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The Fate and Retention of Organic and Inorganic ^{15}N-Nitrogen in an Old-Growth Forest Soil in Western Oregon

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ABSTRACT

Forests in the American Pacific Northwest receive very little nitrogen (N) through atmospheric deposition; therefore, they can provide insights into how the N cycle functioned in other regions before heavy atmospheric deposition of inorganic N began. Our objectives were to determine (a) if the fate of organic N differed from the fate of inorganic N, (b) the effect that polyphenols have on the fate of organic N, and (c) the effect of season of addition on the fate of N inputs. We traced N added to in situ soil cores as ammonium, organic N, tannin-complexed organic N, and the N_2-fixing lichen *Lobaria oregana*. Total ^{15}N recovery was between 74% and 109% for all N additions. Total ^{15}N recovery did not vary significantly from the first sampling date to the last date. The litter/organic horizon, as a bulk pool, was the largest N retention pool for all forms of N addition. Within the litter/organic horizon, the chloroform-extractable microbial biomass initially accounted for nearly all of the added N from the ammonium additions. On a different time scale, microbial biomass also played a noteworthy role in the retention of N from organic N, tannin-complexed organic N, and *Lobaria*. Complexing organic matter with tannin appeared to slow N cycling, but it did not significantly change the ultimate distribution of added organic N. Season of N addition had little effect on the retention of added N; however, where differences did occur, spring additions had lower recoveries than autumn additions.

Key words: ^{15}N; ammonium; forest soil; lichen; microbial biomass; nitrogen; organic nitrogen; polyphenol; tannin.

INTRODUCTION

Nitrogen (N) is usually the nutrient most limiting to terrestrial plant growth in temperate regions (Vitousek and Howarth 1991), including the Pacific Northwest of the United States (Date 1973); therefore, studying N cycling and examining the fate of N inputs in this region could provide insights into how the N cycle functioned before heavy atmospheric deposition of N began. In contrast to many forests in eastern North American (Aber and others 1989; Swank and Vose 1997; Nadelhoffer and others 1999) and central Europe (Emmett and Quarmby 1991; Tietema and others 1998; Johannison and others 1999), where pollution inputs of inorganic N can be in excess of 60 kg N ha^{-1} y^{-1} (Tietema and others 1998), old-growth forests of the Pacific Northwest have inorganic N deposition that is lower than 2 kg N ha^{-1} y^{-1} (Sollins and others 1980). Most new N inputs into these forests are primarily of organic origin, resulting from symbiotic N_2 fixation (Sollins and others 1980).

In early and mid-successional forests of the Pa-

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cific Northwest, symbiotic N_2-fixing trees in the genera *Alnus* and *Ceanothus* are estimated to input between 30 and 300 kg N ha^{-1} y^{-1} (Sollins and others 1980; Binkley and others 1992). However, in mature and late-successional forests, where the presence of *Alnus* and *Ceanothus* is diminished, N inputs from N_2-fixing epiphytic lichens become important. Many different genera of N_2-fixing lichens are found in forests around the globe, including South America (Forman 1975), northern Alaska (Gunther 1989), and Asia. The dominant N_2-fixing epiphytic lichen in most Pacific Northwest old-growth forests is *Lobaria oregana* (Tuck.) Müll. Arg. (hereafter *Lobaria*). *Lobaria* can have a standing biomass of 500 to 1,000 kg ha^{-1} or more in old-growth Douglas fir forests of the Pacific Northwest (McCune 1994; Denison 1979). *Lobaria* can input 2.5 to 4.5 kg N ha^{-1} y^{-1} or more by N_2 fixation (Pike 1978; Denison 1979). This represents approximately 33% to 67% of new N inputs into the ecosystems where *Lobaria* is abundant.

The amount of N input by *Lobaria* exceeds the estimated 1.5 kg N ha^{-1} y^{-1} ecosystem losses of N through leaching (Sollins and others 1980) and denitrification (Vermes and Myrold 1992). The small amount of N that leaches from these forests, as well as from many forests that have minimal anthropogenic N inputs, is more than 90% organic (Sollins and others 1980; Hedin and others 1995; Seely and Lajtha 1997; Stottlemyer 2001; Perakis and Hedin 2002). Combining N inputs from *Lobaria* with precipitation inputs exceeds the 2.8 kg N ha^{-1} y^{-1} requirement for new vegetation growth (Sollins and others 1980). Therefore, the ecosystem losses and plant uptake of N are more than accounted for by the gains from *Lobaria*. This indicates that the ecosystem is retaining at least some N fixed by *Lobaria,* but the specific ecosystem pools that retain *Lobaria* N and the time required for *Lobaria* N to be mineralized are not well known.

Polyphenols (for example, tannin, gallic acid) from fungal, plant, and microbial sources play an important role in soil N dynamics (Schimel and others 1996; Bradley and others 2000; Hättenschwiler and Vitousek 2000; Fierer and others 2001). Polyphenols have generally been shown to have an inhibiting effect on net N mineralization and may affect N retention and alter the distribution of N among different ecosystem pools. Low-molecular-weight phenolics induce N immobilization by acting as a labile carbon source for microbial growth and respiration. In contrast, higher-molecular-weight polyphenols, such as condensed tannins, reduce available N by forming recalcitrant complexes with proteins and other organic N in the soil, which limits N mineralization (Schimel and others 1996; Fierer and others 2001). High polyphenol concentrations have been correlated with slow decomposition of organic material (Benoit and others 1968; Benoit and Starkey 1968; Northup and others 1995) and also with a possible reduction in nitrification (Rice and Pancholy 1973; Baldwin and others 1983; Bradley and others 2000), although their effect on nitrification has not been supported in other studies (for example, Schimel and others 1996). Many theories about the formation of humic substances rely on reactions involving polyphenols or related compounds (Stevenson 1994).

Recovery of added N can be affected by the season of N addition. Large losses of added N have occurred after autumn additions relative to growing-season additions in an agricultural setting (Nyborg and others 1990). Low demand for N in the autumn can contribute to higher leaching losses (Seely and Lajtha 1997) and greater denitrification (Nyborg and others 1990).

The objectives of this study were to determine (a) if the fate of organic N differed from the fate of inorganic N, (b) the effect that polyphenols have on the fate of organic N, and (c) the effect of the season of addition on the fate of N inputs.

We hypothesized that the fate of the initial N solutions would determine the short-term fate of the added N, because the water would carry dissolved N with it as it washed through the forest floor and soil. After the initial hydrologic influence, however, overall retention should be high as abiotic processes and microbial uptake retain the added N. We hypothesized that the rate of absorption into stable pools would be quickest for the ammonium and decrease through organic N, tannin-complexed organic N, and lichen because of their increasing recalcitrance and decreasing initial microbial availability.

We hypothesized that the ammonium added in the spring would reach deeper into the soil than the ammonium added in the autumn because spring soil would be moist from the winter rains and would therefore not absorb as much water as the dryer autumn soil. The *Lobaria* added in the spring was hypothesized not to leach as much N over the short term because the lichen would decay more slowly in the summer months following the addition, compared to the lichen in the autumn, which had wet winter months following its addition.

Methods

Site Description

We chose a mid-elevation (550 m), old-growth site in the H. J. Andrews Experimental Forest Long

Table 1. Soil and Organic Matter Characteristics of the Site[a]

Ecosystem Pool	Total N (%) (SE)	Total C (%)[b] (SE)	Dry Mass (g/m^2) (SE)	Bulk Density (g/cm^3) (SE)	Stone Content (%) (SE)
Forest floor plants	1.34 (0.12)	47.34 (0.38)	4.8 (1.0)	NA	NA
Moss	0.911 (0.018)	44.63 (0.73)	91.1 (4.1)	NA	NA
Litter/organic horizon	0.915 (0.016)	36.53 (0.58)	5406 (278)	0.129 (0.004)	NA
0–5 cm soil	0.205 (0.003)	5.67 (0.34)	30105 (534)	0.765 (0.012)	20.74 (0.43)
5–15 cm soil	0.148 (0.003)	2.78 (0.34)	71870 (1002)	0.843 (0.013)	17.74 (0.80)

N, nitrogen; C, carbon; NA, not applicable All mineral soil values are on the less than 4.75 mm fraction, except bulk density and stone content, which are on a whole-soil basis. There were no significant differences among treatments for any of these measures ($P > 0.05$).
Unless otherwise indicated, $n = 96$
[a]Pooled over all collection dates and all treatments.
[b] $n = 6$ for total carbon measurements

Term Ecological Research (LTER) site near Blue River, Oregon (44°13' 53N, 122°13'40W). The site is classified as *Tsuga heterophylla/Rhododendron macrophyllum/Berberis nervosa* habitat in the Western Cascade Province of the Oregon Cascade Range (Franklin and Dyrness 1988). It is dominated by large *Pseudotsuga menziesii* (Mirb.) Franco (Douglas fir), with mid-size *Tsuga heterophylla* (Raf.) Sarg. (western hemlock) and *Thuja plicata* Donn ex D. Don (western red cedar) in the overstory. Many overstory trees have a large amount of epiphytic mosses and lichens growing on them, especially the N_2-fixing lichen *Lobaria oregana* (Tuck.) Müll. Arg. The forest floor is covered with a layer of mosses, including *Eurhynchium oreganum* (Sull.) Jaeg. (Oregon beaked moss) and *Hylocomium splendens* (Hedw.) B.S.G. (step moss).

The climate at the site is Mediterranean, with mild wet winters and warm dry summers. Mean annual temperature (1980–2000) is 9.2°C. Average annual precipitation (1980–2000) is 224 cm (SE = 10). In the 2nd year of the study (2000–2001), rainfall was considerably lower than average (133 cm), but this did not appear to affect the outcome of the experiment. Soils at this site have been classified recently as coarse loamy mixed mesic Typic Hapludands. Soil characteristics across the site did not differ significantly (Table 1).

^{15}N-labeled Materials

Ammonium. A 21.3 μg N ml^{-1} solution of ^{15}N-labeled ammonium chloride ($^{15}NH_4Cl$, 98 Atom% ^{15}N, Aldrich Chemical, St. Louis, MO) was the source of inorganic N in this study. We chose ammonium over nitrate because our preliminary tests showed that the concentrations of ammonium in soil were 10 times higher than nitrate at our site.

Organic Nitrogen. *Saccharomyces cerevisiae* Meyen ex E. C. Hansen (bread yeast) was grown on a ^{15}N-enriched defined media containing 4% glucose, 0.5% ammonium sulfate (10 Atom% ^{15}N), and commonly used macronutrients, micronutrients, and vitamins at a pH of 4.5 (Fiechter and others 1987). Two batches of sterilized broth were inoculated with *S. cerevisiae* obtained from compressed Fleischmann's baker's yeast and incubated at 22°C with continuous stirring for 48 h.

After the incubation period, the broth was allowed to settle and vacuum-filtered. The yeast batches were rinsed with deionized water and dried at 60°C. One batch yielded yeast that was 8.8% N and 9.9 Atom% ^{15}N, whereas the other batch yielded 8.9% N and 10.8 Atom% ^{15}N. Portions of dried yeast (0.4 g) were added to 50 ml deionized water and autoclaved for 20 min to lyse cells and produce nonliving organic N. The yeast solution was then poured into field-ready bottles, and water was added to make a total of 150 ml.

The N concentration of the yeast converts to 55% crude protein (%N × 6.25). This crude protein value is typical of bread yeast (Reed and Nagodawithana 1991). Previous research has further classified *S. cerevisiae* into 47% actual protein, 8% nucleic acids, and 1% cell wall chitin (Reed and Nagodawithana 1991). Proteins are of particular relevance to organic N cycling and decomposition in forest soils because 80% of the organic N in a forest litter layer has been found to consist of amide-peptide N, the same bond found in proteins (Clinton and others 1995).

Tannin-complexed Organic Nitrogen. After autoclaving, half of the yeast bottles had tannic acid (Sigma) added to them to create tannin-complexed organic N. Tannic acid is a high-molecular-weight (MW = 1701.22) polyphenol that is known to complex many forms of organic N, especially proteins (Siebert and others 1996). To favor the complexing

reaction, tannic acid was added to the vials at a ratio of 100 g tannic acid to 1 g yeast N.

Lobaria oregana. The N_2-fixing epiphytic lichen *Lobaria oregana* was labeled with ^{15}N by spraying it with a nutrient solution containing ^{15}N-ammonium chloride. A more detailed description of the procedure can be found in Holub and Lajtha (2003). The entire process was repeated twice to obtain fresh tissue for each season of addition, once in September 1999 for an autumn addition and once in March 2000 for a spring addition. The *Lobaria* from the autumn-labeling experiment contained an average of 16.5 Atom% ^{15}N (SE = 1.2, $n = 10$), whereas the spring addition contained an average of 11.3 Atom% ^{15}N (SE = 0.6, $n = 5$). The autumn-labeled *Lobaria* tissue averaged 2.75% N (SE = 0.09, $n = 10$), whereas the spring-labeled tissue was 2.28% N (SE = 0.08, $n = 5$).

Plot Installation

Large polyvinylchloride (PVC) tubes (15.25 cm diameter × 40 cm length) were driven into the ground to a depth of 35 cm 2 months prior to the spring and autumn ^{15}N additions to act as in situ containers of forest floor and soil. These tubes eliminated lateral movement of added ^{15}N and loss of *Lobaria* so that a mass balance of ^{15}N could be determined within each tube. Similar tubes have been used in other ^{15}N tracer studies (Schimel and Firestone 1989; Preston and Mead 1995; Johannison and others 1999).

Applying ^{15}N-labeled Material

Autumn Addition. On 27 October 1999, we added ^{15}N-ammonium and ^{15}N-labeled *Lobaria* to separate PVC tubes in each of the three autumn-installed blocks, for a total of 21 ammonium tubes and 15 *Lobaria* tubes. Ammonium solution was added to each tube to equal 1 cm additional rainfall and 2 kg N ha^{-1} (3.83 mg ^{15}N per tube), which equals the annual amount of atmospheric N deposition at the site (Sollins and others 1980) and is approximately one-third of the exchangeable ammonium in the litter layer and top 5 cm of mineral soil. Labeled *Lobaria* was added in 0.8-g portions to each tube, which approximates the 5 kg N ha^{-1} added annually by the lichen under ambient conditions. To maintain consistent moisture with the ammonium addition, 1.0 cm (180 ml) deionized water was added over the lichen pieces. One PVC tube in each block was used for background ^{15}N measurements and received only 1.0 cm (180 ml) deionized water to maintain consistent moisture with the other treatments.

Spring Addition. On 27 April 2000, we prepared ^{15}N-labeled *Lobaria,* ^{15}N-ammonium, and background correction tubes as in the autumn addition. We also added ^{15}N-labeled organic N and tannin-complexed ^{15}N-labeled organic N to separate tubes. Both complexed and noncomplexed organic N were added in a 150-ml suspension at a rate of 35 mg N per tube, or approximately 20 kg N ha^{-1}. An additional 30 ml deionized water was used to rinse the original container to ensure complete ^{15}N addition and total the 1.0 cm of water (180 ml) that the other treatments received.

Sample Collection

At each collection date, three tubes were harvested from each treatment. Autumn-addition ammonium tubes were collected at 40 min, 3 days, 14 days, 3 months, 6 months, 1 year, and 2 years after the addition of the label. Spring-addition ammonium tubes were collected after the same time postaddition excluding a 2-year collection. The 40-min collection tubes were labeled under laboratory conditions so that samples could be processed quickly. *Lobaria*-addition tubes in the spring and autumn were collected at the same times as the ammonium addition, excluding 40 min and 3 days, which were omitted because the lichen was not expected to show any considerable decomposition during that time. Organic N and tannin-complexed organic N, only added during the spring addition, were collected after 14 days and 1 year. Background correction tubes were collected on day 0. All collected tubes were enclosed in sealed plastic bags to prevent desiccation, placed on ice as needed to keep cool, and brought back for immediate separation at Oregon State University in Corvallis, Oregon.

Isolation of Ecosystem Pools

Bulk Separation. Materials from each tube were separated into moss, aboveground plant biomass, a combined litter/organic horizon, and mineral soil at depths of 0–5 cm and 5–15 cm. Mineral soils were coarsely sieved to 4 mesh (4.75 mm) prior to further separation and analysis. Remaining *Lobaria* was also collected from the *Lobaria* treatment. Separation was always complete within 24 h and usually complete within 8 h of field collection. During the short time before separation, the tubes were stored at 4°C.

A field moist portion of the litter/organic horizon and the 0–5 cm mineral soil were extracted and fractionated using the chemical and physical methods described below. The field moist and dry weights (60°C) of all pools were determined. The 5–15 cm mineral soil was not fractionated because

results from previous studies indicate that only small amounts of ^{15}N reach that depth (Preston and Mead 1995; Swanston and Myrold 1997).

Extractable Nitrogen. Available ammonium, nitrate, and total dissolved N (TDN) were extracted from the litter/organic horizon and the 0–5 cm mineral soil using 0.5 molar potassium sulfate immediately after separation. Ammonium concentrations were determined using a modified Berthelot reaction on an Orion Scientific (Gallarate, Italy) AC 100 colorimetric autoanalyzer. Nitrate concentrations were determined using a copper/cadmium reduction column with sulfanilamide and N-1-naphthyl-ethylenediamine on an Orion Scientific AC 100 colorimetric autoanalyzer.

Total dissolved N was determined by using an alkaline persulfate digestion (Cabrera and Beare 1993). Using caffeine and ammonium as standards, we found digestion efficiencies to be between 95% and 100%. Dissolved organic N (DON) concentration was determined by subtracting ammonium and nitrate from total dissolved N.

Microbial Biomass. As a measure of microbial biomass, a chloroform fumigation-extraction was performed on field moist litter/organic horizon and the 0–5 cm mineral soil immediately after separation using the method described in Brookes and others (1985), as modified by Martikainen and Palojarvi (1990). The extracts were digested and analyzed for TDN. Net chloroform labile N was calculated by subtracting TDN in the initial potassium sulfate extraction from the total N in the chloroform fumigation-extraction; it represents the most labile portion of the microbial biomass.

No correction factor (k_{EN}) was used in determining the ^{15}N recovery from microbial biomass, because the chloroform labile component of the microbial biomass probably had a different ^{15}N label from the nonchloroform labile components of the microbial biomass. This was especially true during the earliest sampling dates, when much of the ^{15}N taken up by microbes probably had not yet been assimilated into the more recalcitrant components. If a k_{EN} had been used at these early dates, ^{15}N recovery from microbial biomass would be artificially inflated. At later sampling dates, however, the ^{15}N recovery from the chloroform fumigation-extraction certainly underestimates the total ^{15}N in microbial biomass because it excludes the ^{15}N assimilated into the nonextractable components. Because we could not accurately predict the ratio of ^{15}N in the chloroform labile portion to the nonlabile portion of the microbial biomass, we chose to report uncorrected values of ^{15}N recovery across all sampling dates. Typical values for k_{EN} range from 0.18 (Bremer and van Kessel 1990) to 0.38 ± 0.14 (Sparling and Zhu 1993). These correction factors would make recovery of ^{15}N in microbial biomass two to five times higher than the uncorrected chloroform fumigation-extraction values.

Density Fractions. A density fractionation (Strickland and Sollins 1987) was performed on the 0–5 cm mineral soil using a 1.7 g cm^{-3} solution of sodium polytungstate (SPT). The light fraction is classified as a moderate turnover pool of N consisting largely of unmodified or partially modified plant material. The heavy fraction is a slow turnover N pool that consists of N compounds that have become bound to mineral soil particles and are more recalcitrant (Strickland and Sollins 1987). To reduce the consumption of relatively expensive SPT, we made slight modifications to scale down the procedure.

Nitrogen Isotope and Carbon Analyses

All ^{15}N analyses were performed at the University of California–Davis Stable Isotope Facility, Davis, California, using a Europa Scientific Integra continuous flow mass spectrometer equipped with Dumas combustion/reduction to simultaneously determine total N. Solid pools, including density fractions, were dried at 60°C, ground to 40 mesh, and submitted for Atom% ^{15}N and total N analyses. A subset of the solid pools, including density fractions, were simultaneously analyzed for total carbon.

$$\text{Atom\%}\,^{15}\text{N} = \text{atoms of } ^{15}\text{N}/(\text{total atoms of N}(^{14}\text{N} + ^{15}\text{N})) \quad (1)$$

Extractable ammonium and nitrate were prepared for ^{15}N analysis using ammonia diffusion to Teflon-enclosed acid traps, as described by Stark and Hart (1996). Total dissolved N and chloroform labile N were also prepared by this method; but due to low ionic strength of the digestion solution, potassium chloride was added to raise the ionic strength to prevent the acid traps from becoming flooded with water. Diffusion of all of the dissolved N forms was carried out for 7 days. After drying over desiccant and concentrated sulfuric acid, the acid traps were wrapped in tin cups and submitted for ^{15}N and total N analysis.

The Atom% ^{15}N of the dissolved organic N pool and the net chloroform labile pool were determined by solving for weighted average of their constituents for their Atom% ^{15}N value using the following equations:

Table 2. Percent Total ^{15}N Recovery over All Times since N Addition

Season	Ammonium (%) (SE)	Organic N (%) (SE)	Tannin/Organic N (%) (SE)	*Lobaria* (%) (SE)
Autumn	94.7a x(3.2)	—	—	95.0ax(6.0)
Spring	80.3ay[a](2.5)	74.5a[a](5.7)	90.1ab(6.3)	108.7bx(5.5)

Within a single season, across-treatment comparisons indicated by a, b; within a single treatment, across-season comparisons indicated by x, y. Different letters indicate a significant difference using ANOVA, followed by Tukey's HSD ($P < 0.05$)
[a]*Significant differences from 100%*

$$A\%_{DON} = (A\%_{DN} \times C_{TDN} - A\%_{NO_3} \times C_{NO_3} - A\%_{NH_4} \times C_{NH_4}) / C_{DON} \quad (2)$$

$$A\%_{NCN} = (A\%_{TCN} \times C_{TCN} - A\%_{TDN} \times C_{TDN}) / C_{NCN} \quad (3)$$

where, DON is dissolved organic N, TDN is total dissolved N (unfumigated), NCN is net chloroform labile N (representing microbial biomass), and TCN is total N extracted after chloroform fumigation. C_y is the concentration of y in dry soil or litter, $A\%_y$ is the Atom% ^{15}N of y, where y can be DON, TDN, NCN, NH_4^+, or NO_3^-.

Using the total dry mass, %N, and the Atom% ^{15}N of a pool, the total amount of ^{15}N in each pool was calculated. By subtracting the amount of ^{15}N found in unlabeled pools, the net amount of ^{15}N added to each pool as a result of the tracer was determined.

Polyphenol Concentration

We measured potassium sulfate (0.5 M) soluble polyphenols on a subset of the extracted samples using the Folin-Denis method (Allen and others 1974), with catechol as a standard. Polyphenols were measured to ensure that our tannin addition actually increased the polyphenol concentrations of our samples. Only samples collected from the spring addition on days 14 and 365 were analyzed because these were the dates when the tannin-complexed organic N addition tubes were collected.

Bulk Density and Stone Content

We determined unsieved bulk density for the litter/organic horizon, 0–5 cm soil, and 5–15 cm soil. We calculated the percent stone content for the mineral soil horizons using the greater than 4.75 cm fraction.

Statistics and Calculations

We calculated the percent recovery of added ^{15}N in each pool. These percentages were averaged by N addition type and by days since addition. Differences among ecosystem pools were examined using analysis of variance (ANOVA) followed by Tukey's HSD to analyze for differences between pools by N addition type and season of addition:

Results

Total ^{15}N Recovery

There were differences in total ^{15}N recovery among N addition types for the spring addition and between seasons for the ammonium addition (Table 2). Total ^{15}N recovery data were combined over all sampling dates, because they were not different from the first collection through the last collection within each N addition type ($P > 0.05$).

Distribution of ^{15}N in Whole Pools

With the exception of the *Lobaria* additions, the ^{15}N recovery from each individual whole-ecosystem pool did not differ significantly through time. The distribution of ^{15}N in whole pools from autumn and spring ammonium, spring organic N, and spring tannin-complexed organic N additions were similar (Figure 1a). The litter/organic horizon retained the largest amount of added ^{15}N when averaged over all times since addition. The 5–15 cm mineral soil was usually the second largest pool of ^{15}N recovered. The amount of ^{15}N recovery from the 0–5 cm mineral soil was less than that from the litter/organic horizon and usually greater than or similar to moss. Understory plants were the smallest whole pool that was measured and retained only a very small amount (0.3% to less than 0.001%) of the added ^{15}N.

The distribution of ^{15}N in the autumn and spring *Lobaria* additions changed considerably through time (Figure 1b and c). Both seasons of *Lobaria*

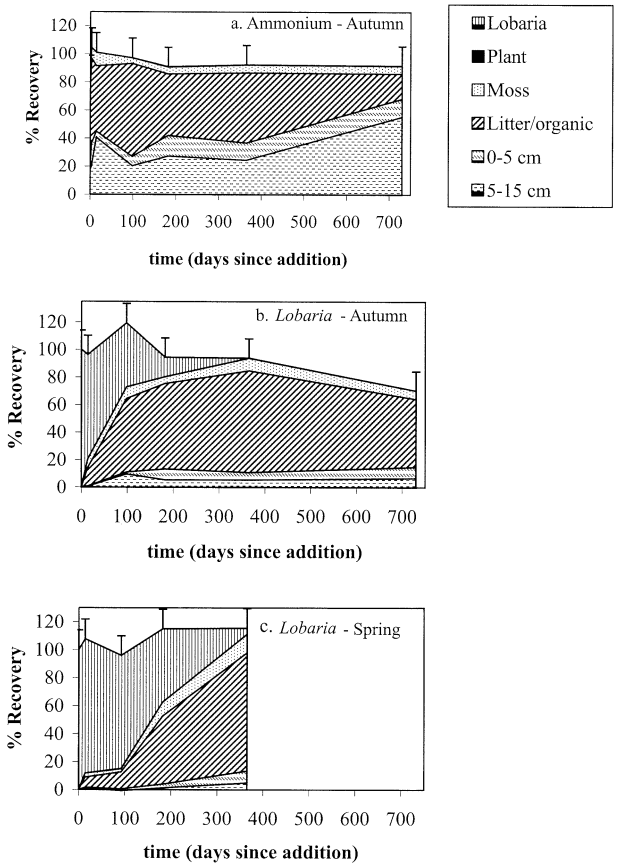

Figure 1. Average distribution of ^{15}N in whole material collected from in situ soil cores. **a** Ammonium in autumn (representative of ammonium in spring, organic nitrogen in spring, and tannin-complexed organic nitrogen in spring). **b** *Lobaria* in autumn. **c** *Lobaria* in spring. At each sampling time, $n = 3$. ^{15}N recovery in plant biomass was measured but the amount was too small (less than 0.3%) to be reflected in the figures.

addition followed a similar sequence of ^{15}N movement out of the labeled lichen, but there was more of a delay in the spring-addition *Lobaria*. The ^{15}N from *Lobaria* moved into the litter/organic horizon primarily, but also into moss and deeper mineral soils. Understory plants were again the smallest whole pool measured and retained only a very small amount (less than 0.2%) of the added ^{15}N.

Extractable Nitrogen Fractions

The average concentrations of potassium sulfate extractable ammonium, nitrate, dissolved organic N, and microbial biomass N did not differ by treatment or time since addition (Table 3). Unlike the whole pools, ^{15}N recovery from many of the extractable N components of the litter/organic horizon and 0–5 cm mineral soil did vary through time. Microbial biomass often contained the largest amount of added ^{15}N among the extractable pools (Figures 2 and 3), whereas recovery of ^{15}N as dissolved organic N and ammonium were much lower. ^{15}N recovery from the nitrate pool was rarely above detection limits.

The ^{15}N recovery in litter/organic horizon extracts from the autumn and spring ammonium additions was dominated by microbial biomass (Figure 2a and b). Immediately after the ^{15}N addition, the microbial biomass pool contained most of the added ^{15}N that was recovered in the litter/organic horizon bulk material. The recovery of ^{15}N in microbial biomass dropped considerably from that high level at later sampling dates, but it continued to be the largest ^{15}N sink among the extractable pools. ^{15}N recovery as ammonium was somewhat high at the earliest collection but also fell to a low level in subsequent samplings.

On the first collection date for the autumn and spring *Lobaria* additions, very little ^{15}N was recovered in any litter/organic matter extractable pool (Figure 3c and d). The added lichen had not yet shown much decay at this early sampling date. By day 98, however, microbial biomass began to dominate the ^{15}N recovery from the extractable pools. ^{15}N recovery as microbial biomass increased further at 6 months; thereafter, it began to decrease.

The ^{15}N recovery from the 0–5 cm soil extracts from the autumn and spring ammonium addition were dominated by extractable ammonium initially, but at later sampling dates microbial biomass was the largest ^{15}N sink (Figure 3a and b). After initial peaks in ^{15}N recovery in the microbial biomass, dissolved organic N, and ammonium, all fell to a plateau that lasted through the 2-year collection for ammonium and dissolved organic N and through the 1-year collection for microbial biomass.

The ^{15}N recovery from all 0–5 cm mineral soil extractable pools in the autumn and spring *Lobaria* additions (Figure 4c and d) was always much lower than the litter/organic horizon extractable pools (Figure 3c and d). On the first sampling date, little if any ^{15}N was recovered from the extractable pools in the 0–5 cm soil. On subsequent collection dates, ^{15}N recovery as microbial biomass dominated the total recovery in extractable pools for the autumn addition but not the spring addition. ^{15}N recoveries from later sampling dates for the spring *Lobaria* addition were variable. Ammonium and dissolved organic N were often higher than microbial biomass.

The ^{15}N recovery in litter/organic horizon and 0–5 cm soil extracts from the spring organic N and tannin-complexed organic N generally did not vary

Table 3. Average Extractable Nitrogen (N) Concentrations[a]

Ecosystem Pool	Microbial Biomass N[b] (mg N/kg) (SE)	Dissolved Organic N[c] (mg N/kg) (SE)	Ammonium[c] (mg N/kg) (SE)	Nitrate[c] (mg N/kg) (SE)
Litter/organic horizon	488.9(34.6)	177.2(7.5)	49.7(2.5)	4.73(1.75)
0–5 cm soil	110.9(12.4)	32.0(1.5)	11.7(0.7)	1.80(0.61)

[a]*Pooled over all collection dates and all treatments*
[b]*Chloroform fumigation extraction minus control on fresh field moist material, n = 96*
[c]*Field moist material extracted with 0.5 molar potassium sulfate, n = 96*

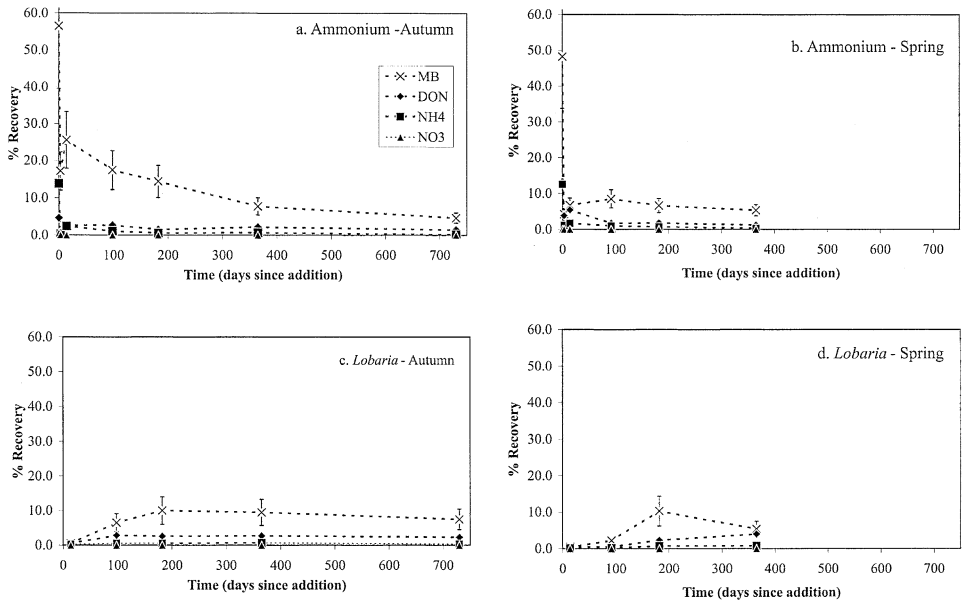

Figure 2. Average recovery of added ^{15}N in potassium sulfate extracts from litter/organic horizons. **a** Ammonium in autumn. **b** Ammonium in spring. **c** *Lobaria* in autumn. **d** *Lobaria* in spring. MB, chloroform-labile microbial biomass N; DON, dissolved organic N; NH4, ammonium; NO3, nitrate. At each sampling time, $n = 3$.

greatly between day 14 and 1 year (Table 4). Where there were apparent, although not statistically significant, differences between organic N and tannin-complexed organic N, ^{15}N recoveries in the tannin-complexed organic N addition extracts usually tended to be lower.

Density Fractions

^{15}N recovery in the light and heavy fractions of 0–5 cm mineral soil varied significantly among sampling dates (Figure 4). ^{15}N recovery in the heavy fraction was generally much higher than ^{15}N recovery in the light fraction for all addition types. The heavy fraction had about 20 times more mass than the light fraction (Table 5). ^{15}N recovery in light and heavy fractions for both seasons of ammonium addition showed similar patterns. ^{15}N recovery gradually increased for most N addition types as time progressed. There were no significant differences in recovery of light or heavy fractions between organic N and tannin-complexed organic N.

Polyphenols

Polyphenol concentrations in the litter/organic horizon were initially elevated as a result of the tannin addition (850 μg catechol equivalents g^{-1}, SE = 220), but they fell to background levels (425 μg catechol equivalents g^{-1}, SE = 22) at the later sampling date. Polyphenol concentrations in the 0–5 cm soil from the tannin-complexed organic N addition were never significantly different from the background levels (120 μg catechol equivalents g^{-1}, SE = 14).

Discussion

Total ^{15}N Recovery

Because there were no changes in total ^{15}N recovery through time, and the total ^{15}N recovery for many N addition types was very near 100%, we inferred that the N added inside tubes, regardless of form, was strongly retained in the whole pools that were measured. Even without active tree roots and

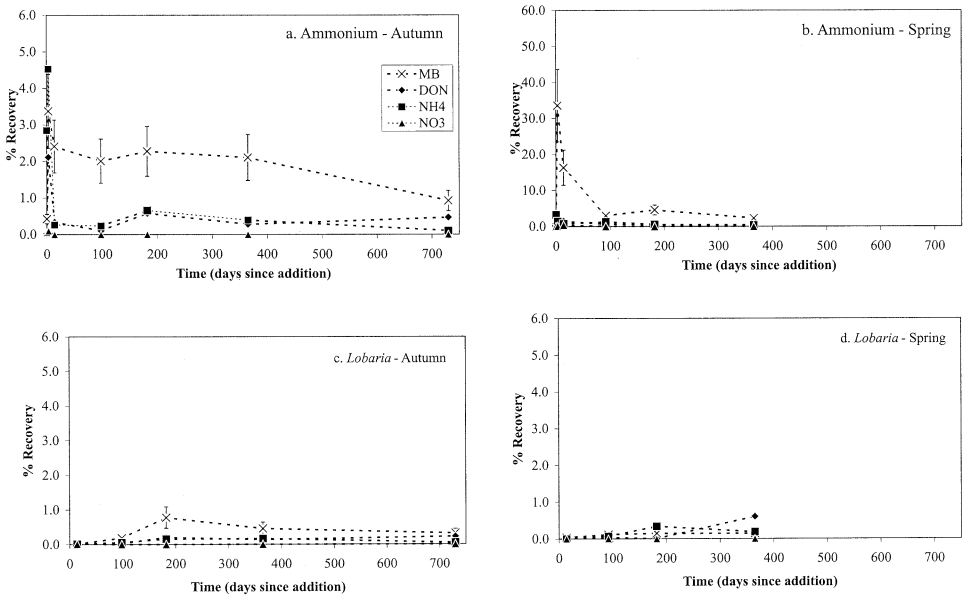

Figure 3. Average recovery of added ^{15}N in potassium sulfate extracts from 0–5 cm mineral soil. **a** Ammonium in autumn. **b** Ammonium in spring (×10 scale increase on y axis). **c** *Lobaria* in autumn. **d** *Lobaria* in spring. MB, chloroform-labile microbial biomass N; DON, dissolved organic N; NH4, ammonium; NO3, nitrate. At each sampling time, $n = 3$.

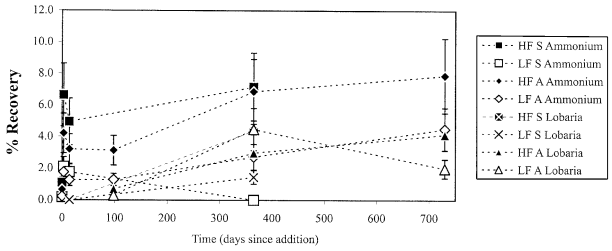

Figure 4. Average recovery of ^{15}N in density fractions of 0–5 cm mineral soils that have had ^{15}N-ammonium or ^{15}N-labeled *Lobaria oregana* additions to in situ soil cores in autumn (A) or spring (S). HF (*filled symbols*), heavy fraction, the fraction that sinks in a 1.7 g cm^{-3} solution of sodium polytungstate; LF (*open symbols*), light fraction, the fraction that floats in a 1.7 g cm^{-3} solution of sodium polytungstate. At each sampling time, $n = 3$.

their mycorrhizal fungal associates, the litter/organic horizon, 0–15 cm mineral soil, moss, and understory plants were able to retain essentially all of the added N at our site for all of the N sources we added during both seasons of addition. For those N additions that had slightly less than 100% ^{15}N recovery, it is possible that during the initial addition of these N forms some of the added ^{15}N reached to lower soil depths that were not measured.

In other ^{15}N tracer studies where tree roots were not excluded, plants accounted for only a small percentage of ^{15}N recovery (Vitousek and Matson 1984; Zak and others 1990; Groffman and others 1993; Nadelhoffer and others 1999; Perakis and Hedin 2001). Compared to microbes, plants are often considered poor competitors for newly added N (Kaye and Hart 1997). Especially because of the high recovery without active plant roots and mycorrhizae, the evidence from our study suggests that plants would have had difficulty competing for added N at this site as well and probably would have had little impact on the distribution of N or total N recovery in the short term.

Neither ammonium, organic N, tannin-complexed organic N, nor *Lobaria* showed any significant change in the total ^{15}N recovery of whole pools among sampling dates. As the initial solution of N was added to the forest floor, it appears that the N quickly (within 40 min for the ammonium additions) reached a stable destination and remained there. This implies that there was some mechanism by which litter and soils at our site retained added N, regardless of source, and limited further leaching. When N was added to the forest floor more slowly, as in the *Lobaria* additions, N retention was not different from 100%. As N left the *Lobaria*, it was found primarily in the litter/organic horizon, with much less ^{15}N recovery in deeper soils.

Past studies have shown that denitrification (Vermes and Myrold 1992) and N leaching (Sollins and others 1980) are small in forests of our region. The high and stable recovery of added ^{15}N provides further evidence that denitrification and leaching are probably not large pathways of N loss in this ecosystem. Indeed, our data showed that net ^{15}N loss from the first samples to the last samples was negligible over the course of our study.

The litter/organic horizon was the largest ^{15}N sink for all forms of added N. Its relatively large mass and proximity to the surface where the N was

Table 4. ^{15}N Recovery from Uncomplexed and Tannin-complexed Organic Nitrogen (N) Fractions[a]

	Days since ^{15}N Addition	Microbial Biomass[b] (SE)	Dissolved Organic N (SE)	Ammonium (SE)	Nitrate (SE)
Litter/organic horizon					
Organic N	14	1.60(1.43)	1.34(0.51)	0.22 (0.02)	0.0157 (0.0112)
	365	1.54(0.79)	1.36(0.17)	0.42 (0.25)	0 (0)
Tannin-complexed organic N	14	1.60(1.54)	0.38(0.02)	0.04 (0.01)	0.0001 (0.0000)
	365	4.79(2.13)	1.95(0.87)	0.38 (0.21)	0 (0)
0–5 cm mineral soil					
Organic N	14	1.31(0.99)	0.39(0.11)	0.13 (0.08)	0.0004 (0.0002)
	365	2.30(0.29)	0.85(0.10)	0.49 (0.25)	0 (0)
Tannin-complexed organic N	14	0.42(0.11)	0.07(0.01)	0.013(0.003)	0.0100 (0.0099)
	365	0.55(0.09)	0.18(0.03)	0.25 (0.11)	0 (0)

[a] *As a percent of ^{15}N added*
[b] *Chloroform fumigation-extraction without a k_{EN} correction factor*

Table 5. Average 0–5 cm Soil Density Fraction Characteristics[a]

Density Fraction	Total N[b] (%) (SE)	Total C[c] (%) (SE)	Dry Mass[b] (g/m^2) (SE)
Light fraction	0.646(0.014)	33.51(0.78)	1,712 (71)
Heavy fraction	0.162(0.003)	3.24(0.08)	27,653(577)

All values are on the less than 4.75 mm fraction. Light and heavy fractions were separated using a 1.7 g cm^{-3} sodium polytungstate solution. Whole 0–5 cm soil values are given in Table 1.
[a] *Pooled over all collection dates and all N addition types*
[b] *n = 72*
[c] *n = 45*

added probably contributed to this result. Relative to their mass, mosses were a strong sink for added N. Because mosses are on the surface, they have the first opportunity to absorb N inputs and may play an important role in regulating N cycling. The mineral soil to 15 cm appeared to sequester most of the N that was not acquired by the litter/organic horizon. With mineral soil extending meters beneath the surface, it seems unlikely that much of the added N will be lost from the system over several decades.

Microbial Biomass ^{15}N Recovery

The main mechanism of initial N retention in the litter/organic horizon was microbial assimilation. At the 40-min collection for the spring and autumn ammonium addition, the ^{15}N recovery in the whole unfractionated litter/organic horizon was approximately equal to the ^{15}N recovered as microbial biomass N (that is, uncorrected net chloroform labile N) extracted from the same litter/organic horizon. This indicates that most of the ^{15}N-ammonium that reached the litter/organic horizon was assimilated into microbial biomass within 40 min of N addition.

In the 0–5 cm soil from the autumn and spring ammonium addition, the ^{15}N recovery in microbial biomass N did not peak at the 40-min collection like the litter/organic horizon microbial biomass did. Microbial biomass ^{15}N recovery in the 0–5 cm soil was higher at day 3 than at 40 min, but the peak could have been sometime between collections.

In contrast to the ammonium additions, ^{15}N-labeled *Lobaria* additions in the spring and autumn both had gradual increases in the ^{15}N recovery as microbial biomass. This delay is almost certainly related to the delay of N release from the *Lobaria* as it decomposed. Microbial biomass still dominated the ^{15}N recovery from the extractable pools of N, even when N was made available more slowly.

These results add to the growing body of evidence that indicates that the microbial biomass pool retains a large portion of added mineral N and acts as a rapid initial N sink (for example, see Zak and others 1990; Emmett and Quarmby 1991; Seely and Lajtha 1997; Perakis and Hedin 2001). The rapid microbial immobilization of added N that we observed in the litter/organic horizon and mineral soil provides evidence that the microbes have the capacity to quickly utilize N as soon as it becomes available. The tendency to rapidly take up added N suggests that the microbes may be limited by N. However, the concentration of total (^{15}N plus ^{14}N) microbial biomass N did not increase over time. If N limited the microbial growth, the pool of microbial biomass should get larger as the limitation is tem-

porarily eased, but phenomenon this was not observed.

Dissolved Organic Nitrogen, Ammonium, and Nitrate

Recoveries of ^{15}N as dissolved organic N, ammonium, and nitrate were always less than ^{15}N recovery as microbial biomass from the litter/organic horizon and almost always less than microbial biomass in the 0–5 cm mineral soil. After a period of stabilization, the amount of ^{15}N recovery in dissolved organic N, ammonium, and nitrate from the litter/organic horizon and 0–5 cm mineral soil was also quite constant, with few exceptions. The absence of a significant decrease in the ^{15}N recovery in these pools was unexpected. We hypothesized that the extractable pools should decrease over time as they were diluted by N from unlabeled sources. There are two possible reasons that ^{15}N recovery in these pools remained so constant: (a) small inputs and withdrawals from the pools or (b) replenishment of the ^{15}N by N from labeled sources.

The dissolved organic N, ammonium, and nitrate pools may not have been subject to large inputs of N from outside sources or uptake of N by sinks. This seems unlikely because past work has shown that despite small pool size, turnover rates can be quite fast (Stark and Hart 1997).

Added ^{15}N could have been slowly released from stable pools or cycled and recycled to these extractable pools. A constant rate of ^{15}N release would have had to have been maintained for 2 years in some cases, but this is improbable, because ^{15}N stores would become depleted after a short time of continued loss. Constant recycling, probably mediated by microbial biomass, is the most likely scenario to explain the relatively constant ^{15}N recovery in the dissolved organic N, ammonium, and nitrate pools.

Density Fractions

The heavy fraction, which has been defined as a less active, more recalcitrant, and older pool of carbon, was a stronger initial sink than the light fraction for added ammonium, organic N, and tannin-complexed organic N. As time progressed, however, the light fraction tended to become a stronger sink for ^{15}N. Ionic exchange or other chemical binding of N could occur initially in the heavy fraction of soil, followed by transport to the light fraction by fungi or other microbes to be used to decompose the light fraction.

Effects of Tannin

Tannin complexation of organic N appeared to delay normal N cycling processes, but not prevent them; however, this trend was not statistically significant. Although many of the effects of tannin were not significant, total ^{15}N recovery from the tannin-complexed organic N additions was somewhat higher than uncomplexed organic N. Tannin complexation tended to have higher ^{15}N recovery as microbial biomass and dissolved organic N at day 365 in the litter/organic horizon than uncomplexed organic N, and tended to decrease recovery as microbial biomass in the 0–5 cm soil. More of the tannin-complexed organic ^{15}N was recovered in the heavy fraction than in the light fraction compared to the uncomplexed organic ^{15}N, but not significantly more. Tannin complexation of organic matter appeared to reduce the immediate availability of N in the short term, but it caused a slow release of N, which resulted in a more sustained N recovery in active pools. However, the general absence of statistical significance warrants further study.

Adding tannin-complexed organic N increased the extractable polyphenols from the litter/organic horizon at day 14 but not at day 365. This indicates that the added tannic acid was degraded over time. After 1 year, and probably much earlier, the tannic acid had been removed from the litter/organic horizon, and polyphenol concentrations in tannin additions were not different from the samples without added polyphenols. The 0–5 cm mineral soil did not show an increase in polyphenols at either day 14 or day 365. Perhaps much of the added tannic acid stayed in the litter/organic horizon and did not leach to the soil initially.

Conclusions

The results of this study suggest that added organic and inorganic N were highly retained in the forest floor and soil at this low-N-deposition, Andic soil site in western Oregon even in the absence of tree roots. The form of N had little effect on the overall N retention over the time period of this study. The fate of added N into specific ecosystem pools was similar among the inorganic and simpler organic N, but the complex N from *Lobaria* was found more toward the surface. Microbial biomass appeared to play a large role in the short-term retention of N as it became available, whereas bulk soil and litter were responsible for longer-term retention.

Polyphenols in the form of tannic acid had little significant effect on the fate of added N, although there were trends to suggest that complexation by

tannins may have slowed the cycling of N. The season of addition had no effect on the recovery of added N from the first sampling date to the last, but overall recovery of added N was lower in the spring, probably due to increased initial leaching of N to soil depths not measured. More research is needed to determine if these findings are applicable to other soils at other sites. In the meantime, caution should be used in extrapolating the data to forests outside this region or to forests within this region on different soil types.

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