Tree Physiology 27, 375–383
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Soil DIC uptake and fixation in *Pinus taeda* seedlings and its C contribution to plant tissues and ectomycorrhizal fungi

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Received March 1, 2006; accepted May 24, 2006; published online December 1, 2006

Summary Plants can acquire carbon from sources other than atmospheric carbon dioxide (CO_2), including soil-dissolved inorganic carbon (DIC). Although the net flux of CO_2 is out of the root, soil DIC can be taken up by the root, transported within the plant, and fixed either photosynthetically or anaplerotically by plant tissues. We tested the ability of *Pinus taeda* L. seedlings exposed to ^{13}C-labeled soil DIC and two NH_4^+ availability regimes to take up and fix soil DIC. We also measured the concentration and distribution of the fixed soil DIC within the plant and mycorrhizal tissues, and quantified the contribution of soil DIC to whole-plant carbon (C) gain. Seedlings exposed to labeled DIC were significantly enriched in ^{13}C compared with seedlings exposed to unlabeled DIC (6.7 versus –31.7‰). Fixed soil DIC was almost evenly distributed between above- and belowground biomass (55 and 45%, respectively), but was unevenly distributed among tissues. Aboveground, stem tissue contained 65% of the fixed soil DIC but represented only 27% of the aboveground biomass, suggesting either corticular photosynthesis or preferential stem allocation. Belowground, soil DIC had the greatest effect (measured as ^{13}C enrichment) on the C pool of rapidly growing nonmycorrhizal roots. Soil DIC contributed ~0.8% to whole-plant C gain, and ~1.6% to belowground C gain. We observed a slight but nonsignificant increase in both relative C gain and the contribution of soil DIC to C gain in NH_4^+-fertilized seedlings. Increased NH_4^+ availability significantly altered the distribution of fixed soil DIC among tissue types and increased the amount of fixed soil DIC in ectomycorrhizal roots by 130% compared with unfertilized seedlings. Increased NH_4^+ availability did not increase fixation of soil DIC in nonmycorrhizal roots, suggesting that NH_4^+ assimilation may be concentrated in ectomycorrhizal fungal tissues, reflecting greater anaplerotic demands. Soil DIC is likely to contribute only a small amount of C to forest trees, but it may be important in C fixation processes of specific tissues, such as newly formed stems and fine roots, and ectomycorrhizal roots assimilating NH_4^+.

Keywords: anaplerotic fixation, carbon cycling, carbon dioxide, corticular photosynthesis, dissolved inorganic carbon, stable isotope.

Introduction

Carbon acquisition by terrestrial plants primarily occurs by aboveground fixation of atmospheric carbon dioxide (CO_2), yet plants gain minor amounts of C from sources other than atmospheric CO_2. For example, although net flux of CO_2 in plant roots is outward into the soil, some inorganic C dissolved in the soil solution (dissolved CO_2, H_2CO_3 and HCO_3^-; hereafter soil DIC) is taken up and fixed by plants (reviewed by Enoch and Olesen 1993). Soil DIC is ubiquitous in terrestrial soil environments. Concentrations of soil DIC are highly dynamic and remain in equilibrium with soil gas phase CO_2 concentrations, which are typically an order of magnitude higher than those in the atmosphere (0–5 versus 0.03%; Dudziak and Halas 1996, Bajracharya et al. 2000). Under scenarios of rising atmospheric CO_2 concentrations, both soil CO_2 and soil DIC concentrations are expected to increase (cf. Andrews and Schlesinger 2001).

It is known that soil DIC influences plant growth (e.g., Birner and Lucanus 1866), either by affecting the $[CO_2]$ gradient from the root to the soil, or as a result of plant uptake and fixation (Enoch and Olesen 1993). The concentration of soil DIC in the rhizosphere influences many physiological processes. High concentrations of rhizosphere DIC lead to decreased root DIC efflux (van der Westhuizen and Cramer 1998), dampened photosynthetic rates, altered patterns of N uptake (van der Merwe and Cramer 2000, Viktor and Cramer 2005) and increased plant growth (Vapaavuori and Pelkonen 1985, Viktor and Cramer 2003). Soil DIC taken up by plant roots can be incorporated into plant tissues in several ways. Soil DIC can be anaplerotically fixed (dark fixation) in mycorrhizal and root tissues (Jackson and Coleman 1959, Wingler et al. 1996) and subsequently translocated within the plant in the form of organic or amino acids (Vuorinen et al. 1992, Cramer et al. 1993), or transported directly as DIC by the transpiration stream and fixed aboveground by light or anaplerotic reactions (reviewed by Cramer 2002). In addition, the uptake and fixation of soil DIC can include the reincorporation of root-respired CO_2. Given the potential for complex physiological interactions, and the difficulty in measuring plant DIC transport,

concentrations and fixation rates in situ (cf. Martin et al. 1994), the direct contribution of soil DIC to plant growth is difficult to quantify. Thus, although exogenous soil DIC is taken up and fixed by plants (Cramer and Richards 1999, Viktor and Cramer 2003, Viktor and Cramer 2005) and contributes up to 2.7% of C gain in hydroponically grown crop species (Voznesenski 1958, Kick et al. 1965*b*, Schäfer 1988), the contributions of soil DIC to the C budgets of forest trees and mycorrhizal fungi are still largely unknown.

Anaplerotic fixation of soil DIC by roots provides tricarboxylic acid (TCA) cycle intermediates needed for subsequent metabolic pathways. When plant uptake of NH_4^+ increases, the demand for anaplerotic generation of TCA cycle intermediates also increases because α-ketoglutarate is diverted to synthesize amino acids (Raven and Farquhar 1990). When NH_4^+ is assimilated in root tissues, root DIC can be anaplerotically fixed and incorporated into the C skeleton of newly generated amino acids. For example, under NH_4^+ nutrition, root incorporation of DIC into amino acids increases relative to that under NO_3^- nutrition (Vuorinen et al. 1992, Cramer et al. 1993, Viktor and Cramer 2005). Therefore, because anaplerotic fixation is more likely to occur in tissues that actively assimilate NH_4^+, such as mycorrhizal roots and fungi (Chalot et al. 2002), more soil DIC may be fixed in these belowground tissues than in aboveground tissues when plants are exposed to a high amounts of NH_4^+.

Our primary objective was to quantify soil DIC uptake in *Pinus taeda* L. seedlings exposed to two NH_4^+ availability regimes. We used an experimental pot system in which seedlings and ECM fungi grew in sand while receiving isotopically labeled soil DIC applied in the irrigation water. We also determined the distribution of fixed DIC and its relative C contribution to plant tissues and ECM fungi. We predicted that seedlings would take up and incorporate soil DIC into biomass, and that this C would contribute relatively more to belowground plant tissues than to aboveground plant tissues. In response to high NH_4^+ availability, we predicted that ECM roots and ECM fungi would contain a greater proportion of fixed soil DIC relative to plants receiving a low amount of NH_4^+.

Methods and materials

Experimental pot and greenhouse conditions

We transplanted 7-week-old, half-sib *P. taeda* seedlings into sand-filled PVC pots three months before treatment application. Pots were 10.2 cm internal diameter (I.D.) and 30.5 cm high pipe sections with clear polycarbonate plates to seal the top and bottom. The top plate was cut into halves with a 5 mm I.D. opening, allowing the plates to be sealed around the seedling stem. Two valves were installed 2.5 cm from the top and bottom of the pot for applying solutions and flushing with gas. Hyphal in-growth pouches (40 µm aperture stainless steel mesh, filled with 130 g sand and 13 g slow-release 19,6,12 N,P,K pellets (Osmocote), with half of the N occurring in ammoniacal form), were placed below the sand surface. Seedlings were watered weekly, irrigated once with a micronutrient solution (Kraus et al. 2004) and inoculated twice with a commercial mix of *Pisolithus tinctorius* (Pers.) Coker & Couch and *Scleroderma sp.* inoculum (Plant Health Care, PA) to promote ectomycorrhizal (ECM) colonization. With the aid of a compound microscope, we confirmed ECM colonization (presence of fungal mantle and Hartig net) on all seedlings before treatment application. The pot top plates were left unsealed and the upper and lower valves were left open until one day before the beginning of the treatment period.

Three weeks before the treatment period, we determined daily O_2 and CO_2 depletion and accumulation rates on a randomly selected pot and seedling. We sealed the upper plate onto the pot and the area around the stem with a gas-impermeable tack adhesive (Qubitac; Qubit Systems, Ontario, Canada). Measurements of soil O_2 concentration ($[O_2]$) in the pot were made with an O_2 microelectrode (Model MI-730; Microelectrodes Inc.; Bedford, NH) installed through the top plate and sealed in place such that the membrane penetrated the surface of the soil. Measurements of soil CO_2 concentration ($[CO_2]$) in the pot were made with a CO_2 probe (Model GMM222; Vaisala; Helsinki, Finland) sealed in a removable, closed, flow-through system that was connected externally to the pot's valves. From these rates, we determined that flushing the pots by connecting the upper valve to a gas tank regulator (set at 0.1 MPa) for 10–15 min with a 30% O_2 and 70% N_2 gas mixture every 72 h prevented anoxia ($[O_2] < 10\%$).

We measured greenhouse air temperature and photosynthetic photon flux (PPF) every 5 min and recorded 30-min means (HMP35C, LI190SB, CR23X, Campbell Scientific, Inc.; Logan, UT). Mean ambient temperature and relative humidity in the greenhouse were maintained at 22.2 ± 2.7 °C and 81 ± 8% (respectively, mean ± one SD). Mean daytime PPF was 199 µmol m^{-2} s^{-1} (788 µmol m^{-2} s^{-1} maximum).

One day before treatments began, six seedlings (Pretreatment plant group; Pre) were randomly selected and destructively sampled for initial biomass, leaf area and $\delta^{13}C$ of the tissue types. Another 20 seedlings were placed randomly into one of four treatments (five replicates per treatment) and their pots sealed. Treatment solutions containing a known concentration of DIC, either labeled with ^{13}C or unlabeled, in the presence and absence of 3.1 mM NH_4Cl (N treatment) were then applied. Hereafter, treatments are denoted $^{13}CO_2$ + N, $^{13}CO_2$, CO_2 + N and CO_2, with the latter two treatments serving as identical unlabeled treatments for the former two, respectively.

Preparation of DIC and N treatments

Each treatment solution was made in a carboy with degassed deionized water containing background alkalinity (4 mM $CaCO_3$). The N treatment carboys also contained 3.1 mM NH_4Cl. Carboys fitted with valves were filled with solution and sealed so that they contained no headspace gas. Subsequently, 1 l of solution was displaced from each carboy with 100% CO_2 gas: the two labeled DIC treatment carboys with 99 atom percent $^{13}CO_2$ (ICON Services; Summit, NJ) and the two unlabeled DIC treatment carboys with 1.06 atom percent

$^{13}CO_2$. The headspace gas was recirculated through the solution for 36 h at room temperature with a diaphragm pump, dispersing gas through a bubble stone at the bottom of the carboy. We estimated DIC in the treatment solutions twice: initially by titration (Langmuir 1997) and calculation of total CO_2 based on the measured solution pH (Franson 1995), and also by total inorganic carbon analysis (Shimadzu TOC-5000A) at the Stable Isotope/Soil Biology Laboratory of the University of Georgia, Institute of Ecology. Treatment solutions contained an average of 0.021 (SE 0.001) g C l^{-1} or 1.782 (SE 0.083) mM C.

Treatment solutions were made and applied once per week during the 4-week period. We applied 200 ml of solution through the upper valve of each pot. Pots were flushed after 72 h. During flushing, pots receiving labeled DIC were connected by way of their lower valve to a wastewater flask with a soda lime gas exhaust to trap CO_2 thus preventing atmospheric enrichment. After each treatment application, seedlings were randomly rearranged. During the treatment period, we also determined rates of CO_2 accumulation in the unlabeled pots with and without treatment solutions present using the removable CO_2 probe system.

Determination of total seedling C gain

At the end of the treatment period, seedlings were harvested and the following measurements made: fresh leaf area, and dry mass of primary and secondary leaves; dry mass of aboveground stem (stems, buds, fascicles); woody root dry mass; nonmycorrhizal root (NM) dry mass; and ECM root dry mass. The ECM roots were identified on each seedling based on the presence of a fungal mantle and Hartig net. For three seedlings from each treatment group, we calculated total leaf surface area (LA) as $LA = L\,(2\pi R/N + 2NR)$, where L is needle length measured with calipers, N is number of needles per fascicle and R is the fascicle radius determined with a hand lens micrometer. For the remaining replicates we used the specific LA relationship (established from LA, and leaf DW) to estimate LA. Because the entire ECM hyphal biomass could not be extracted, we extracted a subsample from the hyphal pouches (Boddington et al. 1999), dried it to constant mass and ground the tissue. All other biomass fractions were dried to constant mass, weighed, and ground to a fine powder using standard precautions to prevent ^{13}C cross-contamination. For all biomass fractions, a 2-mg subsample of ground tissue was analyzed on a continuous flow, combustion, isotope ratio mass spectrometer (Finnigan Delta C; Bremen, Germany) interfaced with an elemental analyzer (NA1500, Carlo Erba Instruments; Milan, Italy) at the Stable Isotope/Soil Biology Laboratory of the University of Georgia, Institute of Ecology to determine the $\delta^{13}C$ and the C concentration (standards 0.04–0.51‰ SD). The amounts of $\delta^{13}C$ and C in the 2-mg subsamples were scaled to the corresponding biomass fraction for each seedling except the hyphal fraction.

Calculations of seedling-acquired soil DIC

To calculate the amount and distribution of fixed soil DIC, we determined the atom percent excess of ^{13}C in the treatment seedlings relative to the mean signal of the corresponding seedlings in the unlabeled treatment as:

$$A^{13}CE_c = A^{13}C_c^T - \left(AC_c^T \frac{\overline{AP}_c^U}{100} \right) \quad (1)$$

$$A^{13}CE_{plant} = \sum_{c=1}^{5} A^{13}CE_c \quad (2)$$

where subscript c denotes tissue category; superscripts T and U denote labeled and the corresponding unlabeled treatments; AC_c and $A^{13}C_c$ denote the number of atoms of C and ^{13}C, respectively; DW_c is dry mass; AP_c is atom percent of ^{13}C in a 2-mg subsample; and $A^{13}CE_c$ denotes the atoms of ^{13}C in the treated plants in excess of the atoms of ^{13}C in unlabeled control plants. The mean of the five seedlings in the corresponding unlabeled treatment is represented by $\overline{AP}_c^U$. We present results as a percentage ($(A^{13}CE_c/\text{total } A^{13}CE_{plant})100$) and as moles of C in excess where appropriate.

Estimation of the contribution of soil DIC to seedling C gain

To model seedling C gain, we estimated C gain over the experimental period for each seedling based on measurements of photosynthesis and respiration, calculated the C gain from soil DIC (as above), and then calculated the percent of total net C gain attributable to soil DIC fixation. Specifically, we developed light response curves across all seedlings by measuring net C assimilation, A_{net} (μmol CO_2 m^{-2} s^{-1}) versus varying PPF (μmol photons m^{-2} s^{-1}) (Figure 1) with all other conditions set to current greenhouse ambient conditions (LI-6400, Li-Cor, Lincoln, NE) for primary ($F = 44.99$, $P < 0.01$, $n = 23$; $A_{net} = 3.76(1 - e^{0.0097\,(PPF42.89)})$) and secondary ($F = 96.34$, $P < 0.01$, $n = 54$; $A_{net} = 7.75(1 - e^{0.0059\,(PPF20.18)})$) leaves. No N effect was detected on C assimilation rate. Because seedlings were only 15 weeks old, and had only primary and secondary needles, we measured gas exchange on these needles throughout the crown during the daytime (1100–1800 h) and at night over six days during the treatment period. Dark needle respiration measurements were made with inside chamber PPF set at 0 μmol m^{-2} s^{-1}, and outside chamber PPF ranging from 0 to 80 μmol m^{-2} s^{-1}. At harvest, respiration measurements on attached stems and attached and excavated ECM roots, NM roots and woody roots were made with inside chamber PPF at 0 μmol m^{-2} s^{-1} and outside PPF < 10 μmol m^{-2} s^{-1}. Mean respiration rates for attached stems (1.89 (SE 0.49) nmol m^{-2} s^{-1}), ECM roots (14.58 (SE 4.30) nmol m^{-2} s^{-1}), NM roots (16.05 (SE 4.11) nmol m^{-2} s^{-1}), and woody roots (14.56 (SE 3.74) nmol m^{-2} s^{-1}) did not differ between fertilized and unfertilized treatment groups ($P = 0.84$, $F = 0.41$). We calculated cumulative daily flux (g C g^{-1} dry mass day^{-1}) for the five measured tissue categories based on these measured rates and greenhouse climate data.

To model daily C gain by each seedling, we started with the final (time t) known C pool sizes of each tissue category. The

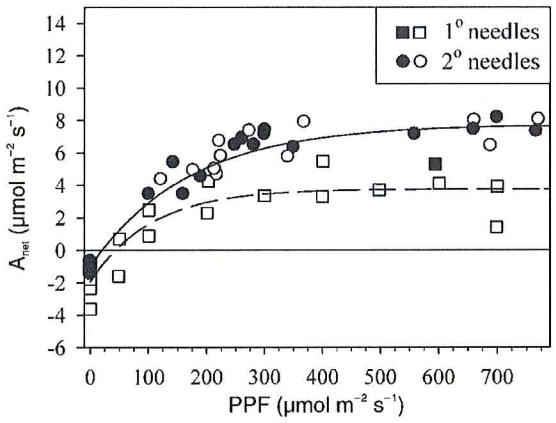

Figure 1. Net assimilation (A_{net}) as a function of incident photosynthetic photon flux (PPF) for primary and secondary needles of fertilized (■, ●) and unfertilized (□, ○) seedlings.

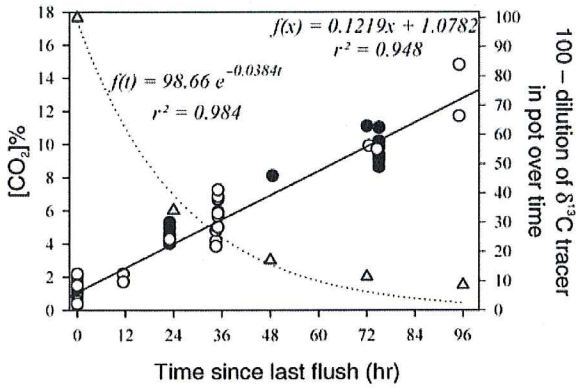

Figure 2. The concentration of CO_2 in a pot containing a *Pinus taeda* seedling growing in sand increased linearly over time but the increase was not significantly influenced by the presence of DIC treatment solution (left *y* axis). Symbols: ● = DIC solution present; ○ = DIC solution absent; ▲ = reciprocals of the cumulative slope of the accumulation line (solid line), which were used to fit the negative exponential function (dotted line), representing the dilution of $^{13}CO_2$ as a result of root respiration (right *y* axis).

pool size at time *t* was multiplied by the cumulative daily C flux rate for each tissue category. New pool sizes were then calculated for the previous day ($t-1$) by taking the pool size at time *t* and subtracting the net C gain at time *t* multiplied by the allocation to that tissue category at time *t*. We separated the total net C gain from atmosphere and soil DIC according to the following steps. First, we summed the modeled net C gain from leaf-fixed CO_2 for individual days when seedlings were exposed to treatment solutions. To this quantity, we added the number of ^{13}C atoms detected in excess of the corresponding unlabeled treatment group ($A^{13}CE_{plant}$), and defined this as the total net C gain from atmosphere and soil DIC. We then calculated the percent that soil DIC contributed to total seedling C gain ($\%C_{total}$). We also calculated the percent that soil DIC contributed to belowground C gain ($\%C_{belowground}$) with Equation 1 to calculate the daily $A^{13}CE_{roots}$ (from soil DIC source) and dividing this number by daily $A^{13}CE_{roots}$ plus the daily number of C atoms gained by the leaves allocated to the root system (C gain from soil DIC and atmospheric sources allocated belowground).

To estimate the ^{13}C content of soil DIC, we accounted for the dilution of ^{13}C by belowground respiration as well as the uptake of the associated, undetected, $^{12}CO_2$ by the seedlings. We derived this function from pot CO_2 accumulation rates (Figure 2). Because the treatment did not affect CO_2 accumulation rates, we concluded that the primary source of CO_2 was from respiration and not from the degassing of applied treatment water (Figure 2). Dilution was modeled as an exponential decay function (Figure 2), where the atom percent of ^{13}C in the pot (AP_{pot}) at time *t* can be described as a mixture of sources (tracer and respiration, with the latter signal equal to the final signal of the leaves) given by (Figure 3):

$$AP^{13}C_{pot} = AP^{13}C_{treatment}\, f(t) + AP^{13}C_{leaves}\,(100 - f(t)) \quad (3)$$

and $AP^{12}C_{pot}$ is given by $1 - AP^{13}C_{pot}$ (Figure 3). We integrated under both curves from 0 to 72 h and multiplied by the number of 72-h exposure periods. We assumed that the area under the $AP^{13}C_{pot}$ curve as a fraction of the total area under both curves (for the entire exposure period) was equivalent to the fraction of signal detected ($A^{13}CE_{plant}$) to the total amount of C fixed from soil DIC. Thus, we added $A^{12}CE_{plant}$ to $A^{13}CE_{plant}$ to estimate the total amount of fixed DIC.

Statistical design

To examine whether the seedlings incorporated soil DIC source into biomass, we tested the effect of soil DIC treatment (pretreatment, labeled and unlabeled DIC) and N regime (pretreatment, unfertilized and fertilized) on whole-seedling stable carbon isotope ratio. We used a two-factor (fixed effects) analysis of variance (ANOVA) with five replicates in the DIC groups and six replicates in the pretreatment group.

To examine whether N availability affected the concentration of the label in the plant tissues ($\delta^{13}C$ of each tissue pool) and the distribution of label within the plant tissues (percent of uptake found in each pool), we performed two separate ANOVAs, each with five replicates. We used a blocked (random effect), two-factor (fixed effects) ANOVA design. The first factor in the design, N treatment, had two levels. The second factor, tissue type, had six levels: ECM roots, NM roots, woody roots, hyphae, leaves, and stem. We used a blocking factor because tissue samples taken from the same seedling (block) were not independent.

To evaluate whether N availability affected total C gain during the experiment, and to determine the percent of soil DIC that contributed to either total seedling C gain ($\%C_{total}$) or belowground C gain ($\%C_{belowground}$) we performed three separate *t*-tests with five replicates. The response variable for test-

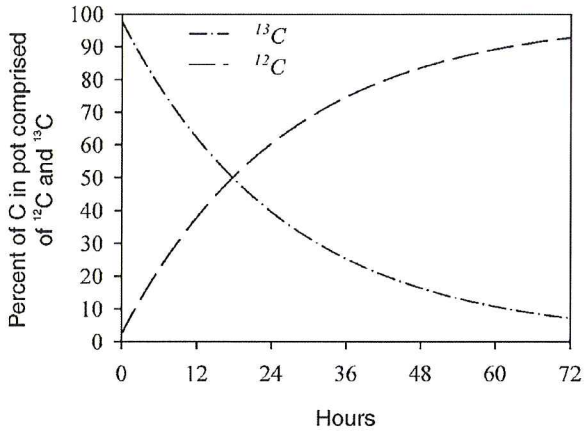

Figure 3. Time course of changes in atom percent of ^{13}C and ^{12}C in a pot containing a *Pinus taeda* seedling growing in sand. Atom percent of ^{13}C decayed exponentially over time and atom percent of ^{12}C increased logarithmically over time. Uptake and fixation of undetected ^{12}C was estimated from the areas under these curves.

ing differences in total C gain was relative C gain during the treatment days, calculated as total C gain during the treatment days (from above- and belowground sources) divided by the ending seedling biomass. This calculation compensated for uptake by plants differing in size (see above).

All data were analyzed with SAS software (SAS Institute, Inc., Cary, NC) using PROC GLM with a post-hoc Duncan's NMR means separation procedure where appropriate, or PROC TTEST. All tests of significance were conducted at the $\alpha = 0.05$ level.

Results

Carbon gain and water use

Compared with the mean biomass of seedlings in the pretreatment group (2.69 g DW), labeled seedlings increased in biomass by 49–148% (Table 1) and unlabeled seedlings increased in biomass by 146–175% (data not shown) during the 4-week treatment. Mean seedling LA at the end of the treatment was 767.5 (SE 65.3) cm^2, compared with 370 (SE 27.9) cm^2 for seedlings in the pretreatment group. During the treatment, water use averaged 23.2 (SE 1.5) and 17.9 (SE 2.4) mg day^{-1} for both unfertilized and N-treated seedlings, and was positively related to leaf area. Considerable variance existed among seedlings in total biomass and leaf area at both the start and end of the study. Partitioning of biomass above- and belowground was consistent between treatment groups (Table 1).

Whole-seedling uptake of soil DIC

Seedlings in the labeled DIC treatment groups were significantly enriched in ^{13}C compared with seedlings in the unlabeled treatment groups, whereas seedlings in the pretreatment and unlabeled DIC groups did not differ in enrichment ($F = 128.33$, $P < 0.01$; Figure 4). This indicated uptake and fixation of soil DIC, as well as no evident $^{13}CO_2$ contamination from pot leakage or from aboveground respiratory loss of $^{13}CO_2$ from labeled plants. No N effects or interaction effects between ^{13}C and N were detected on plant $\delta^{13}C$, indicating that, at the plant level, seedlings receiving additional N did not fix more soil DIC than unfertilized seedlings (Figure 4).

Concentration and distribution of assimilated label among tissues

The concentration of label among tissue pools ($\delta^{13}C$) differed by treatment group. Within the unlabeled and pretreatment seedlings, tissues fell into three groups based on their $\delta^{13}C$ signal: leaves were more depleted than all other tissues, hyphae were more enriched than all other tissues, and all other tissues were intermediate and not significantly different from one another (Figure 5). After the treatment, the $\delta^{13}C$ of all tissues, except leaves, were significantly greater in labeled seedlings ($P < 0.01$) than in the corresponding tissues from unlabeled seedlings (Figure 5). Within the labeled treatment groups, tissues fell into three groups based on their $\delta^{13}C$ signal: NM roots were significantly more enriched than all other tissues; leaves were significantly less enriched than all other tissues; and ECM roots, woody roots, hyphae and stems were intermediate and not significantly different from one another.

The distribution of assimilated label within the plant, which is independent of tissue pool size, was inconsistent between fertilized and unfertilized seedlings and between tissue types (N × Tissue interaction, $F = 5.35$, $P < 0.01$; Figure 6). On average, 55% of the ^{13}C ended up in the aboveground tissues. Of the ^{13}C detected belowground, there was significantly more

Table 1. Measured and modeled characteristics of fertilized and unfertilized *Pinus taeda* seedlings. Values are means with 1SE given in parenthesis. An asterisk (*) denotes that only seedlings in the labeled treatment group were used to calculate the reported value. No significant treatment differences were detected for any characteristic at $\alpha = 0.05$.

Characteristic	CO_2 and $^{13}CO_2$	CO_2 + N and $^{13}CO_2$ + N
Aboveground partitioning (fraction)	0.73 (0.14)	0.72 (0.02)
Relative C gain during treatment days (mmol g^{-1})*	0.41 (0.09)	0.53 (0.05)
Relative water use during treatment days (mg g^{-1})	3360 (170)	2970 (190)
Soil DIC contribution to whole-seedling net C gain (%)*	0.75 (0.13)	0.92 (0.11)
Soil DIC contribution to root-system net C gain (%)*	1.59 (0.17)	1.60 (0.15)

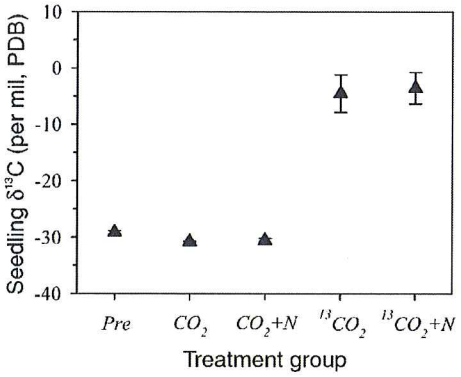

Figure 4. Treatment effects on stable carbon isotope ratios ($\delta^{13}C$) of *Pinus taeda* seedlings. Values are means and bars denote and one standard error of the mean. Abbreviations: Pre = pretreatment plant group; CO_2 = unlabeled DIC, fertilized; CO_2 + N = unlabeled DIC, not fertilized; $^{13}CO_2$ = labeled DIC, not fertilized; and $^{13}CO_2$ + N = labeled DIC, fertilized. Unlabeled seedlings were significantly more depleted in the heavier stable isotope than labeled seedlings at α = 0.05.

^{13}C in ECM roots of fertilized plants compared with unfertilized plants (F = 12.18, P < 0.01), and significantly less soil DIC in woody roots (F = 5.34, P = 0.02) of fertilized plants compared with unfertilized plants.

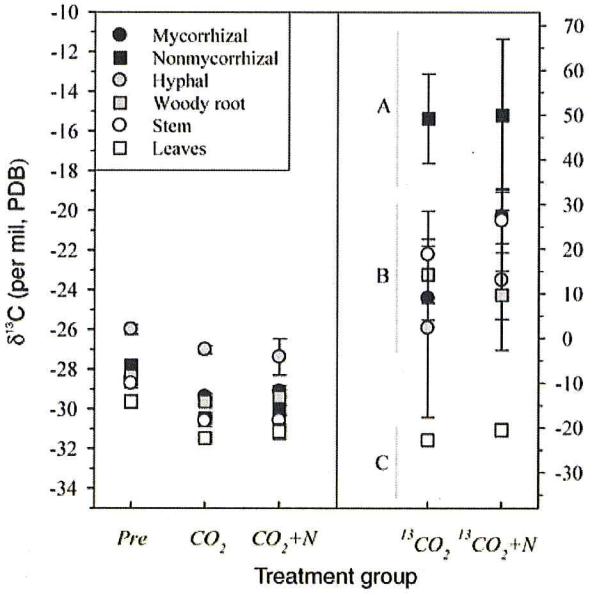

Figure 5. Treatment effects on stable carbon isotope ratios ($\delta^{13}C$) of tissue types of *Pinus taeda* seedlings. Values are means and bars denote and one standard error of the mean. Abbreviations: Pre = pretreatment plant group; CO_2 = unlabeled DIC, fertilized; CO_2 + N = unlabeled DIC, not fertilized; $^{13}CO_2$ = labeled DIC, not fertilized; and $^{13}CO_2$ + N = labeled DIC, fertilized. Different letters denote significant groupings of tissues within the labeled treatment groups. Note different y-axis scales for left and right panels.

Estimates of soil DIC contribution to seedling C gain

Relative C gain from combined above- and belowground sources during the treatment did not differ significantly between seedlings in the $^{13}CO_2$ and $^{13}CO_2$ + N treatments (t = 1.86, P = 0.32; Table 1). Nitrogen fertilization did not significantly increase the contribution of soil DIC to total plant C gain or to belowground C gain, although a trend in this direction was evident on a whole-plant basis (P = 0.15; Table 1). Soil DIC contributed less than 1% to total plant C gain and the contribution was unaffected by the treatments (Table 1). Soil DIC contributed 1.6% to belowground C gain (Table 1).

Discussion

Whole-seedling uptake of soil DIC

The potential for plants to take up and incorporate soil DIC into biomass has been recognized since at least 1866 (Birner and Lucanus). We observed uptake of soil DIC by *P. taeda* tree roots and subsequent incorporation of soil DIC into above- and below-ground seedling biomass. Past studies have shown that tree species are capable of incorporating DIC into excised leaves (Stringer and Kimmerer 1993), hydroponic cuttings (Vapaavuori and Pelkonen 1985) and excised ECM roots (Carrodus 1967, Harley 1964). Previous studies have also exposed plants to soil DIC for short time periods (30 min – 48 h), to determine the initial pathway of fixation (Cramer et al. 1993, Cramer and Lips 1995, van der Merwe and Cramer 2000, Vapaavuori and Pelkonen 1985). We exposed seedlings to 12 days of ^{13}C-enriched soil DIC over 4–6 weeks to determine the ultimate distribution of fixed soil DIC among plant tissues, and its net contribution to plant C gain. The results of our study, and the response to an NH_4^+ treatment, confirm DIC uptake and fixation by *P. taeda* seedlings.

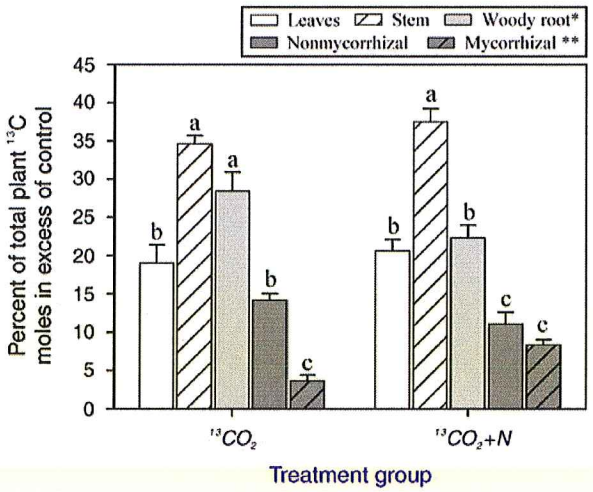

Figure 6. Distribution of ^{13}C in excess by tissue type in *Pinus taeda* seedlings. Different letters over bars denote significant differences among tissues (α = 0.05). Asterisks in the legend denote significant differences (*, P = 0.05*; and **, P = 0.01) in woody root and mycorrhizal root tissues between fertilized and unfertilized seedlings.

Concentration of assimilated label among tissues ($\delta^{13}C$)

The concentration of fixed soil DIC varied among plant tissues but was unaffected by NH_4^+ availability (Figure 5). Fixed soil DIC was the most concentrated in the C pool of NM roots, and least concentrated in the C pool of needles (Figure 5). Similar to our findings, when hydroponic potato plants were exposed to 6 h of ^{14}C-DIC, concentrations of the radioactive label were greater in roots than in stems (Arteca and Poovaiah 1982). In addition, Stringer and Kimmerer (1993) observed a decreasing ^{14}C concentration gradient, from petiole to distal leaf mesophyll, when excised leaves were exposed to $NaH^{14}CO_3$ solutions in the dark. The results of these studies suggest that a concentration gradient of fixed DIC is related to the distance of the tissue from the source. However, distance from the DIC source does not explain all the patterns that we observed in tissue concentration of DIC. We found no significant difference in $\delta^{13}C$ among ECM hyphae, ECM roots, woody roots or stems, and these tissues span a significant distance across the plant (Figure 5). In addition, all fine roots and hyphae were proximal to the source yet differed in concentration of the label: NM roots were more enriched in ^{13}C than ECM roots and ECM hyphae (Figure 5). In our study, greater ^{13}C enrichment of NM roots compared with ECM roots could be a function of relative growth (i.e., sink strength) of the two fine root tissue pools. Over the 4-week experiment, the growth of NM root biomass of treatment seedlings increased 1.6–4.7% compared to pretreatment NM root biomass, whereas ECM root biomass showed no net increase during the experiment (results not shown). If DIC fixation is a function of metabolic activity and C demand, it may be more important in certain tissues and seasons, such as during periodic increases in fine root production.

Distribution of assimilated label among tissues

The distribution of the ^{13}C tracer among plant tissues yielded further insight into the fixation processes of DIC within the plant. In general, the ^{13}C tracer was evenly divided between above- and belowground tissues. Similar to our study, Skok et al. (1962) and Kick et al. (1965*a*) found that the roots of cocklebur and sunflower contained 44–84% and 38–46%, respectively, of the ^{14}C fixed from hydroponic solutions. Although ^{13}C was equally distributed between the above- and belowground pools in our study, it was unequally distributed among the tissues comprising those pools. Aboveground, a greater portion (65%) of ^{13}C was found in the stem compared with the needles (Figure 6), although stem tissue represented only 27% of the aboveground biomass. Conversely, needles contained 35% of the tracer, yet represented 73% of the aboveground biomass.

There are several possible explanations for a greater accumulation of ^{13}C in the stem than in needles. One possibility is that soil DIC is fixed anaplerotically by the root or needles and subsequently allocated to the stem. Another possibility is corticular photosynthetic fixation of ^{13}C in the stem, a process that chiefly utilizes internal C sources rather than external sources (Pfanz et al. 2002). In this process, ^{13}C could have been transported to the stems in the inorganic form and fixed, or transported to the stem in an organic form, decarboxylated and fixed (Müller et al. 1991, Cramer et al. 1993, Hibberd and Quick 2002, Viktor and Cramer 2005). Kursanov et al. (1951) observed greater fixation of soil DIC in bean stems exposed to light compared with stems kept in darkness. Similarly, when tobacco plant roots were exposed to ^{14}C labeled malate, Hibberd and Quick (2002) found greater ^{14}C concentrations in stems compared with leaves. Corticular photosynthesis may well occur in seedling stems; but as it declines with tissue age, it may not be found in older stem tissue (Gartner 1996, Cernusak and Marshall 2000, Aschan et al. 2001). The precise pathway of ^{13}C incorporation into stem tissues will require analysis of ^{13}C in all classes of compounds (e.g., Vuorinen et al. 1992) as well as in specific carbon compounds (e.g., Ceccaroli et al. 2003).

The distribution of ^{13}C among tissues also differed belowground, and was further affected by NH_4^+ availability. Woody roots were the dominant sink for ^{13}C, whereas NM and ECM roots contained the least amount of ^{13}C (Figure 6). Distribution of ^{13}C in belowground plant tissues may be driven by the relative size of biomass pools. For example, woody roots contained nearly 60% of both belowground ^{13}C and belowground biomass. When plant roots assimilate NH_4^+, α-ketoglutarate is diverted from the TCA cycle and used in the synthesis of amino acids (Raven and Farquhar 1990) and as a consequence, anaplerotic fixation rates increase to replenish TCA cycle intermediates. This has been experimentally supported by a study where NH_4^+ fertilized tomato plants contained greater anaplerotic enzyme activity and soil DIC fixation than plants fertilized with NO_3^- (Cramer et al. 1993, Viktor and Cramer 2005). Hence, in response to greater NH_4^+ availability, we predicted greater ^{13}C enrichment in tissues directly assimilating NH_4^+, such as ECM roots (Jackson and Coleman 1959). With greater NH_4^+ availability, we observed significantly less ^{13}C in woody roots and more ^{13}C in ECM roots (Figure 6). Increased NH_4^+ availability did not increase the amount of ^{13}C fixed in NM roots. Similar to our results, Wingler et al. (1996) observed that excised ECM roots had a greater $^{14}CO_2$ fixation rate than NM roots. Our results suggest that small pulses of NH_4^+ may increase anaplerotic demand and thus increase DIC fixation, and that ECM fungi may assimilate a significant portion of the available NH_4^+ relative to other root tissues. Carbon fixation has been induced in ECM roots in the presence of NH_4^+ in other studies (Harley 1964, Martin et al. 1988, Martin et al. 1998), in which the anaplerotic fixation process is dominated by the ECM fungus rather than by the plant (Wingler et al. 1996). Our results indicate that the distribution of fixed DIC among above- and belowground plant tissues may be related to multiple physiological processes, and can be further influenced by NH_4^+ availability and NH_4^+ assimilation.

Contribution of soil DIC to seedling C gain

Numerous studies have measured growth responses of crop plants and the incorporation of soil DIC into labile C products in the plant (i.e., amino and organic acids) (reviewed by Cramer 2002, Enoch and Olesen 1993, Viktor and Cramer 2003, Viktor and Cramer 2005). Of these, few have estimated

soil DIC contributions to whole-plant C gain (Voznesenski 1958, Kick et al. 1965*b*, Schäfer 1988, Stringer and Kimmerer 1993), and each concluded that soil DIC accounted for ~1–3% of total plant or leaf-fixed CO_2. Our estimates of the contribution of soil DIC to *P. taeda* net C gain (~0.8%; Table 1) are at the low end of the published range. Soil DIC was a more important C source for belowground tissues than for the seedling as a whole, contributing ~1.6% to belowground C gain. Soil DIC made a minor, but measurable contribution to total seedling and belowground C gain, which is likely to be the case for trees in natural systems. Although our experimental design included an NH_4^+ treatment to induce greater DIC fixation through the anaplerotic pathway, we detected only a nonsignificant increase in response to increased NH_4^+ availability (Table 1), suggesting that the response of DIC fixation to increased NH_4^+ availability is minor in forest trees and that C gain from DIC may increase in response to NH_4^+ availability only in individual tissues such as ECM roots.

In conclusion, because the net flux of CO_2 is outward from the roots of plants, root uptake and fixation of soil DIC reflects the potential for reacquisition of respired CO_2. In *P. taeda* seedlings, soil DIC contributed a small percentage (< 1%) of C gain, and this C was more important to the C budgets of belowground tissues than of aboveground tissues. Among plant tissues, soil DIC was found in the greatest relative amount in rapidly growing NM roots. However, the stem acquired the most ^{13}C, indicating a possible role of corticular photosynthesis. These results and their consistency with published reports from other species indicate the potential for uptake and fixation of exogenous soil CO_2 and the reincorporation of root-respired CO_2 in forest trees. Rates of soil DIC fixation and allocation among tissues within the plant were affected by rates of tissue growth (e.g., NM roots) and N assimilation (e.g., ECM roots) in *P. taeda* seedlings. Therefore, in forest trees, soil DIC fixation may occur more frequently during increases in fine root production, in newly formed stems, and when ECM roots and fungi assimilate pulses of NH_4^+.

Acknowledgments

This study was supported by the University of Georgia Daniel B. Warnell School of Forestry and Natural Resources, LTER grant DEB-0218001, and EPA STAR Fellowship Agreement No. 91617001–0. The paper has not been formally reviewed by EPA. The views expressed in this document are solely those of the authors and EPA does not endorse any products or commercial services mentioned in this publication. We are grateful to Reinhard Stürzenbecher and Ken Wurzburger for their careful translations of German and Russian literature. We thank Jay Brown, Brian Higgins, Mary Anne McGuire, Tom Maddox, Jon Miniat, Dale Porterfield, Lee Ogden and Leo Sternberg for technical assistance. J.R. Brooks, E.A. Hobbie and J.M. Vose provided helpful comments on an earlier draft of this manuscript.

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