Measuring field-scale isotopic CO2 fluxes with tunable diode laser absorption spectroscopy and micrometeorological techniques

T.J. Griffis\textsuperscript{a,∗}, J.M. Baker\textsuperscript{a,b}, S.D. Sargent\textsuperscript{c}, B.D. Tanner\textsuperscript{c}, J. Zhang\textsuperscript{a}

\textsuperscript{a} Department of Soil, Water, and Climate, University of Minnesota-Twin Cities, Borlaug Hall, 1991 Upper Buford Circle, St. Paul, MN 55108, USA
\textsuperscript{b} USDA-ARS, St. Paul, MN 55108, USA
\textsuperscript{c} Campbell Scientific, Inc., 815 West 1800 North, Logan, UT 84321-1784, USA

Received 11 August 2003; received in revised form 29 January 2004; accepted 29 January 2004

Abstract

The combination of micrometeorological and stable isotope techniques offers a relatively new approach for elucidating ecosystem-scale processes. Here we combined a micrometeorological gradient technique with tunable diode laser absorption spectroscopy (TDLAS) using the Trace Gas Analyzer (TGA100, Campbell Scientific, Inc., Utah, USA) to measure field-scale isotopic CO2 mixing ratios and fluxes of \(^{12}\)CO2 and \(^{13}\)CO2. The experiment was conducted in a recently harvested soybean (\textit{Glycine max}) field that had been in corn (\textit{Zea mays}) production the previous 4 years. Measurements were made over a period of 26 days from October 25 to November 19, 2002. Weather conditions were unusually cold and dry during the experiment. Isotopic gradients were small and averaged \(-0.153\) and \(-0.0018\) mmol mol\(^{-1}\)m\(^{-1}\) for \(^{12}\)CO2 and \(^{13}\)CO2, respectively for \(u^*_v > 0.1 \) m s\(^{-1}\). The average \(^{12}\)CO2 and \(^{13}\)CO2 flux for the period was 1.0 and 0.012 mmol m\(^{-2}\)s\(^{-1}\), respectively. The isotope ratio of respired carbon \((\delta^{13}C_R)\) obtained from the linear intercept of a Keeling plot was \(-27.93\) ‰ \((\pm0.32\) ‰) for the experimental period. The Keeling plot technique was compared to a new flux ratio methodology that estimates \(\delta^{13}C_R\) from the slope of a linear plot of \(^{13}\)CO2 versus \(^{12}\)CO2 flux. This method eliminates a number of potential limitations associated with the Keeling plot and provides a \(\delta^{13}C_R\) value that can be directly related to the flux footprint. In this initial comparison, our analysis showed that the flux ratio method produced a similar \(\delta^{13}C_R\) value (\(-28.67\) ‰), but with greater uncertainty (\(\pm2.1\) ‰). Better results are expected during growing season conditions when fluxes are substantially larger and the signal to noise ratio is improved. The isotope ratio of respired carbon was consistent with C\(_3\) agricultural systems indicating that soybean decomposition was the dominant substrate for respiration. The observed increase in ecosystem respiration \((R_E)\) and decrease in \(\delta^{13}C_R\) following tillage indicated that the incorporation of fresh soybean residue provided the major source for decomposition and further illustrates that the combination of micrometeorological and stable isotope techniques can be used to better interpret changes in carbon cycle processes. Long-term and continuous measurements of isotopic CO2 exchange using tunable diode laser absorption spectroscopy and micrometeorological techniques offers a new opportunity to study carbon cycle processes at the field-scale.

© 2004 Elsevier B.V. All rights reserved.

Keywords: Tunable diode laser absorption spectroscopy (TDLAS); Micrometeorology; Stable isotopes; Keeling plot; Isotopic fluxes; Net ecosystem exchange; Ecosystem respiration
1. Introduction

The combination of micrometeorological and stable isotope techniques offers a relatively new approach for improving the description of ecosystem-scale processes (Yakir and Wang, 1996; Bowling et al., 1999, 2001). The stable isotomers, \(^{12}\text{CO}_2\) and \(^{13}\text{CO}_2\), can be used as natural tracers to study biophysical processes because gross photosynthesis (\(P_g\)) discriminates against \(^{13}\text{CO}_2\) and fixes proportionally more \(^{12}\text{CO}_2\) (Farquhar et al., 1989). Consequently, the isotopic ratio of the atmospheric surface layer can become relatively enriched with \(^{13}\text{CO}_2\) during the daytime. Lin and Ehleringer (1997) reported that on relatively short time-scales (<days), there was no significant evidence of discrimination caused by plant respiration. Recently, however, Durieux et al. (1999) and Ghashghaie et al. (2001) demonstrated that respired \(\text{CO}_2\) from the leaves of a variety of \(\text{C}_3\) plants was enriched in \(^{13}\text{CO}_2\) relative to the leaf metabolites. The amount of discrimination caused by dark respiration processes, however, is likely to be minor compared to that of \(P_g\). Ecosystem respiration (\(R_E\)) therefore, adds relatively more \(^{12}\text{CO}_2\) to the atmospheric surface layer causing it to become depleted in \(^{13}\text{CO}_2\). The isotopic composition of respired carbon will differ from recently photosynthesized carbon because organic substrates, such as lignin and cellulose, exhibit differences in carbon isotope composition caused by enzymatic transformations following \(P_g\) (Ehleringer et al., 2000). The isotopic composition of \(R_E\) and \(P_g\) are not in equilibrium, which provides an opportunity to partition the net ecosystem \(\text{CO}_2\) exchange (NEE) into its components using stable isotope techniques.

Differences in the relative isotopic abundance of ecosystem components can influence the isotopic signature of \(R_E\), which can be used to help interpret changes in \(R_E\) (Lin and Ehleringer, 1997; Flanagan et al., 1999; Ehleringer et al., 2000). Furthermore, the distinct difference between \(\text{C}_3\) and \(\text{C}_4\) species in the degree of discrimination means that temporal changes in the isotope ratio of respired carbon can offer potential insight into the relative source contribution, mechanisms, and biophysical description of \(R_E\) in systems that have experienced known changes in species composition (Flanagan and Ehleringer, 1998; Ehleringer et al., 2002).

In recent years there has been increasing interest in the use of stable isotopes to study carbon cycling processes and to combine stable isotope and micrometeorological techniques to gain greater process information. There remains a need for obtaining continuous data at high temporal resolution to improve the understanding of land-atmosphere carbon cycling dynamics and ecosystem function. Previous experiments (Yakir and Wang, 1996; Bowling et al., 1999, 2001, 2003a) combined micrometeorological and stable isotope methods using flask sample air collection, stable isotope ratio analyses, and Keeling plots (Keeling, 1958) to estimate the isotopic fluxes of \(^{13}\text{CO}_2\) and \(^{12}\text{CO}_2\) and to characterize the isotopic signature of \(R_E\). Bowling et al. (1999, 2001) used mass spectrometry with hyperbolic relaxed eddy accumulation (HREA) and the eddy covariance flask (isoflux) approach to measure isotopic fluxes. Flask measurement methods, however, are not well-suited to continuous and long-term monitoring of ecosystem exchange because they require significant amounts of manual field and laboratory labor. Furthermore, because flasks represent discrete, point-in-time samples, it is difficult to devise a measurement strategy that adequately captures all frequencies contributing to the flux. A new development in micrometeorology and trace gas research is the ability to measure \(^{12}\text{CO}_2\) and \(^{13}\text{CO}_2\) concentrations directly and continuously using tunable diode laser absorption spectroscopy (TDLAS). The Trace Gas Analyzer (TGA100, Campbell Scientific, Inc., Logan, UT) has recently been adapted to measure stable isotomers (Bowling et al., 2003b). The relative high-frequency of isotopic \(\text{CO}_2\) measurement using the TDLAS approach is unprecedented and the increase in temporal resolution should lead to greater insight into the biophysical controls on \(\text{CO}_2\) exchange and ecosystem response to climate variation and land use practices. In particular, the TDLAS technique promises to provide better partitioning of NEE into \(P_g\) and \(R_E\) because a number of key parameters (isotope ratio of respiration and ecosystem discrimination) and quantities (isotopic fluxes) will be obtained at unprecedented frequency and duration. Thus, a better understanding of the diurnal, seasonal and interannual variations of these key parameters will be obtained.

This paper describes the results of a 26 day experiment where a micrometeorological gradient technique
The stable isotopomers, $^{12}\text{CO}_2$ and $^{13}\text{CO}_2$, have unique absorption lines that can be selected and measured using the TGA (Bowling et al., 2003b). In order to measure an individual absorption line without overlapping other absorption lines the sample air pressure of the TGA was reduced to 2.0 kPa to reduce pressure broadening. $^{12}\text{CO}_2$ and $^{13}\text{CO}_2$ infrared absorption was measured at wavenumber frequencies of 2308.225 and 2308.171 cm$^{-1}$, respectively. A CO$_2$ reference gas with approximately 10% $^{12}\text{CO}_2$ and 0.1% $^{13}\text{CO}_2$ was maintained at a flow rate of 10 ml min$^{-1}$ through the reference cell.

Mixing ratios of $^{13}\text{CO}_2$ and $^{13}\text{CO}_2$ were measured above the roughness sublayer at two sampling heights (1.65 and 2.35 m). Each sample inlet consisted of Delrin 25 mm filter holder with Teflon filter membranes (A-06623-32 and EW-02916-72, Cole-Parmer, Illinois, USA) followed by a brass critical flow orifice (Model D-7-BR, O’Keefe Controls Co., Monroe, CT, USA) to set a flow rate of 0.260 l min$^{-1}$ and to maintain a TGA sample cell pressure of 2.0 kPa. Dekabon tubing (Dekabon Type 1300, 1/4 in. o.d. × 0.040 in. wall, Dekoron, Furon Brands, Aurora, Ohio, USA) connected the orifices to the inlets of desiccant cells (Gas Purifier A-01418-50, Cole-Parmer, Illinois, USA) filled with magnesium perchlorate. Dekabon tubing conducted the air streams back to the instrument trailer located at the edge of the field, a distance of approximately 200 m. The lag time for a sample entering the intake and reaching the TGA sample cell was 2 min and 14 s. Within the trailer, the two sample lines and two calibration gas lines were each connected through sample inlet valves (Model LHDA12232-11HA, The Lee Co., Westbrook, CT, USA) mounted on a custom manifold (Campbell Scientific, Inc., Logan, UT, USA) to the TGA sample cell. A rotary vane vacuum pump (RB0021, Busch Inc., Virginia Beach, VA, USA) pulled the sample and calibration gases through the TGA sample cell. The manifold directed the flow from the unselected intakes (i.e. three of the four intakes) to the vacuum pump to maintain a constant flow in each intake.
This reduced pressure transients that could affect the measurements. Nitrogen gas, with flow rate of 10 mL min\(^{-1}\), purged the sample detector housing and the air space between the laser and the sample cell to prevent absorption by ambient CO\(_2\).

The TGA controlled the sampling system, with its parameters set to cycle through the four air streams every 2 min as follows: (1) calibration using ~350 μmol mol\(^{-1}\) CO\(_2\) with known isotope ratio; (2) calibration using ~600 μmol mol\(^{-1}\) CO\(_2\) with known isotope ratio; (3) measurement of CO\(_2\) mixing ratio at height \(z_1\); (4) measurement of CO\(_2\) mixing ratio at height \(z_2\). Within each 2 min cycle each calibration gas was sampled for 20 s, while each of the two measurement heights was sampled for 40 s. Following initial plumbing and leak testing, a zero gradient test was performed by moving both inlets to a common height, to ensure there were no systematic differences in the measured mixing ratios of the two sample lines.

The research trailer temperature was thermostatically controlled at approximately 20 °C to help maintain a stable calibration gain and offset. The TGA itself is enclosed in an insulated housing that is maintained at a constant temperature of approximately 35 °C. A two-point gain (G) and offset (O) correction, specific to each isotopomer, was performed every 2 min in order to obtain the \(^{12}\)CO\(_2\) and \(^{13}\)CO\(_2\) mixing ratios (see Bowling et al., 2003b for a detailed discussion of the gain and offset corrections).

2.3. Isotope convention, calibration standards and measurement precision

Our calibration procedure and data interpretation are referenced to the Vienna Pee Dee belemnite (VPDB) scale as recommended by the International Union of Pure and Applied Chemistry, Commission on Atomic Weights and Isotopic Abundances. The isotope relative abundance (δ\(^{13}\)CO\(_2\)) of our standard gases and atmospheric samples were calculated from

\[
\delta^{13}C = \left( \frac{R_{\text{VPDB}}}{R} - 1 \right) \times 1000
\]

where \(R\) is the molar ratio of the heavy to light isotope (\(^{13}\)C/\(^{12}\)C) of the sample and \(R_{\text{VPDB}}\) the standard molar ratio (\(^{13}\)C/\(^{12}\)C). \(\delta^{13}C\) is reported in parts per thousand (per mil or ‰).

The TGA calibration procedure requires that the mixing ratio of \(^{13}\)CO\(_2\) and \(^{12}\)CO\(_2\) be accurately known for the standard gases. Thus, the molar ratios of \(^{13}\)C and \(^{12}\)C in VPDB is required to determine the mixing ratio of \(^{13}\)CO\(_2\) and \(^{12}\)CO\(_2\) in each standard (see Appendix A for further details). We used R\(_{\text{VPDB}}\) to determine the mixing ratio of \(^{12}\)CO\(_2\) and \(^{13}\)CO\(_2\) in primary standards obtained from the National Oceanic and Atmospheric Administration-Climate Monitoring and Diagnostics Laboratory (NOAA-CMDL). The NOAA-CMDL standards were analyzed for total CO\(_2\) mixing ratio and δ\(^{13}\)CO\(_2\) with values of 348.61 μmol mol\(^{-1}\), 18.35‰, and 568.44 μmol mol\(^{-1}\), −17.22‰, for cylinders A and B, respectively. Mole mixing ratios of \(^{12}\)CO\(_2\) and \(^{13}\)CO\(_2\) of the primary standards were calculated as 343.15, 3.80 and 559.60, 6.15 μmol mol\(^{-1}\) for cylinders A and B, respectively.

Secondary field standards were propagated by using the TGA to calibrate unknown calibration cylinders onsite. A detailed description of the field standards used during this experiment is provided in Table 1. During the period DOY 300 to DOY 318 we observed an increasing trend (~0.95% total CO\(_2\) mixing ratio) in the standard cylinder B for \(^{13}\)CO\(_2\) (calibration standard value 517.49 μmol mol\(^{-1}\) and \(^{13}\)CO\(_2\) (calibration standard value 5.72 μmol mol\(^{-1}\)) mixing ratios. No trend was observed in the corresponding standard cylinder A. The CMDL has reported the potential for trace gases to release from the walls of pressurized cylinders as cylinder pressure decreases (http://www.cmdl.noaa.gov/ccgg/refgases/airstandard.html). Consequently, the total CO\(_2\) mixing ratio can increase with decreasing pressure (time). To correct this we detrended the drift in the \(^{12}\)CO\(_2\) and \(^{13}\)CO\(_2\) standard (cylinder B) prior to applying the two-point gain and offset corrections. This removed the trend that was introduced in the gain and offset factors. The variability and magnitude, however, were greater than expected during the period DOY 300 to DOY 318 as compared to the end of the experimental period when the secondary standards were changed.

The offset correction for each 2 min measurement cycle during the experimental period, shown in Fig. 1, averaged 2.97 (±1.42) and 0.039 (±0.015) μmol mol\(^{-1}\) for \(^{12}\)CO\(_2\) and \(^{13}\)CO\(_2\), respectively (the values in parenthesis indicate the standard deviation). The cor-
Table 1
Calibration standards and the uncorrected mixing ratio measurement of each standard used during the experimental period

<table>
<thead>
<tr>
<th>DOY</th>
<th>Cylinder A</th>
<th>Cylinder B</th>
<th>Cylinder A</th>
<th>Cylinder B</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$^{12}$CO$_2$</td>
<td>$^{13}$CO$_2$</td>
<td>$^{13}$CO$_2$</td>
<td>$^{12}$CO$_2$</td>
</tr>
<tr>
<td>298 (I)</td>
<td>345.42 (0.006)</td>
<td>3.81 (0.00004)</td>
<td>514.23 (0.02)</td>
<td>5.68 (0.0001)</td>
</tr>
<tr>
<td>305 (II)</td>
<td>367.85 (0.007)</td>
<td>4.05 (0.00003)</td>
<td>514.35 (0.01)</td>
<td>5.68 (0.0001)</td>
</tr>
<tr>
<td>318 (III)</td>
<td>367.38 (0.011)</td>
<td>4.05 (0.00006)</td>
<td>580.39 (0.02)</td>
<td>6.40 (0.0001)</td>
</tr>
</tbody>
</table>

*a Indicates the NOAA-CMDL primary standards. The $^{12}$CO$_2$ and $^{13}$CO$_2$ mixing ratios were calculated for NOAA-CMDL standards based on the analyzed total CO$_2$ mixing ratio and $\delta^{13}$CO$_2$ values.
*b Indicates original field standards. All other field standards were propagated from the original field standards. Mixing ratios are expressed in $\text{mol mol}^{-1}$ and the isotopic ratio, $\delta^{13}$CO$_2$, in units of parts per thousand (‰). The values in parenthesis indicate the standard error of the mean. The uncorrected mixing ratios are the average measured values obtained over the time interval that each standard cylinder (A and B) was used during the experiment. I–III designate the time periods that these field standards were used as shown in Figs. 1 and 2. Note that the reported number of significant digits for the mixing ratios is inadequate to reproduce the $\delta^{13}$CO$_2$ values shown.

Fig. 1. Offset factors calculated for $^{12}$CO$_2$ (top panel) and $^{13}$CO$_2$ (bottom panel). The offset was calculated every 2 min using two known standard calibration cylinders. The time intervals I–III correspond to the calibration tank changes as described in Table 1.

Fig. 2. Gain factors calculated for $^{12}$CO$_2$ (top panel) and $^{13}$CO$_2$ (lower panel). The gain was calculated every 2 min using two known standard calibration tanks. The time intervals I–III correspond to the calibration tank changes as described in Table 1.
The precision of the TGA was determined by measuring the $^{12}$CO$_2$ and $^{13}$CO$_2$ mixing ratios and the isotope ratio of two secondary standard cylinders for 34 half-hour periods. The mean and standard deviation of $^{12}$CO$_2$, $^{13}$CO$_2$ and $^{13}$CO$_2$ for each cylinder was $348.87 \pm 0.03$ μmol mol$^{-1}$, $3.8474 \pm 0.0002$ μmol mol$^{-1}$, $-13.55 \pm 0.07$ ‰ and $584.32 \pm 0.05$ μmol mol$^{-1}$, $6.4435 \pm 0.0005$ μmol mol$^{-1}$, $-13.63 \pm 0.03$ ‰, respectively.

2.4. Micrometeorological techniques

A three-dimensional sonic anemometer-thermometer (Model CSAT3, Campbell Scientific, Inc., UT, USA) was used to obtain the eddy diffusivity of the sensible heat flux ($K$). $K$ was derived from

$$K = \frac{\bar{u}_a (z_2 - z_1)}{\phi} \quad (2)$$

where $\bar{u}$ is the von Karman constant, $u_a$ the friction velocity, measured directly with the CSAT3, $z_2$ and $z_1$ are the measurement heights of the two air intakes and $\phi$ is the diabatic correction for atmospheric stability, calculated as described by Wagner-Riddle et al. (1996). Similarity was assumed for the diffusivity of sensible heat, CO$_2$, and its isotopomers.

Half-hour averaged flux calculations of net ecosystem CO$_2$ exchange (NEE) were computed from

$$\text{NEE} = -K \frac{d\bar{X}}{dz} - \rho_d \frac{d}{dz} \int \bar{X}(z) dz \quad (3)$$

where $\rho$ is the density of dry air, $M_a$ the molecular weight of dry air, and $d\bar{X}/dz$ the 30 min time-averaged vertical gradient of the CO$_2$ mixing ratio. The eddy flux is obtained from the first term on the right hand side of Eq. (3). The integral term is the rate of change in storage of CO$_2$ in the air column between the surface and the top of the CO$_2$ profile at height $z$. Corrections for density fluctuations resulting from sensible heat and latent heat fluxes (Webb et al., 1980) were eliminated because the air streams at each level were scrubbed of water vapor and brought to a common temperature at the analyzer.

Eddy covariance flux measurements of total CO$_2$ were obtained using the CSAT3 and an open-path infrared gas analyzer (LI-7500, LI-COR, NE, USA) measured at a height of 2 m. Post-processing of the data included 2-D coordinate rotation followed by simultaneous solution of the Webb et al. (1980) corrections and the Schotanus et al. (1983) equation, modified (Arjan van Dijk, pers. comm.) to account for the fact that the instantaneous sonic temperature measurements have already been corrected for cross-wind fluctuations, to determine the latent and sensible heat fluxes from the measured mean covariances of vertical wind speed with sonic temperature and with water vapor density. These were then used to compute a density-corrected CO$_2$ flux from the mean half-hour covariance of vertical wind speed and carbon dioxide concentration.

2.5. Keeling plot and flux ratio technique

The Keeling plot technique has been used extensively to characterize the isotope ratio of respiration ($^{13}$C R ) at various spatial scales (Keeling, 1958; Buchmann and Ehleringer, 1998; Flanagan et al., 1999; Bowling et al., 2001; Pataki et al., 2003). Keeling (1958) demonstrated that there is a strong linear relationship between $^{13}$CO$_2$ and the reciprocal of the total CO$_2$ mixing ratio. The decrease in $^{13}$CO$_2$ with increasing CO$_2$ mixing ratio results from the addition of CO$_2$ to the atmosphere that has a different isotopic ratio than the background tropospheric value of approximately $-8\%$. In order to be linear, the added CO$_2$ to the atmosphere must have a constant isotopic ratio. If this is true the linear intercept of the Keeling plot can be used to quantify the isotope ratio of the added (respired) CO$_2$. We used this technique to examine the variability in $^{13}$CO$_2$ during this experiment. A geometric mean regression (Model II regression) method was used to estimate the Keeling intercept and slope (Pataki et al., 2003).

The uncertainty of the y-intercept for the Keeling plot technique remains an important issue (Yakir and Sternberg, 2000) because the linear equation must be extrapolated well beyond the range of the observed data. Furthermore, the intercept may be sensitive to changes in the background tropospheric isotope ratio, isotopic disequilibrium between daytime photosynthesis and nighttime respiration (Pataki et al., 2003) and changes in boundary layer depth and mixing (Lloyd et al., 1996). As shown in previous studies (Yakir and Wang, 1996; Bowling et al., 2001), the Keeling intercept provides one of the key parameters needed for partitioning NEE into $R_u$ and $P_T$. One of the potential
advantages of the TDLAS technique is that, in contrast to the traditional Keeling plot approach, it allows a direct measurement of the isotopic fluxes. A flux ratio method, therefore, can be used to quantify the isotope ratio of $R_E$ and can eliminate the potential problems mentioned above.

The traditional mass spectrometry technique fundamentally compares the isotopic ratio of a sample to the isotopic ratio of a reference material, leading to the use of the $\delta$ notation. The TDLAS technique, however, independently measures the mixing ratio of the two isotopomers. Although the $\delta$ notation is easily used (via Eq. (1)), a more direct approach is suggested: to determine the flux ratio of $^{13}\text{CO}_2$ to $^{12}\text{CO}_2$ as the slope of a linear fit. This ratio is then converted to the traditional $\delta$ notation. We obtained $\delta^{13}\text{C}_R$ from Eq. (1) as

$$^{13}\text{CO}_2 = (1 + \frac{\delta^{13}\text{C}_R}{1000}) \times R_{VPDB} \times ^{12}\text{CO}_2 \quad (4a)$$

and taking the first-order derivative of $^{13}\text{CO}_2$ with respect to $^{12}\text{CO}_2$ yields

$$\frac{d^{13}\text{CO}_2}{d^{12}\text{CO}_2} = (1 + \frac{\delta^{13}\text{C}_R}{1000}) \times R_{VPDB} \quad (4b)$$

The term $\delta^{13}\text{CO}_2/d^{12}\text{CO}_2$ is obtained through the flux (gradient) measurement of $^{13}\text{CO}_2$ and $^{12}\text{CO}_2$. Therefore $\delta^{13}\text{C}_R$ can be defined as

$$\delta^{13}\text{C}_R = \left( \frac{F^{13}\text{CO}_2}{F^{12}\text{CO}_2} \times R_{VPDB} - 1 \right) \times 1000 \quad (4c)$$

where $F^{13}\text{CO}_2$ and $F^{12}\text{CO}_2$ are the isotopic effluxes—obtained during non-growing season or nighttime conditions. Application of this method during daytime growing season conditions would provide a flux ratio of NEE, which would include the effects of $P_g$ and $R_E$. It should be noted that the source area influencing the flux ratio and the Keeling plot estimates of $\delta^{13}\text{C}_R$ are not equal. A concentration measured above the surface is associated with a larger source area than a flux measurement made at the same height (Schmid, 2002). Thus, the flux ratio method provides an estimate of $\delta^{13}\text{C}_R$ that can be related directly to the flux footprint.

3. Results

3.1. Climatic conditions

Conditions were unseasonably cloudy, cold, and dry during the measurement period. Fig. 3 shows the surface temperature measured with an infrared thermometer (Everest Interscience, Inc., AZ, USA) and cumulative precipitation recorded during the experiment. The average temperature for the period was $0.8^\circ C$ with an observed minimum and maximum of $-10.6$ and $18.1^\circ C$, respectively. The warmest day coincided with the timing of tillage (DOY 311, November 7). Total cumulative precipitation during the experimental period was 3.4 mm. Approximately 3 mm of rainfall was recorded on DOY 308 (November 4). Conditions were extremely dry prior to the field experiment.

3.2. Variation in CO$_2$ mixing ratio and isotopic ratio

There was considerable variability in the mixing ratios of $^{12}\text{CO}_2$ and $^{13}\text{CO}_2$ (Fig. 4). The changes

![Fig. 3. Half-hour mean surface temperature measured with an infrared thermometer (top panel) and cumulative precipitation (bottom panel) measured during the course of the 26 day experiment. Climatic conditions were cloudy, and relatively cold and dry compared to the climate normal for Rosemount, Minnesota.](image-url)
in each isotopomer were highly correlated. Maximum values of $^{12}$CO$_2$ and $^{13}$CO$_2$ were 435.9 and 4.8/µmol mol$^{-1}$, respectively, during the experimental period. The largest mixing ratios occurred on clear stable nights when $u_*$ was less than 0.1 m s$^{-1}$ (weak turbulent mixing). The buildup of CO$_2$ during these stable conditions was relatively depleted in $^{13}$CO$_2$ resulting in a $\delta^{13}$CO$_2$ that was frequently less than $-9$‰.

Fig. 5 illustrates the relation between total CO$_2$ and $^{13}$CO$_2$ as a function of $u_*$. The strong increase in CO$_2$ and decrease in $^{13}$CO$_2$ at $u_*$ less than 0.15 m s$^{-1}$ reveals the effect of respired CO$_2$ accumulating in the developing nocturnal boundary layer. During prolonged stable conditions the boundary layer $^{13}$CO$_2$ was $-11$‰.
Fig. 6. Half-hour flux estimates of CO₂ (top panel), ¹³CO₂ (middle panel) and ¹⁴CO₂ (bottom panel). Total CO₂ fluxes were measured with a three-dimensional sonic anemometer and an open-path infrared gas analyzer. ¹³CO₂ and ¹⁴CO₂ fluxes were measured using the gradient technique. The largest fluxes were observed following tillage (DOY 311), which also correlated with relatively warm daytime temperatures.

0.80, 1.33, and 0.015 µmol m⁻² s⁻¹, respectively. For $u_*$ > 0.15 m s⁻¹ the corresponding flux values were 0.84, 1.0 and 0.012 µmol m⁻² s⁻¹, illustrating that at low $u_*$ the gradient technique overestimates the flux, presumably because gradient measurements are more sensitive to changes in storage. We also note that the eddy covariance flux measurements are likely underestimated because we did not correct for flux loss due to sensor separation or energy balance closure (Massman and Lee, 2002). The relative error of the gradient flux calculations was determined from the root mean squared error (RMSE) method (Bevington, 1969). Error propagation was estimated by examining the partial derivatives of the flux with respect to each of the dependent variables. We assumed a relative error of 15% for $K$ and that the random error in measuring CO₂ mixing ratio vanishes with time. Furthermore, the systematic error between levels was assumed to be zero (zero gradient test) when calculating the finite differences in CO₂ mixing ratio (Griffis et al., 2000). The relative error propagating into the flux calculations was approximately 24% for ¹³CO₂ and ¹⁴CO₂.

3.4. Isotope ratio of ecosystem respiration

Fig. 7a shows the Keeling plot for all acceptable data ($u_*$ > 0.1 m s⁻¹) collected during the experiment at measurement height $z_1$ (1.65 m). The δ¹³C_R estimate ($y$-intercept of the linear equation) was $-27.93\%$ with a standard error of ±0.32% (Table 2). Similar results were obtained for height $z_2$ (2.35 m). The flux ratio δ¹³C_R value was $-28.67\%$ with a standard error of ±2.1%. Although the flux ratio technique avoids the extrapolation of the Keeling approach, it offered no real advantage in this particular experiment as the uncertainty in δ¹³C_R was large compared to the Keeling method. We further explored the isotope ratio of respired carbon by examining the ratio of the change in storage (integral term of Eq. (3)), which is analogous to calculating a flux in an open-top-chamber. The δ¹³C_R estimated from this approach was $-27.76\%$ with a standard error of ±0.62% (Fig. 7d). Finally, we calculated δ¹³C_R using the ratio of ¹²CO₂ and ¹³CO₂ mixing ratios at each measurement height, which is fundamentally different than the flux ratio method. Although this method also avoids the problem of extrapolating to the $y$-intercept the δ¹³C_R values were significantly less negative than the other approaches. There was generally good agreement between the Keeling plot and the flux ratio techniques.

The temporal variability of δ¹³C_R (Fig. 8) was assessed by applying the Keeling plot and flux ratio method to two time intervals including pre-tillage (298 ≥ DOY ≥ 310) and post-tillage (311 ≥ DOY ≤ 323). Consistent with each approach was the strong decrease in δ¹³C_R following tillage. Further analysis showed that the average surface temperature during the period of flux measurements was 3.8 °C prior to tillage and 1.0 °C following tillage. The decrease in δ¹³C_R, however, correlated with increased surface temperature and the large fluxes following tillage. Ideally, the isotope ratio of an individual half-hour efflux could be measured and used to assess the short-term variation in δ¹³C_R. However, given the small fluxes observed during the experimental period
Fig. 7. Estimates of the isotopic ratio of respired carbon ($\delta^{13}C_R$) using the Keeling plot technique at height $z_1$ (top left panel) and $z_2$ (top middle panel) and alternative approaches using a linear plot of $^{13}$CO$_2$ vs. $^{12}$CO$_2$ flux (for friction velocity $> 0.1 \text{m s}^{-1}$) (top right panel), storage ratio (bottom left panel), and mixing ratios at height $z_1$ (bottom middle panel) and height $z_2$ (bottom right panel). $\delta^{13}C_R$ was quantified using: (1) the intercept of the Keeling plot, (2) the slope of the linear plots (ratio technique). Each plot is based on coincident data in order to eliminate bias resulting from missing or screened values.

Table 2
Keeling plot and ratio techniques for estimating the isotopic signature of respired carbon at the field-scale

<table>
<thead>
<tr>
<th>Technique</th>
<th>Slope</th>
<th>Intercept</th>
<th>$\delta^{13}C_R$</th>
<th>n</th>
<th>$\sigma$ (adj)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Keeling plot ($z_1$)</td>
<td>7454</td>
<td>−27.93</td>
<td>−27.93 (±0.32)</td>
<td>656</td>
<td>0.83</td>
</tr>
<tr>
<td>Keeling plot ($z_2$)</td>
<td>7462</td>
<td>−28.02</td>
<td>−28.02 (±0.34)</td>
<td>656</td>
<td>0.81</td>
</tr>
<tr>
<td>Flux ratio</td>
<td>0.010859</td>
<td>0.001</td>
<td>−28.67 (±2.1)</td>
<td>656</td>
<td>0.99</td>
</tr>
<tr>
<td>Storage ratio</td>
<td>0.010869</td>
<td>0.0000006</td>
<td>−27.76 (±0.62)</td>
<td>656</td>
<td>0.99</td>
</tr>
<tr>
<td>Conc. ratio ($z_1$)</td>
<td>0.010884</td>
<td>0.075</td>
<td>−26.39 (±0.30)</td>
<td>656</td>
<td>0.99</td>
</tr>
<tr>
<td>Conc. ratio ($z_2$)</td>
<td>0.010885</td>
<td>0.079</td>
<td>−26.32 (±0.31)</td>
<td>656</td>
<td>0.99</td>
</tr>
</tbody>
</table>

The comparative analysis was restricted to periods when data was available for each method (i.e. $n = 656$). Data were excluded for periods when friction velocity was $< 0.1 \text{m s}^{-1}$. Geometric mean regression was used for the fitting procedure. The values in parenthesis indicate the standard error of the parameter estimate.

The relative error in the flux ratio approach was significant. Daily estimates of $\delta^{13}C_R$ calculated from individual half-hour periods, for $^{13}$CO$_2$ flux > 1.0 $\mu$mol m$^{-2}$ s$^{-1}$, showed considerable variation over the course of the experimental period, with decreasing values associated with the large effluxes immediately following tillage. We could not conclude with certainty, however, that these daily variations in $\delta^{13}C_R$ were directly related to changes in biophysical factors.

4. Discussion

The combination of micrometeorological and stable isotope techniques offers the ability to gain additional...
process information at the field-scale. In the first study of its kind, Bowling et al. (2003b) compared in situ measurements using tunable diode laser absorption spectroscopy to laboratory analysis of flask samples using a stable isotope ratio mass spectrometer and an infrared gas analyzer. Their study showed that TD-LAS concentration and isotopic ratio measurements compared well with the flask measurements, although they observed a systematic difference of 1.77‰, with the TD-LAS technique underestimating (i.e. more positive) the δ^{13}CO_{2}. They demonstrated that this offset was related to a pressure broadening effect caused by using calibration standards with CO_{2} balanced in nitrogen rather than air. In this study we used TD-LAS over a period of 26 days to measure mixing ratios of 12CO_{2} and 13CO_{2} at two heights to estimate isotopic fluxes from a recently harvested soybean field using the flux gradient method. Calibrations were performed with CO_{2} balanced in dry air. The TGA system ran continuously during this period with data loss due only to system maintenance. Gradients of 12CO_{2} and 13CO_{2} were relatively small given the cold and dry weather conditions, but were larger than the limit of precision of the TGA system. The prospect of applying this approach for continuous year-long measurements shows considerable promise.

Consistent with flask-based measurement studies was an observed buildup of CO_{2} in the nocturnal boundary layer that was relatively depleted in 13CO_{2} due to the contribution of respiration (Bowling et al., 1999, 2001). For stable atmospheric conditions, w/L > 0 and u_{*} < 0.1 m s^{-1}, the maximum total CO_{2} mixing ratio exceeded 430 μmol mol^{-1} with a δ^{13}CO_{2} of −11‰. This amount of depletion is consistent with nocturnal and early morning observations made by Bowling et al. (1999, 2001) for a deciduous Tennessee forest. The temporal variation in δ^{13}CO_{2} showed a consistent pattern of depletion on stable nights and a return to the daytime well-mixed δ^{13}CO_{2} value of approximately −8‰. Isotopic fluxes of CO_{2} were small over the majority of the study period. In general, total flux estimates from the gradient approach (12CO_{2} + 13CO_{2}) were larger than the eddy covariance estimates. However, eddy covariance and the gradient fluxes showed a strong temporal correlation with the largest fluxes observed following tillage on day of year 316. 

**Fig. 8. Variability in the isotopic ratio of respired carbon (δ^{13}C_{R}) using the Keeling plot technique (upper panels) and flux ratio method (lower panels). δ^{13}C_{R} was estimated for the time intervals including pre-tillage ≥ DOY ≥ 310 (left panels) and post-tillage 311 ≥ DOY ≥ 323 (right panels). Each method showed that δ^{13}C_{R} became more negative following tillage. The values in parenthesis indicate the standard error of the parameter estimate.**
extrapolating to the growth and entrainment), eliminates the problem of projecting to the
value that is consistent with the source area of the flux.

We estimated δ¹³C_R to be ~27.93‰ using the Keeling plot technique for the entire experimental period. The Keeling plot technique was compared to a new flux ratio method, which in theory, is less sensitive to changes in background atmospheric CO₂ mixing ratios and δ¹³CO₂ related to boundary layer dynamics (growth and entrainment), eliminates the problem of extrapolating to the y-intercept, and provides a δ¹³C_R value that is consistent with the source area of the flux. The flux ratio estimate of δ¹³C_R is based on the slope of a linear geometric mean regression fit. Thus, it eliminates the problem of projecting to the y-axis (i.e. 1/finite CO₂ concentration), which is well beyond the range of the observed data. This approach produced δ¹³C_R values that were more negative (~0.7‰) than the Keeling approach; however, the uncertainty was large (>2‰) because the fluxes were very small. Better results are expected during growing season conditions when fluxes are significantly larger and the signal to noise ratio is improved. In this first attempt we find that the relatively small difference between the flux ratio and Keeling plot δ¹³C_R values indicates that the Keeling method is very robust despite the potential uncertainty related to projecting to the y-intercept and beyond the range of observed data. Furthermore, it is relatively easier to measure than fluxes. Thus, continuous isotopic mixing ratio measurements using TD-LAS with traditional eddy covariance flux measurements will offer new information for studying carbon cycle dynamics and ecosystem function.

Contrary to our original expectation, the isotopic ratio of respired carbon did not consistently become heavier (less negative δ¹³C_R) with time due to a reduction in fresh soybean residue relative to the 4-year accumulation of corn residue. We found δ¹³C_R values that were consistent with C₃ agricultural systems (Ehleringer et al., 2000) indicating that soybean decomposition dominated respiration. Most intriguing was the change in δ¹³C_R following tillage, which correlated with a short-term increase in temperature. We hypothesize that these changes resulted from: (1) incorporation of surface soybean residue and increased microbial decomposition following tillage on DOY 311, (2) an increase in microbial activity in response to warmer surface temperatures. The analysis illustrates that soybean (C₃) substrates were the main source for microbial decomposition following tillage despite the previous 4 years of corn production. Long-term continuous data are needed to further examine the influence of soil temperature, soil water content, and soil disturbance on the temporal variability δ¹³C_R and the fluxes of ¹²CO₂ and ¹³CO₂. Estimating the contribution of fresh versus old carbon to R_E will be possible by combining atmospheric isotopic measurements with stable isotope plant and soil assays.

The high-frequency measurement of isotopic ratios and fluxes has the potential to offer considerable insight into the controls on R_E. Temporal variation in δ¹³C_R suggests changes in the source contribution of respired carbon. Such changes probably reflect isotopic differences in organic matter and differential rates of microbial activity related to changes in temperature, water availability and disturbance of the soil profile. During growing season conditions, when fluxes are relatively large, we expect that the isotopic ratio of R_E (at night) and NEE (daytime) could be obtained with a relatively small error on an individual half-hour basis using the flux ratio method.

Quantifying the temporal variability in δ¹³C_R has major implications for flux partitioning studies (Yakir and Wang, 1996; Bowling et al., 2001). If the flux ratio of respiration is conservative on diurnal time-scales (>1 day) then the direct partitioning of daytime NEE into P_E and R_E should be possible from daytime flux measurements of ¹²CO₂ and ¹³CO₂ and estimates of ecosystem discrimination. Continuous long-term measurements of isotopic CO₂ exchange are needed to help partition NEE into its components and to better understand the temporal variability of δ¹³C_R and photosynthetic discrimination. Such studies should make a valuable contribution to the regional carbon modeling community (Ciais et al., 1995; Francey et al., 1995; Fung et al., 1997).

5. Conclusions

1. Long-term and relatively high-frequency measurements of isotopic CO₂ mixing ratios and fluxes are possible using tunable diode laser absorption spectroscopy.
2. Eddy covariance flux measurements of total CO₂ and gradient flux measurements of ¹³CO₂ and ¹²CO₂ measured using the TGA system showed a strong temporal correlation.

3. Linear plots of ¹³CO₂ versus ¹²CO₂ fluxes support the Keeling plot technique for estimating the isotope ratio of respired carbon (δ¹³Cᵣ) and provide a more direct means of evaluating the variation in the isotope ratio of ecosystem respiration.

4. The observed increase in ecosystem respiration (Rₑ) and decrease in δ¹³Cᵣ following tillage indicated that the incorporation of fresh soybean residue provided the major source for decomposition and further illustrates how combining micrometeorological and stable isotope techniques can be used to better interpret changes in carbon cycle processes.

Acknowledgements

Funding for this research was provided by the University of Minnesota, Grant-in-Aid-of-Research, Artistry and Scholarship Program (TJG). Field assistance was provided by W.A. Breiter and K. Vang, USDA-ARS Technicians, University of Minnesota. We gratefully acknowledge the logistical support provided by the Rosemount Research and Outreach Center, University of Minnesota and the USDA-ARS. We thank T.A. Black and K. Morgenstern for reviewing an earlier draft of the manuscript. We especially acknowledge the criticisms and helpful suggestions provided by D.R. Bowling and the anonymous reviewers.

Appendix A. Isotope standards and calculation of isotopomer mixing ratios

Most isotope ratio measurements are made with mass spectrometers and the results are reported as a relative difference in the isotope ratio compared to a standard ratio. Calculations are normally performed using delta notation, avoiding the use of the actual value for the standard ratio. The TGA100 fundamentally measures the mixing ratio of individual isotopomers—not their ratio. Therefore we report some results as mixing ratios and fluxes of individual isotopomers. Where appropriate, we also report our results in the familiar delta notation. In this appendix we present our methods for converting into and out of delta notation and discuss the origin of the constants used for these conversions.

Total mixing ratios and isotope ratios are calculated from the mixing ratios of individual isotopomers using Eqs. (A.1) and (A.2):

\[
[\text{CO}_2] = \frac{[\text{¹²CO}_2] + [\text{¹³CO}_2]}{1 - f_{\text{other}}}
\]

\[
\delta^{13}\text{C} = \frac{1000 \times \left(\frac{[\text{¹³CO}_2]}{[\text{¹²CO}_2]} - 1\right)}{R_{VPDB}}
\]

Eqs. (A.1) and (A.2) are rearranged to calculate the mixing ratios of individual isotopomers from the total mixing ratio and the isotope ratio:

\[
[\text{¹²CO}_2] = [\text{CO}_2] \left(1 - f_{\text{other}}\right)\left(1 + \frac{R_{VPDB}}{1 + \delta^{13}\text{C}/1000}\right)
\]

\[
[\text{¹³CO}_2] = [\text{CO}_2] \left(1 - f_{\text{other}}\right) - [\text{¹²CO}_2]
\]

where \(f_{\text{other}}\) is the fraction of CO₂ containing all isotopomers other than \(\text{¹²C}⁰\text{O}_⁰\text{O} \) and \(\text{¹³C}⁰\text{O}_⁰\text{O}\) (predominantly \(\text{¹²C}⁰\text{O}_⁸\text{O} \) and \(\text{¹³C}⁰\text{O}_⁸\text{O}\)). We assume \(f_{\text{other}} = 0.00474\), from the isotopic abundances used for the high-resolution transmission molecular absorption database (HITRAN), based on the work of De Bièvre et al. (1984).

Isotope ratios are reported relative to the Vienna Pee Dee belemnite scale, which is defined by assigning a value of 1.95‰ to the National Institute of Standards and Technology (NIST) reference material (RM) 8544 (NBS19) (Coplen, 1994, 1995). The NIST Report of Investigation for RM8544 gives the carbon isotopic abundance as 98.8922 atom percent ¹²C and 1.1078 atom percent ¹³C (NIST Report of Investigation from Reed, 1992), citing the measurements of Zhang and Li (1990). Zhang and Li measured the isotopic abundance of ¹³C in NBS19 with mass spectrometers calibrated with CO₂ produced from mixtures of nearly pure Ba¹²CO₃ and Ba¹³CO₃, reporting a value for \(R_{13,NBS19} = 11201.5 \times 10^{-6} \pm 4.5 \times 10^{-6}\). Zhang and Li calculated a total uncertainty of 28 \times 10^{-6}, rounded \(R_{13,NBS19}\) to 0.011202, and then converted it to the atom percentages included in the NIST report. To avoid accumulated round-off errors, we base our value of the reference ratio \(R_{VPDB} =\)
of Craig and Nier, suggesting a bias in Nier’s measurements caused by calibrating the mass spectrometer with argon isotopes (with mass 36 and 40) rather than CO2 isotopomers (with mass 44 and 45).

In our terminology, we differentiate the VPDB standard ratio, $R_{VPDB}$, from the ratio reported by Craig, $R_{VPDB-Chicago}$, which refers to the sample of Cretaceous Belemnite from the Pee Dee formation of South Carolina that was used as a calibration standard at the University of Chicago. This sample was exhausted many years ago, but comparisons made between various reference materials, whose $R_{VPDB}$ is known, are also used as working standards. This also demonstrates why the actual isotopic abundance ratio for the VPDB standard, $R_{VPDB}$, is not normally used in the analysis of isotopic ratios. However, we calibrate the trace gas analyzer for the individual $^{12}$CO$_2$ and $^{13}$CO$_2$ mixing ratios, and report our results as mixing ratios and fluxes of each isotopomer. Our primary standards are tanks of CO$_2$ in air supplied and measured by CMDL, which provides calibrations as total CO$_2$ mixing ratios and isotope ratios relative to VPDB. Therefore we use $R_{VPDB}$ in Eqs. (A.3) and (A.4) to convert these values to mixing ratios of each isotopomer. Where we report isotope ratios relative to VPDB, we use this same value for $R_{VPDB}$ in Eqs. (A.1) and (A.2) to convert from mixing ratios of individual isotopomers to total mixing ratio and isotope ratios relative to VPDB. Use of a different value for $R_{VPDB}$, such as that provided by Craig, would change the values we report for isotopomer mixing ratios and fluxes, but it would not change the values we report for isotope ratios.

References


