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Open top chamber microclimate may limit photosynthetic processes in Antarctic lichen: Case study from King George Island, Antarctica

Angelica Casanova-Katny[1], Miloš Barták[2*], Catalina Gutierrez[3]

[1]*Laboratorio de Ecofisiología Vegetal, Facultad de Recursos Naturales, Universidad Católica de Temuco, Campus Luis Rivas del Canto, Rudecindo Ortega #03694- Temuco, Chile*
[2]*Laboratory of Photosynthetic Processes, Plant Physiology and Anatomy Section, Department of Experimental Biology, Faculty of Science, Masaryk University, Kamenice 5, 625 00 Brno, Czech Republic*
[3]*Laboratorio de Micología y Micorrizas, Facultad de Ciencias Naturales y Oceanográficas, University of Concepcion, Víctor Lamas 1290, Concepcion, Chile*

Abstract
Long-term manipulated warming experiments using the open top chamber (OTC) approach tend to mimick the future climate and predict the changes in photosynthesis and production of vegetation under globally changed climate. In Antarctica, several long-term experiments are carried out recently. Here we report to the lichens grown in OTCs installed at the Fildes Peninsula (King George Island). The field study compares primary photochemical processes of photosynthesis in Antarctic lichen *Placopsis antarctica* grown for one year in OTC and compared to outside plot (control). We measured effective quantum yield of photosystem II (Φ_{PSII}) of green algae part of thallus in 10 min. interval for 12 days. We examined the responses of diurnal Φ_{PSII} to PAR in relation to environmental factors through continuous 12-d-long monitoring of chlorophyll fluorescence parameters Φ_{PSII} in particular. Daily courses of Φ_{PSII} and photosynthetic electron transport rate (ETR) to photosynthetically active radiation (PAR) and hydration state of thallus have been assumed to reflect changes in physiological status of *P. antarctica* in changing Antarctic environment. The data indicate that OTC microenvironment may lead to partial limitation of photosynthetic processes in *P. antarctica* during austral summer season. The limitation is caused by accelerated dehydration of thallus in OTC compared to the outside generally colder control plot, and thus shortened physiologically active period of lichens in OTC.

Key words: monitoring fluorometer, cyanolichens, Antarctic tundra, *Placopsis antarctica*, electron transport rate, chlorophyll fluorescence, long-term exposition

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[*]Corresponding author: M. Barták <mbartak@sci.muni.cz>
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Introduction

To study long-term effects of manipulated warming on Antarctic vegetation components, several types of open top chambers (OTC) similar-in-design have been used in Antarctica since the 90-ies of the last century. They are located in a great variety of ecosystems and geographical sites: Signy and Anchorage Islands (Marion et al. 1997, Bokhorst et al. 2007). The OTCs were used to apply passive warming methods to study the responses of Antarctica vegetation to increased air and surface temperature (for review of passive warming method, *see* Bokhorst et al. (2011). Within last two decades, several studies have focused on a variety of aspects of vegetation responses to OTC environment, such as biodiversity changes, altered growth and productivity of vegetation components. The effects of OTCs on photosynthesis and its rate under different climatic/meteorological conditions have been, however, much less studied. In such studies (*see e.g.* Barták and Váczi 2014), mainly the approach of long-term chlorophyll fluorescence measurements is applied. Recent development of automated monitoring fluorometers enabled measurements of large data sets and, consequently, the analyses of particular driving factors effects on primary photosynthesis.

In field photosynthetic and primary production studies, monitoring fluorometric systems with automatic record of the effective quantum yield are used in a great variety of ecosystems and species. In last decades, the technique was applied in several conifer species (*e.g.* Porcar-Castell et al. 2008, Kolari et al. 2014), desert shrubs (Zha et al. 2017), sea macroalgae (Figueroa et al. 2014), cryptogamic vegetation in the Arctic (Sehnal et al. 2014) and Antarctics (Hovenden and Seppelt 1995, Schlensog et al. 2013).

Fluorometric devices available recently for long-term monitoring of fluorescence use the modulated technique principle (Porcar-Castell et al. 2008). The design of such instruments is modular, with a series of measuring heads, each containing the optics and light sources. These components are connected to a data collection unit or a computer. In typical applications, several measuring heads are deployed in vegetation and fixed to a leaf/leaves (see *e.g.* Barták et al. 2012) or lichen thallus (Barták 2014). The head is capable of measuring maximum chlorophyll fluorescence signal reached after a saturation pulse apllied in light-adapted sample (F_M') measured *in situ* during light period of a day. During the night period, the value of F_M' become F_M, *i.e.* maximum chlorophyll fluorescence reached on the dark-adapted sample by a saturation pulse.

Finally, the monitoring fluorometer calculates photosystem II-based photosynthetic electron transport rate (*ETR*) according to the equation: $ETR = 0.5 \times 0.84 \times PPFD \times \Phi_{PSII}$ (Schreiber 2004, Baker 2008), where PPFD is photosynthetically active radiation, and Φ_{PSII} is effective quantum yield of photosynthetic processes in PS II (Genty parameter, $F_M' - F' / F_M'$ Genty et al. 1989). In automatic *ETR* calculation, absorptance (*A*) is assumed 0.84 (Baker 2008) which is valid for a vast majority of green plants. In lichens, however, absorbance is much smaller. Another assumption in the *ETR* calculation is that the light absorbed by photosynthetic pigments is equally distributed between the two photosystems (*i.e.* coefficiet 0.5 in the *ETR* equation). This approximation is reasonable for comparison of *ETR* values between optically similar samples. In samples that change their optical properties or long-term studies, species-specific variation in *A* need to be taken into account when calculating *ETR*. For vascular plants, the absorptance range of 0.551 - 0.902 is reported by Stemke and Santiago (2011). Recently, *A* is being studied across a wide variety of vascular plant species (*e.g.* Ritchie and Runcie 2014). In lichen species, however, knowledge on *A* value is still fragmentary and numeric value of *A* is generally unknown. Ritchie (2014) reports *A* of 0.92 for freshly green thalli of *Dirinaria picta* but for differently-coloured lichen species is not known. Moreover, numeric value of *A* may change during dehydration and rehydration which must be taken into consideration when calculating ETR in lichens in response to hydration status of lichen thalli. Numeric value of Φ_{PSII} recorded during dark period of a day is highest and, in principle, reflects potential yield of photochemical processes (F_V/F_M). Since PPFD equals 0 during night, then ETR is 0 during dark period of a day.

Long-term *in situ* monitoring of Φ_{PSII} and evaluation of *ETR* together the measurements of environmental characteristics, photosynthetically active radiation in particular leads to the evaluation daily courses of *ETR*. Such approach has been applied many times in vascular plants but scarcely in mosses and lichens. In last decade, however, several in field studies have been done on poikilohydric autotrophic organisms such as *e.g.* mosses (Barták and Váczi 2014), and moss-dominated biological soil crusts (Raggio et al. 2014).

In this study, we used a monitoring fluorometer to measure primary photochemical processes of photosynthesis in Antarctic lichen *Placopsis antarctica* and compare *ETR* in the lichen samples grown for 1 year in OTC (experimental warming, manipulated microenvironment) and outside plot (control) exposed to natural variation of environmetal factors. We hypothesized that OTC microenvironment would differ from outside control plot, and may lead, due to altered hydration status to partial limitation of photosynthetic processes in *P. antarctica* during austral summer season. We hypothesized that the limitation would be caused by accelerated dehydration of thallus in OTC thanks to warmer environment.

Material and Methods

Species characteristics

Placopsis antarctica was described as new to science just recently (Galloway et al. 2005). The species is reported for the Maritime Antarctic, especially the South Shetland Island and the Antarctic Peninsula (Olech 2010, Spielman and Pereira 2012). *P. antarctica* is a lichen of the family Agyriaceae, order Agyriales. This is typical Antarctic lichen growing on vulcanic or siliceous rocks on moraine boulders, fellfield and coastal volcanic rocky areas, between 10-550 m (Galloway et al. 2005), and we found it growing on moribound moss species on Collins area on King George Island. *Placopsis antarctica* presents a white medulla, with a green photobiont recently identified as *Stichococcus antarcticus* (Beck et al. 2019) and a cephallodia with the cyanobiont *Scytonema* (Galloway et al. 2005). Apothecia are scattered and frequent on old thalli, the colour vary when moist from rust- brown pale to dark red-brown when dry, and this species is characterized by the presence on laminal dactyls of numerous soredia, which make the visual difference with *Placopsis contortuplicata*, which is growing on the same area (Galloway et al. 2005, Beck et al. 2019).

Site description and OTC

The study was carried out on the Fildes Peninsula, King George Island (62° 00' S, 58° 15' W) in the South Shetland Island Archipelago. Experimental site was La Cruz Plateau (62° 12' S, 58° 57' W, 41 m a. s. l.). The site is located in the interior of the Fildes Bay and characterized by polygonal soils with several small-area rocky outgrowths. Vegetation cover of La Cruz Plateau is formed by a moss-lichen community dominated by *Usnea aurantiaco-atra*, *Himantormia lugubris* and *Placopsis antarctica.* In 2008, several open top chambers (OTCs) were installed at La Cruz Plateau as a part of manipulated warming experiment studying moss (Casanova-Katny et al. 2015, Shortlidge et al. 2017) and lichen growth responses to increased air temperature (for installation details, *see* Casanova-Katny et al. 2016). The OTCs are made of 3 mm thick, 40 cm high, transparent acrylic panels with a basal area of 0.93 m^2 forming a hexagonal frustum with open top. The panels are provided with small perforations to permit air exchange and avoid excessive warming. At the site (both in OTCs and outside control plots), microclimatic characteristics has been measured since 2008: air temperature and relative air humidity in 1 h step (HOBO Pro v2 loggers (Onset, Bourne, Mass). The sensors are placed at 20 cm above the vegetation. Similarly, soil temperature at different soil depths is measured.

Microclimate of measuring spots

Apart from the long-term monitoring of air temperature and relative air humidity (*see* above), soil temperature at the depth of 2 cm (OTC and control sites) and the temperature of the measuring spot (optical window of the fluorometer, control and OTC site – *see* below) were monitored during the experimental period. A set of Cu-Co thermocouples linked to a multichannel EdgeBox V12 datalogger (Environmental Monitoring Systems, Brno, Czech Republic) was used.

Weather

Air temperature in a close proximity of the three experimental thalli (0.5 cm above) was measured by a Cu-Co thermocouple connected to an EdgeBox datalogger (Environmental Monitoring Systems, Brno, Czech Republic), Similarly, soil temperature in the depth of 2 cm was measured in 5 min. interval. Thus, daily courses of the above-specified temperatures were recorded and analyzed for the OTC and the outside control environment.

Field measurements of ETR

Continuous field measurements of chlorophyll fluorescence of *P. antarctica* started at the La Cruz Plateau experimental site on Jan 3rd and ended Jan 16th 2019. A multichannel monitoring fluorometer Moni-PAM (Heinz Walz, Germany) was used to measure diurnal courses of Φ_{PSII} of *Placopsis antarctica*. The system consisted of control and data logging unit (MONI-DA), and two monitoring emitter-detector heads (probes). In our field experiment, we used probes located above two particular measuring spots of *P. antarctica* thalli grown on small stones.

Fig. 1. Experimental set up of the MONI-PAM with measuring probes installed over *Placopsis antarctica* thalli. A - general view on the OTCs located on La Cruz Plateau, B - the same site after snowfall, C, D - detail of the measuring probe on the control plot (C - after a rainfall, D - after a snowfall), E - location of saturation pulse spot on *P. antarctica* thallus, F - detail of the measuring probe and Cu-Co thermocouple measuring air temperature at the measuring spot inside OTC. Photo © M. Barták.

One of the probes measured the epilithic lichen thallus located in open top chamber, the second one measured the thallus located outside OTC (control sample). The probes with sample clips mounted at a distance of 25 mm from the probe´s end formed an optical window arranged in the angle of 45° between the probe longitudal axis and the sample. The optical window was a metal frame with a spot of white area (radiation sensor) measuring incident photosynthetically active irradiance. The optical window was placed directly on the stone surface with *P. antarctica* (*see* Fig. 1). Such set up allowed to use repetitive saturation pulse method. The pulses of light (duration 1 s, intensity 3 500 μmol m^{-2} s^{-1}) were applied on *P. antarctica* thalli in light-adapted state (natural irradiation at the site following daily courses) each 10 min. In such a way, steady state chlorophyll fluorescence (F_S) and maximum chlorophyll fluorescence induced by a saturation pulse applied in light-adapted state (F_M') were measured and stored in the MONI-PAM memory.

The data allowed the calculation of effective quantum yield (Φ_{PSII}) using the equation $\Phi_{PSII} = (F_M' - F_S) / F_M'$. Using particular Φ_{PSII} values, Moni-PAM software calculated photosynthetic electron transport rates (*ETR*) for each single measurement and stored data on PAR and sample temperature (*Ts*). These data were plotted versus time and used for construction of daily courses of *ETR*, PAR and *Ts*.

Fig. 2. Experimental site with OTCs on La Cruz Plateau after snow fall (left). Detailed view on the OTC taken immediatelly after a snowfall (right). Note that majority of freshly depositted snow is over the ground outsite OTC while inside the OTC there is hardly any snow. Photo © M. Barták.

Data processing

To analyze the differences in primary photosynthetic processes in *P. antarctica* between OTC and the outside plot (control), Φ_{PSII} and *ETR* data were analysed by a modification of the diurnal regression method (Durako 2012) for each plot (OTC, control). Light response curves (*ETR* to PPFD) were plotted and the inicial slope (alpha parameter) of the relationship was calculated for the PPFD interval 0-100. The alpha parameter was considered a measure of the light harvesting effectivity of the chloroplastic photosynthetic apparatus (Belshe et al. 2008). The asymptote of the *ETR* to PPFD curve was calculated for OTC thalli to estimate the maximum rate of *ETR* (*ETRmax*) which was considered a measure of PS II capacity to utilize the absorbed light energy in photosynthetic processes (Marshall et al. 2000).

Intrathalline chlorophyll fluorescence heterogeneity

In laboratory experiment, chlorophyll (Chl) fluorescence was measured on *P. antarctica* thalli with a portable kinetic fluorescence camera FluorCam HFC 1000-H (Photon Systems Instruments, Czech Republic) equipped with an imaging system (software FluorCam *v.*7.0). The light source consisted of four panels, each with 42 super-bright orange light-emitting diodes (LEDs, lambda = 620 nm). The LEDs generated measuring light, actinic light and saturation pulses in order to induce (1) basic Chl fluorescence signal (F_0), (2) variable Chl fluorescence on light-adapted sample (F_V) and (3) maximum Chl fluorescence on dark- (F_M) and light-adapted sample (F_M). Temporal variation in Chl fluorescence signals (slow Kautsky kinetics of chlorophyll fluorescence) of *P. antarctica* were detected by a CCD camera with F1/1.4 objective, and recorded as false colour images with maximum recording rate of 20 ms. The images showed the actual level of Chl fluorescence for each pixel. Measurements followed the protocol optimized for lichens (*see e.g.* Barták et al. 2018) and started with F_0 determination in dark-adapted thalli followed by F_M determination after the saturation pulse of high light (PPFD of 1 100 μmol m^{-2} s^{-1}). Then, after 20 s in darkness, 65 μmol m^{-2} s^{-1} of actinic light was applied for 300 s to induce the variable Chl fluorescence to reach a steady-state (F_S) at the end of 5 min. period. Finally, another pulse of saturating light was applied to reach the maximum Chl fuorescence in light-adapted state (F_M'). After switch off the actinic light, another saturation pulse was applied and F_M'' Chl fluorescence level determined. Chlorophyll fluorescence parameters (F_M, F_V/F_M, and Φ_{PSII}), were calculated for each pixel of the area taken by *P. antarctica* thallus in the output image. To analyse the effect of different photobionts in central (*Nostoc commune* in cephalodium) and marginal thallus (possessing green microalga) parts, 5 subareas were selected in central and marginal parts and analyzed. Time courses of slow Kautsky kinetics of Chl fluorescence as well as the values of Φ_{PSII} were related to hydration status of *P. antarctica.*

Results

Field measurements

Microclimate inside OTCs was affected by the construction of the OTC. Photosynthetic photon flux density (PPFD) reached lower values in OTCs thanks to the absorption by the OTC walls (Fig. 2). The OTC-caused decrease in PPFD values was found both for sunny and overcast days. Air temperature of the measuring sites is shown in Table. 1. The OTC effect on the temperature shift is exhibited in daily mean air temperature, as well as daily minima/ maxima. In the period of measurements of photosynthetic activity of *P. antarctica*, the OTC microenvironment was 1.4°C to 3.3°C warmer (difference of daily means taken for the measuring spot, for data *see* Table 1). OTC also increased ground temperature. The difference between ground temperature inside OTC and the outside control plot was more pronounced on sunny calm days (delta T of 2.2°C) while it was lower on overcast windy days (Table 2).

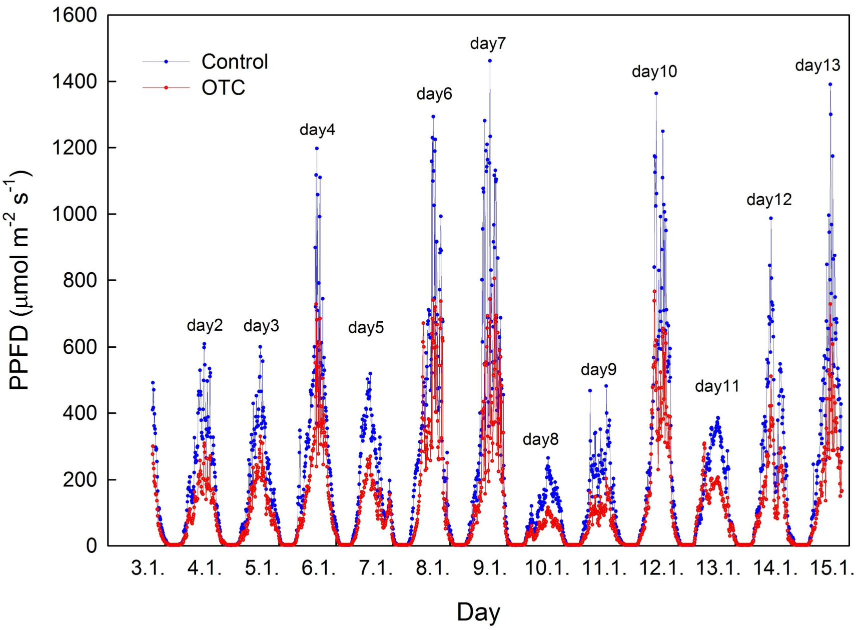

Fig. 3. Daily courses of photosynthetic photon flux density (PPFD) measured 1 mm above the experimental lichen thalli (Chlorophyll fluorescence measuring spot). Blue – outside control, red – inside OTC.

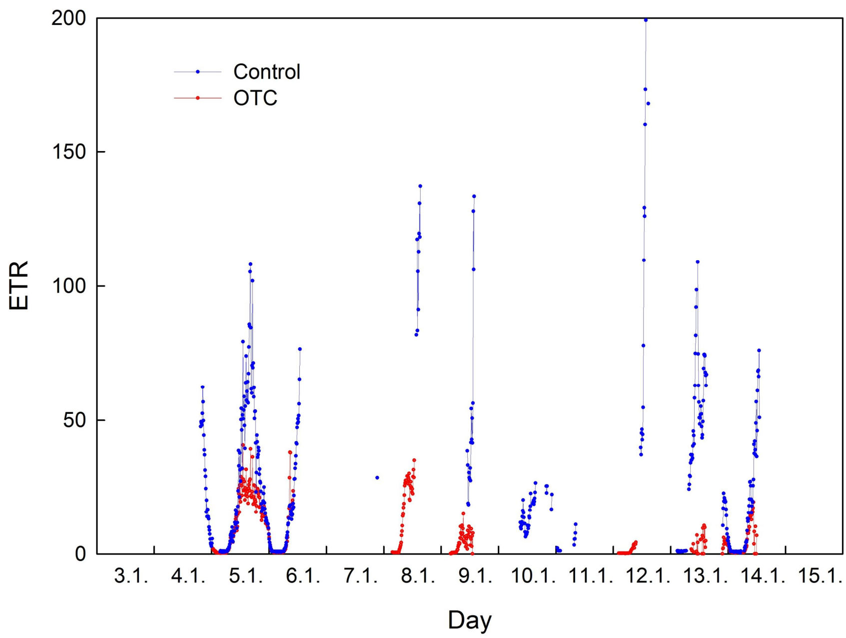

Fig. 4. Photosynthetic electron transport rate (*ETR*) in *Placopsis antarctica* measured for sample thalli located in open top chambers (red symbols) and outside control plots at the (blue symbols) measured in January 2019 at the Fildes peninsula, Antarctica.

		OTC Daily air		Control Daily air		OTC		Control	
Day	Date	mean T	std	mean T	std	min	max	min	max
1	3.1.2019	**0.88***	± 3.24	**-1.56***	± 2.60	n.d.	8.4	n.d.	4.6
2	4.1.2019	**-1.83**	± 2.54	**-3.99**	± 1.66	-5	4.7	-6	0.2
3	5.1.2019	**-1.90**	± 2.98	**-3.80**	± 2.16	-5.2	4.7	-6	0.6
4	6.1.2019	**-1.96**	± 3.32	**-4.16**	± 2.27	-5.6	5.4	-7	1.6
5	7.1.2019	**-2.81**	± 4.14	**-5.08**	± 2.70	-6.5	7.2	-7.5	0.9
6	8.1.2019	**0.46**	± 5.55	**-1.96**	± 3.99	-5.2	15.2	-6.3	7
7	9.1.2019	**0.88**	± 5.94	**-1.95**	± 4.02	-5.2	14.3	-6.3	7
8	10.1.2019	**-3.35**	± 1.24	**-4.74**	± 0.97	-5.2	0.6	-6.5	-2.1
9	11.1.2019	**-3.64**	± 1.51	**-5.08**	± 1.28	-5.2	1.3	-6.5	-1.1
10	12.1.2019	**1.10**	± 7.06	**-2.20**	± 4.37	-5.2	17.9	-6.3	8.5
11	13.1.2019	**-3.44**	± 1.65	**-5.03**	± 1.33	-5.4	0.6	-6.5	-2.7
12	14.1.2019	**-1.94**	± 3.24	**-3.56**	± 2.44	-5.2	6.3	-6	2.7
13	15.1.2019	**-1.25**	± 3.76	**-3.06**	± 2.95	-5.4	7.5	-6.5	4.1

Table 1. Temperature characteristics of the sites of chlorophyll fluorescence measurements (the measuring spots). Daily mean temperature for OTC and control sites, as well as daily maxima and minima are presented. The means are calculated from daily data taken in 10 min. interval. The means with an asterics are not calculated for the whole day data. *Key to the abbreviations*: n.d. - not determined.

Soil temperature (°C) (2 cm depth)	**Fully sunny days** January 8, 9, 12	**Partly sunny days** January 5, 7, 14	**Overcast days** January 10, 11, 13
Control	3.95 ± 0.35	2.2 ± 0.18	1.2 ± 0.09
OTC	6.15 ± 0.05	3.5 ± 0.28	2.17 ±0.12
Difference (OTC-control)	2.2 ± 0.33	1.3 ± 0.29	0.87 ± 0.25

Table 2. Daily mean soil temperature (2 cm) for sunny, intermediate, and overcast days measured in the OTC and outside control plot in January 2019 La Cruz plateau, King George Island.

Measurements of *ETR* documented the fact that during the experimental period, full daily course was recorded only for a single day (Jan 5th, *see* Fig. 4) when lichen thalli remained wet for whole day thanks to previous precipitation (rainfall and snow for 30 h). For the particular day (Jan 5th), values of *ETR* were found higher by the factor of about 2 for control than OTC-located lichen thallus. Maximum *ETR* reached 110 and 40 for control an OTC-located thalli respectively. On the other days of the experimental period that were typical by gradual desiccation of lichen thalli from early morning to midday, *ETR* was found higher and generally longer-lasting in control then OTC-located thalli. Since the OTC -located lichen desiccated faster thanks to higher air temperature and lower relative air humidity (compared to control), the *ETR* values were found typically only in the first part of day (light period) and then, later within a single day, they reached zero (*see* Jan 8th, 9th, and 12th). *ETR* in control lichen thallus was typically higher and lasting longer (until midday). Therefore, thanks to thallus hydration and higher PPFD availability during midday (compared to the early-morning hours), *ETR* reached high values on Jan 8th, 9th, and 12th (140, 135 and 200 – *see* Fig. 3). The key role of sufficient hydration of lichen thalli was demonstrated on Jan 9th, when, thank to only limited precipitation, *ETR* was detectable in hydrated control lichen thallus, while the OTC-located one remained dry and physiologically inactive. This was caused by the fact that vegetation in OTC, thanks to wind direction, did not receive precipitation. It was caused by the OTC construction, specifically by shielding by OTC wall that did not allow precipitation to reach the site of lichen placement (and fluorometric measurements) inside the OTC.

In OTC-located lichen, *ETR* recorded at the day parts with PAR above 100 (partly sunny and sunny days) was limited by unsufficient hydration. Maximum *ETR* at such situations ranged between 25 and 40, while much higher ETR values were reached in control thallus located outside OTC (*see* Fig. 5). Indeed, *ETR* in control *P. antarctica* increased linearly with PAR increase, while OTC-located thallus showed more or less constant *ETR* values at the PPFD above 150. Higher efficiency of the control thallus (compared to the OTC-located one) might be documented by alpha parameter (the initial slope of *ETR* to PPFD relationship which was much higher in control (0.364) than OTC-located thallus (0.218).

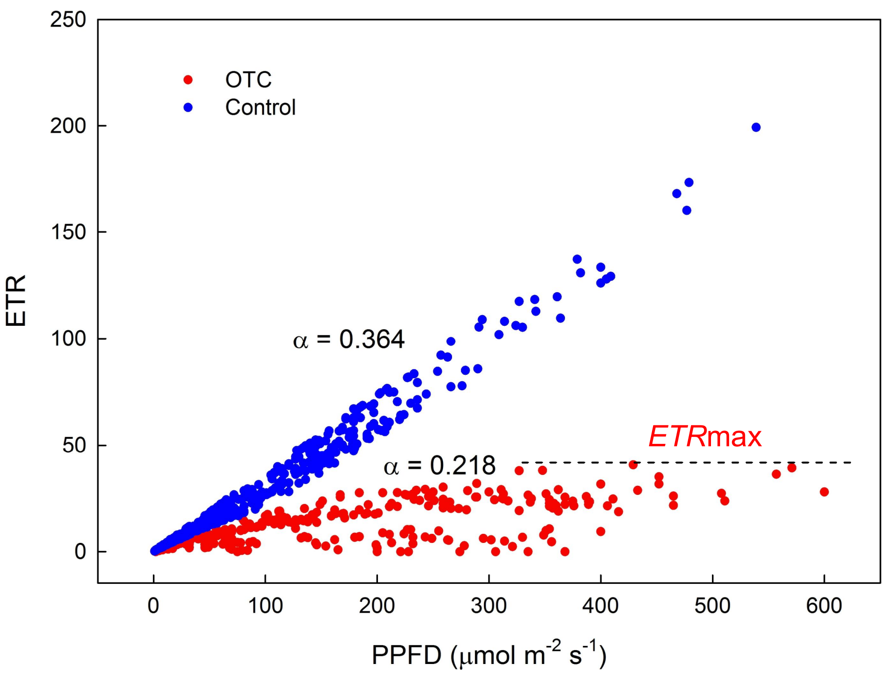

Fig. 5. Relation of photosynthetic electron transport rate (ETR) to light (PPFD) in *Placopsis antarctica* measured for sample thalli located in open top chambers and outside control plots at the Fildes peninsula, Antarctica. *ETRmax* for *P. antarctica* located inside OTC was 40, while it was much higher in control thalli.

Laboratory measurements

Intrathalline heterogeneity of chlorophyll fluorescence

Distribution of photosynthetic processes within the thalli of *P. antarctica* reflected location of the two photobionts: *Nostoc commune* in central cephalodium (deep blue central spot in Fig. 6) and green microalga in marginal thalli parts. Upon rehydration from fully dry state, *P. antarctica* was able to restore photosynthetic activity even after 1 h (the upper panels) which was demonstrated both by maximum chlorophyll fluorescence (F_M of 46.7 and 207.4 mV for cephalodium and algal part, respectively) and the values of effective quantum yield of PS II (0.14 and 0.54 for cephalodium and algal part, respectively).

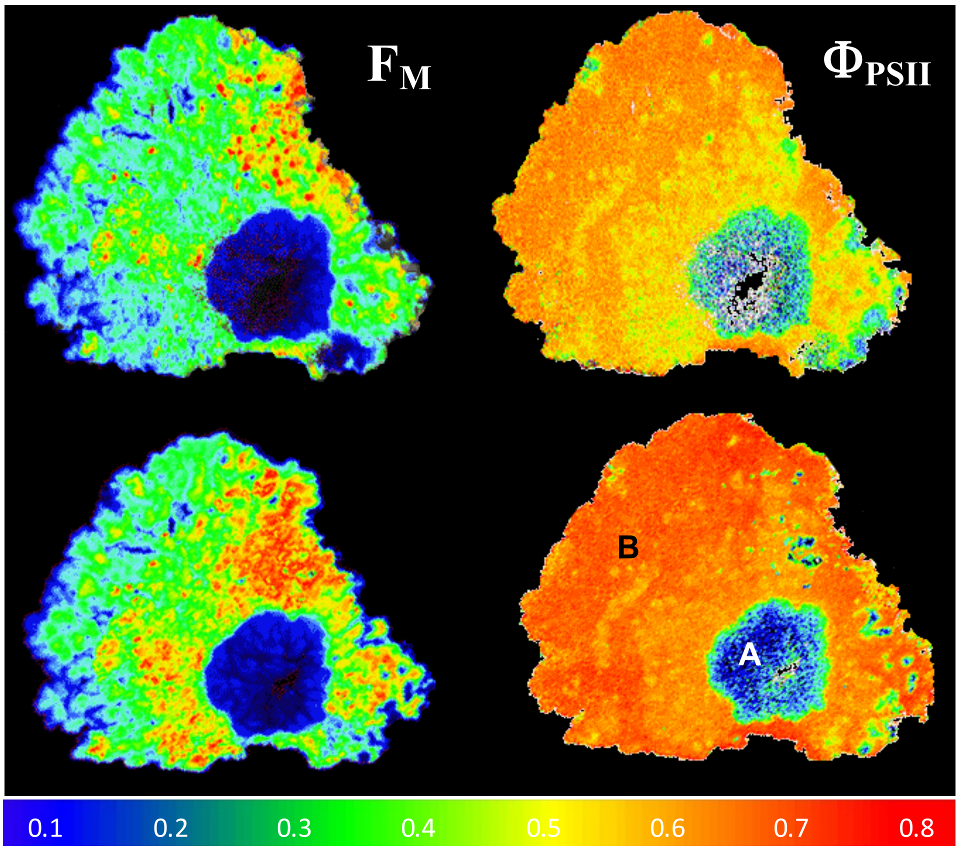

Fig. 6. Chlorophyll fluorescence imaging of *P. antarctica* thallus hydrated for 1 h (upper panels) and 48 h (lower panels). False colour scale indicates low to high values of particular parameters (blue-green-yellow-orange-red), spectrum color bar represents the values of effective quantum yield of PS II (Φ_{PSII}). *Key to the symbols and abbreviations*: F_M - maximum chlorophyll fluorescence induced by saturation pulse, Φ_{PSII} - effective quantum yield of photosystem II, A - central part of thallus (cephalodium) possessing *Nostoc commune*, B - marginal part of the thallus possessing green microalga.

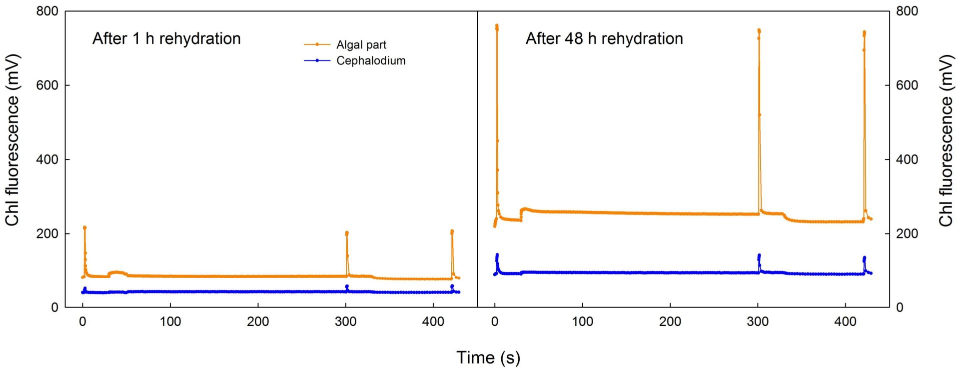

Fig. 7. Kautsky kinetics recorded after 1 h rehydration (left panel) and after 48 h of rehydration (right panel). Orange line - algal part of the thallus, blue line - *Nostoc commune* containing cephalodium.

Discussion

Passive warming by OTC led to an increase in air and ground temperature, which is consistent with earlier observation from OTC site located in Juan Carlos Point, Fildes Peninsula, King George Island (Kim et al. 2018). OTC-induced warming of ground surface and soil profile leads to an increase in bacterial and fungal biomass in OTC. The increase is achieved thanks to increased microbial degradation activity for soil organic matter. For poikilohydric autotrophs forming vegetation cover (mosses, lichens and other biological soil crusts components), passive warming in OTC leads to generally higher temperature of the photosynthetizing organisms, however, it may also reduce the physiologically active time thanks to accelerated dehydration and loss of physiological activity.

We report limitation of primary photosynthetic processes in OTC-located *Placopsis antarctica* in austral summer period (compared to the control thalli outside the OTCs). The phenomenon is caused by faster loss of water from lichen thallus thanks to elevated temperature inside OTC. The other factor is that in outside control, thalli of *P. antarctica* remain hydrated and photosynthetically active in those periods of daytime in which high PPFD values are available. OTC-located thalli are typically dry in such periods. Therefore, control thalli may benefit in terms of higher *ETR* (than OTC thalli) and, consequently, photosynthetic CO_2 fixation. These factors may be considered the main reasons for decreased vigor observed ocularly after 1 year exposition on OTC-located thalli (the exposition time 2018-2019). Therefore, OTC environment, mainly altered precipitation due to OTC construction and generally warmer surface, might be the reason for limitation of growth in OTC-located lichens. Water availability was limiting factor for photosynthesis in OTC-located lichens. Accelerated drying, as a consequence of warming of Antarctic terrestrial ecosystems has been identified by Stanton et al. (2013) as key factor limiting photosynthetic performance of mosses *Ceratodon purpureus* and *Schistidium antarctici*. Similarly, Casanova -Katny et al. (2016) reported decreased production of sporophytes of *Hennediella antarctica* at La Cruz Plateau (9.3 ± 5 in controls compared to 5.5 ± 3.4 in OTCs) which could be due to the decrease in mean daily relative humidity as a consequence of warming induced by the OTCs.

Our study proved that field chlorophyll fluorescence study is an efficient tool to indicate key factors for photosynthesis, its limitation by thallus hydration status, respectively in poikilohydric Antarctic autotrophs. As shown in earlier studies (*e.g.* Leisner et al. 1997, Green et al. 2002), the measurements of Φ_{PSII} and *ETR* may monitor the physiological activity time (Schroeter et al. 2011, 2017) and daily courses of photosynthetic processes in lichens. To quantify net photosynthetic CO_2 uptake from Φ_{PSII} and *ETR* data, however, is not possible from chlorophyll fluorescence data because net CO_2 uptake (measured gasometrically) is not linearly related to *ETR* (chlorophyll fluorescence) - Green et al. (1998). Moreover, in water suprasaturated moss/lichen thalli net photosynthesis is inhibited while *ETR* not (Pannewitz et al. 2005). In higher plants under physiological conditions, effective quantum yield of photosynthetic processes in PS II (Φ_{PSII}) is well correlated to net photosynthetic rate, effective quantum yield of CO_2 fixation in particular (Krall and Edwards 1992). In lichens, however, the relation is linear and with maximum slope only when measured under optimum hydration. If lichen thalli under different degree of desiccation are considered, lower of slope of Φ_{PSII}/Φ_{CO2} ratio and a high variation in Φ_{CO2} values can be seen. Light is another factor affecting CO_2 fixation of lichen thallus. Photosynthetic rates are only proportional to absorbed light energy at low light levels. Under medium to high light, or when desiccation limits photosynthesis, excess light energy can be transferred from photo-excited pigments onto reactive oxygen species (Kranner et al. 2008). Under such circumstances, imbalance between primary photochemical and secondary biochemical processes of photosynthesis appears. Therefore, high *ETR* values found for control *P. antarctica* thalli at the PPFD over 400 μmol m^{-2} s^{-1} may overestimate. In *ETR* calculation, absorption coefficient play an important role. In higher plants, it varies in the range 0.81-0.87 (Rosenqvist and van Kooten 2003) and is used for *ETR* evaluation in field studies (*e.g.* Yang et al. 2014). In lichens, absorption coefficient is generally unkown (Sundberg et al. 1997, Lakatos et al. 2006), however several attempt have been done to evaluate (*e.g.* Ritchie 2014). Lack of knowledge on numeric value of the absorption coefficient in particular lichen species leads to calculation of relative *ETR* which uses the equation without absorption coefficient (ETR_{rel} = Φ_{PSII} * PPFD * 0.5, see *e.g.* Gauslaa et al. 2017). It is believed that absorption coefficient in lichens has lower values than in vascular plants. This is particularly true for non-cortical lichens in which the absorption coefficient of 0.7 is reported (Pardow et al. 2010). The same authors, however, reported 0.84 for cortical lichens. For the lichens of genus *Placopsis,* an information on numeric value of the absorption coefficient is missing. Therefore, we used numerical value of 0.84 for *ETR* calculation as recommended by Nimis et al. (2002).

Our data from the chlorophyll fluorescence imaging visualized photobiont-related heterogeneity of primary photochemical processes of photosynthesis in *P. antarctica* in wet and dry state. Similarly to Schroeter (1994), chlorophyll fluorescence parameters were found different in the green algal (outer) and the cyanobacterial thallus parts (central). In the thallus of lichens of genus *Placopsis*, there are specially modified structures called cephalodia that completely enclose colonies of cyanobacteria as secondary symbionts. Cyanobacterial symbionts have been shown to be capable of both carbon and nitrogen fixation (*e.g.* Rai. 1990). Case study done on three species of *Placopsis* in southern Chile (Raggio et al. 2012) found positive correlation of intrathalline N content to maximum photosynthetic rate of the green alga which is considered to be main dominant photosynthetizing partner. This was documented by our measurements of Φ_{PSII} in fully hydrated *P. antarctica*. Higher Φ_{PSII} values were found in algae-possessing than cyanobacterial (cephalodium) thallus part (*see* Fig. 5). Partial hydration of lichen thallus leads to a decrease in Φ_{PSII}, however, the response depends on species (Barták et al. 2015), intrathalline heterogeneity of photobiont cells, their age and effectivity in particular, and relative water content in a lichen thallus. It is well established that in desiccating lichens, Φ_{PSII} decreases slowly with gradual atmospheric desiccation (*e.g.* Schroeter et al. 1999, Barták et al. 2005). Then, with progressive desiccation, Φ_{PSII} decrease is accelerated at the relative water content below 25% and full inhibition of Φ_{PSII} is found at 5% (critical point) - Barták et al (2018). For *Placopsis* sp., however, such relation has not yet been investigated and is a matter of the forthcoming studies (for *P. antarctica*, Barták and Casanova-Katny, MS in prep.).

References

BAKER, N. R. (2008): Chlorophyll fluorescence: A probe of photosynthesis in vivo. *Annual Review of Plant Biology*, 59: 89-113.

BARTÁK, M., HÁJEK, J., MORKUSOVÁ, J., SKÁCELOVÁ, K. and KOŠUTHOVÁ, A. (2018): Dehydration induced changes in spectral reflectance indices and chlorophyll fluorescence of Antarctic lichens with different thallus color, and intrathalline photobiont. *Acta Physiologiae Plantarum*, 40: (Publication No. 177). https://doi.org/10.1007/s11738-018-2751-3.

BARTÁK M., VÁCZI P. and HÁJEK J. (2012): Photosynthetic activity in three vascular species of Spitsbergen vegetation during summer season in response to microclimate. *Polish Polar Research*, 33(4): 443-462.

BARTÁK, M., TRNKOVÁ, K., HANSEN, E. S., HAZDROVÁ, J., SKÁCELOVÁ, K., HÁJEK, J. and FORBELSKÁ, M. (2015): Effect of dehydration on spectral reflectance and photosynthetic efficiency in *Umbilicaria arctica* and *U. hyperborea*. *Biologia Plantarum*, 59: 357-365.

BARTÁK, M., VÁCZI, P. (2014): Long-term fluorometric measurements of photosynthetic processes in Antarctic moss *Bryum* sp. during austral summer season. *Czech Polar Reports*, 4: 63-72.

BARTÁK, M. (2014): Lichen photosynthesis. Scaling from the cellular to the organism level. *In*: M. F. Hohmann-Marriott (ed.): The Structural Basis of Biological Energy Generation. Advances in Photosynthesis and Respiration. Dordrecht, Springer, pp. 379-400.

BARTÁK, M., GLOSER, J. and HÁJEK, J. (2005): Visualized photosynthetic characteristics of the lichen *Xanthoria elegans* related to daily courses of light, temperature and hydration: a field study from Galindez Island, maritime Antarctica. *Lichenologist*, 37: 433-443.

BARTÁK, M., PLÁTENÍKOVÁ, E. CARERRAS, H., HÁJEK, J., MATEOS, A. C. and MAREČKOVÁ, M. (2018): Effect of UV-B radiation on the content of UV-B absorbing compounds and photosynthetic parameters in *Parmotrema austrosinense* from two contrasting habitats. *Plant Biology* (Stuttg), 20: 808-816.

BECK, A., BECHTELER, J., CASANOVA-KATNY, A. and DZHILYANOVA, I. (2019) The pioneer lichen *Placopsis* in maritime Antarctica: Genetic diversity of their mycobionts and green algal symbionts, and their correlation with deglaciation time. *Symbiosis*, pp. 1-24, https://doi.org/10.1007/s13199-019-00624-4.

BELSHE, F., DURAKO, M. J. and BLUM, J.E. (2008): Diurnal light curves and landscape-scale variation in photosynthetic characteristics of *Thalassia testudinum* in Florida Bay. *Aquatic Botany*, 89: 16-22.

BOKHORST, S., HUISKES, A., CONVEY, P. and AERTS, R. (2007): The effect of environmental change on vascular plant and cryptogam communities from the Falkland Islands and the Maritime Antarctic. *BMC Ecology*, 7: 15.

BOKHORST, S., HUISKES, A. H. L., CONVEY, P., SINCLAIR, B. J., LEBOUVIER, M., VAN DE VIJVER, B. and WALL, D. H. (2011): Microclimate impacts of passive warming methods in Antarctica: implications for climate change studies. *Polar Biology*, 34(10): 1421-1435, https://doi.org/10.1007/s00300-011-0997-y.

CASANOVA-KATNY, A., PIZARRO, M., CABALLERO, M. M., CORDERO, R. and ZÚÑIGA, G. E. (2015): Non-structural carbohydrate content in cryptogamic Antarctic species after two years of passive warming on the Fildes Peninsula. *Czech Polar Reports,* 5: 88-98.

CASANOVA-KATNY, A., TORRES-MELLADO, G. A. and EPPLEY, S. M. (2016): Reproductive output of mosses under experimental warming on Fildes Peninsula, King George Island, maritime Antarctica. *Revista Chilena de Historia Natural*, 89: 13.

DURAKO, M. J. (2012): Using PAM fluorometry for landscape-level assessment of Thalassia testudinum: Can diurnal variation in photochemical efficiency be used as an ecoindicator of seagrass health? *Ecological Indicators*, 18: 243-251.

FIGUEROA, F. L., CONDE-ÁLVAREZ, R., BONOMI BARUFI, J., CELIS-PLÁ, P. S. M., FLORES, P., MALTA, E. J., STENGEL, D. B., MEYERHOFF, O. and PÉREZ-RUZAFA, A. (2014): Continuous monitoring of *in vivo* chlorophyll a fluorescence in *Ulva rigida* (Chlorophyta) submitted to different CO_2, nutrient and temperature regimes. *Aquatic Biology*, 22: 195-212.

GALLOWAY, D. J., LEWIS-SMITH, R. and QUILHOT, W. (2005): A new species of *Placopsis* (Agyriaceae: Ascomycota) from Antarctica. *The Lichenologist*, 37(4): 321-327.

GAUSLAA, Y., SOLHAUG, K. A. and LONGINOTTI, S. (2017): Functional traits prolonging photosynthetically active periods in epiphytic cephalolichens during desiccation. *Environmental and Experimental Botany*, 141: 83-91.

GENTY, B., BRIANTAIS, J. M. and BAKER, N. R. (1989): The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. *Biochimica et Biophysica Acta (BBA) - General Subjects*, 990: 87-92. https://doi.org/10.1016/S0304-4165(89) 80016-9.

GREEN, T. G. A., SCHROETER, B., KAPPEN, L., SEPPELT, R. D. and MASEYK, K. (1998): An assessment of the relationship between chlorophyll fluorescence and CO_2 gas exchange from field measurements on a moss and lichen. *Planta*, 206: 611-618.

GREEN, T. G. A., SCHLENSOG, M., SANCHO, L. G., WINKLER, B., BROOM, F. D. and SCHROETER, B. (2002): The photobiont determines the pattern of photosynthetic activity within a single lichen thallus containing cyanobacterial and green algal sectors (photosymbiodeme). *Oecologia*, 130: 191-198.

HOVENDEN, M. SEPPELT, R. D. (1995): Utility of modulated fluorescence in measuring photosynthetic activity of Antarctic plants: Field and laboratory studies. *Functional Plant Biology*, 22: 321-330.

KIM, D., PARK H. J., KIM, J. H., YOUN, U. J., YANG, Y. H., CASANOVA-KATNY, A., VARGAS, C. M., VENEGAS, E. Z., PARK, H. and HONG, S. G. (2018): Passive warming effect on soil microbial community and humic substance degradation in maritime Antarctic region. *Journal of Basic Microbiology*, 58: 513-522.

KOLARI, P., CHAN, T., PORCAR-CASTELL, A., BÄCK, J., NIKINMAA, E., and JUUROLA, E. (2014): Field and controlled environment measurements show strong seasonal acclimation in photosynthesis and respiration potential in boreal Scots pine. *Frontiers in Plant Science*, 5: 717.

KRALL, J. P., EDWARDS, G. E. (1992): Relationship between photosystem II activity and CO_2 fixation in leaves. *Physiologia Plantarum*, 86: 180-187.

KRANNER, I., BECKETT, R., HOCHMAN, A. and NASH, T. H. (2008): Desiccation-tolerance in lichens: A Review. *The Bryologist*, 111: 576-593.

LAKATOS, M., RASCHER, U. and BÜDEL, B. (2006): Functional characteristics of corticolous lichens in the understory of a tropical lowland rain forest. *New Phytologist*, 172: 679-695.

LEISNER, J. M. R, GREEN, T. G. A. and LANGE, O. L. (1997): Photobiont activity of a temperate crustose lichen: Long-term chlorophyll fluorescence and CO_2 exchange measurements in the field. *Symbiosis*, 23(2): 165-182.

MARION, G. M., HENRY, G. H. R., FRECKMAN, D.W., JOHNSTONE, J., JONES, G., JONES, M. H., LÉVESQUE, E., MOLAU, U., MØLGAARD, P., PARSONS, A.N., SVOBODA, J. and VIRGINIA, R. A. (1997): Open-top designs for manipulating field temperature in high-latitude ecosystems. *Global Change Biology*, 3: 20-32.

MARSHALL, H. J., GEIDER, R. J. and FLYNN, K. J. (2000): A mechanistic model of photoinhibition. *New Phytologist*, 145: 347-359.

NIMIS, P. L., SCHEIDEGGER, CH. and WOLSELEY, P. A. (2002): Monitoring with lichens–monitoring lichens. *The Bryologist*, 105: 408.

OLECH, M. (2010): Responses of Antarctic tundra ecosystem to climate change and human activity. *Papers on Global Change IGBP*, 1(17): 43-52.

PANNEWITZ, S., GREEN, T. G. A., MAYSEK, K., SCHLENSOG, M., SEPPELT, R., SANCHO, L. G., TÜRK, R. and SCHROETER, B. (2005): Photosynthetic responses of three common mosses from continental Antarctica. *Antarctic Science*, 17: 341-352.

PARDOW, A., HARTARD, B. and LAKATOS, M. (2010): Morphological, photosynthetic and water relations traits underpin the contrasting success of two tropical lichen groups at the interior and edge of forest fragments. *AoB Plants*. 2010: plq004.

PORCAR-CASTELL, A., PFUENDEL, E., KORHONEN, J. F. J. and JUUROLA, E. (2008): A new monitoring PAM fluorometer (MONI-PAM) to study the short-and long-term acclimation of photosystem II in field conditions. *Photosynthesis Research*, 96: 173-179.

RAGGIO, J., GREEN, T. G. A., CRITTENDEN, P. D., PINTADO, A., VIVAS, M., PÉREZ-ORTEGA, S., DE LOS RÍOS, A. and SANCHO, L. G. (2012): Comparative ecophysiology of three *Placopsis* species, pioneer lichens in recently exposed Chilean glacial forelands. *Symbiosis*, 56: 55-66. doi: 10.1007/s13199-012-0159-1.

RAGGIO, J., PINTADO, A., VIVAS, M., SANCHO, L. G., BÜDEL, B., COLESIE, C., WEBER, B., SCHROETER, B. and GREEN, T.G. A. (2014): Continuous chlorophyll fluorescence, gas exchange and microclimate monitoring in a natural soil crust habitat in Tabernas badlands, Almería, Spain: Progressing towards a model to understand productivity. *Biodiversity and Conservation*, 23: 1809-1826.

RAI, A. N. (1990): Cyanobacterial ‑ fungal symbioses: the cyanolichens *In*: A. N. Rai (ed.): CRC Handbook of Symbiotic Cyanobacteria. CRC Press, Boca Raton, Florida. pp. 9-41.

RITCHIE, R. J., RUNCIE, J. W. (2014): A portable reflectance-absorptance-transmittance meter for photosynthetic work on vascular plant leaves. *Photosynthetica*, 52: 614-626.

RITCHIE, R. J. (2014): Photosynthesis in an Encrusting Lichen (*Dirinaria picta* (Sw.) Schaer. ex Clem., Physiaceae) and its Symbiont, *Trebouxia* sp., Using Pulse Amplitude Modulation Fluorometry. *International Journal of Plant Science*, 175: 450-466.

ROSENQVIST, E., VAN KOOTEN, O. (2003): Chlorophyll fluorescence: A general description and nomenclature. *In*: J. R. DeEll, P. M. A. Toivonen (eds): Practical Applications of Chlorophyll Fluorescence in Plant Biology, pp. 31-77. https://doi.org/10.1007/978-1-4615-0415-3_2.

SEHNAL, L., BARTÁK, M. and VÁCZI, P. (2014): Diurnal changes in photosynthetic activity of the biological soil crust and lichen: Effects of abiotic factors (Petuniabukta, Svalbard). *Czech Polar Reports*, 4: 158-167.

SHORTLIDGE, E. E., EPPLEY, S. M., KOHLER, H., ROSENSTIEL, T. N., ZÚÑIGA, G. E. and CASANOVA-KATNY, A. (2017): Passive warming reduces stress and shifts reproductive effort in the Antarctic moss, *Polytrichastrum alpinum*. *Annals of Botany*, 119: 27-38.

SCHLENSOG, M., GREEN, T. G. A. and SCHROETER, B. (2013): Life form and water source interact to determine active time and environment in cryptogams: An example from the maritime Antarctic. *Oecologia*, 173: 59-72.

SCHREIBER, U. (2004): Pulse-Amplitude (PAM) fluorometry and saturation pulse method. *In*: G. Papageorgiou and Govindjee (eds.): Chlorophyll fluorescence: A signature of photosynthesis. Springer, Dordrecht, The Netherlands. pp. 279-319.

SCHROETER, B. (1994): *In situ* photosynthetic differentiation of the green algal and the cyanobacterial photobiont in the crustose lichen *Placopsis contortuplicata*. *Oecologia*, 98: 212-220.

SCHROETER, B., GREEN, T. G. A., PANNEWITZ, S., SCHLENSOG, M., and SANCHO, L. G. (2011): Summer variability, winter dormancy: lichen activity over 3 years at Botany Bay, 77°S latitude, continental Antarctica. *Polar Biology*, 34: 13-22.

SCHROETER, B., GREEN, T. G. A., PINTADO, A., TÜRK, R. and SANCHO, L. G. (2017): Summer activity patterns for mosses and lichens in Maritime Antarctica. *Antarctic Science*, 29(6): 517-530. doi: 10.1017/S095410201700027X.

SCHROETER, B., SANCHO, L. G. and VALLADARES, F. (1999): *In situ* comparison of daily photosynthetic activity patterns of saxicolous lichens and mosses in Sierra de Guadarrama, Central Spain. *The Bryologist*, 102 (4): 623-633. doi: 10.2307/3244252.

SPIELMAN, A. A., PERERIA A. B. (2012): Lichens on the Maritime Antarctica (A small field guide for some common species). *GLALIA*, Revista Electrónica del Grupo LatinoAmericano de Liquenólogos, 4: 1-28.

STANTON, D. E., MERLIN, M., BRYANT, G. and BALL, M. C. (2013) Water redistribution determines photosynthetic responses to warming and drying in two polar mosses. *Functional Plant Biology*, 41: 178-186.

STEMKE, J. A., SANTIAGO, L. S. (2011): Consequences of light absorptance in calculating electron transport rate of desert and succulent plants. *Photosynthetica*, 49: 195-200.

SUNDBERG, B., CAMPBELL, D. and PALMQVIST, K. (1997): Predicting CO_2 gain and photosynthetic light acclimation from fluorescence yield and quenching in cyano-lichens. *Planta*, 201(2): 138-145.

YANG, S.-J., SUN, M., ZHANG, Y.-J. and CAO, K.-F. (2014): Strong leaf morphological, anatomical, and physiological responses of a subtropical woody bamboo (*Sinarundinaria nitida*) to contrasting light environments. *Plant Ecology*, 215: 97-109.

ZHA, T.-S., WU, Y. J., JIA, X., ZHANG, M. Y., BAI, Y. J., LIU, P., MA, J. Y., BOURQUE, CH. P.-A. and PELTOLA, H. (2017): Diurnal response of effective quantum yield of PS II photochemistry to irradiance as an indicator of photosynthetic acclimation to stressed environments revealed in a xerophytic species. *Ecological Indicators*, 74: 191-197.