Original article

Important part of urban biodiversity: Lichens in cemeteries are influenced by the settlement hierarchy and substrate quality

Josef P. Halda a, Vladimír P. Janeček b, Jakub Horák a,b,*

a University of Hradec Králové, Faculty of Science, Rokitanského 62, CZ-500 03 Hradec Králové, Czech Republic
b Czech University of Life Sciences Prague, Faculty of Forestry and Wood Sciences, Kamýcká 1176, CZ 165 00 Prague, Czech Republic

ARTICLE INFO
Handling Editor: Gregory Dahle
Keywords:
Species traits
Town greening
Marginal land use
Urban trees
Village environment
City management

ABSTRACT
Cities, towns and villages are important places with almost specific greening in comparison with open landscapes. Cemeteries are one of the most common land-use types in Central European settlements; however, they are still rather marginal regarding their total extent. In this study, we focused on the diversity of lichens in these important artificial habitats.

Our study was done in the Czech Republic and we analyzed 164 substrates (trees and gravestones) in 19 cemeteries located in a city, a town and in villages between them.

Our results reveal that the majority of 65 species of lichens were rock-dwellers, preferring silicate substrates. Notably, ten species were red-listed. We found greater species richness in the town cemeteries, followed by village cemeteries, with those in the city most species-poor. Gravestones hosted a significantly higher number of lichen species than did trees. For tree-associated species, broadleaf species supported greater lichen species richness than did conifers. For rock-dwelling species, sandstone gravestones supported greatest lichen species richness, while those of concrete hosted the fewest species.

The results of this study indicate that cemeteries are important habitats for lichen diversity in human settlements. Cemeteries hosted a diverse community of lichens, including a number of threatened species. Even though trees were not as species rich as gravestones, they did support a different community of lichens in these cemeteries, suggesting that trees, particularly broadleaf species, provide important habitat in cemeteries to support a diverse lichen community. Planting of broadleaf trees and especially the retention and management of existing trees appears to be one of the most important management considerations for cemeteries to support diverse lichen communities.

1. Introduction

Human settlements are places where green areas play many important environmental roles (Moisejevs et al., 2019). Cemeteries are one such place with an important cultural function (Nordh and Swensen, 2018). They, however, can have many potential environmental functions (Rosenvald et al., 2019). One example is the greening within them, which can help to reduce many pollutants and creates specific microclimates (e.g., decreasing wind, airborne dust, traffic noise and solar irradiation; Elmqvist et al., 2015; Vieira et al., 2018). Green urban areas can also host biodiverse communities (Boom and van den, 2015; Horák et al., 2018). Cemeteries are one such place potentially important for biodiversity (Kowarik et al., 2016; Yılmaz et al., 2018).

Little attention has been paid to the diversity of lichen communities in urban cemeteries (Wainwright, 1986). Nevertheless, gravestones have been used in the past to study lichen succession (Hill, 1993) or to study the rate of colonization of certain species (Warren, 2003). Diaspore size-dependent colonization capabilities were studied in the UK (Leger and Forister, 2009). Lichens have limited possibilities to spread in cities due to the lack of suitable substrates. Thus, for example, gravestones may provide suitable permanent substrates for rock-dwelling (i.e., saxicolous) lichens. The composition of epiphytic (e.g., bark-dwelling) lichen flora depends on the choice of planted trees, which might be limited in cities. Solitary trees are slowly disappearing for safety reasons (Horák, 2018), and with them, epiphytes.

Systematic studies of the diversity of lichen communities on gravestones in Europe are rare (Caneva and Bartoli, 2017). Gravestones and trees represent various microhabitats for lichens. Gravestones can be colonized by both calciphilous and flora known from silicates. In
addition to gravestones, trees also provide important habitat—especially due to the known important role of sunlit trees for epiphytic lichens (Horák et al., 2014). As indicated, investigation of the oldest gravestones can confirm the assumption of the importance of cemeteries as biodiversity islands in the surrounding landscape (e.g., Leger and Forister, 2009). The older the gravestone is, the greater the lichen species diversity it hosts (Leger and Forister, 2009). On old gravestones, species emerging in later stages of succession can be found—e.g., Acarospora fuscata, Buellia aethalia and Lecidea fuscoatra. Newer gravestones are typically occupied by fast-growing lichens with a crustose or lobate thallus (Halda et al., 2016).

Cemeteries represent an assortment of diverse areas ready for biodiversity to be studied. Gravestones gradually weather and disintegrate and are colonized by different species of organisms. Nearly the same situation can be found with trees that are getting older and offer a diversity of microhabitats. Therefore, we aimed to determine the species diversity of lichens on gravestones and trees in cemeteries in the Czech Republic.

**Fig. 1.** The effect of the environment on lichens in cemeteries in the Czech Republic. Note that only statistically significant independent variables (settlement hierarchy—left and perimeter—right) are visualized and results are in interaction with other studied independent variables included in GLMM.

**Fig. 2.** Species richness of lichens between trees and gravestones in cemeteries in the Czech Republic. Results are visualized using CTREE.
Republic and to identify the most important factors that influence the diversity of these communities. Namely, we had four particular aims:

(i) Our first aim focused on the difference of specialization of lichens for (a) substrate and (b) nutrients.

(ii) The second aim focused on the main factors that influence lichen diversity on the scale of localities.

(iii) Further, we focused the third aim at the site scale on the difference between the two dominant substrates in cemeteries – trees and gravestones.

(iv) The fourth aim looked at the species composition and individual species’ responses to the categories of material of gravestones and tree species.

2. Methods

2.1. Study area and localities

We studied 19 cemeteries in one city, one town and seven villages in the Czech Republic in 2019. All localities were situated in east Bohemia in the Pardubice region in a flat lowland area. The mean elevation was 264 m a.s.l.

Seven freely accessible cemeteries (Krematorium, Rosice, Svitkov, Pardubicky, Lany na Dlku, Mnětice and Hostovice) were studied within Pardubice. Pardubice is an industrial city with nearly 100,000 inhabitants and an area of 82.66 km². The mean altitude is 220 m a.s.l.

Five cemeteries (Evangelický, Katolický, Úrnový háj, Hemze and Bestovice) were studied in the town of Chocœ. Chocœ is a small town with the motto: Town in park, park in town, indicative of the great extent of urban greening. This green town has nearly 10,000 inhabitants and covers an area of 21.70 km². The mean altitude is 290 m a.s.l.

We also studied cemeteries in seven villages in the transition area between these two settlements. Namely, we studied the cemeteries in Srub (584 inhabitants; area of 6.9 km²; elevation of 280 m a.s.l.), Zámrsk (729; 7.5; 260), Radhošť (174; 4.8; 255), Uhersko (257; 3.7; 252), Slepotice (433; 7.4; 248), Moravany (1869; 16.4; 242) and Kostnice (544; 5.8; 235).

All cemeteries were established before the year 1900 as evidenced by at least one grave from the 19th century in each particular site.

2.2. Study dependent variables

We used a proportional stratified design for the number of samples studied per locality (i.e., cemetery), which means that the number of samples per cemetery was proportional to the size of the cemetery (Hirzel and Guisan, 2002). In total, we studied 164 samples (mean = 9; minimum = 4; maximum = 30). We sampled both gravestones (n = 121) and trees (n = 43), again using a proportional stratified design. Sampled gravestones did not appear to have been cleaned, thus, the lichen community was presumably undisturbed, and most were older than 50 years. Only trees with a trunk without branches below a height of 2.5 m were sampled and lichens were observed up to this height.

All observed lichens were entered into database, but only the first thallus detected on a particular substrate was noted. This means that we have an incidence-based dataset of presence/absence data for each species at each sample. We classified each observed species by specialization based on the terminology of Resl et al. (2018). Namely, we classified lichens as rock-, bark-, or soil-dwellers. We also classified each species by their preference regarding the nutrients in the substrate. Namely, we used four categories of acidophilous, nitrophilous, calciphilous and neutral (no nutrient preference) species (Halda et al., 2016). We further classified species by their red list ranking (Liska and Palice, 2010). Species categorized as at least concern (LC) were counted as 0, those categorized as data deficient (DD) were counted as 1, those categorized as near threatened (NT) were counted as 2, and those categorized as vulnerable (VU) were counted as 3. Species from other red-list categories were not observed in this study.

2.3. Study environmental variables

For each cemetery, we noted category of settlement (7 in city, 5 in town, and 7 in villages), perimeter (which ranged from 101 to 1114 m, with a mean = 273 m), and percent cover of trees (which ranged from 5 to 75 %, with a mean = 20 %). The perimeter was used as a reflection of the cemetery extent as it appears to be more suitable than area for urban studies (Horák, 2016). The cemeteries within the city and town were distributed at the beginning and end, respectively, of easting of GPS. Therefore, we performed an analysis on spatial autocorrelation. There was no significant effect of attraction (i.e., spatial aggregation) or repulsion (i.e. regular spatial distribution). Thus, distribution of all dependent variables was random (Table S1), and the effect of category

<table>
<thead>
<tr>
<th>Specialization</th>
<th>Category</th>
<th>Trend</th>
<th>Statistic</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>All</td>
<td>Grave vs. Tree</td>
<td>+</td>
<td>77.09</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Bark</td>
<td>Grave vs. Tree</td>
<td>–</td>
<td>37.33</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Soil</td>
<td>Grave vs. Tree</td>
<td>–</td>
<td>n.s.</td>
<td></td>
</tr>
<tr>
<td>Rock</td>
<td>Grave vs. Tree</td>
<td>+</td>
<td>117.21</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Acidophilous</td>
<td>Grave vs. Tree</td>
<td>–</td>
<td>n.s.</td>
<td></td>
</tr>
<tr>
<td>Nitrophilous</td>
<td>Grave vs. Tree</td>
<td>–</td>
<td>9.03</td>
<td>0.003</td>
</tr>
<tr>
<td>Calciphilous</td>
<td>Grave vs. Tree</td>
<td>+</td>
<td>111.72</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

Table 1. Comparison of the influence of dominant substrate types on species richness of lichens in cemeteries in the Czech Republic. Results are based on CTREE.
of settlement was used as an appropriate independent variable.

At the site scale, we studied the difference between gravestones and trees as habitats (i.e., substrates) for lichens in cemeteries.

Each tree sampled was identified to the genus level (11 genera) and categorized as broadleaved (23 trees sampled across 5 genera) or coniferous (20 trees sampled across 6 genera). For each gravestone, we estimated the percentage of each composite material. Namely, of the 121 gravestones sampled, concrete was present in 102 cases, granite in 40, sandstone in 36, marble in 23 and iron in 3.

2.4. Statistics

Analyses were done in R 3.5.1 (R Core Team, 2019) and Canoco 4.53 (ter Braak & Šmilauer, 2002).

The spatial autocorrelation of dependent variables was tested by Geary’s C test under randomizations using package spdep.

We analyzed the difference in the number of species incidences regarding substrate and nutrient specializations. The number of incidences was not normally distributed among categories and was analyzed using Kruskal-Wallis ANOVA with Dunn test for multiple comparisons using package FSA.

The influence of the environment at the locality level was analyzed using Generalized linear mixed model (GLMM) with the number of studied sites per locality as a random factor. Category of settlement, perimeter and tree coverage were used as fixed variables. We used appropriate distribution of dependent variable (normal or negative binomial with appropriate 9) using packages nlm and MASS. Species with a neutral relationship to nutrients were not analyzed in this specific analysis due to the low number of incidences. Visualization of the results of the influence of independent variables in interaction with others in the GLMM was done using the package visreg.

The Conditional inference tree method (CTREE) was used for computation and visualization of the difference in species richness between trees and gravestones using package party.

Species composition was analyzed using Canonical correspondence analyses (CCA). We focused the scaling on interspecies distances with biplot scaling. We used the Monte-Carlo permutation test with 9999 permutations under the full model.

For analyses of individual species responses, we used the multiple species response curves analysis in CanoDraw 4.12. We used Generalized linear model (GLM) with binomial distribution. Only species with more than 4 records of occurrence were analyzed.

3. Results

We observed 65 species of lichens. Two species were red listed as vulnerable (Physconia peristidious and Placopyrenium fuscum), five as near threatened (Caloplaca obscurella, Catrillaria chalybeia, Lecanora campestris, Physconia enteraxantha and Stereocaulon nanodes), and three as data deficient (Caloplaca oasis, Candelariella subdeflexa and Lecania suavis).

We inventoried 43 trees (32 with lichens), of which 20 were conifers (10 with lichens) and 23 broadleaved trees (22 with lichens; Table S2). All gravestones were inhabited by some lichen species.

3.1. Substrate and nutrient specialization

Most species detected were classified as rock-dwellers (61.5 %), with approximately one third of species detected classified as bark-dwellers (32.3 %), and just a few species classified as soil-dwellers (6.2 %; Table S3). Incidence of occurrence differed significantly among rock-, soil-, and bark-dwellers (H = 7.70; P = 0.021), with significantly higher occurrences of rock-dwellers compared to soil-dwellers (P = 0.026). Other differences were not significant.

Most species detected were associated with acidic substrates, mainly sandstone and granite (44.6 %). Other species were associated with calcic (30.8 %) or nitrogenous (23.1 %) substrates or had no substrate preference (Table S3). Incidence of occurrence differed significantly (H = 14.73; P = 0.002). The number of incidences of acidophilous specialists was significantly higher than calciphilous specialists (P < 0.001). Other differences were not significant.

3.2. Locality level

The results of GLMM indicated that the number of observed lichen species was significantly influenced by the type of settlement and by the perimeter of the cemetery. Namely, town cemeteries hosted the highest number of species, followed by those in villages, with city cemeteries hosting the fewest lichen species. The difference between city and village cemeteries was not significant. Overall, species richness increased with cemetery perimeter (Fig. 1), and while this relationship was not significant when looking specifically at bark- or soil-dwellers, it did remain significant when looking at rock-dwellers as well as those species with a known preference for acidic substrates (Table S4). There was also a significant positive relationship between red list index and cemetery perimeter (Table S4). Although tree cover had no effect on overall lichen species richness, there was a significant negative relationship between tree cover and rock-dwelling species richness (Table S4).

3.3. Site scale

We found 13 species exclusively on trees and 37 species only on gravestones, with all other species (n = 15) detected on both substrates. Overall, species richness was significantly greater on gravestones than on trees (Fig. 2). For both rock-dwellers and those species with a known preference for calcic substrates, richness was significantly greater on gravestones, but for bark-dwellers and nitrophilous species, richness was significantly greater on trees (Table 1).

3.4. Substrate level

Tree affiliation (broadleaves vs. conifers) had a significant effect on lichen community composition (F = 1.66; P = 0.018). The majority of species were associated with broadleaves. There was no observed trend of preference of species regarding their known nutrient requirements with respect to the tree affiliation (Fig. 3).

We also observed a significant effect of gravestone substrate on species composition (F = 2.34; P = 0.017). Communities on concrete were different to those associated with sandstone and communities on granite were different to those associated with marble. Iron substrate was rather marginal, and its influence was against concrete. There was again no clear trend, but species preferring acidic substrates mainly avoided concrete and species preferring nitrogenous substrate were rather rare on granite (Fig. 3).

In total, 13 species were analyzed regarding trees and 28 regarding gravestones as a substrate. Only one species (Xanthoria parietina) revealed a significant response to the parameter of both studied substrates. This species was positively related to broadleaf trees, and regarding gravestones also to sandstone and negatively to concrete (Table S3).

Amandinea punctata was the most frequent species detected on trees. Eight species were positively related to broadleaves and no species was significantly associated with conifers (Table S3).

Lecanora dispersa was the most frequent lichen overall, including on gravestones. The number of species that were significantly influenced by the particular substrate of gravestones amounted to 22. Five of them preferred and 11 avoided concrete. Two species preferred and the same number avoided granite. Five species preferred iron. Three species preferred and five avoided marble. Nine species preferred and four avoided sandstone. The most complex response was in the case of three Lecanora species. Lecanora dispersa preferred concrete and granite and
avoided marble and sandstone. Lecanora polytropa preferred iron, marble and sandstone and avoided concrete. Lecanora campestris, a red listed species, preferred marble and sandstone and avoided concrete and granite (Table S3).

4. Discussion

The majority of lichens in cemeteries were rock-dwellers and the most abundant were acidophilous specialists. Lichen communities were more species-rich in town cemeteries compared to cemeteries in the countryside. The majority of tree-associated lichens preferred broadleaf tree species. For gravestone-associated lichens, sandstone was the most species-rich substrate while concrete was the most species-poor.

4.1. Urban and rural differences caused by trees

The diversity of substrates of saxicolous species was basically the same in urban and rural cemeteries, and therefore the species diversity of lichens (39 species in an urban environment, 30 species in the countryside) was not very different if we do not divide urban environment into city and town. The difference in species numbers was likely caused by the low diversity of woody plants, which was only represented by cypresses in villages (25 epiphytic lichens in the urban environment, 11 in the countryside) and 14 species of woody plants in urban cemeteries. Furthermore, ten species of lichens occupied coniferous trees in urban areas but only one lichen species in the countryside. Suitable tree species for bark-dwelling (often nitrophilous lichens) were found in urban areas, namely: maple (Acer) and linden (Tilia). The species diversity of lichens associated with trees is generally lower on conifers due to their acidic bark. The pH of damp conifer bark usually ranges from 3.0 – 4.5 (Hlaváč, 2011). This is also indicated by the fact that there were no lichens with fruticose thalli, which are sensitive to factors like water and atmospheric pollutants (mainly SO2 and NH3; Bytnerowicz et al., 2019). For epiphytes, there was a noticeable absence of species with fruticose thalli (e.g., Evernia prunastri or Pseudevernia furfuracea). On the bark of trees, predominate species prefer a nutrient-rich substrate and have either crustose (Amandinea punctata) or foliose (lobate) thalli (Physcia tenella or Xanthoria parietina).

4.2. Lichens and gravestone substrates

In general, lichens can colonize numerous substrates, but most lichens have adapted to a particular substrate type (Nash, 2008; Korkanç and Savran, 2015). This is evident in the case of rock-dwelling lichens. Calcium-rich rocks are often more species-rich than silicate (acidic) rocks (Nash, 2008). Species diversity can also vary greatly on the same rock substrate. The difference is usually caused by differing stages of succession or differing management methods in the case of gravestones. A diversity of substrates has great potential to enrich the whole community.

4.3. Species responses

Many detected lichens were pioneer species with low competitive ability. Among the rock specialists, it is possible to distinguish groups of species that colonize the substrate very quickly. However, in some cases the presence on concrete was striking – e.g., Verrucaria muralis is mainly calciphilous and its presence was probably caused by the use of lime mortar to bind prefabricated concrete blocks (Halda, 2003). Nevertheless, this coincides with our previous statement that lichens can colonize nearly any substrate. This might also be due to the high diversity and combination of substrate types, which enables pioneer lichens to colonize substrates not common in nature.

The recent scientific agenda on lichens mainly focused on species in threatened habitats (e.g., primeval forests and the importance of dead wood substrates for biodiversity; Blasy and Ellis, 2014) or biological crusts (Hernandez and Knudsen, 2012; Ian et al., 2015; Tucker et al., 2019; Weber et al., 2019). Anthropogenic substrates are studied mainly regarding the protection of monuments (e.g., statues or memorials; Hoppert and König, 2019). Focusing on this, the oldest marble gravestones were colonized by species with crustose thallus (e.g., Aspicilia calcarea). The species composition of granite and sandstone gravestones differed, and gravestones made of silicate substrates were colonized by microlichens with crustose and foliose thalli (e.g., Candelariella vitellina).

5. Conclusions and management implications

Our study showed that cemeteries host diverse lichen communities, with several red-listed species and high overall species diversity across substrates and nutrient preferences. Town cemeteries in particular have great potential to host species-rich lichen flora. Trees were not as species-rich as gravestones in these cemeteries, however broadleaved trees increased the number of specialized lichen species detected. Therefore, the planting of broadleaved trees and especially the retention of veteran trees, together with avoiding the cleaning of gravestones are the most important management considerations for urban cemeteries.

CRediT authorship contribution statement

Josef P. Halda: Funding acquisition, Investigation, Methodology, Writing - original draft. Vladimír P. Janeček: Investigation, Methodology. Jakub Horák: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Supervision, Validation, Visualization, Writing - original draft, Writing - review & editing.

Declaration of Competing Interest

Authors declare no conflict of interest.

Acknowledgments

The authors are grateful to Excelence project PIF UHK2208/2019 for the financial support, the editor, Amy Ross-Davis and anonymous reviewer for valuable suggestions, Simon O’Flynn corrected the English.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.ufug.2020.126742.

References