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LICHEN SPECIES DIVERSITY OF *PINUS MUGO* SCRUBS COMPARED TO ADJACENT HABITAT TYPES IN THE RODNEI MTS. NATIONAL PARK (ROMANIA)

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Abstract: We explored: i) lichen species richness and composition of *Pinus mugo* shrubs and adjacent habitat types, alpine vegetation and spruce forests and ii) the distribution of lichen species in the *P. mugo* shrubs in relation to environmental factors. Epiphytic, terricolous, saxicolous and lignicolous lichens were considered. The total number of species and the mean species density in one hectare plots are both lower in the *Pinus mugo* shrubs than in the other two habitat types. However, *P. mugo* shrubs harbour 15 species that are not present in the other habitat types. The measured beta diversity revealed that lichen communities from *P. mugo* shrubs are heterogeneously distributed. The regional to local diversity ratio, shows that the species richness of *P. mugo* shrubs is three times higher than that of a one hectare sampling plot of , which is true for the spruce forest, too. This ratio is also higher than in the alpine vegetation. The total species turnover, which shows the number of species that are present in the entire habitat type in addition to the average number of species for one hectare plot, is also high, about 45 species, as in the alpine vegetation, and lower than in the spruce forests. The beta diversity measured with a multivariate approach had higher values in *P. mugo* shrubs than in spruce forest, when considering the lichens from all substrates. The alpine vegetation had higher species richness of terricolous and saxicolous lichens. Altitude and northing of the slope significantly influence the distribution pattern of lichen species of the *P. mugo* habitat type.

Keywords: Lichen diversity, *Pinus mugo* shrubs, Rodnei Mountains National Park

Introduction

The habitats dominated by *Pinus mugo* (a.k.a., 4070* “Bushes with *Pinus mugo* and *Rhododendron myrtifolium*”) have a high conservation value, the asterisk underlying that it is a habitat of interest in the European Community. *P. mugo* is protected in Romania because of the anthropogenic pressure [7]. This habitat, henceforth called ***PM***, occupies the most ecologically extreme habitats, such as glacial cirques, tall and steep slopes in Rodnei Mts. Resmeriţă and Raţiu [14] pointed out its importance against soil erosion, protection of spruce forests downhill and its ecosystem function for water retention. In habitats with such harsh conditions lichens can have major importance, contributing to the complexity of the food chains, influencing water retention, and soil formation.

Coldea *et al.*, [6] identified *Cetraria islandica* as a “companion” species with relatively high frequency in the *Rhododendro myrtifolii – Pinetum mugi* Borza 1959 em. Coldea 1995 plant association, described in their plant diversity inventory of Pietrosul Mare protected area (part of the Rodnei Mts. National Park). Moreover, studies on the ***PM*** habitat demonstrated that it offered favourable conditions to macromycete abundance and diversity, due to its protective function against the wind power, characterising the mountain climate [15].

In Romania, the lichens from ***PM*** were studied in a comparative study with spruce forests and alpine vegetation in Parâng and Lotru Mts. [4] based on ecological preferences and distribution area of species, showing differences in lichen communities. Kutorga et al. [10] reported 47 lichen species in ***PM*** plantations.

Recently, Ardelean et al. [3] published a checklist of 283 lichen species, and one subspecific taxon (out of which 67 species were new for Romanian lichen flora) from Rodnei Mts., which included this habitat type, too.

The aims of this study were 1) to assess lichen species richness and diversity analyzing alpha and beta diversity of the ***PM*** by comparisons with adjacent habitat types, i.e. alpine vegetation and spruce forests (***SF***), and 2) to analyse the importance of ecological factors that influence the lichen composition in the ***PM***.

Materials and Methods

Study area

The Rodnei Mountains have the highest elevations in the Northern Group of the Eastern Carpathians, reaching 2303 m on Pietrosul Mare Peak. Most of the mountain area is included in the Rodnei Mts. National Park. The importance of this protected area lies in its geology and geomorphology and the presence of many plant and animal species that are either endemic and/or glacial relicts [1]. Pietrosul Mare region was declared a UNESCO Biosphere Reserve in 1980, this status being extended later to the current limits of the national park. It is one of the four scientific reserves within the Park, and among the first protected areas in Romania.

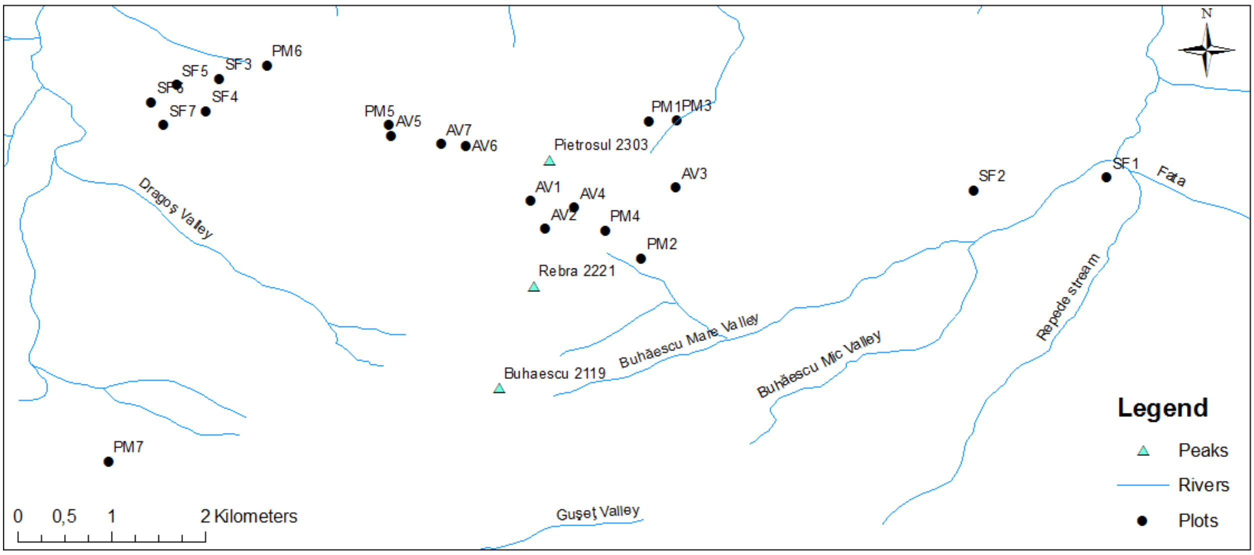

Fig. 1: Sampling plots distribution in Pietrosul Mare region. *AV* – alpine vegetation, *PM* – *Pinus mugo* shrubs, *SF* – spruce forests.

Due to the position and orientation (East–West) of the mountains, the climate is characterised by the Baltic and the Oceanic influences with differences between the southern and northern slopes. The differences are manifested in their thermal regime and precipitations. The mean annual temperature decreases with altitude, ranging between 6 °C at the base of the mountains and -1.5 °C at the highest altitudes. Annual precipitations range from 1300 to 1400 mm [8].

Sampling method

The sampling plots are located within Pietrosul Mare scientific reserve (or its vicinity) and follow a 1000 m altitudinal gradient (between 1150 – 2200 m) (Fig.1). We chose only conserved areas of the three habitats investigated (***PM***, ***AV*** - Alpine vegetation and ***SF***- Spruce forests) thus eliminating the influence of habitat management from these studies. The variables measured at plot level were altitude, slope, and exposition.

A standard diversity inventory method was used for sampling [16]: choosing seven circular plots of one hectare for each habitat type. The minimum distance between the plots was 100 m. Each plot was sampled in 24 relevés, investigating four main substrate types: living trees, wood, soil (including plant detritus), and rocks. If any of these substrates was not available, we replaced that relevé with another, from another substrate bearing lichens. The order of choosing the replacements was: living tree to wood, to soil, to rock, and conversely. Therefore, we investigated the lichens on all the four substrates in ***PM*** and in ***SF***, whereas in ***AV*** only lichens on soil and rocks could be sampled. The surface of a relevé was 0,2 m^2 and all lichen species were considered, except the crustose lichens from rocks.

Data analysis

Species richness for each habitat type was counted for lichen species from all substrates, and subsets formed on the substrate criterion, namely: soil, trees and rocks. The lichen composition was compared between the habitats, to see which species are specific and which of them are shared, illustrating the values in a Venn diagram.

We assessed alpha diversity (mean number of species per plot) and gamma diversity (total number of species) for each habitat type in part. In order to measure the compositional heterogeneity, beta diversity was first calculated with two classic approaches: a) multiple partitioning, first time described by Whittaker in 1960 [18] ($\beta_w = \gamma / \overline{\alpha}$), and also called *regional-to-local diversity ratio*, quantifies how many times as rich in species the entire data set is (in this case habitat type) than the one hectare plot level [17], and b) additive partitioning $\beta_{Add} = \gamma - \overline{\alpha}$ [11] also known as *total amount of species turnover*, shows the number of species that are additionally present in the entire habitat compared to the average number of species present in a one hectare plot.

Another approach for comparing the beta diversity in the three habitat types was in multivariate space, using the *betadisper* function from vegan package, in which case we computed the dispersion of the pairwise beta diversity values to the group centroid [2]. The centroid is defined in the principal coordinate space of the dissimilarity measure. The groups are the three habitat types. Pairwise similarity matrixes were computed with Sørensen similarity coefficient for each of the different data subsets: lichen species on all substrates, species sampled on living trees (in ***PM*** and ***SF***), soil, and rock. In the case of species from wood subset, the analysis was not possible due to a low number of samples in the ***PM*** habitat type and their absence in ***AV***. The logical argument *bias.adjust* was used for biases represented by small unequal numbers of samples in beta diversity estimates. To test if the dispersions (variances) of one or more groups are different, the distances of group members to the group centroid were subjected to ANOVA. Tukey HSD test was implied where the ANOVA showed significant differences, aiming to see which groups differed most.

The variation in species composition from ***PM*** habitat was visualised with the ordination method NMDS (using *metaMDS* function), and environmental variables correlated afterword (using the *envfit* function with 9999 permutations). The analysis was run at relevé level, including all substrate types. Only species that occurred less than five times were considered. 18 species met this criterion.

Statistical analyses were carried out with R version 2.15.2 (2012 The R Foundation for Statistical Computing) using the vegan package [12].

Results

Comparing species richness among the three habitat types considering the lichens on all substrates, and those on soil, trees and rock separately, we see that the results differ among the data sets used. Considering the lichens on all substrates and species on soil, ***AV*** and ***SF*** habitats were richer than ***PM*** in species at habitat level (gamma diversity). Considering the epiphytic species, ***SF*** has three more species than ***PM***, whereas for lichens from rocks, the values are almost the same among the three habitat types at habitat level (Table 1).

The alpha diversity values at the one hectare plot show visible differences from the previous scale in the results of lichen species richness. For species on soil the situation is opposite between ***PM*** and ***SF*,** the first having higher species richness. For species on trees alpha diversity is equal in the ***SF*** and ***PM***. In the case of species on rock, the highest alpha diversity is in ***AV***, then ***PM*** and the lowest is in ***SF***.

Table 1: Gamma diversity - ϒ (the species richness of the habitat type) and alpha diversity – α (as the mean species richness/plot) values for the *AV*, *PM* and *SF* habitat types, counted for species on all substrates, and species on soil, trees and rock separately

	All substrates		Soil		Trees		Rocks	
	ϒ	α	ϒ	α	ϒ	α	ϒ	α
AV	78	33	69	28	-	-	16	6
PM	69	23,7	29	11	41	13	16	4
SF	95	31,5	37	10	44	13	15	3,1

Testing for the lichen community overlap among the three compared habitats, we found that about 10% of the species are present only in the ***PM*** (Figure 2). This confers a particular importance to ***PM*** habitat type, for the lichen species diversity. Due to the fact that ***PM*** share lichen species with the two neighbouring habitat types it increases the distribution area of these species and contributes to the stability of their populations.

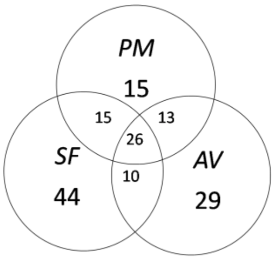

Fig. 2: Venn diagram with species shared between the three habitat types. Sum of all species found in the three habitats equals 152.

The beta diversity completes the diversity assessment and gives new perspectives considering the heterogeneity of species distribution within each habitat type. It is defined as the extent of change in community composition [19] measured with different methods, which provide complementary information.

The regional to local diversity ratio (β_w), shows that the species richness of ***PM*** habitat is three times higher than a sampling plot of one hectare, the same as in the ***SF***, and is higher than in the ***AV*** (Table 2). The total species turnover, which shows what number of species is additionally present in the entire habitat type compared to the average number of species for one hectare plot, is also high for the ***PM*** habitat. Its values are the same as in the ***AV***, about 45 species, but lower than in the ***SF***, where the number of additional species is about 63 (Table 2).

Table 2: Beta diversity measured using the multiplicative method (β_w) and the additive method (β_{Add}) for the three habitat types

	PM	***AV***	***SF***
β_w	2,91	2,36	3,01
β_{Add}	45,3	45	63,43

The multiplicative method (β_w) and the additive method (β_{Add}), show the relations of species richness from habitat level to plot level (one hectare) for each habitat type. Due to the fact that these measures are strongly related to habitat species richness (gamma diversity) we cannot compare values of beta diversity among habitat types [9].

Table 3: The average distance to group centroid in the analysis of multivariate homogeneity of beta diversity dispersions (variances)

	Groups		
	AV	***PM***	***SF***
All substrates	0,39	0,32	0,29
Soil	0,42	0,37	0,23
Tree	-	0,31	0,28
Rock	0,34	0,34	0,24

The multivariate test for homogeneity of variance for pairwise beta diversity, on the other hand, gives us the possibility to compare beta diversity values among different groups of samples, here represented by the habitat types. The higher the average distance to group centroid, the greater the values of beta diversity. For the lichen species from all substrates and the species on soil, ***AV*** has the highest values, followed by ***PM*** shrubs, whereas ***SF*** has the lowest values. The species on rock have equal values in ***PM*** and ***AV***, higher than the value of the ***SF*** group. The species on trees (analysed for ***PM*** and ***SF***) show higher values for the ***PM*** (Table 3). Results of average distance to centroid are represented as box plots in Figure 3 for lichen species on all substrates, soil, trees and rocks. In all data sets analysed in multivariate space, ***PM*** has higher values of beta diversity than the ***SF***, thus having higher habitat heterogeneity for the lichen species.

ANOVA tests showed that in the case of species from all substrates and species on soil there were significant differences in the beta diversity dispersion between land-use types. The Post – hoc Tukey's HSD shows that for the species from all substrates, the groups ***AV*** and ***SF*** have significantly different multivariate dispersion. For the species on soil, the groups ***SF*** – ***AV*** and ***PM*** – ***SF*** have significant differences in the homogeneity of multivariate dispersion at the 0.05 level of significance.

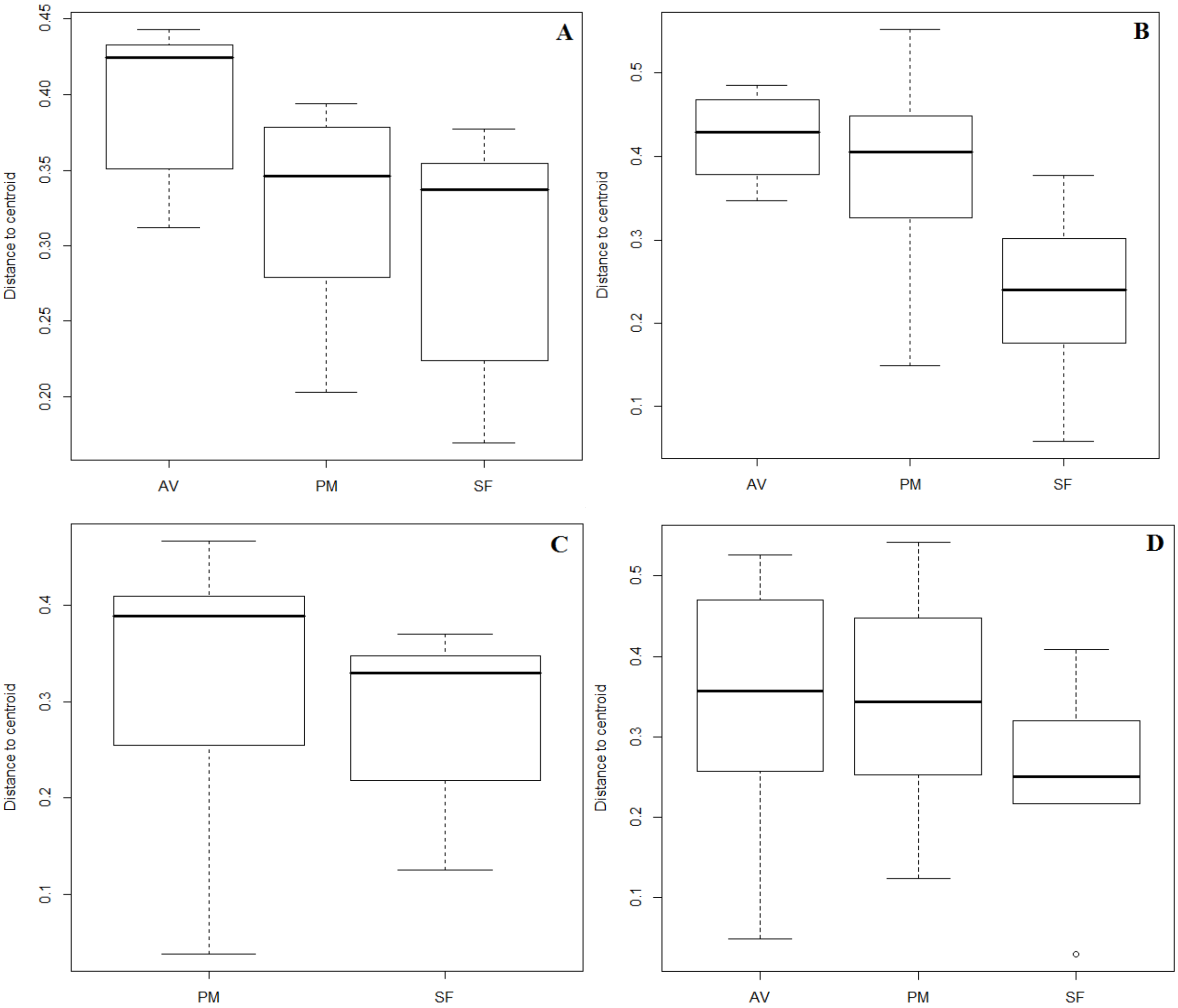

Fig. 3: Graphical representation of variances homogeneity for pairwise beta diversity of species to the group centroid for four data sets: all substrates (A), soil (B), trees (C) and rock (D)

The NMDS ordination of the lichen species composition variability at the relevé level in the ***PM*** was computed in three – dimensional solutions with a final stress of 0.13. Between the two factors that have significant correlations with species distribution, the altitude seems to have a very small influence (r^2 0,05) whereas the northing exposure of slopes has a higher influence (r^2 0,12) (Figure 4). The influence of the northern exposed slopes on lichen composition can be attributed to the different climatic influences: Oceanic and Baltic, for the northern and southern exposures respectively, with differences in the precipitation regimes and temperature. Even though ***PM*** strongly influences the microclimate for the lichen species, this result underlines that the macroclimatic conditions are also important in the lichen species distribution for frequent species from ***PM***.

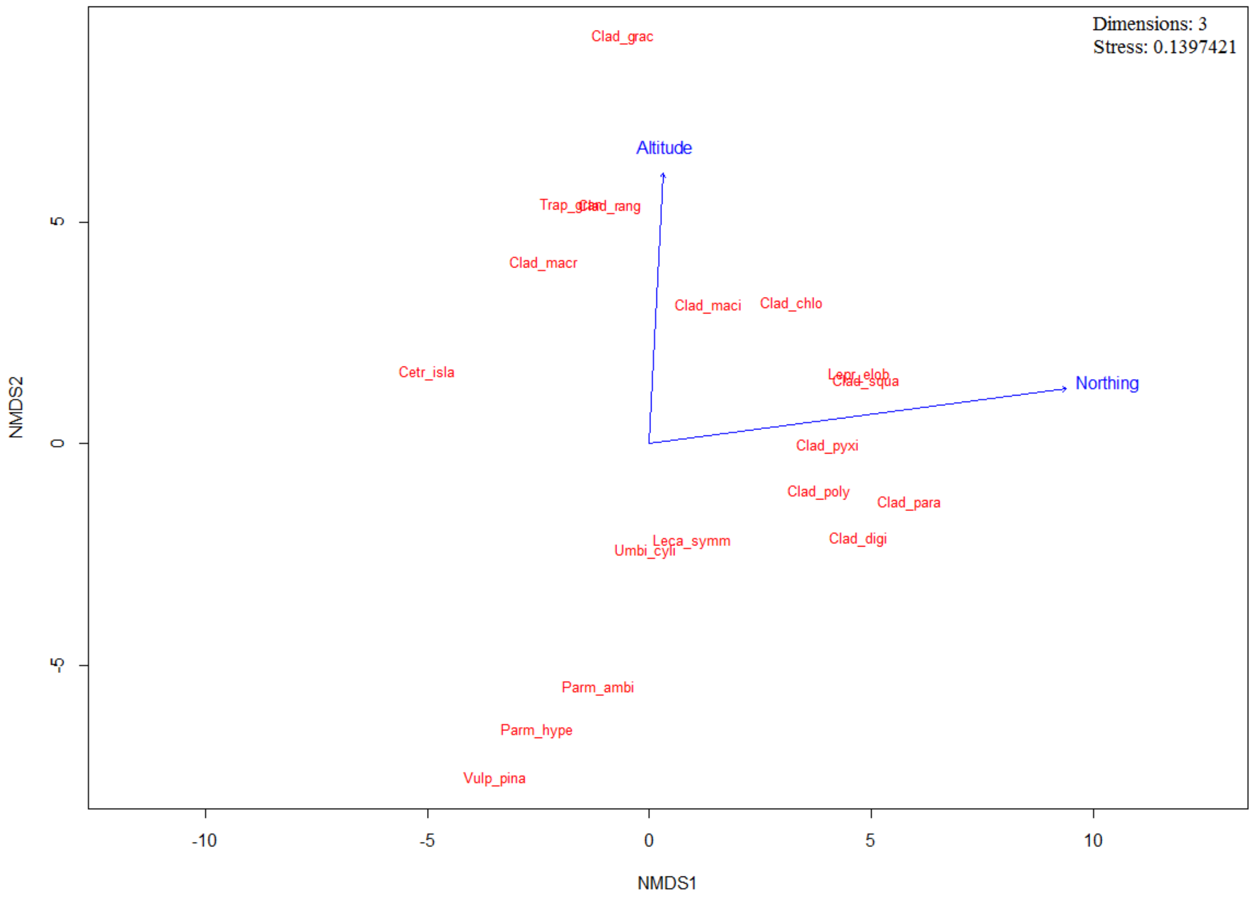

Fig. 4: Non – Metric Multi Dimensional Scaling (NMDS) ordination of lichen species composition at relevé level. Manhattan distance was used. Correlations with environmental variables and responses (only $p < 0.05$) are shown.

Codes for the lichen species: Cetr_isla - *Cetraria islandica*, Clad_chlo - *Cladonia chlorophaea*, Clad_digi - *C. digitata*, Clad_grac - *C. gracilis*, Clad_maci - *C. macilenta*, Clad_macr - *C. macroceras*, Clad_para - *C. parasitica*, Clad_poly - *C. polydactyla*, Clad_pyxi- *C. pyxidata*, Clad_rang - *C. rangiferina*, Clad_squa - *C. squamosa*, Leca_symm - *Lecanora symmicta*, Lepr_elob - *Lepraria elobata*, Parm_ambi - *Parmeliopsis ambigua*, Parm_hype – *P. hyperopta*, Trap_gran - *Trapeliopsis granulosa*, Umbi_cyli - *Umbilicaria cylindrica*, Vulp_pina - *Vulpicida pinastri*.

Discussions

The different levels of biodiversity (alpha, beta and gamma) provide important aspects about lichen communities in the three habitats. The species richness at habitat level (gamma diversity) helps us see how many species can be hosted in each habitat type. We found the higher number in ***SF***, followed by ***AV*** and ***PM***. If we took only this result into account for comparing the biodiversity of this three habitat types, our conclusions would not be valid. The alpha diversity show different results.

A simple comparison of the lichen composition from the three habitat types results in a picture that shows their uniqueness for each of them. From the total of 152 species surveyed in this study in the three habitat types, 10% are restricted to the ***PM*** habitat, 29% to the ***SF***, and 19% to the alpine vegetation. The ***PM*** habitats smooths the transition of lichen communities from forest to alpine vegetation. This result was found also in Parâng and Lotru Mts., by Bartók and Lőkös [4], showing that the boreal flora is characteristic for the spruce forests, whereas the arctic and alpine elements were dominant in *Pinus mugo* shrubs and alpine vegetation.

Considering that compositional heterogeneity (beta diversity) is related to environmental heterogeneity [2] we can state that the ***PM*** habitat type is a favourable habitat for epiphytic, terricolous and saxicolous (foliose and fruticose) lichen species, offering a high variety of microclimate conditions.

The distribution of lichen species in ***PM*** habitat type is significantly influenced by the altitude and northing, even though this habitat type diminishes the extreme environmental conditions, thus creating its own microclimate.

PM habitat type covers the subalpine belt in the study region and harbours specific lichen communities that include rare and threatened species [3]. Due to fact that the ***PM*** habitat is limited to the subalpine belt, thus having a limited distribution, and is exposed to the anthropogenic pressure, we consider its conservation for lichen diversity important.

Conclusions

Habitat type influences lichen diversity and the substrate types have different importance for the species richness as well as for the variation in species composition. Trees have the higher number of species in the ***PM***, followed by soil, and rock. The wood substrate was so sparce that we haven’t assessed it in our analysis. The ***PM*** has a high diversity of lichen species even though, at habitat level, the species richness is higher in the other two habitats considered: ***AV*** and ***SF***. This is confirmed by the three measurements of beta divesity, which indicate a relatively high habitat heterogenity for the lichen species. Moreover, even if it has a high degree of compositional overlap with the adjacent habitats, it still has a 10% unique species, restricted to this habitat, among the three considered.

This study underlines the importance of *Pinus mugo* habitat type for the lichen divesity, and brings another reason for its conservation.

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DIVERSITATEA SPECIILOR DE LICHENI DIN TUFĂRIŞURILE DE *PINUS MUGO* COMPARATĂ CU CEA DIN HABITATELE ADIACENTE DIN PARCUL NAŢIONAL MUNŢII RODNEI

(Rezumat)

Habitatul dominat de *Pinus mugo* are o valoare ridicată de conservare în Romania, fiind protejat din cauza presiunii antropice. De asemenea acesta este habitat de interes Comunitar.

Tufărişurile de *P. mugo* ocupă ecotopuri cu condiţii spre extreme din Munţii Rodnei, cum ar fi circuri glaciare cu pante înalte şi abrupte. Importanţa lor a fost subliniată prin rolul jucat în prevenirea eroziunii solului, în retenţia de apă şi prin aceasta şi protejarea pădurilor de molid din aval. Lichenii pot avea o importanţa majoră în habitate cu condiţii atât de dure de viață, contribuind la complexitatea lanţurilor trofice, influenţând retenţia de apă şi formarea solului.

Specia *Cetraria islandica* a fost menţionată ca fiind *însoţitoare* cu o frecvenţă relativ mare în asociaţia *Rhododendro myrtifolii - Pinetum mugi* Borza 1959 em. Coldea 1995, în Rezervaţia ştiinţifică Pietrosul Mare. Dar, în general, se ştie puţin despre lichenoflora acestor tipuri de habitate.

În acest studiu s-a explorat: i) bogăţia specifică şi compoziţia speciilor de licheni din habitatul cu *P. mugo* şi din habitatele adiacente, reprezentate de vegetaţia alpină şi păduri de molid şi ii) distribuţia speciilor de licheni din habitatul cu *P. mugo* în raport cu factorii de mediu. Am considerat lichenii epifiţi, tericoli, saxicoli şi lignicoli. Numărul total de specii şi densitatea medie a speciilor în ploturi de un hectar sunt mai mici în habitatele cu *P. mugo* decât în celelalte două tipuri de habitate. Cu toate acestea, habitatul adăpostește 15 specii de licheni care nu sunt prezente în habitatele adiacente.

Beta diversitatea măsurată a arătat că comunităţile de licheni din tufărişurile de *P. mugo* sunt distribuite în mod eterogen. Beta diversitatea masurată ca raport dintre diversitatea locală şi cea regională arată că bogăţia specifică din habitatele de *P. mugo* este de trei ori mai mare decât cea găsită într-un plot de un hectar, la fel ca în pădurea de molid, dar mai mare decât în vegetaţie alpină. Beta diversitatea măsurată ca numărul total al speciilor înlocuite, ce calculeaza câte specii sunt în plus în întreagul tip de habitat fata de alfa diversitate (numărul mediu de specii pentru un hectar) este de asemenea mare (aproximativ 45 de specii) ca şi în vegetaţia alpină, şi mai mică decât în pădurile de molid. Beta diversitatea măsurată prin metode multivariate are valori mai mari în comunităţile de *P. mugo* decât în pădurea de molid, atunci când au fost luati în considerare lichenii de pe toate tipurile de substrat. Vegetaţia alpină are o bogăţie specifică de licheni mai mare în cazul speciilor tericole şi saxicole. Altitudinea şi expoziţia nordică influenţează în mod semnificativ distribuţia speciilor de licheni din habitatele de *P. mugo*.