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PLANT ANIMAL INTERACTIONS

Recycling of nitrogen in herbivore feces: plant recovery, herbivore assimilation, soil retention, and leaching losses

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Abstract Herbivores directly and indirectly affect ecosystem functioning in forests. Feces deposition is a direct effect that supplies ephemeral N pulses to soils. Herbivore-mediated changes in plant N allocation and uptake are indirect effects that can also influence soil N availability. These effects may interact if defoliation influences the ability of plants to recover fecal N, and this may affect subsequent generations of herbivores. We added ^{15}N-enriched insect feces (frass) to a series of replicated red oak, *Quercus rubra*, mesocosms that had been damaged experimentally and then followed the frass N over the course of 2 years. In the first season, some frass N was mineralized in the soil and leached in organic form from the mesocosms within 1 week of deposition. Within 1 month, frass N had been acquired by the oaks and enriched the foliage; late-season herbivores assimilated the frass N within the same growing season. In the second season, herbivore damage from the previous year lowered total leaf N contents and ^{15}N recovered in the foliage. A subsequent cohort of early-season herbivores fed on this foliage consequently derived less of their N from the previous year's frass, and feral leaf rollers colonized fewer of these saplings. The 0- to 5-cm soil fraction was the largest N sink measured, and 42% of the frass N was recovered in the soil. The results demonstrate that: (1) some frass N can be recycled rapidly into foliage and assimilated by successive cohorts of herbivore within the same season; (2) damage can affect N allocation in the following year's foliage, influencing N availability to and host selection by herbivores; and (3) leaching losses occur soon after deposition but are buffered by soil pools, which are the largest sinks for frass N.

Keywords Nitrogen-15 · *Anisota senatoria* · Ecosystem processes · *Orgyia leucostigma* · *Quercus rubra*

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Introduction

Herbivores influence terrestrial ecosystem processes in ways that may feedback to affect future herbivores. Observations linking herbivores with energy and nutrient fluxes are long standing (Swank et al. 1981; Schowalter and Crossley 1983; Risley and Crossley 1988), and recent work in grasslands and forests has begun to elucidate the underlying mechanisms. Herbivore damage stimulates changes in foliar chemistry (Schultz and Baldwin 1982) that can persist into litter and alter rates of decomposition and nutrient turnover (Ritchie et al. 1998; Chapman et al. 2003). The complex suite of plant responses to herbivore damage includes changes to C partitioning between above- and below-ground tissues (Hamilton and Frank 2001; Ayres et al. 2004), further affecting inputs to the soil. In addition to these "slow

cycle" effects (sensu McNaughton et al. 1988), excreta (e.g., frass) from herbivores provides rapid, but transient, nutrient pulses that significantly increase N availability and loss from terrestrial systems (Frost and Hunter 2004). However, the temporal dynamics of frass N recovery by plants, the availability of that recycled N to future herbivores, and frass N leaching losses are not well characterized in any system.

Soils are strong sinks for ephemeral pulses of N, whether deposited as inorganic N (Nadelhoffer et al. 1999; Zogg et al. 2000) or frass (Christenson et al. 2002). Despite this, there are multiple field observations linking insect herbivore damage with increases in stream N, presumably as a result of leaching losses (Swank et al. 1981; Eshleman et al. 1998; Reynolds et al. 2000; Townsend et al. 2004). When damage occurs to a plant, herbivore-mediated changes in plant N and C allocation belowground can affect soil processes. Our previous work suggested that, similar to grasses (Hamilton and Frank 2001), oaks may allocate C and N among tissues contingent upon the type of foliar damage experienced (Frost and Hunter 2004). Herbivore-mediated changes in soil processes or plant N allocation patterns may alter the distribution and fate of N in frass, which may then feedback to affect subsequent herbivores. However, no study has yet traced the recycling of frass N back into plants and future herbivores. We report here the results of a 2-year study measuring the dynamics of frass N in red oak, *Quercus rubra* L., field mesocosms using ^{15}N-enriched frass and annual experimental manipulations of insect herbivores.

In the first season, our objective was to test whether frass N recycling was affected by herbivore damage, with particular focus on the temporal dynamics of N recovery in leaf tissue and loss as leachate (Swank et al. 1981; Reynolds et al. 2000). Based on previous results in this system (Frost and Hunter 2004), we predicted that herbivore damage would stimulate recovery of frass N and consequently decrease leaching losses from the system. However, oaks commonly reduce foliar N concentrations throughout a growing season (Boerner 1984), and we did not anticipate significant N recovery in leaf tissue in the first year. In addition to the main objective, we used a serendipitous occurrence of the late-season herbivore *Anisota senatoria* Smith. to explore whether early-season frass N would be assimilated by late-season herbivores in the same season.

In the second season, we introduced another cohort of early-season herbivores to test the availability to the herbivores of N derived from the previous year's frass. Expanding on the hypothesis that herbivore activity would increase plant N recovery, we predicted that second year herbivores would recover more frass N feeding on saplings damaged in the previous year than on undamaged saplings. In addition, we destructively sampled each mesocosm to determine the distribution of frass N remaining in each system 1 year following initial deposition. This allowed us to assess N allocation patterns by *Q. rubra* in response to herbivore damage in the previous year and to estimate the overall recovery and distribution of frass N within the terrestrial system.

Materials and methods

Field site

The experimental array of potted mesocosms is described in detail in Frost and Hunter (2004). Briefly, the array was established in 2002 in a field adjacent to the University of Georgia Atlanta (UGA) Botany greenhouses (Athens, Georgia) using 160 nursery-grown *Q. rubra* saplings (Forest Keeling Nursery, Mo.) transplanted into 7-gallon pots using soil and leaf litter from watershed 27 at the Coweeta Hydrologic Laboratory (CWT; Otto, N.C., elevation 1,300 m). Each mesocosm was suspended above the ground by a wooden stand to facilitate leachate collection. The *Q. rubra* saplings were 1.33 ± 0.14 m tall and averaged 13.71 ± 0.18 mm in diameter (10 cm from base of soil). From the entire array, we randomly selected 45 saplings to receive ^{15}N-enriched frass (15 per damage group described below) and five as unenriched controls to measure natural ^{15}N abundances. The five natural abundance mesocosms were not damaged experimentally.

Generating ^{15}N-labeled frass

In March 2003, ten potted *Q. rubra* saplings separate from the field site saplings were labeled by applying 1 l of 0.05 mol l^{-1} $^{15}NH_4^{15}NO_3$ (99 atom%) directly to the soil of each sapling during the initial stages of budbreak. We stress that the saplings used to generate the ^{15}N frass were not used in the experiment in any other way; they were not part of the experimental array. They were, however, otherwise similar to the experimental saplings. Six additional *Q. rubra* saplings generated unlabeled frass from which to determine natural isotope abundances. This group of saplings (ten enriched, six unenriched) is hereafter called "isotope saplings."

Isotope saplings were transported to the Animal and Plant Health Inspection Service (APHIS) Laboratory in Cape Cod, Massachusetts and defoliated under

controlled conditions by fourth-instar *Lymantria dispar* L. We used *L. dispar* out of necessity because we did not have a sufficient supply of *Orgyia leucostigma* Fitch. to both generate the labeled frass and perform the damage treatments (see Damage treatments and ^{15}N-frass additions). The feeding behavior of the two lymantriids is similar but, more importantly, their gut and frass chemistry are also similar (Kopper et al. 2002; Lovett et al. 1998). In addition, there were no differences in total C or N between frass generated by *O. leucostigma* or *L. dispar*. The isotope saplings were completely covered with Reemay agricultural cloth secured to prevent herbivore escape. Following complete defoliation, the frass was collected, pooled, and stored by type (enriched and unenriched). Subsamples of each type of frass were analyzed for ^{15}N content (see Isotope analysis): isotope abundances for the unenriched and enriched frass were 0.3688 atom% ^{15}N (δ^{15}N = 6.44 ‰) and 27.4 atom% ^{15}N (δ^{15}N = 76,087‰), respectively. Frass total N did not differ between enriched and unenriched samples.

Damage treatment and ^{15}N-frass additions

Fifteen experimental saplings in the field array were selected randomly for each of three treatments (herbivore damage, mechanical damage, undamaged). Herbivore damage was inflicted on the experimental saplings by white-marked tussock moth larvae, *O. leucostigma*, tannin-tolerant defoliators common throughout the eastern United States. Twenty-five herbivores per sapling were contained in branch bags made of Reemay cloth. Bags covered ~60% of foliage and all saplings were bagged to control for any effects of the bags on the saplings (e.g., reduced photosynthesis). Fourth to fifth instar *O. leucostigma* fed continuously from 1 to 10 June 2003. Mechanical damage using scissors occurred on discrete dates (5 June and 10 June 2003) and mimicked herbivore damage as closely as possible in terms of total leaf mass and leaf area removed (LAR), which was measured using a visual scoring technique described in Hunter (1987). Herbivore and mechanical damage treatments generated 21.24 ± 5.71% and 24.3 ± 1.92% LAR, respectively, compared with 5.9 ± 1.52% LAR under undamaged conditions (mean ± SE). All frass generated by the herbivore treatment was collected and prevented from contacting the soil; the only frass additions were from ^{15}N-labeled frass. Prior to frass additions, leaf, soil, and leachate samples were collected to measure natural abundance δ^{15}N.

On 18–20 June 2003, we applied 60 g ^{15}N-enriched frass m^{-2}, which is approximately representative of the leaf mass removed by our experimental treatment and well within the range of ecological relevance (Christenson et al. 2002; Reynolds et al. 2000). This resulted in the addition of ~6 g dry-weight-equivalent frass (2 g day^{-1}) directly to the soil/litter surface of each experimental mesocosm. This level of deposition is approximately 400% and 50% of endemic and outbreak levels at CWT, respectively (Reynolds et al. 2000). Frass was 2.26 ± 0.04% N (mean ± SD), resulting in the addition of ~135.6 mg (1.36 g m^{-2}; 13.6 kg ha^{-1}) total N and ~37.2 mg frass ^{15}N to each mesocosm. The same quantity of unenriched frass would have added ~0.50 mg ^{15}N.

Sample collection 2003

Four leaf samples were collected during the 2003 growing season: pre-treatment (28 May 2003), 1-week post-frass additions (25 June 2003), 1-month post-frass additions (21 July 2003), and litter samples (28 October 2003). During each collection, three to four fully expanded leaves per sapling were collected from terminal stems receiving full sun and pooled for analysis. Litter samples were collected by gently shaking a mainstem and collecting the falling litter, though nine saplings had fully dropped their foliage and collecting litter samples was not possible.

Soil samples were collected 3 times in 2003: pre-frass additions (1 June 2003), 1-week post-frass additions (26 June 2003), and 1-month post-frass additions (17 July 2003). Two-centimeter-diameter cores were taken for each mesocosm to a depth of 10 cm and pooled for analysis (see Soil analysis). All surface litter and detritus were temporarily cleared from the mineral soil surface prior to sampling the soil cores to avoid collecting enriched frass as part of the soil samples. These samples should therefore reflect the N and ^{15}N concentrations of the surface mineral soil.

Leachate samples were collected continuously from the bottom of each mesocosm in a 250-ml plastic nalgene bottle attached with a length of flexible plastic tubing (see Frost and Hunter 2004 for diagram). Due to our watering regime, essentially all leaching occurred as a result of rainfall events, and we collected leachate samples immediately following each rainfall to minimize the time the leachate remained in the collection vials. Leachates were clear and did not require filtering. One pre-frass addition leachate sample was collected on 13 June 2003. Leachate samples collected post-frass additions were pooled by pot into three samples: 0- to 1-week post frass additions, 1- to 4-week, and 4- to 12-week samples. The pooled sample contained subsamples of leachate from each individual collection date in proportion to the total leachate

generated over the entire pooled period. We present leachate data as concentrations (mg l^{-1}) to compare among sampling dates, and also estimate the total amount of frass N lost to the mesocosm as leachate for calculating mass balance. N in leachate samples was analyzed by isotope diffusion (see Isotope diffusion). We were unable to estimate the leachate inorganic N concentrations because of small sample concentrations, though we determined total leachate N and $\delta^{15}N$ following persulfate oxidation (Cabrera and Beare 1993).

Subsequent herbivores

Our original plan was to examine the effects of 2003 frass additions on 2004 herbivores (methods described below). However, we took advantage of an August 2003 emergence of orange-striped oakworm, *A. senatoria*, near the field site to explore the availability of early-season frass N to late-season herbivores within the same growing season. The third instar larvae of *A. senatoria* were brought into the laboratory and raised to the fourth instar on unenriched *Q. rubra* foliage. The larvae were then starved for 24 h to void their guts and fed foliage removed from treatment saplings. Due to the limited number of herbivores available, we randomly selected five herbivore-damaged saplings and five reference (enrichment-free) saplings to feed to the *A. senatoria*. As a result, we were not able to test for treatment effects but rather used *A. senatoria* to test for general availability of frass N. Herbivores fed for 4 days and were given fresh foliage as necessary. After 4 days, any remaining foliage was removed, frass was collected, and the herbivores were starved for 24 h to clear their gut contents (Hunter 1987). Following starvation, herbivores and their frass were dried separately at 60°C, ground into fine powders, and analyzed for total N and $\delta^{15}N$ (see Isotope analysis).

We then examined whether frass N deposited in 2003 was available to herbivores in 2004. During the 2004 growing season, a cohort of *O. leucostigma* were reared from egg masses to fourth instar on an artificial diet in the laboratory and then introduced to the experimental saplings in the field. Once foliage had fully expanded, we randomly selected one terminal stem per sapling that received full sun. Prior to introducing herbivores, the total number of leaves and their leaf area on each stem were measured and two leaves per stem were collected to determine total N and $\delta^{15}N$. Each stem was then enclosed in a small Reemay branch bag and ten *O. leucostigma* larvae were added to the bag. All 50 saplings (45 receiving ^{15}N frass in 2003, five enrichment-free) received herbivores in 2004. Herbivores were added on 5 June 2004 and removed on 10 June 2004. Herbivores, pooled by sapling, were starved for 24 h to clear gut contents. Larva and their frass were dried at 60°C, ground individually in a ball mill, and analyzed for total N and $\delta^{15}N$. In addition to the 2004 herbivore treatment, we also scored each sapling for the presence of feral leaf rollers that had naturally colonized some saplings in the experimental array.

Destructive sampling

All 50 mesocosms were destructively harvested on 23–24 July 2004, with replicates in each treatment group spread between the 2 days. Each mesocosm was harvested individually and all materials used during harvest (e.g., plastic sheets) were replaced between mesocosms to avoid ^{15}N cross-contamination. The mesocosms were separated into the following sections: foliage, new stem (2004 growing season), and soil (and fine root) layers 0–5, 5–15, and 15–25 cm from the surface. All samples were stored at 4°C in the laboratory until processing.

Soil (and root) subsamples were taken from the soil layers within 5 days of harvest. From each soil depth, two ~100-cm^3 ($r = 5$ cm, depth = 5 cm) cores were taken for separate analyses. The first core was used to provide a rough estimate of bulk density, which was used for mass balance calculations. The second core was used for nutrient analyses, following separation from fine roots (see Soil analysis). Fine root (diameter < 2 mm) samples from the soil cores were washed on 1-mm-screen mesh sieves to remove any remaining soil particles. A small amount of root material may have passed through the sieve; the samples we analyzed consisted only of those fine roots that did not pass through the sieve. Fine roots were then dried at 60°C and weighed to calculate dry weight and root density (mg cm^{-3} soil). They were then ground to a powder and analyzed for total N and $\delta^{15}N$ (see Isotope analysis).

Soil analysis

Soil cores were weighed and passed through a 1-mm-screen mesh to exclude fine roots, and were separated into two subsamples for separate analyses. The sieving process mixed rhizosphere and bulk soils. The first subsample of soil was weighed, dried for 48 h at 105°C to determine water content, and the dried sample ground to a fine powder and analyzed for total C and N and their isotopes (see Isotope diffusion). The remaining subsample was extracted with 50 ml of 0.5 M K_2SO_4 for 1 h on an orbital shaker (120 r.p.m.) and filtered through Whatman 42 filter paper. The filtrate was analyzed for extractable inorganic N ($NO_3^- + NH_4^+$) and

$\delta^{15}N$ using isotope diffusion (see Isotope diffusion). We also performed chloroform fumigations on the 2003 samples to measure microbial enrichment (Kaye and Hart 1997; Vance et al. 1987), though the data were unusable due to an error in the elemental analyzer that was out of our control.

Isotope diffusion

Leachate and soil extracts were analyzed for ^{15}N using a modified isotope diffusion method (Sigman et al. 1997; Downs et al. 1999). Acidified glass fiber disks (Whatman 934AH; Whatman, N.J.) were sealed between two squares of teflon tape and added directly to a known volume of sample in the presence of NaOH and Devarda's alloy (Sigma 269484; Sigma–Aldritch, St. Louis, Mo.). Samples with disks were incubated for 5 days with gentle orbital shaking (60 r.p.m.). We used NaOH in place of MgO for all diffusions because MgO did not always raise the pH of the solution adequately following persulfate digest. Following diffusions, packets were dried for 48 h in a desiccating chamber with a small vial of concentrated H_2SO_4. Dry disks were transferred into silver capsules for analysis. For leachate samples, duplicate diffusions per sample were carried out for: (1) inorganic N ($NO_3^- + NH_4^+$), and (2) organic N following persulfate digestion (Cabrera and Beare 1993). Organic N is determined as the difference between the persulfate digested N and inorganic N. For the leachate samples, inorganic N concentrations were not measurable; we report the total leachate $\delta^{15}N$ from the persulfate digest, which, while technically a combination of inorganic and organic N, is essentially all organic N. For soil extracts, only inorganic N diffusions were performed.

Isotope analysis

We follow $\delta^{15}N$ notation, where units are expressed per 1,000 deviations from the atmospheric standard (atom% $^{15}N = 0.3663$), $\delta^{15}N$ (‰) = [(sample atom%^{15}N/standard atom%^{15}N) − 1] × 1,000 (Lajtha and Michener 1994). All stable isotope samples (dry leaf, soil and diffusion samples) were analyzed on a Costech elemental combustion system 4010 (Costech Analytical Technologies, Valencia, Calif.) connected to a ThermoFinnigan ConfloIII interface and Deltaplus continuous flow–stable isotope ratio mass spectrometer (IRMS) (Thermo Electron, Waltham, Mass.) for total N and $\delta^{15}N$. A set of samples was analyzed on two dates to provide an estimate of IRMS and element analyzer errors (Jardine and Cunjak 2005). The coefficient of variation (CV) on this data set was 0.85 ± 1.19% for $\delta^{15}N$ and 2.17 ± 2.06% (mean ± SD) for total N. In addition, frass "standards" within a single run also had a 0.3% CV.

Statistical analyses

Data were analyzed using the GLM procedure of SAS 8.2. Transformations were made when necessary to satisfy assumptions of normality. Repeated measures analysis was used when appropriate to test for within-subjects effects. Following ANOVA models, the Tukey (honest significant difference) post-hoc test was used to determine significant differences among treatment means. In cases where residuals of the models could not be normalized by transforming the raw data, data were analyzed with the GENMOD procedure and differences among treatment means were determined using the Wald χ^2-test (Littell et al. 2002). The count data for 2004 leaf rollers were analyzed with GENMOD using a binomial distribution and logit link function.

Results

Growing season 2003

N deposited via insect frass was assimilated in foliage, and subsequently by late-season herbivores, within the same growing season (Fig. 1). Foliage showed no evidence of ^{15}N enrichment in the 1-week samples, but all mesocosms receiving enriched frass had $\delta^{15}N$-enriched foliage within 1-month post-frass additions (date $F_{2,82} = 57.88$, $P < 0.0001$, Fig. 1a). The late-season herbivores, which fed on foliage between the 1-month and litter samples, showed ^{15}N enrichment in their bodies and frass ($F_{1,7} = 8.00$, $P = 0.0255$; $F_{1,7} = 11.16$, $P = 0.0124$, respectively; Fig. 1b).

Assimilation of frass N into foliage apparently continued between the 1-month and litter samples despite decreases in total foliar N concentrations as the growing season progressed (Fig. 1). Foliar $\delta^{15}N$ enrichment increased significantly between the 1-month sampling and collection of leaf litter (date $F_{1,35} = 26.71$, $P < 0.0001$; Fig. 1a), while senescing foliage was resorbing N (date $F_{1,35} = 127.96$, $P < 0.0001$; Fig. 1c). The $\delta^{15}N$ of the reference samples was unchanged between the 1-month and litter samples ($P = 0.1280$), suggesting that isotope discrimination during senescence was unlikely and the increase between the 1-month and litter samples resulted from continued accumulation of soil N (including frass N).

Consistent with the observed assimilation by saplings and late-season larva, frass N was mineralized within 1 week of deposition (Fig. 2a) and constituted

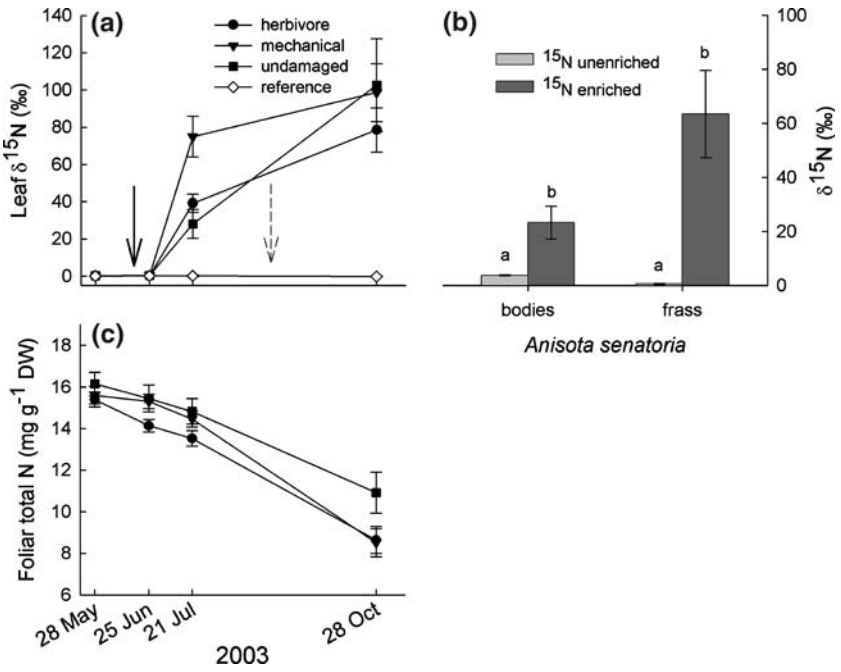

Fig. 1 **a** $\delta^{15}N$ abundances in foliage throughout the 2003 growing season. *Dark points* represent the means ± SE of 15 replicates; *white points* are means ± SE of five enrichment-free replicates. *Dark arrow* indicates date of ^{15}N-frass additions; *dashed arrow* indicates approximate feeding date of *Anisota senatoria*. **b** $\delta^{15}N$ values for *A. senatoria* larva and frass. *Different letters* represent statistically significant treatment means using the Tukey post-hoc test ($\alpha = 0.05$). **c** Total foliar N throughout the 2003 growing season. *Bars* represent the means ± SE of 15 replicates per treatment. Not all replicates could be used at each sampling date, so the number of replicates per bar varies. *Jun* June, *Jul* July, *Oct* October

an increasing portion of the total soil N pool as the season progressed (Fig. 2b). Because frass is almost entirely organic material (Lovett and Ruesink 1995), the significant date effect on the $\delta^{15}N$ of the soil inorganic N pool (date $F_{2,92} = 4.24$, $P = 0.0195$) suggests that frass N was being mineralized by microbial activity. The total soil N from 0 to 10 cm followed a similar pattern of $\delta^{15}N$ enrichment; soils receiving ^{15}N frass were significantly enriched following frass additions (date $F_{6,92} = 3.53$, $P = 0.0068$).

A small portion of frass N was lost as leachate within 1 week of deposition (Fig. 3a), though the amount lost in the first week accounted for ~49% of all frass N lost in leachate during the first 3 months following deposition. The first rainfall following frass additions occurred almost 1 week after additions, and the ^{15}N in mesocosms receiving frass additions showed a pulse ($F_{3,44} = 6.98$, $P = 0.0007$) that also corresponded to total N (Fig. 3b). In the first week, we recovered 13.3 ± 3.3 μg ^{15}N, or 0.035 ± 0.009% of the of initial frass N added (mean ± SE). Leachate $\delta^{15}N$ in mesocosms receiving frass remained enriched relative to baseline mesocosms in both the 1- to 4- and 4- to 12-week sampling periods ($F_{3,35} = 14.03$, $P < 0.0001$; $F_{3,23} = 14.50$, $P < 0.0001$, respectively). In the 1- to 4- and 4- to 12-week samples we recovered 2.9 ± 0.5 μg and 11.1 ± 2.9 μg ^{15}N, or 0.008 ± 0.0006% and 0.03 ± 0.008% (mean ± SE) of the initial frass added, respectively.

By comparing among our damage treatments, we can determine whether or not assimilation and/or loss of frass N was contingent upon aboveground defoliation. Assimilation occurred more rapidly following mechanical damage (date × damage $F_{4,82} = 3.71$, $P = 0.0306$, Fig. 1a), though total N concentrations were unaffected over the same time period (date × damage $F_{4,82} = 1.69$, $P = 0.1679$). There were no treatment effects on either mineral or total soil N pools (date × damage $F_{4,84} = 0.71$, $P = 0.5844$; $F_{4,84} = 0.43$, $P = 0.7405$, respectively). However, during the 1- to 4-week post-frass addition sampling period, more frass N was lost from mesocosms suffering herbivore damage ($F_{3,35} = 14.03$, $P < 0.0001$, Fig. 3a). Nonetheless, total N losses in leachate over the same time interval were similar among treatments ($F_{2,21} = 0.08$, $P = 0.9248$, Fig. 3b). At the end of the 2003 growing season, the measured frass N "losses" from the mesocosms were a modest ~0.08% and ~1.27% of the added ^{15}N in leachate and litter, respectively. Unfortunately, gaseous N losses from the frass and soil were not measured.

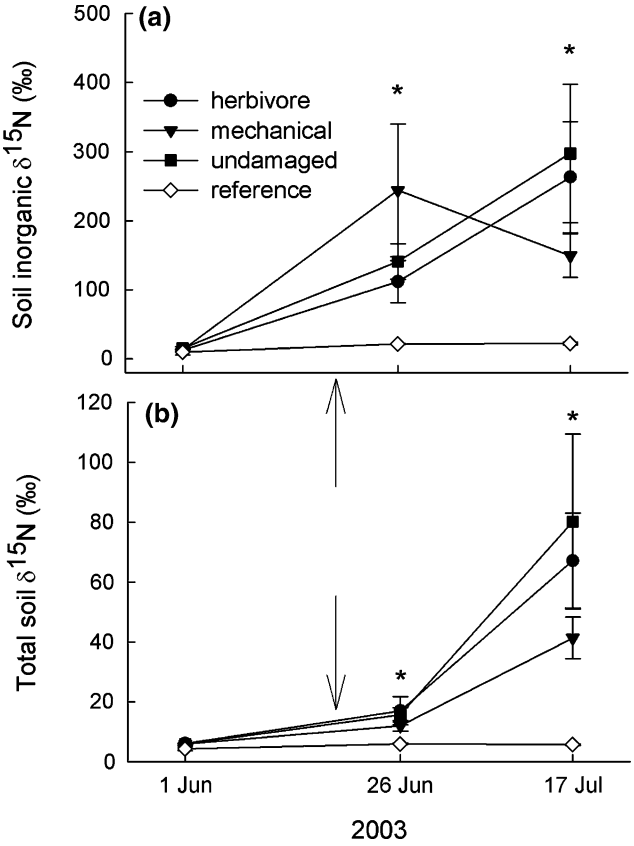

Fig. 2 **a** Soil inorganic $\delta^{15}N$ values throughout the 2003 growing season. **b** Total soil $\delta^{15}N$ values throughout the 2003 growing season. *Dark points* represent the means ± SE of 15 samples; *white points* are means ± SE of five enrichment-free replicates. *Asterisks* indicate the dates with statistically significant enrichment ($\alpha = 0.05$). *Arrows* indicate the date of ^{15}N-frass additions

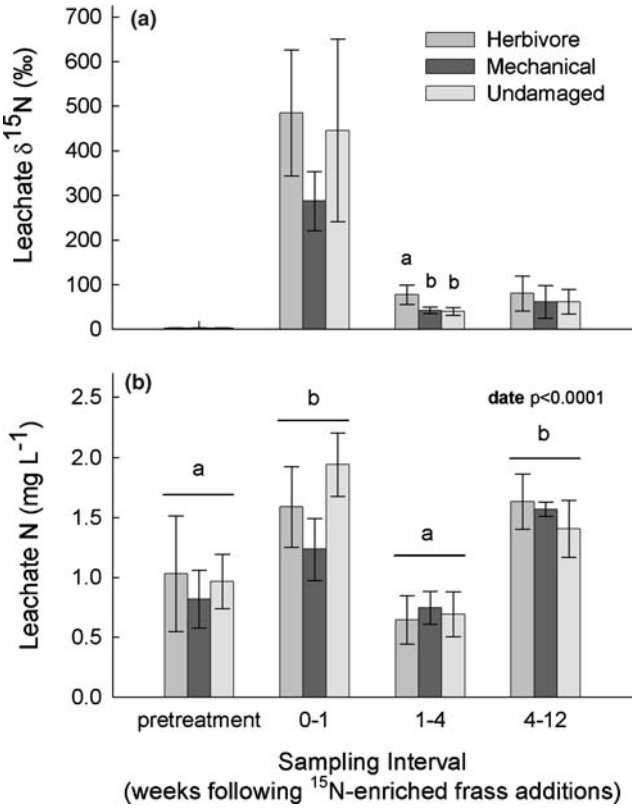

Fig. 3 **a** Leachate $\delta^{15}N$ in 2003 during the first 3 months following ^{15}N-frass additions. *Different letters* above the bars indicate statistical differences based on treatment means. **b** Leachate total N in 2003 during the first 3 months following ^{15}N-frass additions. Statistics for damage treatment are omitted because leachate total N was not influenced by the treatment. *Different letters* above groups of bars indicates statistical differences in sample means based on date, independent of the damage treatment. Because leachate samples could not be collected for each mesocosm on each date, sample sizes are not equal between or within dates. *Bars* are means ± SE for each treatment. Differences among treatment and date means determined using the Tukey post-hoc test ($\alpha = 0.05$)

Growing season 2004

During the 2004 growing season, saplings that had been damaged (herbivore or mechanical) in 2003 had less frass N in their foliage than did undamaged saplings ($F_{2,44} = 3.76$, $P = 0.0314$, Fig. 4). The recovery of frass N in all other subsections of the mesocosms was not affected by the damage treatment. The lower frass N recovery in 2003-damaged sapling foliage was the combined result of lower total N ($F_{2,44} = 6.27$, $P = 0.0041$, Fig. 5a) and lower $\delta^{15}N$ enrichment ($F_{2,44} = 3.84$, $P = 0.0294$, Fig. 5b) relative to previously undamaged saplings. The specific leaf areas and total leaf biomass produced in 2004 were not affected by the 2003 treatments ($P = 0.3244$ and $P = 0.4786$, respectively). The recovery of frass N was not affected by the damage treatment in the new stems or roots, and there were no treatment effects on either the root density or the ratio of frass N recovered in leaf and root (3.08 ± 0.29, mean ± SE). The 0- to 5-cm soil sample comprised the largest pool of recovered frass N (Fig. 4), though it was not the largest pool in terms of total mass (Table 1). When the 2003 "losses" were included, we recovered 61.0 ± 3.1% (mean ± SE) of frass N in the mesocosms (Table 2).

The lower allocation of frass N in the foliage of saplings damaged by herbivores in 2003 translated into lower recovery of frass N in the bodies of *O. leucostigma* larva feeding during spring 2004 (Fig. 5). The $\delta^{15}N$ values of leaf, body, and frass samples correlated tightly (leaf–body $r^2 = 0.310$; leaf–frass $r^2 = 0.718$; body–frass $r^2 = 0.653$; all $P < 0.0001$) and all *O. leucostigma* fed on treatment saplings were significantly ^{15}N enriched compared to reference herbivores ($F_{3,49} = 131.39$, $P < 0.0001$). *O. leucostigma* fed on foliage from herbivore-damaged saplings had lower $\delta^{15}N$ than did those fed on foliage from undamaged saplings

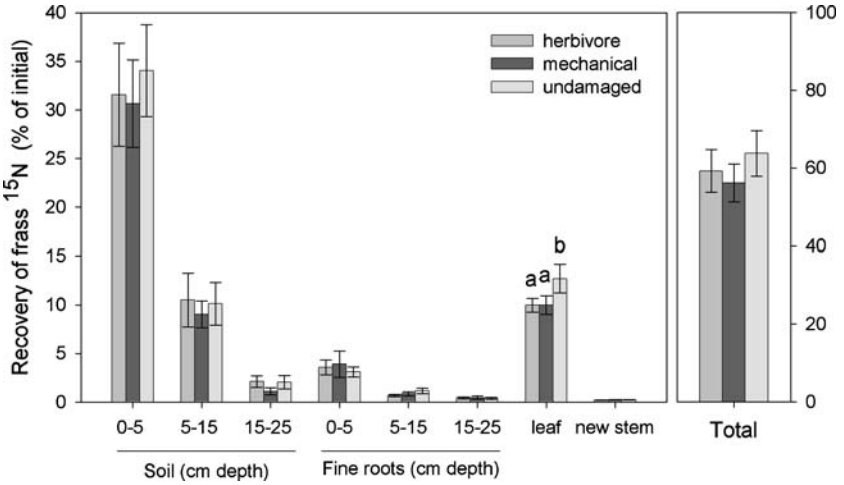

Fig. 4 Total recovery of frass N in mesocosms by 2003 damage treatment. Bars are means ± SE of 15 samples. *Different letters* above bars indicate that the herbivore and mechanical groups were statistically distinct from the undamaged group using the Tukey post-hoc test ($\alpha = 0.05$)

($F_{1,29} = 4.41$, $P = 0.0448$, Fig. 5b) and, consequently, had a lower proportion of their N derived from frass N (Fig. 5c). *O. leucostigma* bodies had a higher frass N recovery expressed per unit biomass than did foliage irrespective of treatment (leaf vs. *Orgyia* body vs. *Orgyia* frass: $F_{2,134} = 4.50$, $P = 0.0129$, Fig. 5d), suggesting that, as expected, they were accumulating foliar N. There were no treatment effects on *O. leucostigma* frass δ^{15}N, though the trend is similar to the leaf and body samples (Fig. 5). Total N in *O. leucostigma* was 11.0 ± 0.1% (mean ± SE); the frass, unexpectedly, also had significantly higher total N than did the foliage (Fig. 5a). In addition, 2004 leaf rollers colonized 13 of 15 saplings that were undamaged in 2003 but only seven of 15 that had been damaged by herbivores and eight of 15 damaged mechanically ($\chi^2 = 6.43$, $df = 2$, $P = 0.0401$).

Discussion

Our study was designed to explore the distribution and allocation of frass N following insect herbivore damage on *Q. rubra*, and our results fall into two broad categories: (1) plant recovery of frass N and its availability to subsequent herbivores, and (2) soil retention and leaching losses of frass N. Considering N recovery in plants and herbivores, our results show that: (1) a portion of frass N is rapidly recovered in oak foliage and late-season herbivores therefore derive a portion of their N from the frass of early-season herbivores, and (2) herbivore damage indirectly affects N allocation (including frass N) by oaks and therefore N availability to subsequent cohorts of herbivores. Considering the soil, the data suggest that a large percentage of frass N lost as leachate escapes relatively quickly in organic form following deposition.

Frass N recovery in oaks and herbivores

Leaf N content plays an important role in caterpillar fecundity, and is often a more important determinant than are putative defensive compounds (reviewed in Nykanen and Koricheva 2004). One ecological outcome for the rapid recovery of early-season herbivore fecal N (Fig. 1a) is the direct link between the nutritional supplies of early- and late-season insect herbivores. Late-season herbivores consume relatively low quality foliage because of the reduction in foliar N concentrations in deciduous trees throughout a growing season (Fig. 1c; Chapin 1980; Chapin and Moilanen 1991; Arco et al. 1991; Killingbeck 1996), though early-season defoliation also influences host selection and fitness of late-season herbivores through changes in foliar quality (Hunter 1987). Our use of *A. senatoria* supports this general paradigm by documenting that N mobilized by early-season herbivores can be recovered in foliage and assimilated by late-season herbivores: the nutritional supply of the late season herbivore is linked with frass deposition earlier in the season. As far as we are aware, this is the first study to document that late-season herbivores can acquire N from early-season herbivore feces. Future research should examine the ecological significance of this link.

From an ecosystem perspective, the recovery of ~1.2% of frass N in leaf litter within the same season of frass deposition directly links the "fast" and "slow" cycles of nutrient dynamics (McNaughton et al. 1988) via plant N recovery following herbivore feeding. A portion of early-season frass N can be recovered by the oak and re-deposited on the forest floor in leaf litter within the same season. The remobilized frass N in leaf litter is then subject to decomposition processes and re-enters soil N pools.

In 2004, foliar total N and δ^{15}N enrichment were the only plant N pools altered by the 2003 herbivore

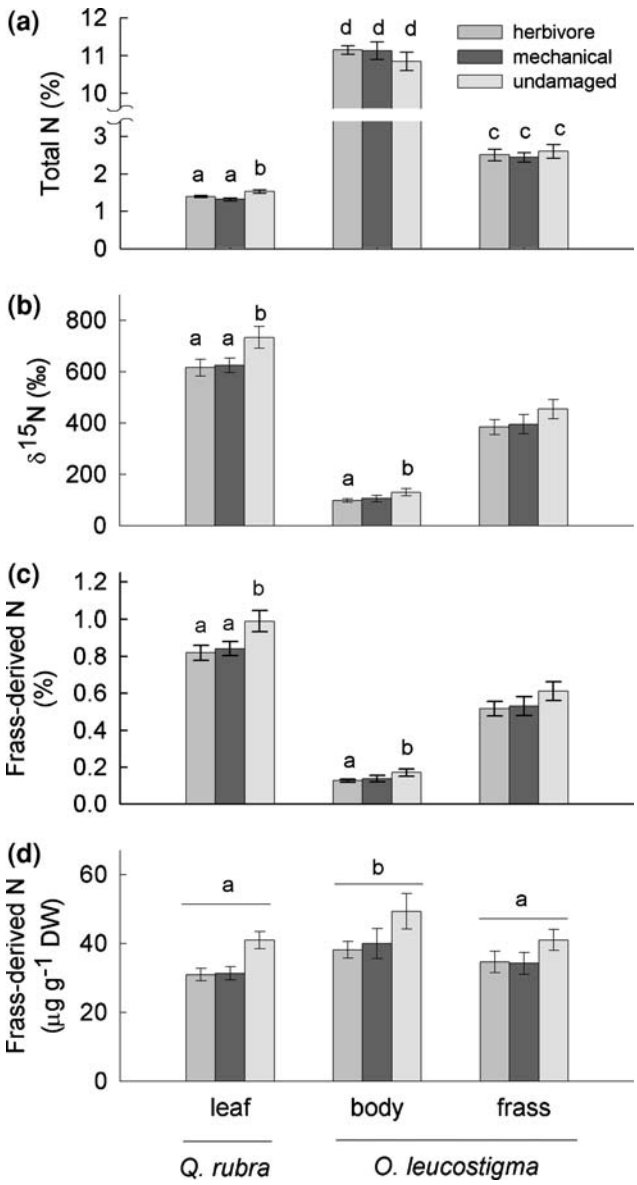

Fig. 5 **a** Total N (%) in *Quercus rubra* foliage and *Orgyia leucostigma* bodies and frass in spring 2004. **b** $\delta^{15}N$ of *Q. rubra* foliage and *O. leucostigma* bodies and frass in spring 2004. **c** Percent of N in *Q. rubra* foliage and *O. leucostigma* bodies and frass in spring 2004 that was derived from frass deposited in spring 2003. *Different letters* above bars indicate statistical significance of treatment means using the Tukey post-hoc test ($\alpha = 0.05$). **d** N expressed per unit biomass in *Q. rubra* foliage and *O. leucostigma* bodies and frass in spring 2004 derived from frass deposited in spring 2003. For graph **d**, treatment-based post-hoc statistical differences are not presented, but are similar to **b** and **c**. In order to highlight that *Orgyia leucostigma* were assimilating leaf N (and previously deposited frass N), *different letters* above the groups of bars indicate statistical significance among tissue types (leaf, body, frass) independent of damage treatment using the Tukey post-hoc test ($\alpha = 0.05$). *Bars* represent means ± SE of 15 replicates

treatment (Fig. 4), suggesting specific N allocation changes based on previous herbivore pressure. There were also no differences among treatments in any growth parameter measured (e.g., budbreak timing, stem expansion rates, total leaf and new stem biomass, photosystem II efficiency, changes in stem width); the reduction in foliar N had neither deleterious nor beneficial effects on plant growth or vigor, at least in the season following damage. This suggests that previously damaged oaks compensated for their lower foliar N, consequently increasing their N use efficiency. Overall, oak saplings in 2004 recovered only ~10% of the frass N deposited in 2003, which then comprised ~1% of their foliar N. The large majority (~90%) of foliar N lost to herbivores was not recovered within the following season. This result stresses that even if frass produces a nutrient pulse in the soil (e.g., Frost and Hunter 2004), such a pulse comes at the expense of a plant that cannot recover the N lost as a result of herbivory.

N acquisition is energetically expensive (Clarkson 1985) and the damage-mediated reduction in foliar N makes intuitive sense if it influences future foliar herbivores. Because herbivore stoichiometry is homeostatic (Sterner and Elser 2002), the ecological outcomes of lower foliar N could be longer herbivore feeding times, lower pupal masses (Tikkanen and Julkunen-Tiitto 2003) and greater susceptibility to natural enemies (Price et al. 1980). Our data provide two independent lines of evidence in support of these hypotheses. First, *O. leucostigma* fed on foliage from previously damaged saplings had lower $\delta^{15}N$ than did those fed on previously undamaged saplings, and consequently derived less of their N content from previous-season's frass deposition (Fig. 5). If the herbivores had been raised from egg masses on the individual saplings, we assume that herbivores feeding on previously damaged saplings would have also required longer feeding times than would those feeding on undamaged saplings. Second, feral leaf rollers in 2004 colonized fewer saplings at our site that had been damaged in 2003 than those that were undamaged. This suggests that leaf rollers were less likely to select previously damaged saplings, possibly as a result of lower foliar quality mediated by the previous damage.

As a caveat, our experiments were conducted in potted mesocosms, which could generate artificially high root densities and uptake rates. However, our estimates of fine root densities are similar to those reported in *Quercus*-dominated forests (Davis et al. 2004). Nadelhoffer et al. (1999) reported recovery of 20–25% of applied inorganic ^{15}N in plant tissue under experimental fertilization but <5% in unfertilized plots. Our mesocosms were fertilized only by the frass additions, which provided a single application similar to the fertilization treatment in Nadelhoffer et al.

Table 1 Masses, N, C, and natural ^{15}N abundances for the *Quercus rubra* mesocosms

Sample type	Mass (g)	N (%)	C (%)	C:N	Reference $\delta^{15}N$
Leaf	121.30 ± 36.77[a]	1.42 ± 0.17	47.89 ± 0.64	34.05 ± 3.76	0.26 ± 1.02
New stem	8.31 ± 2.32	0.53 ± 0.07	46.76 ± 0.63	88.63 ± 14.07	-0.89 ± 1.02
Root					
0–5 cm	38.97 ± 31.72	0.96 ± 0.19	47.68 ± 1.53	51.61 ± 10.48	0.03 ± 0.50
5–15 cm	25.36 ± 17.01	0.97 ± 0.26	46.69 ± 1.97	50.99 ± 11.99	2.03 ± 0.34
15–25 cm	32.10 ± 21.45	1.00 ± 0.23	46.26 ± 2.24	49.02 ± 13.42	2.35 ± 0.91
Soil					
0–5 cm	2,323.88 ± 536.23	0.67 ± 0.11	11.26 ± 2.03	16.77 ± 0.51	4.29 ± 0.78
5–15 cm	3,413.39 ± 559.31	0.51 ± 0.05	9.11 ± 1.08	17.72 ± 0.84	6.41 ± 0.17
15–25 cm	3,712.97 ± 573.01	0.51 ± 0.06	9.29 ± 1.11	18.24 ± 1.02	8.64 ± 0.65

[a] Data are means ± SD of 50 samples for mass, N, C, and C:N, and five samples for reference δ^{15} N

Table 2 Frass ^{15}N-N recovered in *Q. rubra* experimental mesocosms

Sample type	% Recovery (mean ± SE)	
Oak sapling		
Leaf (includes foliar herbivores)		10.88 ± 0.65
New stem		0.24 ± 0.01
Root		4.86 ± 0.54
0–5 cm	3.53 ± 0.53	
5–15 cm	0.90 ± 0.13	
15–25 cm	0.43 ± 0.07	
2003 Leaf litter		1.27 ± 0.26
Soil		43.71 ± 2.84
0–5 cm	32.07 ± 2.75	
5–15 cm	9.88 ± 1.24	
15–25 cm	1.76 ± 0.33	
Leachate loss (June–August 2003)		0.08 ± 0.018
Total recovery (% of initial ^{15}N added)		61.04 ± 3.10

(1999). Our observed recovery of ^{15}N in foliage (~10%, Fig. 4) is therefore reasonable, though recovery in nature would be distributed among multiple plant species.

Soil and leachate

Soils supporting northern deciduous forests are strong sinks for surface deposited N via microbial immobilization followed by incorporation into the soil organic matter (Vitousek and Matson 1985; Zak et al. 1990; Groffman et al. 1993; Seely and Lajtha 1997; Zogg et al. 2000). Based on this well-established literature, our observation that the largest sink for frass N is the top 0–5 cm of soil is no great surprise. Indeed, Christenson et al. (2002) recovered ~17% of ^{15}N-enriched frass in the top 0–5 cm of soil and ~40% from 0 to 30 cm. Our results are similar in terms of total recovery (43.7 ± 2.8% in the entire mesocosm soil, 0–25 cm), though we recovered a larger percentage of the label (32.1 ± 2.7%) in the 0- to 5-cm soil. Considering our soils were highly disturbed (even after 2 years in the pots prior to the experiment), the similar results between the field study of Christenson et al. (2002) and our mesocosm data are auspicious. The absence of any damage treatment effect on the soils in our study suggests that the retention of N in these soils is probably independent of root-mediated changes following aboveground herbivory.

Our results add temporal resolution to frass-N-leaching dynamics. Frost and Hunter (2004) predicted that an immediate, precipitation-dependent mobilization of frass N in leachate would follow deposition following microbial mineralization but before incorporation into stable soil organic matter (SOM), though they had no supporting ^{15}N data. Results reported here support that prediction: ~40% of frass N recovered in leachate during the 3-month sampling period was collected in the first week's sample. Similarly, Seely and Lajtha (1997) found that more than 90% of ^{15}N recovered in lysimeter samples occurred within 2 days following deposition. Christenson et al. (2002) estimated that 0.00004% of frass N was lost via leaching of inorganic N, while calculations based on Frost and Hunter (2004) suggest that ~20% of frass N can be lost via leaching. Leaching losses in our study (~0.08% of added ^{15}N for the first 3 months following deposition) bifurcate the two studies, though there is substantial variation. A pulse of N escapes the system relatively quickly, the magnitude of which may depend on the timing of rainfall events and frass deposition. In our study, rainfall did not occur immediately following deposition and visible fungal hyphae surrounded the frass pellets in most of the mesocosms. This suggests that fungal hyphae were immobilizing N and, while only observational evidence, it is consistent with observations from previous microcosm experiments (Lovett and Ruesink 1995). Of course, despite our best attempts to replicate field conditions, we again stress that our experimental pots do not replicate conditions in nature. With this critical caveat in mind, the data indicate that the potential loss of frass N through leaching is

large but buffered by precipitation-dependent incorporation into stable SOM.

As a final point, the large majority of the frass N leached from the mesocosms was in organic form. We cannot determine from our data whether the organic N has passed through the microbial biomass or leached from the pot unprocessed. However, the recovery of primarily organic N suggests that previous studies of herbivore impacts on inorganic N losses, whether reporting herbivore-linked N export (Swank et al. 1981; Webb et al. 1995; Eshleman et al. 1998; Townsend et al. 2004) or not (Bormann and Likens 1979; Christenson et al. 2002), may have underestimated overall herbivore impacts on terrestrial N losses.

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