

Effect of light stress on maximum photochemical efficiency of photosystem II and chloroplast structure in cryptogams *Cladonia mitis* and *Pleurozium schreberi*

Patrycja Dziurawicz (✉ patrycja.dziurawicz@uj.edu.pl)

Jagiellonian University

Patrycja Fałowska

Jagiellonian University

Karolina Waszkiewicz

Jagiellonian University

Paulina Wietrzyk-Pełka

Jagiellonian University

Michał H. Węgrzyn

Jagiellonian University

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Abstract

Earlier beliefs suggested that lichens thrived in open and warm environments, whereas mosses prefer shaded places with high humidity. However, excessive light can be a stress factor for both lichens and bryophytes, as it can damage their photosynthetic apparatus, particularly the chlorophyll pigments located in the chloroplasts. The measurement of photosynthetic activity and the use of fluorescence microscopy allows the assessment of the influence of light as a stress factor on the state of preservation of the thallus. The main goal of our research was to determine the changes that occur in the photosynthetic activity and structure of chloroplasts of selected species lichen *Cladonia mitis* (Sandst.) and moss *Pleurozium schreberi* (Willd. Ex Brid.) under the influence of different intensity of light in laboratory conditions: FL – 100% light, RL – 30% maximum light, and NL - natural sunlight. The results of the two-factor analysis of variance (ANOVA) tests showed a variation in the activities of the selected species over time depending on the amount of light energy supplied. The results showed that *Cladonia mitis* showed the lowest level of chloroplast fluorescence in the strongest and reduced light, which coincides with its low level of photosynthetic activity. The moss showed high and intense fluorescent activity of the chloroplasts, indicating their much higher resistance to light radiation.

Introduction

Lichens are very common in microhabitats where they can be exposed to adverse environmental conditions, including extreme temperatures, desiccation, or solar radiation (Beckett et al. 2021; Dziurawicz et al. 2022; Nguyen et al. 2013). These tiny poikilohydric organisms have evolved defence mechanisms to protect themselves against changing environmental conditions, including reducing metabolism when water availability decreases, tolerating the loss of virtually all free water without dying, and resuming growth after rehydration. In turn, photoprotection focusses on light scattering, radiation shielding, heat dissipation, activation of antioxidant and macromolecular defences, and membrane repair (Beckett et al. 2021; Dziurawicz et al. 2022; Nguyen et al. 2013). Bryophyte, on the other hand, unlike lichens, prefer moist habitats, which allows them to be fully hydrated and metabolically active for extended periods of time (Dziurawicz et al. 2022; Green et al. 2011). However, due to their distinctive morphology and physiology, bryophyte rely heavily on their external environment and are vulnerable to the impacts of climate change, which can alter the availability of water and nutrients, as well as affect the temperature and humidity conditions in their habitat (He et al. 2016). Therefore, the bryophyte faces great challenges associated with the drying and rehydration cycle and often high temperatures, which causes them to be metabolically inactive (Liu et al. 2003). One way to reduce water loss is for mosses to form dense clusters that reduce water evaporation (Charron and Quatrano 2009). Assessing the physiological state of plants and their activity, in addition to monitoring their physical existence and the intensity of environmental stress, can provide important information about ecosystems. Various experimental and analytical methods have been developed to monitor the physiological state of plants (Chen et al. 2019). One of the methods uses the phenomenon of chlorophyll fluorescence. It is a simple tool widely used to analyse the state and function of the photosystem II (PSII). The maximum photochemical efficiency of

photosystem II (Fv/Fm), otherwise called photosynthetic activity, is easy to measure as a representation of the photosynthetic performance of a studied organism. Changes in the functioning of photosynthetic systems can be detected without any damage to the studied samples by measuring chlorophyll fluorescence. This is because a fraction of the light is absorbed by chlorophyll molecules in PSII and used for energy conversion in photosynthesis, while excess energy can be either dissipated as heat or remitted as light (Maxwell and Johnson 2000). Chlorophyll fluorescence variations reflect the response of photosystem II (PS II) to changes in photosynthetically active radiation (PAR) intensity (Demmig-Adams and Adams 1992; Dziurawicz et al. 2022; Wu et al. 2017). Studies conducted over the years using such measurements have identified several relationships, including the level of hydration and photosynthetic activity. Among the results, we find information that dehydration damages the photosynthetic apparatus and therefore causes inactivation of the photosynthetic process (Liu et al. 2014). Other studies indicate that in the case of desiccated mosses and lichens, wetting the thallus leads to its reactivation (Orehova et al. 2022; Schlensog and Schroeter 2001). Those so-called desiccation-tolerant organisms (DT) can be subdivided into two groups. Homoiochlorophyllous (HDT) are those that maintain their chlorophyll content during periods of desiccation, while poikilochlorophyllous (PDT) lose most or all their chlorophyll during these periods (Bewley 1979; Hamblen 1961; Gaff 1977; 1989; Tuba et al. 1994; Tuba et al. 1996). In opposition to complete desiccation is the equally negative effect of too much water content, one that prevents optimal CO₂ exchange and contributes to stopping the process (Dziurawicz et al. 2022; Lakatos 2011; Lange et al. 1993). Apart from water availability, the efficiency of photosynthesis can be significantly impacted by light conditions. Although light is its integral element, necessary for it to be carried out, the relationship between the amount of available light and the intensity of photosynthesis is not linear since the rate of photosynthesis reaches a saturating point at a consuming certain level. Research over many years has shown that too much light becomes a stressor and can be harmful (Beckett et al. 2021; Dziurawicz et al. 2022; Rochaix 2011). Damage occurs when photon absorption of photons exceeds the capacity of the downstream reactions to consume the products of the light phase, and reaction centers become saturated (Allorent et al. 2013; Barber and Andersson 1992; Wobbe et al. 2016).

To date, most work using photosynthetic activity to assess the state of plant viability has focused on interpreting photosynthetic activity alone. However, the evaluation of photosynthetic activity combined with chloroplast fluorescence imaging by fluorescence microscopy can more accurately show the changes that occur in photosynthetically active organisms under stress. It is very easy to observe chloroplasts by chlorophyll fluorescence using fluorescence microscopy (Wada 2013), and it offers a way to test the viability status of lichen and moss thalli (Kauppi 1980).

Our research carried out consisted of collecting selected lichens and bryophyte in the natural environment and then growing them in strictly defined changing light conditions while maintaining the same other conditions (temperature and humidity). For this study, we selected *Cladonia mitis* (Sandst.) and *Pleurozium schreberi* (Willd. Ex Brid.) Mitt. as representatives of lichens and bryophyte, respectively, which occur in this same type of pine forest habitat. The main goal of our research was to determine the changes that occur in photosynthetic activity and presence and location of chloroplasts of selected

species under the influence of different amounts of light in laboratory conditions. We made the following hypotheses: (1) photosynthetic activity was variable over time, depending on the amount of light energy supplied (2) the photosynthetic activity of the lichen is higher than that of the moss in all light types tested, (3) soaked thalli of lichen *Cladonia mitis* and moss *Pleurozium schreberi* show higher photosynthetic activity compared to non-soaked thalli, and (4) the structure of chloroplasts in both study species degrades under the influence of the strongest light (full light).

Materials And Method

Establishment and maintenance of laboratory cultures of lichen and bryophytes

Species of lichen *C. mitis* and bryophyte *P. schreberi* were randomly collected in Bory Tucholskie National Park within the lichen Scots pine forest community (*Cladonio-Pinetum* Ass.) in February 2022 (Węgrzyn et al, 2020; 2021a; 2021b). The collected samples were cleaned of plant debris and soil particles and divided into 3 study samples, which were later placed in 3 experimental glass containers (aquariums) with different light treatments. Within each container, we placed 6 subsamples of *C. mitis* (C) and 6 subsamples of *P. schreberi* (P) as pseudoreplications (6 chambers per container eq. study sample) (Fig. 1, Table 1). As treatment within containers, we used two types of artificial light conditions: 1) full light (FL) – 100% of artificial light; 2) reduction light (RL) – 30% of artificial light. The reduction was achieved by covering the glass of the aquarium with a dark film. Both light conditions were created by application of artificial lighting which was generated by lamps with the following specifications: type GROWY LED 354, light PAR – 224.3 $\mu\text{mol}/\text{m}^2\text{s}$ and wavelength range 50% max. 450 nm and 50% max. 650 nm. For the control container identified as natural light (NL), we used natural sunlight (Table 1). The substrate for the study species was sterile sand. We kept the boxes in a climatic chamber at 21 ° C.

Table 1

Segregation of six study samples (experimental containers) of *Cladonia mitis* (C) and *Pleurozium schreberi* (P) in fieldwork into types of light: FL – 100% light; RL – 30% maximum light; NL – natural sunlight (control sample), with the values: Light energy (LE) and photosynthetically active radiation (PAR).

Species	Types of Light	LE	PAR
		[W/m ²]	[μmol/m ²]
<i>Cladonia mitis</i>	FL	122.7	506.9
<i>Pleurozium schreberi</i>	FL	125.2	495.1
<i>Cladonia mitis</i>	RL	40.6	151.4
<i>Pleurozium schreberi</i>	RL	42.0	165.3
<i>Cladonia mitis</i>	NL	11.5	178.6
<i>Pleurozium schreberi</i>	NL	11.5	178.5

When FL samples with RL samples, the first ones mentioned were characterized by a more than three times higher light energy. In the case of the RL samples, the energy was about four times higher than in the NL samples (Table 1). As the artificial lighting was active 18 hours a day. The lighting was automatically turned off for 6 hours from 21 pm to 3 am. Lichens and bryophyte were systematically watered so that their thallus and tissues were constantly moist. The experimental cultivation was carried out for 150 days.

Measurements of photosynthetic activity

Photosynthetic activity measurements were performed six times, at different time intervals, to assess the viability of the examined samples. The first measurement was made on the day the culture was established; the next measurements took place after 30, 60, 90, 120 and 150 days from the beginning of the experiment. During measurement, 36 samples were taken from each compartment, three each from all six subspecies, both to measure soaked and non-soaked samples: 1) soaked samples: *Cladonia mitis* (CM-S) and *Pleurozium schreberi* (PS-S) were placed in Eppendorf filled with distilled water for a period of 2 h, and after this, the material was transferred to the HPEA/LC clips for a 15-minute dark adaptation aimed at extinguishing the activity of the photosystem II; 2) non-soaked samples: *Cladonia mitis* (CM-NS) and *Pleurozium schreberi* (PS-NS) were measured directly after being transferred to the HPEA/LC clips without water treatment. That was repeated for both species. Measurements were made using a Handy PEA + fluorometer (Handy PEA, Hansatech Instrument Ltd, King's Lynn, Norfolk, England). The fluorometer light probe emitted the dose of excitation light, which allowed recording the maximum photochemical efficiency of photosystem II (Fv/Fm) (Chowaniec and Rola 2020; Dziurawicz et al. 2022; Węgrzyn et al. 2021b).

Microscopic fluorescence imaging of chloroplasts

At the designated dates of photosynthetic activity measurements, small samples of lichen thalli and bryophyte gametophyte were also collected directly from the culture to make microscopic preparations showing the preservation status of the chloroplasts. In the case of lichens, the top fragments of the thallus were collected, where there are numerous cells of *Asterochloris* sp. algae of the lichen photobiont belonging to the Trebouxiaceae family within the Chlorophyta cluster. The thallus fragments were cut into thin flat sections, which were placed in a water solution of the microscopic preparation. In the case of bryophyte, gametophyte leaflets were collected, from which a water microscope preparation was made directly. In visible and fluorescent light with filter V-2A, EX 380–420, DM 430, BA450, the Nikon Eclipse 7000 microscope was used to observe the state of preservation of chloroplasts in the organisms tested.

Statistical analysis

A two-way analysis of variance (ANOVA) followed by Tukey's HSD test (Honestly Significant Difference) for an equal sample size ($p < 0.05$) was performed to reveal significant differences in Fv/Fm between the duration of the experiment (1, 30, 60, 90 and 150 days) and light types (FL, RL, NL) separately for *C. mitis* (N = 648), and *P. schreberi* (N = 648) as well as soaked or non-soaked. A graphical interpretation of the interaction between the duration of the experiment, the species, and the type of sample (soaked and non-soaked) for different types of light was presented. Before analysis, the normality of the distribution was verified using the Kolmogorov-Smirnov test ($p > 0.05$) and the Levene test ($p > 0.05$) to assess the equality of variances. Box plots were presented to illustrate the differences in individual comparisons.

Results

Photosynthetic activity

The results of the statistical analysis indicated visible differences in the photosynthetic activity of *Cladonia mitis* and *Pleurozium schreberi* depending on the type of light used and the duration of the experiment (Table 2, Fig. 2).

Our results shown in Fig. 1 include four graphs that illustrate the photosynthetic activity of CM-NS (Fig. 2A), CM-S (Fig. 2B), PS-NS (Fig. 2C) and PS-S (Fig. 2D) depending on light intensity (FL, RL, NL) and the duration of the experiment (1, 30, 90, 120, 150 days). CM-NS indicates the lowest values of photosynthetic activity under FL conditions, higher values in the RL sample, and the highest values in the control sample. The photosynthetic activity values range from 0.1 to 0.8 Fv/Fm for the FL sample (Fig. 2A). CM-S indicated the lowest values of photosynthetic activity under FL conditions, the highest in RL, and the highest in NL. As with the CM-NS sample, CM-S was characterised by the highest decrease in photosynthetic activity for FL light, higher values for the RL sample, and the lowest decrease for the NL sample. The values of photosynthetic activity range from 0.38 to 0.8 (Fig. 2B). For the PS-NS sample, photosynthetic activity values range from 0.55 to 0.8. The lowest level of photosynthetic activity was observed for the FL conditions, which are close to the values presented by the RL conditions. The highest values were observed in the NL sample (Fig. 2C). For PS-S, the average values of photosynthetic activity reach the range of 0.55 to 0.78 Fv/Fm. PS-S reaches the lowest activity level in FL conditions, then higher

in the RL sample, and the highest in the NL conditions. Bryophyte (PS-NS, PS-S) and lichens (CM-NS, CM-S) showed the highest level of photosynthetic activity in NL and the lowest levels in FL.

Table 2

Results of multivariate ANOVA for the effects of the following variables on the Fv/Fm of the samples: A) CM-NS, B) CM-S, C) PS-NS, and D) PS-S on the duration of the experiment (1, 30, 90, 120 and 150 days), types of lighting (FL, RL, NL), and interaction between time and types of light. Significant effects ($p < 0.05$) are shown in bold.

Variables		F	p
A	Measurement Day	92.903	p < 0.001
	Type of Light	88.071	p < 0.001
	Measurement Day x Type of Light	8.185	p < 0.001
B	Measurement Day	58.420	p < 0.001
	Type of Light	57.105	p < 0.001
	Measurement Day x Type of Light	5.224	p < 0.001
C	Measurement Day	9.32	p < 0.001
	Type of Light	6.23	p = 0.002
	Measurement Day x Type of Light	5.13	p = 0.003
D	Measurement Day	13.39	p < 0.001
	Type of Light	21.22	p < 0.001
	Measurement Day x Type of Light	2.82	p = 0.002

The graphical interpretation of the interaction for three different types of light (respectively: Fig. 3 - FL, Fig. 4 - RL, Fig. 5 - NL) and the selected species CM-S and CM-NS and PS-S and PS-NS were shown in Figs. 3–5.

On each measurement day, CM-NS showed the lowest photosynthetic activity values among all selected species, and PS-NS species showed the highest values for FL conditions (Fig. 3). CM-NS noted decreasing values from the beginning of the experiment, with the lowest value occurring on day 60, and then increased until the end of the experiment. In turn, for PS-NS, the lowest activity values were observed on day 30 and then increased to day 150. Species PS-S showed activity slightly lower than that of its non-hydrated form while maintaining an increase and decrease trend analogous to that of the PS-NS species. In turn, CM-S showed higher values of photosynthetic activity than its non-hydrated CM-NS species, with a progressive decrease in photosynthetic activity with each day of measurement (Fig. 3).

The highest values of the photosynthetic activity are recorded by the PS-NS species and slightly lower or similar PS-S species for the RL sample (Fig. 4). Species *P. schreberi*, both in soaked and non-soaked samples, showed photosynthetic activity in the range of 0.6–0.7. The lowest values of the photosynthetic activity are shown by CM-NS, which reaches a critical level on day 90 and then increases to day 120. The values F_v/F_m of the CM-S thallus are higher than those of CM-NS, but still lower than those of both samples of *P. schreberi* (soaked and non-soaked (Fig. 4).

The activity level of all samples was very high and oscillated between 0.7–8.0 F_v/F_m for the control sample (Fig. 5). The highest activity values were recorded for PS-NS and PS-S because they did not differ much from each other. In turn, the CM-NS species has the lowest values on each measurement day, with it was lower limit on day 60. The CM-S species showed photosynthetic activity higher than that of the CM-NS and are closely comparable to that of the PS-NS and PS-S species (Fig. 5).

Microscopic fluorescence imaging of chloroplasts

The fluorescence of the chloroplasts of *Cladonia mitis* varied depending on the type of light and the length of the experiment. Initially, in all types of light, the fluorescence of chloroplasts was at a level similar to that of fluorescence. For the FL sample, the lowest fluorescence was on day 60 and day 90, and during the following days, the chloroplast fluorescence increased significantly. For the RL sample, a decrease in fluorescence was observed, with the lowest fluorescence occurring on day 90. On subsequent days, an increase in fluorescence could be observed. The control sample was characterised by similar chloroplast fluorescence for all days. The FL sample had the lowest chloroplast activity, while the control sample had the highest chloroplast activity relative to the rest of the tested groups (Fig. 6).

The fluorescence of chloroplasts of the moss *Pleurozium schreberi* varied over time depending on the type of light. Referring to the FL sample, a decrease in the fluorescence of the chloroplasts was evident from day 30 to day 90. On subsequent days, an increase in the fluorescence of chloroplasts was observed. For the RL sample, slight decreases in fluorescence could be observed on day 30 and day 90, however, there was a resurgence later. For the control sample, there are no major differences in the change in chloroplast fluorescence (Fig. 7).

Discussion

Photosynthetic activity

Bryophyte and lichens are two distinct groups of organisms, often co-occurring in the same habitats that share certain ecological and morphological characteristics. It is well-known that both hydration and access to light are crucial for the photosynthesis and growth of the organisms mentioned. Both groups tolerate full desiccation without suffering photooxidative damage under strong light. The results of two-factor analysis of variance (ANOVA) tests showed a variation in photosynthetic activity in *C. mitis* and *P. schreberi* over time depending on the amount of light energy supplied. The results obtained in the present

work refer to earlier research (Dziurawicz et al. 2022). Current research is another step in understanding the mechanisms which confirmed that changes in photosynthetic activity occur in mosses and lichens depending on the amount of PAR energy supplied (Beckett et al. 2021; Dziurawicz et al. 2022; Wu et al. 2017). Evaluation of the methods and findings resulted in updated methodology and data collection, this time using both non-soaked and soaked samples, as well as fluorescence imaging.

Under non-stressful conditions, F_v/F_m levels usually remain at the same level, but this balance can be disturbed by the influence of stress factors, which include water, high temperature, and excessive radiation (PAR) (Lan et al. 2012). Absorption of more light that can be used for photosynthesis can lead to oxidative reactions that cause damage. This is especially dangerous for poikilohydric photoautotrophs that retain their chlorophyll during periods of dehydration (Heber and Lüttge 2011). In the present study, the photosynthetic activity of *P. schreberi* and *C. mitis* was lowest at the highest light intensity, which consequently coincides with the adaptation of lichens and mosses to perform photosynthesis under reduced sunlight and even shade (Tobias and Niinemets 2010; Wegrzyn et al. 2021). Species that are adapted to photosynthesise in the dark show maximum photochemical potential and the highest solar energy conversion efficiency in PS II when the reaction centres are completely open (Krause 1988; Franklin et al. 1992). On the other hand, the presence of excess light affects the partial illumination of the PS II reaction center, thereby determining its partial closure, resulting in an inevitable reduction in F_v/F_m levels (Lan et al. 2012). In extreme cases, excess energy can activate oxygen, thus creating reactive oxygen species (ROS), which in turn can damage the photosynthetic apparatus and other cellular components leading to photoinhibition and photooxidative stress (Mkhize et al. 2022). The first line of defence against oxidative stress in lichens involves the inactivation of reactive oxygen species (ROS) by enzymatic systems and the accumulation of lipophilic and hydrophilic compounds within cells (Beckett et al. 2021). However, under moderate light conditions, ROS is also produced, and a small amount of it is a signaling element responsible for acclimation and programmed cell death (Pospíšil, 2016). Results from the early 2000s (de la Torre et al. 2002; 2004; de Vera et al. 2002; 2004a; 2004b; de la Torre Noetzel et al. 2007) have shown that the ability to withstand high UV radiation can be credited to the fungus (mycobiont) that protects the more-sensitive photosynthetic active algae (photobiont) of the lichen with a dense cortex it develops (Vera et al. 2010). Furthermore, the concentration of specific secondary metabolites, such as depsidones, and dibenzofurans, often increases in lichens under stress conditions (Holger 2014). Responsible for the radiation shielding effects is the production of metabolites that absorb radiation, such as parietin (Solhaug et al. 2003; Solhaug and Gauslaa 2004; Wynn-Williams et al. 2002) and usnic acid (e.g. Galloway 1993; Quilhot et al. 1994; 1998; Rikkinen 1995; Solhaug and Gauslaa 1996).

Lichens are specialised to live in environments with limited access to water and can quickly recover from dehydration when water becomes available again. On the contrary, bryophytes are better suited for occupying moist habitats, often those that are only available seasonally, but have slower recovery times after experiencing dehydration (Green and Proctor 2016). The higher the PAR energy, the faster the drying of the thallus (Tobias and Niinemets 2010; Veres et al. 2022b), which in turn leads to photoinhibition and confirms the first research hypothesis that photosynthetic activity varies over time depending on the

amount of PAR energy supplied. The response to high PAR is observed in both *C. mitis* and *P. schreberi*, and is more pronounced in lichens (Kappen et al. 1996). Lichens under conditions of water scarcity become dehydrated very quickly and go into a state of anabiosis, limiting or completely stopping photosynthesis (Kappen et al. 1996; Lange et al. 1993). An example is the CM-NS sample for which very low and sometimes critical Fv/Fm values were observed in full and reduced light. The completely opposite situation was observed for the PS-NS trial in which very high levels of photosynthetic activity were recorded throughout the experiment. The indicated differences are due to the different physiology of the studied species, and thus the degree of hydration of their thallus. Single bryophyte shoots have low resistance to water loss, while packed shoots forming turf have an increased capacity to store and transport water, significantly prolongs their physiological activity (Tobias and Niinemets 2010).

As part of our investigation, we not only measured the photosynthetic activity of the lichens in their non-soaked state, but also irrigated them for a period of two hours, since the importance of hydrating the samples under investigation before measuring their physiological parameters has already been shown in the previous study (Węgrzyn et al. 2021b). Exposure to the sunlight of the dry lichen thallus can cause the accumulation of severe damage in PS II, and water is the endogenous factor for the regeneration of the damaged apparatus (Veres et al. 2022a). In the study, the hydrated form of *C. mitis* showed significantly higher levels of photosynthetic activity than the non-soaked thallus, which is a natural process that occurs in lichens (Grimm et al. 2021; Węgrzyn et al. 2021). The process of desiccation is responsible for reducing the amount of light that can pass through the upper cortex of a lichen thallus (Büdel and Lange 1994; Ertl 1951). Additionally, it is believed to create a functional disconnection of components of the photochemical apparatus (Sigfridsson 1980; Bilger et al. 1989). On the other hand, soaked *P. schreberi* thallus mosses showed lower Fv/Fm values in each light, which may be the result of the supersaturation phenomenon (Green et al. 2011) and limited CO₂ diffusion (Green et al. 2011; Möller et al. 2022). The results presented here did not confirm the second research hypothesis that lichens in any type of light show higher values of photosynthetic activity than mosses, however, they partially confirmed the third hypothesis, as we found that the lichens that were soaked in water exhibited a significant increase in photosynthetic activity when compared to the non-soaked specimens. It can be explained by the fact that both lichens and bryophytes are classified as homoiochlorophyllous, which means that they can rapidly resume photosynthesis after rewetting. However, a disadvantage of this trait is that chlorophyll continues to absorb photons even after desiccation, and the energy from the excess light cannot be used for photochemical work (Gasulla et al. 2021; Heber et al. 2006; Kranner et al. 2003). To prevent photooxidative damage in the desiccated state, homoiochlorophyllous poikilohydric cryptogams have evolved a highly efficient mechanism of photoprotection intended to prevent photodamage through controlled dissipation of thermal energy that is assessed by measuring non-photochemical quenching (NPQ) (Hájek et al. 2009; Heber and Lüttge 2011; Mkhize et al. 2022). NPQ is induced rapidly on a time scale of seconds to a few minutes, making it ideal for dealing with sudden fluctuations in light intensity (Goss and Lepetit 2015). In the case of lichens, NPQ is induced only after algae have been acclimated to prolonged light, so it would be a mistake to calculate NPQ values while measuring photosynthetic activity (Goss and Lepetit 2015; Maxwell and Johnson 2000).

Chloroplast structure

Fluorescence microscopy is a technique that allows the study of changes in the viability of single and intact cells with the accompanying autofluorescence of chloroplasts being verifiable with the naked eye (Boluda and Hawksworth 2014; Kauppi 1980; Takahashi 2019). Assessing chloroplast cell viability based on fluorescence microscope images alone is not precise enough (Bolhàr-Nordenkamp and Öquist 1993). However, in combination with the measurement of photosynthetic activity, it provides a reliable picture of the changes that occur in the studied species caused by excessive light exposure (Dutta et al. 2015). Chloroplasts can move depending on the amount of light energy provided to them, and their movement is directly proportional to the amount of PAR energy demand (Davis et al. 2011; Wada 2013). Under conditions of low illumination, chloroplasts carry out an accumulation process, in which case they expose themselves to light, but with excess light, there is an avoidance process in which chloroplasts hide behind each other (Dutta et al. 2015; Suetsugu and Wada 2012). The combination of accumulation and avoidance responses presumably allows plant cells to strike a balance between maximising light uptake and minimizing photodamage (Davis and Hangarter 2012). Photos of the changes under the microscope are an illustration of the trends of photosynthetic changes that occur in *C. mitis* and *P.schreberi* along with their chloroplast structure. At low fluorescence of chloroplasts, the preparation showed low intensity, the dominant colour of the cell walls (blue), while in the situation of full fluorescence the red colour dominated. *C.mitis* showed the lowest level of chloroplast fluorescence in the strongest and reduced light, which coincides with its low level of photosynthetic activity. This observation may indicate that chloroplast degradation or its avoidance reaction is occurring. As noted by other authors (Beckett et al. 2021; Derks et al. 2015; Veres et al. 2022b), high light intensity leads to progressive changes in the cell, which in most cases are irreversible. However, lichens in the sample of natural light show very high photosynthetic activity and thus are accompanied by intense fluorescence, which informs about the normal state of chloroplast behaviour. In *P.schreberi*, the identification of chloroplasts is much simpler, as they are arranged in a single layer, which was a problem in lichens in which chloroplasts were grouped in algal cells. The moss species suffer the greatest loss of chloroplast fluorescence in the trial of intense light. However, in bryophyte, the movement of chloroplasts is more likely to take place than their complete degradation. As photosynthetic activity decreased, fluorescence decreased, but at the final stages of the experiment, activity increased significantly, translating into intense fluorescence of chloroplasts. In a situation where the structure of chloroplasts was destroyed, they would not show fluorescence activity, as the chlorophyll in them is degraded (Otegui 2018). The activity of the repair apparatus depends on the regeneration time and the rate of progressive photodamage. This condition is known as photoinhibition of photosynthesis and occurs when the rate of photodamage exceeds the repair capacity (Derks et al. 2015; Melis 1999). Under reduced and natural light conditions, the mosses showed both high photosynthetic activity and intense fluorescence of the chloroplasts, indicating their much higher resistance to light radiation. Thus, the results presented partially confirm the fourth hypothesis, which assumes that chloroplasts are degraded under the influence of the strongest light. In lichens, this phenomenon is already noticeable in the first days of measurement, and the damage caused by PAR energy leads to the degradation of chloroplast cells. However, the degradation is small, or at least

imperceptible, at a given stage of analysis. More research is needed to assess the viability of chloroplasts along with their photosynthetic activity.

Conclusion

Measurements of photosynthetic activity, combined with fluorescence microscopy imaging, allow observation of the conservation and viability of chloroplasts. In lichens and mosses, there is a phenomenon of displacement and relocation of chloroplasts depending on the given PAR energy demand. The intensification of light causes degradation of the photosynthetic apparatus in lichens, leading to photoinhibition of photosynthesis under oxidative stress. Mosses under conditions of excessive hydration exhibit superstorage properties, which slows down photosynthesis. The adequate hydration of the thallus and the availability of water are essential elements that affects the repair processes that take place in both lichens and mosses.

Declarations

Author Contributions: PD, PF and MHW conceptualization, methodology, supervised the findings of this work. PD performed statistical analysis of the data. PD, PF, MHW and KW field material collection, writing original draft preparation. PD, PF, MHW, KW and PW-P writing review and editing. MHW. project management, performed microscopic fluorescence imaging of chloroplasts. All authors have read and agreed to the published version of the manuscript.

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Data availability: All data generated or analysed during this study are included in this published article. Raw data are available from the corresponding author on reasonable request.

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Figures

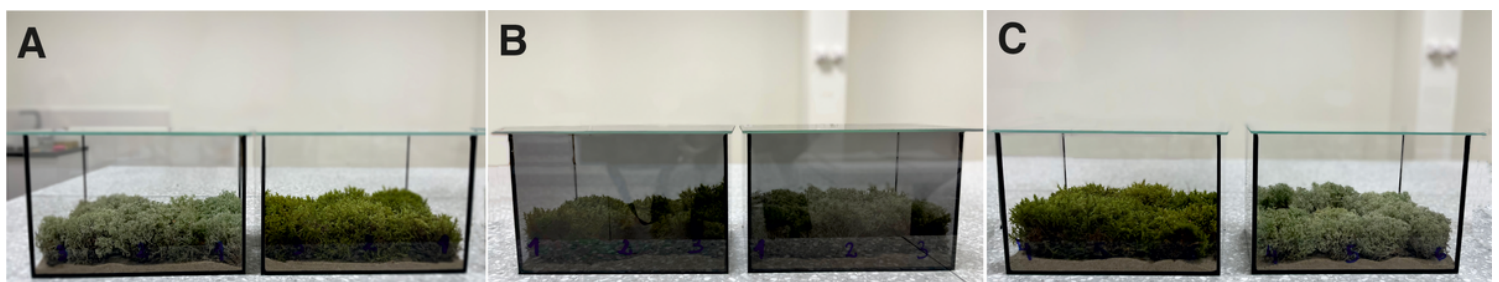


Figure 1

Experiment consisting of three samples (A, B, C), each with two species: *Cladonia mitislichen* and *Pleurozium schreberi* bryophyte. Each of the samples differed in the level of light: A (FL), B (RL) and C (NL) according to the parameters contained in Table 1.

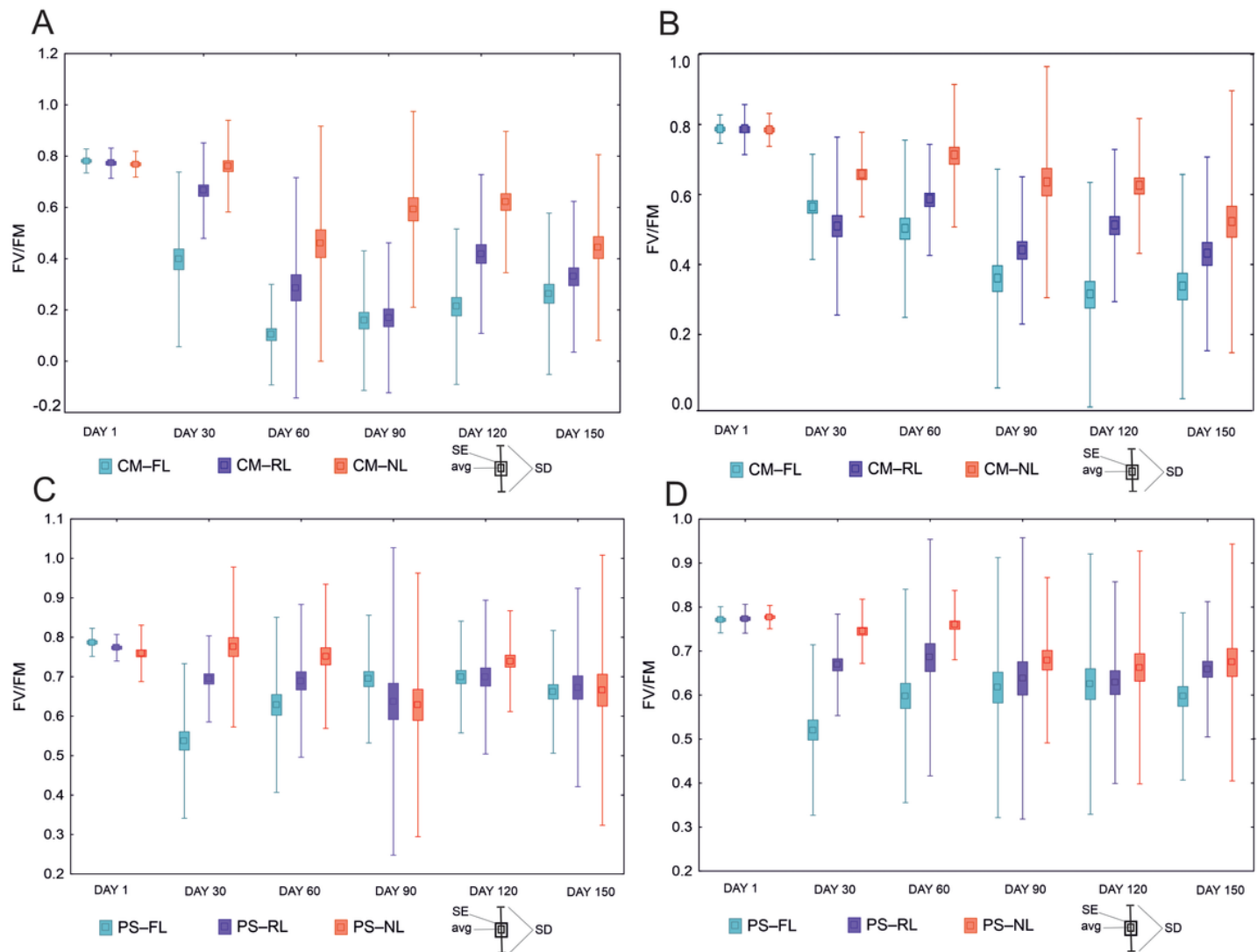


Figure 2

Mean $\pm$ SE and SD of F_v/F_m , including days (1, 30, 60, 90, 120, 150) and type of light (FL, RL, NL) for A) CM-NS (N = 324), B) CM-S (N = 324), C) PS-NS (N = 324), D) PS-S (N = 324). The results of ANOVA ($p < 0.05$) are presented graphically.

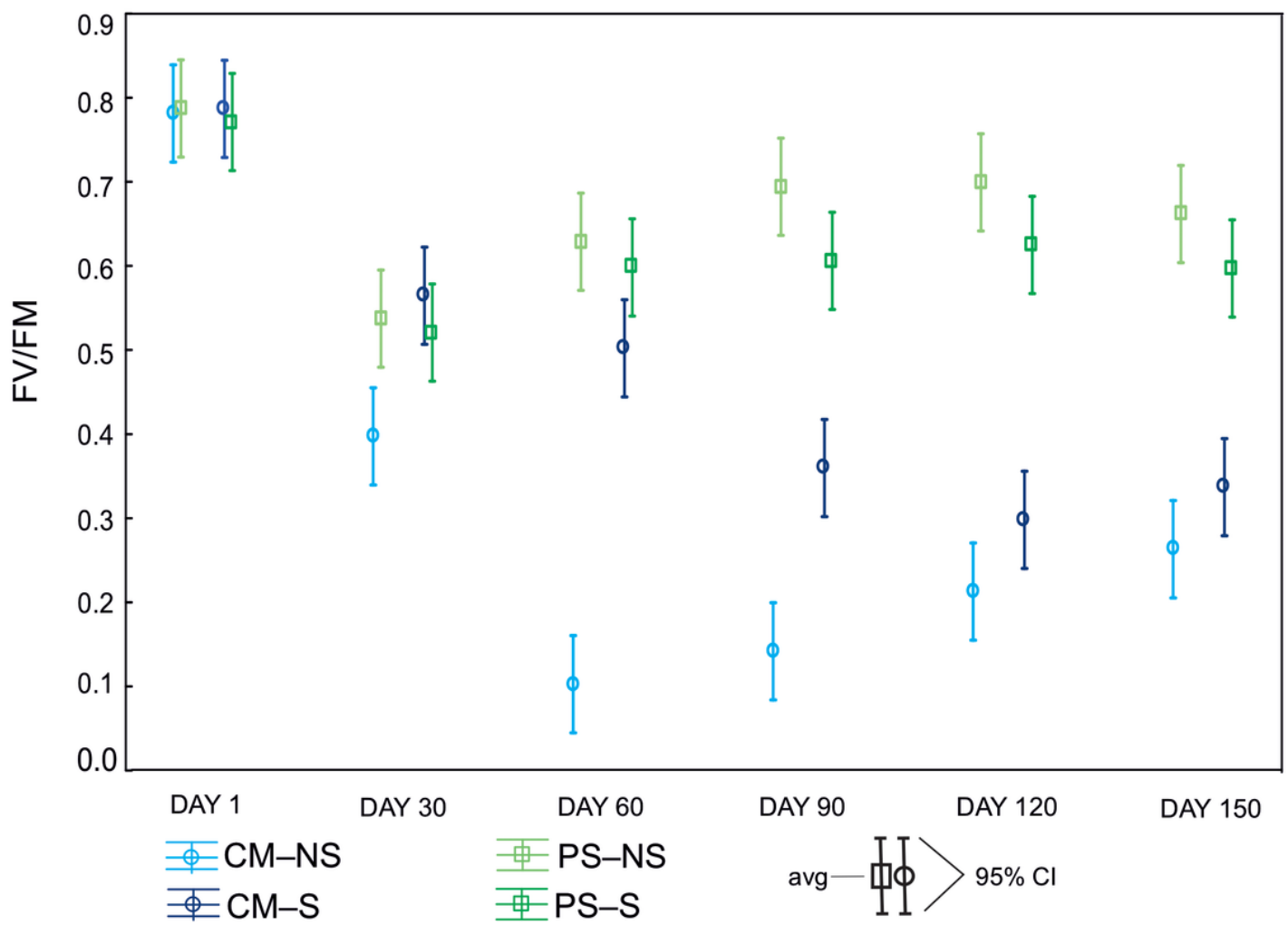


Figure 3

Graphical interpretation for the ANOVA test ($p = 0.026$) including days (1, 30, 60, 90, 120, 150), selected species: CM-NS, PS-NS, CM-S, and PS-S for the FL conditions ($N = 436$).

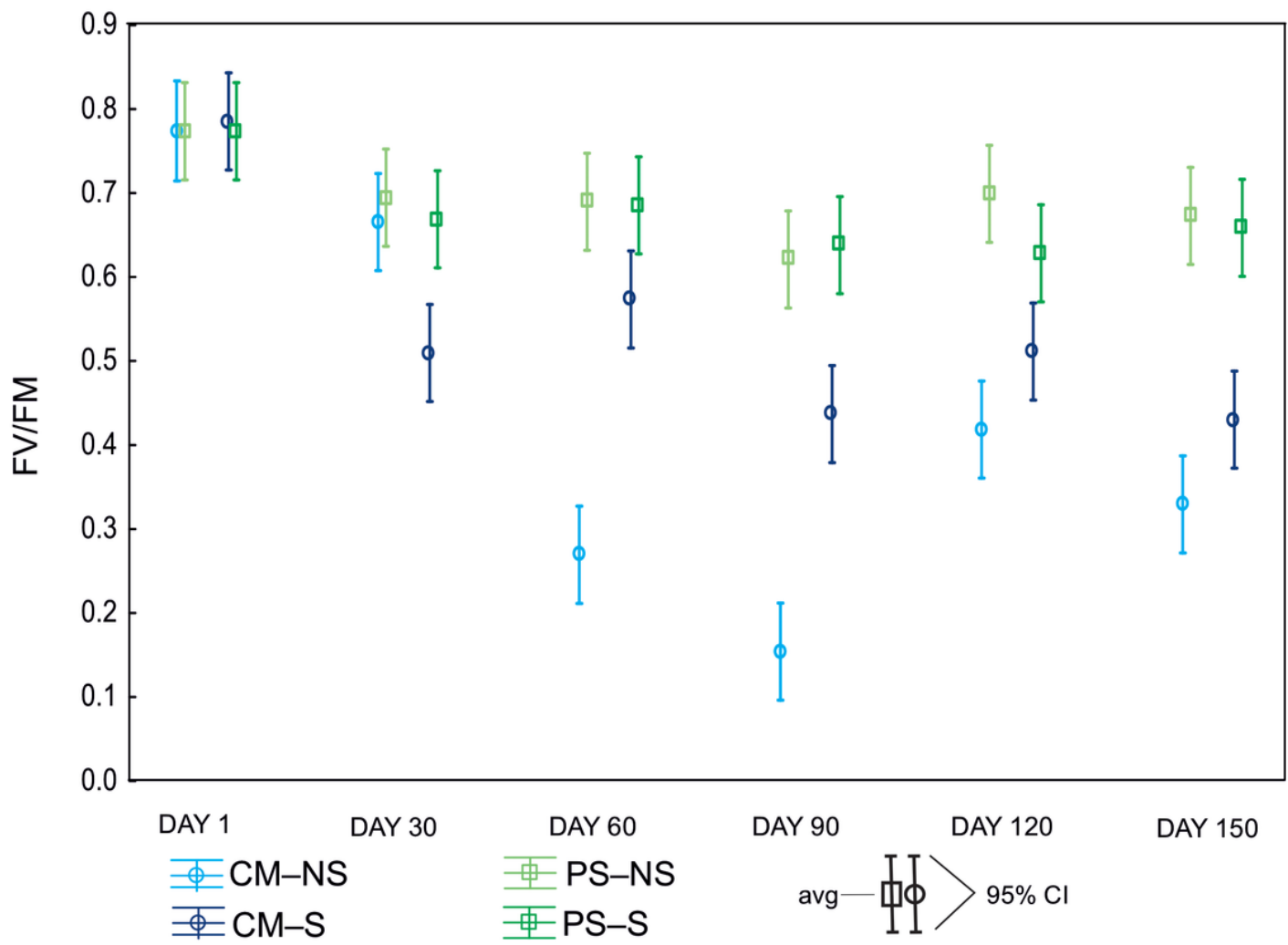


Figure 4

Graphical interpretation for the ANOVA test ($p = 0.026$) including days (1, 30, 60, 90, 120, 150), selected species: CM-NS, PS-NS, CM-S and PS-S for RL conditions ($N = 436$).

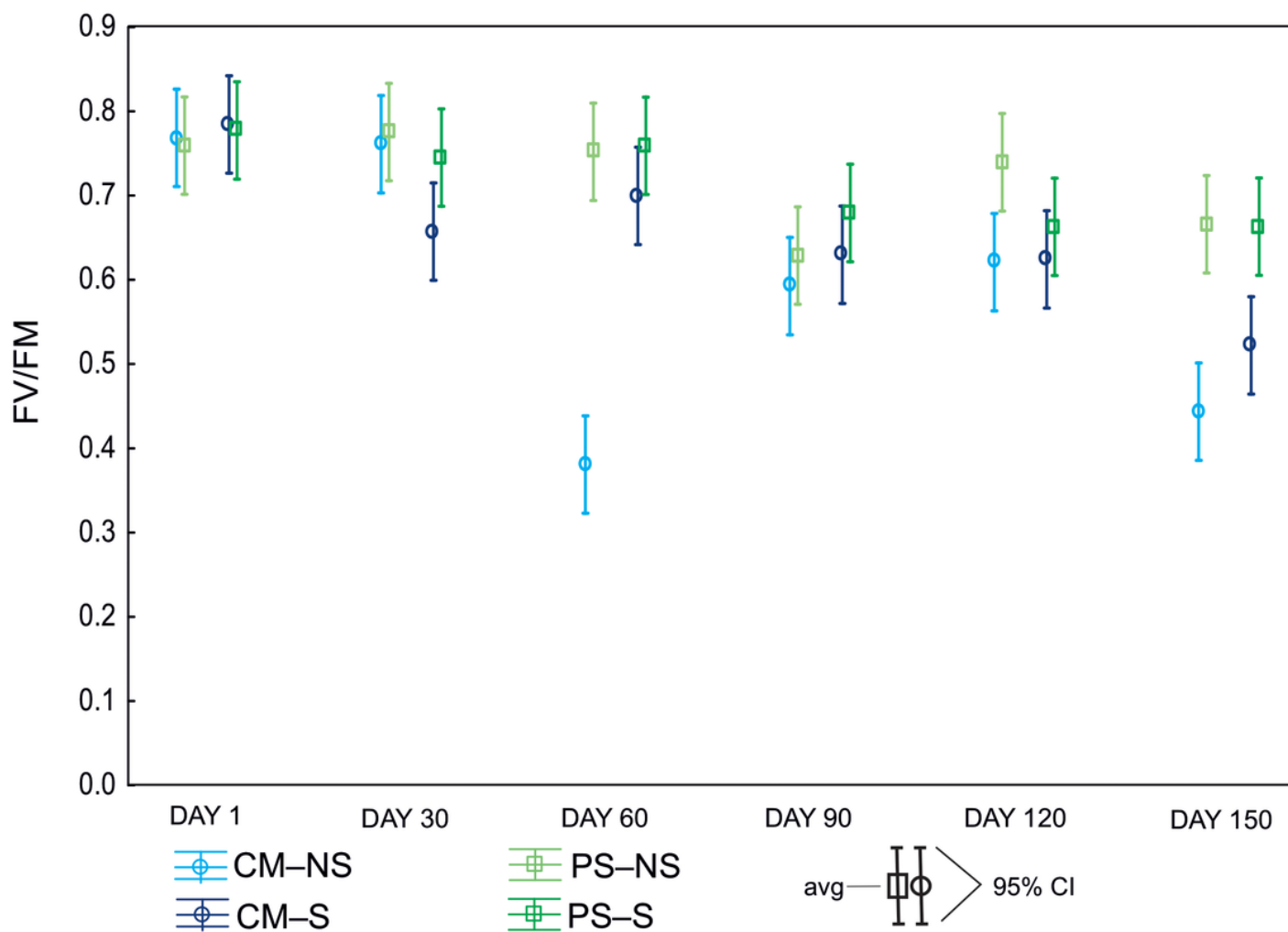


Figure 5

Graphical interpretation for the ANOVA test ($p = 0.026$) including days (1, 30, 60, 90, 120, 150), selected species: CM-NS, PS-NS, CM-S and PS-S for NL conditions ($N = 436$).

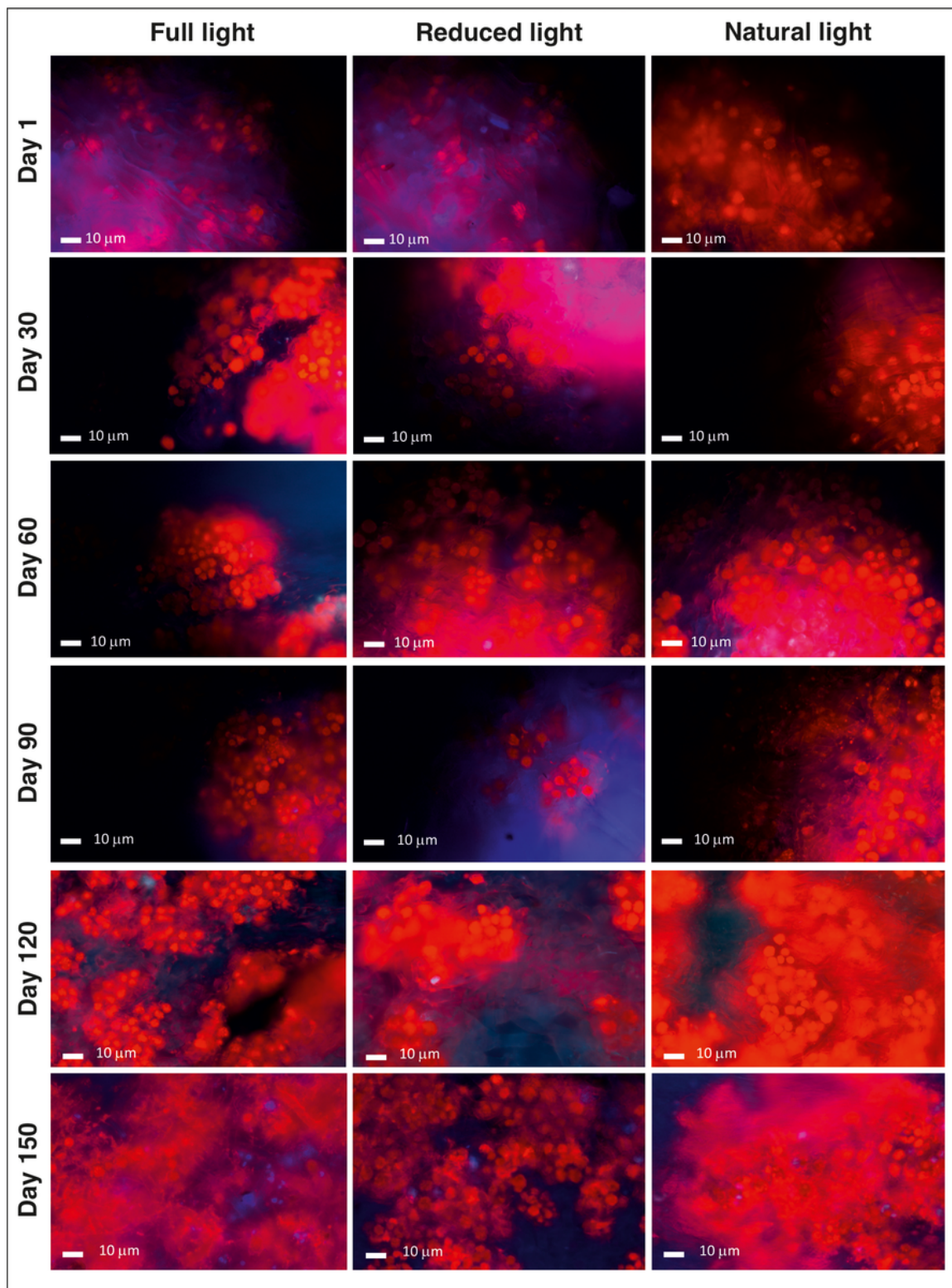


Figure 6

Fluorescence microscope images of the lichen species *Cladonia mitis* for different lights (FL, RL, NL) and duration of the experiment (1, 30, 60, 90, 120, 150). The red refers to the fluorescence of the chloroplasts and the colour blue-violet refers to the fluorescence of the fungi hyphae.

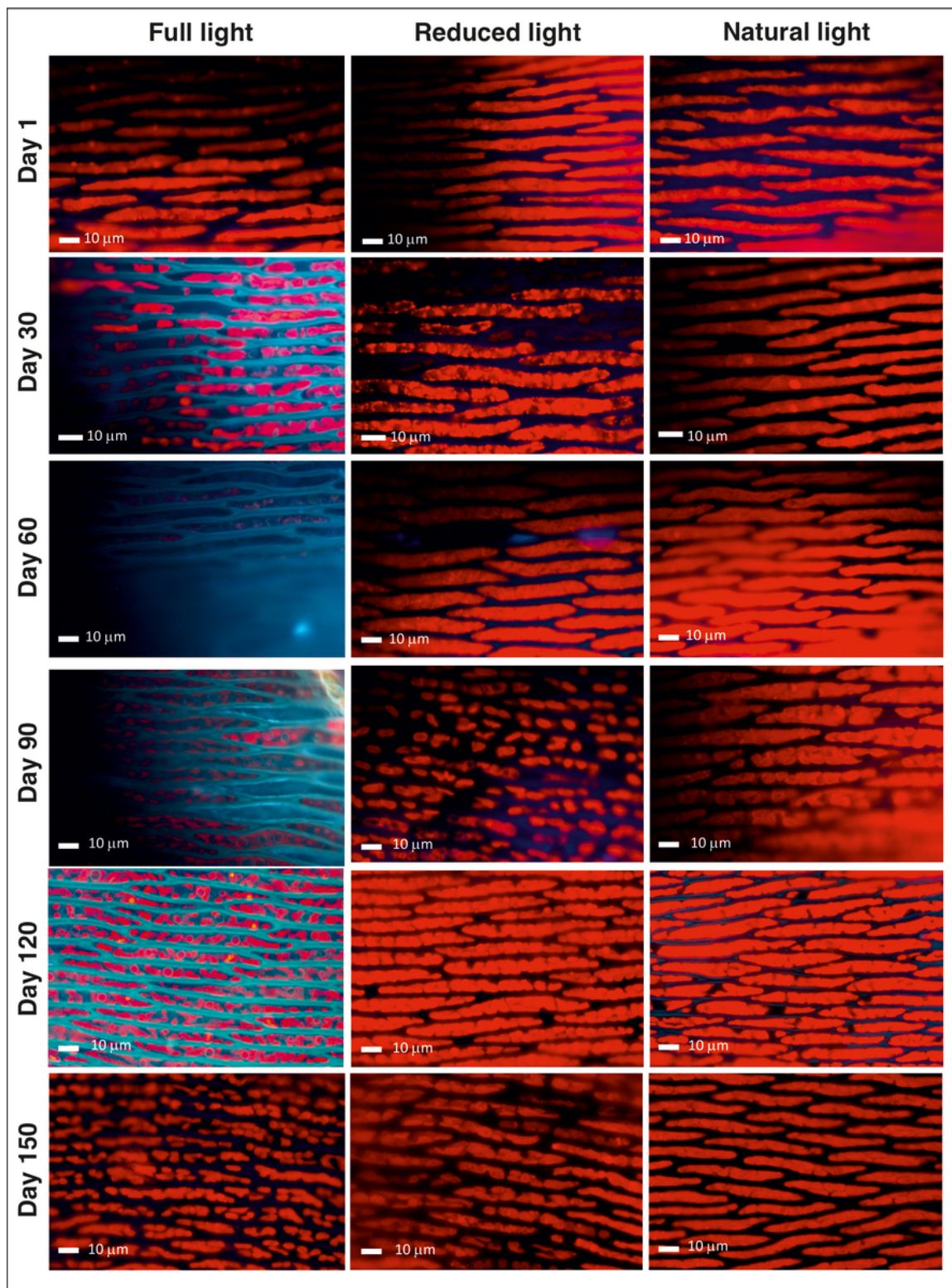


Figure 7

Fluorescence microscope images of the lichen species *Pleurozium schreberi* for different lights (FL, RL, NL) and duration of the experiment (1, 30, 60, 90, 120, 150). The red represents the fluorescence of the chloroplasts and the blue violet refers to the fluorescence of the bryophyte cell walls.