Incorporation of nitrogen from decomposing red alder leaves into plants and soil of a recent clearcut in Oregon[1]

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Abstract: Nitrogen incorporation from red alder (*Alnus rubra* Bong.) into an Oregon upland mesic forest soil was studied by tracing the fate of ^{15}N added as ^{15}N-labeled alder leaf litter. The recovery of ^{15}N in vegetation, litter, light- and heavy-fractions of the soil, the chloroform-labile (microbial biomass) pool, and the whole soil were investigated after a 21-month field incubation of the labeled litter. ^{15}N abundances well in excess of normal values were measured in vegetation growing in the plots, perhaps 3% of the ^{15}N excess initially added. Additionally, the recovery of initial ^{15}N after 21 months was 31% in remaining litter, 34% in the upper 5 cm of soil, and 4% in the 5–15 cm depth class. Alder litter had lost 78% of its mass, 77% of the total initial N ($^{14}N + {}^{15}N$), and 64% of the initial ^{15}N. ^{15}N recovery was higher in the light fraction than in the heavy fraction. The soil heavy fraction accounted for 77 to 88% of the total soil N; however, the concentration of N in the light fraction was 3.5 times that in the heavy fraction. Recovery of excess ^{15}N in the chloroform-labile N fraction was not significantly different from zero. After 21 months of decomposition, alder detritus was a net source of N; most of which remained in the top 5 cm of soil where it was concentrated in the more labile pools of soil N, and some of which was incorporated into growing plant tissue.

Résumé : L'incorporation de l'azote de l'aulne rouge (*Alnus rubra* Bong.) dans un sol forestier mésique des hautes terres de l'Orégon a été étudiée en suivant le devenir de ^{15}N ajouté sous la forme de litière foliaire d'aulne marquée avec ^{15}N. Le recouvrement de ^{15}N dans la végétation, la litière, les fractions légères et lourdes du sol, le pool labile de biomasse microbienne (méthode au chloroforme), et le sol entier a été investigué après 21 mois d'incubation au champ de la litière marquée. Des abondances de ^{15}N bien en excès des valeurs normales ont été mesurées dans la végétation croissant dans les parcelles, peut-être 3% du surplus de ^{15}N initialement ajouté. De plus, le recouvrement du ^{15}N initial après 21 mois était de 31% dans la litière résiduelle, de 34% dans les 5 cm supérieurs du sol et de 4% dans la classe de profondeur 5–15 cm. La litière d'aulne avait perdu 78% de sa masse, 77% de son N total initial ($^{14}N + {}^{15}N$) et 64% de son ^{15}N initial. Le recouvrement de ^{15}N était plus élevé dans la fraction légère que dans la fraction lourde. La fraction lourde du sol comptait pour 77 à 88% de N total du sol; toutefois, la concentration de N dans la fraction légère était 3,5 fois plus élevée que dans la fraction lourde. Le recouvrement du surplus de ^{15}N dans la fraction N labile (méthode au chloroforme) n'était pas significativement différent de zéro. Après 21 mois de décomposition, les débris d'aulne étaient une source nette de N; la majorité de celui-ci se retrouvait dans les 5 premiers cm du sol où il était concentré dans les pools les plus labiles d'azote du sol, et une partie était incorporée dans les tissus des plantes en croissance.
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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 *Frankia*, a N_2-fixing actinomycete (Binkley et al. 1994), produces leaf litter with an N content far in excess of associated tree species (Tarrant et al. 1951; Harmon et al. 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 commercially important interplanted or alternately cropped conifer species (Brozek 1990), it is critical 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 C in the light and heavy fractions of soils under conifer, red alder, and conifer–alder

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sites. Alder and alder–conifer sites showed higher relative concentrations of N and lower 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 ^{15}N enrichment in each fraction. The ^{15}N tracer technique and incubation facilitated the conclusion that N is incorporated into the heavy fraction more quickly than previously thought. These studies show a cumulative enrichment in both C 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 alder 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 an organic source of N on soil fractions and whole soil after nearly 2 years of litter decomposition.

Materials and methods

Research site

The study was conducted in the central western Cascades Range at H.J. Andrews Experimental Forest (44°09′17" N, 122°20′24" W), Willamette National Forest, Oregon, U.S.A. All plots were established on a single slope within a clear-cut area of about 1 ha, 110° aspect, and 7–12% slope. Care was taken to choose sites 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.

Plot establishment

Labeled red alder leaves were produced by injecting artificial sap mixed with 0.675 g of 15.8 atom% $(^{15}NH_4)_2SO_4$ into the xylem (Horwath et al. 1992; Swanston and Myrold 1997) 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. A subset of air-dried leaves were sampled and oven-dried at 75°C for 48 h to correct for water content.

For each microplot, grass sod and other aboveground vegetation was removed to ensure leaf contact with the soil. Labeled leaves (125 g) were placed directly on mineral soil and were 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 microplots, pulled taut, and secured into the ground with stakes. For sampling purposes, the microplots were placed in five groups 50 m apart with each group consisting of four microplots at a 2-m spacing. When the microplots were established in December 1993, soil samples were taken immediately adjacent to each microplot. A "second year" of leaf fall was not placed within the microplots. At the conclusion of the experiment in September 1995, all material recognizable as red alder leaf detritus was collected from the microplots. This detritus was dried at 75°C for 48 h, weighed, and analyzed for N and C. Loss on ignition was measured after 4 h at 550°C.

Litter bags were fashioned of 10 × 10 cm squares of 1-mm nylon mesh on top and nylon sailcloth on bottom (Long-term Intersite Decomposition Experiment Team 1995). Each bag was individually labeled with a metal tag and filled with 10 g of air-dried ^{15}N-labeled red alder leaves, described above. Weights for each bag were individually recorded. Samples were taken and oven-dried at 75°C for 48 h to correct for moisture content. Once collected, bags were oven-dried and their contents weighed and analyzed by mass spectrometry for N and ^{15}N.

Soils

The soils at the study site were classified as fine-loamy mesic Typic Xerumbrepts. Soil samples were collected with a split-tube sampler to 0–5 and 5–15 cm depths in December 1993, and in April and September of both 1994 and 1995. Field-moist soils were frozen at –20°C until analyzed. Unless otherwise stated, weights are oven dry (water content determined at 105°C for 48 h). Soil samples were ground with a roller mill and analyzed by combustion to determine total N and C.

Estimates of bulk density were required to calculate percent recovery of ^{15}N in September 1995. Bulk density ($g \cdot cm^{-3}$), estimated separately for each depth and microplot, was calculated from soil C content using a regression equation reported by Grigal et al. (1989):

$$[1] \quad \text{Bulk density} = 0.075 + 1.301^{(-0.060 \times \text{LOI})}$$

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).

Density fractionation

Soil samples for each depth class were composited by microplot 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). The modified separation procedure was better adapted to the soils at the study site. Rocks and twigs larger than 2 mm in diameter were removed from the field-moist soils by hand. Field-moist soil (20 g) was added to a standard 250-mL beaker containing 100 mL of sodium polytungstate (SPT), an inorganic salt solution (density = 1.60 $g \cdot cm^{-3}$). The SPT and soil were mixed at 13 000 rpm for 30 s with a soil mixer to break up soil and fine-root aggregates. A probe-type sonic disrupter (Branson Sonic Power Company, Danbury, Conn., model 350) was immediately used to further disperse the soil in suspension. About 156 $J \cdot mL^{-1}$ of soil suspension was delivered over a 2-min period, 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, when 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.

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, Milwaukee, Wis.). Digests (15 mL)

Table 1. The mean distribution of mass, N, and ^{15}N in aboveground vegetation growing in microplots 21 months after addition of ^{15}N-labeled red alder litter.

Vegetation type	Mass (g)	N ($g \cdot kg^{-1}$)	^{15}N abundance (atom% ^{15}N)
Grass[a]	25.9 (4.1)	6.6 (0.4)	0.3788 (0.0027)
Herbaceous[b]	14.6 (4.8)	11.3 (0.6)	0.3752 (0.0038)
Woody[a]	17.7 (3.0)	13.3 (0.5)	0.3669 (0.0004)

Note: Standard error is in parentheses.
[a] $n = 20$.
[b] $n = 14$.

were prepared for ^{15}N analysis using 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 (M.L. Cabrera and M.H. Beare, 1993, personal communication). As a control, 25 g of nonfumigated 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 nonfumigated extracts were subtracted from those of the fumigated extracts to determine chloroform-labile N (Brookes et al. 1985).

Vegetation

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

^{15}N recovery in soil

Recovery of ^{15}N was determined by dividing milligrams ^{15}N excess recovered in a given soil pool by the initial milligrams ^{15}N excess in the litter (Hauck et al. 1994). Atom% ^{15}N excess in litter was defined as the initial difference between the atom% ^{15}N of the litter and of the soil pool. Estimates of milligrams ^{15}N excess in each pool were obtained from its N content and atom% ^{15}N excess. Atom% ^{15}N excess in a given soil pool was defined as the atom% ^{15}N greater than that measured in the soil pool in December 1993 (untreated).

Recovery of ^{15}N in litter was determined by dividing total final milligrams ^{15}N by total initial milligrams ^{15}N. Total milligrams ^{15}N was calculated from the total weight, total N, and atom% ^{15}N of the leaves.

Mass spectrometry

Samples for mass spectrometry were ground to pass through a 40-mesh sieve and analyzed for percent N, atom% ^{15}N, and percent C on a Europa Scientific Tracermass isotope ratio mass spectrometer or a Europa Scientific 20/20 isotope ratio mass spectrometer (Europa Scientific Ltd., Crewe, U.K.). Nitrogen concentrations at natural abundance were measured by the Tracermass with a precision of ±0.0003 atom% ^{15}N and the 20/20 with a precision of ± 0.000 07 atom% ^{15}N. Carbon concentrations (as a percent of dry weight) were measured by the Tracermass 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.

Statistical analysis

One-tailed *t*-tests were used to determine whether the percent recovery of ^{15}N in the litter and various soil components was significantly greater than zero. Analysis of variance and Fisher's protected least significant difference (LSD) tests 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 × depths = 6) and all pairwise comparisons were of interest, a multiple range test was considered appropriate. Fisher's protected LSD test was chosen on the basis that the comparisons were planned and the samples sizes were unequal between treatments (Milliken and Johnson 1992). Statistical comparisons of values likely to be correlated (e.g., mg N in light fraction·kg^{-1} soil will increase with decreasing mg N in heavy fraction·kg^{-1} soil) were not conducted, given inadequate degrees of freedom needed to use the proper statistical methods.

Results and discussion

Above ground

A reliable estimate of percent recovery of ^{15}N in vegetation growing on the plots could not be computed because of the lack of controls (i.e., plants growing in areas not receiving ^{15}N). Additionally, only data for vegetation in the second season (1995) are available, because an early snowfall precluded the planned fall sampling of vegetation in 1994. However, even a cursory examination of the data suggests that there was accumulation of excess ^{15}N in the vegetation in the second year (Table 1), given normal atom% ^{15}N values of vegetation. Atom% ^{15}N values in plants typically range from 0.3630 to 0.3670 (e.g., Cabrera and Kissel 1989; Ehleringer and Rundel 1989). The values for grass and herbaceous vegetation collected 21 months after plot establishment were well outside this range, and the atom% ^{15}N of the woody vegetation was at the upper end (Table 1).

If the atom% ^{15}N of the unlabeled vegetation at the site was considered to equal the soil atom% ^{15}N values (0.3680), which were slightly above atmospheric levels (0.3663), the recovery in the weighted-average mass of total vegetation of a microplot would be 2.7% ($n = 20$, SE = 0.46). This may be an underestimate of percent 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). The measurable recovery of ^{15}N indicates that the mineralization of N from alder detritus to plant-available N, and subsequent uptake by plants can occur in meaningful quantities within a 21-month time span.

At the end of this time span, nearly 31% of the initial quantity of ^{15}N was recovered in the litter (Table 2). Yet, of the 125 g of fallen leaves placed in the microplots, on average only 28 g ($n = 20$, SE = 2) were recognizable as alder detritus and collected. Thus, only 22% of the original mass was recovered, whereas 78% was incorporated into the soil organic matter or otherwise lost. This high mass loss and lower loss of ^{15}N was accompanied by only a slight, nonsignificant increase in N concentration, resulting in a rise in atom% ^{15}N (Table 3).

Microbial degradation may have resulted in a slight increase in ^{15}N abundance in remaining litter. Discrimination during decomposition may leave enriched residual substrate (Tiessen et al. 1984; 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 ^{15}N in the remaining litter, it is unlikely they had a large impact because discrimination effects are small.

The most basic and likely explanation for the proportionally higher retention of ^{15}N is that ^{15}N was concentrated in more

Table 2. Percent of whole soil occupied by density fractions, the recovery of ^{15}N in density fractions and other microplot components, and the probability of ^{15}N recovery being greater than zero.

Ring component	% of whole soil	Atom% ^{15}N excess	^{15}N recovery (%)	*n*	*P*-value[a]
Alder detritus	—	—	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

Note: All microplots have received ^{15}N-labeled red alder leaf litter 21 months earlier. Standard error is in parentheses.
[a]Testing H_0: ^{15}N recovery = 0.

Table 3. Changes in mean composition of red alder leaf detrital fractions over time.

		N[b]			C[a]	
Sample time	Ash-free fraction[a]	Concn. (g N·kg^{-1} AF leaves[c])	Total (g N)	^{15}N abundance (atom% ^{15}N)	Concn. (kg C·kg^{-1} AF leaves[c])	Total (g C)
December 1993 (initial values)	0.96 (0.003)	29.90 (0.59)	3.57 (0.077)	0.4195 (0.0054)	0.58 (0.0044)	70.69 (0.55)
September 1995 (final values)	0.93 (0.009)	31.46 (0.55)	0.85 (0.065)	0.5446 (0.0048)	0.49 (0.0042)	16.55 (2.97)
P-value	<0.0001	0.12	<0.0001	<0.0001	<0.0001	<0.0001

Note: Standard error is in parentheses.
[a]$n = 6$ for initial values and $n = 12$ for final values.
[b]$n = 6$ for initial values and $n = 20$ for final values.
[c]AF, ash free.

recalcitrant parts of the leaf even before the commencement of decomposition. Having observed that petioles appeared to be more resistant to breakdown than blades, we separated petioles from blades and analyzed for N and ^{15}N (Swanston 1996). These tests revealed that for both initial and final sampling dates, in both microplots and litter bags, the atom% ^{15}N in excess of natural abundance was over 50% higher in petioles than in blades. Although we have no information on chemical fractions of the leaves, it is also plausible that recalcitrant chemical fractions contained a higher initial proportion of ^{15}N. As these recalcitrant and more highly labeled parts of the leaves persisted and occupied increasing proportions of the litter, they may have raised the overall atom% ^{15}N of the remaining litter.

Substantial import of native N from outside the litter should have contributed to a dilution of ^{15}N in the leaves, likely countering any concentration of ^{15}N in recalcitrant parts of the leaves (Berg 1988). This dilution was not apparent; furthermore, red alder litter may be more likely to lose N than immobilize N. Edmonds (1980) investigated the decomposition of four litter types, including red alder, in four ecosystems. His study corroborated earlier findings (Edmonds 1979) that, in the ecosystems studied, net immobilization of N occurs in decomposing leaves at C:N ratios above 35:1, and net N 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 net N immobilization reported by Edmonds (1980).

Edmonds (1980) also reported that after 24 months of decomposition in litter bags, alder leaves retained 41% of their mass and 67% of their initial N content. In the present study, after 21 months the alder leaves confined in litter bags retained 64% of their mass ($n = 41$, SE = 0.62), 76% of their initial N content ($n = 12$, SE = 1.22), and 79% of their initial ^{15}N content ($n = 12$, SE = 1.97). Recoveries in both of these litter-bag studies are substantially higher than those from the unconfined leaves in the microplots, which retained only 22% of their mass, 24% of their N, and 31% of their ^{15}N. There are several potential reasons for the discrepancies between the litter-bag and microplot studies, including sampling error in the microplots, moisture differences, and exclusion of mycelia and arthropods from the litter bags (Edmonds 1980; St. John 1980; Blair et al. 1992). However, all three studies showed alder litter as a net source of N. With no dilution of ^{15}N apparent in either litter bags or microplots, it is possible to conclude that red alder litter is a net contributor of N to soil within 21 months of leaf fall.

Below ground

The highest recovery of ^{15}N in soil appeared to be from the whole soil in the 0–5 cm depth class, followed by the light fraction in the same depth class (Table 2). Recoveries below

Table 4. Carbon and N concentration (%) of whole soil and density fractions and content (g) of C and N in each density fraction per kilogram of soil in 0–5 and 5–15 cm depth classes.

Density fraction	Depth (cm)	C		N		C/N
		%	g·kg^{-1}	%	g·kg^{-1}	
Light fraction	0–5	33.23*a*	24.5 (6.8)	0.57*a*	0.429 (0.136)	59.72*b*
	5–15	34.87*a*	12.8 (6.5)	0.42*b*	0.154 (0.076)	83.03*a*
Whole soil	0–5	10.90*b*	—	0.31*c*	—	34.67*c*
	5–15	7.08*bc*	—	0.24*cd*	—	28.95*cd*
Heavy fraction	0–5	3.75*cd*	34.6 (3.0)	0.16*de*	1.472 (0.122)	23.54*d*
	5–15	2.58*e*	24.8 (4.9)	0.12*e*	1.142 (0.132)	21.42*d*

Note: Standard error is in parentheses. Fisher's protected LSD test was performed for C and N concentrations; column values not followed by the same letter are significantly different ($\alpha = 0.05$). Parametric statistical tests were not conducted on C and N contents because of violation of the independence assumption. Sample size is too low to conduct nonparametric 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.

Table 5. Recovery of ^{15}N in the $CHCl_3$-N fraction with depth and the probability of ^{15}N recovery being greater than zero.

Depth (cm)	^{15}N excess (atom% ^{15}N)	^{15}N recovery (%)	*n*	*P*-value[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

Note: Standard error is in parentheses.
[a]Testing H_0: ^{15}N recovery = 0.

5 cm were low and variable. In each depth classes the light fraction contained about two-thirds of the ^{15}N recovered in the density fractions. This is in strong contrast with the proportion of soil that is composed of light fraction, about 7% by weight in the top 5 cm, and 3% from 5 to 15 cm. Conversely, though the heavy fractions compose greater than 90% of the soil by weight and the bulk of the soil C and N (Table 4), they have far lower concentrations of C and N as well as lower ^{15}N recoveries.

Although C and N concentrations were clearly influenced by depth in all soil fractions, the soil fractions themselves appeared to be the most important determinants of C and N concentrations (Table 4). The general trend was that light fractions had higher C and N concentrations, followed by the whole soils and the heavy fractions. Within the fractions and the whole soils, the upper depth class generally had higher concentrations of C and N, if there was any statistical difference. This trend was expected and has been reported in other studies (e.g., Christensen 1992; Sollins et al. 1984). The much smaller C:N ratio in the upper light fraction might have resulted from the addition of the low C:N alder leaves, which could have influenced the upper soil much more strongly than the lower soil. This idea is borne out by the higher recovery of ^{15}N in the upper soil and specifically in the upper light fraction.

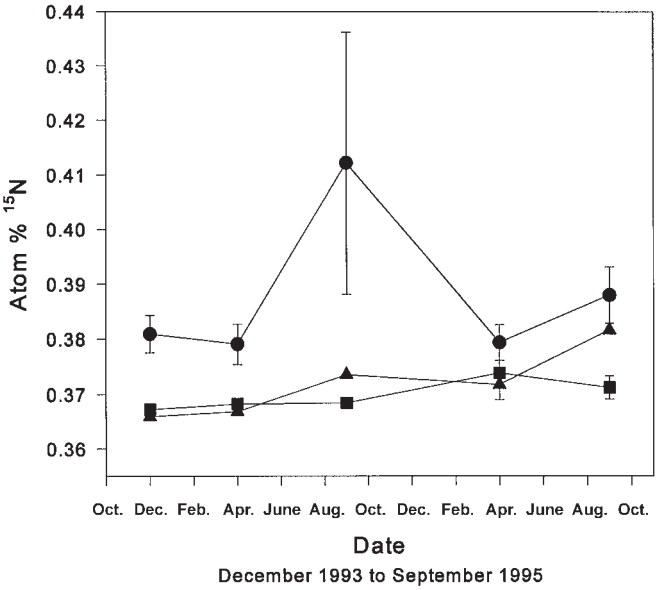
An interesting development in the density fractionation process was the failure of the summed ^{15}N recoveries in the density fractions to equal the values in the whole soil. This inconsistency in the 5–15 cm depth class is partly explained by the atom% ^{15}N excess in the lower soil being both highly variable and near the reliable detection limit of mass spectrometry. However, this discrepancy was also observed in the N and C estimates. Conceptually, summed nutrient values of the light and heavy fractions should equal the values of the whole soil for the same nutrient. It appears that the densimetric separation of the fractions may result in the loss of inorganic N, microbial N and C, soluble organic N and C, and clay. Clay loss, perhaps the greatest source of C loss (Nelson et al. 1994), would tend to result in underestimates of heavy fraction C, N, and ^{15}N. Losses from the other listed sources would most likely cause underestimates of light fraction C, N, and ^{15}N.

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. Unfortunately, most other studies that have used density separation alone have assumed complete recovery of C and N. Values for one of the fractions are most often derived from the difference between the whole soil and the other fraction. Considering the relatively low losses reported by Sollins et al. (1984) and losses as high as 46% in the present study, the assumption of full nutrient recovery does not appear well founded when used with density separation.

Surprisingly, the recovery of excess ^{15}N in the chloroform-labile N pool was not significantly different from zero at either depth (Table 5). Although some microplots showed up to 3% recovery, the mean recovery for all microplots was low and the variability was high. In particular, the upper 5 cm showed high variability in the recovery of ^{15}N. The low recovery could have been due to the abnormally high atom% ^{15}N value of the December 1993 point for the chloroform-labile N (Fig. 1), which was used as the reference to calculate atom% ^{15}N excess and ^{15}N recovery. We do not know the reason for the high atom% ^{15}N value of chloroform-labile N sampled in December 1993, although it may have resulted from ^{15}N contamination during one of the fumigation, extraction, and diffusion procedures. It is interesting to note, however, that a similar, unreplicated study with more highly labeled red alder leaves (Swanston, 1996) showed similar trends to those in Fig. 1 except for the first point of the chloroform-labile fraction, which was close to natural abundance and similar in atom% ^{15}N to the density fractions.

Atom% ^{15}N in the chloroform-labile pool was greater in September 1994 than in September 1995 and may have resulted in statistically significant percent recovery of ^{15}N (Fig. 1). This pulse of 15N into the chloroform-labile pool may represent incorporation into microbial biomass and subsequent turnover of microorganisms. The same temporal periodicity is seen in the light and, to a lesser extent, in heavy density

Fig. 1. Atom% ^{15}N of chloroform-labile N (●), light-fraction N (▲), and heavy-fraction N (■) in the 0–5 cm depth class as measured through time in microplots after the addition of ^{15}N-labeled red alder leaf litter in December 1993. Error bars are ±1 SE.



fractions. The periodic increase in atom% ^{15}N is especially apparent in the fall, during the period of high microbial activity, and is damped over time as less readily available ^{15}N-labeled substrate remains.

Recovery and loss of ^{15}N

Overall, approximately 71% of the added excess ^{15}N was recovered; 29% was not accounted for in measured soil, litter, or vegetation components. Recoveries of excess ^{15}N added to soil or litter as urea or in inorganic forms range from 39 to 100% (e.g., Preston et al. 1990; Hart et al. 1993). Most comparable to the present study is that of Preston and Mead (1995). Using unconfined Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) litter labeled with ^{15}N by the uptake of ^{15}N-labeled fertilizer, they conducted a mass balance over the course of 7 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 our 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 0.5 M K_2SO_4-extractable N (organic + inorganic), the loss of excess ^{15}N due to leaching would be just under 3%. Also, denitrification probably played only a minor role in the loss of ^{15}N from the system. Vermes and Myrold (1992) estimated that the upper limit of denitrification at this site was 0.08 kg $N \cdot ha^{-1} \cdot year^{-1}$, which at most 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 microplot boundary by creeping blackberry; foraging (removal) of labeled grasses by elk or insect herbivores; lateral leaching; and uptake of ^{15}N by roots invading from outside the plot. These losses were not directly quantified, nor are 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. Thus, the majority of the missing ^{15}N may not have been missing from the plot, but from the samples of the plot.

Summary

^{15}N-labeled red alder litter lost most of its mass, N, and ^{15}N during the 21-month incubation, suggesting active decomposition and release of N. Because there was no dilution of ^{15}N in the alder litter, it did not immobilize appreciable amounts of native soil N. Nitrogen from the alder litter was found in plants and soil. The highest recovery of ^{15}N in the soil beneath the litter was in the upper 5 cm of soil and in the light fraction. This study has been able to illustrate a completed cycle of N, begun as organic N in fallen leaves and ultimately traced 21 months later into labile and stable soil organic pools, remaining leaf litter, and growing vegetation.

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