Instruction to Use the Tool for Evaluating Mesophyll Impact on Predicting Photosynthesis (TEMIPP)

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Purpose

TEMIPP is a Microsoft Excel Spreadsheet-based tool used for demonstrating the impact of lacking an explicit representation of mesophyll diffusion in a photosynthetic model on the predicted response of photosynthesis to the increase in CO_2 partial pressures.

Approach

TEMIPP simulates the measurement, analysis and application of curves of photosynthesis *A* against intercellular CO_2 pressures C_i (*i.e.*, the so-called A/Ci curves). A/Ci curves are typically measured at a saturating level of photosynthetic photon flux density (PPFD) and a fixed temperature. TEMIPP generates an A/Ci curve at a set of 'measuring' environmental conditions (PPFD, temperature, atmospheric pressure and oxygen) and a set of fundamental photosynthetic parameters (e.g. V_{cmax}, J_{max}, *TPU*, R_{d}, mesophyll conductance g_{m} etc), all specified by the user. The photosynthetic rate is then calculated by applying the Farquhar – von Caemmerer – Berry (FvCB) model (Farquhar et al. 1980) extended with a finite g_m (Ethier and Livingston 2004, Gu et al. 2010). A g_m-lacking model, which is the FvCB model applied with an assumption of an infinite g_m, is fit to the generated A/Ci curve. The obtained key photosynthetic parameters are then used in the g_m-lacking model as in current carbon cycle models to predict photosynthesis at a new set of conditions that is different from the original set of 'measuring' conditions under which the A/Ci curve for fitting was produced.

Instead of using simulated A/Ci curves, users have the option to apply real A/Ci measurements to TEMIPP. When real A/Ci curves are used, users will need to provide TEMIPP independently estimated photosynthetic parameters including g_m.

Users can examine the impact of lacking an explicit representation of g_m by comparing model performance between the fitting to the original A/Ci curve and the prediction at new conditions. It is important to check the residuals between the actual value and the value calculated by the g_m-lacking model as the residuals can reveal model performance more clearly than a simple direct comparison which can be misleading.

The impact of lacking g_m can also be investigated by comparing the beta (β) factor calculated from the actual data with that from the prediction by the g_m-lacking model via the following ratio *R* of beta factors:

$$R(C_i) = \frac{\beta_c(C_i)}{\beta_i(C_i)} = \frac{\left[A_c(C_i) - A_c(C_{i,ref})\right]A_i(C_{i,ref})}{\left[A_i(C_i) - A_i(C_{i,ref})\right]A_c(C_{i,ref})},$$

where β_c is the actual beta factor and β_i is the beta factor calculated with the g_m-lacking model. $C_{i,ref}$ is a reference intercellular CO_2 partial pressure. A value $R > 1$ indicates that the g_m-lacking model underestimates the actual CO_2 fertilization effect; $R < 1$ the opposite.

The fitting uses the Evolutionary method in the Solver provided by Microsoft Excel. The Evolutionary algorithm is selected because the optimization problem for the FvCB model is a change-point model and is not smooth (Gu et al. 2010). If users don't wish to use the Microsoft Solver, they can use any optimization software they might have or LeafWeb (leafweb.ornl.gov) to estimate the parameters and then input their own parameters directly into the Excel Spreadsheet.

The temperature response functions are from Sharkey et al. (2007). If users wish to use different temperature response functions, they can input their own temperature response funcitons as well.

Detailed instructions

1. Generate a new A/Ci curve. A new A/Ci curve can be generated in any of the following ways:
    - Change the standardized fundamental parameters Vcmax25, Jmax25, TPU25, gm25, Rd25 (Cells E10 to I10). Users can also change the Rubisco kinetic parameters (J10 to L10) or the leaf absorptance parameter (M10) if they wish
    - Change the A/Ci curve 'measuring' conditions of temperature, PPFD, atmospheric barometric pressure and oxygen partial pressure (E20 to H20)
    - If they wish, users can provide their own coefficients in the temperature response functions in the section from E15 to L17.
    - The A/Ci data for fitting are automatically computed from the Cell B36 to B53, depending on the values of Ci from A36 to A53. Users can adjust the Ci values from A36 to A53 as they wish. Leave any unused cell empty.
2. Fit the g_m-lacking model
    - Click the cursor at Data in the top of Excel Spreadsheet
    - Click Solver. You may have to install the Excel Solver first.
    - This brings up the Solver Parameters menu
    - The settings should have been already specified.
    - Click Solve to minimize the value in the Objective Cell F54
    - Wait for the Solver to complete its job. This may take a while.

- When Solver Results menu appears, choose 'Keep Solver Solution' and click OK.

3. Provide a new set of environmental conditions for which the g_m-lacking model will make predictions. Put these values in E21 to H21. Try different conditions to see how the performance of the g_m-lacking model vary as the conditions for prediction deviate from the conditions for which the original A/Ci curve for fitting was produced.
4. Examine the two plots around Row 70
5. The default setting in the Solver Parameters menu is for optimizing Vcmax25, Jmax25 and Rd25 for the g_m-lacking model. TPU25 for the g_m-lacking model is set to be equal to that users provide in the Cell G10 to take advantage of the fact that TPU-limited photosynthesis is $3*TPU - R_d$, which does not depend on CO_2 partial pressures and therefore g_m. This avoids potential over-fitting and unreasonable parameter values. But if you wish to estimate TPU25 for the g_m-lacking model as well, go to the Solver Parameters menu, add ",G11" (without the quotation marks) after "I11" in the box under "By Changing Variable Cells".
6. If they wish, users can also optimize for the Rubisco kinetic parameters for the g_m-lacking model by adding ",J11,K11,L11' under "By Changing Variable Cells" in the Solver Parameters menu. However, A/Ci data generally don't contain enough information to constrain all these parameters.

7. Use real A/Ci measurements with independently estimated parameters. To use real A/Ci measurements with parameters estimated with other means for TEMIPP, do the following:
   - Save a copy of TEMIPP.
   - Manually input the real A/Ci data in the section A36 to B53 and leave any unused cells empty (Do not cut and paste as this will cause disabling the auto-computing functions).
   - Input the standardized fundamental parameters estimated with explicit consideration of g_m into E10 to L10 (TEMIPP can be modified to estimate g_m for the purpose of testing. See Instruction No. 8).
   - Input the A/Ci measuring conditions in E20 to H20.
   - If in their A/Ci curve analysis, users used a different set of coefficients for the temperature response functions than those listed in TEMIPP, input these different coefficients into the section E15 to L17.
   - If users have independent estimates of the corresponding parameters for the g_m-lacking model, input them to E11 to I11 and skip the Microsoft Solver; otherwise, invoke the Solver.

- Check the plots.

8. Modify TEMIPP to estimate g_m and associated fundamental photosynthetic parameters.
   - Save a copy of TEMIPP
   - Manually input the real Ci data in the section A36 to A53 and leave any unused cells empty (Do not cut and paste).
   - Manually input the *A* (net photosynthesis) data in the section M36 to M53 and leave any unused cells empty (Do not cut and paste).
   - If users wish to use a different set of coefficients for the temperature response functions than those listed in TEMIPP, input these different coefficients into the section E15 to L17.
   - Bring up the Solver.
   - Replace the content in "Set Objective:" with "O54" (without the quotation marks)
   - Replace the content in "By Changing Variable Cells:" with "E10,F10,G10,H10,I10"
   - Replace all "$11"s in the box under "Subject to the Constraints" with "$10"
   - Click Solve
   - Wait for the Solver to complete its job
   - The optimized parameters are displayed in the Cells from E10 to I10.

TEMIPP is not meant to be a tool for research-grade A/Ci curve analyses for which the method used in LeafWeb (leafweb.ornl.gov) is more appropriate (Gu et al. 2010).

References

Ethier, G. J. & Livingston, N. J. (2004) On the need to incorporate sensitivity to CO2 transfer conductance into the Farquhar-von Caemmerer-Berry leaf photosynthesis model. *Plant Cell and Environment* 27, 137–153.

Farquhar, G. D., Von Caemmerer, S. & Berry, J. A. (1980) A Biochemical-model of Photosynthetic Co2 Assimilation in Leaves of C-3 Species. *Planta* 149, 78–90.

Gu, L., Pallardy, S. G., Tu, K., Law, B. E. & Wullschleger, S. D. (2010) Reliable estimation of biochemical parameters from C3 leaf photosynthesis-intercellular carbon dioxide response curves. *Plant Cell and Environment* 33, 1852–1874.

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