Seasonal biochemical changes in coniferous forest canopies and their response to fertilization

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Summary

Seasonal changes in concentrations of total nitrogen, free amino acids, chlorophyll, starch and sugar were measured in foliage from fertilized and unfertilized conifer forests in New Mexico and Oregon. In the New Mexico Douglas-fir (Pseudotsuga menziesii var glauca (Beissn.) Franco) forest, fertilization resulted in elevated foliar nitrogen concentrations on all dates, from an average of 9 mg g$^{-1}$ in unfertilized trees to 14 mg g$^{-1}$ in fertilized trees. In the Oregon western hemlock (Tsuga heterophylla (Raf.) Sarg.) forest, fertilization increased total N by only 15%, from 13 mg g$^{-1}$ in unfertilized trees to 15 mg g$^{-1}$ in fertilized trees. Foliar nitrogen concentrations on a weight basis were lowest in winter and spring, but did not vary seasonally when expressed on a leaf area basis.

Chlorophyll concentrations increased with fertilization and had greater seasonal variation than did total nitrogen concentrations. Chlorophyll concentrations were significantly higher during the growing season than in the winter and spring months. Fertilization did not result in major changes in the proportion of total nitrogen in chlorophyll at either the Oregon or the New Mexico site. Concentrations of free amino acids varied with date and fertilization treatment; in New Mexico, amino acids were highest in the winter sample, whereas in Oregon, they were lowest in winter and spring. At both sites, amino acid concentrations were significantly higher in fertilized trees than in control trees on most dates and the ratios of amino acid-N to total N were also significantly higher in fertilized trees. For both sites, starch concentrations were nearly zero for most of the year, but increased sharply just before bud break and initiation of new growth in the spring.

Although fertilization resulted in increased nitrogen concentrations in foliage at both sites, the response in New Mexico was much greater than in Oregon. These results are in agreement with forest productivity data that suggest that growth in the New Mexico site is limited by nitrogen, whereas in the Oregon site it is not.

Keywords: canopy biochemistry, chlorophyll, Douglas-fir, fertilization, free amino acids, nitrogen, seasonal variation, starch, sugars, western hemlock.

Introduction

Plants respond to differences in resource availability through several physiological mechanisms, including alteration in leaf area, changes in carbon and other element allocation patterns, and changes in photosynthetic efficiency, nutrient and water use efficiency (Chapin 1980, 1991). These patterns are mediated by and reflected in foliar nutrient content and concentrations. For example, in forests where productivity is limited by nitrogen availability, trees may be more efficient in their use of nitrogen to fix carbon than trees in high-nutrient sites; trees in these low-fertility sites often have relatively low foliar nitrogen concentrations (Vitousek 1982, Pastor et al. 1984,
Birk and Vitousek 1986, Chapin et al. 1989). In addition, the relative proportions of nitrogen-containing compounds vary with fertility; several studies have demonstrated that concentrations and relative proportions of soluble nitrogen increase in relation to total nitrogen in response to fertilization (van den Driessche and Webber 1975, 1977, Mattson 1980, Matson and Waring 1984, Kim et al. 1987, Näsholm and Ericsson 1990).

Changes in carbon fixation, protein synthesis and growth resulting from resource limitations may also affect the quality, allocation and concentrations of carbohydrates stored in trees. For example, plants growing under conditions of nutrient limitation often have higher concentrations of nonstructural carbohydrates than plants growing under conditions of high nutrient availability (Meyer and Splittstoesser 1971, Etter 1972, Chapin 1980, Shaver and Chapin 1980, Matson and Waring 1984, Birk and Matson 1986). Lignin and carbon-based defensive compounds like terpenes and phenolics may also be higher in sites with low nutrient availability than in sites with high nutrient availability (Matson et al. 1987, Lerdau 1991).

Biochemical changes in response to resource availability are coincident with differences that result from seasonality in photosynthesis, growth, senescence and dormancy. Chlorophyll and specific amino acid concentrations, for example, appear to vary as a function of season, with high values occurring during the growing season and low values during cold winter months (Linder 1973, 1980, Näsholm and Ericsson 1990, Matson et al. 1994). Likewise, starch reserves in conifers are high immediately before bud break and very low early in the growing season, although some evidence suggests that rate of draw-down of starch may vary with resource availability (Birk and Matson 1986, Matson et al. 1987).

To the extent that foliar chemical characteristics reflect physiological activity and resource limitation, their measurement at the individual tree or forest canopy level may be useful in predicting potential growth response to fertilization, in providing information on timing of physiological activity, or in monitoring seasonal changes in water or nutrient availability. Whether made on individual leaves using laboratory-based methods or on whole canopies using aircraft-based high spectral resolution sensor methods (Wessman et al. 1989, Ustin et al. 1991, Matson et al. 1994), such measurements of foliar chemistry may need to account for variations that result from seasonal changes in plant activity as well as from resource differences. The purpose of this study was to determine seasonal variation in foliar biochemicals in fertilized and unfertilized trees in two conifer forests.

Methods

Study sites

To evaluate foliar chemical response to fertilization, we took advantage of two continuing forest fertilization experiments. One was located in an even-aged stand of 50-year-old Douglas-fir (Pseudotsuga menziesii var glauca (Beissn.) Franco)
growing on Mount Taylor in northwestern New Mexico (35°15' N, 107°34' W, elevation 2900 m). In this site, snow melt usually occurs in May and June, and the growing season extends until October. From 1985–1987, a complete fertilizer was applied to two 20 x 20 m plots once yearly immediately following snow melt. Fertilizer was applied at the rate of 200 kg N ha⁻¹ year⁻¹; equal proportions of NO₃-N and NH₄-N were applied. Among other treatments, two control plots were also maintained. For more details on site characteristics and the complete fertilizer, see Gower et al. (1992). For our study, foliage was collected from five trees in each of the two fertilized plots and five trees in each of the two control plots; we considered the 10 trees per treatment as replicates.

The second study site was located in the Cascade Mountains near Scio, Oregon (44°40'30" N, 122°36'40" W, elevation 732 m), in forests dominated by 30-year-old western hemlock (Tsuga heterophylla (Raf.) Sarg.). Snow melt in this site usually occurs in June, with the growing season extending through October. An area encompassing the plot was given an aerial application of urea (300 kg N ha⁻¹) in 1988. Within this area, a complete fertilizer was applied to a 55 x 55 m plot, with nitrogen as urea and ammonium sulfate, at the rate of 160 kg N ha⁻¹ during the winters of 1990 and 1991. Spring applications during this period were entirely urea at a rate of 300 kg N ha⁻¹. A control plot, located approximately 4 km to the east and slightly uphill of the fertilized plot, did not receive the aerial urea application. The control plot was similar to the fertilized plot in species composition, age and density. For further details on the study site, see Runyon et al. (1994) and Matson et al. (1994). For our study, foliage was collected from five trees in the fertilized plot and five trees in the unfertilized plot; for our analysis, we treated the five trees as replicates.

**Foliar sampling**

In the New Mexico site, foliage was collected on June 16, August 18 and October 1, 1986, and on March 30, June 15 and July 25, 1987. Bud break and new foliage growth initiation began just before the June 16, 1986 collection, and just after the June 15, 1987 collection. Fresh foliage samples were collected by hand from the upper third of the canopy of each of the 10 trees per treatment. Samples were returned to the laboratory at Northern Arizona University on ice, frozen at −60 °C and freeze-dried. The samples were mailed to Ames Research Center (ARC) and were separated by foliage age before grinding. Preliminary analyses indicated that chemical concentrations of the 1- and 2-year-old needles did not differ significantly; all analyses were carried out on a weighted composite of the 1 + 2-year-old-age classes in 1986; in the second year, the same cohort was sampled (2 + 3-year-old foliage).

In Oregon, samples were collected by shotgun from five randomly selected trees from each plot. Collections were carried out monthly from August 1989 to October 1990, except for October 1989, and February, April and September 1990. Bud break and new growth initiation began just after the June 1990 collection. For the 1989 collections, foliage was collected from the upper third of the canopy within the plots; starting in 1990, foliage was collected from the upper third of trees at the road edges of the plots to ensure collection of sun leaves. Foliage was returned to the laboratory
at Oregon State University on dry ice, frozen at −60 °C and mailed on dry ice to ARC where the samples were freeze-dried. The one-year-old foliage (previous calendar year’s new growth) was separated from current and older age classes for biochemical analysis.

Chemical analyses
Total nitrogen was measured colorimetrically with a continuous-flow analyzer after block digestion using a sulfuric acid-mercuric oxide catalyst (Technicon Instruments Corporation, Tarrytown, NY). Chlorophyll was extracted in acetone buffered by CaCO₃ and its concentration was determined spectrophotometrically. Free amino acids were extracted with 0.5 M citric acid buffer (pH 5.5) followed by colorimetric analysis with ninhydrin (Lee and Takahashi 1966).

Starch was measured by the method of Matson and Waring (1984). Tissue was extracted in a methanol-chloroform-water (MCW) solution, and the starch-containing residue was dried and treated with a solution of purified α-1,4-glucan glucohydrolase and α-amylase. After incubation, starch concentrations were measured as glucose using glucose oxidase (Sigma Diagnostics, St. Louis, MO). Soluble sugars in the MCW solution were measured by an anthrone colorimetric procedure (Hazid and Neufeld 1964).

For each chemical, reference samples were included with each analytical run. In addition, for the total nitrogen assay, standard samples were included to monitor accuracy of the analysis (Standard Reference Material 1976, 1982).

Water content of freeze-dried samples was determined by oven-drying at 65 °C for 48 h and was used to convert chemical concentrations to an oven-dry basis. Freeze-dried samples generally had less than 6% water content.

Specific leaf area for the New Mexico foliage was determined on subsamples of approximately 50 needles with a video camera coupled to a computer image processing system (Gower et al. 1987, 1992). For the Oregon foliage, specific leaf area was determined on subsamples of approximately 20 needles with a Li-Cor 3100 leaf area meter.

Statistical analyses
Seasonal and fertilization effects were resolved using two-way analysis of variance with trees considered replicates \((n = 10\) for New Mexico, \(n = 5\) for Oregon, Statview II, Abacus Concepts Inc., Berkeley, CA). Comparisons among means were made using the Tukey test (Zar 1984). Statview II was used for correlation analysis. Although measurements were made on individual trees, all of the trees were from one plot per treatment in Oregon and two plots per treatment in New Mexico; thus, the treatments were pseudoreplicated and results should be viewed with this in mind.
Results

Specific leaf area

Specific leaf area (SLA) was measured once at the New Mexico site in July 1987. There was no significant difference between control and fertilized foliage (control mean = 24.0 cm$^2$ g$^{-1}$, fertilized plot mean = 25.0 cm$^2$ g$^{-1}$). At the Oregon site, SLA was significantly higher in fertilized foliage than in unfertilized foliage in the 1989 samples; in 1990 there was no significant difference between treatments (Figure 1). The differences between 1989 and 1990 SLA values may be a result of location of foliage collection; as noted earlier, foliage was collected from within plots on all 1989 collection dates and may have been more shaded than foliage collected at plot edges in the 1990 collections. A marked seasonal reduction in SLA was evident in April, May and June, probably due to the higher foliar starch concentrations during that time (see below).

Nitrogen-containing chemicals

Analysis of variance of total nitrogen concentrations indicated significant effects of fertilization treatment, sampling date and their interaction. Foliar nitrogen concentrations in the fertilized plots in New Mexico were always significantly higher ($P < 0.001$) than N concentrations in the unfertilized plots (Figure 2a). Fertilization increased total foliar nitrogen concentrations by over 50% in the New Mexico site (from an average of 9.0 mg g$^{-1}$ in the control to 14 mg g$^{-1}$ in fertilized trees, Figure 2a). In Oregon, the effect of fertilization was less consistent but significant in August, November and December 1989, and August 1990 ($P < 0.05$, Figure 2b). Overall, fertilization increased total N by 15% (from 13 mg g$^{-1}$ in the control to 15 mg g$^{-1}$ in fertilized trees, Figure 2b) in the Oregon plots. In general, trees in the unfertilized Oregon plot had much higher N concentrations than unfertilized trees in the New Mexico control plot.

In both Oregon plots and in the New Mexico fertilized plots, foliar N concentrations were lowest in the period just before bud break (Figures 2a and 2b). However, when the Oregon data were expressed on an area basis, the increase in weight per area (Figure 1) offset the reduction in N concentration on a weight basis and no

![Figure 1. Average specific leaf area (cm$^2$ g$^{-1}$) for foliage from Oregon collected monthly from August 1989 until October 1990. Each value is the mean (± SE) of five trees. Foliage was from a 1-year-old age class; ● = fertilized and □ = control.](image-url)
significant seasonal trend was apparent.

Chlorophyll concentrations varied significantly in response to both season and fertilization, with a significant interaction effect in the Oregon plots. In both the New Mexico and Oregon plots, chlorophyll concentrations were significantly higher \( (P < 0.001) \) during the growing season than during winter and spring (Figures 3a and 3b). Fertilization resulted in higher chlorophyll concentrations on all dates except the June 1986 collection in New Mexico (Figure 3a). In Oregon, chlorophyll concentrations were significantly higher in fertilized trees than in unfertilized trees during August and November 1989 (Figure 3b). As with total N, chlorophyll concentrations were generally much higher in trees at the Oregon site than at the New Mexico site.

Ratios of chlorophyll-N to total nitrogen in control and fertilized plots were higher in Oregon than in New Mexico, ranging from 4.0 to 6.7 in Oregon and from 2.5 to 4.2 in New Mexico. Although the sampling date effect for the chlorophyll/N ratio was significant \( (P < 0.001) \), with lowest ratios occurring in March and highest ratios occurring in July and August, there was no significant fertilization effect or interaction for the Oregon site. In New Mexico, the chlorophyll/N ratio was significantly \( (P < 0.05) \) higher in the unfertilized trees than in the fertilized trees in August 1986. The effect of sampling date was also significant \( (P < 0.001) \), with the highest ratios occurring in August 1986.

Amino acid concentrations varied significantly with season and fertilization treatment; the interaction between date and treatment was also significant. At the New Mexico site, the highest foliar amino acid concentrations occurred before bud break.
in April and June 1987 (Figure 4a). In Oregon, the lowest amino acid concentrations occurred in March and the highest concentrations were measured in fall and summer (Figure 4b). A fertilization response was evident at both sites; amino acid concentrations were significantly higher \( (P < 0.001) \) in fertilized trees than in unfertilized trees, with the exception of March 1990 in Oregon and June 1986 in New Mexico. Ratios of amino acid-N to total nitrogen were significantly greater in the fertilized trees than in the unfertilized trees at both sites \( (P < 0.001) \).
Nonstructural carbohydrates

Among all of the foliar chemicals examined, foliar starch concentrations showed the most obvious and consistent seasonal pattern (main effect, \( P < 0.001 \)). Large buildups of starch occurred at both sites at or just before bud break; during the rest of the year, foliar starch concentrations were near zero (Figures 5a and 5b). For the New Mexico site, the treatment effect was significant (\( P < 0.001 \)), with significantly higher starch concentrations in unfertilized trees than in fertilized trees in June 1986 and 1987 (just before bud break, \( P < 0.01 \), Figure 5a). In Oregon, the fertilization effect was not significant (Figure 5b).

Total soluble sugar concentrations in foliage of both unfertilized and fertilized trees had a significant (\( P < 0.001 \)) seasonal trend, with highest values in winter at both sites (Figures 6a and 6b). Fertilization main effects were not significant for either the New Mexico or Oregon sites, although there was a significant interaction between date and treatment for the Oregon site, where fertilized trees had consistently higher sugar concentrations than unfertilized trees from January through July.

Discussion

Most studies of nitrogen fertilization in coniferous forests have reported at least a temporary increase in foliar nitrogen concentration in response to fertilization. In this study, fertilization of conifers at both the New Mexico and Oregon sites resulted in elevated total nitrogen concentrations in foliage, but the response was more pronounced and consistent in trees at the New Mexico site. Foliar nitrogen concentrations showed relatively little seasonal variation, whereas chlorophyll and amino acid concentrations had marked seasonal variations.

Chlorophyll concentrations declined over winter, as did the proportion of total

Figure 5. Starch concentrations (mg g\text{DW}^{-1}) in foliage from fertilized (○) and control (□) trees in New Mexico (a) and Oregon (b). See Figure 2 for experimental details.
foliar nitrogen in chlorophyll. Over all dates, the relationships between total nitrogen and chlorophyll were low ($r^2 = 0.32$ for Oregon and $r^2 = 0.44$ for New Mexico). There was, however, a seasonal influence on the nitrogen–chlorophyll relationship at the New Mexico site, with a significant correlation of total nitrogen and chlorophyll during the nongrowing season. Interestingly, the ratio of chlorophyll-N to total N was quite constant between treatments at both sites. At the New Mexico site, the fertilizer treatment significantly increased concentrations of both chlorophyll and total nitrogen, but the proportion of total N allocated to chlorophyll was similar between treatments.

In contrast, fertilized trees at both sites apparently allocated more nitrogen to free amino acids than unfertilized trees. In New Mexico, the highest foliar concentrations of amino acids (and the largest differences between treatments) occurred during the nongrowing season; the lowest free amino acid concentrations (and the smallest differences between treatments) occurred very early in the growing season (June 1986, Figure 4a) at the time when new needle growth was being initiated. It is possible that, before active growth periods, nitrogen is taken up in excess of growth requirements and stored as amino acids in the foliage (Margolis and Waring 1986, Näsholm and Ericsson 1990), or mobilized from proteins to amino forms in preparation for allocation to new growth. High free amino acid concentrations for both New Mexico treatments in early April may also be associated with the maintenance of cold hardness in the harsh high-altitude winter, although results of several studies on frost hardness in conifers suggest this is unlikely (e.g., Kim and Glerum 1988).

In Oregon, on the other hand, free amino acid concentrations were lowest in the winter and spring months, with relatively small differences between fertilized and unfertilized treatments. The seasonal patterns at this mild site were similar to those reported for fertilized and unfertilized Scots pine trees by Näsholm and Ericsson...
The large increase in foliar starch concentration just before bud break and the mobilization of starch at the initiation of growth is in agreement with the results of many other studies. Earlier studies have suggested that the difference in rate of draw-down of starch reserves before bud break to after bud break may provide an index of growth limitation in trees, because plants that are limited by nitrogen or some other resource would presumably exhibit less carbohydrate demand in the early growing season (Birk and Matson 1986). Our limited data do not support this suggestion: at the New Mexico site, the unfertilized trees had higher foliar starch concentrations before bud break and a greater percentage loss during bud break than the fertilized trees (Figure 5a). Concentrations in the fertilized and unfertilized trees changed similarly at the Oregon site (Figure 5b).

Differences in site response to fertilization

Fertilization resulted in increased foliar nitrogen concentrations at both sites, but the magnitude of the response was much greater in the New Mexico Douglas-fir forest. At the New Mexico site, total foliar N in the unfertilized trees was generally less than 1% and fertilization increased it by nearly 50%. In Oregon, on the other hand, total foliar N in the unfertilized trees was on average 1.3% by weight, which was already near the maximum achieved for fertilized western hemlock seedlings (1.39–1.61%, Radwan and DeBell 1980). Fertilization increased the average N concentration to 1.5%. The differences in these foliar N concentrations and their relative responses to fertilization suggest that the New Mexico site is more nitrogen deficient than the Oregon site.

Given the major response in foliar N concentrations to fertilization, it seems reasonable to expect that trees at the New Mexico site would also respond to fertilization with greater increases in leaf area index (LAI, Brix and Ebell 1969, Linder and Axelsson 1982, Waring 1983) and aboveground net primary production (ANPP) than trees at the Oregon site. Gower et al. (1992) measured these growth responses at the New Mexico site and reported that fertilization resulted in a 24% increase in LAI two years after initial fertilization (LAI of 7.4 and 9.2 in the fertilized plots in 1984 and 1986, respectively, 8.2 and 8.6 in the control plots in 1984 and 1986, respectively). Aboveground net primary production ranged from 13 Mg ha\(^{-1}\) year\(^{-1}\) in the unfertilized plots to 17 Mg ha\(^{-1}\) year\(^{-1}\) in the fertilized plots in 1986. Moreover, Gower et al. (1992) estimated that the ratio of belowground net primary production to ANPP was 0.46 in the unfertilized plots and only 0.23 in the fertilized plots.

At the Oregon site, fertilization apparently did not result in a positive growth response. Runyon et al. (1994) found no change in LAI with fertilization (approximately 10 in both plots) and insignificant differences in ANPP between unfertilized and fertilized plots. There were also no significant differences in relative or absolute stem growth between treatments (Runyon and Yoder, personal communication). In Oregon, nitrogen availability was apparently not a limiting factor to tree growth.

In both the New Mexico and Oregon sites, seasonal variation in canopy chloro-
phyll and nonstructural carbohydrates reflected the timing of growth initiation; concentrations of total nitrogen and free amino acids apparently also provide information on potential growth responses to increased N availability. The finding that canopy chemical constituents tend to reflect the interacting effects of season and resource availability suggests that foliar measurements using both ground-based and remote sensing approaches will require sampling in the temporal as well as spatial realms.

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References


