Hungarian University of Agriculture and Life Sciences

Doctoral School of Biological Sciences

PhD Dissertation

The bright and shaded side of duneland life from lichens' point of view

By

Katalin Veres

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This thesis is dedicated to the memory of Dr. Zsolt Csintalan (1963–2019)
and Dr. Klára Verseghy (1930–2020)

The Doctoral School

Discipline:	Biological Sciences
Name of Doctoral School:	Doctoral School of Biological Sciences
Head of school:	Dr. Zoltán Nagy Professor, D.Sc. Department of Plant Physiology and Plant Ecology, Institute of Biological Sciences, Faculty of Agricultural and Environmental Sciences, Hungarian University of Agriculture and Life Sciences
Supervisor 1:	Dr. Edit Farkas Scientific advisor, D.Sc. Institute of Ecology and Botany, Centre for Ecological Research
Supervisor 2:	Dr. Zsolt Csintalan † Professor, dr. habil. Department of Plant Physiology and Plant Ecology, Institute of Biological Sciences, Faculty of Agricultural and Environmental Sciences, Hungarian University of Agriculture and Life Sciences

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Approval of the Head of Doctoral School

Farkas Edit

....

Approval of the Supervisor 1

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1 INTRODUCTION

1.1 Terricolous lichens, their role, environment and the threatening factors

Terricolous lichens, members of biological soil crusts (BSC), are dominant associates of arid and semi-arid ecosystems (Belnap & Lange 2003). At a global scale, terricolous lichen associations are more abundant in dry habitats (Liu *et al*. 2021), where most vascular plants cannot colonise because of their sensitivity (Belnap & Lange 2003). Temperature extremes, intense (UV) radiation and a low amount of precipitation characterise these habitats to which lichens are adapted in different ways (Armstrong 2017). At the same time, terricolous lichen communities are susceptible to disturbances and intense human activities like trampling, heavy traffic (off-roading), grazing or fire that can damage them (Belnap & Eldridge 2001; Condon & Pyke 2018). Because low growth rate and slow biomass production characterise the lichen community, recovery of lichen thallus is restrained after a disturbance event, especially in crustose species, endangering BSC compartments in semi-arid regions (Hale 1973; Palmqvist & Sundberg 2000). During that time, non-native invasive plant species can colonise the free surfaces caused by the damage and suppressing of cryptogams in these ecosystems. Terricolous lichens are also threatened by the effects of climate change, especially the increasing severity and frequency of drought events in semi-arid regions (Bartholy *et al*. 2009; Ferrenberg *et al*. 2015). Therefore, the protection of vulnerable BSC lichen associations is essential. Collecting information on the composition and working processes of the communities is crucial.

1.2 Investigations of terricolous lichens on the world and in Hungary

There are many open questions in the field of terricolous lichens investigation. Around 1% of more than 52,000 publications are available on terricolous lichens involving taxonomy, ecology, physiology, etc., in the 'Recent Literature on Lichens' database (personal communication, Veres 2022. 02. 10). The topics photosynthesis (n = 688) and photoprotection (n = 38) are still total up only a tiny part of the articles in lichen research. While terricolous lichen communities are still underinvestigated (n= 42, involving areas out of semi-arid region).

The investigation of terricolous lichens and their ecophysiological adaptations and acclimations to the unique conditions prevailing in semi-arid sandy grasslands were introduced by the works of Klára Verseghy and Edit Kovács-Láng from the beginning of the 1970s in Hungary. The research of terricolous lichen communities (Gallé 1973; Lőkös & Verseghy 2001) and the main ecophysiological processes (e.g. photosynthesis, water relations, biomass production) of the dominant species had been investigated (e.g. Kalapos & Mázsa 2001; Tuba *et al*. 2008; Verseghy 1971, 1976; Verseghy *et al*. 1987). However, many open questions remained unanswered, requiring the more intense investigation of terricolous lichen assemblages and the environmental factors influencing them.

1.3 The uniqueness of inland dunelands

Microtopography plays an essential role in controlling the lichen environment, affecting the overall microclimate of a lichen association (Kershaw 1985). Dunes provide various habitats by their heterogeneous topography: sides with different exposition or the bottom and top of dune create different micro-environments and vegetation (e.g. Molnár 2003). In Europe, inland dunes can be found in the European Aeolian Sand Belt (Koster 2009), Italy and Hungary. Despite harsh environmental conditions, high species richness can characterise these habitats (Liu *et al*. 2021), and rare species can also be present (Farkas & Lőkös 2006). Continental dunes in open sand steppes are protected by the European Habitat Directive (European Commission 2013) and belong to the Natura 2000 network to ensure their long-term persistence and the survival of their valuable and threatened species assemblages.

Investigations on sand living lichen communities of inland dunelands are known from Denmark, Estonia, the Netherlands, Northern Italy and Poland in Europe. Hungary is also a potential study site with many open grasslands and dunes in the Kiskunság region. The land use has been changed during the last decades (Biró & Molnár 1998; Molnár *et al*. 2012). Therefore, updating the collected Hungarian literature data (Lőkös & Verseghy 2001) on terricolous lichen communities was relevant. Because of the low amount of investigations carried out between inland dunes, we still have limited information on terricolous lichen assemblages and the main drivers, especially soil surface variables, that shape the community in these ecosystems in Europe. Latitude (Büdel *et al*. 2014), climate and microhabitat conditions (Concostrina-Zubiri *et al*. 2014; Escolar *et al*. 2012), soil properties and the characteristics of the neighbouring vegetation (Ochoa-Hueso *et al*. 2011) or the herb and moss layer (Leppik *et al*. 2013) could have an impact on lichen species richness, functional diversity and composition in different ecosystems depending on the habitat features. The species richness and relative abundance of terricolous lichens are related to aridity, pH and vascular plant cover at a global scale (Liu *et al*. 2021).

1.4 Terricolous lichen associations of calcareous sandy grasslands

Two main groups of the soil-inhabiting lichen communities can be determined according to the acidity of the soil and vascular plant association in the open grasslands. Most of the dune lands of the European countries are typified by acidic sand are poor in nutrients (e.g. Ketner-Oostra *et al*. 2012). Since only two studies were known about the lichen communities of calcareous sandy grasslands between inland dunes in Europe, one from Italy (Gheza *et al*. 2020) and one from Estonia (Jüriado *et al*. 2016), the description of these communities from Hungary can significantly contribute to filling this knowledge gap. The effect of environmental conditions caused by human activities (disturbance history at site scale) or the topography (at landscape scale) and micro-environments (at dune scale) of inland dunes on the composition of the lichen communities is poorly known in Pannonian semi-arid sandy grasslands.

1.5 Lichens as miniature ecosystems

A lichen thallus is a complex association, mentioned as a 'self-supporting ecosystem' (Seaward 1988) and redefined as a more complex ecosystem in the past few years (Hawksworth & Grube 2020). Lichens are inhabitants of extreme habitats, where the mycobiont partner could not survive alone in nature, and the life of the photobiont is limited outside the symbiotic partnership. The coexistence can expand the distribution area of the partners alone (Nash 2008) and develop several survival strategies. In this relationship, every member supports the survival of the thallus. The photobiont may constitute as little as 10% of the total lichen biomass (Sundberg *et al*. 1999), supplies the primary carbon source for the mycobiont by carbon fixation due to photosynthesis. The mycobiont has a crucial role in protecting the photobiont and shaping the thallus structure. The three main lichen growth forms are crustose, foliose and fruticose (with many transitional forms), differing in the surface area to volume ratio (Kershaw 1985), which correlates with water relations in the thallus (Larson 1981). Lichens also produce unique fungal secondary metabolites with diverse functional roles (Molnár & Farkas 2010).

1.6 The main limiting environmental factors and the protecting strategies against them

1.6.1 Water

While the photosynthetic process and capacity in lichens are very similar to the plants (Palmquist *et al*. 2008), the poikilohydric nature of lichens strongly influences the period when photosynthesis is possible. Water is the main factor affecting lichen metabolism because thalli can photosynthesise only in the hydrated state, but they can not actively control water uptake or loss. Lichens developed several strategies to speed up water uptake or slow down water loss according to the environmental conditions, especially in dry environments (Büdel & Scheidegger 2008; Gauslaa 2014). The surface area to volume ratio correlates closely with the time required to achieve maximum thallus water content (Larson 1981). The amount of liquid water absorbed by the thallus is directly proportional, and the rate of water loss is inversely proportional to the thickness of the cortical layer

(Verseghy 1971). Growing on moss cushions prolongs the hydrated and metabolically active periods of lichens during dry days (Colesie *et al*. 2012).

1.6.2 Light

A minimum amount of light is needed for photosynthesis, but too high irradiation can cause irreversible damage to the photosynthetic system (PS) (Beckett *et al*. 2021; Heber *et al*. 2000). The protection of the PS is essential because the symbiotic partners are dependent on the primary production of the photobiont (Sadowsky & Ott 2016). In hydrated thalli, the excitation energy absorbed by the antenna system may be used for photochemical charge separation in the reaction centres (RCs) in the presence of water. When the electron transport chain is saturated, the excitation energy can be re-emitted as fluorescence or dissipated as heat. Information about the seasonal change of partition of the incoming light between the photochemical and photoprotective processes was not found in lichenological literature. The changes of the parameters in sun and shade populations are also poorly documented.

Both the mycobiont and photobiont contribute to protection against the damaging effect of excess radiation by various mechanisms (Gasulla *et al*. 2012; Nguyen *et al*. 2013; Sadowsky & Ott 2016). Reversible dehydration can be considered one of the protecting processes in preserving proteins of vital importance from long term damage (Veerman *et al*. 2007). Curling during desiccation prevents the thallus from photodamage (Barták *et al*. 2006). Algae change their shape and are more aggregated after desiccation to prevent themselves from the long term effect of desiccation (de los Rios *et al*. 2007; Scheidegger *et al*. 1995). The lower transmittance (Dietz *et al*. 2000) or higher density (Gauslaa *et al*. 2017) of the cortex also supports the protection of photobiont. Solar radiation protectant lichen secondary metabolites (LSMs) help the thallus avoid damage in the essential compartments. Substances can reflect the incoming light (e.g. atranorin) while others absorb them (e.g. usnic acid). Some substances are deposited in the cortex (e.g. atranorin, usnic acid), others deeper in the photosynthetic and medullary layer (e.g. fumarprotocetraric acid) (Molnár & Farkas 2010; Nguyen *et al*. 2013).

Both non-photochemical quenching (NPQ) via zeaxanthin (Demmig-Adams & Adams 1990; Färber *et al*. 1997) and desiccation induced (Heber *et al*. 2001, 2006; Kopecky *et al*. 2005) fluorescence quenching are also known in lichens. The process increases the thermal dissipation of excess light energy to protect the photosystem in the photobiont (Heber *et al*. 2001; Paoli *et al*. 2010; Vráblikova *et al*. 2006). The protection of PSII is critical since photodamage can also occur in an air-dry thallus (Gauslaa & Solhaug 1999; Heber *et al*. 2010; Solhaug *et al*. 2003). Other important protective mechanisms, such as enzymatic and non-enzymatic antioxidants, repairing of

PSII or chlorophagy (Beckett *et al*. 2021), are only mentioned briefly. In the absence of effective thermal energy dissipation, the production of damaging reactive oxygen species (ROS) as by-products can cause irreversible damage in the PSII (Krieger-Liszkay 2005; Müller *et al*. 2001). Conformational change in the chlorophyll-protein complex is also an effective way to dissipate excess light energy due to desiccation (Heber *et al*. 2007) in the photobiont.

The protective processes all presume efficient communication and precise regulations between the symbiotic partners (Kranner *et al*. 2005); they can regulate the photoprotective system of the other symbiotic component (Kranner *et al*. 2005; Solhaug & Gauslaa 2004). By the adaptation and acclimation, shade and sun forms of the species can develop (Piccotto & Tretiach 2010).

1.7 Adaptation and acclimation to the changing environment

At a shorter time scale, the seasonally changing weather creates fluctuating environmental conditions. Thallus anatomy (e.g. Atala *et al*. 2015; Tretiach *et al*. 2013), pigment composition (e.g. Färber *et al*. 2014), physiological functioning (e.g. Lange & Green 2003; Pirintsos *et al*. 2011), photoprotection (e.g. Gauslaa & McEvoy 2005; Vráblíková *et al*. 2006) of lichens are all influenced by both microenvironment conditions and seasonality. These studies indicate that lichens can adapt and acclimate to changing environments in different ways. However, the investigations were mostly carried out on epiphytic, forest-living or saxicolous lichens. Only a few works focused on the investigation of acclimation of terricolous lichens to the changing environment in semi-arid grasslands (Kalapos & Mázsa 2001; Tuba *et al*. 2008; Verseghy 1976).

At a long term scale, microtopography also plays an essential role in controlling the lichen environment by affecting the overall microclimate of dunes (Tilk *et al*. 2018). Description of microclimate is necessary for evaluating relations between primary processes of photosynthesis and abiotic factors (Sehnal *et al*. 2014). Only several works followed up the climate or microclimate over one year and investigated them concerning lichen functioning (e.g. Leisner *et al*. 1996; Vrábliková *et al*. 2006). The investigation of Lange (2003a, 2003b) was an important milestone. Palmquist and Sundberg (2000) investigated the seasonal acclimation of more than one species. At the same time, Raggio and co-workers (2014) monitored different soil crust types. The result of our extensive literature research revealed that studies on the seasonal change of microclimate lacked in inland dunelands. Therefore, the topic can provide a new perspective from lichens point of view. Microenvironmental conditions involving vegetation (by density or moss cover) can influence the lichen composition too (Ketner-Oostra & Sýkora 2000; Leppik *et al*. 2013; Verseghy 1983); however, the information is sparse in the literature (e.g. Concostrina-Zubiri *et al*. 2014).

2 THE AIMS OF THE PhD STUDY

2.1 Community structure of terricolous lichens in semi-arid sandy grasslands

The main goal of my PhD work was to explore the lichen community of an inland duneland ecosystem on calcium-rich sand, the main factors influencing the community at different scales (disturbance history, microclimate, soil surface variables) and the habitat preference of the species.

2.2 Acclimation strategies of the most abundant species

The second goal was to reveal the acclimation of the photosynthetic activity and photoprotection to the changing environment in space (microhabitat) and time (seasonality) combined in the seven most abundant species of the lichen community. The third goal was to describe the changing proportion of absorbed light between photosynthesis and the different photoprotection mechanisms in lichens.

Usually, only one species is grabbed from the lichen community of a given area and point in time for a study object. We intended to expand the circle of research objects deriving from the same habitat in different seasons to get closer to the entire picture of the mechanisms working in lichens and avoid any wrong generalisation.

Based on the main goals, further detailed groups of questions led the research resulting in three journal publications (Veres *et al.* 2020, 2021a, 2021b). The questions and hypotheses, then the applied methods and the achieved results are presented as a summary of these publications:

VERES, K., CSINTALAN, ZS., KOVÁCS, B., FARKAS, E. (2021a): Factors at multiple scales influence the composition of terricolous lichen communities in temperate semi-arid sandy grasslands. In: *The Lichenologist* 53(6) 467–479

Article 1 (Veres *et al.* 2021a) demonstrates the terricolous lichen community structure of semi-arid sandy grassland and the main drivers that shape them in an inland duneland ecosystem at different scales. We aimed to answer the following questions:

- What is the difference in species richness and composition at a coarse scale (site); and, does disturbance history impact this spatial level?
- How different are the terricolous lichen communities at smaller scales (dune, microhabitat)?
- What kind of environmental variables could cause this difference?

We hypothesised that disturbance history might cause differences in the species pool. In addition, we supposed that there could be a difference in environmental variables at the scale of the dune side and even smaller scales because of different light and humidity conditions. We hypothesised that this could lead to a difference in terricolous lichen species composition and that the coverage of the various growth forms would be different at different dune aspects.

VERES, K., FARKAS, E., CSINTALAN, ZS. (2020): The bright and shaded side of duneland life: the photosynthetic response of lichens to seasonal changes is species-specific. In: *Mycological Progress* 19(6) 629–641

Article 2 (Veres *et al.* 2020) focused on the influence of both microhabitat conditions and seasonal changes on photosynthetic activity and photoprotection of the most abundant terricolous lichen species (with various growth forms) of the community. We were going to reveal the main differences between microhabitat types by the continuous recording of micrometeorological data. We aimed to answer the following questions:

- How do the heterogenous terricolous lichen species acclimate in their photosynthetic activity and photoprotection during seasons and between different microhabitats?
- How is seasonality in photosynthetic activity and photoprotection of different species influenced by microhabitat conditions?
- What kind of microclimatic conditions prevail between the dunes? What are the main microclimatic differences between the opposite side of dunes, and how is it changing over seasons?

We hypothesised that sun and shade populations of the different species would differ in their responses to seasonal and spatial climatic changes because of their particular adaptation strategies. We assumed a characteristic difference in environmental conditions between the dune sides with opposite directions.

VERES, K., CSINTALAN, ZS., LAUFER, ZS., ENGEL, R., SZABÓ, K., FARKAS, E. (2021b): Photoprotection and high-light acclimation in semi-arid grassland lichens – a cooperation between algal and fungal partners. In: *Symbiosis* – doi.org/10.1007/s13199-021-00823-y

The main objective of Article 3 (Veres *et al.* 2021b) was to reveal the contributions of the photobiont and mycobiont to light acclimation and photoprotection within the lichen thallus. We aimed to reveal the influence of the seasonal light conditions and exposed versus shaded microhabitats. We also focused on the responses of the various lichen species exhibiting different thallus structures to the changing environment. We aimed to answer the following questions:

- Do the microhabitat and seasonality influence the photoprotection of the photobiont?

- Do the exposed and shaded thalli differ in the level of their solar radiation protectant lichen substances during the seasons?
- Does the utilisation of incoming light show seasonality?
- Do photobiont and mycobiont contribute equally to the photoprotection during the whole year?

We hypothesised that the investigated lichen species acclimate differently to the changing light conditions in space and time because of the different anatomy and thallus structure. We also assumed that both algae and fungi contribute more or less equally to protecting against excessive solar radiation during the whole year. The investigation extends the previous knowledge of terricolous lichens in semi-arid regions. Our results also contribute to the limited lichenological data on the proportion of absorbed excitation energy available in the literature.

3 MATERIALS AND METHODS

3.1 Study sites

The two investigated sites are located in the Kiskunság National Park near Bugacpusztaháza ('BU') (46° 39' 11.81" N, 19° 35' 45.17" E; Natura 2000 site code HUKN20024) and near Fülöpháza (Fig. 1) ('FH') (46° 52' 21.45" N, 19° 24' 18.29" E; Natura 2000 site code HUKN20011) in Central Hungary. The climate is moderately continental with a sub-Mediterranean influence on both study sites (Péczely 1967). The mean regional annual air temperature is 10.4 °C, and the yearly precipitation is 505 mm based on a 30-year-average (see in detail in Biró *et al*. 2013; Biró & Molnár 1998; Lellei-Kovács *et al*. 2011). This region is mainly covered by calcareous sand from the deposits of the Danube River. As a secondary effect, the wind reshaped the landscape resulting in North-East (NE) and South-West (SW) facing dune sides with interdune depressions between them (Pécsi 1967). The natural vegetation has been exposed to intensive human impacts for at least two millennia in the region (Biró & Molnár 1998; Molnár *et al*. 2012). The dominant plants in the semi-arid sandy grassland (*Festucetum vaginatae 'danubiale'* Rapaics ex Soó em. Borhidi association) are *Festuca vaginata* W. et K., *Stipa borysthenica* Klokov, *Fumana procumbens* (Dun.) Gren. et Godr., *Alcanna tinctoria* (L.) Tausch, *Dianthus serotinus* W. et K., *Onosma arenaria* W. et K. and *Sedum hillebrandtii* Fenzl (Fekete 1997; Borhidi *et al*. 2012) at the investigation sites. Most of the terricolous lichen species found here are members of the 'Bunte Erdflechten-Gesellschaft' (Gams 1938), also known as the complex of *Fulgensietum fulgentis* Gams and *Cladonietum symphycarpiae* Doppelb. associations, typical for this region (Büdel 2001).

Fig. 1 The study site at Fülöpháza

3.2 Micrometeorological data collection

Two micrometeorological stations were placed at the Bugacpusztaháza site, on the opposite sides of a dune with NE and SW expositions. It was hypothesised that the conditions on the selected dune represent the prevailing conditions on other dunes of similar morphology and aspects found in the territory. Two HOBO Micro Station H21-002 (Onset Computer Corporation, U. S. A.) were used with sensors (Onset Computer Corporation, U. S. A.) of air temperature (T_{air}), relative humidity (RH), and photosynthetically active radiation (PAR, 400–700 nm) at 0.5 m height and soil temperature (T_{soil}) and soil water content (WC_{soil}) at 5 cm deep in the sand. Data were continuously recorded each minute for four years. Vapour pressure deficit (VPD) was also calculated from measured data. We also used meteorological and precipitation data of the meteorological station of the Department of Plant Physiology and Plant Ecology of the Hungarian University of Agriculture and Life Sciences at Bugacpusztaháza and the Centre for Ecological Research at Fülöpháza. Unfortunately, some data are missing because of an unexpected battery discharge and damage to some wires by wild animals. We also estimated a hypothetical metabolically active period during nights (PAR = 0 µmol m^{-2} s^{-1} and RH > 80%) and a period when weather conditions could be suitable for photosynthesis (30 µmol m^{-2} s^{-1} < PAR <1,000 µmol m^{-2} s^{-1} and RH > 80% during days, based on Lange (2003) and Verseghy (1971).

3.3 Lichen material

The most abundant species have been collected for physiological measurements: the large, squamulose *Cladonia foliacea* (Huds.) Willd. (Fig. 2a); the fruticose *Cladonia furcata* (Huds.) Schrad. (Fig. 2b), the cup lichen *Cladonia magyarica* Vain. (Fig. 2c); the crustose *Diploschistes muscorum* (Scop.) R. Sant. (Fig. 2d), the placodioid *Gyalolechia fulgens* (Sw.) Søchting, Frödén and Arup (Fig. 2e), the squamulose *Thalloidima physaroides* (Opiz) Opiz. (Fig. 2f) and the foliose *Xanthoparmelia pokornyi* (Körb.) O. Blanco, A. Crespo, Elix, D. Hawksw. and Lumbsch (Fig. 2g). The species hosted eukaryotic green algae: various species of *Asterochloris* in *Cladonia* species (Škaloud *et al*. 2015; Smith *et al*. 2009), and *Trebouxia* species in *G. fulgens* and *X. pokornyi* (Leavitt *et al*. 2015; Škaloud and Peksa 2008; Stenroos *et al*. 2011). *Diploschistes muscorum* switches photobiont during its lifespan (Friedl 1986; Piercey-Normore and DePriest 2001; Škaloud *et al*. 2015; Wedin *et al*. 2015). In the early stage, it has *Asterochloris* captured from *Cladonia* species, later replaced by various species of *Trebouxia*. In the early stages, the photosynthetic partner of *Thalloidima physaroides* is a cyanobacterium switched to coccoid green algae over time (Smith *et al.* 2009; Timdal 1991).

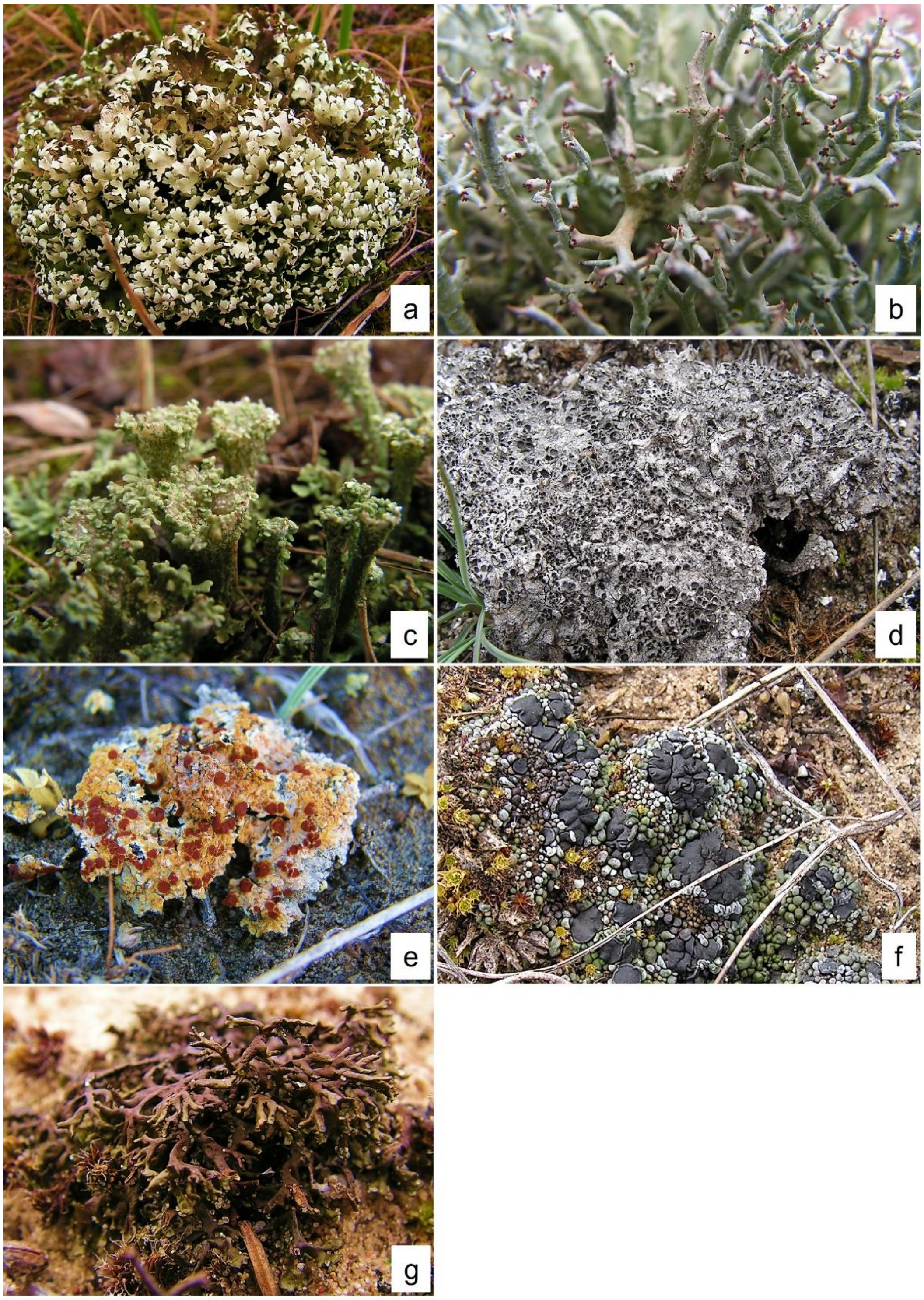

Fig. 2a-g The most abundant species: a.) *Cladonia foliacea* (Huds.) Willd., b.) *Cladonia furcata* (Huds.) Schrad., c.) *Cladonia magyarica* Vain., d.) *Diploschistes muscorum* (Scop.) R. Sant., e.) *Gyalolechia fulgens* (Sw.) Søchting, Frödén and Arup, f.) *Thalloidima physaroides* (Opiz) Opiz. and g.) *Xanthoparmelia pokornyi* (Körb.) O. Blanco, A. Crespo, Elix, D. Hawksw. and Lumbsch

Lichens were identified according to Smith *et al*. (2009), Verseghy (1994) and Wirth *et al*. (2013). The nomenclature follows the Index Fungorum CABI (2020) and Farkas & Lőkös (1994). After measurements, voucher specimens were deposited in Lichen Herbarium VBI, Hungary.

3.4 Sampling method

3.4.1 Coenology

Overall, ten stands were randomly selected, representing the duneland vegetation of the area. At Bugacpusztaháza six and at Fülöpháza four stands were located on randomly selected SW and NE aspects of different dunes according to the characteristics of the aggregated vegetation (Scheidegger *et al*. 2002). On each stand (i.e. dune side), one 1 m × 1 m sampling quadrat was placed (10 quadrats in total). The quadrats had 10 × 10 sections (100 micro-quadrats per quadrat). In each micro-quadrat, the coverages of lichen growth forms (% fruticose, foliose and crustose), moss (%), vascular plant (%), litter (%) and bare soil (%) were evaluated with Braun-Blanquet method (1964). The average height of vascular plants (mm) and the presence or absence of each lichen species were also recorded. Many lichen species were identified in the field, while others were transported to the laboratory and then examined with HPTLC using the method of Arup *et al*. (1993). Lichen species that fell out of the sampling units were also recorded and added to a complete species list at both sites. The species list represents only a part of the protected area: 300/11488 ha at Bugacpusztaháza and 60/1992 ha at Fülöpháza.

3.4.2 Physiological measurements

Thalli were collected from populations living on both the SW and NE oriented microsites in each of four seasons (spring, summer, autumn, winter) when possible (Table 1). Ten replicates per season per stand were measured in each species. Because of the low abundance, only five *G. fulgens* samples per season per stand were analysed. Altogether almost 1200 specimens were collected during the investigation period. At Bugacpusztaháza, some data were missing, especially from the summer period, because one of our investigation stands was destroyed by heavy equipment/diggers, and lichen thalli were challenging to find and collect. After that, the results of only two arid and two humid stands of Bugacpusztaháza were taken into account. *Gyalolechia fulgens* and *X. pokornyi* occurred only on SW sides of the dunes. After sample collection, thalli were transported to the laboratory, dried out under semi-natural conditions (i.e. seasonal temperature, humidity, and light regime, Csintalan *et al*. 1999), and cleaned with particular attention to plant and moss particles.

Table 1 The number of measured thalli per measurement types per species

species	chlorophyll *a* fluorescence kinetics	plastid pigment concentration	LSMs concentration
Cladonia foliacea	160	160	80
Cladonia furcata/rangiformis	160	160	80
Cladonia magyarica/pocillum	320	160	80
Diploschistes muscorum	266	160	
Gyalolechia fulgens	80	40	
Thalloidima physaroides	80		
Xanthoparmelia pokornyi	80	80	
sum	**1146**	**760**	**240**

3.5 Laboratory measurements

3.5.1 Chlorophyll *a* fluorescence kinetics

A few days after collection, specimens were rehydrated and kept under low light conditions at seasonal ambient temperature for 1–2 days until the photosynthetic system regenerated. After dark adaptation, chlorophyll fluorescence of lichen thalli was measured using a portable pulse amplitude modulated fluorometer (FMS 2 Hansatech Instruments Ltd. U. K.; Modfluor software) as Jensen (2002) described. The maximum quantum yield of PSII photochemistry and Stern-Volmer non-photochemical quenching were calculated according to Kitajima & Butler (1975) and Bilger & Björkman (1990). The yield of photochemical electron transport (φPSII), non-photochemical quenching (φNPQ), and the yield of non-regulated excitation dissipation (φNO) were also calculated according to Klughammer & Schreiber (2008). The φPSII gives insight into the effective photochemical quantum yield of PSII and shows the proportion of use excitation energy for charge separation. When the electron transport chain is saturated, the excitation energy can be re-emitted as fluorescence or dissipated as heat. The φNPQ represents the quantum yield of light-induced (ΔpH- and zeaxanthin-dependent) non-photochemical fluorescence quenching. The φNO describes the combined pathways of radiative and non-radiative deexcitation reactions, which do not lead to photochemical energy conversion and are not involving the NPQ-mechanisms (Klughammer & Schreiber 2008). The parameters φPSII, φNPQ, and φNO are competitive processes, and their sum equals 1 (Kramer *et al*. 2004).

3.5.2 Measurement of photosynthetic pigments and LSMs concentration

The chemicals used were of analytical grade quality. The concentration of plastid pigments was measured by spectrophotometry (Shimadzu UV-1601) according to Pfeifhofer *et al*. (2002) with minor modifications based on Lichtenthaler & Buschmann (2001). The concentrations of lichen secondary metabolites (LSMs) were measured by high-performance liquid chromatography (HPLC) based on Ji & Khan (2005). A Waters HPLC (e2695) equipped with Photodiode Array Detector (2998 PDA Detector) was used. The amount of atranorin and fumarprotocetraric acid in *C. furcata* and *C. magyarica*, and usnic acid and fumarprotocetraric acid in *C. foliacea* were analysed.

3.6 Data analysis

3.6.1 Micrometeorological data

The Shapiro-Wilk test was applied to test the normality of the data. A pairwise t-test was performed to compare the means of micrometeorological data, taking the average of every 10 minutes between the SW and NE facing sides of the investigated dune (R Core Team 2020). In the case of seasonal means of parameters, the average of the month preceding sample collection was taken. For the calculation of direct illumination reaching the dune sides during a year, the geographical characteristics (latitude, longitude), angle of the slopes and solar coordinates were used. For the calculation of the seasonal duration of direct illumination and the hypothetical metabolically active periods, a 1-month average preceding the sampling was used. For creating the figures, the R environment (R Core Team 2020) was used.

3.6.2 Coenological data

We compiled a detailed species list of the two study sites, where the previous and present occurrences were combined and compared. Only lichen data collected in micro-quadrats were used for quantitative analyses. The effects of the site ('BU' or 'FH') and dune side ('arid' or 'humid' side) on the environmental (cover of bryophytes, vascular plants, soil, litter and height of vascular plants) and lichen-related (species number, total lichen cover, cover of three growth forms) variables were evaluated. First, Non-metric Multidimensional Scaling (NMDS, Borcard *et al.* 2018) using Jaccard-dissimilarity was applied to unfold the species composition among sites and dune sides based on all sampled micro-quadrats; additionally, Permutational Multivariate Analysis of Variance (PERMANOVA, Anderson 2001) was performed to test the separation using the Manhattan metrics on pooled (i.e. quadrat-level) frequency data. Then species richness and cover of lichen growth forms, as well as the different substrate forms as potential drivers of lichen species, were further investigated by generalised mixed-effects models (GLMMs, Bolker *et al.* 2009; Dunn & Smyth 2018), where site and dune side were set as fixed effects and quadrat as random term. Finally, the relationships between the presence of the eight most abundant lichen species, the site, the dune side and the environmental variables were tested by separate logistic mixed-effects models. Prior to these analyses, all tested fixed factors were centred and scaled (z-score standardization). All analyses were carried out with R version 4.0.3 (R Core Team 2020). Graphs were also prepared in R (using ggplot2 package, Wickham 2016).

3.6.3 Chlorophyll *a* fluorescence kinetic parameters, LSM and plastid pigment concentrations

The effect of seasons and exposition of microhabitats on plastid pigment concentration, solar radiation screening LSMs and values of quenching mechanisms (response variables) were statistically evaluated. All statistical analyses were carried out with the R software version 4.0.3 (R Core Team 2020). The influence of species, seasons and microhabitat exposition were tested by the three-way ANOVA followed by a Tukey HSD test. The combination of six species, four seasons and two microhabitats resulted in 40 groups. During the analysis, each group were compared to each group. The normality of data distribution was checked visually by Q-Q plot (quantiles of the residuals are plotted against the quantiles of the normal distribution with a 45° degree reference line) and by Shapiro-Wilk normality test. Our data followed a normal distribution. Levene's test was used to check the homogeneity of variances. The variances across groups were homogeneous. A level of $p < 0.05$ was considered for a significant difference. Graphs were prepared in R environment and MS Excel.

4 MAIN RESULTS

4.1 Micrometeorological data

According to the four-year data series, the microclimatic conditions differed significantly between the NE- and SW-facing sides over the long term (Table 2). The T_{air}, PAR (corrected with slope and orientation), T_{soil} and VPD values were significantly higher ($p < 0.001$) on the SW than on the NE oriented side, where the RH and WC_{soil} proved to be significantly higher ($p < 0.001$) at 95% confidence. The microhabitats situated on the SW sides of the dunes can be characterised as arid, whereas the NE oriented sides as humid. The daily fluctuation of differences for every micrometeorological parameter changed seasonally. The highest daily fluctuation was observed in summer, but a balanced difference in light and humidity conditions between dune sides was detected in winter. Figure 3 shows how the soil temperature (from which we can deduce the degree of irradiation) differs between the dune sides during the seasons.

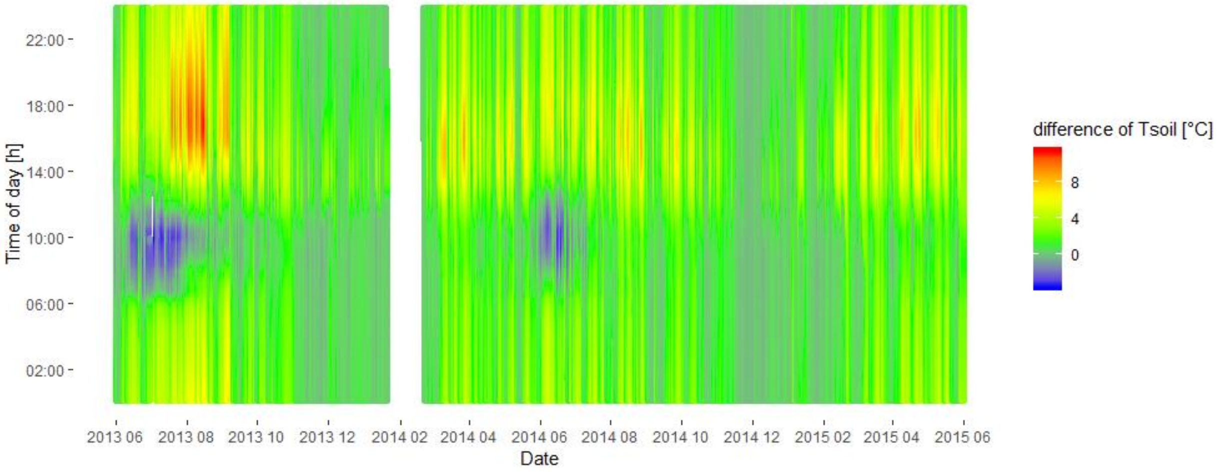

Fig. 3 The extent and direction of difference between SW and NE side of a dune in soil temperature (T_{soil}) during days in different seasons (row data) during a two year investigation period (01. 06. 2013–01. 06. 2015). The Y-axis represents the time within a day (the hours); meanwhile, the X-axis represents the time within two years by date (months). The false colour scale indicates the degree and direction of difference between SW and NE facing dune sites (difference = SW-NE). Red colour indicates higher values on the SW than on the NE side; meanwhile, blue colour indicates lower value on the SW than on the NE side. Some data are lacking (white strip) because of wire damage by wild animals.

Table 2 The average differences in micrometeorological parameters between the southwest (SW) and northeast (NE) oriented slopes of a dune based on a four-year data series.

parameter	average difference (4 years)	p-value	result
T_{air}	0.2 °C	$p < 0.001$	SW > NE
VPD	0.2 kPa	$p < 0.001$	SW > NE
PAR	3.8 μmol m^{-2} s^{-1}	$p < 0.001$	SW > NE
T_{soil}	2 °C	$p < 0.001$	SW > NE
RH	0.5%	$p < 0.001$	SW < NE
WC_{soil}	0.06%	$p < 0.001$	SW < NE

A clear difference in direct illumination was recorded between the arid (annual average 11 h 30 min) and humid (9 h 30 min) dune sides at a long term scale. The quality and quantity of incoming irradiation varied among the seasons. In the winter and autumn months, the direct illumination lasted 3–4 h longer on the arid than on the opposite side because there was a remarkable difference between 'sunset' time (Article 3 Fig. 1). During the summer months, the direct illumination reached the humid side 1–2 hours longer because of the significant difference between 'sunrise' time (Article 3 Fig. 1). The incoming irradiation varied among the seasons from 14 h 35 min in summer to 4 h 55 min in winter.

The average time when active metabolism was possible for lichens also varied among seasons during the day and at night (Fig. 4). The average daily time when photosynthesis may occur was remarkably lower in spring (mean arid: 39 min, mean humid: 3 h 53 min) and summer (mean arid: 3 h 15 min, mean humid: 3 h 7 min) than in autumn (mean arid: 4 h 48 min, mean humid: 4 h 31 min) and winter (mean arid: 7 h 5 min, mean humid: 4 h 10 min). The overnight rehydration lasted twice as long in winter (mean arid: 14 h 44 min, mean humid: 12 h 26 min) and autumn (mean arid: 13 h 3 min, mean humid: 13 h 2 min) than in spring (mean arid: 39 min, mean humid: 6 h 52 min) and summer (mean arid: 4 h 58 min, mean humid: 5 h 18 min).

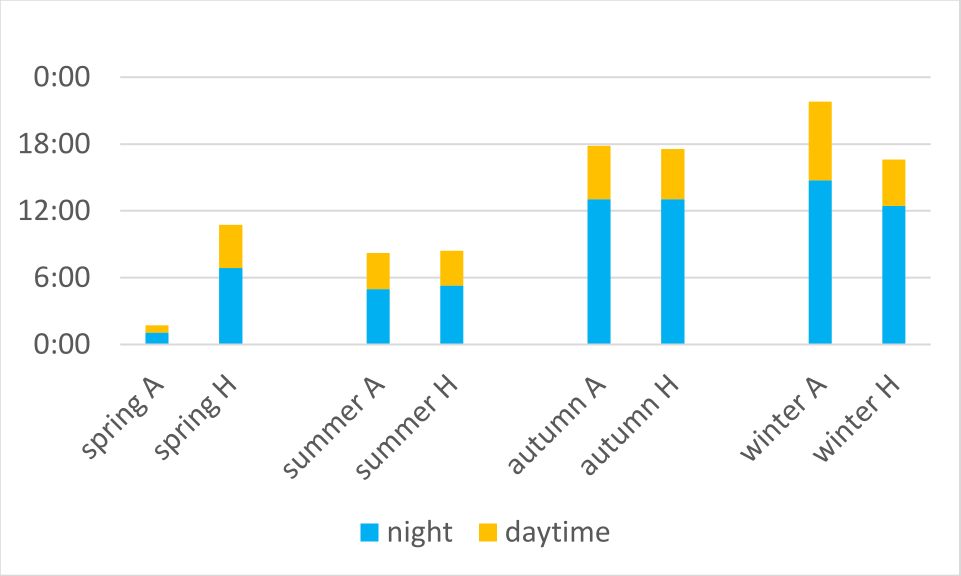

Fig. 4 The length of periods (hours) when rehydration and active metabolism of lichens was possible (relative humidity > 80% and 30 µmol m^{-2} s^{-1} < photosynthetically active radiation < 1000 µmol m^{-2} s^{-1}) during daytime and nights in the different seasons on the arid (A) and humid (H) side of a dune.

4.2 Soil surface variables on the two investigated sites and dune sides

There was no remarkable difference in the moss, vascular plant, litter and open soil surface cover between the sites (Article 1 Fig. 4). The average moss cover was higher ('FH': $p < 0.05$), but the vascular plant cover ('FH': $p < 0.01$) and height ('BU': $p < 0.01$, 'FH': $p < 0.001$) was lower on arid than on humid dune sides (Article 1 Fig. 4).

4.3 Inland duneland terricolous lichen community

4.3.1 Influence of soil surface variables on the species appearance

Almost all abundant species (found in > 50 micro-quadrats and at both sites) were negatively influenced by a higher moss cover (Article 1 Table 2). The cover of disturbed topsoil negatively affected the abundance of *C. magyarica/pyxidata* ($p < 0.001$) and *D. muscorum* ($p = 0.041$). *Cladonia foliacea* was negatively affected by higher vegetation ($p = 0.001$). The position of the species in the ordination space indicated that the species has heterogeneous preferences. Higher coverage of leaf litter had a strong association with *P. canina* (Article 1 Fig. 2). *Diploschistes muscorum*, on the other hand, preferred growing in and on moss patches. The presence of *C. foliacea*, *C. furcata*, *C. rangiformis*, *Xanthoparmelia* species, *S. lentigera*, *D. muscorum* and *G. fulgens* were more abundant in patches where vascular plant height was lower (Article 1 Fig. 2).

4.3.2 Lichen communities of the two investigated sites and dune sides

In total, 22 terricolous lichen taxa were recorded: 20 at the Bugacpusztaháza and 19 at the Fülöpháza site (Article 1 Table 1). Five of them were new to the sites from this investigation, and eight formerly reported species were not confirmed during the present study. Nine species were only found on humid dune sides (e.g. *C. chlorophaea, E. pusillum, P. canina*). Nine other species occurred only on arid sides of the dunes (e.g. *G. fulgens, X. pokornyi*). Seven species occurred on both dune sides. The core lichen species composition of Fülöpháza arid dune sides is much different from the arid core species of Bugacpusztaháza (Article 1 Fig. 2). In contrast, humid core lichen species composition showed a notable overlap between the sites (Article 1 Fig. 2).

The site, dune side and environmental factors were important factors determining terricolous lichen assemblages. The site had higher explanatory power than the aspect of the dunes in species composition ($p < 0.0046$ and $p = 0.0231$, respectively) (Article 1 Fig. 2). The NMDS (stress = 0.102) showed a separation among dune sides and sites. The ordination using two orthogonal dimensions cumulatively represented 59.61% of the variation of species composition (Mantel tests; NMDS 1: $r^2 = 0.349$, $p < 0.001$; NMDS 2: $r^2 = 0.247$, $p < 0.001$). The species richness was more affected by the site than the side. Species richness was significantly higher at 'FH' than at 'BU' ($p < 0.001$; Article 1 Fig. 3). At the 'FH' site, the total species richness was significantly higher ($p < 0.001$) in arid micro-quadrats (mean: 3.66; range: 0–9) compared to humid micro-quadrats (1.55 [0–4]; Article 1 Fig. 3). The opposite was detected at 'BU', where the humid sides (1.69 [0–4]) hosted more species ($p = 0.056$) than arid ones (1.16 [0–4]; Article 1 Fig. 3). The cover of fruticose species was higher on humid than on arid sides (Article 1 Fig. 3).

4.4 The influence of seasonality and microhabitat conditions on chlorophyll fluorescence kinetics

For each of the investigated species, both seasonality and aspect had an effect on Fv/Fm ($p < 0.001$), as well as on NPQ ('BU' season: $p < 0.001$, aspect: $p = 0.006$; 'FH': $p < 0.001$ for both season and aspect). Higher Fv/Fm values were measured in humid than in arid microhabitats. Usually, the spring and autumn samples showed higher values than the summer (and winter) samples (Article 2 Fig. 3). The results also revealed that the aspect influenced the extension of seasonal fluctuation in Fv/Fm, reaching a greater extent on arid than on humid sides (Article 2 Fig. 3). In general, a significant ('BU': $p = 0.036$, 'FH': $p < 0.001$) difference was detected among species. The thalli of *Cladonia* species (each containing *Asterochloris* algae) and *X. pokornyi* (with *Trebouxia* algae) showed higher Fv/Fm than was measured in *D. muscorum* or *G. fulgens* with *Trebouxia* photobiont.

Usually, higher NPQ was detected in humid compared to arid samples and the autumn samples showed the highest values between the seasons (Article 2 Fig. 4). The exposition of microhabitat also impacted the seasonality of NPQ (Article 2 Fig. 4). Significantly higher ($p < 0.001$) NPQ was measured in *C. foliacea* than in other investigated species. Species with *Asterochloris* algae (*Cladonia* species) reached higher values than species with *Trebouxia*. *Cladonia foliacea* and *C. furcata* exhibited an extended seasonal fluctuation, especially in arid populations.

4.5 The influence of seasonality and microhabitat conditions on photoprotection and high-light acclimation

4.5.1 Lichen secondary metabolites

A seasonal change in fumarprotocetraric acid concentration was detected in *C. furcata* ($p = 7.55e^{-12}$) (spring, summer < autumn, winter) and *C. foliacea* ($p = 0.03$), unlike in *C. magyarica* ($p = 0.44$). (Article 3 Fig. 2a). Sun thalli showed higher fumarprotocetraric acid concentration compared to shade thalli in *C. furcata* ($p = 0.02$) and *C. foliacea* ($p = 0.04$), while microhabitat exposition did not affect the amount of fumarprotocetraric acid in *C. magyarica* ($p = 0.36$).

Atranorin showed significant seasonal ($p = 6.2e^{-10}$) (spring, summer < autumn, winter) and microhabitat ($p = 0.007$) variation only in *C. furcata* (Article 3 Fig. 2b). In *C. magyarica* atranorin did not vary across microhabitats ($p = 0.58$) or seasons ($p = 0.32$).

A significant seasonal change in usnic acid concentration was found in *C. foliacea* ($p = 0.0004$) (spring, summer < autumn, winter) (Article 3 Fig. 2c). Meanwhile, usnic acid did not differ between sun and shade thalli ($p = 0.98$).

Compared to *C. furcata*, *C. magyarica* showed a higher concentration of atranorin. *Cladonia furcata* showed approximately two times higher fumarprotocetraric acid concentration than the other two *Cladonia* species ($p < 2e^{-16}$). *Cladonia magyarica* showed a constant high value during the year compared to the seasonal fluctuation in *C. furcata*. The concentrations of usnic acid reached three times higher values (mean 12.16 mg g^{-1}) than the other UV-protectant cortical pigment, atranorin (mean 3.99 mg g^{-1}).

4.5.2 Photosynthetic pigments

The seasonal change of photosynthetic pigment concentrations differed among the species (Article 3 Fig. 3a-d). *Cladonia furcata* showed the most apparent response to the seasonally changing environment in each parameter. In species where seasonality existed, the minimum chlorophyll concentrations occurred during winter (on average 155–326 µg g^{-1} in *Cladonia* species and *D. muscorum*), except for *G. fulgens* showing maximum (204 µg g^{-1}) in the season. *Gyalolechia* *fulgens* and *X. pokornyi* occurred only in arid microhabitats. Therefore, the comparison was only possible between sun and shade populations of *Cladonia* species and *D. muscorum*. The concentration of chlorophylls was significantly higher in sun than in shade populations in most species (Article 3 Fig. 3a). Usually, the chlorophyll *a+b* concentration was lower in *G. fulgens* and *D. muscorum* than in other species.

There was a specie specific seasonal trend in the mean chlorophyll a/b ratio; however, no significant difference was shown between arid and humid dune sides in any species (Article 3 Fig. 3b). The chlorophyll a/b was higher in *G. fulgens* and lowered in *C. foliacea* than in the other species.

Usually, a significant seasonality of the carotenoid concentration occurred in the species (Article 3 Fig. 3c). In general, the highest mean carotenoid concentration was detected in summer (43–145 µg g^{-1}), whereas the lowest values occurred in winter (71–105 µg g^{-1}) for *Cladonia* species and in autumn for the other three species (36–44 µg g^{-1} in *D. muscorum*; 106 µg g^{-1} in *X. pokornyi*; 222 µg g^{-1} in *G. fulgens*). The sun populations contained significantly more carotenoids than shade populations in most of the species. The carotenoid concentration was lower in *D. muscorum* and higher in *G. fulgens* than in the three *Cladonia* species or *X. pokornyi* in each season.

Significant seasonal differences were detectable in the chlorophylls/carotenoids ratio in most of the species (Article 3 Fig. 3b). The mean chlorophylls/carotenoids ratio usually peaked in autumn (2.12–4.74) and was the lowest in spring (0.59–4.01). Usually, the parameter was significantly higher in shade than in sun populations. The ratio of chlorophylls to carotenoids was the lowest in *G. fulgens* and the highest in *D. muscorum*.

4.5.3 Partition of incoming irradiation between photochemical quenching, regulated non-photochemical quenching and non-regulated excitation dissipation

The φPSII was significantly lower in summer than in autumn or winter in each species, except for *G. fulgens*. However, the response of photochemical quenching to seasonal environmental changes seemed to be species-specific (Article 3 Table 3). Still, it differed significantly between the sun and shade populations in only a few cases.

A significantly lower φNPQ was measured in summer than in other seasons in *C. foliacea*, *C. furcata* and *X. pokornyi*, whereas *G. fulgens* exhibited the opposite seasonal pattern (Article 3 Table 3). *Cladonia magyarica* and *D. muscorum* did not show remarkable differences among the seasons. Generally, the φNPQ was higher in the humid (mean 28–52%) compared to the arid (mean 17–49%) microhabitats. This difference was always significant during winter in each species, whereas a species-specific response was characteristic for the other seasons.

The φNO was significantly higher in summer than in other seasons in each species except for *G. fulgens*, where winter samples showed the highest values (Article 3 Table 3). The φNO was usually higher in the arid (mean 20–70%) than in the humid (mean 18–52%) microhabitats.

4.6 The species-specific differences

Cladonia foliacea showed high Fv/Fm (mean: 0.65), the highest NPQ (mean: 1.93) and φNPQ (mean: 43%) among the species. The amount of cortical UV-protectant pigment, usnic acid, was three times higher (mean: 12.16 mg g^{-1}) than the amount of atranorin (cortical pigment) in the other two *Cladonia* species.

Cladonia furcata/rangiformis exhibited the highest values of Fv/Fm (mean: 0.66) and φPSII (mean: 27%), relatively high NPQ (mean: 1.37), and a moderate level of φNPQ (mean: 35%) and φNO (mean: 38%) among the species. The highest concentration of fumarprotocetraric acid (mean 8.8 mg g^{-1}) and relatively low level of atranorin (mean: 3.7 mg g^{-1}) was measured in *C. furcata/rangiformis*.

Cladonia magyarica produced a constantly high amount of atranorin (mean: 4.1 mg g^{-1}) but the lowest amount of fumarprotocetraric acid (mean: 4.7 mg g^{-1}) among the species during the year. The species maintained relatively high NPQ (mean: 1.33) during the seasons.

Diploschistes muscorum showed relatively low Fv/Fm (mean: 0.60) and NPQ (mean: 1.08). The proportion of φPSII (mean: 26%) was relatively high among the species. The chlorophyll concentration showed low (mean: 186 μg g^{-1}), and the carotenoid concentration showed the lowest values (mean: 46.6 μg g^{-1}). The highest chlorophyll/carotenoid ratio (mean: 4.1) characterised the species.

Gyalolechia fulgens represented the significantly highest Fv/Fm (mean: 0.71), φNPQ (mean: 29%), φNO (mean: 51%) and the lowest φPSII (mean: 20%) and chlorophyll concentrations (mean: 172.6 μg g^{-1}) among the species. The carotenoid concentration was two times higher (mean: 247.8 μg g^{-1}) than it was measured in the other species.

The highest amount of chlorophyll (mean: 351.6 μg g^{-1}) and high level of carotenoid (mean: 119.6 μg g^{-1}) and Fv/Fm (mean: 0.69) was measured in *X. pokornyi*. The proportion of φNO was relatively low (mean: 32%).

5 DISCUSSION

5.1 The effect of side and site on lichen community composition and the comparison with other European communities of dunelands

Our results showed that the exposition of microhabitat could impact the composition of dune lichen communities. The revealed difference in herb height and cover between sides could cause a difference in the light level reaching lichen thalli, resulting in different microclimatic conditions where lichens with different environmental needs could find their preferred niche. It was also shown that at lower vegetation cover and height, the chance of competition could increase between mosses and lichens on arid sides, explaining the lower total lichen cover in arid compared to humid microhabitats. Our results agreed with the observation that vegetation generally becomes more open with increasing aridity (Walter & Breckle 1984) and lichens compete with mosses for free patches (Ketner-Oostra & Sýkora 2000). Our results showed that the soil surface variables exhibited a very important role in shaping terricolous lichen communities. The increased density of the herb layer was one of the main drivers for the development of a terricolous lichen community, and a shift was detected in the composition of lichen growth form from the dominance of crustose (and squamulose) to fruticose lichens along an aridity scale Leppik *et al*. (2013).

The site had a high explanatory power explaining community composition in our study. Since we could not find any significant difference in the climate between the two study sites, and the investigated soil surface variables differed significantly only in open soil cover, we hypothesise that the disturbance history played an important role in developing different lichen communities (c.f. Leppik *et al*. 2013). The noticeable spread of juniper and poplar (Molnár *et al*. 2012) could facilitate the appearance of tree-related species (e.g. *C. chlorophaea, C. coniocraea* or *Peltigera* species) on the Bugacpusztaháza site. At the Fülöpháza site, the later abandonment of grazing likely helped the occurrence of terricolous lichen species (e.g. *G. fulgens, X. subdiffluens*) preferring more open vegetation, increasing lichen diversity (Ketner-Oostra & Sýkora 2000). Besides the lack of fire events and low-intensity grazing, the total BSC cover was not reduced (e.g. Johansen 2001; Warren & Eldridge 2001), and species richness could reach a higher value (Boch *et al.* 2016) at Fülöpháza. The Bugacpusztaháza site appears to have had a higher lichen species turnover and less species retention than Fülöpháza. Seven formerly mentioned species (Lőkös & Verseghy 2001) were not confirmed during the present investigation at the Bugacpusztaháza site. The earlier common *B. bagliettoana*, *C. symphycarpa* and *C. subulata* and the frequent *G. fulgens* are serious losses. Because the vegetation is more open at the Fülöpháza site (Molnár 2003), the effect of topography could prevail to a greater degree than at Bugacpusztaháza, where the vegetation is further progressing to a climax state (Molnár 2003), and the more balanced microclimate (influenced by the vegetation) can mitigate the contrast between environmental conditions prevailing on arid and humid dune sides.

Slightly fewer terricolous lichen taxa were found in the present research (Bugacpusztaháza 20; Fülöpháza 18; overall 22 species) than in other European countries (e.g. Estonia 28 (Jüriado *et al*. 2016); Italy 33 (Gheza *et al*. 2020)) and half of the species also recorded belong to the genus *Cladonia*. The communities found in the present study differed from communities presented in dunelands of other European countries mainly because of the acidity of the soil (sand) and vascular plant vegetation (Molnár 2003). Our data contributed significantly to the knowledge on the lichen communities in calcareous sandy grasslands because limited research has focused on the exploration of these associations between inland dunes (Jüriado *et al*. 2016; Gheza *et al*. 2020). *Cladonia foliacea, C. furcata, C. magyarica/pyxidata* and *Diploschistes muscorum* were the most frequent species in the present study. The species were found on acidic and calcareous soils and in arid and humid microhabitats proving their broad ecological tolerance (e.g. Adamska 2010; Balogh *et al.* 2017). Five of the lichen species recorded in the study area are listed on the Red List of lichens as occurring in Hungary and lacking in other mentioned European areas *(C. magyarica, X. pulvinaris, X. subdiffluens, X. pokornyi* and *X. ryssolea* (Farkas & Lőkös 2006; [Hungarian] Ministry of Rural Development 2013). The four *Xanthoparmelia* species were found only on arid dune sides and were more abundant in more open vegetation.

5.2 Influence of the spatially and temporary changing environment on photosynthesis and photoprotection

Usually, lower photosynthetic activity, chlorophyll/carotenoid ratio, LSM concentrations and regulated non-photochemical quenching were characteristic during brighter and warmer seasons and on arid dune sides in the investigated species (Fig. 5). At the same time, the chlorophyll and carotenoid concentration and the non-regulated excitation dissipation were higher than on humid seasons and on humid dunes sides. A seasonal trend was detected previously in photosynthetic activity, photoprotection and in the concentration of lichen secondary metabolites and plastid pigments in *Lobaria pulmonaria* (L.) Hoffm. and *Xanthoria parietina* (L.) Th. Fr. (Gauslaa & McEvoy 2005; MacKenzie *et al*. 2002; Vráblíková *et al*. 2006). The daily average PAR and VPD were relatively high in summer and late spring. Therefore, the average time lichens presumably spent metabolically active was lowered during the day and at night. However, the shading effect of vascular plants could influence light and humidity conditions (Kalapos & Mázsa 2001; Serpe *et al*. 2013). The cover and the average height of vascular plants were lower on arid compared to humid sides.

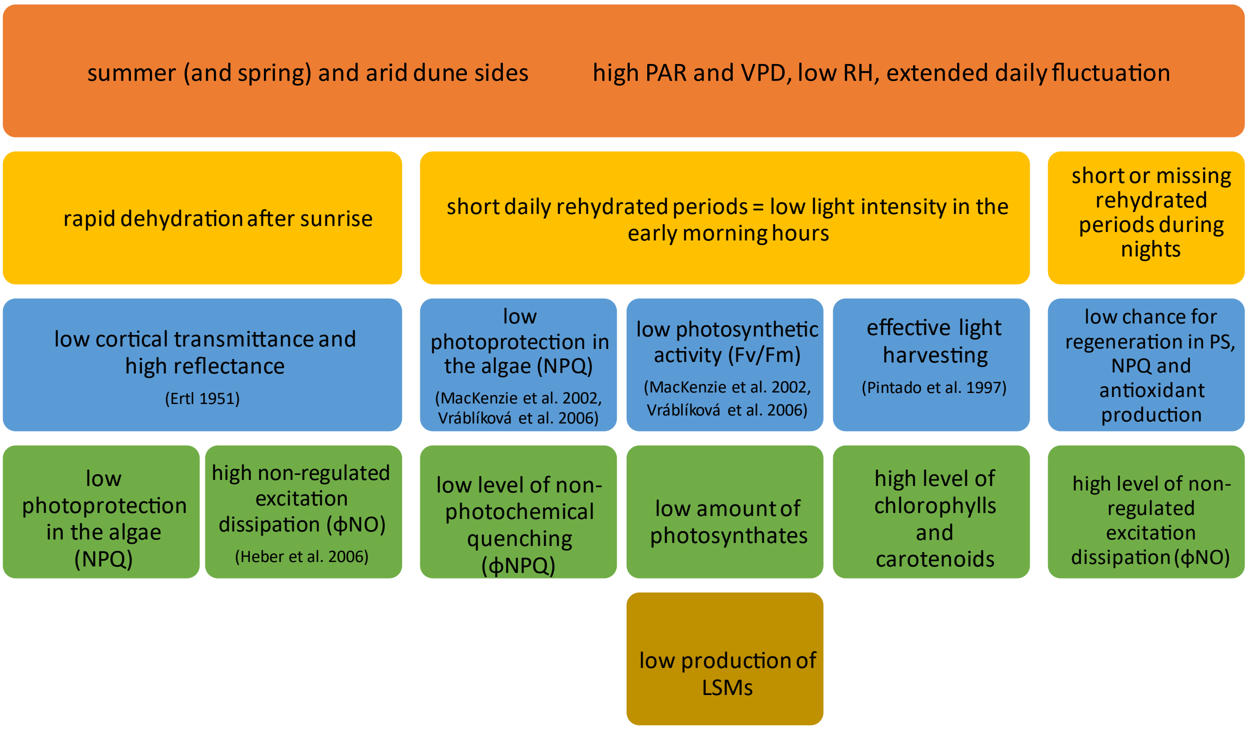

Fig. 5 Connection between the values of the different variables measured during summer, spring and arid dune sides. LSM = lichen secondary metabolites, NPQ = non-photochemical quenching, PAR = photosynthetically active radiation, PS = photosynthetic system, VPD = vapour pressure deficit

Because of the short moistened active periods of lichens, the amount of incoming irradiation reaching photobiont was lower in the early morning hours. Therefore, wet lichen thalli needed less protection against harmful excess light energy, resulting in lower NPQ (Barták *et al*. 2000), but other protection mechanisms were also working. Rapid desiccation a few hours after sunrise protects lichens from photodamage (Heber *et al*. 2007). Because the lichen can only produce solar radiation protectant substances in the hydrated state (Solhaug *et al*. 2003), the decreased level of these metabolites in the dry summer may result from short metabolically active periods insufficient to provide the required amounts of photosynthates for LSM synthesis (Solhaug & Gauslaa 2004). Our results showed that despite the most precipitation in summer, only 13–19% of incoming solar radiation was used for photochemical quenching in lichens, suiting the results of Verseghy (1976). Furthermore, the short hydrated periods can increase the need for more efficient harvest and use of light due to the higher amount of chlorophylls and carotenoids (Demmig-Adams & Adams 1992; Pintado *et al*. 1997). The concentration of carotenoids can indicate both the size of the antenna complex and the xanthophyll pool as they could participate both in light-harvesting and in photoprotection (Demmig-Adams 1990; Müller *et al*. 2001). Our results showed a higher amount of carotenoids measured in arid populations, and the NPQ was lower in these thalli, indicating that carotenoids were mainly used in the light-harvesting antenna complex. It seemed that in summer, the φNPQ was less involved in dissipating excess light energy (φNO > φNPQ), especially in arid microhabitats. Combined pathways of radiative and non-radiative deexcitation reactions were probably dominant in the form of heat and fluorescence, mainly due to closed PSII reaction centres (Klughammer & Schreiber 2008). Furthermore, there were nights when RH could not reach 80%, significantly reducing the chance for rehydration and starting active metabolism. Therefore, the regeneration of the PS, recovery of NPQ or the antioxidant enzyme production responsible for ROS deactivation (Aoussar *et al*. 2018; Veerman *et al*. 2007) were also reduced.

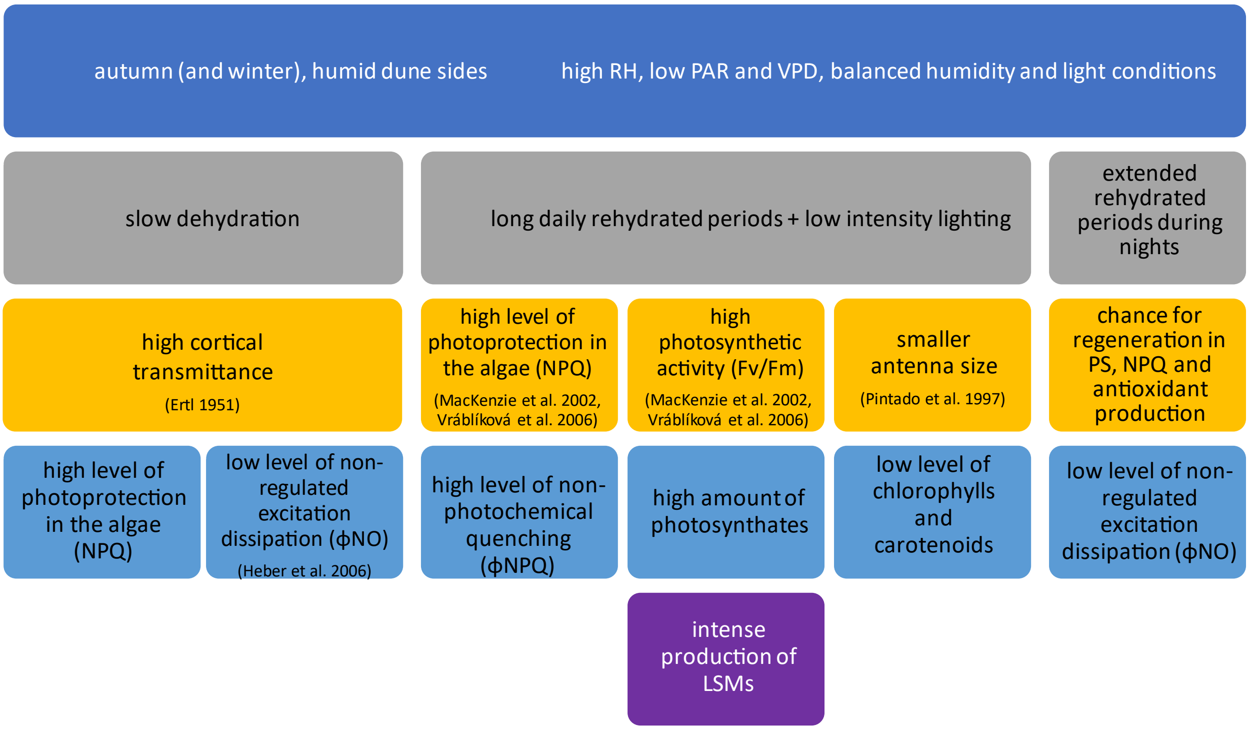

Fig. 6 Connection between the values of the different variables measured during autumn, winter and on humid dune sides. LSM = lichen secondary metabolites, NPQ = non-photochemical quenching, PAR = photosynthetically active radiation, PS = photosynthetic system, VPD = vapour pressure deficit

During winter and autumn, high and more stable air humidity and lower irradiation frequently occurred (Lange 2003b), creating a longer favourable environment for lichen metabolism and photosynthesis (Fig. 6). During seasons when RH was higher and on humid sides, prolonged wet periods occurred (during days and nights), increasing the cortical transmittance (Dietz *et al*. 2000) and the amount of light reaching algal cells. Therefore, the photosynthetic apparatus needed more protection against the harmful excess light energy than in dried thalli (Heber *et al*. 2006), where most of the radiation is reflected from the surface of the thallus (Ertl 1951). The phenomenon explains the low amount of chlorophylls because the higher amount of incoming light reaching photobiont required a smaller chlorophyll antenna size for effective light-harvesting (Jin *et al*. 2001) but also increased the production of light screening fungal metabolites (BeGora & Fahselt 2001; Farkas *et al*. 2020; Solhaug *et al*. 2003) and NPQ (Barták *et al*. 2000). During autumn, more than one-third (on average) of the excitation energy was utilised by photochemical processes leading to the most productive period of the year in semi-arid sandy grasslands suiting the results of other studies (MacKenzie *et al*. 2001; Raggio *et al*. 2014; Verseghy 1976). Furthermore, φNPQ was higher than φNO, indicating that the antenna complex had safely dissipated the excessive light as heat and the energy flux of the photosynthetic apparatus worked efficiently during winter and autumn. The desiccation-induced quenching decreased in humid seasons and increased in late spring and summer (Heber *et al*. 2006). Our results also showed that the proportion of φNPQ was the most sensitive to the microhabitat and seasonally changing environmental conditions among the quenching parameters. The proportion of φNPQ was significantly impacted by the exposure of thalli, particularly in summer and winter.

Our work pointed out a significant phenomenon in photoacclimation of lichen thalli. Cooperation between photo- and mycobiont changed between seasons in *C. foliacea* and *C. furcata*. Since the synthesis of light screening LSMs requires more time (days, Solhaug & Gauslaa 2004; Verma *et al*. 2012) than is necessary for the activation of non-photochemical quenching mechanisms (e.g. VAZ cycle 5–10 min, Müller *et al*. 2001), the latter seems to be a more efficient process during the short metabolically active period in drier and brighter seasons in some species. Dehydrated thalli were mainly defended by the solar screening metabolites produced by the mycobiont and curling during desiccation (caused by the mycobiont). In humid seasons and microhabitats, more extended active periods lead to increased photosynthesis and production of solar radiation protectant fungal metabolites, allowing a lower level of photoprotection in the form of regulated non-photochemical quenching by the photobiont. In contrast, *C. magyarica* maintained relatively high photoprotection by both of the partners.

5.3 The species-specific response to the changing environment

The species-specific acclimation mechanisms were confirmed by earlier studies on plastid pigment concentrations across different habitat types (e.g. Balarinová *et al*. 2014; Paoli *et al*. 2017) and seasons (Higgins *et al*. 2015). Our results agreed with that: a remarkable species-specific response was observed to seasonality and exposition of the microhabitat in photosynthesis, photoprotection, occurrence and abundance of the different species. Lichens are poikilohydric organisms that cannot actively regulate their water content. However, they can extend or shorten their metabolically active period and protect themselves due to different long term adaptations besides the short term acclimation strategies.

Cladonia foliacea contains *Asterochloris* algae and exhibits a high surface area to volume ratio and thin cortical and photosynthetic layer. The species can avoid photoinhibition due to curling during desiccation (Barták *et al*. 2006) and produce a high amount of usnic acid. Usnic acid plays a significant role when the species is rehydrated and hence unfolded since the thalli have only a dense, upper cortex; otherwise, the thick, white medulla covers them (Verseghy 1971). Usnic acid absorbs light and functions as a very effective solar radiation screening pigment (Rancan *et al*. 2002) that protects against PAR (McEvoy *et al*. 2007). Besides, the algal component plays a major role in photoprotection by the highest measured NPQ and φNPQ among the species. The presence of *C.a foliacea* was negatively affected by higher herb height, probably because the spread of thalli is hindered due to scrolling by the wind.

The branching *C. furcata* and *C. rangiformis* (containing *Asterochloris* algae) were more tolerant of the drought and light stress of the arid sides and could reach higher cover on that dune sides, where the plant cover was more sparse. *Cladonia furcata/rangiformis* exhibit a thin cortical layer and high surface area to volume ratio allowing rapid water uptake and fast water loss causing remarkable seasonal differences in physiological parameters (Colesie *et al*. 2017; Dietz *et al*. 2000; Verseghy 1971). Therefore, higher protection of PSs against the harmful excess light energy reaching the photobiont layer and effective light harvesting were needed (Heber *et al*. 2006), which may explain the high Fv/Fm, NPQ, fumarprotocetraric acid and low atranorin concentration among the species. The moderate proportion of φNPQ and φNO supports the efficiency of absorbing light (Nguyen *et al*. 2013) of the fumarprotocetraric acid concentration. On the other hand, *C. furcata* can benefit from the thin cortex due to rapid air moisture uptake in humid periods (Verseghy 1971), explaining the highest φPSII among the species. The fluctuating cooperation in photoprotection between the partners also facilitates the survival of the species.

Cladonia magyarica and *C. pyxidata* (containing *Asterochloris* algae) often grew on moss cushions and earned higher cover on humid than arid sides, probably preferring more stable microclimatic conditions prevailing on humid dune sides (e.g. Blum 1973; Büdel & Scheiddeger 2008). The high water retention ability of moss cushions (Colesie *et al*. 2012) and the species (Verseghy 1971) allowed a more extended hydrated period compensating for the lower incoming irradiation caused by a higher herb cover and height on humid sides (e.g. Pintado *et al*. 2005). Our results suggested that among the most abundant species, *C. magyarica* and *C. pyxidata* were the most sensitive species, negatively affected by the measured soil surface variables. The thalli showed an increased level of photoprotection by producing a high amount of atranorin (Heber *et* *al*. 2006; Solhaug *et al*. 2003), explained by the longer hydrated periods compared to other species. A constantly high level of NPQ was also needed in the photobiont. In *C. magyarica/pyxidata*, both partners contributed to the photoprotection during the whole year. The seasonal fluctuation of Fv/Fm was also less pronounced because of the stable humidity conditions.

The crustose *D. muscorum* (containing *Trebouxia* algae) is characterised by different photobiont types compared to *Cladonia* species in their mature lifespan (e.g. Smith *et al*. 2009). The moderate seasonal fluctuation was detected in Fv/Fm and NPQ, indicating relatively rapid acclimation ability to changing environmental conditions. The relatively low chlorophyll, the lowest carotenoid concentration among the species, and the relatively high proportion of φPSII suggest that the species received enough photons for assimilation due to the prolonged hydration period on moss cushions (Colesie *et al*. 2012). This lichen can protect itself by mechanisms other than φNPQ and φNO. The pale colour (high albedo) and the pruinose surface likely increase in light reflectance, thus protecting the thallus from excessive light. The highest chlorophyll/carotenoid ratio among the investigated species suggests that the mycobiont provides strong screening pigments (Gauslaa & Goward 2020) or structure (Gauslaa *et al*. 2017) to protect the photobiont, which likely is adapted to the low light conditions inside the thallus (Demmig-Adam & Adams 1992). *Diploschistes muscorum* can acclimate relatively rapidly to changing environmental conditions helping the species to be the most abundant crustose species at both investigated sites and on arid and humid dune sides.

Gyalolechia fulgens thalli were only be found on arid dune sides. The species with *Trebouxia* photobiont showed the lowest proportion of φPSII, φNPQ and chlorophyll concentration and the highest proportion of φNO and carotenoid level, suggesting that this species experiences a shorter period in the wet state compared to other species (Pintado *et al*. 1997). The highest carotenoid level likely contributes more to photoprotection due to the direct defence of the thylakoid membrane (Müller *et al*. 2001) than the anthraquinone compound or whitish pruina in the cortex.

Xanthoparmelia pokornyi, (exhibiting *Trebouxia* photobiont) occurring only in arid microhabitats showed the highest chlorophyll and relatively high carotenoid concentration. Since the φNPQ was relatively low, the carotenoids could participate mainly in an effective light-harvesting caused by the rapid desiccation in the exposed microhabitats.

6 CONCLUSIONS, RECOMMENDATIONS AND PERSPECTIVES

Based on the received information on microclimate, we concluded that it strongly influenced lichen functioning and abundance at a large and a fine scale. It was established that lichens could significantly contribute to the biodiversity of dry calcareous sandy grasslands due to their heterogeneous community structure, having high conservation importance. Some lichen species were to be found only on arid dune sides or more open sites (e.g. the protected steppe species or the key species of the *Fulgensietum fulgentis* association), while others only on humid dune sides or on the site hosting more closed vegetation and trees (e.g. the wood-correlated species). Therefore, it is important to protect and maintain a diverse topography and landscape structure hosting various lichen communities when considering habitat-specific conservation and monitoring in inland dunelands. It is worth considering that the different species forming duneland communities have various microenvironmental needs and are very sensitive to different environmental factors. We can postulate that as a result of climate change (drier and warmer conditions are projected in this region), lichens can gain more space due to the shrinkage of vascular plant vegetation. However, lichens have to compete with mosses for these free surfaces. These changes will supposedly lead to higher cryptogamic biodiversity.

In our extensive study, we showed that the effect of microhabitat conditions and seasonal changes on photosynthetic functioning and photoprotection is species-specific and varies in space and time. We suggest future workers study more than one species to investigate the response of lichens to changing environmental conditions in space or in time. Additionally, because photosynthetic activity and a part of photoprotection derive from the photobiont, future work should focus on species-specific differences of chlorophyll fluorescence in the different photobiont partners found in lichens (e.g. *Asterochloris*, *Trebouxia*).

The different species can acclimate to the changing environmental conditions by taking advantage of each season in various ways. The regulated non-photochemical quenching seemed the most species- and microhabitat-specific protection mechanism. Our results showed that only c. 25% of the absorbed light energy was utilised in photochemical processes. It confirms that the balance between energy conservation and dissipation is shifted towards dissipation in poikilohydric organisms compared to vascular plants (Heber *et al.* 2006). The proportion of intensity in the protection of lichen thalli against the excessive radiation between algal and fungal partners is a promising field for further studies, for example, from antioxidant enzyme activity point of view.

7 NEW SCIENTIFIC RESULTS

The following new scientific results have been achieved:

1. The T_{air}, PAR (corrected with slope and orientation), T_{soil}, and VPD values were significantly higher on the SW than on the NE oriented dune side, where the RH and WC_{soil} proved to be significantly higher.
2. The daily fluctuation of differences for every micrometeorological parameter was changing during the seasons. The daily fluctuation was higher in summer and lowered in winter.
3. The quality and quantity of incoming irradiation on dune sides varied among the seasons. In winter and autumn, the direct illumination lasted 3–4 h longer on the exposed than on the opposite side because of the later sunset. During the summer months, the direct illumination reached the shaded side 1–2 hours longer because of the significantly earlier sunrise.
4. The site (by the disturbance history), dune side (by the microclimate) and soil surface variables (as microenvironment) were found to be important factors determining terricolous lichen assemblages and species richness in calcareous semi-arid sandy grasslands.
5. The cover and the average height of vascular plants were lower, and the moss cover was higher on arid compared to humid dune sides.
6. The abundant species differed in their habitat and microhabitat preference, and almost all abundant species were negatively influenced by a high moss cover.
7. Lower photosynthetic activity, chlorophyll/carotenoid ratio, NPQ and φNPQ were characteristic during brighter and warmer seasons and on arid dune sides. The chlorophyll and carotenoid concentration and the φNO were higher in arid than humid seasons and dunes sides.
8. The proportion of absorbed light between photosynthesis and the different photoprotection quenching mechanisms is species-specific and changes seasonally in lichens.
9. A significant seasonal change in atranorin, fumarprotocetraric acid, and usnic acid concentration was found in *C. foliacea* and *C. furcat*a (spring, summer < autumn, winter). The level of LSMs constantly remained high in *C. magyarica* during the year.
10. Higher levels of LSMs were found in shade compared to sun populations in *C. foliacea* and *C. furcata*. It did not differ between arid and humid dune sides in *C. magyarica*.
11. Since the synthesis of light screening LSMs requires more time than is necessary to activate non-photochemical quenching mechanisms, the latter seems to be a more efficient process during the short metabolically active period in drier and brighter seasons in some species (e.g. *C. furcata*). In other species (e.g. *C. magyarica*), the mycobiont and photobiont increase the production of these substances simultaneously to achieve a more effective excess energy dissipation.

8 SUMMARY

As members of BSC, terricolous lichens are dominant associates of arid and semi-arid ecosystems. Dunes are ecosystems of high conservation interest because of their unique, vulnerable and threatened features. Terricolous lichens adapted in different ways in extreme habitats. However, the communities are sensitive to disturbances and intense human activities (e.g. climate change, trampling, heavy traffic, off-roading, intense grazing, fire). Because of the low growth rate and slow biomass production, recovery of lichen thalli is restrained after a disturbance event. Therefore, the protection of BSC associations is essential. Collecting information on the composition of the communities and working physiological acclimation processes (photosynthesis, photoprotection) is indispensable. Many open questions remained unanswered, requiring a more intense investigation of terricolous lichen assemblages and the environmental factors influencing them at different scales in space and time and outlining our aims accordingly.

For a better understanding of processes, we collected micrometeorological data during a four years period. We studied the structure of the terricolous lichen assemblages of calcareous grassland in an inland duneland ecosystem by comparing the lichen communities and soil surface variables of opposite dune sides on two sites with different disturbance histories. We also revealed the habitat and microhabitat preferences of the community members. We followed the acclimation of the photosynthetic activity and photoprotection to the changing environment in space (exposition of microhabitat) and time (seasonality) combined in the seven most abundant terricolous lichen species. We also investigated the changing proportion of absorbed light between photosynthesis and the different photoprotection mechanisms in terricolous lichens because the literature data is sparse on this topic. We aimed to expand the number of research objects deriving from the same habitat in different seasons to get closer to the entire view of the mechanisms working in lichens, unlike former studies where often only one species was grabbed from the lichen community of a given area and point in time for a study object, also considering that the growth form (surface area/volume ratio) significantly impact the water relations in poikilohydric organisms.

Since only two studies were known about the lichen communities of calcareous sandy grasslands between inland dunes in Europe, the description of these assemblages from Hungary significantly contributed to our knowledge. We found that the disturbance history of the sites had a significant effect on species richness and composition. In general, at a smaller, 'dune' scale, lower herb cover and height and a higher moss cover were characteristic for arid dune sides. Most of the frequent species were negatively affected by the high moss cover. Some lichen species were more abundant or found only on arid dune sides, while others preferred or occurred only on humid sides resulting in a species rich community at a landscape scale.

Our results showed that opposite dune sides exhibited significantly different microclimates, and the difference fluctuated during seasons. The T_{air}, PAR, T_{soil} and VPD were significantly higher on arid than on humid microhabitats, where RH and WC_{soil} showed higher values. The arid side was exposed to direct illumination 3–4 hours longer in winter and 1–2 hours shorter in summer compared to the humid side of the dune, influencing the metabolism of sun and shade populations of various species. Higher photosynthetic activity and a higher level of regulated photoprotection (in photobiont and mycobiont) were detected in lichens from humid microhabitats and seasons compared to exposed populations and arid seasons. In addition, the exposure of microhabitat influenced the extension of seasonal fluctuation. Our results indicated that different lichens acclimated to the changing environments differently. Both the mycobiont and photobiont contributed to protection against the damaging effect of excess radiation. The contribution of the partners changed during seasons in some species, while in other species, partners equally shared the protecting role during the whole year. Since a strong species-specific physiological reaction and distribution pattern to microhabitat and season was detected, we propose to investigate more than one species of different growth forms to reveal the response of lichens to the changing environment in space and time.

9 ÖSSZEFOGLALÁS

A talajlakó zuzmók a biológiai kéreg részeként domináns tagjai a száraz és félszáraz élőhelyek élőlényközösségeinek. A homokdűnék egyedülállóan értékes, ámde sérülékeny természetük miatt természetvédelmi szempontból is figyelemreméltó ökoszisztémák. A talajlakó zuzmók különbözőképpen képesek alkalmazkodni az extrém élőhelyek környezeti viszonyaihoz. Ugyanakkor ezek a közösségek igen érzékenyek a zavarásokra és az intenzív emberi tevékenységekre (pl. klímaváltozás, taposás, terepjárózás, intenzív legeltetés, tűz). Alacsony növekedési rátájuk és biomassza produkciójuk miatt a zuzmótelepek regenerációja igen lassú egy-egy zavarási eseményt követően. Ezért a talajlakó zuzmók (és biológiai kéreg) közösségek védelme alapvető fontosságú. Ehhez nélkülözhetetlen adatokat gyűjteni a közösség összetételéről és működéséről, alkalmazkodóképességéről (fotoszintézis, fényvédelmi mechanizmusok). A sok, nemzetközi szinten is nyitott kérdés megköveteli ezen talajlakó közösségek és környezetük még intenzívebb kutatását. Ezért célul tűztük ki annak feltárását, hogy mely környezeti ható tényezők befolyásolják leginkább a közösség életét különböző térbeli és időbeli skálákon.

Hogy jobban megérthessük ezeket a folyamatokat, mikrometeorológiai adatokat gyűjtöttünk (1 perces felbontásban) 4 éven keresztül. Két eltérő diszturbancia-történettel bíró tájon és a homokbuckák ellentétes oldalain tanulmányoztuk a meszes homoki gyepek talajlakó zuzmóközösségének összetételét és a talajfelszíni változókat egy homokbuckás tájon különböző térléptékben. A közösség tagjainak élőhely- és mikrohabitat-preferenciáját is feltártuk. Nyomonkövettük a hét leggyakoribb faj fotoszintetikus aktivitásának és fényvédelmi mechanizmusainak akklimatizációját térben (mikrohabitatok kitettsége) és időben (évszakosság) és ezek kombinációjában. Azt is megvizsgáltuk, hogy a beérkező fény mennyisége hogyan oszlik meg a fotoszintézis és fényvédelmi mechanizmusok között talajlakó zuzmókban, mert erre eddig nem találtunk irodalmi adatokat. A korábbi vizsgálatok nagy részében egy fajt ragadnak ki egy adott élőhelyről, adott időben, hogy tanulmányozzák a bennük zajló folyamatokat, pedig a zuzmók vízháztartása és így metabolizmusuk nagyban függ vetületi terület/térfogat arányuktól. Ezért célunk volt, hogy kiterjesszük a vizsgált objektumok számát (ugyanarról az élőhelyről évszakonkénti ismétlésben és egyidejűleg több faj gyűjtésével), hogy egy teljesebb képet kapjunk arról, milyen mechanizmusok működnek zuzmókban, továbbá, hogy elkerüljük a téves általánosításokat is.

Mivel eddig csak két tanulmány foglalkozott meszes homokgyepek talajlakó zuzmóközösségeinek összetételével Európában, ezen közösségek magyarországi feltárása, bemutatása szignifikánsan hozzá járul a nemzetközi ismeretekhez. Vizsgálatunkban azt találtuk, hogy egy terület tájtörténete (korábbi diszturbanciák) szignifikánsan meghatározza a terület talajlakó zuzmóinak fajgazdagságát és a közösség összetételét. Kisebb, „bucka”-léptékű skálán általában alacsonyabb edényes növényzeti borítás és magasság és magasabb mohaborítás volt jellemző a szárazabb buckaoldalakon. A leggyakoribb fajok többségére negatív hatással volt a magas mohaborítás. Egyes fajok inkább a szárazabb, míg mások a nedvesebb buckaoldalakat preferálták vagy csak azokon fordultak elő, így táji léptékben a buckák nyújtotta diverz mikorkörnyezetek egy igen fajgazdag zuzmóközösségnek adnak otthont.

Eredményeink azt mutatják, hogy az ellentétes buckaoldalakat eltérő mikroklíma jellemzi és ez a különbség évszakosan változik. A levegő és a talaj hőmérséklete, PAR, és vízgőz telítési hiány szignifikánsan magasabb volt az aridabbbuckaoldalon, míg a relatív páratartalom és talajnedvesség a humidabb buckaoldalon volt magasabb. Az aridabb oldalon ősszel és télen a direkt besugárzás 3–4 órával hosszabb volt, míg télen 1–2 órával rövidebb, mint az árnyékosabb oldalon, ami meghatározta a különböző fajok fény- és árnyékpopulációinak metabolizmusát. Magasabb fotoszintetikus aktivitás és fokozottabb szabályozott fényvédelem (a fotobiontában és a mikobiontában egyaránt) volt jellemző a humidabb buckaoldalakról és évszakokban gyűjtött telepekre, mint az aridabb mikroélőhelyeken és évszakokban gyűjtött példánykra. Továbbá az élőhely ariditása befolyásolta az évszakos ingadozást a vizsgált paraméterekben. Eredményeink azt mutatták, hogy a különböző zuzmófajok különbözőképpen alkalmazkodtak a változó környezeti viszonyokhoz. Mind a mikobionta, mind a fotobionta hozzájárult a telepet érő túlzott besugárzás elleni fényvédelemhez. Egyes fajoknál a partnerek hozzájárulásának mértéke az évszakokkal változott, míg más fajokban a két partner egész évben egyenlő mértékben járult hozzá a telep fényvédelméhez. Mivel a zuzmók egyaránt erős faj-függő fiziológiai és elterjedésbeli választ adtak a mikrohabitatonként és évszakosan változó környezeti viszonyokra, a jövőbeni vizsgálatok tervezésénél egynél több, különböző telepfelépítésű faj vizsgálatát javasoljuk, ha fel szeretnénk tárni a zuzmók környeztre adott válaszát.

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12.2 S.2. Article 1: Factors at multiple scales influence the composition of terricolous lichen communities in temperate semi-arid sandy grasslands

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Factors at multiple scales influence the composition of terricolous lichen communities in temperate semi-arid sandy grasslands

Katalin Veres[1], Zsolt Csintalan[2], Bence Kovács[1] and Edit Farkas[1]

[1]Institute of Ecology and Botany, Centre for Ecological Research, H-2163 Vácrátót, Alkotmány u. 2–4, Hungary

[2]Department of Plant Physiology and Ecology, Institute of Biological Sciences, Hungarian University of Agriculture and Life Sciences, H-2100 Gödöllő, Páter K. u. 1, Hungary

Author for correspondence: Katalin Veres. E-mail: veres.katalin@ecolres.hu

Abstract

Inland dune ecosystems are rich in terricolous lichen species. However, these communities are sensitive to human activities, both locally and globally. Since terricolous lichens have a dominant role in semi-arid sandy grasslands, it is important to explore the composition of their communities and the environmental factors affecting them. We studied the structure of the terricolous lichen assemblages of calcareous grassland in an inland duneland ecosystem by comparing the lichen communities of arid and humid dune sides on two sites with different disturbance histories. Micro-coenological data were collected according to the Braun-Blanquet method. Environmental variables include the cover of bare soil, moss, litter, herb cover and height of herbs. We investigated the relationship of these variables and the presence and absence data of terricolous lichen species to sites and dune sides. We found that the site had a significant effect on species richness that might reflect the different types and severity of previous disturbance events at the studied sites. On a smaller, 'dune' scale, lower herb cover and height and a higher moss cover were generally characteristic of arid dune sides. Most of the frequent species were negatively affected by higher moss cover. Some lichen species were more abundant (e.g. *Cladonia furcata*) or found only (e.g. *Xanthoparmelia subdiffluens*, *Gyalolechia fulgens*) on arid dune sides, while others preferred (e.g. *C. pyxidata*) or occurred only on (e.g. *Peltigera* species, *C. rei*) humid sides. It was observed that the impact of the dune side on several variables differed between sites. The diverse microhabitat types, microclimate and landscape structure results in species-rich and valuable terricolous lichen communities forming in inland dune ecosystems.

Keywords: diversity, functional traits, inland dunes, lichen community ecology, microhabitat

Introduction

Inland dunes provide a unique but threatened environment for drought-tolerant organisms. In Europe, in addition to the European Aeolian Sand Belt, located in the northern European lowlands from Great Britain to Belarus and beyond Moscow (Koster 2009), they can also be found in Italy and Hungary. Depending on their origin, they could be morainic or fluvioglacial and are extremely oligotrophic (Schmidt 1986). Some of them are drift dunes; soil crusts and vascular plants bind others. Despite harsh environmental conditions (extreme irradiation, high temperature variability), the species richness is typically high in these habitats and rare specialists can also be present (Molnár 2003). Investigations on lichens are known from the Netherlands (Ketner-Oostra & Sýkora 2008; Sparrius 2011; Ketner-Oostra *et al.* 2012), Northern Italy (Gheza *et al.* 2015, 2016), Denmark (Schmidt 1986), Poland (Zielińska 1967; Adamska 2010; Adamska & Adamski 2014) and Estonia (Jüriado *et al.* 2016; Tilk *et al.* 2018).

Although terricolous lichens, which are a relevant part of the biological soil crusts (BSC), are essential elements of the semi-arid sandy grassland community (Belnap & Lange 2003), we still have limited information on their assemblages and the main drivers that shape them in these ecosystems. In a European-wide study, it was demonstrated that latitude has a strong effect on inter- and intraspecific variability within BSC communities (Büdel *et al.* 2014). The richness and diversity of BSC communities are significantly affected by both climate and microhabitat conditions (Escolar *et al.* 2012; Concostrina-Zubiri *et al.* 2014). Moreover, soil properties and the characteristics of the neighbouring vegetation (Ochoa-Hueso *et al.* 2011), as well as the herb and moss layer, have a detectable effect on species richness, for example, in alvar grasslands (Leppik *et al.* 2013). The increased density of the herb layer can cause a shift in the composition of lichen growth forms.

Disturbances such as those caused by fire and livestock in the Great Basin (North America) (Condon & Pyke 2018) have been shown to decrease the cover of BSC lichens and impact the lichen communities. Ullmann & Büdel (2001) gathered information about the relationships between environmental factors and the floristic composition of BSCs at local to regional scales in

Africa and Australia. They emphasized soil characteristics (chemical, physical), vegetation cover, geological attributes and climate variables as the most critical drivers of BSC floristic variation. The studies mentioned above revealed that numerous variables could have an impact on lichen species richness, functional diversity and composition in different ecosystems. However, there is limited information available on the effect of soil surface variables on terricolous lichen cover and species composition in inland dune ecosystems in Europe.

Inland dune ecosystems are considered to be vulnerable and extremely sensitive to human impact (e.g. trampling, fire, grazing). They are also threatened by the effects of climate change, especially the increasing severity and frequency of drought events in this region (Bartholy *et al*. 2009; Ferrenberg *et al*. 2015). According to the European Red List of Habitats, grasslands of the Pannonian and Pontic sandy steppe habitat types (E1.1a) are critically endangered (Janssen *et al*. 2016). As with the majority of European dry habitats, continental dunes in open sand steppes are protected by the European Habitat Directive (European Commission 2013) and belong to the Natura 2000 network to ensure their long-term persistence and the survival of their valuable and threatened species assemblages. Cryptogamic species are important in dry ecosystems; however, as Gheza *et al*. (2020) stressed, these organism groups are often neglected during both habitat characterization and conservation management planning. Nevertheless, for conservation purposes, it is essential to discover the composition of the lichen communities, as well as the environmental factors affecting them, because they dominate large areas between vascular plant patches in these vulnerable habitats.

The open sand steppes, providing habitats for terricolous lichens in duneland ecosystems, cover *c*. 10 840 ha (0.12%) in Hungary (MÉTA database: Molnár *et al*. 2007). This vegetation type (EU habitat directive code 6260 – Pannonic sand steppes: European Commission 2013) is of high conservation value. This habitat also provides ideal environmental conditions for several lichen species that are rare and protected in Hungary. Some of them are regarded as endemic by Verseghy (1958, 1994). Most (71%) of the lichen species protected in Hungary are terricolous (Farkas & Lőkös 2006; Farkas *et al*. 2012; Hungarian Ministry of Agriculture 2013; Sinigla *et al*. 2014, 2015). Five of these were found at the investigated sites: *Cladonia magyarica*, *Xanthoparmelia pokornyi*, *X. pulvinaris*, *X. ryssolea*, and *X. subdiffluens* (authorities can be found in Table 1). *Xanthoparmelia pulvinaris* and *X. subdiffluens* are regarded as endangered (Lőkös & Tóth 1997; Lőkös &Verseghy 2001), and all except *X. ryssolea* were described from the Pannonian steppe areas in Hungary. Comprehensive floristic surveys of lichens in the region have been performed by Gallé (1973) and Lőkös & Verseghy (2001). Nevertheless, there is only limited information about the effect of environmental conditions caused by the topography of inland dunes on the composition of lichen communities in Pannonian semi-arid sandy grasslands.

The present study aims to fill the knowledge gaps mentioned above by asking the following questions. What is the difference in species richness and composition on a coarse scale, and does disturbance history have an impact at this spatial level? How different are the terricolous lichen communities at smaller scales? What kind of environmental variables could cause this difference? To answer these questions, total species number, species composition and coverage of the three main growth forms (crustose, fruticose and foliose) were analyzed by site, by dune side and by micro-quadrat environment. We hypothesized that disturbance history might cause differences in the species pool. In addition, we supposed that because of different light and humidity conditions, there could be a difference in environmental variables at the scale of dune side and even smaller scales. We hypothesized that this could lead to a difference in terricolous lichen species composition and that the coverage of the various growth forms would be different with different dune aspects.

Table 1 The list of terricolous lichen species at Bugacpusztaháza ('BU') and Fülöpháza ('FH') according to previous authors and collectors (Lőkös & Verseghy 2001; Gallé 1973), with results from the present investigation. Protected species are highlighted in bold.

Species	Occurrence in BU	Occurrence in FH	Habitat
Bacidia bagliettoana (Massal. et De Not.) Jatta	0		–
Cladonia chlorophaea (Flörke ex Sommerf.) Spreng.	1	0	humid
C. coniocraea (Flörke) Spreng.	2		humid
C. fimbriata (L.) Fr.	1		humid
C. foliacea (Hudson) Willd.	1	1	humid / arid
C. furcata subsp. furcata (Huds.) Schrad.	1	1	humid / arid
C. furcata subsp. subrangiformis (L. Scriba ex Sandst.) Pišút	1	1	humid / arid
C. glauca Flörke	1*		humid
***C. magyarica* Vain.**	1	1	humid / arid
C. pocillum (Ach.) O.J. Rich.	2*	2*	humid
C. phyllophora Ehrh. ex Hoffm.	0		–
C. pyxidata (L.) Hoffm.	2	2	humid / arid
C. rangiformis Hoffm.	1	1	humid / arid
Cladonia rei Schaer.	2*	2*	humid
C. subulata (L.) Weber ex F. H. Wigg.	0		–
C. symphycarpa (Flörke) Fr.	0	0	–

Species	Occurrence in BU	Occurrence in FH	Habitat
Diploschistes muscorum (Scop.) R. Sant.	1	1	humid / arid
Enchylium bachmanianum (Fink) Otálora, P.M. Jørg. & Wedin		1*	arid
E. tenax (Sw.) Gray	1	1	arid
Endocarpon pusillum Hedw.	2*		humid
Gyalolechia fulgens (Sw.) Søchting, Frödén & Arup	0	1	arid
Peltigera canina (L.) Willd.	1		humid
P. rufescens (Weiss) Humb.	1		humid
Psora decipiens (Hedw.) Hoffm.		1*	arid
Squamarina lentigera (Weber) Poelt	0	1	arid
Thalloidima physaroides (Opiz) Opiz	1	1	humid / arid
***Xanthoparmelia pokornyi* (Körb.) O. Blanco, A. Crespo, Elix, D. Hawksw. & Lumbsch**	1	1	arid
***X. pulvinaris* (Gyeln.) Ahti & D.Hawksw.**	1	1	arid
***X. ryssolea* (Ach.) O. Blanco, A. Crespo, Elix, D. Hawksw. & Lumbsch**		1	arid
***X. subdiffluens* Hale**	0	1	arid

Blank cells – species not yet recorded at this site or the habitat preference is not known.

0 - species was not found in the present study.

1 - species was also found in the present study.

2 - species was discovered in the present study for the first time at the site.

* species was found in a studied stand but only outside the sampled quadrats

Materials and Methods

Study sites

The two sites investigated are located in the Kiskunság National Park near Bugacpusztaháza ('BU') (46°39′11.81″N, 19°35′45.17″E; Natura 2000 site code HUKN20024) and near Fülöpháza ('FH') (46°52′21.45″N, 19°24′18.29″E; Natura 2000 site code HUKN20011), in Central Hungary (Fig. 1A). Sites are located 28 air km from each other. Both sites are situated in nature conservation areas and have been protected with no agricultural activity since 1975.

The natural vegetation has been exposed to intensive human impact for at least two millennia in the Kiskunság region (Biró & Molnár 1998; Molnár *et al*. 2012). Trees were absent from both territories in the 19th century (earlier data are not available). The extensive grazing of Hungarian grey cattle followed by rural grazing of sheep resulted in open, strongly degraded grasslands in both sites and moving dunes in Fülöpháza. After the grazing was gradually abandoned in the 1960s, both sites were used for military purposes (1952–1974), where digging and trampling were characteristic. Nevertheless, these areas (especially the inner parts) were also protected from other activities (Molnár 2003). At the Bugacpusztaháza site, the spread of juniper (*Juniperus communis* L.) and poplar (*Populus* spp.) after extensive grazing was abandoned (until the mid-19th century and then after the 1950s) is noticeable (Biró & Molnár 1998). However, the increased population of the European rabbit (*Oryctolagus cuniculus* L.) reshaped the landscape at Bugacpusztaháza (1985–1994; Ónodi *et al*. 2006). Low intensity (only a goat herd) grazing was present until 1994 at the Fülöpháza site (Biró *et al*. 2015). Another difference between the two sites is the two anthropogenic, accidental fire events at Bugacpusztaháza, when the area was partly burned. The first was in 1976 (*c*. when 80% of damage occurred), and the second happened in 2012. The investigation site was partly damaged during the second fire (Szatmári *et al*. 2016); however, one year later, there were no visible signs of burns in the open grasslands (Szatmári *et al*. 2016). Lichens seemed healthy without any signs of burning in our study site during the data recording. The Bugacpusztaháza site had more dense tree vegetation (consisting of poplar and juniper); meanwhile, Fülöpháza, surrounded by pine (*Pinus nigra* J. F. Arnold and *P. sylvestris* L.) plantations, exhibits a more open landscape where signs of grazing were still visible in the 2000s and trees still grow sparsely (Molnár 2003).

The area's climate is moderately continental with a sub-Mediterranean influence on both study sites (Péczely 1967). The mean regional annual air temperature is 10.4 °C and the yearly precipitation is 505 mm based on a 30-year average (for details see: Biró & Molnár 1998; Lellei-Kovács *et al*. 2011; Biró *et al*. 2013). The region is mostly covered by calcareous sand from the deposits of the Danube River. The wind, as a secondary effect, reshaped the landscape, resulting in north-east, south-west facing dune sides with interdune depressions between them (Pécsi 1967).

The Kiskunság National Park varies in topography and consists of nine separated areas surrounded by fields, plantations (*Pinus* spp., *Robinia pseudoacacia* L.) and fallows (Molnár 2003). The duneland in Bugacpusztaháza is double the size of Fülöpháza (2000 ha). Our knowledge of the similarities and differences between these dune fields before the 19th century is incomplete. However, it is generally accepted that Bugacpusztaháza dunes are closer to the natural state and became forested (as climax association) after grazing was abandoned, while the Fülöpháza site bears the signs of deforestation and grazing (Molnár 2003).

Dominant plants in the semi-arid sandy grassland (*Festucetum vaginatae 'danubiale'* Rapaics ex Soó em. Borhidi association) at the investigation sites are *Festuca vaginata* W. et K., *Stipa borysthenica* Klokov, *Fumana procumbens* (Dun.) Gren. et Godr., *Alcanna tinctoria* (L.) Tausch, *Dianthus serotinus* W. et K., *Onosma arenaria* W. et K. and *Sedum hillebrandtii* Fenzl (Fekete 1997; Borhidi *et al*. 2012). Most of the terricolous lichen species found here are members of the

'Bunte Erdflechten-Gesellschaft' (Gams 1938), also known as the complex of *Fulgensietum fulgentis* Gams and *Cladonietum symphycarpiae* Doppelb. associations, which are typical for this region (Büdel 2001).

Sampling method

Overall, ten stands of open sand grasslands were randomly selected, representing the duneland vegetation of the area.

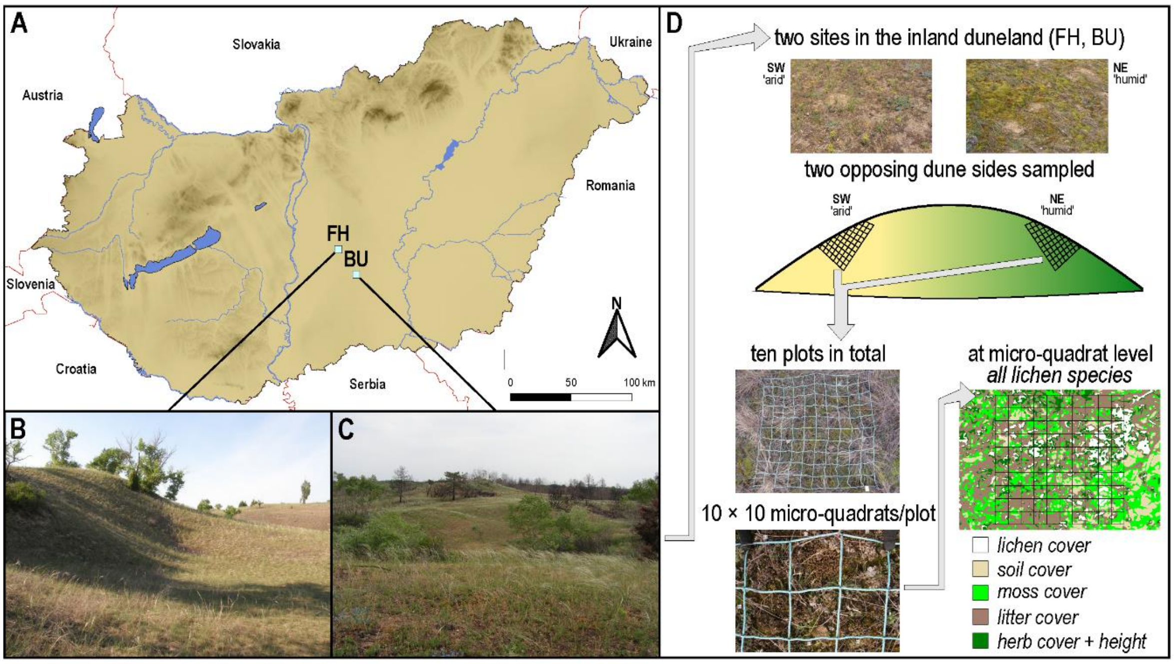

Fig. 1. A, location of the two study sites in Hungary. B, the landscape of Fülöpháza (FH). C, the landscape of Bugacpusztaháza (BU). D, a diagramatic representation of the study design; 'plots' = quadrats. In colour online.

Sampling was carried out in late autumn and during wintertime because it was more advantageous to estimate the cover of the different lichen growth forms than in summer when they are usually in a dry condition. Our previous long-term study (Veres *et al*. 2020) revealed a significant difference in microclimate between the north-east (NE) and south-west (SW) facing dune sides or aspects of the sites. The more exposed, SW-facing slopes are considered arid; the NE-facing sides are considered less arid (hereafter 'humid') dune sides. At Bugacpusztaháza, three stands were located on randomly selected SW and three on NE aspects of different dunes (six dunes in total). Two stands for each dune side were similarly selected at Fülöpháza (four dunes in total). On each stand (i.e. dune side), one 1 m × 1 m sampling quadrat was placed in the central part of the stand

(10 quadrats in total). The quadrat has 10 × 10 sections (100 contiguous 10 cm × 10 cm micro-quadrats per quadrat) (Fig. 1). The size of the quadrat was selected based on the characteristics of the aggregated vegetation (Scheidegger *et al*. 2002). In the present study, only soil surface attributes were investigated. In each micro-quadrat, the coverages of lichen growth forms (% fruticose, foliose and crustose), moss (%), herb (%), litter (%) and bare soil (%) were evaluated with the Braun-Blanquet method (Braun-Blanquet 1964). The average height of the herbs (mm) was also measured with a measuring tape. The presence or absence of each lichen species was recorded in each micro-quadrat. Thus in this study, lichen data and the potential explanatory variables were recorded at the micro-quadrat level (n = 100 micro-quadrats / quadrat for each of 10 quadrats) across two studied sites and two types of dune side at each site.

Many lichen species were identified in the field. Species that were difficult to identify in the field were transported to the laboratory and examined with HPTLC using the method of Arup *et al*. (1993). They were then identified using Verseghy (1994), Smith *et al*. (2009), and Wirth *et al*. (2013). Nomenclature follows Index Fungorum (CABI 2020) and Farkas & Lőkös (1994). Lichen species that occurred outwith the sampling units were also recorded and added to a complete species list, including authorities (Table 1).

Data analysis

First, we compiled detailed species lists for the two study sites, incorporating previous and present occurrences. Besides study site, dune side where a certain species occurred (i.e. 'arid' or 'humid' preference) was also recorded.

Only data for lichens in micro-quadrats were used for quantitative analyses. For these analyses, data for some species were merged, because of their very similar thallus structure, when they occurred in the same microhabitat type or their abundance were low (*Cladonia chlorophaea* and *C. fimbriata* only on humid dune sides; *C. furcata* and *C. rangiformis*, and *C. magyarica* and *C. pyxidata* in both types of microhabitats; and only occurring on arid dune sides were *Xanthoparmelia pokornyi* and *X. ryssolea*, and *X. pulvinaris* and *X. subdiffluens*). These five pairs are hereafter known as 'groups' and are presented, for example, as follows: *Cladonia chlorophaea / fimbriata*. *Cladonia rangiformis* occurred only in a small number of micro-quadrats (1%); therefore, we decided to merge the species together with the more frequent *C. furcata* (9%). They were the only two several-branched to many-branched *Cladonia* species in our study. Both species were represented by only 1–3 podetia in the micro-quadrats.

The effects of site ('BU' or 'FH') and dune side ('arid' or 'humid') on the environment (cover of bryophytes, herbs, bare soil, litter and height of herbs) and lichen-related (species number, total lichen cover, cover of three growth forms) variables, as well as the relationship of environmental variables to lichen variables, were evaluated. Non-metric multidimensional scaling ordination and generalized mixed-effects models (random intercept models; Zuur *et al*. 2009) were performed on data in micro-quadrats. PERMANOVA was performed on lichen frequency by quadrat. All analyses were carried out using R version 4.0.3 (R Core Team 2020).

Lichen assemblages were the first to be studied. A global non-metric multidimensional scaling (NMDS) was performed on the data set of all single lichen species (13) and groups (five) present in the micro-quadrats (with a Jaccard dissimilarity matrix) to visualize micro-quadrat separation along with the dune sides and sites simultaneously using the 'metaMDS' function of *vegan* (Oksanen *et al*. 2019); details can be found in Supplementary Material Section 1 (available online). A two-dimensional solution was selected based on stress as a function of dimensionality (Supplementary Material Fig. S1A, available online). The final stable solution was the lowest stress from 100 random starting configurations. The final configuration was centred and rotated to maximize orthogonality with the highest point dispersion on the first axis (see Borcard *et al*. 2018; Oksanen *et al*. 2019), goodness-of-fit was assessed by Kruskal's stress formula one (Legendre & Legendre 2012) and by a Shepard diagram, and proportion of original variation was estimated (Supplementary Material Fig. S1B). Mantel tests were used to estimate the proportion of variation explained by individual NMDS dimensions, and environmental variables were fitted passively onto the ordination space (both using functions in *vegan* (Oksanen *et al*. 2019).

Besides ordination, separation of the lichen assemblages among sites and dune sides as main questions were tested at quadrat-level; therefore, samples were pooled and frequency data were calculated for the analysis. Permutational multivariate analysis of variance (PERMANOVA; Anderson 2001) based on Manhattan metrics with 9999 permutations was applied to test the aspect and site effects in one model. PERMANOVA is a flexible, dissimilarity-based, semi-parametric method to test differences in centroids and dispersions of groups with no assumption of multivariate normality. The method compares within-group to between-group variances using an F-test, distribution-free inferences achieved by random permutations of the objects between the compared groups. PERMANOVA was performed with the 'adonis' function in *vegan* (Oksanen *et al*. 2019). Species richness and cover of lichen growth forms, as well as the different substratum forms as potential drivers of lichen communities, were further investigated. In the mixed-effects models, site (two sites) and dune side (two dune aspects at each site) were fixed factors, quadrat was a random factor (10 states), with response variable values for each micro-quadrat (100 per quadrat, 1000 in total). Since the distributions of most of the studied variables were highly skewed, generalized mixed-effects models (GLMMs) (random intercept models) with a restricted maximum likelihood (REML) method were used (Bolker *et al*. 2009; Dunn & Smyth 2018). Prior to the analyses, all fixed factors were centred and scaled (*z*-score standardization). GLMMs were fit for most using *lme4* (Bates *et al*. 2015) with different link functions depending on the distribution of a variable. For some, Tweedie models were built using *glmmTMB* (Brooks *et al*. 2017) with the *tweedie* package (Dunn 2017). *P*-values were provided by *lmerTest* (Kuznetsova *et al*. 2017). Details of the models are provided in Supplementary Material Section 2 (Tables S1.1 and S1.2, respectively, available online). Site and dune side (within separate sites) contrasts were then evaluated by estimated marginal means that were based on the constructed GLMMs using *emmeans* (Lenth 2021).

Finally, the presence of each of the six most abundant individual lichen species or groups (*Cladonia foliacea*, *C. furcata / rangiformis*, *C. magyarica / pyxidata*, *Diploschistes muscorum*, *Enchylium tenax*, *Xanthoparmelia pokornyi* / *ryssolea*) was tested by logistic mixed-effects models using *lme4* (Bates *et al*. 2015). Site, dune side and substratum cover variables were used as fixed effects in micro-quadrats (details for logistic mixed-effects models provided in Supplementary Material Table S1.3, available online). Prior to these analyses, all tested explanatory variables as fixed factors were centred and scaled (*z*-score standardization). Details for logistic GLMMs can be found in Supplementary Material Table S3.

Results

Lichen communities of the two investigated sites and dune sides

In the present study, a total of 20 terricolous lichen taxa were recorded at the Bugacpusztaháza site and 19 at the Fülöpháza site, including species outside of the quantitatively measured quadrats (Table 1). Five of these are new to the sites from this investigation (Table 1). While *Cladonia pocillum*, *C. pyxidata* and *C. rei* were new occurrences to both sites, *C. coniocraea* and *Endocarpon pusillum* were found only at the Bugacpusztaháza site. A previous study (Lőkös & Verseghy 2001) documented 22 terricolous lichen taxa from Bugacpusztaháza and 18 from the Fülöpháza site. Though *C. convoluta* and *C. foliacea* were mentioned as separate species, they are currently considered conspecific (Pino-Bodas *et al*. 2018). Eight formerly reported species were not confirmed during the present investigation (Table 1). *Cladonia subulata* was not detected at either site in the present study, although the morphologically very similar but chemically distinguishable *C. rei* was verified. Twelve species (13 taxa) were shared between the two sites, and six species were found outside of the quantitatively measured quadrats (Table 1).

Nine species were found only on humid dune sides (Table 1): *Cladonia chlorophaea*, *C. coniocraea*, *C. fimbriata*, *C. glauca*, *Endocarpon pusillum*, *Peltigera canina* and *P. rufescens* were found only at Bugacpusztaháza, while *C. pocillum* and *C. rei* occurred at both sites. Nine other species occurred only on arid sides of the dunes: *Enchylium bachmanianum*, *Gyalolechia fulgens*, *Psora decipiens*, *Squamarina lentigera*, *Xanthoparmelia ryssolea* and *X. subdiffluens* were found only at Fülöpháza, while *Enchylium tenax*, *Xanthoparmelia pokornyi* and *X. pulvinaris* occurred at both sites. The seven species (8 taxa) that were recorded on both dune sides were the same at the two sites (Table 1).

The lichen communities of the two sites differed, especially the arid lichen communities of Bugacpusztaháza from the arid lichen communities of Fülöpháza (Fig. 2). The core species composition of the Fülöpháza arid dune sides varied more among micro-quadrats along both NMDS1 and NMDS2 (Fig. 2) than the other three groups (FH humid, BU arid, BU humid), and is very different from the other three groups along NMDS1. In a weaker pattern expressed on both axes, Fülöpháza humid and Bugacpusztaháza humid core lichen species composition show a notable overlap, with core Bugacpusztaháza arid composition mainly being a subset of both.

The site, dune side and environmental factors in micro-quadrats were found to be important factors determining terricolous lichen assemblages in these sandy areas. According to the pseudo F statistics, the site had higher explanatory power than the aspect of the dunes at a quadrat level ($P < 0.0046$ and $P = 0.0231$, respectively). The NMDS (stress = 0.102) showed a separation among dune sides and site (Fig. 2). The ordination using two orthogonal dimensions cumulatively represented 59.61% of the variation of species composition at the micro-quadrat scale (Mantel tests, NMDS 1: $R^2 = 0.349$, $P < 0.001$; NMDS 2: $R^2 = 0.247$, $P < 0.001$).

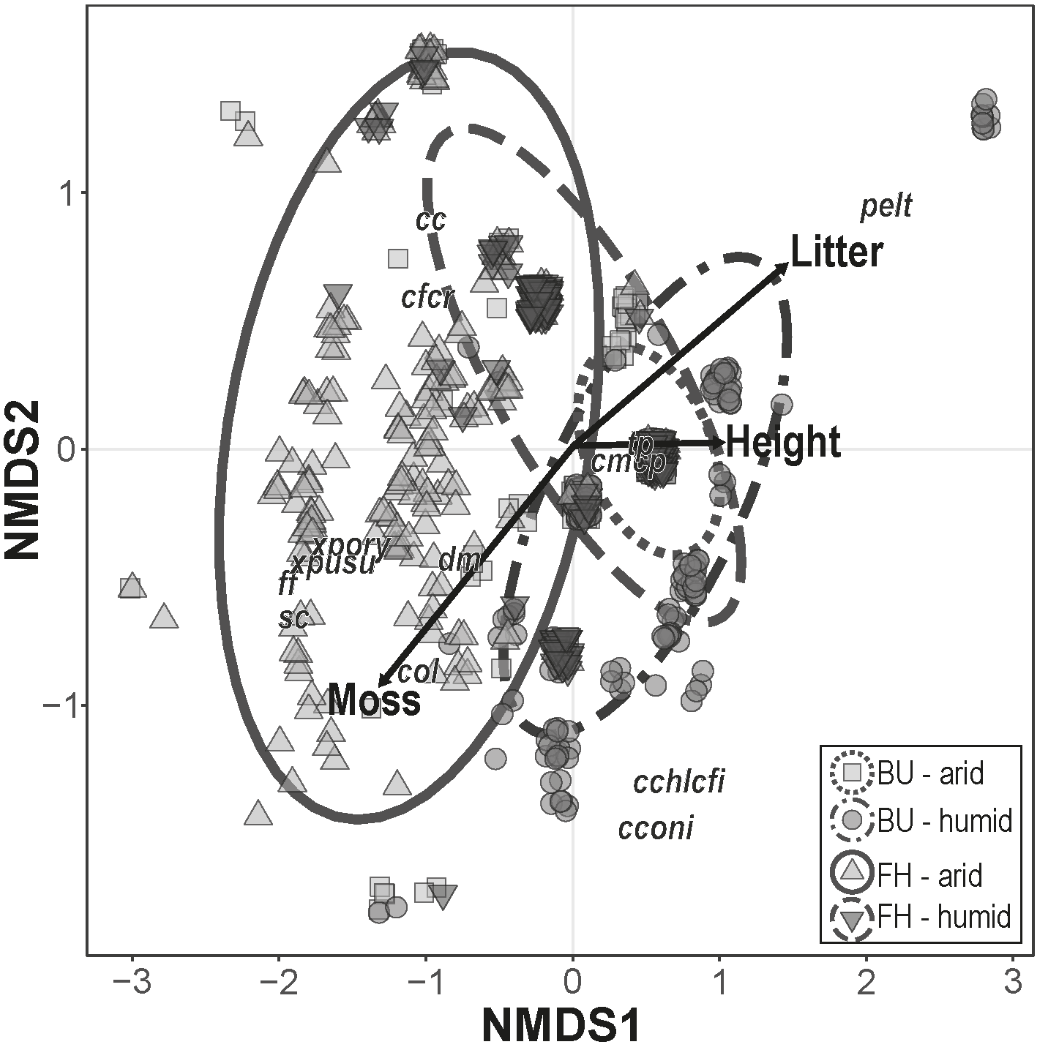

Fig. 2. Ordination diagram from the non-metric multidimensional scaling (NMDS, stress = 0.102) showing the pattern of terricolous lichen assemblages and micro-quadrats based on sites and the dune side with passively projected explanatory variables. Black arrows represent significant correlations ($P < 0.05$) with the sample scores. PERMANOVA statistics: Site, $R^2 = 0.343$, $F(1,7) = 5.429$, $p = 0.0046$; Dune side, $R^2 = 0.215$, $F(1,7) = 3.399$, $p = 0.0231$. BU = Bugacpusztaháza site; FH = Fülöpháza site. Abbreviations of species and group names: cc – *Cladonia foliacea*; cchlcfi – *Cladonia chlorophaea / fimbriata*; cconi – *Cladonia coniocraea*; cfcr – *Cladonia furcata / rangiformis*; cmcp – *Cladonia magyarica / pyxidata*; col – *Enchylium tenax*; dm – *Diploschistes muscorum*; ff – *Gyalolechia fulgens*; pelt – *Peltigera canina* and *P. rufescens*; sc – *Squamarina lentigera*; tp – *Thalloidima physaroides*; xpory – *Xanthoparmelia pokornyi / ryssolea*; xpusu – *Xanthoparmelia pulvinaris / subdiffluens*.

The position of the species in the ordination space indicated that a higher coverage of leaf litter had a strong association with *P. canina*. *Enchylium tenax* and *Diploschistes muscorum*, on the other hand, preferred growing in and on moss patches. The NMDS emphasized that *C. foliacea*, *C. furcata* / *rangiformis*, *Xanthoparmelia* species (*Xanthoparmelia pokornyi* / *ryssolea* and *X. pulvinaris* / *subdiffluens*), *Squamarina lentigera* and the crustose *Diploschistes muscorum* and *Gyalolechia fulgens* were more abundant in patches where herb height was lower.

The variation related to herb height along the first axis is distinct from variation attributable to a stronger factor correlated with litter and moss cover because their vectors are divergent (Fig. 2). The variation in herb height is correlated with both site and dune side, each varying greatly along NMDS1. In contrast, variation in a micro-quadrat environmental factor linked to litter and moss cover varies almost equally with NMDS1 and NMDS2.

Species richness of the two investigated sites and dune sides

Species richness at the microplot scale was significantly higher at FH than at BU ($t_{994} = -3.502$, $P < 0.001$) (Fig. 3A). At the FH site, the total species richness was significantly higher ($t_{994} = 3.671$, $P < 0.001$) in arid micro-quadrats (mean 3.66, range 0–9) compared to humid micro-quadrats (1.55 [0–4]) (Fig. 3A). The opposite was detected at BU, where humid sides (1.69 [0–4]) hosted more species ($t_{994} = -1.909$, $P = 0.056$) than arid ones (1.16 [0–4]) (Fig. 3A).

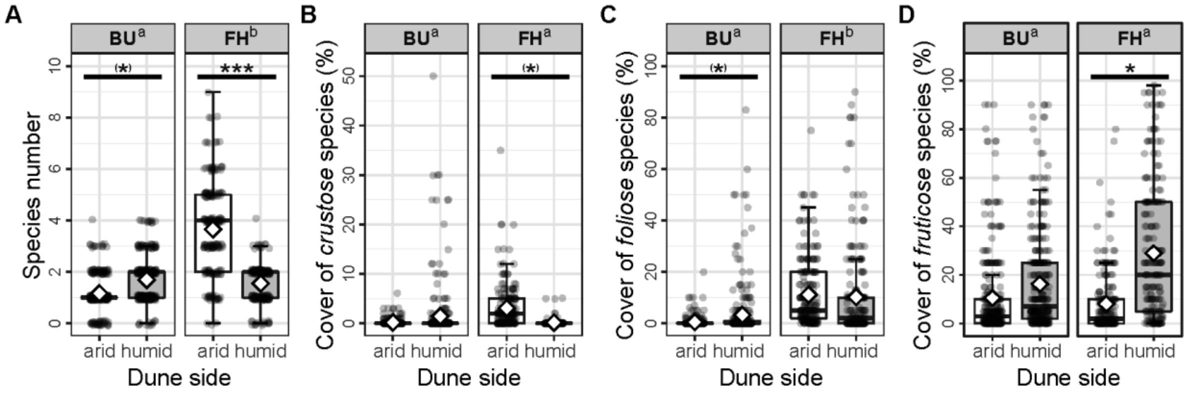

Fig. 3. Species richness of the two investigated sites and dune sides. The number of species per micro-quadrat (A), coverage (%) of crustose (B), foliose (C) and fruticose (D) species, by site (BU = Bugacpusztaháza, FH = Fülöpháza) and dune side (arid, humid). On the boxplots (median with the interquartile range = box, range = whiskers), both raw data (dots) and mean values (diamonds) are represented. Summary statistics of the Wald's Chi^2 tests (Chi^2 and *P*-values) are also shown on each graph. Different letters ('a' and 'b') indicate the statistical difference ($P < 0.05$) between the sites. The asterisks code the significant difference between the dune sides ($^{(*)}$ = $P < 0.1$, * = $P < 0.05$, *** = $P < 0.001$).

The site effect was not significant ($t_{994} = -0.389$, $P = 0.697$) on the coverage of crustose species (Fig. 3B). Dune side had a marginally significant effect on the crustose abundance at FH ($t_{994} = 1.931$, $P = 0.054$), where the cover in arid micro-quadrats was higher (3.01% [0–35%]) compared to humid ones (0.11% [range 0–5]), but not at BU ($t_{994} = 0.264$, $P = 792$; arid 0.18% [0–6%], humid 1.45% [0–50%]).

At the FH site, foliose species had a significantly higher ($t_{994} = -3.280$, $P = 0.001$) cover (arid 11.13% [0–75%]; humid 10.27% [0–90%]) than at BU (arid 0.52% [0–20%], humid 3.22% [0–83%]) (Fig. 3C). Contrary to the BU site, cover among dune sides at FH did not differ ($t_{994} = -1.777$, $P = 0.077$ and $t_{994} = 0.753$, $P = 0.452$, respectively) (Fig. 3C).

The coverage of fruticose species did not differ ($t_{994} = -1.026$, $P = 0.3049$) between the two localities (Fig. 3D). In FH micro-quadrats, fruticose species reached significantly higher ($t_{994} = -2.087$, $P = 0.037$) cover on humid (28.92% [0–98%]) than on arid (8.08% [0–80%]) sides, while at BU their coverage was similar ($t_{994} = -0.743$, $P = 0.458$) on both dune sides (arid 10.48% [0–90%], humid 16.24% [0–90%]) (Fig. 3D).

Soil surface variables on the two investigated sites and dune sides

The cover of bare soil was significantly lower ($t_{994} = 4.075$, $P < 0.001$) in FH than in the BU micro-quadrats (Fig. 4A). At the FH site, the average bare soil cover was significantly higher ($t_{994} = 3.599$, $P < 0.001$) in arid (4.02% [0–40%]) compared to humid (0.2% [0–35%]) micro-quadrats (Fig. 4A). The two dune sides did not differ significantly ($t_{994} = -0.228$, $P = 0.8198$) in their bare soil cover (arid 8.37% [0–90%], humid 13.33% [0–90%]) at BU.

There was no remarkable difference detectable in the moss cover (t_{994}, $P = 0.6877$) between the two localities (Fig. 4B). However, at the FH site, the average moss cover was significantly higher (t_{994}, $P = 0.0109$) in arid (63.89% [1–98%]) compared to humid (44.52% [0–98%]) micro-quadrats, while at BU the coverage was similar ($t_{994} = 1.287$, $P = 0.1984$) on both dune sides (arid 57.84% [0–100%], humid 46.95% [0–100%]) (Fig. 4B).

Site effect was not significant on the cover of litter (t_{994}, $P = 0.8512$) between the two sites, and it was similar on arid and humid dune sides at BU ($t_{994} = -0.076$, $P = 0.9396$; arid 18.18% [0–95%], humid 15.12% [0–80%]) as well as at FH ($t_{994} = -1.546$, $P = 0.1225$; arid 10.72% [0.1–75%], humid 20.70% [2–95%]) (Fig. 4C).

The herb cover did not differ significantly ($t_{994} = 1.469$, $P = 0.1422$) between the two sites (Fig. 4D). The average herb cover was significantly lower ($t_{994} = -2.649$, $P = 0.0082$) in arid (5.24% [0–60%]) compared to humid (13.00% [0–100%]) micro-quadrats at the FH site (Fig. 4D). The two dune sides did not differ significantly ($t_{994} = -0.802$, $P = 0.4227$) in their herb cover (arid 9.99% [0–80%], humid 13.78% [0–80%]) at BU.

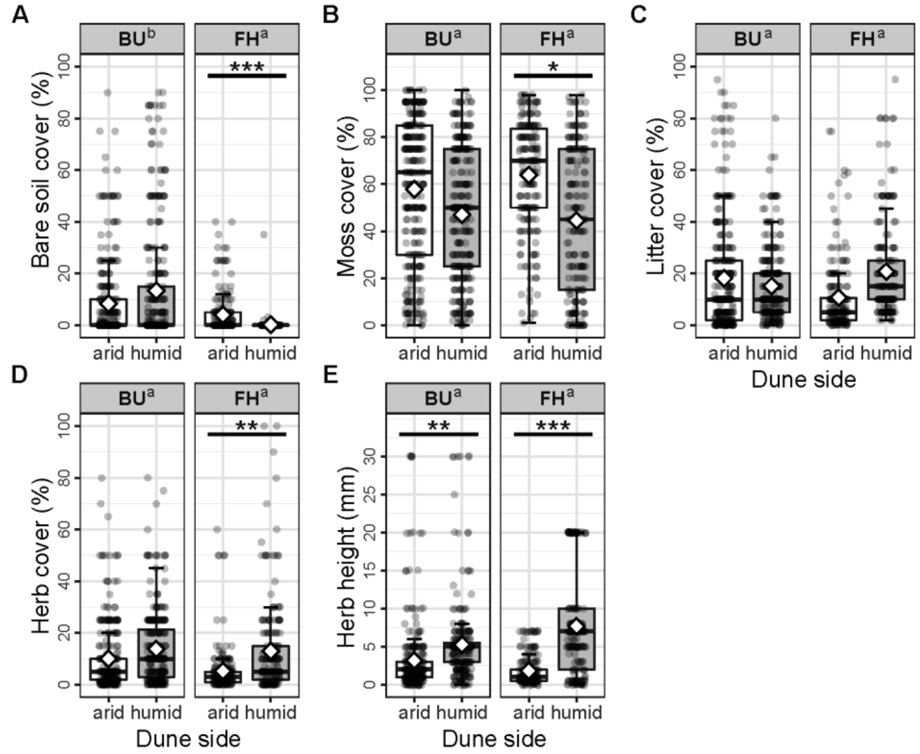

Fig. 4. Cover (%) of the studied soil surface types per micro-quadrat by site (BU = Bugacpusztaháza, FH = Fülöpháza). A, soil. B, moss. C, litter. D, herb. E, mean height (mm) of herbs. On the boxplots (median with the interquartile range = box, range = whiskers), both raw data (dots) and mean values (diamonds) are represented. Summary statistics of the Wald's Chi^2 tests (Chi^2 and *P*-values) are also indicated on each graph. Different letters ('a' and 'b') indicate the statistical difference ($P < 0.05$) between the sites. The asterisks code the significant difference between the dune sides (* = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$).

The average height of herbs was similar ($t_{994} = 0.499$, $P = 0.6180$) at the two localities (Fig. 4E). At the same time, herbs reached a significantly lower height ($t_{994} = -6.696$, $P < 0.001$) on arid (1.82 mm [0–7 mm]) than on humid (7.69 mm [0–20 mm]) dune sides at FH (Fig. 4E), as well as at BU ($t_{994} = -2.927$, $P = 0.0034$; arid 3.169 mm [0–30 mm], humid 5.25 mm [0–30 mm]) micro-quadrats (Fig. 4E).

Factors determining abundant species

Abundance of the *C. magyarica / pyxidata* group was higher in the Bugacpusztaháza micro-quadrats ($P = 0.01$) (Table 2). In contrast, the *C. furcata / rangiformis* group ($P < 0.001$) and *C. foliacea* ($P = 0.001$) were more abundant at the Fülöpháza site (Table 2). Almost all abundant species (found in > 50 micro-quadrats and at both sites) were negatively influenced by a higher moss cover; only *Enchylium tenax* benefitted (Table 2). Herb cover negatively affected the *C. magyarica / pyxidata* group ($P < 0.001$), while litter cover negatively influenced the presence of both the former group and *Enchylium tenax* (Table 2). The cover of bare soil negatively affected the abundance of the *C. magyarica / pyxidata* group (*P* < 0.001) and *Diploschistes muscorum* (*P* = 0.041) (Table 2). *Cladonia foliacea* was negatively affected by higher herb height (*P* = 0.001) (Table 2).

Table 2. Summary table of the species-specific logistic generalized mixed-effects models studying the effects of potential explanatory variables on the most abundant (found in > 50 micro-quadrats and at each site) species and groups. For each tested variable, the results of the Wald's likelihood ratio-based Chi^2-tests, estimate signs (Est.) and *P*-values are also presented (for further details see Supplementary Material Table S3, available online). Values in bold are statistically significant at $P < 0.05$. The number of records for each abundant species is also indicated in parentheses after the species name. Estimate sign = '+' when the value is higher at Fülöpháza than at Bugacpusztaháza and '–' when the value is higher at Bugacpusztaháza than at Fülöpháza.

	Site			Moss cover			Herb cover			Litter cover			Bare soil cover			Herb height		
Model	Est.	Chi^2	*P*	Est.	Chi^2	*P*	Est.	Chi^2	*P*	Est.	Chi^2	*P*	Est.	Chi^2	*P*	Est.	Chi^2	*P*
Cladonia foliacea (270)	**–**	**15.983**	**< 0.001**	**–**	**5.493**	**0.019**		0.095	0.758		0.923	0.337		1.440	0.230	**–**	**11.990**	**0.001**
C. furcata / rangiformis (102)	**+**	**10.719**	**0.001**		2.211	0.137		0.266	0.606		0.048	0.827		3.484	0.062		0.773	0.379
C. magyarica / pyxidata (809)	**–**	**6.596**	**0.010**	**–**	**20.169**	**< 0.001**	**–**	**30.712**	**< 0.001**	**–**	**19.144**	**< 0.001**	**–**	**15.024**	**< 0.001**		2.064	0.151
Diploschistes muscorum (199)		0.307	0.579	**–**	**10.885**	**0.001**		1.190	0.275		0.533	0.465	**–**	**4.176**	**0.041**		0.783	0.376
Enchylium tenax (190)		3.723	0.054	**+**	**5.741**	**0.017**		3.381	0.066	**–**	**4.692**	**0.030**		0.006	0.939		2.875	0.090
Xanthoparmelia pokornyi / ryssolea (93)		0.592	0.442	**–**	**4.582**	**0.032**		1.548	0.213		2.756	0.097		0.175	0.676		0.415	0.519

Discussion

Species pools of the two study sites

Fewer species were previously known from Fülöpháza than from the Bugacpusztaháza site, and the species composition of the two localities is slightly different. There may be several reasons for the differences in the species pools of the studied sites, which could affect the species composition separately and/or in interaction. Terricolous lichen communities are mainly soil type-specific but historical disturbances and land-use change also affect their composition (Leppik *et al*. 2013). The natural vegetation has been exposed to intensive human impact for at least two millennia in the Kiskunság region (Molnár *et al*. 2012). At the Bugacpusztaháza site, the noticeable spread of juniper and poplar could provide favourable microhabitat conditions for terricolous lichen species requiring shade, more humidity or increased humus content of the substratum (through the decomposition of wood and litter). This phenomenon could probably explain the significantly higher number of 'tree/shrub-correlated' species (e.g. *Cladonia chlorophaea*, *C. coniocraea* or *Peltigera* species) than at the Fülöpháza site. Two fire events influenced the vegetation and BSC communities of the Bugacpusztaháza site, in contrast to Fülöpháza where grazing was abandoned later.

Our results showed that the site had higher explanatory power than dune side. Since we could not find any significant difference in the climate between the two study site (Veres *et al*. 2020), and the investigated environmental variables differed significantly only in open soil cover, we hypothesize that the disturbance history could also play an important role in developing different lichen communities.

The dunes near Fülöpháza have been affected by several kinds of disturbances in the past (mainly grazing) (Molnár 2003, 2008). The species richness at Fülöpháza could be explained by the lack of fire events, as well as by the later abandonment of grazing. Such activities can dramatically reduce the total BSC cover, lichen cover and species richness (e.g. Johansen 2001; Warren & Eldridge 2001). Grazing can also cause changes in the amount of lichen biomass and species richness in grasslands (Boch *et al*. 2016; Balogh *et al*. 2017). However, low-intensity grazing, especially by sheep, can cause higher species richness (Boch *et al*. 2016) and may explain the higher number of terricolous species at Fülöpháza. The more open vegetation could also provide habitats for more species, as Ketner-Oostra & Sýkora (2000) pointed out in their study where both the terricolous lichen diversity and coverage was the highest in the early, more open vegetation stages on coastal dunes.

The cover of foliose lichens was significantly lower at the Bugacpusztaháza than at the Fülöpháza site. However, *P. canina* occurred only in the Bugacpusztaháza micro-quadrats; the higher abundance of *C. foliacea* and *Xanthoparmelia* species caused the significantly higher cover of foliose species at the Fülöpháza site. The cover of fruticose and crustose species was similar at the two study sites. Thalli of *C. magyarica*, *C. pyxidata* and the wood-correlated, scyphose *Cladonia* species were more abundant at Bugacpusztaháza, whereas *C. furcata* and *C. rangiformis* were more abundant at Fülöpháza. The most abundant crustose species was *Diploschistes muscorum* at both sites.

The Bugacpusztaháza site appears to have had a greater lichen species turnover and less species retention than Fülöpháza. Seven formerly mentioned species (Lőkös & Verseghy 2001) were not confirmed during the present investigation at the Bugacpusztaháza site. The disappearance of the earlier rare *Cladonia phyllophora* and the relatively rare *Squamarina lentigera* is not entirely surprising. However, that of the earlier common *Bacidia bagliettoana*, *C. symphycarpa* and *C. subulata* and the frequent *Gyalolechia fulgens* is a serious loss. Similarly, *C. chlorophaea* and *C. symphycarpa*, previously common at the Fülöpháza site, were not found during the present study, indicating environmental changes in the last decades.

Differences in species composition between arid and humid dune sides

There are many microclimatic differences between the SW- and NE-facing dune sides. In the long-term, incoming light (photosynthetically active radiation), and air and soil temperatures are usually higher on SW- than NE-facing dune sides, whereas the relative air humidity and soil water content are lower (Veres *et al*. 2020). These diverse microenvironmental conditions provide different microhabitat types; therefore, species with various ecological requirements can live together in the same habitat.

We observed that fruticose species could reach a greater cover in the humid micro-quadrats where the cover and height of herbs were usually higher at both localities. Our results are consistent with the findings of Leppik *et al*. (2013), who demonstrated that the increased density of the herb layer was one of the main drivers for the development of a terricolous lichen community. A shift was detected in the composition of lichen growth form from the dominance of crustose (and squamulose) to fruticose lichens. In our study, it appears that the branching *C. furcata* and *C. rangiformis* are more tolerant of the environmental stress of the arid sides than the cup *Cladonia* species. *Cladonia magyarica* and *C. pyxidata* are probably more sensitive to moisture fluctuation and prefer more stable microclimatic conditions on humid dune sides (e.g. Blum 1973; Büdel & Scheiddeger 2008; Veres *et al*. 2020).

Another factor is that the competition with mosses seems to be more pronounced, especially on arid sides, where vegetation was lower and more sparse. Therefore, the lower coverage of plant-derived variables, and significantly higher moss cover in arid compared to humid micro-quadrats, can explain the lower total lichen cover in arid microhabitats. Our results are in agreement with the observation that vegetation generally becomes more open with increasing aridity (Walter & Breckle 1984) and lichens compete with mosses for free patches (Ketner-Oostra & Sýkora 2000).

The higher cover of fruticose species in humid micro-quadrats is mainly due to *C. pyxidata* and *C. magyarica*. They often grow on moss cushions, which prolongs their assimilation time during dry days (Colesie *et al.* 2012) and compensates for the lower incoming irradiation caused by a higher herb cover and amount of litter (e.g. Pintado *et al.* 2005). This might explain why these species can tolerate the disadvantageous light conditions caused by vascular plants.

Our results suggest that the arid and humid lichen communities are rather different at the two sites. An interaction effect between the sites and dune sides was also detectable. In Fülöpháza, arid micro-quadrats vary much more in lichen species composition than their humid counterparts. The opposite is true for Bugacpusztaháza arid and humid sides. The difference in soil surface variables between the arid and humid dune sides was also different at the two localities. While at Bugacpusztaháza only the average height of herbs was significantly higher in the humid compared to arid micro-quadrats, at Fülöpháza every soil surface variable showed significant differences (except litter cover) between the microhabitats. Because the vegetation is more open at the Fülöpháza site (Molnár 2003), the effect of topography could prevail to a greater degree than at Bugacpusztaháza, where the vegetation is further progressing to climax state (Molnár 2003). The more balanced microclimate (Veres *et al.* 2020) can mitigate the contrast between environmental conditions prevailing on arid and humid dune sides.

Differences in species composition at the microhabitat scale

Almost all species were negatively influenced by a higher moss cover because terricolous lichens usually compete with mosses for open patches between vascular plants (e.g. Ketner-Oostra & Sýkora 2000). Only *Enchylium tenax*, which grows in moss cushions, might benefit from the greater moss cover, but this species was negatively influenced by higher litter cover. Our results suggest that among the most abundant species, *C. magyarica* and *C. pyxidata* are the most sensitive species, negatively affected by the measured microenvironmental variables. These species grow on moss cushions, preferring open spaces between vascular plants. The litter cover negatively influenced the presence of both the former group and *Enchylium tenax*, probably because of its shading effect. The higher cover of bare soil negatively affected the abundance of *Diploschistes muscorum*, whose host species are *C. magyarica* and *C. pyxidata*. *Cladonia foliacea* was negatively affected by higher herb height, probably because the spread of thalli is hindered due to scrolling by the wind.

Our results showed that the composition of dune lichen communities could be impacted by both site and dune side. The difference shown in herb height and herb cover between sites and dune sides might cause a difference in the light level reaching lichen thalli, resulting in different microclimatic conditions where lichens with different environmental needs could find their preferred niche. It was also shown that at lower herb cover and height, the chance of competition might increase between mosses and lichens.

We can also assume that individual lichen species are influenced by the progression to climax vegetation as a principal effect of site. The Bugacpusztaháza site is more closed (Molnár 2003), where woody species are more abundant, providing substratum, shade, humidity, humus or other advantages over these harsh conditions for wood-correlated species. Some species were detected only here, such as the wood-correlated species, or the *Peltigera* species and *C. chlorophaea* appearing only at later successional stages (Gallé 1973; Jun & Rozé 2005). In contrast, other species seem to prefer the more open habitats with scarce vegetation, since *Psora decipiens*, *Gyalolechia fulgens* and *Squamarina lentigera* were found only at the Fülöpháza site.

Comparison with communities from other European countries

The number of terricolous lichen taxa found on the dunes of the investigated sites (Bugacpusztaháza 20; Fülöpháza 18; overall 22 species) is slightly lower than those reported from other European countries (Denmark 23 (Schmidt 1986); Poland 29 (Adamska 2010); Netherlands 34 (Ketner-Oostra *et al*. 2012); Estonia 28 (Jüriado *et al*. 2016); Italy 33 (Gheza *et al*. 2020)). In line with those countries, half of the species recorded in these habitats in Hungary belong to the genus *Cladonia*.

The communities found in the present study differed from communities presented in dunelands of other European countries mainly because of the characteristics of the soil (sand) and vascular plant vegetation. The sandy soil of the Kiskunság region (and most of the Hungarian sandy soils) is calcium-rich, contains more nutrients because of the extended grazing (Molnár 2003) and hosts different lichen associations compared to most of the cited study sites that are acidic and poor in nutrients (e.g. Ketner-Oostra *et al*. 2012). Limited research has focused on the exploration of lichen associations in calcareous sandy grasslands between inland dunes (Jüriado *et al*. 2016; Gheza *et* *al*. 2020), therefore our data contribute significantly to the knowledge of the lichen communities of these habitats. *Enchylium tenax*, *Gyalolechia fulgens*, *Psora decipiens* and *Thalloidima sedifolium* (Scop.) Kistenich *et al*., very similar to *Thalloidima physaroides*, were also detected by Gheza *et al*. (2020) in Italian calcareous grasslands. Even though most of the cited studies were performed on lichen associations of acidophilous sand, several species overlap with the current research. *Cladonia chlorophaea*, *C. fimbriata*, *C. foliacea*, *C. furcata*, *C. glauca*, *C. pyxidata*, *C. rangiformis*, *C. subulata*, *C. rei*, *Diploschistes muscorum*, *Peltigera canina* and *P. rufescens* are also present in Denmark, Estonia, Italy, Netherlands or Poland. *Cladonia foliacea*, *C. furcata*, *C. rangiformis*, *C. pyxidata* and *Diploschistes muscorum* were the most frequent species in the present study. These species have a broad ecological tolerance and can be found on both acidic and calcareous soils, and in arid as well as humid microhabitats. Besides the Hungarian record, *C. coniocraea* was detected only on Estonian dunes.

Five of the lichen species recorded in the study area are listed on the Red List of lichens as occurring in Hungary and lacking in other mentioned European areas. *Cladonia magyarica*, *Xanthoparmelia pulvinaris* and *X. subdiffluens* have been protected since 2005, while *X. pokornyi* and *X. ryssolea* since 2013 (Farkas & Lőkös 2006; [Hungarian] Ministry of Rural Development 2013). The four *Xanthoparmelia* species were found only on arid dune sides, and their thalli were more abundant at Fülöpháza in the present study.

Conservation implications

From a conservation perspective, our results indicate that lichens could significantly contribute to the biodiversity of dry calcareous grasslands due to their heterogeneous community structure. In relevés where the cover of bare soil was higher (due to previous, low-intensity wildlife disturbances, e.g. trampling or rooting) the number of lichen species was lower, therefore activities resulting in more open surfaces (trampling, removal of vegetation etc.; Belnap & Eldridge 2001) should be reduced to avoid species loss. However, low-intensity grazing can lead to a higher terricolous lichen species richness (Boch *et al*. 2016), as was exhibited at the Fülöpháza site. Since the closing vegetation at Bugacpusztaháza is at an advanced stage towards the climax association, the territory can provide a less favourable environment for the steppe species moved by wind or animals.

It was also pointed out that arid and humid dune sides host special lichen communities (nine species occurred only on arid and another nine only on humid sides), unambiguously influenced by site. Disturbances in the past, which have been greater at Fülöpháza, might be required to maintain open dune habitat facilitating establishment and growth of the endangered and protected species (*Xanthoparmelia ryssolea, X. subdiffluens*) and of the characteristic species of the *Fulgensietum fulgentis* association (*Gyalolechia fulgens, Psora decipiens*, *Squamarina lentigera*). Our results indicate that the further progression to climax vegetation shown at Bugacpusztaháza might eliminate those endangered species that should be protected. Nevertheless, the wood-correlated species were more abundant at the Bugacpusztaháza site, and they preferred humid dune sides, increasing species diversity. Determining the various needs of the different species provides further proof for the importance of habitat diversity.

Another threat for the cryptogamic communities is the introduction of non-native annual plant species (e.g. *Asclepias syriaca* L. in the studied regions). The probability of the adverse effects of biological invasions in these habitats is higher after soil disturbances when bare soil areas are created and, in general, these effects are more pronounced in the case of crustose species (Belnap & Eldridge 2001).

We found that past disturbances can play a significant role in the species composition of terricolous lichen communities in inland duneland ecosystems and may result in an alteration in lichen species richness. The ability to compete with herbs and mosses may also be a crucial factor that determines the occurrence of different terricolous lichen species.

From a conservation and restoration point of view, these results could contribute to the development of conservation recommendations for achieving a more diverse terricolous lichen community (and therefore BSC community). In setting the 'target state' of an ecosystem, it is essential to determine the past disturbance history of the region and also be aware of the present state.

Conclusions

It was revealed, as a proxy for local species pool and land-use history, that the effect of site was stronger on terricolous lichen community composition than was the impact of dune aspect, and the effect of the side was distinct at the two localities, probably due to the different disturbance history and successional stage of the vegetation under the same climate. The dune aspect leads to distinct microclimatic patterns that induce differences in soil surface variables (especially those which are connected to the herbaceous layer) and lichen species composition. At sites with more open vegetation, cover of the fruticose growth form and herbs, as well as their height, decreased with aridity, unlike the cover of moss. Therefore, we can postulate that as a result of climate change (drier and warmer conditions are projected in this region), lichens can gain more space due to the shrinkage of vascular plant vegetation (Kröel-Dulay *et al*. 2018). However, lichens have to compete with mosses for these free surfaces. These changes will supposedly lead to higher cryptogamic biodiversity. On the other hand, species preferring arid microhabitats could appear, leading to a higher terricolous lichen diversity. Conversely, in microhabitats which might become more humid or where grazing is abandoned, lichen communities can be suppressed due to the closing vegetation. Some lichen species were found only on arid dune sides or more open sites (e.g. the protected steppe species or the key species of the *Fulgensietum fulgentis* association), while others occurred only on humid dune sides or on the site hosting more closed vegetation and trees (e.g. the wood-correlated species), therefore it is important to protect and maintain a diverse topography and landscape structure hosting various lichen communities when considering habitat-specific conservation and monitoring (Gheza *et al*. 2020) in inland dunelands. It is worth considering that the different species forming duneland communities have various microenvironmental needs and are very sensitive to different environmental factors.

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Author ORCIDs Katalin Veres, 0000-0001-5818-4891; Zsolt Csintalan, 0000-0001-7744-3393; Bence Kovács, 0000-0002-8045-8489; Edit Farkas, 0000-0002-5245-1079.

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Supplementary Material

Factors at multiple scale influence the composition of terricolous lichen communities in temperate semi-arid sandy grasslands

Katalin Veres[1], Zsolt Csintalan[2], Bence Kovács[1], Edit Farkas[1]

[1]Institute of Ecology and Botany, Centre for Ecological Research, H-2163 Vácrátót, Alkotmány u. 2–4, Hungary

[2]Department of Plant Physiology and Ecology, Institute of Biological Sciences, Hungarian University of Agriculture and Life Sciences, H-2100 Gödöllő, Páter K. u. 1, Hungary

Section 1: Additional information about the non-metric multidimensional scaling

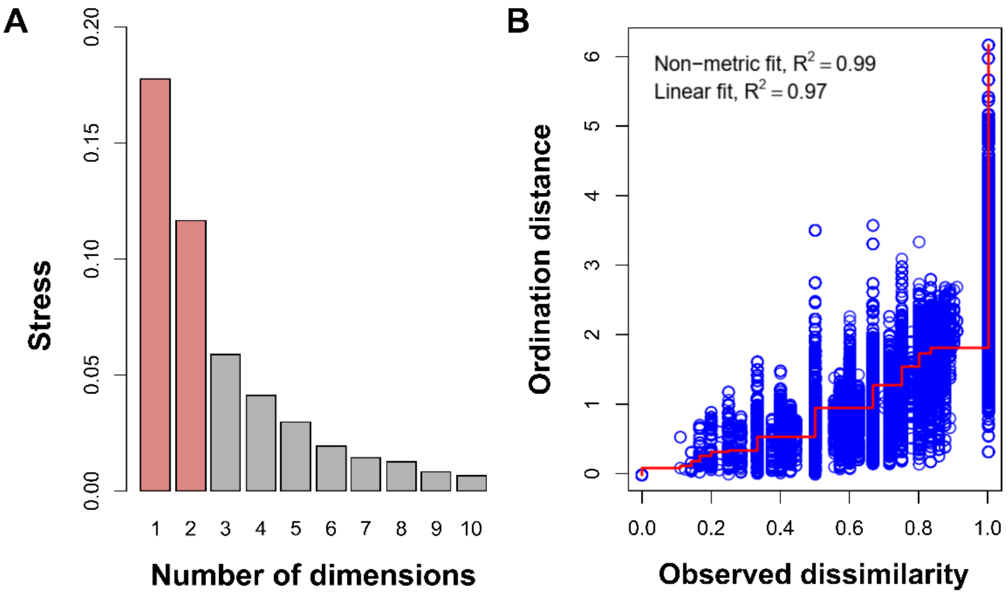

Fig. S1. A) Scree plot displaying the stress as a function of dimensionality; red shading highlights the first two dimensions where stress decreased substantially. B) Shepard diagram showing the relationship between the original distances of objects (from the original dissimilarity matrix) and the ordination distances; two correlation-like statistics on the goodness-of-fit (non-metric and metric fit) are also displayed in the plot. The fit between the observed dissimilarities and ordination distances is indicated by a red monotonic step line.

The unconstrained ordination method, non-metric multidimensional scaling (NMDS), iteratively arranges the objects (points) in a small and predefined number of dimensions (i.e. axes) so that the rank order of ordination distances is as close to the rank order of the original distances of the objects as possible (Podani 2000, McCune & Grace 2002, Legendre & Legendre 2012). The rank order of original distances and the rank order of the distances in the reduced-space configuration is compared by monotone regression, and the goodness-of-fit of the configurations is quantified as stress. Stress is the sum of squared differences between ordination-based distances and the distances predicted by the regression.

The number of dimensions of NMDS is arbitrary and is set *a priori* by the users, but dimensionality has a high impact on the stability of the solution. In general, stress decreases as dimensionality increases; however, there is a trade-off between dimensionality and interpretability. Stress-reduction does not necessarily mean to maximise interpretation capability, and low-dimensional projections (two or three) are often easier to interpret and are so preferable for interpretation issues. Therefore, it is important to find a compromise between a summary of the data (information) and an accurate representation of the original distances. As for example, Podani (2000) or Legendre and Legendre (2012) suggest, it is useful to plot and examine the relationship between stress and the number of dimensions in order to determine the 'optimal' number of dimensions. With such scree plots, the decrease of stress can be inspected as dimensionality increases, and for the final solution, the dimensionality where the change in stress considered as 'small' can be applied. Additionally, several rules of thumbs can be found (e.g. McCune & Grace 2002) using stress value – besides goodness-of-fit of the NMDS ordination – as a simplistic measure of its appropriate dimensionality.

To support our decision regarding the number of dimensions visually, we executed global NMDSs (based on the Jaccard dissimilarity matrix) from random sets of 30 starting configurations with several different dimensions (1–10) to extract their lowest stress. Stress values were plotted as the function of dimensionality (Fig. S1A). Since stress decreased substantially by the first two dimensions (coloured red) and while adding subsequent dimensions produce smaller changes, the two-dimensional configuration was chosen ($0.1 < \text{stress} < 0.2$, which indicates a fair solution for ecological data). Moreover, for illustration purposes, the 2D solution is also ideal.

Besides stress value, Shepard diagram is often used for the evaluation of a NMDS configuration. This plot displays ordination distances and a monotone or linear fit line against original dissimilarities (Podani 2000, Borcard *et al*. 2018). Two often-used correlation-like statistics can be computed: the non-metric fit is based on stress (S) and is defined as $R^2 = 1 - S \times S$ (Oksanen *et al*. 2019), while the 'linear fit' is the squared correlation between fitted values and ordination distances. As it can be seen in Fig. S1B, the final 2D NMDS solution in our data set (Fig. S1A) had a sufficient goodness-of-fit (non-metric fit: $R^2 = 0.99$; linear fit: $R^2 = 0.97$), thus can be accepted.

To fulfil the recommendations of McCune & Grace (2002) and estimate proportion of variation explained by individual NMDS dimensions, Mantel tests were used, and the squared correlations of Euclidean distance matrices of the constructed axes with the original community matrix were calculated.

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Section 2: Detailed model descriptions

In this study, lichen data and environmental variables were recorded on the micro-quadrat level (N = 1000 in total) that formed quadrats (N = 10) across the studied sites (Bugacpusztaháza and Fülöpháza) and dune sides (arid or humid). For the analyses, we used this micro-quadrat-level data, but quadrats as individual sampling units were also taken into account. Quadrat was treated as a random factor. Since micro-quadrats were nested within quadrats and the distributions of most of the studied variables were highly skewed, generalised mixed-effects models (GLMMs) – random intercept models – were used (Bolker *et al*. 2009, Dunn and Smyth 2018, Zuur *et al*. 2009). For modelling, mainly the *lme4* package (Bates *et al*. 2015) was used, except for the Tweedie models, which were built using *glmmTMB* (Brooks *et al*. 2017) with *tweedie* (Dunn 2017). Variance homogeneity and distribution were checked by *DHARMa* package (Hartig 2018).

(i) The effects of dune sides (factor with two values), site (factor with two values) and their interaction were tested within each GLMMs on the species richness and the cover of the three focal functional groups (crustose, fruticose and foliose). Species number data (counts) was analysed by Poisson GLMM, while for cover data (positive continuous with many exact zeros), Tweedie GLMMs were created (Dunn and Smyth 2018). Contrasts between sites and

dune sides within sites were tested by estimated marginal means using *emmeans* package (Lenth 2021).

(ii) The effects of dune sides and sites on the cover of bare soil, bryophytes, litter, vascular plants and on the average height of vascular plants were also studied by individual models. Moss cover was analysed using Beta GLMM, the bare soil cover was evaluated by Tweedie GLMM, while the other three variables were modelled by Gamma GLMMs. Contrasts between sites and dune sides within sites were tested by estimated marginal means using *emmeans*.

(iii) Responses of the most abundant lichen species and species groups based on the logistic generalised linear regression models (link function of the binomial GLMMs are also noted). We did not apply model selection to find minimum adequate models by selection procedures (e.g. using AIC or based on the F statistics), but the effects of all tested potential variables were tested and presented. Prior to the analyses, explanatory variables were scaled (*z*-score standardisation) because of the different ranges of the cover and height data. The models' goodness-of-fit values were measured by likelihood-ratio tests based on the conditional and marginal coefficient of determination (R^2_c and $R^2_{m,}$ respectively; Bartoń 2020). The explanatory power of the fix factors was evaluated by the analysis of deviance (Chi^2 and P values; Zuur *et al*. 2009), their effects were assessed by the likelihood-ratio based Wald's Chi^2 tests by *car* package (Fox & Weisberg 2019). Additionally, the accuracy of the constructed models was characterised by Receiver Operator Characteristic (ROC) plots with the calculated Area Under Curve (AUC) values (Fielding and Bell 1997).

Model summaries of the generalized mixed-effects models used for Fig. 3.

Table S1.1. Type II Analysis of Deviance Table (Wald's Chi^2 tests). Df = degrees of freedom based on Satterthwaite's approximation; *P* = *P*-value.

Response variable	Family and link function	site (Df = 1)		dune side (Df = 1)		site : dune site (Df = 1)	
		Chi^2	*P*	Chi^2	*P*	Chi^2	*P*
Species number	Poisson with log link	12.574	0.0004	0.797	0.3719	16.326	< 0.0001
Crustose cover (%)	Tweedie with log link	0.272	0.6020	1.883	0.1700	1.887	0.1695
Foliose cover (%)	Tweedie with log link	10.702	0.0011	0.772	0.3797	2.938	0.0865
Fruticose cover (%)	Tweedie with log link	1.068	0.3015	3.599	0.0578	1.308	0.2528

Table S1.2. Estimated marginal means and group contrasts using GLMMs in Table S1.1: 'site' – comparing Bugac-pusztaháza (BU) and Fülöpháza (FH) means; 'dune side within sites' – comparing arid (a) and humid (h) dunes sides using site as stratum. Abbreviations: EMM (S.E.) – estimated marginal means and standard error; Df = degrees of freedom based on Satterthwaite's approximation; *t* = *t*-score of the test statistic; *P* = *P*-value.

Model	Site					Dune side within sites									
	BU	FH	Df	*t*	*P*	BUa	BUh	Df	*t*	*P*	FHa	FHh	Df	*t*	*P*
	EMM (S.E.)	EMM (S.E.)				EMM (S.E.)	EMM (S.E.)				EMM (S.E.)	EMM (S.E.)			
Species number	1.37 (0.13)	2.32 (0.27)	994	-3.502	0.0005	1.14 (0.16)	1.65 (0.22)	994	-1.909	0.0562	3.55 (0.57)	1.52 (0.25)	994	3.671	0.0002
Crustose cover (%)	0.059 (0.07)	0.126 (0.19)	994	-0.389	0.6974	0.082 (0.14)	0.043 (0.08)	994	0.264	0.7917	2.59 (5.23)	0.01 (0.02)	994	1.931	0.0537
Foliose cover (%)	0.713 (0.33)	7.371 (4.03)	994	-3.280	0.0011	0.318 (0.21)	1.602 (1.02)	994	-1.773	0.0766	11.123 (8.57)	4.884 (3.79)	994	0.753	0.4518
Fruticose cover (%)	9.92 (2.53)	15.01 (4.68)	994	-1.026	0.3049	8.21 (2.97)	12.00 (4.33)	994	-0.743	0.4577	7.83 (3.47)	28.76 (12.6)	994	-2.087	0.0371

Table S1.3. Model output of the GLMMs. Abbreviations: Estimate = intercept (for the 'Intercept' term) and slope values of the tested variables; S.E. = standard error; *t* = *t*-score of the test statistic; *P* = *P*-value. For each of the tested categorical variables (site and dune side), one level was used to create the baseline and absorbed into the term 'Intercept'; therefore, site ('FH') and dune side ('H') refer to the slopes of the remaining levels of these factors ('FH' = Fülöpháza, 'H' = humid).

Response variable	Explanatory variables	Estimate	S.E.	t	*P*	Family and link function
Species number	Intercept	0.1303	0.1384	0.942	0.3464	Poisson with log link
	Site ('FH')	1.1362	0.2119	5.363	< 0.0001	
	Dune side ('H')	0.3692	0.1934	1.909	0.0562	
	Site ('FH'):Dune side ('H')	-1.2173	0.3013	-4.041	0.0001	
Crustose cover (%)	Intercept	-2.506	1.679	-1.493	0.1360	Tweedie with log link
	Site ('FH')	3.456	2.638	1.310	0.1900	
	Dune side ('H')	-0.653	2.471	-0.264	0.7920	
	Site ('FH'):Dune side ('H')	-5.388	3.922	-1.374	0.1700	
Foliose cover (%)	Intercept	-1.147	0.652	-1.759	0.0786	Tweedie with log link
	Site ('FH')	3.556	1.009	3.522	0.0004	
	Dune side ('H')	1.618	0.913	1.773	0.0763	
	Site ('FH'):Dune side ('H')	-2.441	1.424	-1.714	0.0865	
Fruticose cover (%)	Intercept	2.105	0.362	5.822	< 0.0001	Tweedie with log link
	Site ('FH')	-0.047	0.572	-0.083	0.9340	
	Dune side ('H')	0.379	0.511	0.743	0.4580	
	Site ('FH'):Dune side ('H')	0.922	0.806	1.144	0.2530	

Model summaries of the generalized mixed-effects models used for Fig. 4.

Table S2.1. Type II Analysis of Deviance table (Wald's Chi^2 tests). Df = degrees of freedom based on Satterthwaite's approximation; *P* = *P*-value.

Response variable	Family and link function	site (Df = 1)		dune side (Df = 1)		site : dune site (Df = 1)	
		Chi^2	*P*	Chi^2	*P*	Chi^2	*P*
Bare soil cover (%)	Tweedie with log link	14.734	0.0001	3.994	0.0470	9.062	0.0026
Moss cover (%)	Beta with probit link	0.162	0.6874	6.808	0.0091	1.355	0.2445
Litter cover (%)	Gamma with log link	0.035	0.8512	1.076	0.3001	1.322	0.2503
Herb cover (%)	Gamma with log link	2.159	0.1417	5.276	0.0216	2.386	0.1224
Herb height (mm)	Gamma with log link	0.249	0.6182	42.268	< 0.0001	11.124	0.0009

Table S2.2. Estimated marginal means and group contrasts using GLMMs in Table S2.1: 'site' – comparing Bugac-pusztaháza (BU) and Fülöpháza (FH) means; 'dune side within sites' – comparing arid (a) and humid dunes (h) sides using site as stratum. Abbreviations: EMM (S.E.) – estimated marginal means and standard error; Df = degrees of freedom based on Satterthwaite's approximation; *t* = *t*-score of the test statistic; *P* = *P*-value.

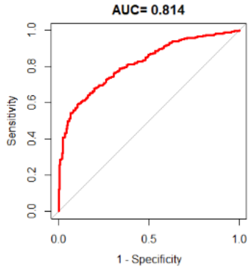
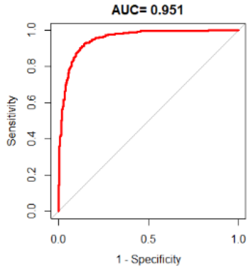
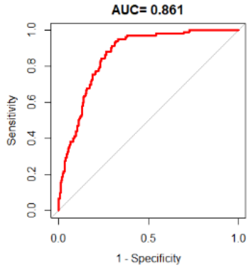
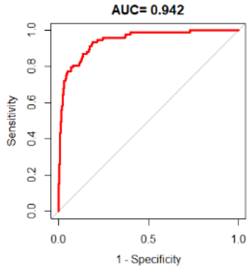
Model	Site					Dune side within sites									
	BU	FH	Df	*t*	*P*	BUa	BUh	Df	*t*	*P*	FHa	FHh	Df	*t*	*P*
	EMM (S.E.)	EMM (S.E.)				EMM (S.E.)	EMM (S.E.)				EMM (S.E.)	EMM (S.E.)			
Bare soil cover (%)	7.293 (2.55)	0.689 (0.32)	994	4.075	<0.0001	6.736 (3.32)	7.897 (3.89)	994	-0.228	0.8198	3.638 (2.22)	0.131 (0.09)	994	3.599	0.0003
Moss cover (%)	50.8 (0.04)	48.2 (0.05)	994	0.402	0.6877	56.0 (0.06)	34.7 (0.57)	994	1.287	0.1984	60.7 (0.07)	35.9 (0.07)	994	2.552	0.0109
Litter cover (%)	14.8 (2.86)	14.0 (3.31)	994	0.188	0.8512	14.57 (3.99)	15.00 (4.11)	994	-0.076	0.9396	9.68 (3.24)	20.13 (6.75)	994	-1.546	0.1225

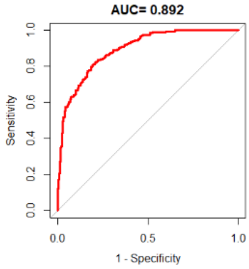
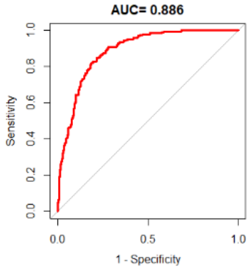
Herb cover (%)	11.04 (1.46)	8.12 (1.32)	994	1.469	0.1422	9.93 (1.86)	12.28 (2.30)	994	-0.802	0.4227	5.28 (1.21)	12.47 (2.86)	994	-2.649	0.0082
Herb height (mm)	3.96 (0.35)	3.69 (0.40)	994	0.499	0.6180	3.05 (0.39)	5.14 (0.65)	994	-2.927	0.0034	1.77 (0.27)	7.66 (1.18)	994	-6.696	<0.0001

Table S2.3. Model output of the GLMMs. Abbreviations: Estimate = intercept (for the 'Intercept' term) and slope values of the tested variables; S.E. = standard error; *t* = *t*-score of the test statistic; *P* = *P*-value. For each of the tested categorical variables (site and dune side), one level was used to create the baseline and absorbed into the term 'Intercept'; therefore, site ('FH') and dune side ('H') refer to the slopes of the remaining levels of these factors ('FH' = Fülöpháza, 'H' = humid).

Response variable	Explanatory variables	Estimate	S.E.	*t*	*P*	Family and link function
Bare soil cover (%)	Intercept	1.907	0.494	3.863	0.0001	Tweedie with log link
	Site ('FH')	-0.616	0.784	-0.786	0.4321	
	Dune side ('H')	0.159	0.698	0.228	0.8198	
	Site ('FH'):Dune side ('H')	-3.486	1.158	-3.010	0.0026	
Moss cover (%)	Intercept	0.150	0.143	1.045	0.2960	Beta with probit link
	Site ('FH')	0.122	0.227	0.539	0.5900	
	Dune side ('H')	-0.261	0.203	-1.287	0.1980	
	Site ('FH'):Dune side ('H')	-0.373	0.321	-1.164	0.2450	
Litter cover (%)	Intercept	2.679	0.274	9.788	<0.0001	Gamma with log link
	Site ('FH')	-0.409	0.433	-0.945	0.3440	
	Dune side ('H')	0.029	0.387	0.076	0.9400	
	Site ('FH'):Dune side ('H')	0.703	0.612	1.149	0.2500	
Herb cover (%)	Intercept	2.295	0.187	12.255	<0.0001	Gamma with log link
	Site ('FH')	-0.631	0.296	-2.131	0.0331	
	Dune side ('H')	0.213	0.265	0.802	0.4225	
	Site ('FH'):Dune side ('H')	0.647	0.419	1.545	0.1224	
Herb height (mm)	Intercept	1.115	0.126	8.831	<0.0001	Gamma with log link
	Site ('FH')	-0.541	0.200	-2.711	0.0067	
	Dune side ('H')	0.522	0.178	2.927	0.0034	
	Site ('FH'):Dune side ('H')	0.941	0.282	3.335	0.0009	

Table S3. Responses of the most abundant lichen species and species groups based on the logistic generalised linear regression models (link function of the GLMMs are also noted). Estimates (intercept and slopes), standard errors (S.E.) and test statistics for all variables are presented in the table. The models' goodness-of-fit values were measured by likelihood-ratio tests based on the conditional and marginal coefficient of determination (R^2_c and $R^2_{m,}$ respectively). The explanatory power of the fix factors were evaluated by analysis of deviance (Chi^2 and *P*-values). Additionally, receiver operating characteristic (ROC) curves were created as a graphical diagnostics of the constructed logostic GLMMs depicting the relation between sensitivity (or true positive rate) 1 – specificity (or false positive rate). AUC: Area Under Curve values. Df_{num} = numerator degrees of freedom; Df_{den} = denumerator degrees of freedom; *t* = *t*-score of the test statistic; *P* = *P*-value. For each of the tested categorical variables (site and dune side), one level was used to create the baseline and absorbed into the term 'Intercept'; therefore, site ('FH') and dune side ('H') refer to the slopes of the remaining levels of these factors ('FH' = Fülöpháza, 'H' = humid).

Explanatory variables	Estimate	S.E.	*t*	*P*	ROC
Cladonia magyarica / pyxidata (logit link)			*(Df_{num}: 7, Df_{den}: 801, Chi^2: 56.662, P: < 0.001, R^2_c: 0.426, R^2_m: 0.299)*		
Intercept	1.843	0.480	3.835	0.0001	
Site ('FH')	1.708	0.603	2.834	0.0046	
Dune side ('H')	-1.566	0.610	-2.568	0.0102	
Moss cover (%)	-0.695	0.155	-4.491	< 0.0001	
Herb cover (%)	-0.638	0.115	-5.542	< 0.0001	
Litter cover (%)	-0.530	0.121	-4.375	0.0000	
Bare soil cover (%)	-0.528	0.136	-3.876	0.0001	
Herb height (mm)	0.161	0.112	1.437	0.1509	
Cladonia foliacea (logit link)			*(Df_{num}: 7, Df_{den}: 262, Chi^2: 30.723, P: < 0.001, R^2_c: 0.521, R^2_m: 0.451)*		
Intercept	-3.597	0.974	-3.694	0.0002	
Site ('FH')	-1.624	1.161	-1.399	0.1619	
Dune side ('H')	4.714	1.179	3.998	0.0001	
Moss cover (%)	-0.361	0.154	-2.344	0.0191	
Herb cover (%)	0.053	0.171	0.308	0.7578	
Litter cover (%)	-0.145	0.151	-0.961	0.3366	
Bare soil cover (%)	-0.206	0.171	-1.200	0.2301	
Herb hight (mm)	-0.510	0.147	-3.463	0.0005	
Cladonia furcata / rangiformis (probit link)			*(Df_{num}: 7, Df_{den}: 94, Chi^2: 16.278, P: 0.023, R^2_c: 0.483, R^2_m: 0.396)*		
Intercept	-1.701	0.273	-6.238	< 0.0001	
Site ('FH')	-1.106	0.344	-3.218	0.0013	
Dune side ('H')	1.100	0.336	3.274	0.0011	
Moss cover (%)	-0.127	0.086	-1.487	0.1370	
Herb cover (%)	-0.044	0.086	-0.516	0.6061	
Litter cover (%)	-0.016	0.074	-0.219	0.8266	
Bare soil cover (%)	-0.280	0.150	-1.867	0.0620	
Herb height (mm)	-0.092	0.105	-0.879	0.3792	
Xanthoparmelia pokornyi / ryssolea (log-log link)			*(Df_{num}: 7, Df_{den}: 85, Chi^2: 14.788, P: 0.039, R^2_c: 0.764, R^2_m: 0.440)*		
Intercept	-3.004	0.887	-3.387	0.0007	
Site ('FH')	-3.371	1.235	-2.730	0.0063	
Dune side ('H')	0.922	1.199	0.769	0.4418	
Moss cover (%)	-0.371	0.173	-2.141	0.0323	
Herb cover (%)	0.229	0.184	1.244	0.2134	
Litter cover (%)	-0.350	0.211	-1.660	0.0969	
Bare soil cover (%)	-0.076	0.182	-0.418	0.6757	
Herb height (mm)	-0.133	0.206	-0.645	0.5192	

Explanatory variables	**Estimate**	**S.E.**	***t***	***P***	**ROC**
Diploschistes muscorum (logit link)			*(Df_{num}: 7, Df_{den}: 191, Chi^2: 14.477, P: 0.043, R^2_c: 0.638, R^2_m: 0.157)*		
Intercept	-1.827	1.117	-1.635	0.1020	
Site ('FH')	-2.103	1.401	-1.501	0.1333	
Dune side ('H')	0.782	1.412	0.554	0.5795	
Moss cover (%)	-0.469	0.142	-3.299	0.0010	
Herb cover (%)	-0.152	0.140	-1.091	0.2753	
Litter cover (%)	-0.086	0.118	-0.730	0.4653	
Bare soil cover (%)	-0.244	0.119	-2.044	0.0410	
Herb height (mm)	-0.140	0.159	-0.885	0.3764	
Enchylium tenax (logit link)			*(Df_{num}: 7, Df_{den}: 182, Chi^2: 38.087, P: < 0.001, R^2_c: 0.987, R^2_m: 0.018)*		
Intercept	-3.763	0.996	-3.778	0.0002	
Site ('FH')	0.323	1.166	0.277	0.7820	
Dune side ('H')	2.282	1.183	1.929	0.0537	
Moss cover (%)	0.313	0.131	2.396	0.0166	
Herb cover (%)	-0.332	0.181	-1.839	0.0660	
Litter cover (%)	-0.307	0.142	-2.166	0.0303	
Bare soil cover (%)	-0.010	0.135	-0.077	0.9388	
Herb height (mm)	-0.329	0.194	-1.695	0.0900	

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12.3 S.3. Article 2: The bright and shaded side of duneland life: the photosynthetic response of lichens to seasonal changes is species-specific

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The bright and shaded side of duneland life: the photosynthetic response of lichens to seasonal changes is species-specific

Katalin Veres[1]*, Edit Farkas[1], †Zsolt Csintalan[2]

[1]Institute of Ecology and Botany, MTA Centre for Ecological Research, H-2163 Vácrátót, Alkotmány u. 2–4, Hungary

[2]Institute of Botany and Ecophysiology, Szent István University, H-2100 Gödöllő, Páter K. u. 1, Hungary

*Corresponding author. Tel: + 36 28 360 122; +36 20 2316411; Fax: +36 28 360 110

E-mail address: veres.katalin@okologia.mta.hu

Abstract

Terricolous lichens are relevant associates of biological soil crusts in arid and semiarid environments. Dunes are ecosystems of high conservation interest, because of their unique, vulnerable and threatened features. The function of lichens is affected by the changing seasons as well as different microhabitat conditions. At the same time, inland dunes are less investigated areas from the terricolous lichens point of view. We explored the effect of seasonal variation and different micro-environmental conditions (aspect) on the metabolic activity of five terricolous lichen species, representing various growth forms, in temperate semiarid grasslands. Populations of *Cladonia foliacea*, *C. furcata*, *C. pyxidata* group, *Diploschistes muscorum* and *Thalloidima physaroides* were investigated. Thalli sampled from the south-west and north-east facing microhabitats were studied by chlorophyll fluorescence analysis for two years. The present study aims to understand how changing climate (during the year) and aspect affect photosynthetic activity and photoprotection. Microclimatic data were also continuously recorded to reveal the background of the difference between microhabitat types. As a result, the air temperature, photosynthetically active radiation, soil temperature and vapour pressure deficit were significantly higher on south-west than on north-east facing microsites, where relative humidity and water content of soil proved to be considerably higher. Higher photosynthetic activity, as well as a higher level of photoprotection, was detected in lichens from north-east oriented microsites compared to south-west populations. In addition, the difference between sun and shade populations varied seasonally. Since a species-specific response to both aspect and season was detected, we propose to investigate more than one species of different growth forms, to reveal the response of lichens to the changing environment in space and time.

Keywords: terricolous lichens, aspect, microclimate, photosynthetic activity, photoprotection, temperate semiarid sandy grassland

Introduction

Dunes are dynamic and threatened ecosystems in temperate regions of Central-Europe. These habitats are characterised by extreme environmental conditions, where the majority of vascular plants are not able to colonize the available substrates because of their sensitivity (Belnap & Lange 2003). Terricolous lichen associations are more abundant because lichens are able to adapt to these harsh and complex conditions in different ways (e.g. Armstrong 2017). Among other factors, microtopography plays an essential role in controlling the lichen environment as it affects the overall microclimate between dunes (Tilk *et al*. 2018). Thalli derived from sun and shade populations of the same lichen species may differ in several aspects, for example in thallus anatomy (e.g. Atala *et al*. 2015; Dietz *et al*. 2000; Pintado *et al*. 2005), pigment composition (e.g. Färber *et al*. 2014; Gauslaa & Solhaug 2004) and physiological functioning. The photosynthetic activity (Fv/Fm) can be lower in sun than in shade lichen populations (e.g. Gauslaa *et al*. 2006; Pirintsos *et al*. 2011) or in thalli exposed to sudden changes in light conditions, especially in smaller thalli (Bianchi *et al*. 2019a).

Besides the long-term effect of different microhabitat conditions, seasonal variation of climate may also cause significant changes in thallus anatomy (e.g. Holopainen 1982; Tretiach *et al*. 2013) and in photosynthetic functioning (Lange & Green 2003). Schroeter *et al*. (2011) showed that in Antarctic vegetation, the Fv/Fm of *Umbilicaria aprina* Nyl. was lower in winter compared to summer values. An inverse correlation was found in lichens living in submediterranean oak forests (e.g. Baruffo & Tretiach 2007). In southern Norwegian forests, a peak was observed in this parameter in late autumn and a minimum in spring in the case of *Xanthoria parietina* (L.) Th. Fr. Similarly, photoprotection (NPQ) also showed a seasonal pattern (Vráblíková *et al*. 2006). MacKenzie *et al*. (2002) measured a higher level of photoprotection in *Lobaria pulmonaria* (L.) Hoffm.

in spring than in late summer, but the opposite was observed by Vráblíková *et al*. (2006). These studies clearly indicate that lichens can adapt to changing environments in different ways. However, these investigations were carried out mostly on epiphytic, forest-living or saxicolous lichens. Terricolous lichens can cover large areas; however, there are only a few studies about how different microenvironmental conditions affect the seasonal changes of photosynthetic functioning of different species in semiarid (temperate zone) grasslands (e.g. Kalapos & Mázsa 2001; Lange & Green 2003). Different climatic conditions fundamentally affect the photosynthetic process of lichens; therefore, it is important to collect information from as many diverse areas and habitat types as possible.

The aim of the present study was to investigate the influence of both microhabitat conditions (aspect) and seasonal changes on photosynthetic activity and photoprotection of several terricolous lichen species. Species of various growth forms were selected because the water household capacity greatly depends on the morphology of these poikilohydric organisms (e.g. Larson 1981; Petruzzellis *et al*. 2018) and is closely correlated with metabolic functions (e.g. Büdel & Scheidegger 2008; Gauslaa 2014). We hypothesized that populations of the different species would differ in their responses to seasonal climatic changes, because of their special adaptation strategies. To investigate our hypothesis, chlorophyll *a* fluorescence kinetic measurements were applied, as an adequately sensitive but non-destructive method, for studying the response of photosynthetic organisms to environmental factors (Schreiber *et al*. 1994). From the measured data, Fv/Fm and NPQ were calculated as the most frequently used chlorophyll fluorescence variables in ecological investigations. The recorded micrometeorological data revealed the main differences between microhabitat types. This paper presents how terricolous lichen species of different growth forms were able to acclimate to seasonal changes in their photosynthetic activity and photoprotection under different microhabitat conditions.

Materials and methods

Study sites

Semiarid sandy grasslands (*Festucetum vaginatae danubiale* association Rapaics ex Soó 1929 em. Borhidi 1996) with many endemic lichens are native vegetation types of the Kiskunság region of Hungary (Gallé 1972–1973), where sandy dunes provide diverse lichen microhabitats. This region is mostly covered by calcareous sandy soil from the Pleistocene deposit of Danube River. The prevailing wind (from north-west to south-east), as a secondary effect, reshaped the landscape resulting in a dune land (Pécsi 1967) with dune sides of north-east (NE) and south-west (SW) aspects. The vegetation is dominated by *Festuca vaginata* W. et K., *Stipa borysthenica* Klokov, *Fumana procumbens* (Dun.) Gren. et Godr., *Alcanna tinctoria* (L.) Tausch, *Dianthus serotinus* W. et K., *Onosma arenaria* W. et K. and *Sedum hillebrandtii* Fenzl (Borhidi *et al*. 2012; Fekete 1997). The study area is characterised by a moderately continental climate with a submediterranean influence (Péczely 1967). Three dunes near Bugacpusztaháza (46° 42' 7.9" N, 19° 38' 8.3" E) were chosen for investigation in the first year. Unfortunately, heavy equipment traffic increased near the study site because of a fire event during the first year. Therefore, for the second year, two more dunes near Fülöpháza were chosen for the study (46° 53' 29" N, 19° 26' 35.6" E) with the same environmental characteristics (Fig 1). On each selected dune, the NE and the SW facing sides (henceforth microsites) were chosen for collection of lichen material.

Fig. 1 Location of the study sites in Hungary

Lichen material

Lichen samples were collected in the Kiskunság region, Central Hungary, in the territory of Kiskunság National Park, to exclude the effect of human activity as much as possible. On both the SW and NE slopes of the dunes in the sampling area, the following species have been collected (from the association): the large, squamulose *Cladonia foliacea* (Huds.) Willd.; the fruticose *Cladonia furcata* (Huds.) Schrad., the cup lichens *Cladonia magyarica* Vain. and *Cladonia pyxidata* (L.) Hoffm.; and the crustose lichens *Diploschistes muscorum* (Scop.) R. Sant. and *Thalloidima physaroides* (Opiz) Opiz. Among others, these six lichen species are also members of the "Bunte Erdflechten-Gesellschaft" (Gams 1938), also known as the *Fulgensietum fulgentis* association, typical for this region (Büdel 2001). Thalli were collected from populations living on both the SW and NE oriented microsites in 10 replicates per species, per aspect type, per microsite, in each of four seasons (Bugacpusztaháza: March, July and October of 2013, January of 2014;

Fülöpháza: July and November of 2014, February and May of 2015) when possible. Altogether almost 1200 specimens were measured during the investigation period. Due to the large number of the samples, specimens were collected at the same time, but measured later under nearly constant laboratory conditions. In the first year, some data are missing, especially from the summer period, because one of our investigation microsites was destroyed by heavy equipment/digger and lichen thalli were challenging to find and collect. After sample collection, thalli were transported to the laboratory where they dried out under semi-natural conditions (i.e. seasonal temperature, humidity, and light regime) (Csintalan *et al*. 1999). To avoid measuring the chlorophyll fluorescence of other photosynthesizing organisms living directly beside the lichen thalli, they were cleaned with special attention to these particles. Lichens were identified according to Smith *et al*. (2009), Verseghy (1994) and Wirth *et al*. (2013). A few days after collection, specimens were rehydrated by spraying with distilled water twice a day (in the morning and the afternoon) and kept under low light conditions (about 10 μmol m^{-2} s^{-1}) at seasonal ambient temperature for 1–2 days, until the photosynthetic system regenerated (i.e. until Fv/Fm became constant). In summer samples, this preparation process required more time (3 days) than for thalli collected in other seasons. After measurements were completed, voucher specimens were deposited in Lichen Herbarium VBI, Hungary.

Chlorophyll *a* fluorescence kinetic measurements

Only healthy thalline lobes (without any sign of parasites or lichenicolous fungus) were selected for our studies. Chlorophyll *a* fluorescence kinetics were measured on fully water-saturated lichen thalli after 30 minutes of dark adaptation at room temperature (T = 20–22 °C) using a portable pulse amplitude modulated fluorometer (FMS 2 Hansatech Instruments Ltd.; Modfluor software) as described in Jensen (2002). The maximum quantum yield of PSII photochemistry (Fv/Fm), and Stern-Volmer non-photochemical quenching (NPQ), were calculated according to Kitajima & Butler (1975) and Bilger & Björkman (1990). These are the most frequently used chlorophyll fluorescence variables in ecological investigations. Fv/Fm gives insight into the condition of the photosynthetic systems within the thalli, and shows how efficiently the photochemical reaction is proceeding. Similar to vascular plants, the photobiont algae of the lichen also protect themselves from the harmful effects of high light intensity, which cannot be used for photosynthesis (Demmig-Adams *et al*. 1989). The non-photochemical quenching represents the degree of these protective mechanisms (photoprotection) while photosynthesis is running.

Micrometeorological data collection

Two micrometeorological stations were placed at the Bugacpusztaháza site, on the opposite sides of a dune with NE and SW faces, in order to record changes of microclimate during the investigation period as well as to observe the main differences between the two microhabitat types. It was hypothesized that the conditions on the selected dune represent the prevailing conditions on other dunes of similar morphology and aspects found in the territory. For collecting micrometeorological data, two HOBO Micro Station H21-002 (Onset Computer Corporation, USA) were used with sensors of air temperature (T_{air}), relative humidity (RH), and photosynthetically active radiation (PAR) at 0.5 m height; and soil temperature (T_{soil}) and soil water content (WC_{soil}) at 5 cm deep in the sand. Data were continuously recorded each minute. Vapour pressure deficit was calculated from measured data as follows: $VPD = 0.6108*\exp(17.27*T_{air}\ (°C)/(T_{air}\ (°C)+237.3))*(1-RH/100)*10$. We also used meteorological and precipitation data derived from the meteorological station of Plant Ecology Research Group of Hungarian Academy of Sciences, Szent István University, which is located 4.2 km from the Bugacpusztaháza study site. The precipitation data were corrected by the locally recorded soil water content data. A meteorological station owned by the MTA Centre for Ecological Research, not far from the Fülöpháza sites (1.3 km), also recorded meteorological data. It provided information about environmental conditions and precipitation on the second study site. Micrometeorological data from the first spring are lacking, because data loggers were only installed after the first spring sampling. However, based on the spring data, recorded by the meteorological station of Plant Ecology Research Group, a rough estimation of environmental conditions before spring sampling was possible. Unfortunately, some micrometeorological data are missing because of an unexpected battery discharging, as well as damage to some wires by wild animals. Although a large amount of micrometeorological data were recorded, due to space restrictions, only the most relevant are presented here.

Statistical analysis

All statistical analyses were performed in the R statistical environment (R Core Team, 2018). Sufficient sample size for a correct statistical comparison from the first year was available only for the *C. pyxidata* group and *D. muscorum*. The values of Fv/Fm and NPQ fluorescence parameters (response variables) related to seasons and different microhabitat types (explanatory variables) were statistically evaluated by a linear model, followed by ANOVA to find differences between species. Because there were no significant differences in Fv/Fm and NPQ between the microsites with the same direction within one site and one season, data were handled as one group, and therefore, each SW dataset was compared to each NE data within one site and one season for each species. The two (SW, NE aspect) and four (seasons) levels of the two explanatory variables (Fv/Fm, NPQ) generated eight groups according to the combination of variables at each site (Bugacpusztaháza, Fülöpháza), where each group was compared to each group. The differences between these groups were analysed by a multiple comparison Tukey-test on least square means by sites using the *lsmeans* package (Lenth 2016) in the R environment (R Core Team, 2018). The Shapiro-Wilk test was applied to test the normality of the samples, and a pairwise t-test was performed to compare the means of micrometeorological data, taking the average of every 10 minutes between the SW and NE facing sides of the investigated dune (R Core Team, 2018). In case of seasonal means, the average of the month preceding sample collection was taken using the *dplyr* package (Wickham *et al*. 2018). For creating figures, the R environment was used.

Results

Micrometeorological data

Seasons. The values of micrometeorological parameters usually differed not only among seasons but also among years (Table 1). The environmental variables observed from 01. 06. 2013 to 01. 06. 2015 showed clear seasonal patterns. During summers, the daily mean T_{air} was between 21–22 °C and 35–39.5 °C maximum values were recorded; meanwhile, the daily mean RH was 67.1–70.2% depending on aspect. The mean PAR was 483–516 µmol m^{-2} s^{-1}, resulting in an average 23.6–26.6 °C soil temperature and a 2–9% average daily soil water content depending on aspect. The daily mean VPD reached relatively high values between 10.4–11.4 kPa. During the first summer, there were ten rainy days, with altogether 35.2 mm precipitation, with different intensities (light, intermediate, shower). In the second summer, the precipitation was 70.7 mm over ten rainy days, with varying intensity. During autumns, 8.1–12 °C mean daily T_{air} was recorded, and the air temperature dropped below 0 °C on 5 (1st year) and 6 (2nd year) days in this season. The daily mean RH was higher than in the previous season, between 75.7–86.9%, and the mean PAR was lower, 111–240 µmol m^{-2} s^{-1}. The mean daily T_{soil} was 8.8–15.8 °C, and 3.9–10.6% WC_{soil} was measured, depending on aspect. The daily mean vapour pressure deficit was 1.8–4.3 kPa. During the first autumn, there were 14 rainy days, with a total of 31.4 mm precipitation, with different intensities of the rain (light, intermediate). In the second autumn, the precipitation was 11.2 mm over seven rainy days, all as light rain.

During autumns, 8.1–12 °C mean daily T_{air} was recorded, and the air temperature dropped below 0 °C on 5 (1st year) and 6 (2nd year) days in this season. The daily mean RH was higher than in the previous season, between 75.7–86.9%, and the mean PAR was lower, 111–240 µmol

m^{-2} s^{-1}. The mean daily T_{soil} was 8.8–15.8 °C, and 3.9–10.6% WC_{soil} was measured, depending on aspect. The daily mean vapour pressure deficit was 1.8–4.3 kPa. During the first autumn, there were 14 rainy days, with a total of 31.4 mm precipitation, with different intensities of the rain (light, intermediate). In the second autumn, the precipitation was 11.2 mm over seven rainy days, all as light rain.

Table 1 The average values of T_{air}, RH, VPD, PAR, T_{soil}, WC_{soil}, and precipitation recorded in different seasons during a two year investigation period measured on the south-west (SW) and north-east (NE) facing microhabitats located on a dune. Mean of differences between SW and NE facing slopes in the present micrometeorological parameters during the whole measuring period are also presented. *differences significant between SW and NE facing dune side (Pairwise t-test, $p < 0.05$)

direction/ season	T_{air} [°C]	RH [%]	VPD [kPa]	PAR [µmol m^{-2} sec^{-1}]	T_{soil} [°C]	WC_{soil} [%]	precipitation [mm]
1st Year							
spring 1)	4.46	81.1	1.6	161	5.3	NA	78.4
summer NE	21.7	70.2	11.2	516	24.4	8	35.2
summer SW	21.8	69.9	11.4	527	26.6	2	
autumn NE	12	76.1	4.2	240	13.7	10.6	31.4
autumn SW	12.3	75.7	4.3	263	15.8	4.2	
winter NE	2.6	88.1	1.1	59.8	2.8	9.7	4.2
winter SW	2.7	88.4	1	66.8	3.5	3.1	
2nd Year							
spring NE	16.4	73.9	6.6	401	17.7	9.2	58
spring SW	16.5	73.2	6.7	363	20.5	3.4	
summer NE	20.9	67.7	10.4	483	23.6	9	70.7
summer SW	20.9	67.1	10.5	494	25.8	2.8	
autumn NE	8.1	86.6	1.8	111	8.8	10.3	11.2
autumn SW	8.2	86.9	1.8	121	10	3.9	
winter NE	2.4	86.5	1.1	76.8	1.7	12.9	69.6
winter SW	1.1	88	0.9	86.8	2.6	3.4	
mean of the differences	0.171*	-0.494*	0.169*	14.089*	2.047*	-6.28*	

Abbreviations: T_{air}–air temperature, RH–relative humidity, VPD–vapour pressure deficit, PAR–photosynthetically active radiation, T_{soil}–soil temperature, WC_{soil}–soil water content.

1) meteorological data from the meteorological station of the Plant Ecology Research Group of Hungarian Academy of Sciences, Szent István University near the investigation site

The winters were cold, averaging 1.1–2.7 °C T_{air}, and 4–22 days were recorded, when the temperature dropped below 0 °C depending on year and aspect. The mean daily RH was relatively high during this season (81–88%); meanwhile, the PAR remained relatively low (59.8–86.8 μmol m^{-2} s^{-1}), resulting in a moderate degree of soil warming (daily mean T_{soil}: 1.7–3.5 °C) depending on aspect. The water content of the soil was higher than recorded in summer and autumn, averaging 3.1–12.9% according to year and direction. The daily mean VPD was 0.9–1.1 kPa during winters. In the first winter, there were nine rainy/snowing days, with altogether 4.2 mm precipitation. During the second winter, the precipitation was 69.6 mm over 17 days.

In the first spring, the mean daily air temperature was 4.5 °C; additionally, 81.8% mean RH, 161 μmol m^{-2} s^{-1} PAR and 1.6 kPa VPD were recorded. The daily mean T_{soil} was 5.3 °C. During the first spring, there were 19 rainy days, with altogether 78.4 mm precipitation. In the second spring, the mean daily T_{air} was around 16.5 °C, although on one day the temperature dropped below 0 °C. The daily mean RH was between 73.2 and 79.9%; meanwhile, the mean PAR was 363 and 401 μmol m^{-2} s^{-1}, resulting in 17.7 and 20.5 °C daily mean T_{soil} and 3.4% and 9.2% WC_{soil} depending on aspect. The daily mean VPD was 6.6 kPa (NE) and 6.7 kPa (SW). In the second spring, the precipitation was 58 mm over 16 days with different rain intensities.

Aspect. The application of pairwise t-test showed that the means of micrometeorological data between the SW and NE facing sides of the investigated dune were significantly different for every parameter during the two-year measuring period (Table 1). The T_{air}, PAR (corrected with slope and orientation according to Olmo *et al.* 1999), T_{soil} and VPD values were significantly higher ($p < 0.001$) on the SW than on the NE oriented side, where the RH and WC_{soil} proved to be significantly higher ($p < 0.001$) at 95% confidence (Table 1). The most remarkable differences among the SW and NE sides were observed in T_{soil} (mean 60% higher on SW slope) and in WC_{soil} (mean 75% higher on NE slope). The daily fluctuation of differences for every micrometeorological parameter changed seasonally. The higher daily fluctuation was observed in summer, whereas on winter days a more balanced difference in light and humidity conditions was detected (except WC_{soil}, where the difference between the two microhabitat types was close to constant over each year) (Fig 2a–f). According to the results, the microhabitats situated on the SW sides of the dunes can be characterised as arid, whereas the NE oriented sides as less arid. Figure 2 shows the differences between the SW and NE facing dune sides in micrometeorological parameters changing during the day in different seasons (row data). The false colour scale indicates the degree and direction of difference between SW and NE facing dune sites. Red indicates that the value of the given parameter is higher on the SW than on the NE side, whereas the blue colour indicates that the value is lower on the SW than on the NE side. For example, the T_{soil} is higher on the NE than on the SW side of the dune in summer mornings. Then, in the afternoon, the T_{soil} was higher on the SW than on the opposite side of the dune. Interestingly, in winter, the difference between the SW and the NE side in T_{soil} is nearly constant during the day.

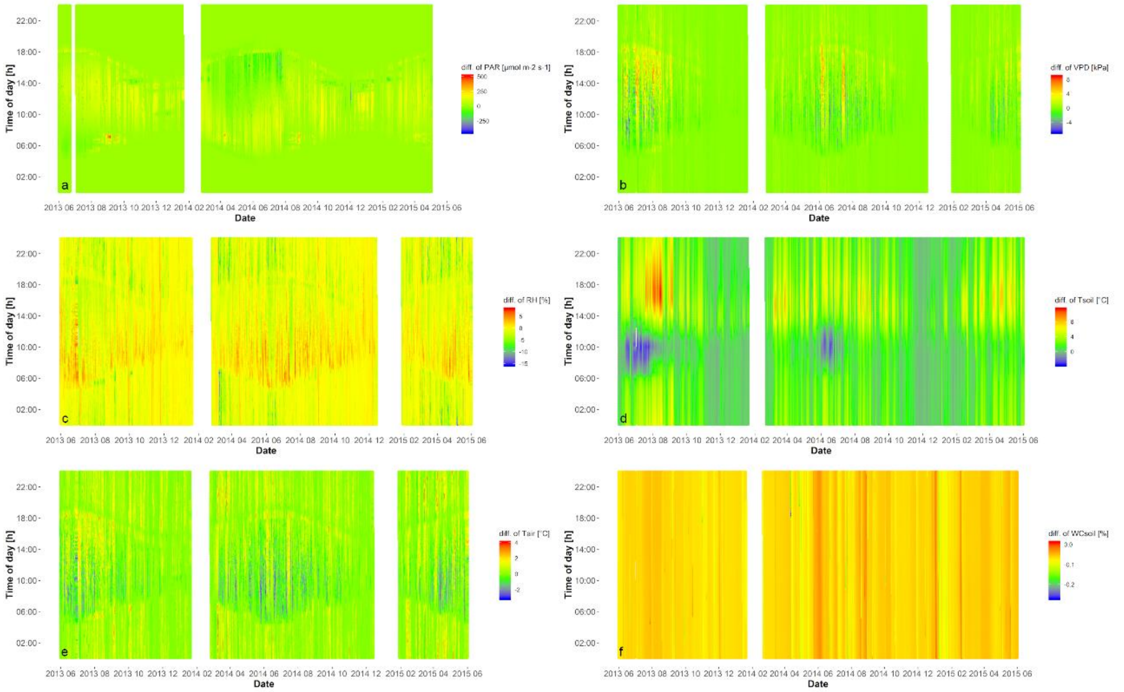

Fig. 2 a–f The extent and direction of difference between SW and NE side of a dune in micrometeorological parameters during days in different seasons (row data). During the two year investigation period scale (01. 06. 2013–01. 06. 2015) PAR (a), VPD (b), RH (c), T_{soil} (d), T_{air} (e), and WC_{soil} (f) are depicted. The Y-axis represents the time within a day (the hours of the day), meanwhile the X-axis the time within two years by date (months). The false colour scale indicates the degree and direction of difference between SW and NE facing dune sites. Red colour indicates higher values on the SW than on the NE side, meanwhile blue colour signs lower value on the SW than on the NE side

Chlorophyll *a* fluorescence parameters

The maximum quantum yield of photosystem II photochemistry (Fv/Fm)

Since we could not observe any significant differences (Fv/Fm $p = 0.576$; NPQ $p = 0.902$) in the physiological properties of the morphologically very similar *C. magyarica* and *C. pyxidata* (both represent the same growth form), we grouped them together as the "*C. pyxidata* group".

For all five different terricolous lichen species/species groups investigated, both seasonality and aspect had an effect on Fv/Fm ($p < 0.001$), as well as on NPQ (1st year season: $p < 0.001$, aspect: $p = 0.006$; 2ndyear $p < 0.001$ for both season and aspect), each year (Figs 3 and 4).

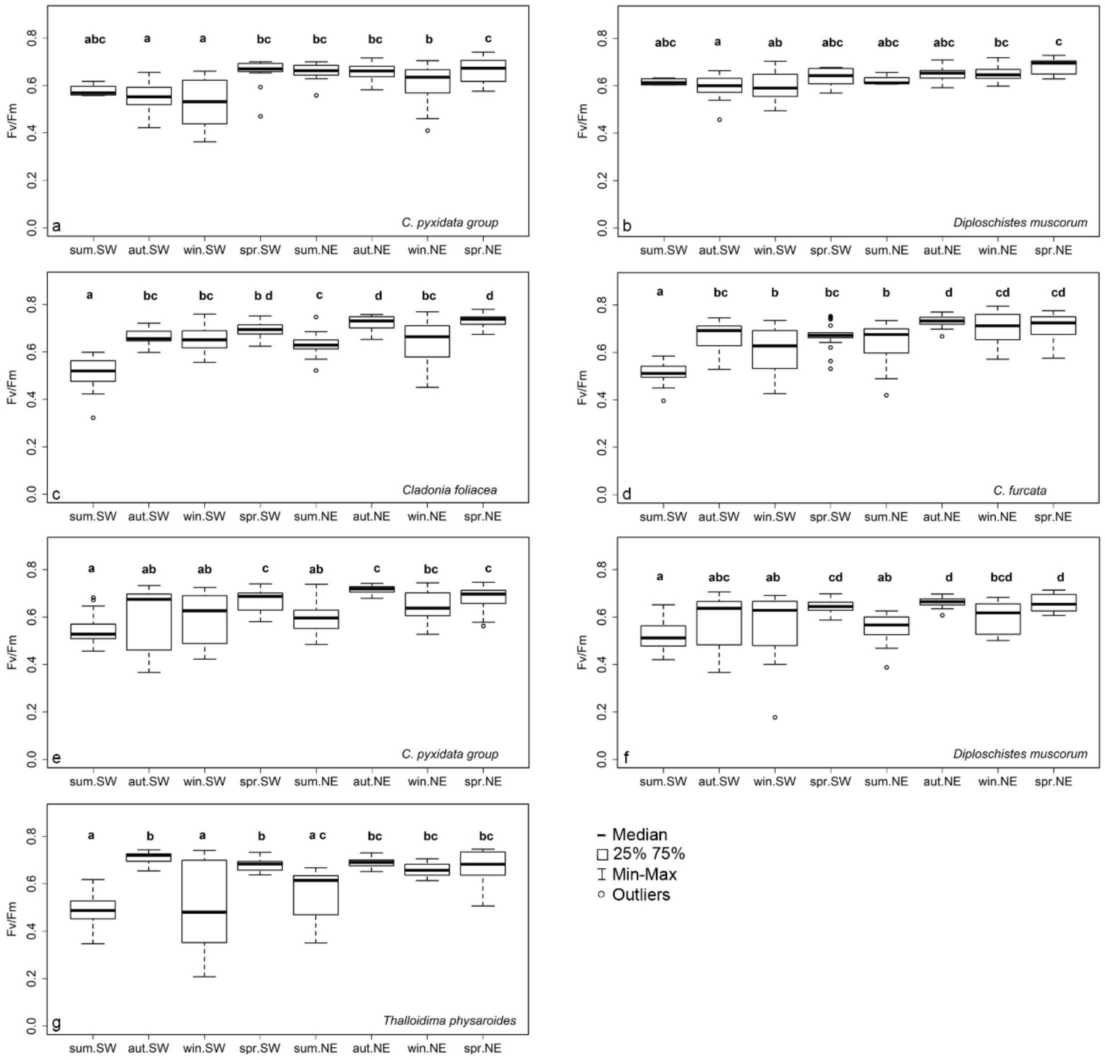

Fig. 3 a–g Variation in Fv/Fm [relative unit] within terricolous lichen species/species group living both on SW and on NE facing microhabitats in different seasons (spr = spring, sum = summer, aut = autumn, win = winter) during the two year investigation period. From the first year, only values of the *Cladonia pyxidata* group (a) and *Diploschistes muscorum* (b) are presented. In the second year, data of *Cladonia foliacea* (c), *C. furcata* (d), the *C. pyxidata* group (e), *Diploschistes muscorum* (f) and *Thalloidima physaroides* (g) are presented. Within one year and species / species group, each group was compared to each group by a multiple comparison Tukey-test on least square means by sites. Means with the same letter are not significantly different at 95% confidence

In the *C. pyxidata* group, the average of Fv/Fm was higher in NE than in SW exposed thalli; however, the difference was significant only in autumn and winter (Fig 3a, 3e). Regarding seasons, spring samples showed significantly higher values than winter ones on NE facing microhabitats, and winter and autumn ones in SW facing microhabitats. Meanwhile, in the second year on SW facing microsites, this value is significantly higher in spring samples than in samples from other seasons. On NE facing microsites autumn and spring samples showed significantly higher values than summer ones. The Fv/Fm was usually higher in NE than in SW exposed thalli in *D.*

muscorum; however, the differences were not significant (Fig 3b, 3f). Neither the seasonal fluctuation of Fv/Fm was remarkable in this species. Significantly higher Fv/Fm was measured in the NE than in the SW populations of *C. foliacea* in summer and autumn. In contrast, in winter and spring, this value did not differ significantly among microhabitats (Fig 3c). In summer, considerably lower values were recorded than in other seasons on SW facing microhabitats. Still, it showed a slightly different picture on NE facing microhabitats: significantly higher Fv/Fm was measured in autumn and spring than in summer and winter. Significantly higher Fv/Fm was found in NE than in SW exposed thalli of *C. furcata* in every season, except in spring (Fig 3d). For both types of microhabitat, significantly lower values were measured in summer than in other seasons. The NE populations of *T. physaroides* did not usually show significantly higher Fv/Fm values than thalli of SW populations, only in winter (Fig 3g). The fluctuation of Fv/Fm between seasons, however, was markedly higher in SW than in NE exposed thalli. On SW facing microhabitats, significantly higher values were measured in spring and autumn samples than in summer or winter ones, but there were no significant differences detected on NE sides between seasons in this parameter.

In general, a significant (1st year: $p = 0.036$, 2nd year: $p < 0.001$) difference was detected among species in both years. The thalli of large squamulose and fruticose species (*C. foliacea*, *C. furcata*) showed higher Fv/Fm than was measured in crustose species (*D. muscorum*, *T. physaroides*). Meanwhile, within the same growth form, there were no remarkable interspecific differences in this parameter. *C. pyxidata* showed transitional Fv/Fm values between those two groups.

Non-photochemical Quenching (NPQ)

There were significant differences between the NE and SW populations in NPQ values, mostly in the second year in the case of the *C. pyxidata* group (Fig 4e). The values of NPQ were significantly higher in NE than in SW exposed thalli in summer, winter and autumn. In the first year, the NPQ was significantly higher in spring samples than in winter samples in SW facing microhabitats; meanwhile, in NE exposed thalli, the spring samples showed significantly higher NPQ values than was measured in other seasons (Fig 4a).

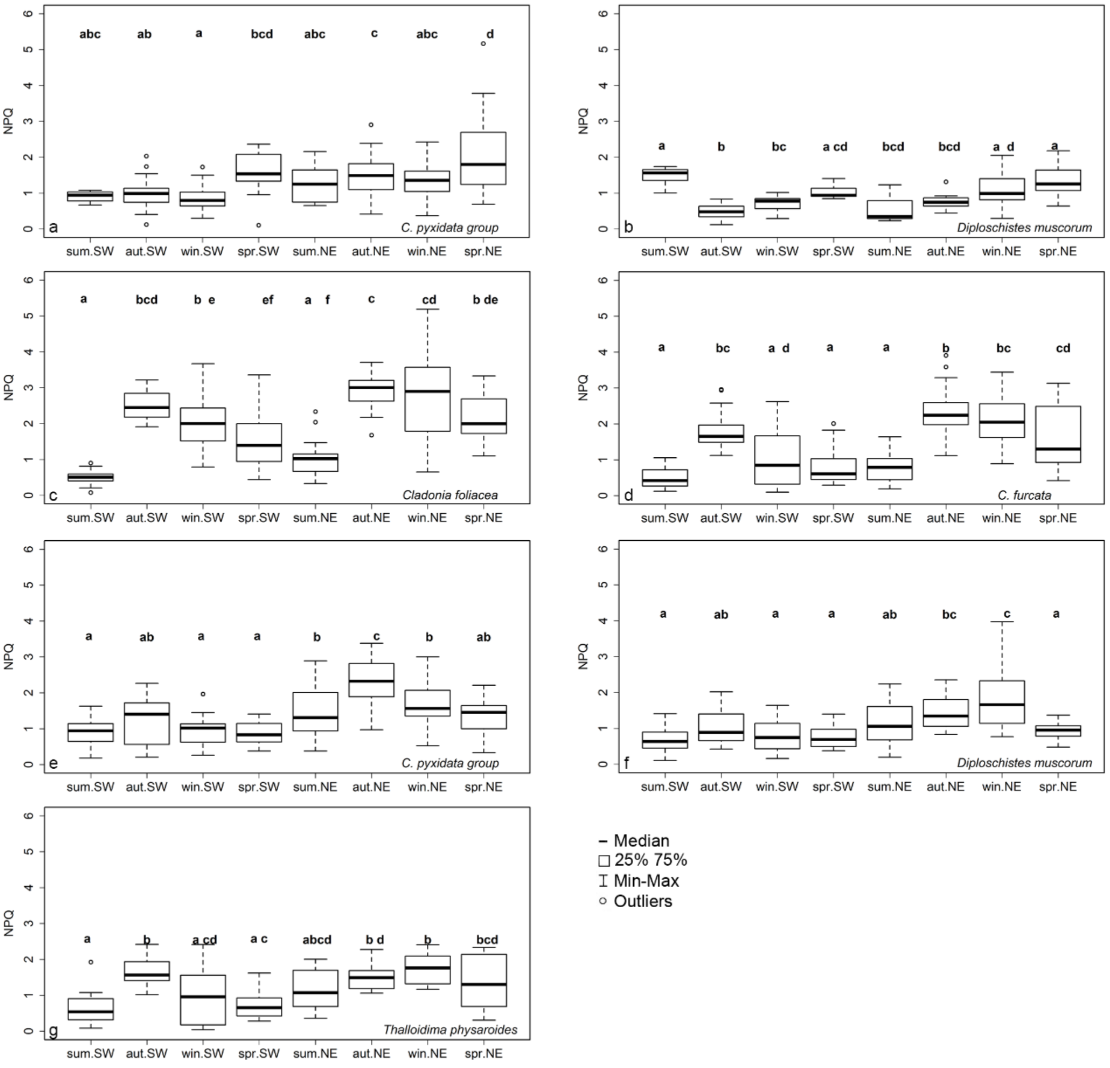

Fig. 4 a–g Variation in NPQ [relative unit] within terricolous lichen species living both on microhabitats with SW and NE aspects in different seasons (spr = spring, sum = summer, aut = autumn, win = winter) during the two year investigation period. From the first year, only data of the *Cladonia pyxidata* group (a) and *Diploschistes muscorum* (b) are presented. In the second year, data of *C. foliacea* (c), *C. furcata* (d), the *C. pyxidata* group (e), *Diploschistes muscorum* (f) and *Thalloidima physaroides* (g) are presented. Within one year and species / species group, each group was compared to each group by a multiple comparison Tukey-test on least square means by sites. Means with the same letter are not significantly different at 95% confidence

In the second year, there was no remarkable difference between seasons on SW facing microsites; however, on microsites with opposite aspect, significantly higher values were measured in autumn samples than in other seasons (Fig 4e). Higher NPQ was usually measured in NE compared to SW exposed thalli of *D. muscorum*; however, the difference was significant only in the summer and winter in the first year (Fig 4b), and winter in the second year (Fig 4f). The seasonal fluctuation was more remarkable in the first year in both type of microhabitats; however, in the second year, a significant difference between seasons was detected only in thalli of NE facing populations.

Higher NPQ was measured in the NE than in the SW populations of *C. foliacea*, although this difference was significant only in winter (Fig 4c). Significantly higher NPQ was measured in autumn than in summer, or in spring on both types of microhabitats; additionally, NE populations showed significantly lower values in summer than in other seasons. However, higher NPQ was measured in the NE than in the SW populations of *C. furcata* in every season; it was more pronounced in winter and spring (Fig 4d). It was true for both NE and SW populations that in autumn samples, significantly higher NPQ was recorded than in summer or spring. The NE exposed thalli of *T. physaroides* usually did not show significantly higher NPQ values than thalli of SW facing microsites, except in the winter (Fig 4g). There was no significant difference between seasons in NE populations; meanwhile, significantly higher values were measured in autumn than in other seasons in SW populations.

Significantly higher ($p < 0.001$) NPQ was measured in *C. foliacea* than in other investigated species. Specimens of *T. physaroides*, *D. muscorum* and the *C. pyxidata* group showed moderate changes among seasons in this parameter compared to *C. foliacea* and *C. furcata*, especially for SW facing populations (Fig 4).

Discussion

The maximum quantum yield of photosystem II photochemistry (Fv/Fm)

The effect of microhabitat conditions. The results clearly indicate that Fv/Fm is usually higher on less arid than on arid microhabitats for each species studied. The results also show a remarkable species-specific seasonality. From a micrometeorological point of view, there was an unambiguous contrast between arid and less arid microsites. The most remarkable difference was observed in soil temperature, which was higher for SW than for NE facing sides during the daytime (Fig 2), indicating a higher amount of incoming solar radiation on SW than on NE oriented microsites. Additionally, higher soil water content and lower VPD on NE compared to SW side provided a higher amount of humidity for rehydration/activation for terricolous lichens. The shading effect of vascular plants is an additional factor influencing light and humidity conditions, which can generate a higher Fv/Fm value in lichens (Kalapos & Mázsa 2001; Serpe *et al*. 2013). On less arid microsites, both the cover and average height of vascular plants were higher than on arid ones (NE: 14% cover, 6 cm height; SW: 8% cover, 2.5 cm height, (Veres *et al*. unpublished data)). As previously revealed for epiphytic lichen species (e.g. Atala *et al*. 2015; Gauslaa *et al*. 2006; Pirintsos *et al*. 2011), there is a more remarkable, long-term reduction in Fv/Fm in lichens living in sunny, open places compared to shade populations. This also explains the lower measured Fv/Fm values in the present study.

The effect of seasonal changes. The seasonal changes also had a characteristic effect on photosynthetic activity. In general, similar to previous studies (MacKenzie *et al*. 2002; Vráblíková *et al*. 2006), higher Fv/Fm values were detected in spring and autumn samples compared to summer and winter ones in both types of microhabitats. This result indicates that the environmental conditions during spring and autumn were more favourable from a photosynthetic point of view than in the other two seasons. High relative humidity and also increasing intensity of incident light were representative for spring and autumn. During winter and summer, the investigated species showed lower Fv/Fm values, indicating that lichen thalli had been exposed to stress (Maxwell & Johnson 2000). In summer, it could be explained by heat as well as by light stress, which had a photoinhibition effect on lichen thalli both separately and combined (Gauslaa & Solhaug 1999). The daily average PAR and VPD were relatively high, creating a challenging environment for lichens in the summer. The second-lowest Fv/Fm values were usually measured in winter samples. In this season the low temperature, days below freezing (4–22 during the present study), and thick snow coverage could have an inhibitory effect on metabolic activity and photosynthesis in lichens (Leisner *et al*. 1996; Schroeter *et al*. 2011). According to Hájek *et al*. (2009; 2016), Fv/Fm is a cold-sensitive parameter, which shows a species-specific decrease during declining temperature, confirming the findings of the present study.

The species-specific response. Comparing the different species, the Fv/Fm was usually higher in the large, squamulose *C. foliacea* and fruticose *C. furcata* than in the other two, crustose species, *D. muscorum* and *T. physaroides* for every season in the second year. Meanwhile, the thalli of the *C. pyxidata* group showed transitional values between the two above-mentioned groups.

The former two groups differ from each other mainly in their photosynthetic partners (Beck *et al*. 2002; Friedl 1986; Smith *et al*. 2009; Timdal 1991; Wedin *et al*. 2015), which probably could explain the differences in Fv/Fm values (Demmig-Adams *et al*. 1990). Their different morphology could also explain the variability in Fv/Fm between growth forms due to water household (Lange & Green 2003). The thalli of the *C. foliacea* and *C. furcata* have a higher surface area to volume ratio; have loose, less compact thalli; and live higher above the ground than those of the *C. pyxidata group*, *D. muscorum* or *T. physaroides*. The latter species usually live on moss cushions, ensuring more humid micro-conditions than lichens living on bare soil (Colesie *et al*. 2012). Because of these reasons, the air ventilation and boundary layer, radiation and temperature could have a more substantial influence on the water household of *C. foliacea* and *C. furcata* thalli than on the *C. pyxidata* group, *D. muscorum* or on *T. physaroides* under the same atmospheric humidity conditions. Lange & Green (2003) revealed a species-specific duration of metabolic activity depending on the thallus growth form. It was also shown that *C. foliacea* could activate rapidly even between different humidity conditions, represented here by the different seasons. Lichens could utilize dew and heavy rain showers during summer, or high and frequently saturated air humidity, dew, fog and rain in spring and autumn, as a water source (e. g. Lange & Green 2003), as well as melting snow and frost in winter (e.g. Colesie *et al*. 2016; Schlensog *et al*. 2013). All investigated species vary considerably in seasonal fluctuation of their average Fv/Fm values; however, this fluctuation is more pronounced in *C. foliacea*, *C. furcata* and *T. physaroides* than in the *C. pyxidata* group or in *D. muscorum* because of the above-mentioned possible reasons.

The combined effect of microhabitat and seasonal changes. Regarding seasonality, the most significant difference in Fv/Fm between SW and NE populations of *C. foliacea* and *C. furcata* appeared in the summer season. The microhabitat conditions (radiation, humidity) probably had a more pronounced effect on the water household of these species. Because of fruticose habit and thin cortical layer (*C. furcata*) (Verseghy 1971), or the lack of lower cortical layer (*C. foliacea*), the thalli of these two species presumably dry out more rapidly on arid than on less arid microhabitats compared to other investigated species. *D. muscorum* is able to begin photosynthetic activity even at low water content (Lange *et al*. 1997), which can explain why this species did not show any significant difference between sun and shade populations in summer.

Non-photochemical Quenching (NPQ)

The effect of microhabitat conditions. Our results suggest that NPQ is usually higher on shaded than on exposed microhabitats in the different investigated lichen species. One possible explanation of this apparent paradox phenomenon is that populations living on NE oriented slopes are exposed to wet conditions in the early morning time, and light can reach their surface more intensely than those growing on the opposite, SW facing slopes. The wet lichen thallus is more translucent; therefore, the photosynthetic apparatus needs more protection against the harmful excess light energy than in dried thalli (Heber *et al*. 2006), where most of the radiation is reflected from the surface of the thallus (Ertl 1951).

The effect of seasonal changes. The seasonal fluctuation of NPQ was usually more pronounced on less arid than on arid microsites. In autumn and winter, higher humidity and incoming light was typical. Under these conditions (prolonged moistened active periods as well as solar radiation), wet lichen thalli needed more protection against harmful excess light energy, resulting in higher NPQ (Barták *et al*. 2000). In addition to other factors, more frequent desiccation-hydration cycles might increase the NPQ values in these seasons (Vráblíková *et al*. 2006). Seasonal differences in NPQ have also been found in other lichen species, for example, *Xanthoria parietina* (Gauslaa & McEvoy 2005; Vráblíková *et al*. 2006).

The species-specific response. Our results also show a clear species-specific seasonality of NPQ. The highest values were measured among species of the *C. pyxidata* group in summer. This could be explained by the fact that these species are usually growing on moss thalli, ensuring prolonged hydration during dry days (Colesie *et al*. 2012). However, these lichens are even more threatened by the intensive radiation, and are unable to protect themselves due to desiccation-induced defense mechanisms (Heber *et al*. 2006, Kranner *et al*. 2008). Therefore, wet thalli needed more protection against harmful excess light energy by increasing NPQ. Favourable humidity conditions (lower VPD), as well as irradiation, could result in the higher NPQ values in autumn (e.g. *C. foliacea*) than were measured in summer (e.g. *C. foliacea*). In winter time, a species-specific response to cold (e.g. *C. foliacea*; the *C. pyxidata* group) was detected; this was also observed by Mishra and co-workers (2015), who found that NPQ values decreased simultaneously with decreasing temperatures.

The seasonal fluctuation of NPQ was different in the investigated species. It was notable in *C. foliacea* and *C. furcata,* but less remarkable in the *C. pyxidata* group, or *D. muscorum*. This finding could be explained by the thin (or absent) cortical layer on the lower side of the thallus in the former two species, causing lower water household capacity, but also by the higher amount of incoming light reaching the photobiont cells. Samples of *T. physaroides* also showed moderate changes in NPQ among seasons compared to *Cladonia* species. The NPQ values of *D. muscorum* differed among seasons, and also among microhabitats and years, indicating relatively rapid adaptation ability of this species to changing light conditions. The highest NPQ values were usually observed in *C. foliacea* thalli. The reason for this phenomenon could be that because of the thinner gonidial layer (Verseghy 1971) the self-shading effect of photobiont cells could be lower, compared to the other investigated *Cladonia* species (Wu *et al*. 2014), which could result in an increased photoprotective mechanism in the hydrated state. Additionally, because of the thin cortical layer, a more frequent desiccation-hydration period could also increase the level of NPQ (Vráblíková *et al*. 2006). Lower values of NPQ found in the other investigated species probably indicate that other protection mechanisms are also working in the background. These species-specific responses could be, e.g. the light-reflecting white colour of the thallus (*D. muscorum*); thick cortical layer (e.g. Beckett *et al*. 2008; Gauslaa *et al*. 2017; Váczi *et al*. 2018); hairiness (Bianchi *et al*. 2019b); production of UV-reflective lichen substances (e.g. usnic acid, fumarprotocetraric acid in *C. foliacea*); light-reflecting calcium oxalate crystals (*T. physaroides*); or biochemical protective mechanisms (Gasulla *et al*. 2018).

Conclusion. In this extensive study, we investigated the effect of microhabitat conditions and seasonal changes on photosynthetic functioning simultaneously in five terricolous lichen species/species groups (representing different growth forms). These effects have not widely studied before. Therefore, our results provide new information on how these species can adapt to varying conditions with regards to photosynthetic activity and photoprotection. Directional aspect had a species-specific effect on seasonal photosynthetic function due to different light and humidity conditions. The different values of Fv/Fm and NPQ of the five lichen species/species groups under the same humidity and light conditions can be explained by their different morphologies, and thus their water household capacity, photobiont, or lichen substances. We suggest future workers study more than one species to investigate the response of lichens to changing environmental conditions in space and/or in time. Additionally, because both photosynthetic activity, and a part of photoprotection, derive from the algal cells in lichens, future work should focus on species-specific differences of chlorophyll fluorescence in the different chlorobiont partners found in lichens (e.g. *Asterochloris, Trebouxia*).

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ORCIDs First author: https://orcid.org/0000-0001-5818-4891; Second author: https://orcid.org/0000-0002-5245-1079; Third author: https://orcid.org/0000-0001-7744-3393

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12.4 S.4. Article 3: Photoprotection and high-light acclimation in semi-arid grassland lichens – a cooperation between algal and fungal partners

Symbiosis

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Photoprotection and high-light acclimation in semi-arid grassland lichens – a cooperation between algal and fungal partners

Katalin Veres[1], †Zsolt Csintalan[2], Zsanett Laufer[3], Rita Engel[1], Krisztina Szabó[1], Edit Farkas[1]

[1]Institute of Ecology and Botany, Centre for Ecological Research, H-2163 Vácrátót, Alkotmány u. 2–4, Hungary

[2]Department of Plant Physiology and Ecology, Institute of Biological Sciences, Hungarian University of Agriculture and Life Sciences, H-2100 Gödöllő, Páter K. u. 1, Hungary

[3]Bükk National Park Directorate, H-3304 Eger, Sánc u. 6, Hungary

corresponding author: Katalin Veres, veres.katalin@ecolres.hu, telephone number: +36 28 360-122

Abstract

In lichens, each symbiotic partner cooperates for the survival of the symbiotic association. The protection of the susceptible photosynthetic apparatus is essential for both participants. The mycobiont and photobiont contribute to the protection against the damaging effect of excess light by various mechanisms. The present study investigated the effect of seasonality and microhabitat exposure on photoprotection and photoacclimation in the photo- and the mycobiont of six lichen species with different thallus morphology in inland dune system in the Kiskunság region (Hungary) with shaded, more humid and exposed, drier dune sides. High-Performance Liquid Chromatography, spectrophotometry, chlorophyll *a* fluorescence kinetic technique were used, and micrometeorological data were collected. The four years data series revealed that the north-east-facing side was characterized by higher relative humidity and lower light intensities compared to the south-west-facing drier and more exposed sides. The south-west facing side was exposed to direct illumination 3–4 hours longer in winter and 1–2 hours shorter in summer than the north-east facing side of the dune, influencing the metabolism of sun and shade populations of various species. Because rapid desiccation caused short active periods of lichens during bright and drier seasons and on exposed microhabitats, the rapid, non-regulated non-photochemical quenching mechanisms in the photobiont had a significant role in protecting the photosynthetic system in the hydrated state. In dehydrated conditions, thalli were mainly defended by the solar screening metabolites produced by the mycobiont and curling during desiccation (also caused by the mycobiont). Furthermore, the efficacy of light use (higher chlorophyll and carotenoid concentration) increased because of short hydrated periods. Still, a lower level of received irradiation was appropriate for photosynthesis in dry seasons and on sun exposed habitats. In humid seasons and microhabitats, more extended active periods lead to increased photosynthesis and production of solar radiation protectant fungal metabolites, allowing a lower level of photoprotection in the form of regulated non-photochemical quenching by the photobiont. Interspecific differences were more pronounced than the intraspecific ones among seasons and microhabitat types.

Keywords: photoacclimation, seasonality, microhabitat, species-specific response, lichen secondary metabolites, plastid pigments

Introduction

Lichens are unique and complex ecosystems in which each symbiotic partner can contribute to the survival of extreme environmental conditions. The photobiont supplies the primary carbon source for the mycobiont; accordingly, the protection of the susceptible photosynthetic apparatus is essential for both participants (Sadowsky and Ott 2016). Light is indispensable to photosynthesis, although light can also cause irreversible photodamage in the algal photosystem (PS) II (Heber *et al*. 2000). In hydrated lichens, the excitation energy absorbed by the antenna system may be used for photochemical charge separation in the reaction centres (RCs). When the electron transport chain is saturated, the excitation energy can be re-emitted as fluorescence or dissipated as heat. Both non-photochemical quenching via zeaxanthin (Demmig-Adams and Adams 1990; Färber *et al*. 1997) and desiccation induced fluorescence quenching (Heber *et al*. 2001, 2006; Kopecky *et al*. 2005) are known in lichens. In the absence of effective thermal energy dissipation, the production of damaging reactive oxygen species (ROS) as by-products can cause irreversible damage in the PSII (Müller *et al*. 2001; Krieger-Liszkay 2005).

Both the mycobiont and photobiont can defend against solar radiation damage by various mechanisms (Beckett *et al*. 2021; Gasulla *et al*. 2012; Kranner *et al*. 2005; Nguyen *et al*. 2013; Sadowsky and Ott 2016). Lack of water is a main limiting factor for lichen metabolism. On the other hand, reversible drying out for a short period is one way of protecting poikilohydric organisms from excess light (Veerman *et al*. 2007). The lower transmittance (Dietz *et al*. 2000) or higher density (Gauslaa *et al*. 2017) of the cortex, the increased accumulation of solar radiation screening pigments (e.g. BeGora and Fahselt 2001; Singh *et al*. 2011; Solhaug and Gauslaa 1996; Solhaug *et al*. 2010), and curling during desiccation (Barták *et al*. 2006) are defence strategies offered by the mycobiont under high light exposure. The photobiont has also developed mechanisms to protect its photosynthetic apparatus, such as the aggregation and the change in shape during desiccation (de los Rios *et al*. 2007; Scheidegger *et al*. 1995). The enhanced non-photochemical quenching (ΔpH- and zeaxanthin dependent and desiccation induced) increases the thermal dissipation of excess light energy to protect the photosystem in the algae (Heber *et al*. 2001; Paoli *et al*. 2010; Vrábliková *et al*. 2006). Conformational change in the chlorophyll-protein complex is also an effective way to dissipate excess light energy due to desiccation (Heber *et al*. 2007). These protecting mechanisms require accurate coordination of both partners (Kranner *et al*. 2005), since a separated mycobiont and photobiont suffer from oxidative stress during desiccation. In the lichen thallus, the partners can regulate the photoprotective system of the other symbiotic component (Kranner *et al*. 2005; Solhaug and Gauslaa 2004).

Several studies have also demonstrated the seasonal change of solar radiation protectant lichen secondary metabolites (LSMs) and plastid pigments. The LSM and carotenoid concentration in lichens are higher, and the chlorophyll concentration is lower in brighter seasons in the northern or arctic-alpine region (Bjerke *et al*. 2005; Gauslaa and McEvoy 2005; Gauslaa *et al*. 2013; Vráblíková *et al*. 2006), which is contrary to the situation in continental, semi-arid (Farkas *et al*. 2020) and coastal areas (Higgins *et al*. 2015). The sun exposure of lichen microhabitats also influences the photoprotection mechanisms. Compared to shady microhabitats, higher sun exposure induces the production of UV protecting LSMs (BeGora and Fahselt 2001; Bjerke *et al*. 2002; Nybakken *et al*. 2007), and may also cause changes in the plastid pigment concentration (Czeczuga and Krukowska 2001). The concentration of photoprotective carotenoids in lichen populations is higher in the sun than in the shade (Demmig-Adams and Adams 2006; Gautam *et al*. 2011; Paoli *et al*. 2010). According to some studies, shade-adapted thalli exhibit a higher concentration of chlorophyll than exposed ones (Pintado *et al*. 2005; Paoli *et al*. 2010; Pirintsos 2011), but not always (Picotto and Tretiach 2010; Pintado *et al*. 1997).

The main objective of the present study was to reveal the contributions of the photo- and mycobiont to light acclimation and photoprotection within the lichen thallus. We aimed to reveal the influence of the seasonal light conditions and exposed versus shaded microhabitats. We also focused on the responses of the various lichen species exhibiting different thallus structures to the changing environment. We hypothesized that because of the different anatomy and thallus structure, the investigated lichen species acclimate differently to the changing light conditions in space and time. We also assumed that both algae and fungi contribute equally to the protection of excessive solar radiation during the whole year.

The investigation extends the previous knowledge of terricolous lichens in semi-arid regions where they cover large areas between patches of vascular plants (Belnap and Lange 2003). Our results also contribute to the limited lichenological data on the proportion of absorbed excitation energy available in the literature.

Materials and methods

Study site

The study site is located in the Kiskunság region of Hungary near Fülöpháza (46° 52' 21.45" N, 19° 24' 18.29" E). The moderately continental climate with a submediterranean influence (Péczely 1967) provides habitats for semi-arid sandy grasslands (*Festucetum vaginatae danubiale* association Rapaics ex Soó 1929 em. Borhidi 2012). The landscape is characterized by an inland dune system with calcareous sand derived from the deposit of the Danube River. The wind, as a secondary effect, reshaped the landscape resulting in a dune system (Pécsi 1967). The prevailing wind has a northwest direction, and the yearly average wind speed is between 2.5–3 m s^{-1} (Péczely 1967).

The investigation was completed with micrometeorological data deriving from two micrometeorological stations. The stations were situated at Bugacpusztaháza, a similar duneland system, 28 air km from the present study site and had been already in operation during the present study. According to macrometeorological data (National Meteorological Service), landscape structure and soil surface variables at microenvironmental scale (Veres *et al.* 2021), the conditions at Bugacpusztaháza seemed to be representative of Fülöpháza. The method for micrometeorological data recording is specified in Veres *et al.* (2020). Changes in microclimate were recorded during the investigation period, including the main differences between the two microhabitat types (humid: northeast-facing (NE) and arid: southwest-facing (SW)). Two HOBO Micro Station H21-002 (Onset Computer Corporation, U.S.A.) with sensors (Onset Computer Corporation, U.S.A.) measuring photosynthetically active radiation (PAR) and relative humidity (RH) were placed at 0.5 m height. Data were continuously recorded each minute. PAR data were aligned with the seasonal change of angle in light incidence and the dune side inclination and exposition. Only the PAR and RH data are reported in the present study. We also estimated a hypothetically active period during nights (PAR = 0 µmol m^{-2} s^{-1} and RH > 80%), and a period when weather conditions could be suitable for photosynthesis (30 µmol m^{-2} s^{-1} < PAR <1,000 µmol m^{-2} s^{-1} and RH > 80%, based on Lange (2003). PAR data of the arid dune side are lacking because of some wire damage caused by wild animals in the spring.

Sampling method

Samples were taken from both sun populations on SW-facing side and shade populations on NE-facing side of foliose *Cladonia foliacea* (Huds.) Willd., the fruticose *Cladonia furcata* (Huds.) Schrad., the cup *Cladonia magyarica* Vain. and the crustose *Diploschistes muscorum* (Scop.) R. Sant. Additionally, populations of placodioid *Gyalolechia fulgens* (Sw.) Søchting, Frödén and Arup and foliose *Xanthoparmelia pokornyi* (Körb.) O. Blanco, A. Crespo, Elix, D. Hawksw. and Lumbsch were collected but found only in the exposed SW-facing microhabitats. The species hosted eukaryotic green algae: various species of *Asterochloris* in *Cladonia* species (Smith *et al*. 2009, Škaloud *et al*. 2015), and *Trebouxia* species in *G. fulgens* and *X. pokornyi* (Škaloud and Peksa 2008, Stenroos *et al*. 2011, Leavitt *et al*. 2015). *Diploschistes muscorum* switches photobiont during its lifespan (Friedl 1986, Piercey-Normore and DePriest 2001, Škaloud *et al*. 2015, Wedin *et al*. 2015). In the early stage, it has *Asterochloris* captured from *Cladonia* species; later replaced by various species of *Trebouxia*. These six lichen species are members of the "Bunte Erdflechten-Gesellschaft" (Gams 1938), also known as the *Fulgensietum fulgentis* Gams and *Cladoniaetum symphycarpae* Doppelb. associations, typical for this region (Büdel 2001). Thalli were collected in four seasons (July and November of 2014, February and May of 2015).

Samples were taken from one SW- and one NE-facing side (= microhabitats) of a dune. In every season, new thalli were collected from the same dune sides during the investigation period. After collection, thalli were transported to the laboratory, where they dried out under semi-natural conditions (i.e. seasonal temperature, humidity and light regime) (Csintalan *et al*. 1999) and then cleaned from plant and moss debris. The samples were stored for a maximum of 14 days at room temperature (Tair = 20–22 ºC, RH = 23% all year) in darkness before measuring chlorophyll fluorescence kinetics and 21 days before plastid pigment analysis. LSMs were quantified after all samplings had been completed in October 2017. Samples were stored at room temperature (Tair = 20–22 ºC, RH = 23% all year) in the darkness.

Ten samples per species per season per microhabitat were measured during plastid pigment and HPLC analysis. Because of its low abundance, *G. fulgens* was sampled in 5 replicates only. Twenty samples per species per season per microhabitat were measured by chlorophyll fluorescence kinetic technique, except for *G. fulgens* with only ten samples. *Gyalolechia fulgens* and

X. pokornyi occurred only in the SW-facing microhabitat, and only seasonal changes were investigated.

Extraction and measurement of lichen secondary metabolite concentration

Quantification of lichen secondary metabolites in the three *Cladonia* species from each microhabitat type and season was performed with a high-performance liquid chromatography (HPLC, Alliance e2695, Waters Corporation, Milford, MA, U.S.A.) system including a Photodiode Array Detector (2998 PDA Detector, Waters Corporation, Milford, MA, U.S.A). Ten thalli were chosen from the randomized material of each microhabitat type sampled in four seasons. Lichen material was pulverized with liquid nitrogen. 50 mg of the homogenized material were dissolved in 10 ml pure acetone and sonicated for 10 min. in an ultrasonic water bath. Samples were centrifuged for 20 min. After separation, the supernatant was filtered through a Cronus Ø 25 mm PTFE syringe filter (0.22 µm). For calibration, standard stock-solutions (1 mg ml^{-1}) were prepared from reference standards dissolved in acetone. Usnic acid, fumarprotocetraric acid and atranorin were quantified based on a five-point (5, 10, 20, 50, 100 µg ml^{-1}) calibration. The chromatographic design of Ji and Khan (2005) was modified for the application. A Phenomenex Luna 5 µm C18, 150 × 4.6 mm, column was used for chromatographic separation. The sample injection volume was 10 µl. The temperature was 40 °C in the column oven and 5 °C in the sample cooler. Baseline separation of the compounds investigated was achieved with a gradient elution program where solvent A consisted of ortho-phosphoric acid and deionized (Milli-Q ultrapure) water (0.5 : 99.5), and solvent B contained ortho-phosphoric acid and acetonitrile (0.5 : 99.5). All the chemicals used were HPLC grade. The linear gradient started with 60% of solvent A after the volume decreased to 10% within 20 min. and then to 0.5% in 30 sec., it remained constant for 9.5 min. The volume of solvent A was changed back to 60% within 1 min. The flow rate of solvents was 1 ml min^{-1}. Lichen metabolites were detected at 280 nm (usnic acid), 240 nm (fumarprotocetraric acid) and 233 nm (atranorin).

Extraction and measurement of photosynthetic pigments

The concentration of plastid pigments was measured by spectrophotometry (Shimadzu UV-1601) according to Pfeifhofer *et al*. (2002). A cooled mortar and a pestle were used to grind c. 100 mg of clean, air dry (max. 23% RH in the laboratory) thalli of each sampled lichen with c. 7–8 mg of L-sodium ascorbate to prevent chlorophyll degradation (Calatayud *et al*. 2000). Then liquid nitrogen was added, and the samples were pulverized. The material was mixed with 500 µl pure acetone

(HPLC grade) in the mortar then poured quickly into cold centrifuge tubes. The rinse of the remnant was performed by adding 500 µl pure acetone and pouring it into the sample. The last step was repeated two times. The homogenate was stored on ice in darkness (in an isolated box with a lid) until the analysis. The samples were centrifuged at 20,000 g for 10 min. at 4 °C. After separation, the supernatant was decanted into clean, cold centrifuge tubes. The pellet was re-suspended with 1,000 µl acetone and vortexed. Then the centrifuge procedure was repeated, and the supernatants were combined. Spectrophotometrical quantification of pigments in the acetone extracts was conducted following Lichtenthaler and Buschmann (2001). Preliminary investigations showed that two extraction steps were adequate for quantitative extraction. The samples were filtered through Ø 13 mm PTFE syringe filter (0.45 µm) and measured in a glass cuvette on 470, 645, 662, 710 nm against a blank (100% acetone). The chemicals used were of analytical grade quality. The concentration was calculated from the recorded absorbance (A) on specified wavelengths completed with A710 (presence of sediment in the samples):

chlorophyll *a* (µg g^{-1}) = (11.24 * A662 - A710) - 2.04 * (A645 - A710)) * extract volume/thalli weight

chlorophyll *b* (µg g^{-1}) = (20.13 * A645 - A710) - 4.19 * (A662 - A710)) * extract volume/thalli weight

carotenoids (µg g^{-1}) = (1,000 * (A470 - A710) * extract volume/thalli weight - 1.9 * chlorophyll *a* - 63.14 * chlorophyll *b*) / 214

Chlorophyll *a* fluorescence kinetic measurements

After specimens were dried out and cleaned, they were rehydrated by spraying with distilled water twice a day (in the morning and the afternoon). Thalli were kept under low light (about 10 µmol m^{-2} s^{-1}) at seasonal ambient temperature for 1–2 days until the photosynthetic system regenerated (i.e. until Fv/Fm became constant). In summer samples, this preparation process required more time (3 days) than for thalli collected in other seasons. Chlorophyll *a* fluorescence kinetics were measured (described by Jensen 2002) on fully water-saturated lichen thalli after 30 min. of dark adaptation at room temperature (T = 20–22 °C) with a portable pulse amplitude modulated fluorometer (FMS 2 Hansatech Instruments Ltd. U.K.; Modfluor software) in the laboratory.

After dark adaptation, minimum fluorescence yield in dark-adapted state (Fo) was obtained, using a weak measuring beam for 3 sec. The maximum fluorescence yield of the dark-adapted sample (Fm) was determined with a saturation pulse of 7,500 µmol m^{-2} s^{-1} light intensity for 800 msec. From these parameters, maximum variable chlorophyll fluorescence yield in dark-adapted state (Fv = Fm - Fo) and maximum quantum yield of PSII photochemistry (Fv/Fm = (Fm - Fo) / Fm; Kitajima and Butler 1975) was calculated. The maximum fluorescence yield of the light-adapted sample (Fm') was determined with a saturation pulse of 7,500 µmol m^{-2} s^{-1} light intensity at actinic light 500 µmol m^{-2} s^{-1}. Before the maximum (Fm') and the steady-state (Ft) fluorescence yields were determined, two saturating pulses were added for 800 msec. The yield of photochemical electron transport (φPSII), non-photochemical quenching (φNPQ), and the yield of non-regulated excitation dissipation (φNO) were calculated according to Klughammer and Schreiber (2008) (the last Ft and Fm' values were used for calculations):

φPSII = (Fm' - Ft) / Fm'

φNPQ = Ft / Fm' - Ft / Fm

φNO = Ft / Fm.

Measurement of chlorophyll fluorescence is a non-invasive method, and the measured data can give important information on energy dissipation. Fv/Fm provides information about the condition of the photosynthetic systems within the thalli and shows how efficiently the photochemical reaction is proceeding. φPSII gives insight into the effective photochemical quantum yield of PSII and shows the proportion of use excitation energy for charge separation. φNPQ represents the quantum yield of light-induced (ΔpH- and zeaxanthin-dependent) non-photochemical fluorescence quenching. The φNO describes the combined pathways of radiative and non-radiative deexcitation reactions, which do not lead to photochemical energy conversion and are not involving the NPQ-mechanisms (Klughammer and Schreiber 2008). These are competitive processes, and their sum is equal to 1 (Kramer *et al.* 2004).

Statistical analysis

A pairwise t-test was performed to compare the means of micrometeorological data (PAR, RH), taking the average of every 10 min. between the SW- and NE-facing sides of the investigated dune (R Core Team 2020). For seasonal means, the average of a month preceding sample collection was taken using the *dplyr* package (Wickham *et al.* 2018). For the calculation of direct illumination reaching the dune sides during a year, the geographical characteristic (latitude, longitude), angle of the slopes and solar coordinates were used. 'Sunrise' time means when the direct radiation first reaches the dune side, and 'sunset' is when the last direct beam reaches the side. These are the possible light conditions that can be still affected by humidity and clouds. For the calculation of the seasonal duration of direct illumination and the hypothetical active periods, a 1-month average preceding the sampling was used. The period when relative humidity is higher than 80% is regarded as a hypothetical active period for lichens. The daily activity was calculated after sunrise.

The effect of seasons (summer, autumn, winter, spring) and microhabitats (SW, NE) on plastid pigment concentration, solar radiation screening LSMs and values of quenching mechanisms (response variables) were statistically evaluated. All statistical analyses were carried out with the R software version 3.6.3 (R Core Team 2020). The influence of species, seasons and microhabitats was tested by the three-way ANOVA followed by a Tukey HSD test with 'TukeyHSD' in *agricolae*. The combination of six species, four seasons and two microhabitats resulted in 40 groups. During the analysis, each group were compared to each group. Normality of data distribution was checked visually by Q-Q plot (quantiles of the residuals are plotted against the quantiles of the normal distribution with a 45º degree reference line) with 'qqnorm' and 'qqline' functions of *car* package and by Shapiro-Wilk normality test using 'shapiro.test' of *dplyr*. Our data followed a normal distribution. Levene's test was used to check the homogeneity of variances with 'leveneTest' in *car*. The variances across groups were homogeneous. A level of $p < 0.05$ was considered for a significant difference. Graphs were prepared in R environment (Fig. 3) and MS Excel (Fig. 1 and Fig. 2).

Results

Micrometeorology

According to the four years data series, the microclimatic conditions differed significantly between the NE- and SW-facing sides over the long term. The NE-facing sides were characterized by higher relative humidity ($p < 2e^{-16}$) and lower light intensities ($p < 2e^{-16}$) compared to the drier and more exposed SW-facing sides. Hence, lichen thalli at NE sides are regarded as shade/humid (H) and as sun/arid (A) populations at SW sides (Veres *et al*. 2020). A clear difference in direct illumination was recorded between the exposed (annual average 11 h 30 min) and shaded (9 h 30 min) dune sides on a long term scale.

The quality and quantity of incoming irradiation varied among the seasons (Table 1). In the winter and autumn months, the direct illumination lasted 3–4 h longer on the exposed than on the opposite side because there was a remarkable difference between 'sunset' time, while there was no significant difference in 'sunrise' time (Fig. 1). During the summer months, the direct illumination reached the shaded side 1–2 hours longer because of the significant difference between 'sunrise' time (Fig. 1). The direct illumination reaching the dune side was ca. 90% of the total irradiation on the arid and 93% on the humid slope during summer, 97% (A) and 57% (H) during winter and

96% (A) and 56% (H) autumn. In spring, no significant difference was detected in direct illumination between arid and humid sides: 91% (A) and 88% (H).

Table 1 Average length (hours) of direct illumination and the length of the hypothetical active period during a year in different seasons in the two humid (NE) and arid (SW) microhabitat types on a dune. Lenght of active period: the period when lichens could be metabolically active (RH > 80% during nights and by 30 μmol m^{-2} s^{-1} < PAR < 1000 μmol m^{-2} s^{-1} (Lange 2003) during days). diff. Start: the difference (SW-NE) in the direct illumination starts between the dune sides (NE = humid, A = arid); diff. End = difference between the direct illumination ended on the dune side. Seasons (1 month periods preceding sampling): summer = 17.07–17.06. 2014, autumn = 23.11–22.10. 2014, winter = 02.02–02.01. 2015, spring = 27.05–27.04. 2015.

	diff. Start	diff. End	Duration of direct illumination		Lenght of active period	
Season			**NE**	**SW**	**NE**	**SW**
summer day	-1:31	1:09	14:36	14:12	3:07	3:15
summer night					5:18	4:58
autumn day	0:25	4:19	5:31	9:21	4:31	4:48
autumn night					13:02	13:03
winter day	0:19	4:14	4:56	8:46	4:10	7:05
winter night					12:26	14:44
spring day	1:23	1:41	13:03	13:30	3:53	0:39
spring night					6:52	NA

*NA: data are lacking because of wire damage on PAR sensor caused by wild animals

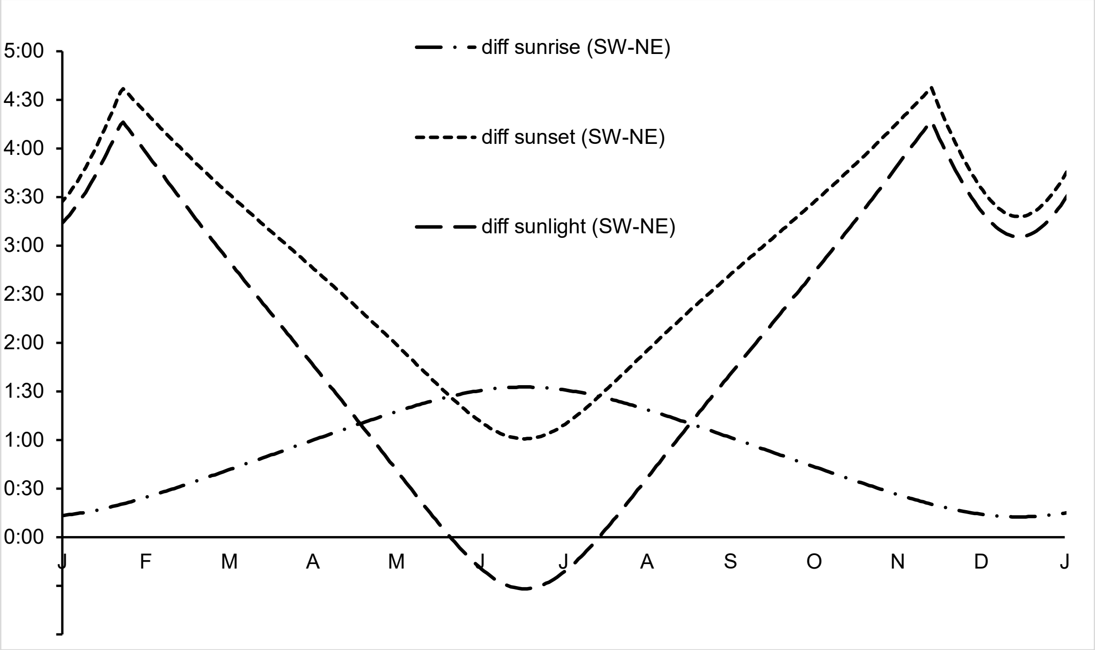

Fig. 1 The mean difference in direct illumination time between the two, exposed south-west (SW) facing and shaded north-east (NE) facing dune sides from January to December corrected with the seasonal solar coordinates and inclination of the slopes. Difference (SW-NE) between 'sunrise' of direct light (diff sunrise) and 'sunset' of direct light (diff sunset) and the difference between the direct illumination length (diff sunlight) between the dune sides.

The incoming irradiation varied among the seasons (Table 1) from 14 h 35 min in summer to 4 h 55 min in winter. The average time when the relative humidity rose above 80% (when the water content of lichen thalli shows a significant increase, Blum 1973) also varied among seasons both during the day and at night (Table 1). The average daily time when photosynthesis may occur was remarkably lower in spring (mean arid: 39 min, mean humid: 3 h 53 min) and summer (mean arid: 3 h 15 min, mean humid: 3 h 7 min) than in autumn (mean arid: 4 h 48 min, mean humid: 4 h 31 min) and winter (mean arid: 7 h 5 min, mean humid: 4 h 10 min). The overnight rehydration lasted twice as long in winter (mean arid: 14 h 44 min, mean humid: 12 h 26 min) and autumn (mean arid: 13 h 3 min, mean humid: 13 h 2 min) than in spring (mean arid: no data, mean humid: 6 h 52 min) and summer (mean arid: 4 h 58 min, mean humid: 5 h 18 min).

Lichen secondary metabolites

Compared to *C. furcata*, *C. magyarica* showed higher concentration of atranorin (Fig. 2b, Table S.1). Atranorin showed significant seasonal variation only in *C. furcata* ($p = 6.2e^{-10}$) (Fig. 2b, Table S.1), being lower in summer and spring than in winter and autumn. In *C. magyarica* atranorin did not vary across microhabitats (p = 0.58) or seasons (p = 0.32) (Table S.1). The microhabitat had a remarkable effect on the amount of atranorin in *C. furcata* (p = 0.007, Table S.1). Atranorin was usually higher in arid than humid microhabitats in spring and autumn and vice versa in summer and winter.

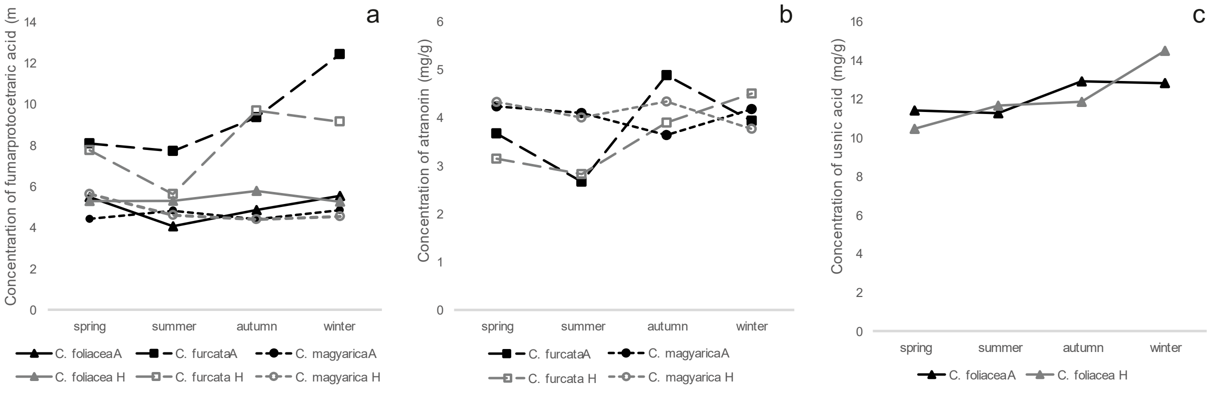

Fig. 2a–c The seasonal and microhabitat variation in mean concentrations of fumarprotocetraric acid (a), atranorin (b) and usnic acid (c) in three *Cladonia* species during a one year investigation period. Populations of *C. foliacea*, *C. furcata* and *C. magyarica* were investigated from exposed arid (A) and shaded humid (H) microhabitats. sd = standard deviation of means, n = 10 / species / season / microhabitat. The different letters sign significant differences (season * microhabitat interaction) in *C. furcata*.

Cladonia furcata showed approx. two times higher fumarprotocetraric acid concentration compared to the other two *Cladonia* species (Fig. 2a, Table S.2). A seasonal change in fumarprotocetraric acid concentration was detected in samples of *C. furcata* ($p = 7.55e^{-12}$) (lower concentration in summer and spring than in winter and autumn) and *C. foliacea* ($p = 0.03$), unlike in *C. magyarica* ($p = 0.44$) (Table S.2). Sun thalli showed higher fumarprotocetraric acid concentration compared to shade thalli in *C. furcata* ($p = 0.02$) and *C. foliacea* ($p = 0.04$), while microhabitat did not affect the amount of fumarprotocetraric acid in *C. magyarica* ($p = 0.36$) (Table S.2).

A significant seasonal change in usnic acid concentration was found in *C. foliacea* ($p = 0.0004$) (Fig. 2c, Table S.3), being lower in summer and spring than in winter and autumn. Meanwhile, usnic acid did not differ between sun and shade thalli ($p = 0.98$, Table S.3).

Photosynthetic / plastid pigment concentration

The seasonal change of plastid pigment concentrations differed among the species (Fig. 3a–d, Table S.4–7). Usually, the chlorophyll *a* + *b* concentration was lower in *G. fulgens* and *D. muscorum* than in other species (Fig. 3a, Table S.4). *Cladonia foliacea* ($p = 1.58e^{-6}$), *C. furcata* ($p = 0.003$), *G. fulgens* ($p = 0.05$), and *X. pokornyi* ($p = 0.05$) showed significant differences among the seasons (Table S.4). Usually, the minimum values occurred during winter (on average 155–326 $\mu g\ g^{-1}$ in *Cladonia* species and *D. muscorum*), except for *G. fulgens* showing maximum (204 $\mu g\ g^{-1}$) during winter. *Gyalolechia fulgens* and *X. pokornyi* occurred only in SW-facing microhabitats; therefore, the comparison was only possible between sun and shade populations of *Cladonia* species and *D. muscorum* (Fig. 3a–d). The concentration of chlorophylls was significantly higher in sun than in shade populations in *C. furcata* ($p = 0.0006$), *C. magyarica* ($p = 0.002$) and *D. muscorum* ($p = 0.04$) (Table S.4).

The chlorophyll *a* / *b* showed higher values in *G. fulgens* and lower ones in *C. foliacea* than in the other species (Fig. 3b, Table S.5). There was a seasonal trend in the mean chlorophyll *a* / *b* ratio of *C. foliacea* ($p = 3.0e^{-12}$), *C. furcata* ($p = 0.01$), *D. muscorum* ($p = 9.6e^{-5}$), and *X. pokornyi* ($p = 0.02$) (Table S.5). There was no significant difference between arid and humid dune sides in any of the species (Table S.5).

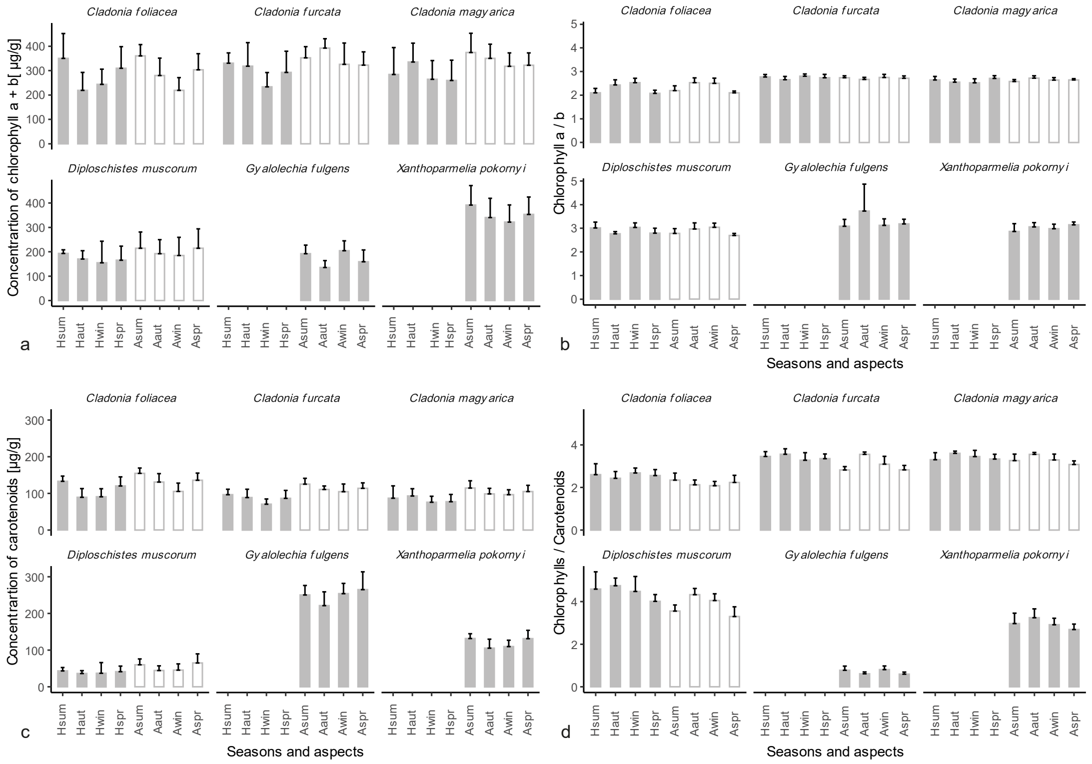

Fig. 3a–d The mean concentration of photosynthetic pigments in six terricolous lichen species. Chlorophyll *a* + *b* (a), chlorophyll *a* / *b* (b), the total concentration of carotenoids (c), chlorophylls / carotenoids (d) of shade (H) and sun (A) populations were investigated from two sides (microhabitat = arid, humid) of a dune in different seasons (sum = summer, aut = autumn, spr = spring, win = winter). The standard deviation of means is also depicted. The columns (groups = species / season / population) with different letters are significantly different within one variable. n = 10 / species / season / microhabitat, except *G. fulgens*, n = 5 / season / microhabitat

The carotenoid concentration was lower in *D. muscorum* and higher in *G. fulgens* than in the three *Cladonia* species or *X. pokornyi* in each season (Fig. 3c, Table S.6). A significant seasonality of the carotenoid concentration occurred in *C. foliacea* (p = 6.41e^{-9}), *C. furcata* (p = 0.001), *D. muscorum* (p = 0.03) and *X. pokornyi* (p = 0.006) (Table S.6). Usually, the highest mean carotenoid concentration was detected in summer (43–145 µg g^{-1}), whereas the lowest values occurred in winter (71–105 µg g^{-1}) for *Cladonia* species and in autumn for the other three species (36–44 µg g^{-1} in *D. muscorum*; 106 µg g^{-1} in *X. pokornyi*; 222 µg g^{-1} in *G. fulgens*). The sun populations contained significantly more carotenoids than shade populations, in *C. foliacea* (p = 4.29e^{-6}), *C. furcata* (p = 4.74e^{-10}), *C. magyarica* (p = 2.44e^{-5}) and *D. muscorum* (p = 0.002) (Table S.6).

The ratio of chlorophylls to carotenoids was the lowest in *G. fulgens* and the highest in *D. muscorum* (Fig. 3d). The mean chlorophylls / carotenoids ratio usually peaked in autumn (2.12–4.74) and was the lowest in spring (0.59–4.01). Significant seasonal differences were detectable in *C. furcata* (p =1.80e^{-7}), *C. magyarica* (p = 4.2e^{-5}) and *D. muscorum* (p = 1.9e^{-7}) (Table S.7).

Significant differences were detected in the ratio of chlorophylls / carotenoids between sun and shade populations (shade > sun) of *C. foliacea* ($p = 1.48e^{-6}$), *C. furcata* ($p = 6.07e^{-8}$), *C. magyarica* ($p = 0.02$) and *D. muscorum* ($p = 1.68e^{-7}$) (Table S.7).

Partition of photochemical, regulated and non-regulated non-photochemical quenching in the different seasons and microhabitat types

The Fv/Fm values of the species (Table 2) provide information about the state of PSII. In general, Fv/Fm was higher in autumn, spring, and in shade thalli than in summer, winter and sun thalli of most species (see also Veres *et al*. 2020).

Table 2 The seasonal average values (± standard deviation of means) of maximum quantum yield of photosystem II photochemistry (Fv/Fm) measured in the sun and shade population of different lichen species during a year. (n = 20 / species / season / microhabitat, *Gyalolechia fulgens* n = 10 / season / microhabitat). *Gyalolechia fulgens* and *Xanthoparmelia pokornyi* occurred only on arid dune sides.

	ARID				**HUMID**			
Species	**spring**	**summer**	**autumn**	**winter**	**spring**	**summer**	**autumn**	**winter**
Cladonia convoluta	0.69 ± 0.03	0.51 ± 0.07	0.66 ± 0.03	0.65 ± 0.06	0.73 ± 0.03	0.63 ± 0.05	0.72 ± 0.03	0.64 ± 0.09
Cladonia furcata	0.67 ± 0.05	0.51 ± 0.05	0.67 ± 0.06	0.61 ± 0.09	0.71 ± 0.05	0.64 ± 0.09	0.73 ± 0.02	0.7 ± 0.06
Cladonia magyarica	0.67 ± 0.04	0.54 ± 0.06	0.59 ± 0.14	0.59 ± 0.1	0.68 ± 0.05	0.6 ± 0.07	0.72 ± 0.02	0.65 ± 0.06
Diploschistes muscorum	0.64 ± 0.03	0.52 ± 0.06	0.58 ± 0.11	0.56 ± 0.13	0.66 ± 0.04	0.56 ± 0.06	0.66 ± 0.02	0.6 ± 0.06
Gyalolechia fulgens	0.63 ± 0.02	0.53 ± 0.03	0.71 ± 0.02	0.45 ± 0.11				
Xanthoparmelia pokornyi	0.63 ± 0.06	0.6 ± 0.05	0.69 ± 0.02	0.64 ± 0.05				

The proportion of absorbed excitation energy transformed to photochemical quenching (φPSII) was significantly lower ($p < 0.00001$) in summer than in autumn or winter in each species, except for *G. fulgens* (Table 3, Table S.8). The photochemical quenching was also considerably higher during wintertime compared to summer and spring in *C. furcata* ($p < 0.00001$) and *X. pokornyi* ($p < 0.00001$) (Table 3, Table S.8). The photochemical quenching differed significantly between the sun and shade populations in only a few cases. However, the response of photochemical quenching to seasonal environmental changes seemed to be species-specific (Table 3, Table S.8).

Table 3 Mean partition (%) of photochemical (φPSII), regulated non-photochemical quenching (φNPQ) and non-regulated non-photochemical quenching (φNO) (%) in the different species, seasons and microhabitat types (A = arid, H = humid). Standard errors (%) are also shown. The sum of cells with the same background (white for A and grey for H) in a row is 100%. * = different letters indicating a significant difference between seasons within each species (n = 20 / species / season / microhabitat, *Gyalolechia fulgens* n = 10 / species / season / microhabitat)

	φPSII				**φNPQ**				**φNO**			
Species	**A**	*****	**H**	*****	**A**	*****	**H**	*****	**A**	*****	**H**	*****
Cladonia convoluta												
springing	21±4%	de	23±5%	cde	46±1%	ab	52±6%	a	33±1%	c	25±6%	cde
summer	13±5%	f	18±5%	e	17±9%	d	32±14%	c	70±13%	a	32±16%	b
autumn	31±5%	ab	32±6%	a	49±5%	ab	51±6%	a	20±02%	e	50±2%	e
winter	27±7%	abc	26±7%	bcd	42±1%	b	51±9%	a	32±12%	cd	23±11%	de
Cladonia furcata												
spring	18±5%	d	22±4%	cd	34±12%	bcd	45±13%	a	48±12%	b	32±11%	cd
summer	19±6%	d	20±8%	d	18±11%	e	28±13%	d	64±15%	a	34±17%	ab
autumn	39±6%	a	37±9%	a	39±5%	abc	44±7%	ab	22±4%	d	52±3%	d
winter	28±1%	bc	35±6%	ab	29±15%	cd	43±6%	ab	44±23%	bc	22±5%	d
Cladonia magyarica												
spring	22±7%	b	22±6%	bc	36±07%	bcd	42±1%	abcd	42±07%	ab	36±13%	bc
summer	16±5%	c	19±7%	bc	32±13%	d	44±13%	ab	52±14%	a	31±11%	bc
autumn	31±5%	a	33±7%	a	33±11%	cd	46±8%	a	36±16%	bc	37±3%	d
winter	22±5%	b	29±7%	a	33±12%	d	43±9%	abc	45±15%	ab	28±7%	cd
Diploschistes muscorum												
spring	24±5%	cd	23±4%	cd	33±8%	bc	37±5%	ab	43±09%	bc	40±6%	bcd
summer	19±4%	d	19±3%	d	26±13%	c	36±14%	ab	55±14%	a	36±14%	abc
autumn	33±4%	b	39±4%	a	31±7%	bc	35±6%	ab	35±1%	cde	45±4%	e
winter	25±1%	c	27±5%	c	28±9%	bc	42±8%	a	47±18%	ab	31±9%	de
Fulgensia fulgens												
spring	20±5%	b			47±6%	a			34±7%	b		
summer	15±4%	b			17±12%	b			67±13%	a		
autumn	32±2%	a			42±6%	a			25±2%	b		
winter	14±12%	b			08±18%	b			78±28%	a		
Parmelia pokornyi												
spring	14±7%	c			26±12%	b			60±14%	a		
summer	17±3%	c			18±12%	b			65±14%	a		
autumn	35±5%	a			42±8%	a			23±4%	b		
winter	27±7%	b			42±8%	a			30±11%	b		

A significantly lower ($p < 0.00001$) amount of the absorbed excitation energy could be dissipated in a regulated way (φNPQ) in summer than in other seasons for *C. foliacea*, *C. furcata* and *X. pokornyi* (Table 3, Table S.9). Meanwhile, *C. magyarica* ($p = 0.87$) did not show remarkable differences among the seasons, whereas *G. fulgens* and *D. muscorum* exhibited the opposite seasonal pattern (Table 3, Table S.9). Generally, the φNPQ was higher in the shaded (mean 28–52%)

compared to the exposed (mean 17–49%) microhabitats ($p < 2e^{-16}$). This difference was always significant during winter in each species, whereas a species-specific response was characteristic for the other seasons (Table 3, Table S.9).

The proportion of absorbed excitation energy transformed to φNO was significantly higher in summer ($p < 0.01$) than in other seasons in each species except for *G. fulgens,* where winter samples showed the highest values (Table 3, Table S.10). The φNO was usually higher in the exposed (mean 20–70%) than in the shaded (mean 18–52%) microhabitats (Table 3).

Discussion

Seasons

A significant seasonal trend was detectable in the concentration of lichen secondary metabolites, plastid pigments and the proportion between ways of excitation energy absorbed by the antenna complex. Higher irradiation and lower relative humidity (RH) characterized the climate in summer and late spring (Veres *et al*. 2020). In bright and warm seasons, thallus WC can reach even 90% at dawn, but the fast dehydration after sunrise (1–1.5 hours, Verseghy 1971) can partly protect the lichen thalli against the harmful effects of the high irradiation (Heber *et al*. 2001, Veerman *et al*. 2007). During hydration periods in the morning, repair of damage from the previous day may occur (Weissman *et al*. 2005). Low RH after sunrise shortens the rehydrated state, and therefore the duration of active metabolism in summer. During drought periods, RH does not exceed the critical level for active metabolism even at dawn (Verseghy 1971). Without nocturnal hydration, the damage could slowly accumulate (Mayaba and Beckett 2001). Besides, the water uptake of the species is also lower in summer in the region (Verseghy 1971). During the night, dew and high RH (e.g. Lange *et al*. 1990; Lange 2003; Raggio *et al*. 2014) can provide a water source for active metabolism including synthesis of solar radiation protectant LSMs. Moisture conditions were not favourable for both metabolite synthesis and repairing mechanisms during nights in drier seasons. The results of Rajczy (1982) supports our findings revealing that *C. furcata* and *C. foliacea* became wet between 22–23 p.m. and took up water until 1 a.m. between dunes at Fülöpháza in the middle of summer. One hour before sunrise, they took up water again. The morning dew was formed at sunrise (4.15 a.m.), and from that time, lichens lost water until 6–7 a.m. when the critical hydration level was reached. Because the lichen can only produce solar radiation protectant substances in the hydrated state (Solhaug *et al*. 2003), the decreased level of these metabolites in the dry summer may result from short active periods insufficient to provide required amounts of photosynthates for LSM synthesis (Solhaug & Gauslaa 2004) in summer. Our results showed that despite the fact that the highest amount of precipitation was measured in summer, only 13–19% of incoming radiation was used for photochemical quenching. Verseghy (1976) pointed out that lichens exhibited the lowest biomass production during summer in the Kiskunság region. The lowest biomass increase occurs in summer and the highest in autumn (Lange 2003b, Verseghy 1976). Days with dew and nocturnal rain contributes more to the annual net primary production than heavy rain (Lange 2003a, 2003b, Veste *et al*. 2001).

Furthermore, the short hydrated periods can increase the need for more efficient use of light due to the higher amount of chlorophylls and carotenoids (Demmig-Adams and Adams 1992; Pintado *et al*. 1997). However, the seasonal changes in chlorophyll concentrations could also reflect fluctuations in the photobiont populations (Tretiach *et al*. 2013). Vráblikova *et al*. (2006) and MacKenzie *et al*. (2001) also demonstrated seasonal variation in the content of lichen chlorophyll and xanthophyll which was related to seasonally changing light conditions. Our lichens were mostly dehydrated during summer. The protection of PSII is critical since photodamage can also occur in an air-dry thallus (Gauslaa and Solhaug 1999; Heber *et al*. 2010; Solhaug *et al*. 2003), when the RCs are also active (PSI is not or partially inhibited by desiccation). The dissipation of excess light energy is essential since excitation energy flow used for charge separation is not possible (Heber *et al*. 2006). It seems that in summer, the φNPQ was less involved in dissipating excess light energy (φNO > φNPQ), especially in arid microhabitats. Combined pathways of radiative and non-radiative deexcitation reactions were probably dominant in the form of heat and fluorescence, mainly due to closed PSII reaction centres (Klughammer and Schreiber 2008). Lichens probably possess mechanisms to protect PSII while desiccated or they can repair it upon rehydration. Lichens can protect the PSII due to reversible desiccation of the thallus. In the desiccated state, the energy quenching is mainly through non-regulated non-photochemical quenching mechanisms rather than by conformation changes in the chlorophyll-protein complex (Flores-Bavestrello *et al*. 2016). Furthermore, in summer, there were nights (8 days before sampling) when RH could not reach 80%, significantly reducing the chance for rehydration and starting active metabolism, and thus the regeneration of the PS, recovery of NPQ or the antioxidant enzyme production responsible for the deactivation of ROS (Aoussar *et al*. 2018; Veerman *et al*. 2007).

During winter and autumn, high and more stable air humidity and lower irradiation frequently occur (Lange 2003b, Veres *et al*. 2020), creating a more extended favourable environment for lichen metabolism and photosynthesis. The temperature dropped below the freezing point for only two days during the whole measuring period, one month before the winter sampling, allowing higher net photosynthesis than in harsh winters (Lange 2003b, Tuba *et al*. 2008). These may require a smaller chlorophyll antenna size for effective light-harvesting (Jin *et al*. 2001). In the hydrated state, the cortical transmittance (Dietz *et al*. 2000) and the production of light screening fungal metabolites increase (Solhaug *et al*. 2003). Similar results for *C. foliacea* were found by Farkas *et al*. (2020): the amount of usnic acid and fumarprotocetraric acid showed higher concentrates in winter than summer. The concentration of usnic acid in *Cladina mitis* was also significantly higher in autumn and winter (BeGora and Fahselt 2001). Gauslaa and McEvoy (2005) observed the reverse seasonal courses for the cortical solar radiation screening pigment, parietin in *Xanthoria parietina* peaked in summer. Furthermore, φNPQ was higher than φNO in our study, indicating that the antenna complex had safely dissipated the excessive light as heat and the energy flux of the photosynthetic apparatus worked efficiently during winter and autumn. Heber *et al*. (2006) also showed that the desiccation-induced quenching decreased in humid seasons and increased in late spring and summer. During autumn, on average, more than one-third of the excitation energy was utilized by photochemical processes leading to the most productive period of the year in semi-arid sandy grasslands, as also found by MacKenzie *et al*. (2001), Raggio *et al*. (2014) and Verseghy (1976). Tuba *et al*. (2008) pointed out that temperature had a significant role for the chlorophyll content of lichens and mosses. Our results suit to the measured values of Tuba *et al*. (2008) in *C. foliacea* and *C. furcata*. The temperature data originating from our measurements justify that there was a mild winter during the study period (Veres *et al*. 2020).

Aspect (microhabitats)

A more contrasting environmental difference may be required, for example, different forest stands (Gauslaa *et al*. 2006), to induce a clear difference between sun and shade lichen populations. The detected significant differences in photoacclimation and -protection between the sun and shade populations could be explained by factors characterizing arid and humid seasons (see the section on 'Seasons' above). However, the proportion of φNPQ was significantly impacted by the exposure of thalli, particularly in summer and winter. Less time is available for photosynthesis in sun populations during summer and in shade populations during winter. In winter, the direct illumination lasts longer on the exposed than on the shaded side of the dune because of later sunset time. In summer, the direct illumination on the arid side is shorter than that on the humid side because of the later sunrise. An effective light-harvesting (Pintado *et al*. 1997) and excess light dissipation (Vráblikov á *et al*. 2006), mainly via φNO (Flores-Bavestrello *et al*. 2016), is required in both seasons. The higher level of φNPQ (and lower proportion of φNO) on humid microhabitats indicates a longer active period. The more variable weather conditions (clear and overcast) result in a less contrasting difference between the sun and shade populations in spring and autumn. Our results also showed that the non-photochemical quenching parameters are more affected by the microhabitat conditions than that the photochemical quenching in most seasons.

Species-specific response

The seasonal variations of investigated parameters showed species-specific differences. Lichens are poikilohydric organisms that cannot actively regulate their water content. However, they can extend or shorten their metabolically active period and protect themselves due to different long term adaptation strategies (e.g. morphological traits). *Cladonia furcata* exhibits a thin cortical layer allowing rapid water uptake and fast water loss (Colesie *et al*. 2017; Dietz *et al*. 2000; Verseghy 1971). A thin cortex rapidly becomes more translucent during rehydration than a thick cortex. A thin cortex presumably contains less solar radiation protectant atranorin than a thicker cortex (needs further evidence). Therefore, a higher protection of PSs against the harmful excess light energy reaching the photobiont layer is needed (Heber *et al*. 2006), which may explain the high concentration of fumarprotocetraric acid accumulating on the surface of the hyphae in the photobiont layer (Honegger 1986). The moderate proportion of φNPQ and φNO (less non-photochemical quenching needed) supports the efficiency of absorbing light (Nguyen *et al*. 2013) of the metabolite. On the other hand, *C. furcata* can benefit from the thin cortex due to rapid air moisture uptake in humid periods (Verseghy 1971), explaining the highest φPSII among the species.

Cladonia magyarica showed a constant high atranorin concentration during the year compared to the seasonally changing level in *C. furcata*. The more stable humidity conditions beneath *C. magyarica* thalli due to the water retention ability of moss cushions (Colesie *et al*. 2012) and the higher water retention capacity of the species (Verseghy 1971) may allow a continued high level of solar radiation screening in the upper cortex (Heber *et al*. 2006; Solhaug *et al*. 2003).

Among the cortical solar radiation protective pigments, usnic acid in *C. foliacea* exhibited approximately four times higher concentrates than those measured for atranorin (*C. magyarica*, *C. furcata*). Furthermore, the species can avoid photoinhibition due to curling during desiccation (Barták *et al*. 2006). Usnic acid plays a significant role when the species is rehydrated and hence unfolded since the thalli have only a dense, upper cortex; otherwise, the thick, white medulla covers them (Verseghy 1971). The thicker cortex may contain a higher amount of cortical pigment (needs further evidence). Meanwhile, during the extended dehydrated period, long-lasting protection is required against harmful excess light indicated by the highest level of φNPQ. The working mechanisms of the two LSMs are also different. Atranorin is a more labile metabolite, reflects the incoming light (Nguyen *et al*. 2013) and has a low or moderate SPF (sun protection factor) (Nguyen *et al*. 2013); however, usnic acid absorbs light and functions as a very effective solar radiation screening pigment (Rancan *et al*. 2002) that also protects against PAR (McEvoy *et al*. 2007). Färber *et al*. (2014) also pointed out the difference in the efficiency of cortical pigments in pendulous lichen species.

The concentration of carotenoids can indicate both the size of the antenna complex and the xanthophyll pool as they could participate both in light-harvesting and in photoprotection (Demmig-Adams 1990; Müller *et al*. 2001). Although our investigation did not cover the qualitative analysis of the carotenoid composition, it did acquire useful information on the proportion of excessive light energy. In *D. muscorum,* the relatively low chlorophyll, the lowest carotenoid concentration among the species, and the relatively high proportion of φPSII suggest that this species can receive enough photons for assimilation due to the prolonged hydration period on moss cushions (Colesie *et al*. 2012). This lichen can protect itself by mechanisms other than φNPQ and φNO. The pale colour (high albedo) and the pruinose surface likely increase in light reflectance, thus protecting the thallus from excessive light. The highest chlorophyll / carotenoid ratio among the investigated species suggests that the mycobiont in this species provides strong screening pigments (Gauslaa and Goward 2020) or structure (Gauslaa *et al*. 2017) to protect the photobiont, which likely is adapted to the low light conditions inside the thallus (Demmig-Adam and Adams 1992).

Gyalolechia fulgens showed the lowest proportion of φPSII, φNPQ and chlorophyll concentration and the highest proportion of φNO and carotenoid level, suggesting that this species experiences a shorter period in the wet state compared to other species (Pintado *et al*. 1997). The highest carotenoid level likely contributes more to photoprotection due to the direct defence of the thylakoid membrane (Müller *et al*. 2001) than the anthraquinone compound or whitish pruina in the cortex.

Xanthoparmelia pokornyi, occurring only in arid microhabitats, showed the highest chlorophyll concentration and relatively high carotenoid concentration as well. Since the φNPQ was relatively low, the carotenoids could participate mainly in an effective light-harvesting caused by the rapid desiccation in the exposed microhabitats. The species-specific acclimation mechanisms were also confirmed by earlier studies on plastid pigment concentrations across different habitat types (Balarinová *et al*. 2014, Cempírková and Večeřová 2018, Dymova and Kuzivanova 2018; Paoli *et al*. 2010, 2017) and seasons (Higgins *et al*. 2015).

Conclusions

The different species can acclimate to the changing environmental conditions (humidity, light) by taking advantage of each season in various ways, as formerly documented by Paoli *et al*. (2017). During drier and brighter seasons, the mycobiont (by fungal screening in the cortex and fungal induced curling) in *C. furcata* and *C. foliacea*, has a significant role in the protection of the photosynthetic system. Meanwhile, in wetter seasons, under lower light intensity, the protecting role of the photobiont increases. Since the synthesis of light screening LSMs requires more time (days, Solhaug and Gauslaa 2004; Verma *et al*. 2012) than is necessary for the activation of non-photochemical quenching mechanisms (e.g. VAZ cycle 5–10 minutes, Müller *et al*. 2001), the latter seems to be a more efficient process during the short metabolically active period in drier and brighter seasons. In other species (e.g. *C. magyarica*), the mycobiont and photobiont increase the production of these substances simultaneously to achieve a more effective excess energy dissipation. The regulated non-photochemical quenching showed the highest variance among the species and the highest difference between sun and shade populations (compared to seasonal changes) and is thus the most species- and microhabitat-specific protection mechanism. Our results showed that only c. 25% of the absorbed light energy was utilized in photochemical processes. It confirms that the balance between energy conservation and dissipation is shifted towards dissipation in poikilohydric organisms compared to vascular plants (Heber *et al*. 2006).

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Declarations

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ORCIDs Katalin Veres: https://orcid.org/0000-0001-5818-4891; †Zsolt Csintalan: https://orcid.org/0000-0001-7744-3393; Edit Farkas: https://orcid.org/0000-0002-5245-1079

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Supplementary Material

Photoprotection and high-light acclimation in semi-arid grassland lichens – a co-operation by algal and fungal partners

Katalin Veres[1], †Zsolt Csintalan[2], Zsanett Laufer[3], Rita Engel[1], Krisztina Szabó[1], Edit Farkas[1]

[1]Institute of Ecology and Botany, Centre for Ecological Research, H-2163 Vácrátót, Alkotmány u. 2–4, Hungary

[2]Department of Plant Physiology and Ecology, Institute of Botany and Ecophysiology, Szent IstvánBiological Sciences, Hungarian University of Agriculture and Life Sciences, H-2100 Gödöllő, Páter K. u. 1, Hungary

[3]Bükk National Park Directorate, H-3304 Eger, Sánc u. 6, Hungary

Table S.1 Output of the three-way ANOVA analysis showing the combined effect of species, seasons and microhabitats on atranorin concentration. df = degrees of freedom, sum sq = sum of squares, mean sq = mean of the sum squares, signif. level = Significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	signif. level
species	1	1.72	1.722	3.955	0.048620	*
season	3	15.32	5.107	11.728	$6.40e^{-07}$	***
habitat	1	1.41	1.406	3.230	0.074416	.
species:season	3	19.07	6.357	14.598	$2.36e^{-08}$	***
species:habitat	1	2.74	2.741	6.294	0.013224	*
season:habitat	3	3.09	1.032	2.369	0.073150	.
species:season:habitat	3	8.91	2.969	6.819	0.000249	***
Cladonia furcata						
season	3	33.15	11.051	21.163	$6.2e^{-10}$	***
habitat	1	4.04	4.037	7.730	0.00692	**
season:habitat	3	8.64	2.880	5.516	0.00182	**
Cladonia magyarica						
season	3	1.238	0.4125	1.183	0.3223	
habitat	1	0.110	0.1103	0.316	0.5756	
season:habitat	3	3.363	1.1208	3.214	0.0278	*

Table S.2 Output of the three-way ANOVA analysis showing the combined effect of species, seasons and microhabitats on fumarprotocetraric acid concentration. df = degrees of freedom, sum sq = sum of squares, mean sq = mean of the sum squares, signif. level = Significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	signif. level
species	2	685.3	342.6	274,873	$< 2e^{-16}$	***
season	3	67,2	22,4	17,968	$1{,}9e^{-10}$	***
habitat	1	0,1	0,1	0,044	0,83397	
species:season	6	73,6	12,3	9,845	$1{,}35e^{-09}$	***
species:habitat	2	14,8	7,4	5,924	0,00313	**
season:habitat	3	36,6	12,2	9,795	$4{,}38e^{-06}$	***
species:season:habitat	6	38,7	6,5	5,18	$5{,}34e^{-05}$	***
Cladonia foliacea						
season	3	12.72	4.240	3.166	0.02951	*
habitat	1	5.86	5.864	4.379	0.03990	*
season:habitat	3	19.00	6.335	4.730	0.00455	**
Cladonia furcata						
season	3	125.79	41.93	27.129	$7.55e^{-12}$	***
habitat	1	8.23	8.23	5.325	0.0239	*
season:habitat	3	48.73	16.24	10.510	$8.15e^{-06}$	***
Cladonia magyarica						
season	3	2.31	0.7698	0.901	0.4453	
habitat	1	0.73	0.7277	0.851	0.3593	
season:habitat	3	7.63	2.5438	2.976	0.0372	*

Table S.3 Output of the three-way ANOVA analysis showing the combined effect of species, seasons and microhabitats on usnic acid concentration. df = degrees of freedom, sum sq = sum of squares, mean sq = mean of the sum squares, signif. level = Significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Cladonia foliacea	Df	Sum Sq	Mean Sq	F value	Pr(>F)	signif. level
season	3	86.51	28.835	6.836	0.000406	***
habitat	1	0.00	0.002	0.000	0.983553	
season:habitat	3	25.28	8.427	1.998	0.121924	

Table S.4 Output of the three-way ANOVA analysis showing the combined effect of species, seasons and microhabitats on chlorophyll *a* + *b* concentration. df = degrees of freedom, sum sq = sum of squares, mean sq = mean of the sum squares, signif. level = Significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	signif. level
species	5	1301323	260265	54.188	< 2e^{-16}	***
season	3	173178	57726	12.019	1.72e^{-07}	***
habitat	1	108342	108342	22.557	3.03e^{-06}	***
species:season	15	178994	11933	2.484	0.00176	**
species:habitat	3	27980	9327	1.942	0.12260	
season:habitat	3	1237	412	0.086	0.96774	
species:season:habitat	9	52180	5798	1.207	0.28947	
Cladonia foliacea						
season	3	191323	63774	12.164	1.58e^{-06}	***
habitat	1	2050	2050	0.391	0.534	
season:habitat	3	20198	6733	1.284	0.286	
Cladonia furcata						
season	3	69203	23068	5.086	0.002999	**
habitat	1	59296	59296	13.073	0.000552	***
season:habitat	3	17504	5835	1.286	0.285627	
Cladonia magyarica						
season	3	42409	14136	2.453	0.07021	.
habitat	1	60996	60996	10.583	0.00174	**
season:habitat	3	14298	4766	0.827	0.48335	
Diploschistes muscorum						
season	3	12802	4267	0.993	0.4017	
habitat	1	18003	18003	4.188	0.0446	*
season:habitat	3	2315	772	0.180	0.9099	
Gyalolechia fulgens						
season	3	14633	4878	3.208	0.0514	.
Xanthoparmelia pokornyi						
season	3	0.414	0.13801	2.832	0.0519	.

Table S.5 Output of the three-way ANOVA analysis showing the combined effect of species, seasons and microhabitats on chlorophyll *a* / *b* ratio. df = degrees of freedom, sum sq = sum of squares, mean sq = mean of the sum squares, signif. level = Significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	signif. level
species	5	25.981	5.196	119.839	< 2e^{-16}	***
season	3	1.313	0.438	10.093	2.21e^{-06}	***
habitat	1	0.017	0.017	0.383	0.5366	
species:season	15	4.540	0.303	6.980	2.94e^{-13}	***
species:habitat	3	0.076	0.025	0.586	0.6247	
season:habitat	3	0.346	0.115	2.660	0.0482	*
species:season:habitat	9	0.365	0.041	0.935	0.4951	
Cladonia foliacea						
season	3	2.9498	0.9833	28.471	3,00e^{-12}	***
habitat	1	0.0428	0.0428	1.239	0.269	
season:habitat	3	0.0533	0.0178	0.515	0.673	
Cladonia furcata						
season	3	0.1477	0.04923	3.857	0.0128	*
habitat	1	0.0113	0.01128	0.884	0.3503	
season:habitat	3	0.0116	0.00387	0.303	0.8231	
Cladonia magyarica						
season	3	0.1001	0.03335	2.391	0.07568	.
habitat	1	0.0324	0.03240	2.323	0.13189	
season:habitat	3	0.2221	0.07402	5.306	0.00232	**
Diploschistes muscorum						
season	3	0.8757	0.29190	8.244	9.56e^{-05}	***
habitat	1	0.0030	0.00301	0.085	0.7714	
season:habitat	3	0.4236	0.14121	3.988	0.0113	*
Gyalolechia fulgens						
season	3	1.368	0.4561	1.219	0.335	
Xanthoparmelia pokornyi						
season	3	1.562	0.5208	3.642	0.0216	*

Table S.6 Output of the three-way ANOVA analysis showing the combined effect of species, seasons and microhabitats on the concentration of carotenoids. df = degrees of freedom, sum sq = sum of squares, mean sq = mean of the sum squares, signif. level = Significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	signif. level
species	5	697733	139547	331.997	$< 2e^{-16}$	***
season	3	29237	9746	23.186	$1.16e^{-13}$	***
habitat	1	35866	35866	85.329	$< 2e^{-16}$	***
species:season	15	17394	1160	2.759	0.000492	***
species:habitat	3	1988	663	1.577	0.194739	
season:habitat	3	359	120	0.285	0.836391	
species:season:habitat	9	4747	527	1.255	0.260772	
Cladonia foliacea						
season	3	24213	8071	18.282	$6.41e^{-09}$	***
habitat	1	10928	10928	24.753	$4.29e^{-06}$	***
season:habitat	3	2322	774	1.753	0.164	
Cladonia furcata						
season	3	5371	1790	5.927	0.00113	**
habitat	1	15652	15652	51.823	$4.74e^{-10}$	***
season:habitat	3	369	123	0.407	0.74814	
Cladonia magyarica						
season	3	2300	767	1.853	0.145	
habitat	1	8426	8426	20.364	$2.44e^{-05}$	***
season:habitat	3	1599	533	1.288	0.285	
Diploschistes muscorum						
season	3	3117	1039	3.101	0.03245	*
habitat	1	3538	3538	10.556	0.00181	**
season:habitat	3	816	272	0.812	0.49178	
Gyalolechia fulgens						
season	3	5190	1730	1.328	0.3	
Xanthoparmelia pokornyi						
season	3	5753	1918	4.865	0.00609	**

Table S.7 Output of the three-way ANOVA analysis showing the combined effect of species, seasons and microhabitats on chlorophylls / carotenoids ratio. df = degrees of freedom, sum sq = sum of squares, mean sq = mean of the sum squares, signif. level = Significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	signif. level
species	5	239.09	47.82	459.130	< 2e^{-16}	***
season	3	7.75	2.58	24.817	1.59e^{-14}	***
habitat	1	10.21	10.21	98.061	< 2e^{-16}	***
species:season	15	7.31	0.49	4.682	3.46e^{-08}	***
species:habitat	3	2.27	0.76	7.278	9.64e^{-05}	***
season:habitat	3	0.93	0.31	2.991	0.0311	*
species:season:habitat	9	1.85	0.21	1.977	0.0412	*
Cladonia foliacea						
season	3	0.392	0.1305	1.307	0.279	
habitat	1	2.753	2.7528	27.563	1.48e^{-06}	***
season:habitat	3	0.359	0.1196	1.198	0.317	
Cladonia furcata						
season	3	2.724	0.9079	14.468	1.80e^{-07}	***
habitat	1	2.292	2.2916	36.519	6.07e^{-08}	***
season:habitat	3	1.270	0.4234	6.748	0.000448	***
Cladonia magyarica						
season	3	1.556	0.5186	8.926	4.2e^{-05}	***
habitat	1	0.320	0.3200	5.509	0.0217	*
season:habitat	3	0.152	0.0505	0.869	0.4610	
Diploschistes muscorum						
season	3	8.891	2.964	14.678	1.90e^{-07}	***
habitat	1	6.887	6.887	34.109	1.68e^{-07}	***
season:habitat	3	1.007	0.336	1.662	0.184	
Gyalolechia fulgens						
season	3	0.1782	0.05941	2.961	0.0637	.
Xanthoparmelia pokornyi						
season	3	26254	8751	1.539	0.221	

Table S.8 Output of the three-way ANOVA analysis showing the combined effect of species, seasons and microhabitats on the yield of photochemical electron transport (φPSII). df = degrees of freedom, sum sq = sum of squares, mean sq = mean of the sum squares, signif. level = Significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	signif. level
species	5	0.209	0.0419	11.154	$2.09e^{-10}$	***
season	3	3.179	1.0597	282.197	$< 2e^{-16}$	***
habitat	1	0.071	0.0712	18.962	$1.51e^{-05}$	***
species:season	15	0.355	0.0237	6.302	$9.69e^{-13}$	***
species:habitat	3	0.004	0.0014	0.361	0.781344	
season:habitat	3	0.012	0.0040	1.060	0.365203	
species:season:habitat	9	0.112	0.0125	3.325	0.000532	***
Cladonia foliacea						
season	3	0.5623	0.18744	58.586	$< 2e^{-16}$	***
habitat	1	0.0177	0.01770	5.533	0.0197	*
season:habitat	3	0.0244	0.00814	2.544	0.0576	.
Cladonia furcata						
season	3	1.0323	0.3441	65.868	$< 2e^{-16}$	***
habitat	1	0.0222	0.0222	4.248	0.0409	*
season:habitat	3	0.0503	0.0168	3.213	0.0245	*
Cladonia magyarica						
season	3	0.4866	0.16220	41.437	$< 2e^{-16}$	***
habitat	1	0.0347	0.03474	8.874	0.00333	**
season:habitat	3	0.0268	0.00893	2.282	0.08102	.
Diploschistes muscorum						
season	3	0.6283	0.20942	74.212	$< 2e^{-16}$	***
habitat	1	0.0097	0.00974	3.452	0.0649	.
season:habitat	3	0.0227	0.00758	2.687	0.0482	*
Gyalolechia fulgens						
season	3	0.2126	0.07088	15.76	$1.04e^{-06}$	***
Xanthoparmelia pokornyi						
season	3	0.6028	0.20094	61.15	$< 2e^{-16}$	***

Table S.9 Output of the three-way ANOVA analysis showing the combined effect of species, seasons and microhabitats on the non-photochemical quenching (φNPQ). df = degrees of freedom, sum sq = sum of squares, mean sq = mean of the sum squares, signif. level = Significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	signif. level
species	5	1.193	0.2387	22.794	$< 2e^{-16}$	***
season	3	2.358	0.7861	75.074	$< 2e^{-16}$	***
habitat	1	1.413	1.4125	134.903	$< 2e^{-16}$	***
species:season	15	2.570	0.1713	16.364	$< 2e^{-16}$	***
species:habitat	3	0.018	0.0061	0.585	0.62508	
season:habitat	3	0.142	0.0475	4.533	0.00368	**
species:season:habitat	9	0.118	0.0131	1.249	0.26147	
Cladonia foliacea						
season	3	1.9248	0.6416	69.841	$< 2e^{-16}$	***
habitat	1	0.3287	0.3287	35.783	$1.14e^{-08}$	***
season:habitat	3	0.1201	0.0400	4.358	0.00542	**
Cladonia furcata						
season	3	0.8289	0.2763	23.239	$1.34e^{-12}$	***
habitat	1	0.4695	0.4695	39.488	$2.83e^{-09}$	***
season:habitat	3	0.0480	0.0160	1.346	0.261	
Cladonia magyarica						
season	3	0.0078	0.0026	0.234	0.873	
habitat	1	0.4585	0.4585	41.457	$1.24e^{-09}$	***
season:habitat	3	0.0238	0.0079	0.717	0.543	
Diploschistes muscorum						
season	3	0.0807	0.02689	2.893	0.0370	*
habitat	1	0.2963	0.29631	31.886	$6.89e^{-08}$	***
season:habitat	3	0.0682	0.02273	2.446	0.0657	.
Gyalolechia fulgens						
season	3	1.0485	0.3495	26.36	$3.37e^{-09}$	***
Xanthoparmelia pokornyi						
season	3	0.9157	0.30524	29.17	$6.89e^{-13}$	***

Table S.10 Output of the three-way ANOVA analysis showing the combined effect of species, seasons and microhabitats on the yield of non-regulated excitation dissipation (φNO). df = degrees of freedom, sum sq = sum of squares, mean sq = mean of the sum squares, signif. level = Significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	signif. level
species	5	1.296	0.259	18.316	$< 2e^{-16}$	***
season	3	9.739	3.246	229.394	$< 2e^{-16}$	***
habitat	1	2.118	2.118	149.668	$< 2e^{-16}$	***
species:season	15	3.855	0.257	18.161	$< 2e^{-16}$	***
species:habitat	3	0.037	0.012	0.869	0.45689	
season:habitat	3	0.225	0.075	5.299	0.00128	**
species:season:habitat	9	0.328	0.036	2.576	0.00631	**
Cladonia foliacea						
season	3	4.237	1.4122	125.569	$< 2e^{-16}$	***
habitat	1	0.499	0.4990	44.367	$3.09e^{-10}$	***
season:habitat	3	0.205	0.0684	6.078	0.000577	***
Cladonia furcata						
season	3	3.0143	1.0048	56.849	$< 2e^{-16}$	***
habitat	1	0.6958	0.6958	39.367	$2.97e^{-09}$	***
season:habitat	3	0.1919	0.0640	3.619	0.0145	*
Cladonia magyarica						
season	3	0.5633	0.1878	14.419	$2.16e^{-08}$	***
habitat	1	0.7457	0.7457	57.259	$2.50e^{-12}$	***
season:habitat	3	0.0755	0.0252	1.931	0.126	
Diploschistes muscorum						
season	3	0.8589	0.2863	22.052	$4.43e^{-12}$	***
habitat	1	0.4135	0.4135	31.849	$7.00e^{-08}$	***
season:habitat	3	0.0806	0.0269	2.069	0.106	
Gyalolechia fulgens						
season	3	1.9493	0.6498	25.63	$4.76e^{-09}$	***
Xanthoparmelia pokornyi						
season	3	2.772	0.9242	69.59	$< 2e^{-16}$	***