PRESENCE OF UNDERSTORY SHRUBS CONSTRAINS CARBON GAIN IN SUNFLECKS BY ADVANCE-REGENERATION SEEDLINGS: EVIDENCE FROM *QUERCUS RUBRA* SEEDLINGS GROWING IN UNDERSTORY FOREST PATCHES WITH OR WITHOUT EVERGREEN SHRUBS PRESENT

E. T. Nilsen,[1],* T. T. Lei,† and S. W. Semones‡

*Department of Biological Sciences, Virginia Tech, Blacksburg, Virginia 24061-0406, U.S.A.; †Department of Environmental Solution Technology, Faculty of Science and Technology, Ryukoku University, Otsu, Japan; and ‡Roots Plant Care Group, Novozymes Biologicals, Salem, Virginia 24153, U.S.A.

We investigated whether dynamic photosynthesis of understory *Quercus rubra* L. (Fagaceae) seedlings can acclimate to the altered pattern of sunflecks in forest patches with *Rhododendron maximum* L. (Ericaceae), an understory evergreen shrub. Maximum photosynthesis (A) and total CO_2 accumulated during lightflecks was greatest for 400-s lightflecks, intermediate for 150-s lightflecks, and lowest for 50- and 75-s lightflecks. For the 400-s lightflecks only, maximum A and total CO_2 accumulated were significantly lower for seedlings in forest patches with shrubs present (SF) than for seedlings in forest patches without shrubs (F). These effects were found only when A was calculated on a leaf-area basis because the specific leaf area of seedlings in F patches was 16% lower than it was for seedlings in SF patches. Photosynthesis reached 50% induction in 159 s for seedlings in F patches compared with 226 s for seedlings in SF patches. The faster induction of A for seedlings in F patches resulted in a significantly higher lightfleck use efficiency than for seedlings in SF patches. The inefficient use of lightflecks by *Q. rubra* seedlings in SF patches may be a primary mechanism by which *Q. rubra* seedlings are inhibited by subcanopy thickets of *R. maximum*.

Keywords: dynamic photosynthesis, sunfleck responses, transient light, advance regeneration, deciduous forest, *Quercus rubra*, *Rhododendron maximum*.

Introduction

Canopy tree seedlings that are present in the understory before forest disturbance, referred to as advance-regeneration seedlings, replace many canopy tree species following disturbance (Dillaway et al. 2007; Frey et al. 2007; Madsen and Hahn 2008). Important forest traits such as resilience to disturbance, productivity, and biodiversity are significantly affected by the health and abundance of advance-regeneration seedlings (Canham 1989; Ashton and Larson 1996). Also, advance-regeneration trees refill canopy gaps created by the death of individual or small groups of canopy trees in many different forest ecosystems (Shure et al. 2006; Collet et al. 2008). Therefore, understanding the processes that regulate the survival of advance-regeneration seedlings is valuable in understanding forest dynamics and appropriately managing forest resources.

Advance regeneration is inhibited by understory evergreen plants in tropical forests (Veblen 1989; Denslow et al. 1991), temperate deciduous forests (Taylor and Qin 1992; Beckage et al. 2000; Lei et al. 2002), coniferous forests (Klinka et al. 1989; Messier 1993), and boreal forests (Mallik 2003). In fact, some understory evergreen species can completely preclude any canopy tree reestablishment in the understory following large-scale disturbance to the canopy (Mitchell et al. 1997; Ito and

Hino 2004) or small canopy-gap disturbance (Beckage and Clark 2003). Therefore, the influence of understory vegetation on advance-regeneration seedlings will have significant consequences for forest regeneration following disturbance worldwide. Learning the mechanisms by which understory vegetation inhibits advance regeneration will lead to a better understanding of forest dynamics and to more effective forest management programs.

Advance-regeneration seedlings can contribute to forest structure when they are released from suppression by an increase in photon flux density (PFD) following disturbance to the forest canopy. However, advance-regeneration seedlings can respond to disturbance only if they can survive and persist in the understory before the disturbance occurs. Recruitment and persistence of canopy tree seedlings into the advance-regeneration pool can depend on the relationship between resource availability in the forest understory and physiological and structural traits of the seedlings (Walters and Reich 1996). Resources such as light, nutrients, and water can be limiting and are spatially and temporally heterogeneous in the forest understory. Hence, the ability of advance-regeneration seedlings to alter their physiological traits among microsites that have different patterns of resource availability will help the seedlings persist in the understory (Runkle 1990; Royo and Carson 2006).

Spatial and temporal availability of PFD, nutrients, and water depend on a complex relationship among soil parent material, topography, climate, disturbance, and biotic factors (Abe et al. 2002; Griscom and Ashton 2003; Boerner 2006). In par-

[1] Author for correspondence; e-mail: enilsen@vt.edu.

ticular, the PFD and its heterogeneity in understory patches of temperate forest are contingent on the composition of tree and shrub canopies over the patches (Messier et al. 1989; Nilsen et al. 2001; Lei et al. 2002, 2006). Patches with both canopy trees and understory evergreen shrubs can result in as little as 1% of full-sun PFD reaching the height of advance-regeneration seedlings (Nakashizuka 1987; Kelly and Canham 1992; George and Bazzaz 1999; Aubin et al. 2000; Lei et al. 2002). Moreover, the frequency, duration, and intensity of transient periods of high light (sunflecks) may be less in understory patches with trees and understory evergreen shrubs compared with those in forest patches with canopy trees but no understory evergreen shrubs (Nilsen et al. 2001; Beier et al. 2005; Lei et al. 2006).

The availability of belowground resources in different patches could influence the efficiency of utilizing transient high light (dynamic photosynthesis) by advance-regeneration seedlings. Nutrient availability in forest soils is heterogeneous (Jackson and Caldwell 1993; Gross et al. 1995; Gallardo 2003; Messaoud and Houle 2006). Moreover, understory shrubs in mature forest stands reduce nutrient resource availability (Nilsen et al. 2001; Beier et al. 2005; Royo and Carson 2006; Wurzburger and Hendrick 2006), which creates some of the heterogeneity of nutrient availability among understory patches. Soils in locations with evergreen shrubs in the understory often have a lower soil pH, a lower cation availability, a lower available nitrogen, a higher carbon-to-nitrogen ratio, and a higher concentration of aluminum and iron ions (Monk et al. 1985; Nilsen et al. 2001; Beier et al. 2005) than do soils in forest locations without shrubs. In addition, water availability in the seedling rooting zone can be lower in sites with understory shrubs than in sites without understory shrubs (Nilsen et al. 2001), although this is not consistently true (Clinton 2003). Therefore, the presence of understory shrubs in a forest patch is associated with both reduced belowground nutrient availability and reduced PFD compared with forest patches without understory evergreen shrubs.

In previous research at our research site, we have determined the negative influence of subcanopy thickets of *Rhododendron maximum* L. (Ericaceae) on nutrients (Beier et al. 2005) and PFD (Lei et al. 2006). The objective of this research project was to evaluate dynamic photosynthetic responses to heterogeneous light (sunflecks) for seedlings in patches that differed in the presence of understory evergreen shrubs. On the basis of our previous research at the same location, we predicted that *Quercus rubra* L. (Fagaceae) seedlings growing in patches without understory evergreen shrubs would utilize transient high PFD more efficiently than seedlings growing in patches where understory evergreen shrubs are present. Seedlings that do not use dynamic light efficiently are not likely to persist in the understory (Chazdon 1988). Therefore, we propose that shrubs in the understory inhibit advance-regeneration seedlings by impairing the ability of seedlings to utilize dynamic light in the understory. On the basis of this proposal, we formulated the following hypotheses.

1. The response of maximum CO_2 assimilation (A) and total CO_2 gain per leaf area to increasing intensity and duration of PFD during transient periods of high light (lightflecks) is greater for seedlings growing in patches without understory evergreen shrubs present (F) compared with seedlings growing in patches with understory evergreen shrubs present (SF).

2. Seedlings growing in F patches have a more rapid increase in induction state (IS), which results in higher light-

fleck use efficiency (LUE), compared with seedlings growing in SF patches.

3. As total PFD of a forest patch increases (on the basis of weighted canopy openness [WCO]), the total carbon gain per leaf area during transient light will be greater for seedlings in F patches compared with seedlings in SF patches.

We focus on dynamic photosynthesis because efficient use of dynamic light is crucial for carbon gain and survival of understory seedlings (Pearcy et al. 1987; Chazdon 1988; Montgomery and Givnish 2008). Our goal was to understand the effects of different forest patch types on advance-regeneration seedling response to non–steady state PFD. The systems we selected were naturally occurring and artificially planted *Q. rubra* seedlings growing in a deciduous forest with or without patches of *R. maximum* L. in the understory. We selected *Q. rubra* as our example of an advance-regeneration species because it is the most important component of advance regeneration in the southern Appalachian forest (Monk et al. 1985; Beckage and Clark 2003). We selected *R. maximum* as our understory shrub because it is the most abundant evergreen shrub in the southern Appalachian forest (Elliott et al. 1999).

Material and Methods

Study Species and Sites

This study was conducted at Coweeta Hydrologic Laboratory, a 2185-ha U.S. Forest Service experimental station that is a member site of the Long Term Ecological Research (LTER) network. The basin is located in the Nantahala Mountains of the Blue Ridge Mountain physiographic province near Otto, North Carolina (lat. 35°02′N, long. 83°24′W). Vegetation has been described as including northern hardwood, cove hardwood, oak, and oak-pine communities (Day et al. 1988). Elevations range from 675 to 1592 m. Yearly average precipitation is 1800 mm at the base climate station, and it increases with elevation to >2200 mm (Swank and Crossley 1988; Yeakley et al. 1998). High moisture levels and mild temperatures, which are characteristic of the basin, place Coweeta in the marine, humid, temperate category of Koppen's climatic classification scheme. The study site selected was a mature, mixed-hardwood forest located on a north-facing slope at an elevation of 1000 m. Patches of *Rhododendron maximum* shrubs are found in the understory throughout the site. Dominant tree species at our site include mature reproductive *Quercus rubra*.

Quercus rubra, northern red oak, is a large (18.3–24.4 m in height) deciduous canopy tree in the eastern deciduous forest of the northeast and north central United States. Northern red oak occurs in mixed forests at low to moderate elevations. It can be a dominant forest tree, but usually *Q. rubra* grows in association with other codominant trees of the southeastern mixed forest. In Coweeta Hydrologic lab, *Q. rubra* is common at middle elevations on north- and east-facing slopes (Elliott and Swank 2008). Acorns are dropped in the fall and germinate the following spring (Garcia and Houle 2005). Seedlings of *Q. rubra* are scattered in the understory and have relatively high survivorship in comparison with seedlings of other important canopy trees (Elliott et al. 1999; Beckage and Clark 2003).

Experiment 1: photosynthetic response to lightfleck duration and intensity. We identified all regions of the research

site with the same aspect (northeast) and slope (26°). Six locations were randomly selected from the identified regions, and one 25×25-m plot was installed in each of the six locations. Three of the plots did not have understory evergreen shrubs present (F patches), and three plots were located where understory evergreen shrubs were present (SF patches). In each plot, 15 2×2-m experimental subplots were placed in a regular design, with a 2-m buffer around each subplot.

Quercus rubra acorns were collected from parent trees in the vicinity of the study site and subsequently cold stratified for 3 mo. In March of 1998, 15 randomly chosen *Q. rubra* acorns were directly planted in each subplot. Emerged seedlings were tagged and utilized for growth analysis (Lei et al. 2002) and gas exchange (Lei et al. 2006). The second year after planting acorns, 72 *Q. rubra* seedlings were randomly selected from F subplots, and 72 were selected from SF subplots. All leaves used were those that were produced on seedlings in May 1999, and they were fully mature when photosynthetic measurements were taken (June–July).

Each *Q. rubra* seedling was assigned one of three lightfleck intensities (PFD = 100, 500, or 1000 μmol m^{-2} s^{-1}) and one of four lightfleck durations (50, 75, 150, or 400 s). These values were selected to represent the range of maximum PFD and the duration of many sunflecks at these plots, as previously reported (Lei et al. 2002, 2006). Each lightfleck duration and intensity were replicated six times for a total of 144 analyses (two forest patch types × three PFD intensities × four lightfleck durations × six replicate seedlings = 144 lightfleck simulations).

Lightflecks were created and net photosynthesis (*A*) of seedling leaves was measured using a portable gas-exchange system fitted with an LED light source (model 6400; LI-COR, Lincoln, NE). An internal program was coded in the gas-exchange system to create lightflecks by the LED source and to analyze gas-exchange response to lightfleck conditions. No correction for the response time of the gas-exchange system to transient light was necessary because the response of this gas-exchange system is fast enough for determining IS and LUE for lightflecks of 50–300 s. Carbon dioxide concentration within the leaf chamber (C_a) was set to 390 ppm CO_2 using an external CO_2 source assembly (model 6400-01, LI-COR). Relative humidity of the leaf chamber and vapor pressure deficit were maintained within 1% over the duration of the simulation by manually adjusting the system flow rate through the desiccant. Leaves were exposed to PFD = 10 μmol m^{-2} s^{-1} light (a common diffuse radiation value in the understory) for 600 s before each lightfleck. Each lightfleck response recording consisted of (1) the final 120 s of the preliminary exposure to PFD = 10 μmol m^{-2} s^{-1} and (2) a transient high-PFD period (lightfleck) of a randomly selected maximum PFD value (100, 500, or 1000 μmol m^{-2} s^{-1}) and duration (50, 75, 150, or 400 s), followed by (3) 120 s at PFD = 10 μmol m^{-2} s^{-1}. Net photosynthetic rate (*A*) was logged every 2 s during the entire lightfleck response recording. Total CO_2 uptake from the lightfleck + 120 s at 10 μmol m^{-2} s^{-1} following the lightfleck was determined for all measured leaves. A second batch of 144 seedlings was randomly selected for the same analysis as described for experiment 1, except these seedlings were dark adapted overnight before the initiation of the lightfleck protocols.

In the late summer, after all gas-exchange measurements were complete, 15 seedlings from each plot (one per subplot, including some of the seedlings used for gas-exchange measurements) were harvested and separated into leaves, stems, and roots. We measured the following parameters: (1) seedling height, (2) total leaf area, (3) total leaf dry mass, (4) specific leaf area (SLA), (5) total root mass, and (6) total plant mass.

Experiment 2: IS and LUE. We selected different research sites for our second experiment. Paired sites (20 pairs) were randomly chosen in the broader region of our research region such that in one part of each paired site there were no understory evergreen shrubs present (F) and in another part of the same paired site there were understory evergreen shrubs present (SF). Paired sites were selected so they were at least 20 m apart from each other and possessed naturally regenerating *Q. rubra* seedlings. Five even-sized *Q. rubra* seedlings, located at least 10 m apart from each other, were randomly assigned for measurements in the two designated parts of each paired site. Of the five assigned seedlings, three were randomly selected for lightfleck simulations, giving a sample size of 60 seedlings (i.e., three in each of 10 F patches and three in each of 10 SF patches).

The availability of PFD above each subject seedling was estimated using hemispherical canopy photography. Photographs were taken at a height of 0.5 m using a leveled Nikon 8-mm fisheye lens (180° field of view) mounted on a Nikon F3HP body directly above each seedling used for lightfleck simulations. The lens was fitted with a compass for indicating magnetic north. A high-contrast black-and-white film (ASA 400, Tri X; Eastman Kodak, Rochester, NY) and a red built-in filter were used for taking pictures with enhanced contrast. Black-and-white negatives were digitized using a slide scanner (Polaroid SprintScan 35 ES) and saved as bitmap files until they were analyzed. Canopy photos were analyzed using FEW4.0 (Lei et al. 2006) to obtain WCO, an index that represents light availability to seedlings and that weights canopy gaps above the seedling higher than those close to the horizon (Chazdon and Field 1987).

All gas-exchange measurements were performed on recently mature leaves of the subject seedlings during June–July 2000. A repetition of eight identical lightflecks was used to ensure that complete photosynthetic activation was attained during the analysis without photoinhibition. Following exposure to low-intensity light (PFD = 10 μmol m^{-2} s^{-1}) for 600 s, leaves were exposed to a sequence of eight replicate lightflecks. This lightfleck duration is representative of that which commonly occurs in the forest understory of this region. Each lightfleck simulation in the series consisted of (1) 60 s at low PFD (10 μmol m^{-2} s^{-1}), (2) 90 s at high PFD (500 μmol m^{-2} s^{-1}), and (3) 60 s at low PFD (10 μmol m^{-2} s^{-1}). The 60-s period at low PFD following each lightfleck was also the 60-s period of low PFD before the next lightfleck, resulting in a total of 60 s between lightflecks. After the sequential lightfleck simulations, we checked that maximum *A* had been attained by allowing leaves to reach maximum steady state *A* values at PFD = 500 μmol m^{-2} s^{-1}. We determined that maximum *A* had been achieved after the lightfleck simulation when *A* remained constant for at least 300 s. Net photosynthetic rate (*A*), stomatal conductance (g_{st}), and intercellular CO_2 concentration (C_i) was logged every 2 s for the entire sequential lightfleck simulation.

Maximum *A* and total CO_2 uptake during the lightfleck + 30 s of low PFD was determined for each lightfleck in the series. In addition, total CO_2 uptake encompassing the entire sequence of eight lightflecks was determined. Leaf IS values at

three time periods (IS_0, IS_{60}, and IS_{90}) were calculated using the equation

$$IS = \frac{A_{LF} - A_L}{A_H - A_L} \times 100,$$

where A_{LF} is A at 0, 60, or 90 s and A_L and A_H are steady state A values at low and high PFD, respectively (Chazdon and Pearcy 1986a). PFD during lightflecks was greater than light saturation, as determined from steady state light-response curves generated for *Q. rubra* seedlings at our research site in a previous study (Lei et al. 2006), but it did not induce photoinhibition. Also, low PFD was just above the light compensation point as measured previously (Lei et al. 2006), and it represented common diffuse PFD found in these understory plots.

The LUE during each lightfleck was calculated by comparing the measured total CO_2 uptake during the lightfleck plus 30 s at low PFD following the lightfleck with the predicted total CO_2 uptake for a square wave calculated from steady state rates measured at low and high PFDs (Chazdon and Pearcy 1986b):

$$LUE = \frac{measured\ CO_2\ uptake + 30\,s}{predicted\ CO_2\ uptake} \times 100.$$

Data Analysis

Of the six randomly selected, noncontiguous blocks (three inside the *R. maximum* thickets [SF patches] and three where *R. maximum* was absent [F patches]) used for experiment 1, we found no systematic bias of edaphic and environmental characteristics among blocks within each canopy type (Lei et al. 2002, 2006). Hence, the blocks were treated as replicates of the main effect in the ANOVA. All statistical analyses were completed using SAS PROC GLM (SAS/STAT system, ver. 8 for Windows; SAS Institute, Cary, NC). We acknowledge (Lei et al. 2002) that the two factors in the main effect (i.e., F or SF) are not strictly experimental treatments but represent naturally occurring heterogeneity in the forest environment. Therefore, the main effects of treatment (F vs. SF) on plant traits were determined using the following sums of square (SS) ratio to calculate the F statistic, with df = 1, 4:

$$F = \frac{SS\ canopy\ type/1}{SS\ main\ plot(canopy\ type)/4}.$$

Two-way ANOVA was used for experiment 1 to determine the effects of treatment (F or SF), lightfleck duration in seconds (25, 50, 150, or 400 s), and their interaction on maximum photosynthesis, and total carbon gain for each lightfleck PFD individually. Data were transformed when necessary to meet normality and equal-variance tests. Differences among means of all lightfleck durations, within PFD levels, were tested with the Tukey method. We utilize a significance level of 0.10 for all analyses because of the inherent heterogeneity of environmental conditions within forest understory patch types. The growth data were analyzed by a Kruskal-Wallace ANOVA on ranks because normality tests could not be met.

All parameters attained during the sequential lightflecks of experiment 2 were analyzed for each individual lightfleck, and not across lightflecks, using ANOVA with F and SF as the treatment factor. Linear regression was used to relate WCO derived from canopy photographic analysis with total CO_2 uptake from the sequence of eight lightflecks in experiment 2.

Results

Experiment 1: Gas Exchange

We detected no significant effects of dark adaptation versus ambient adaptation on maximum A or total carbon gain within any sunfleck intensity or duration. Therefore, results for dark-adapted and ambient-adapted seedlings were lumped ($n = 288$) for all further analyses.

For lightflecks of 100 μmol $m^{-2}\ s^{-1}$. The effect of forest patch type on mean maximum A per leaf area was significant ($P = 0.009$), as was the effect of lightfleck duration ($P < 0.001$), but their interaction was not significant ($P = 0.727$). Seedlings in both patch types had a significantly higher mean maximum A value during 400-s lightflecks than did seedlings experiencing all other shorter-duration lightflecks (fig. 1A). There were no significant differences in mean maximum A value among any other lightfleck durations. For lightflecks of a 400-s duration only, seedlings in F patches accumulated a significantly higher amount of CO_2 than did seedlings in SF patches (fig. 1B).

In order to meet the Kolmogorov-Smirnov normality test, the data for total CO_2 accumulation were transformed by the square root. The effect of forest patch type on total CO_2 accumulated during the lightflecks per leaf area was significant ($P = 0.009$), as was the effect of lightfleck duration ($P < 0.001$), but their interaction was not significant ($P = 0.244$). For both forest types, mean total CO_2 accumulated by seedlings during the 400-s lightfleck was significantly higher than mean total CO_2 accumulated by seedlings experiencing all other shorter-duration lightflecks (fig. 1B). In addition, for both forest types, mean total CO_2 accumulated by seedlings during the 150-s lightfleck was significantly higher than mean total CO_2 accumulated by seedlings during lightflecks of 50 and 75 s. For lightflecks of a 400-s duration only, seedlings in F patches accumulated a significantly higher amount of CO_2 than did seedlings in SF patches (fig. 1B).

For lightflecks of 500 μmol $m^{-2}\ s^{-1}$. The effect of forest patch type on mean maximum A per leaf area was significant ($P = 0.002$), as was the effect of lightfleck duration ($P < 0.001$), but their interaction was not significant ($P = 0.418$). For both forest types, mean maximum A attained during a 400-s lightfleck was significantly higher than mean maximum A attained by seedlings experiencing all other shorter-duration lightflecks (fig. 1A). For lightflecks of a 400-s duration only, seedlings in F patches attained a significantly higher mean maximum A value than did seedlings in SF patches (fig. 1A).

In order to meet the equal-variance test, the data for total CO_2 accumulation during lightflecks were transformed by the natural log. The effect of forest patch type on mean total CO_2 accumulated during the lightflecks per leaf area was not significant ($P = 0.192$), but the effect of lightfleck duration on mean total CO_2 accumulated during the lightflecks was significant ($P < 0.001$). The interaction between forest patch type and
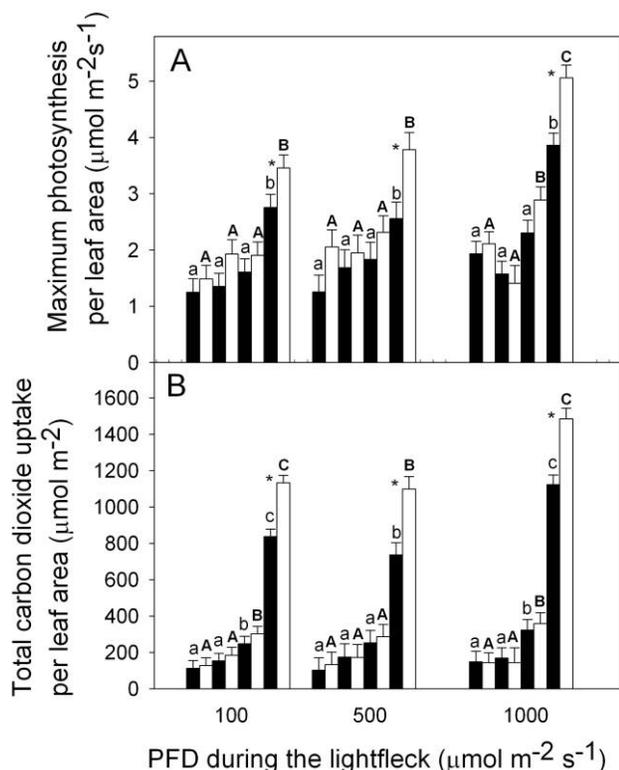
Fig. 1 Mean net photosynthetic rate (A) or mean total CO_2 uptake (B) for *Quercus rubra* seedlings growing in the understory of a southern Appalachian forest in lightflecks of photon flux density (PFD) = 100 μmol m^{-2} s^{-1}, PFD = 500 μmol m^{-2} s^{-1}, and PFD = 1000 μmol m^{-2} s^{-1}. Open bars represent seedlings growing in forest patches without understory evergreen shrubs (F patches). Filled bars represent seedlings growing in forest patches with understory evergreen shrubs (SF patches). Error bars represent 1 SEM. The first two bars in each group represent 50-s lightflecks, the second pair represents 75-s lightflecks, the third pair represents 150-s lightflecks, and the fourth pair represents 400-s lightflecks. Filled bars with a different lowercase letter are significantly different from each other within each PFD group. Open bars with different uppercase letters are significantly different from each other within each PFD group. An asterisk represents a significant difference between treatments (F vs. SF) at the α = 0.05 level.

lightfleck duration on mean total CO_2 accumulated by seedlings during the lightflecks was not significant ($P = 0.781$). For both forest types, mean total CO_2 accumulated by seedlings during the 400-s lightfleck was significantly higher than mean total CO_2 accumulated by seedlings for shorter-duration lightflecks (fig. 1B). For lightflecks of a 400-s duration only, seedlings in F patches accumulated a significantly higher amount of CO_2 than seedlings in SF patches (fig. 1B).

For lightflecks of 1000 μmol m^{-2} s^{-1}. The effect of forest patch type on mean maximum A per leaf area was significant ($P = 0.009$), as were the effect of lightfleck duration ($P < 0.001$) and the interaction of the two effects ($P = 0.032$). For both forest types, mean maximum A attained by seedlings during a 400-s lightfleck was significantly higher than mean maximum A attained by seedlings experiencing all other shorter-duration lightflecks (fig. 1A). In addition, mean

maximum A attained by seedlings in F patches during 150-s lightflecks was significantly higher than mean maximum A of seedlings in F patches experiencing 50- or 75-s lightflecks. For lightflecks of a 400-s duration only, seedlings in F patches attained a significantly higher mean maximum A value than did seedlings in SF patches (fig. 1A).

In order to meet the Kolmogorov-Smirnov normality test, the data for total CO_2 accumulation during lightflecks were square-root transformed. The effect of forest patch type on total CO_2 accumulated during the lightflecks per leaf area was significant ($P = 0.100$), as were the effect of lightfleck duration ($P < 0.001$) and the interaction of the two effects ($P = 0.011$). For both forest types, mean total CO_2 accumulated by seedlings during the 400-s lightfleck was significantly higher than mean total CO_2 accumulated by seedlings experiencing all other shorter-duration lightflecks (fig. 1B). Moreover, for both forest types, mean total CO_2 accumulated by seedlings during the 150-s lightfleck was significantly higher than mean total CO_2 accumulated by seedlings for 50- and 75-s lightflecks (fig. 1B). For lightflecks of a 400-s duration only, seedlings in F patches accumulated a significantly higher amount of CO_2 than did seedlings in SF patches (fig. 1B).

Experiment 1: Plant Growth

Total plant leaf area and all measures of plant mass were significantly lower for seedlings in SF plots compared with seedlings in F plots (table 1). Plant height was not significantly different between forest patch types. SLA was significantly higher for seedlings in SF plots compared with seedlings in F plots (table 1).

Experiment 2: Photosynthesis and Carbon Gain

The general patterns of gas exchange were the same for leaves on seedlings in F or SF patches during the sequence of eight repeated lightflecks. Here we present an example of a gas-exchange response for a leaf on a seedling in an F patch, which represents the general patterns observed for all leaves (fig. 2). Maximum net photosynthesis (A) during each lightfleck increased as the lightfleck sequence progressed, until lightfleck 5 (fig. 2A); following this, A gradually increased through lightfleck 8. Stomatal conductance (g_{st}) gradually increased from the initiation of lightfleck 2 until the end of lightfleck 4 (fig. 2A). Stomatal conductance (g_{st}) gradually decreased from lightfleck 5 through lightfleck 8. The ratio of intercellular CO_2 concentration to ambient CO_2 concentration (C_i/C_a) decreased rapidly at the initiation of each lightfleck and increased rapidly at the end of each lightfleck (fig. 2B). Between each successive lightfleck, the C_i/C_a ratio was the same as it was before the lightfleck sequence began. The lowest attained C_i/C_a ratio in each lightfleck increased during the first four lightflecks and decreased from lightfleck 5 to lightfleck 8 (fig. 2B). Patterns similar to these were observed in all other F and SF samples (data not shown).

Mean total CO_2 uptake per leaf area, measured in each lightfleck, increased as the lightfleck sequence progressed (fig. 3A). Most of the increase in total CO_2 uptake per leaf area (calculated as the percentage of maximum attained CO_2 uptake following each lightfleck) occurred by lightfleck 5 (80% for F, 72% for SF). Across all eight lightflecks, mean total CO_2 uptake per leaf area during each lightfleck was significantly higher for seed-

Table 1

Mean and Standard Error of Growth Characteristics for *Quercus rubra* Seedlings Grown in Forest Locations With (SF) or Without (F) an Understory of *Rhododendron maximum* in the Southern Appalachian Forest

Growth trait	F	SF
Total leaf area (cm^2)	110.3 ± 9.6	45.8 ± 5.2[a]
Total leaf mass (g)	.44 ± .04	.16 ± .02[a]
Specific leaf area (cm^2 g^{-1})	254 ± 5.0	302 ± 11.0[a]
Plant height (cm)	18.8 ± .5	19.7 ± .6
Total stem mass (g)	.47 ± .03	.29 ± .02[a]
Total root mass (g)	.49 ± .04	.21 ± .02[a]
Total plant mass (g)	1.22 ± .08	.62 ± .04[a]

[a] Significant difference between forest patch types using the Kruskal-Wallace ANOVA on ranks at the $P < 0.10$ level.

lings in F sites than for seedlings in SF sites (fig. 3A). An exponential function of the form $y = a(1 - b^x)$ could be used to describe the relationship between fleck sequence number (x) and seedling total CO_2 uptake per leaf area (y) for seedlings growing in both patch types (seedlings in F patches: $y = 34(1 - 0.717^x)$, $R^2 = 0.99$, $P < 0.0001$; seedlings in SF patches: $y = 460(1 - 0.657^x)$, $R^2 = 0.99$, $P < 0.0001$). Similar exponential relationships were found for maximum A in each lightfleck and A during low PFD between lightflecks (data not shown).

Experiment 2: LUE and IS

Seedling LUE increased as the lightfleck simulation progressed in both F and SF patches (fig. 3B). An exponential function of the form $y = a(1 - b^x)$ could be used to describe the relationship between lightfleck sequence number (x) and seedling LUE (y) for seedlings growing in both patch types (seedlings in F patches: $y = 92.6(1 - 0.726^x)$, $R^2 = 0.99$, $P < 0.0001$; seedlings in SF patches: $y = 92.5(1 - 0.650^x)$, $R^2 = 0.99$, $P < 0.0001$). Seedlings in both patch types attained a LUE at or just above 80% by lightfleck 7. For lightflecks 1–5 only, LUE for seedlings in F patches was significantly higher than it was for seedlings in SF sites. There were no significant interactions between patch type and paired site (data not shown).

Leaf IS of seedlings during each lightfleck in the sequence increased from 0 to 60 to 90 s. All three IS values for seedlings increased as the lightfleck sequence progressed (fig. 4). For all IS indices, seedlings in F patches tended to maintain higher IS relative to that of seedlings in SF patches. The mean IS_0 values were low (<20%) and not significantly different between seedlings growing in F or SF patches, regardless of lightfleck sequence number. For lightflecks 1–4 only, mean IS_{60} values for seedlings located in F patches were significantly higher compared with mean IS_{60} values for seedlings in SF patches (fig. 4). Mean IS of seedlings during lightfleck 1 increased by 20% from IS_0 to IS_{60} and by 10% from IS_{60} to IS_{90} in both patch types. However, for seedlings in both patch types, mean IS of seedlings during lightfleck 8 increased by 60% from IS_0 to IS_{60} and by 8% from IS_{60} to IS_{90}. Therefore, as lightfleck sequence progressed, mean IS_{60} and mean IS_{90} increased in parallel for seedlings in both patch types, while mean IS_0 remained fairly constant.

Experiment 2: WCO and Total Carbon Gain

There was a significant effect of the different paired sites on WCO (table 2), indicating that there was a significant variation in WCO among different forest locations for reasons other than the effect of shrubs on WCO. This forest scale spatial variation in WCO resulted in a high degree of overlap in WCO between F and SF patches (fig. 5). The range of WCO was 4.7%–17.2% in F patches (only one measurement was >12%) and 3.1%–12.3% for seedlings in SF sites. Most seedlings of both patch types (60% of all seedlings for F and 73% of all seedlings for SF) experienced a similar range of canopy openness (between 5% and 10%). There was a significant effect of forest patch type (F vs. SF) on WCO (table 2). Also, the interaction term between forest paired sites and patch type was significantly different (table 2). This significant interaction indicates that the relationship between WCO and patch type was significantly different among forest paired sites.

Paired sites had a significant effect on mean maximum A (table 2). Also, the mean maximum A value attained during the multifleck sequence was significantly higher for seedlings growing in F patches compared with seedlings growing in SF patches (table 2). The range in maximum A logged for *Quercus rubra* seedlings in F patches was 7.84–0.339 μmol CO_2 m^{-2} s^{-1},
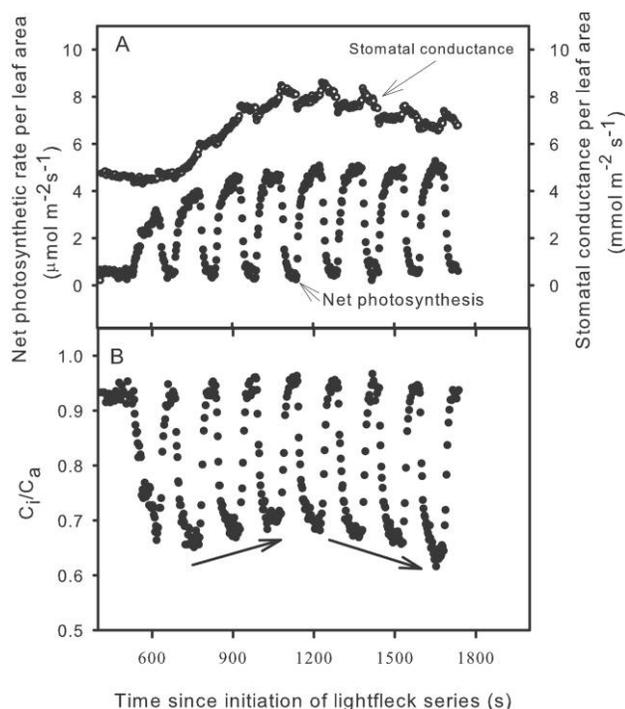

Fig. 2 Gas-exchange dynamics for a representative *Quercus rubra* seedling experiencing a series of eight replicate lightflecks growing in an understory patch without understory shrubs (F patch) in a southern Appalachian forest. *A*, Net photosynthetic rate and stomatal conductance; *B*, ratio of intercellular (C_i) and ambient (C_a) carbon dioxide concentrations. Each lightfleck response curve in the sequence consisted of (1) 60 s at low photon flux density (PFD; 10 μmol m^{-2} s^{-1}), (2) 90 s at high PFD (500 μmol m^{-2} s^{-1}), and (3) 60 s at low PFD (10 μmol m^{-2} s^{-1}). Arrows represent trends in minimum C_i/C_a in each lightfleck across the lightfleck sequence.
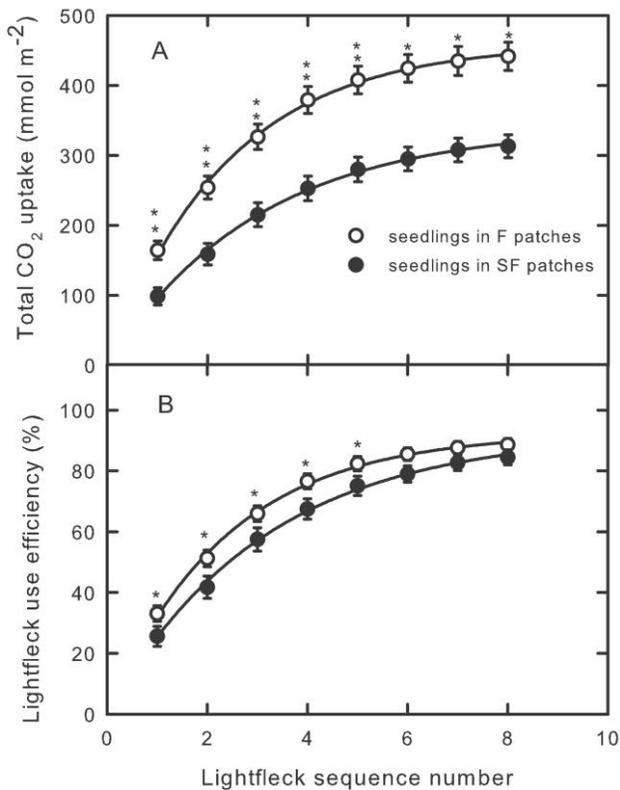
Fig. 3 Mean total CO_2 uptake (*A*) and lightfleck use efficiency (*B*) for each lightfleck in a series of eight replicate lightflecks experienced by *Quercus rubra* seedlings growing in a southern Appalachian forest. Each lightfleck response curve in the sequence consisted of (1) 60 s at low photon flux density (PFD; 10 μmol m^{-2} s^{-1}), (2) 90 s at high PFD (500 μmol m^{-2} s^{-1}), and (3) 60 s at low PFD (10 μmol m^{-2} s^{-1}). Open circles represent seedlings growing in forest patches without understory evergreen shrubs (F patch). Filled circles represent seedlings growing in forest patches with understory evergreen shrubs (SF patch). Error bars represent 2 SEM. One asterisk represents a significant difference at the $\alpha = 0.05$ level; two asterisks represent a significant difference at the $\alpha = 0.01$ level between the two forest patch types.

while for seedlings in SF patches it was 6.38–0.06 μmol CO_2 m^{-2} s^{-1}. The interaction term between paired sites and forest patch type was not significant (table 2).

The mean maximum total CO_2 uptake (μmol CO_2 m^{-2}) was significantly different among paired sites (table 2). Also, seedlings in F patches accumulated significantly greater amounts of CO_2 compared with seedlings in SF patches during the entire multilightfleck sequence (table 2). The range in maximum total CO_2 uptake for seedlings in F patches was 4459–552 μmol CO_2 m^{-2}. The range in maximum total CO_2 uptake for seedlings in SF patches was 3832–274 μmol CO_2 m^{-2}. The interaction between paired site and forest patch type on total CO_2 uptake was not significant (table 2).

The average WCO above seedlings in F patches was significantly higher than that above seedlings in SF patches when a *t*-test was applied to the data (table 3). Also, the maximum *A* attained at the end of the eight-lightfleck simulation was

significantly lower for seedlings in SF patches compared with seedlings in F patches (table 3). The total CO_2 accumulated during the entire eight-fleck simulation was significantly lower for seedlings growing in SF patches compared with seedlings growing in F patches (table 3).

The regressions of total CO_2 uptake per leaf area during the entire multilightfleck simulations were only slightly positively related to WCO (SF: $R^2 = 0.0529$; F: $R^2 = 0.112$) for seedlings in both patch types (fig. 5). The independent variable, WCO, failed to explain a significant amount of the variation present in the dependent variable, mean total CO_2 uptake. Therefore, there was no reason to test for the effect of patch type on the slopes or intercepts of the regressions using ANCOVA. However, in the region of the plot where WCO overlaps between the two patch types (5%–10% WCO), total CO_2 uptake of most seedlings in F patches (77%) was >2500 μmol m^{-2}, and total CO_2 uptake of most seedlings in SF patches (95%) was <2500 μmol m^{-2}. A linear regression formulated with only the data in the region of WCO overlap (5%–10%) resulted in a positive slope for seedlings in F patches ($R^2 = 0.088$; slope = 190.9, $P = 0.2047$) and a negative slope ($R^2 = 0.041$; slope = −88.2, $P = 0.4194$) for seedlings in SF understory patches.
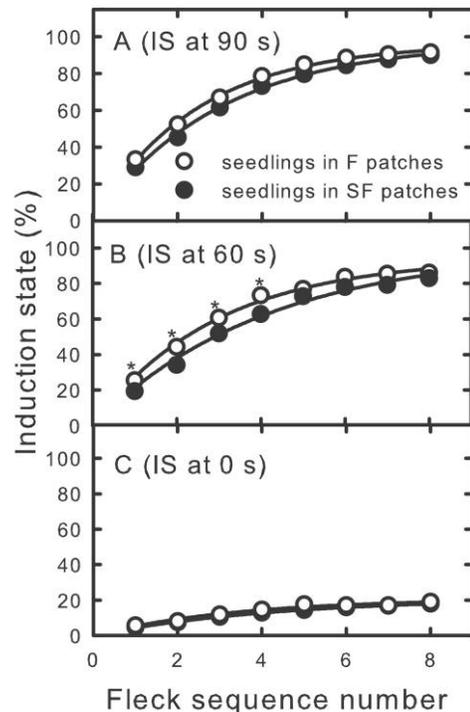

Fig. 4 Photosynthetic induction state at 90 s (*A*), 60 s (*B*), and 0 s (*C*) for each lightfleck in a series of eight replicate lightflecks experienced by *Quercus rubra* seedlings growing in a southern Appalachian forest. Each lightfleck response curve in the sequence consisted of (1) 60 s at low photon flux density (PFD; 10 μmol m^{-2} s^{-1}), (2) 90 s at high PFD (500 μmol m^{-2} s^{-1}), and (3) 60 s at low PFD (10 μmol m^{-2} s^{-1}). Open circles represent seedlings growing in forest patches without understory evergreen shrubs (F patches). Filled circles represent seedlings growing in forest patches with understory evergreen shrubs (SF patches). An asterisk represents a significant difference (paired *t*-test) at the $\alpha = 0.05$ level between the two forest patch types.

Table 2

ANOVA Results for the Effects of Paired Site and Patch Type and the Interaction of the Two on Weighted Canopy Openness (WCO), Maximum CO_2 Assimilation, and Total CO_2 Uptake during Eight Repeated Lightflecks

Source	df	F	P
WCO above seedlings used for lightfleck simulations:			
Paired site	9	3.99	.0011
Patch type	1	10.4	.0029
Paired site × patch type	9	3.66	.0020
Maximum A (μmol m^{-2} s^{-1}) rate attained during the lightfleck simulation:			
Paired site	9	4.5	.0004
Patch type	1	43.60	.0001
Paired site × patch type	9	1.39	.2252
Total CO_2 uptake (μmol m^{-2}) from the lightfleck simulation:			
Paired site	9	2.95	.0088
Patch type	1	28.43	.0001
Paired site × patch type	9	1.25	.2920

Note. Paired site refers to different forest locations in which pairs of patch types were studied. Patch type refers to the effect of the different understory patch types. Patch types differ by the presence or absence of the understory evergreen shrub *Rhododendron maximum*. Results are presented for degrees of freedom, F statistic, and P values for *Quercus rubra* seedlings growing in the southern Appalachian forest. Each lightfleck response curve, in the sequence of eight repeated lightflecks, consisted of (1) a 60-s period at low light (photon flux density [PFD] = 10 μmol m^{-2} s^{-1}), (2) 90 s at high light (PFD = 500 μmol m^{-2} s^{-1}), and (3) a 60-s period postlightfleck at low light (PFD = 10 μmol m^{-2} s^{-1}).

Discussion

Site Dynamic Light Environment

In the forest understory, 90% of daily PFD occurs during transient periods of high light (Chazdon 1988; Naumburg et al. 2001a; Montgomery and Givnish 2008). Dynamic photosynthetic response to transient light can account for >65% of total carbon gain for understory plants (Pfitsch and Pearcy 1989). Therefore, it is critical to understand the relationship between understory heterogeneous light and dynamic photosynthesis when evaluating the persistence of advance-regeneration seedlings. In previous research, we established that total PFD in our forest sites where understory shrubs were not present (F sites) averaged 0.5 mol m^{-2} d^{-1} (Lei et al. 2006) and that total PFD in forest sites where understory shrubs were present (SF sites) averaged 0.1 mol m^{-2} d^{-1}. These values correspond to light penetration through the canopy of 0.9%–1.9% (Lei et al. 2006), and they are similar to the WCO values we recorded in this study. These total PFD and WCO values are comparable with those measured in tropical rain forests (Chazdon and Fetcher 1984; Clark et al. 1996) and are lower than those measured in most other forests (Leakey et al. 2004; Montgomery and Givnish 2008). Hence, the PFDs of our research sites are reflective of tropical rain forest and are dim compared with those of other deciduous forests.

Diffuse radiation during the growing season at our research site averaged 40 μmol m^{-2} s^{-1} in F sites and 10 μmol m^{-2} s^{-1} in SF sites (Lei et al. 2006). These values are close to the compensation point for *Quercus rubra* seedlings growing in this forest (Lei et al. 2006). Therefore, *Q. rubra* seedlings are unlikely to persist in the understory without efficiently using the transient periods of high radiation that they experience. Similarly, the survival of many understory seedlings in other forests is dependent on efficient use of sunfleck radiation (Chazdon and Pearcy

1986b; Chazdon 1988; Naumburg and Ellsworth 2002). Moreover, sunfleck PFD is of greater importance to daily leaf carbon gain for seedlings in deeply shaded microhabitats like those of our sites (Naumburg and Ellsworth 2002; Montgomery and Givnish 2008), compared with that of seedlings in higher-light understory locations. Thus, it is most likely that dynamic light is critical to *Q. rubra* seedling persistence.

Sunflecks measured at our research site in previous studies were more abundant and often had higher PFD values in F patches than in SF patches. In fact, an average of 18.3 sunflecks d^{-1} reached PFD of 100 μmol m^{-2} s^{-1} in F patches, compared with only 0.79 sunflecks d^{-1} in SF patches (Nilsen et al. 2001; Lei et al. 2006). Therefore, the lightfleck simulations we performed at 100 μmol m^{-2} s^{-1} PFD in this study represent sunfleck PFD values experienced by seedlings in both F and SF patches. However, lightfleck simulations performed at or above 500 μmol m^{-2} s^{-1} PFD represent light-saturating sunflecks commonly experienced by seedlings growing in F patches and rarely experienced by seedlings in SF patches (*Q. rubra* light saturation points; F = 400 ± 68 μmol m^{-2} s^{-1} PFD; SF = 360 ± 54 μmol m^{-2} s^{-1} PFD; Lei et al. 2006). We found that seedlings in both understory patch types were not able to attain their maximum A values in light-saturating lightflecks <400 s or when exposed to fewer than five sequential lightflecks of a 90-s duration. Therefore, natural sunflecks must be above light saturation (400 μmol m^{-2} s^{-1} PFD), last longer than 400 s, or be very frequent (more than five sunflecks of 90 s in duration within 600 s) for *Q. rubra* seedlings to attain full A induction in the forest understory.

The number of sunflecks that seedlings encountered in F understory patches ranged from 2 to 43 (mean of 19.3) and from 0 to 10 (mean of 0.75) per 12 h in SF understory patches (Nilsen et al. 2001). Also, during diurnal cycles in the growing season, our previous research found that 180 min of light was
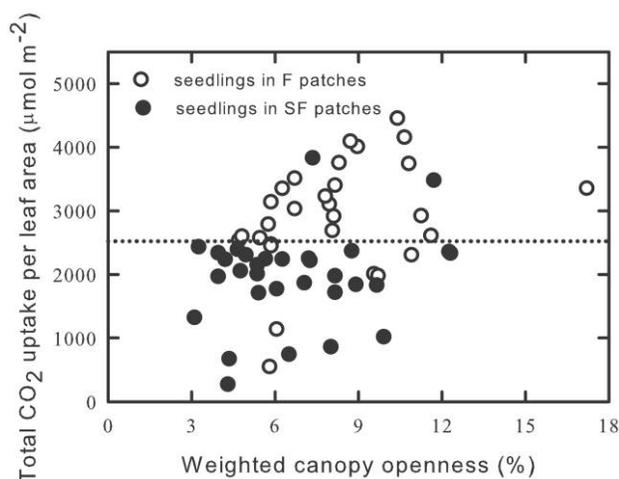
Fig. 5 Total CO_2 uptake from a series of eight replicate lightflecks plotted against the weighted canopy openness (WCO) above *Quercus rubra* seedlings growing in a southern Appalachian forest. Each lightfleck response curve in the sequence consisted of (1) 60 s at low photon flux density (PFD; 10 μmol m^{-2} s^{-1}), (2) 90 s at high PFD (500 μmol m^{-2} s^{-1}), and (3) 60 s at low PFD (10 μmol m^{-2} s^{-1}). Open circles represent seedlings growing in forest patches without understory evergreen shrubs (F patches). Filled circles represent seedlings growing in forest patches with understory evergreen shrubs (SF patches). Dotted line represents total CO_2 uptake of 2500 μmol m^{-2}.

>100 μmol m^{-2} s^{-1} in F patches, while in SF patches only an average of 8 min was >100 μmol m^{-2} s^{-1} (Nilsen et al. 2001). These values correspond to the lowest frequencies of sunflecks for forests in Hawaii (Montgomery and Givnish 2008) and are similar to sunfleck frequencies in tropical rain forests (Chazdon and Fetcher 1984). Given the light intensity and duration of sunflecks in understory environments (Nicotra et al. 1999; Nilsen et al. 2001), rapid photosynthetic induction and optimization of LUE is important for persistence of advance-regeneration seedlings in the understory of deciduous forest. Seedlings in patches with evergreen shrubs will experience fewer sunflecks of lower PFDs. Therefore, to maximize growth and

survival, seedlings in SF patches should respond more efficiently to fewer sunflecks than seedlings in F patches.

Dynamic Photosynthesis of Q. rubra Seedlings

We utilized lightflecks that ranged in duration from 50 s (0.8 min) to 400 s (6.7 min). These durations correspond to the range of durations experienced by seedlings in the southern Appalachian forest (Lei et al. 2006). The sunfleck durations we used correspond to sites with PFD <7 mol m^{-2} d^{-1} in Hawaiian forests (Montgomery and Givnish 2008), which is comparable with the total PFD found at our sites (Lei et al. 2006). Dynamic photosynthesis is affected by both dynamic light and total PFD. Both of these were relatively low at our site. Thus, our research sites are relatively dim compared with those used in many other studies of dynamic photosynthesis.

We found that values of maximum attained A and total CO_2 uptake during lightflecks were relatively constant at lightflecks of 50–150 s. However, for seedlings experiencing 400-s lightflecks, A and total CO_2 uptake per leaf area were significantly higher than they were for seedlings experiencing shorter-duration lightflecks. This result is comparable to dipterocarp seedlings, for which maximum A in 360–480-s sunflecks was twice that of seedlings in 120–240-s lightflecks (Leakey et al. 2004).

The effect of subcanopy shrubs on A and total CO_2 uptake per leaf area during lightflecks was only significant at the 400-s lightfleck duration. The significant difference in dynamic gas exchange between seedlings in F and SF sites observed for longer-duration lightflecks could have been due to a lower IS attained during 400-s sunflecks for seedlings in SF sites. These results agree with previous studies that have demonstrated that IS increases with an increase in site PFD (Naramoto et al. 2001). However, the reverse relationship (decreasing induction with increasing PFD) has been found in other studies of dynamic photosynthesis (Montgomery and Givnish 2008). The relatively low-range PFD experienced by all the seedlings in our study may be the reason why our results seem to oppose those found when a large range of PFDs is used.

If A is calculated on a mass basis rather than an area basis, there are no differences in dynamic photosynthesis between seedlings in F and in SF. The 16% higher SLA value for seed-

Table 3

Weighted Canopy Openness (WCO), Maximum Net Photosynthetic Rate (A_{max}), and Total CO_2 Uptake for *Quercus rubra* Seedlings Exposed to Eight Sequential Lightflecks

| Characteristic | Unit | Patch type | | P ($T \leq t$) |
		F	SF	
WCO	%	8.17 ± .49	6.17 ± .48	.0011
A_{max}	μmol m^{-2} s^{-1}	5.49 ± .20	4.11 ± .18	.0004
Total CO_2 uptake	mmol m^{-2}	2907 ± 156	1955 ± 136	.0088

Note. Each lightfleck response curve in the sequence consisted of (1) a 60-s period at low light (photon flux density [PFD] = 10 μmol m^{-2} s^{-1}), (2) 90 s at high light (PFD = 500 μmol m^{-2} s^{-1}), and (3) a 60-s period postlightfleck at low light (PFD = 10 μmol m^{-2} s^{-1}). Means ± SE are presented for each characteristic. Patch type refers to the effect of the different understory patch types. Patch types differ by the presence or absence of the understory evergreen shrub *Rhododendron maximum*. F = forest locations without an understory thicket of evergreen shrubs; SF = forest locations with an understory of evergreen shrubs. P values denote significant differences between means of the two understory patch types (two-tailed t-test).

lings in SF patches negated the significant difference in maximum A attained during 400-s sunflecks. Therefore, the difference we found in total CO_2 uptake on a leaf area basis was partly due to a difference in leaf thickness.

It takes between 10 and 30 min for A to reach its maximum value during a single sunfleck (Chazdon 1988; Naramoto et al. 2001). Therefore, the sunfleck durations we used in experiment 1 (maximum of 6.5 min) did not cause our seedlings to reach their maximum attainable photosynthesis (full induction). It is well known that a series of shorter sunflecks can induce maximum photosynthesis in a time frame comparable to a single long sunfleck (Pearcy et al. 1985; Leakey et al. 2004). It is better to use a sequence of moderate-length lightflecks to induce maximum A to avoid leaf overheating and photoinhibition (Leakey et al. 2004). Therefore, we utilized a sequence of eight lightflecks of a 90-s duration each to ensure that our seedlings would reach maximum A during lightflecks.

Our multilightfleck simulations indicated that seedlings in F sites and SF sites were at the same IS before experiencing a lightfleck. During lightflecks, seedlings in F patches attained a high IS faster than seedlings growing in SF patches. The time to 50% of maximum A (A_{50}) in transient light is highly correlated with the rate of photosynthetic induction (Allen and Pearcy 2000). We calculated A_{50} for our sequential lightfleck simulation. This is an unconventional but not unprecedented (Horton and Neufeld 1998) technique, because A_{50} is usually determined for a single lightfleck and not a sequence of lightflecks. Therefore, our measurements of A_{50} are longer than those in studies of individual lightflecks because IS decreases during the period of low irradiance between each lightfleck in the sequence. However, we use this determination to compare A_{50} between treatments rather than to compare A_{50} with data from other studies. Seedlings in F patches attain A_{50} significantly faster (mean time to A_{50} in F = 159 s; mean time to A_{50} in SF = 226 s; $P = .04117$, two-tailed t-test with $n = 30$) than do seedlings growing in SF patches during the lightfleck sequence. The faster increase in IS during lightflecks for seedlings in F patches was associated with higher LUE compared with seedlings in SF patches. Therefore, our second hypothesis was supported because seedlings growing in F patches attain a higher IS more quickly in lightflecks, resulting in higher LUE compared with seedlings in SF patches.

Implications for Advance-Regeneration Carbon Gain

Our data suggest that differences in response to heterogeneous light will be important to carbon accumulation by advance-regeneration seedlings in forest understory patches affected by canopy gaps. Canopy gaps cause longer sunflecks with higher PFD in the forest understory than do nongap forests. We found a significantly higher carbon accumulation per leaf area by seedlings in F patches than by seedlings in SF patches when they experience long (400-s) sunflecks. Therefore, shrub presence in the understory under canopy gaps may significantly reduce carbon gain per leaf area of advance-generation seedlings compared with seedlings in canopy gaps without evergreen shrubs present. Studies of forest understory in or out of canopy gaps support this concept (Beckage et al. 2000) because shrubs in gaps have a larger effect on advance-regeneration seedlings than does the gap itself.

We hypothesized that the slope of the relationship between WCO and total carbon gain in a multilightfleck sequence would be greater for seedlings in F patches compared with seedlings growing in SF patches. The slopes of these regressions were weakly positive and not significantly different between seedlings in different patch types. The lack of significance was most likely due to a large variation in total CO_2 accumulation in the range of WCO overlap between the two forest patch types. Our data showed that WCO above seedlings in F patches was often higher compared with that above seedlings in SF patches, but there was a large overlap in WCO between the two understory patch types. Seventy percent of seedlings in F patches and 60% of seedlings in SF patches had WCO values between 5% and 10%. This means that there are many locations under the shrub canopy that have similar canopy openness to sites in forests without shrubs. Therefore, factors other than WCO in SF forest patches contribute to inhibition of advance regeneration. Within that range of WCO overlap, most seedlings in F patches had higher total CO_2 uptake per leaf area compared with seedlings in SF patches. These data suggest that other factors associated with the presence of shrubs inhibit efficient use of sunflecks for carbon gain by advance-regeneration shrubs.

Simulation models for seedling total carbon gain in forest understory have focused on dynamic photosynthesis on a leaf area basis (Naumburg et al. 2001b). These models calculate total carbon gain on the basis of site dynamic light, dynamic photosynthesis, and plant leaf architecture (Pearcy and Yang 1996; Naumburg and Ellsworth 2000; Naumburg et al. 2001a). On the basis of our results, seedlings in F patches should have greater carbon gain per seedling per day because they have faster induction of photosynthesis and more leaf area than seedlings in SF patches. Even if canopy openness is equivalent between seedlings in SF and F patches, our results suggest that the seedling in the F patch should still have a higher daily carbon gain than the seedling in the SF patch. Also, seedling carbon gain could be affected by nocturnal respiration of leaves and the mass of nonphotosynthetic, respiring tissues. Leaf respiration rates were found to be similar for seedlings in F and SF patches (Lei et al. 2006). The higher leaf area of seedlings in F patches would result in a higher nocturnal leaf respiratory loss per plant than that of seedlings in SF patches. Moreover, seedlings in F patches had a higher mass of roots and stems than did seedlings in SF patches. The carbon loss by respiration due to the combination of more nonphotosynthetic mass and higher leaf area of seedlings in F patches could reduce the difference in seedling carbon gain between seedlings in F and SF patches.

Models of annual carbon gain for understory seedlings are normally parameterized by dynamic photosynthesis and dynamic light during the growing season when the tree canopy is closed. However, understory plants can gain a significant amount of their annual carbon gain in the early spring (spring carbon gain subsidy) before the tree canopy closes above them (Lei and Koike 1998; Augspurger 2008) if the seedlings flush leaves before the tree canopy closes. During the early spring, canopy openness in F patches is 2.5 times that in SF patches at our research site (Lei et al. 2006). Advance-regeneration seedlings in F patches would be able to take advantage of the spring subsidy more than seedlings in SF patches would. *Quercus rubra* seedlings did leaf out before the canopy at our site (Lei

et al. 2006) and would be able to take advantage of a spring carbon gain subsidy.

Implications for Shrub Inhibition of Advance Regeneration

Advance-regeneration seedlings are inhibited by understory vegetation in forests worldwide (Royo and Carson 2008). Reduction in PFD associated with the understory vegetation has been considered to be the main factor that reduces survivorship of seedlings in the understory (Beier et al. 2005). However, other factors may contribute to the inhibition of advance-regeneration seedlings by understory vegetation. Many abiotic and biotic processes in the understory may affect advance-regeneration seedlings (Garcia and Houle 2005) at fine scales. The availability of belowground resources such as nutrients and water and the ability of seedlings to access them through mycorrhizae is a possible augmentation to low PFD inhibition of advance-regeneration seedlings (Nilsen et al. 2001; Beckage and Clark 2003; Beier et al. 2005).

Many researchers have proposed descriptive models for the interaction of multiple resource limitations to understory seedlings, among which the best supported is the additive model, where growth limitation by shade is augmented in an additive way by drought or nutrient limitation (Sack and Grubb 2002). This additive interaction between resource limitation induced by understory shrubs and seedling response to transient high PFD may be an important mechanism by which understory shrub vegetation inhibits advance-regeneration seedlings.

On the basis of our results, we envision a descriptive model that involves a spiral of events that leads to mortality of advance-regeneration seedlings. Advance-regeneration seedlings that germinate beneath understory vegetation establish their initial flush from seed reserves. The low PFD, infrequent sunflecks, and reduced LUE would decrease aboveground carbon gain by the initial leaf area. The resultant reduction in transportable carbon from the leaves to the roots would cause reduced root growth and a reduced association with mycorrhizae (Walker and Miller 2002; Walker et al. 2008). Plants that have only a small amount of carbon stored (seed carbon subsidy) will expire after the first growing season. Plants that have a large seed carbon subsidy (e.g., *Quercus* species) can continue to flush leaves during subsequent years of growth but will not be able to keep growing roots. The small root growth and minimal mycorrhizal association would reduce the growth potential of seedlings further than would be expected by low PFD alone because fewer nutrients could be accumulated. The number of annual cycles that the seedling could survive would depend on the size of the seed carbon subsidy relative to the additive effects of light and belowground limitations to carbon gain. This model would predict that tree seedlings with a large seed subsidy could survive longer in the presence of understory vegetation than seedlings with small or no seed subsidy. However, all seedlings would eventually perish. In addition, advance-regeneration seedlings, which depend upon mycorrhizal associations for accessing belowground resources, would be inhibited more than seedlings that do not need mycorrhizae when in the presence of understory vegetation. This descriptive model can provide the framework to design other studies for improving our understanding of the mechanisms by which subcanopy vegetation inhibits advance regeneration.

Acknowledgments

Literature Cited

Abe M, J Izaki, H Miguchi, T Masaki, A Makita, T Nakashizuka 2002 The effects of *Sasa* and canopy gap formation on tree regeneration in an old beech forest. J Veg Sci 13:565–574.

Allen MT, RW Pearcy 2000 Stomatal versus biochemical limitations to dynamic photosynthetic performance in four tropical rainforest shrub species. Oecologia 122:479–486.

Ashton MS, BC Larson 1996 Germination and seedling growth of *Quercus* (section *Erythrobalanus*) across openings in a mixed-deciduous forest of southern New England, USA. For Ecol Manag 80:81–94.

Aubin I, M Beaudet, C Messier 2000 Light extinction coefficients specific to the understory vegetation of the southern boreal forest, Quebec. Can J For Res 30:168–177.

Augspurger CK 2008 Early spring leaf out enhances growth and survival of saplings in a temperate deciduous forest. Oecologia 156: 281–286.

Beckage B, JS Clark 2003 Seedling survival and growth of three forest tree species: the role of spatial heterogeneity. Ecology 84: 1849–1861.

Beckage B, JS Clark, BD Clinton, BL Haines 2000 A long-term study of tree seedling recruitment in southern Appalachian forests: the effects of canopy gaps and shrub understories. Can J For Res 30:1617–1631.

Beier CM, JL Horton, JF Walker, BD Clinton, ET Nilsen 2005 Carbon limitation leads to suppression of first year oak seedlings beneath evergreen understory shrubs in southern Appalachian hardwood forests. Plant Ecol 176:131–142.

Boerner REJ 2006 Unraveling the Gordian knot: interactions among vegetation, topography, and soil properties in the central and southern Appalachians. Bull Torrey Bot Club 133:321–361.

Canham CD 1989 Different responses to gaps among shade-tolerant tree species. Ecology 70:548–550.

Chazdon RL 1988 Sunflecks and their importance to forest understorey plants. Adv Ecol Res 18:1–63.

Chazdon RL, N Fetcher 1984 Photosynthetic light environments in a lowland tropical rain forest in Costa Rica. J Ecol 72:553–564.

Chazdon RL, CB Field 1987 Photographic estimation of photosynthetically active radiation: evaluation of a computerized technique. Oecologia 73:525–532.

Chazdon RL, RW Pearcy 1986a Photosynthetic responses to light variation in rain forest species. 1. Induction under constant and fluctuating light conditions. Oecologia 69:517–523.

——— 1986b Photosynthetic responses to light variation in rain forest species. 2. Carbon gain and photosynthetic efficiency during lightflecks. Oecologia 69:524–531.

Clark DB, DA Clark, PM Rich, S Weiss, SF Oberbauer 1996 Landscape scale evaluation of understory light and canopy structure: methods and application in a Neotropical lowland rain forest. Can J For Res 26:747–757.

Clinton BD 2003 Light, temperature, and soil moisture responses to elevation, evergreen understory, and small, canopy gaps in the southern Appalachians. For Ecol Manag 186:243–255.

Collet C, A Piboule, O Leroy, H Frochot 2008 Advance *Fagus sylvatica* and *Acer pseudoplatanus* seedlings dominate tree regeneration in a mixed broadleaved former coppice with standards forest. Forestry 81:135–150.

Day FPJ, DL Phillips, CD Monk 1988 Forest communities and patterns. Pages 141–150 in WT Swank, D Crossley, eds. Forest hydrology and ecology at Coweeta. Springer, New York.

Denslow JS, E Newell, AM Ellison 1991 The effect of understory palms and cyclanths on the growth and survival of *Inga* seedlings. Biotropica 23:225–234.

Dillaway DN, JW Stringer, LK Rieske 2007 Light availability influences root carbohydrates, and potentially vigor, in white oak advance regeneration. For Ecol Manag 250:227–233.

Elliott KJ, WT Swank 2008 Long-term changes in forest composition and diversity following early logging (1919–1923) and the decline of American chestnut (*Castanea dentata*). Plant Ecol 197:155–172.

Elliott KJ, JM Vose, WT Swank, PV Bolstad 1999 Long-term patterns in vegetation-site relationships in a southern Appalachian forest. Bull Torrey Bot Club 126:320–334.

Frey BR, MS Ashton, JJ McKenna, D Ellum, A Finkral 2007 Topographic and temporal patterns in tree seedling establishment, growth, and survival among masting species of southern New England mixed deciduous forests. For Ecol Manag 245:54–63.

Gallardo A 2003 Spatial variability of soil properties in a floodplain forest in northwest Spain. Ecosystems 6:564–576.

Garcia D, G Houle 2005 Fine-scale spatial patterns of recruitment in red oak (*Quercus rubra*): what matters most, abiotic or biotic factors? Ecoscience 12:223–235.

George LO, FA Bazzaz 1999 The fern understory as an ecological filter: emergence and establishment of canopy tree seedlings. Ecology 80:833–845.

Griscom BW, PMS Ashton 2003 Bamboo control of forest succession: *Guadua sarcocarpa* in southeastern Peru. For Ecol Manag 175:445–454.

Gross KL, KS Pregitzer, AJ Burton 1995 Spatial variation in nitrogen availability in three successional plant communities. J Ecol 83:357–367.

Horton JL, HS Neufeld 1998 Photosynthetic responses of *Microstegium vimineum* (Trin.) a. Camus, a shade tolerant, C_4 grass, to variable light environments. Oecologia 114:11–19.

Ito H, T Hino 2004 Effects of deer, mice and dwarf bamboo on the emergence, survival and growth of *Abies homolepis* (Piceaceae) seedlings. Ecol Res 19:217–223.

Jackson RB, MM Caldwell 1993 The scale of nutrient heterogeneity around individual plants and its quantification with geostatistics. Ecology 74:612–614.

Kelly VR, CD Canham 1992 Resource heterogeneity in oldfields. J Veg Sci 3:545–552.

Klinka K, RE Carter, MC Feller, Q Wang 1989 Relations between site index, salal, plant communities, and sites in coastal Douglas-fir ecosystems. Northwest Sci 63:19–28.

Leakey ADB, JD Scholes, MC Press 2004 Physiological and ecological significance of sunflecks for dipterocarp seedlings. J Exp Bot 56:469–482.

Lei TT, T Koike 1998 Some observations of phenology and ecophysiology of *Daphne kamtschatica* Maxim. Var. Jezoensis (Maxim.) Ohwi, a shade deciduous shrub, in the forest of northern Japan. J Plant Res 111:207–212.

Lei TT, ET Nilsen, SW Semones 2006 Light environment under *Rhododendron maximum* thickets and estimated carbon gain of regenerating forest tree seedlings. Plant Ecol 184:143–156.

Lei TT, SW Semones, JF Walker, BD Clinton, ET Nilsen 2002 Effects

of *Rhododendron maximum* thickets on tree seed dispersal, seedling morphology, and survivorship. Int J Plant Sci 163:991–1000.

Madsen P, K Hahn 2008 Natural regeneration in a beech-dominated forest managed by close-to-nature principles: a gap cutting based experiment. Can J For Res 38:1716–1729.

Mallik AU 2003 Conifer regeneration problems in boreal and temperate forests with ericaceous understory: role of disturbance, seedbed limitation, and keystone species change. Crit Rev Plant Sci 22:341–366.

Messaoud Y, G Houle 2006 Spatial patterns of tree seedling establishment and their relationship to environmental variables in a cold temperate deciduous forest of eastern North America. Plant Ecol 185:319–331.

Messier C 1993 Factors limiting early growth of western redcedar, western hemlock and sitka spruce seedlings on ericaceous dominated clear cut sites in coastal British Columbia. For Ecol Manag 60:181–206.

Messier C, TW Honer, JP Kimmins 1989 Photosynthetic photon flux density, red : far-red ratio, and minimum light requirement for survival of *Gaultheria shallon* in western redcedar–western hemlock stands in coastal British Columbia. Can J For Res 19:1470–1477.

Mitchell RJ, RH Marrs, MG Le Duc, MHD Auld 1997 A study of succession on lowland heaths in Dorset, southern England: changes in vegetation and soil chemical properties. J Appl Ecol 34:1426–1444.

Monk CD, DT McGinty, FP Day 1985 The ecological importance of *Kalmia latifolia* and *Rhododendron maximum* in the deciduous forest of the southern Appalachians. Bull Torrey Bot Club 112:187–193.

Montgomery RA, TJ Givnish 2008 Adaptive radiation of photosynthetic physiology in the Hawaiian lobeliads: dynamic photosynthetic responses. Oecologia 155:455–467.

Nakashizuka T 1987 Regeneration dynamics of beech forests in Japan. Vegetatio 69:169–175.

Naramoto M, Q Han, Y Kakubari 2001 The influence of previous irradiance on photosynthetic induction in three species grown in the gap and understory of a *Fagus crenata* forest. Photosynthetica 39:545–552.

Naumburg E, DS Ellsworth 2000 Photosynthesis sunfleck utilization potential of understory saplings growing under elevated CO_2 in FACE. Oecologia 122:163–174.

——— 2002 Short term light and leaf photosynthetic dynamics affect estimates of daily understory photosynthesis in four tree species. Tree Physiol 22:393–401.

Naumburg E, DS Ellsworth, GG Katul 2001*a* Modeling dynamic understory photosynthesis of contrasting species in ambient and elevated carbon dioxide. Oecologia 126:487–499.

Naumburg E, DS Ellsworth, RW Pearcy 2001*b* Crown carbon gain and elevated [CO_2] responses of understorey saplings with differing allometry and architecture. Funct Ecol 15:263–273.

Nicotra AB, RL Chazdon, SVB Iriarte 1999 Spatial heterogeneity of light and woody seedling regeneration in tropical wet forests. Ecology 80:1908–1926.

Nilsen ET, BD Clinton, TT Lei, OK Miller, SW Semones, JF Walker 2001 Does *Rhododendron maximum* L. (Ericaceae) reduce the availability of resources above and belowground for canopy tree seedlings? Am Midl Nat 145:325–343.

Pearcy RW, O Bjorkman, MM Caldwell, JE Keeley, RK Monson, BR Strain 1987 Carbon gain by plants in natural environments. BioScience 37:21–29.

Pearcy RW, K Osteryoung, HW Calkin 1985 Photosynthetic response to dynamic light environments by Hawaiian trees. Plant Physiol 79:896–902.

Pearcy RW, WM Yang 1996 A three-dimensional crown architecture model for assessment of light capture and carbon gain by understory plants. Oecologia 108:1–12.

Pfitsch WA, RW Pearcy 1989 Daily carbon gain by *Adenocaulon*

bicolor (Asteraceae), a redwood forest understory herb, in relation to its light environment. Oecologia 80:465–470.

Royo AA, WP Carson 2006 On the formation of dense understory layers in forests worldwide: consequences and implications for forest dynamics, biodiversity, and succession. Can J For Res 36: 1345–1362.

——— 2008 Direct and indirect effects of a dense understory on tree seedling recruitment in temperate forests: habitat-mediated predation versus competition. Can J For Res 38:1634–1645.

Runkle JR 1990 Gap dynamics in an Ohio *Acer-Fagus* forest and speculations on the geography of disturbance. Can J For Res 20: 632–641.

Sack L, PJ Grubb 2002 The combined impacts of deep shade and drought on the growth and biomass allocation of shade-tolerant woody seedlings. Oecologia 131:175–185.

Shure DJ, DL Phillips, PE Bostick 2006 Gap size and succession in cutover southern Appalachian forests: an 18 year study of vegetation dynamics. Plant Ecol 185:299–318.

Swank W, D Crossley 1988 Forest hydrology and ecology at Coweeta. Springer, New York.

Taylor AH, ZS Qin 1992 Tree regeneration after bamboo dieback in Chinese *Abies-Betula* forests. J Veg Sci 3:253–260.

Veblen TT 1989 Tree regeneration responses to gaps along a transandean gradient. Ecology 70:541–543.

Walker JF, OK Miller 2002 Ectomycorrhizal sporophore distributions in a southeastern Appalachian mixed hardwood/conifer forest with thickets of *Rhododendron maximum*. Mycologia 94:221–229.

Walker JF, OK Miller, JL Horton 2008 Seasonal dynamics of ectomycorrhizal fungus assemblages on oak seedlings in the southeastern Appalachian mountains. Mycorrhiza 18:123–132.

Walters MB, PB Reich 1996 Are shade tolerance, survival, and growth linked? low light and nitrogen effects on hardwood seedlings. Ecology 77:841–853.

Wurzburger N, RL Hendrick 2006 *Rhododendron* thickets alter N cycling and soil extracellular enzyme activities in southern Appalachian hardwood forests. Pedobiologia 50:563–576.

Yeakley JA, WT Swank, LW Swift, GM Hornberger, HH Shugart 1998 Soil moisture gradients and controls on a southern Appalachian hillslope from drought through recharge. Hydrol Earth Syst Sci 2: 41–49.