Long-term fluorometric measurements of photosynthetic processes in Antarctic moss *Bryum* sp. during austral summer season

Miloš Barták, Peter Váczi

Department of Plant Physiology and Anatomy, Institute of Experimental Biology, Masaryk University, Kamenice 5, 625 00 Brno, Czech Republic

Abstract

Photosynthetic activity pattern of *Bryum* sp. was monitored for 28 days using a chlorophyll *a* fluorescence measuring system installed in the field. For the study, long-term research plot, a moss-dominated vegetaiton oasis at seashore located close to the J.G. Mendel station (James Ross Island, Antarctica) was selected. In this study, two measuring sites were used: (1) control plot with moss cover and (2) moss located inside open top chamber (OTC). At both sites, effective quantum yield of photosynthetic processes in photosyntem II (Φ_{PSII}) was measured and relative photosynthetic electron transport rate (ETRrel) evaluated each 15 min. Simultaneously, microclimate of the sites was measured including air and moss surface temperature, relative air humidity and photosynthetically active radiation. The length of photosyntetically active period depended mainly on hydration of moss cushion. Water availability, however, was not limiting in the measuring period (Jan 8 - Feb 18, 2009), because the sites were well suplied by melt water from neighbouring snowfield. Thus, daily courses of ETRrel were dependent on incident PAR. On sunny days, ETRrel reached values over 400. Inhibition of primary photosynthetic processes due to below-zero temperature and resulting freezing of moss cushions appeared two times within the measuring periods thanks to rapid decreases in air temperature. The effect of low air temperature on ETRrel was less apparent in OTC site since moss cushion freezing period was shorter and less pronounced than in control site thanks to OTC-induced shift in air temperature. For future photosynthetic studies in Antarctic mosses, simultaneous measurements of gas exchange- and chlorophyll fluorescence-related parameters is recommended so that the effects of particular limiting factors for photosynthesis and photosynthetic productivity can be distinguished and evaluated.

Key words: Antarctics, daily courses, effective quantum yield, electron transport rate, chlorophyll fluorescence, James Ross Island

DOI: 10.5817/CPR2014-1-7

Received April 25, 2014, *accepted* July 29, 2014.

*Corresponding author: Miloš Barták <mbartak@sci.muni.cz>

Acknowledgements: The authors thank J. G. Mendel station for providing infrastructure for field research at the James Ross Island.

Symbols and abbreviations: *e* – electron, ETRrel – photosynthetic electron transport rate, Φ_{PSII} – effective quantum yield of photosynthetic processes in photosystem II, LTRP – long-term research plot, PAR – photosynthetically active radiation, PS II – photosystem II

Introduction

Since the 80-ies of the last century, *in situ* photosynthesis in Antarctic mosses and lichens has been measured using a CO_2 exchange by infra-red gas analysers (*e.g.* Kappen 1989, Kappen et al. 1998, Kennedy 1993). Over last two decades, however, chlorophyll fluorescence covering a wide range of methods has been increasingly involved into photosynthetic research (*e.g.* Roháček et al. 2008) and numerous portable fluorometers developed. Recently, they are available for long-term field measurements (Schreiber et al 1994, Schlensog et Schroeter 2001). However, for extreme conditions, Antarctic environments in particular, no commercial instrument has yet been produced. In spite of the fact that a long-term measurement of photosynthetic activity in polar regions, is challenging for plant ecologists and physiologists, there is a high demand for a robust instrumentation. However, several attempts have been made to adapt commercial instruments for the measurements at harsh conditions. Among them, several instrumental set ups made by prof. Kappen and Assoc. Prof. Schroeter must be mentioned. They pioneered this particular field of science and improved pulse amplitude modulated fluorometers for field conditions (Kappen et al. 1998). Later, some more attempts were made by the international group formed by Australian and German scientists who measured *in situ* photosynthesis in Antarctic lichens using simultaneous gas exchange (CO_2 consumption by infra-red gas analysers) and fluorometric measurements. In the Antarctica, gasometric (*e.g.* Kappen 1989), chlorophyll fluorescence, and combined (*e.g.* Kappen et al. 1998) approach to study *in situ* daily courses of photosynthetic processes in Antarctic mosses has been used only sporadically. To our best knowledge, only several long-term fluorometric studies were published since 2000 focused on Antarctic lichens and mosses (Pannewitz et al. 2005, Schlensog et Schroeter 2001, Schlensog et al. 2013, Schroeter et al., 2010, 2011, 2012).

After the completion of construction of the Czech Antarctic Scientific Station (Mendel station) in 2006 (Prošek et al. 2013), Czech scientist started to measure chlorophyll fluorescence characteristics of Antarctic lichens on long-term basis. Chlorophyll fluorescence approach has been applied both in field- and laboratory-based experiments. The measurements are based on the application of strong light (saturation pulse) on plant material that is adapted to environmental light conditions. Before that date, the measurements performed by the Czech group, were time-limited and dependent on hand operation of instruments in field set ups (Barták 2005, Barták et al. 2005, Barták et al. 2007). Within last couple of years, Czech specialists tested several instrumental set ups and gained experience in technical solutions for field set ups of instruments. Here we report overview and first results from *in situ* fluorometric measurements of photosynthetic processes in *Bryum* sp., a moss species abundant in wet part of Antarctic vegetation oasis at James Ross Island, Antarctica.

Material and Methods

Overview of in situ fluorometric measurements (James Ross Island)

Fluorometric measurements of photosynthesis of Antarctic mosses are a part of the long-term research program aimed to the evaluation of their physiological response to manipulated warming of air and soil temperature. For such a purpose, open top chambers (OTCs) are used to increase air temperature. The OTCs are installed at three different coastal ecosystems of the James Ross Island (coastal terrace, top of a table mountain, glacier forefield) in order to evaluate site-specific response (for more details, *see* Barták et al. 2009). In this paper, we focused on photosynthetic processes in *Bryum* sp., a dominant species of small-area vegetation oasis at a coastal terrace (63° 48′ 00″ S, 57° 52′ 56″ W, 6 m a.s.l.) of the northern coast of James Ross Island. The vegetation oasis is a part of a long-term research plot (LTRP), that was established in January 2007. The first *in situ* measurements of primary photosynthetic processes in *Bryum* sp. started in 2007/2008 austral summer. A modified PAM-210 fluorometer (Heinz Walz, Germany) was installed at LTRP and measured effective quantum yield of photosynthetic processes in photosystem II (Φ_{PSII}) as dependent on hydration/dehydration and microclimatic parameters. In this set up, a tiny fiberoptic cable was used as a probe. The probe end was fitted into a holder placed into a moss cushion. The end of fiberoptics was positioned on the top of the cushion touching gently its upper surface. Values of Φ_{PSII} were measured in 15 min. step for 28 days. However, the measuring system was not found robust enough to perform long-term measurement since the probe suffered from vibrations caused by a wind. Thus, new fluorometric systems were prepared for the 2008/2009 austral summer.

Pulse amplitude modulated fluorometery

In 2009, two different fluorometers were used to measure diurnal courses of Φ_{PSII}: (1) a multichannel monitoring fluorometer Moni-PAM (Heinz Walz, Germany) and (2) FluorPen FL-100 fluorometer (Photon Systems Instruments, Czech Republic) – *see* below. In this paper, results gained by the Moni-PAM fluorometers (Fig. 1) for February 2009 are presented. General technical description of the Moni-PAM system and its operation in the field conditions is given by Porcar-Castell et al. (2008). In our field experiment, we used 2 monitoring heads (located above two particular measuring spots of *Bryum* sp. cusion) connected to a data-collecting unit and an on-line linked PC. Such set up allowed us to use repetitive saturation pulse method. The pulses of light (duration 1 s, intensity 3 500 μmol m^{-2} s^{-1}) were applied on moss thalli in light-adapted state under natural irradiation each 15 min. In such a way, Φ_{PSII} was evaluated repeatedly (Eqn. 1).

$$\Phi_{PSII} = (F'_M - F_S) / F'_M \qquad \textit{Eqn. 1}$$

In Eqn. 1, values of steady state chlorophyll fluorescence (F_S) and maximum chlorophyll fluorescence in light adapted state (F'_M) were used.

Together with data available on photosynthetically active radiation (PAR) incident to measuring spots (Moni-PAM reflective plate and a PAR sensor), photosynthetic electron transport rate (ETRrel) was evaluated and stored in a Moni-PAM data logger.

$$ETRrel = PAR * \Phi_{PSII} \qquad \textit{Eqn. 2}$$

Simultaneously with Moni-PAM fluorometers, FluorPen (FL-100, Photon Systems Instruments, Czech Republic) fluorometers were installed at two additional spots to measure Φ_{PSII}, its long-term changes in particular. Since 2009, Φ_{PSII} in *Bryum* sp. has been measured continuously to evaluate seasonal changes in physiological activity, duration of winter dormancy in particular (Barták 2013).


Fig. 1. View on a fluorometer head installed in the field at the long-term research plot (LTRP). The metallic frame with reflexive white plate (for PAR measuremenst) delimits measuring spot on *Bryum* sp. cushion.

Microclimatological measurements

Microclimate measurements were taken both at the LTRP using EdgeBox V12 data loggers (Environment Measuring Systems, Brno, Czech Republic) equipped with several sensors. Air temperature sensors were located at the height of 30 cm, moss and/or lichen surface, and in soil at the depths of 5, and 10 cm. The sensors were copper-constantan thermocouples for air and moss surface measurements, and platinum sensors (Pt100) for soil temperature measurements. Apart of microclimatic data measured at the LTEP, local climate, specifically global, photosynthetically-active, UV-A, UV-B radiation, reflected radiation, relative air humidity, wind speed and direction, soil temperature at different depths were monitored by a weather station located 250 m away from the LTEP as described elsewhere (Láska et al. 2011).

Results and Discussion

For control plot, temperature of the upper surface of the moss varied within -8.0 and +11.8°C (Fig. 2) for control plot which is a typical range for summer season at the LTRP at James Ross Island (unpublished data). Within the measuring period, proportion of days with maximum moss surface temperature above 5°C was 57.9% (22 out of 38 days). Proportion of days with maximum temperature above 0°C was 84.2%. Therefore, photosynthetic processes in *Bryum* sp. were not inhibited by temperature *per se* during a light period of majority of days. There were two periods of several consecutive days with surface temperature below zero, the first one from Feb. 2nd to Feb 5th, the second one from Fab. 10th to Feb 14th. The first one was typical by medium to high daily doses of PAR and partial negative temperature effect on ETRrel, the second one was typical by low PAR (overcast days – *see* Fig. 3, and freezing temperature). Positive effect of surface temperatute on is apparent also from the differences found between control and OTC measuring site (*see* Fig. 2), where moss surface temperature was higher throughout the whole measuring period. Such conclusion can be supported by data of Davey et Rothery (1996, 1997) who reported substantial photosynthetic rates in Antarctic mosses during summer season at temperature above 5°C. Photosynthetic optima for majority of mosses in Antarctica, however, are higher, ranging typically between 10 and 22°C (Collins 1976). Even wider range for optimum photosynthesis (7 - 23°C) was found in *Sanionia uncinata* at Svalbard by Uchida et al. (2002).

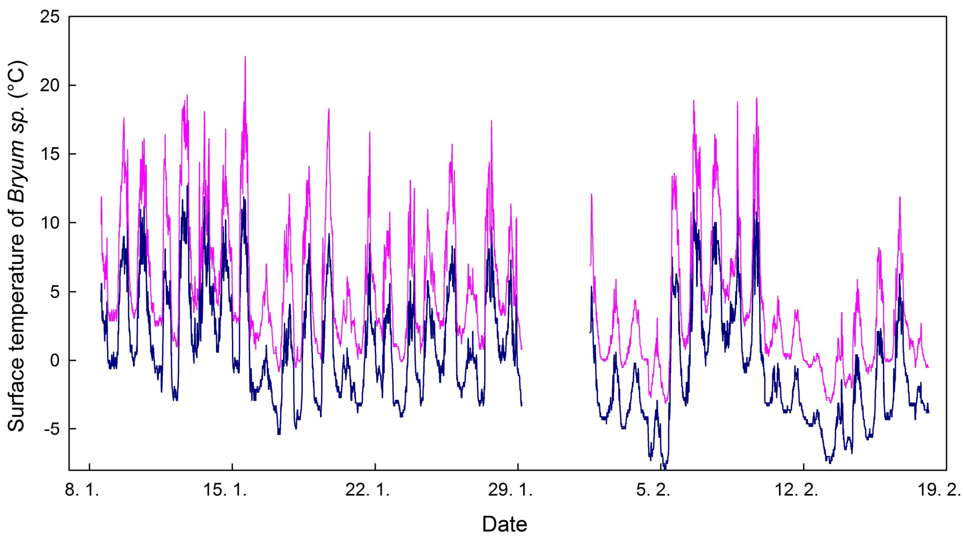

Fig. 2. Surface temperature of *Bryum* sp. moss cushions recorded at the costal experimental plot at the James Ross Island between January 8th and February 18th 2009. Blue line – moss cushion on control plot, violet line – moss cushion inside open top chamber (OTC).

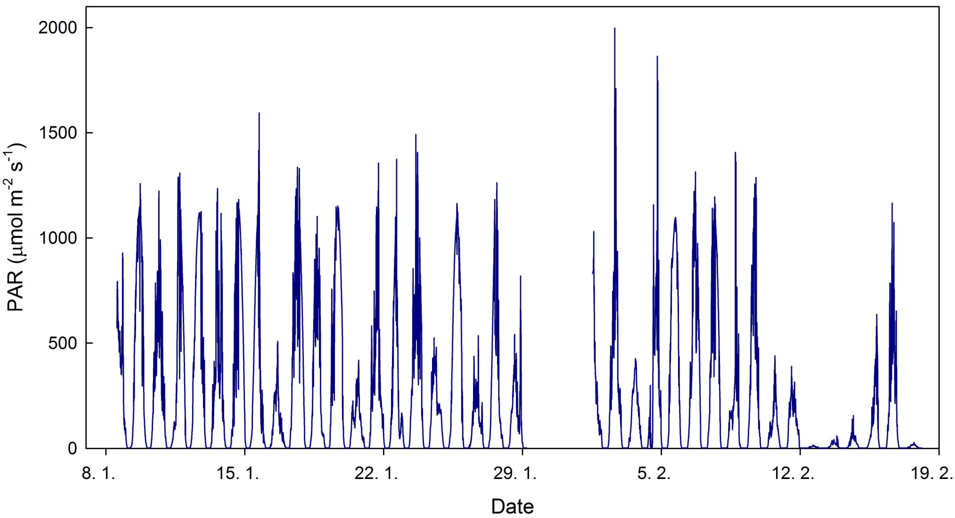

Fig. 3. Diurnal courses of photosyntehtically active radiation (PAR) incident on at the costal experimental plot at the James Ross Island, Antarctica, between January 8th and February 18th 2009.

In January – February 2009, ETRrel in partly/fully hydrated thalli of *Bryum* sp. showed daily courses delimited by night-related zero values (at the site, there is a dark period lasting for 3 h in January – February) and maxima reached during a midday. Within the periods abbreviated A (Jan 8th – Jan 15th) and C (Feb 2nd – Feb 6th, 2009), daily maxima of ETRrel for control site reached the values above 400 in majority of cases (Fig. 4). Such high daily peaks of ETRrel indicated that both values of incident PAR and surface temperature were high enough to allow substantial net photosynthetic rate. The ETRrel maxima values are well comparable to the data reported by Schroeter et al. (2012) who, using an approach of light-response curves of ETRrel, found ETRrel as high as 400 µmol m^{-2} s^{-1} at 900 µmol m^{-2} s^{-1} PAR in sun form of *Bryum argenteum.* In our ETRrel measurements, no water limitation of photosynthetic processes occurred in the A and C periods. Moss cushions were fully hydrated and, especially during the C period, covered by a several centimeters thick column of stagnant melt water.

Throughout the whole period of ETRrel measurements, two drops in air temperature to below zero values were apparent in the periods of January 30 – February 2nd, and February 7th – 15th, 2014. Such rapid and several days lasting drops caused partial and or full inhibition of ETRrel in *Bryum* sp. Photosynthetic processes were inhibited thanks to freezing temperature-dependent decrease of an effective quantum yield of photosynthetic processes in photosystem II to zero. Moreover such situation led to a complete freezing of moss cushions that had been fully wet immediately before the freezing temperature came. The cushions of *Bryum* sp. remained frozen for several consecutive days. At the freezing days, only the upper surface of the cushions exhibited temperature slightly above zero for limited time during a midday (*see* Fig. 2). Such small and short-term increase to above zero temperature, however, did not lead to a substantial increase in ETRrel.

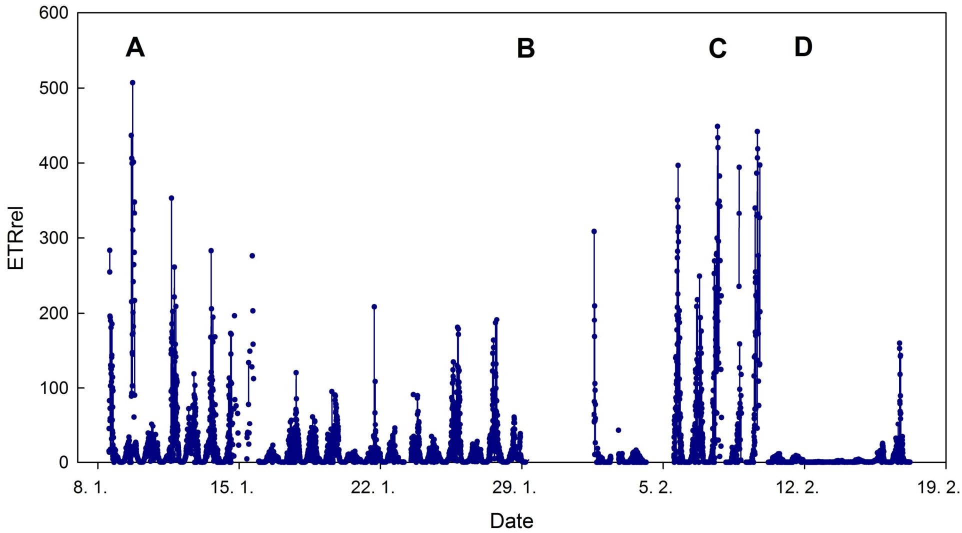

Fig. 4. Diurnal courses of photosynthetic electron transport rate (ETRrel) recorded by a Moni-PAM fluorometer on *Bryum* sp. moss cushions located at the costal experimental plot at the James Ross Island between January 8^{th} and February 18^{th} 2009. Main factors affecting photosynthetic performance are: sunny days with sufficient water availability (A, C), sunny days with partial water and temperature limitation (B), D (cloudy days with freezing temperature and resulting strong inhibition of ETRrel).

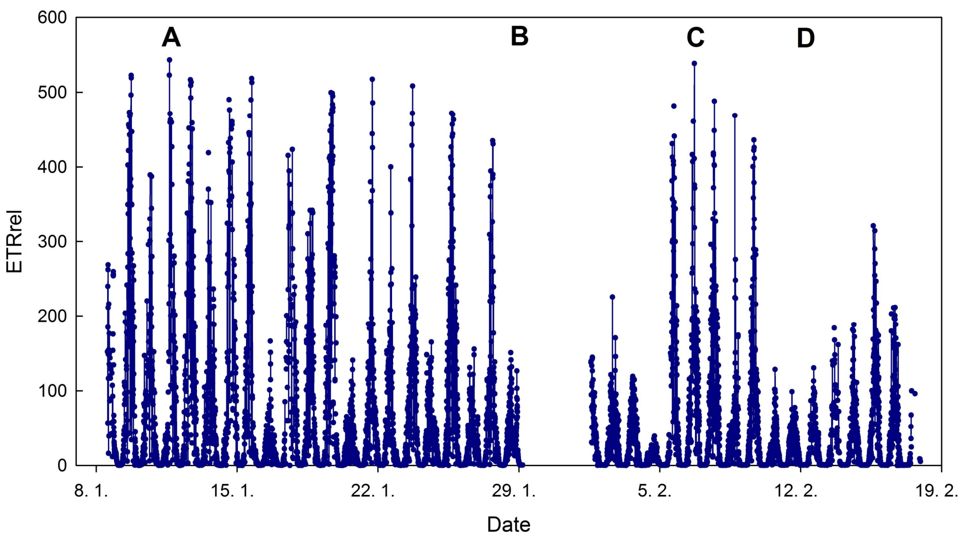

Fig. 5. Diurnal courses of photosynthetic electron transport rate (ETRrel) recorded on *Bryum* sp. moss cushions in open top chamber (OTC) by a Moni-PAM fluorometer (costal experimental plot at the James Ross Island) between January 8^{th} and February 18^{th} 2009.

Concluding remarks

Long-term measurements of photosynthetic activity in *Bryum* sp. gained diurnal courses of electron transport rate (ETRrel) that indicated subperiods of pronounced and/or inhibited photosynthesis in the moss species. From data followes that photosynthetic processes within the period of Jan 8th – Feb 18th 2009 were not limited too much by water unavialibility. That was caused by two main reasons. First, the experimental plot was located within large carpet of *Bryum* sp. of eliptic sape and dimensions 15/4 m in length/width. Such a large area represented important water retention that kept liquid water for quite long time even during a period without precipitation. Second, at the beginning of January, there was small water source available from meeting snowfield in close vicinity of experimental plot. Therefore, water was not limiting factor at least at the beginning of January 2009. Maximum of ETRrel were thus found during the days with highest values of incident PAR (data not shown here).

As shown earlier (*e.g.* Leisner et al. 1997, Green et al. 2002), the measurement Φ_{PSII} and ETRrel represents a tool to monitor the physiological activity time courses of lichens especially hydration and/or dehydration of lichen thalli. To quantify net photosynthesis from Φ_{PSII} and ETRrel data, however, is not possible because net CO_2 uptake (measured gasometrically) is not linearly related to ETRrel (chlorophyll fluorescence) - Green et al. (1998). Moreover, in water suprasaturated lichen thalli net photosynthesis is inhibited while ETRrel not (Pannewitz et al. (2006). Thus, for photosynthesis studies in Antarctic mosses and lichens, simultaneous gas exchange and chlorophyll fluorescence measurements can be recommended. Such trend in photosynthetic studies carried out *in situ* is expected to increase in future (Barták 2014) in orded to evaluate importance of particular physical factors for limitation of photosynthesis in Antarctic mosses and lichens.

References

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., LÁSKA K., PROŠEK P., HÁJEK J. and VÁCZI P. (2009): Long-term study on vegetation responses to manipulated warming using open top chambers installed in three contrasting Antarctic habitats. *In:* M. Barták, J. Hájek, P. Váczi (eds.): Structure and Function of Antarctic Terrestrial Ecosystems. *Book of Abstracts and Contributed Papers*. Conference, Brno, October 22th-23th, 2009. Masaryk University, Brno, Czech Republic, pp. 48-51.

BARTÁK, M., VÁCZI, P., HÁJEK, J. and SMYKLA, J. (2007): Low temperature limitation of primary photosynthetic processes in Antarctic lichens *Umbilicaria antarctica* and *Xanthoria elegans*. *Polar Biology,* 31: 47-51.

BARTÁK, M. (2005): Photosynthesis in Antarctic Lichens in extreme Environments: Plant Stress Physiology Approaches. *In:* Investigating Life in Extreme Environments. Brusel: European Science Foundation, 2005. E-publication, p. 14-14. Sant Feliu de Guixols.

BARTÁK, M. (2013): The effect of climate warming on Antarctic vegetation and its stress physiology. *In*: P. Prošek (ed.): Antarctica. Academia Publishing House, Prague, Czech Republic, pp. 318-322 (in Czech).

COLLINS, N. J. (1976): The growth of mosses in two contrasting communities in the Maritime Antarctic: Measurement and prediction of net annual production. *In*: G. A. Llano, (ed.): Adaptations within Antarctic Ecosystems, Proc. 3rd Symposium on Antarctic Biology, pp. 921-933.

DAVEY, M. C, ROTHERY, P. (1996): Seasonal Variation in Respiratory and Photosynthetic Parameters in Three Mosses from the Maritime Antarctic. *Annals of Botany*, 78: 719-728.

DAVEY, M. C, ROTHERY, P. (1997): Interspecific variation in respiratory and photosynthetic parameters in Antarctic bryophytes. *New Phytologist*, 137: 231-240.

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.

KAPPEN, L. (1989): Field measurements of carbon dioxide exchange of the Antarctic lichen *Usnea sphacelata* in the frozen state. *Antarctic Science*, 1: 31-34.

KAPPEN, L., SCHROETER, B., GREEN, T. G. A. and SEPPELT, R. D. (1998): Chlorophyll *a* fluorescence and CO_2 exchange of *Umbilicaria aprina* under extreme light stress in the cold. *Oecologia*, 113: 325-331.

KENNEDY, A. D. (1993): Photosynthetic response of the Antarctic moss *Polytrichum alpestre* Hoppe to low temperatures and freeze-thaw stress. *Polar Biology*, 13: 271-279.

LÁSKA, K., BARTÁK, M., HÁJEK, J., PROŠEK, P. and BOHUSLAVOVÁ, O. (2011): Climatic and ecological characteristics of deglaciated area of James Ross Island, Antarctica, with a special respect to vegetation cover. *Czech Polar Reports*, 1: 49-62.

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: 165-182.

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.

PANNEWITZ, S., GREEN, T. G. A., SCHLENSOG, M., SEPPELT, R., SANCHO, L. G., and SCHROETER, B.(2006): Photosynthetic performance of *Xanthoria mawsonii* C. W. Dodge in coastal habitats, Ross Sea region, continental Antarctica. *Lichenologist*, 38: 67–81.

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.

PROŠEK, P., BARTÁK, M., LÁSKA, K., SUCHÁNEK, A., HÁJEK, J. and KAPLER, P. (2013): Facilities of J. G. Mendel Antarctic station: Technical and technological solutions with a special respect to energy sources. *Czech Polar Reports*, 3: 38-57.

ROHÁČEK, K., SOUKUPOVÁ, J. and BARTÁK, M. (2008): Chlorophyll fluorescence: A wonderful tool to study plant physiology and plant stress. *In*: B. Schoefs (ed.): Plant Cell Compartments – Selected Topics. Research Signpost, Kerala, India, pp. 41-104.

SCHLENSOG, M., SCHROETER, B. (2001): A new method for the accurate *in situ* monitoring of chlorophyll *a* fluorescence in lichens and bryophytes. *Lichenologist*, 33: 443-452.

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., BILGER, W. and NEUBAUER, C. (1994): Chlorophyll fluorescence as a nonintrusive indicator for rapid assessment of *in vivo* photosynthesis. *In*: E. D. Schulze, M. M. Caldwell, (eds.): Ecophysiology of photosynthesis. Berlin, Springer, pp. 49-70.

SCHROETER, B., GREEN, T. G. A., PANNEWITZ, S., SCHLENSOG, M. and SANCHO, L. G. (2010): Fourteen degrees of latitude and a continent apart: comparison of lichen activity over two years at continental and maritime Antarctic sites. *Antarctic Science*, 22: 681-690.

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., KULLE, D., PANNEWITZ, S., SCHLENSOG, M. and SANCHO, L. G. (2012): The moss *Bryum argenteum* var. *muticum* Brid. is well adapted to cope with high light in continental Antarctica. *Antarctic Science*, 24: 281-291.

UCHIDA, M., MURAOKA, H., NAKATSUBO, T., BEKKU, Y., UENO, T., KANDA, H. and KOIZUMI, H. (2002): Net photosynthesis, respiration, and production of the moss *Sanionia uncinata* on a glacier foreland in the High Arctic, Ny-Ålesund, Svalbard. *Arctic Antarctic and Alpine Research*, 34: 287-292.