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Factors influencing spatial variability in soil nitrogen (N) dynamics in N-treated and untreated watersheds of the Fernow Experimental Forest, West Virginia

by

Nikki Lenore Lyttle

Department of Biological Sciences

Marshall University

Date: 26 June 2001

Approved:

Frank S. Gilliam, Co-Advisor

Leonard J. Deutsch, Dean

Charles C. Somerville, Co-Advisor

Jeffrey May

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in the Department of Biological Sciences in the Graduate School of Marshall University

ABSTRACT

Factors influencing spatial variability in soil nitrogen (N) dynamics in N-treated and untreated watersheds of the Fernow Experimental Forest, West Virginia

By

Nikki Lenore Lyttle

The central Appalachian region of the United States receives some of the highest inputs of nitrogen (N) due to acidic deposition in the nation. It is believed that these high could levels contribute to a decline in forest soils within the next 50 to 70 yrs. This study examines factors that influence spatial variability in N-treated and untreated watersheds of the Fernow Experimental Forest, Parsons, West Virginia. Within each of the two watersheds [WS4 untreated control, > 100 yr.; WS3 N-treated, acidified, clear cut, ~ 31 yr.], two 0.04 ha plots, one high N and one low N, were selected for study. Three subplots were chosen from within each of the two sample plots, for a total of 12 subplots. Soil samples were collected with a hand trowel. Nitrogen extractions were performed using 10 g of soil and 100 ml of 1 N KCl. Litter samples were ground using a Wiley Mill and analyzed for foliar lignin concentration, C:N, and %N. Nitrogen extracts were analyzed with a Bran + Luebbe TrAAcs 2000 automatic analysis system. Bacterial DNA was analyzed using primer sets. The primer sets (1-6) were designed for the specific detection of ammonia oxidizing bacteria in forest soils by PCR. DNA was purified and amplified by PCR.

All plots detected AMO gene groups during pre-incubation, but by day 7 only WS4/low N had detectable gene groups present. On day 14 all plots, with the exception of WS4/high N, had detectable groups present. At days 21 and 28 only one plot for each

day had detected AMO genes. On day 21 WS4/low N had group 2 present and on day 28 WS3/high N had group 2 present. Overall, it is clear that the nitrifying bacterial community is very dynamic. AMO genes were readily detected at pre-incubation, but were nearly absent after seven days of incubation. By fourteen days of incubation the communities had shifted. Only AMO genes of the group 2 were detected after three and four weeks of incubation. The data suggest either that incubation conditions were not suitable for nitrifying bacteria, or that previously uncharacterized AMO genes were dominant after prolonged incubation.

The first hypothesis posed was that soil incubation temperature and net mineralization and nitrification were correlated. This hypothesis was supported. The lowest rates for both net mineralization and nitrification were seen at the 10 C incubation temperature. The 30 C incubation temperature allowed the highest rates. This was true of all the study plots within WS3 and WS4.

The second hypothesis, that the lack of net nitrification on WS4/low N was caused by a lack of nitrifying bacteria at that site, was rejected. The opposite was found to be true. The high N plot of WS4 did have bacterial communities present. The lack of nitrification be attributed to the inactivity of the bacterial communities due to an unknown environmental limitation.

ACKNOWLEDGEMENTS

Ah, I can now finally sit down and write something in this thesis that a "non-science" person can understand, which to me is very important, especially since it's those "non-science" people who have helped me maintain my sanity over the past two years (especially this last semester). It is extremely important to have the kind of support you guys have provided me with and for that, I would like to take a couple of pages to thank you guys.

Thanks to Dr. Gilliam and Dr. Somerville for the countless hours of help and for teaching me how to collect and analyze my data. Your vast knowledge of ecology and microbiology has been important in helping me make this thesis a reality. Again, thanks for taking the time out of your schedules to help.

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I can't write this acknowledgments section without mentioning my Eco-Office parter Beth Burdette. We've had some fun times over the past two years and I couldn't have picked a better person to share office space with, though I must admit, when we

were both in the office, more gossip was going on than actual work! I'll never forget the trips to Chattanooga (the city that never sleeps) and New Orleans, both have provided us with countless hours of laughter. It's a wonder we haven't been on America's Most Wanted! I could sit here and write all day about our antics, but I think it would look really bad if the acknowledgements were longer than my thesis (YIKES!).

I would also like to thank Jessica Wooten for giving me thesis advice and looking over my thesis. Also, thanks for being such a good friend and may you never get caught "borrowing" cinderblocks from video stores anytime in the near future! If you do, I guess I'll see you on America's Most Wanted too!

I also have to give a huge thanks to my boyfriend Kevin McIlwain who probably deserves a gold medal for putting up with my neurosis over the past two years. I can't even begin to thank you enough for all the support and help you've provided me with. You always know how to cheer me up and lower my stress level, even if it involves driving 8 hours to South Carolina to take me to the beach for the weekend and then standing in the Build-A-Bear Workshop for 2 hours while I try to decide on what stuffed animal I should build. Thanks again for everything.

OK, now it's time to thank the family. Special thanks to my brother Boogie. All the years of torment and abuse have paid off in making me a stronger person (plus if I didn't mention you in this I'm sure I would get put in a head lock and I think we're both just getting a little too old for that). Another special thanks needs to go out to my nephew Nathan who, while only being 20 months old, has taught me that there's more to life than just school, work, or a thesis.

Last, but not least, a huge thanks to the two people that I owe it all to, my mom and dad. I definitely believe that without you, none of this would have been possible. Thanks for the undying support, the encouragement, and never allowing the thought of failure to enter my mind. While you've always taught me to excel academically, you've also instilled the value of living life to the fullest (in other words, while school is very important and degrees are nice, it's not what defines you). Thanks for all the sacrifices you've made for me and I promise I won't let you down.

Now, after seven long years as a college student, I'm ready to enter the real world (and pay off all those student loans). While I've had lots of good times here at Marshall and I've made some great friends, I'm ready to start a new chapter in my life...hopefully one that doesn't involve a thesis!

N.L.L

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

Chapter I
Introduction

This thesis examines the spatial variability of soil nitrogen (N) processing, temperature manipulation, and bacterial communities at the Fernow Experimental Forest (FEF), Parsons, West Virginia. The objectives of this study are to:

(1) evaluate the spatial variability of N-treated WS3 and untreated WS4 with respect to net N mineralization and net nitrification;

(2) study the influence that temperature manipulation has on net N mineralization and net nitrification;

(3) characterize the microbial community of two watersheds (WS3 and WS4) using the polymerase chain reaction (PCR).

Two hypotheses are tested:

(1) soil incubation temperature and rates of net mineralization and nitrification are correlated.

(2) the lack of net nitrification on the low N plot of WS4 is caused by a lack of nitrifying bacteria.

In 1988, the United States Department of Agriculture (U.S.D.A.) Forest Service began a project at FEF, Parsons, West Virginia to examine ecosystem responses to the aerial applications of $(NH_4)_2SO_4$ on a watershed (WS3). Responses examined included the response of soils and herbaceous layer plants (Gilliam and Turrill 1993, Gilliam et al. 1994) and changes in stream chemistry (Adams 1999). Adams and Angradi (1996) performed a study examining the decomposition and nutrient dynamics of hardwood leaf litter on WS3 (the acidified watershed). They found that rates of decay did vary between

the treatment and control watersheds and that there were differences in the concentrations of nitrogen (N), calcium (Ca), and potassium (K), but those differences disappeared after 2 years.

Nitrogen Saturation: A Review

Industrialized and developing regions of the world are experiencing an increase in nitrogen (N) emissions to the atmosphere from human activity, a pattern not likely to improve in the near future. Currently, in the northeastern United States N amounts accrued through atmospheric deposition are 10 to 20 times higher than historic background levels (Magill et al. 1997). Concern about increasing N emissions is on the rise because of its potential effects on air and water quality and the health of forest ecosystems (Aber et al. 1989). Human alteration of the nitrogen cycle has increased dramatically, causing unwanted and unintended increases in N availability in terrestrial, freshwater, and marine ecosystems worldwide.

In the 1980s, the United States acid deposition effects program (National Atmospheric Precipitation Assessment Program, or NAPAP) was funded primarily to study the effects of sulfur deposition rather than those of N deposition (Aber et al. 1998). A 10-year study done by NAPAP concluded that acidic deposition was not a significant factor contributing to forest health problems in North America, except for high–elevation red spruce stands in the northern Appalachians (Adams 1999). The report further stated that sensitive forest soils should be monitored carefully due to their possible future impacts on forest health. Since the NAPAP report was published, interest in the effects of air pollution on forest soils and nutrient cycling processes has continued to grow. In

1990, the Clean Air Act Amendments were passed, although the United States regulatory community had not supported additional substantial research on acidic deposition (Aber et al. 1998). Unlike many European countries that have formed active and well–coordinated programs, efforts in the United States have yet to emphasize research on N deposition effects. Despite the lack of effort in the United States, many American researchers have continued to express concern about the long–term effects of N deposition on forests, grasslands, streams, and estuaries (Fenn et al. 1998).

Nitrogen saturation has also been defined as the availability of ammonium and nitrate in excess of total plant and microbial demand (Aber et al. 1989, Adams 1999). Thus, an N-saturated ecosystem is one in which the biota cannot utilize all of the N that is added to the system. These excess N additions enter into the ecosystem as atmospheric N deposition.

Nitrogen saturation has become a concern in recent years. The threat of N saturation to forest ecosystems was first recognized as a cause for concern 15 years ago (Aber et al. 1989). Temperate, humid forest ecosystems that were once N limited are now experiencing increased N deposition (Aber et al. 1989). Some forests in the central Appalachian mountain region have already become N saturated (Gilliam et al.1996). The central Appalachian region of the United States receives the highest inputs of N due to acidic deposition in the nation (Gilliam and Adams 1996). It is believed that within the next 50 to 70 years, acidic deposition along the humid east coast could contribute to a decline in forest soils (Gilliam and Adams 1999).

Early warning signs that a forest may be N saturated include increases in nitrate leaching losses, increases in net nitrification rates (Magill et al. 1996), decreases in both

foliar lignin concentration and soil C/N ratios (Gilliam et al. 1996), soil acidification (Laverman et al. 2000; Magill et al. 1997), micronutrient impoverishment, reductions in net carbon uptake (Asner et al. 1997), altered fluxes of trace gases (Magill et al. 1997), and forest decline (Gilliam et al. 1996; Laverman et al. 2000; Magill et al. 1997; Ohrui and Mitchell 1997). Although N deposition may lead to N saturation of forest ecosystems, there are a few factors that predispose forests to N saturation. Characteristics that may make a forest prone to N saturation include forest type, stand vigor, successional stage, previous land use, topography, and climate (Fenn et al. 1998). Forests containing trees that do not conserve N and exhibit a high N turnover in the canopy are characteristically found in many N saturated forests (Fenn et al. 1998). Consequently, different forest types will react differently to N saturation. Coniferous forests move rapidly through the stages of N saturation in comparison to broad-leaved deciduous forests (Magill et al. 2000).

Nitrogen Inputs

The principle pathways for N inputs in a forest ecosystem include atmospheric (wet and dry) deposition, symbiotic N_2 fixation (e.g. *Rhizobium* and *Frankia)*, and fertilization (Fenn et al. 1998). Fundamentally, there is a difference between precipitation and fertilizers as nitrogen sources. Atmospheric wet deposition (precipitation) is chronic, while fertilizations are one–time applications (Aber et al. 1989). Fertilizer may lead to short-term increases in N cycling and forest productivity, but will decline within several years as vegetation and soil use the additional N. Atmospheric wet deposition is a continuous addition of N to an already available supply of N for the process of

mineralizing organic matter. In time, these additions can exceed the demand of plants, soils, and microbes, causing N saturation. Also, in some ecosystems, such as the one in the Hubbard Brook sandbox ecosystem study, associative N_2 fixation by free – living soil organisms, and N_2 fixation by cyanolichens in the forest canopy are considered to be important sources of N (Fenn et al. 1998).

Soil age plays a major role in how soil retains nitrogen. For example, young soils are soils that have experienced a reduction in carbon (C) and N pools because of a disturbance (i.e. logging, fire). These soils actively accumulate organic matter and N, whereas older soils are prone to leach high levels of NO_3 (Fenn et al. 1998).

Soil Microorganisms

Soil microorganisms play an integral role in N cycling within terrestrial ecosystems (Holmes and Zak 1994). However, the spatial distribution of soil bacteria within an ecosystem is not well understood (Morris and Boerner 1998). Even though the spatial distribution of soil microorganisms is unclear, microbial communities do not pattern themselves within an ecosystem by chance. Some potential causes for their distribution may include vegetation, water potential, soil temperature, and C availability.

An example of how vegetation can alter the pattern of a microbial community is that some plants, such as the ericaceous shrub *Kalmia angustifolia,* produce substances that inhibits the microbial communities responsible for soil nutrient cycling and plant uptake (Bradley et al. 2000). The assimilation of N by microbes appears to be limited in forest soils by the availability of C (Fenn et al. 1998; Holmes and Zak 1994; Magill and Aber 2000). It is believed that because soil microbial growth is limited by C availability,

inputs of organic matter could increase gross N immobilization within an ecosystem (Holmes and Zak 1999). This process would decrease the NH_4^+ that is available to nitrifying bacteria, resulting in a decrease of NO_3^- leaching and denitrification.

If soil temperature and moisture are at levels characteristic of an ecosystem they will not be the factor that regulates the distribution of bacteria. In this case, litter quality will be the regulating factor (Magill and Aber 2000). Also, if an ecosystem is experiencing a period of increased substrate availability, such as a C flux, then microbial populations could potentially increase, as long as soil temperatures and water potential do not limit growth. This would cause an increase in microbial biomass and a decline in net N mineralization because N would be assimilated to form new microbial biomass (Holmes and Zak 1994).

Spatial Variability of Nitrogen

Nitrogen cycling, as well as the cycling of other nutrients in soils of terrestrial ecosystems, is controlled by spatially heterogeneous processes (Gilliam et al. in press). These processes are important in determining an ecosystem's response to disturbances such as fire, logging, acidification, and N deposition. Landscape components can also contribute to an ecosystem's spatial variability (Ohrui et al. 1999). Other factors of spatial variability in forest soils include the distribution of bacteria and fungi (Morris and Boerner 1998), topography, and microclimate (Walley et al. 1996).

Forested watersheds in Japan have experienced large differences in nutrient cycling at different locations within a watershed (Ohrui and Mitchell 1998). Types of nutrient cycling affected by the spatial variability of N included the decomposition of

organic matter and vegetation uptake. Increased N mineralization and nitrification rates were reported for moist soils at lower slope positions in the near-stream zone when compared to well-drained soils near ridges at higher slope positions (Ohrui and Mitchell 1997). Concentrations and contents of soil inorganic and organic N also show distinct spatial patterns in Japanese watersheds.

Studies done in the Adirondack Mountains have shown that within a watershed, different landscape components, such as lakes, streams, forested wetlands, and upland conifer and hardwood forests, may possess distinctive roles as sinks or sources for N (Ohrui et al. 1999). Each of these components has distinctive N cycling characteristics. Within the Adirondack region, the interrelationships between these landscape components are anticipated to contribute to the area's spatial patterns of N dynamics (Ohrui et al. 1999).

Whereas little is known about the influence of topography on the spatial distribution of soil variables (such as N cycling) in forest landscapes, it is known that topography does play a critical role in the modification of a landscape's microclimate and hydrological conditions (Walley et al. 1996). The topographic control of moisture and nutrient redistribution through microbial response has the potential to alter nutrient cycling processes and influence nutrient losses. Topography does influence the movement of water and along, with that, causes the redistribution of materials that are carried by the water (Walley et al. 1996).

At FEF, a three year study showed that extractable NH_4^+ and NO_3^- pools and nitrification were highest on the treated watershed (WS3) than on the untreated watersheds (WS4 and WS7) for the majority of the incubation periods (Gilliam et al. in

press). The spatial patterns of NO_3^- in the soil water of WS4 (untreated) suggest that even though there are signs of N saturation, microenvironmental variability could limit N processing rates. Soil water spatial patterns of NO_3^- in WS3 (treated) suggest that the later stages of N saturation may cause higher concentrations of NO_3^-, as well as less spatial variability. The study concluded that responses to earlier N additions to WS3 are the typical responses of an N-saturated forest ecosystem to further N inputs (Gilliam et al. in press). Nearly 100% of all mineralized N is nitrified, along with having elevated NH_4^+ and NO_3^- pools in the soil. This has lead to the current study evaluating factors influencing spatial variability and temperature manipulation on soil N processes at FEF, as well as the characterization of the bacterial community.

Chapter II.
Materials and Methods

Study Site

The Fernow Experimental Forest is an outdoor laboratory that comprises 1900 ha of the Northern Forest Experiment Station (Figure 1). It is located in Tucker County, West Virginia in the Allegheny Mountain section of the unglaciated Allegheny Plateau. The climate is cool and rainy with an average yearly precipitation of ~ 145 cm, occurring mainly during the growing season (Gilliam and Adams 1996). Precipitation is highest in June, lowest in January, and increases with higher elevations. The mean annual temperature is 9 C (Gilliam and Adams 1996). Snow is common from December through March. Soils are Incepticols of Berks and Calvin series, both loamy-skeletal, mixed, mesic Typic Dystrochrepts (Gilliam et al. 1995). These soils are derived from sandstone and are course-textured sandy loams that are well drained and 1 m in depth.

Two watersheds (WS3 and WS4) were selected for this study (Table 1). WS3 is an even-aged stand, ~ 31 years old, that was clear-cut to 2.5 cm diameter at breast height (dbh) in 1970. The basal area [BA] = 25m^2/ha and the density of the watershed is 2410 stems/ha. Beginning in 1989, the watershed received aerial applications of $(NH_4)_2SO_4$ three times per year for four years, as part of the U.S.D.A. Forest Service Watershed Acidification Study. The average amount of ambient deposition of N is about 15 kg/ha^{-1} year $^{-1}$ respectively (Gilliam et al. 1995). During March and November, the application amounts were 33.6 kg/ha^{-1} of fertilizer (7.1 kg N/ha^{-1}) and the July application

Figure 1. Map of FEF study watersheds (WS3 and WS4) with location of sample plots shown.

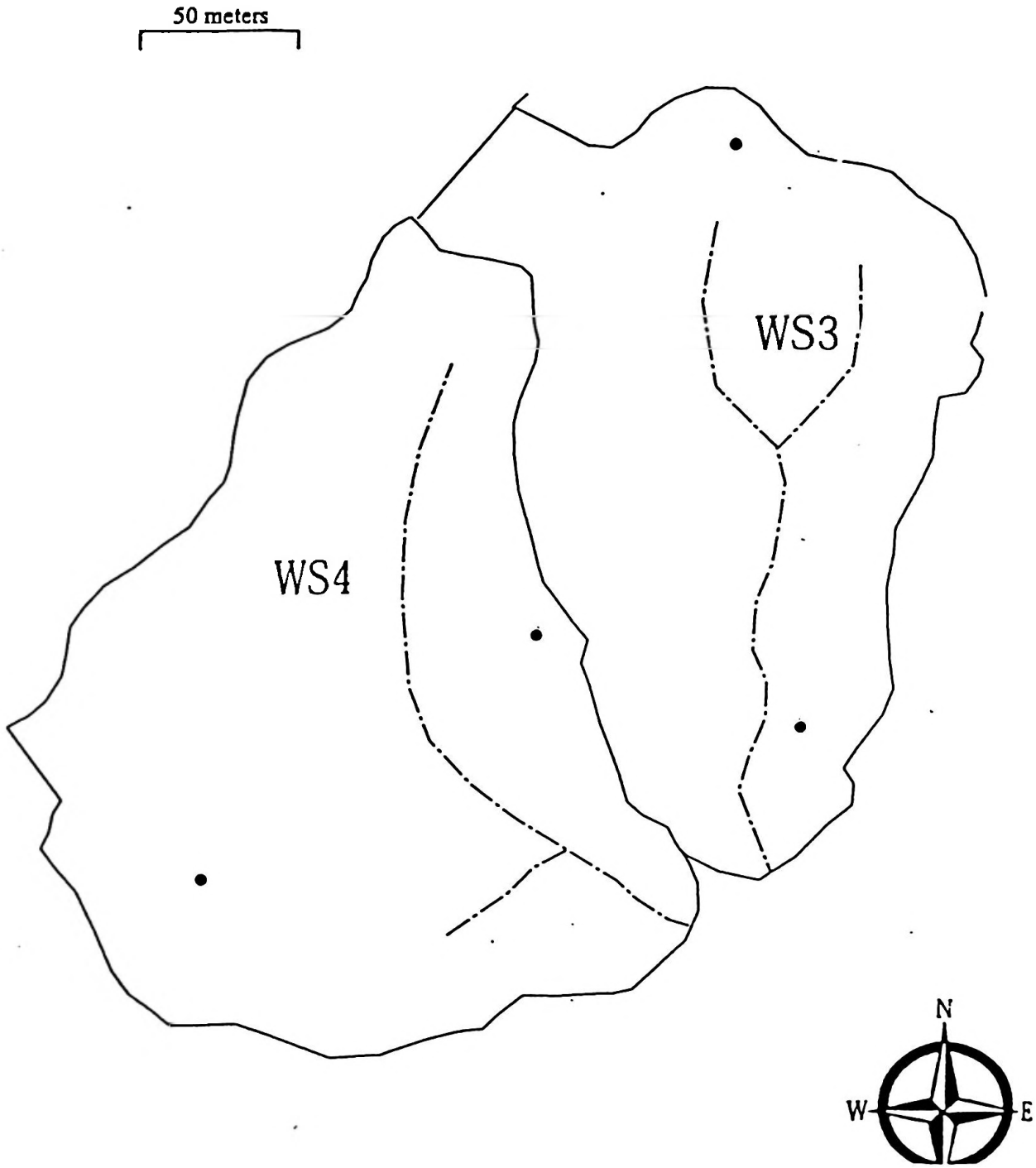

50 meters
WS3
WS4
N
W
E

Table 1. Characteristics from two study watersheds (WS) at the Fernow Experimental Forest, West Virginia, USA. Nomenclature follows Gleason and Cronquist (1991). Data taken from Adams et al. 1993 and Gilliam et al.1995.

Variable	WS3	WS4
Stand age (yr)	~ 31; even-aged	>100; mixed-aged
Stand history	clear-cut; acidification	select cut
Density (stems/ha)	2410	948
Basal area (m^2/ha)	25.0	40.0
Area (ha)	34.3	38.7
Aspect	S	S-SE
Minimum elevation (m)	735	750
Maximum elevation (m)	860	870
Dominant tree species	*Prunus serotina* *Acer saccharum* *Quercus rubra* *Liriodendron tulipifera*	*Acer saccharum* *Quercus rubra* *Fagus grandifolia* *Prunus serotina*

100.8 kg/ha^{-1} of fertilizer (21.2 kg N/ha^{-1}) (Gilliam et al. 1996). These rates were chosen because they are twice the ambient rates of N deposited on the watershed via throughfall. The total amount of N that was deposited on WS3 (application plus atmospheric deposition) was approximately 54 kg/ha^{-1} year^{-1}, or roughly three times the pretreatment levels.

Watershed 4 is a mixed-aged stand, > 100 years old. The watershed is 38.7 ha in size and was selectively cut in 1910 (Gilliam and Turrill 1993). More of the large stems (~950 stems/ha, basal area [BA] = 38m^2/ha) are found in WS4 overall when compared to WS3.

Similarities between WS3 and WS4 include a S-SE slope, elevational ranges of ~740 m - 865 m, and the mixed hardwood stand composition. Dominant hardwood species consist of *Acer saccharum* (sugar maple), *Quercus rubra* (northern red oak), *Liriodendron tulipifera* (yellow-poplar), *Fagus grandifolia* (American beech), *Betula lenta* (black birch), and *Prunus serotina* (black cherry) (Gilliam and Turrill 1993). The herbaceous layers are spatially heterogeneous and dominated by *Laportea canadensis* (stinging nettle) and *Viola* spp. (species of violet) (Gilliam et al. 1996).

Plot Location and Sampling Methods

Within each of the two watersheds (WS3 and WS4), two 0.04 ha plots, one high N (plot with more available N) and one low N (plot with less N available) were chosen for study. Three subplots were randomly selected within each watershed plot and labeled either A, B, or C. A total of 12 subplots were used in this study.

A hand trowel and latex gloves were used to collect the samples. Soils were examined for any unwanted debris and then placed into the appropriate polyethylene bag. The trowel and gloves were cleaned with antibacterial wipes between each sample collected. Each plot had three samples taken from it, for a total of 12 samples. Forest litter samples were also taken from each plot and were placed in a small brown paper bag.

Upon return from FEF to Huntington, West Virginia, the 12 sample bags were taken to the laboratory for pre-incubation extractions. From each bag, 20 g of soil was placed into 100 ml beakers. Soil and forest litter samples were dried in a heated oven. The 12 sample bags were divided into 36 smaller bags and were placed in three incubators. The incubation temperatures were 10 C, 20 C, and 30 C. Each of the 12 sample bags represented a subplot .

Laboratory Analysis

Nitrogen extractions were performed using 10 g samples of soil with the addition of 100 ml of 1 N KCL. The flasks were shaken for 15 minutes and filtered. Extract samples were frozen until analysis for NH_4^+ and NO_3^- (see below).
Litter samples taken from the forest floor were ground to pass through a 40 mesh screen using a Wiley Mill. Foliar lignin concentrations were determined by the analytical lab at the University of Maine, Orono, Maine.

Block digestion of plant material was performed by placing ground leaf samples in reflux tubes with three Teflon boiling stones. A digestion solution was dispersed into the tubes and then placed in a block digester at room temperature. Samples were cooled,

filtered, and diluted to 50 ml using distilled H_2O. This procedure allowed for the determination of % N.

Forest floor samples were ashed using cuvettes filled three-fourths full with ground leaf litter samples to determine C:N. The cuvette weights along with the cuvette plus sample weights were recorded. The cuvettes containing the forest floor samples were placed in a muffle furnace and dried at 500 C for approximately five hrs. The weights of the cuvette plus ash were recorded.

The N extractions were analyzed colorimetrically for $NH_4^+$and NO_3^- with a Bran+Luebbe TrAAcs 2000 (BLT) automatic analysis system. Samples were placed in a BLT sample cup. Net N mineralization and nitrification were calculated using linear regression.

Microbial Community Analyses

Oligomer DNA primer sets were designed for the specific detection of ammonia oxidizing bacteria in forest soils by polymerase chain reaction (PCR). Gene sequences were obtained from the GenBank database and were aligned using ClustalX (Thompson et al. 1994). Once the alignments were complete, phylogenetic trees were displayed using TreeView (Page 1996). The phylogenetic trees were reviewed and the primers were selected by inspection of the aligned sequences. Information obtained on GenBank allowed for the identification of the highly conserved regions of the bacterial DNA. Selected primers were made by the Marshall University DNA Core Facility, Huntington, West Virginia.

The direct lysis method of Burlage et al. (1998) was used in this study. Total microbial DNA was purified by placing 5 g of soil in a 50 ml Oak Ridge tube and washing with 10 ml of washing buffer (120 mM NaH_2PO_4/ NaH_2PO_4, pH 8). Samples were centrifuged at 8000 rpm for 10 minutes, the supernatant was poured off, and the process was repeated. Lysis Solution I (150 mM NaCl, 100 mM EDTA (pH 8), 10 mg/ml lysozyme) was added followed by 1-2 hrs. of incubation at 37 C. Lysis Buffer II (100mM NaCl, 500 mM Tris-Cl (pH 8), 10% SDS) was added before three cycles of freezing at –70C for 20 minutes followed by thawing at 65 C for 20 minutes.

The samples were centrifuged for 10 minutes at 8,000 rpm and the pellet was discarded. The supernatant was filtered with a wet Kimwipe to remove any particulates. To the supernatant, 5 M NaCl and 10%CTAB (dexadecyltrimethyl ammonium bromide in 0.7 M NaCl) was added. Samples were incubated for 10 minutes at 65 C. An equal amount of $CHCl_3$ was added and mixed by inversion. The samples were centrifuged at 5,000 rpm for 5 minutes and the upper phase was transferred to a clean Corex tube. An equal volume of 13% polyethylene glycol was added to the upper phase and mixed. Samples were kept on ice for 10 minutes, then centrifuged at 10,000 rpm for 15 minutes. The supernatant was discarded and the pellet washed with 70% ethanol and air dried.

Nucleic acids were dissolved in 750 µl TE buffer (10 mM Tris HCl, 1 mM EDTA, pH 8) the sample was transferred to a 1.5 ml microfuge tube containing 110 µl 10M ammonium acetate. The samples were kept on ice for 10 minutes and centrifuged at 14,000 rpm for 15 minutes. The supernatant was transferred to a clean microfuge tube with 2 volumes of 95% ethanol. Samples were again centrifuged at 14,000 rpm for 15

minutes. The supernatant was discarded and the pellet was washed with 70% ethanol. The pellet was dried and dissolved in 200 µl TE buffer.

The DNA was amplified by PCR and separated by electrophoresis. PCR was performed by using a small, thin walled microfuge tube and placing inside the necessary components for DNA replication (40 µl sterile double distilled H_2O, 5 µl PCR 10X buffer, 1 µl dNTP mix, 0.5 µl forward primer, 0.5 µl reverse primer, 2 µl template, and 0.5 µl TAQ polymerase to be added while hot (94 C)). The tube was placed in a gene cycler which carried out the three parts of the polymerase chain reaction. The first part of the process required the separation of the two strands of DNA which comprise the double helix. Denaturation occurred at 94 C. The second step was to anneal the primers to the DNA. This involves cooling the vial so the annealing can occur. The annealing temperature for this study was 10 C below the melting temperature of each set of primers (approximately 63 C). The final step involves making a complete copy of the template (elongation). The elongation temperature used for this study was 72 C. The entire process of PCR for one set of primers took approximately 7.5 hours.

Separation of the DNA was done by gel electophoresis. The 1.5 % gels were made using 50 ml of 1 X TAE and 0.75 g of agarose (molecular biologically certified agarose). This was mixed in a beaker and microwaved for 40 seconds. The mixture was then swirled and microwaved for an additional 10 seconds to ensure homogeneity of the mixture. The beaker was then placed in a 48 C waterbath and cooled to touch. After the waterbath, 1 µl of ethidium brodmide was added. The mixture was then swirled and poured. An eight tooth comb was used to make the wells in the gels. The samples were added and the samples were electrophoresed. After approximately 45 minutes the gels

were viewed in the darkroom under an ultraviolet lamp to check for DNA bands. A picture was then taken of the gels.

Chapter III.
Net Nitrogen Mineralization and Nitrification

Extractable NO_3^- exhibited a significant increase over time on both the high and low N plots of WS3 at all three incubation temperatures. The amount of extractable NO_3^- on the low N plots of WS3 remained steady between day 21 and 28 of the study. High N plots at 10 C and 20 C reached a plateau from day 7 until day 21, followed by an increase from day 21 until the end of the experiment at day 28 (Figure 2). The highest amount of extractable NO_3^- on WS3 occurred on the high N plot at 30 C and the lowest amount was on the low N plot at 10 C.

In contrast to WS3, only the high N plots of WS4 showed a significant increase in extractable NO_3^- over time and at all incubation temperatures (Figure 3). The low N plots exhibited no increase in the amount of extractable NO_3^-. Again, of the plots that did give values for NO_3^-, the lowest values corresponded with the 10 C incubation temperature and the highest values were found at 30 C.

Total mineral N for WS3 was highest on the high N plot at 30 C. The low N plot of WS3 at 10 C produced the lowest amount of total mineral N. Total mineral N appeared to level off at day 21 of the incubation for all plots, with the exception of WS3/high N at 20 C and 30 C. These plots increased until the end of the incubation at day 28 (Figure 4). Soil temperature and total mineral N are related.

Figure 2. Extractable NO_3-N (mg N/kg soil) for low and high N plots of WS3 at FEF.

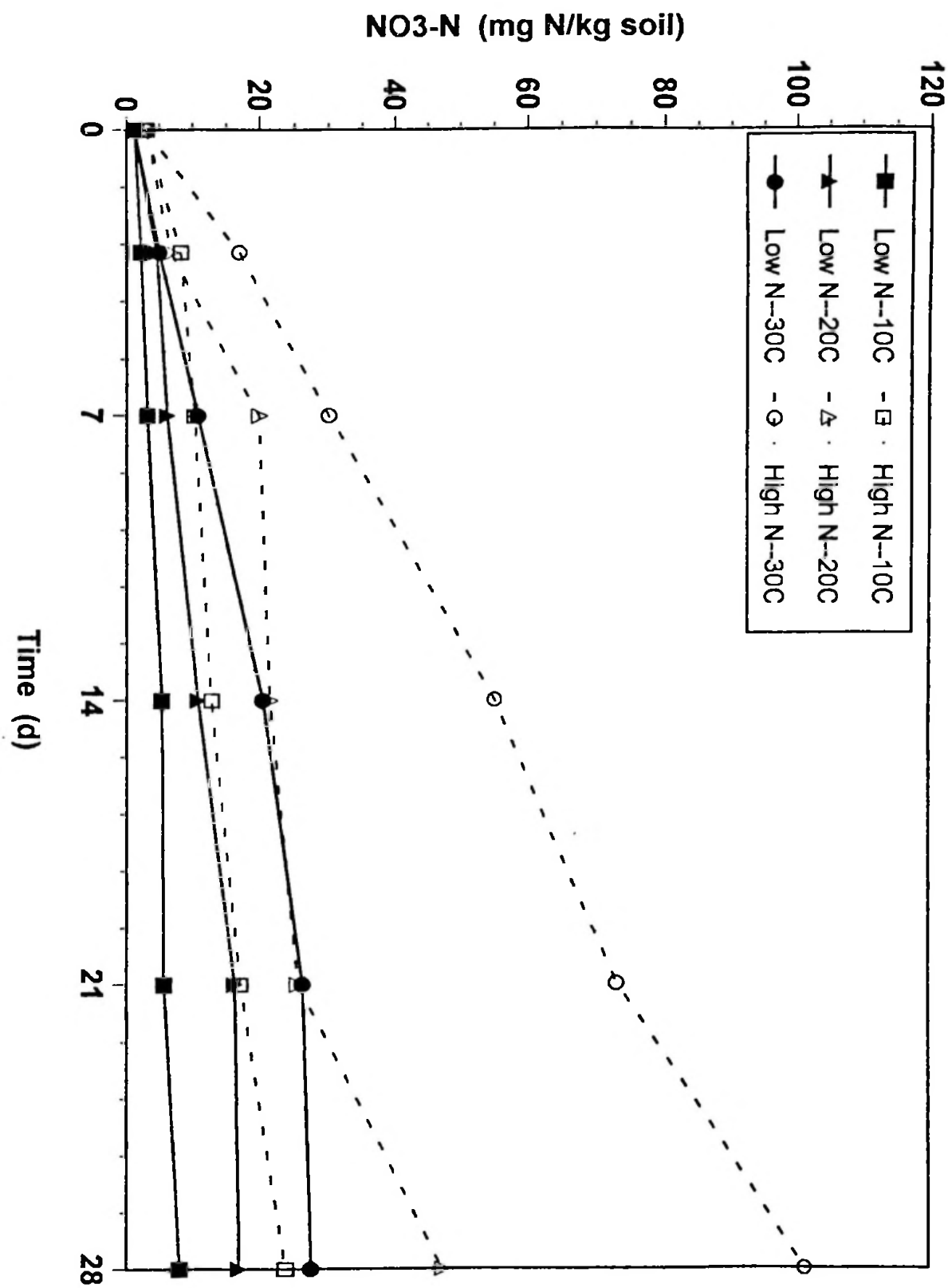

NO3-N (mg N/kg soil)
0
20
40
60
80
100
120
Time (d)
0
7
14
21
28
Low N--10C
High N--10C
Low N--20C
High N--20C
Low N--30C
High N--30C

Figure 3. Extractable NO_3-N (mg N/kg soil) for low and high N plots of WS4 at FEF.

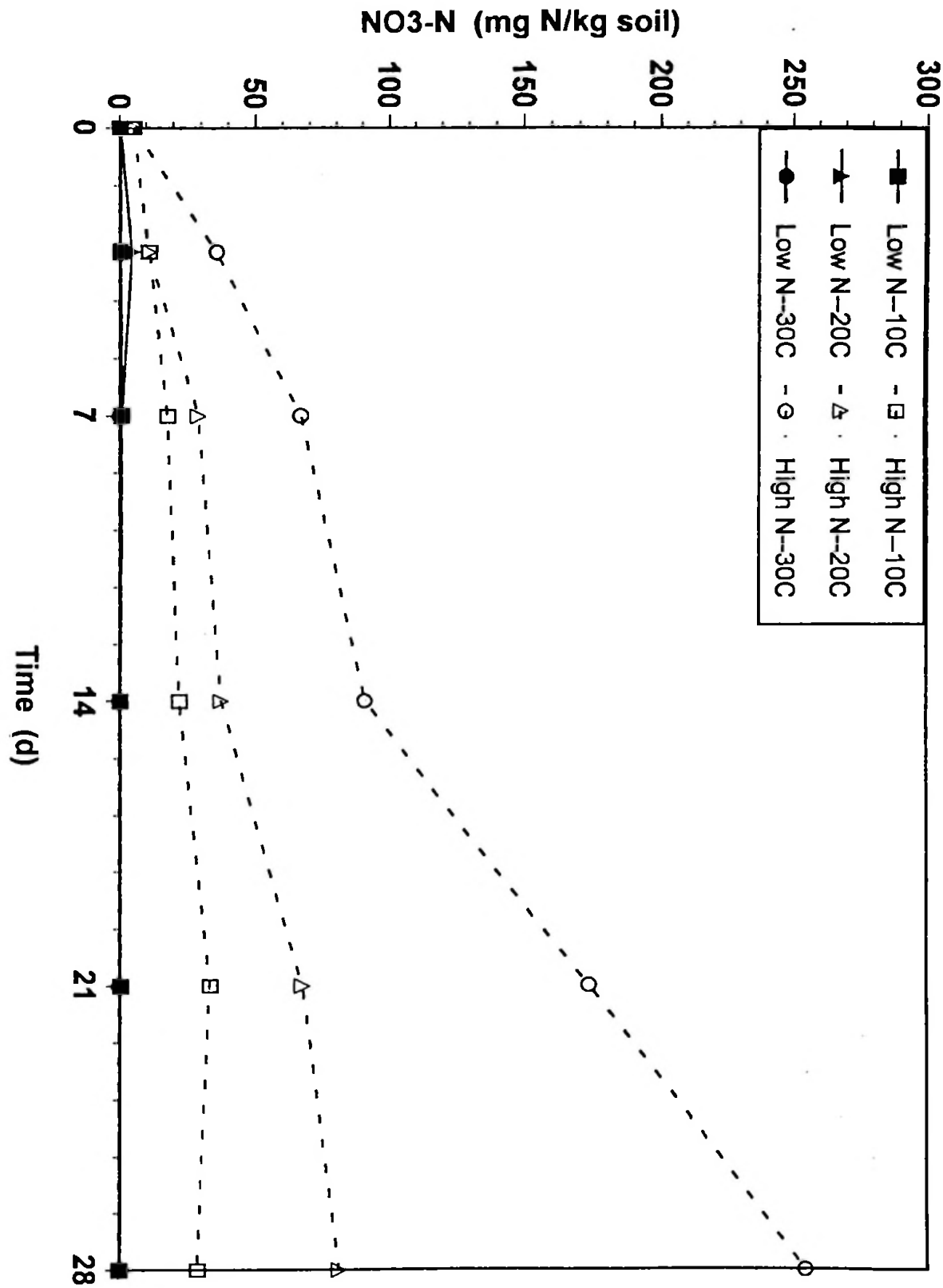
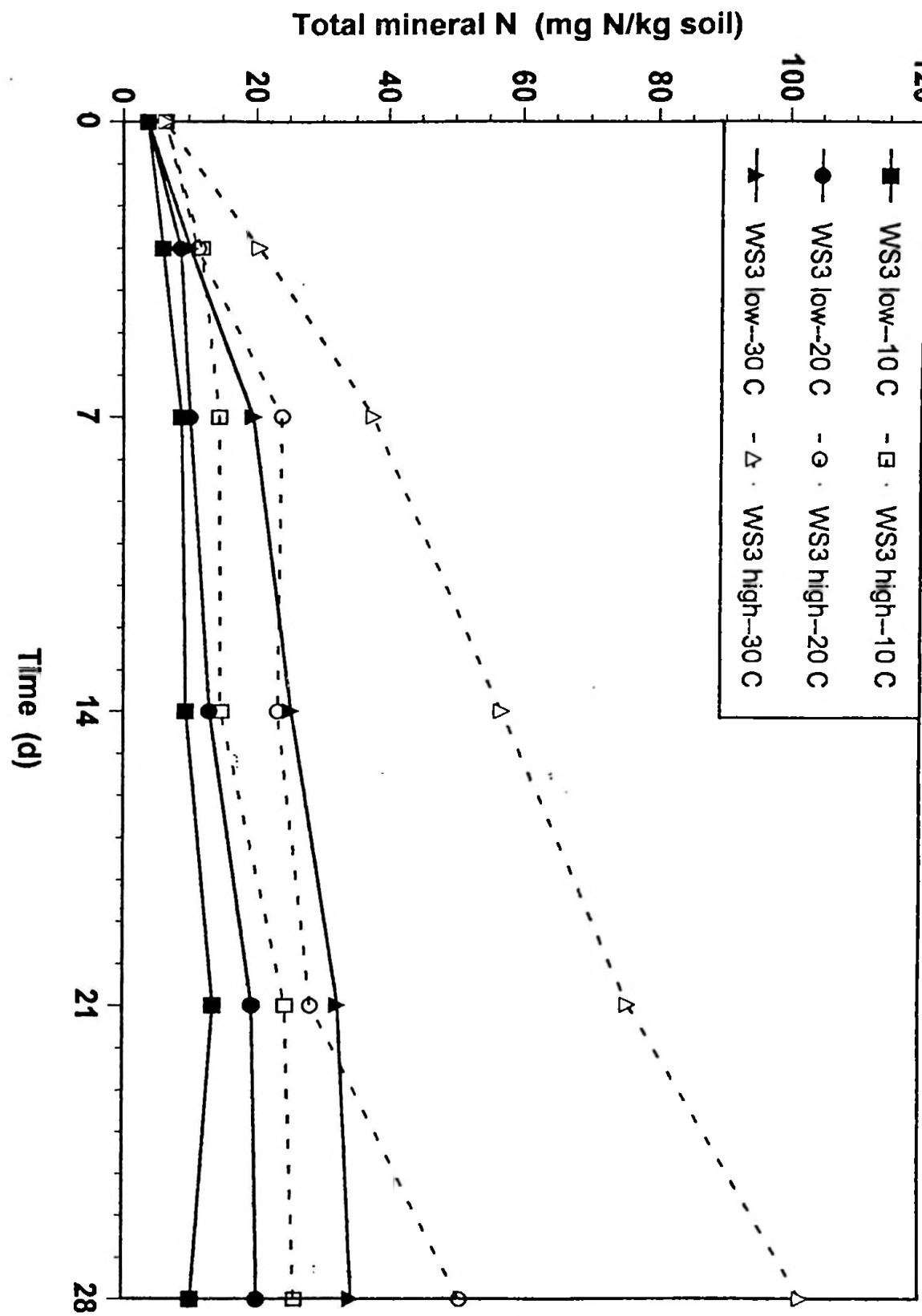

NO3-N (mg N/kg soil)
0
50
100
150
200
250
300
Time (d)
0
7
14
21
28
Low N--10C
High N--10C
Low N--20C
High N--20C
Low N--30C
High N--30C

Figure 4. Total mineral N (mg N/kg soil) over time for the low and high N plots of WS3 at FEF.


Total mineral N (mg N/kg soil)
0
20
40
60
80
100
120
Time (d)
0
7
14
21
28
WS3 low--10 C
WS3 high--10 C
WS3 low--20 C
WS3 high--20 C
WS3 low--30 C
WS3 high--30 C

Soils of WS4 also exhibited the same reaction to incubation temperature as those of WS3. The higher temperatures produced the highest total mineral N values and the lowest temperature produced the lowest values. Plot type also played an important role in total mineral N. Again, high N plots produced higher values and the low N plots yield lower values (Figure 5). The main difference between WS3 and WS4 is that WS4 has much higher total mineral N values. The highest value on WS4 is approximately 265 mg N/kg soil. This value is over two times higher than the highest value on WS3 (110 mg N/kg soil). However, the lowest amount of total mineral N for both WS3 and WS4 is roughly the same. The low N plots of both watersheds are roughly 5 mg N/kg soil.

The lowest net mineralization rates were found on the low N plots at both WS3 and WS4. The highest rates of net mineralization are at the 30 C incubation temperature of the high N plots (Figure 6). Net mineralization rates are nearly identical for the low N plots at all three incubation temperatures, with the exception of the low N plots at 10 C. The low N plot of WS3 is slightly higher at 10 C in comparison to the low N plot of WS4. The high N plot on WS4 is consistently higher at all three incubation temperatures.

Net mineralization rates increased among soils at all incubation temperatures (10 C, 20 C, and 30 C) at both watersheds (Figure 6). The most dramatic increase occurred at the high N plot of WS4 at an incubation temperature of 30 C. All net mineralization values responded significantly ($p<0.05$) to all three incubation temperatures. This is in contrast to the findings of Niklinska et al. (1999) which found that N mineralization at study sites in Sweden and Poland did not significantly respond to temperatures below 15 C, but did generally respond to temperatures above 15 C.

Figure 5. Total mineral N (mg N/kg soil) over time for the low and high N plots of WS4 at FEF.

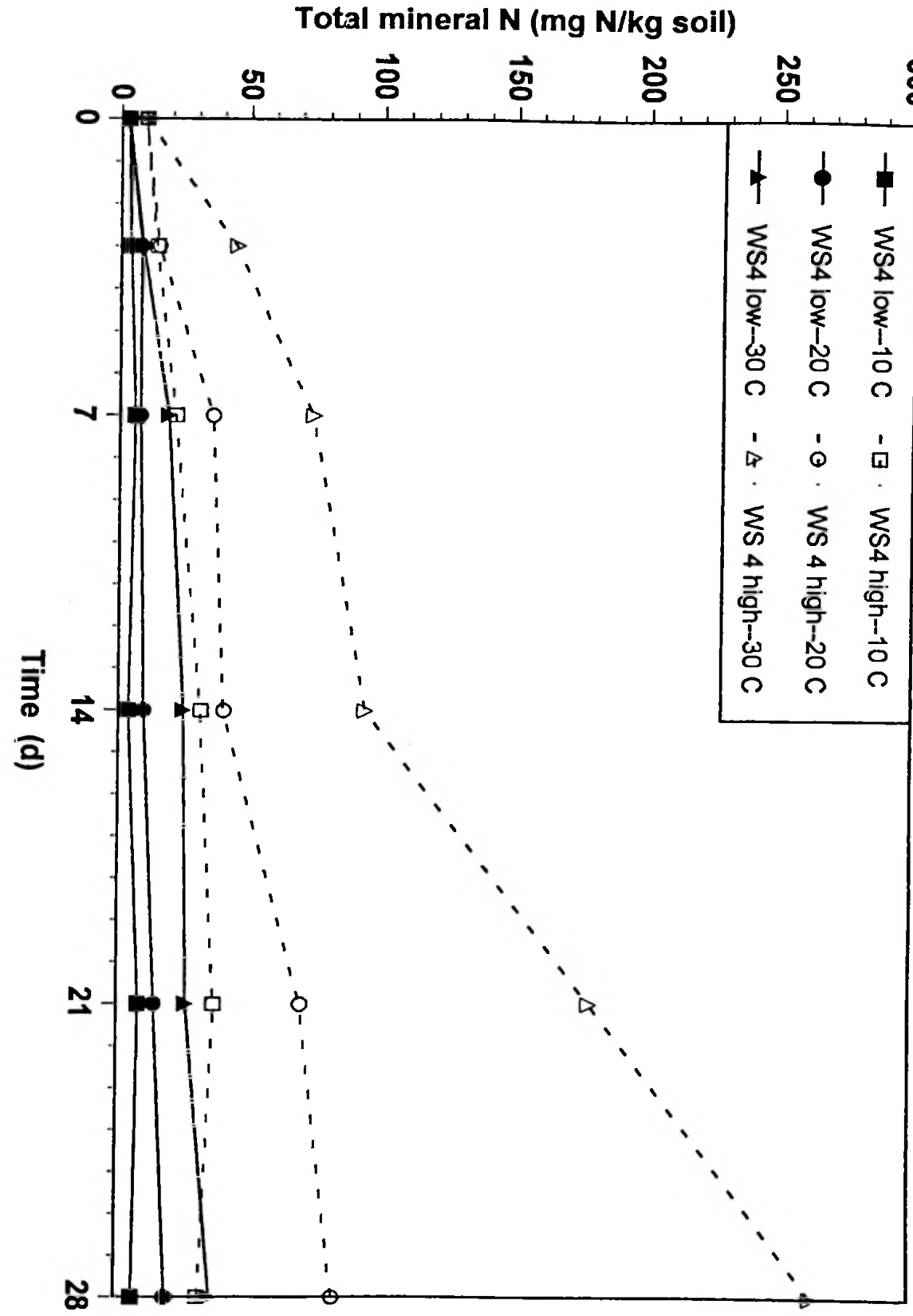
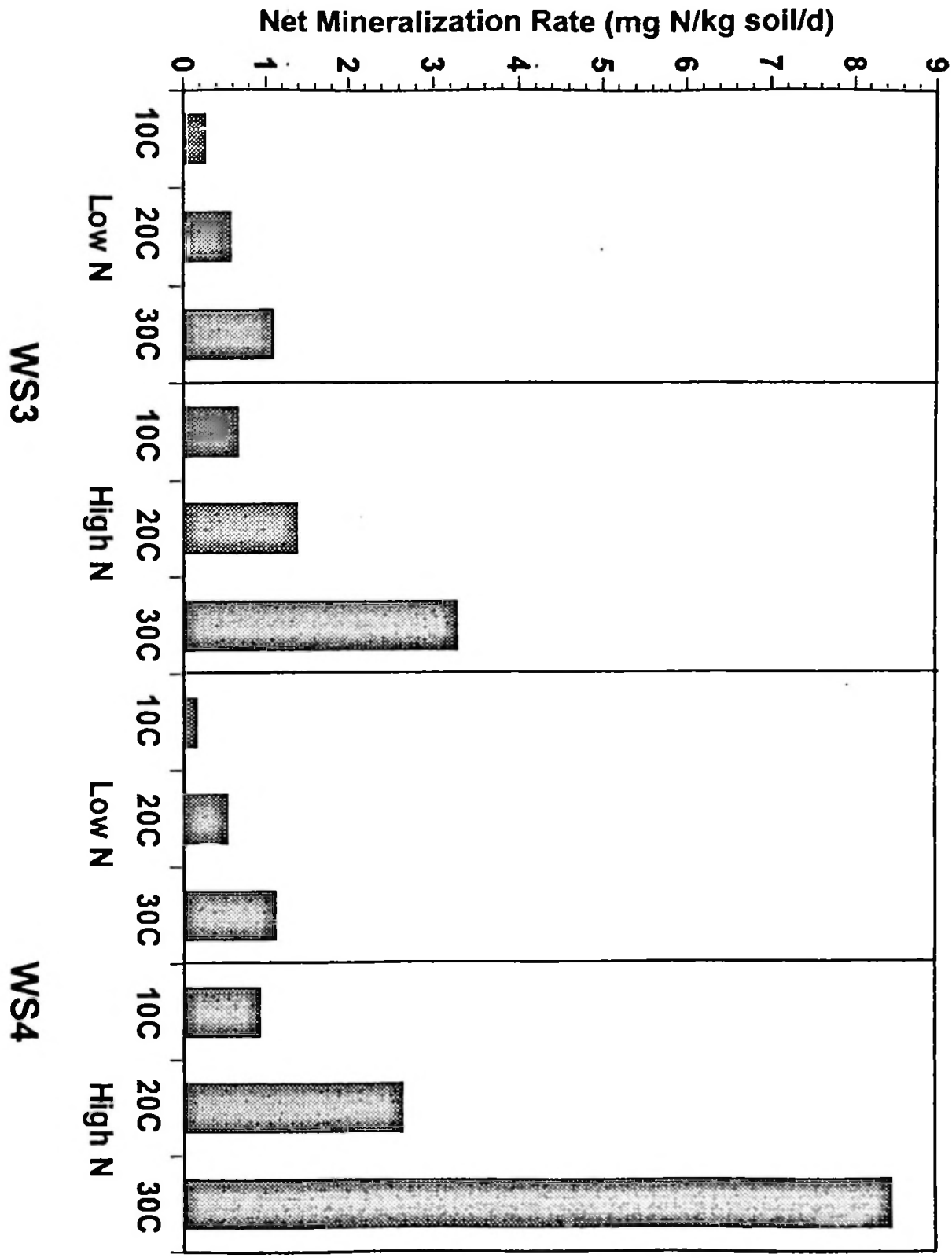

Total mineral N (mg N/kg soil)
0
50
100
150
200
250
300
Time (d)
0
7
14
21
28
WS4 low--10 C
WS4 high--10 C
WS4 low--20 C
WS 4 high--20 C
WS4 low--30 C
WS 4 high--30 C

Figure 6. Net N mineralization rate (mg N/kg soil/d) determined by linear regression for low and high N plots of WS3 and WS4 of FEF at three incubation temperatures.


Net Mineralization Rate (mg N/kg soil/d)
0
1
2
3
4
5
6
7
8
9
WS3
WS4
Low N
High N
Low N
High N
10C
20C
30C
10C
20C
30C
10C
20C
30C
10C
20C
30C

Similar rates of net mineralization were exhibited in low N plots on both watersheds. These plots also exhibited lower net mineralization rates than the high N plots at both WS3 and WS4. The lowest rates of mineralization appear to correlate with the lowest temperature for all plots. As the temperature increased, so does mineralization. As a general rule, soil organic N decreases as the average temperature of soil increases (Stevenson 1982). A rise in temperature of 10 C should cause soil N to become two or three times lower (Stevenson 1982). This decrease in organic soil N is partially attributed to the fact that for every 10 C increase in temperature in the 20 C to 30 C range, microbial N mineralization doubles. Mineralization will continue to rise until a temperature is reached that will affect microbial growth.

Net nitrification increased with increasing temperature among WS3 and WS4, with the exception of WS4/low N. The low N plot of WS4 showed no net nitrification. In WS4, the high N plot exhibited the highest values of net nitrification. Rates for net nitrification of the high N plot of WS3 were approximately twice the value of the low N plot. The high N plot of WS4 was nearly two and a half times larger than the high N plot of WS3 (Figure 7). Increases in net nitrification rates were seen in both high N plots and in the low N plot of WS3. Regardless of temperature, WS4/low N exhibited no net nitrification throughout the 28 day experiment.

The pH values (Table 2) for the soils in the study plots were determined. The pH value for soils of WS3/low N were the most acidic, while the soils of WS4/high N were the least acidic. Low N plots had more acidic soils in comparison to the high N plots. This is also true of WS3 when compared to WS4. Overall, WS3 had more acidic soils,

Figure 7. Net nitrification rates (mg N/kg soil/d) determined by linear regression for low and high N plots of WS3 and WS4 of FEF at three incubation temperatures.

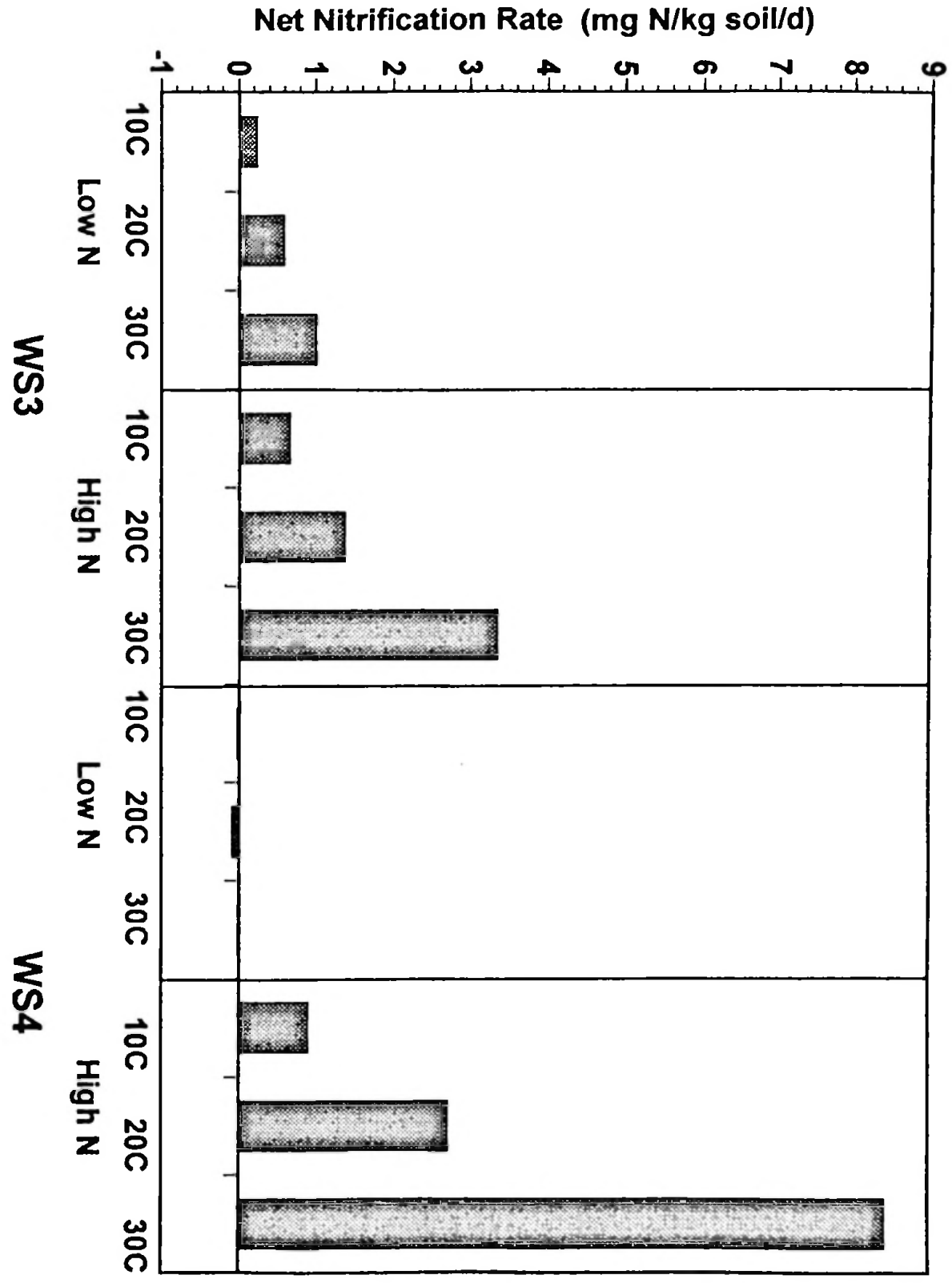

Net Nitrification Rate (mg N/kg soil/d)
-1
0
1
2
3
4
5
6
7
8
9
WS3
WS4
Low N
High N
Low N
High N
10C
20C
30C
10C
20C
30C
10C
20C
30C
10C
20C
30C

Table 2. Least significant difference test (LSD T) comparison of means by study plots at the Fernow Experimental Forest. Means with different superscripts are significantly different at $P < 0.1$.

Plot	Initial NO_3-N (mg N/kg soil)	Initial NH_4-N (mg N/kg soil)	N (%)	Lignin (%)	Lignin:N	C:N	pH
WS3 low N	1.26 ± 1.40^{b}	2.55 ± 0.16^{b}	1.82 ± 0.05^{ab}	35.90 ± 1.28^{a}	19.78 ± 1.22^{b}	24.73 ± 0.80^{b}	3.46 ± 0.04^{c}
WS3 high N	$3.10 \pm 1.23_{ab}$	3.41 ± 0.62^{ab}	1.97 ± 0.04^{a}	33.77 ± 1.86^{ab}	$17.17_{c} \pm 0.97^{c}$	23.17 ± 0.91^{b}	3.75 ± 0.05^{b}
WS4 low N	0.28 ± 0.14^{c}	2.58 ± 0.52^{b}	1.44 ± 0.19^{c}	32.77 ± 1.40^{b}	22.90 ± 2.38^{a}	32.37 ± 3.56^{a}	3.47 ± 0.10^{c}
WS4 high N	5.37 ± 2.96^{a}	4.36 ± 1.09^{a}	1.73 ± 0.06^{b}	33.07 ± 1.06^{b}	19.08 ± 0.44^{b}	25.97 ± 0.74^{b}	4.00 ± 0.08^{a}

which are most likely caused by the application of $(NH_4)_2SO_4$.

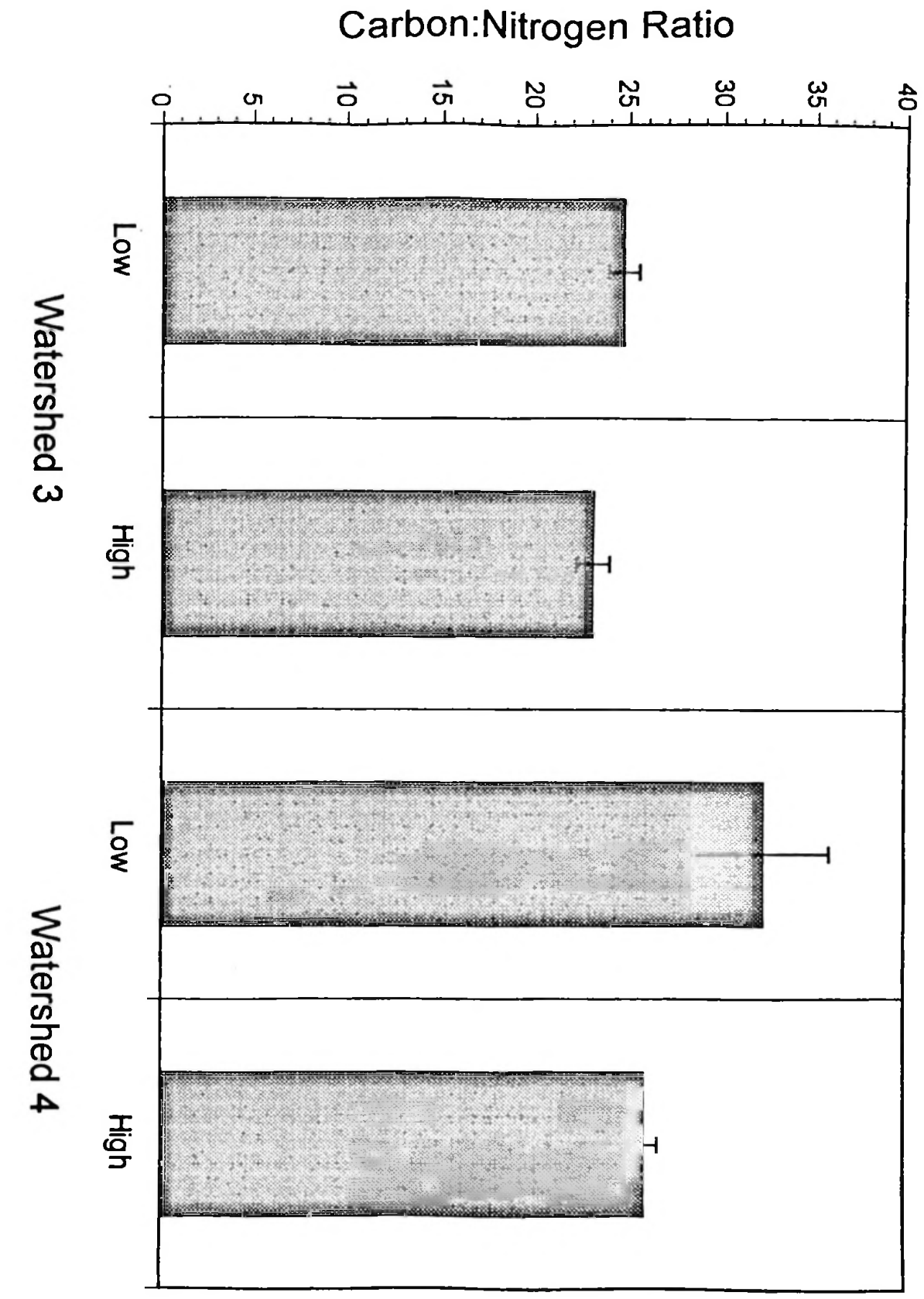
C:N ratios were also determined for this study (Figure 8). Comparison of soil C:N among the plots for this experiment were determined to be in the order of WS4/low N > WS4/high N > WS3/low N > WS3/high N. Here, WS4 had higher C:N ratios than WS3 and the low N plots had higher values than the high N plots.

The amount of extractable NO_3^- corresponded with temperature as well as plot type (high N or low N). The highest temperature of 30 C gave the highest values, while the lowest temperature had the lowest values. In general, high N plots at 30 C had the highest extractable NO_3^- values and low N plots at 10 C had the lowest. It should also be noted that the WS4 (control watershed), high N plot had extractable NO_3^- values nearly twice that of the WS3 (fertilized with $(NH_4)_2SO_4$), high N plot.

The larger amount of net mineralization on the high N plot of WS4 could be attributed to stand age. WS4 is older than WS3 and has only been selectively cut, whereas WS3 is younger and has been clear-cut. Goodale and Aber (2001) reported that N mineralization rates are greater in old-growth forests than in forests that have been disturbed throughout history.

A study done by Emmer and Tietema (1990) found no significant amount of nitrification to occur at temperatures of 0 C and 5 C. However, at 20 C and 25 C, nitrification increased considerably before seeing a significant decrease in nitrification at 30C. The data differ slightly from the data found at FEF. Emmer and Tietema (1990) found the temperature optimum for nitrification to be 25 C and argued that data produced at temperatures higher than that would be of limited use. Generally, nitrification rates are

Figure 8. Comparison of C:N for the low and high N plots of WS3 and WS4.


Carbon:Nitrogen Ratio
0
5
10
15
20
25
30
35
40
Low
High
Low
High
Watershed 3
Watershed 4

very low below 5 C and above 40 C due to the accumulation of NH_4^+. Nitrification is more susceptible to changes in environmental conditions, such as temperature, than is mineralization.

Researchers have stated that nitrification rates in old-growth forests (such as WS4) nearly double the rates of nitrification in forests that have been clear-cut (such as WS3) (Goodale and Aber 2001). This finding correlates with plots in WS3 (clear-cut site) when compared to the high N plot of WS4 (old-growth site). However, this does not explain why the low N plot of WS4 shows no net nitrification. Work by Donaldson and Henderson (1990a) may explain the high net nitrification rates on the high N plot of WS4. They found that the highest potential for nitrification occurs in soils that are sampled from sites that have mature vegetation. This offers no explanation for the phenomenon occurring at the low N plot.

One possible explanation for the absence of nitrification in the low N plot of WS4 is allelopathic inhibition. A study done by Davies et al. (1964) found that vegetation that grows in sites with low N concentrations produce high concentrations of polyphenolic compounds. The high concentration of polyphenolics in oak foliage increases during the growing season (Feeny and Bostock 1968). These polyphenolic compounds inhibit nitrifying organisms, hence decreasing nitrification. Inhibitory compounds are produced in larger quantity in mature stands, coupled with high soil N levels, enhancing decomposition and increasing nitrification. Again, this explains the high nitrification rates in the high N plot of WS4 and it also offers a plausible reason as to why the low N plot has no nitrification. The low N plot is a mature stand, which has the potential to produce a large amount of oak polyphenolic compounds. The plot is also low in N, which would

not allow for the enhancement of decomposition, thus making the nitrification rate very low or null. It is important to keep in mind that one of the dominant tree species of both watersheds is *Quercus rubra* (red oak).

Another possible cause of allelopathic inhibition is the shrub *Vaccinium* sp. *Vaccinium* belongs to the Ericale family and includes other members such as *Calluna, Kalmia, and Rhododendron* (Straker 1996). *Vaccinium* was found repeatedly in WS4 during the soil sampling for this study. A study in the northern European *Calluna* heathlands proved that low mineralization and nitrification rates were related to the allelopathic effects of *Calluna* (Read 1985). The toxic phenolics and aromatic and aliphatic organic acids in the *Calluna* heathland soils produced an inhibitory effect on the microbial community; specifically those associated with mineralization and nitrification. The high accumulation of organic matter causes a release of carboxylic acids, which help to acidify the soil and cause the inhibition of nitrification due to NH_4^+ becoming the main source of inorganic N (Read 1985). *Kalmia angustifolia* has also been documented to produce substances that inhibit microbial communities that aid in soil nutrient cycling and plant uptake (Bradley et al. 1997; Yamasaki et al. 1998).

A previous study at FEF (Gilliam et al. in press), found that *Vaccinium vacillans* constituted approximately 29% of the herb layer aboveground biomass on WS4. This species can maintain and increase soil acidity through the preferential uptake of NH_4^+, instead of NO_3^- (Gilliam et al. in press). Roots of *V. vacillans* are also capable of secreting organic acids that limit N-mineralizing microbes, particularly nitrifying bacteria. The study concluded that the herbaceous layer, not tree species could possibly be responsible for the limitation of N mineralization and nitrification.

The lack of net nitrification on the low N plot of WS4 could also be attributed to the low pH and high C:N of the soil (Table 2), consistent with a study done in the Huntington Forest located in the Adirondack Mountains of New York (Ohrui et al. 1999). The upland conifer zone of the Huntington Forest exhibited low net nitrification rates that were determined to be linked to the low soil pH and high C:N. Similarly, the high N plot of WS4 resembled the upland hardwood zone of the Huntington Forest. Both had high net nitrification rates, which could be caused by a lower C:N and low pH. Net nitrification occurs at the low and high N plots of WS3, despite the low soil pH.

A study performed in Skimikin, British Columbia reported similar results as those found at FEF (Thomas and Prescott 2000). All three study plots of the British Columbia study had low soil pH. The lowest nitrification rate occurred in the lodgepole pine site which had the lowest soil pH and highest C:N. The highest rates of nitrification was expressed in the Douglas fir site, again a low pH site, but the site with the lowest C:N. The occurrence of nitrification at low soil pH has also been reported in forests throughout Europe and the northeastern United States (Ohrui et al. 1999). With excess N availability in these forest soils, it has allowed for nitrification to take place, even though the pH is low.

Chapter IV.
Characterization of the Soil Bacterial Community

The polymerase chain reaction (PCR) is a powerful DNA amplification technique capable of providing a sensitive and specific method for characterizing microorganisms in an environment (Watson and Blackwell 2000). In order to perform DNA amplification using PCR, a small partially purified sample of DNA is prepared from a sample taken from the field. The sample is then examined for the DNA sequence unique to the study organism. Although this procedure is used quite often for characterizing microorganisms such as *Cercospora caricis* (Inglis et al. 2001), *Pichia kluyveri* Bedford and Kudrjavzev (Ganter and de Barros Lopes 2000), and *Campylobacter jejuni* (Englen and Kelley 2000), there can be complications with amplifying DNA from soil microorganisms. The major complication is the presence of PCR inhibitory compounds, particularly those found in soils and sediments (Watson and Blackwell 2000; Aakra et al. 2000). Cell extraction preceded by lysis and DNA extraction would combat this problem, but could possibly introduce bias inherent in the cell extraction (Aakra et al. 2000). Recently, a few studies have been done with methane oxidizing bacteria in forest soils that have avoided this type of complication (Jensen et al. 2000; Steinkamp et al. 2001).

Six groups of nitrifying bacteria were defined, based on unique sequences of their ammonia monooxygenase (AMO) genes (Figure 9). Each group was represented by different set of primers (Table 3). The groups were chosen based on their how closely their AMO genes clustered to each other. Closely related genes were placed in the same

Figure 9. Phylogenetic tree depicting the relationship of genes for known ammonia monooxygenases. Bacterial DNA primers were selected from these organisms. "Unidentified bacterium" refers to the AMO of an uncharacterized bacterial strain.

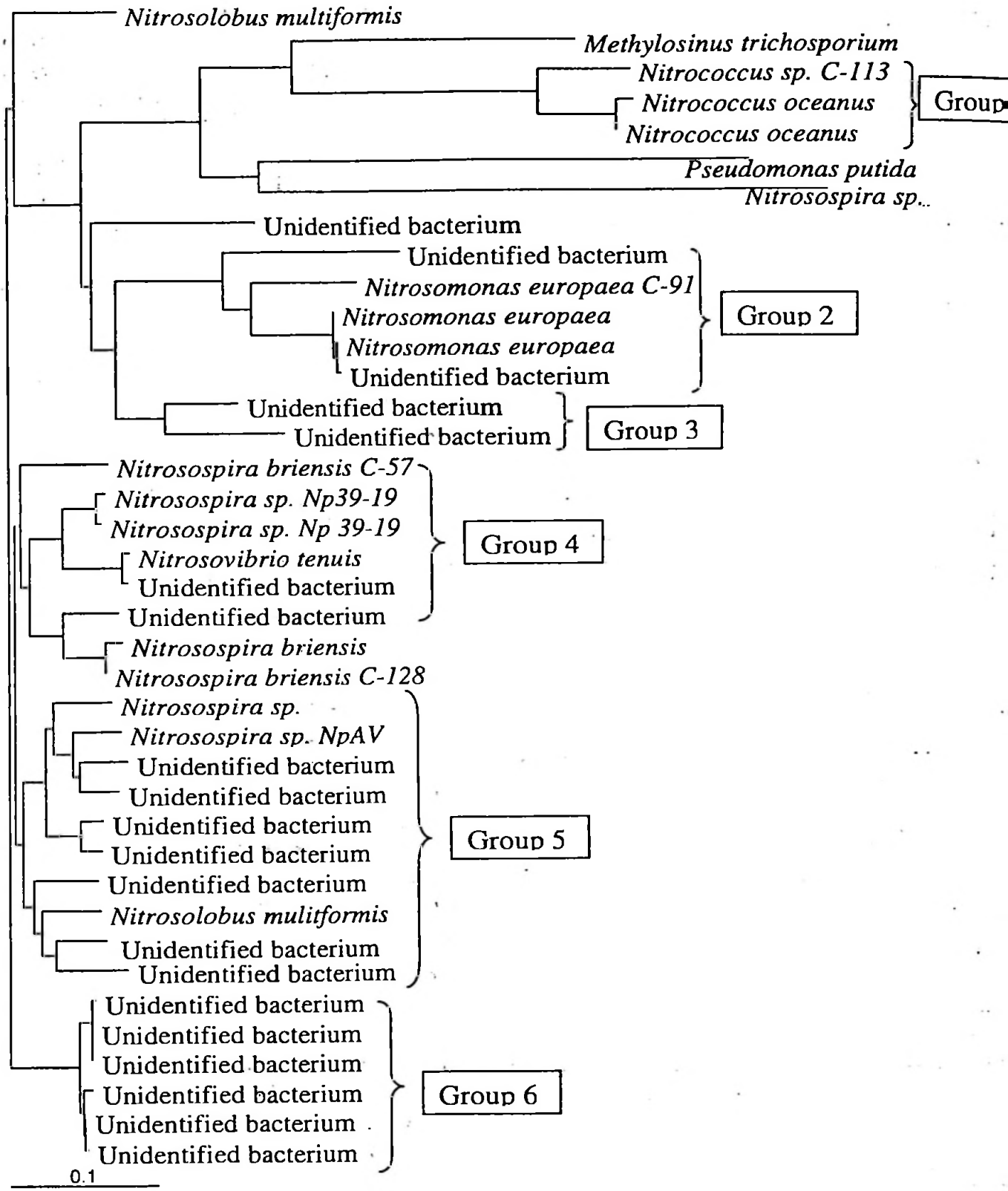

Nitrosolobus multiformis
Methylosinus trichosporium
Nitrococcus sp. C-113
Nitrococcus oceanus
Nitrococcus oceanus
Group
Pseudomonas putida
Nitrosospira sp..
Unidentified bacterium
Unidentified bacterium
Nitrosomonas europaea C-91
Nitrosomonas europaea
Nitrosomonas europaea
Unidentified bacterium
Group 2
Unidentified bacterium
Unidentified bacterium
Group 3
Nitrosospira briensis C-57
Nitrosospira sp. Np39-19
Nitrosospira sp. Np 39-19
Nitrosovibrio tenuis
Unidentified bacterium
Group 4
Unidentified bacterium
Nitrosospira briensis
Nitrosospira briensis C-128
Nitrosospira sp.
Nitrosospira sp. NpAV
Unidentified bacterium
Unidentified bacterium
Unidentified bacterium
Unidentified bacterium
Unidentified bacterium
Nitrosolobus mulitformis
Unidentified bacterium
Unidentified bacterium
Group 5
Unidentified bacterium
Unidentified bacterium
Unidentified bacterium
Unidentified bacterium
Unidentified bacterium
Unidentified bacterium
Group 6
0.1

Table 3. Host organisms and forward and reverse primers found in each primer group used in this study. Unidentified bacterium refers to unidentified bacterium DNA from ammonia monooxygenase.

Group	Host Organisms	Primers (Forward and Reverse)
1	Unidentified bacteria *Nitrosolobus multiformis* *Nitrosospira sp.* *Nitrosospira sp. NpAV*	5'ACTACCCNATCAACTTCGT3' 5'GANCCTTGTTCCATCAG3'
2	*Nitrosospira briensis C-57* *Nitrosolobus multiformis* *Nitrosospira sp. Np39-19* *Nitrosovibrio tenuis* Unidentified bacteria *Nitrosospira briensis C-128*	5'CGCAACTGGATGATCAC3' 5'ACCAGCGGCAGGTGGGTC3'
3	Unidentified bacteria	5'TACCCCATCAACTTTGTATTCCC3' 5'GCCAAATGCGGTGACGTCGTTCT3'
4	*Nitrosomonas europaea C-91* *Nitrosomoas europaea* Unidentified bacteria	5'CACTACCCATCAACTTCGTAACA3' 5'GCGATGTACGATACGACCTCTTTT3'
5	*Nitrococcus oceanus* *Nitrococcus sp. C-113*	5'ATGAGTGCACTTACGTCTGCGGTT3' 5'TCCTTGGAGAACCTGCGGCC3'
6	Unidentified bacteria	5'TTTGTACTGCCATCGACCATGAT3' 5'CGCAGAACATCAGATAGATAC3'

group. Each group shares a region of highly conserved DNA from which the primers were made.

The group 2, group 4, and group 5 AMO genes were detected over the 28 day soil incubation period of WS3/low N. Group 2 and group 5 appeared at pre-incubation. At day 7, no AMO genes were detectable. After 14 days of incubation, genes from primer group 4 were detected. No AMO genes were detected after 21 or 28 days of incubation. Over the course of the 28 day incubation period, the microbial community changed. The lack of detectable AMO genes may indicate conditions that select against nitrifying bacteria, but could also indicate a selection for a nitrifying community that is no detected by any of the primer pairs used.

Primer group 5 and group 2 were the only groups present in the high N plot of WS3. Group 5 first appeared in these soils during the pre-incubation stage of the study. At day 7, the AMO genes for group 5 were no longer detectable. However, on day 14, the AMO genes for group 5 were again detected. No AMO genes of group 5 were detected in soils incubated for 21 or 28 days. Group 2 AMO genes were only detected at day 28 of the study. These data indicated that the microbial community did change during the incubation period.

WS4/low N had detectable AMO genes for groups 2, 6, and 4. At pre-incubation, groups 2 and 6 were detected. By day 7, only group 6 was detectable. On day 14, neither group 2 or 6 were detected, but group 4 was present. On day 21 group 2 was again detected (groups 4 and 6 were not). At the end of the 28 day incubation period, no AMO genes were detected. Again, these data indicate that the microbial community did change during the course of the 28 day incubation period.

Only the pre-incubation stage of incubation had detectable AMO gene groups present on WS4/high N. At pre-incubation group 1, group 2, and group 6 were all detected. However, days 7, 14, 21, and 28 had no detectable AMO genes. This indicates that the microbial community did not change after the pre-incubation stage. It should be noted, that even if a band is not detected at a particular time throughout the experiment, that does not mean that there are no bacteria present. The absence of a DNA band simply means that the method of detection used in this study did not detect it or that it is a bacterial community that is not known about.

All plots detected AMO gene groups during pre-incubation, but by day 7 only WS4/low N had detectable gene groups present (Table 4). On day 14 all plots, with the exception of WS4/high N, had detectable groups present. At days 21 and 28 only one plot for each day had detected AMO genes. On day 21 WS4/low N had group 2 present and on 28 WS3/high N had group 2 present. The group that was detected the most throughout the experiment was group 2, followed by group 5 and group 6. The group that was least detected was group 1. Overall, it is clear that the nitrifying bacterial community is very dynamic. AMO genes were readily detected at pre-incubation, but were nearly absent after seven days of incubation. By fourteen days of incubation the communities had shifted. Only AMO genes of the *Nitrosomonas* group (group 2) were detected after three and four weeks of incubation. The data suggest either that incubation conditions were not suitable for nitrifying bacteria, or that previously uncharacterized AMO genes were dominant after prolonged incubation.

Table 4. Presence of primer groups in study watershed plots over a 28 day period.

Primer	Pre				D7				D14				D21				D28			
	3L[b]	3H	4L	4H	3L	3H	4L	4H	3L	3H	4L	4H	3L	3H	4L	4H	3L	3H	4L	4H
1[a]				+																
2	+		+	+												+				+
3																				
4											+		+							
5		+	+									+								
6				+	+			+												

[a] Refers to groups of ammonia monooxygenase.
[b] Refers to study plots within the watersheds (WS3/low N, WS3/high N, WS4/low N, and WS4/high N).

Microbial soil activity is limited mainly by C input from plant litter production (Holmes and Zak 1994). If substrate availability is increased it could also cause an increase in microbial populations. However, extremes in soil temperature and water potential could cause a growth limitation. During the autumn months, microbial populations would increase followed by a decline in net N mineralization as the N is used to assimilate new microbial populations. However, in midsummer (the approximate time of sampling) C availability is low and N is mineralized from a declining microbial population (Holmes and Zak 1994). This could explain why only a relatively low amount of soil samples actually yielded a positive response to the DNA primers, while mineralization rates were relatively high.

As mentioned previously in this study, the ericaceous shrub *Vaccinium sp.* was found in the study sites of WS4. These inhibitory substances can cause the inhibition of microbial populations that are responsible for soil nutrient cycling and plant uptake. This provides another possible scenario as to why only a few soil samples had tested positive for the ammonia oxidizing bacteria. If the shrub was in certain sampling areas it could have caused a decline in microbial populations affecting the outcome of this portion of the study.

Chapter V.
Synthesis

Both extractable NO_3^- and total mineral N for WS3 and WS4 expressed a tendency to increase starting at day 14. This coincides with bacterial data, in that at day 14 the detectable presence of ammonia oxidizing bacterial DNA bands is decreasing. At the pre-incubation stage (day 0), the diversity of the bacterial community is rather large. By day 7 the community diversity has decreased and there is evidence for a shift in the nitrifying. In this study, extractable NO_3^- and total mineral N appear to decrease when microbial demand is high and increase when microbial demand is low. The bacterial communities appear to be utilizing the extractable NO_3^- and total mineral N present in the soil samples. It should be noted that the low N plot of WS4 did not illustrate an increase in extractable NO_3^- and therefore, no nitrification was occurring. However, genes for the bacteria responsible for the initiation of nitrification were located in the plot. Therefore, the genetic potential was present, but not expressed. In WS4/high N, no detectable AMO genes were seen however, the plot was highly active, indicating activity from uncharacterized AMO genes.

Net N mineralization rates and net nitrification rates appear to be directly related to each other. The amount of net mineralizaton at a plot is nearly identical for that same plot when compared to the nitrification rates, except at WS4/low N. The low N plot of WS4 exhibits no net nitrification, but does exhibit mineralization. Something is happening in soil processing between mineralization and nitrification, which is causing the inhibition of nitrification at the low N plot of WS4. Again, genes for nitrifying bacteria were detected on WS4/low N and therefore the lack of nitrifiers could not be the

reason why there is no net nitrification at this plot. A possible explanation for what is occurring is that while the bacteria are in this plot, they may not be active. This would cause nitrification to stop on WS4/low N.

The bacterial community is also changing over time (Table 5). As time passes, the community shifts in all soils. WS3 and WS4 start off with fundamentally different communities and over time, both watersheds change and the communities present as NO_3^- accumulates are not detected by the primers used in this study. It should again be noted that if a band is not present, it does not mean that no community is present in that plot at that time. It is possible that bacterial communities are there, but they may be ones that are not known.

In conclusion, the first hypothesis posed by this thesis was that soil temperatures and rates of net mineralization and nitrification, were correlated. This hypothesis was supported. The lowest rates for both net mineralization and nitrification were seen at the 10 C incubation temperature. The 30 C incubation temperature allowed the highest rates. This was true of all the study plots within WS3 and WS4.

The second hypothesis, that the lack of net nitrification on WS4/low N was caused by a lack of nitrifying bacteria at that site, was rejected. The opposite was found to be true. The low N plot of WS4 did have bacterial communities present and therefore, something other than the absence of nitrifying bacteria has caused the lack of net nitrification on WS4/low N. What appears to be happening is that the bacterial communities have become inactive and stopped the process of nitrification. What is not known is why the communities became inactive.

This study expresses the need for more bacterial work to be done at FEF to provide a better understanding of the spatial variability of soil N processes of WS3 and WS4. Future work should be done trying to characterize the bacterial communities by looking for specific bacteria and exploring the role of other bacterial communities involved in the process of nitrification.

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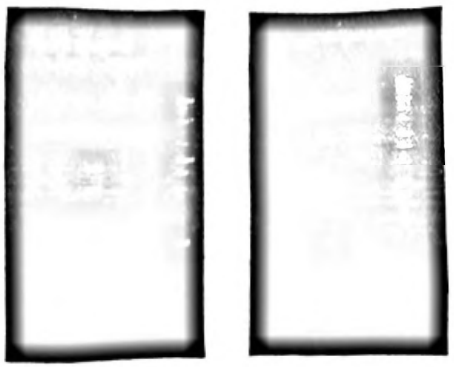
APPENDIX

Primer Group 1



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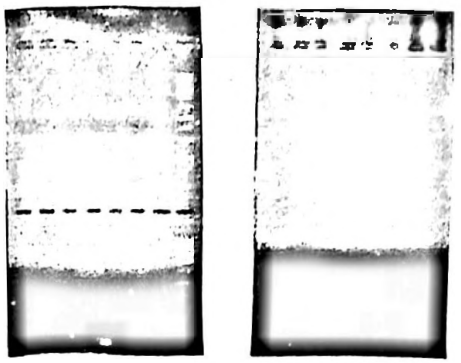

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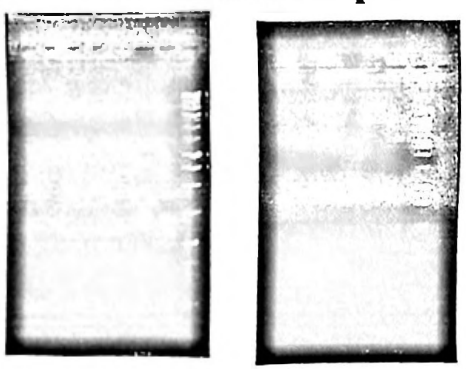

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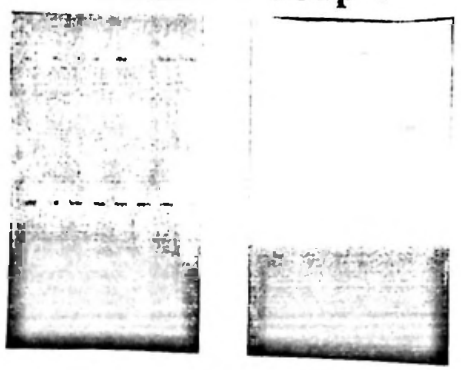

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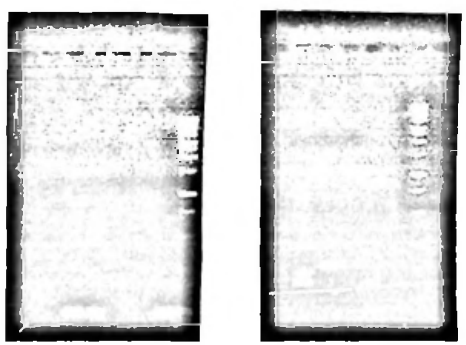

Primer Group 6