Carbon and oxygen isotope ratios of ecosystem respiration along an Oregon conifer transect: preliminary observations based on small-flask sampling

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Summary  Isotope ratio analyses of atmospheric CO₂ at natural abundance have significant potential for contributing to our understanding of photosynthetic and respiration processes in forest ecosystems. Recent advances in isotope ratio mass spectrometry allow for rapid, on-line analysis of small volumes of CO₂ in air, and open new research opportunities at the eco-physiological, whole-organism, and atmospheric levels. Among the immediate applications are the carbon and oxygen isotope ratio analyses of carbon dioxide in atmospheric air. Routine analysis of carbon dioxide in air volumes of approximately 50–300 µl is accomplished by linking a commercially available, trace gas condenser and gas chromatograph to an isotope ratio mass spectrometer operated in continuous-flow mode. Samples collected in the field are stored in either gastight syringes or 100-ml flasks. The small sample volume makes it possible to subsample the air for CO₂ and then to sample the remaining air volume for the analysis of the isotopic composition of either methane or nitrous oxide. Reliable δ¹³C and δ¹⁸O values can be obtained from samples collected and stored for 1–3 days. Longer-term storage, on the order of weeks, is possible for δ¹³C measurements without drift in the isotope ratio signal, and should also be possible for δ¹⁸O measurements.

When linked with an infrared gas analyzer, pump and flask sampling system, it is feasible to sample CO₂ extensively in remote forest locations. The air-sampling system was used to measure the isotope ratios of atmospheric CO₂ and to conduct a regression analysis of the relationship between these two parameters. From the regression, we calculated the δ¹³C of ecosystem respiration of four coniferous ecosystems along a precipitation gradient in central Oregon. The ecosystems along the coast-to-interior Oregon (OTTER) gradient are dominated by spruce-hemlock forests at the wet, coastal sites (> 200 cm precipitation annually) to juniper woodlands (20 cm precipitation) at the interior, dry end of the transect. The δ¹³C values of ecosystem respiration along this transect differed by only 1.3‰ (range of -25.2 to -23.9‰) during August at the peak of the summer drought. Following autumn rains in September, the δ¹³C of ecosystem respiration in the four stands decreased; overall the difference in the carbon isotope ratio of ecosystem respiration among sites increased to 3.9‰ (-26.8 to -22.9‰).

Introduction

An understanding of the interactions among the atmosphere, biosphere, and pedosphere is essential for interpreting the role of terrestrial ecosystems on global carbon dynamics (Tans et al. 1990, 1996, Keeling et al. 1995, 1996, Lloyd et al. 1996). Current anthropogenic CO₂ emissions continue to exceed the capacity of the earth’s ecosystems to fix that carbon photosynthetically, resulting in continual net increases in global atmospheric CO₂ concentration. Although there are strong indications that terrestrial ecosystems are currently a net carbon sink for a significant fraction of the annual anthropogenic emissions, there is still debate over which specific ecosystems are primary sinks for this carbon and how this sink strength varies on an inter-annual basis (Tans et al. 1990, Ciais et al. 1995a, Keeling et al. 1995, 1996). The analysis of both the concentrations and stable isotope ratios of CO₂ provides one of the most important signals for quantifying these ecosystem–atmosphere interactions (Tans et al. 1990, 1996, Farquhar et al. 1993, Ciais et al. 1995b). Yet acquiring large numbers of samples to analyze has been a challenge, in part because of the large volumes of air needed for each analysis.

In photosynthesis, fractionation events occur, resulting in an organic carbon isotope composition that is different from that of the atmosphere. Farquhar et al. (1989) showed carbon isotope discrimination at the leaf level (Δ) as:

\[ \Delta = a + (b - a) \frac{c_i}{c_a} \]  

where \( a \) is the isotopic fractionation associated with the slower diffusion of ¹²CO₂ in air, \( b \) is the net fractionation associated with RuBP carboxylase, and \( c_a \) and \( c_i \) are the atmospheric and intercellular CO₂ concentrations, respectively. Expressing the carbon isotope composition in terms of discrimination (Δ) has the advantage that it allows one to factor out the influences of canopy air, the ¹³C source (δ¹³C), from the product, the organic carbon isotope ratio (δ¹³Cp), and instead to focus on the bio-
logical processes influencing the product. Conversion between \( \Delta \) and leaf carbon isotope ratio (\( \delta^{13}C_p \)) follows a simple algebraic relationship:

\[
\Delta = \frac{\delta^{13}C_a - \delta^{13}C_p}{1 + \delta^{13}C_p}.
\]  

(2)

Variations in the \( \delta^{13}C_p \) values of tree species range more than 8‰, which is equivalent to a change in the intercellular CO\(_2\) concentration of ~75 µl l\(^{-1}\). Such large changes in \( c_i \) and \( c_l/c_a \) have significant effects on leaf-level gas exchange, including photosynthetic rate, stomatal limitation on photosynthesis, and water-use efficiency (Farquhar et al. 1989). Subsequent to carbon fixation by the leaf, there is no significant discrimination during carbon loss as mitochondrial respiration (Lin and Ehleringer 1997), leading to the expectation that the isotope ratio of leaf respiration (\( \delta^{13}C_R = \delta^{13}C_{R,p} \)) should be similar to \( \delta^{13}C_p \). Although measuring \( \delta^{13}C_p \) of individual leaves has become routine, measurements of the isotope ratio of respiration within an ecosystem or incorporating all components of the ecosystem are far less common.

Given an overall objective of linking biosphere–atmosphere processes, we should be measuring the isotope ratio of all respiratory CO\(_2\) losses by the ecosystem or the overall carbon isotope discrimination by the ecosystem after phototrophic CO\(_2\) uptake. Although Lin and Ehleringer (1997) have recently shown that there is no significant discrimination during carbon loss as mitochondrial respiration, spatial and temporal mismatches in carbon gain and carbon loss reduce the likelihood that simple measurements of \( \delta^{13}C_p \) can be extrapolated directly to ecosystem-level gas exchange. For instance, turnover rates of soil carbon are slower than turnover rates of aboveground leaf materials (Bird et al. 1996, Trumbore et al. 1996), and, therefore, a temporal disequilibrium in the isotope ratio of respiration must exist between above- and belowground respiration components.

In early studies, Keeling (1958) showed that analyses of atmospheric concentration and isotope ratio of CO\(_2\) in a canopy could be used to determine the isotope ratio of respiration. The \( \delta^{13}C \) of CO\(_2\) currently respired by an ecosystem (\( \delta^{13}C_R \)) will integrate both the carbon isotope composition of current-year aboveground components as well as the litter and soil organic matter deposited in previous periods. We can partition \( \delta^{13}C_R \) into its two components as:

\[
\delta^{13}C_R = \delta^{13}C_{R,p}f + \delta^{13}C_{R,s}(1 - f),
\]  

(3)

where the subscripts p and s refer to aboveground canopy and belowground soil respiration components, respectively, and \( f \) represents the fraction of the total respiratory flux coming from aboveground canopy sources.

Buchmann et al. (1998) defined ecosystem carbon isotope discrimination as (\( \Delta_e \)):

\[
\Delta_e = \frac{\delta^{13}C_i - \delta^{13}C_R}{1 + \delta^{13}C_R},
\]  

(4)

where \( \delta^{13}C_i \) is the carbon isotope ratio of bulk tropospheric CO\(_2\) and \( \delta^{13}C_R \) is the integrated carbon isotope ratio of respired CO\(_2\) from the ecosystem. This equation is similar to the definition of leaf-level carbon isotope discrimination (discrimination between leaf and air beyond its boundary layer), but is scaled to incorporate the appropriate end members for ecosystem isotope discrimination. This is because the ultimate gas exchange and discrimination of interest is that between the free troposphere (beyond the surface planetary boundary layer, PBL) and the terrestrial ecosystem. Measurements in the PBL will be a mix of \( \delta^{13}C_i \) and \( \delta^{13}C_s \) values, although they will often be close to that of the upper atmosphere.

The value of \( \delta^{13}C_R \) is determined by sampling atmospheric air collected within the canopy over a range of CO\(_2\) concentrations (Keeling 1958). When \( \delta^{13}C \) of the atmospheric CO\(_2\) is plotted as a function of the inverse of the CO\(_2\) concentration, the y-intercept is the integrated isotope ratio of the CO\(_2\) contributing to ecosystem respiration. What limits our ability to extend such estimates is that 15–25 air samples are needed for a reliable estimate of \( \delta^{13}C_R \). In the traditional approaches, 2-l flasks of air are collected for each isotope ratio analysis, creating a requirement for large amounts of space to accommodate the flasks and preventing such analyses from becoming routine.

In this paper, we show that reliable estimates of ecosystem respiration and ecosystem carbon isotope discrimination can be obtained from analysis of small amounts of canopy air, allowing for extensive field sampling without large amounts of equipment or taking cryogenic extraction lines to the field. This small-flask approach allows for the sampling of ecosystem isotope characteristics over a broad geographical scale, and will be particularly important for field sampling in remote locations.

**Methods**

To measure the \( \delta^{13}C \) value of CO\(_2\) in air on small sample volumes (50–300 µl of air), we used an isotope ratio mass spectrometer (MS) operating in continuous-flow (CF) mode. A helium carrier gas was used in conjunction with a gas chromatograph (GC) to separate CO\(_2\) and N\(_2\)O, and this is preceded by a trace gas condensing device (PreCon) to separate CO\(_2\) and N\(_2\)O from the other gases in atmospheric air. The PreCon was originally developed by Finnigan MAT (Bremen, Germany) for condensing typical abundances of N\(_2\)O and CH\(_4\) from 100-ml volumes of atmospheric air for isotope ratio analysis, but it also provides a convenient means of extracting and condensing small volumes of CO\(_2\).

**Set-up of the PreCon-GC-CF-MS**

A Varian GC (Model 3400, Walnut Creek, CA) was arranged so that its effluent was sent through a 1:2 fixed post column split interface via a capillary to an isotope ratio mass spectrometer (Model 252, Finnigan MAT) operating in CF mode. The GC has a 25-m POROPLOT column and was operated at a column temperature of 25 °C, which was sufficient to separate the CO\(_2\) and N\(_2\)O peaks. The helium stream (at 0.06 MPa)
entering the GC came from an automated condensing trap (PreCon, Finnigan MAT), which was used as a convenient means of introducing a mixture of CO₂ and N₂O that had been frozen from the air sample. Use of the PreCon involved attaching 12-mm Y-shaped tubing with a septum port to the helium carrier stream (at 0.14 MPa) of the PreCon before the liquid nitrogen condenser loops, which froze and isolated CO₂ and N₂O from the air.

A 500 µl gas-tight, locking syringe (Model A-2, VICI Precision Sampling, Baton Rouge, LA) was then used to inject 50–300 µl of air into the helium carrier stream for analysis. Before injection, the syringe was over-pressurized slightly to ensure that the needle dead volume was cleansed before sample injection. In sampling field and laboratory flasks, a presample was collected and spent to ensure that the needle dead volume was clean before flask subsampling. Blanks were run every tenth sample to adjust for any laboratory air CO₂ that might have been introduced by the valve-switching system on the PreCon; this correction was generally ~2–3% of the gas volume.

All isotope ratio data are expressed in delta notation (δ) and are presented relative to the Pee Dee Belemnite standard for δ¹³C and relative to the standard mean ocean water (SMOW) scale standard for δ¹⁸O.

Comparison of isotope ratios of CO₂ from small and large samples

To test the accuracy and precision of using small volumes (~100–300 µl) of atmospheric air in place of large volumes (1–2 l), a series of experiments was conducted in which small volumes were extracted and analyzed from large flasks. To do this, a series of 1.7-l flasks were individually prepared by filling the flasks with atmospheric air, removing all of the CO₂ by circulating the flask air through an Ascarite trap in a closed loop fashion, and then adding CO₂ containing isotope ratios to the flasks to recreate an atmospheric concentration of ~350 ppmv. A septum was attached to each flask next to the inlet stopcock, which allowed 500-µl air samples to be extracted with a gas-tight syringe, but did not result in a significant decrease in the flask pressure. After all of the syringe sampling had been completed, the CO₂ was cryogenically extracted from each 1.7-l flask and analyzed for δ¹³C and δ¹⁸O by traditional off-line approaches (Buchmann et al. 1997a).

Flask collection in the field

To evaluate the potential of a small-flask system for use in the field, we constructed flasks that had a volume of 100 ml. This volume was a sufficient buffer to obtain a steady CO₂ reading when air was analyzed immediately after it had flowed through the flask. Each flask had a vacuum stopcock (with Viton® O-ring) at the entry and exit openings. In the middle of the flask was a septum port, which provided a convenient means of extracting small volumes of air from the flask.

During the summer of 1996, four forest stands along the Oregon coast-to-interior (OTTER) transect (Peterson and Waring 1994, Runyon et al. 1994) were sampled to determine if differences in the isotope ratio of ecosystem respiration (δ¹³CR) were detectable. These stands spanned the entire conifer precipitation gradient and included spruce-hemlock (Cascade Head, 44°3’ N, 123°57’ W, elev. 49 m), Douglas-fir (Corvallis, 44°6’ N, 123°16’ W, elev. 60 m), ponderosa pine (Metolius, 44°25’ N, 121°40’ W, elev. 1027 m) and juniper (Sisters, 44°17’ N, 121°19’ W, elev. 930 m). Air samples were collected in late May, early August, and mid-September, 1996. However, May samples at all sites were lost except those from Cascade Head.

Canopy air was sampled from different heights within the canopy (0.3–55 m, depending on the forest) during both daytime and nighttime. Dry air (by using magnesium perchlorate) was drawn through tubing (Dekoron 1300, 0.625 cm O.D., non-buffering ethylene copolymer coating: Aurora, OH), which was attached to portable masts. With the aid of a battery-operated 12-V pump (TD-3LS, Brailsford and Company Inc., Rye, NY), air was drawn for 5 min at a flow rate of 10 ml s⁻¹ through each 100-ml glass flask (with two high-vacuum stopcocks) before both stopcocks were closed. Up to four flasks were collected at the same time using separate lines.

Air was sampled on the coast (Cascade Head), in order to estimate free tropospheric values. During each sampling period, there were strong onshore breezes at the time of sample collection.

Results

Evaluation of PreCon-GC-CF isotope ratio analyses of small quantities of CO₂

Precision of the measurements of δ¹³C and δ¹⁸O from 300-µl air samples was determined by sequential measurements from the same sample flasks (Figure 1). The standard errors for δ¹³C and δ¹⁸O measurements for the data in Figure 1 were 0.021 and 0.028‰, respectively, leading to 95% confidence intervals of ±0.041 and ±0.056‰. When the same measurements were repeated on a different day, the standard errors for δ¹³C and δ¹⁸O were slightly higher at 0.028 and 0.059‰, respectively, suggesting some day-to-day variations in overall precision.
Subsequent analyses have indicated that small variations in column or capillary temperatures, or both, associated with changes in laboratory air temperatures may affect overall precision.

To compare isotope ratios of CO2 measured using 300-μl samples (analyzed in CF mode) with traditional off-line analysis of the entire 1.7-l flasks (analyzed in dual inlet mode), three flasks were created containing CO2 at atmospheric concentrations, but varying in δ13C from −8.49 to −16.85‰ and in δ18O from −23.39 to −41.71‰. When syringe-extracted subsamples of the air from these flasks were analyzed, two observations were clear. First, there were no memory effects between sequential gas samples of high to low isotope ratio and, second, there were no statistically significant differences between the two measurement approaches.

Flasks were also sampled over time to determine if there might be isotopic exchange between the small volume of CO2 present in the flask (typically 1.6 nmol CO2) and either the glass walls or the teflon plunger of the stopcock. Sixty-five field-collected flask samples were randomly chosen and analyzed 26–30 days following initial sample collection; these samples were then re-analyzed 10–29 days after the initial isotope ratio analysis. These samples had changed in either δ13C or δ18O values between the initial and second measurements (r2 < 0.03, not significant). However, when all 65 samples are considered there was a mean shift in δ13C of 0.06‰ and a mean shift in δ18O of −0.18‰. Most of these changes can be attributed to eight of the 65 flasks that stood out from the remainder of the samples in the scatter-diagram analysis. These samples had changed in δ13C value by more than 0.5 between sampling periods. Most probably the size of the needle puncture through the septum varied among flasks providing an opportunity in some flasks for an exchange with atmospheric air in the laboratory. In our sampling, we observed no time trend in δ18O values over the 10–29-day period for the other 57 samples.

Because of possible exchange between the Viton® O-rings and CO2, Revesz and Coplen (1991) have cautioned that an increase in δ18O ratios of CO2 (but not δ13C values) can occur with small gas volumes. However, in our flasks, the Viton® O-rings were on the central portion of the stopcock plunger, above the terminal portion of the plunger that made a contact seal with the glass flask. Thus, the O-rings were not directly exposed to the stored gas volume and possible isotopic exchange with the O-rings would not have posed a problem.

### Isotopic composition of ecosystem respiration along the OTTER transect

The four forest stands along the OTTER transect were sampled during early spring (late May), during the summer drought period in early August, and again in September following a precipitation event that occurred at the three wettest sites. Unfortunately, flask samples from three of the four sites during the May sampling period were lost.

At Cascade Head, the carbon isotope ratio of ecosystem respiration (δ13C_R) was most negative in the early spring and late fall and more than 1‰ more positive during the summer drought period (Figure 2). The changes in δ13C_R values during the season could indicate changes in the fractional contribution of aboveground and belowground components to overall respiration rates or changes in the isotope ratio of aboveground respiration in response to drought. Without isotope ratio values of aboveground and soil respiration components, it is not possible to decide between the probable causes.

The carbon isotope ratio of tropospheric CO2 (δ13C_t) increased from −8.55‰ in late May to −7.92‰ in early August and finally to −7.87‰ by mid-September. When δ13C_R and δ13C_t values were combined to calculate ecosystem carbon isotope discrimination values (Δ), the lowest discrimination values occurred in midsummer (16.5‰), associated with the most positive δ13C_R values (Figure 2). However, we calculated that the highest Δ values (18.4‰) occurred in late fall, as a result of large changes in δ13C, values that occurred as the growing season progressed.

With 15–23 flasks collected and analyzed per site, we obtained highly significant linear relationships in a Keeling-plot analysis at the three other sites along the OTTER transect (Figure 3). These strong regression coefficients persisted regardless of whether the δ13C variations in atmospheric CO2 spanned 5‰ as at the ponderosa pine site or 1.2‰ as at the juniper site.

The δ13C_R values at the three driest sites increased as the mean precipitation at the site decreased (Figure 3). During peak summer drought, which occurred during the August sampling period, δ13C_R values differed by 1.3‰ between the Douglas-fir and juniper sites. Following the autumn rains at the Douglas-fir and ponderosa pine sites, δ13C_R decreased by 1.6
and 0.9‰, respectively. However, rains had not yet occurred at the juniper site and δ13C value continued to decrease by 1‰ from its August value.

The δ18O values in CO2 should vary with changes in both plant water source and humidity conditions, whereas δ13C values should change with ci/ca ratios. At all but the juniper site in September, the carbon and oxygen isotope ratios of canopy CO2 were highly correlated (Figures 4 and 5). Oxygen isotope ratios were uniformly higher in September than in August. This covariance of isotope ratio values suggested a common source was responsible for the changes in both isotope ratios. A Keeling-plot analysis of the relationship between the oxygen isotope ratio of CO2 and the inverse of canopy CO2 concentration values at the spruce-hemlock site in September predicted that the δ18O value of the source CO2 was 27‰ (Figure 6). The source was likely to be soil-respired CO2, canopy-respired CO2, or a combination of the two. Assuming a mean temperature of 20 °C and complete equilibration, soil at Cascade Head (soil water value of −7‰) was predicted to release CO2 with a δ18O value of 26‰, whereas leaves (leaf water value of 0‰) were predicted to release CO2 with a δ18O value of 34‰ (J. Roden, University of Utah, Salt Lake City, and J. Ehleringer, unpublished data). Similar patterns were evident for δ18O values at other sites along the transect.

**Discussion**

Changes in the concentration and stable isotope ratio of atmospheric CO2 can be used to study variations in ecosystem-level processes at forest, stand, and regional spatial scales (Ciais et al. 1995b, 1997, Flanagan and Ehleringer 1997, Nakazawa et al. 1997). Recently measured changes in the magnitude and the
timing of seasonal fluctuations in atmospheric CO₂ concentration (Keeling et al. 1995, 1996) imply an imbalance between terrestrial ecosystem photosynthesis and respiration associated with a lengthening of the growing season in northern latitudes. However, based on changes in CO₂ concentration alone, it is difficult to separate these ecosystem effects, because variations in CO₂ concentration are the net result of differences in large one-way CO₂ fluxes associated with photosynthesis and respiration. Stable isotope techniques can be used to separate the effects of photosynthesis and respiration, because these processes have contrasting effects on both the carbon and oxygen isotope ratios of atmospheric CO₂. Extensive measurements of the isotope ratios of ecosystem respiration are needed to characterize CO₂ fluxes between the ecosystem and the atmosphere. The Keeling-plot approach of measuring local variations in CO₂ concentration simultaneously with the isotopic composition of that CO₂ has been a productive approach for quantifying the isotopic composition of CO₂ fluxes into and out of ecosystems (Keeling 1958, 1961, Sternberg et al. 1989, Broadmeadow et al. 1992, Flanagan et al. 1996, Buchmann et al. 1997a, 1997b).

One factor that has limited application of the Keeling-plot approach on a broad geographical basis has been the bulkiness of traditional air sampling flasks (2- to 4-l flasks), which prevents obtaining large numbers of field samples, especially from remote locations, on a routine basis. Modification of the traditional approach for sampling and analyzing air for determination of isotope characteristics at the ecosystem level is possible if some sacrifice in the stable isotope precision of the individual flask measurement is acceptable. That is, if we can accept a decrease in precision from 0.006‰ to 0.03‰, which is the basic loss in precision in going from dual inlet to continuous flow isotope ratio analyzers for atmospheric CO₂, much smaller gas volumes are required for isotope ratio analysis. As shown earlier, we were able to get sufficient amounts of CO₂ from only 200–300 µl of atmospheric air for δ¹³C and δ¹⁸O measurements of CO₂ that are adequate for determination of a Keeling plot. However, when a 100-ml flask of atmospheric air is collected, the isotope ratios of methane and nitrous oxide can also be determined on the remainder of the same sample. By substituting 100-ml flasks for 1-2-l flasks we were able to collect >200 flasks in the field for sampling without requiring extensive storage space and eliminating the need for CO₂ to be cryogenically extracted from flasks in the field. Thus, compared with the traditional sampling method, the use of 100-ml flasks allowed much more extensive field sampling over a shorter period and enabled the samples to be processed more quickly.

Because stomata tend to restrict gas exchange under drier atmospheric or soil-drought conditions (Schulze 1986, 1994), δ¹³C values might be expected to increase under conditions of low water availability or high evaporative demand (Farquhar et al. 1989, Ehleringer et al. 1993, Lloyd and Farquhar 1994). Therefore, based on the finding that there is no isotopic fractionation during respiration (Lin and Ehleringer 1997), the δ¹³C values should be similar to leaf δ¹³C values. Preliminary data from the OTTER transect confirm a pattern of decreasing carbon isotope ratios from ecosystems along a geographical gradient of increasing water availability. Moreover, the δ¹³C values decreased further at each of the sites following autumn rains. Because the spruce-hemlock ecosystem at Cascade Head is at the location with the highest water input, we expected δ¹³C values to be the most negative at that site. However, they were not. A possible explanation is that the forest sampled at Cascade Head is very mature, whereas the other stands were less than a century old. Runyon et al. (1994) noted that net primary productivity of this particular spruce-hemlock site was less than that of the Douglas-fir site, even though the Douglas-fir stand received less precipitation. Yoder et al. (1994) suggested that coniferous trees in these older stands had reduced photosynthetic rates. They also observed that older trees had increased leaf δ¹³C values, indicating that hydraulic constraints had impacted both photosynthetic rates and leaf intercellular CO₂ values. If increased δ¹³C values typify older conifers at a site, then this finding provides a possible explanation for the reduced δ¹³C values at the Cascade Head spruce-hemlock site.

We conclude that small-flask sampling offers the potential for rapid acquisition of a large number of field samples for Keeling-plot analyses of ecosystem respiration. Our preliminary isotope ratio data obtained using small flasks suggest that an isotopic analysis of how coniferous forest ecosystems respond to site water balance over time and across space will yield new insights into constraints on ecosystem function and will also provide data of interest to global modelers conducting inverse modelling of CO₂ sources and sinks.

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