

ANALYSIS OF THE SAXICOLOUS LICHEN COMMUNITIES IN MĂCIN MOUNTAINS NATIONAL PARK

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Abstract: The assemblage of saxicolous lichenized fungal communities in Măcin Mountains National Park was assessed during a biodiversity study developed between 2006 and 2008. Fifty three species of saxicolous lichenized fungi were identified on Hercynic granites and granitoid outcrops characterized by intense weathering process. Apparently, competition was not the main mechanism in community assemblage as calculated C score showed (non-significant difference between mean calculated and simulated score). Niche overlap assessment showed that lichens avoided competition by spatial niche partition (mean Pianka index of 0.07 for sampling quadrats and 0.20 for locations). The estimation of nestedness index (N=0.63 at local scale and N=0.88 at sampling quadrat scale) indicated that local communities were subsets of a larger, regional scale metacommunity. Similarities in community composition across locations were assessed by means of Ward algorithm, results indicating that the most dissimilar communities were encountered at Pietrele Mariei, a residual inselberg and Suluc foothill. Conservation of saxicolous communities containing endangered species such as Umbilicaria grisea, critically endangered Ramalina obtusata and vulnerable Acrocordia gemmata, Pertusaria hemisphaerica, Pertusaria pertusa will be challenged in the future by anthropogenic impact coming from agriculture, sheep grazing and quarries operating in the proximity of the reserve area.

Keywords: community assemblage, saxicolous lichenized fungal community, Hercynic granites, C-score, niche overlap, nestedness, conservation of endangered species

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Introduction

One of the central goals of ecology is to identify the mechanisms that govern community assembly and structure (Horner-Devine et al. 2007, Beaudrot & Marshall 2011). Whether the co-occurrence of species is random or dictated by species interaction or abiotic factors is still a matter of debate. Assembly rules were advocated as driving the community composition, at the present day state of art, being understood as any constraint imposed to species association to establish a community (Diamond 1975, Connor & Simberloff 1979, Weiher & Keddy 1999, Maestre et al. 2008, Götzenberger et al. 2012) and describe co-occurrence patterns. In a larger context, metacommunity models advocate two categories of structuring forces: a) ecological niche differentiation through species interaction sand species sorting along ecological

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gradients and b) dispersal limitation (Leibold & Norberg 2004). Another structural feature of any community is species richness, maybe the simplest way to describe community and regional diversity (Magurran 1988). Species richness and species cooccurrence within communities are both modeled by environmental envelope, dispersion and species interactions.

Nestedness is a particular case of beta diversity exploring the level of among site variation in terms of species richness (Ulrich & Almeida-Neto 2012). However, nestedness and co-occurrence patterns estimated by some metric are both important in the quest of community assembly mechanisms and devised to be used as alternatives to explain the site or regional variation in species richness.

Saxicolous lichens are intimately linked to geochemical composition of the rocks they colonize as substrates, rock composition playing an important role in lichen community assembly (Purvis & Halls 1996) but other forces as competition or parasitism can be tested for community structuring. In a large area such as a mountain range, saxicolous lichens establish a metacommunity (*sensu* Leibold et al. 2004), with distinct local patches or local communities, Măcin Mountains National Park serving as an example for this concept.

Măcin National Park is a heterogeneous area with a variety of unique biotopes surrounded by agricultural landscape. A highly fragmented land use types represent the matrix of the reserve mosaic of biotopes. One may refer to the reserve as to an island in an agricultural ocean. The protected biotopes are of climax type or close to a climax state (Hansson & Angelstam 1991) therefore sensitive to anthropogenic stress. Măcin Mountains National Park margin represents a sharp transition to different types of anthropogenic ecosystems. Consequently, the various annual agricultural crops as wheat, barley, sunflower, canola and corn represent a threat rated as of medium risk due mostly to nitrate pollution (Badiu et al. 2014).

The present study addresses the topic of saxicolous lichens richness, community structure and the assembly rules leading to the current structure of spatially distinct communities developing on acidic substrate represented by granitic and granitoid rocks in Măcin Mountains National Park in South-Eastern Romania and establishing a metacommunity at regional scale. The study of saxicolous lichen communities was performed between 2006 and 2008 in the frame of GEF/UNDP project: Strengthening the National System of Protected Areas in Romania by Demonstrating the Best Management Practices in the Măcin Mountains National Park. The analysis should indicate whether sets of spatially distinct local communities were established by competition or some other species segregation/aggregation mechanism as main driving force (Diamond 1975) or/and are nested meaning that the assemblages in less speciesrich sites form non/random subsets of those at progressively species richer sites, a model proposed initially in island biogeography (Patterson & Atmar 1986). Both hypotheses on species assembly rules were tested for statistical significance using a hierarchy of null models which generally help in understanding which factors contribute mostly to the observed pattern in species assemblages as compared to a null, randomly assembled community (Feeley 2003). Two scales were employed in the analysis: the small scale of sampling quadrats and the scale of local communities covering different types of habitats within the investigated area (steppe versus woodland boulders and outcrops embedding vegetation, the foothills versus mountain ridges).

A sound ecological theory concerning community structure could serve for sustainable conservation of saxicolous lichen communities in Măcin Mountains National Park in the context of rare habitats established by the oldest rocks in Europe.

Material and methods

1. Description of the site and sampling

Măcin Mountains correspond to Northern Dobrogea orogenesis with Hercynic foundation of diverse geological composition dominated by Permo-Carboniferous formations (Carpelit strata) Hercynic granites and granitoid outcrops are characteristic for this area (Seghedi 2012) which harbor specific saxicolous lichen communities adapted to acidic substrate, with high silica content. Măcin Mountains unfold as parallel ridges oriented in North-Western to South-Eastern direction generating a characteristic ruiniform landscape. An important feature is the advanced erosion of the cliffs and rocks which determined the modest contemporary altitude of these mountains, the highest peak reaching only 467 m (Ţuţuiatu Peak) composed of ridges, pyramidal peaks, and steep slopes resulted from erosion. The geology of the area is characterized by Paleozoic weakly metamorphosed rocks, intruded by various igneous rocks in the Western part and by Mezosoic rocks predominating in the Eastern part of the range. The Paleozoic schists are protruded by intrusive granite and granodiorite rocks (Gavrilă 2012). Precambrian rocks are represented by amphibolites, schists and quartzites. Within the main basin, inselbergs represent an important geomorphologic feature.

The climate is temperate continental with hot summers, frequent droughts and cold winters with little snow. The average annual temperature is 11-11.5°C and average annual rainfall varies between 350-400 mm/m²/year. The main vegetation covers are represented by forests (forested steppe, mesophilic forests and Mediterranean forests) and xerophilous grasslands (Popescu & Doniță 2010, Gavrilă 2012).

Other protected habitats within this area are: transition between steppe and woodland areas and wetlands. However, most remarkable habitats are established by granitic and granitoid outcrops which harbor specific saxicolous associations such as *Koelerio lobatae-Semperviretum ruthenicae* Popescu & Doniță, *Moehringio grisebachi-Alyssetum saxatile* Popescu & Doniță and *Diantho nardiformis – Campanulo romanicae* Popescu & Doniță (Popescu & Doniță 2010) and lichen communities.

Locations of sampling sites were selected within the area of Măcin Mountains National Park (45°05'- 45°16'N: 28°12'-28°16'E). Sampling on rocks and boulders occurred in 14 locations, both in forested areas and on peaks where steppe vegetation prevails: A – Osmanului Ridge: B – Căpușa Peak: C - Arsu Peak: D – Caramalău Peak: E – Şerparu Saddle: F – Suluc foothill: G – Stănilă Hill: H – Cheia Peak: I – Vraju Peak: J – Chediu Canyon: K – Chediu Plateau: L – Pietrele Mariei Inselbergs: M-Sulucu Mare Peak: N – Crapcea Peak).

Caramalău and Vraju are residual peaks developed on granitoids containing the entire spectrum of weathering morphologies such as boulders, tors and exfoliated rocks (Gavrilă & Anghel 2013). Chediu canyon was formed by Chediu creek activity and is characterized by a milder local climate being composed of conglomerates and sandstone of Carboniferous origin. Pietrele Mariei is a specific type of geomorphosite for Măcin Mountains, consisting in isolated and eroded formations (inselbergs) which dominate the surrounding steppe (Fig. 1A). Caramalău Peak is characterized by steep slopes and advanced erosion of rocks generating a ruiniform landscape with tors (Gavrilă 2012). Same ruiniform geomorphology is encountered in Sulucu Mare ridge and Stănilă Hill.

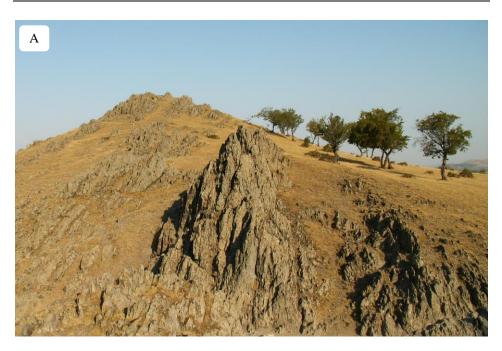




Fig. 1. Pietrele Mariei inselbergs (A); Granite quarry seen from Vraju Peak (B) (Photos Ovidiu Hâruţa)

2. Stressful factors affecting the protected area

The activity of several granite quarries (Fig. 1B) in the past represented the main disturbance in the area to which agriculture and sheep breeding contributed as stressful factors.

3. Species identification and matrix construction

Sampling was performed at random in the above mentioned locations and separately, in sampling quadrats *in situ*, also on photographs and collected material (rock fragments).

The identification was performed in laboratory conditions, mainly based on microscopic analysis and color reactions. For lichen identification various keys were employed and confronted with existing Romanian literature synthesized by Ciurchea (1998). A series of photographic images was taken to asses lichen species as additional identification tool. Nomenclature follows *Index Fungorum* specifications.

Photographs of standard areas were employed and observations from all mentioned locations were merged for the construction of the incidence matrix. The considered area for each plot corresponded to a microhabitat with homogenous lichen distribution, varying between 1m² and 0.25cm² (in accordance with Hale 1982), approximately 5 sampling quadrats per site. Another matrix was assembled for separate locations merging all observation made *in situ* and on photographs. The species resolution is generally high when sampling quadrats are considered still giving an incomplete projection of local community composition while inventory of species on random walk, in different locations has a low resolution but permits a more inclusive species inventory as a part of metacommunity composition.

4. Quantitative analysis

In order to make robust inferences about community structure and following previous recommendations (Feeley 2003), we employed a co-occurrence metric (C score) in combination with nestedness metric (incidence matrix temperature) to which additional information provided by ordination methods (non-metric multidimensional scaling and cluster analysis) was added. A hierarchy of null models, with increasing constraint degree was employed to better explore the significance of community structure testing results. The null models were introduced by Connor & Simberloff (1979) to test for non-random co-occurrence patterns. Incidence data were used to assess the sampling effort and completeness of species richness account.

4.1. Species accumulation curve

Sample rarefaction depicted by the species accumulation curve performed on incidence data is a tool for the assessment of sampling effort (Colwell et al. 2004) and species potential richness in the area. The sample based rarefaction computes the number of species s when a number of m samples are drawn at random from a set of samples (locations) (Gotelli & Colwell 2001) preserving the spatial aggregation or segregation between species. The illustrating accumulation curve in which on x axis are represented the samples or locations and on y axis, the accumulated number of species permits a graphical display of increasing number of species as function of area. The matrix with species identified in locations was employed for the assessment of the species accumulation curve.

The analytical solution is given by Mao's tau with standard deviations.

$$\tau(h) = S_{obs} - \sum_{i=1}^{H} \alpha_{ih} S_{j}$$

where: S_{obs} stands for number of observed species, j for samples, S_I the number of species found exactly in one sample, etc. The calculations and graphical representation were performed in PAST (Hammer et al. 2001). For species richness estimation, nonparametric Chao2 index was used devised for incidence data and more or less homogenous samples.

$$S_{Chao2} = S_{obs} + \frac{{q_1}^2}{2q_2}$$

Where: q_1 stands for number of species found exactly in one sample, q_2 is the number of species found exactly in 2 samples.

4.2. Co-occurrence C score

Presence-absence matrices (incidence matrices) are considered as fundamental units of analysis in community ecology (McCoy & Hech 1987), accordingly the present analysis was developed based on the presence- absence matrix of lichen species in selected locations across the national park.

The co-occurrence C score (Stone & Roberts 1990, Gotelli 2001) was employed to test the hypothesis of aggregation/segregation of the saxicolous lichens in the study area. However, initially, the index was devised for testing competition as driving force, yet there are opinions that a better interpretation of the results should be focused on aggregation due to some habitat characteristic or facilitation (Götzenberger et al. 2011). This algorithm was previously employed for lichens composing the biotic crusts of gypsum outcrops in Central Spain (Maestre et al. 2008). Co-occurrence was calculated over all possible pairs of species assuming the null model of random species assembly. If the number of non-co-occurring species combinations differs from the observed co-occurrences, the assembly is considered non-random and structured by competition (Sanderson et al. 1998) or other force driving specific species aggregation. C score represents the number of checkerboard units (CU) in species presence/absence matrix:

$$CU = (r_i - S)(r_j - S)$$

where: S is the number of shared sites (sites containing both species) and r_i and r_j are the row totals for species i and j. The C-score is the average of all possible checkerboard pairs, calculated for species that occur at least once in the matrix. The checkerboard units form sub-matrices that are exchanged using a swap algorithm (Gotelli & Enstminger 2001a).

A presence-absence matrix of sampling quadrats was constructed for the calculation of C score, rows corresponding to species and columns to unit samples. Another matrix was constructed on data collected from different locations across the investigated protected area.

Significance of calculated C score indices was tested using different null models. The null model is conceived as one that should generate a stochastic pattern with respect to an ecological process of interest such as community assembly (Moore & Swihart 2007). Models with insufficient structure are rejected as non-significant.

EcoSim software ver. 7 was employed (Gotelli & Enstminger 2001b) on 5000 simulated matrices to test the fixed rows and fixed columns sequential swap algorithm, rff (Connor & Simberloff 1979). Swapping creates a new matrix configuration without altering the row and column totals (Gotelli & Entsminger 2001a). Software R, package vegan (Oksanen et al. 2013) was employed to test alternative null models. The employed null models were: r00 null model - maintains the number of presences but fills them anywhere so that neither species nor sites are preserved, a maximally relaxed constrain on 1000 simulated matrices is known to be the least restrictive of the null models employed also in testing nestedness results (Wright et al. 1998); r0 - fills the presences anywhere on rows with no respect to species frequencies, assuming that species are observed with similar frequencies, testing mostly for species commonness; r1 - uses column marginal frequencies as probabilities which are proportional to observed species incidence frequencies, and r2 - fixes the row sums and assigns presences to each columns with probabilities proportional to squared incidence frequencies (Jonsson 2001). There was criticism in literature on r1 model as being prone to type I error (Simberloff & Martin 1991) which has led to r2 model by Jonsson in 2001, supposed to be a better model with respect to significance testing errors. Null communities (Gotelli & Graves 1996) are generated in this manner and test statistic or index is calculated for each generated null community. Null model is tested against the hypothesis that community is structured by interspecific competition or other form of aggregation triggered by some environmental factor.

A measure of departure from the null model of the observed index is given by SES (standardized effect size) which is basically the difference between calculated index (I_{obs}) and the mean of simulated indices (I_{sim}) obtained by sequential swap of the sub-matrices of the form 01/10 divided by the standard deviation of the simulated indices, (I_{obs} - I_{sim})/SD_{sim}. Significant departure from null model means that SES must be different from 0 (Sanders et al. 2003) with the probability generated by simulations reported.

4.3. Niche overlap

It is a community attribute that can be estimated using different indices, among which Pianka (1973) is one of the most utilized indices. Niche overlap predicts the degree of species interaction in sharing or competing for a food resource and was initially introduced by McArthur & Levins (1967). It is a symmetric index taking values in the range (0:1). EcoSim is testing the calculated index over 1000 simulations providing SES and a probability for assessing the significance of the calculated index using rff as null model, under the sequential swap algorithm.

4.4. Nestedness

It is a community univariate metric that illustrates a particular type of asymmetry in species interactions (Bascompte et al. 2003, Vásquez et al. 2005) and is one of the proposed assembly rules which states that subsets of species extracted from samples or local communities are nested within a larger community, at larger spatial scales (Patterson & Atmar 1986). Nestedness is calculated as T or the matrix temperature, a measure of how the presence/absence pattern departs from perfect nestedness; it is testing concomitantly for species incidence and species composition. The basic idea behind nestedness calculation is to assess the state of ordering (Ulrich & Gotelli 2007, Podani 2000) being performed for the present work with software Binmatnest

(Rodríguez-Gironés & Santamaría 2006) which finds the best minimum temperature matrix using a genetic algorithm. Matrix temperature is a normalized sum of squared distances of absences above and presences below the hypothetical isocline that separates occupied from non-occupied area in a perfectly nested matrix. Nestedness is a non-dimensional index and is calculated as N=100-T/100 for a matrix which contains lichen species on rows and sample units on columns. The matrix of interest is ordered according to marginal row and column totals, with most common species placed in the upper rows and species-rich sites placed in the left hand column. The nestedness is expressed as the concentration of presences in the left triangle of the matrix (Ulrich et al. 2009).

Statistical validation was performed against the null matrices using r00, r0, r1 and r2 (for explanations see table 2) in R package *vegan* version 2.0-10 (Oksanen et al. 2013). Null models were considered to exclude the constraint of interest in community assemblage (Gotelli 2001a) being liberal models such as r00 and with increasing degree of constraint in r0, r1 and r2. The interaction matrix was considered nested if the matrix temperature of the observed matrix was below or above the mean temperature of the 1000 simulated random matrices generated under the specified null model (Rodríguez-Gironés & Santamaría 2006).

4.5. Species ordination

NMDS: ordination of locations with respect to species composition was performed using non metric multidimensional scaling in PAST software. The method is based on a distance matrix such Euclidean distance and places the points iteratively in 2D in such a way that ranked differences are preserved (does not take absolute distances into account).

Clustering: similarities in species composition across unit samples and separately across different locations were analyzed using clustering, Ward algorithm (dendrogram generation and all the calculations were performed in PAST). In Ward algorithm the clusters are joint to minimize the within group variance, Euclidean distance measure being inhered in the algorithm.

Results

1. Identified species

Granite containing substrates generally harbor highly acidophilic crustose, umbilicate, fruticose and foliose lichens (Wolseley et al. 2006). Rocks can be compared to islands considering space discontinuities. The composition differs according to lichens positioning on the rock (southward as opposed to northward, on sunny expositions of the rock as opposed to shadowed expositions). However, the main characteristic is the rock type and the plots in this study were selected from granitic or granitoid rocks, rich in silica. Local saxicolous lichen communities are parts of the metacommunity established on granitic rocks within the Măcin Mountains range.

Species richness estimation produced different results at two scales of investigation: at microsite scale, using random sampling quadrats (not more than 5-7 per location) there were identified 34 species and at location scale, using observations made on random walk track, 53 species were identified (Table 1). Sample rarefaction indicates that more intense sampling should be performed at location scale (Fig. 2).

Table 1
Saxicolous lichen species identified in Măcin Mountains National Park. 2006-2008

Saxicolous lichen species identified in Măcin Mountains National Park, 2006-2008				
Species	Sampling locations [*]			
Acarospora badiofusca (Nyl.) Th. Fr.	CILM			
Acarospora bullata Anzi	HKL			
Acarospora cervina (Ach.) A. Massal	I			
Acarospora fuscata (Nyl.) Th. Fr	CEFHKL			
Acarospora smaragdula (Wahlenb.) A. Massal.	CIL			
Aspicilia caesiocinerea (Nyl. ex Malbr.) Arnold	DEFGIKLM			
Aspicilia cinerea (L.) Körb	BCFGHIJK			
Caloplaca crenularia (With.) R. Laudon	CL			
Caloplaca holocarpa (Hoffm ex Ach.) Wade	L			
Candelariella reflexa (Nyl.) Lettau	A			
Candelariella vitellina f. vitellina (Hoffm.) Müll.Arg	ABCDEFGHIJKLM N			
Chrysotrix chlorina (Ach.) J.R. Laundon	J			
Cladonia foliacea (Huds.) Willd	AC			
Collema flaccidum (Ach.) Ach.	J			
Dermatocarpon miniatum var. miniatum(L.)W. Mann	ACEHIK			
Dimelaena oreina (Ach.) Norman	DN			
Diploschistes actinostomus (Ach.)Zahlrb.	C			
Diploschistes scruposus (Schreb.) Norman	DEFHIKL			
Haematomma ochroleucum var. ochroleucum (Neck.) J.R.	F			
Laudon				
Lecanora argopholis (Ach.) Ach	ΑΕΗ			
Lecanora gangaleoides Nyl.	AEFKL			
Lecanora garovaglioi (Körb.) Zahlbr.	EL			
Lecanora muralis (Schreb.) Rabenh	FGHJN			
Lecanora silicea var. silicea Gyeln.	K			
Lecidea fuscoatra (L.) Ach.	EFKL			
Lepraria incana (L.) Ach	I K M			
Melanelia panniformis (Nyl.) Essl.	A C			
Melanelia stygia (L.) Essl.	G			
Melanelia tominii (Oxner) Essl.	ABDEFGHIKM			
Micarea erratica (Körb.) Hertel, Rambold & Pietschm.	L			
Parmelia saxatilis (L.) Ach.	BEFGHIKLM			
Pertusaria lactea (L.) Arnold	AFHIL			
Pertusaria pertusa (L.) Tuck	DH			
Physcia caesia (Hoffm.) Hampe ex Fürm.	F L M			
Physconia grisea (Lam.) Poelt	A			
Protoparmelia badia (Hoffm.) Haffelner	EΗ			
Ramalina farinacea (L.) Ach.	F			
Ramalina fastigiata (Pers.) Ach.	BEGH			
Ramalina obtusata (Arnold) Bitter	CGH			
Ramalina pollinaria (Westr.)Ach.	CFIL			
Ramalina polymorpha (Lilj.) Ach.	F			
Ramalina sp.	N			
Rhizocarpon geographicum (L.) DC	ABCDEFGHIL			
Squamarina cartilaginea (With.) P.James	C			

Tephromela atra (Huds.) Hafellner	LN
Umbilicaria grisea Ach.	A
Umbilicaria polyphylla (L.) Baumg.	E
Umbilicaria pustulata (L.) Hoffm	AC
Umbilicaria spodochroa Erh. ex Hoffm.	DEM
Xanthoparmelia conspersa (Ehrh. ex Ach.) Hale	ABFHIL
Xanthoparmelia pulla (Ach.) O. Blanco, A. Crespo, Elix, D.Hawksw. & Lumbosch	BDFH
Xanthoria elegans (Link.) Th. Fr.	J
Xanthoria parietina (L.) Beltr.	JL

*Sampling locations: A - Osmanului Ridge: B - Căpușa Peak: C - Arsu Peak: D - Caramalău Peak: E - Şerparu Saddle: F - Sulucu Mare foothill: G - Stănilă Hill: H - Cheia Peak: I - Vraju Peak: J - Chediu Canyon: K - Chediu Plateau: L - Pietrele Mariei Inselbergs: M- Sulucu Mare Peak: N - Crapcea Peak

Mean number of species in a sampling quadrat was 2.09 and, mean species number per location was 12.71.

Several of the identified species are included in the IUCN list of vulnerable and endangered species:

- regionally extinct in several countries in Europe is *Umbilicaria grisea*.
- critically endangered: Ramalina obtusata
- vulnerable: Acrocordia gemmata, Pertusaria pertusa

The compiled lichen lists in Măcin are very similar to lists reported from Turkey for Bursa district (Yazici & Aslan 2006) and Şirvan Mountain (Halici & Askoy 2006). In this context, *Melanelia stygia* is cited as being rare for Romania, while *Rhizocarpon geographicum* and *Xanthoparmelia pulla* considered being widespread (Cobanoğlu et al. 2011).

2. Species accumulation curve (sample rarefaction)

The accumulation curve of locations' matrix showed that species richness did not reach an asymptote, several infrequent or rare species still being absent from performed censuses (Fig. 2). Sample rarefaction is recommended to be used when smaller samples or sets of species are nested subsets of larger assembly of species (Gotelli & Colwell 2001).

Since asymptote was not reached, additional sampling would bring new species, contributors to regional species pool. In conjunction with Chao 2 species richness estimator which provides a stopping rule for species richness sampling, our data indicate that further studies will be needed to census fully saxicolous lichen diversity in the investigated area. Chao 2 index yielded a result that indicated that the species richness of the area should have been reached 65.91±8.12 saxicolous lichen species under a more extensive sampling effort.

3. Species co-occurrence

Species co-occurrence estimation at two scales, sampling quadrats and locations indicated that the restrictive *rff* null model discarded competition or other type of species interaction as the mechanism in community assembly (species co-occur randomly) (Table 2).

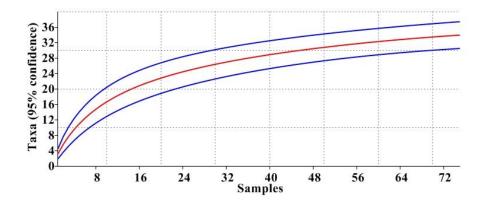


Fig. 2. Sample rarefaction curve of saxicolous lichen communities in Măcin Mountains National Park performed on locations matrix. Graphical representation generated in PAST. Blue curves correspond to confidence interval.

 $Table\ 2$ Results of C-score index calculation at small scale of sampling quadrats and larger scale of local communities across the Măcin Mountains National Park

C-score of local communities (incidence matrix)						
Null model	Calculated C	Mean simulated C	Z score (SES)	P_{simul}		
	score	score				
Rff	3.68	3.69	-0.27	0.57NS		
R00	52.62	88.05	-14.18	0.01**		
R0	52.62	54.24	0.50	0.67NS		
R1	52.62	53.64	-0.39	0.59NS		
R2	52.62	43.75	2.85	0.01**		
C-score of sampling quadrats (incidence matrix)						
Rff	34.69	34.78	-0.29	0.58NS		
R00	5.29	7.07	-18.53	0.01**		
R0	5.29	7.08	-19.99	0.01**		
R1	5.29	4.76	3.32	0.01**		
R2	5.29	7.04	3.97	0.01**		

On the contrary, null model r00, the least restrictive of the employed null models yielded a different result: species co-occur at small and local scale driven by competition or some aggregation promoting interaction factor (SES values were negative), a result yielded by the more restrictive r2 model also. The restricted null model being considered the most appropriate for assessing C-score results due to its robustness in type I errors (Ulrich et al. 2009) competition or other biotic interactions can be considered as less important mechanisms in community assembly of saxicolous lichens. Species interaction and habitat filtering are more likely to select species at microsite level since the different null models display stable significant results. Being pioneer organisms, lichens colonize the favorable substrata obeying to the preemptive model of competition, first colonizers arriving in a random sequence.

4. Niche overlap

Under the null model rff, niche overlap estimated by Pianka index yielded the observed mean index of 0.07 for sampling quadrats (simulated mean of 0.063 after 5000 iterations, SES=4.09, p \leq 0.001), and of 0.20 for locations (simulated mean of 3.69, SES=-0.27, p \leq 0.006). Both results are significant and indicate a relative separation of species' niches, more clearly in the case of microsite level observations. The results support the non-competitive nature of community assembly (still, the presumed mechanism to be considered, preemptive competition).

5. Nestedness

Nestedness temperature (N_{local com}=0.63; N_{sampling quad.}=0.88; see table 3 for nestedness temperature values) reflects the fact that at microsite level communities appear to be more structured than at local communities' level (Fig. 3A). However, the results are unstable according to employed null models (Table 3). The recommended r2 null model for testing nestedness significance (Jonsson 2001) yielded a significant result in the case of local communities and highly significant in the case of sampling quadrats. The richest local community was located at Pietrele Mariei inselbergs while the poorest, appeared to be at Stănilă Hill (Fig. 3B). Pietrele Mariei inselbergs are massive, relatively continuous rock formations while other locations are characterized by fragmented rocks, tors, exfoliated and weathered rocks supporting different abundance of saxicolous lichen species. The most frequently encountered species were: Candelariella vitellina, Parmelia saxatilis, Rhizocarpon geographicum, Lecanora gangaleoides, Pertusaria lactea, Acarospora bullata, Physcia caesia, Ramalina polynaria, Acarospora smaragdula, Tethromela atra, Xanthoria parietina, Lecanora garovaglioi, Caloplaca crenularia and Caloplaca holoplaca.

Table 3
Results of nestedness temperature calculation at small scale of sampling quadrats and larger scale of local communities across the Măcin Mountains National Park

Nestedness temperature of local communities incidence matrix							
null	NT _{calc.}	Mean simulated	Z score	Matrix	P _{simul} (1000		
model		NT	(SES)	fill	iterations)		
R00	36.908	53.23	-3.17	0.23	0.0009***		
R0	36.908	22.202	4.116	0.23	0.0009***		
R1	36.62	33.49	0.77	0.23	0.46 NS		
R2	36.94	22.75	4.22	0.23	0.02**		
Nestedness temperature of sampling quadrats incidence matrix							
R00	11.03	25.25	-5.74	0.09	0.0009***		
R0	11.13	10.12	0.78	0.09	0.44NS		
R1	11.19	7.64	3.64	0.09	0.0009***		
R2	11.11	5.34	7.19	0.09	0.0009***		

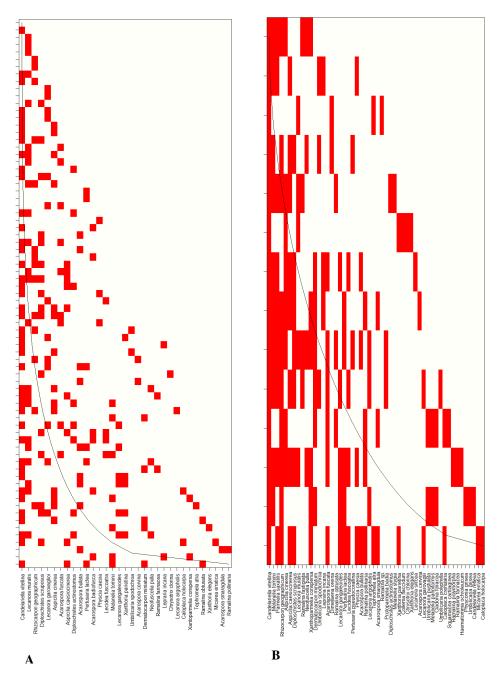


Fig. 3. Nested matrices of sampling quadrats (A) and local communities (B). Curves correspond to isoclines of perfect nestedness

6. Species ordinations: NMDS and clustering

Locations characterized by dissimilar lichen communities according to the performed ordinations (clustering analysis and non-metric multidimensional scaling, were: Osmanului Ridge, Arsu Peak, Pietrele Mariei Inselbergs, Sulucu Mare foothill, Cheia Peak, Chediu Canyon and Şerparu Saddle. The site differences are indeed significant: Chediu Canyon is more humid and harbors abundant woody vegetation interspersed with cliffs and outcrops, other locations are more xerophytic, with steppe and sparse woody vegetation, with exposed rocks and various degrees of anthropogenic influences. The dissimilar locations in terms of species composition cluster in two separate clusters merging Pietrele Mariei inselbergs with Sulucu Mare Foothill and a separate cluster merging Osmanului Ridge and Arsu Peak characterized by xerophytic steppe vegetation and sparse woody vegetation exposed to anthropogenic pressure from surrounding agricultural lands, Chediu Canyon and Crapcea Peak with alternate mesophytic forests and steppe interspersed with sparse woody vegetation (Figs 4, 5).

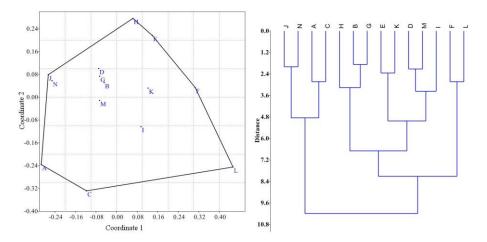


Fig. 4. NMDS ordination (using Euclidean distance) performed on saxicolous lichen communities identified in 14 different locations in Măcin Mountains National Park. Notations same as in Table 1

Fig. 5. Clustering of locations according to saxicolous lichen species composition in Măcin Mountains (Ward agglomerative algorithm). Notations as in Table

The remaining locations harbor lichen communities sharing many common species under more similar environmental conditions (exposed peaks with ruiniform landscape, scattered outcrops and steppe vegetation).

Discussion

Saxicolous lichens in Măcin Mountains establish a metacommunity (sensu Leibold et al. 2004), with local patches whose composition vary according to substrate (various granitic rocks), by the nature of the rock, light availability, competition with

other lichen species or plants, moisture level (Will-Worf et al. 2004), and various anthropogenic influences such as eutrophication. Metacommunities are characterized by species with unequal local abundances, with more frequent species in the area to be more likely to colonize a new patch of habitat, larger sites harboring many species (Ulrich et al. 2009). Same pattern was observed in the case of saxicolous lichens in Măcin Mountains range.

A section of granitic boulders harbors 10-15 species of lichens as cited for Canada (Hale 1982). It is considered that lichens occupy surface at scales corresponding to microhabitat variation. At scales larger than 0.01-0.25 m² the distribution of lichens will be heterogeneous. Our observations performed on quadrats *in situ*, *ex situ* (on photographs) and on small rock fragments indicated that the average number of species was lower, around 2.09. Also, community composition differed strongly among microhabitats, the degree of structuring being correlated to higher nestedness highlighting the non-random assembly of species at small scale.

The local communities displayed nestedness considering that rocks and boulders represent a highly fragmented habitat. It is a pattern of species co-occurrence intrinsically related to species aggregation but different mechanisms can lead to nested patterns, stochastic and deterministic (Ulrich et al. 2009), lichens displaying the both. Propagula dispersed by wind, random arrival on a substrate and then the influence of preemptive competition between thalli are the mechanisms leading to community establishment on rocks. The demonstrated competition in lichen guilds (Armstrong & Welch 2007) is mostly preeemtive (Hestmark et al. 2007) in nature but it is not the only species interaction observed among lichen thalli. Competition is largely overrated since biotic interactions include other negative and positive types such as parasitism or facilitation. Diploschistes scruposus, Candelariella vitellina and Rhizocarpon geographicum are initially lichenicolous, parasitizing other lichens (Will-Wolf et al. 2004). So far, these species are among the most frequently identified on granitic rocks of Măcin Mountains. At small scale, community appears to be assembled by dispersal and the ability to establish given the abiotic combination of factors, a mechanism observed in other types of communities also (Lindo et al. 2008) being an explanation for the nested community structure. Modest overlap of species niches is consistent with non-significant competition in the case of saxicolous lichens, the most probable competition mechanism to be considered being preemptive competition.

Testing nestedness under the assumptions of several null models with increasing constrain in randomness generating process in order to comply to some biological criterion indicated that r00 null model, the least restrictive provided significance to nestedness result in both matrices, one to summarize species richness at local scale and the one based on sampling quadrates. R0 null model test the community structure with focus on species commonness, and nestedness of the empirical matrices again departed significantly from random. This null model generates pattern under the assumption that species vary in terms of commonness and rarity but sites are characterized by the same species richness (Jonsson 2001). Null models that incorporate constraints (r1 and r2) on row and column totals address more subtle structural characteristics than previous models namely the mechanisms generating the actual composition of community such as competition, niche partition and nestedness. The most conservative of the models with fixed row and column sums was successfully applied to test the significance of co-

occurrence metrics (C-score is one example) (Gotelli 2001a) and more recently nestedness (Moore & Swihart 2007). However, in testing C-score the rff null model is largely employed because it is robust to type I errors and in testing nestedness r00 and r0 are the preferred null models to be tested. According to the more stringent rff model, saxicolous lichen communities in Măcin area are not driven by competition but are significantly nested at both scales (under the null model r2).

Lichens are considered stress tolerant organisms according to Grime classification (1979) and competition plays an important role in community structure (Armstrong & Welch 2007) but co-occurrence analysis has failed to extract competition mechanism as important for saxicolous lichen communities. If equiprobable null models are used, nestedness and co-occurrence scores such as C-score produce similar, significant results; under less liberal null models this correlation does not hold (Ulrich et al. 2009) as our results indicate too.

Cliffs and rocks support diverse lichenological guilds together with rare and diverse vascular plants (Matthes et al. 2004). Umbilicate lichens as *Umbilicaria spodochroa*, *Umbilicaria grisea*, *Umbilicaria pustulata* or *Dermatocarpon miniatum* are prone to destruction due to climbers (on rocks) and sheep grazing (on boulders). Among the crustose lithophilous lichens, species display different degrees of tolerance toward anthropogenic stress: *Acarospora fuscata* is tolerant, the infrequent *Melanelia panniformis* is sensitive (Paukov & Trapeznikova 2004).

Among fruticose lichens building extended covers on boulders and rocks was observed *Xanthoparmelia conspersa* at Şerparu Saddle, a species known to react with the substrate, leading to the rock exfoliation (Schatz 1963), one of important geomorphological characteristics of the area.

For lichens vegetating on acidic rocks, one of the most threatening factors are nitrogen deposits. Nitrogen enrichment is caused by grazing sheep and their excrements. Frequently, in saxicolous lichen thalli were found parasitical Ixodidae demonstrating the intense grazing in the boulders' vicinity. Agricultural activities influence lichen guilds composition directly by destruction or indirectly by eutrophication; consequently lichen communities in agricultural landscapes are severely impoverished (Motiejûnaitë & Faútynowicz 2005). Most diverse saxicolous lichen communities are associated in Măcin with peaks and plateau rocky environment were grazing pressure is not so high. For instance species from genera such as Parmelia and Ramalina are relatively indifferent to levels of acidification or nitrogen addition, Xanthoria parietina is nitrophyte (Sparrius 2004) and is found on rocks in the majority of locations being accordingly, tolerant to stresses induced by anthropogenic activities in the area. However, R. geographicum is one of the nitrophilous and acidophilous species encountered also on man-made substrates such as walls, consequently tolerant to anthropogenic disturbance. Parmelia saxatilis is acidophilous and Candelariella vitellina, Acarospora fuscata, Tephromela atra are nitrophilous their association being linked elsewhere with granite boulders in pastures and arable lands (Wolseley et al. 2006). The nitrophilous associations could be used as indicators for agricultural pressure on adjacent natural habitats harboring saxicolous lichen communities. It was previously stressed that the agriculture based on ploughing is a serious threat to protected areas in Măcin Mountains (Badiu et al. 2014) due to the specificity of the

landscape: the mountains are immersed in a matrix of cultivated land and are intensely grazed by sheep lately.

Because of the slow growth and the environmental sensitivity of most lichen species, a habitat approach to their conservation is a practical approach (Knudsen & Magney 2006). Lichen guilds cover the substrate in various proportions which is sometimes an indication of successional stage in rock colonization. The cover for different crustose species on rocks depends on life history, initial growth rate, maximal size possible to be attained, maturation, death rate and propagule type. Covers in the investigated area differ significantly depending also on exposition and disturbance regime. High mortality was observed on top areas of the boulders (*Melanelia tominii, Kanthoparmelia saxatilis* thalli) where the desiccation is most severe, proving once more that rocks represent an extreme environment.

It is worth to make efforts toward saxicolous lichens' conservation for their multiple functions: increase of structural complexity, influence on nutrient cycles, providing habitats for various invertebrates and nesting material for mammals and birds (Perez et al. 2004).

Advocating lichens' conservation, one argument refers to their scenic beauty, argument that Knudsen & Magney (2006) consider that has been neglected in the past and must be taken in consideration in the present in conservation plans.

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