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Carbon isotope discrimination in photosynthetic bark

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Abstract We developed and tested a theoretical model describing carbon isotope discrimination during photosynthesis in tree bark. Bark photosynthesis reduces losses of respired CO_2 from the underlying stem. As a consequence, the isotopic composition of source CO_2 and the CO_2 concentration around the chloroplasts are quite different from those of photosynthesizing leaves. We found three lines of evidence that bark photosynthesis discriminates against ^{13}C. First, in bark of *Populus tremuloides*, the $\delta^{13}C$ of CO_2 efflux increased from –24.2‰ in darkness to –15.8‰ in the light. In *Pinus monticola*, the $\delta^{13}C$ of CO_2 efflux increased from –27.7‰ in darkness to –10.2‰ in the light. Observed increases in $\delta^{13}C$ were generally in good agreement with predictions from the theoretical model. Second, we found that $\delta^{13}C$ of dark-respired CO_2 decreased following 2–3 h of illumination ($P<0.01$ for *Populus tremuloides*, $P<0.001$ for *Pinus monticola*). These decreases suggest that refixed photosynthate rapidly mixes into the respiratory substrate pool. Third, a field experiment demonstrated that bark photosynthesis influenced whole-tissue $\delta^{13}C$. Long-term light exclusion caused a localized increase in the $\delta^{13}C$ of whole bark and current-year wood in branches of *P. monticola* ($P<0.001$ and $P<0.0001$, respectively). Thus bark photosynthesis was shown to discriminate against ^{13}C and create a pool of photosynthate isotopically lighter than the dark respiratory pool in all three experiments. Failure to account for discrimination during bark photosynthesis could interfere with interpretation of the $\delta^{13}C$ in woody tissues or in woody-tissue respiration.

Keywords Carbon isotope discrimination · Corticular photosynthesis · *Pinus monticola* · *Populus tremuloides* · Refixation

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Introduction

Photosynthetic bark in woody trees re-assimilates respired CO_2 that would otherwise be lost to the atmosphere (Schaedle 1975; Sprugel and Benecke 1991; Nilsen 1995). Although bark photosynthesis has been studied most extensively in young twigs (e.g., Perry 1971; Coe and McLaughlin 1980; Han and Suzaki 1981; Larcher et al. 1988; Comstock and Ehleringer 1990; Langenfeld-Heyser et al. 1996), photosynthetic refixation is expected wherever bark surfaces transmit sufficient light for such activity to occur (Sprugel and Benecke 1991); this includes older branches, main stems, and even coarse roots that have been exposed to sunlight (Benecke 1985). Because stomata are typically absent in the epidermal layer of woody tissues (Nilsen 1995), photosynthetic bark relies predominantly on internally produced CO_2 as its substrate. Light-dependent recycling of respired CO_2 in such a manner has been termed refixation (Sprugel and Benecke 1991) or corticular photosynthesis (Strain and Johnson 1963; Nilsen 1995).

Because photosynthesis discriminates against ^{13}C, the $^{13}C/^{12}C$ ratio of plant carbon is less than that of atmospheric CO_2. For plants with the C_3 photosynthetic pathway, the discrimination has been related primarily to differential diffusivities of $^{13}CO_2$ and $^{12}CO_2$ in air, as well as an intrinsically lower reactivity of ^{13}C during initial fixation by photosynthetic enzymes (O'Leary 1981; Farquhar et al. 1982, 1989; Farquhar and Richards 1984;

O'Leary 1988). Measurement of stable carbon isotope ratios has become increasingly common in studies of plant physiological ecology, owing to its utility in identifying photosynthetic pathways, estimating photosynthetic water-use efficiency, and identifying sources of CO_2 used for photosynthesis (Ehleringer and Osmond 1989; Ehleringer 1991; Lajtha and Marshall 1994).

An opportunity for photosynthetic discrimination against ^{13}C exists wherever there is more than one possible fate for CO_2. During refixation in woody tissues, CO_2 may either be re-assimilated or diffuse to the atmosphere. Theory suggests a possibility for discrimination during this process. Moreover, the re-assimilated carbon could have an isotopic signature quite different from that of leaf-assimilated carbon. Assuming re-assimilated carbon can be incorporated into plant tissues, photosynthetic refixation may bias interpretation of carbon isotope ratios in photosynthetic bark and underlying wood.

We developed and tested a theoretical model that describes discrimination against ^{13}C in refixing tissues. The model is similar in concept to existing models of carbon isotope discrimination during CAM and C_4 photosynthesis (Farquhar et al. 1989; Luo 1992); in these models isotopic discrimination by carboxylating enzymes is modified by the leakage of CO_2 from the photosynthetic tissue, rather than diffusion of CO_2 into the tissue. The model was tested with measurements of respired CO_2 from two phylogenetically divergent tree species. In addition, we conducted a light-exclusion experiment in the field to quantify the effects of refixation on carbon isotope ratios of bark and underlying wood.

Theory

We developed a simple model describing refixation in a branch or stem with photosynthetic bark (Fig. 1). In the model, D represents dark respiration, P is corticular photosynthesis, F_{ia} is the unidirectional diffusive flux of CO_2 from the bark to the atmosphere, F_{ai} is the unidirectional diffusive flux from the atmosphere into the bark, C_i is the CO_2 pool in the bark cortex, and C_a is the CO_2 pool in the surrounding atmosphere. Each box in Fig. 1 represents a distinct pool and the arrows represent fluxes between the pools.

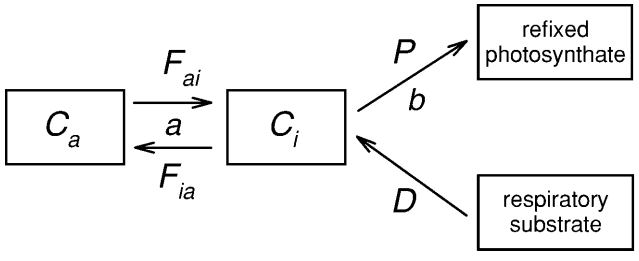

Fig. 1 A simple model of photosynthetic refixation in bark. D is dark respiration, P is corticular photosynthesis, F_{ia} is the diffusive flux from the cortex to the surrounding atmosphere, F_{ai} is the diffusive flux from the atmosphere into the cortex, C_i is the CO_2 pool inside the cortex, and C_a is the CO_2 pool in the atmosphere. The terms a and b represent carbon isotope discrimination constants for diffusion and carboxylation, respectively

The terms a and b are carbon isotope discrimination constants that apply to the designated fluxes. The term 'discrimination' refers to the deviation of an isotope effect (α) from unity, where α is defined as the carbon isotope ratio of reactant divided by that of product (i.e. $\alpha = R_{reactant}/R_{product}$) (Farquhar et al. 1989).

The C_i pool is assumed to be at steady state with respect to both total mass ($dC_i/dt=0$) and isotopic composition ($dR_i/dt=0$; R_i is the $^{13}C/^{12}C$ ratio of C_i). The refixed photosynthate pool is a sink with isotopic composition determined by P. The isotope ratio of P (R_p) is determined by b, the discrimination against ^{13}C during carboxylation, and R_i. A net accumulation of material is allowed in the refixed photosynthate pool. The respiratory substrate pool contributes CO_2 with a defined isotopic composition (R_D); it is assumed able to provide material without being exhausted. Dark respiration itself, at the level of the mitochondria, is assumed to show no discrimination against ^{13}C. It is further assumed that all fluxes entering the pools are instantaneously mixed with each other.

Initially, we consider a branch respiring in a closed chamber, such as a gas-exchange cuvette attached to a closed system. In this context, C_a is a sink allowed to accumulate CO_2. Under these conditions, the discrimination between respiratory substrate and refixed photosynthate ($\Delta_{P(c)}$) can be expressed as

$$\frac{R_D}{R_P} - 1 = \Delta_{P(c)} = \left(1 - \frac{P}{D}\right)\left(b - a\frac{C_i - C_a}{C_i}\right) \quad (1)$$

Similarly, discrimination between respiratory substrate and net CO_2 efflux ($\Delta_{a(c)}$) can be expressed as

$$\frac{R_D}{R_a} - 1 = \Delta_{a(c)} = \frac{P}{D}\left(\frac{1}{b+1}\right)\left(a\frac{C_i - C_a}{C_i} - b\right) \quad (2)$$

where R_a is the $^{13}C/^{12}C$ ratio of the cuvette CO_2. Note that in this case R_a is equal to R_n, the carbon isotope ratio of the net CO_2 efflux. Details of the derivation of Eqs. 1–4 are given in the Appendix.

Equations 1 and 2 do not apply to a branch or stem in the forest. Under natural conditions, R_a is largely independent of R_n. Thus, there are two distinct sources of CO_2 of differing isotopic composition that could provide substrate for refixation: CO_2 released by dark respiration and CO_2 diffusing into the bark from the atmosphere. For a branch under field conditions, the discrimination between respiratory substrate and refixed photosynthate ($\Delta_{P(f)}$) becomes

$$\frac{R_D}{R_P} - 1 = \Delta_{P(f)} = \frac{D - P}{D + (D - P)\dfrac{C_a}{C_i - C_a}} \cdot \left(b\frac{C_i}{C_i - C_a} - a - \Delta_A\frac{C_a}{C_i - C_a}\right) \quad (3)$$

where Δ_A is discrimination between atmospheric CO_2 and leaf-assimilated carbon ($\Delta_A = R_a/R_A - 1$; R_A is the

$^{13}C/^{12}C$ ratio of leaf-assimilated carbon). It is assumed that no fractionation of leaf-assimilated carbon occurs during translocation from leaves to woody tissues. It is further assumed that leaf-assimilated carbon forms the sole substrate for dark respiration.

Rather than write an expression for discrimination between dark respiration and net CO_2 efflux for a tree under field conditions, we wrote an expression for the carbon isotope ratio of net CO_2 efflux using small delta (δ) notation, where

$$\delta_X = \frac{R_X}{R_S} - 1 \tag{4}$$

δ_X is the δ value of material X, R_X is the $^{13}C/^{12}C$ ratio of material X, and R_S is the $^{13}C/^{12}C$ ratio of a standard. The following equation predicts the carbon isotope ratio of net CO_2 efflux (δ_n) under field conditions:

$$\delta_n = \frac{D\delta_D + Db\delta_D + P\left[b - a - \frac{C_a}{C_i}(\delta_a - a)\right]}{D - P\frac{C_a}{C_i} + (D-P)b + Pa\frac{C_i - C_a}{C_i}} \tag{5}$$

where δ_a is the carbon isotope ratio of atmospheric CO_2. The big delta values (Δ) and small delta values (δ) are easily inter-converted by the following relationship:

$$\Delta_{\text{reactant:product}} = \frac{\delta_{\text{reactant}} - \delta_{\text{product}}}{\delta_{\text{product}} + 1} \tag{6}$$

Note that in this theoretical treatment, delta values have not been scaled to the familiar units of per mil (Farquhar et al. 1989).

Equations 1–4 are approximate insofar as they ignore isotope effects associated with absorption of CO_2 at the air-liquid interface and liquid-phase diffusion. Taking these effects into account, Eq. 1 can be written as

$$\frac{R_D}{R_P} - 1 = \Delta_{P(c)} = \left(1 - \frac{P}{D}\right)\left[(e_s + a_l)\frac{C_i - C_c}{C_i} + b\frac{C_c}{C_i} - a\frac{C_i - C_a}{C_i}\right] \tag{7}$$

where e_s is the equilibrium discrimination during dissolution of CO_2 into water, a_l and a are kinetic discrimination constants for liquid- and gas-phase diffusion, and C_c is the CO_2 concentration at the active sites of ribulose-1,5-bisphosphate carboxylase oxygenase (Rubisco) in the bark chloroplasts. A description of the derivation of Eq. 7 will be published elsewhere (Comstock, in press); Eqs. 2–4 can be similarly expanded. However, the discriminations associated with dissolution and liquid-phase diffusion are small [1.1 and 0.7‰, respectively; reviewed by Farquhar et al. (1989)], relative to those associated with carboxylation and gas-phase diffusion. Additionally, theoretical considerations suggest only a minor draw-down from C_i to C_c in refixing tissues (Comstock 1989). Therefore, we suggest that errors associated with omitting e_s and a_l from Eqs. 1–4 are negligible.

A theoretical value for the isotope effect associated with gas-phase diffusion can be derived by taking the ratio of the diffusivities of $^{13}CO_2$ and $^{12}CO_2$ in dry air, yielding 1.0044 (Craig 1954a). The discrimination against $^{13}CO_2$ during diffusion can then be expressed as a=0.0044 or 4.4‰. The isotope effect associated with carboxylation is usually taken as b=1.029, or 29‰ (Farquhar et al. 1989). By assuming these values for a and b, and a constant value of –25‰ for δ_D, we made representative model predictions of δ_D and δ_P as functions of P/D, which we term the proportional refixation rate (Fig. 2). For simplicity in this example, we assumed a zero flux from the atmosphere into the bark; this assumption is not made in any of our subsequent analyses. $\Delta_{a(c)}$ and $\Delta_{P(c)}$ are indicated conceptually in Fig. 2 as the difference between δ_D and δ_a, and between δ_D and δ_P, respectively. These definitions are good approximations for those given by Eq. 6.

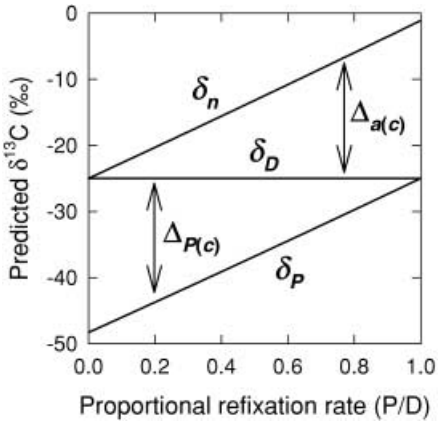

Fig. 2 Representative model predictions of δ_P and δ_n as functions of P/D. Calculations were performed after assuming δ_D=–25‰, a=4.4‰, and b=29‰. For simplicity in this example, we assumed that F_{ai}=0. The discrimination terms $\Delta_{P(c)}$ and $\Delta_{a(c)}$ can be approximated by δ_D-δ_P and δ_D-δ_n, respectively, as indicated on the graph

Materials and methods

Plant material

To establish the generality of the proposed model, we tested it with measurements on two phylogenetically divergent tree species: *Pinus monticola* Dougl. (western white pine), a needle-leaved, evergreen gymnosperm, and *Populus tremuloides* Michx. (trembling aspen), a broad-leaved, deciduous angiosperm. The capacity of *P. tremuloides* bark for photosynthetic refixation is well documented (Pearson and Lawrence 1958; Strain and Johnson 1963; Foote and Schaedle 1976, 1978; Brayman and Schaedle 1982; Schaedle and Brayman 1986; Kharouk et al. 1995). Photosynthetic refixation approaching 80% of dark respiration was recently reported in *Pinus monticola* branches (Cernusak and Marshall 2000).

Branches were collected from two field sites. One was a 17-year-old *P. monticola* plantation located on the University of Idaho Experimental Forest. This site has been recently described (Cernusak and Marshall 2000). The other site comprised a stand of both mature and juvenile *Populus tremuloides*. It was located 24 km north of Moscow, ID (46°48′30″ N, 116°59′30″ W) at an elevation of 869 m. Mean annual temperature was approximately 8.3°C and mean annual precipitation 648 mm. The stand was positioned next to a small stream, which ceased to flow in late summer.

Experiments with respired CO_2

In order to test the model of carbon isotope discrimination during refixation, we measured the isotopic composition of CO_2 respired from branches in the dark and at several irradiances. Measurements were made on excised branch sections; the cut surfaces were covered with melted paraffin to minimize disruption of normal CO_2 diffusion pathways. We found previously that excision had no apparent effect on branch gas exchange for *Pinus monticola* (Cernusak and Marshall 2000); likewise, we had no reason to expect that it would affect *Populus tremuloides*. Respired CO_2 was collected and gas exchange measured in a one-liter Plexiglas cuvette (Li-Cor, Lincoln, Neb., USA). The cuvette was fitted with an attachment through which water from a controlled temperature bath circulated, thereby allowing regulation of the air temperature inside the cuvette (Cernusak and Marshall 2000). Two artificial light sources (Quantum Devices, Barneveld, Wis., USA) provided illumination. One was positioned above the cuvette and one below it, such that branches within the cuvette were illuminated from the top and the bottom. A portable LI-6200 photosynthesis system (Li-Cor, Lincoln, Neb., USA) measured CO_2 efflux.

Before collection of respired CO_2, the closed system was scrubbed free of CO_2 by directing flow through a soda lime trap on the LI-6200. The system was then allowed to refill with respired CO_2 until the air inside reached a CO_2 concentration of approximately 360 µmol mol^{-1}. At that time, gas-tight, locking syringes (VICI Precision Sampling, Baton Rouge, La., USA) were used to extract two air samples from the closed system through a glass tee fitted with a septum. For branches with rapid respiration rates, it was not possible to scrub the CO_2 concentration all the way to zero. In these cases the scrub was maintained until we felt confident that the residual CO_2 pool was at steady state (usually 15–20 min). The rate of CO_2 efflux from branch sections was measured just prior to extraction of air samples.

Carbon isotope ratios of extracted air samples were measured on an isotope ratio mass spectrometer (MS) (Delta Plus, Finnegan MAT, Bremen, Germany). Before entering the MS, air samples were swept into a trace-gas condensing device (PreCon, Finnegan MAT) by a helium carrier gas. The PreCon condensed CO_2 and N_2O from the air samples. The condensate then passed through a gas chromatograph (GC, Finnegan MAT) that separated CO_2 from N_2O. The system was configured as described by Ehleringer and Cook (1998), except that we used a 50 m POROPLOT Q column in the GC to separate CO_2 from N_2O, rather than a 25 m column. Values obtained from the PreCon were corrected for the presence of a blank; the blank was measured every tenth sample. PreCon injections consisted of 400 µl air samples and all samples were run in duplicate using two different syringes. The overall precision of the analyses, based on multiple sets of repeated injections of a standard gas (ISU-720 C, Oztech Trading, Dallas, Tex., USA; $\delta^{13}C$=–10.98‰), was ±0.2‰ (SD). Carbon isotope ratios are presented in δ notation with respect to the Pee Dee Belemnite standard.

We corrected the respired CO_2 values for leakage of laboratory air into the cuvette. The leak conductance of the empty cuvette and the carbon isotope ratio of laboratory air were measured twice daily. We assumed the carbon isotope ratio of leaked CO_2 was 4.4‰ less than that of laboratory air. The amount of time required for each respired CO_2 collection to reach a CO_2 concentration of 360 µmol mol^{-1} was recorded; the change in CO_2 concentration gradient from laboratory to cuvette was assumed to decrease linearly over that time period. The leakage was estimated in 5 µmol mol^{-1} increments by multiplying the concentration gradient by the leak conductance and the approximate amount of time spent at that gradient. On average, the volume of leaked CO_2 was less than 5% of the total CO_2 volume; however, at high refixation rates (i.e. low CO_2 efflux) the magnitude of the leak correction increased.

Two experiments were conducted in which respired CO_2 was collected and measured for its carbon isotope ratio. In the first experiment, respired CO_2 was collected in the dark and then incrementally at irradiances of 50, 100, 250, and 500 µmol photosynthetically active radiation (PAR) m^{-2} s^{-1}. At each irradiance, the proportional refixation rate was calculated by assuming the dark respiration rate did not change during illumination. After each change in light intensity, branches were allowed to reach steady-state CO_2 efflux before data were collected (usually about 30 min). All measurements were made at bark surface temperatures of 23±2°C. For some branches, CO_2 efflux rates at the highest light intensities were so low that respired CO_2 could not be collected. Branches were measured on the same day they were collected from the field. The ten measured *Populus tremuloides* branches ranged from 2.0 to 2.9 cm in diameter, and the six measured *Pinus monticola* branches ranged from 1.9 to 3.1 cm in diameter.

In the second experiment, we wished to ascertain whether or not the carbon isotope ratio of dark-respired CO_2 would change following a period of illumination. We expected that if refixed photosynthate were converted to respiratory substrate, it might alter the $\delta^{13}C$ of dark-respired CO_2. We stored the branches measured in the first experiment in darkness overnight (approximately 10 h) at laboratory temperature. The following day, we collected dark-respired CO_2 and measured its isotopic composition. We then exposed the branches to 500 µmol PAR m^{-2} s^{-1} for 2 to 3 h. Following the period of illumination, branches were placed in darkness and allowed to return to steady-state CO_2 efflux. When steady state was reached, dark-respired CO_2 was again collected and its carbon isotope ratio measured. The experiment was repeated on seven branches for *Populus tremuloides* and six branches for *Pinus monticola*.

We measured the surface conductance of a subset of branches used in the light response experiment. Five *Populus tremuloides* branches and three *Pinus monticola* branches were randomly selected and their surface conductance to water vapor measured as described by Cernusak and Marshall (2000). Vapor conductance was converted to CO_2 conductance by dividing by 1.6 and used in conjunction with CO_2 efflux rates to estimate CO_2 concentrations within the bark cortex:

$$C_i = \frac{F_n}{g} + C_a \tag{8}$$

where F_n is net CO_2 efflux, and g is the bark conductance to CO_2. Mean g values for each species were used to estimate C_i for branches on which g was not measured.

We tested for significant differences between species in the light response of bark photosynthesis using repeated measures analysis of variance (Potvin et al. 1990). To estimate light-saturated bark photosynthetic rates and proportional refixation rates, we fit the following nonlinear curve to the light response data:

$$P_{gi} = P_{g\,max}(1 - \exp[-s(I)]) \tag{9}$$

where P_{gi} is instantaneous gross bark photosynthesis, calculated as the difference between CO_2 efflux in the dark and that in the light, P_{gmax} is the light saturated rate of bark photosynthesis, s is the initial slope of the light response curve, and I is irradiance. The same equation was used to analyze the light response of proportional refixation rates.

The isotopic discrimination between dark respiration and net CO_2 efflux ($\Delta_{a(c)}$) was predicted for each of the measurements in the light response experiment using Eq. 2; predicted $\Delta_{a(c)}$ values were then compared to observed $\Delta_{a(c)}$ values calculated from Eq. 6. For calculating predicted values, δ_D and D measured before illumination were assumed constant during illumination. We calculated P as the difference between CO_2 efflux in the dark and that in the light. We used a C_a value of 350 µmol mol^{-1} in the calculations of C_i because that was the concentration at which F_n was measured.

Differences in the carbon isotope ratio of dark-respired CO_2 before and after illumination were analyzed with paired *t*-tests. All statistical analyses in the study were performed in SYSTAT 9.0 (SPSS, Chicago, Ill., USA).

Light exclusion experiment

In order to determine the influence of refixation on whole-tissue carbon isotope ratios, we excluded sunlight from ten *Pinus monticola* branch sections for the duration of the 1999 growing season.

Branches for the experiment were selected in pairs; each pair consisted of two branches growing in the same whorl of the same tree. In each pair, one branch was randomly selected for treatment while the other served as an untreated control. The treated branches were loosely wrapped in aluminum foil and the foil ends taped to the bark. Foil-covered sections comprised one needle-free internode and ranged from 15 to 30 cm in length. A similarly aged internode was taken from the control branch of each pair at the conclusion of the experiment. The treatments were applied on 18 May 1999, before bud-break occurred. Because cambial activity usually occurs after bud-break in conifers, we hoped to ensure that the new annual ring would be formed after the foil was in place. Diameters of branch sections ranged from 1.1 to 3.0 cm.

The branches were harvested between 8 and 11 September 1999. Dark-respired CO_2 was collected from a subset of the branch sections (six pairs) to determine whether prolonged light exclusion influenced its isotopic composition. Dark-respired CO_2 was also collected from foliage on the main terminus of each branch to ensure that differences between treatments were not due to differences in the isotopic composition of imported, leaf-assimilated carbon. Leaf respired CO_2 was collected as described for branch sections; whole, current-year shoots were excised and placed inside the 1-l cuvette. Dark-respired CO_2 was collected from branch sections and current-year shoots on the same day that branches were harvested.

Immediately following collection of dark-respired CO_2, shoots and branch sections were frozen and stored for isotopic analysis of leaf, bark, and wood tissue. Ten needle fascicles were removed from each current-year shoot and air-dried at 70°C. A cross-section was removed from each branch section; whole bark and current-year wood were separated and air-dried at 70°C. The current-year wood ring was removed with a razor blade. Dried samples were ground to a fine powder in a rotating ball mill. A 1 mg subsample of each was combusted in an elemental analyzer (NC2500, CE Instruments, Milan, Italy); the combustion products were swept via a helium carrier gas and continuous-flow interface into the MS, where their carbon isotope ratio was measured. The analytical precision, based on repeated measurements of a working standard (Idaho flour), was ±0.08‰. Differences in $\delta^{13}C$ of dark-respired CO_2, bark, and wood between foil-covered and uncovered branches were analyzed with paired *t*-tests.

Comparison of inner and outer bark

We collected *Populus tremuloides* branches from upper and lower crown positions at the *P. tremuloides* site from which branches were sampled for respired CO_2. Branches were collected in late September 1998. In 12 branches we separated the outer, chlorophyll-containing bark from the inner, non-chlorophyll-containing bark and measured the two fractions separately for $\delta^{13}C$. Whole-tissue carbon isotope ratios were determined as described for the light-exclusion experiment. Carbon isotope ratios of inner and outer bark fractions were compared with a paired *t*-test.

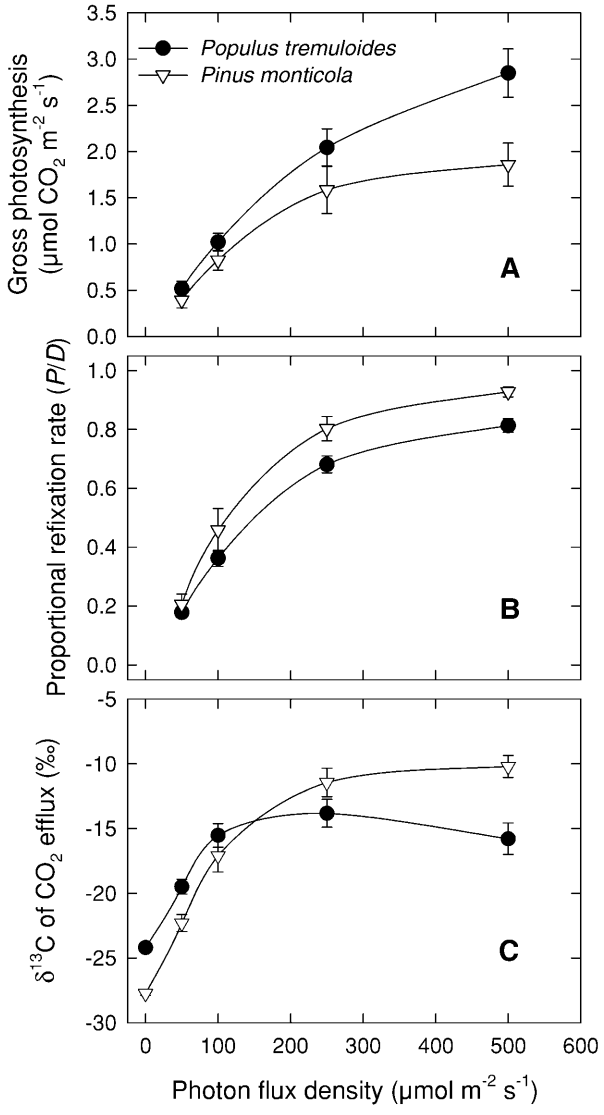

Fig. 3 Light response of **A** gross photosynthesis, **B** proportional refixation rate (*P*/*D*), and **C** carbon isotope ratio of CO_2 efflux (δ_n) for branches of *Populus tremuloides* and *Pinus monticola*. Branches were illuminated with two artificial light sources such that the illumination was approximately uniform. Measurements were made at bark surface temperatures of 23±2°C. *Error bars* represent 1 standard error

Results

Experiments with respired CO_2

The light response of gross photosynthesis in bark differed between branches of *Populus tremuloides* and branches of *Pinus monticola* (*P*=0.02, Fig. 3A). *Populus tremuloides* had a higher light-saturated rate of bark photosynthesis than *Pinus monticola* (3.45 vs 2.04 μmol CO_2 m^{-2} s^{-1}). However, the initial slope of the light response curve, also referred to as the apparent quantum yield, was slightly higher for *Pinus monticola* than for *Populus tremuloides* (0.0053 vs 0.0035 μmol CO_2 μmol^{-1} photons). The light response of the proportional refixation rate also differed between species (*P*=0.01, Fig. 3B); the light-saturated proportional refixation rate of *Pinus monticola* was higher than that of *Populus tremuloides* (0.99 vs 0.89). Thus, *Populus tremuloides* branches had higher maximum rates of bark photosynthesis, but also had higher dark respiration rates; as a result, light-saturated photosynthesis was a smaller proportion of dark respiration for *Populus tremuloides* than for *Pinus monticola* (Fig. 3B).

The carbon isotope ratio of dark-respired CO_2, collected on the day of branch excision, was more negative for *Pinus monticola* than for *Populus tremuloides* (*P*<0.0001, Fig. 3C). The mean value for *Pinus montico-*

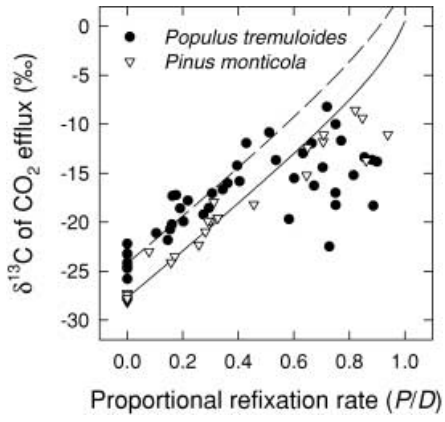

Fig. 4 Carbon isotope ratio of CO_2 efflux (δ_n) plotted against the proportional refixation rate (*P*/*D*) for branches of *Populus tremuloides* and *Pinus monticola*. Lines represent model predictions. The *solid line* corresponds to *Pinus monticola*, and the *broken line* to *Populus tremuloides*

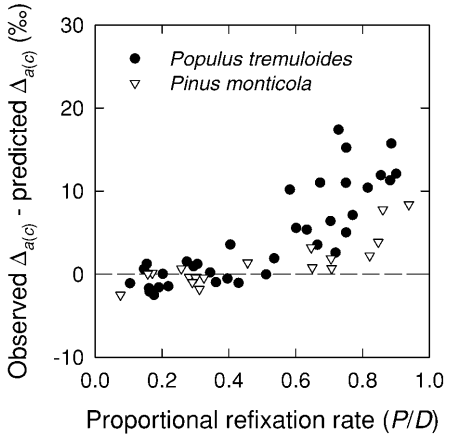

Fig. 5 Discrepancies between predicted and observed discrimination from dark respiration to CO_2 efflux ($\Delta_{a(c)}$) plotted as a function of the proportional refixation rate (*P*/*D*)

la was –27.7‰; the mean value for *Populus tremuloides* was –24.2‰. The $\delta^{13}C$ of CO_2 efflux (δ_n) increased for both species (became less negative) as light intensity increased. At the highest light intensities, CO_2 respired by *P. tremuloides* branches appeared to decrease slightly in its carbon isotope ratio, whereas that of *Pinus monticola* appeared to remain relatively constant (Fig. 3C). Consequently, δ_n at the highest light intensity was more negative for *Populus tremuloides* than for *Pinus monticola* ($P<0.01$). Mean values at 500 µmol PAR m^{-2} s^{-1} were –15.8‰ and –10.2‰, respectively.

When observed δ_n was plotted against *P*/*D*, its distribution agreed well with that predicted by the model for refixation rates less than ~0.5 (Fig. 4). At *P*/*D* greater than 0.5 the observed increase in δ_n with increasing *P*/*D* appeared to be less than that predicted by the model for both species. This discrepancy between observed and predicted $\Delta_{a(c)}$ continued to increase as *P*/*D* approached unity (Fig. 5). There was considerably more scatter in

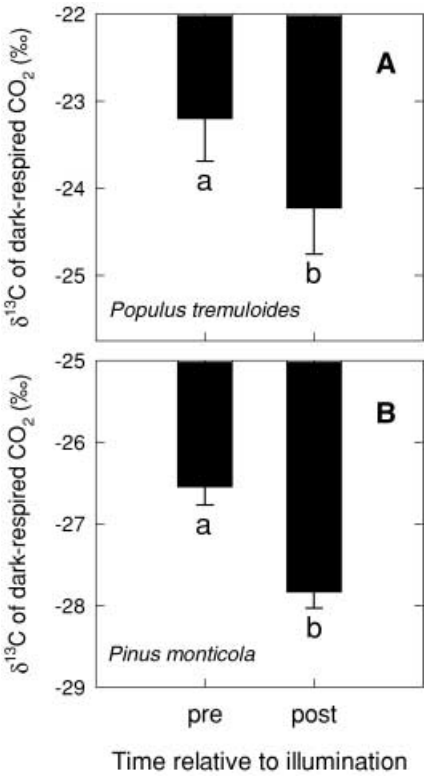

Fig. 6 Carbon isotope ratio of CO_2 respired in the dark for branches of **A** *Populus tremuloides* and **B** *Pinus monticola*. Dark-respired CO_2 was collected before a 2- to 3-h illumination period and again afterward. Prior to the experiment, branches were kept in darkness for approximately 10 h. *Bars* within a panel followed by different letters are significantly different at $P<0.05$. *Error bars* represent 1 SE. For *Populus tremuloides*, $n=7$; for *Pinus monticola*, $n=6$

the data at high *P*/*D* than at low *P*/*D*, particularly for *Populus tremuloides*. This scatter may have been caused by reduced CO_2 efflux from branches at high refixation rates, which resulted in longer periods of CO_2 collection. With increasing amounts of time required for collection of respired CO_2, errors associated with fluctuating gas-exchange rates, or CO_2 leakage, may have increased.

The mean CO_2 conductance of *Populus tremuloides* bark was more than twice that of *Pinus monticola* bark. The mean value for *Populus tremuloides* was 1.4± 0.6 mmol CO_2 m^{-2} s^{-1} (mean±SE), whereas that for *Pinus monticola* was 0.6±0.1 mmol CO_2 m^{-2} s^{-1}. However, sample sizes ($n=5$ for *Populus tremuloides*, $n=3$ for *Pinus monticola*) were too small to allow a meaningful statistical comparison between species. Mean C_i estimates for darkened branches were 3,230±564 µmol mol^{-1} for *Populus tremuloides* and 3,814±642 µmol mol^{-1} for *Pinus monticola*. For illuminated branches, C_i estimates ranged from 6,020 to 530 µmol mol^{-1} for *Populus tremuloides* and from 3,967 to 577 µmol mol^{-1} for *Pinus monticola*.

When dark-respired CO_2 (δ_D) was collected after a period of illumination and compared to δ_D collected before illumination, the post-illumination CO_2 had a more negative $\delta^{13}C$ for both species ($P<0.01$ for *Populus tremuloides*, $P<0.001$ for *Pinus monticola*; Fig. 6). This was

Table 1 Mean carbon isotope ratios for dark-respired CO_2 and corresponding whole tissues. For branches, $\delta^{13}C$ of respired CO_2 was compared to that of whole bark. Dark-respired CO_2 was collected after tissues were kept in darkness for several hours. Values were compared with paired *t*-tests

Species	Tissue	*n*	Mean carbon isotope ratio (‰)				
			Dark-respired CO_2	Whole tissue	Mean difference	SD difference	*P*-value
Populus tremuloides	Bark	7	–23.20	–26.09	2.89	0.93	0.0002
Pinus monticola	Foliage	12	–22.72	–24.92	2.20	1.27	0.0001
P. monticola	Bark	12	–25.51	–26.52	1.01	0.91	0.003

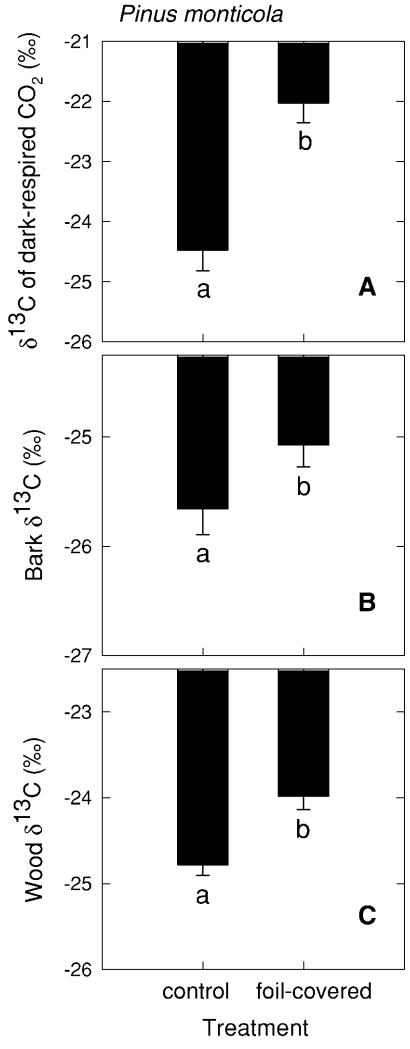

Fig. 7 Carbon isotope ratio of **A** dark-respired CO_2, **B** whole bark, and **C** current-year wood for branches of *Pinus monticola* that were either covered with foil throughout the growing season or left uncovered. Carbon isotope ratios for bark and wood are for whole tissues. *Bars* within a panel followed by different letters are significantly different at $P<0.05$. *Error bars* represent 1 standard error. For dark-respired CO_2, $n=6$; for bark and wood, $n=10$

consistent with the expectation that refixed photosynthate could alter the carbon isotope ratio of dark-respired CO_2. We presume that as refixed photosynthate became a substrate for dark respiration, δ_D decreased, owing to the depleted nature of δ_P.

Light exclusion experiment

Four months of light exclusion by foil wrapping strongly influenced $\delta^{13}C$ of dark-respired CO_2, bark, and current-year wood in *Pinus monticola*. Dark respiration from the foil-wrapped branches was 2.5‰ less negative than dark respiration from control branches ($P=0.001$, Fig. 7A). The carbon isotope ratio of whole bark was 0.6‰ less negative in foil-wrapped branches than in control branches ($P<0.001$, Fig. 7B); that of current-year wood was 0.8‰ less negative ($P<0.0001$, Fig. 7C). The $\delta^{13}C$ of CO_2 produced in the dark by subtending foliage was similar between foil-wrapped and control branches ($P=0.29$); likewise, the whole-tissue carbon isotope ratio of current-year foliage did not differ ($P=0.32$). This suggests similar $\delta^{13}C$ of leaf-assimilated carbon between foil-wrapped and control branches. Thus, refixation decreased $\delta^{13}C$ in woody tissues and woody-tissue respiration.

We compared the carbon isotope ratio of dark-respired CO_2 collected after several hours of darkness to that of whole tissues. On average, dark-respired CO_2 was less negative than whole tissue by about 2‰ (Table 1). The $\delta^{13}C$ of dark-respired CO_2 for foliage and woody tissues of both species combined was a linear function of whole-tissue $\delta^{13}C$ ($R^2=0.51$, $P<0.0001$, $n=31$). The slope of the relationship did not differ from unity ($P=0.17$), and the intercept did not differ from zero ($P=0.10$).

Comparison of inner and outer bark

When we compared the $\delta^{13}C$ of outer, chlorophyll-containing bark to the $\delta^{13}C$ of inner, non-chlorophyll-containing bark for *Populus tremuloides*, we found that the outer bark was more negative than the inner bark ($P<0.0001$). Mean $\delta^{13}C$ of outer bark was –24.9‰; mean $\delta^{13}C$ of inner bark was –24.3‰. Bark strata from the full circumference of the branches were compared; thus self-shading on the north- and downward-facing bark sections may have decreased the overall difference. Nevertheless, these data are consistent with the expectation that δ_P should have a greater influence over whole-tissue $\delta^{13}C$ in the photosynthetic strata of the bark than in the non-photosynthetic strata.

Discussion

We tested a theoretical model that predicts carbon isotope discrimination during photosynthetic refixation in the bark of woody trees. We found strong evidence of discrimination in two phylogenetically divergent tree species, *Populus tremuloides* and *Pinus monticola*. Moreover, the nature of the discrimination was in general agreement with the theoretical model, particularly at low to moderate refixation rates. In addition, we found isotopic evidence that refixed carbon rapidly enters substrate pools for respiration and tissue synthesis.

The theoretical model proposes that carbon isotope discrimination occurs during photosynthetic refixation, and that this discrimination is modified by the proportional refixation rate (P/D). The result (according to the model) is a decline in discrimination between respiratory substrate and refixed photosynthate ($\Delta_{P(c)}$) and an increase in discrimination between respiratory substrate and net CO_2 efflux ($\Delta_{a(c)}$) as P/D increases (Fig. 2). Note that $\Delta_{a(c)}$ is effectively a negative discrimination; it favors the heavier isotope. We tested the model by measuring $\Delta_{a(c)}$ over a range of refixation rates. The model performed well at low refixation rates, but appeared to over-predict $\Delta_{a(c)}$ at high refixation rates (Fig. 5).

The assumption of steady state was not strictly met during our measurements. In collecting the CO_2 efflux, we first removed all of the CO_2 from the closed system and then let the system refill with respired CO_2. The abrupt change in C_a while the system was scrubbed free of CO_2 would have resulted in a change in the diffusion gradient from the bark cortex to the atmosphere (C_i-C_a), thus changing C_i. The error associated with this assumption can be constrained by considering the proportional difference in diffusion gradient induced by setting C_a to zero, and the difference in isotopic composition between C_i and C_a. We estimated C_i to range from several thousand µmol mol^{-1} in darkened branches to mean values of 1,001±177 and 734±172 µmol mol^{-1} at the highest irradiance for *Populus tremuloides* and *Pinus monticola*, respectively. Using Eq. A6, we generated estimates of δ_i-δ_a, the isotopic difference between C_i and C_a. Values ranged from 4.1 to 1.4‰ for *Populus tremuloides* and from 4.0 to 1.6‰ for *Pinus monticola*; highest values occurred in darkened branches and lowest values in branches under maximum irradiance.

It seems possible that the lack of steady state could have caused a model bias at high refixation rates. However, given the proportionally small changes in diffusion gradient associated with a 350 µmol mol^{-1} reduction in C_a and the relatively small isotopic differences between C_a and C_i (especially at high refixation rates), it seems unlikely that the prediction errors resulting from non-steady state conditions could have exceeded 1 or 2‰. Moreover, if heavier CO_2 were drawn from the bark cortex into the chamber as a result of the abrupt reduction in C_a, the error would be in the wrong direction to explain the discrepancies observed in Figs. 4 and 5.

Four additional assumptions were required for predicting $\Delta_{a(c)}$. These were (1) a constant dark respiration rate in illuminated branches, (2) constant P/D at a given irradiance, (3) a constant carbon isotope ratio of dark-respired CO_2 during refixation, and (4) an enzymatic discrimination of 29‰. This last assumption implies that light-induced fixation is exclusively by Rubisco.

In addition to Rubisco, PEP carboxylase also fixes substantial amounts of CO_2 in plant tissues. Not surprisingly, this "dark fixation" has been observed in woody tissues (Langenfeld-Heyser 1989). Because of dark fixation, net enzymatic discrimination by photosynthetic leaves is usually assumed to equal 27‰ (Farquhar et al. 1989). [Note that this is not the case for species that assimilate nitrogen only in the roots (Livingston et al. 1999)]. However, if rates of dark fixation in illuminated woody tissues are similar to those that occur in darkness, our estimate of δ_D should have already accounted for discrimination by PEP carboxylase. Therefore our assumption of 29‰ for enzymatic discrimination is appropriate.

We suggest that the departure of observed $\Delta_{a(c)}$ from predicted $\Delta_{a(c)}$ at high refixation rates resulted from mixing of refixed photosynthate into the respiratory substrate pool. This would have caused a decrease in the carbon isotope ratio of dark respiration and a resulting decrease in the apparent $\Delta_{a(c)}$, consistent with our observations. Continued recycling of refixed photosynthate for dark respiration at higher refixation rates would have lead to a cyclical depletion of ^{13}C in CO_2 efflux and increased departure from model predictions. Again, this is consistent with our observations (Fig. 5).

Our second experiment with respired CO_2 (the comparison of δ_D collected before illumination with δ_D collected after illumination) further supports this suggestion. However, the changes (1.0‰ for *Populus tremuloides*, 1.3‰ for *Pinus monticola*; Fig. 6) were smaller than one might predict given the preceding discussion. We had to wait approximately 1 h for the CO_2 efflux rates to return to steady state following the period of illumination. Thus, the majority of refixed photosynthate that entered the respiratory substrate pool may have been consumed before the post-illumination period for CO_2 collection began.

The assumption of no carbon isotope fractionation during dark respiration has frequently been applied by ecologists. For example, it is implicit in any analysis wherein the carbon source for respiration is inferred from the carbon isotope ratio of respired CO_2 (e.g. Jacobson et al. 1970; Smith 1971; Walker et al. 1983; Tang et al. 1987). Relatively few data are available for evaluating this assumption. Lin and Ehleringer (1997) concluded that δ_D is precisely indicative of the $\delta^{13}C$ of respiratory substrates, based on experiments with protoplasts isolated from *Phaseolus vulgaris* and *Zea mays*. Similarly, Guy et al. (1989) reported nearly identical carbon isotope ratios for respired CO_2 and starch in the green alga *Selenastrum minutum*. However, this conclusion has not been catholic. Duranceau et al. (1999) re-

cently reported a discrepancy of 6‰ between δ_D and that of sucrose in cotyledon leaves of *Phaseolus vulgaris*; δ_D was enriched relative to sucrose.

In our study, there was a linear relationship between δ_D (collected after tissues were held in darkness for several hours) and $\delta^{13}C$ of whole tissues. The δ_D was about 2‰ less negative, on average, than the $\delta^{13}C$ of whole tissue (Table 1). Carbohydrates are frequently observed to be 1–2‰ less negative than whole tissue (Deines 1980; Galimov 1985). Thus, our results appear to be consistent with the suggestion of little or no fractionation during dark respiration for the two species examined in this study. However, because we did not measure the $\delta^{13}C$ of the respiratory substrate directly, we cannot draw a firm conclusion.

Prolonged exclusion of sunlight from *Pinus monticola* branches produced marked changes in the carbon isotope ratios of dark-respired CO_2, whole bark, and current-year wood (Fig. 7). Interestingly, the δ_D from foil-wrapped branches was about 2.5‰ less negative than the δ_D from control branches. In contrast, the $\delta^{13}C$ of current-year wood from foil-wrapped branches was only about 1‰ less negative than that of wood from controls. Because the wood was produced after the application of the light exclusion treatment, and assuming no carbon isotope fractionation during dark respiration, one might expect the two values to be more similar in magnitude. We speculate that the current-year wood was partially constructed from stored photosynthate, assimilated before the application of the light exclusion treatment. If this were the case, the change in $\delta^{13}C$ of wood following light exclusion would be expected to approach the magnitude of the change in δ_D within 2 or 3 years. This change in $\delta^{13}C$ with light exclusion may have important implications for studies which attempt to reconstruct long-term trends in the physiology of leaf photosynthesis from the $\delta^{13}C$ of tree-ring chronologies (e.g., Marshall and Monserud 1996; Bert et al. 1997; Duquesnay et al. 1998; Feng 1998, 1999; Tang et al. 1999).

An increase in the carbon isotope ratio of stem wood, or stem-wood cellulose, with increasing tree age has been observed in many tree species (Craig 1954b; Francey 1981; Yoder et al. 1994; Bert et al. 1997; Duquesnay et al. 1998); this tendency has been termed the juvenile effect. Hypothesized mechanisms underlying the juvenile effect include increased C_i/C_a in leaves of young trees resulting from low irradiance in the understory (Francey and Farquar 1982), refixation of soil-respired CO_2 (Schleser and Jayasekera 1985), and decreased C_i/C_a in leaves of old trees resulting from reduced hydraulic conductance (Waring and Silvester 1994; Yoder et al. 1994; Panek and Waring 1995). We now add a fourth possible cause to this list: a decreasing contribution of photosynthate from bark refixation to stemwood synthesis as trees age.

Cernusak and Marshall (2000) reported a linear decrease in the photosynthetic capacity of *Pinus monticola* bark with decreasing specific bark area (ratio of bark area to bark mass); this indicates reduced refixation as bark becomes older and thicker. Shading by the canopy would also tend to reduce refixation on stems in older trees. Our light-exclusion experiment demonstrated the capacity of photosynthetic bark for altering the carbon isotope ratio of underlying wood. Moreover, this appears to be a localized effect, as the foil-covered branch sections measured only 15–30 cm in length. While it is likely that each of the proposed mechanisms contributes to the juvenile effect to some extent, consideration of each one independently may be important for interpreting long-term trends in the $\delta^{13}C$ of tree rings. For example, in a recent analysis of several $\delta^{13}C$ tree-ring chronologies (Feng 1999), it was assumed that because individuals were selected from open-grown environments, they were not subject to the juvenile effect. Presumably, changes in hydraulic conductance and bark refixation with tree age would continue, regardless of a tree's position in relation to other trees.

In conclusion, we developed and tested a model describing carbon isotope discrimination during photosynthetic refixation in the bark of woody trees. This discrimination influences the carbon isotope ratio of CO_2 respired from such woody tissues in the dark and in the light. A field experiment showed that this process contributes measurably to the whole-tissue carbon isotope ratios of photosynthetic bark and underlying wood. If unaccounted for, carbon isotope discrimination by photosynthetic bark may jeopardize meaningful interpretation of the isotopic composition of woody tissues and woody-tissue respiration.

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Appendix

Part 1: $\Delta_{P(c)}$

The one-way diffusional fluxes F_{ia} and F_{ai} can be defined as follows:

$$F_{ia}=gC_i, \tag{A1}$$

and

$$F_{ai}=gC_a, \tag{A2}$$

where g is the conductance to CO_2 from the bark cortex to the atmosphere. The net CO_2 efflux can then be defined as

$$F_n=F_{ia}-F_{ai}=g(C_i-C_a) \tag{A3}$$

Equation A3 can be written for ^{13}C as

$$R_nF_n = \frac{g}{1+a}(R_iC_i - R_aC_a) \tag{A4}$$

Combining Eqs. A3 and A4 yields

$$R_n F_n = \frac{F_n}{C_i - C_a}\left(\frac{1}{1+a}\right)(R_i C_i - R_a C_a) \quad \text{(A5)}$$

For the situation of a branch respiring into a closed-system cuvette, we assume no sources of CO_2 other than the branch. The isotopic composition of C_a is then defined by that of the net CO_2 efflux, such that R_n=R_a. Replacing R_n with R_a in Eq. A5 and rearranging yields

$$\frac{R_i}{R_a} - 1 = a\frac{C_i - C_a}{C_i} \quad \text{(A6)}$$

Asuming steady state with respect to total mass,

$$D - P = g(C_i - C_a) \quad \text{(A7)}$$

and with respect to isotopic composition,

$$R_D D - R_P P = \frac{g}{1+a}(R_i C_i - R_a C_a) \quad \text{(A8)}$$

Using the definition R_i/R_p=1+b and substituting from Eqs. A6 and A7, Eq. A8 can be rewritten as

$$R_D D - R_P P = \frac{D-P}{C_i - C_a}\left(\frac{1}{1+a}\right)\left[R_p(1+b)C_i - \frac{R_p(1+b)C_a}{a\frac{C_i - C_a}{C_i}+1}\right] \quad \text{(A9)}$$

Isolating R_D/R_P on the left side of the equation yields

$$\frac{R_D}{R_P} = \frac{P}{D} + \left[\frac{(D-P)(1+b)C_i}{D(C_i - C_a)(1+a)}\right] - \left[\frac{(D-P)(1+b)C_a}{D(C_i - C_a)(1+a)a\frac{C_i - C_a}{C_i}+1}\right] \quad \text{(A10)}$$

Subtracting one from each side, finding a common denominator, and rearranging leads to

$$\frac{R_D}{R_P} - 1 = \left(1 - \frac{P}{D}\right)\left[\frac{b + a\frac{C_a - C_i}{C_i}}{a\frac{C_i - C_a}{C_i}+1}\right] \quad \text{(A11)}$$

Equation A11 is approximated to within 0.1‰ by Eq. 1 of the main text.

Part 2: $\Delta_{a(c)}$

Incorporating the assumptions stated for the derivation of $\Delta_{P(c)}$, we begin with Eq. A8. Substituting from Eqs. A6 and A7, Eq. A8 can be rewritten as

$$R_D D - \frac{R_a\left(a\frac{C_i - C_a}{C_i}+1\right)}{1+b}P = \frac{D-P}{C_i - C_a}\left(\frac{1}{1+a}\right)\left[R_a\left(a\frac{C_i - C_a}{C_i}+1\right)C_i - R_a C_a\right] \quad \text{(A12)}$$

Isolating R_D/R_a on the left side of the equation yields

$$\frac{R_D}{R_a} = \frac{D-P}{C_i - C_a}\left(\frac{1}{1+a}\right)\left(a\frac{C_i - C_a}{C_i}+1\right) \cdot C_i - \frac{D-P}{C_i - C_a}\left(\frac{1}{1+a}\right)C_a + \frac{a\frac{C_i - C_a}{C_i}+1}{1+b} \quad \text{(A13)}$$

Subtracting one from each side, finding a common denominator, and rearranging leads to Eq. 2 of the main text.

Part 3: $\Delta_{P(f)}$

For discrimination between dark respiration and refixed photosynthate in the situation where R_a is independent of R_n, we again start with Eq. A8. However, rather than substitute for R_a from Eq. A6, we use the definition R_a/R_D=Δ_A+1, where Δ_A is the discrimination between atmospheric CO_2 and leaf photosynthesis. As noted in the main text, we assume no fractionation during dark respiration or translocation and that leaf-assimilated carbon forms the sole substrate for dark respiration. Making these assumptions allows one to equate R_D with R_A, the isotope ratio of leaf-assimilated carbon. Equation A5 can then be rewritten as

$$R_D D - R_P P = \frac{D-P}{C_i - C_a}\left(\frac{1}{1+a}\right)[R_P(1+b)C_i - R_D(1+\Delta_A)C_a] \quad \text{(A14)}$$

Isolating R_D/R_P on the left side of the equation yields

$$\frac{R_D}{R_P} = \frac{P + (D-P)\frac{C_i}{C_i - C_a}\left(\frac{1+b}{1+a}\right)}{D + (D-P)\frac{C_a}{C_i - C_a}\left(\frac{1+\Delta_A}{1+a}\right)} \quad \text{(A15)}$$

Subtracting one from each side of the equation yields

$$\frac{R_D}{R_P} - 1 = \frac{P + (D-P)\frac{C_i}{C_i - C_a}\left(\frac{1+b}{1+a}\right) - \left[D + (D-P)\frac{C_a}{C_i - C_a}\left(\frac{1+\Delta_A}{1+a}\right)\right]}{D + (D-P)\frac{C_a}{C_i - C_a}\left(\frac{1+\Delta_A}{1+a}\right)} \quad \text{(A16)}$$

Factoring 1/(1+a) from both the numerator and denominator leads to

$$\frac{R_D}{R_P} - 1 = \frac{P(1+a) + (D-P)\frac{C_i}{C_i - C_a}(1+b) - \left[D(1+a) + (D-P)\frac{C_a}{C_i - C_a}(1+\Delta_A)\right]}{D(1+a) + (D-P)\frac{C_a}{C_i - C_a}(1+\Delta_A)} \quad \text{(A17)}$$

Expanding and rearranging:

$$\frac{R_D}{R_P} - 1 = \frac{(D-P)\left(\frac{C_i}{C_i - C_a} - 1 - \frac{C_a}{C_i - C_a}\right) + (D-P)\left(b\frac{C_i}{C_i - C_a} - a - \Delta_A\frac{C_a}{C_i - C_a}\right)}{D + (D-P)\frac{C_a}{C_i - C_a} + Da + (D-P)\Delta_A\frac{C_a}{C_i - C_a}} \quad \text{(A18)}$$

The left-most term in the numerator is equal to zero, and thus drops out

$$\frac{R_D}{R_P} - 1 = \frac{(D-P)\left(b\frac{C_i}{C_i - C_a} - a - \Delta_A\frac{C_a}{C_i - C_a}\right)}{D + (D-P)\frac{C_a}{C_i - C_a} + Da + (D-P)\Delta_A\frac{C_a}{C_i - C_a}} \quad \text{(A19)}$$

Equation A19 is approximated to within 0.15‰ by Eq. 3 of the main text.

Part 4: δ_n

We begin by solving Eq. A5 for R_i:

$$R_i = R_n(1+a)\frac{C_i - C_a}{C_i} + R_a\frac{C_a}{C_i} \quad \text{(A20)}$$

Assuming steady state,

$$R_n F_n = R_D D - P\frac{R_i}{1+b} \quad \text{(A21)}$$

Combining Eqs. A20 and A21:

$$R_n F_n = R_D D - \frac{P}{1+b}\left[R_n(1+a)\frac{C_i - C_a}{C_i} + R_a\frac{C_a}{C_i}\right] \quad \text{(A22)}$$

Rearranging to isolate R_n on the left side of the equation:

$$R_n = \frac{R_D D - \left(\frac{P}{1+b}\right) R_a \frac{C_a}{C_i}}{F_n + P\left(\frac{1+a}{1+b}\right)\frac{C_i - C_a}{C_i}} \quad \text{(A23)}$$

Factoring $1/(1+b)$ from both the numerator and denominator yields

$$R_n = \frac{R_D D(1+b) - P R_a \frac{C_a}{C_i}}{F_n(1+b) + P(1+a)\frac{C_i - C_a}{C_i}} \quad \text{(A24)}$$

Dividing both sides of the equation by R_S, the carbon isotope ratio of some standard, and substituting from the equation $\delta_X = R_X/R_S - 1$:

$$\delta_n + 1 = \frac{D(\delta_D + 1)(1+b) - P(\delta_a + 1)\frac{C_a}{C_i}}{F_n(1+b) + P(1+a)\frac{C_i - C_a}{C_i}} \quad \text{(A25)}$$

Expanding and replacing F_n with D-P:

$$\delta_n + 1 = \frac{D(\delta_D + b + \delta_D b + 1) - P\delta_a \frac{C_a}{C_i} - P\frac{C_a}{C_i}}{D - P\frac{C_a}{C_i} + (D-P)b + Pa\frac{C_i - C_a}{C_i}} \quad \text{(A26)}$$

Subtracting one from both sides of the equation yields

$$\delta_n = \frac{D\delta_D + D\delta_D b - P\delta_a \frac{C_a}{C_i} + Pb - Pa\frac{C_i - C_a}{C_i}}{D - P\frac{C_a}{C_i} + (D-P)b + Pa\frac{C_i - C_a}{C_i}} \quad \text{(A27)}$$

which can be rearranged to give Eq. 4 of the main text.

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