AN ABSTRACT OF THE THESIS OF

James Y. Tang for the degree of Master of Science in Soil Science presented on October 8, 1997. Title: Nitrogen Fixation and Cycling in a Mixture of Young Red Alder and Douglas-fir

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Abstract approved: _____

David D. Myrold

Red alder (*Alnus rubra* Bong.) plays an important role in many Pacific Northwest forest ecosystems because of its input of N, a major limiting factor for the growth of Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco). Although many studies of red alder have been done, few have studied young (< 10 years old) stands. This thesis evaluated the ^{15}N dilution method and the effects of young red alder on soil N dynamics. The soil around pairs of red alder and Douglas-fir trees was labeled with $(^{15}NH_4)_2SO_4$ to measure N_2 fixation by ^{15}N dilution. We found that, when N_2 fixation rate is high, the atom % ^{15}N of red alder foliage was not affected by the ratio of labeling area to tree crown area (LA/CA) but that of Douglas-fir was. The roots of Douglas-fir trees were concentrated mainly within 1.5 crown areas. The percentage of N derived from N_2 fixation (PNDF) of red alder was estimated to be over 95 % and was not very sensitive to reference plant selection. The total N derived from N_2 fixation (TNDF) was estimated to be about 22 kg N ha^{-1} yr^{-1} for mixed stands (25% of red alder and 75%

Douglas-fir) and about 90 kg N ha^{-1} yr^{-1} for pure red alder stands. This study was the first to use the ^{15}N dilution method to estimate the PNDF and TNDF of young red alder trees under field conditions in the Pacific Northwest. Our estimated PNDF and TNDF of red alder were in good agreement with previous studies. The evaluation of the PNDF sensitivity to reference plant selection and to LA/CA provides important information for future N_2 fixation studies of red alder using the ^{15}N dilution method. Periodic soil sampling and resin core incubation showed no significant differences in soil inorganic N, net N mineralization, and net nitrification under red alder and Douglas-fir trees, probably because of the young age of the red alder trees and the close spacing of red alder and Douglas-fir seedlings. Ammonium was found to be the main form of soil inorganic N. Plant N uptake of red alder and Douglas-fir showed a seasonal pattern, spring > summer/fall > winter. Uptake of N by Douglas-fir as measured by using resin cores was in good agreement with that calculated from its allometric regression equation, which suggested that the in situ resin core incubation technique is a reliable method for estimating plant N uptake in the field.

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Nitrogen Fixation and Cycling in a Mixture of Young Red Alder and Douglas-fir

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James Y. Tang

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For their love, care and moral support throughout my many years

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Chapter I

Introduction

I-1. Topics of Study

The general goal of this study was to advance the state of knowledge of N cycling in red alder (*Alnus rubra* Bong.), of which two topics were selected for this thesis. The first evaluated the ^{15}N dilution method and estimated the percentage of N derived from the N_2 fixation (PNDF) by young red alder. This was important because of the increasing concerns on the selection of a reference plant in ^{15}N dilution method (Chalk, 1985; Fried et al., 1983; Witty, 1983) and the lack of studies that have measured PNDF by young red alder in the Pacific Northwest. An accurate and reliable method to measure PNDF of young red alder is certainly important in estimating the annual N_2 fixation input by red alder to the soil ecosystem.

The second topic was to measure soil net N mineralization, nitrification, and plant N uptake in young red alder and Douglas-fir stands, targeting the effects of red alder on soil N dynamics. This was to provide an answer to an important open question of whether the extra input N from young red alder would affect the soil inorganic N pools and speed up the N processes.

I-2. Roles of the N_2 Fixation by Red Alder in the Soil Ecosystem

Nitrogen is thought to be a major limiting factor for the growth of economically important Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) (Gessel et al., 1969) and to be the most commonly limiting nutrient in the Northwest Pacific region (Gessel et al., 1973). Previous studies showed that red alder can significantly increase the growth rate of associated trees or later planted trees (Dawson and Funk, 1981; Tarrant, 1961; DeBell and Radwan, 1979; Kurdali et al., 1990) and serve as a "biological barrier" to the spread of the disease caused by a root rot fungus *Phellinus weirii* (Nelson et al., 1978). Because of the large amount of N input from fallen N-rich alder leaves and from root and nodule turnover following decay and regeneration (Cote and Camire, 1985; Hansen and Dawson, 1982), soils under alders usually have more total N and inorganic N than soil under conifers (Bollen and Lu, 1968; Tarrant and Miller, 1963). Some studies showed that the symbiotic N_2 fixation between red alder and *Frankia* can increase the N mineralization and nitrification rates compared to Douglas-fir stands (Binkley et al., 1993; Van Miegroet et al., 1989; Van Miegroet et al., 1990). However, other studies indicated that soil inorganic N, net mineralization, and nitrification were not different under alder and Douglas-fir or associated trees (Cole and Newton, 1986; Cole et al., 1993; Van Miegroet et al., 1993).

I-3. Methods to Measure the Rates of N_2 Fixation by Red Alder

Previous estimates of annual N_2 fixation input by red alder in the Pacific Northwest forests have ranged more than ten-fold, from 23 to 320 kg N ha^{-1} yr^{-1} (Binkley et al., 1994). This variation is partly caused by using different measuring methods.

There are five approaches that have been used to estimate rates of N_2 fixation by alder: (1) N accretion, (2) acetylene reduction, (3) ^{15}N natural abundance, (4) $^{15}N_2$ reduction, and (5) ^{15}N dilution. Each method has its own advantages and disadvantages under some circumstances.

The N accretion approach has the advantage of integrating the rate of N accumulation over time, however, it does not actually estimate N_2 fixation, just the net effect of all N inputs and outputs in red alder stands.

The acetylene reduction method proposed by Burris (1974), is well suited to laboratory studies and is useful for measuring short-term temporal dynamics. However, disturbance of nodules usually decreases nitrogenase activity and the error in estimating total nodule biomass on an areal basis can be very large. Similarly, a yearly estimate of the N_2 fixation integrated from hourly or daily rates of acetylene reduction requires extensive temporal sampling to provide a good yearly estimate.

The ^{15}N natural abundance method, first used by Bardin et al. (1977), has the advantage of being an integrative measure to estimate annual N_2 fixation without any disturbance of the ecosystem. It is, however, restricted by the requirement of a large

difference in ^{15}N abundance between the soil N and atmospheric N_2, the selection of a suitable reference plant, and the high cost of high-resolution mass spectrometry.

The $^{15}N_2$ reduction method represents a powerful tool in the fundamental research on many qualitative and quantitative aspects of the translocation and fate of biologically fixed N because of its direct measurement of N_2 fixation. The use of this method is, however, limited due to its destructive, complicated, and repetitive sampling over long-term experiments.

The ^{15}N dilution method, first described by McAuliffe et al. (1958), is integrative and non-destructive, and gives good estimates of N_2 fixation under controlled conditions in both lab and field study (Domenach and Kurdali, 1989b; Sougoufara et al., 1990). However, the selection of a reference plant with a similar pattern of soil N uptake as the N_2-fixing plant is the most important factor affecting the accuracy and sensitivity of the method (Witty, 1983; Fried et al., 1983; Sanginga et al., 1990). The absence of non-nodulated isolines of alder and the ubiquitous nature of *Frankia* have made the selection of the non-fixing reference tree very difficult in the field study. Because of the high costs and limitations of the ^{15}N dilution method, there have no field studies using the ^{15}N isotope dilution to measure the PNDF of young red alder in the field, to evaluate the effect of the selection of a reference tree on PNDF.

I-4. Objectives

Each of the two chapters has specific objectives. The objectives of the research reported in Chapter II were to evaluate the importance of the selection of a reference plant in the ^{15}N dilution method, to use the ^{15}N dilution method to estimate the PNDF of young red alder trees (7- to 8-year-old) under field conditions, and to estimate the total N derived from N_2 fixation (TNDF) by red alder in stands containing young red alder and Douglas-fir in the Cascade Range of Oregon. The objectives of the research reported in Chapter III were to investigate the effect of interplanted red alder on soil inorganic N pools, net N mineralization, and net nitrification in red alder/ Douglas-fir mixtures.

Chapter II

Using ^{15}N Dilution to Measure the Annual N_2 Fixation Rate of a Young Red Alder Stand in Oregon

James Y. Tang and David D. Myrold

II-1. Abstract

Previous estimates of annual N_2 fixation input by red alder (*Alnus rubra* Bong.) in Pacific Northwest forests have ranged more than ten-fold, from 23 to 320 kg N ha^{-1} yr^{-1}. This variation is partly caused by using different measuring methods. This study was designed to evaluate the ^{15}N dilution method, to use it to estimate the percentage of N derived from N_2 fixation (PNDF) by 7- to 8-year-old red alder trees under field conditions, and to estimate the total N derived from N_2 fixation (TNDF) in stands containing young red alder in the Cascade Range of Oregon. Soils under paired red alder and Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) trees were labeled with ^{15}N fertilizer at several ratios of labeling area to tree crown area (LA/CA). Foliage samples were collected and analyzed for atom % ^{15}N. Allometric regression equations were developed to calculate red alder biomass and used to estimate the TNDF by red alder. When the N_2 fixation rate of young red alder is high, the foliage atom % ^{15}N of red alder was not affected by LA/CA but Douglas-fir was. The PNDF by red alder was estimated to be over 95 % and TNDF was estimated to be about 22 kg N ha^{-1} yr^{-1} for mixed stands (25% of red alder and 75% Douglas-fir) and about 90 kg N ha^{-} yr^{-1} for pure red alder stands. This study was the first to use the ^{15}N dilution method to estimate PNDF and TNDF of the young red alder trees under field conditions in the Pacific Northwest. Our estimated PNDF and TNDF of red alder were in good agreement with previous studies. The evaluation of the PNDF sensitivity to reference plant selection and to LA/CA provides important information for future N_2 fixation studies of red alder using the ^{15}N dilution method.

II-2. Introduction

Red alder (*Alnus rubra* Bong.) is a major deciduous (hardwood) forest tree of the Pacific Coast of the United States and Canada. Red alder is well known for its capacity to fix atmospheric N_2 and to dominate sites on which growth of conifers is desired. Red alder had been described variously as a pioneer species with a major role in soil development, a source of protection for emerging conifers, a commercial forest type, and a brush species of no appreciable beneficial impact (Neal et al., 1965). Not many years ago, red alder was regarded by most the Pacific Northwest forest managers as a weed to be eradicated with herbicides. Today, red alder provides the raw material for a thriving industry, producing fine furniture, cabinetry, specialized veneers and plywoods, shipping pallets, and paper products.

The ability of alder (*Alnus spp.*) to enrich soils has been recognized for centuries (Tarrant and Trappe, 1971). The symbiosis between red alder and *Frankia*, a N_2-fixing actinomycete, provides significant additional input of N to soils. The benefits of using alders to enrich forest productivity are most clearly seen in studies of short-rotation interplantings of alders with other trees (Nelson et al., 1978). For example, *Populus*-alder mixes yielded 71% greater biomass than when *Populus* was grown alone (DeBell and Radwan, 1979). This additional biomass came solely from the *Populus*, suggesting a response to N added by the alder. Similar results were obtained by Kurdali et al. (1990), who also measured an increase in the proportion of alder N derived from N_2 fixation. These results not only show that interplanting alders can provide a N

fertilizing effect, but suggest that the mixtures act synergistically with the non-fixing trees scavenging more soil N, which stimulates the alder to fix more N_2. Studies also have shown that red alder is resistant to conifer root diseases and can be used in alternate rotations with conifers to suppress root rot fungi or landed as a "biological barrier" to the spread of disease (Nelson et al., 1978).

There are five approaches that have been used to estimate rates of N_2 fixation by alder: (1) N accretion, (2) acetylene reduction, (3) ^{15}N natural abundance, (4) $^{15}N_2$ reduction, and (5) ^{15}N dilution.

The accumulation of N can be measured either by comparing the N content of stands belong to a chronosequence or by following the N content of a stand through time. The time sequence method involves contrasting the total N content of an ecosystem at two points in time, any increase (or accretion) of N during the period is used as an estimate of N_2 fixation. A variation on the accretion method uses a time sequence of plots at different locations to approximate the accretion that would be observed in a single site through time. It was found that the greater N content of red alder relative to conifer forests gave an average annual soil accretion over 40 years of 140 kg N ha^{-1} in the mineral soil to a depth of 0.9 m at Cascade Head, Oregon, and 27 kg N ha^{-1} at Widow Creek, Oregon (Franklin et al., 1968). Other scientists have also used the N accretion method to estimate N_2 fixation rates by red alder (Table II-1): in pure stands ranging from 50 to 320 kg N ha^{-1}; in red alder/conifer mixed stands ranging from 0 to 73 kg N ha^{-1}. The N accretion approach has the advantage of integrating the

Table II-1. Field estimates of N_2 fixation rates by red alder in Pacific Northwest forests.

Location	Age	Method	Rate	Reference
	yr		kg N ha^{-1} yr^{-1}	
Red alder				
Western Oregon	0-30	Accretion, plants, forest floor, soil to 0.6 m	320	Newton et al., 1968
Cascade Head, OR	0-40	Accretion, mineral soil to 0.9 m	140	Franklin et al., 1968
Widow Creek, OR	0-30	Accretion, mineral soil to 0.9 m	27	Franklin et al., 1968
Hoh River, WA	0-14	Accretion, forest floor, soil to 0.6 m	164	Luken and Fonda, 1983
	15-24		58	
	25-65		25	
Thompson Res. Site, WA	0-38	Accretion, plants, forest floor, soil to 0.6 m	85	Cole et al., 1978
	0-50		78	Van Miegroet et al., 1989
Western Washington	0-40	Accretion, forest floor, soil to 0.2 m	50	Bormann and DeBell, 1981
Centralia, WA	0-30	Accretion, soil to 0.18 m	59-65	Heilman, 1982
Centralia, WA	0-4	Acetylene reduction	150	Heilman and Ekuan, 1982
West Washington	2-4	Acetylene reduction	62	Tripp et al., 1979
Lady Island, WA	0-4	Accretion, soil to 0.15 m	80	DeBell and Radwan, 1979
Rainier, OR	5	Acetylene reduction	60-80	Bormann and Gordon, 1984
Red alder / conifer				
Cascade Head, OR	0-40	Accretion, forest floor, soil to 0.9 m	28	Franklin et al., 1968
	0-55	Accretion, plants, forest floor, soil to 0.9 m	73	Binkley et al., 1993
	55	Acetylene reduction	85	Binkley et al., 1993
Wind River	0-26	Accretion, soil to 0.9 m	40	Tarrant and Miller, 1963
	0-55	Accretion, plants, forest floor, soil to 0.9 m	54	Binkley et al., 1993
	55	Acetylene reduction	75	Binkley et al., 1993
Mt. Benson, B. C.	0-23	Accretion, plants, forest floor, soil to 0.5 m	65	Binkley, 1983
	23	Acetylene reduction	130	Binkley, 1981
Skykomish, WA	0-23	Accretion, plants, forest floor, soil to 0.6 m	42	Binkley, 1983
Western Oregon	0-17	Accretion, soil to 0.15 m	13-51	Berg and Doerksen, 1975
Coast Range, OR	0-4	Accretion, soil to 0.15 m	0	Cole and Newton, 1986
Red alder / poplar				
Toutle River, WA	0-6	Accretion, forest floor, soil to 0.3 m	56	Heilman, 1990
Lady Island, WA	0-4	Accretion, soil to 0.15 m	32	DeBell and Radwan, 1979

rate of N accumulation over time. It should be recognized, however, that this method does not actually estimate N_2 fixation, just the net effect of all N inputs and outputs.

The acetylene reduction method, proposed by Burris (1974), is a sensitive and relatively simple measure of nitrogenase activity. The acetylene reduced by nitrogenase activity of nodules is used to calculate the rate of the N_2 fixation. Because individual nodules are measured, the total nodule biomass on an areal basis must be scaled up to a per-hectare figure. Similarly, the hourly or daily rate of acetylene reduction must be integrated over time to get a yearly measurement. Tripp et al. (1979) used the acetylene reduction method to measure N_2 fixation rates of 2- to 4-year-old red alder seedlings in Washington and obtained a N_2 fixation rate of about 62 kg N ha^{-1} yr^{-1}. Heilman and Ekuan (1982) used the same method to measure the N_2 fixation rates of young red alder seedlings in the same area and got an estimate of 150 kg N ha^{-1} yr^{-1}. The difference in these acetylene reduction estimates of N_2 fixation came primarily from the greater nodule biomass per tree (about 270 kg ha^{-1} compared with 40 kg ha^{-1} in former study). For 20-year-old black alder (*Alnus glutinosa* (L.) Gaertn.) stands, Akkermans and van Dijk (1976) found that the contribution of symbiotic N_2 fixation to range from 22 % to 64 % of the total annual N demand.

The acetylene reduction method is well suited to laboratory studies and is useful for measuring short-term temporal dynamics. However, the disturbance of nodules greatly affects (usually decreases) nitrogenase activity and the error in estimating of total nodule biomass on an areal basis can be very large. Similarly, a

yearly estimate of the N_2 fixation integrated from hourly or daily rates of acetylene reduction requires extensive temporal sampling to provide a good yearly estimate.

The ^{15}N natural abundance method uses the natural variations in ^{15}N that result from isotope discrimination between the atmosphere, plant, and soil to estimate the fractional contribution of the atmosphere N and soil N to the plant. Due to the isotope discrimination, the soil ^{15}N natural abundance is often higher than the atmosphere ^{15}N natural abundance, therefore the natural abundance of N_2-fixing plant is somewhere between the soil ^{15}N natural abundance and the atmosphere ^{15}N natural abundance. How close the ^{15}N natural abundance of N_2-fixing plant is to the atmosphere ^{15}N natural abundance reflects the fractional contribution of the atmosphere N to the plant. The PNDF of a N_2-fixing plant can be calculated by the following equation (Bardin at al., 1977):

$$PNDF = [\, \delta\, {}^{15}N_{non\text{-}fixing} - \delta\, {}^{15}N_{fixing} \,/\, \delta\, {}^{15}N_{non\text{-}fixing} - \delta\, {}^{15}N_{fixing\ in\ N\text{-}free\ medium} \,] * 100$$

Where $\delta\, {}^{15}N$ is the part per thousand deviation in ^{15}N relative to the natural abundance of ^{15}N.

Studies of red alder stands in Pacific Northwest forests demonstrated that the PNDF were above 90 % using the ^{15}N natural abundance method (Binkley et al., 1985). The PNDF of a mature black alder stand (*Alnus glutinosa* (L.) Gaertn.) based on ^{15}N natural abundance was estimated to be 94 % (Beaupied et al., 1990), which was in

agreement with those of Domenach and Kurdali (1989b) for gray alder (*Alnus incana*) using the same method under semi-controlled conditions and in a climax community.

The ^{15}N natural abundance method has the advantage of being an integrative measure to estimate annual N_2 fixation without any disturbance of the ecosystem. However, it can only be used when certain conditions exist. First, the difference in ^{15}N abundance between soil N and atmospheric N_2 must be sufficiently great. This is not true for all sites and is probably less likely to be the case at sites with a long history of sizable N_2 fixation inputs. Second, suitable reference plants must be available so that the ^{15}N abundance of soil N available for plant uptake is known. Lastly, this method depends upon being able to detect extremely small (parts per thousand) differences in ^{15}N abundance, which can be technically difficult.

Soon after ^{15}N became available, $^{15}N_2$ was used to provide evidence for N_2 fixation (Burris and Miller, 1941). The principle of the N_2 reduction method is to use $^{15}N_2$ as a tracer to label the plant. The whole plant is exposed to an atmosphere enriched in $^{15}N_2$ for a specified period of time, the ratio of the atom % ^{15}N excess in the plant to the atmospheric atom % ^{15}N excess represents the fractional contribution of the atmospheric N to the plant. The PNDF of the N_2-fixing plant can be calculated by using equation:

$$PNDF = [{}^{15}N \text{ atom \% excess}_{plant} / {}^{15}N \text{ atom \% excess}_{atmosphere}] * 100$$

A study of N_2 fixation by alder species using the $^{15}N_2$ reduction method was conducted in a closed-system, flow-through enclosure apparatus (McNeill et al., 1994), in which the PNDF of the 1-year-old black alder seedlings was estimated to be only 16%.

The $^{15}N_2$ reduction method represents a powerful tool in fundamental research because it is the only direct method of measuring N_2 fixation. Moreover, many qualitative and quantitative aspects of the translocation and fate of biologically fixed N can be investigated only through the use of $^{15}N_2$. However, a sophisticated and expensive apparatus is required to prevent leaks and maintain normal environmental conditions because the N_2-fixing system must be exposed to a $^{15}N_2$ atmosphere of constant enrichment. Even with maximum precautions, leakage can occur, especially when working with higher plants. So the main limitation of the $^{15}N_2$ reduction method is that it is technically difficult, especially working with tall trees in the field. In addition, the method is destructive, complicating repetitive sampling over long-term experiments.

The basic principle of the ^{15}N dilution method, first described by McAuliffe et al. (1958), is similar to that of the $^{15}N_2$ reduction method. The only difference is that the soil is enriched in ^{15}N instead of the atmosphere. By adding ^{15}N fertilizers to soils, one ensures that the plants, both N_2-fixing and non-fixing reference plants, will take up N from soils with higher ^{15}N content than that of the atmosphere. The extent to which atmospheric N in the N_2-fixing plant dilutes this ^{15}N enrichment reflects the magnitude of N_2 fixation. Use of ^{15}N -enriched fertilizer results in access of N_2-fixing plants to

three N sources: soil N, fertilizer N, and atmospheric N. By using a non-fixing plant as a reference, the PNDF of the N_2-fixing plant can be calculated by the following equation (Fried and Middleboe, 1977):

$$PNDF = [1 - (FAP^{fixing} - NA^{fixing}) / (FAP^{non\text{-}fixing} - NA^{non\text{-}fixing})] * 100$$

FAP^{fixing}: the foliage atom % ^{15}N of the N_2-fixing plant

NA^{fixing}: the ^{15}N natural abundance of the N_2-fixing plant foliage

$FAP^{non\text{-}fixing}$: the foliage atom % ^{15}N of the non- N_2-fixing plant

$NA^{non\text{-}fixing}$: the ^{15}N natural abundance of the non- N_2-fixing plant foliage

A study on 1-year-old black alder seedlings using the ^{15}N dilution method reported that the PNDF was 74 % in monoculture and 92 % in association with poplar (*Populus nigra*) (Kurdali et al., 1990). The results of Parrotta et al. (1994) showed that the PNDF of *Casuarina equisetifolia* using the ^{15}N dilution method were in close agreement with estimates made by using the N accretion method and proved that the ^{15}N dilution method works well for large woody perennial plant species. Other studies showed that the ^{15}N dilution method is integrative, non-destructive, and gives good estimates of N_2 fixation under controlled conditions in both lab and field study (Domenach and Kurdali, 1989b; Sougoufara et al., 1990). However, the selection of a reference plant with a similar pattern of soil N uptake as the N_2-fixing plant is the most important factor affecting the accuracy and sensitivity of the method (Witty, 1983;

Fried et al., 1983; Sanginga et al., 1990). The absence of non-nodulated isolines of alder and the ubiquitous nature of *Frankia* have made the selection of the non-fixing reference tree very difficult in field studies. Because of the high costs and limitations of the ^{15}N dilution method, there have been no field studies using the ^{15}N isotope dilution to measure the PNDF of young red alder in the field.

The objectives of this study were to evaluate the importance of the selection of a reference plant in the ^{15}N dilution method, to use the ^{15}N dilution method to estimate the PNDF of young red alder trees (7- to 8-year-old) under field conditions, and to estimate the TNDF in stands containing young red alder and Douglas-fir (*Pseudotsuga menziesii*) in the Cascade Range of Oregon.

II-3. Materials and Methods

II-3-1. Site Description

The study area was located at the H. J. Andrews Experimental Forest, which is located on the western slopes of the Cascade Range of Oregon (44°09' N, 122°22' W). The elevation ranges from 700 m to 750 m. At the primary meteorological station, which is at 430 m elevation, mean monthly temperature ranges from 1.0 °C in January to 18.0 °C in July. The average annual precipitation is 2600 mm, falling mainly in November through March. Soils of this area are mostly well-drained Inceptisols with local areas of Alfisols and Spodosols. Soil pH ranges form 5.74 to 5.91, total N from

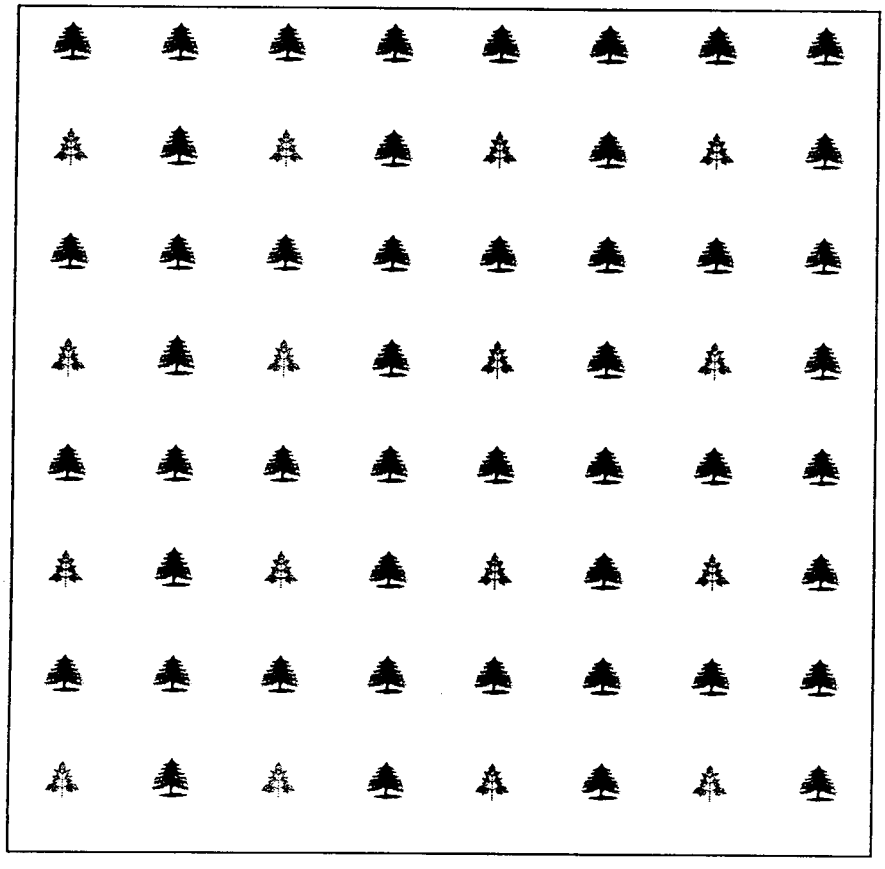
1.36 to 1.57 g kg^{-1} soil, total C from 28.1 to 35.3 g kg^{-1} soil (David Hibbs, unpublished data, Forest Science, Oregon State University).

The study site was once an old-growth conifer forest. After clear cutting in 1986, the plantation was established in May 1987 using containerized seedlings of red alder and Douglas-fir. The red alder and Douglas-fir seedlings were planted as a mixture at 3.0 m x 3.0 m spacing, with 25 % red alder and 75 % Douglas-fir. Within each plot, 16 red alder and 48 Douglas-fir seedlings were planted. The arrangement of red alder and Douglas-fir seedlings is showed in Figure II-1. In October of 1994, the size of young red alder ranged from 175 cm to 324 cm in height, from 26.5 mm to 65.4 mm in DBH (diameter at breast height); the size of Douglas-fir ranged from 152 cm to 204 cm in height, from 6.7 mm to 34.7 mm in DBH.

II-3-2. Experimental Design

In order to estimate the PNDF of red alder trees, Douglas-fir was used as a reference tree. A paired-tree technique, one red alder tree and an adjoining Douglas-fir tree, was used. A total of eight pairs of trees were selected and used in each year of the study. As an alternative to using a reference tree, the average atom % ^{15}N of soil inorganic N (NH_4^+ and NO_3^-) under each Douglas-fir tree was also used to calculate the PNDF of red alder trees.

Figure II-1. Arrangement of red alder and Douglas-fir seedlings at the study site.



red alder

Douglas-fir

II-3-3. ^{15}N Soil Labeling

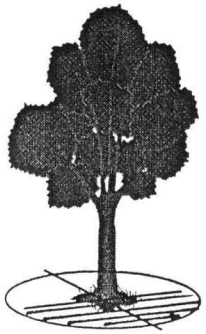
On 29 April and 1 July 1993, ^{15}N-enriched ammonium sulfate (16 atom % ^{15}N excess) was applied to soils surrounding each of the eight paired trees at a rate of 0.45 g N m^{-2}. This amount did not greatly alter the size of soil inorganic N pool, but was labeled highly enough to assure that trees would have measurable increase in ^{15}N abundance. We labeled the area within a radius of 1.5 m around the base of each tree, which was about 1.5 to 2.0 times the crown area for the Douglas-fir trees and 1.5 times the crown area for most of the red alder trees. A total amount of 3.2 g N fertilizer was applied to the soils around each tree.

On 28 April and 6 July of 1994, ^{15}N-enriched ammonium sulfate (16 atom % ^{15}N excess) was applied to soils surrounding a different set of eight paired trees at the same rate as 1993. However, the total amount of 3.2 g N fertilizer was applied to a designated crown area for each tree. Of the eight paired trees, four were labeled at 0.25, 0.5, 1.0, and 3.0 crown areas for each tree. Two paired trees were labeled at 1.5 crown areas for each tree, another two paired trees were labeled at 2.0 crown areas for each tree. The labeling areas for each labeling treatment and the ratio of the labeling area to the crown are (LA/CA) are shown in Figure II-2.

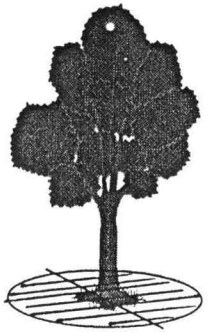
The ammonium sulfate was spread uniformly on the soil by applying as an aqueous solution using a garden sprayer.

Figure II-2. Labeling areas of 0.25, 0.5, 1.0, 1.5, 2.0, and 3.0 LA/CA for each tree in the 1994 study.

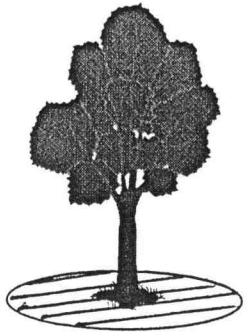
0.25 LA/CA



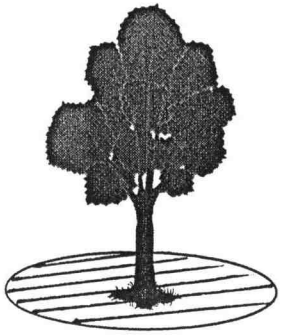
0.5 LA/CA



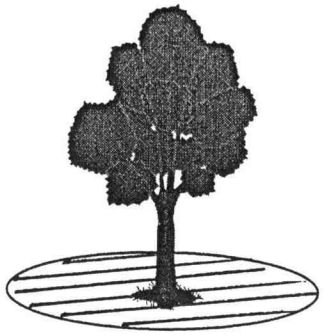
1.0 LA/CA



1.5 LA/CA



2.0 LA/CA



3.0 LA/CA

II-3-4. Foliage Sampling

Following each ^{15}N fertilizer application, foliage samples were collected from each labeled tree: three times in 1993 and twice in 1994. For red alder trees, 10-15 leaves were collected randomly from different parts of the canopy. For Douglas-fir trees, the tips of 5-6 twigs (< 5 mm diameter) with current year needles were collected from lower, middle, and upper portions of the canopy.

In 1994, non-labeled red alder and Douglas-fir leaves (at least 30 m away from labeled trees) were also collected to estimate the ^{15}N natural abundance of red alder and Douglas-fir foliage.

II-3-5. Soil Sampling and Analysis

Soil samples were taken periodically starting in April of each year. At each sampling time, four auger samples evenly spread within a radius of 1.5 m were taken from the soil around each tree to a depth of 15 cm and mixed together thoroughly by hand. Soils were stored below 4 ^{o}C for no longer than 72 h prior to analysis. Gravimetric soil water content was determined at 105 ^{o}C.

Inorganic N was extracted using 50 ml of 2.0 M KCl per 25 g wet soil. The KCl extracts were analyzed for NH_4^+ and NO_3^- colorimetrically using an Alpkem auto-analyzer (Alpkem, Co., Clackamas, OR).

II-3-6. ^{15}N Analysis

Leaf samples were dried at 65 ^{o}C to a constant weight and ground to a fine powder using a roller mill. For Douglas-fir foliage samples, needles were removed from twigs before they were ground. Analysis of the foliage atom % ^{15}N and total N of red alder and Douglas-fir were performed with an automated isotope ratio mass spectrometer (Europa Scientific, Crewe, UK).

Soil NH_4^+ and NO_3^- were prepared for ^{15}N analysis by diffusion onto filter paper disks (Brooks, 1989) and then analyzed by mass spectrometry.

II-3-7. Biomass Estimation (Red Alder)

Allometric regression equations for the biomass of leaves (B^L), stems (B^S), and roots (B^R) of 7- to 9-year-old red alder trees were developed from a data set provided by Dr. David Hibbs (Forest Science, Oregon State University). This data set was collected by destructive harvest of other red alders planted at the same site. We found DBH (diameter at breast height, 1.58 cm) to be the best predictor of biomass.

$$\ln B^L = -1.247 + 2.126 * \ln DBH \qquad (df = 3, R^2 = 0.997) \qquad \text{(Equation II-1)}$$

$$\ln B^S = -0.695 + 2.442 * \ln DBH \qquad (df = 3, R^2 = 0.955) \qquad \text{(Equation II-2)}$$

$$\ln B^R = 0.004 + 1.959 * \ln DBH \qquad (df = 2, R^2 = 0.973) \qquad \text{(Equation II-3)}$$

The annual total N accumulation (TN^{AC}) of a red alder tree is the sum of the N increment in the standing biomass and the amount of N lost in litter-fall during the year.

$$TN^{AC} = N^{sd}_{T1} + N^{lf}_{T1} - N^{sd}_{T0} \qquad \text{(Equation II-4)}$$

N^{sd}_{T1} is the amount of N in the standing biomass of a red alder tree at the end of year.

N^{sd}_{T0} is the amount of N in the standing biomass of a red alder tree at the beginning of the year.

$$N^{sd} = (N\%^{R} * B^{R} + N\%^{S} * B^{S}) \qquad \text{(Equation II-5)}$$

N^{lf}_{T1} is the amount of N lost for one red alder tree in the form of litter fall during the year.

$$N^{lf} = N\%^{L} * BS^{L} \qquad \text{(Equation II-6)}$$

$N\%^{R}$, $N\%^{S}$, and $N\%^{L}$ are the N percentages of red alder roots, stems, and leaves. In this study, the $N\%^{L}$ was measured to be 2.74 %. The value of 0.98 %, from the N percentage of black alder roots (Camire et al., 1991), was used to represent $N\%^{R}$. The N percentage of 0.17 % for red alder stems was provided by Dr. Mark Harmon (Forest Science, Oregon State University).

II-3-8. Calculation of PNDF and TNDF

The PNDF of red alder tree was calculated by using the equation of Fried and Middleboe (1979).

$$PNDF = [1 - (FAP^{alder} - NA^{alder}) / (FAP^{fir} - NA^{fir})] * 100 \quad \text{(Equation II-7)}$$

FAP^{alder}: the foliage atom % ^{15}N of red alder

NA^{alder}: the ^{15}N natural abundance of red alder foliage

FAP^{fir}: the foliage atom % ^{15}N of Douglas fir

NA^{fir}: the ^{15}N natural abundance of Douglas-fir foliage

A study of N_2 fixation by black alder using ^{15}N natural abundance showed that leaves were the most practical tissue to collect to estimate annual N_2 fixation (Domenach and Kurdali, 1989a) and a ^{15}N dilution study reported that the PNDF calculated from the alder leaves can be used to represent the whole plant PNDF when estimating the annual N_2 fixation in a natural ecosystems (Kurdali et al., 1990).

The yearly TNDF in red alder-Douglas-fir stands was calculated as:

$$TNDF = TN^{AC} * PNDF * A\# \quad \text{(Equation II-8)}$$

A# is the number of red alder trees per hectare in red alder-Douglas-fir stands.

II-3-9. Statistic Analysis

The data were analyzed statistically using Statgraphics Plus 2.0 for Windows. One-way analyses of variance (ANOVA) were carried out for the atom % ^{15}N abundance of red alder foliage, Douglas-fir foliage, and soil inorganic N for different sampling times, the PNDF using Douglas-fir as reference for different sampling times, and the PNDF using soil inorganic N as reference for different sampling times. When the ANOVA was significant ($p < 0.05$), the least significant difference (LSD) was calculated in order to compare the influence of different sampling times. Student's *t*-tests were used to examine differences in the atom % ^{15}N abundance between red alder and unlabeled red alder, between Douglas-fir and unlabeled Douglas-fir. Paired *t*-tests were performed to examine differences in the PNDF using Douglas-fir and soil inorganic N as reference, in the PNDF between 1.5 LA/CA and 2.0 LA/CA treatments, and in the foliage atom % ^{15}N abundance of red alder and Douglas-fir between 1.5 LA/CA and 2.0 LA/CA treatments. Data of the PNDF were transformed to the square root of (100-PNDF) before statistical analysis.

II-4. Results and Discussion

II-4-1. 1993 Experiment

The atom % ^{15}N of red alder foliage at the three sampling times (Table II-2) was significantly greater than that of unlabeled red alder at the study site ($t = 6.054$, $p <$

0.001 with $df = 15$ for 1 July 1993; $t = 4.360$, $p < 0.001$ with $df = 15$ for 3 August 1993; $t = 2.174$, $p < 0.05$ with $df = 15$ for 28 October 1993), which indicated that the foliage of red alder was labeled successfully. The average atom % ^{15}N excess of red alder foliage for the three sampling times was only 0.0097 %, 0.0012 %, and 0.0007 %, indicating that young red alder trees got almost all of their N from symbiotic N_2 fixation. The deep roots of the young red alders could have taken up unlabeled N from soils, but because soil inorganic N generally decreases dramatically with depth in soil profiles, this was not likely to have affected the ^{15}N abundance of young red alders.

The atom % ^{15}N of red alder foliage on 1 July 1993 was significantly higher than that on 3 August and 28 October 1993, however, the coefficient variation (CV) of the foliage atom % ^{15}N in July (1.3 %) was much higher than those in August (0.2 %) and October (0.3 %). The ^{15}N fertilizer in the soils during the April - July period was probably more available to the plants than later in the season because soil moisture was higher then, which likely enhanced ^{15}N uptake and caused the atom % ^{15}N of red alder foliage in July to be higher than that in August and October.

With 16 atom % excess ^{15}N fertilizer labeling the soils around each tree, the Douglas-fir foliage and soil inorganic N were highly labeled with ^{15}N compared to the red alder foliage (Table II-2). The atom % ^{15}N of Douglas-fir foliage was not significantly different among the three sampling times because of high variation (CVs 25 - 35 %). There also were no significant differences in the atom % ^{15}N of soil inorganic N among different sampling times in the 1993 study (Table II-2), mainly due to the large sampling variation.

Table II-2. The average ^{15}N abundance of red alder foliage, Douglas-fir foliage, and soil inorganic N for different sampling times in the 1993 and 1994 studies, the PNDF using Douglas-fir as reference in the 1993 and 1994 studies, and the PNDF using soil inorganic N as reference in the 1993 study. All numbers in parentheses are standard deviations. n = 8, except n = 7 for the ^{15}N abundance of soil inorganic N and the PNDF using soil inorganic N as reference. The foliage atom % ^{15}N of unlabeled red alder at the study site was 0.3644 % (0.0003 %) and Douglas-fir was 0.3638 % (0.0002 %). Within each column for the 1993 and 1994 studies, the values followed by the same lower case letter are not significantly different at the 0.05 level as determined by using one factor ANOVA LSD multiple-range tests.

	N Content		^{15}N Abundance			PNDF	
Sampling time	Red alder foliage	Douglas-fir foliage	Red alder foliage	Douglas-fir foliage	Soil inorganic N	Douglas-fir as reference	Soil inorganic N as reference
	------ N % ------		------ Atom % ^{15}N ------			------ % ------	
07/01/93	2.63 (0.36)	1.05 (0.09)	0.3741 (0.0048)[a]	0.6372 (0.2201)[a]	1.7721 (0.6744)[a]	95.12 (3.54)[a]	99.18 (0.38)[a]
08/03/93	2.86 (0.49)	1.13 (0.14)	0.3655 (0.0008)[b]	0.6388 (0.2132)[a]	2.1727 (0.3812)[a]	99.44 (0.37)[b]	99.93 (0.04)[b]
10/28/93	2.07 (0.26)	2.04 (0.17)	0.3651 (0.0010)[b]	0.6067 (0.1489)[a]	1.8950 (0.7217)[a]	99.69 (0.45)[b]	99.96 (0.04)[b]
07/13/94	2.66 (0.18)	1.09 (0.14)	0.3676 (0.0032)[a]	0.5896 (0.1484)[a]	ND*	97.97 (2.01)[a]	ND
09/23/94	2.39 (0.26)	1.32 (0.11)	0.3673 (0.0043)[a]	0.5723 (0.1821)[a]	ND	98.59 (0.79)[a]	ND

*ND not determined

By using the paired Douglas-fir tree as a reference tree, the PNDF on 1 July 1993 was significantly lower than the PNDF on 3 August and 28 October 1993 (Table II-2). The atom % ^{15}N of the soil inorganic N was used as an alternate to the atom % ^{15}N of Douglas-fir foliage to estimate the PNDF of the young red alder trees. By using the atom % ^{15}N of the soil inorganic N as a reference, the PNDF on 1 July 1993 was significantly lower than the PNDF on 3 August and 28 October 1993, which is similar to the results of PNDF using the paired Douglas-fir tree as a reference. By using either the paired Douglas-fir tree or the soil inorganic N as a reference, the pattern of the significant differences in PNDF among different sampling times corresponded directly to the changes in the atom % ^{15}N of red alder foliage among the different sampling times rather than the atom % ^{15}N of Douglas-fir foliage or the atom % ^{15}N of the soil inorganic N.

The PNDF using the atom % ^{15}N of Douglas-fir foliage as a reference was significantly lower than the PNDF using the atom % ^{15}N of the soil inorganic N among the three sampling times ($t_{paired} = 3.523$, $p = 0.012$ with $df = 6$ for 1 July 1993; $t_{paired} = 5.090$, $p = 0.002$ with $df = 6$ for 3 August 1993; $t_{paired} = 2.960$, $p = 0.025$ with $df = 6$ for 28 October 1993). However, the PNDF estimated by using either the atom % ^{15}N of Douglas-fir foliage or the atom % ^{15}N of the soil inorganic N as a reference were all above 95 %, which demonstrates that the 7- to 8-year-old young red alder trees got almost all of their N from symbiotic N_2 fixation. Therefore, there were no biological differences in using the atom % ^{15}N of Douglas-fir foliage or the atom % ^{15}N of the soil inorganic N as a reference to estimate the PNDF of the young red alder trees.

II-4-2. 1994 Experiment

The soil ^{15}N labeling areas for each red alder and Douglas-fir tree in the 1993 study were all equal, which could be a potential shortcoming to estimating the values of the foliage atom % ^{15}N of red alder and Douglas-fir in the field. If the labeling area for a tree is smaller than the area of the plant root system, the roots will get N outside of the labeling area. Therefore, the atom % ^{15}N of the plant foliage will be underestimated. Underestimation of the atom % ^{15}N of the plant foliage would also happen when the labeling area for a tree is larger than that of its main root system, because some of the applied N fertilizer is beyond its reach. In order to estimate the PNDF of red alder trees more accurately, we designed the 1994 experiment to study the effects of LA/CA on foliage atom % ^{15}N and the PNDF. Due to the high cost of a ^{15}N field study, only the paired trees of the 1.5 and 2.0 LA/CA labeling treatments were replicated.

The average atom % ^{15}N of red alder foliage on 13 July 1994 and 23 September 1994 were very close to that of unlabeled red alder in the field, as they were in the 1993 study (Figure II-3 and Table II-2). The atom % ^{15}N value of red alder foliage at the two sampling times were significantly greater than that of unlabeled red alder at the study site ($t = 3.118, p = 0.007$ with $df = 15$ for 12 July 1994; $t = 2.103, p = 0.053$ with $df = 15$ for 23 September 1994), however, which indicated that the soil labeling was also successful in the 1994 study. The small difference in atom % ^{15}N between leaves of labeled and unlabeled red alder means that they derived most of their N from N_2 fixation. No LA/CA effect on red alder suggests that there was little soil N uptake, at least from surface roots.

There were no significant differences in the atom % ^{15}N of red alder foliage between the 1.5 and 2.0 LA/CA treatments for the two sampling times ($t = 0.699$, $p = 0.557$ with $df = 2$ for 13 July 1994; $t = 0.633$, $p = 0.641$ with $df = 2$ for 23 September 1994). The atom % ^{15}N of red alder foliage for other LA/CA treatments were very close to that of the 1.5 and 2.0 LA/CA treatments (Figure II-3). In general, the atom % ^{15}N of red alder foliage were similar among the different LA/CA treatments. Because the PNDF of the young red alder trees was over 95 %, the atom % ^{15}N of red alder foliage was not very sensitive to LA/CA treatments. There were also no significant differences in the atom % ^{15}N of red alder foliage between the two sampling times.

With the same mass of ^{15}N fertilizer applied to the soils around each tree as we applied in the 1993 study, the Douglas-fir foliage was also highly labeled with ^{15}N compared to the red alder foliage (Figure II-4 and Table II-2). There were no significant differences in the foliage atom % ^{15}N of Douglas-fir between the two sampling times in the 1994 study, as found in the 1993 study. The atom % ^{15}N of Douglas-fir foliage of the 1.5 LA/CA treatment was significantly higher than that of the 2.0 LA/CA treatment for the two sampling times ($t = 3.359$, $p = 0.039$ with $df = 2$ for 13 July 1994; $t = 4.319$, $p = 0.025$ with $df = 2$ for 23 September 1994). The atom % ^{15}N of Douglas-fir foliage for 1.5 LA/CA treatment was higher than that measured for the rest of LA/CA treatments for the two sampling times (Figure II-4), which indicated that the roots of Douglas-fir trees were concentrated mainly within 1.5 crown areas.

The PNDF for the different LA/CA treatments, using the atom % ^{15}N of Douglas-fir foliage as a reference, are shown in Figure II-5. There were no significant

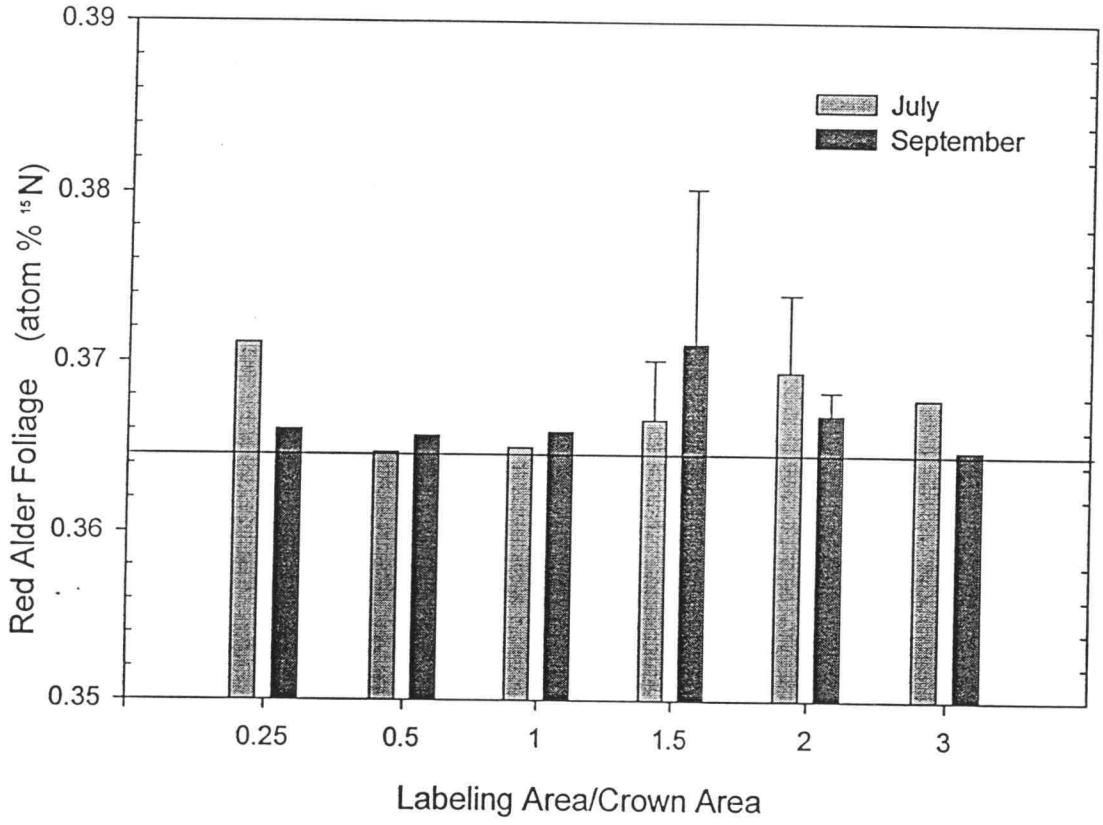


Figure II-3. The ^{15}N abundance of red alder foliage for different LA/CA treatments. n = 2 for 1.5 and 2.0 LA/CA, n = 1 for other LA/CA treatemnts. The ^{15}N of unlabeled red alder foliage (solid line) is 0.3644 %.

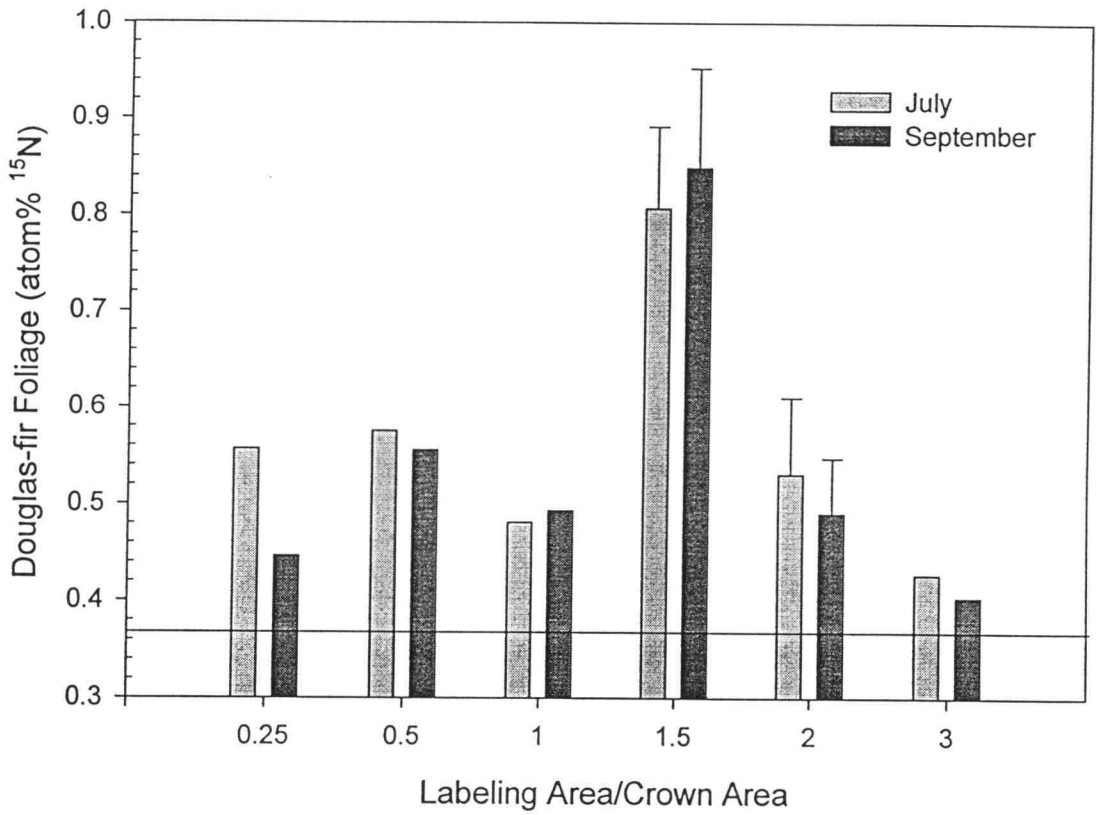


Figure II-4. The ^{15}N abundance of Douglas-fir foliage for different LA/CA treatments. n = 2 for the 1.5 and 2.0 LA/CA, n = 1 for other LA/CA treatments. The ^{15}N abundance of unlabled Douglas-fir folaige (solid line) is 0.3638 %.

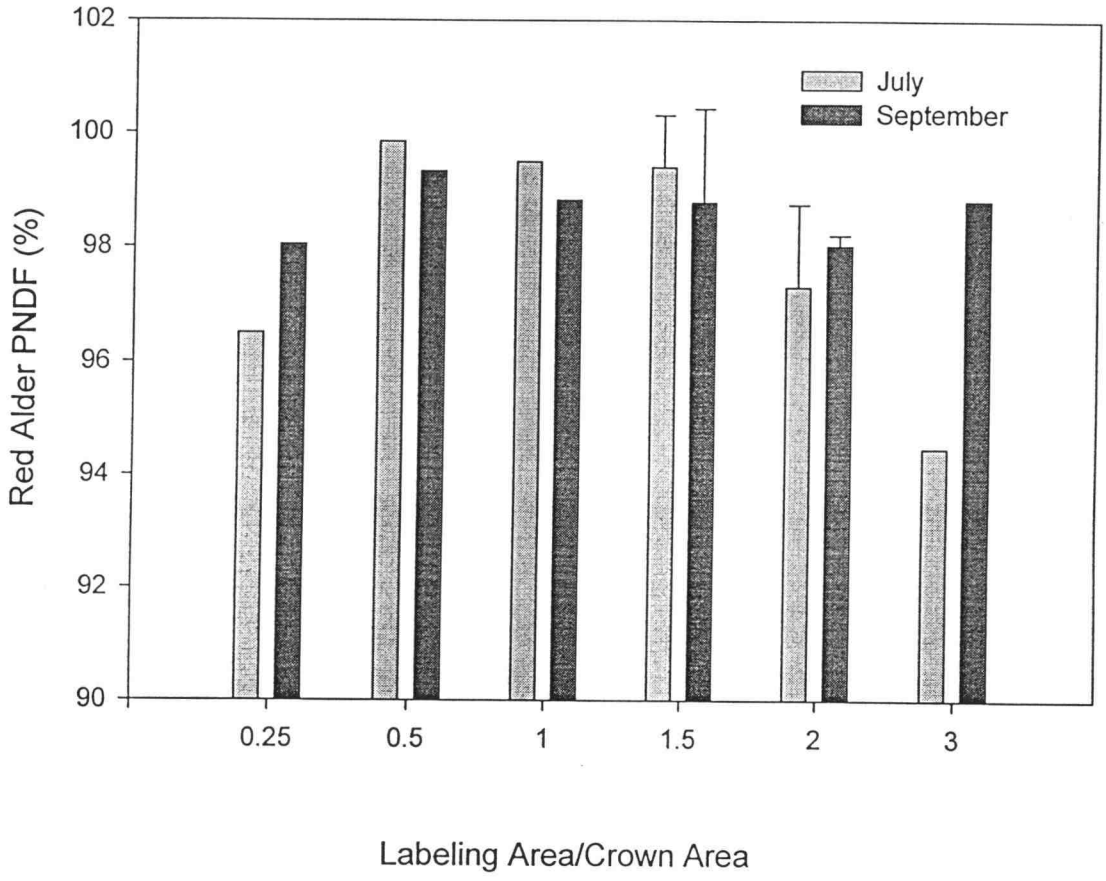


Figure II-5. Red alder PNDF using Douglas-fir as the reference tree for different LA/CA treatments. n = 2 for 1.5 and 2.0 LA/CA, n = 1 for other LA/CA treatments.

differences in the PNDF between the 1.5 and 2.0 LA/CA treatments for both samplings ($t = 1.738$, $p = 0.224$ with $df = 2$ for 13 July 1994; $t = 0.654$, p = 0.631 with $df = 2$ for 23 September 1994). There seemed to be no differences in the PNDF among the different LA/CA treatments at the two sampling times (Figure II-5). Because the 7- to 8-year-old young red alder trees got most of their N from symbiotic N_2 fixation, LA/CA did not play a significant role on the PNDF of red alder trees. There were no significant differences in the PNDF between the two sampling times simply because there were no significant differences in the atom % ^{15}N of red alder foliage between the two sampling times.

To further check the accuracy of the high PNDF values, we sampled foliage from blackberry vines growing on the plots as an alternative reference plant. On 8 December 1994, two blackberry leaf samples were taken from the 0.25 and 1.0 LA/CA treatments. The atom % ^{15}N of blackberry foliage ranged from 0.6150 to 1.1673 %, giving a range of PNDF of 99.4 to 99.8 %. Even with this large range in the foliage atom % ^{15}N of blackberry, the range of the PNDF by red alder was still between 99 to 100 %. Therefore, the average PNDF by red alder was similar in the two years of this study. All of the average PNDF using different references in both 1993 and 1994 studies were over 95 %, which indicated that the selection of the reference was not biologically important for estimating the PNDF of young red alder trees if N_2 fixation rate is high.

In previous studies using the ^{15}N dilution method to estimate PNDF, the importance of selecting a reference plant with a similar pattern of soil N uptake as the N_2-fixing plant has been emphasized (Chalk, 1985; Chatarpaul and Lachance, 1989;

Fried et al., 1983; Sanginga et al., 1990; Witty, 1983). The requirements for a satisfactory reference plant for the isotope dilution are: (1) the reference plant should not fix N_2; (2) both the fixing and the non-fixing plants must always absorb N from the same nutrient pool; and (3) the reference plant should be able absorb available N from the soil and fertilizer in the same proportion (Fried et al., 1983).

We modeled the sensitivity of the PNDF to the foliage atom % ^{15}N of the reference tree using the average foliage atom % ^{15}N of red alder in the 1993 and 1994 studies and three higher ^{15}N abundance (Figure II-6). The PNDF using the foliage atom % ^{15}N of red alder in the 1993 and 1994 studies were not sensitive to changes of the foliage atom % ^{15}N of the reference tree when the foliage atom % ^{15}N of the reference tree was greater than 0.40 %. Once the foliage atom % ^{15}N of the reference tree reached 0.44 %, the PNDF based on the foliage atom % ^{15}N of red alder in both 1993 and 1994 studies are already over 95 %. If the red alder foliage was more highly labeled ($\geq$ 0.38 atom % ^{15}N), then the PNDF would be much more sensitive to the foliage atom % ^{15}N of the reference tree. Therefore, when the atom % ^{15}N of red alder foliage is very close to that of unlabeled red alder, the PNDF calculated from Equation II-5 is very insensitive to the foliage atom % ^{15}N of the reference tree, particularly when the reference foliage atom % ^{15}N is relatively high.

Using lodgepole pine, herbs, and shrubs as non-fixing reference trees, the PNDF of 8-year-old Sitka alder (*Alnus sinuata* (Reg.) Rydb.) in Pacific Northwest forests was estimated to be 94 - 99% (Mead and Preston, 1992) based on the ^{15}N dilution method. The PNDF of a mature black alder in Europe was estimated to be 94 $\pm$ 15 % using the

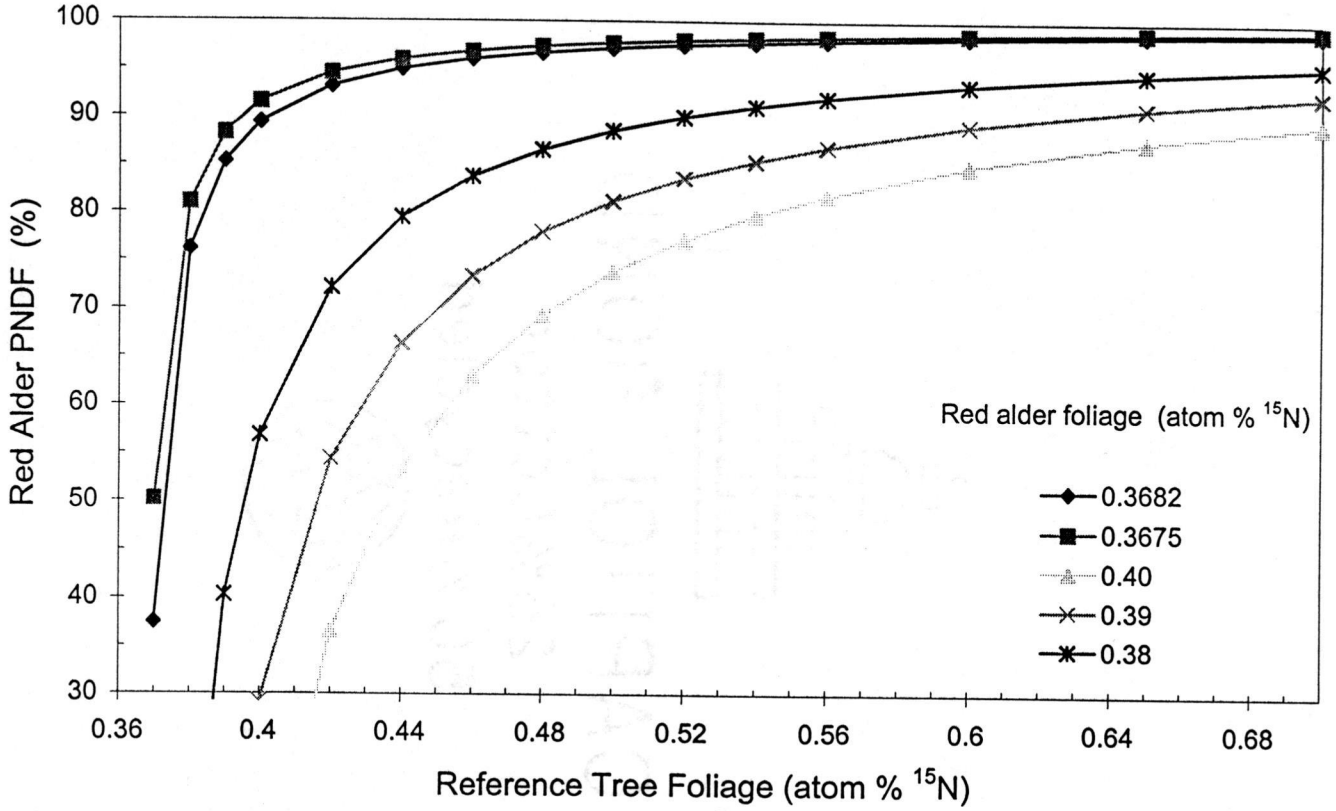


Figure II-6. Sensitivity of the PNDF of red alder to the atom % ^{15}N of the reference tree foliage for different atom % ^{15}N of red alder foliage. The average atom % ^{15}N of red alder foliage was 0.3682 % in 1993 and 0.3675 % in 1994.

^{15}N natural abundance method (Beaupied et al., 1990). Therefore, the PNDF estimated in our study was in good agreement with the previous studies on other alder species using the ^{15}N dilution method and the ^{15}N natural abundance method.

However, our PNDF results differ from some other studies using the acetylene reduction and the $^{15}N_2$ reduction method. Using the acetylene reduction method, Zavitkovski and Newton (1968) estimated that N_2 fixation contributed only 12 -50 % of the total annual N demand of young red alder stands and Akkermans and Van Dijk (1976) estimated that the PNDF of 20-year-old black alder trees ranged from 36 - 88 %. The PNDF of the 1-year-old black alder seedlings was estimated to be 33 % with non-nodulated black alder reference tree seedlings and 58 % with red fescue (*Festuca rubra* L.) reference using the $^{15}N_2$ reduction method (McNeill et al., 1994).

For the foliage N of a perennial N_2-fixing plant, like red alder, there is another source of N that is stored mainly in the root system. In this study, we were unable to estimate the proportion N translocated from this reserve N to red alder foliage. A study on the influences of reserve N on leaf formation of 5- to 6-year-old black alder trees showed that reserve N accounted for 10 % of leaf N (Domenach and Kurdali, 1989b). Assuming the same contribution of reserve N as the young black alder trees, the PNDF of young red alder would be 85 - 90 %. By using the ^{15}N dilution method and poplar (*Populus alba* L.) as a reference tree, the PNDF of black alder was estimated to be 87 % taking into account the N reserves in the above study (Domenach and Kurdali, 1989b), which is in good agreement with our results. Taking N reserves into account, the PNDF of red alder we calculated would have been overestimated on a yearly base, however,

the PNDF of red alder on the long-term basis would still be very close to 95 - 99 % because 85 - 90 % of the reserve N in the current year's foliage was fixed in the previous year.

II-4-3. Estimation of Total N_2 Fixation

The average DBH of red alder trees measured in October 1993 was 46.0 mm and 72.3 mm in October 1995. Based on the average DBH of red alder trees measured in 1993 and 1995, the average TN^{AC} of a mixed stand (25% red alder and 75% Douglas-fir) during 1993 to 1995 was estimated to be 22.8 kg N ha^{-1} yr^{-1}. The average PNDF for both 1993 and 1994 study using Douglas-fir as a reference tree was 98.2 %. Therefore, the average TNDF of a mixed stands was estimated to be 22.3 kg N ha^{-1} yr^{-1}. For a pure red alder stand, the TNDF would be about 90 kg N ha^{-1} yr^{-1}, without considering the effect of alder/fir mixed stands on the biomass of red alder trees. In general, the average TNDF estimated in our study was in the range of most previous studies in the Pacific Northwest (Table II-1).

This study was the first to use the ^{15}N dilution method to estimate the PNDF and TNDF of red alder under field conditions in the Pacific Northwest. Our estimated PNDF of the young red alder trees was in good agreement with some former studies on other alder species, which suggests that the ^{15}N dilution method is reliable method to estimate PNDF of red alder under field conditions. The evaluation of PNDF sensitivity to reference plant selection and to LA/CA provides information important to future N_2 fixation studies of red alder using the ^{15}N dilution method.

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Chapter III

Measurement of Soil Net N Mineralization, Nitrification and Plant N Uptake in Young Red Alder and Douglas-fir Plots

James Y. Tang and David D. Myrold

III-1. Abstract

Previous studies discovered that the prolonged presence of red alder (*Alnus rubra* Bong.) increased total and available N in the soil and caused greater N mineralization and nitrification rates because of inputs from symbiotic N_2 fixation between red alder and *Frankia*. It is still an open question, however, whether the extra input of N from young red alder trees would affect the soil inorganic N pools and speed up the processes of N mineralization-immobilization and nitrification. The objectives of this study were to investigate the effect of 7- to 8-year-old interplanted red alder on soil inorganic N pools, net N mineralization, and net nitrification under Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) trees in the Cascade Range of Oregon. Resin cores were installed in the soil under adjacent red alder and Douglas-fir trees and replaced one or two times during one-year incubations to determine annual net N mineralization, net nitrification, and plant N uptake rates under field conditions. An allometric regression equation for Douglas-fir biomass was developed and used to validate its N uptake. There were no significant differences in soil inorganic N, net N mineralization, and net nitrification under red alder and Douglas-fir trees, probably because of the young age of the red alder trees and the close spacing of red alder and Douglas-fir seedlings. Ammonium was the main form of soil inorganic N. Plant N uptake by red alder and Douglas-fir showed a seasonal pattern, spring > summer/fall > winter. Plant N uptake by Douglas-fir as measured using resin cores was in good agreement with that calculated from its N accumulation using allometric regression

equation, which suggested that the in situ resin core incubation technique is a reliable method to estimate plant N uptake in the field.

III-2. Introduction

Nitrogen is thought to be a major limiting factor to growth of Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) (Gessel et al., 1969) and to be the most commonly limiting nutrient in the Northwest Pacific region (Gessel et al., 1973). Nitrogen fixation by *Frankia* in root nodules of red alder (*Alnus rubra* Bong.) primarily provides N in a usable form for the host, but the fixed N is eventually cycled into the ecosystem. Not all N_2 fixed by red alder is immediately available to the soil or other plants; the natural processes of litter decomposition and soil N transformation must occur first.

Many reports indicate that alders can significantly increase the growth rate of associated trees or later planted trees. Tarrant (1961) found that current diameter and height growth of 30-year-old Douglas-fir trees interplanted with red alder was better than diameter and height growth in an adjacent pure plantation of Douglas-fir of the same age. A subsequent study on the same two stands showed that the mixed stand had accumulated 159 m^3 ha^{-1} of wood (88 in fir and 71 m^3 ha^{-1} in red alder) as compared to 82 m^3 ha^{-1} in the pure Douglas-fir stand (Miller and Murray, 1978). Some nutritional differences and biomass changes were also observed between Douglas-fir seedlings

grown in adjacent former Douglas-fir and red alder stands, partly due to the greater total N and organic matter accumulated in the soil by the former red alder forest (Brozek, 1990). The high capacity of alders to fix atmospheric N_2 in symbiosis with *Frankia* and the large amount of N in alder litter (Dawson and Funk, 1981) offered good possibilities for increasing the productivity of poplar (*Populus nigra*) associated with alders. Other studies (DeBell and Radwan, 1979; Hansen and Dawson, 1982; Kurdali et al., 1990) with similar results indicated that red alder can improve the growth of associated species.

Because of the large amounts of N input from fallen N-rich alder leaves and from root and nodule turnover following decay and regeneration (Cote and Camire, 1985; Dawson et al. 1983; Hansen and Dawson 1982; Zou et al., 1995), soils beneath alders usually have more total N and greater inorganic N than soils beneath conifers (Binkley, 1983; Bollen and Lu, 1968; Binkley et al., 1984; Cole, et al. 1978; Tarrant and Miller, 1963; Van Miegroet and Cole, 1984). Bormann and DeBell (1981) found that N concentration in soil beneath red alder stands (5- to 41-year-old on the same soil type in the same area) was 41% higher than in soil beneath Douglas-fir. When N content was expressed in kg N ha^{-1}, the differences were still significant but not as pronounced, because soil bulk densities under alder were lower than under Douglas-fir. Total N in mineral soil averaged about 3200 kg ha^{-1} for red alder stands, 16% more than the 2800 kg ha^{-1} for Douglas-fir stands. Nitrogen was accumulating in the 0- to 20-cm soil horizon of alder stands at a rate of about 35 kg ha^{-1} yr^{-1}. A study on 3- or 4-year-old gray alder (*Alnus incana* (L.) Moench) trees showed an increase in soil N

corresponding to 17 % of the N_2 fixed by alder trees (Huss-Danell and Ohlsson, 1992). In Pacific Northwest forests, N accretion by *Alnus* spp. amounts to 2 - 320 kg ha^{-1} yr^{-1} (Hibbs and Cromack, 1990).

The extra input of N in red alder stands increases the rates of the soil net N mineralization and nitrification. Studies from two pairs of adjacent, 55-year-old forests showed that the net N mineralization of forest floor plus 0-0.15 m mineral soil of alder-conifer stands was about 124 kg N ha^{-1} yr^{-1}, more than four times the rate of conifer stands dominated primarily by Douglas-fir in the Cascade Head (Binkley et al., 1992). Several studies showed that the symbiotic N_2 fixation between *Frankia* and red alder increase the N mineralization and nitrification rates compared to pure Douglas-fir stands (Binkley et al., 1993; Van Miegroet et al., 1989; Van Miegroet et al., 1990), but some studies showed total soil N soil inorganic N, net N mineralization, and net nitrification were not different beneath alder and Douglas-fir or associated trees (Cole and Newton, 1986). There have been no studies to estimate net N mineralization and nitrification under young red alder soils in the Pacific Northwest under field conditions. Thus, it is still an open question whether the extra input of N in young red alder stands would increase the soil inorganic N pools and speed up the net N mineralization and nitrification in the field.

Traditionally, plant N uptake by perennial plants, such as Douglas-fir, was measured from the annual N accretion of plant biomass (Turner, 1981). Subsequently, the resin core technique was introduced to estimate plant N uptake (Nadelhofer et al.,

1984). There have been no reported studies comparing these two methods to measure the plant N uptake of young Douglas-fir in the field.

The objectives of this study were to investigate the effect of interplanted red alder on soil inorganic N pools, net N mineralization, and net nitrification in red alder/ Douglas-fir mixtures in the Cascade Range of Oregon.

III-3. Materials and Methods

III-3-1. Site Description

The study area was located at the H. J. Andrews Experimental Forest, which is located on the western slopes of the Cascade Range of Oregon (44°09' N, 122°22' W). The elevation ranges from 700 m to 750 m. At the primary meteorological station, which is at 430 m elevation, mean monthly temperature ranges from 1.0 °C in January to 18.0 °C in July. The average annual precipitation is 2600 mm, falling mainly in November through March. Soils of this area are mostly well-drained Inceptisols with local areas of Alfisols and Spodosols. Basic characteristics of the soils at the study site are listed on Table II-1. Soil pH ranges form 5.74 to 5.91, total N from 1.36 to 1.57 g kg^{-1} soil, total C from 28.1 to 35.3 g kg^{-1} soil (David Hibbs, unpublished data, Forest Science, Oregon State University).

The study site was once an old-growth conifer forest. After clear cutting in 1986, the plantation was established in May 1987 using containerized seedlings of red

alder and Douglas-fir. The red alder and Douglas-fir seedlings were planted as a mixture at 3.0 m x 3.0 m spacing following a pattern to give 25 % red alder and 75 % Douglas-fir (Figure II-1). In October of 1994, the size of young red alder ranged from 175 cm to 324 cm in height, from 26.5 mm to 65.4 mm in DBH (diameter at breast height); the size of Douglas-fir ranged from 152 cm to 204 cm in height, from 6.7 mm to 34.7 mm in DBH.

III-3-2. Experimental Design

To compare the rates of soil net N mineralization and nitrification under red alder and Douglas-fir, a paired-tree technique, one red alder tree and an adjoining Douglas-fir tree, was used. A total of eight pairs of trees were selected in each year of the study. Ammonium sulfate fertilizer (3.2 g N), at a rate of 0.45 g N m^{-2} ha^{-1}, was applied to the soil surrounding each of the eight paired trees twice a year. In the 1994 study, the same amount and rate of N fertilizer was applied to the designated soil area based on the ratios of the labeling area to the crown area (LA/CA) treatments (Chapter II).

III-3-3. Resin-core Incubation

Net N mineralization, net nitrification, and plant N uptake in the forest floor and mineral soil (0 - 15 cm) horizons under red alder and Douglas-fir were estimated through using in situ, open-top resin core incubation (DiStefano and Gholz, 1986). Resin cores were made of PVC pipe (7.5 cm diameter by 20 cm length, and 0.5 cm

thickness), which had been sharpen at one end. The resin bags were made from nylon netting, which enclosed 20 g of mixed bed (cation and anion) exchange resin. The resin bags were saturated with 50 ml of 2 M NaCl and shaken for 1 hr before use to reduce interference during colorimetric analysis. The core was pounded through the forest floor into the mineral soil to a total depth of about 20 cm, removed, a resin bag was implanted at the bottom of the core, and the core was placed back into the soil. Under each tree, one resin core was installed close to the main stem of the tree, with the exact location shifted to avoid large rocks and roots.

In the 1993 study, the soil resin cores were incubated from 29 April 1993 to 28 April 1994, and were replaced on 1 July and 7 December during the one-year incubation. In the 1995 study, the soil resin cores were incubated from 28 April 1994 to 27 April 1995, and were replaced on 6 July 1994. We were unable to replace the resin cores in December 1994 due to an early snowfall.

III-3-4. Soil Sampling and Analysis

Soil samples were taken periodically starting in April of each year. Soil samples were taken before and after soil N fertilization. For each sampling time, four auger samples (2.5 cm in diameter) were evenly taken from the soil around the main stem of each tree to the depth of 15 cm.

Soils were stored at 4 ^{o}C for no longer than 72 h prior to analysis. Composited soils were mixed thoroughly by hand. A 12-g wet soil sample was dried to constant weight at 105 ^{o}C to measure soil water content.

Soil samples (25 g wet) were put into 100-ml specimen cups and extracted with 50 ml of 2 M KCl solution. After resin core in situ incubation, soil (25 g wet) from the cores were extracted with 50 ml of 2 M KCl. Resin bags were extracted with 100 ml of 2 M KCl. The extraction procedure recovered all the exchangeable NH_4^+ and NO_3^- from the soil and core soil samples, but only 82 ± 4 % for NH_4^+ and 78 ± 3 % for NO_3^- from the resin, as determined by the recovery of spike. Therefore, the resin bag values were divided by the recovery rates for NH_4^+ and NO_3^-, respectively, to account for the incomplete recovery. The KCl extracts were filtered and analyzed for NH_4^+ and NO_3^- colorimetrically using an Alpkem auto-analyzer (Alpkem, Co., Clackamas, OR).

III-3-5. Estimation of Net N Mineralization, Nitrification, and Plant N Uptake

Estimates of inorganic N from forest floor and surface mineral soil were used to extrapolate net N mineralization, nitrification, and plant N uptake values to a hectare basis using the core radius (Nadelhoffer et al., 1984).

The difference in the sum of NH_4^+ and NO_3^- accumulated in the resin core soil and on the resin bag at the end of incubation minus the initial amount of soil NH_4^+ and NO_3^- is an estimate of net N mineralization.

$$\textit{Net N Mineralization} = [\, N_{T1}^{Core} + N_{T1}^{Bag} - N_{T0}^{Soil} \,]$$

N_{T1}^{Core}: NH_4^+ and NO_3^- in the resin core soil at the end of incubation

N_{T1}^{Bag}: NH_4^+ and NO_3^- on the resin bag at the end of incubation

N_{T0}^{Soil}: Soil NH_4^+ and NO_3^- outside the resin core at the beginning of incubation

Annual net N mineralization was calculated by summing the net N mineralization through the year.

The difference in the sum of NO_3^- in the resin core soil and on the resin bag at the end of incubation minus the initial amount of soil NO_3^- is an estimate of net nitrification.

$$\textit{Net N Nitrification} = [\, NO_3^-{}_{T1}^{Core} + NO_3^-{}_{T1}^{Bag} - NO_3^-{}_{T0}^{Soil} \,]$$

$NO_3^-{}_{T1}^{Core}$: NO_3^- in the resin core soil at the end of incubation

$NO_3^-{}_{T1}^{Bag}$: NO_3^- on the resin bag at the end of incubation

$NO_3^-N_{T0}^{Soil}$: Soil NO_3^- outside the resin core at the beginning of incubation

Annual net nitrification was calculated by summing the net nitrification through the year as we did in net N mineralization estimation.

Plant N uptake was estimated to be the difference in the sum of NH_4^+ and NO_3^- in the resin core soil and on the resin bag at the end of incubation minus the soil NH_4^+ and NO_3^- at the end of incubation based on the assumption that there were no NH_4^+ and NO_3^- leaching in the soil outside the core.

$$\textit{Plant N Uptake} = [\, N_{TI}^{Core} + N_{TI}^{Bag} - N_{TI}^{Soil} \,]$$

N_{T1}^{Core} : NH_4^+ and NO_3^- in the resin core soil at the end of incubation

N_{T1}^{Bag} : NH_4^+ and NO_3^- on the resin bag at the end of incubation

N_{T1}^{Soil} : Soil NH_4^+ and NO_3^- outside the resin core at the end of incubation

Annual plant N uptake was calculated by summing seasonal uptake through the year as we did in net N mineralization estimation.

III-3-6. Estimation of Douglas-fir Biomass

Allometric regression equations for the biomass of leaves (B^L), stems (B^S), and roots (B^R) of 7- to 9-year-old Douglas-fir trees were developed from a data set provided by Dr. David Hibbs (Forest Science, Oregon State University). This data set was collected by destructive harvest of other Douglas-fir trees planted at the same site. DBH (diameter at breast height, 1.58 cm) was found to be the best predictor of biomass.

$$\ln B^{L} = 2.416 + 1.388 * \ln DBH \qquad (df = 3, R^2 = 0.898)$$

$$\ln B^{S} = -0.096 + 2.101 * \ln DBH \qquad (df = 3, R^2 = 0.803)$$

$$\ln B^{R} = 1.041 + 1.878 * \ln DBH \qquad (df = 3, R^2 = 0.952)$$

The annual total N accumulation (TN^{AC}) of a Douglas-fir tree is the sum of the N increment in the standing biomass and the amount of N lost in litter-fall during the year.

$$TN^{AC} = [\, N^{sd}_{T1} + 0.2 * N^{lf}_{T1} - N^{sd}_{T0} \,] * A\#$$

N^{sd}_{T1} is the amount of N in the standing biomass of a Douglas-fir tree at the end of year.

N^{sd}_{T0} is the amount of N in the standing biomass of a Douglas-fir tree at the beginning of the year.

N^{lf}_{T1} is the amount of N lost for one Douglas-fir tree in the form of litter fall during the year. Because the average life of Douglas-fir needles is about five years, the yearly percentage of Douglas-fir litter fall is 0.2.

A# is the number of Douglas-fir trees per hectare in the field.

$$N^{sd} = (N\%^{R} * B^{R} + N\%^{S} * B^{S} + N\%^{L} * B^{L})$$

$$N^{lf} = N\%^{L} * B^{L}$$

$N\%^R$, $N\%^S$, and $N\%^L$ are the N percentages of Douglas-fir tree roots, stems, and leaves, respectively. In this study, the $N\%^L$ was measured to be 1.09 % (Chapter II). The N percentage of Douglas-fir stems ($N\%^S$ = 0.11 %) and roots ($N\%^R$ = 0.58 %) was provided by Dr. Mark Harmon (Forest Science, Oregon State University).

III-3-7. Statistic Analysis

The data were analyzed statistically using Statgraphics Plus 2.0 for Windows. One-way analyses of variance (ANOVA) were carried out for soil NH_4^+ and NO_3^- under red alder and Douglas-fir for different sampling times, net N mineralization and net nitrification of red alder and Douglas-fir soils, and plant N uptake of red alder and Douglas-fir for different incubation periods. When the ANOVA was significant ($p < 0.05$), the least significant differences (LSD) were calculated in order to compare the influence of different sampling times and incubation periods. Student's *t*-test was used to examine whether plant N uptake of red alder and Douglas-fir during the second incubation period were different from zero in the 1994 study. A paired *t*-test was performed to examine differences in soil NH_4^+ and NO_3^-, net N mineralization, net nitrification, and plant N uptake between red alder and Douglas-fir or red alder and Douglas-fir soils.

III-4. Results and Discussion

III-4-1. Soil Inorganic N Pools

Compared to soil NH_4^+, the values of soil NO_3^- were much smaller and less variable under both red alder and Douglas-fir in the 1993 and 1994 studies, which indicated that NH_4^+ was the main form of soil inorganic N. The values of soil NH_4^+ under both red alder and Douglas-fir in the spring (from April to May) were significantly higher than those in the other seasons of the year in both 1993 and 1994 (Figure III-1a and III-1b). The values of soil NH_4^+ in the spring of 1993 ranged from 72 to 94 kg N ha^{-1}, which were unusually high. In 1993 the values of soil NO_3^- under both red alder and Douglas-fir in the winter (from November to April) were significantly higher than in the other seasons of the year (Figure III-2a), probably because of the lower plant N uptake. There were no significant differences in soil NO_3^- values among different seasons under either red alder or Douglas-fir in the 1994 study (Figure III-2b).

After N fertilizing at a rate of 4.5 kg N ha^{-1}, the values of soil NH_4^+ increased about 6 ± 2 kg N ha^{-1} in the 1993 study, and 7 ± 3 kg N ha^{-1} in the 1994 study (Figure III-2a and Figure III-2b). Therefore, the effect of soil N fertilization was observed under field conditions.

There were no significant differences in soil inorganic N pools between red alder and Douglas-fir soils in 1993 and 1994 ($t_{paired} = 0.937$, $p = 0.351$ for NH_4^+ in

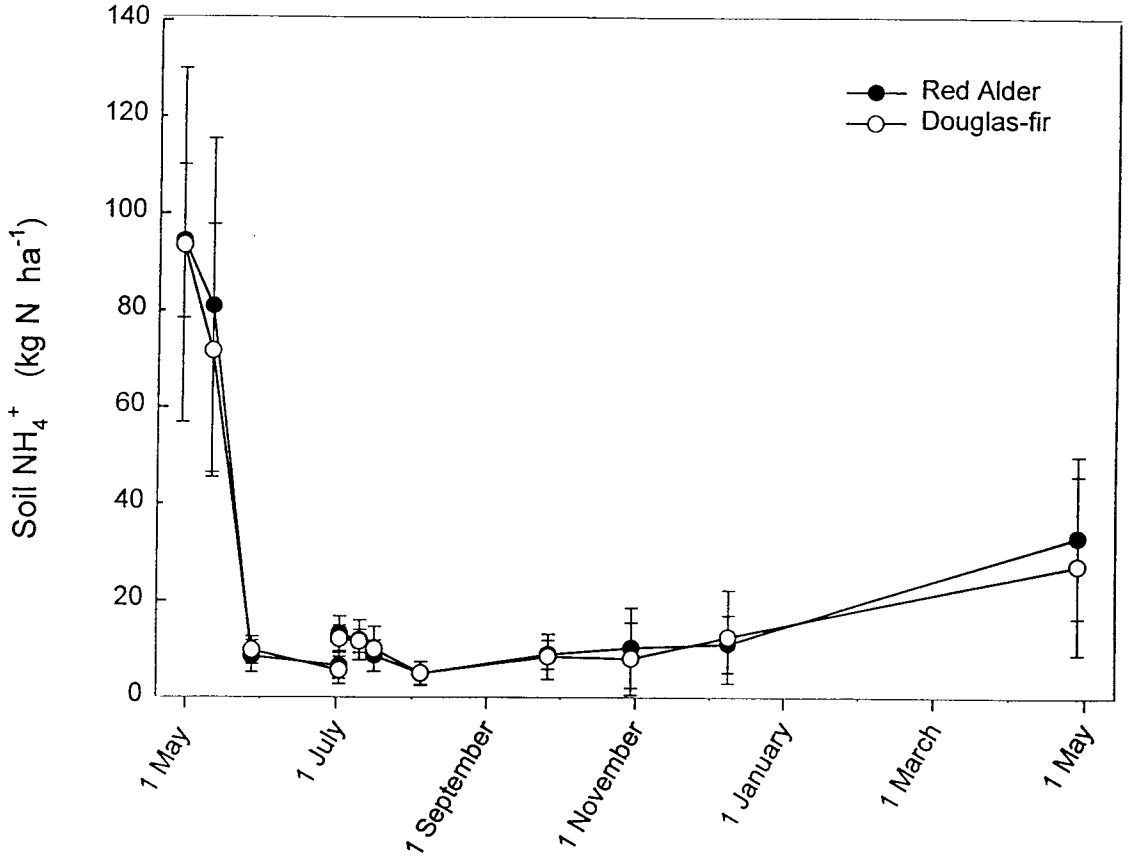

Figure III-1a. Soil NH_4^+ under red alder and Douglas-fir for different sampling times in the 1993 study. Bars are standard deviations, n = 8.

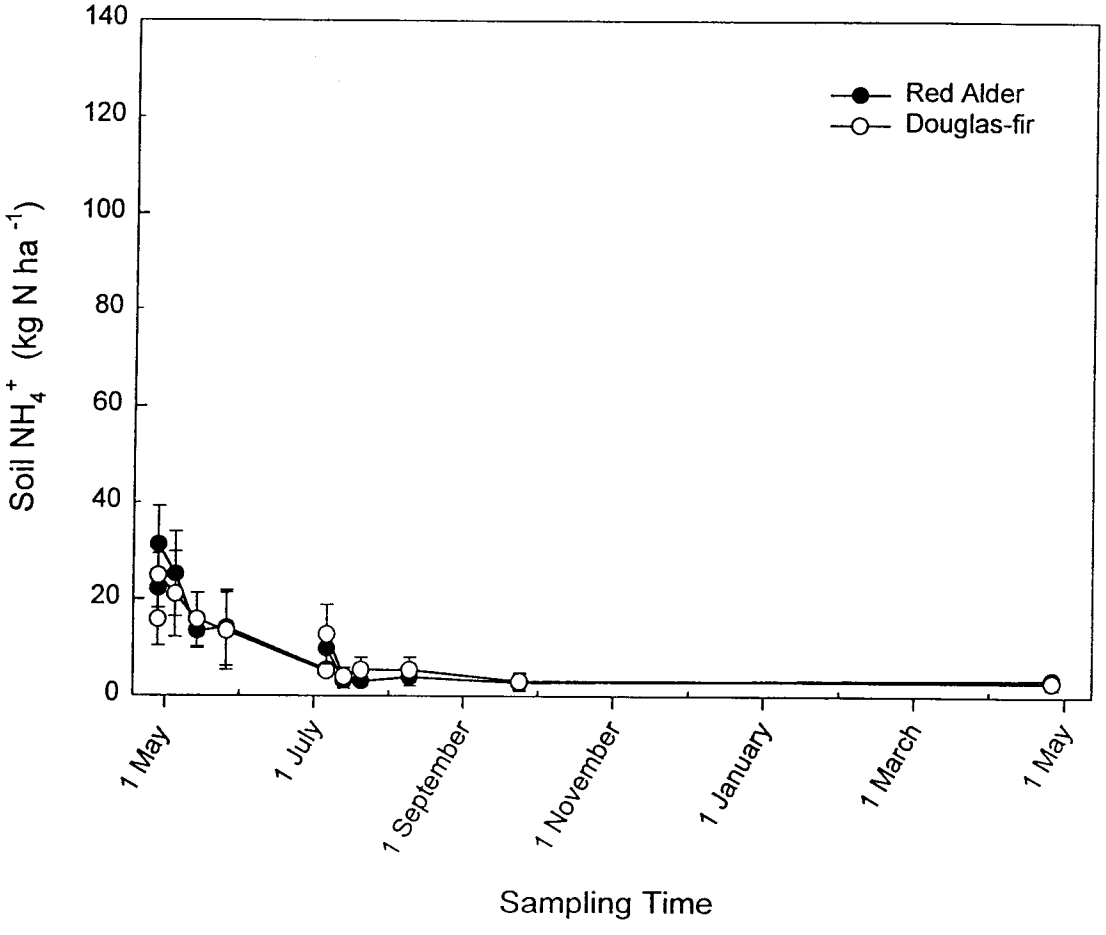

Figure III-1b. Soil NH_4^+ under red alder and Douglas-fir for different sampling times in the 1994 study. Bars are standard deviations, n = 8.

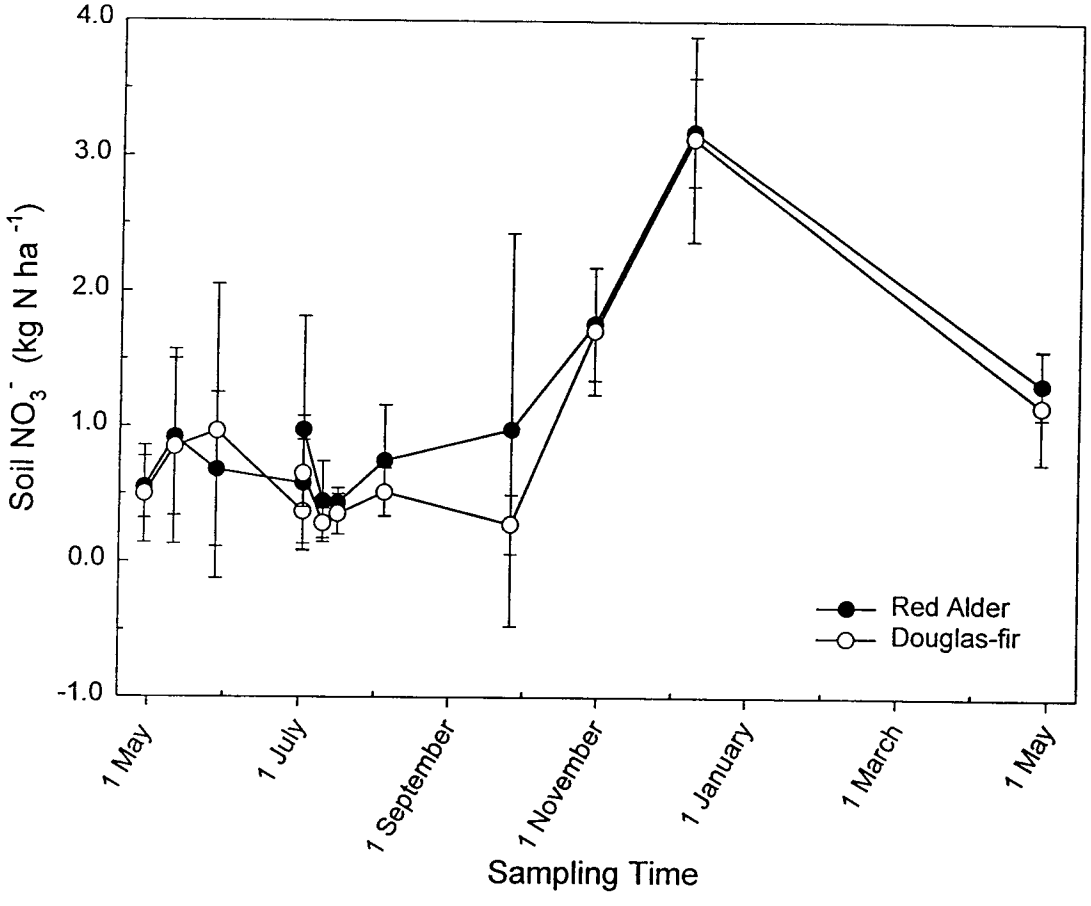

Figure III-2a. Soil NO_3^- under red alder and Douglas-fir for different sampling times in the 1993 study. Bars are standard deviations, n = 8.

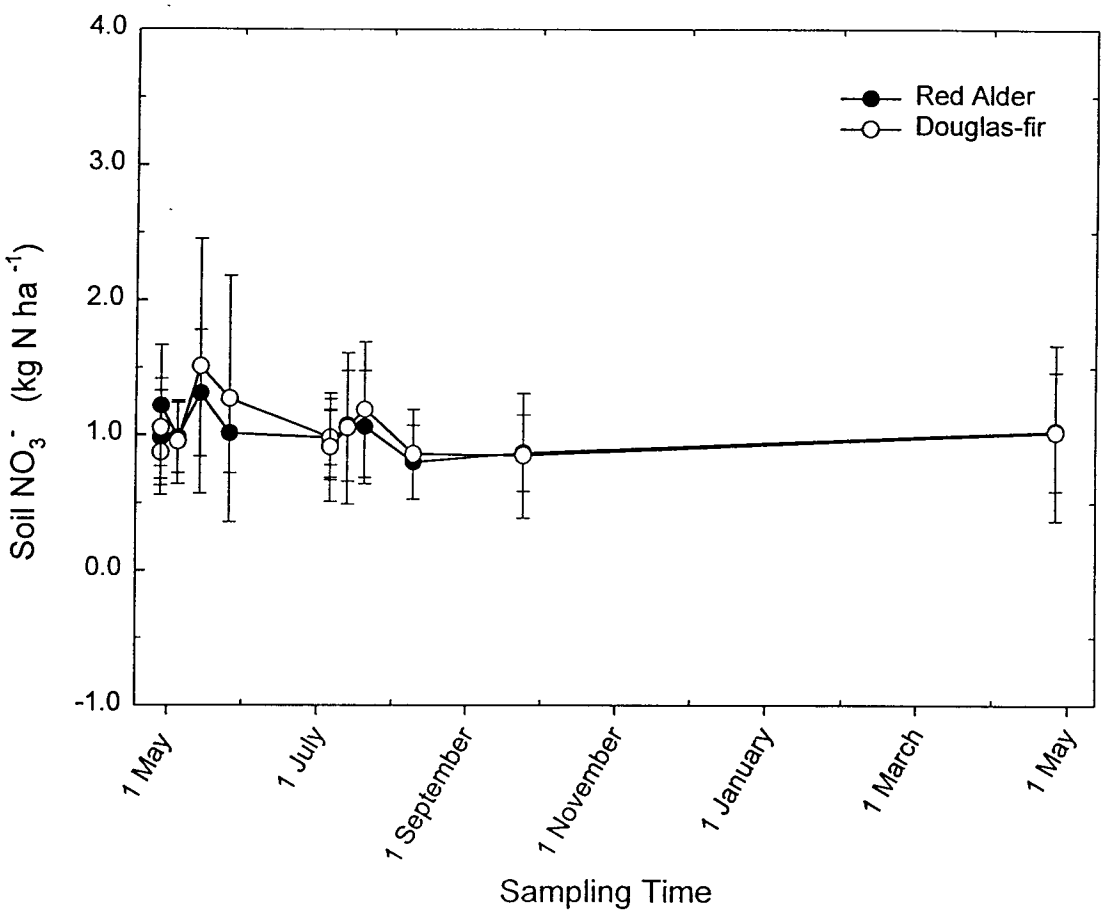

Figure III-2b. Soil NO_3^- under red alder and Douglas-fir for different sampling times in the 1994 study. Bars are standard deviations, n = 8.

1993; t_{paired} = 1.112, p= 0.269 for NH_4^+ in 1994; t_{paired} = 1.894, p= 0.394 for NO_3^- in 1993; t_{paired} = 1.068, p= 0.288 for NO_3^- in 1994).

Previous studies showed that the soil inorganic N pools under alders were greater than under Douglas-fir or other associated trees (Tarrant and Miller, 1963; Bormann and DeBell, 1981; DeBell et al., 1983). The larger soil inorganic N pool under alders was mainly due to the large amounts of N input from fallen alder leaves, and from root and nodule turnover following decay and regeneration (Cote and Camire, 1984; Dawson et al. 1983; Edmonds, 1980; Hansen and Dawson 1982). We found no differences in soil NH_4^+ and NO_3^- between red alder and Douglas-fir soil for different sampling times in our study, however, probably because it may take many years of symbiotic N_2 fixation by red alder to build up soil N content sufficiently to alleviate the competition for available N (soil inorganic N) among microorganisms, understory vegetation, and other tree species.

The alder trees used in the previous studies (Bormann and DeBell, 1981; DeBell et al., 1983; Tarrant and Miller, 1963) were more than 20 years old. The red alder used in our study were only 7 to 9 years old. The effect of red alder symbiotic N_2 fixation may not have accumulated enough to significantly increase the soil inorganic N pools under red alder. Also, red alder and Douglas-fir seedlings were planted so closely that even if red alder added extra N, the experimental design might not have detected a difference in soil inorganic N between under red alder and Douglas-fir. The leaves of red alder can fall on the ground of adjacent Douglas-fir, and be decomposed and transformed into soil inorganic N. The roots of red alder can spread to the soils under

adjacent Douglas-fir. Therefore, the additional N input from red alder can easily spread in the soils under Douglas-fir.

Two studies on 4-year-old young red alder stands also showed no differences in soil inorganic N between red alder and Douglas-fir (Cole and Newton ,1986; Cole et al. 1993). Our results were also in agreement with results from Van Miegroet et al. (1992), in which they found there were no significant increases in soil inorganic N dynamics, by using in situ buried-bag incubation technique, between the paired adjacent 3-year-old red alder and Douglas-fir stands converted from former Douglas-fir stands.

III-4-2. Net N Mineralization and Net Nitrification

In both years, there were no differences in net N mineralization between red alder and Douglas-fir soils ($t_{paired} = 0.159$, $p = 0.875$ in 1993; $t_{paired} = 0.994$, $p = 0.338$ in 1994).

In both years, the net N mineralization under both red alder and Douglas-fir in the spring (May to July) were negative (Figure III-3a and Figure III-3b), indicating that net N immobilization took place for this period of time. The net N immobilization rates in the spring of 1993 were much higher than those in the other seasons of the year, which made the overall yearly net N mineralization negative. However, the net N immobilization rates in the spring of 1994 were very close to the net N mineralization rates of the subsequent incubation period. Because the second incubation period was much longer than the first, the overall yearly net N mineralization rates in the 1994 study were positive for both red alder and Douglas-fir soils.

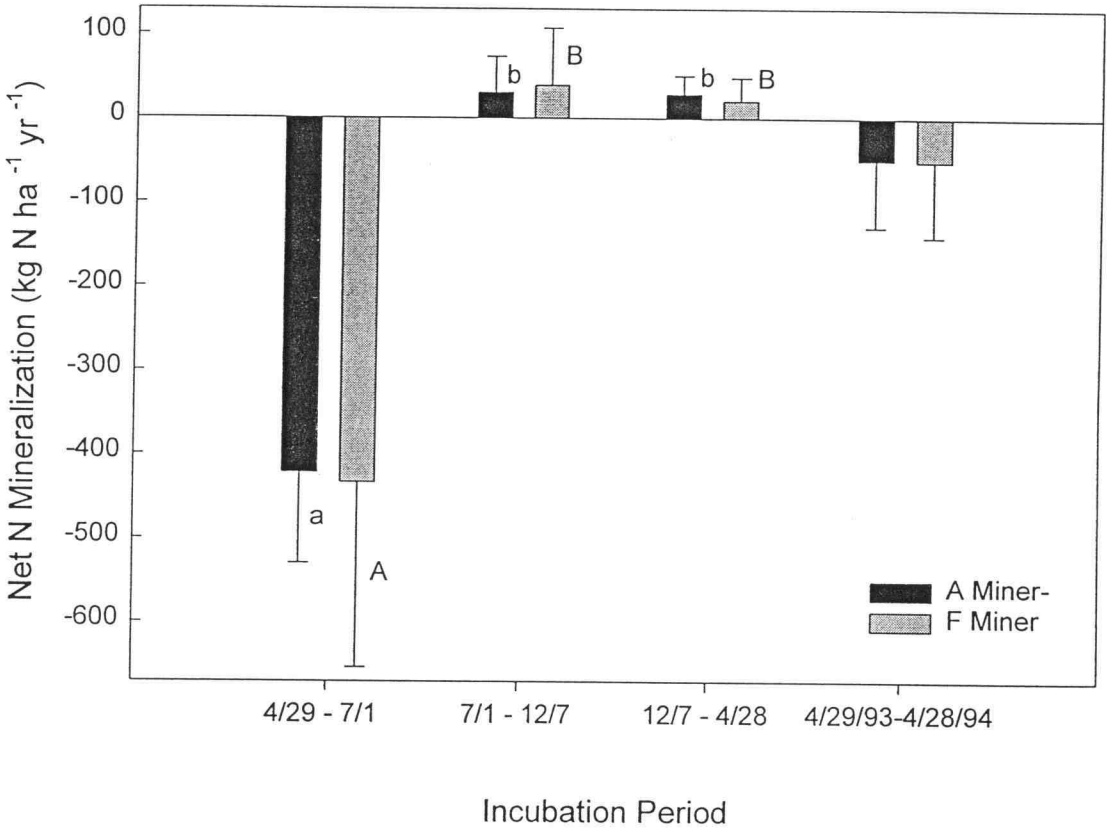

Figure III-3a. Soil net N mineralization under red alder and Douglas-fir in the the 1993 study. Bars are standard deviations, n = 8.

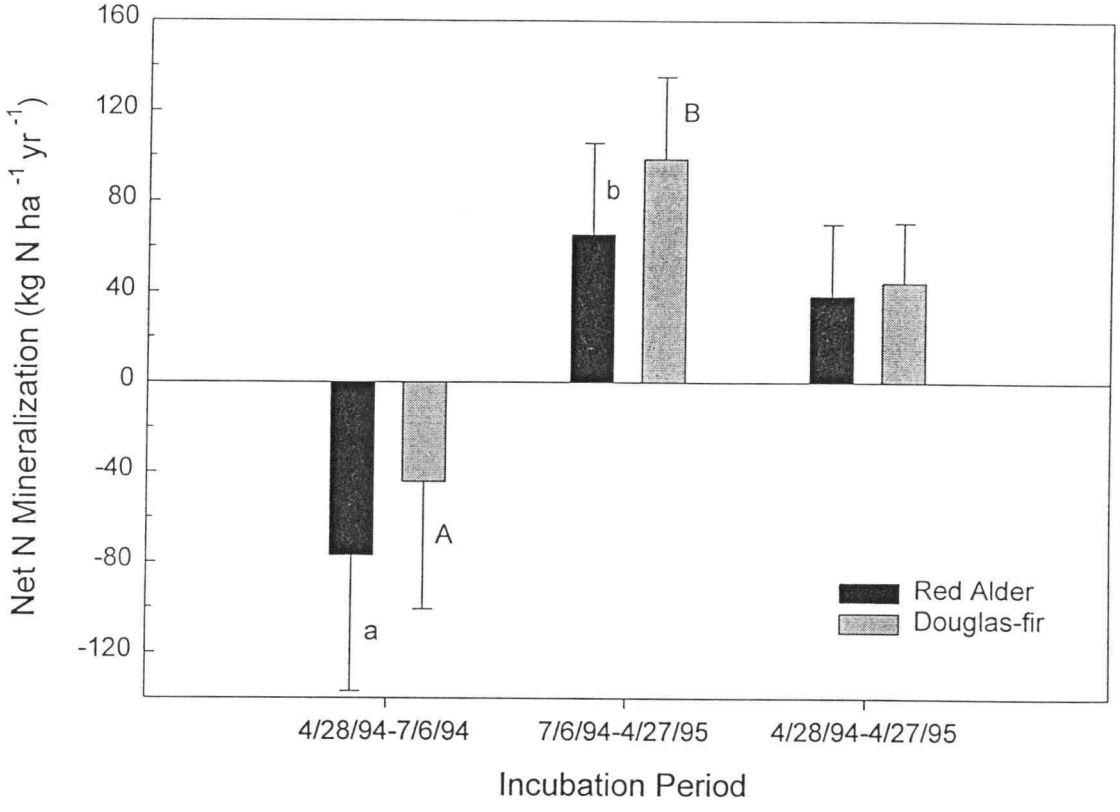

Figure III-3b. Soil net N mineralization under red alder and Douglas-fir in the the 1994 study. Bars are standard deviations, n = 8.

In 1993 and 1994, there were no differences in net nitrification between red alder and Douglas-fir soils (t_{paired}= 0.208, p= 0.837 in 1993; t_{paired} = 1.413, p= 0.181 in 1994).

In 1993, the net nitrification rates in the spring were significantly higher than in the other seasons of the year for both red alder and Douglas-fir soils (Figure III-4a). The higher net nitrification rates in the spring could be caused by the greater soil NH_4^+, and higher soil moisture and temperature during that season. There were no significant differences in net nitrification between the two incubation periods for both red alder and Douglas-fir soils in 1994 (Figure III-4b), mainly due to the large variation and the longer incubation time of the second incubation period.

Negative net N mineralization may reflect methodological limitations of the in situ resin core incubation method. Severing of roots by placing cores in the soils may cause underestimation of net N mineralization by enhancing N immobilization (Stump and Binkley, 1993). Because the artifacts introduced by containment become more pronounced as the incubation time is increased, the longer incubation may make soil net N mineralization and net nitrification more vulnerable to error (Adams et al., 1989). It is therefore possible that some underestimation of actual net N mineralization and nitrification rates occurred in this study, especially during the spring incubation period for both red alder and Douglas-fir soils. Because of heavy early snow in 1994, we could not sample resin cores. The second incubation period in the 1994 study lasted 295 days, which certainly made soil net N mineralization and net nitrification estimates tenuous.

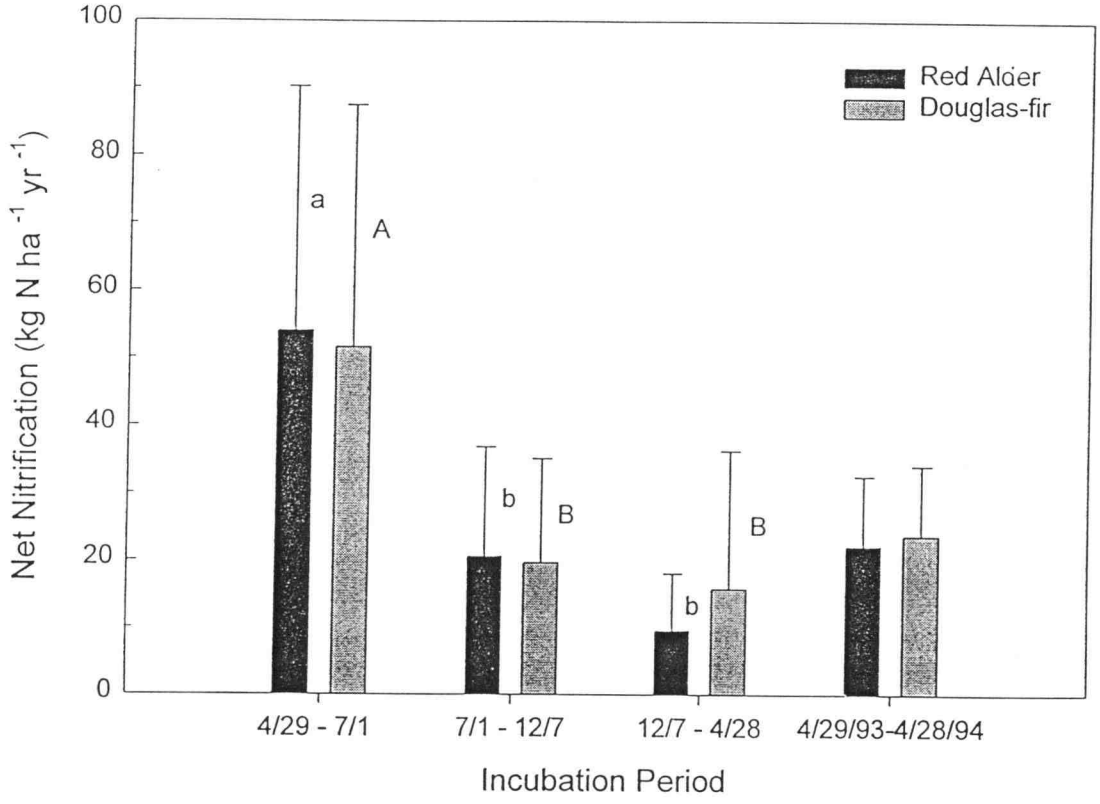

Figure III-4a. Soil net nitrification under red alder and Douglas-fir in the 1993 study. Bars are standard deviations, n = 8.

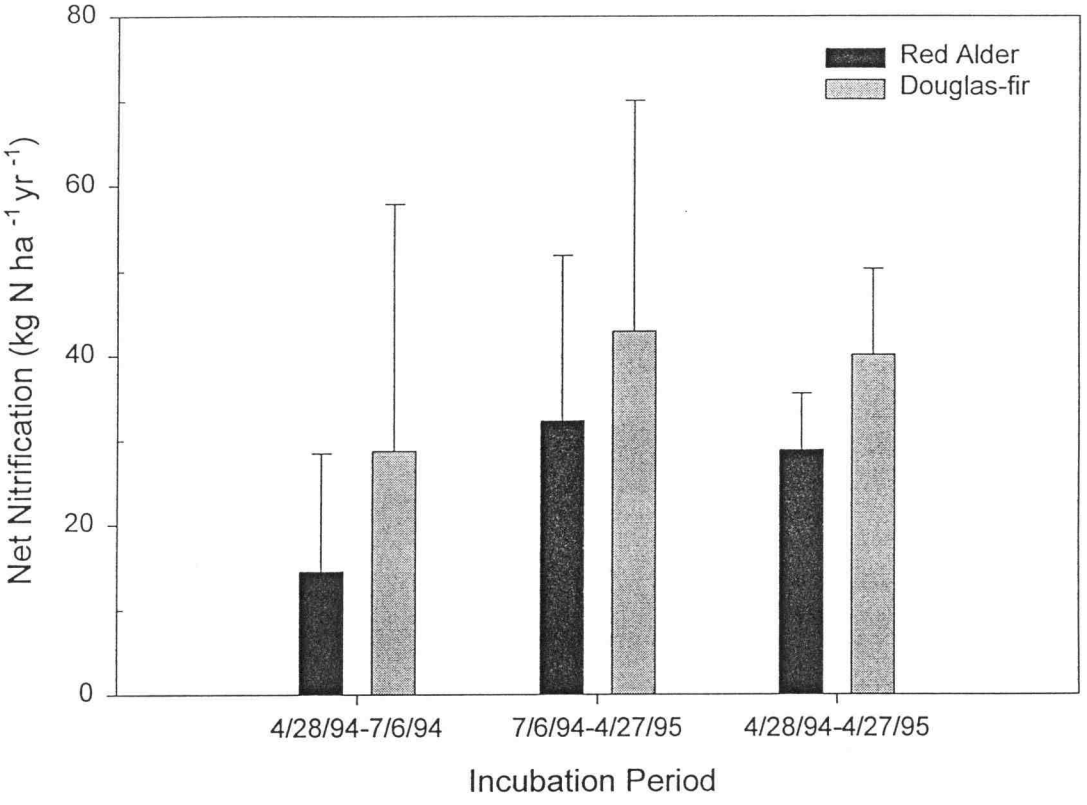

Figure III-4b. Soil net nitrification under red alder and Douglas-fir in the 1994 study. Bars are standard deviations, n = 8.

A study from two pairs of adjacent, 55-year-old forests showed that the net N mineralization in a year-long series of resin-core incubations of forest floor plus 0-0.15 m depth soil of alder-conifer stands was much higher than that of conifer stands (Binkley et al., 1992). Some other studies indicated that the symbiotic N_2 fixation between red alder and *Frankia* caused greater net N mineralization, higher net nitrification rates compared to the associated species (Binkley et al., 1993; Van Miegroet et al., 1989; Van Miegroet et al., 1990).

The young age of red alder trees and the close spacing of red alder and Douglas-fir seedlings in our study could be the main reasons why we could not detect a difference in net N mineralization and net nitrification between red alder and Douglas-fir soils.

III-4-3. Plant N Uptake

There were no differences in plant N uptake between red alder and Douglas-fir plots in 1993 or 1994 studies ($t_{paired} = 0.105$, $p = 0.917$ in 1993; $t_{paired} = 0.084$, $p = 0.935$ in 1994). The failure to detect a difference could be caused by high variability.

In 1993, plant N uptake showed a significant seasonal pattern for all the plants in the system, with spring > summer/fall > winter (Figure III-5a). The seasonal plant N uptake pattern mainly reflected the seasonal growth of red alder and Douglas-fir. The plant N uptake of red alder and Douglas-fir plots in the winter (December to May) was negative, however, it was not significantly different from zero.

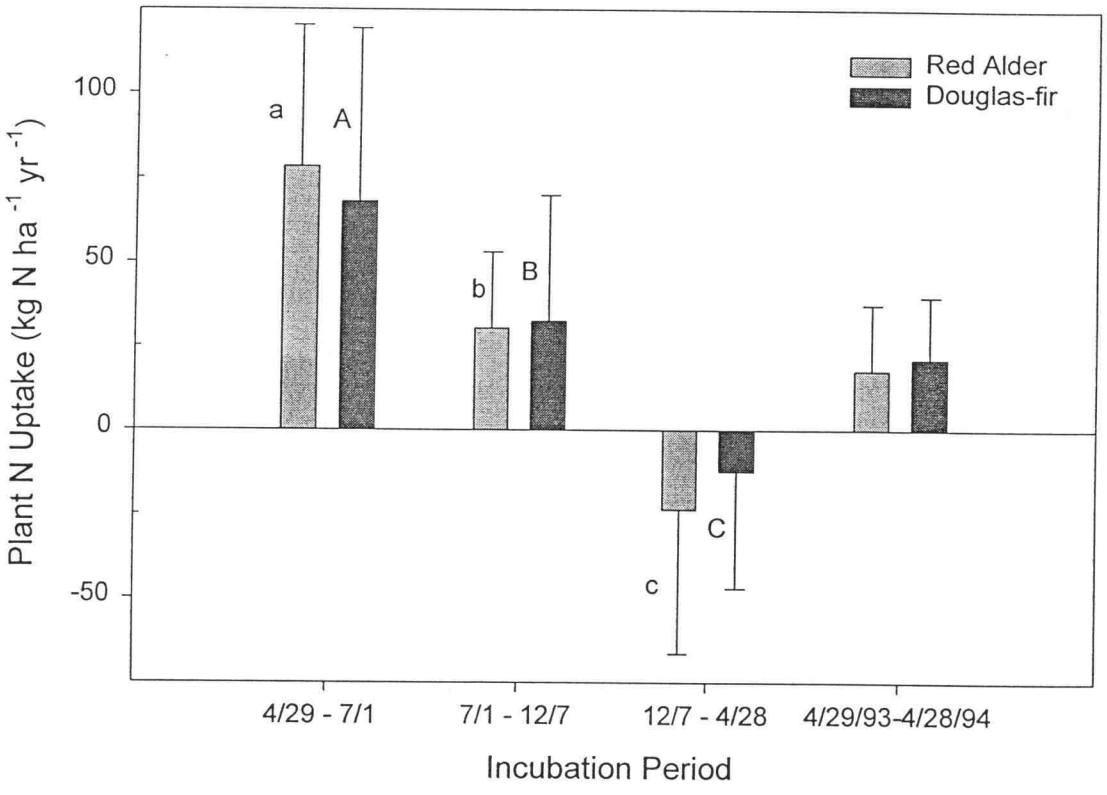

Figure III-5a. Plant N uptake of red alder and Douglas-fir plots in the 1993 study. Bars are standard deviations, n = 8.

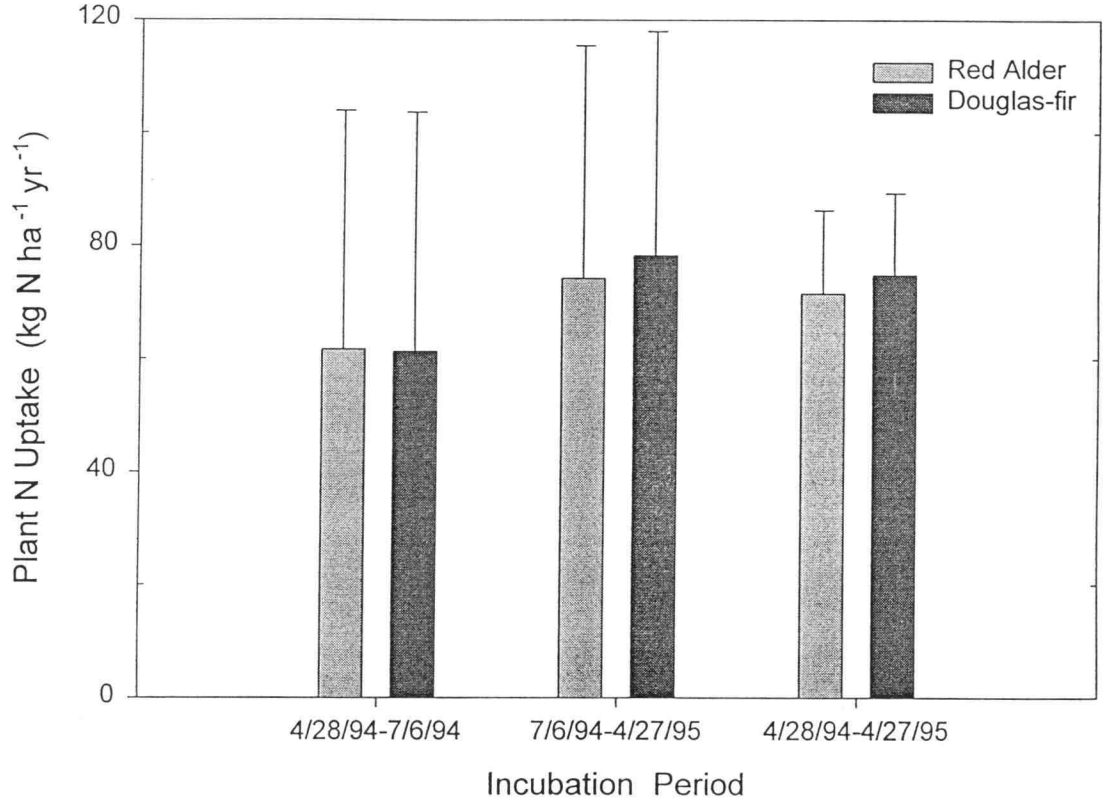

Figure III-5b. Plant N uptake of red alder and Douglas-fir plots in the 1994 study. Bars are standard deviations, n = 8.

Results from our N_2 fixation study (Chapter II) demonstrated that the average percentage nitrogen derived from N_2 fixation of red alder was 98 ± 2 %. For pure red alder stands, the total nitrogen derived from N_2 fixation was estimated to be about 90 kg N ha^{-1} yr^{-1}. Therefore, the actual N uptake from soil of pure red alder stands would be only 2 kg N ha^{-1} yr^{-1}. Using the resin core method, the overall yearly plant N uptake of red alder plots in 1993 was estimated to be 18 kg N ha^{-1} yr^{-1}. Therefore, about 16 kg N ha^{-1} yr^{-1} (18 minus 2 kg N ha^{-1} yr^{-1}) were taken up by the understory vegetation. Peter Homann (personal communication) estimated that the understory vegetation in 8-year-old red alder stands in western Washington contained 37 kg N ha^{-1}. Assuming that the understory vegetation biomass turns over once every two years, the annual N uptake of the understory vegetation would be about 18 kg N ha^{-1}, which was close to our estimation.

There was understory vegetation growing under both red alder and Douglas-fir. Assuming that the rate of plant N uptake by other plants under red alder was the same as under Douglas-fir, the actual plant N uptake of Douglas-fir would be 5 kg N ha^{-1} yr^{-1} (21 minus 16 kg N ha^{-1} yr^{-1}). Based on the calculation of Douglas-fir biomass, the yearly N gain of young Douglas-fir was 6 kg N ha^{-1} yr^{-1} (range from 4 to 8 kg N ha^{-1} yr^{-1}), which fits well with the N uptake of Douglas-fir measured by the resin core incubation method.

The young age of red alder trees and the close spacing of red alder and Douglas-fir seedlings could be the main reasons why we could not detect a difference in soil inorganic N, net N mineralization, and net nitrification under red alder and Douglas-fir

trees. Ammonium was the main form of soil inorganic N. Plant N uptake by red alder and Douglas-fir showed a seasonal pattern, spring > summer/fall > winter. Plant N uptake of Douglas-fir measured by using resin cores was in good agreement with that calculated from its allometric regression equation, which suggested that the in situ resin core incubation technique is reliable method to estimate plant N uptake in the field.

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Chapter IV

Summary and Conclusion

Red alder plays an important role in many Pacific Northwest forest ecosystems because of its input of N, a major limiting factor for the growth of Douglas-fir. Studies on red alder have increased significantly in the past three decades. Many of these are focused on the measurement of N_2 fixation rates of red alder and its influences on the ecosystem. This thesis provided the opportunity to advance the state of knowledge regarding the evaluation of the ^{15}N dilution method and the effects of young red alder on soil N dynamics.

In the first part of our study, we found that the atom % ^{15}N of red alder foliage was not affected by the ratio of labeling to tree crown area (LA/CA) when the N_2 fixation rate of young red alder is high but Douglas-fir was. The roots of Douglas-fir trees were concentrated mainly within 1.5 crown areas. The percentage of N derived from N_2 fixation (PNDF) of red alder was estimated to be over 95 %, and the total N derived from N_2 fixation (TNDF) was estimated to be about 22 kg N ha^{-1} yr^{-1} for mixed stands (25% of red alder and 75% Douglas-fir) and about 90 kg N ha^{-1} yr^{-1} for pure red alder stands. This study was the first to use the ^{15}N dilution method to estimate PNDF and TNDF of the young red alder trees under field conditions in the Pacific Northwest. Our estimated PNDF and TNDF of young red alder were in good agreement with previous studies. The evaluation of the PNDF sensitivity to reference plant selection

LA/CA provides important information for future N_2 fixation studies of red alder using the ^{15}N dilution method.

In the second part of our study, we found no significant differences in soil inorganic N, net N mineralization, and net nitrification under red alder and Douglas-fir trees, probably because of the young age of the red alder trees and the close spacing of red alder and Douglas-fir seedlings. Ammonium was found to be the main form of soil inorganic N. Plant N uptake of red alder and Douglas-fir showed a seasonal pattern, spring > summer/fall > winter. Uptake of N by Douglas-fir as measured by using resin cores was in good agreement with that calculated from its allometric regression equation, which suggested that the in situ resin core incubation technique is reliable method to estimate N plant uptake in the field.

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