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Periodic carbon flushing to roots of *Quercus rubra* saplings affects soil respiration and rhizosphere microbial biomass

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Abstract Patterns of root/shoot carbon allocation within plants have been studied at length. The extent, however, to which patterns of carbon allocation from shoots to roots affect the timing and quantity of organic carbon release from roots to soil is not known. We employed a novel approach to study how natural short-term variation in the allocation of carbon to roots may affect rhizosphere soil biology. Taking advantage of the semi-determinate phenology of young northern red oak (*Quercus rubra* L.), we examined how pulsed delivery of carbon from shoots to roots affected dynamics of soil respiration as well as microbial biomass and net nitrogen mineralization in the rhizosphere. Young *Q. rubra* exhibit (1) clear switches in the amount of carbon allocated belowground that are non-destructively detected simply by observing pulsed shoot growth above-ground, and (2) multiple switches in internal carbon allocation during a single growing season, ensuring our ability to detect short-term effects of plant carbon allocation on rhizosphere biology separate from longer-term seasonal effects. In both potted oaks and oaks rooted in soil, soil respiration varied inversely with shoot flush stage through several oak shoot flushes. In addition, upon destructive harvest of potted oaks, microbial biomass in the rhizosphere of saplings with actively flushing shoots was lower than microbial biomass in the rhizosphere of saplings with shoots that were not flushing. Given that plants have evolved with their roots in contact with soil microbes, known species-specific carbon allocation patterns within plants may provide insight into interactions among roots, symbionts, and free-living microbes in the dynamic soil arena.

Keywords *Quercus rubra* · Rhizosphere · Allocation · Microbial biomass · Mineralization

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Introduction

The flux of photosynthetically fixed carbon from plant shoots through roots to mycorrhizae and soil supports substantial microbial activity in the rhizosphere. This rhizosphere microbial activity can in turn influence the availability of nutrients to plants and the turnover of soil organic matter (SOM) in ecosystems (Paul and Clark 1996). Though patterns of carbon allocation within plants have been examined extensively (Bazzaz and Grace 1997) and are very well understood for some tree and crop species (e.g. Dickson 1989; Friend et al. 1994; Liljeroth et al. 1994), the extent to which the timing and quantity of carbon allocation from plant shoots to roots can influence the quantity, quality, and timing of carbon release to mycorrhizae and to rhizosphere soil remains unclear.

Because of its importance for plant nutrition and ecosystem function, the rhizosphere has been the focus of extensive applied and basic research. Researchers have, for example, quantified the carbon “costs” of mycorrhizal infection (Whipps 1990), characterized the flux of organic compounds from roots under the influence of various environmental conditions (e.g. Barber and Martin 1976; Christiansen-Weniger et al. 1992; Kraffczyk et al. 1984; Smith 1969, 1976), and mapped sugars around roots in non-sterile soil (Bringhurst et al. 2001; Jaeger et al. 1999). Also, a number of studies have explored whether rhizodeposition (the deposition of organic compounds by roots to soil) stimulates or suppresses microbial breakdown of SOM, and under what conditions (e.g. Badalucco and Kuikman 2001; Bradley and Fyles 1995;

Cardon et al. 2001; Cheng and Johnson 1998; Clarholm 1985; Liljeroth et al. 1994; Sallih and Bottner 1988). Still, it remains unclear to what extent plants can modulate microbial activity around their roots. Certainly plants release signaling molecules that encourage interactions with mycorrhizal and nitrogen fixing symbionts (e.g. Long 1996). But, can plants adjust the amount or type of more common carbon compounds moving to mycorrhizae or to rhizosphere soil, or adjust the timing of that loss, encouraging growth and activity of particular microbial communities? There is some evidence supporting this idea. For example, Johnson (1993) suggests that increased loss of soluble carbohydrate from roots of slightly nutrient stressed *Andropogon gerardi* Vitm. may support establishment of mycorrhizal associations with vesicular-arbuscular fungal species that are superior mutualists.

The potential for plant species-specific differences in below-ground rhizosphere activity to influence ecosystem processes is intriguing. It is clear that the presence of particular species or phenological groups in plant communities can influence ecosystem processes such as soil nutrient availability and nitrogen cycling (e.g. Binkley and Giardina 1998; Finzi et al. 1998; Hooper and Vitousek 1998; Vinton and Burke 1995; Wedin and Tilman 1990). In these studies, however, a focus on consequences of species-specific root function for below-ground ecosystem processes (without interference from, for example, leaf litter chemistry) is rare. Vitousek and Walker (1989) have convincingly demonstrated a significant change in nitrogen (N) dynamics in Hawaiian ecosystems after the invasion of the N_2 fixer *Myrica faya*. Also, Bradley and Fyles (1995) suggest that the 'soil-improving' nature of paper birch in silviculture is not derived from the quality of its leaf litter but instead stems from a higher rate of rhizodeposition from birch roots than from other forest species. Bradley and Fyles conclude that enhanced rhizodeposition by paper birch may drive increased nutrient cycling in forest systems, providing the nutrients necessary for quick growth of this early successional species.

As noted above, there is a wealth of information in the plant literature about shoot/root allocation patterns. Generally, these patterns are not interpreted from the point of view that roots are embedded in soils surrounded by, and interacting with, active microbes potentially important for ecosystem function. A first step toward exploring whether known internal plant carbon allocation patterns can be reinterpreted within this larger system context is simply to test whether a known carbon allocation pattern can be correlated with effects in the rhizosphere.

We took advantage of natural, repeating, short-term phenological fluctuations in carbon allocation below-ground that are associated with semi-determinate growth in young *Quercus rubra* L. (northern red oak, Dickson 1991a). We examined how this semi-determinate phenology affects light-saturated photosynthesis and soil respiration, as well as microbial biomass and net N mineralization in the rhizosphere. Semi-determinate growth in plants is characterized by rhythmic expansion of new shoot tissue during the growing season (Borchert 1991). Roots may or may not grow rhythmically, depending on the species (Borchert 1991). As young red oaks grow, they send carbon to their roots in distinct phases that correspond to lulls between flushes of shoot growth. During these lulls, increased root growth generally occurs. The switch in allocation below-ground can be dramatic. Using saplings, Dickson (1991a) showed that after the first spring flush of oak leaves had fully matured, 95% of photosynthate being fixed by those leaves was translocated downward to the lower stem and roots. Several weeks later, during the second flush of shoot extension and leaf production, 90% of new photosynthate from mature first-flush leaves was instead translocated upward to support the newly developing portions of the shoot. These switches in allocation continued as further shoot flushes and lag periods occurred. Fortunately for our study, this striking switching of internal carbon allocation (up to expanding shoots or down to roots) is correlated with the easily visible developmental stages of the shoot above-ground.

If internal root/shoot allocation patterns can influence microbial activity (mycorrhizal or free-living), population sizes, and/or community structure in the rhizosphere, then plant carbon allocation strategies already well-documented in the literature may provide a base for exploring the function of the plant/microbial/soil system as a whole. As noted above, we do not know whether carbon allocation to below-ground plant parts can be directly linked to the quantity, quality, and timing of carbon release from roots to soils. However, in young *Q. rubra*, the timing of switches in carbon allocation to roots is on the order of weeks, a period sufficiently long that if fluctuating carbon flux to the rhizosphere accompanies periodic carbon allocation to roots, then microbial biomass and activity in the rhizosphere may change during shoot flush and shoot lag periods. The timing of switches in carbon allocation is also sufficiently short that microbial changes in response to plant phenology can be observed during multiple switches in shoot-root allocation independent of longer-term seasonal effects. Most generally, the timing of carbon allocation below-ground in dominant plant species of an ecosystem may be important for understanding dynamics of soil respiration, which encompasses respiration from root activity and growth as well as from associated heterotrophic soil organisms.

Materials and methods

We conducted two experiments, one in summer of 1998 examining northern red oaks grown outside in pots, and another in summer of 1999 focusing on soil respiration around northern red oaks rooted directly in soil.

Pot experiment

Two-year-old bare-root seedlings (shoots approximately 45–60 cm tall) were purchased dormant from Musser Forests (Indiana, Pa.)

and potted in 7.6 l, 15.2×40.6 cm tree pots in April 1998. Growth medium was a 2:1:1 by volume mix of sandy loam soil: perlite: sand. Pots were placed into slightly larger, similarly shaped containers that had been sunk individually into holes dug in the ground outside the greenhouses at University of Connecticut, Storrs. Plants were kept well watered.

Measures of shoot growth

Growth rates of saplings were assessed in two ways. Total leaf area of young trees was determined approximately every 2 days from early July through late August. Using a LiCor 3000a leaf area meter (LiCor, Lincoln, Neb.), we developed a regression based on leaf length that was used to estimate leaf area (n=38 with leaf length ranging from 3 to 15 cm, r^2=0.948, Area = 0.336–0.23× length + 0.388×length^2). Area of all leaves on all trees was assessed using this regression within a 2-h measurement period on any one day. Another indicator of shoot growth was gathered quickly (but more sporadically) by measuring the length of lateral and apical shoots; this information allowed identification of shoot flush stages, and is useful for interpreting results from the 1999 summer garden experiment (see below).

Measurements of photosynthesis and soil respiration

We measured photosynthesis of first-flush leaves, soil respiration rates, and shoot and leaf expansion rates during the second and third shoot flushes in July and August. Photosynthesis was measured using a LiCor 6400 gas exchange system, at saturating light (800 μmol photons m^{-2} s^{-1}), temperature (25°C), and leaf internal CO_2 concentration (c_i between 240 and 260 ppm), provided by a blue/red LED light source, temperature controlled leaf cuvette, and CO_2 mixer attachment, respectively. Measurements were made only between 11 a.m. and 2 p.m. to avoid complications caused by circadian rhythms in photosynthesis and stomatal conductance. One fully expanded first-flush leaf on each of the 14 oak trees was tagged early in the season; the photosynthetic rates of these 14 leaves were followed through time. Leaves were allowed to equilibrate at least ten minutes at constant conditions, or until steady photosynthetic rates were achieved, before rates were recorded. (Photosynthesis was determined at saturating, not ambient, light conditions because natural variation in ambient light levels within and among days would have dominated photosynthetic measurements and obscured comparisons of photosynthetic capacity within and among groups of oaks at different flush stages.)

Soil respiration from the 14 oaks was measured using a closed system with a 17- l chamber and pump circulating air through a LiCor 6262 infrared gas analyzer connected to a laptop computer. The depth of soil in each pot was measured before soil respiration measurements. Each pot was put into the 17-l chamber, the top of the chamber was sealed around the stem of the oak (a diffusion tube allowed for pressure equilibration), and a variable CO_2 scrub (soda lime) was used to maintain CO_2 concentration in the chamber at ambient for at least 5 min. The scrub system was then switched out of the pump loop, and the LiCor 6262 measured CO_2 change through time. Though pots sat with their volume almost entirely below-ground in the garden, we found that in companion pots (not part of this study), soil temperature measured using the LiCor 6400 temperature probe varied day to day with ambient temperature and weather. Temperatures of all pots, however, behaved similarly day to day. Unfortunately, we did not measure temperature of the soil in pots used for this study because we did not want to make deep holes in the soil with our LiCor 6400 temperature probe, disturbing the soil, and potentially changing the gas flux. Because soil temperature was likely varying day to day in the oak pots, and we do not have a measure of that variation, soil respiration rate for each oak each day was expressed relative to the average soil respiration rate for all oaks measured that day. We measured soil respiration from 2:00 to 5:00 p.m.

Final harvest soil measurements

On 28 August 1998, we unpotted five randomly selected oaks whose shoots were flushing, and five randomly selected oaks whose shoots were not flushing. Bulk soil was gently moved away from root systems (which were ectomycorrhizal), and rhizosphere soil associated directly with roots was then gently shaken from roots and immediately taken to the laboratory. There, it was passed through a 2 mm sieve, roots and organic debris were removed, and soil was divided for use in assays of microbial biomass, net N mineralization, and immediate determination of soil moisture content.

Soil moisture content was determined by weighing a sample of rhizosphere soil, then drying that sample to constant weight at 65°C.

Microbial biomass was assessed with the chloroform fumigation incubation technique using 20 g fresh soil and incubation in the dark at 24°C (Anderson and Domsch 1978; Voroney and Paul 1984). Over the course of 12 weeks after fumigation, CO_2 concentrations in closed 0.95- l mason jars were measured by removing a 100-μl sample of jar air through a septum and injecting that sample into a CO_2-free air stream flowing through a LiCor 6262 analyzer at a constant rate. Data from the LiCor 6262 was ported directly to computer and peaks associated with each injection were quantified using a calibration based on injection peaks associated with known CO_2 concentrations generated by a LiCor 6400 CO_2 mixing system. Analysis of covariance was used to test whether microbial biomass differed around roots of oaks with shoots flushing versus those with shoots not flushing; the covariate was tree size quantified as total leaf area for each harvested tree.

The potential for net N mineralization was assessed using 2 M KCl extraction (Binkley and Vitousek 1989) and aerobic incubation in the dark. For each oak, a 20 g (fresh weight) rhizosphere soil sample taken during harvest was immediately extracted with 100 ml of 2 M KCl, and another 20 g sample was incubated aerobically in the laboratory for 1 week at 24°C before similar extraction. (All incubated samples were brought to a soil moisture content of 20% 1 day after harvest, when soil moisture content had been determined from other dried soil samples.) Amount of nitrate and ammonium in each KCl solution after extraction was determined using a Scientific Instruments Continuous Flow Analysis System (Orion Scientific, Hawthorne, New York). The difference in amount of N in the two KCl solutions, corrected for dry weight of soil added to each extraction, indicated net mineralization (or immobilization) over the course of the incubation time.

Garden experiment

Ten 2-year-old bare-root seedlings were purchased dormant from Musser Forests (Indiana, Pa.) and planted into soil outside greenhouses at University of Connecticut, Storrs, in May 1998. During summer of 1999, we measured soil respiration rates and shoot extension rates during several shoot flushes.

Shoot flush measurements

Because thousands of leaves developed on the soil-planted oaks during their second year in the garden, we did not measure leaf area as we had with the potted oaks. We only measured shoot extension rates, and noted when leaf expansion on any given shoot was complete. These measurements gave us the data we needed to assess flush stage without laborious leaf area determinations.

Soil respiration measurements

A LiCor 6400 soil respiration chamber was used to measure soil respiration. So that the soil surface would not be disturbed during

each measurement, 10-cm-diameter PVC rings, each 5 cm tall, were buried 2.5 cm into the soil during spring, 1998, and the LiCor 6400 soil chamber was settled on top of each ring during measurements in summer 1999. The closest edge of each ring was buried approximately 5 cm from the base of each oak tree so that during soil respiration measurements the hardware on top of the soil respiration chamber did not damage lateral shoots of trees. Weeds that germinated sporadically within the rings and in soil around the rings were removed throughout the summer. Soil respiration was never measured directly following weed removal. Soil respiration was measured between 10:00 a.m. and 2:00 p.m., and temperature was determined outside the ring using the LiCor 6400 soil temperature probe. For comparison of respiration rates among days, a Q10 of 2 was used to calculate respiration rates at 20°C from the soil respiration and soil temperature measurements for each oak each day (for a range of Q10s, see Raich and Schlesinger 1992). A Moisture Point single diode TDR probe (Environmental Sensors, Victoria, British Columbia, Canada) tracked soil moisture over the course of the season; moisture never dropped below 27%.

Results

Pot experiment

Above-ground growth during the second and third shoot flushes of the 14 oaks was quantified in several ways (Fig. 1). Trajectories of leaf areas through time for each of the14 oaks are plotted in Fig. 1a. Measures were taken approximately every 2 days, but individual symbols have been removed from lines in panel (a) for clarity. Note that on any individual oak trajectory, there is a small break in the curve mid-way through the season to indicate the separation of second and third shoot flushes. Note also that oaks flushed in groups. All 14 oaks flushed a second set of leaves during “Flush 2” (labeled on the figure). The shaded areas in Fig. 1a show the two time periods during which groups of oaks flushed that second set of leaves. Seven oaks began flushing during the “early flush 2” period, and seven began later, during the “late flush 2” period. Only six oaks flushed a third time (“Flush 3”) during the 1998 season, three in early August and three in late August. These groups are easily seen in Fig. 1a so are not highlighted with shading.

To ease viewing on subsequent graphs, instead of presenting all data for all oaks individually, we combined oaks into the groups based on above-ground flush dynamics visible in Fig. 1a. The mean change (±SE) in leaf area for each group of oaks is shown in Fig. 1b. Note that error bars on these changes in leaf area are large mostly because different oaks expanded leaf area to different extents during the flush, though there are some differences in flush timing within groups (e.g. in the early flush 3 group). In Fig. 1c, we plot average change in total shoot length of the different groups of oaks through time. Comparison of panels 1b and 1c reveals that shoot extension precedes leaf expansion by several days, and shoot extension finishes before leaf expansion is complete. This relative timing is important for interpreting results from soil-rooted garden oaks in summer 1999 (see below, Fig. 4), because only shoot extension was measured in those larger saplings.

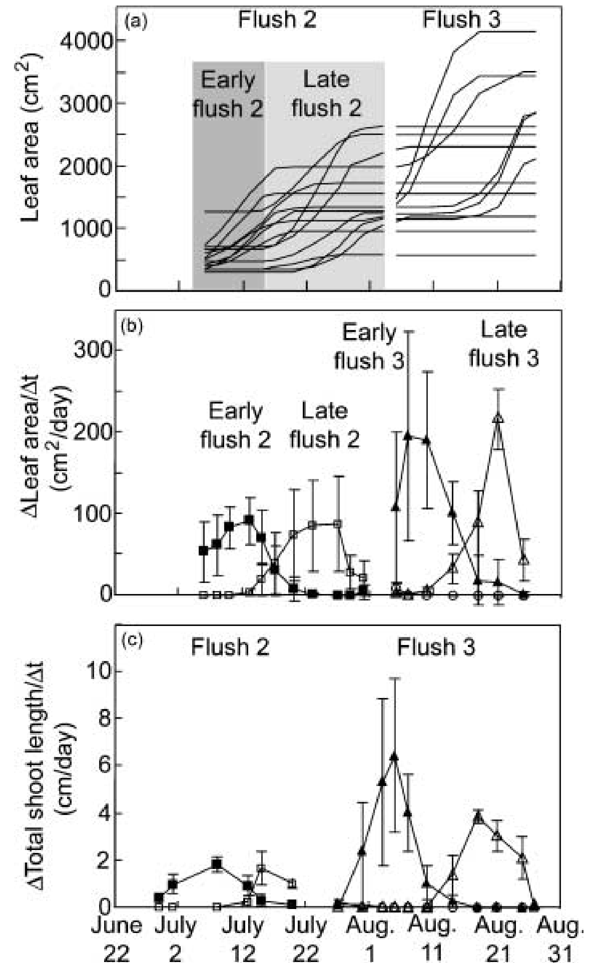

Fig. 1 Time courses of **a** leaf area, **b** leaf expansion rate (mean±SE), and **c** shoot extension rate (mean±SE) of individual (*panel a*) and grouped oaks (*panels b, c*) during summer 1998. Groups of oaks were determined by the timing of shoot flushes visible in *panel a*. Groups include early flush 2 (*filled square*), late flush 2 (*open square*), early flush 3 (*filled triangle*), late flush 3 (*open triangle*), no flush 3 (*open circle*)

Relationships among dynamics of soil respiration, photosynthesis (of first flush leaves), and timing of shoot flushing are shown in Fig. 2. Average normalized soil respiration rates (Fig. 2a) and average photosynthetic rates (Fig. 2c) for groups of oaks are plotted through time. As noted above, oaks were grouped based on flush 2 and flush 3 dynamics. Groups are indicated with different symbols. For easy comparison with shoot flush stages, Fig. 2b again shows average change in leaf area through time. During flush 2, soil respiration (Fig. 2a) was maximum when shoots were not flushing (i.e., when Δleaf area/Δt was zero, Fig. 2b), and minimum when shoots were expanding new leaves. Also, during August, oaks that did not flush a third time (Δleaf area/Δt=0 throughout August) had the highest soil respiration rates (Fig. 2a). Oaks that flushed a third time early in August had lower soil respiration rates during the flush than after the flush ceased (Fig. 2a). Similarly, oaks that

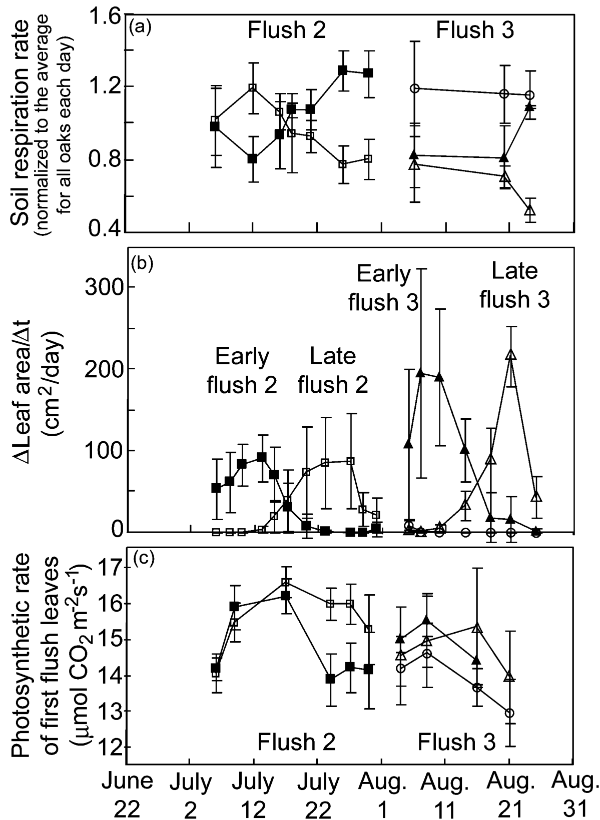

Fig. 2 Time courses of **a** normalized soil respiration, **b** leaf expansion rate, and **c** photosynthetic rates (of first-flush leaves) in oaks grouped as in Fig. 1. All panels show mean±SE for each measure. Symbols as in Fig. 1

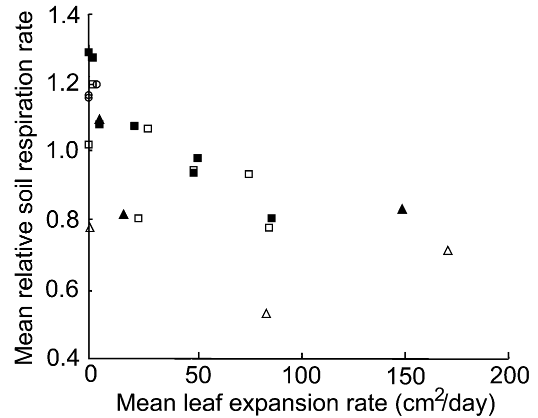

Fig. 3 Inverse relationship between mean relative soil respiration rates (data from Fig. 2a) and mean leaf expansion rate (Fig. 2b) on the same days. Symbols as in Fig. 1

flushed a third time late in August had higher soil respiration rates in early August before the shoot flush than during the flush (Fig. 2a).

Figure 3 further illustrates this inverse relationship using the 14 soil respiration points from flush 2 and 9 points from flush 3 found in Fig. 2a. These soil respiration values are plotted against the corresponding mean leaf expansion rates on those same days. (Error bars for the means are not shown in Fig. 3, though they are shown in Fig. 2a, b.) With increasing leaf expansion rate, soil respiration decreased; linear regression on the means yields r^2=0.45, P<0.001. We were not comfortable with assuming a linear relationship, with regressing means, or with the independence of data being analyzed from all time points. To test the basic hypothesis that higher soil respiration rates were associated with periods when oak shoots were not flushing, we performed paired, one-tailed t-tests using data from dates when oaks were flushing vigorously and from dates when oaks were not flushing at all. (Data from dates when flushes were just starting, or were just ending, were not used in this paired analysis.) For example, on 12 July, seven of 14 oaks were flushing maximally (solid squares, Fig. 2b), and seven were not flushing at all (open squares, Fig. 2b). On 26 July, the seven that had been flushing on 12 July were finished flushing, and those that had been quiescent on 12 July were expanding new leaves vigorously (Fig. 2b). For each oak, then, we had one measurement of soil respiration from a date that the oak was expanding leaves at maximal rate, and another from a date when the oak shoot was not flushing. Because of incomplete sampling caused by late afternoon thunderstorms, paired comparisons of soil respiration rates under non-flushing and flushing conditions were performed on nine of 14 oaks. A paired one-tailed t-test indicated soil respiration was higher when oaks were flushing and expanding new leaves compared with soil respiration when all leaves were mature and expanded (n=9, P<0.002). For the time period labeled flush 3 (Fig. 2b), eight oaks never flushed a third time (Fig. 1a, flat leaf area curves). For the six oaks that did flush, three early and three late (Figs. 1a, 2b), the paired one-tailed t-test conducted using data gathered 6 August and 24 August again indicated soil respiration was higher in pots when oak shoots were not flushing (n=6, P<0.03).

Photosynthetic rates also tended to vary with flush stage as expected based on Hanson et al. (1988), but patterns were not as clear as those for soil respiration. During August, oaks that did not flush a third time tended to have the lowest photosynthetic rates (Fig. 2b, c). Oaks that flushed a third time early in August had higher photosynthetic rates during the flush and then they dropped off as the flush ended. Oaks that flushed late in August showed the highest photosynthetic rates during early shoot flush. Similarly, during flush 2 in July, oaks that flushed early had their highest photosynthetic rates during flushing, and those rates decreased as flushing stopped (Fig. 2b, c). Oaks that flushed late had high photosynthetic rates during the flush, but note that mid-July photosynthetic rates were also high. Paired t-tests detected no significant differences related to flush stage.

Final measurements from destructive harvest revealed higher microbial biomass in the rhizosphere soil surrounding roots of oaks whose shoots were not flushing (Table 1). From our single harvest we cannot rule out that the increased microbial biomass was unrelated to shoot flush status, and instead correlated to some other

Table 1 Microbial biomass and net N mineralization rates from rhizosphere soil harvested on 28 August 1998 from around roots of oaks growing in pots

	Oak shoots flushing	Oaks shoots not flushing
Microbial biomass carbon* (μg C/g_{soil})	237±20	301±8
Net mineralization rate (mg N/g_{soil})	0.027±0.004	0.028±0.005

* Significantly different at $P<0.05$; see Table 2

Table 2 Analysis of covariance for microbial biomass in harvested rhizosphere soil

Source of variation	*df*	SS	MS	*F*s	*F* 0.05 [1, 6]
Adjusted means	1	9,788	9,788	7.67	5.99
Error	6	7,661	1,277		

characteristics of the flushing and non-flushing oaks we used. However, during our analysis, we did take into account tree size, another likely influence on microbial biomass, by including total leaf area as a covariate. (Only nine oaks, five flushing and four not flushing, were used in microbial biomass determinations because soil samples from one non-flushing oak were lost during fumigation.) Still we detected higher microbial biomass around roots of oaks whose shoots were not flushing (ANCOVA, with tree size as a covariate, $P<0.05$, Table 2), though flushing oaks tended to have higher leaf area than non-flushing oaks (mean±SE, 2,568±353 cm^2 flushing and 1,868±240 cm^2 non-flushing trees). Net N mineralization rates, however, were identical in the rhizosphere soil around oaks no matter the shoot flush status (Table 1). (Ammonium N was negligible compared with nitrate N; data not shown.) There is a suite of papers in the soils literature addressing the best way to carry out and analyze fumigations, each producing slightly different results (Martens 1995). Our measurements were of soil samples gathered from around oaks growing under identical environmental conditions in well-mixed soil, so we feel confident in our ability to make comparisons between our samples. Given the very different opinions in the literature about the exact quantification of microbial biomass, we feel less confident about the absolute magnitude of our microbial biomass numbers, but this absolute magnitude, while necessary, for example, in modeling efforts, is not as important in our comparative assays.

Garden experiment

During summer 1999, we expanded our investigation to include ten trees planted directly into soil. For this garden experiment, actual, not relative, soil respiration rates are shown, normalized to 20°C to allow comparison through time (Fig. 4). Note that these data were taken from rings partially buried in soil. Because we could not know where roots were in the soil, rings were likely positioned over different amounts of root biomass from oak to oak. However, rings were left in place throughout

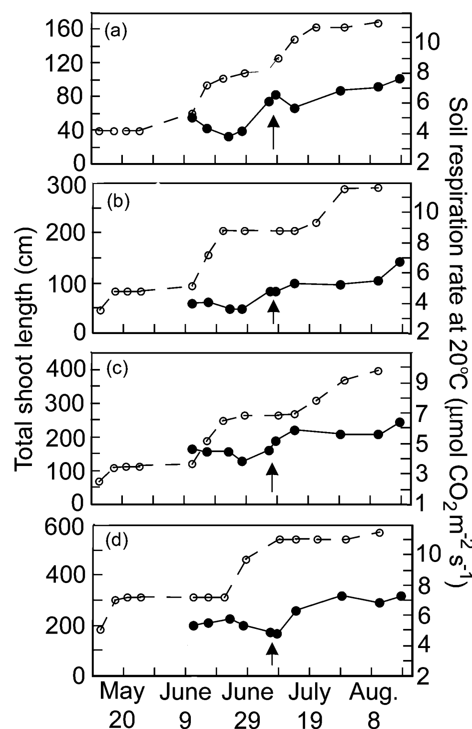

Fig. 4 Time courses of total shoot length (*clear circles*) and soil respiration rates (*filled circles*) for oaks rooted in soil. Results from four individual oaks, flushing with slightly different timing, are shown. *Arrows* indicate data taken on 6 July 1999, during or after shoot flushes in the various oaks; see text for discussion of data from this date

the experiment, so soil respiration from any one ring could be followed through the season to see whether variation correlated with shoot flush stage could be detected for each oak.

These oaks did not flush simultaneously, so results from four individual oaks, selected for their varied flush timing, are shown in Fig. 4. Given that leaf expansion continues for several days after shoot extension finishes in this species (Fig. 1b, c), Fig. 4 shows that soil respiration rates were lowest as leaves expanded during the final stages of shoot extension. For example, arrows mark data taken in early July 1999, and emphasize differences in flush timing for different oaks. Figure 4a shows soil respiration peaking on 6–8 July at the end of a shoot lag phase for one oak, just before shoot extension begins anew. Figure 4b, c shows soil respiration increasing through 6–8 July peaking later just before a new shoot flush. Figure 4c shows low soil respiration on 6–8 July during maximum leaf expansion at the end of the shoot extension phase.

Discussion

Variations in shoot/root carbon allocation have been well documented in a number of tree species at diurnal, phenological, and seasonal time scales (e.g. Dickson 1987, 1991b; Gower et al. 1995; Isebrands and Nelson 1983; Reich et al. 1980). These known allocation patterns may have implications for rhizosphere microbial (including mycorrhizal) activity and for ecosystem processes. We took advantage of the striking carbon allocation switches during a single growing season in northern red oak saplings to begin investigating this possibility.

The patterns shown in Figs. 2, 3 and 4 match those expected if photosynthate is preferentially sent to roots during the shoot lag phase (when leaves are expanded and mature), stimulating below-ground activity. When new shoots were flushing and leaves were expanding, photosynthetic rates tended to be high, and soil respiration rates were low. When leaves had matured and shoots had entered a resting phase, soil respiration increased and photosynthesis tended to decrease slightly (though the high photosynthetic rate of late flush 2 oaks during the early season, Fig. 2, was higher than might be expected in this scheme). Interestingly, a slight increase in photosynthesis during the shoot flush phase (also noted by Hanson et al. 1988) may suggest that the expanding shoot is a stronger sink for current photosynthate than the below-ground root and mycorrhizal system expanding between shoot flushes.

Increased soil respiration during the shoot lag phase could have been caused simply by an increase in root activity and/or biomass, but it may also have reflected increased microbial respiration associated with larger microbial biomass. To test this possibility, we measured microbial biomass by chloroform fumigation incubation. This measure reports biomass of free-living microbial inhabitants of the rhizosphere as well as mycorrhizal hyphae inevitably loosened from the root system during harvest. We measured increased microbial biomass around roots of oaks that were sending the majority of photosynthate below-ground during a shoot lag period (Tables 1, 2, $P<0.05$). Increased shoot to root carbon allocation may have increased carbon available to support ectomycorrhizal biomass and activity. Also, though we did not directly measure rhizodeposition, the active root growth typical in red oaks between shoot flushes may have increased rhizodeposition in the form of mucilage production and root cell sloughing. Any microbes in the rhizosphere, free-living or mycorrhizal, could benefit from this organic carbon flux if sufficient mineral nutrients were present.

We did not detect significant differences in net N mineralization in the rhizosphere soil of groups of oaks in the shoot flush and shoot lag stages (Table 1). Since net N mineralization is the balance of gross mineralization and immobilization, a constant net mineralization rate can result either from no change in gross mineralization and microbial immobilization rates, or from balanced changes in microbial gross mineralization and immobilization rates. These two possibilities can be quite different from the point of view of nutrient cycling and nutrient availability to the plant. Their implications would also be quite different depending on whether the observed increase in microbial biomass stemmed from increased ectomycorrhizal hyphae, increased free-living microbial biomass, or both.

Considering free-living microbes first, with no change in gross mineralization and immobilization, the active pool of potentially plant-available mineral nutrients in soil solution and microbial biomass remains unchanged. If, however, gross mineralization of organic matter increases, though immobilization must also increase to produce the observed constant net mineralization change, nutrients are being moved from a less active SOM pool to an active microbial pool. A mechanism, either abiotic or biotic, eventually promoting turnover of the increased microbial biomass could enhance the availability of nutrients in dead microbes for plant root uptake as the next flush of shoot extension and leaf expansion begins (e.g. Clarholm 1985). We speculate that the oak's internal reallocation of photosynthate away from roots to developing shoots (to support a subsequent shoot flush) could provide such a mechanism by limiting supply of labile carbon from roots to soil. A boom of microbial growth associated with root proliferation could be followed by a microbial population bust (or a slowing of microbial growth) as the carbon flow to roots, and the rhizodeposition simply associated with root growth through soil, decreases during full shoot flush. Detecting such rhythms in boom and bust of free-living microbes, and any associated changes in nutrient availability to plant roots, will require a number of destructive harvests spaced throughout the shoot flush and lag cycle of multiple oaks, and separation of changes in mycorrhizal hyphal biomass from biomass of free living microbes. A major difference, however, between mycorrhizal proliferation and proliferation of free-living microbes around roots is that mycorrhizal proliferation could provide immediate nutrient benefit for the plant. No ectomycorrhizal turnover is necessary for nutrients gathered by hyphae from decomposition of SOM to be made available to roots. Whether mycorrhizal hyphae or free-living microbes proliferated in our experiment, the question still remains whether gross mineralization and immobilization remained unchanged around roots of oaks with different shoot flush status, or simply changed in parallel.

At the organismal scale, our data from potted and soil-grown young oaks suggest that endogenous rhythms of cyclic root/shoot allocation were linked with cyclic changes in soil respiration, as well as altered microbial biomass in the rhizosphere. At the ecosystem scale, the potential for variations in internal carbon allocation of dominant plant species to influence soil respiration, and thus carbon cycling, has not been thoroughly explored, though the effects of longer-term seasonal dynamics of root production and senescence on soil respiration rates have been recognized

(Singh and Gupta 1977). A focus on shorter time-scales is developing. Recently, Johnson et al. (2002) showed, using carbon isotopic techniques, that newly fixed photosynthate can move from roots to mycorrhizal hyphae easily within half a day in the field, and that environmental conditions enhancing photosynthesis also enhance the flux to fungal mycelium. Respiration of the labeled carbon from soils can also occur quickly, completing a rapid pathway for carbon cycling through plants, to soil microorganisms, and back to the atmosphere. Also, in a recent paper based on data taken at the Duke FACE site, Luo et al. (2001) emphasized that accurately modeling soil respiration in the loblolly pine plantation ecosystem required biotic drivers, including shoot phenology, as well as abiotic drivers such as soil temperature and moisture. Our results suggest that the synchronicity with which dominant plants in an ecosystem allocate carbon below-ground will be key in determining whether local variations in soil respiration rate around roots of individual plants translate into larger ecosystem fluctuations.

It remains to be seen under what circumstances species-specific variation in carbon allocation below-ground (including, but not limited to, fine root growth and senescence, e.g. Zak and Pregitzer 1998) can influence bulk soil processes at the ecosystem level. At either organismal or ecosystem scales, given that plants have evolved with microbial populations in soils around their roots, our challenge now is to understand internal root/shoot allocation patterns not as if plants function as isolated organisms, but, instead, as they function with their roots in the dynamic soil arena.

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