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IG: participation in the research designing, identification and analysis of microfungal communities, writing the manuscript; MT: research designing, measurements of abiotic factors, sample collection, preparation of photos, identification and preparation of lichens, participation in the manuscript writing

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ORIGINAL RESEARCH PAPER

Interior of saxicolous lichens on different types of rocks as a habitat for microfungal communities in Upper Galilee, Israel

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Abstract

We examined the diversity and composition of fungi from the interior of saxicolous (rock inhabiting) lichens covering basaltic and chalk rocks at the Alma–Har-Ben-Zimra area of Upper Galilee, Israel. We also compared the composition of lichen-associated and soil microfungal communities inhabiting the two contrasting soil types in the area to trace possible sources of formation of endolichenic fungal assemblages. In the course of the study, 39 fungal species were isolated from the interior of 13 lichen species. Species richness of the endolichenic fungal communities was associated, to some extent, with the growth form of lichens, being higher in those lichens with thick, warted, and wrinkled thalli. Species composition of the communities was characterized by the dominance of melanin-containing microfungi with large, multicellular, and thick-walled spores that significantly increased in abundance in the summer. Dominant species were also known as endophytes and phylloplane-inhabiting fungi; at the same time, typical soil-borne species were extremely rare components of the isolated endolichenic communities. Some endolichenic melanized microfungi were comprised by coprophilous species prevailing in some lichen thalli; this observation was probably due to a long period of use of the studied area for cattle grazing. Protective morphological features are important for fungi inhabiting the interior of lichen thalli characterized by limited nutrient sources, low-water availability, and restricted aeration. In addition, endolichenic fungi should resist the activity of various extracellular secondary metabolites produced by their host lichen species.

Keywords

basaltic rocks; chalk rocks; endolichenic fungi; lichen thalli; melanin-containing fungi

Introduction

Fungi represent a group of organisms which are extremely diverse taxonomically, functionally, and ecologically. Fungi typically live in highly heterogeneous communities colonizing a great variety of substrates. Lichen thalli are one of such substrates supporting the formation of specific communities of endolichenic fungi. Endolichenic fungi are considered to be analogous to endophytic fungi of vascular plants because they inhabit the interior of a lichen thallus without causing any visible disease symptoms and are spread horizontally (e.g., [1,2]). During the last decades, the group of endolichenic fungi has been the subject of many studies in different geographical regions and ecosystems involving various lichen species on a variety of substrates using both culture-based and culture-independent molecular methodologies (e.g., [3–9]). Moreover, endolichenic fungi were found to produce a range of bioactive metabolites which makes them promising subjects for study in pharmacology and biotechnology (e.g., [10,11]).

In Israel, lichen thalli, as niches for endolichenic fungi, have not yet been explored, although lichen biota in the country is rich and diverse [12–14]. For our study of endolichenic fungi, we have chosen the Alma–Har-Ben-Zimra area located in the eastern Upper Galilee (Fig. 1). Here, undulating plateau surfaces are composed of calcareous rocks, mostly Cenomanian and Senonian chalk and marls, scattered with occasional basaltic rocks, including vesicular basalt and tuff of the Plio-Pleistocene age [15]. Two types of rocks dispersed on the soil surface, chalk and basaltic, are covered by rich and dense saxicolous (rock inhabiting) lichen communities. The bedrock lithology in the area also determines the key differences in mineralogical composition of the soils on chalk and basaltic rocks [16].

The present study focuses on the composition of endolichenic fungi associated with saxicolous lichen species inhabiting chalk and basaltic rocks in the Alma–Har-Ben-Zimra area of the Upper Galilee. The main goal of the study addresses the influence of different environmental aspects – growth form of lichen, type of rock, and season (sampling was conducted in the summer of 2017 and the winter of 2018) – on the composition and richness of endolichenic fungal communities. We also compared the composition of lichen-associated and soil microfungal communities inhabiting two contrasting soil types developed on different bedrock lithology to trace possible sources of formation of endolichenic fungal assemblages.

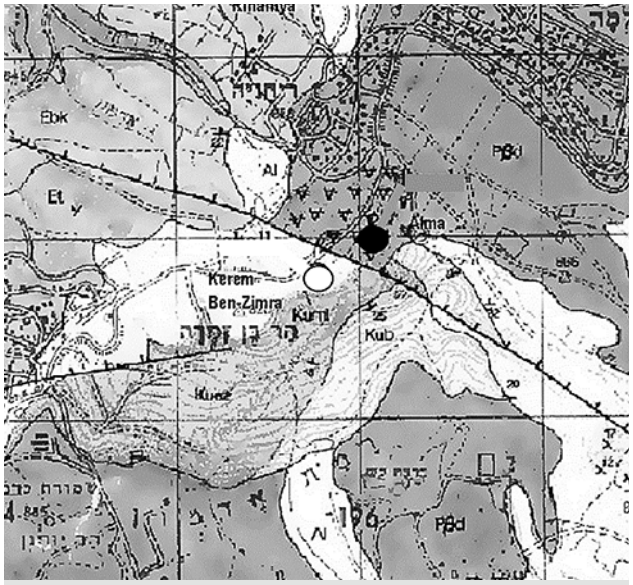

Fig. 1 Geological map of the Alma–Har-Ben-Zimra area, eastern Upper Galilee, Israel, showing the location of the sites with basaltic rocks on basaltic protovertisol (black circle) and chalk rocks on pale rendzina soil (white circle). The image is based on Levitte [46].

Material and methods

Site description

The study area (Fig. 1, Fig. 2A) is located in the northern part of Israel (eastern Upper Galilee, 33°02′ N, 35°29′ E). The climate of the region is Mediterranean, with a mean annual precipitation about 600 mm, with warm, dry summers (the mean temperature of the hottest months, July–August, is 22–27°C) and humid winters (the mean temperature of the coldest months, January–February, is 6–12°C) [17]. The sites chosen for sampling are characterized by contrasting soils developed on different bedrock lithology at slightly different altitudes [16]. The pale rendzina soil on Senonian soft chalk lies at 840 m above mean sea level (a.m.s.l.) and is covered, mainly, by prickly burnet (*Sarcopoterium spinosum*). Alternately, the site on basaltic rock occupies the plateau-like surface of the same slope at nearly 830 m a.m.s.l. and is covered mainly by carline thistle (*Carlina hispanica*) plant communities. Taxonomically, the soil belongs to basaltic protovertisols [16].

Chalk and basalt rocks are both alkaline, but they vary in mineralogical composition and physical properties. Chalk rocks are relatively soft (hardness ≈ 3), with a dense texture (Fig. 2B); while basalt rocks are hard (hardness = 6), with a vesicular texture containing many small holes [18,19] (Fig. 2C), and with a high resistance to weathering [20,21]. The porosity of basaltic rocks considerably exceeds that of chalk, resulting in different water holding capacities [22,23]. Because of the above differences, these rocks are characterized by a different composition of saxicolous lichen species (Fig. 2D,E). Notably, the studied area has been used for cattle grazing for, at least, 100 years and is still irregularly used for this. Therefore, most of the rock-inhabiting lichens found here belong to nitrophilous species that prefer dust- or nutrient-enriched substrates.

Sampling

Thirteen lichen species were collected from chalk and basaltic rocks in the summer of 2017 and the winter of 2018. Among them, three species (*Aspicilia calcarea*, *Caloplaca*

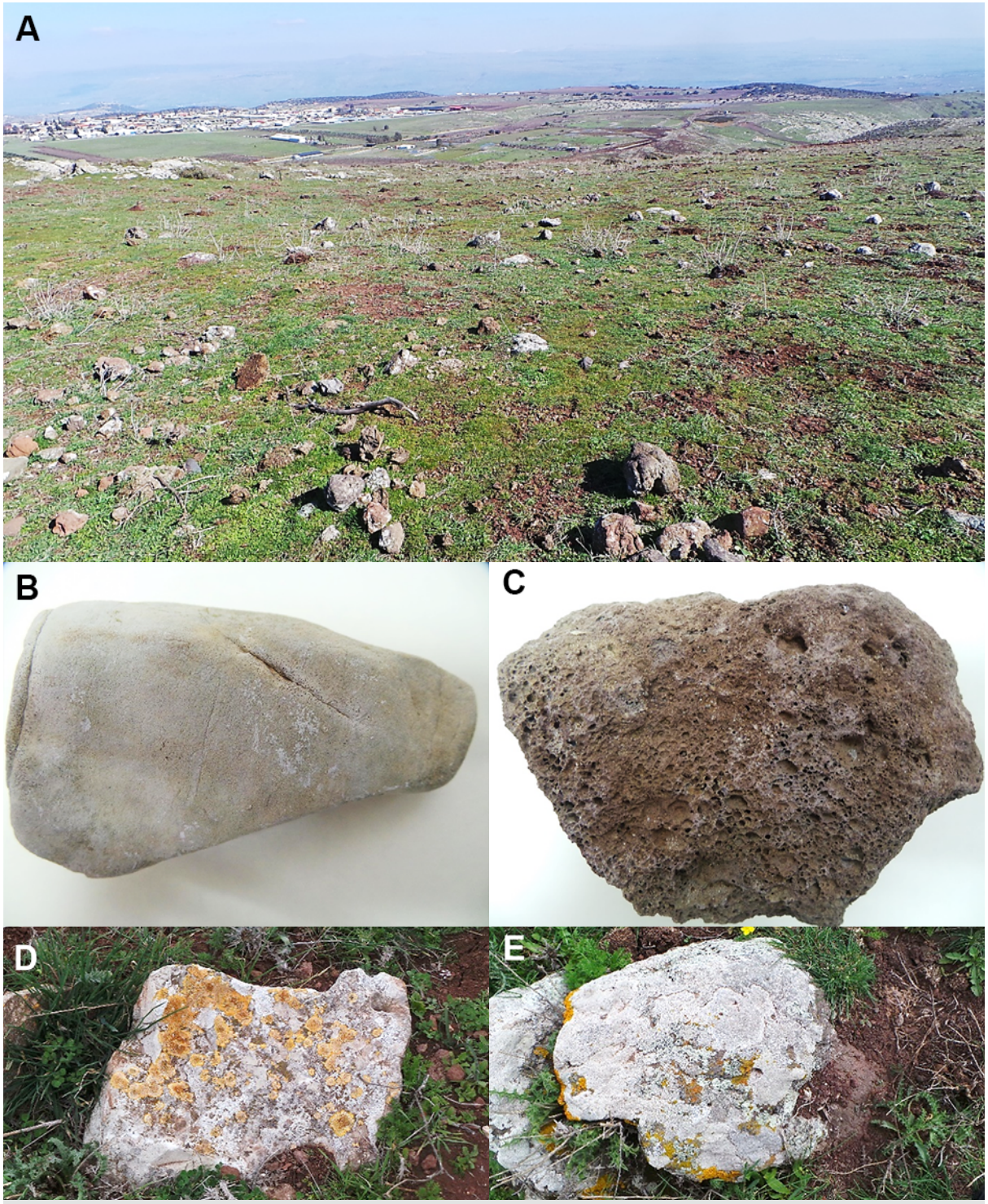

Fig. 2 General view of the research site at the Alma–Har-Ben-Zimra area, eastern Upper Galilee (**A**), containing chalk rocks with dense texture (**B**), basaltic rocks with vesicular texture (**C**), and lichen communities growing on chalk (**D**) and basaltic (**E**) rocks.

oasis, and *Lecanora albescens*) were collected only on chalk rocks, six species (*A. caesiocinerea*, *C. crenulatella*, *Diploschistes actinostomus*, *L. muralis*, *Neofuscelia verruculifera*, and *Tephromela grumosa*) only on basaltic rocks, and four species (*C. aurantia*, *C. lactea*, *Candelariella aurella*, and *Xanthoria calcicola*) on both substrates. In the study area, *C. aurantia*, *L. albescens*, *L. muralis*, and *X. calcicola* are the most common species with a rather wide coverage of the rock surface. Three randomly chosen samples of these lichens were collected in both seasons. The other nine species are less common, with a limited rock coverage; one to three samples of these species were collected only in the winter. Lichen specimens were sampled together with their substratum by chiseling

off pieces of the rocks covered with lichens. The samples were immediately placed in plastic bags, labeled, and taken to the laboratory. Samples were stored in a dry, cool place and processed within 5 days after collection.

Soil samples were collected from the uppermost horizons of the soils on chalk and basaltic lithology (randomly over an area of approximately 100 m^2 on each lithology) both in summer and winter. Samples were taken from sunny, open localities; three samples were obtained from each soil type and in each season. Samples were collected at a depth of 1 to 2 cm in sterile paper bags and stored before processing (1–5 days) in a dry, cool place.

Measurement of temperature, water-holding, and water release capacities of rocks

The surface temperature of ten randomly selected rocks of each type was measured at the warmest time of day (from 11 a.m. to 2 p.m.) with a Micron infrared thermometer (model M102HTL, USA) twice during the period of investigation: in winter (February) and in summer (July). Water-holding capacity (WHC) was determined as the increase in rock mass between dehydrated and saturated states [22,24]. Ten pieces of each type of rock were cleaned, oven-dried at 70°C for 48 hours and weighed to the nearest 0.01 mg. Rocks were then submerged in water for 15 minutes and reweighed to get wet weights. The maximum substrate water-holding capacity was determined as $(c - g)100/g$, where c is the wet weight of the rock, and g is the dry weight of the rock. To measure the rate and duration of water release, water-saturated rocks were weighed repeatedly while drying at room conditions (26°C and 60% relative humidity). The loss of weight of the rocks was recorded to the nearest 0.01 mg after 3 and 24 hours of drying.

Preparation of lichen thalli

Lichen samples were cleaned in tap water to remove earth, dirt, and debris from the surfaces, and then thoroughly washed under running tap water. Lichen thalli were surface-sterilized by consecutive immersion for 1 minute in 75% ethanol, 3 minutes in 2% sodium hypochlorite, and 30 seconds in 75% ethanol [3]. Lichen thalli were then dried by placing over sterile filter paper, exposed to ultraviolet (UV-C) light for 30 minutes for additional surface sterilization, and cut into three segments of approximately 1 mm^2 size using a sterile razor blade.

Isolation of fungal strains

Surface-sterilized pieces of lichen thalli were evenly spread over the surface of Malt Extract Agar (MEA) (Pronadisa, Laboratorios Conda S.A., Spain) in Petri dishes (90 mm diameter; one lichen sample per two Petri dishes). For isolation of soil fungi, the soil dilution plate method [25] was employed. Briefly, 1 mL of soil suspension, from a dilution 1:1,000 (soil:sterile water), was mixed with MEA at 40°C in Petri dishes. Streptomycin (Spectrum Chemical Mfg. Corp, USA) was added to the medium (100 µg/mL) to suppress bacterial growth. All plates were incubated at 25°C in darkness for 10–14 days.

After incubation, the emerging fungal colonies were transferred to MEA for purification and further taxonomic identification. In an attempt to induce sporulation, all nonsporulating isolates were also grown on oatmeal agar (Sigma-Aldrich Inc., USA) as recommended by Bills et al. [26]. Taxonomic identification was mainly based on morphological characteristics of fungal isolates. Four of the most frequently occurring nonsporulating strains of endolichenic fungi were subjected to molecular identification at Hy Laboratories Ltd., Israel. DNA was extracted from pure cultures according to the procedure described by Graham et al. [27], and each of the 18S-28S rRNA regions were amplified by PCR using a Hy-FID PCR kit (cat. No. 505; Hylabs, Israel). The resulting amplicons were sequenced using an ABI BigDye V1.1 Terminator Cycle Sequencing kit (Applied Biosystems, USA) and an ABI 3730 automated DNA gene analyzer according

to the manufacturer's instructions. Obtained sequences were aligned and compared to sequences available at the National Centre for Biotechnology Information (NCBI) (http://www.ncbi.nlm.nih.gov/) using BLASTN search [28]. All names of the identified species are cited according to the database of Kirk et al. (http://www.indexfungorum.org/).

Data analysis

For each endolichenic species, two parameters were calculated: frequency of occurrence as the percent of all lichen samples in which a particular species was observed, and relative abundance as the number of isolates of a particular species in the lichen sample/total number of all isolates in the sample.

For comparing abiotic parameters between chalk and basalt rocks, temperature and WHC, the nonparametric Kruskal–Wallis median test (STATISTICA 7.0) was used. To evaluate similarity between endolichenic fungal communities from different lichen species, types of rocks, and seasons, as well as between endolichenic and soil fungal communities, the clustering of communities based on species' relative abundance was made by the unweighted pair-group average method with Chi-squared distance as the distance coefficient. A nonmetric multidimensional scaling (NMDS) plot with Bray–Curtis dissimilarity matrix was performed for exploring the pattern in the distribution of endolichenic fungal communities. The ordination was performed using R version 3.5.1 (http://www.R-project.org/) with the package vegan. A three-way unbalanced ANOVA with interactions (XLSTAT; http://www.xlstat.com/) was used to test the effect of some environmental aspects, growth form of lichen, type of rock, and season, separately and in interaction, on species richness of the endolichenic communities and relative abundance of their dominant group: melanin-containing species with large multicellular and thick-walled spores.

Results

Abiotic conditions of substrates

Average summer and winter temperatures and WHC of substrates are shown in Tab. 1. The temperature of basaltic rocks in both seasons was higher than the temperature of chalk rocks by 3°C (Kruskal–Wallis test, $p = 0.001$ and $p = 0.0001$ in summer and winter, respectively). Our measurements of WHC showed that basaltic rock absorbed a sixfold higher amount of water than chalk rock after a simulated saturation event ($p = 0.0002$). The greater WHC allowed them to retain absorbed water over much longer periods of time than chalks. Basalts retained 60% of absorbed water after 3 hours of drying while chalks retained only 20%, but both kept 20% after 24 hours. Higher water absorbing and retention power of basalt is due to its great porosity. Pitted basalt surface (Fig. 2B) soaks up water like a sponge and provides channels for capillary absorption and retention of liquid water in numerous pores inside the rock.

Tab. 1 Measured abiotic parameters of basaltic and chalk rocks (mean ±*SD*, $n = 10$) at the Alma–Har-Ben-Zimra area, eastern Upper Galilee, Israel.

	Surface temperature (°C)			Water-holding capacity (g water/g rock)	
Rock type	Summer	Winter	Water retention (%)	After 3 h drying	After 24 h drying
Basalt	42.5 ±0.50	19.3 ±0.15	0.0585 ±0.0057	60 ±1.4	20 ±0.8
Chalk	39.6 ±0.75	16.3 ±0.15	0.0096 ±0.0028	20 ±0.6	20 ±0.5

Species composition

In the course of the study, 39 endolichenic species belonging to 24 genera were isolated from 13 lichen species (Tab. 2). Two types of nonsporulating strains remained unidentified. Except for one species from the phylum Basidiomycota, *Sporotrichum* sp., all other species represent the phylum Ascomycota, 10 of them are teleomorphic, producing sexual fruit bodies in culture.

The great majority of isolated fungi were melanin-containing: 33 species. The dominant core of endolichenic communities was formed by the following species: *Alternaria alternata*, *Pleospora tarda*, *A. atra*, and *Cladosporium cladosporioides*. They occurred in the studied lichens with high frequencies (79.2%, 79.2%, 75%, and 66.7%, respectively) and at comparatively high relative abundances (especially the first two species; Tab. 2). A set of melanized fungi with large multicellular and thick-walled spores dominated almost all endolichenic communities: they comprised 47–100% of the isolates. Relative abundance of this microfungal group was significantly influenced by season ($p = 0.046$). Moreover, this group also contained species belonging to the teleomorphic genera *Sordaria*, *Preussia*, and *Sporormiella* (Tab. 2), that are known by their coprophilous nature (e.g., [29]).

The number of species isolated from the interior of different lichen species varied from 5 to 13 (we only took into account lichen species represented by three samples, collected from a definite type of rock, in a specific season; Tab. 2). Maximal species richness was found in the thalli of *X. calcicola*, followed by *L. muralis*, both collected from basaltic rocks in the summer (13 and 12 species, respectively) (Tab. 3). The minimal number of species was isolated from the thalli of *A. caesiocinerea* and *C. crenulatella*, collected from basaltic rocks in the winter (five species). None of the environmental aspects, either separately or in interaction, significantly affected species richness of the endolichenic communities.

The clustering of endolichenic microfungal communities was based on those communities isolated only from lichen species represented by three samples, collected from a definite type of rock, in a specific season (Fig. 3). The majority of microfungal communities comprised one homogenous group except for the basalt winter communities from *L. muralis* and *A. caesiocinerea*, which clustered separately due to the dominance of different *Preussia* species (Tab. 2). Cluster analysis divided summer and winter communities, mainly due to the dominance of different species: *A. alternata* and *P. tarda* in the summer and winter communities, respectively (Tab. 2). Similarly, an NMDS ordination (Fig. 4) revealed seasonal differences in the composition of endolichenic microfungal communities, with some species rather confined to summer communities (e.g., *A. alternata*, *S. fimicola*, and *Fusarium oxysporum*) or winter communities (e.g., *P. tarda*, *C. cladosporioides*, and *Boeremia exigua*), or irrespective of a specific season (e.g., *A. atra*, *A. phragmospora*, and *Setophoma terrestris*). At the same time, the type of rock or lichen species apparently played a minor role in communities' divergence.

Comparison between endolichenic and soil microfungal communities

Microfungal communities isolated from two types of soil – pale rendzina and basaltic vertisol – contained several species that were also part of the endolichenic communities, these species are underlined in Tab. 4. In summer soil communities, *C. cladosporioides* prevailed; this species was also frequently isolated from lichen thalli (Tab. 2). Notably, species from the genus *Penicillium*, which comprised a substantial part of soil microfungal communities, with *P. simplicissimum* overwhelmingly dominating in the soil on chalk in summer (Tab. 4), were almost entirely absent in the tendolichenic communities (Tab. 2). Similarly, endolichenic communities did not contain any zygomycetous species, while *Rhizopus arrhizus* was frequently isolated from the soil of the studied area (Tab. 4). On the other hand, coprophilous teleomorphic ascomycetes, characteristic for the endolichenic mycobiota, did not appear in soil microfungal communities. Cluster analysis based on the relative abundance of species isolated in winter (Fig. 5) showed that soil fungal communities formed a separate group and clustered apart from the endolichenic communities.

Tab. 2 Endolichenic fungi isolated from different saxicolous lichens covered basaltic (bas) and chalk (ch) rocks in summer (s) and winter (w) at the Alma–Har-Ben-Zimra area, eastern Upper Galilee, with their relative abundance (%)[a]. Melanin-containing species are underlined; melanin-containing species with large multicellular spores are in bold.

	C.a.[b]				X.c.		L.a.		L.m.		Can.a.		A.c.	C.l.	C.o.	C.c.	N.v.	T.g.	A.ca.
	bas	ch	bas	ch	bas		ch		bas		ch	bas	ch	ch	bas	bas	bas	bas	bas
Species	s		w		s	w	s	w	s	w	w		w	w	w	w	w	w	w
Alternaria alternata	64.2	62.5	5	17.6	42	30.4	48	5.9	56.6	-	40.6	25	-	9.5	27.8	30	26.3	-	-
A. atra	8.5	13.7	-	-	8.2	8.7	9.3	17.6	6	6.7	10.8	-	8.3	4.8	11.1	10	-	-	8.3
A. phragmospora	4.7	10.2	-	-	4.8	8.7	18.7	5.9	3	-	5.4	-	-	-	-	-	-	-	-
Apiospora montagnei	-	-	-	-	-	-	-	-	3	-	-	-	-	-	-	-	-	-	-
Aspergillus alliaceus	-	-	-	-	1.6	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A. niger	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	5.3	-	-
A. terreus	-	-	-	-	-	4.3	-	-	-	-	-	-	-	-	-	-	-	-	-
Aureobasidium pullulans	-	-	-	5.9	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Boeremia exigua	1.9	-	-	5.9	3.2	4.3	-	-	-	6.7	-	-	-	-	-	10	-	-	-
Chaetomium murorum	-	-	-	-	-	4.3	-	-	-	-	-	-	-	-	-	-	-	-	-
Chaetomium sp.	-	-	5	-	-	-	-	-	-	-	-	25	-	-	-	-	-	-	-
Chaetomium sp.1	-	-	-	5.9	-	4.3	-	-	-	-	-	-	-	-	-	-	-	-	-
Cladosporium cladosporioides	4.7	-	5	11.8	1.6	8.7	1.3	17.6	11.9	20	8.1	25	-	47.6	16.7	-	-	27.3	-
C. sphaerosper-mum	-	-	-	-	-	-	-	-	3	-	-	-	-	-	-	-	-	-	-
C. spongiosum	-	-	-	-	-	-	-	-	-	-	2.7	-	-	-	-	-	-	-	-
Curvularia clavata	-	-	-	-	1.6	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Epicoccum nigrum	-	-	-	-	1.6	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Fusarium equiseti	-	1.1	-	-	1.6	-	-	-	-	-	-	-	-	-	-	-	-	-	-
F. oxysporum	-	3.4	-	-	1.6	-	-	5.9	1.5	-	-	-	-	-	-	-	-	-	-
Juxtiphoma eupyrena	-	-	-	-	-	-	-	-	-	-	-	-	-	9.5	-	-	21	-	-
Metarhizium carneum	-	-	-	-	-	-	1.3	-	-	-	-	-	-	-	-	-	-	-	-
Neocucurbitaria cava	-	-	-	-	-	-	-	5.9	-	-	-	-	-	-	-	-	-	-	-
Nigrospora oryzae	-	-	-	-	-	4.3	-	-	-	-	-	-	-	-	-	-	-	-	-
Papulaspora pannosa	-	1.1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Tab. 2 Continued

Species	C.a.[b]				X.c.		L.a.		L.m.		Can.a.		A.c.	C.l.	C.o.	C.c.	N.v.	T.g.	A.ca.
	bas	ch	bas	ch	bas		ch		bas		ch	bas	ch	ch	bas	bas	bas	bas	bas
	s		w		s	w	s	w	s	w	w		w	w	w	w	w	w	w
Paraboeremia putaminum	-	-	-	-	-	-	-	-	-	13.3	-	-	-	-	-	-	-	-	-
Penicillium simplicissimum	-	-	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Pleospora tarda	0.9	3.4	75	23.5	1.6	-	14.7	41.2	1.5	6.7	27	25	30	28.6	38.9	30	36.8	27.3	8.3
Preussia africana[c]	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	27.3	25
P. cymatomera[d]	-	-	-	-	-	-	-	-	-	40	-	-	-	-	-	-	-	18.1	-
Preussia sp.[e]																			41.7
Setophoma terrestris[f]	14.2	4.6	-	-	12.9	-	5.3	-	6	-	-	-	-	-	-	-	-	-	16.7
Scytalidium thermophilum	-	-	-	5.9	-	-	-	-	-	-	5.4	-	-	-	5.5	-	10.5	-	-
Sordaria fimicola	0.9	-	5	5.9	17.7	-	-	-	4.5	-	-	-	-	-	-	-	-	-	-
Sporormiella minima	-	-	-	-	-	-	-	-	-	6.7	-	-	-	-	-	-	-	-	-
Sporotrichum sp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	20	-	-	-
Trichoderma harzianum	-	-	-	-	-	-	-	-	1.5	-	-	-	-	-	-	-	-	-	-
Immature fruit bodies	-	-	-	17.6	-	4.3	-	-	1.5	-	-	-	-	-	-	-	-	-	-
Nonsporulated dark strains	-	-	-	-	-	17.4	1.3	-	-	-	-	-	-	-	-	-	-	-	-

[a] Lichen species represented by only one sample are excluded from the table.
[b] Abbreviation of lichen species: A.ca. – *Aspicilia caesiocinerea*; A.c. – *A. calcarea*; C.a. – *Caloplaca aurantia*; C.c. – *C. crenulatella*; C.l. – *C. lactea*; C.o. – *C. oasis*; Can.a. – *Candelariella aurella*; L.a. – *Lecanora albescens*; L.m. – *L. muralis*; N.v. – *Neofuscelia verruculifera*; T.g. – *Tephromela grumosa*; X.c. – *Xanthoria calcicola*.
[c–f] Identified by molecular analysis, GenBank accession: [c] MH878448; [d] KX710252; [e] MG977003; [f] MH046778.

Tab. 3 Number of fungal species isolated from different lichens at the Alma–Har-Ben-Zimra area, eastern Upper Galilee.

Lichen species	Lichen growth form[a]	Number of fungal species			
		Basalt		Chalk	
		Summer	Winter	Summer	Winter
Aspicilia caesiocinerea	3	-	5(3)[b]	-	-
A. calcarea	2	-	-	-	3(2)
Caloplaca aurantia	2	8(3)	6(3)	8(3)	9(3)
C. crenulatella	1	-	5(3)	-	-
C. lactea	1	-	3(1)	-	5(2)
C. oasis	1	-	-	-	5(2)
Candelariella aurella	1	-	4(2)	-	7(3)
Diploschistes actinostomus	3	-	2(1)	-	-
Lecanora albescens	2	-	-	8(3)	7(3)
L. muralis	4	12(3)	7(3)	-	-
Neofuscelia verruculifera	4	-	5(2)	-	-
Tephromela grumosa	3	-	4(2)	-	-
Xanthoria calcicola	4	13(3)	11(3)	-	-

[a] Growth forms: 1 – very thin, smooth, and crustose thallus; 2 – thick, rounded or placodioid, and more or less cracked areolated thallus; 3 – thick, warted, and areolated thallus; 4 – thick, wrinkled, and foliose thallus.
[b] In parenthesis – number of lichen samples.

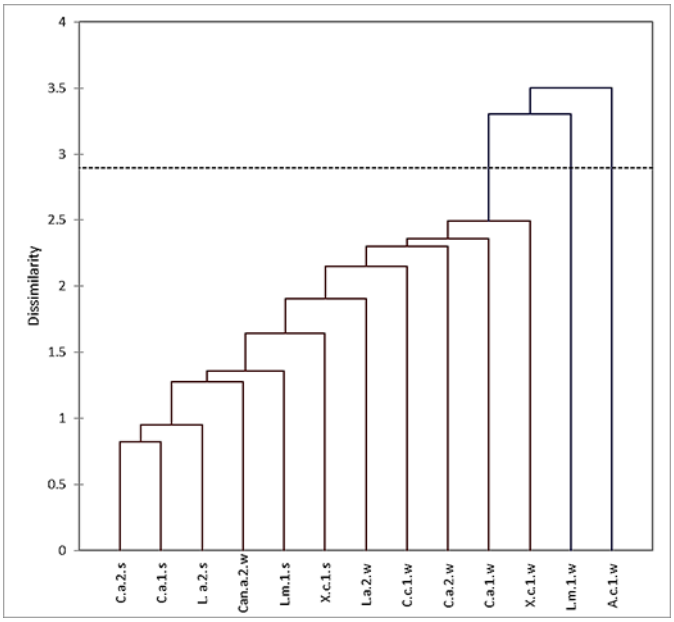

Fig. 3 Clustering of the endolichenic microfungal communities from different lichen species. A.c. – *Aspicilia caesiocinerea*; C.a. – *Caloplaca aurantia*; C.c. – *C. crenulatella*; Can.a. – *Candelariella aurella*; L.a. – *Lecanora albescens*; L.m. – *L. muralis*; X.c. – *Xanthoria calcicola* collected on basaltic (1) and chalk (2) rocks in summer (s) and winter (w) at the Alma–Har-Ben-Zimra area, eastern Upper Galilee, based on species relative abundance.

Discussion

Our study has shown that the thalli of saxicolous lichens (13 species) inhabiting basaltic and chalk rocks in the Upper Galilee harbor comparatively rich diversity of endolichenic microfungi: almost 40 species representing 24 genera. By species richness, this mycobiota can be compared, for example, with the endolichenic fungal assemblage (33 species) isolated from 11 species of epiphytic lichens collected in the forests of Uttarakhand, a northern state in India [8].

We did not find any significant influence of the studied environmental aspects – type of rocks, growth form of lichens, and season – on species richness of the endolichenic communities. Nevertheless, some relationships between growth form of lichens and the number of endolichenic fungi can be outlined. Species richness of fungi was higher in the lichens with thick, warted, and wrinkled thalli in comparison with the number of fungal species in lichens possessing rather thin, smooth, or cracked-areolated thalli (see Tab. 3).

The prevalence of melanin-containing microfungi with large, multicellular, and thick-walled spores, which significantly increased their abundance in summer, is the most prominent characteristic of the studied endolichenic communities. Such microfungi, as well as *Cladosporium* spp., are also known as endophytes (e.g., [30,31]) and belong to the group of phylloplane-inhabiting species [32,33].

Microfungi producing dark-colored multicellular spores dominated topsoil communities in the most microclimatically and edaphically stressful localities of the Negev

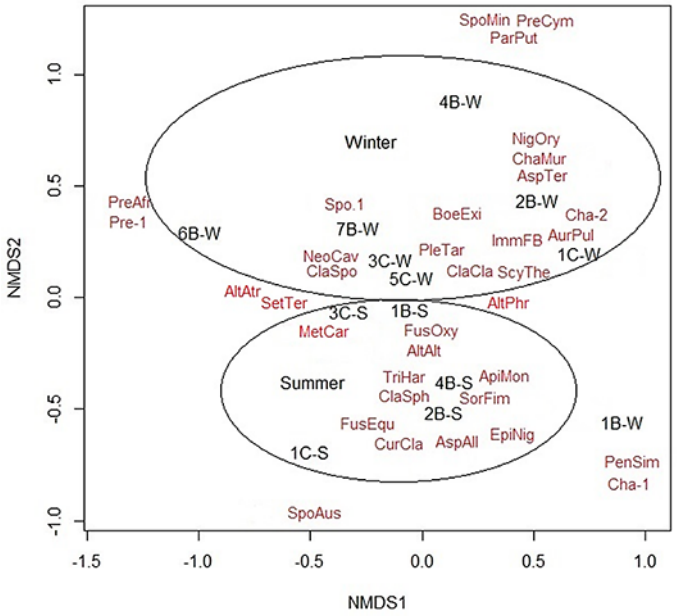

Fig. 4 NMDS ordination of endolichenic fungal communities by fungal species collected on basaltic (B) and chalk (C) rocks in summer (S) and winter (W) at the Alma–Har-Ben-Zimra area, eastern Upper Galilee. Fungal species abbreviations – AltAlt – *Alternaria alternata*; AltAtr – *A. atra*; AltPhr – *A. phragmospora*; ApiMon – *Apiospora montagnei*; AspAll – *Aspergillus alliaceus*; AspTer – *A. terreus*; AurPul – *Aureobasidium pullulans*; BoeExi – *Boeremia exigua*; ChaMur – *Chaetomium murorum*; Cha-1 – *Chaetomium* sp.; Cha-2 – *Chaetomium* sp.1; ClaCla – *Cladosporium cladosporioides*; ClaSph – *C. sphaerospermum*; ClaSpo – *C. spongiosum*; CurCla – *Curvularia clavata*; EpiNig – *Epicoccum nigrum*; FusEqu – *Fusarium equiseti*; FusOxy – *F. oxysporum*; MetCar – *Metarhizium carneum*; NeoCav – *Neocucurbitaria cava*; NigOry – *Nigrospora oryzae*; ParPut – *Paraboeremia putaminum*; PenSim – *Penicillium simplicissimum*; PleTar – *Pleospora tarda*; PreAfr – *Preussia africana*; PreCym – *P. cymatomera*; Pre-1 – *Preussia* sp.; ScyThe – *Scytalidium thermophilum*; SetTer – *Setophoma terrestris*; SorFim – *Sordaria fimicola*; SpoAus – *Sporormiella australis*; SpoMin – *S. minima*; Spo.1 – *Sporotrichum* sp.; TriHar – *Trichoderma harzianum*; ImmFB – immature fruit bodies. Lichen species: 1 – *Caloplaca aurantia*; 2 – *Xanthoria calcicola*; 3 – *Lecanora albescens*; 4 – *L. muralis*; 5 – *Candelariella aurella*; 6 – *Aspicilia caesiocinerea*; 7 – *Caloplaca crenulatella*.

and Arava deserts in Israel (e.g., [34–36]), where their spores apparently carry out both dispersal and resting functions. Moreover, melanized fungi with large many-celled conidia substantially also contributed to the communities at 10–50 cm of the fine-textured playa profiles at Wadi Nizzana, western Negev [37]. It indicates that melanin, which is known to protect fungal cells from various kinds of stresses (e.g., [38] and references therein), together with many-celled spore morphology, can support microfungi surviving under severe pressure of strongly limited aeration and high salinity. All of the above-mentioned protective morphological features are important for fungi inhabiting the interior of lichen thalli, an environment characterized by limited nutrient sources, low water availability, and restricted aeration. A harsh interior situation in saxicolous lichens is associated with harsh exterior conditions, with severe and sometimes rapid changes in temperature and hydration, as well as UV radiation. In addition, lichen-associated fungi should resist the activity of various extracellular secondary metabolites produced by their host species [39]. Israeli lichens are also known as sources of such metabolites, possessing significant antibacterial and antifungal activities [40–42].

Notably, a part of the endolichenic melanized microfungi was comprised by coprophilous teleomorphic species, *Sordaria fimicola*, *Sporormiella*, and *Preussia* spp.; in some lichen thalli, these species prevailed (see Tab. 2). Such a comparatively rich presence of

Tab. 4 Most frequently isolated microfungal species from the soil of the Alma–Har-Ben-Zimra area, eastern Upper Galilee, with their relative abundance (%). Underlined species are also found in the endolichenic communities.

Species	Pale rendzina		Basaltic vertisoil	
	Summer	Winter	Summer	Winter
Alternaria alternata	2.4	0.9	1.1	9.5
A. atra	9.1	5.3	6.0	12.1
A. phragmospora	7.0	1.8	2.5	5.8
Aspergillus alliaceus	-	-	0.8	-
A. niger	-	0.9	-	0.5
Aureobasidium pullulans	-	-	-	0.5
Beauveria bassiana	-	-	-	3.7
Boeremia exigua	-	-	6.9	1.0
Chaetomium sp.	-	-	1.1	-
Cladosporium cladosporioides	47.0	2.6	42.6	25.8
Clonostachys roseus	-	-	3.3	-
Fusarium equiseti	3.8	-	5.5	-
F. oxysporum	1.3	-	-	-
Humicola grisea	3.8	-	3.6	-
Mortierella humilis	0.5	-	-	-
Penicillium aurantiogriseum	-	-	-	3.7
P. simplicissimum	14.7	60.4	15.6	16.3
Pleospora tarda	2.7	-	-	-
Rhizopus arrhizus	0.5	13.2	1.1	4.2
Setophoma terrestris	6.7	12.3	9.9	11.1

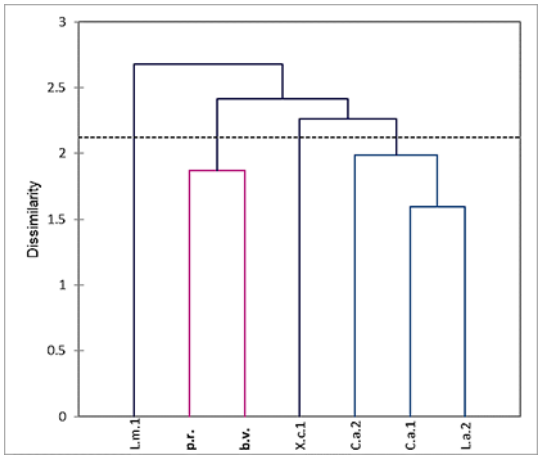

Fig. 5 Clustering of the endolichenic and soil microfungal communities isolated in winter at the Alma–Har-Ben-Zimra area, eastern Upper Galilee, based on species relative abundance. Abbreviations: C.a. – *Caloplaca aurantia*; L.a. – *Lecanora albescens*; L.m. – *L. muralis*; X.c. – *Xanthoria calcicola*; 1 – basaltic rocks; 2 – chalk rocks; p.r. – pale rendzina; b.v. – basaltic vertisol.

coprophilous microfungi, both in number of species and their abundance, might be caused by cattle grazing in the studied area over a long period of time.

By contrast, typical soil-borne fungi, such as fast reproducing *Penicillium* and *Aspergillus* species, as well as very fast-growing zygomycetes and mycoparasitic *Trichoderma* and *Clonostachys* spp., were extremely rare components of the studied endolichenic communities. Penicillii and aspergilli were considered as epithalline, unspecific, and ubiquitous contaminants which could be isolated from lichen thalli after insufficient surface sterilization [9,43]. At the same time, representatives of *Aspergillus*, *Penicillium*, and *Trichoderma* were recorded from epiphytic and terricolous macrolichens characterized by leaf-like or fruticose thalli [44]. Previous studies support the idea that the growth form of lichen hosts affects not only species richness but also the composition of associated fungi: species belonging to Eurotiomycetidae (includes *Penicillium* and *Aspergillus*) have been mainly isolated from foliose and fruticose macrolichens [1,2], while taxa from Dothideomycetes (includes the aforementioned melanin-containing species with large, multicellular, and thick-walled spores) are mainly recovered from epilithic crustose lichen thalli [39,45]. On a whole, our findings are in agreement with the assumption [44] that slow growing (and slow-reproducing) fungi might be more adapted to the conditions of the interior of lichens than the ubiquitous species, which are characterized by faster growth (and/or reproduction rate) and might experience nutrient deficiency in lichen thalli.

Our study revealed a comparatively rich diversity of fungi, 39 species, that inhabits the interior of saxicolous lichens (13 species) covering the basaltic and chalk rocks at the Alma–Har-Ben-Zimra area of Upper Galilee, Israel. Species richness of the endolichenic fungal communities, to some extent, was associated with growth form of lichens, being higher in the lichens with thick, warted, and wrinkled thalli. Species composition of the communities was characterized by the dominance of melanin-containing microfungi with protective spore morphology – large, multicellular, and thick-walled – with significant increases in their abundance in the summer. Dominant species were also known as endophytes and phylloplane-inhabiting fungi; at the same time, typical soil-borne species were extremely rare components of the isolated endolichenic communities. The present study is the first one devoted to lichen-associated fungi in Israel, but it unraveled some important regularities in the diversity and composition of their communities. Undoubtedly, this research should be continued involving new substrates and environments (e.g., desert areas), as well as employing both culture-based and culture-independent molecular approaches.

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