Plant Ecophysiology
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Supplementary Material S1

The Cryothermocycler Device

The device consists of a set of thermoelectric coolers (Peltier cells), with their upper surface attached to an aluminium plate serving as a thermal stage, and their lower surface connected to an aluminium heat sink that facilitates the dissipation of heat generated by the Peltier cells (Fig. S1.1). These components together form the thermoelectric block. The thermal stage is surrounded by a lateral insulator, which ensures thermal homogeneity while also serving as a mounting and securing system for a set of interchangeable thermal plates that constitute the thermal block. These plates are specifically designed with recesses to accommodate the samples to be analyzed. Above the thermal block, the device features an innovative main lid equipped with a set of interchangeable optical fibers. This lid enables fluorescence measurements on the samples under experimentation via a pulse-amplitude modulation (PAM) fluorometer, while simultaneously shielding them from external influences that could compromise their thermal stability during the experiments.

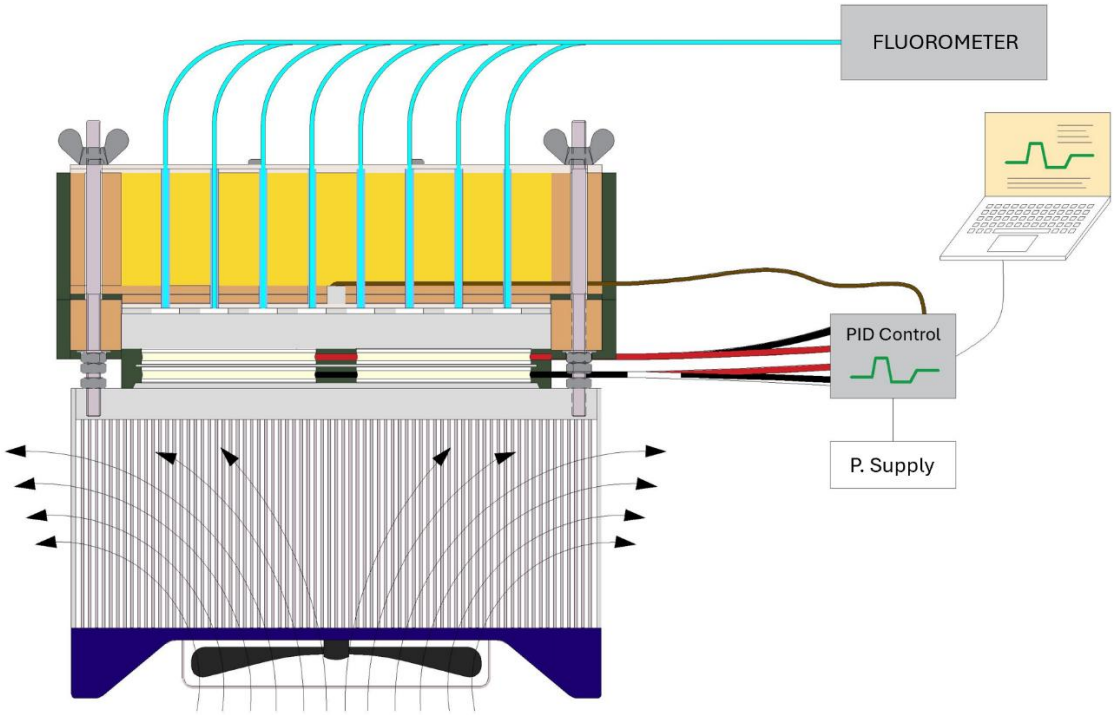

Fig. S1.1. Schematic of the Cryothermocycler Device with Measurement and Control Elements. The cryothermocycler is connected to a PID (Proportional–Integral–Derivative) control system, enabling the precise programming of thermal cycles. A fluorometer for measuring the maximum quantum efficiency of photosynthesis (F_v/F_m) is also connected to the device via a set of optical fibers (represented by blue lines). The entire system is powered by a direct current power supply (P. Supply).

Thermoelectric Block

The thermoelectric block consists of a CP-121HT thermoelectric cooling module (TE Technology Inc., Michigan, USA), based on a set of Peltier cells with a dual functionality that allows for both cooling and heating, depending on the configuration selected via a bipolar thermal controller. The device operates within a temperature range of -26 °C to 100 °C under standard ambient temperature and pressure (SATP) conditions. It contains a 152 × 152 mm aluminium thermal plate, on which the thermal block is placed. It also includes an aluminium heat sink equipped with two low-noise fans (42 dBA), providing the necessary airflow for efficient heat transfer. The typical thermoelectric power is 9.2 A at 24 VDC, while the maximum thermoelectric power reaches 11.2 A at 24 VDC. The fan power consumption is 0.30 A at 24 VDC. The device weighs 4.2 kg.

With a thermal load close to 0 W—which is practically achieved through the device's thermal insulation—the thermoelectric block follows the transfer curve:

$$T_{min} = 0.7605T_a - 45.053$$

where:

- T_{min} (ºC): Minimum achievable temperature.
- T_a (ºC): Ambient temperature.

Thermal Block

Directly placed on the thermal stage, one or more stackable and interchangeable aluminium plates form the thermal block. These 150 mm × 150 mm plates are available in various thicknesses to accommodate different sample types. They feature pre-cut recesses in various shapes, creating sample wells or compartments. Above each well, and through the main lid, optical fibers are positioned to measure chlorophyll fluorescence, enabling the evaluation of photosystem II (PSII) status. The variety of well shapes and sizes across different interchangeable thermal blocks allows for the accommodation of samples of various types and dimensions. The maximum number of wells per thermal block is 68, allowing for the simultaneous examination of up to 68 samples.

Main Lid

The main lid represents the most innovative feature of the cryothermocycler device. It consists of a stainless-steel lower plate that, in the measurement position, serves as the upper seal for the thermal block wells, allowing for the insertion of one or more optical fibers per sample, depending on their size. Its primary function is to direct a set of optical fibers or other elements, such as temperature sensors or microtubes, to each sample while minimizing external thermal loads that could affect the experiment. This ensures that the samples undergo stable and precisely preprogrammed thermal cycles. To minimize atmospheric load on the samples and enhance stability, the stainless-steel lower plate is covered with a 4 cm polystyrene layer, providing efficient insulation. Inside the main lid, and in close contact with the stainless-steel plate, a temperature sensor is embedded to enable precise thermal control of the device. A peripheral insulator, made of wood or polymer, acts as an adjustment and securing system.

Thermal Control System

The TC-36-25 RS232 thermal control system (TE Technology Inc., Michigan, USA) is a high-precision temperature controller designed to regulate thermoelectric devices in both heating and cooling modes. It utilizes high-efficiency solid-state switching devices of the N-channel MOSFET (Metal-Oxide-Semiconductor Field-Effect Transistor) type, arranged in an H-bridge configuration, allowing polarity inversion of the applied current. This enables the device to seamlessly operate in either heating or cooling mode as needed. The system supports a switching capacity of 25 A with a supply voltage ranging from 12 to 36 VDC, handling loads of up to 900 W. Power control is achieved through Pulse Width Modulation (PWM) at 2.7 kHz, ensuring precise power delivery. It features two advanced control algorithms: a PID (Proportional-Integral-Derivative) controller, with adjustable proportional, integral, and derivative parameters (used in this application), and a dead-zone (on/off) controller with adjustable hysteresis for simpler configurations. The precise preprogrammed temperature value, set via a computer, is transmitted to the control system through an RS232 (Recommended Standard 232) communication port.

Control Software

The device is managed through a desktop application based on Microsoft's open-source .NET platform. This application provides a graphical interface that allows the user to program temperature cycles, visualize recorded temperature curves, store results, and adjust PID controller parameters. The communication between the computer and the control system is established through a serial port, connecting a USB port on the computer to the RS-232 interface of the controller via an adapter. This system follows a Master-Slave communication scheme, where the application acts as the Master and the device as the Slave.

The user interface has been developed using the WinAppSDK framework and the WinUI3 library, both part of the .NET ecosystem, making the application compatible exclusively with Windows platforms. The application offers three operating modes.

- Mode 1 (Basic). Allows the user to set a target temperature, which the device maintains indefinitely until manually deactivated.
- Mode 2 (Thermal Cycle). This is the mode used in the application referenced in this manuscript. It enables the execution of a preprogrammed temperature cycle (Fig. S1.2). Programs can be configured from a dedicated screen and stored in memory for easy reuse. The resulting thermal cycle and related data are saved in a CSV file.

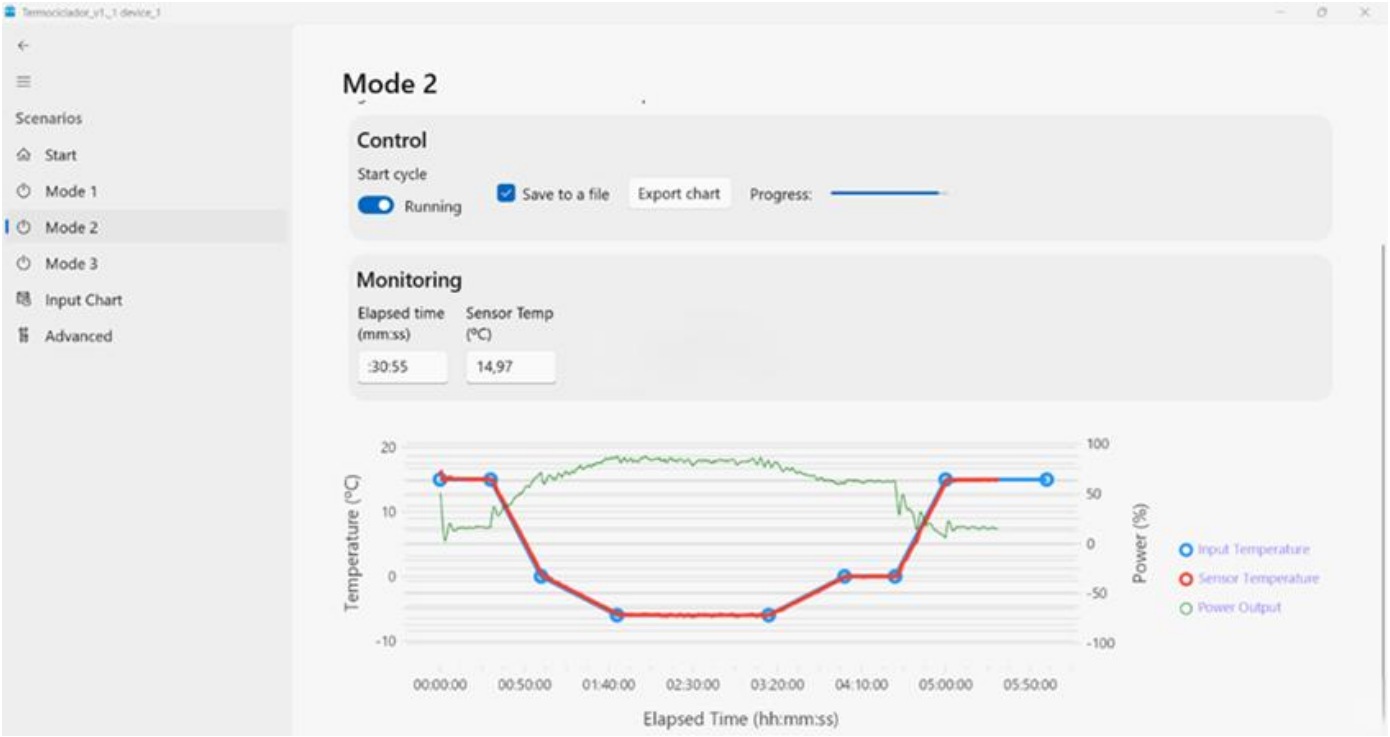

Fig. S1.2. Control software view during the execution of a thermal cycle in Mode 2. The figure shows a screenshot of the computer display while running a thermal cycle in Mode 2.

- Mode 3 (Power Cycle). Allows programming a power cycle, which is recommended for PID controller tuning.

Supplementary Material S2

Thermocouple Placement for Measuring Device's Thermal Homogeneity

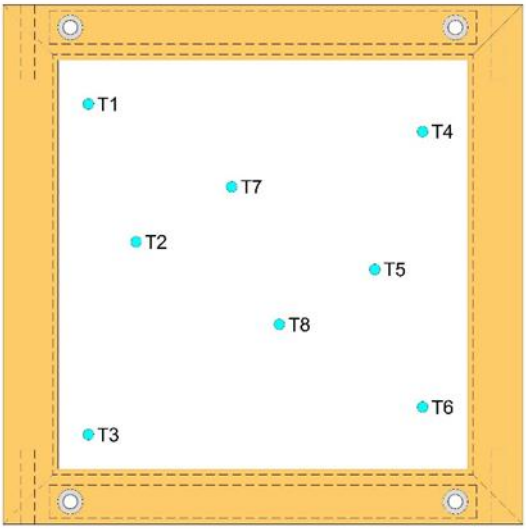

Fig. S2.1. Placement of the different thermocouples used to measure the device's thermal homogeneity. These were positioned in the designated area at three different locations (see details in the main text).

Supplementary Material S3

Species Used

Below is a complete list of the species used in all the experiments conducted.

Antarctic ochrophyta (brown algae)

- ***Desmarestia menziesii* J.Agardh**

Antarctic rhodophyta (red algae)

- ***Iridaea cordata* (Turner) Bory**
- ***Palmaria decipiens* (Reinsch) R.W.Ricker**
- ***Pyropia endiviifolia* (A.Gepp & E.Gepp) H.G.Choi & M.S.Hwang**

Antarctic chlorophyta (non-streptophyte green algae)

- ***Acrosiphonia arcta* (Dillwyn) Gain**
- ***Monostroma hariotii* Gain**
- ***Prasiola* sp.**

Antarctic charophyta (streptophyte green algae)

- ***Klebsormidium* sp.**

Antarctic lichens

- ***Himanthormia lugubris* (Hue) I.M.Lamb**
- ***Leptogium* sp.**
- ***Mastodia tessellata* (Hook.f. & Harv.) Hook.f. & Harv.**
- ***Ramalina terebrata* Hook.f. & Taylor**
- ***Sphaerophorus globosus* (Huds.) Vain.**
- ***Stereocaulon alpinum* Laurer**
- ***Usnea antarctica* Du Rietz**
- ***Usnea aurantiaco-atra* (Jacq.) Bory**

Antarctic bryophyta (Mosses)

- ***Andreaea gainii* Cardot**
- ***Bartramia patens* Brid.**
- ***Brachythecium austrosalebrosum* (Müll.Hal.) Kindb.**
- ***Bryum pseudotriquetrum* (Hedw.) P.Gaertn., B.Mey. & Scherb.**
- ***Ceratodon purpureus* (Hedw.) Brid.**
- ***Hymenoloma crispulum* (Hedw.) Ochyra**
- ***Pohlia cruda* (Hedw.) Lindb.**
- ***Polytrichastrum alpinum* (Hedw.) G.L.Sm.**
- ***Polytrichum piliferum* Hedw.**
- ***Polytrichum strictum* Menzies ex Brid**
- ***Sanionia uncinata* (Hedw.) Loeske**
- ***Schistidium rivulare* (Brid.) Podp.**
- ***Syntrichia filaris* (Müll.Hal.) R.H.Zander**
- ***Warnstorfia sarmentosa* (Wahlenb.) Hedenäs**

Antarctic anthophyta (tracheophytes)

- ***Colobanthus quitensis* (Kunth) Bartl.**
- ***Deschampsia antarctica* É.Desv.**

Arctic anthophyta (tracheophytes)

- ***Bistorta vivipara* (L.) Delarbre**
- ***Cerastium alpinum* L.**
- ***Dryas octopetala* L.**
- ***Equisetum arvense* L.**
- ***Erigeron humilis* Graham**
- ***Oxyria digyna* (L.) Hill**
- ***Papaver dahlianum* Nordh.**

- ***Poa pratensis* L.**
- ***Salix polaris* Wahlenb.**
- ***Saxifraga cernua* L.**
- ***Saxifraga cespitosa* L.**
- ***Saxifraga hirculus* L.**
- ***Saxifraga oppositifolia* L.**

Supplementary Material S4

Experimental Procedure:

1. Insert the replicates of each sample into the cryothermocycler device programmed for the -18 °C thermal treatment.
2. Close the device and activate it to maintain the samples at 15 °C.
3. Repeat steps 1 and 2 with the cryothermocycler device programmed for the -12 °C thermal treatment.
4. Repeat steps 1 and 2 again with the cryothermocycler device programmed for the -6 °C thermal treatment.
5. Measure the maximum photochemical efficiency of PSII (F_v/F_m) in the -18 °C treatment replicates and activate the corresponding thermal cycle.
6. Measure the maximum photochemical efficiency of PSII (F_v/F_m) in the -12 °C treatment replicates and activate the corresponding thermal cycle.
7. Measure the maximum photochemical efficiency of PSII (F_v/F_m) in the -6 °C treatment replicates and activate the corresponding thermal cycle.
8. After 180 minutes from the moment the device reached 15 °C again following the -6 °C thermal treatment, measure F_v/F_m again in the replicates from this treatment.
9. After 180 minutes from the moment the device reached 15 °C again following the -12 °C thermal treatment, measure F_v/F_m again in the replicates from this treatment.
10. After 180 minutes from the moment the device reached 15 °C again following the -18 °C thermal treatment, measure F_v/F_m again in the replicates from this treatment.
11. Turn off all devices, remove the samples and laboratory paper, and clean the devices to prepare them for the next experiment.

For algae measurements, the cryothermocycler devices were pre-cooled to 5 °C (Wienckel and Dieck, 1990) before inserting the samples. Once the samples were placed, the same protocol was followed, with the only difference being that the initial and final temperature was 5 °C, instead of 15 °C, as used for the other organisms.

Handling and Preparations

Based on our experience with the handling of cryothermocycler devices and the application of different techniques, various errors and operational difficulties were identified. Listing and documenting these incidents are essential not only to prevent future losses of time and resources but also to foster collective learning, enabling the optimization of both device usage and technical implementation. This effort will allow future research to build upon our accumulated experience, maximizing reproducibility and efficiency in the results obtained through this innovative approach.

- **Device Assembly:**
  1. Fiber insertion: Fibers should be inserted on a flat surface to ensure they reach the end of the designated holes precisely. The lower plate of the lid must be in direct contact with the flat surface to prevent fibers from protruding or falling short.
  2. Fiber irregularities: Due to potential manufacturing variations, some fibers may not fit easily into certain holes. If this occurs, try inserting them into a different hole.
  3. Hole blockage: Every hole must contain a fiber. If not all wells are used, a fiber or a fiber fragment should be inserted into the unused ones. Cut fibers must be polished again to ensure proper function.
  4. Thermal block placement: Always verify that a thermal block is correctly positioned on the thermal plate; otherwise, temperature fluctuations may occur, preventing the system from reaching the desired setpoints.
  5. Lid adjustment: Ensure that the lid is tightly secured on the thermal block. A loose fit can lead to undesired thermal fluctuations.
- **Pre-use Checks:**
  1. Connections and configuration: Confirm that all connections are properly established and that the correct serial port is configured.
  2. Initial thermal cycle: Before use, run a test thermal cycle, including at least one isothermal phase at approximately 15 °C for 30 minutes and a thermal step decrease of around 4 °C. This allows verification of PID values.
  3. Computer sleep mode: Disable automatic sleep mode on the computer to prevent cycle interruptions, which could compromise the experiment.
- **Sample Handling and Measurement Process:**
  1. Sample collection: Follow standard precautions during sample collection and ensure that samples are not collected too far in advance of their intended use.
  2. Insertion into wells: Place samples into the wells as quickly as possible to minimize dehydration, which could significantly impact results. It is recommended that multiple operators perform this task simultaneously to optimize time.
  3. Fiber adjustment: For improved measurement sensitivity, fibers can be inserted slightly toward the sample, ensuring that tissue integrity is not compromised.

- **Device Disassembly and Storage:**
  1. Cleaning: Clean the device and remove any residual materials after use.
  2. Moisture removal: If moisture is present, dry the device completely before storing it.
  3. Fiber removal and storage: Carefully remove the fibers and store them in their designated case.
  4. Lid positioning for storage: Place the main lid in an inverted position on its base and maintain this configuration during storage.