The application and interpretation of Keeling plots in terrestrial carbon cycle research
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[1] Photosynthesis and respiration impart distinct isotopic signatures to the atmosphere that are used to constrain global carbon source/sink estimates and partition ecosystem fluxes. Increasingly, the “Keeling plot” method is being used to determine the carbon isotope composition of ecosystem respiration (δ13C_R) in order to better understand the processes controlling ecosystem isotope discrimination. In this paper we synthesize emergent patterns in δ13C_R by analyzing 146 Keeling plots constructed at 33 sites across North and South America. In order to interpret results from disparate studies, we discuss the assumptions underlying the Keeling plot method and recommend standardized methods for determining δ13C_R. These include the use of regression calculations that account for error in the x variable, and constraining estimates of δ13C_R to nighttime periods. We then recalculate δ13C_R uniformly for all sites. We found a high degree of temporal and spatial variability in C3 ecosystems, with individual observations ranging from −19.0 to −32.6‰. Mean C3 ecosystem discrimination was 18.3‰. Precipitation was a major driver of both temporal and spatial variability of δ13C_R, suggesting (1) a large influence of recently fixed carbon on ecosystem respiration and (2) a significant effect of previous climatic effects on δ13C_R. These results illustrate the importance of water availability as a key control on atmospheric 13CO2 and highlight the potential of δ13C_R as a useful tool for integrating environmental effects on dynamic canopy and ecosystem processes.

INDEX TERMS: 0315 Atmospheric Composition and Structure: Biosphere/atmosphere interactions; 0322 Atmospheric Composition and Structure: Constituent sources and sinks; 1615 Global Change: Biogeochemical processes (4805); 1694 Global Change: Instruments and techniques; 3322 Meteorology and Atmospheric Dynamics: Land/atmosphere interactions; KEYWORDS: carbon cycle, carbon isotopes, ecosystem respiration, carbon dioxide, terrestrial ecosystems


1. Introduction

[2] The existing and potential feedbacks between terrestrial ecosystem processes and atmospheric CO2 concentrations remain one of the largest uncertainties in our understanding of the global carbon cycle. The balance of photosynthesis and ecosystem respiration appears to be strongly influenced by interannual variability in climate with discernable effects on global CO2 concentrations [Bousquet et al., 2000], although the exact mechanisms remain unclear. In order to explain the current regional distribution of terrestrial carbon sources and sinks we must gain a better understanding of the factors controlling Net Ecosystem Exchange (NEE) of CO2 both on short timescales and over long periods.

[3] NEE is now measured around the world in numerous ecosystems as part of FLUXNET, the international network of eddy covariance sites [Baldocchi et al., 2001]. Key products of FLUXNET studies include responses of NEE and its components to environmental variables. Methods to partition NEE into Gross Primary Production (GPP) and respiration are numerous, and include estimating ecosystem respiration from nighttime measurements [Goulden et al., 1996], scaling cuvette measurements [Law et al., 1999], and applying process models [Baldocchi and Meyers, 1998]. Because of the potentially different effects of photosyn-
thesis and respiration on the isotopic composition of CO₂, analysis of air samples for the carbon and oxygen isotope ratios of carbon dioxide within and above the canopy has also been proposed as a tool for understanding the components of NEE [Baldocchi et al., 1996; Flanagan and Ehleringer, 1998; Yakir and Wang, 1996]. This isotopic approach has now been combined with eddy covariance measurements [Bowling et al., 2001] and offers an additional and independent means of partitioning photosynthesis and ecosystem respiration interannually and across FLUXNET sites.

[4] A comprehensive effort to study the biophysical processes that control whole-canopy and whole-ecosystem discrimination at a number of sites around the world may contribute not only to ecosystem-scale carbon cycle studies, but also to global estimates of carbon sources and sinks. The carbon isotope ratios of CO₂ in the atmosphere are used to partition the regional carbon sources/sinks inferred from atmospheric CO₂ measurements and physical transport models [Battle et al., 2000; Ciais et al., 1995; Francey, 1985; Keeling et al., 1989]. The mechanistic basis for this approach is the influence of photosynthetic enrichment and respiratory depletion of 13CO₂ on the isotopic composition of the atmosphere. Therefore, C₃ and C₄ photosynthetic discrimination as well as the isotopic disequilibrium between current photosynthetic fixation and respired CO₂ must be modeled for terrestrial ecosystems at large spatial scales [Fung et al., 1997; Kaplan et al., 2002; Lloyd and Farquhar, 1994]. Process models that predict the isotopic composition of ecosystems are based on the leaf-level discrimination equations given by Farquhar et al. [1989] and Farquhar and Sharkey [1982], and are just beginning to be tested with ecosystem-scale data [Buchmann and Kaplan, 2001]. As modeling efforts continue, a new understanding of the processes controlling isotopic discrimination at ecosystem and larger scales is likely to emerge. To facilitate this process, the Global Change and Terrestrial Ecosystems (GCTE) core project of the International Geosphere Biosphere Programme (IGBP) has initiated an international network to work in concert with the existing FLUXNET effort. The Biosphere-Atmosphere Stable Isotope Network (BASIN) consists of studies measuring the isotopic composition of ecosystems and their trace gas fluxes at FLUXNET sites as well as other sites around the world. Like FLUXNET, BASIN serves as an archive for network data and initiates international synthesis and modeling efforts on key ecosystem processes (http://getefocus1.org/basin.html).

[5] A consideration of experimental protocols, sampling schemes, and common data interpretation is essential to any effort to integrate data across disparate studies. General reviews of the application of isotopic techniques to ecosystem gas exchange measurements have been published previously [Flanagan and Ehleringer, 1998; Yakir and Sternberg, 2000]. In this paper we focus on one of the primary methods of extracting information on the isotopic composition of ecosystem fluxes. The “Keeling plot” method, first employed by Keeling [1958, 1961], is now commonly used and often cited, but seldom explicitly analyzed. We discuss the assumptions underlying the Keeling plot method in detail using data compiled from the literature and the BASIN online database in order to propose a common framework for the collection and interpretation of Keeling plot data. We then calculate Keeling plot intercepts in a uniform manner across all sites and present a synthesis of the patterns of the carbon isotope ratio of ecosystem respiration.

2. Keeling Plot Approach

[6] The basis of the Keeling plot method is conservation of mass. The atmospheric concentration of a gas in the canopy and adjacent boundary layer reflects the combination of some background atmospheric concentration and variable amounts of that gas added by sources in the ecosystem,

\[ \begin{align*}
  c_a &= c_b + c_S, \\
  \delta^{13}C_{a} &= \delta^{13}C_{b} + \delta^{13}C_{S},
\end{align*} \]

where \( c_a \), \( c_b \), and \( c_S \) are, respectively, the atmospheric CO₂ concentration measured in the ecosystem, the background CO₂ concentration, and the additional concentration component produced by the source, which has raised atmospheric CO₂ concentration above background. In this paper, we focus on CO₂ as the atmospheric gas and respiration as the source, but the same arguments and application will apply to other gases such as water vapor [e.g., Moreira et al., 1997], methane [e.g., Thom et al., 1993], and other isotope ratios including \(^{18}O/^{16}O\) and D/H (each application may have its own caveats).

[7] Given conservation of mass,

\[ \begin{align*}
  \delta^{13}C_{a} &= \delta^{13}C_{b} \delta^{13}C_{S}^{C_{S}},
\end{align*} \]

where \( \delta^{13}C \) represents the carbon isotope ratio of each CO₂ component. Combining equations (1) and (2),

\[ \begin{align*}
  \delta^{13}C_{a} &= c_b (\delta^{13}C_{a} - \delta^{13}C_{S}^{C_{S}}) + \delta^{13}C_{S},
\end{align*} \]

where \( \delta^{13}C_{S} \) is the integrated value of the CO₂ sources in the ecosystem. This is illustrated graphically in Figure 1.

[8] The linear regression approach was first used by Keeling [1958, 1961] to interpret fluctuations in the \( \delta^{13}C \) values of ambient CO₂ and to identify the sources that contributed to increases in atmospheric CO₂ in a forest canopy. Later studies extended this approach to other forest ecosystems [Buchmann et al., 1997a, 1997b; Flanagan et al., 1996; Harwood et al., 1999; Lancaster, 1990; Quay et al., 1989; Sternberg et al., 1989] and agricultural sites [Buchmann and Ehleringer, 1998]. Estimates of the carbon isotope composition of ecosystem respiration (\( \delta^{13}C_{R} \)) are essential for approaches using concentration and isotope measurements to partition net ecosystem fluxes of CO₂ into their photosynthetic and respiratory components [Bowling et al., 2001; Yakir and Wang, 1996]. Similar partitioning approaches have been used to distinguish root versus microbial respiration [Lin et al., 1999; Rochette and Flanagan, 1997; Rochette et al., 1999] and transpiration versus evaporation [Harwood et al., 1999; Moreira et al., 1997].
The CBL integrates the effects of photosynthesis, respiration, and turbulent transport of CO₂ over landscapes, thus increasing the spatial scale of discrimination measurements [Lloyd et al., 1996; Nakazawa and Sugawara, 1997].

[11] It is important to recognize that the model described by equations (1)–(3) involves two basic assumptions. First, we assume that a simple mixing of only two gas components is considered (a source S and the bulk background B). Second, we assume that the isotope ratio of these two components does not change over the course of the observation. It is rare for these two assumptions to hold true in a strict sense under natural field conditions. Rather, researchers have found appropriate points in time and space for which these assumptions are acceptable. Below we outline recommendations for minimizing error in the use of the Keeling plot for assessing the carbon isotope composition of respiration.

3. Statistical Analysis

[12] An important consideration in constructing Keeling plots is which regression formulation to use. The standard (“Model I”) linear regression assumes that the independent variable (1/CO₂ concentration in this case) has no errors associated with it, or that these errors are under the experimenter’s control [Sokal and Rohlf, 1995]. Furthermore, Model I regression also assumes that errors in the dependent variable (δ₁³C of atmospheric CO₂ in this case) are unrelated to the independent variable [Laws, 1997; Sokal and Rohlf, 1995]. These assumptions are violated when constructing a Keeling plot, as there are likely to be errors in estimating both δ₁³Cᵣ and CO₂ concentration that are related, as one is present in the other. In this case, if Model I regression is applied to determining the functional relationship between y and x, the slope is biased to smaller values, such that the intercept is biased to less negative values [Friedli et al., 1987; Sokal and Rohlf, 1995; Angleton and Bonham, 1995; Laws, 1997].

[13] There are several alternative regression formulations that attempt to account for errors in both x and y variables (for discussions see Sokal and Rohlf [1995] and Laws [1997]). A simple and widely used technique in this class is the Model II or geometric mean regression (GMR, also known as the reduced or standard major axis regression [Laws, 1997; Ricker, 1973; Sokal and Rohlf, 1995]). The slope of a Model II regression is simply calculated as the slope of a Model I regression divided by the R-coefficient of the x and y variables. To illustrate the effect of regression method on the Keeling plot intercept, δ₁³Cᵣ was calculated (inappropriately) with Model I and (correctly) with Model II regression for 146 individual Keeling plots in the BASIN database (see Figure 2 for a map and list of all sites used in the analysis). Intercepts were sorted into plots where the r² of the Model II results was less than 0.95 and plots where r² was greater than 0.95. As r² approaches unity, the slope and therefore the intercept values from Model I and Model II converge, as shown in Figure 3. This is because Model II effectively splits the difference between the two Model I predictive slopes that can be calculated for any set of 1/CO₂ and δ₁³C data: a regression of δ₁³C on 1/CO₂ (i.e., y on x) or a regression of 1/CO₂ on δ₁³C (x on y). Laws [1997] discusses this in further detail. However, as the correlation

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**Figure 1.** Graphical illustration of the Keeling plot method given as equation (3) in the text. The carbon isotope composition of two endpoints of source CO₂ (δ₁³Cₛ) and well-mixed, background atmospheric CO₂ (δ₁³Cᵣ) are shown in solid circles. The carbon isotope composition of sampled air (δ₁³Cₐ) is shown by open circles. Isotope ratios are plotted against the inverse of CO₂ concentration (c). Note the distance of the samples from the intercept.
of nighttime air samples to estimate the $\delta^{13}C$ value of respired CO$_2$ in an ecosystem. It is assumed in this case that when photosynthesis has ceased at night, only $^{13}C$-depleted, respired CO$_2$ is added to the atmosphere, and that $\delta^{13}C$ of both background and respired CO$_2$ are constant during that time. Many of the potential sources of error in applying the Keeling approach can be considered under these conditions.

4.1. Multicomponent Systems

Clearly, respired CO$_2$ originates from different sources including above and below ground respiration, and autotrophic and heterotrophic components. This may potentially violate the assumption of mixing only two compo-

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**Figure 2.** (opposite) Location of sites used in this analysis. Data sources: 1 and 2: C$_3$ primary forest and mixed C$_3$-C$_4$ pasture in Manaus, Brazil, details given by Ometto et al. [2002]; 3 and 4: C$_3$ primary forest and mixed C$_3$-C$_4$ pasture in Santarem, Brazil, details given by Ometto et al. [2002]; 5 and 6: C$_3$ primary forest and mixed C$_3$-C$_4$ pasture in Ji Parana, Brazil, details given by Ometto et al. [2002]; 7: coniferous forest in Yosemite National Park, California, details given by Lancaster [1990]; 8: mixed coniferous-deciduous forest in Cuyamaca Rancho State Park, California, details given by Lancaster [1990]; 9: deciduous forest in Red Butte canyon, Utah, details given by Buchmann et al. [1997b]; 10: deciduous and coniferous forests near Kanas, Utah, details given by Buchmann et al. [1997b]; 11: coniferous forest near Hamilton, Montana, details given by Lancaster [1990]; 12: C$_3$ grassland near Lethbridge, Alberta, from L.B. Flanagan (unpublished data, 1999); 13: coniferous and deciduous forests at the BOREAS Southern Study Area, details given by Flanagan et al. [1996, 1998]; 14: coniferous and deciduous forests at the BOREAS Northern Study Area, details given by Flanagan et al. [1996, 1998]; 15: mixed C$_3$-C$_4$ grassland at the Konza Prairie Long-Term Ecological Research Site, Kansas, from L.B. Flanagan (unpublished data, 1998); 16 and 17: coniferous forest and corn crop near Ottawa, Ontario, from L.B. Flanagan (unpublished data, 1998); 18: deciduous forest near Barnard, Vermont, details given by Lancaster [1990]; 19: deciduous forest in Harvard Forest, Massachusetts, from J.R. Ehleringer (unpublished data, 1991); 20: deciduous forest in Scotia Range, Pennsylvania, details given by Lancaster [1990]; 21: tropical forest in Chamela, Mexico, details given by Lancaster [1990]; 22: tropical forest in Barro-Colorado Island, Panama, details given by Lancaster [1990]; 23: tropical forest in Paracou, French Guiana, details given by Buchmann et al. [1997a]; 24: tundra ecosystem near Bethel, Alaska, details given by Lancaster [1990]; 25: tundra ecosystem near Toolik Lake, Alaska, details given by Lancaster [1990]; 26: coniferous forests near Seattle, Washington, details given by Buchmann et al. [1998b]; 27: coniferous forests at the Wind River Canopy Crane site, Washington, details given by Fessenden and Ehleringer [2002]; 28–33: various coniferous forests along the Oregon Transect for Ecological Research (OTTER), details given by Bowling et al. [2002]. See color version of this figure at back of this issue.
ponents in the linear model, but is not a concern if the $\delta^{13}C$ values of all these components are the same, or if their contributions to the total flux do not change proportionally over the time of sampling. In this case the spatial distribution of these fluxes dictates the locations of sample collection, depending on the question of interest. For instance, if we are interested in soil respiration and sample very near the ground (but not in the canopy), we will still have at least three CO$_2$ sources (background, heterotrophic respiration, and autotrophic respiration), but autotrophic and heterotrophic respiration with different $\delta^{13}C$ values may be represented by one well-mixed source of “soil” respired CO$_2$ above the ground. The estimated $\delta^{13}C_{R}$ in this case would be a flux-weighted mean value of the combined autotrophic and heterotrophic respiration belowground. If we have independent estimates of the specific $\delta^{13}C$ values of autotrophic and heterotrophic respiration, we could use equations (1)–(3) to partition soil respiration [e.g., Yakir and Sternberg, 2000]. Above and within the forest canopy, belowground and aboveground fluxes are combined in an estimate of whole ecosystem respiration; however, turbulent conditions often prevent a large buildup of CO$_2$ concentrations over rough canopies.

4.2. Footprint

[18] In the absence of herbaceous ground cover, measurements near the soil surface largely reflect the activity of a small patch of soil (small footprint) which may or may not be representative of the whole ecosystem. Although sampling footprint area increases with sampling height, maintaining the validity of the Keeling model assumptions may be difficult within the canopy. Under relatively stable conditions associated with canopy CO$_2$ buildup we may sample at different heights that reflect different mixtures of soil-respired, plant-respired and atmospheric CO$_2$ that may have distinct $\delta^{13}C$ values. If this is the case, the weighting of each value on the final Keeling plot intercept may be affected by the chosen locations of sample collection. Such complications are usually ignored as differences in sampling various height profiles have not been well distinguished empirically. Additional research on sampling profiles is encouraged; currently, researchers should be aware of potential influence of sampling profile on the estimate of $\delta^{13}C_{R}$ and describe their sampling scheme accordingly.

[19] For regional-scale estimates representing very large footprints, aircraft measurements in well-mixed portions of the convective boundary layer (CBL) look to be promising. In a recent study in Siberia, Lloyd et al. [2001] found a correlation between $\delta^{13}C$ and 1/CO$_2$ from aircraft flask measurements over very small CO$_2$ ranges of less than 3 ppm. As discussed below, when the Keeling plot is applied to small CO$_2$ ranges, the standard error around the intercept may be relatively large (1.6–5.6$\%$ in the Lloyd et al. study).

4.3. Temporal Variations in Isotopic Signals

[20] Recent studies question that the $\delta^{13}C$ in soil respired CO$_2$ is constant over timescales of days [Ekblad and Högberg, 2001]. Different organic substrates in the same plant have distinct $\delta^{13}C$ values, e.g., lipids are more depleted than carbohydrates by several per mil [Benner et al., 1987], and transition between different substrates for respiration may occur over short timescales (e.g., the diurnal cycle) [Durmanau et al., 1999; Ghashghaie et al., 2001]. Other recent studies also show that large changes in photosynthetic discrimination (on the order of 5$\%$) due to changes in environmental conditions are reflected in the $\delta^{13}C$ of respired CO$_2$ within a few days [Bowling et al., 2002; Ekblad and Högberg, 2001]. If the sampling period spans these temporal changes, the Keeling intercept will represent the intermediate of a family of lines with different slopes and intercepts. The best fit line may give a seemingly

![Figure 3. Comparison of Keeling plot intercepts from numerous study sites in North and South America as calculated with Model I (standard) regression and Model II (geometric mean) regression. (a) Data with Model II R-squares <0.95. (b) Data with Model II R-squares >0.95. The solid line is the 1:1 line.](image-url)
“reasonable” value for the source, but one that may fall outside of the range of the actual value of the sources due to nonlinearities and other violations of the two-ended linear mixing model assumptions. To date, published Keeling plot data has generally been collected during time periods spanning 2–8 hours, although some have been collected over consecutive nights [Bowling et al., 1999; Flanagan et al., 1996]. We know of no reports of dynamic changes in the carbon isotope composition of ecosystem or soil respiration within these short periods, but additional studies are necessary to resolve the possibility of dynamic changes at this temporal scale.

[21] These recent findings of large changes in Δ13CR over timescales of several days and longer provide examples of the potential complications in applying the Keeling-plot approach over long periods. However, they also demonstrate the importance of estimating nighttime Δ13CR in ecosystems as a dynamic indicator of plant physiological response to change. Repeated Keeling plot sampling over time can provide valuable information on plant physiological function in response to environmental variables, as well as dynamic changes in heterotrophic substrates that influence the isotopic composition of soil respiration [Bowling et al., 2002; Ekblad and Högberg, 2001].

4.4. Daytime Sampling

[22] Studies of the isotopic composition of ecosystem respiratory CO2 should be limited to nighttime sampling. In the daytime, it is difficult to avoid the problems caused by multicomponent systems and isotopic signals that vary over time. Firstly, as the land surface heats in the morning and cools at night, the planetary boundary layer (PBL) becomes more dynamic, such that there may be rapid changes in the background signal during periods of rapid boundary layer depth change. Advection of air masses that have passed over other ecosystems (plant, marine, urban, industrial) will influence the Keeling mixing line if changes in δ13C of background air occur during the measurement period. Respired CO2 from the soil surface may also be re-fixed by the lower canopy, particularly in tropical forests [Lloyd et al., 1996; Sternberg et al., 1997, 1989]. For example, this occurs if photosynthesis begins before turbulent mixing disrupts the nighttime CO2 profile, and it is likely to change over the time span of Keeling plot measurements.

[23] In addition, in many cases, photosynthesis and respiration may be at isotopic disequilibrium; that is, the change in the isotope ratio of atmospheric CO2 caused by a unit of respiratory flux is not equivalent to the opposite change caused by a unit of photosynthetic fixation. This may be particularly true over short time periods such as one diurnal cycle; indeed, this is the basis for using isotopes to partition photosynthesis and respiration in ecosystem-scale flux studies [Bowling et al., 2001; Yakir and Wang, 1996]. If photosynthetic and respiratory isotope effects on the atmosphere are not equal, combining nighttime and daytime measurements may create considerable uncertainty in the resulting intercept. To demonstrate the effect of diurnal sampling on the determination of Δ13CR, we have estimated Δ13CR in two ways for the data in the BASIN database, both with daytime-only and nighttime-only data. Daytime and nighttime measurements were distinguished by calculating sunrise and sunset for each location by its latitudinal and longitudinal coordinates [Campbell and Norman, 1998], and sorting measurements based on their time of collection (there may be carryover effects of storage following the initiation or cessation of photosynthesis at dawn and dusk but these are difficult to determine). The results showed considerable scatter around the 1:1 line, such that the daytime estimate may differ from the nighttime-only value by as much as 5% in either direction (Figure 4). Hence, it appears that respiratory effects on atmospheric CO2 commonly differ from recent photosynthetic effects, complicating the use of daytime data in Keeling plot estimates.

4.5. CO2 Range

[24] The exclusion of daytime data or sampling over very short time periods (several hours) may present another difficulty: that of obtaining a sufficiently large CO2 range for a Keeling plot analysis. A disadvantage of the Keeling plot approach is that it requires extrapolation far beyond the measured range of data to obtain the intercept (Figure 1), such that small uncertainties in the regression slope may lead to large uncertainties in the intercept [Tans, 1998]. In order to determine possible sampling strategies that reduce the standard error of the intercept, we analyzed the errors in the Keeling plot database using nighttime-only calculations with Model II regression (GMR).

[25] Assuming that measurement error has been minimized, two factors associated with sampling strategy are likely to influence the intercept. Intuitively, a broader CO2 range in the air samples for a given Keeling plot should provide a better estimate of the intercept, as the distance from the data points to the y axis decreases with a larger x range. Secondly, simply increasing sample size (n) may provide a smaller error in Δ13CR.

[26] We plotted the standard error of the intercept as a function of the CO2 range of the samples, sorting the data into categories corresponding to the number of air samples used in each plot (Figure 5). An inverse relationship was apparent. (Note that some data sets yielded very large standard errors exceeding 3%.) A multiple, second-order, curvilinear regression showed that CO2 range influenced the standard error of the intercept (p < 0.0001), but despite the autocorrelation between n and the standard error, the number of samples did not (p = 0.57). Thus, the error in the Keeling plot intercept is minimized by maximizing the range of CO2 collected, regardless of how many samples are involved. The data in Figure 5 suggest that, in general, to reliably maintain a standard error in Δ13CR below 1%, a CO2 range of approximately 75 ppm should be obtained, which occurs most commonly under stable conditions with relatively high respiratory fluxes.

4.6. Other Sampling Considerations

[27] Air samples for Keeling plot analysis have been collected in flasks ranging from 100 mL to 2 L in volume, such that the data set compiled for the present study represents a variety of air sample sizes. Previous work has shown that flask volume does not impact the quality of Keeling plot results [Ehleringer and Cook, 1998], so we
have treated the Keeling plot intercept as independent of flask volume in this analysis. It is also apparent that analytical methods and instrument precision are not constant across studies. For $d_{13}C$, precision values ranging from 0.03 to 0.13% have been reported, depending on whether dual inlet or continuous flow IRMS methods were used to measure $^{13}C_O_2$ [Buchmann et al., 1997b; Ehleringer and Cook, 1998; Flanagan et al., 1999]. As more than 95% of the Keeling plots analyzed in this study yielded standard errors exceeding 0.2% with a mean of 1.2%, we consider the reported values for instrument precision to be of an acceptable magnitude.

5. Interpreting $d_{13}C_R$

[28] A major objective of a number of recent studies was to use estimates of $d_{13}C_R$ as a proxy for measurements of ecosystem-scale photosynthetic discrimination [Buchmann et al., 1998b; Flanagan et al., 1996; Flanagan and Ehleringer, 1998]. We have shown that applying the Keeling plot method during daytime conditions can be problematic on short, diurnal timescales (Figure 4). On longer timescales, this application is based on a number of assumptions of ecosystem processes and their associated isotopic fractionation. As discussed below, data from the BASIN network and information from several other recent studies can be used to address these assumptions and evaluate the consequences of using Keeling plot data to estimate ecosystem discrimination.

[29] First, it is critical to determine whether fractionation occurs during respiration. If this is not the case, the carbon isotope composition of respired $CO_2$ is dependent on the isotopic composition of the substrate molecules. While isotope secondary plant products are generally depleted in $^{13}C$ compared to carbohydrates [Schmidt and Gleixner, 1998], on very long timescales, conservation of mass dictates that all fixed carbon will eventually return to the atmosphere. On shorter timescales relevant to ecosystem carbon exchange, i.e., days to years, we need to know the magnitude and consequence of secondary fractionation processes that might occur during plant and ecosystem respiration.

[30] Lin and Ehleringer [1997] observed that the $d_{13}C$ value of respired $CO_2$ released by incubated leaf protoplasts was not significantly different than that of the source carbohydrate (fructose, glucose, sucrose) supplied as a respiratory substrate. In such an experiment the source of the respiratory substrate is clear and unambiguous. For autotrophic respiration in a living leaf containing various substrates, it can be much more difficult to assess fractionation effects. Duranceau et al. [1999] and Ghashghaie et al. [2001] have shown that $CO_2$ respired in the dark was enriched in $^{13}C$ compared to leaf sucrose by 3–6%. In work by Ghashghaie et al. [2001] the observed fractionation was variable with environmental conditions and differed between species. It is difficult to interpret the significance of these studies for an understanding of Keeling plot data, because it is unknown if the apparent fractionation results from isotope effects during decarboxylation reactions or isotopic differences among respiratory substrates. Similar difficulties apply to assessing fractionation in microbial respiration, which has
been proposed in several studies [Henn and Chapela, 2000; Schweizer et al., 1999]. Currently we suggest that at the ecosystem scale, there is insufficient evidence to conclude that fractionation during respiration has a large influence on the interpretation of Keeling plot data. However, this remains an active area of research.

[31] Second, the majority of recently respired carbohydrate was presumably fixed by sun leaves at the top of a plant canopy, and so its isotopic composition is not significantly affected by gradients in light intensity within the canopy, or source CO$_2$ that is depleted in $^{13}$C [Berry et al., 1997; Buchmann et al., 2002]. The $\delta^{13}$C of CO$_2$ respired by an entire ecosystem is more closely associated with that of sun foliage than with shade foliage across a variety of ecosystems (Figure 6). In addition, $\delta^{13}$C$_{R}$ of an entire ecosystem can be either more enriched or more depleted in $^{13}$C than sun foliage. This is consistent with the idea that $\delta^{13}$C$_{R}$ may vary with short-term changes in environmental conditions, while leaf biomass $\delta^{13}$C largely represents the value of photosynthetic discrimination at the time of leaf development. In other words, there is no reason to expect that the isotopic composition of ecosystem pools will be exactly equivalent to that of ecosystem fluxes.

[32] Ecosystems with a similar range of $\delta^{13}$C values for soil organic matter also show a wide range of variation in ecosystem $\delta^{13}$C$_{R}$ (Figure 7). Soil organic matter is a carbon pool that represents an even longer temporal integration of primary production processes than that of individual leaves. In studies where the $\delta^{13}$C of respired CO$_2$ was measured for both the entire ecosystem and the respiration flux from the soil, the soil flux was consistently enriched in $^{13}$C compared to the entire ecosystem flux (Figure 7). Although enrichment of total soil organic matter (SOM) relative to litter inputs has been observed in many studies [Ehleringer et al., 2000], the carbon isotope composition of the pool of total SOM is a poor predictor of $\delta^{13}$C of soil CO$_2$ flux [Buchmann et al., 1997a; Fessenden and Ehleringer, 2002], a large fraction of which originates from autotrophic respiration and heterotrophic respiration of a small, labile pool of carbon [Trumbore, 2000]. Root and stem tissues tend to be slightly $^{13}$C enriched relative to leaf tissues, on the order of 1–3‰ in woody, C$_3$ plants [Boutton, 1996]. To the extent that $\delta^{13}$C of soil CO$_2$ flux is influenced by the isotopic composition of root biomass, some enrichment in soil CO$_2$ efflux is expected relative to aboveground respiration.

6. Spatial and Temporal Patterns in $\delta^{13}$C$_{R}$

[33] Spatial variability in $\delta^{13}$C$_{R}$ has long been observed within C$_3$ ecosystems as a result of species-specific effects and environmental conditions. In this analysis, individual values for $\delta^{13}$C$_{R}$ in all studies containing only C$_3$ plants ranged from $-19.0$ to $-32.6‰$, with a mean of $-26.2 \pm 0.2‰$ for 137 plots. The nighttime-only average over time obtained for each C$_3$ study ranged from $-21.4$ to $-28.9‰$, illustrating the large spatial variability in $\delta^{13}$C$_{R}$. Using the global average of $-8‰$ as a rough estimate of $\delta^{13}$C$_T$ in equation (4), the arithmetic mean $\Delta_e$ for the C$_3$ only data set was $18.3‰$. This is somewhat lower than estimates of global C$_3$ discrimination of $20.0 \%$ by Quay et al. [1992] and Fung et al. [1997], but compares favorably with C$_3$ only estimates of $17.6–18.0‰$ by Keeling et al. [1989], Tans et al. [1993], Lloyd and Farquhar [1994], and Buchmann and Kaplan [2001]. However, it is worth emphasizing that this is an ensemble average of data collected at various times, in some studies over multiple years. The range of $\Delta_e$ estimates from individual plots ranged from $11.2–25.5‰$, with differences of up to $7‰$ at a single study site from one year to the next. Thus, applying static estimates of biospheric discrimination to seasonal or interannual inversions of atmospheric CO$_2$ and $^{13}$CO$_2$ data may add to the uncertainty of temporal variability in source/sink estimates.

[34] Factors affecting the magnitude of $\delta^{13}$C$_{R}$ include the range of environmental parameters that control leaf $c_i/c_o$, such as light, temperature, and water availability, in addition to soil parameters that influence the isotopic composition of soil respiration, and the balance between above- and belowground respiration. Models of photosynthetic discrimination have generated reasonable fits between predicted values and biomass or $\delta^{13}$C$_{R}$ data [Kaplan et al., 2002]. These models are based on leaf-level equations of environmental controls on photosynthesis and stomatal conductance [Lloyd and Farquhar, 1994] and the release of older, isotopically heavier carbon from soils and long-lived plant material [Buchmann et al., 1998a; Ciais et al., 1999; Fung et al., 1997]. However, considerable unexplained variability still exists, particularly as canopy-level estimates of photosynthetic discrimination are difficult to obtain in the field. It is useful to consider the key controls on $\delta^{13}$C$_{R}$ in a range of

![Figure 6. Carbon isotope composition ($\delta^{13}$C) of sun and shade foliage in relation to the carbon isotope composition of ecosystem respiration ($\delta^{13}$C$_{R}$) for a number of ecosystems throughout North and South America. Sun foliage values are the mean of measurements taken at the top of the canopy, and shade foliage is the mean of measurements taken in the bottom of the canopy and/or the understory. The solid line is the 1:1 line.](image)
ecosystems both to improve modeling estimates and to further our understanding of ecosystem function.

[35] Trends among various biomes may highlight the importance of climate or plant functional type in influencing $\delta^{13}C_R$. We grouped the values for forest sites in the BASIN data set according to tropical, temperate broadleaf, temperate conifer, or boreal type to determine if latitude or biome was an important predictor of $\delta^{13}C_R$. No clear separations among biomes emerged (Figure 8). While differences in leaf $\delta^{13}C$ have been correlated with plant functional type within a given ecosystem [Brooks et al., 1997], between broadleaf tropical forests and coniferous forests [Broadmeadow and Griffiths, 1993], and by latitude [Körner et al., 1991], no simple and clear biome-dependent patterns emerged when all data were analyzed. Given the large range of studies and the additional variation introduced by the influence of soil respiration on $\delta^{13}C_R$, latitudinal and species effects may be obscured by other factors affecting spatial variability.

[36] Water availability is a major driver of plant productivity, and is also likely to be an important control of $\delta^{13}C_R$. Factors which cause short-term stomatal closure without a proportional reduction in photosynthesis should cause isotopic enrichment of organic material and subsequently $\delta^{13}C_R$. This has been demonstrated experimentally at the leaf-level as a positive relationship between water use efficiency (the ratio of carbon gain to water loss) and $\delta^{13}C$ of leaf material [Brugnoli et al., 1998; Farquhar et al., 1989; Farquhar and Richards, 1984]. We investigated the role of long-term water availability in influencing $\delta^{13}C_R$ by plotting mean $\delta^{13}C_R$ for each site versus mean annual precipitation (for sites which were only measured in a single year we plotted the annual precipitation for that year). This approach expands on the study by Bowling et al. [2002]. In the current study, a highly linear relationship was found for 17 study sites where average precipitation ranged from 230–2250 mm yr$^{-1}$, showing a large, long-term influence of water availability on the carbon isotope composition of respired CO$_2$. However, in seven coniferous forest observations from the U.S. Pacific northwest region, where precipitation averaged 2400 mm yr$^{-1}$ or greater, $\delta^{13}C_R$ was more enriched than predicted from the trend at other locations. Notably, of these forests the most enriched value was measured at the oldest forest, a 450-year-old stand at the Wind River Canopy Crane site in Washington, United States. Ryan and Yoder [1997] proposed that age-related factors other than drought stress cause stomatal closure in older forests (e.g., hydraulic limitation). The observations of enriched $\delta^{13}C_R$ in older coniferous forests are consistent with this observation [Bowling et al., 2002; Fessenden and Ehleringer, 2002], although this has not been observed in all studies of these forests [Buchmann, 2000].

[37] Excluding the seven Pacific northwest conifer stand observations, precipitation explained 88% of the spatial

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**Figure 7.** Carbon isotope composition ($\delta^{13}C$) of soil organic matter (SOM) at 0–20 cm and soil CO$_2$ flux in relation to the carbon isotope composition of ecosystem respiration ($\delta^{13}C_R$) for a number of ecosystems throughout North and South America. The isotopic composition of soil CO$_2$ flux was determined by sampling air from closed, flow-through soil respiration measurement systems with a number of different protocols. For further details see work by Flanagan et al. [1996], Buchmann et al. [1997a, 1998b], Flanagan et al. [1999], and Fessenden and Ehleringer [2002]. The solid line is the 1:1 line.

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**Figure 8.** A box plot of data distribution of the carbon isotope composition of ecosystem respiration ($\delta^{13}C_R$) for four forest biome types in North and South America. The boxes enclose 50% of the data population, with the centerline showing the median value. The error bars show the upper/lower quartile $\pm$ (1.5 $\times$ interquartile distance). Points that lie outside of this range are shown by open circles. Sample sizes ranged from 6 to 17 for the boreal forests and temperate broadleaf forests, respectively.
variability in $\delta^{13}C_R$, with a slope of $-2.0 \pm 0.2\% \text{ m}^{-1} \text{ yr}^{-1}$ and an intercept of $-24.5 \pm 0.2\%$ ($p < 0.01$). Bowling et al. [2002] propose water stress-induced stomatal closure as the most likely explanation for this relationship across the forest types measured in that study. Further testing will be necessary to explicitly reject other potential mechanisms, such as moisture-related trends in the composition of soil respiration, or changes in the proportion of photosynthesis and respiration.

To determine if current mechanistic models of isotope fractionation can reproduce the observed effect of precipitation across all ecosystems, the equilibrium global vegetation model BIOME4 was used to plot $\Delta_e$ (see equation (4)) against temperature and precipitation according to Kaplan et al. [2002]. The model predictions showed a correlation between $\Delta_e$ and both precipitation and temperature (Figure 9). Although there was more scatter and some curvilinearity in the precipitation relationship in contrast to the pattern shown in Figure 10, it should be noted that this exercise was global in scope and included a variety of biomes rather than forest ecosystems alone. Given this range, it appears that variations in $\Delta_e$ and $\delta^{13}C_R$ as a function of water availability may be expected based on both measurements and modeling output.

Recent studies have shown that a large fraction of respired CO$_2$ comes from the metabolism of recently fixed carbohydrates (fast cycling carbohydrate pools). Ekblad and Högberg [2001] observed a strong lagged correlation between atmospheric humidity and the $\delta^{13}C$ value of CO$_2$ released from the soil surface 2–4 days later in a boreal coniferous forest. Bowling et al. [2001] also have shown that the carbon isotope composition of CO$_2$ respired in coniferous forests in western Oregon was strongly correlated to the vapor pressure deficit ($D$) of air, consistent with expected responses of stomatal conductance and carbon isotope discrimination to humidity changes in these forests. The observed changes in $\delta^{13}C_R$ lagged behind shifts in $D$ by 5–10 days, possibly because of the time necessary for recently fixed carbohydrate to be transported and metabolized in aboveground and belowground plant parts.

Consistent with these observations, Ometto et al. [2002] have shown that seasonal changes in $\delta^{13}C_R$ values in a tropical evergreen forest were correlated with seasonal variation in precipitation inputs (Figure 11). The $\delta^{13}C_R$...
values within Amazonian rain forests became more enriched in $^{13}$C as monthly precipitation declined below 300 mm. Lower precipitation either reduced soil moisture and led to an increase in the stomatal limitation of photosynthesis during drought stress (lower $c_i/c_a$), or was associated with an increase in air temperature and $D$ during the dry season, which would also contribute to an increase in the $\delta^{13}$C of carbohydrates synthesized during photosynthesis. In addition, during a month of higher than normal precipitation (February 2000, 463 mm), $\delta^{13}$CR was enriched in $^{13}$C (Figure 11). This was likely due to saturation of the soil and possible anaerobic stress effects on plants. Stomatal closure and reduced carbon isotope discrimination is a general stress response and has been demonstrated for plants exposed to flooding [Guy and Wample, 1984].

[41] The studies discussed above indicate that within an ecosystem, $\delta^{13}$CR appears to represent a multiple-day integration of carbon isotope discrimination during ecosystem photosynthesis, providing an excellent tool for analyzing canopy responses to fairly short-term changes in environmental conditions. These results have implications for inverse calculations of regional carbon sinks that use $^{13}$CO$_2$ to partition oceanic and terrestrial contributions. In these studies, estimates of disequilibrium effects are often based on the age of respired carbon in box models of ecosystem carbon pools [Ciais et al., 1995; Francey et al., 1995; Fung et al., 1997]. Two trends discussed here may change the interpretation of these results: (1) a larger than expected influence of recently fixed carbon and (2) a detectable impact of previous climatic events on $\delta^{13}$C$_R$.

[42] Intersite comparisons offer an opportunity to evaluate the controls over $\delta^{13}$C$_R$ on larger spatial and temporal scales. In this study, an analysis of the spatial distribution of $\delta^{13}$C$_R$ revealed an influence of long-term differences in climatic conditions (Figure 10). While these data were insufficient to analyze the impacts of large-scale climatic event such as ENSO on discrimination, the finding that water availability has a large influence on mean $\delta^{13}$C$_R$ across a variety of ecosystems suggests that such patterns may indeed be observable at regional scales. Dynamic rather than static estimates of regional and global scale discrimination in inverse calculations may provide new information about seasonal and interannual variability in carbon sources and sinks.

7. Conclusions

[43] Spatially and temporally integrated values of ecosystem carbon isotope discrimination can be obtained from measurements of $\delta^{13}$C of CO$_2$ respired by the entire ecosystem [Buchmann et al., 1998a; Flanagan and Ehleringer, 1998; Keeling, 1958]. There is accumulating evidence to suggest that a large fraction of respired CO$_2$ comes from the metabolism of recently fixed carbohydrates [Bowling et al., 2002; Ekblad and Högb erg, 2001; Högb erg et al., 2001; Malhi et al., 1999]. The $^{13}$C/$^{12}$C ratio of this carbohydrate records information about plant physiological characteristics during the time that it was fixed, assuming no significant fractionation occurs during respiratory processes [Lin and Ehleringer, 1997]. Over longer periods, we expect that measurements of the carbon isotope composition of CO$_2$ respired from the entire ecosystem will represent an integrated measure of whole ecosystem discrimination [Bowling et al., 2002; Buchmann et al., 1998a; Flanagan et al., 1996; Flanagan and Ehleringer, 1998]. Analysis of trends across large regions can provide insight into biological controls over the isotopic composition of atmos-
phere. Further study is needed to elaborate the mechanisms influencing the magnitude of δ¹³C and the disequilibrium between δ¹³C and photosynthetic discrimination in a variety of ecosystems.

To facilitate the integration of numerous data sets into a comprehensive understanding of terrestrial biosphere function, it is essential that methodology, analytical techniques, and interpretation be applied consistently. We have shown that standard, Model I regression introduces bias into the estimation of the Keeling plot intercept, such that techniques that account for error in the CO₂ parameter, such as Model II regression, must be applied. A large range (greater than 75 ppm) of CO₂ concentration during measurement period reduces the standard error in the intercept, and this standard error should always be reported. Although daytime data generally extends the measured CO₂ range, daytime measurements should only be included in Keeling plot analyses explicitly and with caution. Despite these caveats, we have shown that the isotopic composition of ecosystem carbon pools, such as leaf or soil organic material, is not equivalent to δ¹³C, so that Keeling plot estimates are the preferred method for assessing the carbon isotope composition of ecosystem fluxes. By applying common techniques to the estimation of the Keeling plot intercept, we can gain the maximum benefit from integrating estimates of δ¹³C and Δd into a common framework for terrestrial carbon research.

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Figure 2. Location of sites used in this analysis. Data sources: 1 and 2: C3 primary forest and mixed C3-C4 pasture in Manaus, Brazil, details given by Ometto et al. [2002]; 3 and 4: C3 primary forest and mixed C3-C4 pasture in Santarem, Brazil, details given by Ometto et al. [2002]; 5 and 6: C3 primary forest and mixed C3-C4 pasture in Ji Parana, Brazil, details given by Ometto et al. [2002]; 7: coniferous forest in Yosemite National Park, California, details given by Lancaster [1990]; 8: mixed coniferous-deciduous forest in Cuyamaca Rancho State Park, California, details given by Lancaster [1990]; 9: deciduous forest in Red Butte canyon, Utah, details given by Buchmann et al. [1997b]; 10: deciduous and coniferous forests near Kamas, Utah, details given by Buchmann et al. [1997b]; 11: coniferous forest near Hamilton, Montana, details given by Lancaster [1990]; 12: C3 grassland near Lethbridge, Alberta, from L.B. Flanagan (unpublished data, 1999); 13: coniferous and deciduous forests at the BOREAS Southern Study Area, details given by Flanagan et al. [1996, 1998]; 14: coniferous and deciduous forests at the BOREAS Northern Study Area, details given by Flanagan et al. [1996, 1998]; 15: mixed C3-C4 grassland at the Konza Prairie Long-Term Ecological Research Site, Kansas, from L.B. Flanagan (unpublished data, 1998); 16 and 17: coniferous forest and corn crop near Ottawa, Ontario, from L.B. Flanagan (unpublished data, 1998); 18: deciduous forest near Barnard, Vermont, details given by Lancaster [1990]; 19: deciduous forest in Harvard Forest, Massachusetts, from J.R. Ehleringer (unpublished data, 1991); 20: deciduous forest in Scotia Range, Pennsylvania, details given by Lancaster [1990]; 21: tropical forest in Chamel, Mexico, details given by Lancaster [1990]; 22: tropical forest in Barro-Colorado Island, Panama, details given by Lancaster [1990]; 23: tropical forest in Paracou, French Guiana, details given by Buchmann et al. [1997a]; 24: tundra ecosystem near Bethel, Alaska, details given by Lancaster [1990]; 25: tundra ecosystem near Toolik Lake, Alaska, details given by Lancaster [1990]; 26: coniferous forests near Seattle, Washington, details given by Buchmann et al. [1998b]; 27: coniferous forests at the Wind River Canopy Crane site, Washington, details given by Fessenden and Ehleringer [2002]; 28–33: various coniferous forests along the Oregon Transect for Ecological Research (OTTER), details given by Bowling et al. [2002].