DIRECT AND INDIRECT EFFECTS OF INSECT HERBIVORES ON TERRESTRIAL ECOSYSTEM PROCESSES

by

CHRISTOPHER JOHN FROST

(Under the Direction of MARK D. HUNTER)

ABSTRACT

Herbivores can influence terrestrial ecosystem functioning through a number of mechanisms. Deposition of waste products ('frass') introduces labile nitrogen (N) and other nutrients directly to the soil, potentially altering soil N availability. Herbivores stimulate changes in the chemistry and nutrient allocation patterns of plant tissues, which may indirectly affect N uptake dynamics and ecosystem N cycling. These processes occur rapidly and are "fast" cycle components of the terrestrial N cycle. Herbivore-mediated changes to the chemistry of foliage may alter rates of leaf litter decomposition, which is a "slow" cycle component of the terrestrial N cycle. This dissertation explores the direct and indirect effects of insect herbivores on "fast" and "slow" cycle N dynamics in forest ecosystems.

We first present evidence from a field mesocosm manipulation experiment demonstrating that insect herbivores can facilitate the export of N from terrestrial to aquatic components of a watershed via feces (frass) deposition. This is the first study to provide mechanistic evidence that aqueous N export from terrestrial systems can result from frass deposition. Using the same experimental design, we examined the influence of herbivore damage and frass deposition on foliar quality and litter decomposition.

Despite expected herbivore-mediated changes to leaf chemistry, leaf litter decomposition rates were not affected by herbivore activity. Collectively, our results indicate that herbivore damage and frass deposition affected "fast" but not "slow" cycle N dynamics.

We then discuss an experiment in which we generated and applied ^{15}N-labeled frass to oak mesocosms to explore the distribution and plant recovery of frass N. Surprisingly, oaks accumulated new N in their foliage throughout the growing season despite declines in total N as a result of senescence. Herbivore damage reduced the recovery and apparently the allocation of frass N to oak foliage, which affected the N supply to the next cohort of spring-feeding insect herbivores. In addition, only a small percentage of N in frass was exported via leachate, with rainfall as a likely factor influencing the loss. The majority of N in frass was retained in the surface soil, suggesting that surface soils are strong sinks for exogenous, labile N.

Finally, we report the results of a dual-isotope (^{13}C and ^{15}N) microcosm experiment designed to examine the effects of foliar herbivore damage on belowground carbon allocation (BCA) and N uptake. In the presence of herbivores, BCA in oak seedlings was lower and stem storage of new N was higher. The data are the first to show that BCA in oaks is altered by foliar herbivores and have implications for C cycling and sequestration in forest ecosystems.

INDEX WORDS: belowground carbon allocation, decomposition, ecosystem function, feces, frass, herbivores, insects, nutrient cycling, oaks, *Quercus*, stable isotopes, tannins

DIRECT AND INDIRECT EFFECTS OF INSECT HERBIVORES ON TERRESTRIAL ECOSYSTEM PROCESSES

by

CHRISTOPHER JOHN FROST

B.A., New College of Florida, 1997

M.S., University of Florida, 2000

A Dissertation Submitted to the Graduate Faculty of The University of Georgia in Partial Fulfillment of the Requirements for the Degree

DOCTOR OF PHILOSOPHY

ATHENS, GEORGIA

2005

DIRECT AND INDIRECT EFFECTS OF INSECT HERBIVORES ON TERRESTRIAL ECOSYSTEM PROCESSES

by

Christopher John Frost

Major Professor:	Mark D. Hunter
Committee:	Miguel Cabrera David Coleman Lisa Donovan Paul Hendrix

Electronic Version Approved:

Maureen Grasso
Dean of the Graduate School
University of Georgia
August 2005

DEDICATION

Jack Kenneth Cooke
Glenah Hathaway Cooke
Meryll Maurice Frost, Sr.
Pauline MacKenzie Frost

ACKNOWLEDGMENTS

Mark Hunter deserves more credit than words can give. He has been generous with his time, energy, resources, and intellect over the course of my time here and has earned my utmost respect as a colleague and friend. My other committee members Miguel Cabrera, David Coleman, Lisa Donovan, and Paul Hendrix were also remarkably generous with their time and expertise, and I am fortunate to have had the opportunity to be advised by each of them.

Tom Maddox is the best analytical lab manager that I have ever met. His attention to detail and rigorous understanding of the mechanics of mass spectrometers, elemental analyzers, and Alpkem continuous flow autoanalyzers ensured complete confidence in all data leaving his lab. The Analytical Laboratory at the Institute of Ecology is fortunate to have such a detail-oriented manager.

Bob McCron at the Canadian Forest Service kindly supplied *Orgyia leucostigma* egg masses throughout the project.

When my first field season was interrupted by a family emergency, members of the Hunter lab, Carmen Hall, Mike Madritch, Sandy Helms, Oren Kleinberger, and Star Scott, collectively helped me set up the experimental treatments. In so doing, they made Chapters 2 and 3 possible.

John Tanner at the USDA APHIS Lab in Cape Cod generously allowed me to travel to his lab with ^{15}N-enriched trees to generate ^{15}N-enriched frass. Without his help,

Chapter 4 would not have been possible.

Jane Rogers, Mike Madritch, Scott Connelly, Ashok Ragavendren, and Bryan Nuse provided support at the beginning of my career. Carmen Hall and I have supported each other throughout. Caralyn Zehnder, Kyle Wickings, and Becky Ball provided advice (and occasionally a K-Bob) towards the second half of my career here.

My parents, Meryll and Susan Frost, are a constant source of support and inspiration. They are foundation of any success I am fortunate to enjoy.

My friend, Raysun Goergen, has been my support structure throughout this process, from helping suck frass out of bags to calming me down with a good game of cribbage.

TABLE OF CONTENTS

LIST OF TABLES

Page

LIST OF FIGURES

CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

The term "ecosystem function" describes the transformations of matter and flows of energy and nutrients through ecological systems ("ecosystems"). Since ecosystems are composed of organisms and their abiotic environment, all organisms simultaneously influence and depend on ecosystem functioning (Odum 1969). In terrestrial ecosystems, the cycling of carbon (C) and nitrogen (N) have received substantial attention due to the concerns of apparent human alteration of both cycles (Ollinger *et al.* 1993;Keeling *et al.* 1996). While the ways in which C and N enter terrestrial systems differ, their biological transformations are interdependent. Nitrogen fertilization almost universally increases net primary production (NPP) (Reich *et al.* 1997), which supplies labile, organic C to soil microorganisms (Cheng *et al.* 1996). Labile C is required for aerobic transformations of N by microbes (Cardon *et al.* 2002) and for uptake by plants (Chapin 1980).

The suggestion that consumers may regulate ecosystem functioning is longstanding (Chew 1974), and Wilson (1987) went further to suggest that invertebrates specifically were "(running) the world." For example, insect herbivores influence the uptake dynamics of N and the C-fixation rates of plants (Lovett & Tobiessen 1993) and have been linked with ecosystem N fluxes (Swank *et al.* 1981;Schowalter *et al.* 1991). The loss of N from the terrestrial ecosystem following herbivore activity disrupts the N cycle (Lovett *et al.* 2002), and the consequent decreased leaf area can reduce ecosystem

NPP. However, the mechanisms underlying the herbivore-mediated effects are not always clear.

This dissertation investigates mechanisms by which insect herbivores influence the dynamics of N and C in oak ecosystems. This introductory chapter summarizes the previous literature and develops the set of hypotheses that are explored in the subsequent chapters. The literature review begins by considering the general dynamics of N in terrestrial ecosystems, then incorporates the influence of plants, and finally considers the impacts of insect herbivores on plants and ecosystem function.

Nitrogen Pathways Into and Out of Terrestrial Ecosystems

Whether by natural or anthropogenic sources, N enters a forest ecosystem by one of three broad mechanisms: atmospheric deposition, fixation by symbiotic microbes, fixation by non-symbiotic microbes (Figure 1). The amount of N deposited in atmospheric deposition has increased since the industrial revolution, and it generally occurs as nitrate (Zogg *et al.* 2000) or ammonium (Zak *et al.* 1990), though low molecular weight organic forms are also possible. Atmospheric deposition usually occurs in the form of precipitation, but can also occur as dry deposition (EPA 2001). Fixation by soil microbes, on the other hand, is a natural process. The observation that N fertilization increases NPP in forests suggests that N is a limiting nutrient in most terrestrial ecosystems (Reynolds *et al.* 2000) and thus it tends to be highly conserved (Lovett & Ruesink 1995). However, N can also be lost from terrestrial ecosystems via a number of pathways. Anaerobic microsites in soils can result in denitrification (Barton *et*

al. 1999); abiotic ammonia volatilization is possible since NH_3 is in equilibrium with NH_4^+ (Mahendra & Ogden 1973). In addition, the processes of N mineralization and denitrification both have the potential for gaseous loss of NO and N_2O (Firestone & Davidson 1989). While these are trace gasses, their abundances can represent significant pools of N (Groffman & Tiedje 1991). Nitrogen can also be transported to lower soil depths (Walvoord *et al.* 2003) and from terrestrial to stream ecosystems via leaching (Berntson & Aber 2000), particularly as NO_3^- (Vitousek & Matson 1985;Nadelhoffer *et al.* 1999a).

The fact that N is highly conserved in forests suggests that forest organisms partition available N efficiently and competitively (Clarkson 1985;Berntson & Aber 2000;Bardgett *et al.* 2003). The soil micro-organisms ("microbial biomass") and inorganic soil matrix are major factors preventing N loss due to leaching (Bossier & Fontvieille 1995). Coleman & Crossley (1996) suggest that >90% of the NPP in forest ecosystems enters the soil system as dead organic litter (e.g., leaves, stems, trunks, roots). The physical and biological properties of soils must therefore be able to immobilize ephemeral inorganic nutrient pulses and decompose recalcitrant biological material. As might be expected, the labile forms of N are substantially more susceptible to export than are recalcitrant forms such as leaf litter (Christenson *et al.* 2002)

The first component of N retention in the soil (or barrier against leaching) is the soil microbial biomass, which functions as the initial sink for labile N in the soil (Zogg *et al.* 2000;Vitousek & Matson 1985;Groffmann *et al.* 1993;Seely & Lajtha 1997). For example, Zak *et al.* (1990) determined that microbial immobilization of ^{15}N was 10-20

times greater than uptake by the herbaceous groundcover *Allium tricoccum.* Zogg *et al.* (2000) followed the flow of N through an *Acer saccharum* hardwood forest using $^{15}NO_3^-$ and found that there was an immediate (within 2 hr) uptake of at least one third of the labeled N by soil microorganisms. While almost no ^{15}N was found in the roots in the first two hours, roots of <5mm diameter had begun to acquire the label within two days. After 6 weeks, the total recovery of ^{15}N did not change significantly (~20% of initial concentration) and the relative concentrations of the sinks (roots, organic matter, microbial biomass, forest floor) also varied slightly, which suggested that homeostasis was achieved. Zogg *et al.* (2000) hypothesized than the unrecoverable portion of the initial ^{15}N (approximately 80%) was likely due to a combination of leaching and plant uptake, neither of which was measured in that experiment.

Seely & Lajtha (1997) found that, after 5-20 days, there was an increase in recoverable tracer in both litter and fine roots. After application, there was little increase in tracer in the O2 soil horizon, but 30-45% recovery in two of three sites in the mineral soil. Further, the concentration of tracer in the mineral soil appeared to decrease more slowly during tracer application in the fall than in the spring. Very little tracer was recovered from the litter in following fall applications, though there appeared to be a precipitous increase in fine root accumulation five days after application. This result also supports the hypothesis that microbes outcompete plants for N when conditions for microbial growth are adequate.

While soil microbes appear to be an immediate sink for novel N added to the soil, the biological transformation of N (e.g., immobilization) under aerobic conditions is

mediated by carbon availability (Hart *et al.* 1994). Carbon is used by microbes for growth and respiration, yielding increased biomass and CO_2 (Rodrigo *et al.* 1997). The link between carbon utilization by soil microbes and N immobilization and mineralization is related to the C:N ratio of the substrate and the microorganisms involved in the processes (Magill & Aber 2000). Carbon availability in the soil is influenced (if not controlled) by plants (Cheng *et al.* 1996;Kuzyakov & Cheng 2001;Cardon *et al.* 2002;Farrar *et al.* 2003), and so consideration of N and C dynamics in ecosystems must consider the impact of plants.

Plants as Conduits Between Aboveground/Belowground Processes

The processes described thus far have all occurred in the soils. Plants, by their physiology and morphology, provide a conduit between these belowground nutrient dynamics and aboveground processes in terrestrial ecosystems and therefore have the potential to influence ecosystem function (Knops *et al.* 2002). Carbon fixed through photosynthesis is allocated belowground to roots and exudates into the rhizosphere, and rhizosphere microorganisms depend on these C inputs (Cheng *et al.* 1996;Cardon *et al.* 2002;Farrar *et al.* 2003). Nitrogen is translocated from the soil to the shoots and foliage (Rajaniemi & Reynolds 2004). At the community level, different dominant plant species partition C and N differently and have significant impact on N dynamics in their respective soils (Verchot *et al.* 2001;Templer *et al.* 2003;Fitzhugh *et al.* 2003;Lovett *et al.* 2004). In addition, studies exploring the fate of inorganic N deposition in terrestrial ecosystems have measured plant uptake. For example, Nadelhoffer *et al.* (1999b)

recovered up to 24% of applied N in plant tissue. Nitrogen uptake is energetically expensive (Clarkson 1985) and a percentage of the C allocated into the roots is used as the energy source to fuel N uptake. The opportunistic nature of roots (Eissenstat & Yanai 1997) and general response to fertilization (Lovett & Tobiessen 1993) suggest that C may be allocated when pulses of N occur. Plants can therefore compete for N, though they must allocate C to do so.

In addition to uptake of inorganic forms of N, plant roots can also accumulate organic N (ON) directly from the soil and its organic matter (Lipson & Näsholm 2001). Reports of plants directly accumulating organic N have been around since the 40's (Virtanen & Linkola 1946;Miettinen 2001), though this observation was discounted because it was assumed that soil microbes effectively out-compete plants for soil ON (Bardgett *et al.* 2003). Amino acids and small proteins are excellent C and N sources for microbes, and so it was thought that only inorganic N in excess of microbial requirements would be available to plants (Lipson & Näsholm 2001). Recent studies have shown that there is more complexity to the system than previously thought. A variety of terrestrial plant communities from Alaskan tundra (Kielland 1994) to Colorado shortgrass steppe (Raab *et al.* 1999) to tropical savanna woodland (Schmidt & Stewart 1999), and agricultural systems (e.g., Jones & Darrah 1994;Yamagata & Ae 1996;Näsholm *et al.* 2000), have demonstrated the importance of ON to plant nutrition. Indeed, there are some cases in which the rate of ON uptake is comparable or greater than uptake of inorganic N (Kielland 1994;Raab *et al.* 1999;Schmidt & Stewart 1999;Lipson *et al.* 2002). Considering that soil ON may account for 20-50% of total labile N (Senwo &

Tabatabai 2001), plants likely compete for such a large pool of an important but limited resource. Regardless of the form, N acquisition by plants results in translocation of N to foliage. Phytophages access that N during feeding and release a percentage of it back into the ecosystem.

The Relationship between Phytophagous Insects and Nutrient Cycling

Insect herbivores influence plants in a number of ways that may affect N and C dynamics. Phytophagous insects have long been recognized as contributors to forest nutrient cycling, with longstanding suggestions that they may act as regulators (Chew 1974). While regulation of nutrient cycling by herbivores is difficult to support experimentally, the hypothesis that herbivores may at least influence ecosystem function is receiving renewed attention (Belovsky & Slade 2000;Hunter 2001) based on field observations (Swank *et al.* 1981;Reynolds & Crossley 1997;Reynolds *et al.* 2000;Reynolds 2000). In the absence of large-scale dispersal, insect herbivores do not directly import or export N from terrestrial systems. Rather, their influence on forest N cycling results from the indirect effects of their byproducts and/or changes in plant physiology/demography that result from their activity (Figure 1.2). For example, Hunter (2001) recently reviewed seven general ways in which phytophagous insects can affect forest nutrient cycling: (i) defoliator-mediated changes in the chemistry of precipitation, (ii) inputs of insect cadavers, (iii) effects upon root exudation and root/mutualist interactions, (iv) changes in nutrient uptake by the plant community, (v) changes in the quality and quantity of litter inputs, (vi) deposition of frass, and (vii) effects upon canopy

structure and subsequent microclimate. The first four and (vi) roughly correspond to what McNaughton *et al.* (1988) termed "fast" cycle dynamics in African savannas. The inputs following these herbivore-mediated changes are primarily labile and result in immediate, or "fast," rates of N turnover. In contrast, leaf litter decomposition is a "slow" cycle process that releases nutrients over the course of months to years depending on the conditions of the terrestrial systems (McNaughton *et al.* 1988). Herbivores may influence both fast and slow cycle N dynamics, though the mechanisms may be very different. For this review, the changes to canopy structure following herbivory are omitted, and throughfall and cadaver inputs are only treated briefly (but see Hunter (2001) for more detail).

Throughfall

Foliage damaged by herbivores is susceptible to N (Stadler *et al.* 2001) and C (Stadler & Michalzik 1998a) losses during rainfall events. Such changes in throughfall can influence soil micro-organisms (Stadler & Michalzik 1998b) and meso-fauna (Reynolds *et al.* 2003). However, such increases in N and C in throughfall are not always observed (Hollinger 1986), and it appears that throughfall changes may be system-dependent (Schowalter 2000).

Insect Cadavers

Generally a small amount of research has been dedicated to the impact of insect cadavers in soil processes, which is probably a result of the small amount of input

cadavers make in terms of biomass and nutrient additions (Fogal & Slansky Jr 1984). Nonetheless, insect cadavers per unit biomass are N-rich, accumulate on an annual basis, and may be an important source of N on a localized scale.

Effects on Roots, Root Exudation, and Plant Nutrient Uptake

Plant-mediated links between aboveground and belowground processes in terrestrial ecosystems have received considerable research focus in the last few years (Wardle 2002). Much of the focus has been on grasses (Frank & Groffman 1998;Hamilton & Frank 2001;Mikola *et al.* 2001a;Mikola *et al.* 2001b) and agricultural crops (Holland 1995;Holland *et al.* 1996). There are four potential mechanisms that might, independently or interactively, contribute to belowground C and N fluxes following aboveground herbivory in the absence of surface soil inputs: (i) root turnover, (ii) increased root growth, (iii) increased root activity (independent of growth and rhizodeposition), and (iv) increased root rhizodeposition. Each mechanism has unique implications for soil C and N dynamics, and for developing a predictive framework for ecosystem-wide responses to foliar herbivory.

Root turnover and resulting microbially-mediated decomposition can occur when herbivory hinders a plant's ability to sustain fine root biomass; roots die and are colonized by microbial decomposers. Such a mechanism was demonstrated following mammalian browsing in the Alaskan taiga (Ruess *et al.* 1998), though not after 50% defoliation in graminoid microcosms (Mikola *et al.* 2000). Alternatively, aboveground damage might stimulate root growth, exploration, and biomass accumulation (Ritchie *et*

al. 1998). Increased root activity can alter soil respiration and N dynamics without adding root biomass. For example, roots can actively increase kinetic rates of nutrient uptake. Ion transport is an energetic process (Clarkson 1985), with nitrate assimilation estimated at 20% of NPP (Bloom *et al.* 1992). Finally, rhizodeposition of C-rich compounds can provide a substrate for microbial activity that can result in a positive feedback of N mineralization and subsequent plant uptake in nutrient rich soils (Hamilton & Frank 2001). Carbon allocation to rhizodeposition in oaks is linked to increased rhizosphere microbial biomass and soil N mineralization (Cardon *et al.* 2002), but the impact of aboveground herbivory has not been studied. However, stimulation of rhizodeposition by foliar herbivory is common among grasses, particularly those that suffer heavy grazing damage by ungulate herbivore populations (McNaughton *et al.* 1988;Frank & Groffman 1998). Aboveground biomass is stimulated in these grass communities following such herbivory (Frank & McNaughton 1993), in part due to increased rhizodeposition that stimulates soil nutrient mineralization. Hamilton & Frank (2001) argue that the benefit of C allocation to rhizodeposition is increased nutrient availability, suggesting facilitation between grasses and their rhizosphere micro-organisms. In fact, all four mechanisms result in changes in soil N distribution.

Foliar Quality and Litter Inputs

Most forest trees change the quality of their foliage in response to herbivores (Schultz & Baldwin 1982;Rossiter *et al.* 1988), and substantial reviews on the theories of plant defense (Stamp 2003;Gershenzon 1994;Hamilton *et al.* 2001) and meta-analyses

(Nykanen & Koricheva 2004;Koricheva *et al.* 2004) already exist. The changes, which include increasing tannin concentrations and lowering foliar N concentrations, lower the quality of foliage for the herbivore. The relevance of these chemical changes for this review is the influence that they may have on the process of leaf litter decomposition (Choudhury 1988;Findlay *et al.* 1996).

Herbivore activity can either "accelerate" or "decelerate" leaf litter decomposition depending on the types of chemical changes induced by the feeding (Ritchie *et al.* 1998). For example, tannin induction, while affecting leaf palatability for herbivores, may also decelerate nutrient loss from the resulting leaf litter (Hättenschwiler & Vitousek 2000), either by the inhibitory effect of the tannins themselves (Northup *et al.* 1998) or by the change in C:N stoichiometry they may produce (Magill & Aber 2000). Ritchie *et al.* (1998) showed that herbivores in an oak savanna can decelerate decomposition via their effects on plant community composition by reducing the abundance of plant species with high foliar quality.

Chapman *et al.* (2003) found that herbivores accelerated decomposition via increased litter N contents (and decreased C:N and Lignin:N ratios) in a *Pinus edulis* system. In this case, the effect resulted from herbivore-mediated acceleration of litter fall and incomplete nutrient resorption following premature leaf abscission. Hunter (2001) also suggested that resorption proficiency (Killingbeck 1996) could be significantly reduced following herbivory, which should increase litter N and accelerate decomposition. Whether the difference in resorbed N is sufficient to alter the quality of the litter may be a function of the C:N ratio, lignin:N ratio, or the tannin:N ratio.

Herbivore-induced phytochemical induction is also influenced by nutrient availability (Glynn *et al.* 2003). Hunter & Schultz (1995) found that chemical fertilization mitigated the induction of tannins in oak foliage. Insect herbivore densities were skewed away from damaged leaves in unfertilized trees, which led the authors to support the hypothesis that nutrient availability affected phytochemical induction and herbivore preference. Nutrient availability may also influence rates of litter decomposition.

Frass Deposition

The most direct method by which herbivores influence N dynamics is through frass (feces) deposition. Substantial quantities of frass can be deposited on the forest floor by insect herbivores (Grace 1986), and can actually exceed the mass of leaf litter. For example, Fogal & Slansky (1984) found that 804 and 1255 kg/ha of frass were deposited on two 25-year-old Scots pine plantations compared to 1107 and 929 kg/ha of litterfall. Nitrogen returned to the soil by frass can even double overall rates of return of N from plants to soil (Hollinger 1986).

Lovett & Ruesink (1995) determined that 94.5% of gypsy moth frass is organic matter and, of that, approximately 45% is C. As mentioned above, the biological transformation of N (e.g., immobilization) under aerobic conditions is mediated by carbon availability (Hart *et al.* 1994); frass C may be an immediate source for microbial respiration, which could increase the rate of N-immobilization. As this source of carbon is depleted, the microbial efficiency may decrease and previously accumulated N may be

released in latent pulses sometime after its initial introduction to the forest floor. While Lovett & Ruesink (1995) found that frass treatments representative of an insect outbreak resulted in increased microbial respiration, Reynolds (2000) found that field additions of endemic levels of frass did not significantly increase soil respiration. It appears that additions of frass that correspond to insect outbreaks may be required to see a quantifiable change in rates of soil respiration and N-mineralization.

Literature investigating the role of frass in nutrient cycling is scarce, and there are likely a number of reasons for its absence. Because phytophagous insects are an intermediate trophic level, their populations are thought to be controlled by both top-down (Hairston *et al.* 1960;Hunter & Price 1992) and bottom-up (Power 1992;Hunter & Price 1992;Hunter *et al.* 1997) forces. Population densities below carrying capacity should result in a fairly consistent and small annual frass production relative to other inputs of N in forest ecosystems in terms of biomass. For example, Risley & Crossley (1988) found that around 4 g m^{-2} yr^{-1} of frass was produced under non-outbreak conditions on four sites at the Coweeta Hydrologic Laboratory, compared to 300 g m^{-2} yr^{-1} of leaf litter on the same site. The difference between gross frass and litterfall biomass reaching the forest floor at Coweeta was two orders of magnitude. This frass production is comparable to, though slightly higher than, frass production of 2.4 g m^{-2} yr^{-1} at Coweeta during a different year (Risley 1986) and 2.6-2.9 g m^{-2} yr^{-1} in a British woodland (Carlisle et al. 1966). Further, Lovett & Ruesink (1995) determined that N comprised 2.4% of gypsy moth frass under laboratory conditions and 1.5% under field conditions in a hardwood forest in upstate New York. This means that frass inputs under non-outbreak

conditions may provide 0.096 g m^{-2} yr^{-1} N. The C:N ratio of *Periclista sp.* frass in a northern hardwood forest at high elevation in western North Carolina was approximately 25 (Hunter *et al.* 2003), which is on the N immobilization/mineralization border.

Foliar N is variable among plant species and also within species under different conditions. An estimate, for the purposes of comparing foliar to frass N, is that roughly 2% of leaf mass is N. Assuming that 50-75% of leaf N is resorbed before abscission under non-outbreak conditions (Killingbeck 1996;Hunter 2001) the amount of N deposited on the forest floor in leaf litter is about 1-1.5 g m^{-2} yr^{-1}. These calculations indicate a substantial difference in the gross amount of N introduced to the soil from foliage compared to frass, so it may be easy to dismiss the contribution of frass N based on gross input alone. However, a recent field experiment found that, during an outbreak event, soil and stream NO_3^- concentrations increased significantly in an herbivore-affected watershed (Reynolds 2000).

Frass decomposes much faster than leaf litter and, because of its relatively low C:N ratio, is thought to have a fairly labile pool of N. Therefore, frass N may act similarly to labile NO_3^- or NH_4^+ in previous studies mimicking human-mediated atmospheric deposition (e.g., (Groffmann *et al.* 1993;Zogg *et al.* 2000). As such, we would expect microbes to be efficient competitors for frass N in the presence or absence of plants. Lovett & Ruesink (1995) demonstrated, in a soil microcosm experiment, that essentially all labile frass N is rapidly immobilized by the microbial biomass, and this result was reproduced in a field experiment in a northern hardwood forest in upstate New York (Christenson *et al.* 2002). It might further be expected that the microbial

population will deplete labile C during respiration, while suffering predation (e.g., collembola, nematodes, protozoa). Both processes may release N in the form of dissolved organic N (DON), which can undergo mineralization to NH_4^+ and nitrification via chemoautotropic nitrifiers (e.g., *Nitrosomonas sp.* and *Nitrobacter sp.*). Nitrification releases NO_3^-, which may be taken up by plants or surviving microbes, denitrified, or leached from the system (Zeller *et al.* 2000). This process may occur at some point after the initial frass deposition. Reynolds *et al.* (2000) reported that soil nitrate concentrations increased one month following a *Periclista* sp. outbreak at the Coweeta Hydrologic Laboratory. In addition, NO_3^- concentrations from stream samples that drained the defoliated catchment basin showed a latent "pulse" during a period 1-3 months following the defoliation period. In another study at Coweeta, Swank *et al.* (1981) also found that nitrate export from a defoliated forest increased following an insect outbreak with a latency period. Neither study identified an explicit mechanism, though they do suggest that insect herbivores can have demonstrable effects on N cycling in a forest ecosystem.

The observations of NO_3^- pulses following herbivory may only be the beginning of the story. For example, Seely & Lajtha (1997) found that the majority of N collected in tension lysimeters following experimental deposition of NH_4^+ occurred during the first two days and was in the form of DON rather than NO_3^-. The source of the DON pulse is not known, but no DON measurements were taken in the studies at Coweeta, opening the possibility that the story between frass and N dynamics is even richer.

CONCLUSIONS

Nitrogen and C dynamics are a fundamental component of ecosystem functioning, and the processes that control their cycles are influenced by microbial, plant, and herbivore populations. However, the degree to and mechanisms by which herbivores influence the processes are not well understood. This dissertation addressed the mechanisms underlying herbivore effects on forest N and C dynamics. Because we were exploring mechanisms to explain previous field observations, the majority of the research was conducted in mesocosms. The mesocosm experimental designs, while in some sense artificial, allowed us to increase replication and control experimental manipulations.

I conducted a set of field, mesocosm, and microcosm experiments to explore the patterns described above. Chapter 2 reports the results of a one-year experiment designed to test the hypothesis that frass deposition would result in N export (Figure 1.2E). We manipulated potted *Quercus rubra* mesocosms with soil from the Coweeta Long-Term Ecological Research (LTER) site in a factorial experimental design that crossed frass additions with varying levels of herbivore damage. Chapter 3 expands upon the mesocosm study from Chapter 2 and reports damage- and frass-mediated changes in foliage and litter chemistry and subsequent effects on decomposition. Frass deposition may essentially "fertilize" trees similar to inorganic deposition (Hunter & Schultz 1995;Glynn *et al.* 2003), leading to the hypothesis that frass may mitigate herbivore-mediated chemical changes in oaks and subsequently alter rates of leaf litter decomposition (Figure 1.2D). Chapter 4 describes a two-year experiment to trace the fate of frass N using ^{15}N-labeled frass. This experiment used a stable isotope tracer to

measure the distribution of frass N in a soil-oak system and the feedback between herbivore activity and N recovery in oak foliage (Figure 1.2C&E). We hypothesized that soils would be strong sinks for frass N, though substantial frass N would be lost via leaching. Chapter 5 describes a short-term, dual-isotope microcosm experiment designed to test the hypothesis that aboveground herbivory on oak seedlings would increase C allocation into roots and rhizosphere soils and affect N uptake (Figure 1.2C). Chapter 6 draws some general conclusions from the results of the previous chapters and suggests directions for future research.

REFERENCES

Bardgett R.D., Streeter T.C. & Bol R. (2003) Soil microbes compete effectively with plants for organic-nitrogen inputs to temperate grasslands. *Ecology* 84, 1277-1287

Barton L., McLay C.D.A., Schipper L.A. & Smith C.T. (1999) Annual denitrification rates in agricultural and forest soils: a review. *Australian Journal of Soil Research* 37, 1073-1093

Belovsky G.E. & Slade J.B. (2000) Insect herbivory accelerates nutrient cycling and increases plant production. *Proc Natl Acad Sci (USA)* 97, 14412-14417

Berntson G.M. & Aber J.D. (2000) Fast nitrate immobilization in N saturated temperate forest soils. *Soil Biology and Biochemistry* 32, 151-156

Bloom A.J., Sukrapanna S.S. & Warner R.L. (1992) Root respiration associated with ammonium and nitrate absorption and assimilation by barley. *Plant Physiology* 99, 1294-1301

Bossier J.M. & Fontvieille D. (1995) Biological characteristics of forest soils and seepage waters during simulated rainfalls of high intensity. *Soil Biology and Biochemistry* 27, 139-145

Cardon Z.G., Czaja A.D., Funk J.L. & Vitt P.L. (2002) Periodic carbon flushing to roots of *Quercus rubra* saplings affects soil respiration and rhizosphere microbial biomass. *Oecologia* 133, 215-223

Chapin F.S., III (1980) The Mineral Nutrition of Wild Plants. *Annual Review of Ecology and Systematics* 11, 233-260

Chapman S.K., Hart S.C., Cobb N.S., Whitham T.G. & Koch G.W. (2003) Insect herbivory increases litter quality and decomposition: An extension of the acceleration hypothesis. *Ecology* 84, 2867-2876

Cheng W., Zhang Q., Coleman D.C., Carroll R.C. & Hoffman C.A. (1996) Is available carbon limiting microbial respiration in the rhizosphere? *Soil Biology and Biochemistry* 28, 1283-1288

Chew R.M. (1974) Consumers as regulators of ecosystems: an alternative to energetics. *Ohio Journal of Science* 74, 359-370

Choudhury D. (1988) Herbivore induced changes in leaf-litter resource quality: a neglected aspect of herbivory in ecosystem nutrient dynamics. *Oikos* 51, 389-393

Christenson L.M., Lovett G.M., Mitchell M.J. & Groffman P.M. (2002) The fate of nitrogen in gypsy moth frass deposited to an oak forest floor. *Oecologia* 131, 444-452

Clarkson D.T. (1985) Factors affecting mineral nutrient acquisition by plants. *Annual Review of Plant Physiology* 36, 77-115

Coleman D.C. & Crossley D.A.Jr. (1996) *Fundamentals of Soil Ecology*. Academic Press, San Diego.

Eissenstat D.M. & Yanai R.D. (1997) The ecology of root lifespan. *Advances in Ecological Research* 27, 1-60

EPA. Clean Air Status and Trends Network (CASTNet). 1999 Annual Report. 2001. Research Triangle Park, NC, U.S. Environmental Protection Agency, Office of Air Quality Planning and Standards.

Farrar J., Hawes M., Jones D. & Lindow S. (2003) How roots control the flux of carbon to the rhizosphere. *Ecology* 84, 827-837

Findlay S., Carreiro M., Krischik V. & Jones C.G. (1996) Effects of damage to living plants on leaf litter quality. *Ecological Applications* 6, 269-275

Firestone M.K. & Davidson E.A. (1989) Microbiological basis of NO and N2O productions and consumption in soil. In: *Exchange of Trace Gases between Terrestrial*

Ecosystems and the Atmosphere (eds Andreae M.O. & Schimel D.S.), pp. 7-21. John Wiley & Sons, Chichester.

Fitzhugh R.D., Lovett G.M. & Venterea R.T. (2003) Biotic and abiotic immobilization of ammonium, nitrite, and nitrate in soils developed under different tree species in the Catskill Mountains, New York, USA. *Global Change Biology* 9, 1591-1601

Fogal W.H. & Slansky Jr F. (1984) Contribution of feeding by European pine sawfly larvae to litter production and element flux in Scots pine plantations. *Canadian Journal of Forest Research* 15, 484-487

Frank D.A. & Groffman P.M. (1998) Ungulate versus landscape control of soil C and N processes in grasslands of Yellowstone National Park. *Ecology* 79, 2229-2241

Frank D.A. & McNaughton S.J. (1993) Evidence for the promotion of aboveground grassland production by native large herbivores in Yellowstone National Park. *Oecologia* 96, 157-161

Gershenzon J. (1994) Metabolic costs of terpenoid accumulation in higher plants. *Journal of Chemical Ecology* 20, 1281-1328

Glynn C., Herms D.A., Egawa M., Hansen R. & Mattson W.J. (2003) Effects of nutrient availability on biomass allocation as well as constitutive and rapid induced herbivore resistance in poplar. *Oikos* 101, 385-397

Grace J.R. (1986) The influence of gypsy moth on the composition and nutrient content of litter fall in a Pennsylvania oak forest. *Forest Science* 32, 855-870

Groffman P.M. & Tiedje J.M. (1991) Relationships between denitrification, CO2 production and air-filled porosity in soils of different texture and drainage. *Soil Biology & Biochemistry* 23, 299-302

Groffmann P.M., Zak D.R., Christensen S., Mosier A. & Tiedje J.M. (1993) Early spring nitrogen dynamics in a temperate forest landscape. *Ecology* 74, 1579-1585

Hairston N.G., Smith F.E. & Slobodkin L.B. (1960) Community structure, population control, and competition. *American Naturalist* 94, 421-425

Hamilton E.W.I. & Frank D.A. (2001) Can plants stimulate soil microbes and their own nutrient supply? Evidence from a grazing tolerant grass. *Ecology* 82, 2397-2404

Hamilton J.G., Zangerl A.R., DeLucia E.H. & Berenbaum M.R. (2001) The carbon-nutrient balance hypothesis: its rise and fall. *Ecology Letters* 4, 86-95

Hart S.C., Nason G.E., Myrold D.D. & Perry D.A. (1994) Dynamics of gross nitrogen transformation in an old-growth forest: the carbon connection. *Ecology* 75, 880-891

Hättenschwiler S. & Vitousek P.M. (2000) The role of polyphenols in terrestrial ecosystem nutrient cycling. *Trends in Ecology and Evolution* 15, 238-243

Holland J.N. (1995) Effects of above-ground herbivory on soil microbial biomass in conventional and no-tillage agroecosystems. *Appied Soil Ecology* 2, 275-279

Holland J.N., Cheng W. & Crossley D.A.Jr. (1996) Herbivore-induced changes in plant carbon allocation: assessment of below-ground C fluxes using carbon-14. *Oecologia* 107, 87-94

Hollinger D.Y. (1986) Herbivory and the cycling of nitrogen and phosphorous in isolated California oak trees. *Oecologia* 70, 291-297

Hunter M.D. (2001) Insect population dynamics meets ecosystem ecology: effects of herbivory on soil nutrient dynamics. *Agricultural and Forest Entomology* 3, 77-84

Hunter M.D., Linnen C.R. & Reynolds B.C. (2003) Effects of endemic densities of canopy herbivores on nutrient dynamics along a gradient in elevation in the southern appalachians. *Pedobiologia* 47, 231-244

Hunter M.D. & Price P.W. (1992) Playing chutes and ladders: heterogeneity and the relative roles of bottom-up and top-down forces in natural communities. *Ecology* 73, 724-732

Hunter M.D. & Schultz J.C. (1995) Fertilization mitigates chemical induction and herbivore responses within damaged oak trees. *Ecology* 76, 1226-1232

Hunter M.D., Varley G.C. & Gradwell G.R. (1997) Estimating the relative roles of top-down and bottom-up forces on insect herbivore populations: a classic study revisited. *Proceedings of the National Academy of Sciences (USA)* 94, 9176-9181

Jones D.L. & Darrah P.R. (1994) Amino acid influx at the soil-root interface of *Zea mays* L. and its implications in the rhizosphere. *Plant and Soil* 163, 1-12

Keeling C.D., Chin J.F.S. & Whorf T.P. (1996) Increased activity of northern vegetation inferred from atmospheric CO_2 measurements. *Nature* 382, 146-149

Kielland K. (1994) Amino acid absorption by artic plants: implications for plant nutrition and nitrogen cycling. *Ecology* 75, 2373-2383

Killingbeck K.T. (1996) Nutrients in senesced leaves: keys to the search for potential resportion and resorption proficiency. *Ecology* 77, 1716-1727

Knops J.M.H., Bradley K.L. & Wedin D.A. (2002) Mechanisms of plant species impacts on ecosystem nitrogen cycling. *Ecology Letters* 5, 454-466

Koricheva J., Nykanen H. & Gianoli E. (2004) Meta-analysis of trade-offs among plant antiherbivore defenses: Are plants jacks-of-all-trades, masters of all? *American Naturalist* 163, E64-E75

Kuzyakov Y. & Cheng W. (2001) Photosynthesis controls of rhizosphere respiration and organic matter decomposition. *Soil Biology and Biochemistry* 33, 1915-1925

Lipson D. & Näsholm T. (2001) The unexpected versatility of plants: organic nitrogen use and availability in terrestrial ecosystems. *Oecologia* 128, 305-316

Lipson D.A., Raab T.K., Schmidt S. & Monson R.K. (2002) An empirical model of amino acid transformations in an alpine soil. *Soil Biology and Biochemistry* 33, 189-198

Lovett G.M., Weathers K.C., Arthur M.A. & Schultz J.C. (2004) Nitrogen cycling in a northern hardwood forest: Do species matter? *Biogeochemistry* 67, 289-308

Lovett G.M., Christenson L.M., Groffman P.M., Jones C.G., Hart J.E. & Mitchell M.J. (2002) Insect defolation and nitrogen cycling in forests. *BioScience* 52, 335-341

Lovett G.M. & Ruesink A.E. (1995) Carbon and nitrogen mineralization from decomposing gypsy moth frass. *Oecologia* 104, 133-138

Lovett G.M. & Tobiessen P. (1993) Carbon and nitrogen assimilation in red oaks (*Quercus rubra* L.) subject to defoliation and nitrogen stress. *Tree Physiology* 12, 259-269

Magill A.H. & Aber J.D. (2000) Dissolved organic carbon and nitrogen relationships in forest litter as affected by nitrogen deposition. *Soil Biology and Biochemistry* 32, 603-613

Mahendra M.K. & Ogden E.D. (1973) Patterns of ammonia volatilization from a forest soil. *Plant and Soil* 38, 257-265

McNaughton S.J., Ruess R.W. & Seagle S.W. (1988) Large mammals and process dynamics in African ecosystems. *BioScience* 38, 794-800

Miettinen K.J. Assimilation of amino acids in higher plants. 210-230. 2001. New York, Academic Press. Symposium of the Society of Experimental Biology #13.
Ref Type: Conference Proceeding

Mikola J., Barker G.M. & Wardle D.A. (2000) Linking above-ground and below-ground effects in autotrophic microcosms: effects of shading and defoliation on plant and soil properties. *Oikos* 89, 577-587

Mikola J., Yeates G.W., Barker G.M., Wardle D.A. & Bonner K.I. (2001a) Effects of defoliation intensity on soil food-web properties in an experimental grassland community. *Oikos* 92, 333-343

Mikola J., Yeates G.W., Barker G.M., Wardle D.A. & Bonner K.I. (2001b) Response of soil food-web structure to defoliation of different plant species combinations in an experimental grassland community. *Soil Biology and Biochemistry* 33, 205-214

Nadelhoffer K., Downs M., Fry B., Magill A. & Aber J. (1999a) Controls on N retention and exports in a forested watershed. *Environmental Monitoring and Assessment* 55, 187-210

Nadelhoffer K.J., Downs M.R. & Fry B. (1999b) Sinks for N-15-enriched additions to an oak forest and a red pine plantation. *Ecological Applications* 9, 72-86

Näsholm T., Huss-Danell K. & Högberg P. (2000) Uptake of organic nitrogen by four agriculturally important plant species. *Ecology* 81, 1155-1161

Northup R.R., Dahlgren R.A. & McColl J.G. (1998) Polyphenols as regulators of plant-litter-soil interactions in northern California's pygmy forest: a positive feedback? *Biogeochemistry* 42, 189-220

Nykanen H. & Koricheva J. (2004) Damage-induced changes in woody plants and their effects on insect herbivore performance: a meta-analysis. *Oikos* 104, 247-268

Odum E.P. (1969) The strategy of ecosystem development. *Science* 164, 262-270

Ollinger S.V., Aber J.D., Lovett G.M., Millham S.E., Lathrop R.G. & Ellis J.M. (1993) A spatial model of atmospheric deposition for the northeastern U.S. *Ecological Applications* 3, 459-472

Power M.E. (1992) Top-down and bottom-up forces in food webs: do plants have primacy. *Ecology* 73, 733-746

Raab T.K., Lipson D. & Monson R.K. (1999) Soil amino acid utilization among the Cyperaceae: plant and soil processes. *Ecology* 80, 2048-2419

Rajaniemi T.K. & Reynolds H.L. (2004) Root foraging for patchy resources in eight herbaceous plant species. *Oecologia* 141, 519-525

Reich P.B., Walters M.B. & Ellsworth D.S. (1997) From tropics to tundra: Global convergence in plant functioning. *Proceedings of the National Academy of Sciences (USA)* 94, 13730-13734

Reynolds B.C. & Crossley D.A.Jr. (1997) Spatial variation in herbivory by forest canopy arthropods along and elevation gradient. *Environmental Entomology* 26, 1232-1238

Reynolds B.C., Crossley D.A.Jr. & Hunter M.D. (2003) Response of soil invertebrates to forest canopy inputs along a productivity gradient. *Pedobiologia* 47, 1-13

Reynolds B.C., Hunter M.D. & Crossley D.A.Jr. (2000) Effects of canopy herbivory on nutrient cycling in a northern hardwood forest in western North Carolina. *Selbyana* 21, 74-78

Risley L.S. & Crossley D.A.Jr. (1988) Herbivore-caused greenfall in the southern Appalachians. *Ecology* 69, 1118-1127

Ritchie M.E., Tilman D. & Knops J.M.H. (1998) Herbivore effects on plant and nitrogen dynamics in oak savanna. *Ecology* 79, 165-177

Rodrigo A., Recous S., Neel C. & Mary B. (1997) Modelling temperature and moisture effects on C-N transformations in soils: comparison of nine models. *Ecological Modelling* 102, 325-339

Rossiter M., Schultz J.C. & Baldwin I.T. (1988) Relationships among defoliation, red oak phenolics, and gypsy moth growth and reproduction. *Ecology* 69, 267-277

Ruess R.W., Hendrick R.L. & Bryant J.P. (1998) Regulation of fine root dynamics by mammalian browsers in early successional Alaskan taiga forests. *Ecology* 79, 2706-2720

Schmidt S. & Stewart G.R. (1999) Glycine metabolism by plant roots and its occurence in Australian plant communities. *Australian Journal of Plant Physiology* 26, 253-264

Schowalter T.D. (2000) *Insect Ecology: An Ecosystem Approach*. Academic Press, San Diego.

Schowalter T.D., Sabin T.E., Stafford S.G. & Sexton J.M. (1991) Phytophage effects on primary production, nutrient turnover, and litter decomposition of young Douglas-fir in western Oregon. *Forest Ecology and Management* 42, 229-243

Schultz J.C. & Baldwin I.T. (1982) Oak leaf quality declines in reponse to defoliation by gypsy moth larvae. *Science* 217, 149-151

Seely B. & Lajtha K. (1997) Application of a 15N tracer to simulate and track the fate of atmospherically deposited N in the coastal forests of the Waquoit Bay Watershed, Cape Cod, Massachusetts. *Oecologia* 112, 393-402

Senwo Z.N. & Tabatabai M.A. (2001) Amino acid composition of soil organic matter. *Biol Fertil Soils* 26, 235-242

Stadler B. & Michalzik B. (1998a) Aphid infested Norway spruce are "hot spots" in throughfall carbon chemistry in coniferous forests. *Canadian Journal of Forest Research* 28, 1717-1722

Stadler B. & Michalzik B. (1998b) Linking aphid honeydew, throughfall, and forest floor solution chemistry of Norway spruce. *Ecology Letters* 1, 13-16

Stadler B., Solinger S. & Michalzik B. (2001) Insect herbivores and the nutrient flow from the canopy to the soil in coniferous and deciduous forests. *Oecologia* 126, 104-113

Stamp N. (2003) Out of the quagmire of plant defense hypotheses. *Quarterly Review of Biology* 78, 23-55

Swank W.T., Waide J.B., Crossley D.A.Jr. & Todd R.L. (1981) Insect defoliation enhances nitrate export from forest ecosystems. *Oecologia* 51, 297-299

Templer P., Findlay S. & Lovett G.M. (2003) Soil microbial biomass and nitrogen transformations among five tree species of the Catskill Mountains, New York, USA. *Soil Biology and Biochemistry* 35, 607-613

Verchot L.V., Holmes Z., Mulon L., Groffman P.M. & Lovett G.M. (2001) Gross vs net rates of N mineralization and nitrification as indicators of functional differences between forest types. *Soil Biology and Biochemistry* 33, 1889-1901

Virtanen A.I. & Linkola H. (1946) Organic nitrogen compounds as nitrogen nutrition for higher plants. *Nature* 157, 515

Vitousek P.M. & Matson P.A. (1985) Disturbance, nitrogen availability, and nitrogen loss in an intensively managed loblolly pine plantation. *Ecology* 66, 1360-1376

Walvoord M.A., Phillips F.M., Stonestrom D.A., Evans R.D., Hartsough P.C., Newman B.D. & Striegl R.G. (2003) A Reservoir of Nitrate Beneath Desert Soils. *Science* 302, 1021-1024

Wardle D.A. (2002) *Communities and Ecosystems: Linking the Aboveground and Belowground Components*. Princeton University Press, Princeton.

Wilson E.O. (1987) The little things that run the world (the importance and conservation of invertebrates). *Conservation Biology* 1, 344-346

Yamagata M. & Ae N. (1996) Nitrogen uptake response of crops to organic nitrogen. *Soil Science and Plant Nutrition* 42, 389-394

Zak D.R., Groffman P.M., Pregitzer K.S., Christensen S. & Tiedie J.M. (1990) The vernal dam: plant-microbe competition for nitrogen in northern hardwood forests. *Ecology* 71, 651-656

Zeller B., Colin-Belgrand M., Dambrine E., Martin F. & Bottner P. (2000) Decomposition of 15N-labeled beech litter and fate of nitrogen derived from litter in a beech forest. *Oecologia* 123, 550-559

Zogg G.P., Zak D.R., Pregitzer K.S. & Burton A.J. (2000) Microbial immobilization and the retention of anthropogenic nitrate in a northern hardwood forest. *Ecology* 81, 1858-1866

Figure 1.1 A Simplified Schematic of the Terrestrial Nitrogen Cycle. The gray box contains the terrestrial ecosystem, boxes outside represent either inputs or exports from the system.

Figure 1.1

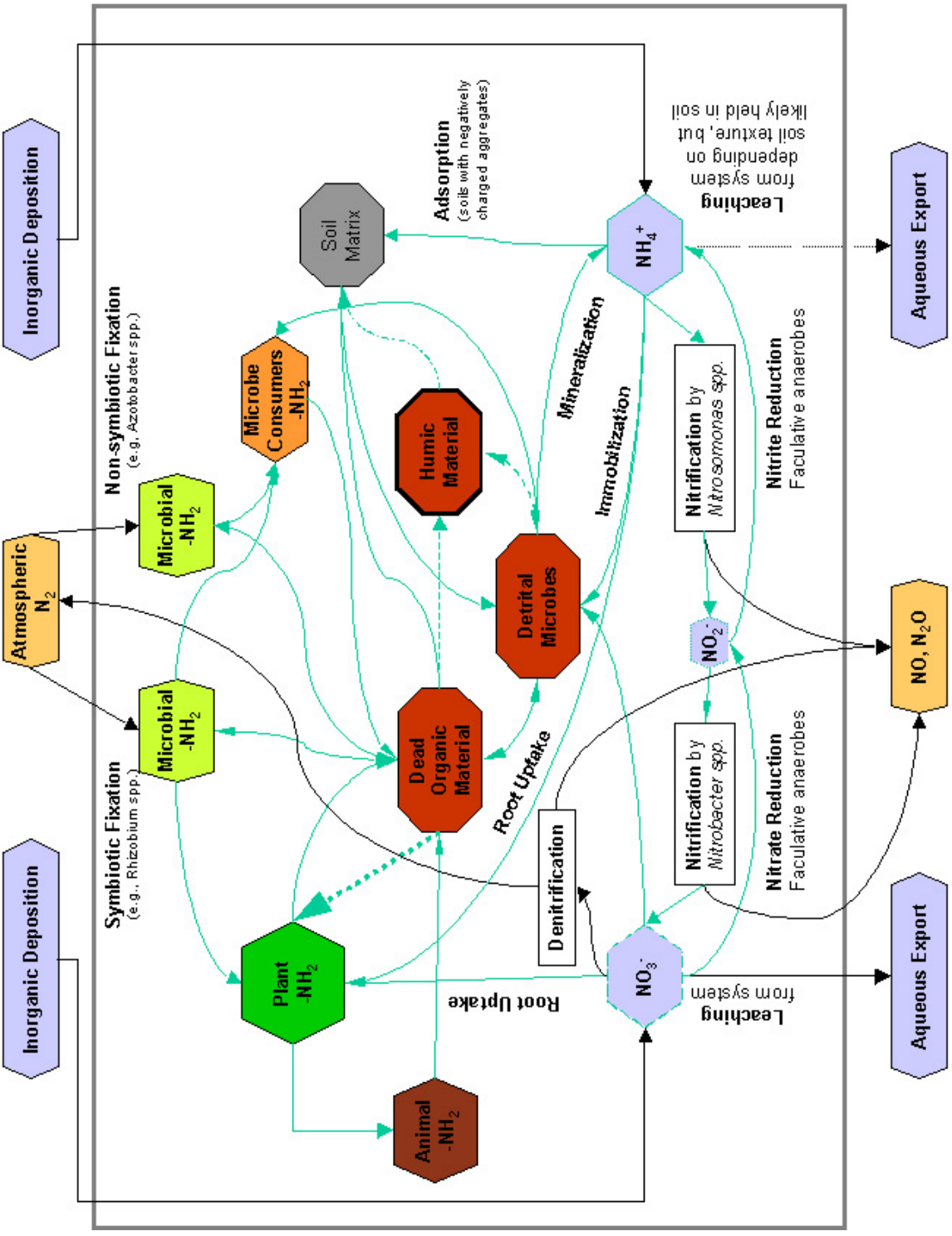
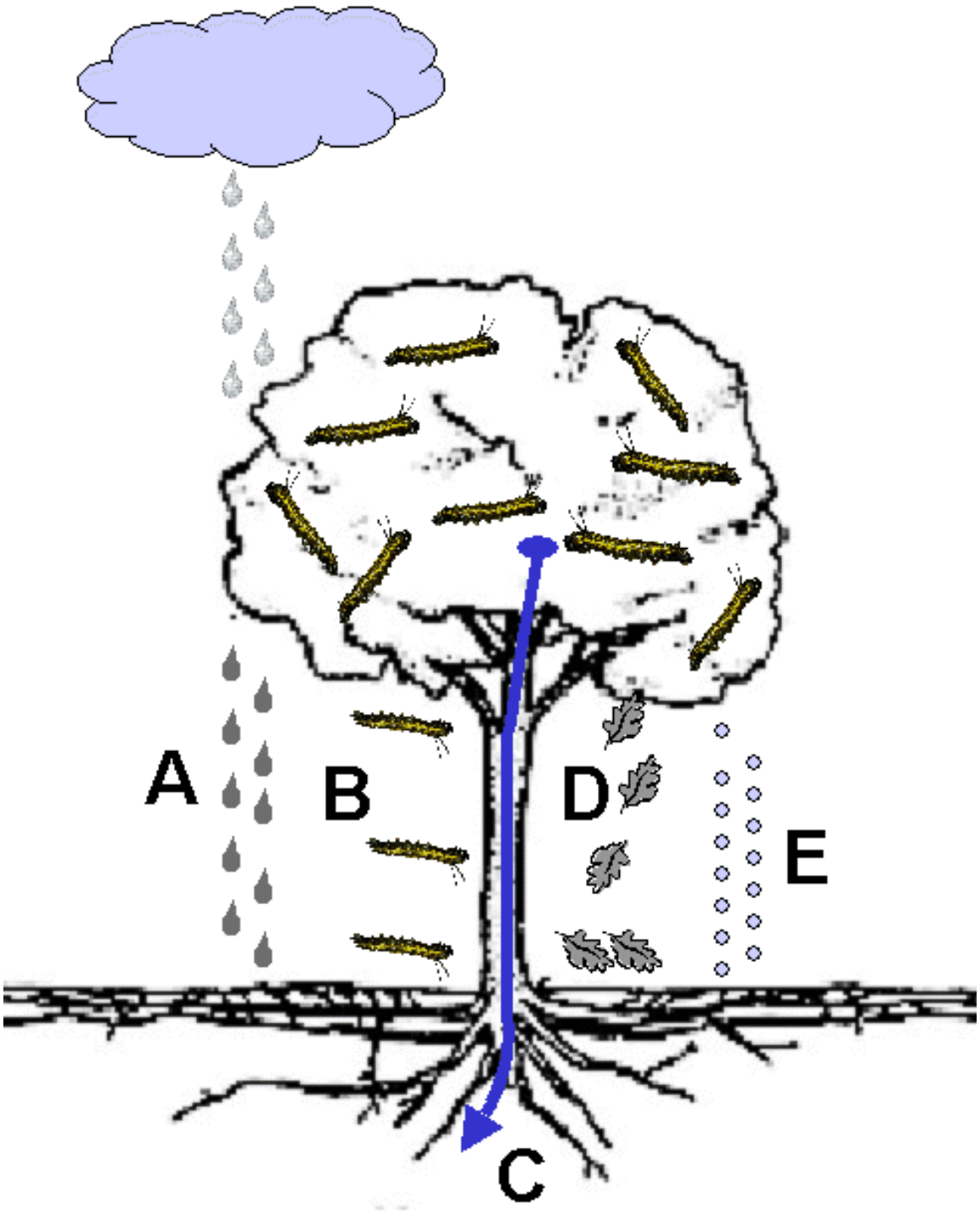

Inorganic Deposition
Inorganic Deposition
Atmospheric N_2
Non-symbiotic Fixation
(e.g. Azotobacter spp.)
Symbiotic Fixation
(e.g., Rhizobium spp.)
Soil Matrix
Adsorption
(soils with negatively charged aggregates)
Microbe Consumers -NH_2
Humic Material
Microbial -NH_2
Microbial -NH_2
Detrital Microbes
Dead Organic Material
Plant -NH_2
Animal -NH_2
Root Uptake
Root Uptake
Mineralization
Immobilization
Denitrification
NH_4^+
NO_2^-
NO_3^-
Leaching from system depending on soil texture, but likely held in soil
Leaching from system
Nitrification by *Nitrosomonas spp.*
Nitrite Reduction
Facultative anaerobes
Nitrification by *Nitrobacter spp.*
Nitrate Reduction
Facultative anaerobes
Aqueous Export
Aqueous Export
NO, N_2O

Figure 1.2 Herbivore Inputs to the Terrestrial Nitrogen Cycle. Insect herbivores cause a number of disturbances to the N cycle: A) Changes in throughfall chemistry; B) deposition of insect cadavers; C) Plant-mediated responses that alter root growth and activity, rhizodeposition, and N uptake; D) changes in the quality of litter fall; E) deposition of fecal pellets (frass).

Figure 1.2



CHAPTER 2

INSECT CANOPY HERBIVORY AND FRASS DEPOSITION AFFECT SOIL NUTRIENT DYNAMICS AND EXPORT IN OAK MESOCOSMS [1]

[1] Frost, C.J. and M.D. Hunter. 2004. Ecology 85(12):3335-3347

ABSTRACT

Increased nitrogen (N) mobilization and export from terrestrial forest ecosystems following canopy herbivory have been well documented, though the mechanism behind the loss is not clear. Because carbon (C) and N dynamics are closely linked, herbivore activity may also affect C distribution. We initiated a replicated mini-ecosystem experiment to test the hypothesis that insect frass (feces) influences soil C and N dynamics following insect defoliation. One hundred and sixty red oak (*Quercus rubra*) saplings were transplanted to seven-gallon (26.5-L) pots with soil and litter from the Coweeta Hydrologic Laboratory (CWT) (Otto, North Carolina, USA) and overwintered in experimental pot stands. During the 2002 growing season, trees were subjected to a 3 x 2 factorial experimental design with three damage groups (herbivore, mechanical, "undamaged") and two frass depositions (frass, no frass).

Frass deposition increased soil total C, total N, and the soil NH_4^+ pool. Leachate NO_3^- export also increased following frass additions. We suggest that herbivore frass mobilizes sufficient C and N to affect soil pools and N export, though abiotic factors may influence the ultimate fate of the nutrients in frass. In addition, herbivory increased soil respiration and decreased total soil N relative to "undamaged" controls independent of frass deposition. While we discuss four possible mechanisms for this observation, we hypothesize that the increased soil respiration results from enhanced root-exudate C and subsequent microbial oxidation. This mechanism has implications for C sequestration and N retention in forest soils. In addition, the effects of mechanical damage consistently did not match those of real herbivory, suggesting that differential responses of *Q. rubra* to

damage types also may affect soil nutrient dynamics. Our results demonstrate that the feeding activity of insect herbivores can have direct and indirect effects on the cycling of C and N within the season of defoliation.

Keywords: ammonium, carbon, dissolved organic nitrogen, frass, herbivory, microbial biomass, nitrate, nitrogen cycling, *Quercus rubra*, real vs. simulated herbivory, red oak, soil respiration

INTRODUCTION

In terrestrial ecosystems, aboveground and belowground processes are fundamentally linked by the availability and flow of energy and nutrients (Swift et al. 1979, Wardle 2002). Carbon (C) and nitrogen (N) have received considerable attention, and the dynamics of their distributions are tightly coupled (BassiriRad et al. 2003). Any disturbance that alters the retention and flow of C and/or N in a terrestrial ecosystem can affect the feedback mechanisms between the above- and belowground subsystems. The activity of insect canopy herbivores can alter nutrient dynamics between above- and belowground subsystems (Bardgett et al. 1998, Bardgett and Wardle 2003), and thus may affect the dynamics of the feedbacks between the two (Holland et al. 1996).

The idea that herbivores may affect ecosystem dynamics is not new (Chew 1974), and has received renewed attention (Schowalter et al. 1991). However, the effects of herbivory on ecosystem processes in forests have often been considered inconsequential. Herbivore population densities in forests are often kept low by the activity of natural enemies (Hairston et al. 1960, Slobodkin et al. 1967), the low nutritional quality of the foliage (Haukioja et al. 1985a, Haukioja et al. 1985b, Herms and Mattson 1992, Zvereva et al. 1997, Glynn et al. 2003), or both (Hunter and Price 1992). Despite these forces, foliar herbivory has been linked to soil C and N dynamics. The effects of herbivore activity on soil processes have been most pronounced during outbreak events, where N export has been reported (Swank et al. 1981, Reynolds et al. 2000). However, recent evidence also suggests that even endemic levels of herbivory have significant effects on soil processes (Hunter et al. 2003). The loss of N from the terrestrial ecosystem

following herbivore activity, largely in the forms of aqueous NO_3^- and dissolved organic nitrogen (DON) (Townsend et al. 2004), disrupts the N cycle and may have aboveground repercussions in N-limited systems. Despite the apparent stimulation of N cycling and export following herbivory, only recently have efforts been made to evaluate the underlying mechanisms (Bardgett and Wardle 2003), particularly within forests (Ritchie et al. 1998, Lovett et al. 2002). Of the seven possible mechanisms by which insect herbivores can affect soil nutrient dynamics (Hunter 2001), five occur during the season of defoliation (the so-called "fast cycle," *sensu* McNaughton et al. (1988)): (i) frass (feces) deposition, (ii) turnover of insect cadavers, (iii) changes in throughfall chemistry, (iv) modified plant nutrient uptake rates, and (v) changes in root dynamics including rhizodeposition (exudation) and root-soil microbe interactions. The first three mechanisms are surface soil inputs, while the last two are plant-mediated, belowground responses to herbivory.

The composition of frass has made it an appealing candidate to explain the ecosystem-wide fluxes of C and N following herbivory. Frass is almost entirely organic material and is primarily a labile substrate (Lovett and Ruesink 1995). While frass deposition generally accounts for only 1-4% of annual N deposition in forests (Risley 1986, Risley and Crossley 1988, Hunter et al. 2003), nitrogen returned to soil in frass can exceed that of leaf litter during severe outbreaks (Fogal and Slansky 1984, Grace 1986, Hollinger 1986). Despite this, relatively little is known about the importance of insect frass in nutrient cycling (Bardgett and Wardle 2003), and the few studies explicitly discussing it have offered mixed results. Frass deposition has resulted in increased N

mineralization (Lightfoot and Whitford 1990, Reynolds et al. 2000), increased microbial immobilization of N in microcosm and field experiments (Lovett and Ruesink (1995) and Christenson et al. (2002), respectively), and no effect (Reynolds and Hunter 2001).

One interpretation for the inconsistent results is that the effects of canopy herbivory on soil nutrient dynamics may not be entirely explained by increased frass deposition. For example, plants respond to foliar herbivory with a host of species- and condition-specific defenses (Schultz and Baldwin 1982, Rossiter et al. 1988, Hunter and Schultz 1995), and these active defensive responses can extend belowground (Holland 1995, Holland et al. 1996, Ruess et al. 1998). It is known that rhizosphere processes are constrained by the input of photosynthetically-derived C (Cheng et al. 1996, Kuzyakov and Cheng 2001), and that these C inputs affect N dynamics (Knops et al. 2002). Thus, the quality and quantity of rhizodeposition in response to herbivore damage may interact with frass deposition to influence the fate of the C and N in the soil system.
In this context, one of the proposed mechanisms by which herbivore damage influences N dynamics and plant performance is through insect-specific signals including saliva (Dyer et al. 1995). To account specifically for the differences between herbivores and damage *per se*, our experiment manipulated both real and simulated (mechanical) damage.

We report here the results of a controlled, factorial experiment to isolate the effects of frass deposition and damage on soil C and N dynamics in a *Quercus rubra* mini-ecosystem. The unique aspect of our study is the ability to explore the effects of frass deposition on soil C and N dynamics independent of aboveground damage and the

effects of herbivore damage (real and simulated) on soil C and N dynamics independent of frass deposition. We tested the following hypotheses: 1) frass deposition increases soil mineral N availability; 2) frass deposition increases N export as NO_3^- and DON independent of damage; 3) frass deposition increases soil respiration independent of damage via microbial oxidation of frass C; 4) insect, but not mechanical, damage alters soil C and N pools independent of frass deposition, and 5) herbivore-induced belowground responses by oaks interact with frass deposition to influence soil C and N dynamics.

METHODS

Field Site

A field site was prepared in Autumn 2001 adjacent to the UGA Botany greenhouses (Athens, GA) using 160 nursery-grown *Quercus rubra* saplings (Forest Keeling Nursery, Missouri), transplanted into 7-gallon pots using soil and leaf litter from watershed 27 ("WS27") at the Coweeta Hydrologic Laboratory ("CWT," Otto, NC, elevation 1300 m). The *Q.rubra* saplings were 1.33±0.14 m tall and averaged 13.71±0.18 mm in width (10 cm from base of soil). Initial height and width measurements did not differ among treatment groups, which are described below. Before collecting soil, the depth of freshly-fallen leaf litter at WS27 was first estimated and then we removed and saved all litter from the soil surface area from where soil was collected. The litter was a mixture of different species present in proportion to their representation in the community. At the collection site, the most abundant tree species was *Quercus*

rubra, though the litter contained leaf material from other taxa. The soil, classified as Typic Haplubrept (Risley 1986), was then collected to a depth of 20-25 cm to match the depth of the experimental pots (Figure 1). Because of the large volume of soil, soil was roughly mixed and only large roots were removed prior to transplanting. No other additions or deletions were made to the soil. Each experimental pot contained approximately 21.5 kg of CWT soil. Following transplanting, the surface of the soil was covered to a depth of 5 cm with the litter removed from above the soil at CWT. The mass of leaf litter in each pot was 45-50 g dry weight equivalent, corresponding to 450-500 g litter m^{-2} and approximating the leaf litter coverage on WS 27 at CWT (Risley and Crossley 1988). Once established, the experimental units were allowed to equilibrate for 8 months prior to experimental manipulations in 2002. To isolate the experimental units, each was suspended in a triangular stand constructed of pressure-treated pine (Figure 1). The pots were elevated approximately 25 cm above the ground to facilitate leachate collection and exclude fire ants, *Solenopsis invicta*, whose presence is ubiquitous in the field. All links to the pots (e.g., legs, irrigation lines) were frequently treated with Tanglefoot® to further prevent fire ant infiltration. We installed an automated irrigation system (Rain Bird Inc.) with a 1 gallon hr^{-1} (3.785 L hr^{-1}) emitter and ring-shaped, 1.5 cm soaker hose attached to each pot to ensure consistent and uniform watering. While we irrigated with potentially N-rich tap water, all pots received similar watering regimes. Watering was timed to maintain the soil moisture near field capacity and minimize irrigation-based leaching. As a result, most leaching losses occurred during rainfall events.

Experimental Manipulations

We used a 3x2 factorial experimental design with three damage (herbivore, mechanical, "undamaged") and two frass (frass, no frass) groups. The control group for the damage treatment, "undamaged," is written in quotation marks because the trees experienced low background levels of defoliation (approximately 6% leaf area removed). Twenty experimental units were randomly selected for each of six treatments, totaling 120 experimental units. Because 60 trees required frass additions but only 40 experimental trees received herbivores, 20 additional trees also received herbivores in order to generate sufficient frass to reflect the level of damage.

Herbivore damage was inflicted by the Eastern Tent Caterpillar, *Malacosoma americanum*, in early June 2002. Individuals of *M.americanum* preferentially feed on *Prunus* sp., and we used them as defoliators only because our laboratory supply of white marked tussock moth, *Orgyia leucostigma*, failed to hatch prior to the experiment. Leaf counts and area measurements were made prior to herbivore additions (June 1-3, 2002) with a LI-3000A portable area meter (LiCor Inc., Lincoln, NE). Total leaf area per tree averaged 9053±3260 cm^3, and was not significantly different among treatment groups.

Herbivores were contained within hand-sewn bags of Reemay® agricultural cloth, and all trees were covered with the bags so that any effects of the bags on trees (e.g., reduced photosynthesis) would occur across all treatments and controls. The bag, a 1.0x0.8m rectangle double sewn on three sides, was placed over the top of each tree and extended downward to cover between 65-85% of the total leaf area. Air temperatures

inside the bags were approximately 3-5^{o}C higher than outside air temperatures during the day, though no trees showed any signs of heat stress. The bags were secured around the main stem using rope such that herbivores could not escape and frass would collect at the base of the bag without falling through.

Herbivores were added to trees on June 5, 2002 and removed on June 15, 2002. Thirty 4th-5th instar herbivores were initially added per tree, and visual estimates of mortality were made on a daily basis. The large majority of defoliation by *M. americanum* occurs in the final two instars, and the experimental period of 10 days covers approximately the length of those instars in the field. We estimated damage as leaf area removed (LAR) within two days of the discontinuation of herbivore treatments using techniques described in Hunter (1987). Herbivore activity roughly doubled background levels of defoliation (14.3% LAR versus 6.7% LAR; Figure 2). While the damage level was substantially lower than the target value of 40% LAR to replicate outbreak conditions (Reynolds et al. 2000), it was nonetheless significantly higher than "undamaged" controls (t_{73}=10.54; p<0.0001, Figure 2) .

We also simulated herbivory by mechanically removing leaf tissue with scissors. Mechanical damage was imposed simultaneously with herbivore damage and mimicked herbivory in terms of the total leaf area removed (t_{75}=-0.69; p=0.4952, Figure 2) and the manner in which the foliage was removed. Herbivore damage removed portions of leaf tissue rather than entire leaves; the mechanical damage removed similar amounts of leaf tissue from damaged leaves. Specifically, herbivore-damaged foliage was most often categorized in the 5-30% or 30-50% LAR groups (Hunter 1987), and the mechanical

treatment generated foliage within these two damage classes. We also replicated as closely as possible the proportion of leaves damaged, and only leaves that were contained a bag were clipped by the mechanical treatment. Mechanical damage occurred on three discrete dates (June 7, 10, and 15), while the herbivores fed continuously throughout the ten-day period.

Frass from each herbivore-infested tree was collected by making a small incision in the bag, inserting one end of a modified aspirator (termed "frasspirator"), and sucking all the frass pellets into the collection vial. The small incision was sealed following frass extraction to prevent herbivore escape. On the same day, the frass collected from each tree (60 total trees) was individually weighed, pooled, and redistributed evenly among the appropriate experimental units. Thus, the amount of frass deposition was representative of the average level of damage experienced by the trees. Frass was collected and distributed twice during the experiment (June 10 and June 15). There were no rainfall events during the period between herbivore additions and the first frass collection from bags. Rainfall (. 1.5cm) did occur on the day after the first frass collection, which might have leached some of the nutrients from the frass in the bags. However, only 1 of 5 days was influenced by rain and the C and N values in frass were not different between the two collection dates. There was another rainfall event (. 2.5cm) on the day following the second frass additions to the soil in pots, following which frass pellets were no longer visible on the soil surface. Overall, the frass additions totaled approximately 0.982 g frass per tree (dry weight equivalent), corresponding to a deposition of 10 g m^{-2}, substantially lower than the target "outbreak" value of 60 g m^{-2} (Reynolds et al. 2000).

The frass was composed of 49.13±0.94% C, 3.10±0.05% 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. While this level of frass deposition is roughly 3 times the average annual input of frass at CWT (Hunter et al. 2003), it is only approximately 17% of that observed under outbreak conditions (Swank et al. 1981, Reynolds et al. 2000). As a result, our experiment tested the hypotheses using herbivore activity and inputs closer to endemic than to outbreak levels of herbivory.

Sampling

Soil N was measured twice (pre-treatment and post-treatment) with ion exchange resin bags and one month post-treatment with a single soil sample. A pair of ion exchange resin bags, consisting of positively and negatively charged resin contained in lengths of nylon stocking, were used to estimate available inorganic N in the surface soil (Binkley and Matson 1983). On May 4, 2002, the pre-treatment set of bags were installed approximately 3 cm underneath the surface of the soil via insertion holes cut at an angle to minimize the disturbance to the soil above the bags. While placement of the resin bags may have missed nitrification occurring at lower depths and closer to the rhizosphere, we chose the shallow depth because root infiltration can affect the nutrient retention the ion exchange matrix and limit the utility of the resin bag technique for understanding rhizosphere processes (Binkley and Matson 1983). Resin bags were removed on June 4, 2002, the day before experimental manipulations, and replaced with a new set of bags that were left for one additional month (until July 5, 2002). The

contents of each bag was extracted with 100 mL 1M KCl and analyzed for NO_3^- or NH_4^+ using the automated cadmium reduction and phenate assays, respectively, on an Alpkem Segmented Flow Autoanalyzer. Following extraction, the resin was washed with DI H_2O, air dried, and weighed.

Bulk surface soil samples were taken to a depth of 5 cm on July 15, 2002. While the soil sample excluded any short-term soil C and N dynamics, we chose to sample the soil one month following the end of treatments for three reasons. First, the small soil surface area in the pots precluded multiple soil samples. Second, resin bags were installed to estimate N availability during that time. Finally, we were able to collect one month of post-treatment leachate without disturbance to the surface soil that might have affected the results.

Bulk surface soils were passed through a 1x1mm screen mesh to exclude fine roots and separated into three subsamples for separate analyses. The sieving process mixed rhizosphere and bulk soils. Since we did not destructively sample the replicates at the end of the season, the proportion of root biomass collected during soil sampling is unknown. The first subsample of soil was dried for 48 h at 60ºC to determine water content, and the dried sample subsequently ground to a fine powder and analyzed for total C and N on a Carlo Erba 1500 C/N Analyzer (Milan, Italy). The remaining two samples were analyzed for dissolved organic C (DOC) and bulk surface soil microbial biomass via the fumigation-extraction method (Vance et al. 1987). Briefly, one subsample was immediately extracted with 50ml 0.5M K_2SO_4 on an orbital shaker (150rpm) and subsequently filtered through Whatman 42 filter paper. We here define

this K_2SO_4-extractable soil sample as the soil DOC fraction, which represents a soluble fraction that has been correlated with the labile pool of soil C (Powlson and Jenkinson 1976, Cook and Allan 1992a,b). The other subsample was subjected to chloroform fumigation for 48 h under reduced pressure, and then extracted as above. Non-fumigated (NF) and fumigated (F) samples were analyzed with the Shimadzu TOC-500 Total Carbon Autoanalyzer (Columbia, MD). Total microbial biomass C was estimated from the difference between F and NF: Microbial Biomass C = (F - NF) / k_{ec}, where k_{ec} is a correction factor based on the efficiency of chloroform fumigation (Sparling and West 1988). Because the mass of soil was low (6-7 g dry weight equivalent), no correction factor (i.e., k_{ec} = 1) was used when calculating microbial C (Vance et al. 1987).

Leachate was collected directly from a drainage hole at the bottom of each pot with a collection bottle attached with a length of flexible plastic tubing (Figure 1). Samples were collected following rainfall events and pooled to correspond with resin bag data (above). Therefore, leachate collected from May 5 to June 4, 2002 is "pre-treatment" and leachate collected from June 5 to July 5, 2002 is "post-treatment." Pooled samples were frozen until analysis. Leachates were clear and did not require filtering. Leachate samples were analyzed for total C (TC) on the Shimadzu TOC-500 (above), and for NO_3^- and NH_4^+ as above. Total N (TN) in leachate samples was estimated following persulfate oxidation (Cabrera and Beare 1993), and DON was calculated as the difference between TN and the sum of NO_3^--N and NH_4^+-N.

Soil respiration was measured weekly from June 4, 2002 to August 1, 2002 with an EGM-2 Environmental Gas Monitor (PP Systems, Haverhill, MA), which uses a non-

dispersive infrared measurement technique combined with a soil respiration chamber. We also took three pre-treatment measurements over the course of 8 weeks (April-May 2002). The first post-treatment measurement was taken after herbivore additions but before frass deposition (June 10, 2002), and all remaining post-treatment measurements were subsequent to frass additions. Soil respiration is highly sensitive to soil moisture and temperature. We therefore restricted respiration measurements to between 11:30 am and 3:30 pm on rain-free days. Once the chamber was set on the soil, the IRGA measured the change in CO_2 concentrations for 60 seconds. Therefore, one replicate could be sampled in approximately 1.5 minutes, and the array of 120 soil samples required roughly 3.5 hours to sample. We monitored soil temperatures in a random subset of pots during respiration measurements to ensure that soil temperatures were consistent during the sampling period. Soil temperatures and respiration rates were higher than those recorded in the forest from which the soils were collected (see Reynolds and Hunter (2001) for CWT soil respiration rates). Since we were not manipulating soil temperature, all pots experienced the same environmental conditions. Comparing absolute rates of soil respiration between this study and the field is not possible; only comparisons among treatments are meaningful.

Data Analyses

Data were analyzed using ANOVA models generated by the GLM procedure of SAS 8.2; the residuals of the models were tested for normality (Kery and Hatfield 2003). Parametric repeated measures models were used when appropriate (signified by "RM_P").

The data sets whose residuals failed the test of normality were $\log_n$ and/or square-root transformed and reanalyzed. Data that failed these tests were analyzed with the GENMOD procedure of SAS 8.2, using Poisson distributions and log link functions (SAS Inst. 1999). The development of generalized estimating equations (GEE) allows GENMOD to be used as a non-parametric alternative that includes repeated measures analysis (Littell et al. 2002). The GENMOD procedure reports the χ^2 statistic, and we use "RM_{NP}" to denote non-parametric repeated measures analyses. Posthoc analyses of treatment means are not performed with GENMOD. We used the Student-Neuman-Keuls (SNK) post hoc test (α=0.05) to distinguish among treatment means following parametric analyses. In all cases, the outcomes of the statistical analyses were identical regardless of whether parametric or non-parametric tests were used on the non-transformed data.

RESULTS

1) Soil mineral nitrogen pools

Despite the small addition of frass, significant effects on soil and leachate N pools were detected independent of damage. The concentration of NH_4-N in soil was higher relative to frass-free controls following frass deposition (RM_{NP} χ^2= 4.38, p=0.0364, Figure 3). Total soil N was also higher in pots with frass deposition ($F_{1,104}$=7.76, p=0.0064, Figure 4A). Based on previous results (Reynolds et al. 2000, Hunter et al. 2003), we expected elevated soil NO_3-N concentrations following frass deposition.

Contrary to this expectation, frass deposition did not increase soil NO_3-N concentrations (RM_{NP} χ^2=1.08, p=0.2983, data not shown).

In addition to frass effects, herbivory affected soil N pools independent of frass deposition. There was a date*damage effect on soil NO_3^- (RM_{NP} χ^2=7.01, p=0.0301, Figure 5A). Total soil N was also lower in herbivore-damaged systems ($F_{2,104}$=8.35, p=0.0004, Figure 4B). There were no significant damage*frass interactions with soil N in any form. As expected, between-date soil NO_3^- and NH_4^+ measurements were positively correlated (r=0.432, p<0.001 for NO_3^-, r=0.418, p<0.001 for NH_4^+).

2) Nitrogen leaching as nitrate and DON

Leachate NO_3^- concentrations positively correlated with soil NO_3^- concentrations on both dates (r=0.272, p=0.0035 for pre-treatment, r=0.492, p<0.001 for post-treatment), and the leachate measurements were themselves positively correlated (r=0.250, p=0.007). Nitrate concentrations in leachate increased across treatments between May and June 2002, but concentrations were significantly greater following frass deposition relative to frass-free controls (RM_P $F_{1,107}$=6.47, p=0.0124, Figure 5B). There were no treatment effects on leachate NH_4-N, and concentrations were low. Low NH_4-N concentrations in leachate is parsimonious with NH_4^+ adsorption to negatively charged soil particles (Quemada et al. 1997), which effectively abiotically immobilizes the NH_4-N and prevents its loss via leaching. While the total concentration of DON-N increased between pre- and post-treatment samples (from 1.20±0.13 to 3.03±0.26 mg L^{-1}, p<0.0001), percentage of DON-N in leachate was consistent (24.0% and 27.5%, respectively, p=0.2328).

Neither the total amount nor percentage of DON-N were affected by frass deposition (p=0.8776 and p=0.5576, respectively) or damage (p=0.8092 and p=0.4553, respectively). Some DON values were slightly negative (because DON is calculated as a difference between TN and [NO_3-N + NH_4-N]) and thus treated as 0 for purposes of calculations. There were no significant damage*frass interactions with leachate N in any form.

3) Soil respiration, DOC, and total carbon

Surface soil microbial biomass C was higher in frass-treated systems ($F_{1,102}$=5.70, p=0.0191, Figure 6). However, frass deposition did not increase soil respiration over the course of the experiment using our method of weekly measurements (RM_P $F_{10,1050}$=0.54, p=0.8003, data not shown). Since we did not measure soil respiration in the hours following frass additions, it is possible that we missed a finer resolution of soil respiration responses to frass additions. This seems likely considering the apparent frass-derived increase in microbial C.

Soil respiration increased following herbivore damage relative to "undamaged" controls independent of frass deposition (RM_P $F_{20,1050}$= 2.07, p=0.0118, Figure 7). The herbivore-mediated increase in respiration was significant within 10 days of herbivory and was maintained throughout the duration of respiration measurements (almost 2 months). While the effect was pronounced following herbivory, mechanically-damaged systems also responded with increased soil respiration on two post-treatment measurement dates (Figure 7). However, the date*damage interaction was not significant

when just mechanically damaged and "undamaged" control systems were considered (data not shown).

Soil DOC was higher in herbivore and mechanical damage treatments relative to "undamaged" controls in the absence of frass deposition (Damage*Frass $F_{2,103}$=3.78, p=0.0260, Figure 8 "no frass"). The effect disappeared in frass addition treatments (Figure 8, "frass"). Conversely, total soil C was lower in herbivore, but not mechanically-damaged, pots relative to "undamaged" controls independent of frass deposition ($F_{2,104}$=6.87; p=0.0016, Figure 4D). Total soil C was higher in frass-treated pots relative to frass-free pots ($F_{1,104}$=7.56, p=0.0071, Figure 4E). When total soil C and N data were taken together, the higher concentrations following frass deposition and lower concentrations following herbivory resulted in C:N ratios that were not significantly different from either frass-free or "undamaged" controls ($F_{2,104}$=0.020, p=0.888, Figure 4E and $F_{2,104}$=1.11, p=0.3337, Figure 4F, respectively). In addition, there was a strong positive correlation between soil total C and N values (r=0.90, p<0.0001).

These results suggest that foliar herbivory stimulated some form of belowground biological activity independent of surface inputs (i.e., frass deposition). Leachate samples were tested for total C and, while there was a decline in leachate C over time (p=0.0274), the decline was not mediated by damage (p=0.3372), frass (p=0.7543), or the interaction of the two (p=0.0904). Therefore, the stimulation of belowground C fluxes following herbivory did not increase C export in leachate.

DISCUSSION

This experiment was designed to test the hypotheses that frass deposition from canopy herbivory increases soil N, aqueous N exports, and soil respiration. While we were unable to replicate the level of damage (and thus frass deposition) of outbreak conditions, the data tell three equally-interesting stories about canopy herbivory and nutrient dynamics at closer to endemic levels. First, frass deposition increases soil N pools and accelerates N export from the terrestrial system. Second, mechanical damage did not generate the same belowground responses as did real herbivory. Third, aboveground herbivory on *Q.rubra* affects soil C and N pools independent of frass (or other surface) additions.

Frass Deposition

Our results provide evidence that frass deposition from herbivore activity can account for increases in the soil inorganic N pool and the N lost from the terrestrial system via leaching during the season in which the defoliation occurs. Since the differences in soil mineral N and leachate NO_3^- were observed between pots receiving frass and those that did not, we can say that frass deposition has an effect on soil N dynamics. While we assume that the N in the frass is the N observed in leachate NO_3^- and soil NH_4^+, we are currently conducting a stable isotope ^{15}N-labeled frass experiment to confirm this hypothesis.

Contrary to increased soil NO_3^- levels found in the field under endemic (Hunter et al. 2003) and outbreak (Reynolds et al. 2000) conditions, our data indicate relative

increases in NH_4^+, but not NO_3^-, in response to frass deposition. The difference in form may be an artifact of the oak mesocosm, because *Q.rubra* tend to inhibit nitrification (Verchot et al. 2001). Another possibility is that the resin bags buried at 3 cm did not detect increases NO_3^- because nitrification may have occurred at lower soil depths, especially considering increased leachate NO_3^- implies increased soil NO_3^- concentrations. While the form of inorganic N is different, the overall message is the same: insect herbivores influence soil inorganic N availability via frass deposition. From our perspective, the interesting result is that the systems responded to relatively small frass additions. Forest soil N pools are dependent on annual surface inputs of leaf litter (Risley 1986, Risley and Crossley 1988, Hunter et al. 2003), and it is increasingly clear that insect herbivore activity influences litter quality and subsequent decomposition (Chapman et al. 2003). Nitrogen mobilized in frass is the same N that would enter the soil as leaf litter in the absence of herbivores. Herbivore activity consequently can change the timing and/or form of N deposition but may not affect the total amount of N inputs to the soil. However, the measurable increase in soil N following even modest frass deposition apparently has consequences for how the total N is ultimately distributed.

Considering N loss from the terrestrial system, our data are consistent with results from previous field observations at CWT (Swank et al. 1981). Reynolds et al. (2000) linked frass deposition from an outbreak event at CWT with elevated NO_3^- levels in the stream that drained the defoliated watershed. Nitrogen lost as leachate NO_3^- cannot be recycled through the terrestrial system in which it had been immobilized as leaf tissue.

As far as we know, our data are the first to demonstrate that frass deposition represents a mechanism by which herbivore activity can increase stream nitrate concentrations. Even at relatively low levels, insect herbivore activity may therefore simultaneously influence N dynamics in a forest and its associated aquatic ecosystem. However, "loss" from a pot may not equal "loss" from a forest ecosystem if other conservation mechanisms operate.

Stadler et al. (2001) report that excreta from aphid and lepidopteran larval feeding did not change the size of mineral N pools in the soil. They suggested that the effects of aboveground herbivory were obscured in the soil by "buffering biological processes." A similar conclusion was reached by Lovett and Ruesink (1995), who found net N immobilization of substantial amounts of frass N relative to soil following incubation in closed microcosms. In contrast, Hunter et al. (2003) found increased soil NO_3^- concentrations following endemic levels of frass deposition in the field, and the data reported here support those results. Our fifth hypothesis, interaction between frass deposition and below-ground responses to damage affecting soil C and N dynamics, was conceived to explain the variation in reported results of frass deposition on soil N. The data do not support the hypothesis: frass deposition and herbivory influenced soil C and N dynamics independently.

Instead of the interaction between frass deposition and belowground responses to aboveground herbivory, variable results among published studies might be reconciled by considering the interaction of precipitation and frass deposition. For example, Lovett and Ruesink (1995) maintained high moisture contents inside their microcosms, did not flush them with solution, and visible fungal hyphae infiltrated the frass pellets. We have

observed similar phenomena in recently conducted microcosm and field studies (personal observations). Conditions of high humidity and low rainfall apparently allow rapid immobilization of frass N by soil fungi, and we would correspondingly predict minimal leaching losses of N. In the absence of rainfall, frass pellets deposited on the soil surface of a hybrid poplar stand in Michigan remained visible and undecomposed, and no increases in soil mineral N were observed (referenced as a "personal communication" in Hunter (2001)). During our experimental manipulations, rainfall events occurred within two days of frass additions, and the frass pellets were not visible the day after the rainfall. Historical precipitation data at CWT show rainfall events during the peak periods of herbivore activity in the studies that have correlated defoliation and N export (Swank et al. 1981, Reynolds et al. 2000). Assessing our data in the context of the other published studies, herbivore activity evidently mobilizes sufficient N as frass to affect soil N pools and export even at endemic herbivore densities, though the ultimate fate may depend on the relative timing of frass deposition and precipitation.

Our third hypothesis, that frass deposition would increase soil respiration, was not supported using our sampling regime despite an apparent stimulation of the surface soil microbial community. Similarly, a field study at CWT by Reynolds and Hunter (2001) found no relationship between soil respiration and frass deposition at double background levels. Soil micro-organisms are thought to be C-limited (Cheng et al. 1996), and our experimental frass deposition may not have supplied enough labile C to elicit a noticeable difference. Our experimental additions supplied approximately 482 mg C to the soil in the form of frass, some of which was in the form of recalcitrant phenolics

(Kopper et al. 2000). The additional respiration measured following herbivory alone roughly amounted to 100 mg C hr^{-1}, which would exceed the total amount of C added as frass within 5 hours. Therefore, it is not surprising that the direct contribution of frass C to soil respiration was not detected with our sampling regime. In our system, the contribution of roots to total soil respiration possibly masked any frass effect. Lovett and Ruesink (1995) found a positive effect of frass on respiration using large quantities of frass relative to soil in a root-free controlled environment, though the effect was short-lived. These results collectively suggest that (1) a threshold level of frass deposition may be required to elicit a response in soil respiration above background levels, (2) the response will be ephemeral, and (3) the C added as frass remains in the surface soil horizons.

Real versus Simulated Herbivory

To our knowledge, this is the first reported study to test the responses of belowground nutrient fluxes to real and simulated herbivory. In our experiment, mechanical damage failed to generate soil C and N fluxes comparable to real herbivore activity. Mechanical damage can elicit a number of tangible responses, including increased photosynthetic rate in oaks (Lovett and Tobiessen 1993) and reduction in seed pod production in vetch (Koptur et al. 1996). However, differential effects of real and simulated herbivory have long been recognized (Baldwin 1988a, Baldwin 1988b). The focus of the studies testing for differences between real and simulated herbivory have been limited to aboveground direct (Litvak and Monson 1998) or indirect (Alborn et al.

1997, Kainoh et al. 1999, Hoballah and Turlings 2001, Oppenheim and Gould 2002) defensive responses. The mechanisms underlying the differences have been identified in some cases (Bergman 2002). We are unable to provide a specific mechanism for our observation, though we hypothesize that there is a signaling pathway by which oaks recognize the presence of insect herbivores and engage herbivore-specific defensive responses (Dyer et al. 1995; Dyer et al. 1993) that extend belowground. This result suggests that the response of oaks to disturbance is contingent upon the type of disturbance and, more importantly, that the conditional response has implications for system-wide C and N dynamics. In addition, herbivore-damaged trees had higher foliar tannin concentrations than did mechanically-damaged trees in our experiment, suggesting a whole-plant differential response by oaks (C.J. Frost and M.D. Hunter, unpublished data).

Herbivore Activity and Soil C and N Dynamics

Herbivore activity stimulated changes in soil C and N independent of frass deposition, which implicates a root-mediated reaction to herbivore activity. Unfortunately, the experiment was not designed to address the rhizosphere, and the data do not clearly differentiate between potential mechanisms. There are four mechanisms that might, independently or interactively, contribute to belowground C and N fluxes following aboveground herbivory independent of surface soil inputs: (i) root turnover, (ii) increased root growth, (iii) increased root activity (independent of growth and rhizodeposition), and (iv) increased root rhizodeposition.

The effects of root turnover on soil nutrient dynamics are not well known (Eissenstat and Yanai 1997, Matamala et al. 2003), though herbivore-mediated root turnover and resulting microbial-mediated decomposition can occur when herbivory hinders a plant's ability to sustain fine root biomass, which die and are colonized by microbial decomposers. Ruess et al. (1998) demonstrated such a mechanism following mammalian browsing in the Alaskan taiga. In our system, this mechanism seems unlikely because soil respiration remained positively correlated with total leaf area following treatments and total soil N declined in herbivore-damaged systems.

Alternatively, aboveground damage might stimulate root growth, exploration, and biomass accumulation, as suggested by the deceleration effect hypothesis (Ritchie et al. 1998). Increased root activity can alter soil respiration and N dynamics without adding root biomass. For example, roots can actively increase kinetic rates of nutrient uptake. Ion transport is an energetic process (Clarkson 1985), with nitrate assimilation estimated at 20% of NPP (Bloom et al. 1992). In our experiment, reduction in total soil N and C in herbivore-damaged systems supports these hypotheses.

Finally, rhizodeposition of C-rich compounds can provide a substrate for microbial activity that can result in a positive feedback of N mineralization and subsequent plant uptake in nutrient rich soils (Hamilton and Frank 2001). Soil micro-organisms are often limited by the quantity and quality of rhizodeposited C from plant roots (Cheng et al. 1996, Knops et al. 2002), and rhizodeposited C influences belowground nutrient dynamics (McNaughton et al. 1988, Cheng 1996, Frank and Groffman 1998, Kuzyakov et al. 2001, Kuzyakov and Cheng 2001, Cardon et al. 2002,

Farrar et al. 2003). The reduction in total soil N and the increase in soil DOC following herbivore activity supports this hypothesis. Understanding the root-mediated effects of herbivory in forests and distinguishing between the competing mechanisms will be important for our understanding of the relationship among foliar herbivory, root dynamics, and ecosystem processes in temperate forests.

CONCLUSIONS

This study factorially manipulated herbivore damage (real and simulated) and subsequent frass deposition to test the hypothesis that frass deposition would increase aqueous export of N from soil independent of damage. While the data supported this hypothesis, herbivore damage also affected belowground N pools independent of frass additions. Statistical interaction between herbivore damage and frass additions seldom occurred, suggesting that herbivory and frass deposition have significant, but largely independent, effects on soil processes. The feeding activity of insect herbivores, even at endemic population densities, can therefore have direct and indirect effects on the cycling of C and N within the season of defoliation.

ACKNOWLEDGEMENTS

We would like to thank B.Ball, C.Hall, S.Helms, O.Klienberger, M.Madritch, T.Muenz, B.Nuse, S.Scott, K.Wickings, C.Zehnder, and especially R.Goergen for field assistance and/or comments on earlier drafts of the manuscript. Additionally, comments by Dr. Patrick Bohlen and two anonymous reviewers greatly strengthened the

manuscript. We thank G.H. and J.K. Cooke for logistical support. This research is supported by NSF grant DEB 9815133 to MDH.

REFERENCES

Alborn H.T., T. C. J. Turlings, T. H. Jones, G. Stenhagen, J. H. Loughrin and J. H. Tumlinson. 1997. An elicitor of plant volatiles from beet armyworm oral secretion. Science **276**: 945-949.

Baldwin I.T. 1988a. Short-term damage-induced increases in tobacco alkaloids protect plants. Oecologia **75**: 367-370.

Baldwin I.T. 1988b. The alkaloidal responses of wild tobacco to real and simulated herbivory. Oecologia **77**: 378-381.

Bardgett R.D. and D. A. Wardle. 2003. Herbivore-mediated linkages between aboveground and belowground communities. Ecology **84**: 2258-2268.

Bardgett R.D., D. A. Wardle and G. W. Yeates. 1998. Linking above-ground and below-ground interactions: how plant responses to foliar herbivory influence soil organisms. Soil Biology and Biochemistry **30**: 1867-1878.

BassiriRad H., J. V. H. Constable, J. Lussenhop, B. A. Kimball, R. J. Norby, W. C. Oechel, P. B. Reich, W. H. Schlesinger, S. Zitzer, H. L. Sehtiya and S. Silim. 2003. Widespread foliage $\delta^{15}N$ depletion under elevated CO_2: inferences for the nitrogen cycle. Global Change Biology **9**: 1582-1590.

Bergman M. 2002. Can saliva from moose, *Alces alces*, affect growth responses in the sallow, *Salix caprea*? Oikos **96**: 164-168.

Binkley D. and P. A. Matson. 1983. Ion exchange resin bag method for assessing forest soil nitrogen availability. Soil Science Society of America Journal **47**: 1050-1052.

Bloom A.J., S. S. Sukrapanna and R. L. Warner. 1992. Root respiration associated with ammonium and nitrate absorption and assimilation by barley. Plant Physiology **99**: 1294-1301.

Bringhurst R.M., Z. G. Cardon and D. J. Gage. 2001. Galactosides in the rhizosphere: utilization by *Sinorhizobium meliloti* and development of a biosensor. Proceedings of the National Academy of Sciences (USA) **98**: 4540-4545.

Cabrera M.L. and M. H. Beare. 1993. Alkaline persulfate oxidation for determining total nitrogen in microbial biomass extracts. Soil Science Society of America Journal **57**: 1007-1012.

Cardon Z.G., A. D. Czaja, J. L. Funk and P. L. Vitt. 2002. Periodic carbon flushing to roots of *Quercus rubra* saplings affects soil respiration and rhizosphere microbial biomass. Oecologia **133**: 215-223.

Chapman S.K., S.C. Hart, N.S. Cobb, T.G. Whitham and G.W. Koch. 2003. Insect herbivory increases litter quality and decomposition: an extension of the acceleration hypothesis. Ecology **84**: 2867-2876.

Cheng W. 1996. Measurement of rhizospheric respiration and organic matter decomposition using natural ^{13}C. Plant and Soil **183**: 263-268.

Cheng W., Q. Zhang, D. C. Coleman, R. C. Carroll and C. A. Hoffman. 1996. Is available carbon limiting microbial respiration in the rhizosphere? Soil Biology and Biochemistry **28**: 1283-1288.

Chew R.M. 1974. Consumers as regulators of ecosystems: an alternative to energetics. Ohio Journal of Science **74**: 359-370.

Christenson L.M., G. M. Lovett, M. J. Mitchell and P. M. Groffman. 2002. The fate of nitrogen in gypsy moth frass deposited to an oak forest floor. Oecologia **131**: 444-452.

Clarkson D.T. 1985. Factors affecting mineral nutrient acquisition by plants. Annual Review of Plant Physiology **36**: 77-115.

Cook B.D. and D. L. Allan. 1992a. Dissolved organic carbon in old field soils: compositional changes during the biodegradation of soil organic matter. Soil Biology and Biochemistry **24**: 595-600.

Cook B.D. and D. L. Allan. 1992b. Dissolved organic carbon in old field soils: total amounts as a measure of available resources for soil mineralization. Soil Biology and Biochemistry **24**: 585-594.

Dyer M.I., A.M. Moon, M.R. Brown, and D.A. Crossley 1995. Grasshopper crop and midgut extract effects on plants - an example of reward feedback. Proceedings of the National Academy of Sciences of the United States of America **92**: 5475-5478

Dyer M.I., C.L. Turner and T.R. Seastedt 1993. Herbivory and its consequences. Ecological Applications **3**: 10-16

Eissenstat D.M. and R. D. Yanai. 1997. The ecology of root lifespan. Advances in Ecological Research **27**: 1-60.

Farrar J., M. Hawes, D. Jones and S. Lindow. 2003. How roots control the flux of carbon to the rhizosphere. Ecology **84**: 827-837.

Fogal W.H. and F. Slansky Jr. 1984. Contribution of feeding by European pine sawfly larvae to litter production and element flux in Scots pine plantations. Canadian Journal of Forest Research **15**: 484-487.

Frank D.A. and P. M. Groffman. 1998. Ungulate versus landscape control of soil C and N processes in grasslands of Yellowstone National Park. Ecology **79**: 2229-2241.

Frank D.A. and S. J. McNaughton. 1993. Evidence for the promotion of aboveground grassland production by native large herbivores in Yellowstone National Park. Oecologia **96**: 157-161.

Glynn C., D. A. Herms, M. Egawa, R. Hansen and W. J. Mattson. 2003. Effects of nutrient availability on biomass allocation as well as constitutive and rapid induced herbivore resistance in poplar. Oikos. **101**: 385-397.

Grace J.R. 1986. The influence of gypsy moth on the composition and nutrient content of litter fall in a Pennsylvania oak forest. Forest Science **32**: 855-870.

Hairston N.G., F. E. Smith and L. B. Slobodkin. 1960. Community structure, population control, and competition. American Naturalist **94**: 421-425.

Hamilton E.W.I. and D. A. Frank. 2001. Can plants stimulate soil microbes and their own nutrient supply? Evidence from a grazing tolerant grass. Ecology **82**: 2397-2404.

Haukioja E., P. Niemela and S. Siren. 1985a. Foliage phenols and nitrogen in relation to growth, insect damage, and ability to recover after defoliation, in the moutain birch *Betula pubescens* ssp *tortuosa*. Oecologia **65**: 214-222.

Haukioja E., J. Soumela and S. Neuvonen. 1985b. Long-term inducible resistance in birch foliage: triggering cues and efficacy on a defoliator. Oecologia **65**: 363-369.

Herms D.A. and W. J. Mattson. 1992. The dilemma of plants: to grow or defend. Quarterly Review of Biology **67**: 283-335.

Hoballah M.E.F. and T. C. J. Turlings. 2001. Experimental evidence that plants under caterpillar attack may benefit from attracting parasitoids. Evolutionary Ecology Research **3**: 553-565.

Holland J.N. 1995. Effects of above-ground herbivory on soil microbial biomass in conventional and no-tillage agroecosystems. Appied Soil Ecology **2**: 275-279.

Holland J.N., W. Cheng and D. A. Jr. Crossley. 1996. Herbivore-induced changes in plant carbon allocation: assessment of below-ground C fluxes using carbon-14. Oecologia **107**: 87-94.

Hollinger D.Y. 1986. Herbivory and the cycling of nitrogen and phosphorous in isolated California oak trees. Oecologia **70**: 291-297.

Hunter M.D. 1987. Opposing effects of spring defoliation on late season caterpillars. Ecological Entomology **12**: 373-382.

Hunter M.D. 2001. Insect population dynamics meets ecosystem ecology: effects of herbivory on soil nutrient dynamics. Agricultural and Forest Entomology **3**: 77-84.

Hunter M.D., C. R. Linnen and B. C. Reynolds. 2003. Effects of endemic densities of canopy herbivores on nutrient dynamics along a gradient in elevation in the southern appalachians. Pedobiologia **47**: 231-244.

Hunter M.D. and P. W. Price. 1992. Playing chutes and ladders: heterogeneity and the relative roles of bottom-up and top-down forces in natural communities. Ecology **73**: 724-732.

Hunter M.D. and J. C. Schultz. 1995. Fertilization mitigates chemical induction and herbivore responses within damaged oak trees. Ecology **76**: 1226-1232.

Jaeger C.H., S. E. Lindow, W. Miller, E. Clark and M. K. Firestone. 1999. Mapping of sugar and amino acid availability in soil around roots with bacterial sensors of sucrose and tryptophan. Applied and Environmental Microbiology **65**: 2685-2690.

Kainoh Y., C. Tanaka and S. Nakamura. 1999. Odor from herbivore-damaged plant attracts the parasitoid fly *Exorista japonica* Townsend (Diptera:Tachinidae). Applied Entomology and Zoology **34**: 463-467.

Kery M. and J. S. Hatfield. 2003. Normality of raw data in general linear models: the most widespread myth in statistics. Bulletin of the Ecological Society of America **82**: 92-94.

Knops J.M.H., K. L. Bradley and D. A. Wedin. 2002. Mechanisms of plant species impacts on ecosystem nitrogen cycling. Ecology Letters **5**: 454-466.

Kopper B.J., V. N. Jakobi, T. L. Osier and R. L. Lindroth. 2000. Effects of paper birch condensed tannin on whitemarked tussock moth (Lepidoptera: Lymantriidae) performance. Physiological and Chemical Ecology **31**: 10-14.

Koptur S., C. L. Smith and J. H. Lawton. 1996. Effects of artificial defoliation on reproductive allocation in the common vetch, *Vicia sativa* (Fabaceae: Papilionoideae). American Journal of Botany **83**: 886-889.

Kuzyakov Y. and W. Cheng. 2001. Photosynthesis controls of rhizosphere respiration and organic matter decomposition. Soil Biology and Biochemistry **33**: 1915-1925.

Kuzyakov Y., H. Ehrensberger and K. Stahr. 2001. Carbon partitioning and below-ground translocation by *Lolium perenne*. Soil Biology and Biochemistry **33**: 61-74.

Lightfoot D.C. and W. G. Whitford. 1990. Phytophagous insects enhance nitrogen flux in a desert creosotebush community. Oecologia **82**: 18-25.

Littell R.C., W. W. Stroup and R. J. Freund. 2002. SAS for Linear Models. **4**Cary, NC, SAS Institute, Inc.

Litvak M.E. and R. K. Monson. 1998. Patterns of induced and constitutive monoterpene production in conifer needles in relation to insect herbivory. Oecologia **114**: 531-540.

Lovett G.M., L. M. Christenson, P. M. Groffman, C. G. Jones, J. E. Hart and M. J. Mitchell. 2002. Insect defolation and nitrogen cycling in forests. BioScience **52**: 335-341.

Lovett G.M. and A. E. Ruesink. 1995. Carbon and nitrogen mineralization from decomposing gypsy moth frass. Oecologia **104**: 133-138.

Lovett G.M. and P. Tobiessen. 1993. Carbon and nitrogen assimilation in red oaks (*Quercus rubra* L.) subject to defoliation and nitrogen stress. Tree Physiology **12**: 259-269.

Matamala R., M. A. Gonzalez-Meler, J. D. Jastrow, R. J. Norby and W. H. Schlesinger. 2003. Impacts of Fine Root Turnover on Forest NPP and Soil C Sequestration Potential. Science **302**: 1385-1387.

McNaughton S.J., R. W. Ruess and S. W. Seagle. 1988. Large mammals and process dynamics in African ecosystems. BioScience **38** : 794-800.

Oppenheim S.J. and F. Gould. 2002. Is attraction fatal? The effects of herbivore-induced plant volatiles on herbivore parasitism. Ecology **83**: 3416-3425.

Powlson D.S. and D. S. Jenkinson. 1976. The effects of biocidal treatments on metabolism in soil: II. Gamma irradiation, autoclaving, air-drying and fumigation. Soil Biology and Biochemistry **8**: 179-188.

Quemada M., M. L. Cabrera and D. V. McCracken. 1997. Nitrogen release from surface-applied cover crop residues: evaluating the CERES-N submodel. Agronomy Jounral **89**: 723-729.

Reynolds B.C. and M. D. Hunter. 2001. Responses of soil respiration, soil nutrients, and litter decomposition to inputs form canopy herbivores. Soil Biology and Biochemistry **33**: 1641-1652.

Reynolds B.C., M. D. Hunter and D. A. Jr. Crossley. 2000. Effects of canopy herbivory on nutrient cycling in a northern hardwood forest in western North Carolina. Selbyana **21**: 74-78.

Risley L.S. 1986. The influence of herbivores on seasonal leaf-fall: premature leaf abcission and petiole clipping. Journal of Agricultural Entomology **3**: 152-162.

Risley L.S. and D. A. Jr. Crossley. 1988. Herbivore-caused greenfall in the southern Appalachians. Ecology **69**: 1118-1127.

Ritchie M.E., D. Tilman and J. M. H. Knops. 1998. Herbivore effects on plant and nitrogen dynamics in oak savanna. Ecology **79**: 165-177.

Rossiter M., J. C. Schultz and I. T. Baldwin. 1988. Relationships among defoliation, red oak phenolics, and gypsy moth growth and reproduction. Ecology **69**: 267-277.

Ruess R.W., R. L. Hendrick and J. P. Bryant. 1998. Regulation of fine root dynamics by mammalian browsers in early successional Alaskan taiga forests. Ecology **79**: 2706-2720.

Schowalter T.D., T. E. Sabin, S. G. Stafford and J. M. Sexton. 1991. Phytophage effects on primary production, nutrient turnover, and litter decomposition of young Dougas-fir in western Oregon. Forest Ecology and Management **42**: 229-243.

Schultz J.C. and I. T. Baldwin. 1982. Oak leaf quality declines in reponse to defoliation by gypsy moth larvae. Science **217**: 149-151.

Slobodkin L.B., F. E. Smith and N. G. Hairston. 1967. Regulation in terrestrial ecosystems, and the implied balance of nature. American Naturalist **101**: 109-124.

Sparling G.P. and A. W. West. 1988. A direct extraction method to estimate soil microbial C: calibration *in situ* using microbial respiration and ^{14}C labeled cells. Soil Biology and Biochemistry **20**: 337-343.

Stadler B., S. Solinger and B. Michalzik. 2001. Insect herbivores and the nutrient flow from the canopy to the soil in coniferous and deciduous forests. Oecologia **126**: 104-113.

Swank W.T., J. B. Waide, D. A. Jr. Crossley and R. L. Todd. 1981. Insect defoliation enhances nitrate export from forest ecosystems. Oecologia **51**: 297-299.

Swift M.J., O. W. Heal and J. M. Anderson. 1979. Decomposition in Terrestrial Ecosystems. Berkeley, CA, University of California Press.

Townsend P.A., K.N. Eshleman and C. Welcker 2004. Remote sensing of gypsy moth defoliation to assess variations in stream nitrogen concentrations. Ecological Applications **14**: 504-516

Vance E.D., P. C. Brookes and D. S. Jenkinson. 1987. An extraction method for measuring soil microbial biomass C. Soil Biology and Biochemistry **19**: 703-707.

Verchot L.V., Z. Holmes, L. Mulon, P. M. Groffman and G. M. Lovett. 2001. Gross vs net rates of N mineralization and nitrification as indicators of functional differences between forest types. Soil Biology and Biochemistry **33**: 1889-1901.

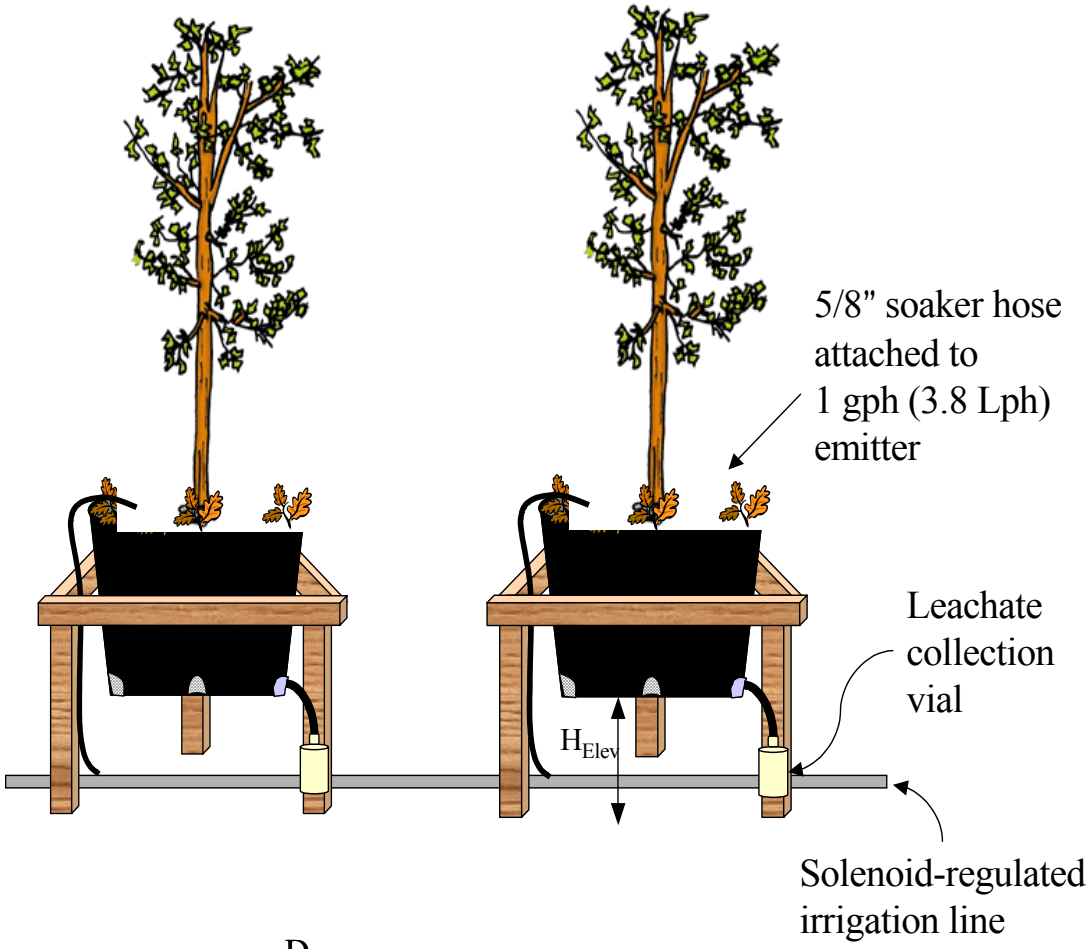
Wardle D.A. 2002. Communities and Ecosystems: Linking the Aboveground and Belowground Components. Princeton, Princeton University Press. Monographs in Population Biology. Levin, Simon. A. and Horn, Henry. S.

Zvereva E.L., M. V. Kozlov, P. Niemela and E. Haukioja. 1997. Delayed induced resistance and increase in leaf fluctuating asymmetry as responses of Salix borealis to insect herbivory. Oecologia **109**: 368-373.

Figure 2.1 Design of *Quercus rubra*-soil experimental units. A. Each sapling was established in a 14-gallon pot elevated (H_{elev}) approximately 25 cm above the ground by hand-made stands of pressure-treated pine. The experimental array contained 16 rows and ten columns, totaling 160 experimental units. Two experimental units are shown here, connected by irrigation line. Each unit was irrigated with a 5/8" (1.5 cm) ring-shaped soaker hose that was attached to a 1 gallon-per-hour (3.8 Lph) emitter to ensure uniform watering of the replicates. B. The diameter of each pot (D_{pot}) was 14" (35.6 cm). The depth of the soil (H_{soil}) in each experimental unit was approximately 24 cm, and the litter depth (H_{litter}) was approximately 5 cm. See text for descriptions of soil and litter collection and preparation. To collect leachate, one of the drain holes was fitted with a tube connector and sealed with silicon caulk. The remaining three drain holes were plugged with caulk. The replicates were used for a 3x2 factorial experiment manipulating real and simulated herbivory, and frass deposition. Herbivore damage was inflicted by the eastern tent caterpillar, *Malacosoma americanum*, while mechanical damage occurred on three days during herbivore feeding. Frass was collected from the herbivore-infested trees (plus an additional 20 trees subjected to herbivore feeding) and distributed among the replicates receiving frass additions. The level of frass deposition was representative of the level of damage experienced by the defoliated saplings. There were 20 individual replicates per treatment; 120 replicates in the experiment.

Figure 2.1

A


5/8" soaker hose
attached to
1 gph (3.8 Lph)
emitter
Leachate
collection
vial
H_{Elev}
Solenoid-regulated
irrigation line

B

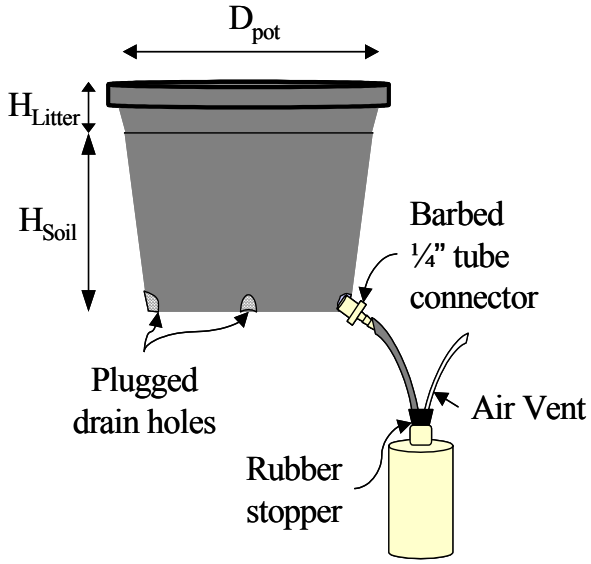

D_{pot}
H_{Litter}
H_{Soil}
Barbed
¼" tube
connector
Plugged
drain holes
Air Vent
Rubber
stopper

Figure 2.2 Estimated Leaf Area Removed (LAR) from *Q.rubra* saplings in experimental damage groups. Herbivore manipulations roughly doubled background levels of defoliation. Mechanical damage was conducted three times during herbivore feeding. Damage levels generated by the herbivores guided the severity of mechanical damage, resulting in similar LAR between real and simulated herbivory treatments. In addition, the type of damage generated by the herbivores was replicated by the mechanical damage such that similar percentages of individual leaves were removed. Bars are the means of 40 saplings per damage class and error bars represent +/- 1 SE.

Figure 2.2

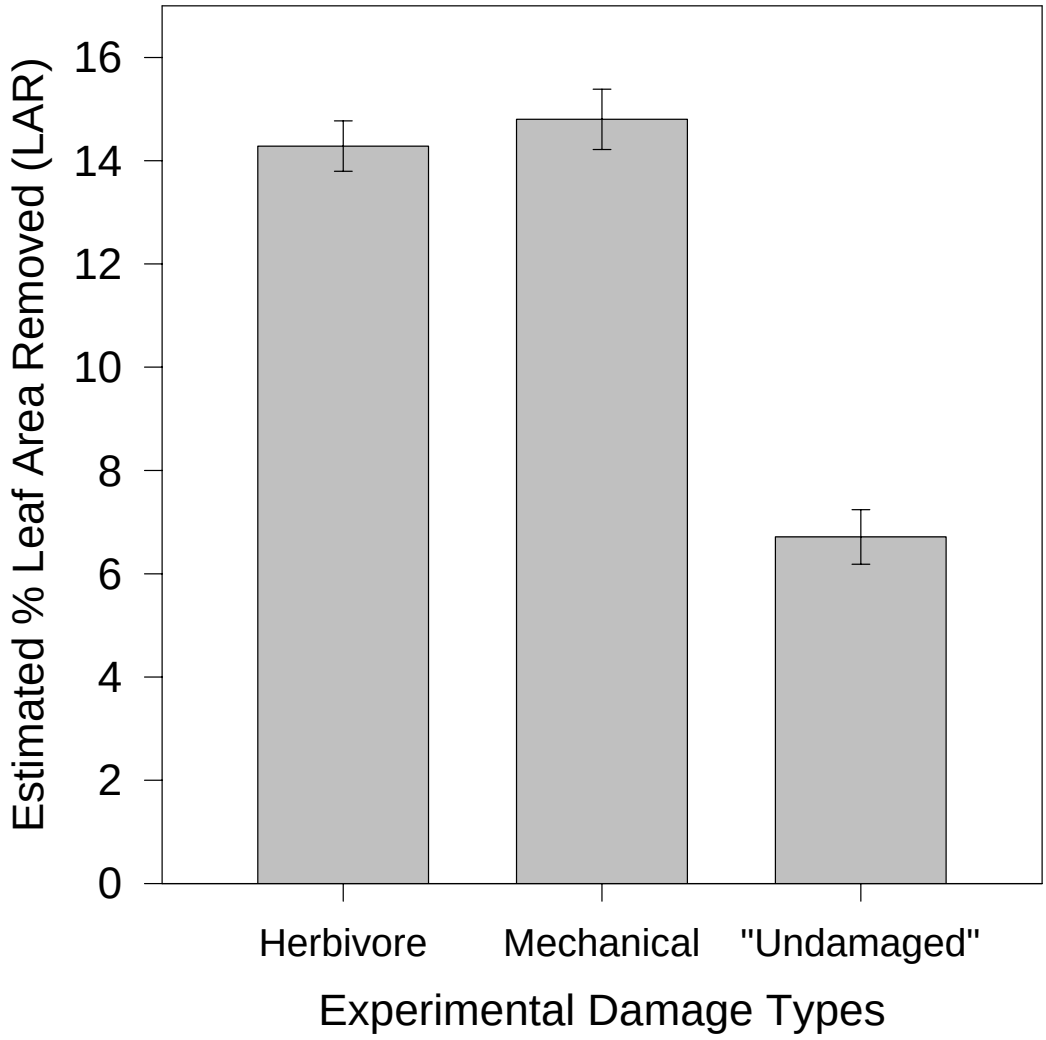

Estimated % Leaf Area Removed (LAR)
0
2
4
6
8
10
12
14
16
Herbivore
Mechanical
"Undamaged"
Experimental Damage Types

Figure 2.3 Soil NH_4-N concentrations in the presence and absence of insect frass as estimated by ion-exchange resin bags. Bags were in the pots for one month (both pre- and post-treatment) and extracted with 1M KCl. "RM_{NP}" indicates that the data were analyzed as repeated measures with the non-parametric GENMOD procedure. Points are means of 60 samples and error bars are provided for visual reference only.

Figure 2.3

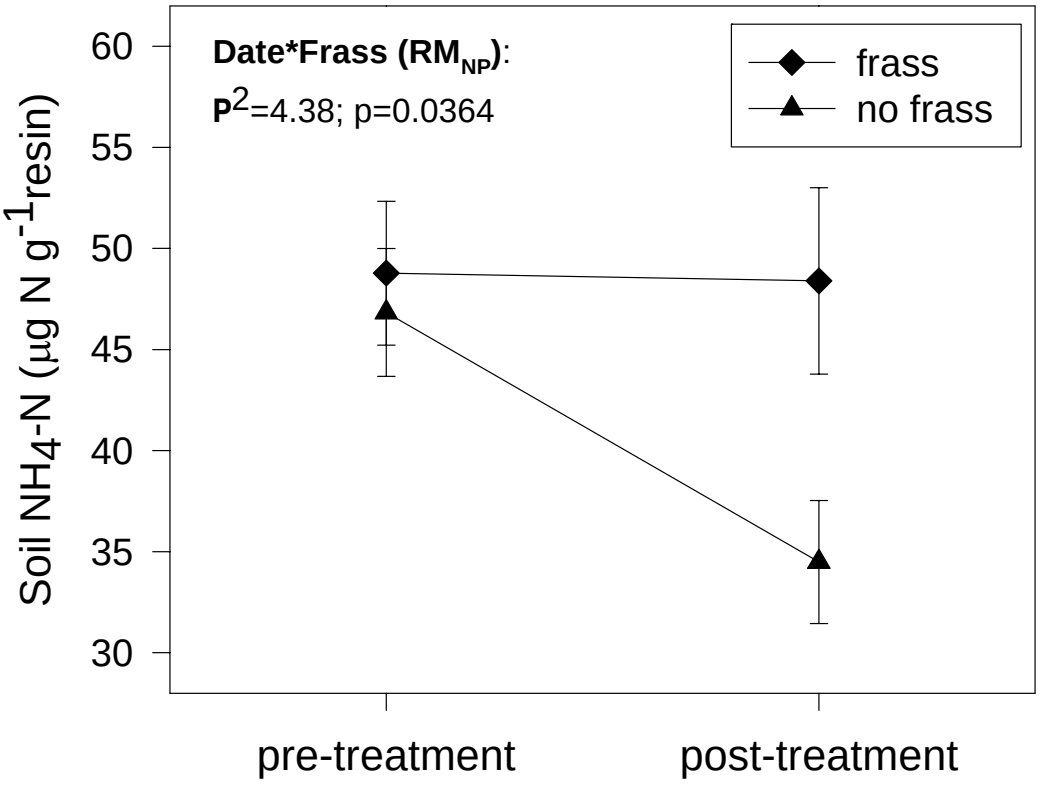

Date*Frass (RM$_{NP}$):
P^2=4.38; p=0.0364
frass
no frass
Soil NH$_4$-N (μg N g^{-1}resin)
60
55
50
45
40
35
30
pre-treatment
post-treatment

Figure 2.4 Total C and N concentrations from soil extracts under frass and defoliation treatments on *Q.rubra*. Different letters within a graph indicate statistically significant differences between treatment groups using the SNK post hoc test (α=0.05). Bars are the means of 60 samples (frass treatments) and 40 samples (damage treatments); error bars represent +/- 1 SE.

Figure 2.4

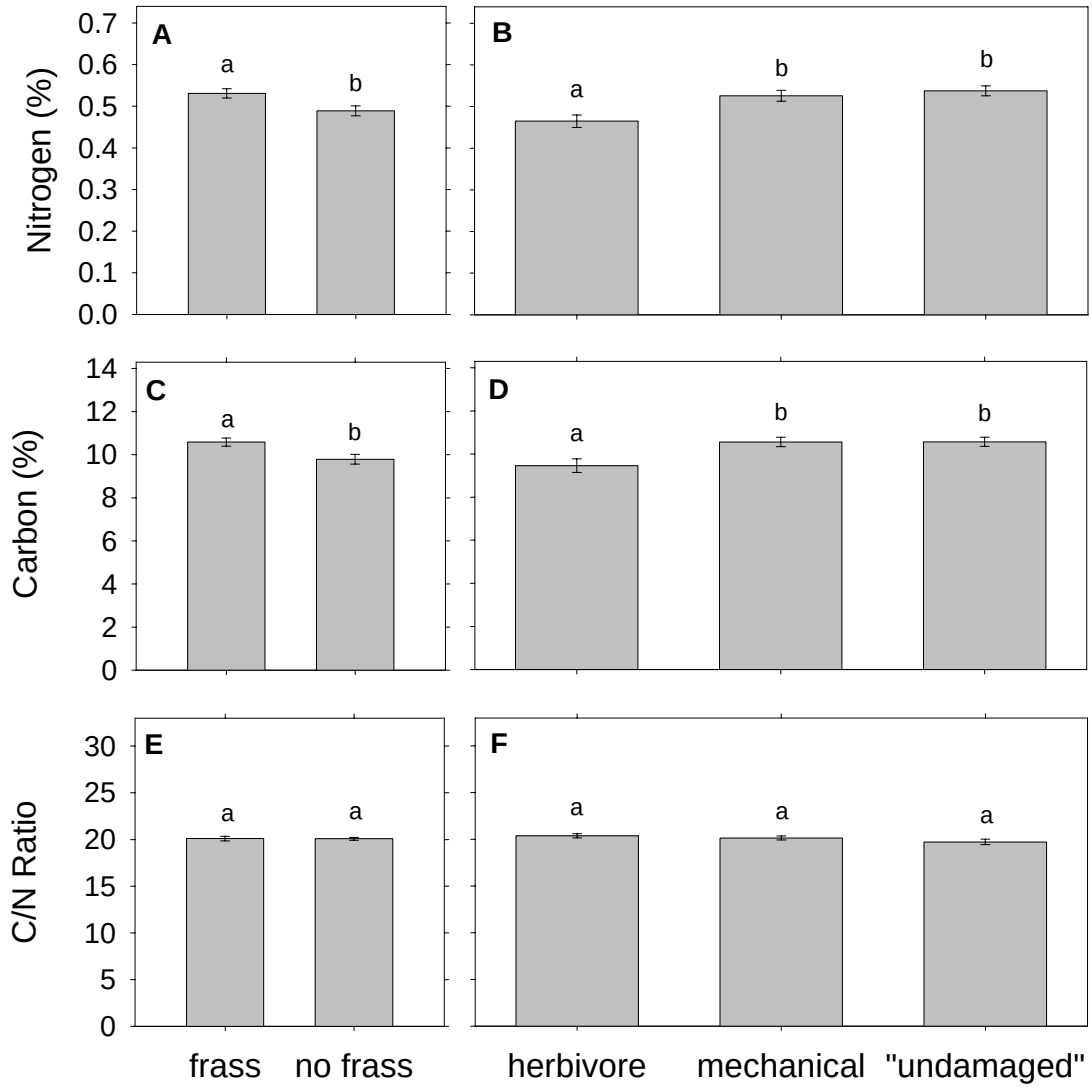

A
B
C
D
E
F
Nitrogen (%)
Carbon (%)
C/N Ratio
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0
2
4
6
8
10
12
14
0
5
10
15
20
25
30
a
b
frass
no frass
herbivore
mechanical
"undamaged"

Figure 2.5 Concentrations of NO_3-N in soil extracts and leachate from *Q.rubra* miniecosystems. (A) Soil NO_3-N concentrations under 3 defoliation treatments were estimated by ion-exchange resin bags extracted with 1M KCl. "RM_{NP}" indicates that the data were analyzed as repeated measures with the non-parametric GENMOD procedure. Points are the means of 40 samples; error bars are provided for visual reference only. (B) Leachate NO_3-N compared between frass treatment groups. "RM_P" indicates that data were analyzed as repeated measures using the parametric ANOVA. Different letters within each date indicate statistically significant differences between treatment groups using the SNK post hoc test (α=0.05). Points are the means of 60 samples and error bars represent +/- 1 SE.

Figure 2.5

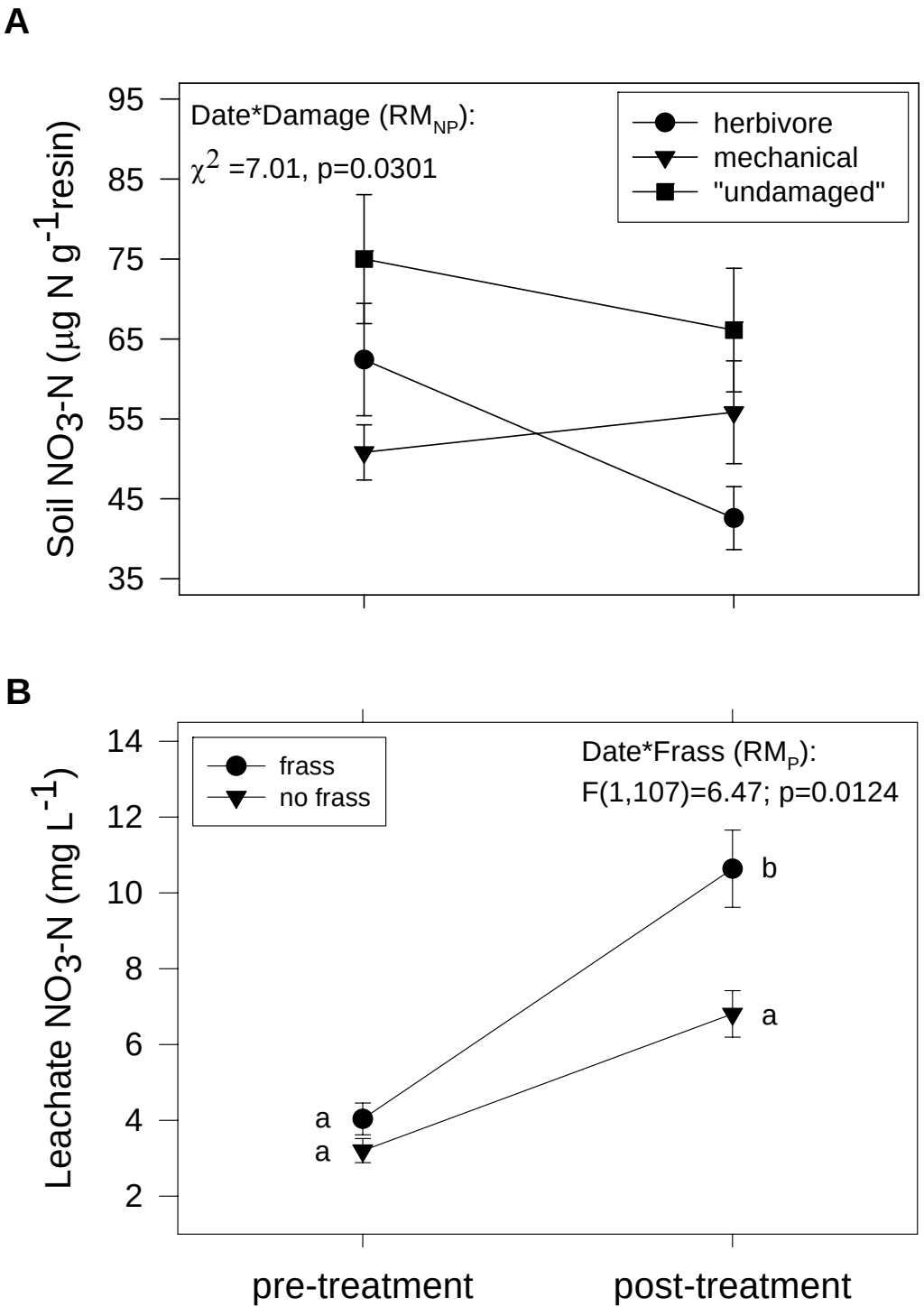

A
Date*Damage (RM_NP):
χ2 =7.01, p=0.0301
herbivore
mechanical
"undamaged"
Soil NO3-N (µg N g-1resin)
95
85
75
65
55
45
35
B
frass
no frass
Date*Frass (RM_P):
F(1,107)=6.47; p=0.0124
Leachate NO3-N (mg L-1)
14
12
10
8
6
4
2
b
a
a
a
pre-treatment
post-treatment

Figure 2.6 Soil microbial biomass C, as estimated using the fumigation-extraction method, compared between frass treatment groups. Different letters above the bars indicate statistically significant differences between treatment groups using the SNK post hoc test (α=0.05). Bars are the means of 60 samples and error bars represent +/- 1 SE.

Figure 2.6

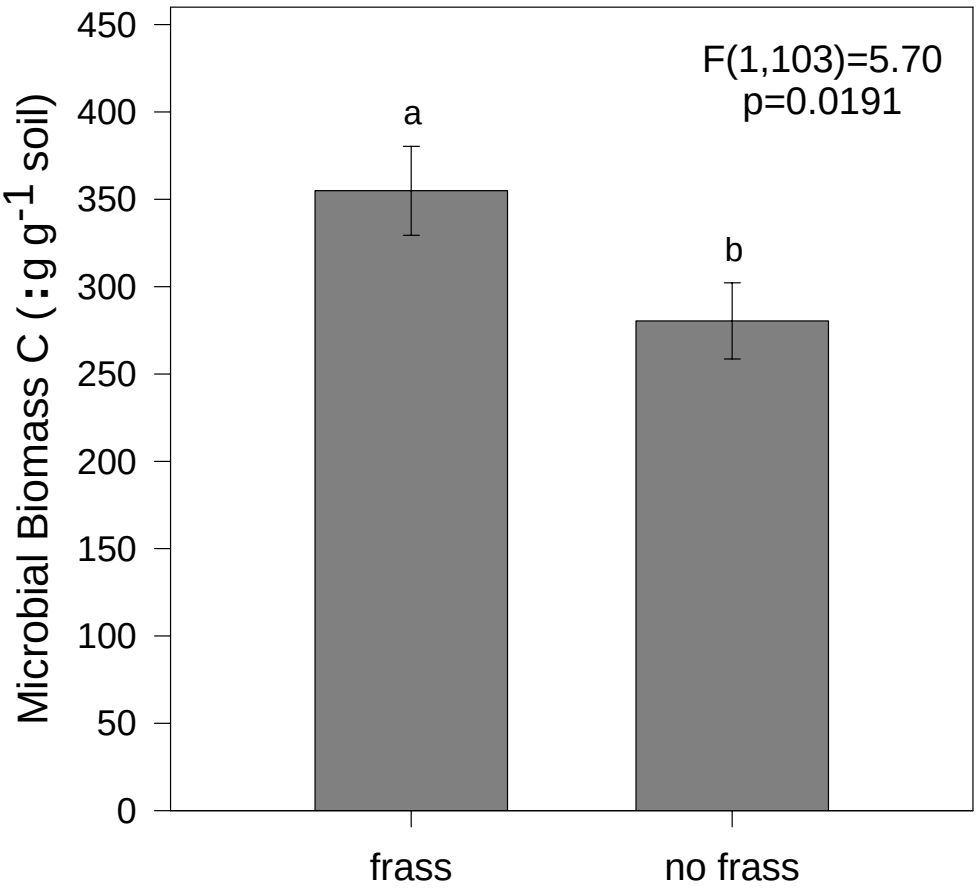

F(1,103)=5.70
p=0.0191
a
b
Microbial Biomass C (μg g-1 soil)
450
400
350
300
250
200
150
100
50
0
frass
no frass

Figure 2.7 The response of soil respiration to defoliation treatments on *Q.rubra*. Soil respiration measurements were made *in situ* with a portable soil respiration meter. "RM_P" indicates that data were analyzed as repeated measures using the parametric ANOVA. The herbivore damage group was statistically different (α=0.05) from the control group for each of the last six sampling dates presented. The vertical reference line represents the initiation of experimental manipulations (June 5, 2003, Julian Date 156). The mechanical damage group was statistically different (α=0.05) from the control group on the dates indicated with a double asterisk (**). Points are the means of 40 samples and error bars represent +/- 1 SE.

Figure 2.7

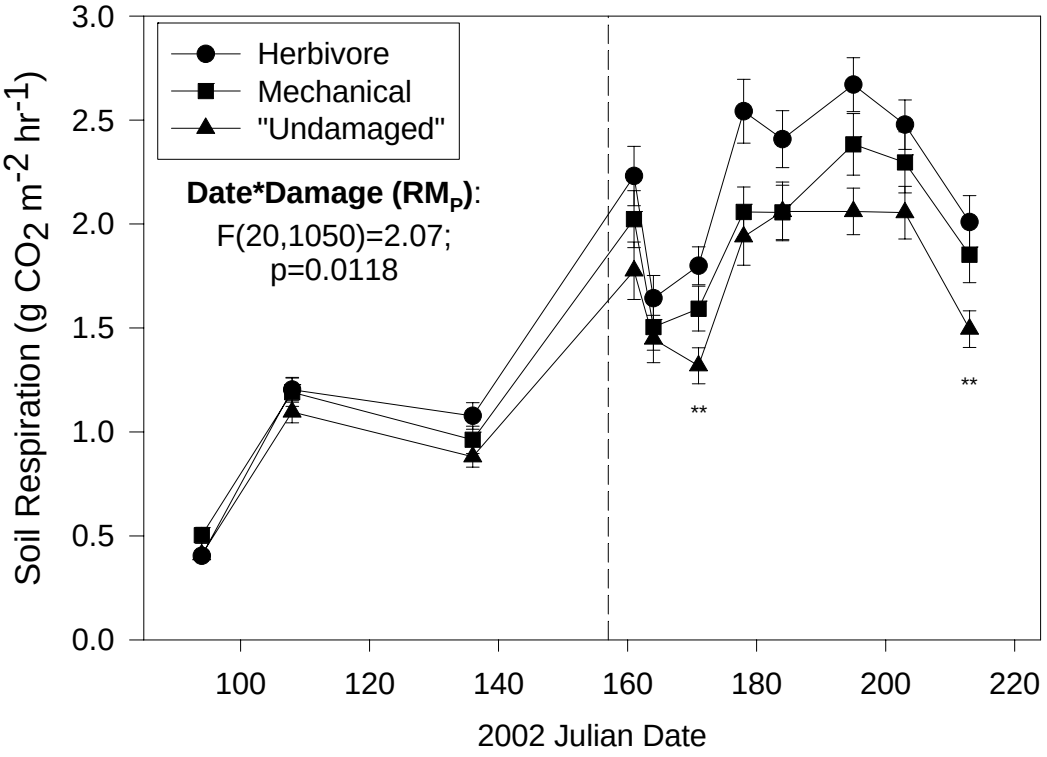
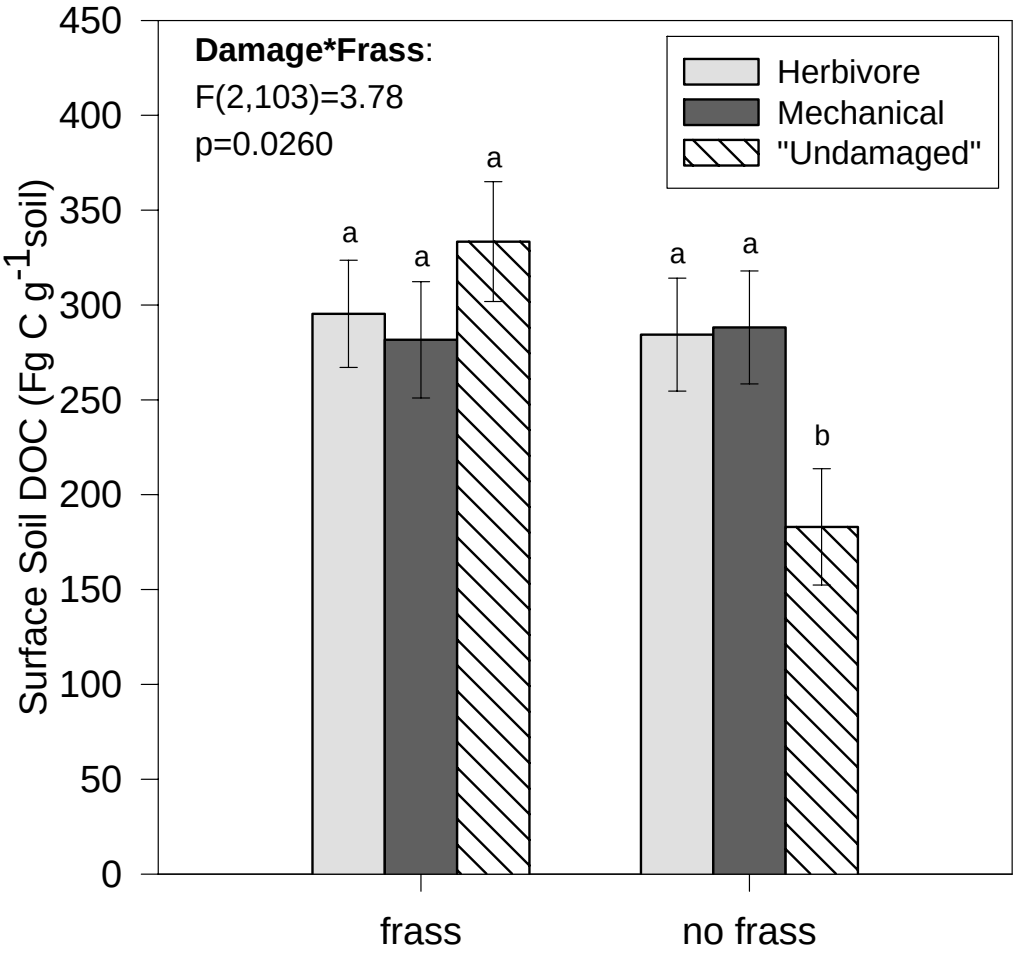

Herbivore
Mechanical
"Undamaged"
Date*Damage (RM$_P$):
F(20,1050)=2.07;
p=0.0118
Soil Respiration (g CO$_2$ m^{-2} hr^{-1})
3.0
2.5
2.0
1.5
1.0
0.5
0.0
100
120
140
160
180
200
220
2002 Julian Date
**
**

Figure 2.8 Surface soil DOC measurements in *Q.rubra* mini-ecosystems. Soil samples were taken 1 month following damage treatments and frass additions. DOC is here defined as the K_2SO_4-extractable fraction of soil C. Similar letters above the bars indicate statistical similarity via the SNK post-hoc test (α=0.05). Bars are the means of 20 samples and error bars represent +/- 1 SE.

Figure 2.8


Damage*Frass:
F(2,103)=3.78
p=0.0260
Herbivore
Mechanical
"Undamaged"
Surface Soil DOC (Fg C g-1soil)
450
400
350
300
250
200
150
100
50
0
a
a
a
a
a
b
frass
no frass

CHAPTER 3

INSECT HERBIVORES AND THEIR FRASS AFFECT LEAF AND LITTER QUALITY BUT NOT DECOMPOSITION RATES OF *QUERCUS RUBRA*[1]

[1]Frost, C.J. and M.D. Hunter. Submitted to Oecologia May 5, 2005

ABSTRACT

Defoliation-induced changes in plant foliage are ubiquitous, though factors mediating induction and the extent of their influence on ecosystem processes are poorly understood. Soil nitrogen (N) availability, which can be affected by insect herbivore frass (feces), influences phytochemical induction. We conducted an experiment to test the hypotheses that insect frass deposition would (1) reduce phytochemical induction following herbivory and (2) increase the decomposition rate of the subsequent leaf litter. During the 2002 growing season, 120 *Quercus rubra* saplings were subjected to a 3x2 factorial experimental design with 3 damage groups (herbivore, mechanical, undamaged) and 2 frass levels (frass, no frass). One pre-treatment and three post-treatment leaf samples were collected for chemical analysis. Following senescence, litter samples from the treatment groups were decomposed in litterbags for 18 months at the Coweeta Hydrologic Laboratory, NC. Herbivore feeding, not mechanical wounding, increased total phenolics and tannins, but frass deposition did not mitigate the induction of tannins or total phenolics. Higher tannin concentrations in herbivore-damaged foliage were short-lived; tannin concentrations among treatments were similar by the late season. Herbivore activity resulted in higher specific leaf masses, presumably as a result of phytochemical induction of condensed tannins. Damage (independent of type) reduced foliar N concentrations throughout the growing season and foliar lignin concentrations in the late-season foliage. Despite phytochemical induction in foliage, tannin concentrations in litter samples were lower in the damaged groups and higher in the frass addition group than in their respective controls. Mechanical damage had largest effect on

litter chemistry, though decomposition rates among treatments were similar. We discuss the results in the context of feedbacks between herbivores and ecosystem processes.

INTRODUCTION

The ubiquity of induced chemical responses by plants to herbivore activity is now widely recognized (Karban and Baldwin 1997). Despite large advances in our understanding of the biochemistry and molecular biology underlying phytochemical induction (Kessler and Baldwin 2002; Qu et al. 2004; Kessler et al. 2004; Schultz and Appel 2004; Allison and Schultz 2005), basic questions remain regarding the ecological factors mediating induction (Nykanen 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). Processes that affect N availability may therefore influence phytochemical induction (Kyto et al. 1996).

Feedback loops exist between above- and below-ground components of terrestrial ecosystems (Wardle 2002), some of which are influenced by herbivores (Frost and Hunter 2004). If soil N availability is important for induced responses of plants to herbivory (Bjorkman et al. 1998), then herbivore-mediated feedback loops affecting soil nutrient dynamics may influence the intensity and duration of induced chemical changes in plant foliage. Herbivore activity converts leaf N into herbivore biomass and frass, with up to 86% of leaf N consumed by insect herbivores excreted as frass (Hollinger

1986; Lightfoot and Whitford 1990; Lovett et al. 2002). The potential that frass may essentially act as a fertilizer has been suggested previously (Haukioja et al. 1985), and field (Reynolds et al. 2000; Christenson et al. 2002) and lab (Lovett and Ruesink 1995) experiments generally support this hypothesis. For example, frass deposition following herbivore feeding can increase soil N pools (Frost and Hunter 2004), generating a transient nutrient supply that could affect induced responses to damage. This study investigates the hypothesis that frass deposition following insect herbivore activity will affect herbivore-mediated phytochemical induction in *Q. rubra*. Based on previously published studies (e.g. Bryant et al. 1993; Hunter and Schultz 1995; Kyto et al. 1996), we expected that frass additions would reduce the induction response as has been shown with inorganic fertilizer. Phytochemical induction can also be damage-specific (Dyer et al. 1993; Dyer et al. 1995; Alborn et al. 1997; De Moraes et al. 2001; Oppenheim and Gould 2002), and our study therefore included a mechanical wounding treatment in addition to real herbivory to explore the effect of frass deposition on induction following damage per se as well as herbivore-specific damage.

One less-explored potential effect of herbivore activity is 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). Herbivore damage in oaks generally increases tannin concentrations, which should decelerate decomposition

(Hättenschwiler & Vitousek 2000). However, herbivore activity may also alter the timing of senescence and leaf drop, which may affect resorption efficiency and the nutrient content of the litter (Killingbeck 1996). High nutrient concentrations in litter following premature leaf drop would be predicted to accelerate decomposition, and potentially counteract the effect of higher tannin content (Cornelissen et al. 1999). Any lasting effects of frass deposition on leaf and subsequent litter quality may also affect decomposition and alter the predicted outcomes of the deceleration effect (Hunter 2001).

We report here the results of a controlled, factorial experiment to isolate the effects of frass deposition and damage on phytochemical induction in *Quercus rubra* saplings and subsequent decomposition of the senesced litter. The experimental design allowed us to explore the independent and interactive effects of aboveground damage and frass deposition on phytochemical induction and subsequent leaf litter decomposition.

METHODS

Field Site for Experimental Manipulations

Detailed description of the potted *Quercus rubra* experimental array can be found in Frost and Hunter (2004). Briefly, 160 nursery-grown *Q. rubra* saplings (Forest Keeling Nursery, Missouri), were established in August 2001 in 14-gallon pots suspended in individual stands using soil and litter from watershed 27 ("WS27") at the Coweeta Hydrologic Laboratory Long Term Ecological Research (LTER) Site ("CWT," Otto, NC, elevation 1300 m). The *Q.rubra* saplings were 1.33±0.14 m tall and averaged 13.71±0.18 mm in width (an average of two measurements made 10 cm from base of

soil) at the beginning of the 2002 growing season. We repeated width measurements following complete leaf drop in December 2002 as an estimate of seasonal growth rate.

Experimental Manipulations

Specific details of the experimental manipulations are reported in Frost and Hunter (2004). Briefly, we used a 3x2 factorial experimental design with three damage (herbivore, mechanical, undamaged) and two frass (frass, no frass) levels. Twenty trees (replicates) were randomly selected for each of the six treatments (N=120), with 20 supplemental trees receiving herbivore in order to generate enough frass for the experiment. The supplemental trees were not used or sampled subsequently.

Herbivores were contained within hand-sewn bags of Reemay® agricultural cloth, and all trees were covered with the bags so that any effects of the bags on trees (e.g., reduced photosynthesis) would occur across all treatments and controls. Each bag enclosed 65-85% of the total leaf area of the sapling. Fourth-fifth instar insect herbivores were added to trees on June 5, 2002 and removed on June 15, 2002. The large majority of defoliation by early-season Lepidopteran defoliators occurs in the final two instars, and the experimental period of 10 days covers approximately the length of those instars in the field. We estimated damage as leaf area removed (LAR) within two days of the discontinuation of herbivore treatments using techniques described in Hunter (1987). Herbivore activity significantly increased estimated leaf area removed (14.3% LAR versus 6.7% LAR; $t_{73}=10.54$; $p<0.0001$; Frost and Hunter 2004). We also simulated herbivory by mechanically removing leaf tissue with scissors. Mechanical damage was

imposed concurrently with herbivore damage and mimicked herbivory in terms of the total leaf area removed (t_{75}=-0.69; p=0.4952) and the manner in which the foliage was removed. We also replicated as closely as possible the proportion of leaves damaged, and only leaves that were contained in a bag were clipped by the mechanical treatment. Mechanical damage occurred on three discrete dates (June 7, 10, and 15).

Frass from each herbivore-infested tree was collected by making a small incision in the bag, transferring frass pellets to collection vials using a modified insect aspirator, and resealing the incision. On the same day, the frass collected from each tree (60 total trees) was individually weighed, pooled, and redistributed evenly among the appropriate experimental units. Thus, the amount of frass deposition was representative of the average level of damage experienced by the trees. Frass was collected and distributed twice during the experiment (June 10 and June 15). The resulting frass additions totaled approximately 0.982 g frass per tree (dry weight equivalent), corresponding to a deposition of 10 g m^{-2}. The frass was composed of 49.13±0.94% carbon (C), 3.10±0.05% 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.

Leaf Sampling and Analysis

There were four foliage sampling dates over the course of the field season: 1. pre-treatment ("PT", May 29, 2002), 2. post-treatment early-season greenleaf ("EG", July 6, 2002), 3. late-season greenleaf ("LG", September 25, 2002), 4. senescing litter ("SL", October 27, 2002). The rationale for the four sampling dates is described below.

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 3 weeks following complete expansion of all first-flush foliage and 6 days before experimental manipulations. All leaf samples for the pre-treatment cohort were categorized in the 0-5% LAR class. We restricted samples to leaves in terminal stems receiving full sun. Three leaf disks were taken from a single leaf per tree (trees as replicates) in the field directly into 70:30 acetone-water with 1mM ascorbic acid and stored in the dark on dry ice during collection. On each sampling date, 15 leaf disks were also taken from 2-3 additional leaves per tree and pooled to prepare a single bulk standard for the phenolic assays (described below). On the same day, the area of the sample leaves was measured with a LiCor 3000A area meter (LiCor, Lincoln, NE, USA) and wet weight was measured for each sample leaf. Leaves were then dried for 48 h at 60^{o}C and dry weights were taken to calculate leaf water content. The leaf area and dry weight measurements were also used to determine the specific leaf mass ("SLM," mg cm^{-2}) and its reciprocal, specific leaf area ("SLA," cm^2 mg^{-1}), for each leaf sample (Yin 2002). The dried leaves were then finely ground in a ball mill and the resulting powder was used to measure total carbon (C), N, and three fiber measurements (lignin, cellulose, hemicellulose).

The EG sample date was approximately 1 month following the initiation of experimental manipulations, which is sufficient time for the mineralization of frass N deposited as a consequence of the herbivore feeding (Frost and Hunter 2004). The LG sample (late September) was in the middle of the natural feeding phenology of fall-

feeding insect herbivores of oak (Hunter and Schultz 1995). The LG sampling date was chosen to determine the effect of early season herbivory on the quality of foliage available to late season herbivores. The EG and LG samples were collected and processed similarly to PT samples, though the damage classifications chosen for chemical analysis necessarily differed. The majority of the damaged foliage was in the 5-30% damage class, and we haphazardly selected leaves in this damage class from both herbivore and mechanical damage groups. Leaf samples from the undamaged group remained in the 0-5% LAR damage class. All sample leaves were from the area of trees that had been bagged during defoliation.

The senescing litter samples (SL) were used as a link between the greenleaf samples and the litter samples used in the decomposition experiment described below. Leaves were brown and apparently senesced (though not abscised) when this sample was taken, though N contents suggested that senescence was incomplete (see *Results*). Samples were collected directly from each tree by gently shaking each tree and collecting the material that fell. We maintained the convention of using damaged foliage from herbivore and mechanical damage groups and undamaged litter from the undamaged group. Specific leaf mass could not be determined because the foliage had deformed during senescence. Senescing litter was first air dried for 2 weeks in the laboratory. After grinding each sample in the ball mill, approximately 20 mg of dry powder per sample was removed and stored at -80^{o}C for phenolic assays. Samples were then dried overnight at 60^{o}C before removing sub-samples for C:N and fiber analyses.

Total C and N were measured with a Carlo Erba 1500N total CHN analyzer

(Carlo Erba Instrumentazione, Milan, Italy). The three fiber measurements were determined by sequential acid digestions using an ANKOM A200 Fiber Analyzer (ANKOM Technology, Macedon, NY). Phenolics were assayed colorimetrically following well-established methods (Bate-Smith 1977). The Folin-Denis assay was used to estimate total phenolics, as the focus of this study on a single taxon overcame the limitations of the redox-based assay (Appel et al. 2001). Condensed tannins were assayed following acid depolymerization in a polar solvent (N-butanol) at 100ºC (Hagerman and Butler 1980; Hagerman 1988), and hydrolyzable 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. For the litter samples, 5 g of litter was amassed by pooling equal subsamples from each tree and used, after extraction, to generate standard curves.

Decomposition Experiment

Leaf litter was again collected in mid-November 2002. By this point, foliage remaining on the trees was visibly dead and buds had formed. During this collection, litter was collected by gently shaking the mainstem to release the litter. Because the amount of litter per tree was not sufficient for individual replicates, litter was collected and pooled in five replicate groups per experimental treatment. Litter was air-dried in the laboratory in closed, suspended paper bags, and subsequently partitioned into 1x1 cm

mesh bags (Crossley and Hoglund 1962). Each bag initially contained approximately 3 g of dry leaf litter. The litter bags were established on February 1, 2003 in a randomized grid design on watershed 27 at CWT (Elevation 1374m [4598 ft], N 35.03143° W 083.45997°). For each litter bag, the randomized grid location was first cleared of fresh litterfall, the bag was placed on the remaining O horizon and impaled in one corner with a labeled field flag. The surface litter was then replaced on top of the litterbag. Replicate packs from each of the six treatment groups were collected at 3, 9, 12, and 18 months. In addition, a set of replicate packs was immediately collected to determine initial litter quality parameters.

For each collection date, thirty bags (5 replicates x 6 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 gently cleared and the litterbag was placed into a plastic ziplock bag. Samples were stored at ambient temperature in a cooler during transport to the laboratory.

Dried leaf tissue was weighed to determine mass loss and ground to a fine powder that was used for analysis of phenolics, fiber, and total C and N. Approximately 20 mg of powder was removed from the sample and stored at -80°C for phenolic assays. The remaining litter powder was dried overnight at 60°C, and subsamples were analyzed for total C and N and three fiber measurements. Samples were processed as described above.

Statistical Analyses

Data were analyzed using ANOVA models generated by the GLM procedure of SAS 8.2 (SAS Institute, Cary, NC); the residuals of the models were tested for normality (Kery and Hatfield 2003). The two fixed factors in the model were "damage" (herbivore, mechanical, undamaged) and "frass" (frass, no frass); the interactive "damage*frass" term was also included. Data were transformed when necessary to satisfy model assumptions. A repeated-measures framework was used when appropriate to test for within-subjects as well as between-subjects effects. We used the Student-Neuman-Keuls (SNK) test (α=0.05 unless otherwise noted) to distinguish among treatment means.

Stepwise regression (PROC STEPWISE) was used to identify leaf or litter quality parameters that were the best predictors of SLM and decomposition rate. A p-value of 0.05 was established for parameters of remain in the stepwise model. Stepwise regressions for SLM were performed for each of the three greenleaf sampling dates using date-specific leaf quality parameters from each tree. In addition to leaf quality parameters, the SLM stepwise regressions also included budbreak date, total leaf area, and soil available NO_3^- and NH_4^+. Soil nutrient concentrations were reported in Frost and Hunter (2004). Budbreak date was independently assessed as the date at which half of a set of twenty buds had (1) initiated expansion and (2) formed visible green tissue. We estimated total leaf area by multiplying the total number of leaves per tree by the average area of 20 randomly-selected leaves non-destructively measured using a LiCor LI3000 portable leaf area meter (LiCor, Lincoln, NE).

We analyzed litter decomposition rate using methods modified from population

time series analysis (Royama 1992; Madritch and Hunter 2002; Madritch and Hunter 2005). The rate of change (Δ) 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 through the number of months in the time interval and performing the stepwise regressions for each ΔMR against our estimates of litter quality for the initial samples of that time interval.

RESULTS

Phytochemical Induction following Damage

As expected, condensed and hydrolyzable tannin concentrations increased in the green foliage following herbivore, but not mechanical, damage (Date*Damage $F_{4,200}$=2.66, p=0.0429; $F_{4,198}$=6.17, p=0.0001, respectively; Table 1). Total phenolics were also induced by herbivory (Date*Damage $F_{4,200}$=3.62, p=0.0072, Table 1). Total phenolics in the mechanical damage group were consistently higher than those in the undamaged group, including in the pretreatment. However, there was no Date*Damage effect when comparing just the mechanical and undamaged groups ($F_{2,130}$=0.14, p=0.8556, Table 1), suggesting that mechanical damage did not induce increases in total phenolics. In all cases, induction was only apparent in the EG sample and the tannin concentrations in the LG samples were similar among treatment groups. Phenolic concentrations in the senescing leaf litter were slightly lower in both herbivore and mechanical damage groups (Table 1), an effect that reached statistical significance in the initial decomposition samples (see below).

Both herbivore and mechanical damage treatments affected lignin, N, and C:N ratios. Foliar lignin concentrations were lower in herbivore and mechanical damage groups relative to the undamaged group, but only in the late season green foliage ($F_{6,285}$=3.01, p=0.0076, Table 1). Leaf N concentrations were lower in both herbivore and mechanical damage groups relative to the undamaged group following the treatment (Date*Damage $F_{4,200}$=2.81, p=0.0338), and the effect lasted throughout the growing season (Table 1). The damage effect on N resulted in elevated C:N ratios that also lasted throughout the growing season (Date*Damage $F_{4,200}$=5.65, p=0.0003). Leaf N in the senescing litter was not affected by damage, though there were treatment effects on the initial litter chemistry in the decomposition experiment (see below). Premature leaf abscission was not apparent on the damaged trees relative to the undamaged trees (data not shown).

Frass Effects

Frass additions did not strongly influence any response variables in the foliage (Table 1). However, frass additions did apparently mediate higher condensed and hydrolyzable concentrations in the leaf litter relative to frass-free controls ($F_{1,104}$=4.97, p=0.0280; $F_{1,104}$=3.97, p=0.0489, respectively; Table 1). This result was confirmed in litter chemistry of the initial decomposition samples (see below).

Neither damage nor frass treatments affected the cellulose, hemicellulose, or water contents of the foliage on any sampling date, and there were no treatment interactions among these variables (Table 1, water content data not shown). The same is

true for final stem width measurements and the overall growth rate of the saplings as measured by the difference between stem width measurements before leaf flush and following complete leaf drop for the 2002 growing season (data not shown). There were no damage*frass interactions on any index of green leaf quality.

Specific Leaf Mass

Specific leaf mass (SLM) increased in the herbivore and mechanical groups in the first month following damage, but induction was lost in late-season foliage (Figure 1a). The herbivore group had a stronger effect on SLM than did the mechanical group, though both were statistically different from the undamaged group (Date*Damage $F_{4,200}$=5.18, p=0.0005). Therefore, the rate of leaf toughening increased following damage but the overall change was the same among groups. Specific leaf area (SLA), the inverse of SLM, has been strongly linked to both leaf N and photosynthetic potential (Reich et al. 1997). However, the results of the stepwise regression did not suggest that foliar N was influential on SLM (Figure 1b-d). The timing of budbreak was strongly correlated with SLM in the pre-treatment sample, while leaf N and total phenolics were weaker predictors (Figure 1b). Following the treatment, SLM was consistently, positively correlated with condensed tannin concentrations (Figure 1c,d). In addition, positive correlations between the rates of change of SLM and condensed tannin concentrations between the PT-EG and EG-LG sampling periods were statistically significant (r^2=0.134, p<0.0001; r^2=0.126, p<0.0001, respectively, data not shown). Lignin and cellulose failed to enter any of the models.

Decomposition Experiment

Damage and frass treatments independently and interactively affected our measures of initial litter chemistry prior to decomposition, though biologically-meaningful patterns were not obvious. In general, hydrolyzable and condensed tannins were higher with frass additions ($F_{1,29}$=26.58, p<0.0001; $F_{1,29}$=40.115, p<0.0001, respectively) and lower with damage ($F_{2,29}$=15.21, p<0.0001), while the effect of frass on tannins was strongest in the undamaged group (Figure 2a,b). Total phenolics followed the same pattern, though effects were weaker (Figure 2c). Treatment effects on litter N concentrations were statistically significant but idiosyncratic ($F_{5,29}$=22.51, p<0.0001, Figure 3a), and both the lignin:N and C:N ratios mirrored total N (Figure 3b,c).

Treatment-based changes in litter chemistry were also observed during decomposition (Figure 3, Table 2). Condensed tannins were lower in the frass addition group in the 3-month samples (Date*Frass $F_{4,96}$=17.91, p<0.0001; Figure 3a), though they were higher in the initial samples. Lignin content was lower in the frass addition group in the three month sample (Table 2). All other apparent frass effects on initial leaf chemistry disappeared by the 3 month sample. Hydrolyzable tannins and total phenolics, though lower initially in both damage groups, were higher than those in the undamaged group in the 3rd month (Date*Damage $F_{8,96}$=6.84, p<0.0001; $F_{8,96}$=14.11, p<0.0001, respectively; Figure 3d,f). Tannins remained higher in the mechanical damage group in the 9th month. The mechanical damage group also had higher N and lower C:N and Lignin:N in later stages of decomposition when the herbivore and undamaged groups were similar (Table 2).

Despite apparent damage- and frass-mediated differences in the chemistry and stoichiometry of leaf litter, rates of mass loss were unaffected by either damage or frass treatments (date*damage $F_{8,96}$= 0.95, p=0.4745; date*frass $F_{4,96}$= 0.64, p=0.6103, respectively, Figure 4a). Overall, the data fit a standard first order decomposition model (k=-0.0217 $month^{-1}$, r^2 = 0.90). However, significant deviations from the overall decomposition constant were observed when we calculated the rate of change in mass loss (ΔMR) between consecutive sampling dates (Figure 4a). Compared to the overall k, ΔMR was faster during the 3-9 month interval and slower during the 9-12 and 12-18 month intervals ($F_{3,119}$=9.49, p<0.0001). Total lignin concentration correlated negatively with ΔMR in the 9-12 and 12-18 month intervals (r^2= 0.2108, p= 0.0107; r^2= 0.2103, p= 0.0108, respectively; Figure 4c). Lignin was the only litter quality variable that entered any of the stepwise regression models.

DISCUSSION

Our previous research in this system has demonstrated that insect herbivore frass deposited on forest soil can result in ephemeral increases in soil mineral N availability (Frost and Hunter 2004). The purpose of the current study was to explore the relative influence of those frass additions and the herbivore activity that produced them on phytochemical induction during the season of defoliation and through the first 18 months of litter decomposition. The results did not support our initial hypotheses: frass deposition following moderate levels of herbivore feeding neither mitigated phytochemical induction nor influenced subsequent leaf litter decomposition.

Effects of Frass Deposition on Phytochemical Induction

Previous studies demonstrating reductions in phytochemical induction following additions of exogenous inorganic fertilizer (e.g., Bryant et al. 1993; Hunter and Schultz 1995) were designed primarily to test the carbon-nutrient balance hypothesis (Bryant et al. 1983), which is no longer widely accepted (Hamilton et al. 2001). Our current study was not a test of the carbon-nutrient balance hypothesis. Rather, 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 similar reductions in phytochemical induction would be observed following herbivore-mediated changes in soil N. There are two obvious differences between inorganic fertilizer applications and frass deposition: the magnitude of additions and the source of N. Hunter and Schultz (1995) reported mitigation of tannin induction when 18.2 g N per tree was applied in a time-release fertilizer, which is three orders of magnitude more N than that applied as frass in the current experiment. Bryant et al. (1993) fertilized in discrete intervals over the course of several years to achieve an N supply "exceeding that found under natural conditions" (p.2075) in order to remove all N limitation, again greatly exceeding our N additions. Even a large herbivore outbreak event (e.g., Reynolds et al. 2000) would only contribute 1 to 2 g N m^{-2} during the outbreak, which is half of a single fertilizer application in Bryant et al. (1993).

Secondly, 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 (Frost and Hunter,

unpublished data); the overall mass balance of herbivory is a loss in N for the defoliated plant. This is a general trend for any level of herbivory. Standard induction experiments without explicit fertilization treatments (reviewed in Nykanen & Koricheva 2004) remove N via damage without "replacing" it to the soil, as occurs with frass deposition. 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. There were no damage*frass effects on any metric of leaf quality (e.g., tannins, N, fiber) in our experiment; frass did not mitigate tannin induction. In fact, saplings receiving frass additions had higher tannin concentrations in their leaf litter (Figure 2). The event of damage on tannin induction evidently overwhelms any potential mitigating effect of N returned to the oak as frass. Fertilization events independent of damage that does 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.

Independent Dynamics of Foliar Tannins and Nitrogen following Damage

While foliar tannin and N concentrations were affected by damage, their dynamics were largely independent. Our manipulation of both real and simulated herbivory demonstrated that both classes of tannins and total phenolics were selectively induced by herbivore - not mechanical - damage. These data are consistent with the view that induction of tannins is an active response to herbivores (reviewed in Nykanen and Koricheva 2004). However, apparently only the rate of tannin production was increased

following herbivore feeding rather than the overall seasonal maximum, a result also consistent with other studies of tannin concentrations within a single growing season (Rossiter et al. 1988; Hunter and Schultz 1993; Hunter and Schultz 1995).

The decline in foliar N (and increase in C:N ratios) occurred regardless of damage type, which suggests that the effect was not an active response to herbivore activity. Previous work with oaks has not consistently shown damage-induced reductions in foliar N (Schultz and Baldwin 1982; Lovett and Tobiessen 1993), and effects on N across multiple plant taxa are also idiosyncratic (Nykanen and Koricheva 2004). In our case, the decline in N could result from a damage-mediated nutrient deficiency if the damage inhibited the plant's ability to acquire new N (Hunter and Schultz 1995), or N could be actively resorbed from the damaged foliage and translocated to other foliage or stored in stem or roots. The higher litter N contents in our study following damage suggest that resorption may have been hindered by damage.

One unexpected result was the apparent reduction in lignin content in fall green foliage in both damage treatments relative to the undamaged group. We are unaware of other studies that have reported changes in leaf fiber throughout a growing season following herbivore manipulations. Lignin is one of the most recalcitrant compounds produced by plants (Boerjan et al. 2003) and is not degraded in vivo. One possible explanation for our observation is that the decline in foliar N following damage reduced lignin biosynthesis, though this hypothesis requires further experimentation.

Specific Leaf Mass/Area

One common response of oak foliage to damage is increasing toughness (Schultz and Baldwin 1982) related to increases in SLM. SLA is the inverse of SLM and declines following herbivory. For example, van Kleunen et al. (2004) simulated herbivory by clipping and additions of jasmonic acid (JA) to *Solidago canadensis* and found a reduction in SLA (increase in SLM) following JA application but not mechanical wounding. SLM has been negatively correlated with net photosynthesis and leaf N across a broad range of taxa (Reich et al. 1997), herbivore spatial abundances (Peeters 2002; Poorter et al. 2004), and litter decomposition rates (Cornelissen et al. 1999). Our data suggest that intraspecific variation in SLM is influenced by foliar characteristics other than N, primarily condensed tannins, once the effects of budbreak have faded. This is consistent with the findings of Hattenschwiler and Schafellner (1999), who measured decreased SLA (increased SLM) and increasing condensed tannins under elevated CO_2.

Ecosystem Consequences of Phytochemical Induction

Choudhury (1988) argued that herbivores could affect ecosystem function by altering the quality of leaf litter as a substrate for decomposition, and initial laboratory analyses supported the hypothesis (Findlay et al. 1996). The acceleration/deceleration effect hypothesis formalized a mechanistic framework for predicting the direction of herbivore-mediated changes in decomposition in terrestrial systems (Ritchie et al. 1998). This framework essentially applies to any effect on substrate quality, such as changes in intra- or inter-specific plant diversity (Schweitzer et al. 2004; Madritch and Hunter 2005)

or the effects of elevated CO_2 on litter chemistry (Peñuelas and Estiarte 1998; Norby et al. 2001). The emerging synthesis is that decomposition is initially influenced by litter N and tannin contents (Kraus et al. 2003) and later by lignin (Wang et al. 2004). Our stepwise regression results agree with the latter half of this synthesis (Figure 4c), where lignin concentrations correlate negatively with decomposition rate. However, early in decomposition, substrate quality effects were absent, perhaps overshadowed by abiotic factors (e.g., rainfall) consistent among all experimental treatments.

Two independent long-term studies provide further insight into the effect of herbivores on litter decomposition. Ritchie et al. (1998) showed that herbivores in an oak savanna can decelerate decomposition via their effects on plant community composition by reducing the abundance of plant species with high foliar quality. Chapman et al. (2003) found that herbivores accelerated decomposition via increased litter N contents (and decreased C:N and Lignin:N ratios) in a *Pinus edulis* system. In this case, the effect resulted from herbivore-mediated acceleration of litter fall and incomplete nutrient resorption following premature leaf abscission.

Our study is a short-term analog of Chapman et al. (2003), where the focus was on individuals in a single plant species rather than an entire community. Both treatments in our study apparently affected initial litter tannin and N contents, though not in the expected directions: tannins were lower in damaged groups and higher in the frass group, while nitrogen was higher in damaged groups and lower in the frass group. The higher N in damaged groups could result from incomplete resorption despite no apparent changes in timing of leaf drop. It is 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). This initial effect, as well as the majority of the treatment effects, were short lived and largely limited to the first three months of decomposition. The effects that apparently extended into later stages of decomposition were exclusively in the mechanical damage group. This effect could be driven by tannin type if the specific identity of tannins produced following mechanical damage resulted in greater recalcitrance and binding activity (Maie et al. 2003), which would sequester more N in the litter and reduce C:N and lignin:N stoichiometries. While the result that mechanical damage affects litter quality more than herbivore damage alone is intriguing, the bottom line remains that none of these effects accelerated or decelerated decomposition. Our data therefore suggest that herbivore damage does not affect "slow" cycle nutrient dynamics (*sensu* McNaughton et al. 1988) under all conditions.

CONCLUSIONS

Our data do not support the hypothesis that frass deposition from endemic levels of insect herbivore activity mitigates the defensive response in oaks within the season of defoliation. Such an effect may depend on large N deposition events independent of herbivore activity. Moreover, neither herbivore damage nor frass deposition in the early season affected overall decomposition rates, though both altered litter chemistries during the decomposition process. The influence of moderate herbivore activity on ecosystem processes may therefore be largely limited to the "fast cycle" effects of excreta

production on nutrient dynamics (Frost and Hunter 2004) rather than through the “slow cycle” process of leaf litter decomposition.

ACKNOWLEDGEMENTS

We would like to thank M.Ardon, B.Ball, M.Cabrera, D.Coleman, L.Donovan, C.Hall, P.Hendrix, S.Helms, O.Klienberger, M.Madritch, T.Muenz, B.Nuse, S.Scott, K.Wickings, C.Zehnder, and especially R.Goergen for field assistance and/or comments on earlier drafts of the manuscript. We thank G.H. and J.K. Cooke for logistical support. This research was supported by the Coweeta LTER and NSF grants DEB-9815133 to and DEB-0404876 to MDH. All experiments reported here comply with the current laws of the United States of America.

REFERENCES

Alborn HT, Turlings TCJ, Jones TH, Stenhagen G, Loughrin JH, Tumlinson JH (1997) An elicitor of plant volatiles from beet armyworm oral secretion. Science 276:945-949

Allison SD, Schultz JC (2005) Biochemical responses of chestnut oak to a galling cynipid. J Chem Ecol 31:151-166

Appel HM, Govenor HL, D'Ascenzo M, Siska E, Schultz JC (2001) Limitations of Folin assays of foliar phenolics in ecological studies. J Chem Ecol 27:761-778

Bate-Smith EC (1977) Detection and determination of ellagitannins. Phytochemistry 11:1153-1156

Belovsky GE, Slade JB (2000) Insect herbivory accelerates nutrient cycling and increases plant production. Proc Natl Acad Sci USA 97:14412-14417

Bjorkman C, Kyto M, Larsson S, Niemela P (1998) Different responses of two carbon-based defences in Scots pine needles to nitrogen fertilization. Ecoscience 5:502-507

Boerjan W, Ralph J, Baucher M (2003) Lignin biosynthesis. Ann Rev Plant Biol 54:519-546

Bryant JP, Chapin FS, Klein DR (1983) Carbon nutrient balance of boreal plants in relation to vertebrate herbivory. Oikos 40:357-368

Bryant JP, Reichardt PB, Clausen TP, Werner RA (1993) Effect of Mineral Nutrition on Delayed Inducible Resistance in Alaska Paper Birch. Ecology 74:2072-2084

Chapman SK, Hart SC, Cobb NS, Whitham TG, Koch GW (2003) Insect herbivory increases litter quality and decomposition: An extension of the acceleration hypothesis. Ecology 84:2867-2876

Choudhury D (1988) Herbivore induced changes in leaf-litter resource quality: a neglected aspect of herbivory in ecosystem nutrient dynamics. Oikos 51:389-393

Christenson LM, Lovett GM, Mitchell MJ, Groffman PM (2002) The fate of nitrogen in gypsy moth frass deposited to an oak forest floor. Oecologia 131:444-452

Cornelissen JHC, Perez-Harguindeguy N, Diaz S, Grime JP, Marzano B, Cabido M, Vendramini F, Cerabolini B (1999) Leaf structure and defence control litter decomposition rate across species and life forms in regional floras on two continents. New Phytol 143:191-200

Crossley DA, Hoglund MP (1962) A litter-bag method for the study of microarthropods inhabiting leaf litter. Ecology 43:571-573

De Moraes CM, Mescher MC, Tumlinson JH (2001) Caterpillar-induced nocturnal plant volatiles repel conspecific females. Nature 410:577-580

Dyer MI, Moon AM, Brown MR, Crossley DA (1995) Grasshopper crop and midgut extract effects on plants - an example of reward feedback. Proc Natl Acad Sci USA 92:5475-5478

Dyer MI, Turner CL, Seastedt TR (1993) Herbivory and its consequences. Ecol Appl 3:10-16

Findlay S, Carreiro M, Krischik V, Jones CG (1996) Effects of damage to living plants on leaf litter quality. Ecol Appl 6:269-275

Fox CW, Waddell KJ, Groeters FR, Mousseau TA (1997) Variation in budbreak phenology affects the distribution of a leafmining beetle (*Brachys tessellatus*) on turkey oak (*Quercus laevis*). Ecoscience 4:480-489

Frost CJ, Hunter MD (2004) Insect canopy herbivory and frass deposition affect soil nutrient dynamics and export in oak mesocosms. Ecology 85:3335-3347

Glynn C, Herms DA, Egawa M, Hansen R, Mattson WJ (2003) Effects of nutrient availability on biomass allocation as well as constitutive and rapid induced herbivore resistance in poplar. Oikos 101:385-397

Hagerman AE (1988) Extraction of tannin from fresh and preserved leaves. J Chem Ecol 14:453-461

Hagerman AE, Butler LG (1980) Condensed tannin purification and characterization of tannin-associated proteins. J Agric Food Chem 28:947-952

Hamilton JG, Zangerl AR, DeLucia EH, Berenbaum MR (2001) The carbon-nutrient balance hypothesis: its rise and fall. Ecol Letters 4:86-95

Hattenschwiler S, Schafellner C (1999) Opposing effects of elevated CO_2 and N deposition on *Lymantria monacha* larvae feeding on spruce trees. Oecologia 118:210-217

Hättenschwiler S, Vitousek PM (2000) The role of polyphenols in terrestrial ecosystem nutrient cycling. Trends Ecol Evol 15:238-243

Haukioja E, Soumela J, Neuvonen S (1985) Long-term inducible resistance in birch foliage: triggering cues and efficacy on a defoliator. Oecologia 65:363-369

Hollinger DY (1986) Herbivory and the cycling of nitrogen and phosphorous in isolated California oak trees. Oecologia 70:291-297

Hunter MD, Schultz JC (1993) Induced plant defenses breached - phytochemical induction protects an herbivore from disease. Oecologia 94:195-203

Hunter MD, Watt AD, Docherty M (1991) Outbreaks of the winter moth on Stika spruce in scotland are not influenced by nutrient deficiencies of trees, tree budburst, or pupal predation. Oecologia 86:62-69

Hunter MD (1987) Opposing effects of spring defoliation on late season caterpillars. Ecol Entomol 12:373-382

Hunter MD (2001) Insect population dynamics meets ecosystem ecology: effects of herbivory on soil nutrient dynamics. Agric For Ento 3:77-84

Hunter MD, Linnen CR, Reynolds BC (2003) Effects of endemic densities of canopy herbivores on nutrient dynamics along a gradient in elevation in the southern appalachians. Pedobiologia 47:231-244

Hunter MD, Schultz JC (1995) Fertilization mitigates chemical induction and herbivore responses within damaged oak trees. Ecology 76:1226-1232

Hutchens JJ, Benfield EF (2000) Effects of forest defoliation by the gypsy moth on detritus processing in southern Appalachian streams. Am Mid Nat 143:397-404

Karban R, Baldwin IT (1997) Induced Responses to Herbivory. University of Chicago Press, Chicago, IL

Kery M, Hatfield JS (2003) Normality of raw data in general linear models: the most widespread myth in statistics. Bull Ecol Soc Am 84:92-94.

Kessler A, Baldwin IT (2002) Plant responses to insect herbivory: the emerging molecular analysis. Ann Rev Plant Biol 53:299-328

Kessler A, Halitschke R, Baldwin IT (2004) Silencing the jasmonate cascade: induced plant defenses and insect populations. Science 305:665-668

Killingbeck KT (1996) Nutrients in senesced leaves: keys to the search for potential resportion and resorption proficiency. Ecology 77:1716-1727

Kraus TEC, Dahlgren RA, Zasoski RJ (2003) Tannins in nutrient dynamics of forest ecosystems - a review. Plant Soil 256:41-66

Kyto M, Niemela P, Larsson S (1996) Insects on trees: Population and individual response to fertilization. Oikos 75:148-159

Lightfoot DC, Whitford WG (1990) Phytophagous insects enhance nitrogen flux in a desert creosotebush community. Oecologia 82:18-25

Lou Y, Baldwin IT (2004) Nitrogen Supply Influences Herbivore-Induced Direct and Indirect Defenses and Transcriptional Responses in Nicotiana attenuata. Plant Physiol 135:496-506

Lovett GM, Christenson LM, Groffman PM, Jones CG, Hart JE, Mitchell MJ (2002) Insect defoliation and nitrogen cycling in forests. BioScience 52:335-341

Lovett GM, Ruesink AE (1995) Carbon and nitrogen mineralization from decomposing gypsy moth frass. Oecologia 104:133-138

Lovett GM, Tobiessen P (1993) Carbon and nitrogen assimilation in red oaks (*Quercus rubra* L.) subject to defoliation and nitrogen stress. Tree Physiol 12:259-269

Madritch MD, Hunter MD (2002) Phenotypic diversity influences ecosystem functioning in an oak sandhills community. Ecology 83:2084-2090

Madritch MD, Hunter MD (2005) Phenotypic variation in oak litter influences short- and long-term nutrient cycling through litter chemistry. Soil Biol Biochem 37:319-327

Maie N, Behrens A, Knicker H, Kogel-Knabner I (2003) Changes in the structure and protein binding capacity of condensed tannins during decomposition. Soil Biol Biochem 35:577-589

McNaughton SJ, Ruess RW, Seagle SW (1988) Large mammals and process dynamics in African ecosystems. BioScience 38:794-800

Norby RJ, Cotrufo MF, Ineson P, O'Neill EG, Canadell JG (2001) Elevated CO_2, litter chemistry, and decomposition: a synthesis. Oecologia 127:153-165

Nykanen H, Koricheva J (2004) Damage-induced changes in woody plants and their effects on insect herbivore performance: a meta-analysis. Oikos 104:247-268

Ollinger SV, Aber JD, Lovett GM, Millham SE, Lathrop RG, Ellis JM (1993) A spatial model of atmospheric deposition for the northeastern U.S. Ecol Appl 3:459-472

Oppenheim SJ, Gould F (2002) Is attraction fatal? The effects of herbivore-induced plant volatiles on herbivore parasitism. Ecology 83:3416-3425

Peeters PJ (2002) Correlations between leaf structural traits and the densities of herbivorous insect guilds. Biol J Linn Soc 77:43-65

Peñuelas J, Estiarte M (1998) Can elevated CO_2 affect secondary metabolism and ecosystem function? Trends Ecol Evol 13:20-24

Poorter L, de Plassche MV, Willems S, Boot RGA (2004) Leaf traits and herbivory rates of tropical tree species differing in successional status. Plant Biol 6:746-754

Qu N, Schittko U, Baldwin IT (2004) Consistency of *Nicotiana attenuata*'s Herbivore- and Jasmonate-Induced Transcriptional Responses in the Allotetraploid Species *Nicotiana quadrivalvis* and *Nicotiana clevelandii*. Plant Physiol 135:539-548

Reich PB, Walters MB, Ellsworth DS (1997) From tropics to tundra: Global convergence in plant functioning. Proc Natl Acad Sci USA 94:13730-13734

Reynolds BC, Hunter MD (2001) Responses of soil respiration, soil nutrients, and litter decomposition to inputs form canopy herbivores. Soil Biol Biochem 33:1641-1652

Reynolds BC, Hunter MD, Crossley DAJr (2000) Effects of canopy herbivory on nutrient cycling in a northern hardwood forest in western North Carolina. Selbyana 21:74-78

Ritchie ME, Tilman D, Knops JMH (1998) Herbivore effects on plant and nitrogen dynamics in oak savanna. Ecology 79:165-177

Rossiter M, Schultz JC, Baldwin IT (1988) Relationships among defoliation, red oak phenolics, and gypsy moth growth and reproduction. Ecology 69:267-277

Royama T (1992) Analytical Population Dynamics. Chapman & Hall, New York

Schultz JC, Appel HM (2004) Cross-kingdom cross-talk: Hormones shared by plants and their insect herbivores. Ecology 85:70-77

Schultz JC, Baldwin IT (1982) Oak leaf quality declines in reponse to defoliation by gypsy moth larvae. Science 217:149-151

Schweitzer JA, Bailey JK, Rehill BJ, Martinsen GD, Hart SC, Lindroth RL, Keim P, Whitham TG (2004) Genetically based trait in a dominant tree affects ecosystem processes. Ecol Letters 7:127-134

Swank WT, Waide JB, Crossley DAJr, Todd RL (1981) Insect defoliation enhances nitrate export from forest ecosystems. Oecologia 51:297-299

van Kleunen M, Ramponi G, Schmid B (2004) Effects of herbivory simulated by clipping and jasmonic acid on *Solidago canadensis*. Basic Appl Ecol 5:173-181

Wang WJ, Baldock JA, Dalal RC, Moody PW (2004) Decomposition dynamics of plant materials in relation to nitrogen availability and biochemistry determined by NMR and wet-chemical analysis. Soil Biol Biochem 36:2045-2058

Wardle DA (2002) Communities and Ecosystems: Linking the Aboveground and Belowground Components. Princeton University Press, Princeton

Yin XW (2002) Responses of leaf nitrogen concentration and specific leaf area to atmospheric CO2 enrichment: a retrospective synthesis across 62 species. Glob Change Biol 8:631-642

Zogg GP, Zak DR, Pregitzer KS, Burton AJ (2000) Microbial immobilization and the retention of anthropogenic nitrate in a northern hardwood forest. Ecology 81:1858-1866

Table 3.1 Independent Effects of Damage and Frass Treatments on *Quercus rubra* Foliar Chemistry

	Date[1]	Damage Treatment Only			Frass Treatment Only	
		Herbivore	Mechanical	Undamaged	Frass	No Frass
Condensed Tannin (% DW)	PT	6.33 ± 0.58[a]	5.54 ± 0.47[a]	6.08 ± 0.64[a]	5.89 ± 0.43[a]	6.08 ± 0.49[a]
	EG	**14.77 ± 0.88[b]**	12.89 ± 1.02[a,b]	11.32 ± 0.72[a]	13.88 ± 0.77[a]	12.28 ± 0.71[a]
	LG	7.44 ± 0.42[a]	6.53 ± 0.56[a]	6.60 ± 0.57[a]	7.27 ± 0.45[a]	6.46 ± 0.39[a]
	SL	11.02 ± 1.00[a]	9.59 ± 0.92[a]	12.07 ± 1.16[a]	**12.13 ± 0.87[b]**	9.54 ± 0.77[a]
Hydrolyzable Tannin (% DW)	PT	**3.21 ± 0.12[b]**	3.67 ± 0.10[a]	3.71 ± 0.18[a]	3.51 ± 0.11[a]	3.55 ± 0.11[a]
	EG	**8.63 ± 0.46[b]**	7.18 ± 0.45[a]	6.81 ± 0.45[a]	7.97 ± 0.39[a]	7.19 ± 0.37[a]
	LG	9.26 ± 0.30[a]	9.25 ± 0.30[a]	8.71 ± 0.45[a]	9.13 ± 0.31[a]	8.74 ± 0.28[a]
	SL	13.44 ± 1.05[a]	13.44 ± 1.04[a]	14.20 ± 1.07[a]	**14.73 ± 0.82[b]**	12.44 ± 0.78[a]
Total Phenolics (% DW)	PT	5.20 ± 0.21[a,b]	**5.80 ± 0.29[b]**	4.65 ± 0.18[a]	5.18 ± 0.19[a]	5.27 ± 0.24[a]
	EG	**8.82 ± 0.43[b]**	**8.05 ± 0.33[b]**	6.63 ± 0.24[a]	8.14 ± 0.31[a]	7.63 ± 0.30[a]
	LG	8.52 ± 0.30[a,b]	**9.53 ± 0.44[b]**	8.20 ± 0.52[a]	8.95 ± 0.35[a]	8.61 ± 0.35[a]
	SL	8.79 ± 0.62[a]	8.46 ± 0.62[a]	8.70 ± 0.61[a]	9.08 ± 0.48[a]	8.21 ± 0.52[a]
Lignin (% DW)	PT	11.12 ± 0.32[a]	10.63 ± 0.32[a]	10.71 ± 0.30[a]	10.74 ± 0.25[a]	10.91 ± 0.26[a]
	EG	15.20 ± 0.28[a]	14.51 ± 0.29[a]	15.43 ± 0.38[a]	15.32 ± 0.29[a]	14.78 ± 0.23[a]
	LG	**12.96 ± 0.28[b]**	**13.85 ± 0.35[b]**	14.99 ± 0.43[a]	13.67 ± 0.26[a]	14.10 ± 0.35[a]
	SL	15.62 ± 0.32[a]	15.91 ± 0.39[a]	15.96 ± 0.41[a]	15.89 ± 0.28[a]	15.77 ± 0.32[a]
Nitrogen (% DW)	PT	2.30 ± 0.05[a]	2.28 ± 0.04[a]	2.40 ± 0.05[a]	2.31 ± 0.03[a]	2.35 ± 0.04[a]
	EG	**1.82 ± 0.03[b]**	**1.91 ± 0.03[b]**	2.08 ± 0.04[a]	1.97 ± 0.03[a]	1.90 ± 0.03[a]
	LG	**1.76 ± 0.03[b]**	**1.78 ± 0.02[b]**	1.89 ± 0.03[a]	1.83 ± 0.03[a]	1.78 ± 0.02[a]
	SL	1.36 ± 0.08[a]	1.52 ± 0.09[a]	1.36 ± 0.09[a]	1.37 ± 0.06[a]	1.47 ± 0.08[a]
Carbon (% DW)	PT	49.13 ± 0.10[a]	49.28 ± 0.12[a]	49.22 ± 0.10[a]	49.24 ± 0.10[a]	49.19 ± 0.08[a]
	EG	48.76 ± 0.28[a]	49.06 ± 0.23[a]	48.36 ± 0.26[a]	48.89 ± 0.20[a]	48.59 ± 0.22[a]
	LG	50.13 ± 0.32[a]	50.00 ± 0.13[a]	49.82 ± 0.16[a]	50.16 ± 0.23[a]	49.83 ± 0.12[a]
	SL	49.91 ± 0.31[a]	50.25 ± 0.11[a]	50.00 ± 0.13[a]	50.06 ± 0.10[a]	50.06 ± 0.21[a]
C:N	PT	21.71 ± 0.42[a]	21.89 ± 0.39[a]	20.88 ± 0.49[a]	21.61 ± 0.32[a]	21.39 ± 0.39[a]
	EG	**27.05 ± 0.47[b]**	**25.94 ± 0.46[b]**	23.50 ± 0.45[a]	25.14 ± 0.40[a]	25.95 ± 0.44[a]
	LG	**28.86 ± 0.45[b]**	**28.23 ± 0.35[b]**	26.45 ± 0.38[a]	27.61 ± 0.33[a]	28.32 ± 0.36[a]
	SL	40.69 ± 2.31[a]	38.06 ± 2.48[a]	41.77 ± 2.50[a]	40.56 ± 1.77[a]	39.58 ± 2.18[a]

[1]Sampling dates in 2002 were: PT = pretreatment, May 29; EG = post-treatment early-season, July 6; LG = late-season greenleaf, September 25; SL = senescing litter, Oct 27. Data were analyzed by ANOVA and differences in treatment means identified by the Student-Neuman-Keuls post-hoc test. Values in bold and with different letters are statistically different from their respective controls (α=0.05). There were no date*damage*frass or damage*frass interactions with any response variable. Data are means (n=40 for damage groups; n=60 for frass groups) " 1SE.

Table 3.2 Independent Effects of Damage and Frass Treatments on *Quercus rubra* Litter Chemistry

	Month[1]	Damage Treatment Only			Frass Treatment Only	
		Herbivore	Mechanical	Undamaged	Frass	No Frass
Nitrogen	0	**1.50 ± 0.13** [b]	**1.67 ± 0.05** [c]	1.35 ± 0.06 [a]	**1.38 ± 0.08** [b]	1.63 ± 0.06 [a]
(% DW)	3	0.61 ± 0.05 [a]	0.71 ± 0.06 [a]	0.78 ± 0.08 [a]	0.65 ± 0.05 [a]	0.76 ± 0.06 [a]
	9	1.73 ± 0.07 [a]	**2.10 ± 0.10** [b]	1.65 ± 0.04 [a]	1.75 ± 0.06 [a]	1.91 ± 0.09 [a]
	12	1.52 ± 0.08 [a]	1.70 ± 0.06 [a]	1.53 ± 0.05 [a]	1.56 ± 0.04 [a]	1.61 ± 0.07 [a]
	18	1.87 ± 0.04 [a]	1.90 ± 0.06 [a]	1.83 ± 0.04 [a]	1.83 ± 0.04 [a]	1.90 ± 0.03 [a]
Carbon	0	49.36 ± 0.05 [a]	49.70 ± 0.13 [a]	49.70 ± 0.11 [a]	49.49 ± 0.08 [a]	49.68 ± 0.10 [a]
(% DW)	3	47.39 ± 0.26 [a]	48.55 ± 0.13 [a]	47.27 ± 0.38 [a]	47.54 ± 0.28 [a]	47.93 ± 0.25 [a]
	9	47.86 ± 0.20 [a]	47.92 ± 0.24 [a]	47.96 ± 0.27 [a]	47.64 ± 0.18 [a]	48.19 ± 0.16 [a]
	12	47.98 ± 0.39 [a]	48.95 ± 0.26 [a]	48.61 ± 0.18 [a]	48.85 ± 0.20 [a]	48.18 ± 0.27 [a]
	18	49.64 ± 0.24 [a]	49.47 ± 0.18 [a]	49.90 ± 0.22 [a]	49.80 ± 0.17 [a]	49.54 ± 0.18 [a]
Lignin	0	15.72 ± 0.33 [a]	15.66 ± 0.43 [a]	16.92 ± 0.48 [a]	16.25 ± 0.43 [a]	15.96 ± 0.29 [a]
(% DW)	3	**17.47 ± 0.48** [b]	**17.94 ± 0.33** [b]	19.89 ± 0.53 [a]	**17.46 ± 0.38** [b]	19.40 ± 0.38 [a]
	9	21.30 ± 0.76 [a]	21.95 ± 0.60 [a]	21.26 ± 0.52 [a]	20.91 ± 0.39 [a]	22.10 ± 0.57 [a]
	12	23.46 ± 0.91 [a]	22.60 ± 0.78 [a]	24.63 ± 1.03 [a]	23.80 ± 0.88 [a]	23.33 ± 0.62 [a]
	18	28.58 ± 0.83 [a]	**23.83 ± 0.79** [b]	26.68 ± 0.93 [a]	26.71 ± 0.92 [a]	26.02 ± 0.79 [a]
C:N	0	35.40 ± 3.20 [a]	**29.97 ± 1.05** [b]	37.60 ± 1.88 [a]	**37.68 ± 2.19** [b]	30.97 ± 1.20 [a]
	3	84.33 ± 7.84 [a]	72.48 ± 6.47 [a]	65.72 ± 6.23 [a]	80.21 ± 6.45 [a]	68.13 ± 4.75 [a]
	9	28.08 ± 1.19 [a]	**23.27 ± 1.06** [b]	29.18 ± 0.77 [a]	27.62 ± 0.91 [a]	26.07 ± 1.17 [a]
	12	32.76 ± 2.52 [a]	29.21 ± 1.23 [a]	32.10 ± 1.01 [a]	31.61 ± 0.86 [a]	31.10 ± 1.84 [a]
	18	26.71 ± 0.63 [a]	26.26 ± 0.68 [a]	27.42 ± 0.55 [a]	27.34 ± 0.54 [a]	26.25 ± 0.45 [a]
Lignin:N	0	**11.22 ± 0.96** [b]	**9.45 ± 0.43** [c]	12.85 ± 0.81 [a]	**12.39 ± 0.79** [b]	9.96 ± 0.44 [a]
	3	30.54 ± 2.26 [a]	26.80 ± 2.43 [a]	27.48 ± 2.51 [a]	29.13 ± 2.06 [a]	27.42 ± 1.85 [a]
	9	12.50 ± 0.69 [a]	**10.63 ± 0.48** [b]	12.96 ± 0.54 [a]	12.12 ± 0.45 [a]	11.94 ± 0.61 [a]
	12	16.03 ± 1.38 [a]	13.54 ± 0.86 [a]	16.32 ± 0.97 [a]	15.50 ± 0.87 [a]	15.09 ± 1.00 [a]
	18	15.38 ± 0.57 [a]	**12.60 ± 0.39** [b]	14.62 ± 0.47 [a]	14.62 ± 0.48 [a]	13.79 ± 0.49 [a]

[1]Samples were extracted on five dates: 0 (initial conditions), 3, 9, 12, and 18 months following experiment establishment. Data were analyzed with ANOVA models with differences in treatment means identified by the Student-Neuman-Keuls post-hoc test. Values in bold and with different superscript letters are statistically different from their respective control groups (α=0.05). Data are means (n=10 for damage groups; n=15 for frass groups) " 1SE.

Figure 3.1 (a) Effects of foliar damage on specific leaf mass (SLM). Double asterisk over July 6, 2002 indicates that all treatments were significantly different (SNK posthoc test, α=0.01) from one another at that sampling date. Data points represent means (n=40 samples) ± 1SE. Stepwise regression analyses of SLM at each of three sampling dates are also presented: (b) May 29, 2002, pre-treatment sample; (c) July 6, 2002, 1-month post-treatment sample; (d) September 25, 2002, late-season green leaf sample. Bars indicate that variables were significant contributors to the stepwise model. Dark shaded bars indicate that the correlation between the variable and SLM was positive; light shaded bars indicate that the correlation was negative.

Figure 3.1

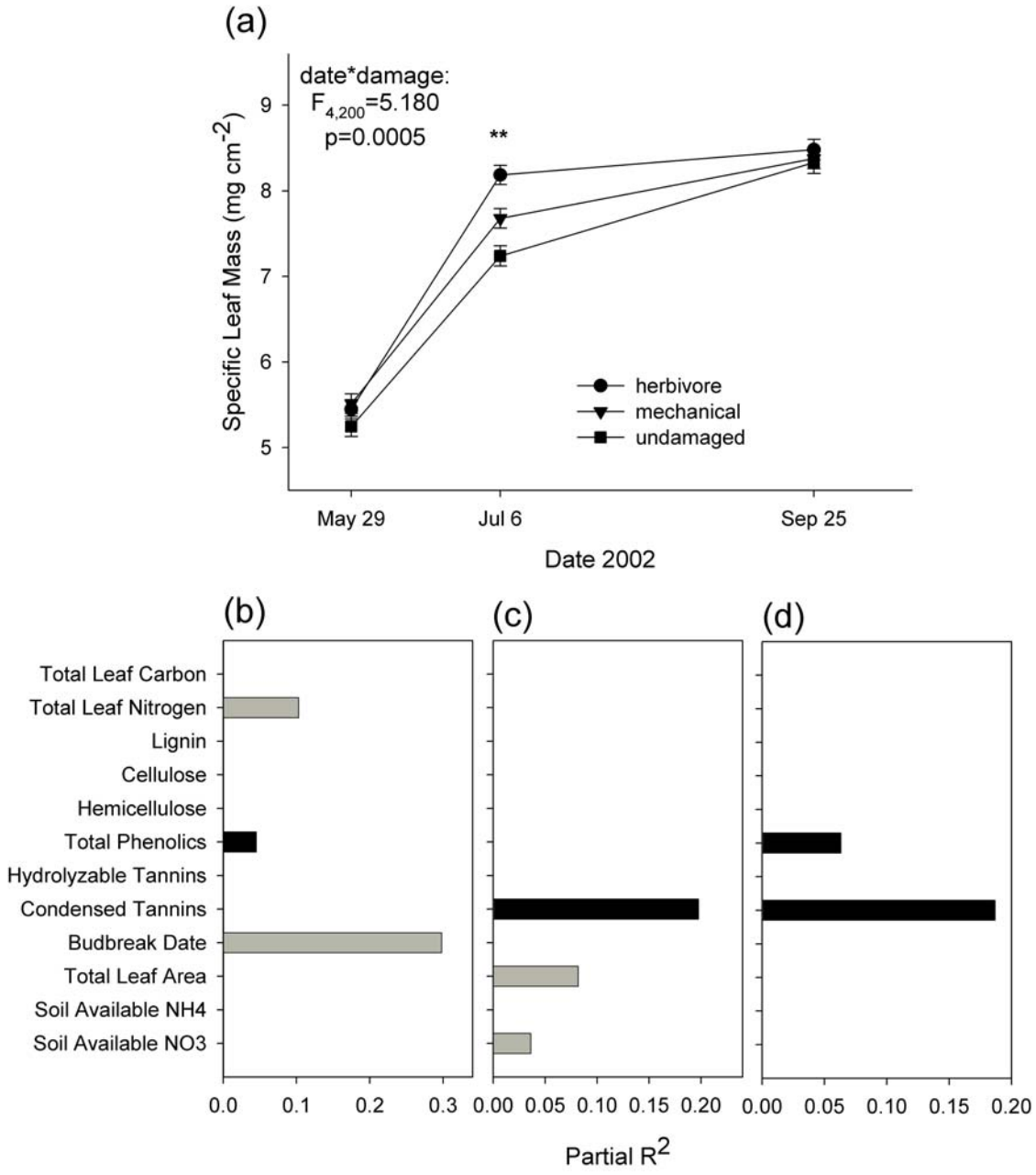

(a)
date*damage:
$F_{4,200}$=5.180
p=0.0005
**
Specific Leaf Mass (mg cm^{-2})
9
8
7
6
5
herbivore
mechanical
undamaged
May 29
Jul 6
Sep 25
Date 2002
(b)
(c)
(d)
Total Leaf Carbon
Total Leaf Nitrogen
Lignin
Cellulose
Hemicellulose
Total Phenolics
Hydrolyzable Tannins
Condensed Tannins
Budbreak Date
Total Leaf Area
Soil Available NH4
Soil Available NO3
0.0
0.1
0.2
0.3
0.00
0.05
0.10
0.15
0.20
0.00
0.05
0.10
0.15
0.20
Partial R^2

Figure 3.2 Interactive effects of damage and frass on the concentrations of initial litter quality parameters (a) hydrolysable tannins, (b) condensed tannins, (c) total phenolics, (d) nitrogen, (e) lignin:nitrogen ratio, (f) carbon:nitrogen ratio. Bars are means (n=5) ± 1 SE. Different letters within each graph are significantly different using the Student-Neuman-Keuls post-hoc test (α=0.05).

Figure 3.2

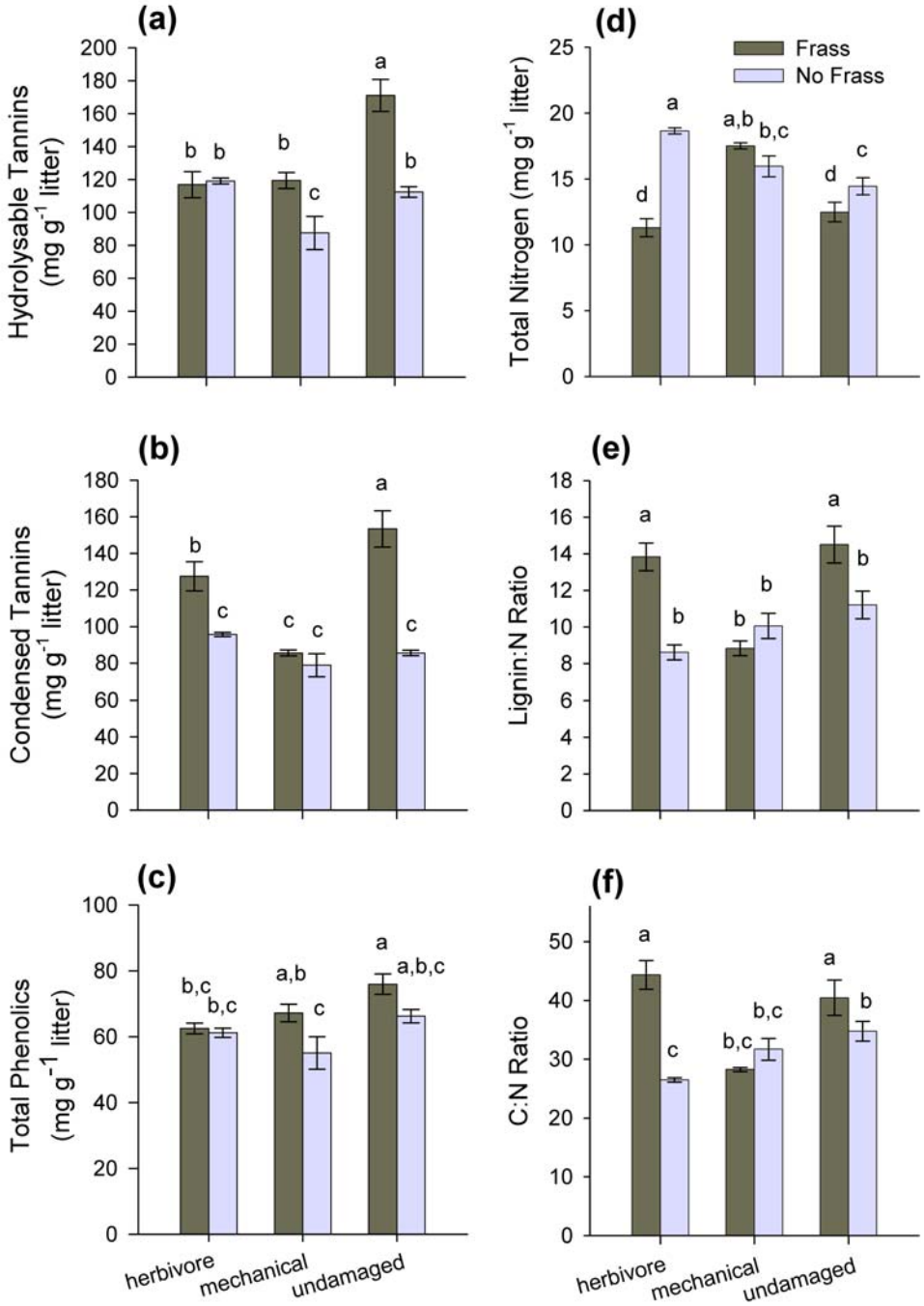

(a)
Hydrolysable Tannins (mg g-1 litter)
(b)
Condensed Tannins (mg g-1 litter)
(c)
Total Phenolics (mg g-1 litter)
(d)
Total Nitrogen (mg g-1 litter)
Frass
No Frass
(e)
Lignin:N Ratio
(f)
C:N Ratio
herbivore
mechanical
undamaged

Figure 3.3 (a-b) Changes in condensed tannin concentrations during decomposition as influenced by frass additions and damage. (b-c) Changes in hydrolyzable tannin concentrations during decomposition as influenced by frass additions and damage. (e-f) Changes in total phenolics concentrations during decomposition as influenced by frass additions and damage. The points represent the means of 15 data points ± 1 SE and 10 data points ± 1 SE for frass (a,c,e) and damage (b,d,f) graphs, respectively. An asterisk above a date indicates statistical difference (α=0.05) between or among treatments on that date using the Student-Neuman-Keuls post-hoc test.

Figure 3.3

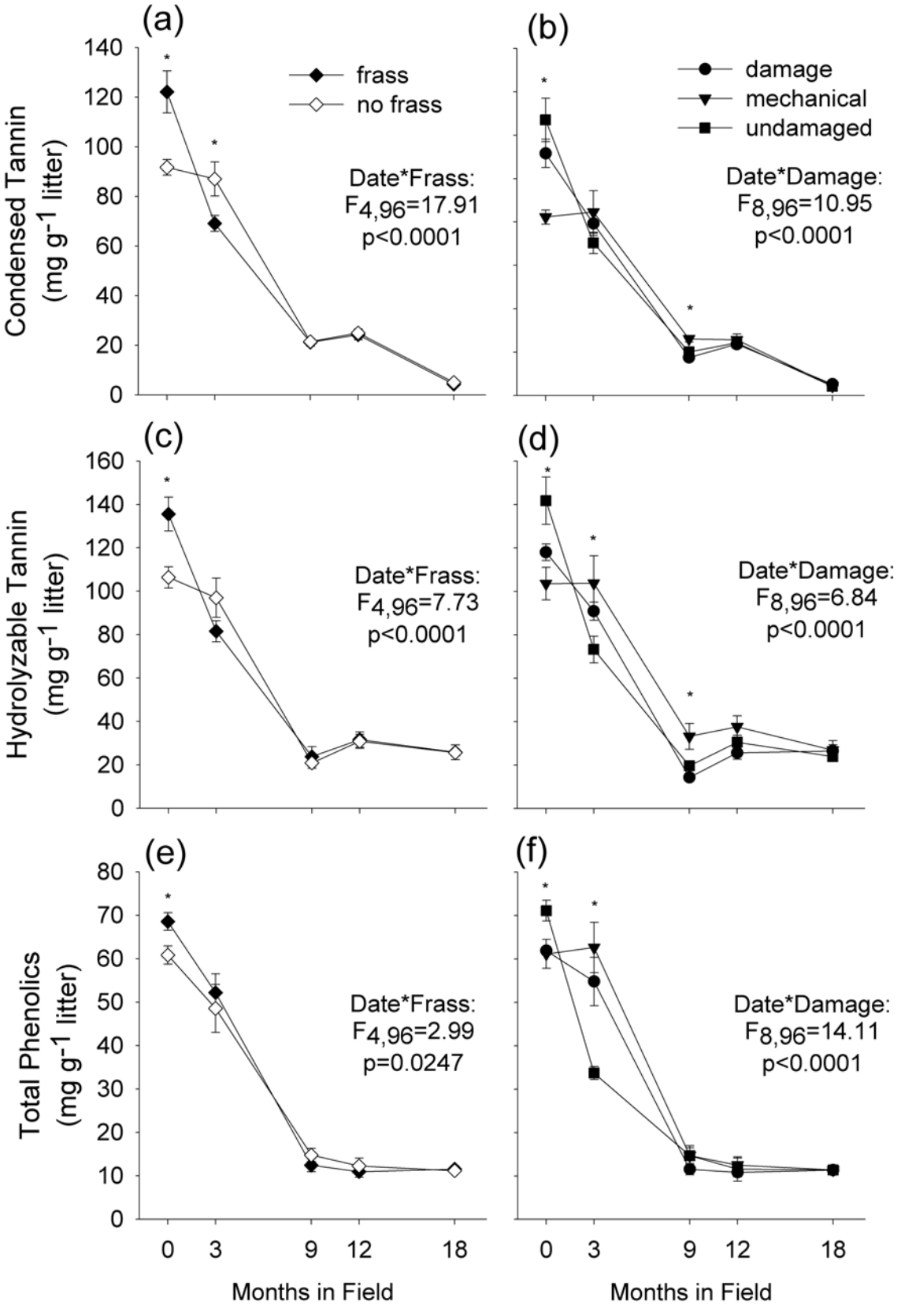
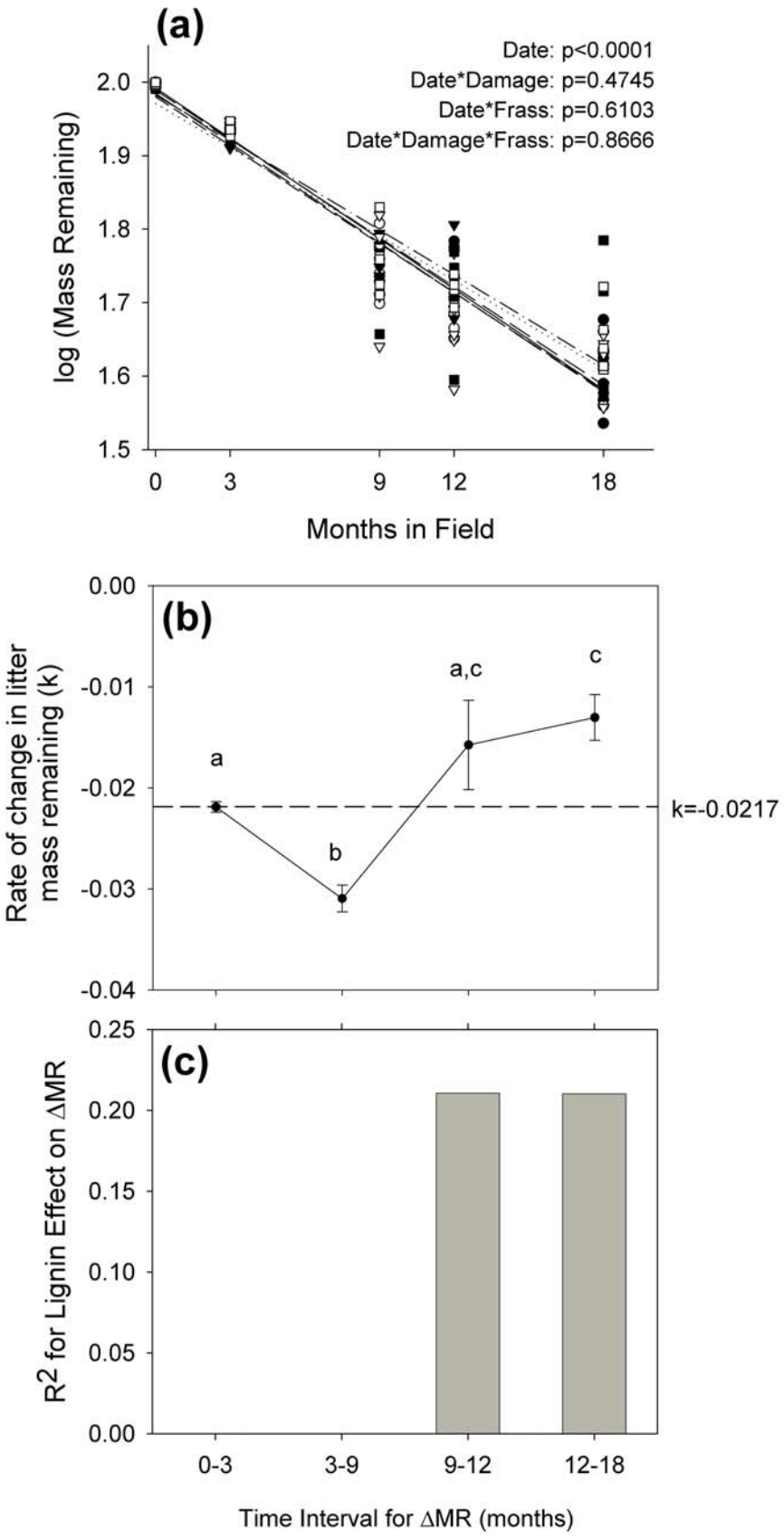

(a)
(b)
(c)
(d)
(e)
(f)
frass
no frass
damage
mechanical
undamaged
Condensed Tannin (mg g-1 litter)
Hydrolyzable Tannin (mg g-1 litter)
Total Phenolics (mg g-1 litter)
Date*Frass: F4,96=17.91 p<0.0001
Date*Damage: F8,96=10.95 p<0.0001
Date*Frass: F4,96=7.73 p<0.0001
Date*Damage: F8,96=6.84 p<0.0001
Date*Frass: F4,96=2.99 p=0.0247
Date*Damage: F8,96=14.11 p<0.0001
Months in Field
Months in Field

Figure 3.4 (a) Regression lines of log (mass remaining) by months in the field for each of the six possible treatment combinations. Dark symbols represent samples receiving frass additions, clear symbols represent samples without frass additions; circles, triangles, and squares represent the herbivore, mechanical, and undamaged groups respectively. The solid and long-dash regression lines represent the herbivore damage group with and without frass additions, respectively. The medium-dash and short-dash regression lines represent the mechanical damage group with and without frass additions, respectively. The dotted and dash-dot regression lines represent the undamaged group with and without frass additions, respectively. The overall decomposition rate (k) for all data points was -0.0217 $month^{-1}$, r^2=0.90. (b) Rates of change in litter mass remaining over specific time intervals. Interval-specific changes in litter mass remaining (ΔMR) deviated from the overall decomposition rate (k=-0.0217 $month^{-1}$), which is represented by the dashed, horizontal line. The points represent the means of 30 rates of change ± 1 SE during the specific time interval. Different letters above each point indicate statistical difference (α=0.05) using the Student-Neuman-Keuls post-hoc test. (c) Influence of lignin concentration on ΔMR. Initial lignin concentration had a negative effect on ΔMR for both 9-12 and 12-18 month intervals. Only lignin concentration entered any of the stepwise models, with the other following regressors included for each date: condensed tannins, hydrolyzable tannins, total phenolics, cellulose, hemicellulose, total N, C:N, and lignin:N.

Figure 3.4


(a)
Date: p<0.0001
Date*Damage: p=0.4745
Date*Frass: p=0.6103
Date*Damage*Frass: p=0.8666
log (Mass Remaining)
2.0
1.9
1.8
1.7
1.6
1.5
0
3
9
12
18
Months in Field
(b)
Rate of change in litter mass remaining (k)
0.00
-0.01
-0.02
-0.03
-0.04
a
b
a,c
c
k=-0.0217
(c)
R2 for Lignin Effect on ΔMR
0.25
0.20
0.15
0.10
0.05
0.00
0-3
3-9
9-12
12-18
Time Interval for ΔMR (months)

CHAPTER 4

HERBIVORES ALTER NITROGEN UPTAKE PATTERNS IN OAKS AND INDIRECTLY REDUCE THE RECOVERY OF NITROGEN IN THEIR EXCRETA[1]

[1]Frost, C.J. and M.D. Hunter For Submission to Ecology Letters

ABSTRACT

Herbivores directly and indirectly affect ecosystem functioning in forests. Feces (frass) deposition is a direct effect that supplies ephemeral nitrogen (N) pulses to soils. Herbivore-mediated changes in plant carbon (C) and N allocation and/or uptake are indirect effects that may also influence soil N availability. These direct and indirect effects may interact if defoliation influences the ability of plants to recover nitrogen deposited in frass. We added ^{15}N-enriched insect frass to a series of replicated red oak, *Quercus rubra,* mesocosms that had been damaged experimentally (herbivore, mechanical, undamaged controls). We then followed the frass N over the course of two years. Some frass N was mineralized within one week of deposition. Within one month, frass N had been assimilated by the oaks and had enriched the foliage. Late-season herbivores were then able to assimilate the frass-derived N within the same growing season. Enrichment levels in the foliage continued to increase despite reductions in total N during senescence, and leaf litter was more enriched than was the foliage. In the second season, herbivore damage from the previous year resulted in lower total leaf N contents and less recovery of the enriched frass N, and a subsequent generation of early-season herbivores fed on this foliage consequently derived less of their N from the previous year's frass. Approximately 10% of the initial frass N was recovered in leaf tissue. Neither soil nor root pools were affected by the damage treatment, and 42% of the frass N was recovered in the soil. The results suggest that plants mediate the rapid cycling of N through the herbivore subloop of the terrestrial N cycle.

INTRODUCTION

Herbivores influence terrestrial ecosystem processes. Observations linking herbivores with energy and nutrient fluxes are long standing (Swank *et al.* 1981;Schowalter & Crossley 1983;Risley & Crossley 1988), and recent work in grasslands and forests has begun to elucidate the underlying mechanisms. Herbivore damage stimulates changes in foliage that can persist into litter and alter rates of decomposition and nutrient turnover (Ritchie *et al.* 1998;Chapman *et al.* 2003). Plants respond to herbivore damage in complex ways that result in changes to carbon (C) partitioning between above- and below-ground tissues (Hamilton & 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 (frass) from herbivores provides rapid, but transient, nutrient pulses that significantly alter nitrogen (N) availability and loss from the terrestrial system (Frost & Hunter 2004). Frass deposition is therefore a double-edged sword: providing transient nutrient pulses that may stimulate plant growth while facilitating the loss of N from the terrestrial system.

Attempts to predict the fate of frass N have focused on soil properties. Soils are typically 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). When damage occurs to a plant, any herbivore-mediated changes in plant N and C allocation belowground may also affect soil processes, including the recovery of frass N by plants. Our previous work suggested that, similar to grasses (Hamilton & Frank 2001), oaks may allocate C and N among tissues contingent upon foliar damage (Frost & Hunter 2004)

We report here the results of a two-year study exploring the dynamics of frass-derived N in red oak, *Quercus rubra*, field mesocosms using ^{15}N-enriched frass.

In the first season, our objective was to explore whether uptake of frass N and subsequent allocation patterns within trees were affected by herbivore damage, with particular focus on recovery in leaf tissue and loss as leachate (Swank *et al.* 1981;Reynolds *et al.* 2000). Based on previous results in this system, we predicted that herbivore damage would stimulate belowground activity that would increase immediate recovery of frass N and consequently decrease loss from the system as leachate. However, oaks commonly reduce foliar N concentrations throughout a growing season (Boerner 1984), and we did not anticipate significant 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* to explore whether early-season frass N would be assimilated by late-season herbivores within the same growing season.

In the second season, we introduced a second cohort of early-season herbivores to test the availability to the herbivores of N derived from frass from the previous year. Expanding on the hypothesis that herbivore activity would increase N recovery, we predicted that a second generation of herbivores would recover more frass-derived N feeding on a tree damaged in the previous year than on an undamaged tree. In addition, we destructively sampled each mesocosm to determine the amount of recoverable frass N held in the system one year following initial deposition. This allowed us to examine N allocation patterns by *Q. rubra* in response to herbivore damage in the previous year and to estimate total recovery and distribution of frass-derived N within the terrestrial system.

METHODS

Field Site

The experimental array of potted mesocosms is described in detail in (Frost & Hunter 2004). Briefly, the array was established in a field adjacent to the UGA Botany greenhouses (Athens, GA) using 160 nursery-grown *Q. rubra* saplings (Forest Keeling Nursery, Missouri) transplanted into 7-gallon pots using soil and leaf litter from watershed 27 ("WS27") at the Coweeta Hydrologic Laboratory ("CWT," Otto, NC, elevation 1300 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 width (10 cm from base of soil). From the entire array, we randomly selected forty-five saplings to receive ^{15}N-enriched frass (15 per damage group described below) and five an unenriched controls to measure natural ^{15}N abundances.

Generating ^{15}N-Labeled Frass

In March 2003, ten potted *Q. rubra* saplings separate from the field site trees were labeled by applying dissolved 99 atom% $^{15}NH_4{}^{15}NO_3$ directly to their soil 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. Six additional *Q. rubra* saplings were used to generate unlabeled frass for baseline isotope abundances. This group of 16 trees (10 enriched, 6 unenriched) is hereafter called "isotope trees."

Isotope trees were transported to the Animal and Plant Health Inspection Service (APHIS) Lab in Cape Cod, MA and defoliated under controlled conditions by 4th-instar *Lymantria dispar*. The isotope trees were completely covered with 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 (*Methods: 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.

Damage Treatment and ^{15}N-Frass Additions

Fifteen experimental trees in the field array were randomly selected for each of three treatments (herbivore damage, mechanical damage, undamaged). Herbivore damage was inflicted on the experimental trees by white-marked tussock moth larvae, *Orgyia leucostigma*, tannin-tolerant defoliators common throughout the eastern United States. Twenty-five herbivores per tree were contained in branch bags made of agricultural cloth. Bags covered ~60% of foliage and all trees were bagged to control for any effects of the bags on the trees (e.g., reduced photosynthesis). Fourth-to-fifth instar *O. leucostigma* fed continuously from 1 Jun 2003 to 10 Jun 2003. Mechanical damage occurred on discrete dates (5 Jun and 10 Jun 2003) and mimicked herbivore damage in terms of total leaf area removed (LAR, Frost & Hunter 2004), 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 baseline isotope abundances.

On June 18, 2003, we applied 60 g m^{-2} (60 kg ha^{-1}) frass, which is approximately representative of the LAR generated by our experimental treatment (Frost & Hunter 2004). This resulted in the addition of ~6 g dry-weight-equivalent frass to the surface of each experimental mesocosm. Frass was 2.26 ± 0.04 % N (mean ± SD), resulting in the addition of ~135.6 mg total N and ~37.2 mg frass-derived ^{15}N to each mesocosm. By comparison, the same total quantity of non-enriched frass would add ~497 μg ^{15}N.

2003 Sample Collection

Four leaf samples were collected during the 2003 growing season: pre-treatment (28 May 2003), 1-week post-frass additions (25 Jun 2003), 1-month post-frass additions (21 Jul 2003), and litter samples (28 Oct 2003). During each collection, 3-4 fully-expanded leaves per tree were collected from terminal stems receiving full sun and pooled for analysis. Foliage sampled on each date was of similar age. Litter samples were collected by gently shaking a mainstem and collecting the falling litter, though 9 trees had fully dropped their foliage and collecting litter samples was not possible.

Soil samples were collected three times in 2003: pre-frass additions (1 Jun 2003), 1-week post-frass additions (26 Jun 2003), and 1-month post-frass additions (17 Jul

2003). Two 2 cm diameter cores were taken for each mesocosm to a depth of 10 cm and pooled for analysis (*Methods: Soil Analysis*).

Leachate samples were collected directly from the bottom of each mesocosm in a 250 ml plastic nalgene bottle attached with a length of flexible plastic tubing (see Frost & Hunter (2004) for diagram). Leachates were clear and did not require filtering. One pre-frass addition leachate sample was collected on 13 Jun 2003. Leachate samples collected post-frass additions were pooled into three samples: 0-1 week post frass additions, 1-4 week, and 4-12 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. This allowed us to estimate the total amount of frass-derived N lost to the mesocosm as leachate during each time period. Nitrogen in leachate samples was analyzed by isotope diffusion (*Methods: Isotope Diffusion*). We were unable to estimate the leachate inorganic N pool because of small sample concentrations, though we determined total leachate N following persulfate oxidation (Cabrera & 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, *Anisota senatoria*, near the field site to explore the availability of early-season frass N to late season herbivores within the same growing season. The 2nd instar larvae of *A. senatoria* were brought into the lab and raised to the fourth instar on unenriched *Q. rubra* foliage. The larvae were then starved for 24 hr to

void their guts and fed foliage from treatment trees. Due to the limited number of herbivores collected, we randomly selected 5 treatment trees and 5 reference (enrichment-free) trees to feed 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-derived 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 hours to clear their gut contents. Following starvation, herbivores and their frass were dried at 60°C, separately ground into fine powders, and analyzed for total N and $\delta^{15}N$ (*Methods: 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 4th instar on artificial diet in the lab and then introduced to the experimental trees in the field. Once foliage had fully expanded, we randomly selected one terminal stem per tree that received full sun. Prior to introducing herbivores, the total number of leaves and their leaf area on each stem were measured and 2 leaves per stem were collected to determine total N and $\delta^{15}N$. Each stem was then enclosed in a small branch bag and 10 *O. leucostigma* larvae were added to the appropriate bag. All 50 trees (45 receiving ^{15}N frass in 2003, 5 enrichment-free) received herbivores in 2004. Herbivores were added on 5 Jun 2004 and removed on 10 Jun 2004. Herbivores, pooled by tree, were starved for 24 hours to clear gut contents. Larvae 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 tree for the presence of feral leaf rollers that had naturally colonized some trees in the field.

Destructive Sampling

All 50 mesocosms were destructively harvested on July 23-24, 2004, with replicates in each treatment group spread between the two 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 layers from 0-5 cm, 5-15 cm, and 15-25 cm from the surface. All samples were stored at 4ºC in the lab.

Soil (and root) subsamples were taken from the soil layers within five days of harvest. From each soil depth, two ~100 cm^3 (r= 5cm, depth= 5 cm) cores were taken for separate analyses. One core was used to provide a rough estimate of bulk density. The other core was used to extract soil nutrients. This core was weighed and then passed through a 2 mm sieve to exclude fine roots, which were also collected and analyzed (described below). The sieved soil was then separated into two groups for separate analyses (*Methods: Soil Analysis*).

Fine root samples from the soil cores were washed in a 2 mm sieve to remove all soil particles. Fine roots were then dried at 60ºC and weighed to calculate dry weight and root density (mg cm^{-3} soil). They were then ground into a fine powder and analyzed for total N and $\delta^{15}N$ (*Methods: Isotope Analysis*).

Soil Analysis

Soil cores were passed through a 2x2mm screen mesh to exclude fine roots and 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^{o}C to determine water content, and the dried sample ground to a fine powder and analyzed for total C and N and their isotopes (*Methods: Isotope Diffusion*). The remaining subsample was extracted with 50 ml 0.5M K_2SO_4 for 1 hr on an orbital shaker (120rpm) and filtered through Whatman 42 filter paper. The filtrate was analyzed for extractable inorganic N (NO_3^- + NH_4^+) and their $\delta^{15}N$ with via isotope diffusion (*Methods: Isotope Diffusion*).

Isotope Diffusion

Soil extracts and leachate 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 Inc., New Jersey, USA) were sealed between two squares of teflon tape and added directly to a known amount of sample in the presence of NaOH and Devarda's alloy (Sigma 269484, Sigma-Aldritch, St. Louis, MO, USA). Duplicate diffusions per sample were carried out for (1) inorganic N (NO_3^- + NH_4^+) and (2) organic N following persulfate digestion (Cabrera & Beare 1993). Samples with disks were incubated for 5 days with gentle orbital shaking (60 rpm). 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

hr in a desiccating chamber with a small vial of concentrated H_2SO_4. Dry disks were transferred into silver capsules for analysis.

Isotope Analysis

We follow the notation of $\delta^{15}N$, where units are expressed per thousand deviations from the atmospheric standard (atom% 15N = 0.3663), $\delta^{15}N$ = [(sample atom%^{15}N / standard atom%^{15}N) - 1] x 1000 (Lajtha & Michener 1994). All stable isotope samples (dry leaf, soil and diffusion samples) were analyzed on a Costech Elemental Combustion System 4010 (Costech Analytical Technologies, Inc., Valencia, CA) connected to a ThermoFinnigan ConfloIII Interface and Deltaplus Continuous Flow-Stable Isotope Ratio Mass Spectrometer (IRMS) (Thermo Electron, Waltham, MA) 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. The coefficient of variation (CV) on this data set was 0.85 ± 1.19 % (Mean ± SD) 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 Analysis

Data were analyzed using the GLM and MIXED procedures 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. The Student Neuman Keuls (SNK) post-hoc test was used to determine significant differences among treatment means. In cases where data could not be normalized by

transformation, data were analyzed with the GENMOD procedure and differences among treatment means were determined using the Wald Chi-Squared test (Littell *et al.* 2002). The count data for 2004 leaf rollers were analyzed with a χ^2 frequency table (Proc FREQ).

RESULTS

2003 Growing Season

Nitrogen deposited via insect frass was assimilated in foliage, and subsequently by late-season herbivores, within the same growing season (Figure 4.1). Foliage showed no evidence of ^{15}N enrichment in the 1-week samples, but a majority of mesocosms had $\delta^{15}N$-enriched foliage within 1-month post-frass additions (date $F_{2,82}=57.88$, $p<0.0001$). 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; Figure 4.1b).

Surprisingly, 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 (Figure 4.1). Foliar $\delta^{15}N$ enrichment increased significantly between the 1-month sampling and collection of leaf litter (date $F_{1,35}=26.71$, $p<0.0001$; Figure 4.1a), while senescing foliage was resorbing N (date $F_{1,35}=127.96$, $p<0.0001$; Figure 4.1c). 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 newly acquired N.

Consistent with the observed assimilation by trees and late-season larvae, frass N was mineralized within one week of deposition (Figure 4.2a) and constituted an increasing portion of the total soil N pool as the season progressed (Figure 4.2b). Because frass is almost entirely organic material (Lovett & 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 followed a similar pattern of $\delta^{15}N$ enrichment; soils in mesocosms receiving ^{15}N-frass were significantly enriched following additions (date $F_{6,92}=3.53$, $p=0.0068$).

A small portion of frass N was lost as leachate within one week of deposition (Figure 4.3a), though the amount lost in the first week accounted for ~49% of all frass N lost in leachate during the first three months following deposition. The first rainfall following frass additions occurred almost one week following frass additions and the $\delta^{15}N$ in mesocosms receiving frass additions showed a pulse ($F_{3,44}=6.98$, $p=0.0007$). We estimated a recovery 13.3 ± 3.3 μg of ^{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 1-4 and 4-12 week sampling periods ($F_{3,35}=14.03$, $p<0.0001$; $F_{3,23}=14.50$, $p<0.0001$, respectively). In the 1-4 and 4-12 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 appeared to occur more rapidly following mechanical damaged (date*damage $F_{4,82}$=3.71, p=0.0306, Figure 4.1a), 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 1-4 week post-frass addition sampling period, more frass N was lost from mesocosms suffering herbivore damage ($F_{3,35}$=14.03, p<0.0001, Figure 4.3a). Nonetheless, total N losses in leachate over the same time interval were similar among treatments ($F_{2,21}$= 0.08, p=0.9248, Figure 4.3b). At the end of the 2003 growing season, the measured frass N "losses" from the mesocosms were ~0.08% and ~1.22% of the added ^{15}N in leachate and litter, respectively.

2004 Growing Season

During the 2004 growing season, trees that had been damaged (herbivore or mechanical) in 2003 recovered less frass N in their foliage than did undamaged trees ($F_{2,44}$=3.76, p=0.0314, Figure 4.4). The lower recovery in foliage from previously damaged trees resulted from lower total N and $\delta^{15}N$ concentrations in their foliage relative to previously undamaged trees ($F_{2,44}$=6.27, p=0.0041; $F_{2,44}$=3.84, p=0.0294, respectively; Figure 4.5a,b). Overall, we recovered 59.7 ± 3.1 % (mean ± SE) of frass N in the mesocosms. The 0-5 cm soil sample comprised the largest pool of recovered frass

N (Figure 4.4), though it was not the largest pool in terms of total mass (Table 4.1). The recovery of frass N was not affected by the damage treatment in any belowground pool, including the roots. There were also no treatment effects on either the root density or the ratio of N recovered in leaf and root (3.08 ± 0.29, mean ± SE).

The lower recovery of frass N in the foliage of trees damaged by herbivores in 2003 translated into lower recovery of frass N in the bodies of *O. leucostigma* larvae feeding during spring 2004 (Figure 4.5b,c). The $\delta^{15}N$ values of leaf, body, and frass samples were all tightly correlated (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 trees 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 trees had lower $\delta^{15}N$ than those fed on foliage from undamaged trees ($F_{1,29}$= 4.41, p= 0.0448, Figure 4.5b) and, consequently, had lower proportion of N derived from frass N (Figure 4.5c). *O. leucostigma* bodies had a higher frass N recovery expressed per unit biomass than did foliage irrespective of treatment (Figure 4.5d), suggesting that, as expected, they were accumulating foliar N. There were no treatment effects on *O. leucostigma* frass $\delta^{15}N$, though the trend is similar to the leaf and body samples (Figure 4.5). 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 (Figure 4.5a). In addition, 2004 leaf rollers colonized 13 of 15 trees that were undamaged in 2003 but only 7 of 15 that had been damaged by herbivores (χ^2= 5.4, d.f.=1, p= 0.0201).

DISCUSSION

Our study was designed to explore the distribution and allocation of frass N following insect herbivore damage on oaks. The salient results fall into two broad categories: (a) plant recovery of frass N and its availability to subsequent herbivores and (b) soil retention and loss as leachate 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, (2) late-season herbivores derive a portion of their N from the frass of early-season herbivores, (3) the effects of damage on frass recovery may influence the nutrient supply of late-season herbivores, and (4) herbivore damage indirectly reduces the recovery of frass N by oaks and therefore N availability to subsequent cohorts of herbivores. Considering the soil, our data support the hypotheses that (1) soils are strong sinks for frass N and (2) a small, but significant, portion of frass N is lost via leachate immediately following deposition before labile N can be immobilized.

Frass N Recovery in Oaks and Herbivores

The most surprising result of our study was the increasing $\delta^{15}N$ content in 2003 foliage and litter (Figure 1a), which suggests continued allocation of newly acquired N despite reductions in total foliar N. Nitrogen allocation patterns in plants are complex and are both influenced by and affect herbivores (Hunter 1987). In general, late-season herbivores consume lower quality foliage because of the reduction in foliar N concentrations in deciduous trees throughout a growing season (Chapin 1980;Chapin & Moilanen 1991;Arco *et al.* 1991;Killingbeck 1996), which implies reduction in allocation

of newly-acquired N into foliage. Our study differed from previous ones (e.g., Nadelhoffer *et al.* 1999;Christenson *et al.* 2002) in that we used a single deposition event that allowed us to follow the N incorporation into foliage throughout the growing season; any ^{15}N above background was necessarily derived from that deposition event. Our data demonstrate that foliar uptake of new N continues despite reduction in total N. Some N is likely derived from passive mass flow (Chapin 1980), but the apparent difference in uptake among damage treatments (Figure 4.3a) implies active control of N loading. The uptake dynamics of N in the late season are evidently more complex than previously thought.

Early-season defoliation influences host selection and fitness of late-season herbivores through changes in foliar quality (Hunter 1987). Our use of *A. senatoria* further supports the argument that N mobilized by early-season herbivores can be recovered in foliage and available to late-season herbivores. In addition, the recovery of ^{15}N in *A. senatoria* frass and ~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 (*sensu* McNaughton *et al.* 1988) via plant N recovery following herbivore feeding. A portion of N mobilized by early-season herbivores as frass can be recovered by the oak and re-deposited on the forest floor in the leaf litter within the same growing season.

Oak saplings in 2004 recovered ~10% of the frass N deposited in 2003, which then comprised ~1% of their foliar N. 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.* (1999). Our observed recovery of ^{15}N in foliage is therefore reasonable, though recovery in the field is likely distributed among multiple plant species.

On the flip side, ~90% of the N lost to herbivore feeding was not recovered by the oaks. Nitrogen acquisition is energetically expensive (Clarkson 1985) and the observed reduction in foliar N following damage makes intuitive sense in light of our estimated recovery efficiency if such a reduction influences future herbivores. *O. leucostigma* fed on foliage from previously damaged trees had lower $\delta^{15}N$ than did those fed on previously undamaged trees. Because herbivore stoichiometry is homeostatic (Sterner & Elser 2002), the lower foliar N could result in longer feeding times, lower pupal masses (Tikkanen & Julkunen-Tiitto 2003) and greater susceptibility to natural enemies (Price *et al.* 1980). In addition, leaf rollers in 2004 colonized fewer trees that had been damaged in 2003 than those that were undamaged, suggesting that leaf rollers were less likely to oviposit on previously damaged trees. Lastly, similar fine root ^{15}N concentrations among damage treatments suggests that the reduction in foliar N was an active response to damage. There were no differences among treatments in any growth parameter measured (budbreak timing, stem expansion rates, total leaf and new stem biomass, changes in stem width); the reduction in foliar N did not have deleterious effects on plant growth, at least in the season following damage.

Soil and Leachate

Soils supporting northern deciduous forests are strong sinks for surface deposited N via microbial immobilization followed by incorporation into the soil abiotic matrix (Vitousek & Matson 1985;Zak *et al.* 1990;Groffmann *et al.* 1993;Seely & Lajtha 1997;Zogg *et al.* 2000). Zak *et al.* (1990) reported that immediate microbial immobilization of N can be 10-20 times greater than plant uptake. Zogg *et al.* (2000) recovered over 33% of $^{15}NO_3^-$ added to an *Acer saccharum* hardwood forest in soil microorganisms within two hours following deposition. Fine roots had begun to acquire the label within two days, and homeostasis was achieved in ~6 weeks. Seely & Lajtha (1997) reported that the surface mineral soil retained 10-25% and 19-45% of applied $^{15}NO_3^-$ in the spring and fall, respectively; the label was detected in the fine roots within 5-20 days of application. The apparent mineralization of frass N within one week of deposition in our study is consistent with the hypothesis that soil microorganisms are an immediate sink for exogenous N.

Christenson *et al.* (2002) recovered ~17% of ^{15}N-enriched frass in the top 0-5 cm of soil and ~40% from 0-30 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 0-5 cm soil. The absence of any damage treatment effect on the soils in our study suggests that the retention of N in these soils is probably (1) the result of surface fungi and bacteria and the physical properties of the soil matrix and (2) largely independent of root-mediated changes following aboveground herbivory. The one statistically significant effect of herbivore damage belowground, greater loss of ^{15}N

from herbivore-damaged mesocosms, does suggests that roots may have been negatively effected and uptake subsequently reduced. However, the effect was small and likely biologically unimportant in terms of forest N dynamics because it did not affect the mobilization of frass N in the week following deposition.

The estimates of N leaching losses among studies provides an interesting contrast. Christenson *et al.* (2002) estimated that only 0.00004% of frass N was lost via leaching of inorganic N through the soil. Calculations based on Frost & Hunter (2004) suggest that ~20% of frass N was lost via leaching in the first month following deposition, though they suggested that rainfall events within hours of frass deposition leached the majority of the mobile N. The total estimated leachate loss in this study (~0.08% of added ^{15}N for the first three months following deposition) is in between the two studies. Unlike Frost & Hunter (2004), rainfall did not occur immediately following deposition in this study and visible fungal hyphae surrounded the frass pellets in most of the mesocosms. While only observational evidence, this is consistent with observations reported by Lovett & Ruesink (1995). Frost & Hunter (2004) predicted that an immediate, precipitation-dependent mobilization of frass N in leachate would follow deposition before microbial or abiotic immobilization occurred. Seely & Lajtha (1997) found that more than 90% of ^{15}N recovered in lysimeter samples occurred within 2 days following deposition. The data from this study are similar: ~40% of frass N recovered in leachate was collected in the first week's sample. Whether deposited as inorganic or frass N, an immediate "mobilized" pulse of unmineralized N escapes the system before microbial immobilization, the magnitude of which may depend on the relative timing of rainfall

events and frass deposition. Frass N, in contrast to mineral N (Seely & Lajtha 1997), continued to be leached at much smaller levels over the course of months. Evidently, the potential loss of frass N through leaching is large but buffered by biological immobilization when rainfall does not immediately proceed deposition.

Acknowledgments

We would like to thank B. Ball, M. Cabrera, R. Canon, D. Coleman, L. Donovan, R. Goergen, C. Hall, S.Helms, P. Hendrix, C. Jennison, J. Malloway, R. Malloy, J. Nelson, K. Wickings, and C. Zehnder for field/lab assistance and/or comments on previous versions. John Tanner at the Gypsy Moth Rearing Facility of APHIS supplied space and voracious larvae. Bob McCron from the Canadian Forest (Service Sault Ste. Marie, Ontario) provided tussock moth egg masses. Tom Maddox processed all isotope samples in the Analytical Lab of the Institute of Ecology. This research is supported by NSF grants DEB-9815133 and DEB-0404876 to MDH and a UGA University-Wide Assistantship to CJF.

REFERENCES

Arco J.M.D., Escudero A. & Garrido M.V. (1991) Effects of Site Characteristics on Nitrogen Retranslocation From Senescing Leaves. *Ecology* 72, 701-708

Ayres E., Heath J., Possell M., Black H.I.J., Kerstiens G. & Bardgett R.D. (2004) Tree physiological responses to above-ground herbivory directly modify below-ground processes of soil carbon and nitrogen cycling. *Ecol Letters* 7, 469-479

Boerner R.E.J. (1984) Foliar nutrient dynamics and nutrient use efficiency of four deciduous tree species in relation to site fertility. *J App Ecol* 21, 1029-1040

Cabrera M.L. & Beare M.H. (1993) Alkaline persulfate oxidation for determining total nitrogen in microbial biomass extracts. *Soil Sci Soc Am J* 57, 1007-1012

Chapin F.S., III (1980) The Mineral Nutrition of Wild Plants. *Ann Rev Ecol Syst* 11, 233-260

Chapin F.S., III & Moilanen L. (1991) Nutritional Controls Over Nitrogen and Phosphorus Resorption From Alaskan Birch Leaves. *Ecology* 72, 709-715

Chapman S.K., Hart S.C., Cobb N.S., Whitham T.G. & Koch G.W. (2003) Insect herbivory increases litter quality and decomposition: An extension of the acceleration hypothesis. *Ecology* 84, 2867-2876

Christenson L.M., Lovett G.M., Mitchell M.J. & Groffman P.M. (2002) The fate of nitrogen in gypsy moth frass deposited to an oak forest floor. *Oecologia* 131, 444-452

Clarkson D.T. (1985) Factors affecting mineral nutrient acquisition by plants. *Ann Rev Plant Phys* 36, 77-115

Davis J.P., Haines B.L., Coleman D.C. & Hendrick R.L. (2004) Fine root dynamics along an elevational gradient in the southern Appalachian Mountains, USA. *For Ecol Mgmt* 187, 19-34

Downs M., Michener R., Fry B. & Nadelhoffer K.J. (1999) Routine measurement of dissolved inorganic 15N in precipitation and streamwater. *Env Monitor Assess* 55, 211-220

Frost C.J. & Hunter M.D. (2004) Insect canopy herbivory and frass deposition affect soil nutrient dynamics and export in oak mesocosms. *Ecology* 85, 3335-3347

Groffmann P.M., Zak D.R., Christensen S., Mosier A. & Tiedje J.M. (1993) Early spring nitrogen dynamics in a temperate forest landscape. *Ecology* 74, 1579-1585

Hamilton E.W.I. & Frank D.A. (2001) Can plants stimulate soil microbes and their own nutrient supply? Evidence from a grazing tolerant grass. *Ecology* 82, 2397-2404

Hunter M.D. (1987) Opposing effects of spring defoliation on late season caterpillars. *Ecol Ento* 12, 373-382

Killingbeck K.T. (1996) Nutrients in senesced leaves: keys to the search for potential resportion and resorption proficiency. *Ecology* 77, 1716-1727

Lajtha K. & Michener R.H. (1994) *Stable Isotopes in Ecology and Environmental Science*. Blackwell Scientific Publications, Cambridge, MA.

Littell R.C., Stroup W.W. & Freund R.J. (2002) *SAS for Linear Models*, 4 edn. SAS Institute, Inc., Cary, NC.

Lovett G.M. & Ruesink A.E. (1995) Carbon and nitrogen mineralization from decomposing gypsy moth frass. *Oecologia* 104, 133-138

McNaughton S.J., Ruess R.W. & Seagle S.W. (1988) Large mammals and process dynamics in African ecosystems. *BioScience* 38, 794-800

Nadelhoffer K.J., Downs M.R. & Fry B. (1999) Sinks for N-15-enriched additions to an oak forest and a red pine plantation. *Ecol App* 9, 72-86

Price P.W., Bouton C.E., Gross P., McPheron B.A., Thompson J.N. & Weis A.E. (1980) Interactions Among Three Trophic Levels: Influence of Plants on Interactions Between Insect Herbivores and Natural Enemies. *Ann Rev Ecol System* 11, 41-65

Reynolds B.C., Hunter M.D. & Crossley D.A.Jr. (2000) Effects of canopy herbivory on nutrient cycling in a northern hardwood forest in western North Carolina. *Selbyana* 21, 74-78

Risley L.S. & Crossley D.A.Jr. (1988) Herbivore-caused greenfall in the southern Appalachians. *Ecology* 69, 1118-1127

Ritchie M.E., Tilman D. & Knops J.M.H. (1998) Herbivore effects on plant and nitrogen dynamics in oak savanna. *Ecology* 79, 165-177

Schowalter T.D. & Crossley D.A.Jr. (1983) Forest canopy arthropods as sodium, potassium, magnesium, and calcium pools in forests. *For Ecol Mgmt* 7, 143-148

Seely B. & Lajtha K. (1997) Application of a 15N tracer to simulate and track the fate of atmospherically deposited N in the coastal forests of the Waguoit Bay Watershed, Cape Cod, Massachusettes. *Oecologia* 112, 393-402

Sigman D.M., Altabet M.A., Michener R., McCorkle D.C., Fry B. & Holmes R.M. (1997) Natural abundance-level measurement of the nitrogen isotopic composition of oceanic nitrate: an adaptation of the ammonia diffusion method. *Marine Chem* 57, 227-242

Sterner R.W. & Elser J.J. (2002) *Ecological Stoichiometry*. Princeton University Press, Princeton.

Swank W.T., Waide J.B., Crossley D.A.Jr. & Todd R.L. (1981) Insect defoliation enhances nitrate export from forest ecosystems. *Oecologia* 51, 297-299

Tikkanen O.-P. & Julkunen-Tiitto R. (2003) Phenological variation as protection against defoliating insects: the case of *Quercus robur* and *Operophtera brumata*. *Oecologia* 136, 244-251

Vitousek P.M. & Matson P.A. (1985) Disturbance, nitrogen availability, and nitrogen loss in an intensively managed loblolly pine plantation. *Ecology* 66, 1360-1376

Zak D.R., Groffman P.M., Pregitzer K.S., Christensen S. & Tiedie J.M. (1990) The vernal dam: plant-microbe competition for nitrogen in northern hardwood forests. *Ecology* 71, 651-656

Zogg G.P., Zak D.R., Pregitzer K.S. & Burton A.J. (2000) Microbial immobilization and the retention of anthropogenic nitrate in a northern hardwood forest. *Ecology* 81, 1858-1866

Table 4.1 Masses, Nitrogen, Carbon, and Natural ^{15}N Abundances for the *Quercus rubra* Mesocosms

Sample Type	Mass (g)[1]	N(%)	C (%)	C:N	Reference $\delta^{15}N$
Leaf	121.30 " 36.77	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 " 0.36
Fine Roots					
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	2323.88 " 536.23	0.67 " 0.11	11.26 " 2.03	16.77 " 0.51	4.29 " 0.78
5-15 cm	3413.39 " 559.31	0.51 " 0.05	9.11 " 1.08	17.72 " 0.84	6.41 " 0.17
15-25 cm	3712.97 " 573.01	0.51 " 0.06	9.29 " 1.11	18.24 " 1.02	8.64 " 0.65

[1] Data are means ± SD of 50 samples for Mass, N, C, and C:N, and 5 samples for reference $\delta^{15}N$

Figure 4.1

(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 5 enrichment-free replicates. Asterisk (*) marks indicate that treatment means were significantly different using the SNK post-hoc test (α=0.05). 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* larvae and frass. Different letters represent statistically significant treatment means using the SNK post-hoc test (α=0.05). (c) Total foliar N throughout the 2003 growing season. Bars represent the means ± SE 0f 15 replicates per treatment. Not all replicates could be used at each sampling date, so the number of replicates per bar varies.

Figure 4.1

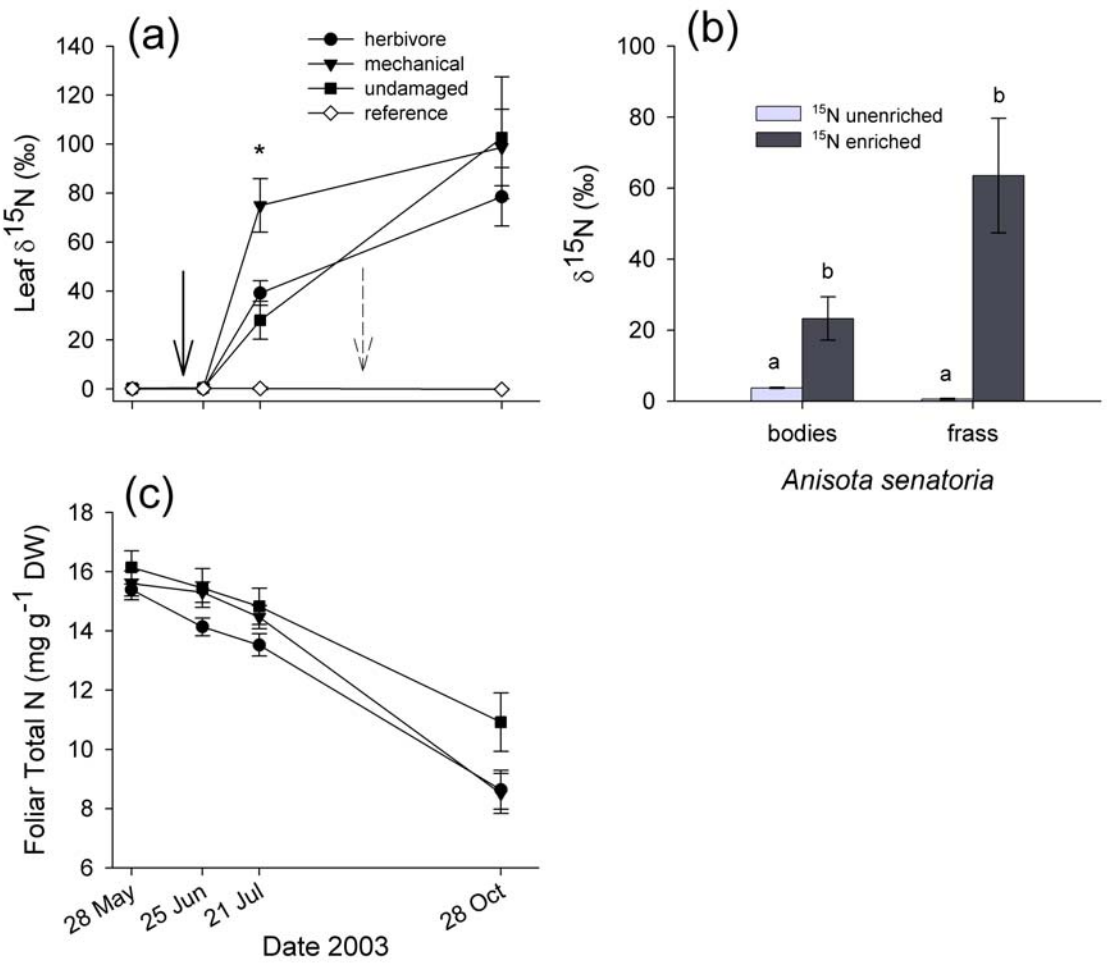

(a)
herbivore
mechanical
undamaged
reference
Leaf δ15N (‰)
140
120
100
80
60
40
20
0
*
(b)
15N unenriched
15N enriched
δ15N (‰)
100
80
60
40
20
0
a
b
a
b
bodies
frass
Anisota senatoria
(c)
Foliar Total N (mg g-1 DW)
18
16
14
12
10
8
6
28 May
25 Jun
21 Jul
28 Oct
Date 2003

Figure 4.2

(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 5 enrichment-free replicates. Asterisks (*) indicate the dates with statistically significant enrichment (α=0.05). Arrows indicate the date of ^{15}N-frass additions.

Figure 4.2

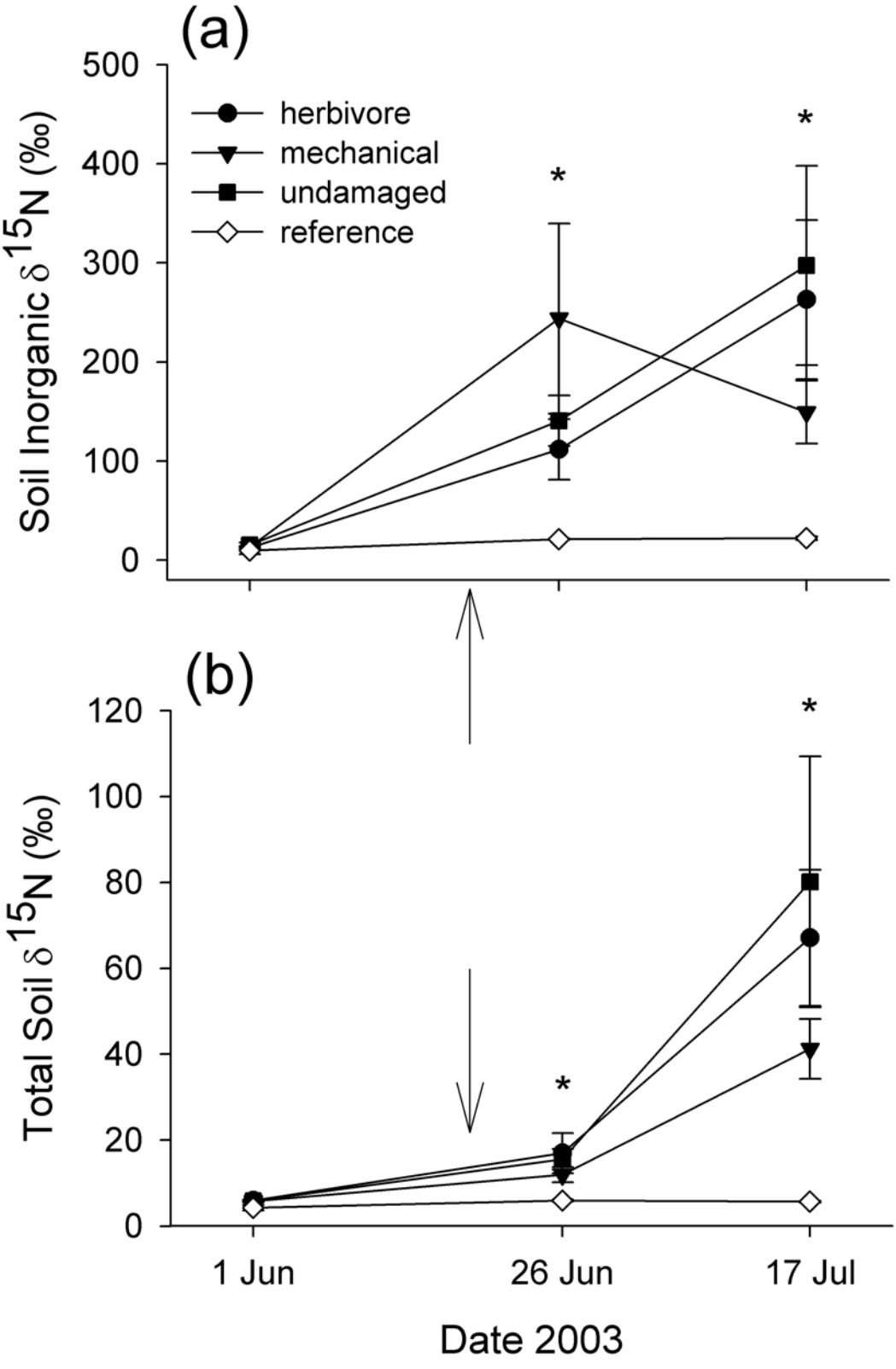

(a)
herbivore
mechanical
undamaged
reference
Soil Inorganic δ15N (‰)
500
400
300
200
100
0
*
*
(b)
Total Soil δ15N (‰)
120
100
80
60
40
20
0
*
*
1 Jun
26 Jun
17 Jul
Date 2003

Figure 4.3

(a) Leachate $\delta^{15}N$ in 2003 during the first three months following ^{15}N-frass additions. (b) Leachate total N in 2003 during the first three months following ^{15}N-frass additions. Bars are means ± SE for each treatment. Because leachate samples could not be collected for each mesocosm on each date, sample sizes are not equal between or within dates. Different letters above individual bars for (a) or the groups for (b) indicate statistical significance of treatment means using the SNK post-hoc test (α=0.05).

Figure 4.3

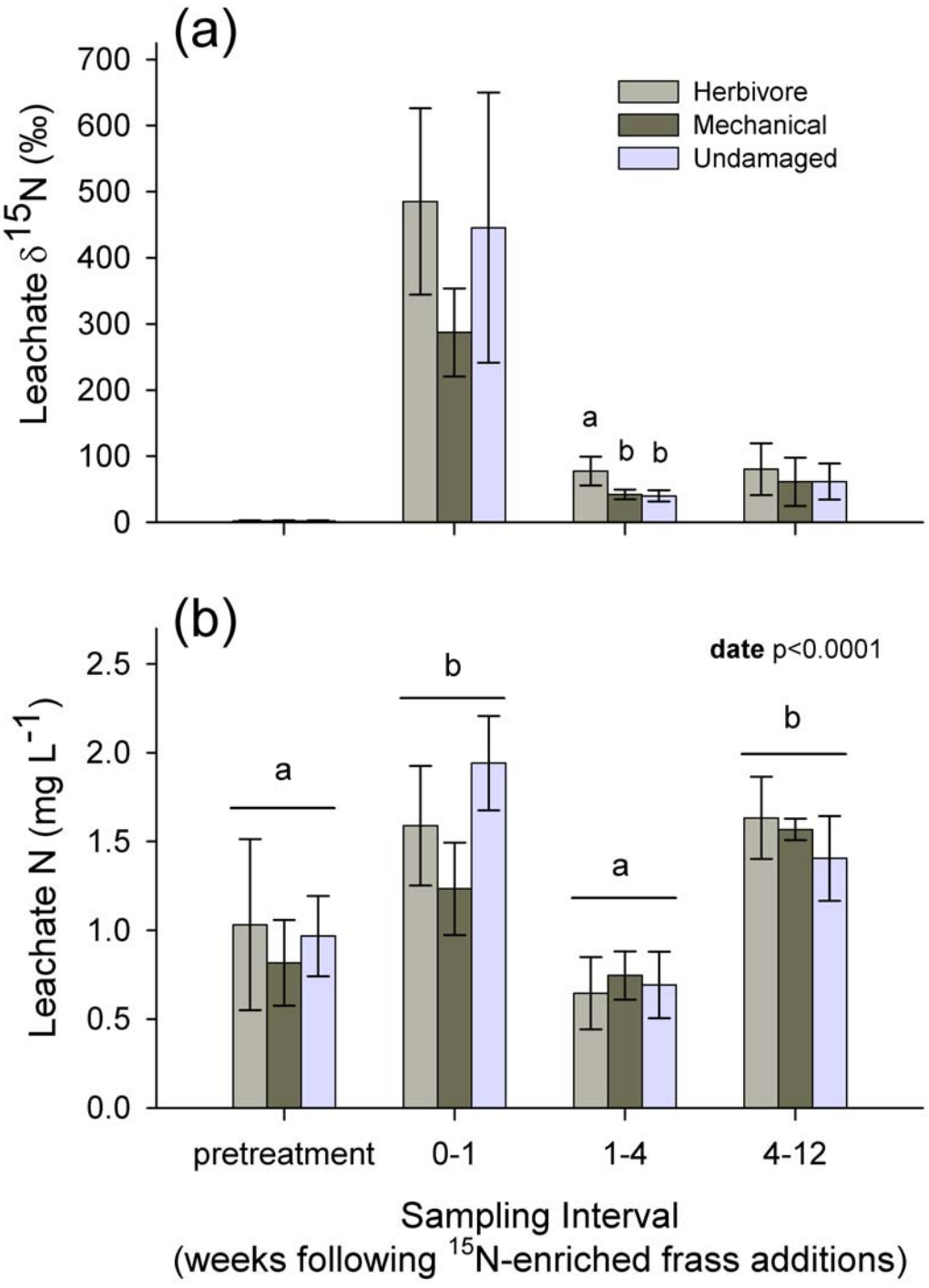

(a)
Herbivore
Mechanical
Undamaged
Leachate δ15N (‰)
700
600
500
400
300
200
100
0
a
b
b
(b)
date p<0.0001
Leachate N (mg L-1)
2.5
2.0
1.5
1.0
0.5
0.0
a
b
a
b
pretreatment
0-1
1-4
4-12
Sampling Interval
(weeks following 15N-enriched frass additions)

Figure 4.4

Total recovery of frass-derived N in mesocosms by 2003 damage treatment. Bars are means " SE of 15 samples. The asterisk (*) indicates that the herbivore and mechanical group are statistically distinct from the undamaged group using the SNK post-hoc test (α=0.05).

Figure 4.4

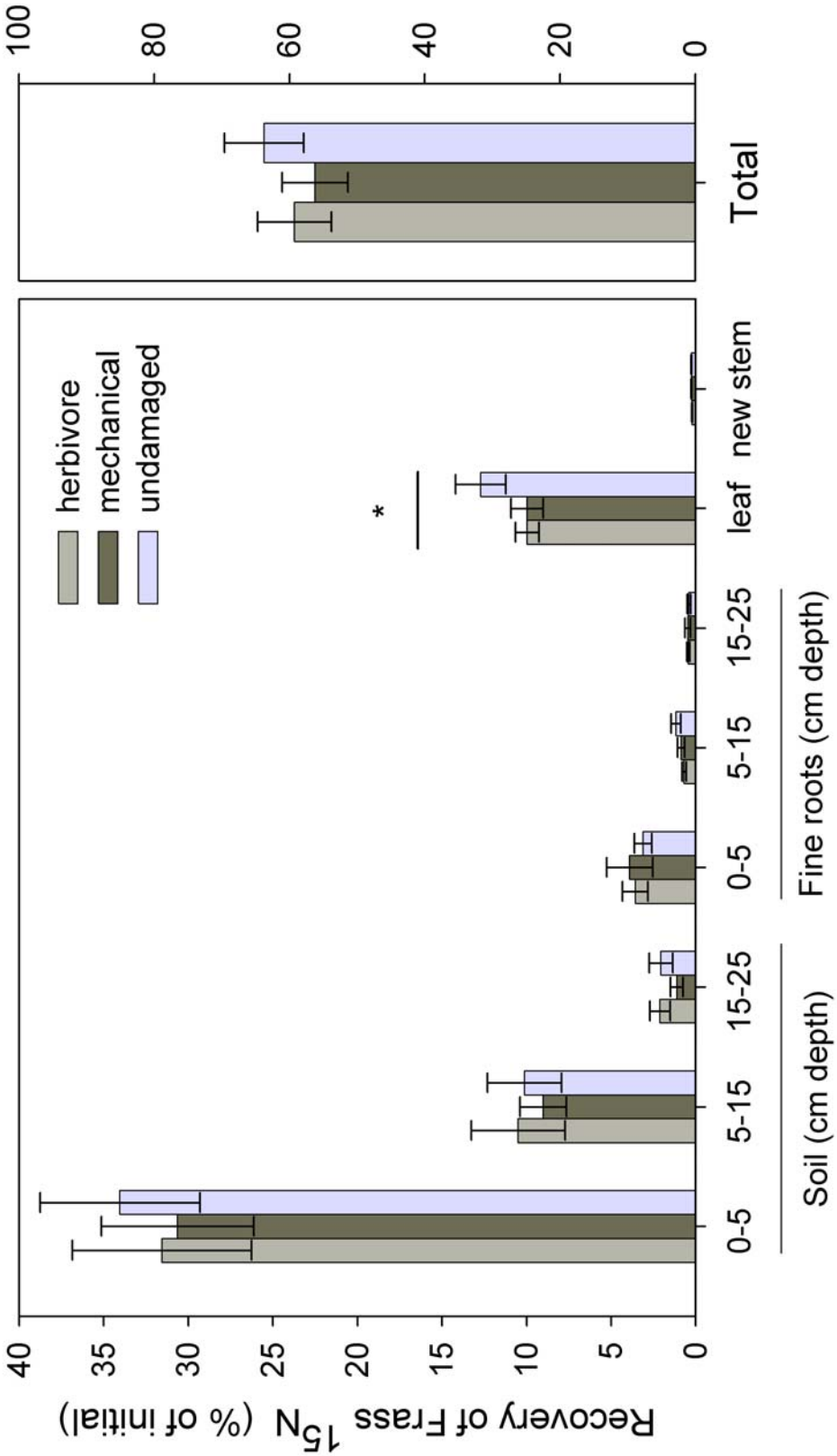
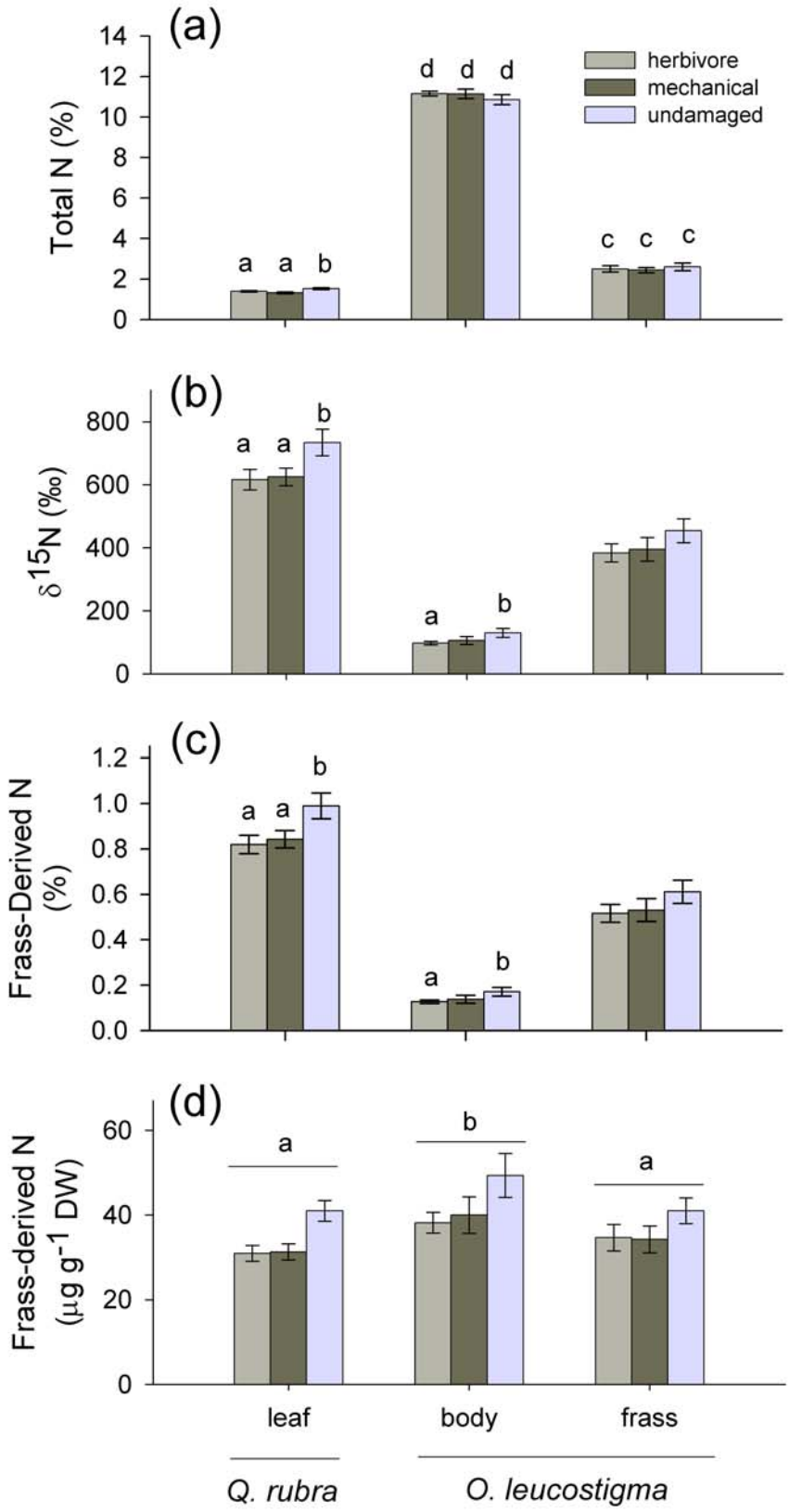

Recovery of Frass ^{15}N (% of initial)
0
5
10
15
20
25
30
35
40
herbivore
mechanical
undamaged
*
0-5
5-15
15-25
0-5
5-15
15-25
leaf
new stem
Soil (cm depth)
Fine roots (cm depth)
0
20
40
60
80
100
Total

Figure 4.5

(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. (d) Nitrogen, expressed per unit biomass, in *Q. rubra* foliage and *O. leucostigma* bodies and frass in spring 2004 derived from frass deposited in spring 2003. Bars represent means ± SE of 15 replicates. Different letters above bars or groups of bars indicate statistical significance of treatment means using the SNK post-hoc test (α=0.05).

Figure 4.5


(a)
Total N (%)
herbivore
mechanical
undamaged
(b)
δ15N (‰)
(c)
Frass-Derived N (%)
(d)
Frass-derived N (μg g-1 DW)
leaf
body
frass
Q. rubra
O. leucostigma

CHAPTER 5

OAK SEEDLINGS REDUCE BELOWGROUND CARBON ALLOCATION FOLLOWING FOLIAR HERBIVORE DAMAGE[1]

[1]Frost, C.J. and M.D. Hunter For Submission to Ecology Letters

ABSTRACT

Belowground carbon allocation (BCA) by plants links soil ecosystems and aboveground processes and influences ecosystem function. However, the environmental factors that affect BCA and the extent of their influences are not well understood. Grazing-tolerant grasses can increase BCA in response to foliar herbivore damage, and recent evidence suggests that woody plant taxa might respond similarly to aboveground herbivore damage. We conducted a microcosm experiment with red oak, *Quercus rubra*, seedlings to test the hypothesis that foliar herbivory would increase BCA, C rhizodeposition (root exudation), and nitrogen (N) uptake. Thirty-six microcosms, containing soil collected from the Coweeta Hydrologic Laboratory in western North Carolina, were housed in a single, environmentally-regulated growth chamber. The tops of the microcosms were sealed with wax to isolate the soil and roots from the chamber environment. Seedlings were subjected to an experimental treatment of either herbivore or mechanical damage, or left as undamaged controls. Immediately following the damage treatment, microcosms were injected with ^{15}N-enriched glycine and the chamber was pulsed with ^{13}C-enriched CO_2. Herbivore damage reduced BCA and consequently increased the percentage of new C in the foliage. However, the proportion of new C belowground that was recovered in the rhizosphere was higher, suggesting that roots from damaged seedlings were "leakier" than were roots from undamaged seedlings. While total recovery of ^{15}N was not affected by damage, allocation of new N to stems was higher in herbivore-damaged seedlings. This is the first study to demonstrate that allocation of newly assimilated C and N in oaks is affected by aboveground herbivory.

INTRODUCTION

Plants provide a direct conduit between above and belowground processes in terrestrial ecosystems and, as a result, influence ecosystem function (Wardle 2002;Knops *et al.* 2002). Some carbon (C) assimilated through photosynthesis is allocated belowground to roots and exudates into the rhizosphere (Whipps & Lynch 1983;Martin & Merckx 1992). This C source is critical to the regulation of soil organic matter (Giardina *et al.* 2005) and as an energy source for rhizosphere microorganisms (Martens 1990;Cheng *et al.* 1996;Kuzyakov & Cheng 2001;Cardon *et al.* 2002;Farrar *et al.* 2003). Rhizosphere micro-organisms decompose soil organic matter (Cheng & Coleman 1990), mineralizing nitrogen (N) that is then available to plants (BassiriRad *et al.* 2001;Hamilton & Frank 2001;Bronstein 2001;Rudgers *et al.* 2004;Kula *et al.* 2005). Nitrogen translocated from the soil to the shoots and foliage (Rajaniemi & Reynolds 2004) strongly influences relative growth rates (RGR) and net primary production (NPP) (Chapin, III 1980;Reich *et al.* 1997). Because belowground C allocation (BCA) and rhizodeposition are critical in this process, factors that alter BCA may influence the feedback loop. BCA is the third largest biologically-mediated C flux on a global scale, and may comprise some 50% of NPP (Giardina *et al.* 2005). However, despite its magnitude and importance, the environmental controls on BCA and their consequences for ecosystem function are not well understood. Plant-mediated links between aboveground and belowground processes in terrestrial ecosystems have received considerable attention recently (Wardle 2002), with particular focus on the influence of herbivores (Hunter 2001).

Herbivores can influence plant allocation patterns. Plants respond to herbivore damage with a complex suite of direct (Schultz & Baldwin 1982;Rossiter *et al.* 1988;Schultz & Appel 2004) and indirect (De Moraes *et al.* 1998;Hoballah & Turlings 2001;De Moraes *et al.* 2001;Ament *et al.* 2004) chemical changes to their foliage. These chemical changes reflect changes in C allocation patterns in the foliage that are likely regulated by wound-induced genes (Davis *et al.* 1991). Such herbivore-mediated shifts in plant C allocation patterns also extend belowground and therefore influence BCA. Much of the focus of this research has been on grasses (Frank & Groffman 1998;Hamilton & Frank 2001;Mikola *et al.* 2001a;Mikola *et al.* 2001b) and agricultural crops (Holland 1995;Holland *et al.* 1996). Stimulation of BCA and rhizodeposition by foliar herbivory is evidently common among grasses, particularly those that suffer severe damage by grazers (McNaughton *et al.* 1988;Frank & Groffman 1998). Aboveground biomass stimulated in these grass communities following such herbivory (Frank & McNaughton 1993) is, in part, due to increased rhizodeposition that presumably stimulates soil N mineralization (Hamilton & Frank 2001). However, herbivore-mediated changes in BCA may not be limited to grazing-tolerant grasses.

Oaks periodically allocate and rhizodeposit C belowground and their rhizosphere microbial communities respond to these inputs (Cardon *et al.* 2002). Recent evidence suggests that responses to herbivore damage similar to those observed in grasses may also occur in woody plants (Ayres *et al.* 2004;Frost & Hunter 2004;Babst *et al.* 2005). While the benefit to BCA could be C storage, Hamilton & Frank (2001) argued that one benefit of C allocation to rhizodeposition is increased nutrient uptake, possibly facilitated

by rhizosphere micro-organisms. However, no study to date has used a dual isotope approach to explore herbivore-mediated increases in rhizodeposition resulting in acquisition of new soil N.

Our objective in this study was to explore the effects of aboveground insect herbivore grazing on BCA, C rhizodeposition, and N uptake patterns in *Q. rubra* seedlings and their rhizosphere microbial populations. Based on our previous results (Frost & Hunter 2004) and those from other systems (Holland *et al.* 1996;Hamilton & Frank 2001;Babst *et al.* 2005), we predicted that foliar herbivory on oaks would increase BCA and result in increased uptake of soil N into root or stem tissue for storage. We report here the results of a short-term, dual-isotope (^{13}C, ^{15}N), pulse-chase experiment using *Q. rubra* seedlings subjected to real and simulated herbivore damage in controlled microcosms.

METHODS

Methods for isolating aboveground and belowground components in microcosm are well established (Cheng 1996), and we modified the design to include side injection ports for the addition of labeled N (Figure 5.1). The microcosms were constructed of 15 x 5 cm clear polycarbonate tubing with ~250 g of a sieved soil/sand mix (1:1 v/v). Soil used in the experiment was collected from watershed 27 at the Coweeta Hydrologic Laboratory ("CWT") in western North Carolina. Soil was passed through a 2.5 x 2.5 mm sieve to remove roots and other debris. This soil was then mixed 1:1 (v:v) with acid-

washed, autoclaved sand. The soil was at ~70% field capacity when the microcosms were established.

The experiment was contained entirely in a Conviron E15 growth chamber controlled with CMP 4030 v.4.0 software (Conviron, Winnepeg, Manitoba, Canada). The chamber was maintained on a 12 h photoperiod with "daytime" and "nighttime" temperatures of 25ºC and 16ºC, respectively. There was a 2-hour ramping transition period for both light and temperature regimes in order to simulate sunrise and sunset. The maximum photon flux density at the level of the microcosms was ~500 μmol m^{-2} s^{-1}.

Seedlings were grown from wild seeds collected underneath a single parent red oak. Seeds were planted in 2002 in potting soil with time-release fertilizer and spent the 2003 growing season in a shaded, outdoor facility adjacent to the University of Georgia Botany greenhouses, Athens, GA, USA. In February 2004, the dormant seedlings were transplanted to the experimental microcosms. The seedlings were carefully removed from their germinating plugs and the potting soil gently brushed from the roots. The bare roots of the seedlings were then placed inside the microcosms and filled with CWT soil/sand mix. Soil was gently packed around the roots and the microcosm filled completely. Microcosms were then brought to field capacity and wet weights of the entire microcosm recorded. All microcosms then equilibrated to the growth chamber for 4 months prior to experimental manipulations, during which time the seedlings broke dormancy and fully expanded their foliage.

Each microcosm was assigned randomly to one of two damage groups (herbivore, mechanical) or left undamaged as a control. There were five replicates of each treatment

per date, with destructive sampling 2 and 7 days following the end of damage treatments and isotope additions (N= 30 total). In addition, 2 microcosms were isotope-free controls and 2 microcosms per date were seedling-free controls against isotope contamination. The isotope-free controls were removed and destructively sampled immediately following the damage treatment. Seedlings began to break bud on 19 Apr 2004 and were fully expanded by mid-May 2004. On 13 Jun 2004, each microcosm was brought to field capacity (by weight) and the top sealed with molten paraffin wax separated from the soil with a layer of aluminum foil to prevent decomposition of the wax by soil micro-organisms. Fourth-instar herbivores (4 per seedling) were added on 16 Jun 2004 and removed on 18 Jun 2004. Herbivores and seedlings were completely enclosed in small branch bags tied to the microcosms. All seedlings were covered to control for any effects of the bags (e.g., reduced photosynthesis). Mechanical damage was imposed on 16 and 17 Jun 2004 by using a single hole puncher to remove leaf tissue from the edges of the leaves. Mechanical damage mimicked herbivore damage as closely as possible; herbivore and mechanical damage removed 22.2 ± 1.1 and 20.6 ± 1.2 % leaf area (mean ± SE), respectively, using a common visual damage estimate technique (Hunter 1987). Undamaged seedlings suffered no damage.

On 18 Jun 2004 following herbivore removal, the two enrichment-free microcosms were destructively sampled (described below) and their isotope ratios used to represent background abundances. We then injected each microcosm with a total of 3.0 ml of a 0.27 M solution of 98 atom% ^{15}N-Glycine into the three vertically-distributed injection ports sealed with rubber septa (Figure 1). This added 11.2 mg of highly

enriched but dilute ^{15}N to minimize the potential for priming effects (Jenkinson *et al.* 1985). While plants can directly acquire organic forms of N (Lipson & Näsholm 2001), we added glycine instead of mineral ^{15}N to promote microbial mineralization followed by plant uptake of the mineralized ^{15}N. Immediately following ^{15}N injections, the chamber was sealed and injected with 1 L of 99 atom% $^{13}CO_2$. Two more equivalent pulse labeling events occurred in the subsequent 2 hours. Following treatments, the chambers remained sealed for 48 hr when 2-day microcosms were removed. The 7-day microcosms were watered through the injection ports as necessary until they were destructively sampled on 25 Jun 2004.

All microcosms were destructively sampled and sorted into the following categories for analysis: foliage, new stem (during 2004 growing season), old stem, fine roots, rhizosphere soil, and bulk soil. Foliage was clipped at the stem; stems were clipped at the surface of the wax layer. The soil and roots were then pushed out the bottom of the microcosm into a plastic bag with as little disturbance to the soil structure as possible. The main root was then gently lifted from the soil with some soil clinging to the roots. The "bulk soil" was defined as the soil collected in the plastic bag; "rhizosphere soil" was defined as the soil clinging to the roots. Rhizosphere soil was immediately brushed from the root material into a separate bag. The roots were then washed in a 2 x 2 mm sieve to remove remaining soil particles and the fine roots were separated from the tap root. Leaf, stem, and root samples were dried separately for 48 h at 60^{o}C and ground into a fine powder for isotope analysis (*Methods: Isotope Analysis*).

We also sampled soil respiration continuously during the experiment using NaOH traps. However, the traps were compromised by atmospheric contamination, and they will not be considered further.

Soil Analysis

Rhizosphere and bulk soils were analyzed separately. Soils were passed through a 2x2 mm screen mesh and separated into three subsamples for separate analyses. The first subsample of soil was weighed, dried for 48 h at 60°C to determine water content, and the dried sample ground to a fine powder and analyzed for total C and N and their isotopes (*Methods: Isotope Analysis*). The remaining two soil samples were used to analyze extractable microbial and non-microbial C and N via the fumigation-extraction method with 0.5 M K_2SO_4 (Vance *et al.* 1987). Briefly, one subsample was immediately extracted with 50ml 0.5M K_2SO_4 on an orbital shaker (150rpm) and subsequently filtered through Whatman 42 filter paper (Frost & Hunter 2004). The filtrate represents a soluble, labile pool of soil C and N (Powlson & Jenkinson 1976;Cook & Allan 1992a;Cook & Allan 1992b). The third subsample was subjected to chloroform fumigation for 48 h under reduced pressure, and then extracted as above. Non-fumigated (NF) and fumigated (F) samples were analyzed for total organic C (TOC) and $\delta^{13}C$ (*Methods: Isotope Analysis*). Total microbial biomass C was estimated from the difference between F and NF: Microbial Biomass C = (F - NF) / k_{ec}, where k_{ec} is a correction factor based on the efficiency of chloroform fumigation (Sparling & West 1988).

Isotope Analysis

We use both the atom% and δ notations, where δ units are expressed per thousand deviations from the atmospheric standard (atom% ^{15}N = 0.3663, atom% ^{13}C = 1.106), δ (‰) = [(sample atom% / standard atom%) - 1] x 1000 (Schimel 1993;Lajtha & Michener 1994). All dry stable isotope samples (i.e., leaf, stems, roots, soils) were analyzed on a Costech Elemental Combustion System 4010 (Costech Analytical Technologies, Inc., Valencia, CA) connected to a ThermoFinnigan ConfloIII Interface and Deltaplus Continuous Flow-Stable Isotope Ratio Mass Spectrometer (IRMS) (Thermo Electron, Waltham, MA) for total N, total C, $\delta^{15}N$ and $\delta^{13}C$. Soil extracts were analyzed on an OI 1010 Total Organic Carbon (TOC) analyzer (OI Analytical, College Station, TX) connected to the above IRMS via a Scrubber Interface designed and built at the G.G. Hatch Isotope Laboratories (University of Ottowah, Ontario, Canada). Sets of samples were analyzed on two dates to provide estimates of IRMS, elemental analyzer, and TOC errors. The coefficient of variation on these data sets were 0.85 for $\delta^{15}N$, 2.17 for total N, 0.02 for ^{13}C, and 1.13 for total C.

Statistical Analysis

Data were analyzed using the GLM procedure of SAS 8.2 with Student-Neuman-Keuls (SNK) post-hoc tests to determine significant differences among treatment means (Littell *et al.* 2002). Data were transformed as necessary to satisfy the assumption of normality (Kery & Hatfield 2003).

RESULTS

Herbivore and mechanical damage reduced new C allocation to fine roots (Figure 5.2). There were no date effects on $\delta^{13}C$ abundances or allocation patterns, so we pooled data for analysis. Damage (irrespective of type) resulted in higher allocation of ^{13}C in leaves ($F_{2,27}$=3.54, p=0.0441) and lower in the fine roots ($F_{2,27}$=4.92, p=0.0158) of the seedlings (Figure 5.2a). Allocation in the new stem, old stem, and rhizosphere soil was not affected by damage ($F_{2,27}$=3.28, p=0.0545; $F_{2,27}$=1.55, p=0.2329; $F_{2,27}$=0.45, p=0.6427, respectively). However, damaged seedlings had lower $\delta^{13}C$ in their new stems than did undamaged seedlings ($F_{2,29}$=4.35, p=0.0244, Figure 5.2b). The general pattern of $\delta^{13}C$ enrichment, as expected, was strongest in the foliage and new stem and weakest in the soil, with bulk soils showing no evidence of $\delta^{13}C$ enrichment (Figure 5.2b). We recovered 1.82 ± 0.20 mg ^{13}C in the microcosms, 72.0 ± 13.9 μg of which was in the rhizosphere soil.

A greater percentage of newly-fixed C allocated belowground was recovered in the rhizosphere following aboveground herbivore activity (Figure 5.3). Since bulk soils were not $\delta^{13}C$ enriched, the fine roots and rhizosphere soils comprised the belowground elements of the microcosm. Despite the lower allocation of ^{13}C to fine roots following damage (Figure 5.2), the ^{13}C in the rhizosphere was similar among treatments ($F_{2,27}$=0.45, p=0.6427). This combination resulted in a greater percentage of belowground C in the rhizosphere of the damaged relative to undamaged microcosms ($F_{2,27}$=3.60, p=0.0421, Figure 5.3a). However, neither the total microbial C nor ^{13}C-enrichment were affected by the damage treatment ($F_{2,28}$= 0.08, p= 0.9228, Figure 3b; $F_{2,28}$= 1.97, p= 0.1629, Figure

5.3c, respectively). While the ^{13}C recovered in the rhizosphere microbial biomass was not affected by damage ($F_{2,28}$=0.76, p=0.4771, Figure 5.3d), it correlated positively with the total rhizosphere ^{13}C-enrichment (r^2= 0.4232, p<0.0001, data not shown).

The microbial biomass was the principal labile pool for newly assimilated and rhizodeposited C (Figure 5.4). The total and net ^{13}C enrichment of the microbial biomass were significantly greater than that of the non-microbial, extractable DOC ($F_{1,57}$= 5.89, p= 0.0186, Figure 5.4a; $F_{1,57}$= 22.07, p<0.0001, Figure 5.4b, respectively). Significantly more total ^{13}C was therefore recovered in the microbial biomass than the extractable DOC (2.14 ± 0.69 μg ^{13}C in microbes v. 0.69 ± 0.14 μg ^{13}C DOC; $F_{1,57}$=20.38, p<0.0001, Figure 5.4c). The ^{13}C recovered in the microbial biomass accounted for ~70-80% of all ^{13}C recovered in the extractable fraction of the rhizosphere soil. In addition, though the total microbial C was <1% of the total rhizosphere C, the ^{13}C recovered in the microbial biomass was 4-8% of the ^{13}C recovered in the rhizosphere ($F_{1,57}$= 86.43, p<0.0001, Figure 5.3d). The proportion of microbial new ^{13}C to rhizosphere new ^{13}C declined significantly sampling dates ($F_{1,28}$= 5.24, p=0.0316, Figure 5.3d), suggesting that the micro-organisms were utilizing a less-enriched source of C by day 7. Overall, we estimated that 93.2 ± 5.5 % (mean ± SD) of rhizosphere new ^{13}C was in non-extractable form.

Nitrogen allocation to new stem tissue was higher in herbivore-damaged seedlings 7 days following treatment (Figure 5.5). Seedlings acquired ^{15}N within two days; approximately 37% of the accumulated N had been translocated to and enriched significantly the aboveground tissue (Figure 5.5a,b). Seedlings continued to acquire ^{15}N over the course of the experiment, resulting in significant increases in $\delta^{15}N$ in all four

seedling tissues between sampling dates (leaf $F_{1,29}$=38.65, p<0.0001; new stem $F_{1,29}$=33.29, p<0.0001; old stem $F_{1,29}$=46.76, p<0.0001; fine roots $F_{1,29}$=11.57, p=0.0023, Figure 5.4b,d). Two and seven days following ^{15}N additions, the seedlings accumulated 126.8 ± 13.8 μg (1.10 ± 0.11 % of added ^{15}N) and 340.4 ± 39.1 μg ^{15}N (2.84 ± 0.33 % of added ^{15}N), respectively. The distribution of new N within seedlings changed over the course of the experiment, with significant increases in all three aboveground tissues (leaf $F_{1,29}$=22.10; new stem $F_{1,29}$=30.89; old stem $F_{1,29}$=29.75; p<0.0001 for all, Figure 5.5a,c). The damage treatment did not affect total recovery of ^{15}N ($F_{2,29}$=0.68, p=0.5173). However, as aboveground biomass accumulated new N, a significantly higher percentage of ^{15}N was allocated to the new stem in the seedlings suffering herbivore damage ($F_{2,14}$=3.91, p=0.0492, Figure 5.5b). This could suggest greater allocation to storage following herbivore damage, though the new stem tissue was the smallest pool of N (Table 1).

DISCUSSION

This is the first study to demonstrate that the allocation patterns of newly assimilated C and N in oak seedlings can be altered by foliar herbivore damage. Specifically, belowground C allocation was lower and N allocation to new stems was higher in the herbivore-damaged seedlings than undamaged seedlings. Despite the lower BCA following herbivore damage, the total amount of newly-assimilated C in the rhizosphere soil was not affected by damage. This suggests that herbivore damage mediated a shift that allocated new C to foliage that otherwise would have been used for

root growth or belowground C storage. While belowground C allocated to rhizodeposition was apparently higher in herbivore-damaged seedlings relative to undamaged seedlings, the effect was evidently compensatory. Irrespective of foliar damage, the rhizosphere microbial biomass disproportionately used rhizodeposited C relative to their total biomass. In addition, although there were no effects of damage on overall plant acquisition of new N, the higher allocation of new N to new stem tissue following herbivore damage suggests that storage of N was stimulated by herbivory.

Our data suggest that oak seedlings divert C away from root growth or storage, but apparently not rhizodeposition, in the presence of foliar herbivores. While this is opposite our prediction, it may be consistent with the tendency of oak seedlings to allocate significant C to root biomass in the absence of foliar herbivores (Maillard *et al.* 2001). The additional C allocated to foliage may be converted into defensive compounds (e.g., increased tannin production), which are typically induced following herbivore feeding (Schultz & Baldwin 1982;Rossiter *et al.* 1988;Allison & Schultz 2004). These data suggest that a tradeoff may occur in oak seedlings whereby foliar damage necessitates reduction in BCA to conserve foliar C. Oak seedlings have minimal C stores (Maillard *et al.* 2001), which may demand such a tradeoff. Reduction in BCA may have deleterious consequences for the growth of herbivore-damaged seedlings if root systems are compromised relative to undamaged seedlings. Unfortunately, our short-term experiment was not designed to account for effects on total root biomass. As oaks mature and root systems develop with C storage, it is possible that an opposite response of oaks to foliar herbivores might be observed.

A number of studies suggest that BCA increases in woody plants following foliar damage. Ayres *et al.* (2004) found increases in soil C sequestration, but decreases in course and fine root biomass, following mechanical damage to leaves of *Abies* and *Fagus* seedlings. Frost & Hunter (2004) measured increased soil respiration from oak-soil mesocosms following foliar herbivory but not mechanical damage, suggesting that herbivores specifically triggered the potential C allocation shifts. Babst *et al.* (2005) elegantly showed that *Populus tremuloides* seedlings increase export of newly assimilated ^{11}C to roots following application of jasmonic acid (JA). JA is a well-known signaling hormone that stimulates chemical defenses in plants (Farmer & Ryan 1990;McConn *et al.* 1997;Baldwin 1998;Engelberth *et al.* 2004;Ament *et al.* 2004). Other studies using *Populus* sp. also observed similar responses (Bassman & Dickmann 1985;Kosola *et al.* 2001;Kosola *et al.* 2004). While it is tempting to infer a general conclusion that foliar damage stimulates BCA, our data present a cautionary note that the BCA can also be reduced following foliar damage.

Changes in BCA can occur actively or passively. Passive reduction of BCA following herbivore damage can occur if such damage reduces total C assimilation and the ability of plants to sustain fine root biomass (Ruess *et al.* 1998). The theoretical rationale for active reduction of BCA following herbivore damage is maintenance and/or induction of foliar defenses. We suspect that this is the mechanism that operated in our oak seedlings since there was no significant reduction in N assimilation following foliar damage. On the other hand, increases in BCA imply active control by plants, and there are two theoretical rationales underlying active increases of BCA following foliar

damage: C storage and nutrient acquisition. Belowground C storage would not affect rhizosphere processes or N uptake, at least while the C was in storage. Rather, storage would provide an energy source from which to recover from the foliar damage at a later date. Increased nutrient acquisition resulting from herbivore-mediated increases in BCA can occur by changes in root morphology, direct root N uptake, or facilitation with soil microorganisms. Aboveground damage might stimulate root growth, exploration, and biomass accumulation (Ritchie *et al.* 1998). Increased root activity can alter soil respiration and N dynamics without adding root biomass; roots can actively increase kinetic rates of nutrient uptake. Ion transport is an energetic process (Clarkson 1985), with nitrate assimilation reaching ~20% of NPP (Bloom *et al.* 1992). Finally, rhizodeposition of C-rich compounds can provide a substrate for microbial activity that can result in a positive feedback of microbially-mediated N mineralization and subsequent plant uptake (Hamilton & Frank 2001). These four outcomes correspond roughly to the four mechanisms by which plant belowground responses to foliar herbivory could affect belowground C and N fluxes in the absence of surface soil inputs identified by Frost & Hunter (2004).

Root exudation of photosynthetically-derived organic C can also occur actively or passively. The gradient of C concentrations between roots and rhizosphere soil (Table 5.1) provides for the potential for passive diffusion of C into the rhizosphere (Kuzyakov 2002). There is substantial evidence that, in addition to passive diffusion, plants actively regulate rhizodeposition (Holland *et al.* 1996;Knops *et al.* 2002;Farrar *et al.* 2003;Jones *et al.* 2004;Thelen *et al.* 2005). In our study, a greater proportion of belowground C

apparently entered the rhizosphere following herbivore damage. This suggests that the roots were "leakier" following damage. With no evidence that N uptake was affected by damage, "leakier" roots provide evidence for active control of rhizodeposition by the oak seedlings. (Hamilton & Frank 2001) reported increases in microbial biomass following similar active rhizodeposition, though the entire pool of rhizosphere C increased (Hamilton & Frank 2001). In our case, the "active" rhizodeposition did not increase the pool of rhizodeposited C but rather apparently compensated for reduced overall belowground allocation of new C following herbivore damage.

The rhizosphere microbial biomass (as estimated by the fumigation-extraction technique) was apparently actively incorporating and using root rhizodeposits. Cardon *et al.* (2002) reported a correlation between periodic C flushes to the roots of *Q. rubra* and the rhizosphere microbial biomass. Our results are consistent with their observations. In our study, there was a positive correlation between C allocation to rhizodeposition and new C recovered in the microbial biomass. The rhizosphere microbes comprised ~52% of the extractable C in the rhizosphere, but they contained 70-80% of the newly rhizodeposited C. In addition, though the rhizosphere microbes comprised <1% of the total rhizosphere C, they accounted for over 8% of the newly rhizodeposited C within 2 days of isotope additions. This suggests that rhizosphere micro-organisms were disproportionately utilizing newly rhizodeposited C from the roots. By the end of 7 days, they accounted for only ~4% of the newly rhizodeposited C. Presumably, roots continued to rhizodeposit C but with diminishing ^{13}C enrichment proportional to the number of days following the $^{13}CO_2$ pulse-labeling event. If microbes utilize the C

rhizodeposited days following the labeling treatment, their $\delta^{13}C$ would be diluted relative to that of the total rhizosphere pool. This is consistent with our results, suggesting that the rhizosphere microbial biomass continually utilized newly rhizodeposited C as their primary C source (Cheng *et al.* 1996). In our study, aboveground damage did not affect absolute rhizodeposition of ^{13}C. However, based on our results of microbial utilization of rhizodeposited C, any affect of aboveground damage on rhizodeposition would be predicted to influence the microbial community (Hamilton & Frank 2001).

Interestingly, over ~93% of the ^{13}C recovered in the rhizosphere was not K_2SO_4-extractable and therefore neither labile nor microbial. The forms of rhizodeposited C are probably species specific, but include simple sugars and amino acids (Whipps & Lynch 1983;Jaeger III *et al.* 1999;Bringhurst *et al.* 2001) that are presumably suitable substrates for microbial activity. Soil microbes utilize ephemeral C and nutrients within hours of deposition (Seely & Lajtha 1997;Zogg *et al.* 2000), and it is possible that the rhizodeposited C had been utilized and converted into recalcitrant material by the 2-day sampling period. However, it is also possible that the physical properties of the soil organic matter abiotically bound the large majority of the rhizodeposits (Paul & Clark 1996).

Herbivore damage in the oak seedlings stimulated N allocation in new stems, which we suggest represents increased storage of newly assimilated N. While the N in new stems could also be in transport in xylem sap, there were no treatment-based differences in enrichment levels or allocation of new ^{15}N in the foliage. This suggests that the N was not in transport but rather incorporated into the new stem tissue. This

partially supports our hypothesis. We predicted that, herbivore-damaged oaks would allocate more N to storage tissues than would undamaged oaks. This differs from the results of Hamilton & Frank (2001), who showed increases in foliar N in grazed grasses relative to controls. Interestingly, N storage in new stems evidently occurred independently of C dynamics. We also found no evidence for increases in assimilation of new N following foliar damage, which suggests that the reduced BCA following foliar herbivory nevertheless did not apparently affect N uptake dynamics. Indeed, the higher allocation of N to new stem storage following herbivore damage may result in higher foliar N concentrations in the next growing season.

The use of seedlings in all of the studies explicitly exploring BCA in woody plants underscores the difficulty of measuring BCA in woody taxa and highlights an important pitfall. Seedlings often do not behave similarly to saplings or mature trees when confronted with herbivores (reviewed in Nykanen & Koricheva 2004). As a result, while our data and those from other studies outlined above are important as a first step, they may not reflect responses to herbivory of mature trees in a forested landscape. Clearly, short-term microcosm experiments with oak seedlings do not scale to mature oaks, and we do not intend for these results to be scaled to individual mature oaks or forested landscapes. Rather, we designed the experiment to explore the hypothesis that foliar herbivores would influence oak BCA and the feedback loops that depend on BCA. Further experiments will be required to determine if the patterns observed in our study are seedling specific or applicable to oaks in general. There is ample evidence that different tree species partition uniquely C and N and have significant impact on C and N

dynamics in their respective soils (Verchot *et al.* 2001;Templer *et al.* 2003;Fitzhugh *et al.* 2003;Lovett *et al.* 2004), which further broadens the importance of understanding the effects of foliar herbivory on BCA at a landscape scale. Future studies, though expensive and logistically difficult, should test for similar herbivore-mediated changes in BCA of mature woody taxa. Our data provide a compelling case that herbivores influence BCA and N allocation patterns in at least one ontogenetic stage of oak development.

ACKNOWLEDGMENTS

We would like to thank M. Cabrera, D. Camp, D. Coleman, L. Donovan, R. Goergen, P. Hendrix, and C. Jennison for assistance and/or comments on previous versions. Bob McCron from the Canadian Forest Service (Sault Ste. Marie, Ontario) provided tussock moth egg masses. Tom Maddox processed all isotope samples in the Analytical Lab of the Institute of Ecology. This research is supported by NSF grants DEB-9815133 and DEB-0404876 and a UGA University-Wide Assistantship.

REFERENCES

Allison S.D. & Schultz J.C. (2004) Differential activity of peroxidase isozymes in response to wounding, gypsy moth, and plant hormones in northern red oak (Quercus rubra L.). *J Chem Ecol* 30, 1363-1379

Ament K., Kant M.R., Sabelis M.W., Haring M.A. & Schuurink R.C. (2004) Jasmonic Acid Is a Key Regulator of Spider Mite-Induced Volatile Terpenoid and Methyl Salicylate Emission in Tomato. *Plant Physiol* 135, 2025-2037

Ayres E., Heath J., Possell M., Black H.I.J., Kerstiens G. & Bardgett R.D. (2004) Tree physiological responses to above-ground herbivory directly modify below-ground processes of soil carbon and nitrogen cycling. *Ecol Letters* 7, 469-479

Babst B.A., Ferrieri R.A., Gray D.W., Lerdau M., Schlyer D.J., Schueller M., Thorpe M.R. & Orians C.M. (2005) Jasmonic acid induces rapid changes in carbon transport and partitioning in Populus. *New Phytol* 167, 63-72

Baldwin I.T. (1998) Jasmonate-induced responses are costly but benefit plants under attack in native populations. *Proc Natl Acad Sci (USA)* 95, 8113-8118

BassiriRad H., Gutschick V.P. & Lussenhop J. (2001) Root system adjustments: regulation of plant nutrient uptake and growth responses to elevated CO_2. *Oecologia* 126, 305-320

Bassman J.H. & Dickmann D.I. (1985) Effects of defoliation in the developing leaf zone on young Populus x euramericana plants 2. distribution of photosynthate-C-14 after defoliation. *For Sci* 31, 358-366

Bloom A.J., Sukrapanna S.S. & Warner R.L. (1992) Root respiration associated with ammonium and nitrate absorption and assimilation by barley. *Plant Physiol* 99, 1294-1301

Bringhurst R.M., Cardon Z.G. & Gage D.J. (2001) Galactosides in the rhizosphere: utilization by *Sinorhizobium meliloti* and development of a biosensor. *Proc Natl Acad Sci (USA)* 98, 4540-4545

Bronstein J.L. (2001) The exploitation of mutualisms. *Ecol Letters* 4, 277-287

Cardon Z.G., Czaja A.D., Funk J.L. & Vitt P.L. (2002) Periodic carbon flushing to roots of *Quercus rubra* saplings affects soil respiration and rhizosphere microbial biomass. *Oecologia* 133, 215-223

Chapin F.S., III (1980) The Mineral Nutrition of Wild Plants. *Ann Rev Ecol System* 11, 233-260

Cheng W.X. & Coleman D.C. (1990) Effect of living roots on soil organic matter decomposition. *Soil Biol Biochem* 22, 781-787

Cheng W. (1996) Measurement of rhizospheric respiration and organic matter decomposition using natural 13C. *Plant Soil* 183, 263-268

Cheng W., Zhang Q., Coleman D.C., Carroll R.C. & Hoffman C.A. (1996) Is available carbon limiting microbial respiration in the rhizosphere? *Soil Biol Biochem* 28, 1283-1288

Clarkson D.T. (1985) Factors affecting mineral nutrient acquisition by plants. *Ann Rev Plant Physiol* 36, 77-115

Cook B.D. & Allan D.L. (1992a) Dissolved organic carbon in old field soils: compositional changes during the biodegradation of soil organic matter. *Soil Biol Biochem* 24, 595-600

Cook B.D. & Allan D.L. (1992b) Dissolved organic carbon in old field soils: total amounts as a measure of available resources for soil mineralization. *Soil Biol Biochem* 24, 585-594

Davis J.M., Gordon M.P. & Smit B.A. (1991) Assimilate movement dictates remote sites of wound-induced gene expression in poplar leaves. *Proc Natl Acad Sci (USA)* 88, 2393-2396

De Moraes C.M., Lewis W.J., Pare P.W., Alborn H.T. & Tumlinson J.H. (1998) Herbivore-infested plants selectively attract parasitoids. *Nature* 393, 570-573

De Moraes C.M., Mescher M.C. & Tumlinson J.H. (2001) Caterpillar-induced nocturnal plant volatiles repel conspecific females. *Nature* 410, 577-580

Engelberth J., Alborn H.T., Schmelz E.A. & Tumlinson J.H. (2004) Airborne signals prime plants against insect herbivore attack. *Proc Natl Acad Sci (USA)* 101, 1781-1785

Farmer E.E. & Ryan C.A. (1990) Interplant communication: airborn methyl jasmonate induces synthesis of proteinase inhibitors in plant leaves. *Pro Natl Acad Sci (USA)* 87, 7713-7716

Farrar J., Hawes M., Jones D. & Lindow S. (2003) How roots control the flux of carbon to the rhizosphere. *Ecology* 84, 827-837

Fitzhugh R.D., Lovett G.M. & Venterea R.T. (2003) Biotic and abiotic immobilization of ammonium, nitrite, and nitrate in soils developed under different tree species in the Catskill Mountains, New York, USA. *Global Change Biol* 9, 1591-1601

Frank D.A. & Groffman P.M. (1998) Ungulate versus landscape control of soil C and N processes in grasslands of Yellowstone National Park. *Ecology* 79, 2229-2241

Frank D.A. & McNaughton S.J. (1993) Evidence for the promotion of aboveground grassland production by native large herbivores in Yellowstone National Park. *Oecologia* 96, 157-161

Frost C.J. & Hunter M.D. (2004) Insect canopy herbivory and frass deposition affect soil nutrient dynamics and export in oak mesocosms. *Ecology* 85, 3335-3347

Giardina C.P., Coleman M.D., Binkley D., Hancock J.E., King J.S., Lilleskov E.A., Loya W.M., Pregitzer K.S., Ryan M.G. & Trettin C.C. (2005) The response of belowground carbon allocation in forests to global change. In: *Tree Species Effects on Soils: Implications for Global Change* (eds Binkley D. & Menyailo O.), pp. 119-154. Kluwer Academic Publishers, Dordrecht.

Hamilton E.W.I. & Frank D.A. (2001) Can plants stimulate soil microbes and their own nutrient supply? Evidence from a grazing tolerant grass. *Ecology* 82, 2397-2404

Hoballah M.E.F. & Turlings T.C.J. (2001) Experimental evidence that plants under caterpillar attack may benefit from attracting parasitoids. *Evol Ecol Res* 3, 553-565

Holland J.N. (1995) Effects of above-ground herbivory on soil microbial biomass in conventional and no-tillage agroecosystems. *Appl Soil Ecol* 2, 275-279

Holland J.N., Cheng W. & Crossley D.A.Jr. (1996) Herbivore-induced changes in plant carbon allocation: assessment of below-ground C fluxes using carbon-14. *Oecologia* 107, 87-94

Hunter M.D. (1987) Opposing effects of spring defoliation on late season caterpillars. *Ecol Entomol* 12, 373-382

Hunter M.D. (2001) Insect population dynamics meets ecosystem ecology: effects of herbivory on soil nutrient dynamics. *Ag For Entomol* 3, 77-84

Jaeger III C.H., Lindow S.E., Miller W., Clark E. & Firestone M.K. (1999) Mapping of sugar and amino acid availability in soil around roots with bacterial sensors of sucrose and tryptophan. *Appl Environ Microbiol* 65, 2685-2690

Jenkinson D.S., Fox R.H. & Rayner J.H. (1985) Interactions between fertilizer nitrogen and soil nitrogen - the so-called priming effect. *J Soil Sci* 36, 425-444

Jones D.L., Hodge A. & Kuzyakov Y. (2004) Plant and mycorrhizal regulation of rhizodeposition. *New Phytol* 163, 459-480

Kery M. & Hatfield J.S. (2003) Normality of raw data in general linear models: the most widespread myth in statistics. *Bull Ecol Soc Am* 92-94

Knops J.M.H., Bradley K.L. & Wedin D.A. (2002) Mechanisms of plant species impacts on ecosystem nitrogen cycling. *Ecol Letters* 5, 454-466

Kosola K.R., Dickmann D.I., Paul E.A. & Parry D. (2001) Repeated insect defoliation effects on growth, nitrogen acquisition, carbohydrates, and root demography of poplars. *Oecologia* 129, 65-74

Kosola K.R., Durall D.M., Robertson G.P., Dickmann D.I., Parry D., Russell C.A. & Paul E.A. (2004) Resilience of mycorrhizal fungi on defoliated and fertilized hybrid poplars. *Canadian J Botany-Revue Canadienne de Botanique* 82, 671-680

Kula A.A.R., Hartnett D.C. & Wilson G.W.T. (2005) Effects of mycorrhizal symbiosis on tallgrass prairie plant-herbivore interactions. *Ecol Letters* 8, 61-69

Kuzyakov Y. (2002) Review: factors affecting rhizosphere priming effects. *J Plant Nut Soil Sci* 165, 382-396

Kuzyakov Y. & Cheng W. (2001) Photosynthesis controls of rhizosphere respiration and organic matter decomposition. *Soil Biol Biochem* 33, 1915-1925

Lajtha K. & Michener R.H. (1994) *Stable Isotopes in Ecology and Environmental Science*. Blackwell Scientific Publications, Cambridge, MA.

Lipson D. & Näsholm T. (2001) The unexpected versatility of plants: organic nitrogen use and availability in terrestrial ecosystems. *Oecologia* 128, 305-316

Littell R.C., Stroup W.W. & Freund R.J. (2002) *SAS for Linear Models*, 4 edn. SAS Institute, Inc., Cary, NC.

Lovett G.M., Weathers K.C., Arthur M.A. & Schultz J.C. (2004) Nitrogen cycling in a northern hardwood forest: Do species matter? *Biogeochemistry* 67, 289-308

Maillard P., Guehl J.M., Muller J.F. & Gross P. (2001) Interactive effects of elevated CO2 concentration and nitrogen supply on partitioning of newly fixed C-13 and N-15 between shoot and roots of pedunculate oak seedlings (Quercus robur). *Tree Physiol* 21, 163-172

Martens R. (1990) Contribution of rhizodeposits to the maintenance and growth of soil microbial biomass. *Soil Biol Biochem* 22, 141-147

Martin J.K. & Merckx R. (1992) The partitioning of photosynthetically fixed carbon within the rhizosphere of mature wheat. *Soil Biol Biochem* 24, 1147-1156

McConn M., Creelman R.A., Bell E., Mullet J.E. & Browse J. (1997) Jasmonate is essential for insect defense in *Arabidopsis*. *Proc Natl Acad Sci (USA)* 94, 5473-5477

McNaughton S.J., Ruess R.W. & Seagle S.W. (1988) Large mammals and process dynamics in African ecosystems. *BioScience* 38, 794-800

Mikola J., Yeates G.W., Barker G.M., Wardle D.A. & Bonner K.I. (2001a) Effects of defoliation intensity on soil food-web properties in an experimental grassland community. *Oikos* 92, 333-343

Mikola J., Yeates G.W., Barker G.M., Wardle D.A. & Bonner K.I. (2001b) Response of soil food-web structure to defoliation of different plant species combinations in an experimental grassland community. *Soil Biol Biochem* 33, 205-214

Nykanen H. & Koricheva J. (2004) Damage-induced changes in woody plants and their effects on insect herbivore performance: a meta-analysis. *Oikos* 104, 247-268

Paul E.A. & Clark F.E. (1996) *Soil Microbiology and Biochemistry*, 2nd edn. Academic Press, San Diego.

Powlson D.S. & Jenkinson D.S. (1976) The effects of biocidal treatments on metabolism in soil: II. Gamma irradiation, autoclaving, air-drying and fumigation. *Soil Biol Biochem* 8, 179-188

Rajaniemi T.K. & Reynolds H.L. (2004) Root foraging for patchy resources in eight herbaceous plant species. *Oecologia* 141, 519-525

Reich P.B., Walters M.B. & Ellsworth D.S. (1997) From tropics to tundra: Global convergence in plantáfunctioning. *Proc Natl Acad Sci (USA)* 94, 13730-13734

Ritchie M.E., Tilman D. & Knops J.M.H. (1998) Herbivore effects on plant and nitrogen dynamics in oak savanna. *Ecology* 79, 165-177

Rossiter M., Schultz J.C. & Baldwin I.T. (1988) Relationships among defoliation, red oak phenolics, and gypsy moth growth and reproduction. *Ecology* 69, 267-277

Rudgers J.A., Koslow J.M. & Clay K. (2004) Endophytic fungi alter relationships between diversity and ecosystem properties. *Ecol Letters* 7, 42-51

Ruess R.W., Hendrick R.L. & Bryant J.P. (1998) Regulation of fine root dynamics by mammalian browsers in early successional Alaskan taiga forests. *Ecology* 79, 2706-2720

Schimel D.S. (1993) *Theory and Application of Tracers*. Academic Press, San Diego, CA.

Schultz J.C. & Appel H.M. (2004) Cross-kingdom cross-talk: Hormones shared by plants and their insect herbivores. *Ecology* 85, 70-77

Schultz J.C. & Baldwin I.T. (1982) Oak leaf quality declines in reponse to defoliation by gypsy moth larvae. *Science* 217, 149-151

Seely B. & Lajtha K. (1997) Application of a 15N tracer to simulate and track the fate of atmospherically deposited N in the coastal forests of the Waguoit Bay Watershed, Cape Cod, Massachusettes. *Oecologia* 112, 393-402

Sparling G.P. & West A.W. (1988) A direct extraction method to estimate soil microbial C: calibration *in situ* using microbial respiration and ^{14}C labeled cells. *Soil Biol Biochem* 20, 337-343

Templer P., Findlay S. & Lovett G.M. (2003) Soil microbial biomass and nitrogen transformations among five tree species of the Catskill Mountains, New York, USA. *Soil Biol Biochem* 35, 607-613

Thelen G.C., Vivanco J.M., Newingham B., Good W., Bais H.P., Landres P., Caesar A. & Callaway R.M. (2005) Insect herbivory stimulates allelopathic exudation by an invasive plant and the suppression of natives. *Ecol Letters* 8, 209-217

Vance E.D., Brookes P.C. & Jenkinson D.S. (1987) An extraction method for measuring soil microbial biomass C. *Soil Biol Biochem* 19, 703-707

Verchot L.V., Holmes Z., Mulon L., Groffman P.M. & Lovett G.M. (2001) Gross vs net rates of N mineralization and nitrification as indicators of functional differences between forest types. *Soil Biol Biochem* 33, 1889-1901

Wardle D.A. (2002) *Communities and Ecosystems: Linking the Aboveground and Belowground Components*. Princeton University Press, Princeton.

Whipps J.M. & Lynch J.M. (1983) Substrate flow and utilization in the rhizosphere of cereals. *New Phytol* 95, 605-623

Zogg G.P., Zak D.R., Pregitzer K.S. & Burton A.J. (2000) Microbial immobilization and the retention of anthropogenic nitrate in a northern hardwood forest. *Ecology* 81, 1858-1866

Table 5.1 Masses, Nitrogen, Carbon, and C:N Ratios for the *Quercus rubra* Microcosms

Sample Type	Mass (g)[1]	N (%)	C (%)	C:N
Leaf	0.529 ± 0.209	2.305 ± 0.372	46.105 ± 0.479	20.529 ± 3.410
New Stem	0.053 ± 0.024	1.390 ± 0.331	44.638 ± 0.717	33.795 ± 7.461
Old Stem	0.220 ± 0.102	1.336 ± 0.389	46.588 ± 0.699	37.140 ± 8.837
Fine Roots	0.125 ± 0.069	2.365 ± 0.477	41.262 ± 3.837	17.920 ± 3.021
Rhizosphere Soil	18.879 ± 6.777	0.237 ± 0.045	3.526 ± 0.760	14.881 ± 1.245

[1] Data are means ± SD of 30 samples

Figure 5.1 Diagram of the experimental microcosm. The microcosm design was modified from Cheng (1996) to include side injection ports for the introduction of ^{15}N-glycine following damage treatments. Soil and roots were isolated from stems with paraffin wax and caulk.

Figure 5.1

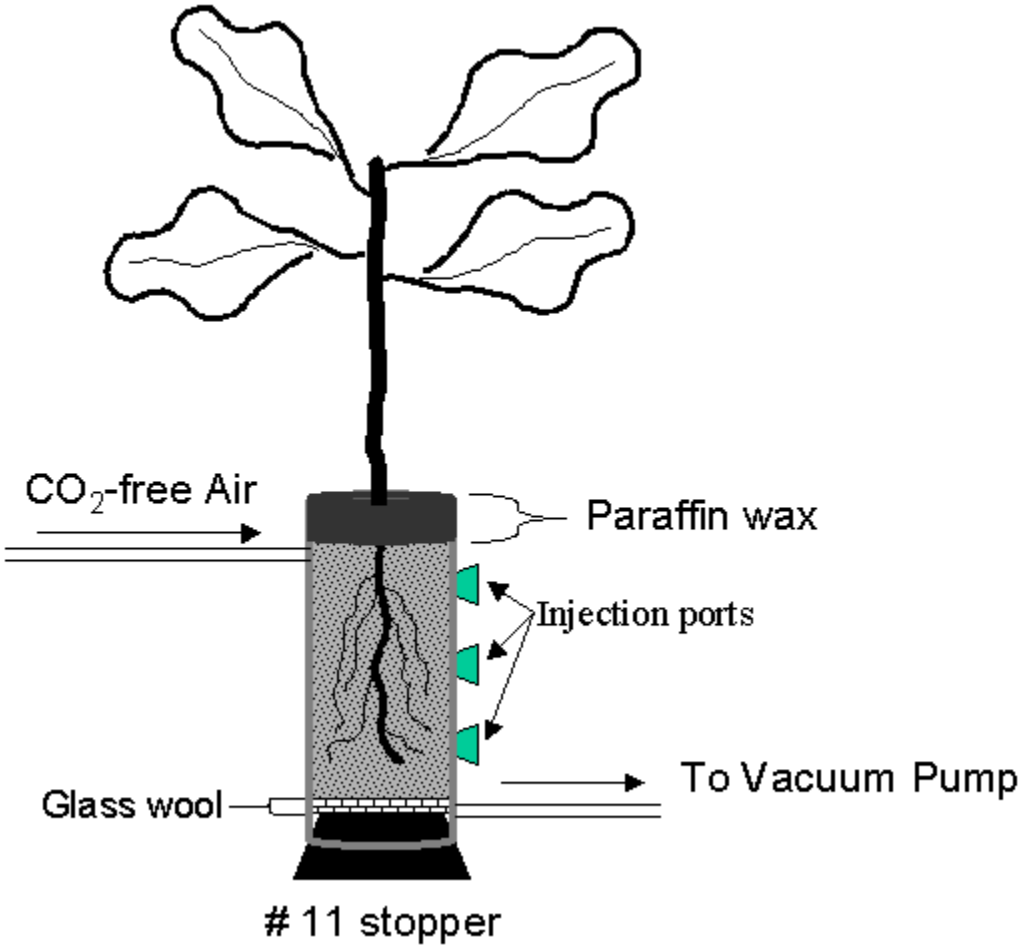

CO_2-free Air
Paraffin wax
Injection ports
To Vacuum Pump
Glass wool
11 stopper

Figure 5.2 (a) Distribution of assimilated 13C (%) and (b) $\delta^{13}C$ (‰) values among leaf, new stem, old stem, fine roots, rhizosphere soils, and bulk soils. Bars are means ± SE of 10 samples for the herbivore, mechanical, and undamaged groups. Pretreatment $\delta^{13}C$ values are means of the 2 pre-enrichment controls. Bars with different letters indicate statistical significance using the SNK post hoc test (α=0.05).

Figure 5.2

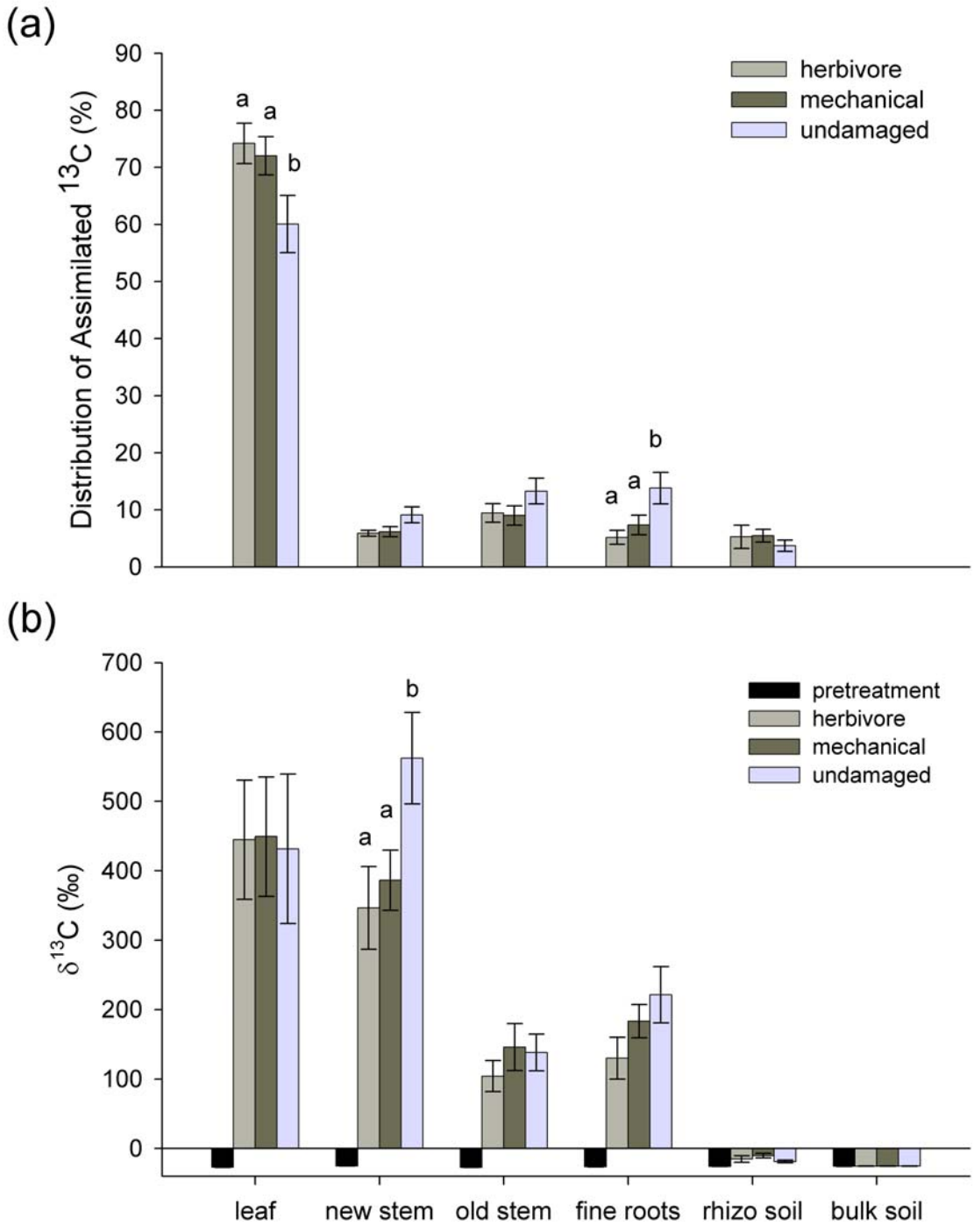

(a)
Distribution of Assimilated ^{13}C (%)
herbivore
mechanical
undamaged
a
a
b
a
a
b
(b)
$\delta^{13}C$ (‰)
pretreatment
herbivore
mechanical
undamaged
a
a
b
leaf
new stem
old stem
fine roots
rhizo soil
bulk soil

Figure 5.3 (a) Percentage of belowground new ^{13}C in the rhizosphere soil by damage treatment. (b) Total microbial C by damage treatment. (c) Net enrichment of microbial C by damage treatment. (d) Total new ^{13}C recovered in microbial biomass by damage treatment. Bars are means ± SE of 10 samples. Bars with different letters indicate statistical significance using the SNK post hoc test (α=0.05).

Figure 5.3

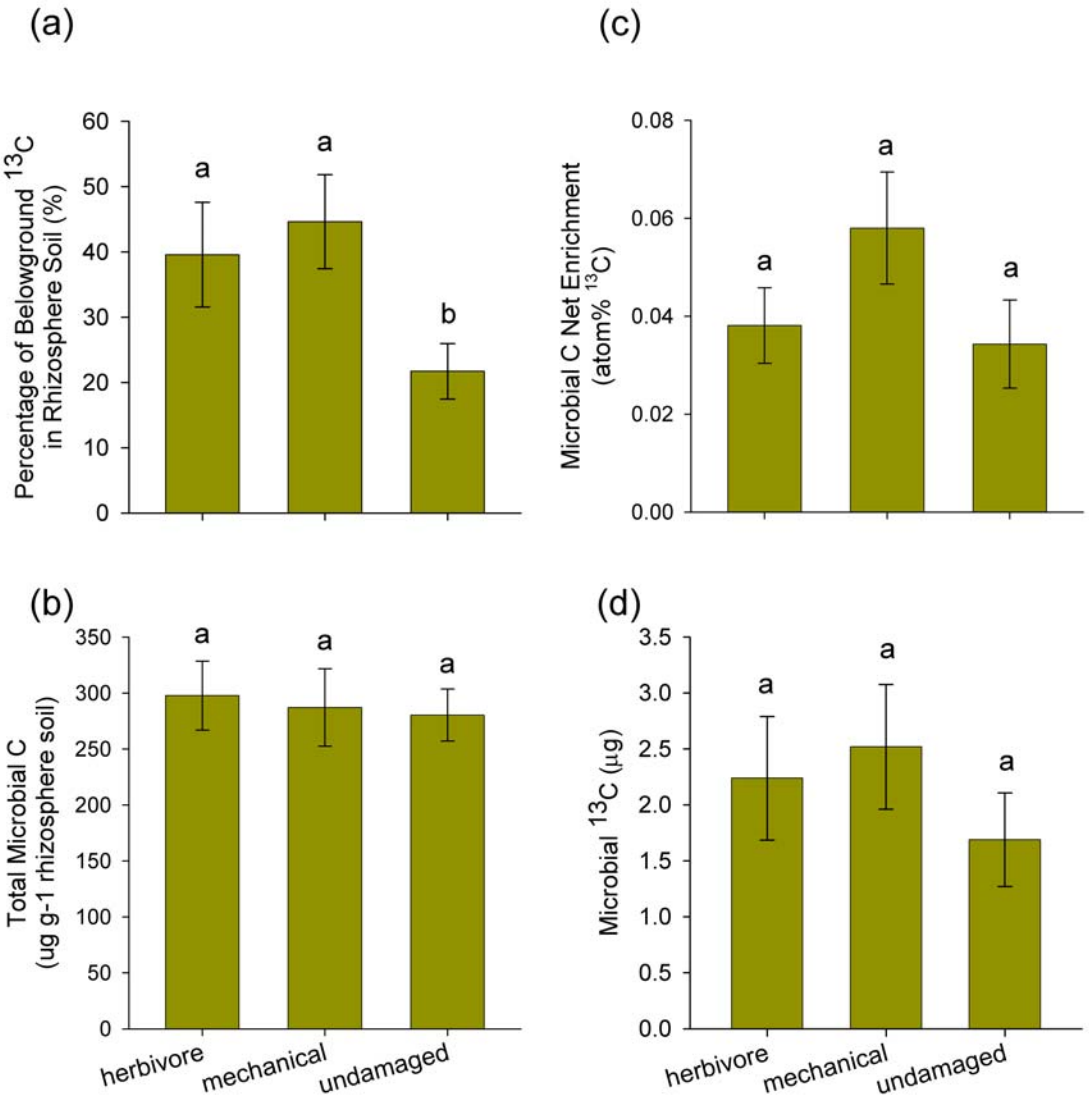

(a)
Percentage of Belowground ^{13}C in Rhizosphere Soil (%)
60
50
40
30
20
10
0
a
a
b
(c)
Microbial C Net Enrichment (atom% ^{13}C)
0.08
0.06
0.04
0.02
0.00
a
a
a
(b)
Total Microbial C (ug g-1 rhizosphere soil)
350
300
250
200
150
100
50
0
a
a
a
herbivore
mechanical
undamaged
(d)
Microbial ^{13}C (µg)
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
a
a
a
herbivore
mechanical
undamaged

Figure 5.4 (a) Total C, (b) net ^{13}C enrichment, and (c) total new ^{13}C recovery in the extractable DOC and microbial biomass by date. (d) The percent of rhizosphere total C and new ^{13}C contained in the microbial biomass. Bars are means ± SE of 15 samples. Bars with different letters indicate statistical significance using the SNK post hoc test (α=0.05).

Figure 5.4

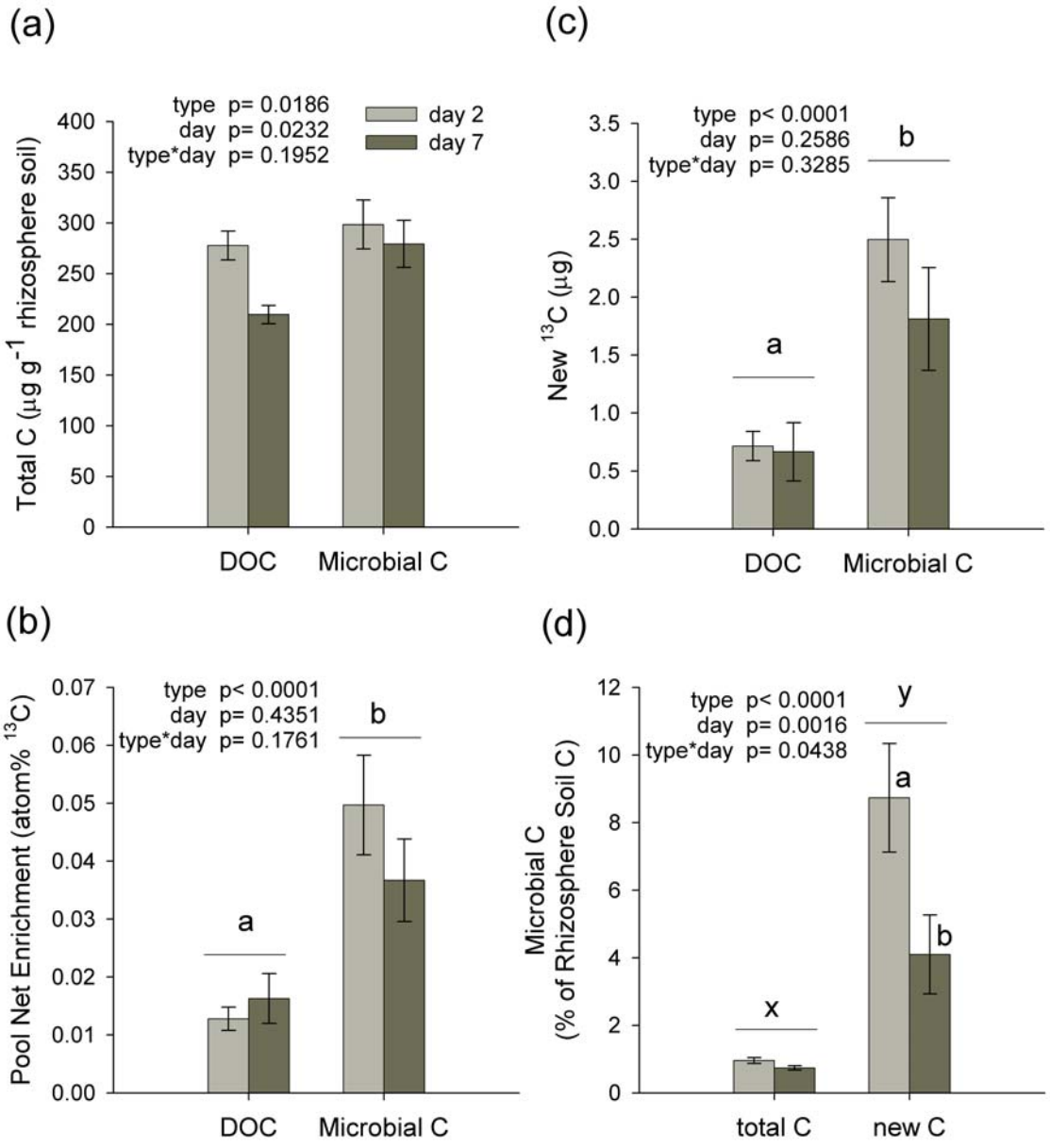
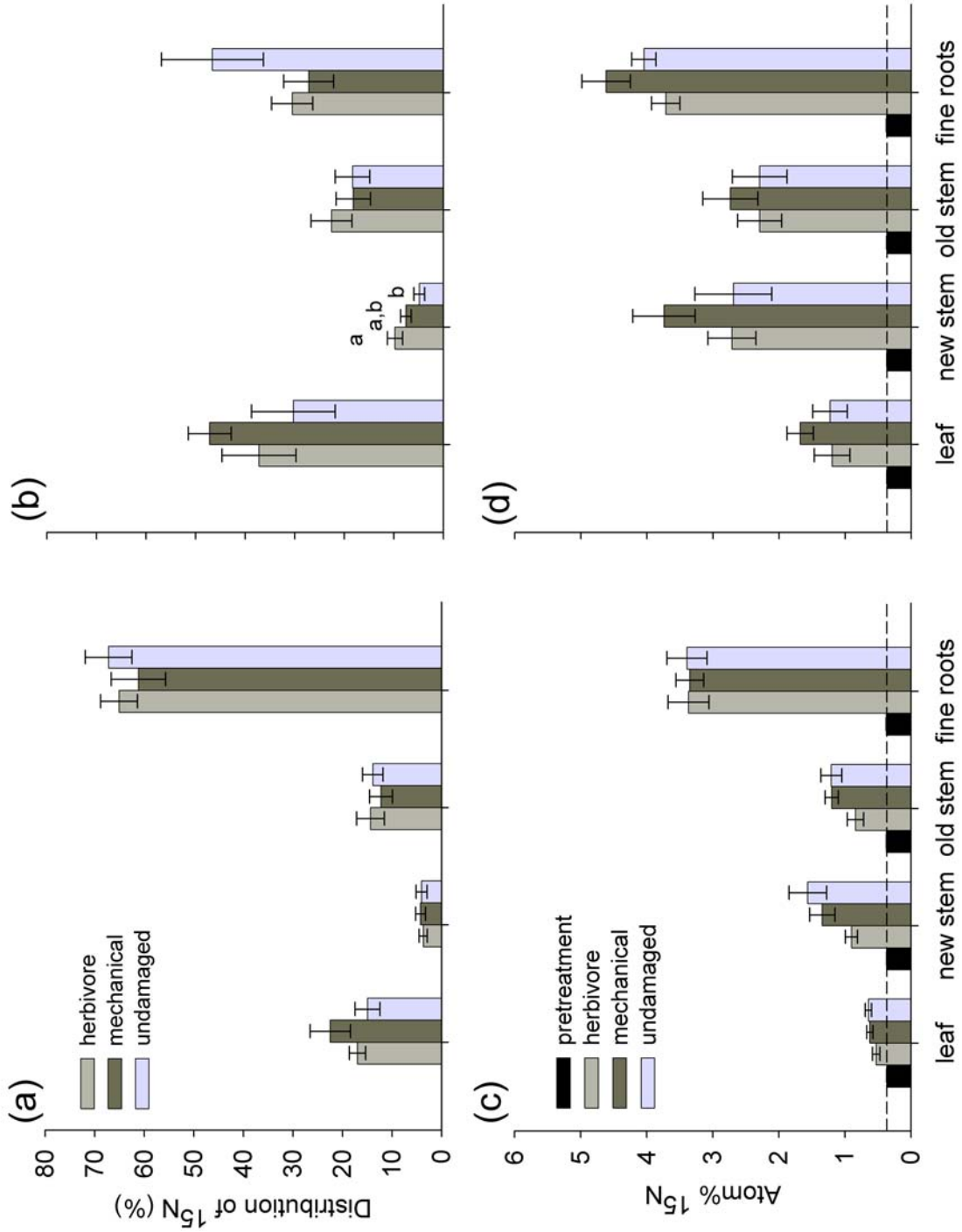

(a)
type p= 0.0186
day p= 0.0232
type*day p= 0.1952
day 2
day 7
Total C (μg g-1 rhizosphere soil)
DOC
Microbial C
(c)
type p< 0.0001
day p= 0.2586
type*day p= 0.3285
New 13C (μg)
a
b
DOC
Microbial C
(b)
type p< 0.0001
day p= 0.4351
type*day p= 0.1761
Pool Net Enrichment (atom% 13C)
a
b
DOC
Microbial C
(d)
type p< 0.0001
day p= 0.0016
type*day p= 0.0438
Microbial C (% of Rhizosphere Soil C)
x
a
y
b
total C
new C

Figure 5.5 Distribution of new ^{15}N (%) among leaf, new stem, old stem, and fine roots of *Q. rubra* seedlings (a) 2 days and (b) 7 days following experimental damage treatments in ^{15}N-glycine additions to the soil. Enrichment (atom% ^{15}N) in leaf, new stem, old stem, and fine roots of *Q. rubra* seedlings (c) 2 days and (d) 7 days following experimental damage treatments in ^{15}N-glycine additions to the soil. Bars are means ± SE of 5 samples for the herbivore, mechanical, and undamaged groups. Pretreatment $\delta^{13}C$ values are means of the 2 pre-enrichment controls. Bars with different letters indicate statistical significance using the SNK post hoc test (α=0.05). Dashed line in (c) and (d) represents the atmospheric abundance of ^{15}N (0.3663 atom%).

Figure 5.5


(a)
herbivore
mechanical
undamaged
Distribution of 15N (%)
80
70
60
50
40
30
20
10
0
(b)
a
a,b
b
(c)
pretreatment
herbivore
mechanical
undamaged
Atom% 15N
6
5
4
3
2
1
0
(d)
leaf
new stem
old stem
fine roots

CHAPTER 6

CONCLUSIONS AND FUTURE DIRECTIONS

In general, our data show that insect herbivores directly and indirectly influence ecosystem function. The direct effect that we measured was the deposition of feces (frass) and the resulting pulses in soil and leachate nitrogen (N). The significant indirect effects in our studies resulted largely from oak responses to the herbivores that resulted in modifications to allocation patterns of carbon (C) and N with oak tissues. Links between herbivores and ecosystem function have long been observed (Swank *et al.* 1981) or suggested (Choudhury 1988), but mechanisms underlying the observations were not always apparent. McNaughton *et al.* (1988) defined "fast" and "slow" cycle processes in relation to nutrient cycling and herbivores. Fast cycle, as the name implies, are those that occur quickly (e.g., mineralization of nutrients in excreta, immediate changes to plant nutrient dynamics, increased nutrient content of throughfall). The "slow" cycle effects refer to processes that require months or years to cycle nutrients (e.g., leaf litter decomposition). This dissertation addressed the effects of herbivores on both fast and slow cycle N and C dynamics in terrestrial systems. We began with a focus on the ecosystem consequences of herbivory, with particular consideration on the effects of frass deposition. However, the prevalence of indirect effects of herbivore activity via changes in assimilation and allocation by oaks inspired us to integrate our ecosystem focus with plant physiological responses to herbivory. As a result, the dissertation offers

four principal results: (1) frass from herbivores represents a labile pool of N that may be lost from terrestrial systems, (2) herbivore-mediated changes in litter chemistry do not always translate into accelerated or decelerated decomposition rates, (3) oaks unexpectedly increase N uptake throughout the course of a growing season and overall patterns of N uptake and allocation are affected by herbivores, (4) belowground C allocation in oak seedlings is reduced following herbivory.

From an ecosystem perspective, the herbivore-mediated loss of terrestrial N is a substantial perturbation to the terrestrial N cycle. We first designed an experiment to test the hypothesis that insect herbivores feeding on red oak would increase N export via feces (frass) deposition. Chapter 2 showed that the deposition of frass during herbivore feeding can increase soil NH_4^+ and NO_3^- lost as leachate. These results are mechanistic evidence that support the field observation that insect herbivore activity leads to N export from terrestrial systems (Reynolds *et al.* 2000). The results supported the predictions from the discussion of Chapter 2: a pulse of frass N was lost in the first week following deposition, followed by a steep reduction in the amount of frass N lost over the course of the subsequent 3 months. This suggests that a portion of the labile N in frass is transported quickly from the system before microbial immobilization occurs. A similar experiment was conducted by Christenson *et al.* (2002), who estimated three orders of magnitude less leaching losses of frass N than we report in Chapter 4. The discrepancy between total amount of N lost in leachate between studies in Chapter 2 and 4 also spans orders of magnitude. This suggests that herbivores mobilize sufficient N in frass to represent substantial losses to the terrestrial system, but other factors must be involved to

determine whether the N is lost or retained. We suggest in both chapters that the timing of rainfall relative to frass deposition may determine the amount of N lost, and this remains an untested hypothesis. Because of the importance of N as a limiting nutrient in most terrestrial systems, the determinants of the fate of N in insect frass are important for understanding the ecosystem consequences of herbivore activity. On the flip side, loss of terrestrial N via leaching potentially leads to gain of N in stream ecosystems. This is an important point because linkages between terrestrial and aquatic ecosystems have received increasing attention in recent years (Grimm *et al.* 2003). Frass deposition by insect herbivores is a tangible, albeit unlikely, connective link between the biogeochemistry of terrestrial and aquatic components of a forested landscape that should be considered when estimating N flows throughout entire watersheds.

Chapter 3 addressed the effects of insect herbivores on slow cycle processes in oak foliage/litter. The mechanism by which herbivores could influence the decomposition of foliage on which they feed is by their indirect effects on the chemistry of the foliage. We expected and observed changes in the chemistry of oak foliage following herbivore damage: tannin concentrations increased and N concentrations decreased. We further expected these changes to persist into the litter and potentially affect rates of decomposition and slow cycle nutrient turnover. While there were treatment effects on the initial litter quality, none of the differences translated into changes in decomposition rates. Others have shown that herbivore damage can both decelerate (Ritchie *et al.* 1998) and accelerate (Chapman *et al.* 2003) decomposition rates in terrestrial systems. Similar observations have been made when leaf litter decomposes

in streams (Findlay *et al.* 1996; Hutchens & Benfield 2000). So, Choudhury (1988) was correct to suggest that there are instances where herbivore-mediated changes in plant chemistry can alter decomposition rates. Our data introduce a cautionary note to the story: moderate levels of herbivore damage did not influence litter decomposition rates of a single species of plant.

The data presented in Chapter 4 revealed unexpected N uptake patterns in the oak saplings. We applied a damage treatment effect to our study of the distribution of ^{15}N-enriched frass to test the hypothesis that aboveground damage would affect the recovery and distribution of the frass N. The single deposition event occurred in mid-June, after foliar total N concentrations had begun to decline. We therefore expected little, if any enrichment in the foliage in that growing season. On the contrary, ^{15}N-enrichment of the foliage was apparent after 1 month of deposition and continued to increase into the senescing litter. Despite declines in foliar N as the saplings resorbed N, new N was incorporated into the leaf tissue. The total amount was relatively small (ca. 1% of added frass N) and it is possible that mass flow accounted for some of the enrichment. However, the continued accumulation of the label suggests that the new N was incorporated into the leaf tissue in proteins, amino acids, or other N-containing compounds.

In the following growing season, oaks damaged by herbivores recovered less frass N in their foliage than did undamaged oaks. Did this occur because damaged oaks were hindered from assimilating new N? Our data do not provide a clear answer. On one hand, no independent measure of growth (e.g., budbreak timing, stem expansion rates,

total leaf area, relative growth rates, stem widths, photosystem II efficiency) differed among damage treatments. Nitrogen recovery in the fine roots at all three depths was also unaffected by the damage treatment. This suggests that the damage-mediated reduction in foliar N did not apparently affect plant performance. On the other hand, there was no evidence that differential allocation occurred based on ratios of new N among oak tissues. Irrespective of whether or not the damage-mediated reduction in foliar N was an active response by the oaks, previously damaged trees were evidently less preferred for feral leaf rollers and presumably reduced herbivore performance.

Finally, we showed that short-term C and N allocation in oak seedlings can be affected by herbivore damage. Our observation in Chapter 2 that foliar herbivore damage increased soil respiration independent of frass additions led us to the experiment reported in Chapter 5. In our microcosm experiment, oak seedlings subjected to herbivore damage reduced belowground allocation of newly assimilated C. However, the proportion of belowground new C that was recovered in the rhizosphere was higher following herbivore damage, suggesting that herbivore-damaged roots were "leakier." Was this the result of active rhizodeposition? There were no differences in total recovery of new C in the rhizosphere or rhizosphere micro-organisms, so it is difficult to answer that question. However, during the course of our study, two other groups also demonstrated that aboveground damage/signaling could alter belowground C allocation. Hamilton & Frank (2001) measured higher concentrations of newly assimilated C in rhizodeposition and rhizosphere microbial biomass C following simulated herbivory on a common prairie grass, *Poa pratensis*. Babst et al. (2005) showed that the allocation and rate of transport

of newly assimilated C (using ^{11}C) to stem and roots increased significantly following application of jasmonic acid to leaves. Jasmonic acid is a well-known signaling hormone that stimulates chemical defenses in plants (Farmer & Ryan 1990; McConn *et al.* 1997; Baldwin 1998; Engelberth *et al.* 2004; Ament *et al.* 2004). Previous work with corn (*Zea mays*) likewise showed that belowground carbon allocation was stimulated by herbivore damage (Holland *et al.* 1996). Each of these studies implied active control of belowground C allocation, including rhizodeposition. Our data presented in Chapter 2 also suggested that foliar herbivore damage stimulated belowground C mineralization, though we again could not suggest active C allocation by the oaks. Interestingly, the results from Chapter 5 are apparently opposite all previous studies. It is possible that the difference could be the result of ontogeny (Nykanen & Koricheva 2004), though the mechanisms for allocation shifts are not well known. This suggests two future lines of research. First, experiments using isotope tracers to measure assimilate allocation in saplings and more mature oaks would reveal if the patterns of resource allocation are similar to those observed in our study. The next step would logically be to use saplings similar to those in Chapters 2-4, though even scaling to this level is a logistical hurdle. However, belowground assimilate transport influences soil ecosystems (Cheng *et al.* 1996; Cardon *et al.* 2002; Farrar *et al.* 2003) and feeds back to aboveground productivity (Bardgett *et al.* 1998; Bardgett & Wardle 2003), and studies focusing on mature trees would reveal if herbivores do indeed influence C allocation patterns and if their influence has consequences for ecosystem processes. In addition, belowground C allocation to soils can sequester atmospheric C (Zak *et al.* 1993; Cheng & Johnson 1998; Cheng

1999), and herbivores may therefore indirectly influence global patterns of C cycling. Second, research at the molecular level to understand the mechanisms underlying changes in aboveground/belowground C allocation would be useful, particularly with the potential of soils as C sinks. One approach would be the development of cDNA libraries of genes that are upregulated following herbivore damage and targeting those responsible for the shift in allocation. Some promising work has been conducted with aboveground plant direct (Constabel *et al.* 2000; Christopher *et al.* 2004) and indirect (Arimura *et al.* 2004) defenses. The genes that regulate allocation belowground, however, remain largely unexplored. Recent studies have shown that inter and intraspecific genetic diversity can influence ecosystem function (Madritch & Hunter 2002; Schweitzer *et al.* 2004; Madritch & Hunter 2005). Genes that increase C allocation belowground have the potential to influence ecosystem function directly when they are upregulated. The study of plant-herbivore interactions is becoming increasingly molecular (Kessler & Baldwin 2002), and the consequences of those interactions for ecosystems may also be understood in the context of transcriptional regulation of herbivore-induced responses in trees.

REFERENCES

Ament K., Kant M.R., Sabelis M.W., Haring M.A. & Schuurink R.C. (2004) Jasmonic acid is a key regulator of spider mite-induced volatile terpenoid and methyl salicylate emission in tomato. *Plant Physiology* 135, 2025-2037

Arimura G., Huber D.P.W. & Bohlmann J. (2004) Forest tent caterpillars (*Malacosoma disstria*) induce local and systemic diurnal emissions of terpenoid volatiles in hybrid poplar (*Populus trichocarpa* x *deltoides*): cDNA cloning, functional characterization, and patterns of gene expression of (-) -germacrene D synthase, *PtdTPS1*. *The Plant Journal* 37, 603-616

Babst,B.A., Ferrieri,R.A., Gray,D.W., Lerdau,M., Schlyer,D.J., Schueller,M., Thorpe,M.R. & Orians,C.M. (2005) Jasmonic acid induces rapid changes in carbon transport and partitioning in Populus. *New Phytologist*, 167**,** 63-72.

Baldwin I.T. (1998) Jasmonate-induced responses are costly but benefit plants under attack in native populations. *Proceedings of the National Academy of Sciences (USA)* 95, 8113-8118

Bardgett R.D. & Wardle D.A. (2003) Herbivore-mediated linkages between aboveground and belowground communities. *Ecology* 84, 2258-2268

Bardgett R.D., Wardle D.A. & Yeates G.W. (1998) Linking above-ground and below-ground interactions: how plant responses to foliar herbivory influence soil organisms. *Soil Biology and Biochemistry* 30, 1867-1878

Cardon Z.G., Czaja A.D., Funk J.L. & Vitt P.L. (2002) Periodic carbon flushing to roots of *Quercus rubra* saplings affects soil respiration and rhizosphere microbial biomass. *Oecologia* 133, 215-223

Chapman S.K., Hart S.C., Cobb N.S., Whitham T.G. & Koch G.W. (2003) Insect herbivory increases litter quality and decomposition: An extension of the acceleration hypothesis. *Ecology* 84, 2867-2876

Cheng W. (1999) Rhizosphere feedbacks in elevated CO_2. *Tree Physiology* 19, 313-320

Cheng W. & Johnson D.W. (1998) Elevated CO_2, rhizosphere processes, and soil organic matter decomposition. *Plant and Soil* 202, 167-174

Cheng W., Zhang Q., Coleman D.C., Carroll R.C. & Hoffman C.A. (1996) Is available carbon limiting microbial respiration in the rhizosphere? *Soil Biology and Biochemistry* 28, 1283-1288

Choudhury D. (1988) Herbivore induced changes in leaf-litter resource quality: a neglected aspect of herbivory in ecosystem nutrient dynamics. *Oikos* 51, 389-393

Christenson L.M., Lovett G.M., Mitchell M.J. & Groffman P.M. (2002) The fate of nitrogen in gypsy moth frass deposited to an oak forest floor. *Oecologia* 131, 444-452

Christopher M.E., Miranda M., Major I.T. & Constabel C.P. (2004) Gene expression profiling of systemically wound-induced defenses in hybrid poplar. *Planta* 219, 936-947

Constabel C.P., Yip L., Patton J.J. & Christopher M.E. (2000) Polyphenol oxidase from hybrid poplar. Cloning and expression in response to wounding and herbivory. *Plant Physiology* 124, 285-295

Engelberth J., Alborn H.T., Schmelz E.A. & Tumlinson J.H. (2004) Airborne signals prime plants against insect herbivore attack. *Proceedings of the National Academy of Sciences (USA)* 101, 1781-1785

Farmer E.E. & Ryan C.A. (1990) Interplant communication: airborn methyl jasmonate induces synthesis of proteinase inhibitors in plant leaves. *Proceedings of the National Academy of Sciences (USA)* 87, 7713-7716

Farrar J., Hawes M., Jones D. & Lindow S. (2003) How roots control the flux of carbon to the rhizosphere. *Ecology* 84, 827-837

Findlay S., Carreiro M., Krischik V. & Jones C.G. (1996) Effects of damage to living plants on leaf litter quality. *Ecological Applications* 6, 269-275

Grimm N.B., Gergel S.E., McDowell W.H., Boyer E.W., Dent C.L., Groffman P.M., Hart S.C., Harvey J., Johnston C., Mayorga E., McClain M.E. & Pinay G. (2003) Merging aquatic and terrestrial perspectives of nutrient biogeochemistry. *Oecologia* 137, 485-501

Hamilton E.W.I. & Frank D.A. (2001) Can plants stimulate soil microbes and their own nutrient supply? Evidence from a grazing tolerant grass. *Ecology* 82, 2397-2404

Holland J.N., Cheng W. & Crossley D.A.Jr. (1996) Herbivore-induced changes in plant carbon allocation: assessment of below-ground C fluxes using carbon-14. *Oecologia* 107, 87-94

Hutchens J.J. & Benfield E.F. (2000) Effects of forest defoliation by the gypsy moth on detritus processing in southern Appalachian streams. *American Midland Naturalist* 143, 397-404

Kessler A. & Baldwin I.T. (2002) Plant responses to insect herbivory: the emerging molecular analysis. *Annual Review of Plant Biology* 53, 299-328

Madritch M.D. & Hunter M.D. (2002) Phenotypic diversity influences ecosystem functioning in an oak sandhills community. *Ecology* 83, 2084-2090

Madritch M.D. & Hunter M.D. (2005) Phenotypic variation in oak litter influences short- and long-term nutrient cycling through litter chemistry. *Soil Biology & Biochemistry* 37, 319-327

McConn M., Creelman R.A., Bell E., Mullet J.E. & Browse J. (1997) Jasmonate is essential for insect defense in *Arabidopsis*. *Proceedings of the National Academy of Sciences (USA)* 94, 5473-5477

McNaughton S.J., Ruess R.W. & Seagle S.W. (1988) Large mammals and process dynamics in African ecosystems. *BioScience* 38, 794-800

Nykanen H. & Koricheva J. (2004) Damage-induced changes in woody plants and their effects on insect herbivore performance: a meta-analysis. *Oikos* 104, 247-268

Reynolds B.C., Hunter M.D. & Crossley D.A.Jr. (2000) Effects of canopy herbivory on nutrient cycling in a northern hardwood forest in western North Carolina. *Selbyana* 21, 74-78

Ritchie M.E., Tilman D. & Knops J.M.H. (1998) Herbivore effects on plant and nitrogen dynamics in oak savanna. *Ecology* 79, 165-177

Schweitzer J.A., Bailey J.K., Rehill B.J., Martinsen G.D., Hart S.C., Lindroth R.L., Keim P. & Whitham T.G. (2004) Genetically based trait in a dominant tree affects ecosystem processes. *Ecology Letters* 7, 127-134

Swank W.T., Waide J.B., Crossley D.A.Jr. & Todd R.L. (1981) Insect defoliation enhances nitrate export from forest ecosystems. *Oecologia* 51, 297-299

Zak D.R., Pregitzer K.S., Curtis P.S., Teeri J.A., Fogel R. & Randlett D.L. (1993) Elevated atmospheric CO_2 and feedback between carbon and nitrogen cycles. *Plant and Soil* 151, 105-117