Tellus (1999), 51B, 367–384
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TELLUS
ISSN 0280–6495

Spatial and temporal variation in the carbon and oxygen stable isotope ratio of respired CO_2 in a boreal forest ecosystem*

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(Manuscript received 12 December 1997; in final form 3 September 1998)

ABSTRACT

We measured the stable isotope ratio of respired carbon dioxide at two spatial scales in a black spruce forest in northern Canada: CO_2 released from the forest floor and CO_2 released from the entire ecosystem at night. Despite wide variation in the $\delta^{13}C$ values of organic matter among above-ground plant species, and along a continuum from moss through to the mineral soil, the carbon isotope ratio of respired CO_2 was quite similar to the $\delta^{13}C$ value for the dominant black spruce foliage. The CO_2 released from the forest floor during the fall was slightly enriched in ^{13}C compared to CO_2 respired by the entire ecosystem, perhaps because soil respiration contributes a larger percentage to total ecosystem respiration later in the year as the soil warms. Short-term changes in the oxygen isotope ratio of precipitation and variation in enrichment of ^{18}O during evaporation and transpiration had significant effects on the $\delta^{18}O$ value of respired CO_2. Changes in the oxygen isotope ratio of water in moss tissue can have a large effect on total ecosystem respired CO_2 both because a large surface area is covered by moss tissue in this ecosystem and because the equilibration between CO_2 diffusing through the moss and water in moss tissue is very rapid. During the summer we observed that the $\delta^{18}O$ value of CO_2 respired from the forest floor was relatively depleted in ^{18}O compared to CO_2 respired from the entire ecosystem. This was because water in black spruce foliage had higher $\delta^{18}O$ values than moss and soil water, even at night when transpiration had stopped.

1. Introduction

Anthropogenic changes in the composition of our atmosphere have the potential to alter physiological responses of terrestrial vegetation with subsequent feedback effects on our global climate system (Sellers et al., 1996; Betts et al., 1997). Analyses of the stable isotope ratio of atmospheric carbon dioxide can be used to study the effects of global changes on terrestrial ecosystem function on large spatial scales (Ciais et al., 1995a,b; Francey et al., 1995). Changes in the stable isotope ratio of atmospheric CO_2 result from isotope effects that occur during ecosystem–atmosphere CO_2 exchange (Farquhar et al., 1993; Ciais et al., 1995b). Photosynthesis and respiration have very different and contrasting effects on the stable isotope ratio of atmospheric CO_2, which potentially allows the magnitude of these two fluxes to be determined separately at regional scales (Yakir and Wang, 1996).

The interpretation of the stable isotope signal

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recorded by the atmospheric boundary layer requires a detailed understanding of fractionation processes that occur during total ecosystem photosynthesis and respiration. For the leaf level, mechanistic models have been developed that successfully explain the carbon isotope effects that occur during photosynthetic gas exchange in C_3, C_4, and CAM plants (Farquhar et al., 1989). During photosynthesis, plants preferentially assimilate $^{12}CO_2$, resulting in an increase in the $^{13}C/^{12}C$ ratio of CO_2 remaining in the atmosphere. Current models can be used to determine the effect of different terrestrial ecosystems on the $^{13}C/^{12}C$ ratio of atmospheric CO_2, because of differences in photosynthetic pathway, genotypic variation, and environmental influences (Lloyd and Farquhar, 1994).

At the ecosystem scale we have a much more limited understanding of factors influencing the carbon isotope ratio of respired CO_2 in terrestrial systems. There is no significant isotope effect associated with respiration (Lin and Ehleringer, 1997), so the carbon isotope ratio of respired CO_2 is dependent on the isotopic composition of the carbon substrate broken down during respiration. Plant carbon compounds have a lower $^{13}C/^{12}C$ ratio than atmospheric CO_2 because of fractionation during photosynthesis, so respiration releases CO_2 that is relatively depleted in ^{13}C, resulting in a reduction in the $^{13}C/^{12}C$ ratio of atmospheric CO_2. However, there is a great deal of variation among the carbon isotope ratios of different carbon compounds that are broken down during plant and soil respiration (O'Leary, 1981; Benner et al., 1987). Many enzymes involved in secondary carbon metabolism have isotope effects; thus, different chemical components have different carbon isotope ratios (for example lipids have lower ^{13}C contents than whole plant tissue (O'Leary, 1981)). Because of differences in the timing and magnitude of plant and soil respiratory processes, and associated variation in the chemical nature of the carbon-containing molecules that get broken down, there may be significant spatial and temporal variation in the carbon isotope ratio of respired CO_2 in terrestrial ecosystems.

While much of the previous work with stable isotopes of atmospheric CO_2 has focused only on carbon isotopes, ecosystem CO_2 exchange also has a significant influence on the oxygen isotope ratio of atmospheric CO_2 (Francey and Tans, 1987; Farquhar et al., 1993; Ciais et al., 1997a,b; Flanagan et al., 1997). Discrimination against $C^{18}O^{16}O$ during photosynthesis acts to enrich atmospheric CO_2 in ^{18}O. Similar to what occurs for carbon isotope discrimination, discrimination against $C^{18}O^{16}O$ is controlled by differences in plant physiological characteristics, and the mechanisms are fairly well understood at the leaf level (Farquhar and Lloyd, 1993; Flanagan, 1997). Ecosystem respiration acts in opposition to photosynthesis by releasing CO_2 depleted in ^{18}O to the atmosphere. The primary factor influencing the oxygen isotope ratio of respired CO_2 is an equilibrium isotope effect that occurs between oxygen in CO_2 and oxygen in plant and soil water (Farquhar et al., 1993). The oxygen isotope ratio of water in plants will depend on the source water taken up from the soil (ground water or recent precipitation (White et al., 1985)), and isotope effects that occur during evaporation of soil water and plant transpiration (Farquhar et al., 1993; Ciais et al., 1997a,b; Flanagan et al., 1997). Significant spatial and temporal variability in the oxygen isotope ratio of ecosystem respired CO_2 may occur because of variation in: (i) the isotope ratio of precipitation inputs; (ii) evaporation of water in soils; (iii) water sources used by different plants; and (iv) isotope effects during transpiration.

Our objective in this study was to analyze potential mechanisms contributing to spatial and temporal variation in the stable isotope ratio of respired CO_2 in a boreal forest ecosystem. Such information is necessary in order to apply stable isotope techniques in a rigorous manner to study variation in ecosystem photosynthesis and respiration on large spatial scales.

2. Methods

2.1. Study site

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). We conducted our research in the southern study area of the BOREAS project at the *Picea mariana* (black spruce) site (53.99°N, 105.12°W). The forest was approximately 110–120 years old. Average tree height was appoximately

12 m with a leaf area index of approximately 5 (Steele et al., 1997). This black spruce forest had a near continuous understory of mosses on the forest floor that was dominated by two distinct moss communities.

The first moss community type (feather moss community) had an overstorey of black spruce (4150 live stems/ha), with scattered individuals of jack pine (*Pinus banksiana* Lamb.) and tamarack (*Larix laricina* (Du Roi) K. Koch). The black spruce ranged in height up to 12 m, and the understorey was dominated (covering approximately 75% of the ground area) by feather mosses (predominantly *Pleurozium schreberi*) and the vascular evergreen shrub *Ledum groenlandicum* Oeder. In the second moss community type (*Sphagnum* spp. community) the black spruce overstorey was shorter (up to 6 m) and less dense (3700 live stems/ha), and the understorey contained a greater proportion of *Sphagnum* spp. moss (covering approximately 30% of the ground area). Vascular plant species common in the understorey were *L. groenlandicum*, *Vaccinium vitis-idaea* L. and *Equisetum* spp. The soils in the two areas of the forest also differed and have been studied in association with the BOREAS project by Dr. Darwin Anderson of the University of Saskatchewan. A brief description of Dr. Anderson's soil classification (Canadian System of Soil Classification) is described below. In the feather moss community, 7 separate horizons were recognized: (1) living moss, pH 4.1; (2) LFH, weakly decomposed, pH 3.4; (3) Ae, single grain, pH 4.3, texture coarse sand; (4) AB, single grain, pH 4.3, texture coarse sandy loam; (5) Bt, moderate medium, pH 4.9, texture coarse sandy loam; (6) Bfj, moderate fine, pH 5.8, texture coarse sand; (7) Ck, amorphous, pH 6.6–7.0, texture coarse sand. In the *Sphagnum* area, the first 30–60 cm of the forest floor consisted of *Sphagnum* moss or peat in various stages of decomposition. At the base of the peat was coarse sand of the C horizon described above.

2.2. *Air sample collection and analysis*

We collected samples of air within the forest canopy at intervals during a 2–3 day period on separate dates during the summer and fall of 1996. Sample lines (Bev-a-line tubing, 6 mm outer diameter, Warehoused Plastic Sales, Toronto, Ontario, Canada) were located at different heights in and above the forest canopy (25, 13, 8 and 3 m) by attachment to a scaffold tower at the site. An inverted funnel was connected to the inlet to prevent water from entering the tubing. Air was pulled down through the tubing, through a desiccant tube (6200DP, Li-Cor, Lincoln, Nebraska, USA) containing magnesium perchlorate, and into 100 ml glass flasks by a battery-operated pump (TD-4N pump, Brailsford & Co. Inc., Rye, New York, USA) located in an instrument hut approximately 10 meters away from the scaffold tower. The CO_2 concentration of the air was measured using an infrared gas analyzer (LI-6250 CO_2 analyzer, Li-Cor, Lincoln, Nebraska, USA). Air was passed through the flasks for approximately 10 minutes before a CO_2 concentration measurement was recorded and the high vacuum stopcocks on the flask were closed. The flasks were then returned to a lab for stable isotope analysis of the CO_2.

A dynamic closed-chamber soil respiration system, similar to that described by Rochette et al. (1997), was used to collect CO_2 respired from the ground surface for isotopic analysis. The chamber top (60×60 cm $\times$ 15 cm) contained a Li-Cor sensor head and was connected to a Li-Cor 6200 portable photosynthesis system for CO_2 concentration measurements. The chamber top was clamped onto collars that were installed in the ground. In this study we sampled two collars installed in the feather moss area and two collars installed in the *Sphagnum* area of the black spruce forest. An evacuated 100 ml glass flask could be connected to the respiration chamber via a port with an Ultra-Torr connector. After connecting the soil chamber top to the collar installed in the ground, the rise in CO_2 concentration inside the closed chamber was monitored using the infrared gas analyzer of the Li-Cor photosynthesis system. At intervals during the rise in CO_2 concentration, samples of the chamber air were collected by opening a high vacuum stopcock on the glass flask. Air passing into the sample flask, first passed through a small tube of magnesium perchlorate. Air samples were collected at intervals of approximately 10–15 μmol mol^{-1} change in chamber CO_2 concentration. The respiration chamber had a volume of approximately 100 liters. The flask volume (100 ml) was small relative to the chamber volume, resulting in negligible changes in overall pressure within the system. After collection

the flasks were returned to a lab for stable isotope analysis of the CO_2.

Stable isotope ratios of CO_2 in air samples collected in 100 ml glass flasks were analyzed using a gas isotope ratio mass spectrometer (Model 252, Finnigan MAT, Bremen, Germany) operating in continuous flow mode. A trace gas condensing device (PreCon, Finnigan MAT) was used to separate CO_2 and N_2O from air samples. A 500 µl gas-tight, locking syringe (Model A-2, VICI Precision Sampling, Baton Rouge, Louisiana, USA) was used to remove a subsample of air via a septum port on the sample flasks. A pre-sample was collected and spent in order to ensure that the needle dead volume was clean prior to flask subsampling. After removal of a subsample from the flask, the syringe was slightly over-pressurized to ensure that the dead volume of the needle was cleansed prior to injection of the sample into the PreCon. Our use of the PreCon involved attaching a 12 mm Y-shaped tube with a septum port to the helium carrier stream (20 PSI) of the PreCon before the cryogenic sample loops for separation of CO_2 and N_2O from the air sample. A helium carrier gas (at 8 PSI) moved the sample from the PreCon cryogenic traps through a gas chromatograph (Model 3400, Varian, Walnut Creek, California, USA) to separate CO_2 and N_2O before introducing the CO_2 into the mass spectrometer. The gas chromatograph was arranged so that its effluent was sent to the mass spectrometer through a 1:2 fixed post column split interface via a capillary. The gas chromatograph had a 25 m POROPLOT column operated at 25°C. An analysis of the accuracy and precision of this method for determining stable isotope ratios of atmospheric CO_2 is presented by Ehleringer and Cook (1998). The precision of the technique was estimated to be 0.13‰ for ^{13}C and 0.28‰ for ^{18}O (Ehleringer and Cook, 1998).

Isotope ratios in delta notation are calculated as:

$$\delta = \left[\frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right], \tag{1}$$

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

We used a simple mixing model developed by Keeling (1958, 1961) to calculate the isotope ratio of CO_2 respired by a forest ecosystem:

$$\delta_f = \frac{[CO_2]_o}{[CO_2]_f} (\delta_o - \delta_R) + \delta_R, \tag{2}$$

where $[CO_2]$ is concentration of CO_2 and δ is the stable isotope ratio of CO_2, and the subscripts f and o represent the atmosphere within a forest boundary layer and the atmosphere above (outside) the forest boundary layer, respectively. It can be seen from eq. (2) that a plot of $1/[CO_2]_f$ versus δ_f gives a straight line relationship with a slope, $[CO_2]_o$ $(\delta_o - \delta_R)$, and an intercept, δ_R. We used this model to estimate δ_R, the isotope ratio of CO_2 respired by plants and soils. Estimates were obtained from the y-intercept of a geometric mean linear regression (Sokal and Rohlf, 1981) between δ and $1/[CO_2]$ values measured on air samples collected at different heights above the forest floor during the night when photosynthesis was not active. The same mixing model (eq. (2)) was used to calculate the isotope ratio of respired CO_2 from air samples collected during the increase in CO_2 in the moss/soil respiration chamber. It is possible, therefore, to use the "Keeling plot" approach to calculate separately the isotope ratio of respired CO_2 at different spatial scales. This allows determination of the relative contribution of different ecosystem components to total ecosystem respiration rate.

2.3. *Plant and soil collection for carbon isotope analysis*

Leaf samples were collected from the dominant species at the study site. From each of 5 individual plants per species, 4–5 mature leaves were collected from the top of the plant and bulked into a single sample. For the black spruce trees, samples of current-year foliage were collected. Foliage was dried at 65°C, and ground to a fine powder with a tissue grinder or a mortar and pestle.

Samples of the forest floor (soil, and the overlying moss and undecomposed litter) were collected from three locations within the study site in October 1996. These samples were collected at approximately 3 cm depth increments from the top of the moss on the forest floor down to the

mineral soil. The samples were dried in an oven at 65°C. Roots were removed from the samples, and the soil and plant tissue was ground to a fine powder with a mortar and pestle. Carbonates were removed from soil samples (Rask and Schoenau, 1993) prior to stable isotope analysis by incubating 25 g of soil with 0.1 M HCl for 48 h 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 motar and pestle.

The above-ground plant and forest floor (soil/moss) samples were prepared for measurements of carbon isotope composition by combustion. A subsample of ground tissue was sealed in a tin capsule and loaded into an elemental analyzer for combustion. The carbon dioxide generated from the combustion was purified cryogenically. For leaf samples the purified CO_2 was passed directly to the inlet of a gas isotope ratio mass spectrometer (Delta S, Finnigan Mat, San Jose, CA, USA) at the University of Utah. For the forest floor organic samples, the purified CO_2 was analyzed on a Sira 12 gas isotope ratio mass spectrometer (VG Isotech, Middlewich, Cheshire, UK) at the Ottawa–Carleton Stable Isotope Facility.

2.4. *Water collection and oxygen isotope analysis*

Water samples from black spruce stem xylem tissue, black spruce leaf tissue, and moss tissue were collected for stable isotope analysis. Small non-green stem samples (approximately 7×60 mm) were cut from trees near the top of the canopy. One sample from each of 3 trees was collected at each sampling interval. Tree foliage samples (from three separate trees) were collected from a variety of positions within a tree crown and combined for one tree. Moss samples were collected from three different areas within the forest (two dominated by *Pleurozium* moss and one dominated by *Sphagnum* moss). The plant samples were immediately placed in a glass tube which was sealed with a rubber stopper and wrapped with Parafilm. The glass tubes containing plant samples were placed in a small cooler in the field, and returned to the laboratory where they were stored in a freezer until water was extracted from the tissue using a cryogenic vacuum distillation apparatus (Ehleringer and Osmond, 1989). Water samples were analyzed using the CO_2 equilibrium method modified for small samples (Socki et al., 1992). The oxygen isotope ratios were expressed using delta notation relative to Vienna Standard Mean Ocean Water (V-SMOW).

3. Results and discussion

3.1. *Plant and soil carbon isotope ratios*

Since the carbon isotope ratio of respired CO_2 is dependent on the isotopic composition of the substrate molecule broken down (Lin and Ehleringer, 1997), we measured the leaf $\delta^{13}C$ values of different plant species at the study site in order to examine the potential sources of variation in the isotope ratio of respired CO_2 at the ecosystem level. There was a large, 4.8‰ variation in the leaf $\delta^{13}C$ values among different species at the black spruce site (Table 1). The black spruce foliage was the most enriched in ^{13}C, while the *Pleurozium* moss was the least enriched ^{13}C. The observed patterns of variation among species were consistent with previous measurements of leaf $\delta^{13}C$ values made at this site (Brooks et al., 1997b), and are consistent with vertical gradients in leaf $\delta^{13}C$ observed in a range of other forest environments (Ehleringer et al., 1986; Schleser, 1990; Garten and Taylor, 1992; Broadmeadow and

Table 1. *Comparison of leaf carbon isotope ratio ($\delta^{13}C$, ‰) among the major plant species within a black spruce forest in Saskatchewan during 1996; values are the mean ± SE, n = 5*

Species	$\delta^{13}C$ (‰)
Tree	
Picea mariana	-26.5 ± 0.66
Shrub (deciduous)	
Rosa acicularis	-28.18 ± 0.80
Potentilla fructicosa	-28.84 ± 0.67
Shrub (evergreen)	
Ledum groenlandicum	-28.44 ± 0.86
Vaccinium vitis idaea	-30.02 ± 0.73
Herb (deciduous)	
Petasites palmatus	-31.24 ± 0.95
Rubus chamaemorus	-28.30 ± 1.04
Mosses	
Pleurozium schreberi	-31.35 ± 0.23
Sphagnum spp.	-29.31 ± 0.53

Griffiths, 1993; Berry et al., 1997; Buchmann et al., 1997). The majority of the differences in leaf $\delta^{13}C$ values among species has been attributed to variation in leaf physiological characteristics (ratio of intercellular to ambient CO_2 concentration; Farquhar et al., 1989) associated with light gradients in forest canopies, rather than variation in the $\delta^{13}C$ of source CO_2 available for photosynthesis (Berry et al., 1997; Brooks et al., 1997a,b).

There was also a substantial change in the $\delta^{13}C$ values along the continuum from live moss at the top of the forest floor, down through to the mineral soil (Fig. 1). In an area of the forest floor dominated by *Pleurozium* moss, there was a significant increase in $\delta^{13}C$ values with increases in depth through dead (brown) moss, in addition to a large change in $\delta^{13}C$ values observed during the transition between recognizable plant material (brown moss) and humus (Fig. 1). In an area of the forest floor dominated by *Sphagnum* moss, a large change was observed in $\delta^{13}C$ values during the transition between recognizable plant material (brown moss) and humus. However, no change was observed in the $\delta^{13}C$ values between live and dead *Sphagnum* moss (Fig. 1). The humus and mineral soil had carbon isotope ratios similar to, but slightly enriched in ^{13}C compared to the dominant black spruce tree foliage. 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 (Keeling et al., 1989; Ciais et al., 1995a,b; Francey et al., 1995).

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 (Benner et al., 1987). An alternative explanation for the progressive enrichment of ^{13}C in moss/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. Several other studies of forest soils have also observed a consistent increase in the ^{13}C content of organic carbon with an increase in soil depth (Nadelhoffer and Fry, 1988; Melillo et al., 1989; Balesdent et al., 1993).

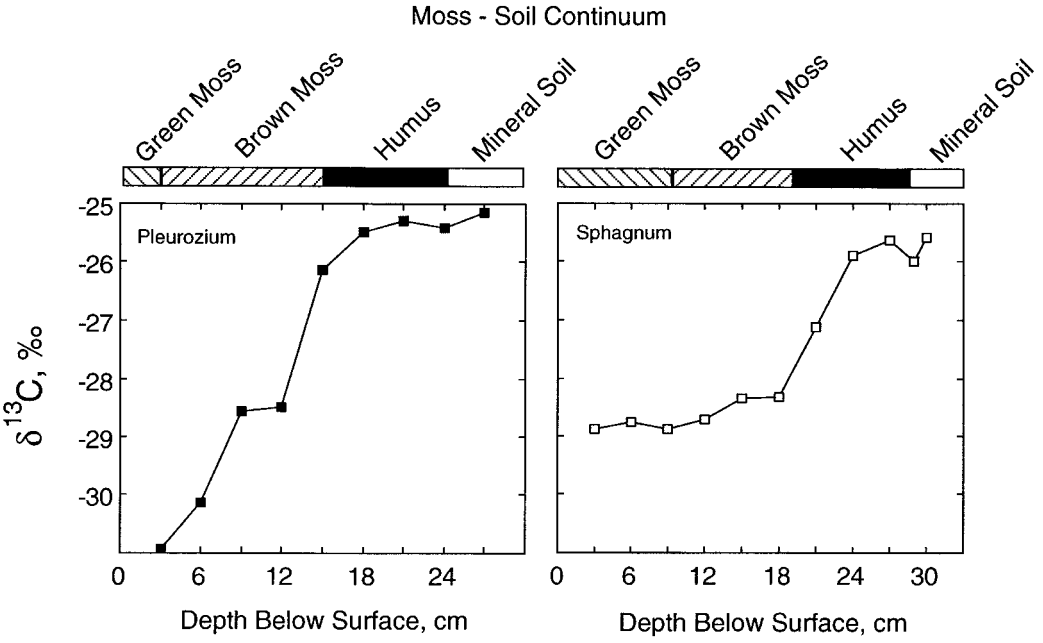

Fig. 1. Changes in the carbon isotope ratio of organic matter along a continuum from the top of the forest floor down to the mineral soil at two locations within a black spruce forest in Saskatchewan.

The large range of variation in $\delta^{13}C$ values within the moss/soil at the forest floor presents the possibility of substantial variation in the $\delta^{13}C$ values of respired CO_2 released from the forest floor, depending on the relative magnitude of moss, tree root, and heterotrophic soil respiration rates.

3.2. *Carbon isotope ratio of respired CO_2*

We used the "Keeling plot" technique (eq. (2)) to calculate the isotope ratio of respired CO_2 at two spatial scales, the total ecosystem and the forest floor. New analytical approaches made it possible for us to collect and analyze the $\delta^{13}C$ values of small subsamples of CO_2 taken from a soil respiration chamber headspace. Air samples were collected at intervals of approximately 10–15 μmol mol^{-1} change in chamber CO_2 concentration. The rise in CO_2 concentration remained linear during the time required to collect 10 flask samples (Fig. 2). We also observed significant linear relationships between $\delta^{13}C$ and $1/CO_2$ concentration measured on samples collected from the respiration chamber (Figs. 3–5). The estimates we obtained for the carbon isotope ratio of CO_2 respired (δ_R values) from the forest floor varied among chambers placed in different locations on the forest floor (Table 2). In the summer sampling

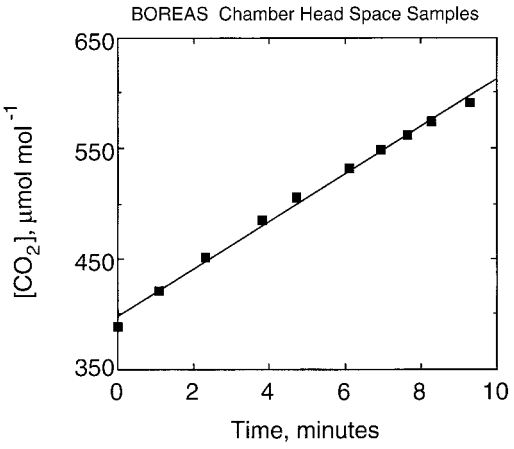

Fig. 2. Changes in the CO_2 concentration within a moss/soil respiration chamber after the chamber was closed. Subsamples of the air were taken at intervals, marked by the points, during the rise in CO_2 concentration. Air samples were collected using evacuated 100 ml flasks attached to the chamber via a port and the carbon dioxide was later analyzed for stable isotope ratio.

Table 2. *The carbon isotope ratio ($\delta^{13}C$, ‰) of respired carbon dioxide at different spatial scales within a black spruce forest in Saskatchewan during 1996; values for the moss/soil respiration chambers represent the mean ± standard deviation ($n = 4$ for summer and $n = 8$ for fall)*

Scale	Summer	Fall
ecosystem	−24.92	−25.56
moss/soil chambers	−24.45 ± 1.33	−23.56 ± 1.50

period there was a 3.14‰ range of varation among four separate chamber measurements, and in the fall sampling period there was a 4.06‰ range of variation among eight separate chamber measurements. The mean δ_R values of forest floor respired CO_2 (Table 2) were similar to the $\delta^{13}C$ values for humus and the C mineral horizon at the site (Fig. 1). This suggests that moss respired CO_2 contributes only a small fraction of the carbon dioxide released from the forest floor surface. In areas dominated by *Pleurozium* moss, gas exchange measurements indicated that the moss contributed approximately 7% to the total respiratory flux of CO_2 from the forest floor during the summer. In areas dominated by *Sphagnum* moss, moss respiration contributed 21% of the total respiratory flux of CO_2 from the forest floor during the summer (R. Swanson and L. B. Flanagan 1999, unpublished data). The gas exchange and isotope measurements demonstrate that tree root and heterotrophic respiration are the major contributors to forest floor respiratory CO_2 flux in summer and fall periods at this site. Analysis of our gas exchange measurements also suggests that an upper limit to the contribution of black spruce tree roots is approximately 90% and 70% of the respiratory flux from the forest floor during the summer in areas dominated by *Pleurozium* moss and *Sphagnum* moss, respectively.

During the summer, the average $\delta^{13}C$ values of forest floor respired CO_2 were very similar to the δ_R values measured for the entire ecosystem (Table 2, Fig. 6). In contrast, during the fall the average carbon isotope ratio of forest floor respired CO_2 was slightly enriched in ^{13}C compared to the δ_R values measured for the entire ecosystem (Table 2, Fig. 6). This may result because soil respiration contributes a larger percentage to total ecosystem respiration later in the

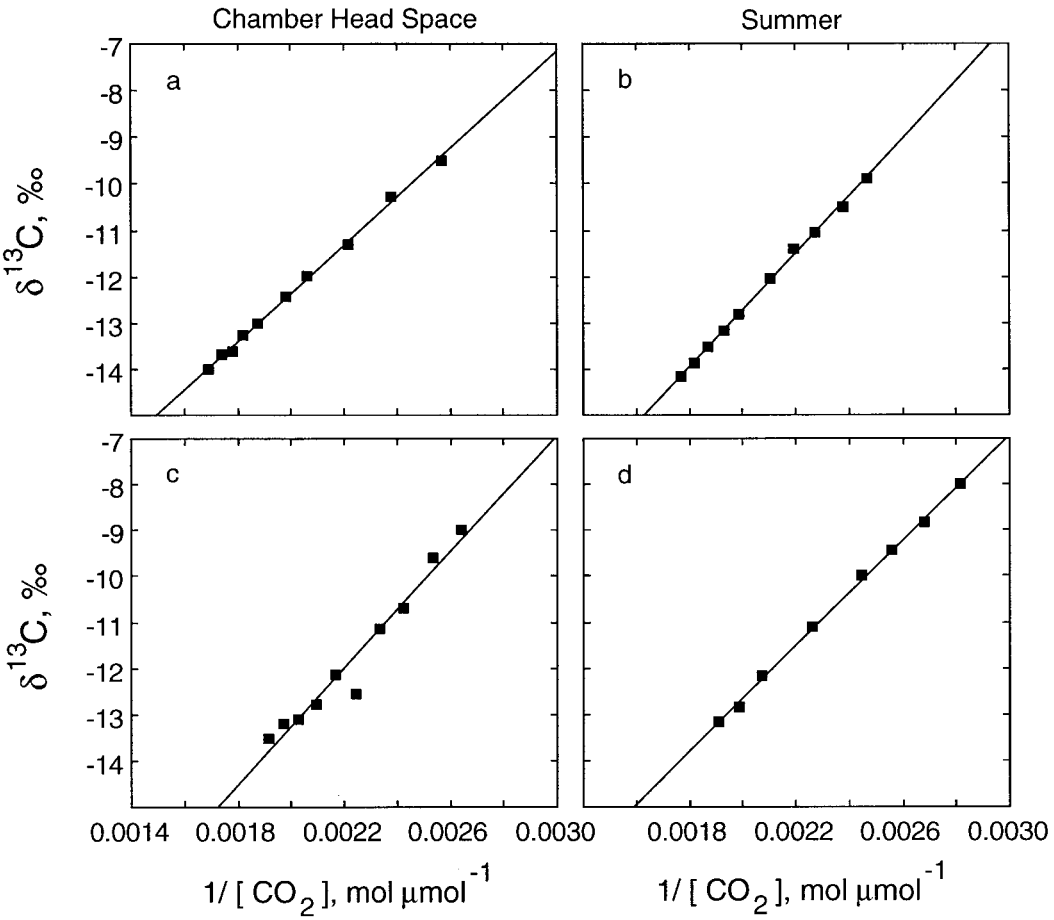

Fig. 3. Comparison of the relationships between $1/CO_2$ concentration and the carbon isotope ratio of CO_2 in air samples collected from moss/soil respiration chambers in a black spruce forest in Saskatchewan during the summer of 1996. The different panels represent replicate measurements made on four different collars installed in the forest floor. Panels (a) and (b) represent collars in the feather moss area and panels (c) and (d) represent collars in the *Sphagnum* area.

year as the soil warms (Goulden et al., 1998), and the deep soil organic carbon is more enriched in ^{13}C relative to the moss layers (Fig. 1). The older carbon at depth within the mineral soil is also likely enriched in ^{13}C because of past changes in the $\delta^{13}C$ values of atmospheric CO_2 (Keeling et al., 1989; Ciais et al., 1995a,b; Francey et al., 1995). A greater proportion of this older carbon may be respired in the fall after the deep soil warms contributing to the more enriched ^{13}C in CO_2 released from the forest floor. In addition, the total ecosystem δ_R values were slightly enriched in ^{13}C compared to the black spruce tree foliage $\delta^{13}C$ values (Table 1), and to total ecosystem δ_R values measured at the same site during 1994 (Flanagan et al., 1996). Variation in environmental conditions between different study years could influence changes in carbon isotope discrimination during photosynthetic gas exchange and be observed in the total ecosystem δ_R values if tree foliage and tree root respiration are major contributors to the total CO_2 respired by a forest. Alternatively, interannual variability in environmental conditions and shifts in the relative magnitude of CO_2 fluxes from foliage, roots, moss and heterotrophic soil organisms could contribute to

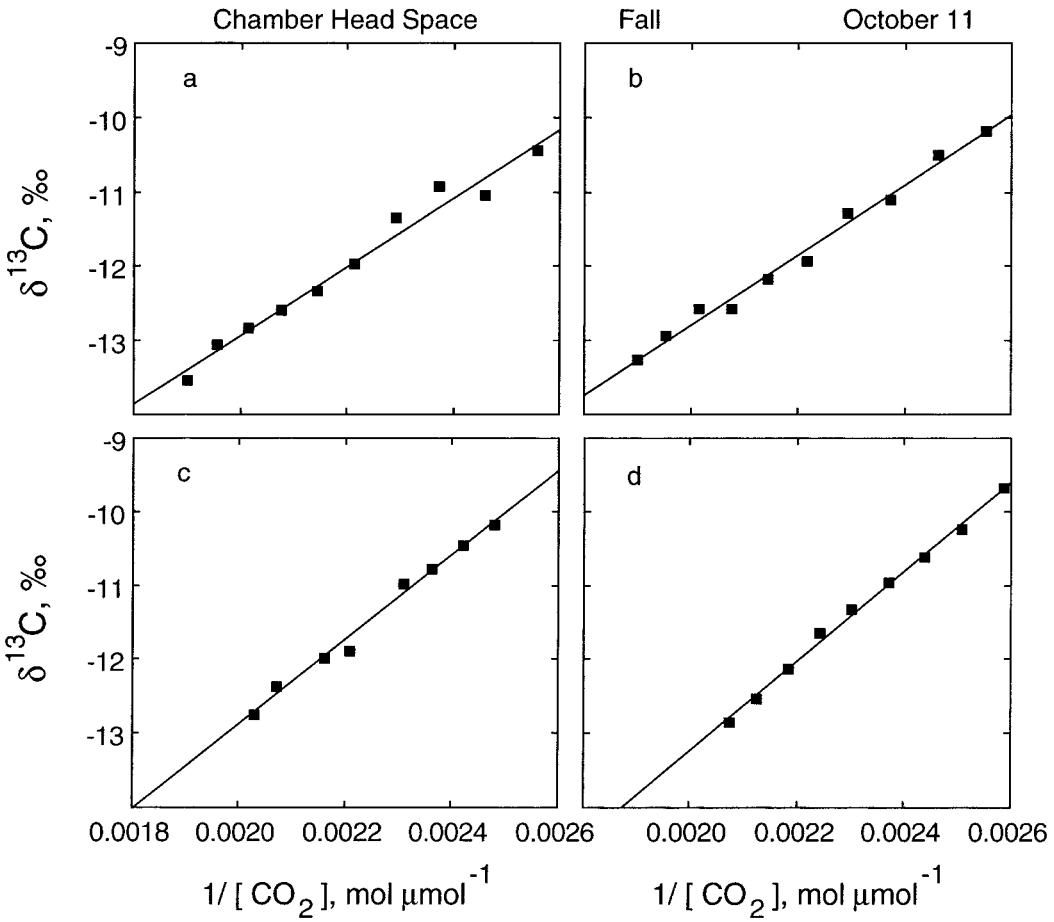

Fig. 4. Comparison of the relationships between $1/CO_2$ concentration and the carbon isotope ratio of CO_2 in air samples collected from moss/soil respiration chambers in a black spruce forest in Saskatchewan during 11 October 1996. Format of the figure is the same as that described in Fig. 3.

variation in total ecosystem δ_R values, because of the observed differences among the $\delta^{13}C$ values of the different components.

3.3. *Oxygen isotope ratio of plant and soil water*

The oxygen isotope ratio of respired CO_2 in terrestrial ecosystems is strongly affected by an equilibrium isotope effect that occurs between oxygen in CO_2 and oxygen in plant and soil water (Francey and Tans, 1987; Farquhar et al., 1993; Hesterberg and Siegenthaler, 1991; Ciais et al., 1997a; Flanagan et al., 1997). Therefore we made diurnal measurements of the $\delta^{18}O$ value of plant and soil water in order to determine potential sources of variation in the oxygen isotope ratio of ecosystem respired CO_2. The $\delta^{18}O$ values of black spruce stem water remained quite constant during both the summer and fall sampling periods (Figs. 7, 8), and were consistent with the trees taking up groundwater. We collected groundwater at a nearby site and it had a $\delta^{18}O$ value of $-16.3‰$ (Flanagan et al., 1997). Previous studies at this site have shown, however, that the isotope ratio of stem water in black spruce trees can be influenced by short-term changes in the $\delta^{18}O$ values of surface inputs (summer precipitation and melting snow) (Flanagan et al., 1997).

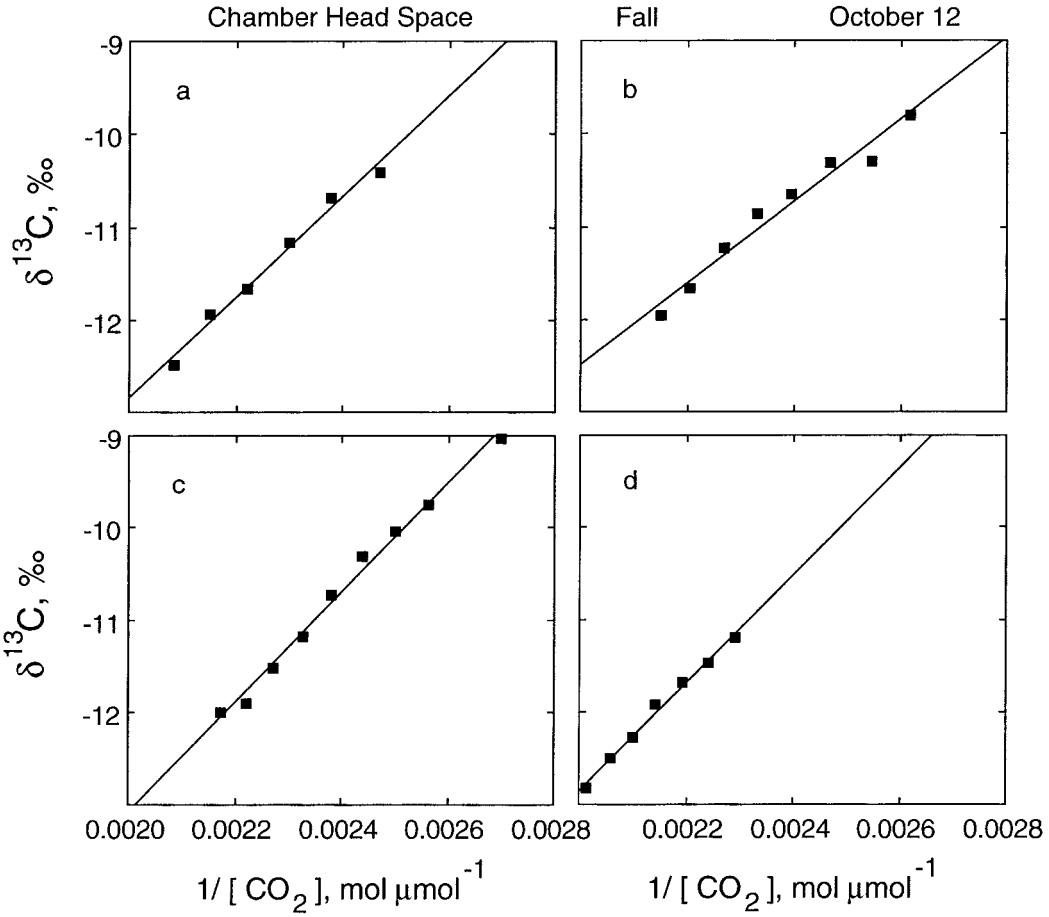

Fig. 5. Comparison of the relationships between $1/CO_2$ concentration and the carbon isotope ratio of CO_2 in air samples collected from a moss/soil respiration chamber in a black spruce forest in Saskatchewan during 12 October 1996. Format of the figure is the same as that described in Fig. 3.

The oxygen isotope ratio of black spruce leaf water was enriched in ^{18}O relative to stem water and the extent of evaporative enrichment varied on a diurnal basis (Figs. 7, 8). Tree leaf water $\delta^{18}O$ values were similar during both summer and fall sampling periods. Our observed leaf water $\delta^{18}O$ values were consistent with expectations based on the environmental conditions (temperature and humidity) and the Craig and Gordon (1965) model of evaporative enrichment. We did not observe the $\delta^{18}O$ value of black spruce leaf water to return to the value of stem water even in leaf samples collected at night or in the early morning (Figs. 7, 8), as has also been observed for juniper tree foliage (Flanagan et al., 1993). Water extracted from moss tissue collected during the summer sampling period had $\delta^{18}O$ values enriched in ^{18}O above that of the black spruce stems, but moss water was not as enriched in ^{18}O as black spruce foliage water (Fig. 7). Water in moss tissue was likely affected by evaporative enrichment of ^{18}O during transpiration, as is suggested by the observed diurnal variation in the moss $\delta^{18}O$ values. However, moss also intercepts and absorbs summer precipitation, which generally has higher $\delta^{18}O$ values than groundwater (Flanagan et al., 1997). Short frequent rain storms occurred during our summer sampling period, which may have

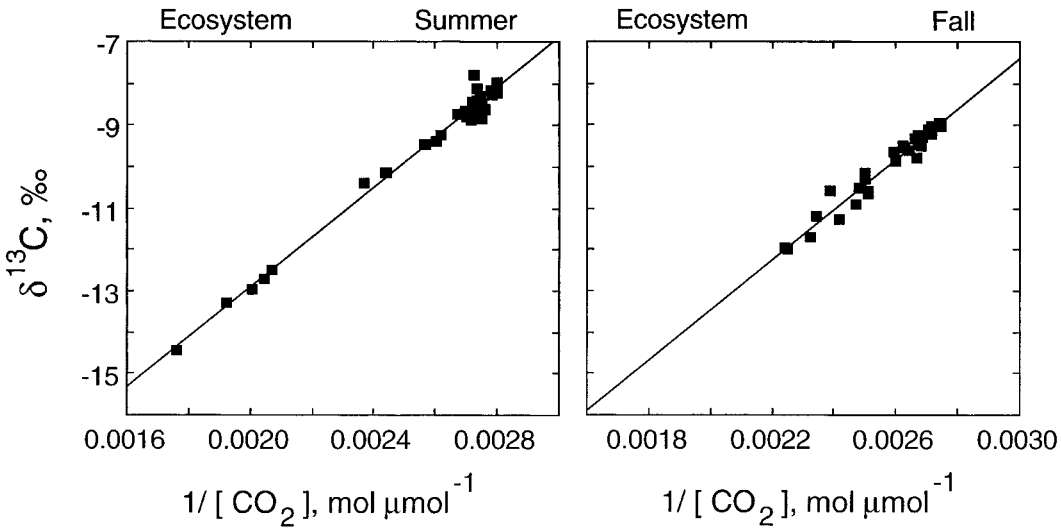

Fig. 6. Comparison of the relationships between $1/CO_2$ concentration and the carbon isotope ratio of CO_2 in air samples collected from different heights above ground at night in a black spruce forest in Saskatchewan during the summer and fall 1996.

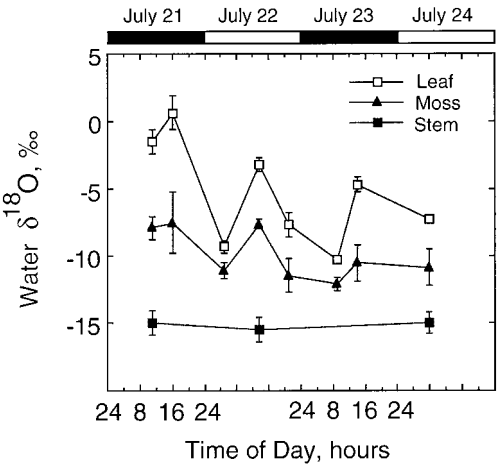

Fig. 7. Daily variation in the oxygen isotope ratio of water extracted from black spruce leaf and stem tissue, and moss tissue collected in a black spruce forest in Saskatchewan during summer 1996.

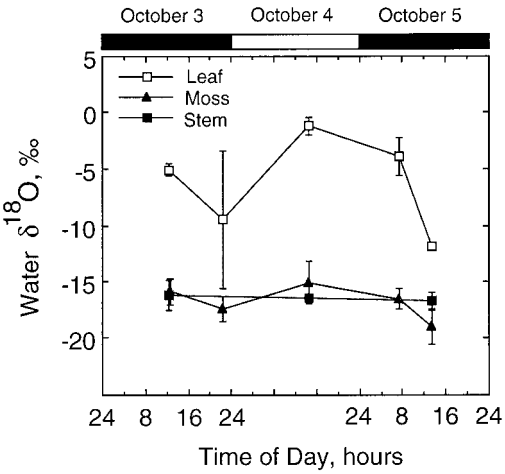

Fig. 8. Daily variation in the oxygen isotope ratio of water extracted from black spruce leaf and stem tissue, and moss tissue collected in a black spruce forest in Saskatchewan during fall 1996.

also influenced the oxygen isotope ratio of moss water. During the fall sampling period moss water had $\delta^{18}O$ values very similar to that of the tree stems.

These isotope measurements on the different water pools indicate that CO_2 respired by tree foliage, moss and soils could have substantially different $\delta^{18}O$ values and be variable on a daily basis. The short-term changes in the isotope ratio of water in tree foliage and moss/soil should result

Table 3. *Comparison of measured and predicted oxygen isotope ratios ($\delta^{18}O$, ‰) of respired CO_2 from moss/soil using field respiration chambers in a black spruce forest in Saskatchewan during the summer of 1996; the predicted values assume a 8.8‰ diffusion isotope effect*

Chamber	Measured $\delta^{18}O$ (‰)	Predicted $\delta^{18}O$ (‰)
Pleurozium 1	−19.2	−17.2
Pleurozium 10	−19.0	−17.1
Sphagnum 11	−29.1	−22.4

in greater variation among the $\delta^{18}O$ values of atmospheric CO_2 sampled in and above the forest canopy than is observed for the $\delta^{13}C$ values of atmospheric CO_2.

3.4. *Oxygen isotope ratio of respired CO_2*

We observed significant linear relationships between $\delta^{18}O$ and $1/CO_2$ concentration measured on samples collected from the respiration chamber (Figs. 9–11). These relationships (Keeling plots) were used to estimate the oxygen isotope ratio of CO_2 (δ_R values) respired from the forest floor. We observed substantial variation in δ_R values among chambers placed at different locations within the forest (Table 3, Figs. 9–11). This variation was likely associated with differences in the $\delta^{18}O$ values of water in moss tissue, as Flanagan et al. (1997) previously noted that changes in moss water $\delta^{18}O$ values can significantly affect the oxygen isotope ratio of respired CO_2 in black spruce ecosystems. We compared our observed δ_R values with those predicted based on CO_2 in equilibrium with moss water (Table 3). The predicted δ_R values also assumed a 8.8‰ fractionation factor for diffusion of CO_2 during passage out of the moss/soil. The observed δ_R values were similar to but more negative than the predicted values. This result may have occurred because of the linear regression technique used in the calculation of our observed values. Tans (1998) has shown that penetration of atmospheric CO_2 into the soil can influence the equilibration between soil CO_2 and water, a result that causes curvature in the $\delta^{18}O$ and $1/CO_2$ relationship. As a result the linear regression technique we used should result in calculated δ_R values that were slightly more negative than actual δ_R values (Tans, 1998). We note that the difference between the measured and predicted δ_R value in the *Sphagnum* chamber was larger than was observed for the feather moss chambers, but we are unsure of the cause of the difference (Table 3).

During the summer period, the respiration chamber samples were collected immediately after completion of the flask air sampling for the entire forest ecosystem. This allowed direct comparison between the calculated δ_R values for the two spatial scales, forest floor and entire ecosystem. The average δ_R for forest floor samples was -23.6 ± 6.1‰ compared to a δ_R of -16.2‰ for the entire ecosystem (Fig. 12). This difference was consistent with expectations because water in moss and soil was depleted in ^{18}O relative to tree foliage water (Fig. 7), and tree foliage respiration is a significant fraction of the total ecosystem respiration rate at night (Ryan et al., 1997).

In the fall, the respiration chamber samples were collected seven days after the ecosystem-level flask sampling had finished. In addition it rained in the time period between sample collection at the different spatial scales. Therefore, it was not possible to make direct comparison between δ_R values measured at the two spatial scales. An example to illustrate the short-term variation in calculated δ_R values associated with precipitation inputs is described below. We made collections of samples from the respiration chamber at four different locations on 11 October and repeated measurements at the same locations on 12 October. There was a substantial change in the average ($\pm$standard deviation, $n = 4$) δ_R values from -21.1 ± 4.6‰ on 11 October to -26.6 ± 5.0‰ on 12 October. This change was likely caused by the input of precipitation depleted in ^{18}O during the night of 11 October, which caused a change in the oxygen isotope ratio of moss water. This short-term variation in the $\delta^{18}O$ values of moss water make it difficult to compare δ_R values separated in time when rain has occurred. The δ_R value we calculated for the entire ecosystem was -31.3‰, based on measurements made during 3–5 October. The $\delta^{18}O$ values we measured for atmospheric CO_2 were quite variable and the linear regression for the $\delta^{18}O$ and $1/CO_2$ relationship did not explain a high proportion of the variation in the data (Fig. 12). This variability

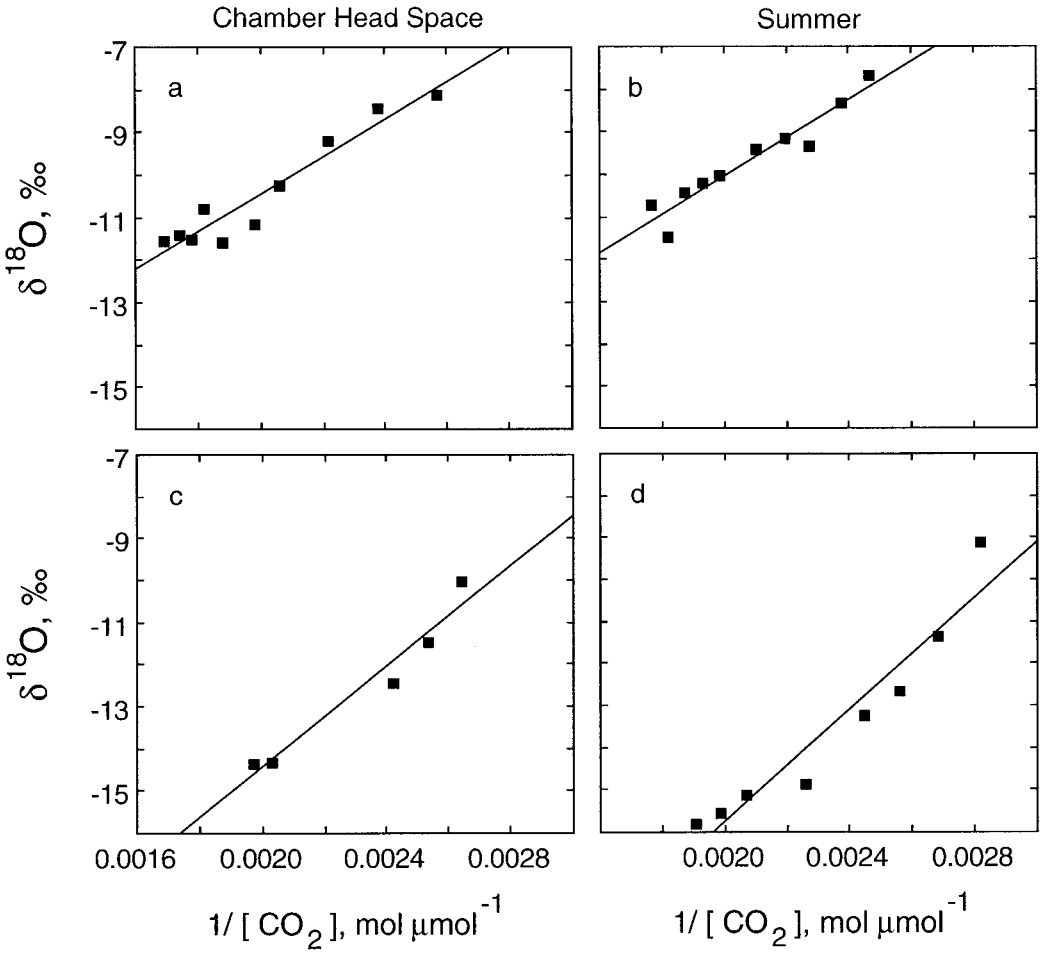

Fig. 9. Comparison of the relationships between $1/CO_2$ concentration and the oxygen isotope ratio of CO_2 in air samples collected from moss/soil respiration chambers in a black spruce forest in Saskatchewan during the summer of 1996. Format of the figure is the same as that described in Fig. 3.

was likely caused by rain that occurred during the collection of air sample flasks on 3–5 October. Air temperature was near 0°C during the collection of samples at night in early October, and so the black spruce tree foliage respiration rate was likely very low. This low leaf respiration would result in less input of CO_2 relatively enriched in ^{18}O from foliage respiration compared to the summer sampling period. As a consequence the expected δ_R value for the entire ecosystem during the fall should be lower than δ_R values observed during the summer.

The values we observed for the oxygen isotope ratio of respired CO_2 for the entire ecosystem were more depleted in ^{18}O than our δ_R values determined at the same site in 1994 (Flanagan et al., 1997). During 1994, the moss had not received precipitation inputs for some time before we collected samples, and moss water was relatively enriched in ^{18}O (Flanagan et al., 1997). The ^{18}O-enriched water signal in the moss can get passed on to respired CO_2 diffusing through the moss layer to the forest floor surface and affect the δ_R values for the entire ecosystem.

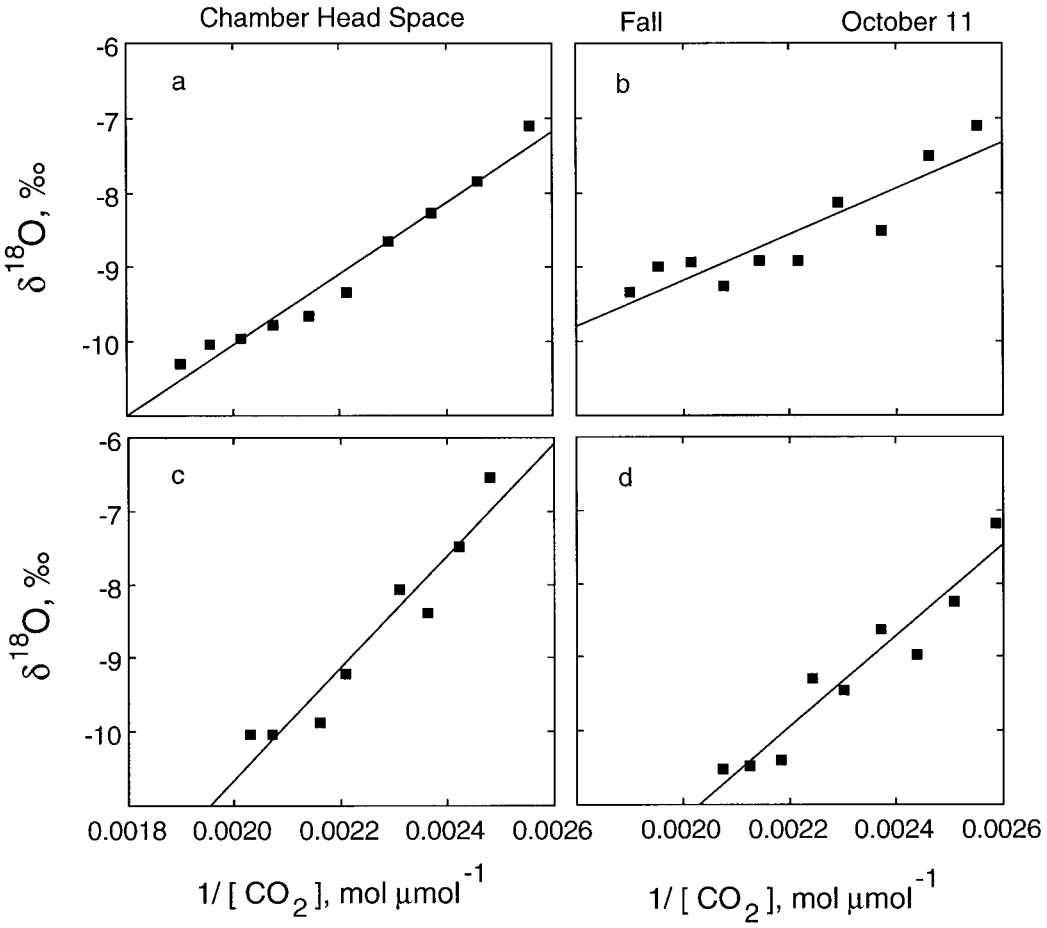

Fig. 10. Comparison of the relationships between $1/CO_2$ concentration and the oxygen isotope ratio of CO_2 in air samples collected from moss/soil respiration chambers in a black spruce forest in Saskatchewan during 11 October 1996. Format of the figure is the same as that described in Fig. 3.

4. Conclusions

Despite wide variation in the $\delta^{13}C$ values of organic matter among above-ground plant species, and along a continuum from moss through to the mineral soil, the carbon isotope ratio of respired CO_2 was quite similar to the $\delta^{13}C$ value for the dominant black spruce foliage. The CO_2 released from the forest floor during the fall was slightly enriched in ^{13}C compared to CO_2 respired by the entire ecosystem. This may be related to the fact that older carbon in soil organic matter was fixed during times when atmospheric CO_2 was more enriched in ^{13}C. There was minor temporal variation the $\delta^{13}C$ value of respired CO_2, perhaps associated with shifts in environmental conditions that can alter discrimination against $^{13}CO_2$ during photosynthesis and change the relative proportions of CO_2 originating from respiration by tree foliage, tree root, moss, and soil heterotrophic soil processes.

Short-term changes in the oxygen isotope ratio of precipitation and variation in enrichment of ^{18}O during evaporation and transpiration had significant effects on the $\delta^{18}O$ value of respired CO_2. Changes in the oxygen isotope ratio of water in moss tissue can have a large effect on total ecosystem respired CO_2 because of the large surface area covered by moss tissue in this ecosystem, and the fast equilibra-

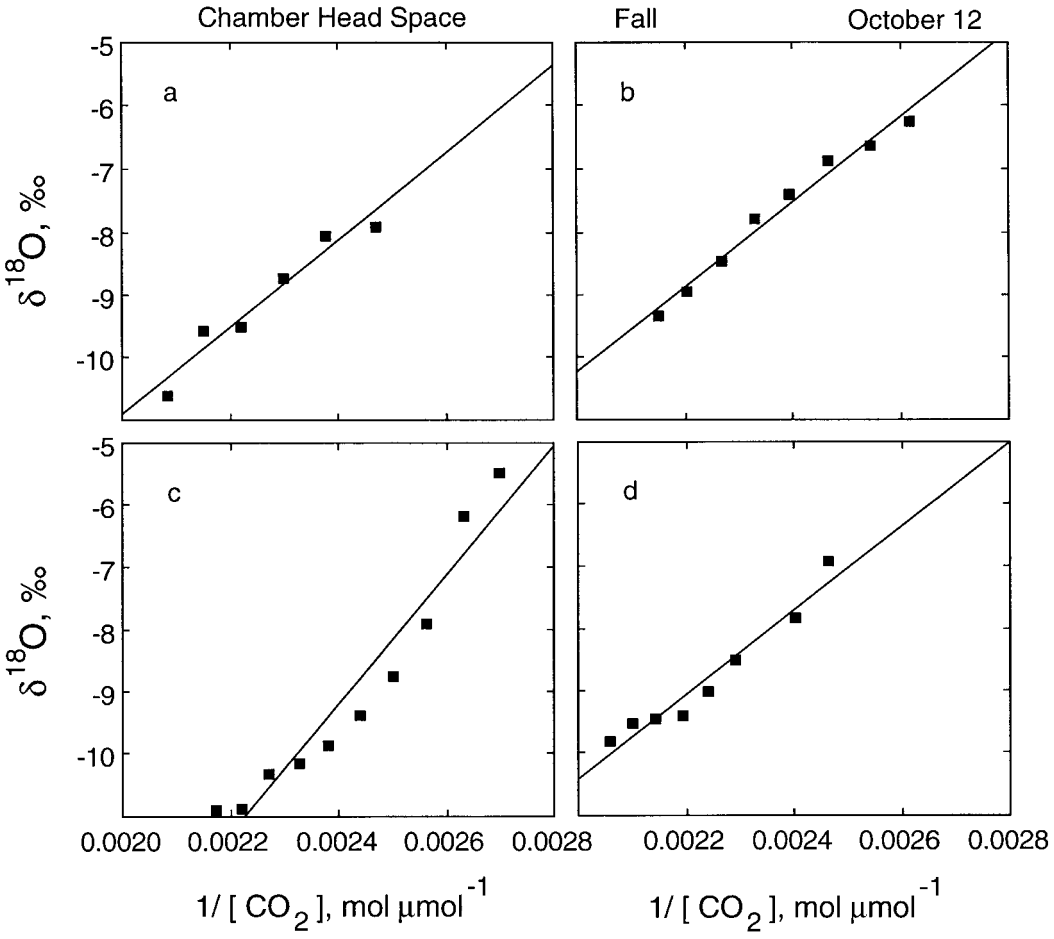

Fig. 11. Comparison of the relationships between $1/CO_2$ concentration and the oxygen isotope ratio of CO_2 in air samples collected from moss/soil respiration chambers in a black spruce forest in Saskatchewan during 12 October 1996. Format of the figure is the same as that described in Fig. 3.

tion between CO_2 diffusing through the moss and water in moss tissue. We observed during the summer that the $\delta^{18}O$ value of CO_2 respired from the forest floor was relatively depleted in ^{18}O compared to CO_2 respired from the entire ecosystem. This was because water in black spruce foliage had higher $\delta^{18}O$ values than moss and soil water, even at night when transpiration had stopped.

Large environmental changes may occur within a season or between years that strongly influence a number of isotopic fractionation processes during CO_2 exchange between the terrestrial biosphere and the atmosphere. Global models attempting to use stable isotope signals for separating the effects of terrestrial photosynthesis and respiration need to incorporate realistic, dynamic models of physical and physiological processes to deal with this temporal and spatial variation in isotopic fractionation processes.

5. Acknowledgments

This work was supported by the Natural Sciences and Engineering Research Council of Canada through grants to LBF and by grants to JRE from NASA BOREAS. We thank Craig Cook, Julie Forget, Rob Swanson and Tim Williams for help with the field work and preparation of isotope samples.

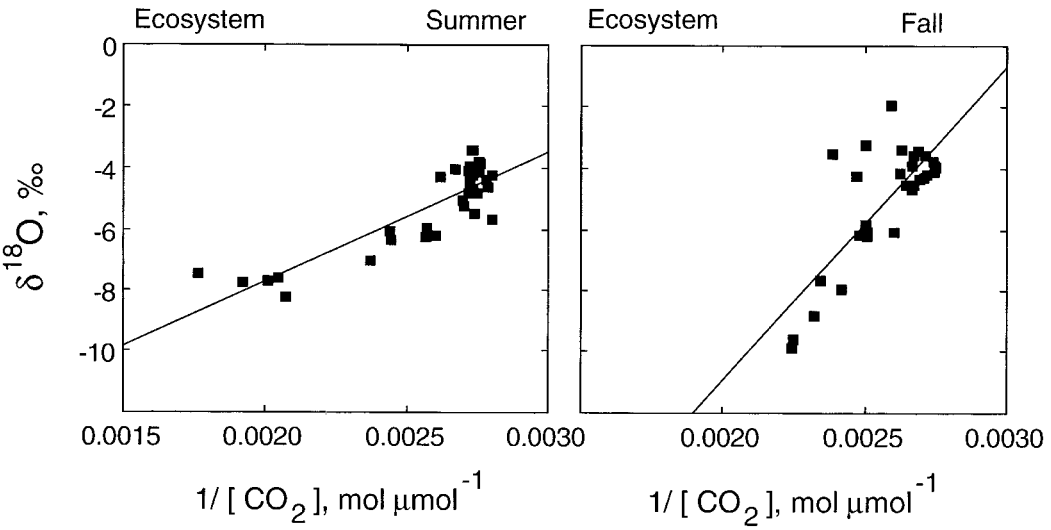

Fig. 12. Comparison of the relationships between $1/CO_2$ concentration and the oxygen isotope ratio of CO_2 in air samples collected from different heights above ground at night in a black spruce forest in Saskatchewan during the summer and fall 1996.

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