

An injection method for measuring the carbon isotope content of soil carbon dioxide and soil respiration with a tunable diode laser absorption spectrometer

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We present a novel technique in which the carbon isotope ratio ($\delta^{13}\text{C}$) of soil CO_2 is measured from small gas samples (<5 mL) injected into a stream of CO_2 -free air flowing into a tunable diode laser absorption spectrometer (TDL). This new method extends the dynamic range of the TDL to measure CO_2 mole fractions ranging from ambient to pure CO_2 , reduces the volume of sample required to a few mL, and does not require field deployment of the instrument. The measurement precision of samples stored for up to 60 days was 0.23‰. The new TDL method was applied with a simple gas well sampling technique to obtain and measure gas samples from shallow soil depth increments for CO_2 mole fraction and $\delta^{13}\text{C}$ analysis, and subsequent determination of the $\delta^{13}\text{C}$ of soil-respired CO_2 . The method was tested using an artificial soil system containing a controlled CO_2 source and compared with an independent method using the TDL and an open soil chamber. The profile and chamber estimates of $\delta^{13}\text{C}$ of an artificially produced CO_2 flux were consistent and converged to the $\delta^{13}\text{C}$ of the CO_2 source at steady state, indicating the accuracy of both methods under controlled conditions. The new TDL method, in which a small pulse of sample is measured on a carrier gas stream, is analogous for the TDL technique to the development of continuous-flow configurations for isotope ratio mass spectrometry. While the applications presented here are focused on soil CO_2 , this new TDL method could be applied in a number of situations requiring measurement of $\delta^{13}\text{C}$ of CO_2 in small gas samples with ambient to high CO_2 mole fractions. Copyright © 2010 John Wiley & Sons, Ltd.

Tunable diode laser (TDL) absorption spectrometers have been utilized for a variety of ecosystem stable isotope applications due to their portability, fast response, and relatively low cost compared with mass spectrometers.^{1–6} However, most TDL-based methods reported to date have required enough volume of sample to flow through the instrument to flush the sample cell and for measurements to remain stable during an averaging period. This typically requires flow rates of 100–400 sccm, which limits the potential applications of the instrument. Tunable diode laser-based applications for CO_2 isotopes have also been restricted by poor instrument performance when the CO_2 mole fractions are outside the range of 350–700 $\mu\text{mol mol}^{-1}$.⁶

We have developed a new TDL-based method in which the carbon isotope content ($\delta^{13}\text{C}$) of CO_2 is calculated from independent, time-varying $^{12}\text{CO}_2$ and $^{13}\text{CO}_2$ peaks produced by a small injection of sample gas into a flow of CO_2 -free air. Engel *et al.*⁷ recently presented a method in which CO_2 -free air flowed through small (<60 mL) arthropod chambers and mixed with respired CO_2 for TDL measurement of $\delta^{13}\text{C}$. They showed how the carbon isotope ratio of a pulse of CO_2

could be measured with the instrument by averaging the ratios of $^{13}\text{CO}_2$ and $^{12}\text{CO}_2$ over a period of a few seconds while the CO_2 mole fractions were within an acceptable measurement range. In contrast, the method presented in the current study entails calculation of $\delta^{13}\text{C}$ from a specific moment within a CO_2 peak produced by a manual syringe injection of sample gas through a septum port. With this configuration, gas samples of a few mL or smaller can be sampled, stored, and measured on the TDL instrument at a later date. This method extends the measurement capability of the TDL to include CO_2 mole fractions from 350 $\mu\text{mol mol}^{-1}$ to pure CO_2 without additional dilution, and has the potential to expand TDL isotope applications in a way similar to the development of continuous-flow isotope ratio mass spectrometry.

Tunable diode laser instruments have been used to measure the isotopic content of soil respiration with open soil chambers,^{4,5} by sampling air during nocturnal accumulation just above ground,^{4,8,9} or from diluted flows from above and within snow and soil.¹⁰ The injection method described here enables a new soil respiration measurement application for the TDL which was previously limited to mass spectrometers: measurement of $\delta^{13}\text{C}$ of CO_2 in small gas samples collected from soil pore spaces.

Profiles of CO_2 mole fraction and $\delta^{13}\text{C}$ within the soil pore space have been used to calculate the carbon isotope content of

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soil-respired CO₂ using a two-source mixing model.¹¹ This approach assumes that the CO₂ in the soil column is composed of a diffusively mixed combination of (1) atmospheric CO₂ and (2) a weighted mean of CO₂ produced by all soil sources. Under these circumstances, a plot of $\delta^{13}\text{C}$ vs. $1/[\text{CO}_2]$ (a Keeling plot¹²) from soil profile measurements will be linear, with an intercept that reflects the $\delta^{13}\text{C}$ of the soil surface CO₂ flux (net $\delta^{13}\text{C}$ from all soil sources) plus a $\sim 4.4\%$ offset caused by diffusive enrichment.^{13,14} The 4.4% enrichment is theoretically based, and advection within the soil may mean enrichment of the soil gas profile is somewhat less than the theoretical value under some conditions.¹⁰

In biologically and/or geologically active soils a steep $\delta^{13}\text{C}$ gradient typically exists near the soil surface, as the CO₂ mole fraction increases with the composite soil source over the first few cm to m of depth.^{13,15,16} In the absence of geological sources, localization of biological activities near the surface frequently leads to little variation in $\delta^{13}\text{C}$ below approximately 10 cm depth. Thus, to obtain maximum biologically driven CO₂ and $\delta^{13}\text{C}$ measurement ranges for isotopic analysis with the Keeling plot method, multiple measurements from the upper 10 cm of soil depth are often ideal, with the use of additional deeper measurements as needed.

The central objective of the present study was to test the TDL injection technique for the analysis of soil gas samples collected from shallow depth increments, for use in determination of the $\delta^{13}\text{C}$ of the soil surface CO₂ flux using the Keeling plot approach. Breecker and Sharp¹⁷ developed a method to obtain high-resolution profiles of CO₂ mole fractions and $\delta^{13}\text{C}$ in shallow soil depths by inserting gas wells horizontally into the soil, accessible by lengths of 2 mm diameter tubing which extended above the surface. Sample collection required initially purging this volume of tubing by drawing gas up from the gas well with a syringe. A secondary objective of the present study was to test a modified approach in which the gas well tubing (0.64 cm o.d.) extends above the soil surface, allowing for immediate sample collection (no purging necessary). This approach may reduce disturbance to diffusive soil profiles, but requires that the entire gas well volume remains equilibrated with the CO₂ mole fraction and isotope content at the depth of the open gas well end. We evaluated this assumption and the performance of the TDL injection method in two laboratory experiments with an artificial soil. In the first experiment, samples from vertically inserted gas wells were compared with gas from perforated, horizontally buried tubes as CO₂ diffused from a reservoir through an artificial soil medium. In the second experiment we compared the Keeling plot based soil profile method with an independent method where the carbon isotope ratio and rate of an artificially induced CO₂ efflux were measured continuously using the TDL with an open, flow-through soil respiration chamber.

EXPERIMENTAL

Tunable diode laser injection technique

Three CO₂ isotope standard cylinders were prepared by spiking empty compressed-gas cylinders with variable amounts of pure CO₂ having $\delta^{13}\text{C}$ values of -31.63 and $+12.15\%$, and using an air compressor to pressurize and

dilute the cylinders with air from outside our laboratory. The cylinders were spiked to achieve a final CO₂ mole fraction of approximately $3000 \mu\text{mol mol}^{-1}$ and carbon isotope ratios across the range of values typically observed in soils (~ -8 to -25%). Standard cylinders were measured for the $\delta^{13}\text{C}$ of CO₂ by purifying approximately $150 \mu\text{mol}$ of CO₂ away from all other cylinder constituents for subsequent dual-inlet IRMS analysis (DeltaPlus Advantage, ThermoFinnigan, Bremen, Germany). Cylinder air was moved at a known flow rate through a dry ice/ethanol slush trap for water removal and a liquid nitrogen trap for CO₂ collection under high vacuum. The contents of the liquid nitrogen trap were inserted into a helium stream for CO₂ and N₂O separation via gas chromatography (GC). A thermal conductivity detector was monitored for peak elution. The CO₂ was collected in a second liquid nitrogen trap downstream of the GC column. Once the CO₂ peak had completely eluted, the helium carrier was routed to vent. The CO₂ was vacuum-transferred to a glass cold finger, which was then flame-sealed. The CO₂ was introduced into a dual-inlet IRMS instrument for analysis against NIST-traceable CO₂ isotope standards (OzTech Trading Corporation, Safford, AZ, USA) that had previously been standardized to the Vienna Pee Dee Belemnite (VPDB) scale by measurement against the calcite standard NBS19. The $\delta^{13}\text{C}$ values of CO₂ in the cylinders from three repeated cryogenic extractions and analyses were -7.576 ± 0.002 , -19.560 ± 0.006 , and $-31.275 \pm 0.023\%$.

The tunable diode laser (TDL, model TGA100A, Campbell Scientific, Logan, UT, USA) was described in detail by Bowling *et al.*⁶ The absorption lines used for ¹²CO₂ and ¹³CO₂ in this study were 2299.642 and 2299.795 cm^{-1} , respectively. The TDL was configured with a continuous flow of CO₂-free air (ambient air chemically scrubbed of CO₂ with soda lime) at a flow rate of 50 sccm (Fig. 1). At this flow rate, the sample cell pressure was 2.3 kPa. Samples were injected manually through a septum (Microseep F-138, Alltech, Deerfield, IL, USA) placed within a union tee fitting (316L-400-3, Swagelok, Solon, OH, USA) downstream of the CO₂ trap, but upstream of a $15 \mu\text{m}$ filter (SS-4FW-15, Nupro, Willoughby, OH, USA) and mass flow controller (1179A, MKS, Andover, MA, USA). Injections were performed using gas-tight syringes with capacities ranging from 0.5 to 5.0 mL (Pressure-Lok, VICI, Baton Rouge, LA, USA). Before entering the TDL system, water vapor was removed from the sample and carrier stream with a Nafion membrane counterflow system (PD 625, Campbell Scientific).

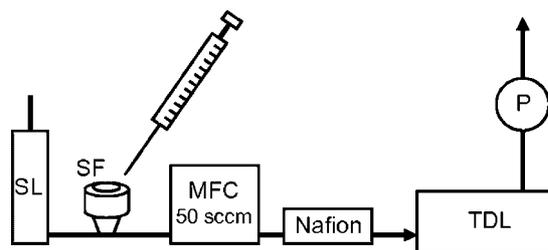


Figure 1. Schematic of syringe injection plumbing for the tunable diode laser (TDL). SL = soda lime, SF = septum fitting, MFC = mass flow controller, Nafion = a counterflow drying assembly to remove water vapor, and P = pump.

To achieve appropriately sized $^{12}\text{CO}_2$ and $^{13}\text{CO}_2$ peaks using our method, measurement of CO_2 mole fraction was required prior to sample injection into the TDL. The CO_2 mole fraction of all gas samples was determined a few minutes to one hour prior to TDL measurement, using an infrared gas analyzer (IRGA, Li-7000, Licor, Lincoln, NE, USA) as described by Davidson and Trumbore.¹⁸ From each sample 0.5 mL of gas at ambient pressure was injected using a gas-tight syringe into a closed, CO_2 -free air stream, just upstream of the IRGA. The integrated voltage peak from the injected sample was used to calculate the mole fraction of CO_2 in the sample by applying a calibration produced from injections of standard gases from custom-prepared cylinders or flasks. Standard cylinders for CO_2 mole fraction calibration were prepared using an air compressor as described above. Standard flasks were prepared as volumetric combinations of CO_2 -free air and pure CO_2 by (1) flushing glass flasks of precisely known volume (nominally 2 to 2.5 L) in a loop containing a soda lime trap, (2) disconnecting the flask and attaching a septum fitting, and then (3) removing a calculated volume of gas with a syringe and replacing the same volume with pure CO_2 .

The volumes of sample injected into the CO_2 -free carrier stream for TDL measurement were adjusted based on CO_2 mole fraction to consistently introduce 0.88 nmol of CO_2 . A factorial examination of variable injection volumes and CO_2 mole fractions showed that measurement precision was optimized when the amount of CO_2 injected was near this value. During a routine sample run, gas samples were injected into the TDL every 2 min. Calibration gas from each of the three standard cylinders was injected in the same manner between sets of 3–5 unknown samples. This frequency of calibration was required to correct for instrumental drift of unknown origin. The $^{12}\text{CO}_2$ and $^{13}\text{CO}_2$ peaks were measured individually by the TDL at a frequency of 10 Hz (Fig. 2(a)). The slope of the change in mole fraction vs. time was calculated for successive 0.5-s windows (Fig. 2(b)). The maximum and

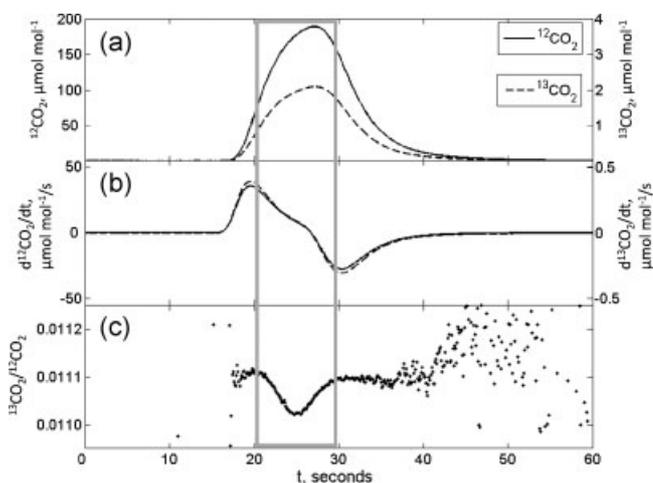


Figure 2. 10-Hz data from one representative injection into the TDL inlet stream. $^{12}\text{CO}_2$ and $^{13}\text{CO}_2$ peaks were measured by the instrument (a), within which the maximum and minimum slopes vs. time were identified (b), and used to find and define (grey lines) a persistent local minimum feature within the ratio of $^{13}\text{CO}_2/^{12}\text{CO}_2$ vs. time (c).

minimum slopes were found and used to define a highly repeatable, local minimum feature apparent in the molar ratio of $^{13}\text{CO}_2/^{12}\text{CO}_2$ vs. time (Fig. 2(c), grey window). A second-order polynomial was fitted to the data within this feature and the minimum value of the fit line was used to calculate a raw carbon isotope ratio ($\delta^{13}\text{C}$, ‰) following convention:

$$\delta^{13}\text{C} = \left(\frac{R_{\text{sample}}}{R_{\text{std}}} - 1 \right) * 1000 \quad (1)$$

where R_{sample} is the minimum value of a line fitted to the $^{13}\text{CO}_2/^{12}\text{CO}_2$ feature and R_{std} is 0.01124, the ratio of $^{13}\text{C}/^{12}\text{C}$ in PDB carbonate.¹⁹ The shape of this feature is probably determined by a kinetic fractionation of CO_2 isotopologues during gas flow from the injection port to the TDL. However, calculating a ratio based on either an average or minimum value for this feature within peaks consistently produced better precision than using a ratio determined from individual $^{12}\text{CO}_2$ and $^{13}\text{CO}_2$ peak maxima or integrated peak areas (data not shown). The advantage of using a $^{13}\text{CO}_2/^{12}\text{CO}_2$ ratio calculated from a specific point within the peaks, rather than a ratio of integrated $^{13}\text{CO}_2$ and $^{12}\text{CO}_2$ peak areas, may be due to the limitations of TDL performance at very low mole fractions (with fewer IR-absorbing molecules).

A linear fit between isotope measurements of the calibration standard cylinders and known $\delta^{13}\text{C}$ of CO_2 in each cylinder was used to calibrate all samples within a run to the VPDB scale. Instrument drift within each run was then identified by interpolating between the average difference between measured and known tank isotope ratios of each calibration set vs. injection number. This interpolated offset function was subtracted from the calibrated measurements to correct for changes in instrument response during a run.

Gas sample collection and storage

When not sampled with a syringe immediately before injection into the TDL, gas well samples were collected in 12-mL, septum-capped, evacuated vials (Exetainer, Labco, High Wycombe, UK) using a two-ended blood-collection needle (22G1 Vacutainer, Becton Dickinson, Franklin Lakes, NJ, USA). Storage in vials was tested for the isotope effects of leaking or CO_2 exchange with septum polymer material. Vials were filled from two of the isotope standard cylinders (−7.58 and −31.28‰) and an additional gas preparation mixed from a cylinder of pure CO_2 at +12.15‰ and laboratory air to a CO_2 mole fraction of $3000 \mu\text{mol mol}^{-1}$. The vials were stored at room temperature. Subsets of vials from each source were randomly selected and measured on the day of filling and then at various intervals for up to 60 additional days (each vial was measured only once).

Laboratory experiments

A method based on the design of Pumpanen *et al.*²⁰ was used to generate an artificial soil respiration source in the laboratory (an alternate design was recently used for a soil CO_2 isotope study by Kayler *et al.*¹¹). An artificial soil was prepared using oven-dry desert sand derived from the Cedar Mesa sandstone formation of southern Utah containing very low organic content,²¹ sieved to remove particles larger than $500 \mu\text{m}$ diameter. A 17-cm column of this medium was suspended on a perforated platform within an 89 cm tall,

58 cm wide polyethylene barrel. The surface of the sand was open to air in the laboratory and the bottom of the barrel was plumbed to allow a flow of pure CO₂ with a δ¹³C value of -31.63‰ from a gas cylinder, using a mass flow controller (1179A, MKS, Andover, MA, USA).

Two parallel lengths of 0.64 cm diameter tubing (Bev-A-Line IV, Cole-Parmer, Vernon Hills, IL, USA) were perforated by piercing multiple times with a needle and laid horizontally through the sand medium at 1, 7.5, and 13 cm depths. These tubes were accessible by septum fittings on the outside of the barrel. A second set of gas-sampling wells was installed vertically in the sand from the surface. These were 15-cm lengths of 0.64-cm diameter stainless steel tubing inserted to 0.5, 1, 2, 3, 4, 5, and 7.5 cm, with straight unions (SS-400-6, Swagelok) containing septa on the protruding ends.

In the first laboratory experiment, mole fraction and isotope gradients were initially produced in the sand medium by flowing pure CO₂ into the lower portion of the barrel. The rise in the CO₂ mole fraction inside the barrel was monitored with a solid-state CO₂ analyzer (GMT222, Vaisala, Woburn, MA, USA). The flow was stopped when the CO₂ mole fraction in the gas below the sand column reached 12 000 μmol mol⁻¹, and the flow then remained off. Gas samples were collected from both sets of gas wells approximately 2 h later, and again on the following day. Gas from the vertically inserted tubes was sampled directly with syringes immediately before measurement of CO₂ mole fraction and carbon isotope content, whereas gas from the horizontal tubes was collected in evacuated vials before measurement.

The second laboratory experiment began with the barrel and soil medium at ambient CO₂ mole fraction and δ¹³C. Profile and chamber measurements were made over a period of about 3 days after a continuous, 5 sccm flow of pure CO₂ of -31.63‰ was initiated into the lower barrel compartment from a gas cylinder. The open chamber was run continuously and gas samples were collected from the inserted and buried tubes into evacuated vials at 0.5, 2, 6, 23, 36, 58, and 70 h after the start of CO₂ flow into the barrel.

Chamber measurements of soil surface CO₂ flux

During the second laboratory experiment, an open, flow-through soil chamber²² was plumbed to the TDL (Fig. 3) and inserted about 3 cm into the barrel sand column from the

surface. The chamber was made of transparent acrylic, 33 cm tall and 26 cm in diameter, with a specialized lid assembly²² containing a funneled aluminum inlet tube. The chamber design allowed measured pressure differentials (inside to outside the chamber) to remain below 0.2 Pa at flow rates of up to 5000 sccm. These low-pressure differentials at particular flow rates were verified by direct measurement of the differential pressure (PX653-0.05BD5V, Omega Engineering Inc., Stamford, CT, USA) with the chamber attached to an impermeable slate laboratory bench, as recommended by Xu *et al.*²³

A continuous flow through the chamber was driven by a pump and controlled by a mass flow controller (1179A, MKS). A second pump pulled sample flows of gas continuously from the chamber inlet and outlet flows, each set to 300 sccm with an additional mass flow controller. A solenoid valve manifold was used to select between sampling chamber inlet and outlet flows and four calibration gas cylinders. A datalogger (CR5000, Campbell Scientific) controlled measurement cycling between these six flows, with each cycle (inlet, outlet, and 4 calibration tanks) lasting 10 min. The flow to the TDL was maintained at 150 sccm by an additional mass flow controller and dried with a Nafion counterflow system (PD 625, Campbell Scientific). All flows were filtered to 15 μm. Standard gases were prepared and calibrations conducted as described by Schaeffer *et al.*²⁴ Soil surface flux rates were calculated from the chamber using:

$$\text{Flux} = \frac{(\text{CO}_{2o} - \text{CO}_{2i}) \times \text{Flow}}{\text{Area}} \quad (2)$$

where CO_{2o} and CO_{2i} are the mole fractions of CO₂ in the outlet and inlet flows from the chamber, Flow is the number of moles of air passing through the chamber per second, and Area is the soil surface area enclosed by the chamber. The carbon isotope ratio of the soil surface CO₂ flux in the chamber (δ¹³C_F) was calculated as:

$$\delta^{13}\text{C}_F = \frac{(\text{CO}_{2o} \times \delta_o) - (\text{CO}_{2i} \times \delta_i)}{\text{CO}_{2o} - \text{CO}_{2i}} \quad (3)$$

where δ_o and δ_i are the δ¹³C values, respectively, of the CO₂ in the outlet and inlet flows in ‰. The chamber flow was adjusted periodically to keep the mole fraction difference between inlet and outlet flows around 50 μmol mol⁻¹. The chamber data were averaged for 3-h periods.

RESULTS AND DISCUSSION

The injection method for the TDL

Vials filled with ~3000 μmol mol⁻¹ CO₂ spanning a range of δ¹³C values and stored at room temperature showed no directional drift for up to 60 days of storage time after filling (Fig. 4). This lack of drift represents an improvement over similar tests performed with the same commercial vials.^{25,26} This improvement is probably due in part to the higher mole fraction of CO₂ in our samples of about 3000 μmol mol⁻¹. For the calculation of a Keeling plot intercept from soil CO₂, this suggests that any effect of storage on the calculated intercept will be reduced when the sample mole fractions are at least 3000 μmol mol⁻¹. In soil gas profiles collected in the field, this mole fraction is likely to be reached within the first few

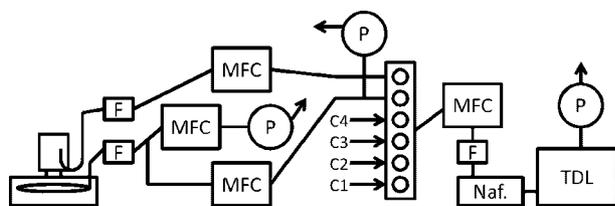


Figure 3. Plumbing diagram of soil chamber measurement with the tunable diode laser (TDL). Mass flow controllers (MFCs) regulated (from left to right) total chamber flow, inlet and outlet sampling flows, and TDL sampling flow, using three separate pumps (P). A solenoid manifold selected between the calibration tanks (C1–4) and the chamber inlet and outlet flows. All flows were filtered (F) and samples entering the TDL were dried using Nafion (Naf.).

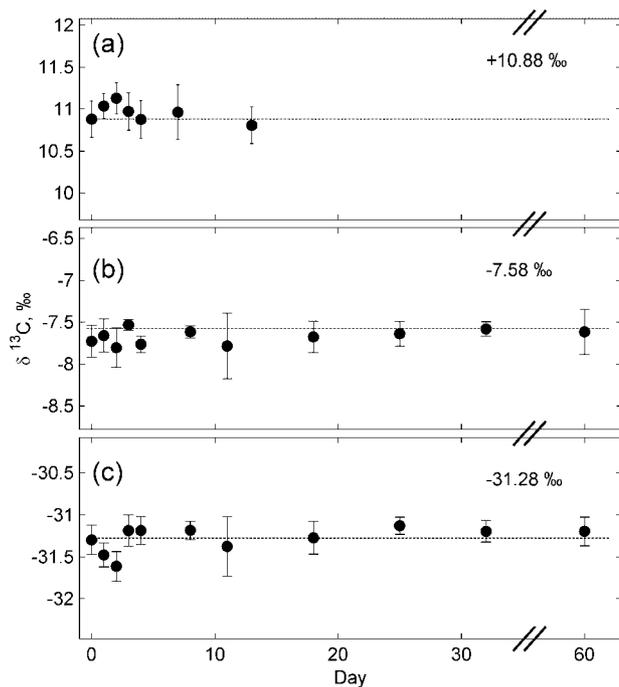


Figure 4. Repeated measurements of sample vials filled on day 0 with one of three prepared gases containing $\sim 3000 \mu\text{mol mol}^{-1}$ CO_2 at different carbon isotope ratios (labeled on each panel). Points are means and standard deviations of 5–11 randomly selected vials from each group.

centimeters of soil depth. The standard deviation of the $\delta^{13}\text{C}$ value of the CO_2 of all the vials sampled during the storage test (calculated on the population of measured – known values for each vial) was 0.23‰. This value incorporates errors associated with the collection, storage, measurement, and calibration of samples and represents a good indication of the overall performance of this method as it applies to field studies. While this measurement uncertainty is larger than typical precision associated with mass spectrometry, the small sample volumes required may enable repeated measurements, and the error of Keeling plot intercepts is usually more dependent on the range of CO_2 mole fractions of each sample population.²⁷ Estimates of precision and accuracy of the TDL measurement alone (without sampling and storage errors) were obtained by injecting gas directly from standard cylinders into the TDL and treating some standard gas injections as unknowns. In these tests the accuracy was within $\pm 0.1\%$ of the $\delta^{13}\text{C}$ value of the CO_2 in the cylinder, with standard deviations of 0.15‰ or less.

The new TDL measurement capability made possible by this method is analogous to the extended capabilities brought to isotope ratio mass spectrometry (IRMS) by continuous-flow (CF) configurations. In both cases a small sample is introduced into a carrier stream and peak measurements are used to calculate an isotope ratio, enabling a larger range of potential applications.²⁸ For example, CF-IRMS techniques have been developed to measure the carbon isotope ratios of atmospheric CO_2 ²⁹ and methane,³⁰ oxygen isotope ratios in dissolved and gaseous O_2 ,³¹ sulfur isotope ratios in mineral sulfides and sulfates,³² and nitrogen and

oxygen isotope ratios of N_2O .³³ Similarly, the new TDL method described in this paper could be directly applied to measure the $\delta^{13}\text{C}$ of CO_2 in a variety of research situations, such as gas exchange studies with plants,³⁴ animals,³⁵ or microbial incubations;³⁶ analysis of gases processed by reacting acid with fossil teeth³⁷ or pedogenic carbonates,³⁸ or measurement of samples collected from volcanic emissions in remote areas.³⁹ In addition, modifications of this method could probably be developed for TDL instruments configured to measure isotopologues of other trace gases.

Laboratory experiments

The CO_2 profiles in the sand 2 and 24 h after turning off the pure CO_2 source in the first experiment were linear (Fig. 5(a)), reflecting a diffusive flux through a homogeneous medium that did not contain any source activity. As CO_2 diffused out of the barrel through the sand over the course of the experiment, the CO_2 gradient decreased and the $\delta^{13}\text{C}$ profile became more enriched (Fig. 5(b)). Without a CO_2 source, the system underwent an isotopic distillation as the lighter $^{12}\text{CO}_2$ molecules left the barrel at a slightly higher rate than the heavier $^{13}\text{CO}_2$ molecules. Because the room was large and ventilated, the atmospheric boundary condition was held relatively constant, which was apparent in the isotope mixing lines that reflected a progressively enriched flux from the sand (Figs. 5(c) and 5(d)). The Keeling plot intercept from samples collected at 2 h (Fig. 5(d)) was -29.03% , reflecting a CO_2 surface flux $\delta^{13}\text{C}$ of -33.43% (subtracting 4.4‰ from -29.03%) and the intercept from the 24-h sample set was -23.16% , indicating a CO_2 surface flux $\delta^{13}\text{C}$ of -27.56% .

Samples collected with evacuated 12-mL vials from the horizontal tubing wells and with gas-tight syringes from the vertical tubing wells produced the same relationships of CO_2 mole fractions and carbon isotope content with depth (Fig. 5). This indicates that the gas within the volume of the vertically inserted tubing was effectively equilibrated with the CO_2 at the depth of the open end, although the profiles were changing over time. Numerous measurements spanning relatively large gradients of CO_2 mole fraction and $\delta^{13}\text{C}$ within the top 10 cm of sand were made possible by the small gas well design, enabling relatively precise calculations of Keeling intercepts. In the Keeling plot calculation from the profiles sampled 2 h after flow was stopped, the standard error of the intercept was 0.11‰, smaller than the errors associated with the individual isotope measurements in the regression (typically 0.25‰). In the profiles collected at 24 h, the measured ranges of CO_2 mole fraction and $\delta^{13}\text{C}$ were smaller and the standard error of the intercept was 0.28‰.

In the second laboratory experiment a CO_2 flux began to develop from the sand surface shortly after initiation of the flow of pure CO_2 into the space below the sand (Fig. 6(a)). The $\delta^{13}\text{C}$ of the flux quickly became about 4‰ more depleted in $^{13}\text{CO}_2$ than the source in both chamber and profile estimates, followed by a gradual, asymptotic increase towards the source value of -31.63% (Fig. 6(b)). This transient increase towards a steady-state flux $\delta^{13}\text{C}$ followed theoretical predictions based on isotopic diffusion¹⁵ and lasted approximately 3 days. The equilibration time for this

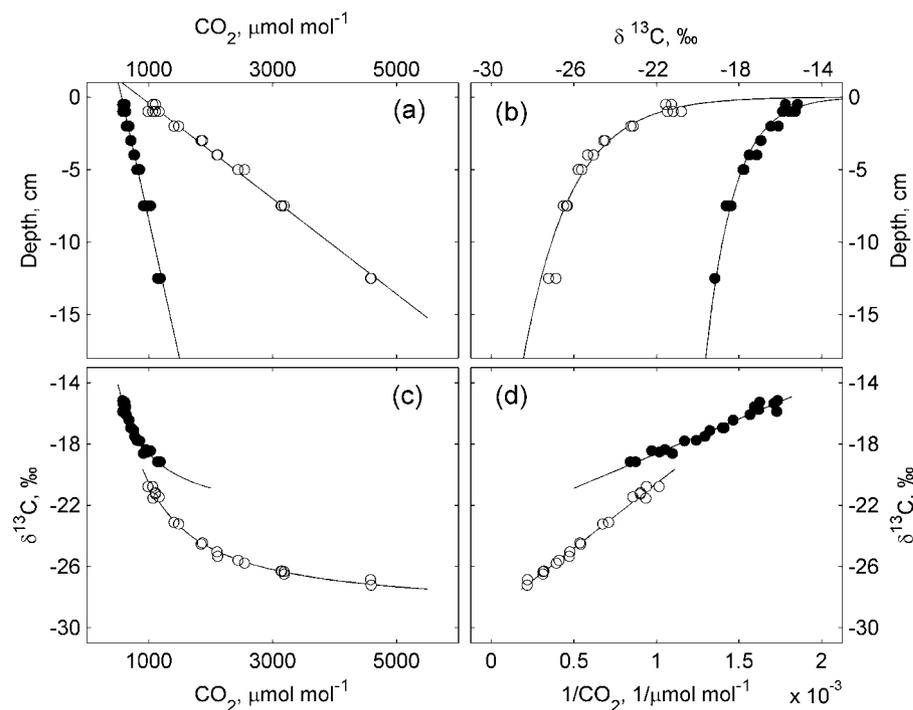


Figure 5. Combined data from vertical (at 0.5, 1, 2, 3, 4, 5, and 7.5 cm) and horizontal (at 1, 7.5, and 13 cm) gas wells from within 17 cm of sand 2 h (open symbols) and 24 h (closed symbols) after stopping flow of pure CO₂ into an air space below the sand layer. Fit lines are: (a) linear, (b) calculated from a linear fit of $\delta^{13}\text{C}$ vs. log depth, and (c) calculated from the linear Keeling plot fit shown in (d).

system was so long because of the large storage volume of air beneath the sand platform (~150 L). The calculated $\delta^{13}\text{C}$ of the CO₂ flux produced in the medium over time was similar for the Keeling plot approach using combined data from

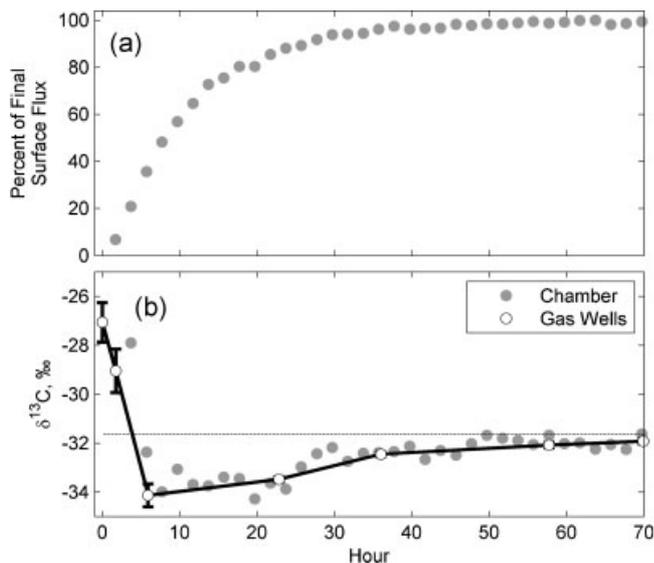


Figure 6. (a) Open chamber surface flux of CO₂ and (b) $\delta^{13}\text{C}$ of CO₂ in the soil surface flux measured using open chamber and gas well methods, plotted vs. time since initiation of pure CO₂ flow under 17 cm of sand. The dashed line is the $\delta^{13}\text{C}$ of CO₂ in the source tank (-31.63‰). Gas well derived flux isotope ratios were adjusted by 4.4‰ for diffusive enrichment and error bars are 1 standard error of the intercept (smaller than symbols in some cases).

vertical and horizontal gas well samples and the open chamber approach (Fig. 6(b)). This agreement, and the convergence towards a steady-state CO₂ flux $\delta^{13}\text{C}$ matching the measured value for the tank CO₂ source, provide evidence of the accuracy of both methods for determining the $\delta^{13}\text{C}$ of soil-respired CO₂ under these controlled conditions, and a measure of confidence for the use of these methods in the field.

For this experiment evacuated 12-mL vials were used for the collection of sample gas from the vertical and horizontal gas wells, although the internal volume of the vertical gas wells was only ~3.5 mL. This means that gas in the pore spaces surrounding the open end of the gas well was drawn into the sample. Because the CO₂ mole fraction and $\delta^{13}\text{C}$ profiles were a result of diffusive mixing of two sources, the use of horizontal gas wells equilibrated at precise depths or vertical gas wells drawing from a volume of pore spaces surrounding the gas well ends produced the same calculated Keeling plot intercepts.

CONCLUSIONS

This paper outlines a new syringe injection approach for a tunable diode laser absorption spectrometer, and its application to measure the $\delta^{13}\text{C}$ of soil-respired CO₂. With this injection method, small volumes of soil gas with high CO₂ mole fractions can be delivered to the TDL and measured every 2 min with approximately 0.25‰ precision. The injection technique was applied to measure profiles of mole fractions and the $\delta^{13}\text{C}$ of CO₂ in an artificial soil medium in laboratory experiments using a known, con-

trolled CO₂ source. Keeling plot derived $\delta^{13}\text{C}$ measurements of the induced CO₂ flux from depth profile samples compared well with expectations based on theory and with a second TDL method, which employed an open chamber on the surface of the medium. Keeling plot intercept calculations were unaffected by the gas-sampling methods including (1) drawing gas from within vertical gas wells with a small syringe, (2) drawing gas from the vertical gas well plus pore spaces around the submerged end of tubing using an evacuated vial and a double-ended needle, and (3) sampling gas from within horizontal perforated tubing.

Soil profile samples can be collected, transported, and stored for later measurement for the calculation of the $\delta^{13}\text{C}$ of soil-respired CO₂, but this method offers limited temporal resolution. Alternatively, the open chamber method has an advantage of providing continuous measurements of the $\delta^{13}\text{C}$ and rates of CO₂ efflux, but with limited spatial replication and requiring field deployment of the instrument. The independence of the profile and chamber methods, while making use of the same instrument, provides a unique validation opportunity for researchers with access to a tunable diode laser. We encourage researchers reporting open chamber $\delta^{13}\text{C}$ data in future studies to take such a validation step.

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