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# Insect herbivores and their frass affect *Quercus rubra* leaf quality and initial stages of subsequent litter decomposition

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Defoliation-induced changes in plant foliage are ubiquitous, though factors mediating induction and the extent of their influence on ecosystem processes such as leaf litter decomposition are poorly understood. Soil nitrogen (N) availability, which can be affected by insect herbivore frass (feces), influences phytochemical induction. We conducted experiments to test the hypotheses that insect frass deposition would (1) reduce phytochemical induction following herbivory and (2) increase the decomposition and nutrient release of the subsequent leaf litter. During the 2002 growing season, 80 *Quercus rubra* saplings were subjected to a factorial experiment with herbivore and frass manipulations. Leaf samples were collected throughout the growing season to measure the effects of frass deposition on phytochemical induction. In live foliage, herbivore damage increased tannin concentrations early, reduced foliar N concentrations throughout the growing season, and lowered lignin concentrations in the late season. Frass deposition apparently reduced leaf lignin concentrations, but otherwise did not influence leaf chemistry. Following natural senescence, litter samples from the treatment groups were decomposed in replicated litterbags for 18 months at the Coweeta Hydrologic Laboratory, NC. In the dead litter samples, initial tannin concentrations were lower in the herbivore damage group and higher in the frass addition group relative to their respective controls. Tannin and N release rates in the first nine months of decomposition were also affected by both damage and frass. However, decomposition rates did not differ among treatment groups. Thus, nutrient dynamics important for some ecosystem processes may be independent from the physical loss of litter mass. Overall, while lingering effects of damage and even frass deposition can therefore carry over and affect ecosystem processes during decomposition, their effects appear short lived relative to abiotic forces that tend to homogenize the decomposition process.

Plants respond to herbivore attack with a diverse array of chemical changes to their foliage (Karban and Baldwin 1997). These changes include the production of specific direct (Schultz and Baldwin 1982) and indirect (De Moraes et al. 1998) chemical defenses that can reduce herbivore damage and increase plant fitness (Nykänen and Koricheva 2004). Such defensive chemicals are often energetically expensive (Baldwin 1998, Koricheva et al. 2004) and, as a result, plants have some ability to differentiate between types of damage and induce herbivore-specific responses (Baldwin 1988, Dyer et al. 1993, 1995, Alborn et al. 1997, De Moraes et al. 1998, Oppenheim and Gould 2002). However, despite large advances in our understanding of the biochemistry and molecular biology underlying phytochemical induction (Qu et al. 2004, Kessler et al. 2004, Schultz and Appel 2004), basic questions remain regarding the ecological factors mediating induction (Nykänen and Koricheva 2004) and the extent to which induction influences ecosystem processes (Choudhury 1988). For example, nitrogen (N) availability affects phytochemical induction at both molecular (Lou and Baldwin 2004) and organismal scales (Hunter and Schultz 1995, Glynn et al. 2003); and processes that affect N availability may in turn affect phytochemical induction (Kyto et al. 1996).

Herbivores can directly and indirectly influence soil N availability through feedback loops between their activity and soil processes (Wardle 2002, Frost and Hunter 2004). There are several mechanisms through which herbivores can affect ecosystem processes (Hunter 2001), and they can be categorized roughly into ''fast'' and ''slow'' cycle effects (sensu McNaughton et al. 1988). Fast cycle effects occur within the same growing season as the herbivore; examples include the direct effect of feces deposition (Hollinger 1986, Lightfoot and Whitford 1990, Frost and Hunter 2007) and the indirect effect of changes in root exudation (Fu and Cheng 2004). Slow cycle effects span more than a single season and are the result of herbivore-mediated indirect changes to individuals or populations of plants. For example, herbivore-mediated changes in leaf chemistry could persist into dead litter and alter nutrient release and decomposition rates in subsequent seasons (Choudhury 1988, Schweitzer et al. 2005b, Chapman et al. 2006).

Since soil N availability can influence phytochemical induction (Bryant et al. 1993, Hunter and Schultz 1995, Björkman et al. 1998), herbivore-mediated feedback loops affecting soil nutrient dynamics may influence the intensity and duration of induced chemical changes in plant foliage. For example, up to 86% of leaf N consumed by insect herbivores can be excreted as frass (Hollinger 1986, Lightfoot and Whitford 1990, Lovett et al. 2002), and the potential for frass to fertilize trees has been suggested previously (Haukioja et al. 1985). However, while field (Reynolds et al. 2000, Christenson et al. 2002, Frost and Hunter 2004, 2007) and lab (Lovett and Ruesink 1995) experiments generally agree that frass deposition affects soil N, the relationship between frass deposition and phytochemical induction has not been tested empirically. This led us to ask whether a frass nutrient pulse could affect phytochemical induction in a manner similar to that previously observed with inorganic N fertilization (Bryant et al. 1993, Hunter and Schultz 1995, Kyto et al. 1996). Our first objective was therefore to explore herbivore-induced phytochemistry independent of and interactive with frass deposition, testing the hypothesis that frass deposition would reduce phytochemical induction.

Our second objective was to test empirically a less-explored potential effect of herbivore activity: modification of litter quality as a substrate for decomposition and resulting changes to nutrient dynamics (Choudhury 1988). Herbivore-mediated changes that reduce the quality of leaf litter may decelerate decomposition and nutrient turnover (Findlay et al. 1996), while changes that increase litter quality may accelerate decomposition (Ritchie et al. 1998, Belovsky and Slade 2000, Hutchens and Benfield 2000, Chapman et al. 2003, 2006). Herbivore damage in oaks generally increases tannin concentrations, which should decelerate decomposition (Hättenschwiler and Vitousek 2000), though lasting effects of frass deposition on leaf and subsequent litter quality may also affect decomposition and nutrient release. However, few studies have followed herbivore manipulation experiments through to the subsequent decomposition of leaf litter either with or without insect frass as an experimental variable (Schweitzer et al. 2005b). For our second objective, we hypothesized that (1) herbivore damage on oak foliage would decelerate decomposition of the subsequent litter via differences in tannin concentrations release rates and (2) mitigating effects of frass deposition on tannin induction or N concentration would accelerate decomposition and nutrient release.

## Methods

### Field site for experimental manipulations

Detailed description of the potted *Quercus rubra* experimental array and a figure of representative experimental units can be found elsewhere (Frost and Hunter 2004). Briefly, we built a field site in a fenced, open area adjacent to the Plant Sciences Greenhouses, Athens, GA, in an area receiving full sun (PAR $\sim$1900–2000 $\mu$mol $m^{-2}$ $s^{-1}$). Mesocosms were established using 160 nursery-grown *Q. rubra* saplings planted in pots and suspended in individual wooden stands using soil and litter from watershed 27 ("WS27") at the Coweeta Long Term Ecological Research (LTER) site ("CWT," Otto, NC, elevation 1300 m). The *Q. rubra* saplings were $1.33 \pm 0.14$ m tall and averaged $13.71 \pm 0.18$ mm in diameter (an average of two measurements made 10 cm from base of soil) at the beginning of the 2002 growing season. We repeated stem diameter measurements following complete leaf drop in Dec 2002 as an estimate of seasonal growth rate.

### Experimental manipulations

Half of the saplings at the field site were chosen at random for this experiment. We used a full factorial experimental design with herbivore damage (herbivore, undamaged) and frass (frass, no frass) treatments. Individual oak mesocosms served as replicates: twenty experimental units were randomly selected for each of four treatments, totaling 80 saplings. Prior to herbivore additions (1–3 June 2002), leaf counts and area measurements were made on each tree with an LI-3000A. Total leaf area per tree averaged $9053 \pm 3260$ $cm^3$ (mean $\pm$ SD), and was not significantly different among treatment groups.

Herbivore damage was inflicted by *Malacosoma americanum* collected from the field. This herbivore was chosen because of its local abundance and ease of collection. Herbivores were contained within hand-sewn bags ($1.0 \times 0.8$ m rectangles double sewn on three sides) of Reemay agricultural cloth. A bag was placed over the top of each tree and extended downward to cover 65–75% of the total leaf area. All trees, including those free of herbivores, were covered with the bags to control for any potential bag effects (e.g. reduced photosynthesis). Air temperatures inside the bags were $\sim$ 3–5°C higher than outside the bag, though no trees showed visible signs of stress. The bags were secured around the main stem using rope to prevent herbivore escape.

Thirty 4th-5th instar *M. americanum* were added to each sapling on 5 June 2002 and removed on 15 June 2002. The large majority of defoliation by *M. americanum* occurs in the final two instars; the ten-day feeding period covers approximately the length of those instars in the field. We estimated damage as leaf area removed by the herbivore ($LAR_{herbivore}$) within two days following herbivore treatments using techniques described in Hunter (1987), here denoting leaf area removed by the herbivore as "$LAR_{herbivore}$" to avoid confusion with leaf area ratio (LAR), a common abbreviation in plant growth analysis. As expected, our herbivore addition increased defoliation levels on the herbivore-damaged saplings vs controls (14.3% vs 6.7% $LAR_{herbivore}$, respectively; $t_{73} = 10.54$; $p < 0.0001$).

The frass used in the experiment was generated in real time by the herbivores. Frass from each herbivore-infested sapling was collected on 10 and 15 June 2002 by manually sucking frass pellets into a modified aspirator ("frasspirator") inserted through a small incision in the bag. The incision was sealed following frass collection to prevent herbivore escape. On each day, frass collected from each sapling (40 total saplings) was individually weighed, pooled, and redistributed evenly among those trees designated for frass addition. Thus, the amount of frass deposition was representative of the average level of damage experienced by

the saplings. Frass was added to the surface of the soil without mixing to simulate natural frass deposition. Overall, the frass additions totaled $\sim$0.982 g frass per tree (dry weight equivalent), corresponding to a deposition of $\sim$10 g $m^{-2}$. The frass was composed of 49.1 $\pm$ 0.9% carbon (C), 3.1 $\pm$ 0.1% N, yielding frass-derived C and N additions of 482 mg (48.2 kg $ha^{-1}$) and 30 mg (3.0 kg $ha^{-1}$), respectively. This level of frass deposition is $\sim$3 times the average annual input of frass at CWT (Hunter et al. 2003), and $\sim$17% of that observed under outbreak conditions (Swank et al. 1981, Reynolds et al. 2000). As a result, our experiments tested the hypotheses at near endemic levels of defoliation and frass deposition; we therefore avoid generalizing the results to insect outbreak conditions, which may induce stronger defensive responses and deposit more frass per unit area.

## Leaf sampling and analysis

We collected live leaf samples three times over the course of the field season: (1) pre-treatment (''PT'', 29 May 2002), (2) post-treatment early-season greenleaf (''EG'', 6 July 2002), and (3) late-season greenleaf (''LG'', 25 September 2002). The experimental treatments occurred toward the end of the natural spring-feeding period of insect herbivore activity when most defoliation occurs. The PT leaf samples were taken approximately three weeks following complete expansion of all first-flush foliage and six days before experimental manipulations. The EG sample date was $\sim$1 month following the experimental manipulations, by which point frass N had been mineralized (Frost and Hunter 2004). The LG sample was in the middle of the natural feeding phenology of fall-feeding insect herbivores of oak (Hunter and Schultz 1995), and was chosen to determine the effect of early season herbivory on the quality of foliage available to late season herbivores.

All leaves collected within the pre-treatment sample were within 0-5% $LAR_{herbivore}$; that is, they were all essentially undamaged. The post-treatment (EG and LG) samples were collected and processed similarly to PT samples, though the damage levels differed. The majority of leaves in the herbivore damage treatment suffered 5–30% $LAR_{herbivore}$, and we selected leaves in this range for chemical analysis. Leaf samples from the undamaged group necessarily remained 0–5% $LAR_{herbivore}$. In other words, damaged leaves were sampled from the herbivore treatment saplings, while undamaged leaves were sampled from the undamaged saplings. We chose this sampling regime to focus on induced chemical changes to leaves suffering physical damage, and our results cannot address chemical effects of systemic induction. For all collections, we restricted samples to leaves on terminal stems receiving full sun from the area of trees that had been bagged during treatments.

For chemical analyses, a standard hole puncher was used to collect three leaf disks ($\sim$0.28 $cm^2$ and $\sim$2.0 mg dry weight per disk) a single leaf per tree (trees as replicates) in the field directly into 70:30 acetone-water with 1 mM ascorbic acid. Samples were stored in the dark on dry ice during collection. Additional leaf disks were collected and used to estimate wet weight/dry weight ratios; all data are reported per unit dry weight. On each sampling date, 15 leaf disks were also pooled from 2–3 additional leaves per tree to prepare a bulk standard for the phenolic assays (described below). Remaining leaf material was then dried for 48 h at 60°C and ground in a ball mill; the resulting powder was used for total C, N and three fiber measurements (lignin, cellulose, hemicellulose).

Total C and N were measured with a Carlo Erba 1500N total CHN analyzer. The three fiber measurements were determined by sequential acid digestions using an ANKOM A200 Fiber Analyzer according to manufacture's instructions. Phenolics were assayed colorimetrically following well-established methods (Bate-Smith 1977a, 1977b). The Folin-Denis assay was used to estimate total phenolics (Appel et al. 2001). Condensed tannins were assayed following acid depolymerization in a polar solvent (N-butanol) at 100°C (Hagerman and Butler 1980, Hagerman 1988), and hydrolysable tannins were measured using the standard potassium iodate assay (Rossiter et al. 1988). Standards for phenolic assays were date-specific composite samples of all the individual trees (described above) and were extracted similarly to the individual samples. The bulk aqueous extract was frozen and lyophilized, and the resulting powder was used to make dilutions for the standard curves.

## Decomposition experiment

Naturally-senesced leaf litter was collected in mid-November 2002, by which point the foliage remaining on the trees was visibly dead. Litter was collected by gently shaking the mainstem to dislodge the dead material. Because the amount of litter per tree was not sufficient for individual replicates, litter from each of four trees within a treatment group was collected and pooled to generate five replicate groups per experimental treatment. When considering the effects of herbivores on leaf litter decomposition, comparing the overall litter composition was essential; in nature, both damaged and undamaged foliage falls as leaf litter from trees suffering herbivore attack. As a consequence, experiments using only damaged litter will overestimate the importance of herbivore activity on subsequent decomposition. Therefore, litter samples from each damage treatment contained the natural mixture of damaged and undamaged leaves that resulted from our herbivore manipulations. Litter was air-dried in the laboratory in closed, suspended paper bags, and subsequently partitioned into 15 $\times$ 15 cm bags made of commonly available 1 mm nylon mesh screening (Crossley and Hoglund 1962). Each bag initially contained $\sim$3 g of dry leaf litter. The litter bags were established on 1 February 2003 in a randomized grid design on WS 27 at CWT (elevation 1374 m, 35°01′53″N, 83°27′35″W). For each litter bag, a randomized grid location was first cleared of fresh litterfall; the bag was then placed on the surface soil, secured in one corner with a labeled field flag, and covered with the surface litter. Replicate bags from each of the four treatment groups were collected at 3, 9, 12 and 18 months, and a set of replicate bags was also collected immediately to determine initial litter quality (5 dates $\times$ 5 replicates $\times$ 4 treatments = 100 litterbags). All litterbags were recovered, and data collected from all litterbags were used in the analyses unless otherwise noted.

For each collection date, 20 bags (5 replicates × 4 experimental groups) were collected. Each bag was removed from the soil surface by first gently removing surface litter, removing the stake with minimal disturbance to the bag itself, and then removing the bag. Any remaining external debris was carefully cleared and the litterbag was transported to the lab in a sealed plastic ziplock bag stored at ambient temperature.

Litter tissue was dried under incandescent light for 48 h (for microarthropod collection, data not shown), weighed to determine mass loss, and then ground to a fine powder that was used for analysis of phenolics, fiber, and total C and N. Samples underwent chemical analyses as described above for green leaves, except that ~20 mg of dry powder per sample was used for phenolic assays. In addition, we pooled ~5 g of material from equal subsamples of each replicate to generate bulk phenolic standards for the analyses.

### Statistical analyses

Data were analyzed using the GLM procedure of SAS 8.2 to generate models containing two fixed factors and their interactive term: "damage" (herbivore, undamaged) and "frass" (frass, no frass). Data were transformed when necessary to satisfy model assumptions (Kery and Hatfield 2003). A repeated-measures framework was used when necessary and appropriate to test for within-subjects effects. For repeated measures analyses, we accounted for the assumption of sphericity using Huynh-Feldt (H-F) epsilon to reduce the error degrees of freedom and yield more conservative hypothesis tests (Littell et al. 1998, 2002). We used the Tukey HSD post hoc test ($\alpha = 0.05$) to distinguish among treatment means.

Litter decomposition and chemical rates of change were analyzed using methods modified from population time series analysis (Royama 1992, Madritch and Hunter 2002). The rate of change ($\Delta$) in litter mass remaining (MR) was calculated as $\ln(MR_{t+1}/MR_t)$ for each time step (0–3, 3–9, 9–12, 12–18 months), correcting for the length of the time step by dividing the number of months in the time interval. Similar analyses were conducted for tannins, total phenolics, N and lignin. This allowed us to determine treatments affects on rates of chemical release and decomposition separately.

## Results

### Damage and frass effects on phytochemical induction

As expected, condensed tannins, hydrolysable tannins and total phenolics increased in the green foliage following herbivore damage (date × damage $F_{2,130} = 4.67$, $p = 0.0162$; $F_{2,128} = 6.42$, $p = 0.0022$, $F_{2,130} = 5.26$, $p = 0.0064$, respectively; Fig. 1). However, herbivore-induced differences in tannin concentrations were relatively short-lived. In all cases, Tukey post-hoc analysis indicated that tannin induction was only apparent in the EG samples and not in the LG samples (Fig. 1a-c). Interestingly, treatment effects were also apparent in the initial litter samples (Decomposition experiment).

In addition to tannin induction, the oak saplings reduced specific leaf area (SLA), foliar N, and lignin concentrations in response to the herbivores. Lower SLA values indicate tougher leaves; SLA was significantly reduced following herbivory, though only in the EG samples (date × damage $F_{2,130} = 6.51$, $p = 0.0022$; Fig. 1d). Thus, herbivore damage increased the rate but not the overall magnitude of leaf toughening. In contrast, the herbivore treatment increased the rate and overall magnitude of leaf N decline. Foliar N concentrations declined following herbivore damage relative to the undamaged group in both post-treatment green leaf samples (date × damage $F_{2,130} = 4.86$, $p = 0.0137$; Fig. 1e). The damage effect on N resulted in elevated C:N ratios that also lasted throughout the growing season (date × damage $F_{2,130} = 10.10$, $p < 0.0001$). In addition, foliar lignin concentrations declined following herbivory, but only in the LG samples (date × damage $F_{2,130} = 7.82$, $p = 0.0007$, Fig. 1f).

In comparison to the effects of defoliation, the effects of frass deposition on leaf chemistry were minimal. Frass deposition did not affect N or tannin concentrations in either of the two post-treatment leaf samples, and there were no significant treatment interactions to indicate that frass mitigated herbivore-induced condensed or hydrolysable tannin concentrations either in the overall dataset (date × damage × frass $F_{2,130} = 0.68$, $p = 0.4810$; $F_{2,128} = 0.06$, $p = 0.9389$, respectively, Fig. 1) or in date-specific analyses (damage × frass EG $F_{1,65} = 0.36$, $p = 0.5491$, LG $F_{1,65} = 0.00$, $p = 0.9621$; EG $F_{1,64} = 0.00$, $p = 0.9661$, LG $F_{1,64} = 0.26$, $p = 0.6097$, respectively). The only statistically significant effect of frass additions was higher lignin concentrations in the early season following deposition independent of herbivore damage, which was strong enough to affect lignin accumulation during the growing season relative to controls (date × frass $F_{2,130} = 4.02$, $p = 0.0215$, Fig. 1f).

Neither treatment affected tree growth rate as measured by change in stem width (damage $p = 0.3912$, frass $p = 0.3946$, damage × frass $p = 0.1363$), and there was no visual evidence of treatment-mediated premature leaf abscission. There were also no damage × frass interactions on any index of green leaf quality.

### Decomposition experiment

Initial litter chemistries were influenced by both damage and frass treatments. Contrary to our expectations, concentrations of hydrolysable and condensed tannins were higher with frass additions ($F_{1,19} = 18.81$, $p = 0.0005$; $F_{1,19} = 43.20$, $p < 0.0001$, respectively) and lower with herbivory ($F_{1,19} = 13.29$, $p = 0.0022$; $F_{1,19} = 5.73$, $p = 0.0293$, Fig. 2a-b). For hydrolysable tannins, there was a statistical interaction such that frass additions resulted in higher tannin concentrations in undamaged treatment litter relative to either damage type (damage × frass $F_{1,19} = 21.88$, $p = 0.0003$; Fig. 2b). Total phenolics followed a similar pattern, though effects were weaker with only a moderate statistical interaction (Fig. 2c). Effects on litter N concentrations also showed treatment interactions (damage × frass $F_{1,19} = 19.54$, $p = 0.0004$). Initial litter N concentrations were higher in the herbivore group than the undamaged group in the absence of frass (Fig. 2d), suggesting that herbivore damage lowered resorption

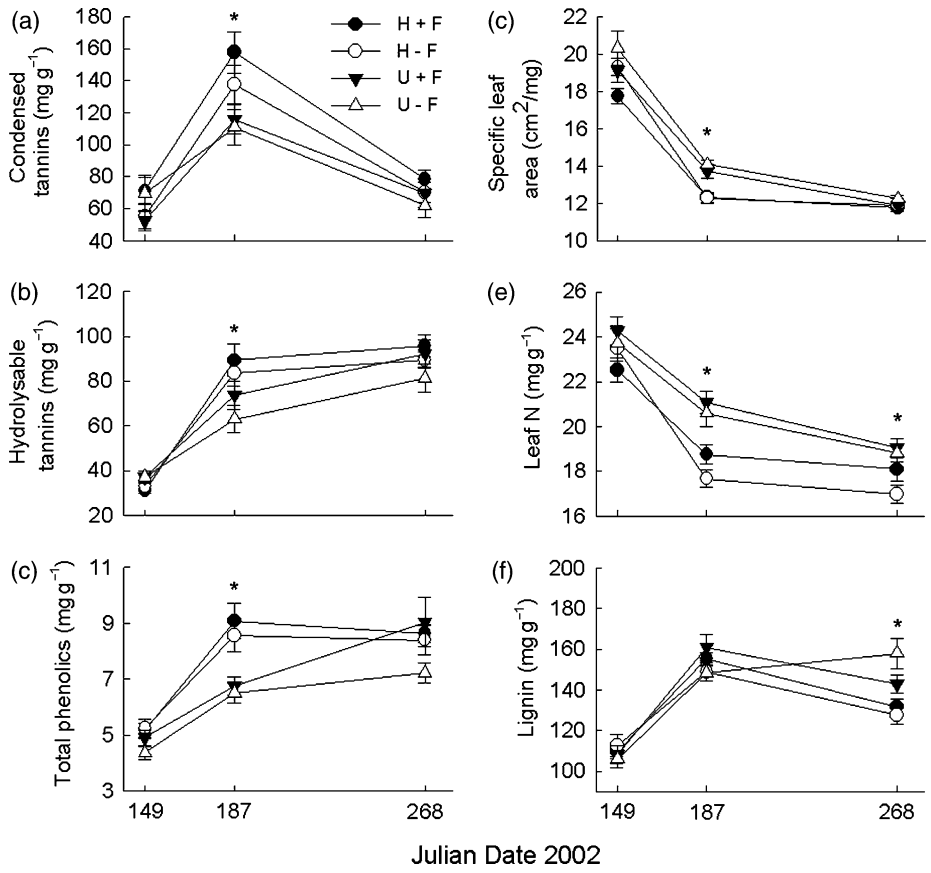


Fig. 1. Effects of frass and damage on changes in concentrations of (a) condensed tannins, (b) hydrolysable tannins, (c) total phenolics, (d) specific leaf area, (e) foliar N, and (f) lignin in *Quercus rubra* foliage in experimental mesocosms. Points represent the means of 20 samples ± SE. Shaded and unshaded symbols represent samples receiving and not receiving frass additions, respectively; circles and triangles represent the herbivore and undamaged groups, respectively. All data are presented per unit dry mass. Dates marked with an asterisk (*) indicate significant differences between the herbivore and control treatments (Tukey, $\alpha = 0.05$). 2002 Julian dates: 149 = May 29 (PT); 187 = July 6 (EG); 268 = September 25 (LG).

proficiency. However, in the presence of frass, N concentrations were similar between herbivore and undamaged groups (Fig. 2d). Lignin concentrations also showed statistical interaction such that with frass, lignin concentrations were lower with herbivory (Fig. 2e). Lignin:N essentially mirrored total N (Fig. 2f).

Chemical release rates from the decomposing litter were influenced by both herbivore and frass treatments, but only during the first nine months of decomposition. When considering the rates of change between collections, condensed and hydrolysable tannins and total phenolics declined more slowly (CT: $F_{1,19} = 7.02$, $p = 0.0175$; HT: $F_{1,19} = 9.45$, $p = 0.0073$; TP: $F_{1,19} = 85.84$, $p < 0.0001$), and N concentration declined more rapidly ($F_{1,19} = 7.31$, $p = 0.0156$) in the herbivore-damaged litter relative to controls in the first three months (Fig. 3a-d). These rates were all reversed in the 3–9 month interval (CT: $F_{1,19} = 4.96$, $p = 0.0406$; HT: $F_{1,19} = 9.45$, $p = 0.0073$; TP: $F_{1,19} = 85.84$, $p < 0.0001$; N: $F_{1,19} = 4.52$, $p = 0.0495$). The herbivore treatment had no influence on the rate of change in lignin concentrations in the first three months, but the lignin concentration increased at a significantly higher rate in the herbivore-damaged litter during the 3–9 month interval ($F_{1,19} = 6.93$, $p = 0.0181$, Fig. 3e).

Frass deposition during the growing season also had a detectable effect on the dynamics of decomposition. Condensed and hydrolysable tannins declined faster in the frass addition group compared to controls from 0–3 months in the field ($F_{1,19} = 28.47$, $p < 0.0001$; $F_{1,19} = 9.39$, $p = 0.0074$, respectively, Fig. 3a-b). Interestingly, total phenolics in the frass addition group appeared to decline slower in the first three months and faster between 3–9 months ($F_{1,19} = 16.18$, $p = 0.0010$; $F_{1,19} = 26.38$, $p < 0.0001$, respectively; Fig. 3c), though these results were driven by a statistical interaction (Fig. 4c). The frass treatment resulted in a marginal increase in the rate of N accumulation in litter during the 3–9 month interval ($F_{1,19} = 4.31$, $p = 0.0544$, Fig. 3d), and significantly lowered the rate of change in litter lignin concentration in the first three months of decomposition ($F_{1,19} = 28.54$, $p < 0.0001$, Fig. 3e).

While there were clear treatment-based differences in rates of change in litter chemistry during the early stages of decomposition, rates of litter mass loss were unaffected by the treatments at any time interval (Fig. 3f). The statistically significant effects of defoliation and frass on rates of change in litter tannins, N, and lignin over the course of decomposition were driven largely by effects during the first nine

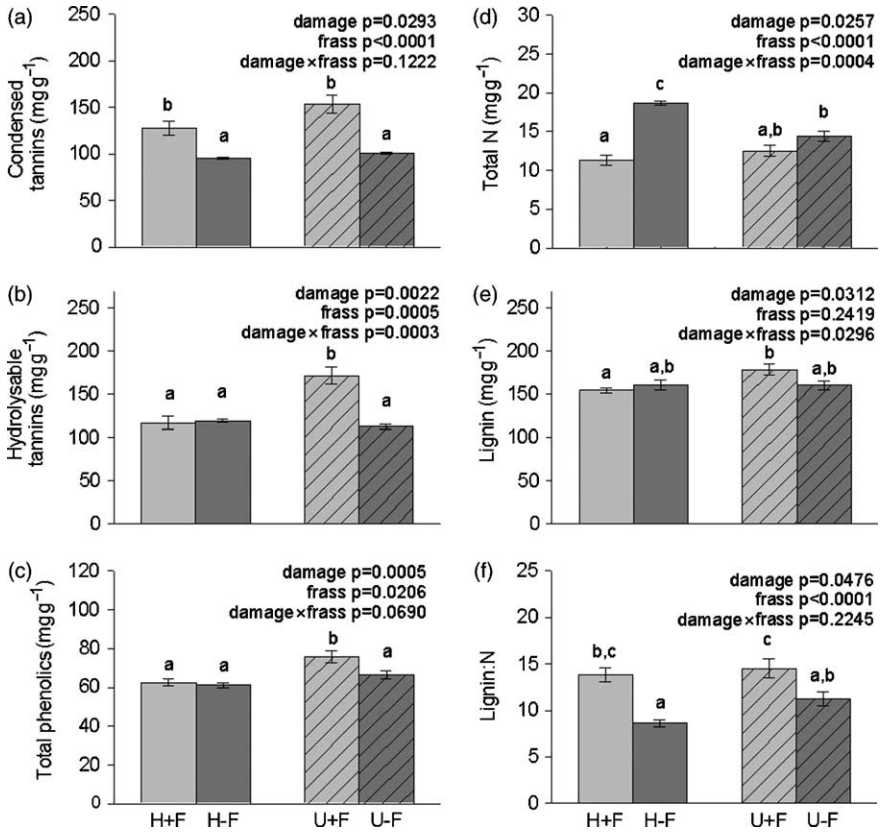


Fig. 2. Initial chemical composition of *Quercus rubra* leaf litter before the decomposition experiment. Bars are means ± SE of five samples. Data are presented per unit dry mass. Different letters over bars within a graph indicate statistical differences among means (Tukey, $\alpha = 0.05$).

months of decomposition. From month 9 through month 18, there was an overall convergence of litter chemistries among treatments (Fig. 4a-e). In addition, leaf litter mass loss was unaffected by either damage or frass treatments (date × damage $F_{4,96} = 0.95$, $p = 0.4745$; date × frass $F_{4,96} = 0.64$, $p = 0.6103$, respectively, Fig. 4f), and mass loss data fit a standard first order decomposition model ($k = -0.0217$ month$^{-1}$, $r^2 = 0.90$). Our results therefore suggest that the processes controlling tannin, N and lignin dynamics in decomposing litter are not tightly linked with actual rates of litter mass loss.

## Discussion

### Phytochemical induction and frass deposition

Our first objective was to test the hypothesis that frass deposition would mitigate phytochemical induction in response to herbivores. Our data do not support this hypothesis. Field observations of frass-derived increases in soil mineral N pools from mesocosm (Frost and Hunter 2004) and field (Reynolds and Hunter 2001, Hunter et al. 2003) experiments inspired us to ask whether reductions in phytochemical induction would be observed following herbivore-mediated changes in soil N, as have been observed following inorganic fertilization (Bryant et al. 1993, Hunter and Schultz 1995). Our experimental design allowed us to compare undefoliated and defoliated oak saplings in the presence and absence of frass deposition to determine if the N returned in frass affected phytochemical induction. The absence of independent or interactive effects indicates that frass deposition did not affect the chemistry of the live foliage during the growing season, at least at endemic levels of defoliation. Based on well-established literature (Karban and Baldwin 1997), we expected – and observed – induction of tannins following herbivore damage. In addition, herbivore-damaged trees exhibited increased leaf toughness and reduced foliar N content, both of which have been observed to varying degrees in other studies (Nykänen and Koricheva 2004). Thus, oak leaf nutritional quality declined following herbivory as a combined result of induced increases in tannins and toughness as well as reductions in N concentrations. The frass treatment did not influence any of the herbivore-induced changes to the green-leaf quality.

While our data do not support our initial hypothesis, the results allow us to highlight two obvious differences between inorganic fertilizer applications and frass deposition: the magnitude of additions and the source of N. First, in an experiment that demonstrated mitigation of tannin induction by the addition of fertilizer, Hunter and Schultz (1995) added 18.2 g N per tree, three orders of magnitude more N than that applied as frass in our experiment. Moreover, our frass additions were equivalent to only one half of a single fertilizer application in the study of Bryant et al. (1993) on nutrient availability and induction. These previous studies were designed to provide saturating doses

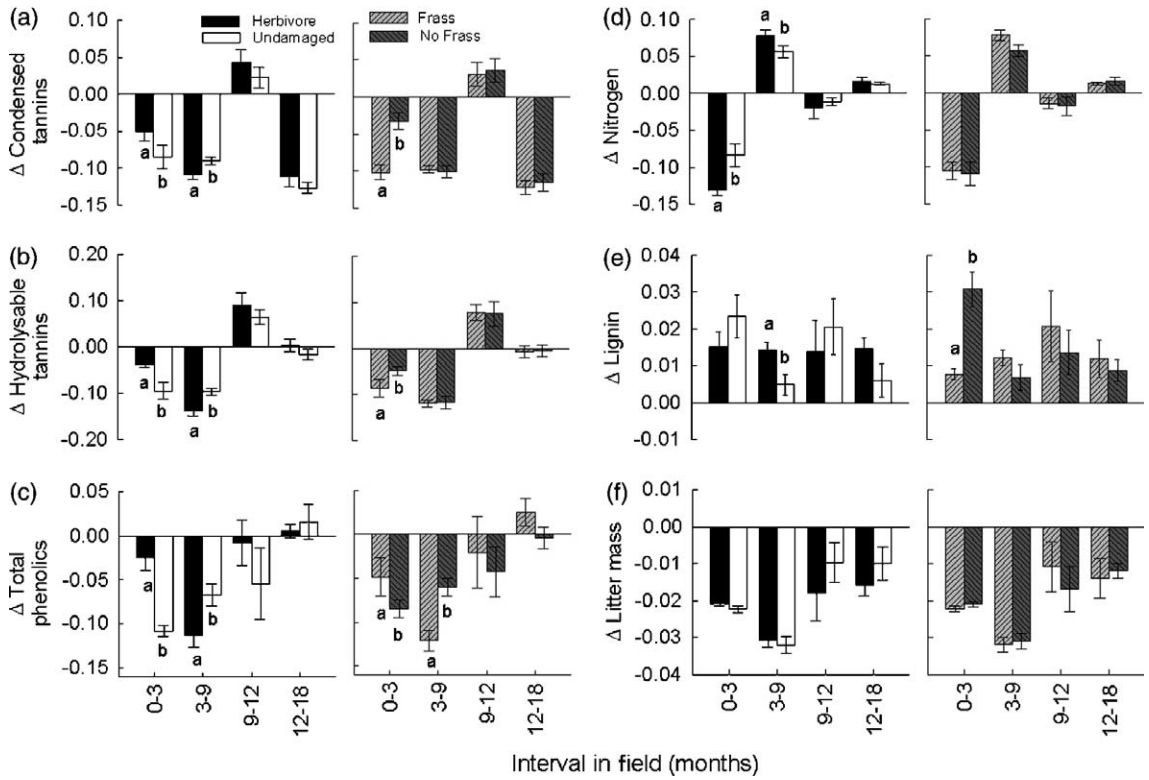


Fig. 3. Rates of change of (a) condensed tannins, (b) hydrolysable tannins, (c) total phenolics, (d) nitrogen, (e) lignin, and (f) litter mass in decomposing *Quercus rubra* leaf litter as a result of herbivore damage or frass deposition. We determined rates of change between each time point in the decomposition process using methods from population time series analysis (Methods). Bars are means ($n = 5$) $\pm$ 1 SE. Post-hoc analysis compared the treatment vs control groups within each time interval, and different letters within time interval are significantly different (Tukey, $\alpha = 0.05$).

of N. Since our applications were $\sim 3\times$ endemic levels, the experimental manipulation of frass was biologically relevant but delivered a small amount of N relative to inorganic fertilization experiments. In addition, even outbreak defoliation levels would not produce anywhere near the level of N supplied during the fertilization treatments (Swank et al. 1981, Reynolds et al. 2000). Second, frass N is necessarily derived from foliage of defoliated trees, representing a tangible loss of acquired N. Although oaks can take up N mineralized from herbivore frass, they do not regain all N lost through defoliation; the overall mass balance of herbivory is a loss in N for the defoliated plant (Frost and Hunter 2007). This is a general trend for any level of herbivory. The overall result is clear: the amount of N returned to soil as frass is not sufficient to affect phytochemical induction during the season in which defoliation occurs. Large, unnatural fertilization events independent of damage that do not directly reduce the pool of foliar N, such as anthropogenic N deposition (Ollinger et al. 1993, Zogg et al. 2000), may be required to provide sufficient "free" N to affect phytochemical induction within the same season.

## Ecosystem consequences of phytochemical induction and frass deposition

Our second objective was to examine whether a single herbivore damage event or its associated frass deposition could affect subsequent leaf litter decomposition. Herbivore damage and frass additions during the growing season influenced initial litter chemistries and subsequent tannin and N release dynamics in unexpected and complex ways. In contrast, herbivore activity did not influence overall rates of mass loss. Choudhury (1988) argued that herbivores could affect ecosystem function by altering the quality of leaf litter as a substrate for decomposition, and initial experimental work supported the hypothesis (Findlay et al. 1996). In our study, the differences in initial litter qualities were contrary to expectations and opposite from those in green leaves: litter tannins were lower in the damaged group and higher in the frass group, while litter nitrogen was higher in the damaged group and lower in the frass group. This apparent contradiction between effects on green leaves and subsequent effects on litter has been observed previously in oaks (Hall et al. 2005). In our study, the higher N in litter from damaged leaves could result from incomplete resorption despite no apparent changes in timing of leaf drop. It is also possible that differences in senescence were overlooked, since oaks can retain their foliage long after completing resorption (Madritch and Hunter 2002) and all initial litter N values were higher than those reported for red oak in the field (Killingbeck 1996). Nonetheless, the central point is that both herbivore damage and frass additions affected initial litter quality.

Herbivore damage and its associated frass deposition also independently affected nutrient dynamics in the early stages of decomposition. Moreover, our data indicate that nutrient

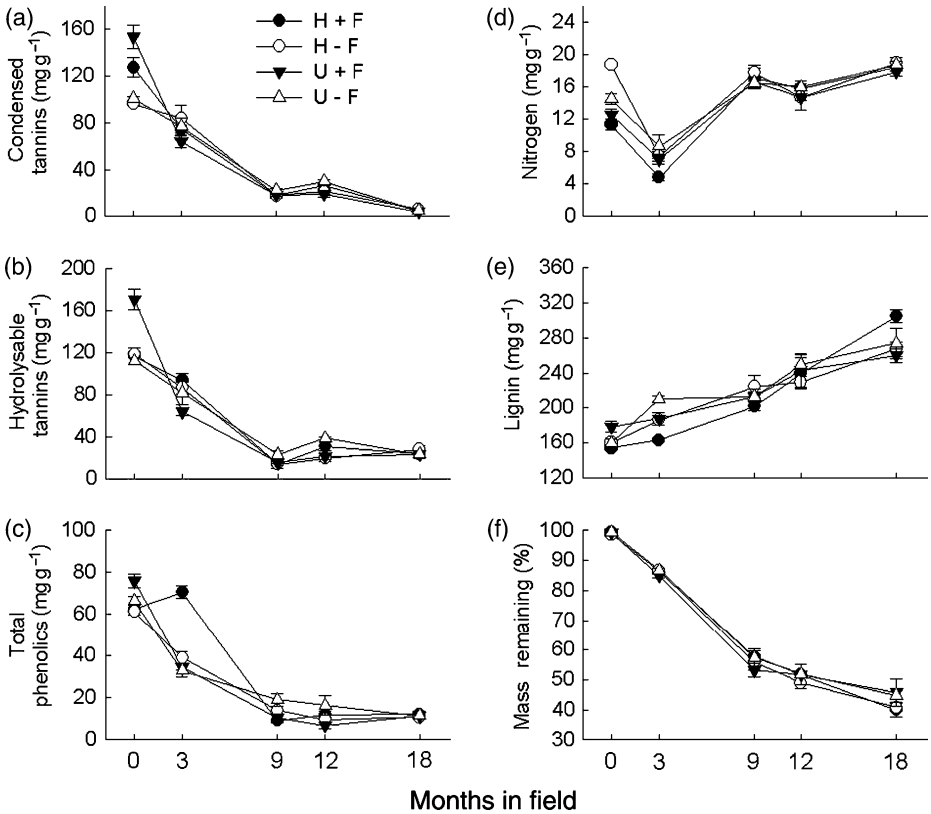


Fig. 4. Effects of frass and damage on changes in (a) condensed tannins, (b) hydrolysable tannins, (c) total phenolics, (d) nitrogen, (e) lignin, and (f) mass loss during *Quercus rubra* litter decomposition. Points represent the means of 5 samples ± SE. Data are presented per unit dry mass (a-e) and dry mass (f). Shaded and unshaded symbols represent samples receiving and not receiving frass additions, respectively. Circles and squares represent the herbivore-damaged and undamaged groups, respectively. The overall decomposition rate (k) for all data points was $-0.0217$ month$^{-1}$, $r^2 = 0.90$, and was not affected by either treatment.

release is, at least in part, decoupled from physical mass loss. It is therefore useful to consider herbivore effects on nutrient release and mass loss separately since those processes have different effects on ecosystem dynamics. The acceleration/deceleration hypothesis (Ritchie et al. 1998, Chapman et al. 2006) provides a framework for predicting the direction of herbivore-mediated changes in decomposition in terrestrial systems. While this framework essentially applies to any effect on substrate quality, such as changes in intra- or inter-specific plant diversity (Schweitzer et al. 2004, 2005a, Madritch and Hunter 2005) or the effects of elevated $CO_2$ on litter chemistry (Peñuelas and Estiarte 1998, Norby et al. 2001, Hall et al. 2005, 2006), it can also be applied to any specific component of decomposition. A key finding from our data is that herbivore activity influences the timing of nutrient release, though sometimes in opposing fashions (Fig. 3). For example, during the first three months of decomposition, damaged litter releases tannins more slowly than does undamaged litter. In contrast, during the same time period, litter grown under frass addition releases tannins more rapidly, and rates of N loss are higher. During months 3 to 9, herbivore damage increases the rate at which tannins are lost from litter. In combination, these results indicate that there is no simple relationship between herbivore activity and decomposition; some processes accelerate, some decelerate, and it varies with the form of herbivore activity (e.g. leaf damage versus frass deposition).

Are the release rates of tannins and nitrogen important for subsequent ecosystem processes? Previous work has shown that tannin release into the soil can hinder microbial activity (Northup et al. 1998, Maie et al. 2003), and decomposer organisms are known to respond to ephemeral pulses of resources and nutrients (Hättenschwiler and Gasser 2005). Thus, the herbivore-mediated changes in initial litter quality that we observed could potentially impact short-term dynamics of microbial organisms and associated soil processes in significant ways. Indeed, our N data suggest that the herbivore damage increased both the immediate loss of N and also the subsequent increases that would result from detritivore importation of exogenous N.

The differences in nutrient release rates during the early stages of decomposition did not translate into treatment-based changes in overall mass loss. In addition, we observed a general convergence among litter chemistries over time that was largely independent of treatments or the litter quality parameters measured. Recalcitrant fractions of leaf litter (e.g. lignin) form an important element of soil organic matter (SOM, Swift et al. 1979), so it is useful to know the extent to which these fractions are affected by abiotic and biotic factors. In our case, mass losses in our litters were consistent and, as a result, our decomposition rate constant estimates were not influenced by the herbivore or frass treatments. These results indicate that ecosystem processes such as SOM formation may not be influenced by foliar herbivores through changes in litter chemistry, at least when

considering such effects from a single damage event on a single tree species. The bottom line is that the abiotic environment more strongly influenced mass loss than did the biotic factors of litter quality or our treatments over the course of the full 18 months.

As a final point, herbivores are pervasive in most terrestrial systems, and it is therefore useful to consider our results in a broader context. Chronic herbivory may affect ecosystem processes through decomposition by acting as an agent of selection and therefore have a more dramatic effect on decomposition and nutrient release than indicated here (Schowalter 2000). For example, intraspecific variation in susceptibility to herbivores results in differences in decomposition between litters from susceptible and resistant genotypes (Chapman et al. 2003). In addition, there are clear and obvious differences in litter decomposition dynamics among tree species; herbivores can therefore affect decomposition by altering the community composition of the plants through preferential feeding on specific tree species (Schowalter 1981, Ritchie et al. 1998). Our results reinforce the importance of chronic herbivory by showing that even single herbivore damage events have detectable effects in at least early stages of decomposition that affect nutrient dynamics and possibly detritivores as well, even if overall decomposition is controlled ultimately by abiotic factors.

## Summary

We tested, but could not support, the hypothesis that frass deposition following endemic levels of insect herbivore feeding would mitigate the induced defensive response in oaks within the season of defoliation. However, both herbivore damage and subsequent frass deposition in the growing season did alter initial litter chemistries and tannin and N release during decomposition, suggesting that the dynamics important for some ecosystem processes are decoupled from actual rates of litter mass loss. Despite this, the equilibration of litter chemistries across treatments over the course of decomposition indicates that the immediate effects of single herbivore events on ecosystem processes are likely short lived.

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