MS44 Protocol for response measurements

Note: The information in this MS document is also relevant to MS43.

Protocols for plant physiological measurements in C3 of ÉCLAIRE

This protocol is designed to give some guidance to experimentalists of the data and associated plant physiological measurements that would ideally be collected from ECLAIRE experimental sites to help inform the validation and development of models (predominantly DO3SE, FORSPACE and the plant physiology algorithms of ecosystem models such as JULES and LPJ) used in C3.

This protocol is also designed to help experimentalists complete the **'ECLAIRE_C3_Experiment_Data_DO3SE'** template that allows experimentalists to upload their data to the ECLAIRE data management database. Therefore, throughout the document the appropriate worksheets of this template to which these measurements relate is clearly defined.

The experimental data will be used for 3 main aspects of modelling: i. as input data; ii. to provide parameterisation to the models and iii. as evaluation data. This document is divided according to these three areas with a focus on the last two in relation to protocols for physiological measurements.

i. Input data.

The models require standard information about the site and treatment; key details are requested in the **'Site'** worksheet whilst information on the vegetation characteristics (including the response of vegetation to treatments) is requested in the **'Veg'** and **'Species'** worksheet. These data should be provided per treatment and per component (plant species) so that combinations of treatments and multiple species will be listed accordingly in the spread sheet. Information on the soil properties (especially important for field grown plants or experiments with drought treatments are requested in **'Soil prop'** worksheet. The **'Depos'** worksheet allows for additional treatment information (i.e nitrogen additions) to be added if appropriate. The DO3SE model (as well as other models that estimate aspects of plant physiology) requires core environmental (meteorology and gas concentration) input data to run; for DO3SE these are required on an hourly time-step. These input data are requested in the **'Met'** and **'Gas conc'** worksheets of the template.

ii. Parameterisation data.

A/Ci (internal CO_2) and A/Q (light response) curves for parameterisation of photosynthesis-based modelling

The models will have a focus on improving our understanding of how multiple stresses may influence key fundamental plant processes such as photosynthesis, stomatal conductance, respiration and C allocation. We will use and develop coupled photosynthesis-stomatal conductance algorithms (including possible de-coupling effects of ozone) therefore it is essential that we get as

much information as possible to parameterise these models for the experimental conditions and species under investigation. A key component of this will be A/Ci and A/Q curves to allow parameterisation of the terms V_{cmax} (maximum Rubisco activity) and J_{max} (maximum rate of electron transport) as well as dark respiration. It would also be important to have measurements of leaf nitrogen content and specific leaf area, and ideally, leaf rubsico and leaf chlorophyll contents.

While V_{cmax} and J_{max} tend to co-vary according to a standard ratio, there is increasing evidence that this might not always be the case depending on factors such as plant and leaf age, N content, and ozone levels etc. Therefore, A/Ci and A/Q curves should be carried out for all treatments (e.g. different O3, N, drought, CO2 treatments); the A/Q curves will be particularly helpful in determining the definition of the J_{max}/V_{cmax} relationship since light drives the electron transport rate (J).

We suggest that A/Ci and A/Q curves be measured:

- At a constant optimal temperature
- The A/Ci curves should be carried out under light saturated conditions (e.g. Q or > 800 µmol m-2 s-1 PPFD)
- The A/Q at ambient CO2 level (e.g. in ambient CO2 at 390 ppm or at in elevated CO2 at the elevated CO2 level 510 ppm at the CLIMAITE site)
- During the physiologically active part of the day which may vary with experimental location but before midday depression in gs (e.g. mid-morning)
- Three times during the treatment period, beginning, middle and end, as far as possible measurements for A/Ci and A/Q should be conducted at the same time during the treatment period,for both young fully expanded and older leaves.
- At least 3 replicates per treatment
- Where ever possible the measurements should be made according to the protocol of Long & Bernacchi, 2003 (further information can be found in Kosugi et al. 2003)

The worksheet **'AciAQ'** provides the opportunity for experimentalists to log these data (as raw data describing the curves from which V_{cmax} and J_{max} can then be extracted).

Mitochondrial (dark) respiration (R_d)

- The measurement of mitochondrial (dark) respiration (R_d) is required to calculate V_{cmax} and assess the cost of damage caused by stresses such as O_3. R_d occurs both during the daylight and at night, however, during daylight photo-respiration also occurs. Therefore to ensure the Rd measured is truly mitochondrial respiration two different methods can be used:
    I. To produce A/Ci curves at different light intensities with the true Rd value being the Rd that does not change with light intensity (also known as the Laisk method).
    II. Take measurements in the dark (either created artificially by keeping the leaves in the dark for at least 10 to 30 mins before the measurement or by making the measurements at night).
- Ideally, R_d measurements should be carried out for all O_3 and N treatments and should be repeated several times during the growing season for leaves of different ages to capture the seasonal variation.

- For further details see: http://5e.plantphys.net/article.php?ch=e&id=480%5C

iii. Evaluation data

It will be important to be able to compare observed with modelled predictions of both leaf level stomatal conductance and photosynthesis and canopy estimates of CO_2 and H_2O vapour flux (if available) between different treatments and over the course of the experimental period. These measurements can then be used to assess the predictive capability of the model and should be made under ambient conditions. Below we identify the different apparatus that can be used to make such measurements, issues of inter-calibration and the ideal frequency with which these measurements would be made. These data can be uploaded into the template: H2O vapour fluxes defined as soil evaporation (Es), canopy transpiration (Et) and system evapotranspration (E) in the **'Evap'** worksheet; leaf level stomatal conductance and photosynthesis measurements in the **'Gas flux'** worksheet.

In addition, we also allow uploading of data that might help us evaluate key plant and plant related characteristics (i.e. soil water content, LAI and height) that are simulated by the model according to certain assumptions but to which the models are likely to be particularly sensitive. Therefore, if we can obtain additional information about how these characteristics might vary from time-to-time over the course of the experimental period this would be extremely valuable. These data are requested in worksheet **'Eval'**.

Stomatal conductance (g_s)

Porometer and IRGA (e.g. LiCor, CIRAS etc.))

- Individual measurements on fully-developed leaves, representative of 2 different leaf ages and 2 or 3 different canopy (predominantly sunlit *vs* predominantly shaded) positions if possible.
- Conducted for entire diurnal periods under a range of environmental conditions (sunny and overcast); ideally in excess of 20 to 30 measurements made over the course of each day
- These diurnal campaigns ideally to be made at the beginning, middle and end of the treatment periods so that a total of in excess of 5 days' worth of data be provided
- Conducted for all treatments
- Recording of ambient environmental variables (e.g. PAR, CO2, temperature etc…) to be made simultaneously (as indicated in **'Gas flux'**)
- Where possible g_{max} measurements (i.e. measurements of g_s under optimal environmental conditions during the height of the growing season) should be made using both a porometer and IRGA (where possible) to also allow intercomparison of recorded gs values between instruments

Photosynthesis (A)

IRGA (e.g. LiCor, CIRAS etc.)

- Individual measurements on fully-developed leaves, representative of 2 different leaf ages and 2 or 3 different canopy (predominantly sunlit *vs* predominantly shaded) positions if possible.
- Conducted for entire diurnal periods under a range of environmental conditions (sunny and overcast); ideally in excess of 20 to 30 measurements made over the course of each day
- These diurnal campaigns ideally to be made at the beginning, middle and end of the treatment periods so that a total of in excess of 5 days' worth of data be provided
- Conducted for all treatments
- Recording of ambient environmental variables (e.g. PAR, CO2, temperature) to be made simultaneously (as indicated in **'Gas flux'**)
- Ideally, where possible, A and gs measurements would be recorded simultaneously

Other leaf level measurements

- Leaf nitrogen (Leaf_N) content (especially if conducting N addition treatments)
- Specific Leaf Area (SLA)
- Leaf Rubisco content (Leaf_Rub) (if possible)
- Leaf chlorophyll content (Leaf_Chl) (if possible)

N.B. **Please, whenever possible and plant sizes are big enough, always measure SLA and N content for each of the physiological measurements mentioned above!** For SLA the leaf area should be measured directly after the plant physiological measurements are made, on sun and shade leaves if applicable; the leaf can then be removed and stored in a freezer. This would allow the dry weight of leaves and leaf nitrogen content measurements to be performed in 1 batch at the end of the experiment. Caution is needed, however, in deciding how many leaves cdan be removed without having a detrimental effect on subsequent growth and final biomass.

It is acknowledged that following these methods could lead to a large number of measurements being required. One way to reduce the number of measurements would be to make detailed measurements of Leaf_Chl, Leaf_Rub, J_{max} and V_{cmax} on a limited number of leaves, but covering a sufficiently large range of conditions (i.e. different treatments). This would then allow calibration of Leaf_Chl to Leaf_Rub and total Leaf_N and allow Leaf_Chl to be used as a quick proxy in subsequent measurements. Since Leaf_Chl can be relatively easily measured (using a chlorophyll content SPAD meter) this then provides the opportunity of recording additional data more easily. Importantly, application of this method avoids the assumption that the ratio of Leaf_Chl to Leaf_N is constant (and subsequently that Jmax to Vcmax is constant); the constancy or otherwise of these ratios under O_3 and N stress are exactly what needs to be tested by these experimental investigations.

Further Information on plant physiological measurements

If you require further guidance in how to make any of the measurements described above please see: Cornelissen et al. (2003).

References

Cornelissen, J. H. C., S. Lavorel, et al. (2003). "A handbook of protocols for standardised and easy measurement of plant functional traits worldwide." Australian Journal of Botany 51(4): 335-380. http://www.cedarcreek.umn.edu/biblio/fulltext/t1936.pdf

Long, S.P., and Barnacchi, C.J. (2003) Gas exchange measurements, what they can tell us about the underlying limitations to photosynthesis? Procedures and sources of error. J. of Exp. Bot. 54 (392): 2393-2401

Kosugi, Y., Shibata, S., Kobashi, S. (2003) Parameterization of the CO2 and H2O gas exchange of several temperate deciduous broad-leaved trees at the leaf scale considering seasonal changes. Plant, Cell and Environ. 26: 285-301