INTRODUCTION

For various physio-ecological investigations of soil-plant-atmosphere systems, the accurate determination of the fluxes of gases such as CO₂, H₂O, and other greenhouse gases is required. The quantification of CO₂ fluxes by photosynthesis and respiration of a plant canopy, for example, is crucial not only for understanding crop productivity but also for analyzing a global carbon balance. The quantification of H₂O fluxes by evapotranspiration is required to estimate irrigation requirement and water use efficiency in crop fields. The fluxes of CH₄ and N₂O from agricultural lands have been widely investigated for evaluating their effects on global warming. To measure these gas fluxes, several methods can be used, each with advantages and limitations.

Micrometeorological techniques such as the eddy covariance or Bowen ratio methods have a clear advantage in continuous measurements without disturbing the micro-environment of a measuring field (Müller et al., 2009). These methods, however, are not applicable to experiments in small-scale fields where certain assumptions of these methods, such as sufficient fetch length or stable conditions, are not satisfied (Stamnard, 1997; Baldocchi, 2003).

Conversely, chamber methods, where a transparent chamber is placed over vegetation or soil and gas fluxes are estimated from the concentration changes of the gases in the chamber, have remained the sole method in small-scale fields (Steduto et al., 2002). Typically, chamber methods are classified into two categories: (i) closed chamber method and (ii) open chamber method (Livingston and Hutchinson, 1995). The closed chamber method can be advantageous over the open chamber method in terms of system simplicity and has been widely used in numerous studies related to gas flux measurements of different subjects and scales (Wheeler, 1992; Kitano et al., 1997; Scott et al., 1999; Hoffmann et al., 2015; Lesmeister and Koschorreck, 2017).

The closed-chamber method estimates gas fluxes by measuring the rate of change in gas concentrations in the chamber air in a short time during chamber closure. To measure these gas fluxes, several methods can be used, each with advantages and limitations.

Keywords : curve fitting, dead time, gas exchange, rate of change, regression, response lag

A New Method of Evaluating Gas Fluxes in a Closed Chamber System with Theoretical Consideration for Dynamic Characteristics of a Concentration Sensor

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fluxes (Wagner et al., 1997; Kutzbach et al., 2007; Langensiepen et al., 2012). To avoid this underestimation of fluxes, Wagner et al. (1997) proposed the quadratic regression function (QR), where a concentration change is expressed by the quadratic function and the flux can be estimated by the first derivative of the function at time zero (i.e., immediately after chamber closure).

Although LR and QR apparently fit well to observed concentration changes, these conventional regression functions lead to underestimations of fluxes owing to the dynamic characteristics of a concentration sensor (i.e., response lag and dead time). To reduce the error caused by the response lag and dead time of a concentration sensor, concentration changes measured immediately after chamber closing have frequently been omitted from the regression (Reicosky, 1990; Grau, 1995; Wagner et al., 1997; Steduto et al., 2002; Heijmans et al., 2004; Kutzbach et al., 2007; Langensiepen et al., 2012; Pérez-Priego et al., 2010; Koskinen et al., 2014; Pérez-Priego et al., 2015). However, this simple omission does not guarantee the accurate estimation of gas fluxes because the effects of the response lag and dead time of a concentration sensor persist during the entire period of the chamber closure.

To minimize the errors of flux estimation caused by the response lag and dead time of a concentration sensor, the responsiveness of concentration sensors has been required in flux measurements using a closed chamber system. Rapid-response concentration sensors, however, tend to be expensive, and this has limited the applicability of the closed chamber method; for example, achieving the high spatial resolution of flux estimations in a field study requires multiple closed chambers, but this can be very costly if expensive, rapid-response sensors are employed. The development of a method for gas flux estimation with low-cost yet slow-response concentration sensors, therefore, is desired.

The purpose of this study is to develop a new regression function that can consider the dynamic characteristics of a concentration sensor. The newly proposed function is derived theoretically by solving a differential equation of a first-order lag relation between the sensor output and actual gas concentration inside a closed chamber. The validity of the newly proposed function is examined based on the data of CO₂ concentration changes in a closed chamber induced by the photosynthesis of a leafy-vegetable canopy.

MATERIALS AND METHODS

First-order lag relation between actual gas concentration and sensor output

Typically, the closed-chamber method estimates gas flux by the product of the rate of change in the gas concentration and the volume of the chamber air, and the rate of change in the gas concentration is derived from the first derivative of a regression function (e.g., linear or quadratic function) fit to the output of a concentration sensor. However, this procedure can cause an underestimation of the gas flux because the change in sensor output, \( X_s \), lags behind the change in the actual gas concentration inside the chamber, \( X_a \), owing to the dynamic characteristics of the sensor. This lag between \( X_s \) and \( X_a \) can be approximated by the first-order lag relation as follows:

\[
\frac{dX(t)}{dt} = k \left( X_d(t-\Delta t) - X_s(t) \right)
\]

(1)

where \( t \) is the elapsed time after the chamber closing, \( \Delta t \) is the dead time of the system, \( X_s(t) \) is the sensor output at time \( t \), \( X_d(t-\Delta t) \) is the actual gas concentration inside the chamber at time \( t-\Delta t \), and \( k \) is the rate constant of the sensor response. This equation indicates that the rate of change in sensor output is proportional to the difference between the actual gas concentration at \( t-\Delta t \) and the sensor output at \( t \). Equation (1) can be solved if \( X_s \) is expressed by an appropriate function.

Conventional regression functions

Conventionally, regression functions have been applied directly to change in the sensor output, \( X_s \), by regarding \( X_s \) as the actual gas concentration, \( X_a \). Among several regression functions, the linear regression function (LR) has frequently been used to express the change in gas concentration in a closed chamber system (Reicosky et al., 1990).

\[
X_s(t) = at + b
\]

(2)

where \( a \) is the rate of change in the gas concentration during the chamber closure and \( b \) is the initial gas concentration at \( t = 0 \); both \( a \) and \( b \) can be obtained by the regression of Eq. (2) to sensor output. This function assumes that sensor output during chamber closure changes at a constant rate \( a \) with no response lag and dead time. If response lag and dead time exist, however, LR leads to underestimations of the gas fluxes.

Another regression function, the quadratic regression function (QR), has also been applied (Wagner et al., 1997).

\[
X_s(t) = a't^2 + b't + c'
\]

(3)

where \( a' \), \( b' \), and \( c' \) are fitting parameters. The use of QR is appropriate when the rate of change in gas concentration decreases during chamber closure due to diminishing concentration difference between the chamber air and intercellular space in leaves. QR can be fit well to such nonlinear changes in concentration, and taking the first derivative of QR at \( t = 0 \) allows for the estimation of the gas flux immediately after the chamber closing (i.e., the gas flux that is not affected by the chamber closing). QR, however, is susceptible to the dead time and response lag of a concentration sensor because the shape of the fit quadratic curve can be altered by such dead time and response lag. Therefore, QR can cause a serious underestimation of a gas flux, especially when a concentration sensor with slow response is used.

Using Eqs. (2) or (3), the gas flux \( Q \) is evaluated by

\[
Q = \frac{dX(0)}{dt} \frac{pV}{RT}
\]

(4)

where \( V \) is the volume of the chamber air (m³), \( p \) is the air pressure inside the chamber (Pa), \( R \) is the universal gas constant (8.31 m³ Pa mol⁻¹ K⁻¹), and \( T \) is the air temperature inside the chamber (K).

From Eq. (2), the rate of change in the gas concentration can be evaluated as
where the lag relation (Eq. (1)) and that relation between the gas fluxes. If response lag and dead time exist, however, the parameters \(a\) and \(b\)' are obtained from the rate of change in sensor outputs, \(\frac{dX(t)}{dt}\), rather than the rate of change in actual gas concentration, \(\frac{dX_a(t)}{dt}\) (i.e., the assumption of \(X_t = X_a\) no longer holds and Eqs. (2) and (3) are fit to \(X_t\) rather than \(X_a\)). In this case, the parameters \(a\) and \(b\) underestimate the rate of change in the actual gas concentration because \(X_t\) changes more slowly than \(X_a\) due to the response lag and dead time.

New regression function based on the first-order lag relation

The underestimation of the rate of change in the gas concentration caused by a sensor’s dynamic characteristics such as initial dead time and response lag can be corrected by distinguishing \(X_a(t)\) from \(X_t(t)\). Assuming that the relation between \(X_t(t)\) and \(X_a(t)\) is expressed by the first-order lag relation (Eq. (2)) and that \(X_a(t)\) changes linearly in the closed chamber (Eq. (2)), Eqs. (1) and (2) can be combined as follows:

\[
\frac{dX_a(t)}{dt} = k[a(t-\Delta t)+b] - X_a(t) \tag{7}
\]

This differential equation can be solved analytically using a Laplace transform and eventually becomes

\[
X_a(t) = at + c + d \exp(-kt) \tag{8}
\]

where

\[
c = \frac{a}{k} a \Delta t + b \tag{9}
\]

\[
d = -c + X_a(0) \tag{10}
\]

Equation (8) represents \(X_a(t)\) with the parameter \(a\), which is the rate of change in the actual gas concentration, \(\frac{dX_a(t)}{dt}\), as indicated in Eq. (5). The parameter \(a\), with the other parameters \(c\), \(d\), and \(k\), can be obtained by fitting Eq. (8) to the observed sensor output, \(X_a(t)\). The gas flux can be derived from Eq. (4) by substituting \(\frac{dX(t)}{dt} = a\). In Eq. (8), the exponential term, \(d \exp(-kt)\), expresses the response lag of a sensor, and as \(t\) increases, this term goes to zero.

In this study, Eq. (8) was newly applied as a regression function and hereafter referred to as \(LR_a\), where the subscript “d” in \(LR_a\) indicates a linear regression (LR) with the consideration of the dynamic characteristics of the sensing system.

Closed chamber system

To test the validity of the new regression function, \(LR_a\), CO2 concentration change, which was induced by photosynthesis or respiration of a crop canopy, was measured using a closed chamber system (Fig. 1). The chamber unit was made of 5 mm-thick acrylic plates with a basal area of 0.5 m x 0.6 m. The height was adjustable depending on crop heights to three different heights (0.24, 0.36, or 0.48 m) by inserting or removing adapter frames between the chamber top and base. The sidewalls of the chamber had two circular openings of 80 mm diameter, where ventilation fans (San Ace model 9GA0812P7G001, Sanyo Denki, Tokyo, Japan) were installed. For the evaluation of the gas fluxes, these openings could be temporarily closed or opened by an acrylic cover at arbitrary intervals. The fans were used for both ventilation and air mixing, and the flow rate through the openings was approximately 0.50 m³ min⁻¹, which corresponds to an air change rate of 8.3 times min⁻¹ when the chamber height is set to 0.24 m. At the base of the chamber, a nutrient solution was supplied at a depth of approximately 25 mm to grow a canopy of leafy vegetables. The canopy was planted on the hydroponic panel that separated the root zone from the chamber air space, and all the gaps between the sidewalls and the hydroponic panel were sealed with putty to prevent the evaporation of the nutrient solution from entering the chamber air space. The nutrient solution was circulated

Fig. 1 Schematic view of closed-type chamber system used for experiments. The chamber is ventilated by the fans attached on the chamber wall such that continuous cultivation of a plant canopy is possible. It is only closed during gas flux estimation.
The change in CO₂ concentration was measured by two concentration sensors (IRGAs) with different dynamic characteristics, i.e., GMT222 (Vaisala, Helsinki, Finland) and LI820 (LI-COR Biosciences, Nebraska, USA) (Table 1). The GMT222 is low cost; however, it is relatively slow in response (30 seconds to achieve 63% of the steady-state value). Therefore, this sensor has been widely used for studies where sudden change in CO₂ concentration is not expected (e.g., measurement of diurnal change in CO₂ concentration, Tang et al., 2005). The LI820 is relatively high cost; however, it offers a quick response. This sensor has been used in studies where dynamic change in CO₂ concentration is expected (e.g., Hoffmann et al., 2015, Speckman et al., 2015). In our chamber system, the sensing probe of the GMT222 was placed at approximately 50 mm above the canopy top, whereas the sensing probe of the LI820 was placed outside the chamber and the chamber air was introduced into the LI820 and circulated back to the chamber by a pump and tubing. The airflow rate to the LI820 was set high (1.0 L min⁻¹) such that the time required for the air to reach the LI820 was negligibly short (0.72 seconds). The outputs of the two CO₂ sensors, together with the sensors of photosynthetic photon flux density (PPFD) (LI190R, LI-COR Biosciences), temperature (T-type thermocouple), and humidity (HMP76, Vaisala, Helsinki, Finland), were recorded at 1-second intervals by a datalogger (ZR-RX40, OMRON, Kyoto, Japan). Hereafter, the two CO₂ sensors, GMT222 and LI820, are referred to as “slow IRGA” and “rapid IRGA”, respectively.

**Table 1. CO₂ sensors (IRGAs) used for experiments.**

| CO₂ Sensor | GMT222 | LI820 |
|------------|--------|-------|
| Manufacturer | Vaisala | LI-COR |
| Response | Slower | Faster |
| Response time (s) | 30 (63%) | Negligible* |
| Cost | Lower | Higher |
| Accuracy | 30 ppm±2% of reading | 3% of reading |
| Measurement range (ppm) | 0~2,000 | 0~20,000 |

*No specific value is offered in the manufacturer’s catalog.

The sensor output of the slow IRGA was used for the comparative analysis of the LR, QR, and LRᵣ (Eqs. (2), (3), and (8), respectively); the output of the rapid IRGA was regarded as actual concentration inside the chamber and used as the reference. The measurement of CO₂ fluxes from a canopy of spinach plants (*Spinacia oleracea*, c.v. “Summertop”) was conducted on three days (November 17, 29, and December 1 in 2017) using the closed chamber system. The leaf area index, which was estimated from the sampling of three plants, was 1.0 on November 17 and 2.5 on December 1. The plant canopy in the closed chamber with chamber air volume of 0.06 m³ was placed under light emitting diodes (LEDs) in an environmentally controlled room (Ta = 20°C, RH = 60%). The PPFD at the canopy top increased stepwise by approximately 150 μmol m⁻² s⁻¹ every hour from dark (0 μmol m⁻² s⁻¹) to saturating light (1,300 μmol m⁻² s⁻¹) condition. Before the measurement of the gas concentration change, the plants were exposed to each light intensity for thirty minutes. After thirty minutes, the chamber was closed for 1.5 minutes and the change in CO₂ concentration was measured. The chamber was then opened for ventilation for 4.5 minutes. This cycle of closing and opening was repeated five times under each light intensity.

**Software package**

To obtain the fitting parameters in the regression functions of LR, QR, and LRᵣ (i.e., Eqs. (2), (3), and (8)), nonlinear least-squares fitting was performed using the Lmfit package (version 0.9.7) in Python.

**RESULTS AND DISCUSSION**

Figure 2 (a) displays a typical change in CO₂ concentration measured by the slow and rapid IRGAs during chamber closure. The output of the slow IRGA changed nonlinearly, whereas the output of the rapid IRGA decreased virtually linearly. The nonlinear change of the slow IRGA’s outputs can be attributed to the dynamic characteristics of the sensor (i.e., response lag and dead time), which is formulated by the first-order lag relation (Eq. (1)).

Because of the nonlinear change of the outputs of the slow IRGA, fitting LR (Eq. (2)) to the output of the slow IRGA can cause an underestimation of the rate of change in CO₂ concentration. In Fig. 2 (a), LR was fit to the outputs of the slow IRGA between 60 and 90 seconds after the chamber closure. The initial 60 seconds of the sensor output, which indicated gradual change owing to the response lag and dead time of the sensor, were omitted from the fitting data, as has been common practice (Reicosky, 1990; Grau, 1995; Wagner et al.,1997; Steduto et al., 2002; Heijmans et al., 2004; Kutzbach et al., 2007; Langensiepen et al., 2012; Pérez-Priego et al., 2010; Koskinen et al., 2014; Pérez-Priego et al., 2015). The slope of the fit regression line, however, was less than the slope of the output of the rapid IRGA, suggesting an underestimation of the rate of change in CO₂ concentration and hence CO₂ flux.

QR (Eq. (3)) was fit well to the outputs of the slow IRGA (Fig. 2 (a)) between 60 and 90 seconds; however, the rate of change in the gas concentration estimated by QR was inaccurate because QR estimates the rate of change from the initial slope of the fit curve, which is very small in the case of the slow IRGA owing to the response
lag and dead time. In general, QR provides a more accurate estimation of the gas fluxes compared to LR when the rate of change in gas concentration declines owing to diminishing concentration difference between the gas inside the chamber air and that of the vegetation or soil (Wagner et al., 1997); however, this function is not applicable to the output of slow-response sensors that have large response lag and dead time because the change in CO₂ concentration is small. Figure 4 displays the relationship between fitting durations and the average Root Mean Square Errors (RMSE) of the fluxes estimated by LRd fit to the output of the slow IRGA, where fluxes estimated by QR fit to the slow IRGA output, where fluxes estimated by QR fit to the output of the rapid IRGA.

Whereas the conventional regression functions such as LR and QR could not provide appropriate estimations of the rate of change in CO₂ concentration from the output of the slow IRGA, the newly developed regression function, LRd (Eq. (8)), could estimate the rate of change that was compatible to the change in the output of the rapid IRGA. Figure 2 (b) indicates the regression curve of LRd fit to the output of the slow IRGA and the rate of change in CO₂ concentration estimated by LRd (i.e., straight line with the slope a). The slope a of the straight line is identical to the parameter a in LRd (Eq. (8)) and was obtained by fitting LRd to the output of the slow IRGA. The straight line obtained by LRd passed through multiple points of the rapid IRGA outputs. This acceptable agreement between the concentration change estimated by LRd and the change in the output of the rapid IRGA, together with the well-fit curve of LRd to the output of the slow IRGA, indicates the validity of the newly proposed LRd function.

Figure 2 (c) summarizes the rates of change in CO₂ concentration estimated by LR, QR, and LRd fit to the outputs of the rapid and slow IRGAs in Fig. 2 (a) and (b). When fit to the output of the rapid IRGA, all three functions offered similar values of the estimated rates of change. This suggests that all three functions can be applicable to flux estimations in the closed chamber method, if the response lag and dead time of the employed sensor are negligible. When fit to the slow IRGA, however, the conventional regression functions (LR and QR) provided inappropriate estimations of the rates of change in CO₂ concentration: LR and QR underestimated the rate of change by 23% and 65%, respectively. Conversely, LRd provided an estimation of the rate of change considerably closer to the estimations from the output of the rapid IRGA. This result highlights the advantage of the newly proposed function LRd in flux estimations with a slow-response sensor.

Figure 3 (a) displays typical changes in the sensor output of the slow and rapid IRGAs under consecutive open-close operations with six-minute intervals (close duration = 1.5 minutes and open duration = 4.5 minutes). Whereas the output of the rapid IRGA started to change immediately after the chamber closings and openings, the outputs of the slow IRGA had appreciable lags. Accordingly, the fluxes estimated by LR fit to the output of the slow IRGA were significantly less than the fluxes estimated from the outputs of the rapid IRGA (Fig. 3 (b)). Conversely, the fluxes estimated by LRd fit to the slow IRGA were much closer to the fluxes estimated from the rapid IRGA. Compared to the fluxes estimated from the rapid IRGA, the average underestimation of the fluxes by the proposed LRd fit to the slow IRGA was only by 9%, whereas that by the conventional LR was by 39%.

The fitting duration of LRd in the flux estimations can affect estimated fluxes because the relative influence of sensor noise on the shape of a fit curve is large if the fitting duration is short and change in the output of the concentration sensor is small. Figure 4 displays the relationship between fitting durations and the average Root Mean Square Errors (RMSE) of the fluxes estimated by LRd fit to the slow IRGA output, where fluxes estimated by QR fit to
the rapid IRGA were used as the reference values. Extending the fitting durations of LR from 60 to 90 seconds reduced the RMSE from 1.9 to 1.3 μmol s⁻¹, indicating the advantage of longer fitting durations.

In LR (Eq. 8), the rate of decrease in the nonlinear exponential term, \(d \exp(-kt)\), is governed by the rate constant of the sensor response, \(k\). If the value of \(k\) is small, the sensor output changes slowly because \(d \exp(-kt)\), which acts as a brake on the change in the sensor output, goes to zero slowly (i.e., in Eq. 8 the terms \(at \) and \(d \exp(-kt)\) have opposite signs such that they cancel out each other until \(d \exp(-kt)\) diminishes). In such a case, the relative contribution of sensor noise to the change in the sensor output becomes large because the change in the sensor output occurs mainly due to the sensor noise rather than the change in the gas concentration. Therefore, if the response lag of a concentration sensor is expected to be large (i.e., \(k\) is expected to be small), longer duration of chamber closure is required so that the influence of \(d \exp(-kt)\) and the relative contribution of the sensor noise become small. Overly long chamber closure, however, must be avoided because it can cause a large change in the chamber environment and inversely affect the gas flux. Reducing the volume of the chamber air might increase the accuracy of the flux estimations with a slow response sensor because the rates of change in the gas concentration and sensor output increase by reducing the volume of the chamber air.

**CONCLUSION**

A new regression function, LRd, that can consider the dynamic characteristics of a gas concentration sensor, was developed for the estimations of gas fluxes in a closed chamber method. LRd was theoretically derived by solving a differential equation of a first-order lag relation between the sensor output and actual gas concentration inside the chamber. The validity of the newly proposed function was examined by comparative analysis using the CO₂ concentration change measured by a slow and rapid-response sensor (slow and rapid IRGAs, respectively). Even from the slow IRGA output, LRd provided estimations of CO₂ fluxes that were compatible to the fluxes estimated by the rapid IRGA, while the conventional linear and quadratic regression functions (LR and QR, respectively) underestimated the gas fluxes considerably. The newly proposed function enables, for example, multipoint evaluations of gas fluxes using automated closed chambers, where several low-cost concentration sensors are required.

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