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Belowground carbon dynamics in loblolly pine (*Pinus taeda*) immediately following diammonium phosphate fertilization

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Summary Forest soils store an immense quantity of labile carbon (C) and a may be a large potential sink for atmospheric C. Forest management practices such as fertilization may enhance overall C storage in soils, yet changes in physiological processes following nutrient amendments have not been widely investigated. We intensively monitored belowground C dynamics for nearly 200 days following diammonium phosphate fertilization of pot-grown loblolly pine (*Pinus taeda* L.) seedlings in an effort to examine the short-term effects of fertilization on processes involved in soil C sequestration. Soil respiration rates initially increased in fertilized pots relative to controls, followed by a brief reversal in this trend and then a final sustained pattern of elevated rates of soil respiration in the fertilized treatment. Patterns in soil respiration rates over time reflected changes in autotrophic (root) and heterotrophic (microbial) components of soil respiration. Root respiration rates were greater in the fertilized treatment 49 days following fertilization and returned to control rates by the end of the study. In contrast, microbial respiration rates and microbial activity per soil C concentration remained depressed over the same time period. Compared with control seedlings, total root biomass was 27% greater in fertilized seedlings harvested at the end of the study, indicating that the elevated soil respiration rates observed toward the end of the study were a result of increased respiring root biomass. We conclude that fertilization, at least over the short-term, may increase soil C sequestration by increasing belowground biomass production and reducing microbial driven C turnover.

Keywords: autotrophic respiration, heterotrophic respiration, microbial respiration, root respiration, soil CO_2 efflux, soil respiration.

Introduction

Short-term changes in belowground carbon (C) dynamics associated with fertilization have not been intensively examined in loblolly pine (*Pinus taeda* L.). Yet in terms of C sequestration, belowground C storage may account for over 70% of the total C stored in forest ecosystems (Schlesinger et al. 2000). Common forest management practices, such as fertilization, may shift the C balance and enhance the total amount of C stored both above- and belowground (Valentini et al. 2000). Although aboveground C-biomass management has been of prime importance to forest managers for decades, the potential implementation of C credits may present opportunities for managing C storage belowground. Thus, there is increasing interest in maximizing ecosystem C sink and storage strength (Woodwell et al. 1983, Turner et al. 1995, Field and Fung 1999, Banfield et al. 2002, Liski et al. 2002). A detailed knowledge of how components of the belowground C cycle are altered when fertilization occurs will allow for greater understanding of the potential for enhancing belowground C storage through fertilization.

Generally, soil microbial respiration, or heterotrophic respiration, declines shortly after mineral nutrient additions (Kowalenko et al. 1978, Söderström et al. 1983, Smolander et al. 1994, Lovell and Hatch 1998, Thirukkumaran and Parkinson 2000). Root respiration per unit area, or autotrophic respiration, on the other hand, frequently increases in response to fertilization (Zogg et al. 1996, Griffin et al. 1997, Lu et al. 1998). Several studies have shown that fertilization has no effect on soil respiration, the sum of autotrophic and heterotrophic respiration (Castro et al. 1994, Vose et al. 1995, Maier and Kress 2000), indicating that any decrease in microbial respiration could be offset by increases in root respiration. Other investigators report reductions in soil respiration following fertilization (Haynes and Gower 1995, Butnor et al. 2003). If fertilization has no effect or suppresses microbial-driven C turnover, net belowground C sequestration may be enhanced when belowground biomass increases in response to fertilization. Increases in belowground biomass associated with fertilization have been demonstrated in loblolly pine (Albaugh et al. 1998, King et al. 1999).

Although the literature offers insight into the likely changes in belowground C dynamics following fertilization, there has been little investigation into autotrophic, heterotrophic and total soil respiration simultaneously. In this study, we inten-

sively examined changes in belowground C dynamics for over 190 days following diammonium phosphate (DAP) fertilization of potted loblolly pine (*Pinus taeda* L.). We monitored root and microbial respiration, and examined changes in belowground biomass at the end of the study. We hypothesized that soil respiration would reflect a changing balance between root respiration and microbial respiration following fertilization, initially increasing as a result of increased root respiration per unit area, then decreasing because of suppressed microbial respiration rates. We expected that, over the long-term, soil respiration rates would increase in the fertilized pots relative to the controls because respiring root biomass would increase over time.

Materials and methods

Plant material, growing medium and study design

Forty container-grown, 1-year-old, half-sibling loblolly pine seedlings (family: WV-3; Meade Westvaco, Summerville, SC) were transplanted to 29-liter pots (dimensions: 36 × 29 × 28 cm) containing about 24 liters of sieved (6.4 mm screen) and well-mixed sandy, siliceous, thermic Psammentic Hapludult soil of the Wakulla series (USDA Forest Service, unpublished data), which was collected from the Forest Service's Southeastern Tree Research and Education Site located in Scotland County, North Carolina (35° N, 79° W). The soil is low in native fertility (Albaugh et al. 1998). A low dose of fertilizer in the form 20:20:20 N,P,K was applied at the outset of the experiment at a rate equivalent to 15 kg ha^{-1} of each of the following: elemental nitrogen (N), P_2O_5 and K_2O. The potted seedlings were randomly assigned to pairs, which were placed side by side on a greenhouse bench, each pair serving as an experimental block.

Growth conditions

The greenhouse in which the plants were grown provided supplementary illumination extending the daily photoperiod to 16 h. The containers were watered frequently, but leaching was avoided.

Fertilizer treatment

About 1 year after seedlings had been transplanted, they were randomly assigned (within blocks) to either the fertilized or control treatments. Each seedling assigned to the fertilized treatment received 1.85 g DAP (0.35 g N and 0.37 g phosphorous (P)), which is equivalent to 280 kg DAP ha^{-1} or 50 kg N and 106 kg P ha^{-1}, the recommended rate for nursery-grown loblolly pine seedlings (Jokela and Long 1999).

Soil respiration measurements

Soil carbon dioxide (CO_2) efflux was measured over a 190-day period with a Li-Cor LI-6200 infrared gas analyzer (IRGA) (Li-Cor, Lincoln, NE) equipped with a dynamic closed cuvette chamber system (Janssens et al. 2000). The chamber was created by placing a plastic trash can lid over the measurement pot. A hole in the center and an incision from the center hole to the perimeter made it possible to seal the trash can lid around the stem of the seedling with insulating foam. Foam insulation around the perimeter of the plant pot ensured a good seal. The total chamber headspace was approximately 12 liters and was the sum of the volume above the soil and below the rim of the pot (5 l) and the volume of the chamber itself (7 l). A gas sampling and a return-air port allowed air to be circulated from the chamber through the IRGA. The chamber isolated the soil and the entire seedling root system, thereby trapping CO_2 emitted by microbes and roots. Soil CO_2 efflux rates were determined by measuring change in CO_2 concentration (ΔC) over a 30-s period, from which respiration rate per unit ground area was calculated:

$$\text{Soil } CO_2 \text{ efflux} = \frac{(\Delta C/\Delta t)(PV_1/RT)}{S} \quad (1)$$

where t is time, P is atmospheric pressure, V_1 is the system volume, R is the universal gas constant, T is temperature and S is the surface area of the soil.

Soil respiration rates were measured in the same sequential blocking order during every measurement period. The IRGA was zeroed before each sampling period.

Root respiration

Root respiration was measured 47 and 197 days after fertilization on a sample of detached fine roots from each pot with the Li-Cor LI-6200 equipped with a 0.25-l cuvette chamber. An area of about 10 cm^2 of soil was gently excavated to not more than a 5 cm depth and fine roots (< 2 mm) were removed. Shaken free of soil, the roots were placed in the cuvette on a moist paper towel. Respiration was measured over 30-s as described above. Root surface area was determined with WinRhizo 5.0A software (Regent Instruments, Québec, Canada) and all measurements were expressed on a per unit root surface area basis.

Microbial respiration

Microbial respiration rates were measured 49 and 197 days after fertilization on a sample of detached fine roots from each pot with a Li-Cor LI-6200 equipped with a 0.25-l leaf cuvette chamber. A push tube (2.5 cm diameter) was used to extract three soil core samples from each pot over the full vertical soil profile. The three samples were combined resulting in an approximate total sample volume of 350 cm^3 per pot. All roots were removed and the soil was mixed and placed in an open aluminum weighing boat (10 × 2 cm) that was immediately positioned in the leaf cuvette chamber. After respiration measurements, soil was oven-dried for 2 days at 65 °C and reweighed. Microbial respiration measurements were expressed on a soil mass basis. To provide an index of microbial activity in relation to soil C, we compared absolute and relative microbial activity (i.e., soil respiration) per gram of soil C (Nohrstedt et al. 1989). Soil CO_2 efflux was calculated as described previously.

Soil carbon percent

Carbon percent was determined for soil collected during microbial respiration measurements. Samples were analyzed for

C percent by the USDA Forest Service Southern Research Station laboratory (RTP, NC) with a Carlo-Erba elemental analyzer (Model NA 1500, Carlo Erba/Thermo Electron, Milan, Italy).

Root biomass measurements

Seedlings were harvested 211 days after fertilization. Roots were washed to remove soil. Organs were oven-dried at 65 °C for 1 week and weighed to determine absolute and relative dry biomass.

Statistical analysis

Effects of fertilization on soil respiration, specific root respiration, specific soil respiration and soil C percent were examined by a repeated measures analysis of variance (ANOVA). Multiple comparisons of means at $\alpha = 0.05$ were made with Tukey's HSD test. Effects of fertilization on root and aboveground biomass were subjected to analysis of variance based on a randomized complete block design. Statistical analyses were performed with the SAS statistical software package (SAS, Cary, NC).

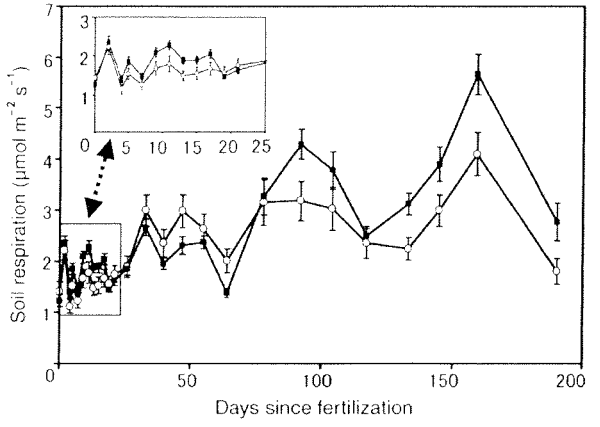

Figure 1. Soil respiration in containers with either fertilized (■) or non-fertilized (○) loblolly pine seedlings. Seedlings were grown in 29-l pots in sandy, infertile soil. Fertilized seedlings received 1.85 g diammonium phosphate (DAP) per pot (280 kg DAP ha^{-1}). The inset shows soil respiration rates during the first 25 days following fertilization. Error bars show 1 standard error from the mean; $n = 20$.

Results

Soil respiration measurements

Soil respiration rates were significantly greater ($P < 0.05$) in the fertilized pots than in the controls beginning 4 days after fertilizer application (Figure 1). This trend persisted for 13 days, followed by a reversal in which non-fertilized pots had significantly higher soil respiration rates on days 40, 47 and 64 ($P < 0.05$). Soil respiration rates became significantly greater in the fertilized pots 90 days following the initial fertilization ($P < 0.05$) and this trend persisted for the remainder of the study. Soil respiration rates among measurement dates ranged from about 1 to 6 µmol m^{-2} s^{-1}. This range likely reflects changes in soil temperature over the study along with increasing root biomass and alterations in microbial activity, making quantitative comparisons between dates less meaningful.

Root respiration and microbial respiration

Forty-nine days after fertilization, root respiration rates were 32% higher in fertilized seedlings than in controls ($P < 0.05$; Figure 2). No significant difference between treatments was apparent 197 days following fertilization. Microbial respiration rates were significantly lower in the fertilized pots both at 49 and 197 ($P < 0.05$) days after fertilization, with mean rates 42 and 32% less than the controls, respectively.

Percent soil carbon and microbial activity

Percent soil C (dry mass) from samples collected concurrently with microbial respiration measurements were not significantly different between the two dates or treatments (Table 1) and ranged from 0.48 to 0.73%. Microbial activity per gram of soil C was significantly lower in fertilized pots than in control pots on both sampling dates ($P < 0.05$; Table 1), reaching 44 and 66% of the maximum observed in the non-fertilized treat-

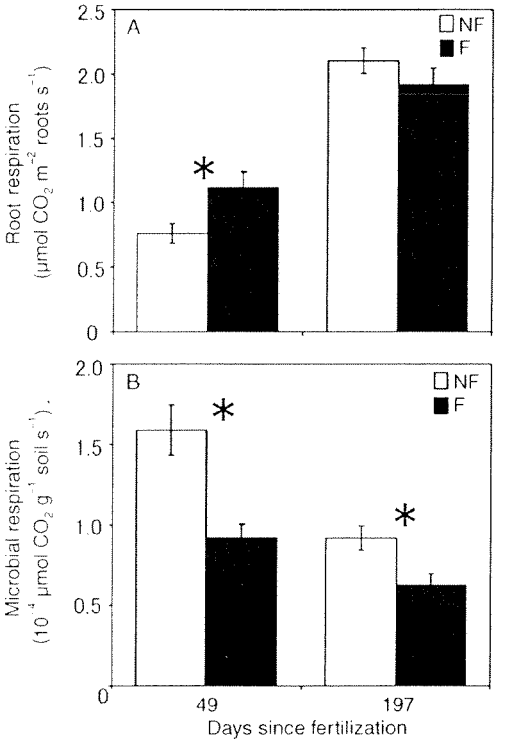

Figure 2. Mean root respiration rate per unit surface area (A) and mean microbial respiration rate (B) in non-fertilized (NF) and fertilized (F) loblolly pine on two dates. Asterisks above bars indicate significant differences between treatment means on the given measurement date, as determined by Tukey's HSD ($\alpha = 0.05$) following repeated measures ANOVA. Error bars show 1 standard error from the mean; $n = 20$.

ments 49 and 197 days following fertilization, respectively. Comparisons were made in relative terms because absolute microbial activity is greatly influenced by environmental conditions such as temperature.

Root biomass measurements

Mean belowground dry biomass of fertilized seedlings was 27% greater (74.2 g) than that of control seedlings (54.1 g) (Figure 3; $P < 0.05$). Fertilized seedlings had 40% (210.2 versus 126.8 g) greater aboveground biomass at harvest than controls (Figure 3; $P < 0.05$).

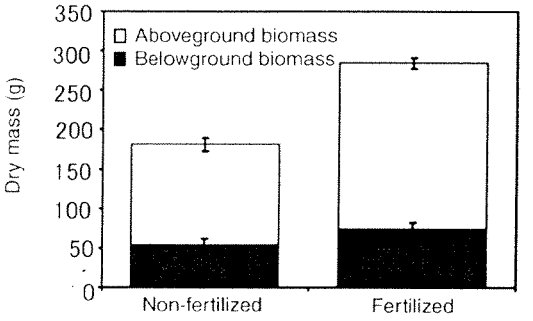

Figure 3. Aboveground and belowground dry biomass in fertilized and non-fertilized loblolly pine seedlings 207 days after treatment application. Seedlings were grown in 56.8-l pots in sandy, infertile soil. Fertilized seedlings received 1.85 g diammonium phosphate (DAP) per pot, which is equivalent to 280 kg DAP ha^{-1}. Dry biomass differences between treatments were significant ($P < 0.05$) for both above- and belowground tissues. Error bars show 1 standard error from the mean; $n = 20$.

Discussion

Effects of fertilization on microbial respiration

The reduction in microbial respiration rates that we observed in response to fertilization is supported by several previous reports showing both short- and long-term reductions in microbial activity following nitrogen (N) additions. Thirukkumaran and Parkinson (2000) monitored microbial respiration rates in the laboratory from soil collected in a lodgepole pine (*Pinus contorta* Dougl.) forest over a 120-day period. They determined that ammonium nitrate and urea additions (of 188 and 300 kg N ha^{-1}, respectively) decreased microbial respiration, and speculated that osmotic changes or ammonium toxicity were responsible. Microbial respiration was temporarily reduced over the short-term (5 weeks) when grazed *Lolium perenne* L. received 200 kg N ha^{-1} in the form of ammonium nitrate (Lovell and Hatch 1998). Similarly, Gorissen and Cotrufo (2000) reported a reduction in decomposition of leaf and root tissues of three perennial grasses for 222 days following ammonium nitrate (270 kg N ha^{-1}) fertilization, that may have been caused by a reduction in microbial biomass. Numerous studies have linked reductions in microbial respiration following N fertilization with both a reduction in microbial biomass (Söderström et al. 1983, Nohrstedt et al. 1989, Thirukkumaran and Parkinson 2000) and a decline in metabolic activity per unit microbial biomass (Thirukkumaran and Parkinson 2000). Fertilization has also been shown to impact microbial respiration over the long-term, reducing activity for up to 5 years (Kowalenko et al. 1978, Söderström et al. 1983, Smolander et al. 1994). The duration and extent of the effect of N on long-term soil C storage deserves attention.

In contrast to our findings, some studies have reported an increase in microbial activity following N fertilization, primarily when nutrient-deficient soils are amended with N. Gallardo et al. (1994) reported an increase in microbial respiration in temperate deciduous forest soils fertilized with ammonium nitrate. Kelly and Henderson (1978) reported increased decomposition following urea additions to a temperate hardwood forest floor.

The variable response of microbes to N additions may be explained partially by differences in substrate quality and existing soil nutrition prior to fertilization. Litter decomposition rates have been shown repeatedly to be greater when C/N ratios are lower (Cotrufo et al. 1998, De Angelis et al. 2000), suggesting that N fertilization may enhance decomposition by shifting C/N ratios downward. Soil from the current study was collected from a nutrient-poor N-limited site (Albaugh et al. 1998). Although we did not measure C/N of our soils, C/N likely decreased following fertilization, yet we observed a reduction rather than an increase in microbial activity per unit C. Our results are consistent with findings by Nohrstedt et al. (1989), who reported reduced microbial respiration per gram of soil C in Swedish Scots pine (*Pinus sylvestris* L.) forests

Table 1. Mean ± SE percent soil carbon (C) and microbial respiration rate per gram of soil C in non-fertilized (NF) and fertilized (F) seedlings 49 and 197 days after treatment application. Superscript letters represent multiple comparisons grouping (Tukey's HSD, $\alpha = 0.05$, $n = 20$).

Day	Treatment	Soil C (%)	Microbial respiration per gram of soil C	
			Absolute (10^{-4} μmol CO_2 g^{-1} soil C s^{-1})	Relative to maximum (%)
49	NF	0.55 ± 0.028 a	2.88 ± 0.238 a	100 ± 8.25 a
	F	0.73 ± 0.085 a	1.27 ± 0.113	44 ± 8.90 c
197	NF	0.48 ± 0.013 a	1.90 ± 0.117 ab	100 ± 6.14 a
	F	0.50 ± 0.016 a	1.26 ± 0.096	66 ± 7.68 b

that received two N additions in the form or urea or ammonium nitrate twice over a 7-year period. Nohrstedt and colleagues concluded that increased soil C content in the fertilized plots was a result of reduced microbial C turnover.

In contrast to N fertilization, P has generally been shown to enhance microbial respiration. Dilly (1999) reported an initial increase in microbial activity in forest and field mineral soils amended with P. Cotrufo et al. (1999) attributed variable rates of downy oak (*Quercus pubescens* Willd.) litter decomposition to P concentrations in the native litter. The addition of sodium phosphate resulted in a reduction in microbial biomass in temperate deciduous forest soils (Gallardo and Schlesinger 1994). Also, Kelly and Henderson (1978) reported a reduction in deciduous litter decomposition following high rates of P fertilization (275 and 550 kg P ha^{-1}).

Effects of fertilization on root respiration

Our data are consistent with previous reports indicating that N and P fertilization increase root respiration. In 155-day-old loblolly pine and ponderosa pine (*Pinus ponderosa* Dougl. ex Laws.) seedlings, above-optimal ammonium additions increased specific root CO_2 efflux rates (Griffin et al. 1997). These authors concluded that root respiration was primarily driven by N uptake and detoxification of ammonia rather than growth, because growth was stunted in plants receiving high N. Maier and Kress (2000) found that elevated root respiration in response to N may depend on root size in loblolly pine, citing an increase in coarse root respiration, but no increase in fine root respiration in response to N. In Douglas-fir (*Pseudotsuga menziesii* Franco) seedlings grown in root boxes, specific root respiration and total root respiration increased with enhanced N (Lu et al. 1998). Zogg et al. (1996) determined that N fertilization did not significantly alter specific fine root respiration rates in sugar maple (*Acer saccharum* Marsh.); however, total root respiration was highly correlated with root tissue N concentration. Although the direct impact of P fertilization on root respiration has not been as extensively reported, a positive relationship between P fertilization and root respiration has been observed. For example, Conjeaud et al. (1996) reported a positive relationship between root tissue P content and root respiration in maritime pine (*Pinus pinaster* Ait.), and others have cited similar findings in herbaceous species (Klock et al. 1997, Hansen and Lynch 1998).

There are several potential reasons for increased specific root respiration in response to N and P fertilization. High metabolic (i.e., respiratory) costs are associated with the conversion of NO_3^- to NO_2^- by nitrate reductase and the subsequent conversion of NO_2^- to NH_4^+ by nitrite reductase (Zogg et al. 1996). Also, growth respiration costs of incorporating NH_4^+ into amino acids are significant (Vessey and Layzell 1987). Furthermore, maintenance costs increase with increasing protein concentration. When N additions increase whole-plant biomass, total maintenance respiration costs also increase (Ryan 1991). Similarly, reports suggest that P uptake is metabolically demanding, relying on active transport and expenditure of ATP (Kochian 2000).

Effects of fertilization on soil respiration

Soil respiration during the latter half of our 190-day experiment was greater in fertilized pots relative to the controls as a result of an increase in total respiring root biomass. Previous investigators found increases in loblolly pine seedling root biomass following fertilization (Gebauer et al. 1996, Griffin et al. 1997). We observed a continued depression in microbial respiration rates over the study period along with the return of root respiration rates per unit area to control values, indicating that the increased root biomass associated with fertilization is responsible for the enhanced soil respiration observed during the second half of the study. Therefore, despite greater soil respiration rates, there should be a long-term accumulation of belowground C following fertilization if microbial rates remain depressed and belowground root growth remains enhanced in fertilized plants.

Our results also indicate that the timing of measurements may be important in the detection of effects of fertilizer on soil respiration, because we observed a reduction, an increase, and no difference in soil respiration rates following fertilization at different times during the study. Transient soil respiration patterns following fertilization over the short-term reflected relatively rapid changes in the contributions of autotrophic and heterotrophic components to total soil CO_2 efflux. Some authors report that soil respiration remains unchanged in response to fertilization, which may be the result of a simultaneous decrease in microbial respiration and an increase in total root respiration. Maier and Kress (2000) measured soil respiration in 11-year-old loblolly pine stands fertilized for 4 years and found that soil CO_2 evolution was typically the same as rates recorded in control stands with low native fertility. Similarly, Castro et al. (1994) found that 4 years of urea-N fertilization in mature slash pine (*Pinus elliottii* Engelm.) forests did not result in changes in soil CO_2 evolution. No changes in soil respiration were detected in 3-year-old ponderosa pine fertilized with 10 and 20 g m^{-2} of ammonium sulfate (Vose et al. 1995). These authors concluded that the lack of response to N might be associated with the counteracting effects of autotrophic and heterotrophic respiration. In our study, soil respiration rates did not differ significantly between treatments for a short period centered around 25 days following fertilization. Shifts in microbial and root respiration following fertilization indicated that opposing changes in the two soil respiration components resulted in no detectable difference in soil CO_2 efflux for a brief period. In particular, we found that root surface area expressed and total pot root respiration rates were elevated whereas microbial respiration rates were depressed.

Other studies report an increase or decrease in soil respiration rate when soils are fertilized. Johnson et al. (1994) reported an increase in soil respiration rates in response to N fertilization of chamber-grown ponderosa pine seedlings and attributed the increases to increased respiring root biomass and enhanced specific root respiration immediately following fertilization. In contrast to our findings, however, Johnson et al. (1994) observed no reduction in microbial respiration following fertilization, although they detected an increase in soil respiration beginning six months following nutrient applications, which is consistent with our observations. Similarly, Gallardo

and Schlesinger (1994) reported an increase in soil respiration persisting for several months following N and P fertilization of a temperate deciduous forest. In contrast, Butnor et al. (2003) reported a reduction in soil respiration following annual fertilizations (11.2 g N m^{-2}) for 3 years in loblolly pine stands in the North Carolina piedmont. Haynes and Gower (1995) observed decreased soil respiration in continuously fertilized red pine (*Pinus resinosa* Ait.) stands and attributed the reductions to decreased root or mycorrhizal growth or respiration rather than to a reduction in bacterial respiration. Soil respiration rates of a eucalypt forest (*Eucalyptus pauciflora* Sieber) remained depressed for 8 months following P fertilization (103.5 kg P ha^{-1}) (Keith et al. 1997). The relationship between soil respiration and fertilization frequency and intensity remains uncertain and, along with measurement timing following fertilization application, may explain some of the inconsistencies among reports in the literature. In the context of management, it is unclear whether repeated fertilization provides greater suppression of microbial activity, and therefore soil respiration, than a single fertilizer event.

Summary of short-term effects of fertilization

Fertilization with N and P may have the dual effect of increasing belowground biomass production and reducing soil C turnover thereby enhancing soil C sequestration. If fertilization has no effect or if it suppresses soil C turnover through a reduction in microbial activity, net belowground C sequestration will be enhanced when belowground biomass increases in fertilized stands (Figure 4). Our results demonstrate the transient nature of increased soil C efflux following fertilization and further exemplify the need to simultaneously monitor the heterotrophic and autotrophic components of the C cycle over time. Long-term studies will be crucial in understanding whether common forest management practices such as fertilization allow for soil C conservation or even increases in soil C capture and storage over typical rotation lengths.

Acknowledgments

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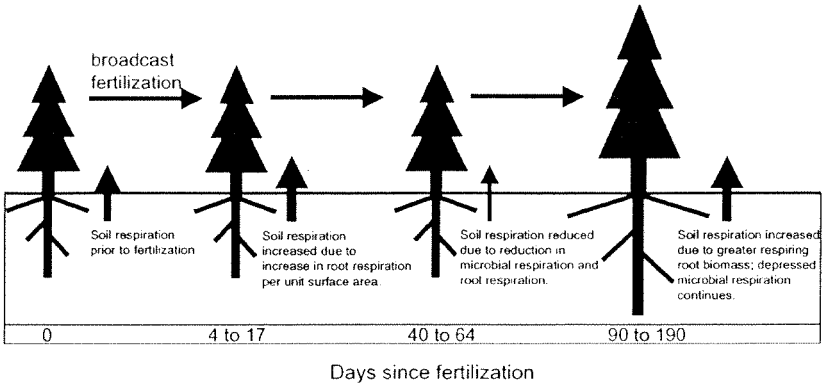

Figure 4. Belowground changes in carbon (C) flux following diammonium phosphate fertilization. Fertilization increased belowground C storage over the short-term by reducing microbial activity and simultaneously enhancing belowground biomass production. The timeline is based on observations summarized in Figures 1, 2 and 3. Note that, although soil respiration rate is greater than the pre-fertilization rates over the longer-term, overall belowground C sequestration increased because C efflux was primarily a result of root respiration rather than microbial-mediated C turnover.

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