Critical evaluation of micrometeorological methods for measuring ecosystem–atmosphere isotopic exchange of CO₂

David R. Bowling*, Diane E. Pataki, James R. Ehleringer

Department of Biology, University of Utah, 257 South 1400 East, Salt Lake City, UT 84112-0820, USA

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Abstract

Isotopic net ecosystem exchange (isofluxes, or flux densities of 13CO₂) can be combined with standard eddy covariance methods to partition net ecosystem exchange of carbon dioxide (F) into its component one-way fluxes, photosynthesis and respiration. At present, the approaches used to estimate isotopic fluxes are labor-intensive and dependent on several assumptions. To assess the relative utility of the available methods, we studied an ecosystem associated with large CO₂ fluxes and maximal isotopic exchange. Three independent techniques were used to measure isotopic flux densities over an irrigated alfalfa field: (1) a combination of standard eddy covariance and flask sampling; (2) the flux-gradient method; and (3) hyperbolic relaxed eddy accumulation (HREA). Consistent isotopic flux results were obtained via the three methods, with similar diurnal patterns and peak midday isotopic flux densities of 600–700 ‰.

Air samples were collected over a wide range of CO₂ mole fractions (325.3–597.5 ‰) and isotopic composition (−5.9 to −15.4 ‰). The relationship between isotopic composition (δ¹³C) and CO₂ mole fraction was consistent among types of samples, except for HREA samples during the morning boundary layer transition.

Total ecosystem respiration was estimated based on a regression against soil temperature, and the flux and isotopic flux measurements were used to examine whole-canopy photosynthetic discrimination (Δcanopy) and the isotopic composition of the photosynthetic flux. Δcanopy weighted by net ecosystem exchange was 17.9 ‰. The isotopic content of total ecosystem respiration, soil respiration, and foliar respiration, and δ¹³C of various organic components (leaves, roots, soil organic matter) were examined and evaluated relative to Δcanopy. The δ¹³C of organic components does not appear to be a good predictor of δ¹³C of ecosystem CO₂ fluxes.

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Keywords: Net ecosystem exchange; Isotopic fluxes; Isoflux; Photosynthesis; Respiration; Hyperbolic relaxed eddy accumulation

1. Introduction

Stable isotopes of carbon dioxide provide a unique way to investigate aspects of the carbon cycle within terrestrial ecosystems. Uptake of atmospheric CO₂ by photosynthesis is associated with a change in the relative amounts of ¹³C and ¹²C. The ¹³C/¹²C isotope ratio of CO₂ in air (expressed as δ¹³C, in dimensionless “units” of ‰, Farquhar et al., 1989) is roughly −8 ‰ during the daytime, and δ¹³C of C₃ plant materials and ecosystem components is −22 to −32 ‰. The process of photosynthesis removes relatively more of the lighter ¹²CO₂ isotope, and leaves the air enriched in the heavy ¹³CO₂ isotope (δ¹³C of photosynthetic sugars is more negative than that of the air, and δ¹³C of air becomes more positive during photosynthesis as the
Nomenclature

- $a$: intercept of a regression between $\delta^{13}C$ and [CO$_2$] (‰)
- $b$: HREA empirical coefficient
- $C$: CO$_2$ mole fraction [ ] within the canopy (µmol mol$^{-1}$)
- $d$: zero-plane vertical displacement height (m)
- $F$: net ecosystem exchange of CO$_2$ (mol CO$_2$ m$^{-2}$ s$^{-1}$)
- $F_a$: assimilation flux density of CO$_2$ (µmol CO$_2$ m$^{-2}$ s$^{-1}$)
- $F_R$: respiratory flux density of CO$_2$ (µmol CO$_2$ m$^{-2}$ s$^{-1}$)
- $F_{13}$: net ecosystem exchange of $^{13}$CO$_2$ (µmol $^{13}$CO$_2$ m$^{-2}$ s$^{-1}$)
- $F_{\delta}$: isoflux, analogous to net ecosystem exchange of $^{13}$CO$_2$, but expressed using δ notation (µmol CO$_2$ m$^{-2}$ s$^{-1}$‰)
- $G$: soil heat flux density (W m$^{-2}$)
- $H$: sensible heat flux density (W m$^{-2}$)
- $k$: von Karman constant (dimensionless)
- $K$: eddy diffusivity (m$^2$ s$^{-1}$)
- $L$: Obukhov length (m)
- $L_E$: latent heat flux density (W m$^{-2}$)
- $m$: slope of a regression between $\delta^{13}$C and [CO$_2$] (mol µmol$^{-1}$‰)
- $R$: molar ratio of heavy to light isotope (13C/12C)
- $R_{ac}$: net radiation flux density (W m$^{-2}$)
- $SOM$: soil organic matter
- $T$: temperature (°C)
- $u^*$: friction velocity (m s$^{-1}$)
- $v_{pd}$: vapor saturation deficit of air (kPa)
- $w$: vertical wind velocity (m s$^{-1}$)
- $z$: height within the canopy (m)

Greek symbols

- $\delta^{13}C$: carbon isotopic composition (‰)
- $\delta^{18}O$: oxygen isotopic composition (‰)
- $\Delta$: carbon isotope discrimination
- $\rho$: air density (mol m$^{-3}$)
- $\sigma_w$: standard deviation of vertical wind velocity (m s$^{-1}$)
- $\Phi$: universal profile function (dimensionless)

Subscripts

- $a$: air within the canopy
- $A, P$: photosynthetic assimilation flux
- $c$: flux of CO$_2$
- $dn$: downdraft
- $R$: respiration flux
- $R$-branch: branch respiration flux
- $R$-soil: soil respiration flux
- $up$: updraft
- 1, 2: height 1 or 2

Respiration then releases CO$_2$ back to the atmosphere which is $^{13}$C depleted, causing the carbon isotope ratio of CO$_2$ in the atmosphere to decrease. As a result, there is significant diurnal variation in the isotopic content of CO$_2$ within terrestrial ecosystems (Keeling, 1958; Quay et al., 1989; Flanagan et al., 1996; Buchmann et al., 1997; Bowling et al., 1999a).

The isotopic composition of organic matter and of ecosystem CO$_2$ fluxes are roughly in balance, but can differ due to temporal separations in photosynthetic and respiratory activities or because of longer-term carbon dynamics. To date there is no definitive evidence of carbon isotope fractionation with mitochondrial respiration (Lin and Ehleringer, 1997). However, some authors define fractionation in different ways, leading to debate in the literature. Isolated leaf sugars can differ isotopically from CO$_2$ respired from those leaves (Duranteau et al., 1999; Ghosh et al., 2001), but it is difficult to establish the exact chemical substrate for leaf respiration. Plant secondary compounds do show systematic variation in isotopic composition (Gleixner et al., 1998), and preferential degradation of organic compounds in plant respiration or microbial oxidation might lead to isotopic differences in the bulk organic substrate and respired CO$_2$ (e.g. Ehleringer et al., 2000). There is some evidence of isotopic changes upon fungal uptake of sugars (Henn and Chapela, 2000), but in an ecosystem context such apparent fractionations could not be sustained indefinitely and still conserve mass.
Total ecosystem respiration is always depleted (more negative $\delta^{13}$C) in $^{13}$C relative to the air, and photosynthesis always leaves the air more enriched. These labels mean that the ecosystem-scale processes of photosynthesis and respiration can be studied by examination of the isotopic content of $^{13}$CO$_2$ in terrestrial ecosystems (Yakir and Wang, 1996; Lloyd et al., 1996; Flanagan et al., 1996; Bowling et al., 2001b). These equations were initially derived with the intention of using Lloyd et al. (1996) derived a useful suite of equations that have been used to investigate isotopic fractionation during net carbon assimilation, which is defined as gross leaf carbon uptake minus leaf respiration. This definition of leaf-level net discrimination (defined as isotopic fractionation during net carbon assimilation, which is defined as gross leaf carbon uptake minus leaf respiration), we must include foliar respiration in the assimilation flux during the day ($F_A$) and in the respiration flux at night ($F_R$) (Lloyd et al., 1996; Bowling et al., 2001b).

Ideally, $F_R$ could be measured directly via eddy covariance of $^{13}$CO$_2$, and appropriate characterization of the isotopic storage flux. The former would require a field-based instrument that can accurately measure $^{13}$CO$_2$ mole fractions at a sampling interval on the order of 100 ms, with considerable precision (corresponding to an isotope ratio of 0.05‰). In this regard, there are several promising spectroscopic technologies. These include tunable diode lasers (TDL, Beeker et al., 1992), a combination of TDL with cavity ringdown spectroscopy (Crosson et al., 2002), and Fourier-transform infrared spectroscopy (Elder et al., 2000). At present no instruments meet these requirements, and we are limited to three indirect methods for assessing $F_R$ at the ecosystem-scale. These are the flux-gradient technique (Yakir and Wang, 1996), hyperbolic relaxed eddy accumulation (Bowling et al., 1999b), and a combination of eddy covariance and flask sampling called the IC/flask technique (Bowling et al., 2001b).

The flux-gradient or gradient profile technique (e.g. Businger, 1986) relates a vertical gradient of an atmospheric constituent (such as CO$_2$) to a flux via:

$$ F = \rho K (\delta^{13}C_1 - \delta^{13}C_2) $$

where $F$ is the flux of interest, $\rho$ the air density, and $\delta^{13}C_1$ and $\delta^{13}C_2$ the CO$_2$ mole fractions at two heights, $z_1$ and $z_2$. $K$ is an empirical parameter called the eddy diffusivity, and can be determined by measurements of the vertical wind profile, Monin-Obukhov similarity theory, or by assuming similarity with another scalar that can be measured via eddy covariance (sensible heat, water vapor, etc.) and rearranging Eq. (3). This equation can be modified for $^{13}$CO$_2$ flux by multiplying each mole fraction or flux term by the isotope ratio of CO$_2$ at that height:

$$ h \delta F = \rho K (\delta^{13}C_1) \delta^{13}C_1 - (\delta^{13}C_2) \delta^{13}C_2 $$

Thus, the isotopic flux ($h \delta F$) can be determined by measuring $\delta^{13}C$ and [CO$_2$] (where [ ] denotes mole fraction) at two heights above a plant canopy, and samples can be returned to a laboratory for analysis on any time scale. The gradient method is attractive for its simplicity. Further, $K$ can be determined entirely...
independently of the $\delta^{13}C$ and [CO$_2$] measurements. (As with net ecosystem exchange, the isotopic flux $\delta F_c$ must be combined with an isotopic storage flux to produce $F_1$ in Eq. (2)—see Appendix A for details.)

The gradient technique assumes that mole fraction variation in the atmosphere follows well-defined vertical profiles. Such profiles are observed only in the surface layer above a rough plant canopy can extend many (5–10) canopy heights above the canopy top. In the roughness sublayer, and within the canopy itself, serious problems with this technique emerge (Raupach, 1979; Cellier and Brunet, 1992), and fluxes can even be in a direction opposite the observed mole fraction gradient (Demme and Bradley, 1985). In practice, this restricts use of the gradient method to sites such as crops and grasslands that are aerodynamically smooth and short-statured.

To develop a method that could be used to estimate isotopic fluxes over forests, Bowling et al. (1999a,b) merged the relaxed eddy accumulation technique (Businger and Oncley, 1990) with hyperbolic hole analysis (Shaw, 1985) to produce the hyperbolic relaxed eddy accumulation (HREA) method. This method relates the flux to mole fraction differences in relaxed eddy accumulation (HREA) method. This method relates the flux to mole fraction differences in relaxat

\[ F = \rho b \sigma_a (C_{up} - C_{dn}) \]  
\[ (5) \]

where $\sigma_a$ is the standard deviation of the vertical wind velocity and $b$ an empirical coefficient. Sampling decisions are made every 100 ms and samples are collected in updraft or downdraft containers, or discarded. Samples are accumulated in the containers over a 30–45 min time period and then the bulk sample is analyzed at leisure. Eq. (5) can be extended for isotopic fluxes as:

\[ b \delta F_c = \rho b \sigma_a (\delta^{13}C_{up} - \delta^{13}C_{dn}) \]  
\[ (6) \]

where $\delta^{13}C_{up}$ and $\delta^{13}C_{dn}$ are the carbon isotope ratios of updrafts and downdrafts. In HREA, only updrafts and downdrafts exceeding a certain threshold are sampled, and their identification is dependent on measurements of wind and [CO$_2$] (Bowling et al., 1999a,b).

While this technique provides maximal isotopic differences in updrafts and downdrafts, it suffers from several problems. One disadvantage of HREA is that the majority of air (80%) is discarded, and roughly 10% of the original volume is sampled into each of the updraft and downdraft containers. The “information” contained in the discarded air must be reconstructed through the $b$ coefficient. The determination of $b$ is problematic—this has been done by assuming similarity with [CO$_2$], measuring [CO$_2$] in updrafts and downdrafts, and CO$_2$ flux by eddy covariance, and rearranging Eq. (5) to solve for $b$ (Bowling et al., 1999a). This is somewhat circular; if the eddy covariance fluxes ($F_c$) are used to determine the isotopic flux (i.e. if the HREA fluxes are calculated using $b$ derived from eddy covariance), and then $F$ and $F_c$ are used together (Eqs. (1) and (2)), then they are not strictly independent. Further, determining $b$ in this fashion assumes that CO$_2$ and $\delta^{13}$CO$_2$ act identically in the atmosphere, which is likely false when there are major differences in sources and sinks (such as in a forest with photosynthesis and respiration occurring). A recent examination of this and other conditional-sampling techniques by Ruppert (2002) showed that under some conditions large errors can result from violations of scalar similarity using HREA.

The simplest of the methods to determine isotopic fluxes, which we will refer to as the EC/flask technique, involves establishing a regression between $\delta^{13}C$ and [CO$_2$]. This is somewhat circular; if the eddy covariance fluxes are calculated using $b$ derived from eddy covariance), and then $F$ and $F_c$ are used together (Eqs. (1) and (2)), then they are not strictly independent. Further, determining $b$ in this fashion assumes that CO$_2$ and $\delta^{13}$CO$_2$ act identically in the atmosphere, which is likely false when there are major differences in sources and sinks (such as in a forest with photosynthesis and respiration occurring). A recent examination of this and other conditional-sampling techniques by Ruppert (2002) showed that under some conditions large errors can result from violations of scalar similarity using HREA.

The simplest of the methods to determine isotopic fluxes, which we will refer to as the EC/flask technique, involves establishing a regression between $\delta^{13}C$ and [CO$_2$]. Then, using this regression combined with 10 Hz measurements of [CO$_2$] to calculate $\delta^{13}$CO$_2$ flux via:

\[ \delta F_c = \rho w (\delta^{13}C_{up} - \delta^{13}C_{dn}) \]  
\[ (7) \]

where $w$ is vertical wind velocity, $m$ and $a$ the slope and intercept of the regression $\delta^{13}C = mC_{up} + a$, and overbars denote Reynolds averaging, and primes denote deviation from that average. The EC/flask method has been described in detail elsewhere (Bowling et al., 1999a, 2001b). It has the major advantage that the only measurements required beyond standard eddy flux instrumentation are flask samples of $\delta^{13}$C and [CO$_2$] in air. Isotopic flux studies are too few at present to firmly establish just how frequently these samples need to be collected, but there can be important ecophysiological variation in $\delta^{13}C$ on a time scale of days to weeks (Buchmann et al., 1997; Bowling et al., 2002; Ometto et al., 2002), which is likely a good proxy.

A disadvantage of the EC/flask technique is that it assumes a regression between $\delta^{13}C$ and [CO$_2$] is valid at all time scales associated with turbulent exchange (100 ms to 30 min, or longer), but the regression is established with flask samples collected relatively slowly. Bowling et al. (2001b) showed that
Table 1
Geometric mean regressions of $\delta^{13}C$ vs. $1/[CO_2]$ and $\delta^{13}C$ vs. $[CO_2]$ (last row only)

<table>
<thead>
<tr>
<th>Sample type</th>
<th>Intercept $\pm$ Slope $r^2$</th>
<th>n</th>
<th>a</th>
</tr>
</thead>
<tbody>
<tr>
<td>EC/flask</td>
<td>$-26.60 \pm 0.12$ 6757.3 $\pm 49.0$ 1.00 19</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gradient</td>
<td>$-26.63 \pm 0.37$ 6834.5 $\pm 142.1$ 0.98 42</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HREA</td>
<td>$-26.90 \pm 0.13$ 6916.6 $\pm 52.6$ 1.00 59</td>
<td></td>
<td></td>
</tr>
<tr>
<td>EC/flask and gradient, nocturnal$^a$</td>
<td>$-27.12 \pm 0.17$ 7026.6 $\pm 75.4$ 1.00 27</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Soil</td>
<td>$-24.73 \pm 0.46$ 5998.3 $\pm 195.6$ 0.98 20</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Branch</td>
<td>$-23.79 \pm 0.50$ 5759.6 $\pm 247.3$ 0.98 14</td>
<td></td>
<td></td>
</tr>
<tr>
<td>EC/flask$^b$</td>
<td>$6.62 \pm 0.29$ $-0.040 \pm 0.001$ 0.98 61</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Errors are presented as the standard error of the slope or intercept.

$^a$ Regression used to calculate $\delta^{13}C_R$ and $\delta^{13}C_a$.
$^b$ Regression used to calculate EC/flask fluxes.

this may not be a problem for samples collected over time scales varying from 500 ms to 30 min. A more serious issue is the choice of a regression of $^{13}C$ versus $[CO_2]$ instead of $^{13}C$ versus $1/[CO_2]$ (Keeling, 1958). Both are satisfactory in predicting isotope ratio variation based on $[CO_2]$ in the range 330–500 mol mol$^{-1}$, but only the latter matches observations at higher $CO_2$ values (see Fig. 4 and Table 1). However, on a theoretical basis, Bowling et al. (2001b) argued that using the Keeling relationship in Eq. (7) ($mC_a + a^2$ instead of $mC_a + a$) forces isotopic equilibrium (where $\delta^{13}C_R = \delta^{13}C_a - \Delta_{canopy}$ in Eq. 2). In this case Eq. (2) becomes a multiple of Eq. (1), and there is no unique information in $^{13}CO_2$ that is not already contained in $CO_2$ fluxes. Ecosystems seem to operate very near the condition of isotopic equilibrium; thus, indirect empirical techniques such as this one should be examined quite critically.

3. Methods

3.1. Site

This study was conducted in an irrigated field of alfalfa (Medicago sativa L.) in the Cache Valley, Utah (41°53’N, 111°50’W, 1380 m elevation) between 11 and 25 August 2000. Since a land-use history of C$_4$ crops would confound our isotopic measurements, we selected a site that contained strictly C$_3$ plants for more than 20 years. The site was flat and measured 400 m × 400 m (16 ha in area). Following local agricultural practice, the field was heavily irrigated roughly 2 weeks prior to measurements and not irrigated again. Rain fell during a single afternoon (23 August), otherwise the weather was sunny, hot, and dry.

3.2. Eddy covariance and meteorology

Fluxes of sensible heat, latent heat, and carbon dioxide were measured using the eddy covariance technique at a height equal to three canopy heights (3h, where $h = 54.8 \pm 12.6$ cm on 24 August). Instrumentation included an open-path infrared gas analyzer (LI-7500, Licor Inc., Lincoln, NE) and a sonic anemometer (CSAT3, Campbell Scientific Inc., Logan, UT). Fluxes were averaged over 30 min periods, and standard corrections for density were applied (Webb et al., 1980). Data were examined for stationarity by comparing 5 and 30 min covariances—if the mean of the six 5 min $w-CO_2$ covariances differed from the 30 min covariance by more than 30%, the flux data were excluded from some analyses (Foken and Wichura, 1996). $CO_2$ mole fraction was measured at several heights (3h, 3h, 0.3h, 0.5h, and 0.05h) using a second IRGA (LI-6262, Licor Inc., Lincoln, NE), and used to compute the storage component of F (Wofsy et al., 1993). All $CO_2$ measurements in this study are referenced to WMO [CO$_2$] standards.
3.3. Isotopic fluxes

Isotopic fluxes of $^{13}$CO$_2$ were measured using the EC/flask technique, the flux-gradient technique, and hyperbolic relaxed eddy accumulation. While the method of sampling in each flux measurement technique differed, in all cases the samples were ultimately stored in 100 ml glass flasks (34–5671, Kontes Glass Co., Vineland, NJ) in the field and then analyzed in our laboratory. Carbon isotope ratios of CO$_2$ in the flasks were measured using a continuous flow isotope ratio mass spectrometer (IRMS, Finnigan MAT, San Jose, CA), as described by Ehleringer and Cook (1998). Precision for $\delta^{13}$C was determined daily by comparison to known standards and was typically ±0.15‰. Corrections for the presence of $^{18}$O were applied, and CO$_2$ was separated from N$_2$O by gas chromatography before analysis. We report all carbon isotope ratio values in this paper relative to the international PDB standard. $\delta$ values in this paper relative to the international PDB standard. 

3.3.1. EC/flask

We established the relationship between $\delta^{13}$C and [CO$_2$] using samples collected over a range of time periods, both at night and during the day. Nineteen samples (which we denote EC/flask samples) were collected in 100 ml flasks by pulling air at 1000 ml min$^{-1}$ through a Mg(ClO$_4$)$_2$ trap to remove water vapor with a pump (UNMP50KNDC, KNF Neuberger Inc., Trenton, NJ) downstream of the flask. These were collected at a variety of heights (5h, 3h, 0.8h, 0.5h, and 0.05h) within the canopy, with the intention of maximizing the range of [CO$_2$] in the samples (which minimizes the standard error of the Keeling intercept, Pataki et al., 2003). The EC/flask samples were combined with 42 gradient samples (described below) and a geometric mean regression of $\delta^{13}$C versus [CO$_2$] was performed (Table 1). This regression was used to compute isotopic fluxes via Eq. (7).

3.3.2. Flux-gradient

We collected air samples every 4 h (except at 2 a.m.) over a 5-day period (20–24 August) at two heights (3h and 5b). These heights were intentionally chosen above the roughness sublayer to avoid complications associated with counter-gradient fluxes. Air was pumped from the two sampling heights through 10.4 l glass buffer volumes at 347 ml min$^{-1}$ to provide a 30 min residence time in the buffers. The air was dried using Mg(ClO$_4$)$_2$, and pulled through a 100 ml sampling flask via a diaphragm pump downstream of the flask.

Gradient isotopic fluxes were calculated using Eq. (4), and the eddy diffusivity for CO$_2$ ($K$) was computed from Monin–Obukhov similarity theory via:

$$K = \frac{\ln^*(z-d)}{\Phi}.$$  

$$\Phi = \begin{cases} 
1 + 10 \left( \frac{z-d}{L} \right)^{1/2}, & \quad -2 \leq \frac{z-d}{L} \leq 0, \\
1 + 5 \left( \frac{z-d}{L} \right), & \quad 0 < \frac{z-d}{L} \leq 1, 
\end{cases}$$

where $k$ is von Karman’s constant, $\alpha^*$ the friction velocity, $z$ the measurement height, $d$ the zero-plane displacement height, $\Phi$ is the universal function describing scalar profiles as a function of the stability parameter ($z-d/\kappa L$), and $L$ the Obukhov length (Raupach, 1979; Kaimal and Finnigan, 1994). Implicit in this approach is the assumption that the eddy diffusivities for momentum and scalars are identical (i.e. the turbulent Schmidt number equals unity). Flesch et al. (2002) suggest that the Schmidt number can be smaller (0.6 on average in their study), which would cause our gradient CO$_2$ measurements to underestimate the true flux.

3.3.3. HREA

The HREA method has been used once before with a sampling system involving cryogenic purification of CO$_2$ during sample collection (Bowling et al., 1999a). For the present study, we used a much simpler system involving flexible bags as collection reservoirs (Fig. 1). Since the simplified technique is more practical, we describe our new design in detail here.
is similar in design to the relaxed eddy accumulation systems of Oncley et al. (1993) and Bowling et al. (1998). HREA sampling decisions, based on 10Hz wind and CO2 measurements, were made as described by Bowling et al. (1999a). We used an asymmetric hyperbolic threshold of 1.1, and a recursive filter (McMillen, 1988) to estimate relevant turbulent parameters ($w', \sigma_w, CO'_2, \sigma_{CO_2}$). These parameters are described in detail in other papers (Bowling et al., 1999a,b). HREA isotopic fluxes were calculated according to Eq. (6).

Air was drawn (Fig. 1) from within 10 cm of the sonic anemometer path at 5000 ml min$^{-1}$ through a primary flow path consisting of 25 cm × 0.64 cm o.d. stainless steel tubing, a 15 µm sintered ceramic filter (F, SS-4FW-15, Swagelok/NUPRO Co., Willoughby, OH), 7 m of polymer tubing (0.64 cm o.d., Dekoron, Syntex Specialty Products, Mantua, OH), and a mass flow controller (MFC1, 1179A, MKS Instruments, Andover, MA) by two pumps in parallel (P1, PC-X76-001/7, Charles Austen Pumps Ltd., Surrey, UK). A subsample of this airstream was drawn at 620 ml min$^{-1}$ through a Nafion counterflow drying membrane (MD-070-48S, Perma-Pure Inc., Toms River, NJ) to remove water vapor, and a second mass flow controller (MFC2, 1179A, MKS Instruments, Andover, MA) by a pump (P2, UNMP50KNDC, KNF Neuberger Inc., Trenton, NJ). Downstream of the pump (P2) the flow was routed via a three-way Teflon manifold valve (V3, P-01367-81, Cole-Parmer Instrument Co., Vernon Hills, IL) into updraft/downdraft bags or vented.

Internal volume of various system components was measured manometrically in the lab and the appropriate delay (2.3 s) applied to valve control signals to synchronize an event at the sonic/IRGA to the associated plumbing change. Flow rate in the longer (7 m) sampling line (5620 ml min$^{-1}$) was sufficient to maintain turbulent flow (Reynolds number = 2466), but flow in the shorter secondary path (tens of cm in length) was laminar.

The bags (party balloons, Anagram International Inc., Minneapolis, MN) were flexible and made of four layers, which (from the inside) were polyethylene, nylon, aluminum flake, and ink. The bags were sealed after collection using stainless steel toggle valves (V, SS-1GS4, Swagelok/Whitey Co., Highland Heights, OH). Stainless steel filter tubes (25 cm × 0.64 cm o.d.) were drilled with multiple holes and inserted into the flexible valves on the bags and sealed with rubber bands. Bags were flushed with dried ambient air and pumped flat immediately prior to filling. Samples were collected over 30 min then the samples were immediately transferred to 100 ml glass flasks and stored until analysis. Samples did not reside in the bags for more than 35 min.

Several laboratory tests were performed to assess the integrity of gas samples stored in the bags. Results of some of these tests are shown in Table 2. Air from a compressed cylinder with known isotope ratio was introduced into the bags. Some samples were immediately transferred to flasks (0 min), and others sat...
in bags for varying periods of time, up to 30 h. After aging, samples were transferred to pre-evacuated 1.7 l glass flasks, the CO₂ was purified cryogenically under vacuum, and analyzed via dual inlet mass spectrometry (Finnigan MAT 252, Finnigan, San Jose, CA). A shift in both \( \delta^{13}C \) and \( \delta^{18}O \) of CO₂ was apparent over time (Table 2), but within a 60 min time period the shift in \( \delta^{13}C \) was insignificant. Tests were also performed with the full HREA system as it was used in the field, using dry and humidified compressed air of known isotope ratio, with similar results (not shown).

There was an immediate change in \( \delta^{18}O \) measured in the bags that increased dramatically over time (Table 2). We attempted to identify the cause of this shift without success. Based on these results, we do not recommend using these bags to collect samples that will be analyzed for \( \delta^{18}O \) of CO₂; at present, the cryogenic system of Bowling et al. (1999a) is the only alternative for HREA sampling with oxygen isotopes of CO₂. However, recent tests in other laboratories have shown promising results with \( \delta^{13}C \) and \( \delta^{18}O \) in bags after they have been conditioned (Ruppert and Brand, Max-Planck-Institut für Biogeochemie, personal communication).

3.4. Soil respiration rate

Fourteen PVC collars were installed every 7 m along a 100 m transect on 19 August. Each collar measured 9.5 cm in diameter and was inserted to 5 cm soil depth. Collars were installed in small patches of bare soil between aboveground plant parts, immediately adjacent to the stems. Aboveground plant components were excluded from the chambers. Respiration rates were measured using a portable photosynthesis system (LI-6200, Licor Inc., Lincoln, NE) configured in a closed loop, by examining the rate of a \( 20 \mu \text{mol} \text{mol}^{-1} \) change in [CO₂] over roughly 1 min. Due to an undetected probe malfunction during the experiment, soil temperature data measured concomitantly at each chamber were unreliable. We present soil temperature data measured at a central location along the transect using a Cu-Co thermocouple at 0.04 m depth (5 s data averaged every 30 min). This is not necessarily the most appropriate depth for this crop if most respiration occurs in deeper roots, but is a suitable indicator of the diurnal pattern.

3.5. \( \delta^{13}C \) of ecosystem respiration

The intercept of a geometric mean regression between \( \delta^{13}C \) and 1/[CO₂] (a Keeling plot) was used on nocturnal EC/flask and gradient samples (Table 1) to calculate \( \delta^{13}C_R \), the isotope ratio of ecosystem respiration (Keeling, 1958). Outliers were selected and removed as necessary as described by Bowling et al. (2002).

3.6. Calculation of \( \Delta_{\text{canopy}} \)

\( F_A \) was prescribed as a function of soil temperature, and Eqs. (1) and (2) were solved for \( F_A \) and \( \Delta_{\text{canopy}} \). For this analysis, \( F_1 \) and \( F_2 \) were measured using eddy covariance and the EC/flask technique, respectively, with appropriate storage fluxes included. \( \delta^{13}C_A \) was derived from a Keeling plot as described above, and \( \delta^{13}C_{\text{a}} \) was calculated from the Keeling regression (Table 1) and measured [CO₂] at 0.4 m height (0.8 h). We then compared this estimate of \( \Delta_{\text{canopy}} \) to isotopic content of ecosystem organic components and respired CO₂.

3.7. \( \delta^{13}C \) of leaves, roots, and soil organic matter

Sun leaf samples were saved from the plants used for foliar respiration measurements (described below). Bulk soil and root samples were collected from three separate pits at depths of 4, 9, 20, and 32 cm in thin (2 cm) layers. All organic samples were dried to constant mass at 60 °C. Roots were removed from soil samples and tap root and fine roots (<2 mm diameter) saved. Root-free soil samples were acid-washed to remove carbonates (0.5N HCl). Leaves, roots, and bulk soil were ground with mortar and pestle to #20 mesh and subsamples (2 mg leaf and root, 20 mg soil) were flash-combusted and analyzed for \( \delta^{13}C \) on an IRMS (deltaS, Finnigan MAT, San Jose, CA). Measurement precision was 0.2‰, and data are presented as means and standard errors of three or more replicates.

3.8. \( \delta^{13}C \) of foliar respiration

On the evening of 22 August, measurements of the isotope ratio of foliar respiration were made. This was done by adding an assembly of five 100 ml flasks (connected to each other in parallel) to the closed loop soil
respiration chamber. The chamber was used simply as a gas-exchange enclosure to insert detached foliar components. First, all flask stopcocks were opened, and the pump run for several minutes to fill the flasks with ambient air near the ground. Next, the plastic protective cap for the soil respiration chamber was attached, and the empty chamber and flask assembly were flushed for several minutes, to fully mix the internal volume. Next, the pump was turned off, and three to five alfalfa stems, roughly 60 cm long, were excised at the base of the plants, inserted into the chamber, and immediately the cap was replaced and the pump turned on. Attention was paid to avoid contaminating the sample with human breath. As the excised stems and leaves respired, \([\text{CO}_2]\) increased in the chamber and was monitored using the LI-6200. In roughly 50 ppm increments, the stopcocks were closed on individual flasks. This provided a set of five flasks per measurement, collected over several minutes, with \([\text{CO}_2]\) varying from near ambient at the start to 200 ppm above ambient at the end of the measurement. In total, five replicate measurements were made (five different foliage samples) between 21:00 and 22:00 h local time. All foliar measurements were pooled together and a single Keeling plot was constructed for the set (Table 1).

3.9. \(\delta^{13}C\) of soil respiration

On 22 August, at 16:00–18:00 h local time, estimates of the isotope ratio of soil-respired \(\text{CO}_2\) were made. The procedure was identical to that for the foliar measurements, except that once the empty chamber and flask assembly was flushed and mixed, the cap was removed and the chamber gently placed on one of the soil respiration collars. As \([\text{CO}_2]\) increased in the system, flasks were closed in 50 ppm increments. Five chambers were measured in total, and a single Keeling plot was constructed (Table 1). A single measurement of five flasks was completed in several minutes.

4. Results and discussion

4.1. Energy and \(\text{CO}_2\) fluxes

A major limitation of isotopic flux studies to date has been a lack of sampling frequency. Typically only a few days of measurements are possible even with considerable effort (Yakir and Wang, 1996; Bowling et al., 1999a, 2001b). For this reason, we present as much data as possible. The alfalfa crop exhibited rapid growth during the 15 days (11–25 August) of our measurements, with mean canopy height changing from 34 to 55 cm. Isotopic fluxes were measured using the EC/flask technique on all 15 days, but due to labor and analytical requirements, the gradient and HREA methods were only employed during 20–24 August. We were concerned that fluxes at the beginning of this time period might be markedly different from the end, so we present data for the entire period as well as the 5-day subset when all isotopic techniques were used.

Energy fluxes are shown in Fig. 2. Available energy (the difference between net radiation \((R_n)\) and soil heat flux \((G)\)) was consistent over the 15 days. Latent heat \((LE)\) fluxes (Fig. 2B) showed little day to day variation and closely followed available energy. Sensible heat fluxes \((H)\), however, showed a contrast in diurnal pattern, with a negligible mid-morning peak and downward (negative) flux during the afternoon. The direction of flux was confirmed by independent measurements of air temperature at the two flux-gradient heights (data not shown), and prevented us from using those temperature measurements and \(H\) to compute the eddy diffusivity for \(\text{CO}_2\) (as is common with the flux-gradient technique). The pattern of negative afternoon \(H\) has been observed by others, where irrigated and aerodynamically smooth crops in hot dry climates can transpire more energy as latent heat than is available as incoming radiation (Brakke et al., 1978; Rosenberg and Verma, 1978). This suggests that substantial horizontal advection of energy as sensible heat flux occurred at our site from the surrounding area.

Net ecosystem exchange \((F)\) of \(\text{CO}_2\) is shown in Fig. 3. Peak carbon uptake of \(\sim 25\) to \(\sim 30 \mu\text{mol m}^{-2}\text{s}^{-1}\) occurred at about 12:30 h local time, which is earlier than the \(R_n\) and LE peaks at 13:00 and 14:00 h, respectively. These peak values of \(F\) are similar to other alfalfa studies reported (Verma and Rosenberg, 1976; Asseng and Hsiao, 2000). We observed no discernible difference in the fluxes measured over the 15-day period and the 5-day subset. Nocturnal respiration was higher in the evening than in the morning (see the line in Fig. 3), which is consistent with measured air and soil temperatures (not shown).
4.1.1. Isotopic relationships and fluxes

The relationship between δ13C and [CO2] was generally consistent among the various types of samples (Fig. 4). Flask air samples were obtained at midday with [CO2] as low as 325.3 μmol mol⁻¹, which is evidence of strong photosynthetic removal of CO2 within the canopy. Such low values represent a maximal isotopic signal associated with photosynthetic enrichment of canopy air (δ13C was −5.9‰ at 325.3 μmol mol⁻¹). Similarly extreme values have been reported in alfalfa.
Fig. 3. Diurnal pattern of net ecosystem exchange of CO₂. Data are shown for all measurement periods from 11 to 25 August (filled circles), and for stationary periods only during 20–24 August (empty circles) for comparison. The latter period coincided with isotopic flux measurements. The line represents the stationary ensemble mean of all 15 days.

Midday minimum [CO₂] values differed strongly with height, with lowest mean (±S.D.) values of 321.2 ± 5.9 μmol mol⁻¹ at 0.5 m canopy heights, and higher ones above the canopy, 353.0 ± 3.2 μmol mol⁻¹ at 5.0 m (data not shown). These are substantially lower than background CO₂ at this latitude. (The Niwot Ridge, CO monthly means during August 2000 were 367.6 μmol mol⁻¹ and −7.92 ‰; data from NOAA/CMDL website: http://www.noaa.cmdl.gov.)

High [CO₂] (597.5 μmol mol⁻¹) and very negative δ¹³C (−15.4 ‰) were observed in nocturnal samples, indicating respiratory buildup of carbon dioxide that was depleted in ¹³C. The ranges in δ¹³C observed in our study are among the highest ever reported. Bowling et al. (1999a) reported a difference in the slopes of the δ¹³C versus [CO₂] relationship between whole-air samples and HREA samples (updrafts or downdrafts), and suggested the difference was possibly due to differing time scales associated with the sampling strategies of each method. HREA samples consist of 100 ms air samples representing extreme updrafts and downdrafts that are accumulated over time.

Bowling et al. (2001b) showed that whole-air samples collected over varying time periods (500 ms to 30 min) did not differ in their slopes, leaving the cause of the difference still unresolved. All samples analyzed for isotopic content are shown in Fig. 4, and these were collected over the entire diel cycle. The majority of samples showed a consistent pattern, but all HREA samples collected during the time period when the nocturnal boundary layer is breaking up (8:00 h local time) deviated from this pattern in a more positive isotopic direction (Fig. 4). We have no reason to suspect an experimental artifact unique to this sampling time period.
Fig. 4. The relationship between $\delta^{13}C$ and CO$_2$ for samples associated with the EC/flask (filled squares), flux-gradient (filled triangles), and HREA (filled circles) isotopic flux measurement techniques. The former two types represent whole-air samples, while the HREA are updraft or downdraft samples. HREA samples collected during the morning boundary layer transition (08:00–08:30 h local time, empty circles) are plotted separately. The line represents the Keeling relationship for nocturnal EC/flask and gradient samples ($\delta^{13}C = 7026.6/\left[CO_2\right] - 27.12$‰; Table 1).

very negative $\delta^{13}C_a$ due to respiratory buildup might cause substantial enrichment of above-canopy air relative to the normal mixing line, since the photosynthetic flux would have a very negative isotope ratio ($\Delta = \delta^{13}C_a - \delta^{13}C_o$). If so, this should be observed in whole-air samples as well. Unfortunately no gradient or EC/flask samples were collected during this time period for comparison. Second, the shallower slope is indicative of a lower discrimination relationship in general, consistent with some contribution from C$_4$ photosynthesis. It is possible that corn crops growing in the Cache Valley may have some influence that is apparent during the period of rapid boundary layer growth.

Measured isotopic fluxes are shown in Fig. 5A, including both stationary and non-stationary periods. Midday $F_\delta$ peaked at 600–700 $\mu$mol m$^{-2}$ s$^{-1}$‰, and nighttime values roughly averaged $-100$ $\mu$mol m$^{-2}$ s$^{-1}$‰. These values are similar to those reported over a deciduous forest in Tennessee (Bowling et al., 1999a, 2001b). Isotopic fluxes measured by the three methods were consistent in both diurnal pattern and magnitude, particularly when stationary atmospheric conditions were present (Fig. 5B). At night, there was a tendency for the gradient technique to overestimate $F_\delta$ relative to the others (Fig. 5A). However, we do not expect either the HREA or gradient techniques to be especially robust at night. The assumptions on which these methods are based are likely to fail within a neutral or stable nocturnal boundary layer.

4.1.2. Estimation of $\Delta_{\text{canopy}}$ from fluxes and isotopic fluxes

At the leaf level, carbon isotope discrimination has long been used by plant physiologists and ecologists as an indicator of plant carbon and water relations (Farquhar et al., 1989; Ehleringer et al., 1993). A conceptually identical quantity is used at the regional and global scales in large-scale carbon cycle studies that focus on the nature and timing of terrestrial carbon exchange with the atmosphere (Tans et al., 1993; Fung et al., 1997; Battle et al., 2000). Similarly, at the ecosystem-scale, $\Delta_{\text{canopy}}$ is proving to be a useful parameter in investigations of carbon and water cycling via ecosystem physiological processes (Lloyd
Fig. 5. Diurnal pattern of isotopic flux density \( \delta \) measured via EC/flask (filled circles), flux-gradient (empty squares), and HREA (empty circles) methods. Panel (A) shows all data available, which included stationary and non-stationary periods from 11 to 25 August (EC/flask technique) and from 20 to 24 August (gradient and HREA techniques). Panel (B) is a subset of panel (A) but with non-stationary periods removed. Isotopic storage fluxes have been incorporated into \( \delta \).

et al., 1996; Yakir and Sternberg, 2000; Bowling et al., 2001b; Baldocchi and Bowling, 2003).

Bowling et al. (2001b) showed that flux and isotopic flux measurements such as those in Figs. 3 and 5 can be used to partition \( F \) into \( F_A \) and \( F_R \) using Eqs. (1) and (2). This approach requires an estimate of flux-weighted, whole-canopy integrated photosynthetic discrimination \( \Delta_{\text{canopy}} \), which cannot be obtained by direct measurement. In fact, a primary conclusion of Bowling et al. (2001b) was that the isotopic approach to partitioning net ecosystem exchange is quite sensitive to \( \Delta_{\text{canopy}} \), and thus further exploration of \( \Delta_{\text{canopy}} \) is warranted. In this study, we chose to specify the total ecosystem respiration flux \( F_R \) in another fashion, then use Eqs. (1) and (2) to solve for \( F_A \) and \( \Delta_{\text{canopy}} \) as unknowns (rather than \( F_A \) and \( F_R \) as in Bowling et al., 2001b).

A common method to estimate total ecosystem respiration at eddy flux sites is examination of nocturnal \( F \), when photosynthesis is absent (Goulden et al., 1996; Valentini et al., 2000). Since at night the flux is strictly respiratory, an exponential dependence of measured fluxes on temperature \( T \) is expected (Lloyd and Taylor, 1994). Nighttime \( F \) is shown as a function of measured soil \( T \) in Fig. 6, along with spatially averaged soil chamber measurements. Despite an 8 °C range in soil \( T \), there was not a clear dependence of either \( F \) or chamber respiration measurements on soil \( T \) (Fig. 6), even when the chambers were evaluated individually (data not shown). The chamber measurements generally showed higher respiration than \( F \). Despite the homogenous plant canopy, more spatial variability was evident in the chamber measurements than variability due to \( T \) (not shown). We did not measure soil

Fig. 5 Diurnal pattern of isotopic flux density \( \delta \) measured via EC/flask (filled circles), flux-gradient (empty squares), and HREA (empty circles) methods. Panel (A) shows all data available, which included stationary and non-stationary periods from 11 to 25 August (EC/flask technique) and from 20 to 24 August (gradient and HREA techniques). Panel (B) is a subset of panel (A) but with non-stationary periods removed. Isotopic storage fluxes have been incorporated into \( \delta \).

Fig. 6 Respiration as a function of soil temperature (at 0.04 m depth). Data are shown for nocturnal \( F \) during stationary periods (filled circles), and for soil chambers measured throughout the diel period (shaded circles). Soil chamber data are spatial averages along >100 m transect (mean ± S.D.). Also shown are the \( F \) data averaged within 1 °C bins (shaded squares, mean ± S.D.). The line is an exponential regression through the binned \( F \) data \( \delta F_R(T) = 0.466 e^{0.107 T} \), \( r^2 = 0.730 \).
moisture along the soil respiration transect, but we did observe variation in volumetric soil moisture (9–13% at 0.15±0.02 m depth) in the soil pits used for organic sample collection. The high degree of spatial variability in soil respiration was likely a consequence of soil moisture variation (Davidson et al., 1998; Law et al., 2001). Since $F$ also includes the foliar component of total ecosystem respiration, it should be larger than soil chamber estimates. We attempted to measure total ecosystem respiration (including the foliage) with chambers, which would have possibly resolved this discrepancy. Unfortunately, this measurement was unsuccessful due to instrument problems in the field.

Only when the nocturnal $F$ data were bin-averaged in $1^\circ C$ increments was an exponential relationship apparent (Fig. 6). This is common in eddy flux studies (Greco and Baldocchi, 1996). Since our goal is to examine $\Delta_{canopy}$, we make the assumption that this regression provides an adequate representation of the true total ecosystem respiration flux. We acknowledge that this is a weak assumption. However, it facilitates a comparison with independent isotopic measurements that provide some confidence for our interpretation of $\Delta_{canopy}$ and the isotopic content of the photosynthetic flux (below).

Fluxes of $F$, $F_R$ (defined by the regression), and $F_A$ (where $F_A = F - F_R(T)$) are shown in Fig. 7. $F_R(T)$ peaked in late afternoon (16:00–16:30 h) at about $5 \mu mol m^{-2} s^{-1}$, while the assimilation peak ($\approx 30 \mu mol m^{-2} s^{-1}$) occurred earlier (14:00 h). Combining these fluxes with the EC/flask isotopic fluxes of Fig. 5B, specifying $\delta^{13}C_R$ using the Keeling plot (Table 1), and estimating $\delta^{13}C_a$ using measured [CO$_2$] at 0.4 m (0.8 h) and the $\delta^{13}C$ versus 1/[CO$_2$] regression (Table 1), we can solve for $\Delta_{canopy}$ using Eqs. (1) and (2).

The daytime diurnal pattern of $\Delta_{canopy}$ is shown in Fig. 8A. There was a general peak of about 19‰ around 11:00 h, and a gradual decrease to near 15‰ at the end of the day. This pattern differs somewhat from the diurnal pattern of discrimination reported by Bowling et al. (2001) over a deciduous forest, which was similar (19‰) in the early morning, and decreased rapidly to about 17‰ at 12:00 h, then stayed constant until sunset. In their study, $\Delta_{canopy}$ was estimated by inverting the Penman-Monteith equation and using a derived canopy conductance. The $F$-weighted average for $\Delta_{canopy}$ in the present study was 17.9‰. This is lower than $\Delta_e$, the ecosystem discrimination defined by Buchmann et al. (1998), which was $-19.7$‰ for
Fig. 8. (A) Whole-canopy carbon isotope discrimination (empty circles), derived using the fluxes in Fig. 7 and the EC/flask estimates of $F_{\delta}$ in Fig. 5B, and Eqs. (1) and (2). Half-hourly means (±S.D.) are also shown (filled circles). Data are shown only for daylight periods ($R_n > 50$ W m$^{-2}$) when $F_A < -5$ μmol m$^{-2}$ s$^{-1}$. (B) The isotopic composition of the photosynthetic flux (empty circles), and of the respired flux (filled circles). The latter was calculated from the Keeling plot (Table 1).

Using an ecophysiological canopy model, Baldocchi and Bowling (2003) have shown that considerable seasonal variation in $\Delta_{\text{canopy}}$ can be expected based on the response of photosynthesis and stomatal conductance to environmental variation (in particular light and humidity). Bowling et al. (2002) showed that $\delta^{13}$C$_{R}$ varies in response to freezing air temperatures and variations in vapor pressure deficit of air, implying a change in $\Delta_{\text{canopy}}$ on a scale of days to
weeks. Hence, we expect that our present estimates of $\Delta_{\text{canopy}}$ may not be representative of a time period of more than several days.

4.1.3. $\delta^{13}$C of the photosynthetic and respiratory fluxes and organic materials

The quantity $\delta^{13}$C$_{\text{P}} = \delta^{13}$C$_{\text{a}} - \Delta_{\text{canopy}}$ in Eq. (2) (which we will call $\delta^{13}$C$_{\text{P}}$) describes the isotopic composition of carbon removed by the photosynthetic assimilation flux throughout the day. This quantity is shown in Fig. 8B, along with $\delta^{13}$C$_{\text{R}}$, the isotopic composition of the respiration flux, for comparison. There was a strong diurnal change in $\delta^{13}$C$_{\text{P}}$, from $-27.5$ ‰ at 09:00 h to $-22.3$ ‰ at 18:00 h. At isotopic equilibrium, $\delta^{13}$C$_{\text{R}} = \delta^{13}$C$_{\text{a}} - \Delta_{\text{canopy}}$, and Eq. (2) is simply a multiple of Eq. (1). Equilibrium occurred only very early in the morning, and in fact the $F$-weighted average of $\delta^{13}$C$_{\text{P}}$ was $-25.1$ ‰, a full $2$ ‰ offset from $\delta^{13}$C$_{\text{R}}$ (Table 1). It is only through this time-varying disequilibrium that Eqs. (1) and (2) can be used as independent equations.

Bowling et al. (2002) showed that there is a link between $\delta^{13}$C$_{\text{a}}$ and vapor pressure saturation deficit (vpd) of the air, implying indirectly that $\Delta_{\text{canopy}}$ responds to changes in vpd. The diel pattern of the isotopic composition of the photosynthetic flux ($\delta^{13}$C$_{\text{P}}$) in Fig. 8B follows the diel pattern in vpd closely, with the most positive $\delta^{13}$C$_{\text{P}}$ at the end of the day when vpd was the highest. $\delta^{13}$C$_{\text{P}}$ changed by only $2.4$ ‰ over this time period (from $-9.2$ ‰ at 09:00 h to $-6.8$ ‰ at 18:00 h, not shown in Fig. 8), which is not enough to account for the full change in $\delta^{13}$C$_{\text{P}}$. This provides support for the hypothesis that $\Delta_{\text{canopy}}$ varies on diel time scales in response to environmental variables, possibly through diel changes in stomatal conductance.

The isotopic composition of ecosystem carbon stocks and fluxes is shown in Fig. 9. Sun leaves exhibited the most negative $\delta^{13}$C ($-28.0$ ‰) of all measured samples, and the CO$_2$ respired from whole branches was the most positive ($-23.8$ ‰). (Shade leaves are typically quite negative but they were not measured.) All measured stocks except sun leaves were more enriched than the total respiration flux. There was only marginal isotopic enrichment in bulk SOM with depth, a phenomenon commonly observed in natural soils (e.g. Ehleringer et al., 2000). Since total ecosystem respiration is a combination of soil

![Fig. 9: Isotopic composition of various ecosystem organic components (left panel), and ecosystem fluxes (right panel). Data are means and standard errors (for the organic), or Keeling plot intercepts (Table 1) with error bars equal to the standard error of the intercepts (for the fluxes). The theoretical point (filled circle) is equal to the soil-respired value minus $4.4$ ‰, and represents the maximum possible measurement error associated with disturbance of the isotopically enriched soil gas profile.](image-url)
respiration (all belowground processes including root respiration) and foliar respiration, we expected that \( \delta^{13}C_R \) would fall between the isotopic compositions of the soil-respired and branch-respired fluxes. Conservation of mass dictates that:

\[
\delta^{13}C_R (F_R) = \delta^{13}C_{R-soil} (F_{R-soil}) + \delta^{13}C_{R-branch} (F_{R-branch}),
\]

where the subscripts ‘R-soil’ and ‘R-branch’ denote the soil-respired and branch-respired fluxes, respectively, and \( F_R = F_{R-soil} + F_{R-branch} \). However, both component fluxes were more enriched than \( \delta^{13}C_R \) (Fig. 9), which violates conservation of mass. We suspect that our soil chamber isotopic measurements might be in error. A pool of enriched CO2 normally resides in the undisturbed soil profile (Cerling et al., 1991), and a measurement which disturbs this profile could be in error by as much as 4.4‰, the fractionation factor associated with binary diffusion of CO2 in air. The filled circle in Fig. 9 displays the theoretical lower limit for the soil-respired flux, which is considerably lower than \( \delta^{13}C_R \). This is not an issue with the branch-flux measurements since the volume of air where diffusion is dominant is very small. Perhaps the most important result of our study is the demonstration that \( \delta^{13}C \) of ecosystem carbon stocks (leaves, roots, SOM) is a poor predictor of ecosystem carbon fluxes (\( \delta^{13}C_P \) and \( \delta^{13}C_R \)). \( \delta^{13}C_P \) was always more enriched than bulk sun leaf tissue, and more enriched than \( \delta^{13}C_R \) for the majority of the day. Neither fine roots, tap roots, nor SOM matched \( \delta^{13}C_{R-soil} \), although it is possible that the 4.4‰ error might not be fully expressed.

5. Conclusions

Despite potential problems with each technique, we have demonstrated that the EC/flask, flux-gradient, and HREA methods provide similar estimates of ecosystem isotopic fluxes. Since the EC/flask method is fairly easy to apply, a survey of ecosystem isotope dynamics at a variety of ecosystem eddy flux sites is a realistic and attainable goal. However, these methods remain estimates of ecosystem isotopic fluxes, and are dependent on unresolved assumptions. These assumptions include scalar similarity and well-defined vertical profile relationships for CO2 and its isotopic forms (HREA and gradient techniques), and validity of the relationship in Fig. 4 at all time scales relevant to turbulent transport (EC/flask technique). Robust determination of isotopic fluxes will require development of instrumentation that can directly measure eddy covariance of \( ^{13}CO_2 \).

The relationship between \( \delta^{13}C \) and [CO2] was consistent except during the morning boundary layer transition, but reasons for this discrepancy are unclear. Values for \( \delta^{13}C_{assim} \) were obtained that are consistent with other canopy-level studies, and realistic from a leaf-level physiological perspective. The isotopic composition of the assimilation flux (\( \delta^{13}C_P \)) was not in equilibrium with the respiration flux except in the early morning. \( \delta^{13}C_P \) changed over the diel pattern, becoming more enriched at the end of the day when vpd was highest. Finally, the carbon isotope ratio of ecosystem carbon stocks does not appear to be a good predictor of the isotopic content of ecosystem carbon fluxes.

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Appendix A. Isoflux and net ecosystem exchange of 13CO2

Differing terminology in different fields, and in our own papers (Bowling et al., 1999a, 2001b), has led to some confusion in discussions with colleagues about isotopic net exchange fluxes. In particular, the isoflux (Bowling et al., 2001b) is similar in context to net ecosystem exchange of 13CO2, but they are not equal. Here we discuss the pertinent equations and introduce terminology that we hope will provide clarity in future analyses of ecosystem net isotopic exchange.

The “iso-” prefix generally refers to a constant quantity in meteorology (e.g. isotherms), analytical chemistry (isocentration), and fluid dynamics (isoflux, constant flux). However, these terms are also used in the carbon cycle community to refer to the product of isotopic composition and CO2 concentration or mole fraction (isocentration, Raupach, 2001), or the product of isotopic composition and CO2 flux (isoflux, Bowling et al., 2001b). These terms, especially isoflux, are common parlance within the global carbon cycle community, but are used more frequently in discussions than in the scientific literature (e.g. Rayner et al., 1999). All scientific approaches that use conservation of mass for total CO2 and 13CO2 are similar in context to net ecosystem exchange of 13CO2, but they are not equal.

Formally, net ecosystem exchange (F) of total CO2 is described by conservation of mass in a three-dimensional context (e.g. Baldocchi et al., 1988). With appropriate site selection, terms associated with flux divergence and horizontal heterogeneity can be neglected, reducing the description of F to:

\[ F = F_c + \frac{dC}{dt} = F_R + F_A, \]  

(A.1)

where \( F_c \) is total CO2 flux density (measured by eddy covariance), and \( dC/dt \) is the storage flux density, which is the time rate of change of CO2 mole fraction (C\text{\textsubscript{a}}) within the canopy between ground level and the measurement height, both expressed on a ground area basis (\( \mu \text{mol CO2 m}^{-2} \text{s}^{-1} \)). Eq. (A.1) applies to a suitable control volume where spatial averaging masks any localized near-field effects (Raupach, 1989). F can be interpreted in a biological context (on the right-hand side of Eq. (A.1)) as the sum of the CO2 flux densities of total ecosystem respiration (\( F_R \)) and photosynthetic assimilation (\( F_A \)), where \( F_A \) is negative.

Multiplying each term in Eq. (A.1) by its respective isotope ratio, we obtain an expression for the net ecosystem exchange of 13CO2:

\[ R_c F_c + \frac{d(R_c C_{\text{a}})}{dt} = R_R F_R + R_p F_p. \]  

(A.2)

where the R terms are the molar isotope ratios of the net flux (\( R_c \)), the air within the canopy (\( R_p \)), and the CO2 removed by photosynthesis (\( R_p \)). When the isotope ratios are expressed as \( ^{13}{\text{C}}/^{12}{\text{C}} \) isotope ratios and the CO2 removed by photosynthesis is equal to the product of the molar isotope ratios of air (\( R_p \)) and photosynthetic fractionation factor \( \alpha_{\text{canopy}} \) to the ratio of the molar isotope ratios of air (\( R_p \)) and photosynthetic fractionation factor \( \alpha_{\text{canopy}} \).

Following the leaf level definitions of Farquhar et al. (1989), we now define a whole-canopy, flux-weighted photosynthetic fractionation factor \( \alpha_{\text{canopy}} \) as the ratio of the molar isotope ratios of air (\( R_p \)) and photosynthetic fractionation factor \( \alpha_{\text{canopy}} \) to:

\[ \alpha_{\text{canopy}} = R_p/R_c. \]  

(A.3)

and substitute into Eq. (A.2) to obtain:

\[ F_{13} = R_c F_c + \frac{d(R_c C_{\text{a}})}{dt} = R_R F_R + R_p F_p + \frac{R_p}{\alpha_{\text{canopy}}} F_A. \]  

(A.4)

This equation represents conservation of mass for 13CO2, and states that the net ecosystem exchange of 13CO2 (denoted \( F_{13} \)) is equal to the sum of a flux term (\( R_c F_c \)) and a storage term (\( d(R_c C_{\text{a}})/dt \)) and photosynthesis (\( R_p F_p \)) times the flux (\( F_A \)). On the RHS of Eq. (A.4), the 13CO2 produced by total ecosystem respiration is equal to the product of the isotope ratio of the respiratory flux (\( R_R \)) times the flux (\( F_A \)), and the 13CO2 removed by photosynthesis is the product of the isotope ratio of the photosynthetic fractionation factor \( \alpha_{\text{canopy}} \) and the flux (\( F_A \)).
where the molar ratio of the sample and isotopic standard are $R_m$ and $R_{PDB}$, respectively. Eq. (A.6) in our context represents the discrimination associated with the entire canopy. Dividing Eq. (A.4) through by $R_{PDB}$ and substituting Eq. (A.6):

\[
\frac{R_c}{R_{PDB}} F_c = \frac{R_k}{R_{PDB}} F_k + \frac{R_a}{R_{PDB}} \left(\frac{1}{\Delta_{canopy} / 1000} + 1\right) F_A.
\]

This equation can be reduced to:

\[
\left(\frac{\Delta_k 1000}{F_c + \frac{d}{dt} \left(\frac{\Delta_k 1000}{C_a}\right)}\right) + \left(F_t + \frac{dC_a}{dt}\right) = \left(\frac{\Delta_k 1000}{F_k} + \frac{\Delta_{canopy} 1000}{F_A}\right) + (F_k + F_A).
\]

by neglecting the intermediate terms ($\Delta_{canopy}/1000)^2$ and $\Delta_{canopy} \cdot 6(1000)^2$. In Eq. (A.8), terms II and IV are equal (Eq. (A.1)), so they can be subtracted away to obtain:

\[
\Delta_k F + \frac{d(\Delta_k C_a)}{dt} = \Delta_k F_k + (\delta_k - \Delta_{canopy}) F_A.
\]

Eq. (A.9) is simply the delta-notation form of Eq. (A.4).

We summarize the three relevant equations here. Net ecosystem exchange is expressed for total CO$_2$ as:

\[
F = F_t + \frac{dC_a}{dt} = F_k + F_A,
\]

and is expressed for $^{13}$CO$_2$ as:

\[
F_{13} = R_c F_t + \frac{d(R_c C_a)}{dt} = R_k F_k + \frac{R_a}{\Delta} F_A.
\]

In common isotopic notation, Eq. (A.11) is expressed as an isoflux ($F_{13}$):


