



ELSEVIER

Available online at [www.sciencedirect.com](http://www.sciencedirect.com)

SCIENCE @ DIRECT®

Environmental and Experimental Botany 51 (2004) 103–110

Environmental  
and Experimental  
Botany

[www.elsevier.com/locate/envexpbot](http://www.elsevier.com/locate/envexpbot)

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

J.T. Baker<sup>a,\*</sup>, S.-H. Kim<sup>b</sup>, D.C. Gitz<sup>b</sup>, D. Timlin<sup>b</sup>, V.R. Reddy<sup>b</sup>

<sup>a</sup> USDA-ARS, Cropping Systems Research Laboratory, Big Spring, TX 79720, USA

<sup>b</sup> USDA-ARS, Alternate Crops and Systems Laboratory, Beltsville, MD 20705, USA

Received 22 April 2003; received in revised form 23 July 2003; accepted 4 August 2003

## 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 CO<sub>2</sub> 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 CO<sub>2</sub> leak quantification system which used N<sub>2</sub>O as a tracer gas to estimate chamber CO<sub>2</sub> leakage rates in a recently constructed outdoor, controlled-environment chamber facility at Beltsville, MD. Chamber CO<sub>2</sub> leakage rates as determined by the loss of CO<sub>2</sub> ( $C_L$ ) from the chamber were compared with CO<sub>2</sub> leakage rates determined using N<sub>2</sub>O as a tracer gas ( $C_{LN}$ ). 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  $C_L$  with  $C_{LN}$  indicated that  $C_{LN}$  was an excellent predictor of  $C_L$ . However, over a wide range of internal to external concentration gradients, the analysis did show a slight but consistent overestimation of  $C_L$  by  $C_{LN}$  that averaged 0.3, 1.4, and 1.1  $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$  for the Daylit chambers, the SPAR chambers and all data combined, respectively. These results indicate that N<sub>2</sub>O can be used as a tracer gas to accurately and reliably estimate chamber CO<sub>2</sub> leakage rates in real time during experiments in the presence of plants and when it is necessary to maintain specific chamber CO<sub>2</sub> treatment set points that make estimation of  $C_L$  difficult. Published by Elsevier B.V.

**Keywords:** Controlled-environment chamber; CO<sub>2</sub> enrichment; N<sub>2</sub>O; 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 precisely

control the aerial environment and continuously monitor canopy scale gas exchanges. Accurately determining chamber CO<sub>2</sub> 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 CO<sub>2</sub> during the day and a source at night, and it is necessary to distinguish these fluxes from chamber leakage rate. One way of estimating

\* Corresponding author. Tel.: +1-915-263-0293;

fax: +1-915-263-0293.

E-mail address: [jtbaker@lbk.ars.usda.gov](mailto:jtbaker@lbk.ars.usda.gov) (J.T. Baker).

chamber leakage rate is to measure leakage before and/or after an experiment in the absence of plants and assume that CO<sub>2</sub> 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 CO<sub>2</sub> concentration alters the CO<sub>2</sub> 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 CO<sub>2</sub> concentration can vary from 350 to over 500  $\mu\text{mol mol}^{-1}$  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 (N<sub>2</sub>O) to account for CO<sub>2</sub> leakage rates from greenhouses during CO<sub>2</sub> assimilation measurements. In addition to being inert, N<sub>2</sub>O has the advantage of being a good tracer gas for estimating CO<sub>2</sub> leakage rates because both CO<sub>2</sub> and N<sub>2</sub>O have the same molecular weight which would cause these two gases to have the identical molecular diffusivity.

Other research groups have since utilized N<sub>2</sub>O to estimate chamber CO<sub>2</sub> leakage rates (Oechel et al., 1992; Baker et al., 2000; Sakai et al., 2001) while Tingey et al. (2000) used sulfur hexafluoride (SF<sub>6</sub>) in a similar fashion. Our goal was to evaluate a new N<sub>2</sub>O leak quantification system for a recently constructed outdoor, controlled-environment chamber facility at Beltsville, MD. This evaluation was conducted by comparing leakage rates calculated during simultaneous CO<sub>2</sub> and N<sub>2</sub>O draw-down tests in the absence of plants and soil in these chambers. We tested the hypothesis that N<sub>2</sub>O can be used to estimate chamber CO<sub>2</sub> leakage rates.

## 2. Materials and methods

The outdoor, controlled-environment chambers are located at the USDA-ARS Alternate Crops and Sys-

tems Laboratory 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 m<sup>2</sup> in cross-sectional area with a total chamber volume of 3360 l. Excluding the internal ducting, the space available for growing plants in the Daylit chambers is 1.0 m<sup>2</sup>. 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 × 1.4 m × 2.5 m (length × width × height) constructed of 0.0127 m thick Plexiglas G. Each SPAR chamber top is mounted to a steel soil bin measuring 2.0 m × 0.5 m × 1.0 m (length × width × depth). A heavily insulated sheet metal air handler is attached to 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<sup>-1</sup>.

The facility includes a dedicated Sun SPARC 5 work station (Sun Microsystems, Inc., Mountainview, CA)<sup>1</sup> used to control chamber atmospheric carbon dioxide concentration and record plant responses (photosynthesis, evapotranspiration) and chamber environmental data (air and soil temperatures, humidity, CO<sub>2</sub> 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

<sup>1</sup> Mention of this or other proprietary products is for the convenience of the readers only, and does not constitute endorsement or preferential treatment of these products by USDA-ARS.

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 CO<sub>2</sub> concentration were run underground from each chamber to the field laboratory building. The sample lines were composed of Nylon-12 Nylo tube (NewAge Industries, Southampton, PA). Gas sample line length from the chambers to the field laboratory building was approximately 30 m and gas sample flow rate was approximately 2 l min<sup>-1</sup>. CO<sub>2</sub> 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 CO<sub>2</sub> concentration was maintained by supplying pure CO<sub>2</sub> from a compressed gas cylinder to mass flow controllers (Model FMA-766-V-CO<sub>2</sub> Omega Engineering, Inc, Stamford, 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 CO<sub>2</sub> infrared gas analyzers were zeroed and spanned with pure nitrogen and a span gas of 974 μmol CO<sub>2</sub> mol<sup>-1</sup> air, respectively. Each IRGA was then calibrated with standard gases of 0, 339, 484, 680, and 974 μmol CO<sub>2</sub> mol<sup>-1</sup> 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 instrument and entered into the system control computer file to allow calculation of CO<sub>2</sub> concentration.

To determine CO<sub>2</sub> leakage rates, N<sub>2</sub>O was pulsed into each chamber once a day in order to drive chamber N<sub>2</sub>O concentration to over 1000 μmol mol<sup>-1</sup>. The subsequent loss of N<sub>2</sub>O in each chamber was used to calculate CO<sub>2</sub> leakage rates. To continuously monitor this loss of N<sub>2</sub>O, a single time-shared nitrous oxide IRGA (Fisher-Rosmont NGA 2000, Rosemount Analytical Inc., Anaheim, CA) was used. In order to time-share the N<sub>2</sub>O IRGA, the air sample streams

from the chambers were branched in the field building with one branch going to the CO<sub>2</sub> IRGAs and the other branch going to the N<sub>2</sub>O IRGA. The N<sub>2</sub>O branch was physically multiplexed using solenoid valves to divide the 18 chambers into three groups of six chambers, each with a dedicated measurement channel for recording chamber N<sub>2</sub>O concentration. Prior to the leak tests, the N<sub>2</sub>O infrared gas analyzer was zeroed and spanned with pure nitrogen and a span gas of 2000 μmol N<sub>2</sub>O mol<sup>-1</sup> air.

In order to compare the ability of the system to measure CO<sub>2</sub> leakage rates as determined by the loss of both CO<sub>2</sub> and N<sub>2</sub>O, leakage test for the Daylit chambers was conducted on April 26, 2002 in the absence of both plants and soil. At 08:00 h, N<sub>2</sub>O was pulsed into each chamber by opening computer-controlled solenoid valves on the N<sub>2</sub>O injection lines in order to drive chamber N<sub>2</sub>O concentration to excess of 1000 μmol mol<sup>-1</sup>. From 08:00 to 09:00 h, CO<sub>2</sub> concentration was controlled to 1200 μmol mol<sup>-1</sup>. At 09:00 h, the CO<sub>2</sub> concentration set point was reset to 0 μmol mol<sup>-1</sup>, stopping further injections of CO<sub>2</sub>. After 09:00 h the draw-downs of both chamber CO<sub>2</sub> and N<sub>2</sub>O 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 CO<sub>2</sub> and N<sub>2</sub>O 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 CO<sub>2</sub> leakage rate ( $C_L$ ) was calculated from the CO<sub>2</sub> loss as:

$$C_L = VD/A \times (dC/dt) \quad (1)$$

where,  $V$  is the chamber volume (l),  $D$  is density of chamber air at chamber air temperature setpoint, and pressure (mol air l<sup>-1</sup> m<sup>-2</sup> (air)),  $A$  is chamber floor area (m<sup>2</sup>),  $C$  is chamber atmospheric CO<sub>2</sub>

concentration ( $\mu\text{mol CO}_2 \text{ mol}^{-1}$  (air)),  $t$  is the time (s), and  $C_L$  is chamber leakage rate ( $\mu\text{mol CO}_2 \text{ s}^{-1}$ ).  $C_L$  was expressed on a chamber ground area basis ( $1 \text{ m}^2$ ) to allow  $C_L$  to be expressed in the same units used to calculate canopy net photosynthesis measured in subsequent experiments ( $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ). Similarly,  $\text{N}_2\text{O}$  leakage rate ( $N_L$ ) was calculated as:

$$N_L = VD/A \times (dN/dt) \quad (2)$$

where,  $N_L$  is the  $\text{N}_2\text{O}$  leakage rate ( $\mu\text{mol N}_2\text{O s}^{-1} \text{ m}^{-2}$ ) and  $N$  is the chamber atmospheric  $\text{N}_2\text{O}$  concentration ( $\mu\text{mol N}_2\text{O mol}^{-1}$  (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  $N_L$  measurements, first a resistance to the loss of  $\text{N}_2\text{O}$  ( $r$ ) was calculated as the ratio of the concentration gradient between chamber and ambient  $\text{N}_2\text{O}$  concentration and the rate at which  $N$  is lost from the chamber:

$$r = (N - N_a) / [VD/A \times (dN/dt)] \quad (3)$$

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

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

$$C_{LN} = \frac{C - C_a}{r} \quad (4)$$

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

During an experiment, it is difficult to use (1) to estimate  $\text{CO}_2$  leakage rate directly because plants and

respiring soil are typically present in the chambers, and it is necessary to maintain a particular treatment  $C$ . Thus, it is more advantageous to calculate  $\text{CO}_2$  leakage rates from  $C_{LN}$  (4) rather than  $C_L$  (1). The ability of the leak detection system to estimate  $\text{CO}_2$  leakage was evaluated by regressing  $C_{LN}$  versus  $C_L$  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  $C_L$  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):

$$\text{Bias} = \frac{1}{N} \sum_{i=1}^N (C_{LN(i)} - C_{L(i)}) \quad (5)$$

$$\text{RMSE} = \left( \frac{1}{N} \sum_{i=1}^N (C_{LN(i)} - C_{L(i)})^2 \right)^{1/2} \quad (6)$$

where  $C_{LN(i)}$  and  $C_{L(i)}$  are the  $C_{LN}$  and  $C_L$  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  $C_L$  plotted against the  $\text{CO}_2$  concentration gradient ( $C - C_a$ ). There was a tendency for  $C_{LN}$  to be slightly higher than  $C_L$  in both chamber types. Due to their inherent leakiness, the SPAR chambers had much higher  $C_{LN}$  and  $C_L$  than the Daylit chambers at any given  $\text{CO}_2$  concentration gradient.

A plot of  $C_L$  versus  $C_{LN}$  and compared to a 1:1 line is shown in Fig. 3. Here again, the SPAR chambers

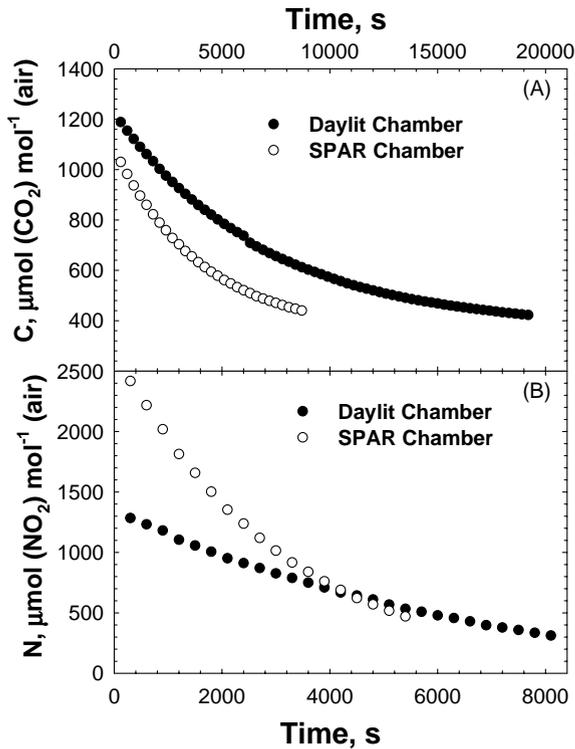


Fig. 1. Example of carbon dioxide ( $C$ ) and nitrous oxide ( $N$ ) concentrations vs. time during leakage tests for Daylit and SPAR chambers.

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

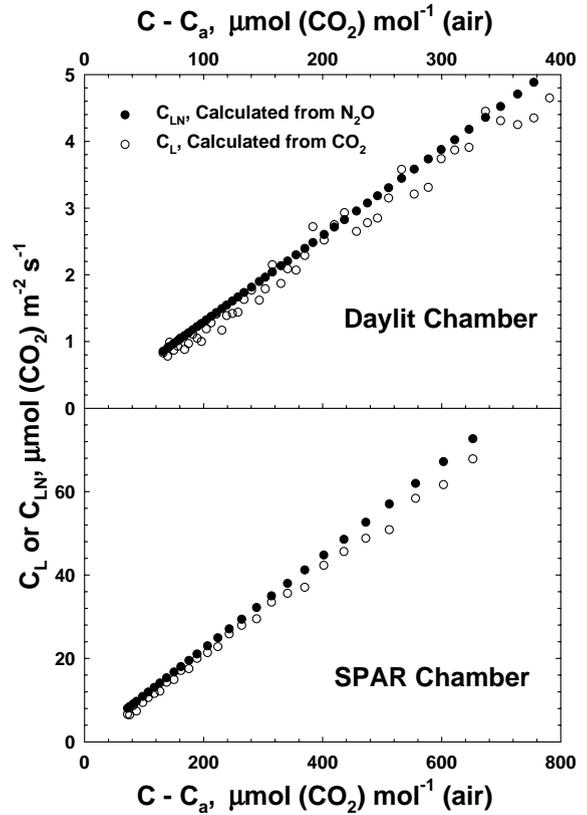


Fig. 2. Comparison of chamber  $CO_2$  leakage rates calculated either by  $CO_2$  loss ( $C_L$ ) or by  $N_2O$  loss ( $C_{LN}$ ) for Daylit and SPAR chambers.

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  $1.4 \mu mol CO_2 m^{-2} s^{-1}$  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 ( $y = b_1x + b_0$ ) of  $C_L$  estimated from  $CO_2$  loss ( $y$ ) vs.  $C_{LN}$  estimated from  $N_2O$  loss ( $x$ ) for 4 Daylit and 12 SPAR outdoor controlled-environment chambers

Chamber type	$b_1 \pm S.E.$	$b_0 \pm S.E.$	$n$	$R^2$	RMSE	Bias
Daylit	$0.77^* \pm 0.024$	$0.31^* \pm 0.074$	163	0.86***	0.42	0.33
SPAR	$0.95^* \pm 0.006$	$-0.07 \pm 0.181$	554	0.98***	1.71	1.36
Combined	$0.95^* \pm 0.004$	$-0.15 \pm 0.101$	717	0.99***	1.52	1.13

Notes. 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  $b_2$ ; asterisks (\*\*\*) indicate the regression model was significant at  $P < 0.001$ .

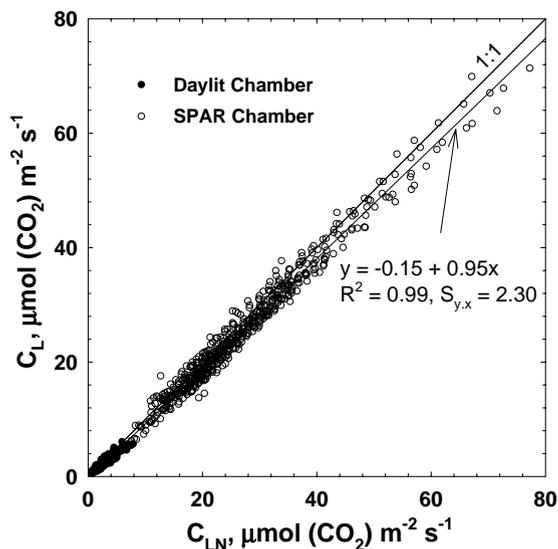


Fig. 3. 1:1 comparison of CO<sub>2</sub> leakage rates calculated either by CO<sub>2</sub> loss ( $C_L$ ) or by N<sub>2</sub>O loss ( $C_{LN}$ ) for Daylit and SPAR chambers.

timating  $C_L$  from  $C_{LN}$  at  $0.33 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$  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  $356 \pm 0.15 \mu\text{mol CO}_2 \text{ mol}^{-1}$  and  $349 \pm 0.25 \mu\text{mol CO}_2 \text{ mol}^{-1}$  on 26 April for the Daylit chambers and 28 May for the SPAR chambers, respectively. Ambient  $N_a$  is much lower ( $\sim 310 \text{ nmol mol}^{-1}$ ) than  $C_a$  ( $\sim 360 \mu\text{mol mol}^{-1}$ ) 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 gasses 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<sub>2</sub>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<sub>2</sub>O is an important greenhouse gas, several studies have attempted to quantify N<sub>2</sub>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<sub>2</sub>O emission 21 days after fertilizer application. They reported maximum emission rates of  $4 \text{ g N}_2\text{O-N ha}^{-1} \text{ per day}$  ( $1.65 \times 10^{-4} \mu\text{mol N}_2\text{O m}^{-2} \text{ s}^{-1}$ ). Denmead et al. (1979) found that emission of N<sub>2</sub>O peaked one day after flooding at  $0.01 \text{ kg N ha}^{-1} \text{ per day}$  ( $4.14 \times 10^{-4} \mu\text{mol N}_2\text{O m}^{-2} \text{ s}^{-1}$ ) and declined to undetectable levels by day 13. Reasons for the extremely low N<sub>2</sub>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  $N_2O$  in the soil. A thorough review of this topic is provided by Granli and Bøckman (1994).

In aerobic soil,  $N_2O$  is formed by microbial activity during the processes of nitrification and denitrification. Rates of  $N_2O$  evolution are determined by soil organic matter, soil fertility, temperature and soil moisture status. The highest rates of  $N_2O$  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  $N_2O$  emission rates of  $46 \text{ mg } N_2O\text{-N m}^{-2} \text{ h}^{-1}$  which is equivalent to about  $0.3 \text{ } \mu\text{mol } N_2O \text{ m}^{-2} \text{ s}^{-1}$ . While this rate is much lower than the leakage rates reported in this experiment, soil  $N_2O$  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  $N_2O$  can be used to estimate  $C$  leakage rates with an acceptable level of reliability and precision.

## Acknowledgements

The authors gratefully acknowledge the technical assistance of Jackson Fischer, Geetha Reddy and Bob Jones.

## References

- Acock, B., Acock, M.C., 1989. Calculating air leakage rates in controlled-environment chambers containing plants. *Agron. J.* 81, 619–623.
- Acock, B., Charles-Edwards, D.A., Hern, A.R., 1977. Growth response of a chrysanthemum crop to the environment. 1. Experimental techniques. *Ann. Bot.* 41, 41–48.
- Baker, J.T., Allen Jr., L.H., Boote, K.J., Pickering, N.B., 2000. Direct effects of atmospheric carbon dioxide concentration on whole canopy dark respiration of rice. *Global Change Biol.* 6, 275–286.
- Denmead, O.T., Freney, J.R., Simpson, J.R., 1979. Nitrous oxide emission during denitrification in a flooded field. *Soil Sci. Soc. Am. J.* 43, 716–718.
- Freney, J.R., Denmead, O.T., 1992. Factors controlling ammonia and nitrous oxide emissions from flooded rice fields. *Ecol. Bull.* 42, 188–194.
- Granli, T., Bøckman, O.C., 1994. Nitrous oxide from agriculture. *Norwegian J. Agric. Sci.* 12, 1–125.
- Hua, X., Guangxi, X., Cai, Z., Tsuruta, H., 1997. Nitrous oxide emissions from three rice paddy fields in China. *Nutr. Cycl. Agroecosyst.* 49, 23–28.
- Ineson, P., Coward, P.A., Hartwig, U.A., 1998. Soil gas fluxes of  $N_2O$ ,  $CH_4$ , and  $CO_2$  beneath *Lolium perenne* under elevated  $CO_2$ : The Swiss free air carbon dioxide enrichment experiment. *Plant Soil* 198, 89–95.
- Kimball, B.A., 1990. Exact equations for calculating air leakage rates from plant growth chambers. *Agron. J.* 82, 998–1003.
- Lindau, C.W., Patrick Jr., W.H., Delaune, R.D., Reddy, K.R., 1990. Rate of accumulation and emission of  $N_2$ ,  $N_2O$  and  $CH_4$  from a flooded rice soil. *Plant Soil* 129, 269–276.
- Lake, J.V., 1966. Measurement and control of carbon assimilation by glasshouse crops. *Nature* 209, 97–98.
- Oechel, W.C., Reichers, G., Lawrence, W.T., Prudhomme, T.L., Grulke, N., Hastings, J.S., 1992. 'CO<sub>2</sub>LT' an automated, null-balance system for studying the effects of elevated  $CO_2$  and global climate change on unmanaged ecosystems. *Funct. Ecol.* 6, 86–100.
- Pickering, N.B., Allen Jr., L.H., Albrecht, S.L., Jones, P., Jones, J.W., Baker, J.T., 1994. Environmental plant chambers: control and measurement using CR-10 data loggers. In: Watson, D.G., Zazueta, F.S., Harrison, T.V. (Eds.), *Computers in Agriculture. Proceedings of the Fifth International Conference, Orlando, Florida, February 5–9. American Society of Agricultural Engineers, St. Joseph, Michigan*, pp. 29–35.
- Reddy, K.R., Baker, J.T., Reddy, V.R., McKinion, J., Tarpley, L., Read, J.J., 2001. Soil-Plant-Atmosphere-Research (SPAR) facility: a tool for plant research and modeling. *Biotronics* 30, 27–50.
- Sakai, H., Yagi, K., Kobayashi, K., Kawashima, S., 2001. Rice carbon balance under elevated  $CO_2$ . *New Phytologist* 150, 241–249.
- Smith, C.J., Brandon, M., Patrick Jr., W.H., 1982. Nitrous oxide emission following urea-N fertilization of wetland rice. *Soil Sci. Plant Nutr.* 28, 161–171.
- Steel, R.D., Torrie, J.H., Dickey, D.A., 1997. *Principles and Procedures of Statistics: A Biometrical Approach*, 3rd. ed. McGraw-Hill, New York, NY.
- Tingey, D.T., McVeety, B.D., Waschmann, R.S., Johnson, R.M., Phillips, D.L., Rygielwicz, R.T., Olszyk, D.M., 1996. A versatile sun-lit controlled-environment facility for studying plant and soil processes. *J. Environ. Qual.* 25, 614–625.
- Tingey, D.T., Waschmann, R.S., Phillips, D.L., Olszyk, D.M., 2000. The carbon dioxide leakage from chambers measured using sulfur hexafluoride. *Environ. Exp. Bot.* 43, 101–110.

- Tsuruta, H., Kanda, K., Hirose, T., 1997. Nitrous oxide emission from a rice paddy field in Japan. *Nutr. Cycl. Agroecosyst.* 49, 51–58.
- Willmott, C.J., 1982. Some comments on the evaluation of model performance. *Am. Meteorol. Soc. Bull.* 63, 1309–1313.
- Ziska, L.H., Ghannoum, O., Baker, J.T., Conroy, J., Bunce, J.A., Kobayashi, K., Okada, M., 2001. A global perspective of ground level, “ambient” carbon dioxide for assessing the response of plants to atmospheric CO<sub>2</sub>. *Global Change Biol.* 7, 789–796.