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Irrigation and fertilization effects on foliar and soil carbon and nitrogen isotope ratios in a loblolly pine stand

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Abstract

We examined $\delta^{13}C$ and $\delta^{15}N$ in needle (current and 1-year-old) and soil samples collected on two occasions (July and September 1999) from a 15-year-old loblolly pine (*Pinus taeda* L.) stand in an irrigation and fertilization experiment to investigate whether these treatments leave specific isotope signals in the samples and thus to infer the effects of treatments on C and N cycling. Irrespective of foliar age or sampling date, carbon isotope discrimination (Δ) was increased by irrigation, and decreased by fertilization. The carbon isotope discrimination model suggested that increased Δ by irrigation was due to decreased water use efficiency (WUE) through increased stomatal and/or mesophyll conductance. The decreased Δ by fertilization and published gas exchange measurements at the same site suggest that water stress resulting from increased water demand in the fertilized plots improved WUE through increased stomatal control of water loss. Foliar $\delta^{15}N$ values were not affected by irrigation, but were increased by fertilization. The $\delta^{15}N$ value of soil NH_4^+ were higher than those of NO_3^- and were positively correlated with foliar $\delta^{15}N$, suggesting that tree uptake of NH_4^+ was one of the factors affecting foliar $\delta^{15}N$. We conclude that irrigation and fertilization affected $\delta^{13}C$ and $\delta^{15}N$ values in plant and soil samples, reflecting changed C and N cycling patterns and water use efficiency in the studied loblolly pine stand.

Keywords: $^{13}C/^{12}C$; $^{15}N/^{14}N$; Carbon isotope discrimination; Nitrification; Water use efficiency; *Pinus taeda*

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1. Introduction

Loblolly pine (*Pinus taeda* L.) is native in southeastern United States and is widely planted in

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this region because of its high plasticity to environmental conditions and high productivity (Albaugh et al., 2004). Much of the planted loblolly pine forests in southeastern United States occur on relatively sandy sites that have low water holding capacity and soil fertility (Allen et al., 1990). To improve stand productivity by manipulating soil resource availability, silvicultural treatments such as fertilization and irrigation are often used in this area (Albaugh et al., 1998; Allen, 2001).

Fertilization and irrigation increase loblolly pine stand productivity that can be measured as total height, basal area, leaf area index, or total biomass production (Albaugh et al., 1998, 2004). Fertilization is generally assumed to increase net photosynthesis through changing both the photosynthetic apparatus and CO_2 assimilation capacity of pine species (Evans, 1989; Kellomaki and Wang, 1997; Strand, 1997). For example, fertilization-induced increase in foliar N concentration was positively correlated with rates of net photosynthesis of loblolly pine seedlings (Green and Mitchell, 1992; Gough et al., 2004a), and such a correlation was also observed for 9-year-old loblolly pine trees during the first 2 years of fertilization (Murthy et al., 1996, 1997). However, fertilization effects on net photosynthesis have been shown to decrease or disappear as trees grow or the time since fertilization lapses. For example, after a relatively longer period of time after fertilization (>4 years), intensive gas exchange measurements showed that fertilization increased foliar N concentration, but did not improve rates of net photosynthesis of loblolly pine trees (Tang et al., 1999; Samuelson et al., 2001; Gough et al., 2004b). The mechanisms underlying the changing photosynthetic responses of loblolly pine to fertilization remain unclear; it has been ascribed to downward regulation of net photosynthesis after achieving maximum leaf area (Gough et al., 2004b), to increasing amount of mutual shading in the canopy (Zhang et al., 1997), or to improved water use efficiency (WUE) as trees grow faster after fertilization (Ewers et al., 1999).

Irrigation is believed to increase tree growth mainly by improving stomatal conductance, which facilitates CO_2 diffusion into photosynthetic cells (Green and Mitchell, 1992); however, measurements of stomatal conductance of loblolly pine trees have not always provided consistent indication of such improvement, probably due to the shortcoming of instantaneous measurements which do not integrate the physiological process over the growing season (Murthy et al., 1996, 1997). Hence, alternative measures of photosynthetic performance may contribute to the better understanding of the physiological responses of loblolly pine stands to silvicultural treatments.

The relative abundance of ^{13}C and ^{12}C (expressed as $\delta^{13}C$) in foliar tissue could serve as a time-integrating measure of gas exchange response of loblolly pine to fertilization and irrigation, because altered WUE, the ratio of net photosynthesis to transpiration, by the treatments is directly related to carbon isotope discrimination against ^{13}C (Δ) during photosynthesis (e.g., Livingston et al., 1999; Ripullone et al., 2004). Several studies observed increases in Δ in pine species under well-watered conditions and decreases in Δ under N-sufficient conditions relative to the untreated (Korol et al., 1999; Livingston et al., 1999; Warren et al., 2001). In combination with $\delta^{13}C$, stable nitrogen isotope abundance ($\delta^{15}N$) of tree tissue is expected to provide insights into tree responses to changing nutrient and water conditions. It is generally accepted that $\delta^{15}N$ values of plant or soil samples cannot be used as sole indicators of specific processes because the values are affected not only by N isotope composition of external sources but also by several N isotope fractionation processes associated with N dynamics in the soil–plant system (Högberg, 1997; Chang and Handley, 2000; Choi et al., 2003). However, since such factors are susceptible to changing conditions such as nutrient and water availability, repeated imposition of such treatments may produce systematic $\delta^{15}N$ patterns in plant and soil samples.

In spite of the potential usefulness of the stable C and N isotope techniques, as far as we know there has been no study on the variations in $\delta^{13}C$ and $\delta^{15}N$ in loblolly pine stands as affected by fertilization and irrigation. Through extensive sampling of various ecosystems and studying the effects of ecosystem manipulations, we expect to gain a better understanding of $\delta^{13}C$ and $\delta^{15}N$ patterns in soil–plant systems. In this study, we investigated the variations of $\delta^{13}C$ and $\delta^{15}N$ signatures in foliar and soil samples collected from a loblolly pine stand which has been subjected to long-term (over 7 years) fertilization and irrigation treatments. The specific objectives were to examine (1) whether fertilization and irrigation leave specific $\delta^{13}C$ signals reflecting their effects on WUE, and (2) whether changed N dynamics induced by the silivicultural treatments could be inferred from the $\delta^{15}N$ of foliage and soil samples.

2. Materials and methods

2.1. Study site

The study was conducted in a loblolly pine fertilization and irrigation trial at the Southeast Tree Research and Education Site (SETRES) at Sandhills (35°N, 79°W), Scotland County, NC, USA. The site is flat, infertile, excessively drained, sandy, and siliceous. The soil is a thermic Psammentic Hapludult. The soil has a water holding capacity of 0.18 m in the top 2 m profile. Annual precipitation averages 1210 mm with roughly even distribution throughout the year, but extended droughts are possible during the growing season. Mean annual temperature is 17 °C with an average of 303 frost-free days. Mean winter and summer temperatures are 9 and 26 °C, respectively.

In 1992, sixteen 50 m × 50 m treatment plots with the center 30 m × 30 m as the measurement plot were set up in an 8-year-old loblolly pine stand, and a 2 × 2 factorial combination of fertilization (no addition and complete nutrition) and irrigation (no addition and well watered) experiment with four replications was established. The treatments are control (Control), irrigation (IRR), fertilization (FER), and irrigation plus fertilization (IRR + FER). Fertilizer was applied annually to attain a foliar N concentration of 1.3% with other nutrients (P, K, Ca, Mg, S, and B) in balance. Foliar nutrient status was monitored monthly and fertilizer was applied as necessary throughout the year to meet the above target. The cumulative amount of each nutrient (in kg ha^{-1}) added from 1992 to 1998 was N (777), P (151), K (337), Ca (168), Mg (164), S (208), and B (3.9). Fertilizer urea ($\delta^{15}N = -2.7‰$) was used as the N source. Irrigation was targeted to maintain soil water content at greater than 3 cm in the surface 50 cm of soil (the equivalent of 40% (v/v) available water content, with water holding capacity about 6.5 cm in the upper 50 cm of the profile) as determined from volumetric soil water content measured with time domain reflectometry on biweekly intervals during the growing season (from March to October). More details of the site and treatments are described in Albaugh et al. (1998, 2004).

2.2. Sampling and chemical analyses

In 1999, 10 average-sized trees were randomly selected in each plot, and three branches at the upper crown position of each tree were located and marked. These branches were from different whirls and different directions. Current and 1-year-old needle samples were collected from the branches on two occasions (July 27 and September 27) to examine potentially different responses of foliage with different age and to explore their temporal changes along leaf-developmental stage. Ten fully expanded needles were collected from each branch and composited for each plot. All plant samples were oven dried at 60 °C, ground to fine powder in a ball mill (MM 200, Retsch GmbH & Co. KG, Hann, Germany), and analyzed for N concentration, $\delta^{15}N$, and $\delta^{13}C$ using methods described below.

At each sampling, soil samples were collected from 0 to 20 cm mineral soil layer, where most of the tree roots are found. A sub-sample was air-dried, ground to fine powder in the ball mill, and analyzed for C and N concentrations, $\delta^{15}N$, and $\delta^{13}C$ as described below. To determine available N concentration and to obtain enough sample size for $\delta^{15}N$ analysis, fresh soil samples (600 g in dry basis) were extracted with 2 M KCl at 1:1.25 ratio, and steam-distilled with MgO for NH_4^+; thereafter the samples in the flasks were distilled again after addition of Devarda's alloy to determine NO_3^- on a steam distillation system (Vapodest 20, C. Gerhardt GmbH & Co. KG, Königswinter, Germany). The liberated NH_3 was collected in 0.01N H_2SO_4 solution (Keeney and Nelson, 1982). To prevent isotopic cross-contamination between samples, 25 ml of reagent-grade ethanol was added to distillation flasks and steam-distilled for 3 min between each sample distillation (Hauck, 1982). Nitrogen concentrations were determined by titration with 0.01N NaOH using an automatic potentiometric titrator (719s Titrino, Metrohm, Herisau, Switzerland). The H_2SO_4 solution containing NH_4^+ was evaporated to dryness at 65 °C in an oven after adjustment of the solution to pH 3 using 0.1N H_2SO_4 and analyzed for $\delta^{15}N$ (Hauck, 1982; Feast and Dennis, 1996). The accuracy and reproducibility of the analytical procedure checked with reference materials, IAEA-N2 (ammonium sulfate, +20.3‰) and IAEA-N3 (potassium nitrate, +4.6‰), were better than 0.3 and 0.2‰, respectively.

Nitrogen concentration, $\delta^{13}C$, and $\delta^{15}N$ (in foliage and soil samples) were determined using a continuous-flow stable isotope ratio mass spectrometer (IsoPrime-EA, Micromass, UK) linked to a CN analyzer (NA Series 2, CE Instruments, Italy). In this study, isotope compositions of whole foliage samples were investigated because a study comparing whole tissue, cellulose, and lignin (Loader et al., 2003) showed that whole tissue retains the strongest C isotope signal for environmental conditions as its composition is closest to that of leaf sugars. Korol et al. (1999) and Warren et al. (2001) also found similar C isotope compositions between whole tissue and cellulose.

Carbon and nitrogen isotope compositions were calculated as:

$$\delta\,(‰) = \left(\frac{R_{\text{sample}}}{R_{\text{standard}}} - 1\right) \times 1000 \qquad (1)$$

where R is the ratio of $^{13}C/^{12}C$ or $^{15}N/^{14}N$, and the standards are the Pee Dee Belemnite standard (PDB) for carbon and atmospheric N_2 for nitrogen. Pure CO_2 ($\delta^{13}C = -28.2 \pm 0.1‰$) and N_2 ($\delta^{15}N = -2.1 \pm 0.1‰$) gases calibrated against standard CO_2 (−10.38‰) and N_2 (−0.22‰) gases (Oztech Trading Co. Dallas, TX, USA) using a dual-inlet stable isotope ratio mass spectrometer (Optima, Micromass, UK) served as reference gases for $\delta^{13}C$ and $\delta^{15}N$, respectively. The accuracy and reproducibility of the measurements of $\delta^{13}C$ and $\delta^{15}N$ checked with an internal reference material, cabbage (*Brassica campestris* L.) sample (−28.3 ± 0.1‰ for $\delta^{13}C$ and +3.4 ± 0.1‰ for $\delta^{15}N$), calibrated against NIST SRM 8542 (sucrose, −10.5‰) for $\delta^{13}C$ and against IAEA-N2 for $\delta^{15}N$, were better than 0.2 and 0.1‰ for $\delta^{13}C$ and 0.3 and 0.2‰ for $\delta^{15}N$, respectively.

2.3. Statistical analyses

For statistical analysis, data were tested first for homogeneity of variance and normality of distribution. Transformation of data was not needed as no heterogeneity was detected in the data set and distribution was normal. Analysis of variance (ANOVA) was performed for a 2 × 2 factorial design with four replications on all experimental variables using the general linear models (GLM) procedure in the SPSS 11.5 package (SPSS Inc., Chicago, IL, USA) to assess the significance of the effects of fertilization, irrigation, and their interactions. A level of significance at $\alpha = 0.05$ is used for inferring statistical significance.

Pearson correlation analysis was performed to examine relationships between parameters (e.g., foliar $\delta^{13}C$ versus foliar N concentration, foliar N concentration versus foliar $\delta^{15}N$, $\delta^{15}N$ of soil mineral N versus foliar $\delta^{15}N$) using the SPSS 11.5 package.

3. Results

3.1. Soil C and N concentrations, and $\delta^{13}C$ and $\delta^{15}N$

Because soil C and N contents, and $\delta^{13}C$ and $\delta^{15}N$ values were virtually the same between the July and September samplings, the average values were reported for comparison among treatments (Table 1). Total soil C and N concentrations and their corresponding isotope abundances were neither significantly affected by fertilization nor by irrigation, showing values that range from 12.9 to 14.4 g kg^{-1} for total C concentrations and −26.1 to −25.6‰ for the corresponding $\delta^{13}C$ values, and 0.42 to 0.44 g kg^{-1} for total N concentration and +2.2 to +2.8‰ for the corresponding $\delta^{15}N$ values (Table 1).

3.2. Concentration and $\delta^{15}N$ of soil mineral N

At both sampling dates, neither irrigation nor fertilization affected NH_4^+ concentration, but NO_3^- concentration was significantly affected by irrigation and fertilization (Table 2). Fertilization increased NO_3^- concentration from 0.1 to 1.5 mg kg^{-1} in July and from 0.1 to 1.7 mg kg^{-1} in September within the non-irrigation treatments. On the other hand, irrigation decreased NO_3^- concentration; for example, irrigation with fertilization decreased NO_3^- concentration from 1.5 to 0.6 mg kg^{-1} in July and 1.7 to 0.5 mg kg^{-1} in September. Overall, the mineral N ($NH_4^+ + NO_3^-$) concentration showed an increasing pattern in the following order: FER > IRR + FER > Control > IRR, with the main difference ($P < 0.05$) between the FER and other three treatments.

Table 1
Carbon and nitrogen concentrations, and $\delta^{15}N$ and $\delta^{13}C$ (mean with S.E. in parentheses) of the total soil pool of a loblolly pine stand as affected by irrigation and fertilization

Treatment[a]	Carbon		Nitrogen	
	Concentration (g kg^{-1})	$\delta^{13}C$ (‰)	Concentration (g kg^{-1})	$\delta^{15}N$ (‰)
Control	13.7 (1.3)	−25.8 (0.2)	0.42 (0.02)	+2.5 (0.2)
IRR	14.4 (0.6)	−26.1 (0.2)	0.42 (0.01)	+2.8 (0.3)
FER	12.9 (0.5)	−25.6 (0.2)	0.42 (0.02)	+2.8 (0.1)
IRR + FER	13.7 (0.6)	−25.7 (0.1)	0.44 (0.01)	+2.2 (0.3)
Effect	Probability > *F*			
IRR	0.394	0.343	0.454	0.497
FER	0.370	0.106	0.515	0.461
IRR × FER	0.977	0.639	0.603	0.053

[a] IRR, irrigation; FER, fertilization; IRR + FER, irrigation plus fertilization.

In contrast to the mineral N concentration data, $\delta^{15}N$ of NH_4^+ rather than NO_3^- was significantly affected by irrigation and fertilization (Table 3). Irrigation tended to decrease $\delta^{15}N$ of NH_4^+ and that was significant in September; $\delta^{15}N$ of NH_4^+ decreased by irrigation from +0.3 to −3.3‰ within the unfertilized treatments and from +5.2 to +1.9‰ within the fertilization treatments. Fertilization significantly increased $\delta^{15}N$ of NH_4^+ by around 5‰ units on both sampling dates, regardless of irrigation. Irrespective of the treatment and sampling date, NO_3^- (ranges from −8.6 to −5.8‰) showed consistently lower $\delta^{15}N$ value than NH_4^+ (ranges from −4.6 to +5.2‰). The integrated mean $\delta^{15}N$ values of soil mineral N ($NH_4^+ + NO_3^-$) calculated using the isotope mass balance approach did not show any significant variations among treatments.

3.3. Foliar $\delta^{13}C$, N concentration, and $\delta^{15}N$

On both sampling dates, the $\delta^{13}C$ values of both current and 1-year-old needles showed that irrigation increased Δ during photosynthesis, while fertilization decreased Δ (Table 4). The most significant effect of irrigation or fertilization was observed for 1-year-old needle samples collected in July; irrigation changed foliar $\delta^{13}C$ from −28.5 to −29.2‰ within the unfertilized and from −27.7 to −28.5‰ within the fertilized treatments, while fertilization changed foliar $\delta^{13}C$ from −28.5 to −27.7‰ within the non-irrigated and from −29.2 to −28.5‰ within the irrigated treatments.

Overall, N concentrations were higher in the current year than in the 1-year-old needle, and they decreased from July to September (Table 5). Fertilization significantly increased nitrogen concentration of

Table 2
Concentrations (mean with S.E. in parentheses) of soil mineral N in a loblolly pine stand as affected by irrigation and fertilization

Treatment[a]	Sampling time and N species					
	July			September		
	NH_4^+ (mg N kg^{-1})	NO_3^- (mg N kg^{-1})	$NH_4 + NO_3$ (mg N kg^{-1})	NH_4^+ (mg N kg^{-1})	NO_3^- (mg N kg^{-1})	$NH_4 + NO_3$ (mg N kg^{-1})
Control	0.5 (0.2)	0.1 (0.1)	0.6 (0.2)	0.7 (0.1)	0.1 (0.0)	0.8 (0.2)
IRR	0.5 (0.1)	0.1 (0.1)	0.6 (0.1)	0.6 (0.2)	0.0 (0.0)	0.6 (0.2)
FER	1.0 (0.4)	1.5 (0.3)	2.5 (0.5)	0.3 (0.1)	1.7 (0.2)	2.0 (0.3)
IRR + FER	0.5 (0.1)	0.6 (0.2)	1.1 (0.2)	0.3 (0.1)	0.5 (0.3)	0.8 (0.4)
Effect	Probability > *F*					
IRR	0.586	0.039	0.097	0.488	0.022	0.041
FER	0.385	0.001	0.008	0.059	0.003	0.092
IRR × FER	0.271	0.115	0.086	0.506	0.055	0.224

[a] Treatment codes are described in Table 1 footnote.

Table 3

The δ^{15}N (mean with S.E. in parentheses) of soil mineral N in a loblolly pine stand as affected by irrigation and fertilization

Treatment[a]	Sampling time and N species					
	July			September		
	NH_4^+ (‰)	NO_3^- (‰)	$NH_4 + NO_3$ (‰)	NH_4^+ (‰)	NO_3^- (‰)	$NH_4 + NO_3$ (‰)
Control	−4.6 (0.7)	−8.3 (0.7)	−5.6 (0.9)	+0.3 (0.6)	−5.8 (1.7)	+0.1 (0.7)
IRR	−3.6 (1.5)	−8.6 (1.2)	−3.9 (1.6)	−3.3 (0.9)	−5.9 (1.7)	−3.3 (0.9)
FER	+1.7 (2.5)	−7.4 (1.3)	−3.8 (2.2)	+5.2 (1.2)	−6.7 (0.8)	−4.8 (1.1)
IRR + FER	+0.2 (0.4)	−8.6 (1.5)	−4.9 (1.4)	+1.9 (1.2)	−8.2 (0.8)	−2.8 (1.0)
Effect	Probability > *F*					
IRR	0.921	0.589	0.647	0.020	0.582	0.660
FER	0.017	0.762	0.588	0.002	0.277	0.099
IRR × FER	0.461	0.775	0.304	0.969	0.615	0.056

[a] Treatment codes are described in Table 1 footnote.

both current and 1-year-old needles ($P < 0.001$) on both sampling dates by around 25%. However, irrigation effects were observed only for the 1-year-old needle. As inferred from the significant interaction, irrigation significantly decreased N concentration only within the fertilized treatments from 13.7 to 12.0 g kg^{-1} in July and from 12.8 to 11.2 g kg^{-1} in September. The δ^{15}N of neither current nor 1-year-old needles was affected by irrigation, while the values were significantly increased by fertilization from below −3.0 to about −1.5‰, regardless of needle age and sampling date (Table 6).

3.4. Correlations between variables

While the statistical significance varied, foliar δ^{13}C became less negative with increasing foliar N concentration (Fig. 1). The relationship between δ^{13}C and N concentrations in the current year needle was not significant ($P > 0.05$), but this correlation was significant for the 1-year-old needle ($P < 0.001$; Fig. 1).

The δ^{15}N values of both current and 1-year-old needles were positively correlated with δ^{15}N of soil NH_4^+ ($P < 0.05$) but not with soil NO_3^-, regardless of sampling date (Fig. 2). Foliar δ^{15}N values were also positively correlated with the corresponding foliar N concentrations (Fig. 3).

4. Discussion

4.1. Soil and foliar N concentration

Irrigation and fertilization changed soil N dynamics as shown by soil mineral N concentrations

Table 4

The δ^{13}C (mean with S.E. in parentheses) of current and 1-year-old foliage in a loblolly pine stand as affected by irrigation and fertilization

Treatment[a]	Sampling time and foliage type			
	July		September	
	Current (δ^{13}C (‰))	1-year-old (δ^{13}C (‰))	Current (δ^{13}C (‰))	1-year-old (δ^{13}C (‰))
Control	−27.6 (0.3)	−28.5 (0.1)	−28.1 (0.2)	−28.3 (0.1)
IRR	−28.6 (0.1)	−29.2 (0.2)	−28.6 (0.2)	−28.6 (0.1)
FER	−27.1 (0.3)	−27.7 (0.1)	−27.3 (0.3)	−27.8 (0.2)
IRR + FER	−28.1 (0.1)	−28.5 (0.2)	−28.3 (0.2)	−28.2 (0.1)
Effect	Probability > *F*			
IRR	<0.001	0.001	0.007	0.045
FER	0.040	0.001	0.045	0.011
IRR × FER	0.513	0.949	0.283	0.472

[a] Treatment codes are described in Table 1 footnote.

Table 5
Foliar N concentration (mean with S.E. in parentheses) of current and 1-year-old foliage in a loblolly pine stand as affected by irrigation and fertilization

Treatment[a]	Sampling time and foliage type			
	July		September	
	Current (g N kg^{-1})	1-year-old (g N kg^{-1})	Current (g N kg^{-1})	1-year-old (g N kg^{-1})
Control	12.0 (0.5)	10.5 (0.5)	11.2 (0.2)	9.5 (0.2)
IRR	11.8 (0.4)	10.4 (0.2)	10.7 (0.2)	9.7 (0.1)
FER	15.7 (0.5)	13.7 (0.1)	14.8 (0.5)	12.8 (0.1)
IRR + FER	15.6 (0.3)	12.0 (0.2)	14.3 (0.3)	11.2 (0.1)
Effect	Probability > *F*			
IRR	0.762	0.009	0.174	0.001
FER	<0.001	<0.001	<0.001	<0.001
IRR × FER	0.941	0.013	0.922	0.000

[a] Treatment codes are described in Table 1 footnote.

(Table 2). A decrease in NO_3^- concentration by irrigation suggests NO_3^- leaching loss due to the coarse-textured nature of the study soil; and this effect was more apparent within the fertilization treatment. Meanwhile, N fertilization promoted nitrification and thus increased NO_3^- concentrations in the fertilization treatment. Such effects of fertilization on nitrification are further discussed below in relation to $\delta^{15}N$ variations of soil mineral N.

Many studies (Murthy et al., 1996; Maier et al., 2002; Albaugh et al., 2004), including this research (Table 5), have shown that N fertilization consistently increases foliar N concentration of loblolly pine; however, irrigation does not show consistent effects on foliar N concentration (e.g., Murthy et al., 1996; Albaugh et al., 2004). In this study, irrigation decreased foliar N concentration of the 1-year-old needles within the fertilization treatment (Table 5). This result is consistent with Jose et al. (2003) who found a significant decrease in foliar N concentration of longleaf pine (*Pinus palustris* Mill.) by improved water availability, and they attributed that to a dilution effect resulting from enhanced biomass production by irrigation.

4.2. Foliar $\delta^{13}C$

The overall effects of irrigation or fertilization on $\delta^{13}C$ were consistent for the two foliage cohorts (current and 1-year-old) and season (July and September) we sampled (Table 4). The effect of irrigation on $\delta^{13}C$ variations is theoretically predict-

Table 6
The $\delta^{15}N$ (mean with S.E. in parentheses) of current and 1-year-old foliage in a loblolly pine stand as affected by irrigation and fertilization

Treatment[a]	Sampling time and foliage type			
	July		September	
	Current (‰)	1-year-old (‰)	Current (‰)	1-year-old (‰)
Control	−3.1 (0.7)	−3.7 (0.2)	−4.8 (0.2)	−3.4 (0.1)
IRR	−3.4 (0.5)	−3.5 (0.3)	−4.3 (0.6)	−3.0 (0.2)
FER	−0.8 (1.2)	+0.2 (0.6)	+0.5 (0.7)	−0.6 (0.5)
IRR + FER	−1.3 (0.9)	−1.2 (0.4)	−0.6 (0.4)	−1.5 (0.6)
Effect	Probability > *F*			
IRR	0.633	0.168	0.551	0.489
FER	0.026	<0.001	<0.001	<0.001
IRR × FER	0.950	0.081	0.155	0.113

[a] Treatment codes are described in Table 1 footnote.

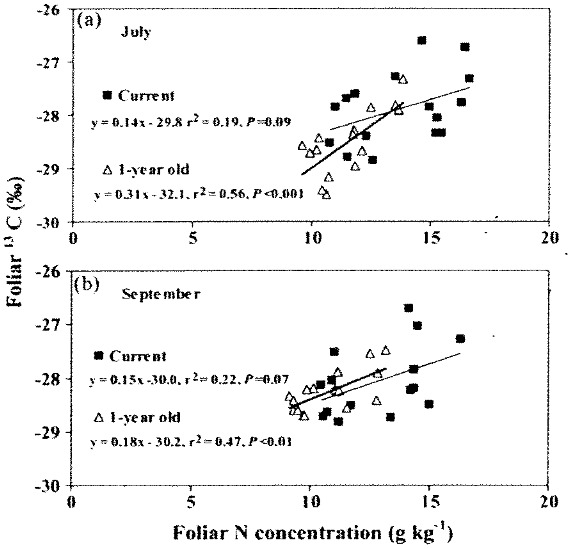

Fig. 1. Relationship between foliar δ^{13}C and N concentrations of loblolly pine needles collected in (a) July and (b) September 1999.

able from the carbon isotope discrimination model of C_3 photosynthetic pathway (Farquhar et al., 1989) that is described as:

$$\Delta = a + (b - a)\frac{C_i}{C_a} \tag{2}$$

where Δ is the carbon isotope discrimination, C_i/C_a the ratio of intercellular to atmospheric CO_2 concentration, and a and b are the discriminations against ^{13}C during CO_2 diffusion through stomata (normally 4.4‰) and during CO_2 fixation (normally 27‰), respectively. Although discrimination during CO_2 dissolution, diffusion in liquid phase, and (photo-) respiration may also affect Δ, this simple equation is widely used to represent the relationship between Δ and C_i/C_a. Increase in Δ results in more negative δ^{13}C value and vice versa because of its relationship with C_i/C_a ratio (Farquhar et al., 1989):

$$\delta^{13}C_{\text{plant}} = \delta^{13}C_{\text{air}} - a - (b - a)\frac{C_i}{C_a} \tag{3}$$

where $\delta^{13}C_{\text{plant}}$ and $\delta^{13}C_{\text{air}}$ are the carbon isotope abundances of plant and atmospheric CO_2, respectively.

According to this model, the more negative δ^{13}C in the irrigated treatment resulted from increased C_i/C_a by facilitated gas exchange through stomata and/or mesophyll cell. An estimation of WUE can be made from the δ^{13}C variations based on the relationship between WUE and C_i/C_a ratio (Farquhar et al., 1989):

$$\text{WUE} = \frac{C_a(1 - C_i/C_a)}{1.6v} \tag{4}$$

where v is the water vapor pressure difference between the intercellular space and the atmosphere.

Increases in Δ (decrease in WUE) of plants with a favorable water status have been observed for different tree species including conifers (e.g., Högberg et al., 1995; Korol et al., 1999; Warren et al., 2001). In this study, the δ^{13}C values show that the effects of irrigation on gas exchange over the growth period were at least as significant as that of fertilization (Table 4), although studies reported that productivity responses to irrigation was much lower than that to fertilization (Albaugh et al., 1998, 2004; Samuelson et al., 2001). Therefore, by using the stable C isotope technique, we are able to show that loblolly pine trees in non-fertilized plots at the SETRES site responded to the irrigation treatment, but limitation in the availability of other resources reduced the usefulness of the increased water availability to increase stand productivity (Albaugh et al., 2004).

Water use efficiency of loblolly pine trees was greater in the fertilized plots as indicated by the less negative δ^{13}C values in the current and 1-year-old needles on both sampling dates (Table 4). A positive correlation between foliar δ^{13}C values and foliar N concentrations (Fig. 1) also indicates that fertilization-induced increases in WUE resulted in a smaller Δ by lowering the C_i/C_a ratio. Sparks and Ehleringer (1997) and Ripullone et al. (2004) suggested that the decrease in Δ with increasing foliar N concentration reflects a positive effect of high N availability on photosynthetic rate. Many other studies also reported that N fertilization tends to decrease Δ by lowering C_i/C_a ratio mainly through enhanced carboxylation efficiency, particularly for seedlings or natural stands where N is the primary limiting factor (e.g., Högberg et al., 1993, 1995; Sparks and Ehleringer, 1997; Livingston et al., 1999; Ripullone et al., 2004). Recently, Ripullone et al. (2004) also found that, for Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco), N addition increased WUE (or less negative δ^{13}C) but neither transpiration nor stomatal conductance was affected in a greenhouse experiment. Hence, increased

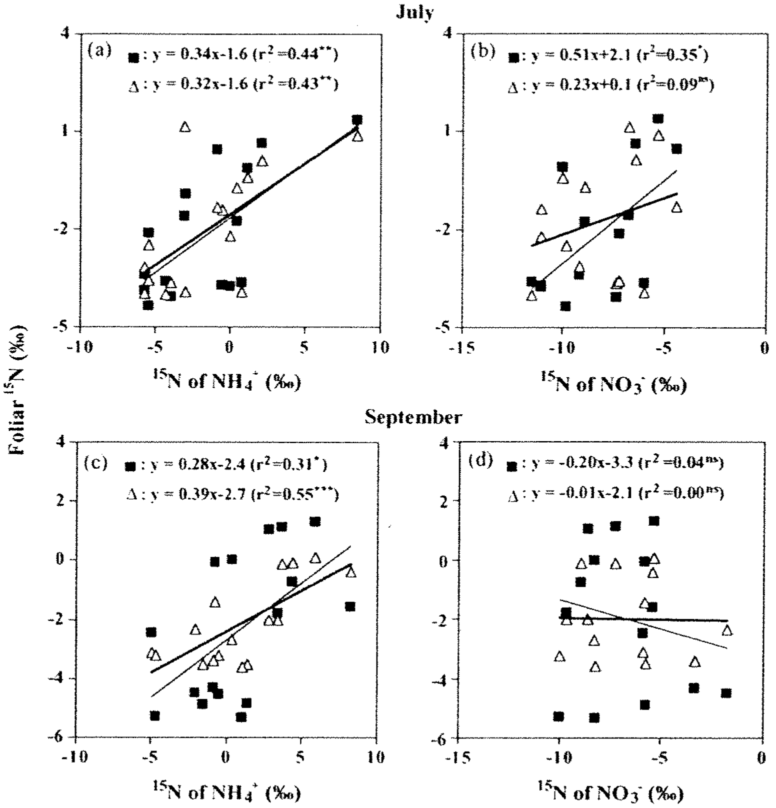

Fig. 2. Relationship between $\delta^{15}N$ of soil mineral N (NH_4^+ and NO_3^-) and foliage (current and 1-year-old needles) collected in July and September: (a) NH_4^+ vs. foliage $\delta^{15}N$ in July, (b) NO_3^- vs. foliage $\delta^{15}N$ in July, (c) NH_4^+ vs. foliage $\delta^{15}N$ in September, and (d) NO_3^- vs. foliage $\delta^{15}N$ in September. Closed square (■) indicates current and open triangle (Δ) indicates 1-year-old needle. The solid and dotted lines represent the regression for current and 1-year-old needles, respectively. The regression equations are shown in each figure. Symbols for statistical significance of the regression are: ns, non-significant; $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

WUE shortly after N fertilization could be primarily attributed to increased carboxylation efficiency.

Similar to other pine species, nitrogen supply could increase leaf level net photosynthesis of loblolly pine shortly after fertilization (Green and Mitchell, 1992; Murthy et al., 1996, 1997; Gough et al., 2004a) without changing stomatal conductance (Green and Mitchell, 1992). However, the increased WUE of loblolly pine which has been subjected to long-term fertilization treatment should be carefully interpreted because N fertilization effects on net photosynthesis have been shown to be negligible or even negative with increasing length of N fertilization (Tang et al., 1999; Samuelson et al., 2001; Gough et al., 2004b) and because a decrease in Δ (increase in WUE) could be caused by increasing carboxylation efficiency or decreasing CO_2 diffusion rate (see review by Ripullone et al., 2004). Ewers et al. (1999) reported that fertilization decreased transpiration rate per unit leaf area for loblolly pine at the SETRES site 4 years after initiation of fertilization, even though soil moisture availability was not different from that of the non-fertilized treatment. They suggested that increased water stress resulting from high leaf area index in the fertilization treatment led trees to use water more efficiently through stomatal control of water loss. Recently, Munger et al. (2003) found that 4 years of fertilization did not increase net photosynthesis, but did decrease stomatal conductance in loblolly pine trees. They suggested that the increased demand for water resulting from greater leaf biomass in fertilized stands might have led trees to acclimate to

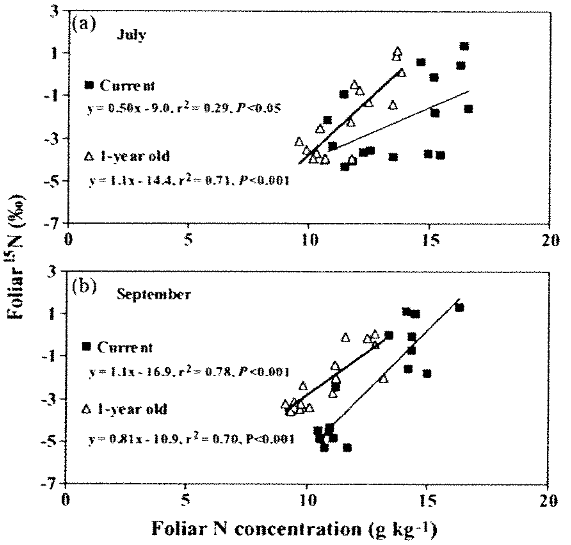

Fig. 3. Relationship between foliar $\delta^{15}N$ and N concentrations of needles collected in (a) July and (b) September 1999.

water stress via stomatal control. Increased hydraulic resistance, and decreased sapwood to leaf area or root to leaf area ratios might also contribute to fertilization-induced water stress by decreasing water supply to foliage (Ewers et al., 2000). We thus suggest that increased WUE by fertilization in this study might have resulted from decreased stomatal and/or mesophyll conductance rather than increased carboxylation efficiency. Munger et al. (2003) also found that foliar $\delta^{13}C$ increased as stomatal conductance decreased in fertilized loblolly pine stands. In this study, the limitation on CO_2 diffusion caused by water stress was ameliorated by water supply in the IRR + FER treatment, as indicated by more negative $\delta^{13}C$ as compared with the FER treatment (Table 3), suggesting that water stress rather than nutrient availability was the limiting factor in the fertilized treatments. This study shows that the silivicultural treatments directly affected WUE of loblolly pine mainly through stomatal control of water loss, and suggests that fertilization should be combined with irrigation to achieve maximum stand productivity.

4.3. Soil and foliar $\delta^{15}N$

Irrigation did not affect $\delta^{15}N$ of current or 1-year-old needles, whereas fertilization significantly increased these values (Table 6). Since foliar $\delta^{15}N$ largely depends on the $\delta^{15}N$ of N sources (soil-N, fertilizer-N, N_2-fixation, and mycorrhizal N), and N isotopic fractionation (^{15}N enrichment of the remaining substrate) accompanying N transformations in soil, N assimilation by plant, N loss from the forest ecosystem, and N translocation within plant (Högberg, 1997), we can not attribute the $\delta^{15}N$ variations to a sole factor. Despite the relatively low $\delta^{15}N$ (−2.7‰) of the urea-N applied, fertilization-induced ^{15}N enrichments of foliage have often been suggested to reflect loss of applied N through ammonia volatilization, denitrification, and NO_3^- leaching from the soil, as those processes leave isotopically heavier N in the soil (Högberg et al., 1992; Högberg and Johannisson, 1993). This corresponds with the observation that foliar $\delta^{15}N$ is positively correlated with rates of nitrification, which produces NO_3^- that are susceptible to loss through leaching or denitrification (Garten, 1993; Garten and Miegroet, 1994).

In this study, the consistently higher $\delta^{15}N$ values of NH_4^+ than those of NO_3^- across all the treatments and the two sampling dates (Table 2) strongly indicate the contribution of nitrification in enriching ^{15}N of the remaining NH_4^+ (Choi et al., 2003). Furthermore, the consistently higher NO_3^- concentration and higher $\delta^{15}N$ of NH_4^+ in fertilized (+0.2 to +5.2‰) than those in unfertilized treatments (−4.6 to +0.3‰) suggest that higher nitrification rates in fertilized soils eventually led to increases in $\delta^{15}N$ of NH_4^+ and foliage. Meanwhile, irrigation decreased $\delta^{15}N$ of NH_4^+ in September, but did not affect foliar $\delta^{15}N$, in disagreement with Austin and Vitousek (1998) and Handley et al. (1999) who found that foliar $\delta^{15}N$ decreased with increasing soil water availability. A positive correlation between foliar $\delta^{15}N$ and $\delta^{15}N$ of NH_4^+ (Fig. 2) suggests that increases in foliar $\delta^{15}N$ are strongly associated with increasing $\delta^{15}N$ of NH_4^+ (the substrate of nitrification) rather than NO_3^- (the product). This correlation could primarily be attributed to conifer preference of NH_4^+ over NO_3^- (BassiriRad et al., 1997; Kronzucker et al., 1997; Constable et al., 2001). Therefore, $\delta^{15}N$ values of NH_4^+ rather than NO_3^- have value in predicting foliar $\delta^{15}N$ of loblolly pine in this study. Increasing foliar $\delta^{15}N$ as foliar N concentration increases (Fig. 3) supports again that increasing contribution of ^{15}N-enriched N (particularly NH_4^+ pool) to foliar N concentration led to ^{15}N-enrichment of foliar N. A few other studies also observed such a relationship (Kielland et al., 1998; Hobbie et al., 2000).

5. Conclusions

This study found that irrigation increased Δ (more negative foliar $\delta^{13}C$) and fertilization decreased Δ (less negative foliar $\delta^{13}C$) of loblolly pine trees. The carbon isotope discrimination model suggests that irrigation decreased WUE through increased stomatal and/or mesophyll conductance. Decreases in Δ by fertilization suggest that fertilization increased WUE. Published gas exchange data suggest that the increased WUE would be mainly due to decreased stomatal and/or mesophyll conductance, resulting from increased demand for water by increased growth rates that result in water supply limitations. Fertilization-induced water stress was lowered by irrigation as indicated by more negative $\delta^{13}C$ in the IRR + FER treatment than those in the FER treatment. Hence, fertilization should be combined with irrigation to achieve maximum potential stand productivities. Foliar $\delta^{15}N$ was not affected by irrigation, but was increased by fertilization. A positive correlation between foliar $\delta^{15}N$ and N concentration suggested that the available N source was enriched in ^{15}N through N loss in the fertilization treatments. The higher $\delta^{15}N$ of soil NH_4^+ than those of NO_3^- and the positive correlation between $\delta^{15}N$ of soil NH_4^+ and foliar $\delta^{15}N$ suggested that nitrification played an important role in the foliar $\delta^{15}N$ variations by increasing $\delta^{15}N$ of NH_4^+ which tends to be preferentially assimilated by conifers. Although the $\delta^{13}C$ measurement is useful in examining WUE responses to the silvicultural treatments, it could not reveal the exact mechanisms associated with the responses of trees. Instantaneous measurements of gas exchange do not integrate the physiological processes over the growing season. Hence, a combination of $\delta^{13}C$ measurement and the conventional gas exchange measurements would allow us to better understand the physiological mechanisms underlying the productivity and photosynthetic responses to the treatments.

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References

Albaugh, T.J., Allen, H.L., Dougherty, P.M., Kress, L.W., King, J.S., 1998. Leaf area and above- and belowground growth responses of loblolly pine to nutrient and water additions. For. Sci. 44, 317–328.

Albaugh, T.J., Allen, H.L., Dougherty, P.M., Johnsen, K.H., 2004. Long-term growth responses of loblolly pine to optimal nutrient and water resource availability. For. Ecol. Manage. 192, 3–19.

Allen, H.L., 2001. Silvicultural treatments to enhance productivity. In: Evans, J. (Ed.), The Forests Handbook, vol. II. Blackwell Science Publications, Oxford, pp. 382.

Allen, H.L., Dougherty, P.M., Campbell, R.G., 1990. Manipulation of water and nutrients-practice and opportunity in southern US pine forests. For. Ecol. Manage. 30, 437–453.

Austin, A.T., Vitousek, P.M., 1998. Nutrient dynamics on a precipitation gradient in Hawaii. Oecologia 113, 519–529.

BassiriRad, H., Griffin, K.K., Rynolds, J.F., Strain, B.R., 1997. Changes in root NH_4^+ and NO_3^- absorption rates of loblolly and ponderosa pine in response to CO_2 enrichment. Plant Soil 190, 1–9.

Chang, S.X., Handley, L.L., 2000. Site history affects soil and plant ^{15}N natural abundances ($\delta^{15}N$) in forests of northern Vancouver Island, British Columbia. Funct. Ecol. 14, 273–280.

Choi, W.J., Ro, H.M., Hobbie, E.A., 2003. Patterns of natural ^{15}N in soils and plants from chemically and organically fertilized uplands. Soil Biol. Biochem. 35, 1493–1500.

Constable, J.H., BassiriRad, H., Lussenhop, J., Zerihun, A., 2001. Influence of elevated CO_2 and mycorrhizae on nitrogen acquisition: contrasting responses in *Pinus taeda* and *Liquidambar styraciflua*. Tree Physiol. 21, 83–91.

Evans, J.R., 1989. Photosynthesis and nitrogen relationships in leaves of C3 plants. Oecologia 78, 9–19.

Ewers, B.E., Oren, R., Sperry, J.S., 2000. Influence of nutrient versus water supply on hydraulic architecture and water balance in *Pinus teada*. Plant Cell Environ. 23, 1055–1066.

Ewers, B.E., Oren, R., Albaugh, T.J., Dougherty, P.M., 1999. Carryover effects of water and nutrient supply on water use of *Pinus taeda*. Ecol. Appl. 9, 513–525.

Farquhar, G.D., Ehleringer, J.R., Hubick, K.T., 1989. Carbon isotope discrimination and photosynthesis. Ann. Rev. Plant Physiol. Plant Mol. Biol. 40, 503–537.

Feast, N.A., Dennis, P.E., 1996. A comparison of methods for nitrogen isotope analysis of groundwater. Chem. Geol. 129, 167–171.

Garten Jr., C.T., 1993. Variation in foliar ^{15}N abundances and the availability of soil nitrogen on Walker Branch Watershed. Ecology 74, 2098–2113.

Garten Jr., C.T., Miegroet, H.V., 1994. Relationships between soil nitrogen dynamics and natural ^{15}N abundance in plant foliage from Great Smoky Mountains National Park. Can. J. For. Res. 24, 1636–1645.

Gough, C.M., Seiler, J.R., Maier, C.A., 2004a. Short-term effects of fertilization on loblolly pine (*Pinus taeda* L.) physiology. Plant Cell Environ. 27, 876–886.

Gough, C.M., Seiler, J.R., Johnsen, K.H., Sampson, D.A., 2004b. Seasonal photosynthesis in fertilized and non-fertilized loblolly pine. For. Sci. 50, 1–9.

Green, T.H., Mitchell, R.J., 1992. Effects of nitrogen on the response of loblolly pine to water stress. I. Photosynthesis and stomatal conductance. N. Phytol. 122, 627–633.

Handley, L.L., Austin, A.T., Robinson, D., Scrimgeour, C.M., Raven, J.A., Heaton, T.H.E., Schmidt, S., Stewart, G.R., 1999. The ^{15}N natural abundance ($\delta^{15}N$) of ecosystem samples reflects measures of water availability. Aust. J. Plant Physiol. 6, 185–199.

Hauck, R.D., 1982. Nitrogen-isotope ratio analysis. In: Page, A.L. (Ed.), Methods of Soil Analysis. Part 2. Chemical and Microbiological Properties. Agron. Monogr. 9. ASA and SSSA, Madison, pp. 735–779.

Hobbie, E.A., Macko, S.A., Williams, M., 2000. Correlations between foliar $\delta^{15}N$ and nitrogen concentrations may indicate plant–mycorrhizal interactions. Oecologia 122, 273–283.

Högberg, P., Tamm, C.-O., Högberg, M., 1992. Variations in ^{15}N abundance in a forest fertilization trial: Critical loads of N, N saturation, contamination and effects of revitalization fertilization. Plant Soil 142, 211–219.

Högberg, P., Johannisson, C., 1993. ^{15}N abundance of forests is correlated with losses of nitrogen. Plant Soil 157, 147–150.

Högberg, P., Johannisson, C., Hällgren, J.E., 1993. Studies of ^{13}C in the foliage reveal interactions between nutrients and water in forest fertilization experiments. Plant Soil 152, 207–214.

Högberg, P., Johannisson, C., Högberg, M., Högbom, L., Näsholm, T., Hällgren, J.E., 1995. Measurements of abundances of ^{15}N and ^{13}C as tools in retrospective studies of N balances and water stress in forests: a discussion of preliminary results. Plant Soil 168–169, 125–133.

Högberg, P., 1997. ^{15}N natural abundance in soil–plant systems. New Phytol. 137, 179–203.

Jose, S., Merritt, S., Ramsey, C.L., 2003. Growth, nutrition, photosynthesis and transpiration responses of longleaf pine seedlings to light, water and nitrogen. For. Ecol. Manage. 180, 335–344.

Keeney, D.R., Nelson, D.W., 1982. Nitrogen–inorganic forms. In: Page, A.L. (Ed.), Methods of Soil Analysis. Part 2. Chemical and Microbiological Properties. Agron. Monogr. 9. ASA and SSSA, Madison, pp. 643–698.

Kellomaki, S., Wang, K.Y., 1997. Photosynthetic response of Scots pine to elevated CO_2 and nitrogen supply: results of a branch-in-bag experiment. Tree Physiol. 17, 231–240.

Kielland, K., Barnett, B., Schell, D., 1998. Intraseasonal variation in the $\delta^{15}N$ signature of taiga trees and shrubs. Can. J. For. Res. 28, 485–488.

Korol, R.L., Kirschbaum, M.U.F., Farquhar, G.D., Jeffreys, M., 1999. Effects of water status and soil fertility on the C-isotope signature in *Pinus radiate*. Tree Physiol. 19, 551–562.

Kronzucker, H.J., Siddiqi, M.Y., Glass, A.D.M., 1997. Conifer root discrimination against soil nitrate and ecology of forest succession. Nature 385, 59–61.

Livingston, N.J., Guy, R.D., Ethier, G.J., 1999. The effects of nitrogen stress on the stable carbon isotope composition, productivity and water use efficiency of white spruce (*Picea glauca* (Moench) Voss) seedling. Plant Cell Environ. 22, 281–289.

Loader, N.J., Robertson, I., McCarroll, D., 2003. Comparison of stable carbon isotope ratios in the whole wood, cellulose and lignin of oak tree-rings. Palaeogeogr. Palaeoclimatol. Palaeoecol. 196, 395–407.

Maier, C.A., Johnsen, K.H., Butnor, J., Kress, L.W., Anderson, P.H., 2002. Branch growth and gas exchange in 13-year-old loblolly pine (*Pinus taeda*) trees in response to elevated carbon dioxide concentration and fertilization. Tree Physiol. 22, 1093–1106.

Munger, G.T., Will, R.E., Borders, B.E., 2003. Effects of competition control and annual nitrogen fertilization on gas exchange of different-aged *Pinus taeda*. Can. J. For. Res. 33, 1076–1083.

Murthy, R., Dougherty, P.M., Zarnoch, S.J., Allen, H.L., 1996. Effects of carbon dioxide, fertilization, and irrigation on photosynthetic capacity of loblolly pine trees. Tree Physiol. 16, 537–546.

Murthy, R., Zarnoch, S.J., Dougherty, P.M., 1997. Seasonal trends of light-saturated net photosynthesis and stomatal conductance of loblolly pine trees grown in contrasting environments of nutrition, water, and carbon dioxide. Plant Cell Environ. 20, 558–568.

Ripullone, F., Lauteri, M., Grassi, G., Amato, M., Borghetti, M., 2004. Variation in nitrogen supply changes water-use efficiency of *Pseudotsuga menziesii* and *Populus* × *euroamericana*; a comparison of three approaches to determine water-use efficiency. Tree Physiol. 24, 671–679.

Samuelson, L., Stokes, T., Cooksey, T., McLemore, P., 2001. Production efficiency of loblolly pine and sweetgum in response to 4 years of intensive management. Tree Physiol. 21, 369–376.

Sparks, J.P., Ehleringer, J.R., 1997. Leaf carbon isotope discrimination and nitrogen content for riparian trees along elevational transects. Oecologia 109, 362–367.

Strand, M., 1997. Effect of mineral nutrient content on oxygen exchange and chlorophyll *a* fluorescence in needles of Norway spruce. Tree Physiol. 17, 221–230.

Tang, Z., Chambers, J.L., Guddanti, S., Barnett, J.P., 1999. Thinning, fertilization, and crown position interact to control physiological responses of loblolly pine. Tree Physiol. 19, 87–94.

Warren, C.R., McGrath, J.F., Adams, M.A., 2001. Water availability and carbon isotope discrimination in conifers. Oecologia 127, 476–486.

Zhang, S., Hennessey, T.C., Heinemann, R.A., 1997. Acclimation of loblolly pine (*Pinus taeda*) foliage to light intensity as related to leaf nitrogen availability. Can. J. For. Res. 27, 1032–1042.