Herbivore-induced shifts in carbon and nitrogen allocation in red oak seedlings

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Summary

- A dual-isotope, microcosm experiment was conducted with *Quercus rubra* (red oak) seedlings to test the hypothesis that foliar herbivory would increase belowground carbon allocation (BCA), carbon (C) rhizodeposition and nitrogen (N) uptake. Plant BCA links soil ecosystems to aboveground processes and can be affected by insect herbivores, though the extent of herbivore influences on BCA is not well understood in woody plants.
- Microcosms containing 2-yr-old *Q. rubra* seedlings and soil collected from the Coweeta Hydrologic Laboratory (NC, USA) were subjected to herbivory or left as undamaged controls. All microcosms were then injected with ^{15}N-glycine and pulsed with $^{13}CO_2$.
- Contrary to our hypothesis, herbivore damage reduced BCA to fine roots by 63% and correspondingly increased allocation of new C to foliage. However, ^{13}C recoveries in soil pools were similar between treatments, suggesting that exudation of C from roots is an actively regulated component of BCA. Herbivore damage also reduced N allocation to fine roots by 39%, apparently in favor of storage in taproot and stem tissues.
- Oak seedlings respond to moderate insect herbivore damage with a complex suite of allocation shifts that may simultaneously increase foliar C, maintain C rhizodeposition and N assimilation, and shift N resources to storage.

Key words: carbon allocation, herbivory, nitrogen allocation, *Orgyia leucostigma* (white marked tussock moth), *Quercus rubra* (red oak), rhizodeposition, stable isotopes.

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Introduction

Plants provide a direct conduit between aboveground and belowground processes in terrestrial ecosystems and, as a result, influence ecosystem function (Knops *et al.*, 2002; Wardle, 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 development (Giardina *et al.*, 2005) and as an energy source for rhizosphere microorganisms (Martens, 1990; Cardon *et al.*, 2002). Rhizosphere microorganisms decompose soil organic matter (Cheng & Coleman, 1990), mineralizing nitrogen (N) that is then available to plants (Hamilton & Frank, 2001; Kula *et al.*, 2005). Nitrogen translocated from the soil to the shoots and foliage strongly influences relative growth rates (RGR) and net primary production (NPP) (Chapin, 1980; Reich *et al.*, 1997), completing a feedback loop between aboveground and belowground processes. Belowground C allocation (BCA), which includes C allocated to roots as well as rhizodeposition of C exudates from roots to surrounding soil, is a critical link in the loop. Extrinsic factors

that alter BCA may therefore influence a wide range of aboveground and belowground processes. Belowground C allocation 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; Bezemer & van Dam, 2005), with particular focus on the influence of herbivores (Hunter, 2001; Frost & Hunter, 2004). Herbivores can influence plant energy and nutrient allocation patterns aboveground and belowground. Plants respond to herbivore damage with a complex suite of chemical changes to their foliage (Karban & Baldwin, 1997; Zangerl, 2003). These chemical changes reflect changes in C allocation patterns in the foliage that are likely regulated by wound-induced gene expression (Davis *et al.*, 1991). Such herbivore-mediated shifts in plant C allocation patterns also extend belowground and therefore influence BCA and soil faunal and floral communities (Bardgett & Wardle, 2003). Much of the previous research in this area has focused on grasses (Hamilton & Frank, 2001; Mikola *et al.*, 2001a,b) and agricultural crops (Holland, 1995; Holland *et al.*, 1996). Stimulation of BCA and rhizodeposition by foliar herbivory may be common among grasses, particularly those that suffer severe damage by grazers (McNaughton *et al.*, 1988; Frank & Groffman, 1998). While the benefit to plants of herbivore-induced BCA could be C storage, another potential benefit is increased nutrient uptake, facilitated by rhizodeposition and subsequent responses of rhizosphere microorganisms. For example, accumulation of aboveground biomass in some grass communities following herbivory (Frank & McNaughton, 1993) may be due, in part, to increased rhizodeposition that presumably stimulates soil N mineralization (Hamilton & Frank, 2001).

Herbivore-mediated changes in BCA may not be limited to grazing-tolerant grasses. 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). Oaks allocate and release C-rich rhizodeposits belowground in periodic cycles and their rhizosphere microbial communities respond to these inputs (Cardon *et al.*, 2002), indicating that such microbes might also respond to herbivore-induced changes in BCA. Our objective in this study was to explore the effects of aboveground insect herbivore grazing on BCA, C rhizodeposition, and N uptake and distribution in red oak (*Quercus rubra*) seedlings and their rhizosphere microbial populations. We reasoned that oak seedlings would have limited C or N storage reserves and would therefore depend on newly assimilated C and N for responses to herbivores, the allocation of which we could measure with stable isotopes. In addition, seedlings are an important ontogenic stage in oaks and other woody plants because of high seedling mortality rates (Chaar *et al.*, 1997; Murakami & Wada, 1997); herbivore-mediated allocation patterns in seedlings may therefore have a large effect on overall recruitment. Based on our previous results with oak saplings (Frost & Hunter, 2004) and those from other woody plants (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 herbivore damage in controlled microcosms. The dual isotope approach was important to explore simultaneous changes in C and N assimilation and allocation in response to foliar insect herbivores.

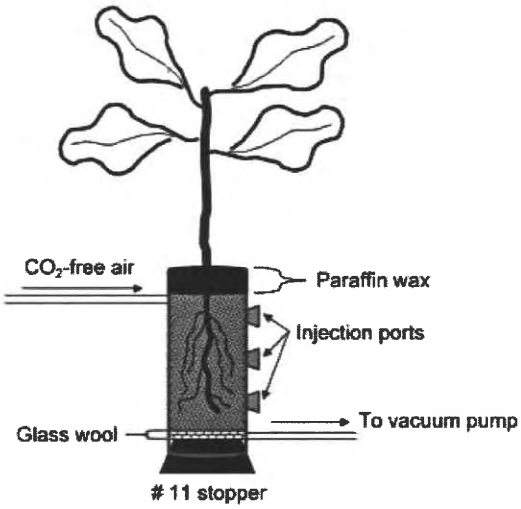

Fig. 1 Diagram of the microcosm units used to grow 2-yr-old *Quercus rubra* (red oak) seedlings. Microcosm design was modified from Cheng (1996). Microcosms were 15 × 5 cm (length × internal diameter) polycarbonate tubes with vertical side injection ports for the introduction of ^{15}N-glycine. Soil and roots were isolated from atmospheric gases with paraffin wax, and caulk was used to ensure an airtight seal. The soil was aerated with CO_2-free air, driven by a vacuum pump and regulated with individual flow meters to ensure consistent flow among microcosms.

Materials and Methods

Methods for isolating aboveground and belowground C components in microcosms are well established (Cheng, 1996). The soil and roots are isolated from direct atmospheric exchange using a wax seal on the soil surface; thus any new C gained by roots or soil must be derived from photosynthate. The design was modified to include side injection ports for the addition of ^{15}N-glycine (Fig. 1). The microcosms were constructed of 15 × 5 cm (length × internal diameter) clear polycarbonate tubing with approx. 200 g of a sieved soil–sand mix (1:1 v : v). The soil used in the experiment was collected from watershed 27 at the Coweeta Hydrologic Laboratory

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(CWT) in western North Carolina (USA) to ensure that soil microorganisms would be representative of field conditions. Soil was passed through a 1 mm sieve to remove roots and other debris. This soil was then mixed 1:1 (v : v) with acid-washed, autoclaved sand, which increased soil porosity and facilitated homogeneous watering in the microcosms. The soil mixture was at approx. 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 'night-time' temperatures of 25°C and 16°C, respectively. There was a 1-h ramping transition period for both light and temperature regimes to simulate sunrise and sunset. The maximum photon fluence rate at the level of the microcosms was approx. 500 μmol m^{-2} s^{-1}. Thus, all microcosms were exposed to the same environmental conditions.

Red oak (*Q. rubra* L.) 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 glasshouses (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 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 with deionized (DI) water and the outside wrapped with aluminum foil. All microcosms (26 total) were then equilibrated to the growth chamber for 4 months before experimental manipulations, during which time the seedlings broke dormancy and fully expanded their foliage.

Each microcosm was randomly assigned to either receive herbivores or be left undamaged as a control. There were five replicates of each treatment per sampling date, with destructive sampling 2 and 7 d following the end of damage treatments and isotope additions ($n = 20$ total). In addition, two microcosms were isotope-free controls and two microcosms per date (four total) 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 April 2004 and were fully expanded by mid-May 2004. On 13 June 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 microorganisms. Four fourth-instar white-marked tussock moth larvae (*Orgyia leucostigma* Smith; egg masses from Canadian Forest Service, Sault Ste Marie, Ontario) were added per seedling on 16 June 2004 and removed on 18 June 2004. *Orgyia leucostigma* are tannin-tolerant defoliators common throughout the eastern USA and can generally be found on red oaks at CWT. They inflict damage by physically chewing leaf tissue and therefore remove leaf area as they feed. Herbivores and seedlings were completely enclosed in small branch bags made of Reemay agricultural cloth tied to the microcosms, and all seedlings were covered to control for any effects of the bags (e.g. reduced photosynthesis). Herbivores removed 22.2 ± 3.5% leaf area (mean ± SD) using a common visual damage estimate technique (Hunter, 1987); undamaged seedlings suffered no damage.

On 18 June 2004 following herbivore removal, the two enrichment-free microcosms were destructively sampled (described later) and their isotope ratios represented background abundances. Each remaining microcosm was then injected with a total of 3.0 ml of a 0.27 M solution of 98atom% ^{15}N-glycine into the three vertically-distributed injection ports sealed with rubber septa (Fig. 1). This added approx. 11.2 mg of highly enriched but dilute ^{15}N to minimize the potential for priming effects (Jenkinson *et al.*, 1985). Glycine was added instead of mineral ^{15}N to promote microbial mineralization followed by plant uptake of the mineralized ^{15}N. While plants can directly acquire organic forms of N (Lipson & Näsholm, 2001), plant uptake of organic N may be low (Jones *et al.*, 2005) and, to our knowledge, there are no reports of organic N uptake in red oaks. Nonetheless, we cannot be certain that all ^{15}N acquired by the seedlings was derived from microbial processing. Immediately following ^{15}N injections, the chamber was sealed and injected with 1 l of 99atom% $^{13}CO_2$. Two more equivalent pulse-labeling events occurred in the subsequent 2 h. Following treatments, the chambers remained sealed for 48 h when day 2 microcosms were removed. The day 7 microcosms were watered through the injection ports as necessary until they were destructively sampled on 25 June 2004.

All microcosms were destructively sampled and sorted into the following categories for analysis: foliage, new stem (during 2004 growing season), old stem, main (tap) root, fine roots, rhizosphere soil and bulk soil (Table 1). Foliage was clipped at the stem; stems were clipped at the surface of the wax layer. The soil and roots were then pushed gently out the bottom of the microcosm into a plastic bag with as little disturbance to the soil as possible. The main root was then lifted gently 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 intact roots were then washed with DI water in a 2 mm sieve to remove remaining soil particles; fine roots were separated from the tap root. Leaf, stem, and root samples were dried separately for 48 h at 65°C and ground into fine powders for isotope analysis (described later in 'Isotope analysis').

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Soil analysis

Rhizosphere and bulk soils were analysed separately. Soils were passed through a 1-mm screen mesh and separated into three subsamples for separate analyses. The first subsample of soil was weighed, dried for 48 h at 105°C, and then ground to a fine powder and analysed for total C and N and their isotopes. The remaining two soil samples (approx. 7 g per sample) were used to analyse extractable microbial and nonmicrobial C via the fumigation-extraction method with 0.5 M K_2SO_4 (Vance *et al.*, 1987). Briefly, one subsample was immediately extracted with 50 ml 0.5 M K_2SO_4 on an orbital shaker (150 r.p.m.) and subsequently filtered through Whatman 42 filter paper. The filtrate represents a soluble, labile pool of soil C (Powlson & Jenkinson, 1976; Cook & Allan, 1992). The third subsample was subjected to chloroform fumigation for 48 h under reduced pressure, and then extracted as already described. Nonfumigated (NF) and fumigated (F) samples were analysed for total organic C (TOC) and $\delta^{13}C$ (described in 'Isotope analysis'). Total microbial biomass C was estimated from the difference between F and NF:

$$\text{Microbial biomass C} = (F - NF)/k_{ec}$$

($k_{ec} = 0.33$ and is a correction factor based on the efficiency of chloroform fumigation) (Sparling & West, 1988).

Isotope analysis

All dry stable isotope samples (i.e. leaf, stems, roots, soils) were analysed on a Costech Elemental Combustion System 4010 (Costech Analytical Technologies, Inc., Valencia, CA, USA) 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 analysed on an OI 1010 total organic carbon (TOC) analyser (OI Analytical, College Station, TX, USA) connected to the above IRMS via a scrubber interface designed and built at the G. G. Hatch Isotope Laboratories (University of Ottawa, Ontario, Canada). Sets of samples were analysed on two dates to provide estimates of IRMS, elemental analyser, and TOC errors (Jardine & Cunjak, 2005). 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

Distribution of newly assimilated C and N in the plant tissues and soils was determined using standard isotope mixing models (Lajtha & Michener, 1994; Dawson *et al.*, 2002). All data were analysed using the GLM procedure of SAS 8.2 with Tukey HSD *post hoc* tests to determine significant differences among treatment means (Littell *et al.*, 2002). Since tissue samples were collected from the same experimental units, a nested analysis that treated tissue type as a within-subjects effect was used. To account for violations of sphericity in the within-subjects models, Huynh–Feldt epsilon corrections (generated by PROC GLM) were used to adjust df to avoid overestimates of significance. Damage (herbivore, undamaged) and sampling date (days 2 and 7) were treated as between-subjects effects and allowed to interact.

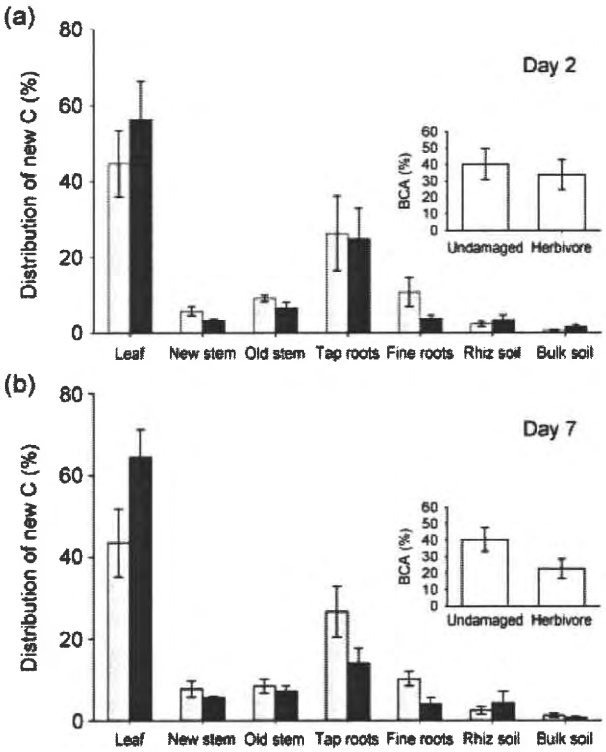

Fig. 2 Distribution of assimilated ^{13}C (% of total recovered ^{13}C) among leaf, new stem, old stem, tap roots, and fine roots in *Quercus rubra* (red oak) seedlings exposed to undamaged control conditions (light tinted bars) or insect herbivore damage (dark tinted bars) on (a) day 2 and (b) day 7 following herbivory. Inset graphs represent belowground C allocation (sum of roots + soil pools) as a percentage of total C allocation for each sampling date. In all cases, bars are means ± SE ($n = 5$ for the herbivore day 7 and undamaged day 2; $n = 4$ for herbivore day 2 and undamaged day 7).

Results

Herbivore damage altered C allocation patterns between aboveground and belowground tissues of the oak seedlings (Fig. 2). In the complete within-subjects model, there was a significant tissue × damage interaction ($F_{4,64} = 3.85$, $P = 0.0190$) indicating that C allocation patterns differed between tissue types as a function of damage; C allocation was highest in the foliage and taproots, and lowest in stem material and fine roots. Contrary to our hypothesis, the allocation of new ^{13}C-C to

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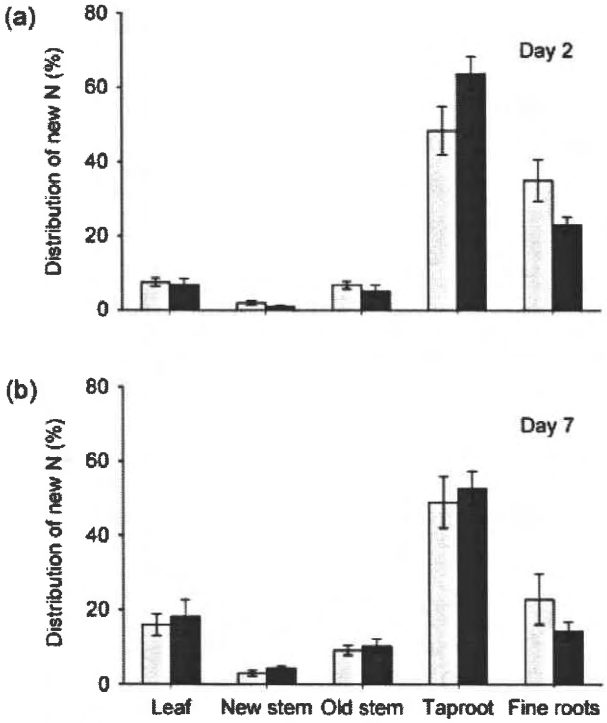

Fig. 3 Distribution of ^{15}N (% of total recovered ^{15}N) among leaf, new stem, old stem, tap roots and fine roots in *Quercus rubra* (red oak) seedlings exposed to undamaged control conditions (light tinted bars) or insect herbivore damage (dark tinted bars) on (a) day 2 and (b) day 7 following herbivory and isotope additions. Bars are means ± SE ($n = 5$ for the herbivore day 7 and undamaged day 2; $n = 4$ for herbivore day 2 and undamaged day 7).

fine roots was 63% lower in herbivore-damaged seedlings relative to controls independent of sampling date ($F_{1,16} = 13.45$, $P = 0.0021$, Fig. 2). The allocation of new C to leaf tissue was correspondingly higher in the seedlings suffering herbivore damage relative to controls ($F_{1,16} = 5.02$, $P = 0.0396$). Carbon allocation to new stems, old stems, and taproots was not affected by damage when considered individually ($F_{1,16} = 2.95$, $P = 0.1053$; $F_{1,16} = 2.14$, $P = 0.1626$; $F_{1,16} = 1.25$, $P = 0.2806$, respectively). However, allocation of new C to total (old + new) stem tissue was significantly lower in seedlings damaged by herbivores (6.09 ± 0.60% vs 8.06 ± 0.75% in undamaged controls; $F_{1,16} = 5.76$, $P = 0.0308$). In addition, when we considered date-specific analyses, BCA (tap roots + fine roots) was significantly lower in the herbivore-damaged seedlings relative to controls on day 7 ($F_{1,8} = 6.55$, $P = 0.038$; Fig. 2b inset) but not day 2 ($F_{1,8} = 0.42$, $P = 0.535$; Fig. 2a inset); the effect on day 7 was weaker when the two soil pools were included in the estimate of BCA ($F_{1,8} = 3.64$, $P = 0.098$).

Nitrogen allocation patterns in the oak seedlings were also affected by herbivory (Fig. 3). Significantly (39%) less of the ^{15}N was allocated to fine roots of herbivore-damaged seedlings relative to controls ($F_{1,14} = 4.79$, $P = 0.0461$); in the complete model, there was a marginal within-subjects tissue × damage interaction ($F_{4,56} = 2.90$, $P = 0.0675$). Instead of allocation to fine roots, herbivore-damaged seedlings appeared to allocate new N to taproots as indicated by a moderate tissue × damage interaction when analysing fine roots against taproots alone ($F_{1,14} = 4.47$, $P = 0.0506$). Moreover, a significant negative correlation between allocation of new N to fine roots and taproots ($r = -0.6815$, $P < 0.0001$) supports the suggestion that herbivory favored a shift in the allocation of new N from fine roots towards taproots. In addition, there was a significant day × damage interaction in new stem tissue ($F_{1,14} = 5.33$, $P = 0.0367$); there was a greater increase from day 2 to day 7 in assimilated N in the new stem tissue of the herbivore-damaged seedlings relative to the undamaged seedlings (Fig. 3). There were no significant treatment effects on new N accumulation in the leaf tissue.

There was no difference in total assimilated C between herbivore-damaged and control seedlings ($F_{1,16} = 0.23$, $P = 0.6193$). We recovered 2.28 ± 0.28 mg ^{13}C per microcosm (*c.* 1.42% of the ^{13}C added to the chamber), 95.4% of which was recovered in plant tissue. We also recovered 67.3 ± 13.7 μg ^{13}C in the rhizosphere soil and 24.8 ± 5.0 μg ^{13}C in the bulk soil, which represents *c.* 3.4% and *c.* 1.2% of the newly assimilated C, respectively. For N, 11.4 ± 0.6 mg of the ^{15}N in the microcosms (*c.* 102% of the ^{15}N added to the microcosms) was recovered, 87.0% of which was in the bulk soils. Seedlings continued to acquire ^{15}N in all tissue types over the course of the experiment: 2 d and 7 d following ^{15}N additions, the seedlings accumulated 297.4 ± 87.1 μg (*c.* 2.6% of added ^{15}N) and 808.9 ± 276.4 μg ^{15}N (*c.* 7.2% of added ^{15}N), respectively. A significant date × tissue interaction indicated that the distribution of new N within seedlings changed over the course of the experiment, with significant increases in all three aboveground tissues (leaf $F_{1,14} = 10.46$, $P = 0.006$; new stem $F_{1,14} = 16.35$, $P = 0.001$; old stem $F_{1,14} = 5.84$, $P = 0.030$) balanced by a significant decrease in the allocation to fine roots ($F_{1,14} = 4.96$, $P = 0.043$). However, there were no differences in total N assimilation between herbivore and undamaged groups at either time-point (day 2: $F_{1,14} = 0.01$, $P = 0.988$; day 7: $F_{1,14} = 1.68$, $P = 0.236$).

When considering the soil fractions of the microcosms, rhizodeposition recovered in the soil pools appeared to be buffered from herbivore-induced changes in C allocation. Reduced allocation of new C to fine roots did not reduce the amount of new C exuded from roots ('soil' comparison between damage treatments: $F_{1,16} = 0.67$, $P = 0.427$; Fig. 4). However, a significant interaction was found between the herbivore treatment and the ^{13}C recovery and distribution in fine roots versus soil that was consistent between sampling dates (^{13}C recovery: $F_{1,16} = 5.48$, $P = 0.035$; % distribution: $F_{1,16} = 6.91$, $P = 0.018$). These interactions indicate that, for a given amount of new C allocated to fine roots, proportionally

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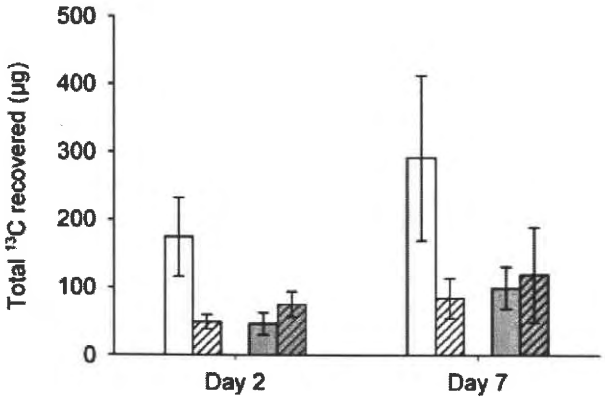

Fig. 4 Total (µg) of photosynthetically derived ^{13}C in fine roots and soils (rhizosphere + bulk) of *Quercus rubra* (red oak) microcosms exposed to insect herbivory. Open bars, fine roots from undamaged seedlings; open bars with hatching, soil from undamaged microcosms. Tinted bars, fine roots from herbivore-damaged seedlings; tinted bars with hatching, soils from herbivore-damaged microcosms. Bars are means ± SE (n = 5 for the herbivore day 7 and undamaged day 2; n = 4 for herbivore day 2 and undamaged day 7).

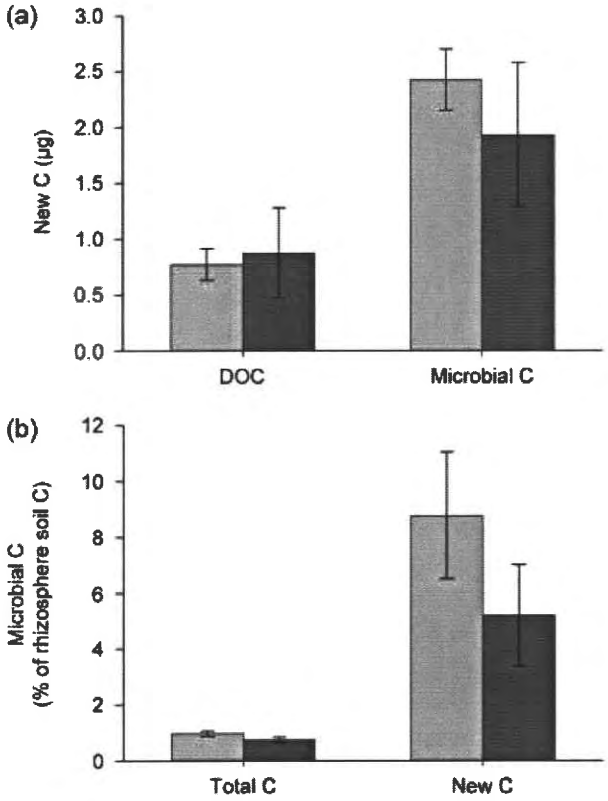

Fig. 5 (a) Total photosynthetically derived ^{13}C recovered as K_2SO_4-extractable dissolved organic carbon (DOC) and microbial biomass. Microbial biomass was estimated by fumigation-extraction. (b) The percentage of rhizosphere soil C in microbial biomass. Bars are means ± SE of nine samples. Light tinted bars, day 2; dark tinted bars, day 7.

more of that ^{13}C was recovered from the rhizosphere of herbivore-damaged than from undamaged seedlings (Fig. 4).

Probably because rhizodeposition to soil pools between herbivore treatments was equal overall, neither the total microbial C nor ^{13}C-enrichment were affected by the damage treatment ($F_{1,16} = 0.16$, $P = 0.6904$; $F_{1,16} = 0.77$, $P = 0.392$ respectively). Also, nonmicrobial total DOC and ^{13}C-DOC were not affected by herbivory ($F_{1,16} = 0.35$, $P = 0.551$; $F_{1,16} = 0.09$, $P = 0.767$ respectively). However, while the ^{13}C recovered in the rhizosphere microbial biomass was not affected by herbivory, the microbial biomass was the principal labile pool for newly assimilated and rhizodeposited C (Fig. 5). The total C in microbial biomass was significantly greater than that in nonmicrobial, extractable DOC ($F_{1,32} = 4.20$, $P = 0.0487$), and significantly more total ^{13}C was recovered in the microbial biomass than the extractable DOC independent of date (2.18 ± 0.35 µg ^{13}C in microbes v. 0.82 ± 0.21 µg ^{13}C in DOC; $F_{1,16} = 14.97$, $P = 0.0014$; Fig. 5a). Thus, the ^{13}C recovered in the microbial biomass accounted for approx. 73% of all ^{13}C recovered in the extractable fraction of the rhizosphere soil. In addition, although the total microbial C was < 1% of the total rhizosphere C, the ^{13}C recovered in the microbial biomass was 7.0 ± 1.5% of the ^{13}C recovered in the rhizosphere ($F_{1,16} = 17.14$, $P = 0.0008$; Fig. 5b). Overall, approx. 93% of ^{13}C recovered in the rhizosphere soil was in nonextractable form and therefore possibly incorporated into soil organic matter.

The experimental design also allowed us to explore correlations between C and N. Surprisingly, there were few correlations between either total C and N or the ^{13}C and ^{15}N recoveries. There were strong correlations between total C and total N in the bulk and rhizosphere soils; in the seedlings, total C and N were correlated only in the fine roots (Fig. 6). Moreover, fine roots were the only pool in which the recovered ^{13}C and ^{15}N were significantly correlated (Fig. 6). When we considered how the newly assimilated C and N was correlated within the microcosms, ^{13}C recoveries were correlated among some of the plant tissues (Table 2), while all of the plant parts were correlated with respect to ^{15}N recovery (Table 3).

Discussion

Our results show that red oak seedlings respond to insect herbivore damage with a suite of C and N allocation shifts. A 63% reduction in allocation of new C to fine roots was coupled with increased allocation to foliage, presumably at the expense of root growth or belowground C storage. Despite lesser C allocation to fine roots, seedlings apparently maintained C exudation to soil at levels similar to those of undamaged controls, suggesting that rhizodeposition by oak seedlings to soils must be buffered to some degree against damage-induced changes in C allocation. Indeed, rhizodeposition

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Table 1 Masses, nitrogen (N), carbon (C), and C : N ratios for the *Quercus rubra* (red oak) microcosms

Sample type	Mass (g)[1]	N (%)	C (%)	C : N
Leaf	0.514 ± 0.239	2.313 ± 0.362	46.192 ± 0.495	20.529 ± 3.410
New stem	0.055 ± 0.028	1.370 ± 0.328	44.522 ± 0.729	34.183 ± 7.592
Old stem	0.235 ± 0.094	1.274 ± 0.264	46.724 ± 0.754	38.198 ± 8.152
Tap roots	0.757 ± 0.344	2.337 ± 0.678	45.946 ± 0.698	21.066 ± 5.297
Fine roots	0.135 ± 0.078	2.365 ± 0.477	41.262 ± 3.837	17.920 ± 3.021
Rhizosphere soil	19.930 ± 7.648	0.238 ± 0.048	3.541 ± 0.786	14.847 ± 1.281
Bulk soil	181.653 ± 7.421	0.300 ± 0.029	3.252 ± 0.419	10.840 ± 0.658

[1]Data are means ± SD of 18 samples.

Table 2 Pearson correlations among ^{13}C recoveries in *Quercus rubra* (red oak) seedling microcosms

	Leaf	New stem	Old stem	Tap root	Fine roots	Rhizosphere soil
New stem	0.573**					
Old stem	0.290	0.565**				
Tap root	−0.162	0.169	0.655***			
Fine roots	−0.280	0.112	0.417	0.740***		
Rhizosphere soil	−0.135	0.049	0.383	0.217	0.031	
Bulk soil	0.070	0.061	0.383	0.337	0.088	0.242

**$P < 0.05$; ***$P < 0.01$.

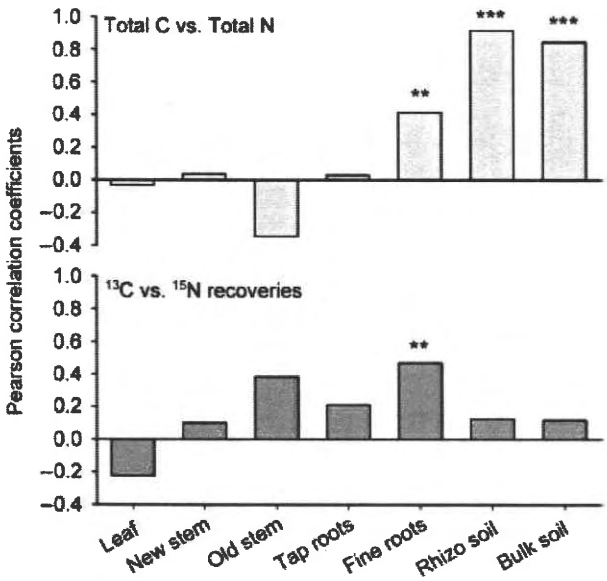

Fig. 6 Pearson correlations between total C and total N, and between ^{13}C and ^{15}N recoveries, in the individual components of *Quercus rubra* (red oak) microcosms. The components of the microcosms were foliage, 'new' stem grown during the experimental growing season, 'old' stem, tap roots, fine roots, rhizosphere (rhizo) soil and bulk soil. **$P < 0.05$; ***$P < 0.01$.

was not a simple and constant proportion of the C allocated belowground, but appeared to be actively maintained (Fig. 4, but see caveat later). The higher allocation of new N to taproot and stem tissue following herbivore damage suggests herbivore-induced N storage. We interpret the results to indicate that oak seedlings respond to moderate insect herbivore damage with a complex combination of allocation shifts that may simultaneously increase leaf C, maintain soil C exudation, maintain N assimilation and prompt N storage for later growth.

Our data suggest that oak seedlings divert C away from root growth or storage in the presence of foliar herbivores. While this is opposite to 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). It is possible that 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; Hunter & Schultz, 1995; Frost & Hunter, 2008). For example, jasmonic acid (JA) application to leaves of poplar saplings stimulates *de novo* synthesis of tannins that increases total foliar tannin concentrations (Arnold & Schultz, 2002). Jasmonic acid is a well-known signaling hormone that stimulates chemical defenses in plants (Farmer & Ryan, 1990). In other words, an aboveground/belowground tradeoff may occur in seedlings whereby foliar damage necessitates reduction in BCA in order to induce defensive foliar chemistry. Oak

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Table 3 Pearson correlations among ^{15}N recoveries in *Quercus rubra* (red oak) seedling microcosms

	Leaf	New stem	Old stem	Tap root	Fine roots	Rhizosphere soil
New stem	0.825***					
Old stem	0.739***	0.893***				
Tap root	0.437*	0.741***	0.730***			
Fine roots	0.479**	0.581**	0.456*	0.703***		
Rhizosphere soil	0.200	0.354	0.194	0.209	0.255	
Bulk soil	−0.204	−0.267	−0.216	−0.177	−0.167	−0.018

*$P < 0.1$; **$P < 0.05$; ***$P < 0.01$.

seedlings have minimal C stores (Murakami & Wada, 1997; Chaar *et al.*, 1997; 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 (Ruess *et al.*, 1998), though the observed N allocation shifts may help compensate. Also, it is possible that different responses of oaks to foliar herbivores might be observed as oaks mature and develop C storage reserves (Frost & Hunter, 2004).

A number of studies have reported effects of foliar damage on BCA and related processes in woody plants. Ayres *et al.* (2004) found increases in soil C sequestration, but decreases in coarse and fine root biomass, following mechanical damage to leaves of *Abies* and *Fagus* seedlings. In our previous work, we measured increased soil respiration from red oak mesocosms following foliar herbivory but not mechanical damage, suggesting that herbivores specifically triggered a shift increasing BCA (Frost & Hunter, 2004). Babst *et al.* (2005) showed that *Populus tremuloides* saplings increase export of newly assimilated ^{11}C to roots following JA application, and similar results were observed using a ^{14}C tracer in a poplar hybrid in the laboratory (Bassman & Dickmann, 1985). However, severe insect herbivore damage on hybrid poplars in the field has been shown to reduce BCA to fine roots (Kosola *et al.*, 2001), though the damage also reduced overall tree growth. Thus, while it is tempting to infer a general conclusion that foliar damage stimulates BCA, our data taken with those of Kosola *et al.* (2001) present a cautionary note that observed herbivore-mediated changes in BCA may depend on ontogeny and the severity of damage.

Despite the reduction in BCA, we nonetheless found evidence for the active maintenance of C exudation, as estimated by the recovery of ^{13}C in the rhizosphere soil. While the gradient of C concentrations between roots and rhizosphere soil (Table 1) provides for the potential for passive diffusion of C into the rhizosphere (Kuzyakov & Cheng, 2001), there is substantial evidence that plants actively regulate rhizodeposition (Holland *et al.*, 1996; Knops *et al.*, 2002; Jones *et al.*, 2004; Thelen *et al.*, 2005). If the ^{13}C recovered in the rhizosphere occurred from passive diffusion alone, then the ^{13}C in the rhizosphere should be proportional to the ^{13}C in the fine roots. This was not the case (Fig. 4), which suggests that the oak seedlings regulated rhizodeposition while reducing overall BCA. This interpretation of the data, however, comes with an important caveat. Respiration of photosynthetically derived C by roots and soil microorganisms constitutes a portion of BCA (Phillips & Fahey, 2005). This is a missing pool in our study that, if included, may affect our conclusions about BCA and rhizodeposition on the whole. However, the results and interpretations are robust for the belowground pools measured, and the prevalent theoretical rationale for active maintenance of root C exudation to soils is facilitative nutrient acquisition by rhizosphere microbes (Hamilton & Frank, 2001).

The constant rates of rhizodeposition to soil between the damage treatments were paralleled by constant levels of new C in microbial biomass. Rhizosphere microbial biomass is known to fluctuate in concert with periodic C flushes from oak roots (Cardon *et al.*, 2002). In cases where aboveground clipping leads to greater net C rhizodeposition, microbial biomass is correspondingly higher (Hamilton & Frank, 2001) over the same time-course of our experiments reported here. In our case, the 'active' C rhizodeposition to soils was compensatory; we would therefore predict (and observed) no differences in microbial biomass. However, the rhizosphere microbial biomass was actively incorporating and using root rhizodeposits, which supports the previous work of Cardon *et al.* (2002). Thus, any effect of aboveground damage on soil rhizodeposition may be predicted to influence the microbial community. Given the herbivore-mediated reduction in C allocation to fine roots, it may be more important to note that there was not a decline in rhizosphere microbial biomass as a result of the herbivore damage. Mycorrhizal fungi play an important role in rhizodeposition in oak seedlings (Dickie *et al.*, 2001), and it is possible that they were an important factor in the maintenance of the C exudation observed (Jones *et al.*, 2004).

Interestingly, approx. 93% of the ^{13}C recovered in the rhizosphere was not K_2SO_4-extractable and therefore neither labile nor microbial. The forms of C in rhizodeposition are species specific but include simple sugars and amino acids (Whipps & Lynch, 1983; Jaeger *et al.*, 1999; Bringhurst *et al.*, 2001) that are presumably suitable substrates for microbial

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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 day 2 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). It is further possible, despite our best efforts to remove them, that fine root contamination in the soil samples accounted for some of the ^{13}C recovered in the nonextractable fraction of soils.

Herbivore damage to the oak seedlings stimulated N allocation away from fine roots and toward tap roots, while new stem tissue from herbivore-damaged seedlings accumulated new N more rapidly than did controls. We interpret these results as increasing storage of newly assimilated N. This differs somewhat from previous results showing increases in foliar N in clipped grasses (Hamilton & Frank, 2001). Life history likely plays a role in the observed differences; while it may be beneficial for an annual grass to reinvest new N immediately into aboveground growth, a more appropriate strategy for long-lived oak seedlings may be to store nutrients for future growth. There were no treatment-based differences in enrichment levels or allocation of new ^{15}N in the foliage, which suggests that the increased N in taproots and stems was not merely in transport. This partially supports our hypothesis that herbivore-damaged oaks would allocate more N to storage tissues than would undamaged oaks. However, the hypothesis that foliar herbivory on oaks leads to N storage yields the prediction that N allocation should shift away from foliage and toward storage tissues, which we did not observe over the time-course of our experiment. Herbivore-induced reductions in foliar N in oaks have been observed previously (Nykanen & Koricheva, 2004), and it is possible that translocation to N storage may account for this effect. Indeed, the higher allocation of N to storage following herbivore damage may affect foliar N concentrations in the next growing season. However, oak saplings damaged by herbivores in one growing season flush foliage with lower N concentrations than do controls in the following growing season (Frost & Hunter, 2007), so predicting how stores of N will be allocated in future remains unclear.

As a final point, 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 earlier are an important step, they may not reflect responses to herbivory of mature trees in a forested landscape. Short-term microcosm experiments with oak seedlings do not scale to mature oaks, and we draw no such inferences. Rather, the experiment was designed to explore the hypothesis that foliar herbivores would influence oak BCA and the feedback loops that depend on BCA. Further experiments are required to determine if the patterns observed in this study are seedling-specific or applicable to oaks in nature, and in particular how use of C and N reserves in mature trees affects the interactions between insect herbivores and tree energy and nutrient allocation. There is ample evidence that different tree species uniquely partition C and N and have significant impacts on C and N dynamics in their respective soils (Templer *et al.*, 2003; Lovett *et al.*, 2004), which further broadens the importance of understanding the effects of herbivory on BCA at a landscape scale. Nonetheless, our data provide evidence that herbivores influence BCA and N allocation patterns during oak development and, taken with previous work, indicate that young oaks from seedlings to saplings change energy and nutrient allocation patterns in response to insect herbivores.

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