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Editorial

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Improving the Quality and Reliability of Gas Exchange Measurements

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Abstract

With the advancement of portable gas exchange systems, measuring foliage gas exchange (photosynthesis, respiration and transpiration) has become common in a wide range of disciplinary areas. Modern instruments are easy to operate and generally require little or no user calibration. In fact some equipment can generate photosynthetic response curves to light and carbon dioxide automatically. The easiness of utilization and the enhanced functions of gas exchange instruments have greatly increased user confidence and the application of gas exchange measurements in both field studies and research in enclosed environments. However, measurement and calculation errors can still occur. For instance, one manuscript submitted to a refereed journal reported net rates of photosynthesis with intercellular concentrations greater than the ambient CO_2 concentration at which the measurements were taken. Erroneous measurements have also been noticed in my own lab, particularly by inexperienced students. Errors can occur for various reasons, such as faulty equipment, improper operations, leaking leaf chambers, leaking or blocked gas lines, or inadequate environmental conditions. Errors can also occur if the sequence or timing of measurements is inappropriate for different treatments within the same experiment. Furthermore, results from different studies even on the same species are not always comparable because differences in measurement protocols or in bases on which results are expressed (e.g., projected, hemi-surface and total surface leaf area). In this paper, I will discuss some of the aspects of foliage gas exchange measurement that are either confusing or need to pay close attention to.

Keywords

Measurement of photosynthesis; Transpiration measurement; Stomatal conductance

The Instrument

There are three common types of portable gas exchange systems: closed system, semi-closed system and open system [1,2]. Some systems can be used as an open system, semi-closed or closed system by re-configuring the gas circuit. In a closed system, there is no air flow into or out of the leaf chamber once the leaf chamber is closed. In such a system, gas exchange rates (photosynthesis and transpiration) of the enclosed foliage are calculated based on the rate of change in CO_2 and H_2O concentration over time. A closed system cannot produce a real steady state measurement because the concentrations of both water and CO_2 change continuously in one direction [2]. Additionally temperature control in a closed system is more difficult because heat will accumulate. A semi-closed is basically a closed system with CO_2 injection to compensate for the CO_2 drawdown by photosynthesis [1,2]. In an open gas exchange system, the air in the leaf chamber is replaced continuously and the rate of replacement is determined by the user-set air flow rate. In such as system, the rates of photosynthesis (or respiration) and transpiration are calculated based on the air flow rate and concentrations differences between input and output air. All modern open gas exchange systems have the capacity to control CO_2 and humidity in the leaf chamber (as detailed in the following two sections), but not all systems can regulate temperature and light levels. It should be noted that the boundary layer resistance in leaf chambers generally is much lower than the values in a natural environment due to the effect of the high speed mixing fan in the leaf chamber.

CO_2 Control

There are two measurements in an open gas exchange system: the analysis CO_2 and reference CO_2 concentration. The reference CO_2 concentration is the CO_2 concentration in the input air into the leaf chamber while the analysis CO_2 is the CO_2 concentration in the air coming out of the leaf chamber. Nearly all modern gas exchange systems control CO_2 concentrations in the leaf chamber by stripping out all of the CO_2 from the intake air and then add pure CO_2 from a pressurized canister at a modulated rate as determined by the rate of air flow and the user-set target CO_2 concentration. It is important to understand that the reference CO_2 concentration is not what the foliage is exposed to. As the input air enters the leaf chamber, it is immediately mixed with the air in the leaf chamber by a high speed mixing fan. Therefore, the CO_2 concentration at the leaf surface is approximately the same as the analysis CO_2. Thus the analysis CO_2 should be used as the Ca for calculating the internal to ambient CO_2 ratio (Ci/Ca).Furthermore, since the rate of photosynthesis tends to differ between samples, particularly between different treatments, and for conifers the amount of foliage enclosed in the leaf chamber also varies between samples, the analysis CO_2 can be different between different samples measured at the same reference CO_2 concentration. The difference (drawdown) between the reference CO_2 and analysisCO_2 concentration is also influenced by the rate of air flow through the leaf chamber: the greater the flow rate is, the smaller the difference will be. Because the measured rate of photosynthesis is a function of the CO_2 concentration at which the measurement is taken [3], different samples and different treatments should be measured at the same or at least similar analysis CO_2 concentrations.

The analysis CO_2 can be controlled directly or indirectly. The direct control is achieved by setting the equipment to control the analysis CO_2 concentration to a target value. The direct control gives consistent analysis CO_2 concentration among measurements but is more time consuming because for each measurement the equipment will have to adjust the rate of CO_2 addition continuously until the analysis reaches the target. This method has two merits: 1) all the measurements are taken at the same CO_2 at the leaf surface and 2) the target CO_2 only needs be set only once for all the measurements. In the indirect method, the equipment is set to control the reference

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CO_2 and the target analysis CO_2 concentration is approximated by increasing or decreasing the reference CO_2 setting based on the actual reading of the analysis CO_2. It should be noted that the magnitude of increase in the analysis CO_2 is generally smaller than the increase in reference CO_2 because the increased photosynthetic rate by increasing CO_2 supply leads to a greater differential between the two CO_2 measurements. This method is more suitable for experienced researchers who are better able to estimate the required adjustment in reference CO_2 to achieve a target analysis CO_2. This method is faster than the direct control method because the instrument determines the CO_2 injection rate mathematically and thus the continuous adjustment of CO_2 addition rate by the equipment is eliminated. The trade off is that it is difficult to achieve exactly the same analysis CO_2 for all measurements. For the measurement of photosynthetic light response curves, the target reference CO_2 concentration must be reset at each light level because the rate of photosynthesis and thus the CO_2 drawdown change with changes in the flux density of the light (i.e., the photosynthetically active radiation). Most of the following discussions focus on the proper control of the environmental conditions in the leaf chamber of an open gas exchange system.

Humidity Control

Humidity influences stomatal conductance, transpiration and photosynthesis. Therefore, it is important to measure different samples under similar humidity. For the purpose of control in gas exchange measurement, humidity can be measured using water vapor (partial) pressure, vapor pressure deficit (i.e., the difference between actual vapor pressure and saturation vapor pressure) and relative humidity (RH: the ratio of actual water vapor pressure (or content) to saturation vapor pressure (or content), expressed as %). Humidity can be controlled directly or indirectly depending on the capacity of the specific equipment, similar to CO_2 control as discussed previously. A common method is to control the RH in the reference air to a fixed value, e.g., 50%. However, the 50% here generally means that 50% of the intake air will enter the leaf chamber directly while the other 50% flows through the desiccant column(s) first, rather than 50% relative humidity in the reference air. Similarly100% RH simply means that all the intake air will enter the leaf chamber directly without going through the desiccant. Modern gas exchange systems (such as those from ADC and PP-Systems) are capable of controlling the reference vapor pressure or reference vapor pressure deficit by regulating the proportion of the intake air that will flow through the desiccant column(s) which removes all the water vapor. Most gas exchange systems can only reduce the humidity in the intake air with the exception of some models from ADC BioScientific Ltd. (U.K.) that can chemically increase the humidity in the reference air.

The reference air is enriched with the water vapor from transpiration immediately after it enters the leaf chamber. For the season described in the section of CO_2 control, the humidity (vapor pressure, VPD or RH) that the foliage is exposed to is the humidity of the analysis air (i.e., the air flowing out of the leaf chamber). Similar to photosynthetic rates, transpiration rates vary between samples and between treatments. The magnitude of humidity increase by transpiration is also influenced by the amount of foliage enclosed in the leaf chamber. For conifers, it is particularly difficult to control the amount of foliage in the chamber to similar levels between samples. Thus the actual humidity experienced by the foliage being measured can be different between samples even if the humidity in the reference air is controlled to the same level. I recommend that the humidity of the analysis air be controlled to similar levels between samples. If increasing humidity is desirable but the equipment does not have the capacity, researchers can construct a simple apparatus to humidify the intake air. Furthermore, the humidity in the analysis air can also be regulated by adjusting the air flow rate. A higher flow rate will lead to a lower humidity because the more humid air in the leaf chamber will be replaced faster by the drier intake air. However, changing the flow rate will also impact the analysis CO_2 concentration because the CO_2 differential between reference and analysis air decreases with increasing flow rates. For the effectiveness of CO_2 and humidity controls and the accuracy of measurements, it is extremely important to replace stale chemicals (desiccants, soda lime, and molecular sieve) promptly. Drierite, silica gel and soda with indicators are readily available in the market, but molecular sieves with indicators are less common. However, not all the systems use molecular sieves but PP-Systems equipment does.

Leaf Area

Gas exchange parameters (e.g., photosynthesis, transpiration and stomatal conductance) are commonly expressed on a leaf area basis. There are three different measurements of leaf area: total surface area, hemi-surface area (50% of the total surface area) and projected area. The selection of a leaf area measurement affects the interpretation of the measurement and the comparability among different studies and different species. For flat leaves, the total surface area includes the area of both sides. For the same measurement, semi-surface area based rates will be twice as high as those that are expressed on a total surface area basis. When selecting the leaf area measurement to use, one should consider the distribution of stomata and the light source for the measurement. For flat leaves with stomata distributed only on one side of the leaf, projected or semi-surface leaf area should be used. On the other hand, if stomata are distributed on both sides of the leaf and the light levels are similar on the two sides, total surface area can be used. If light is supplied from a light unit mounted on the leaf chamber, projected leaf area is recommended because the light normally comes from one direction and the quantum flux density is measured on a projected area basis (i.e., per unit of area perpendicular to the direction of the light). The use of projected leaf area is particularly important in the measurement of photosynthetic light response curves, especially for coniferous species.

Chamber Leakage

Gaskets are used to seal the joints in leaf chambers in all gas exchange systems. Gaskets wear off over time. Once the gasket loses the resilience, the leaf chamber will start to leak air. For cylindrical conifer chambers, the gaskets wear out first at the location where the stem of the branch being measured sits. Minor leakage does not affect the gas exchange measurement as long as a positive air pressure is maintained inside the leaf chamber as indicated by a small amount of air leaking out of the chamber. However, the gaskets should be replaced immediately if a more serious leakage is detected. Efforts should be made to make sure that the gas lines do not leak. All the tubing and fittings should be checked for cracks and loose connections before the start of each measurement session. Problems should be resolved before starting to take measurements.

Diurnal Variations and Its Effects on Your Measurements

The values of gas exchange parameters generally vary through the course of the day both in the field [4] and under a controlled

environment [5]. The pattern of the diurnal variation is affected by the specific weather conditions [4]. Diurnal variations can result in serious errors in the data if not treated properly. I strongly recommend that researchers do a complete diurnal measurement before starting data collection. If a time period can be identified when gas exchange parameters are relatively stable and that time period is sufficiently long, all subsequent measurements should be taken during that period of time. Two or more time periods in the day can be used if the diurnal measurements show that the measurements are comparable. If this approach is not feasible, the sequence of measurements for different treatments should be so arranged that the errors due to diurnal variations will be more or less evenly distributed across different treatments. Using time of the day as a covariate in data analysis may offer a viable alternative. Field researchers should particularly pay attention to diurnal fluctuations, especially when sites are far apart from one another. While it is convenient to complete one site before moving to another and take measurements all day long, you could be sampling different parts of a diurnal curve at different sites, resulting in systemic errors in your data.

Photosynthetic Response Curves

Measuring photosynthetic response curves is a time consuming process because the physiological mechanisms have to adjust to the new conditions at each step before reaching a new steady state. However, the time it takes to adjust to the new conditions depends on the direction of the change, for instance, the stomatal conductance responds faster to a stimulus that decreases it than a change that will increase it [3]. For generating a photosynthetic light response curve, it generally takes less time if the measurements start from a high light level and work downward, but the reverse is true from measuring a photosynthetic response curve to CO_2.

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