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The fate of nitrogen in gypsy moth frass deposited to an oak forest floor

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Abstract Forest defoliation by insects can lead to severe disruptions of the nitrogen (N) cycle resulting in elevated NO_3^- levels in stream water. To trace the movement of insect-mobilized N in a forest soil, ^{15}N-labeled gypsy moth frass or ^{15}N-labeled oak leaf litter was added to trenched plots in an oak forest over 29 months. Nitrogen movement from the frass or litter was measured in the available, mineralizable, microbial and total soil pools. Uptake of ^{15}N by oak seedlings and inorganic N leaching losses were also measured. No significant differences were found between the frass or leaf treatments for total N in any of the pools. Significant differences were found among the treatments in the distribution of the ^{15}N tracer. Forty percent of the ^{15}N added as frass became incorporated in the soils, with less than 1% found in oak seedlings. Almost 80% of ^{15}N added as leaves remained in the undecomposed leaf material after 2 years. Less than 0.001% of the added ^{15}N was leached in both treatments. Our data indicate that N in frass is mobilized more quickly than N in leaf litter. However, this frass N may be largely unavailable to plants and microorganisms as little of it was found in the extractable, microbial, or readily mineralizable pools.

Keywords N cycling · Insect defoliation · Frass · Gypsy moth · ^{15}N

Introduction

Defoliation by insects can be a source of stress for both individual trees and for entire forest ecosystems. Many studies have evaluated impacts of defoliation on trees, but fewer have examined the ecosystem-level consequences (Grace 1986). Price (1997 following Mattson and Addy 1975) listed five effects of insect defoliation: (1) changing the host's physiological status, (2) increasing litterfall, (3) increasing nutrient input to the forest floor through leaching from trees, (4) changing the composition and structure of the forest through death of weakened trees and release of survivor trees, and (5) enhancing soil microbial activity.

Seastedt and Crossley (1984) suggested that large-scale defoliation can result in a larger input of foliar litter and frass to a forest floor with a potential increase in nutrient cycling. Grace (1986) found that heavy defoliation did not change the total quantity of litter produced in an oak forest but did change the composition and seasonal distribution of the litterfall. Over a 1-year period, Grace (1986) found that insect defoliation increased litterfall N from 31 kg N/ha in non-defoliated forests to 52 kg N/ha in defoliated forests. This input of fresh organic matter with high N and labile C may accelerate microbial activity and produce flushes of NO_3^- and NH_4^+ release (Harris and Riha 1991; Lovett and Ruesink 1995). However, Lovett and Ruesink (1995) found that frass from gypsy moth caterpillars (*Lymantria dispar* L.) increased microbial immobilization of N, thereby reducing, at least temporarily, the possibility of N losses from the ecosystem. The labile C in the frass stimulated growth and production of microbial heterotrophs, increasing the demand for available N.

Investigations into large-scale defoliation events have shown varying results. Swank et al. (1981) reported an increase in NO_3^- export in stream water after the fall cankerworm (*Alsophila pometaria* Harris) partially defoliated an area at Coweeta Hydrological Laboratory in North Carolina, although the quantity of N loss was quite low (<0.5 kg N ha^{-1} $year^{-1}$). Webb et al. (1995) and Eshleman et al. (1998) reported an increased level of NO_3^- export in streams from areas defoliated by gypsy moths over 3 years in the Mid-Appalachian mountains of the United States. In contrast, after defoliation by the saddled prominent caterpillar (*Heterocampa guttivitta* Walker), no increase in NO_3^- export in stream water was

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observed at Hubbard Brook in the White Mountains of New Hampshire (Bormann and Likens 1979). These watershed studies indicate that N losses after defoliation are variable, and that there is considerable uncertainty about the ecosystem-scale effects of insect outbreaks.

In this study, we asked the question; how does conversion of an oak canopy into gypsy moth frass affect cycling and loss of N? More specifically, we asked if the conversion to frass would increase or decrease N mineralization, plant uptake and leaching loss of N. To answer these questions, ^{15}N enriched leaf litter and gypsy moth frass were produced and applied to trenched plots in a mature upland oak forest, and the fate of the ^{15}N was followed for 29 months.

Materials and methods

Site description

The research was done in a forested upland site at the Institute of Ecosystem Studies in Millbrook, N.Y. The site was dominated by mature *Quercus rubra* L. (northern red oak), with *Tsuga canadensis* L. (eastern hemlock), *Acer rubrum* L. (red maple), *Q. prinus* L. (chestnut oak), and *Q. velutina* Lam. (black oak) found in the overstory. This site was selected because it had a mature canopy and a species composition that would typically favor gypsy moths. The soils of the study site are classed as the Nassau-Cardigan complex, hilly (15–30% slopes) and very rocky (Dutchess County Soil and Water District 1991). These soils are generally shallow, with many rock outcrops and are considered well drained.

Field methods

To obtain ^{15}N enriched oak leaves a ^{15}N solution was infused into a black oak tree that was 9 m tall with a DBH of 12 cm. The infusion solution was 5 g/l $(^{15}NH_4)_2SO_4$, 99% atom enriched in ^{15}N. To infuse the isotope solution, holes were drilled approximately 1 cm into the bark of the tree 1 m from the ground and neoprene tubes with plastic connector tips were pushed into the holes. The solution flowed by siphon action into the tree, with the tree rapidly taking up the solution. This procedure was carried out in the spring of 1995 (May 31) and again in the spring of 1996 (May 22) for a total of 4 l of solution taken up by the tree. Leaf samples from the summer of 1995 and 1996, 2 weeks after infusion, were collected, dried and ground. These samples were sent to the Department of Crop and Soil Science at Michigan State University for ^{15}N analysis. An atom $\%^{15}N$ of 0.6129–0.6035 was determined for the leaves, well above the background level of ^{15}N of approximately 0.366 atom %.

Senescent leaves from the infused tree were collected from natural leaf fall in October and November each year and air-dried at room temperature for 48 h. These leaves were then applied to the appropriate field plots in November of the same year. The mean atom $\%^{15}N$ of these leaves was 0.859.

To produce ^{15}N-enriched frass, fresh leaves from the ^{15}N-labeled tree were harvested daily and fed to captive gypsy moth caterpillars. Third instars were obtained from the USDA APHIS laboratory in Massachusetts in June 1995, 1996 and 1997. These caterpillars were reared through to pupation. The frass collected was dried at 30°C for 48 h and stored for 1–4 weeks before application to the field plots. The labeled frass had a mean atom $\%^{15}N$ of 0.894.

Twelve 0.49 m^2 plots were established at the field site in November 1994. The plots were trenched around the perimeter to a depth of ~1 m, then the sides were lined with plastic sheeting and the trenches backfilled on three sides. On the fourth side, the plots were lined with plastic and the trench covered with plywood allowing access to the subsoil. The field site had a very sparse ground cover consisting of club moss (*Lycopodium* sp.) an unidentified grass species and seedlings of the overstory trees. These plants were weeded from the plots. It should be noted that the plants removed were few and disturbance to the soil was minimal. The 12 plots were randomly assigned to three groups: reference (no manipulation), leaf (^{15}N-labeled leaf litter added) and frass (^{15}N-labeled frass added). Note that the reference plots were used to assess natural abundance or background ^{15}N levels and to monitor seasonal N-cycling in the absence of frass or leaf manipulations. There were four replicate sub-plots for each treatment and the reference. A 2-month-old, red oak seedling was planted in the center of each of the twelve plots in the spring of 1995 as a "bioassay" for N availability. The seedlings were covered with wire mesh (0.8 cm) cages to prevent browsing by deer. ^{15}N-labeled senescent leaves (203 g dry mass), equivalent to a normal leaf litterfall at this site, were added to each leaf treatment plot in November 1995 and November 1996 for a total of 3,049 mg N and 26.2 mg ^{15}N added. ^{15}N-labeled frass (52 g dry mass) was added to the frass treatment plots at the end of June in 1995, 1996 and 1997 for a total of 3,087 mg N and 27.4 mg ^{15}N added. This amount of frass approximated the amount that would be produced by a 50% defoliation of the canopy. Both frass and the forest floor soils had a 20:1 C/N ratio while the senescent leaves had a C/N ratio of 64:1. Plots were sampled in August 1995 and April and August 1996 and 1997.

For each sampling period, four soil cores were taken from each plot to a depth of 25 cm with a 2-cm-diameter stainless steel corer. Each core was divided into two sub-samples, 0–10 cm and 10–25 cm depth. The four sub-samples at each depth were composited, creating two soil samples per plot per sample period.

Three ion exchange resin bags were placed in each of the 12 plots at a 30-cm depth to index leaching losses of N. These bags were collected at each sampling period, with replacement bags installed. Senescent leaves were also collected from each of the 12 seedlings in October–November in 1995, 1996 and 1997. In November 1997, the entire seedling in each plot, including coarse and fine roots, was harvested for analysis. The 12 plots were excavated to a depth of 10 cm (divided into two sub-horizons of 0–3 and 3–10 cm) in 1997.

Laboratory methods

Soil analysis

After collection all soils were brought directly back to the laboratory where they were passed through an 8-mm sieve. Roots, woody debris and stones, were separated from each soil sample. A sub-sample of fresh soil was removed for extraction of available inorganic N and for the measurement of microbial biomass N and N mineralization potential. Another sub-sample was removed and stored at 4°C for later pH analysis while the remainder of the sample was dried for 48 h at 60°C, and then stored in a sealed plastic bag.

Soil pH was measured potentiometrically in a 2:1 slurry (Nanopure water: field moist soil) 1–3 days after collection. Moisture contents were determined gravimetrically after drying at 60°C for 48 h. After moisture determination, the dried soils were ground to a fine powder in a KLECO pulverizer. Total C% and N% were determined on a Carlo-Erba NA1500 autoanalyzer. KCl extractions were used to measure extractable NH_4^+ and NO_3^- (available inorganic N). A Perstorp Analytical, Flow Solution 3000 autoanalyzer was used to analyze the samples for NH_4^+ (salicylate method) and NO_3^- (cadmium reduction method) (Perstorp Analytical 1994). All results are reported in µg N/g DM (dry mass) soil.

To determine the readily mineralizable pools (N-mineralization potentials), soil samples were incubated in glass quart (946 ml) canning jars with airtight lids fitted with butyl rubber septa (Robertson et al. 1999). Jars were incubated in the dark at room temperature for 10 days. Gas samples were taken for analysis of CO_2 by thermal conductivity gas chromatography (Shimadzu GC

8 A) and inorganic N was extracted and analyzed as described above. All N mineralization and nitrification rates are reported as µg N/g DM soil/day.

Microbial biomass of C and N content was determined using the chloroform fumigation incubation method (Horwath and Paul 1994). Microbial biomass C was calculated by: $MBC=1.73F_C-0.56UF_C$ where MBC is microbial biomass carbon, F_C is the carbon released from the fumigated sample, UF_C is the carbon released from the unfumigated sample and 1.73 and 0.56 are constants calculated by direct microscopy (Horwath et al. 1996). Microbial biomass N was calculated by: $BN=\{MBC\times[0.6\times(F_N/F_C)]\}+0.09$ where BN is biomass nitrogen, MBC and F_C are as described above, F_N is the nitrogen found in the fumigated, incubated soil and 0.6 and 0.09 are constants from direct microscopy (Paul and Clark 1996).

Plant tissue and frass analyses

Plant tissues included senescent leaves collected from the labeled oak tree, senescent and green leaves from the oak seedlings, weeds removed from the treatment plots and seedling stems and roots harvested from the plots at the end of the field experiment. Woody debris was separated from the soil in the final plot harvest. Frass was collected from the reared gypsy moth caterpillars. Material was oven dried at 60°C for 48 h, weighed, and reduced to a powder form using a KLECO pulverizer and stored in sealed plastic bags until analyzed for C and N % using a C-N analyzer (Carlo-Erba NA1500).

Resin bags

Resin bags consisted of two separate resin forms, a chloride (anion) form (DOWEX 20-50 mesh 1-X8) and a hydrogen (cation) form (DOWEX 20-50 mesh 50W-X8). Each bag was made using a pre-washed nylon stocking. The stockings were soaked in 1 M HCl for 2 h and then rinsed with deionized water. Each bag, divided in half by tying a knot in the stocking, contained 17 g moist weight of the chloride form resin and 15 g of the hydrogen form. The resin bags were then soaked in 2 M KCl for 2 h and then rinsed in deionized water. Bags were stored in sealed plastic bags at 4° C until placement in the field. After collecting, the bags were extracted with 100 ml of 2 M KCl. Resin extracts were filtered and analyzed for NH_4^+ and NO_3^- as described above. Results are reported as µg N/g DW resin. To estimate the leaching loss of N from the field plots, the amount of N extracted per bag was divided by the approximate area of the bag (30 cm^2) and then scaled to the area of the plot.

Isotopic analyses

To determine the abundance of ^{15}N in the KCl extracts, a N-diffusion technique (modified from Stark and Hart 1996; Brookes et al. 1989) was used. All N-diffusion samples, plus soil and plant tissues were sent to the stable isotope laboratory at the University of California at Davis for ^{15}N atom % determination. Measurement precision reported by UC Davis is less than 0.2‰ $\delta^{15}N$ between reference and check standards. A frass standard was also run in triplicate with each group of samples submitted to UC Davis. The isotopic values for these standards varied by less than 3%.

Mass balance of ^{15}N

To determine the fate of the ^{15}N added to the leaf and frass treatment plots, a mass balance of the added ^{15}N was calculated. The reference plots provided estimates of the natural abundance or background levels of ^{15}N for all of the N pools sampled. The mean atom %^{15}N measured in the reference plots was subtracted from each of the leaf and frass plot ^{15}N values. This calculation provided the ^{15}N in excess of background levels and was the estimate of the ^{15}N that had been derived from either the labeled leaf or frass material. All data presented for the mass balance calculations are therefore for the leaf and frass treatments only.

Statistical analyses

The Shapiro-Wilk test of distribution was used to determine normality of the data. Analysis of variance was used for normally distributed data to test for differences between the treatments (reference, leaf, frass) using the means taken across sampling dates. Within a treatment, ANOVA was used to test for seasonal differences in the response variables between the dates for the treatments. A Student-Newman-Keuls test was used to determine the significant differences among treatment means. The non-parametric Kruskal-Wallis test was used to compare treatment effects for non-normally distributed data. All statistical analyses were done using the SAS statistical program (UNIVARIATE, GLM, and RANK procedures) (SAS 1989).

Results

N Pools and fluxes

Most of the soil N pools (extractable or available inorganic N, mineralizable, microbial, total soil and inorganic N leaching) showed no significant differences between the treatments and the reference in the two seasons sampled (Table 1). Means were calculated across years for the 1996 and 1997 samples because significant differences between years were not observed (Table 1). However, August 1995 samples were not included in those means because of significant differences in potential net N mineralization between 1995 and the other years (Table 1). No significant differences were found for pH or moisture values between the treatments or control for any sample dates. Estimated loss of dissolved inorganic N below the rooting zone as sampled by ion exchange resins showed no significant differences between treatments or the reference for NH_4^+ or NO_3^- (Table 2).

Plant tissue

Senescent and green leaves of the seedlings showed no significant differences in amounts of %N or %C. Neither stem length nor stem biomass differed between the treatments or reference and there were no differences in total N (Table 2). Root lengths and weights were similar for all seedlings from all treatment plots and there were no differences for total N (Table 2).

To account for potential sinks of ^{15}N added to the plots, plants that were weeded from the plots were analyzed for element %N and atom %^{15}N. “Weeds” included unidentified herbaceous plants, mosses and tree seedlings. The mass of these plants ranged from 0.1 to 0.3 g DW $plot^{-1}$ $year^{-1}$ and there were no differences in %N.

Table 1 Soil N concentrations and rates for the reference, leaf and frass treatment plots. "August" and "April" refer to the means from those months in 1996 and 1997. (SE are given in parentheses following the mean values)

Treatment	Sample date	(μg N g soil^{-1})			(μg N g^{-1} day^{-1})		(μg N g soil^{-1})	(μg N g resin^{-1})	
		Extractable NH_4^+	Extractable NO_3^-	N-min rate	Nitrification rate	Microbial biomass N	Resin bag NH_4^+	Resin bag NO_3^-
Reference plots	August1995	62.85 (18.22)	6.04 (1.97)	1.02 (0.30)	0.253 (0.10)	28.37 (78.64)	42.38 (4.06)	55.8 (31.61)
	August	5.72 (0.65)	0.88 (0.67)	0.40 (0.17)	0.27 (0.08)	50.33 (13.35)	8.03 (2.20)	5.95 (3.08)
	April	10.85 (2.64)	0.62 (0.13)	1.03 (0.28)	0.38 (0.06)	18.74 (22.36)	12.98 (3.19)	30.00 (14.90)
Leaf plots	August1995	54.10 (28.81)	5.08 (1.81)	2.18 (0.63)	0.09 (0.06)	118.86 (32.24)	67.68 (15.06)	28.58 (6.97)
	August	5.34 (1.00)	0.04 (0.01)	0.38 (0.13)	0.13 (0.04)	55.87 (2.25)	6.92 (1.19)	7.82 (5.76)
	April	9.08 (1.56)	0.50 (0.18)	0.80 (0.21)	0.26 (0.05)	50.95 (3.78)	17.87 (4.37)	33.75 (13.37)
Frass plots	August1995	50.24 (18.76)	7.17 (1.22)	0.21* (0.33)	0.136 (0.12)	99.74 (60.93)	36.26 (6.02)	28.36 (14.87)
	August	5.85 (0.87)	0.55 (0.29)	0.19 (0.05)	0.25 (0.06)	64.91 (6.53)	9.01 (4.34)	11.96 (5.39)
	April	9.63 (1.92)	0.83 (0.13)	0.77 (0.45)	0.39 (0.06)	12.96 (27.90)	13.52 (2.75)	47.51 (20.50)

* Statistically significant difference between the treatments and control (P<0.05)

Fig. 1 Mean (±SE) of atom % ^{15}N for surface (0–10 cm) and subsurface (10–25 cm) soils. An *asterisk* indicates a significant (P<0.05) difference

Fig. 2 Mean (±SE) atom % ^{15}N of leaching losses for NO_3^- in ion exchange resin extracts. *Asterisks* (*P<0.05, **P<0.01, ***P<0.001) indicate significant differences between the treatments and reference within a sampling period

^{15}N concentrations, pools and fluxes

In contrast to patterns of total N pools, the concentrations of ^{15}N showed strong treatment effects. The frass plots exhibited higher ^{15}N values in the extractable, potentially mineralizable and microbial pools for all sample dates compared to the reference and leaf plots (Christenson 1999). The frass treatment plots had significantly higher concentrations of ^{15}N than the reference or leaf plots in the total N pool in both surface and subsurface soils on most sampling dates (Fig. 1). There were no significant differences in atom %^{15}N between the reference and leaf treatments.

Significant treatment differences were found for NO_3^- leaching with the frass treatment significantly higher than the leaf treatment or reference in April 1996 and 1997 (Fig. 2). Atom %^{15}N analysis for NH_4^+ leaching loss showed no significant differences between the treatments and reference plots.

Green leaves, stems and roots (collected only in 1997) from the frass treatment plots had significantly

Table 2 Total N in mg N m^{-2}. Mean values of each treatment or sums of leaching losses across the five sample dates are presented for each treatment. Standard errors are shown in parentheses. The percentage of the measured N pools are calculated from the total soil N pool

Treatment	Soil 0–10 cm	Extractable N	% of total soil N	Mineralizable N	% of total soil N	Microbial N	% of total soil N	Leaching loss (NH_4^+)	Leaching loss ($N0_3^-$)	Seedlings		
										Senescent leaves	Stems	Roots
Reference	60,755 (11,347)	392 (110)	0.64	149 (51)	0.24	1,771 (398)	2.9	169 (20)	243 (108)	27 (12)	55 (29)	108 (53)
Leaf	67,037 (7,588)	400 (149)	0.59	202 (61)	0.30	1,882 (310)	2.8	231 (37)	233 (31)	31 (6)	73 (8)	190 (39)
Frass	63,667 (8,045)	349 (92)	0.54	86 (49)	0.13	1,869 (308)	2.9	165 (29)	292 (102)	38 (8)	53 (12)	145 (14)

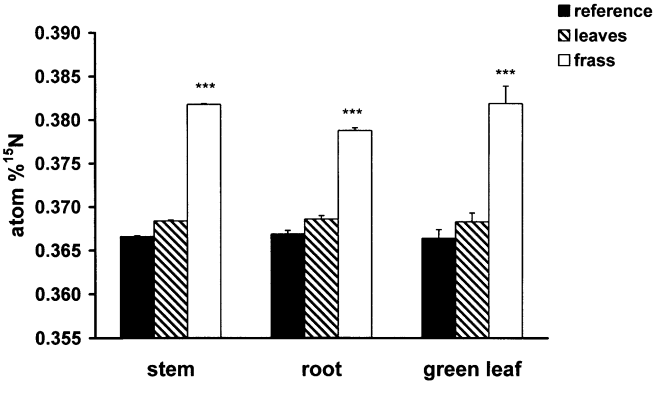

Fig. 3 Mean (±SE) atom %^{15}N for green leaves, stems and roots. Green leaves were sampled in July 1997 and treatment seedlings were harvested in November 1997. *Asterisks* (*P<0.05, **P<0.01, ***P<0.001) indicate significant differences between the treatments and reference

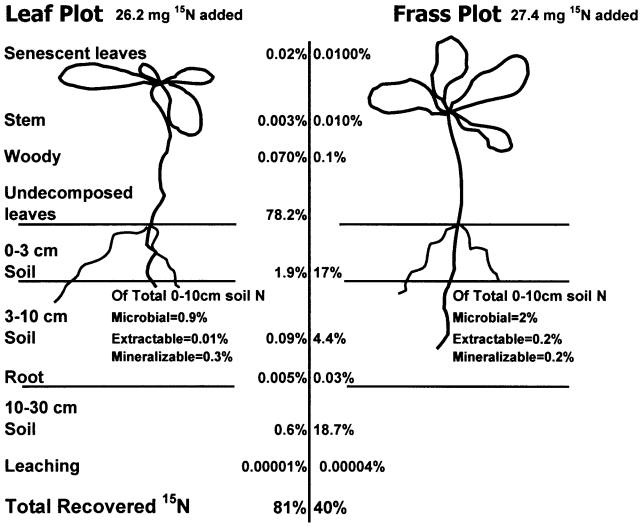

Fig. 4 Percent recovery of ^{15}N added as frass or leaves at the end of the experiment (November 1997). Percentages shown represent the amount recovered relative to the total amount added over the duration of the experiment

higher atom %^{15}N than leaves, stems and roots from the reference and leaf treatments (Fig. 3). Atom %^{15}N values in weeds were also higher (P<0.001) in the frass plots compared to the control and leaf plots (no weeds grew in the leaf plots). There were no differences in atom %^{15}N values for the planted oak seedling senescent leaves for the reference or treatment plots.

Mass balance of ^{15}N

Mean overall recovery of the added ^{15}N at the end of the experiment was 81% for the leaf treatment and 40% for the frass treatment. A large fraction (~78%) of the added ^{15}N in the leaf treatment plots remained in the undecomposed leaves (Fig. 4). The mass of leaf litter remaining on the leaf treatment plots was 5–30% less than the total amount added over the course of the 29-month experiment. Compared to the leaf litter added to these plots, the litter harvested from the plots contained an average of 15% more N but 22% less ^{15}N, indicating a bidirectional exchange of N between leaf litter and soil. It should be noted that native leaf litter was excluded from the frass and leaf treatment plots, while native litter could accumulate on the reference plots. In the frass treatment plots, no visible frass remained on the soil surface at the time of harvest, and the recovered ^{15}N was primarily in the soil pools (~17% in the 0–3 cm soil layer, ~5% in the 3–10 cm layer and ~19% in the 10–30 cm layer; Fig. 4).

In both the leaf and frass treatments, the oak seedlings took up very little of the added ^{15}N (Fig. 4). The largest differences in ^{15}N pool distributions were in the soils. In the leaf treatment, ^{15}N recovered in the soil (0–30 cm depth) accounted for only 2.5% of the added ^{15}N, compared to 40% in the frass treatment plots. Finally, leaching losses for both treatments were very small (0.00004% of the recovered ^{15}N), with no statistically significant difference between the frass and leaf treatments (Fig. 4).

To compare the fate of "mobilized" ^{15}N, we calculated the distribution of ^{15}N that was recovered but not in the undecomposed leaf litter (Table 3). We use the term "mobilized" to mean that the N was moved from the soil surface, probably by water, but not necessarily mineralized. Undecomposed leaf litter was still present at the

Table 3 Percent distribution of ^{15}N that was "mobilized" (i.e., not remaining in undecomposed leaves) and recovered in the leaf and frass plots and t-test comparison of leaf versus frass treatment. (SE for the means are given in parentheses)

N pools	Treatment		
	Leaves	Frass	*P* value
0–3 cm soil	71.7 (9.3)	45.4 (12.1)	0.09
3–10 cm soil	5.1 (3.2)	11.1 (1.72)	0.10
10–30 cm soil	19.6 (11.9)	42.8 (11.61)	0.16
Seedling stem	0.1 (0.01)	0.03 (0.008)	0.05*
Seedling roots	0.2 (0.09)	0.07 (0.01)	0.16
Seedling senescent leaves	0.7 (0.57)	0.03 (0.006)	0.30
Woody material	2.3 (1.93)	0.3 (0.07)	0.38
Loss of NH_4^+	0.02 (0.01)	0.01 (0.005)	0.50
Loss of NO_3^-	0.2 (0.11)	0.3 (0.22)	0.70

* Statistically significant difference between treatments ($P<0.05$)

end of the experiment, while all frass had "dissolved" into the plots. There was a tendency for greater recovery of mobilized N in the surface soils and seedlings in the leaf plots, and greater recovery in the deeper soils in the frass plots, although this difference was not statistically significant. The only significant treatment effect was found in seedling stems (leaf treatment >frass treatment). Leaching losses of mobilized $^{15}NH_4^+$ and $^{15}NO_3^-$ were also not significantly different between the leaf and frass treatments.

Discussion

The cycling and use of N in a forest ecosystem following insect defoliation is complex and linked to the local topography, climate, edaphic conditions and existing plant and animal communities. The results of this study indicate that N in gypsy moth frass had a different fate than N in senescent oak leaves. These differences were not distinguishable in most cases for total N pools and fluxes but were clearly observable using the ^{15}N tracer. In general, the ^{15}N from the frass was quickly mobilized then primarily retained in the soil in pools that were not exchangeable (in 2 M KCl), readily mineralizable (10 day incubation) or microbial. In contrast, the litter ^{15}N was primarily retained in the undecomposed litter.

The soil organic matter was the strongest sink for mobilized, recovered ^{15}N in both leaf litter and frass plots. There was a tendency, though not statistically significant, for the frass ^{15}N to be retained deeper in the soil profile than leaf ^{15}N. Other ^{15}N tracer experiments investigating the fate of N deposition to forests have also found soils to be strong N sinks (Currie et al. 1999; Nadelhoffer et al. 1999a, b).

The disturbance created by the initial plot trenching may have affected our measurements early in the experiment. The highest concentrations of extractable, potentially mineralizable and to some extent microbial N, were found on our first sampling date (August 1995). Both treatments and the reference exhibited high concentrations during this time, with concentrations then declining as the experiment continued. Other workers have also reported enhanced mineralization and nitrification leading to increased leaching losses following soil disturbance (e.g., Dhamala and Mitchell 1996). Even though disturbance is the probable reason for the elevated concentrations and rates, the frass treatment plots still had a significantly lower N-mineralization rate than the leaf treatment or reference plots.

Lovett and Ruesink (1995) proposed that microbial immobilization of N liberated from frass could act to conserve N in a system defoliated by phytophagous insects. They saw decreased potential N mineralization rates in frass-amended soil over 120 days, which they attributed to increased N immobilization by microbes. One of the questions posed by Lovett and Ruesink (1995) was whether this decreased N-mineralization rate would be observable in the field and if it would continue with repeated frass additions. Our data do not conclusively answer this question. The N-mineralization rate was lower in the frass plots than in the reference or leaf plots on some dates but not all dates, and was significantly lower only in August 1995, when rates were elevated in all plots due to the initial disturbance. Averaged across all dates, however, mineralizable N in the frass plots was less than half the level found in the leaf plots (Table 2).

One of the questions addressed in our study was whether N derived from frass differed in its availability to plants and microbes compared to N derived from leaf litter. Again, the data suggest a rather complex answer. Both microbial biomass and plant tissue were more enriched in ^{15}N in the frass plots than in the leaf plots. This occurred largely because the frass ^{15}N was completely mobilized in the soil, whereas the leaf ^{15}N remained mostly bound in undecomposed leaf material on the soil surface (Fig. 4). Therefore more N and ^{15}N were potentially available to the system in the frass treatment during the period of this experiment. If we compare the fate of "mobilized" ^{15}N however, it appears that a larger percentage was taken up by plants in the leaf plots than in the frass plots, whereas frass plots had more in the deeper soil pool (Table 3).

Although the seedlings in the frass plots did not show higher biomass or N concentrations, they were significantly higher in atom $\%^{15}N$ than the seedlings from the reference and leaf plots, probably because so much more ^{15}N was mobilized in the frass plots. Surprisingly, there were no differences in atom $\%^{15}N$ in the senescent leaves among the treatments. This may have been due to the preferential re-translocation of ^{14}N, leaving slightly higher ^{15}N levels in the senesced leaves of all the seedlings and perhaps obscuring the treatment effect (Virginia and Delwiche 1982; Garten 1993).

The low ^{15}N mobilization in the leaf treatment plots was partly a result of the slow decomposition of the oak litter, which ranged from 5.5% to 30% mass loss over the 1- to 2-year period that leaves were on the plots. These low values are similar to those obtained in other studies of oak leaf decomposition (e.g. Mudrick et al.

1994), and probably result from high levels of lignin and secondary compounds such as tannins in oak leaves (Cornelissen 1996). During the initial phase of decomposition, N immobilization acts to increase leaf litter N concentrations (Melillo et al. 1982; Mudrick et al 1994; Nadelhoffer et al. 1995). Analysis of the leaf litter remaining on the leaf treatment plots showed a gain in total N but a loss in ^{15}N amounts. This indicates a bidirectional transfer of N across the litter/soil interface resulting in a dilution of the ^{15}N in the leaves. Zeller et al. (2000) described a similar fate in decomposing beech litter. The ^{15}N that was mobilized from the decomposing leaf litter was observed in all of the measured soil and plant pools, resulting in atom $\%^{15}N$ concentrations that were usually slightly higher than the reference, but not significantly different (Figs. 1, 2, 3).

Several studies have reported increased NO_3^- leaching in stream water from watersheds experiencing defoliation (Swank and Crossley 1988; Eshleman et al. 1998). MacDonald et al. (1992) attributed high NO_3^- concentrations (~ 0.3–0.4 mmol/l) in soil solution from Michigan forests to episodic defoliation by the forest tent caterpillar. In this study we did not measure leaching losses directly but estimated them from resin bags placed in the subsoil of the plots. We did not observe increases in leaching losses of N from the frass treatment compared to the leaf treatment or the reference plots. The mean accumulated loss rate observed in the trenched field plots was 143 mg NO_3^--N/plot over 28 months in the frass treatment, which is equivalent to 1,269 g N ha^{-1} $year^{-1}$. Elevated N leaching losses that occurred in the August 1995 sample period for all treatments (reference, leaf and frass) were attributed to disturbance in establishing the study site. If leaching losses are calculated excluding the August 1995 data, the cumulative NO_3^--N loss from the frass plots was 26 mg NO_3^--N/plot over 24 months, equivalent to 236 g N ha^{-1} $year^{-1}$. This is less than the values reported by Swank and Crossley (1988) (450 g N ha^{-1} $year^{-1}$) and much less than the amounts measured by Eshleman et al. (1998) (980–4,900 g N ha^{-1} $year^{-1}$). Our study differed from the defoliation events studied by Swank and Crossley (1988), Eshleman et al. (1998) and McDonald et al. (1992) because in their studies frass, green leaf litter, dead insects and molts all contribute to N loss. In our study, only insect frass was added. Defoliation events may also increase soil temperatures and movement of soil water as the canopy cover is removed. These factors may accelerate decomposition of surface organic matter, releasing nutrients into solution and potentially increasing leaching loss (Perry 1994). In our study, plant uptake capacity was also minimal, as the planted seedlings were primarily intended for use as a bioassay of N availability to plants. The uptake by a seedling would be small compared to an actively growing forest. Because all other root systems were cut off from our trenched plots, leaching losses were expected to be potentially higher than from intact forests or from defoliated systems which retain some plant uptake capacity for N. We observed enhanced leaching of NO_3^- to the 30-cm depth in the frass plots as shown by significantly higher atom $\%^{15}N$ values in this treatment (Fig. 2). However, the leaching loss rates were very low, accounting for <0.0001% of the ^{15}N applied (Fig. 4).

We observed differences in the fate of ^{15}N between the frass and leaf treatments, despite the fact that few significant differences were found in the distribution of total N. In general, the ^{15}N from the frass was more quickly mobilized then primarily retained in the soil in pools that were not exchangeable, readily mineralizable or microbial. In contrast, the leaf litter ^{15}N was primarily retained in the undecomposed litter.

We observed important treatment differences in total recovery of applied ^{15}N, with 81% recovery in the leaf litter plots and only 40% recovery in the frass plots. Because we harvested all the soil and litter in these plots to a depth of 10 cm, and intensively sampled below that depth, we believe that the ^{15}N not recovered was lost from the system. The difference in total recovery between the leaf and frass plots indicates that whatever the mechanism of loss, at first glance it appears to operate more strongly on the frass N than on the leaf N. However, the percentage of ^{15}N that was mobilized (i.e. released from the frass or litter in which it was applied) and then not recovered was actually higher in the leaf plots (84%) then in the frass plots (60%). This suggests that, after the N is mobilized, the unmeasured loss mechanism may in fact be operating more strongly on leaf litter N than frass N. The long-term retention of leaf litter N would depend on whether the N continued to be lost at the same rate during the longer-term decomposition of the leaves.

One unmeasured loss mechanism may be through gas flux. Gaseous losses of N from forests through denitrification are variable, but are thought to be small in well-drained soils. Work in Europe investigating N saturation (NITREX project) reported small N_2O emission rates, 0.5 kg ha^{-1} $year^{-1}$ to 4 kg ha^{-1} $year^{-1}$ (Tietema et al. 1998) from northern forests. In our study a total of 62–63 kg N ha^{-1} $year^{-1}$ over 2.3 years or approximately 27 kg N ha^{-1} $year^{-1}$ of leaves and frass were added to the experimental plots. Using the 4 kg N ha^{-1} $year^{-1}$ reported by Tietema et al. (1998), we calculated that 0.72 mg ^{15}N $plot^{-1}$ $year^{-1}$ might be denitrified (this calculation based on atom % ^{15}N values of 0.36 for natural abundance and 0.38 for enriched levels). Of the 27.2 mg ^{15}N added in the leaf plots and 27.4 mg ^{15}N added to the frass plots, this equals only 2.6% of the ^{15}N added. According to Groffman and Teidje (1989) and Davidson et al. (1990), N_2O emission underestimates actual rates of denitrification that can be as high as 40 kg ha^{-1} $year^{-1}$ in poorly drained temperate forest ecosystems. This level of denitrification is not likely in the well drained soils of our field plots. There are other possible mechanisms of gaseous loss from forest soils. Ammonia volatilization is possible, although Lovett et al. (1998) report very low rates of volatilization from gypsy moths and their frass. Gaseous N loss can also occur during nitrification (e.g. Bremner and Führ 1966; Firestone and Davidson 1989).

Another potential mechanism for unmeasured N loss from these plots is leaching of dissolved organic N (DON) that is not retained in our resin bags. DON losses reported by the NITREX experiments indicate that 0.5–9.4 kg N ha^{-1} $year^{-1}$ leach from northern forests as DON (Tietema et al. 1998). Magill et al (1997) reported similar values (4–6 kg N ha^{-1} $year^{-1}$) for temperate forests in the NE United States. Using the low (0.5 kg N) and high (9.4 kg N) values, and assuming natural abundance of ^{15}N at 0.36 atom % and enriched at 0.38 atom %, loss of ^{15}N as DON could be 0.09 to 1.7 mg ^{15}N over the experimental period, or 3–6% of the applied ^{15}N. However, it is possible that DON could have been leached to soils below our maximum sampling depth (30 cm) and retained there.

In conclusion, this study has shown that in these trenched field plots, the fate of N from gypsy moth frass was different than the fate of N from leaf litter. Frass N was quickly mobilized by microbial activity or direct dissolution. Most of the recovered ^{15}N was in the soil organic matter, but the majority of the ^{15}N was not recovered, suggesting an unmeasured loss mechanism. In contrast, the leaf litter ^{15}N remained largely in undecomposed leaves. The proportion of mobilized and recovered N found in seedlings suggests that mobilized frass N was less available to plants than mobilized leaf N. Lowered N availability over the long term could result in reduced recovery and growth of defoliated trees. It would be interesting to examine an actual forest defoliation event using the tracer techniques employed in this study to determine ecosystem level responses and pathways of N movement. The long-term and cascading effects of insect defoliation should be quantified and modeled if forest managers are to maintain forest health and productivity in an environment that includes defoliating insects.

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