

Automated system for simultaneous analysis of $\delta^{13}\text{C}$, $\delta^{18}\text{O}$ and CO_2 concentrations in small air samples

Miquel Ribas-Carbo[†], Chris Still[‡] and Joe Berry^{*}

Carnegie Institution of Washington, Department of Plant Biology, 260 Panama Street, Stanford, CA 94305, USA

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SPONSOR REFEREE: Pieter Tans, National Oceanic Atmospheric Administration, Climate Monitoring and Diagnostics Laboratory, Boulder, Colorado, USA

In this paper we present an automated system for simultaneous measurement of CO_2 concentration, $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ from small (<1 mL) air samples in a short period of time (\approx 1 hour). This system combines continuous-flow isotope ratio mass spectrometry (CF-IRMS) and gas chromatography (GC) with an inlet system similar to conventional dual-inlet methods permitting several measurement cycles of standard and sample air. Analogous to the dual-inlet method, the precision of this system increases with the number of replicate cycles measured. The standard error of the mean for a measurement with this system is 0.7 ppm for the CO_2 concentration and 0.05‰ for the $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ with four replicate cycles and 0.4 ppm and 0.03‰ respectively with nine replicate cycles. The mean offset of our measurements from NOAA/CMDL analyzed air samples was 0.08 ppm for the CO_2 concentration, 0.01‰ for $\delta^{13}\text{C}$ and 0.00‰ for $\delta^{18}\text{O}$. A specific list of the parts and operation of the system is detailed as well as some of the applications for micrometeorological and ecophysiological applications. Copyright © 2002 John Wiley & Sons, Ltd.

The application of stable isotope analyses is widespread in the environmental sciences, particularly carbon cycle research.^{1–4} For example, the $\delta^{13}\text{C}$ of atmospheric CO_2 has been used at a global scale to partition the net sink between oceans and land.⁵ At an ecosystem scale, the $\delta^{18}\text{O}$ of CO_2 is used to partition net carbon exchanges into photosynthetic and respiratory components,⁶ and the $\delta^{13}\text{C}$ is used to determine the fractional contribution of C_3 and C_4 species to net ecosystems CO_2 exchange.⁷ In addition, isotope analysis of air is used for studies of isotopic fractionation in photosynthesis.^{8–10} Along with the increased emphasis on understanding biological and physical controls on the concentration and isotopic composition of atmospheric CO_2 , there has been renewed interest in improvement of sampling and measurement techniques.^{4,11} The most precise and widely used technique for these studies is dual-inlet isotope ratio mass spectrometry (DI-IRMS). This requires ca. 500 mL of air per determination. Furthermore, the CO_2 concentration (also required for most analysis) must be determined separately.

CF-IRMS has several potential advantages: (1) it requires smaller sample sizes (500 μL of air), (2) it separates N_2O from CO_2 , and (3) it can be used for simultaneous measurements of CO_2 concentrations and isotope ratios. Recently, Ferretti *et*

*al.*¹² described a method based on continuous-flow isotope ratio mass spectrometry (CF-IRMS) methodology.

The system we describe here takes advantage of the CF-IRMS methodology while retaining a functional similarity to the conventional dual-inlet approach. The system is fully automated and allows simultaneous measurements of both the CO_2 concentration and the $^{13}\text{C}/^{12}\text{C}$ and $^{18}\text{O}/^{16}\text{O}$ ratios of CO_2 of very small air samples (0.5–0.7 mL) with highly variable CO_2 concentrations (250–1200 ppm). A key feature of this design is that a whole air standard of known CO_2 concentration and isotope ratios is used as the reference. As with the dual-inlet method, the CO_2 from the sample and reference air are alternatively directed to the mass spectrometer. Repeated cycles of sample and reference measurements are used to enhance the precision of the CF-IRMS method. To further increase the precision of isotope ratio and CO_2 concentration measurements, the system adjusts the amount of air analyzed so that the size of the m/z 44 peaks are similar for the reference and sample and for successive samples. In the rest of this paper, we describe the design of this technique, its uncertainty and accuracy, and examples of its application to ecophysiological studies.

System overview

A quantity of air predetermined to contain ca. 20×10^{-9} mol of CO_2 is metered into a pre-evacuated volume. The air is first dried by passing through a dry ice/ethanol trap, then CO_2 is condensed on a liquid nitrogen trap. The CO_2 is then vaporized into flowing helium, further purified of water vapor and passed through a capillary GC column to separate CO_2 and N_2O before entering a continuous-flow isotope ratio mass spectrometer.

*Correspondence to: J. Berry, Carnegie Institution of Washington, Department of Plant Biology, 260 Panama Street, Stanford, CA 94305, USA.

E-mail: mribas@biosphere.stanford.edu

†Current address: Departament de Biologia, Area de Fisiologia Vegetal, Universitat de les Illes Balears, Ctra de Valldemossa, km 7.5., 07071. Illes Balears, Spain.

‡Current address: Berkeley Atmospheric Sciences Center, University of California, Berkeley, CA, USA.

Table 1. Sample data and calculations for five measurement cycles. These are the data from a single flask analysis. The flask was filled from a NOAA/CMDL air mixture tank (CA04434) ($[\text{CO}_2] = 328.90$ ppm) and run against our own primary NOAA/CMDL standard air mixture tank (CA03893) ($[\text{CO}_2] = 365.13$ ppm). The individual values of each sample measurement compared to its standard pair and their respective (SDEV) values are shown in parentheses

a) Primary data					
Peak#		μ mol air	nmol CO_2	$\delta^{18}\text{O}$	$\delta^{13}\text{C}$
1	Standard	49.868	19.972	-1.16	-8.21
2	Sample	49.852	15.929	-1.74	-8.51
3	Standard	55.767	20.394	-1.08	-7.94
4	Sample	63.029	20.750	-1.38	-8.27
5	Standard	55.661	20.355	-1.17	-8.04
6	Sample	62.407	20.507	-1.61	-8.12
7	Standard	55.682	20.250	-1.09	-8.06
8	Sample	61.684	20.230	-1.59	-8.19
9	Standard	57.573	21.040	-0.94	-7.91
10	Sample	61.081	20.013	-1.58	-8.23

b) Internal calculations					
$[\text{CO}_2]$ ppmv		$\delta^{18}\text{O}$		$\delta^{13}\text{C}$	
Standard	Sample	Standard	Sample	Standard	Sample
365.70	329.21 (328.70)	-1.08	-1.38 (-1.74)	-7.94	-8.27 (-8.35)
365.70	328.60 (328.09)	-1.17	-1.61 (-1.87)	-8.04	-8.12 (-8.10)
363.67	327.96 (329.28)	-1.09	-1.59 (-1.94)	-8.06	-8.19 (-8.15)
365.45	327.65 (327.36)	-0.94	-1.58 (-2.08)	-7.91	-8.23 (-8.34)
Means					
365.14	328.36 (328.36)	-1.07	-1.54 (-1.91)	-7.99	-8.20 (-8.23)
SEM (SDEV)					
0.48	0.35 (0.71)	0.05	0.04 (0.14)	0.05	0.03 (0.13)

c) Final calculation of the sample CO_2 concentration:

$$[\text{CO}_2]_{\text{SA}} = \frac{(\text{area}_{m/z44}/\mu\text{mol air})_{\text{SA}}}{(\text{area}_{m/z44}/\mu\text{mol air})_{\text{ST}}} * [\text{CO}_2]_{\text{ST}} = (328.36/365.14) * 365.13 = 328.4 \text{ ppm} \pm 0.3$$

d) Final normalization of the sample delta values to the CMDL standard:

$$\delta_{\text{SA}} = \frac{(\delta_{\text{SAM}} + 1000) * (\delta_{\text{STR}} + 1000)}{(\delta_{\text{STM}} + 1000)} - 1000; \quad \text{where } \begin{array}{l} \delta_{\text{SAM}} = \delta \text{ sample measured} \\ \delta_{\text{STM}} = \delta \text{ standard measured} \\ \delta_{\text{STR}} = \delta \text{ standard real} \end{array}$$

$$\delta^{18}\text{O sample} = ((-1.54 + 1000) * (-1.44 + 1000)) / (-1.07 + 1000) - 1000 = -1.91\%$$

$$\delta^{13}\text{C sample} = ((-8.20 + 1000) * (-8.02 + 1000)) / (-7.99 + 1000) - 1000 = -8.23\%$$

In order to obtain the CO_2 concentration of a given air sample, both the total amount of CO_2 and the amount of air must be measured. The total amount of CO_2 is obtained from the integrated area of the peak at m/z 44. The amount of air is measured manometrically from the increase in pressure in an expansion volume downstream of the trapping loop. Each flask analysis consists of alternative cycles measuring the sample air and a whole air standard reference air mixture. Our laboratory standard is a tank of known CO_2 concentration, $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ prepared at NOAA/CMDL. The sample and standards are alternatively processed as with a dual-inlet system. Each cycle takes approximately 12 min. For a typical analysis, five cycles of each sample and reference air are analyzed. As mentioned above, the system is programmed to attain a target amount of CO_2 (peak area m/z 44). The amount (pressure) of air used to obtain the targeted amount of CO_2 is calculated from the amount of CO_2 obtained by integration of the m/z 44 peak of the previous cycle. Consequently, regardless of the sample CO_2 concentration, the sizes of the CO_2 peaks (m/z 44) are similar for

both the sample and the standard (see Table 1), as is typically done for the dual-inlet method. The CO_2 concentration and $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ of the sample are then calculated with reference to the NOAA/CMDL standard reference air mixture. The precision of the method is a function of the number of replicate cycles (sample-standard) analyzed. A full example of these calculations is shown in Table 1.

Automation

A detailed description of all the parts of this system is reported in the Appendix and a diagram of the system is shown in Fig. 1. The entire system operation is controlled by a datalogger (Campbell Scientific CS1-21X). The datalogger communicates with the mass spectrometer (Finigan Delta S), operating the standard ISODAT 7.2 software intended for automated combustion analysis. A contact closure signal from the mass spectrometer is used to initiate each flask analysis. In addition, the datalogger performs some on-line calculations as described below. All valves and the cylinder for submerging the trapping loop are air-actuated and

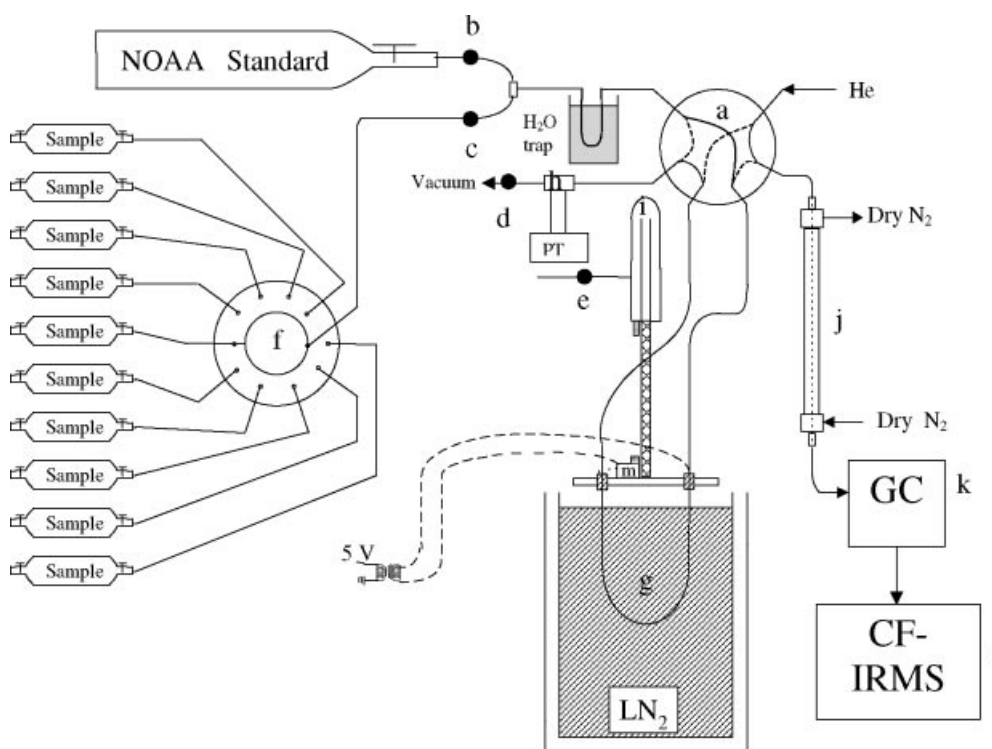


Figure 1. Description of the automated system.

- a) Two-position 6-port valve controlling the flows of the system. Trapping position (solid lines) and releasing position (dotted lines).
 - b) Pneumatically activated on/off microvalve controlling the flow of the NOAA/CMDL standard air.
 - c) Pneumatically activated on/off microvalve controlling the flow of the sample air.
 - d) Pneumatically activated on/off microvalve controlling the vacuum.
 - e) Pneumatically activated on/off microvalve controlling the flow of compressed air to the stainless steel non-rotating cylinder (i).
 - f) Ten-position valve controlling the sample flask to be analyzed.
 - g) Trapping loop 50 cm capillary tube (0.020" i.d., 1/32" o.d.).
 - h) Expansion chamber next to the pressure transducer (PT).
 - i) Stainless steel non-rotating cylinder that moves the trapping loop in and out of the liquid nitrogen dewar (LN_2).
 - j) 20-cm long Nafion drier with N_2 as a drier in a countercurrent flow.
 - k) Gas chromatograph with a 25-m long capillary Poraplot Q column (10 μm i.d., 0.32 mm o.d.) that separates CO_2 from N_2O at 40°C.
 - l) Delta S isotope ratio mass spectrometer with dual-inlet and continuous-flow setups.
- PT) Pressure transducer (0–100 Torr) that measures the increase in pressure in the trapping loop (g) and the expansion volume (h).

controlled by solenoids linked to the datalogger.

System operation

The following is a description of the system operation for a single measurement.

Step 1 The 6-way Valco valve (a) is in the 'trapping' position with the trapping loop (g) out of the liquid nitrogen dewar. Both inlet valves closed (b, c) and the vacuum valve open (d). The system is held in this state for at least 30 s in each measurement cycle.

Step 2 The trapping loop (g) is submerged under liquid

nitrogen by an air-actuated cylinder (i) and permitted to cool for 30 s.

Step 3 The vacuum valve (d) is closed, the pressure in the trapping loop (g) plus the expansion chamber (h) is recorded by the pressure transducer (PT). Valve b (standard) or valve c (sample), depending on the air to be measured, is opened and air is allowed to flow through a 50-cm capillary tube (stainless steel tubing 0.005" i.d., 1/16" o.d.), through a water trap (stainless steel tubing 0.020" i.d., 1/32" o.d., immersed in dry ice/ethanol); through the CO_2 trapping loop (stainless steel tubing 0.020" i.d., 1/32" o.d.) in liquid N_2 and into the expansion volume (h). The amount of air allowed into the trapping system is controlled by the datalogger which closes

the inlet valve as soon (ca. 15 s) as the pressure transducer reaches a target 'trapping pressure'. After the trapping pressure (ca. 50 mbar) is equilibrated (15 s) and recorded, the amount of air (in μmol) is calculated from the pressure increase and corrected for any small leaks of He.

Step 4 The vacuum valve (d) is opened and all non-condensed gases are evacuated from the expansion volume and the trapping loop. All the air ultimately passes through both traps.

Step 5 The 6-way Valco valve (a) is then switched to the 'release' position. The He flow that goes to the inlet of the CF-IR mass spectrometer, then passes through the loop where the CO_2 , N_2O and H_2O are being held at liquid nitrogen temperature.

Step 6 The condensed gases (including CO_2 and N_2O) are released when the loop is fully withdrawn from the liquid nitrogen by the action of the air-actuated cylinder (i) and heated up to 80°C by an electrical current (5 V) controlled by a micro-switch (m) which closes when the cylinder is fully retreated. The He flow carrying the sample of CO_2 and N_2O passes through a Nafion drier (j) and through a capillary GC column (Poraplot Q, $25\text{ m} \times 0.32\text{ mm o.d.}$, $10\text{ }\mu\text{m i.d.}$) where CO_2 , N_2 and N_2O are separated at 40°C (k). The column eluate (1.5 mL/min) goes into the isotope ratio mass spectrometer (l) by an open split inlet of the GC combustion interface.

Step 7 Sixty seconds later, the 6-way Valco valve (a) is switched to the 'trapping' position and the vacuum valve (d) is opened, returning to step 1. Trapping of the next air sample (steps 1 to 5) occurs simultaneously with the GC separation (step 6) and MS analysis (step 8).

Step 8 Peak integration: the retention time of the CO_2 peak is 300 s. An output of the signal from the cup measuring m/z 44 is connected to the datalogger and the CO_2 peak is integrated. This is then used for sample size adjustment. The CO_2 (m/z 44) for the sample and the reference air CO_2 concentration, $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ are obtained from the isotope ratio mass spectrometer.

Step 9 Determination of the amount of air to be trapped: the datalogger is programmed to control the amount of air trapped (trapping pressure) to obtain very similar CO_2 peaks (m/z 44 $\approx 3.5 \pm 0.1\text{ V}$). For the first measurement of each sample and reference flask, the trapping pressure is set using an initial estimate of the CO_2 concentration (usually 370 ppm). In subsequent cycles the trapping pressures are calculated to obtain a target area applying the following correction:

$$\begin{aligned} \text{Next trapping pressure} \\ = (\text{target area}/\text{measured area}) * \text{previous trapping pressure} \end{aligned}$$

The pressure adjustment is performed independently for the sample and the standard air measurements.

Flask change

After all the measurement cycles of a flask have been performed, the 10-position Valco valve (f) is activated and steps to the next position to analyze a new flask. The line is purged under vacuum for 2 min, after which the trapping volume is evacuated for 30 s. The Isodat software, working

in sequence mode, will close that run and start a new run by sending a contact closure to re-start the datalogger program.

Data acquisition

The datalogger and the mass spectrometer data files are collected separately and transported to an Excel spreadsheet for analysis.

Calculation of the CO_2 concentration

Like standard dual-inlet analysis, in this system the sample and NOAA/CMDL standard air samples are alternatively measured. The first cycle is discarded as the peak sizes are not equal. The remaining values are averaged.

The CO_2 concentration of the sample air $[\text{CO}_2]$ is obtained from the area of m/z 44 peak area (MS44) divided by the quantity of air (μmol air) for the sample flask compared to the same ratio for the whole air standard NOAA/CMDL flask, using the following equation:

$$[\text{CO}_2]_{\text{sample}} = \frac{\text{MS44}_{\text{sample}}/\mu\text{mol air}_{\text{sample}}}{\text{MS44}_{\text{standard}}/\mu\text{mol air}_{\text{standard}}} * [\text{CO}_2]_{\text{standard}}$$

Calculation of $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$

The $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ values are calculated from the average 45/44 and 46/44 ratios from the sample and the standard peaks. The ISODAT software calculates delta values with reference to a working standard placed in the dual-inlet bellows (OzTech). A correction for ^{17}O is incorporated in these calculations.

The delta values for sample flasks are then referenced to the NOAA/CMDL whole air standard using the following formula:

$$\delta_{\text{SA}} = \frac{(\delta_{\text{SAM}} + 1000) * (\delta_{\text{STR}} + 1000)}{(\delta_{\text{STM}} + 1000)} - 1000$$

where δ_{SAM} is the δ value of the sample measured against the OzTech standard, δ_{STM} is the δ value of the whole air standard NOAA/CMDL measured against the OzTech standard and the δ_{STR} is the δ value of the whole air standard reported by NOAA/CMDL. The correction to the NOAA/CMDL standard is ca. 0.03‰ and 0.7‰ for $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$, respectively.

RESULTS

In this work we have tested the accuracy and precision of this technique for measuring CO_2 concentrations, $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$. We present one of its applications for measurements of experimental samples and determined the influence of the measurement's error to the calculation of photosynthetic isotope fractionation from an open system gas exchange measurement (using the equation from Evans *et al.*⁹).

Single run example

Table 1 shows an example of a single flask analysis with five replicate determination cycles for both the sample and the whole air standard NOAA/CMDL. A sample flask (100 mL) was flushed with air from a NOAA (CA04434) air tank,

Table 2. Test of accuracy. Results of CO_2 concentration, $\delta^{13}\text{C}$, and $\delta^{18}\text{O}$ measurements of nine flasks filled from a NOAA standard air tank (CAO 3893)

Flask #	$[\text{CO}_2]$	$\delta^{18}\text{O}$ (‰)	$\delta^{13}\text{C}$ (‰)
1	364.83	-1.39	-8.00
2	364.73	-1.46	-7.99
3	365.33	-1.49	-8.03
4	365.45	-1.41	-8.04
5	365.22	-1.41	-7.99
6	365.10	-1.41	-8.02
7	365.01	-1.44	-7.99
8	365.02	-1.47	-7.99
9	364.73	-1.46	-8.03
Avg.	365.05	-1.44	-8.01
SEM	0.09	0.01	0.01
NOAA	365.13	-1.44	-8.02
Diff	-0.08	0.00	-0.01

which had a CO_2 concentration of 328.9 ppm, at a flow rate of 500 mL/min for 5 min, as described by Lang.¹³

Part (a) of Table 1 shows the data as it is output by the datalogger and Isodat software. Shown are the amount of air (in μmol) from which the CO_2 was extracted; the area of the m/z 44 peak (expressed in nmols); and the $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ referred to the CO_2 standard (OzTech). Note that the first measurement cycles of the standard and sample air have a different amount of air trapped and consequently a different peak area. These data were excluded from further calculation. The average amount of CO_2 of all the air samples (standard and sample) measured was 20.442 nmol with a standard deviation of 0.302 nmol.

Part (b) of Table 1 shows the intermediate calculations for the standard and sample measurements (after discarding the initial set of measurements). Parts (c) and (d) show the final CO_2 concentration, $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ calculations.

System precision

The fundamental precision of the system is the standard deviation for the measurement of a pair of peaks or cycle (standard and sample). The standard deviation for a cycle was 0.71 ppm for the CO_2 concentration measurement and 0.14 and 0.13‰ for $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$, respectively (Table 1). The precision of a flask analysis can be improved by increasing the number of measurement cycles, as with the dual-inlet analysis method. Analysis carried out with five replicates (average of four peaks) of both the sample and standard gases had a SEM of 0.7 ppm for the CO_2 concentration and 0.05‰ for both $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ while, with nine cycles, the SEM were 0.4 ppm for CO_2 concentrations and 0.03‰ for both $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$.

System accuracy

Determination of the system's accuracy was obtained from two sets of analysis. In the first analysis, ten flasks were filled with a NOAA/CMDL standard reference air mixture (CA03893) with a $[\text{CO}_2] = 365.13$ ppm, $\delta^{18}\text{O} = -1.44\text{‰}$ and $\delta^{13}\text{C} = -8.02\text{‰}$. These flasks were analyzed against the same NOAA/CMDL reference air mixture as a reference gas with

Table 3. Determination of the accuracy of the system for measuring CO_2 concentration. Nine flasks were filled from three different NOAA/CMDL standard air mixture tanks with different CO_2 concentrations. Flasks were analyzed using air from the CA0389 tank as a standard

Tank #	$[\text{CO}_2]$	Measured	Average	Diff.
CA04434	328.90	329.36 328.69 329.15	329.07	0.17
CA04404	387.46	388.39 387.56 386.57	387.51	0.05
CA03893	365.13	364.73 365.45 365.01	365.06	-0.07

five replicates of both sample and reference gases. The first flask analyzed was always discarded and the data of the remaining nine flasks were averaged (Table 2). The differential between the measured and the real values was 0.08 ppm for the CO_2 concentration, 0.00 for $\delta^{18}\text{O}$ and 0.01 for $\delta^{13}\text{C}$.

The accuracy of the CO_2 concentration measurements was further analyzed with a second set of experiments in which three flasks were filled with different NOAA/CMDL reference air mixtures with CO_2 concentrations of 328.90 ppm (CA04434), 365.13 ppm (CA03893), and 387.46 ppm (CA04404). The flasks were run against the NOAA/CMDL reference air mixture (CA03893). The measured averages of the CO_2 concentrations obtained for each tank were 329.07, 365.06 and 387.51 ppm, respectively, producing differentials of 0.17, -0.07 and 0.05 ppm (Table 3). The $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ of CA04434 and CA04404 were not available.

TECHNICAL APPLICATIONS

Photosynthetic isotope fractionation

One of the applications of this system in our laboratory is the measurement of the photosynthetic isotope fractionation by open flow gas analysis.^{9,10} In these measurements a plant leaf is placed in a gas exchange chamber where H_2O and CO_2 are exchanged between the leaf tissue and the air under controlled temperature, light intensity and humidity.

The isotope fractionation is obtained from the following equation:⁹

$$\Delta^{13}\text{C} = \frac{\xi(\delta^{13}\text{C}_e - \delta^{13}\text{C}_o)}{1000 + \delta^{13}\text{C}_o - \xi(\delta^{13}\text{C}_o - \delta^{13}\text{C}_e)}$$

where $\xi = C_e/(C_e - C_o)$, C_e , C_o are the CO_2 concentrations of the air entering and leaving the chamber, respectively, and $\delta^{13}\text{C}_e$, $\delta^{13}\text{C}_o$ are their isotopic composition.

The uncertainty of the calculated fractionation can be obtained from the fundamental uncertainty of the concentration and isotope ratio as described in Daniels *et al.*¹⁴ As shown in Fig. 2, the error in the calculation of $\Delta^{13}\text{C}$ decreases

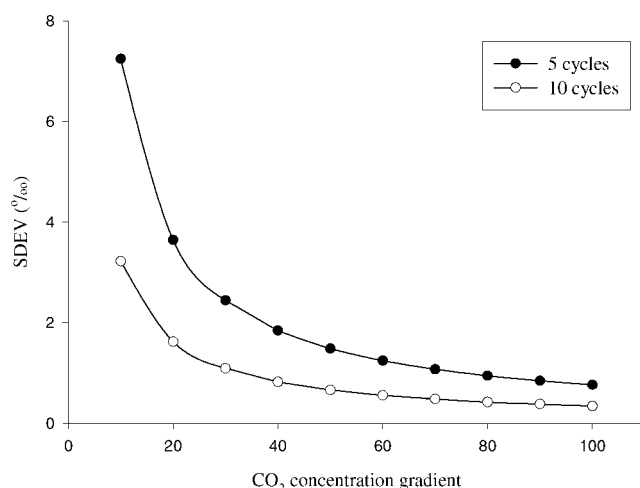


Figure 2. Calculation of the standard deviation of a single measurement of the isotope discrimination during photosynthesis (C_3 plant, $\Delta = 20\text{‰}$) by open flow gas analysis^{9,10} as a function of the CO_2 gradient. The errors of the CO_2 concentration, $\delta^{18}O$ and $\delta^{13}C$ used for these calculations were 0.7 ppm and 0.05‰ for five replicates and 0.4 ppm and 0.3‰ for (the) ten replicates, respectively.

as the gradient ($C_e - C_o$) and the number of replication cycles increase. Typically, for on-line photosynthesis discrimination, the CO_2 concentration gradient ($C_e - C_o$) is around 50 ppm. Using ten measurement cycles would be adequate to obtain a precision of 1‰ (discrimination of a C_3 plant, $\Delta \approx 20\text{‰}$).

Ecosystem isotope fractionation

The application of this technique to ecosystem studies is demonstrated in a field experiment conducted in a tallgrass prairie in Oklahoma. Samples were taken from a 4.5-m tower over the course of 3 days. The sampling interval was 3 h during the night and 1.5 h during the day, totalling 38 100-mL air samples. The relatively small size of these flasks facilitated easy packing and shipment to the laboratory (Stanford, CA, USA) and the time required for the analytical measurement (~ 1 h per flask) made this experiment technically feasible in a reasonable time.

Figure 3 shows the diurnal changes in CO_2 concentration, $\delta^{13}C$ and $\delta^{18}O$, of these samples (Still *et al.*, unpublished). There was a wide range of CO_2 concentrations, from 350 ppm to 485 ppm. $\delta^{13}C$ varied from -7.8‰ to -10.5‰ , while the range for $\delta^{18}O$ was between 0.9‰ and -1.4‰ . The intercept of a Keeling plot of these data, that gives an estimate of the $\delta^{13}C$ of the CO_2 respired by this ecosystem, was $-17.32\text{‰} \pm 0.33\text{‰}$.

CONCLUSIONS

This fully automated system measures simultaneously the CO_2 concentration, $\delta^{13}C$ and $\delta^{18}O$ of very small air samples (≈ 1 mL) of widely variable CO_2 concentrations in a short time (≈ 1 h). The accuracy and precision of the system have been tested by using gases of known CO_2 concentrations,

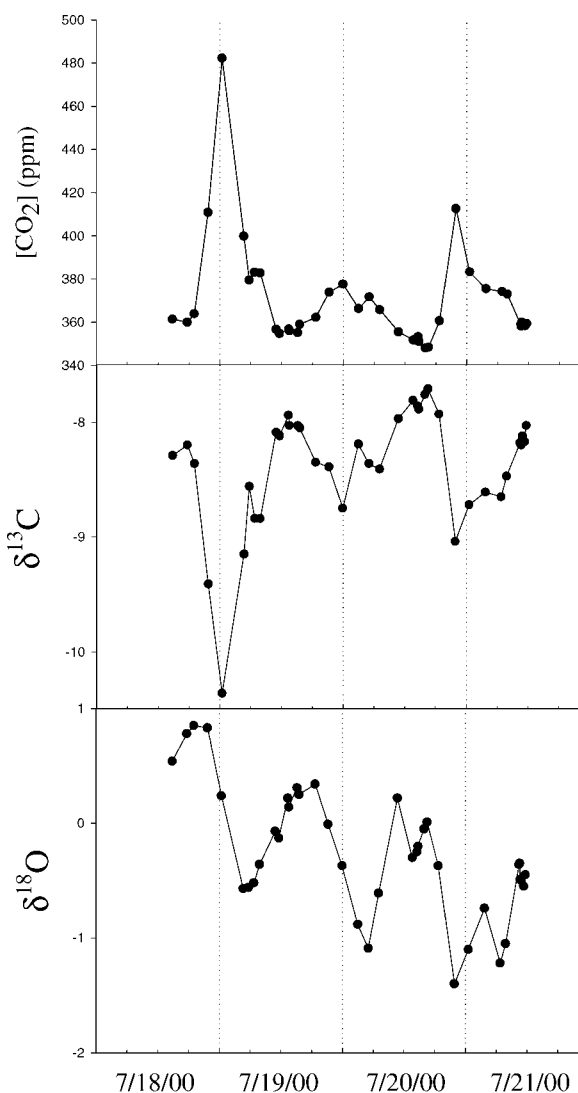


Figure 3. Diurnal changes in CO_2 concentration, $\delta^{13}C$, and $\delta^{18}O$ of air samples collected above a tallgrass prairie canopy in Oklahoma. The variations in CO_2 concentration and $\delta^{13}C$ primarily reflect variations in net photosynthesis (daytime) and respiration interacting with a diurnally varying boundary layer. The $\delta^{18}O$ of CO_2 also reflects these processes, but with added complications due to oxygen isotope exchanges with soil and leaf water pools. This accounts for the phase lags between $\delta^{18}O$ and both CO_2 and $\delta^{13}C$.

$\delta^{13}C$ and $\delta^{18}O$. Its precision is adequate for micrometeorological and ecophysiological applications. While the precision is not quite equal to that attained with dual-inlet analysis, it can be increased by running more replicates. Furthermore, as this technique is developed and improved, its precision should also improve. The small sample size and ease of analysis provided by this system are an advantage of this method.

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APPENDIX

Description of all the parts of this system:

- (a) Low-pressure valve (6-port UW type) with air actuator. VICI (Houston, TX, USA).
- (b) and (c) Valco on/off valve with air actuator. VICI.
- (d) MINI-MYTE interface valves *Humphrey* (Kalamazoo, MI, USA).
- (e) MINI-MYTE interface valves *Humphrey*.
- (f) 10-Position dead-ended flowpath – SD configuration with microelectric actuator. VICI.
- (g) Stainless steel tubing 0.020", 1/32" (50 cm). VICI.
- (i) Stainless steel cylinder: non-rotating 3/4" bore. *Clippard* (Cincinnati, OH, USA).
- (j) Nafion drying tube. *Permapure*, (Toms River, NJ, USA).
- (k) 25-m long capillary Poraplot Q column (10 μm i.d., 0.32 mm o.d.), *Hewlett-Packard* (Palo Alto, CA, USA).
- (l) Delta S isotope ratio mass spectrometer with dual-inlet and continuous-flow setups. *Thermo-Finnigan* (San Jose, CA).
- (PT) Pressure transducer (0-100 Torr), *MKS, Baratron* (Andover, MA, USA).
- (Other parts) Datalogger, 21x Micrologger, *Campbell Scientific Inc.*, (Logan, VT, USA).

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