AN ABSTRACT OF THE THESIS OF

Michael H. McClellan for the degree of Master of Science in Forest Science presented on May 20, 1987.

Title: Denitrification Potential in Forest Riparian Soils of the Western Oregon Cascades: Spatial and Temporal Variation

Abstract approved: _____

Kermit Cromack Jr.

Denitrification, the biological or chemical reduction of ionic nitrogen oxides to nitrous oxide or dinitrogen, has not been widely studied in forest ecosystems despite widespread interest in other facets of the forest nitrogen economy. This study had three main objectives: to determine whether potential for denitrification exists in forest riparian and hill slope soils in the western Oregon Cascade Mountains; to test the hypothesis that denitrification potential within riparian forests varies with slope position, soil depth, and season; and to contrast denitrification potential in old-growth Douglas-fir (Pseudotsuga menziesii (Mirb.) Franco.) and secondary-successional red alder (Alnus rubra Bong.) forests.

Denitrification potential was assessed by anaerobically incubating freshly-collected soils in the presence of acetylene and measuring nitrous oxide emission with electron capture gas chromatography. Soils were amended with deionized water, but not with nitrate or carbon sources. Soil moisture, pH, nitrate, ammonium, and total nitrogen, carbon, and phosphorus were measured. Soils were collected during May-June 1984 and August-September 1983 and 1984.

After 1 h of incubation, average N-losses from the upper mineral soil (0-15 cm) ranged from 0-11.3 ng N g^{-1} h^{-1} for the Douglas-fir soils, and from 3.4-24.4 ng N g^{-1} h^{-1} for the red alder soils. N-loss was significantly less ($p < 0.001$) from the lower mineral soil (15-30 cm); averages ranged from 0-0.4 ng N g^{-1} h^{-1} for the Douglas-fir soils, and from 1.1-16.0 ng N g^{-1} h^{-1} for the red alder soils.

Flood plain soils usually lost more N (3.6-24.4 ng N g^{-1} h^{-1}) than did hill slope soils (0-2.3 ng N g^{-1} h^{-1}); intermediate N losses were measured in toe slope soils (0.8-4.2 ng N g^{-1} h^{-1}). The effect of slope position was significant ($p < 0.001$) only in 1983. Seasonal variation was not significant. Multiple regression of N-loss on soil nitrate, total N, and pH accounted for 61% of the N-loss rate variation. Denitrification potential was highly variable, with coefficients of variation ranging between 53 and 281%, and appeared to fit a lognormal distribution.

Denitrification Potential in Forest Riparian Soils of the Western Oregon Cascades: Spatial and Temporal Variation

by

Michael H. McClellan

A THESIS

submitted to

Oregon State University

in partial fulfillment of
the requirements for the
degree of

Master of Science

Completed May 20, 1987
Commencement June 1988

ACKNOWLEDGMENTS

I would like to thank all of those who helped in so many ways over the course of this project. My parents, Mrs. Viola McClellan and Mr. Howard McClellan, deserve special thanks for their love, support, and encouragement through the years. As my major advisor, Dr. Kermit Cromack, Jr. guided and supported my work with valuable insights, advice, and encouragement, and I am very grateful for the remarkable opportunities he has provided me. My good friend John Price assisted me through all phases of this work.

Dr. Cliff Dahm and Dr. John Gordon reviewed the research proposal, and Dr. Bernard Bormann, Dr. David Myrold, and Dr. Bruce Coblentz provided valuable and constructive criticism of the thesis. Dr. Richard Morita graciously provided facilities and equipment for the early stages of this project, and I would like to thank Dr. Robert Griffiths and Dr. Bruce Caldwell for introducing me to the arcane world of gas chromatography. Carol Glassman performed many of the soil analyses and provided facilities and technical advice for others.

I am grateful to Dr. Stan Gregory and the OSU Stream Team for their support. This study was supported by the National Science Foundation's Division of Ecosystem Studies through grant BSR-8112455, 'The role of riparian vegetation in the metabolism and structure of stream ecosystems'.

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INTRODUCTION

Nitrogen may be lost from natural or managed forest ecosystems as dissolved or particulate N in streams or groundwater, as transported plant or animal biomass, or as a gas escaping to the atmosphere. Nitrogen is lost in the gas phase as ammonia, dinitrogen, or as any one of several oxides of nitrogen (Wollum and Davey 1975).

Denitrification is the biological and abiotic reduction of the ionic nitrogen oxides, nitrate and nitrite, to gaseous nitrogen oxides, nitric oxide and nitrous oxide, and dinitrogen. Biological denitrification, in the most restricted sense, is the reduction of nitrate (NO_3^-) to dinitrogen (N_2) by facultatively anaerobic bacteria respiring under anaerobic conditions. In addition, nitrogen may be lost as a gas, primarily nitrous oxide, during nitrification (Freney et al. 1979, Blackmer, Bremner, and Schmidt 1980, Goreau et al. 1980) and during dissimilatory nitrate reduction by nondenitrifying bacteria (Smith and Zimmerman 1981, Smith 1982). The general ecological significance of the last two processes is open to question. The nitrous oxide is produced as a by-product of the main reactions and the total amount produced is small.

The transformation of nitrogen oxides from ionic to gaseous forms represents a potentially significant loss of ecosystem nitrogen. Because of this, denitrification has been intensively studied in terrestrial agricultural systems, where over one-half of the nitrogen applied as inorganic fertilizer may be lost under suitable conditions (Delwiche 1981, Knowles 1982). Prior to the early 1980's, denitrification in forest ecosystems received little attention, as it was commonly asserted that forest soils were too low in pH, water content, and nitrate to support denitrification. The high organic matter content of forest soils was thought to restrict nitrification, a prerequisite for denitrification, due to the inability of nitrifying chemoautotrophs to compete with heterotrophs for the limited supply of soil ammonium (Vitousek et al. 1982).

Although it is accepted that denitrification is a significant factor in the nitrogen economy of some agricultural soils, only recently have studies begun to quantify denitrification in forest soils by direct methods (e.g. Melillo et al. 1983; Robertson and Tiedje 1984; Matson et al. 1987; Robertson et al. 1987).

During 1983 and 1984, I conducted research to determine the potential for denitrification in two typical forested riparian ecosystems in the western Cascade Range of Oregon: an old-growth conifer stand and a secondary-successional deciduous stand. Within these stands, I studied the variation of denitrification potential with slope position, soil depth, season, and soil physical and chemical properties. Before presenting the detailed study objectives, I will discuss the denitrifying bacteria, their biochemistry and ecology, and selected methods for measuring denitrification.

The Denitrifiers

A diverse group of bacteria share the functional descriptor denitrifier. The denitrifiers are relatively rare, a few species representing less than thirty genera. All are free-living bacteria and only one of the genera, Propionibacterium, contains obligate anaerobes (Bryan 1981). The denitrifiers include heterotrophs and autotrophs, chemotrophs and phototrophs, Gram-positive and Gram-negative bacteria, and some are considered nitrifiers or N_2-fixers (Bryan 1981, Knowles 1982, Ingraham 1981).

Of all the denitrifiers, the genera Pseudomonas and Alcaligenes (many formerly considered Achromobacters) are most abundant based on frequency of isolation data (Knowles 1982, Hattori 1973) using standard methods, but greatest abundance does not necessarily imply greatest physiological activity or ecological importance (Ingraham 1981).

The ability to denitrify is genetically unstable. In culture these bacteria may lose or regain the ability to synthesize the nitrogen oxide reductases. The ability to synthesize nitrous oxide reductase is most frequently lost in culture, but its absence has not been noted in wild-types of strains normally possessing this enzyme. This genetic instability and the wide distribution of denitrifying ability led some investigators to speculate that the gene for denitrifying enzymes was located on a plasmid. It has since been shown that at least one of these genes is located on the chromosome (Ingraham 1981).

Biochemistry of Denitrification

The biochemistry of denitrification reflects some of the diversity of the group, but I will present only the features most representative of the entire group and most pertinent to an understanding of the ecology of denitrification. This subject has been reviewed by Bryan (1981), Ingraham (1981), Firestone (1982) and Knowles (1982).

Denitrification is a rapid process of respiration, not fermentation, for the purpose of providing useful energy to the organism in the form of ATP high-energy bonds. This is in contrast to microbial assimilatory nitrate reduction, an energy-demanding process that incorporates N into cellular ammonium and amine groups. During denitrification, ATP is generated by oxidative phosphorylation coupled with electron transfer. The electron source is usually an organic carbon compound, while a few denitrifiers obtain energy from reduced sulfur compounds or light. When oxygen is absent from the environment, the denitrifiers use nitrogen oxides instead of oxygen as terminal electron acceptors.

In accepting an electron, nitrate is reduced to nitrite which in turn may act as a terminal electron acceptor, and so on until fully-reduced dinitrogen is produced. Each step along this respiratory sequence is coupled to ATP generation. The efficiency of this respiratory process is not fully known, but it is thought to yield less energy than aerobic respiration (Bryan 1981, Knowles 1982). Skinner (1975) claimed this pathway was 67.9% as efficient as aerobic respiration.

Ingraham (1981) presented the following pathway for denitrification (enzymes catalyzing each step are listed within brackets):

Nitrate (NO_3) [Nitrate reductase] -->
Nitrite (NO_2) [Nitrate reductase] -->
Nitric Oxide (NO) [Nitric oxide reductase] -->
Nitrous Oxide (N_2O) [Nitrous oxide reductase] -->
Dinitrogen (N_2)

There has been some controversy whether nitric oxide exists as a free intermediate produced by whole cells (Bryan 1981, Payne 1981, Knowles 1982).

Each step is catalyzed by a different enzyme, one of the nitrogen oxide reductases. The enzymes appear to be membrane-bound for the most part and Fe and S are required for activation. Mo is an essential constituent of nitrate reductase (NaR), while Cu is incorporated into or required for the synthesis of two types of nitrite reductases (NiR) (Bryan 1981). The reductases vary in their induction times; logically, NaR derepresses first when O_2 becomes limiting. A particular denitrifier may not carry out the full series of N reductions for one of several reasons: some intermediate, but not nitrate, may be available; pH, $_O2$ concentration, or intermediate concentration may limit some step; due to the varied derepression times, a cell may not possess the full complement of reductases; or the strain may lack the gene for one or more reductases. Seventy-one genera of bacteria contain strains that are capable of reducing nitrate to nitrite but no further (Ingraham 1981). It is questioned whether members of this

last group, often called nitrate respirers, should be considered true denitrifiers (Smith and Zimmerman 1981).

Factors Affecting Denitrification

In general, denitrification has been shown to increase with decreased oxygen availability and increased organic substrate availability, nitrate concentration, pH, and temperature. Soil moisture affects denitrification largely through its effect on O_2 availability (Hattori 1973, Focht 1978, Knowles 1982). Addition of acetylene blocks the reduction of nitrous oxide to dinitrogen (Yoshinari and Knowles 1976). This inhibitory action is exploited in several techniques for measuring denitrification rates (Yoshinari et al. 1977; Knowles 1982).

Moisture and Aeration

Denitrification is usually associated with water-saturated soil. Alexander (1977) states that no N losses from this process occur when soils are at less than 60% of their water-holding capacity. Moisture itself has little effect on denitrification as long as the bacteria do not dry out (Focht 1978). It is mostly by controlling oxygen availability that soil water affects the rate and amount of N loss (Knowles 1982). When studying the effect of moisture increases in field soils, timing of denitrification measurements is critical, since denitrification rates have been shown to peak within 1 to 12 h after rainfall or irrigation and to decline to previous levels after

12 to 60 h (Sexstone et al. 1985a). Coarse-textured soils responded faster than fine-textured soils.

The presence of O_2 directly inhibits denitrification by reducing enzyme activity and by repressing enzyme synthesis. Oxygen will out-compete nitrate as an electron acceptor and, in addition, oxygen may temporarily inactivate the reductases by oxidizing them. The enzymes do not appear to be destroyed by oxygen. Evidence for this comes from observations on a mutant in which oxygen cannot act as a terminal electron acceptor. This strain continues to denitrify in the presence of oxygen, its enzymes unaffected by oxygen (Bryan 1981). Perhaps this mutant prompted the early reports of aerobic denitrification.

Oxygen represses the synthesis of denitrification enzymes, but the precise mechanism of repression is not clear. As a soil becomes saturated with water, oxygen is depleted, the denitrifiers switch from aerobic respiration to anaerobic respiration. The NaR and NiR derepress in 40 minutes to 3 hours. During this time, evolution of N_2 from the soil will reflect the degree of anaerobiosis in the soil prior to saturation, other controlling factors being constant. Once the NaR and NiR are fully derepressed, N_2O will become the major gas produced. It takes longer to derepress nitrous oxide reductase (N_2OR) so this intermediate product accumulates and escapes from the soil. After one to two days, the N_2OR is fully-operational and nitrous oxide is reduced to dinitrogen, which now becomes the major product (Knowles 1982).

Oxygen may interact with the nitrogen oxides in controlling enzyme synthesis, but the role of the N oxides is unclear. Their effect varies among species studied and with oxygen concentration (Bryan 1981, Knowles 1982).

Organic Matter

After aeration, the abundance of available organic carbon is frequently the most rate-limiting environmental factor for denitrification (Alexander 1977, Focht 1978). The quality and quantity of organic matter in the soil affects the denitrification rate directly, acting as a substrate for the bacteria, and indirectly by controlling the carbon and nitrogen mineralization activities of other heterotrophs (and hence, the amount of available oxygen and ammonium-N) and by promoting the formation of stable soil aggregates.

Field studies of simultaneous additions of organic matter and nitrate fertilizers to agricultural soils have demonstrated rapid losses of N through denitrification and plants are thought to increase denitrification rates through their input to the soil of root debris and exudates (Rolston 1981).

In well-aerated soils, the presence of abundant organic C with limited N may allow heterotrophic microbes to immobilize N and potential denitrification rates will drop. Johnson and Edwards (1979) reported that no nitrification occurred at C mineralization to N mineralization ratios of 100 or more. Working with soils with high nitrate contents, Myrold and Tiedje (1985) found that additions of alfalfa straw increased denitrification capacities by 40-63%. They

attributed the increase to growth in total microbial biomass, of which denitrifiers were a constant fraction.

During anaerobiosis, denitrification is proportional to the amount of available organic C, and once the available C is consumed, the rate of denitrification depends upon the rate of C mineralization (Reddy et al. 1982). Available organic C, with respect to denitrification, has been defined in several ways. A first criterion is that the carbon must be available within the anoxic region of the soil along with nitrate (Focht 1978). In arid land soils with low total soil C, the soil organic matter (SOM) and denitrification activity were limited to a layer near the surface, even though other factors were suitable for denitrification lower in the profile.

Good correlations of water soluble C (WSC) and denitrification have been obtained (Focht 1978, Reddy et al. 1982) but WSC concentrations in the soil are rarely enough to fully reduce the existing nitrate. Sollins et al. (1984) state that soluble C represents less than 1% of the total organic C input to the soil. Maximum available organic C has been defined as C evolved as CO_2 during a 2-week aerobic incubation (Rolston 1981, Reddy et al. 1982) and attempts have been made to correlate denitrification potential with total soil organic C, with generally poor results (Muller et al. 1980, Reddy et al. 1982).

Cycles of wetting and drying or freezing and thawing have been observed to increase C mineralization and subsequent N loss via denitrification. This has been interpreted in terms of disruption of organomineral associations that physically or chemically protect OM from decomposition (Hattori 1973, Focht 1978). Foster (1981) used

selective staining of carbohydrates and electron microscopy to demonstrate that carbohydrates could be physically protected from microbial breakdown within soil aggregates by mineral occlusion or residence in pores too small to admit bacteria.

It is apparent that total organic C will be a poor measure of potential denitrification. The location, C/N ratio, and the degree of physical or chemical resistance to microbial breakdown will all determine the relative contribution of each SOM fraction to the rate of nitrate reduction and loss.

Nitrogen Oxide Concentration

If other factors are not limiting, denitrification will increase with nitrate availability. There does not seem to be a minimum level of nitrate required for denitrification to occur. Nitrate reduction will proceed until essentially all the nitrate is depleted, or at least to the level of our ability to measure nitrate (Skinner 1975). It is common to find that other factors, e.g. organic C, are limiting denitrification and that reaction rates are independent of nitrate concentration within some range (Focht 1978, Rolston 1981). The diffusion rate of nitrate to the reaction site is another complicating factor to consider when trying to interpret denitrification kinetics.

As previously mentioned, nitrate and the other N oxides may interact with oxygen to control the synthesis of denitrifying enzymes. At less than totally anoxic conditions, the presence of nitrate will often accelerate reductase synthesis (Knowles 1982).

The mixture of potential electron acceptors present will affect the ratio of products evolved by the bacterium. Generally the ionic N oxides nitrate and nitrite will be reduced before the gaseous N oxides NO and N_2O. Nitrate may be a better electron acceptor than N_2O or the reductases involves may have different specific activities. When nitrate concentration is high, reduction of NO_2, NO, and N_2O may be inhibited; the mole fraction of N_2O evolved will increase. If little nitrate is present, denitrification goes to completion; dinitrogen will be the major product (Knowles 1982). Vitousek et al. (1979) have reviewed the factors influencing the accumulation and loss of nitrate in terrestrial systems.

pH

Denitrifying bacteria are sensitive to low pH. The magnitude of their response varies, depending on the strain of bacteria and the soil type (Alexander 1977), but generally denitrification is repressed at low pH. Optima of pH 7-8 (Knowles 1982) and 5.8 to 9.2 (Bryan 1981) have been reported. Interpretation of N oxide evolution at low pH is confounded by the occurrence of abiological denitrification. Muller et al. (1980), in one of the very few studies of the denitrification potential of acid peats and spodosols, reported rates of N evolution of 0.12-53.8 ug $N/cm^3/day$. The O-horizon (pH 5.7) of a deciduous forest soil produced 45.6 ug N/cm3/day. These data represent maximal values, measured from nitrate-enriched, anaerobically-incubated samples. They illustrate the point that considerable denitrification is possible at moderately low pH. At pH

4.5, 3-10% of the added nitrate was evolved as N_2O during the 21-day incubation period. Samples from the B-horizons of the spodosols tested yielded higher N losses than did the samples from the O and A horizons. This was attributed to the higher pH of the B-horizons.

The pH influences the types as well as the amount of gases produced. At high pH, the reaction equilibrium shifts toward completion and dinitrogen is the major product. The gross production of nitrous oxide increases with the reaction rate, but most of it is reabsorbed and reduced to dinitrogen. Thus the net production of nitrous oxide decreases at high pH (Delwiche 1981, Knowles 1982). At pH less than 7, nitrous oxide is the major product of denitrification. At pH 4.5, N_2O and NO are produced in equal volume. The nitric oxide is very reactive and would be observed as a free product only in very anoxic environments. Nitric oxide reacts with oxygen to form NO_2, which is very toxic (Delwiche 1981, Bryan, 1981).

Temperature

Denitrifiers are physiologically active over a wide temperature range. Denitrification has been reported to occur at temperatures as low as 0-5° C. The rate increases with temperature to 60-65° C. and decreases thereafter. The relatively high optimum temperature may be due to thermophilic Bacilli (Knowles 1982). Bryan (1981) reported 30° C. as an optimum temperature and attributed the higher optimum to abiological reactions. Within the 10-35° C. range, the temperature coefficient Q_{10} is 1.5 to 3.0 (Knowles 1982), i.e. the denitrifica-

tion reaction rate increases by a factor of 1.5 to 3.0 for each 10° C. increase in temperature. Temperature has an indirect influence on denitrification through its influence on the oxygen consumption rate of the soil biota. All other factors being equal, proportionally more dinitrogen will be evolved at higher temperatures.

Denitrification in Well-Aerated Soils

Limited anaerobic areas could develop in an otherwise well-aerated soil if favorable levels of temperature, moisture, and available carbon allowed soil respiration to exceed the rate of oxygen diffusion into a site (Stolzy and Fluhler 1978). Anaerobic microsites may occur within the rhizosphere and the interior of soil aggregates.

The rhizosphere typically contains an abundance of organic matter such as root exudates, sloughed-off root caps and root hairs, and dead cortical cells (Hattori 1973). The rhizosphere oxygen concentration may be low due to uptake by respiring roots and microorganisms. In certain chronically anaerobic soils and sediments, specially-adapted plant roots will act as oxygen sources rather than sinks; the oxygen diffuses from the atmosphere through the stem and roots into the soil (Knowles 1982).

The ratio of microbes found within the rhizosphere to those found outside of the rhizosphere is called the rhizosphere effect (R:S). It has been taken as a measure of the effect the root has on a microbe population. For spring wheat the rhizosphere effect was 1260:1 for denitrifiers (Hattori 1973) and for oak seedlings the denitrifier R:S was 10-100:1 (Knowles 1982). This in itself is not

evidence for increased denitrification within the rhizosphere, but it has been demonstrated with ^{15}N tracer studies that the presence of plants increased the rate of N losses and that the denitrification occurred within the rhizosphere, not within the root itself. Plant root uptake of nitrate or ammonium can reduce denitrification rates under N-limited conditions (Knowles 1982, Rolston 1981).

A well-aggregated soil could have a significant anaerobic volume even though it appeared, on average, to be relatively well-drained. Small amounts of organic matter or water may occupy the micropores and limit gas diffusion. The maximum-sized aggregate that would be entirely aerobic would be determined by the respiratory activity within the aggregate, the resistance to O_2 diffusion into the aggregate, and the concentration of O_2 within the macropores surrounding the aggregate. The maximum size for an aerobic aggregate and the size distribution of aggregates within the soil would determine the total anoxic volume in the profile (Stolzy and Fluhler 1978). Sexstone et al. (1985b) used a microelectrode to measure within-aggregate oxygen profiles and usually found anaerobic interiors when aggregate radii exceeded 10 mm. All aggregates with measurable denitrification activity (whole aggregate incubated in air with acetylene) had anaerobic zones, but not all aggregates with anaerobic zones had measurable denitrification activity.

Foster (1981) used electron microscopy to demonstrate the presence of bacteria within the pores of microaggregates. As mentioned earlier, carbohydrates also were present, but were often inaccessible to the bacteria. Bacteria within the organomineral matrix had fewer stored food reserves than did bacteria isolated from the rhizosphere.

Juxtaposition of aerobic and anaerobic conditions in time or space may increase denitrification. Typically the greatest losses of soil N occur during alternating wet and dry cycles (Alexander 1977). Nitrate that accumulates during aerobic nitrification is reduced to gaseous nitrogen oxides when the conditions in the microsite shift to favor anaerobiosis, or, alternatively, when nitrate diffuses from an oxygenated microsite to an oxygen-depleted microsite.

Denitrification in Forest Soils

Elevated nitrate concentrations found in stands of pure or mixed nitrogen-fixing species could encourage denitrification. In studies of alder stands and mixed Douglas-fir/alder stands, Miller (1974 Ph.D. thesis, OSU) and Bormann and DeBell (1981) report high SOM, high total N, rapid ammonification and nitrification, and leaching of nitrate into streams at alder-dominated sites. The low pH typical of soils under alder may inhibit denitrification to some degree. Miller reported that soils within his study area could remain nearly saturated until May and he suggested that denitrification could occur within this system.

Vegetation disturbance studies (Sollins et al. 1981; Johnson and Edwards 1979; Vitousek et al. 1979) have generally demonstrated accelerated decomposition and, after some delay, increased nitrification. Reduced evapotranspiration usually leads to an increase in soil moisture. The appearance and behavior of nitrate varies. In recent work by Robertson et al. (1987), denitrification activity in Pinus taeda L. plantations in North Carolina increased, relative to an untreated

control, 5 to 8 times after clearcutting, piling, and disking. Denitrification activity also increased after herbicide treatment. In the Sierra Nevada, Strauss and Firestone (1982) measured denitrification rates in soil cores taken from clearcut and neighboring undisturbed coniferous forest and they failed to report any difference in rates. Only 16 of the 238 cores incubated had detectable nitrous oxide production.

Urea fertilizer increases the pH and increases the rate of nitrification in some conditions. Overrein (1969) detected small quantities of $^{15}N_2$ over stands fertilized with 100 g m^{-2} of urea-^{15}N. Applications of urea to saturated soils or to soils with large aggregates and moderate moisture levels may lead to losses of gaseous N.

Prescribed fire or wildfire may create soil conditions that promote denitrification. Fire is known to increase soil pH and although some N may be volatilized during the burn, following the fire an increase in nitrification and the availability of nitrate is often observed (Boyer and Dell 1980).

Denitrification Measurement

Acetylene inhibition of nitrous oxide reduction to dinitrogen has been demonstrated in culture and in soil (Yoshinari et al. 1977). This provides a valuable tool for studying denitrification because, with the addition of acetylene, the sole denitrification end product is nitrous oxide, a gas with a normal atmospheric concentration of 300 ppb, as opposed to dinitrogen's 78.1%. Relatively small increas-

es in nitrous oxide are easily detected. The quantitative reduction of nitrate to nitrous oxide under acetylene inhibition was confirmed with ^{13}N studies (Smith et al. 1978). At very low nitrate concentrations or after long periods (greater than 160 hours) of acetylene treatment, inhibition has been observed to diminish (Firestone and Tiedje 1979). Acetylene is a strong inhibitor of the oxidation of ammonium to nitrate. This could cause underestimation of rates in systems where nitrification and denitrification are closely linked (Knowles 1982). This limitation of the acetylene inhibition technique would not affect short-term anaerobic incubations.

The measurement of denitrifier enzyme concentration, often called denitrification potential, involves preparing a soil-water slurry which is amended with nitrate and incubated anaerobically in the presence of 10-20% acetylene in the headspace (Smith and Tiedje 1979). Protein synthesis inhibitors may be added to the slurry. Some workers (e.g. Firestone and Tiedje 1979) have also added simple organic carbon sources to the soil slurry. The object of this manipulation is to remove all substrate constraints and oxygen inhibition so that rates of nitrous oxide emission are directly proportional to enzyme concentration. With this method, Smith and Tiedje (1979) identified an early linear phase of nitrous oxide production lasting 1-3 h (phase I). Nitrous oxide evolution then increased, due to enzyme synthesis, until a second linear phase was reached, usually 4-8 h after the onset of anaerobiosis. They stated that phase I rates, which precede the synthesis of new denitrifier enzymes, should relate to field soil aeration and denitrification activity.

In another approach (Tiedje 1982), soil or soil cores are put into gastight containers and 10% of the container atmosphere is replaced with acetylene. The aerobic atmosphere is maintained and no nitrate, carbon, or water is added. By recirculating air within the incubation vessels, Parkin et al. (1984) were able to reduce the time required to determine denitrification rates, presumably by speeding diffusion of acetylene and nitrous oxide to and from active denitrification sites.

The denitrification potential measurement used in the current study differs from that of others (Smith and Tiedje 1979; Firestone and Tiedje 1979) in that I did not amend the soil with nitrate or carbon, relying instead upon the native levels of soil nitrate and carbon. Protein synthesis inhibitors were not added, but should not have been necessary due to the short (1 h) initial incubation period. The denitrification activity assay described by Tiedje (1982) has the advantage over potential measurements in that measured rates are more closely related to field rates measured by ^{15}N methods (Parkin et al. 1985), but I was concerned that forest soil N-loss rates would be below the detection limits of the activity assay, as was the case in the Sierra Nevada study of Strauss and Firestone (1982). As a compromise, I selected the denitrification potential method and deleted the substrate amendments. My original intent was to follow the potential measurements with soil cover measurements of gaseous N fluxes in the field.

Study Objectives

The major objectives of this study were to:

1. determine whether denitrification potential exists in riparian forest soils in the western Cascades of Oregon.
2. determine whether denitrification potential increases with increasing incubation time.
3. test the hypothesis that denitrification potential varies with slope position within the riparian zone.

Additionally, there were three minor objectives. They were to:

1. test the effect of soil sampling depth on denitrification potential.
2. contrast rates in systems dominated by either conifers or N_2-fixing hardwoods.
3. determine whether seasonal (spring vs. late summer) variations occur in denitrification potential.

MATERIALS AND METHODS

Site Description

Two riparian forest sites in the Cascade Mountains of Oregon were studied. The first site was along Mack Creek (44° 13' N., 122° 10' W.), about 15 km ENE of the town of Blue River, Oregon. This site is 760 m above sea level and is part of the H.J. Andrews Experimental Forest, a forest study area located on the Blue River Ranger District of the Willamette National Forest. The second site, along the North Fork of Quartz Creek (44° 12' N., 122° 19' W.), was within the Blue River Ranger District, outside the experimental forest. The Quartz Creek site was about 5 km NNE of Blue River, Oregon, at 490 m above sea level.

The climate of this area is mild, with a total annual precipitation of over 2500 mm. The winters are wet, with temperatures generally remaining above freezing, but a snowpack can develop. Summers are cool and dry, and drought is typical in July and August (Shumway 1978).

The flood plain soils of both sites show little or no profile development, but seasonal ebbs and floods have left layers of organic and mineral matter. The flood plain of Mack Creek has been relatively stable for 100 to 500 years, while the Quartz creek flood plain has been heavily disturbed within the past 50 years.

At the study site, Mack Creek flows through a coniferous forest over four hundred years old, dominated by Douglas-fir, Pseudotsuga menziesii (Mirb.) Franco, and western hemlock, Tsuga heterophylla

(Raf.) Sarg. The abundant fallen boles from this riparian forest have provided structure and stability to the Mack Creek flood plain. A thick forest floor and numerous fallen logs covered soil surfaces upslope from the flood plain.

A 35-40 year-old stand of red alder, Alnus rubra Bong., established following logging and burning of the site, dominated the Quartz Creek study area. Remnants of old logging roads and skid trails were apparent. The Quartz Creek site forest floor was far thinner than that of Mack Creek.

Experimental Design

Although this was an observational, not experimental, study, the experimental design approach was an appropriate framework for the study layout and data analyses. A mixed model randomized complete block design formed the fundamental arrangement of experimental units and treatments, while certain hypotheses were tested in a split-block framework. The dependent variable was denitrification potential, measured as the amount of nitrous oxide-N released per unit time per unit mass of dry soil under anaerobic conditions.

The experimental units were elongated rectangular plots with their long axes parallel to the stream channel. Denitrification potential measurements and other soil analyses were performed on four soil samples per depth per experimental unit. The samples were obtained from soil pits, the sampling units, randomly selected within the experimental units and new sets of sampling units were selected for each sampling period. I subsampled experimental units for three

reasons: from results of an exploratory study, I expected that within-site variability would render single point estimates unreliable, I wanted to obtain estimates of within-experimental unit variability to aid the design of future studies, and finally, subsampling would provide an error term for testing block x treatment interactions with ANOVA .

The experimental units were arranged in four blocks corresponding to two sides of two creeks. At each creek the sides differed in aspect, slope, and disturbance history. Blocks were used for allocation of sample points and for grouping of lab analyses; I considered blocks to be random factors in the ANOVA model.

The classification variables used as fixed treatments included slope position, soil layer, seasonal variation, and duration of incubation period. Slope position, which I call ZONE, integrated effects of distance from and elevation above the stream channel. ZONE had three levels: flood plain, toe slope , and hill slope. The flood plain was a vegetated zone of highly-disturbed alluvial soils, lying outside the unvegetated active stream channel, but this zone was subject to seasonal flooding. The toe slope was a region of accumulated colluvium lying between the flood plain and the valley wall hill slope. The riparian hill slope was primarily a zone of colluvial soils. At each site the hill slope extended for several hundred meters above the stream channel, but only points within 40 meters of the creek were sampled. All sample points were considered to be within the riparian zone, the source of plant material providing energy and structure to the forest stream ecosystem (Waring and Schlesinger 1985).

Soil layer, which I called DEPTH, had two levels: mineral soil from depths of 0-15 cm and mineral soil from 15-30 cm. The distances were measured from the organic layer-mineral soil boundary. The layers did not correspond to soil horizons observed in the field. During 1984, sampling was limited to the upper soil layer. Seasonal variation, called SEASON, was limited to two levels: spring and fall of 1984. The methods used in fall 1983 were different enough from those used in 1984 to warrant separate analyses. Duration of incubation period, called TIME, had three possible levels: one hour, eight hours, and twenty-four hours. At these times gas was sampled from the headspace of the soil incubation flasks.

Sampling Design

A sampling scheme developed by M. Gillham was adopted with some modification for the present study. At two creeks, 100 meter-long study areas were established and zone boundaries were delineated and mapped. Forty-five points were randomly-located within the toe slope zone, and a transect perpendicular to the stream channel was established through each point. Flood plain sample points were located along each transect, as close to the 'bank full width' as possible. Hill slope points were selected approximately 20 m uphill from each toe slope point, on the transect or as close as possible. Actual distances upslope varied between 10 and 40 m.

For the current study I sampled a subset of sampling points established by Gillham, taking care to collect soil within 1 m of the soil pits used in Gillham's study. In the Fall 1983 series, I selected

four transects at random from each side of each creek and collected soil samples from flood plain, toe slope, and hill slope points along those transects. Samples were taken from 0-15 cm and 15-30 cm depths. (2 creeks x 2 sides x 4 transects per side x 3 zones x 2 depths = 96 samples). A slightly different design was used for the spring and fall series in 1984 after I discovered that detailed soil data would be available for only a subset of the original set of transects. From this subset four sample points per zone were selected on each side of the creeks, without regard to the transects. At each point a single sample of the soil from the 0-15 cm layer was collected. Sampling of the lower soil layer was abandoned for 1984 after analysis of the results of the 1983 experiment showed that N-loss rates obtained from the lower soil layer samples closely paralleled the upper soil layer rates.

Sample Collection and Handling

At the time of soil sample collection, simple weather observations such as air temperature, wind speed, and presence or absence of clouds or rain were recorded. At several soil pits within each zone, I measured the soil temperature at 5 and 15 cm below the surface of the A horizon. Unusual or remarkable characteristics at any sampling point were recorded. Where possible, soil was collected from previously dug soil pits by exposing a fresh soil surface at least 30 cm uphill from the old soil pit face. The O1 and O2 layers were removed where present, and the remaining mineral soil was dug out with a trowel. Approximately 400 cm^3 of soil was collected from each layer

(0-15 or 15-30 cm, measured from the top of the A horizon) sampled and then placed within doubled 17 x 21 cm polyethylene bags and sealed with a twist closure. Polyethylene bags were chosen for their relatively high permeability to oxygen and low permeability to water vapor.

To reduce soil respiration, and thus limit changes in soil oxygen or carbon dioxide concentrations, I kept the soil samples cool until they were incubated. In the field, I stored bags of soil in the shade for less than 1 hour until they were placed in an ice-filled insulated chest for transport to the lab. At the lab, samples were stored in darkness at 4° C. for two to three days before incubation.

Soil Preparation

The soils were minimally processed before being incubated. Fresh soil was passed through a 4 mm sieve and all retained material was discarded. In addition, I removed any visible plant roots greater than 1 mm in diameter. To reduce contamination, between samples I rinsed the sieve with distilled water and dried (Fall 1983) or flamed the sieve with 95% ethyl alcohol (Spring and Fall 1984). After all of the soils were sieved, I prepared one incubation flask for each sample: I added 30 g (fresh weight) of sieved soil to a weighed 125 ml flask (Pyrex 4980), reweighed the combined soil and flask, capped the flask loosely with aluminum foil, and returned the prepared flask to the cooler to await incubation. Prior to use, the flasks were washed with lab cleaner, rinsed three times with distilled water, and air-dried in a dust-free environment. The flasks were not sterile.

Incubations

Just prior to the start of the incubation, I removed the sample from the cooler, replaced the aluminum foil cap with a white rubber septum stopper (Z10,145-1, Aldrich Chemical Co., Milwaukee, WI), and wired the stopper to prevent a blowout. The headspace of the stoppered flask was then evacuated for 2 minutes via a needle connected to a water aspirator. The flask was then refilled to (5 lbs psi) with Ultra-High Purity (UHP) N_2. Subsequently, the flask was twice evacuated for 1 minute and refilled with UHP N_2, for a total of three evacuation and purge cycles. Degassed, deionized water at the incubation temperature was added to the flask to yield 75 ml of soil-water slurry. To prepare the water, I boiled deionized water for two minutes under a continuous UHP N_2 purge and then cooled the water to the incubation temperature while maintaining a low flow of UHP N_2 through the water. An anaerobic media dispenser was employed to prevent O_2 or N_2O contamination of the water. After adding water, I adjusted the flask headspace pressure to equal ambient pressure, replaced 10% of the headspace gas with acetylene (C_2H_2), and shook the flask. The acetylene was supplied from a pressurized cylinder of laboratory quality acetylene or was generated by mixing calcium carbide (CaC_2) and water. Prior to use, the acetylene flowed through a purification apparatus consisting of a deionized water bubbler, a concentrated sulfuric acid (H_2SO_4) bubbler, a soda lime (NaOH with CaO) cartridge, and finally an anhydrous magnesium perchlorate ($Mg[ClO_4]_2$) drying cartridge. The incubation starting time was taken to be the time of the acetylene addition.

Immediately following the acetylene addition I sampled the headspace for N_2O analysis and placed the flask in a temperature-controlled water bath. An inefficient water bath used during the Fall 1983 series of incubations allowed the Mack Creek-East incubation temperatures to vary from 13 to 15 °C., while the Mack Creek-West incubation temperatures varied from 10 to 13 °C. A higher-quality water bath was employed for the Quartz Creek sample incubations and temperatures were maintained at 12 °C. All samples processed during 1984 were incubated at 10 °C in a large recirculating water bath with excellent temperature control.

I chose to incubate the soil slurries at temperatures close to the soil temperatures measured at the time the samples were taken. There is evidence that a population of soil microbes adapts to the temperature range found in its natural environment and that rates of microbial physiological processes may decline if the temperature strays too far from the natural range (Lynch 1979).

Gas Analyses and Calculation of Nitrogen Loss Rates

After one hour of incubation the headspace of each flask was sampled for N_2O analysis. In Fall 1983, further samples were taken eight and twenty-four hours after the start. Each flask was removed from the water bath at the appropriate time and was shaken by hand for 10 seconds. A 0.5 ml (0.3 ml in Fall 1983) headspace gas sample was removed through the septum using a 0.5 ml gastight valved syringe (Pressure-Lok A-2, Precision Sampling Corp., Baton Rouge, LA). The gas sample was injected into a gas chromatograph immediately or after

a short delay. The locking syringes used prevented sample contamination or loss during delays.

The nitrous oxide concentration in the headspace was determined by gas-solid chromatography. All analyses were performed on a gas chromatograph (H-P 5840A or H-P 5834A, Hewlett-Packard Co., Avondale, PA) equipped with an electronic integrator (H-P 18850A) and an electron capture ionization detector (H-P 18803B). The detector used contains a 15 mCi ^{63}Ni radioactive source and operates in the variable pulse frequency (constant current) mode. The detector temperature was 300 °C. (Fall 1983) or 325 °C. (1984). Argon (95%) + methane (5%) at a 30 ml/minute flow rate served as the carrier gas. The separative column was a 4 m length of 3.2 mm (o.d.) stainless steel tubing packed with a porous polymer stationary phase (Porapak-Q, 50/80 mesh or 80/100 mesh, Water Associates, Inc., Milford, MA). The column oven temperature was 50 °C. (isothermal mode) and the injection port temperature was 50 °C. (250 °C. in Fall 1983).

The headspace nitrous oxide ppm (vol/vol) was calculated from the integrated chromatogram peak area using the external standard calibration method (Rowland 1974). Commercial standard gas mixtures of nitrous oxide in dinitrogen (1000 ppm N_2O [±2%] & 100 ppm N_2O [±2%], Alltech Associates, Deerfield, IL; 0.984 ppm N_2O [±5%] and 0.0906 ppm N_2O [±5%], Scott Specialty Gases, San Bernardino, CA) were analyzed before, during, and after each series of sample analyses. I generally measured a set of standards after every 25 to 35 gas sample injections. I used the integrated peak areas and stated concentrations of the standard gas mixtures to develop working curves, rather than the automatic calibration feature of the electron-

ic integrator. The lack of detector response linearity over the wide nitrous oxide concentration range observed in sample headspaces, coupled with declining detector sensitivity over the course of an analytical series, rendered the automatic calibration grossly inaccurate. The decline in detector sensitivity was most likely due to water vapor interacting with the radioactive source within the detector (ASTM Committee E-19 on Chromatography 1979). Equations for the working curves were developed with curve fitting routines in an H-P 41CV programmable calculator equipped with a Stat Pac (Hewlett-Packard Co., Corvallis, OR). The equations were used to calculate the sample headspace nitrous oxide concentration from the integrated area of the sample nitrous oxide peak.

Calculating the total volume of nitrous oxide produced by the soil slurry required that I account for nitrous oxide dissolved in water, as nitrous oxide is highly soluble in water. I used the Bunsen absorption coefficient method outlined in Tiedje (1982):

$$M = C_g (V_g + V_l a) \quad (1)$$

where M is the total N_2O content in ml at STP (273 °K., 1 atm), C_g is the concentration of N_2O in the gas phase (headspace), V_g is the volume of the gas phase in ml at STP, V_l is the volume of the liquid phase (water) in ml, and a is the Bunsen absorption coefficient, the ml of gas (in this case N_2O) at STP dissolved in one ml of water at a given temperature. The absorption coefficient varies with the gas and decreases as the temperature of the water increases;

a ranges from 0.743 to 0.882 for N_2O in water at the incubation temperatures used.

The volume of the liquid phase had to be determined indirectly by subtracting the volume of the soil solids from the total volume of soil slurry, 75 ml. The soil solid volume was calculated by dividing the dry weight of soil added to the incubation flask by the average particle density of mineral soil, 2.65 g cm^{-3} (Brady 1974).

The Ideal Gas Law:

$$PV = nRT \quad (2)$$

(where P is gas pressure in atm, V is gas volume in liters, n is the number of moles of gas, R is the gas constant, 0.082054 l atm/mol °K, and T is the gas temperature in °K) was used to convert the volume of nitrous oxide produced by the soil slurry to moles of nitrogen lost. The nitrogen loss rate in ng N g^{-1} h^{-1} was then calculated using this value, along with a molar conversion factor, the dry weight of the incubated soil, and the elapsed time of the incubation. It was this nitrogen loss rate that I used as a measure of a sample's denitrification potential. All preliminary calculations were performed with Symphony or 123 (Lotus Development Corp., Cambridge, MA) microcomputer electronic spreadsheets .

Soil Physical and Chemical Analyses

Soil temperature at the time of collection and soil moisture were determined during each sampling period. The soil temperatures were obtained with a mercury lab thermometer. Gravimetric soil moisture content was determined by oven-drying the sample at 105 °C. to a constant weight. Several chemical analyses were performed only for the set of samples collected in Fall 1984, including: pH, KCl-extractable nitrate and ammonium, total nitrogen, total phosphorous, and total carbon.

Soil pH was measured by mixing 10 g of undried soil with 20 ml of distilled water. The mixture was stirred and then allowed to sit for 1 hour, after which the pH of the supernatant was determined with a pH meter equipped with glass electrodes. The meter was standardized with pH 7.00 and pH 4.00 buffer solutions before and during use.

Mineral nitrogen was determined from 2 M KCl-extracts of fresh soil (3:5 soil:extractant ratio, 1 h shaking time, followed by filtration). Nitrate was determined by an automated cadmium reduction colorimetric method and ammonium was determined by an automated salicylate-hypochlorite colorimetric method.

Total nitrogen and total phosphorous were determined from micro-Kjeldahl digests of dried soil. Total carbon was measured by the dry combustion method (Nelson and Sommers 1982) in a LECO dry combustion induction furnace.

Statistical Methods

Data Cleanup

The N-loss rates appeared to be highly variable; rates within groups varied by several factors of ten and extreme values, usually high, were common. For this reason I decided to screen the values before calculating descriptive statistics or testing hypotheses. Screening included a formal test for outliers, tests of variance homogeneity, and tests for normality. The microcomputer program SAS-PC (SAS Institute Inc. 1985a) was used for all statistical analyses, unless noted otherwise.

For outlier detection I used a Pascal microcomputer program (McClellan, M. QTEST. Available from the author on request) to perform Dixon's Q test on each dependent variable grouped by date, creek, zone, and depth. I checked the accuracy of each outlying variate by reviewing field and lab notes for errors in transcription or computation, unusual field conditions, or nonstandard methods.

Plots were made of sample variance or standard deviation over the mean N-loss rate. Hartley's F-max test was used to test the homogeneity of within-zone variances by creek. Valid conclusions can be drawn from Hartley's test even when the underlying data distribution is nonnormal (Sokal and Rohlf 1981). I suspected that the distribution was lognormal; for this reason I chose Hartley's test over the more common Bartlett's test of homogeneity of variance.

Tests for normality of small data sets can be unreliable, so I applied a combination of tests, including the Shapiro-Wilk W test for

normality, tests for significant skewness and kurtosis, and examination of the location of mean and median values. My intention was to compensate for the weakness of individual tests by considering several independent tests in unison. The Shapiro-Wilk test of normality is considered more appropriate for small data sets than are other goodness of fit tests (SAS Institute, Inc. 1985b). The rate data were grouped by experiment, incubation period, creek, soil layer, and zone, for a total of 47 groups of 7 or 8 observations each. Blocks, the sides of creeks, were not used as classification variables in this analysis because this would have yielded only three to four observations per group, preventing the calculation of some statistics. This may have introduced some extra within-group heterogeneity, but t-tests of block mean rates within creeks revealed very few significant differences. In one group, all rates were zero, leaving 47 groups where normality statistics were calculable.

Test results indicated a logarithmic transformation of N-loss rates to ensure the validity of subsequent analyses of variance. Because zero values were present, the selected transformation was:

$$Y' = \ln (Y + 1) \qquad (3)$$

where Y represents the original variate and Y' represents the transformed variate.

Descriptive Statistics

Tables of descriptive statistics were prepared from untransformed variates for measured soil physical and chemical properties. Means and confidence intervals for N-loss rates were first calculated from log-transformed data and then converted to the original measurement units, as suggested in Sokal and Rohlf (1981).

Tests of Hypotheses

The first step was to test whether significant denitrification potential existed. To do this, I used a two-tailed t-test of the null hypothesis that the mean log-transformed N-loss rate was zero. To better understand the pattern of N-loss, several breakdowns of the data were tested. Rates from different incubation periods and depths were always separately considered. I tested Fall 1983 values by depth averaged over all zones, by depth and zone averaged over both creeks, by depth and creek averaged over all zones, and finally within creeks by depth and zone. Since only the upper soil layer was sampled in 1984, the analysis scheme for Spring and Fall was simpler: I tested means by zone averaged over both creeks, by creek averaged over all zones, and by each zone within creeks.

To test whether N-loss rates differed between the 1, 8, and 24-hour incubation periods used in the Fall 1983 experiment, I used the univariate repeated measures ANOVA of SAS-General Linear Models (GLM) with a randomized complete block design. Sides of the creeks formed the blocks and data for the upper and lower soil layers were

analyzed independently. A model factor TIME represented the within-subject change, in this case within soil pit change, in N-loss rate over the three incubation periods. The current and subsequent ANOVAs used the natural log of the N-loss rate as the independent variable.

Gas chromatograph malfunctions prevented taking some gas samples at the planned 8 and 24 h sampling times; usually the 8 h sampling was delayed, yielding a long second period and short third period. Since the test for an incubation time effect might be affected by nonstandard incubation times, I removed any observation with an incubation time more than one standard deviation greater or less than the mean. Of 94 observations, I removed 17. Whether these observations were absent or present did not affect the significance of tests of zone and block effects.

I tested the overall time effect and the time x block and time x zone interaction terms with F-test probabilities adjusted by the Huyhn-Feldt estimator. F values all used the time x block x zone mean square as the error term. Unlike standard ANOVA, this type of analysis accounts for the correlation between successive measurements taken on a single experimental or observational unit (SAS Institute, Inc. 1986). To determine if one hour N-loss rates were different from eight hour rates, and eight hour rates different from twenty four hour rates, I specified a set of planned linear contrasts using the time x block x zone mean square as the error term.

To test whether N-loss rates varied between the upper (0-15 cm) and lower (15-30 cm) soil layers, I analyzed Fall 1983 rate data with the SAS-GLM univariate repeated measures ANOVA. The factor layer represented the within soil pit change in rates between layers. Data

from each incubation period were analyzed separately in randomized complete block experiments. The layer main effect and the layer x block and layer x zone interaction terms were tested for significance using the layer x block x zone mean square as the error term. After finding a significant layer x block interaction in the 8 and 24 hour rates, I performed a series of paired t-tests of the depth effect by block.

To assess the effect of slope position on denitrification potential, I first calculated descriptive statistics and produced scatter plots for N-loss rates by zone. I then tested the significance of observed trends within each sampling period using randomized complete block GLM ANOVA. The block x zone interaction mean square was used as the error term for testing block and zone effects. Fall 1983 zone effects were tested by depth and incubation period; spring and fall 1984 zone effects were tested separately in RCB ANOVAs and were then tested jointly in a split-block ANOVA (described below) designed to test the season effect. To further develop details of zonal changes in N-loss rates, I used a pair of planned comparisons with each RCB ANOVA: flood plain versus toe slope and hill slope combined and toe slope versus hill slope. The block x zone interaction mean square was used as the linear contrast error term.

I used unpaired t-tests to evaluate the null hypothesis that Mack Creek and Quartz Creek denitrification potentials were equal. For each incubation period and depth, tests were performed by zone and with all zones combined. Fall 1983 rates were also tested with all zones and depths combined.

Testing the season effect in the 1984 data set required a change in the experimental design. The repeated measures ANOVA used to test depth and incubation time effects was inappropriate in the testing of season effects because different sets of sample points were used in spring and fall. Experiments with measurements repeated over time have been analyzed as split-plot designs, but the split-block design is more appropriate (Steel and Torrie 1980). Zones and seasons are of equal rank; in a split-plot design the factor assigned to the subplots would be necessarily subordinate. The split-block design avoids this, giving zone and season equal rank.

I used zone within block means for this test, ignoring the subsampling. This was necessary to avoid overloading the microcomputer memory with the GLM design matrix. The season effect was tested using the block x season mean square as an error term. In addition, I tested the block and zone effects using the block x zone mean square as an error term. Three ANOVAs were generated: one with the two sites combined and one for each site alone.

Correlating N-Loss Rates with Soil Properties

Fall 1984 data from Mack and Quartz Creeks were combined to test for linear correlations between the 1 h N-loss rates and the following soil physical and chemical properties: moisture, pH, KCl-extractable nitrate, and ammonium, total nitrogen, carbon, and phosphorus, and carbon:nitrogen ratio. The Pearson product-moment correlation statistic was calculated for each soil property paired

with the N-loss rate expressed in both the original units and in the log-transformed units.

I used a stepwise linear regression method to construct a model relating N-loss to the measured soil properties. The F statistic significance level for inclusion or deletion of an independent variable was set at 0.10 and the set of potential independent variables included linear, quadratic, and cubic terms for each soil property. I evaluated models using either linear or log-transformed N-loss rates as the dependent variable and selected the model yielding the highest R^2, adjusted for the number of independent variables included. Four outlying observations were deleted out of 47 total.

RESULTS

Tests of Normality

The data were highly variable: untransformed 1 h N-loss rates had coefficients of variation ranging from 53 to 281%. Mack Creek rates varied more than Quartz Creek rates. Most data sets contained outliers significant at probabilities of 5% or less. After checking the accuracy of each outlying variate, I concluded that most outliers were not the result of errors or unusual conditions so I retained them. Only one point, from the Fall 1983 sample set, was removed during this review; standing water was present in the soil pit.

Plots of sample variance and standard deviation over the mean N-loss rates showed that variation increased with the mean and that standard deviation was most closely proportional to the mean. Hartley's F-max test revealed significant heteroscedasticity of N-loss rate variance in over half of the groups.

All groups were skewed to the right; all means were greater than the median, all g_1 (skewness statistic) were positive, and 27 of 47 cases were significantly skewed at 5% or less. Most g_2 (kurtosis statistic) were positive, indicating a tail-heavy population. Of 47 cases, 26 were significantly kurtotic. The Shapiro-Wilk test deemed 32 cases significantly nonnormal ($p < 0.05$).

The heterogeneous variance, standard deviations proportional to means, and the apparent nonnormality of the underlying distribution indicated a logarithmic transformation of N-loss rates, previously described in Methods.

The log-transformation significantly increased the normality of the N-loss rate data. Mean and median values differed less and many means equalled or were slightly less than median values. Skewness was not significant in 35 of 47 cases, an improvement in 15 cases. In no case did skewness increase. Kurtosis was not significant in 37 cases, an improvement of 16, and kurtosis was never made more significant by the transformation. The Shapiro-Wilk normality statistic was not significant in 31 cases, an improvement of 16. In four cases the Shapiro-Wilk statistic increased, but never so much as to make the result significant. The log-transformation of N-loss rate data was justifiable and yielded real improvements in data set normality.

Nitrogen Loss Rates

Tables 1 and 2 contain N-loss rate means and 95% confidence limits for 1983 and 1984, respectively. Means of the log-transformed rates were calculated and were then back-transformed to the original units (ng N/g soil/h) for this table. The confidence limits were calculated from means and standard errors of log-transformed rates and were then converted to the original measurement scale. For this reason, the confidence intervals presented are asymmetric about the means.

Table 1. Fall 1983 — Rate of N-loss (ng N/g soil/h) from soil samples after incubation for 1 to 24 h.

Creek	Zone	Depth	n	Mean	LCL	UCL *
Fall 1983 - 1 h incubations:						
Mack	Flood plain	0-15 cm	7	5.17	0.65	22.02
		15-30 cm	7	0.44	-0.15	1.43
	Toe slope	0-15 cm	8	0.76	-0.46	4.73
		15-30 cm	8	0.30	-0.04	0.76
	Hill slope	0-15 cm	8	0.46	-0.37	2.39
		15-30 cm	8	0.00	0.00	0.00
Quartz	Flood plain	0-15 cm	8	24.38	15.00	39.25
		15-30 cm	8	16.03	8.22	30.45
	Toe slope	0-15 cm	8	6.76	1.77	20.75
		15-30 cm	8	1.08	-0.03	3.47
	Hill slope	0-15 cm	8	3.42	1.12	8.23
		15-30 cm	8	1.18	0.07	3.46
Fall 1983 - 8 h incubations:						
Mack	Flood plain	0-15 cm	7	4.75	0.27	25.02
		15-30 cm	7	3.62	0.99	9.75
	Toe slope	0-15 cm	8	0.85	-0.34	4.19
		15-30 cm	8	1.83	-0.05	7.41
	Hill slope	0-15 cm	8	0.37	-0.28	1.62
		15-30 cm	8	0.06	-0.03	0.17
Quartz	Flood plain	0-15 cm	8	43.52	31.16	60.63
		15-30 cm	8	35.02	19.14	63.40
	Toe slope	0-15 cm	8	20.98	7.84	53.65
		15-30 cm	8	4.75	0.83	17.06
	Hill slope	0-15 cm	8	10.80	3.49	30.01
		15-30 cm	8	3.22	0.33	12.33
Fall 1983 - 24 h incubations:						
Mack	Flood plain	0-15 cm	7	4.60	0.10	27.41
		15-30 cm	7	4.84	0.89	17.05
	Toe slope	0-15 cm	8	0.51	-0.32	2.32
		15-30 cm	8	1.60	-0.16	6.98
	Hill slope	0-15 cm	8	0.26	-0.19	0.97
		15-30 cm	8	0.09	-0.02	0.20
Quartz	Flood plain	0-15 cm	8	75.02	59.36	94.74
		15-30 cm	8	66.49	48.32	91.35
	Toe slope	0-15 cm	8	39.04	16.70	89.59
		15-30 cm	8	12.04	2.90	42.56
	Hill slope	0-15 cm	8	30.47	16.04	57.11
		15-30 cm	8	7.32	1.49	26.80

* Mean: Average of log-transformed rates, transformed to original units; LCL/UCL: Lower/Upper 95% confidence limits, calculated in log units, then transformed to original units.

Table 2. Spring and Fall 1984 — Rate of N-loss (ng N/g soil/h) from soil samples after incubation for 1 h.

Creek	Zone	Depth	n	Mean	LCL	UCL *
Spring 1984 - 1 h incubations:						
Mack	Flood plain	0-15 cm	7	3.60	0.20	16.67
	Toe slope	0-15 cm	7	0.87	-0.35	4.37
	Hill slope	0-15 cm	7	0.02	0.00	0.04
Quartz	Flood plain	0-15 cm	8	8.28	3.58	17.80
	Toe slope	0-15 cm	8	3.97	1.19	10.30
	Hill slope	0-15 cm	8	3.82	1.05	10.33
Fall 1984 - 1 h incubations:						
Mack	Flood plain	0-15 cm	8	11.28	2.89	37.72
	Toe slope	0-15 cm	8	2.60	0.19	9.88
	Hill slope	0-15 cm	8	0.09	-0.04	0.23
Quartz	Flood plain	0-15 cm	8	5.22	1.78	12.94
	Toe slope	0-15 cm	8	4.17	1.51	9.64
	Hill slope	0-15 cm	8	7.63	3.54	15.38

* Mean: Average of log-transformed rates, transformed to original units; LCL/UCL: Lower/Upper 95% confidence limits, calculated in log units, then transformed to original units.

Tests for Denitrification Potential

Denitrification potential was observed in nearly all zones and depths. Average log N-loss rates at 1, 8, and 24 hours were significantly greater than zero (all $p < 0.05$) for both depths, both study sites, and all zones. Some means calculated by depth and zone within creeks were not significantly greater than zero.

For the Fall 1983 experiments, t-tests of the null hypothesis that mean rate equaled zero led to its rejection for most means. At each depth, averaged over all blocks and zones, means for log N-loss 1, 8, and 24 h rates were all highly significant ($p < 0.001$, $n=47$). In the depth-by-zone breakdown, averaged over both creeks, all mean rates were significantly greater than zero ($p < 0.05$, $n=15$ or 16). In the depth by creek breakdown, all mean rates were significantly greater than zero ($p < 0.05$, $n=23$ or 24)

These large-scale breakdowns are not very interesting and are potentially misleading; examining means at a finer scale produced very different results for the two creeks. All Quartz Creek depth-by-zone means (1-24 h) were significantly greater than zero with the exception of the 1 h rate for the toe slope 15-30 cm layer ($p = 0.063$). Only three such groups had samples with no detectable N-loss; all of these were in the 15-30 cm layer. Table 3 contains a summary of the t-test results for all sampling periods.

The level of activity in Mack Creek samples was much lower, and this was reflected in tests for mean rates not equal to zero: none of the toe slope or hill slope mean rates at any depth or incubation

Table 3. T-tests for non-zero N-loss rate: by creek, zone and depth.

	Soil Depth	n	Incubation Time: 1 h	8 h	24 h
Fall 1983					
Mack Creek					
Flood plain	0-15 cm	7	*	*	*
	15-30 cm	7	NS	**	**
Toe slope	0-15 cm	8	NS	NS	NS
	15-30 cm	8	NS	NS	NS
Hill slope	0-15 cm	8	NS	NS	NS
	15-30 cm	8	NS	NS	NS
Quartz Creek					
Flood plain	0-15 cm	8	***	***	***
	15-30 cm	8	***	***	***
Toe slope	0-15 cm	8	**	***	***
	15-30 cm	8	NS	**	**
Hill slope	0-15 cm	8	**	***	***
	15-30 cm	8	*	*	**
Spring 1984					
Mack Creek					
Flood plain	0-15 cm	7	*	NA	NA
Toe slope	0-15 cm	7	NS	NA	NA
Hill slope	0-15 cm	7	NS	NA	NA
Quartz Creek					
Flood plain	0-15 cm	8	***	NA	NA
Toe slope	0-15 cm	8	**	NA	NA
Hill slope	0-15 cm	8	**	NA	NA
Fall 1984					
Mack Creek					
Flood plain	0-15 cm	8	*	NA	NA
Toe slope	0-15 cm	8	*	NA	NA
Hill slope	0-15 cm	8	NS	NA	NA
Quartz Creek					
Flood plain	0-15 cm	8	***	NA	NA
Toe slope	0-15 cm	8	***	NA	NA
Hill slope	0-15 cm	8	***	NA	NA

Significance levels:
Not significant: NS ; 5% or less: * ; 1% or less: ** ;
0.1% or less: *** ; Not Available: NA

period was significant, but, in all but one case, flood plain mean rates were significantly greater than zero. Zero rates were common at Mack Creek, but only in the 1 h rates for the hill slope 15-30 cm soil layer do we see a total absence of activity — not a single sample developed a detectable rate of N-loss. Some modest activity was found in all other groups. See Tables 21 through 23 in the Appendix.

Spring 1984 mean log N-loss rates were measured for only the upper soil layer (0-15 cm) incubated for one hour. All zone means, averaged over both creeks, were significantly different from zero ($p < 0.01$, $n = 15$) as were the creek means ($p < 0.02$, $n = 21$ [Mack] or 24 [Quartz]). Table 24 (in the Appendix) contains mean rates for Spring 1984, broken down by creek and zone. The pattern evident in the data from Fall 1983 reoccurs: flood plain means were significant for both creeks, Mack Creek toe slope and hill slope rates were again nonsignificant, while Quartz Creek toe slope and hill slope were very significant ($p < 0.01$). All Quartz Creek samples lost detectable amounts of N, while all Mack Creek zones had at least one sample without measurable N-loss.

Zone mean rates, averaged over both creeks, for Fall 1984 were all significantly greater than zero ($p < 0.01$, $n = 16$), as were the mean rates for each creek ($p < 0.001$, $n = 24$). Quartz Creek continued to have significant N-loss in all zones, with all samples showing some activity, while Mack Creek showed a slight increase in activity with significant flood plain and toe slope mean rates. The Mack Creek hill slope continued to have very low N-loss rates. Even with

significant activity over all, the toe slope and hill slope at Mack Creek contained some inactive areas (see Table 25, column of minimum rates).

Tests of the Effect of Increasing Incubation Period

Examination of the Fall 1983 mean rates for 1, 8, and 24 hours reveals a general trend where rates increase with increases in the incubation periods (see Tables 21 through 23). The trend holds for both soil layers sampled. The results of repeated measures ANOVA, displayed in Tables 4 and 5, show that this trend is highly significant ($p < 0.001$) in both soil layers, but significant time x block interaction in the upper soil requires that rate trends over time be specified on a block by block basis. Mean N-loss rate declined with time for the Mack-West block (Figure 1) and rates increased from 1 h to 8 h and then held steady in the Mack-East block (Figure 2). This is in contrast with the ever-increasing Quartz Creek rates, seen in Figures 3 and 4. Linear contrasts of 1 h rates versus 8 h rates, and 8 h rates versus 24 h rates, were highly significant for upper and lower soil layers (Tables 4 and 5).

Tests of the Soil Layer Effect

Mean N-loss rates significantly decreased with depth for all three incubation periods. The magnitude of the difference decreased as incubation period increased and as distance from the creek increased. At one hour the mean rates of lower layers averaged 0.74

Figure 1. Rate change with depth and time - Fall 1983, Mack-E

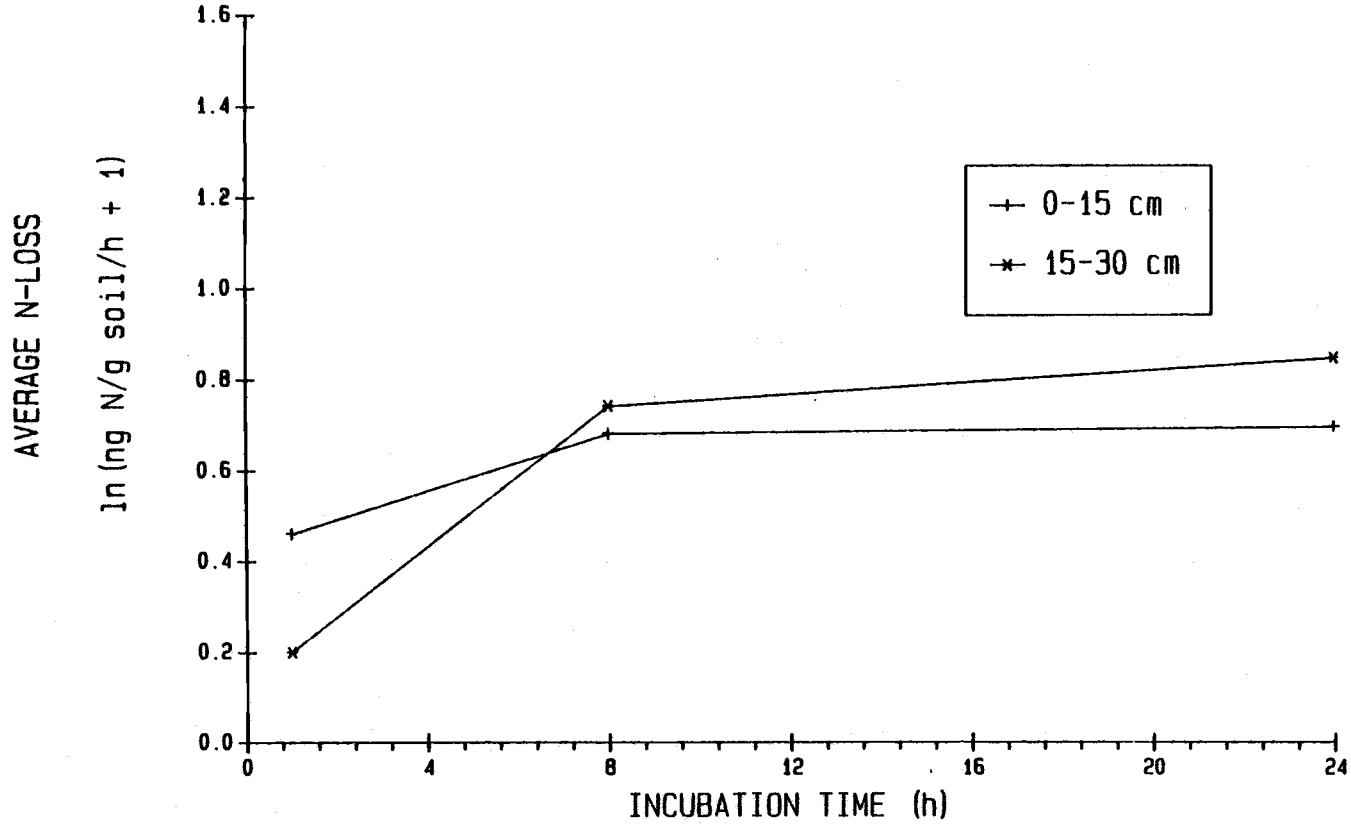


Figure 2. Rate change with depth and time - Fall 1983, Mack-W

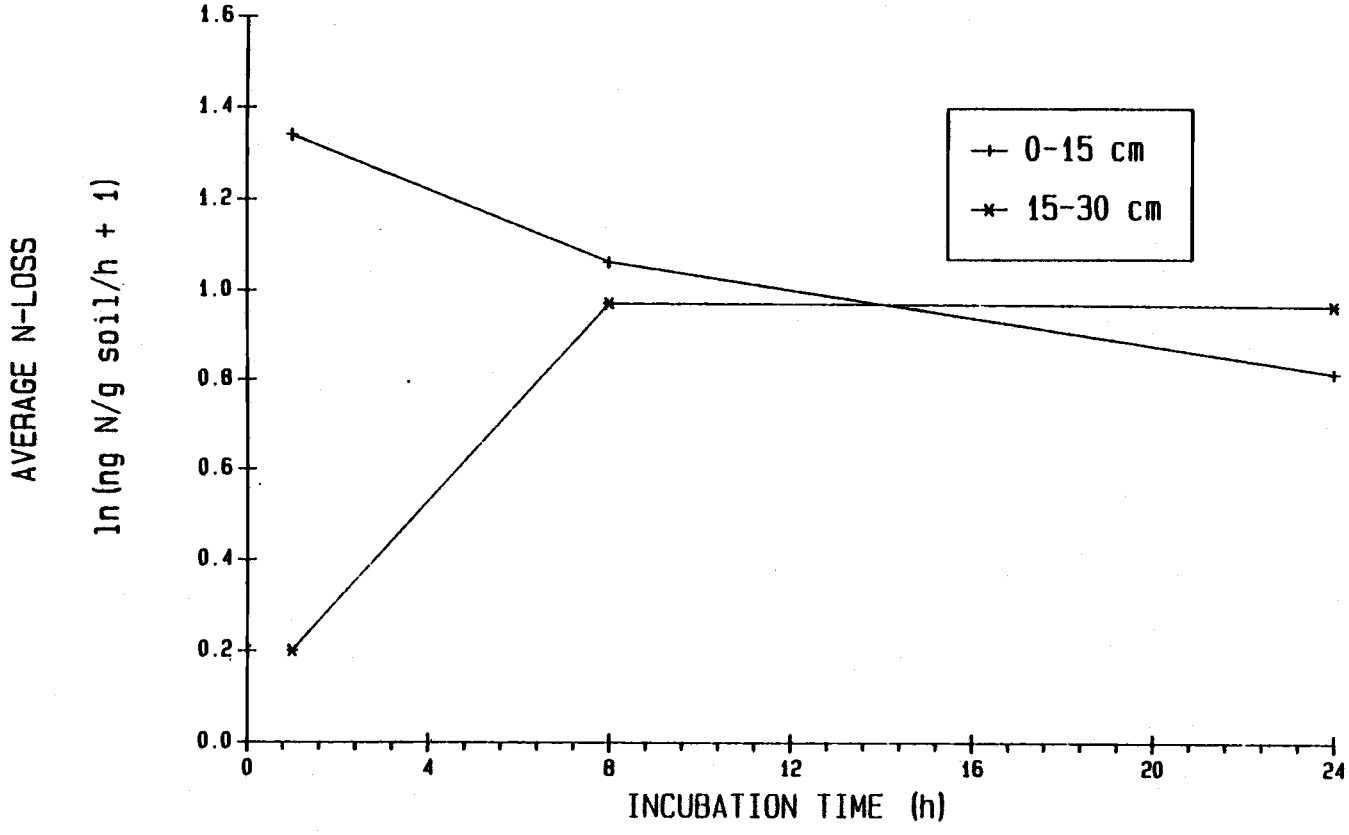


Figure 3. Rate change with depth and time - Fall 1983, Quartz-E

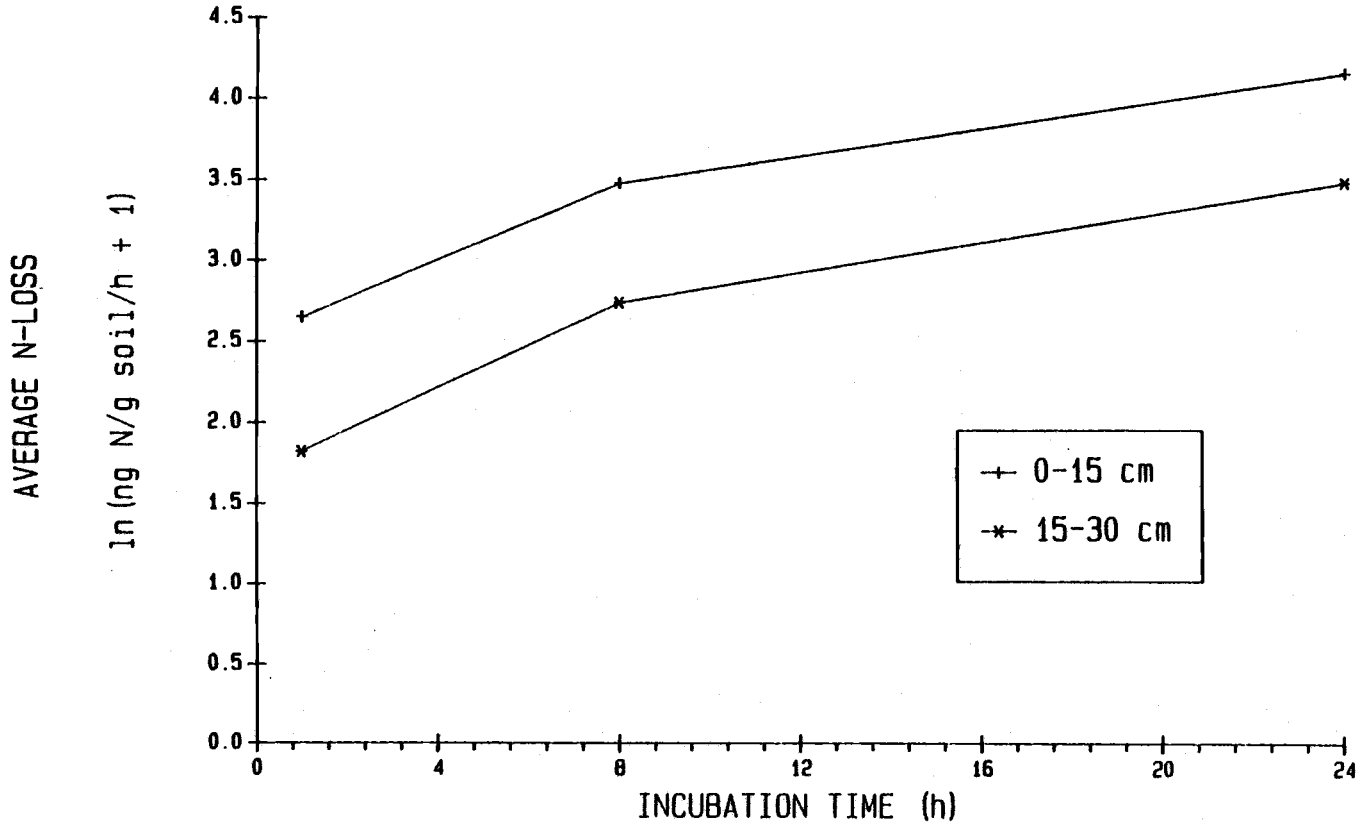


Figure 4. Rate change with depth and time - Fall 1983, Quartz-W

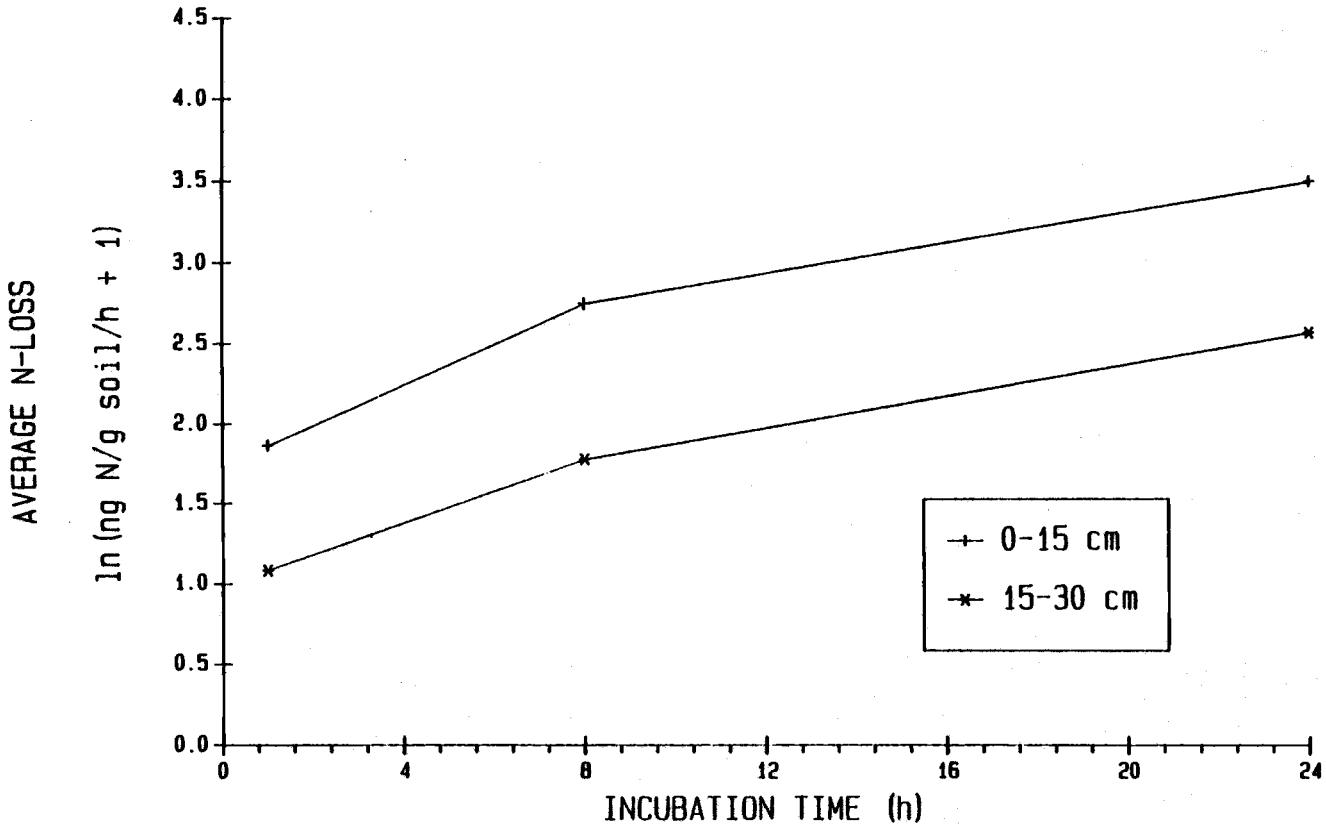


Table 4. Fall 1983 — Rate of N-loss from upper-layer soil samples (0-15 cm): Repeated measures ANOVA table for incubation time effects.

Source of variation	df	SS	MS	F	p*
Time	2	11.01	5.51	54.33	<0.001
Time x block	6	18.61	3.10	30.61	<0.001
Time x zone	4	0.78	0.20	1.93	0.118
Time x block x zone	12	2.49	0.21	2.05	0.036
Error	56	5.68	0.10		

Planned contrast	df	SS	MS	F	p
1 h rate vs. 8 h rate	1	7.06	7.06	40.31	<0.001
8 h rate vs. 24 h rate	1	4.09	4.09	24.76	<0.001

Table 5. Fall 1983 — Rate of N-loss from lower-layer soil samples (15-30 cm): Repeated measures ANOVA table for incubation time effects.

Source of variation	df	SS	MS	F	p*
Time	2	22.88	11.44	31.45	<0.001
Time x block	6	2.66	0.44	1.22	0.316
Time x zone	4	2.12	0.53	1.46	0.233
Time x block x zone	12	3.93	0.33	0.90	0.547
Error	50	18.19	0.36		

Planned contrast	df	SS	MS	F	p
1 h rate vs. 8 h rate	1	18.59	18.59	24.86	<0.001
8 h rate vs. 24 h rate	1	5.56	5.56	24.63	<0.001

* Corrected (Huynh-Feldt) probability.
SS: Type III sums of squares.

Table 6. Fall 1983 — Rate of N-loss from upper and lower layer soil samples: Repeated measures ANOVA tables for depth effects, by incubation time.

Part a: 1 h incubation time

Source of variation	df	SS	MS	F	p
Layer	1	13.91	13.91	21.51	<0.001
Layer x block	3	2.64	0.88	1.36	0.271
Layer x zone	2	0.71	0.36	0.55	0.582
Layer x block x zone	6	4.72	0.79	1.22	0.321
Error	35	22.63	0.65		

Part b: 8 h incubation time

Source of variation	df	SS	MS	F	p
Layer	1	4.31	4.31	10.02	0.003
Layer x block	3	4.42	1.47	3.42	0.028
Layer x zone	2	0.80	0.40	0.92	0.406
Layer x block x zone	6	3.75	0.63	1.45	0.223
Error	35	15.08	0.43		

Part c: 24 h incubation time

Source of variation	df	SS	MS	F	p
Layer	1	2.90	2.90	7.25	0.011
Layer x block	3	6.03	2.01	5.02	0.005
Layer x zone	2	2.00	1.00	2.50	0.097
Layer x block x zone	6	2.68	0.45	1.12	0.373
Error	35	14.01	0.40		

SS: Type III sums of squares.

units (ln of ng N g^{-1} soil h^{-1}) less than did the upper layer ($p < 0.001$, $n = 47$). Of 47 soil pits, 41 had a greater rate in the upper layer. Repeated measures ANOVA showed the layer effect to be highly significant ($p < 0.001$, see Table 6, part a). Block and zone interactions with layer were nonsignificant, so the main effect interpretation is straightforward.

At eight hours, the mean rate of the lower layer was 0.44 units (ln ng N g^{-1} soil h^{-1}) less than in the upper layer ($p = 0.01$, $n = 47$), and 36 out of 47 soil pits had greater rates in the upper soil layer. A significant main effect at $p=0.003$ and a significant layer x block interaction, $p = 0.028$, were detected with the repeated measures ANOVA (Table 6, part b).

In Figures 1 through 4, the effect of the interaction is clearly visible; you can see that for Mack Creek blocks, the upper layer rate is greater than the lower layer rate at 1 h, but 8 and 24 h, rates are essentially equal; the Quartz Creek blocks, in contrast, maintain at least 0.7 unit decrease from the upper to the lower layer over all three incubation periods. Paired t-tests (two layers within one soil pit) averaged over all zones within blocks, showed the depth effect to be small and nonsignificant in the two Mack Creek blocks, but relatively large and very significant in the Quartz Creek blocks (Table 7, compare with 1 h rates).

Average 24 h rates of the lower layers were 0.36 units less than those of the upper layer ($p = 0.02$, $n = 47$), and 32 out of 47 pits had greater activity in the upper layer. Repeated measures ANOVA deemed significant the main effect ($p = 0.011$) and the layer x block interaction ($p = 0.005$, Table 6, part c). As in the case of the 8 h

Table 7. Fall 1983 — Rate of N-loss from soil samples after 1, 8, and 24 h incubation: paired t-tests of soil depth effect, by block.

Time	Block	n	Mean*	SE	t	p
1 h	Mack-E	12	0.26	0.36	0.72	0.488
	Mack-W	11	1.13	0.47	2.41	0.037
	Quartz-E	12	0.83	0.28	2.96	0.013
	Quartz-W	12	0.78	0.17	4.67	0.001
8 h	Mack-E	12	-0.062	0.349	-0.18	0.861
	Mack-W	11	0.086	0.239	0.36	0.727
	Quartz-E	12	0.731	0.249	2.93	0.014
	Quartz-W	12	0.972	0.258	3.76	0.003
24 h	Mack-E	12	-0.159	0.328	-0.48	0.638
	Mack-W	11	-0.143	0.199	-0.72	0.490
	Quartz-E	12	0.781	0.242	3.22	0.008
	Quartz-W	12	0.934	0.293	3.19	0.009

* Mean is the average D within zone, where D = (ln rate for upper layer) - (ln rate for lower layer), by soil pit.

rates, the main effect is not directly interpretable due to layer x block interaction. The pattern is similar to that of the 8 h rates. See Figures 1 through 4 and Table 7.

Tests of the Slope Position Effect

Viewing the pattern of average zone N-loss rates, the overall impression one gets is of a repeated decline in denitrification activity from the flood plain to the hill slope. This pattern is present in data for nearly all blocks, depths, and incubation periods. Exceptions to the pattern occurred in only two data sets, both at 1 h: in the Fall 1983 Quartz Creek 15-30 cm layer, where the rate declined from flood plain to toe slope and then held steady, and in the Fall 1984 Quartz Creek 0-15 cm layer, where the rate declined from flood plain to toe slope and then reached a maximum in the hill slope.

In spite of the striking regularity of the slope effect, significance was not always forthcoming. The ANOVAs for Fall 1983 rates showed significant zone effects in the 0-15 cm layer at 1, 8, and 24 h ($p = 0.001$, 0.018, and 0.015 respectively), and in the 15-30 cm layer at 8 and 24 h ($p = 0.005$ each). At 1 h, the lower layer zone effect was nonsignificant ($p = 0.067$). Fall 1983 ANOVA summaries are contained in Table 8. ANOVA Tables 26 through 31, contained in the Appendix, contain detailed results.

The results of planned comparisons of zone means are included in the summary and detailed ANOVA tables. In each case, the flood plain rates are significantly different — greater in all but one case —

Table 8. Slope position effects: ANOVA summaries.

Time	Depth	F-test probabilities for: Zone	F vs. O	T vs. H
Fall 1983 (n = 47)				
1 h	0-15 cm	< 0.001	< 0.001	0.088
	15-30 cm	0.067	0.026	0.814
8 h	0-15 cm	0.018	0.008	0.207
	15-30 cm	0.005	0.002	0.100
24 h	0-15 cm	0.015	0.006	0.490
	15-30 cm	0.004	0.002	0.098
Spring 1984 (n = 45)				
1 h	0-15 cm	0.168	0.078	0.580
Fall 1984 (n = 48)				
1 h	0-15 cm	0.294	0.151	0.598

Time: Incubation time in hours; Depth: Soil depth; Zone: Slope position effect; F vs. O: Contrast of flood plain vs. others; T vs. H: Contrast of toe slope vs. hill slope; n: number of observations.
Block x Zone mean square was used as an error term.

Note: Detailed ANOVA tables for these tests are available in the Appendix.

from the combined toe slope and hill slope rates. No comparison of toe slope rates and hill slope rates showed them to be significantly different.

ANOVA tests showed no significant slope position effect for Spring or Fall 1984 data ($p = 0.168$ and 0.294). Splitting the data sets by creek and retesting the zone effect did not yield significance. The ANOVA summaries for the full designs are contained in Table 8; details are contained in Tables 32 and 33 in the Appendix. I tested the zone effect as part of the split-block analysis of the season effect and again found zone effect to be nonsignificant ($p = 0.158$, Table 9).

Tests of Between-Site Differences in N-loss Rates

Mack Creek rates were, with one exception, less than Quartz Creek rates, and this difference was nearly always significant in Fall 1983, but 1984 results were mixed. Fall 1983 rates for Quartz Creek were greater than comparable Mack Creek rates in every case; the difference was significant at nearly every level examined. For rates, see Table 1. All unpaired t-test comparisons of 1, 8, and 24 h rates averaged over all depths and zones, and by depth over all zones, were significant at $p < 0.001$. Comparisons of creek mean rates by depth x zone groups yielded only two nonsignificant differences.

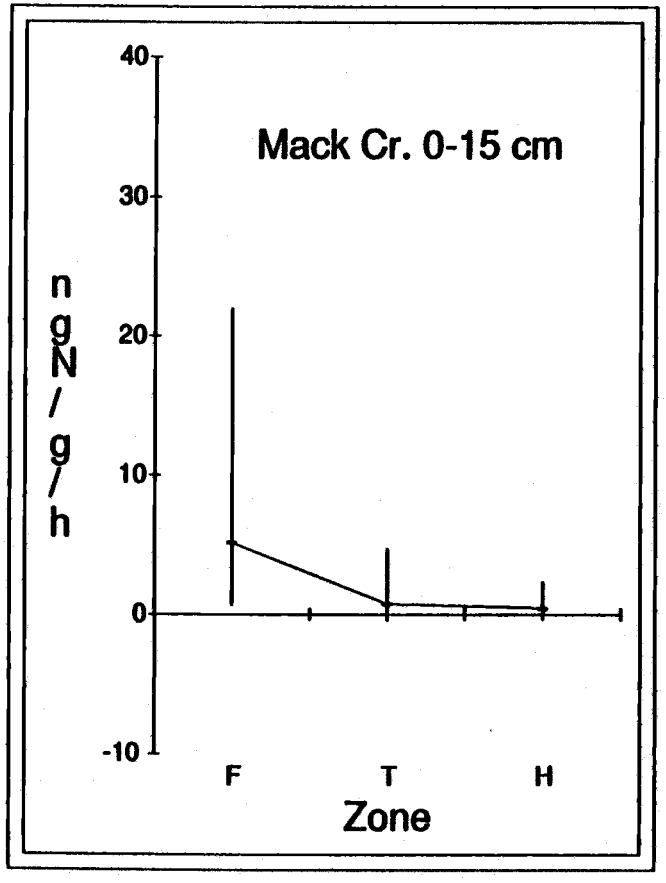
Spring 1984 mean rates for Mack were once again lower than Quartz Creek means in all cases, but fewer subgroup means were significantly different (see Table 2). One hour rates were significantly different ($p = 0.002$, $n = 24$) when averaged over all zones, but only the hill

slope means were significantly different ($p = 0.004$, $n = 8$). In Fall 1984, only the hill slope means were significantly different ($p < 0.001$). The toe slope pattern was reverse of that seen elsewhere -- the Mack Creek rates were greater than at Quartz Creek -- but not significantly so.

Tests of the Season Effect

Seasonal change in mean N-loss rates appears to exist, but a split-block analysis of combined Spring and Fall rate data could not detect a season effect ($p = 0.221$). The block x season interaction was not significant (Table 9, part a). Examination of Figure 13 reveals part of the reason for a nonsignificant main effect. The direction of seasonal changes in N-loss rates depended upon the site and the blocks within sites. Splitting the data set by site and retesting with split-block ANOVA detected a seasonal effect at Mack Creek ($p = 0.044$) but not at Quartz ($p = 0.882$). Thus, denitrification potential in Fall exceeded that of Spring at Mack Creek, except in the hill slope, where denitrification potentials were negligible at either time. No clear trend is present at Quartz Creek.

Figure 5. Fall 1983 - N-loss rates at 1 hour, 0-15 cm layer.



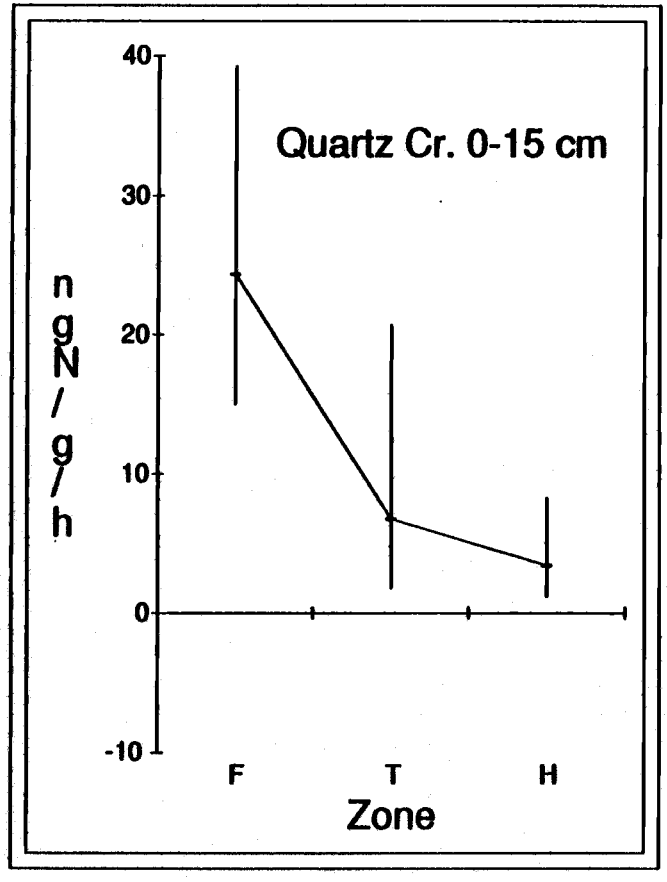


Figure 6. Fall 1983 - N-loss rates at 1 hour, 15-30 cm layer.

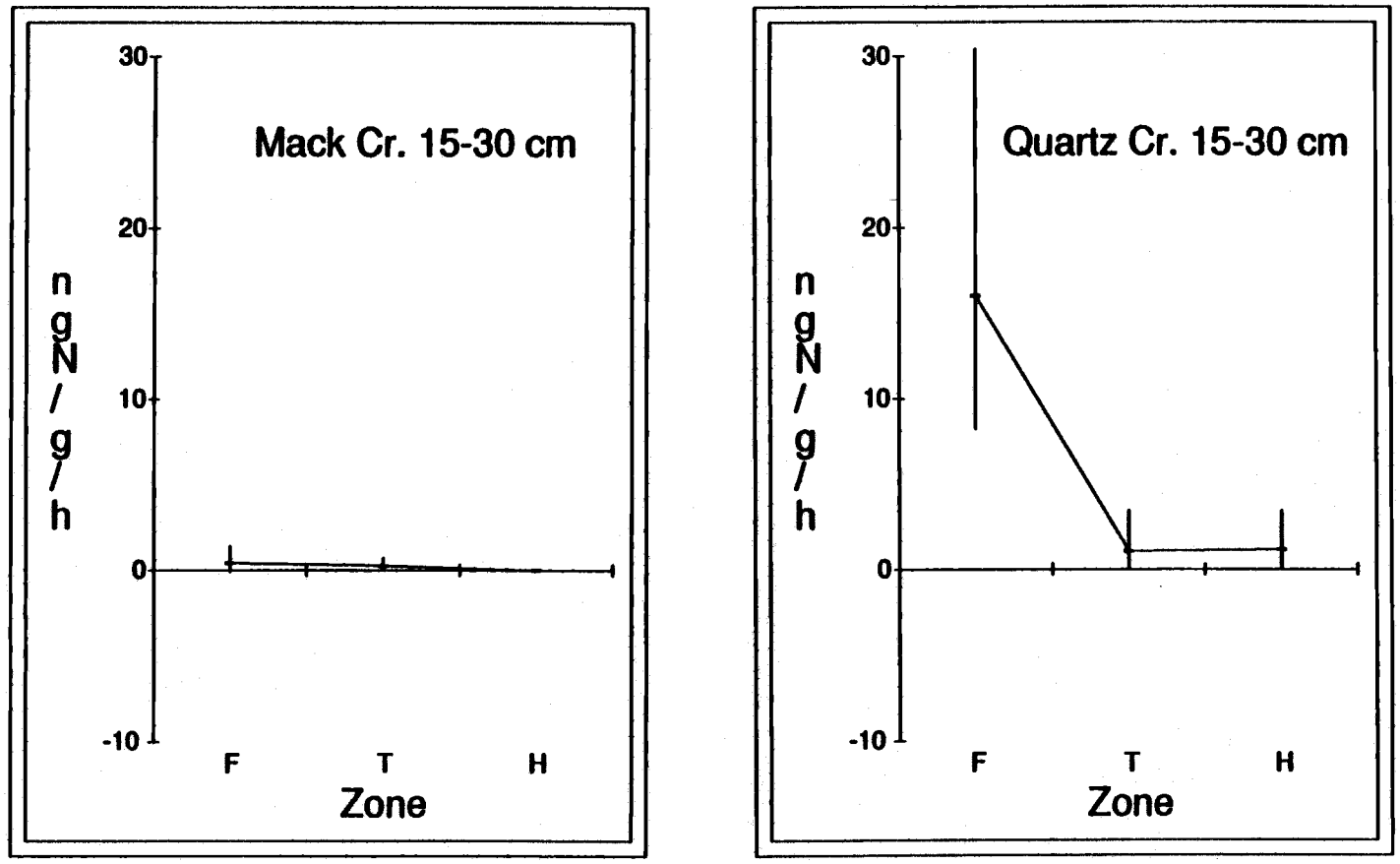
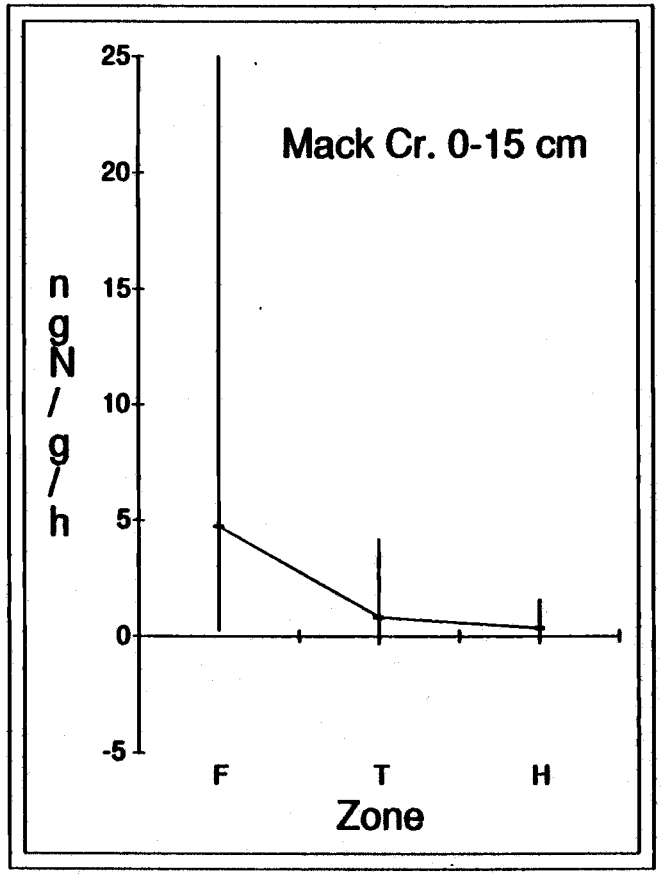


Figure 7. Fall 1983 - N-loss rates at 8 hours, 0-15 cm layer.



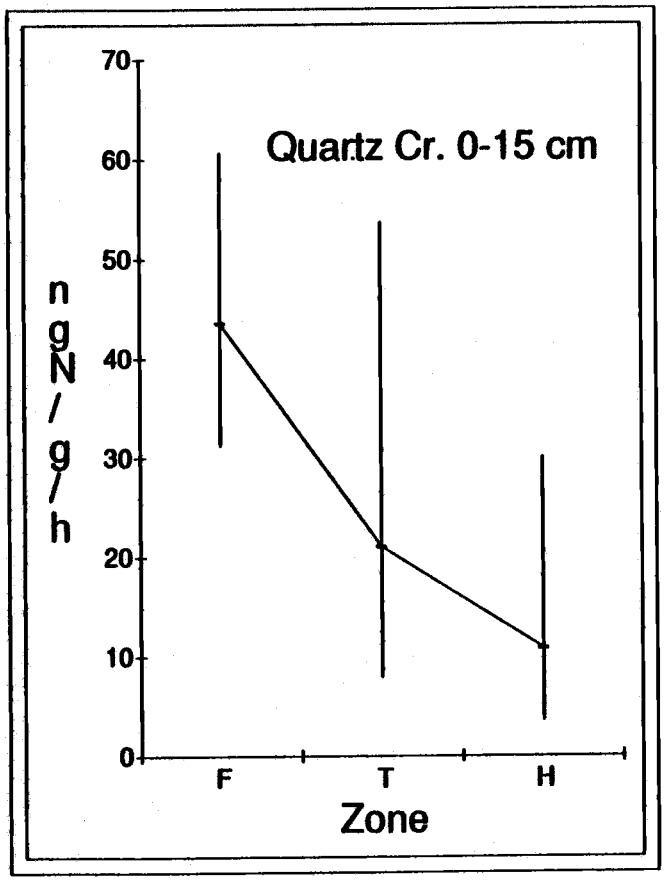
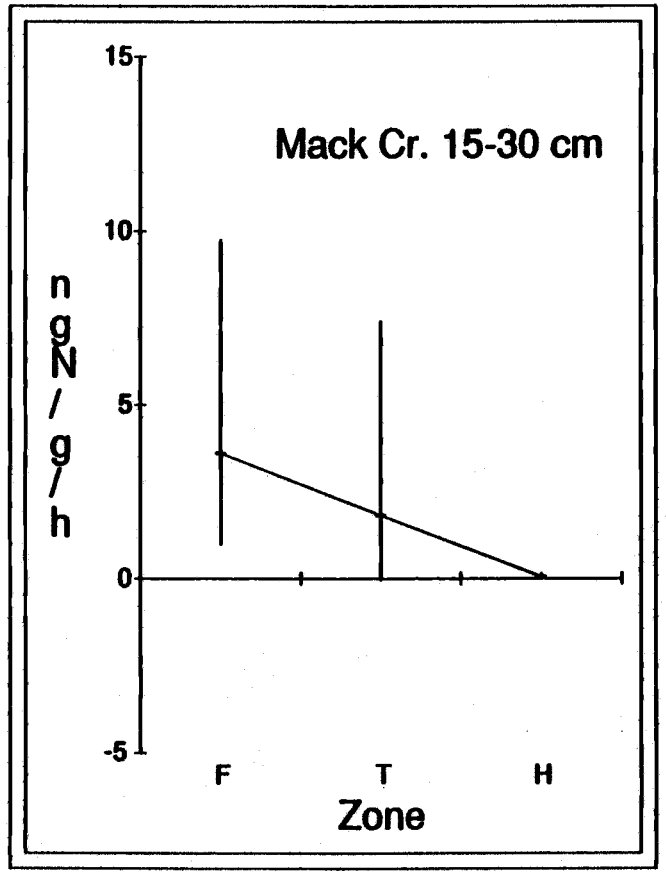


Figure 8. Fall 1983 - N-loss rates at 8 hours, 15-30 cm layer.



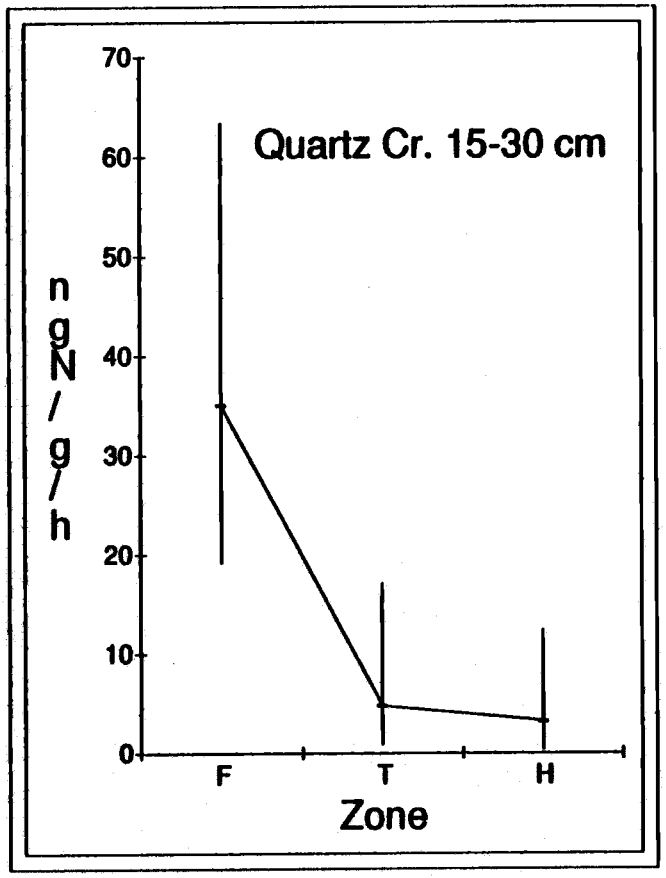
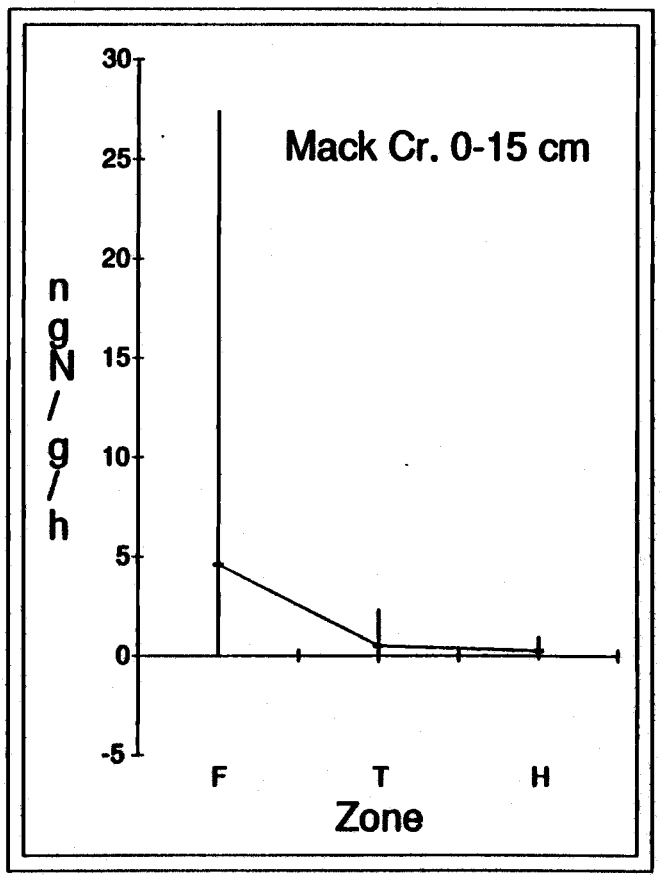


Figure 9. Fall 1983 - N-loss rates at 24 hours, 0-15 cm layer.



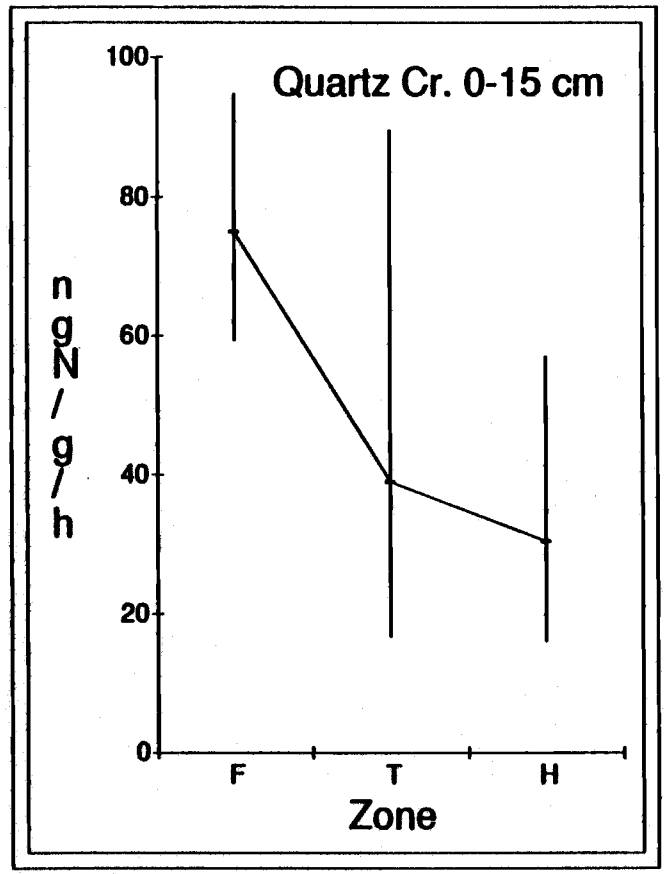


Figure 10. Fall 1983 - N-loss rates at 24 hours, 15-30 cm layer.

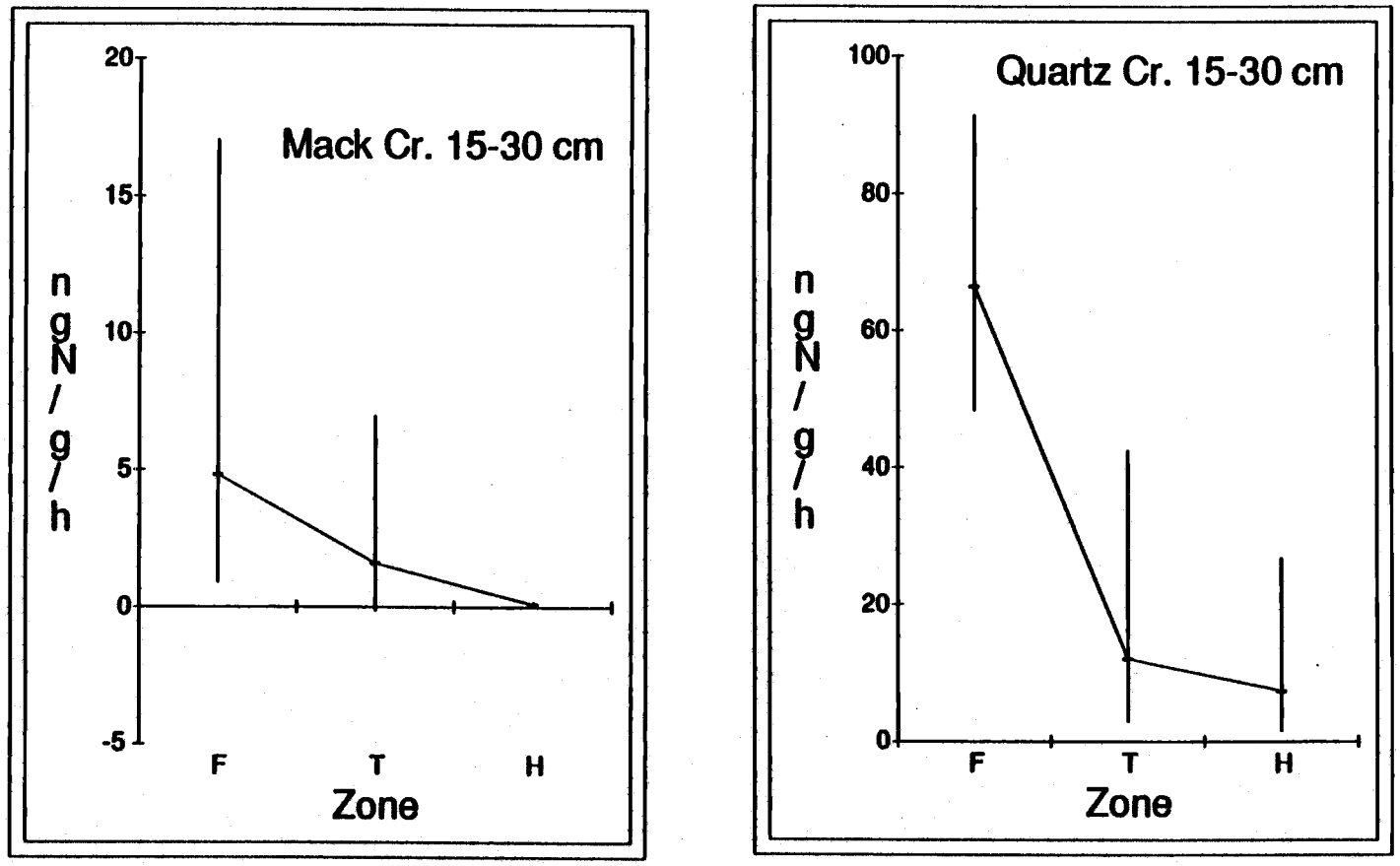


Figure 11. Spring 1984 - N-loss rates at 1 hour, 0-15 cm layer.

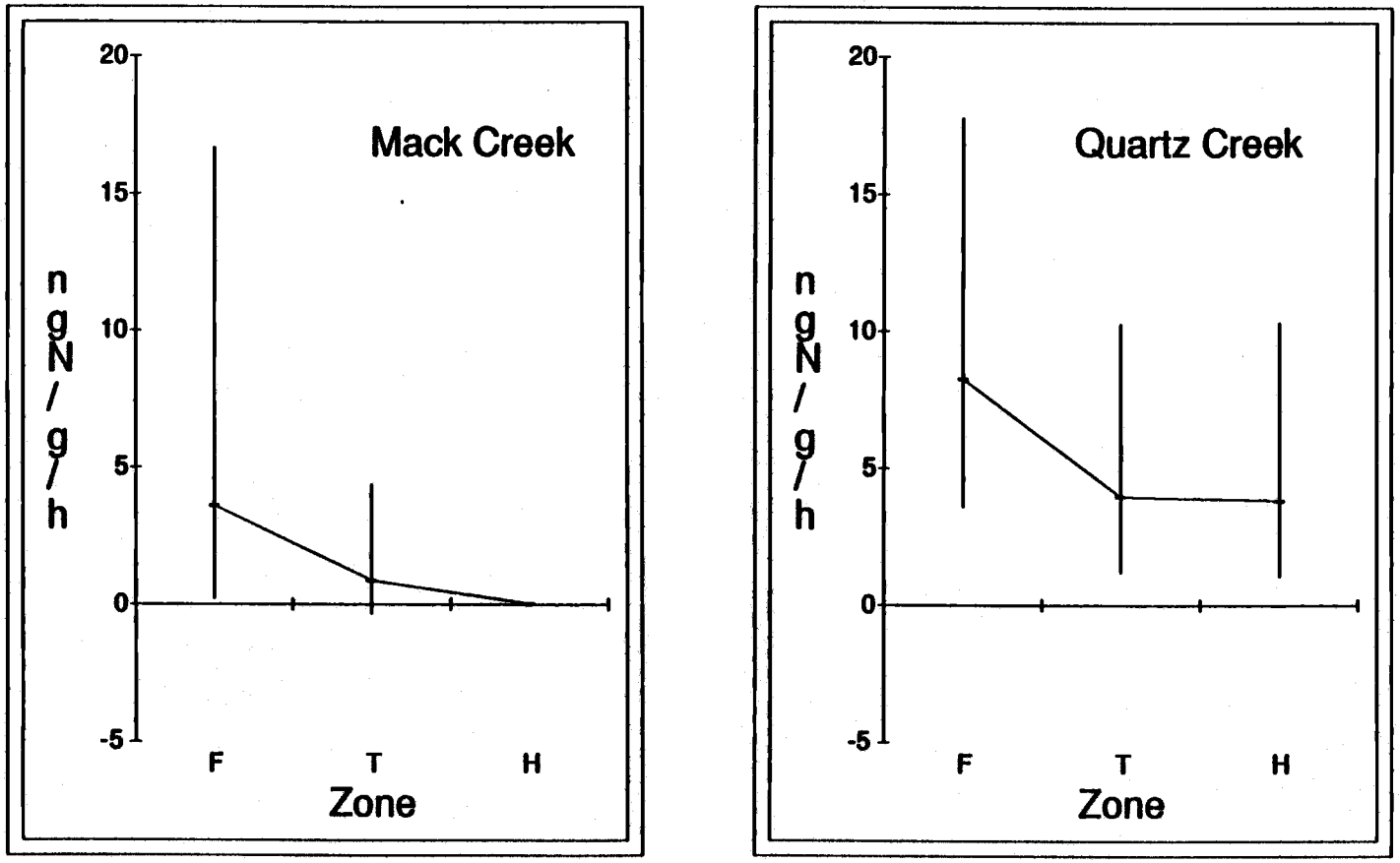


Figure 12. Fall 1984 - N-loss rates at 1 hour, 0-15 cm layer.

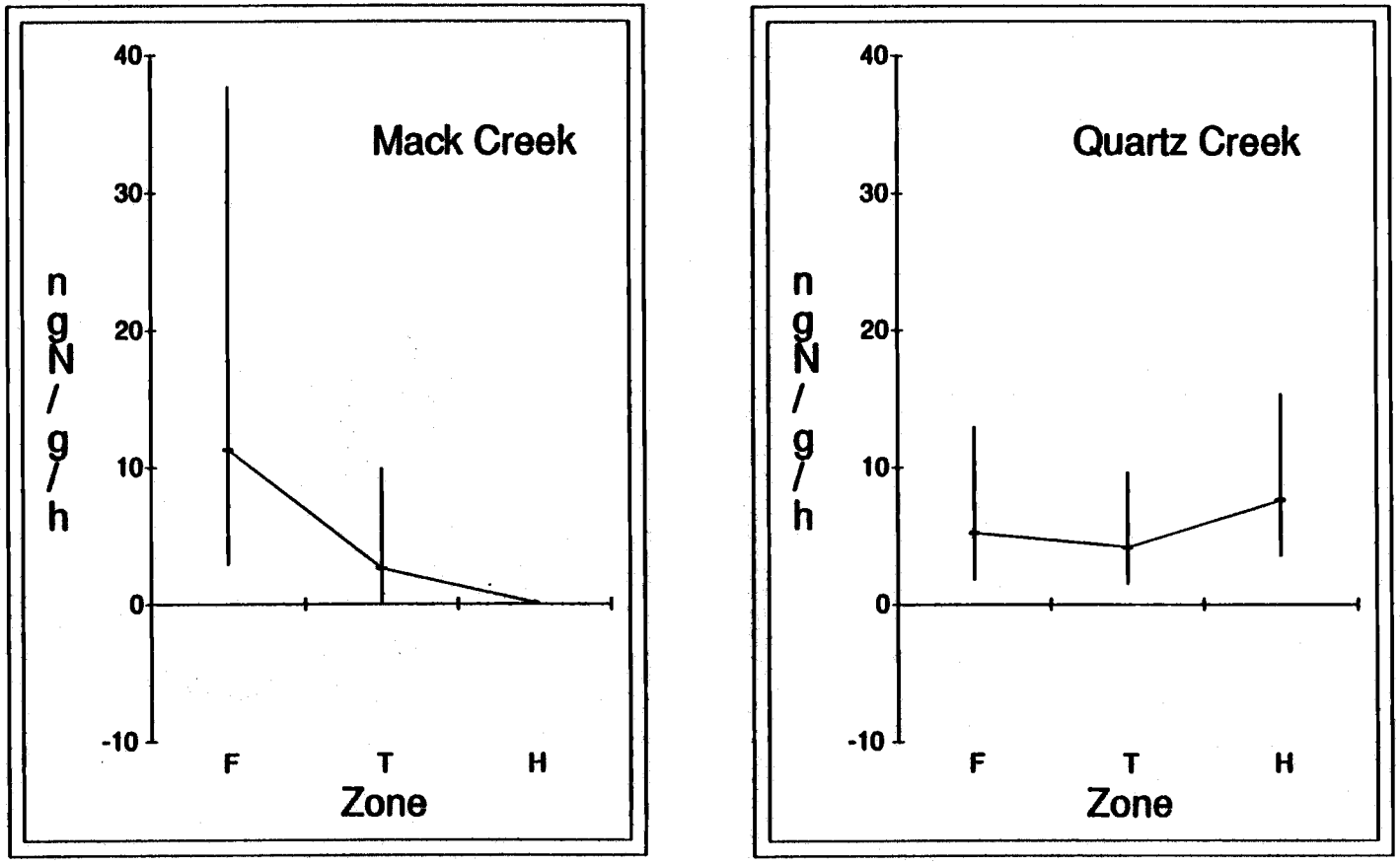


Figure 13. Seasonal change in N-loss rates by block, 1984

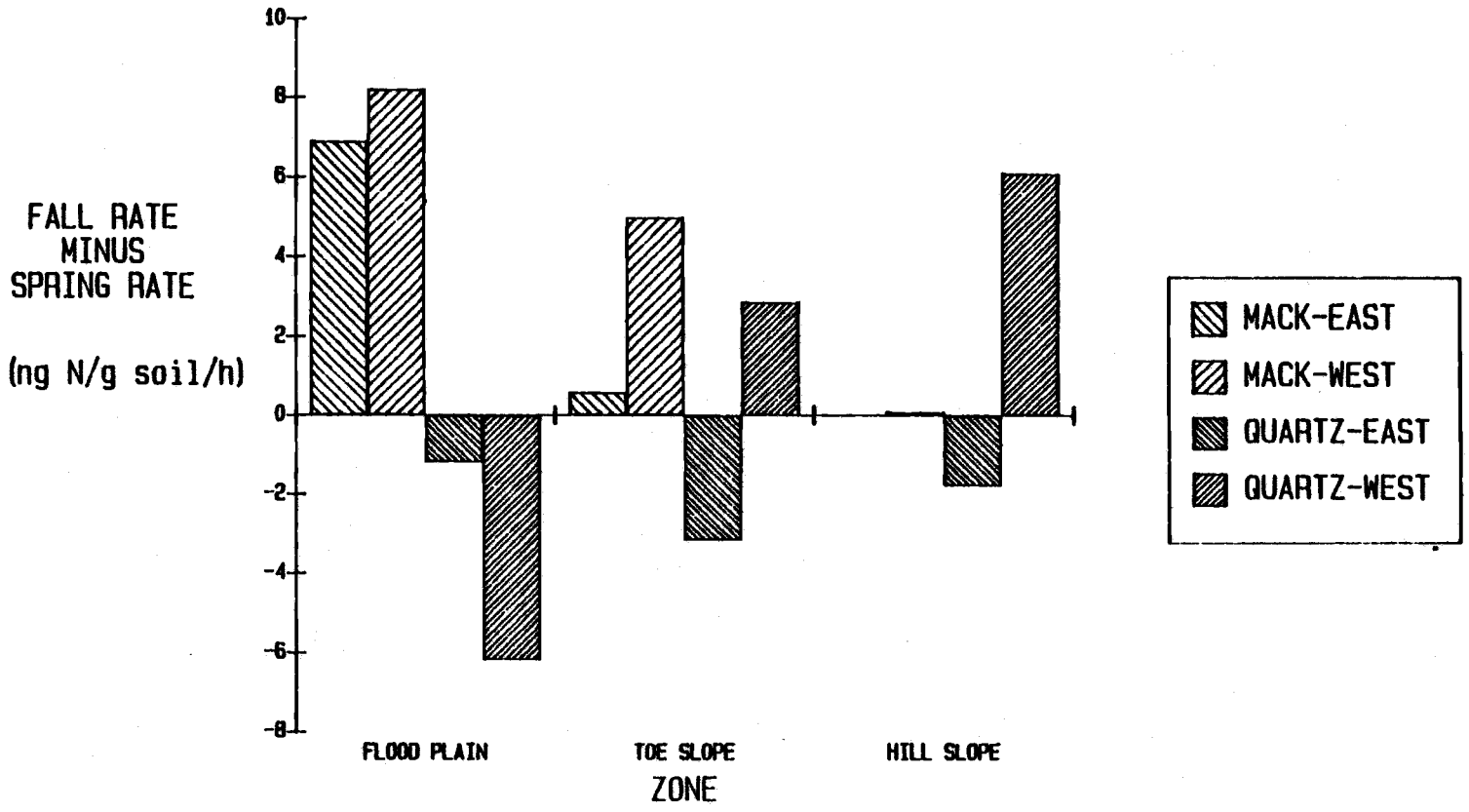


Table 9. Spring and Fall 1984 -- ANOVA table for split-block analysis of season effect on mean ln N-loss rates after 1 h incubation of soil from 0-15 cm layer.

Part a: Mack and Quartz Cr.

Source of variation	df	SS	MS	F	p	
Block	3	6.48	2.16	2.44	0.162	a
Zone	2	4.52	2.26	2.55	0.158	a
Block x zone	6	5.31	0.89	2.96	0.106	
Season	1	0.74	0.74	2.37	0.221	b
Block x season	3	0.94	0.31	1.05	0.438	
Zone x season	2	0.004	0.002	0.01	0.994	
Error	6	1.79	0.30			
Corrected Total	23	19.80				

SS are Type III sums of squares.
a Block x zone mean square used as an error term.
b Block x season mean square used as an error term.

Table 9. Part b: Mack Cr. alone

Source of variation	df	SS	MS	F	p	
Block	1	1.97	1.97	4.02	0.183	a
Zone	2	7.30	3.65	7.44	0.118	a
Block x zone	2	0.98	0.49	3.70	0.213	
Season	1	1.19	1.19	212.66	0.044	b
Block x season	1	0.01	0.01	0.04	0.856	
Zone x season	2	0.55	0.27	2.06	0.327	
Error	2	0.27	0.13			
Corrected Total	11	12.26				

Table 9. cont. Part c: Quartz Cr. alone

Source of variation	df	SS	MS	F	p	
Block	1	0.06	0.06	0.09	0.792	a
Zone	2	0.33	0.17	0.27	0.787	a
Block x zone	2	1.22	0.61	2.44	0.291	
Season	1	0.02	0.02	0.04	0.882	b
Block x season	1	0.47	0.47	1.88	0.304	
Zone x season	2	0.49	0.24	0.97	0.508	
Error	2	0.50	0.25			
Corrected Total	11	3.08				

SS are Type III sums of squares.
a Block x zone mean square used as an error term.
b Block x season mean square used as an error term.

Soil Chemical and Physical Characteristics

Temperature

Quartz Creek temperatures were generally 1 to 4 °C higher than those at Mack Creek. Fall 1983 Mack Creek temperatures varied between 10.5 and 13.5 °C at 5 cm and between 10.0 and 13.0 °C at 15 cm. Little between-zone variation existed. At Quartz Creek, the upper soil layer temperature varied between 13.0 and 14.0 °C and the lower layer varied between 12.8 and 13.3 °C. The upper slopes were slightly warmer than the flood plain. Spring temperatures at Mack Creek varied between 8.0 and 9.6 °C at 5 cm and between 6.9 and 8.5 at 15 cm. Quartz Creek values varied between 11.0 and 14.0 °C at 5 cm and 10.0 to 11.5 at 15 cm. Fall 1984 temperatures at Mack Creek were: 5cm: 11.0 to 15.5, 15cm: 11.2 to 14.0 °C. At Quartz Creek they were 5cm: 14.0 to 17.0, 15cm: 13.0 to 15.0 °C. Upper slopes were generally warmer than flood plain sites.

Moisture

Soil moisture varied widely within zones. Due to the presence of many outliers in the data set, medians, rather than means, best represented the central tendencies of soil moisture. For this reason, medians have been included in the soil moisture statistics summarized in Tables 10 through 12. Mack Creek soil moisture usually exceeded Quartz Creek values by at least 10%; this difference was often as high as 20%.

Table 10. Fall 1983 -- Soil moisture, percent by weight.

Site	n	Min	Max	Mean	Median	Std
Mack Creek						
Flood plain						
0-15 cm	7	20.9	120.3	65.2	61.2	35.50
15-30 cm	7	19.2	121.3	48.4	34.6	35.60
Toe slope						
0-15 cm	8	32.7	232.2	98.5	71.2	67.46
15-30 cm	8	22.4	175.8	83.2	70.8	51.68
Hill slope						
0-15 cm	8	50.4	105.5	70.5	63.4	22.01
15-30 cm	8	48.1	69.6	59.9	60.6	6.62
Quartz Creek						
Flood plain						
0-15 cm	8	21.4	76.3	42.1	38.4	16.40
15-30 cm	8	30.6	84.2	53.8	51.7	18.62
Toe slope						
0-15 cm	8	31.2	81.5	53.0	50.3	20.41
15-30 cm	8	25.1	82.2	45.4	41.6	19.83
Hill slope						
0-15 cm	8	33.5	63.8	44.8	42.5	10.54
15-30 cm	8	29.1	41.8	37.1	38.2	3.90

Table 11. Spring 1984 -- Soil moisture, percent by weight

Site	n	Min	Max	Mean	Median	Std
Mack Creek - 0-15 cm						
F	7	35.4	171.5	82.2	88.2	47.67
T	7	92.0	222.8	144.3	107.5	56.18
H	7	60.4	102.6	80.3	87.1	14.87
Quartz Creek - 0-15 cm						
F	8	21.6	63.6	44.3	46.0	13.41
T	8	53.0	113.3	75.9	71.0	23.02
H	8	52.5	104.7	76.1	77.2	18.24

F: Flood plain; T: Toe slope; H: Hill slope

Table 12. Fall 1984 — Soil moisture, percent by weight.

Site	n	Min	Max	Mean	Median	Std
Mack Creek - 0-15 cm						
F	8	19.1	85.8	52.4	52.1	19.20
T	8	38.0	181.8	82.6	64.4	46.53
H	8	33.4	57.3	44.1	44.1	8.74
Quartz Creek - 0-15 cm						
F	8	11.3	43.2	27.2	23.1	12.33
T	8	22.7	42.6	31.1	29.2	6.01
H	8	26.0	42.9	33.2	33.2	4.86

F: Flood plain; T: Toe slope; H: Hill slope

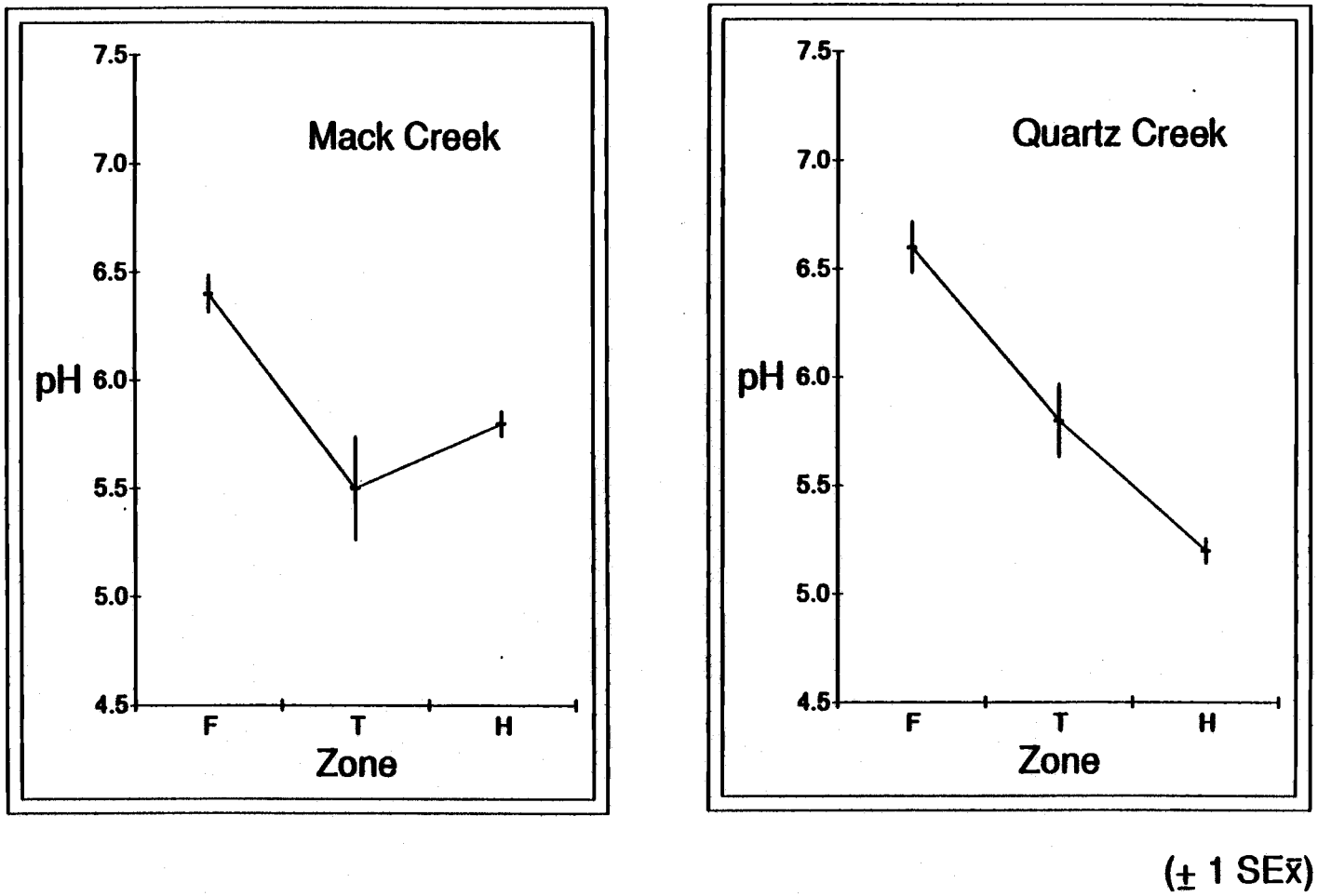
pH

Mean soil pH (measured in Fall 1984 only) varied between 5.5 and 6.4 at Mack Creek and between 5.2 and 6.6 at Quartz Creek. Figure 14 shows that soil pH was highest within the flood plains of both creeks. At Mack Creek, pH dropped within the toe slope and increased again within the hill slope zone. At Quartz Creek the pH declined continuously from flood plain to hill slope. Detailed pH data are presented in Table 13.

Table 13. Fall 1984 — Soil pH by creek and zone.

Creek	Zone	n	Min	Max	Mean	Std	SE
Mack	Flood plain	8	6.1	6.9	6.4	0.26	0.09
	Toe slope	8	4.5	6.4	5.5	0.68	0.24
	Hill slope	8	5.5	6.0	5.8	0.16	0.06
Quartz	Flood plain	8	6.2	7.1	6.6	0.33	0.12
	Toe slope	8	5.1	6.6	5.8	0.47	0.17
	Hill slope	8	5.0	5.5	5.2	0.16	0.06

Figure 14. Fall 1984 - pH by creek and zone, upper 0-15 cm of soil.



Nitrate

Average KCl-extractable soil nitrate concentrations varied from a high of 1.60×10^{-2} cmol/kg within the Quartz Creek hill slope to a low of 2.10×10^{-4} cmol/kg within the Mack Creek hill slope. Figure 15 illustrates the nitrate distribution pattern. At Mack Creek the nitrate concentration declined from the flood plain to the hill slope, while at Quartz nitrate increased from the flood plain to the hill slope. Table 14 contains the soil nitrate data.

Table 14. Fall 1984 -- Soil nitrate by creek and zone.

Creek	Zone	n	Min	Max	Mean	Std	SE
Mack	Flood	8	3.3E-04	7.7E-03	3.0E-03	3.07E-03	1.08E-03
	Toe	8	0.0E+00	1.1E-02	1.9E-03	3.72E-03	1.31E-03
	Hill	8	0.0E+00	4.7E-04	2.1E-04	2.26E-04	8.00E-05
Quartz	Flood	8	2.8E-04	5.4E-03	2.4E-03	2.14E-03	7.55E-04
	Toe	8	3.9E-04	1.3E-02	5.2E-03	4.44E-03	1.57E-03
	Hill	8	3.4E-03	4.3E-02	1.6E-02	1.40E-02	4.93E-03

Concentration in cmol nitrate/kg soil

Table 15. Fall 1984 -- Soil ammonium by creek and zone.

Creek	Zone	n	Min	Max	Mean	Std	SE
Mack	Flood	8	1.7E-03	8.2E-03	4.0E-03	2.01E-03	7.09E-04
	Toe	8	2.5E-03	3.0E-02	1.0E-02	9.76E-03	3.45E-03
	Hill	8	0.0E+00	9.1E-03	2.8E-03	3.20E-03	1.13E-03
Quartz	Flood	8	0.0E+00	4.8E-03	2.0E-03	1.54E-03	5.46E-04
	Toe	8	3.0E-03	2.6E-02	9.7E-03	7.70E-03	2.72E-03
	Hill	8	7.3E-03	5.0E-02	2.1E-02	1.50E-02	5.29E-03

Concentration in cmol ammonium/kg soil

Figure 15. Fall 1984 - Nitrate by creek and zone, upper 0-15 cm of soil.

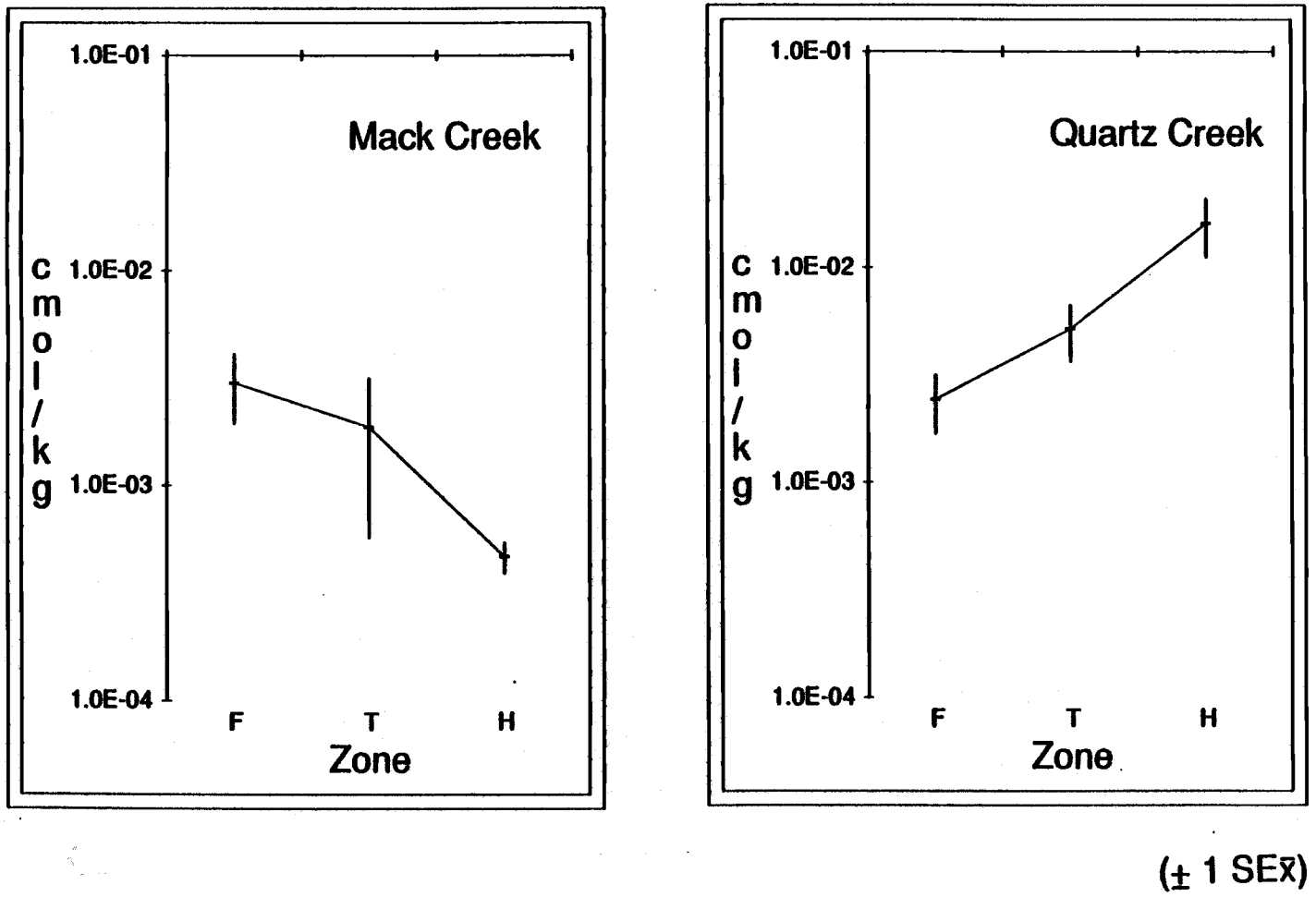


Table 16. Fall 1984 -- Soil total N by creek and zone.

Creek	Zone	n	Min	Max	Mean	Std	SE
Mack	Flood plain	8	6.6	36.9	23.0	10.87	3.84
	Toe slope	8	17.8	55.2	31.3	12.26	4.33
	Hill slope	8	14.5	32.8	22.9	5.76	2.04
Quartz	Flood plain	8	2.1	13.7	9.2	3.64	1.29
	Toe slope	7	13.7	38.9	24.3	8.15	3.08
	Hill slope	8	18.2	39.6	29.8	7.44	2.63

Concentration in cmol nitrogen/kg soil

Ammonium

Average KCl-extractable ammonium concentrations are presented in Table 15. The highest average concentration (2.08×10^{-2} cmol/kg) was measured within the Quartz Creek hill slope, while the lowest average concentration (2.02×10^{-3}) was measured within the Quartz Creek flood plain. Distribution of ammonium paralleled that of nitrate at Quartz Creek. At Mack Creek, ammonium and nitrate both reached their minimum within the hill slope, but ammonium peaked within the toe slope and nitrate peaked within the flood plain.

Total Nitrogen

Average total N varied from a high of 31.3 cmol N/kg soil within the Mack Creek toe slope to a low of 9.2 cmol N/kg soil within the Quartz Creek flood plain. The total N distribution at Quartz Creek paralleled the nitrate and ammonium distributions. At Mack Creek, the average total N did not significantly differ between the flood plain and the hill slope. Table 16 contains a summary of the total N data.

Total Carbon

Total soil C varied widely; values from 54 to 1900 cmol C/kg soil were recorded and outliers were common. Means varied from 255 to 967 cmol C/kg soil. I found charcoal in some soil samples and its presence may have caused inflated estimates of C. Samples with unusually

high C:N ratios probably contained charcoal. A few samples were noted having high C estimates and normal C:N ratios. I suspect that these samples were contaminated with forest floor material.

At Mack Creek the toe slope had the highest average C content, while the flood plain and the hill slope had less C and were roughly equal. At Quartz Creek the total C content increased from flood plain to hill slope in a series of distinct steps (Table 17).

Carbon:Nitrogen Ratio

Zone means ranged from 30.2 to 26.9 and differences between zones were small in relation to the standard errors of the means. While the toe slope at Mack Creek had the highest C:N ratio, the toe slope at Quartz Creek had the lowest. Table 18 contains the carbon:nitrogen values. As noted under the discussion of total C, some samples may have contained charcoal, yielding abnormally high C:N ratios.

Total Phosphorus

Total Kjeldahl phosphorus showed a clear pattern of increase from flood plain to hill slope at both creeks (Table 19). Zone-to-zone differences were greater at Quartz Creek. Zone-by-zone comparisons of average P content reveals higher P at Mack Creek in each case.

Table 17. Fall 1984 -- Soil carbon by creek and zone.

Creek	Zone	n	Min	Max	Mean	Std	SE
Mack	Flood plain	8	160	1050	641	333.2	117.8
	Toe slope	8	500	1900	967	459.4	162.4
	Hill slope	8	384	1140	666	246.0	87.0
Quartz	Flood plain	8	54	355	256	105.8	37.0
	Toe slope	7	331	996	649	233.4	88.2
	Hill slope	8	484	1310	902	312.5	110.5

Concentrations in cmol carbon/kg soil

Table 18. Fall 1984 -- Soil carbon:nitrogen by creek and zone.

Creek	Zone	n	Min	Max	Mean	Std	SE
Mack	Flood plain	8	23.8	36.5	27.4	4.00	1.41
	Toe slope	8	24.6	34.4	30.2	3.63	1.28
	Hill slope	8	22.4	34.9	28.5	4.66	1.65
Quartz	Flood plain	8	21.2	34.0	27.6	4.26	1.51
	Toe slope	7	19.6	39.0	26.9	6.27	2.37
	Hill slope	8	24.8	39.2	29.9	5.13	1.81

Ratio based on molar C and N concentrations

Table 19. Fall 1984 -- Soil total P by creek and zone.

Creek	Zone	n	Min	Max	Mean	Std	SE
Mack	Flood plain	8	4.6	5.6	5.0	0.41	0.14
	Toe slope	8	2.3	7.0	5.2	1.42	0.50
	Hill slope	8	3.8	10.3	6.2	2.32	0.82
Quartz	Flood plain	8	1.8	3.1	2.4	0.56	0.20
	Toe slope	7	2.7	5.6	3.9	1.04	0.39
	Hill slope	8	3.6	9.1	5.8	2.10	0.74

Concentrations in cmol phosphorus/kg soil

Correlation of N-loss Rates and Soil Properties

Table 20 contains the linear correlation test results, ordered by r-values calculated for rates in the original scale. Only total N, total C, and nitrate were significantly correlated with N-loss rate at the 5% level of significance. Only nitrate was significantly correlated with log-transformed N-loss. It should be noted that only linear correlations were tested by this method -- detecting nonlinear correlations requires other methods.

Equation 4 is the regression model relating N-loss rate to soil properties. The unadjusted R^2 for this model was 0.66; the R^2 adjusted for the number of independent variables included in the model was 0.61. The independent variables had significance levels of less than 2%, except for the nitrate cubic term, which was significant only at the 7% level.

$$(4) \qquad \ln(\text{N-loss rate} + 1) = -6.24 + 669(\text{nitrate}) - 7.06\times10^{2}(\text{nitrate})^{2} + 2.03\times10^{6}(\text{nitrate})^{3} + 1.00(\text{pH}) + 4.61\times10^{-2}(\text{total N})$$

(Where: N-loss rate is in ng N/g soil/h; nitrate and total N contents are in cmol/kg soil).

Table 20. Correlations of soil properties with N-loss rates: combined Fall 1984 data from Mack and Quartz Creeks.

Soil Property	N-loss rate used in calculation: log-transformed r	log-transformed p	original scale r	original scale p *
Total N	0.26	0.08	0.34	0.02
Total C	0.20	0.17	0.30	0.04
Nitrate	0.41	<0.01	0.29	0.05
Moisture	0.19	0.20	0.26	0.08
pH	0.22	0.13	0.23	0.11
C:N Ratio	0.01	0.96	0.07	0.65
Ammonium	0.19	0.19	0.07	0.65
Total P	-0.13	0.40	-0.01	0.97

* r: Pearson's product-moment correlation statistic
p: Probability of obtaining an r with a greater absolute value under the null hypothesis that the correlation is zero.

DISCUSSION

Distribution and Variability of N-loss Rates

The significance of the tests for non-zero N-loss rates showed that potential for denitrification existed in at least some of the soils studied. The distribution of denitrification potential was patchy and highly-variable: zones with significant overall activity yielded samples with no detectable denitrification potential, while very active sites appeared in zones with little overall activity. The variability was higher than that reported in many other studies. Anaerobic nitrate-amended soil slurry methods for measuring denitrification enzyme activity typically yield coefficients of variation (CV) of 5 to 25% (Tiedje 1982). Parkin et al. (1985) report that CVs of 50 to 100% are common in aerobic-intact soil core field studies of denitrification activity. I calculated a CV of 29% for control plot rates obtained by this method and presented by Robertson et al. (1987). Folorunso and Rolston (1984) reported CVs of 161 to 508% for agricultural field N_2+N_2O fluxes measured with a closed-chamber method. The CVs of 53 to 281% observed in this study fall within reported upper ranges, but due to differences in methods it is impossible to attribute the large variation to either technique or natural variability.

The apparent lognormal distribution of denitrification potential in this study has parallels in other work. Parkin et al. (1985), after extensive field sampling, concluded that denitrification

rates were log-normally distributed, as were many other soil properties known to affect denitrification rates, e.g. soil aggregate size, nitrate and carbon dioxide contents, and nitrification rates. The data of Folorunso and Rolston (1984) and Robertson et al. (1987) were log-transformed prior to statistical analysis.

Correlation of N-loss Rates and Soil Properties

Nearly two-thirds of the variation in N-loss rates was explained by soil nitrate, pH, and total N. The significant correlation of N-loss with soil nitrate was expected given the role of nitrate as a substrate for denitrification. Denitrifiers are known to respond to pH changes (Knowles 1982), but the strength of the correlation observed here is surprising. It is possible that pH and N-loss are both responding to some other environmental gradient. Total N and total C correlations with N-loss were similar, which was to be expected since they are both indexes of soil organic matter. This is demonstrated by the r of 0.94 ($p < 0.001$) for the correlation of total N with total C. In the regression model, similar results were obtained by using either C or N as an independent variable, but use of total N yielded a slightly higher R^2. Measurement of particular readily-decomposed C fractions, e.g. the water soluble C of Hu et al. (1972) might have produced stronger correlations between C and N-loss. The presence of charcoal in some samples may have contributed to weaker correlations as well.

Gravimetric soil moisture, as used here, would have suited a study conducted on very uniform textured soils, but in this study it proved to be inadequate for describing apparent differences in soil moisture regime. Flood plain soils were coarser-textured than hill slope soils, and a given gravimetric soil moisture yielded very different degrees of water saturation. Percent air-filled porosity would have been a more appropriate measure of soil water status.

Rate Differences with Incubation Time

If you accept the premise that the N-loss rate during the 1 h (phase I) incubation is an indicator of the field soil anaerobic volume (Smith and Tiedje 1979) and substrate sufficiency, and that sample storage and manipulation did not affect existing levels of denitrifying enzymes, the 1 h rates should correlate well with the actual rate of denitrification in the field at the time the soil was sampled. Actual rates could be 10 to 100 times lower than those measured in the current study (Tiedje 1982). The N-loss rates measured during the 8 and 24 h incubations could be predictors of the rate of denitrification found under field conditions should oxygen supply become limited, after heavy rainfall for example. There is no guarantee that such limits to oxygen availability will occur. The general increase in N-loss rate with incubation time suggests that oxygen availability under field conditions is such that denitrification rates rarely achieve the maximum possible for given levels of substrates and other rate-controlling factors.

The patterns of denitrification potentials observed could be divided into four classes: first consider denitrification potential to be either low or high, and then contrast the 1 h denitrification potential (early) with the average 8 and 24 h denitrification potential (late). In case 1, both early and late rates are low; this response is likely when substrates are limited, denitrifiers are absent, or if pH is unfavorably low. In case 2, a low early rate is followed by a high late rate. When substrates are sufficient, pH is tolerable, but the soil has few anaerobic microsites, this pattern should appear. Case 3 has a high early rate followed by low late rates; here the soil must have been at least partly anaerobic because the denitrification enzyme system is active at the start, pH is tolerable, but substrates — nitrate, carbon, or both — are limited. The system runs down. This could happen if the pool of substrate is small, or in the case of nitrate, if denitrification is tightly linked with nitrification. During the incubation, acetylene and anaerobiosis would both limit nitrification. In case 4, early and late rates are both high. In this case substrates are available, pH is tolerable, and the soil must have been partly anaerobic. This is an over-simplification, but it may provide a framework for interpreting the results.

Slope Position Effects on N-loss

Slope position differences in denitrification potential appear to be related to pH, which was always highest in the flood plain, nitrate levels, and total N. As I previously stated, the total N effect on N-loss rates probably represents the effect of soil organic matter and total C. Although available C was not measured, many flood plain sites are populated with small herbs, whose foliage litter may provide to denitrifying bacteria a C source that is more readily-oxidized than lignified shrub and tree litter. Elevated soil moisture within the flood plain may also contribute to increased levels of denitrification.

The general trend of declining denitrification potential from flood plain to hill slope was not present in Fall 1984 data from Quartz Creek. In 1984, there was no precipitation for 33 days prior to my sampling at Quartz Creek, while in 1983, 2.8 mm of rain were received in the 5 days prior to sampling, and, in general, the summer of 1983 was wetter than that of 1984: 126 mm versus 8 mm of precipitation in July and August (data from H.J. Andrews Experimental Forest Climatic Station WS-2, courtesy of Alfred Levno, USFS). The dry conditions may have led to accumulation of nitrate in the hill slope soils at Quartz Creek, which may explain the high rates of N-loss during incubation of these soils.

Depth Effects on N-loss

The decline in denitrification potential with depth can be attributed to lower levels of available carbon and nitrate in the 15-30 cm layer. Soil analyses show less nitrate, mineralizable-N, and total C in the lower layers, but pH increased with depth (Marla Gilham, personal communication). Although the pH increase might be expected to increase denitrification potential, the overwhelming influences appear to be nitrate and carbon. This is consistent with results described by Tiedje et al. (1982) and Myrold and Tiedje (1985), where increased denitrification capacity followed organic matter additions to soils.

Differences in N-loss Between Sites

The denitrification potential difference between Mack and Quartz Creek cannot be attributed to differences in vegetation without further study. The conifer and N_2-fixing hardwood treatments were not replicated, therefore differences in denitrification potential could be attributed to any other variable that changes between these sites. In the interim, however, it is reasonable to assume that between-site differences in denitrification potential are due to vegetation type: red alder fixes nitrogen, has been shown to increase soil nitrate content, and red alder leaf litter may be a better source of simple carbon compounds. This is demonstrated by the fact that flood plain leaf litter at Quartz Creek decomposed 2

to 4 times faster than did Mack Creek leaf litter (Kermit Cromack, personal communication).

In Situ Rate Estimates

If the hill slope N-loss rates are representative of the denitrification potential to be found in upslope Douglas-fir/western hemlock forests, we can expect generally low denitrification potential over much of the forest land in the western Cascades of Oregon, with higher denitrification potential expected in red alder stands. Actual rates of denitrification will be much lower than potential estimates. Riparian zones in both types of forest should exhibit higher denitrification potentials, and possibly, higher in situ denitrification. A study with much broader scope would be required to confirm this.

Estimating field rates from potential measurements is risky: it is necessary to account for spatial and temporal variation and artifacts due to the estimation technique (Tiedje 1982). Tiedje et al. (1982) state that the anaerobic slurries amended with nitrate and C yield rates 40 or more times higher than field rates. By ignoring denitrification in the litter layer or in mineral soil below 30 cm, assuming bulk densities of 0.85 and 1.10 g cm^{-1} in the 0-15 and 15-30 cm soil layers respectively, and assuming constant denitrification potential for one third of the year, I estimated that my potential measurements represent annual N losses of 0-0.2 kg N ha^{-1} y^{-1} in the Douglas-fir system and 0.2-1.7 kg N ha^{-1}

y^{-1} in the red alder system. I assumed that real rates were 100 times less than the potential rates.

Bowden (1986) recently reviewed estimates of gaseous N-losses from forests and other natural terrestrial ecosystems. Estimates of denitrification in temperate zone hardwood forests ranged from less than 1 to 10 kg N ha^{-1} y^{-1}. Temperate zone conifer forests were said to lose less than 1 kg N ha^{-1} y^{-1}. Robertson et al. (1987) estimated losses in southeastern pine plantations of 0.4-0.7 kg N ha^{-1} y^{-1} under undisturbed conditions and 3-6 kg N ha^{-1} y^{-1} after disturbance. My estimates, although based on several untested assumptions, seem to fit within ranges reported elsewhere.

IMPLICATIONS FOR MANAGEMENT

The hypotheses to be tested in this study stated that denitrification occurs in forest soils, and that geomorphological features, soil structure, and vegetation can act singly or in concert to create sites suited in various degrees for denitrification. The sample set represented typical western Oregon Cascade forest conditions, and the results showed that areas of high denitrification potential existed within the riparian forest. Field measurements of denitrification should be made in these areas to determine whether high potentials yield equally high real rates.

If significant in situ denitrification occurs in forest soils, its significance lies in its effect on the availability of endogenous or added nitrogen and in its effect on the form and rate of nitrogen exports from the system. As silviculture moves toward more intensive management, the potential for nitrogen loss increases. Total utilization, short rotations, site preparation, and soil compaction from repeated entries may all take their toll on an already growth-limiting nitrogen capital.

Silviculturists must weigh management alternatives in light of the nutrient capital of the site, the potential for loss due to the treatment, and the potential for amelioration of the nutrient status. The latter is most commonly achieved through nitrogen fertilization or, less commonly, by deliberately including nitrogen-fixing species in the stand. The biological and economic

efficiency of these efforts may be limited if they caused denitrification losses to increase.

Further studies of denitrification in forest soils are needed to address nitrous oxide production as a pollution source. The proportion of dinitrogen and nitrous oxide produced by denitrifiers varies; in acidic conditions N_2O production exceeds N_2 production. Atmospheric scientists have observed that as nitrous oxide moves into the stratosphere from below, it is photochemically changed to nitric oxide. Nitric oxide catalyzes the destructive reaction of ozone (O_3) to oxygen (O_2). Knowledge of a similar reaction involving fluorocarbon compounds led some nations to outlaw fluorocarbon aerosol propellants in order to protect the ozone layer of the stratosphere. This layer shields Earth from intense solar ultraviolet radiation. Similar concerns have brought nitrous oxide and nitrogen fertilizer use under similar scrutiny (Knowles 1982).

Many natural and man-caused sources of nitrous oxide have been identified. Tropospheric nitrous oxide is increasing at 0.2% yearly (Sahrawat and Keeney 1986), but the major sources and sinks for nitrous oxide have not yet been quantified. For instance, very little information exists for the large tracts of forest that cover the globe. The results of this study clearly show that forests, at least riparian forests, could constitute a source of nitrous oxide.

Denitrification may be a mechanism for removal of excess nitrate from groundwater and surface water (Knowles 1982), and it is possible that denitrification in riparian zones limits nitrogen inputs to streams and thus limits their productivity.

With regard to Pacific Northwest forest soils and denitrification, many questions remain unanswered:

1. Does denitrification occur in situ?
2. If so, how are potential measurements related to actual rates?
3. How much nitrogen is lost yearly and how does the loss rate vary throughout the year?
4. How much nitrous oxide is evolved to the atmosphere from forested areas?
6. How do management activities affect denitrification rates and can their effects be controlled?

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APPENDIX

Table 21. Fall 1983 — Rate of N-loss from upper and lower-layer soil samples after 1 h incubation: t-tests by creek, zone and depth of Ho: mean rate = 0.

	n	Min	Max	Mean	SE	t	p
Mack Creek							
Flood plain							
0-15 cm	7	0.000	3.376	1.819	0.557	3.27	0.017
15-30 cm	7	0.000	1.543	0.366	0.221	1.66	0.148
Toe slope							
0-15 cm	8	0.000	4.135	0.565	0.512	1.10	0.307
15-30 cm	8	0.000	0.892	0.263	0.132	1.99	0.087
Hill slope							
0-15 cm	8	0.000	2.935	0.376	0.366	1.03	0.337
15-30 cm	8	0.000	0.000	0.000	0.000	.	.
Quartz Creek							
Flood plain							
0-15 cm	8	2.521	3.962	3.234	0.200	16.14	<0.001
15-30 cm	8	1.418	3.796	2.835	0.266	10.65	<0.001
Toe slope							
0-15 cm	8	0.539	4.219	2.049	0.447	4.58	0.003
15-30 cm	8	0.000	2.788	0.732	0.332	2.21	0.063
Hill slope							
0-15 cm	8	0.335	2.995	1.487	0.319	4.66	0.002
15-30 cm	8	0.000	2.340	0.780	0.310	2.52	0.040

Values are natural logarithms of rate in ng N/h/g soil.

Table 22. Fall 1983 — Rate of N-loss from upper and lower-layer soil samples after 8 h incubation: t-tests by creek, zone and depth of Ho: mean rate = 0.

	n	Min	Max	Mean	SE	t	p
Mack Creek							
Flood plain							
0-15 cm	7	0.000	3.815	1.750	0.638	2.74	0.034
15-30 cm	7	0.091	3.187	1.531	0.357	4.29	0.005
Toe slope							
0-15 cm	8	0.000	3.679	0.616	0.447	1.38	0.211
15-30 cm	8	0.000	3.376	1.039	0.473	2.20	0.064
Hill slope							
0-15 cm	8	0.000	2.258	0.318	0.279	1.14	0.292
15-30 cm	8	0.000	0.344	0.062	0.042	1.49	0.181
Quartz Creek							
Flood plain							
0-15 cm	8	3.259	4.453	3.769	0.141	26.81	0.000
15-30 cm	8	2.463	4.555	3.584	0.252	14.25	0.000
Toe slope							
0-15 cm	8	1.531	4.812	3.090	0.395	7.82	0.000
15-30 cm	8	0.000	3.809	1.750	0.496	3.53	0.010
Hill slope							
0-15 cm	8	0.204	4.109	2.468	0.419	5.88	0.001
15-30 cm	8	0.038	3.353	1.439	0.499	2.88	0.023

Values are natural logarithms of rate in ng N/h/g soil.

Table 23. Fall 1983 -- Rate of N-loss from upper and lower-layer soil samples after 24 h incubation: t-tests by creek, zone and depth of Ho: mean rate = 0.

	n	Min	Max	Mean	SE	t	p
Mack Creek							
Flood plain							
0-15 cm	7	0.000	4.722	1.722	0.687	2.50	0.046
15-30 cm	7	0.050	4.090	1.765	0.477	3.70	0.010
Toe slope							
0-15 cm	8	0.000	2.794	0.409	0.343	1.19	0.272
15-30 cm	8	0.000	4.009	0.954	0.487	1.96	0.091
Hill slope							
0-15 cm	8	0.000	1.586	0.231	0.194	1.19	0.273
15-30 cm	8	0.000	0.280	0.082	0.043	1.90	0.100
Quartz Creek							
Flood plain							
0-15 cm	8	3.963	4.868	4.331	0.100	43.25	<0.001
15-30 cm	8	3.712	4.849	4.212	0.136	30.96	<0.001
Toe slope							
0-15 cm	8	2.341	5.392	3.690	0.354	10.42	<0.001
15-30 cm	8	0.021	4.743	2.568	0.523	4.91	0.002
Hill slope							
0-15 cm	8	2.339	4.631	3.449	0.266	12.94	<0.001
15-30 cm	8	0.213	3.981	2.119	0.523	4.05	0.005

Values are natural logarithms of rate in ng N/h/g soil.

Table 24. Spring 1984 — Rate of N-loss from upper-layer soil samples after 1 h incubation: t-tests by creek and zone of Ho: mean rate = 0.

	n	Min	Max	Mean	SE	t	p
Mack Creek							
Flood plain	7	0.000	3.369	1.526	0.569	2.68	0.036
Toe slope	7	0.000	3.430	0.624	0.477	1.31	0.239
Hill slope	7	0.000	0.049	0.018	0.009	2.10	0.080
Quartz Creek							
Flood plain	8	1.041	3.635	2.228	0.306	7.27	0.000
Toe slope	8	0.294	2.862	1.604	0.356	4.50	0.003
Hill slope	8	0.149	3.258	1.572	0.371	4.24	0.004

Values are natural logarithms of rate in ng N/h/g soil.

Table 25. Fall 1984 — Rate of N-loss from upper-layer soil samples after 1 h incubation: t-tests by creek and zone of Ho: mean rate = 0.

	n	Min	Max	Mean	SE	t	p
Mack Creek							
Flood plain	8	0.053	3.994	2.508	0.498	5.04	0.002
Toe slope	8	0.000	3.384	1.282	0.479	2.68	0.032
Hill slope	8	0.000	0.409	0.085	0.053	1.59	0.156
Quartz Creek							
Flood plain	8	0.328	3.068	1.828	0.350	5.23	0.001
Toe slope	8	0.457	3.069	1.643	0.313	5.25	0.001
Hill slope	8	0.798	3.029	2.155	0.278	7.75	0.000

Values are natural logarithms of rate in ng N/h/g soil.

Table 26. Fall 1983 -- Anova table for zone effect on log-transformed N-loss rates after 1 h incubation of soil from 0-15 cm layer.

Source of variation	df	SS	MS	F	p
Block	3	29.92	9.97	36.51	<0.001 a
Zone	2	22.22	11.11	40.68	<0.001 a
Flood vs. others	1	21.10	21.10	77.24	<0.001 a
Toe vs. hill	1	1.13	1.13	4.12	0.088 a
Error A (Block x zone)	6	1.64	0.27	0.22	0.969 b
Error B	35	43.99	1.26		
Corrected total	46	98.30			

Table 27. Fall 1983 -- Anova table for zone effect on log-transformed N-loss rates after 1 h incubation of soil from 15-30 cm layer.

Source of variation	df	SS	MS	F	p
Block	3	21.28	7.09	4.58	0.054 a
Zone	2	13.58	6.79	4.39	0.067 a
Flood vs. others	1	13.49	13.49	8.71	0.026 a
Toe vs. hill	1	0.09	0.09	0.06	0.814 a
Error A (Block x zone)	6	1.64	0.27	0.22	0.969 b
Error B	35	14.78	0.42		
Corrected Total	46	60.37			

SS are Type III sums of squares.
a Error A mean square used as an error term.
b Error B mean square used as an error term.

Table 28. Fall 1983 — Anova table for zone effect on log-transformed N-loss rates after 8 h incubation of soil from 0-15 cm layer.

Source of variation	df	SS	MS	F	p
Block	3	62.60	20.87	24.73	<0.001 a
Zone	2	14.43	7.21	8.55	0.018 a
Flood vs. others	1	12.74	12.74	15.09	0.008 a
Toe vs. hill	1	1.69	1.69	2.00	0.207 a
Error A (Block x zone)	6	5.06	0.84	0.68	0.666 b
Error B	35	43.37	1.24		
Corrected Total	46	127.06			

Table 29. Fall 1983 — Anova table for zone effect on log-transformed N-loss rates after 8 h incubation of soil from 15-30 cm layer.

Source of variation	df	SS	MS	F	p
Block	3	28.64	9.55	10.83	0.008 a
Zone	2	25.58	12.79	14.52	0.005 a
Flood vs. others	1	22.26	22.26	25.27	0.002 a
Toe vs. hill	1	3.32	3.32	3.76	0.100 a
Error A (Block x zone)	6	5.29	0.88	0.75	0.614 b
Error B	35	41.12	1.17		
Corrected Total	46	102.50			

SS are Type III sums of squares.
a Error A mean square used as an error term.
b Error B mean square used as an error term.

Table 30. Fall 1983 — Anova table for zone effect on log-transformed N-loss rates after 24 h incubation of soil from 0-15 cm layer.

Source of variation	df	SS	MS	F	p
Block	3	112.04	37.35	57.71	<0.001 a
Zone	2	11.72	5.86	9.05	0.015 a
Flood vs. others	1	11.37	11.37	17.57	0.006 a
Toe vs. hill	1	0.35	0.35	0.54	0.490 a
Error A (Block x zone)	6	3.88	0.65	0.66	0.682 b
Error B	35	34.31	0.98		
Corrected Total	46	164.32			

Table 31. Fall 1983 — Anova table for zone effect on log-transformed N-loss rates after 24 h incubation of soil from 15-30 cm layer.

Source of variation	df	SS	MS	F	p
Block	3	53.19	17.73	19.56	0.002 a
Zone	2	27.60	13.80	15.23	0.004 a
Flood vs. others	1	24.12	24.12	26.61	0.002 a
Toe vs. hill	1	3.48	3.48	3.84	0.098 a
Error A (Block x zone)	6	5.44	0.91	0.69	0.661 b
Error B	35	46.18	1.32		
Corrected Total	46	134.95			

SS are Type III sums of squares.
a Error A mean square used as an error term.
b Error B mean square used as an error term.

Table 32. Spring 1984 -- Anova table for zone effect on log-transformed N-loss rates after 1 h incubation of soil from 0-15 cm layer.

Source of variation	df	SS	MS	F	p
Block	3	18.47	6.16	3.62	0.084 a
Zone	2	8.27	4.14	2.43	0.168 a
Flood vs. others	1	7.69	7.69	4.53	0.078 a
Toe vs. hill	1	0.58	0.58	0.34	0.580 a
Error A (Block x zone)	6	10.20	1.70	1.93	0.104 b
Error B	33	29.01	0.88		
Corrected Total	44	66.51			

Table 33. Fall 1984 -- Anova table for zone effect on log-transformed N-loss rates after 1 h incubation of soil from 0-15 cm layer.

Source of variation	df	SS	MS	F	p
Block	3	8.04	2.68	0.89	0.500 a
Zone	2	9.13	4.57	1.51	0.294 a
Flood vs. others	1	8.20	8.20	2.71	0.151 a
Toe vs. hill	1	0.94	0.94	0.31	0.598 a
Error A (Block x zone)	6	18.13	3.02	2.95	0.019 b
Error B	36	36.90	1.02		
Corrected Total	47	72.19			

SS are Type III sums of squares.
a Error A mean square used as an error term.
b Error B mean square used as an error term.