A method for estimating carbon dioxide leakage rates in controlled-environment chambers using nitrous oxide

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Abstract

Naturally sunlit, outdoor controlled-environment chambers provide an important research tool for studying the effects of environmental variables on crop physiological processes. Typically these types of chambers are semi-closed and are capable of continuously monitoring canopy scale gas exchanges. Accurately determining chamber CO2 leakage rate is essential for correcting measurements of photosynthesis and respiration in these kinds of chambers. The purpose of this study was to evaluate the ability of a new CO2 leak quantification system which used N2O as a tracer gas to estimate chamber CO2 leakage rates in a recently constructed outdoor, controlled-environment chamber facility at Beltsville, MD. Chamber CO2 leakage rates as determined by the loss of CO2 (CL) from the chamber were compared with CO2 leakage rates determined using N2O as a tracer gas (CLN). These two methods of determining leakage rates were compared in two different types of chambers: smaller and more tightly sealed Daylit chambers and larger more leaky Soil-Plant-Atmosphere-Research (SPAR) chambers. Comparisons of CL with CLN indicated that CLN was an excellent predictor of CL. However, over a wide range of internal to external concentration gradients, the analysis did show a slight but consistent overestimation of CL by CLN that averaged 0.3, 1.4, and 1.1 μmol CO2 m−2 s−1 for the Daylit chambers, the SPAR chambers and all data combined, respectively. These results indicate that N2O can be used as a tracer gas to accurately and reliably estimate chamber CO2 leakage rates in real time during experiments in the presence of plants and when it is necessary to maintain specific chamber CO2 treatment set points that make estimation of CL difficult.

Keywords: Controlled-environment chamber; CO2 enrichment; N2O; Assimilation

1. Introduction

Crop modelers as well as researchers studying the effects of environment on plant physiological processes often use semi-closed, controlled-environment plant growth chambers that are equipped to control the aerial environment and continuously monitor canopy scale gas exchanges. Accurately determining chamber CO2 leakage rates is essential for correcting measurements of photosynthesis and respiration rates in these types of chambers. Measurement of chamber leakage rate is complicated by the presence of plants in the chamber because plants are normally a sink for CO2 during the day and a source at night, and it is necessary to distinguish these fluxes from chamber leakage rate. One way of estimating
chamber leakage rate is to measure leakage before and/or after an experiment in the absence of plants and assume that CO₂ leakage is either constant or varies linearly with time during the experiment (Acock et al., 1977; Acock and Acock, 1989). However, the intervening plant growth cycle may be more than 120 days. Kimball (1990) notes that chamber leakage rates may vary over short periods of time due to changes in ambient conditions. For example, a change in ambient CO₂ concentration alters the CO₂ concentration gradient between the chamber atmosphere and ambient air and this in turn changes chamber leakage rate. 

Examining data from several sites around the world, Ziska et al. (2001) reported that ambient CO₂ concentration can vary from 350 to over 500 µmol mol⁻¹ in a single 24-h period. Changes in the manner in which chamber doors are sealed following plant sampling or instrument recalibration as well as deterioration of chamber seals with time can also alter chamber leakage rates (Acock and Acock, 1989).

Lake (1966) first recommended the use of an inert tracer gas such as nitrous oxide (N₂O) to account for CO₂ leakage rates from greenhouses during CO₂ assimilation measurements. In addition to being inert, N₂O has the advantage of being a good tracer gas for estimating CO₂ leakage rates because both CO₂ and N₂O have the same molecular weight which would cause these two gases to have the identical molecular diffusivity.

Other research groups have since utilized N₂O to estimate chamber CO₂ leakage rates (Oechel et al., 1992; Baker et al., 2000; Sakai et al., 2001) while Tingey et al. (2000) used sulfur hexafluoride (SF₆) in a similar fashion. Our goal was to evaluate a new N₂O leak quantification system for a recently constructed outdoor, controlled-environment chamber facility in Beltsville, MD. A total of 18 outdoor chambers are available at this facility and are of two types, referred to here as either ‘Daylit’ (six chambers) and/or Soil-Plant-Atmosphere-Research (SPAR, 12 chambers) chambers. This facility is comparable in design and operation to similar experimental systems at the University of Florida (Pickering et al., 1994), Corvallis, OR (Tingey et al., 1996), and Mississippi State University (Reddy et al., 2001).

The Daylit chambers are constructed of clear acrylic and are 2.3 m tall and 1.5 m² in cross-sectional area with a total chamber volume of 3360 l. The SPAR chambers are very similar in design to those in use at Mississippi State University (Reddy et al., 2001). The SPAR chambers consist of transparent chamber tops, 2.2 m x 1.4 m x 2.5 m (length x width x height) constructed of 0.0127 m thick Plexiglas G. Each SPAR chamber top is mounted to a steel soil bin measuring 2.0 m x 0.5 m x 1.0 m (length x width x depth). A heavily insulated sheet metal air handler is attached to the north side of each SPAR chamber while the air handler in the Daylit chambers is mounted at the base of the chambers. Total SPAR chamber volume, including the air handler is 9494 l. In both chamber types, the air handler contains a squirrel cage fan that draws air from the chambers and forces it past resistive heaters and liquid cooled heat exchanger on the return path back to the chamber. These heating and cooling elements are used to control air temperature and humidity. Air is constantly re-circulated in a closed loop at about 3 m s⁻¹.

The facility includes a dedicated Sun SPARC 5 work station (Sun Microsystems, Inc., Mountainview, CA) used to control chamber atmospheric carbon dioxide concentration and record plant responses (photosynthesis, evapotranspiration) and chamber environmental data (air and soil temperatures, humidity, CO₂ concentration, and solar radiation) every 300 s. Air temperature and relative humidity were monitored and controlled with TC2 controllers (Environmental Growth Chambers, Inc. Chagrin Falls, OH). Constant relative humidity was maintained at 70% by operating solenoid valves that injected chilled water through...
the cooling coils located in the air handler of each Daylit chamber while the same relative humidity was controlled in the SPAR chambers by driving a 3-way valve that regulated the flow of chilled water through a heat exchanger. These cooling coils condensed excess water vapor from the chamber air in order to regulate relative humidity.

Gas sample lines for measuring chamber and ambient air CO2 concentration were run underground from each chamber to the field laboratory building. The sample lines were composed of Nylon-12 Nylotube (NewAge Industries, Southampton, PA). Gas sample lines length from the chambers to the field laboratory building was approximately 30 m and gas sample flow rate was approximately 2 l min−1. CO2 concentrations in each chamber as well as ambient air was measured with a bank of dedicated infrared gas analyzers (IRGA) (LI-COR Model LI-6252, Lincoln, NE) every 30 s and averaged and recorded every 300 s. Moisture was removed from the gas sample by running the sample lines through a refrigerated water trap (4°C) that was automatically drained once each hour. Chamber CO2 concentration was maintained by supplying pure CO2 from a compressed gas cylinder to mass flow controllers (Model FMA-766-V-CO2 Omega Engineering, Inc, Stanford, CT) located in the air ducting in each chamber using a feed-forward, feed-back proportional-integral-differential (PID) control algorithm similar to the one described by Pickering et al. (1994). Prior to the leak tests, the CO2 infrared gas analyzers were zeroed and spanned with pure nitrogen and a span gas of 974 mol CO2 mol−1 air, respectively. Each IRGA was then calibrated with standard gases of 0, 339, 484, 680, and 974 mol CO2 mol−1 air. This data, consisting of millivolt output of the infrared gas analyzer versus standard gas concentration, was fit with a second order polynomial for each infrared gas analyzer versus standard gas concentration, air. This data, consisting of millivolt output of the infrared gas analyzer versus standard gas concentration, was fit with a second order polynomial for each infrared gas analyzer versus standard gas concentration.

In order to compare the ability of the system to measure CO2 leakage rates as determined by the loss of both CO2 and N2O, leakage test for the Daylit chambers was conducted on April 26, 2002 in the absence of both plants and soil. At 08:00 h, N2O was pulsed into each chamber by opening computer-controlled solenoid valves on the N2O injection lines in order to drive chamber N2O concentration to excess of 1000 μmol mol−1. From 08:00 to 09:00 h, CO2 concentration was controlled to 1200 μmol mol−1. At 09:00 h, the CO2 concentration set point was reset to 0 μmol mol−1, stopping further injections of CO2. After 09:00 h the draw-downs of both chamber CO2 and N2O concentration were monitored at 30 s intervals and averaged and recorded at 300 s intervals. Solenoid valves for two of the Daylit chambers failed to open so the results reported here are for four of the Daylit chambers. Air temperatures were maintained at the setpoints of 23, 27, 27, and 35°C to be used in a subsequent experiment for these four chambers.

The leak tests for the SPAR chambers was conducted on May 28, 2002 in the same fashion as that described for the Daylit chambers except that chamber CO2 and N2O draw-downs were conducted twice beginning at 11:00 h and again at 17:00 h. Air temperature set points for the 12 SPAR units were also maintained at the set points required in a subsequent experiment: two chambers each maintained at 20, 25, 35, and 40°C and four chambers at 30°C.

Chamber CO2 leakage rate (Ct) was calculated from the CO2 loss as:

\[ C_t = \frac{V D}{A} \times \frac{dc}{dt} \]  

where, V is the chamber volume (l), D is density of chamber air at chamber air temperature setpoint, and pressure (mol air l−1 m−3 (air)), A is chamber floor area (m²), C is chamber atmospheric CO2 concentration.
concentration (μmol CO$_2$ mol$^{-1}$ (air)), $t$ is the time (s), and $C_1$ is chamber leakage rate (μmol CO$_2$ s$^{-1}$).

$C_1$ was expressed on a chamber ground area basis (1 m$^2$) to allow $C_1$ to be expressed in the same units used to calculate canopy net photosynthesis measured in subsequent experiments (μmol CO$_2$ m$^{-2}$s$^{-1}$). Similarly, N$_2$O leakage rate ($N_L$) was calculated as:

$$N_L = VD/A \times (dN/dr)$$

(2)

where, $N_L$ is the N$_2$O leakage rate (μmol N$_2$O s$^{-1}$ m$^{-2}$) and $N$ is the chamber atmospheric N$_2$O concentration (μmol N$_2$O mol$^{-1}$ (air)).

In these types of chambers, gas leakage occurs mostly due to mass flows past imperfect seals rather than via pure molecular diffusion through chamber wall materials (Tingey et al., 2000; Baker et al., 2000). A primary driver determining the leakage rate of a gas from a particular chamber is the concentration gradient of that particular gas between the chamber atmosphere and ambient air. To calculate $C_L$ from $N_L$, the chamber atmospheric N$_2$O concentration ($N_{2\text{O}}$) measured in subsequent experiments was expressed on a chamber ground area basis (1 m$^2$) to allow $C_L$ to be expressed in the same units used to calculate canopy net photosynthesis measured in subsequent experiments.

$$C_L = (N - N_{2\text{O}})/(VD/A \times (dN/dr))$$

(3)

where $C_L$ is chamber leakage rate from measurements of N$_2$O loss (s m$^{-2}$ mol$^{-1}$ (air)), $N$ is chamber atmospheric N$_2$O concentration (μmol N$_2$O mol$^{-1}$ (air)), $N_{2\text{O}}$ is ambient atmospheric N$_2$O concentration (μmol N$_2$O mol$^{-1}$ (air)) and assumed to be 0, $V$ is chamber volume (l), $D$ is density of chamber air at chamber air temperature setpoint and pressure (mol air 1$^{-1}$ (air)), $A$ is chamber floor area (m$^2$), and $t$ is the time (s).

To calculate chamber CO$_2$ leakage rate from measurements of N$_2$O loss, $r$ was applied to the chamber to ambient air CO$_2$ concentration gradient:

$$C_{LN} = \frac{C - C_1}{r}$$

(4)

where $C_{LN}$ is the CO$_2$ leakage rate calculated from the loss of N$_2$O (μmol CO$_2$ m$^{-2}$s$^{-1}$) and $C_1$ is the measured ambient CO$_2$ concentration (μmol CO$_2$ mol$^{-1}$ (air)).

During an experiment, it is difficult to use (1) to estimate CO$_2$ leakage rate directly because plants and respiring soil are typically present in the chambers, and it is necessary to maintain a particular treatment. Thus, it is more advantageous to calculate CO$_2$ leakage rates from $C_{LN}$ (4) rather than $C_1$ (1). The ability of the leak detection system to estimate CO$_2$ leakage was evaluated by regressing $C_{LN}$ versus $C_1$ values. In cases where these regression models were significant ($P < 0.05$), $t$-tests were conducted to determine whether the slope and intercepts were significantly different from 1.0 and 0.0, respectively (Steel et al., 1997). Good statistical agreement between $C_{LN}$ and $C_1$ values was inferred when the regression $F$-value was significant, slope and intercept not significantly different from 1.0 and 0.0, respectively, and the regression yielded a high coefficient of determination ($R^2$). Bias and regression root mean square error (RMSE) were calculated to determine overall system performance (Willmott, 1982):

$$\text{Bias} = \frac{1}{N} \sum_{i=1}^{N} (\hat{C}_{LN(i)} - C_{LO(i)})$$

(5)

$$\text{RMSE} = \left( \frac{1}{N} \sum_{i=1}^{N} (\hat{C}_{LN(i)} - C_{LO(i)})^2 \right)^{1/2}$$

(6)

where $C_{LN(i)}$ and $C_{LO(i)}$ are the $C_{LN}$ and $C_1$ values for the $i$th observation and $N$ is the total number of observations.

3. Results

An example of $C$ and $N$ versus time during measurement periods is shown in Fig. 1 for both types of chambers. In both types of chambers rates of N loss were greater than that for $C$. The rate of both $C$ and N loss was much higher for the SPAR compared with the Daylit chambers (Fig. 1) due to a much higher inherent leakiness of the SPAR chambers.

Shown in Fig. 2 are examples of $C_{LN}$ and $C_1$ plotted against the CO$_2$ concentration gradient ($C - C_1$). There was a tendency for $C_{LN}$ to be slightly higher than $C_1$ in both chamber types. Due to their inherent leakiness, the SPAR chambers had much higher $C_{LN}$ and $C_1$ than the Daylit chambers at any given CO$_2$ concentration gradient.

A plot of $C_1$ versus $C_{LN}$ and compared to a 1:1 line is shown in Fig. 3. Here again, the SPAR chambers
had much higher leakage rates than the Daylit chambers and there was a slight tendency of $C_{LN}$ to overestimate values of $C_L$. The regressions of $C_L$ versus $C_{LN}$ were highly significant (Table 1). The $t$-tests for testing the hypothesis that the slopes were equal to one was rejected at the 0.05 level of confidence while the intercepts were not significantly different from zero for the SPAR chamber and for both the Daylit and SPAR data combined (Table 1). The bias estimates in Table 1 indicate an average ability of $C_{LN}$ to estimate $C_L$ to within less than 1.4 μmol CO₂ m⁻² s⁻¹ across a wide range of both $C$ and $N$ concentration gradients. The bias estimates also indicate even less error in es-

Table 1
Statistics for the regression ($y = b_1 x + b_0$) of $C_L$ estimated from CO₂ loss ($y$) vs. $C_{LN}$ estimated from N₂O loss ($x$) for 4 Daylit and 12 SPAR outdoor controlled-environment chambers.

<table>
<thead>
<tr>
<th>Chamber type</th>
<th>$b_1$ ± S.E.</th>
<th>$b_0$ ± S.E.</th>
<th>$\alpha$</th>
<th>$R^2$</th>
<th>RMSE</th>
<th>Bias</th>
</tr>
</thead>
<tbody>
<tr>
<td>Daylit</td>
<td>0.77* ± 0.024</td>
<td>0.31* ± 0.074</td>
<td>163</td>
<td>0.86***</td>
<td>0.42</td>
<td>0.33</td>
</tr>
<tr>
<td>SPAR</td>
<td>0.95* ± 0.006</td>
<td>-0.07 ± 0.181</td>
<td>554</td>
<td>0.98***</td>
<td>1.71</td>
<td>1.36</td>
</tr>
<tr>
<td>Combined</td>
<td>0.95* ± 0.004</td>
<td>-0.15 ± 0.101</td>
<td>717</td>
<td>0.99***</td>
<td>1.52</td>
<td>1.13</td>
</tr>
</tbody>
</table>

*Note. 0.05 level of significance of the $t$-statistic for testing the hypothesis: $H_0: b_1 = 1.0$ vs. $H_a: b_1 \neq 1.0$ and the hypothesis: $H_0: b_0 = 0.0$ vs. $H_a: b_0 \neq 0.0$. Values with the asterisk (*) indicate the values are significantly different from 1.0 for $b_1$ and 0.0 for $b_0$; asterisks (***), indicate the regression model was significant at $P < 0.001$. 

Fig. 1. Example of carbon dioxide ($C$) and nitrous oxide ($N$) concentrations vs. time during leakage tests for Daylit and SPAR chambers.

Fig. 2. Comparison of chamber CO₂ leakage rates calculated either by CO₂ loss ($C_L$) or by N₂O loss ($C_{LN}$) for Daylit and SPAR chambers.
timating $C_L$ from $C_{LN}$ at 0.33 mol CO$_2$ m$^{-2}$ s$^{-1}$ for the less leaky Daylit compared to the SPAR chambers.

4. Discussion

The reason for the higher rate of rates of $N$ depletion compared to those for $C$ was because of the differences in concentration gradients between the chamber atmosphere and ambient air for these two gasses (Fig. 1). During these measurement periods, ambient $C_a$ averaged 356 ± 0.15 mol CO$_2$ mol$^{-1}$ and 349 ± 0.25 mol CO$_2$ mol$^{-1}$ on 26 April for the Daylit chambers and 28 May for the SPAR chambers, respectively. Ambient $N_a$ is much lower (∼310 nmol mol$^{-1}$) than $C_a$ (∼360 mol CO$_2$ mol$^{-1}$) which created a much steeper concentration gradient for $N$ than $C$ at a common chamber concentration for both gasses and hence higher leakage rates of $N$ than $C$. In the methods described here, $N_a$ was not measured and was assumed to be zero. Eventually, during these draw-downs, both $C$ and $N$ will approach their respective ambient $C_a$ and $N_a$ concentrations as these gases come close to equilibrium with ambient air concentrations.

The primary driver of leakage rate is the concentration gradient between the chamber atmosphere and ambient air (Fig. 2). Chamber air temperature set points had no noticeable effect on chamber leakage rates (data not shown) since temperature effects on air density are accounted for in the equations used to calculate leakage rate (Eqs. (1), (2), and (3)). Indeed, Tingey et al. (2000) found that chamber leakage rates were unaffected by changes in temperature, wind speed, dew point or atmospheric pressure. Baker et al. (2000) concluded that the primary determinants of chamber leakage rates were the inherent leakiness of a given chamber and the magnitude of the concentration gradient between the chamber atmosphere and ambient air.

Although estimates of $C_{LN}$ and $C_L$ agreed quite well (Fig. 3), there are several reasons to expect this agreement to be less than perfect. The calculations of both $C_L$ and $C_{LN}$ depend on measurements from two IRGAs and a thermocouple while $C_{LN}$ requires an additional N$_2$O IRGA. All these instruments have their own electronic noise and measurement error while the readings from IRGAs also depend on the quality of the respective calibration curves.

Another potential source of error could occur during experiments where soils used to grow plants are exposed to the chamber atmosphere. Under anaerobic conditions nitrous oxide can be utilized by anaerobic bacteria as an electron acceptor to form nitrogen. Because N$_2$O is an important greenhouse gas, several studies have attempted to quantify N$_2$O emissions from flooded rice fields. These studies report either extremely low emissions or no emission or uptake because the flux rates were below detectable levels (Denmead et al., 1979; Smith et al., 1982; Lindau et al., 1990; Freney and Denmead, 1992; Hua et al., 1997; Tsuruta et al., 1997). For example, Lindau et al. (1990) used flooded microplots without rice plants and measured N$_2$O emission 21 days after fertilizer application. They reported maximum emission rates of 4 g N$_2$O-N ha$^{-1}$ per day (1.65 × 10$^{-4}$ mol N$_2$O m$^{-2}$ s$^{-1}$). Denmead et al. (1979) found that emission of N$_2$O peaked one day after flooding at 0.01 kg N ha$^{-1}$ per day (4.14 × 10$^{-4}$ mol N$_2$O m$^{-2}$ s$^{-1}$) and declined to undetectable levels by day 13. Reasons for the extremely low N$_2$O exchange of flooded rice paddies compared with measured exchange rates in dryland cropping systems include differences in gas diffusion rates, with molecular diffusion in soil water being a major restriction, and the physical and
biological processes involved in the production and consumption of $\text{N}_2\text{O}$ in the soil. A thorough review of this topic is provided by Granli and Bøckman (1994).

In aerobic soil, $\text{N}_2\text{O}$ is formed by microbial activity during the processes of nitrification and denitrification. Rates of $\text{N}_2\text{O}$ evolution are determined by soil organic matter, soil fertility, temperature and soil moisture status. The highest rates of $\text{N}_2\text{O}$ evolution recorded for any terrestrial ecosystem are reported by Ineson et al. (1998) following a fertilizer application during a free air carbon dioxide enrichment experiment. Under these conditions, Ineson et al. (1998) report $\text{N}_2\text{O}$ emission rates of 46 mg $\text{N}_2\text{O-N} \, \text{m}^{-2} \, \text{h}^{-1}$ which is equivalent to about 0.3 $\mu$mol $\text{N}_2\text{O} \, \text{m}^{-2} \, \text{s}^{-1}$. While this rate is much lower than the leakage rates reported in this experiment, soil $\text{N}_2\text{O}$ emissions could be a factor in situations where soil microbial activity is high and in chambers that are very tightly sealed.

In semi-closed chamber systems that control $C$ while measuring canopy or whole plant CO$_2$ exchanges, accurate determination of chamber $C$ leakage rate is essential. Failing to correct for $C$ leakage rate will result in the underestimation of nighttime respiration rates and overestimation of daytime photosynthetic rates. We conclude that $\text{N}_2\text{O}$ can be used to estimate $C$ leakage rates with an acceptable level of reliability and precision.

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References


