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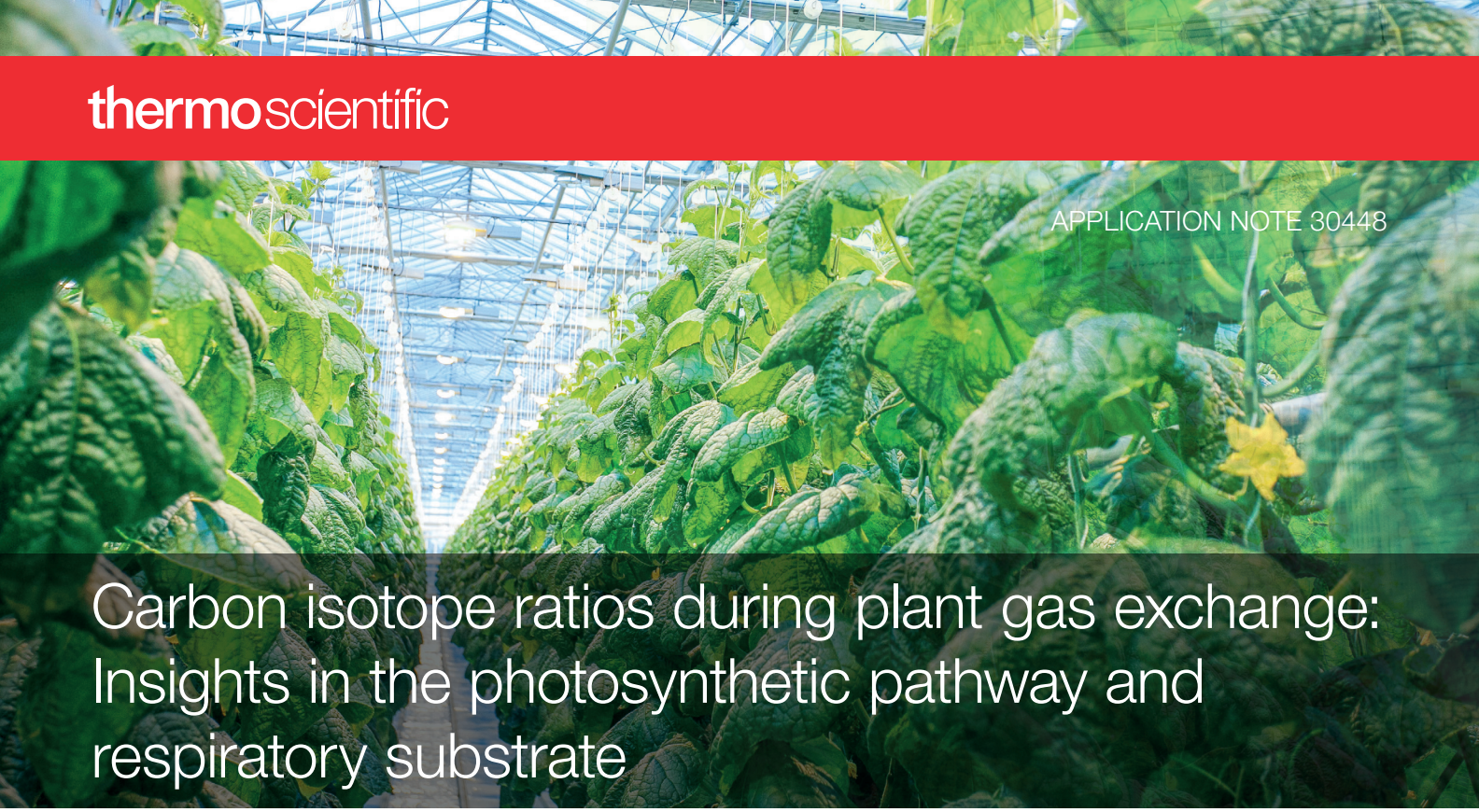

APPLICATION NOTE 30448

Carbon isotope ratios during plant gas exchange: Insights in the photosynthetic pathway and respiratory substrate

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Keywords

Carbon, Gas Exchange, Infrared Spectrometry, Photosynthetic Pathway, Plants, Respiratory Substrate

Goal

Identify short-term and long-term changes in plant metabolism with Isotope Ratio Infrared Spectrometry.

Introduction

The carbon cycle of plants is highly sensitive to changing environmental conditions. Responses of the two main fluxes, photosynthesis (CO_2 uptake) and respiration (CO_2 loss) including its isotopic signal can be determined in high resolution by integrating the Thermo Scientific™ Delta Ray™ IRIS in a plant chamber gas exchange setup. This allows to assess plant stress responses including mesophyll conductance (i.e, the conductance for CO_2 diffusion from intercellular airspaces to the chloroplast) an important process during photosynthesis (Warren 2006) that is challenging to measure.

Analytical instrumentation to measure CO_2 isotopes

In the mid-infrared range absorption lines are about 8000 times stronger than in the near-infrared. This enables a simple direct absorption approach requiring laser path length of 5m. The raw spectrum is scanned 500 times a second, performing simultaneous measurement of all three isotopolgues.

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The gas is continuously flowing through the analyzer and has a gas exchange time of about 30 seconds. The Universal Reference Interface (URI) is an integral part of the Delta Ray IRIS (Figure 1), which includes an internal calibration. In addition the URI can serve as a sample dilution device to match sample gas and reference gas concentrations for ultimate precision measurements using a sample/standard. A micro machined mixing and switching core (MITCH) together with a flow controller dilutes pure CO_2 reference gases to match the sample concentration to minimize the effect of instrument non-linearity.

Figure 1. The Thermo Scientific Delta Ray IRIS.

The sample gas is dried in the URI to prevent matrix effects and interaction of water and CO_2 (oxygen isotope exchange) with the added benefit of providing dry mole fraction concentration data (Figure 2).

Method and experimental set-up

The measurement setup is shown in Figure 2. Transparent plant chambers (Figure 3) were supplied with standardized measurement air of known CO_2 and H_2O concentrations at a constant flow rate (chamber in). The air leaving the chambers (chamber out) was heated to avoid condensation. Two infrared gas analyzers (one in absolute mode, the other in differential mode) measured the in- and outgoing air continuously, which allowed for accurate calculations of CO_2 and H_2O plant gas exchange. The Delta Ray IRIS is integrated into the measurement set-up using a 3/2 valve that switches between the air entering and leaving the chambers controlled via the Delta Ray IRIS Multiport. Because several plant chambers were measured in a sequence, trigger signals (0 Volt and 5 Volt) allowed synchronizing the Delta Ray IRIS with the automated chamber program (see Table 1).

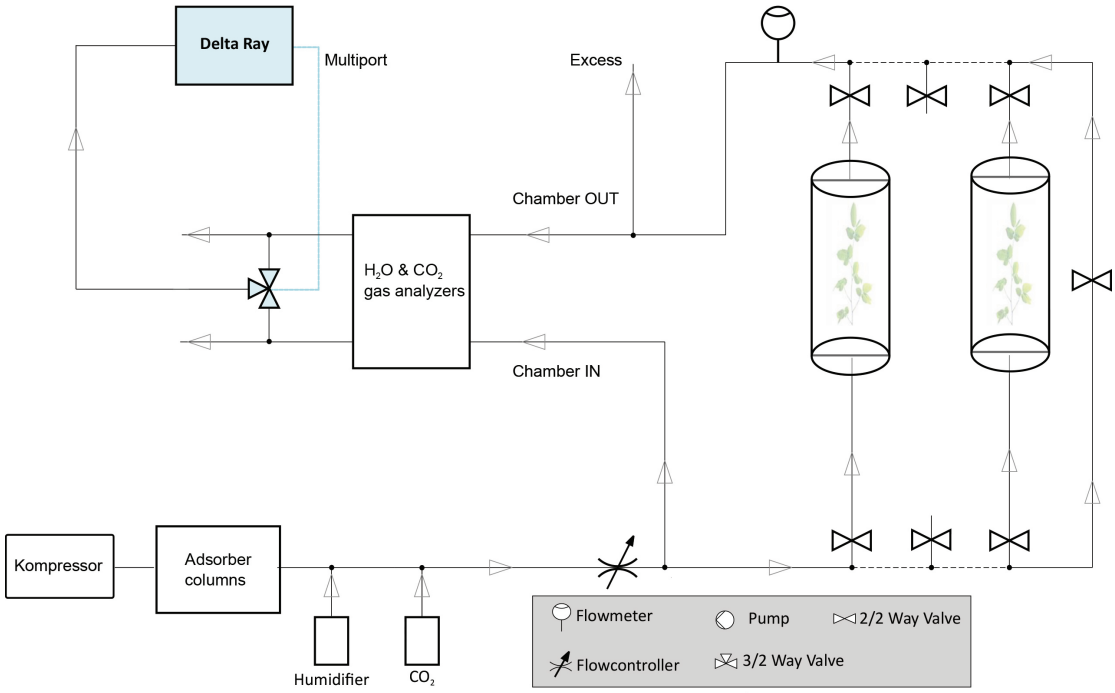

Figure 2. Simplified schematic of the plant chamber experiment.

Figure 3. Plant chamber used in the experiment.

Table 1. Sequence for the plant chamber measurements with the Delta Ray IRIS. The sequence continues until the final chamber in the setup is measured and is then repeated. Every third chamber a reference gas (Ref 1) is measured. The trigger signal send form the automated chamber program indicated the Delta Ray IRIS the beginning of sequence (trigger=0) and when the next chamber is measured (trigger=1). The Multiport triggered a valve that switched between the air entering and leaving the chambers. Chamber out was measured two times and only the final measurement (F) was used to compute $\delta^{13}CO_2$ and CO_2 values.

	Label	Flush time [s]	Measurement time [s]	Trigger	Multiport
Sequence	REF 1	60	180		
	Chamber in 1	60	180	0	1
	Chamber out 1	60	240		0
	Chamber out 1F	0	120		0
	Chamber in 2	60	180	1	1
	Chamber out 2				0
	Chamber out 2F	0	120		0
	Chamber in 3	60	180	1	1
	Chamber out 3				0
	Chamber out 3F	0	120		0
	REF 1	60	240		
	Chamber in 4	60	180	1	1
	...				

Results

Plant CO_2 exchange affects the isotopic CO_2 signal of the air inside the chamber: the larger the CO_2 flux, the larger the isotopic difference between air entering and leaving the chamber. Exemplary results of day- and nighttime measurements from one of the plant chambers during three consecutive days are given in Figure 4. During night, plants emit CO_2 to the atmosphere, which contains more $^{12}CO_2$ and causes depletion of the $\delta^{13}CO_2$ signal in the air. Because the respiratory CO_2 flux of the plant was rather small, the corresponding change in the isotopic signal was not very distinct (here -0.6‰ on average). Using a simple mixing model one can calculate the C isotope signal of the respiratory CO_2 efflux, and a change over time indicates a switch in the respiratory substrate, which could be stress related. During the day, however, CO_2 uptake changes the isotopic signal of the air in the other direction. Due to preferential uptake of $^{12}CO_2$ during photosynthesis of C3 plants the air inside the chamber becomes enriched in ^{13}C, in our example by +2‰ on average. The photosynthetic discrimination (Δ) can be calculated from the change in the isotopic signal and CO_2 concentration following Evans et al (1986) and was in this case study 13.4‰ on average. In combination with simultaneously measured plant CO_2 and H_2O gas exchange, mesophyll conductance can be estimated (see Tazoe et al. 2011 for an example).

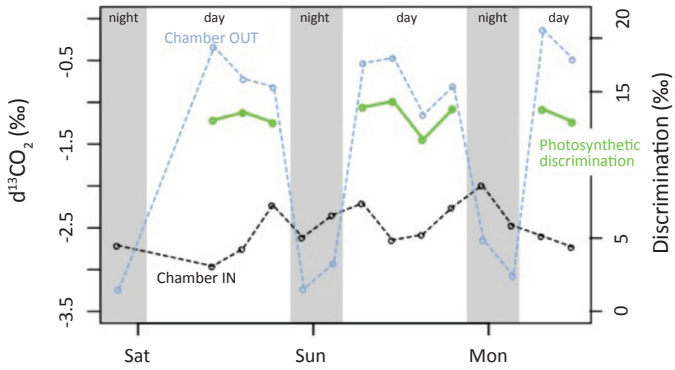

Figure 4. Time series of final averaged $\delta^{13}CO_2$ for air entering (chamber IN) and air leaving (chamber OUT) one of the plant chambers in the experiment. Photosynthetic discrimination is given during daytime and was calculated from the differences in $\delta^{13}CO_2$ and CO_2 concentrations of chamber IN and chamber OUT. The variation in chamber IN results from incomplete removal of CO_2 from ambient air.

Conclusion

The Thermo Scientific Delta Ray IRIS can be easily integrated in a gas exchange setup to measure variations in the $\delta^{13}C$ and $\delta^{18}O$ in CO_2. This results in a high-resolution dataset of plant gas exchange and its isotopic signature, which allows to identify short-term and long-term changes in plant metabolism. During nighttime, a change in the isotopic signal of the respiratory CO_2 efflux over time may indicate a shift in the respiratory substrate related to environmental effects. During daytime, information on photosynthetic discrimination provides insights in changes of the photosynthetic pathway including mesophyll conductance.

Apart from using the natural abundance signal of $\delta^{13}CO_2$, the experimental setup could further be used for labeling studies to trace the faith of recently assimilated CO_2 from plants back to the atmosphere. Using a CO_2 source isotopically enriched in $^{13}CO_2$, recently assimilated carbon can be distinct from previous photosynthetic products with the Delta Ray IRIS. This allows novel insights on turnover times of the carbon pool in plants and ecosystems under changing environmental conditions.

References

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