AN ABSTRACT OF THE THESIS OF

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Red alder (*Alnus rubra* Bong.) is recognized as an important source of nitrogen to ecosystems that it inhabits. I examined N dynamics within alder trees, alder leaf litter, and the soil beneath alder leaf litter. $^{15}$Nitrogen, a stable isotope of N, was used as a tracer to follow the movement of N through the various systems of interest. Red alder trees were labeled with $^{15}NO_3^-$ and $^{15}NH_4^+$ using the stem-injection method. Leaves were sampled 3 and 15 mo subsequent to injection within several crown positions, including top, bottom, proximal, medial, and distal. Stem injection of both $^{15}NH_4^+$ and $^{15}NO_3^-$ at levels approaching one percent of crown N effectively labeled red alder trees. Although more variable, $^{15}NO_3^-$ may have been more efficient in initial labeling. The distribution of $^{15}$N was uniform at the time of the first sampling, but was diluted in the distal and top positions by the second sampling. There was a clear increase in total N concentration toward the periphery of the tree. This increase became more pronounced with increasing crown size and crown closure. Crown position with respect to light availability may be the most important determinant in crown N allocation in red alder foliage. To study the transfer of N from red alder trees to the soil, $^{15}$N-labeled red alder foliage was allowed to decompose in the field for 21 mo. The concentration of $^{15}$N was measured in remaining detritus and at 0-5 and 5-15 cm depths in four soil fractions below the detritus. The soil fractions investigated included the light- and heavy-fractions of the soil, the chloroform-labile (microbial biomass) pool, and the whole-soil. Some recovery of $^{15}$N was noted in vegetation growing in the plots. The alder litter lost 78 % of its mass, 77 % of the total initial N, and only 64 % of the total initial $^{15}$N. Although the heavy-fraction contained

77 to 88 % of the total nitrogen, the concentration of N in the light-fraction was 3.5 times that in the heavy-fraction. Whole-soil recoveries were higher than the summed fractions for total N and for $^{15}N$ in the top 5 cm. Light-fractions exhibited higher percent recoveries of $^{15}N$ than heavy-fractions. Percent recovery of $^{15}N$ in the chloroform-labile N fraction was not significant. The majority of nitrogen released from the leaves was concentrated within the top five centimeters of soil. After 21 mo of decomposition, alder detritus acted as a net source of N, most of which remained in the labile pools of the fractionated soil.

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# Nitrogen Dynamics in Red Alder

by

Christopher W. Swanston

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Christopher W. Swanston, Author

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# **Chapter 1:** Introduction

## 1.1 Topics of study

The general goal of the research in this thesis was to advance the state of knowledge of N cycling in red alder (*Alnus rubra* Bong.). Two topics of study were chosen to do this. The first was more ecological in nature, a characterization of the decomposition of red alder leaves and the incorporation of N from those leaves into the active and recalcitrant soil pools. This was deemed a worthy topic of study because of the high N status of alder litter (Trappe and Bollen, 1981) and the observation that organic soil amendments, such as straw and glucose, release different amounts of N into 'light,' silt, and clay soil organic fractions (Ladd *et al.*, 1977). These fractions, in turn, are associated with plant-available N to differing degrees.

The second topic was more applied. It was designed to test the efficacy of a isotope labeling technique for red alder, and to characterize the subsequent distribution of the isotope within the crowns of the labeled trees. It was hoped that this study would provide basic information for consideration in future studies. The fundamental question was whether the technique actually worked in alder. Also of interest was the distribution of retranslocated N and leaf N concentration in the crown, both important factors in $N_2$-fixation studies as well as other alder-associated studies. The following is a general introduction to some of the effects of alder on ecosystems and some of the techniques and rationales used in this research.

## 1.2 Red alder

Red alder obtains N from the soil solution and from a symbiotic relationship with *Frankia*, a $N_2$-fixing actinomycete (Virtanen *et al.*, 1954; Molina *et al.*, 1994; Myrold, 1994). Alder leaf litter has a high N content, generally higher than associated tree species (Tarrant *et al.*, 1951; Harmon, 1990). Red alder is thought to produce more litter than Douglas-fir (Cole *et al.*, 1993). This N-rich litter provides a ready source of

mineralizable N (Edmonds, 1980). Upon decomposition alder litter can significantly increase the fertility, N content, and organic matter content of soils, while decreasing bulk density (Franklin *et al.*, 1967; Radwan *et al.*, 1984; Huss-Danell and Ohlsson, 1991; Bormann *et al.*, 1993).

Alder, where growing in mixed stands with conifers such as Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco), may significantly impact the growth of conifers (Bollen and Lu, 1968). On many sites, the faster growth habit of alder may result in suppression of conifers (Newton *et al.*, 1968). Where competition has been mitigated or does not otherwise cause a net loss of coniferous growth, alder may actually benefit conifers. Interplanting with alder has been shown to significantly increase Douglas-fir growth (Tarrant, 1961; Bollen and Lu, 1968). Because the effects of alder on soil productivity will significantly influence the growth of important interplanted conifer species, or conifers planted on sites previously dominated by alder (Brozek, 1990), it is important to understand the precise nature of those effects.

## 1.3 Soil organic matter fractions

Nitrogen is commonly the most limiting nutrient to plant growth in Douglas-fir ecosystems (Trappe and Bollen, 1981). The availability of N for plant uptake is largely determined by the activities of soil organisms (Paul and Clark, 1989). Soil organisms, in turn, depend on soil organic matter as their primary source of energy (Youngberg, 1981). Soil organic matter has been divided into two relatively distinct fractions related to density and resistance to decomposition (Christensen, 1992). Although these fractions interact to some degree, separate processes govern their formation and maintenance (Sollins *et al.*, 1983; Christensen, 1992).

The less dense of the two fractions, referred to as the light-fraction, is typified by relatively labile nutrient pools and quick cycles of mineralization and immobilization (Sollins *et al.*, 1983). The light-fraction is variously defined as having a density of less than 1.6 to 2.2 g/cm$^3$ (Sollins *et al.*, 1983; Strickland and Sollins, 1987; Theng *et al.*, 1989; Christensen, 1992; Strickland *et al.*, 1992). The heavy-fraction is associated with mineral particles and is composed of organic components less readily available or easily

decomposed (Strickland *et al.*, 1992). Offering more resistance to disintegration, the heavy-fraction has a far slower rate of cycling (Christensen, 1992).

The light-fraction is comprised primarily of plant debris and soil organisms (Theng *et al.*, 1989). The abundance and diversity of soil biota regulate the decomposition of organic residues, rates of mineralization, and immobilization. These processes, in turn, essentially determine the availability of N to plants (Paul and Clark, 1989).

Plant debris occurs predominantly as decomposing roots and leaves. The bulk of soil organic matter is composed of decomposing root material (Fogel and Hunt, 1979). Although leaf litter also plays a significant role in the addition of nutrients to the soil organic matter (Radwan *et al.*, 1984), it may affect nutrient fluctuations in the light-fraction to a lesser degree than root decomposition (Sollins *et al.*, 1983).

Materials in the light-fraction are relatively quickly degraded by soil biota. This is due in part to the accessibility and the quality of the organic matter (Sollins *et al.*, 1996). Accessibility, as defined by Sollins *et al.* (1996), refers to how the location of the substrate affects the ability of microbes and enzymes to physically reach and decompose it. If organic matter is inaccessible to microbes, they cannot aid in its decomposition.

The quality of the organic matter refers, in part, to its C:N ratio, lignin:N ratio, and cellulose and nutrient content (Theng *et al.*, 1989). The C:N ratio and nutrient content of the substrate affect the microbial population attempting to mineralize it and use the released nutrients for respiration (Sollins *et al.*, 1996). Different C:N ratios and nutrient contents will affect different decomposing populations to varying degrees. The lignin:N ratio and cellulose content appear to be inversely related to the rate at which organic matter is decomposed (Meentemeyer, 1978; Melillo *et al.*, 1982; Harmon *et al.*, 1990). Further, high lignin and N concentrations may result in slower decomposition rates than substrates with the same lignin:N ratio, but lower lignin and N concentrations (Harmon *et al.*, 1990). Nonetheless, even high lignin and N substrates in the light-fraction decompose at a faster rate than those in the heavy-fraction pools.

Heavy-fraction N pools are far more stable. Nitrogen in the heavy-fraction is complexed within organo-mineral complexes, occluded within microaggregates, and

adsorbed to soil colloids. Additionally, humic substances decompose very slowly. Readily mineralizable C has already been removed from these complex molecules, and complete mineralization may take decades or even centuries (Theng *et al.*, 1989). Interaction of soil organic matter with polyvalent metal cations and clay micelles contribute to resistance to microbial breakdown (Theng *et al.*, 1989; Sollins *et al.*, 1996). Occlusion within organo-mineral microaggregates limits the accessibility of decomposers to the soil organic matter, further limiting the rate of decomposition (Sollins *et al.*, 1984; Theng *et al.*, 1989; Strickland *et al.*, 1992).

The resistance of heavy-fraction to decomposition contributes to a slow rate of C cycling and a consequent accumulation of N. Much of this N, perhaps initially in excess of that required to maintain a proper microfloral C:N ratio, will also become unavailable. Thus, the heavy-fraction generally contains a majority of the soil N (Sollins *et al.*, 1983; Spycher *et al.*, 1983). The high N content leads to a narrower C:N ratio in the heavy-fraction than in the light-fraction (Spycher *et al.*, 1983; Christensen, 1992), although the light-fraction is more likely to yield plant available N at a given time.

## 1.4 Methods of soil organic matter fractionation

Several methods have been established to separate the light- and heavy-fractions. Christensen (1992) groups these methods of separation into two categories, primary particle size fractionation and density fractionation. Both categories disperse the soil aggregates prior to soil organic matter fractionation with techniques such as sonification, stirring, or shaking. Primary particle size fractionation usually involves sieving, sedimentation, and sometimes centrifugation. The resultant size-separates commonly have different C:N ratios and are thought to be associated with different rates of nutrient cycling. Density fractionation involves separation of the soil organic matter using organic liquids or inorganic salt solutions. Similar to the size-separates, the density-separates have different C:N ratios and appear to correspond with different levels of nutrient availability and cycling.

Density fractionation may entail dispersion of aggregates within the separation medium. Following dispersion, separation of soil organic matter within the separation

medium may be facilitated by using a centrifuge (Spycher *et al.*, 1983), by prolonged settling (Strickland and Sollins, 1987; Strickland *et al.*, 1992; Cambardella and Elliott, 1993), or by repeated floatation (Sollins *et al.*, 1984). Separated light- and heavy-fraction residues can then be analyzed for N and C content.

Strickland and Sollins (1987) analyzed several soils using both the centrifuge method and the suction method (prolonged settling, then removal of the light-fraction by aspiration) and compared the results. The methods yielded similar results, though the suction method was more precise when differences occurred. In addition, the suction method is less expensive, and labor and equipment intensive. The suction method, given its time and equipment requirements, was determined to be most appropriate for the needs of this study.

Both fauna and flora in the soil and forest floor are crucial mediators in the rate of litter and light-fraction cycling. Exclusion of microarthropods has been shown to significantly decrease the rate of litter decompostion (Seastedt and Crossley, 1984). However, soil biomass and the process of mineralization are dominated by microflora (Paul and Clark, 1989). Chloroform ($CHCl_3$) fumigation methods are the most common chemical methods for estimating soil microbial biomass. Chloroform lyses soil organisms, releasing bound N and C into the soil. Chloroform affects other soil fractions little, if at all (Jenkinson, 1976; Jenkinson and Powlson, 1976). The most widely used methods of fumigation are the chloroform-incubation method and the chloroform-extraction method.

The chloroform-incubation method proposed by Shen *et al.* (1984) estimates N held in microbial biomass ($B_N$) by utilizing both fumigation and incubation. $B_N$ is based on the net flush of N ($F_N$) mineralized after incubation, given that 68 percent ($k_N$) of the original $B_N$ is also mineralized. $F_N$ is the N mineralized in a fumigated soil during incubation minus the N mineralized in a non-fumigated control soil during similar incubation.

Brookes *et al.* (1985) sought to avoid the 10-d incubation period and problems associated with incubation by developing a direct extraction technique. The formula for estimating $B_N$ is similar, but excludes incubation. The fumigation-extraction method

fumigates the soil with $CHCl_3$, then extracts immediately with $K_2SO_4$. The extract is analyzed for N content. A control soil is subjected to extraction alone and its extracted N compared with that of the fumigated-extracted soil to determine $F_N$. Brookes *et al.* (1985) found that although the 24-hour fumigation-extraction appeared to measure the same N pools as fumigation-incubation, the extraction $CHCl_3$-N was only 79 % of the incubation $CHCl_3$-N. Thus, they adjust the constant ($k_N$) proposed by Shen *et al.* (1984) from 0.68 to 0.54 when using the 24-hour fumigation-extraction procedure. Brookes *et al.* (1985) assert that their method and constant are applicable to most forest soils, possibly excluding strongly acid soils with a pH of < 4.5. Because of the relative accuracy and lesser time requirements, the fumigation-extraction technique was used in this study.

## 1.5 Stable isotopes

Nason and Myrold (1991) describe the uses of the stable isotopes of N, $^{14}N$ and $^{15}N$, in ecological research. Atom % $^{15}N$ abundance is commonly used to express the proportion of $^{15}N$ to total N ($^{14}N + ^{15}N$) in N pools. Increase in atom % $^{15}N$ abundance in soil or vegetation after the addition of a tracer indicates incorporation of $^{15}N$ into the pool in question.

The use of stable isotopes such as $^{15}N$ can greatly facilitate the study of the effects of N release from alder litter on soil properties. Davidson *et al.* (1992) traced $^{15}N$ added to the soil as it was incorporated into and lost from soil N pools by analyzing the fluctuations in $^{15}N$ concentration. Measurements of the fluctuations in the various N pools through time can provide a reasonable estimate of the cycling of the added $^{15}N$ (Jones and Richards, 1977). Given that sufficient care is taken to maintain accuracy (Harris and Paul, 1989; Liu and Mulvaney, 1992), stable isotope research has great potential to clarify soil N relationships which could previously be only crudely quantified.

1.6 Nitrogen and alder

As stated earlier, alder is known to acquire fixed $N_2$ from *Frankia* (Virtanen *et al.*, 1954; Molina *et al.*, 1994; Myrold, 1994). However, the rate at which $N_2$ is fixed and the influence of environmental factors on fixation are still a major topic of study (Wheeler *et al.*, 1981; Prégent and Camiré, 1985; Côté *et al.*, 1989; Samuelson *et al.*, 1990; and Vogel and Dawson, 1991). Studies involving $^{15}N$ at tracer and natural abundance levels have been especially useful in determining rates of fixation and sources of N. (Domenach and Kurdali, 1989; Beaupied *et al.*, 1990; McNeill *et al.*, 1994). Using black alder (*Alnus glutinosa* (L.) Gaertn), Domenach and Kurdali (1989) demonstrated the danger of ignoring leaf age in N fixation studies. However, their study focused on the source of N to the leaves, an important consideration in $N_2$-fixation studies but of less importance in other studies.

Many studies have sought to characterize the content or concentration of N in alder crowns to estimate resorption (Dawson and Funk, 1981; Chapin *et al.*, 1983; Côté and Dawson, 1986; Côté *et al.*, 1989) or for various other reasons (DeBell and Radwan, 1983; Joseph *et al.*, 1991). The method of leaf sampling often goes unmentioned (Joseph *et al.*, 1991) or unjustified (Rodríguez-Barrueco *et al.*, 1984; Côté and Dawson, 1986). Improper sampling of crown foliage may lead to inaccurate estimations of N concentration or content, as studies of foliar N distribution in peach and apple canopies would indicate (Porpiglia and Barden, 1980; Sanchez and Righetti, 1990).

With increasing availability of mass spectrometers, isotope studies have become a major avenue of exploring N transformations within alder trees and litter. Several methods for labeling alder trees have been tested, including root and foliar fertilization with $^{15}N$ (González-Prieto *et al.*, 1995) and fixation of $^{15}N_2$ (McNeill *et al.*, 1994). Stem-injection (Horwath *et al.*, 1990) of alder remains untried. Perhaps the main benefit of the stem-injection technique is that it specifically labels the tree. Root fertilization results in soil contamination with $^{15}N$, complicating estimations of reserve use and $N_2$-fixation. Foliar fertilization is limited to trees small enough to adequately and uniformly cover with the urea-$^{15}N$ spray.

There is some question as to the most appropriate form of N to inject into alder. Even small levels of $NH_4^+$ can be toxic in plant leaves (Waring and Schlesinger, 1985). Alternatively, the vast bulk of nitrate reductase activity (NRA) occurs in the roots of alder, with little or no $NO_3^-$ measurable in the stem (Blacquière and Troelstra, 1986; Pizelle and Thiery, 1986). It is unclear how well the constitutive NRA in the leaves and shoots would adjust to even a small influx of $NO_3^-$ (Benamar *et al.*, 1989).

## 1.7 Objectives

Each of the two following chapters has specific objectives. The objective of the research reported in Chapter 2 was to use $^{15}N$ tracer techniques to characterize the loss of N from decomposing $^{15}N$-labeled red alder leaf-litter and the subsequent incorporation of the $^{15}N$ into the whole-soil and the soil light- and heavy-fractions and chloroform-labile N (as and index of microbial biomass). The objectives of the research reported in Chapter 3 were to evaluate the viability of using the stem-injection procedure to label red alder foliage with $^{15}NO_3^-$ and $^{15}NH_4^+$, and to assess the uniformity of foliar $^{15}N$-labeling and N concentration among several different crown positions 3 and 15 mo after injection.

# CHAPTER 2

## Incorporation of Nitrogen from Decomposing Red Alder Leaves into a Mesic Oregon Forest Soil

Christopher W. Swanston and David D. Myrold

## 2.1 Abstract

Nitrogen is typically the most limiting nutrient to conifer growth in forest soils. Red alder (*Alnus rubra* Bong.), through the senescence and deposition of N-rich foliage, often provides a net input of N to soils. The contribution of red alder litter to several labile and non-labile N pools in an Oregon upland mesic forest soil was characterized using $^{15}N$ tracer techniques. The concentration of $^{15}N$ was measured after 21 mo in remaining $^{15}N$-labeled red alder detritus and at 0-5 and 5-15 cm depths in four soil fractions. The soil fractions investigated included the light- and heavy-fractions of the soil, the chloroform-labile (microbial biomass) pool, and the whole-soil. Some recovery of $^{15}N$ was noted in vegetation growing in the plots. The alder litter lost 78 % of its mass, 77 % of the total initial N, and only 64 % of the total initial $^{15}N$. Although the heavy-fraction contained 77 to 88 % of the total N, the concentration of N in the light-fraction was 3.5 times that in the heavy-fraction. Whole-soil recoveries were higher than the summed fractions for total N and for $^{15}N$ in the top 5 cm. Light-fractions exhibited higher percent recoveries of $^{15}N$ than heavy-fractions. Percent recovery of $^{15}N$ in the chloroform-labile N fraction was not significant. The majority of N released from the leaves was detained within the top five centimeters of soil. After 21 mo of decomposition, alder detritus acted as a net source of N, most of which remained in the labile pools of the fractionated soil.

## 2.2 Introduction

Nitrogen deficiency is commonly one of the most limiting factors to tree growth in Pacific Northwest forests (Gessel *et al.*, 1973). Red alder (*Alnus rubra* Bong.), able to obtain N from a symbiotic relationship with a $N_2$-fixing actinomycete (Binkley *et al.*, 1994), produces leaf litter with a N content far in excess of associated tree species (Tarrant *et al.*, 1951; Harmon, 1990). This N-rich litter provides a ready source of mineralizable N (Edmonds, 1980). Upon decomposition, alder leaf-litter can significantly increase the productivity of N deficient soils (Franklin *et al.*, 1968; Radwan *et al.*, 1984; Huss-Danell and Ohlsson, 1992; Bormann *et al.*, 1993). Because the effects of alder on soil productivity may significantly influence the growth of important

interplanted or alternately-cropped conifer species (Brozek, 1990), it is important to understand the nature of those effects.

Although the connection between alder and increased soil N has been demonstrated, there is still a dearth of literature characterizing the actual fate of N once it is released from alder leaf-litter. Previous studies have measured the rate and amount of decomposition and N loss from alder leaf-litter (Edmonds, 1980; Harmon *et al.*, 1990; Fyles and Fyles, 1993; Cole *et al.*, 1995) and the overall effects of alder habitation on soil organic matter and N content (Tarrant and Miller, 1963; Bormann and Sidle, 1990; Binkley *et al.*, 1994). Yet, these studies did not directly show the impact of the mineralized N on specific soil organic matter pools.

Sollins *et al.* (1984) measured N and carbon in the light- and heavy-fractions of soils under conifer, red alder, and conifer/alder sites. Alder and alder/conifer sites showed higher relative concentrations of N and narrower C:N ratios in both the labile light-fraction and recalcitrant heavy-fraction. Strickland *et al.* (1992) attempted to find the source and rate of the N accumulation in the fractions by incubating soils with $^{15}NH_4Cl$ for 60 d and measuring the fractions for $^{15}N$ enrichment. The $^{15}N$ tracer techniques and incubation facilitated the conclusion that N is incorporated into the heavy-fraction more quickly than previously thought. The findings of these studies show a cumulative enrichment in both carbon and N in specific soil fractions under alder, and enrichment of the soil fractions from an inorganic source during a short time period. What remains to be shown is the effect of N from decomposing leaf-litter on the different soil fractions.

The goal of this study was to use $^{15}N$ tracer techniques to characterize the loss of N from decomposing $^{15}N$-labeled red alder leaf-litter and the subsequent incorporation of the $^{15}N$ into the whole-soil and specific soil organic pools. Our findings generally agreed with those of Sollins *et al.* (1984) and Strickland *et al.* (1992), but we were able to examine the influence of a single organic source of N on the soil fractions after two years.

## 2.3 Materials and methods

### *2.3.1 Research site*

The study was conducted in the central western Cascades Range at H.J. Andrews Experimental Forest, Willamette National Forest, Oregon, USA. All plots were established on a single slope within a clear-cut area of about 1 ha. Care was taken to choose microsites with homogeneous characteristics such as absence of large woody debris or nearby seedlings. Vegetation consisted primarily of Pacific blackberry (*Rubus ursinus* Cham. & Schlect.) and grasses.

### *2.3.2 Plot establishment*

Labeled red alder leaves were produced by injecting artificial sap mixed with 0.675 g of 15.8 atom % $^{15}N$-$(NH_4)_2SO_4$ into the xylem (Horwath et al., 1992; Chapter 3) of each of six 7-year-old red alder trees in July 1993. Fallen leaves were captured in 1.25-cm mesh bird netting surrounding the crown and tied at the base of each tree. The leaves were collected in November, air dried, and analyzed by mass spectrometry for N and C content. The labeled leaves from the six trees varied little in $^{15}N$ content and were mixed together (mean $^{15}N$ content = 0.4195 atom % $^{15}N$, SE = 0.0054). The initial concentrations of N and C, as a percent of dry weight, were 2.89 % N and 55.40 % C. The ash content, measured by loss on ignition for 4 h at 550°C, was 4 % of total dry weight. Air-died leaves were subsampled and oven-dried at 75°C for 48 h to correct for water content.

Labeled leaves were placed directly on mineral soil scraped bare of grass sod and other vegetation; 125 g of labeled leaves were deposited on the soil and bound within a plastic ring 0.4 m in diameter and extending from about 6 cm above the soil surface to 3 cm below. Mesh netting (1.25-cm) was draped over each of the 20 rings, pulled taut, and secured into the ground with stakes. For sampling purposes, the rings were placed in five groups at a 50-m spacing with each group including four rings at a 2-m spacing. When the rings were established in December 1993, soil samples were taken immediately adjacent to each ring. No 'second year' of leaf fall was placed within the

rings. At the conclusion of the experiment in September 1995, all material recognizable as red alder leaf detritus was collected from the rings. This detritus was dried at 75°C for 48 hr, weighed, and analyzed for N and C. Loss on ignition was measured after 4 h at 550°C.

### *2.3.3 Soils*

Soil samples were taken at 0-5 and 5-15 cm. Field-moist soils were frozen at -20°C until analyzed. Unless otherwise stated, weights are oven dry. Subsamples of soils were oven dried at 105°C for 48 hr to determine water content. Soil samples were ground with a roller mill and analyzed by combustion to obtain estimates of total N and C. Bulk density, estimated separately for each depth and ring, was calculated from soil C content using a regression equation,

$$Bulk\ density\ (g\ cm^{-1}) = 0.075 + (1.301)^{(-0.060 \times LOI)}, \quad (1)$$

reported by Grigal *et al.* (1989). Loss on ignition (LOI) was assumed to be two times the value of organic C (Nelson and Sommers, 1982), and given the lack of carbonates in the acidic soils of the Pacific Northwest, organic C was assumed to equal total C (Homann *et al.*, 1995).

### *2.3.4 Density fractionation*

Soil samples for each depth class were composited by ring group. The composite samples for each group were fractionated. Light- and heavy-fractions of the soil were separated by using a density separation method modified from that proposed by Strickland and Sollins (1987). Rocks and twigs larger than 2 mm in diameter were removed from the field-moist soils by hand. Soil (20 g) was added to a standard 250-ml beaker with 100 ml of sodium polytungstate (SPT), an inorganic salt solution (density = 1.60 g $cm^{-3}$). The SPT and soil were mixed for 30 s with a Scovil soil mixer, set at "low," to break up soil and fine-root aggregates. A probe-type sonic disrupter (Branson Sonic Power Company, Danbury, CT, Model 350) was immediately used to further disperse the soil in suspension. About 156 J/ml of soil suspension was delivered over

the course of 2 minutes, at which time the samples were set aside. After 1 d, the suspended light-fraction and top centimeter of SPT were aspirated and rinsed with distilled water. The remaining soil suspension was again mixed, sonicated and refilled with SPT to the pre-aspiration level before being set aside for a day. Then, the light-fraction was aspirated, rinsed, and added to the previously collected light-fraction. The rinsing process for the heavy fraction consisted of repeated mixings with distilled water, settling of the heavy fraction, and aspirations of the clear supernatant into a waste container. Oven-dried light- and heavy-fractions were weighed, ground, and analyzed by combustion for N and C content. Loss on ignition was measured after 4 h at 550°C.

### *2.3.5 Chloroform-labile N*

The fumigation-extraction method established by Brookes *et al.* (1985) was used to measure chloroform-labile N, as an index of microbial biomass. Field-moist soil (10 g) was fumigated for 5 d. Fumigated soils were extracted with 37.5 ml of 0.5 *M* $K_2SO_4$, and the extracts were digested for total N by using the persulfate oxidation method optimized by Cabrera and Beare (1993). Nitrate concentration in the digest solutions was determined colorimetrically on a Lachat Autoanalyzer (Lachat Instruments, Milwaulkee, WI ). Atom % $^{15}N$ in the digests was determined through the diffusion method suggested by Brooks *et al.* (1989) with the modification of adding 1.0 ml of 10 *M* NaOH instead of MgO to raise pH to adequate levels for ammonia volatilization (Cabrera and Beare, personal communication). As a control, 25 g of non-fumigated field-moist soil was extracted with 75 ml of 0.5 *M* $K_2SO_4$ and the extracts were digested and diffused with the same methods as the fumigated extracts. Values from the non-fumigated extracts were subtracted from those of the fumigated extracts to determine chloroform-labile N (Brookes *et al.*, 1985).

### *2.3.6 Vegetation*

All vegetation growing within the rings was collected in September 1995. Vegetation was separated and grouped as grass, woody, or herbaceous. Each class was dried, weighed, and analyzed separately for N.

*2.3.7 $^{15}N$ recovery in soil*

Recovery of $^{15}N$ was determined by dividing mg $^{15}N$ excess recovered in a given soil component by the initial mg $^{15}N$ excess in the litter. Estimates of mg $^{15}N$ excess were obtained using N concentration (N as percent of dry weight) and atom % $^{15}N$ excess. Atom % $^{15}N$ excess in a given soil component was defined as the atom % $^{15}N$ greater than that measured in the soil component in December 1993 (untreated). Atom % $^{15}N$ excess in litter was defined as the difference between the atom % $^{15}N$ in the litter and in the given soil component.

*2.3.8 Mass spectrometry*

Samples submitted for mass spectrometry were ground to pass through a 40-mesh sieve and analyzed for % N, atom % $^{15}N$, and % C on a Europa Scientific ANCA-MS automated mass spectrometer or a Europa Scientific 20/20 automated mass spectrometer (Europa Scientific Ltd., Crewe, UK). Nitrogen concentrations at natural abundance were measured by the ANCA-MS with a precision of ± 0.0003 atom % $^{15}N$ and the 20/20 with a precision of ± 0.00007 atom % $^{15}N$. Carbon concentrations (as a percent of dry weight) at natural abundance were measured by the ANCA-MS with a precision of ± 0.2 % C and the 20/20 with a precision of ± 0.01 % C. Duplicate samples were run for N and C both within and between the mass spectrometers to ensure consistency. Results of the duplicate runs were tested for differences using paired t-tests at the 0.05 level of significance. Without exception, estimates of N, $^{15}N$, and C were in agreement between the two mass spectrometers.

*2.3.9 Statistical analysis*

T-tests were used to evaluate the significance of the percent recovery of $^{15}N$ in the litter and various soil components. Analysis of variance and Tukey's Studentized Range test (HSD) were used to compare the N and C concentrations and the C:N ratios in the light- and heavy-fractions in both depth classes. Because the number of variables was small (fractions x depths = 4) and all pairwise comparisons were of interest, a multiple range test was considered appropriate. The Tukey HSD test was chosen as a

conservative test able to protect against experiment-wise error in comparisons involving groups with different sample sizes. Statistical comparisons of values likely to be correlated (e.g., mg N in light-fraction/kg soil will increase with decreasing mg N in heavy-fraction/kg soil) were not conducted, given inadequate degrees of freedom needed to use the proper statistical methods.

## 2.4 Results

### *2.4.1 Above ground*

A reliable estimate of percent recovery of $^{15}N$ of vegetation growing on the plots could not be computed because of the lack of controls. However, a cursory examination of the data suggests that there was accumulation of $^{15}N$ in the vegetation in the second year (Table 2.1), given standard atom % $^{15}N$ values of vegetation (Ehleringer and Rundel, 1989). Only data for vegetation in the second season (1995) is available, because an early snowfall precluded the planned fall-sampling of vegetation in 1994.

TABLE 2.1. The distribution of N and $^{15}N$ in vegetation growing in ring plots. Standard error in parentheses.

| Vegetation type | Mass (g) | N (g $kg^{-1}$) | atom % $^{15}N$ |
|---|---|---|---|
| Grass[a] | 25.86 (4.12) | 6.6 (0.4) | 0.3788 (0.0027) |
| Herbaceous[b] | 14.58 (4.82) | 11.3 (0.6) | 0.3752 (0.0038) |
| Woody[a] | 17.74 (3.00) | 13.3 (0.5) | 0.3669 (0.0004) |

[a] $n = 20$
[b] $n = 14$

Because the vegetation was still growing at the time of collection in 1995, there was little, if any, non-alder detritus from 1995 vegetation. Non-alder detritus from 1994 vegetation had decomposed beyond recognition, although there was very little vegetation growing in the plots by October, 1994.

Alder detritus collected from the rings after 21 mo lost 69 % of original $^{15}N$ ($p < 0.0001$). Of the 125 g of fallen leaves placed in the rings, on average only 28 g ($n = 20$, SE = 2) were recognizable as alder detritus and collected. Thus, 78 % of the original mass was incorporated into the soil organic matter or otherwise lost. Ash-free N and C concentrations, and atom % $^{15}N$, N, C, and total ash-free contents are provided in Table 2.2; all comparisons resulted in significant differences ($p < 0.0001$), except N concentration which was not different between initial and final detritus measurements ($p = 0.12$). Non-recognizable organic debris was usually partly incorporated into the surface soil, and was included in soil sampling.

TABLE 2.2. Initial and final values of several leaf detrital fractions and the probability of a significant difference between them. Standard error in parentheses.

| Detrital fractions[a] | Initial (December 1993) | Final (September 1995) | *n* (I, F) | Probability of Type I error |
|---|---|---|---|---|
| Ash-free fraction[b] | 0.96 (0.003) | 0.93 (0.009) | 6, 12 | < 0.0001 |
| Nitrogen | | | | |
| mg N/g AF-lvs | 29.90 (0.59) | 31.46 (0.55) | 6, 20 | 0.12 |
| Total g N | 3.57 (0.077) | 0.85 (0.065) | 6, 20 | < 0.0001 |
| Atom % $^{15}N$ | 0.4195 (0.0054) | 0.5446 (0.0048) | 6, 20 | < 0.0001 |
| Carbon | | | | |
| g C/g AF-lvs | 0.58 (0.0044) | 0.49 (0.0042) | 6, 12 | < 0.0001 |
| Total g C | 70.69 (0.55) | 16.55 (2.97) | 6, 12 | < 0.0001 |

[a]'Ash-free' is represented by 'AF'; 'leaves' is represented by 'lvs'.
[b]Fraction of dry weight.

*2.4.2 Below ground*

There were discrepancies in both depths between the recovery in the whole soil and the summed recovery in the soil fractions (Table 2.3). In the 0-5 cm depth class, the whole-

TABLE 2.3. Probability of significant % recovery of $^{15}N$ in several above and below ground components of ringed plots. Standard error in parentheses.

| Ring component | % of whole soil | Atom % $^{15}N$ excess | $^{15}N$ recovery (%) | *n* | Probability of Type I error[a] |
|---|---|---|---|---|---|
| Alder detritus | --- | 0.1783 (0.0367) | 30.6 (6.27) | 20 | 0.0001 |
| 0-5 cm depth | | | | | |
| whole-soil | --- | 0.0044 (0.0010) | 34.2 (6.42) | 14 | 0.0001 |
| light-fraction | 7.4 (0.037) | 0.0157 (0.0090) | 11.7 (3.21) | 4 | 0.02 |
| heavy-fraction | 92.6 (0.022) | 0.0021 (0.0012) | 7.4 (3.60) | 4 | 0.07 |
| 5-15 cm depth | | | | | |
| whole-soil | --- | 0.0006 (0.0002) | 3.7 (1.60) | 20 | 0.01 |
| light-fraction | 3.4 (0.017) | 0.0108 (0.0063) | 7.0 (3.25) | 4 | 0.08 |
| heavy-fraction | 97.2 (0.012) | 0.0004 (0.0003) | 2.2 (1.67) | 5 | 0.13 |

[a]Associated with % recovery of $^{15}N$.

soil recovery was greater than the summed fractions. Conversely, the whole-soil returned a lower recovery than the summed fractions in the 5-15 cm depth class. Mainly due to low sample size, the p-values for the 0-5 cm heavy-fraction and both the 5-15 cm fractions were not significant at the 0.05 alpha level (Table 2.3). These values were, however, not unreasonably high and allowed for guarded inferences. The ash content in the 0-5 cm light-fraction was 42 %. The 5-15 cm light-fraction had an ash content of 34 %. The recovery in the chloroform-labile N fraction after 21 mo was small, highly variable, and not significant in either depth (Table 2.4).

In whole-soils, N and C concentration, and the C:N ratio were lower in the 5-15 cm depth class (Table 2.5). When N and C concentrations were considered in the

TABLE 2.4. Probability of significant % recovery of $^{15}N$ in the $CHCl_3$-N fraction with depth. Standard error in parentheses.

| Depth (cm) | Atom % $^{15}N$ excess | $^{15}N$ recovery (%) | *n* | Probability of Type I error[a] |
|---|---|---|---|---|
| 0-5 | 0.0061 (0.0055) | 0.46 (2.69) | 10 | 0.43 |
| 5-15 | 0.0040 (0.0020) | 0.26 (0.34) | 20 | 0.23 |

[a]Associated with % recovery of $^{15}N$.

TABLE 2.5. Soil N and C concentrations with depth class. Standard error in parentheses.

| Depth (cm) | C ($g\ kg^{-1}$) | N ($g\ kg^{-1}$) | C:N |
|---|---|---|---|
| 0-5[a] | 109.0 (9.1) | 3.0 (0.14) | 36 |
| 5-15[b] | 70.8 (6.5) | 2.4 (0.12) | 30 |

[a]$n = 14$
[b]$n = 20$

TABLE 2.6. Comparison of C and N concentrations and C:N ratios in density fractions at different depths.

| Density fraction | Depth (cm) | C (%) | N (%) | C:N |
|---|---|---|---|---|
| Light-fraction | 0-5 | 33.23a | 0.57a | 59.72b |
| | 5-15 | 34.87a | 0.42a | 83.03a |
| Heavy-fraction | 0-5 | 3.75b | 0.16b | 23.54c |
| | 5-15 | 2.58b | 0.12b | 21.42c |

NOTE: Tukey's Studentized Range test was performed for density fractions. Column values not followed by the same letter are significantly different ($\propto = 0.05$).

fractions, depth played a less important role, as indicated in Table 2.6. Light-fractions had higher N and C concentrations than heavy-fractions. The C:N ratio was lower in the heavy-fractions, and highest in the light-fraction at 5-15 cm. When mg of light- or heavy-fraction N per kg soil were considered, the heavy-fractions in both depth classes contained far greater amounts of N/kg soil than the light-fractions (Table 2.7). But when g of light- or heavy-fraction C per kg soil were considered, there appeared to be little difference between fractions or depths.

TABLE 2.7. The amount of C and N contained in light- and heavy-fractions per kg of soil in 0-5 and 5-15 cm depth classes. Standard error in parentheses.

| Density fraction | Depth (cm) | C ($g\ kg^{-1}$) | N ($g\ kg^{-1}$) |
|---|---|---|---|
| Light-fraction | 0-5 | 24.5 (6.8) | 429 (136) |
| | 5-15 | 12.8 (6.5) | 154 (76) |
| Heavy-fraction | 0-5 | 34.6 (3.0) | 1472 (122) |
| | 5-15 | 24.8 (4.9) | 1142 (132) |

NOTE: Parametric statistical tests were not conducted because of violation of the independence assumption. Sample size is too low to conduct non-parametric tests; $n = 4$ in the light-fraction, $n = 4$ in the 0-5 cm heavy-fraction and $n = 5$ in the 5-15 cm heavy-fraction.

## 2.5 Discussion

### *2.5.1 Above ground*

Normally, atom % $^{15}N$ values in plants range from 0.3630 to 0.3670 atom % $^{15}N$ (e.g. Ehleringer and Rundel, 1989). The atom % $^{15}N$ measured in the grass was 0.3788, the herbaceous vegetation was 0.3752, and the woody vegetation was 0.3669. The values for grass and herbaceous vegetation were well outside the range given by Ehleringer and Rundel (1989) for plants, as well as values reported by other researchers (Virginia and Delwiche 1982; Nadelhoffer and Fry 1988; Gebauer and Schulze, 1991;

Garten 1993; Nadelhoffer *et al.*, 1995). The lower atom % $^{15}N$ measured in the woody vegetation is not surprising given the habit of the primary species encountered, *R. ursinus*. Pacific blackberry is a trailing perennial that may grow adventitious roots where in contact with the soil. Only stems which were rooted in the rings were collected. Even these were generally invaders from outside the rings. It is possible that the initially higher $^{15}N$ signal from N acquired within the ring was diluted by native N translocated from outside the ring.

If the atom % $^{15}N$ of the unlabeled vegetation at the site were considered to equal the soil atom % $^{15}N$ values, which were just slightly above atmospheric levels (0.3680), the recovery in the weighted-average mass of total vegetation in a ring would be 2.69 % ($n = 20$, SE = 0.46). This might be an underestimate of % recovery because lower $^{15}N$ values in plants, relative to soils, have been observed in other studies (Delwiche and Steyn, 1970; Virginia and Delwiche, 1982; Legard *et al.*, 1984). A measurable recovery of $^{15}N$ indicates that the mineralization of leaf-N from alder detritus to plant-available N, and subsequent uptake by plants can occur in meaningful quantities within a 21-mo time span.

The mass loss of alder detritus was accompanied by only a slight, non-significant increase in N concentration and a steep rise in the atom % $^{15}N$ excess in the remaining detritus. The lack of significant change in N concentration indicates a proportional net loss of N and mass. Conversely, the increase in atom % $^{15}N$ excess indicates that as N was lost, $^{15}N$ and $^{14}N$ were lost disproportionately, given the original ratio of $^{15}N$:$^{14}N$. In order to maintain the initial atom % $^{15}N$ of 0.4195, $^{15}N$ and $^{14}N$ had to be lost at a ratio of about 1:237. Any increase in the loss ratio would result in an increase in the atom % $^{15}N$ in the remaining detritus.

Microbial degradation may have resulted in a slight increase in $^{15}N$ concentration in remaining litter. Discrimination during decomposition may leave enriched residual substrate (Tiessen *et al.*, 1984; e.g. Ehleringer and Rundel, 1989; Domenach *et al.*, 1989; Blair *et al.*, 1992) and microbial biomass (Delwiche and Steyn, 1970; Macko *et al.*, 1987). Although these processes may have contributed to the increased enrichment of the remaining litter, it is unlikely that this accounted for all of the enrichment observed.

Berg (1988), after observing changes in $^{15}N$-labeled Scotts pine (*Pinus sylvestris* L.) needle litter in a forested and a clear-cut site, reported increased concentration of $^{15}N$ in the litter in the forested site. The increase was attributed to lower N turnover in the forested site, even though mass loss was higher. It was suggested that $^{15}N$ was incorporated early into recalcitrant leaf fractions and became more concentrated as labile fractions were mineralized. This is also the most plausible explanation for the observed atom % $^{15}N$ in the alder litter. Unfortunately, leaves were not fractionated to test this hypothesis.

Even though $^{15}N$ was apparently conserved in or on the remaining detritus, most of the N content in the alder detritus was lost proportionally to the mass. An increase in N concentration would have indicated general N conservation, but the observed increase was not significant. The atom % $^{15}N$ increase and lack of increase in N concentration indicate little or no immobilization of native soil N or N from through-fall or precipitation. Immobilization from these sources would have contributed toward a smaller increase, or even a decrease, in atom % $^{15}N$ as well as an increase in N concentration. Edmonds (1980) investigated the decomposition of four litter types, including red alder, in four ecosystems. His findings corroborated earlier findings (Edmonds 1979) that, in the ecosystems studied, immobilization of N occurs in decomposing leaves at C:N ratios above 35:1, and mineralization is the dominant process between 23:1 and 35:1. The red alder litter in the present study had an initial C:N ratio of 20:1 and a final ratio of 19:1, both well below the threshold of immobilization reported by Edmonds (1980). Other investigators (Berg, 1988; Blair *et al.*, 1992; Downs *et al.*, 1996) have used litter labeled with $^{15}N$ to examine N processes in forest floors. In these cases N immobilization and mineralization occurred simultaneously, although net immobilization and mineralization varied with litter type. Hart and Firestone (1991) reported that although the forest floor acted as both a source and a sink for N, it was a net source of N. Berg (1988) hypothesized that a minimal amount of N import accompanies mycelial invasion into even N-rich litters. This import may have been partly responsible for the slight increase in N concentration in alder litter

in the present study, although ultimately too small to counter the proportional increase in $^{15}N$.

Edmonds (1980) reported that after 24 mo of decomposition in litter bags, alder leaves retained 67 % of the initial N content. That recovery is substantially higher than the 24 % recovery of total N and 31 % recovery of $^{15}N$ in the litter of the present study. The designation of unrecognizable litter fragments as part of the soil organic matter pool, and sampling error in the collection of reconizable fragments, likely elevated mass loss estimates above those of litter bags, which would retain many of the same fragments. A litter bag study conducted adjacent to the rings (Appendix 2) estimated remaining mass to be 64 % after 21 mo ($n = 41$, SE = 0.62). However, lower moisture and at least partial exclusion of mycelia and arthropods are likely to lower the mass loss of leaves decomposing in litter bags (Edmonds, 1980; St. John, 1980; Blair *et al.*, 1992). Preston and Mead (1995) mixed unconfined $^{15}N$-labeled Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) needles with non-labeled surface litter and tracked changes in N and $^{15}N$ for about seven years. In the recognizable-litter layer (L) they recovered 50 % of the added excess $^{15}N$ after the first year, and 15 % after the third year of decomposition. Edmonds (1980) recovered 112 % of the initial N after 1 yr and 88 % after 2 years in bagged Douglas-fir litter. Blair *et al.* (1992), in a litter basket study in which leaves were in direct contact with the soil, reported mass loss of 40 % for *Quercus prinus* L. and 90 % for *C. florida* leaves. In an earlier litter bag study Blair *et al.* (1992) reported only 32 % mass loss in *Q. Prinus* and 56 % in *C. florida*. Though differences in location likely affected the relative rates of decomposition in the above studies, litter bags generally appear to produce lower estimates of mass loss.

The forest floor is commonly considered a net sink for N (e.g. Hart and Firestone, 1991). However, a 'forest floor' comprised primarily of alder litter is not necessarily a net sink of N. Others who have studied the forest floor have concluded it to be a net source of N (Hart and Firestone, 1991). Although Edmonds (1980) reported less mass and N loss, he did note that even as N concentration increased in alder litter, N content dropped continuously throughout the course of the study. Red alder litter appears to immobilize little native N, acting as a net source of N.

*2.5.2 Below ground*

Conceptually, the light- and heavy-fractions together should comprise the organic matter of the whole-soil (e.g. Christensen, 1992). However, the densimetric separation of the fractions may result in the loss of inorganic N, microbial N and C, and soluble organic N and C. Thus, once the separates are analyzed for N and C content, the sum of the contents in the two fractions, supposedly representative of the whole-soil, may be somewhat to substantially depleted in N and C when compared to whole-soils analyzed for the same nutrients. When nutrient concentrations in the whole-soil from Table 2.5 are expressed in mg N $kg^{-1}$ soil, the 0-5 and 5-15 cm depth classes contained 3000 mg N $kg^{-1}$ soil and 2400 mg N $kg^{-1}$ soil, respectively. Estimates of the soil N from the summed density fractions in each depth (Table 2.7) are 1900 mg N $kg^{-1}$ soil in the 0-5 cm depth class and 1300 mg N $kg^{-1}$ soil in the 5-15 cm depth class. These represent losses of about 37 % of the N in the upper depth class and 46 % in the lower. Similarly, the summed fractions in the two depth classes under-represented g C $kg^{-1}$ soil by 45 and 43 %, respectively. In all likelihood, the N and C not accounted for in the light- and heavy-fractions was leached into the SPT supernatant. Unfortunately, at the time of this writing no accurate method of analyzing the SPT for N concentration has been developed.

Sollins *et al.* (1984) separately analyzed the whole-soil, light-fraction, and heavy-fraction for C and N. Summed density fractions of the surface soil amounted to 88 % of the C and 93 % of the N in the whole-soil for soils sampled in H.J. Andrews Experimental Forest. A crucial difference in otherwise similar fractionation techniques was that Sollins *et al.* (1984) used 'tall form' beakers to maximize head space between floating light-fraction and the sedimentary heavy-fraction (personal communication, Carol Glassman). The greater head space minimized disturbance of the settled heavy-fraction and thereby avoided incorporation of clay and silt into light-fraction, where it could be lost during repeated rinsings. The beakers used in the present study did not allow for adequate head space to aspirate light-fraction without disturbing the heavy-fraction, which resulted in uptake of heavy-fraction fines. However, the ash contents of the light-fractions are comparable to other studies using like methods (e.g. Christensen, 1992). The light-fraction in the present study was rinsed by vacuum filtration on 2.7-$\mu$m

pore size filters (Whatman # 50), potentially allowing incorporated clay to escape during rinsing. Slightly discolored supernatant was commonly noted. Nelson *et al.* (1994) presented data on separately analyzed whole-soil and size and density fractions. With 102 % of the whole-soil organic C accounted for in the fractions, the clay-sized fraction comprised 49.5% of the whole-soil organic C, although only 18.5 % of the soil by weight. The soluble fraction, only 1 % of the soil weight, represented 3 % of the whole-soil organic C. Soluble organic C and N were certainly lost given the methods of the present study. These losses, coupled with even higher losses of clay from the light-fraction, were likely the primary factors in the observed discrepancies between whole soil data and density fraction data. Because few papers have provided enough information or data with which to calculate recoveries, a comparison of these findings to most other studies with similar methods is not possible. Future studies should take pains to maintain adequate head space by manipulating the diameter to height ratio of the fractionation container, or the ratio of soil to separation medium. Additionally, C and N contents should be measured in light- and heavy-fractions as well as the whole-soil. This would facilitate both reliable comparisons between studies and evaluation of the usefulness of the fractionation technique in characterizing soil organic matter.

The summed recovery in the density fractions in the 0-5 cm depth class represented a 44 % loss of $^{15}N$ when compared to the recovery in the whole-soil. This amount of loss is comparable to the 37 % loss of total N during the process of density fractionation.

Below ground recovery of $^{15}N$ was highest in the 0-5 cm depth in general and the whole-soil specifically. The light-fraction contained about two thirds of the $^{15}N$ recovered in the density fractions. This is in strong contrast to the proportion of soil that is comprised of light-fraction, about 7 % by weight. However, not all of this N was necessarily available for mineralization or uptake.

Much of the light-fraction N may in fact have been occluded within microaggregates of the heavy-fraction before the soil was mixed and sonicated (Sollins *et al.*, 1984; Strickland *et al.*, 1992; Golchin *et al.*, 1994). This 'occluded' light-fraction would not have been physically available to most plants and microorganisms. Even if

available, though, its lability would not necessarily match that of the free light-fraction. Strickland *et al.* (1992) exposed soils to inorganic $^{15}N$ for 60 d and found that 7 % of the added $^{15}N$ had been incorporated into the heavy-fraction. Because subsequent incubations of sonicated and non-sonicated heavy-fractions revealed little chloroform-labile N in the 'active-protected' pool, it was concluded that little N in this pool exists as microbial biomass. NMR spectroscopy of occluded light-fraction released from sonicated heavy-fraction revealed increased alkyl and decreased O-alkyl, indicating that the occluded light-fraction had undergone some humification and would be somewhat more recalcitrant than the free (non-occluded) light-fraction (Golchin *et al.*, 1994).

The recovery in the density fractions in the 5-15 cm depth class appears suspect when compared to the whole-soil recovery (Table 2.3), especially given the preceding discussion. Namely, the recovery in the fractions is greater than that measured in the whole-soil. This inconsistency is partly explained by the high variability in the density fractions. The atom % $^{15}N$ excess in the heavy-fraction is both highly variable and near the reliable detection limit of the mass spectrometers. The values for recovery in the light-fraction are probably more representative of the actual population, but still too variable to make confident inferences. General conclusions that can be reached are that relatively little $^{15}N$ was incorporated into the 5-15 cm depth fraction after 21 mo, and that most of the incorporation was probably into the free and occluded light-fraction.

Recovery of $^{15}N$ in the chloroform-labile N pool was negligible. The initial atom % excess of the leaves was not adequate to maintain a signal in the microbial biomass for 21 mo. However, a concurrent study of similar design (data not shown) using more highly labeled red alder leaves (0.8004 atom % $^{15}N$) revealed some interesting similarities in the seasonal trends in the atom % $^{15}N$ of the 0-5 cm density fractions and the whole-soil chloroform-labile N (Fig. 2.1). Both chloroform-labile $^{15}N$ and the atom % $^{15}N$ in the density fractions were lowest in the spring and highest in the fall. The atom % $^{15}N$ of the light-fraction and chloroform-labile N was similar at the time of pre-treatment sampling in December 1993 and again during the last two samplings in April and September 1995, perhaps suggesting an equilibration of one to the other. Spycher *et*

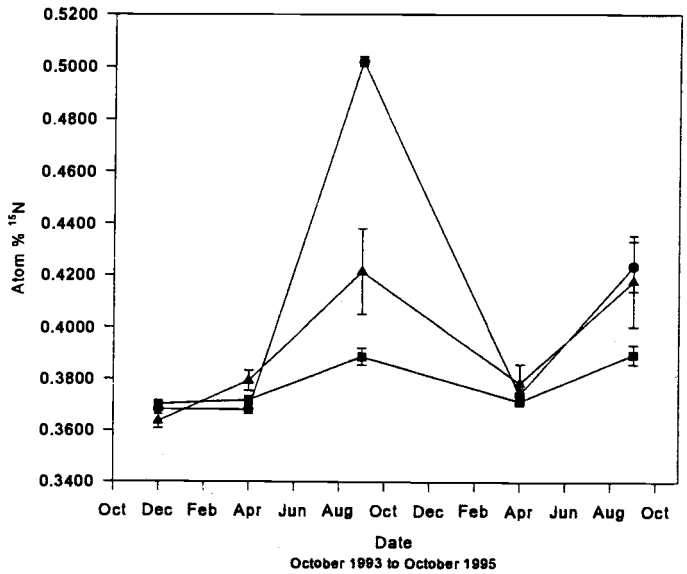


FIG. 2.1. Atom % $^{15}N$ of chloroform-labile N (●), light-fraction N (▲), and heavy-fraction N (■) in the 0-5 cm depth class. Error bars are 1 standard error.

*al.* (1983) observed similar seasonal fluctuations in light-fractions and noted that corresponding patterns in fungal biomass and soil solution chemistry had been observed by others (Fogel and Hunt, 1979; Sollins and McCorison, 1981). Pretreatment estimates of the atom % $^{15}N$ of chloroform-labile N and heavy-fraction N were marginally higher than those of the light-fraction. Thereafter, the heavy-fraction was less responsive to seasonal inputs of $^{15}N$. The drop in atom % $^{15}N$ in April 1995 in the heavy-fraction suggests a more active component of the heavy-fraction more susceptible to seasonal fluxuations.

*2.5.3 Recovery and loss of $^{15}N$*

Overall, approximately 71 % of the added $^{15}N$ was recovered, with 29 % not accounted for in measured soil, litter, or vegetation components. Recoveries of $^{15}N$ added to soil or litter as urea or in inorganic form range from 39 to 100 % (eg Preston *et al.*, 1990; Hart *et al.*, 1993). Most comparable to the present study, however, is that of Preston and Mead (1995). Using Douglas-fir litter labeled with $^{15}N$ by the uptake of $^{15}N$-labeled fertilizer, they conducted a mass balance over the course of seven years to determine the fate of the excess $^{15}N$. After the first year they recovered a total of 54 % of

the excess $^{15}N$. The next sampling, in the third year of the study, recovered only 25 % of the excess $^{15}N$. They suggested that the unrecovered $^{15}N$ may have been leached through the profile or lost by denitrification. Leaching was likely exacerbated by the exclusion of vegetation from their plots.

It is unlikely that leaching can account for the bulk of the missing $^{15}N$ in this study. Even assuming no evaporation (complete leaching) of rainfall during the course of the study, and using the highest recorded atom % $^{15}N$ levels of the total extractable N, the loss of excess $^{15}N$ due to leaching would be just under 3 %. Also, denitrification probably played only a minor role in loss of $^{15}N$ from the system. Vermes and Myrold (1992) estimated that the upper limit of denitrification in this site was 0.08 kg N $ha^{-1}$ $yr^{-1}$. This would amount to about 0.6 mg N per plot during the course of the 1.75 yr study. At most, that would account for < 0.05 % of the missing $^{15}N$. Other possible modes of $^{15}N$ loss include translocation of $^{15}N$ beyond the ringed plot boundary by creeping blackberry; foraging (removal) of labeled grasses by elk; latteral leaching; and uptake of $^{15}N$ by roots invading from outside the plot. These modes of loss were not directly quanified, nor is data available to estimate possible losses. Although minor individually, the sum of the losses discussed here may have been significant.

The greatest "loss" of $^{15}N$ most likely occurred during the collection of the decayed alder leaves at the termination of the field study. Namely, labeled litter inadvertently missed during litter collection contained excess $^{15}N$ not included in the final balance. As mentioned earlier, this would have resulted in an underestimate of the mass, and consequently the amount of $^{15}N$ retained in the litter. Additionally, it was assumed that unidentifiable fragments of alder litter not collected as litter would be adequately represented in the light-fraction. This assumption may have been erroneous, resulting in an underestimate of the actual enrichment of the light-fraction. Thus, the majority of the missing $^{15}N$ may not have been missing from the plot, but from the samples of the plot.

## 2.6 Conclusions

Plants collected from the rings contained levels of $^{15}N$ in excess of standard atom % $^{15}N$ values reported for vegetation in the literature. This suggests that after 21 mo, a measurable quantity of N had been transformed from fresh organic N on the soil surface to N within living plants. The litter from which the N was originally derived lost most of its mass, N, and $^{15}N$ during the same period of time, although $^{15}N$ became concentrated in the remaining detritus. In contact with the soil, this litter lost more mass than similar litter in nearby litterbags. This trend is consistent with comparisons of bagged and non-bagged decomposition of other litter types. A closer examination of the efficacy of litterbags and of the rates and constants derived from them is warranted.

Summed light- and heavy-fractions in both depth classes contained less N and C $kg^{-1}$ soil than did whole-soils. This could be partly explained by incorporation of clay into and subsequent loss from light-fraction. Additionally, loss of inorganic N, soluble organics and microbial and fungal biomass almost certainly contributed to lower recoveries in the density fractions. This trend was mirrored in the 0-5 cm depth where most of the $^{15}N$ was recovered, but deviated in the 5-15 cm depth where too little $^{15}N$ was incorporated into the soil at 21 mo for reliable values.

Light-fractions in both depths had higher N concentrations and higher recovery of $^{15}N$, but lower N content than heavy-fractions. Carbon was also more concentrated in the light-fraction, but did not appear substantially lower in C content. Chloroform-labile N in the whole-soil, as an index of microbial biomass, did not yield a significant percent recovery of $^{15}N$, but did exhibit similar seasonal fluxuations as the heavy-, and especially the light-fraction. The similar trends highlight the labile nature of the light-fraction. The findings of the present study support the concept of alder litter as a net source of N, and light-fraction as a labile soil component.

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# CHAPTER 3

## Evaluation of the Stem Injection Technique and Subsequent $^{15}N$ Partitioning in Red Alder Crowns

Christopher W. Swanston and David D. Myrold

## 3.1 Abstract

Red alder (*Alnus rubra* Bong.) trees were labeled with $^{15}NO_3^-$ and $^{15}NH_4^+$ using the stem-injection method. Leaves were sampled 3 and 15 mo subsequent to injection within several crown positions, including top, bottom, proximal, medial, and distal. Stem injection of both $^{15}NH_4^+$ and $^{15}NO_3^-$ at levels approaching one percent of crown N effectively labeled red alder trees. Although more variable, $^{15}NO_3^-$ may have been more efficient in initial labeling. The distribution of $^{15}N$ in the crown was uniform 3 mo after labeling, but was diluted in the distal and top positions by the following year. There was a clear increase in total N concentration toward the periphery of the tree. This increase became more pronounced with increasing crown size and crown closure. Crown position with respect to light availability may be the most important determinant in crown N allocation in red alder foliage.

## 3.2 Introduction

Increased interest the economic, edaphic, and silvicultural benefits of alder (*Alnus* spp. Ehrhart) has helped to heighten interest in the ecology and biology of red alder (Hibbs *et al.*, 1994; Tarrant *et al.*, 1994). Much of this interest has centered around the fixation of N in alder nodules and on the high levels of foliar N in alder. Studies have sought to determine environmental influences on $N_2$-fixation in alder (Wheeler *et al.*, 1981; Prégent and Camiré, 1985; Côté *et al.*, 1989; Samuelson *et al.*, 1990; Vogel and Dawson, 1991), often using changes in $^{15}N$ concentrations in live alder leaves as determinates (Domenach and Kurdali, 1989; Domenach *et al.*, 1989; Beaupied *et al.*, 1990; McNeill *et al.*, 1994). Using black alder (*Alnus glutinosa* (L.) Gaertn), Domenach and Kurdali (1989) demonstrated the danger of ignoring leaf age in N fixation studies. However, their study focused on the source of N to the leaves, an important consideration in $N_2$-fixation studies but of less importance in other studies.

Many studies have sought to characterize the content or concentration of N in alder crowns to estimate resorption (Dawson and Funk, 1981; Chapin and Kedrowski, 1983; Côté and Dawson, 1986; Côté *et al.*, 1989) or for various other reasons (DeBell and Radwan, 1984; Joseph *et al.*, 1991). The method of leaf sampling often goes

unmentioned (Joseph *et al.*, 1991) or unjustified (Rodríguez-Barrueco *et al.*, 1984; Côté and Dawson, 1986). Improper sampling of crown foliage may lead to inaccurate estimations of N concentration or content, as studies of foliar N distribution in peach and apple canopies would indicate (Porpiglia and Barden, 1980; Sanchez and Righetti, 1990). Dawson and Funk (1981) measured N concentration in size classes of leaves in black alder, but found no significant differences. Small, young, peripheral leaves should have the highest concentration of N, which should decrease with greater size and age (Kramer and Kozlowski, 1979; Waring and Schlesinger, 1985). The inability to differentiate between the N concentration of different size classes in alder would seem to suggest a uniformity of foliar N concentration throughout the crown. This may not be a valid assumption.

With increasing availability of mass spectrometers, isotope studies have become a major avenue of exploring N transformations within alder trees and litter. Several methods for labeling alder trees have been tested, including root and foliar fertilization with $^{15}N$ (González Prieto *et al.*, 1995) and fixation of $^{15}N_2$ (McNeill *et al.*, 1994). Stem-injection (Horwath *et al.*, 1990) of alder remains untried. Perhaps the main benefit of the stem-injection technique is that it specifically labels the tree. Root fertilization results in soil contamination with $^{15}N$, complicating estimations of reserve use and $N_2$-fixation. Foliar fertilization is limited to trees small enough to adequately and uniformly cover with the urea-$^{15}N$ spray.

There is some question as to the most appropriate form of N to inject into alder. Even small levels of $NH_4^+$ can be toxic in plant leaves (Waring and Schlesinger, 1985). Alternatively, the vast bulk of nitrate reductase activity (NRA) occurs in the roots of alder, with little or no $NO_3^-$ measurable in the stem (Blacquière and Troelstra, 1986; Pizelle and Thiery, 1986). It is unclear how well the constitutive NRA in the leaves and shoots would adjust to even a small influx of $NO_3^-$ (Benamar *et al.*, 1989).

The objectives of this study were to evaluate the viability of using the stem-injection procedure to label red alder (*Alnus rubra* Bong.) foliage with $^{15}NO_3^-$ and $^{15}NH_4^+$, and to assess the uniformity of foliar $^{15}N$-labeling and N concentration between several different crown positions 3 and 15 mo after injection.

3.3 Methods

This study was conducted in the Cascades Range at the H.J. Andrews Experimental Forest, Willamette National Forest, Oregon. Red alder and Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) were planted on the clear-cut site 7 yr prior to the study. Ten single-stemmed alder trees, fairly uniform in size and habit at about 5 m in height and 12 cm diameter at breast height, were injected with an artificial sap solution containing $^{15}NO_3^-$ or $^{15}NH_4^+$ using the method described by Horwath *et al.* (1992). The sap solution, composed of 5.0 m*M* KCl and 0.4 m*M* malic acid , was adjusted to pH 5.4 with HCl or KOH (Dickson *et al.*, 1985). $^{15}NO_3^-$ was injected as $KNO_3$ at 66.0 atom % $^{15}N$ and $^{15}NH_4^+$ as $(NH_4)_2SO_4$ at 69.2 atom % $^{15}N$. Five trees received the $KNO_3$ and five received the $(NH_4)_2SO_4$. The injection procedure consisted of drilling a hole at the base of the tree downward at a 45° angle through about 75% of the diameter of the tree. A tube stoppered with a septum was inserted 1 cm into the hole. Air was removed from the hole by injecting sap solution through the septum, thereby forcing the air through an open-ended needle also inserted through the septum. When the air was expelled both needles were removed and a final needle connected by a tube to the sap reservoir was inserted. The sap reservoir consisted of a 1-L bottle hung above the drilled hole and filled with 250 ml of sap solution containing either 12 m*M* $(NH_4)_2SO_4$ or 27 m*M* $KNO_3$. When most of the sap solution was taken up by a given tree, the reservoir was refilled with N-free sap solution and allowed to drain.

Prior to the injection of each tree, leaves were collected from the entire crown and combined into a composite sample. The trees were injected on July 6, 1994. The length of time the trees assimilated the initial 250 ml of sap solution varied from 15 min to 2.5 h. Most trees did not fully drain the secondary, N-free sap solution until the following day. After the stem injection, the crowns were sampled twice: the first time on September 20, 1994 and the second on September 18, 1995. The post-injection sampling involved dividing the crowns into 12 partitions (Fig. 3.1). A composite sample of 10 to 20 leaves was collected from each partition during each sampling. The oven-dried samples were ground to pass through a 40-mesh sieve and analyzed for N concentration and atom % $^{15}N$ on a Europa Scientific ANCA-MS automated mass spectrometer or a

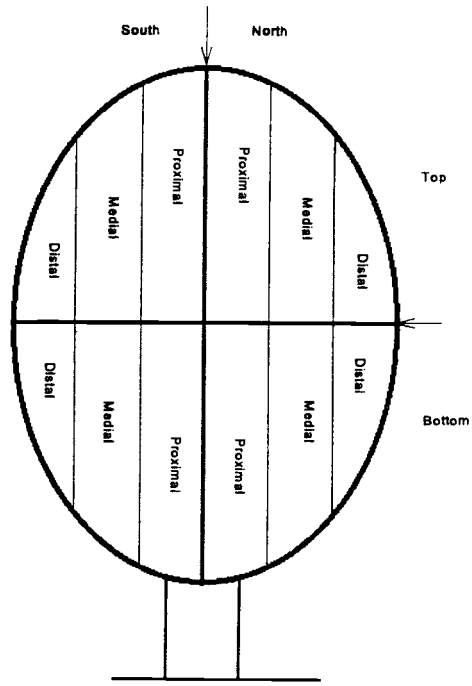


FIG. 3.1. Crown partitions as collected. For example, 'BNP' refers to bottom half, north side, proximal to stem.

Europa Scientific 20/20 automated mass spectrometer (Europa Scientific Ltd., Crewe, UK). The precision levels at natural abundance of the ANCA-MS and 20/20 are ± 0.0003 and 0.00007 atom % $^{15}N$, respectively. Duplicate samples were run within and between the mass spectrometers to ensure consistency. Results of the duplicate runs were tested for differences using a paired t-test at the 0.05 level of significance. Without exception, samples run on each machine and repeatedly on the same machine were consistent.

Information on crown size and leaf area index was not collected. Therefore, statistical tests involving $^{15}N$ were conducted using transformed data to control for high variation within groups resulting from differences in crown densities. Namely, for a given tree, the atom % $^{15}N$ of each partition was divided by the highest atom % $^{15}N$ measured in that tree. This transformation maintained the relationships of atom % $^{15}N$

allocated to the various partitions of the crown, while controlling for the effect of crown size on the level of $^{15}N$ enrichment. Nitrogen concentration and the relative distribution of $^{15}N$ in crown partitions was examined with a repeated measures ANOVA and contrasts to control for possible correlations between partitions. Given the number of experimental units and degrees of freedom, the analysis was limited to two comparisons: differences in lateral and in vertical distribution of N and $^{15}N$ within trees and between treatments, as illustrated in Fig. 3.2. Unless otherwise stated, an alpha level of 0.05 or less was deemed significant.

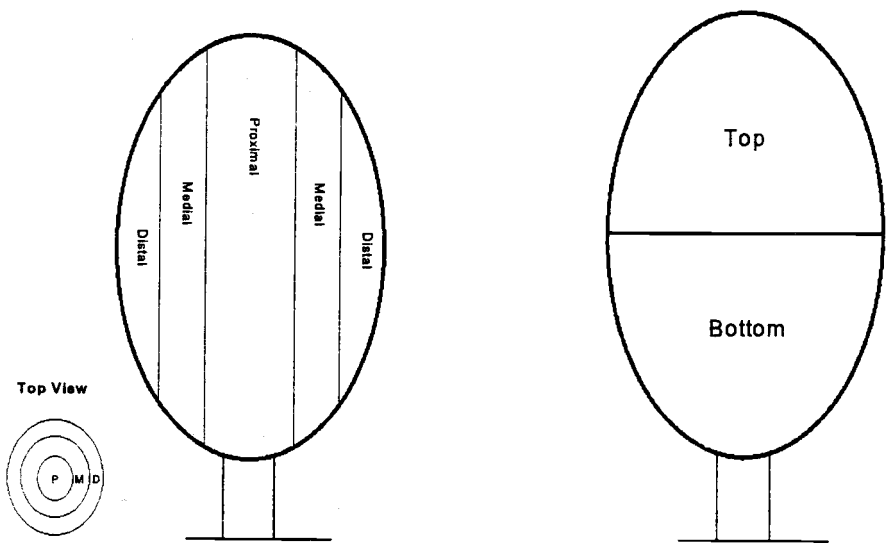


FIG. 3.2. Partitions for statistical comparisons of lateral (left figure) and vertical (right figure) N and $^{15}N$ distribution.

## 3.4 Results

Three injected trees were dropped from the data analysis due to procedural errors. The remaining trees included three treated with $^{15}NO_3^-$ and four treated with $^{15}NH_4^+$. Both the $^{15}NO_3^-$ and $^{15}NH_4^+$ treatments clearly raised atom % $^{15}N$ levels above the pre-injection levels of the alder trees, which were near natural abundance (Fig. 3.3). Because actual enrichment and not merely relative distribution of $^{15}N$ is of interest in Fig. 3.3, statistical tests were not run on these data because we could not correct for the

confounding effects of crown size on enrichment. Qualitative observations of height, crown size, and leaf area index showed no distinct patterns, except that height had increased in nearly all trees by September 1995. The one exception was a tree in the $NH_4^+$ treatment that lost its top half from snow breakage. The bottom half of the tree

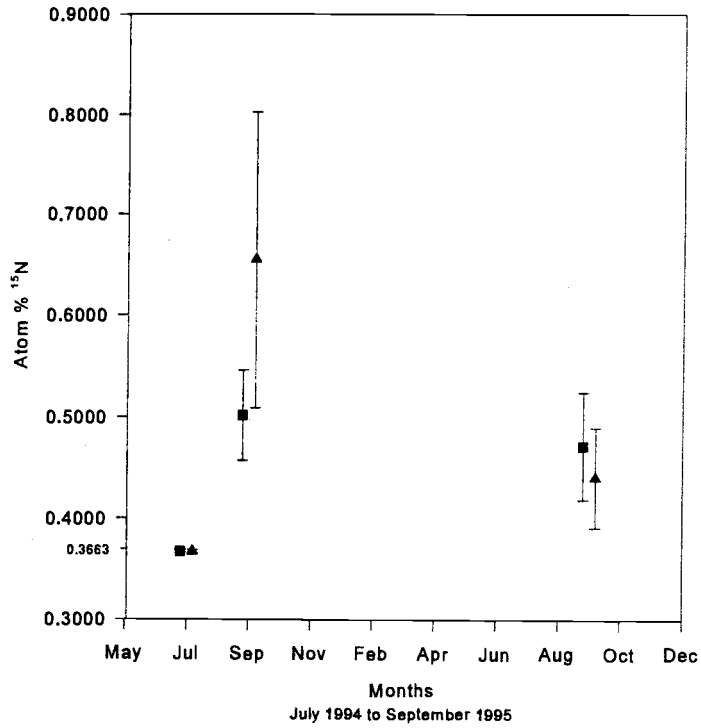


FIG. 3.3. Atom % $^{15}N$ enrichment over time for $^{15}NH_4^+$ (■) and $^{15}NO_3^-$ (▲) treatments, uncorrected by crown size. Error bars are 1 standard deviation.

was intact and appeared healthy. Although the $NO_3^-$ treatment appeared to result in both higher and more variable enrichment in September 1994, these data should be viewed with caution given the lack of information on crown size. By the September 1995 4sampling, $^{15}N$ enrichment of the crowns in both treatments had decreased. The decrease in $^{15}N$ enrichment in the $NO_3^-$ treatment appeared to be substantially greater.

A comparison of first-year relative allocation of $^{15}N$ in lateral partitions (proximal/medial/distal) showed no significant effects at the treatment or individual tree level ($NO_3^-/NH_4^+$ treatment, $p = 0.49$; tree, $p = 0.53$; interaction, $p = 0.51$). Similarly, a comparison of first-year relative allocation of $^{15}N$ in vertical partitions (top/bottom) also

failed to show significant effects by treatment ($p = 0.47$) or tree ($p = 0.33$), although there was some degree of interaction between the two ($p = 0.06$). The lateral partitions were not significantly correlated with one another with respect to relative $^{15}N$ allocation, nor were the vertical partitions.

By September of 1995, however, the relative distribution of $^{15}N$ in each of the lateral partitions was significantly correlated with distribution in the other partitions ($p \leq 0.01$), although vertical partitions were still not significantly correlated. There was still no treatment effect in relative $^{15}N$ distribution (p values $\geq 0.47$), although a comparison of vertical partitions at the tree level revealed a significantly higher proportion of $^{15}N$ in the bottom half of the tree crowns ($p = 0.03$) (Fig. 3.4). Lateral $^{15}N$ distribution was not significantly different ($p = 0.07$), but changed enough from 1994 to merit further investigation (Fig. 3.4). A contrast between proximal and distal crown partitions suggested a lower relative distribution of $^{15}N$ in the distal partition ($p = 0.08$). A second

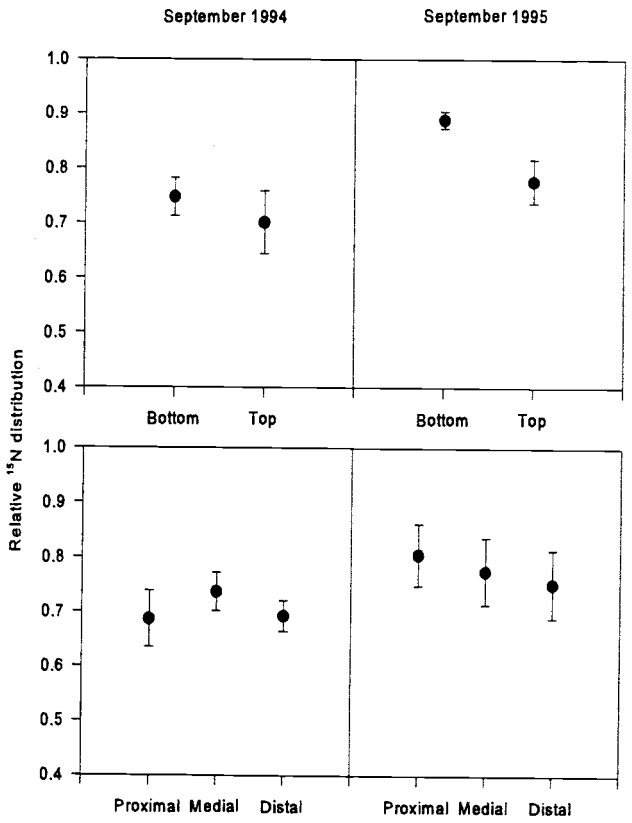


FIG. 3.4. Relative distribution of $^{15}N$ ($at\%_{partition}/at\%_{high}$) in vertical and lateral partitions in September 1994 and September 1995. Error bars are standard error.

contrast, between the proximal and medial partitions, revealed no difference in relative $^{15}N$ distribution ($p = 0.29$).

Nitrogen concentration generally showed the opposite trends as those of atom % $^{15}N$. The first sampling in September 1994 revealed no significant differences in N concentration between treatments (p values $\geq 0.1$), but did show differences between partitions at the tree level. Namely, although the distal partitions did not differ significantly in N concentration from the medial partitions ($p = 0.12$), they exhibited higher N concentrations than the proximal partitions ($p = 0.002$). There were no significant interactions between treatment and tree-level responses ($p = 0.89$). Comparisons of the top and bottom partitions at the treatment and tree level revealed no significant responses (p values $\geq 0.08$).

By the second sampling in September 1995 the trees still had not separated by treatment in the middle ($p = 0.19$) and distal ($p = 0.42$) partitions, but the N concentrations in the proximal partitions of the $NO_3^-$ trees was significantly lower than those in the proximal partitions of the $NH_4^+$ trees ($p = 0.008$) (Fig. 3.5). Similarly, N concentration did not differ by treatment in the top partitions of the trees ($p = 0.71$), but the bottom partitions of the $NO_3^-$ trees were significantly lower in N concentration than

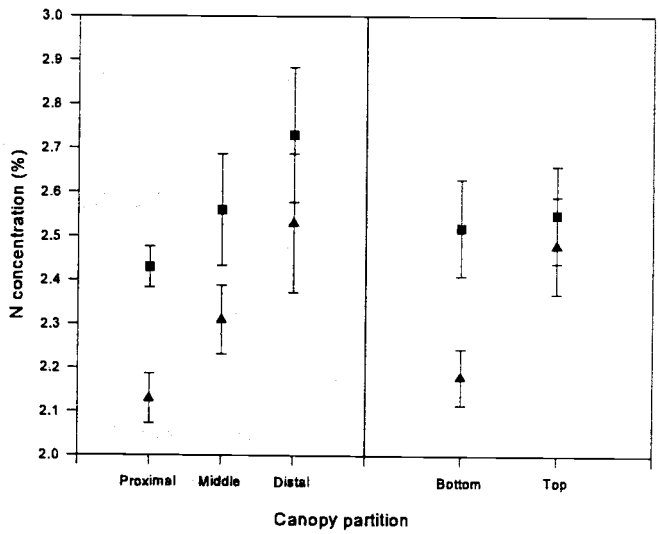


FIG. 3.5. N concentration (as percent of dry weight) in crown partitions in the $NO_3^-$ (▲) and $NH_4^+$ (■) treatments in September 1995. Error bars are standard error.

those of the $NH_4^+$ trees ($p = 0.03$) (Fig. 3.5). At the tree level, the distal partitions showed a higher relative N concentration than both the medial ($p = 0.03$) and the proximal ($p = 0.008$) partitions (Fig. 3.5). There were no significant interactions between treatment and tree-level responses in lateral or vertical partitions ($p = 0.71$).

## 3.5 Discussion

Both $^{15}NO_3^-$ and $^{15}NH_4^+$ treatments were effective in labeling red alder trees. Horwath *et al.* (1992) reported that adding greater than 5-10 % of the crown N through stem injection resulted in toxic effects in their experiments. Using a rough estimate of the crown N, it was estimated that about 1 % of the crown N was injected into the alder trees. This amount, though low, adequately labeled the crown and had no readily discernable negative side effects.

The apparent differences in atom % $^{15}N$ values in Fig. 3.3 could have resulted from a dilution effect due to differences in crown size. The high variation in the $^{15}NO_3^-$ treatment in September 1994 is mostly due to one tree planted at a wider spacing than the others. This tree (N3) had a larger crown, resulting in greater dilution of the added $^{15}NO_3^-$. Excluding N3, the variation is reduced and the $^{15}NO_3^-$ treatment is labeled substantially higher than the $^{15}NH_4^+$ treatment. González-Prieto *et al.* (1995) reported that root fertilization of black alder with $^{15}NO_3^-$ resulted in higher, but more variable foliar labeling than fertilization with $^{15}NH_4^+$. Ultimately, because of a lack of quantitative information on crown size, no definitive statement can be made regarding the relative efficiency of labeling between treatments. In the September 1995 sampling, the crown averages of $^{15}N$ concentration in the $^{15}NO_3^-$-trees were more comparable to those of the $^{15}NH_4^+$-trees. For unknown reasons, the initially higher labeling by the $^{15}NO_3^-$ treatment appear to be coupled with a greater dilution in the second year.

Comparisons of relative N concentrations and atom % $^{15}N$ estimates are far less prone to crown bias. These revealed no significant differences in $^{15}N$ distribution among partitions in the first year (3 mo after injection), indicating a uniform distribution of $^{15}N$ throughout the crown. This is similar to the results of Sanchez and Righetti (1990), who

found no differences in $^{15}N$ distribution in different crown partitions until immediately before leaf fall in autumn.

The lack of a treatment response in the distribution of crown N in the first year was not surprising. The amount of N added by either treatment was so small that the total N patterns of the crown should not have been affected. The lack of uniformity in N concentration among various crown positions was also not surprising. As leaves age and increase in size, the low-N structural components should comprise an increasing proportion of the leaf weight, effectively decreasing N concentration as specific leaf weight increases (Kramer and Kozlowski, 1979; Waring and Schlesinger, 1985; Sanchez and Righetti, 1990).

However, leaf size and age may not be the best indicator of leaf N concentration in alder. Dawson and Funk (1981) randomly collected branches from black alder and separated leaves of each branch by size class, ignoring the potential impact of vertical and lateral crown positions. They found no significant differences in N concentration among leaf size classes. Conversely, ignoring leaf size class and focussing on crown position, Sanchez and Righetti (1990) found increasing levels of N toward the periphery of apple trees. DeJong and Doyle (1985) linked the higher leaf N concentration at the edges of peach crowns with duration and intensity of light exposure, both of which are highest at the crown edge and decrease toward the interior. Kull and Niinemets (1993) found a significant positive correlation between N concentration and irradiance in a shade-intolerant tree (*Betula pendula* Roth.) and significant negative correlations between N concentration and irradiance in shade-tolerant understory shrubs (*Corylus avellana* L., *Lonicera xylosteum* L.). They described photosynthetic activity as being highest in the upper crown of the intolerant tree and the lower crown of the tolerant shrubs. Red alder, an extremely intolerant tree (Shainsky *et al.*, 1994), has been noted to exhibit depressed leaf area in the lower and middle crown due to peripheral shading (DeBell and Giordano, 1994). The peripheral crown positions in alder probably contain a greater proportion of younger, high-N leaves than interior crown positions. It is likely that this, coupled with greater light availability, led to a gradation of higher N concentrations toward the peripheral crown positions.

The second year of the study was notable for a decrease in the uniformity in $^{15}N$ distribution and for a further differentiation in the N concentration within partitions. The linear, significant correlations between relative $^{15}N$ distribution in crown partitions in the second year indicate similar retranslocation patterns among partitions. The lower concentration of $^{15}N$ in the distal partition is mirrored by a slightly lower correlation between proximal and distal partitions. Both responses may be explained by a greater incorporation in the distal partition of soil and fixed N at lower atom % $^{15}N$ abundance levels than the labeled N from the reserves (Sanchez, 1990). Leaves grown in the latter third of the season, and most abundantly in the distal and top partitions of the trees, use little or no reserve N (Domenach and Kurdali, 1989). Further, leaves of any age growing in these partitions are likely to be more photosynthetically active (Porpiglia and Barden, 1980) and a greater sink for N (DeJong, 1982; Weinbaum *et al.*, 1989, Kull and Niinemets, 1993). Thus, although older leaves in the distal and top partitions initially had similar $^{15}N$ signals to leaves in the interior crown, the distal and top leaves eventually exhibited a diluted $^{15}N$ signal later in the season with increased native N uptake over and above that of interior leaves.

The increased concentration of N in peripheral partitions was measured in both top and distal partitions in the second year. As the stand canopy closed during the second year of the experiment, increased competition for light led to greater differentiation within both lateral and vertical canopy groupings (DeJong and Doyle, 1985). Nitrogen concentrations in the top and bottom partitions, not different under adequate light conditions in the first year of the experiment, differentiated as the canopy closed. Distal and medial partitions, not different before canopy closure, were significantly different under more shaded conditions.

An unexpected development in the second year was the significant difference in N concentrations attributable to the $NO_3^-$ and $NH_4^+$ treatments. A toxicity response was considered as an explanation. However, such a small addition to total crown N should not have affected the relative N response even in the first year; it seems especially unlikely that it would elicit a response in the second year. Only a slight, if any, indication of a treatment difference in resorption or retranslocation efficiency was

observed in the isotope measurements, where the difference should have been most apparent. It seems unlikely that this was the cause. The observed differences between treatments were consistently in the form of lower N concentrations within the bottom or inside crown positions of the $NO_3^-$ trees (Fig. 3.5). This is suggestive of greater growth in the $NO_3^-$ trees, resulting in lower N concentrations in shaded partitions of those trees. Yet again, it is difficult to attribute a growth response, positive in the case of the $NO_3^-$ treatment or negative with the $NH_4^+$ treatment, to such a small input of N. Thus, although an actual treatment response should not be ruled out, it is possible that the observed treatment difference was an artifact of stand dynamics which favored faster growth of the $NO_3^-$ trees.

## 3.6 Conclusions

Stem injection of both $^{15}NH_4^+$ and $^{15}NO_3^-$ at levels approaching 1 % of crown N effectively labeled red alder trees. $^{15}NO_3^-$ may have been more efficient in initial labeling, though second-year effects are difficult to interpret. There was a clear increase in N concentration toward the periphery of the tree. This increase became more pronounced with increasing crown size and crown closure. Although the importance of leaf age should not be discounted in determining the source of N (Domenach and Kurdali, 1989), crown position with respect to light availability may be the most important determinant in overall N allocation in red alder foliage.

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## **Chapter 4:** Summary

Responsible management of nutrients in forest ecosystems is becoming increasingly important as the demand for forest products grows and the land base available to provide them shrinks. Red alder is an important component in many Pacific Northwest forest ecosystems for its input of N, a commonly limiting nutrient to the growth to economically important conifer species. This thesis provided the opportunity to advance the state of knowledge regarding the effect of red alder leaf-litter on a forest soil and the efficacy of an $^{15}N$-labeling technique on alder.

The contribution of red alder litter to several labile and non-labile N pools in an Oregon upland mesic forest soil was characterized using $^{15}N$ tracer techniques. The concentration of $^{15}N$ was measured after 21 mo at 0-5 and 5-15 cm depths in remaining $^{15}N$-labeled red alder detritus and four soil fractions, including the light- and heavy-fractions of the soil, the chloroform-labile (microbial biomass) pool, and the whole-soil. Some recovery of $^{15}N$ was noted in vegetation growing in the plots. The alder litter lost 78 % of its mass, 77 % of the total initial N, and only 64 % of the total initial $^{15}N$. Although the heavy-fraction contained 77 to 88 % of the total N, the concentration of N in the light-fraction was 3.5 times that in the heavy-fraction. Whole-soil % recoveries were higher than the summed fractions for total N and for $^{15}N$ in the top 5 cm. Light-fractions exhibited higher % recoveries of $^{15}N$ than heavy-fractions. Percent recovery of $^{15}N$ in the chloroform-labile N fraction was not significant. After 21 mo the majority of N released from the leaves was detained in the top five centimeters of soil within the light-fraction, though a significant amount was incorporated into the heavy-fraction. Alder litter appears to be a net source of N to this forest soil.

Red alder trees were labeled with $^{15}NO_3^-$ and $^{15}NH_4^+$ using the stem-injection method. Leaves were sampled 3 and 15 mo subsequent to injection in several crown partitions, including top, bottom, proximal, medial, and distal. Stem injection of both $^{15}NH_4^+$ and $^{15}NO_3^-$ at levels approaching 1 % of crown N effectively labeled red alder trees. $^{15}NO_3^-$ may have been more efficient in initial labeling, but less efficient in resorption or retranslocation. The distribution of $^{15}N$ was uniform at the time of the first

sampling, but was diluted in the distal and top partitions by the second sampling. There was a clear increase in N concentration toward the periphery of the tree. This increase became more pronounced with increasing crown size and canopy closure. Crown position with respect to light availability may be the most important determinant in crown N allocation in red alder foliage.

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# Appendices

**Appendix A:** Comparison of leaf parts from rings and litterbags

## A.1 Methods and results

### *A.1.1 Research Site*

The study was conducted in the Cascades Range at the H.J. Andrews Experimental Forest, Willamette National Forest, Oregon. All plots were established on a single slope within a clearcut area of about 1 ha. Care was taken to choose microsites with homogeneous characteristics such as absence of large woody debris or nearby seedlings. Vegetation consisted primarily of Pacific blackberry (*Rubus ursinus* Cham. & Schlect.) and grasses.

### *A.1.2 Mass Spectrometry*

Samples submitted for mass spectrometry were ground to pass through a 40-mesh sieve and analyzed for %N, atom % $^{15}N$, and %C on a Europa Scientific ANCA-MS automated mass spectrometer or a Europa Scientific 20/20 automated mass spectrometer (Europa Scientific Ltd., Crewe, UK). Nitrogen contents at natural abundance are measured by the ANCA-MS and 20/20 at precision levels of $\pm$ 0.0003 and 0.00007 atom % $^{15}N$, respectively. Carbon contents at natural abundance are measured by the ANCA-MS and 20/20 at precision levels of $\pm$ 0.2 and 0.05 delta, respectively. Duplicate samples were run for N and C both within and between the mass spectrometers to ensure consistency. Results of the duplicate runs were tested for differences using paired t-tests at the 0.05 level of significance. Without exception, comparisons were not significantly different.

### *A.1.3 Labeled alder leaves*

Labeled red alder leaves were produced by injecting artificial sap mixed with 0.675 g of 15.8 atom % $^{15}N$-$(NH_4)_2SO_4$ into the xylem of each of six 7-year-old red alder trees (Horwath *et al.*, 1992; Chapter 3) in July 1993. The crown of each injected tree was confined within 1.25-cm bird netting. The netting, tied conically at the base of the

tree, captured the leaves after abscision. Abscessed leaves were collected in November 1993, air dried, and analyzed by mass spectrometry for N and carbon content. The labeled leaves from the six trees varied little in $^{15}N$ content and were mixed together (mean $^{15}N$ content = 0.4234 atom % $^{15}N$, SE = 0.0054). Air-dried leaves were subsampled to correct for moisture content.

*A.1.4 Rings and bags*

In December 1993, labeled leaves were placed directly on mineral soil scraped bare of grass sod and other vegetation; 125 g of labeled leaves were deposited on the soil and bound within a plastic ring 0.4 m in diameter and extending from about 6 cm above the soil surface to 3 cm below. Netting (1.25-cm) was draped over each of the 20 rings, pulled taut, and secured into the ground with stakes.

Nine litterbags were placed adjacent to each ring. The 10x10 cm litterbags consisted of nylon sailcloth on bottom and 1-mm mesh on top. Each bag was filled with 10 g of air-dried labeled leaf material from the same source as the leaves placed within the rings. A single bag next to each ring was collected periodically.

*A.1.5 Sample collection and preparation*

In September 1995 all material recognizable as red alder leaf detritus was collected from the rings and two to three of the remaining bags next to each ring were also collected. The petioles of all leaves were detached at the base of the leaf blade in both bagged- and ringed-detritus. Blade and midrib fragments without petioles and petioles alone were included with the artificially separated blade and petiole groups. Twenty-five petioles from each bag and ring were measured for length and weight, then collectively ground and analyzed by mass spectrometry for N and C content. A Licor Digital Area Meter (Decagon Devices, Inc., Pullman, WA) was used to measure approximately 200 $cm^2$ of dried blade material from each litter bag and ring. The blade material was then separately weighed, ground, and analyzed by mass spectrometry for N and C. In addition to the samples from rings and litterbags, five samples were collected as a control from the intitial non-treated source leaves and prepared according to the

above procedure. Values for leaf parts from the initial, litter bags and rings were compared.

*A.1.6 Statistical Analysis*

Nutrient and weight status of the initial material and the bagged- and ringed-detritus were compared using analysis of variance and Tukey's Studentized Range (HSD) Test with a probability of a type I error of 0.05. Although the comparisons were planned, the Tukey test was chosen as a conservative test with tight controls for experimentwise error rate relative to other multiple comparison tests.

TABLE A.1. Percent C in blades and petioles, mg C $cm^{-2}$ in blades, and mg C $cm^{-1}$ in petioles, measured in untreated leaves, detritus from litter bags, and detritus from the soil surface within rings.

| | % C | | mg C | |
|---|---|---|---|---|
| Treatment | Blade | Petiole | Blade ($cm^{-2}$) | Petiole ($cm^{-1}$) |
| Initial | 56.31a | 52.64c | 6.05b | 5.46a |
| Litter bag | 48.15b | 53.38b | 6.01b | 3.71b |
| Ring | 48.61b | 54.11a | 7.03a | 5.80a |

NOTE: The Least Significant Difference test was used to identify differences. Column values not followed by the same letter are significantly different ($\propto = 0.05$). All values are ash-free.

TABLE A.2. Percent N in blades and petioles, mg N $cm^{-2}$ in blades, and mg N $cm^{-1}$ in petioles, measured in untreated leaves, detritus from litter bags, and detritus from the soil surface within rings.

| | % N | | mg N | |
|---|---|---|---|---|
| Treatment | Blade | Petiole | Blade ($cm^{-2}$) | Petiole ($cm^{-1}$) |
| Initial | 2.57b | 1.23b | 0.28c | 0.030c |
| Litter bag | 3.31a | 1.97a | 0.41b | 0.044b |
| Ring | 3.25a | 1.99a | 0.47a | 0.050a |

NOTE: The Least Significant Difference test was used to identify differences. Column values not followed by the same letter are significantly different ($\propto = 0.05$). All values are ash-free.

TABLE A.3. Atom % $^{15}N$ in blades and petioles, $\mu g$ $^{15}N$ $cm^{-2}$ in blades, and $\mu g$ $^{15}N$ $cm^{-1}$ in petioles, measured in untreated leaves, detritus from litter bags, and detritus from the soil surface within rings.

| | atom % $^{15}N$ excess | | $\mu g$ $^{15}N$ | |
|---|---|---|---|---|
| Treatment | Blade | Petiole | Blade ($cm^{-2}$) | Petiole ($cm^{-1}$) |
| Initial | 0.0666a | 0.1172a | 0.19a | 0.036a |
| Litter bag | 0.0633a | 0.1084a | 0.21a | 0.055a |
| Ring | 0.0456b | 0.0817b | 0.26a | 0.041a |

NOTE: The Least Significant Difference test was used to identify differences. Column values not followed by the same letter are significantly different ($\propto = 0.05$). All values are ash-free.

### *A.1.7 Brief interpretation of results*

As discussed in Chapter 2 and shown in Appendix 2, the detritus in rings and bags underwent a net loss of weight, N, $^{15}N$, and C. However, an examination of pre- and post-treatment material may reveal more about the nutrient dynamics than simple mass loss estimates.

TABLE A.4. Weights of blades ($cm^{-2}$) and petioles ($cm^{-1}$), and C:N ratios of blades and petioles, measured in untreated leaves, detritus from litter bags, and detritus from the soil surface within rings.

| | C:N | | Mass (g) | |
|---|---|---|---|---|
| Treatment | Blade | Petiole | Blade ($cm^{-2}$) | Petiole ($cm^{-1}$) |
| Initial | 22a | 45a | 11.13c | 11.02a |
| Litter bag | 15b | 27b | 13.20b | 7.03b |
| Ring | 15b | 27b | 15.50a | 11.02c |

NOTE: The Least Significant Difference test was used to identify differences in log-transformed data. Data presented have been back-transformed to median values. Column values not followed by the same letter are significantly different ($\propto = 0.05$). All values are ash-free.

Similar values for initial and ring wt/cm imply no net loss of mass per cm from ring petioles, most likely import of mass per cm was equal to the loss of mass per cm. Bags underwent net loss in wt/cm. Higher % N in bags and rings imply higher net wt loss than net N loss, or net import of N. Dilution of the atom % excess in rings would indicate import of native N at a lower atom % $^{15}N$. The lower decrease in atom % excess in bags indicates less import of native N than rings. Coupled with a decrease in wt/cm, the lower import of N in bags indicates faster weight loss plays a more important role in the increase of % N in bags.

In addition to greater N import, the rings also appear to have undergone greater C import. Carbon import in the rings is implied by the lack of wt/cm loss, the lack of mg C/cm loss, and the higher % C. Greater C import in rings is implied by the net wt/cm loss in bags coupled with lower % C and mg C/cm, compared to a lack of net change in wt/cm and a higher % C and mg C/cm increase in rings.

If only bag petioles were considered, several important processes might have been overlooked or underestimated. The import of C was far more pronounced in rings; data from bags alone might have obfuscated the import of C from soil to detritus. The degree of N import might have also been misjudged.

Blade detritus showed some similar trends to those of petioles. Both the ring and bag blade detritus contained lower C concentrations than the initial, indicating faster C loss than weight loss. But the ring detritus is denser, as illustrated by the higher $g/cm^2$ (assuming similar thicknesses of blade materials among treatments), and can provide more $C/cm^2$ even if its C concentration on a weight basis is lower. Visual observations of the treatments revealed completely intact initial leaves, almost completely intact bag detritus, and highly perforated ring detritus. The method of area measurement ignored gaps, and counted only solid space. Therefore, the detritus in the ring treatment probably contained far less of the original leaf than the bag detritus. However, the remaining leaf fragments were denser and lower in C concentration than the bag and initial material.

Although C import could not be as clearly identified in blades as in petioles, N import clearly took place in ring detritus. Atom % $^{15}N$ excess in bag detritus is slightly lower, but not significantly different than initial leaves, implying minimal native N import. Thus, the increase in % N and mg N in bag detritus can be interpreted as being almost solely a result of faster weight loss than N loss. Ring detritus, however, underwent a significant decrease in atom % $^{15}N$ excess, implying import of native N. A greater weight increase in rings, but similar % N to bags, implies either slower gross N loss from rings, a similar/higher gross N loss coupled with N import, or a combination.

Estimates of total mass loss and mass loss of nutrients derived from litter bag studies may provide indexes of movement of these material from litter. However, they may fail to account for interaction of the litter with soil flora, fauna, and moisture. Thus, the assumption that changes in mass and concentrations are gross, rather than net, is flawed. Although this understanding is implicit in most studies involving litter bags, it should receive more discussion, especially when the study in question seeks to address nutrient 'cycling' and 'ecology.'

## **Appendix B:** Mass, N, $^{15}N$, and C loss from litterbags

### B.1. Methods and results

#### *B.1.1 Research Site*

The study was conducted in the Cascades Range at the H.J. Andrews Experimental Forest, Willamette National Forest, Oregon. All plots were established on a single slope within a clearcut area of about 1 ha. Care was taken to choose microsites with homogeneous characteristics such as absence of large woody debris or nearby seedlings. Vegetation consisted primarily of Pacific blackberry (*Rubus ursinus* Cham. & Schlect.) and grasses.

#### *B.1.2 Mass Spectrometry*

Samples submitted for mass spectrometry were ground to pass through a 40-mesh sieve and analyzed for % N, atom % $^{15}N$, and % C on a Europa Scientific ANCA-MS automated mass spectrometer or a Europa Scientific 20/20 automated mass spectrometer (Europa Scientific Ltd., Crewe, UK). Nitrogen contents at natural abundance are measured by the ANCA-MS and 20/20 at precision levels of $\pm$ 0.0003 and 0.00007 atom % $^{15}N$, respectively. Carbon contents at natural abundance are measured by the ANCA-MS and 20/20 at precision levels of $\pm$ 0.2 and 0.05 delta, respectively. Duplicate samples were run for N and C both within and between the mass spectrometers to ensure consistency. Results of the duplicate runs were tested for differences using paired t-tests at the 0.05 level of significance. Without exception, comparisons were not significantly different.

#### *B.1.3 Labeled alder leaves*

Labeled red alder leaves were produced by injecting artificial sap mixed with 0.675 g of 15.8 atom % $^{15}N$-$(NH_4)_2SO_4$ into the xylem of each of six 7-year-old red alder trees (Horwath *et al.*, 1992; Chapter 3) in July 1993. The crown of each injected tree was confined within 1.25-cm bird netting. The netting, tied conically at the base of the

tree, captured the leaves after abscision. Abscessed leaves were collected in November 1993, air dried, and analyzed by mass spectrometry for N and C content. The labeled leaves from the six trees varied little in $^{15}N$ content and were mixed together (mean $^{15}N$ content = 0.4234 atom % $^{15}N$, SE = 0.0054). Air-dried leaves were subsampled to correct for moisture and ash content.

*B.1.4 Litterbags*

Two hundred 10x10 cm litterbags were fashioned of 1-mm nylon mesh on top and nylon sailcloth on bottom (LIDET, 1995). Each bag was individually labeled with a metal tag and filled with 10 g of air-dried $^{15}N$-labeled leaf. Weights for each bag were individually recorded. Sub-samples were taken and oven-dried to correct for moisture and ash content. Bags were periodically collected, oven dried at 75°C for 48 h, and weighed. All samples were analyzed for N, $^{15}N$, and C on the mass spectrometers previously discussed. Plots of weight, N, $^{15}N$, and C, measurements over time are shown in Fig. B.1. At each point, $n = 20$.

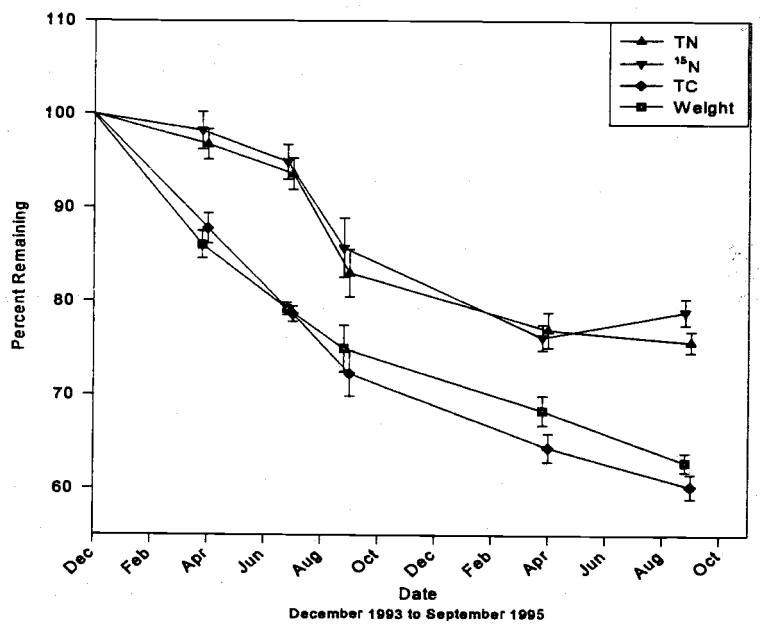


FIG.B.1. Loss of N, $^{15}N$, C and weight from litterbags during a 21 mo period. Error bars are standard error.