Mass loss and nitrogen dynamics during the decomposition of a ^{15}N-labeled N_2-fixing epiphytic lichen, *Lobaria oregana*

Scott M. Holub and Kate Lajtha

Abstract: We studied mass loss and nitrogen dynamics during fall and spring initiated decomposition of an N_2-fixing epiphytic lichen, *Lobaria oregana* (Tuck.) Müll. Arg., using ^{15}N. We developed a method of labeling lichens with ^{15}N for use in a decomposition study that involved spraying lichen material with a nutrient solution containing ^{15}N-enriched ammonium. Through the first 180 days of sampling, lichens placed in the field during the spring had a smaller decay constant (k = 1.24 year^{-1}) than the lichens placed in the field during the fall (k = 3.1 year^{-1}). However, both spring and fall lichen samples were decomposed beyond recognition after 1 year. Patterns in exogenous N uptake and N concentration did not differ by season. Both spring and fall lichens took up N from the surrounding environment during decay while simultaneously losing N to the environment. The N concentration in both sets of lichen additions increased during decay to a peak of around 2.8% N, equal to a C to N ratio of about 16, and then began to decrease. This indicates that early in decay, net N immobilization occurred in the remaining lichen, but this was followed by net N mineralization in later stages of decay.

Key words: decomposition, nitrogen, *Lobaria oregana*, lichen, mineralization, immobilization.

Résumé : Les auteurs ont étudié la perte de masse et la dynamique de l'azote, au cours de la décomposition débutant à l'automne et au printemps, chez un lichen épiphyte fixateur de N_2, le *Lobaria oregana* (Tuck.) Müll. Arg., en utilisant le ^{15}N. Aux fins de cette étude sur la décomposition, ils ont développé une méthode pour marquer les lichens avec du ^{15}N, laquelle consiste à asperger le matériel lichénique avec une solution nutritive contenant de l'ammonium enrichi en ^{15}N. Au cours des premiers 180 jours d'étude, les lichens placés sur le terrain au cours du printemps montrent une constante de décomposition plus faible (k = 1,24 an^{-1}) que les lichens placés sur le terrain en automne (k = 3,1 an^{-1}). Toutefois, les échantillons de lichen du printemps et de l'automne montrent une décomposition au delà de toute reconnaissance, après 1 année. Les patrons d'absorption exogène de N et la teneur en N ne diffèrent pas selon la saison. Les lichens du printemps aussi bien que ceux de l'automne absorbent le N du milieu environnant au cours de la décomposition, tout en perdant simultanément du N au milieu ambiant. Les additions à la teneur en N des deux ensembles de lichen augmentent au cours de la décomposition pour atteindre un sommet autour de 2,8 %, avec un ratio C/N d'environ 16, avant de commencer à décroître. Ceci indique que tôt au cours de la décomposition il y a immobilisation nette de N dans le lichen qui reste, mais qu'il s'ensuit une minéralisation nette au cours des stades avancés de décomposition.

Mots clés : décomposition, azote, *Lobaria oregana*, lichen, minéralisation, immobilisation.

[Traduit par la Rédaction]

Introduction

Lobaria oregana (Tuck.) Müll. Arg. (hereafter *Lobaria*) is the dominant N_2-fixing epiphytic lichen in old-growth Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) forests of the Pacific northwestern United States with up to and exceeding 1000 kg standing biomass·ha^{-1} (McCune 1994). Under field conditions, *Lobaria* can add 2.5–4.5 kg N·ha^{-1} every year (Pike 1978; Denison 1979) or roughly 33–67% 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 10–30% per year (Rhoades 1977; Sillett 1994) and assuming an approximate steady-state biomass of 1000 kg·ha^{-1}, *Lobaria* litterfall is probably around 100–300 kg·ha^{-1}·year^{-1}. The amount of litter can be patchy on the landscape and vary annually.

Nitrogen 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

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S.M. Holub[1,2] and K. Lajtha. Department of Botany and Plant Pathology, Oregon State University, Corvallis, OR 97330, U.S.A.

[1]Corresponding author (e-mail: Holub.Scott@epa.gov).
[2]Present address: U.S. Environmental Protection Agency, Office of Research and Development, National Risk Management Research Laboratory, 919 Kerr Research Drive, P.O. Box 1198, Ada, OK 74820, U.S.A.

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N from *Lobaria* litter has not been extensively studied. Lichen decomposition has been examined in several studies (Wetmore 1982; Moore 1984; Guzman et al. 1990; McCune and Daly 1994; Knops et al. 1996; Esseen and Renhorn 1998; Coxson and Curteanu 2002), but no study has used ^{15}N-labeled material to observe N dynamics through lichen 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 N dynamics in decomposing plant litter (e.g., 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 observe 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 (*Pinus sylvestris* L.) 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 to (*i*) develop an easy method of labeling lichen material with ^{15}N for use in litter decomposition studies, (*ii*) examine the mass loss of *Lobaria* litter as it decomposes on the forest floor, and (*iii*) examine the patterns of N uptake and loss throughout the decay of *Lobaria* litter. For the first objective, we developed a method of labeling *Lobaria* using a nutrient solution that contained ^{15}N-labeled ammonium to label the lichen without using $^{15}N_2$, which can be difficult to manage. With respect to the second objective, we hypothesized that *Lobaria* will decay relatively quickly compared with leaf litter because it has a high N concentration and contains little recalcitrant structural C (e.g., lignin), which can be difficult to decay. We 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 in the spring because wetter conditions in the fall would facilitate faster decomposition. With respect to the third objective, we hypothesized that there would be proportionally more gross N loss relative to 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 C. We 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). Vascular plant 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), so we presumed that a value of 25 would be a good estimate for lichen litter, although we recognize that vascular plants and lichens have different structural C chemistry.

Materials and 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°14′N, 122°14′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 Douglas-fir with smaller western hemlock (*Tsuga heterophylla* (Raf.) Sarg.) and western redcedar (*Thuja plicata* Donn ex D. Don) in the overstory. Many overstory trees have a large amount of epiphytic mosses and lichens growing on them, especially *Lobaria*. The forest floor is covered with a layer of mosses including Oregon beaked moss (*Eurhynchium oreganum* (Sull.) Jaeg.) and step moss (*Hylocomium splendens* (Hedw.) B.S.G.). Soils are andic. For a more detailed site description, see Holub and Lajtha (2003).

Labeling *Lobaria* 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 3–4 weeks. Approximately 30 g of clean *Lobaria* thalli was randomly selected and separated into two equal-mass groups. Each group was spread 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 (S.M. Holub, unpublished data). Cleanliness was necessary because of the importance of labeling *Lobaria* biomass and not organic matter or the algal–bacterial biofilm that covered 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 per spray), the lichens in each tray were gently sprayed with 80 mL of deionized water and placed on separate racks in a growth chamber (model SG 30; Hoffman Manufacturing, Albany, Oreg.) with 12 h of low-strength artificial light (100–140 μmol photosynthetically active radiation$\cdot m^{-2}\cdot s^{-1}$) 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 100–150 mL of deionized water to allow periodic events of high and low thallus moisture as might normally 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 inevitably, some mist was deposited outside the tray. Beginning 4 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 deionized water. The solution contained

566.7 mg ammonium chloride·L^{-1} (98 atom% ^{15}N; Aldrich Chemical), 61.4 mg potassium phosphate·L^{-1} (monobasic), 59.6 mg potassium chloride·L^{-1}, 11.4 mg calcium carbonate·L^{-1}, and 5.0 mg magnesium oxide·L^{-1} and was titrated to pH 5.5 using sulfuric acid to minimize NH_3 volatilization while maintaining an ecologically reasonable pH. The ratios 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 *Lobaria* in the Willamette National Forest. A total of 670 mL of this solution was added over 4 weeks equaling approximately 5.4 mg ^{15}N·g dry *Lobaria*$^{-1}$. Spraying with deionized water continued for an additional 5 days to allow the final ^{15}N additions to be assimilated.

The ^{15}N-labeled lichen pieces were washed in seven successive 250-mL beakers filled with 150 mL of deionized water to remove any readily leachable ^{15}N-labeled ammonium that might occur on the surface of the lichen (Miller and Brown 1999). The water in the beakers was changed after every 2 g of lichen was rinsed. The rinsed lichens were allowed to air-dry overnight at 12°C, which was sufficient to dry the lichens due to their poikilohydric nature. Air-dried lichen mass and oven-dried lichen mass were assumed to be approximately equal because preliminary tests showed that differences in air- versus oven-dry mass were negligible compared with other potential variations in mass such as incomplete recovery of lichen material. The following day, the dried *Lobaria* were weighed and a 50- to 100-mg portion of 10 separate pieces, five from each tray, was ground to 40 mesh and submitted along with three unlabeled *Lobaria* samples for atom% ^{15}N and 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 in diameter by 40 cm in 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 2003). 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 has been shown to increase decomposition at some sites (Blair et al. 1992) and could affect N retention and decomposition. During extraction of the tubes' contents, many individual macroinvertebrates were found including millipedes (*Harpaphe haydeniana haydeniana* (Wood)) as well as unidentified earthworms and centipedes.

The tubes were installed 2 months prior to adding *Lobaria* in October 1999 (fall) and April 2000 (spring). Any naturally occurring *Lobaria* litter was removed from the tube and ^{15}N-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 under ambient conditions. The pieces of lichen thallus that were used appeared to be healthy. No attempt to kill the lichen was made.

Sample collection

Three tubes from both the fall and spring additions were collected at 14, 90, and 180 days and 1 year post-addition. Although the lichen was placed on the forest floor alive, by 14 days it was beginning to show visible browning and other signs of decay. All collected tubes were enclosed in sealed plastic bags to prevent desiccation, placed on ice as needed to keep cool, and brought back to Oregon State University for immediate separation. Any obviously new pieces of nonlabeled *Lobaria* were discarded and *Lobaria* from the additions were collected from the tubes and dried at 60°C. Total dry mass was determined. 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, Calif., 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 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 fitted to values of remaining thallus mass (eq. 1) plotted versus time. Dates with no or a very small amount of mass remaining were omitted from the curve because they had an inordinately large effect on the shape of the curve. Values of zero could not be included in an exponential decay curve because including zero causes the mathematical model to become undefined. Exponential decay only approaches zero and can never actually reach zero. Likewise, values that were more than an order of magnitude less than the other values were given 10–100 times more weight in fitting the curve and drastically skewed the resulting fit. A linear fit was also explored, but in an effort to make our data comparable with existing decomposition data, we elected to use exponential decay. To determine whether the decay rates differed by season, a general linear model was used to test for significant interaction of season and time since placement in the field.

[1] Mass remaining (%) =

(final mass)/(initial mass) × 100

Nitrogen dynamics in the litter were examined by plotting ${}_nN$ (eq. 3) versus M (eq. 2) (see Table 1 for abbreviation definitions) and ${}_gN$ (eq. 4) versus M. The M, ${}_nN$, and ${}_gN$ were expressed as a percentage of the 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 since placement in the field.

[2] M = Mass loss (%) = 100 – (mass remaining)

Table 1. Summary of abbreviations.

Abbreviation	Explanation
M	Mass loss of lichen thallus as a percentage of original mass
${}_{n}N$	Net N loss as a percentage of total original N content
${}_{g}N$	Gross N loss as a percentage of total original ^{15}N content
%N	N concentration in recovered tissue (percentage of total mass remaining)
$\%N_{exog}$	Relative amount (percentage) of exogenous N imported into remaining tissue
Subscripts	
f (e.g., ${}_{n}N_{f}$)	Fall-placed lichen material
s (e.g., ${}_{n}N_{s}$)	Spring-placed lichen material
<10 (e.g., ${}_{n}N_{f<10}$ or $M_{<10}$)	Data points below 10% mass loss*
≥10 (e.g., ${}_{n}N_{f\geq10}$ or $M_{\geq10}$)	Data points equal to or above 10% mass loss*
No subscript	Fall and spring data combined over the whole range of mass loss

*The data suggested that there were two different stages of decomposition above and below 10% mass loss, so these regions were analyzed separately.

[3] ${}_{n}N$ = Net N loss (%) = [1 – (final N content/initial N content)] × 100

[4] ${}_{g}N$ = Gross N loss (%) = [1 – (final ^{15}N content/initial ^{15}N content)] × 100

Data from the two graphs were divided into two data ranges: less than 10% mass loss and greater than or equal to 10% mass loss. 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 $M_{\geq10}$ data often showed clear linear trends, while the $M_{<10}$ data had different and (or) less predictable trends. Using the statistics calculated from the $M_{\geq10}$ data enabled numerical interpretation of overall patterns of N dynamics.

Linear regressions were calculated on the $M_{<10}$ data and the $M_{\geq10}$ data. Since ${}_{n}N$ and ${}_{g}N$ are to a large degree autocorrelated with M, only those relationships that deviated significantly from the 1:1 line are shown on the graphs. Points below the 1:1 line indicate that a net decrease in ${}_{n}N$ or ${}_{g}N$ occurred relative to M. Points above the 1:1 line indicate that a net increase in ${}_{n}N$ or ${}_{g}N$ occurred relative to M. Linear regressions with a significant intercept but with a slope that was not significantly different from the 1:1 line occurred frequently in the $M_{\geq10}$ data. A significant intercept indicates that a difference existed between the net change in the data versus the 1:1 line during the $M_{<10}$ range. The value of the intercept is equal to the magnitude of this difference.

Nitrogen concentration in remaining tissue was plotted as a function of M to observe changes in the concentration of N within the remaining tissue over decay. Nitrogen concentration alone can be a misleading indicator of N dynamics because as C 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 or preferential retention of N could also account for some or all of any apparent increase in N concentration (Holub et al. 2001). Changes in N concentration in the remaining litter also give no indication as to the total amount of N lost from litter through fragmentation because only remaining tissue can be analyzed. To determine if the N concentration versus M data differed by season, a general linear model was used to test for a significant interaction of season and time since the material was placed in the field.

Exogenous N inputs to litter were calculated as a percentage of the total existing N in the remaining tissue (eq. 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. If these assumptions are true, then any decrease in the atom% ^{15}N in the tissue can be attributed to the uptake of exogenous N. The percent exogenous N in the remaining tissue was then plotted as a function of M. To determine if the exogenous N differed by season, a general linear model was used to test for significant interaction of season and time since the material was placed in the field. If the assumptions are violated, especially if the lichen preferentially loses ^{15}N over ^{14}N, then exogenous N inputs could be overestimated by this method.

[5] $\%N_{exog}$ = $[(100 - {}_{n}N) - (100 - {}_{g}N)]/(100 - {}_{n}N) \times 100$

where $\%N_{exog}$ is the percentage of N in the remaining tissue that is from sources outside the tissue (e.g., humus and soil).

Results

Labeling outcome

The method of labeling *Lobaria* with ^{15}N using ^{15}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 from unlabelled lichen (p = 0.4). The total dry mass of fall-labeled lichen was unchanged (±1%) 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 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 (Fig. 1) differed by season of placement in the field ($p < 0.0001$). The spring lichen had a decay constant (k) of 1.24 year^{-1} (SE = 0.09) and an intercept of 100% remaining (SE = 1.03) over the first 180 days and was completely decayed after 1 year. The fall lichen had a larger k of 3.1 year^{-1} (SE = 0.3) over the first 180 days with an intercept of 106% remaining (SE = 1.08) and was also completely decayed after 1 year.

Nitrogen dynamics

The ${}_nN$ versus M (Fig. 2) differed by season ($p < 0.001$). The ${}_nN_{s\geq10}$ was less than what would be expected if ${}_nN$ was equal to M (i.e., it was lower than the 1:1 line) (intercept 19.6%, SE = 1.6, p = 0.0003). This provides evidence that ${}_nN_{s<10}$ was not different from zero, but because ${}_nN_{s\geq10}$ was faster than $M_{s\geq10}$ (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 ${}_nN$ loss occurred. The ${}_nN_{s<10}$ and ${}_nN_{f\geq10}$ were not significantly different from the 1:1 line with M (p = 0.6 and p = 0.72, respectively). The ${}_nN_{f<10}$ was significantly greater than expected as represented by the 1:1 line with M (slope = 2.20, SE = 0.31, n = 3, p = 0.03). The ${}_gN$ versus M (Fig. 3) differed by season of addition ($p < 0.0001$). The ${}_gN_{f\geq10}$ was 13.5% greater than what was predicted by the 1:1 line with M (intercept, SE = 2.8, p = 0.0085), but the rate of ${}_gN_{f<10}$ versus M was not different from the 1:1 line (slope, p = 0.1). The ${}_gN_{f<10}$ versus M did not differ from the 1:1 line (p = 0.1) and ${}_gN_s$ was not different from the 1:1 line with M ($M_{<10}$, p = 0.48; $M_{\geq10}$, p = 0.92).

After excluding the initial N concentrations, N concentration in the remaining tissue versus M (Fig. 4) did not differ by season (p = 0.12), so the data were pooled for analysis. The N concentration in the remaining lichen from both seasons showed a significant quadratic relationship with mass loss (p = 0.003, r^2 = 0.54). Nitrogen concentrations gradually increased to a peak around 2.8% N (SE = 0.09) and then decreased.

The $\%N_{exog}$ in the remaining lichen tissue increased as M increased (p = 0.01 for the linear regression) (Fig. 5). 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

^{15}N-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_2 fixation by its cyanobacterial symbiont, *Nostoc*. Therefore, a logical way to label *Lobaria* with ^{15}N would be to grow it in a ^{15}N-enriched N_2 environment. However, constructing and maintaining a ^{15}N-enriched N_2 environment to grow *Lobaria* would be difficult (see Millbank and Olsen 1981). N_2-fixing lichens are able to take up ammonium when it is available (Rai et al.

Fig. 1. Mass remaining in *Lobaria* over time. Equations are of exponential decay omitting values at 1 year because those values were very close to zero and had inordinate influence on the curves. Note: equations are on an annual basis, while the x-axis is on a daily basis.

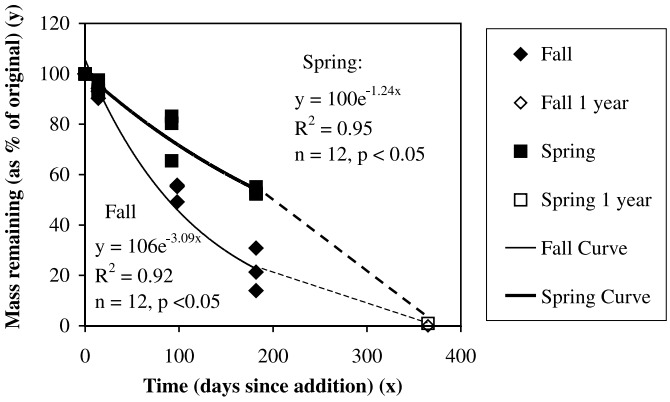


Fig. 2. Net N loss from *Lobaria* tissue versus mass loss. Only regressions that were significantly different from the 1:1 line were plotted ($p < 0.05$). Note: there was a marginally significant (p = 0.03) positive relationship for the ${}_nN_{f<10}$ data that was omitted from the graph because it was likely not meaningful due to small sample size (n = 3, y = 2.20x + 0.19, R^2 = 0.94).

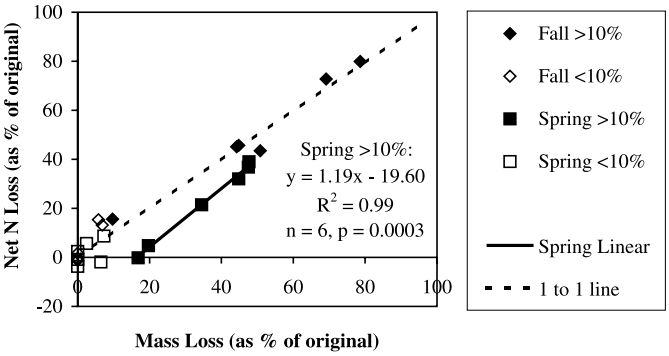


Fig. 3. Gross N lost from *Lobaria* tissue versus mass lost. Only regressions that were significantly different from the 1:1 line were plotted ($p < 0.05$).

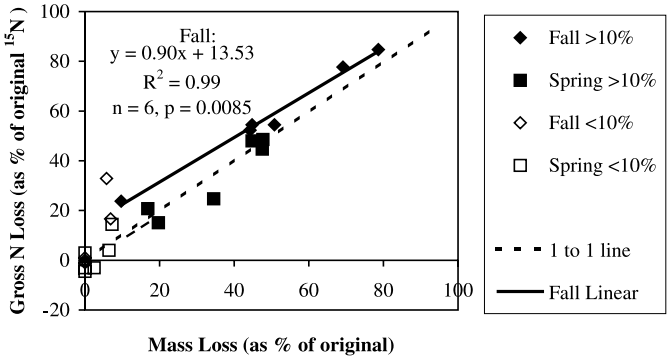


Fig. 4. Nitrogen concentration in *Lobaria* versus mass lost. Spring and fall lichens did not differ in patterns of N concentration and are shown in one regression. Initial N concentrations were omitted from the curve.

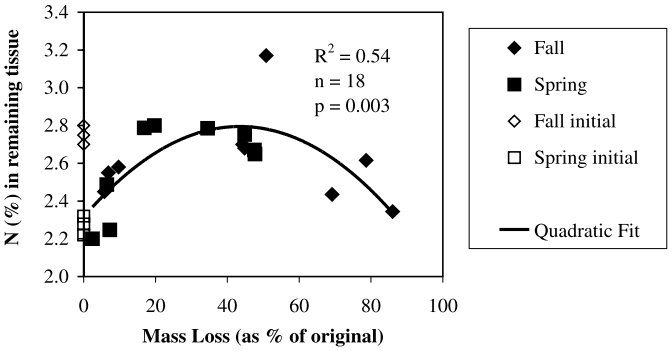
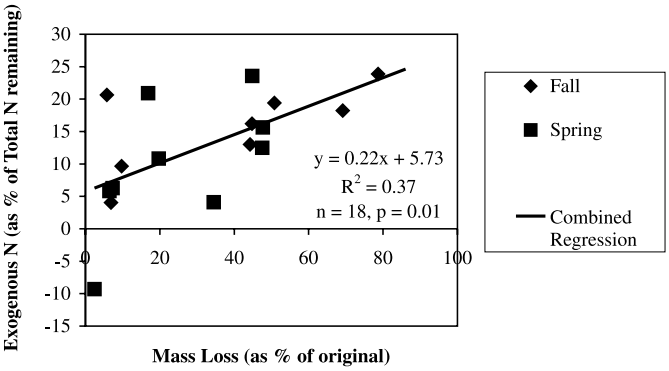


Fig. 5. Exogenous N in remaining lichen tissue versus mass loss. Spring and fall lichens did not differ in patterns of exogenous N concentration.



1983; Rowell et al. 1985; Miller and Brown 1999), and ammonium-derived N and N_2-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). A recent study (Dahlman et al. 2002) has shown with ^{15}N that other tripartite lichens (*Nephroma* and *Peltigera*) do assimilate ammonium into growing tissue, which supports our original assumption that N_2-fixing lichens will assimilate added ammonium. We cannot be certain from our study, however, that the ^{15}N labeling was uniform throughout all forms of N within our lichen material.

The increase in N concentration observed in the fall lichen was probably due to a lack of growth of the fall lichen. Unlike the spring-labeled lichen, the fall lichen was able to take up the ^{15}N but apparently not able to assimilate it into new growth. Muir et al. (1997) showed that *Lobaria pulmonaria*, a closely related species, grew fastest in spring months, so applying ^{15}N to lichens during their growth phase appears to produce lichen material that is labeled with ^{15}N 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 *k* values observed in this study (1.24 year^{-1}) (Fig. 1) were similar to the those reported in past studies of *Lobaria* and other N_2-fixing species. McCune and Daly (1994) reported a half-life (ln 2/*k*) of 7.0 months (k = 1.2 year^{-1}) for unenclosed *L. oregana* placed in the field in late spring at the H.J. Andrews LTER site. Esseen and Renhorn (1998) reported a *k* value of 0.96 year^{-1} for *L. pulmonaria* in litterbags. Guzman et al. (1990) reported *k* values of 2.2–0.45 year^{-1} for *Pseudocyphellaria* species in litterbags.

In contrast, the fall lichen *k* value was much higher (3.1 year^{-1}) than in other studies. 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 year^{-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 *Alectoria* 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* produces chemicals that may inhibit decomposition more than the chemicals in other lichen species. Stictic acid, norstictic acid, and constictic acid are all compounds found in *L. oregana* (Culberson 1969; Culberson 1970; Culberson et al. 1977). While it is not clear that these specific 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 those for *Lobaria* (about 0.4–0.8 and 0.3–0.4 year^{-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 *k* of 0.64 year^{-1}, which is very similar to broadleaf *k* values.

Lichen material in litterbags has been shown to yield 50–90% lower *k* values than free material (McCune and Daly 1994). *Lobaria oregana* falls at the low end of the differences between bagged and unbagged *k* values. While a 50% reduction in the *k* value is large, its relatively small size in comparison with 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) suggested 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 2003), which indicated that nearly 100% of the ^{15}N lost from *Lobaria* into the surrounding environment was recovered in very close proximity to where the li-

chens were placed. This 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 especially toxic or nonpalatable 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) and perhaps less inhibitory secondary compounds are eaten readily by large herbivores.

Nitrogen dynamics

The N data that we presented are strong evidence for the initial net uptake of N in the spring lichen litter but also show that later in decay, the spring lichen had a net loss of N that was19% faster than *M*. The *y*-intercept of the regression line for ${}_{g}N$ versus $M_{\geq 10}$ indicated that the fall lichen lost 13% more ^{15}N-labeled N early in decay than would have been predicted by *M* alone. This could be due to uneven ^{15}N labeling of N in the fall lichen, which was then preferentially lost. After this initial loss of labeled N, the ^{15}N loss slowed and became more constant, which indicates that at least some of the added ^{15}N was assimilated into more recalcitrant N in the lichen. In the spring lichen, where no initial loss of ^{15}N, perhaps much more of the added N was assimilated during the labeling process.

Our data indicate that N from sources outside the lichen was imported into the lichen as it decayed, although the curve did not explain much of the variation in the data. Our study differs from most other decomposition studies because we are able to track the N import and export from our decomposed material using ^{15}N.

The critical value of N concentration (2.8%) that we observed for both the spring- and fall-placed lichen indicates that early in decay of the lichen material at our site, net immobilization of N occurred in the lichen, as seen by the increase in %N, but was followed by net mineralization of N in later stages of decay, as seen by the decrease in %N. 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 very 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 to *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). These authors also discussed the concept of a critical C to N ratio at which the N concentration would cease to increase with *M*. It appears that *Lobaria* litter is prone to reaching this critical C to N ratio quickly 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).

Using ^{15}N-labeled 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 past studies that did not use stable isotope tracers have yielded valuable information about the N dynamics in litter, more studies should undertake the added effort of labeling material, lichen or otherwise, with ^{15}N so that a better understanding of gross and net N dynamics during decomposition can be gained.

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