Tree Physiology 27, 133–140

Physiological and phenological responses of oak seedlings to oak forest soil in the absence of trees

IAN A. DICKIE,[1,2,4] REBECCA A. MONTGOMERY,[1] PETER B. REICH[1] and STEFAN A. SCHNITZER[1,3]

[1] *University of Minnesota, Department of Forest Resources, St. Paul, MN, USA*

[2] *Present address: Landcare Research, Lincoln, New Zealand*

[3] *Present address: University of Wisconsin – Milwaukee, Department of Biological Sciences, Milwaukee, WI, USA*

[4] *Corresponding author (dickiei@landcareresearch.co.nz)*

Received February 13, 2006; accepted April 18, 2006; published online October 2, 2006

Summary Established trees influence the growth and physiology of seedlings by altering above- and belowground conditions; however, tree influences on seedling physiology via belowground interactions are not well understood. We used soil transfers to an open field to examine the belowground influences of a *Quercus ellipsoidalis* E.J.Hill dominated forest on *Q. ellipsoidalis* seedling mycorrhizal infection, nutrient uptake, growth and photosynthesis over three years. After two years, seedlings planted with large quantities of forest soil (HF treatment) had greater leaf mass and foliar N concentrations than seedlings receiving smaller quantities of forest soil (LF) and control treatments. Mycorrhizal infection was greater in the HF treatment after one year compared with the LF and control treatments, with a positive correlation of foliar N and mycorrhizal infection in Year 2. There were marked effects of treatments on seedling spring phenology with HF seedlings breaking bud up to 17 days earlier than seedlings in the other treatments. The HF seedlings also had more rapid leaf expansion and larger leaves, and an increase in net photosynthetic rates. These results highlight complex linkages between above- and belowground physiology: forest soil had substantial effects on seedling physiology, including traits such as phenology that have previously been considered to be under aboveground control. Belowground influences of trees on conspecific seedlings may play a critical role in early seedling establishment.

Keywords: mycorrhiza, phenology, photosynthesis, Quercus ellipsoidalis, tree-seedling interactions.

Introduction

The presence of trees is one of the most important factors influencing the establishment, survival, growth and physiology of seedlings. Tree–seedling interactions influence succession (Pickett et al. 1987), invasion (Callaway et al. 2004, Reinhart and Callaway 2004), diversity (Janzen 1970, Hacker and Gaines 1997) and ecosystem resilience and stability (Perry et al. 1989). Aboveground, trees cast shade, moderate environmental extremes and otherwise alter microenvironments experienced by seedlings (Berkowitz et al. 1995, Finzi and Canham 2000, Danner and Knapp 2001). Trees also modify the belowground environment experienced by seedlings: directly competing with seedlings for nutrients and water (Anderson et al. 2001, Platt et al. 2004, Barberis and Tanner 2005), and indirectly affecting seedlings by altering the biotic and abiotic belowground environment. These influences include increasing the local abundance of soil pathogens (Packer and Clay 2000, Packer and Clay 2003) and mutualists such as mycorrhizal fungi (Haskins and Gehring 2004, Dickie and Reich 2005, Nara 2005) and modifying soil biogeochemistry (Binkley and Giardina 1998, Finzi et al. 1998, Reich et al. 2005) and water availability (Caldwell et al. 1998, Zou et al. 2005).

Seedlings growing under the forest canopy display marked shifts in physiology compared with seedlings growing in the open. Understory seedlings generally have lower maximum photosynthetic rates and lower leaf N, especially on a mass basis, and higher SLA (Walters and Field 1987, Givnish 1988, Walters et al. 1993, Reich et al. 1998, Montgomery 2004). These responses represent a general down regulation of leaf physiology at low irradiances and a shift toward traits that enhance light capture. In addition, understory seedlings can have phenological shifts, with earlier bud break under forest canopies than in the open (McGee 1975). Early leafing under canopies has been attributed to more moderate temperature regimes and more rapid spring warming of air layers near the forest floor (Augspurger 2004), and can be an important adaptation to maximize carbon gain before canopy leaf out (Harrington et al. 1989, Gill et al. 1998). Seedlings growing near established trees may also have a greater extent of mycorrhizal infection, different communities of root symbionts (both mutualistic and pathogenic) and increased nutrient uptake compared with seedlings growing distant from established trees (Packer and Clay 2000, Packer and Clay 2003, Simard and Durall 2004, Dickie and Reich 2005).

How can we distinguish among the myriad factors that affect the physiology of seedlings growing under canopies? To what extent do above- versus belowground factors influence observed responses? In prior studies, we have planted seedlings near and distant from trees and assumed that seedling responses were due to belowground influences (Dickie et al. 2002, Dickie and Reich 2005, Dickie et al. 2005). Here we take an alternative approach by moving soil from under trees into an open field distant from established trees. Our objective was to understand the role of forest soil in influencing seedling establishment, growth and physiology. In addition to providing a test of our earlier assumptions, this study allowed us to examine the physiological responses of seedlings to forest soil in a replicated, randomized design with seedlings growing under uniform microclimatic conditions.

Methods

Study site and treatments

Our study site was located on an old field abandoned from agriculture in 1958 at the Cedar Creek Natural History Area and Long Term Ecological Research (LTER) site of the University of Minnesota, located in east-central Minnesota, USA (45°24′17″ N, 93°11′16″ W). The current dominant vegetation in this field consists of a mix of agricultural (e.g., *Bromus* spp.) and native prairie grasses (e.g., *Andropogon gerardii* Vitman and *Schizachyrium scoparium* (Michx.) Nash). No trees grow near the area of the field used for this research. Soils are flat, sandy, excessively drained Typic Udipsamment, Nymore series soils (94% sand, 5% silt, 1% clay), formed from glacial outwash sediments (Grigal et al. 1974). The area is currently a mix of oak savanna and old fields of various ages.

We collected soil for all treatments from a mature *Quercus ellipsoidalis* E.J.Hill dominated forest (75 ± 3% *Q. ellipsoidalis*, 16 ± 9% *Q. macrocarpa* Michx., 10 ± 7% other tree species; mean percentage of total basal area and SE) adjacent to Field 57 at the Cedar Creek LTER site and on the same soil type as the study field. We used a spade to excavate the top 200 to 300 mm of soil from four locations about 1 m from the bases of *Q. ellipsoidalis* trees. Soils were homogenized and passed through a 12.5-mm wire screen to remove large clumps of roots, with root clumps rubbed against a screen to maximize the number of fine root fragments (and hence mycorrhiza and pathogens) passing through. Soils were used immediately after collecting, with fresh soil collected from the same locations each year.

We established three treatments: low quantity forest soil (LF), high quantity forest soil (HF) and control. From all plots we removed an approximately 300-mm diameter grass "sod" (roots and soil) and dug a hole about 2 l in volume. Removed soil was mixed with treatment soil (except in controls) and returned to the same hole. Control plots received no forest soil, LF plots received 200 ml of forest soil, and HF plots received 2 l of forest soil. Because of the removal of grass sods, all treatments (including controls) had a small amount of additional field soil added to raise the plot level up to the surrounding soil level. The addition of field soil also reduced any potential differences in albedo. Field soil added to all plots was collected from a large established pit adjacent to the research site and at least 100 m distant from any ectomycorrhizal plant. Immediately after mixing and leveling plot soil, two pre-germinated (with root radicle 0 to 40 mm in length) *Quercus ellipsoidalis* seeds were planted in each plot. Plots were protected from herbivores with 12.5-mm mesh, 300-mm diameter, 900-mm tall galvanized "hardware cloth" cages. The entire field has also been fenced to exclude deer. Seedlings were watered periodically as needed during early establishment, particularly in 2002, which was a very dry year.

Seedlings were planted in 2002, 2003 and 2004 allowing one concurrent harvest of seedlings of ages 1, 2 and 3 years. This concurrent harvest allowed comparison of the response (e.g., leaf expansion or photosynthetic rate) for 2- and 3-year-old seedlings under identical weather conditions, reducing cross-year measurement error. The tradeoff is that there may have been cross-year experimental error in the application of treatments, or in the conditions experienced by seedlings during establishment. We established 45 replicates of each treatment, randomly distributed across a rectangular grid with 1-m spacing between plots. Intermediate harvests (data not shown) and natural mortality reduced this number to 19 replicates per treatment for the 3-year-old seedlings, 15 replicates per treatment for the 2-year-old seedlings, and an average of 15 replicates per treatment for the 1-year-old seedlings (range 14 to 16).

Phenology

On May 16, 2003, during a routine survey of seedling survival, we noted that there was a strong treatment effect on spring leaf flush, with bud break having begun in 17 of the 21 then-extant HF treatment plots (buds swelling or broken), but only one plot in other treatments (in the LF treatment). We therefore immediately commenced measurement of leaf flush, with biweekly categorical evaluation of the most fully expanded bud (typically the apical bud) on each seedling into four categories (bud tight, bud swelling, leaves visible, and full leaf out). In 2004, we continued these measurements on a more systematic basis, taking an exact measurement of the length of the most fully expanded bud or leaf on each seedling on a periodic basis (weekly through bud break, less frequently thereafter until full leaf flush had occurred). For 2003, we used the date that leaves were first recorded as visible as the date of bud break. In 2004, we used the date that collection switched from measuring bud length to leaf length as an indication of bud break. For 2004 data, we were able to construct a time series of bud and leaf expansion rates from early spring when buds were tightly closed until full leaf out. This allowed a quantitative analysis of leafing patterns.

We also measured fall phenology as a categorical response in 2003 (0% color change or leaf loss, < 10%, < 50% and > 50%) on three occasions: October 2, October 16, and October 24. Because seedlings were harvested before natural senescence, we did not measure fall phenology in 2004.

Photosynthesis

We measured photosynthesis on 2-year-old seedlings from the control and HF treatments in 2003 and on 2- and 3-year-old control and HF seedlings in 2004 with a CIRAS-1 portable photosynthesis system differential CO_2/H_2O infrared gas analyzer and a Parkinson broadleaf automatic cuvette (PP systems, Amesbury, MA). Measurements were taken in midmorning on sunny days under ambient daylight conditions (photosynthetically active radiation 881 ± 17 μmol s^{-1} m^{-2}, temperature 28.8 ± 0.3 C, CO_2 in chamber 369.2 ± 1.0 ppm; means and standard errors) on the most fully expanded leaf on each plant with minimal herbivore damage. This meant that early in the season we often measured actively expanding leaves. We measured an average of 10 plants (one leaf per plant) per sample date per treatment. We measured photosynthesis about every 3–4 weeks for a total of six censuses in 2003 (May 27, June 11, July 22, August 6, September 4, October 1). In 2004, because of poor weather conditions and equipment failure, we conducted only three censuses (June 22, July 22 and July 29).

Seedling harvests and quantification

We harvested seedlings in September 2004. Roots were excavated with a spade and washed over a wire screen. Root mass was not measured, as it was impossible to harvest 100% of roots intact because of the depth of root growth into the sandy soils. Fine roots were removed from the coarse roots and stored in water under refrigeration up to four weeks before quantification of percent mycorrhizal infected root tips. Large root systems were subsampled with the goal of counting at least 100 root tips per seedling: our final average was 367 root tips per seedling with only two seedlings having fewer than 100 root tips counted. Mycorrhizal morphotypes were not recorded.

Aboveground tissues were measured and dried for biomass and N concentration measurements. Stem diameter, height and leaf numbers were measured. Leaf area was measured on detached fresh leaves with a Li-Cor LI-3000A portable leaf area meter with a LI-3050A belt conveyor (Li-Cor, Lincoln, NE). Dried tissues were weighed and leaves analyzed for foliar N concentrations on an ECS 4010 element analyzer (Costech Analytical, Valencia, CA) at the University of Nebraska.

Statistics

Biomass, N concentration and ECM infection were analyzed independently for each age group as a CRD ANOVA in R (2.0.1; R Foundation for Statistical Computing, Vienna, Austria). Photosynthesis and bud break dates were analyzed by ANOVA in STATISTICA (6.1; StatSoft, Inc., Tulsa, OK). All seedlings in the 2003 photosynthesis analysis were 2 years old. For the 2004 analysis, we pooled data from the 2- and 3-year-old seedlings because of small sample sizes. Leaf expansion rates (2004) were analyzed with repeated measures ANOVA in STATISTICA. This model tested the effects of treatment, seedling age and date (repeated factor) on length of bud or leaf.

Results

Seedling growth, biomass distribution, and nitrogen concentration

Seedling leaf mass was significantly affected by treatment in all three age groups (Table 1). Age 1 seedlings had signifi-

Table 1. Seedling responses to forest soil at ages 1, 2 and 3 years in control (C), low forest soil (LF) and high forest soil (HF) treatments. Ectomycorrhiza is the percentage of total root tips infected by ectomycorrhizal fungi. Values are means with standard errors in parentheses. Within a row, values followed by different letters differ significantly. The *P* value is for overall significance of treatment effects.

Response	C	LF	HF	*P*
Age 1 year				
Leaf mass (g)	0.27 (0.03)[a]	0.24 (0.03)[ab]	0.18 (0.03)[b]	0.040
Stem mass (g)	0.13 (0.02)[a]	0.13 (0.02)[a]	0.09 (0.01)[b]	0.024
Height (mm)	60.9 (7.6)[ab]	64.4 (0.88)[a]	47.5 (0.65)[b]	0.022
Foliar N (%)	0.88 (0.05)	0.86 (0.05)	1.05 (0.10)	0.098
Ectomycorrhiza (%)	8.0 (1.9)[a]	11.1 (2.6)[ab]	22.0 (5.6)[b]	0.024
Age 2 year				
Leaf mass (g)	0.10 (0.03)[ab]	0.08 (0.02)[a]	0.16 (0.04)[b]	0.031
Stem mass (g)	0.14 (0.02)	0.15 (0.04)	0.12 (0.03)	0.76
Height (mm)	58.7 (8.4)	48.7 (9.3)	49.2 (11.6)	0.80
Foliar N (%)	1.05 (0.13)[ab]	0.91 (0.10)[a]	1.49 (0.15)[b]	0.011
Ectomycorrhiza (%)	36.4 (5.0)	28.4 (6.5)	51.5 (7.9)	0.056
Age 3 year				
Leaf mass (g)	0.12 (0.03)[a]	0.12 (0.05)[a]	0.26 (0.06)[b]	0.010
Stem mass (g)	0.17 (0.03)	0.15 (0.03)	0.19 (0.04)	0.41
Height (mm)	57.3 (7.8)	52.6 (11.0)	58.2 (12.0)	0.67
Foliar N (%)	1.65 (0.12)[a]	1.02 (0.12)[b]	1.55 (0.14)[a]	0.006
Ectomycorrhiza (%)	49.1 (4.3)	51.0 (1.8)	50.1 (4.97)	0.94

cantly lower leaf mass in the HF treatment than in the control treatment ($F_{2,37} = 3.53$, $P = 0.040$). At age 2, leaf mass was significantly higher in the HF treatment than in the LF treatment ($F_{2,31} = 3.91$, $P = 0.031$), and at age 3 leaf mass was higher in the HF treatment than in either of the other treatments ($F_{2,37} = 5.28$, $P = 0.010$). Leaf mass declined noticeably in the control and LF treatments from age 1 to age 2, before increasing slightly from age 2 to age 3. Specific leaf area (SLA) was significantly lower in the HF treatment than the LF treatment at age 1 ($F_{2,37} = 4.32$, $P = 0.021$; not shown), but showed no significant treatment effects in age 2 or age 3 seedlings. Stem mass was greater in the control and LF treatments than the HF treatment at age 1 ($F_{2,37} = 4.11$, $P = 0.024$; Table 1) but there were no significant differences between treatments at age 2 or 3.

At age 1, leaf nitrogen concentrations were marginally higher in seedlings in the HF treatment than in other treatments ($F_{2,37} = 2.47$, $P = 0.098$; Table 1), and significantly higher in the HF treatment than the LF treatment at age 2 ($F_{2,27} = 5.36$, $P = 0.011$). At age 3, LF seedlings had significantly lower leaf N than seedlings in the other treatments ($F_{2,34} = 6.053$, $P = 0.006$). In the control and HF treatments, leaf nitrogen concentrations increased with time from around 1% at age 1 to higher but still low concentrations at age 3 (around 1.5%). Symptoms of nitrogen deficiency (chlorotic leaves) were observed but not quantified.

Mycorrhizal infection

At age 1, mycorrhizal infection was significantly higher in HF seedlings than in control seedlings ($F_{2,37} = 4.15$, $P = 0.024$; Table 1). At age 2, there was a marginally significant treatment effect ($F_{2,31} = 3.17$, $P = 0.056$), but no significant differences among treatments in pair-wise post-hoc comparisons. By age 3, there were no significant treatment effects on mycorrhizal infection ($P = 0.94$). Infection rates were low at age 1, and increased with age in all treatments.

There was a strong positive correlation of leaf N concentration with percent ectomycorrhizal infection at age 2 ($P = 0.0002$, $r^2 = 0.38$, Figure 1) but not at ages 1 or 3 ($P = 0.12$ and $P = 0.43$, respectively). In combined data from all years the correlation was also significant ($P < 0.0001$, $r^2 = 0.29$; Figure 1).

Phenology

In 2003, there were marked effects of treatment on phenology of 2-year-old seedlings. The HF seedlings broke bud an average of 17 days earlier than 2-year-old seedlings in other treatments ($F_{2,42} = 24.4$, $P < 0.001$; Table 2). In 2004, 2-year-old HF seedlings again broke bud significantly earlier than 2-year-old seedlings in other treatments ($F_{3,31} = 7.38$, $P = 0.003$; Table 2). There were no significant differences in mean bud break among treatments for 3-year-old seedlings ($P = 0.96$). There were no significant effects of treatments on fall phenology ($P = 0.22$ and $P = 0.74$ for ages 1 and 2, respectively; data not shown).

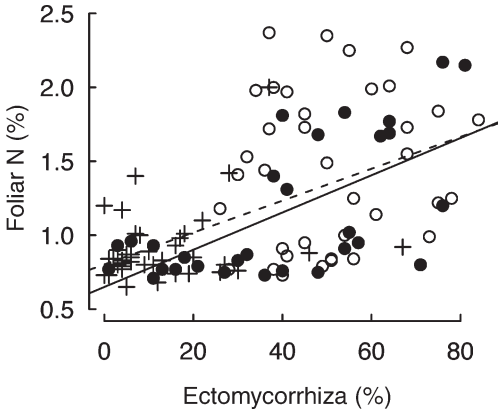

Figure 1. Correlation of foliar N (percentage) with percent of root tips infected by ectomycorrhizal fungi at age 1 year (+), age 2 years (●), and age 3 years (○). The correlation is significant at age 2 years (solid line; $P = 0.0002$, $r^2 = 0.38$) and for combined data across ages (dashed line; $P < 0.0001$, $r^2 = 0.29$), but not for age 1 year or age 3 years.

Patterns of leaf expansion mirrored the population trend in bud break (Figure 2; Table 3). The HF seedlings developed larger and more rapidly expanding leaves than seedlings in the other treatments. Across all treatments, expanded leaves of 3-year-old seedlings were ~ 0.5 cm longer than those of 2-year-old seedlings (Table 3).

Photosynthesis

In 2003, HF seedlings had significantly higher photosynthetic rates (4.17 ± 0.39 μmol m^{-2} s^{-1}) than control (2.90 ± 0.32) seedlings across all dates ($F_{1,103} = 6.30$, $P = 0.014$, Figure 3). In 2004, we found no significant difference in photosynthetic rates between HF and control seedlings but found a significant treatment × date interaction ($F_{2,49} = 4.49$, $P = 0.016$); however post-hoc analyses (Tukey's HSD) revealed no significant contrasts.

Discussion

The responses of oak seedlings to forest soil highlight the complex links between above- and belowground processes. Forest soil in the absence of trees had strong effects at various times on the aboveground physiology of seedlings including

Table 2. Bud break (day of year) as a function of treatment at age 2 years in 2003 and ages 2 and 3 years in 2004 in control (C), low forest soil (LF) and high forest soil (HF) treatments. Values are means with standard errors in parentheses. Within a row, values followed by different letters differ significantly. The *P* value is for overall significance of treatment effects.

	C	LF	HF	*P*
Age 2 year, 2003	157.0 (1.8)[a]	157.8 (1.9)[a]	139.7 (1.9)[b]	< 0.001
Age 2 year, 2004	153.7 (1.9)[a]	151.7 (2.0)[a]	145.7 (2.1)[b]	0.003
Age 3 year, 2004	150.9 (1.7)	150.3 (1.9)	150.2 (1.9)	0.96

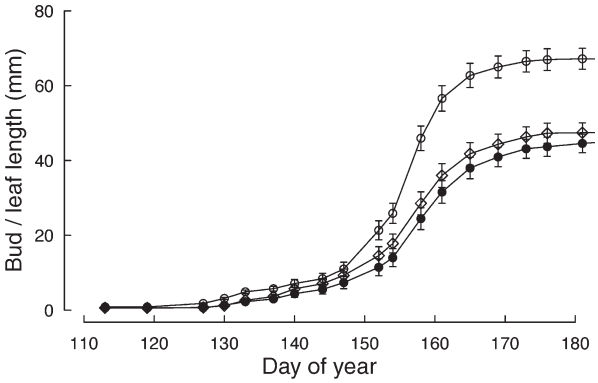

Figure 2. *Quercus ellipsoidalis* bud or leaf length measured from base of bud or petiole to tip on the most expanded bud or leaf of each seedling by soil treatment in 2004. Date is number of days since January 1. Error bars indicate 1 SE. Symbols: ● = control; ◇ = low forest; and ○ = high forest.

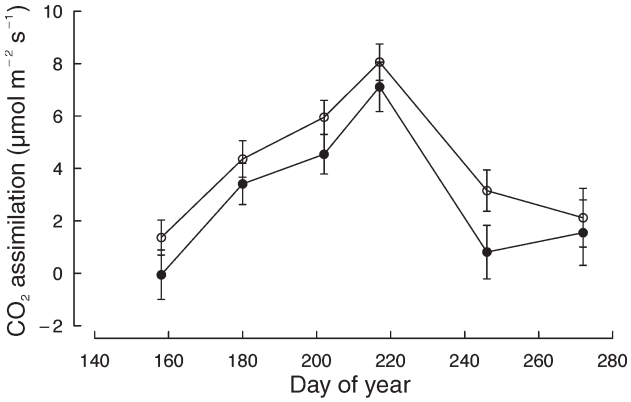

Figure 3. Photosynthetic response of high forest (HF) and control (C) seedlings by date in 2003. Date is number of days since January 1. Error bars indicate 1 SE; there is a significant treatment effect across all dates ($P = 0.014$). Symbols: ● = control and ○ = high forest.

traits generally not considered to be controlled by belowground factors (e.g., phenology). Seedlings receiving large amounts of forest soil had higher initial mycorrhizal infection, increased foliar nitrogen concentration and greater second- and third-year leaf mass than other seedlings. Compared with control and LF seedlings, HF seedlings flushed leaves earlier, expanded leaves more rapidly, had larger leaves and higher photosynthetic rates. Positive effects of trees on seedlings via soil modification are consistent with other studies of ectomycorrhizal seedling establishment (Simard and Durall 2004), but are in direct contrast to reports of negative effects of plants on conspecific seedlings in other systems (Bever 1994, Packer and Clay 2000, Bever 2002, Packer and Clay 2003, Reynolds et al. 2003).

Phenology

The effect of forest soil on bud break was unexpected because bud break has typically been considered to be under environmental, and particularly temperature, control (Lechowicz 1984, Augspurger 2004). Nonetheless, there is at least one prior report of an effect of soil biota on spring phenology—Garbaye (1986) showed that ectomycorrhizal fungal infection sped bud break of *Quercus robur* L. by up to six days. Although mycorrhizal fungi may also influence the timing of fall phenology (Garbaye and Churin 1996), we found no effect of treatment on fall phenology. The effect of soil or mycorrhiza on spring phenology may be related to increased seedling nutrition, because earlier bud break in response to increased nutrients has been reported (Flϕistad and Kohmann 2004). Alternatively, part or all of the effect of forest soil may be associated with the production of signaling compounds or plant hormones by soil biota (including mycorrhiza). This is supported by the lack of treatment differences in both bud break and mycorrhizal infection in 3-year-old seedlings. Breaking of dormancy and cell expansion are influenced by gibberellic acid, which fungi can produce, and other plant hormones may also be produced or influenced by mycorrhiza (Strzelczyk et al. 1994, Lambers et al. 1998).

Early leafing may provide considerable advantages to seedlings as a mechanism to capitalize on the high irradiance of the forest understory before canopy closure (Gill et al. 1998). Nonetheless, there are risks in early leaf out because it makes seedlings more vulnerable to late spring frosts (McGee 1975, Lechowicz 1984), and it should not be assumed that early leaf out would necessarily benefit the plant over the long term. A role for soil biota in signaling leafing phenology deserves further investigation, particularly with a goal of understanding the relative importance of direct seedling control and indirect soil microbial or nutrient mediated controls over phenology.

Table 3. Repeated measures ANOVA results for leaf expansion in 2004. The effects of treatment and date are illustrated in Figure 2.

Effect	SS	df	MS	*F*	*P*
Intercept	11542.87	1	11542.87	1039.87	< 0.0001
Treatment	477.32	2	238.66	21.50	< 0.0001
Age	104.29	1	104.29	9.40	0.0032
Treatment × Age	0.90	2	0.45	0.04	0.9604
Error	699.32	63	11.10		
Date	7499.48	21	357.12	702.85	< 0.0001
Date × Treatment	282.11	42	6.72	13.215	< 0.0001
Date × Age	75.61	21	3.6	7.083	< 0.0001
Date × Treatment × Age	30.80	42	0.73	1.443	0.0344
Error	672.48	1323	0.51		

Carbon assimilation

What was the relative importance of the different physiological responses of seedlings to forest soil? Increased leaf mass, increased photosynthetic rates and earlier spring bud break all probably increase total carbon gain, but the relative importance of these responses for whole-seedling carbon gain is unclear. To estimate this, for each day that a leaf was present, we multiplied leaf size, photosynthetic rate and day length and then summed across this period to estimate seasonal maxi-

mum gross carbon gain for the control and HF seedlings. As our data were not complete in any one year, we combined data on photosynthesis from 2003 with data on leaf expansion rates, plant leaf area and phenology from 2004; as such, our model should be taken as a theoretical construct. To calculate the importance of individual physiological components, we sequentially substituted leaf size, photosynthetic rate and phenology of HF seedlings into the equation for C seedling carbon gain of control seedlings.

The model suggests that leaf size was the most important physiological shift for HF seedlings, contributing 67% of an estimated 80% higher seasonal gross carbon gain in HF seedlings compared with control seedlings. Shifts in photosynthesis contributed 10% and leafing phenology contributed 5.5% of the estimated difference in carbon gain between HF and control seedlings, with the remaining 17.5% due to multiplicative effects. Although phenology did not appear as important as other factors in our open-grown seedlings, we expect that early leafing would provide a substantial advantage in the understory of deciduous forests as a result of the high irradiances experienced by understory plants that leaf out before the canopy (Gill et al. 1998).

Causes of treatment effects

Observed treatment effects may have been a result of either biotic or abiotic properties of forest soil. Trees have substantial effects on soil nutrient status (Reich et al. 2005), and Reich et al. (2001) and Dijkstra et al. (2005) have found that soils from under *Quercus* spp. trees have elevated N availability compared with old field soils at our research site. Nonetheless, some lines of evidence suggest that at least some of the treatment effects were biotic. In year 2, the HF seedlings had much higher ectomycorrhizal infection than seedlings in other treatments. Year 2 is when the largest effects of treatment on foliar N and phenology, and the best correlation of N concentrations and mycorrhiza, were observed.

Mycorrhizal infection rates were quite low in this study, particularly in year 1 (average 13% of root tips infected). For comparison, *Q. macrocarpa* seedlings planted at the edge of two agricultural fields adjacent to the soil collection site had root tip infection rates of around 77% after 1 and 3 years, whereas seedlings planted distant from the forest edge had 8 and 36% of root tips infected at age 1 and 3, respectively (Dickie et al. 2005). The relatively low mycorrhizal infection of seedlings even in the HF treatment may reflect disruption of fungal mycelium during collection of soils, resulting in an increased importance of spore- and sclerotia-based infection (Taylor and Bruns 1999).

The treatment effect on mycorrhizal infection was transient: by year 3 there were no differences among treatments. This was largely a result of an increase in mycorrhizal infection in the LF and control treatments, not a decrease in the HF treatment, and is consistent with the result of other studies showing that initial differences in mycorrhizal infection of seedlings planted near and distant from trees decrease over time (Dickie et al. 2002, Dickie et al. 2005). Although transient, these effects may have an important ecological effect during a critical period in seedling establishment. The increase in leaf mass associated with forest soil is consistent with prior observations of increases in both total growth and relative allocation to aboveground tissues in oak seedlings infected with ectomycorrhizal fungi (Daughtridge et al. 1986, Berman and Bledsoe 1998, Zhou et al. 1998). In all treatments other than HF, there was a decline in leaf mass and height from year 1 to year 2. This is not unusual for oak seedlings, and reflects a high initial growth from acorn reserves that was not sustained.

There were two results that did not fit with the overall picture of a positive effect of forest soil. The HF seedlings had a lower leaf and stem mass than seedlings in the other treatments at age 1: this may have been caused by increased resource allocation to roots or mycorrhizae, or both, at the expense of leaves. It is also possible that insect herbivores removed more mass from the HF treatment than other treatments given the difference in foliar N, although no obvious differences in herbivory were observed (and herbivory was not directly quantified). The other odd result was that LF seedlings, which showed few significant effects of treatment had significantly lower foliar N concentrations at age 3 than seedlings in the other treatments, despite having high ectomycorrhizal infection by age 3. We do not have any compelling explanation for this effect.

In conclusion, the effects of forest soil on seedlings appeared to be largely beneficial, with increased mycorrhizal infection, increased foliar nitrogen, greater leaf mass and elevated rates of photosynthesis. These beneficial effects did not require the physical presence of trees, but only soil from under trees. Although some of these effects were transient, they may still play an important role in the early establishment of seedlings in oak savannas and other areas where the distribution of established plants is patchy. Beneficial tree–seedling interactions may play an important role in the stability of forest ecosystems and the ability of forests to regenerate following disturbance. Conversely, the lack of trees and forest soil may impede tree seedling establishment in grasslands and delay succession on sites where soil has been removed.

Acknowledgments

We thank S. Barrott, R. Guza, M. Goldstien and A. Gunderson for invaluable assistance in measuring photosynthesis, and numerous Cedar Creek research interns who assisted in planting, maintaining and harvesting this experiment. Drs. S. Hobbie and C. Lusk provided valuable input on an earlier version of this manuscript. The US National Science Foundation LTER program funded this research (NSF/DEB 0080382). IAD was also supported by funds from the Foundation for Research Science and Technology (FRST, NSOF) of New Zealand during the analysis and writing of this research.

References

Anderson, L.J., M.S. Brumbaugh and R.B. Jackson. 2001. Water and tree-understory interactions: a natural experiment in a savanna with oak wilt. Ecology 82:33–49.

Augspurger, C.K. 2004. Developmental versus environmental control of early leaf phenology in juvenile Ohio buckeye (*Aesculus glabra*). Can. J. Bot. 82:31–36.

Barberis, I.M. and E.V.J. Tanner. 2005. Gaps and root trenching increase seedling growth in Panamanian semi-evergreen forest. Ecology 86:667–674.

Berkowitz, A.R., C.D. Canham and V.R. Kelly. 1995. Competition vs. facilitation of tree seedling growth and survival in early successional communities. Ecology 76:1156–1168.

Berman, J.T. and C.S. Bledsoe. 1998. Soil transfers from valley oak (*Quercus lobata* Nee) stands increase ectomycorrhizal diversity and alter root and shoot growth on valley oak seedlings. Mycorrhiza 7:223–235.

Bever, J.D. 1994. Feedback between plants and their soil communities in an old field community. Ecology 75:1965–1977.

Bever, J.D. 2002. Negative feedback within a mutualism: host-specific growth of mycorrhizal fungi reduces plant benefit. Proc. Roy. Soc. London Series B-Biol. Sci. 269:2595–2601.

Binkley, D. and C. Giardina. 1998. Why do tree species affect soils? The warp and woof of tree–soil interactions. Biogeochemistry 42:89–106.

Caldwell, M.M., T.E. Dawson and J.H. Richards. 1998. Hydraulic lift: consequences of water efflux from the roots of plants. Oecologia 113:151–161.

Callaway, R.M., G.C. Thelen, A. Rodriguez and W.E. Holben. 2004. Soil biota and exotic plant invasion. Nature 427:731–733.

Danner, B.T. and A.K. Knapp. 2001. Carbon and water relations of juvenile *Quercus* species in tall-grass prairie. J. Veg. Sci. 12:807–816.

Daughtridge, A.T., S.G. Pallardy, H.G. Garrett and I.V. Sander. 1986. Growth analysis of mycorrhizal and nonmycorrhizal black oak (*Quercus velutina* Lam.) seedlings. New Phytol. 103:473–480.

Dickie, I.A., R.T. Koide and K.C. Steiner. 2002. Influences of established trees on mycorrhizas, nutrition, and growth of *Quercus rubra* seedlings. Ecol. Monogr. 72:505–521.

Dickie, I.A. and P.B. Reich. 2005. Ectomycorrhizal fungal communities at forest edges. J. Ecol. 93:244–255.

Dickie, I.A., S.A. Schnitzer, P.B. Reich and S.E. Hobbie. 2005. Spatially disjunct effects of co-occurring competition and facilitation. Ecol. Lett. 8:1191–1200.

Dijkstra, F.S., K. Wrage, S.E. Hobbie and P.B. Reich. 2005. Tree patches show greater N losses but maintain higher soil N availability than grassland patches in a frequently burned oak savanna. Ecosystems 5:385–398.

Finzi, A.C. and C.D. Canham. 2000. Sapling growth in response to light and nitrogen availability in a southern New England forest. Forest Ecol. Manage. 131:153–165.

Finzi, A.C., N. VanBreeman and C.D. Canham. 1998. Canopy tree-soil interactions within temperate forests: species effects on soil carbon and nitrogen. Ecol. Applic. 8:440–446.

Flϕistad, I.S. and K. Kohmann. 2004. Influence of nutrient supply on spring frost hardiness and time of bud break in Norway spruce (*Picea abies* (L.) Karst.) seedlings. New For. 27:1–11.

Garbaye, J. 1986. Effects of mycorrhizal status on bud-break of beech and oak seedlings. *In* Mycorrhizae: Physiology and Genetics—Les Mycorhizes: Physiologie et Genetique. Eds. V. Gianinazzi-Pearson and S. Gianinazzi. INRA, Paris, pp 493–496.

Garbaye, J. and J.L. Churin. 1996. Effect of ectomycorrhizal inoculation at planting on growth and foliage quality of *Tilia tomentosa*. J. Aboric. 22:29–34.

Gill, D.S., J.S. Amthor and F.H. Bormann. 1998. Leaf phenology, photosynthesis, and the persistence of saplings and shrubs in a mature northern hardwood forest. Tree Physiol. 18:281–289.

Givnish, T.J. 1988. Adaptation to sun and shade: a whole-plant perspective. Aust. J. Plant Physiol. 15:63–92.

Grigal, D.F., L.M. Chamberlain, H.R. Finney, D.V. Wroblewski and E.R. Gross. 1974. Soils of the Cedar Creek Natural History Area. University of Minnesota Agricultural Experiment Station Misc Report 123:1–48.

Hacker, S.D. and S.D. Gaines. 1997. Some implications of direct positive interactions for community species diversity. Ecology 78: 1990–2003.

Harrington, R.A., B.J. Brown and P.B. Reich. 1989. Ecophysiology of exotic and native shrubs in Southern Wisconsin I. Relationship of leaf characteristics, resource availability, and phenology to seasonal patterns of carbon gain. Oecologia 80:356–367.

Haskins, K.E. and C.A. Gehring. 2004. Interactions with juniper alter the abundance and composition of pinyon pine ectomycorrhizal fungal communities. Ecology 85:2687–2692.

Janzen, D.H. 1970. Herbivores and the number of tree species in tropical forests. Am. Nat. 104:501–528.

Lambers, H., F.S.I. Chapin and T.L. Pons. 1998. Plant physiological ecology. Springer-Verlag, New York, 540 p.

Lechowicz, M.J. 1984. Why do temperate deciduous trees leaf out at different times? Adaptation and ecology of forest communities. Am. Nat. 124:821–841.

McGee, C.E. 1975. Change in forest canopy affects phenology and development of northern red and scarlet oak seedlings. For. Sci. 21:175–179.

Montgomery, R.A. 2004. Relative importance of photosynthetic physiology and biomass allocation for tree seedling growth across a broad light gradient. Tree Physiol. 24:155–167.

Nara, K. 2005. Ectomycorrhizal networks and seedling establishment during early primary succession. New Phytol. 169:169–178.

Packer, A. and K. Clay. 2000. Soil pathogens and spatial patterns of seedling mortality in a temperate tree. Nature 404:278–281.

Packer, A. and K. Clay. 2003. Soil pathogens and *Prunus serotina* seedling and sapling growth near conspecific trees. Ecology 84: 108–119.

Perry, D.A., M.P. Amaranthus, J.G. Borchers, S.L. Borchers and R.E. Brainerd. 1989. Bootstrapping in ecosystems. Bioscience 39: 230–237.

Pickett, S.T.A., S.L. Collins and J.J. Armesto. 1987. Models, mechanisms and pathways of succession. Bot. Rev 53:335–371.

Platt, K.H., R.B. Allen, D.A. Coomes and S.K. Wiser. 2004. Mountain beech seedling responses to removal of below-ground competition and fertiliser addition. N.Z. J. Ecol. 28:289–293.

Reich, P.B., D.S. Ellsworth and M.B. Walters. 1998. Leaf structure (specific leaf area) modulates photosynthesis-nitrogen relations: Evidence from within and across species and functional groups. Funct. Ecol. 12:948–958.

Reich, P.B., J. Oleksyn, J. Modrzynski, P. Mrozinski, S.E. Hobbie, D.M. Eissenstat, J. Chorover, O.A. Chadwick, C.M. Hale and M.G. Tjoelker. 2005. Linking tree species, litter calcium, earthworms and soil properties: a common garden test with 14 tree species. Ecol. Lett. 8:811–818.

Reich, P.B., D.W. Peterson, D.A. Wedin and K. Wrage. 2001. Fire and vegetation effects on productivity and nitrogen cycling across a forest-grassland continuum. Ecology 82:1703–1719.

Reinhart, K.O. and R.M. Callaway. 2004. Soil biota facilitate exotic *Acer* invasions in Europe and North America. Ecol. Applic. 14: 1737–1745.

Reynolds, H.L., A. Packer, J.D. Bever and K. Clay. 2003. Grassroots ecology: plant-microbe-soil interactions as drivers of plant community structure and dynamics. Ecology 84:2281–2291.

Simard, S.W. and D.M. Durall. 2004. Mycorrhizal networks: a review of their extent, function, and importance. Can. J. Bot. 82:1140–1165.

Strzelczyk, E., M. Kampert and R. Pachlewski. 1994. The influence of pH and temperature on ethylene production by mycorrhizal fungi of pine. Mycorrhiza 4:193–196.

Taylor, D.L. and T.D. Bruns. 1999. Community structure of ectomycorrhizal fungi in a *Pinus muricata* forest: minimal overlap between the mature forest and resistant propagule communities. Mol. Ecol. 8:1837–1850.

Walters, M.B. and C.B. Field. 1987. Photosynthetic light acclimation in two rain forest *Piper* species with different ecological amplitudes. Oecologia 72:449–456.

Walters, M.B., E.L. Kruger and P.B. Reich. 1993. Growth, biomass distribution and CO2 exchange of northern hardwood seedlings in high and low light: relationships with successional status and shade tolerance. Oecologia 94:7–16.

Zhou, M., T.L. Sharik, M.F. Jurgensen, D.L. Richter, M.R. Gale and T.D. Drummer. 1998. Regeneration of northern red oak in relation to ectomycorrhizae in oak and pine stands after overstory and understory manipulations. Northern J. Appl. For. 15:182–190.

Zou, C.B., P.W. Barnes, S. Archer and C.R. McMurtry. 2005. Soil moisture redistribution as a mechanism of facilitation in savanna tree-shrub clusters. Oecologia 145:32–40.