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RESEARCH PAPERS

Photosynthetic and Respiratory Capacity of Foliose Lichen *Lobaria pulmonaria* throughout the Annual Cycle

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Abstract—Lichens are unique phototrophic organisms whose physiology of stress tolerance attracts much attention. Parameters of photosynthetic and respiratory activities of the epiphytic large-leaved lichen *Lobaria pulmonaria* were investigated with an aim to reveal physiological responses to seasonal changes in environmental conditions. The highest accumulation of chlorophylls in thalli (2.3 mg/g dry wt) was noted in autumn (October); the amount of green pigments decreased 2.5 times in spring (April). The chlorophyll/carotenoid ratio varied from 3.1 to 4.4. The extent of deepoxidation of xanthophyll cycle pigments equaled 34% in winter but it was two times as low in summer. When *L. pulmonaria* thalli were hydrated and acclimated shortly under standard laboratory conditions, they exhibited a relatively high photochemical activity and were able to assimilate CO_2 throughout the entire annual cycle. The rate of net CO_2 uptake by thalli under optimal irradiance and temperature ranged from 3 to 5 μmol CO_2/m^2 s and the highest values were recorded in spring. No significant seasonal changes were observed in the total respiration rate of thalli. The proportions of various respiratory pathways were altered in spring and autumn, and metabolic heat production was accelerated due to the activation of an energetically low-efficient alternative respiratory pathway. The results provide evidence that the functional adaptation of photo- and mycobionts in the lichen is implicated in resistance of this complete system to seasonal changes in environmental conditions.

Keywords: *Lobaria pulmonaria*, photosynthetic pigments, xanthophyll cycle, chlorophyll fluorescence, photosystems, CO_2 exchange, respiration, respiratory pathways, heat release, stress tolerance, lichen

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INTRODUCTION

Lichens are a stable symbiotic association of a heterotrophic organism (mycobiont) and a photosynthetic organism (photobiont). The presence of a photobiont (green algae and/or cyanobacteria) converts the lichen into an autotrophic system. Green algae supply sugar alcohols to mycobiont cells, while cyanobacteria act as a source of glucose and nitrogen-fixation products [1]. An outstanding Russian botanist and one of the founders of Russian plant physiology, Andrei S. Faminitsyn, contributed greatly to the development of ideas about the symbiotic nature of lichens [2]. He actually initiated the physiological research of these unique phototrophic organisms and obtained valuable materials for understanding their biology.

Lichens attract great interest for their resistance to dehydration, hypo- and hyperthermia, ionizing radiation, and other adverse factors [3]. They dominate in extreme environments (Arctic, Antarctica, deserts, high mountains), but forests are the most favorable habitats for lichens as evidenced by the high diversity of lichen biota in the boreal zone [4]. The resistance of lichens is supposedly based on constitutive mechanisms for maintaining structural and functional integrity and inducible processes for repairing damage caused by stress [5]. However, the current knowledge is incomplete with regard to physiological bases of resistance in lichens as an integral system in which the mycobiont accounts for >90% of the biomass and the photobiont provides reduced carbon for the entire biotic association.

Lobaria pulmonaria (L.) Hoffm. (lung lichen) is an epiphytic foliose lichen. The species is common in boreal, temperate, mountainous, and oceanic regions of the northern hemisphere and in tropical forests of Eastern and Southern Africa. This species is under protection in most countries of northern and central Europe [6]. The main photobiont of *Lobaria* is the green alga *Symbiochloris reticulata*. Nitrogen fixation is carried out by cyanobacteria of the genus *Nostoc* that are confined in cephalodia inside the thallus. The

Abbreviations: AP—alternative respiratory pathway; Car—carotenoids; Chl—chlorophyll; CP—cytochrome respiratory pathway; DEPS—deepoxidation state of violaxanthin cycle pigments; ETC—electron transport chain; PPFD—photosynthetic photon flux density; PSI—photosystem I; PSII—photosystem II; PSA—photosynthetic apparatus; RH—relative humidity; VXC—violaxanthin cycle.

Table 1. Microclimatic conditions in the habitat of the lichen *Lobaria pulmonaria* at different times of the year

Months	PAR intensity, μmol quanta/m^2 s			Air temperature, ºC			Air relative humidity, %		
	min.	max.	mean	min.	max.	mean	min.	max.	mean
Apr.	57	1300	306 ± 48	0.3	10	5.2 ± 0.5	45	79	61 ± 2
June	11	1300	234 ± 32	23	30	27.3 ± 0.3	36	69	52 ± 2
Oct.	8	137	25 ± 4	5	18	12.0 ± 0.8	58	88	70 ± 2
Jan.	23	78	48 ± 4	−13	−12	−12.5 ± 0.2	43	60	51 ± 5

A full set of data over the entire sampling periods in 2012–2017 was used for statistical treatment.

green algae produce a well-defined algal layer, which is located under the upper cortex consisting of closely appressed fungal hyphae. According to our data, the thickness of the algal layer in mature thalli is 45–50 μm and the cells of green algae have a diameter of approximately 5 μm [7]. Below the algal layer, the fungal hyphae are loosely spaced and form a medulla that determines the thickness of the entire thallus. The lower cortex layer is composed of closely packed hyphae and has outgrowths (rhizines) that ensure the thallus attachment to the substrate.

The aim of this work was to reveal seasonal changes in photosynthesis and respiration in *Lobaria pulmonaria* that are associated with energy and organic matter metabolism and with the resistance of lichen to environmental conditions.

MATERIALS AND METHODS

Experiments were carried out in 2012–2017. Thalli of the lichen *Lobaria pulmonaria* were sampled near the city of Syktyvkar (61°34' N, 50°33' E) in an old-growth aspen forest with an admixture of spruce and fir. Thalli were collected from tree trunks at a height of 1–3 m from the ground. The region is characterized by a temperate continental climate, with an average annual air temperature of approximately +1°C. The average daily temperature in the warmest month (July) is near to 17°C and that in the coldest month (January) is approximately −16°C. The amount of annual precipitation is 600–700 mm. The major part of precipitation falls in summer and autumn (60–80 mm per month), while the monthly amount of precipitation in winter and spring is 1.5–2 times lower. The transition of average daily temperature across the 0°C level occurs in spring in the second ten-day period of April; that in autumn is at the beginning of October. The duration of the frost-free period is 180–190 days, and the period with an average daily temperature above 5°C lasts for approximately 160 days [8].

On the dates of thalli sampling, the irradiance, temperature, and relative humidity in the lichen habitat were measured using an LI-1400 data logger (LI-COR, United States) equipped with a set of meteorological sensors. Microclimatic parameters of the environment varied within a wide range depending on weather conditions and the season (Table 1). In the spring–summer period, the photosynthetically active radiation (PAR) was 250–300 μmol quanta/m^2 s on average, but it could be as high as ≥1000 μmol quanta/m^2 s on the occasions of sunflecks and "windows" in the tree canopy. In autumn and winter, the irradiance was substantially lower. Average values of relative humidity (RH) varied depending on the season within the range of 50–70%.

Photosynthetic pigments were extracted from the peripheral parts of freshly harvested thalli using a mixture of acetone and dimethyl sulfoxide at a 1 : 2 ratio [9]. The pigment content was determined with a UV-1700 spectrophotometer (Shimadzu, Japan) at wavelengths of 662, 644 nm (chlorophylls *a* and *b*), and 478 nm (carotenoids). The composition of carotenoids was analyzed using freeze-dried samples fixed previously in liquid nitrogen. Individual carotenoids (Car) were separated by means of the reverse phase HPLC using a Smartline chromatographic system (Knauer, Germany) and a Diasphere-110-C18-NT column (BioKhimMak ST, Russia). Calibration curves were plotted with the use of standard pigment samples (Sigma and Fluka, United States). The deepoxidation state (DEPS) of violaxanthin cycle (VXC) pigments was characterized by the (Zea + 0.5 Anth)/(Vio + Anth + Zea) ratio, where: Zea, Anth, and Vio designate the content of zeaxanthin, antheraxanthin, and violaxanthin, respectively. The content and composition of photosynthetic pigments was determined with four to six biological replicates.

Low-temperature fluorescence spectra of chlorophyll *a* were studied on thalli collected in January and stored until the end of April at −18°C as well as on thalli freshly collected in April. The measurements were carried out on a Hitachi-850 spectrofluorometer (Hitachi, Japan) at 77 K (−196°C). Fluorescence was excited at 435 nm, and fluorescence emission spectra were recorded in the range of 650–780 nm and normalized to the fluorescence intensity at λ = 735 nm.

Parameters of induced photosystem II chlorophyll *a* fluorescence were measured using a PAM-2100 fluorometer (Walz, Germany) after hydration of thalli and their short-term acclimation to laboratory conditions

(22°C, PAR intensity of 20–30 μmol quanta/m^2 s, acclimation duration 1.5–2 h). The minimum (F_0) and maximum (F_m) fluorescence was measured after keeping the thalli for 30–40 min in darkness. Next, the thallus was adapted for 10–15 min to actinic light of various intensities ranging from 0 to 2000 μmol quanta/m^2 s, and the values of stationary (F_t), background (F_0'), and maximum (F_m') fluorescence were measured. The built-in halogen lamp of the fluorometer was used as a light source. In order to assess the recovery dynamics of the photobiont functional parameters in thalli sampled in winter, the thalli were kept for one day at −16°C; measurements were performed at 22°C at regular intervals.

The potential quantum yield of photosystem II (PSII) was calculated according to [10]:

$$F_v/F_m = (F_m - F_0)/F_m. \quad (1)$$

The effective quantum yield of PSII activity (Φ_{PSII}) in thalli adapted to actinic light was calculated with the following formula:

$$\Phi_{PSII} = (F_m' - F_t)/F_m'. \quad (2)$$

Nonphotochemical quenching of PSII chlorophyll *a* fluorescence (NPQ) was determined by the following formula:

$$NPQ = (F_m - F_m')/F_m'. \quad (3)$$

The relative electron transport rate (ETR) through PSII was calculated as

$$ETR = \Phi_{PSII} \times PPFD \times 0.5, \quad (4)$$

where PPFD is the photosynthetic photon flux density. The plot of ETR as a function of PPFD was approximated with an exponential function:

$$f(x) = a(1 - e^{-bx}) \quad (5)$$

Using this function, we calculated the maximum electron transport rate (ETR_{max}) and the saturating irradiance ($PPFD_{sat}$), at which ETR equals 90% of ETR_{max} [11]. Parameters of the induced chlorophyll fluorescence were determined in five- to tenfold biological replicates.

CO_2/H_2O exchange was measured at 20°C using a portable ADC LCPro$^+$ system (ADC BioScientific, United Kingdom). Prior to measurements, the thalli were incubated under conditions similar to those used for measurements of chlorophyll fluorescence. A thallus lobe with an area of 3–4 cm^2 was placed into a leaf-clip chamber for 2-min incubation, and then a gas exchange rate was recorded at regular intervals of 1 min. The net rate of light-induced CO_2 absorption (P_n) by thalli was determined in the PAR range of 0–2000 μmol quanta/m^2 s at a CO_2 concentration near 0.04%. The light curves were averaged over experiments with 10 to 30 thalli. The quantum yield of photosynthesis (ϕ) and the light compensation point (LCP) of CO_2 exchange in thalli were determined from the regression analysis of the initial portion of the light curve in the PAR range from 0 to 200 μmol quanta/m^2 s.

Respiration rate of thalli was assayed by polarographic measurements of O_2 consumption at 20°C using an Oxytherm system with a Clark electrode (Hansatech, United Kingdom) and expressed in nmol O_2/g dry wt min. The excised fragments from the peripheral functionally active thallus areas with a total weight of 15–20 mg were placed into a 4 mL reaction vessel containing 1.5 mL of 50 mM Hepes buffer (Helicon, Russia), pH 7.2. Measurements were performed under constant stirring of the samples.

Respiration via the cytochrome and alternative pathways was assessed using specific inhibitors [12]. The oxygen uptake rate was expressed as the sum of its individual components:

$$V_t = V_{alt} + V_{cyt} + V_{res}, \quad (6)$$

where V_t is total respiration, V_{alt} is alternative respiration suppressed by an inhibitor of alternative oxidase, V_{cyt} is cyanide-sensitive (cytochrome-mediated) respiration, and V_{res} is the residual respiration recorded in the presence of inhibitors of the alternative and cytochrome pathways.

Salicylhydroxamic acid (Lancaster Synthesis, United Kingdom) was used at a concentration of 6 mM as an inhibitor of alternative oxidase. Cytochrome oxidase activity was inhibited with 2 mM KCN solution (Sigma, United States). The optimal concentrations of mitochondrial oxidase inhibitors were determined in preliminary experiments.

Rates of metabolic heat production were measured with a Biotest-2 isothermal microcalorimeter (Institute of Biological Instrumentation, Russian Academy of Sciences, Pushchino, Russia) at 20°C. The marginal portions of the thalli weighing ~100 mg were put into hermetically sealed containers and placed inside the working cells. In order to eliminate differences in heat fluxes, an empty container was placed into the reference cell. The rate of heat production (Q, μW/mg dry wt) was calculated from the following equation:

$$Q = [q_1 - (q_{n1} + q_{n2})/2 \times 0.22]/m, \quad (7)$$

where q_1 is the metabolic heat release from the sample, q_{n1} and q_{n2} are zero values of heat flux before and after measuring the heat release by the sample (rel. units), 0.22 is the calibration factor of the calorimeter expressed in μW, and m is the sample dry weight expressed in milligrams.

Statistical data processing was carried out using Statistica 10 software (StatSoft Inc., United States). Significant differences between mean values of the measured parameters were revealed using one-way analysis of variance, ANOVA (Duncan's test). The correspondence of data to normal distribution was

Table 2. Content and ratio of photosynthetic pigments in thalli of foliose lichen *Lobaria pulmonaria* in different seasons of the year

Months	Chlorophylls mg/g dry wt	Chl *a*, % of total Chl content	Carotenoids, mg/g dr wt	Chl/Car ratio
Apr.	0.86 ± 0.05^{a}	76.7	0.28 ± 0.01^{a}	3.1 ± 0.1^{a}
June	1.89 ± 0.08^{cd}	72.0	0.40 ± 0.02^{b}	4.7 ± 0.3^{b}
Oct.	2.30 ± 0.30^{d}	75.2	0.70 ± 0.07^{c}	3.3 ± 0.1^{a}
Jan.	1.64 ± 0.21^{bc}	76.2	0.48 ± 0.05^{bc}	3.4 ± 0.7^{a}

Data represent mean values and their standard errors; different superscript letters indicate statistically significant changes in the parameter during the year at $P \le 0.05$ (analysis of variance ANOVA, Duncan's test, $n = 6$).

Table 3. Seasonal dynamics in the content of individual carotenoids in *Lobaria pulmonaria* thalli, mmol/mol Chl

Carotenoids	April	June	October	January
Neoxanthin	45.0 ± 3.0^{a}	15.0 ± 0.7^{a}	108.8 ± 43.2^{b}	20.3 ± 4.8^{a}
Violaxanthin	61.8 ± 1.1^{a}	27.0 ± 2.9^{a}	131.1 ± 57.9^{b}	21.0 ± 2.2^{a}
Antheraxanthin	10.6 ± 1.6^{b}	0.74 ± 0.1^{a}	9.1 ± 4.0^{b}	11.3 ± 2.2^{b}
Lutein	215.5 ± 18.5^{b}	67.5 ± 3.4^{a}	408.8 ± 137.4^{c}	89.8 ± 21.7^{a}
Zeaxanthin	15.9 ± 1.4^{c}	4.0 ± 0.1^{a}	19.6 ± 3.1^{d}	8.0 ± 0.7^{b}
β-carotene	66.5 ± 3.8^{a}	23.7 ± 1.9^{a}	135.4 ± 60.9^{b}	35.5 ± 7.8^{a}
Vio + Anth + Zea	88.3 ± 1.6^{b}	31.8 ± 2.9^{a}	159.8 ± 52.1^{c}	40.3 ± 1.3^{a}
DEPS, %	24 ± 1^{b}	14 ± 1^{a}	17 ± 10^{ab}	34 ± 4^{c}

Vio + Anth + Zea designate the sum of VXC components (% of total Car content); DEPS is the degree of deepoxidation of VXC pigments. Mean values and standard errors are shown; different superscript letters indicate statistically significant changes in the parameter during the year at $P \le 0.05$ (analysis of variance ANOVA, Duncan's test, $n = 4$–6).

assessed by means of the Shapiro–Wilk test. The calculations were carried out at a significance level of $P \le 0.05$. Tables and figures show mean values and standard errors of the means.

RESULTS

Content of photosynthetic pigments and the conversions of violaxanthin cycle pigments. Photosynthetic pigments in the lichen thallus are selective markers of the photobiont. According to our data, the pigment pool of *L. pulmonaria* was subject to seasonal changes (Table 2). The lowest content of green pigments was noted in spring (April). By autumn (September–October), the chlorophyll content increased conspicuously. Chlorophyll *a* (Chl *a*) accounted for 75% of the sum of all green pigments on average. The content of carotenoids (Car) was three to four times lower than that of chlorophylls.

The pool of Car was mainly represented by xanthophylls (Table 3). Lutein accounted for nearly 50%, while neoxanthin made up 10–12% of the Car pool. Violaxanthin cycle (VXC) pigments—violaxanthin, zeaxanthin, and antheraxanthin—constituted 20–25% of the Car pool in total, and the major component was violaxanthin. The extent of VXC pigment conversion averaged 15% in summer and increased 1.5–2 times in winter and spring.

Analysis of low-temperature chlorophyll fluorescence spectra revealed that the ratio of fluorescence intensities at 735 and 685 nm, corresponding to fluorescence maxima of PSI and PSII in light-harvesting complexes, was 1.27 for the thalli collected in spring (Fig. 1a). The long-term storage of thalli at −18°C (from January to April) was accompanied by the increase in the F_{735}/F_{685} ratio to 1.98 (Fig. 1b). After hydration and 30-h acclimation of the same thalli at 22°C, a decrease in the F_{735}/F_{685} ratio was noted toward the values typical of spring-collected thalli.

Parameters of induced PSII chlorophyll *a* fluorescence and CO_2 exchange in thalli. The lichens sampled in winter were photochemically active almost immediately after their hydration and acclimation at 22°C. Under laboratory conditions, the maximal quantum yield of PSII (F_v/F_m) increased during the first hour of acclimation from 0.54 to 0.68 and remained almost unchanged in the subsequent period (Table 4). The effective quantum yield (Φ_{PSII}) showed a similar dynamics, although the time of attaining the Φ_{PSII} stationary level was somewhat longer. After 4-h acclimation of thalli at moderate irradiance (200 μmol quanta/m^2 s) under laboratory conditions, the Φ_{PSII} value was

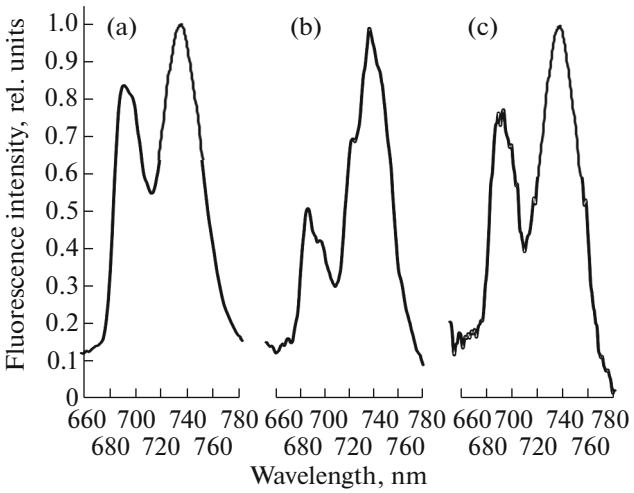

Fig. 1. Chlorophyll *a* fluorescence spectra of the photobiont in *Lobaria pulmonaria*. λ_{exc} = 435 nm, T = 77 K. Spectra were normalized to the fluorescence intensity at 735 nm. Measurements were carried out on (a) thalli collected in April, (b) thalli kept for 5 months at −18°C, and (c) the same as (b) after 30 h acclimation of thalli under laboratory conditions.

approximately 0.4. The value of nonphotochemical quenching (NPQ) of chlorophyll *a* fluorescence characterizes the thermal dissipation of the absorbed light energy in PSII; it did not change appreciably with time and varied within 0.8–1.2 rel. units, tending to decline after 4 h of acclimation. The rate of photochemical electron transport through PSII (ETR) changed insignificantly during thallus acclimation. The ETR recorded in the first minutes after the transfer of thalli to controlled conditions was only 25% less than after 20 h of acclimation. Compared with parameters characterizing the photochemical activity of photosynthetic apparatus (PSA), the CO_2 assimilation rate showed more pronounced changes during acclimation. The net rate of CO_2 assimilation (P_n) increased steadily and exceeded 4 μmol CO_2/m^2 s in 20 h after the onset of thallus acclimation.

The influence of seasonal environmental changes on the condition of PSA was revealed by comparing the functional parameters of thalli sampled in different periods of the year. The thalli were hydrated prior to measurements and acclimated for 1.5 h at 22°C.

The F_v/F_m value varied insignificantly throughout the year, remaining in the range of 0.6–0.7. The effective quantum yield, NPQ, and the rate of electron transport through photosystems depended strongly on irradiance but changed insignificantly depending on the season of the year (Figs. 2, 3). At the same time, a tendency towards the decrease in Φ_{PSII} value and the increase in NPQ in thalli sampled in winter–spring period was noted. Under strong light, the NPQ values reflecting the thermal dissipation of excitation energy reached 4–5 rel. units in "winter" thalli and did not exceed 3 rel. units in "summer" and "autumn" thalli.

The ETR value depends on the effective quantum yield (Φ_{PSII}) and the photosynthetic photon flux density. In the PPFD range from 0 to 400 μmol/m^2 s, the rate of electron transfer through PSII increased linearly ($N = 85$, $F(1.83) = 445$, $P < 0.001$, $R^2 = 0.84$). As can be seen in Table 5, comparatively high ETR_{max} values (88–95 μmol/m^2 s) were recorded in thalli in the warm season. In winter, a significant ($t_{st} = 3.3$, $P \le 0.05$) decrease in ETR_{max} values by 30% was noted. For thalli collected in April–June, the electron transport rate through PSII was saturated at a PPFD of 600–700 μmol quanta/m^2 s (Fig. 3, Table 5). In autumn and winter, the light saturation of PSII photochemical reactions was achieved at lower irradiance; the value of $PPFD_{sat}$ was less than 500 μmol quanta/m^2 s.

Determinations of CO_2 exchange showed that the rate of CO_2 release in darkness was 1–2 μmol CO_2/m^2 s, and a trend to the increase in respiration of thalli sampled in the cold season was noted (Fig. 4, Table 6). The transition of thalli from the net release of CO_2 to net CO_2 uptake was observed at a relatively high PPFD (20–70 μmol quanta/m^2 s). The rate of net CO_2 uptake (P_n) by thalli increased with the irradiance. Under saturating irradiance, the P_n rate of "summer" thalli was almost two times lower than that of thalli sampled in spring, autumn, and even winter.

Table 4. Dynamics of changes in PSII functional parameters of the *Lobaria pulmonaria* photobiont after the transfer of thalli from natural to controlled conditions in winter (January)

Parameters	5 min	60 min	120 min	240 min	20 h
F_v/F_m	0.54 ± 0.02^a	0.68 ± 0.01^b	0.69 ± 0.01^b	0.70 ± 0.01^b	0.72 ± 0.01^b
Φ_{PSII}	0.32 ± 0.02^a	0.34 ± 0.02^a	0.39 ± 0.02^{ab}	0.39 ± 0.02^{ab}	0.43 ± 0.02^b
NPQ	1.18 ± 0.14^a	1.16 ± 0.17^a	1.23 ± 0.19^a	0.84 ± 0.10^a	0.94 ± 0.17^a
ETR, μmol/m^2 s	31 ± 2^a	32 ± 1^a	37 ± 2^{ab}	37 ± 2^{ab}	41 ± 2^b
P_n, μmol CO_2/m^2 s	nd	1.51 ± 0.47^a	2.65 ± 0.39^{ab}	3.18 ± 0.47^{bc}	4.29 ± 0.52^c

Mean values and their standard errors are presented. Measurements of Chl *a* fluorescence and CO_2 net uptake (P_n) were performed at a PPFD of ~200 μmol quanta/m^2 s on hydrated thalli at 18–22°C. Different superscript letters indicate statistically significant parameter changes during the time at $P \le 0.05$ (analysis of variance ANOVA, Duncan's test, $n = 5$–10).

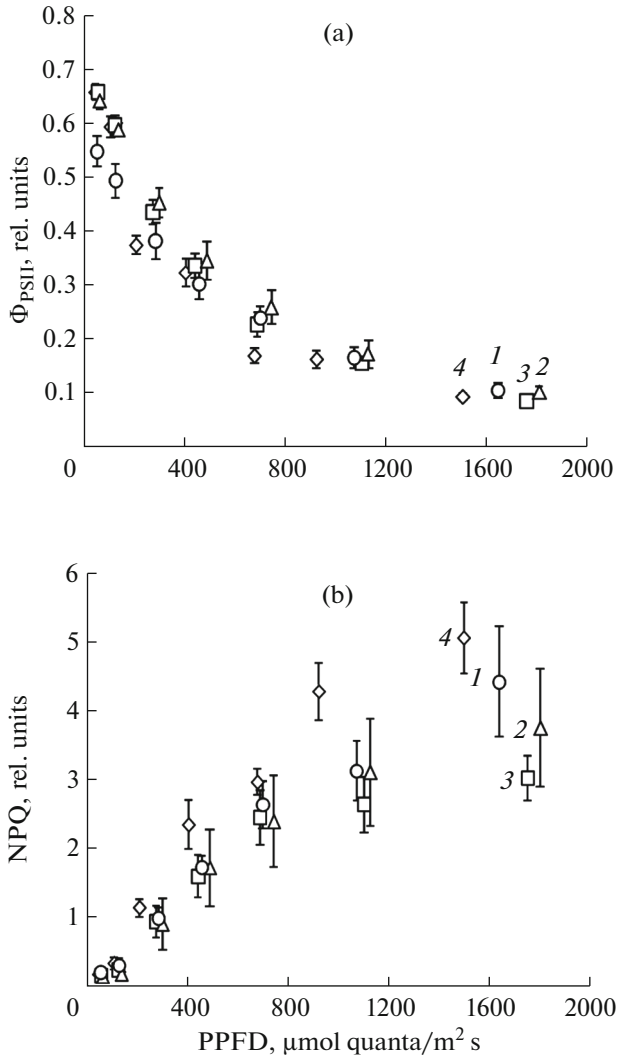

Fig. 2. Dependence of (a) the effective quantum yield of PSII and (b) nonphotochemical quenching of chlorophyll *a* fluorescence on photon flux density in *Lobaria pulmonaria* thalli sampled in different months of the year: (*1*) April; (*2*) June; (*3*) October; (*4*) January. Data show the means and standard errors of the measured parameters. Measurements were carried out during 2012–2014 (n = 5–7 for each PPFD level per year).

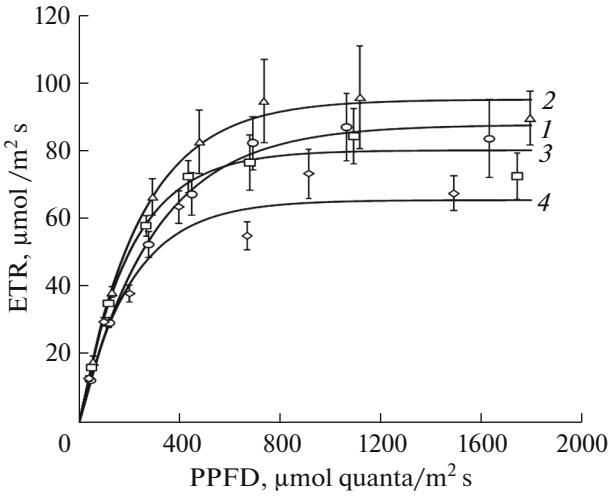

Fig. 3. Electron transport rate through PSII as a function of photon flux density in *Lobaria pulmonaria* thalli sampled in different months of the year: (*1*) April; (*2*) June; (*3*) October; (*4*) January. Symbols and error bars represent mean values and standard errors of ETR for *L. pulmonaria* thalli sampled in different months of 2012–2014 (n = 5–7 for each PPFD per year). Solid curves display the approximation of ETR as a function of PPFD with an exponential function $f(x) = a(1 - e^{-bx})$ [11]. Values of independent parameters a and b for the selected function were statistically significant at $P \leq 0.001$.

Activity and proportions of respiratory pathways in lichen thalli. The thalli of *L. pulmonaria* were characterized by a relatively high respiratory capacity in all seasons of the year (Table 7). The rate of O_2 consumption at 20°C varied in the range of 700–900 nmol/(g dry wt min). The rates of O_2 uptake were slightly higher in spring and autumn than in summer and winter. However, the differences were statistically insignificant. The rate of total O_2 uptake was mostly due to mitochondrial respiration; the contribution of residual (nonmitochondrial) respiration was ≤15%. The activities of respiratory pathways and their contribution to total respiration were subject to changes throughout the year. The contribution of the cytochrome respiratory pathway (CP) varied from 30% (in October) to 56% (in June), while the alternative respiratory pathway (AP) made up 29% in June and reached 56% in October. The yearly average contributions of CP and AP to the total O_2 uptake were 45 and 40%, respectively. The activity ratio for the cytochrome and the alternative respiratory pathways (V_{cyt}/V_{alt}) varied from 0.5 (October) to 1.9 (June).

The rate of heat release (Q) indicative of general metabolic activity varied in the range of 1.7–4.4 μW/mg dry wt; the higher values of this parameter were recorded in spring and autumn. It should be noted that seasonal changes in Q value were similar to those in the activity of alternative respiration.

DISCUSSION

Lichens are poikilohydric organisms that are unable to retain water. Spring and autumn periods with moderate temperatures and high humidity are considered the most favorable for the growth of *L. pulmonaria* [13]. In summer, the thalli often dry out and become physiologically inactive. Our earlier studies showed that the loss of moisture in *L. pulmonaria* was accompanied by the decrease in thallus area; the thallus edges became curled, while the upper cortex layer composed of fungal hyphae turned dense and poorly transmitted light to the algae [7]. Therefore, to obtain compatible data characterizing seasonal changes in photosynthetic and respiratory activity, we first

Table 5. Results of modeling the light dependence of electron transport rate in PSII of the photobiont *Lobaria pulmonaria* in different seasons of the year

Parameter	April	June	October	January
ETR_{max}, μmol/m^2 s	88 ± 5	95 ± 4	80 ± 3	66 ± 3
ETR_{sat}, μmol/m^2 s	79	86	72	59
$PPFD_{sat}$, μmol/m^2 s	678	562	470	476

The exponential function $f(x) = a\ (1 - e^{-bx})$ was used to describe ETR as a function of PPFD; the main parameters (ETR_{max}, $PPFD_{sat}$, and ETR_{sat}) were calculated according to [11]. The values of independent parameters a ($P \le 0.001$) and b ($P \le 0.001$) were statistically significant for the function chosen.

Table 6. Characteristics of the light dependence of CO_2 exchange in *Lobaria pulmonaria* thalli in different seasons

Parameters	April	June	October	January
Quantum yield, ϕ	0.043 ± 0.008[a]	0.011 ± 0.003[b]	0.056 ± 0.009[a]	0.029 ± 0.006[c]
LCP, μmol quanta/m^2 s	20 ± 14[a]	77 ± 59 [a]	24 ± 13[a]	33 ± 22[a]
P_n, μmol CO_2/m^2 s	5.7 ± 0.5[a]	2.5 ± 0.4[b]	4.5 ± 0.5[a]	5.5 ± 0.4[a]
R_d, μmol CO_2/m^2 s	−1.1 ± 0.5[a]	−0.9 ± 0.6[a]	−1.8 ± 0.7[a]	−1.9 ± 0.7[a]

ϕ—quantum yield of photosynthesis; LCP—light compensation point, P_n—maximum rate of net CO_2 uptake; R_d—dark respiration. Different superscript letters indicate statistically significant parameter changes during the year at $P \le 0.05$ (analysis of variance ANOVA, Duncan's test, $n = 5–10$).

hydrated freshly sampled *L. pulmonaria* thalli and allowed them to acclimate under standard conditions for 1.5–2 h. The suitability of such pretreatment of lichen thalli was also noted by other researchers [10].

We found that the photobiont of wintering lichen thalli is able to quickly restore the functional activity of PSA under laboratory conditions (Table 4). One-hour adaptation of hydrated thalli at 22°C sufficed to achieve the F_v/F_m values close to the highest level typical of chlorobiont lichens [10]. After 4 h of acclimation, the Φ_{PSII} and NPQ values for *L. pulmonaria* thalli collected in winter were comparable with the values typical for thalli sampled in summer. The rate of net CO_2 uptake in thalli acclimated for 2–4 h under laboratory conditions was approximately 3 μmol/m^2 s.

The results indicate that the PSA of the photobiont is well preserved in the lichen during overwintering. This view is also supported by low-temperature spectra of chlorophyll *a* fluorescence (Fig. 1). Analysis of these spectra showed that the long-term exposure of lichen to freezing temperatures did not cause the degradation of PSA pigment–protein complexes of the photobiont. In thalli stored for a long time at freezing temperatures, a slight change in the F_{735}/F_{685} ratio was noted in comparison with the samples collected in spring. However, after a 30-h acclimation of these thalli under laboratory conditions, the difference between these samples almost disappeared. Similar observations were made with *L. pulmonaria* during desiccation and under osmotic stress conditions [14]. It is possible that the fast redistribution of energy fluxes between PSII and PSI helps to preserve the integrity of pigment–protein complexes of photosystems at periods with frequent changes in moisture availability or upon the recurrent transitions of air temperature through the 0°C level that influence water condition in thalli.

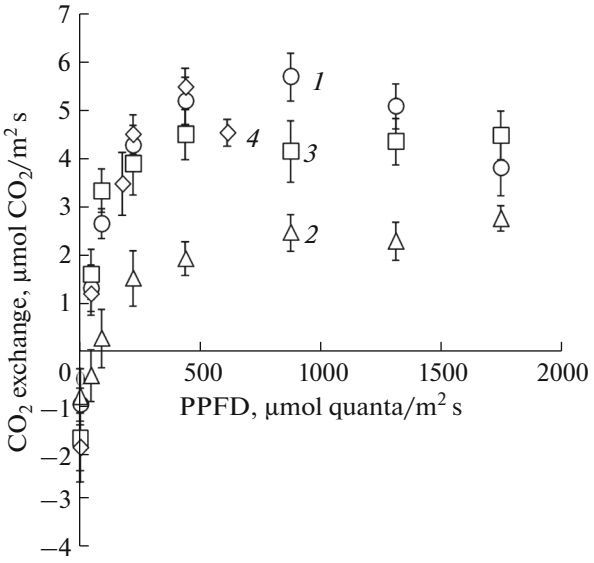

Fig. 4. Dependence of net CO_2 uptake rate on photon flux density in *Lobaria pulmonaria* thalli sampled in different months of the year: (*1*) April; (*2*) June; (*3*) October; (*4*) January. Symbols and error bars represent mean values and standard errors for *L. pulmonaria* thalli sampled in different months during 2012–2014. ($n = 5–7$ for each PPFD per year).

Table 7. Rates of total respiration (V_t), cytochrome-mediated respiration (V_{cyt}), alternative respiration (V_{alt}), and metabolic heat release (Q) in thalli of *Lobaria pulmonaria* in different seasons of the year

Month of sampling	O_2 uptake, nmol/(g dry wt min)				Heat production (Q), μW/mg dry wt
	V_t	V_{cyt}	V_{alt}	V_{cyt}/V_{alt}	
Apr.	885 ± 58^{a}	382 ± 56^{a}	382 ± 25^{bc}	1.0 ± 0.2^{ab}	4.4 ± 0.3^{c}
June	688 ± 47^{a}	387 ± 33^{a}	201 ± 31^{a}	2.4 ± 0.5^{b}	1.7 ± 0.3^{a}
Oct.	945 ± 61^{a}	265 ± 43^{a}	534 ± 76^{c}	0.5 ± 0.1^{a}	3.4 ± 0.4^{bc}
Jan.	796 ± 82^{a}	387 ± 63^{a}	287 ± 31^{ab}	1.5 ± 0.4^{ab}	2.8 ± 0.2^{ab}

Mean values were obtained from several series of independent measurements performed in the period from 2012 to 2017 ($n = 5–11$ per each month). Measurements were conducted at 20°C. Different superscript letters indicate statistically significant parameter changes during the year at $P \le 0.05$ (analysis of variance ANOVA, Duncan's test).

In experiments with the foliose lichen *Umbilicaria aprina*, the presence of three fractions of water in thalli was shown: loosely bound water, tightly bound water, and a fraction of very tightly bound water [15]. Upon gradual cooling of the thalli to −20°C, loosely bound water diffused from the cells to the hypha surface and filled the interstices of the medullary and algal layers [16]. As a result, ice formation occurred in the intercellular spaces and did not damage the thallus. When the thallus thawed, algal and fungal cells absorbed molten water from intercellular spaces and became turgescent. In an earlier study, we used the method of biological calorimetry and showed that the phase transition between liquid water and ice occurred in *L. pulmonaria* thalli in winter at a temperature of −10°C and that nearly 30% of lichen water became frozen [7].

Although the lichen PSA is tolerant to the action of adverse environmental factors in winter, a twofold decrease in the chlorophyll pool was noted in spring time (Table 2). The decay in the content of green pigments can be considered an adaptive response, since it reduces the absorption of quanta in the period when low temperatures restrict the use of light energy for CO_2 assimilation while the excessive insolation may lead to photodegradation of PSA. Some authors believe that changes in the pigment pool of lichens during the growing season are caused by changes in the number of photobiont cells [17]. Apparently, the seasonal regulation of the number of cells of the photobiont allows lichens to adapt to changes in light conditions and to ensure the supply of required organic carbon to the mycobiont during the growing season.

The photodestruction of PSA is mainly prevented by zeaxanthin-dependent quenching of excess energy in PSII of green alga. The light-dependent conversion of violaxanthin into zeaxanthin is carried out by the xanthophyll cycle [18]. However, in contrast to higher plants, the regulatory protein in lichens is LHCSR [19] instead of PSBS. In *L. pulmonaria*, comparatively high DEPS values (24–34%) were recorded in the winter–spring period (Table 3). In summer, the DEPS level was approximately 15%. The elevation of DEPS values in winter is consistent with the seasonal increase in nonphotochemical quenching indicative of thermal energy dissipation (Fig. 2b). This is also in line with a trend towards the decrease in the effective quantum yield of PSII (Fig. 2a). Similar seasonal changes in the activity of photoprotective mechanisms were earlier found in thalli of *L. pulmonaria* inhabiting deciduous forests [20] and in the epilithic lichen *Xanthoria parietina* upon exploring the mechanisms of PSA adaptation to the high light environment [21].

Analysis of the seasonal dynamics of ETR as a function of PPFD indicates that the PSII photochemical processes remain highly efficient in the photobiont at a wide range of irradiance throughout the year. The ETR_{max} values were slightly elevated in summer, while they were reduced insignificantly in the thalli collected in spring and autumn (Table 5). The light saturation of ETR was observed at a PPFD from 470 to 680 μmol/m^2 s, i.e., at photon flux densities that are several times higher than the average irradiance in natural habitats of the lichen. Apparently, the seasonal changes in photochemical activity of PSA do not limit the photosynthetic rate, whereas the CO_2-assimilating capacity is restricted by dark stages of carbon fixation and depends on the environmental factors and the condition of the lichen itself.

Lichens are usually regarded as phototrophic organisms with low photosynthetic activity. Our data suggest that hydrated thalli of *L. pulmonaria* are able to assimilate CO_2 under saturating light at the rates up to 5 μmol/m^2 s (Fig. 4, Table 6). The decrease in P_n rate, the increase in light compensation point, and the suppression of PSII quantum yield in June were most likely due to large losses of moisture from thalli. Under natural conditions at high summer temperatures and low air humidity, *L. pulmonaria* thalli rapidly lost moisture and switched to the release of CO_2 in daytime [7]. The positive CO_2 exchange was recorded only in early mornings, when the thalli were partially hydrated by atmospheric moisture. As a result, the carbon balance of the lichen was negative for the

major part of the day. Our results confirm the opinion of some authors [13, 17] that transitional periods (spring and autumn) with moderately warm and humid weather are most favorable for lichens in the forest zone. In spring and autumn, a positive carbon balance is formed and the thallus growth is activated.

On the other hand, the CO_2 exchange in *L. pulmonaria* in summer might depend on the content of ribulose bisphosphate carboxylase/oxygenase (Rubisco). According to MacKenzie et al. [22], the attenuated irradiance in tree stands with the dominance of deciduous species in summer reduces the content of large Rubisco subunit in *L. pulmonaria* thalli. The authors believed that the content of Rubisco subunits increases in the autumn–winter period due to the adaptation of lichen to the anticipated increase in irradiance in the canopy in the forthcoming spring. This can partly explain the observed high potential activity of thallus photosynthesis in winter. It should be noted that the light saturation of net photosynthesis of *L. pulmonaria* was achieved at a PPFD of at least 500 μmol quanta/m^2 s (Fig. 4), while the actual daily irradiance under natural conditions was significantly (2–5 times) lower (Table 1).

In plant leaves, the dark respiration does not exceed 15–20% of apparent photosynthesis. The prevalence of heterotrophic mycobiont in the lichen biomass is manifested in the proportions of CO_2 uptake and CO_2 release in thalli (Table 6). According to the literature data [23], the respiration of lichens under natural conditions increases in the summer–autumn period and is more sensitive to temperature changes than in winter. We did not reveal significant seasonal changes in respiratory capacity of hydrated *L. pulmonaria* thalli, which was assessed at 20°C from the rates of dark CO_2 release and O_2 consumption (Tables 6, 7). Nevertheless, the cyanide-resistant respiration mediated by alternative oxidase (AOX) was found to be activated in spring and autumn, which altered the proportions of the respiratory pathways. It is known that AOX is present in the mitochondrial electron transport chain (ETC) in plants, algae, and fungi and that the electron transport through AOX during cell respiration is not coupled with energy production and leads to thermal dissipation of energy [24]. The information on the respiratory pathways in lichens has been quite scarce until recently. In *L. pulmonaria*, the activity of the main energy-producing cytochrome pathway (CP) did not change significantly during the seasons. The induction of an alternative respiratory pathway (AP) in plants is considered as one of the main physiological markers of stress [25]. We noted in earlier studies an increase in AP activity in lichen thalli under UV-B irradiation and short-term exposure to high temperatures [26, 27]. It is possible that the activation of AP respiration in lichen thalli in spring and autumn is aimed at preventing the over-reduction of the ubiquinone pool and the excessive ROS production. The role of the alternative respiratory pathway as a component of the antioxidant system has been established for higher plants and nonlichenized fungi [28, 29]. On the other hand, spring and autumn are the most favorable seasons for lichen growth. The high rate of electron transport along the alternative pathway could promote the intense of tricarboxylic acids cycle and facilitate the sustained activity of energetically efficient cytochrome-mediated respiration without over-reduction of mitochondrial ETC. In this case, AP can serve as an indicator of the increased level of lichen metabolism.

The enhanced metabolic activity in *L. pulmonaria* thalli in spring and autumn was also evidenced by the increase in metabolic heat release (Table 7). Moreover, the increase in heat production Q coincided with the activation of AP. According to Beckett et al. [30], the observed significant increase in the rate of heat release in thalli of *Peltigera polydactylon* after rehydration was caused by the acceleration of AP-mediated respiration. However, no direct assay of lichen respiration was performed in that work.

Thus, multiyear studies of functional parameters in the lichen *L. pulmonaria* revealed that, under optimal irradiance and temperature, the hydrated thalli are able to assimilate CO_2 at a rate of 3–5 μmol/m^2 s throughout the entire annual cycle. During overwintering, the lichen photobiont remains photochemically active and is able to assimilate CO_2 immediately after the transfer from natural environments to laboratory conditions, which indicates that the structure and function of PSA are not impaired. A complex of adaptive physiological mechanisms is involved in maintaining the metabolic activity. At the level of the photobiont, the seasonal changes in the content and ratio of pigments take place; the seasonal modulations involve the conversion of VXC pigments as well as the photochemical activity and energy dissipation in PSII. The mycobiont predominates in the biomass and respiration of lichen, regulates the metabolism by involving energetically ineffective alternative respiratory pathway and by maintaining the constitutive level of activity of the main cytochrome respiratory pathway during the annual cycle. The adaptive mechanisms are activated in spring and autumn that are most favorable periods for lichen growth.

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COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interests. The authors declare that they have no conflicts of interest.

Statement on the welfare of humans or animals. This article does not contain any studies involving animals performed by any of the authors.

REFERENCES

1. Honegger, R., Metabolic interactions at the mycobiont-photobiont interface in lichens, in *Plant Relationships*, Carroll, P.D.G.C. and Tudzynski, P.D.P., Eds., Berlin: Springer-Verlag, 1997, p. 209.
2. Manoilenko, K.V., Academician A.S. Famintsyn: from fundamental science to demands of agriculture, *S-kh. Biol.*, 2010, vol. 45, p. 117.
3. Kappen, L. and Valladares, F., Opportunistic growth and desiccation tolerance: the ecological success of poikilohydrous autotrophs, in *Handbook of Functional Plant Ecology*, Pugnaire, F. and Valladares, F., Eds., New York: Marcel Dekker, 1999, p. 9.
4. Pystina, T.N., *Lishainiki taezhnykh lesov Evropeiskogo Severo-Vostoka: podzony yuzhnoi i srednei taigi* (Lichen of Taiga Forests of European Northeast: Subzone of Southern and Middle Taiga), Yekaterinburg: Ural. Otd., Ross. Akad. Nauk, 2003.
5. Beckett, R.P., Kranner, I., and Minibayeva, F.V., Stress physiology and the symbiosis, in *Lichen Biology*, Nash, T.H. III, Ed., 2nd ed., Cambridge: Cambridge Univ. Press, 2008, p. 134. https://doi.org/10.1017/CBO9780511790478.009
6. Yoshimura, I., Lung lichens and their vegetation in Japan and the other regions, in *Lobarion Lichens as Indicators of the Primeval Forests of the Eastern Carpathians*, Kondratyuk, S.Y. and Coppins, B.J., Eds., Kiev: Phytosociocentre, 1998, p. 53.
7. Golovko, T.K., Dal'ke, I.V., Dymova, O.V., Malyshev, R.V., Plyusnina, S.N., Pystina, T.N., Semenova, N.A., Tabalenkova, G.N., and Shelyakin, M.A., Functional ecology of the lichen *Lobaria pulmonaria* (L.) Hoffm. in taiga zone in the European North-East of Russia, *Izv. Komi Nauchn. Tsentra, Ural. Otd., Ross. Akad. Nauk*, 2018, vol. 3, p. 23.
8. *Atlas Respubliki Komi po klimatu i gidrologii* (Atlas of Komi Republic on Climate and Hydrology), Taskaev, A.I., Ed., Moscow: Drofa, 1997.
9. Dymova, O.V. and Kuzivanova, O.A., The optimization of extraction routine of photosynthetic pigments and its content in lichens thalli, *Khim. Rastit. Syr'ya*, 2018, vol. 2, p. 137. https://doi.org/10.14258/jcprm.2018023013
10. Jensen, M., Measurement of chlorophyll fluorescence in lichens, in *Protocols in Lichenology: Culturing, Biochemistry, Ecophysiology and Use in Biomonitoring*, Kranner, I.S., Beckett, R.P., and Varma, A.K., Eds., Berlin: Springer-Verlag, 2002, p. 135. https://doi.org/10.1007/978-3-642-56359-1_9
11. Rascher, U., Liebig, M., and Lüttge, U., Evaluation of instant light-response curves of chlorophyll fluorescence parameters obtained with a portable chlorophyll fluorometer on site in the field, *Plant Cell Environ.*, 2000, vol. 23, p. 1397. https://doi.org/10.1046/j.1365-3040.2000.00650.x
12. Bahr, J.T. and Bonner, W.D., Cyanide-insensitive respiration I. The steady states of skunk cabbage spadix and bean hypocotyl mitochondria, *J. Biol. Chem.*, 1973, vol. 248, p. 3441.
13. Muir, P.S., Shirazi, A.M., and Patrie, J., Seasonal growth dynamics in the lichen *Lobaria pulmonaria, Bryologist*, 1997, vol. 100, p. 458. https://doi.org/10.2307/3244407
14. Chakir, S. and Jensen, M., How does *Lobaria pulmonaria* regulate photosystem II during progressive desiccation and osmotic water stress? A chlorophyll fluorescence study at room temperature and at 77 K, *Physiol. Plant*, 1999, vol. 105, p. 257. https://doi.org/10.1034/j.1399-3054.1999.105210.x
15. Harańczyk, H., Bacior, M., and Olech, M.A., Deep dehydration of *Umbilicaria aprina* thalli observed by proton NMR and sorption isotherm, *Antarct. Sci.*, 2008, vol. 20, p. 527. https://doi.org/10.1017/S0954102008001363
16. Schroeter, B. and Scheidegger, C., Water relations in lichens at subzero temperatures: structural changes and carbon dioxide exchange in the lichen *Umbilicaria aprina* from continental Antarctica, *New Phytol.*, 1995, vol. 131, p. 273. https://doi.org/10.1111/j.1469-8137.1995.tb05729.x
17. Tretiach, M., Bertuzzi, S., Candotto Carniel, F., and Virgilio, D., Seasonal acclimation in the epiphytic lichen *Parmelia sulcata* is influenced by change in photobiont population density, *Oecologia*, 2013, vol. 173, p. 649. https://doi.org/10.1007/s00442-013-2654-3
18. Demmig-Adams, B., Carotenoids and photoprotection in plants: a role for the xanthophyll zeaxanthin, *Biochim. Biophys. Acta, Bioenerg.*, 1990, vol. 1020, p. 1. https://doi.org/10.1016/0005-2728(90)90088-L
19. Pinnola, A., The rise and fall of light-harvesting complex stress-related proteins as photoprotection agents during evolution, *J. Exp. Bot.*, 2019, vol. 70, p. 5527. https://doi.org/10.1093/jxb/erz317
20. MacKenzie, T.D., Król, M., Huner, N.P.A., and Campbell, D.A., Seasonal changes in chlorophyll fluorescence quenching and the induction and capacity of the photoprotective xanthophyll cycle in *Lobaria pulmonaria, Can. J. Bot.*, 2002, vol. 80, p. 255. https://doi.org/10.1139/b02-005
21. Vráblíková, H., McEvoy, M., Solhaug, K.A., Barták, M., and Gauslaa, Y., Annual variation in photoacclimation and photoprotection of the photobiont in the foliose lichen *Xanthoria parietina, J. Photochem. Photobiol. B*, 2006, vol. 83, p. 151. https://doi.org/10.1016/j.jphotobiol.2005.12.019
22. MacKenzie, T.D., MacDonald, T.M., Dubois, L.A., and Campbell, D.A., Seasonal changes in temperature and light drive acclimation of photosynthetic physiology and macromolecular content in *Lobaria pulmonaria, Planta*, 2001, vol. 214, p. 57. https://doi.org/10.1007/s004250100580
23. Lange, O.L. and Green, T.G.A., Lichens show that fungi can acclimate their respiration to seasonal chang-

es in temperature, *Oecologia*, 2005, vol. 142, p. 11. https://doi.org/10.1007/s00442-004-1697-x

24. McDonald, A.E. and Vanlerberghe, G.C., Origins, evolutionary history, and taxonomic distribution of alternative oxidase and plastoquinol terminal oxidase, *Comp. Biochem. Physiol. Part D: Genomics Proteomics*, 2006, vol. 1, p. 357. https://doi.org/10.1016/j.cbd.2006.08.001
25. van Dongen, J.T., Gupta, K.J., Ramírez-Aguilar, S.J., Araújo, W.L., Nunes-Nesi, A., and Fernie, A.R., Regulation of respiration in plants: a role for alternative metabolic pathways, *J. Plant Physiol.*, 2011, vol. 168, p. 1434. https://doi.org/10.1016/j.jplph.2010.11.004
26. Shelyakin, M.A., Zakhozhii, I.G., and Golovko, T.K., Changes of total respiration and respiratory pathways ratio in lichens adaptation to UV-B radiation, *Izv. Ufimsk. Nauchn. Tsentra, Ross. Akad. Nauk*, 2018, vol. 3, p. 100.
27. Shelyakin, M., Zakhozhiy, I., and Golovko, T., The effect of temperature on Antarctic lichen cytochrome and alternative respiratory pathway rates, *Polar Biol.*, 2020, vol. 43, p. 2003. https://doi.org/10.1007/s00300-020-02758-4
28. Maxwell, D.P., Wang, Y., and McIntosh, L., The alternative oxidase lowers mitochondrial reactive oxygen production in plant cells, *Proc. Natl. Acad. Sci. U.S.A.*, 1999, vol. 96, p. 8271. https://doi.org/10.1073/pnas.96.14.8271
29. Joseph-Horne, T., Hollomon, D.W., and Wood, P.M., Fungal respiration: a fusion of standard and alternative components, *Biochim. Biophys. Acta, Bioenerg.*, 2001, vol. 1504, p. 179. https://doi.org/10.1016/S0005-2728(00)00251-6
30. Beckett, R.P., Alyabyev, A.J., and Minibayeva, F.V., Patterns of heat production during desiccation and rehydration in lichens differing in desiccation tolerance, *Lichenologist*, 2011, vol. 43, p. 178. https://doi.org/10.1017/S0024282910000769

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