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Nitrogen dynamics in primary successional soils on the Tanana River of interior Alaska

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NITROGEN DYNAMICS IN PRIMARY SUCCESSIONAL SOILS ON THE TANANA RIVER OF INTERIOR ALASKA

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Fairbanks, Alaska

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NITROGEN DYNAMICS IN PRIMARY SUCCESSIONAL SOILS

ON THE TANANA RIVER OF INTERIOR ALASKA

by

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ABSTRACT

As succession determines the patterns of ecosystem development, it also provides a temporal framework in which to investigate the controls of nitrogen-cycling. The object of this study was to examine patterns of nitrogen-cycling within primary successional soils. Estimates of N mineralization, nitrogen fixation, and denitrification were made within open shrub, alder, and white spruce stands, representing early, mid-, and late successional stages, respectively. Net NH_4^+ mineralization and net nitrification, measured using the polyethylene bag technique, were significantly different between sites and among forest floors and mineral soils. The alder forest floor had the highest observed mineralization rates, <1-21 ug N·g dry $soil^{-1}$·d^{-1}, with the white spruce forest floor exhibiting rates of <1-2 and mineral soils <1. Seasonal patterns of N mineralization were more pronounced in the alder forest floor: high net NH_4^+ mineralization in early summer and high net nitrification in late summer. Immobilization of nitrogen was observed at all sites. Laboratory studies indicate temperature as a limiting factor of N mineralization in early and mid-successional stages, while both temperature and moisture were limiting in later succession. Laboratory studies suggest heterotrophic nitrification may be important in the alder forest floor. Denitrification activity was low to undetectable at all sites, the highest observed rate was in alder forest floor samples,

220 g $N \cdot ha^{-1} \cdot d^{-1}$. Potential denitrification was low, the alder forest floor exhibited the highest rate, 3.4 Kg $N \cdot ha^{-1} \cdot d^{-1}$. Laboratory studies indicated denitrification was more limited by carbon and NO_3^- than temperature or moisture. Nonsymbiotic nitrogen fixation was low to undetectable and highly variable. The highest observed nitrogenase activity was associated with alder root nodules, 163 Kg $N \cdot ha^{-1} \cdot yr^{-1}$.

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CHAPTER 1

INTRODUCTION

NITROGEN DYNAMICS

Nitrogen is an integral part of the biochemical makeup of living matter. It is a key element of amino acids, nucleic acids, and other cellular compounds. The major form of nitrogen within the biosphere is dinitrogen, N_2, which at room temperatures and atmospheric pressures is a highly stable molecule, and is therefore unavailable to most forms of life. Most organisms obtain nitrogen in some combined form: nitrate, ammonia, other reduced or oxidized forms, or organic nitrogen containing compounds. Only a few organisms, among the bacteria and cyanobacteria, are able to utilize N_2 as a source of nitrogen (Postgate 1988).

Biological nitrogen fixation, which occurs symbiotically and non-symbiotically, reduces N_2 to ammonia, NH_3, which subsequently is used in the formation of nitrogenous compounds. The energy cost of breaking the triple molecular bond is high (approximately 18-24 ATP are used in the overall reduction of one mole of N_2, while the photosynthetic fixation of one mole of CO_2 requires 3 ATP, Brock *et al.* 1984), but it is the major process which provides nitrogen for organic matter synthesis. Nitrogen fixation also occurs chemically in the atmosphere,

producing only small amounts of fixed nitrogen, and synthetically through production of fertilizers (Delwiche 1981).

Once N_2 is incorporated into organic matter it becomes available for other plant or microbial activities through cell diffusion, disruption, reabsorption or decomposition. The microbial uptake of nitrogen, immobilization, and the microbial decomposition of organic nitrogenous compounds, mineralization, are processes that act as sinks and sources of available soil nitrogen. Both processes govern the possible rates of nitrification and denitrification. Nitrification, both autotrophic and heterotrophic, is the oxidation of reduced forms of nitrogen to oxidized forms (Focht and Verstaete 1977). Autotrophic nitrification proceeds in two steps, each catalyzed by a different group of aerobic bacteria. Ammonium, NH_4^+, is oxidized to nitrite, NO_2^-, by nitrosofying bacteria, usually followed by the oxidation of nitrite to nitrate, NO_3^-, by nitrifying bacteria. Heterotrophic nitrification is the oxidation of organic nitrogen compounds by fungi or bacteria. The various end-products of heterotrophic nitrification are thought to be used as growth factors, chelators, biocidal agents, and secondary metabolites of constitutive nitrification pathways (Focht and Verstraete 1977; Castignetti and Hollocher 1984; Schimel et al. 1984). Heterotropic nitrification is thought to be unimportant to the net accumulation of oxidized nitrogen, except possibly in acidic environments, (pH $\leq$ 4.5) or highly alkaline environments (pH $\geq$ 8) (Verstraete and Alexander 1973; Tate 1977; Johnsrud 1978; Van de Dijk

and Troelstra 1980; Castignetti and Hollocher 1984; Schimel *et al.* 1984; Adams 1986). Denitrification, a form of anaerobic respiration, is the microbial reduction of NO_2^- or NO_3^- to nitrous oxide, N_2O, or N_2. Denitrification occurs in anaerobic environments and microsites (Tiedje *et al.* 1984; Parkin 1987). Nitrate, a product of nitrification, is repelled by the negatively charged soil particles and is readily leached. Moreover, nitrate is a substrate for denitrification, possibly a major pathway of nitrogen loss from an ecosystem.

Nitrogen has long been recognized as a limiting nutrient in both agricultural and forest productivity. The source and availabilty of nitrogen to plants, often termed a soil fertility index, varies in different ecosystems indicating a range of nitrogen limitation. Soil fertility is dependent on the rates of microbial processes which in turn are controlled by environmental factors. The cyclic nature of nitrogen transformations makes it difficult to identify the rates of single processes and determine how these processes are affected by the physical, chemical, and biological characteristics of an ecosystem.

FLOODPLAIN PRIMARY SUCCESSION ON THE TANANA RIVER

The balsam poplar and white spruce stands of the Tanana River floodplain have some of the highest rates of forest productivity in

Alaska, with the white spruce stands having a high potential as a source of timber for commercial industries (Viereck et al. 1986). Although these forests have been heavily utilized in the past, little is known of the nitrogen-cycling processes within them. This study has proposed to look at nitrogen-cycling within the primary successional sequence of the Tanana River of interior Alaska. As succession determines the patterns of ecosystem structure and function, it also provides a temporal framework in which to investigate the controls of nitrogen cycling and soil fertility. The interior of Alaska is part of the taiga biome. The taiga climate, typified by short, warm summers and long cold winters, is a major factor affecting the range of ecological processes.

The specifics of local climate within interior Alaska and surrounding Fairbanks have been described in detail by Patric and Black (1968), Bowling (1979), Viereck et al. (1983), and Slaughter and Viereck (1986). Interior Alaska receives low average annual precipitation, 300 mm, and has a low average annual temperature, -3.5°C, while average potential evaporation is approximately 450 mm. Winters typically last seven months, and snow pack covers the ground from mid-October to April. In January the mean temperature is -24°C, while in July it is 16°C. The frost free period is approximately 90-100 days. A dominant pedological factor is permafrost which develops in response to the low annual input of solar radiation. Permafrost is discontinuous in interior Alaska and is usually found on north slopes,

valleys with poor soil water drainage, and closed forest canopies where moss layers accumulate and thick organic matter mats are found.

Within interior Alaska, two different successional patterns are found: the uplands and the floodplains (Van Cleve and Viereck 1981; Viereck *et al.* 1983; Viereck *et al.* 1986). The foodplains are topographically flat, and influenced by river meanderings and depositions on the active portion of the floodplain. Floodplain soils, not underlain by permafrost, are within the Salchaket series and are classified as Typic Cryofluvents (Viereck in press). Soil parent material is sandy to silty alluvium and soil profiles are well drained with little soil development. The largest floodplains in interior Alaska are the Yukon, Tanana and Kuskokwim. Flooding usually occurs twice yearly, during spring runoff and during midsummer when increased solar radiation causes glacier melting. Flooding is highly variable; its timing, intensity, and scale determine the overall pattern of floodplain colonization (Walker *et al.* 1986). Silt deposition on the river terraces delays the formation of permafrost by reducing the insulating effects of forest floors.

The upland successional patterns develop on rolling hills, where the parent material consists of a micaceous loess covering schist bedrock. Loess is deep, 30 m, in valleys and at the base of south sloping aspects but is much thinner on ridgetops. Here slope and aspect determine the microclimate: north slopes and valleys being the coldest and wettest, while south slopes are warm and dry. The

allogenic process responsible for the vegetation landscape patterns is fire. Upland, north aspects support the least productive forests, while south aspects and floodplain sites are associated with the most productive forests (Van Cleve and Viereck 1981; Viereck *et al.* 1983; Viereck *et al.* 1986).

The primary successional sequence of the Tanana River, Fig.1.1, has been described by Van Cleve and Viereck (1981), Viereck *et al.* (1983), Viereck *et al.* (1986) and Viereck (in press). This succession begins with the deposition of alluvium (stage I) and early stages (II and III) are dominated by *Hedysarum alpinum*, *Equisetum pratense*, *Calamagrostis canadensis*, willow (*Salix alaxensis* and *S. interior* being the most common), alder (*Alnus tenuifolia*), and balsam poplar (*Populus balsamifera*) in open shrub stands. Mid-succession continues from a closed shrub, stage IV, where dense stands of alder or willow occur, to a mature balsam poplar stand, stage VI. Early-late successional stages are VII and VIII, with white spruce (*Picea glauca*) mixed with the poplar and the later stage dominated by white spruce. Later stages, IX-XII, are found when permafrost formation begins.

Within each stage of the floodplain successional sequence, there are gradations of biogeochemical and physical factors (Van Cleve and Viereck 1981; Viereck *et al.* 1983; Viereck *et al.* 1986). The earliest stages are dominated by the physical and chemical environment; frequent flooding, high surface evaporation resulting in the formation of a $CaSO_4$ and $CaCO_3$ salt crust on the surface of the sandbar, high

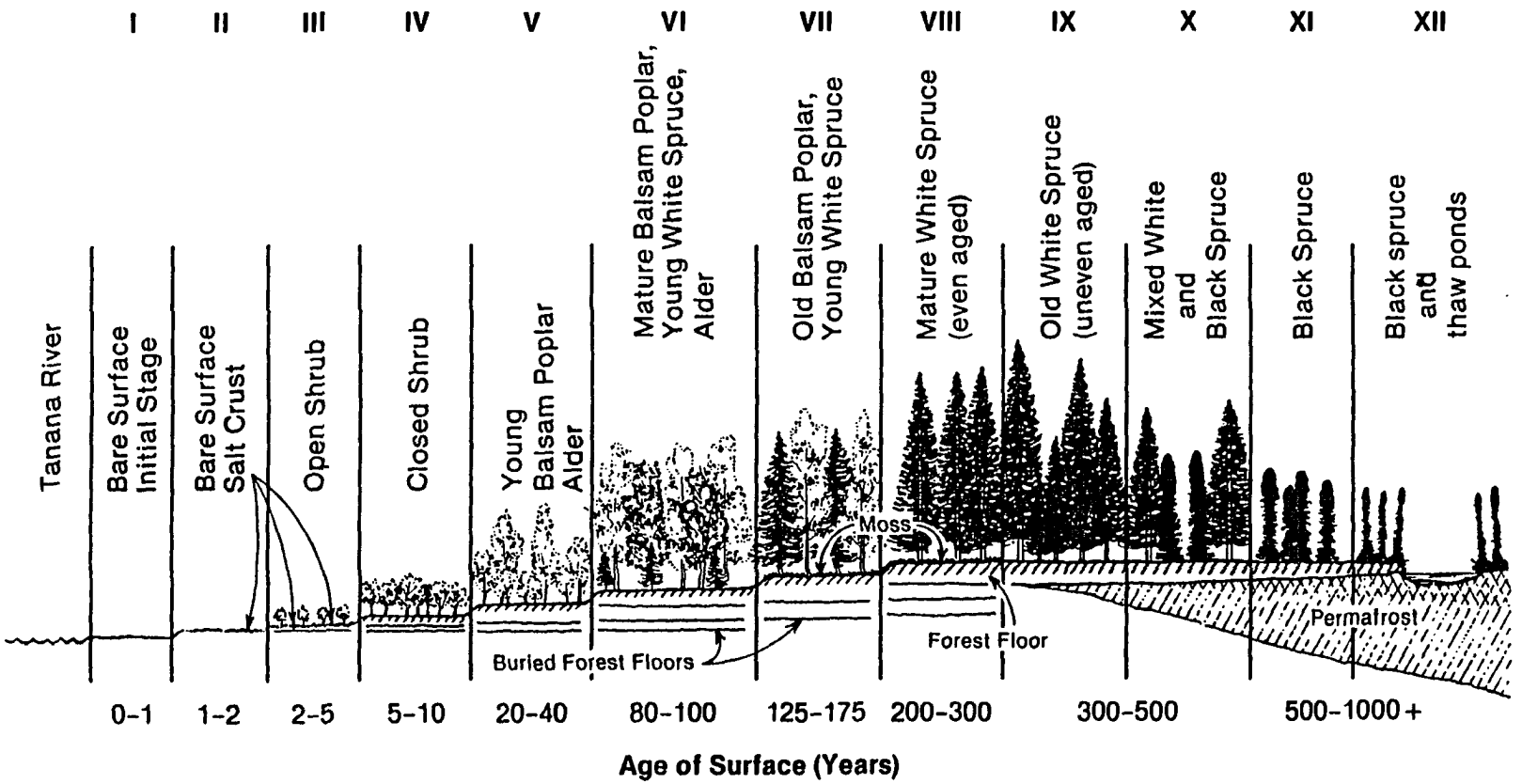

Fig. 1.1. Primary successional stages on the Tanana River floodplain of interior Alaska (Viereck in press).

pH, higher moisture content, and high soil temperatures (Fig. 1.2 and 1.3, and Table 1.1). Mid-successional stages become more biologically influenced with the establishment of plant communities. Capillarity may supply the ground water to the tree rooting zone so that the vegetation has adequate supplies of water throughout the growing season even during drought periods. Plant communities help to stabilize terrace development while also increasing terrace elevation, through the build-up of organic matter, and thus decreasing the likelihood of flooding. The soil nitrogen pool increases from about 50, at stage I, to 500 g m^{-2} at stage V due to nitrogen fixation by the alder and continues to increase in early mid-successional stages only to decline in later successional stages (Van Cleve *et al.* 1971; Van Cleve and Viereck 1981). In the late successional stages terrace elevation has steadily increased so that flooding is infrequent. Forest floor and mineral soil organic matter has also increased but with a higher content of lignin than in earlier successional stages and with an associated reduction in organic matter decomposition rates (Flanagan and Van Cleve 1983; Flanagan 1986). Soil temperatures decrease due to canopy closure, thicker forest floor, and an insulative moss layer, thus increasing the frost depth and delaying the spring thaw. These effects can decrease soil microbial activities, which play an important role in nutrient recycling and in soil fertility.

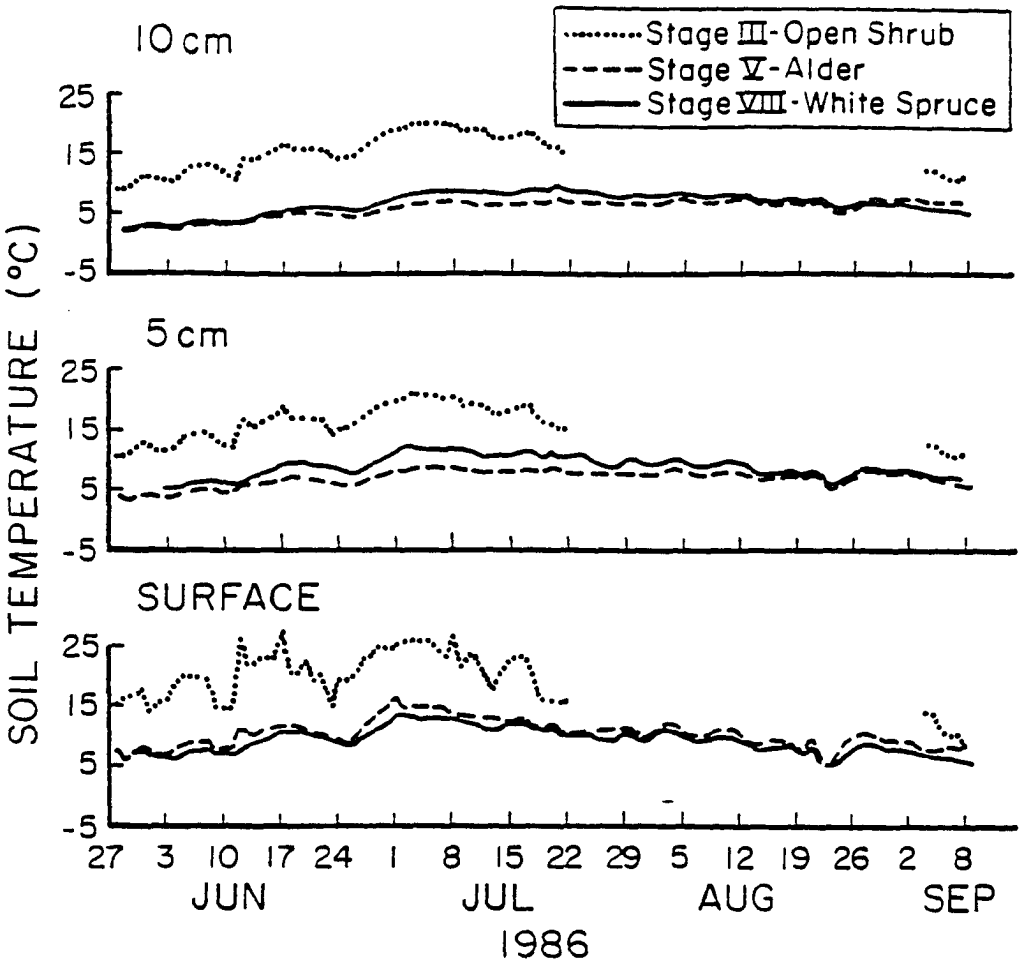

Fig. 1.2. Soil temperatures of the surface and at depths of 5 and 10 cm from the exposed surface of the open shrub stand (stage III), the alder stand (stage V), and the white spruce stand (stage VIII) (K. Van Cleve unpublished).

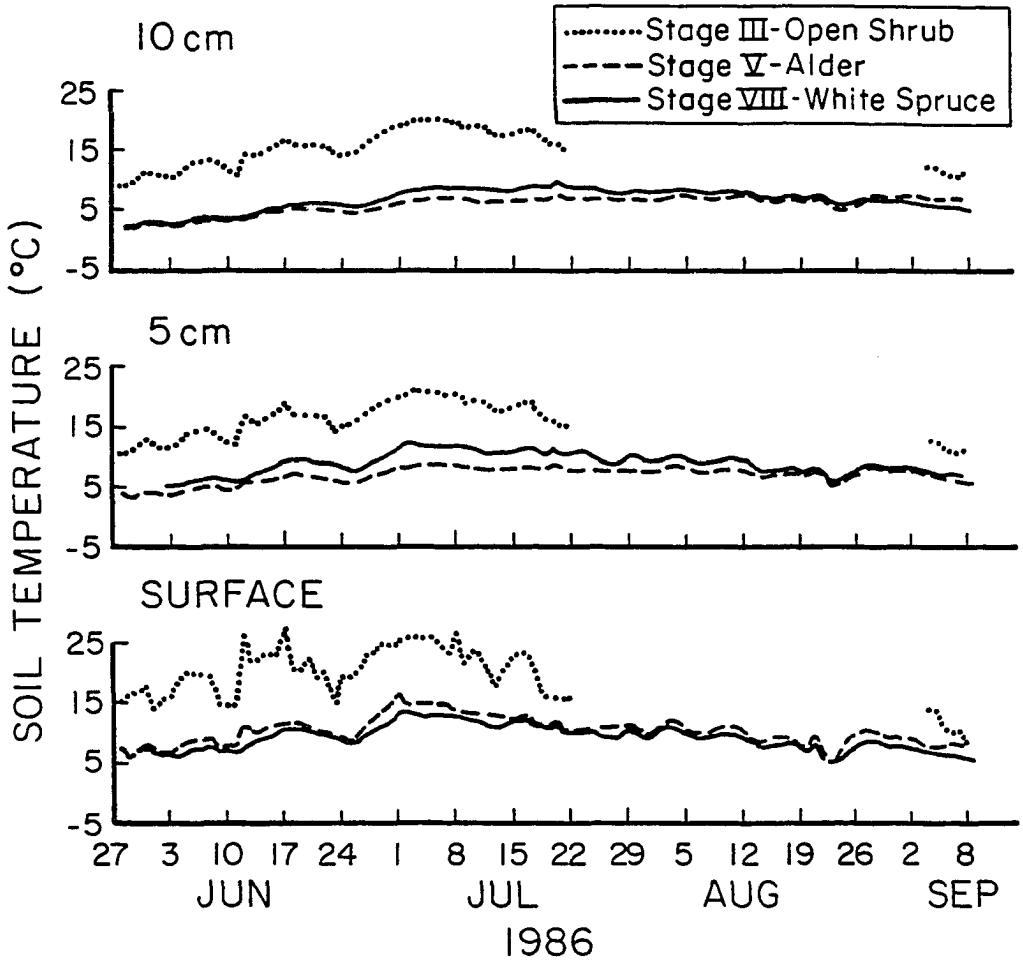

Fig. 1.3. Soil % moisture at depths of 5 and 10 cm from the exposed surface of the open shrub stand (stage III), alder stand (stage V), and the white spruce stand (stage VIII) (K. Van Cleve unpublished).

Table 1.1. General soil characteristics of open shrub (stage III), alder (stage V), and white spruce (stage VIII) stands.

Substrate	Total N %*	Total C %*	C/N*	Organic Matter %	pH	Bulk Density g / m^2*
Open Shrub Mineral Soil	0.03	1.7	48	1.2	7.36	59200
Alder Mineral Soil	0.19	3.3	16	8.2	7.56	42439
Alder Forest Floor	2.28	43.1	18	78.0	6.86	3628
White Spruce Mineral Soil	0.50	16.8	33	2.5	6.70	45843
White Spruce Forest Floor	0.80	36.8	44	44.7	6.68	3625

*K. Van Cleve (unpublished).

HYPOTHESIS

Reiners (1981) proposed a generalized scheme of nitrogen-cycling in successional sequences, suggesting a close relationship between nitrogen-cycle dynamics and successional development. Using a similar generalization, the following central hypothesis was developed as the focus of this study: nitrogen dynamics on the floodplain reflect the influence of forest succession. Because microbial populations responsible for the various nitrogen transformations are directly influenced by temperature, moisture, and quantity and quality of available substrates, a working hypothesis stating was developed: rates of N mineralization and denitrification will steadily increase from early sandbar stages to mid-successional stages following the general accumulation of organic matter and nitrogen but then decrease in older successional stages due to lower temperatures, lower moisture, and increased recalcitrance of forest floor and mineral soil organic matter. Nitrogen fixation would be expected to show the highest rates in early successional stages, although it may be important in later stages that have a high C/N soil ratio. The object of this study is to provide a test of this working hypothesis by measuring the rates of nitrogen fixation, mineralization, nitrification and denitrification in forest floor and mineral soils of selected successional stands. A further objective of this study is to understand the influence of temperature, moisture, and substrate

quantity and quality on nitrogen cycling processes. Laboratory experiments utilizing controlled temperature and moisture regimes, and substrate additions were used to test their effects on nitrogen processes.

STUDY AREA

The study area is located close to the Bonanza Creek Experimental Forest approximately 30 km southwest of Fairbanks and had been previously chosen as part of a larger multidisciplinary study, "The role of salt-affected soils in primary succession on the Tanana River floodplain of interior Alaska," in conjunction with the University of Alaska's Forest Soils Laboratory and the Institute of Arctic Biology, the USDA Forest Service Institute of Northern Forestry, and San Diego State University. Three of the twelve described successional stages, III, V, and VIII, were systematically chosen to represent early, mid-, and late succession and established as permanent plots. These are described as follows: stage III, a 5 yr old willow-poplar-alder (*Salix* spp.-*Populus balsamifera-Alnus tenuifolia*) open shrub stand; stage V, a 27 yr old alder (*Alnus tenufolia*) stand; and stage VIII, a 165 yr old white spruce (*Picea glauca*) stand. Each designated plot is an area of 50 m x 50 m. Transects running perpendicular to the river and spanning the width of each plot were randomly selected for this study.

Both the forest floor and mineral soil of the alder and white spruce stand were sampled and tested independently. The open shrub stand did not have a forest floor, thus only mineral soil was sampled and tested. Soil samples and other measurements of nitrogen processes were collected equidistantly along transects.

THESIS FRAMEWORK

This thesis is presented in chapter form, each chapter explaining a different experimental approach and describing the results of experiments used to test the working hypothesis. The chapters are organized as follows: the second chapter discusses nitrogen mineralization and nitrification, the third chapter characterizes the denitrification and nitrogen fixation results, while the fourth chapter discusses the major conclusions of the hypothesis testing.

CHAPTER 2

NITROGEN MINERALIZATION

ABSTRACT

Seasonal patterns of net NH_4^+ mineralization and nitrification in relatively undisturbed soil core sections were measured in open shrub, alder, and white spruce stands, representing early , mid- and late primary floodplain successional soils. The polyethylene bag technique was used to measure rates of N mineralization utilizing short-term incubations. Laboratory studies were designed to test the effects of temperature and moisture on N mineralization. The chlorate inhibition technique was used to estimate potential nitrification, NH_4^+ limitations, and potential heterotrophic nitrification, while the chloroform fumigation-incubation method was used to estimate a nitrogen availability index. Significant differences between the forest floor layer and the surface mineral soil layers were found. Mineral soil layers had low to undetectable rates of N mineralization and *in situ* concentrations of NH_4^+ and NO_3^-. Forest floors exhibited the highest *in situ* and accumulated NH_4^+ and NO_3^-, with the alder exhibiting the overall highest concentrations. The alder forest floor showed the most pronounced seasonal patterns of mineralization with greater net NH_4^+ mineralization occurring in the early summer, while highest rates of net nitrification were observed in late summer.

Biweekly estimates of net N mineralization, ug N·g dry soil^{-1}·d^{-1}, were as follows: the alder forest floor, <1-21; the white spruce forest floor, <1-2; the alder mineral soil, <1-3; and the white spruce and open shrub mineral soil, <1. The highest net nitrification, 2 ug N·g dry soil^{-1}·d^{-1}, was observed within the alder forest floor. Low to undetectable rates of net nitrification were observed in the white spruce forest floor and mineral soils tested. Laboratory studies indicate temperature as a limiting factor of N mineralization in the early and mid-successional stages, while moisture and temperature are limiting factors in the white spruce forest floor. Ammonium additions increased net nitrification only in the alder forest floor, suggesting NH_4^+ is not a limiting factor for nitrification in the other soils tested. The results of the chlorate inhibition assay indicate that a major portion of the nitrification in the alder forest floor is due to heterotrophic nitrification. The results of the chloroform fumigation-incubation method suggest that the alder forest floor has the highest N availability index.

INTRODUCTION

In undisturbed forested ecosystems, the mineralization of nitrogen-containing organic matter is the major source of available nitrogen for plant use. The process of nitrogen mineralization,

consisting of ammonification and nitrification, results in the accumulation of ammonium, the product of ammonification, and nitrite and nitrate, the products of nitrification. Ammonium is readily adsorbed onto the soil cation exchange sites, decreasing the likelihood of nitrogen loss through leaching. Nitrite rarely accumulates in soils. Both nitrite and nitrate are negatively charged and repelled by soil clay particles, and thus are easily leached and are also available to denitrifiers for conversion to N_2 gas.

The dominant form of mineral nitrogen in later successional stages is ammonium, with nitrate more abundant in early successional stages (Rice and Pancholy 1972; Robertson and Vitousek 1981; Robertson 1982; Gordon and Van Cleve 1983; Rice 1984; Christensen and MacAller 1985). From these observations, researchers have hypothesized that nitrification is either inhibited or limited in mature ecosystems. Some of the proposed mechanisms of this inhibition or limitation are allelopathy (Rice and Pancholy 1972; Rice 1984), physical and chemical soil factors such as temperature, moisture, pH, salinity, and recalcitrant organic material (Laura 1977; Focht and Verstraete 1977; Keeney 1980; Pastor et al. 1984; White and Gosz 1987), and limited availability of ammonium due to immobilization and/or slow rates of mineralization (Robertson and Vitousek 1981; Robertson 1982; Vitousek et al. 1982; Christensen and MacAller 1985). A major limitation to the understanding of the controlling factors of NH_4^+ mineralization and

nitrification are the indirect methods used to estimate these processes.

Ideally, measurements of NH_4^+ mineralization and nitrification should be made directly, but at present there are no known methods that measure mineralization rates accurately in the field. Most studies estimate changes in accumulated and *in situ* nitrogen pool sizes, in the presence and absence of active roots. The difference of these estimates equals the assumed plant uptake (Nadelhoffer *et al.* 1985 and Raison *et al.* 1987). Such methods do not estimate the turnover rates of nutrients or the actual plant uptake. There is also disturbance to the soil physical structure, moisture content, and rhizosphere structure which inadvertently affects the rates of nitrogen mineralization. Isotope tracer studies generally employ the addition of labelled ^{15}N substrate or product (Myrold and Tiedje 1986), also resulting in potential disturbance of the *in situ* mineralization rates and natural chemical gradients. Few studies have attempted to estimate soil nitrogen transformations using natural isotope abundances (Broadbent et al. 1980; Kohl and Shearer 1980; Binkley et al. 1985). Raison et al. (1987) suggests methods which minimize these effects, through the use of relatively undisturbed soil columns in polybags (Nadelhoffer *et al.* 1985; Federer 1983) or isolated soil columns within metal or plastic tubes (Adams and Attiwill 1986). Federer (1983) proposed measuring N mineralization

within distinct soil horizons as a better estimate of total soil mineralization.

Because the soil microbial biomass is responsible for the flow of carbon and nutrients through the soil, it has been postulated that the quantity of the microbial biomass is related to the amount of mineralized N available for plant uptake (Jenkinson and Ladd 1981; Shen *et al.* 1984; Voroney and Paul 1984; Brookes *et al.* 1985; Myrold 1987). Numerous definitions of soil nitrogen availability have been described (Stanford and Smith 1972; Keeney 1980; Powers 1980; Dalal and Mayer 1987). Soil nitrogen availability is generally defined as the quantity of soil organic nitrogen that is susceptible to mineralization, contributing to the N supplying capacity for a given soil. Because the soil microbial biomass is an important aspect of nutrient cycling, estimates of microbial C and N have been used as an index of N mineralization potential and have also been described as a useful index of soil fertility (Jenkinson and Powlson 1976; Jenkinson and Ladd 1981; Carter and Rennie 1982; Shen *et al.* 1984; Voroney and Paul 1984; Carter and Macleod 1987; Dalal and Mayer 1987; Myrold 1987).

Few studies have addressed the importance of heterotrophic nitrification. Most studies assume the accumulation of NO_3^- to be from autotrophic nitrification. Fungal nitrification has been acknowledged since 1896, and many heterotrophic organisms are known to oxidize various nitrogen compounds in culture (Focht and Verstraete 1977;

Schimel et al. 1984). Several studies have reported heterotrophic nitrification as an important source of NO_3^- in strongly acid soils, pH 4.5, (Tate 1977; Van de Dijk and Troelstra 1980; Adams 1986) and in a mature conifer forest (Schimel et al. 1984), but the extent and importance of heterotrophic nitrification in other ecosystems has not been addressed.

Although the taiga is one of the world's major vegetation regions, there is little information on the nitrogen-cycling processes within this environment. This study has proposed to measure net NH_4^+ mineralization and nitrification, in relatively undisturbed soil core sections, using the polyethylene bag technique of Eno (1960) and to examine seasonal patterns of these processes within the Tanana River floodplain primary successional sequence of interior Alaska. Sites chosen for the field work represented early, mid-, and late successional stages. Laboratory studies were designed to measure a nitrogen availability index and test the effects of temperature, moisture, and nitrogen limitation. An experiment was also completed to assay for potential heterotrophic nitrification.

MATERIALS AND METHODS

Study Area

The study area is located close to the Bonanza Creek Experimental Forest approximately 30 km southwest of Fairbanks, and had been previously chosen as part of a larger multidisciplinary study, "The role of salt-affected soils in primary succession on the Tanana River floodplain of interior Alaska," in conjunction with the University of Alaska's Forest Soils Laboratory and the Institute of Arctic Biology, the USDA Forest Service Institute of Northern Forestry, and San Diego State University. Three of the twelve described successional stages, III, V, and VIII, were systematically chosen to represent early, mid-, and late succession and established as permanent plots. These are described as follows: stage III, a 5 yr old willow-poplar-alder (*Salix* spp.-*Populus balsamifera*-*Alnus tenuifolia*) open shrub stand; stage V, a 27 yr old alder (*Alnus tenuifolia*) stand; and stage VIII, a 165 yr old white spruce (*Picea glauca*) stand. Each designated plot is an area of 50 m x 50 m. Transects running perpendicular to the river and spanning the width of each plot were randomly selected for this study. Both the forest floor and mineral soil of the alder and white spruce stands were sampled and tested independently. The open shrub stand did not have a forest floor, thus only mineral soil was sampled and tested at this location. Soil samples were collected equidistantly along

selected transects with a 15 cm diameter soil corer to an approximate depth of 12-15 cm.

Laboratory and Field Methods

Net NH_4^+ mineralization and nitrification were measured using undisturbed soil core sections incubated in polyethylene bags (Eno 1960). Eight soil cores were collected equidistantly along the width of each plot on a randomly chosen transect. Each core was vertically sliced into two sections, one half designated T_0 while the other half was designated T_1. The surface forest floor was left as a distinct layer while the mineral soils were further cut into three horizontal layers, each approximately 3 cm thick. Each soil core section was then placed with as little disturbance as possible into a 0.4 mil polyethylene bag. The samples designated T_0 were brought to the laboratory and frozen that same day, until further chemical analysis could be performed. The T_1 soil core sections were placed in previously made core holes, at each respective depth of sampling, for the duration of the incubation period.

During the first year of the study, incubation times were approximately one month, while during the second year, incubation periods were for two weeks. At the end of each incubation period, T_1 soil core sections were collected, brought to the laboratory, and frozen until chemical analysis could be completed. A new set of soil

cores were processed in a similar fashion, at the end of each incubation period and repeated throughout both summers.

In the laboratory, thawed core sections were separately mixed, large roots removed, and a 15 g fresh weight subsample of each section was extracted with 75 ml 2 N KCl, shaken for 1 h, and then filtered. The filtrate was then analyzed for NH_4^+, using a phenol hypochlorite assay, and NO_3^-, using a Griess-Illosvay method in combination with a Cd reducing column, on a modified Technicon AA II system (Technicon 1973). Dry weights were determined by oven drying for 48 h at 65°C for forest floors and 105°C for mineral soils. Organic matter was determined by ashing subsamples at 400°C for 7 h.

Net nitrification was calculated as the difference in the measured NO_3^- concentrations between T_1 samples (incubated in the field for two weeks) and T_0 samples (*in situ* concentrations, no incubation), both collected on the same sampling date. Net NH_4^+ mineralization was the difference in measured NH_4^+ T_1 and T_0 concentrations plus the net nitrification estimate. Cumulative rates of net N mineralization (net nitrification plus net NH_4^+ mineralization) measured at two week intervals during the 1985 and 1986 field season, were used as an estimate of an average rate.

In September 1986, ten soil cores were collected equidistantly along a randomly selected transect from each site and kept at 4°C until laboratory studies were begun in mid-October. The soil cores were made into a composite mixture, separating the forest floor and the mineral soil of each site.

From each soil composite, quadruplicate subsamples were taken for a factorial (incomplete) temperature and moisture experiment. Subsamples were 100 g fresh weight, for all mineral soils, 20 g fresh weight for alder forest floor, and 50 g fresh weight for white spruce forest floor. Two moisture regimes, the moisture percent at the time of sampling and distilled water added to the water holding capacity of each soil, and four temperatures, 5, 10, 20, and 30°C, were used to test for moisture and temperature effects on mineralization rates. Water holding capacity had been previously determined by saturating soil samples with distilled water for 24 h with subsequent drainage for 24 h (Table 2.1). Soils were placed in plastic 250 ml beakers, covered with a sheet of 0.4 mil polyethylene, and incubated for 20 d at each moisture and temperature. At the end of the incubation period a 15 g fresh weight subsample was removed from each sample, and analyzed for NH_4^+ and NO_3^-, as described previously.

Table 2.1. September 1986 *in situ* and water holding capacity moisture estimates.

	% Moisture	
Substrate	*In Situ*	Water Holding Capacity
Open Shrub Mineral Soil	26.1 ± 4.8	34.1 ± 6.0
Alder Mineral Soil	32.6 ± 5.4	58.6 ± 7.5
Alder Forest Floor	148.2 ± 33.9	339.4 ± 56.8
White Spruce Mineral Soil	6.9 ± 2.3	40.2 ± 3.9
White Spruce Forest Floor	54.7 ± 7.4	243.9 ± 42.6

NOTE: Means ± SE, n=10.

The chloroform fumigation-incubation method, following Jenkinson and Powlson (1976), was carried out using quadruplicate subsamples of each soil composite. In September, 20 g fresh weight of mineral soil and 10 g fresh weight of forest floor were used. Subsamples, with large roots removed, were placed in 50 ml beakers, inside a vacuum desiccator lined with paper towels. Chloroform was placed in a 50 ml beaker at the middle of the desiccator. The desiccator was sealed and placed inside a laboratory hood and evacuated with a vacuum pump, until the chloroform began to boil vigorously for approximately 2-3 minutes, at which time the pump was turned off. The samples were allowed to sit, saturated with chloroform vapor for 24 h. At the end of this time, the desiccator was vacuum-air flushed 5 times in order to remove the $CHCl_3$ vapor. Samples were removed from the desiccator, placed in quart size, wide mouth canning jars, and reinnoculated with 0.5 g of fresh forest floor or mineral soil, respectively. The canning jars were sealed with lids equipped with rubber septa for gas sampling. At the same time unfumigated samples of the test materials were placed in canning jars and treated in similar fashion, serving as controls. In order to keep samples from moisture loss, both the controls and fumigated samples had an additional 5 ml of distilled H_2O added at the begining of the 10-d incubation period. The jars were incubated at room temperature.

Evolved CO_2 was measured on day 2, 3, 4, 5, 6, 9, and 10. The headspace of each jar was sampled with a 5 ml evacuated glass tube and stored for approximately one week at 5°C until further analysis could be completed. Carbon dioxide was measured on a portable Analytical Instrument Development Inc. gas chromatograph, equipped with a 6 ft Poropak Q column with column and detector temperature at 50°C. Biomass C was derived from the relationship $B_c = F_c/K_c$; where F_c = [(CO_2-C evolved from fumigated soil during the 10-d incubation) - (CO_2-C evolved from the control during the 10-d incubation)] and K_c=0.45, the proportion of microbial C evolved as CO_2 for a 10-d incubation at 25°C (Jenkinson and Powlson 1976; Jenkinson and Ladd 1981).

The N flush, 2 N KCl extractable NH_4^+, was measured at the end of the 10-d incubation for both fumigated and unfumigated soil samples. Microbial biomass N, B_n, was derived using the equation $B_n=F_n/K_n$, where F_n=[(N mineralized by a fumigated soil for a 10-d incubation period) - (N mineralized by an unfumigated soil for a 10-d period)]/K_n, the proportion of the soil biomass N that is mineralized, ranging from 0.32 to 0.68 (Adams and Laughlin 1981; Jenkinson and Ladd 1981; Shen et al. 1984; Voroney and Paul 1984; Brookes et al. 1985). A K_n of 0.68 (Shen et al. 1984) was used to determine B_n.

A factorial experiment (incomplete) was also used to measure potential autotrophic and heterotrophic nitrification, and to test NH_4^+ limitation of nitrification using the chlorate inhibition assay of Belser and Mays (1980). This procedure uses chlorate to inhibit the

chemoautotrophic oxidation of nitrite to nitrate, with subsequent accumulation of nitrite when autotrophic nitrification is occurring. Heterotrophic nitrification is unaffected by chlorate, and when occurring, nitrate will accumulate in the presence of chlorate. Quadruplicate 15 g fresh weight subsamples were taken from each soil composite (collected in September 1986 as described above), and separately placed in an erlenmeyer flask with the addition of a treatment. Five treatments were used: A, control; B, 50 ml distilled water; C, 15 ug NH_4^+-N g^{-1} soil; D, 0.5 ml of 1 M $NaClO_3$; E, 15 ug NH_4^+-N g^{-1} soil and 0.5 ml of 1 M $NaClO_3$. Samples were incubated for 24 h on a rotary shaker at room temperature. Ammonium, nitrate and nitrite were measured on KCl extracted soil samples using the previously described methods.

Analyses of variance, using both parametric and nonparametric statistical tests (Zar 1984) were performed utilizing the Statistical Analysis System (SAS 1985). Due to the small sample size, n = 4, 5, or 8, and thus the inability to test reliably for normality and equal variances, the nonparametric and parametric tests were used for comparison. All parametric and nonparametric tests were in agreement (accepting or rejecting the null hypothesis). Scheffe's multiple range test (Zar 1984; SAS 1985) was used to detect significant differences in estimates of nitrogen processes. Ammonium, nitrite, and nitrate were treated separately for each statistical test.

RESULTS

Results of *in situ*, T_0, NH_4^+ and NO_3^- concentrations with depth for both the 1985 and 1986 field season are shown in Table 2.2. The open shrub, stage III, has no forest floor, therefore each core was separated into four mineral soil layers. The open shrub mineral soil layers generally showed no depth variation of NH_4^+ or NO_3^- during both years. There were significant differences ($P<0.05$) between the alder forest floor and the alder mineral soil layers at most sampling periods. Generally, the alder mineral soil layers showed no depth variation in either ammonium or nitrate concentrations. In the white spruce stand, significant differences in NH_4^+ were observed between the forest floor and the mineral soil layers. In all cores the greatest NH_4^+ concentrations were found in the forest floor, while the alder forest floor exhibited the highest concentrations of both NH_4^+ and NO_3^-. In general, the mineral soil layers showed no variation of either NH_4^+ or NO_3^- with depth. There was low to undetectable amounts of NO_3^- in the white spruce forest floor or mineral soil layers.

The depth distribution of organic matter was significantly different within the alder and white spruce core layers. The open shrub core layers showed no organic matter depth variation. The alder forest floor layer was significantly different from its mineral soil layers (Table 2.3). The white spruce stand exhibited significant

Table 2.2. *In situ*, T_0, concentrations of NH_4^+ and NO_3^- in soil layers of open shrub (stage III), alder (stage V), and white spruce (stage VIII) cores.

		ug N / g dry soil					
Sampling		Open Shrub		Alder		White Spruce	
Date	Layer	NH_4^+	NO_3^-	NH_4^+	NO_3^-	NH_4^+	NO_3^-
1985							
Jul 11	1	0.32 ± 0.09^a	0.03 ± 0.01^a	47.33 ± 4.64^a	4.63 ± 0.48^a	32.15 ± 4.56^a	0.63 ± 0.18^a
	2	0.17 ± 0.01^a	ND^a	1.63 ± 0.20^b	0.49 ± 0.04^b	3.10 ± 0.59^b	0.12 ± 0.01^a
	3	ND^a	ND^a	0.78 ± 0.38^b	0.16 ± 0.01^b	10.86 ± 1.59^b	0.18 ± 0.03^a
	4	ND^a	ND^a	0.19 ± 0.02^b	0.14 ± 0.01^b	1.50 ± 0.08^b	0.05 ± 0.01^a
Jul 26	1	0.63 ± 0.05^a	0.07 ± 0.01^a	41.33 ± 4.03^a	7.90 ± 0.57^a		
	2	0.31 ± 0.03^a	0.07 ± 0.03^a	1.70 ± 0.34^b	3.20 ± 0.61^b	No Data	
	3	0.03 ± 0.01^b	0.03 ± 0.01^a	0.41 ± 0.17^b	0.93 ± 0.13^c		
	4	ND^b	ND^a	0.06 ± 0.01^b	0.93 ± 0.13^c		

NOTE: Layers are soil depth increments. Layer 1 of the alder and white spruce stand is forest floor, while layers 2, 3, and 4 are subsequent mineral soil layers. The open shrub layers are all mineral soil, approximately 3 cm thick. The open shrub layer 1 is the surface 0-3 cm. Means ± SE, n=4 for 1985 and n=8 for 1986. Values of each sampling date, in columns, followed by the same letter are not significantly different ($P>0.05$). ND=not detected.

Table 2.2. (continued).

Sampling Date	Layer	ug N / g dry soil: Open Shrub NH_4^+	Open Shrub NO_3^-	Alder NH_4^+	Alder NO_3^-	White Spruce NH_4^+	White Spruce NO_3^-
1985							
Aug 6	1	1.45±0.60^a	2.52±0.59^a	42.00±4.03^a	1.73±0.31^a	6.85±1.24^a	1.97±0.13^a
	2	1.09±0.34^a	2.02±0.22^a	23.24±2.81^b	0.27±0.04^b	1.46±0.23^a	1.22±0.08^a
	3	0.81±0.23^a	1.30±0.24^a	9.75±0.46^{bc}	0.05±0.01^b	4.03±0.40^a	0.85±0.15^a
	4	0.40±0.07^a	1.22±0.21^a	6.74±0.59^c	0.04±0.03^b	3.17±1.25^a	0.87±0.22^a
Aug 20	1	0.88±0.26^a	0.07±0.02^a	51.00±3.92^a	0.45±0.17^a	24.57±2.48^a	1.57±0.07^a
	2	0.22±0.14^a	0.03±0.01^a	3.50±1.36^b	ND^b	4.20±0.22^b	2.02±0.83^a
	3	1.65±0.32^a	0.03±0.02^a	1.73±0.55^b	ND^b	3.02±0.67^b	0.87±0.28^a
	4	0.63±0.10^a	0.03±0.01^a	0.49±0.47^b	ND^b	0.49±0.05^b	0.07±0.03^a
Sep 16	1	1.45±0.17^a	0.19±0.006^a	43.80±4.77^a	71.25±24.9^a	26.50±1.70^a	0.03±0.01^a
	2	0.81±0.05^a	0.10±0.04^a	12.80±1.06^a	0.91±0.20^b	8.50±0.95^{bc}	0.02±0.01^a
	3	1.09±0.23^a	0.09±0.02^a	12.25±2.48^a	0.40±0.04^b	18.25±2.99^b	0.06±0.02^a
	4	0.40±0.04^a	0.04±0.01^a	9.79±1.58^a	0.34±0.02^b	2.97±0.39^c	0.01±0.007^a
1986							
May 25	1	2.03±0.45^a	ND^a	213.25±33.72^a	11.75±4.71^a	50.66±4.08^a	0.23±0.01^a
	2	1.12±0.24^a	0.05±0.01^a	3.50±0.52^b	0.05±0.04^a	12.35±0.58^b	0.14±0.003^c
	3	4.18±0.75^a	0.78±0.06^a	1.73±0.38^b	0.10±0.05^a	22.13±1.45^b	0.18±0.003^a
	4	0.05±0.02^a	ND^a	2.33±0.85^b	0.10±0.05^a	4.15±0.18^b	0.10±0.012^c

Table 2.2. (continued).

Sampling Date	Layer	ug N / g dry soil: Open Shrub NH_4^+	Open Shrub NO_3^-	Alder NH_4^+	Alder NO_3^-	White Spruce NH_4^+	White Spruce NO_3^-
1986							
Jun 10	1	2.32±0.25^a	0.04±0.01^a	268.75±34.64^a	47.12±13.70^a	12.61±1.55^a	0.08±0.01^a
	2	1.16±0.26^a	0.02±0.004^a	28.38±1.22^b	0.46±0.23^b	13.87±1.63^a	0.07±0.01^a
	3	0.53±0.09^a	0.01±0.004^a	6.13±0.95^b	0.11±0.04^b	12.37±0.80^a	0.05±0.01^a
	4	0.76±0.24^a	0.01±0.005^a	4.38±0.29^b	0.17±0.02^b	3.12±0.47^a	0.06±0.02^a
Jun 25	1	1.42±0.20^a	ND^a	100.63±16.06^a	16.51±1.72^a	31.62±2.26^a	0.19±0.01^a
	2	0.85±0.15^a	ND^a	3.13±2.90^b	0.15±0.01^b	6.87±0.44^b	0.11±0.008^a
	3	0.85±0.19^a	ND^a	1.34±0.15^b	0.04±0.01^b	11.25±0.62^b	0.14±0.009^a
	4	0.04±0.01^a	ND^a	0.33±0.06^b	0.06±0.01^b	2.09±0.10^b	0.12±0.01^a
Jul 15	1	0.67±0.10^a	ND^a	100.63±6.58^a	32.50±2.42^a	40.33±4.25^a	0.25±0.08^a
	2	1.23±0.29^a	ND^a	54.25±2.84^b	0.36±0.03^b	3.61±1.01^b	0.03±0.007^b
	3	0.62±0.10^a	ND^a	42.75±2.44^b	0.31±0.04^b	10.14±2.05^b	0.05±0.001^b
	4	0.20±0.04^a	ND^a	41.13±2.47^b	0.30±0.07^b	0.86±0.28^b	0.04±0.01^b
Jul 28	1	0.92±0.08^a	ND^a	68.75±3.30^a	9.50±0.48^a	26.62±2.88^a	ND^a
	2	0.82±0.06^a	ND^a	6.37±1.04^b	0.96±0.09^b	2.82±0.30^b	ND^a
	3	0.61±0.06^a	ND^a	6.30±1.47^b	0.35±0.05^b	5.09±0.46^b	ND^a
	4	0.77±0.06^a	ND^a	4.12±1.70^b	0.25±0.02^b	0.21±0.02^b	ND^a

Table 2.2. (continued).

		ug N / g dry soil					
Sampling		Open Shrub		Alder		White Spruce	
Date	Layer	NH_4^+	NO_3^-	NH_4^+	NO_3^-	NH_4^+	NO_3^-
1986							
Aug 5	1	7.08±0.27[a]	0.01±0.008[a]				
	2	2.67±0.37[b]	ND[a]	No Data		No Data	
	3	1.64±0.16[b]	ND[a]				
	4	0.76±0.07[b]	ND[a]				
Aug 14	1	3.10±0.15[a]	0.22±0.01[a]	16.37±2.11[a]	67.05±11.79[a]	4.45±0.66[b]	ND[a]
	2	2.64±0.43[a]	0.14±0.01[a]	3.61±0.67[b]	0.06±0.01[b]	1.05±0.14[b]	ND[a]
	3	2.04±0.41[a]	0.03±0.02[b]	2.37±0.31[b]	0.03±0.002[b]	15.71±2.28[a]	0.38±0.33[a]
	4	3.10±0.08[a]	0.02±0.006[b]	3.28±2.13[b]	0.01±0.003[b]	0.92±0.06[b]	ND[a]
Aug 28	1	5.42±0.42[a]	0.88±0.25[a]	93.13±7.52[a]	73.75±10.10[a]	46.62±4.01[a]	ND[a]
	2	3.64±0.48[a]	0.10±0.01[a]	7.13±0.75[b]	0.95±0.16[b]	7.20±0.75[b]	ND[a]
	3	4.33±0.34[a]	4.50±0.83[a]	3.50±0.21[b]	0.39±0.01[b]	40.62±2.41[a]	ND[a]
	4	2.92±0.23[a]	4.31±0.40[a]	2.39±0.27[b]	0.43±0.07[b]	8.75±1.00[b]	ND[a]
Sep 9	1	15.77±1.33[a]	0.19±0.09[a]	19.87±1.74[a]	307.25±37.75[a]	7.24±0.90[a]	ND[a]
	2	16.22±1.59[a]	0.05±0.01[b]	3.12±0.40[b]	0.18±0.01[b]	0.79±0.18[a]	ND[a]
	3	7.50±0.64[a]	ND[b]	1.95±0.26[b]	0.10±0.005[b]	8.57±1.31[a]	ND[a]
	4	1.27±0.53[a]	0.09±0.002[b]	0.80±0.12[b]	0.07±0.017[b]	0.42±0.09[a]	ND[a]

Table 2.3. Soil organic matter percent at the depths of the four soil layers sampled.

	% Organic Matter		
Layer	Open Shrub	Alder	White Spruce
1	1.37±0.18[a]	75.62±4.05[a]	64.87±3.68[a]
2	1.43±0.28[a]	8.37±2.07[b]	7.87±2.10[c]
3	0.85±0.05[a]	4.00±1.19[b]	40.34±6.10[b]
4	0.84±0.05[a]	2.59±0.50[b]	5.12±1.91[c]

NOTE: Means ±SE where n=8. Values in each column followed by the same letter are not significantly different (P>0.05).

differences between the forest floor, a buried organic layer, and mineral soil layers.

The 1985 and 1986 seasonal patterns of net NH_4^+ mineralization and net nitrification are shown in Fig. 2.1 and Tables 2.4-2.8. In general, the alder forest floor showed the greatest seasonal variations and the highest rates of N mineralization. Highest rates of net NH_4^+ mineralization, T_1, and *in situ* NH_4^+ concentrations, T_0, were observed in early and mid-summer, whereas highest rates of net nitrification and *in situ* NO_3^- concentrations were observed in late summer. Seasonal variation of net NH_4^+ mineralization was not pronounced in the white spruce forest floor or in any of the mineral soils. Net nitrification was low to undetectable in the white spruce forest floor and all mineral soils. All forest floors and mineral soils exhibited periods of immobilization of NH_4^+ and NO_3^-. Analysis of variance tests indicated that within the mineral soil layers of the open shrub, alder, and white spruce there was no significant depth variation of the layers observed. Therefore, mineral soil net NH_4^+ mineralization and net nitrification estimates at each site are the pooled means of the three mineral soil layers of each core (Fig. 2.1). The lowest N mineralization rates were observed in the mineral soils. The largest pools of nitrogen were observed in the alder mineral soil, due to the high bulk density of the mineral soil. Although rates of net N mineralization were similar for both the alder forest floor and

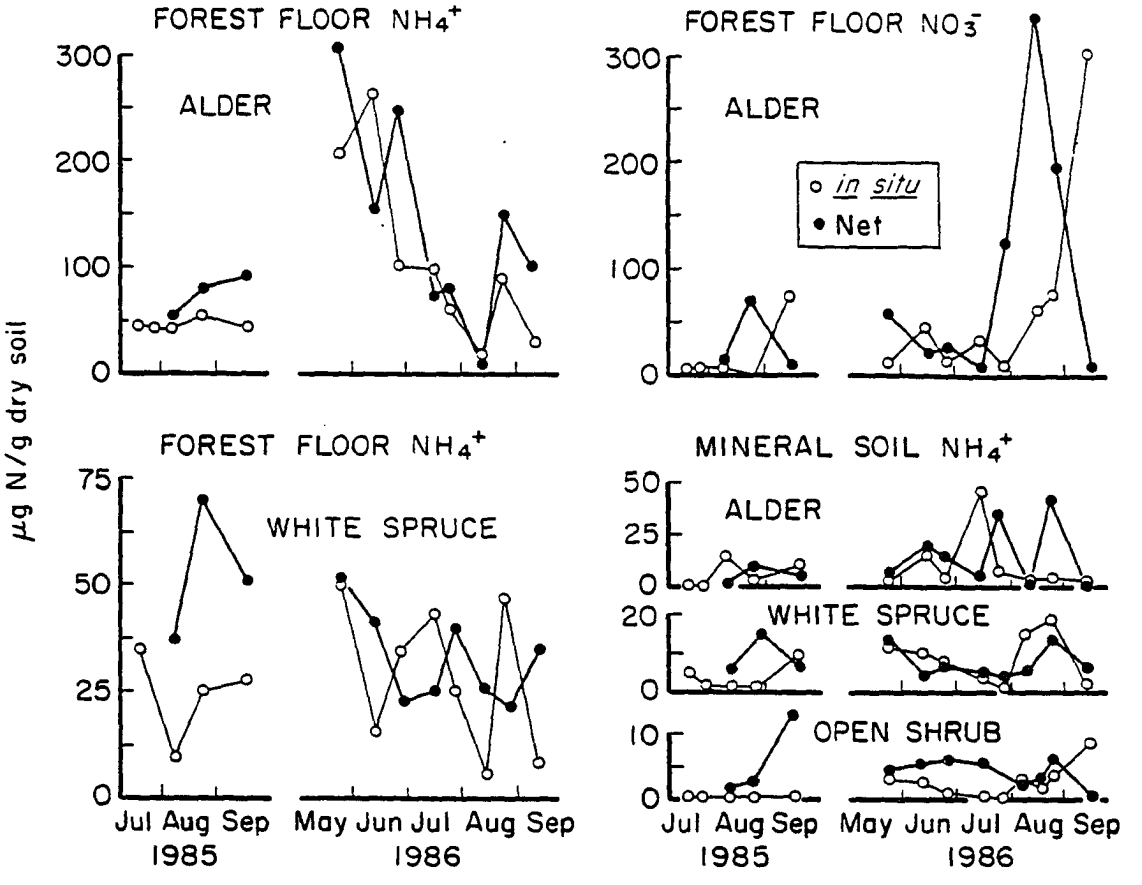

Fig. 2.1. Seasonal patterns of *in situ*, T_0, NH_4^+ and NO_3^- and accumulated, T_1, NH_4^+ and NO_3^- concentrations.

Table 2.4. Open shrub mineral soil concentrations of accumulated, T_1, NH_4^+ and NO_3^- and estimates of net nitrification (NO_3^-) and N mineralization ($NH_4^+ + NO_3^-$).

Sampling Date	ug N / g dry soil Accumulated NH_4^+	ug N / g dry soil Accumulated NO_3^-	ug N / g dry soil · d Net NO_3^-	ug N / g dry soil · d Net $NO_3^- + NH_4^+$
1985				
6 Aug	1.5±0.1	0.030±0.006	-0.161	-0.090
20 Aug	2.8±0.4	0.105±0.040	-0.006	0.038
18 Sep	13.1±2.3	0.154±0.019	0.0004	0.052
1986				
27 May	2.5±0.8	0.037±0.006	0.001	0.045
10 Jun	4.3±0.8	ND	ND	0.020
25 Jun	3.6±0.8	ND	ND	0.139
15 Jul	3.3±0.4	ND	ND	0.203
28 Jul	0.9±0.1	ND	ND	0.015
5 Aug	2.2±0.9	0.030±0.004	0.003	0.082
14 Aug	2.8±0.2	ND	-0.008	0.094
28 Aug	5.2±0.6	ND	-0.085	-0.027
9 Sep	0.8±0.6	ND	-0.005	-0.074
Average g N / ha · d			-18	24

NOTE: Estimates are means ± SE, n=8. ND=not detected.

Table 2.5. Alder mineral soil concentrations of accumulated, T_1, NH_4^+ and NO_3^- and estimates of net nitrification (NO_3^-) and N mineralization ($NH_4^+ + NO_3^-$).

Sampling Date	ug N / g dry soil Accumulated NH_4^+	Accumulated NO_3^-	ug N / g dry soil · d Net NO_3^-	Net $NO_3^- + NH_4^+$
1985				
6 Aug	4.7±1.3	0.02±0.01	0.007	-0.740
20 Aug	9.9±2.1	0.60±0.08	0.020	0.280
18 Sep	6.1±2.1	0.34±0.21	-0.0008	-0.023
1986				
27 May	5.4±5.0	0.19±0.11	0.007	0.188
10 Jun	15.6±7.0	1.21±0.97	0.063	0.208
25 Jun	9.0±6.1	0.17±0.15	0.004	0.358
15 Jul	6.6±4.3	0.24±0.19	-0.006	-3.071
28 Jul	39.6±6.7	1.76±0.41	0.067	1.95
14 Aug	0.8±0.5	3.48±0.82	0.246	0.63
28 Aug	44.5±5.7	2.07±1.92	1.182	3.43
9 Sep	1.7±1.2	0.34±0.15	0.019	0.056
Average g N / ha · d			61	125

NOTE: Estimates are means ± SE, n=8.

Table 2.6. Alder forest floor concentrations of accumulated, T_1, NH_4^+ and NO_3^- and estimates of net nitrification (NO_3^-) and N mineralization ($NH_4^+ + NO_3^-$).

Sampling Date	ug N / g dry soil Accumulated NH_4^+	ug N / g dry soil Accumulated NO_3^-	ug N / g dry soil · d Net NO_3^-	ug N / g dry soil · d Net $NO_3^- + NH_4^+$
1985				
6 Aug	66.8±3.9	1.7±0.3	0.84	2.87
20 Aug	82.0±27.4	0.6±0.1	2.40	3.45
18 Sep	98.3±35.0	74.3±24.9	- 0.27	-0.04
1986				
27 May	305.7±34.6	12.1±4.6	2.64	9.25
10 Jun	154.1±5.6	47.5±4.7	-1.94	-9.54
25 Jun	247.4±14.0	13.9±1.7	0.55	7.84
15 Jul	82.5±3.9	33.0±2.4	-2.00	-3.38
28 Jul	89.9±4.9	10.0±0.4	6.36	7.51
14 Aug	14.8±2.4	67.2±1.1	21.42	21.27
28 Aug	140.9±9.8	74.6±10.1	10.21	14.16
9 Sep	102.3±6.4	307.6±37.6	-27.29	-19.82
Average g N / ha · d			37	111

NOTE: Estimates are means ± SE, n=8.

Table 2.7. White spruce mineral soil concentrations of accumulated, T_1, NH_4^+ and NO_3^- and estimates of net nitrification (NO_3^-) and N mineralization ($NH_4^+ + NO_3^-$).

Sampling Date	ug N / g dry soil Accumulated NH_4^+	ug N / g dry soil Accumulated NO_3^-	ug N / g dry soil · d Net NO_3^-	ug N / g dry soil · d Net $NO_3^- + NH_4^+$
1985				
6 Aug	7.6±3.4	2.01±1.05	0.085	0.484
20 Aug	16.1±2.4	0.08±0.02	-0.031	0.438
18 Sep	9.7±4.6	0.08±0.05	0.0002	-0.001
1986				
27 May	13.4±6.0	0.28±0.27	0.009	0.053
10 Jun	5.7±3.2	0.12±0.11	0.003	-0.029
25 Jun	7.1±5.7	0.19±0.06	0.003	0.007
15 Jul	5.4±3.5	0.09±0.06	0.003	0.043
28 Jul	4.0±3.5	ND	ND	0.073
14 Aug	5.9±1.1	ND	ND	-0.702
28 Aug	16.2±2.7	ND	ND	-0.260
9 Sep	6.3±3.7	ND	ND	0.300
Average g N / ha · d			3	16

NOTE: Estimates are means ± SE, n=8. ND=not detected.

Table 2.8. White spruce forest floor concentrations of accumulated, T_1, NH_4^+ and NO_3^- and estimates of net nitrification (NO_3^-) and N mineralization ($NH_4^+ + NO_3^-$).

Sampling Date	ug N / g dry soil Accumulated NH_4^+	ug N / g dry soil Accumulated NO_3^-	ug N / g dry soil · d Net NO_3^-	ug N / g dry soil · d Net $NO_3^- + NH_4^+$
1985				
6 Aug	35.1±5.2	2.67±0.37	0.03	0.27
20 Aug	73.6±20.3	0.21±0.03	-0.04	1.62
18 Sep	52.4±3.9	0.15±0.02	0.000	0.10
1986				
27 May	52.0±3.9	0.25±0.01	0.001	0.09
10 Jun	40.5±6.3	0.23±0.01	0.009	1.83
25 Jun	24.8±1.5	0.36±0.05	0.008	0.34
15 Jul	25.7±1.3	0.08±0.02	-0.01	1.16
28 Jul	39.7±3.5	0.01±0.04	-0.008	0.80
14 Aug	26.5±3.7	ND	ND	1.56
28 Aug	22.3±2.5	ND	ND	2.06
9 Sep	35.2±3.8	ND	ND	2.54
Average g N / ha · d			-0.1	25

NOTE: Estimates are means ± SE, n=8. ND=not detected.

the alder mineral soil, the alder mineral soil had the highest net nitrification rates.

The results of the temperature and moisture factorial experiment are shown in Fig. 2.2. Among forest floors and mineral soils, mineralization rates were similar at the two moisture regimes, in situ and water holding capacity, except at higher temperatures. The alder forest floor and white spruce forest floors were significantly different from each other and all mineral soils. The mineral soils had similar N mineralization rates. Within mineral soils, similar mineralization rates were observed at 5, 10, and 20°C, but rates at 30°C were the highest. Only the white spruce forest floor at water holding capacity showed higher net nitrification rates at a temperature other than 30°C. An increase in moisture did increase net NH_4^+ mineralization and net nitrification rates in the white spruce forest floor, while only affecting net NH_4^+ mineralization rates in the white spruce mineral soil. There were no significant moisture effects on mineralization rates in the alder forest floor or open shrub mineral soil, or with net NH_4^+ mineralization in the alder mineral soil.

Daily measurements of evolved CO_2-C ug·g dry $soil^{-1}$ on chlorform fumigated and unfumigated, control, (Fig. 2.3 and 2.4) indicated greater respiration rates in the forest floors than any of the mineral soils, with the alder forest floor sample showing the highest amounts of evolved CO_2. The alder mineral soil control sample had higher

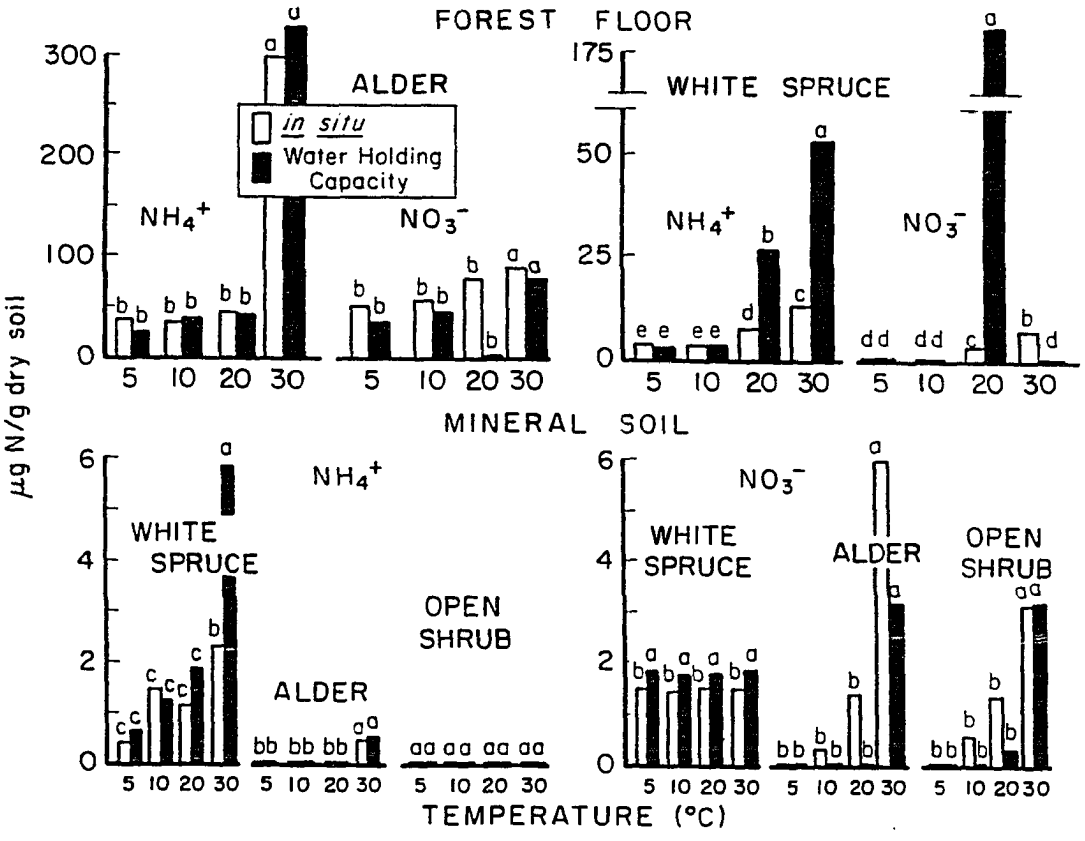

Fig. 2.2. The effects of temperature and moisture on the rates of net NH_4^+ mineralization and net nitrification. Values of NH_4^+ and NO_3^- with the same letter are not significantly different (P>0.05).

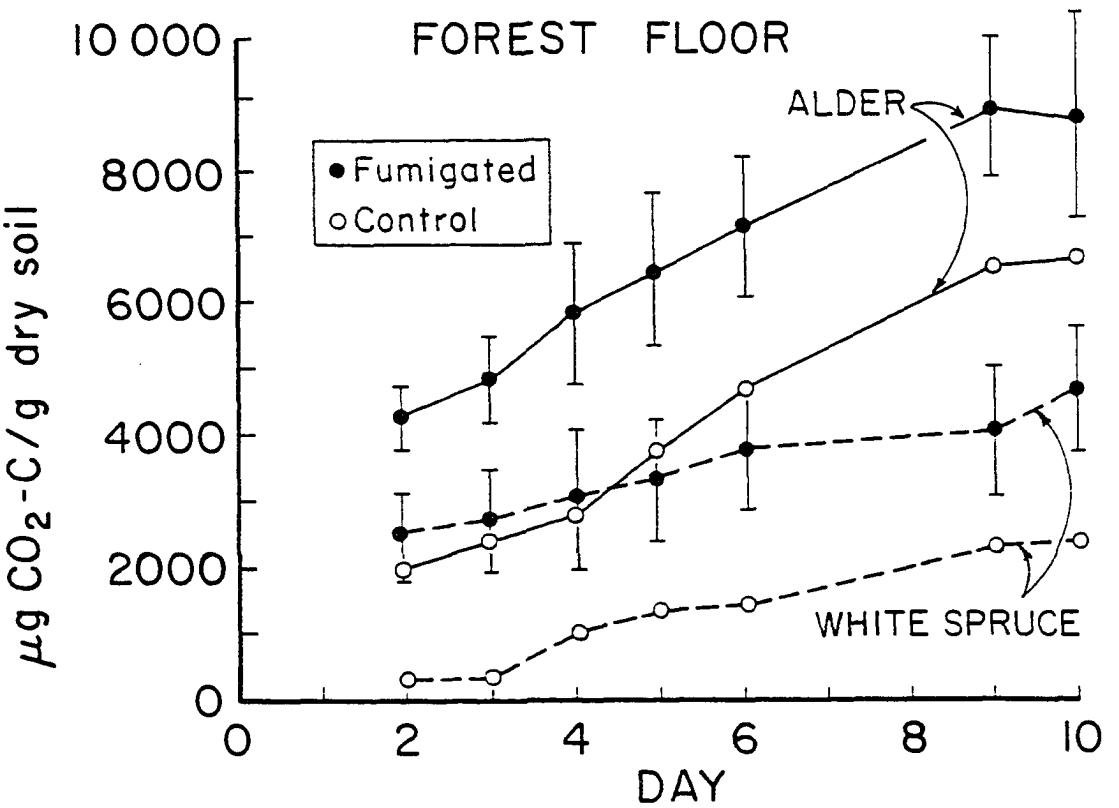

Fig. 2.3. Accumulation of CO_2 on fumigated and nonfumigated (control) alder forest floor and white spruce forest floor samples over the 10-d incubation period. Standard error bars are shown only for fumigated samples.

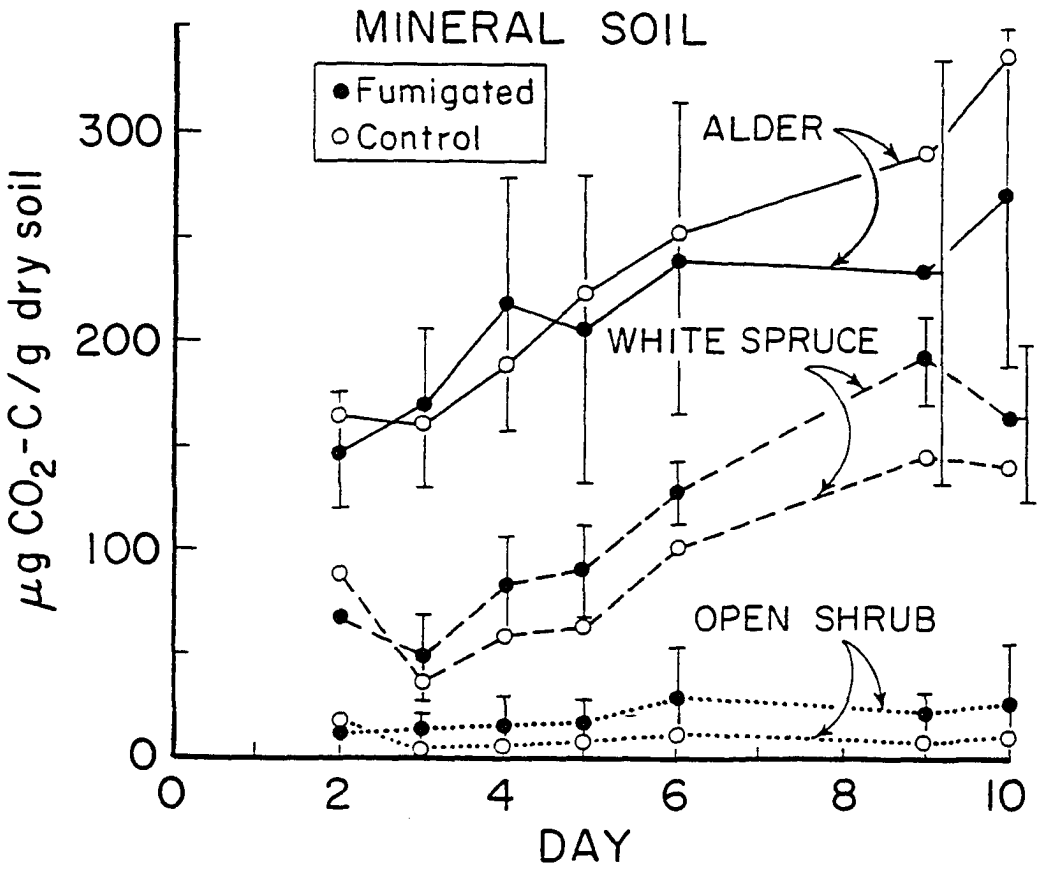

Fig. 2.4. Accumulation of CO_2 on fumigated and nonfumigated (control) mineral soil samples of the open shrub, alder, and white spruce stands over the 10-d incubation period. Standard error bars are shown only for fumigated samples.

respiration rates than its corresponding fumigated samples, while other forest floors and mineral soils tested had greater respiration rates in fumigated samples.

The amounts of the N flush after the 10-d incubation are shown in Table 2.9. The unfumigated values were low to undetectable when compared to similar fumigated samples. The alder forest floor sample exhibited the highest N flush and was significantly different from the white spruce forest floor sample. The white spruce forest floor had a greater N flush than any of the mineral soils tested. The open shrub mineral soil sample did not exhibit a mineral N flush.

Estimates of the microbial C/N ratio, B_C/B_n, (Table 2.9) were lowest in the forest floors, the alder forest floor exhibiting the overall lowest C/N ratios. Because the open shrub mineral soil sample had no detectable mineral N flush due to fumigation and the alder mineral soil sample had a negative B_C value, C/N ratios were not able to be determined for these samples.

Results of the chlorate factorial experiment are shown in Fig. 2.5. Among soils, NH_4^+, NO_3^-, and NO_2^- concentrations are significantly different between the five materials tested (forest floors and mineral soils) and the five treatments. The alder forest floor, the white spruce forest floor, and the white spruce mineral soil had similar NH_4^+ concentrations but were significantly different from the alder and open shrub mineral soils. Nitrate and nitrite were low to undetectable in the white spruce forest floor and in all

Table 2.9. Mean values of the N flush due to fumigation and calculated microbial C/N ratio.

Substrate	N flush ug N /g dry soil	B_c/B_n
Open Shrub Mineral Soil	ND[cd]	ND
Alder Mineral Soil	2.1±0.3[c]	ND
Alder Forest Floor	487.8±16.1[a]	4
White Spruce Mineral Soil	0.1±0.01[c]	76
White Spruce Forest Floor	114.7±10.1[b]	16

NOTE: Means ± SE, n=4. ND=not determined.

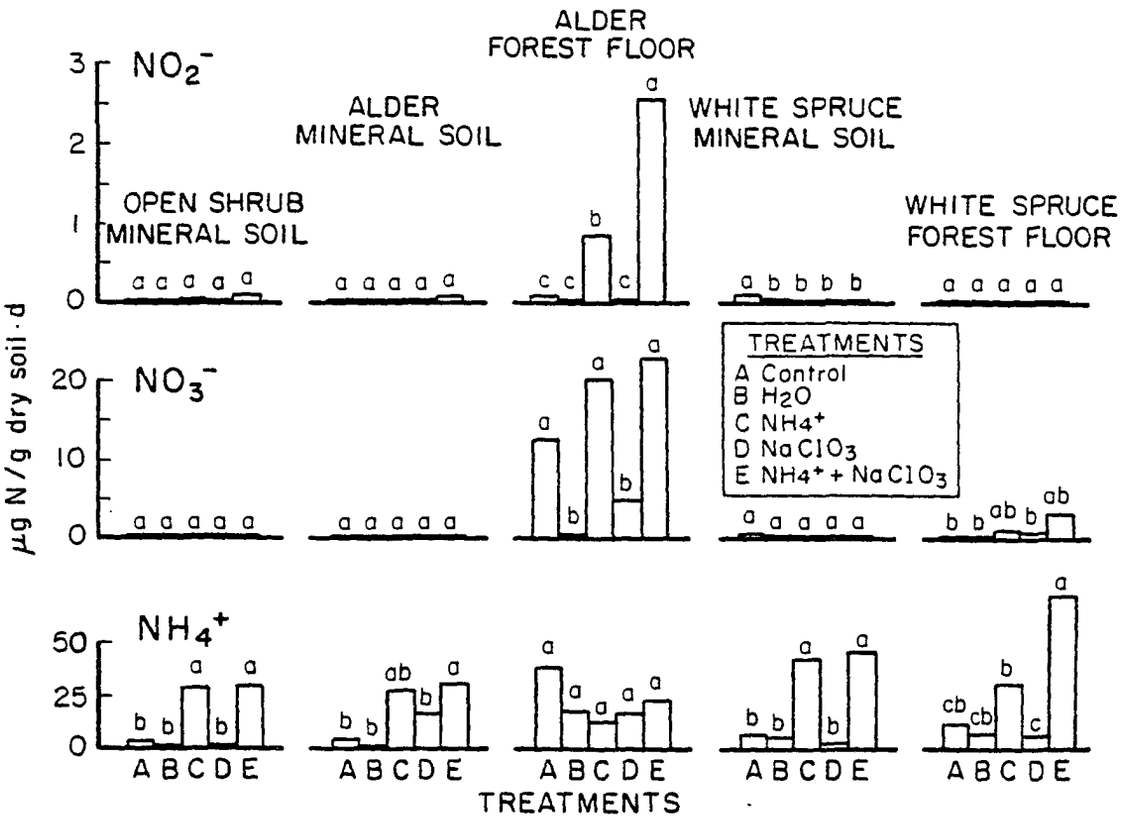

Fig. 2.5. Results of the chlorate inhibition assay. Values of NO_2^-, NO_3^-, and NH_4^+ with the same letter are not significantly different ($P>0.05$).

mineral soils. Ammonium additions in the alder forest floor samples did not increase NH_4^+ or NO_3^- concentrations, but did increase NO_2^- concentrations both in the presence and absence of chlorate. Nitrite production was 3 times greater in the presence of chlorate than in the absence of chlorate in NH_4^+ amended samples.

DISCUSSION

Soil profile features, within the floodplain successional stages, are mainly the result of the depositional history of the alluvium (Viereck et al. 1986). Soil development within these profiles is restricted and soil horizons are not well defined. In most profiles a series of buried forest floor layers in various stages of decomposition are found (Viereck et al. 1986). Each buried organic horizon represents a surface that was exposed for a number of years until a flooding episode occurred, resulting in the deposition of fresh alluvium. Of the sites studied, only the white spruce stand exhibited a buried organic layer, layer three, which was significantly different from the mineral soil layers in organic matter quantity, but at most sampling periods was similar with respect to accumulated and in situ, NH_4^+ and NO_3^- concentrations. These results could reflect a higher C/N ratio, a more recalcitrant organic material, than in the

surface forest floor, resulting in decreased rates of mineralization. For example, Flanagan and Van Cleve (1983) found substrate quality of the organic material within a soil to be the major controlling factor of decomposition.

N mineralization rates in the white spruce forest floor and all mineral soils tested are low when compared to temperate forest soil mineralization rates. Measurements of N mineralization using several different techniques ranged from <1 to 4.51 ug N·g dry soil^{-1}·d^{-1} in temperate forests (Robertson 1982; Christensen and MacAller 1985; Nadelhoffer *et al.* 1985; Raison et *al.* 1987), 4 ug N·g dry soil^{-1}·d^{-1} in a tropical forest (Robertson 1984), and 1 to 34 ug N·g dry soil^{-1}·d^{-1} in agricultural soils (Myrold and Tiedje 1986). Gordon and Van Cleve (1983) reported mineralization rates of a 110 yr old upland white spruce forest floor within the Bonanza Creek Experimental Forest that ranged from <1 to 3.11 ug N·g dry soil^{-1}·d^{-1}. These rates are comparable to the net average mineralization rates estimated in this study, <1 to 3.08 ug N·g dry soil^{-1}·d^{-1} (Tables 2.4-2.8). When comparing biweekly estimates of net mineralization, the alder forest floor ranged from <1 to 21.27 ug N·g dry soil^{-1}·d^{-1}, the alder mineral soil <1 to 3.43 ug N·g dry soil^{-1}·d^{-1}, the white spruce forest floor <1 to 2.45 ug N·g dry soil^{-1}·d^{-1}, and the white spruce mineral soil and open shrub <1 ug N·g dry soil^{-1}·d^{-1}. Although average rates of N mineralization are comparable to other forest soil N mineralization

rates, measured using incubation periods of one month or longer, it is evident that shorter incubations are more sensitive to seasonal fluctuations. In addition, substantial changes in rates of these processes can be estimated using the shorter sampling interval. Negative rates of net nitrification and net NH_4^+ mineralization were observed at all sites at numerous sampling dates indicating possible net immobilization and/or denitrification.

Nitrification patterns observed in this study are similar to those of other successional studies (Rice and Pancholy 1972; Robertson and Vitousek 1981; Robertson 1982; Rice 1984; Christensen and MacAller 1985). The highest net nitrification rates were found in the mid-successional stage, the alder, with low to undetectable rates observed in the white spruce and open shrub stage. It is important to note that net nitrification estimates also showed a pronounced seasonality which may reflect the effects of warmer soil temperatures observed in mid- and late summer (Fig. 2.6).

Temperature had no effect over the range of temperatures that occur naturally (Fig. 2.6), but at higher temperatures, 20°C and 30°C, N mineralization did increase, indicating that the overall effect of cold dominated soils does limit the rates of net N mineralization throughout the successional sequence. Increases of N mineralization rates within mineral soils were relatively small at higher temperatures. The combined effects of temperature and moisture

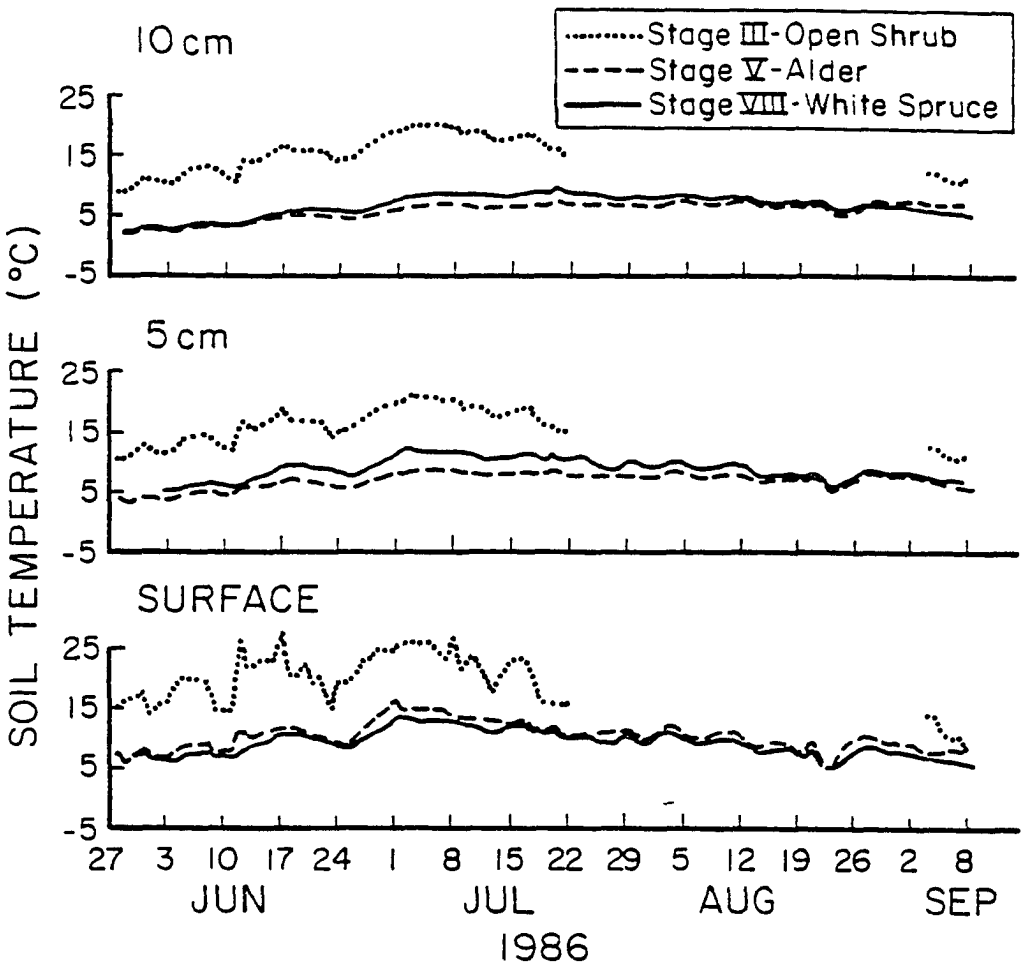

Fig. 2.6. Soil temperatures of the surface and at depths of 5 and 10 cm from the exposed surface of the open shrub stand (stage III), the alder stand (stage V), and the white spruce stand (stage VIII) (K. Van Cleve unpublished).

increased N mineralization in the white spruce stand, which exhibited the lowest soil temperatures and moisture contents for most of the field season (Fig. 2.6 and 2.7). Changes in moisture regimes did not affect mineralization rates of either alder or open shrub stands; both stands exhibited higher mineral soil moisture contents than the white spruce stand. The white spruce forest floor at water holding capacity and 20°C had a net mineralization rate of 10.21 ug N·g dry soil^{-1}·d^{-1} (8.7 ug N·g dry soil^{-1}·d^{-1} due to net nitrification), whereas at water holding capacity and 30°C, a mineralization rate of 3.23 ug N·g dry soil^{-1}·d^{-1} was measured with no observed net nitrification. The inability to detect net nitrification at 30°C may have resulted from denitrification or immobilization. An increase in temperature to 30°C in the alder forest floor increased the N mineralization rates 9 times that of the 5, 10, and 20°C rates. The temperature and moisture study was done using a short-term incubation period. It therefore reflects only a point-in-time estimate of the influences of temperature and moisture on N mineralization rates and does not reflect the possible long-term changes that might be associated with successional stage gradations of temperature and moisture.

Some of the limitations associated with the chloroform fumigation-incubation method are: residual chloroform, low microbial mineralization rates, the inability of the natural microbial population to recolonize, a small surviving microbial population, chloroform effects on the decomposability of other soil organic

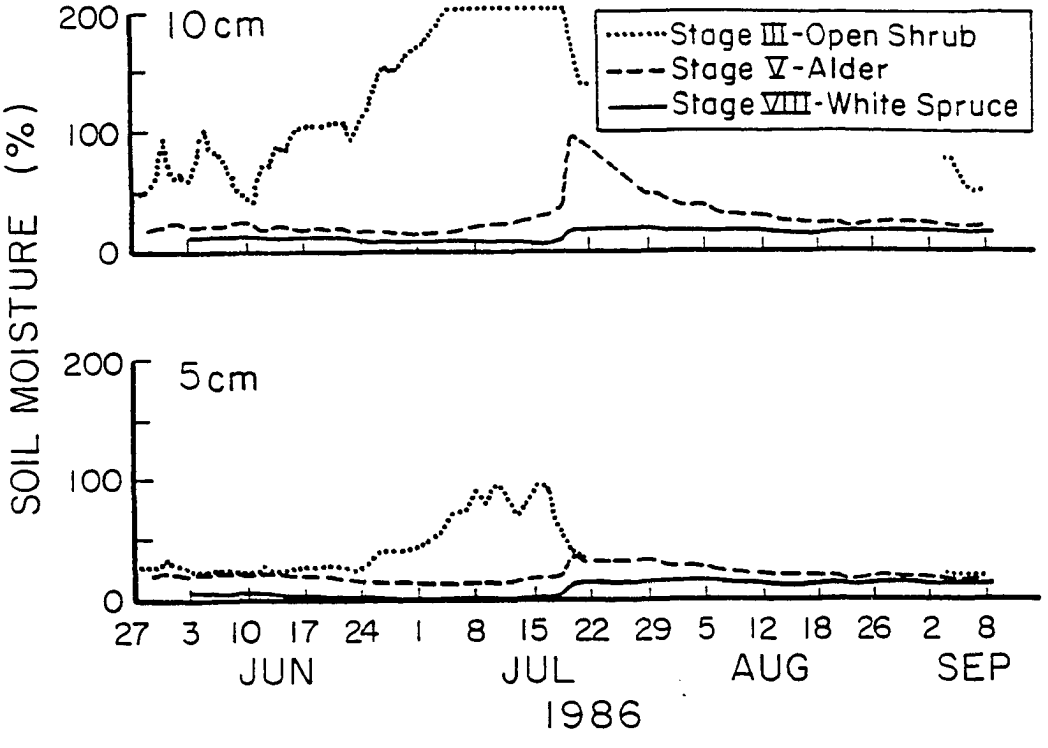

Fig. 2.7. Soil % moisture at depths of 5 and 10 cm from the exposed surface of the open shrub stand (stage III), alder stand (stage V), and the white spruce stand (stage VIII) (K. Van Cleve unpublished).

fractions, and/or masked effects of a large pool of readily mineralizable carbon (as in forest floors with high leaf litter content and the ethanol present in chloroform), and nitrogen immobilization (Jenkinson and Powlson 1976; Voroney and Paul 1984; Scheilds *et al.* 1974; Anderson and Domsch 1978; Brookes *et al.* 1985; West *et al.* 1986; Myrold 1987; Vance *et al.* 1987ab). These limitations may explain the high microbial biomass C/N ratios, the negative B_C calculated for the alder mineral soil sample, and the undetectable N flush in the open shrub sample.

Voroney and Paul (1984), Brookes *et al.* (1985), West *et al.* (1986), Myrold (1987) and Vance *et al.* (1987c) recommended several methods be used to quantify and compare the microbial population of different soils. They also suggested that emphasis should be placed on relative differences within and between soils using data that has not been transformed with K_C or K_n conversion factors. Vance *et al.* (1987b) estimated biomass C in 10 forest mineral soils, where the pH ranged from 7.2 to 3.2. These measurements of evolved CO_2 on fumigated soils over a 10-d incubation period ranged from 618-1710 ug CO_2-C$\cdot g^{-1}$ and were similar to the rates measured in the mineral soils of this study, 28-2115 ug CO_2-C$\cdot g^{-1}$. Both forest floor measurements of evolved CO_2 were higher than any of those found in the literature (Jenkinson and Ladd 1981; Shen *et al.* 1984; Voroney and Paul 1984; Brookes *et al.* 1985; Myrold 1987; Carter and MacLeod 1987; Vance *et al.* 1987b), as

literature values were determined from mineral soils. Measuring biomass C and N in forest floors where a large percentage of the soil is organic material has many limitations, as described above. Soil microbial biomass C generally comprises 1-3% of the total soil organic C (Jenkinson and Ladd 1981). The % biomass C of the total soil C (K. Van Cleve unpublished) was used to calculate the microbial biomass C % of the total organic carbon, assuming that within the forest floor, the majority of the total soil C is primarily due to soil organic C. The % biomass C of the total soil C was 1.9 for the alder forest floor, while the white spruce forest floor was as 1.2, both within range of the expected values.

Relative comparisons of respired C and the mineral N flush on fumigated samples of each soil tested, rather than using the estimates as absolute measurements of the microbial biomass, allows one to estimate a nitrogen availability index (Table 2.3). Total C/N ratios have been used as an indicator of the possible rate of organic matter decomposition, yet it does not indicate differences in the chemical constituents such as % lignin, hemicellulose, or cellulose, of that particular organic material (Alexander 1961). Although the flush of C and N due to fumigation does not quantify individual chemical species, it does estimate a pool of easily mineralized carbon and soil nitrogen and qualitatively characterizes, through the C/N ratio, the C and N status of that particular soil. The results suggest mineral soil samples have the lowest amounts of mineralized C and N and the highest

C/N ratio, resulting in the lowest nitrogen availability. The highest pool of easily mineralizable C and N and the lowest C/N ratio was found in the alder forest floor sample, suggesting the highest nitrogen availability index. The forest floor sample of the white spruce stand is characterized by lower quantities of mineralized C and N and a higher C/N ratio than the alder forest floor sample, suggesting a more recalcitrant organic material and a lower nitrogen availability index. These results also correspond with the N mineralization results, with the alder forest floor exhibiting the highest rates of N mineralization.

Ammonium additions did not stimulate net nitrification in the open shrub or alder mineral soil, or the white spruce stand, suggesting that nitrification is not limited by NH_4^+ availability in these stands. Although the chlorate inhibition method of measuring nitrification (Belser and Mays 1980) is reported to be highly sensitive (Groffman 1987), it gave inconclusive results within the alder forest floor. Difficulties interpreting the results of the chlorate inhibition assay have been reported and are thought to be caused by incomplete blockage of NO_2^- oxidation by chlorate (Belser and Mays 1980; Adams 1986), and/or chlorate reduction to chlorite (Hynes and Knowles 1983). Chlorate is reduced to chlorite by nitrosofying bacteria, which at low concentrations inhibits ammonium oxidation and nitrite oxidations (Hynes and Knowles 1983). This was not evident in this study as both ammonium and nitrate concentrations

were similar in the presence and absence of chlorate. Incomplete blockage may have been responsible for the nitrate accumulation in the presence of chlorate. Ammonium additions in alder forest floor samples did increase nitrite concentrations in both treatment C and E, where 3 times more nitrite was produced in the presence of chlorate. If the following assumptions are correct: nitrate production by heterotrophic nitrifiers is not affected by chlorate, and nitrate production in the presence of chlorate is not due to incomplete blockage, then these results suggest the majority, 90%, of the nitrification potential in the alder forest floor is due to heterotrophic nitrification.

CHAPTER 3

DENITRIFICATION AND NITROGEN FIXATION

ABSTRACT

Forest floors and mineral soils from an open shrub, alder, and white spruce stand, representing early, mid-, and late floodplain primary successional soils, were examined for symbiotic and nonsymbiotic nitrogen fixation, denitrification, and N_2O production. The acetylene reduction and inhibition techniques were used separately and in combination to measure nitrogenase and denitrification activity in laboratory and field studies. N_2O production was estimated using soil inserted gas traps. Low concentrations of N_2O, 2.84 ppm, were detected in the late spring while at all other sampling periods N_2O was undetectable. *In situ* measurements of denitrification were low to undetectable, except after a brief flood on the open shrub stand where increased denitrification activity was measured within the new/old alluvial interface. Intact core assays also had low to undetectable activities, the highest, 220 g $N \cdot ha^{-1} \cdot d^{-1}$, observed in the alder forest floor in September. Potential denitrification assays were greatest, 3.4 kg $N \cdot ha^{-1} \cdot d^{-1}$, in the alder forest floor in September. Laboratory studies indicated substrate limitation, both carbon and NO_3^-, as a major controlling factor of denitrification, although at higher temperatures, substrate and temperature interacted to increase

denitrification activity. Mineral soils and white spruce forest floor had low to undetectable rates of denitrification regardless of substrate additions or temperature increases. Nonsymbiotic nitrogenase activities were highly variable, with the white spruce moss community exhibiting the least amount of variability, 7-50 g $N \cdot ha^{-1} \cdot d^{-1}$. The alder forest floor had nonsymbiotic nitrogen-fixing rates ranging from undetectable to 4 g $N \cdot ha^{-1} \cdot d^{-1}$, while the freshly deposited alluvium had rates ranging from undetectable to 13 g $N \cdot ha^{-1} \cdot d^{-1}$. The highest observed nonsymbiotic nitrogenase activity was observed within the new/old alluvial interface, 66 g $N \cdot ha^{-1} \cdot d^{-1}$, immediately after a brief flood. Alder root nodule nitrogenase activity showed no significant seasonal patterns, but an estimated annual rate of 163 Kg $N \cdot ha^{-1} \cdot yr^{-1}$ contributed a substantial amount of N to the alder stand and to the floodplain ecosystem in general.

INTRODUCTION

Approximately 85% of the combined nitrogen on earth is considered to have been biologically fixed (Brock *et al.* 1984). Nitrogen fixation, both symbiotic and nonsymbiotic, is most abundant when nitrogen availability is limited. In primary succession, nitrogen fixation is thought to be a major mechanism of soil nitrogen build-up

(Crocker and Major 1955; Van Cleve *et al.* 1971; Connell and Slatyer 1977; Reiners 1981; Van Cleve and Viereck 1981; Viereck *et al.* 1983; Viereck *et al.* 1986).

The highest rates of terrestrial nitrogen fixation are reported among symbiotic relationships such as *Rhizobium*/legume, *Frankia*/*Alnus*, and cyanobacteria/lichen. Nitrogen fixation due to nonsymbiotic bacteria and cyanobacteria, and cyanobacteria associations with moss has been reported to be extremely low, ≤ 1 kg $N \cdot ha^{-1} \cdot yr^{-1}$ (Granhall 1981; Nohrstedt 1985; Alexander and Billington 1986; Grant and Binkley 1987). In forest ecosystems where nitrogen-cycling is tightly coupled, low rates of nitrogen fixation may be a significant input of N and may compensate for losses of N due to denitrification or leaching.

As N is considered one of the nutrients most limiting to plants, understanding the patterns and controls of denitrification is most important. Denitrification occurs in response to low oxygen tensions at sites with available nitrate and available organic matter substrates (Tiedje *et al.* 1984). Increased temperatures and moisture regimes, along with an increased amount of available organic matter, results in a greater denitrification potential (Sextone *et al.* 1985; Parkin 1987). Not only does denitrification cause N loss from an ecosystem, but it also results in the production of N_2O, when incomplete reduction of nitrogen oxides occur. This has been observed in ecosystems where high nitrate concentrations and/or high soil acidity are found (Firestone *et al.* 1980; Christensen 1985). Large

releases of N_2O have been reported during spring thawing of frozen soils (Goodroad and Keeney 1984). Nitrous oxide has also been found to be a product of nitrification (Yoshida and Alexander 1970; Blackmer *et al.* 1980; Robertson and Tiedje 1987). Increased atmospheric N_2O has been implicated as a possible cause of increased ultraviolet radiation due to the ability of N_2O to catalytically destroy stratospheric ozone (Weiss 1981; Crutzen 1983). Nitrous oxide can also act as a "greenhouse gas" by absorbing reradiated infrared light (Wang *et al.* 1976).

Denitrification has been found to occur simultaneously with symbiotic and free living nitrogen-fixing Rhizobium (Casella et al. 1984; O'Hara and Daniel 1985; Smith and Smith 1986). As nitrogen fixation is a highly reductive process and the enzyme nitrogenase is inhibited by oxygen, the use of nitrate as an alternative electron acceptor to oxygen would be beneficial to nitrogen-fixers, while reducing the inhibitory effect of NO_3^- on root nodulation (O'Hara and Daniel 1985). In an environment that has large inputs of nitrogen and a readily available carbon source, as in an established alder stand, it seems highly probable that denitrification and nitrogen fixation would occur simultaneously. Few studies have examined patterns of denitrification or nitrous oxide production among successional stages (Melillo *et al.* 1983; Robertson and Tiedje 1984), while also investigating the nitrogen-fixing potentials of each of those communities.

The object of this study was to examine patterns of nitrogen fixation and denitrification in the primary floodplain successional sequence of the Tanana River of interior Alaska. An open shrub, alder, and white spruce stand, representing early, mid-, and late successional stages were chosen as study sites. Several methods were used to estimate nitrogen fixation and denitrification. Gas traps placed at different depths within each site were used to monitor soil N_2O concentrations. ABS, amyl-butyl-styrene, tubes were inserted into the soil and kept in place throughout the study, and were used to simultaneously estimate *in situ* nitrogen fixation and denitrification. Both the acetylene inhibition assay, used to measure denitrification (Tiedje 1982), and the acetylene reduction assay, used to measure nitrogen fixation (Stewart et al. 1967), were used separately and in combination with each other. Measurements of nitrogenase activity within the *Alnus/Frankia* symbiotic relation were conducted on excised alder root nodules. Laboratory studies designed to measure potential denitrification and to test the effects of temperature and moisture on both denitrification and nitrogen fixation were also completed.

MATERIALS AND METHODS

Study Area

The study area is located close to the Bonanza Creek Experimental Forest approximately 30 km southwest of Fairbanks, and had been previously chosen as part of a larger multidisciplinary study, "The role of salt-affected soils in primary succession on the Tanana River floodplain of interior Alaska," in conjunction with the University of Alaska's Forest Soils Laboratory and the Institute of Arctic Biology, the USDA Forest Service Institute of Northern Forestry, and San Diego State University. Three of the twelve described successional stages, III, V, and VIII, were systematically chosen to represent early, mid-, and late succession and established as permanent plots. These are described as follows: stage III, a 5 yr old willow-poplar-alder (*Salix* spp.-*Populus balsamifera-Alnus tenuifolia*) open shrub stand; stage V, a 27 yr old alder (*Alnus tenuifolia*) stand; and stage VIII, a 165 yr old white spruce (*Picea glauca*) stand. Each designated plot is an area of 50 m x 50 m. Transects running perpendicular to the river and spanning the width of each plot were randomly selected for this study. Both the forest floor and mineral soil of the alder and white spruce stand were sampled and tested independently. The open shrub stand did not have a forest floor, thus only mineral soil was sampled and tested. Soil samples were collected equidistantly along selected

transects with a 15 cm diameter soil corer to approximately a 12-15 cm depth. Gas traps and ABS tubes were permanently placed at equal distances along a single selected transect.

Gas traps

Gas traps used to sample the soil atmosphere were constructed from closed-ended, 7.62 cm diameter by 2.0 cm high, plexiglass cylinders. The bottom of each cylinder had twelve equally spaced holes, 0.05 cm diameter, to allow gas exchange with the soil atmosphere. The traps were fitted with plastic capillary tubing, 0.05 cm I.D., which extended from the inside of the cylinder to the surface of the forest floor. The end of the capillary tube extending above the surface of the forest floor was equipped with a rubber septa for ease in sampling the soil atmosphere. In June 1985, triplicate gas traps at each of four depths, 5, 10, 20, and 50 cm from the surface of the forest floor, were inserted equidistantly on a randomly selected transect at each site. Each gas trap was sampled with a 5 ml glass evacuated tube at intervals of two weeks, throughout the summer of 1985 and 1986. Samples were stored at 5°C for approximately one week until analyzed for nitrous oxide content.

Concentrations of N_2O were measured by electron capture (^3H) gas chromatography using an Analytical Instrument Development Inc. gas chromatograph fitted with a 6-ft Poropak Q column. The limit of detection was 0.552 ppm N_2O-N.

ABS Core Tubes

Ten replicate open-ended ABS, amyl-butyl-styrene, tubes (12.09 cm diameter and 15.25 cm high) were placed at uniform intervals, to depths of 6-8 cm below the surface of the forest floor along each site established transect. These were used to measure *in situ* denitrification and nitrogen fixation. Vacuum stopcock grease was applied to the exposed rim of each tube, with subsequent placement of a plexiglass square fitted with a rubber septum, to allow gas sampling of the headspace. The plexiglass square covered the entire exposed opening of the ABS tubes.

For both the *in situ* nitrogen-fixing and denitrification assays, acetylene, generated by the addition of CaC_2 to distilled water, was added to sealed ABS tubes at a concentration of 10 to 20% of the headspace volume. Incubations were from 3 to 5 h, at which time replicate 5 ml gas samples were collected with glass evacuated tubes for N_2O and C_2H_4 analyses. The core tubes were inserted in the soil in July 1986 in both the alder (stage V) and white spruce (stage VIII) stands. Two transects were placed in the open shrub stand (stage III)

in order to sample the newly deposited alluvium resulting from a brief flooding episode and the combined new alluvium/old surface soil interface. The open shrub stage was flooded on July 22, 1986 with approximately 2 m of water. The water receded within 3-4 d and approximately 8-15 cm of fresh alluvium had been deposited. The newly deposited sediment was removed in order to sample this interface layer and immediately replaced after incubations were completed. Core tubes were sampled in this fashion every two weeks until September.

Due to the extremely low to undetectable amounts of N_2O measured, when utilizing the ABS core tubes, an experiment was designed to test the capability of the sealed ABS tubes to accumulate N_2O within their headspace, resulting in the ability to measure denitrification activity. In addition, this experiment was used to estimate an *in situ* potential denitrification rate, without any of the usual substrate limitations of denitrification. Eight ABS core tubes within the alder stand were randomly chosen, four serving as controls (no additions other than acetylene) and the other four saturated with a media containing 1.44 g $KNO_3 \cdot l^{-1}$, 1.00 g dextrose $\cdot l^{-1}$, and 0.25 g chloramphenicol $\cdot l^{-1}$ (Smith and Tiedje 1979). Chloramphenicol is a protein synthesis inhibitor and was added so that only denitification enzymes present were measured. Immediately after the addition of the media, all eight ABS core tubes were sealed with vacuum stopcock grease and plexiglass squares, as described above. At

intervals of 1 h, for a time period of 5 h, a gas sample was collected from each tube and stored for approximately one week in glass evacuated tubes until N_2O analyses could be completed.

Intact Core and Potential Soil Denitrification Assays

Twenty cores (5 cm in diameter including the forest floor and approximately 2-5 cm of mmineral soil) collected equidistantly along a randomly selected transect at each site, were brought to the laboratory for intact core and potential denitrification measurements in May, June, July, and September of 1986. Ten cores kept intact were placed in half pint canning jars that had tops equipped with rubber gas sampling septa. The other 10 cores, used for potential assays, had forest floor and mineral soil of each separated with subsequent mixing of each fraction. Quadruplicate subsamples, 25-50 g fresh weight, of the mixed composites were placed in similar half pint canning jars. Soil samples were then made into a slurry by the addition of a media (media A) containing 1.44 g $KNO_3 \cdot l^{-1}$, 1.00 g dextrose $\cdot l^{-1}$, and 0.25 g chloramphenicol $\cdot l^{-1}$ (Smith and Tiedje 1979) equal in mass to that of the forest floor and mineral soil samples. Potential assay samples were made anaerobic by flushing samples with N_2 five times. Potential assays and one half of the intact core assays were amended with a headspace addition of acetylene, 10% V/V. At the end of the incubation period, 2 h (continually shaken) for the potential assays

and 24 h for the intact cores, a 5 ml gas sample of each jar was taken and stored for approximately one week in a glass evacuated tube at 5°C until N_2O could be analyzed.

A factorial experiment (incomplete) was designed to test the effects of temperature, moisture, nitrate, dextrose, and anaerobiosis on the denitrification potential in early, mid-, and late successional stage soils. Four temperatures, 5, 10, 20, and 30°C, and six treatments were used: A, media containing 1.44 g $KNO_3 \cdot l^{-1}$, 1.00 g dextrose $\cdot l^{-1}$, and 0.25 g chloramphenicol $\cdot l^{-1}$ (Smith and Tiedje 1979); B, control (no treatments); C, distilled water added to water holding capacity; D, anaerobic; E, nitrate addition; and F, dextrose addition. Individual treatments were of the same concentration as that in the media addition. Water holding capacity had been previously determined by saturating soil samples with distilled water for 24 h with subsequent drainage for 24 h (Table 3.1). In mid-September 1986, ten soil cores were randomly collected from each site and kept at 4°C until laboratory studies were begun in early October. The soil cores were made into a composite mixture, separating the forest floor and the mineral soil of each site. Quadruplicate subsamples, 50 g fresh weight, of each soil composite were processed in a similar fashion as potential assays for each temperature and treatment. Incubations were for 6 h at which time a 5 ml gas sample of each jar was taken and stored for approximately one week in a glass evacuated tube at 5°C, until N_2O analyses could be performed.

Table 3.1. September 1986 *in situ* and water holding capacity moisture estimates.

Substrate	% Moisture	
	In Situ	Water Holding Capacity
Open Shrub Mineral Soil	26.1 ± 4.8	34.1 ± 6.0
Alder Mineral Soil	32.6 ± 5.4	58.6 ± 7.5
Alder Forest Floor	148.2 ± 33.9	339.4 ± 56.8
White Spruce Mineral Soil	6.9 ± 2.3	40.2 ± 3.9
White Spruce Forest Floor	54.7 ± 7.4	243.9 ± 42.6

NOTE: Means ± SE, n=10.

Nitrogen Fixation Assays

Nitrogenase activity within alder root nodules, stage V, was measured using excised nodules. Alder root nodules were collected within ten subplots (25 cm by 25 cm) located equidistantly along randomly selected transects, at time intervals of approximately two weeks beginning on August 15 until September 18, 1986. On July 28, 1986 only one subplot was used for nodule collections. Usually several clumps were found within each subplot, and all clumps found within each subplot were collected and treated as one sample. Immediately after excising subplot clumps of nodules, they were placed in a half pint canning jar, sealed with a lid equipped with a gas sampling rubber septa, and approximately a 10% headspace volume of acetylene was added. Incubations were carried out in the field, in a shaded area, for 2 h. At the end of the incubation period a gas sample of the headspace of each jar was taken and stored for approximately one week in a glass evacuated tube at 5°C until acetylene and ethylene analysis could be completed.

Root nodules hand-sorted from cores, in addition to nodules found at each subplot (at each sampling period) were dried at 65°C for 48 h in order to determine nodule weight per area. The cores were collected along three randomly selected transects (ten cores located equidistantly along each), in September 1986.

A factor of 3 was used to convert the C_2H_2 reduction to the amount of N_2-fixed. The theoretical ratio, assuming a complete electron transfer to the substrates, is 3:1 (Stewart 1980). A precise relationship between C_2H_2 reduction and N_2 fixation is not known for these sites. Although there is a wide range of ratios, 1.5:1 up to 25:1 depending on environmental conditions and organisms involved, the 3:1 ratio can be used as an indirect calculation of nitrogenase activity (Stewart 1980; Turner and Gibson 1980). In this study, the conversion of the production of C_2H_4 to N_2-fixed was used as a rough approximation of nitrogen fixation and was not intended to be a direct estimate.

An Analytical Instrument Development Inc. gas chromatograph equipped with a H_2 flame-ionization detector was used for the detection of C_2H_2 and C_2H_4. Nitrogen was used as the carrier gas in conjunction with a 6 ft Poropak R (100-120 mesh) column with the detector at a temperature of 60°C. The limit of detection for C_2H_4 was 0.01 ppm.

A factorial experiment (incomplete) was also used to test the effects of temperature and moisture on nitrogenase activity in the forest floor of the alder and white spruce stands and the surface mineral soil of the open shrub stand. Six intact cores (5 cm diameter) collected equidistantly along a randomly selected transect at each site, were placed in half pint canning jars, with respective moisture

treatments, and sealed with lids equipped with rubber septa. Four temperatures, 5, 10, 20, and 30°C, and two moisture regimes, *in situ* and water holding capacity, were used. Water holding capacity had been previously determined by saturating soil samples with distilled water for 24 h with subsequent drainage for 24 h (Table 3.1). Each temperature and moisture combination was incubated for 24 h in the presence (10% headspace V/V addition) and absence of acetylene. At the end of the incubation period, gas samples were collected from each jar and stored for approximately one week in glass evacuated tubes at 5°C for further C_2H_2 and C_2H_4 analyses.

Another factorial (incomplete) experiment was conducted to test the effects of C_2H_2 on alder forest floor respiration. Four temperatures, 5, 10, 20, and 30°C, and two moisture regimes, *in situ* and water holding capacity, were used. For each temperature and moisture treatment, quadruplicate subsamples, 20 g fresh weight, of soil composites were used and treated in a similar fashion as described above. The incubation period was 6 h, at which time a gas sample was collected in a glass evacuated tube from each jar and stored for approximately one week at 5°C until CO_2 analyses could be completed. Carbon dioxide was measured on a portable Analytical Instrument Development Inc. gas chromatograph that was equipped with a 6 ft Poropak Q column (100-200 mesh) with a detector temperature of 50°C.

For each jar assay the incubated material was dried, forest floor at 65°C for 48 h and mineral soil at 105°C for 48 h, and weighed to determine the rate of reaction per unit sample weight. Total airspace volume in each of the sampling jars was calculated by the addition of water.

Analyses of variance, using both parametric and nonparametric statistical tests (Zar 1984) were performed utilizing the Statistical Analysis System (SAS 1985). Due to the small sample size, n = 4 or 8, and thus the inability to test reliably for normality and equal variances, both nonparametric and parametric tests were used for comparison. All parametric and nonparametric tests were in agreement (accepting or rejecting the null hypothesis). Scheffe's multiple range test (Zar 1984; SAS 1985) was used to detect significant differences in estimates of denitrification and nitrogen fixation.

RESULTS

N_2O Production

More than 40% of the gas traps, inclusive of all depths, within the alder and open shrub stands were saturated with water on any particular sampling date and thus inoperable. After the small flooding episode, on July 22, 1986, all open shrub gas traps were inoperable,

while most alder stand gas traps were also saturated with water. Gas traps located in the white spruce stand were functional throughout both field seasons. There were detectable amounts of N_2O observed in gas traps on only one sampling date, May 27, 1986. On this date N_2O was observed at low concentrations at all three sites. Concentrations of N_2O in the headspace of white spruce gas traps averaged 0.91 ppm (±0.71) at 5 cm, 2.17 ppm (±3.02) at 10 cm, 0.75 ppm (±0.55) at 20 cm, and 2.84 ppm (±3.63) at 50 cm (n=4 for all depths). Of the four operable gas traps in the alder stand, only one trap, at 5 cm, had a detectable amount of N_2O, 3.14 ppm. In the open shrub stand, of the six operable traps, on that particular day, two traps at 5 cm showed similar amounts of N_2O, 3.14 ppm. Because the ambient atmospheric N_2O concentration is 0.333 ppm and the gas chromatograph used to measure N_2O had a limit of detection of 0.552 ppm, small changes in N_2O concentrations would be undetectable.

Denitrification

The addition of acetylene to the alder forest floor samples did not affect forest floor respiration rates at different temperatures or moisture regimes (Table 3.2). An increase in temperature did increase CO_2 evolution at both 20 and 30°C, while an increase in moisture either had no effect on respiration rates, at 10 and 30°C, or decreased respiration at 5 and 20°C.

Table 3.2. The effects of temperature, moisture (water holding capacity, WHC), and acetylene (C_2H_2) on alder forest floor respiration.

	Temperature			
	5°C	10°C	20°C	30°C
Treatment	nmoles CO_2 / g dry soil · d			
Control	51.1±0.2[a]	55.2±1.0[a]	82.5±2.1[a]	193.4±26.5[a]
Control and C_2H_2	53.0±0.8[a]	51.3±0.2[a]	95.5±4.6[a]	144.2±13.3[a]
WHC	47.2±0.9[b]	51.1±1.2[a]	75.6±2.8[b]	138.2±4.9[a]
WHC and C^2H^2	43.2±0.0[b]	51.3±0.8[a]	76.5±3.6[b]	137.4±7.9[a]

NOTE: Means ± SE, n=4. Values in each column followed by the same letter are not significantly different (P>0.05).

The results of the experiment testing the potential of the soil inserted ABS core tubes to measure denitrifying activity did indicate that this technique was able to measure denitrification activity (Table 3.3). There were significant differences ($P<0.05$) between the control core tubes and the potential denitrification amendments at the different sampling times, indicating an accumulation of N_2O.

Estimates of seasonal rates of N_2O production within the *in situ*, ABS core tubes were below the limit of detection at all sites, except for two sampling periods. Accumulation of N_2O within two ABS core tubes sampled on August 15, 1986 were 2.9 and 8.1 g $N \cdot ha^{-1} \cdot d^{-1}$. There were detectable amounts of N_2O production at both the alder and open shrub stage, and the new/old alluvium interface on August 8, 1986. These measurements ranged from 0.8-81.6 g $N \cdot ha^{-1} \cdot d^{-1}$ within the new/old interface and 1.3-2.0 g $N \cdot ha^{-1} \cdot d^{-1}$ within the alder stand.

The results of the laboratory intact core denitrification assays showed no measurable amounts of N_2O production in soil samples that were not amended with acetylene. Intact core samples from mineral soils and white spruce forest floor amended with acetylene showed little or no detectable denitrification activity (Table 3.4). The alder forest floor intact cores had the highest denitrifying activity in September 1986, while showing little or no activity at other sampling times. The potential denitrification rates were substantially higher than the rates in intact cores, with the highest potentials occurring in the alder forest floor on all four sampling dates (Table

Table 3.3. Rates of N_2O accumulation in *in situ* (control) and potential denitrification amended soils within the alder stand ABS tubes.

	Time				
Treatment	1 h	2 h	3 h	4 h	5 h
	g N / ha · d				
Control	0 ± 0^{b}	0 ± 0^{b}	0 ± 0^{b}	0.4 ± 0.2^{a}	1.1 ± 0.3^{a}
Potential	2.4 ± 0.4^{c}	6.9 ± 1.9^{b}	12.2 ± 2.2^{b}	17.0 ± 2.7^{b}	40.5 ± 2.3^{a}

NOTE: Means ± SE, n=5. Values of each row with the same letter are not significantly different (P>0.05).

Table 3.4. Rates of N_2O production in laboratory intact cores, 1986.

	ng N / g dry soil · hr			
Substrate	May	June	July	September
Open Shrub Mineral Soil	ND^{a}	0.4 ± 0.5^{b}	0.7 ± 0.5^{a}	ND^{b}
Alder Mineral Soil	ND^{a}	ND^{c}	12 ± 1^{a}	ND^{b}
Alder Forest Floor	ND^{a}	13 ± 2^{a}	18 ± 8^{a}	259 ± 122^{a}
White Spruce Mineral Soil	ND^{a}	1 ± 1^{b}	0.7 ± 0.7^{a}	ND^{b}
White Spruce Forest Floor	ND^{a}	1 ± 0.7^{b}	4 ± 2^{a}	ND^{b}

NOTE: Means ± SE, n=4. Values in each column with the same letter are not significantly different (P>0.05). ND=not detected.

3.5). In September, the alder forest floor had the highest observable N_2O production rate, with activity approximately 55, 153, and 17 times that measured during May, June, and July.

The highest rates of denitrification were measured in alder forest floor samples in the laboratory factorial denitrification experiment (Fig.3.1 and Table 3.6). The denitrification activity of the alder forest floor was significantly higher from all other soils tested ($P<0.05$). The mineral soils and the white spruce forest floor samples had low to undetectable rates of denitrification. Within soils, other than the alder forest floor, significant differences between treatments were only observed with the potential denitrification amendments, the highest activities occurring at 20 and 30°C. Within the alder forest floor significant differences were observed with the addition of treatments and with an increase in temperature. At 5 and 10°C, all treatments had similar denitrifying activities except for the samples amended with the media A (1.44 g $KNO_3 \cdot l^{-1}$, 1.00 g dextrose $\cdot l^{-1}$, and 0.25 g chloramphenicol $\cdot l^{-1}$, Smith and Tiedje 1979), whereas at 20°C the dextrose and media A treated samples were similar but significantly different from the control, anaerobic, nitrate, and water holding capacity treatments (which were similar). At 30°C, the control had the lowest denitrification rates while the media A, water holding capacity, anaerobic, and dextrose treatments showed similar high rates.

Table 3.5. Rates of N_2O production in potential denitrification assays, 1986.

Substrate	ng N / g dry soil · hr			
	May	June	July	September
Open Shrub Mineral Soil	0.8±0.4[b]	0.7±0.1[a]	13±0.6[b]	1±0.2[b]
Alder Mineral Soil	0.9±0.7[b]	0.2±0.2[b]	54±14[b]	70±10[b]
Alder Forest Floor	77±10[a]	28±47[a]	242±42[a]	4332±996[a]
White Spruce Mineral Soil	ND[c]	0.1±0.1[b]	10±1[b]	1±0.4[b]
White Spruce Forest Floor	1±0.4[b]	4±1[b]	28±2[b]	217±18[b]

NOTE: Means ± SE, n=4. Values in each column with the same letter are not significantly different (P>0.05). ND=not detected.

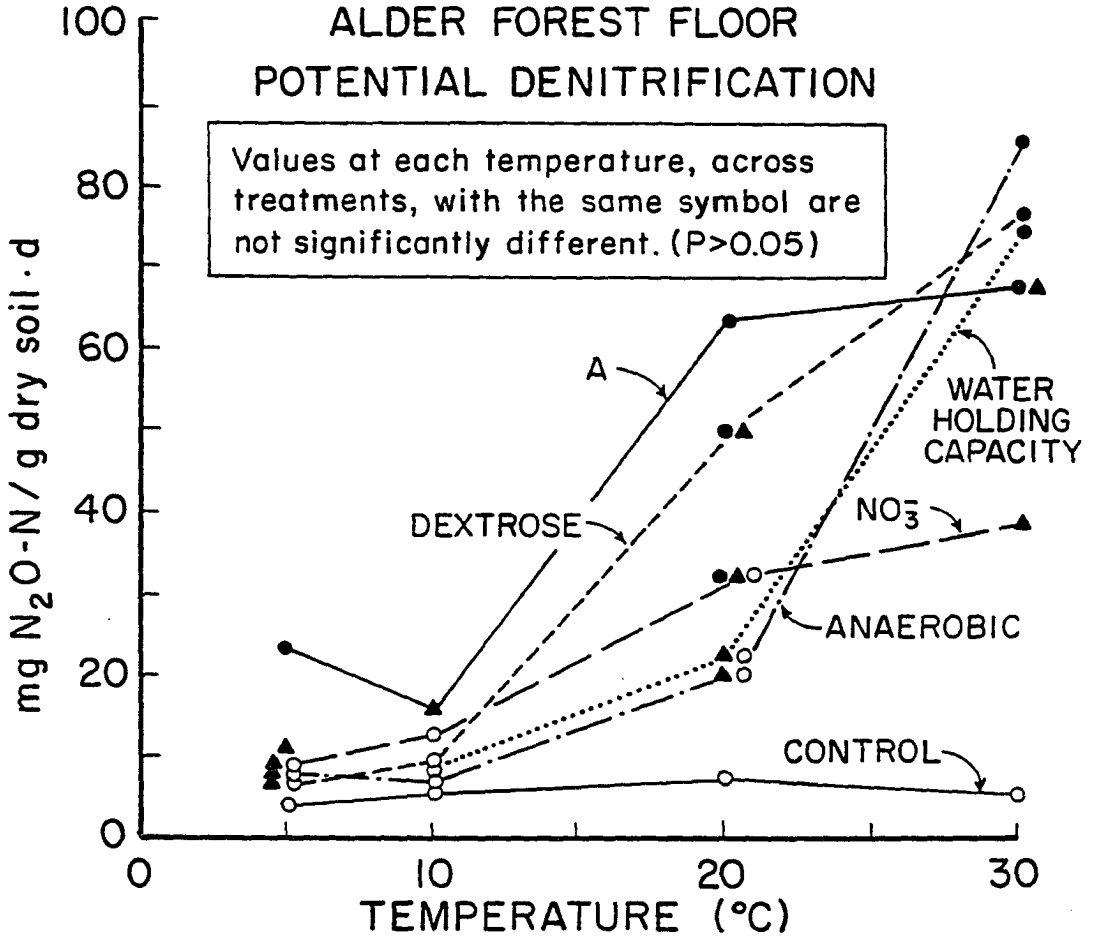

Fig. 3.1. The effects of temperature and treatments on potential denitrification activity in alder forest floor samples. Values at each temperature, across treatments, with the same symbol are not significantly different (P>0.05).

Table 3.6. The effects of temperature and treatments on denitrification activity in mineral soils and white spruce forest floor samples.

		ng N_2O-N / g dry soil · d			
Substrate	Treatment	5°C	10°C	20°C	30°C
Open Shrub	A	ND	7±3	358±20	603±22
Mineral soil	B	ND	ND	ND	ND
	C	ND	ND	11±10	11±5
	D	ND	ND	10±1	10±6
	E	ND	ND	10±1	601±17
	F	ND	ND	ND	4±4
Alder	A	16±0.3	245±48	1988±108	1243±56
Mineral soil	B	ND	ND	ND	129±32
	C	ND	ND	ND	ND
	D	ND	ND	ND	ND
	E	ND	ND	650±69	3015±110
	F	ND	ND	ND	736±19
White Spruce	A	31±3	ND	33±4	33±18
Mineral Soil	B	ND	ND	ND	ND
	C	ND	ND	ND	ND
	D	ND	ND	ND	ND
	E	ND	ND	ND	ND
	F	ND	ND	ND	ND
White Spruce	A	12±2	18±1	49±4	92±8
Forest Floor	B	ND	ND	ND	ND
	C	ND	ND	ND	ND
	D	ND	ND	ND	ND
	E	ND	ND	ND	31±8
	F	ND	ND	ND	ND

NOTE: Treatments are: A, media A; B, control; C, water holding capacity; D, anaerobic; E, NO_3^-; and F, dextrose. Means ± SE, n=4. ND=not detected.

Nitrogen Fixation

Nonsymbiotic nitrogenase activity measured within the three successional stands varied significantly among the soils tested (Table 3.7). The results suggest some seasonal variation among the times sampled. Within the open shrub stage both the newly deposited alluvium and the new/old interface had detectable rates of nitrogen-fixing activity. Highest rates within the freshly deposited alluvium were observed late in the summer, while highest rates in the new/old alluvial interface were observed immediately after the flood. The white spruce stand also showed higher nitrogen-fixing activities in late July and mid-August, while the alder stand exhibited the lowest activities throughout the sampling period.

The effects of temperature and moisture on nonsymbiotic nitrogen-fixing activity are shown in Table 3.8. There were no observed responses to temperature or moisture of any soil tested.

The measured nitrogenase activity of the alder root nodules was approximately 1000 times that of the measured nonsymbiotic nitrogen-fixing activity. The estimated nitrogen-fixing rates were similar at all sampling dates (Table 3.9). The average nitrogen fixation rate of all sampling periods was 1,635 g $N \cdot ha^{-1} \cdot d^{-1}$.

Table 3.7. Nonsymbiotic nitrogenase activity within the open shrub (freshly deposited alluvium and the new/old alluvial interface), the alder and the white spruce forest floor (FF) measured in ABS core tubes, 1986.

	umoles C_2H_4 / m^2 · d			
Sampling Date	Open Shrub Fresh Alluvium	Open Shrub New/Old	Alder FF	White Spruce FF
Jul 30	ND[b]	710±192[a]	ND[b]	351±30[b]
Aug 15	ND[b]	204±25[b]	ND[b]	544±38[a]
Aug 28	148±18[a]	34±11[b]	43±15[a]	38±23[c]
Sep 9	142±14[a]	ND	ND[b]	79±5[c]
g N / ha · d	0-13	0-66	0-4	7-50

NOTE: Means ± SE, n=10. Values in each column with the same letter are not significantly different (P>0.05). ND=not detected.

Table 3.8. Effects of temperature and moisture, *in situ* (IS) and water holding capacity (WHC), on nonsymbiotic nitrogen-fixing activities in forest floors of the alder and white spruce, and the surface mineral soil of the open shrub stand.

	umoles C_2H_4 / m^2 · d					
	Open Shrub		Alder		White Spruce	
Temperature	Mineral Soil		Forest Floor		Forest Floor	
°C	IS	WHC	IS	WHC	IS	WHC
5	28±12[a]	34±11[a]	11±6[a]	ND[b]	103±43[ab]	119±10[ab]
10	49±15[a]	31±14[a]	11±10[a]	6±5[a]	108±21[b]	34±21[b]
20	1810±604[a]	140±56[a]	6±3[a]	13±10[a]	152±32[ab]	117±43[ab]
30	671±614[a]	85±34[a]	3±2[a]	6±3[a]	402±108[a]	97±21[a]

NOTE: Means ± SE, n=6. Values within columns with the same letter are not significantly different (P>0.05). ND=not detected.

Table 3.9. Measured nitrogenase activity of excised alder root nodules, 1986.

Sampling Date	umoles C_2H_4 / g dry wt nodule · d	g N / ha · d
Jul 28	304[a]	2570
Aug 15	422±198[a]	3429
Aug 28	108±25[a]	877
Sep 9	83±12[a]	674
Sep 18	220±88[a]	726

NOTE: Means ± SE, n=10 (July 28, n=1). Values with the same letter are not significantly different (P>0.05).

DISCUSSION

The limitations associated with the acetylene reduction assay (Stewart *et al.* 1967) and the acetylene inhibition assay (Tiedje 1982) are numerous. However, because of the high sensitivity, simplicity, low cost, and high correlation with other more expensive and difficult techniques, they add much to our knowledge of the controls and patterns of nitrogen fixation and denitrification in natural environments. Some of the major difficulties associated with these techniques are acetylene's ability to be used as a carbon source, to inhibit nitrification, and to inhibit endogenous ethylene production (Nohrstedt 1984; Giller 1987; Robertson and Tiedje 1987). Although this study did not specifically address these limitations, the effects of acetylene on alder forest floor respiration were tested (Table 4.2). Acetylene did not significantly increase or decrease soil respiration, suggesting that it was not used as a carbon substrate, or in some way inhibitory to overall soil microbial respiration in the alder forest floor, even at higher temperatures and moistures. As other limitations were not tested, interpretations of the results must be viewed with caution.

The small amounts of N_2O detectable in the gas traps, but not detectable in the intact laboratory assays sampled on the same day, may have been the result of N_2O release from the soils during the spring thaw. Physical removal of the soil cores from the field and an

increase in temperature, may have caused sufficient disturbance to release any N_2O trapped within the soil core prior to conducting the assay. Goodroad and Keeney (1984) observed high rates of N_2O release from soils during spring thaw and postulated this was due to the physical release of accumulated N_2O produced biologically the previous fall. Although they measured N_2O concentrations that ranged from 1082 to 2066 ppm within the soil profile, the highest concentrations occurred early in the spring thaw. The low N_2O concentrations, 0.75-3.14 ppm, observed within the soil profile of this study were measured in late spring and are similar to those that Goodroad and Keeney (1984) observed, 1.3-5.2 ppm, at a similar period.

The highest observed denitrification activities were measured within the alder forest floor at all sampling times. Much lower activities were observed in the other soils tested. Increased soil microbial activity would be expected in the alder forest floor due to the larger reserves of N and organic matter. In addition, forest floors of early and mid- successional stages, such as the alder, would be expected to have lower lignin contents and a higher content of readily mineralizable carbon which could support a greater potential rate of denitrification (Flanagan and Van Cleve 1983; Melillo et al. 1984).

The highest observed intact core denitrification rate, 22 mg N_2O-$N \cdot m^{-2} \cdot d^{-1}$, observed in the alder forest floor is similar to intermediate rates observed by Robertson and Tiedje (1984) in

midsuccessional temperate hardwood stands and old field communities (30-80 mg N_2O-N·m^{-2}·d^{-1}). When this measured denitrification activity is expressed as a rate per organic matter, 371 ng N_2O-N·g organic matter^{-1}·h^{-1}, it is similar to the highest rates observed by Mellilo et al. (1984) in a 2 yr old hardwood clear cut, 560 ng N_2O-N·g organic matter^{-1}·h^{-1}. This high denitrification activity in September may have been in response to the increase in organic matter and nutrients from falling leaf litter while temperatures still remained warm. This was also evident in the factorial denitrification experiment, where individual carbon and nitrogen additions did not stimulate denitrification at 10°C, but did at 20°C. At the higher temperature, 30°C, increased denitrification activity was observed with the additions of carbon, nitrate, water, and an anaerobic atmosphere. Temperature alone did not have an effect without additional treatments, suggesting that denitrification is more limited by carbon, nitrate, and anaerobic conditions, than temperature. When substrates were no longer limiting, an increase in temperature increased the denitrification potential. The highest observed rate was 95,336 ng N_2O-N·g dry soil^{-1}·d^{-1} (3.4 kg N·ha^{-1}·d^{-1}). With an above average rainfall, and/or above average temperatures, flooding may occur on the alder terraces, possibly resulting in large potential losses of N. Potential rates of denitrification did not increase as dramatically in the other soils tested as did the alder forest floor. On an area basis, at 20°C with the addition of water, the open shrub

stand had a potential denitrification rate of 6 g $N \cdot ha^{-1} \cdot d^{-1}$, approximately 25% of the average net N mineralization rate, 24 g $N \cdot ha^{-1} \cdot d^{-1}$ (Chapter 2). Lower terraces, such as the open shrub stand (stage III) are subject to more frequent flooding than the older, higher elevated terraces. The open shrub stand also exhibits the highest moisture contents and the highest surface soil temperatures (Fig. 3.2 and 3.3). The *in situ* assays using the ABS tubes could not have detected these relatively low rates utilizing the short-term incubation periods.

There was no observable N_2O production without acetylene amendments, indicating that N_2O production by nitrification is not an ongoing processes in these soils, or that it is occuring at undetectable rates. It also suggests when denitrification activity is occurring N_2 is the major end-product of denitrification.

Estimates of nonsymbiotic nitrogenase activity were extremely variable. The highest nitrogenase activity was observed in the white spruce moss community (most likely due to a cyanobacteria/moss association) and in the open shrub stand within the new/old alluvium interface. The range of measured nitrogenase activity was higher in the white spruce moss community, 7-50 g $N \cdot ha^{-1} \cdot d^{-1}$ (this study) than in a black spruce cyanobacteria/moss association, 0.028-27 g $N \cdot ha^{-1} \cdot d^{-1}$ (Alexander and Billington 1986). Both studies used the same conversion factors. As the black spruce stand is typically colder and wetter, the higher range of observed rates in the

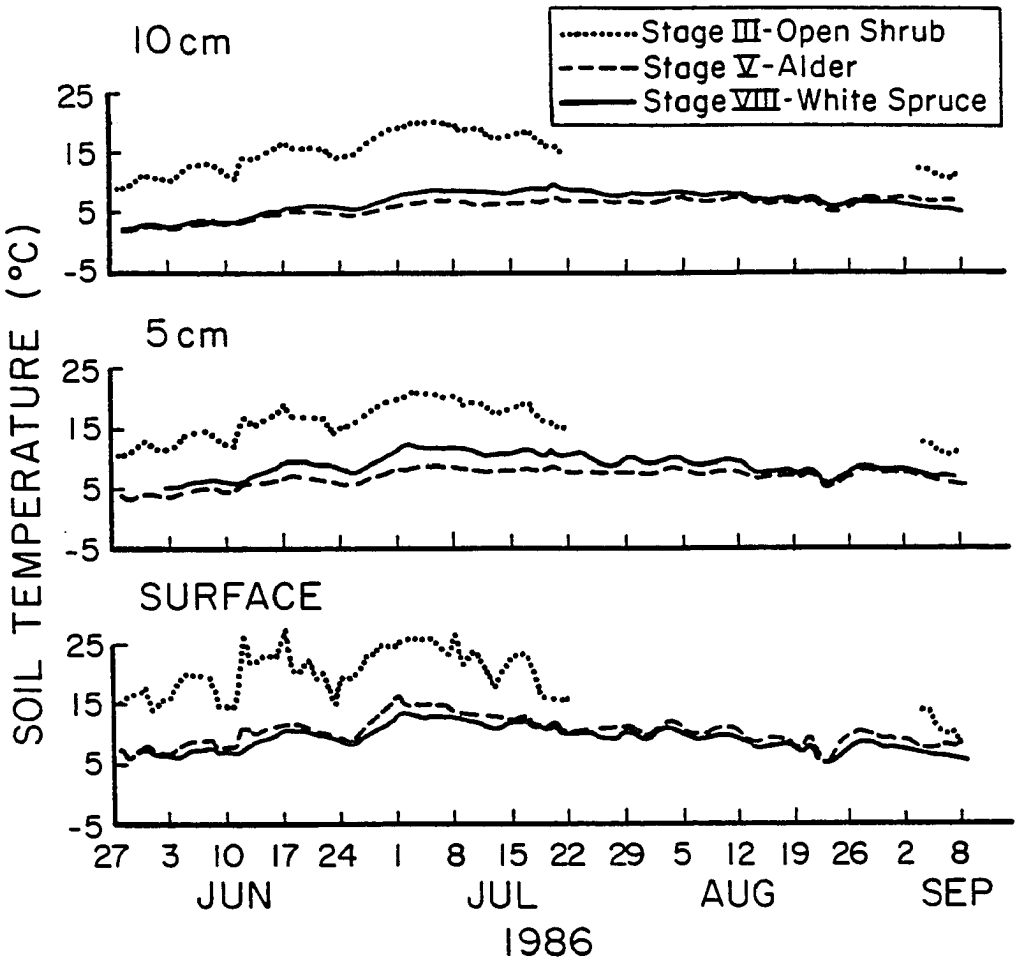

Fig. 3.2. Soil temperatures of the surface and at depths of 5 and 10 cm from the exposed surface of the open shrub stand (stage III), the alder stand (stage V), and the white spruce stand (stage VIII) (K. Van Cleve unpublished).

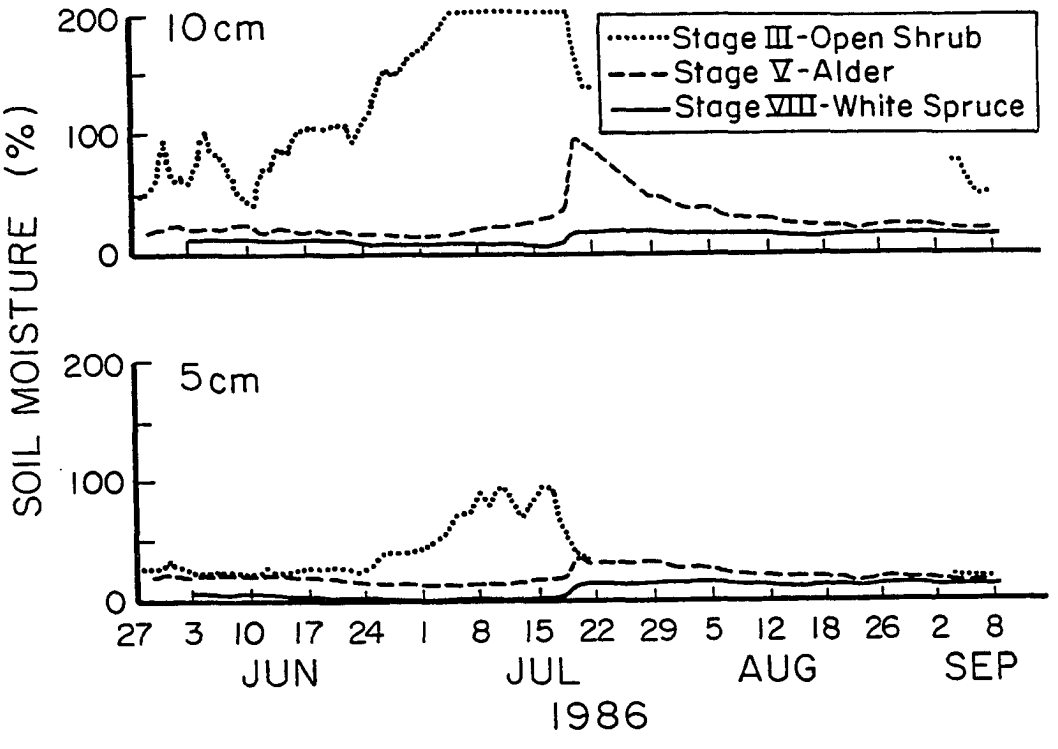

Fig. 3.3. Soil % moisture at depths of 5 and 10 cm from the exposed surface of the open shrub stand (stage III), alder stand (stage V), and the white spruce stand (stage VIII) (K. Van Cleve unpublished).

white spruce stand could be a result of a warmer environment. Although nitrogenase activity showed no response to an increase in temperature or moisture in laboratory studies, the laboratory studies do not necessarily reflect possible rate changes due to long-term temperature or moisture changes. Other possible explanations might include different cyanobacteria species and/or larger population sizes per unit area of moss tissue.

The alder forest floor exhibited the lowest nonsymbiotic nitrogenase activity, undetectable to 4 g $N \cdot ha^{-1} \cdot d^{-1}$. The freshly deposited alluvium, within the open shrub stand showed detectable rates, 13 g $N \cdot ha^{-1} \cdot d^{-1}$, approximately one month after the flood occurred. The new/old alluvial interface had the highest observed nitrogenase activity, 66 g $N \cdot ha^{-1} \cdot d^{-1}$, immediately after the flood with a continual decline with each successive sampling period until there was no detectable nitrogenase activity. Anaerobic conditions may have stimulated free-living heterotrophic nitrogen fixation, which is usually more significant under anaerobic conditions (Tiedje *et al.* 1984). Visual observation of this interface indicated rapid decomposition of the once exposed living organic material along with an obvious sulfide smell, indicating anaerobic conditions. As this carbon source became more limiting with time, heterotrophic activity would have decreased and a pattern of nitrogenase activity similar to that observed would be expected. With the newly deposited alluvium, the increase in nitrogenase activity may have been due to the

establishment of free-living cyanobacteria, as both N and organic matter would be limiting.

Alder root nitrogenase activity did not show any detectable seasonal variation, although measurements taken on the same day exhibited high variation. When the annual mean nitrogenase activity, 1.63 kg $N \cdot ha^{-1} \cdot d^{-1}$, is multiplied by an approximate time of frost free period, 100 d, it results in a nitrogen accumulation rate of 163 kg $N \cdot ha^{-1} \cdot yr^{-1}$. This rate is similar to the average annual N increment for a 20 yr period, 156 kg $N \cdot ha^{-1} \cdot yr^{-1}$, calculated by Van Cleve et *al.* (1971). Although their calculations based on yearly intervals ranged from 313 kg $N \cdot ha^{-1} \cdot yr^{-1}$ at 5 yr to 56 kg $N \cdot ha^{-1} \cdot yr^{-1}$ at 20 yr, these discrepancies may be due to differences in techniques and/or variablity of nitrogenase activity among and within stands. This estimated nitrogen-fixing rate contributes a substantial amount of N to the alder stand and to the floodplain ecosystem in general.

CHAPTER 4

CONCLUSIONS

The results of this study indicate that the nitrogen-fixing alder provides significant amounts of N to the floodplain ecosystem and is most likely a major influencing factor of the high rates of floodplain balsam poplar and white spruce productivity. Not only were the highest nitrogen fixation rates observed in the alder stand, but also the largest pools of NH_4^+ and NO_3^-, the highest rates of net mineralization and net nitrification, and the highest rates of *in situ* and potential denitrification were observed (Table 4.1).

Alder is usually found to be of significance in the soil N build-up of early successional stages (Crocker and Major 1955; Reiners 1981), and it has also been associated with high activities of nitrification (Rice and Pancholy 1972; Hendrickson and Chatarpaul 1984). In this study, the highest rates of nitrification were found within the alder stand, with low to undetectable rates associated with the early and late successional stages. These results follow the nitrification patterns observed in other successional studies (Rice and Pancholy 1972; Robertson and Vitousek 1981; Robertson 1982; Rice 1984; Christensen and MacAller 1985). Laboratory studies indicate that N mineralization rates are generally limited by the cold dominated soils of taiga forests. Ammonium availability may also be a limiting

Table 4.1 Rates of nitrogen-cycling processes in an open shrub (stage III), an alder (stage V), and a white spruce (stage VIII) stand.

Substrate	g N / ha · d			
	Nitrogen Fixation	Mineralization NH_4^+	Mineralization NO_3^-	Denitrification
Open Shrub Mineral Soil	28	24	-18	6
Alder Mineral Soil		125	61	3
Alder Forest Floor	1636	111	37	95
White Spruce Mineral Soil		16	3	0.4
White Spruce Forest Floor	23	25	-0.1	6

factor in the alder stand, while temperature and moisture are limiting factors in the white spruce stand. An increase in temperature increased the N mineralization rates in the open shrub stand but not as markedly as in the alder or white spruce stand. Other factors that may possibly limit nitrification are the quantity and quality of organic material and allelopathy.

Denitrification and potential denitrification rates were highest in the alder forest floor, the site with the highest net mineralization and net nitrification activity. Laboratory studies suggest substrate limitation was greater than temperature or moisture limitation, but once substrates were no longer limiting, temperature increased potential denitrification activities. The intact core assays suggest that when there is a readily available source of carbon, such as fall leaf litter, and when temperatures are still warm, denitrification may be the cause of a significant loss of nitrogen. Although open shrub mineral soil denitrification rates are relatively low, during periods of high moisture content or flooding episodes, increased denitrification activities could result in a substantial amount of nitrogen loss.

Nonsymbiotic nitrogen fixation, although relatively low and highly variable, may be an important input of nitrogen in the early and late successional stages. Lower mineralization rates, higher C/N ratios, and low denitrification activities would substantially increase the value of any N input. In both the open shrub stand and

the white spruce forest floor average nitrogen fixation estimates were similar to the net NH_4^+ mineralization rates (Table 4.1).

Single sites were chosen to estimate several nitrogen cycling transformations at one particular time. In general, ecological studies of nitrogen cycling tend to measure a single nitrogen process due to the considerable amount of time and effort needed to estimate several processes simultaneously (Myrold and Tiedje 1986). Although this study did estimate rates of N mineralization, nitrogen fixation and denitrification within a similar time frame, because replication of sites were not made, significant differences observed in field replicates of each site only give evidence of differences between those three specific sites chosen. In addition, laboratory experiments generally were carried out utilizing subsamples of a single soil composite, therefore significant differences observed only describe differences in soil composite samples.

The object of this study was to provide a test of the working hypothesis: rates of N mineralization and denitrification will steadily increase from early sandbar stages to mid-successional stages following the general accumulation of organic matter and nitrogen but then decrease in older successional stages due to lower temperatures, lower moisture, and increased recalcitrance of forest floor and mineral soil organic matter while rates of nitrogen fixation will be highest in the early stages. Although the results of this study do tend to confirm this hypothesis, because of the lack of successional

stage replication, the testing of this hypothesis is inappropriate. A test of a hypothesis is only valid when the experimental unit or treatment is replicated (Hurlbert 1984).

This study has served as a preliminary study utilizing the working hypothesis as a guide for a detailed description of patterns of nitrogen-cycling found in an open shrub, alder, and white spruce stand, and directs further studies to identifying patterns within the successional sequence. Some specific questions generated by this study that should be addressed are: is heterotrophic nitrification a major portion of the net nitrification in the alder forest floor and to what extent does it occur in other taiga forests; are there other nonsymbiotic and symbiotic nitrogen-fixing associations; what are the patterns of nitrogen cycling within the rapidly growing poplar stages and later black spruce/thaw ponds stages; what are the effects of allelopathy; and how variable are the spatial and seasonal patterns of nitrogen processes.

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