incubation rates were one to four times greater than the *in situ* incubation rates. The differences in magnitude among the three methods were logical, in that the nitrification potential assay supplied essentially unlimited  $\text{NH}_4\text{-N}$  to autotrophic nitrifying microbial communities. The difference in magnitude between the laboratory and *in situ* incubations was also expected as laboratory incubations take place in well mixed soils and higher temperature conditions of a laboratory, in this case  $22^\circ\text{C}$ , and without the diurnal fluctuations in temperature that would be experienced *in situ*. Therefore laboratory incubations are expected to provide more consistent but overestimated rates of microbial activity during the incubation period while *in situ* soils would be limited by cool night time temperatures and perhaps the extreme heat of the warmest of days during the field season. Because of these differences, the laboratory incubation may be more effective at detecting differences in the incipient N dynamics of sites, whereas *in situ* incubations are the logical choice for estimating actual rates of nitrification.

A depiction of *in situ* potential net nitrification (PNN) for the interaction of watershed and dominant forest type for both the O horizons and mineral soils at BBWM is shown in Figure 2.4. The high variability in these data resulted in no significant differences among *in situ* PNN means. West Bear hardwood O horizon *in situ* PNN, while not significant, showed a clear numerical trend towards greater rates than all other soil-treatment combinations in this experimental design. Both the 14-day laboratory

incubation and the nitrification potential assay revealed differences among watershed and forest type as reported in Figure 2.4. A further discussion of watershed and forest type differences can be found in Shah et al., (2002).

### **Nitrification Potential Assay**

The nitrification potential assay (NPA) measures the activity of soil nitrifying microbial communities by providing ideal conditions for nitrification where the limiting factor is no longer  $\text{NH}_4\text{-N}$  availability but the nitrifying population itself. Since subsamples were taken from each soil slurry during the 24-hour incubation period, it is possible to analyze the temporal pattern of nitrate production over time (Figures 2.5 and 2.6). The NPA data confirmed that among O horizon soils, those from the treated West Bear watershed under hardwoods had the highest rates of nitrification and that these rates were higher than mineral soil NPA. Shah et al., (2002) concluded these higher rates in hardwoods from West Bear were due to a lower C/N ratio compared with softwood litter but also a lower C/N ratio compared with the East Bear hardwoods due to the increased N inputs resulting from the amendments. The slope (e.g., the nitrification rate) to the 24 hour incubation time point was positive suggesting that nitrification would likely proceed had the incubation period continued (Figure 2.5). This same pattern is evident in the West Bear softwood mineral soils, where the slope of nitrate production over time continues to increase.

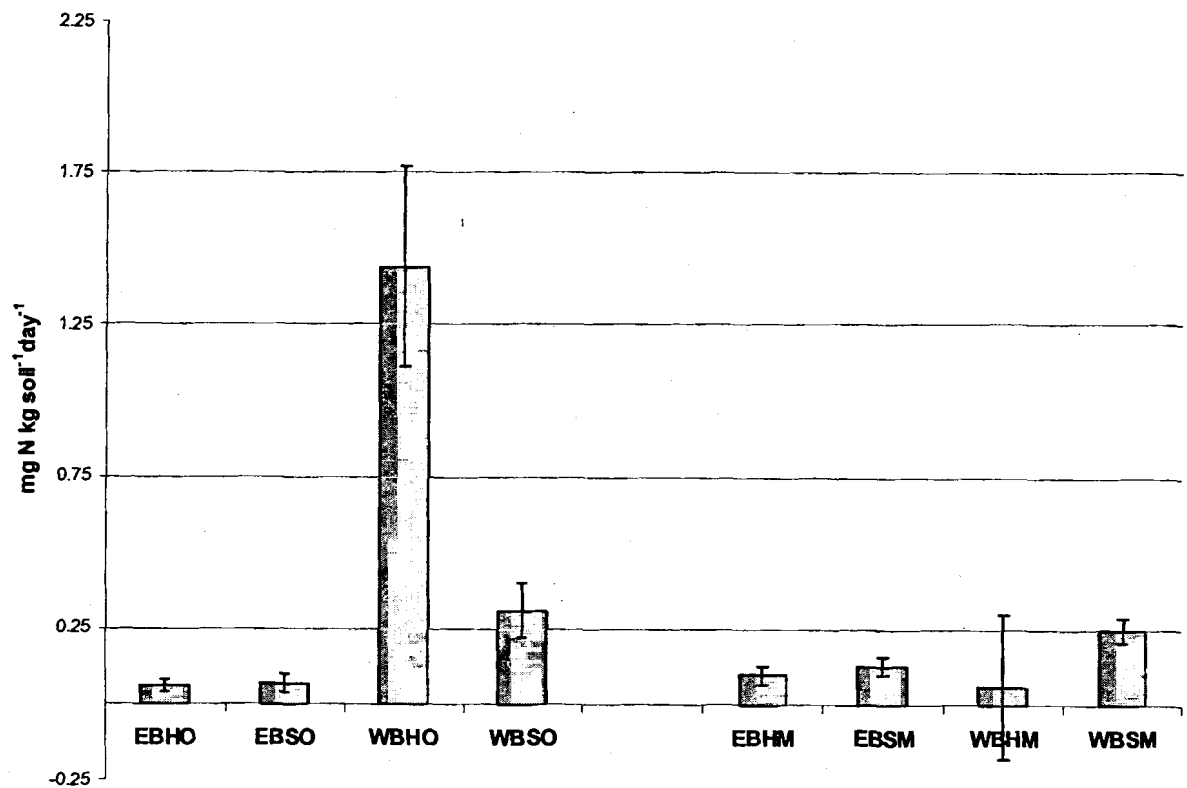


Fig. 2.4. In situ potential net nitrification by compartment with standard error of the mean. Note: EB – the East Bear watershed; WB – the West Bear watershed; S – softwood forest type; H – hardwood forest type; O – O horizons; M – mineral soils.

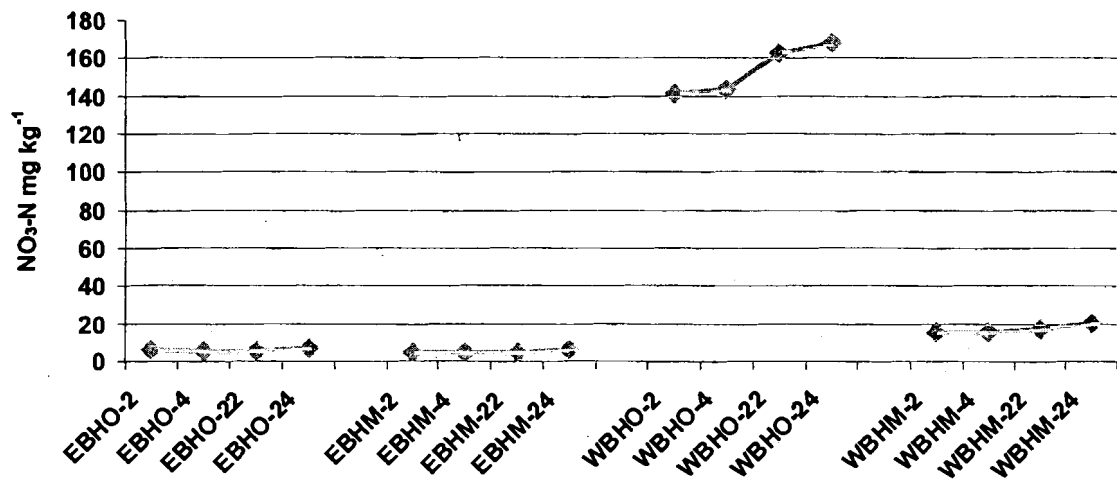


Fig. 2.5. Nitrification potential of hardwood soils over the 24-hour sampling period. Note: EB – the East Bear watershed; WB – the West Bear watershed; H – hardwood forest type; O – O horizons; M – mineral soils. Subsamples removed for analysis at 2, 4, 22, and 24 hours after initiation of incubation.

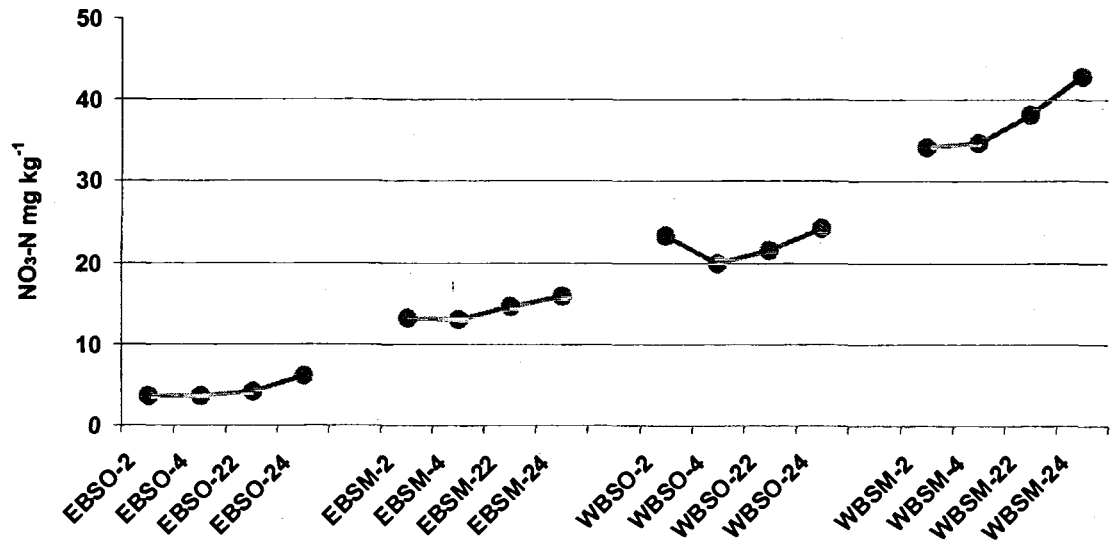


Fig. 2.6. Nitrification potential of softwood soils over the 24-hour sampling period. EB – the East Bear watershed; WB – the West Bear watershed; S – softwood forest type; O – O horizons; M – mineral soils. Subsamples removed for analysis at 2, 4, 22, and 24 hours after initiation of incubation.



## Conclusions

Results from the incubation time trial experiment shows that the 14-day and 28-day laboratory incubation periods provided similar and highly correlated estimates of potential net N mineralization rates. These incubation times produced notably higher rates than the 7-day laboratory incubation period, which may underestimate net N mineralization potentials in these northern forest ecosystem soils. Results demonstrate that all three methods used to assess net nitrification: 14-day laboratory incubation, *in situ* incubation and the nitrification potential assay showed similar trends brought about by either forest cover or the nitrogen treatment. However, the nitrification potential assay was the only method that elucidated differences in net nitrification potential between forest types, particularly between softwood and hardwood mineral soils. Each of the three methods could be suitable choices for determining qualitative differences in N dynamics in various settings. Each also has characteristics that could make it the better choice depending on the scientific objectives. The 14-day laboratory incubation is the easiest to perform, and seems particularly well suited for studies of remote sites where a single collection or site visit is necessary due to logistics and resources. The *in situ* net nitrification is the clear choice when the goal is estimates of actual rates of nitrification in the field. Finally, the less widely used nitrification potential may be more sensitive to emerging differences in soil N dynamics and further evaluation of its efficacy and the merits of additional incubation periods, is warranted.

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## **APPENDIX A**

### **Nitrogen Mineralization and Nitrification**

Table A.1 Net N mineralization ( $\text{mg N kg soil}^{-1} \text{d}^{-1}$ ) for both watersheds and forest types over time  
(standard errors in parenthesis)

Soil Horizon	<i>In-situ</i>		14 day lab incubation		<i>In-situ</i>		14 day lab incubation		<i>In-situ</i>		14 day lab incubation	
	Sep-00	Sep-00	Jun-01	Jun-01	Jul-01	Jul-01	Aug-01	Aug-01	Sep-01	Sep-01		
----- $\text{mg kg}^{-1} \text{d}^{-1}$ -----												
<u>Watershed</u>												
East Bear	Organic	2.03 (0.45)	2.16 (0.82)	4.70 (1.12)	6.80 (1.06)	3.33 (0.91)	4.83 (0.59)	0.98 (0.91)	9.89 (0.59)	0.92 (0.44)	2.45 (1.45)	
	Mineral	0.26 (0.07)	0.59 (0.09)	0.14 (0.03)	1.18 (0.14)	0.42 (0.32)	1.02 (0.22)	0.84 (0.08)	0.89 (0.23)	0.12 (0.08)	0.68 (0.12)	
West Bear	Organic	3.57 (0.63)	11.79 (2.25)	4.66 (1.56)	11.44 (2.37)	6.17 (0.97)	8.41 (1.49)	0.33 (1.14)	12.96 (3.46)	2.03 (0.69)	4.65 (0.71)	
	Mineral	0.07 (0.24)	0.60 (0.63)	0.24 (0.05)	1.65 (0.20)	0.28 (0.07)	0.89 (0.09)	0.37 (0.20)	0.96 (0.22)	0.18 (0.09)	0.6 (0.18)	
<u>Forest Type</u>												
Softwood	Organic	2.29 (0.40)	3.31 (0.90)	2.19 (0.46)	5.39 (0.85)	2.71 (0.52)	4.80 (0.57)	0.62 (1.09)	10.11 (1.62)	1.21 (0.47)	2.53 (1.50)	
	Mineral	0.36 (0.07)	1.20 (0.18)	0.20 (0.05)	1.42 (0.18)	0.43 (0.32)	1.32 (0.17)	0.33 (0.20)	1.26 (0.15)	0.26 (0.05)	0.86 (0.09)	
Hardwood	Organic	3.31 (0.70)	10.63 (2.50)	7.18 (1.62)	12.84 (2.21)	6.75 (1.07)	8.47 (1.49)	0.70 (1.01)	12.75 (3.18)	1.74 (0.79)	4.57 (0.68)	
	Mineral	0.00 (0.22)	0.03 (0.57)	0.19 (0.05)	1.41 (0.18)	0.27 (0.09)	0.59 (0.09)	0.12 (0.11)	0.59 (0.07)	0.04 (0.06)	0.42 (0.09)	

**Appendix A.2 Net N mineralization ( $\text{mg N kg soil}^{-1} \text{ d}^{-1}$ ) for both watersheds by forest types and soil horizons over time (standard errors in parenthesis)**

Watershed	Soil Horizon	Forest Type	$\text{mg kg}^{-1} \text{ d}^{-1}$											
			<i>In-situ</i>		14 day lab incubation		<i>In-situ</i>		14 day lab incubation		<i>In-situ</i>		14 day lab incubation	
			Sep-00	Sep-00	Jun-01	Jun-01	Jul-01	Jul-01	Aug-01	Aug-01	Sep-01	Sep-01		
East Bear	Organic	Hardwood	1.92 (0.67)	2.88 (0.65)	7.27 (1.70)	8.2 (1.31)	4.61 (1.35)	5.47 (0.85)	0 (0.31)	9.51 (0.57)	1.20 (0.60)	4.47 (1.65)		
	Mineral	Hardwood	0.16 (0.06)	0.39 (0.11)	0.11 (0.04)	1.27 (0.18)	0.15 (0.11)	0.54 (0.15)	0 (0.16)	0.56 (0.45)	0.02 (0.03)	0.52 (0.16)		
East Bear	Organic	Softwood	2.15 (0.63)	1.44 (1.53)	2.14 (0.79)	5.4 (1.60)	1.63 (0.79)	4.19 (0.83)	2.00 (1.72)	10.28 (1.22)	0.64 (0.81)	0.44 (1.36)		
	Mineral	Softwood	0.39 (0.13)	0.82 (0.87)	0.17 (0.54)	1.1 (0.21)	0.73 (0.68)	1.51 (0.34)	0.17 (0.06)	1.23 (0.32)	0.23 (0.11)	0.85 (0.10)		
West Bear	Organic	Hardwood	4.71 (1.04)	18.39 (3.02)	7.09 (2.90)	17.49 (3.62)	9.19 (1.18)	11.46 (2.50)	1.40 (2.25)	15.99 (6.28)	2.28 (1.66)	4.69 (0.24)		
	Mineral	Hardwood	-0.21 (0.46)	-0.33 (1.16)	0.27 (0.08)	1.55 (0.31)	0.38 (0.13)	0.64 (0.10)	0.24 (0.13)	0.62 (0.16)	0.06 (0.14)	0.33 (0.03)		
West Bear	Organic	Softwood	2.43 (0.51)	5.19 (0.41)	2.24 (0.53)	5.39 (0.75)	3.52 (0.58)	5.34 (0.77)	-0.74 (0.70)	9.94 (3.78)	1.78 (0.06)	4.61 (1.73)		
	Mineral	Softwood	0.35 (0.07)	1.53 (0.29)	0.22 (0.08)	1.74 (0.26)	0.18 (0.05)	1.13 (0.08)	0.50 (0.44)	1.30 (0.18)	0.30 (0.04)	0.88 (0.20)		

Appendix A.3 Net nitrification (mg N kg soil<sup>-1</sup> d<sup>-1</sup>) for both watersheds by forest types and soil horizons over time (standard errors in parenthesis)

			mg kg <sup>-1</sup> d <sup>-1</sup>											
			<i>In-situ</i>		14 day lab incubation		<i>In-situ</i>		14 day lab incubation		<i>In-situ</i>		14 day lab incubation	
Watershed	Soil Horizon	Forest Type	Sep-00	Sep-00	Jun-01	Jun-01	Jul-01	Jul-01	Aug-01	Aug-01	Sep-01	Sep-01		
East Bear	Organic	Hardwood	0.06 (0.02)	0.08 (0.03)	0.06 (0.03)	0.39 (0.35)	0.21 (0.17)	0.08 (0.05)	0 (0)	0.03 (0.03)	1.20 (0.60)	0 (0)		
	Mineral	Hardwood	0.14 (0.06)	0.13 (0.13)	0.06 (0.02)	0.15 (0.05)	0.18 (0.07)	0.16 (0.08)	0.06 (0.06)	0.16 (0.13)	0.08 (0.08)	0.13 (0.07)		
East Bear	Organic	Softwood	0.06 (0.02)	0.19 (0.09)	0.08 (0.05)	0.06 (0.03)	0.04 (0.03)	0.17 (0.10)	0.04 (0.04)	0 (0)	-0.03 (0.03)	-0.01 (0.01)		
	Mineral	Softwood	0.17 (0.05)	0.36 (0.10)	0.10 (0.04)	0.20 (0.09)	0.14 (0.05)	0.28 (0.12)	0.18 (0.12)	0.26 (0.26)	0.09 (0.05)	0.15 (0.08)		
West Bear	Organic	Hardwood	1.10 (0.16)	4.52 (1.40)	1.78 (0.64)	6.21 (0.72)	1.90 (0.60)	4.00 (0.64)	0.51 (0.15)	2.18 (0.86)	-1.16 (0.12)	0.33 (0.13)		
	Mineral	Hardwood	-0.23 (0.46)	-0.33 (1.19)	0.34 (0.02)	1.07 (0.14)	0.24 (0.03)	0.73 (0.10)	0.18 (0.03)	0.47 (0.18)	0.14 (0.01)	0.27 (0)		
West Bear	Organic	Softwood	0.15 (0.07)	0.67 (0.37)	0.48 (0.15)	1.47 (0.36)	0.33 (0.19)	1.22 (0.28)	0.38 (0.04)	0.18 (0.04)	0.14 (0.07)	0.16 (0.21)		
	Mineral	Softwood	0.25 (0.06)	1.00 (0.14)	0.25 (0.07)	0.81 (0.23)	0.25 (0.03)	1.00 (0.14)	0.25 (0.06)	1.28 (0.27)	0.29 (0.11)	0.67 (0.05)		



Appendix A.4 Net N mineralization (kg N ha soil<sup>-1</sup> d<sup>-1</sup>) for both watersheds by forest types and soil horizons over time  
(standard errors in parenthesis)

			mg kg <sup>-1</sup> d <sup>-1</sup>									
			<i>In-situ</i>	14 day lab incubation	<i>In-situ</i>	14 day lab incubation	<i>In-situ</i>	14 day lab incubation	<i>In-situ</i>	14 day lab incubation	<i>In-situ</i>	14 day lab incubation
Watershed	Soil Horizon	Forest Type	Sep-00	Sep-00	Jun-01	Jun-01	Jul-01	Jul-01	Aug-01	Aug-01	Sep-01	Sep-01
East Bear	Organic	Hardwood	0.19 (0.06)	0.28 (0.06)	0.70 (0.16)	0.79 (0.13)	0.44 (0.13)	0.53 (0.08)	0 (0.03)	0.92 (0.06)	0.12 (0.06)	0.43 (0.16)
	Mineral	Hardwood	0.08 (0.03)	0.21 (0.06)	0.06 (0.02)	0.68 (0.10)	0.08 (0.06)	0.29 (0.08)	0.30 (0.03)	0.01 (0.09)	0.01 (0.02)	0.28 (0.09)
East Bear	Organic	Softwood	0.13 (0.06)	-0.04 (0.09)	0.10 (0.06)	0.29 (0.09)	0.11 (0.06)	0.20 (0.05)	0.05 (0.03)	0.54 (0.29)	0.01 (0.03)	0.07 (0.09)
	Mineral	Softwood	0.16 (0.05)	0.38 (0.06)	0.08 (0.02)	0.45 (0.08)	0.22 (0.22)	0.63 (0.13)	0.07 (0.01)	0.58 (0.30)	0.09 (0.02)	0.39 (0.15)
West Bear	Organic	Hardwood	0.36 (0.08)	1.39 (0.23)	0.54 (0.22)	1.32 (0.27)	0.69 (0.09)	0.87 (0.19)	0.11 (0.17)	1.21 (0.48)	0.18 (0.13)	0.36 (0.02)
	Mineral	Hardwood	-0.11 (0.23)	-0.17 (0.58)	0.13 (0.04)	0.77 (0.15)	0.19 (0.07)	0.32 (0.05)	0.12 (0.06)	0.31 (0.08)	0.03 (0.07)	0.16 (0.01)
West Bear	Organic	Softwood	0.49 (0.10)	1.09 (0.08)	0.44 (0.10)	1.06 (0.15)	0.69 (0.11)	1.05 (0.15)	-0.15 (0.14)	1.94 (0.74)	0.35 (0.01)	0.90 (0.34)
	Mineral	Softwood	0.08 (0.01)	0.36 (0.08)	0.07 (0.02)	0.55 (0.08)	0.06 (0.01)	0.36 (0.03)	0.16 (0.14)	0.41 (0.06)	0.09 (0.01)	0.28 (0.07)

Appendix A.5 Net nitrification ( $\text{kg N ha soil}^{-1} \text{d}^{-1}$ ) for both watersheds by forest types and soil horizons over time (standard errors in parentheses)

Watershed	Soil Horizon	Forest Type	14 day lab		14 day lab		14 day lab		14 day lab		14 day lab	
			<i>In-situ</i>	Incubation	<i>In-situ</i>	Incubation	<i>In-situ</i>	Incubation	<i>In-situ</i>	Incubation	<i>In-situ</i>	Incubation
			Sep-00	Sep-00	Jun-01	Jun-01	Jul-01	Jul-01	Aug-01	Aug-01	Sep-01	Sep-01
East Bear	Organic	Hardwood	0.01 (0.00)	0.01 (0.00)	0.01 (0.00)	0.01 (0.00)	0.01 (0.00)	0.01 (0.00)	0.00 (0.01)	0.00 (0.01)	0.00 (0.00)	0.00 (0.00)
	Mineral	Hardwood	0.08 (0.03)	0.07 (0.07)	0.03 (0.01)	0.08 (0.03)	0.03 (0.04)	0.03 (0.04)	0.03 (0.03)	0.09 (0.07)	0.04 (0.04)	0.07 (0.04)
East Bear	Organic	Softwood	0.00 (0.00)	0.02 (0.01)	0.01 (0.01)	0.00 (0.00)	0.00 (0.01)	0.00 (0.01)	0.01 (0.01)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
	Mineral	Softwood	0.09 (0.03)	0.16 (0.05)	0.04 (0.01)	0.07 (0.03)	0.05 (0.01)	0.07 (0.01)	0.06 (0.03)	0.08 (0.08)	0.03 (0.01)	0.06 (0.02)
West Bear	Organic	Hardwood	0.08 (0.01)	0.34 (0.11)	0.14 (0.05)	0.47 (0.05)	0.14 (0.05)	0.14 (0.05)	0.04 (0.01)	0.17 (0.07)	-0.01 (0.01)	0.03 (0.01)
	Mineral	Hardwood	-0.11 (0.23)	-0.17 (0.59)	0.18 (0.01)	0.53 (0.07)	0.12 (0.02)	0.36 (0.05)	0.09 (0.02)	0.23 (0.09)	0.07 (0.01)	0.14 (0.01)
West Bear	Organic	Softwood	0.05 (0.02)	0.21 (0.12)	0.09 (0.03)	0.29 (0.07)	0.07 (0.04)	0.07 (0.04)	0.08 (0.01)	0.04 (0.01)	0.03 (0.02)	0.03 (0.04)
	Mineral	Softwood	0.05 (0.01)	0.20 (0.03)	0.08 (0.02)	0.26 (0.07)	0.08 (0.01)	0.31 (0.04)	0.08 (0.02)	0.41 (0.09)	0.09 (0.03)	0.21 (0.02)

**APPENDIX B**

**Soil Moisture**

Appendix B.1. Organic and mineral soil moisture content over time (standard errors in parantheses)

Watershed	Soil Horizon	Forest Type	Percent Initial Moisture				
			Sep-00	Jun-01	Jul-01	Aug-01	Sep-01
East Bear	Organic	Hardwood	200.96 (19.88)	188.01 (32.58)	139.03 (20.88)	68.46 (11.81)	65.27 (7.66)
	Mineral	Hardwood	64.35 (8.34)	77.58 (12.90)	55.68 (4.08)	37.33 (3.11)	36.69 (4.37)
East Bear	Organic	Softwood	196.64 (14.28)	158.95 (15.58)	151.66 (11.65)	73.44 (4.80)	46.39 (4.17)
	Mineral	Softwood	66.97 (6.13)	96.66 (10.11)	87.25 (7.60)	54.84 (8.15)	40.91 (5.52)
West Bear	Organic	Hardwood	195.82 (11.94)	151.22 (19.69)	80.64 (11.50)	62.03 (13.06)	45.12 (8.00)
	Mineral	Hardwood	49.06 (4.73)	52.23 (2.11)	41.65 (2.43)	26.55 (4.09)	23.99 (2.60)
West Bear	Organic	Softwood	218.72 (13.71)	193.64 (8.74)	155.77 (8.14)	100.28 (12.19)	83.65 (6.33)
	Mineral	Softwood	114.83 (8.92)	124.63 (6.70)	104.11 (5.48)	86.47 (6.22)	76.50 (3.48)

## **APPENDIX C**

### **Correlation Coefficients**

**Appendix C.1** Pearson correlation coefficients for all potential and in situ net N mineralization and potential and in situ net nitrification vs. soil characteristics  
 † indicates significance at the 0.05 level

	NNM	PNN	NN	NP	Soil H <sub>2</sub> O	Soil T°	pH
PNNM	0.96†	0.88†	0.89†	0.85†	0.49	0.50	-0.22
NNM		0.73†	0.75†	0.70†	0.59	0.70†	-0.19
PNN			0.99†	0.99	0.16	0.19	-0.12
NN				0.99†	0.16	0.21	-0.07
NP					0.10	0.20	-0.09
Soil H <sub>2</sub> O						0.33	-0.71
Soil T°							0.19
pH							

**APPENDIX D**  
**Plot Layout Design**

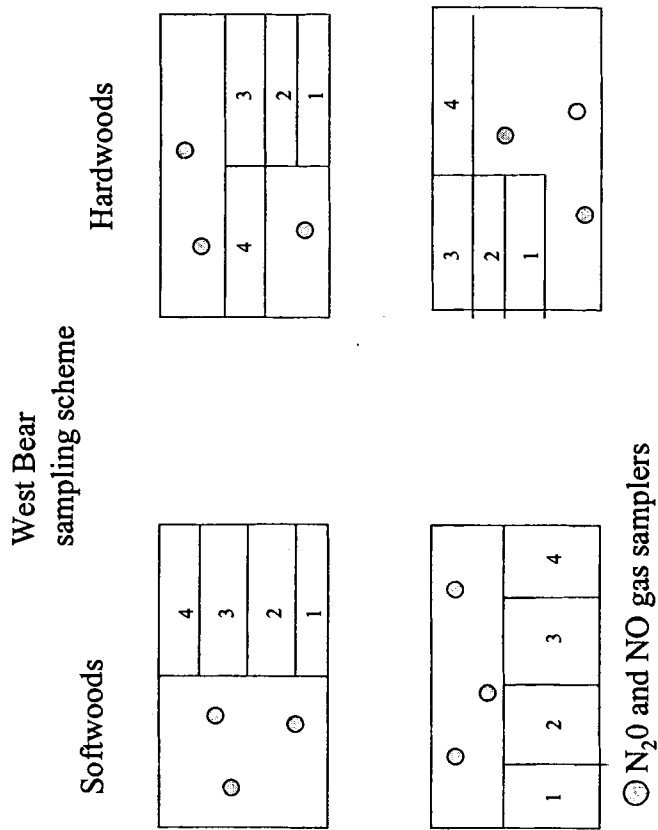


Fig. D.1. Schematic layout of plot designs within each watershed.



## **BIOGRAPHY OF THE AUTHOR**

Sultana Sarvatara Shah was born in Seattle, Washington on January 4, 1977. She was raised in Lynnwood, Washington and graduated from Bothell High School in 1995. She completed her junior and senior high school years at Edmonds Community College through the Running Start program. She attended The Evergreen State College and graduated in 2000 with a Bachelor's of Science degree with an emphasis in Ecology/Biology. After working for the USDA PNW Forest Service Station for one year as a forestry technician, she moved to Maine to attend the University of Maine and attain her Master's degree in Plant, Soil and Environmental Sciences in July 2000. Sultana is a candidate for the Master of Science degree in Plant, Soil, and Environmental Sciences from the University of Maine in December, 2002.