

Winter climate change implications for decomposition in northeastern forests: comparisons of sugar maple litter with herbivore fecal inputs

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Abstract

Forests in northeastern North America are influenced by varying climatic and biotic factors; however, there is concern that rapid changes in these factors may lead to important changes in ecosystem processes such as decomposition. Climate change (especially warming) is predicted to increase rates of decomposition in northern latitudes. Warming in winter may result in complex effects including decreased levels of snow cover and an increased incidence of soil freezing that will effect decomposition. Along with these changes in climate, moose densities have also been increasing in this region, likely affecting nutrient dynamics. We measured decomposition and N release from ^{15}N -labeled sugar maple leaf litter and moose feces over 20 months in reference and snow removal treatment (to induce soil freezing) plots in two separate experiments at the Hubbard Brook Experimental Forest in New Hampshire, USA. Snow removal/soil freezing decreased decomposition of maple litter, but stimulated N transfer to soil and microbial biomass. Feces decomposed more rapidly than maple litter, and feces N moved into the mineral soil more than N derived from litter, likely due to the lower C:N ratio of feces. Feces decomposition was not affected by the snow removal treatment. Total microbial biomass (measured as microbial N and C) was not significantly affected by the treatments in either the litter or feces plots. These results suggest that increases in soil freezing and/or large herbivore populations, increase the transfer rate of N from plant detritus or digested plants into the mineral soil. Such changes suggest that altering the spatial and temporal patterns of soil freezing and moose density have important implications for ecosystem N cycling.

Keywords: climate change, decomposition, herbivores, moose, N cycling, ^{15}N stable isotopes, soil freezing

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Introduction

Forests around the world face rapidly changing environmental conditions, and these conditions are often mediated by changing human activity (Pimm *et al.*, 1995). Climate change is often cited as a contributor to forest change (Campbell *et al.*, 2009; Mohan *et al.*, 2009); however, it is not clearly understood how different feedbacks will ultimately shape the functioning of these forested systems, including decomposition. Historical changes in forest cover and landuse can also influence the functioning of today's forests (Goodale & Aber, 2001; Foster & Aber, 2004). Dynamic links between

trees, browsing animals and nutrient cycling are important factors in how forested systems function (Pastor *et al.*, 1998; Wardle & Bardgett, 2004). The return of large ungulate herbivores to regenerating forests may have important implications for decomposition and nutrient cycling (Harrison & Bardgett, 2004; Christenson, 2007).

Although average global and North American temperatures have been rising in the last century (Hansen, 2005; Brohan *et al.*, 2006), the changes on local scales are more indicative of impacts to regional ecosystems (Hayhoe *et al.*, 2006). Changes in the northeastern United States may include the delay of, or reduction in snowpack development and an increase in winter mean annual temperatures by 1–3 °C (Hayhoe *et al.*, 2006). These increases will result in continued freezing temperatures, especially during nondaylight hours over

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the winter. Snow cover insulates soils from low air temperatures and allows biological activity to continue through the 'dormant season' (Clein & Schimel, 1995; Brooks *et al.*, 1997; Brooks & Williams, 1999). Snow cover can maintain soil temperatures at or above biological freezing ($\geq 5^\circ\text{C}$) while reduced or delayed snow cover can result in disturbed biogeochemical processing. Reports of increased nitrate (NO_3^-) export during the spring melt and subsequent summer period (Mitchell *et al.*, 1996; Fitzhugh *et al.*, 2001; Piatek *et al.*, 2005; Campbell *et al.*, 2006) and loss of tree productivity, both direct tree death and loss of fine root biomass (Boutin & Robitaille, 1995; Tierney *et al.*, 2001) have been attributed to soil freezing events.

The mechanisms for increased NO_3^- export and fine root death with soil freezing are not clearly understood; however, a number of hypotheses have been explored. Investigators have found that frozen soil conditions may reduce microbial biomass (Brookes *et al.*, 1989; Heuer *et al.*, 1999) and freeze-thaw events can also affect microbial activity (Schimel & Clein, 1996). Cleavitt *et al.* (2008) suggested that freezing injury to fine tree roots occurs through physical cellular damage. Other ecosystem processes, such as decomposition, are influenced by soil freezing events and may also be partially responsible for changes in N cycling.

In addition to climate change, a major factor affecting northeastern forests over the past two decades has been an increase in moose (*Alces alces*) populations (K. Bontaitis, personal communication, 2003–2004). Moose have been absent from many forests of the northeastern United States since the late 1800s, when land conversion to agriculture and forest harvesting decreased habitat availability (Snyder & Bontaitis, 1996; Foster and O'Keefe 2000). Hunting pressure during this period also contributed to extirpation of moose from this region. As forests have regenerated in these areas over the last century, moose have slowly returned, interacting with these more mature forested systems. These large herbivores have been shown to affect nutrient cycling in other regions by the short-term deposition of urine and feces, and in the long-term via changes in tree species composition through selective browsing (Brandner *et al.*, 1990; Pastor *et al.*, 1993, 1998). The effect of moose on vegetation can be marked, especially during winter. For example, balsam fir (*Abies balsamea*), which is not a preferred browse species during summer, is heavily browsed in winter when deciduous species cannot satisfy moose food requirements (McInnes *et al.*, 1992; Augustine & McNaughton, 1998). Given that snow protects plants from browsing, there are concerns about multiple stresses caused by climate change (e.g., snow pack depth and duration) and moose population increases in the northern forest.

How forest decomposition will respond to increasing moose densities and changing climate is an important ecological question. Decomposition is primarily driven by three factors: climate, litter quality and soil organisms (Swift *et al.*, 1979). Climate change (warming) will increase decomposition rates (Hobbie, 1996), but only if adequate moisture is available (Robinson *et al.*, 1995). Hayhoe *et al.* (2006) have suggested that within the next 50 years summer temperatures will increase by 1–3°C (under low- and high-emission scenarios, respectively) with no change in summer precipitation. This increase in summer temperature with no change in precipitation would lead to soil moisture deficits during the summer months, thereby reducing decomposition rates. Very little work has investigated how changing snow depths will influence soil freezing events and the subsequent effects on microorganisms and their interactions with macro, meso and microfauna (Swift *et al.*, 1979; Paul & Clark, 1989). Work in the Arctic has found that microbial activity occurs below freezing (Clein & Schimel, 1995), that freeze-thaw events stimulate microbial activity (Schimel & Clein, 1996) and that soil microbial community assemblages change from winter to summer (Schadt *et al.*, 2003; Schmidt & Lipson, 2004). Effects of freezing on physical fragmentation and chemical decomposition of litter and soil fauna, however, have received less attention (Henry, 2007). Soil freezing can decrease species richness of enchytraeids and microarthropods, important detritivores in the forest floor (Sulkava & Huhta, 2003).

Tree species also strongly influence N cycling and decomposition rates in forested ecosystems (Mudrick *et al.*, 1994; Finzi *et al.*, 1998; Lovett *et al.*, 2004), primarily through variation in the chemical quality of the litter that they produce. Conifer species generally have lower quality litter (e.g., higher C:N ratios, higher tannins, slower decomposition) while deciduous species have higher quality litter (e.g., lower C:N ratios, lower tannins, faster decomposition) (Aber & Melillo, 1980; Melillo *et al.*, 1982). To our knowledge, there has been no work on the direct interactions between tree litter and moose feces in the forest floor. There is particular uncertainty about the effects of ambient litter quality on the decomposition of feces.

In our study, we established two experimental manipulations at the Hubbard Brook Experimental Forest (HBEF), in the White Mountains of New Hampshire, USA, to investigate the role of snow depth, soil freezing and litter composition on decomposition and the fate of N in sugar maple leaf litter and moose feces. Our objectives were (1) to determine the effect of soil freezing induced by snow removal on decomposition and N release from sugar maple litter and moose feces and (2) to evaluate the effects of ambient litter quality on feces

decomposition and N release. We hypothesized: (1) that soil freezing would increase decomposition and N release from both litter and feces by increasing physical disruption and fragmentation and (2) that feces would decompose more quickly on the forest floor when in proximity to litter with high decomposition rates. Leaf litter was applied to field plots over two fall seasons to demonstrate how soil freezing may impact decomposition over this entire period, while moose feces was a one time application, simulating how moose feces is naturally distributed across the landscape. Leaf litter was applied during the natural leaf senescence period (October) while application of moose feces occurred in January, the month when moose are most prevalent at mid- to high-elevation forest zones in the White Mountains (Whitaker, 1993).

Methods

Site description

This research was conducted at the HBEF, located in central New Hampshire, USA (43°56'N, 71°45'W). The area is classified as a mature northern hardwood forest with a greater abundance of red spruce and balsam fir at higher elevations (Schwarz *et al.*, 2003). The major overstory species found in the plots used for this study were *Acer saccharum* Marsh. (sugar maple), *Betula alleghaniensis* Britt. (yellow birch), *Picea rubens* Sarg. (red pine), *Fagus grandifolia* Ehrh. (American beech), *Fraxinus americana* L. (white ash) and *Acer rubrum* L. (red maple) while *Viburnum alnifolium* (hobblebush) is a common understory shrub. The snow pack in this area is generally present from mid-November to mid-April (165 days, 30-year average) with average January air temperatures of -9°C and average winter (December–March) temperature of -4.7°C (Hardy *et al.*, 2001). The soil freezing season occurs from the end of November to the end of March and is considered continual (Hardy *et al.*, 2001; Cleavitt *et al.*, 2008). Average summer conditions for Hubbard Brook are 18°C in July and average annual precipitation is 140 cm (Bailey *et al.*, 2003). To study litter decomposition, we used eight, 10×10 m plots located at four sites established for the snow manipulation study described by Cleavitt *et al.* (2008). At each site there were two plots (snow removal and reference). Two of the sites were south facing, located in the Hubbard Brook Valley bottom (elevation 380 and 480 m) and will be referred to as Lower Valley (LV) and Upper Valley (UV). The other two sites referred to as west Kineo (WK) and east Kineo (EK) were located on Mt. Kineo on north-facing slopes at higher elevations (755 and 790 m, respectively). All plots had understory shrubs clipped to facilitate snow shoveling

on the treatment plots. To protect the forest floor and monitoring equipment, and to preserve winter albedo on the plots, 5 cm of snow was left on the shoveled plots. Plots were shoveled commencing with the first significant snowfall of the season (> 5 cm), and shoveled after every major snow event (> 5 cm) until late January in 2003 and 2004 to facilitate soil freezing. Snow removed from the treatment plots was placed at the outside edges of the plots (Cleavitt *et al.*, 2008). Frost depth was measured with frost tubes as described by Hardy *et al.* (2001) and Cleavitt *et al.* (2008). Litter 'corrals' were established on these eight plots to investigate decomposition of litter (see below for litter corral description). A separate series of plots were established in the vicinity of the Mt. Kineo 10×10 m plots to investigate decomposition of moose feces (see below for moose plot description).

Materials: production of ^{15}N -labeled maple litter and moose feces

To obtain ^{15}N -enriched maple leaves, a ^{15}N solution was infused in May 2002 using stemwell injection (Christenson *et al.*, 2002) into a sugar maple tree that was ~ 6 m tall, ~ 12 cm diameter at breast height and located in an open area near the Cary Institute of Ecosystem Studies in Millbrook, NY. The solution was made by mixing 10 g of $(\text{NH}_4)_2\text{SO}_4$ (99% ^{15}N atom enriched) in 2 L of distilled water. To infuse the isotope solution, holes were drilled ~ 1 cm into the bark of the tree 1 m from the ground. The solution was held in a 2 L polyethylene bottle above the holes, and neoprene tubes with plastic connector tips were pushed into the holes. Leaves on the sugar maple were rapidly expanding, and the infusion occurred mid-day under bright sunny conditions and warm temperatures ($\sim 24^{\circ}\text{C}$) with the tree taking up the solution rapidly. Leaf samples from this tree were collected, dried (40°C for 48 h), ground and analyzed for ^{15}N at the University of California (UC) – Davis, Stable Isotope Facility in Davis, CA. The atom ^{15}N atom% value of 0.506 was well above the background levels of ^{15}N of ~ 0.366 atom%. Senescent leaves from the infused tree were collected in October 2002 and air dried at room temperature for 48 h. These senescent leaves were then transported to the HBEF and applied to the appropriate plots in late October 2002 and again in October 2003.

To produce ^{15}N -enriched moose feces, a 30×30 m fenced vegetation plot at the Kenai Moose Research Center in Alaska was labeled with ^{15}N . Tree species in this plot included birch (*Betula* spp.), trembling aspen (*Populus tremuloides*) and white spruce (*Picea glauca*). A total of 27 g of NH_4Cl (^{15}N 99 at.%) mixed with 135 L water was applied with a hand-held sprayer over a

2-day period in May 2003 to this plot. The solution was sprayed at ground level under zero wind conditions, avoiding contact with tree sapling leaves. In October 2003, after leaf senescence, a captive bred, 7-year-old female moose ('Willow') was introduced to the plot to browse on the labeled saplings. Willow had also been prefed approximately 4 kg of pelleted food (aspen sawdust pellets) per day, 2 days before introduction to the pen. This pelleted food was sprayed with ^{15}N -enriched urea (99 at.%) mixed with water and supplemented the total amount of ^{15}N that Willow was consuming as enriched browse saplings. Feeding these two ^{15}N -enriched sources of food ensured highly labeled feces. Pelleted food was fed three times per day ($1.25 \text{ kg} \times 3 = 3.75 \text{ kg}$) for 6 days. At each feeding, approximately 20 mL of ^{15}N -labeled urea solution was sprayed on pelleted food for a total of 4 g of ^{15}N -labeled urea over the 6-day period.

Fecal pellet groupings were collected individually over a 6-day period, weighed and stored at 0°C . Approximately 25 kg of moose feces were collected and packed in a cooler with ice and flown back to the Cary Institute of Ecosystem Studies. Subsamples of each day's collection were dried at 60°C for 48 h, ground and sent to the stable isotope laboratory at UC – Davis for ^{15}N analysis. Labeled feces were significantly higher in ^{15}N (0.493 at.%) compared with native (HBEF) non-labeled feces (0.3665 at.%). The remaining feces were transported to HBEF and applied to the specified plots in January 2004.

Litter 'corrals'

A total of 16 ($0.5 \times 0.5 \text{ m}$) litter 'corrals' consisting of fiberglass window screen (2 mm mesh) secured to fiberglass posts $\sim 35 \text{ cm}$ high were established on each of the eight $10 \times 10 \text{ m}$ study plots. Maple leaf litter was added to each 'corral' and covered with garden netting (mesh size 2.5 cm) to prevent litter from blowing in or out of the small plot. Eight 'corrals' had ^{15}N -labeled maple leaf litter added and eight had nonlabeled maple leaf litter added. Nonlabeled and labeled maple litters (50 g) were added in October 2002 and 2003 to simulate natural litterfall at HBEF (Hughes & Fahey, 1994).

All litter 'corrals' were sampled in May and August in 2003 and 2004 (four sampling times). For each sampling, maple leaf litter was shifted as gently as possible to the side and PVC tubes ($\sim 5 \text{ cm}$ diameter) were used to sample soils beneath each corral. Soils were sampled to a depth of 10 cm, and the cores were separated into organic and mineral horizons. Soils were transported to the laboratory within 4–5 h of collection and stored at 4°C until processing/analysis occurred (within 24 h). Plants growing in the litter 'corrals' were

harvested in August 2003 and 2004, dried, weighed and analyzed for % N and % C and ^{15}N values.

Moose feces plots

As part of a larger investigation (Christenson, 2007), 48, $2 \times 2 \text{ m}$ plots were established on the north facing slope of Mt. Kineo at an elevation of $\sim 640 \text{ m}$. Each plot contained three saplings of the same species (16 plots each of sugar maple, balsam fir and *Viburnum*). Each plot was randomly assigned one of each of the following treatments with two replicates: (a) snow shoveling or no shoveling, (b) mechanical clipping of saplings or no clipping, (c) addition of ^{15}N -labeled moose feces or no feces. Two untreated reference plots were also established for each of the sapling species. For those treatments with ^{15}N -labeled fecal pellets $\sim 500 \text{ g}$ (exact mass recorded) were added in January 2004. This amount of feces is similar to our observations of moose fecal pellet groupings at HBEF.

Porous cup lysimeters (Mitchell *et al.*, 2001) were installed below the major rooting zone (20–40 cm depth) in each plot to monitor dissolved organic nitrogen (DON), NH_4^+ and NO_3^- concentrations, and the ^{15}N fraction in total inorganic N (TIN). Lysimeter solutions were collected monthly from January 2004 through December 2005. Tension was set at 28 MPa 24 h before collection of samples. Samples were analyzed for pH on the day of collection, and subsamples for further chemical analyses were stored at 4°C for up to two weeks before analysis. A second subsample was frozen for later ^{15}N analysis. NH_4^+ (salicylate method) and NO_3^- (cadmium reduction method) were analyzed on a Lachat Quikchem 8100 Flow Injection Analyzer (Hach Company, Loveland, CO, USA). A modified persulfate digestion method (Cabrera & Beare, 1993) was used to determine total dissolved nitrogen concentration. Frozen samples were thawed and bulked into growing season (May through September) and nongrowing season (October through April) composite samples for each sapling plot (96 total samples). A modified diffusion technique (Brookes *et al.*, 1989; Stark & Hart, 1996) was used to determine ^{15}N values in the TIN pool. DON concentrations were low to nondetectable thus not permitting ^{15}N analyses of this solute.

Soils were sampled in May and August of 2004 and 2005 following the same procedures as those used for the litter corrals. Saplings in the plots were harvested in May 2006. Saplings were carefully dug from the ground using a spade, creating a large 'soil plug' around the sapling. As the sapling was lifted from the ground, if resistance was encountered, more excavation around the sapling was performed. Every attempt was made to collect the entire rooting system, but invariably some small diameter root mass was not completely recovered. The saplings were

divided into roots (all size classes), stems/branches and buds/needles. These samples were dried and ground following the same procedure as that used for soil and analyzed for % N and % C and ^{15}N values.

Soil analyses

All soil samples were brought back to the laboratory where they were hand sorted to remove roots, woody debris and stones and homogenized. Subsamples of fresh soil were removed for extraction of inorganic N and for measurements of microbial biomass C and N content, potential net C and N mineralization and nitrification and pH. The remainder of the samples were dried for 48 h at 60 °C to determine moisture content, and then stored in paper coin envelopes placed in sealed plastic bags.

pH. Slurry using field moist soil was created in a 2:1 ratio of nanopure water. The suspension was stirred intermittently for 30 min, then allowed to stand for 1 h (Carter, 1993). An electrode (Fisher Accumet Model 610A, Analytical Instruments, LLC, Golden Valley, MN, USA) was used to measure the pH of the supernatant.

Moisture. Moisture contents were determined gravimetrically after drying at 60 °C for 48 h. The % moisture was calculated by

$$(\text{wet wt} - \text{dry wt}) / (\text{dry wt}) \times 100.$$

Total C and N. After moisture determination, the dried soils were ground to a fine powder in a KLECO[®] pulverizer (Kinetic Laboratory Equipment Company, Visalia, CA, USA) and stored in paper coin envelopes. Total elemental % C and % N were determined on a Carlo-Erba NA1500[®] analyzer (CE Elantech, Inc., Lakewood, NJ, USA).

Extractable N. Ten grams of sieved, field moist soil were weighed into plastic specimen cups and 50 mL of 2 mol L⁻¹ KCl added. Samples were placed on a shaker table at 125 rpm for 1 h and then allowed to stand for 1 h. The supernatant was filtered through Whatman[®] (41) ashless filter paper (Whatman Ltd., UK) into polyethylene sample bottles. Samples were stored at 4 °C until further analysis. A Lachat Quikchem 8100 Flow Injection Analyzer[®] was used to analyze the samples for NH_4^+ (salicylate method) and NO_3^- (cadmium reduction method). All results are reported in $\mu\text{g N g}^{-1}$ dry weight (DW) soil.

Mineralizable N. Soil samples (10 g field moist) were incubated in glass quart (946 mL) mason jars that were fitted with airtight lids with butyl rubber septa to allow

for gas sampling. Jars were incubated in the dark at room temperature for 10 days. After incubation, gas samples were taken by syringe for analysis of CO_2 by thermal conductivity GC (Shimadzu[®] GC 14, Shimadzu Corporation, Kyoto, Japan) and inorganic N was extracted and analyzed as described above. Mineralizable N ($\mu\text{g N g}^{-1}$ DW soil) was calculated as:

$$(\text{final } \text{NH}_4^+ + \text{NO}_3^-) - (\text{initial } \text{NH}_4^+ + \text{NO}_3^-).$$

Microbial C and N. Microbial biomass C and N content were determined using the chloroform fumigation incubation method (Jenkinson & Powlson, 1976). Ten grams of soil were fumigated for 14–16 h, inoculated with 0.1 g of fresh soil and then incubated in 946 mL glass mason jars and sampled as described above. A proportionality constant ($k_C = 0.41$) (Jenkinson & Powlson, 1976) was used to calculate biomass C from the CO_2 produced during the incubation. It was assumed that all recovered N was derived from microbial biomass (e.g., $K_N = 1$). No constant was used to calculate biomass N.

Isotopic analyses

To determine the abundance of ^{15}N in the KCl extracts and lysimeter solutions, a modified N-diffusion technique (Stark & Hart, 1996; Brookes *et al.*, 1989) was used. Acidified glass fiber disks were used to trap NH_3 volatilized in the procedure, and these disks were packed in tin capsules and sealed. Soil, plant and fecal samples were dried at 60 °C, ground to a powder and packed in tin capsules. All samples for ^{15}N determinations were sent to the Stable Isotope Facility at UC Davis.

Statistical analyses

Data were tested for normality of distribution using the Shapiro–Wilk test (PROC UNIVARIATE, SAS) and nonnormally distributed dependent variables were \log_{10} transformed before analysis. To test the hypothesis that soil freezing increases mass loss and N release from maple leaf litter and increases the movement of N into microbial biomass, a paired *t*-test was performed with the JMP statistical package (version 5.0.1a SAS) to determine significant differences between the treatments where site was the replicate ($n = 4$). To test the hypothesis that soil freezing would increase mass loss and N release from feces and increase the movement of N into soils and microbial biomass, a one-way analysis of variance (ANOVA) testing treatment effects was used (PROC GLM, SAS 8.2, SAS Institute, Cary, NC, USA). All statistical analyses were done using $\alpha \leq 0.05$. To test the hypothesis that ambient leaf

litter and/or soil freezing affected moose feces decomposition, a two-way ANOVA followed by a Student–Newman–Keuls *post hoc* test was performed using treatment and species as the independent variables.

Results

The snow manipulation on the large experimental plots (litter corrals) increased the depth of frost penetration in the soils, especially during the winter of 2003–2004 (Table 1). Total soil C and N did not change with the snow removal; however, the east Kineo site had significantly higher soil C and N concentrations compared with the other sites (Table 1). Snow manipulation on the moose feces plots also significantly ($P < 0.0001$) increased depth of frost over the experimental period. Mean depth of soil frost was $22 (\pm 1.2)$ cm on the snow-removal plots and $10 (\pm 1)$ cm on the nonmanipulated plots during the winter of 2003/2004. Frost depths were similar for winter 2004/2005. Total soil C and N were similar to the large experimental plots described above and snow removal did not change the element concentrations (data not shown).

There were distinct chemical composition differences between maple leaf litter and moose feces. Maple leaf litter had lower C, N and moisture contents and higher C:N ratios compared with feces (Table 2). Lignin showed an opposite pattern with feces having higher lignin content than litter. Isotopically labeled litter or feces were chemically similar to the nonlabeled, native forms (Table 2).

Treatment effect on decomposition

Snow removal reduced decomposition resulting in significantly more maple leaf litter mass remaining in litter ‘corrals’ in the treatment plots compared with the reference plots (Fig. 1). By comparison, decomposition of ^{15}N -labeled moose feces was not affected by the snow removal treatment and total mass remaining after 20 months was not significantly different between treatment and reference plots (Fig. 1). In the moose feces plots, neither sapling species composition nor soil freezing significantly influenced decomposition (Fig. 2).

Treatment effect on N dynamics measured with ^{15}N

Soil freezing increased the flux of ^{15}N from sugar maple litter, such that significantly ($P < 0.05$) more ^{15}N was recovered in mineral soils (Fig. 3a) in treatment relative to reference plots. Microbial biomass in the forest floor of treatment plots had significantly ($P < 0.01$) more ^{15}N (Fig. 3a) than reference plots. Snow removal had a similar effect on decomposing moose feces. There was a

Table 1 Hubbard Brook Experimental Freeze Plot environmental characteristics and treatment effect on soil conditions experienced in the litter corrals

| Site | Treatment | Elevation (m) | Aspect (deg.) | Mean frost depth (cm) | | Soil % C | | Soil % N | | Soil pH | |
|-------------------|-----------|---------------|---------------|-----------------------|---------------|------------|-------------|-----------|--------------|------------|-----------|
| | | | | February 2003 | February 2004 | Organic | Mineral | Organic | Mineral | Organic | Mineral |
| Lower valley (LV) | C | 380 | 105 | 5.2 (5)* | 10.5 (1.8)* | 36.2 (4.5) | 6 (3.7) | 1.7 (0.3) | 0.32 (0.18) | 3.8 (0.3) | 4.1 (0.4) |
| | F | | East | 36.2 (1.5) | 35 (0.6) | 35 (5) | 4.4 (1.3) | 1.5 (0.2) | 0.2 (0.08) | 3.9 (0.2) | 4.3 (0.2) |
| Upper valley (UV) | C | 480 | 190 | 2.3 (2.4)* | 15 (5.8)* | 31.8 (4.5) | 4.9 (0.7) | 1.8 (0.3) | 0.33 (0.05) | 3.9 (0.2) | 4.2 (0.1) |
| | F | | South | 35.4 (13.5) | 41 (14.5) | 28.7 (8.8) | 11 (4.8) | 1.7 (0.6) | 0.66 (0.27) | 4.3 (0.07) | 4.7 (0.1) |
| West Kineo (WK) | C | 755 | 352 | 0 (0)* | 8 (0.6)* | 35 (5.7) | 5.3 (1.7) | 1.8 (0.3) | 0.33 (0.12) | 4 (0.09) | 4.1 (0.3) |
| | F | | North | 39.5 (5.1) | 41 (1.9) | 32.7 (8.2) | 8 (0.7) | 1.9 (0.5) | 0.45 (0.05) | 3.9 (0.2) | 4.2 (0.2) |
| East Kineo (EK) | C | 790 | 350 | 0.8 (0.9)* | 10 (1.3)* | 37.9 (2) | 16.5 (8.2)* | 2.1 (0.2) | 0.89 (0.47)* | 3.9 (0.3) | 4.3 (0.4) |
| | F | | North | 48.5 (2.8) | 50 (0) | 41.2 (2.7) | 12.3 (5.2) | 2.1 (0.3) | 0.68 (0.31) | 4 (0.3) | 4.2 (0.3) |

Treatment designation ‘C’ refers to the ‘control’ or reference plot and ‘F’ refers to ‘freeze’ or snow removal treatment. Standard deviations in parentheses ($N = 6$ for Frost Depth February 3, C, N and pH; $N = 8$ for frost depth February 4). Frost depth from Cleavitt *et al.* (2008).

*Significant difference ($P < 0.05$) between treatments within a site.

Table 2 ^{15}N labeled and non-labeled sugar maple litter and ^{15}N labeled and native moose fecal C and N contents, C:N ratio and lignin contents, before and after field incubations (na, not available)

| | Preincubation | | | | | Postincubation | | | |
|--------------------------------|---------------|-----------|------------|-----|------------|----------------|-----------|-----|------------|
| | Moisture (%) | C (%) | N (%) | C:N | Lignin (%) | C (%) | N (%) | C:N | Lignin (%) |
| ^{15}N Labeled Litter | <10 | 47 (0.2) | 0.5 (0.02) | 94 | 12.3 | 46 (0.6) | 1.3 (0.2) | 35 | na |
| Native Litter | <10 | 48 (0.3) | 0.5 (0.02) | 96 | 12.6 | 46 (1.4) | 1.5 (0.5) | 31 | na |
| ^{15}N Labeled Feces | 59 | 52 (0.04) | 1.6 (0.2) | 33 | 20.3 | 50 (3.2) | 2.6 (0.2) | 19 | na |
| Native Feces | 60 | 54 (0.3) | 1.5 (0.1) | 36 | 38 (3.7) | 53 (0.4) | 1.8 (0.1) | 29 | 44 (1.7) |

Standard deviations in parentheses ($N = 4$ for C, N and lignin). Where no standard deviation is presented, only analytical replicate data is available.

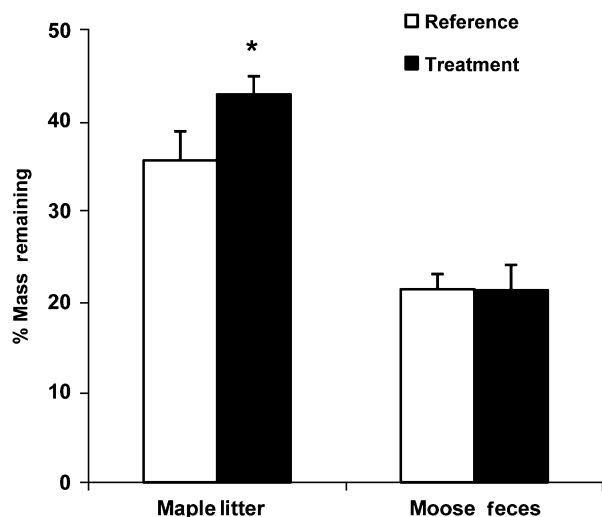


Fig. 1 Percent mass of ^{15}N -labeled sugar maple litter remaining after 22 months of decomposition (October 2002–August 2004) in litter corrals in snow manipulation treatment and reference plots compared with moose feces remaining after 20 months (January 2004–August 2005). Values are mean (\pm SE) of the litter corrals ($n = 4$) and moose plots ($n = 12$). *Significant difference ($P < 0.05$) between treatments.

tendency for more feces ^{15}N to be recovered in the mineral soils in the treatment plots compared with the reference plots, similar to our observations for the sugar maple litter plots (Fig. 3); however, these differences were not statistically significant ($P < 0.08$) (Fig. 3b). Microbial biomass in the mineral soil had significantly ($P < 0.05$) more ^{15}N in when exposed to the freezing treatment (Fig. 3b). Species composition in the moose feces plots did not significantly alter the fate of ^{15}N either in the reference or treatment plots (data not shown).

Estimated total recovery of added ^{15}N either as leaf litter or moose feces exceeded the amount of ^{15}N applied in the snow removal treatment plots, while recovery in the reference plots was $> 88\%$ of total added ^{15}N in both the maple litter and moose feces plots (Table 3). The high (excess) recovery of ^{15}N applied was likely

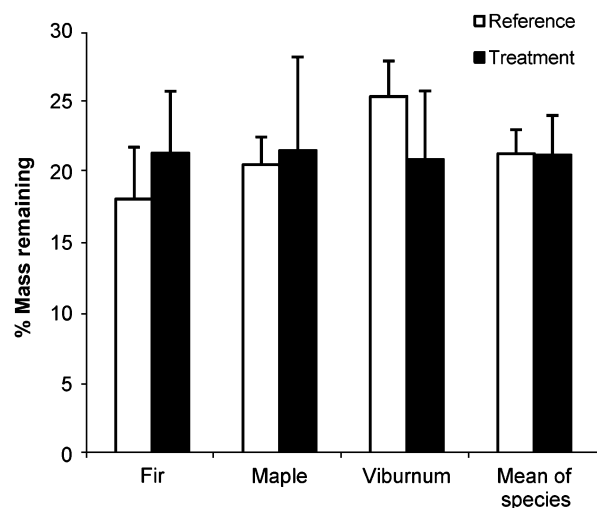


Fig. 2 Percent mass of ^{15}N -labeled moose feces remaining after 20 months (January 2004–August 2005) of decomposition in snow manipulation treatment and reference moose feces study plots dominated by three different sapling tree species. Values are mean (\pm SE) of each species ($n = 4$).

due to variability in soil mass among the plots that affects the calculation of ^{15}N content. For our calculations we used the average HBEF values of 12.8 kg m^{-2} for the forest floor (Oe and Oa) and 74.2 kg m^{-2} for mineral soil (to 10 cm depth) at HBEF as reported by Bohlen *et al.* (2001). Clearly there is considerable variation in soil mass among our plots due to differences in soil coarse fragment content and horizon depths and likely lead to some overestimates of ^{15}N recovery.

Most of the ^{15}N in decomposing maple litter remained in the litter, while more ^{15}N from decomposing moose feces was recovered in the mineral soils (Table 3). The snow removal treatment did not significantly change the fate of ^{15}N from litter or feces, with the exceptions already noted above for the litter corral plots. Treatment did not affect the recovery of ^{15}N in plants measured, but more feces ^{15}N was recovered in the plant pool than litter ^{15}N (Table 3).

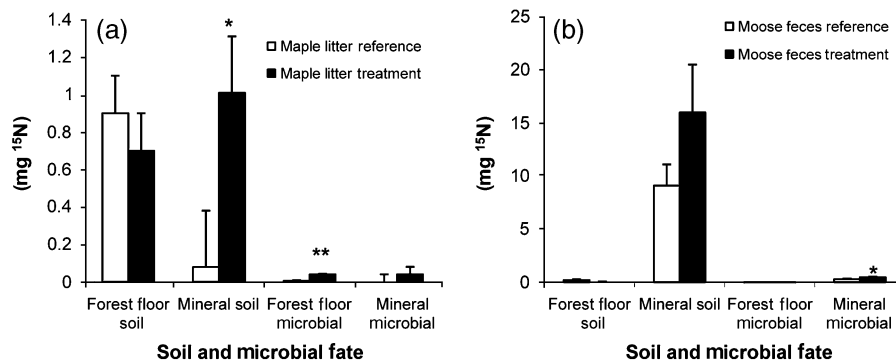


Fig. 3 Recovery of added ^{15}N from decomposing sugar maple litter (a) and moose feces (b) in soils and microbes comparing treatment effect. Values are mean (\pm SE) of the litter corrals ($n = 4$) and moose plots ($n = 12$). Significant treatment differences at $*P < 0.05$, $**P < 0.001$.

Table 3 Recovery of ^{15}N in remaining litter and feces, soil (forest floor and mineral soil), microbial biomass, KCl extractable and plant pools in the snow removal treatment and reference plots after 22 (litter) or 20 (feces) months of decomposition

| | Maple litter | | Moose feces | |
|--|------------------|-----------------|-----------------|-----------------|
| | Frozen | Nonfrozen | Frozen | Nonfrozen |
| mg ^{15}N added | 2.7 | 2.7 | 16.8 | 16.8 |
| mg ^{15}N left in litter or feces | 2.0 | 1.7 | 5.1 | 5.6 |
| mg ^{15}N to trace | 0.64 (0.18) | 1.0 (0.36) | 11.7 (0.91) | 11.2 (0.72) |
| Forest floor (total mg ^{15}N) | 0.7 (0.2) | 0.9 (0.2) | 0 (0.08) | 0.2 (0.07) |
| Extractable | 0.006 (0.002) | 0.005 (0.0006) | 0.002 (0.002) | 0.011 (0.004) |
| Mineralizable | 0.008 (0.001) | 0.004 (0.0004) | 0.004 (0.01) | 0.054 (0.02) |
| Microbial | 0.04* (0.003) | 0.006 (0.003) | 0.01 (0.006) | 0.01 (0.007) |
| Mineral soil (total mg ^{15}N) | 1.01* (0.3) | 0.08 (0.3) | 16 (4.5) | 9.1 (2) |
| Extractable | 0.005 (0.002) | 0 (0) | 0.016 (0.005) | 0.017 (0.007) |
| Mineralizable | 0.001 (0.004) | 0 (0) | 0.065 (0.01) | 0.074 (0.04) |
| Microbial | 0.04 (0.04) | 0 (0.04) | 0.48* (0.06) | 0.3 (0.05) |
| Plant tissue (mg ^{15}N) | 0.0002 (<0.0001) | 0.0003 (0.0001) | 0.002 (<0.0001) | 0.004 (<0.0001) |
| Leaching (mg ^{15}N) L $^{-1}$ soil solution | not measured | | 0 (<0.0001) | 0 (<0.0001) |
| % recovery of added ^{15}N | | | | |
| Remaining litter or feces | 76 | 62 | 30 | 33 |
| Soil N pool | 48 | 37 | 95 | 55 |
| Plant N pool | 0.01 | 0.01 | 0.01 | 0.02 |
| Total | 124 | 99 | 126 | 89 |

Values are mean (\pm SE) of the litter corrals pooled by site ($n = 4$) and moose plots pooled by species with no clipping treatment ($n = 6$).

For plant pool: only herbaceous plants grew in the maple litter plots. Data shown are for total plants harvested in 2004.

Plant values for moose feces plots are calculated with mean mass of one sapling growing in each plot.

*Signifies difference ($P < 0.05$) between treatment within the maple litter or moose feces additions.

Discussion

Snow removal effects on decomposition

Contrary to our original hypothesis, snow removal decreased the rate of decomposition of maple leaf litter (Fig. 1). For the treatment plots $\sim 40\%$ of the litter remained after 2 years of decomposition compared with

$\sim 35\%$ in the reference plots. These results suggest that reduction of biological activity by soil freezing (Brookes *et al.*, 1989; Heuer *et al.*, 1999) may be more important as a regulator of decomposition than any physical effects associated with freezing that could stimulate decomposition. There were, however, no changes in total soil microbial biomass or N mineralization and nitrification rates with soil freezing (data not shown), which is

consistent with results reported by Groffman *et al.* (2001). If microbial biomass was not reduced in forest floor or mineral soils through soil freezing, other biotic members of the soil community (e.g., soil fauna) responsible for initial fragmentation of litter may be influenced by soil freezing and hence be responsible for the reduced rates of decomposition of leaf litter that we observed. Sulkava & Huhta (2003) experimentally manipulated snow levels in pine forests in central Finland and reported decreased species richness of enchytraeids and microarthropods in frozen soils. If these organisms are reduced in number or absent due to freezing events, the initial fragmentation of litter will be slowed, reducing overall mass loss. Mean winter air temperatures for our study plots were -4.7°C (2002/2003 and 2003/2004) and soil temperatures ranged from -0.5°C at the surface (0 cm depth) of control plot soils and -3.7°C for snow manipulation plots in 2003. Our soil frost data indicate that our soils froze during the winter, and similar to results reported by Sulkava & Huhta (2003), these conditions may have limited soil fauna activity. With air temperatures at or below -4.7°C , soil temperatures would have remained above the -5°C limit for microbial activity and this may explain why we did not see changes in overall microbial activity or biomass. Mean soil moisture percentage measured in the litter corals was $65 (\pm 1.03\text{SE})$ in the forest floor soils and $42 (\pm 1\text{SE})$ in the mineral soils. There were no significant differences in soil moisture between the reference and control plots; therefore moisture availability most likely was not an influencing factor on decomposition for these experiments.

In contrast to litter, decomposition of labeled moose feces was not affected by soil freezing (Fig. 1). After 20 months of decomposition, $\sim 20\text{--}25\%$ of the feces mass remained on all the plots. Labeled maple leaf litter lost less mass (i.e., decomposed more slowly) compared with labeled feces, irrespective of treatment (Figs 1 and 2) although it is important to note that litter and feces decomposition measurements were carried out at different times in different locations at HBEF. Litter had a higher C:N ratio and lower moisture content compared with feces (Table 2) and these attributes may partially account for the lower decomposition of litter. Interestingly, the relatively high lignin content of the feces did not appear to inhibit decomposition (Table 2). Many studies have found C:N ratio, moisture content and lignin to be strong drivers of decomposition (Aber & Melillo, 1982; Melillo *et al.*, 1982; Taylor, 1998). The lignin:N ratio was higher in the litter (20.5) than in the feces (12.7) which could explain the lower decomposition rates (Melillo *et al.*, 1982). The differential response to soil freezing between litter and feces may be due to the fact that constituent components of feces were

fragmented by the digestion process and therefore feces decomposition was less dependent on the activity of soil micro and macroarthropods that may have been decreased by soil freezing.

Our studies at HBEF were designed to explicitly investigate the role of soil freezing in altering the fate of N in moose feces produced on a winter diet (principally wood/twig biomass, tree/shrub buds and needles of balsam fir). The distinction in seasonal diet is important. Pastor *et al.* (1993) found that moose feces inputs (from a spring diet with lower C:N) stimulated N mineralization in soils. We did not see increases in net N mineralization or nitrification in our moose feces plots, and we believe that the high amount of C and lignin may have acted to immobilize N even though the labile nature of the feces allowed for faster decomposition compared with sugar maple leaf litter. The contributions of leaf litter and winter moose feces may therefore be viewed as two 'different sources of N' to the forested ecosystem: one from leaf litter (recycled by the tree) and the other from wood biomass that is digested by the moose and returned to the forest floor with additional N from metabolic fecal nitrogen from the digestion process.

Our experiments followed the decomposition of litter and feces over a 20-month integrated period, including the soil freezing winter conditions as well as the summer conditions. While the increase of soil freezing events projected by Hayhoe *et al.* (2006) will likely decrease winter decomposition activity, the ultimate effects of climate change on decomposition will be determined by changes in both winter and summer conditions. Interestingly, Hayhoe *et al.* (2006) suggest that summer temperatures will warm while summer precipitation will remain the same as today and may occur in more intense, punctuated events. Such a change in temperature regime may result in water deficits that could contribute to reductions in decomposition during the summer months.

Ambient litter quality effects on decomposition

We hypothesized that tree litter quality would influence moose fecal decomposition, with higher quality (low C:N) deciduous litter increasing overall decomposition and lower quality (high C:N) litter slowing decomposition. However, tree sapling species presence had no effect on feces decomposition (Fig. 2). Moose utilize sapling size tree and shrub species (generally under 3 m) for both forage and shelter. We were specifically interested in how these smaller plants may influence moose fecal decomposition rates, as moose tend to bed down in these areas and then deposit feces there.

Decomposition of moose feces with relatively high N concentrations may not be limited by N and is not affected by quality of surrounding litter. This idea is partially supported by the amount of N immobilized by the maple leaf litter compared with the feces. Maple leaf litter immobilized 100% of its initial N concentration (Table 2), indicating a high microbial demand for soil N. In contrast, the feces had a reduced immobilization requirement, ~70% for ^{15}N -labeled feces.

Snow removal effects on soil N dynamics – fate of N

Labeled moose feces 'mobilized' more N compared with sugar maple litter. After 20 months of decomposition, 30–33% of the ^{15}N originally added in the moose feces plots was recovered in the undecomposed moose feces (Table 3) and ~70% of the ^{15}N had entered various N pools. In contrast, 62–76% of the initial sugar maple litter ^{15}N added remained in the undecomposed litter after 22 months of decomposition (Table 3) with <40% of litter ^{15}N being transferred from litter. Snow removal did not significantly affect release of N from decomposing moose feces or leaf litter (Table 3).

Snow removal did, however, affect the fate of N in the ecosystem. Though litter decomposition was slowed by snow removal, more ^{15}N was recovered in the total, extractable and microbial biomass N pools in the mineral soil in the snow removal plots than in the reference plots (Table 3, Fig. 3). Soil freezing may therefore play an important role in moving N from the forest floor to deeper mineral horizons where the potential for N retention is lower.

The mobilization of N that we observed was not driven by immobilization treatment differences, as the concentration of N remaining in the litter postincubation did not differ between the treatment and reference plots (data not shown). Also, mobilized N was available to the herbaceous plants growing in the litter corrals, but treatment did not affect plant uptake of this N (Table 3). It is not clear how movement of N to deeper soil horizons through soil freezing will affect tree N uptake, but our results indicate that trees should have access to this N source. Cleavitt *et al.* (2008) showed that soil freezing damages tree root tissues, leading to reduced N uptake. If tree roots are damaged, and a significant amount of N is transported to lower mineral horizons, excess N may leach from the system (Mitchell *et al.*, 1996; Fitzhugh *et al.*, 2001).

A possible mechanism driving increased mobilization of N by soil freezing could be mechanical or physical disruption of the soil environment. Mechanical disruption of the soil through freezing could transport mobilized N down through the soil profile. Steinweg *et al.* (2008) observed that soil freezing increased the move-

ment of organic matter in soil aggregates from organic to mineral soil horizons and increased the N content of mineralizable substrates in mineral soil via physical disruption of aggregates.

Schmidt & Lipson (2004) found that litter decomposition and microbial growth was higher under snow pack in tundra soils, but that mineralized N was immobilized by the winter microbial community. Our data support the finding of higher litter decomposition under snow cover (Fig. 1); however, the retention of ^{15}N in the microbial pool was greater in the snow removal plots (Fig. 3). Schadt *et al.* (2003) found that soil microbial (mostly fungal) biomass in tundra soils reached its annual peak under snow. Our soil sampling occurred during mid spring and mid to late summer (May and August, respectively). We may be observing the effects of a change in microbial community, where soil freezing disruption makes N in maple litter more available for microbial processing, especially in the later winter and early spring.

The fate of moose fecal ^{15}N differed from that of leaf litter ^{15}N . Moose feces N appears to be highly mobile, whether or not they are subjected to freezing (Table 3). Similar to results found for the fate of gypsy moth fecal (frass) N (Christenson *et al.*, 2002), moose fecal N moves through the forest floor and accumulates in the deeper mineral horizons (Fig. 3b) where it is available to plants and microbes (Table 3), but is also susceptible to leaching loss. Our experiment has demonstrated that N cycling and retention in forests undergoing both browsing and soil freezing stress are quite complex and that N can be retained in the soil, limiting N losses often observed in systems experiencing soil freezing.

Ecosystem scale N inputs from leaf litter and moose feces

Large ungulate herbivores influence plant species composition, successional trajectories, nutrient cycling and overall ecosystem productivity (Hobbs, 1996; Augustine & McNaughton, 1998; Pastor *et al.*, 1998). An estimate of the importance of moose feces to total N availability at HBEF can be calculated by assuming a population of five to seven animals within the ~3000 ha Hubbard Brook valley (K. Bontaitis, personal communication, 2003–2004; Buso and Christenson, personal observations) and assuming that each adult moose contributes an estimated 5 kg of feces and three urine events per day (Christenson, observations at Kenai Moose Research Centre, Alaska, October 2003), which represents ~75 g N day⁻¹ (<1 g is TIN) as feces and ~79 g total N day⁻¹ (1.9 mg NH₄⁺-N and 1.2 mg NO₃⁻-N) as urine (Christenson, 2007). Thus, this estimated moose population at HBEF provides ~375–525 g N day⁻¹ (~5–7 g TIN) through feces and ~394–551 g total urine

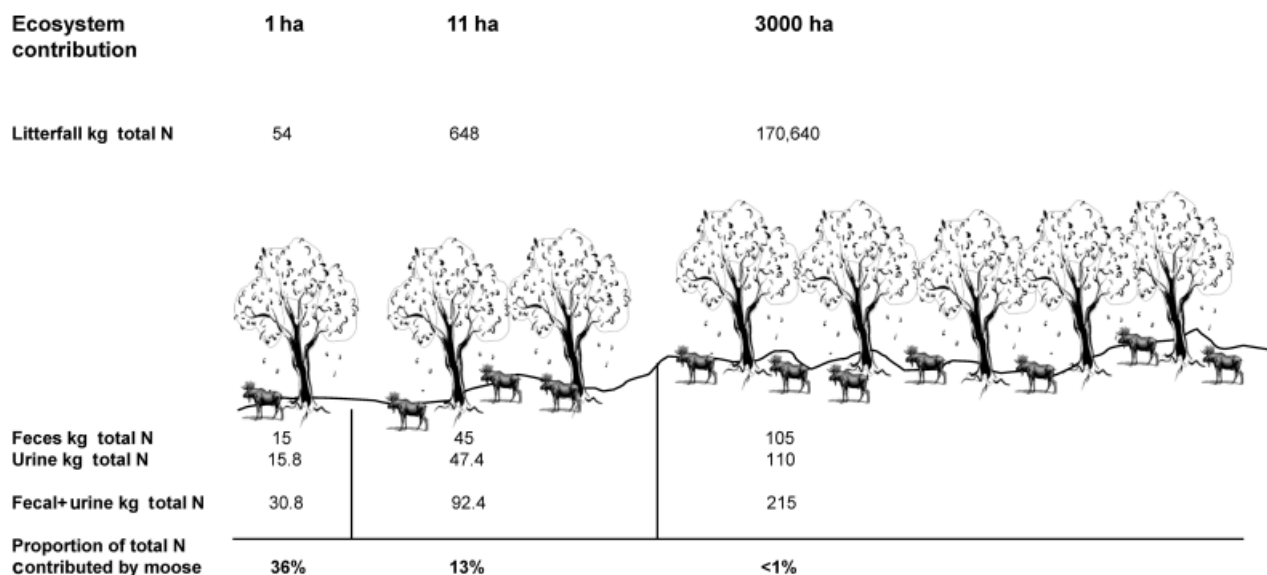


Fig. 4 The relative importance of litterfall compared with moose fecal and urine inputs to the ecosystem at three spatial scales at the Hubbard Brook Experimental Forest. The 3000 ha scale represents the entire watershed, and the 1 and 11 ha scales represent areas utilized intensively by moose during the winter period (200 days). The number of moose illustrated at each spatial scale was used to calculate total N contributions.

N \sim 9.3–13 mg NH_4^+ -N and \sim 6.1–8.5 mg NO_3^- -N). Over 200 days (approximate amount of time moose utilize the 3000 ha Hubbard Brook Valley), \sim 75–105 kg N will be deposited as feces (\sim 1–1.4 kg TIN) while urine will add 79–110 kg total N (1.9–2.6 g NH_4^+ -N and 1.2–1.7 g NO_3^- -N).

By comparison, litterfall accounts for \sim 54 kg N ha^{-1} in a 55-year-old forest at Hubbard Brook (Likens & Bormann, 1995). Over the entire HBEF, litterfall contributes \sim 170 640 kg N (Fig. 4). Thus, litterfall far exceeds the estimate for moose contributions through feces and urine; however, moose contributions are not distributed evenly across the landscape. Instead, moose contribute to N cycling through ‘hotspots’ (McClain *et al.*, 2003) or concentrated areas of deposition. Comparison between litterfall and moose inputs within the small watershed (1–11 ha) area most utilized by moose, litter provides 54–648 kg total N where 1–3 moose can contribute up to \sim 30–92 kg N (Fig. 4). The proportion of total N contributed to the ecosystem by moose over 1 year can be as high as 36% compared with litter inputs (Fig. 4). And these ‘hotspots’ of N contributions are not static. Moose move from location to location in different years, therefore the amount of N coming into localized ‘hotspots’ moves, and this can cover a much larger spatial zone over time, influencing N cycling dynamics.

Many large herbivores utilize habitats over varying spatial and temporal scales (Frank & McNaughton, 1992; Pearson *et al.*, 1995) and our estimates of moose contribution to N dynamics in the northeastern region

are unique. Figure 4 illustrates how important moose fecal and urine inputs can be across the landscape compared with litterfall inputs. Where moose spend their time, as well as how long they stay in various locations, all have implications for N cycling. Moose in northeastern North America occupy smaller areas at higher densities than in other regions of North America (Telfer, 1967a, b, 1970; Kelsall & Prescott, 1971; Crête & Jordan, 1982). These observations are consistent with moose behavior observed at HBEF and support the potential importance for moose at HBEF to significantly contribute to N cycling dynamics through fecal and urine inputs.

Conclusions

Conditions affecting forested ecosystems seldom occur independently. Globally, changes in suitable wildlife habitat as well as changing climate will converge to alter nutrient dynamics, covarying factors that are important to understand as we continue to modify and manage our forest resources. We have demonstrated that both climate change, through soil freezing, and the increasing presence of moose in the northeast, have the capacity to alter decomposition rates and N cycling dynamics. Our study indicates that soil freezing and conversion of forests to feces through moose browsing may influence N cycling through a series of complex interactions, resulting in greater mobilization of N to deeper soil profiles. This increased mobilization of N

may contribute to pulses of NO_3^- -N observed at HBEF (Fitzhugh *et al.*, 2001), where soil freezing may be the catalyst to a series of abiotic and biotic changes within the forested ecosystem.

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