GASEOUS NITROGEN TRANSFORMATIONS IN A MATURE FOREST ECOSYSTEM

by

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Abstract

In mature forests, gains and losses of nitrogen may be dominated by the gaseous transformations, asymbiotic nitrogen fixation and biological denitrification. Both are reduction reactions and are affected by moisture conditions, temperature, pH, supply of organic carbon and the availability of mineral nitrogen.

Gaseous nitrogen inputs, due to asymbiotic nitrogen fixation, and outputs, due to biological denitrification were quantified for a mature coniferous forest in southwestern British Columbia. Forest floor material, mineral soil, decaying wood, foliage and bark were incubated in an atmosphere of 0.1 atm acetylene to allow the simultaneous measurement of \( \text{N}_2\text{O} \) production by denitrifying bacteria and acetylene reduction by free-living bacteria and blue-green algae. Forest floor material accounted for 80% of a total annual input of 0.8 kg N ha\(^{-1}\) a\(^{-1}\). Relatively small amounts of nitrogen were fixed in mineral soil, decaying wood and foliage and no indication of nitrogen fixation activity in bark was detected. Traces of denitrification were found, but gaseous output of nitrogen was effectively 0.0 kg N ha\(^{-1}\) a\(^{-1}\). It is hypothesized that this forest may prevent nitrogen loss by outcompeting other sinks for mineral nitrogen, thereby allowing a slow accretion of nitrogen by asymbiotic nitrogen fixation and bulk precipitation input.
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1. INTRODUCTION

"Nitrogen is the principal limiting factor in the growth of both agronomic and forest crops" (Gordon et al., 1979). The significance of nitrogen in crop production has generated a huge body of literature which can be summarized in abstract form by the nitrogen cycle (Figure 1). While this model is complex, it is interesting to note that outside of industrial fixation, there are only two means of entry into the terrestrial nitrogen cycle. These are electrochemical and photochemical fixation of atmospheric nitrogen ($N_2$), and of far greater significance, biological fixation of $N_2$ by microorganisms (Kormondy, 1976). Similarly, there are a limited number of means by which nitrogen is returned to the atmosphere, thus balancing the global nitrogen cycle. By far the most important of these processes is the bacterial reduction of nitrate, or biological denitrification (Knowles, 1982a).

When nitrogen is the limiting factor to growth in forest soils, nitrogen fertilizers usually stimulate forest growth (Spurr and Barnes, 1973; Gessel, 1959). Forest fertilization is carried out to increase productivity and also to offset a decline in fertility that may occur as a result of nutrients lost in harvested materials (Marion, 1979; Morrison and Foster, 1979). Intensification of utilization and the shortening of rotation ages in response to increased fibre demand will accentuate the potential for soil impoverishment (Kimmins, 1977). While nitrogen fertilizers are effective, they are becoming increasingly expensive in terms of fossil energy, water pollution, and 'hard cash'. As competition for industrially fixed nitrogen develops, it seems clear that forestry will rank a lower priority than agriculture. Biological nitrogen fixation offers two approaches to solving this dilemma. The possibility of producing nitrogen fertilizers using cultured bacteria and waste carbon or photosynthesis exists and is being
Atmospheric nitrogen (N₂)

denitrifying bacteria
(NO₃⁻ → N₂)
eddenitrifying bacteria
(NO₃⁻ → NO₂⁻)

Ammonia (NH₃)

Nitrate (NO₃⁻)

Ammonifying bacteria
(NH₂ → NH₃)

Nitrite bacteria
(NH₃ → NO₂⁻)

Nitrate bacteria
(NO₂⁻ → NO₃⁻)

Electrically and photochemical processes

Industrial fixation

Nitrogen fixing organisms

birds and fish

Shallow marine sediments

Deep sediments

Assimilation and anabolism

Producers

decay and wastes

derbivory

Consumers

decay and wastes

Amino acids

urea, uric acid, organic residues

Figure 1. The nitrogen cycle. Adapted from: Kormondy, 1969.
investigated (Sprent, 1979). Biological nitrogen fixation may also be used as an alternative to chemical fertilizers. Symbiotic nitrogen-fixing systems, such as those involving plants of the Fabaceae (legumes), have been important in agriculture for a long time (Nutman, 1975). Stewart (1977) tells us that nitrogen fixation by blue-green algae has been the mainstay of sustained rice production throughout the ages. Ecosystem studies have suggested that biological nitrogen fixation may be a major source of nitrogen accumulated in forest biomass during succession (Roskoski, 1980; Todd, Waide and Cornaby, 1975).

Much research has focused on the use of nitrogen-fixing symbioses to enhance and preserve forest productivity. Less well understood is the significance of nitrogen fixation by free-living microorganisms. Nitrogen accretion by asymbiotic nitrogen fixation (ANF) is generally small when compared to symbiotic nitrogen fixation. However, when nitrogen is the limiting nutrient factor for plant growth, even small inputs of nitrogen may be significant (Granhall and Lindberg, 1980). Evidence also exists that ANF may be important in the wood decay process (Hendrickson and Robinson, 1982).

Any discussion of biological nitrogen fixation should also consider the significance of denitrification. There is some evidence that the two processes are linked, responding in kind to the nitrogen status of the site (Knowles, 1978; Granhall, 1981). There is one species of bacteria, *Spirillum lipoferum*, that has the capacity to effect both transformations, depending on the conditions present (Neyra et al., 1977).

Denitrification can be a major source of nitrogen fertilizer loss (Rolston, 1981), resulting in decreased fertilizer efficiency. Other reasons for interest in denitrification include:
1. its potential use in the removal of nitrates from waste water (Schroeder, 1981) and other high-nitrogen waste materials.

2. its contribution of nitrous oxide ($N_2O$) to the atmosphere where it is involved in stratospheric reactions which result in the depletion of ozone (Knowles, 1982a).

Our knowledge of these biological nitrogen transformations remains incomplete. The roles of ANF and denitrification in forest ecosystems and the effect of management practices such as harvesting, scarification, prescribed burning and fertilization on them are not well understood. This study was undertaken with the main objective of determining the relative significance of ANF and denitrification in a mature forest ecosystem. A review of the pertinent literature was undertaken to determine more specific objectives and the best methods by which these objectives might be met.
2. LITERATURE REVIEW

2.1 ASYMBIOTIC NITROGEN FIXATION

Biological nitrogen fixation is the reduction of atmospheric nitrogen (N\textsubscript{2}) to ammonia (NH\textsubscript{3}). Organisms capable of this transformation are all members of the kingdom Prokaryota (Sprent, 1979), and include species of eubacteria and cyanobacteria (blue-green algae). They occur as free-living organisms (asymbiotically) or in symbiotic association with certain species of fungi, bryophytes, cycads (Stewart, 1977) and higher plants. These include members of the Fabaceae and non-legumes such as *Alnus* sp., *Arctostaphylos* sp. and *Ceanothus* sp. (Cromack, 1981). Of interest in this study are free-living bacteria and blue-green algae.

Nitrogen fixation by free-living bacteria has been reported in the forest floor, decaying wood and in the phyllosphere. Free-living bacteria capable of fixing nitrogen may be aerobic, anaerobic or facultative anaerobic (Hendrickson and Robinson, 1982). Aerobic nitrogen-fixing bacteria are thought to be rare or absent from generally acidic, forest soils (Jurgensen and Davey, 1971). Anaerobic bacteria that fix nitrogen are generally of the genus *Clostridium* (Hendrickson and Robinson, 1982). Jurgensen and Davey (1971) found the facultative anaerobe *Bacillus polymyxa* to be the predominant nitrogen-fixing bacterium isolated from a variety of forest soils in North Carolina and Washington, and from Alaskan tundra. Other bacteria known to fix nitrogen include members of the genera *Beijerinckia*, *Enterobacter*, *Klebsiella*, *Pseudomonas*, *Achromobacter*, *Spirillum* and *Micrococcus*.

Over 20 species of blue-green algae have been reported to fix nitrogen, all of which are members of the order Nostocales (Jurgensen and...
Davey, 1970). Approximately one third of these are found in soil while others are found associated with certain bryophytes, cycads and ferns, and with fungi as lichens (Hendrickson and Robinson, 1982). Jurgensen and Davey (1968) investigated forest soils and found low numbers of blue-green algae in some soils, but only when pH was above 5.4. They suggest that the contribution of nitrogen-fixing blue-green algae to the nitrogen cycle of established forests is negligible. Of more significance in certain ecosystems may be the contribution of nitrogen-fixing lichens (Denison, 1973; Millbank, 1978).

The reduction of dinitrogen to ammonia,

\[ \text{N}_2 + 3\text{H}_2 \rightarrow 2\text{NH}_3 \]

is an exothermic reaction, but because of the stability of the N\(_2\) molecule, energy is required for the reaction to occur. This energy is provided by adenosine triphosphate (ATP) which is generated by hydrolysis within the organism. The reaction is catalyzed by a highly oxygen sensitive system known as the nitrogenase enzyme complex. The reader is referred to Sprent (1979) for a more thorough discussion of nitrogen fixation metabolism and nitrogen fixation in general.

2.2 DENITRIFICATION

Denitrification may occur abiotically or biologically. "Abiological denitrification which requires high NO\(_3^-\) and low pH is generally low and insignificant" (Wollum and Davey, 1975). Biological denitrification is the microbial reduction of nitrate (NO\(_3^-\)) and nitrite (NO\(_2^-\)) to the gaseous nitrogen oxides (NO, N\(_2\)O) and atmospheric nitrogen:

\[ \text{NO}_3^- \rightarrow \text{NO}_2^- \rightarrow \text{NO} \rightarrow \text{N}_2\text{O} \rightarrow \text{N}_2 \]
Denitrification has been reported in soil and aquatic environments where anaerobic conditions prevail. The transformation is effected by essentially aerobic, but facultative bacteria, that utilize nitrogen oxides as terminal electron acceptors in the absence of oxygen (Knowles, 1982a). The reduction is dissimilatory, in that the nitrogen fails to enter the cell structure of the organisms involved (Alexander, 1977). Nitrate may also undergo assimilatory and dissimilatory reductions to ammonia which do not result in nitrogen loss and will not be discussed further. Nitrous oxide can also be evolved as a byproduct of the microbial oxidation of ammonia (nitrification) which may be a significant contributor of N₂O in certain systems (Aulakh et al., 1982).

The bacterial species that can denitrify come from less than thirty genera (Bryan, 1981). Those from the genera *Pseudomonas* and *Alcaligenes* are the most commonly isolated and may be of greatest significance (Knowles, 1982a). However, in soil systems, *Pseudomonas* and *Achromobacter* may be the most significant (Focht, 1978).

Denitrifying bacteria synthesize a series of reductases that enable them to reduce successively more reduced nitrogen oxides (Knowles, 1981). Most possess all the reductases, while some lack the NO₃⁻ reductase, and are NO₃⁻ dependent. Others lack the N₂O reductase and yield N₂O as the end product, while still others have only the N₂O reductase (Knowles, 1982a). More thorough discussions of denitrification may be found in Knowles (1981,1982a) and Delwiche (1981).
2.3 ENVIRONMENTAL FACTORS AFFECTING ANF AND DENITRIFICATION

Both denitrification and ANF are reduction reactions and are similarly affected by a number of environmental factors. These factors include soil aeration and moisture status, the availability of organic carbon, soil temperature and soil pH. The effects of soil inorganic nitrogen levels on the two processes will be quite different. Few, if any of these factors are independent of each other making it difficult to determine a rate-controlling factor in a particular system. Environmental factors affect not only the overall rate of denitrification but frequently exert differential effects on the successive reductases (Knowles, 1981). In this way the relative amounts of the products that accumulate may be affected.

2.3.1 SOIL AERATION AND MOISTURE

Soil aeration and moisture affect ANF and denitrification as they affect the presence of oxygen which inhibits both processes.

Nitrogen-fixing organisms have developed a number of protective mechanisms against oxygen, including diffusion barriers (as root nodules in symbiotic nitrogen-fixing plants), the formation of heterocysts by blue-green algae and the formation of slimes in some heterotrophic bacteria (Granhall, 1981). Azotobacter uses high respiratory activity to scavenge O₂ and keep it away from the nitrogenase. As oxygen is required for the production of ATP, many bacteria fix nitrogen most effectively under microaerophilic conditions (Silvester et al, 1982). Other organisms, such as Clostridium sp., can fix nitrogen only under anaerobic conditions.

When denitrifying bacteria have access to both nitrate and oxygen, oxygen will be reduced rather than nitrate (Bryan, 1981). The
$N_2O$ reductase is thought to be the most sensitive to $O_2$ and under low concentrations of $O_2$, the overall rate of denitrification will decline but the percentage of $N_2O$ in the end products will increase (Focht, 1974).

Denitrification has been largely associated with very fine-textured soils of poor structure, poorly drained soil, and normal soils of medium or fine texture during periods of excessive rainfall (Allison, 1965). The greatest potential for denitrification would be in completely water-saturated soil (Rolston, 1981), although this may not be true of forest soils, where denitrification capacity is greatest while the soil is drying following saturation (Binstock, 1984).

Evidence that denitrification (Fluehler et al., 1976) and ANF can occur under apparently well-aerated conditions, can be explained by the occurrence of anaerobic microsites in aggregated soils (Currie, 1961; Greenwood, 1961). Gaseous diffusion occurs more readily through the inter-aggregate pores than through the relatively smaller pores within the aggregate (Currie, 1961). Anaerobic conditions may prevail within the aggregate well after the inter-aggregate pores have drained.

The $O_2$ concentration in soil depends on the rates of $O_2$ consumption (respiration), and $O_2$ diffusion and the geometry of the diffusion path (Smith, 1980). The geometry of the diffusion path is complicated by the variation in aggregate size within most aggregated soils. Smith (1980) has attempted to model the extent of anaerobic microsites in aggregated soils, considering the variation in gaseous diffusion coefficients, respiration rate and aggregate size. There is a need to extend this type of study to determine the development of anaerobicity in poorly aggregated soils (Rolston, 1981).
Both ANF and denitrification are promoted in soils that remain saturated for long periods of time due to structure, texture and/or precipitation. Any activity that affects these factors will indirectly affect ANF and denitrification.

2.3.2 AVAILABILITY OF ORGANIC CARBON

Photosynthetic nitrogen-fixing organisms, such as the blue-green algae, depend on light quality and intensity for ATP production. Consequently, daily and seasonal fluctuations in growth and nitrogen-fixing capacity will occur.

Heterotrophic nitrogen-fixing organisms utilize a variety of organic carbon compounds as energy sources, including carbohydrates, alcohols, organic acids and aromatic compounds (Granhall, 1981). Nitrogen fixation, in general, is most common in environments where organic carbon is abundant and combined nitrogen (NH₄⁺, NO₃⁻) is limiting. In forests, relatively high rates of ANF have been reported in decaying wood (Todd et al., 1975; Roskoski, 1980; Granhall and Lindberg, 1980), which has a high carbon to nitrogen ratio.

Most denitrifying bacteria are heterotrophs and require organic carbon as a source of energy. Under anaerobic conditions, denitrification is largely controlled by the supply of readily decomposable organic matter (Burford and Bremner, 1975). Denitrification capacity has been significantly correlated with water-soluble organic carbon (Burford and Bremner, 1975; Bremner and Shaw, 1958) and mineralizable carbon (Burford and Bremner, 1975). Reddy et al. (1982) found denitrification rates to be proportional to concentrations of NO₃⁻ and available carbon and suggested that
Denitrification is influenced by the rate of mineralization of organic carbon. Under some conditions, carbon addition may not affect denitrification, indicating that this factor is not rate limiting (Knowles, 1981).

Denitrification rates will be largely influenced by the amount of carbon in the soil and its position in the profile. Denitrification rates are generally higher near the surface (Knowles, 1981) where organic matter deposition, mineralization and incorporation occur. ANF rates have been demonstrated to decrease significantly with increasing soil depth (Baker and Attiwill, 1984).

2.3.3 TEMPERATURE

Rates of ANF and denitrification fluctuate diurnally and seasonally in response to temperature. In spite of reports that nitrogen fixation occurs at extremely high and low temperatures, most nitrogen-fixing organisms are mesophiles. Evidence exists that nitrogen fixation can be affected more by temperature, than are general growth and photosynthesis (Granhall, 1981).

The optimum range for denitrification is between 60 and 65°C. (Nõmmik, 1956; Bremner and Shaw, 1958). Jacobsen and Alexander (1980) found that nitrate was reduced slowly at 7°C, and the rate increased with increasing temperature. Denitrification has been reported at 4°C, in an anaerobic atmosphere (Limmer and Steele, 1982) although Bailey (1976) reported that denitrification was completely inhibited at 5°C.

The indirect effects of soil temperature on ANF and denitrification may be more important than the direct effects. In
marine sediments, little variation in denitrification rate with temperature has been reported (Knowles, 1982a). Soil temperature affects the rate of other biological processes such as mineralization and nitrification as well as the movement of carbon, water and oxygen in the soil profile. Much of the effect of temperature may be related to the development of anaerobiosis (Rolston, 1981) and the availability of carbon and nitrate.

2.3.4 SOIL REACTION

Optimum pH for denitrification (Nõmmik, 1956; Delwiche and Bryan, 1976) and nitrogen fixation (Granhall, 1981) is generally around 7.

Nitrogen fixation, although optimum at pH 7, can occur over a broad pH range. Certain asymbiotic bacteria (*Bacillus polymyxa* and *Beijerinckia* sp.) and some blue-green algae can fix nitrogen at pH as low as 4 (Granhall, 1981). However, the abundance of nitrogen-fixing free-living bacteria is generally lower in soils more acid than pH 6.

Muller et al. (1980) studied denitrification in low pH soils (minimum 3.6) and found a highly significant correlation between reaction rate and soil pH. While denitrification decreases with decreasing pH, the N\textsubscript{2}O reductase seems to be more sensitive to low pH than the other reductases. Decreasing pH and increasing O\textsubscript{2} concentration tend to decrease the overall rate of denitrification but to increase the mole fraction of N\textsubscript{2}O in the final product (Focht, 1974). Interpretation of these results is complicated by the occurrence of abiological reactions of nitrite at low pH that yield one or more of NO, N\textsubscript{2}O, N\textsubscript{2} and CH\textsubscript{3}NO\textsubscript{2} (Knowles, 1982a).
As with temperature, the indirect effects of soil pH on ANF and denitrification may be significant. Soil pH will affect the availability of mineral nutrients such as phosphorus and molybdenum which are necessary for nitrogen fixation. The availability of both these nutrients is optimum at near neutral pH and declines with decreasing pH. Low pH also seems to inhibit nitrification (Armson, 1977) which will have an important impact on denitrification in systems where nitrate is rate-limiting.

2.3.5 NITROGEN

Nitrogen fixation is most common in environments where organic carbon is abundant and combined nitrogen (NH$_4$+, NO$_3^-$) is limiting. It is a self-regulated process, promoted or inhibited by changes in the levels of inorganic nitrogen (Granhall, 1981). If supplies of combined N are good, nitrogen-fixing organisms are at a competitive disadvantage for carbon with heterotrophs that can utilize combined nitrogen. Nitrogen fixers must use a considerable portion of the energy available to them to fix nitrogen, leaving less for growth (Sprent, 1979).

Denitrification rates are often found to be independent of nitrate concentrations as rates of carbon mineralization will be the limiting factor (Rolston, 1981). At low concentrations, NO$_3^-$ may exert rate-control (Knowles, 1982a). While the forest soil, with relatively high levels of soluble organic matter offers an excellent environment for denitrification, the latter cannot occur without nitrate and little nitrification normally occurs in undisturbed systems (Keeney, 1980). Nitrate concentrations also affect the proportions of the gaseous
products evolved. At high nitrate concentrations, the predominant product is N\textsubscript{2}O (Nõmmik, 1956) while at lower concentrations, more N\textsubscript{2} is produced.

The occurrence of common intermediates (N\textsubscript{2}O, NO\textsubscript{2}⁻) suggests that nitrification and denitrification may be coupled processes (Knowles, 1978; Focht and Verstraete, 1977). Knowles (1978) suggests two ways by which coupling might occur. Nitrification and denitrification may occur successively in time, in response to alternate wetting and drying cycles that provide the appropriate conditions for each process (Binstock, 1984). The two processes may also occur simultaneously, on opposite sides of an anaerobic-aerobic interface, such as may occur in soil aggregates. Simultaneous nitrification and denitrification may also occur in soils with variable moisture content. Nitrification may occur at the surface of a soil profile and nitrate then moves downward to an anaerobic zone where denitrification can take place (Hendrickson, 1981; Knowles, 1978).

Nitrification, and consequently denitrification, may be controlled by competition for NH\textsubscript{4}⁺ among heterotrophic soil organisms. "When NH\textsubscript{4}⁺ is added.....the available C/N ratio is drastically reduced and the nitrifiers have a competitive advantage for NH\textsubscript{4}⁺ supplies"(Johnson and Edwards, 1979).

Nitrification and denitrification may also be coupled with nitrogen fixation. Nitrogenase has the ability to reduce N\textsubscript{2}O as well as N\textsubscript{2}. Denitrification and nitrogen fixation may occur simultaneously in the same or adjacent microenvironments, or even in the same culture of an organism able to catalyze both processes (Knowles, 1978).
2.3.6 OTHER MINERAL NUTRIENTS

Lack of phosphorus affects nitrogen fixation before it affects plant growth, because phosphorus is necessary for the synthesis of ATP. The availability of iron and molybdenum is vital for nitrogen fixation as both are components of the nitrogenase enzyme system. Other nutrients thought to stimulate nitrogen fixation include magnesium, boron, cobalt, copper and zinc (Granhall, 1981).

Copper, molybdenum and magnesium are all required for denitrification. Magnesium is necessary for growth, while molybdenum is an integral part of all the nitrate reductases that have been studied. Copper is involved in the reductase in some organisms while in others it is required for reductase synthesis. Iron and sulphur are both necessary for activity of the denitrification enzymes (Bryan, 1981).

Sulphur compounds have several effects on denitrification. Sulphide appears to inhibit the NO and N\textsubscript{2}O reductases (Knowles, 1982a). Sulphide can also cause relief of acetylene inhibition (discussed in methods section) of the N\textsubscript{2}O reductase which may have consequences for the use of this technique in measuring denitrification.

2.4 DENITRIFICATION AND ASYMBIOTIC NITROGEN FIXATION IN FORESTS

The study of both ANF and denitrification has been limited by the lack of ideal methods for quantifying fluxes. The development of the acetylene-reduction assay as an easy, sensitive and relatively inexpensive method of measuring nitrogen fixation has facilitated a vast increase in information on nitrogen fixation (Hardy et al., 1973). While the acetylene-reduction assay has advantages over other techniques, it can be criticized on a number of grounds which will be discussed in Methods.
A universal method for the measurement of denitrification has proven more elusive (Focht, 1978; Rolston, 1981). Denitrification has most often been estimated by balance from nitrogen cycle studies (e.g. Bormann and Likens, 1979) and is the subject of much speculation.

2.4.1 DENITRIFICATION

Granhall (1981) has suggested that in temperate forests, ANF will more than compensate for generally small losses by denitrification, resulting in slow accumulation of combined nitrogen.

Todd et al. (1975) provided one of the few studies of denitrification in temperate forests. Although their analytical methods allow their results to be considered as rate potentials only, they provided valuable information about the potential rates in different substrates. Highest potential was found in decaying logs on the forest floor and in the upper 10 cm. of soil where available carbon is at a maximum. Highest total flux of nitrogen gas occurred from the soil because of the greater mass of soil. Of less significance, in terms of denitrification capacity, were decaying branches on the forest floor and the forest floor itself.

Melillo et al. (1983) considered denitrification over a successional sequence in organic and mineral soil horizons. They suggested that denitrification potential is greater in young stands following clearcutting. Their results indicated the potential for denitrification in forest soils, in spite of low pH (3.5 to 3.9) and well-drained conditions. The authors speculated that denitrification occurred in pulses following hydrologic events such as spring runoff and major rainstorms. This agrees with the findings of Binstock (1984)
who demonstrated that denitrification capacity in forest soils is
greatest while the soil is drying following saturation. Melillo et al.
(1983) also found a strong correlation between denitrification and
nitrate concentration. In forest soils where nitrification rates are low,
nitrate may be the rate-limiting factor for denitrification.

2.4.2 ASYMBIOTIC NITROGEN FIXATION

Considerably more information exists concerning the role of ANF
in forests, although the picture is still incomplete. Climax forests in
temperate regions have relatively closed nitrogen cycles between
vegetation and soil (Granhall, 1981). Gaseous inputs and outputs may
dominate the total gains and losses in the forest nitrogen cycle (Todd
et al., 1975). A survey of the literature indicates that ANF can account
for 1 to 20 kg Nitrogen ha\(^{-1}\) a\(^{-1}\) which will compensate for generally
small losses of nitrogen caused by leaching and denitrification, and
may yield some accretion (Granhall, 1981).

Paul (1978) suggests that ANF in climax ecosystems may
account for 5 to 10 per cent of the amount of nitrogen cycled
annually between vegetation and soil. While the amounts of nitrogen
fixed asymbiotically seem relatively small, one must consider that 2
kg N ha\(^{-1}\) a\(^{-1}\) accrued over a 90 year rotation is almost equal to one
200 kg N ha\(^{-1}\) application of N fertilizer. This has the advantage of
occurring as slowly released, easily available nitrogen (Granhall, 1981)
in contrast to broadcast fertilizer applications which are subject to
potentially high rates of leaching and denitrification.

Asymbiotic nitrogen fixation in forests is carried out primarily
by heterotrophic bacteria in the soil and forest floor. Other potential
sources include bacteria in decaying wood and on foliage surfaces, and nitrogen-fixing epiphytes living on bark, branches, foliage and the forest floor. Granhall and Lindberg (1980) present nitrogen fixation data for three coniferous forests in Sweden. Their data indicate the relative nitrogen-fixing potentials of different strata. The importance of organic carbon supply was emphasized, as the most important nitrogen-fixing components were decaying wood, the litter layer, and the rhizosphere and humus layer. Todd et al. (1975) reported that while the highest rates of nitrogen fixation occurred in decaying wood and humus, the largest amount of nitrogen fixation occurred in the soil due to the greater mass of soil.

The report by Jones (1970) that nitrogen-fixing bacteria on leaf surfaces of Douglas-fir could fix considerable quantities of nitrogen, focused attention on coniferous foliage as a potential source of nitrogen fixation. Subsequent research by Jones (1982) and others (Granhall and Lindberg, 1980; Caldwell, Hagedorn and Denison, 1979; Todd et al., 1978) failed to support this finding, indicating that foliage may not be a significant source of nitrogen fixation. In a study of forest species in Minnesota and Oregon (Sucoff, 1979), nitrogen fixation on leaf surfaces without epiphytes was considered to be negligible. The presence of epiphytes with a nitrogen-fixing blue-green algae component may make the phyllosphere a significant source of nitrogen fixation (Denison, 1973; Millbank, 1978).

Roskoski (1980) investigated nitrogen fixation in wood litter in the northeastern United States. Fixation was highest in the oldest and youngest stands studied, as a result of higher acetylene reduction rates and larger quantities of wood litter. The higher acetylene
reduction rates were attributed to a greater abundance of large dead logs which provide a more suitable habitat for nitrogen fixation because of their higher moisture content and their ability to retain anaerobic microsites. ANF in the mineral soil in these (Roskoski, 1980) and similar sites (Tjepkema, 1979) was found to be insignificant.

Decomposition of woody material in forests is slow, in part because of the high C/N ratio of the material. It has been suggested that nitrogen fixation may help facilitate the decay of woody substrates (Cornaby and Waide, 1973). Jorgensen (1975) postulated that wood decay fungi will benefit from added nitrogen while nitrogen-fixing bacteria will benefit from the carbon-rich products of wood decay and perhaps from reduced O₂ levels due to fungal respiration. Research indicates that nitrogen fixation may be important in the wood decay process, although not as important as other sources of nitrogen, such as crown wash (Larsen et al., 1982; Silvester et al., 1982).

2.5 THE EFFECTS OF FOREST MANAGEMENT ACTIVITY ON ASYMBIOTIC NITROGEN FIXATION AND DENITRIFICATION

The impact of forest management practices on soil biological processes has received considerably less attention than the effects of the same practices on the physical and chemical properties of soil. Forest fires, clearcutting, scarification and fertilization are examples of treatments that will alter chemical and physical soil properties such as moisture content, temperature, aeration, pH, bulk density and available nutrients (Jurgensen et al., 1979) causing changes in rates of ANF and denitrification.
Site changes resulting from timber harvesting should tend to promote ANF and denitrification. Logging will generally increase soil pH and temperature during the growing season, and cause increased soil saturation due to removal of vegetation. These conditions will favour the anaerobic processes of ANF and denitrification. Logging will also promote more rapid mineralization, resulting in a flush of carbon and mineral nitrogen. These conditions will promote denitrification, but may conceivably inhibit ANF by putting the organisms at a competitive disadvantage for carbon in the presence of high mineral nitrogen availability (Sprent, 1979).

Fire is a natural occurrence in many temperate forests and has been used as a management tool. Jorgensen and Wells (1971) reported significant increases in ANF following prescribed burning in loblolly pine (Pinus taeda L.) stands in South Carolina. They attributed this to increases in soil temperature, soil moisture, pH, available nutrients and available carbon. These conditions will also promote the activity of denitrifying bacteria (Knowles, 1982a).

Denitrification will be influenced by any change in soil nitrate levels. Logging and fire may improve conditions for nitrification and nitrogen fertilizers will increase nitrate concentrations in the soil, resulting in increased denitrification. Rolston (1981) reviewed the literature on nitrogen loss after fertilization on cropped soils and reported losses of 0 to 75 per cent of applied nitrogen fertilizer. This will vary considerably with soil conditions. Fertilizer applications may potentially inhibit nitrogen fixation. This should be considered when evaluating the net benefits of fertilization.

Francis (1982) studied the effects of soil acidity on mineralization, nitrification, nitrogen fixation and denitrification in forest soil. He suggested that increasing acidification of forest soils by acid precipitation will lead
to significant reductions in these microbial processes.

The removal of vegetation and the nutrients contained therein, creates an "ecosystem need". This need may be filled by the use of chemical fertilizers. The use of fertilizers may be unsuitable for economic and ecological reasons. The addition of nitrogen fertilizers may create deficiencies in other plant nutrients, and may also cause imbalances in the natural cycling of organic matter by changing the C/N ratio. "This could lead to a reduction in total nitrogen and organic matter content, so that long-term soil fertility would be decreased, even if a continuous supply of fertilizer were maintained" (Granhall, 1981).

Clearly, a better understanding of ANF and denitrification, and their role in forests, is needed. If artificial means for promoting forest productivity are to be used, the long term effects on the nitrogen cycle must be considered.
3. **OUTLINE OF RESEARCH**

In the fall of 1983 a study was begun, with the specific objectives of:

1. quantifying the net balance between gaseous N inputs to, and outputs from, a mature forest ecosystem and determining the relative significance of the processes contributing to this balance.

2. determining where in the ecosystem the gaseous N transformation processes occur and the relative significance of substrate to the rate at which they occur.

3. studying inputs and outputs in relation to season.

Based on the preceding literature review and some preliminary investigation in the summer of 1983, 11 strata were chosen for study. Foliage and bark of three tree species were sampled for nitrogen-fixing activity and forest floor, mineral soil and 3 classes of decaying wood were sampled for ANF and denitrification.

The ensuing report on the results of this study will include a description of the study area, a discussion of methods and a discussion of the results and their significance.
4. THE STUDY AREA

The University of British Columbia Research Forest is located 7 km. north of Haney, British Columbia, Canada, approximately 40 km. east of Vancouver, B.C. (Figure 2). The 5,151 hectare forest is managed by the Faculty of Forestry, UBC, as an experimental, research and demonstration facility. It was chosen to be the location of this study because:

1. It contained established forest ecosystems that would not be disturbed by management activity during the course of the study.
2. Its proximity to UBC where the analysis was done minimized travel time and handling of sample material.
3. A large amount of mensurational and ecological data exist for various parts of the forest.

The site chosen for this study is in the southern portion of the Research Forest approximately 2.5 km. northwest of the main entrance (Figure 3).

The Research Forest lies in the foothills which mark the transition from the Fraser Valley to the Coast Mountains. The climate, in general, is mesothermal, influenced by the Pacific Ocean to the west and modified by mountainous relief and the inland location in the lower Fraser Valley. The climate has been classified using the Köppen climatic classification as Cfb (Klinka, 1976), ie equable (marine) mesothermal humid to rainy. This is described as a maritime climate characterized by mild temperatures with common cloudiness and a small range of temperatures, wet and mild winters, cool and relatively dry summers, a long frost-free period, and heavy precipitation, most of which occurs during the winter. Mean monthly temperature and monthly precipitation for 1984 and 20 year average mean monthly temperature and monthly precipitation are presented in Table 1. The weather in 1984 was slightly warmer and slightly wetter than the 20 year
Figure 2. Access to the UBC Research Forest.
Figure 3. Location of the study site.
Table 1. Mean monthly temperature and monthly precipitation in 1984 and the 20 year average (last year 1975) for the UBC Research Forest, Marc Station.

<table>
<thead>
<tr>
<th>Month</th>
<th>Mean Daily Max. T. (°C) 20 yr Average</th>
<th>Mean Daily Max. T. (°C) 1984</th>
<th>Mean Daily Min. T. (°C) 20 yr Average</th>
<th>Mean Daily Min. T. (°C) 1984</th>
<th>Monthly Precip. (mm) Average 1984</th>
</tr>
</thead>
<tbody>
<tr>
<td>J</td>
<td>3.9</td>
<td>7.1</td>
<td>-1.6</td>
<td>1.9</td>
<td>289</td>
</tr>
<tr>
<td>F</td>
<td>6.6</td>
<td>9.2</td>
<td>0.2</td>
<td>3.8</td>
<td>219</td>
</tr>
<tr>
<td>M</td>
<td>8.4</td>
<td>12.7</td>
<td>0.8</td>
<td>3.3</td>
<td>231</td>
</tr>
<tr>
<td>A</td>
<td>12.4</td>
<td>13.9</td>
<td>3.3</td>
<td>3.0</td>
<td>154</td>
</tr>
<tr>
<td>M</td>
<td>16.8</td>
<td>15.0</td>
<td>6.6</td>
<td>5.5</td>
<td>111</td>
</tr>
<tr>
<td>J</td>
<td>19.4</td>
<td>18.1</td>
<td>9.4</td>
<td>9.3</td>
<td>92</td>
</tr>
<tr>
<td>J</td>
<td>22.6</td>
<td>23.4</td>
<td>10.9</td>
<td>10.9</td>
<td>68</td>
</tr>
<tr>
<td>A</td>
<td>21.9</td>
<td>22.5</td>
<td>10.9</td>
<td>11.0</td>
<td>80</td>
</tr>
<tr>
<td>S</td>
<td>19.2</td>
<td>18.5</td>
<td>8.8</td>
<td>8.4</td>
<td>128</td>
</tr>
<tr>
<td>O</td>
<td>13.2</td>
<td>11.9</td>
<td>5.6</td>
<td>4.5</td>
<td>244</td>
</tr>
<tr>
<td>N</td>
<td>8.0</td>
<td>7.8</td>
<td>1.8</td>
<td>2.3</td>
<td>275</td>
</tr>
<tr>
<td>D</td>
<td>5.3</td>
<td>3.2</td>
<td>0.0</td>
<td>2.6</td>
<td>315</td>
</tr>
</tbody>
</table>

Yearly Total 2206 2435
average.

The southern part of the forest has a submontane physiography, featuring flat to gently rolling terrain with a few granitic-cored uplands. The entire area is underlain by igneous intrusive rock, predominantly quartzdiorite. Soils have evolved from surficial deposits left by pleistocene glaciation, the most recent event occurring some 10,000 years ago. Glacial till and colluvium are the predominant parent materials (Klinka, 1976). The soil underlying the study site is a mini Humo-Ferric Podzol derived from reworked ablation till overlying basal till. It is a sandy loam in texture with 20-30% coarse fragment content overlain by a 5 cm thick mor humus layer. The study site is located at an elevation of 230 to 235 m asl with a southwest aspect and average slope of 5%.

Vegetation in the forest is dominated by temperate marine coniferous forests, with varying mixtures of Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco var. menziesii), western hemlock (*Tsuga heterophylla* (Raf.) Sarg.) and western redcedar (*Thuja plicata* Donn). A synsystematic classification of the UBC Research Forest has been carried out by Klinka (1976). The study site lies in a transition between his Moss(*Polystichum*)-WRC-WH and Moss-Mahonia-DF-WH plant associations. The vegetation present had developed by natural regeneration following a wildfire in 1868. The overstory was dominated by Douglas-fir while western hemlock and western redcedar occupied places in the lower tree layer. Figure 4 presents a distribution of tree diameters for the study site. The shrub layer was not well developed and higher shrubs were absent. The lower shrub layer (0 to 2 m.) covered approximately 25% of the area and was dominated by *Mahonia nervosa* Pursh with lesser amounts of *Gaultheria shallon* Pursh. Other species present included *Vaccinium parvifolium* Smith, *Acer circinatum*
Figure 4. Diameter distribution of trees in the 30m X 30m sample plot.
Pursh, *Tsuga heterophylla* and *Rosa nutkana* Presl. The herb layer was sparse covering 1 to 2% of the ground with *Polystichum munitum* (Kaulf.)Presl as the major species. Other herb species included *Dryopteris assimilis* Walker, *Trillium ovatum* Pursh, *Triteilalis latifolia* Hook, and *Tiarella trifoliata* L. *Stokesiella oregana* (Sull.)Robins. dominated a moss layer covering roughly 30% of the area, with *Plagiothecium undulatum* (Hedw.)B.S.G. and *Rhizomnium glabrescens* (Kindb.)Koponen common on decaying woody material. *Hylocomium splendens* (Hedw.)B.S.G. and *Rhytidiadelphus loreus* (Hedw.)Warnst. were also present.

Biomass data for the 30m. X 30m. study site are presented in Table 2. The phyllosphere was dominated by Douglas-fir(DFF), foliage biomass of this species amounting to 1,140 kg compared to 800 kg for western redcedar(WRCF) and western hemlock(WHF) combined.

Two species, Douglas-fir and western redcedar, comprised the decaying wood on the study site. These occur as deadfall on the forest floor and as standing stumps and snags. Douglas-fir wood was divided into two classes of decay. The first, referred to as 'white-rot'(WDF), corresponded to an incipient stage of wood decay. The other, referred to as 'red-rot'(RDF), corresponded to an advanced stage of wood decay. The primary decay agent was the brown crumbly rot, *Fomitopsis pinicola* (Schwartz:Fr.)Karst. The two classes of Douglas-fir wood accounted for 9,700 kg of biomass, in roughly equal proportions, while western redcedar(WRCW) accounted for 3,000 kg.

Klinka (1976) describes sites such as this one as having medium productivity, but being well suited for forestry use and suggests that they should be intensively managed as commercial forests.
Table 2. Volume, density and biomass data for the sample strata on the 30m X 30m study site.

<table>
<thead>
<tr>
<th>STRATUM</th>
<th>VOLUME(m³)</th>
<th>DENSITY(g cm⁻³)¹</th>
<th>DRY WEIGHT(kg)</th>
<th>DRY WEIGHT/HA(kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LFH</td>
<td>45</td>
<td>0.29</td>
<td>13,050</td>
<td>145,000</td>
</tr>
<tr>
<td>SOIL¹</td>
<td>270</td>
<td>0.99</td>
<td>159,840</td>
<td>1,776,000</td>
</tr>
<tr>
<td>Decayed Wood RDF</td>
<td>24</td>
<td>0.19</td>
<td>4,565</td>
<td>50,668</td>
</tr>
<tr>
<td>WDF</td>
<td>18</td>
<td>0.29</td>
<td>5,289</td>
<td>58,768</td>
</tr>
<tr>
<td>WRCW</td>
<td>12</td>
<td>0.26</td>
<td>3,065</td>
<td>34,050</td>
</tr>
<tr>
<td>Foliage DF</td>
<td>-</td>
<td>-</td>
<td>1,140</td>
<td>12,670</td>
</tr>
<tr>
<td>WRC</td>
<td>-</td>
<td>-</td>
<td>481</td>
<td>5,339</td>
</tr>
<tr>
<td>WH</td>
<td>-</td>
<td>-</td>
<td>322</td>
<td>3,580</td>
</tr>
</tbody>
</table>

¹ stratum symbols are explained on page 29 of the text.
² density is relative density for wood and LFH and bulk density for soil.
³ soil measurements are for the top 30 cm of mineral soil.
5. METHODS

Gaseous nitrogen fluxes were quantified for a 30m X 30m square plot in the UBC Research Forest. The plot was divided into strata based on ability to fix nitrogen and/or denitrify. The stratification was done with reference to the available literature and to some preliminary sampling done in the summer of 1983. Denitrification and ANF were measured bimonthly for one year beginning in January, 1984 and finishing in November, 1984. Air and soil temperatures on representative sampling days are presented in Table 3.

Measurement of gaseous nitrogen fluxes was done using the acetylene(C\textsubscript{2}H\textsubscript{2})-ethylene(C\textsubscript{2}H\textsubscript{4}) assay for nitrogen fixation (Hardy et al., 1973) and the acetylene inhibition method for denitrification (Yoshinari et al., 1977). These can be done simultaneously, limiting the amount of field sampling.

Nitrogen-fixing organisms transform acetylene to ethylene at a rate proportional to that at which N\textsubscript{2} is reduced to NH\textsubscript{3}:

\[ \text{N}_2 + 3\text{H}_2 \rightarrow 2\text{NH}_3 \]
\[ \text{C}_2\text{H}_2 + \text{H}_2 \rightarrow \text{C}_2\text{H}_4 \]

This suggests a conversion ratio of 3:1, but for a number of reasons "empirically determined ratios seldom equal the theoretical" (Roskoski, 1981; Rice and Paul, 1971). These reasons include:

1. C\textsubscript{2}H\textsubscript{2} is roughly 65 times as water-soluble as is N\textsubscript{2}. Hence, it may saturate the nitrogenase system more than would N\textsubscript{2} (Knowles, 1982b).
2. Oxygen, which is required for the production of ATP, may be depleted during long incubations.
3. As ethylene is not involved in metabolism, nitrogen deficiency may inhibit physiological processes.
Table 3. Air and soil temperature (12 noon) on sampling days.

<table>
<thead>
<tr>
<th>Month</th>
<th>Air Temp(°C)</th>
<th>Soil Temp(°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jan 25</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>Mar 6</td>
<td>12</td>
<td>5</td>
</tr>
<tr>
<td>May 8</td>
<td>10</td>
<td>7</td>
</tr>
<tr>
<td>Jul 4</td>
<td>17</td>
<td>11</td>
</tr>
<tr>
<td>Sep 12</td>
<td>11</td>
<td>11</td>
</tr>
<tr>
<td>Nov 6</td>
<td>9</td>
<td>6</td>
</tr>
</tbody>
</table>
The effects of these factors will be most significant when investigating material, such as forest soil, with low nitrogen-fixing activity, necessitating relatively long incubation times (Sprent, 1979). In some situations, such as in waterlogged soil, nitrogen diffusion may be the limiting factor for nitrogen fixation (Rice and Paul, 1971) and the higher solubility of acetylene will cause overestimates of nitrogen fixation rates. This illustrates the importance of calibrating the acetylene reduction assay for specific conditions. Silvester et al. (1982) investigated the relationship between acetylene and nitrogen reduction in decaying coniferous wood, using $^{15}$N. The ratio was found to increase with time, but averaged 3.5 when samples were incubated less than 7 hours. In this study an incubation time of 8 hours and the stoichiometric conversion ratio of 3:1 were used. The low rates of ANF encountered in this study minimize the potential inaccuracy of this assumption.

Ethylene may also be produced by plant tissue as a result of wounding, by contact between terminating acids and rubber stoppers and by microorganisms, particularly in anaerobic soils (Sprent, 1979). Tests for ethylene production in the absence of acetylene are necessary.

The measurement of $N_2$ evolution as a product of denitrification is made difficult by its occlusion into ambient atmospheric nitrogen (Yoshinari et al., 1977). The acetylene inhibition method for measuring denitrification is based on the principle that acetylene inhibits the reduction of $N_2O$ to $N_2$ (Yoshinari and Knowles, 1976). The evolution of $N_2O$ can then be measured using gas chromatography. From measurements of $N_2O$, estimates can be made of denitrification rate. A serious problem with this method is the report that acetylene inhibits nitrification, which yields $N_2O$ as a byproduct (Aulakh et al., 1982). In systems where nitrification is significant, this will
cause underestimates of N$_2$O production. Nitrification is also the major source of nitrate which may be rate-limiting in certain systems (Knowles, 1982a). However, in a study of northern hardwood forests, Melillo et al. (1983) found that N$_2$O production in the presence and absence of acetylene was not significantly different. This suggests that N$_2$ was not a significant product of denitrification in such systems and that the inhibition of nitrification had no major impact on denitrification.

Alternative methods involve the use of $^{15}$N-labelled tracer compounds. Methods using $^{15}$N have been criticized on the basis of the cost of the marking chemical and the cost and relative insensitivity of mass spectrometric analysis for field samples with low activity (Hardy et al., 1973; Yoshinari et al., 1977). It has also been demonstrated that denitrifying bacteria discriminate between naturally occurring $^{14}$N and isotopic $^{15}$N (Blackmer and Bremner, 1977). For these reasons, techniques involving the use of acetylene offer the easiest and simplest means of measuring ANF and denitrification (Focht, 1978; Hardy et al., 1973).

5.1 FIELD PROCEDURES
The 3 experiments to investigate foliage, bark and decaying wood were designed as randomised blocks. Each experiment involved 3 treatments (species or decay classification) and 6 blocks (month). The forest floor and mineral soil experiment was designed as a 2X6 randomised block but was not analyzed statistically. At each sampling period, 12 samples of each strata were collected. For foliage and bark, 8 samples were incubated with acetylene and 4 samples were incubated without acetylene to monitor endogenous ethylene production. For forest floor, mineral soil and decaying wood, 10 samples were incubated with acetylene and 2 samples without.
Sample sizes were halved for the January and March samplings, as no activity was expected.

Foliage samples were clipped and then wrapped in wet tissue to maintain turgor pressure. Samples were taken from branches on trees that could be reached from the ground. This sampling scheme raised questions as to whether older trees might have better developed lichen components in their crowns and therefore have a higher potential for nitrogen fixation. For this reason, an experiment was done in September/1984 using foliage from mature trees collected at a fresh logging area near the study site. Foliage was collected from the top and bottom of crowns of 3 trees of each of the 3 major species. This experiment was designed as a 3 (species) X 2 (top or bottom) randomised block, with variation between trees nested within species. The experiment was done in an incubation chamber, at room temperature under fluorescent and incandescent lamps, in an attempt to mimic summer conditions.

Bark samples were taken from the dead bark of mature, live trees. Mineral soil and forest floor samples were taken using trowel and shovel. Wood samples were taken from log sections using chainsaw and axe. Samples were taken randomly from all size classes of woody material.

Samples were all placed immediately into 1–L glass mason jars. The jars were equipped with rubber septa to facilitate acetylene amendments and gas sampling. The head space volume of the jar was then amended to 0.1 atm of acetylene using industrial grade acetylene. After allowing approximately 1 hour for equilibration, gas samples were taken using 5–mL vacuum tubes to determine initial N$_2$O and C$_2$H$_4$ concentrations. The jars were then left in the field under ambient conditions for the length of the incubations. Soil and wood samples were kept in boxes to prevent
exposure to light while foliage and bark samples were left on the forest floor to mimic light conditions within the forest.

After 8 hours, vacuum tube samples were taken to measure \( \text{C}_3\text{H}_4 \) production. Preliminary experimentation produced very low rates of denitrification, therefore an incubation time of 24 hours was used.

As the goal of this study was to produce information about real rates of gaseous nitrogen flux, incubations were done under conditions that mimicked natural conditions as closely as possible. Other than acetylene, no amendments or additions were made to the samples. Incubations were aerobic, as anaerobic incubations might exaggerate fluxes.

Two additional experiments were conducted to validate the low results of the denitrification assays. The first involved amending 6 forest floor samples with 60 ml of a 5 mg L\(^{-1}\) nitrate solution to determine if nitrate was rate limiting. The results were compared to denitrification rates for May and July using a \( t \)-test to determine if there was a significant effect. To further test the validity of the denitrification results, an experiment was done in which material known to denitrify, specifically stream sediments, was incubated. Four samples of stream sediment were placed in mason jars and immersed in water to mimic their natural environment. Other experimental procedures were as previously mentioned.

When the incubations were complete, sample wet weight, oven-dry weight, volume and % moisture content were determined.

5.2 LAB ANALYSIS

Analysis for \( \text{N}_2\text{O} \) was done using a Hewlett-Packard 5790A series gas chromatograph equipped with an Ni\(^{4+}\) electron capture detector and using a 1.8 m X 3 mm glass column packed with Porapak Q-5 (80/100 mesh).
Oven temperature was 60 °C, detector temperature was 250 °C and flow rate was 25 ml min⁻¹. Carrier gas was a 95% Argon, 5% Methane mixture. Calibration was by external standard using N₂O in air of 1.5 and 52.5 mg L⁻¹. Response was assumed to be linear based on the investigations of Kaspar and Tiedje (1982).

Concentrations of C₂H₂ and C₂H₄ were measured using a Hewlett-Packard 5830A series gas chromatograph using two 1.8 m X 3 mm stainless steel columns packed with Porapak N (80/100 mesh) and a flame ionization detector. Nitrogen (N₂) carrier gas flowed through the respective columns at rates of 30 and 37 ml min⁻¹. Oven temperature was 50 °C, injection temperature was 105 °C and detector temperature was 130 °C. Calibration was by external standard with a linear response assumed over the range of expected values.

5.3 CALCULATIONS
Gas chromatographic results for denitrification were expressed in mg L⁻¹. This value was multiplied by head space volume and divided by molecular weight and sample dry weight to produce a measure of denitrification rate in nmoles N g⁻¹ day⁻¹. This value was then corrected for dissolved N₂O as recommended by Moraghan and Buresh (1977).

Gas chromatographic results for acetylene reduction were expressed in nmoles ml⁻¹. This value was multiplied by head space volume and divided by sample dry weight to produce a measure of acetylene reduction in nmoles g⁻¹ per 8 hours. This value was multiplied by 3 to produce a per day value and then divided by 3 to convert to nitrogen fixation, producing a value in units of nmoles N g⁻¹ day⁻¹.
Mean rates of nitrogen fixation and denitrification in units of nmoles \(N \text{ g}^{-1} \text{ day}^{-1}\) were calculated and then converted to annual flux rates in units of kg N ha\(^{-1}\) a\(^{-1}\). Multiplying by molecular weight transforms nmoles to units of weight. The number of days of activity per year was calculated from the number of active months in the year-long sample. If 2 out of the 6 monthly samples were active, activity was assumed to occur on \(\frac{2}{6} \times 365\) days of the year. Multiplying by total biomass ha\(^{-1}\) then produced a measure of flux in kg N ha\(^{-1}\) a\(^{-1}\).

5.4 SITE QUANTIFICATION

Foliage biomass was quantified by measuring tree diameters and using regression equations for the UBC research forest relating foliage biomass of tree crowns to their diameters (Table 4). Bark surface area was not quantified as during the course of the experiment no nitrogen fixation was measured in this stratum.

Decayed wood volume was quantified using Smalian's formula,

\[ V = \frac{(A_1 + A_2) \times L}{2} \]

where measurements were made of: \(A_1\)=top end diameter

\(A_2\)=butt end diameter

\(L\)=section length

for each piece of decaying wood present on the study site. Volume was converted to dry weight using densities calculated from selected samples. Density was calculated as \(M/V\) where volume was determined by water displacement and mass was the oven-dry (105 °C) weight of the sample.

Forest floor and mineral soil were quantified by determining volume from horizon depth and area and then converting to dry weight using experimentally calculated densities. Bulk density was calculated for mineral
Table 4. Regression equations relating biomass of foliage (Y) to outside bark diameter at breast height (D), $ln(Y_{kg}) = a + b/nD_{cm}$.  

Source: M.C. Feller (unpublished data).

<table>
<thead>
<tr>
<th>Species</th>
<th>a</th>
<th>b</th>
<th>SE(ln units)</th>
<th>$r^2$</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>DF</td>
<td>-1.216</td>
<td>1.286</td>
<td>.216</td>
<td>.986</td>
<td>10</td>
</tr>
<tr>
<td>WRC</td>
<td>-3.869</td>
<td>2.100</td>
<td>.408</td>
<td>.930</td>
<td>12</td>
</tr>
<tr>
<td>WH</td>
<td>-4.130</td>
<td>2.128</td>
<td>.435</td>
<td>.960</td>
<td>18</td>
</tr>
</tbody>
</table>
soil and relative density for forest floor material. Forest floor volume was determined by water displacement while mineral soil volume was held uniform throughout the experiment. The top 30 cm. of the mineral soil horizon was considered as the area of active microbial activity, as nitrogen fixation has been shown to decrease significantly with depth (Baker and Attiwill, 1984).

Site quantification results and sample densities were presented in Table 2 (page 30).
6. RESULTS AND DISCUSSION

6.1 NITROGEN FIXATION

Nitrogen fixation rates and total annual fluxes are presented in Table 5. Fixation rates are indications of potential nitrogenase activity, based on acetylene reduction rates and the stoichiometric conversion ratio of 3:1. Endogenous ethylene production was not encountered during the course of this experiment.

6.1.1 FOREST FLOOR AND MINERAL SOIL

The most important substrate for nitrogen fixation was the forest floor, which accounted for almost 80% of a total measured input due to biological fixation of 0.8 kg N ha\(^{-1}\) a\(^{-1}\). This finding agrees with other authors (Silvester and Bennett, 1973; Granhall and Lindberg, 1980; Baker and Attiwill, 1984) who found the highest rates of nitrogen fixation in the organic horizons of coniferous forest soils. Forest floor material will have higher nutrient status, higher moisture content and greater available carbon than mineral soil.

Fixation rates in mineral soil were very low. The total input of nitrogen in this substrate is 0.06 kg N ha\(^{-1}\) a\(^{-1}\). This agrees with the reports of Roskoski (1980), Tjepkema (1979), and Granhall and Lindberg (1980) that mineral soil is a relatively insignificant source of nitrogen fixation in northern temperate forests.

An adequate supply of available carbon is often the limiting factor for nitrogen fixation (Sprent, 1979). Forest floor material will have a higher C/N ratio and lower O\(_2\) tension (ie higher moisture content) than mineral soil. Heterotrophic nitrogen fixation rates have
Table 5. Bimonthly nitrogen fixation rates and total annual fixation for 1984.

<table>
<thead>
<tr>
<th>STRATUM</th>
<th>JAN</th>
<th>MAR</th>
<th>MAY</th>
<th>JUL</th>
<th>SEP</th>
<th>NOV</th>
<th>Total Annual Fixation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>kg N ha⁻¹ a⁻¹</td>
</tr>
<tr>
<td>LFH</td>
<td>0.0</td>
<td>0.0</td>
<td>1.439(473)</td>
<td>0.55(16)</td>
<td>0.27(09)</td>
<td>0.23(05)</td>
<td>0.61</td>
</tr>
<tr>
<td>SOIL</td>
<td>0.0</td>
<td>0.0</td>
<td>0.02(01)</td>
<td>0.0</td>
<td>0.01(003)</td>
<td>0.0</td>
<td>0.06</td>
</tr>
<tr>
<td>n</td>
<td>5</td>
<td>5</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>Decayed Wood RDF</td>
<td>0.0</td>
<td>0.04(05)</td>
<td>0.27(08)</td>
<td>0.04(03)</td>
<td>0.05(02)</td>
<td>0.0</td>
<td>0.03</td>
</tr>
<tr>
<td>WDF</td>
<td>0.0</td>
<td>0.04(03)</td>
<td>0.08(03)</td>
<td>0.07(03)</td>
<td>0.01(01)</td>
<td>0.0</td>
<td>0.02</td>
</tr>
<tr>
<td>WRC</td>
<td>0.0</td>
<td>0.03(03)</td>
<td>0.05(02)</td>
<td>0.02(01)</td>
<td>0.10(04)</td>
<td>0.0</td>
<td>0.01</td>
</tr>
<tr>
<td>n</td>
<td>5</td>
<td>5</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>Foliage DF</td>
<td>0.0</td>
<td>0.18(18)</td>
<td>0.47(24)</td>
<td>0.67(29)</td>
<td>0.0</td>
<td>0.0</td>
<td>0.03</td>
</tr>
<tr>
<td>WRC</td>
<td>0.0</td>
<td>0.34(25)</td>
<td>0.13(09)</td>
<td>0.24(12)</td>
<td>0.0</td>
<td>0.0</td>
<td>0.01</td>
</tr>
<tr>
<td>WH</td>
<td>0.0</td>
<td>0.0</td>
<td>0.25(19)</td>
<td>0.29(15)</td>
<td>0.0</td>
<td>0.0</td>
<td>0.00</td>
</tr>
<tr>
<td>n</td>
<td>5</td>
<td>5</td>
<td>10</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>Bark DF</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>WRC</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>WH</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>n</td>
<td>5</td>
<td>5</td>
<td>10</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>TOTAL</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.78</td>
</tr>
</tbody>
</table>

-Standard errors in parentheses.
been correlated with concentrations of organic matter and moisture content and will correspondingly decrease with decreasing soil depth and decreasing soil moisture (Baker and Attiwill, 1984; Granhall and Lindberg, 1980).

Coniferous forest soils generally have low pH. The availability of molybdenum and phosphorus may limit nitrogen fixation under these conditions, as the availability of both these nutrients decreases rapidly with decreasing soil pH (Jurgensen and Davey, 1970).

Highest nitrogen fixation rates occurred in May (Figure 5), when moisture content was highest (Table 6). While nitrogen fixation is positively correlated with temperature, high summer temperatures often correspond to periods when moisture content is relatively low. Moisture content has been found to limit nitrogen fixation rates under apparently optimum temperature conditions (Baker and Attiwill, 1984). The absence of fixation in November when moisture conditions are favourable may be explained by higher mineral nitrogen availability in November than May. Fall increases in nitrate in streamwater have been observed by Feller and Kimmins (1984).

6.12 DECAYING WOOD

Nitrogen fixation by free-living bacteria in decaying wood was relatively insignificant (Table 3). Total annual input due to nitrogen fixation in decaying wood was only 0.06 kg N ha⁻¹ a⁻¹.

Because of the large number of inactive samples, the data had a skewed distribution and statistical analysis was invalid. Transformation failed to correct this problem. The data are compared visually in Figure 6. The greatest flux occurred in the month of May
Figure 5. Nitrogen fixation rates in mineral soil and forest floor for 1984.
Table 6. Average moisture content (by percent) of sample material.

<table>
<thead>
<tr>
<th>STRATA</th>
<th>JAN</th>
<th>MAR</th>
<th>MAY</th>
<th>JUL</th>
<th>SEP</th>
<th>NOV</th>
</tr>
</thead>
<tbody>
<tr>
<td>LFH</td>
<td>208</td>
<td>208</td>
<td>259</td>
<td>154</td>
<td>193</td>
<td>247</td>
</tr>
<tr>
<td>SOIL</td>
<td>22</td>
<td>20</td>
<td>23</td>
<td>21</td>
<td>18</td>
<td>26</td>
</tr>
<tr>
<td>Decayed Wood RDF</td>
<td>341</td>
<td>340</td>
<td>339</td>
<td>346</td>
<td>264</td>
<td>317</td>
</tr>
<tr>
<td>WDF</td>
<td>100</td>
<td>113</td>
<td>117</td>
<td>95</td>
<td>61</td>
<td>67</td>
</tr>
<tr>
<td>WRCW</td>
<td>76</td>
<td>89</td>
<td>148</td>
<td>113</td>
<td>151</td>
<td>63</td>
</tr>
</tbody>
</table>
when, as with forest floor, moisture content was high.

Highest rates of fixation occurred in Douglas-fir wood in the advanced stage of decay. Jurgensen et al. (1984) and Larsen et al. (1978) have reported that nitrogen fixation rates increase as wood decay progresses. Total carbohydrate and soluble sugar have been shown to decrease as wood decay progresses, while total and soluble nitrogen tend to increase, conditions which would tend to inhibit nitrogen fixation (Jurgensen et al., 1984). The increase in nitrogen fixation activity is probably attributable to increased moisture content in the more decayed wood (Table 4) and consequently reduced O₂ levels.

The fact that decay fungi can readily metabolize woody material, in spite of a high C/N ratio, suggests that sources of nitrogen input exist. Nitrogen fixation rates from 0.3 to 1.4 kg N ha⁻¹ a⁻¹ have been reported for decaying wood in northern temperate forests (Roskoski, 1980; Larsen et al., 1978; Larsen et al., 1982; Jurgensen et al., 1984). However, these results were achieved using anaerobic incubations, which may exaggerate the nitrogen fixation by anaerobic bacteria. Silvester et al. (1982) suggested that microaerophilic bacteria may be the most important agent facilitating nitrogen fixation in decaying wood. In an experiment comparing woody material incubated anaerobically and at different O₂ levels, nitrogen fixation rate was greatest under an atmosphere of 5% O₂. Still, Silvester et al. (1982) reported rates of nitrogen fixation in decaying Douglas-fir wood (4.8 nmoles N g⁻¹ day⁻¹, 1.4 kg N ha⁻¹ yr⁻¹) far greater than rates reported in this study. This discrepancy can, to some extent, be explained by differences in incubation temperature. Incubations in the
Figure 6. Nitrogen fixation rates in decaying wood for 1984.
Silvester study were done at 22°C, while in this study incubations were done under ambient conditions which never exceeded 17°C and were lower when moisture conditions were most favourable (Tables 3 and 6). Furthermore, Silvester et al. (1982) emphasized the importance of taking samples of large volume to reduce the surface area/volume ratio and prevent drying during the course of the incubation. In the experiment reported here, condensation was observed on the inside of the mason jars during incubations. This could have decreased nitrogen fixation rates by increasing oxygen exposure to nitrogen-fixing bacteria.

Nitrogen fixation may be less important than other inputs of nitrogen to woody substrates, notably crown wash and litter fall. However, nitrogen fixation may have a significant role in the microfloral and faunal succession in decaying wood and warrants careful study (Silvester et al., 1982).

6.1.3 FOLIAGE

Nitrogen fixation associated with foliage is responsible for an input of 0.04 kg N ha\(^{-1}\) a\(^{-1}\). These low rates are supported by the available literature (page 17). The rates reported here may, in fact, be exaggerated due to the fact that although incubations were done in daytime, fixation rates were assumed to be uniform for 24 hours. Stewart (1974) has reported that day-time nitrogen fixation rates are usually higher than night-time rates. Nitrogenase activity in blue-green algae living in association with sphagnum moss, was reported to respond dramatically to light (Granhall and Lindberg, 1980).
Species and time of year were compared in a randomised block design. The preponderance of inactive samples again caused the distribution to be skewed and statistical analysis was invalid. However, it appeared that nitrogen fixation rate was higher in Douglas-fir than in the other two species (Table 5, Figure 7). Fixation was relatively uniform from May to July. The drop in foliar fixation from July to September may be related to moisture stress and decreases in nutrient uptake by trees.

The presence of epiphytes with a nitrogen-fixing blue-green algae component may make the phyllosphere a significant source of nitrogen fixation (Denison, 1973; Millbank, 1978). Foliage samples for this study were collected primarily from younger trees that could be reached from ground level. This sampling scheme raised questions as to whether older trees might have better developed lichen components in their crowns and therefore have a higher potential for nitrogen fixation. An experiment was carried out in which foliage was collected from mature tree crowns at a fresh logging site. Species and crown position (upper half, lower half) were compared for significant differences (Table 7). The rate of nitrogen fixation in this foliage was similar to that in the younger foliage, supporting the claim that nitrogen fixation in foliage was not significant. The only significant difference found in this experiment was between species. Western hemlock was found to have the highest rate of fixation. In the previous experiment Douglas-fir was found to have the highest rate of nitrogen fixation, suggesting that these differences may be attributable to some cause other than an inherent difference between species or to experimental error.
Figure 7. Nitrogen fixation rates in foliage for 1984.
Table 7. Nitrogen fixation rates (nmols N g\(^{-1}\) day\(^{-1}\)) and total annual flux (kg N ha\(^{-1}\) a\(^{-1}\)) in foliage collected from mature tree crowns (A) compared with foliage collected at ground level (B).

<table>
<thead>
<tr>
<th>Species</th>
<th>Fixation Rate</th>
<th>Annual Flux in A</th>
<th>Annual Flux in B</th>
</tr>
</thead>
<tbody>
<tr>
<td>DF</td>
<td>0.138(.067)</td>
<td>0.001</td>
<td>0.028</td>
</tr>
<tr>
<td>WRC</td>
<td>0.353(.114)</td>
<td>0.001</td>
<td>0.006</td>
</tr>
<tr>
<td>WH</td>
<td>1.018(.259)</td>
<td>0.020</td>
<td>0.004</td>
</tr>
<tr>
<td>n</td>
<td>12</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TOTAL</td>
<td></td>
<td>0.022</td>
<td>0.038</td>
</tr>
</tbody>
</table>

- standard errors in parentheses.
6.1.4 BARK

Several writers have reported nitrogen fixation on tree boles (Todd et al., 1978; Granhall and Lindberg, 1980), and Millbank (1978) has confirmed that nitrogen-fixing lichens occur on coniferous tree trunks in the Pacific Northwest. However, in this study, no nitrogen fixation activity associated with bark was detected.

6.2 DENITRIFICATION

Denitrification results are summarized in Table 8. While the data suggest that some denitrification occurred, the total annual output of nitrogen due to denitrification was effectively 0.0 kg N ha\(^{-1}\) a\(^{-1}\). Rates reported for western redcedar wood and mineral soil were the result of only one active sample (n=10). Similarly, forest floor rates were the result of 3 active samples in May and two active samples in July (n=10, both months). Furthermore, it is unlikely that these measurements are accurate to the 4 decimal places reported. High standard errors support the hypothesis that high spatial variability in denitrification rates is typical even in cultivated systems (Robertson and Tiedje, 1984). Robertson and Tiedje (1984) suggested that denitrification is a highly variable process both among and within temperate forests. Their results, like the ones presented in this study, often consisted of several active samples and a large proportion of inactive samples which diluted the positive effects of active samples. The reasons for this high spatial variability are not well understood, although they may be related to the occurrence of anaerobic microsites and/or nitrification.

The large proportion of '0' results raised concern about the validity of the methods used in this study. To test these methods, stream sediments were assayed for denitrification potential. Denitrification rate in
Table 8. Bimonthly denitrification rates and total annual denitrification for 1984.

<table>
<thead>
<tr>
<th>STRATUM</th>
<th>JAN</th>
<th>MAR</th>
<th>MAY</th>
<th>JUL</th>
<th>SEP</th>
<th>NOV</th>
<th>Total Annual Flux (kg N ha(^{-1}) a(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>LFH</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0005(0.0003)</td>
<td>0.0002(0.0001)</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0003</td>
</tr>
<tr>
<td>SOIL</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0002(0.0002)</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0010</td>
</tr>
<tr>
<td>Decayed Wood RDF</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0000</td>
</tr>
<tr>
<td>WDF</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0000</td>
</tr>
<tr>
<td>WRCW</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0013(0.0013)</td>
<td>0.0002(0.0002)</td>
<td>0.0</td>
<td>0.0001</td>
</tr>
<tr>
<td>n</td>
<td>5</td>
<td>5</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>TOTAL 0.0014</td>
</tr>
</tbody>
</table>

- Standard errors in parentheses.
- n = number of samples per stratum.
this material was 4.93 nmoles N g\(^{-1}\) day\(^{-1}\) (standard error = 0.46) indicating the validity of the low rates determined for soil and decaying wood.

The absence of denitrification in decaying wood may be explained by low levels of mineral nitrogen in such environments. That denitrification did not occur in mineral soil may be explained by the relatively coarse texture of the soil, the lack of available organic carbon and/or the lack of nitrate. Using forest floor, where both carbon supply and moisture content should be suitable for denitrification, an experiment was carried out in which samples (n=6) were amended with 60 ml of a 5-mg L\(^{-1}\) nitrate solution. This treatment significantly increased denitrification rate (p ≤ 0.01) although denitrification rate was still relatively low at 0.012 nmoles N g\(^{-1}\) day\(^{-1}\). Assuming that denitrification occurs for 122 days a\(^{-1}\) this would yield a loss of 0.01 kg N ha\(^{-1}\) a\(^{-1}\). The results of this experiment tend to support the suggestion that "nitrate production may be a principle determinant of N\(_2\)O loss in unfertilized temperate forests" (Robertson and Tiedje, 1984).

Very little information about denitrification in forests exists. In general, forest soils with high levels of soluble organic carbon should provide a favourable environment for denitrification. Several recent studies (Melillo et al., 1983; Robertson and Tiedje, 1984) have suggested that nitrate production may be a dominant factor in controlling N\(_2\)O loss in forests. Melillo et al. (1983) studied denitrification potential over a successional sequence of hardwood stands in New Hampshire. In a 50+ year old stand, rates of nitrogen loss were 9 kg N ha\(^{-1}\) a\(^{-1}\) using anaerobic incubation and 1.4 kg N ha\(^{-1}\) a\(^{-1}\) using aerobic incubation. Melillo et al. (1983) suggested that the "true" rate lies somewhere between the two. Denitrification potential was highest in a recently logged stand. Robertson and Tiedje (1984) studied denitrification potential in a number of sites in Michigan...
forests and reported highest rates in sites with high potential for nitrification and respiration. The most active sites had potential flux rates of 2 to 12 kg N ha\(^{-1}\) a\(^{-1}\), although a number of sites had very low rates of denitrification (<0.1 kg N ha\(^{-1}\) month\(^{-1}\)). In both studies discussed here (Melillo et al., 1983; Robertson and Tiedje, 1984) denitrification was found to be highest in spring.

In forests, little nitrification is thought to occur in unamended systems (Keeney, 1980). Forestry practices such as clearcutting and slash burning will alter the physical and biological environment and may promote denitrification by increasing nitrate availability and soil saturation (Vitousek, 1981). Melillo et al. (1983) and Martin (unpublished) have demonstrated that denitrification may be more significant following clearcutting. Vitousek et al. (1982) suggested a model of nitrate mobility in forests where nitrate mobility in forests may be greater following clearcutting for two reasons; 1. mineralization rates are higher, 2. trees are not actively taking up nitrogen. As succession proceeds, competition for nitrogen should increase, populations of nitrifying bacteria may be outcompeted and plants may return to a predominantly ammonium-based nutrition (Vitousek et al., 1982). The implication is that as the site becomes fully occupied, forest trees will take up mineral nitrogen before nitrification takes place, thereby preventing denitrification losses. If nitrification is occurring, the forest may still be able to outcompete denitrifying bacteria for available nitrate. Significant increases in nitrate in soil leachate following clearcutting have been demonstrated in the UBC Research Forest (M. C. Feller\(^{1}\), pers. comm.).

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\(^{1}\)Assistant Professor, Faculty of Forestry, University of British Columbia, Vancouver, B. C.
Martin (unpublished) found increased nitrate fixation by anion absorption resin bags in forest floor, but not in forest floor leachate supporting the evidence that nitrate in forest floor solution was being denitrified. Martin (unpublished) has determined denitrification rates for a recent clearcut and an old growth forest on Vancouver Island, B.C. Rates in the clearcut (40 kg N ha\(^{-1}\) a\(^{-1}\)) were significantly greater than in the old growth forest (10 kg N ha\(^{-1}\) a\(^{-1}\)). However, old growth denitrification rates were much higher than the rates measured in this study. While some of this difference can be attributed to differences in methodology, they may also be due to stand dynamics. The old growth stand may become less efficient in taking up available nitrogen, allowing mineral nitrogen to become more accessible to nitrifying bacteria and consequently denitrifying bacteria.

The results presented in this study indicate that denitrification is an insignificant factor in the functioning of this ecosystem at its present state of maturity. However, any perturbation such as clearcutting, that might increase nitrate levels, might also increase the potential for denitrification. This potential may also be greater at future stages in stand development.

6.3 **TOTAL FLUX**

Figure 8 illustrates the total annual gaseous nitrogen flux for the study site. Denitrification rates were effectively zero (values are magnified 10X) while ANF accounts for an input of 0.8 kg N ha\(^{-1}\) a\(^{-1}\). This is comparable with other published data for northern temperate forests (Table 9).

This small input may be significant in balancing potential losses of nitrogen, and may contribute to a slow accumulation of nitrogen in this ecosystem. Feller and Kimmins (1984) reported nitrogen fluxes due to precipitation and streamwater runoff in the UBC Research Forest. Their data
Figure 8. Total annual nitrogen flux by strata.
Table 9. Asymbiotic nitrogen fixation rates in temperate forests.

<table>
<thead>
<tr>
<th>Forest type</th>
<th>Estimated kg N fixed ha(^{-1}) a(^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pine/spruce, 160 yrs old (Sweden)</td>
<td>3.8</td>
</tr>
<tr>
<td>Pine, 120 yrs old (Sweden)</td>
<td>0.3</td>
</tr>
<tr>
<td>Pine, 15–20 yrs old (Sweden)</td>
<td>0.3 (Granhall and Lindberg, 1980)</td>
</tr>
<tr>
<td>Pine (South Carolina)</td>
<td>1.0 (Jorgensen and Wells, 1971)</td>
</tr>
<tr>
<td>Deciduous (North Carolina)</td>
<td>12.0 (Todd et al., 1978)</td>
</tr>
<tr>
<td>Deciduous (Massachusetts)</td>
<td>0.2 (Tjepkema, 1979).</td>
</tr>
<tr>
<td>Deciduous, 4 yrs old (New Hampshire)</td>
<td>2.0</td>
</tr>
<tr>
<td>Deciduous, 57 yrs old (New Hampshire)</td>
<td>0.4</td>
</tr>
<tr>
<td>Deciduous, &gt;200 yrs old (New Hampshire)</td>
<td>1.6 (Roskoski, 1980)</td>
</tr>
<tr>
<td>Mixed conifer (British Columbia)</td>
<td>0.8 (this study)</td>
</tr>
</tbody>
</table>
suggest a net input of 3 kg N ha\(^{-1}\) a\(^{-1}\). Coupled with the input from ANF reported here this yields an annual input of 4 kg N ha\(^{-1}\) a\(^{-1}\). Over an 80 year rotation, this amount will more than compensate for losses due to log export. However, losses due to the combined treatment of log export and slash burning may result in a net loss of nitrogen (Feller and Kimmins, 1984, Table 8).

The assumption that flux rates are uniform during succession may not be justified. Some of the evidence discussed in this thesis allows us to speculate on changes in gaseous nitrogen flux during secondary forest succession. Similar successional trends can be suggested for both processes. Flux will be greatest following clearcutting, decrease as succession proceeds and then increase, as the forest degrades in the later stages of succession. Vitousek and Reiners (1975) have hypothesized that nitrate losses may be controlled by net ecosystem productivity which shows a pattern similar to that described above. Evidence has been cited for increases in ANF (Roskoski, 1980) and denitrification (Melillo et al., 1983; Martin, unpublished) following clearcutting. Nitrate availability is increased following clearcutting due to vegetation removal and/or increased nitrification. As succession proceeds, competition for nitrogen will increase and populations of nitrifying bacteria may be outcompeted for available mineral nitrogen. As the forest grows old and becomes less productive due to senescence and mortality, nitrate may again become more readily available for denitrification.

Gorham et al. (1979) have suggested a similar trend for ANF, related to the availability of phosphorus, an important requirement for biological nitrogen fixation (Granhall, 1981). There is speculation that the availability of phosphorus in forms available to plants decreases as succession
Table 10. Nutrient losses, nutrient inputs in precipitation and nutrient reserves for logged (A), logged and slash burned (B) and undisturbed (C) watersheds in the UBC Research Forest. Values are in kg/ha for the two year period 1973–1975.

<table>
<thead>
<tr>
<th></th>
<th>A</th>
<th>B</th>
<th>C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Streamwater export</td>
<td>11</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Log export</td>
<td>234</td>
<td>308</td>
<td></td>
</tr>
<tr>
<td>Atmospheric export</td>
<td>-</td>
<td>982</td>
<td></td>
</tr>
<tr>
<td>Total export</td>
<td>245</td>
<td>1293</td>
<td>1</td>
</tr>
<tr>
<td>Forest floor content</td>
<td>1632</td>
<td>2180</td>
<td>1490</td>
</tr>
<tr>
<td>Mineral soil content</td>
<td>4566</td>
<td>4647</td>
<td>3924</td>
</tr>
<tr>
<td>Total reserve</td>
<td>6198</td>
<td>6827</td>
<td>5414</td>
</tr>
<tr>
<td>Average annual precipitation input</td>
<td>4</td>
<td>4</td>
<td>4</td>
</tr>
</tbody>
</table>

proceeds (Gorham et al., 1979). Similar to nitrogen, phosphorus availability will be greatest following clearcutting. ANF may also be higher after clearcutting and in old growth forests because of an increase in the accumulation of organic matter on the site (Roskoski, 1980). Increases may also be related to net ecosystem productivity as nutrients important for nitrogen fixation may become more readily available as the forest becomes less efficient.

Nitrogen fixation after clearcutting may help to offset potentially high nitrogen losses due to denitrification and leaching. As the site becomes reestablished, denitrification will become less significant and ANF may make a modest contribution to reestablishing the nitrogen pool.

6.4 SOURCES OF ERROR

During the course of this experiment a number of potential sources of error were considered.

The decision to use the stoichiometric conversion factor of 3:1, for acetylene reduction to nitrogen fixation was based on the work of Silvester et al. (1982). Experimentally-determined ratios generally vary from the theoretical suggesting that comparative $\text{C}_2\text{H}_2-^{15}\text{N}$ fixation studies should be done (Hardy et al., 1973; Roskoski, 1981). Because of the low fixation rates encountered in this study, it was felt that the use of the theoretical ratio would not create too great an inaccuracy. Using a ratio of 4:1, total nitrogen fixation in this study would be 0.6 kg N ha$^{-1}$a$^{-1}$ while using a ratio of 2:1 would result in a total flux of 1.2 kg N ha$^{-1}$a$^{-1}$. Silvester et al. (1982) found the ratio to be 3.5:1 when incubations were kept under 7 hours. Using this ratio, total flux in this study would be 0.7 kg N ha$^{-1}$a$^{-1}$. 
The incubation technique necessitates disturbing samples. Wood samples were cut from larger pieces and fragmented to make them fit into the incubation chambers. Similarly, mineral soil and forest floor samples were sampled destructively. These disturbances result in increased surface area/volume and consequently a greater exposure to $O_2$ than might occur under natural conditions. Although aerobic incubations were done to avoid overestimating these reduction processes, aerobic incubation may, in turn, underestimate gaseous fluxes. Silvester et al. (1982) found acetylene reduction rate to be higher under low $O_2$ concentrations than under ambient or anaerobic conditions. Oxygen diffusion is strongly affected by moisture content of the material. Increased surface area/volume will make the sample material more susceptible to drying and may cause artifactual effects.

Future studies of denitrification should consider doing incubations with and without acetylene to determine the full effect of inhibiting nitrification. If nitrification/ denitrification processes are coupled (Knowles, 1978), inhibition of nitrification will uncouple these processes and effectively halt denitrification. In addition, nitrification may produce significant quantities of $N_2O$ in some systems (Aulakh et al., 1982).

Both the acetylene reduction assay and the acetylene inhibition method are indications of nitrogen transformation at a specific point in time. Extrapolating nitrogen fixation rates measured over 8 hours to a per day measurement will cause a decrease in accuracy. Extrapolating flux rates per day to rates per month or year will create an even greater inaccuracy. The high spatial variability reported in this study and elsewhere (Robertson and Tiedje, 1984) suggest that this source of error should not be overlooked.
The methods used in this study are inexpensive and easy to use, but are not ideal. Work directed to improving these methods or developing other techniques should be continued.
7. SUMMARY AND CONCLUSIONS

Evidence has accumulated indicating the importance of biological nitrogen transformations in forest ecosystems (Todd et al., 1975). Increased demand for wood fibre and fuel wood throughout the world and the realization that supplies of cheap chemical nitrogen fertilizers are not inexhaustable have kindled interest in biological nitrogen fixation as a means of augmenting nitrogen supplies in biological systems.

Ecosystem studies have suggested that biological nitrogen fixation may be a major source of nitrogen accumulated in forest biomass and the forest floor during succession (Borman et al., 1977; Todd et al., 1975). While nitrogen inputs due to asymbiotic nitrogen fixation are generally small, coupled with inputs of nitrogen by bulk precipitation, they may be significant in contributing to nitrogen accretion in forests and offsetting nutrient losses due to log export and/or slash burning (Feller and Kimmins, 1984).

Interest exists in denitrification because it is a major mechanism of fertilizer nitrogen loss (Rolston, 1981) and it contributes $N_2O$ to the atmosphere where it is involved in stratospheric reactions which result in the depletion of ozone (Knowles, 1982a). Denitrification in forests is generally thought to be minimal because it does not occur without nitrate and little nitrification normally occurs in undisturbed systems (Keeney, 1980). However, clearcutting may significantly increase nitrate availability (Feller, pers. comm.) and denitrification (Martin, unpublished; Melillo et al., 1983).

The objectives of this study were to quantify gaseous nitrogen fluxes due to asymbiotic nitrogen fixation and denitrification in a mature coniferous forest. Eleven strata were sampled bimonthly for the year of
1984. Gaseous nitrogen losses due to denitrification were effectively zero. Forest floor material was responsible for 80% of a nitrogen input of 0.8 kg N ha\(^{-1}\) a\(^{-1}\). Nitrogen fixation in decaying wood and foliage was relatively small. Fixation rates were greater in more decayed wood, probably due to its greater moisture holding capacity.

Assuming an equal rate of nitrogen fixation over the course of an eighty year rotation, a net input of 64 kg of nitrogen will result. While this amount is small it may contribute to balancing nitrogen losses due to log export, slash burning and other forest management treatments. This input also has the advantage of occurring as a steady accumulation of slowly released, easily available nitrogen (Granhall and Lindberg, 1980).

Future research on ANF and denitrification should focus on the effects of site treatments such as clearcutting, slash burning, scarification and fertilization. Changes in rates of ANF and denitrification may also occur during the course of forest succession. Research must also focus on improving techniques for measuring both processes. The use of acetylene provides an inexpensive and easy method for measuring ANF and denitrification but the potential for inaccuracies exists.

Industrial nitrogen fixation has increased the fixed pool of nitrogen in terrestrial environments (Delwiche, 1981). While there is no concern that atmospheric nitrogen will be depleted, there is concern regarding the increase of nitrates in food and water and increased N\(_2\)O concentrations in the atmosphere.

Nitrogen fertilizers are becoming increasingly expensive (Beuter, 1979) and are subject to potentially high denitrification losses. Biological nitrogen fixation offers an interesting alternative to the forest manager, as a means of maintaining or enhancing forest productivity.
Our knowledge of biological nitrogen transformations such as asymbiotic nitrogen fixation and denitrification is incomplete. The role of these processes in forests, their response to treatment and their relationship to each other and other nitrogen transformations warrants further attention.
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