

## The effect of upper cortex absence on spectral reflectance indices in Antarctic lichens during thallus dehydration

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### Abstract

In maritime Antarctica, lichens and mosses represent dominant autotrophs forming community structure of vegetation oases. In our study, we selected 4 most common lichen species (*Xanthoria elegans*, *Rhizoplaca melanophthalma*, *Leptogium puberulum*, *Physconia muscigena*) and monospecific colony of *Nostoc commune* typical for James Ross Island (Antarctica) for detailed physiological experiments. We investigated their spectral characteristics in response to hydration status of their thalli. In samples desiccating from fully wet (RWC, relative water content of 100%) to dry state (RWC = 0), photochemical reflectance index (PRI), and normalized difference vegetation index (NDVI) were evaluated for control thalli and those with removed upper cortex. In this way, the effect of presence/absence of the upper cortex on PRI, NDVI was studied. PRI showed either no change or species-specific an increase/decrease with dehydration. Removal of the upper cortex caused both PRI decrease (*N. commune*, *P. muscigena*) and increase (*R. melanophthalma*, *L. puberulum*). Removal of the upper cortex led to increase in NDVI in all species, typically within the RWC range of 20-100%. Species-specific differences of hydration-response curves of PRI and NDVI are discussed as well as the role of the absence of the upper cortex in the evaluation of spectral characteristics in desiccating lichens.

**Key words:** PRI, NDVI, cyanolichens, chlorolichens, *Nostoc commune*

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## Introduction

Since the late 80-ies, spectral reflectance curves of lichens (Ager et Milton 1987) have been used in remote sensing to determine lichen-covered and lichen-free rock surfaces. Recently, the attempts are made to distinguish substrate from a lichen cover (*e.g.* Zhang et al. 2005) and identify individual lichen species from a complex spectrum taken by satellite cameras (Morison et al. 2014). Application of spectral reflectance methods in physiological studies in lichens is, however, much less frequent in comparison to the studies dealing with remote sensing and mapping of lichens in forest-free lands. Within last three decades, several physiological studies have focused on spectral characteristics of lichens addressing different aspects of lichen biology such as species-specificity (*e.g.* Van der Veen et Csatho 2005), thallus dehydration (Jupa et al. 2012, Barták et al. 2015a), effects of heavy metals on lichen reflectance spectra (Regan et al. 2016), and manipulated amount of secondary metabolites in the upper cortex (Barták et al. 2016).

Appart of spectral reflectance curves, spectral reflectance indices are used in lichens recently. Normalized difference vegetation index (NDVI), and the photochemical reflectance index (PRI) are the most frequently exploited in lichen biology recently in remote sensing (Marcinkowska-Ochtyra et al. 2018), field studies (*e.g.* Petzold et Goward 1988, Van der Veen et Csatho 2005, Kleefeld et al. 2018), and laboratory experiments (*e.g.* Bechtel et al. 2002, Neta et al. 2010). Generally, the estimation of daily, seasonal, and interseasonal changes of NDVI and PRI in lichen vegetation has a great potential for ecophysiological studies in subpolar and polar regions because such approach may combine field and satellite data. Moreover, laboratory PRI and NDVI data measured at different physical conditions (temperature and hydration in particular) might be used for explanation of PRI, NDVI variability in natural li-

chen-dominated ecosystems. In field research, however, NDVI and PRI would be interpreted carefully because the changes might be driven by a variety of local factors, such as durability of snow cover, precipitation pattern, microrelief-dependent differences in desiccation of lichens, *etc.*

In higher plants, NDVI reaches the highest value for green leaves, *i.e.* those with an optimal amount of chlorophyll. Any decrease in chlorophyll content is demonstrated as a decrease in NDVI. Therefore, NDVI is widely used as indicator of stresses that affect chlorophyll contents in plant tissues negatively. In lichens, NDVI was shown to decrease with thallus dehydration. However, the decrease is species-specific (Neta et al. 2010). NDVI is an indicator of negative effects of heavy metals (*e.g.* Garty et al. 2000). The photochemical reflectance index (PRI) is considered an indicator of the early stress in photosynthetic apparatus, photochemical processes in particular (Gamon et al. 1997). It is because of PRI calculation exploits the wavelengths that are related to photosynthetic pigments. The reflectance at the wavelength of 531 nm evaluates the amount of xanthophyll cycle pigments which are closely linked to radiation use efficiency and protective mechanism. The reflectance at 531 nm is associated with the de-epoxidation of violaxanthin to zeaxanthin via antheraxanthin (Gamon et al. 1990). The 570 nm reflectance is, however, unaffected by xanthophyll cycle pigments. It is, therefore, used as reference value in PRI calculation.

PRI has been exploited for different scales studies in higher plants ranging from a single leaf level to remote sensing of large areas covered by vegetation. For lichen-dominated tundra ecosystems, PRI measurements are used in complex studies focusing interannual changes of spectral characteristics, *e.g.* in North America and Eurasia Arctic transects, specifically at Isachsen Island and Krenkel Island (*see*

Walker et al. 2011). In laboratory studies with lichens, PRI has been used recently in the experiments evaluating PRI changes with progressive thallus dehydration (e.g. Singh et al. 2013, Barták et al. 2018). Since optical properties of the upper cortex change during lichen desiccation, they may affect the amount of photosynthetically active radiation reaching photobiont layer, as well as reflectance signal attributed to photosynthetic pigments. Therefore, the main objective of the present study was to as-

sess the effect of presence/absence of the upper cortex layer on PRI and NDVI in response to gradual dehydration. We hypothesized that species-specific sensitivity would exist in lichen species with different color and photobiont (alga, cyanobacterium). In this study, we measured spectral reflectance indices to describe the effect of absence of upper cortex (scraped) spectral characteristics of particular experimental species in response to dehydration.

## Material and Methods

### Collection of samples

Lichens (*Xanthoria elegans*, *Leptogium puberulum*, *Physconia muscigena*, and *Rhizoplaca melanophthalma*), and colonies of *Nostoc commune* were collected at the northern part of James Ross Island (Ulu peninsula), Antarctica. Collection site was the Long-Term Research Plot (LTRP, 63°48'03'' S, 57°52'50'' W) located close to the coast in between the confluxes of the Bohemian and Algal streams. The area was described by Barták et al. (2015b). The LTRP is dominated by *Bryum pseudo-*

*triquetrum* that forms carpets, a longitudinal axis of which follows the line of thawing water pathway from a temporary snowfield located hillside 50 m away from the area. The LTRP is composed of two sub-areas, both of them rich in mosses, lichens, and microbial mats formed by *Nostoc* sp. colonies, algae (prevalently *Zygnema* sp.) Outside of moss carpets, a stony surface is covered patchily by several lichen species, such as e.g. *Rhizoplaca melanophthalma*, *Xanthoria elegans*.

### Species characteristics

Overview of the characteristics for individual experimental species used in this study was compiled from several web sources listed in References ([1, 2] - Other sources).

*Xanthoria elegans* – epilithic foliose lichen, forming the right, yellow-orange to reddish-orange rosettes 2-4 cm in diameter or larger colonies, tightly adnate, lobate. Lobes are up to 6-7 mm long and 0.5-1 mm wide. Lower surface covered with short rhizoids, light brown or whitish color. Medulla is white, reticulate, with short to elongate hyphae. Apothecia are numerous, sedentary, up to 1-2 mm in diameter,

orange, usually with a concave or flat disc, with a solid, sometimes curved lamellar edge. Photobiont is alga *Trebouxia*. Spores are bipolar, two-cells, thickened septum with a well-visible canal. It is characterized by the predominance of parietin, which in the form of crystals covers the upper cortex layer. Presence of parietin acid, emodin and teloschistin is reported as well. The species is widespread in mountainous and Arctic areas.

*Rhizoplaca melanophthalma* (Ram.) Leuckert & Poelt – thallus lobate, forming rosettes 1-3 cm in diameter. It is attached to the substrate only by the central part.

The lobes are up to 0.3 mm wide, light yellowish, yellowish-green, to grayish-green, often shiny. Apothecia are 1-3 mm in diameter, numerous. The disc from light yellow and pale brown to black, concave, with age becomes flat. Thallus edge is thick, the same color as the disk, one-piece. Secondary metabolites in the upper cortex are with usnic acid only; in medulla - with no substances or various fatty acids (pertusaric/constipatic acid complex), or with psoromic and/or occasionally lecanoric acid chemosyndromes and occasionally other unidentified substances. *R. melanophthalma* inhabits siliceous rocky substrates rich in nitrogen compounds. Cosmopolitan, in mountainous and Arctic areas.

*Leptogium puberulum* – thallus is usually foliose, but also crustose to squamulose to dwarf fruticose, usually lobate. The cortical layer is developed on both sides. The thallus is usually lead-gray, dark-olive, brownish to almost black, dull or shiny, smooth or often strongly wrinkled, sometimes with isidia, phyllidia or lobules but soredia and pseudocyphellae absent. Photobiont – cyanobacteria *Nostoc*. Lower surface has the same color as the upper surface but usually lighter, smooth or wrinkled, sometimes with a dense white tomentum of cylindrical or spherical hairs or otherwise with scattered tufts of rhizines or hapters. Apothecia are located over the entire surface or along the edges of the lobes. Spores are cross-multicellular, fusiform to ellipsoid or ovoid. Secondary metabolites were not detected. Widely distributed from the tropics to the polar regions.

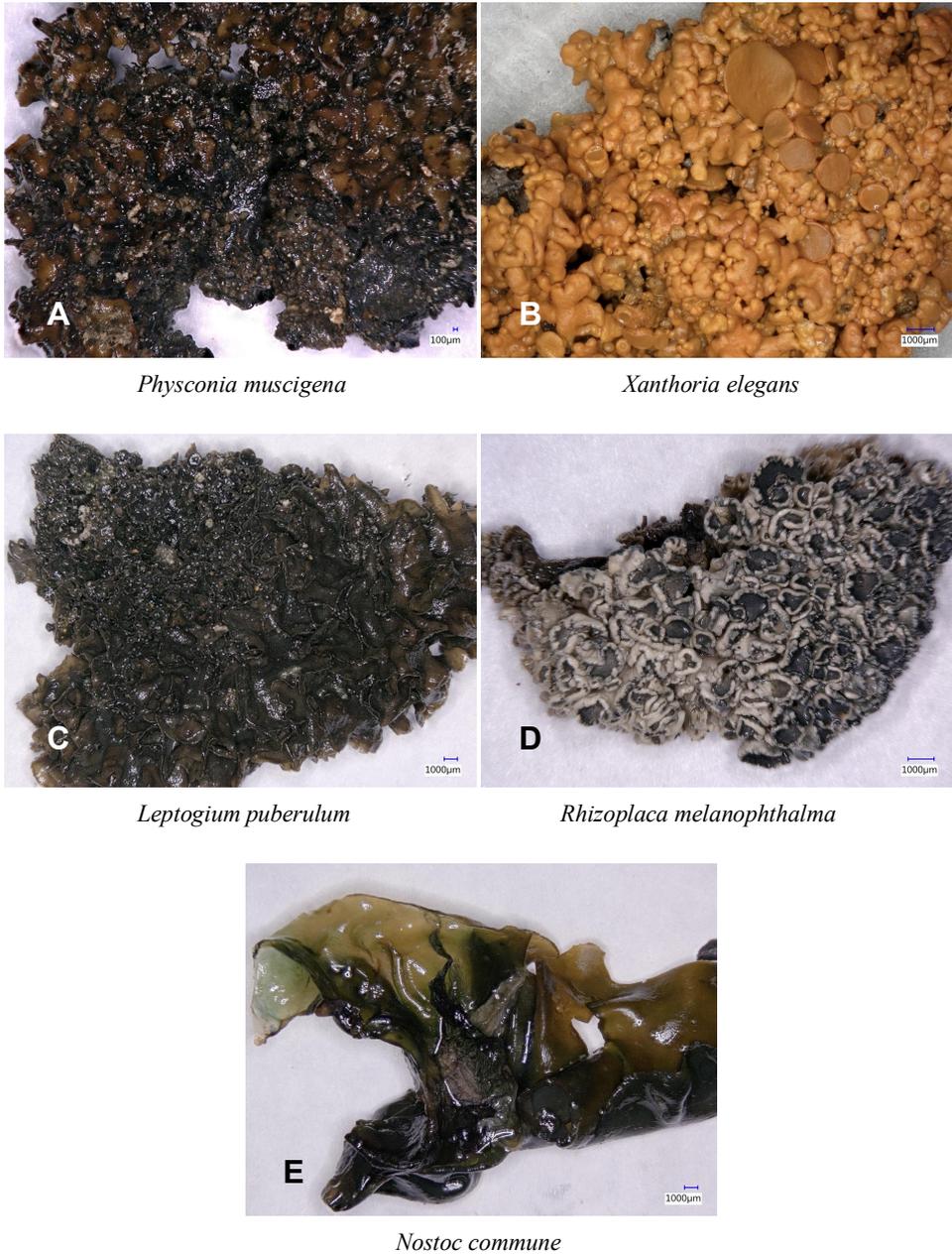
### ***Thallus characteristics***

Fully hydrated samples of experimental lichen species and *Nostoc commune* were cut into thin slices, then placed in a water droplet and observed under a digital light microscope (Keyence, WHX-900, Japan). Digital photographs were taken at the mag-

*Physconia muscigena* (Ach.) Poelt – a foliose lichen, usually irregular and often coalescent and entangled with other thalli, to 5-12 cm wide, cushion-like, loosely attached to substrate, without soredia. The lobes are up to 2 mm wide, generally linear, discrete, at the edges are usually raised up. The upper surface of the gray-brown to dark brown, covered with plaque. Medulla is white. The lower surface is light brown on the periphery, brown-black in the central part. The rhizoids are black, hairy. Secondary metabolites are often absent, but sometimes producing secalonic acid A (accessory pigment) and/or variolaric acid (accessory). *P. muscigena* grows on soil among mosses, on mossy stones and rocks. It is found in both hemispheres from the Arctic to the temperate regions in the mountains.

*Nostoc commune* is a representative of unbranched filamentous cyanoprokaryota having heterocyst. It forms macroscopic colonies, first in the form of spheres of yellow or olive color, then forms a dense sprawling strata, which grows into large, up to several tens of centimeters colonies. Frequently found in terrestrial and semi-aquatic Antarctic habitats, on various substrates, mainly in conditions of excessive moisture. Generally, the ability of *N. commune* to fix atmospheric nitrogen and, to resist desiccation may explain its dominance in many terrestrial habitats in Antarctica. It thrives well in wet Antarctic ecosystems, such as margins of freshwater terrestrial lakes, temporal ponds, streams and seepages, rich in melt water.

nification of 2000. Thicknesses of a lichen cross section (TT), and upper cortex thickness (UCT) were measured, using the 10 µm and ImageJ 1.48 software (National Institute of Health, Bethesda, USA).



**Fig. 1.** Photographs of thalli of 4 experimental lichen species and a cyanobacterial colony collected at the James Ross Island (Antarctica) and used in this study: A - *Physconia muscigena*, B - *Xanthoria elegans*, C - *Leptogium puberulum*, D - *Rhizoplaca melanophthalma*, E - colony of *Nostoc commune*. The photographs were taken by a digital optical microscope Keyence WHX-900 (Japan).

Means of at least 120 measurements of TT and UCT were calculated for each species. For experiments, control thalli and those with removed upper cortex were used. In

individual species, the upper cortex was removed manually by a steel blade under an optical microscope (Olympus BX, Japan).

**Relative water content measurements during desiccation**

The thalli segments were placed on a wet disc of paper on the bottom of Petri dishes and hydrated. Fully-hydrated segments (*i.e.* those exhibiting maximum individual weight after 24 h - tested on laboratory scales) were let to dry at room temperature (18°C, 40% RH). During desiccation, relative water content (RWC) was evaluated gravimetrically before each measurement of spectral indices: NDVI,

PRI – *see* below. During desiccation, individual samples were weighed on an analytical scales (Mettler, Germany) and RWC calculated according to the equation:  $RWC (\%) = [(Fm - Dm) / (Wm - Dm)] * 100$ , where Fm is the actual fresh mass (weight) of a sample, Dm is the mass of the fully dry sample (oven-dried sample at 35°C for 24 h), and Wm is the mass of the fully wet sample.

**Measurements of spectral reflectance indices**

NDVI (normalized difference vegetation index) and PRI (photochemical reflectance index) were measured repeatedly during gradual desiccation from fully wet (RWC = 100%) to dry (RWC = 0%) state. For NDVI measurements, a PlantPen NDVI 300 reflectometer (Photon System Instruments, Czech Republic) was used. For PRI meas-

urements, a PlantPen PRI 200 reflectometer (Photon System Instruments, Czech Republic) was used. Both instruments use particular spectral reflectances (abbreviated R) for calculation of the indices using the following equations:  
 $NDVI = (R740 - R660) / (R740 + R660)$ ,  
 $PRI = (R570 - R531) / (R570 + R531)$ .

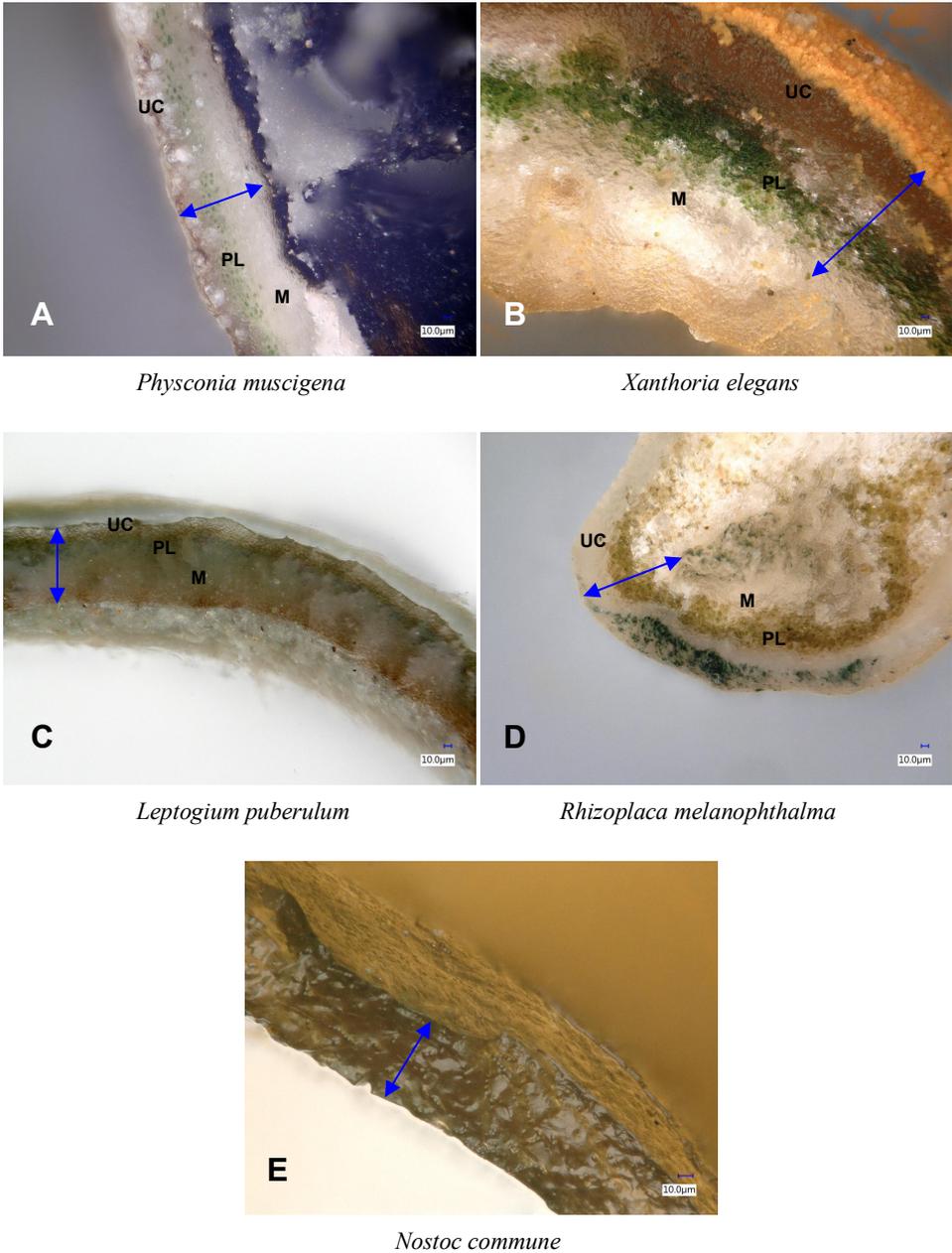
**Results and Discussion**

Thallus thickness (TT) and the upper cortex thickness (UCT) of the experimental species is summarized in Fig. 2 and Ta-

ble 1. The differences are attributed to species-specific peculiarities of morphology and growth strategy of particular species.

Species	Thalus thickness (TT)		Upper cortex thickness (UCT)	
	average	SD	average	SD
<i>Xanthoria elegans</i>	<b>344.10</b>	±52.30	<b>83.69</b>	±22.93
<i>Rhizoplaca melanophthalma</i>	<b>305.16</b>	±77.63	<b>53.00</b>	±8.68
<i>Physconia muscigena</i>	<b>112.23</b>	±12.78	<b>26.16</b>	±5.93
<i>Leptogium puberulum</i>	<b>323.58</b>	±22.62	<b>79.93</b>	±4.69
	<b>Thickness</b>			
<i>Nostoc commune</i>	<b>39.63</b>	±8.65	-	-

**Table 1.** Biometrical characteristic of the cross sections of the studied species.



**Fig. 2.** Thalli cross sections of A - *Physconia muscigena*, B - *Xanthoria elegans*, C - *Leptogium puberulum*, D - *Rhizoplaca melanophthalma*, E - colony of *Nostoc commune* with the indication of thallus thickness (TT - blue line, the arrows indicate the distance between the upper and lower thallus surface). Key to the abbreviations: UC - upper cortex layer, PL - photobiont layer, M - medulla. The photographs were taken by a digital optical microscope Keyence WHX-900 (Japan).

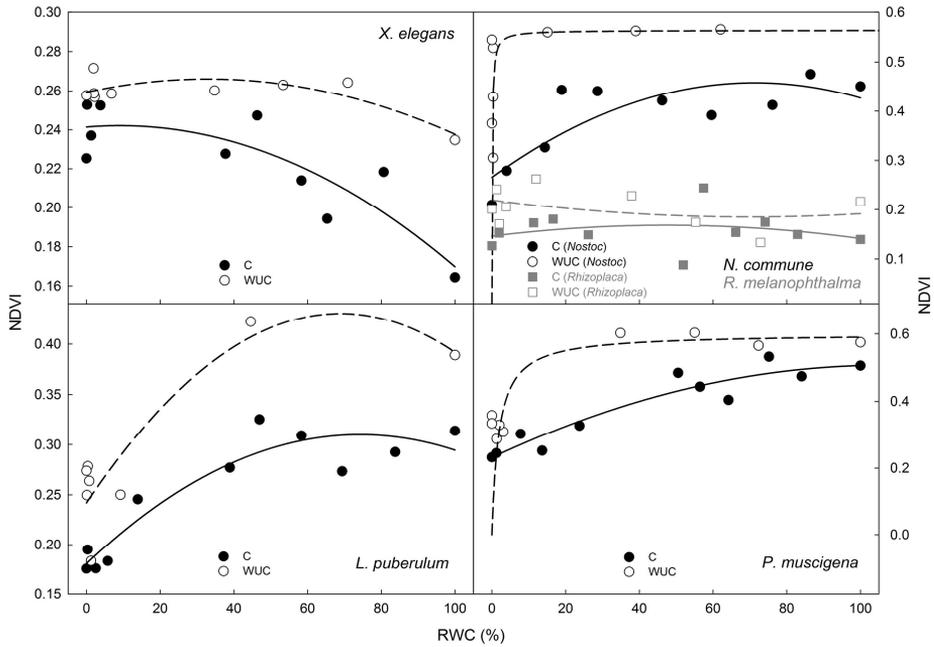
Except of *R. melanophthalma*, removal of the upper cortex led to an increase in NDVI values throughout the whole RWC range (see Fig. 3) in all experimental species. Such response might be attributed to missing absorption in the lichen samples without the upper cortex (*i.e.* scraped out). It is well established that many biomolecules with screening effects of particular wavelengths of solar radiation are located in upper cortex of lichens (Meesen *et al.* 2013). The upper cortex protects the photosynthetic cells of algal/cyanobacterial photobiont located below the upper cortex, slowing evaporation of water from a thallus and filtering harmful or excessive radiation with the assistance of pigments and secondary substances. Amount of secondary metabolites allocated in the upper cortex is species-specific (see *e.g.* Rankovic 2015) and depends on environmental factors, mainly on the availability of light at particular location. It has been shown that the upper cortex thickness increased in lichens highly exposed to sunlight, and even an epinecral layer is formed as protection to high sun exposition in some cases (Büdel 1990, Gaya 2009). Similarly, an increase in the amount of lichen metabolites in the upper cortex have been reported as a part of UV radiation screening system (Beckett *et al.* 2008). High intensity light incident on lichen thallus might induce the synthesis of melanic compounds and other colored metabolites with antioxidative properties. Thickness of the upper cortex and the amount of secondary metabolites located in there may vary even within a single species, and populations grown under different radiation regimen, respectively (Atala *et al.* 2015). Recently, the distribution of secondary metabolites in the upper cortex might be measured by non-invasive methods such as *e.g.* LDI-MSI (Mass Spectrometry Imaging) – Le Pogam *et al.* (2016).

Absence of the upper cortex leads to

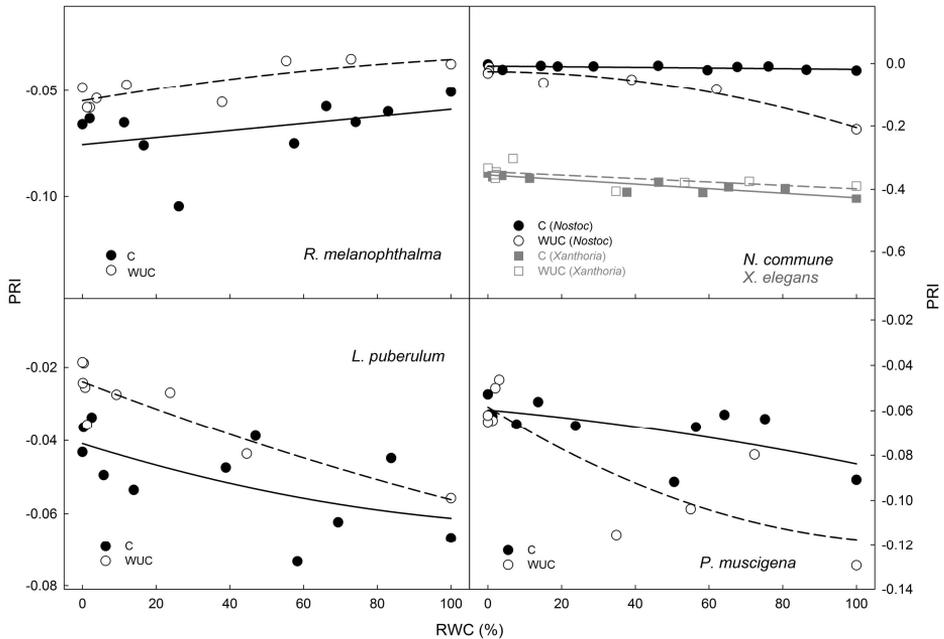
changes of optical properties of lichen thallus. In our study, we evaluated spectral reflectance indices in several species during desiccation and estimated the effect of presence/absence of the upper cortex.

Compared to control, PRI did not show any general response in the samples without the upper cortex (Fig. 4). It showed either no change (*X. elegans*), a decrease in the RWC range of 20-100% (*P. muscigena*, *N. commune*), and an increase in *R. melanophthalma* in the RWC range of 10-100%. Since PRI is well related to (1) non-photochemical quenching of access light energy absorbed by photosynthetic pigments in chloroplasts, and (2) the amount of xanthophyll cycle pigments, de-epoxidation of violaxanthin to zeaxanthin via antheraxanthin, in particular, we may conclude that these characteristics are altered in majority of the experimental species after the upper cortex removal.

PRI values measured in control samples corresponded well to the experimental evidence gained for the same species during dehydration from fully wet (RWC = 100%) to dry state (RWC = 0%). In general, the differences found in NDVI and PRI for control samples recorded in wet (RWC = 100%) and dry (RWC = 0%) are comparable to your earlier study (Barták *et al.* 2016). In recent study, absence of the upper cortex led to an increase in NDVI and both increase/ decrease in PRI (see Figs. 3 and 4). The reason for such different response seems not be easily identified. It might be associated with species-specific structure and optical properties of the upper cortex. However, quantitative data on these properties of the experimental species are rather fragmentary. Therefore, there is a need of follow up studies on the optical/spectral properties of the upper cortex of the three species. We may hypothesize, that the absence of the upper cortex may contribute to changed response of the lichen species to dehydration.



**Fig. 3.** Normalized difference vegetation index (NDVI) as dependent on the relative water content (RWC) in 5 experimental species. Data points are means of three replicates.



**Fig. 4.** Photochemical reflectance index (PRI) as dependent on the relative water content (RWC) in 5 experimental species. Data points are means of three replicates.

In *N. commune*, similar response can be expected since Deng *et al.* (2008) reports different ecophysiological properties of the upper layer of *N. sphaeroides* colonies. On the other hand, *N. commune* represents different optical systems than the lichen thalli examined in our study. It is due to the gelatinous matrix of polysaccharides and many

other organic substances in which cells are allocated. Whole system forms a complex sheet-like 3-D structures (Sand-Jensen 2014). Similarly, in *L. puberulum*, optical co-effect of exopolysaccharidic envelopes of individual *N. commune* cells (a photobiont in *L. puberulum*) might be expected.

### Concluding remarks

In our study study, we report the changes in NDVI, PRI related to presence/absence of the upper cortex in particular lichens. Removal of the upper cortex led to an increase in NDVI values within the whole range of RWC in desiccating thalli (RWC from 100 to 0 %). Shapes of the NDVI to RWC relationships for particular species (control) and particular NDVI values were comparable to a previous study (Barták *et al.* 2018). The same is true for PRI courses. For *X. elegans*, however,

slightly higher values (about 0.40) were found than in the previous study (about 0.36). In *L. puberulum*, a rapid decrease in PRI was reported by (Barták *et al.* 2018) in the thallus desiccating from 10 to 0% RWC which was not found in our study (*see* Fig. 4). This could be attributed to a high degree of intraspecific variability of ecophysiological characteristics of *L. puberulum* in response to environmental factors.

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