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Carbon isotope discrimination during photosynthesis and the isotope ratio of respired CO_2 in boreal forest ecosystems

Lawrence B. Flanagan,[1] J. Renee Brooks,[2] Gregory T. Varney,[1] Stephanie C. Berry,[1] and James R. Ehleringer[2]

Abstract. Our objective was to measure the carbon isotope ratio of CO_2 released by respiration (δ_r) within forest canopies at different times during the growing season and to use this information to estimate forest ecosystem carbon isotope discrimination. We made measurements in the three major forest types (black spruce, jack pine, and aspen) at the southern and northern ends of the boreal forest in central Canada. This research was part of a larger study, the Boreal Ecosystem-Atmosphere Study (BOREAS). The δ_r values, calculated from measurements of change in the concentration and carbon isotope ratio of atmospheric CO_2 in air samples collected at night, ranged from -28.1‰ to -25.9‰ with an average (± s.d.) of -26.8‰ ± 0.5‰. There was good correlation between calculated δ_r values and measurements of (1) the carbon isotope ratio of CO_2 released directly from the soil and (2) the $\delta^{13}C$ values of foliage collected from the dominant tree species at each site. Carbon isotope discrimination during photosynthetic gas exchange (Δ_A) by each forest ecosystem was estimated as the difference between the carbon isotope ratio of atmospheric CO_2 at the top of the canopy (δ_a) and the isotopic composition of respired CO_2: $\Delta_A = \delta_a - \delta_r$. All three of the major forest types had similar values of Δ_A, with an average (± s.d.) of 19.1‰ ± 0.5‰. However, a seasonal change in forest discrimination was observed for aspen forests in both the northern and southern study areas, with an increase in Δ_A occurring between the middle and end of the growing season. In contrast, the evergreen conifer canopies exhibited relatively constant discrimination values throughout the active growing season.

1. Introduction

Current models of the global carbon budget indicate an imbalance between sources and sinks of carbon dioxide, since not all of the CO_2 released into the atmosphere by fossil fuel combustion and land use changes can be accounted for [*Sundquist,* 1993]. In addition, there is controversy about the relative roles of the oceans and terrestrial biosphere as net sinks for anthropogenic CO_2 emissions [*Sundquist*, 1993]. Measurements of the stable isotope ratio of atmospheric CO_2 can provide information relevant to resolving these uncertainties in the global carbon budget, since CO_2 exchange processes that occur between ocean and atmosphere and between terrestrial vegetation and atmosphere have very different associated isotope effects [*Keeling et al.*, 1989; *Farquhar et al.*, 1993]. During photosynthesis plants preferentially assimilate $^{12}CO_2$ and discriminate against $^{13}CO_2$ [*Farquhar et al.,* 1989], while there is only a small isotopic change associated with dissolution of CO_2 into the ocean [*Tans et al.*, 1993]. A consequence of the preferential accumulation of ^{12}C in plant tissues is that ^{13}C becomes enriched in atmospheric CO_2 during net uptake of carbon dioxide by terrestrial ecosystems. By monitoring shifts in the concentration and stable isotope ratio of atmospheric CO_2 over time, it is possible to partition uptake due to ocean processes and that due to terrestrial processes [*Keeling et al.*, 1989; *Ciais et al.*, 1995; *Francey et al.*, 1995]. This application requires, however, knowledge of temporal and spatial variation in carbon isotope discrimination that takes place during terrestrial biosphere-atmosphere CO_2 exchange.

There is a great deal of variation in carbon isotope discrimination among plants in different terrestrial ecosystems, as a result of differences in photosynthetic pathway, genotypic variation and environmental influences [*Farquhar et al.*, 1989; *Lloyd and Farquhar*, 1994]. Detailed mechanistic models have been developed that successfully explain the isotope effects that occur during photosynthetic gas exchange at the leaf level [*Farquhar et al.*, 1989; *Ehleringer et al.*, 1993]. It is complicated, however, to relate these leaf-level models to canopy and forest ecosystem processes because of the heterogeneity in environmental conditions and variation in source CO_2 that can occur within forest canopies [*Broadmeadow and Griffiths*, 1993].

Keeling [1958, 1961] was the first to study ecosystem isotope discrimination, using a simple technique that was based on diurnal measurements of the concentration and isotopic composition of atmospheric CO_2 near a plant canopy. When photosynthetic CO_2 uptake exceeds respiration during the daytime, CO_2 concentrations are reduced, and the atmospheric ^{13}C content becomes slightly enriched because of isotopic discrimination by the vegetation. In contrast, when respiration dominates at night, atmospheric CO_2 concentration is increased and ^{13}C content is depleted. The CO_2 released from soils and plants at night through respiration and decomposition is depleted in ^{13}C because the original carbon fixed by plants is depleted in ^{13}C relative to atmospheric CO_2. If it is assumed that the CO_2 in the vegetation canopy is primarily a result of mixing between two sources, the atmosphere above the canopy and the CO_2 respired by plants and soils, it is possible to use a mass balance approach to calculate the isotope ratio of CO_2 respired into the forest

[1]Department of Biology, Carleton University, Ottawa, Ontario, Canada.

[2]Department of Biology, Stable Isotope Ratio Facility for Environmental Research, University of Utah, Salt Lake City.

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during diurnal cycles. Since no significant isotope effect occurs during respiration [*Farquhar et al.*, 1989], the $^{13}C/^{12}C$ ratio of respired CO_2 should represent an integrated measure of the isotopic composition of plant tissue in the entire forest system. The temporal component integrated by this measurement of the ^{13}C content of respired CO_2 depends, however, on the relative contribution that differently aged carbon pools make to total system respiration, and the difference in isotopic composition among carbon pools with different turnover times [*Ciais et al.*, 1995].

Our objective in this study was to measure the isotopic composition of CO_2 released by respiration within forest canopies at different times during the growing season and to use this information to estimate forest ecosystem isotopic discrimination. We made measurements in the three major forest types in the boreal forest of Canada, mature black spruce, jack pine and aspen dominated forests at two locations at the southern and northern limits of the boreal forest in central Canada. This research was part of a larger study, the Boreal Ecosystem-Atmosphere Study (BOREAS), that aims to improve our understanding of the interactions between the atmosphere and the boreal forest, a globally important biome [*Sellers et al.*, 1995].

2. Materials and Methods

2.1. Study Sites

Two study areas were located at the northern and southern limits of the boreal forest in central Canada. The southern study area (SSA) was located 40 km north of Prince Albert, Saskatchewan and covered an area 130 km wide by 90 km. The northern study area (NSA) was 100 km by 80 km and included the town of Thompson, Manitoba. We conducted our research in both SSA and NSA in forests dominated by each of the following species: *Picea mariana* (black spruce), *Pinus banksiana* (jack pine), and *Populus tremuloides* (aspen).

In the southern study area the *Picea mariana* site (53.99° N, 105.12° W) occurred on poorly drained clay-sand soil with a continuous coverage of feather moss (predominantly *Pleurozium schreberi*) and *Sphagnum* moss. The forest was approximately 140 years old with 4300 stems per hectare. Average tree height was 12 m with a leaf area index of approximately 6.2 (S.T. Gower, unpublished data, 1996). The *Pinus banksiana* site (53.92° N, 104.69° W) occurred on a well-drained, sandy soil. Stand age was approximately 60-75 years, and stand density was approximately 1300 trees per hectare. Tree heights ranged from 11-15 m with a leaf area index of 1.4 (S.T. Gower, unpublished data, 1996). *Alnus crispa* (Ait.) Pursh was the dominant shrub in the understory, and the ground cover was largely lichens and feather moss. The *Populus tremuloides* site (53.63° N,106.20° W) occurred on a well-drained clay-loam soil. Stand age was approximately 60 years, with stand density close to 900 trees per hectare. Tree heights ranged from 12-20 m with a leaf area index of 3.0 (S.T. Gower, unpublished data, 1996). The understory was dominated by *Corylus cornuta* Marsh. and *Rosa woodsii* Lindl.

In the northern study area, the *Picea mariana* site (55.91° N, 98.52° W) occurred in an upland area with poorly drained clay soils. This stand was younger (approximately 50 years) and more productive than its southern counterpart. Tree heights ranged from 6-12 m, with stand density of 9300 trees/hectare and a leaf area index of 8.4 (S.T. Gower, unpublished data, 1996). Similar to the southern *Picea mariana* site, the ground was covered with a deep layer of feather moss. The northern *Pinus banksiana* site (55.93° N, 98.62° W) was similar to the southern pine site, both forests occurring on sandy, well-drained soils with an understory of *Alnus crispa* and a lichen ground cover. The northern stand was younger (40-60 years old)) and more dense (2000-3000 tree per hectare), and the trees were shorter (8-11 m) than the jack pine site in the SSA, but leaf area index was similar. The northern *Populus tremuloides* site (55.89° N, 98.68° W) was approximately 60 years of age, with a dense understory of *Alnus crispa* and a leaf area index of 2.3 (S. T. Gower, unpublished data, 1996). Tree heights ranged from 7-18 m, and stand density was approximately 2000 trees/hectare in the site.

Field measurements were made during three intensive field campaigns during the 1994 growing season. The first campaign (spring) was from May 24 through June 12, just at bud break for both conifers and early leaf out for *Populus tremuloides*. The second campaign (summer) was at the peak of the growing season, between July 26 and August 8. The third study period (fall) was at the onset of dormancy from August 30 to September 15.

2.2. Air Sample Collection

At each site we collected samples of air within the forest canopy at intervals during a 2-3 day period on three separate dates during the summer of 1994. Sample lines (Bev-a-line or Dekoron tubing, 6-mm outer diameter, Warehoused Plastic Sales, Toronto, Ontario, Canada) were located at different heights in the canopy (9 m and 0.5 m) by attachment to a 9-m mast (Rohn E20 telescoping mast). An inverted funnel was connected to the inlet to prevent water from entering the tubing, and a small filter was placed over the inlet to prevent the entry of insects. Air was pulled down through the tubing, through a desiccant tube (6200DP, Li-Cor, Lincoln, Nebraska) containing magnesium perchlorate, and into glass flasks (either 1.7 or 2.0 L) by a battery-operated pump (TD-4N pump, Brailsford and Co. Inc., Rye, New York) on the ground approximately 10 m away from the mast. The CO_2 concentration of the air was measured using an infrared gas analyzer (LI-6250 CO_2 analyzer, Li-Cor, Lincoln, Nebraska). Air was passed through the flasks for approximately 20 min before a CO_2 concentration measurement was recorded and the high vacuum stopcocks on the flask were closed. The flask was then returned to a lab for cryogenic extraction of the CO_2.

In order to extract CO_2, air sample flasks were attached to a stainless steel vacuum line that consisted of two ethanol-dry ice traps to remove water vapor and two liquid nitrogen traps to collect CO_2. After evacuating the vacuum line to less than 10^{-3} torr, the vacuum pump (E2M8 rotary pump, Edwards High Vacuum, Burlington, Ontario, Canada) was isolated from the line by closing a valve, and a stopcock on a sample flask was opened to allow the sample gases to enter the vacuum line. The needle valve isolating the pump from the vacuum line was then opened slightly to slowly remove the incondensable gases. After the vacuum returned to 10^{-3} torr, the two traps containing the CO_2 were isolated by closing toggle valves. The purified CO_2 was released by warming the traps to the temperature of an ethanol-dry ice bath, and the CO_2 was transfered to a tube, connected to a sidearm of the vacuum line, and sealed with a torch. The flame-sealed tubes were stored until stable isotope analysis was performed. The carbon dioxide from forest air samples were analyzed on a gas isotope ratio mass spectrometer (Sira 12, VG Isotech, Middlewich, Cheshire, United Kingdom) at the Ottawa-Carleton Stable Isotope Facility.

2.3. Isotope Ratio of CO_2 Respired From the Soil

A Li-Cor soil respiration system (Model 6299) was used to collect CO_2 respired from the ground surface for isotopic analysis. The respiration chamber was clamped on top of polyvinyl chloride collars (area, 71 cm^2, and depth, 13 or 23 cm) that were installed in the ground at a site the day before measurements were made. In this application, a water trap and a glass flask were connected to the air line exiting the respiration chamber (thus maintaining the closed loop of the system). The glass flask had been previously filled with pure nitrogen gas. After connecting the glass flask, the respiration system was scrubbed of carbon dioxide by passing air exiting the chamber through a column of soda lime. Using this technique, it was possible to reduce the CO_2 concentration in the chamber and sample loop to approximately 20-50 μmol mol^{-1}. The soda lime was then switched out of the system, and the CO_2 concentration was allowed to rise back up to the concentration that was apparent before the start of the collection procedure (approximately 360 μmol mol^{-1}). The high vacuum stopcocks on the sample flask were then closed, and the flask was returned to a lab for cryogenic extraction of the CO_2 and subsequent isotopic analysis.

At the southern jack pine site, we also collected soil CO_2 at several different depths during September 1994 for isotopic analysis. Soil CO_2 was collected by slowly opening the stopcock on an evacuated glass flask that was connected to stainless steel tubes inserted at 20, 40, 60, and 90 cm depth. The flask was then returned to the lab for cryogenic extraction of the CO_2.

2.4. Leaf and Soil Organic Matter Collection

Mature aspen leaves and current-season and one-year-old conifer foliage samples were collected from the dominant trees at all sites during each study period. Foliage was dried at 65°C and ground to a fine powder with a tissue grinder or a mortar and pestle.

The leaf organic tissue samples were prepared for measurements of carbon isotopic composition by combustion. A 1-2 mg subsample of ground leaf tissue was sealed in a tin capsule and loaded into an elemental analyzer for combustion (Carla Erba). The carbon dioxide generated from the combustion was purified cryogenically and passed directly to the inlet of a gas isotope ratio mass spectrometer (Delta S, Finnigan Mat, San Jose, California) at the University of Utah.

Samples of soil, excluding live plant material and undecomposed litter (recognizable brown plant material), were collected from the three sites in the southern study area. Samples were separated into 0-10 cm and 10-20 cm depth increments and dried in an oven at 60°C for 24 hours. Roots were removed from the samples, and the soil was ground to a fine powder with a mortar and pestle. Carbonates were removed from soil samples prior to stable isotope analysis by incubating 25 g of soil with 0.1 *M* HCl for 48 hours at room temperature. The soil samples were then washed five times with pure water, and excess water was removed by filtration. Aftering drying at 65°C, the samples were finely ground with a mortar and pestle, and approximately 20 mg were sealed in an evacuated quartz tube with cupric oxide wire and silver foil. The tubes were heated to 850°C for 6 hours followed by a 8-9 hour period of cooling to room temperature. The carbon dioxide generated from the combustion was purified cryogenically and analyzed on a SIRA 12 mass spectrometer in Ottawa.

2.5. Isotopic Analysis

Isotope ratios in delta notation are calculated as

$$\delta = \left[\frac{R_{sample}}{R_{standard}} - 1 \right] \quad (1)$$

where R is the molar ratio of heavy to light isotope ($^{13}C/^{12}C$), the international standard for atmospheric CO_2 samples and leaf organic samples is CO_2 from Pee Dee Belemnite limestone [*Ehleringer and Osmond,* 1989]. The δ values are conveniently presented in parts per thousand (‰).

Isotopic ratios in atmospheric CO_2 samples were corrected for the presence of N_2O using the procedure described by *Friedli and Siegenthaler* [1988]. This procedure required determining the mass spectrometer sensitivity to N_2O by measuring pure N_2O, pure CO_2, and CO_2-N_2O gas mixtures. We mixed different ratios of N_2O and CO_2 in a vacuum line with a calibrated volume and a pressure transducer. By measuring the isotopic compositions of the N_2O-CO_2 mixtures, we determined that the corrections for our mass spectrometer were similar to those reported by *Mook and van der Hoek* [1983] and *Friedli and Siegenthaler* [1988]:

$$\delta^{13}C_{correct} = \delta^{13}C_{measured} + \rho\ 256‰ \quad (2)$$

$$\delta^{18}O_{correct} = \delta^{18}O_{measured} + \rho\ 385‰ \quad (3)$$

where ρ is the N_2O/CO_2 concentration ratio. Measurements of the mass ratio 30:28, in addition to normal mass ratios 45:44 and 46:44, were used to estimate the N_2O/CO_2 concentration ratio (ρ) on gas samples purified from forest air, as described by *Friedli and Siegenthaler* [1988]. The average corrections applied to the $\delta^{13}C$ and $\delta^{18}O$ values were +0.19‰ (s.d. = 0.03) and +0.29‰ (s.d. = 0.04), respectively.

We used a simple mixing model developed by *Keeling* [1958, 1961] to calculate the isotope ratio of CO_2 respired by the forest system:

$$\delta_f = \frac{[CO_2]_a}{[CO_2]_f} (\delta_a - \delta_r) + \delta_r \quad (4)$$

where $[CO_2]$ is concentration of CO_2 and δ is the stable isotope ratio of CO_2, and the subscripts f and a represent the atmosphere within the forest and the atmosphere above the forest, respectively; δ_r is the isotope ratio of CO_2 respired by plants and soil. It can be seen from (4) that a plot of $1/[CO_2]_f$ and δ_f gives a straight line relationship with slope, $[CO_2]_a (\delta_a - \delta_r)$, and an intercept δ_r. We used this model to estimate values for δ_r, the isotopic composition of CO_2 respired by plants and soil. Estimates for δ_r were obtained from the y intercept of a geometric mean linear regression [*Sokal and Rohlf,* 1981] between $\delta^{13}C$ and $1/[CO_2]$ values measured on air samples collected at two heights in the forest canopy during the night when photosynthesis was not active [*Keeling,* 1958, 1961]. The geometric mean regression is a model II regression that should be implemented when both variables used in the regression have a measurement error associated with them and are not controlled by the investigator [*Sokal and Rohlf,* 1981]. Our estimates of δ_r from the regression technique were

compared to the isotope ratio of CO_2 respired directly from the soil surface (CO_2 collected with the Li-Cor respiration chamber) and to the $\delta^{13}C$ values of leaf tissue and soil organic material.

The CO_2 concentration $[CO_2]_a$ and carbon isotope ratio δ_a of the atmosphere above the forest were estimated in both northern and southern study areas during each field campaign from measurements made on flasks collected at 9 m in black spruce and jack pine sites during midday (10:30 -14:30), when conditions were turbulent. Our estimates of $[CO_2]_a$ and δ_a were compared to measurements made on a limited number of samples that were collected at higher altitude by the National Research Council of Canada Twin Otter flux aircraft. Air samples collected by the Twin Otter were pumped into 6.5 L bags made of Curlam 9300 (Curwood Inc., New London, Wisconsin). The opening to the bag was equipped with a double end shut-off, quick-connect fitting (QC Series, Swagelok, Ottawa Valve and Fitting, Ottawa, Ontario, Canada).

3. Results

There were large changes in CO_2 concentration and $\delta^{13}C$ values observed during diurnal time courses on most sample dates (Figures. 1-6). When CO_2 concentration in the forest increased at night, the $\delta^{13}C$ values declined. There were increases in the $\delta^{13}C$ values associated with decreases in CO_2 concentration during the day. In addition, there was a strong vertical stratification of CO_2 concentration and isotopic composition, with higher concentrations and lower $\delta^{13}C$ values observed closer to the soil surface. (Figures. 1-6). These patterns were consistent in all forests, with the extent of the changes in CO_2 concentration and $\delta^{13}C$ value being dependent on weather conditions (through effects on photosynthesis and respiration) and the extent of turbulent exchange between the forest and the atmosphere affecting the amount of CO_2 storage in the canopy at night.

There was a decline in the CO_2 concentration and an increase in the $\delta^{13}C$ value in air samples collected near the top of the forest canopy during the transition from spring to summer (Table 1). The concentration and isotopic composition of CO_2 remained quite constant, however, between the summer and fall study periods. For comparison, two air samples collected by the NRC Twin Otter during the spring campaign at approximately 1850 m over the SSA had $\delta^{13}C$ values of -8.31‰ and -8.46‰. These aircraft-collected samples had values more negative (by approximately 0.3‰) than those measured at 9 m above ground, likely because the air sampling vessels used on the plane were not equipped with high-vacuum stopcocks and so were not as leak proof as the glass flasks used in the ground sampling. Observations made by the National Oceanic and Atmospheric Administration Climate Monitoring and Diagnostics Laboratory global air sampling network also showed a decline in CO_2 concentration and an increase in the $\delta^{13}C$ value in air samples during the transition from spring to summer. Samples collected at the Mould Bay station (latitude 76°14' N, longitude 119°20' W; 57.6 m above mean sea level) during the summer of 1994 showed the following monthly mean values for background tropospheric CO_2 mixing ratios (μmol mol^{-1}) and $\delta^{13}C$ values (parts per thousand): April, 364.0 and -8.26; May, 363.9 and -8.24; June, 362.2 and -8.20; July, 356.5 and -7.81; August, 350.2 and -7.50; September, 350.3 and -7.55; respectively. We feel justified in using the values in Table 1 as estimates for the atmosphere above the forest canopy because comparisons of our CO_2 concentration measurements made at 9 m and simultaneous measurements made at 24 m above ground were virtually identical. The CO_2 concentration measurements at 24 m were made by P. Jarvis and colleagues in association with their BOREAS eddy covariance flux studies.

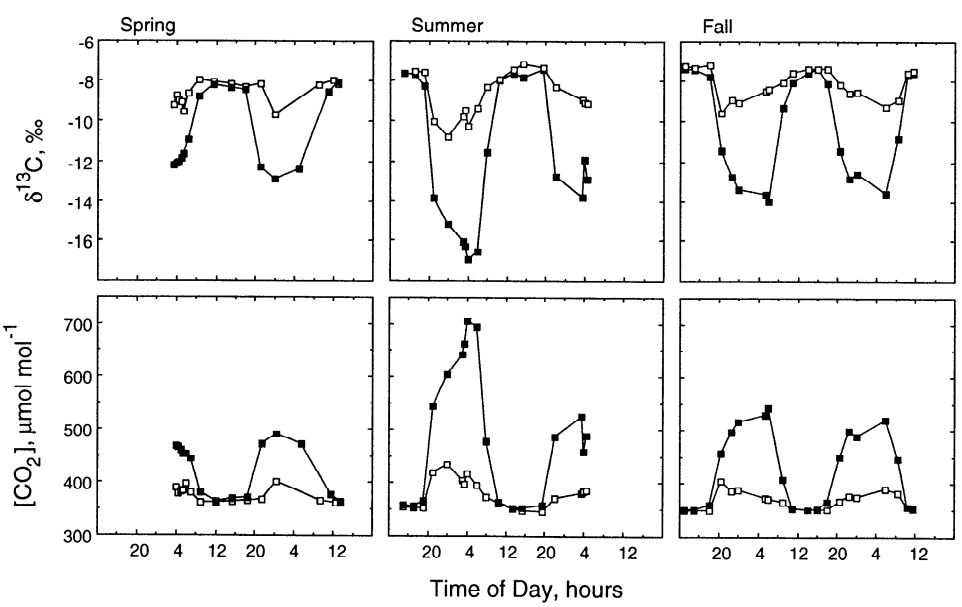

Figure 1. Diurnal variation in the concentration and carbon isotope ratio of CO_2 in air samples collected within the canopy of a black spruce (*Picea mariana*) forest in the Boreal Ecosystem-Atmosphere Study (BOREAS) southern study area during 1994. Air samples where collected at two heights within the canopy, 9 m (open squares) and 0.5 m (solid squares) above ground.

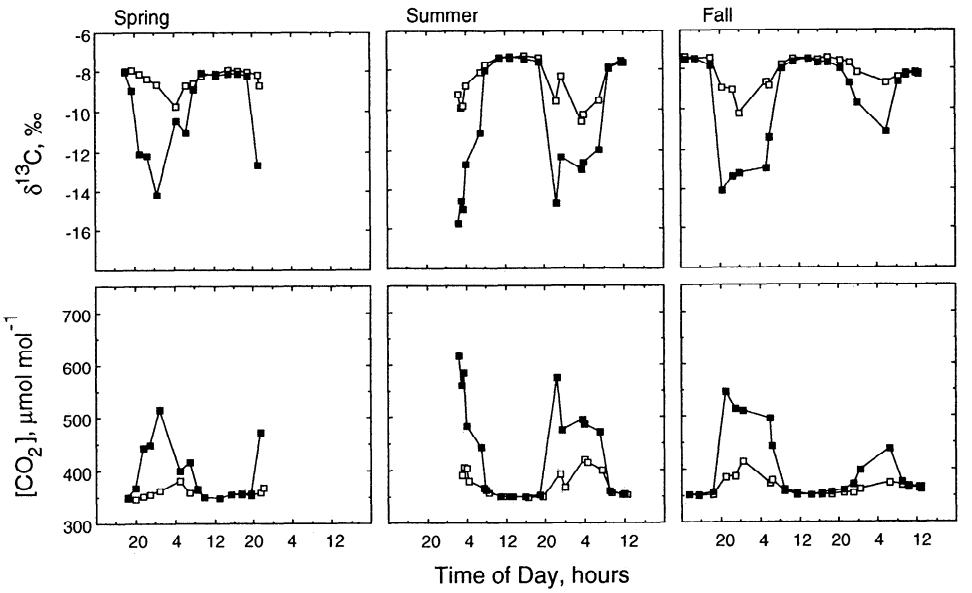

Figure 2. Diurnal variation in the concentration and carbon isotope ratio of CO_2 in air samples collected within the canopy of a jack pine (*Pinus banksiana*) forest in the BOREAS southern study area during 1994. Air samples where collected at two heights within the canopy, 9 m (open squares) and 0.5 m (solid squares) above ground.

Strong linear relationships occurred between CO_2 concentration and $\delta^{13}C$ values measured on air samples collected at night (Figures. 7 and 8). The values calculated for δ_r using the linear regression between $1/[CO_2]$ and $\delta^{13}C$ values ranged from -28.1‰ to -25.9‰ (Table 2). There was a consistent pattern of seasonal increase in the calculated δ_r value for the conifer-dominated sites, while the two aspen-dominated sites showed an increase from spring to summer and then a decline at the end of the active growing season. There was a good correlation between δ_r values estimated with the linear regression technique and the isotopic compositions of (1) CO_2 released from the soil and (2) soil organic matter for the southern study sites (Table 3). In addition, the carbon isotope ratio of soil CO_2 at the southern jack pine site in September 1994 (20 cm depth, -18.7‰; 40 cm, -19.4‰; 60 cm, -19.61‰; 90 cm, -19.91‰; and 200 cm, -20.24 ‰) was on average enriched in ^{13}C by approximately 3.6‰ relative to CO_2 released from the surface (-23.16‰), which is close to the theoretical value of 4.4‰ that was expected because of

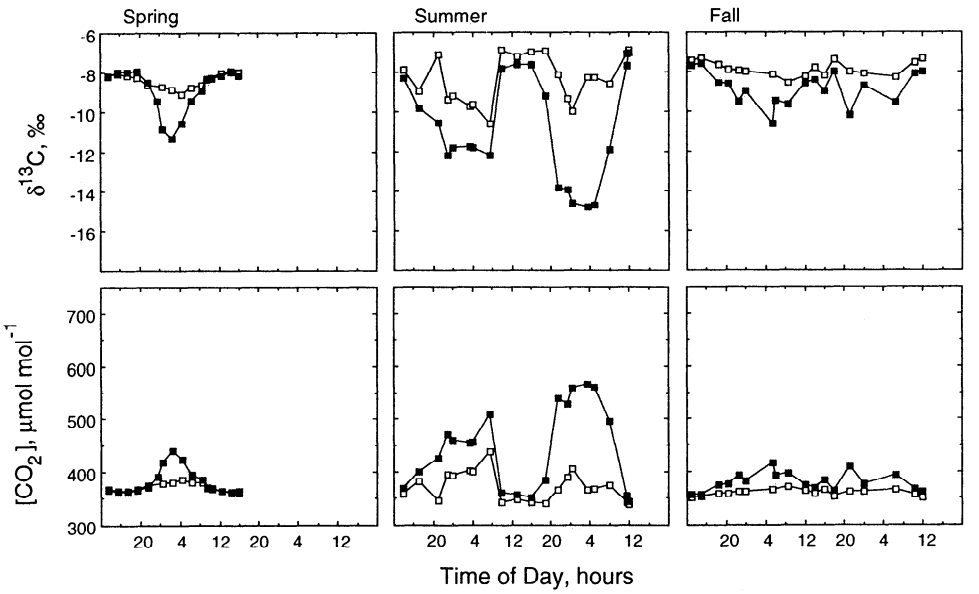

Figure 3. Diurnal variation in the concentration and carbon isotope ratio of CO_2 in air samples collected within the canopy of an aspen (*Populus tremuloides*) forest in the BOREAS southern study area during 1994. Air samples where collected at two heights within the canopy, 9 m (open squares) and 0.5 m (solid squares) above ground.

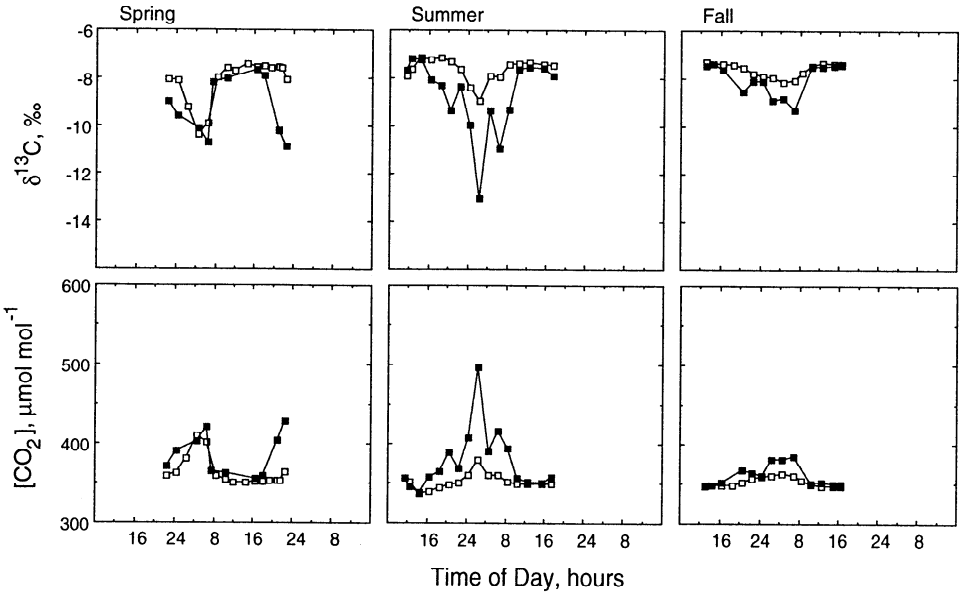

Figure 4. Diurnal variation in the concentration and carbon isotope ratio of CO_2 in air samples collected within the canopy of a black spruce (*Picea mariana*) forest in the BOREAS northern study area during 1994. Air samples where collected at two heights within the canopy, 9 m (open squares) and 0.5 m (solid squares) above ground.

fractionation that occurs during diffusion of CO_2 through the soil [*Dorr and Munnich*, 1980; *Cerling et al.*, 1991]. The values calculated for δ_r using the linear regression technique were also comparable to foliage $\delta^{13}C$ values measured on the dominant tree species at each site (Table 4) but differed from the very depleted $\delta^{13}C$ values measured on the understory herbs and mosses (J.R. Brooks et al., unpublished data, 1996).

In total, these observations provide strong support that the y intercept, from the regression of $1/[CO_2]$ and $\delta^{13}C$ values of forest air, provides an integrated measure of the isotopic composition of CO_2 respired from plants and soil within the footprint of the sampling mast. Assuming smooth surface conditions, air samples collected at 9 m are primarily affected by sources at an upwind distance of approximately 140 m

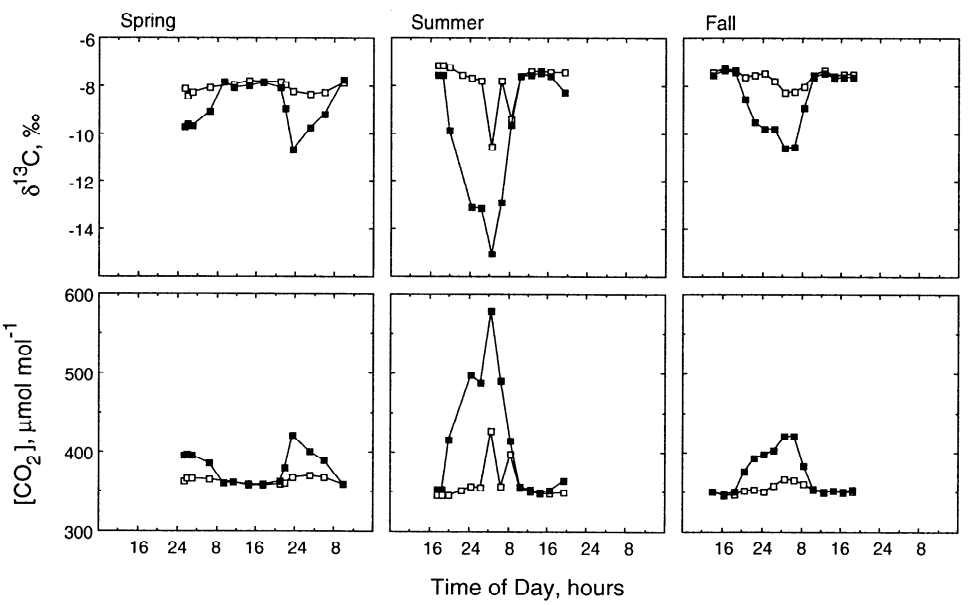

Figure 5. Diurnal variation in the concentration and carbon isotope ratio of CO_2 in air samples collected within the canopy of a jack pine (*Pinus banksiana*) forest in the BOREAS northern study area during 1994. Air samples where collected at two heights within the canopy, 9 m (open squares) and 0.5 m (solid squares) above ground.

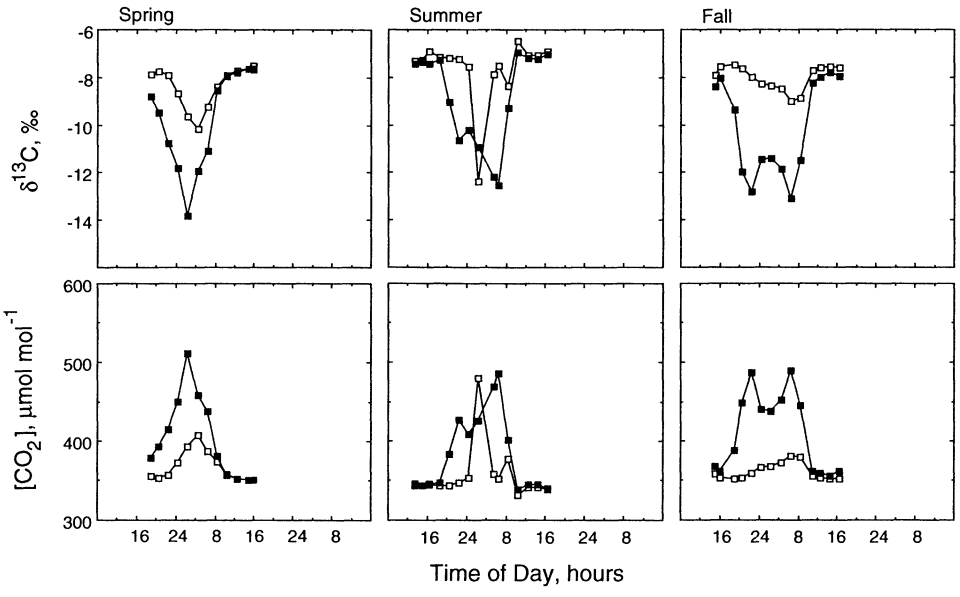

Figure 6. Diurnal variation in the concentration and carbon isotope ratio of CO_2 in air samples collected within the canopy of an aspen (*Populus tremuloides*) forest in the BOREAS northern study area during 1994. Air samples where collected at two heights within the canopy, 9 m (open squares) and 0.5 m (solid squares) above ground.

[*Schuepp et al.*, 1990], although the true distance is likely much smaller due to the roughness created by the forest canopy. The air samples collected at 0.5 m are affected primarily by sources in the close vicinity of the sample inlet.

It is possible to estimate discrimination during photosynthetic gas exchange by the whole forest system from diurnal changes in CO_2 concentration and $\delta^{13}C$ values of atmospheric CO_2 within forest canopies. This forest discrimination estimate was calculated by assuming that no isotope effect occurs during respiration by plants and soils, so that the isotope ratio of CO_2 respired by the forest system (δ_r) represents an integrated measure of the isotopic composition of forest organic matter. Forest carbon isotope discrimination was then calculated as $\Delta_A = \delta_a - \delta_r$ [*Lloyd and Farquhar*, 1994], where the δ_a and δ_r values that were used in the calculations are shown in Tables 1 and 2, respectively. The spatial area integrated by the calculation depends on the footprint of the air sample mast, while the temporal component integrated depends on the relative contribution that different

Table 1. Comparison of the Concentration and Carbon Isotope Ratio of Carbon Dioxide in Air Samples Collected in the BOREAS Study Areas During 1994

	Spring	Summer	Fall
	Northern Study Area		
$[CO_2]$, µmol mol^{-1}	359.2 ± 1.6	350.6 ± 2.7	349.4 ± 2.3
$\delta^{13}C$, ‰	-7.88 ± 0.08	-7.51 ± 0.20	-7.44± 0.10
$\delta^{18}O$, ‰	+0.39 ± 0.38	-0.72 ± 0.33	-1.63 ± 0.62
n	3	7	8
Julian Days	154-162	208-213	245-249
	Southern Study Area		
$[CO_2]$, µmol mol^{-1}	362.0 ± 1.1	351.8 ± 2.0	351.5 ± 2.0
$\delta^{13}C$, ‰	-8.09 ± 0.10	-7.48 ± 0.09	-7.45 ± 0.10
$\delta^{18}O$, ‰	-0.10 ± 0.32	-0.89 ± 0.81	-1.27 ± 0.28
n	4	5	7
Julian Days	146-150	202-209	244-249

Samples were collected at midday (10:30-14:30, local time) at 9 m above ground in jack pine and black spruce sites.

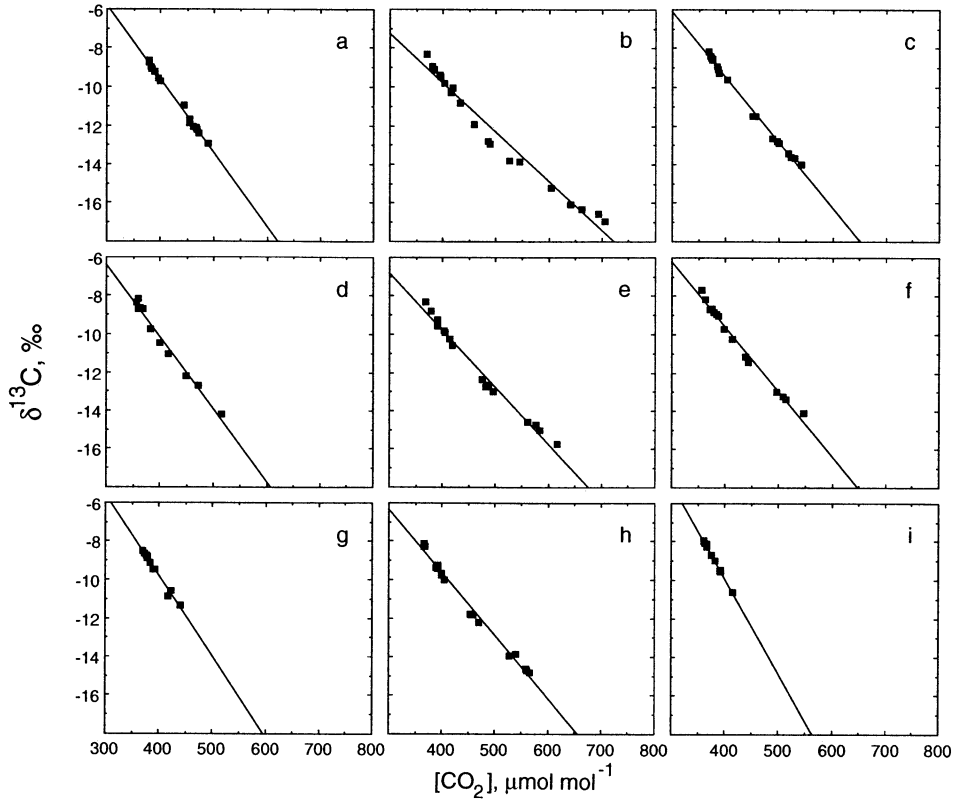

Figure 7. Comparison of the relationships between CO_2 concentration and the carbon isotope ratio of CO_2 in air samples collected at night in the BOREAS southern study area during 1994. The black spruce site during (a) spring, (b) summer and (c) fall sampling periods. The jack pine site during (d) spring, (e) summer, and (f) fall sampling periods. The aspen site during (g) spring, (h) summer, and (i) fall sampling periods.

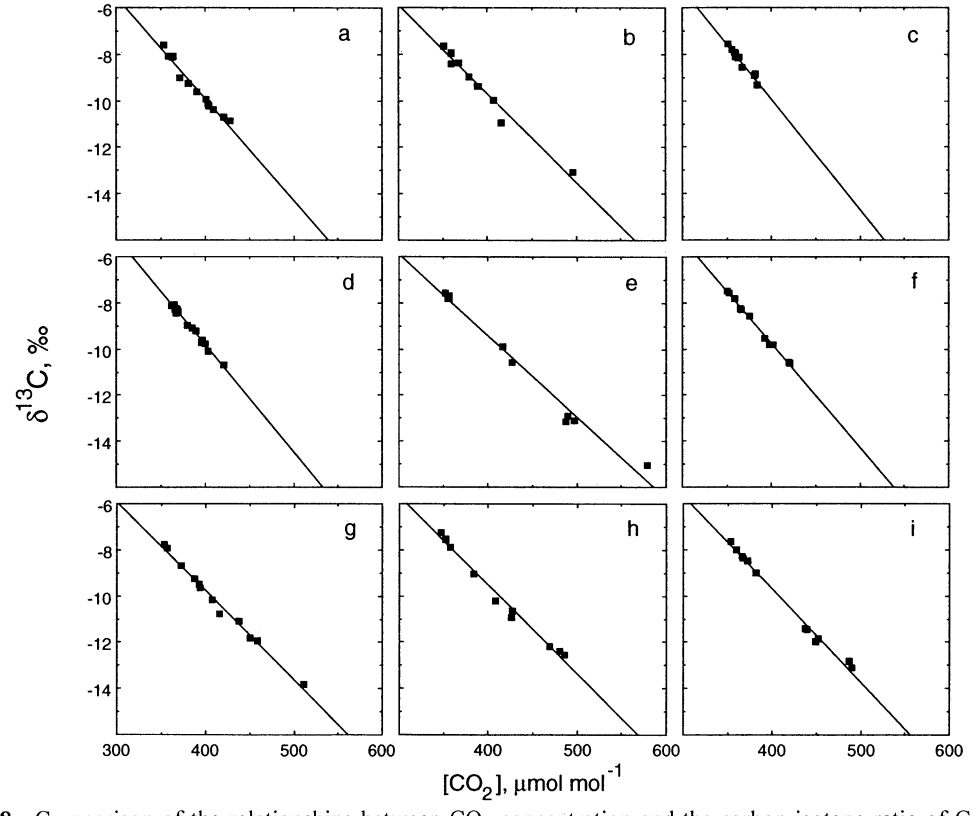

Figure 8. Comparison of the relationships between CO_2 concentration and the carbon isotope ratio of CO_2 in air samples collected at night in the BOREAS northern study area during 1994. The black spruce site during (a) spring, (b) summer and (c) fall sampling periods. The jack pine site during (d) spring, (e) summer, and (f) fall sampling periods. The aspen site during (g) spring, (h) summer, and (i) fall sampling periods.

Table 2. The Isotopic Composition of CO_2 Respired by Plants and Soil (δ_r Values), Calculated Using a Linear Regression Technique

	Spring	Summer	Fall
	Northern Study Area		
Black spruce	-26.74 ± 0.75 (0.968)	-26.63 ± 0.71 (0.971)	-26.38 ± 1.16 (0.957)
Jack pine	-27.23 ± 0.50 (0.989)	-26.79 ± 0.49 (0.984)	-26.48 ± 0.32 (0.996)
Aspen	-26.94 ± 0.43 (0.989)	-25.94 ± 0.43 (0.986)	-27.23 ± 0.25 (0.991)
	Southern Study Area		
Black spruce	-26.99 ± 0.41 (0.992)	-26.32 ± 0.17 (0.972)	-26.14 ± 0.15 (0.996)
Jack pine	-27.23 ± 0.48 (0.982)	-26.55 ± 0.15 (0.982)	-26.16 ± 0.17 (0.988)
Aspen	-27.06 ± 0.78 (0.979)	-26.80 ± 0.17 (0.989)	-28.15 ± 0.43 (0.998)

Values are expressed in parts per thousand (‰) ± SE. Also shown (in parentheses) is the proportion of the variation in the data accounted for by the linear regression (r^2 value).

aged carbon pools make to total system respiration (and to δ_r). If the majority of respired CO_2 comes from recently fixed carbon (within days), the calculated forest Δ_A could be expected to show seasonal changes associated with environmental influences on leaf-level gas exchange and isotopic discrimination.

Our data illustrate a seasonal change in discrimination in the aspen-dominated sites, with an increase in Δ_A occurring between the middle and the end of the growing season, possibly because of a reduction in photosynthetic capacity relative to stomatal conductance associated with leaf senescence (Figure 9). A decline in temperature and vapor pressure deficit experienced by leaves would also have contributed to a reduction in stomatal limitation of photosynthesis (and resulted in an increase in discrimination) in the aspen leaves during the fall. In contrast, the evergreen conifer canopies exhibited relatively constant discrimination values throughout the active growing season. The observed forest Δ_A values were consistent with observations of the sun foliage $\delta^{13}C$ values of the dominant tree species (Table 4).

4. Discussion

Analysis of net partitioning of CO_2 between ocean and terrestrial ecosystems using stable isotope techniques, is dependent on knowledge of carbon isotope discrimination during photosynthetic gas exchange in terrestrial ecosystems, and the isotope ratio of CO_2 released from ecosystem carbon pools [*Ciais et al.*, 1995; *Fung*, 1995]. Our data illustrate that the three main types of mature forest in the boreal forest biome influence the carbon isotope ratio of atmospheric CO_2 in similar manners. Measurements of total system carbon isotope discrimination averaged 19.1‰ ± 0.5‰, which is slightly higher than values calculated from δ_r measurements made by *Lancaster* [1990] in a lodgepole pine forest at Rock Lake, Alberta, Canada (δ_r = -25.3±0.4‰, approximately Δ_A = 17.9‰) and a large-scale modeling study estimate for boreal latitudes (approximately Δ_A = 17.5‰ [*Ciais et al.*, 1995]). Our total system isotopic discrimination values are also slightly higher than values predicted for boreal regions by the global model of *Lloyd and Farquhar* [1994] (northern black spruce forest, Δ_A

Table 3. Comparison of the Carbon Isotope Ratio ($\delta^{13}C$, ‰) of Carbon Dioxide Respired From the Soil Surface and Soil Organic Matter From Forest Sites in the Southern BOREAS Study Area During 1994

Site	Soil Respired Carbon Dioxide		Soil Organic Matter	
	Summer	Fall	0-10 cm	10-20 cm
Spruce	-25.16 ± 0.19	-25.66 ± 0.70	-26.64 ± 0.15	-25.50 ± 0.26
Pine	-24.04 ± 0.15	-23.16 ± 0.36	-26.22 ± 0.23	-25.48 ± 0.35
Aspen	-27.37 ± 0.30*	-25.37 ± 0.96	-26.90 ± 0.17	-26.19 ± 0.69

Values are the mean ± s.d., n = 4.
*Value is the mean ± s.d., n = 2.

Table 4. Seasonal Variation in the Carbon Isotope Ratio of Leaf Tissue ($\delta^{13}C$, ‰) Collected From the Dominant Tree Species in the BOREAS Study Areas During 1994

	Spring	Summer	Fall
	Northern Study Area		
Black spruce	-26.35 ± 0.47	-25.86 ± 0.58	-26.65 ± 0.72
n	6	6	5
Jack pine	-27.02 ± 0.70	-24.66 ± 0.73	-25.63 ± 0.50
n	6	6	5
Aspen	-27.67 ± 1.06	-27.73 ± 0.59	-28.41 ± 0.96
n	5	9	5
	Southern Study Area		
Black spruce	-27.64 ± 0.48	-26.67 ± 0.62	-26.53 ± 0.66
n	5	5	5
Jack pine	-27.16 ± 0.41	-27.12 ± 0.49	-26.63 ± 0.44
n	5	5	5
Aspen	-27.98 ± 0.11	-26.22 ± 1.28	-27.76 ± 0.42
n	2	4	4

During the spring period, 1-year-old conifer foliage samples were collected. During summer and fall, needles that had been produced during the current season were chosen as conifer foliage samples. All samples were "sun leaves" collected near the top of the canopy. Values are the mean ± s.d.

= 16.3‰; southern black spruce forest, Δ_A = 17.4‰), although their estimates cover much larger areas of land than our measurements represent. Using an experimental approach similar to ours, *Quay et al.* [1989] and *Lloyd et al.* [1996] measured δ_r values slightly more negative (approximately -28‰ and -27.1‰, respectively) for tropical forests in Amazonia, a difference that would be expected for comparison between tropical and boreal forests [*Farquhar et al.*, 1989].

While the evergreen coniferous canopies (black spruce and jack pine) had relatively constant discrimination during the growing season, the broadleaf deciduous canopy (aspen) showed distinct seasonal changes in leaf isotopic composition (Table 4) and total system discrimination (Figure 9). It is likely that the increase in discrimination from summer to fall study periods in the aspen sites was primarily caused by leaf senescence and a decline in photosynthetic capacity relative to stomatal conductance. The ability to detect a seasonal change in total forest ecosystem discrimination, that was correlated with expected environmental influences on leaf photosynthetic gas exchange, suggests that a large fraction of the respired CO_2 comes from recently produced carbohydrates in aboveground plant material and roots. In other forest systems, autotrophic respiration has been estimated to contribute 70% of the total respiratory flux, consistent with this suggestion [*Edwards et al.*, 1989]. In addition, *Hesterberg and Siegenthaler* [1991] have shown that the $\delta^{13}C$ values of soil carbon dioxide can vary 4‰ throughout a summer growing season in a soil covered by grass, a result they hypothesized was caused by shifts in the relative magnitude of root respiration and soil organic matter decomposition, with the two CO_2 sources having different isotopic compositions.

The isotopic signature of carbon dioxide released from heterotrophic processes in soils is expected to represent a weighted average of CO_2 released from sources with a range of $\delta^{13}C$ values. Soil organic matter is composed of different chemical fractions that turn over at different rates (for example, lignin decomposes quite slowly) [*Schimel et al.* 1994]. These different carbon pools should have different isotopic compositions because of changes in the $\delta^{13}C$ value of source atmospheric CO_2 over time [*Keeling et al.*, 1989; *Francey et al.*, 1995], and because various chemical components of plant tissues can have dissimilar ^{13}C contents (for example lignin is depleted in ^{13}C relative to whole cellulose [*Benner et al.*, 1987]). It is also possible that some isotopic fractionation can occur during microbial respiration leading to a ^{13}C enrichment in microbial carbon compared to plant derived carbon in soils [*Maco and Estep*, 1984]. In all the forests sampled in our study, we observed a consistent increase in the ^{13}C content of soil organic carbon with an increase in soil depth (Table 3). Such a pattern has been previously observed in several other studies of forest soils [*Balesdent et al.*, 1993; *Melillo et al.*, 1989; *Nadelhoffer and Fry*, 1988]. We also observed a higher $\delta^{13}C$ value in partially decomposed *Sphagnum* moss at depth compared with moss tissue collected near the surface. These patterns are consistent with older carbon at depth being enriched in ^{13}C because of past changes in the ^{13}C content of atmospheric CO_2. The observed pattern is contrary, however, to predicted declines in $\delta^{13}C$ values associated with increases in the relative proportion of lignin in soil carbon as decomposition progresses. An alternative explanation for the progressive enrichment of ^{13}C in soil carbon with changes in depth is that mixing between plant-derived carbon and carbon originating in soil microbial biomass changes the $\delta^{13}C$ value of soil organic matter during decomposition [*Wedin et al.*, 1995]. This mixing process would offset decreases in $\delta^{13}C$ values of soil organic matter associated with an increase in the proportion of lignin during decomposition.

In general, we observed close agreement among $\delta^{13}C$ values for (1) δ_r calculated from flask samples, (2) soil-respired CO_2 collected with a respiration chamber, (3) soil organic matter,

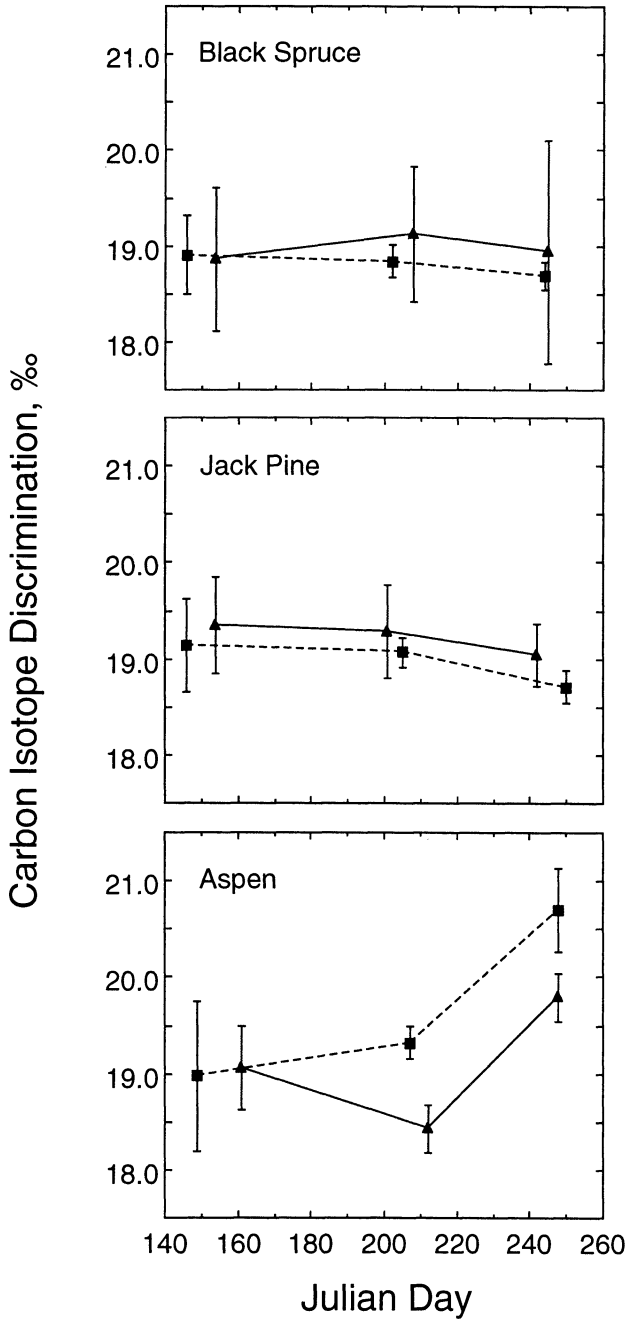

Fig. 9. Seasonal variation in total ecosystem carbon isotope discrimination. Values were calculated using $\Delta_A = \delta_a - \delta_r$ [*Lloyd and Farquhar*, 1994], where the δ_a and δ_r values are shown in Tables 1 and 2, respectively. The square symbols and dotted lines represent measurements for forests in the BOREAS southern study area. The triangle symbols and solid lines represent measurements for forests in the BOREAS northern study area. The error bars represent the SE of the δ_r measurement shown in Table 2.

and (4) tree leaf material. This provides strong support for using the linear regression technique to obtain an integrated measurement of the isotopic composition of CO_2 respired from plants and soil within a forest. This data also illustrates the lack of a major influence of the very depleted ^{13}C content of the moss and other understory plants on the $\delta^{13}C$ values of soil carbon and respired CO_2 (J.R. Brooks et al., unpublished data, 1996). An exception, to the general pattern of agreement among the four parameters listed above, occurred in the jack pine site where soil-respired CO_2 collected with the respiration chamber was enriched in ^{13}C relative to soil organic matter, tree leaf material, and δ_r calculated from flask samples. We do not know the reason for this discrepancy. It was interesting to note, however, that the $\delta^{13}C$ value for soil respired CO_2 was approximately 4‰ lower than the values measured for CO_2 within the soil. This difference is consistent with a theoretically expected 4.4‰ fractionation caused by diffusion [*Dorr and Munnich*, 1980; *Cerling et al.*, 1991] and provides some support for the $\delta^{13}C$ values we measured for soil respired CO_2 in the jack pine site.

Our measurements of temporal variation in total forest carbon isotopic discrimination support the cautionary note expressed by *Lloyd and Farquhar* [1994] for interpreting annual variations in the latitudinal gradient and rates of change in the isotope ratio of atmospheric CO_2. Many previous studies attempting to partition net uptake of atmospheric CO_2 between the ocean and terrestrial biosphere have used a single, constant value for isotopic discrimination by land plants [e.g., *Keeling et al.*, 1989; *Francey et al.*, 1995]. Large environmental differences may occur within a season or between years in a given biome that strongly influence isotopic exchange processes between the terrestrial biosphere and the atmosphere. Global models attempting the use of stable isotopes in partitioning ocean and land uptake of CO_2 should incorporate realistic, dynamic models of biosphere physiological processes to deal with this temporal variation in isotopic discrimination [*Ciais et al.*, 1995].

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S.C. Berry, L.B. Flanagan, and G.T. Varney, Department of Biology, Carleton University, 1125 Colonel By Drive, Ottawa, Ontario, Canada K1S 5B6. (e-mail: lflana@ccs.carleton.ca)

J.R. Brooks and J.R. Ehleringer, Department of Biology, Stable Isotope Ratio Facility for Environmental Research, University of Utah, Salt Lake City, UT 84112.

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