Forests in the Pacific Northwest receive very little nitrogen through atmospheric deposition and thus studying the nitrogen cycle in this region can provide insights into how the unpolluted nitrogen cycle functions. I examined the fate of organic nitrogen versus inorganic nitrogen and the effect of tannins on N retention by tracing N from $^{15}$N-labeled ammonium, organic nitrogen, tannin-complexed organic nitrogen, and the $N_2$-fixing lichen *Lobaria oregana* to *in situ* soil cores. The litter/organic horizon was the largest nitrogen retention pool for all forms of nitrogen added. Within the litter/organic horizon, the microbial biomass initially accounted for most of the added nitrogen from the ammonium additions. On a different time scale, microbial biomass also played a significant role in the retention of nitrogen from other N forms.

I also studied mass loss and nitrogen dynamics during the decomposition of *Lobaria oregana* using $^{15}$N. Lichens placed in the field during the spring had a smaller decay constant ($k=1.24 \text{ yr}^{-1}$) than the lichens placed in the field during the fall ($k=3.1 \text{ yr}^{-1}$). The spring rate is similar to some labile leaf litters, but the fall rate is among the fastest decomposition rates measured for complex organic matter.
Lichen from both seasons took up N from the surrounding environment during decay, while simultaneously losing N.

In an additional project I ran several simple models to explore the effects that added N could have on carbon sequestration. If CO\textsubscript{2} emissions can not be reduced globally, other methods of sequestering carbon need to be explored. Because N is usually the most limiting nutrient in temperate terrestrial ecosystems, I hypothesized that adding N to land plants should cause the plants to grow bigger and therefore sequester more carbon. Various model runs showed that adding N, especially to the most N limited forests, could sequester large amounts of carbon. Although excess N in the environment has a variety of deleterious effects, the wise use of N\textsubscript{2}-fixing plants and N fertilizer on the most N-limited sites, especially high C:N forests, would increase C sequestration, at least temporarily.
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The Fate of Organic and Inorganic Nitrogen Inputs in an Old-growth Forest of the Central Oregon Cascade Range

by
Scott M. Holub

A DISSERTATION

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Dean of Graduate School

I understand that my dissertation will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my dissertation to any reader upon request.

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Scott M. Holub, Author
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TABLE OF CONTENTS

Chapter 1: Introduction........................................................................................................1
  Background.......................................................................................................................1
  \(N_2\)-fixing Lichens - *Lobaria oregana*.................................................................2
  Polyphenols and Nitrogen Cycling..............................................................................4
  Organic and Inorganic Nitrogen.................................................................................5
  \(^{15}\)N Tracer Experiments.......................................................................................6
  Nitrogen Effects on Carbon Sequestration.............................................................9
  Objectives....................................................................................................................10
  References....................................................................................................................11

Chapter 2: The Fate of \(^{15}\)N-labeled Ammonium, Organic Nitrogen, Tannin-complexed Organic Nitrogen, and \(N_2\)-fixing Lichen in an Old-Growth Coniferous Forest Soil.........................................................18
  Abstract.......................................................................................................................18
  Introduction..................................................................................................................19
  Methods......................................................................................................................24
  Results.........................................................................................................................38
  Discussion....................................................................................................................64
  Conclusion..................................................................................................................70
  References....................................................................................................................71
**TABLE OF CONTENTS (CONTINUED)**

<table>
<thead>
<tr>
<th>Chapter 3: Mass Loss and Nitrogen Dynamics During the Decomposition of a $^{15}$N-labeled N$_2$-fixing Epiphytic Lichen, <em>Lobaria oregana</em> (Tuck.) Müll. Arg.</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abstract</td>
<td>78</td>
</tr>
<tr>
<td>Introduction</td>
<td>79</td>
</tr>
<tr>
<td>Methods</td>
<td>82</td>
</tr>
<tr>
<td>Results</td>
<td>90</td>
</tr>
<tr>
<td>Discussion</td>
<td>99</td>
</tr>
<tr>
<td>Conclusion</td>
<td>107</td>
</tr>
<tr>
<td>References</td>
<td>108</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Chapter 4: Modeling the Effects of Added Nitrogen on Terrestrial Carbon Sequestration</th>
<th>113</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abstract</td>
<td>113</td>
</tr>
<tr>
<td>Introduction</td>
<td>114</td>
</tr>
<tr>
<td>Nitrogen Sources and Carbon Sequestration</td>
<td>116</td>
</tr>
<tr>
<td>Modeling Cumulative Carbon Sequestration (NBP)</td>
<td>123</td>
</tr>
<tr>
<td>Conclusions</td>
<td>128</td>
</tr>
<tr>
<td>References</td>
<td>129</td>
</tr>
<tr>
<td>TABLE OF CONTENTS (CONTINUED)</td>
<td></td>
</tr>
<tr>
<td>-----------------------------</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>Chapter 5: Conclusions</td>
<td>133</td>
</tr>
<tr>
<td>Thesis summary</td>
<td>133</td>
</tr>
<tr>
<td>References</td>
<td>136</td>
</tr>
<tr>
<td>Bibliography</td>
<td>137</td>
</tr>
</tbody>
</table>
## LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.1</td>
<td>Average recovery of $^{15}\text{N}$ in whole material collected from \textit{in situ} soil cores to which $^{15}\text{N}$-ammonium had been added in fall..........................41</td>
</tr>
<tr>
<td>2.2</td>
<td>Average recovery of $^{15}\text{N}$ in whole material collected from \textit{in situ} soil cores to which $^{15}\text{N}$-ammonium had been added in spring.........................42</td>
</tr>
<tr>
<td>2.3</td>
<td>Average recovery of $^{15}\text{N}$ in whole material collected from \textit{in situ} soil cores to which $^{15}\text{N}$-labeled organic N had been added in spring.............43</td>
</tr>
<tr>
<td>2.4</td>
<td>Average recovery of $^{15}\text{N}$ in whole material collected from \textit{in situ} soil cores to which tannin-complexed $^{15}\text{N}$-labeled organic N had been added in spring..................................................44</td>
</tr>
<tr>
<td>2.5</td>
<td>Average recovery of $^{15}\text{N}$ in whole material collected from \textit{in situ} soil cores to which $^{15}\text{N}$-labeled \textit{Lobaria oregana} had been added in fall........45</td>
</tr>
<tr>
<td>2.6</td>
<td>Average recovery of $^{15}\text{N}$ in whole material collected from \textit{in situ} soil cores to which $^{15}\text{N}$-labeled \textit{Lobaria oregana} had been added in spring.....46</td>
</tr>
<tr>
<td>2.7a</td>
<td>Average recovery of $^{15}\text{N}$ in potassium sulfate extracts from litter/organic horizons that have had $^{15}\text{N}$-ammonium additions to \textit{in situ} soil cores in spring.................................................................................49</td>
</tr>
<tr>
<td>2.7b</td>
<td>Average recovery of $^{15}\text{N}$ in potassium sulfate extracts from 0 to 5 cm mineral soils that have had $^{15}\text{N}$-ammonium additions to \textit{in situ} soil cores in spring.................................................................................50</td>
</tr>
<tr>
<td>2.8a</td>
<td>Average recovery of $^{15}\text{N}$ in potassium sulfate extracts from litter/organic horizons that have had $^{15}\text{N}$-ammonium additions to \textit{in situ} soil cores in fall.................................................................................51</td>
</tr>
<tr>
<td>2.8b</td>
<td>Average recovery of $^{15}\text{N}$ in potassium sulfate extracts from 0 to 5 cm mineral soils that have had $^{15}\text{N}$-ammonium additions to \textit{in situ} soil cores in fall.................................................................................52</td>
</tr>
<tr>
<td>Figure</td>
<td>Description</td>
</tr>
<tr>
<td>--------</td>
<td>-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>2.9a</td>
<td>Average recovery of $^{15}$N in potassium sulfate extracts from litter/organic horizons that have had $^{15}$N-labeled organic N additions and tannin-complexed $^{15}$N-labeled organic N additions to in situ soil cores in spring.</td>
</tr>
<tr>
<td>2.9b</td>
<td>Average recovery of $^{15}$N in potassium sulfate extracts from 0 to 5 cm mineral soils that have had $^{15}$N-labeled organic N additions and tannin-complexed $^{15}$N-labeled organic N additions to in situ soil cores in spring.</td>
</tr>
<tr>
<td>2.10a</td>
<td>Average recovery of $^{15}$N in potassium sulfate extracts from litter/organic horizons that have had $^{15}$N-labeled Lobaria oregana additions to in situ soil cores in spring.</td>
</tr>
<tr>
<td>2.10b</td>
<td>Average recovery of $^{15}$N in potassium sulfate extracts from 0 to 5 cm mineral soils that have had $^{15}$N-labeled Lobaria oregana additions to in situ soil cores in spring.</td>
</tr>
<tr>
<td>2.11a</td>
<td>Average recovery of $^{15}$N in potassium sulfate extracts from litter/organic horizons that have had $^{15}$N-labeled Lobaria oregana additions to in situ soil cores in fall.</td>
</tr>
<tr>
<td>2.11b</td>
<td>Average recovery of $^{15}$N in potassium sulfate extracts from 0 to 5 cm mineral soils that have had $^{15}$N-labeled Lobaria oregana additions to in situ soil cores in fall.</td>
</tr>
<tr>
<td>2.12</td>
<td>Average recovery of $^{15}$N in density fractions of 0 to 5 cm mineral soils that have had $^{15}$N-ammonium or $^{15}$N-labeled Lobaria oregana additions to in situ soil cores in fall and spring.</td>
</tr>
<tr>
<td>2.13</td>
<td>Heavy fraction A% $^{15}$N excess to Light fraction A% $^{15}$N excess ratios of 0 to 5 cm mineral soils that have had $^{15}$N-ammonium additions to in situ soil cores in fall or spring or $^{15}$N-labeled organic N or tannin-complexed $^{15}$N-labeled organic N additions to in situ soil cores in spring.</td>
</tr>
<tr>
<td>3.1</td>
<td>Mass remaining in Lobaria over time.</td>
</tr>
<tr>
<td>3.2</td>
<td>Net N lost from Lobaria tissue versus mass loss.</td>
</tr>
<tr>
<td>Figure</td>
<td>Description</td>
</tr>
<tr>
<td>--------</td>
<td>-------------</td>
</tr>
<tr>
<td>3.3</td>
<td>Gross N lost from <em>Lobaria</em> tissue versus mass lost</td>
</tr>
<tr>
<td>3.4</td>
<td>Gross N lost from <em>Lobaria</em> tissue versus net N lost</td>
</tr>
<tr>
<td>3.5</td>
<td>Nitrogen concentration versus mass lost</td>
</tr>
<tr>
<td>3.6</td>
<td>Exogenous N in remaining lichen tissue versus mass loss</td>
</tr>
</tbody>
</table>
## LIST OF TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.1</td>
<td>Soil and organic matter characteristics of the site, pooled over all collection dates and all treatments</td>
<td>26</td>
</tr>
<tr>
<td>2.2</td>
<td>Total $^{15}$N Recovery over all times from N addition</td>
<td>39</td>
</tr>
<tr>
<td>2.3</td>
<td>Average extractable nitrogen concentrations pooled over all collection dates and all treatments</td>
<td>47</td>
</tr>
<tr>
<td>2.4</td>
<td>Average characteristics of the 0 to 5 cm soil density fractions pooled over all collection dates and all N addition types</td>
<td>61</td>
</tr>
<tr>
<td>4.1</td>
<td>Effective carbon in plant biomass per nitrogen added</td>
<td>120</td>
</tr>
<tr>
<td>4.2</td>
<td>Worst case scenario - NBP increment per nitrogen added</td>
<td>125</td>
</tr>
<tr>
<td>4.3</td>
<td>Percent change in steady state carbon stores with added nitrogen</td>
<td>127</td>
</tr>
</tbody>
</table>
THE FATE OF ORGANIC AND INORGANIC NITROGEN INPUTS IN AN OLD-GROWTH FOREST OF THE CENTRAL OREGON CASCADE RANGE

CHAPTER 1: INTRODUCTION

BACKGROUND

Interest in the nitrogen (N) cycle of forested ecosystems has been heightened in the past decade because of increased atmospheric N deposition, primarily in eastern North America (Nadelhoffer et al. 1995, Seely and Lajtha 1997, Nadelhoffer et al. 1999) and central Europe (Emmett et al. 1991, Tietema et al. 1998, Johannison et al. 1999). Pollution inputs of inorganic N in these areas can be in excess of 60 kg-N ha\(^{-1}\) yr\(^{-1}\) (Tietema et al. 1998). This large amount of N exceeds the amount that some ecosystems can retain and can lead to a condition known as nitrogen saturation (Ågren and Bosatta 1988, Aber et al. 1989, 1998).

The presence of extra nitrogen is an unusual situation for many terrestrial ecosystems, because nitrogen is usually the nutrient most limiting to plant growth in temperate regions (Vitousek and Howarth 1991) including the Pacific Northwest United States (Date 1973). Nitrogen saturation can be detrimental to plants, and can result in high amounts of nitrate leaching into surrounding ground-water and rivers where it can have toxic effects on humans and the environment (Vitousek et al. 1979).
In old-growth forests of the Pacific Northwest United States, however, inorganic N deposition is low. Measured inputs to the H.J. Andrews Long Term Ecological Research site (HJA) in the coniferous forests of the western Oregon Cascades are less than 2 kg-N ha\(^{-1}\) yr\(^{-1}\) (Sollins et al. 1980). The small amount of N that leaches from these old forests, as well as from most other unpolluted old-growth forests that have been studied, is greater than 90% organic (Sollins et al. 1980, Hedin et al. 1995, Perakis and Hedin 2002). The majority of new N input into these ecosystems is primarily of organic origin resulting from N\(_2\)-fixation, with the remainder added as atmospheric deposition (Sollins et al. 1980).

In early and mid-successional forests of the Pacific Northwest symbiotic N\(_2\)-fixing trees in the genera *Alnus* and *Ceanothus* are estimated to input between 30 and 300 kg-N ha\(^{-1}\) yr\(^{-1}\) (Sollins et al. 1980, Binkley et al. 1992). In late successional forests, where the presence of *Alnus* and *Ceanothus* is diminished, N inputs from N\(_2\)-fixing epiphytic lichens become significant. The fate of N from these organic N sources remains unclear.

**N\(_2\)-FIXING LICHENS - Lobaria oregana**

The dominant N\(_2\)-fixing epiphytic lichen in many Pacific Northwest old-growth forests is *Lobaria oregana* (Tuck.) Müll. Arg. (hereafter *Lobaria*). *Lobaria* can have 500 to 1000 kg ha\(^{-1}\) or more standing biomass in old-growth Douglas-fir forests of the Pacific Northwest (McCune 1994, Denison 1979) and can input 2.5 to
4.5 kg-N ha\(^{-1}\) yr\(^{-1}\) or more from N\(_2\)-fixation (Pike 1978, Denison 1979, Antoine 2001). Although N inputs from *Lobaria* are only 12 to 23% of the 20 kg-N ha\(^{-1}\) yr\(^{-1}\) total litterfall N in old-growth forests (Sollins *et al.* 1980), they represent approximately 33% to 67% of ‘new’ N inputs to ecosystems where *Lobaria* is abundant.

Most litterfall N is recycled through the system from previous N inputs and is not actually a source of new N for the forests. While recycled N is important in maintaining available N pools in the short term, new N is necessary to replace N that has been lost from the ecosystem through leaching, denitrification, logging or through sequestration in recalcitrant soil organic matter (Prescott *et al.* 2000). Inputs of new N are therefore required to sustain long-term growth and production in aggrading forest ecosystems.

The amount of N input by *Lobaria* exceeds the estimated 1.5 kg-N ha\(^{-1}\) yr\(^{-1}\) ecosystem losses of N through leaching (Sollins *et al.* 1980) and denitrification (Vermes and Myrold 1992). Combining N from *Lobaria* with precipitation N inputs exceeds the 2.8 kg-N ha\(^{-1}\) yr\(^{-1}\) requirement for new vegetation growth (Sollins *et al.* 1980). Therefore, the ecosystem losses and plant uptake of N are more than accounted for with 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. N\(_2\)-fixing lichens are found in many forests including South
America (Forman 1975, Guzman et al. 1990), northern Alaska (Gunther 1989), and Asia so the importance of N$_2$-fixing lichens as a new N source has worldwide applicability.

POLYPHENOLS AND NITROGEN CYCLING

Some species of N$_2$-fixing lichens, like Pseudocyphellaria, are known to produce secondary metabolites that could inhibit predation and decomposition (Guzman et al. 1990). Many of these secondary metabolites are polyphenols. Polyphenols (e.g. tannin, gallic acid) from plant and microbial sources are influential in soil N dynamics (Horner et al. 1988, Bending and Read 1996, Schimel et al. 1996, Bradley et al. 2000, Hättenschwiler and Vitousek 2000, Fierer et al. 2001). Polyphenols have generally been shown to have an inhibiting effect on net N mineralization. Depending on the structure and molecular weight of the polyphenol molecules, the mechanism for this observed outcome can vary.

Low molecular weight phenolics induce N uptake and immobilization by acting as a carbon source for microbial growth and respiration in a mechanism similar to the addition of other available carbon forms such as glucose, while 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 et al. 1996, Fierer et al. 2001). High polyphenol concentrations have been correlated with slow decomposition of
organic material (Benoit et al. 1968; Benoit and Starkey 1968; Northup et al. 1995, 1998) and also with a possible reduction in nitrification (Rice and Pancholy 1973, Baldwin et al. 1983, Bradley et al. 2000) although their effect on nitrification has not been supported in other studies (e.g. Schimel et al. 1996). Many theories about the formation of humic substances rely on reactions involving polyphenols or related compounds (Stevenson 1994).

ORGANIC AND INORGANIC NITROGEN

The role of organic N, such as amino acids or peptides, as a direct source of N for plants has recently come to the forefront of N cycling literature (Abuzinadah et al. 1986, Kielland 1994, Chapin 1995, Schimel and Chapin 1996, Nasholm et al. 1998, Lipson et al. 1999, Raab et al. 1999). Studying the processes involved in the production of available N, either as small organic N compounds decomposed from proteins or as inorganic compounds, is necessary to understand soil N dynamics. The rate at which proteins and protein-polyphenol complexes are broken down in an ecosystem could limit N availability to plants and thus limit primary productivity. 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 was found to be amide-peptide N, the same bond found in proteins (Clinton et al. 1995).
While the fates of organic N compounds, including *Lobaria*, are clearly important, inorganic N compounds are still thought to be the major source of directly available N for trees and other plants. Therefore, the fate of inorganic N should not be overlooked. Including ammonium, the most common form of inorganic N in forest soils with low N deposition (Buchmann *et al.* 1995, Perakis and Hedin 2002), would be useful for comparing and contrasting the fate of organic N forms with the fate of inorganic N.

**15N TRACER EXPERIMENTS**

Tracer experiments using 15N have been performed extensively to elucidate N transformations and retention pools in forested ecosystems (e.g. Berg 1988, Schimel and Firestone 1989, Zak *et al.* 1990, Hart and Firestone 1991, Preston and Mead 1995, Buchmann *et al.* 1996, Koopmans *et al.* 1996, Seely and Lajtha 1997, Swanston and Myrold 1997, Tietema *et al.* 1998, Johannison *et al.* 1999, Nadelhoffer *et al.* 1999, Perakis and Hedin 2001). These studies show that the litter and upper layers of soil are the largest pool for added N. Soil and microbial biomass are the major sinks of added ammonium and nitrate (Buchmann *et al.* 1996). While microbes appear to prefer ammonium to nitrate (Davidson *et al.* 1990), occasionally microbes show no preference for one over the other (Groffman *et al.* 1993). Plants are capable of both ammonium and nitrate uptake, but have not been found to be a major short term sink for added N (Vitousek and Matson 1984).
Zak et al. 1990, Groffman et al. 1993, Nadelhoffer et al. 1999). Although in some studies, plants appeared to be a significant sink of added N in the long term (Buchmann et al. 1995). For a more complete review of N retention, see Johnson (1992) and Fenn et al. (1998).

Few studies have used organic $^{15}$N compounds as tracers in forest ecosystems (Swanston and Myrold 1997, Berg 1988, Preston and Mead 1995). Swanston and Myrold (1997) used $^{15}$N-labeled red alder (Alnus rubra Bong.) leaves to track the fate of organic N in a Pacific Northwest forest ecosystem at H.J. Andrews experimental forest. They observed the whole soil and light and heavy fraction $^{15}$N along with chloroform-labile $^{15}$N, and $^{15}$N in aboveground vegetation. They found that after 21 months, the leaves lost 76% of their initial N content and of that, 71% was recovered in the pools they observed. In the 0-5 cm soil pools, they found the light fraction to be the most highly labeled, followed by whole soil, and then heavy fraction. Aboveground vegetation also became labeled over the 21 month period as well. The chloroform-labile pool showed no statistical evidence of being labeled.

Berg (1988) tracked $^{15}$N-labeled Scots pine (Pinus sylvestris L.) needle litter in a forested as well as a clear-cut site in central Sweden using litterbags. He observed sulfuric acid-soluble and acid-insoluble pools of litter in both studies. Other soil or microbial pools were not observed. He observed an uptake of total N from the soil into the needles through the first seven months that resulted in a
doubling of the amount of total N initially present. This was followed by a loss of total N back to near initial values. The $^{15}$N mass, however, was lost at a relatively constant rate over this one year period. This indicates a constant loss of needle N, with variable immobilization of N from the soil. Similar results were observed at both field sites.

Preston and Mead (1995) used $^{15}$N-labeled Douglas-fir ($Pseudotsuga menziesii$ (Mirb.) Franco) needles in combination with urea and NH$_4$NO$_3$ added inside 15 cm diameter steel cylinders to study N dynamics on Vancouver Island. They did not find any significant difference in $^{15}$N recovery among any of the combined inorganic-organic treatments or the organic addition alone. Total $^{15}$N recovery decreased over the 7.5 year time period of the experiment from 54% after one year to 20% after 7.5 years indicating low retention of N, although the fate of the lost N was unclear. Throughout the study they found very little $^{15}$N in inorganic forms, which emphasized the role of organic N forms in N retention.

Organic $^{15}$N additions have also been performed in laboratory settings and other non-forested ecosystems to study N dynamics. Most work was carried out with the goal of understanding the role of organic N in agricultural productivity and examined microbial immobilization of organic N (e.g. Gibbs and Barraclough 1998, Sørensen et al. 1994, Hadas et al. 1992, Cortez and Cherqui 1991, Barak et al. 1990, Schnurer and Rosswall 1987, Lethbridge and Davidson 1983, Marumoto et al. 1982). This work is particularly relevant to my study, because we expect
similar mechanisms to be controlling N dynamics in our forest soils. Studies in
arctic tundra observed amino acid uptake by plants (e.g. Schimel and Chapin 1996,
Kielland 1994) and production of proteins and amino acids from microbes (e.g.
Lipson et al. 1999). Recognizing the possible 'short circuiting' of the N cycle
(Chapin 1995) could be important in understanding patterns I observe in my study.

NITROGEN EFFECTS ON CARBON SEQUESTRATION

In my final chapter, I expanded the scale of my research from the plot to the
terrestrial biosphere, where I modeled the effect that added N would have on
carbon dioxide (CO₂) sequestration. Atmospheric CO₂ concentrations have been
increasing since the industrial revolution. Because CO₂ is a greenhouse gas, its
continued emission to the atmosphere is expected to result in global warming or
other climate changes. However, the amount of atmospheric CO₂ has not increased
as much as known sources have emitted (Tans et al. 1990). Models indicate that
oceanic CO₂ uptake is responsible for some, but not all of this discrepancy, and that
an additional carbon exists on land, possibly North America (Fan et al. 1998).

Many possibilities exist to explain the missing carbon sink, but I will focus
on N fertilization, in particular. Added N can positively effect plant growth and
carbon sequestration because nitrogen is widely accepted to be the limiting nutrient
to plant growth in most temperate terrestrial ecosystems (Vitousek and Howarth
1991). Too much N, however, can be harmful to plants, thereby reducing primary
productivity (Aber et al. 1998, Ågren and Bosatta 1988). Decomposition can also be affected positively or negatively by added N (Fog 1988, Carreiro et al. 2000). By modeling the combined effects of added N on carbon sequestration, we can estimate the overall usefulness of adding N to reduce atmospheric CO₂ concentrations.

OBJECTIVES

Thesis Objectives

The underlying objective of this dissertation was to investigate the fate of added organic and inorganic N in forest soils of the Pacific Northwestern United States.

Chapter 2

The objectives of Chapter 2, "The Fate of ¹⁵N-Labeled Lobaria oregana, Organic Nitrogen, Tannin-Complexed Organic Nitrogen, and Ammonium in an Old-Growth Coniferous Forest Soil," were 1) to determine if the fate of organic N differed from the fate of inorganic N, 2) to determine the effect that polyphenols have on the fate of organic N, and 3) to determine the effect of season of addition on the fate of N inputs.
Chapter 3

The objectives of Chapter 3 "Mass Loss and Nitrogen Dynamics During the Decomposition of a $^{15}$N-Labeled N$_2$-fixing Epiphytic Lichen, *Lobaria oregana* (Tuck.) Müll. Arg.," were 1) to develop an easy method of labeling lichen material with $^{15}$N, 2) to examine the mass loss of *Lobaria* litter as it decomposes on the forest floor, and 3) to examine the patterns of N uptake and loss throughout the decay of *Lobaria* litter.

Chapter 4

The objectives of Chapter 4, "Modeling the effects of added nitrogen on terrestrial carbon sequestration" were 1) to investigate the effect of added N, in its various forms, on terrestrial carbon sequestration and 2) to explore the possible use of N fertilizer to offset carbon emissions.

REFERENCES


Buchmann, N., G. Gebauer, and E.D. Schulze. 1996. Partitioning of $^{15}$N labeled ammonium and nitrate among soil, litter, below- and above-ground biomass of


tannin fractions on carbon and nitrogen dynamics in Alaskan taiga floodplain


Forman, R.T.T. 1975. Canopy lichens with blue-green algae: a nitrogen source in a

decomposition of leaf protein I (Ribulose 1,5-diphosphate carboxylase) in the
presence or absence of sucrose. Soil Biology and Biochemistry 30(13): 1821-
1827.

spring nitrogen dynamics in a temperate forest landscape. Ecology 74(5):
1579-1585.


Guzman, B., W. Quilhot, and D.J. Galloway. 1990. Decomposition of species of
Pseudocyphellaria and Sticta in a southern Chilean forest. Lichenologist 22:
325-331.

of nitrogen by soil microbial population: NH₄⁺ versus organic N. Soil Biology
and Biochemistry 24(2): 137-143.


Hättenschwiler, S., and P.M. Vitousek 2000. The role of polyphenols in terrestrial

unpolluted, old-growth temperate forests: evaluation of biogeochemical theory.

Homer, J.D., J.R. Gosz, and R.G. Cates 1988. The role of carbon-based plant
secondary metabolites in decomposition in terrestrial ecosystems. American


CHAPTER 2

THE FATE OF $^{15}$N-Labeled Ammonium, Organic Nitrogen, Tannin-Complexed Organic Nitrogen, and N$_2$-Fixing Lichen in an Old-Growth Coniferous Forest Soil

Scott M. Holub and Kate Lajtha

ABSTRACT

Forests in the Pacific northwestern region of North America receive very little nitrogen through atmospheric deposition and thus can provide insights into how the nitrogen cycle functioned before heavy atmospheric deposition of inorganic nitrogen began in other regions. New nitrogen inputs from N$_2$-fixing trees and lichens are much greater than inorganic nitrogen inputs in the Pacific Northwest. These organic nitrogen sources contribute nitrogen to the nitrogen-limited forests of this region. Our objectives were 1) to determine if the fate of organic nitrogen differed from the fate of inorganic nitrogen, 2) to determine the effect that polyphenols have on the fate of organic nitrogen, and 3) to determine the effect of season of addition on the fate of nitrogen inputs. We added $^{15}$N-labeled ammonium, organic nitrogen, tannin-complexed organic nitrogen, and the N$_2$-fixing lichen Lobaria oregana (Tuck.) Müll. Arg. to in situ soil cores to follow the fate of added nitrogen in forest soils in the central Cascade Range in Oregon. Materials collected and analyzed for $^{15}$N included moss, above-ground plant
biomass, a combined litter/organic horizon, and mineral soil by depth. Microbial biomass, dissolved organic nitrogen, ammonium, and nitrate were analyzed from the litter/organic horizon and mineral soil. Density fractionations were also performed on the soil. Total $^{15}\text{N}$ recovery was near 100% for all nitrogen additions. The litter/organic horizon, as a bulk pool, was the largest nitrogen retention pool for all forms of nitrogen addition. Within the litter/organic horizon, the chloroform-extractable microbial biomass initially accounted for nearly all of the added nitrogen from the ammonium additions. Although on a different time scale, microbial biomass also played a significant role in the retention of nitrogen from organic nitrogen, tannin-complexed, organic nitrogen, and *Lobaria*. Complexing organic matter with tannin appeared to slow nitrogen cycling, but did not significantly change the ultimate distribution of added organic nitrogen. Season of nitrogen addition had little effect on the retention of added nitrogen. Our study provides further evidence that microbial biomass plays an active role in initial and continued nitrogen retention from both organic and inorganic sources in low atmospheric deposition sites.

**INTRODUCTION**

where pollution inputs of inorganic nitrogen (N) can be in excess of 60 kg-N ha⁻¹ yr⁻¹ (Tietema et al. 1998), old-growth forests of the Pacific northwestern United States have inorganic N deposition that is lower than 2 kg-N ha⁻¹ yr⁻¹ (Sollins et al. 1980). The majority of new N inputs into Pacific Northwest forests are primarily of organic origin resulting from N₂-fixation (Sollins et al. 1980).

In early and mid-successional forests of the Pacific Northwest, symbiotic N₂-fixing trees in the genera *Alnus* and *Ceanothus* are estimated to input between 30 and 300 kg-N ha⁻¹ yr⁻¹ (Sollins et al. 1980, Binkley et al. 1992). However, in mature and late-successional forests, where the presence of *Alnus* and *Ceanothus* is diminished, N inputs from N₂-fixing epiphytic lichens become significant. N₂-fixing lichens are found in forests around the globe including South America (Forman 1975, Guzman et al. 1990), northern Alaska (Gunther 1989) and Asia. The dominant N₂-fixing epiphytic lichen in most Pacific Northwest old-growth forests is *Lobaria oregana* (Tuck.) Müll. Arg. (hereafter *Lobaria*). *Lobaria* can have 500 to 1000 kg ha⁻¹ or more standing biomass 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 yr⁻¹ or more by N₂-fixation (Pike 1978, Denison 1979). Although N inputs from *Lobaria* are only 12 to 23% of the 20 kg-N ha⁻¹ yr⁻¹ total litterfall N in old-growth forests in the central Cascades (Sollins et al. 1980), they represent approximately 33% to 67% of new N inputs into the ecosystems where *Lobaria* is abundant.
N is usually the nutrient most limiting to terrestrial plant growth in temperate regions (Vitousek and Howarth 1991), including the Pacific Northwest United States (Date 1973), so studying N cycling and examining the fate of N inputs in this region could provide insights into how the nitrogen cycle functioned before heavy atmospheric deposition of N began. Most of the N in litterfall has been recycled through the ecosystem from previous N inputs and is not actually a source of new N for the forests. While recycled N is important in maintaining available N pools in the short term, inputs of new N are required for long-term growth in aggrading forest ecosystems. New N is also necessary to replace N lost from the ecosystem through leaching and denitrification or through sequestration in recalcitrant soil organic matter (Prescott et al. 2000).

The amount of N input by Lobaria exceeds the estimated 1.5 kg-N ha\(^{-1}\) yr\(^{-1}\) ecosystem losses of N through leaching (Sollins et al. 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 greater than 90% organic (Sollins et al. 1980, Hedin et al. 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\(^{-1}\) yr\(^{-1}\) requirement for new vegetation growth (Sollins et al. 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 (e.g. tannin, gallic acid) from fungal, plant, and microbial sources play an important role in soil N dynamics (Horner et al. 1988, Bending and Read 1996, Schimel et al. 1996, Bradley et al. 2000, Hättenschwiler and Vitousek 2000, Fierer et al. 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, limiting N mineralization (Schimel et al. 1996, Fierer et al. 2001). High polyphenol concentrations have been correlated with slow decomposition of organic material (Benoit et al. 1968; Benoit and Starkey 1968; Northup et al. 1995, 1998) and also with a possible reduction in nitrification (Rice and Pancholy 1973, Baldwin et al. 1983, Bradley et al. 2000) although their effect on nitrification has not been supported in other studies (e.g. Schimel et al. 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 season of N addition. Large losses of added N have occurred following fall additions relative to growing-season additions in an agricultural setting (Nyborg et al. 1990). Low demand for N in the fall can contribute to more leaching losses (Seely and Lajtha 1997) and more denitrification (Nyborg et al. 1990).

The objectives of this study were 1) to determine if the fate of organic N differed from the fate of inorganic N, 2) to determine the effect that polyphenols have on the fate of organic N, and 3) to determine the effect of 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 fall, because spring soil would be moist from the winter rains and would therefore not soak up as much water as the dryer fall soil. The Lobaria added in the spring was hypothesized to not 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 fall, 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 Term Ecological Research (LTER) site near Blue River, Oregon (44° 13' 53" N, 122° 13' 40" W). 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). This site 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 N₂-fixing lichen *Lobaria oregana* (Tuck.) Müll. Arg. *Tsuga heterophylla* saplings, *Taxus brevifolia* Nutt. (pacific yew), *Rhododendron macrophyllum* G. Don, and *Acer circinatum* Pursh (vine maple) make up the majority of the understory vegetation. Plants on the forest floor include *Polystichum munitum* (Kaulf.) Presl. (sword fern), *Berberis nervosa* Pursh (Oregon grape), and *Chimaphila umbellata* (L.) Barton (prince's pine). The forest floor is covered with a layer of mosses
including *Eurhynchiwn oreganum* (Sull.) Jaeg. (Oregon beaked moss) and *Hylocomium splendens* (Hedw.) B.S.G. (step moss).

Soil characteristics across the site did not differ significantly (Table 2.1). Although soils at H.J. Andrews LTER were formally classified as Inceptisols (Humic Dystrodept at our site), more recent physical and chemical analyses suggest that the soils meet the requirements for classification as Andisols (Keys to Soil Taxonomy, 1998). The soils appear to contain volcanic ash and pumice. Bulk densities are less than 0.9 g cm$^{-3}$, averaging 0.8 g cm$^{-3}$. Percent carbon is >2% in surface samples, averaging about 3.5%. The oxalate extracted-Al plus one-half oxalate extracted Fe totals more than 2% (Spears and Lajtha 2002).

$^{15}$N-labeled materials

**Ammonium**

$^{15}$N-labeled ammonium chloride ($^{15}$NH$_4$Cl, 98 Atom% $^{15}$N, Aldrich Chemical 29,925-1) was the source of inorganic nitrogen in this study. A 21.3 μg N ml$^{-1}$ solution of ammonium chloride was prepared and acidified with hydrochloric acid to a pH of 5.5 to minimize NH$_3$ volatilization. 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. Ammonium is commonly found at much higher concentrations than nitrate in
unpolluted forest soils (Johnson 1992, Perakis and Hedin 2002). We chose to use ammonium chloride instead of ammonium sulfate, because there is some evidence that sulfur may limit growth in a small number of forest stands in the Pacific Northwest (Turner et al. 1979).

Table 2.1
Soil and organic matter characteristics of the site, pooled over all collection dates and all treatments.

<table>
<thead>
<tr>
<th>Ecosystem Pool</th>
<th>Total N (%) (SE)</th>
<th>Total C (%) (SE)</th>
<th>Dry Mass (g/m²) (SE)</th>
<th>Bulk Density (g/cm³) (SE)</th>
<th>Stone Content (%) (SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Forest Floor Plants</td>
<td>1.34 (0.12)</td>
<td>47.34 (0.38)</td>
<td>4.8 (1.0)</td>
<td>na</td>
<td>na</td>
</tr>
<tr>
<td>Moss</td>
<td>0.911 (0.018)</td>
<td>44.63 (0.73)</td>
<td>91.1 (4.1)</td>
<td>na</td>
<td>na</td>
</tr>
<tr>
<td>Litter/Organic Horizon</td>
<td>0.915 (0.016)</td>
<td>36.53 (0.58)</td>
<td>5406 (278)</td>
<td>0.129 (0.004)</td>
<td>na</td>
</tr>
<tr>
<td>0-5 cm Soil</td>
<td>0.205 (0.003)</td>
<td>5.67 (0.34)</td>
<td>30105 (534)</td>
<td>0.765 (0.012)</td>
<td>20.74 (0.43)</td>
</tr>
<tr>
<td>5-15 cm Soil</td>
<td>0.148 (0.003)</td>
<td>2.78 (0.34)</td>
<td>71870 (1002)</td>
<td>0.843 (0.013)</td>
<td>17.74 (0.80)</td>
</tr>
</tbody>
</table>

Notes: All mineral soil values are on the <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. Unless otherwise indicated n = 96. * n = 6 for total carbon measurements na = not applicable
**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 (Fiechter et al. 1987). The pH was adjusted to 4.5. A total of 4.5 L of nutrient broth was made in two flasks. The sterilized broth was inoculated with *S. cerevisiae* obtained from compressed Fleischmann's® baker's yeast and incubated at 22 °C with continuous stirring for 48 hours.

After the incubation period the broth was allowed to settle, vacuum filtered, rinsed three times with excess deionized water, and dried at 60°C. One flask yielded yeast that were 8.8 %N and 9.9 Atom% $^{15}$N while the other flask yielded 8.9 %N and 10.8 Atom% $^{15}$N. The %N values convert to 55% crude protein (%N x 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 was found to be amide-peptide N, the same bond found in proteins (Clinton et al. 1995).

Portions of dried yeast (0.4 g) were added to 50 ml of deionized water and autoclaved for 20 minutes to lyse cells and produce non-living organic N. The
yeast solution was then poured into field-ready bottles and water was added to make a total of 150 ml.

**Tannin-complexed organic nitrogen**

Half of the autoclaved 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 *et al.* 1996). Tannic acid was added to the vials at a ratio of 100g of tannic acid to 1 g of yeast N to favor the complexing reaction.

**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 (2002). The entire process was repeated twice to have fresh tissue for each season of addition, once in September 1999 for a fall addition and once in March 2000 for a spring addition. The *Lobaria* from the fall labeling experiment contained an average of 16.5 atom % $^{15}$N (SE = 1.2, n=10) while the spring addition contained an average of 11.3 atom % $^{15}$N (SE = 0.6, n=10). The fall labeled *Lobaria* tissue averaged 2.75% N (SE=0.09, n=10) while the spring labeled tissue was 2.28% N (SE=0.08, n=5).
Plot installation

Large PVC tubes (15.25 cm diameter by 40 cm length) were driven into the ground to a depth of 35 cm to act as in situ containers of forest floor and soil. To facilitate installation and removal the tubes were sharpened on the bottom and had two 1.3 cm (0.5 inch) holes drilled 3 cm from the top of the tube. The tubes were installed approximately two months prior to the spring and fall $^{15}$N additions to allow the soil to recover from the mild disturbance caused by installation. The tubes went in without visible soil compaction. 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 et al. 1999), but we recognize that they may have some minor drawbacks. The installation of the tubes severs tree roots, eliminating $N$ uptake by trees. However, many studies have found trees to be a poor short term sink for added $N$ (Vitousek and Matson 1984, Zak et al. 1990, Groffman et al. 1993), so we did not expect the lack of tree roots to have a large impact on our relatively short term results.

The tubes probably did not inhibit the movement of soil fauna, which have been shown to increase decomposition (Blair et al. 1992) and could affect $N$ retention. Macrofauna were free to climb into or out of the tubes by climbing over the small amount of tube that remained above the soil. During extraction of the tubes' contents, many individual macroinvertebrates were found including
Harpaphe haydeniana haydeniana (Wood) (millipedes) as well as unidentified earthworms and centipedes.

Applying $^{15}$N-labeled material

**Fall addition**

On October 27, 1999, we added $^{15}$N-ammonium and $^{15}$N-labeled Lobaria to separate PVC tubes in each of the three fall 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 *et al.* 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) of deionized water was added over the lichen pieces.

One PVC tube in each block had no $^{15}$N additions and received only 1.0 cm (180 ml) of deionized water to maintain consistent moisture with the other treatments. These tubes were used to determine the background $^{15}$N signature of the ecosystem pools to be measured in the treated tubes resulting in greater accuracy in sparsely labeled pools.
Spring addition

On April 27, 2000, we added $^{15}$N-labeled Lobaria, $^{15}$N-ammonium, and background correction tubes as in the fall addition. We also added $^{15}$N-labeled organic N and tannin-complexed $^{15}$N-labeled organic N to separate tubes. Both complexed and non-complexed organic nitrogen 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 of 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 harvesting

At each collection date, three tubes were collected from each treatment. Fall addition ammonium tubes were collected at 40 minutes, 3 days, 14 days, 3 months, 6 months, 1 year, and 2 years following addition of the label. Spring addition ammonium tubes were collected after the same time post-addition excluding a two-year collection. The 40 minute collection tubes were labeled under lab conditions so that samples could be processed quickly. Lobaria addition tubes in the spring and fall were collected at the same times as the ammonium addition excluding 40 minutes and three 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 one 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, above-ground plant biomass, a combined litter/organic horizon, and mineral soil at depths of 0 to 5 cm and 5 to 15 cm. Mineral soils were coarsely sieved to 4 mesh (4.75 mm) prior to further separation and analyses. Remaining Lobaria was also collected from the Lobaria treatment. Separation was always complete within 24 hours and usually complete within 8 hours of field collection. During the short amount of time before separation, the tubes were stored at 4 °C.

A field moist portion of the litter/organic horizon and the 0 to 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 to 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 using 0.5 molar potassium sulfate. Ammonium concentrations were determined using a modified Berthelot reaction on an Orion Scientific 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 nitrogen (TDN) 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 to 5 cm mineral soil using the method described in Brookes et al. (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 and represents the most labile portion of the microbial biomass.

No correction factor (kEN) was used in determining the 15N recovery from microbial biomass, because the chloroform labile component of the microbial biomass probably had a different 15N label than non-chloroform labile components of the microbial biomass. This was especially true during the earliest sampling dates, where much of the 15N taken up by microbes probably had not yet been assimilated into the more recalcitrant components. If a kEN was used at these early dates, 15N recovery from microbial biomass would be artificially inflated. At later sampling dates, however, the 15N recovery from the chloroform fumigation-extraction certainly underestimates the total 15N in microbial biomass because it excludes the 15N assimilated into the nonextractable components.

Because we could not accurately predict the ratio of 15N in the chloroform labile portion to the non-labile portion of the microbial biomass, we chose to report uncorrected values of 15N recovery across all sampling dates. Values for kEN 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 15N in microbial biomass 2 to 5 times higher than the uncorrected chloroform fumigation-extraction values.
Density fractions

A density fractionation (Strickland and Sollins 1987) was performed on the 0 to 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). We made slight modifications to scale down the procedure to reduce the consumption of relatively expensive SPT.

To determine a mass corrected sink strength for the density fractions, we divided the Atom% excess \(^{15}\text{N}\) of the heavy fraction by the Atom% excess \(^{15}\text{N}\) of the light fraction. We called this the HF to LF ratio. HF to LF ratios greater than one indicate that the heavy fraction was a stronger sink of added \(^{15}\text{N}\) per total N. HF to LF ratios that are less than one indicate that the light fraction was a stronger sink of added \(^{15}\text{N}\) per total N.

N isotope and C analyses

All \(^{15}\text{N}\) analyses were performed at the U.C. 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\(^{\circ}\)C, ground to 40 mesh, and submitted for Atom % \(^{15}\text{N}\) (eq. 2.1) and total N analyses. A
subset of the solid pools, including density fractions, were simultaneously analyzed for total carbon.

Eq. 2.1  \[
\text{Atom}\% \ ^{15}\text{N} = \text{atoms of } ^{15}\text{N}/(\text{total atoms of N} \ (^{14}\text{N} + ^{15}\text{N}))
\]

Extractable ammonium and nitrate were prepared for \(^{15}\text{N}\) analysis using ammonia diffusion to acid traps as described by Stark and Hart (1996). Total dissolved nitrogen and chloroform labile nitrogen, but due to low ionic strength of the digestion solution potassium chloride was added to make the solution closer to one molar potassium to prevent the acid traps from becoming flooded with water. Diffusion of all of the dissolved nitrogen forms was carried out for seven days. Following drying over desiccant and concentrated sulfuric acid, the acid traps were wrapped in tin cups and submitted for \(^{15}\text{N}\) and total N analysis.

The Atom\% \(^{15}\text{N}\) of the dissolved organic N pool and the net chloroform labile pool were determined by solving the equation for weighted average of their constituents for their Atom\% \(^{15}\text{N}\) value using eq. 2.2 and eq. 2.3.
Eq. 2.2 A\%_{DON} = (A\%_{TDN} \times C_{TDN} - A\%_{NO3} \times C_{NO3} - A\%_{NH4} \times C_{NH4})/C_{DON}

Eq. 2.3 A\%_{NCN} = (A\%_{TCN} \times C_{TCN} - A\%_{TDN} \times C_{TDN})/C_{NCN}

where,

\( C_y \) 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, NH4\(^+\), or NO3\(^-\). DON is dissolved organic nitrogen, TDN is total dissolved nitrogen (unfumigated), NCN is net chloroform labile nitrogen (representing microbial biomass), and TCN is total nitrogen extracted following chloroform fumigation.

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 *et al.* 1974) with catechol as a standard. Polyphenols were measured to ensure that our tannin addition did increase polyphenol concentrations in 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 nitrogen addition tubes were collected.

**Bulk density and stone content**

We determined unsieved bulk density for the litter/organic horizon, 0 to 5 cm soil and 5 to 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 (eq. 2.4). These percentages were averaged by N addition type, and by days since addition. Differences among ecosystem pools were examined using ANOVA followed by Tukey's HSD to analyze for differences between pools by N addition type and season of addition.

Eq. 2.4. $^{15}$N recovered (%) = $^{15}$N in pool above control / $^{15}$N added to tube x 100

**RESULTS**

$^{15}$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 2.2). 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 ($p>0.05$).

Table 2.2
Total $^{15}$N Recovery over all times from N addition

<table>
<thead>
<tr>
<th>N addition type</th>
<th>Ammonium (SE)</th>
<th>Organic N (SE)</th>
<th>Tannin/Organic N (SE)</th>
<th>Lobaria (SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fall (SE)</td>
<td>94.7% a x</td>
<td>-</td>
<td>-</td>
<td>95.0% a x</td>
</tr>
<tr>
<td>Spring (SE)</td>
<td>80.3% a y **</td>
<td>74.5% a **</td>
<td>90.1% a b</td>
<td>108.7% b x</td>
</tr>
</tbody>
</table>

Note: Within a single season, across treatment comparisons use a,b,c and within a single treatment across season comparisons use x,y,z. Different letters indicate a significant difference using ANOVA, followed by Tukey's HSD ($p<0.05$). Significant differences from 100% are indicated by **.

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 (Figure 2.1 through Figure 2.6). For example, the amount of $^{15}$N recovered in 0 to 5 cm mineral soil in the fall ammonium addition was about 5 to 10 percent of the added $^{15}$N at every collection date from day 0 to day 730 (Figure 2.1).

The recoveries of $^{15}$N in whole pools from fall and spring ammonium, spring organic N, and spring tannin-complexed organic N additions were similar. The litter/organic horizon had the largest recovery of added $^{15}$N when averaged
over all times since addition. The 5 to 15 cm mineral soil was usually the second largest pool of $^{15}$N recovered. The amount of $^{15}$N recovery from the 0 to 5 cm mineral soil was less than 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 <0.001%) of the added $^{15}$N (Figure 2.1 through Figure 2.4).

The fall and spring Lobaria additions had much greater variation in recovery pools over time when compared to the other N additions. Both seasons of Lobaria addition followed a similar sequence of $^{15}$N movement out of the labeled lichen, but differed in the timing. The $^{15}$N from fall Lobaria moved more quickly than the spring Lobaria 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 (<0.2%) of the added $^{15}$N (Figure 2.5 and Figure 2.6).

**Extractable nitrogen fractions**

The average concentrations for potassium sulfate extractable ammonium, nitrate, dissolved organic N, and microbial biomass N, did not differ by treatment or time since addition (Table 2.3).
Figure 2.1. Average recovery of $^{15}$N in whole material collected from *in situ* soil cores to which $^{15}$N-ammonium had been added in fall. n=3 at each sampling time.
Figure 2.2. Average recovery of $^{15}$N in whole material collected from *in situ* soil cores to which $^{15}$N-ammonium had been added in spring. $n=3$ at each sampling time.
Figure 2.3. Average recovery of $^{15}$N in whole material collected from *in situ* soil cores to which $^{15}$N-labeled organic N had been added in spring. $n=3$ at each sampling time.
Figure 2.4. Average recovery of $^{15}$N in whole material collected from *in situ* soil cores to which tannin-complexed $^{15}$N-labeled organic N had been added in spring. n=3 at each sampling time.
Figure 2.5. Average recovery of $^{15}$N in whole material collected from *in situ* soil cores to which $^{15}$N-labeled *Lobaria oregana* had been added in fall. n=3 at each sampling time.
Figure 2.6. Average recovery of $^{15}$N in whole material collected from in situ soil cores to which $^{15}$N-labeled Lobaria oregana had been added in spring. $n=3$ at each sampling time.
Table 2.3
Average extractable nitrogen concentrations pooled over all collection dates and all treatments. (n = 96)

<table>
<thead>
<tr>
<th>Ecosystem Pool</th>
<th>Microbial Biomass N† (SE)</th>
<th>Dissolved Organic N‡ (SE)</th>
<th>Ammonium‡ (SE)</th>
<th>Nitrate‡ (SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Litter/Organic Horizon</td>
<td>488.9 (34.6)</td>
<td>177.2 (7.5)</td>
<td>49.7 (2.5)</td>
<td>4.73 (1.75)</td>
</tr>
<tr>
<td>0-5 cm Soil</td>
<td>110.9 (12.4)</td>
<td>32.0 (1.5)</td>
<td>11.7 (0.7)</td>
<td>1.80 (0.61)</td>
</tr>
</tbody>
</table>

† Chloroform fumigation extraction minus control on fresh field moist material
‡ Field moist material was extracted with 0.5 molar potassium sulfate.

Unlike the whole pools, many of the extractable N components of the litter/organic horizon and 0 to 5 cm mineral soil varied in $^{15}$N recovery through time. Microbial biomass often contained the largest amount of added $^{15}$N among the extractable pools (Figure 7 through Figure 11), while recovery of $^{15}$N as dissolved organic N and ammonium were much lower. $^{15}$N recovery from the nitrate pool was rarely larger than zero.

The $^{15}$N recovery in litter/organic horizon extracts from the fall and spring ammonium additions was dominated by microbial biomass (Figure 2.7a and Figure 2.8a). 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 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.

The $^{15}$N recovery from the 0 to 5 cm soil extracts from the fall and spring ammonium addition were dominated by extractable ammonium initially, but at later sampling dates microbial biomass was the largest $^{15}$N sink (Figure 2.7b and Figure 2.8b). 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 two year collection for ammonium and dissolved organic N and through the one year collection for microbial biomass.

The $^{15}$N recovery in litter/organic horizon extracts from the spring organic N and tannin-complexed organic N did not vary between collection dates, with one exception. The $^{15}$N recovery as microbial biomass in the tannin-complexed organic N addition increased from day 14 to day 365 (Figure 2.9a). $^{15}$N recovery from microbial biomass and dissolved organic N were higher in the uncomplexed organic N addition than in the tannin-complexed organic N. $^{15}$N recovery as ammonium was not different between tannin-complexed organic N and uncomplexed organic N.
Figure 2.7a. Average recovery of $^{15}$N in potassium sulfate extracts from litter/organic horizons that have had $^{15}$N-ammonium additions to in situ soil cores in spring. MB: chloroform-labile microbial biomass N, DON: dissolved organic N, NH4: ammonium, and NO3: nitrate. n=3 at each sampling time. Inset graph shows expanded view of early collection times.
Figure 2.7b. Average recovery of $^{15}$N in potassium sulfate extracts from 0 to 5 cm mineral soils that have had $^{15}$N-ammonium additions to in situ soil cores in spring. MB: chloroform-labile microbial biomass N, DON: dissolved organic N, NH4: ammonium, and NO3: nitrate. n=3 at each sampling time. Inset graph shows expanded view of early collection times.
Figure 2.8a. Average recovery of $^{15}$N in potassium sulfate extracts from litter/organic horizons that have had $^{15}$N-ammonium additions to in situ soil cores in fall. MB: chloroform-labile microbial biomass N, DON: dissolved organic N, NH4: ammonium, and NO3: nitrate. n=3 at each sampling time. Inset graph shows expanded view of early collection times.
Figure 2.8b. Average recovery of $^{15}$N in potassium sulfate extracts from 0 to 5 cm mineral soils that have had $^{15}$N-ammonium additions to *in situ* soil cores in fall. MB: chloroform-labile microbial biomass N, DON: dissolved organic N, NH4: ammonium, and NO3: nitrate. n=3 at each sampling time. Inset graph shows expanded view of early collection times.
Figure 2.9a. Average recovery of $^{15}$N in potassium sulfate extracts from litter/organic horizons that have had $^{15}$N-labeled organic N additions and tannin-complexed $^{15}$N-labeled organic N additions to in situ soil cores in spring. MB: chloroform-labile microbial biomass N, DON: dissolved organic N, NH$_4$: ammonium, and NO$_3$: nitrate. n=3 at each sampling time.
Figure 2.9b. Average recovery of $^{15}$N in potassium sulfate extracts from 0 to 5 cm mineral soils that have had $^{15}$N-labeled organic N additions and tannin-complexed $^{15}$N-labeled organic N additions to in situ soil cores in spring. MB: chloroform-labile microbial biomass N, DON: dissolved organic N, NH4: ammonium, and NO3: nitrate. n=3 at each sampling time.
Figure 2.10a. Average recovery of $^{15}$N in potassium sulfate extracts from litter/organic horizons that have had $^{15}$N-labeled *Lobaria oregana* additions to *in situ* soil cores in spring. MB: chloroform-labile microbial biomass N, DON: dissolved organic N, NH$_4$: ammonium, and NO$_3$: nitrate. n=3 at each sampling time.
Figure 2.10b. Average recovery of \(^{15}\text{N}\) in potassium sulfate extracts from 0 to 5 cm mineral soils that have had \(^{15}\text{N}\)-labeled *Lobaria oregana* additions to *in situ* soil cores in spring. MB: chloroform-labile microbial biomass N, DON: dissolved organic N, NH4: ammonium, and NO3: nitrate. \(n=3\) at each sampling time.
Figure 2.11a. Average recovery of $^{15}$N in potassium sulfate extracts from litter/organic horizons that have had $^{15}$N-labeled Lobaria oregana additions to in situ soil cores in fall. MB: chloroform-labile microbial biomass N, DON: dissolved organic N, NH4: ammonium, and NO3: nitrate. n=3 at each sampling time.
Figure 2.11b. Average recovery of $^{15}$N in potassium sulfate extracts from 0 to 5 cm mineral soils that have had $^{15}$N-labeled Lobaria oregana additions to in situ soil cores in fall. MB: chloroform-labile microbial biomass N, DON: dissolved organic N, NH4: ammonium, and NO3: nitrate. $n=3$ at each sampling time.
The $^{15}$N recovery in 0 to 5 cm mineral soil extracts from the spring organic N and tannin-complexed organic N also did not vary significantly between collection dates, with the exception of dissolved organic N (Figure 2.9b). The $^{15}$N recovery as dissolved organic N from both the uncomplexed organic N and the tannin-complexed organic N increased from day 14 to day 365. The $^{15}$N recovery from the microbial biomass of the uncomplexed organic N addition increased, but this increase was not significant. $^{15}$N recovery from uncomplexed organic N extracts was generally higher than from tannin-complexed organic N.

On the first collection date for the fall and spring Lobaria additions, very little $^{15}$N was recovered in any litter/organic matter extractable pool (Figure 2.10a and Figure 2.11a). 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 six months after which it began to decrease.

The $^{15}$N recovery from all 0 to 5 cm mineral soil extractable pools in the fall and spring Lobaria additions was always much lower than the litter/organic horizon extractable pools (Figure 2.10b and Figure 2.11b). On the first sampling date, little if any $^{15}$N was recovered from the extractable pools in the 0 to 5 cm soil. On subsequent collection dates $^{15}$N recovery as microbial biomass dominated the total recovery in extractable pools for the fall 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.

Density Fractions

$^{15}$N recovery in the light and heavy fractions of 0 to 5 cm mineral soil varied significantly among sampling dates (Figure 2.12). $^{15}$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 light fraction (Table 2.4). $^{15}$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 fraction between organic N and tannin-complexed organic N.

The heavy fraction to light fraction ratio (HF to LF ratio) generally decreased though time (Figure 2.13). Both seasons of ammonium addition had rapid decreases in the HF to LF ratio, but the HF to LF ratio leveled off after day 14. The HF to LF ratio of tannin-complexed and uncomplexed organic N decreased between day 14 and day 365. At day 14 and day 365, the HF to LF ratio of tannin-complexed and uncomplexed organic N was higher than any ammonium additions.
Table 2.4
Average characteristics of the 0 to 5 cm soil density fractions pooled over all collection dates and all N addition types.

<table>
<thead>
<tr>
<th>Density fraction</th>
<th>Total N (%)</th>
<th>Total C (%)</th>
<th>Dry Mass (g/m²)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(SE)</td>
<td>(SE)</td>
<td>(SE)</td>
</tr>
<tr>
<td>Light Fraction</td>
<td>0.646</td>
<td>33.51</td>
<td>1712.3</td>
</tr>
<tr>
<td></td>
<td>(0.014)</td>
<td>(0.78)</td>
<td>(70.8)</td>
</tr>
<tr>
<td>Heavy Fraction</td>
<td>0.162</td>
<td>3.24</td>
<td>27653</td>
</tr>
<tr>
<td></td>
<td>(0.003)</td>
<td>(0.08)</td>
<td>(577)</td>
</tr>
</tbody>
</table>

Notes: All values are on the <4.75 mm fraction. Whole 0 to 5 cm soil values are in Table 2.1. 
n = 72 for Total N and Dry mass, and n = 45 for Total C. Light and heavy were separated using a 1.7 g cm⁻² sodium polytungstate solution.

Polyphenols

Polyphenol concentrations in the litter/organic horizon were elevated initially as a result of the tannin addition (850 µg catechol equivalents g⁻¹, SE=220), but fell to background levels (425 µg catechol equivalents g⁻¹, SE=22) at the later sampling date. Polyphenol concentrations in the 0 to 5 cm soil from the tannin-complexed organic N addition were never significantly different than the background levels (120 µg catechol equivalents g⁻¹ SE=14).
Figure 2.12. Average recovery of $^{15}$N in density fractions of 0 to 5 cm mineral soils that have had $^{15}$N-ammonium or $^{15}$N-labeled *Lobaria oregana* additions to *in situ* soil cores in fall (F) 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. n=3 at each sampling time.
Figure 2.13. Heavy fraction A% $^{15}$N excess to Light fraction A% $^{15}$N excess ratios of 0 to 5 cm mineral soils that have had $^{15}$N-ammonium additions to in situ soil cores in fall or spring or $^{15}$N-labeled organic N or tannin-complexed $^{15}$N-labeled organic N additions to in situ soil cores in spring. n=3 at each sampling time. Note: heavy fraction is the fraction that sinks in a 1.7 g cm$^{-3}$ solution of sodium polytungstate. Light fraction is the fraction that floats in a 1.7 g cm$^{-3}$ solution of sodium polytungstate. Results from Lobaria additions were not shown because of highly variable results, caused by very low levels of $^{15}$N reaching the 0 to 5 cm mineral soil.
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 N added inside tubes, regardless of form, was strongly retained in the whole pools that were measured. Even without active tree roots, the litter/organic horizon, 0 to 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 may be 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.

Neither ammonium, organic N, tannin-complexed organic N nor Lobaria showed any significant change in the $^{15}$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 minutes 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, such as in the Lobaria additions, N retention was always close to 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 et al. 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 only slightly less than zero 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 added probably contributed to this result. Mosses were a strong sink for added N relative to total pool mass. Mosses may therefore play an important role in controlling nutrient inputs, because they had the first opportunity to absorb N inputs. 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 minute collection for the spring and fall
ammonium addition, the $^{15}\text{N}$ recovery in the whole unfractionated litter/organic horizon was approximately equal to the $^{15}\text{N}$ recovered as microbial biomass N (i.e. uncorrected net chloroform labile N) extracted from the same litter/organic horizon. This indicates that most of the $^{15}\text{N}$-ammonium that reached the litter/organic horizon was assimilated into microbial biomass within 40 minutes of N addition. Perakis and Hedin (2001) also found that the microbial biomass pool retained a large portion of added $^{15}\text{N}$ as ammonium and acted as an initial N sink.

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

In contrast to the ammonium additions, $^{15}\text{N}$-labeled Lobaria additions in the spring and fall, both had a gradual increase in the $^{15}\text{N}$ 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 $^{15}\text{N}$ recovery from the extractable pools of N, even when N is made available more slowly.

The paucity of sampling dates for the organic N and tannin-complexed organic N made trends through time difficult to examine. From day 14 to day 365, there were few changes in the amount of $^{15}\text{N}$ recovered in extractable pools for the organic N addition. The tannin-complexed organic N, however, did have some
increases in $^{15}$N recovery as microbial biomass and dissolved organic nitrogen from day 14 to day 365. Perhaps the organic N addition had a peak in $^{15}$N recovery that we missed with our sampling, while the tannin-complexed organic N $^{15}$N recovery was elevated at day 365 because N was slowly being released from tannin-complexes.

The rapid microbial immobilization of added N that we observed in the litter/organic horizon and in the 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 is evidence 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 temporarily eased, but this was not observed.

**Dissolved organic N, 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 to 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 to 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: 1) small inputs and withdrawals from the pools or 2) 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 very unlikely, because it is widely accepted that mineralization and immobilization occur in soils. Indeed, other research has shown that Despite small pool size, turnover rates can be quite fast (Stark and Hart 1997).

Added $^{15}$N could have been either 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 two years in some cases, which 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**

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. The heavy fraction to light fraction ratio of Atom% excess $^{15}$N in 0 to 5 cm soil from the organic N and tannin-complexed organic N additions shows that, even after accounting for the larger total N content in the heavy fraction, the heavy fraction is still a stronger N sink per N molecule than the light fraction. However, the heavy fraction to light fraction ratio of Atom% excess $^{15}$N for 0 to 5 cm soils from the ammonium addition shows that the sink strength per N molecule got stronger in light fraction. As time progressed, light fraction tended to become a stronger sink for $^{15}$N than heavy fraction relative to total N pool size for all N additions. Ionic exchange or other chemical binding of N could occur initially in the heavy fraction of soil. Fungi and other microbes could then acquire N from the heavy fraction to be used to decompose light fraction.

**Effects of Tannin**

Tannin complexation of organic N generally acted to delay normal N cycling processes, but not prevent them. Total $^{15}$N recovery from the tannin-complexed organic N additions was higher than uncomplexed organic N. Tannin complexation increased $^{15}$N recovery as microbial biomass and dissolved organic N at day 365 in the litter/organic horizon over uncomplexed organic N, while it decreased recovery as microbial biomass in the 0 to 5 cm soil. The tannin-complexed organic N addition had more recovery in heavy fraction versus light
fraction than the uncomplexed organic N addition. Tannin complexation of organic matter appeared to reduce the immediate availability of N in the short term, but caused a slow release of N, which resulted in a more sustained N recovery in active pools.

Adding tannin-complexed organic N increased the extractable polyphenols from the litter/organic horizon at the day 14 collection, but not at the day 365 collection. This indicates that the added tannic acid was degraded over time. After a year, and probably much earlier, the tannic acid had been reduced in the litter/organic horizon and polyphenol concentrations in tannin additions were not different than samples without added polyphenols. The 0 to 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.

CONCLUSION

Total $^{15}$N recovery was near 100% for all N additions. Therefore, the forest floor and soil at our site in the H.J. Andrews Experimental Forest have a strong tendency to retain added N, regardless of N form, even in the absence of active tree roots. The litter/organic horizon, as a bulk pool, was the largest N retention pool for all N additions. Within the litter/organic horizon, the chloroform-extractable microbial biomass initially accounted for nearly all of the added N from the
ammonium additions. Although on a different time scale, microbial biomass also played a significant role in the retention of N from organic N, tannin-complexed organic N, and Lobaria. Complexing organic matter with tannin appeared to slow nitrogen cycling, but did not significantly change the ultimate fate of added organic nitrogen. Season of nitrogen addition had little effect on the retention of added nitrogen, although when differences occurred spring $^{15}$N recovery was slightly lower. Our study provides further evidence that microbial biomass plays an active role in initial and continued N retention in low atmospheric deposition sites.

REFERENCES


Bremer, E. and C. van Kessel. 1990. Extractability of microbial $^{14}$C and $^{15}$N following addition of variable rates of labeled glucose and $(\text{NH}_4)_2\text{SO}_4$ to soil. Soil Biology and Biochemistry 22: 707-713.


CHAPTER 3


Scott M. Holub

ABSTRACT

We studied mass loss and nitrogen dynamics during the decomposition of an $\text{N}_2$-fixing epiphytic lichen, Lobaria oregana (Tuck.) Müll. Arg. using $^{15}$N. The use of $^{15}$N-labeled lichen litter enabled the exploration of patterns in N dynamics that could not otherwise be examined without the ability to track the fate of exogenous versus endogenous N. No easy method of labeling lichens with $^{15}$N was available so we developed a method. We sprayed lichen material with a nutrient solution containing $^{15}$N-ammonium on two separate occasions, one in the fall and the other in the spring. This resulted in two batches of lichen that were labeled with $^{15}$N ($>10$ atom % $^{15}$N), but the N concentration in the fall-labeled lichen was slightly elevated by the procedure. Through the first six months of sampling, lichens placed in the field during the spring had a smaller decay constant ($k=1.24 \text{ yr}^{-1}$) than the lichens placed in the field during the fall ($k=3.1 \text{ yr}^{-1}$). Both spring and fall lichens were decomposed beyond recognition after one year in the field. We attributed the higher decay rate of the fall lichen primarily to the effect of
season, because the N concentration of the fall lichen quickly dropped to match the N concentration of the spring lichen.

The fall lichen had a gross loss of N that was proportionally greater than mass loss early in decay. The spring lichen, however, had no gross N loss relative to mass loss early in decay and instead had a net uptake of N. Despite the difference in initial N concentration patterns in exogenous N uptake, gross N loss relative to net N loss, and N concentration did not differ by season. Both spring and fall lichens took up N from the surrounding environment during decay. The N concentration in both lichen additions increased during decay to a peak of 2.8% N, equal to a C to N of 16, and then began to fall. This indicates that early in decay, net immobilization was occurring in the lichen, but later net mineralization was occurring.

INTRODUCTION

*Lobaria oregana* (Tuck.) Müll. Arg. (hereafter *Lobaria*) is the dominant *N₂*-fixing epiphytic lichen in old-growth forests of the Pacific Northwestern United States with up to and exceeding 1000 kg standing biomass per hectare (McCune 1994). Under field conditions, *Lobaria* can add 2.5 to 4.5 kg-N ha⁻¹ every year (Pike 1978, Denison 1979) or roughly 33 to 67% of the total new incoming N to these ecosystems (Sollins *et al.* 1980). *Lobaria* contributes its fixed N to the ecosystem through litterfall decomposition and through leaching (Cooper and
Carroll 1978, Millbank 1985). Based on a growth rate of 10 to 30% per year (Rhoades 1977, Sillett 1994) and assuming an approximate steady state biomass of 1000 kg ha\(^{-1}\), *Lobaria* litterfall is probably around 100 to 300 kg ha\(^{-1}\) yr\(^{-1}\). The amount of litter can be patchy on the landscape and vary from year to year.

N limits plant growth on most terrestrial sites in the Pacific Northwest (Date 1973), so N made available by *Lobaria* could increase or maintain ecosystem productivity by stimulating plant growth. However, the rate of release of N from *Lobaria* litter has not been extensively studied. Lichen decomposition has been examined in a small number of studies (Wetmore 1982, Guzman et al. 1990, McCune and Daly 1994, Knops et al. 1996, Esseen and Renhorn 1998), but no study has used \(^{15}\)N-labeled material to observe N dynamics through decomposition.

\(^{15}\)N-labeled organic material has proven useful for a variety of ecological and agricultural studies of organic matter decomposition and N cycling, because it provides an effective way to follow the fate of N from and within a particular source. Past studies have used \(^{15}\)N as a tracer for N from a variety of sources, including tree leaves (e.g. Berg 1988, Preston and Mead 1995, Swanston and Myrold 1997), agricultural crops (e.g. Jawson et al. 1989, Nicolardot et al. 1995), microbial biomass (e.g. Marumoto et al. 1982, Schnurer and Rosswall 1987), and animal wastes (e.g. Sørensen et al. 1994, Clough et al. 1998).

Many researchers have examined nitrogen dynamics in decomposing plant litter (Melillo et al. 1982, Berg and McClaugherty 1987, Vestgarden 2001). These
studies primarily observed changes in the N concentration or net N dynamics in litter as it decomposed. Authors of these studies were not able to calculate gross rates of transformation, because they had no way of determining the source of the N that they measured in the litter. It was unclear whether endogenous N in the litter had been entirely retained or whether some portion of the endogenous N had been lost and subsequently replaced by exogenous N from the surrounding humus and soil. In contrast, Berg (1988) used $^{15}$N-labeled Scots pine needles to observe both net and gross N dynamics and found that a portion of endogenous litter N is indeed lost during decomposition and replaced by N from other sources.

The objectives of this study were: 1) to develop an easy method of labeling lichen material with $^{15}$N, 2) to examine the mass loss of Lobaria litter as it decomposes on the forest floor, and 3) to examine the patterns of N uptake and loss throughout the decay of Lobaria litter. For objective 1, we tried a new method of labeling Lobaria using a nutrient solution that contained $^{15}$N-labeled ammonium in order to label the lichen without using $^{15}$N$_2$, which can be difficult to manage. With respect to objective 2, we hypothesized that Lobaria will decay relatively quickly compared to leaf litter, because it has a high N concentration and contains little recalcitrant structural carbon (e.g. lignin), which can be difficult to decay. I also hypothesized that there would be an effect of starting date on the annual decay rate of Lobaria litter. Litter placed out in the fall should decay more quickly than the spring because wetter conditions in the fall would facilitate faster decomposition.
With respect to objective 3, I hypothesized that there would be proportionally more gross N loss than mass loss initially as labile N is leached out of the lichen. However, N immobilization was also expected to occur simultaneously as decomposer organisms import N to decompose carbon. I hypothesized that net mineralization would occur because Lobaria is 2.1% N, which represents a C to N ratio of approximately 22 (Assuming 45% C). Litter with an initial C to N ratio that is lower than about 25 tends to result in net N mineralization (Paul and Clark 1996).

METHODS

Site description

This study was performed at a mid-elevation (535 m), old-growth site in the H.J. Andrews Experimental Forest Long Term Ecological Research (LTER) site near Blue River, Oregon (44° 13' 53'' N, 122° 13' 40'' W). The site is classified as a Tsuga heterophylla/Rhododendron macrophyllum/Berberis nervosa habitat type in the Western Cascade Province of the Oregon Cascade Range (Franklin and Dyrness 1988) and is dominated by large Pseudotsuga menziesii (Mirb.) Franco (Douglas-fir) with smaller 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 N₂-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). Soils are andic. For a more detailed site description see Holub and Lajtha (2002).

**Labeling *Lobaria oregana* with **₁⁵**N**

Two months prior to setting out the lichen material to study its decomposition, *Lobaria* litterfall was collected for labeling with **₁⁵**N. Lichen litter was collected from the research site and allowed to air dry at room temperature and then stored for three to four weeks. Approximately 30 grams of clean *Lobaria* thalli were randomly selected and separated into two equal mass groups. Each group was spread out evenly on separate plastic trays. Only the cleanest outer portions of the lichens were used because they had a more consistent baseline **₁⁵**N signal (Holub, unpublished data). Cleanliness was necessary because of the importance of labeling *Lobaria* biomass and not the algal/bacterial biofilm apparent on the older more centrally located portions of lichen.

The thalli were placed in the trays with the green side up and the white side down as they are normally found growing in the field. Using a calibrated spray bottle (1.10 ± 0.03 ml spray⁻¹), the lichens in each tray were gently sprayed with 80 ml of deionized (DI) water and placed on separate racks in a growth chamber (Hoffman Manufacturing, Model SG 30; Albany, Oregon) with 12 hours of low
strength artificial light (100 to 140 μmol PAR m\(^{-2}\) s\(^{-1}\)) at 5-10 °C. Wet paper towels were laid flat on several unused racks in the growth chamber to maintain higher humidity.

Each tray was sprayed daily for 5 weeks with 100 to 150 ml of DI water to allow periodic events of high and low thallus moisture as would occur in the field. Prior to the daily water additions, the lichens were visually dry. The exact amount of water added was difficult to determine because some mist inevitably floated out of the tray. Beginning four days after placement in the growth chamber, and continuing three times a week throughout the study, the final 30 ml of spray per tray was a \(^{15}\)N nutrient solution instead of DI water. The solution contained 566.7 mg L\(^{-1}\) ammonium chloride (98 Atom% \(^{15}\)N, Aldrich Chemical), 61.4 mg L\(^{-1}\) potassium phosphate (monobasic), 59.6 mg L\(^{-1}\) potassium chloride, 11.4 mg L\(^{-1}\) calcium carbonate, and 5.0 mg L\(^{-1}\) magnesium oxide, and was titrated to pH 5.5 using sulfuric acid to minimize NH\(_3\) volatilization while maintaining an ecologically reasonable pH. The ratio of nutrients in the solution on a weight basis were 10 N: 1 P: 3.5 K: 1.7 S: 0.33 Ca: 0.22 Mg and were determined using averages of data from United States Forest Service (1999) on lichen chemistry for \textit{Lobaria} in the Willamette National Forest. A total of 670 ml of this solution was added over four weeks equaling 5.4 mg \(^{15}\)N per g of dry \textit{Lobaria}. Spraying with DI water continued for an additional five days to allow the final \(^{15}\)N additions to be assimilated.
The $^{15}$N-labeled lichen pieces were dipped in seven successive 250 ml beakers filled with 150 ml DI water to remove any residual unassimilated $^{15}$N that might occur on the surface of the lichen (Miller and Brown 1999). The water in the beakers was changed after every two grams of lichen were rinsed. The rinsed lichens were allowed to air dry overnight at 12 °C. The following day the dried *Lobaria* were weighed and a 50 to 100 mg portion of 10 separate pieces, 5 from each tray, were ground to 40 mesh and submitted along with three unlabelled *Lobaria* samples for Atom% $^{15}$N and percent total N analysis. The entire process was repeated twice to have fresh tissue for each season of addition, once in September 1999 (fall) and once in March 2000 (spring).

**Plot installation**

Large PVC tubes (15.25 cm diameter by 40 cm length) were driven into the ground to a depth of 35 cm to act as in situ containers of *Lobaria*, forest floor and soil. These tubes were installed as part of a concurrent $^{15}$N-tracer study (see Holub and Lajtha 2002). Using these tubes, as opposed to using the more standard nylon litter bags, has the advantage of not inhibiting the movement of soil fauna, which have been shown to increase decomposition at some sites (Blair *et al.* 1992) and could affect N retention and decomposition. The tubes were installed two months prior to adding *Lobaria* in October 1999 (fall) and April 2000 (spring). Labeled *Lobaria* was added in 0.8 g portions to each tube in the fall and spring, which
approximates the 5 kg N ha\(^{-1}\) added annually by the lichen under ambient conditions.

**Sample collection**

Three tubes from both the fall and spring addition were collected at 14 days, 3 months, 6 months, and 1 year post addition. 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. Visible *Lobaria* was removed from the tubes at dried at 60 °C. Total dry mass was determined and samples were then ground to 40 mesh in a Wiley mill and submitted for Atom% \(^{15}\)N and total N analyses.

\(^{15}\)N and total N analyses

Nitrogen isotope (\(^{15}\)N) analyses were performed at the U.C. David Stable Isotope Facility, Davis California using a Europa Scientific Integra continuous flow mass spectrometer equipped with Dumas combustion/reduction to simultaneously determine total N and thus N concentration in the samples.

Calculations and statistics

All statistics were performed using The SAS System version 8.01. To determine if the lichen was sufficiently labeled with \(^{15}\)N, Atom% \(^{15}\)N values of the
labeled lichen were compared with unlabeled control lichens using ANOVA. The N concentration in the $^{15}$N-labeled lichen tissue was also compared with unlabelled control lichens to determine if any increases in %N had occurred as a result of the $^{15}$N labeling.

The rate of mass loss or decay rate of *Lobaria* litter was determined using an exponential decay curve that was fit to the mass remaining data (eq. 3.1) plotted versus time. Dates with no mass remaining or very small mass remaining were omitted from the curve, because they had an inordinately large effect on the shape of the curve. To determine if the decay rates differed by season, a general linear model was used to test for significant interaction of season and time.

$$
\text{eq. 3.1 Mass remaining (\%) = \frac{\text{final mass}}{\text{initial mass}} \times 100}
$$

Nitrogen dynamics in the litter were examined by plotting net N lost (eq. 3.3) versus mass lost (eq. 3.2), gross N lost (eq. 3.4) versus mass lost, and gross N lost versus net N lost. Mass lost, net N lost, and gross N lost were expressed as a percent of original amount to account for fragmentation losses and for slight differences in starting mass. To determine if any of the data differed by season a general linear model was used to test for significant interaction of season and time.
eq. 3.2 Mass loss (%) = 100 - (Mass remaining)

eq. 3.3 N_{Net} lost (%) = (1 - (final N content / initial N content)) x 100

eq. 3.4 N_{Gross} lost (%) = (1 - (final \textsuperscript{15}N content / initial \textsuperscript{15}N content)) x100

Data from the three graphs were divided into two data ranges: less than 10 percent mass lost (<10% mass lost) and greater than or equal to 10 percent mass lost (≥10% mass lost). This distinction was made because patterns differed between the two data ranges. Decomposition data that show different patterns over different regions are common (Aber et al. 1990). The ≥10% mass lost data often showed clear linear trends, while the <10% mass lost data had different and/or less predictable trends. Using the statistics calculated from the ≥10% mass lost data enabled numerical interpretation of overall patterns of N dynamics.

Linear regressions were calculated on the <10% mass lost data and the ≥10% mass lost data. Only those regression lines that were significantly different from a 1:1 line were shown on the graphs. The regressions were compared to a 1:1 line, because the amount of N lost (gross or net) is autocorrelated with the amount of mass lost. The total mass of litter consists of nitrogen in a matrix of other material, so when a portion of litter mass is lost, N is also lost. Comparing the regressions with a 1:1 line allows observation of deviation from the expected autocorrelation. Points below the 1:1 line indicate that a net decrease in the y-axis variable occurred at some time during decomposition as compared to the x-axis.
variable. Points above the 1:1 line indicate that a net increase in the y-axis variable has occurred as compared to the x-axis variable.

Linear regressions with a significant intercept, but with a slope that was not significantly different from the 1:1 line occurred frequently in the ≥10% mass lost data. A significant intercept indicates that a difference existed between the net change in the data versus the 1:1 line during the <10% mass loss range. The value of the intercept is equal to the magnitude of this difference. A statistically significant slope indicates that the data changed at a rate different than the 1:1 rate.

N concentration in remaining tissue was plotted as a function of mass lost to observe changes in the concentration of N within the remaining tissue over decay. N concentration alone can be a misleading indicator of nitrogen dynamics, because as carbon and mass are lost through decomposition, N concentrations could increase. This increase in N concentration may indicate net immobilization, but a preferential loss of C relative to N could also account for some or all of any apparent increase in N concentration. (Holub et al. 2001). To determine if the N concentration versus mass lost data differed by season, a general linear model was used to test for a significant interaction of season and time.

Exogenous N inputs to litter were calculated as a percent of the total existing N in the remaining tissue (eq. 3.5). The calculation of exogenous N in the tissue depends heavily on the assumptions that the N in litter tissue is evenly labeled with $^{15}$N, and that any loss of N from the lichen has the same $^{15}$N signature.
as the remaining lichen. Any increase in the net N amount over the gross N (i.e. $^{15}\text{N}$) amount can be interpreted as an increase in exogenous N. The percent exogenous N in remaining tissue was then plotted as a function of mass lost. To determine if the exogenous N differed by season a general linear model was used to test for significant interaction of season and time.

\[
\text{eq. 3.5} \quad \%N_{\text{exog}} = \frac{(100 - N_{\text{Net} \text{ lost}}) - (100 - N_{\text{Gross} \text{ lost}})}{(100 - N_{\text{Net} \text{ lost}})} \times 100
\]

Where: $\%N_{\text{exog}}$ is the percent of N in the tissue that is from sources outside the tissue (e.g. humus, and soil).

RESULTS

Labeling outcome

The method of labeling Lobaria with $^{15}\text{N}$ using $^{15}\text{N}$-enriched ammonium was successful, although it increased the N concentration of the fall-labeled lichen to abnormally high levels. Fall-labeled Lobaria tissue averaged 2.75% N (SE=0.09, n=10), which was a significant increase ($p=0.04$) over unlabeled lichen (2.31% N, SE=0.05, n=3). The spring-labeled tissue was 2.28% N (SE=0.08, n=5) and was not significantly different than unlabelled lichen ($p=0.4$). The total dry mass of fall-labeled lichen was unchanged ($\pm1\%$) from the beginning of the labeling process (n=1). However, the total dry mass of spring-labeled lichen
increased by about 5% (from 28.0 g to 29.5 g) during labeling (n=1). *Lobaria* from the fall labeling experiment contained an average of 16.5 Atom% $^{15}$N (SE = 1.2, n=10) while the spring addition contained an average of 11.3 Atom% $^{15}$N (SE = 0.3, n=5). Both Atom% $^{15}$N values were significantly greater (p<0.0001) than the unlabeled lichen (0.3664 Atom% $^{15}$N, SE=0.0001, n=3).

**Mass loss**

The decay curves (Figure 1) differed by season of placement in the field (p<0.0001). The spring lichen had a decay constant (k) of 1.24 yr$^{-1}$ (SE=0.09) and an intercept of 100% remaining (SE=1.03) over the first six months, and was completely decayed after one year. The fall lichen had a larger decay constant (k) of 3.1 yr$^{-1}$ (SE=0.3) over the first six months with an intercept of 106% remaining (SE=1.08), and was also completely decayed after one year.

**Nitrogen dynamics**

The net nitrogen versus mass lost data (Figure 3.2) were different between the two seasons of addition (p<0.001). The net N lost from the ≥10% mass lost data for the spring lichen was below what would be expected if net N loss was equal to mass loss (i.e. it was lower than the 1:1 line) (intercept 19.6%, SE=1.6, p=0.0003). This provides evidence for no net loss of N prior to 10% mass loss, but because N was lost at a faster rate than carbon after 10% mass loss (slope=1.19, SE=0.04,
p=0.01), the y-intercept could not be used directly to determine the scale of the loss. The x-intercept, however, indicates that 17% of the mass was lost before any significant N loss occurred. The <10% mass lost data for the spring lichen and the \( \geq 10\% \) mass lost data for the fall lichen were not significantly different than the 1:1 line (\( p=0.6, p=0.72 \) respectively). However, the <10% mass lost data for the fall lichen showed a net loss of N that was significantly greater than mass loss as represented by the 1:1 line (slope=2.20, SE=0.31, \( p=0.03 \)).

The gross N versus mass loss data (Figure 3.3) were different between the two seasons of addition (\( p<0.0001 \)). The \( \geq 10\% \) mass lost data for the fall lichen showed a gross loss of N that was 13.5% greater than what was predicted by the 1:1 line (SE=2.8, \( p=0.0085 \)), and the rate of gross N lost versus mass lost was not different than the 1:1 line over this range (\( p=0.1 \)). The <10% mass loss data for the fall lichen as well as the <10% and \( \geq 10\% \) mass lost data for the spring lichen showed no difference between the observed gross N lost versus mass lost and the 1:1 line (\( p=0.1, p=0.48, p=0.92 \) respectively). There were no significant differences between seasons for gross N loss versus net N loss data (\( p=0.58 \), Figure 3.4), so data were pooled for the regression analysis. The <10% mass lost data for both seasons showed no differences between the 1:1 line for gross N lost versus net N lost (\( p=0.1 \)). However, the \( \geq 10\% \) mass lost data indicated that a 14% greater gross loss of N versus net loss of N had occurred for both seasons (SE=2.3, \( p=0.0002 \)). The slope was not different from the 1:1 line (\( p=0.06 \)).
Figure 3.1. Mass remaining in *Lobaria* over time. Equations are of exponential decay omiting values at one year because those values were very close to zero an had inordinate influence on the curves.
Figure 3.2. Net N lost from *Lobaria* tissue versus mass loss. Only regressions that were significantly different than the 1:1 line were plotted (p<0.05).
Figure 3.3. Gross N lost from *Lobaria* tissue versus mass lost. Only regressions that were significantly different than the 1:1 line were plotted (p<0.05).
Figure 3.4. Gross N lost from *Lobaria* tissue versus net N lost. Spring and Fall lichen did not differ from each other (p>0.05), but did differ from the 1:1 line. They are shown in the combined regression line.
Figure 3.5. Nitrogen concentration versus mass lost. Spring and Fall lichens did not differ in patterns of N concentration and are shown in one regression. Initial N concentrations were omitted from the curve.
Figure 3.6. Exogenous N in remaining lichen tissue versus mass loss. Spring and Fall lichens did not differ in patterns of exogenous N concentration.
After excluding the initial N concentrations, the N concentration versus mass lost data (Figure 3.5) did not differ by season (p=0.12) so data were pooled for analysis. The N concentration in remaining lichen from both seasons showed a significant quadratic relationship with mass lost (p=0.003, \( r^2 = 0.54 \)). Nitrogen concentrations gradually increased to a peak around 2.8% N (SE=0.09) and then decrease.

The percentage of exogenous N in the remaining lichen tissue increased as mass loss increased (p=0.01 for the linear regression, Figure 3.6). The rate of increase was not significantly different by season (p=0.3). The slope of the combined regression line was 0.22 (SE=0.07). The intercept was 5.7%, but was not significantly different from zero (p=0.07). The regression line explained only 37% of the variation in the data.

**DISCUSSION**

15N-labeling method

Since N deposition is low in Pacific Northwest old-growth forests where *Lobaria* is found, the lichen probably obtains much of its N from N\(_2\) gas via N fixation by its cyanobacterial symbiont, *Nostoc*. Therefore, a logical way to label *Lobaria* with 15N, would be to grow it in a 15N enriched N\(_2\) environment. However, constructing and maintaining a 15N enriched N\(_2\) environment to grow *Lobaria*
would be difficult (see Millbank and Olsen 1981). N₂-fixing lichens are able to take up ammonium when it is available (Rai et al. 1983, Rowell et al. 1985, Miller and Brown 1999) and ammonium derived N and N₂ derived N are likely to have very similar fates in a lichen thallus, because the N fixing symbiont passes N to the fungal symbiont as ammonium (Rai et al. 1983, Rowell et al. 1985).

The labeling procedure, when performed on fall-collected lichen, produced material that, in addition to the desired increase in Atom% ¹⁵N, had an undesired increase in N concentration. The increase in N concentration was probably due to a lack of growth of the fall lichen. Although the fall lichen was able to take up the ¹⁵N, it apparently was not able to assimilate it into new growth. The spring collected lichen, however, had an increase in Atom% ¹⁵N without the undesired increase of N concentration seen in the fall-labeled lichen. Perhaps the spring lichen was able to utilize the added ¹⁵N to form new growth and maintain a constant N concentration. Muir et al. (1997) showed that Lobaria pulmonaria, a closely related species, grew fastest in spring months, so applying ¹⁵N to lichens during their growth phase appears to produce lichen material that is labeled with ¹⁵N, yet still has normal concentrations of N. Because the spring lichen had initial N concentrations that were not elevated, it probably best represents the decomposition patterns and N dynamics of naturally occurring Lobaria.
Mass loss

The spring lichen decay constants observed in this study (1.24 yr⁻¹, Figure 3.1) were similar to decay constants reported in past studies of *Lobaria* and other N₂-fixing species. McCune and Daly (1994) report a half-life (ln 2/k) of 7.0 months (k=1.2 yr⁻¹) for unenclosed *Lobaria oregana* placed in the field in late spring at the H.J. Andrews LTER. Esseen and Renhorn (1998) report a k of 0.96 yr⁻¹ for *Lobaria pulmonaria* in litterbags. Guzman *et al.* (1990) report k values of 2.2 to 0.45 yr⁻¹ for *Pseudocyphellaria* species in litterbags.

In contrast, the fall lichen decay constant was much higher (3.1 yr⁻¹) than in other studies and than the spring lichen. Although season of addition was confounded by a slightly higher initial N concentration, we attribute the increase in the fall lichen decay rate primarily to the effect of season, because the N concentration of the fall lichen quickly dropped to match the N concentration of spring lichen. The wetter conditions in the fall may have made conditions more favorable for decomposition. Statistical analyses of the current data, however, make it impossible to positively conclude that the higher decay rate was due to the wetter winter weather, because the high initial N concentration and the season of addition are confounding factors.

The decay constants for other lichens are usually much higher than the spring decay constant for *Lobaria* observed in this study. Decay constants as high as 5.5 and 3.3 yr⁻¹ for unbagged *Alectoria sarmentosa* and *Hypogymnia inactiva*
have been reported (McCune and Daly 1994). These high rates would not be predicted based on N concentration alone, because Alectorria and Hypogymnia have N concentrations around 0.4% N, while Lobaria has an N concentration of 2.2% N. One possible reason for this deviation from the standard C to N correlation, is that Lobaria produce chemicals that may inhibit decomposition. Stictic acid, norstictic acid, and constictic acid are all compounds found in Lobaria oregana (Culberson 1969, Culberson 1970, Culberson et al. 1977). While it is not clear that these compounds inhibit decomposition, they all contain at least one phenolic group. As a general chemical group, polyphenols have been shown to slow decomposition (Hättenschwiler and Vitousek 2000).

Broadleaf and coniferous tree leaves have lower k values than the values for Lobaria (about 0.4 to 0.8 yr⁻¹ and 0.3 to 0.4 yr⁻¹ respectively (Aber et al. 1990)). The k values cited are, however, from litterbag studies and are probably inherently lower than unbagged k values. Lobaria decomposed in litterbags by McCune and Daly (1994) had a decay constant of 0.64 yr⁻¹, which is very similar to broadleaf decay constants.

Lichen material in litterbags has been shown to yield 50 to 90% lower decay constants than free material (McCune and Daly 1994). Lobaria oregana falls at the low end of the differences between bagged and unbagged decay constants. While a 50% reduction in the decay constant is large, its relatively small size in comparison to other litterbag-related reductions indicates that, of the lichens
examined by McCune and Daly (1994), *Lobaria* was the least affected by the litterbags. McCune and Daly (1994) suggest that this indicates little or no consumption by larger organisms. Their inference is supported by data from the other portion of this study (Holub and Lajtha 2002), where nearly 100% of the $^{15}$N lost from *Lobaria* into the surrounding environment was recovered. Recovery of all of the N in very close proximity to where the lichens were placed provides substantial evidence that the lichen was not carried away by larger organisms. The same lichen compounds that were hypothesized to inhibit decomposition in *Lobaria*, may likewise discourage browsing by larger animals. The existence of phenolics would also explain why such an apparently nutrient rich (>2% N) lichen is not commonly eaten by any known vertebrates, while *Alectoria spp.* with lower N concentrations (~0.4% N) are eaten readily.

**Nitrogen dynamics**

As previously discussed, the reasons for differences between the fall- and spring lichens for many of the measures of nitrogen dynamics are unclear. They are probably due to wetter conditions in the fall or to the elevated initial N concentration in the fall-labeled lichen or to an interaction of those two factors.

The spring lichen lost 17% of its mass before any significant net loss of total N occurred. Yet, gross losses of N, were proportional to mass loses over the entire range of mass lost in the spring lichen. These data are strong evidence for
the net uptake of N in the spring lichen litter during the early stage of decay. In later stages of decay, however, the spring lichen had a net loss of N that was 19% faster than mass loss. Recall that gross N losses were proportional to mass loss over the entire range of mass loss. The combination of gross and net N loss data indicates that the spring lichen must have lost some of the exogenous N it originally gained. Therefore, early in decay, the spring lichen litter, or its decomposers, acquired N from the surrounding litter and soil and then lost some of the N it acquired later in decay.

The fall lichen litter had a net loss of N that was 120% faster than mass loss early in decay. This yielded a 12% net N loss relative to mass loss during the first 10% mass lost. The net loss of N relative to mass was concurrent with a gross loss of N relative to mass. The y-intercept of the regression line for the ≥10% mass lost region of the gross N lost versus mass lost graph indicates that the fall lichen lost 13% more endogenous N early in decay than would have been predicted by mass loss alone. The net and gross loss of N relative to mass, lowered the N concentration of the remaining lichen very quickly, and may have been the result of the lichens' elevated initial N concentration. After the early stage of decay, which lowered the N content of and N concentration in the litter the fall lichen behaved similarly to the spring lichen.

The results of the gross N lost versus net N loss graph, were not different by season of lichen addition. The intercept in the gross N lost versus net N lost graph
was the same for both seasons and showed that gross N losses occurred at a faster rate than net N losses initially, but the gross N losses occurred at the same rate as net N losses later in decay. The mechanisms of attaining this pattern, however, were quite different between the season of lichen addition and thus between initial N concentrations. The spring lichen had a net gain of N relative to mass initially, but had a gross loss of N that was equal to mass loss later in decay. An inverse pattern was seen in the fall lichen, which had no change in net N relative to mass initially, and instead had an initial gross loss of N relative to mass.

The percentage of exogenous N in remaining litter increased linearly as mass was lost. This rate of increase was not different between season of addition and therefore was not different between the initial N concentrations. These data indicate that N from sources outside the lichen were imported into the lichen as it decayed, although the curve did not explain much of the variation in the data. A calculation of exogenous N was reported in lieu of raw Atom% $^{15}$N values, because both presentations of the data demonstrate the same phenomenon. A decrease in the Atom%$^{15}$N was observed as the lichens decayed, presumably because exogenous N was diluting the $^{15}$N signature with unlabeled N. Import of exogenous N into the lichen is the only way the Atom% $^{15}$N values could decrease in remaining lichen material, provided that two assumptions are met. The lichen must be evenly labeled with $^{15}$N and there must be no isotope fractionation during the loss of endogenous N.
N concentrations, for both the spring and fall data, excluding initial N concentrations, increased in the remaining litter as mass was lost up to a critical value of about 2.8% \( N \), equal to a C to N of 16, where the N concentration levels off and then begins to fall. This provides evidence that the critical C to N value appears to be the same regardless of original N concentration of the lichen.

Although the fall lichen had a higher initial N concentration, it had lost N relative to mass early in decay, which caused N concentrations to decrease to a spring level. The critical C to N of 16 may only apply at this site in the H.J. Andrews LTER and only be applicable for *Lobaria* litter. Further investigation of the critical C to N ratios of other litter types at other sites may support or refute this number.

Reaching and surpassing the critical C to N ratio is unusual among litter decomposition studies. Other studies usually find that N concentrations increase linearly as mass is lost (e.g. Aber and Melillo 1980, Melillo *et al.* 1982, McClaugherty *et al.* 1985, Aber *et al.* 1990). These studies however are on material that contains lignin, which lichens do not. The maintenance of a continuous linear increase in N concentration as mass is lost has been questioned by McClaugherty *et al.* (1985). McClaugherty *et al.* (1985) also discuss the concept of a critical C to N ratio at which the N concentration would cease to increase with mass loss. It appears that *Lobaria* is prone to reaching this critical C to N ratio as it decomposes. The moderately high decay rates, high initial N concentration, and lack of lignin-type compounds in *Lobaria* may have contributed to reaching this
critical level so quickly. Carbon and N characteristics of the environment
surrounding the litter being studied certainly play a role as well (Aber and Melillo
1980).

CONCLUSION

Labeling the N₂-fixing lichen Lobaria oregana with ¹⁵N using a nutrient
solution containing ¹⁵N-enriched ammonium was successful, with one caveat. To
avoid an undesired increase in the N concentration of the lichen, additions of ¹⁵N
should be applied during the lichen's growing season, which in this case was the
spring.

Lobaria litter placed out in the fall decayed faster (k=3.1 yr⁻¹) than litter
placed out in the spring (k=1.24 yr⁻¹). Although, the season effect was confounded
by the higher initial N concentration in the fall lichen, we attributed the effect
primarily to the wetter conditions in the fall, because the N concentration of the fall
lichen dropped to spring lichen levels at the first sampling date.

There was proportionally more gross N loss than mass loss early in decay
for the fall lichen as hypothesis 3 stated, but this gross loss did not occur in the
spring addition lichen. However, the spring lichen demonstrated a net
immobilization of N, while the fall lichen did not. Patterns in exogenous N uptake,
gross N lost versus net N lost, and N concentration did not differ by season. Both
lichens, regardless of season of addition or initial N concentration, had a positive
trend in exogenous N uptake over decay. Both lichen additions showed more gross N loss relative to net N loss initially, with equal rates of gross N loss relative to net N loss later in decay. Both lichen additions had a peak in N concentration during decay at 2.8% N, equal to a C to N of 16. This non-linear trend in N concentration during decay was often not seen in past studies, but it had been predicted (McClaugherty et al. 1985).

Using $^{15}$N-labeled Lobaria litter enabled the exploration of patterns in N dynamics that could not otherwise be examined without the ability to track the fate of exogenous and endogenous N. While the ingenuity of study design and creativity of data analyses in past studies has yielded valuable information about the N dynamics in litter, more studies should undertake the added effort of labeling material with $^{15}$N, so that a better understanding of N dynamics during decomposition can be gained.

REFERENCES


Holub, S.M. and K. Lajtha. 2002. The fate of $^{15}$N-labeled ammonium, organic nitrogen, tannin-complexed organic nitrogen, and N$_2$-fixing lichen in an old-growth coniferous forest soil. This volume Chapter 2.


CHAPTER 4:

MODELING THE EFFECTS OF ADDED NITROGEN ON TERRESTRIAL CARBON SEQUESTRATION

Scott M. Holub

ABSTRACT

The concentration of carbon dioxide (CO$_2$) in Earth's atmosphere has been increasing since the industrial revolution. While many factors contribute to this increase, fossil fuel burning is widely believed to be the primary cause. If CO$_2$ concentrations continue to rise, widespread climate changes are predicted to occur. A reduction in CO$_2$ emissions would be the ideal method to control CO$_2$ concentrations. However, if CO$_2$ emissions can not be reduced globally, other methods of sequestering carbon need to be explored. The objectives of this paper were 1) to investigate the effect of added N on terrestrial carbon sequestration and 2) to explore the possible use of N fertilizer to offset carbon emissions. Because N is usually the most limiting nutrient in temperate terrestrial ecosystems, I hypothesized that adding N to land plants should cause the plants to grow bigger and therefore sequester more carbon. To test this hypothesis I ran several simple models to examine how much C would be sequestered by adding N, taking into account that fertilizer production also releases carbon. The results of various model
runs show that adding N, especially to the most N limited forests, could sequester large amounts of carbon. This does not mean that clean air standards should be lowered or that widespread fertilization should begin immediately to sequester more CO$_2$, because excess N in the environment has a variety of deleterious effects. However, if a significant reduction in CO$_2$ emissions cannot be accomplished by other means, adding N may be one possible method to remove CO$_2$ from the atmosphere to reduce the risk of global warming. Although further study is needed, these results indicate that the wise use of N$_2$-fixing plants and N fertilizer on the most N-limited sites, especially high C:N forests, would increase C sequestration, at least temporarily, yet limit the potentially harmful effects of excess N.

**INTRODUCTION**

Global atmospheric CO$_2$ concentrations have been increasing since the industrial revolution. The preponderance of evidence suggests that the main cause of this increase is fossil fuel burning (Tans et al. 1990, Keeling et al. 1996), although other factors such as deforestation and intensive agriculture also play a role (Post et al. 1990, Tans et al. 1990). Since CO$_2$ is a greenhouse gas, its continued emission to the atmosphere is expected to result in global warming or other climate changes. However, the amount of atmospheric CO$_2$ has not increased as much as the 5.7 to 7.9 Pg C yr$^{-1}$ that known sources have emitted (Tans et al. 1990). Models indicate that oceanic CO$_2$ uptake is responsible for some, but not all
of this discrepancy, and that a carbon sink of as much as 2.0 to 3.4 Pg C yr\(^{-1}\) (Tans et al. 1990) or as little as 1.5 to 1.9 Pg C yr\(^{-1}\) (Nadelhoffer et al. 1999) exists on land, possibly North America (Fan et al. 1998).

Several mechanisms of terrestrial CO\(_2\) sequestration have been investigated, but no real consensus has been reached. Many possibilities exist to explain the missing carbon sink including land-use changes (Casperson et al. 2000), carbon burial (Stallard 1998), CO\(_2\) fertilization (Idso and Kimball 1993) and N fertilization (e.g. Schindler and Bayley 1993, Townsend et al. 1996, Holland et al. 1997, Nadelhoffer et al. 1999). N fertilization, in particular, can positively affect plant growth and carbon sequestration because N is widely accepted to be the limiting nutrient to plant growth in most temperate terrestrial ecosystems (Vitousek and Howarth 1991). Additions of N may allow plants to better utilize increasing CO\(_2\) concentrations (Oren et al. 2001).

N is added to ecosystems through many pathways, but humans have added more N (about 140 Tg N yr\(^{-1}\)) to the biosphere than all other natural methods combined (Galloway et al. 1995, Vitousek et al. 1997). Intentional fertilization of fields and forests by humans is perhaps the most obvious pathway of N addition, but others, such as N\(_2\)-fixation and atmospheric deposition, occur as well. Fertilizing fields with animal manure has been performed since early agriculture began and more recently industrial production of N fertilizers from N\(_2\) gas has increased dramatically (Smil 2001). Additionally, humans plant N\(_2\)-fixing crops to
increase N in the soil. This N adds to the N-fixing capacity already present in the biosphere from many naturally occurring N-fixing trees, plants, and lichens. Atmospheric deposition from N pollution is an unintentional anthropogenic source of N, but can contribute 20 Tg N yr\(^{-1}\) or more globally (Vitousek et al. 1997). Asymbiotic N\(_2\)-fixation and lightning-fixed N are also potential sources of N, but they are negligible compared to other anthropogenicly influenced sources.

The objectives of this paper are 1) to investigate the effect of added N, in its various forms, on terrestrial carbon sequestration and 2) to explore the possible use of N fertilizer to offset carbon emissions. N additions can affect net primary production and heterotrophic respiration in different ways. Although both processes are influenced by a variety of environmental and physiological variables, this paper will focus primarily on N effects. By combining the individual effects of N on net primary production (NPP) and heterotrophic respiration (R\(_h\)) using simple models, a range of net carbon sequestration resulting from N additions will be presented in the form of net biome production (NBP) estimates. I will also use a more complex carbon sequestration model to explore the effects of added N. By using two approaches more robust inferences can be made.

**NITROGEN SOURCES AND CARBON SEQUESTRATION**

When N-limited terrestrial plants are provided with more N their response is to grow faster and larger and therefore sequester more CO\(_2\) into their living
biomass, provided that other growth limiting factors are not encountered. Sequestration of C in biomass, however, is somewhat temporary and often fleeting, because plants die and decompose returning a portion of the CO₂ to the atmosphere. The amount of carbon in soil is about 2/3 of all terrestrial carbon storage (Schimel et al. 1994). Therefore, any process that might control carbon fluxes into and out of soil needs to be examined closely and all C expenditures included.

The gain in soil carbon achieved by adding N could be due not only to an increase in C inputs, but also to a direct effect on soil organic matter decomposition. Adding outside N to organic matter has been shown to have mixed effects on the rate of organic matter decomposition (Fög 1988). By lowering the C:N ratio or lignin:N ratio of organic matter, added N could increase decomposition rates by making organic matter more palatable for microbes. However adding N can cause shifts in enzyme production that could reduce decomposition of more recalcitrant organic matter (Carreiro et al. 2000). To understand and model the effect of added N on decomposition it is important to know the characteristics of the organic matter of interest.

Schlesinger (2000) reviewed several long-term agricultural studies of net carbon sequestration in soil with added fertilizer N. Results were variable, but data from a variety of continuous and rotational cropping systems showed that, even after accounting for CO₂ emissions from N fertilizer (1.2 g C per g N), adding N
often resulted in net C sequestration in soil. However, the expenditure of carbon on
the acquisition of N lowered the apparent C sequestration by around 50%, with
results ranging from a low of 20% (still a gain of carbon) to 465% (large net loss of
carbon). Because cultivation generally causes large reductions in soil C (Parton et
al. 1987), any method to create a net gain of carbon in agricultural soil could be
potentially important not only to sequester CO₂, but also to mitigate further
reductions in soil carbon. Similarly, in forested ecosystems adding N as fertilizer
or through N-fixing trees often increases total soil carbon (Johnson and Curtis
2001, Johnsen et al. 2001). The pathway in which N is added to the ecosystem (N
fertilizer, N deposition, or N₂-fixation) as well as the form of N (NH₃, NO₃⁻, or
various organic N forms) could affect overall C sequestration. If N is only weakly
limiting adding N may cause other factors to limit growth and would not increase C
sequestration.

Nitrogen fertilizer

Rates of fertilizer application to farmland are around 100 to 300 kg N ha⁻¹
yr⁻¹ (Brady 1990) or about 80 Tg N yr⁻¹ worldwide (Galloway et al. 1995, Vitousek
et al. 1997). The per hectare rate may seem excessive, but much of the added N is
removed during crop harvest (Brady 1990). Manure has been a common fertilizer
in agriculture. While it adds valuable organic matter and nutrients to croplands, it
is not a true biosphere source of either. The organic matter and N contributed to
soil from manure had to pass through animals as feed from another field. So while manure supplements one field, it draws nutrients from another field resulting in no net gain of biosphere N and a loss of CO₂ compared to if it was left on the original field as plant residue (Schlesinger 2000).

The Haber-Bosch process of industrial ammonia production was initially developed in 1909 (Smil 2001). This process results in a stoichiometric release of 0.375 moles of C as CO₂ per mole of N (Eq. 4.1 and Eq. 4.2, Schlesinger 2000) or about 0.321 kg of C released per kg of N produced.

\[
\text{Eq. 4.1} \quad 3\text{CH}_4 + 6\text{H}_2\text{O} \rightarrow 3\text{CO}_2 + 12\text{H}_2 \\
\text{Eq. 4.2} \quad 4\text{N}_2 + 12\text{H}_2 \rightarrow 8\text{NH}_3
\]

There are, however, other energy costs associated with the high temperature, high pressure Haber-Bosch process, although these costs have been decreasing as the process has been made more efficient (Smil 2001). The most efficient ammonia production plants use about 27 GJ of energy per ton of NH₃, while the global average is about 45 GJ per ton of NH₃ (Smil 2001). Cole et al. (1993) estimated that 1.5 kg of C is released per kg of N after accounting for the full CO₂ emissions from production, transport, and application of N. If we assume that the average plant is 2% N and 50% C by mass (C:N=25, which is conservative considering that some trees can have C to N ratios of greater than 500) and that N
added as NH₃ is from 10 to 50% available to plants, then for every kilogram of N fertilizer added per hectare effectively 1 to 11 kg of C would be sequestered into plant biomass after accounting for N production cost, provided that no other nutrient limitations become important (see Table 4.1 for other possibilities). Table 4.1 shows that with poor N utilization and low plant C to N ratios, fertilizing with ammonia can cause a net loss of CO₂ even without accounting for decomposition losses of CO₂. At high N utilization and high C:N, however, a substantial amount of C could be sequestered in living biomass by adding as little as 10 kg-N ha⁻¹. There are limitations to how long N additions would continue to produce a large positive effect on plant growth. As more N is added to plants, the growth response to N will be reduced.

Table 4.1 Effective carbon in plant biomass per nitrogen added (kg/ha) †

<table>
<thead>
<tr>
<th>Total Plant C:N Ratio †</th>
<th>10</th>
<th>20</th>
<th>30</th>
<th>50</th>
<th>100</th>
<th>200</th>
<th>500</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitrogen</td>
<td>1</td>
<td>-1.4</td>
<td>-1.3</td>
<td>-1.2</td>
<td>-1.0</td>
<td>-0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>Utilization by Plants</td>
<td>10</td>
<td>-0.5</td>
<td>0.5</td>
<td>1.5</td>
<td>3.5</td>
<td>8.5</td>
<td>18.5</td>
</tr>
<tr>
<td>(%)</td>
<td>20</td>
<td>0.5</td>
<td>2.5</td>
<td>4.5</td>
<td>8.5</td>
<td>18.5</td>
<td>38.5</td>
</tr>
<tr>
<td>50</td>
<td>3.5</td>
<td>8.5</td>
<td>13.5</td>
<td>23.5</td>
<td>48.5</td>
<td>98.5</td>
<td>248.5</td>
</tr>
<tr>
<td>100</td>
<td>8.5</td>
<td>18.5</td>
<td>28.5</td>
<td>48.5</td>
<td>98.5</td>
<td>198.5</td>
<td>498.5</td>
</tr>
</tbody>
</table>

Note: The line separates a net loss or no change of carbon from carbon gain † Includes total CO₂ emissions associated with N production, transportation, and application (1.5 kg C/1.0 kg N). Assumes no other nutrient limitation. ‡ weighted average of the ratio of carbon mass to N mass for all tissues ≠ ratio of plant N uptake to total N added multiplied by 100 (See Table 4.2 for estimates Net Biome Production resulting from added N)
Nitrogen deposition

Atmospheric N deposition is another pathway N is made available for plant growth. N deposition can occur as N oxides, ammonia, or organic N. Measuring the amount of N input to the biosphere by these sources can be difficult, but is relatively straightforward compared to calculating all of the CO$_2$ emissions related to each source. The amount of NO$_x$ and N$_2$O produced during fossil fuel or biomass burning can vary with temperature, fuel to oxygen ratios, catalytic converter efficiency, and other factors, so an exact ratio of CO$_2$-C released to N oxide - N produced is difficult to calculate, although estimates from total emissions of CO$_2$-C and NO$_x$-N show ratios of about 200:1 (EPA 2000) to 300:1 (Akimoto and Narita 1994). These relatively high ratios indicate that the N produced through combustion is not enough to re-sequester the CO$_2$ produced. The lack of carbon sequestration could be due, in part, to harm caused by combustion related pollutants like tropospheric ozone (Skärby et al. 1998, Matyssek and Innes 1999) and acid rain (Likens et al. 1996) or to detrimental effects of too much N. Any of these factors could reduce plant growth, cause plant death, or lead to community changes that may reduce C sequestration. Furthermore, N$_2$O can have a direct effect on global warming because it is a greenhouse gas as well as a source of N (Hansen et al. 2000).
Ammonia from animal feedlots and organic N carried on dust particles is not an actual source of new N to the biosphere, because, similar to manure additions, the N inputs by these pathways should have been counted as N\textsubscript{2}-fixation or as inorganic fertilizer added to crops which livestock ate. Moving N from one place to another on dust particles would not be expected to cause any net increase in biosphere carbon sequestration. However, a reduction in N availability, and thus a potential decrease in C sequestration, could occur if dust or other forms of N were to fall on inhospitable or non-N-limited sites, or if N were otherwise transported to a non-biologically available location. Moving N from indefinite storage in a large manure pile or holding pond where little plant growth occurs onto the terrestrial landscape (via ammonia volatilization) where vegetation can utilize the N for growth could be important in sequestering carbon, especially since manure storage can cause N to leach from the system or to be denitrified back to N\textsubscript{2}.

\textbf{N\textsubscript{2}-fixation}

Symbiotic N\textsubscript{2}-fixation is the process where a plant and microbes work together to reduce relatively inert N\textsubscript{2} gas into plant available NH\textsubscript{3}. Symbiotic N\textsubscript{2}-fixation is ubiquitous in the legume family, which includes beans, peas, soybeans, clovers, alfalfa, and vetches. The bacteria involved in leguminous N\textsubscript{2}-fixation are in the genus \textit{Rhizobium}. Several tree species, including alder (\textit{Alnus}) and \textit{Albizia} also have N\textsubscript{2}-fixing symbionts.
The N fixed through symbiotic N$_2$-fixation can range from less than 10 kg N ha$^{-1}$ yr$^{-1}$ in forests and prairies, where N$_2$-fixers are not abundant (Brady 1990), to up to 300 kg N ha$^{-1}$ yr$^{-1}$ in dense alder stands (Binkley et al. 1992). The average N$_2$-fixation rate on cropland is about 140 kg N ha$^{-1}$ yr$^{-1}$ (Brady 1990). Similar to industrial produced N fertilizer, N$_2$-fixation requires energy to manufacture N. Plants, however, get their energy from the sun rather than burning fossil fuels. Under low to moderate soil N, plants with the capability to fix N$_2$ show a net benefit in growth and crop yield and thus show increases in biomass carbon sequestration over non-N$_2$-fixing plants.

**MODELING CUMULATIVE CARBON SEQUESTRATION (NBP)**

Several authors have addressed the possible effects of N deposition on carbon sequestration using mathematical models. Some estimates of carbon fixation due to added N are 1.0 to 2.3 Pg C yr$^{-1}$ (Schindler and Bayley 1993), 0.44 to 0.74 Pg C yr$^{-1}$ (Townsend et al. 1996), and 0.25 Pg C yr$^{-1}$ (Nadelhofer et al. 1999). These estimates indicate that as little as 7% (Nadelhofer et al. 1999) to over 100% (Schindler and Bayley 1993), of the estimated 1.5 to 3.4 Pg C yr$^{-1}$ sink that exists on land (Tans et al. 1990) could be accounted for by added N. This indicates that added N does indeed play a role in increasing the terrestrial carbon sink, but the magnitude of the role is still unclear. In sites that have been receiving N deposition for decades, such as the site in Nadelhofer et al. (1999), perhaps the
easing of N limitation has reduced the potential for N to contribute to C sequestration.

To help resolve the role of added N in terrestrial carbon sequestration, a decomposition component was added to Table 4.1 data to yield results in Table 4.2. An estimate of the amount of biomass remaining when decomposition is nearly completed was derived from Berg (2000). The “limit value” estimates ranged from 50% to 99% of original mass missing (Berg 2000), so a relatively conservative value of 90% was chosen to conservatively estimate the potential carbon sequestration in soil organic matter. The total amount of biome-sequestered carbon was calculated by taking 10% (from 100% - “limit value”) of the predicted plant biomass (from Table 4.1 before subtracting C cost) to determine soil carbon. Then adding back 1/2 of soil carbon to account for non-soil (i.e. plant) storage (2/3 of carbon is in soil (Schimel et al. 1994), so 1/3 or 1/2 of 2/3 is in plants) and then subtracting N production costs (Table 4.2).

As in Table 4.1, if we assume that the average agricultural crop or grassland plant is 2% N and 50% C by mass (C:N=25) and that N added as NH₃ is from 10 to 50% available to plants, then for every kilogram of N fertilizer added per hectare, 1.1 to 0.4 kg of C would be sequestered after accounting for N production cost and decomposition losses, provided that no other nutrient limitations become important (Table 4.2). This is a conservative estimate (on the side of too little carbon sequestered), especially because most trees have a much lower N concentration and
a higher C:N ratio and will thereby sequester more carbon per unit mass. The N not usable by plants would likewise contribute some C sequestration from microbial biomass in soil. This model also omits the potentially important pool of transient detritus (e.g. dead trees) that would likely remain in the system for many years during the course of decomposition. Despite the conservative nature of this method, many reasonable scenarios still show net sequestration of carbon, especially when high C:N forests are considered. This provides strong indication that adding N in these areas would result in net sequestration of carbon. Furthermore, since less than 10% of the atmospheric deposition occurs on forests (Holland et al. 1997), most temperate forests probably still have a high capacity to utilize added N to sequester carbon.

Table 4.2
Worst case scenario - NBP increment per nitrogen added (kg/ha)†

<table>
<thead>
<tr>
<th>Total Plant C:N Ratio*</th>
<th>10</th>
<th>20</th>
<th>30</th>
<th>50</th>
<th>100</th>
<th>200</th>
<th>500</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitrogen</td>
<td>1</td>
<td>-1.48</td>
<td>-1.47</td>
<td>-1.45</td>
<td>-1.42</td>
<td>-1.35</td>
<td>-1.2</td>
</tr>
<tr>
<td>Utilization by Plants‡</td>
<td>10</td>
<td>-1.35</td>
<td>-1.2</td>
<td>-1.05</td>
<td>-0.75</td>
<td>0</td>
<td>1.5</td>
</tr>
<tr>
<td>(%)</td>
<td>50</td>
<td>-0.75</td>
<td>0</td>
<td>0.75</td>
<td>2.25</td>
<td>6.0</td>
<td>13.5</td>
</tr>
<tr>
<td>100</td>
<td>0</td>
<td>1.5</td>
<td>3.0</td>
<td>6.0</td>
<td>13.5</td>
<td>28.5</td>
<td>73.5</td>
</tr>
</tbody>
</table>

Note: The line separates a net loss or no change of carbon from carbon gain
† Includes total CO₂ emissions associated with N production, transportation, and application (1.5 kg C/1.0 kg N). Assumptions: a) no other nutrient limitation, b) plant residues eventually reach 10% of original biomass as soil organic matter (Berg 2000).
‡ weighted average of the ratio of carbon mass to nitrogen mass for all tissues
§ ratio of plant N uptake to total N added multiplied by 100
If we divide the total unknown terrestrial carbon sink of 1.5 to 3.4 Pg C yr\(^{-1}\) (Tans et al. 1990, Nadelhoffer et al. 1999) by the approximately \(8.5 \times 10^9\) ha of usable land on earth (Brady 1990), 175 to 400 kg C ha\(^{-1}\) yr\(^{-1}\) need to be sequestered. Based on Table 4.2, with low C:N ratios and low N utilization by plants no amount of commercially produced fertilizer would be able to account for all of the carbon sequestered. However, assuming an average whole forest C:N of 50 and 100% N utilization (as in Nadelhoffer et al. 1999), about 30 to 67 kg N ha\(^{-1}\) yr\(^{-1}\) would be needed to sequester the extra carbon. If N\(_2\)-fixing plants could add the N, the production cost of fertilizer could be removed and the estimate falls to 23 to 53 kg N ha\(^{-1}\) yr\(^{-1}\), well within the capability of N\(_2\)-fixing plants. Required N additions could be even lower based on critiques of the C:N estimates that Nadelhoffer et al. (1999) used (Jenkinson et al. 1999), but the outcome depends largely on how optimistic the modeler is.

Taking a different approach, I used a 100 year rotation model of forest carbon sequestration (Harmon 2001). This model included more aspects of carbon storage, including wood products. I used nine different model runs determining steady state carbon stores for each simulated condition. There was not an explicit nutrient component to this model, so I adjusted the variables based on what the effect of nutrient additions were expected to be. A 20% increase, a 20% decrease, and no change in maximum biomass and growth rate were combined in all possible ways with 20% increases and 20% decreases in litter and soil decomposition rates.
A 20% increase in maximum biomass and growth rate represents positive effects of N from fertilizer, N deposition, or N$_2$-fixation fertilization. A 20% decrease in maximum biomass and growth rate represents negative effects due to pollution or N saturation. Increases and decreases in decomposition rates represents possible positive or negative effects due to added N. Results are shown as percent difference from the standard run in Table 4.3.

Table 4.3
Percent change in steady state carbon stores with added nitrogen$^+$

<table>
<thead>
<tr>
<th></th>
<th>Decomposition Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+20%</td>
</tr>
<tr>
<td></td>
<td>No Change</td>
</tr>
<tr>
<td></td>
<td>-20%</td>
</tr>
<tr>
<td>Growth Rate</td>
<td>Decrease 20%</td>
</tr>
<tr>
<td>and and</td>
<td>-40.2</td>
</tr>
<tr>
<td>No Change</td>
<td>-35.2</td>
</tr>
<tr>
<td>Max. Biomass</td>
<td>Increase 20%</td>
</tr>
<tr>
<td></td>
<td>28.5</td>
</tr>
<tr>
<td></td>
<td>39.0</td>
</tr>
<tr>
<td></td>
<td>55.9</td>
</tr>
</tbody>
</table>

Note: The line separates a net loss or no change of carbon from carbon gain
$^+$ Calculated using a model from M.E. Harmon (2001).

The positive effects of increased growth rate and maximum biomass outweighed the negative effects of decomposition on carbon sequestration due to added N using this model. It is important to point out that few sites currently show decreases in growth rate and maximum biomass due to N saturation (Aber et al. 1998). The exact amount of N needed to attain the results in Table 4.3 probably varies by site, but the percent changes are reasonable examples of what could be obtained with N fertilization or atmospheric deposition in areas without high concentrations of phytotoxic pollutants. Because the amount of N added was not
known, potential CO₂ costs associated with N fertilizer production could not be evaluated, but using N₂-fixing plants could mitigate these costs.

CONCLUSIONS

These analyses suggest that adding N through fertilization, N₂-fixation, or atmospheric deposition could increase net biome production in N limited biomes, even after accounting for CO₂ emissions from fertilizer production provided that other factors do not limit plant growth. This does not mean that clean air standards should be lowered or that extensive fertilization should begin immediately to sequester more CO₂, because excess N in the environment, such as high nitrate in streams and groundwater or high NOₓ and N₂O in the atmosphere, has a variety of harmful effects (Vitousek et al. 1997). Too much N can also increase the susceptibility of some plants to disease and insect defoliation (Waring and Running 1998). However, if a significant reduction in CO₂ emissions cannot be accomplished by other means, adding N, alone, or in combination with other nutrients, may be one possible method to remove CO₂ from the atmosphere to reduce the risk of global warming. Although further study is needed, it appears that the wise use of N₂-fixing plants and N fertilizer on the most N-limited sites, especially high C:N forests, would increase C sequestration, at least temporarily, yet limit the deleterious effects of excess N.
REFERENCES


Akimoto, H., and H. Narita. 1994. Distribution of SO$_2$, NO$_x$ and CO$_2$ emissions from fuel combustion and industrial activities in Asia with $1^\circ \times 1^\circ$ resolution. Atmospheric Environment 28(2): 213-225.


CHAPTER 5: CONCLUSIONS

Thesis summary

The forest floor and soil at our site in the H.J. Andrews Experimental Forest have a strong tendency to retain added N, regardless of N form, even in the absence of active tree roots. The litter/organic horizon, as a bulk pool, was the largest N retention pool for all N additions. Within the litter/organic horizon, the chloroform-extractable microbial biomass initially accounted for nearly all of the added N from the ammonium additions. Although on a different time scale, microbial biomass also played a significant role in the retention of N from organic N, tannin-complexed organic N, and Lobaria. Complexing organic matter with tannin appeared to slow nitrogen cycling, but did not significantly change the ultimate fate of added organic nitrogen. Season of nitrogen addition had little effect on the retention of added nitrogen, although when differences occurred spring $^{15}$N recovery was slightly lower. Our study provides further evidence that microbial biomass plays an active role in initial and continued N retention in low atmospheric deposition sites.

Labeling the N$_2$-fixing lichen Lobaria oregana with $^{15}$N using a nutrient solution containing $^{15}$N-enriched ammonium was successful, with one caveat. To avoid an undesired increase in the N concentration of the lichen, additions of $^{15}$N should be applied during the lichen's growing season, which in this case was the spring.
Lobaria litter placed out in the fall decayed faster (k=3.1 yr⁻¹) than litter placed out in the spring (k=1.24 yr⁻¹). Although, the season effect was confounded by the higher initial N concentration in the fall lichen, we attributed the effect primarily to the wetter conditions in the fall, because the N concentration of the fall lichen dropped to spring lichen levels at the first sampling date.

There was proportionally more gross N loss than mass loss early in decay for the fall lichen as hypothesis 3 stated, but this gross loss did not occur in the spring addition lichen. However, the spring lichen demonstrated a net immobilization of N, while the fall lichen did not. Patterns in exogenous N uptake, gross N lost versus net N lost, and N concentration did not differ by season. Both lichen additions, regardless of season or initial N concentration, had a positive trend in exogenous N uptake over decay. Both lichen additions showed more gross N loss relative to net N loss initially, with equal rates of gross N loss relative to net N loss later in decay. Both lichen additions had a peak in N concentration during decay at 2.8% N, equal to a C to N of 16. This non-linear trend in N concentration during decay was often not seen in past studies, but it had been predicted (McClugherty et al. 1985).

Using ¹⁵N-labeled Lobaria litter enabled the exploration of patterns in N dynamics that could not otherwise be examined without the ability to track the fate of exogenous and endogenous N. While the ingenuity of study design and creativity of data analyses in past studies has yielded valuable information about
the N dynamics in litter, more studies should undertake the added effort of labeling material with $^{15}$N, so that a better understanding of N dynamics during decomposition can be gained.

The analyses from Chapter 4, suggest that adding N through fertilization, N$_2$-fixation, or atmospheric deposition could increase net biome production in N limited biomes, even after accounting for CO$_2$ emissions from fertilizer production provided that other factors do not limit plant growth. This does not mean that clean air standards should be lowered or that extensive fertilization should begin immediately to sequester more CO$_2$, because excess N in the environment, such as high nitrate in streams and groundwater or high NO$_x$ and N$_2$O in the atmosphere, has a variety of harmful effects (Vitousek et al. 1997). Too much N can also increase the susceptibility of some plants to disease and insect defoliation (Waring and Running 1998). However, if a significant reduction in CO$_2$ emissions cannot be accomplished by other means, adding N, alone, or in combination with other nutrients, may be one possible method to remove CO$_2$ from the atmosphere to reduce the risk of global warming. Although further study is needed, it appears that the wise use of N$_2$-fixing plants and N fertilizer on the most N-limited sites, especially high C:N forests, would increase C sequestration, at least temporarily, yet limit the deleterious effects of excess N.
REFERENCES


BIBLIOGRAPHY


Bremer, E. and C. van Kessel. 1990. Extractability of microbial $^{14}$C and $^{15}$N following addition of variable rates of labeled glucose and (NH$_4$)$_2$SO$_4$ to soil. Soil Biology and Biochemistry 22: 707-713.


