Long-term and Continuous Measurement of Canopy Photosynthesis and Growth of Spinach

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(Received August 4, 2019; Accepted September 25, 2019)

INTRODUCTION

Photosynthesis is one of the most important determinants in crop growth and yield because photosynthesis is virtually the only means for crops to obtain carbon substrates required for the growth and maintenance of their bodies (Amthor, 2000). A number of models for crop growth and yield, therefore, have been constructed based on carbon balance (i.e., net photosynthesis: gross photosynthesis minus respiration) (e.g., Spitters et al., 1989; Jones et al., 1991; Bouman et al., 1996; Marcelis et al., 2006; Thornley, 2011).

To construct models of crop growth and yield, photosynthesis at the canopy scale, rather than at the single-leaf scale, needs to be evaluated, because crops usually constitute a canopy in an agricultural field. Canopy photosynthesis differs from single-leaf photosynthesis: canopy photosynthesis depends not only on environmental elements (e.g., photosynthetically active radiation, CO₂ concentration, temperature, humidity, wind, etc.) but also on the structure of the canopy (e.g., leaf area index (LAI), which is the total area of one side of the leaf per unit ground area; Chen and Black, 1992) (Medlyn et al., 2003; Monsi and Saeki, 2005). Variation in LAI in a crop canopy is indeed a much more important determinant of variation in the growth rate of the canopy than is variation in the photosynthetic rate per unit leaf area (Gifford and Evans, 1981; Lawlor, 1995).

Canopy photosynthesis can be assessed by micro-meteorological methods (e.g., eddy covariance and aerodynamic methods) or chamber methods. Micro-meteorological methods have the advantage of not disturbing the microclimate around a crop canopy (Müller et al., 2009). However, these methods are not applicable to greenhouse studies, because prerequisites of the methods, such as enough fetch length and homogeneous vegetation, are not met in a typical greenhouse environment (Baldocchi, 2003; Jones, 2014). In contrast, the use of chamber methods, where crops are enclosed by a small transparent chamber to measure a change in CO₂ concentration in the chamber, is the only way to estimate canopy photosynthesis in a greenhouse environment. Chamber methods are classified into two types: open and closed chamber methods. In the closed chamber method, complete closure of a chamber is temporarily needed to estimate the photosynthetic rate of enclosed crops; photosynthetic rate is estimated by multiplying the rate of change in CO₂ concentration by the chamber volume. However, this temporary closure of the chamber disturbs the microclimate around the enclosed crops (e.g., increase in temperature by solar radiation, increase in humidity by transpiration, decrease in CO₂ con-
centration by photosynthesis; Garcia et al., 1990). In the open chamber method, on the contrary, continuous airflow is maintained from the inlet to the outlet of the chamber to estimate the photosynthetic rate of enclosed crops; photosynthetic rate is estimated by multiplying the airflow rate and difference of CO₂ concentrations in the inlet and outlet air. The environment around the enclosed crop is more stable in the open chamber than in the closed chamber; hence, the open chamber method is more suitable for continuous, long-term measurement of photosynthetic rate (Garcia et al., 1990). However, there have been only a few studies on the long-term assessment of photosynthesis of a crop canopy using an open chamber system (Müller et al., 2005; Burkart et al., 2007).

LAI is one of the most important properties of a crop canopy because many of the interactions (e.g., gas exchange) between a crop canopy and the surrounding environment occur on the surface of leaves. Also, as a measure of canopy size, LAI is advantageous over other measures, such as dry weight and fresh weight, because LAI can be estimated by non-destructive, non-contact methods, whereas other measures require destruction of crops (Welles and Cohen, 1996; Jonckheere et al., 2004).

Most of the non-destructive, non-contact methods of LAI estimation are based on theories of light extinction through a canopy, where the probability of sunflecks (direct radiation) at the bottom of the canopy is related to LAI by probabilistic models (Nelson, 1971). Based on the light extinction theories, several devices (e.g., LAI-2200, LI-COR Biosciences, Nebraska, USA) have also been developed to estimate LAI. Digital photography using a time-lapse digital camera is advantageous over other methods in that it is less expensive, easy to install, and automatically operative. These advantages allow, for example, multi-point observation of crop growth in an agricultural field such as a large-scale greenhouse. Utilization of digital photography in agricultural and ecological studies has also been a target of active research with advancement of new image processing techniques (e.g., the random forest classifier, convolutional neural network) (Sakamoto et al., 2011; Ryu et al., 2012; Sakamoto et al., 2012; Liu et al., 2013; An et al., 2017; Knox et al., 2017; Perez-Sanz et al., 2017).

Since a crop canopy undergoes gradual but significant changes in LAI throughout its growth period, long-term measurement of canopy photosynthesis and LAI is necessary to understand their association. Also, such long-term and simultaneous measurement of canopy photosynthetic rate and LAI is indispensable for the construction and validation of models of the photosynthesis and growth of a crop canopy for a long-term period. Thus far, however, few studies have reported such long-term measurement of the photosynthesis and LAI of a crop canopy. In this study, a method was proposed, which enables long-term, continuous, and simultaneous measurement of net photosynthetic rates and LAI of a leafy crop canopy by combining the open chamber method and top-of-view photography. The proposed method was applied to long-term measurement of a spinach canopy, and the relationship between the canopy photosynthesis and LAI was assessed.

MATERIALS AND METHODS

Estimation of canopy photosynthesis

An open-type chamber system for measuring the photosynthetic rate of a canopy of leafy vegetables was developed (Fig. 1). The chamber was constructed using a 0.25 mm-thick transparent polyvinyl chloride film supported by a steel frame, which was 0.8 m long, 0.5 m wide, and 0.6 m tall. Airflow from the chamber inlet to the outlet was induced by a blower (San Ace 80 9HVA0812P1G001, SANYO DENKI, Tokyo, Japan), which was attached to the chamber inlet, where the airflow rates were continuously measured by an ultrasonic flow meter (TRX32D-C/5P, Aichi Tokei Denki, Aichi, Japan). Air at the inlet and the outlet of the chamber was automatically and alternatively sampled and introduced to an infrared gas analyzer (LI850, LI-COR Biosciences, Nebraska, USA) to measure the CO₂ and H₂O concentrations. These automatic, and alternative air samplings between the chamber inlet and outlet were switched at one-minute intervals by the

Fig. 1 Schematic view of the open-type chamber system for measuring the net photosynthetic rate and growth of the leafy-vegetable canopy. The canopy net photosynthetic rate was estimated by multiplying the airflow rate with difference in CO₂ concentrations between inlet and outlet air (eq. (1)). The inlet and outlet air was introduced alternatively to the infrared gas analyzer by the built-in pump, and the air sampling paths for the inlet and outlet air were automatically switched by the on/off actions of the two solenoid valves. The growth of the leafy-vegetable canopy was evaluated as increase in the leaf area index estimated by the image analysis of photographs taken using a time-lapse digital camera positioned above the canopy. Nutrient solution was intermittently aerated to the root zone using an aeration pump controlled by an on/off controller.
on/off actions of two solenoid valves attached to the respective sampling paths using a programmable controller/ datalogger (CR1000, Campbell Scientific, Utah, USA). Within the one-minute interval for each air sampling, CO$_2$ gas concentrations were measured every second, and the CO$_2$ gas concentrations of the last 20 s were averaged. The averaged values of the CO$_2$ gas concentrations in inlet and outlet air ($C_{a, in}$ and $C_{a, out}$ ($\mu$mol mol$^{-1}$, respectively) were used for estimating canopy net photosynthetic rates, $A_\ell$ ($\mu$mol m$^{-2}$ s$^{-1}$), as follows:

$$ A_\ell = \frac{Q(C_{a, in} - C_{a, out})}{S} $$

where $Q$ is the flow rate of the air introduced to the chamber (mol s$^{-1}$) and $S$ is the ground surface area below the canopy (m$^2$). $Q$ was adjusted between 8 m$^3$ h$^{-1}$ and 12 m$^3$ h$^{-1}$ to induce significant difference of CO$_2$ concentrations between the chamber inlet and outlet even under low photosynthetic rate. To mitigate inconvenient large fluctuations of CO$_2$ concentrations of entering air ($C_{a, in}$), a buffer tank 200 L in volume was installed at the inlet of the chamber. As with $A_\ell$, canopy transpiration rates can also be estimated by multiplying $Q$ with concentration differences of H$_2$O in inlet and outlet air. Air temperature at the inlet and outlet of the chamber, leaf temperature, and photosynthetic photon flux density (PPFD) were measured by two T-type thermocouples, an infrared thermometer (CS-LT; Optris, Berlin, Germany), and a quantum sensor (PARtosynthetic photon flux density (PPFD) were measured by two T-type thermocouples, an infrared thermometer (CS-LT; Optris, Berlin, Germany), and a quantum sensor (PARtosynthetic photon flux density (PPFD) were measured by two T-type thermocouples, an infrared thermometer (CS-LT; Optris, Berlin, Germany), and a quantum sensor (PAR

Estimation of canopy growth

Canopy growth was evaluated as increase in LAI estimated from top-of-view digital photographs of a canopy (Fig. 2) (Liu and Pattey, 2010; Liu et al., 2013). In this method, the proportion of a non-leaf area in a photograph (gap fraction, $P_G$) was probabilistically related to LAI.

The theory of the LAI estimation from a gap fraction in a canopy photograph is analogous to the transmission of direct sunlight in a canopy (Fig. 3 (a)) (Nilson, 1971; Lang and Xiang, 1986).

In this theory, a canopy is divided into a large number (N) of horizontal layers, each having a partial leaf area index ($\Delta L_i$ for $i = 1, 2, \cdots, N$). The thickness of each layer can be chosen such that each $\Delta L_i$ has the same value (i.e., $\Delta L_i = \Delta L$, for $i = 1, 2, \cdots, N$). Then, the partial LAI projected onto a horizontal plane ($\Delta L_{\text{hor}}$) is as shown below:

$$ \Delta L_{\text{hor}} = \frac{G(\theta)}{\cos \theta} \Delta L $$

(2)

where $\theta$ is the view zenith angle and $G(\theta)$ is a function representing the mean projection of a unit leaf area in the direction $\theta$. In other words, the fraction of the horizontal plane shaded by the projection of $\Delta L$ is $\Delta L_{\text{hor}}$, while the fraction of the unshaded part (i.e., gap) is $1 - \Delta L_{\text{hor}}$. As many layers of $\Delta L$s accumulate downward, $\Delta L_{\text{hor}}$’s shade and decrease gaps on the horizontal plane with some overlap (Fig. 3 (b)). Then, after the accumulation of $N$ layers, the probability ($P_0$) that an arbitrary point in the horizontal plane remains unshaded can be given as shown below:

$$ P_0 = (1 - \Delta L_{\text{hor}})^N = \left(1 - \frac{G(\theta)}{\cos \theta} \Delta L\right)^N $$

(3)

assuming random spatial distribution of leaves. By substituting $\Delta L = \text{LAI} / N$ and taking the limit of $N$ to infinity, eq. (3) becomes:

$$ P_0 = \exp \left( -\frac{G(\theta) \text{LAI}}{\cos \theta} \right) $$

(4)

Equation (4) is the Poisson distribution expressing the probability of unshade (i.e., sunflecks) over an arbitrary point on the horizontal plane. In other words, $P_0$ is the expected value of the fraction of the projected non-leaf area (i.e., gap fraction) on the horizontal plane. From eq. (4), values of LAI can be estimated if the values of $G(\theta)/\cos \theta$ and $P_0$ are provided.

In this study, $\cos \theta$ was unity because photography was taken from right above the canopy (i.e., $\theta = 0$). Furthermore, $G(\theta)$ in eq. (4), representing the mean projection of a unit leaf area in the direction $\theta$, was set to be 0.5, assuming that leaf angle distribution follows the spherical angle distribution (Ross, 1981). The assumption of the spherical leaf angle distribution would be justified as the
first approximation for most crop canopies (Goudriaan, 1988; Liu et al., 2013).

The other value required to quantify LAI is the gap fraction \( P_0 \). \( P_0 \) can be derived from the projected leaf area \( a_L \) and non-leaf area \( a_{NL} \) in the photograph (Fig. 2), as given below:

\[ P_0 = \frac{a_{NL}}{a_L + a_{NL}} \quad (5) \]

To quantify \( a_L \) and \( a_{NL} \), a photograph needs to be segmented into the leaf and non-leaf areas. In this study, this segmentation was done using an open-source software called ilastik (version 1.3.2) (Sommer et al., 2011). The software ilastik can divide an image into several segments called ilastik (version 1.3.2) (Sommer et al., 2011). The validity of eq. (6) for estimating LAI of a spinach canopy was confirmed by comparing LAIs estimated from top-of-view photographs (LAI_{top}), and LAIs measured using the destructive scanning method (LAI_{scan}) in the flatbed scanner (400-SCN025, Sanwa Supply, Okayama, Japan). For this validation, several spinach canopies consisting of 30 to 54 plants with the same planting density (200 plants m\(^{-2}\)) and planting pattern (staggered pattern) were grown hydroponically, and at different growth stages, these canopies were photographed and then harvested to scan all leaves in the canopy.

Long-term and continuous assessment of canopy photosynthetic rate and leaf area index

Long-term and continuous assessment of canopy photosynthetic rate and leaf area index of a spinach canopy was performed using the open type chamber and the top-of-view time-lapse digital camera (Fig. 1). The assessment was performed in an environmentally controlled room (air temperature \( 15 \degree C \) and relative humidity \( 70\% \)) in a phytootron located at the Ito campus of Kyushu University, Japan, for about 50 d, from Novem-
A canopy of spinach plants (Spinacia oleracea L. ‘Wase Krone’) was cultivated hydroponically in the open type chamber system. A total of 54 plants were planted on a 600 mm × 440 mm hydroponic panel (i.e., 200 plants m⁻²) with staggered 18 holes (i.e., three plants per hole), below which nutrient solution was filled at a depth of 50 mm in a plastic tray that was 75 mm deep, 600 mm long, and 370 mm wide. Gaps between the hydroponic panel and the tray were sealed with putty so that no gas exchange between the above-ground and root zone spaces occurred. The nutrient solution was intermittently aerated by an aeration pump (Na-3000, Marukan, Osaka, Japan) with an air flow rate of 2.0 L min⁻¹. The nutrient solution, Otsuka-A formula solution (OAT Agrio Co., Ltd., Tokyo, Japan), with an electric conductivity (EC) of 2.5 dS m⁻¹ was supplied and replaced once a week to maintain the composition of the solution.

To estimate LAI, Raspberry Pi 3 Model B+ with Camera Module v2 was positioned 500 mm above the canopy and top-of-view photographs of the canopy were taken every 10 min automatically. The photographs taken at 8:00 were used for the LAI estimation because of the easiness of segmentation (i.e., the illuminations were almost unity (0.98), indicating the validity of the assumption of the spherical leaf angle distribution for a spinach canopy). The value of LAI was greater with larger LAI kept increasing as PPFD increased, while LAI at smaller LAI seemed to be light-saturated in small PPFD. These results were attributed to the mutual shading of leaves in canopies; when LAI is large, many leaves receive more light and use it for photosynthesis.

Figure 4 shows diurnal changes in PPFD at the top of the canopy, Cᵦᵢ and Cᵦᵢ, and Aᵦ on a typical sunny day in winter. PPFD showed a typical diurnal pattern in the location, reaching the daily maximum of about 500 μmol m⁻² s⁻¹ around midday with some fluctuations. In response to the increase in PPFD, both Cᵦᵢ and Cᵦᵢ decreased, but Cᵦᵢ was lower than Cᵦᵢ due to absorption of CO₂ by the spinach canopy inside the chamber. Accordingly, Aᵦ calculated by eq. (1) showed a diurnal change, the pattern of which was similar to that of PPFD.

Figure 5 shows the relationship between LAI estimated from canopy photographs (LAIₜₚₒₜₜ) and LAI measured using the destructive scanning method (LAIₜₚₒ). The 95% prediction interval is indicated by the gray hatch.
leaves are shaded by upper leaves and remain light-unsaturated even under strong light, and such shaded, light-unsaturated leaves still have the capacity to increase the photosynthetic rate if they can receive more light. In such a case, as PPFD at the top of the canopy increases, more light can penetrate down toward the bottom of the canopy, and eventually these shaded, light-unsaturated leaves can receive stronger light and increase the photosynthetic rate, contributing to increase in the value of $A_c$. This contribution of shaded, light-unsaturated leaves to the increase of $A_c$ is relatively small in a canopy of small LAI; thus, light-saturation occurs at relatively small PPFD.

Figure 7 shows the changes in PPFD, $A_c$, cumulative canopy net photosynthesis ($\Sigma A_c$), and LAI$_{photo}$ of the spinach canopy through the entire growth period (i.e., from transplantation to harvest). PPFD showed diurnal and daily changes depending on weather conditions. $A_c$ also showed a pattern of change similar to that of PPFD, but the change of $A_c$ was gradually amplified as time passed. This amplification in $A_c$ was caused by the increase in LAI, as LAI increased, the canopy absorbed more light and performed more photosynthesis. Consequently, $\Sigma A_c$ increased exponentially, which was similar to the exponential increase in LAI. This exponential increase in $\Sigma A_c$ and LAI indicates that there is a positive feedback loop between photosynthesis and growth; the growth of leaves amplifies photosynthesis, and the amplified photosynthesis further accelerates the growth of leaves. As a result, a crop canopy grows exponentially during its vegetative growth stage (Thornley and Johnson, 1990).

As LAI and $A_c$ increases with time, canopy respiration also increases. Figure 8 shows daily cumulative photosynthetic photon flux density (PPFD$_{daily}$), daily daytime canopy net photosynthetic rate ($A_c$$_{day}$), daily nighttime respiration ($R_c$$_{night}$), and the ratio of $R_c$$_{night}$ to $A_c$$_{day}$. $A_c$$_{day}$ and $R_c$$_{night}$ were calculated by integrating $A_c$ from sunrise to sunset, and vice versa. The value of $R_c$$_{night}$ gradually increased from nearly zero at the transplantation to about 50 mmol m$^{-2}$ d$^{-1}$ near the harvesting. This increasing trend in $R_c$$_{night}$ was caused by the increase in LAI and other resiping organs (i.e., petiole). In addition, daily change in $R_c$$_{night}$ showed similar pattern to the change in $A_c$$_{day}$. This indicates that $R_c$$_{night}$ is dependent on the amount of available carbon substrates, which is produced by photosynthe-
sis during the daytime (i.e., at night, crops consume the carbon substrates synthesized during the daytime) (Gifford, 1995). As a result, the ratio $R_{c, \text{night}} / A_{c, \text{day}}$ was maintained relatively constant with an average value of 14% during the experimental period. This relative stability of $R_{c, \text{night}} / A_{c, \text{day}}$ is convenient in modeling carbon balance in a crop canopy because it allows the estimation of nighttime canopy respiration from daytime net canopy photosynthesis (van Oijen et al., 2010).

As expected from Fig. 7(c) and (d), $A_{c}$ and LAI were linearly correlated (Fig. 9). The relationship between $A_{c}$ and LAI can be empirically written as follows:

$$\text{LAI} = a \Sigma A_{t} + \text{LAI}_{0}$$  \hspace{1cm} (7)

where slope $a$ expresses the increase in leaf area per canopy net photosynthesis ($m^{2}_{\text{leaf mol}^{-1}}$), and LAI$_0$ is the initial LAI at the transplantation. The linearity between $\Sigma A_{t}$ and LAI shown in Fig. 9 indicates that the coefficient $a$ can be regarded as a constant, and this implies that the following two conditions were satisfied throughout the experimental period: first, the proportion of photosynthate (or dry matter) partitioned to leaves was constant; and second, the leaf area was expanded in proportion to the partitioned dry matter (i.e., leaf area per dry matter (specific leaf area; SLA) was constant). The first condition, the constancy of the proportion of dry matter partitioned to leaves, indicates that leaves gain dry weight in proportion to $\Sigma A_{t}$. This is consistent with observations in previous studies, which reported that in the early stage of vegetative growth, shoot dry mass increases proportionally with increase in root dry mass (Brouwer, 1962; Acock et al., 1979; Thornley and Johnson, 1990).

With regard to the second condition of the constant SLA, there have been few previous studies that examined the time-course change in SLA within a canopy, but the experimental result in Fig. 9 suggests that SLA within a crop canopy would be relatively constant under a stable condition. Presumably, in the modern horticultural facilities where environment, irrigation, and fertilization are well controlled, these two conditions of constant dry matter partition and constant SLA may be satisfied during the
stage of vegetative crop growth, and the slope $a$ can be treated as a constant. In such a case, differentiating eq. (7) by time $t$ gives the below equation:

$$\frac{dLAI}{dt} = a \cdot d_L$$

(8)

Equation 8 suggests that the growth of a leafy-vegetable canopy can be predicted if $d_L$ is provided. In general, the direct measurement of $d_L$ in an agricultural field is difficult; hence, $d_L$ must be estimated by some other means, that is, applying a model of canopy photosynthesis (e.g., de Pury and Farquhar, 1997). A canopy photosynthesis model, like any other mathematical models, needs model validation, which requires the simultaneous measurement of dependent and the explanatory variables of the model (i.e., $d_L$, LAI and environmental elements). This measurement is possible using an open chamber and a time-lapse camera, as this study reported. Once the canopy photosynthesis model is validated, it would be possible to predict the growth of leafy vegetables only by the measurement of environmental variables and LAI, which can be done in an actual agricultural field.

ACKNOWLEDGMENTS

This study was conducted as a part of a joint-research project between Fujitsu Limited and Kyushu University and was financially supported by the Grants-in-Aid for Scientific Research (JP17H03895) from the Japan Society for the Promotion of Science and a research project “Advanced Next-Generation Greenhouse Horticulture by IoP (Internet of Plants)”.

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