Soil nitrogen availability influences seasonal carbon allocation patterns in sugar maple (*Acer saccharum*)

MARIANNE K. BURKE,[1] DUDLEY J. RAYNAL, AND MYRON J. MITCHELL

College of Environmental Science and Forestry, State University of New York, Syracuse, NY 13210, U.S.A.

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The influence of soil N availability on growth, on seasonal C allocation patterns, and on sulfate-S content in sugar maple seedlings (*Acer saccharum* Marsh.) was tested experimentally. Relative to controls, the production of foliage doubled in response to high N availability, and the production of foliage, stems, coarse roots, and fine roots was halved in response to N deprivation. The period of foliage production was lengthened by fertilization and the period of fine root production was shortened by N deprivation compared with controls. In August, a shift in priority C allocation from foliage to roots occurred in the N-deprivation treatment. Therefore, during this month alone, the shoot to root ratio was greater in fertilized plants (1.0) than in N-deprived plants (0.5). Allocation to storage reserves was highest in N-deprived and lowest in fertilized plants (average 160 vs. 125 mg glucose/g biomass produced), and storage in roots of unfertilized plants commenced earlier (August) than in fertilized plants (after September). This resulted in unfertilized plants having higher fine root starch concentrations (5.2%) than fertilized plants (4.0%) in December, although sugar concentrations were similar (5.7%). The lengthened season of shoot growth and the low starch to sugar ratios in fine roots of fertilized plants are symptoms consistent with a higher risk of frost injury and microbial pathogen infection. Although soil N availability did not influence the sulfate-S content in foliage, N deprivation resulted in higher organic S to N ratios. This suggests that more S-containing proteins are produced when N availability is poor.

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L'influence de la disponibilité en N dans le sol sur la croissance, sur les patrons saisonniers d'allocation du C et sur le contenu en S sous forme de sulfates chez les semis d'érable à sucre (*Acer saccharum* Marsh.) a été déterminée expérimentalement. Comparativement aux témoins, la production de feuillage a doublé en réponse à une disponibilité élevée en N alors que la production de feuillage, de pousses, de grosses racines et de racines fines a été réduite de moitié en réponse à une carence en N. La période de production de feuillage était allongée par la fertilisation alors que la période de production de racines fines était raccourcie par une carence en N. En août, l'allocation prioritaire de C changeait du feuillage vers les racines dans le traitement comportant une carence en N. Par conséquent, seulement pendant ce mois, le ratio tige:racine était plus élevé chez les plants fertilisés (1,0) que chez les plants carencés en N (0,5). L'allocation vers les réserves pour fin d'emmagasinage était la plus forte chez les plants carencés et la plus faible chez les plants fertilisés (une moyenne de 160 contre 125 mg de glucose/g de biomasse produite). L'emmagasinage dans les racines des plants non fertilisés commençait plus tôt (août) que chez les plants fertilisés (après septembre). Cela provoquait une concentration en amidon dans les racines fines plus élevée chez les plants non fertilisés (5,2%) que chez les plants fertilisés (4,0%) en décembre même si la concentration en sucre était semblable (5,7%). L'allongement de la saison de croissance au niveau des parties aériennes et le faible ratio amidon:sucre dans les racines fines chez les plants fertilisés sont des indices fiables d'un risque plus élevé de dommage causé par le gel et d'infection par des microorganismes pathogènes. Même si la disponibilité en N dans le sol n'a pas influencé le contenu en S sous forme de sulfates dans le feuillage, la carence en N a provoqué une augmentation du ratio S organique : N. Ces résultats suggèrent qu'il y a une plus forte production de protéine contenant du S lorsque la disponibilité en N est faible.

[Traduit par la rédaction]

Introduction

Growth and survival of plants depend on maintaining a positive carbon (C) balance (Mooney 1972; Chapin *et al.* 1987; Tilman 1988). To maintain a positive C balance and optimize growth, plants must allocate assimilates appropriately among various competing sinks. These sinks include roots, shoots, and when plants are exposed to large seasonal climatic changes, storage reserves for maintenance during and after the period of dormancy. If environmental conditions change, C allocation patterns must change if a positive C balance is to be maintained (Osmond *et al.* 1987). Hence, plant survival depends on whether C allocation is appropriate and flexible enough to withstand the many and variable environmental stresses.

The study of C allocation in trees has focused on the production of harvestable yield, although the importance of examining yield as part of the total annual C budget has also been established (Cannell 1985). Carbon allocation among tissues in trees was shown to be a sensitive indicator of the response to environmental changes (Adams *et al.* 1990), but seldom has the seasonal allocation of photosynthate between structural and reserve compounds been used to measure responses to environmental changes in naturally growing plants (Chapin *et al.* 1986). To date, there is little quantitative information available for allocation in deciduous tree species (Dickson 1989), although some descriptive data exist for *Populus* (Isebrands and Nelson 1983; Dickson 1986).

A low C balance has been linked with reduced vigor in some stands of sugar maple (*Acer saccharum* Marsh.). Trees of low vigor are more susceptible to frost injury (Gregory and Wargo 1986; Gregory *et al.* 1986) and microbial pathogens

[1]Present address: Horn Point Environmental Laboratory, University of Maryland, P.O. Box 775, Cambridge, MD 21613, U.S.A.

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(Wargo and Houston 1974; Wargo 1981), and this may be related to nutrient imbalances that interfere with C metabolism caused by excess nitrogen (N) availability (Nihlgård 1985; Aber *et al.* 1989; Stanturf *et al.* 1989; McNulty *et al.* 1990). However, we presently have a poor understanding of how N availability influences C metabolism and allocation in sugar maple trees and of how these processes are linked with the symptoms of low vigor.

In addition to effects on C allocation, N availability may influence sulfur (S) cycling in sugar maple. In conifers, S absorbed in excess of that needed to balance N cycles as sulfate rather than organic S (Lambert and Turner 1977; Turner *et al.* 1977, 1980). Plant sulfate concentration therefore can be a means for diagnosing N sufficiency. The influence of N availability on sulfate-S cycling has not been established for deciduous trees.

Before we can determine whether excess N availability influences the vigor of sugar maple trees, we must understand how N availability influences growth, C allocation, and sulfate cycling. Of particular interest is the effect of N availability on C allocation to carbohydrate reserves, because the quantity of stored reserves is related to vigor in sugar maple (Wargo 1972; Wargo *et al.* 1972; Gregory and Wargo 1986; Gregory *et al.* 1986). The objectives of the present study were to determine the influence of N availability on seasonal C allocation patterns in sugar maple seedlings and to determine whether N availability influenced the form in which S cycled.

Study site

The experiment was conducted at the Lafayette Experiment Station of the State University of New York, College of Environmental Science and Forestry, near Syracuse, New York, from May through December 1987. Soil on the site is a Glossoboric Hapludalf, a Wassaic sandy loam (Hutton and Rice 1977). The uniformly illuminated site has a southwest exposure.

Materials and methods

Soil amendments

Soil on the site was plowed, disked, and analyzed to determine nutrient status. In a systematic design, 25 soil samples were collected to a depth of 15 cm. Samples were air dried for 1 week, passed through a 2-mm sieve, and divided into subsamples. Soil mass was determined by oven-drying (at 105°C) subsamples of air-dried soil to a constant mass and determining moisture correction factors for air-dried soil. Air-dried soil was analyzed for organic matter (using Walkley–Black wet oxidation) and N concentrations (using the macro-Kjeldahl method). Exchangeable calcium, magnesium, and potassium were extracted with ammonium acetate and measured using atomic absorption spectroscopy. Available phosphorus was extracted using sulfuric acid and $(NH_4)SO_4$ solution and was measured spectrophotometrically. All soil analyses were done according to Wilde *et al.* (1972).

Soil nutrient deficiencies were corrected by applying $MgSO_4$ at 3060 $kg \cdot ha^{-1}$, K_2O (0:0:60, N–P–K) at 560 $kg \cdot ha^{-1}$, $CaSO_4$ at 1480 $kg \cdot ha^{-1}$, and P_2O_4 (0:20:0, N–P–K) at 3430 $kg \cdot ha^{-1}$ to an appropriate level for hardwood nursery soil (Stone 1980). To incorporate the fertilizer homogeneously, the top 15 cm of the soil was rototilled twice to a depth of 15 cm.

Four hundred sixty plastic pots (9 L) were numbered, filled with the amended soil, and then randomly assigned to one of three N availability treatments (N deprivation, +N, or +N+C) or the control. Soil N availability was either reduced or increased in pots assigned to the treatments by manipulating the C:N ratios. Soil N availability was reduced in the pots assigned to the N-deprivation treatment by adding 4.7 g glucose and 13.8 g sawdust per kilogram of soil. For the +N treatment, 4.5 g NH_4NO_3 per kilogram of soil was added, and for the +N+C treatment, 9.1 g of both glucose and sawdust and 18.0 g NH_4NO_3 per kilogram of soil was added. The +N+C treatment was used to test the effect of very high plant N availability, and the C source was added in this treatment to prevent rapid leaching of the added N from the soil by providing an energy source for microbes. The target C:N ratios of the mineral soil in the N-deprivation, control, +N, and +N+C treatments were 36, 18, 5, and 3, respectively. Organic matter contents were 3.8, 2.0, 2.0, and 3.8% dry mass, respectively.

Index of N availability

The amount of ammonium and nitrate available to plants was indexed using a method modified from Keeney (1980) and Gosz and White (1986). Approximately 100 g of soil was collected from six randomly chosen pots from each treatment on May 20, June 15, July 15, and August 15, 1987. The samples were stored at 1°C in paper bags until processed (within 24 h of collection), whereupon they were thoroughly mixed by hand.

Approximately 10 g of fresh soil from each sample was dried at 105°C for 1 day, and the differences in masses were used to estimate the moisture content in fresh soil. Seven grams of fresh soil (the dry mass equivalents) were extracted for nitrate and ammonium using 35 mL of 2 M KCl. After 1 h of vigorous shaking, the mixtures and blanks were filtered using Whatman 1 qualitative filter paper and a vacuum filtration system. Extractants were stored at 1°C in capped plastic bottles, then analyzed for nitrate according to Markus *et al.* (1985) using a Technicon auto-analyzer I and for ammonium using a Wescan ammonium analyzer (Scott *et al.* 1989).

Immediately after collection, approximately 40 g of fresh soil was placed in a 125-mL Erlenmeyer flask, and the soil moisture was adjusted to 18% (by mass). Each flask was then covered with Parafilm (which had been perforated for air exchange), incubated for 20 days, and the soil moisture was adjusted biweekly. After incubation, extractable ammonium and nitrate were determined and used as indices of N availability (Johnson and Edwards 1979; Birk and Vitousek 1986). In addition, total extractable inorganic N was used as an availability index, as in Shumway and Atkinson (1978) and Kelly and Johnson (1982).

Experimental design

Four hundred 2-year-old sugar maple seedlings were purchased from a commercial nursery. Before planting, seedlings were ranked by height and labelled to maintain identity, and the stem length and root-collar diameter were measured. Every tenth seedling was removed as a subsample for estimating the original mass of the planted seedlings. Seedlings were randomly assigned to the pots and planted on May 9, 1987. Between the time of unpacking and planting, seedlings were immersed in cold water to their root collars to insure maximum hydration of root cells.

Pots of soil had been buried to their rims before seedlings were planted in order to maintain natural soil temperatures. Soil moisture was kept at ca. −10 kPa using a drip irrigation system; soil moisture was monitored with tensiometers.

Pots were weeded and insect defoliation minimized by regular manual removal of weeds and insects. In addition, deer were restricted from the plot by a baited electric fence (Porter 1983).

Harvest technique

Ten seedlings from each treatment were randomly selected on May 20, June 15, August 15, September 13, and December 15, 1987. During harvest, the pots were unearthed, seedlings were removed from soil, soil was carefully inspected to insure that all the fine roots were collected, roots were rinsed in tap water, and root systems were wrapped individually in brown paper bags. Harvested seedlings were then stored at 1°C until processing (within 24 h) and were kept on ice during processing.

The total stem height, stem height increment (new stem), root length, and root-collar diameter of each seedling were recorded. Leaves were severed at the base of the petiole, stems were severed from the root systems at the root collars, and the shoot was separated into new and old stem at the bud scar. Roots were sorted into coarse

(>2-mm) and fine (≤2-mm) fractions. Leaf areas were measured on a Decagon Devices Incorporated Delta-T area meter after calibration with standards made from photocopied sugar maple leaves. Foliage on the seedlings left for the December 15 harvest was allowed to naturally senesce and abscise while encased in cheesecloth bags. This effectively collected the litter before it fell to the ground.

All tissues were washed with tap water, chopped into small pieces, placed in vials, frozen, freeze-dried, and ground with a Wiley mill with a 20-mesh screen.

Tissue analysis

Foliage obtained during the September 13 harvest and leaf litter collected during the autumn were analyzed for N and C concentrations using a Perkin–Elmer 240C CHN analyzer. The samples were analyzed for total S (David *et al.* 1989) using an automated apparatus (Leco model SC-132), and for sulfate using hydriodic acid (HI) reduction (Landers *et al.* 1983). Organic S was determined by subtracting HI-reducible S from total S.

Nonstructural carbohydrate concentrations of all tissues were measured for seedlings obtained during the initial (May 9) and subsequent harvests (August 15, September 13, and December 15). Soluble sugars were extracted from the tissue using hot 80% ethanol (Association of Official Analytical Chemists 1980) and a thorough deionized water rinse. The residue left after the ethanol extraction was evaporated to dryness and was extracted for starch using cold (0°C) 30% perchloric acid in a modified version of the Hansen and Møller (1975) method. This acid concentration insured complete starch extraction but minimized the potential for hydrolysis of structural carbohydrates as with a stronger acid solution (Hassig and Dickson 1979). Sugar and starch concentrations in the extractants were then measured using the phenol-sulfuric acid colorimetric method (Hodge and Hofneiter 1962) with an absorbance of 490 nm. This method was chosen over other methods because it is rapid, sensitive, accurate, specific for carbohydrates, and is not affected by phenolics or perchloric acid (Hansen and Møller 1975). Moreover, preliminary tests with reagent grade corn starch showed the method was highly accurate for starch concentrations. Apparently, the concentrated sulfuric acid hydrolyzed the starch into monosaccharides, simplifying interpretation of the results over those that would have been obtained using the iodine method. Both sugar and starch concentrations were expressed in glucose equivalents.

Carbon allocation

Carbon allocation to each tissue between sampling dates was determined as the change in mass of that tissue divided by the total change in plant mass. Allocations to storage reserves at the whole-plant level were calculated by dividing each of the whole-plant starch and the starch plus sugar pools by the change in total mass (Chapin *et al.* 1990) since the last sampling date.

Statistical analyses

A completely randomized design with a fixed effects model was used for the ANOVA. For each variable, homogeneity of variance among treatments was tested using Bartlett's test (Sokal and Rohlf 1981). When there was heteroscedasticity, the data were transformed to natural logarithms and then analyzed with Bartlett's tests. Once homogeneity of variance was determined, ANOVA were performed using a completely randomized design. When only two treatments were compared, *t*-tests were used. In the occasional cases where natural log transformations were not successful in producing homoscedastistic data, the data were analyzed with the analogous nonparametric Kruskal–Wallis one-way ANOVA (Sokal and Rohlf 1981).

Means were compared for all variables showing treatment effects using Tukey's honestly significantly different test, except when missing data required the use of Sheffé's test (Kirk 1982). Relationships between soil N availability and foliage N, C, and S concentrations were tested using regression analyses.

Most of the analyses were conducted using SYSTAT (Wilkinson 1988), but all Scheffé's tests were conducted using SAS (SAS Institute Inc. 1985).

Results

Soil N availability

Fertilized treatments had higher KCl-extractable ammonium and nitrate before incubation than unfertilized treatments. In addition, fertilized treatments had the largest declines in KCl-extractable ammonium and rises in extractable nitrate during incubations (Fig. 1).

Extractable nitrate and ammonium before incubation declined in all treatments as the season progressed, and the magnitude of the decline was greatest in the +N+C treatment (Fig. 1). The seasonal mean nitrate and ammonium availabilities, measured as the average amounts of extractable N after incubation, were 4.2, 4.6, 102.2, and 133.9 mg NO_3-N/kg soil and 4.2, 0.7, 63.7, and 114.0 mg NH_4-N/kg soil for the N-deprivation, control, +N, and +N+C treatments, respectively.

Foliage nutrient analyses

Patterns of soil N availability were reflected in the N concentrations of foliage. Soil N availability (indexed as the seasonal mean of extractable nitrate and ammonium after incubation) was reflected in green foliage and leaf litter N concentrations ($r^2 = 0.96$, $p = 0.05$; $r^2 = 0.99$, $p = 0.01$, respectively). Nitrogen concentrations of green foliage were lower in the N-deprived seedlings (1.94 ± 0.1% (mean ± 1 SEM)) than in the +N+C seedlings (2.3 ± 0.1%) or in the +N seedlings (2.5 ± 0.1%), and lower in the control (2.2 ± 0.1%) than in the +N seedlings. Concentrations of N in leaf litter did not differ among treatments ($p = 0.07$, average 1.0%). There were greater amounts of N resorbed in the fertilized treatments, but a similar proportion (50–58%) of the initial N was resorbed from foliage in all treatments.

Carbon concentrations declined with increasing N availability in green foliage and leaf litter, but were not correlated with soil N availability in either case. Concentrations in green foliage did not differ among treatments (average 46.6%), but the C:N ratio was greater in the N-deprivation treatment (24.8 ± 0.8) than in the +N or +N+C treatment (18.5 ± 0.4 and 19.0 ± 1.5, respectively). Carbon concentrations in litter from the N-deprived seedlings (47.10 ± 0.4%) were greater than in the +N or +N+C treatments (44.2 ± 0.4 and 45.1 ± 0.6%, respectively), and concentrations in the control (46.66 ± 0.42) were greater than in the +N+C treatment ($p = 0.001$). After abscission, C:N ratios were greater in the control (54.0 ± 3.7) than in the +N+C treatment (40.3 ± 4.0), but not the N-deprived (51.2 ± 3.4) or +N seedlings (41.8 ± 3.2).

Sulfur in green foliage averaged 0.27% and did not differ among treatments ($p = 0.18$). Similarly, there were not significant differences in the N:S ratios, but there was the expected trend of the highest (10.1) and lowest (7.0) values being found in the +N and N-deprivation treatments, respectively. There were also no differences in either sulfate (average 0.029%) or organic-S foliar sulfate (average 0.26%) concentrations. However, there was a higher ($p = 0.05$) organic S : N ratio in the N-deprived (1.34 ± 0.09) than in the +N+C (1.00 ± 0.07, $p = 0.05$) seedlings.

Biomass production

Soil N availability influenced both the amount of foliage produced and the timing of production. Foliage production was considerably lower for seedlings in the N-deprivation treatment than in the other treatments until August 15 ($p = 0.001$, Fig. 2). Between August and September, foliage

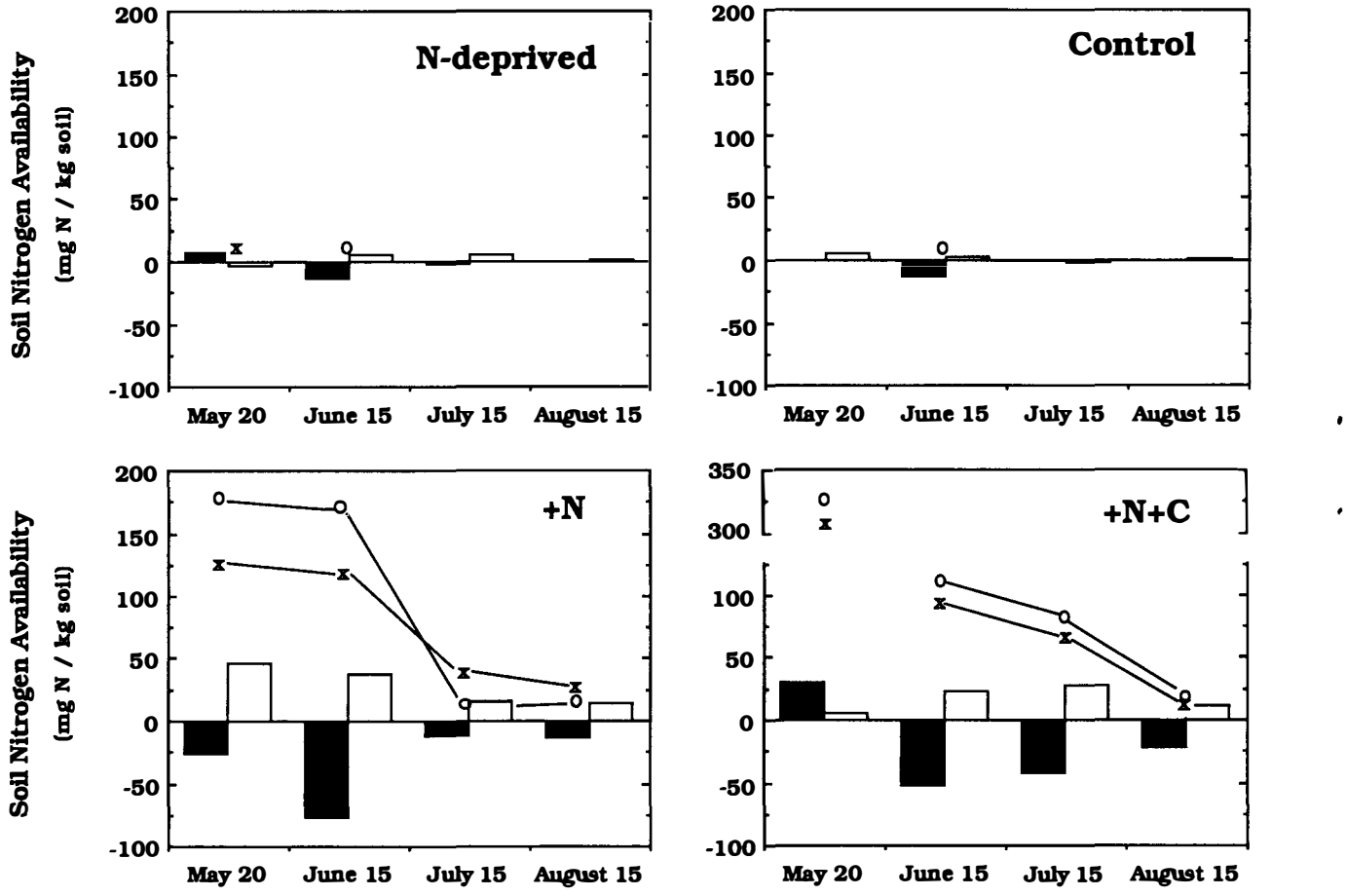

FIG. 1. Seasonal changes in soil N availability for each treatment and the control. Shown are the KCl-extractable ammonium (○) and nitrate (x) before each incubation, and the change in KCl-extractable nitrate (white bars) and ammonium (black bars) after 20-day incubations ($n = 6$).

production stopped in the unfertilized seedlings but continued in the fertilized seedlings until October (M. Burke, personal observation). By September 13, foliage biomass on the N-deprived seedlings was < control < fertilized seedlings ($p = 0.001$). Foliage senescence began in September in the unfertilized treatments but was delayed until October in the fertilized treatments.

In August, the leaf area of the N-deprived seedlings was lower (221.4 ± 27.4 cm^2) than in the control (476.9 ± 61.2), +N (491.0 ± 67.3), and +N+C (549.3 ± 107.7) treatments ($p = 0.001$). In September, leaf areas of the N-deprived and control seedlings (194.4 ± 22.3 and 417.0 ± 100.7) were less than the +N and +N+C treatments (920.7 ± 198.0 and 924.1 ± 206.0, $p = 0.001$).

Stem biomass in the N-deprived seedlings was less than in other treatments in August ($p = 0.02$), September ($p = 0.03$), and December ($p = 0.003$, Fig. 2). Coarse root biomass was least in the N-deprivation treatment, but there were significant differences only in December ($p = 0.05$).

Fine root biomass did not differ among treatments until August ($p = 0.03$, Fig. 2). In the N-deprived seedlings, net fine root production stopped in August and after September, was lower than in the +N+C seedlings. By mid-December, fine root biomass in the control and fertilized seedlings were not different but were greater than in the N-deprived plants ($p = 0.004$).

Nonstructural carbohydrates

Foliar sugar concentrations were higher in the N-deprived than in the +N+C plants in August ($p = 0.004$) and higher than in all other treatments in September ($p = 0.001$, Fig. 3). Starch concentrations were higher in unfertilized than in fertilized seedlings in August ($p = 0.001$), and concentrations in +N+C plants were lower than in controls in September ($p = 0.04$, Fig. 4).

At all sampling times, sugar concentrations in new shoots of fertilized seedlings were greater than in the N-deprivation treatment, and during December, concentrations in the control and +N seedlings were lower than in the +N+C plants ($p = 0.001$, Fig. 3). In contrast, starch concentrations in new shoots were not significantly different in August and December, but during September, concentrations in new shoots in N-deprived seedlings were slightly lower than in the +N treatment ($p = 0.06$, Fig. 4).

Stem sugar concentrations were greater in +N+C than in control plants in August ($p = 0.03$) and greater than in N-deprived plants in December ($p = 0.05$) (Fig. 3*c*). In September, starch concentrations were greater in the control than in the +N+C treatment ($p = 0.04$, Fig. 4).

Sugar concentrations in coarse roots were greater in the +N+C than in the N-deprivation treatment in August ($p = 0.02$), and there were no differences among treatments in September. By December, sugar concentrations in coarse roots of N-deprivation and +N+C treatments were lower than in the

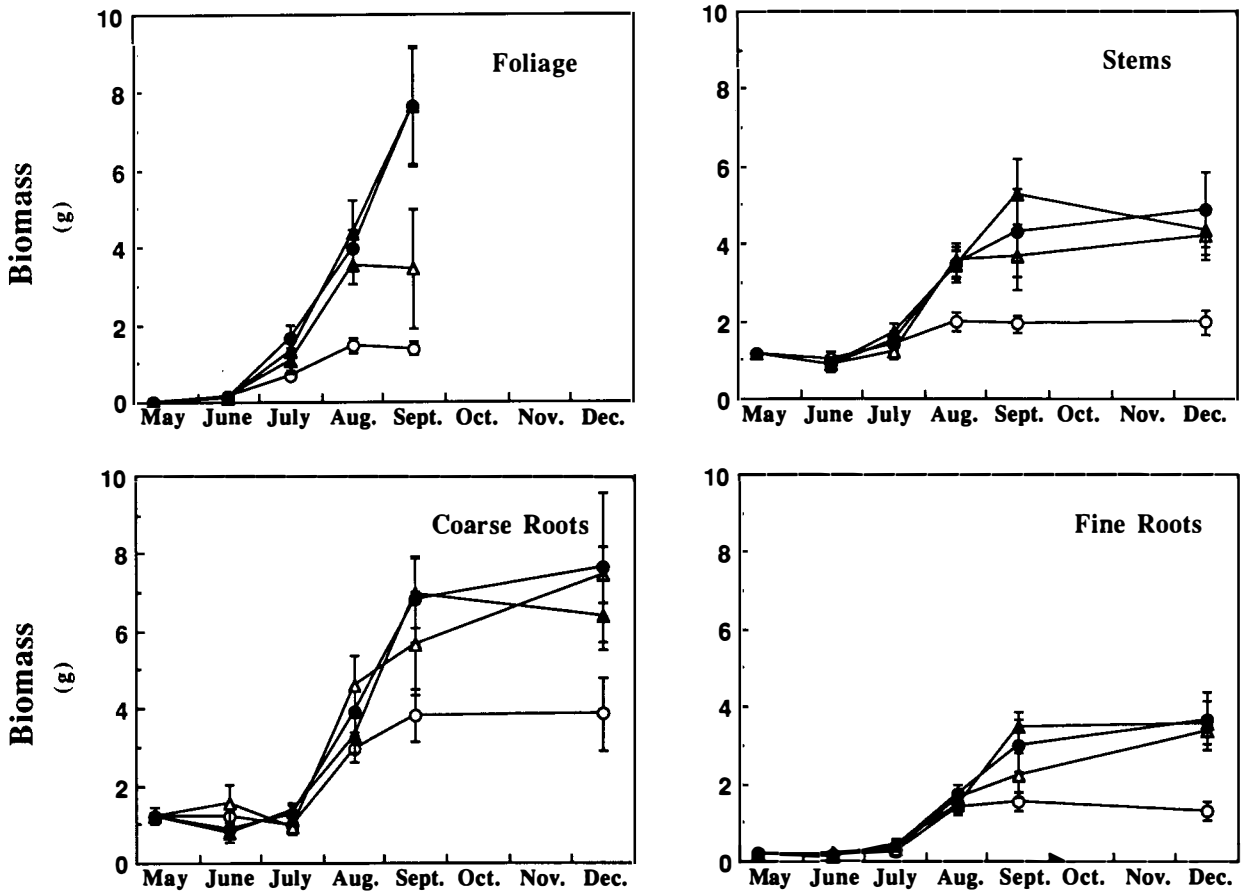

FIG. 2. Seasonal changes in biomass for N-deprived (○), control (△), +N (●), and +N+C (▲) plants (means ± 1 SE, n = 10).

control ($p = 0.005$). In August, starch concentrations in coarse roots were highest in N-deprived seedlings, and concentrations in the control and +N seedlings were similar but greater than in the +N+C seedlings ($p = 0.001$). In September, starch concentrations in N-deprived seedlings were greater than in fertilized treatments ($p = 0.005$), but differences were not significant in December ($p = 0.06$). Starch concentrations in coarse roots fluctuated the most in the fertilized seedlings, and the least in the N-deprived seedlings (Fig. 3).

Sugar concentrations in fine roots declined in all treatments between May and August, but rose during August and September in the unfertilized but not in the fertilized seedlings (Fig. 3). Sugar concentrations in unfertilized seedlings were greater than fertilized seedlings in September ($p = 0.003$). By December, sugar concentrations were not different among treatments, suggesting that the rise in fine root sugar concentrations was merely delayed in the fertilized seedlings. In contrast, starch concentrations changed little in any treatment until after September (Fig. 4). Nevertheless, concentrations in the control seedlings were greater than in the fertilized treatments in August ($p = 0.03$), and starch concentrations in unfertilized seedlings were greater than in fertilized seedlings in December ($p = 0.03$).

Seasonal C allocation

Carbon allocation changed as the season progressed. Allocation to foliage production was greatest early in the season (Table 1), and declined in unfertilized but not fertilized seedlings as the season progressed, resulting in a longer period of allocation to foliage in fertilized plants. Allocation to root production increased with time in all treatments, but allocation to roots in fertilized seedlings was proportionally less. The magnitude of the shift in allocation from foliage to fine roots was greatest in the N-deprived and least in the fertilized plants.

Because allocation to shoots was greatest in the spring, the shoot to root ratios were highest in June (average 1.8), but there were no differences among treatments. By August, shoot to root ratios were lower in the N-deprivation (0.5 ± 0.1) than in the +N and +N+C treatments (1.0 ± 0.2 and 1.0 ± 0.1), but did not differ from the control (0.8 ± 0.2, $p = 0.004$). In September, there was again no difference among treatments (average 0.6).

There were no significant differences in ratios of foliage to fine roots (average 5.0) in June, but the N deprived seedlings had the lowest ratio, as was expected. In August, the N-deprived plants had lower ratios (0.4 ± 0.09) than +N (1.0 ± 0.2) and +N+C (1.0 ± 0.1) plants, but these ratios were not different from those of the control plants (0.9 ± 0.3, $p = 0.006$). By September, ratios again did not differ among treatments (average 0.6).

In August, C allocation to storage reserves in the N-deprivation, control, +N, and +N+C treatments was 126, 110, 98, and 102 mg glucose/g biomass produced, respectively. In September it was 136, 108, 93, and 95 mg and in December, 214, 196, 165, 197 mg, respectively. Much of this pattern

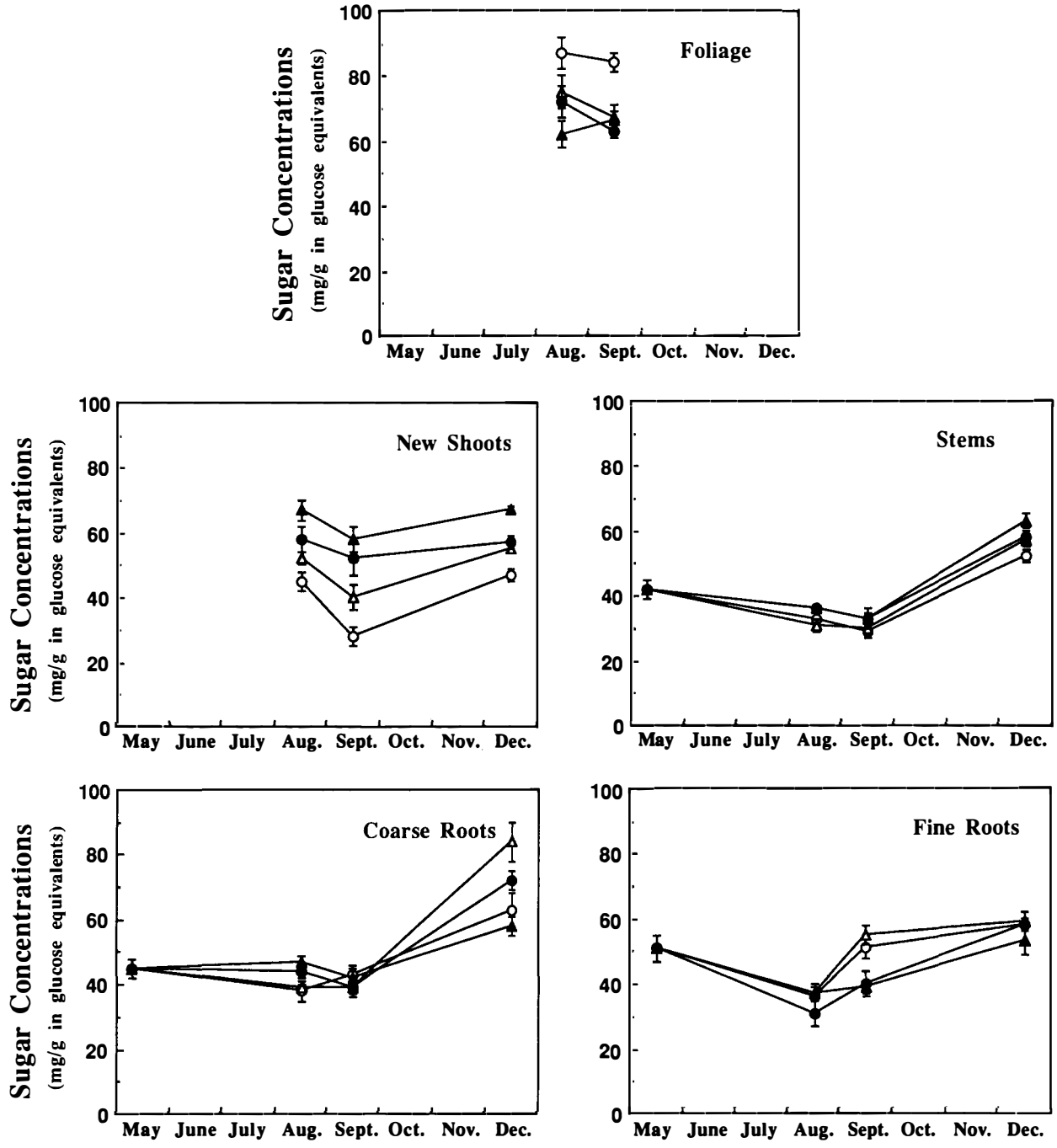

FIG. 3. Seasonal changes in sugar concentrations (n = 10). Symbols are described in Fig. 2.

can be attributed to a smaller allocation to starch reserves as N availability increased at the August (59, 52, 41, and 42 mg glucose/g biomass produced respectively), September (69, 53, 39, 38 mg, respectively), and December (92, 85, 76, 71 mg, repectively) sampling times.

Discussion

Soil N availability

Nitrogen availability in northern hardwood forests is highest early in the growing season and becomes lowest just prior to leaf senescence (Nadelhoffer *et al.* 1984; Pastor *et al.* 1984; Shepard *et al.* 1990). This same pattern of N availability was simulated in the present experiment. However, the N manipulations in this study differed from natural conditions in that pools of ammonium and nitrate were similar and varied concomitantly with season. In unmanipulated forests, ammonium pools are generally larger and more variable than nitrate pools (Nadelhoffer *et al.* 1984; Gosz and White 1986).

We expected additions of sawdust and glucose to soil to reduce N availability (Turner and Olson 1976; Olayinka and Adebayo 1985) by allowing heterotrophic microorganisms to immobilize N. Instead, we detected no differences in N availability between control and N-deprivation treatments, but this may have been due to the low N levels present in the control.

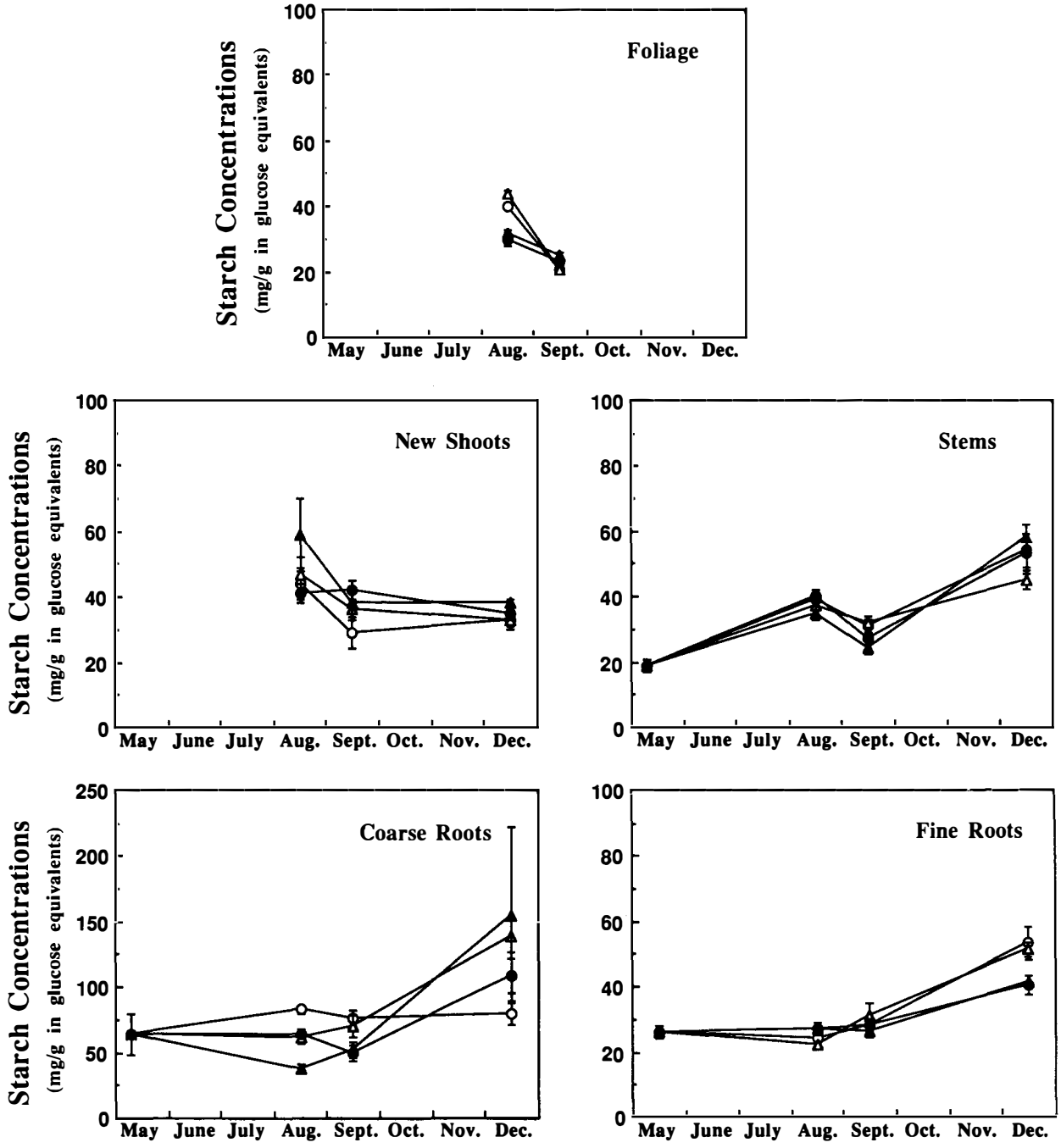

FIG. 4. Seasonal changes in starch concentrations (n = 10). Symbols are described in Fig. 2.

Moreover, control plants assimilated more N in biomass than N-deprived plants, suggesting lower N availability in the N-deprivation treatments. The addition of both C and N (+N+C plants) initially resulted in very high levels of N availability, followed by a fairly rapid reduction in available N during the first month, indicating that C additions provided substrate for microbial immobilization of N.

Foliar nutrients

The patterns of N cycling were similar to those reported in other studies of sugar maple. For example, Lea *et al.* (1980) and Lennon *et al.* (1985) also showed that foliar N concentrations were related to soil N availability. In addition, retranslocation of N from the leaves to perennial tissue during senescence was similar to values reported by Ryan and Bormann (1982, 50–58%) but lower than those reported by Lennon *et al.* (1985, 72–76%). Although this method of conserving N allows trees to be relatively independent of soil supplies during the periods of highest N demand (Ryan and Bormann 1982), the advantages or possible disadvantages of N resorption in a N-rich system are not known.

Foliar S concentrations in the present study were almost three times the concentrations in sugar maple foliage in natural stands (0.10%, David *et al.* 1987) and relative to N concentrations, three times that required for the adequate nutrition of trees (Kelly and Lambert 1972; Johnson *et al.*

TABLE 1. Seasonal C allocation to each tissue

Date and treatment	Foliage	Stem	Coarse roots	Fine roots
June 15				
N deprivation	65	25	0	10
Control	85	5	0	10
+N	75	15	0	10
+N+C	60	25	5	10
August 15				
N deprivation	10	10	35	45
Control	30	30	25	15
+N	30	25	30	15
+N+C	40	20	25	15
September 13				
N deprivation	0	0	100	0
Control	0	5	60	35
+N	40	10	35	15
+N+C	30	15	35	20

NOTE: Values are expressed as the percent of total production since May 9 for June 15 harvest, June 15 for August 15 harvest, and August 15 for September 13 harvest. They have been rounded to the nearest 5%.

1982). Nevertheless, sulfate-S concentrations were only 10% of the total S, compared with 8% in the natural stands. The low sulfate-S concentrations in both N-deprived and N-fertilized seedlings indicate that sugar maple trees do not absorb excessive amounts of sulfate relative to N (Lambert and Turner 1977; Turner *et al.* 1977; Kelly and Johnson 1982).

Although N availability influenced neither S nor sulfate concentrations in the foliage, N availability did affect foliar S biochemistry: i.e., the organic S to N ratios were lower in the fertilized than in the N-deprived seedlings. Because organic S is found primarily in proteins (Anderson 1975), smaller proportions of S containing amino acids may be produced when N is in excess and (or) S availability is low. In some cases, S deficiency causes preferential synthesis of proteins with lower methionine and cysteine content (Wrigley *et al.* 1980).

Biomass production

The enhanced production of foliar biomass in response to N fertilization has been found previously in sugar maple trees (Mitchell and Chandler 1939; Safford 1973; Lea *et al.* 1979; Stanturf *et al.* 1989), but in this study fertilization also lengthened the period of foliage production. Sugar maple seedlings are apparently flexible in their form of shoot development, and the form is at least partly dependent on the soil N availability. Although the determinate growth pattern is considered to be normal for sugar maple trees (Kramer and Kozlowski 1979), indeterminate growth has also been reported previously for sugar maple seedlings (Gregory 1980). These flexible growth forms may be limited to young trees (Kramer and Kozlowski 1979).

Nitrogen availability also affected the periodicity and amount of fine root production. Although the initiation of fine root production coincided in all treatments, the cessation of growth occurred earliest in the N-deprived plants and resulted in a lower fine root biomass production under low N conditions. Our findings support the hypothesis of Nadelhoffer *et al.* (1985) that fine root production increases with N availability in northern hardwood forests. Similarities in the fine root mass of fertilized and control plants in December could be explained if fine root mortality as well as production increase when N is in great excess. However, we did not measure fine root turnover.

Seasonal C allocation

Foliage was the largest single C sink early in the growing season, but became less important compared with other sinks as the season progressed. This pattern contrasts with that described for *Pinus taeda* L. (Adams *et al.* 1990), in which foliage remained the largest C sink until late autumn, with midsummer being the peak period of allocation. Nevertheless, sugar maple and *P. taeda* were similar in two other respects: in both species, allocation to fine roots increased as the summer progressed and allocation to stems did not vary with experimental conditions.

Regardless of treatment, the periods of high C allocation to foliage coincided with the timing of high N availability. In unfertilized plants, N was most available early in the season when allocation to foliage predominated. High N availability during most of the experimental period resulted in a sustained allocation to foliage in fertilized plants. These results support the hypothesis (Bloom *et al.* 1985; Ingestad and Agren 1988; Tilman 1988) that plants maximize growth by allocating more C to leaves when photosynthate is more limiting to growth and to roots when nutrients are more limiting. It follows that if nutrients such as N are not limiting, then C should be allocated to leaves.

Differences in seasonal C allocation among treatments are reflected in shoot:root ratios and can be explained in the context of differences in N availability. Allocation was similar in all treatments early in the season, when N availability was highest and allocation patterns changed little in the treatments where N availability remained high. In contrast, where N availability remained low throughout the experimental period, shoot:root ratios decreased as the season progressed. Regardless of the N availability, the shoot:root ratios became similar at the end of the growing season, suggesting that functional equilibrium develops in these plants regardless of N availability. As described by Cannell (1985), this equilibrium is adaptive and results in assimilates being used preferentially by the roots if conditions limit nutrient uptake and preferentially by the shoots if conditions limit photosynthesis. Apparently, this functional equilibrium is reached late in the growing season and results in plants of the same proportion but different size.

In all treatments, the initiation of fine root production was delayed relative to shoot production. Although the reason for this is not clear, it is probably the result of both physical and physiological factors. Low soil temperatures likely limited the initiation of fine root production because the rate of production has been shown to be correlated with soil temperature in natural stands (Burke 1988). Nevertheless, competition with shoots for photoassimilate during periods of rapid production also may limit fine root production (Webb 1976; Drew 1982). Allocation to foliage production was inversely related to both root production and reserve storage in the present study. However, because substantial foliage and fine root production coincided in fertilized plants, competition for photoassimilate did not always limit fine root production.

During this single year of treatment, there was a distinct trade-off between C allocation to structural growth and storage as reserves, with the priority for allocation similar to that predicted by Waring and Pitman (1985): foliage production

has highest priority, then root production, with storage reserves the lowest priority. The low nonstructural carbohydrate concentrations in both foliage and roots of fertilized seedlings reflected the lower priority of storage relative to foliage and root production, results similar to those reported for N-fertilized *P. taeda* (Adams *et al.* 1986; Birk and Matson 1986). In addition, the initiation of translocation of photosynthate to roots was delayed, probably as a result of shoot growth, and resulted in reduced allocation to stored reserves in roots, but not reduced allocation to fine root production.

The long-term effect of these changes in C allocation patterns is not known, but may influence the ability of trees to withstand natural environmental stresses. For example, the reduction in reserve storage under high N regimes was small compared with the reduction caused by defoliation (e.g., Wargo 1972; Wargo *et al.* 1972), but the combination of low starch and high sugar concentrations in roots has been linked in sugar maple with predisposition to infections of *Armillaria mellea* (Vahl:Fr.) Kummer (Wargo 1972). In addition, low starch reserves in fine roots may cause greater fine root mortality if starch reserves in sugar maple determine root longevity, as has been demonstrated for *Pseudotsuga menziesii* (Mirb.) Franco (Marshall and Waring 1985).

Finally, these changes in growth and C allocation may contribute to an increased risk of frost damage. Because frost hardening is accomplished using sugar that is mainly derived from the digestion of starch (Kramer and Koslowski 1979), a low allocation to starch in N-fertilized trees may limit their ability to become cold tolerant. In addition, because cold hardening begins only after shoot production ends (Larcher 1980), the extended season of shoot production associated with high N availability may contribute to a delay in the initiation of the cold-hardening process and may increase susceptibility to early or very cold winter temperatures.

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