
Relationship Between Foliar $\delta^{13}\text{C}$ and Hydraulic Pathway Length in *Pinus palustris*

Lisa J. Samuelson, Price C. McLemore III, and Greg L. Somers

ABSTRACT. Because of the importance of hydraulic architecture in understanding physiological differences between small and large trees, we tested the hypothesis that foliar $\delta^{13}\text{C}$ increased with aboveground flow-path length in longleaf pine (*Pinus palustris* Mill.) trees of varying age and size at two sites in the lower Coastal Plain. This species offered a unique opportunity to examine variability in foliar $\delta^{13}\text{C}$ in relation to pathway length because of the open, well-mixed canopy in savanna-like stands and concentration of foliage in splayed tufts on branch ends. At both sites, models with total flow-path length (bole + branch) explained 78–81% of the variation in foliar $\delta^{13}\text{C}$, and a full model with separate slopes and intercepts for each tree was required. Branch flow-path length was highly correlated with foliar $\delta^{13}\text{C}$ and the models accounted for 76–88% of the variation in foliar $\delta^{13}\text{C}$, and a reduced model with one slope was suitable for each site. Flow-path length was not as well correlated to foliar N concentration, specific leaf area or tuft leaf area. Greater $\delta^{13}\text{C}$ enrichment with increasing flow-path length indicates the need for mechanistic studies to better understand the role of hydraulic resistance in controlling leaf-level physiology within the canopy and among different size longleaf pine trees. *FOR. SCI.* 49(5):790–798.

Key Words: Flow-path length, longleaf pine, carbon isotopic signature.

INCREASED HYDRAULIC RESISTANCE in response to greater flow-path length may reduce leaf stomatal conductance and net photosynthesis in large trees (Bond 2000, Smith and Long 2001). Structural changes such as an increase in the ratio of sapwood area or fine root area to leaf area may maintain a functional homeostasis in water transport in taller trees (Magnani et al. 2000), but these changes in allocation may only partially compensate for reductions in hydraulic conductance and leaf gas exchange may still decline in response to increasing flow-path length (Schäfer et al. 2000).

The carbon isotopic signature ($\delta^{13}\text{C}$) of foliage, which is a measure of the ratio of substomatal to ambient CO_2 concentration (ci/ca) (Farquhar et al. 1982), provides a time integrated measure of leaf water use efficiency (Warren and

Adams 2000). The carbon isotopic signature of wood and foliage has been shown to be sensitive to changes in hydraulic conductance and flow-path length. For example, Yoder et al. (1994) and Hubbard et al. (1999) proposed that the 30% reduction in net photosynthetic rate in 245-yr-old ponderosa pine (*Pinus ponderosa* Laws) relative to younger trees was a result of decreased hydraulic conductance, and they observed that earlywood $\delta^{13}\text{C}$ enrichment was positively correlated with total tree height. Similarly, Schäfer et al. (2000) reported a 60% decline in mean canopy stomatal conductance with a 30 m increase in tree height in beech (*Fagus sylvatica* L.). Significant positive correlations between branch length and foliar $\delta^{13}\text{C}$ and negative relationships between branch length and leaf transpiration, net photosynthesis, and water poten-

Lisa J. Samuelson, Professor, School of Forestry and Wildlife Sciences, Auburn University, Auburn, AL 36849-5418—Phone: 334-844-1040, Fax: (334) 844-1084; E-mail: samuelj@auburn.edu. Price C. McLemore III is a former graduate student, School of Forestry and Wildlife Sciences, Auburn University, Auburn, AL. Greg L. Somers, Associate Professor, School of Forestry and Wildlife Sciences, Auburn University, Auburn, AL 36849-5418—Phone: 334-844-1059; E-mail: somergl@auburn.edu.

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tial were observed in Monterey pine (*Pinus radiata* D. Don.) (Waring and Silvester 1994, Walcroft et al. 1996). Foliar $\delta^{13}\text{C}$ of maritime pine (*Pinus pinaster* Ait.) in open stands was positively correlated to branch length, which in turn was negatively correlated with branch hydraulic conductance (Warren and Adams 2000). Thus, flow-path length may account for variation in leaf-level physiology between different size trees and within the canopy of large trees particularly on drought prone sites (Fessenden and Ehleringer 2002).

Because of the importance of hydraulic architecture in understanding physiological differences between small and large trees and within the canopy of large trees, the objective of this work was to examine the relationship between foliar $\delta^{13}\text{C}$ and flow-path length in different size trees of longleaf pine (*Pinus palustris* Mill). The sandhills longleaf pine wiregrass (*Aristida stricta*) ecosystem, described as a temperate savanna with scattered individuals in park-like stands, is considered an endangered plant community (Means and Grow 1985). This species offered a unique opportunity to examine foliar $\delta^{13}\text{C}$ in relation to pathway length because of the characteristic open and well-mixed canopy, concentration of foliage in splayed tufts at branch ends, and predominance of summer drought. Although cause and effect relationships were not examined, the hypothesis that foliar $\delta^{13}\text{C}$ increases with aboveground flow-path length was tested.

Materials and Methods

Study Sites

The two research sites were located at Eglin Air Force Base in Florida, 11 km northeast of Fort Walton Beach, Florida, in the Gulf Coastal Plain physiographic province. The Eglin area receives an average of 1624 mm of precipitation a year, and temperatures range from an average low of 3.8°C in January to an average high of 34.4°C in July, with an average annual temperature of 21.8°C (National Oceanic and Atmospheric Administration). Annual precipitation and av-

erage temperature were 2386 mm and 19.9°C, respectively, in 1998 and 1278 mm and 19.1°C, respectively, in 1999.

Site 1 was located at 30°39'41.6" N lat. and 86°39'48.8" W long. and site 2 was located at 30°39'26.2" N lat. and 86°44'3.0" W long. Trees were selected from 0.20 ha plots established to describe stand characteristics. Site indices, base age 50, for sites 1 and 2 were 12.0 m and 16.4 m, respectively. Average diameter at breast height (dbh) (1.37 m) was 22.9 cm and 17.6 cm for sites 1 and 2, respectively. Soils at both sites were deep, excessively well drained sands in the Lakeland soil series, and the sites were not burned within the last 5 yr prior to sampling.

The sites were natural, open-canopy, longleaf pine savannahs with wiregrass (*Aristida* spp.) dominating the understory. Basal area and density were 18.2 m² ha⁻¹ and 340 trees ha⁻¹, respectively, at site 1 and 11.6 m² ha⁻¹ and 280 trees ha⁻¹, respectively, at site 2. No crown overlap with neighboring trees was observed at either site, and trees of all age classes were open grown. Foliage from grass stage seedlings (only at site 2), saplings, and mature trees ranging in age from 80 to 120-yr-old was sampled (Table 1). Site 1 consisted of longleaf pine trees from sapling to mature sizes, and at site 2, longleaf pine trees ranged in age from grass stage to mature.

A target number of 200 tufts were collected from each site. At each site, tufts were sampled from branches of four mature trees (1–3 branches per tree) and from branches of three to four small trees (1–7 branches per tree) (Table 1). Two of the small trees at site 2 were grass stage seedlings. Site 1 was sampled May 3–5, 1999, and site 2 was sampled November 21–22, 1999. All tufts from an intact branch were immediately removed from felled trees and stored in ice coolers to reduce needle respiration and stabilize $\delta^{13}\text{C}$. Entire branches were transported to Auburn, AL for branch length measurement. Branch height on the bole was recorded in the field to determine the total flow-path length (bole + branch) to each individual tuft.

Table 1. Diameter at breast height (1.3 m, dbh), total height, number of branches sampled per tree, range in bole heights at sample branches, range in branch lengths to sample tufts, and total number of tufts sampled per tree for longleaf pine trees at two sites at Eglin Air Force Base, Florida.

dbh (cm)	Total height (m)	No. branches	Branch heights (m)	Branch lengths (m)	No. tufts
Site 1: Large trees					
27.3	21.6	1	18.5	1.7–3.3	28
29.3	16.6	3	12.9–15.7	0.6–3.8	47
17.7	16.4	3	13.0–15.7	0.5–3.3	23
na*	na	1	11.4	4.5–7.5	66
Site 1: Small trees					
12.0	10.2	2	4.4–5.9	0.5–2.5	20
3.5	3.2	1	2.7	1.2–1.7	4
6.0	6.7	3	5.6–6.6	1.0–1.2	5
Site 2: Large trees					
21.8	16.1	3	12.6–14.3	0.8–2.8	53
28.9	19.1	3	14.4–16.8	1.1–3.5	51
32.8	22.7	3	10.9–18.7	0.7–3.4	47
31.0	22.0	3	19.2–19.7	0.5–2.1	41
Site 2: Small trees					
4.4	5.1	7	2.0–5.1	0.0–5.1	7
2.2	1.5	2	1.0–1.5	0.2–1.5	2
0.9 [†]	0.1	1	0.1	0.1	1
1.3 [†]	0.1	1	0.1	0.1	1

* Total height and dbh were not available due to a data recording error.

[†] Indicates grass stage longleaf pine and corresponding diameters refer to groundline diameters.

Foliar Analyses

Foliage from the May trip (site 1) was 1 yr old, and foliage from the November trip (site 2) was fully expanded, current year, first flush needles. Longleaf pine typically produces one flush with an occasional smaller second flush each growing season, and needles persist for two growing seasons. Projected needle area was measured on five fascicles from each tuft using an area meter (Licor-3000, Licor, Inc., Lincon, NE). Needle-specific leaf area was determined and used to calculate total tuft leaf area as the product of specific needle area and total tuft dry weight. For the May sampling period 1-yr-old needles were senescencing from some tufts, and current year foliage was still developing. In order to gain an accurate estimate of the relationship between total tuft leaf area and hydraulic pathway length, total tuft leaf weight and area was determined only for current year needles collected during the November sampling at site 2.

Within 24 hr of collection, 10–20 fascicles per tuft were removed and dried between 65–70°C. Bulk needle tissue was finely ground in steel vials with a Spex ball mill 8000 (Metuchme, NJ) to a 0.2 mm particle size. Between 1.8–2.2 mg of ground powder from each sample representing a whole tuft was combusted, and the resulting gases swept by a helium gas carrier into an isotope ratio mass spectrometer (Delta Plus, Finnigan MAT, Bremen, Germany) operated by the University of Georgia Institute of Ecology. All carbon isotope ratios were expressed in delta notation relative to the Pee Dee Belemnite standard ($\delta^{13}\text{C} = (R_{\text{sample}}/R_{\text{standard}} - 1) 1000\%$). Total foliar nitrogen (N) concentration was determined on a composite sample of 10–20 fascicles per tuft using LECO analysis (Jones and Case 1990).

Statistical Analyses

Linear regression analysis with dummy variables for each tree was used to describe the relationship between foliar $\delta^{13}\text{C}$, foliar N concentration, specific leaf area, or tuft leaf area and total flow-path length or branch flow-path length by site. For total flow-path length, the full model was:

$$Y = \alpha_0 I_\alpha + \beta_0 I_\beta + \gamma_0 I_\gamma + \delta_0 I_\delta + \eta_0 I_\eta + \alpha_1 X_\alpha I_\alpha + \beta_1 X_\beta I_\beta + \gamma_1 X_\gamma I_\gamma + \delta_1 X_\delta I_\delta + \eta_1 I_\eta I_\eta + \varepsilon$$

where X and Y were measurements per tuft, α_0 , β_0 , γ_0 , and δ_0 were intercept terms for individual trees, α_1 , β_1 , γ_1 , and δ_1 were slope terms for each of those trees, η_0 and η_1 were slope terms for small trees, and I was the indicator function (dummy variable). Because of the limited number of tufts from small trees (as low as one tuft per tree in several cases) and correspondingly bias estimates in the full model, small trees were grouped as one tree at each site.

In an effort to test models that combined the slope and intercept terms, and the two size classes (small and large), reduced models were tested against the full model using a likelihood-ratio test (Neter et al. 1990, p. 306). The first reduced model tested against the full model was a model with two slopes, with one slope term for the large trees and one slope term for the small trees. The second model tested in the model reduction process had one slope as a general slope term for all the trees, with each tree still having unique intercept

terms. The third model consisted of one general slope, one intercept term for the large trees, and one intercept for the small trees. The most reduced model tested against the full model had one slope term and one intercept term. The null hypothesis was set as the reduced model ($\alpha_1 = \beta_1 = \gamma_1 = \delta_1 = \eta_1$) and the alternative hypothesis was set as the full model. If the F -value was not significant at the 0.05 level, then the reduced model was considered to be as good an estimator as the full model.

Other methods of model reduction were originally used in the reduction process, such as collapsing the intercepts first and then the slopes, or attempting to reduce the model by determining which tree intercepts or slopes could be combined with each other for the simplest model. Neither of these reduction processes proved advantageous over the previously described model reduction process, nor could they be easily explained biologically.

In order to describe the influence of branch flow-path length on foliar $\delta^{13}\text{C}$, foliar N concentration, specific leaf area and tuft leaf area, branches within 1 m of each other were selected from the sample branches. The 1 m restriction was used to minimize any effects of bole height on branch length relationships. The full model used to describe the four branches (two branches per tree and two trees at each site) was:

$$Y = \alpha_0 I_\alpha + \beta_0 I_\beta + \alpha_1 X_\alpha I_\alpha + \alpha_2 X_\alpha I_\alpha + \beta_1 X_\beta I_\beta + \beta_2 X_\beta I_\beta + \varepsilon$$

where α_1 and α_2 are slope terms for two different branches from the same tree and β_1 and β_2 are slope terms for two different branches from tree β . Due to bias estimators from overparameterization, we were not able to create individual intercept terms for each branch and were forced to use general intercept terms for each tree.

Following the likelihood ratio tests for flow-path length as the independent variable X and foliar $\delta^{13}\text{C}$ as the dependent variable Y , a number of other models were investigated. First, foliar N concentration and specific leaf area were used as dependent variables to investigate relationships between foliar N concentration, specific leaf area, and flow-path length. Second, foliar N concentration and specific leaf area were added as independent variables along with flow-path length to investigate whether foliar N concentration and specific leaf area added significantly to the explanation of foliar $\delta^{13}\text{C}$. In all cases, the full model consisted of separate intercepts for each tree and separate slopes for each tree or branch. As before, likelihood ratio tests were used to reduce slope terms to a minimum for each dependent variable.

Results and Discussion

In longleaf pine, foliar $\delta^{13}\text{C}$ values ranged between -25 and -31% with generally less depletion in seedlings and saplings relative to mature trees (Figure 1). This range of 6% in foliar $\delta^{13}\text{C}$ was similar to the 6% range reported for foliage of an open grown walnut tree (*Juglans regia* L.) (Le Roux et al. 2001) and to the 6% range observed in sun foliage of Monterey pine (Waring and Silvester 1994). Foliar $\delta^{13}\text{C}$ values in longleaf pine were lower than the range reported for

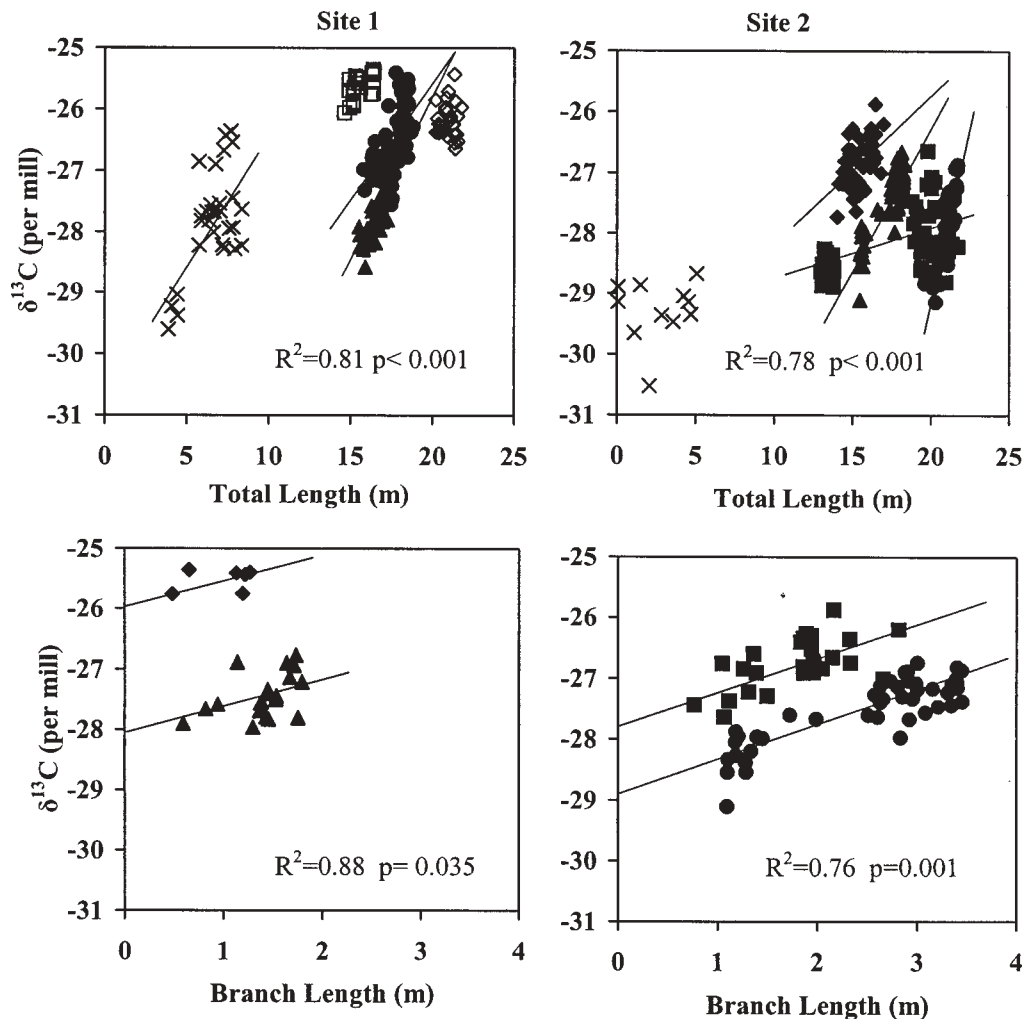


Figure 1. Results of linear regression analyses between foliar $\delta^{13}\text{C}$ and total (bole + branch) or branch flow-path length in longleaf pine at two sites. Different symbols denote different trees or different branches of two trees. The level of significance for the slopes is indicated by the P -value. Solid symbols indicate specific large trees or branches with significant slopes, and x indicates small trees.

other *Pinus* species (-19% to -28%) (Warren et al. 2001) and may reflect lower photosynthetic capacity of this species in response to low soil N availability typical of the longleaf pine wiregrass ecosystem (McGuire et al. 2001). For example, Ellsworth (1999) reported a value of -27% for current-year sun foliage collected from 15-yr-old loblolly pine (*Pinus taeda* L.) and an average foliar N concentration of 12 mg g^{-1} was reported for the same stands (Myers et al. 1999). In contrast, foliar N concentration ranged between $6\text{--}12 \text{ mg g}^{-1}$ in mature longleaf pines (see Figure 4).

Grulke and Retzlaff (2001) determined that total flow-path length was a significant predictor of foliar $\delta^{13}\text{C}$ in sun foliage of ponderosa pine ranging in age from 3 to over 200 yr old. Similarly, flow-path length was positively related to foliar $\delta^{13}\text{C}$ in longleaf pine, and the models with total or branch flow-path length explained over 75% of the variation in foliar $\delta^{13}\text{C}$. At site 1, the full model indicated a significant positive slope between foliar $\delta^{13}\text{C}$ and total flow-path length for the small trees and two large trees (average slope of $0.47\% \text{ m}^{-1}$), and the model accounted for 81% of the variation in foliar $\delta^{13}\text{C}$ (Figure 1). Two mature trees showed no relationship between foliar $\delta^{13}\text{C}$ and total flow-path length,

possibly because ranges in total flow path length were small ($< 3 \text{ m}$). At site 2 the full model indicated a significant positive relationship between foliar $\delta^{13}\text{C}$ and total flow-path length for all the large trees (average slope of $0.45\% \text{ m}^{-1}$) and the model accounted for 78% of the variation in foliar $\delta^{13}\text{C}$ (Figure 1). A significant slope was not detected between foliar $\delta^{13}\text{C}$ and total flow-path length for small trees at site 2 that exhibited a maximum flow-path length of 5 m. The large root to shoot ratio typical in young longleaf pine may have prevented water limitation in juvenile trees.

Because pathway length to each individual tuft was a function of height on the bole and branch length, the relationship between foliar $\delta^{13}\text{C}$ and branch length was examined on trees with branches at a similar height. Sample branches ranged in height from 15.2 to 15.7 m at site 1, and from 14.2 to 14.9 m at site 2 (Table 1). The reduced model with one slope (0.44 and $0.50\% \text{ m}^{-1}$ at sites 1 and 2, respectively) and individual tree intercepts indicated a significant positive relationship between branch length and foliar $\delta^{13}\text{C}$ at both sites (Figure 1). The models with branch flow-path length explained 88% of the variation in foliar $\delta^{13}\text{C}$ at site 1 and 76% of the variation in foliar $\delta^{13}\text{C}$ at site 2. As observed in longleaf

pine, branch length was positively correlated to foliar $\delta^{13}\text{C}$ under varying soil and water availability in Monterey pine and the rate of enrichment was $0.33\% \text{ m}^{-1}$ (Waring and Silvester 1994, Walcroft et al. 1996) The higher enrichment rate in longleaf pine compared to Monterey pine may be in response to greater water limitation in the longleaf pine ecosystem or to greater sapwood permeability (Pothier et al. 1989) in 12- to 20-yr-old Monterey pine compared to 40 to 80-yr-old longleaf pine. Although Cernusak and Marshall (2001) concluded that for notched western white pine branches (*Pinus monticola* Dougl) the adjustment of current year leaf area was a more important response to reduced branch conductivity than adjustment of c_i/c_a , no relationship between branch length and current year leaf area was observed in longleaf pine (Figure 2).

Average slopes for the equations describing the relationship between foliar $\delta^{13}\text{C}$ and total or branch flow-path length were similar between sites, most likely because of the hot dry summers that characterize the longleaf pine sandhills habitat in the lower Coastal Plain (Meldahl et al. 1999). The relationship between flow-path length and needle physiology has been shown to be dependent on site water availability in some species. For instance, a strong negative correlation between foliar carbon isotope discrimination and tree height was reported for four black spruce (*Picea mariana* [Mill.] B.S.P.) families on a dry site but not on more productive sites (Flanagan and Johnsen 1995). Similarly, correlation between foliar $\delta^{13}\text{C}$, branch length and branch hydraulic conductivity in *Pinus pinaster* was significant only on drier sites (Warren and Adams 2000). Foliar $\delta^{13}\text{C}$ of Douglas-fir (*Pseudotsuga menziesii* [Mirb.] Franco) and western hemlock (*Tsuga heterophylla* [Raf.] Sarg.) increased with forest age but differences in foliar $\delta^{13}\text{C}$ among needles from different aged forests were greatest during dry periods (Fessenden and Ehleringer 2002).

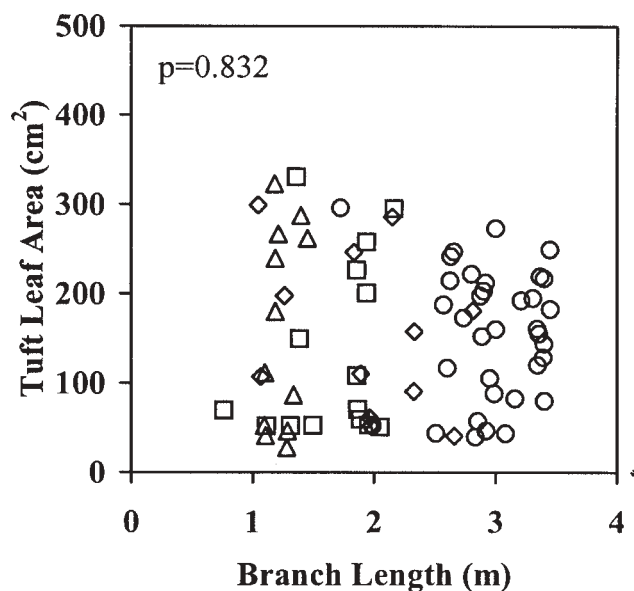


Figure 2. Results of linear regression analyses between current-year total tuft leaf area and branch flow-path length in longleaf pine at site 2. Different symbols denote different branches of two trees. The level of significance for the slopes is indicated by the P-value. Solid symbols indicate branches with significant slopes.

Williams et al. (2001) using a process-based model of the soil-plant-air continuum concluded that differences in sap flow between different size ponderosa pine trees were best explained by increasing hydraulic resistance with increasing tree height. We were unable to isolate bole height effects from branch length effects, because of the difficulty in finding branches of the same length at different stem heights. It is feasible that branch effects may vary with tree height, which may explain the need for different slopes in describing the relationship between total pathway length and $\delta^{13}\text{C}$ for each tree. However, Tyree and Ewers (1991) concluded that the greatest resistance to water transport is over the last meter of branch and Hubbard et al. (2002) concluded that height alone did not explain differences in hydraulic conductance between upper and lower canopy branches of open-grown ponderosa pine. Other potential sources for individual tree slopes include differential root access to soil resources and ontogenetic effects (Percy et al. 1987).

Thomas and Winner (2002) concluded that large trees consistently exhibit lower leaf area per unit leaf mass than smaller trees, and based on work with Scots pine (*Pinus sylvestris* L.) and Norway spruce (*Picea abies* [L.] Karst.) Niinemets (2002) proposed that increases in needle mass per unit area with increasing tree height result in a higher leaf elastic modulus that allows for larger water potential gradients between the soil and foliage, and subsequently increased soil water extraction. A negative relationship between specific leaf area and total flow-path length was indicated for small trees at both sites and large trees as a group at site 2 (the reduced model with one slope and intercept for small trees and one slope and intercept for large trees) (Figure 3). Variation in specific leaf area in large trees was not well explained by branch flow-path length (at site 2 the reduced model with one slope and intercept explained only 7% of the variation in specific leaf area). The majority of change in specific leaf area with increasing flow-path length occurred between 0–10 m and a strong relationship between specific leaf area and flow path length was not indicated for large trees. Rates of change in foliar morphology have been shown to be greater between the juvenile stage and early reproductive maturity than between mid-aged and old-growth stages (Day et al. 2002).

A direct test was not performed to determine if decreased hydraulic conductance was the source of increased foliar $\delta^{13}\text{C}$, but we suggest that correlations between flow-path length and foliar $\delta^{13}\text{C}$ indicate that hydraulic resistance may increase with increasing flow-path length in longleaf pine as reported for other conifers, and the relationship between foliar $\delta^{13}\text{C}$ and flow-path length was a function of stomatal limitation and increased water use efficiency rather than decreased photosynthetic capacity (Waring and Silvester 1994, Yoder et al. 1994, Panek 1996, Walcroft et al. 1996, Warren and Adams 2000). Although foliar $\delta^{13}\text{C}$ can be an integrative indicator of water stress and water use efficiency in conifers (Korol et al. 1999), it is important to note that other factors may also influence foliar $\delta^{13}\text{C}$. For example, Livingston et al. (1998) concluded that along with environmental conditions, shoot developmental processes that influ-

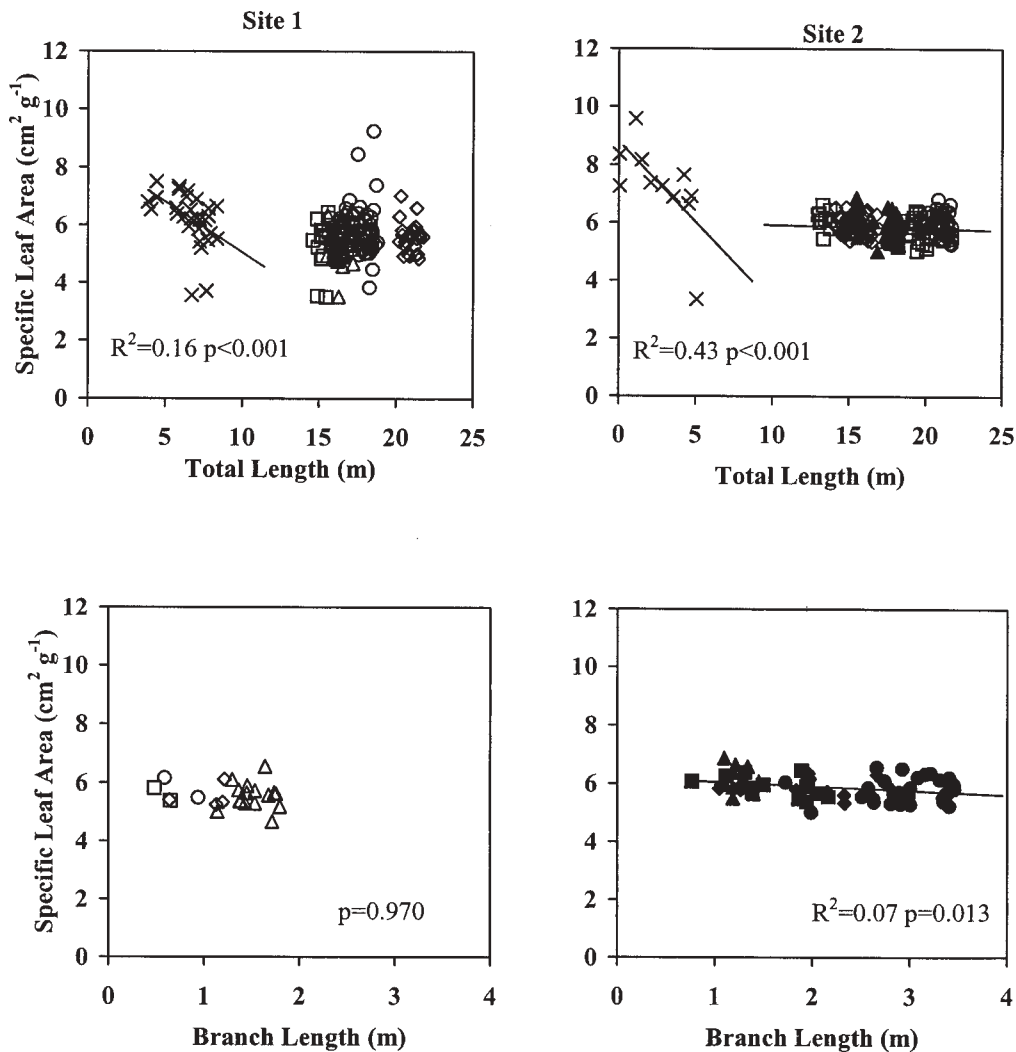


Figure 3. Results of linear regression analyses between specific leaf area and total (bole + branch) or branch flow-path length in longleaf pine at two sites. Different symbols denote different trees or different branches of two trees. The level of significance for the slopes is indicated by the p -value. Solid symbols indicate specific large trees or branches with significant slopes, and x indicates small trees.

ence carbon and nutrient allocation also influence foliar $\delta^{13}\text{C}$ and shoots representing major carbon sinks strongly influence photosynthetic capacity. Additionally, recycling of respired CO_2 may influence variation in foliar $\delta^{13}\text{C}$, but in these open stands turbulent mixing likely eliminated gradients in source air $\delta^{13}\text{C}$. Buchmann et al. (1997) determined that although the most depleted $\delta^{13}\text{C}$ values were found near the forest floor, source air changes ranged from 5–14% in open lodgepole pine (*Pinus contorta* Dougl. ex Loud.) stands and variation in source air affected $\delta^{13}\text{C}$ less than ecophysiological responses.

Foliar N concentration and specific leaf area have been shown to be surrogates for variation in canopy light environment and photosynthetic capacity (Pierce et al. 1994, Reich et al. 1995); however, some literature reports little or no correlation between photosynthesis and foliar N concentration in pines (Korol et al. 1999). In longleaf pine, foliar N concentration was not significantly related to total or branch flow-path length at site 1, but at site 2 the reduced model with one general slope and individual tree intercepts indicated that total or branch flow-path length models explained only 27 to

33% of the variation in foliar N concentration (Figure 4). Although foliar N and specific leaf area were not strongly related to flow-path length, both terms explained additional variation in foliar $\delta^{13}\text{C}$ (Table 2). At site 1, the inclusion of foliar N concentration and specific leaf area allowed a reduced model with a single slope for foliar N, specific leaf area, and total flow-path length (Table 2), indicating that light may co-vary with canopy height. At site 2, single slopes for foliar N and specific leaf area were sufficient, but separate slopes for total flow-path length were still needed. At the branch level, foliar N and specific leaf area were significant in the model only at site 2, and single slopes were sufficient for all independent variables (Table 2). The t values for each slope indicate that flow-path length was relatively more important than either foliar N or specific leaf area in describing foliar $\delta^{13}\text{C}$ (Table 2). We are not suggesting that light environment is not significant in understanding variation in $\delta^{13}\text{C}$, and, indeed, light environment may account for some of the variation between trees observed for flow-path length alone, only that flow-path length is an important limitation to leaf gas exchange in savanna longleaf pine.

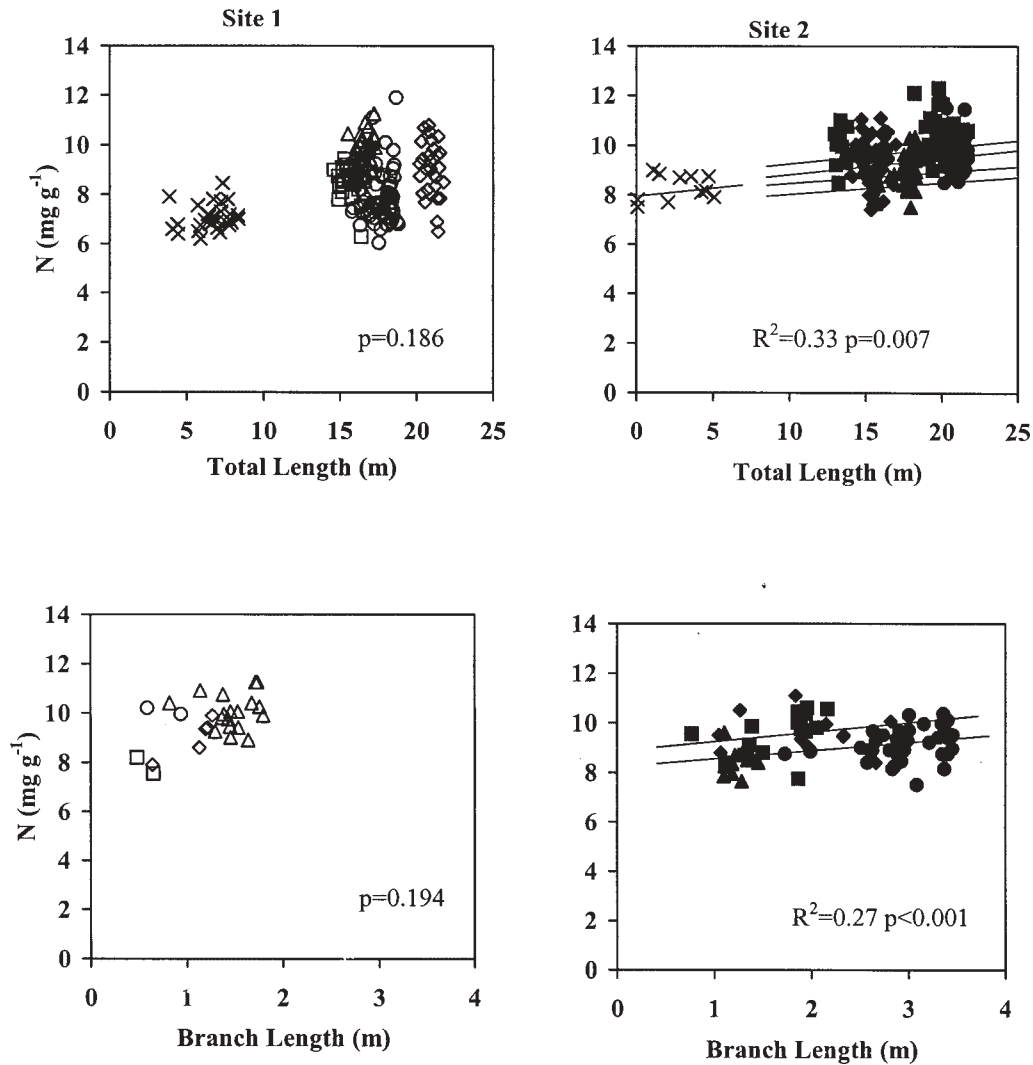


Figure 4. Results of linear regression analyses between foliar nitrogen concentration (N) and total (bole + branch) or branch flow-path length in longleaf pine at two sites. Different symbols denote different trees or different branches of two trees. The level of significance for the slopes is indicated by the *P*-value. Solid symbols indicate specific large trees or branches with significant slopes, and x indicates small trees.

In conclusion, high correlation between foliar $\delta^{13}\text{C}$ enrichment and total or branch flow-path length was observed in longleaf pine at two separate sites. We cannot rule out the exogenous and endogenous factors that may have influenced foliar $\delta^{13}\text{C}$, but the strong relationships reported here support the

importance of hydraulic architecture in understanding variation in leaf carbon gain in conifers. These results indicate the need for mechanistic studies of hydraulic conductance in longleaf pine to better understand the ecophysiology of this unique ecosystem and to facilitate scaling between large and small trees.

Table 2. Results of multiple linear regression analyses between foliar $\delta^{13}\text{C}$ and foliar nitrogen concentration (N), specific leaf area (SLA), and flow-path length (total or branch) for longleaf pine at two sites.

Variable	<i>t</i> value	<i>P</i> > <i>t</i>	Model <i>R</i> ²
Site 1: Trees			
N	6.51	<0.001	0.85
SLA	-2.10	0.004	
Total flow-path length	9.67	<0.001	
Site 2: Trees			
N	6.58	<0.001	0.84
SLA	-4.52	<0.001	
Total flow-path length	3.4-9.8	<0.001	
Site 2: Branches			
N	4.90	<0.001	0.83
SLA	-3.15	0.002	
Branch flow-path length	8.70	<0.001	

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