Epiphytic lichens on *Juniperus communis* – an unexplored component of biodiversity in threatened alvar grassland

Inga Jüriado, Ede Leppik, Piret Lõhmus, Tiina Randlane and Jaan Liira

I. Jüriado (inga.juriado@ut.ee), E. Leppik, P. Lõhmus, T. Randlane, J. Liira, Dept of Botany, Inst. of Ecology and Earth Sciences, Univ. of Tartu, Lai 40 51005 Tartu, Estonia.

Semi-natural grasslands, among them thin-soil calcareous grasslands (alvars), have great conservation value but have become increasingly rare in Europe. The main threat to alvar grasslands is the encroachment by juniper *Juniperus communis* and therefore it is usually removed during the restoration practice. Juniper can also be a host plant for many epiphytic lichens, but its role as a phorophyte is poorly known. We studied epiphytic lichen diversity on 126 junipers in 17 sites in western Estonia and found 140 lichenized taxa including several rare and red-listed species. Using indirect and direct multivariate analyses (DCA, pCCA) and general linear models we revealed that both habitat and phorophyte properties affect lichen assemblies on juniper. Lichen species richness per site showed a unimodal relationship with compound factors of site productivity and juniper characteristics (stem circumference and juniper width). Lichen species richness per phorophyte was increasing with its size and with the proportion of dead branches, and was twice higher in plate alvars than in ryhk alvars. Also, the species composition in plate alvars differed from ryhk alvars by having 42 characteristic lichen species in plate alvars vs three indicators of ryhk alvars. The composition of lichens was significantly influenced by encroachment of alvars, e.g. by high juniper cover and shrub layer height, as well as by the proportion of dead branches and stem circumference of juniper. We conclude that the epiphytic lichen assemblies on junipers are threatened by grassland encroachment similarly to ground layer lichen assemblies. We suggest that some old and scencent junipers should be preserved during the restoration of alvar grasslands.

Alvars are calcareous grasslands formed through non-intensive human management during several thousand years (Laasimer 1965, Rosén 1982). They have limited regional distribution, as they mainly occur in northern Europe, particularly in the coastal regions and islands in the Baltic Sea (Rosén 1982, Pärtel et al. 2007). As a result of continuous land use, mostly grazing by domestic animals, alvars have become species-rich communities (Rosén 1982, Poschlod and WallisDeVries 2002, Pärtel et al. 2007, Gazol et al. 2012). In addition to historical grazing pressure, alvar vegetation is influenced by environmental stress and natural disturbances caused by extreme droughts, frost-induced soil movements or small-scale flooding (Rosén 1995).

Traditionally managed grasslands are becoming increasingly rare in Europe (Poschlod and WallisDeVries 2002). During the 20th century, the cessation of traditional management has caused the extensive encroachment of calcareous grasslands resulting in formation of dense brushwood and natural climax communities, i.e. forest (Pärtel et al. 2005). Juniper *Juniperus communis* L. encroachment has been considered as the main threat for alvar vascular plant communities (Rosén 1982, Van der Maarel 1988, Pärtel et al. 1999b). Also, epigeic lichen communities are strongly affected by increasing shrub cover in alvar grasslands as reflected by a decrease in species richness and changes in the proportions of lichen growth forms (Leppik et al. 2013).

Previous studies of lichen diversity on alvars have dealt mostly with epilithic or epigeic species (Fröberg 1988, Ott et al. 1996, 1997, Dengler et al. 2006, Leppik et al. 2013, 2015), while epiphytes have been almost ignored (Fröberg et al. 2009). At the same time, epiphytes are a valuable part of biodiversity (Ellis 2012), and in grasslands juniper could be an important phorophyte for epiphytic lichens (Ellis and Coppins 2009). Lichen records on juniper are often incorporated into the group of epiphytes on coniferous trees (Martin et al. 2000, Jüriado et al. 2003, 2006) and no special studies concerning the significance of substrate qualities on epiphytic lichens on junipers exist.

Although there are no studies about the effect of shrub encroachment in alvar grassland on epiphytes, it can be assumed that epiphytic lichens on juniper could also be affected by diminished light conditions within a dense shrub layer as has been demonstrated in other semi-natural habitats (Rose 1992, Sanderson and Wolseley 2001, Aragón et al. 2010, Leppik et al. 2011). On alvars, a study about the effect of phorophyte properties on epiphytic lichens on the dwarf shrub *Helianthemum oelandicum* (L.) DC. established that species richness was correlated with the phorophyte age and was greater on dead compared with living phorophytes (Fröberg et al. 2009). Moreover, the formation of an epiphytic community depends on large-scale factors (Bartels and Chen 2012, Ellis 2012) as,

for example, it has been shown that present day epiphytic species diversity on juniper is influenced by the effect of historic woodland structure (Ellis and Coppins 2009).

In this study, we analyze the effect of phorophyte properties and grassland conditions on the composition and richness of lichens associated with juniper in thin-soil calcareous grasslands (alvars). We evaluate the significance of juniper as a phorophyte for epiphytes in alvar grassland and propose practical measures to conserve the epiphytic component of biodiversity in this semi-natural habitat.

Material and methods

Study area and study sites

Alvars are semi-natural calcareous grasslands occurring on shallow skeletal soil formed from Ordovician or Silurian calcareous sediments or from monolithic calcareous bedrock (Laasimer 1965, Rosén 1982). Ecological condition of alvars is primarily determined by the character and weathering of the limestone bedrock, the relief of the bedrock surface and the shallow depth of soils (thickness < 20 cm) (Albertson 1950, Rosén 1982, Zobel 1987). In Estonia, alvars are mainly distributed on the islands on the eastern coast of the Baltic Sea (57.8–59.5°N, 21.7–28.0°E), in a region where rendzinas occur as the dominant soil type on limestone (Laasimer 1965, Pärtel et al. 2007). The alvar region has a mild maritime climate with a mean annual temperature of 6.2°C and a precipitation of 600 mm (<www.emhi.ee>). The area of alvars in Estonia has diminished dramatically over the last century. Only 30% of the former territory (ca 50 000 ha) is preserved

(Kukk and Sammul 2006), while the remaining area is mostly overgrown by juniper or Scots pine *Pinus sylvestris* L.

We selected 17 study sites within the main alvar distribution area in western Estonia (Fig. 1). The site selection was based on the data obtained from the grassland database of the Estonian Seminatural Community Conservation Association, from various maps provided by the Web Map Server of the Estonian Land Board (<http://xgis.maaamet.ee>: land cover maps, soil maps, historical maps and orthophotos), and from field data (shrub height and cover values) gathered in previous projects (Leppik et al. 2013, 2015). The study sites represented alvar grasslands with shrub cover from 10 to 80%, consisting mainly of juniper, and with only insignificant proportion of other shrub species such as *Berberis vulgaris* L., *Frangula alnus* Mill., *Lonicera xylosteum* L., *Rhamnus catharticus* L., *Ribes alpinum* L. and *Sorbus aucuparia* L. Solitary young pines (1–2 trees, max height 6 m) were present in some study plots. In terms of standard habitat classification (Zobel 1987), the studied alvar grasslands belonged to plate (4 study plots) and ryhk alvars (13 study plots). In syntaxonomic terms, the studied grassland plant associations can be classified as the *Festucetum alvarense* and *Avenetum alvarense* of Pärtel et al. (1999a). In the EU Habitat Directive, these habitats are coded as 5130, *8240 and *6280 (<http://ec.europa.eu/environment/nature/legislation/habitatsdirective>).

Data collection

Field work was performed during the summer 2011. At each site, the lichen species composition on up to 10 junipers (min 6 junipers) was described in a circular plot of 0.1 ha
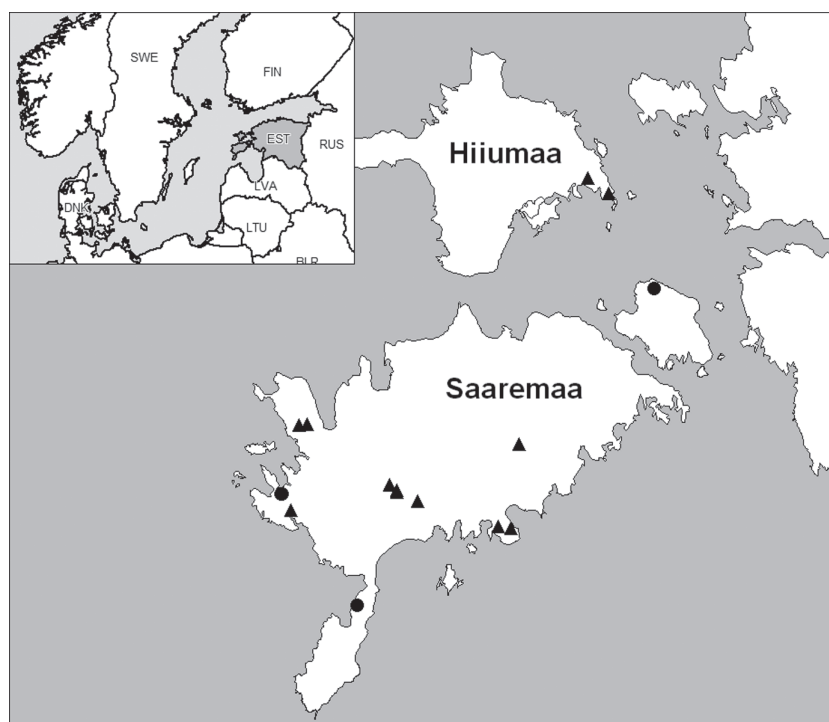

Figure 1. Distribution map of study sites on the western islands of Estonia. Dots denote the plate alvars (n = 4) and triangles denote the ryhk alvars (n = 13).

established by a previous study (Leppik et al. 2013). In total, species composition of lichens on 126 junipers in 17 study plots was registered.

Each juniper was examined comprehensively, including main stem, branches of all diameters, as well as dead branches (both their bark and wood) and the presence/absence of lichen species was recorded. For each examined individual juniper, its vitality status (living/dead) was recorded on a 3-grade scale: 1 = only living branches, 2 = dead branches less than 50%, 3 = dead branches 50% or more. In each study plot, junipers of all different states existing in the site were sampled. The height and width of each juniper and the circumferences of a main stem or 2–3 low positioned large branches was measured.

In addition, for each 0.1 ha plot various environmental parameters were investigated (details in Leppik et al. 2013): shrub (juniper) cover, shrub layer height, soil depth, soil pH, N and Ca content (averaged per plot). Geographical coordinates and the distance of the sample plot from the sea were also measured and considered in data analyses.

Lichen specimens that were difficult to identify in the field were collected for lab-determination. A stereomicroscope, light microscope, UV light and standardized thin-layer chromatography (Orange et al. 2001) were used for the identification of lichen specimens in the laboratory. Nomenclature of lichenized taxa follows Randlane et al. (2013). Some morphologically similar specimens of *Cladonia* that were difficult to identify due to the lack of podetia were combined for data analysis as *Cladonia* spp. Two specimens of *Rinodina* and one *Lecanora* were unidentified and treated as *Rinodina* sp. 1, *Rinodina* sp. 2 and *Lecanora* sp. 1, accordingly. Data on lichen species frequency in Estonia was derived from Randlane and Saag (1999), the eSamba database of Estonian lichens (<www.eseis.ut.ee/>) and the eBiodiversity database (<http://elurikkus.ut.ee/>). Information on red-listed taxa originates from Randlane et al. (2008). The voucher specimens are deposited in the lichenological herbarium of the Natural History Museum at the Univ. of Tartu (TU).

Statistical analyses

Prior to statistical analyses, variables with skewed distribution were log-transformed to approach normality and in order to reduce the influence of a few nominally large values. These variables were shrub layer height, Ca content of soil and distance of the sample plot from the sea shore. Square-root transformation was used for juniper cover. For data analyses, N content of soil was multiplied with average soil depth in the sample plot to describe soil productivity (N-pool in soil). Vitality status of juniper was treated as a continuous variable.

The main gradient of species composition was examined using Detrended Correspondence Analysis (DCA) (Hill and Gauch 1980) implemented in the program PC-ORD (McCune and Mefford 1999). For data analysis species presence–absence data was used. Species occurring on fewer than three junipers were removed from the dataset in order to reduce noise caused by rare species. Therefore 90 species (out of a total of 140) were used in the analy-

sis. The proportion of variance represented by the ordination axes was estimated by the after-the-fact method using relative Euclidean distance (McCune and Mefford 1999). A Multi-Response Permutation Procedure (MRPP) (Mielke 1984) with Euclidean distance was used to test for differences in lichen species composition between alvar types. An Indicator Species Analysis (ISA) (Dufrêne and Legendre 1997) among alvar types was performed in the program PC-ORD, and species pattern randomness was tested using a Monte Carlo simulation test (4999 runs). In order to examine the effect of environmental conditions and substrate properties on species composition we used a Partial Canonical Correspondence Analysis (pCCA) (ter Braak 1986) implemented in the program package CANOCO (ter Braak and Šmilauer 2002). Variance in the composition of the lichen community, caused by the geographical location of the sample plots, was taken into account by setting the variable 'subregion' as the covariable. 'Subregion' was defined according to the 20-km distance of the sample plots from each other and regional landscape properties. The forward selection procedure with randomization tests (Monte-Carlo permutation test, 1000 unrestricted permutations) was employed to select the most important environmental variables influencing species composition, retaining the variables with an independent significant contribution at the $p < 0.05$ level. A Monte-Carlo permutation test was also used to determine the statistical significance of the first and thereafter all canonical axes together.

We tested the response of species richness to the influence of the environmental conditions and phorophyte properties using a General Linear Model analysis (GLM) implemented in the program Statistica (StatSoft Inc.). The model was built using the two-way stepwise selection procedure (backward and forward selection combined), and predictor variables were tested for linear and square effects.

Epiphytic species richness was analysed at two scales: 1) the number of lichen species on junipers per plot (plot level) and 2) the number of lichen species per juniper (phorophyte level). For the model of the plot level, in order to minimize redundancy and reduce the number of correlated environmental parameters in the analyses, we calculated the combined factors (Varimax rotated principal components in Factor Analysis in Statistica) of the dominant ecological gradients. A varimax-normalized rotation was applied in a set of three principal components with eigenvalues greater than one (Table 1). Factor-1 had high loadings (> 0.6) of six environmental variables which described the productivity of the site, and was positively correlated with soil productivity (characterized by N × soil depth), juniper cover, soil depth, height of shrub layer and height of junipers in the study plot, and negatively correlated with soil pH (Table 1). Factor-2 represented variables connected with juniper properties including circumference of the main stem and juniper width. Factor-3 represented a gradient related to the influence from the sea on the study sites, with a high negative correlation with distance from sea and positive correlation with Ca content of soil (Table 1). The scores of these three generalized environmental factors were used in the model at plot level.

Table 1. Varimax-normalized factor loadings of three principal components, their eigenvalues and the variance they describe. The factor loadings of environmental variables higher than 0.6 are shown in bold.

	Factor-1 (Site productivity)	Factor-2 (Circumference)	Factor-3 (Site location)
Eigenvalue	5.1	2.6	1.6
% of total variance	46.5	23.7	15.2
Variables			
Juniper cover (sq-root)	**0.919**	0.100	0.168
Shrub layer height (log)	**0.791**	0.416	−0.084
Soil pH	**−0.878**	0.289	0.225
Soil depth	**0.861**	0.002	−0.361
N × soil depth	**0.951**	−0.196	0.142
Ca content (log)	−0.349	0.144	**0.819**
Distance from sea (log)	−0.204	0.048	**−0.929**
Vitality status of juniper	−0.558	0.447	0.384
Juniper height	**0.769**	0.564	−0.094
Juniper width	−0.085	**0.898**	0.128
Stem circumference	0.078	**0.951**	−0.027

At phorophyte level, 'Alvar type' and juniper properties (height, width, stem circumference and vitality status of a phorophyte) were included in the model directly, along with the number of lichen species per plot, to reflect the species pool of the habitat. The post hoc Fisher's Least Significant Difference (LSD) test was performed to compare species richness between the two alvar types.

Results

Lichen species composition

A total of 140 lichenized taxa were recorded from 126 junipers growing on alvar grasslands. The most frequent lichen species were *Lecanora pulicaris*, *Buellia griseovirens*, *Phlyctis argena* and *Hypogymnia physodes* occurring on more than 80% of juniper individuals (Table 2). Ten species were either red-listed or rare in Estonia, with fewer than ten localities, e.g. *Caloplaca herbidella*, *Lecanora impudens*, *L. intumescens*, *Micarea nitschkeana*, *Opegrapha ochrocheila*, *Parmelia ernstiae* and *Pseudosagedia aenea* (Table 2).

In the DCA ordination, the first axis described 23.1%, the second axis 11.9% and the third axis 7.4% of variation in species data. Junipers of plate and ryhk alvars, both formed compact clusters in the DCA ordination plot of the first and second axes (Fig. 2a). The cluster of ryhk alvars was 2–3 times wider than the cluster of plate alvars, however. The MRPP test detected significant differences in lichen species composition between plate and ryhk alvars (A = 0.045, p < 0.0001). The distinction in species composition was supported by the results of Indicator Species Analysis where 42 lichen species showed preference for junipers in plate alvars whereas only three lichen species grew more frequently in ryhk alvars (Table 2). The characteristic species of plate alvars, e.g. *Anaptychia ciliaris*, *Buellia erubescens*, *Caloplaca ferruginea*, *Lecanora chlarotera*, *Physcia stellaris*, *Physconia distorta* are predominantly in the left side of the ordination plot of the first and the second axes (Fig. 2a). Lichens favouring ryhk alvars or sites further away from the sea shore, e.g.

Cladonia fimbriata, *Micarea prasina*, *Lecidella subviridis* and *Lecanora expallens*, are located in the lower right side of the ordination plot (Fig. 2a). The third ordination axis correlated negatively with the variables juniper height and cover, and shrub layer height reflecting the overgrowing conditions in the study site (Fig. 2b).

We used pCCA with study location as a covariable to reveal a specific effect of the substrate and site factors on the lichen community. The eigenvalue of the first ordination axis of pCCA was 0.10, of the second axis 0.08 and of the third axis 0.07. According to the forward selection procedure, the variation in lichen assemblages on junipers can be explained by the phorophyte properties (juniper height, stem circumference and vitality status of juniper) and by the environmental variables describing the site conditions of the grassland (juniper cover, shrub layer height, soil productivity, pH and Ca content). Juniper width had only very low contribution to ordination results and was dropped from the analysis.

The biplot of species and environmental variables shows that the gradient along the first axis is mainly related to site productivity (Fig. 3). In the (upper) left part of the biplot are the species that occurred on tall junipers in overgrown grasslands with relatively deep and productive soil, e.g. *Micarea prasina*, *M. denigrata* and *Normandina acroglypta*. Species in the right part of the biplot are lichens such as *Anaptychia ciliaris*, *Caloplaca ferruginea*, *Ramalina farinacea* and *Xanthoria parietina*, which prefer less tall junipers in less overgrown grasslands. Along the second axis of the pCCA plot, the change in the composition of the lichen species is determined by the juniper characteristics 'vitality status of juniper' and 'stem circumference', and also by the Ca-content of the soil (Fig. 3). In the lower right side of the pCCA ordination plot are the species which occurred on senescent or dead junipers (e.g. *Cladonia coniocraea*, *C. furcata* and *Ochrolechia microstictoides*) and in the lower left part are the species growing on larger stems of juniper (e.g. *Coenogonium pineti*, *Imshaugia aleurites* and *Parmelia ernstiae*) (Fig. 3). The third axis was negatively correlated with soil pH and Ca-content, and positively related to vitality status of junipers (results not shown).

Table 2. List of lichen taxa (n = 140) found on *Juniperus communis* in alvars of Estonia, abbreviation of species names presented in ordination diagrams, their number of observations at all studied junipers (n = 126), indicator species for plate (P) and ryhk alvars (R) according to Indicator Species Analyses (p ≤ 0.05), frequency status of rare species in Estonia (st r = rather rare, 6–10 localities; r = rare, 3–5 localities) and/or red list category in Estonia (NT = near threatened, VU = vulnerable).

Taxa	Abbreviation	No. of obs. (n = 126)	Indicator species	Status
Acrocordia cavata (Ach.) R. C. Harris	Acr cav	5	P	
Agonimia globulifera M. Brand & Diederich		1		
Agonimia tristicula (Nyl.) Zahlbr.		1		
Amandinea punctata (Hoffm.) Coppins & Scheid.	Ama pun	5		
Anaptychia ciliaris (L.) Körb. var. *ciliaris*	Ana cil	10	P	
Arthonia radiata (Pers.) Ach.		1		
Bacidia arceutina (Ach.) Arnold	Bac arc	22	P	
Bacidia bagliettoana (A. Massal. & De Not.) Jatta		1		
Bacidia beckhausii Körb.		1		
Bacidia fraxinea Lönnr.		1		
Bacidia rubella (Hoffm.) A. Massal.	Bac rub	9		
Bacidia subincompta (Nyl.) Arnold	Bac sub	8		
Bacidina arnoldiana (Körb.) V. Wirth & Vězda		2		
Biatora globulosa (Flörke) Fr.		1		
Bilimbia microcarpa (Th. Fr.) Th. Fr.		1		
Bilimbia sabuletorum (Schreb.) Arnold		1		
Buellia arnoldii Servít	Bue arn	3		
Buellia disciformis (Fr.) Mudd	Bue dis	5		
Buellia erubescens Arnold	Bue eru	25	P	
Buellia griseovirens (Turner & Borrer ex Sm.) Almb.	Bue gri	116		
Caloplaca cerina (Ehrh. ex Hedw.) Th. Fr.	Calo ce	3		
Caloplaca chrysophthalma Degel.		2		
Caloplaca ferruginea (Huds.) Th. Fr.	Calo fe	34	P	
Caloplaca flavorubescens (Huds.) J. R. Laundon	Calo fl	15	P	
Caloplaca herbidella (Hue) H. Magn.	Calo he	33	P	st r
Caloplaca holocarpa (Hoffm. ex Ach.) A. E. Wade	Calo ho	15	P	
Candelariella reflexa (Nyl.) Lettau	Can ref	5	P	st r
Candelariella vitellina (Hoffm.) Müll. Arg.		1		
Candelariella xanthostigma (Ach.) Lettau	Can xan	57		
Catillaria nigroclavata (Nyl.) Schuler	Cat nig	29		
Cetraria islandica (L.) Ach. ssp. *islandica*		1		
Cetraria sepincola (Ehrh.) Ach.	Cet sep	11		
Cladonia arbuscula coll.		1		
Cladonia cenotea (Ach.) Schaer.		2		
Cladonia chlorophaea (Flörke ex Sommerf.) Spreng.	Cla chl	31	R	
Cladonia coniocraea (Flörke) Spreng.	Cla con	11		
Cladonia cornuta (L.) Hoffm.		1		
Cladonia cryptochlorophaea Asahina		1		
Cladonia digitata (L.) Hoffm.		1		
Cladonia fimbriata (L.) Fr.	Cla fim	31	R	
Cladonia furcata (Huds.) Schrad.	Cla fur	3		
Cladonia gracilis (L.) Willd. ssp. *gracilis*		1		
Cladonia ochrochlora Flörke		1		
Cladonia parasitica (Hoffm.) Hoffm.	Cla par	4		NT
Cladonia pocillum (Ach.) Grognot	Cla poc	27		NT
Cladonia subulata (L.) F. H. Wigg.		2	P	
Cladonia sp.	Cla sp	13		
Cliostomum griffithii (Sm.) Coppins	Cli gri	5	P	
Coenogonium pineti (Ach.) Lücking & Lumbsch	Coe pin	12		
Diploschistes muscorum (Scop.) R. Sant.		2		
Evernia prunastri (L.) Ach.	Eve pru	39	P	
Fuscidea arboricola Coppins & Tønsberg		1		
Hypocenomyce scalaris (Ach.) M. Choisy		2		
Hypogymnia physodes (L.) Nyl.	Hyp phy	101	P	
Hypogymnia tubulosa (Schaer.) Hav.	Hyp tub	24		
Imshaugia aleurites (Ach.) S. L. F. Meyer	Ims ale	4		
Lecania cyrtella (Ach.) Th. Fr.	Len cyr	12		
Lecania naegelii (Hepp) Diederich & Van den Boom	Len nae	23	P	
Lecanora albella (Pers.) Ach.	Lec alb	9		
Lecanora allophana Nyl.		1		
Lecanora carpinea (L.) Vain.	Lec car	12	P	

(Continued)

Table 2. (Continued).

Taxa	Abbreviation	No. of obs. (n = 126)	Indicator species	Status
Lecanora chlarotera Nyl.	Lec chl	17	P	
Lecanora compallens Herk & Aptroot	Lec com	47	P	
Lecanora expallens Ach.	Lec exp	15	R	
Lecanora hagenii (Ach.) Ach.	Lec hag	34	P	
Lecanora impudens Degel.		2		VU, r
Lecanora intumescens (Rebent.) Rabenh.		1		VU, st r
Lecanora norvegica Tønsberg		2		
Lecanora phaeostigma (Körb.) Almb.		2		
Lecanora pulicaris (Pers.) Ach.	Lec pul	123		
Lecanora strobilina (Spreng.) Kieff.	Lec str	21		
Lecanora sp. 1		1		
Lecanora symmicta (Ach.) Ach.	Lec sym	94		
Lecanora varia (Hoffm.) Ach.	Lec var	22		
Lecidea nylanderi (Anzi) Th. Fr.	Lec nyl	36		
Lecidella elaeochroma (Ach.) M. Choisy	Led ela	48	P	
Lecidella euphorea (Flörke) Hertel		1		
Lecidella flavosorediata (Vězda) Hertel & Leuckert	Led fla	66	P	
Lecidella subviridis Tønsberg	Led sub	9		
Lepraria eburnea J. R. Laundon		1		
Lepraria elobata Tønsberg	Lep elo	9		
Lepraria incana (L.) Ach.	Lep inc	7		
Lepraria lobificans Nyl.	Lep lob	6		
Leptogium imbricatum P. M. Jørg.	Lep imb	4	P	
Melanelixia glabratula (Lamy) Sandler & Arup	Mel gla	5		
Melanelixia fuliginosa (Fr. ex Duby) O. Blanco et al.	Mel ful	2		
Melanelixia subaurifera (Nyl.) O. Blanco et al.	Mel sub	77		
Melanohalea exasperata (De Not.) O. Blanco et al.		1		
Melanohalea exasperatula (Nyl.) O. Blanco et al.	Mel exl	4		
Micarea denigrata (Fr.) Hedl.	Mic den	7		
Micarea nitschkeana (Lahm ex Rabenh.) Harm.		2		st r
Micarea prasina Fr.	Mic pra	18		
Mycobilimbia carneoalbida (Müll. Arg.) S. Ekman & Printzen		1		
Mycobilimbia epixanthoides (Nyl.) Vitik. et al.		1		
Mycobilimbia hypnorum (Lib.) Kalb & Hafellner		1		
Normandina acroglypta (Norman) Aptroot	Nor acr	10		
Ochrolechia androgyna (Hoffm.) Arnold		1		
Ochrolechia arborea (Kreyer) Almb.	Och arb	56	P	
Ochrolechia microstictoides Räsänen	Och mic	6		
Ochrolechia szatalaensis Verseghy	Och sza	6	P	
Opegrapha atra Pers.		1		NT
Opegrapha ochrocheila Nyl.		1		VU, st r
Opegrapha rufescens Pers.		1		
Parmelia ernstiae Feuerer & A. Thell	Par ern	4	P	st r
Parmelia saxatilis (L.) Ach.	Par sax	7	P	
Parmelia sulcata Taylor	Par sul	78		
Parmeliopsis ambigua (Wulfen) Nyl.	Parm am	22		
Pertusaria albescens (Huds.) M. Choisy & Werner	Per alb	23	P	
Pertusaria amara (Ach.) Nyl.	Per ama	15		
Pertusaria coccodes (Ach.) Nyl.	Per coc	8		
Pertusaria hemisphaerica (Flörke) Erichsen		1		
Phlyctis argena (Spreng.) Flot.	Phl arg	110		
Physcia adscendens (Fr.) H. Olivier	Phy ads	67	P	
Physcia dubia (Hoffm.) Lettau		2		
Physcia stellaris (L.) Nyl.	Phy ste	7	P	
Physcia tenella (Scop.) DC. var. tenella	Phy ten	29	P	
Physconia distorta (With.) J. R. Laundon	Phys di	4	P	
Physconia enteroxantha (Nyl.) Poelt		2		
Placynthiella dasaea (Stirt.) Tønsberg	Plac da	3		
Platismatia glauca (L.) W. L. Culb. & C. F. Culb.		2		
Pseudevernia furfuracea (L.) Zopf	Pse fur	40	P	
Pseudosagedia aenea (Wallr.) Hafellner & Kalb		1		r
Pyrrhospora quernea (Dicks.) Körb.	Pyr que	32	P	
Ramalina farinacea (L.) Ach.	Ram far	26	P	
Ramalina fastigiata (Pers.) Ach.	Ram fas	13	P	
Ramalina fraxinea (L.) Ach.	Ram fra	3		

(Continued)

Table 2. (Continued).

Taxa	Abbreviation	No. of obs. (n = 126)	Indicator species	Status
Rinodina exigua Gray	Rin exi	22	P	
Rinodina pyrina (Ach.) Arnold	Rin pyr	7		
Rinodina sp. 1	Rin sp1	3	P	
Rinodina sp. 2		1		
Scoliciosporum chlorococcum (Stenh.) Vězda	Sco chl	11		
Tephromela atra (Huds.) Hafellner ex Kalb	Tep atr	4	P	
Trapeliopsis flexuosa (Fr.) Coppins & P. James	Tra fle	8		
Usnea hirta (L.) F. H. Wigg.	Usn hir	11	P	
Vulpicida pinastri (Scop.) J.-E. Mattsson & M. J. Lai	Vul pin	5		
Vulpicida juniperinus coll.	Vul jun	4		
Xanthoparmelia conspersa (Ach.) Hale		1		
Xanthoria candelaria (L.) Th. Fr.		2		
Xanthoria parietina (L.) Th. Fr.	Xan par	23	P	
Xanthoria polycarpa (Hoffm.) Th. Fr. ex Rieber	Xan pol	45	P	

Lichen species richness

The number of lichen species on junipers at plot level varied from 22 to 66 (n = 17), and at phorophyte level from 3 to 35 (n = 126).

The GLM analysis of the number of lichen species on junipers at plot level revealed the importance of two generalized environmental factors (Table 3). A compound factor for site productivity (Factor-1) had a unimodal relationship with total species richness (Fig. 4a), indicating species loss in high productivity alvars. In addition, the number of species per site increased with a compound factor joining the effect of stem circumference and juniper width (Factor-2) when estimates were small to medium, but the increase flattened out when junipers become really large (Fig. 4b, Table 3).

The results of GLM analyses at the phorophyte level showed that lichen species richness on juniper was statistically different between the two alvar types, with greater richness in plate alvars (Table 4). The strongest effect on species richness had the proportion of senescent branches of a juniper (Fig. 5a, Table 4). In addition, the species richness increased with phorophyte height and width (Fig. 5b–c, Table 4). Species pool size (species richness at plot level) was, as expected, also positively related to species richness on the individual phorophyte (Fig. 5d, Table 4).

Discussion

The diversity of lichens on a phorophyte is a complex phenomenon determined by intercorrelated factors functioning at multiple scales, including substrate properties, stand characteristics, geographical aspects, disturbances and air pollution (Giordani 2006, Hauck 2011, Bartels and Chen 2012, Ellis 2012). In a wide range of habitat conditions the number of epiphytic lichens found on a certain phorophyte species can be very high, for example in Great Britain, 235 species are reported on *Betula* spp. (Coppins 1984), more than 300 on *Quercus* spp. (Rose 1974), and 239 lichenized taxa on *Juniperus communis* (Ellis and Coppins 2009). Although this study represents a comparatively small geographic area and narrow range of climatic conditions, a rather high number of lichens (140 taxa) growing on juniper was recorded. This is a hundred species more than previously recorded on junipers in Estonia and as much as species number reported on other conifers (*Picea abies* (L.) H. Karst. and *Pinus sylvestris*) in this region (Lõhmus 2003). This is remarkable as in most cases the numbers of epiphytic lichens recorded on shrubby species are rather low (Barkman 1958). The very high species richness revealed by this study indicates that the alvar grassland is a significant and specific habitat not only for epilithic or epigeic lichens (Fröberg 1988, Ott et al. 1996, 1997, Leppik et al. 2013, 2015), but also for epiphytes, a fact that has been overlooked previously.

We showed that on juniper the composition and richness of lichens was influenced by the vitality of the phorophyte (proportion of dead branches) and its size (height and width, and stem circumference), indicating the intercorrelated effects of juniper age and size as well as indirect effects of exogenous disturbances (like flooding, drought, fire), which are known to influence juniper vitality in calcareous grasslands (Rosén 1982, 1995). Increasing number of epiphytes with increasing size of the juniper is evidently caused by an enlarging habitat area and by temporal effects as the colonisation is expected to increase on a substratum which is available for colonisation over a longer period (Ellis 2012, Schei et al. 2013). However, the effect of ageing in terms of changed bark quality may also play an important role in determining species composition, as has been described for older trees (Bartels and Chen 2012). Ageing influences the vitality of phorophytes and we revealed that the epiphytic species richness increased with the proportion of dead branches on a juniper. Also, the composition of epiphytic lichens was specific for scencent junipers, similar to dwarf shrub *Helianthemum oelandicum* in alvars of Öland (Fröberg et al. 2009).

Unique species-rich lichen assemblages occurred on junipers in plate alvars. We found that the epiphytic species richness was twice higher in plate alvars than in ryhk alvars (Table 4). Also, the species composition in plate alvars differed from ryhk alvars by having 42 characteristic lichen species, while ryhk alvars had only three, indicating a more stochastic appearance of lichens in ryhk alvars. Junipers in plate alvars hosted a rich assemblage of acidophobic epiphytes commonly usually on sub-neutral bark of broad-leaved trees (e.g.
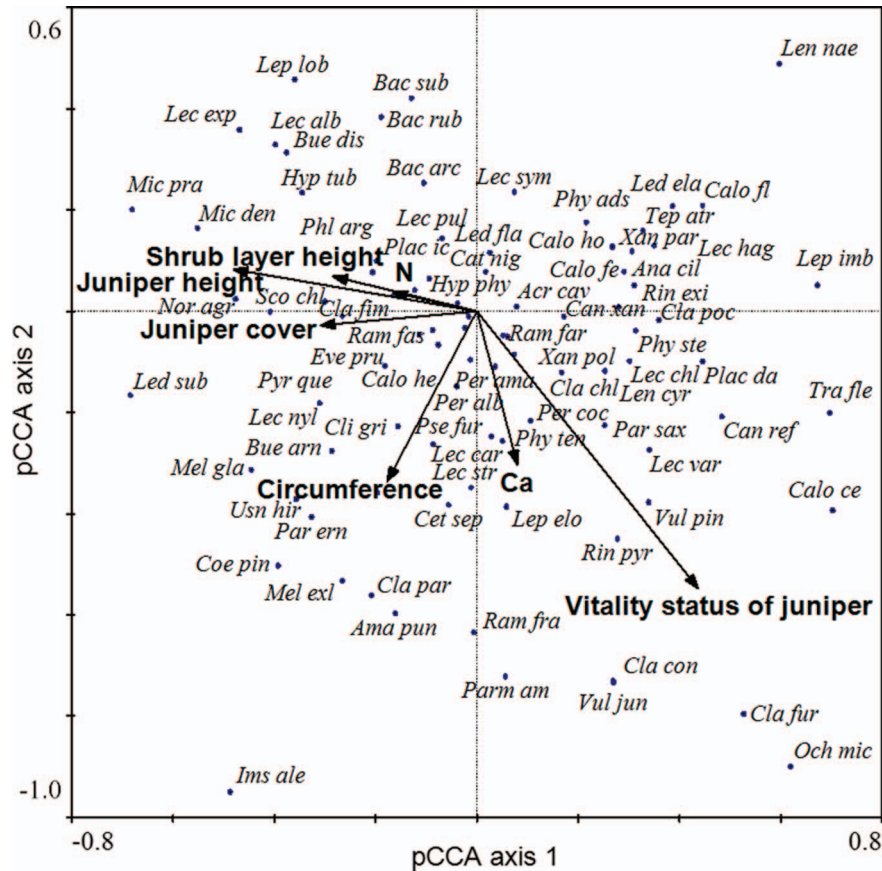
Figure 2. DCA ordination diagram of lichen species and significantly correlated environmental variables (cut off value – 0.2) (a) of the first and the second axes and (b) of the first and the third axes. Samples are grouped according to habitat types: junipers from plate alvars = empty triangle, junipers from ryhk alvars = solid triangle. For abbreviations of lichen species see Table 2.

135

Figure 3. Lichen species and the environmental variables on the biplot of partial canonical correspondence analysis (pCCA) for the first and the second axes. Variance in the composition of the epiphytic community, caused by the geographical location of the study sites, is taken into account by setting variable describing geographical location as the covariable. For abbreviations of lichen species see Table 2. Circumference = stem circumference of juniper, N = soil N content in the sample plot multiplied with soil depth describing soil productivity, Ca = soil Ca content in the sample plot, Vitality status of juniper = the parameter of vitality of juniper reflecting the increasing proportion of senescent branches on a phorophyte.

Anaptychia ciliaris, *Caloplaca flavorubescens*, *C. herbidella*, *Physcia stellaris*, *Physconia distorta* and *Ramalina fastigiata*), although juniper is known to have relatively acid bark (pH ca 5.2) (Barkman 1958, Ellis and Coppins 2009). In addition, several species usually growing on ground mosses in calcareous soil, such as *Agonimia* spp., *Bilimbia* spp. and *Diploschistes muscorum* (Leppik et al. 2013), were found on epiphytic bryophytes on juniper stems as well (Table 2). Particles of calcareous dust from thin soil and bedrock may significantly

Table 3. The results of general linear model analysis (GLM) of the number of lichen species (log-transformed) on junipers per sample plot (n = 17). Alvar types (plate and ryhk) and three generalized environmental factors (Factors 1, 2 and 3, see Table 1) are included in the model. Statistically significant p-values are in bold.

Effect	DF	F	p	Slope
Intercept	1	2721.8	**< 0.00001**	1.74
Alvar type	1	0.7	0.4035	
Factor-1 (site productivity)	1	0.4	0.5228	0.01
Factor-2 (circumference)	1	4.3	0.0641	0.05
Factor-3 (site location)	1	0.8	0.3772	0.02
(Factor-1)^2 (site productivity)	1	6.5	**0.0287**	−0.04
(Factor-2)^2 (circumference)	1	6.4	**0.0292**	−0.05

increase the bark pH of a phorophyte (Gilbert 1976, Gauslaa and Holien 1998) and accordingly, the junipers in calcareous grassland can host both common acidophilic and unusual for juniper neutrophytic epiphytes, depending on the site conditions.

At the plot scale, we documented a unimodal relationship between epiphytic lichen species richness and site productivity (Fig. 4a), which reflects the apparently unsuitable conditions for epiphytic lichen growth on tall junipers in dense shrublands. The light conditions for epiphytes on juniper might be determined by juniper average height and cover, and the limiting light effect is apparently accelerated with rapid overgrowth of grasslands in more productive sites (Rosén 1982). In less overgrown sites (on plate alvars and ryhk alvars on very thin soil) junipers host lichen species adapted to high solar radiation, while in more overgrown sites, lichen species with contrasting light preferences were found (Fig. 3). This indicates more heterogeneous light conditions on the phorophyte, from shade inside clumps, to more well-lit edge branches of an individual, thereby allowing the coexistence of light and shade demanding species (Valladares 2003).

The traditional land-use of alvars, i.e. sheep grazing, is nowadays maintained only in limited areas, and therefore,
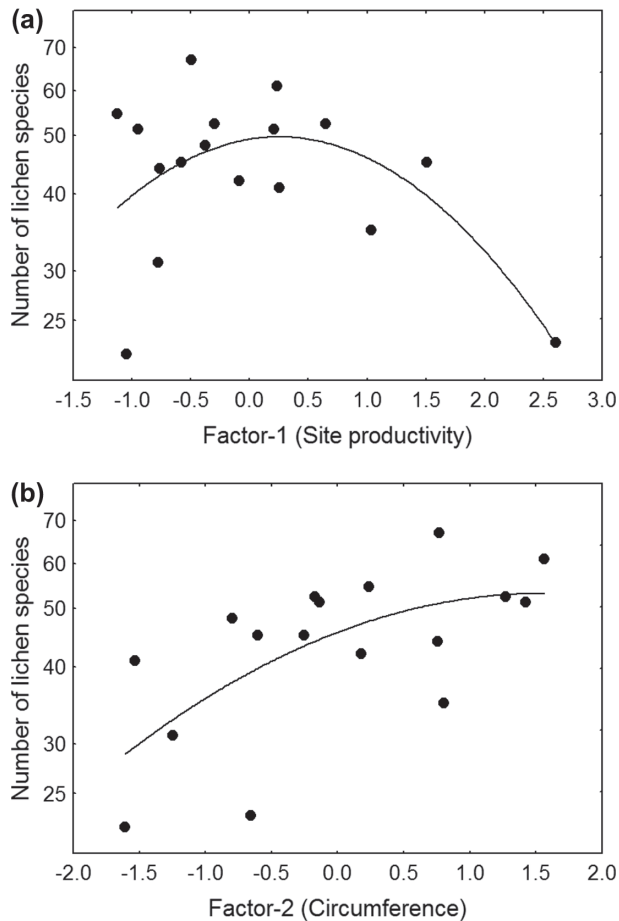
(a)

(b)

Figure 4. Relationship between the number of lichen species per study plot and generalized environmental factors (A) Factor-1 (site productivity) and (B) Factor-2 (circumference) (Table 3).

the cutting of trees and shrubs is often instead used to control grassland encroachment (Rosén and van der Maarel 2000). Our study supports the need to control grassland encroachment as we found that the increasing cover of juniper negatively influence epiphytic lichen assemblies, but we want to highlight the importance of well-lit old and senescent junipers for epiphytic lichens. Therefore we would advocate protection of some old and senescent junipers during restoration activities on overgrown calcareous grasslands. Retaining some

trees or bushes in a grassland is important also for other fungi, for example for basidiomycetes, including many nationally red-listed species (Kalamees 2004). In addition, the presence of some shrubs increases the variability of microhabitats, for example, juniper shade creates suitable growth conditions for some terricolous lichen and moss species that are intolerant to high-irradiance (Rosén 1982, Kalapos and Mázsa 2001).

For vascular plants, a moderate shrub cover can promote plant species coexistence at small spatial scales (Gazol et al. 2012, Reitalu et al. 2014). Unfortunately, small groups of juniper in a grassland may increase the invasion of other woody species in these habitats, for example, *Prunus spinosa* L. and *Rosa* spp. often colonize within juniper shrubs (Rosén 1982), thus accelerating the conversion of grassland to forest (Pärtel and Helm 2007). Moreover, juniper seedlings appear mainly in half-open areas between already existing junipers (Rosén and van der Maarel 2000). Nevertheless, we still would recommend preserving some old or senescent junipers for epiphytes in restored alvars, and do not consider these to be a serious threat to alvar communities as old and big shrubs expand more slowly than intermediate sized junipers (30–50 yr old) (Rosén and van der Maarel 2000).

In conclusion, we can generalize that drivers of epiphytic lichen diversity on a shrub phorophyte (juniper) correspond to drivers of epiphytic communities in forest ecosystems (Bartels and Chen 2012, Ellis 2012). The epiphytic component of biodiversity on juniper is equally endangered by grassland encroachment as is ground layer species assemblies (Leppik et al. 2013, 2015). The significance of juniper as a diverse substratum for epiphytes should be recognized, and this is especially important in semi-natural habitats where the conservation of species diversity should consider different scales and different taxonomic groups (Brown et al. 1994, WallisDeVries et al. 2002).

Table 4. The results of general linear model analysis (GLM) of the number of lichen species per juniper (n = 126). Vitality status of juniper is reflecting the increasing proportion of senescent branches on a phorophyte and species pool is the number of lichen species per plot, reflecting the species pool of the habitat. Statistically significant p-values are in bold.

Effect	DF	F	p	Mean (± SD)	Slope
Intercept	1	4.4	**0.037**		−12.85
Alvar type	1	52.5	**<0.00001**		
Plate alvar				27.1 (± 1.11)[a]	
Ryhk alvar				14.8 (± 0.59)[b]	
Juniper height	1	19.0	**0.00002**		0.02
Juniper width	1	4.9	**0.0275**		0.01
Vitality status of juniper	1	84.7	**<0.00001**		4.40
Species pool	1	6.7	**0.0107**		9.69

Slope estimates are presented for continuous variables; within-group mean values are presented for categorical variables, letter labels denote homogeneity groups according to the results of Fisher's Least Significant Difference (LSD) test.
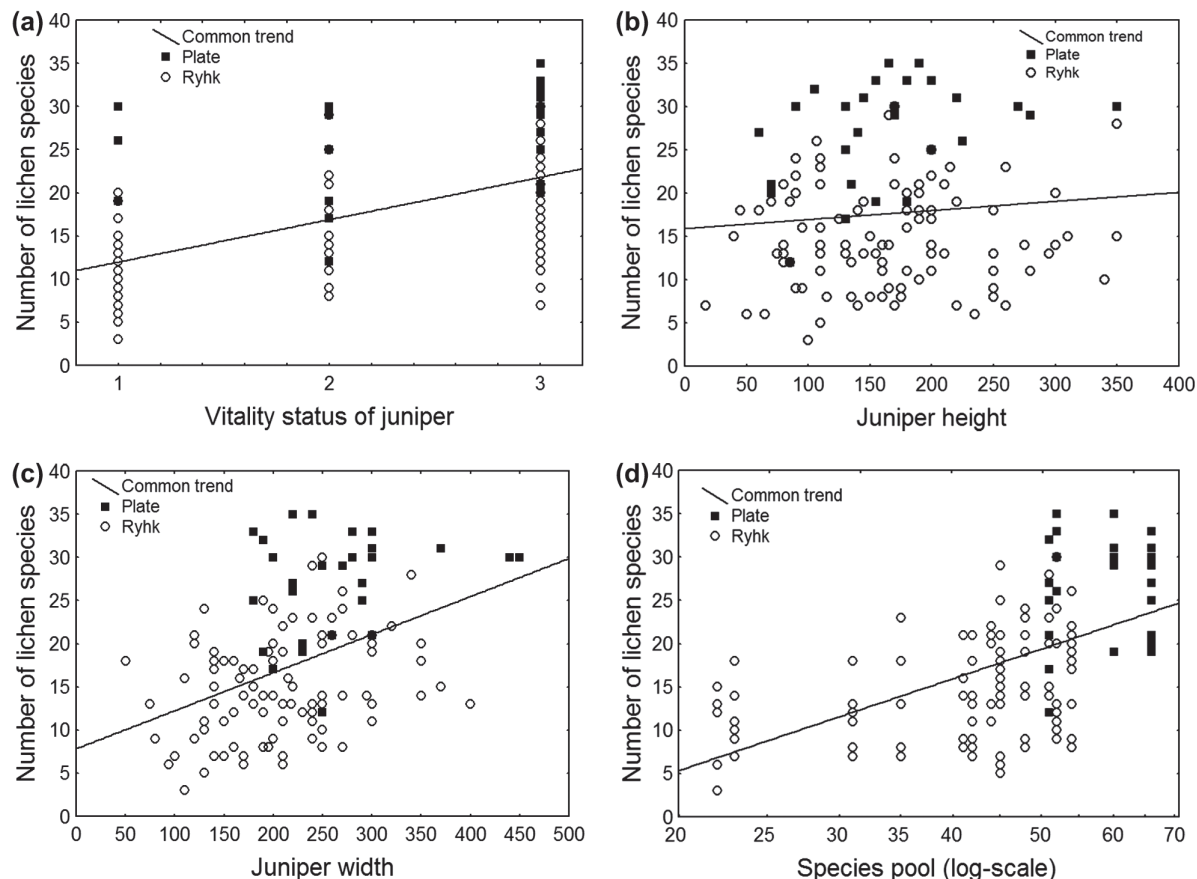
Figure 5. Relationship between the number of lichen species per juniper and (a) vitality status of juniper, (b) juniper height, (c) juniper width and (d) species pool (Table 4). Vitality status of juniper was treated as a continuous variable: 1 = only living branches, 2 = dead branches less than 50%, 3 = dead branches 50% or more. Species pool = the number of lichen species per plot, to reflect the species pool of the habitat. Black quadrates denote the junipers from plate alvar and circles denote the junipers from ryhk alvars.

References

Albertson, N. 1950. Das grosse sudliche alvar der Insel Öland. Eine pflanzensoziologische Übersicht. – Svensk Bot. Tidskr. 44: 269–331.

Aragón, G. et al. 2010. Effects of Mediterranean dehesa management on epiphytic lichens. – Sci. Tot. Environ. 409: 116–122.

Barkman, J. J. 1958. Phytosociology and ecology of cryptogamic epiphytes. – Van Gorcum and Company.

Bartels, S. F. and Chen, H. Y. H. 2012. Mechanisms regulating epiphytic plant diversity. – Crit. Rev. Plant Sci. 31: 391–400.

Brown, M. J. et al. 1994. Conservation and reservation of non-vascular plants in Tasmania, with special reference to lichens. – Biodivers. Conserv. 3: 263–278.

Coppins, B. J. 1984. Epiphytes of birch. – Proc. R. Soc. Edinb. 85: 115–128.

Dengler, J. et al. 2006. Dry grassland communities of shallow, skeletal soils (Sedo-Scleranthenea) in northern Europe. – Tuexenia 26: 159–190.

Dufrêne, M. and Legendre, P. 1997. Species assemblages and indicator species: the need for a flexible asymmetrical approach. – Ecol. Monogr. 67: 345–366.

Ellis, C. J. 2012. Lichen epiphyte diversity: a species, community and trait-based review. – Perspect. Plant Ecol. Evol. Syst. 14: 131–152.

Ellis, C. J. and Coppins, B. J. 2009. Quantifying the role of multiple landscape-scale drivers controlling epiphyte composi-

tion and richness in a conservation priority habitat (juniper scrub). – Biol. Conserv. 142: 1291–1301.

Fröberg, L. 1988. Calcicolous lichens and their ecological preferences on the Great Alvar of Öland. – Acta Phytogeogr. Suec. 76: 47–52.

Fröberg, L. et al. 2009. Age and epiphytic lichen diversity of the dwarf shrub *Helianthemum oelandicum* on the island of Öland, Sweden. – Lichenologist 41: 537–545.

Gauslaa, Y. and Holien, H. 1998. Acidity of boreal *Picea abies* – canopy lichens and their substratum, modified by local soils and airborne acidic depositions. – Flora 193: 249–257.

Gazol, A. et al. 2012. Landscape- and small-scale determinants of grassland species diversity: direct and indirect influences. – Ecography 35: 944–951.

Gilbert, O. L. 1976. An alkaline dust effect on epiphytic lichens. – Lichenologist 8: 173–178.

Giordani, P. 2006. Variables influencing the distribution of epiphytic lichens in heterogeneous areas: a case study for Liguria, NW Italy. – J. Veg. Sci. 17: 95–206.

Hauck, M. 2011. Site factors controlling epiphytic lichen abundance in northern coniferous forests. – Flora 206: 81–90.

Hill, M. O. and Gauch, H. G. 1980. Detrended correspondence analysis: an improved ordination technique. – Vegetatio 42: 47–58.

Jüriado, I. et al. 2003. Epiphytic and epixylic lichen species diversity in Estonian natural forests. – Biodivers. Conserv. 12: 1587–1607.

Jüriado, I. et al. 2006. Biogeographical determinants of lichen species diversity on islets in the west-Estonian Archipelago. – J. Veg. Sci. 17: 125–134.

Kalamees, K. 2004. Seenestik. – In: Kukk, T. (ed.). Pärandkooslused. Õpik-käsiraamat. Pärandkoosluste Kaitse Ühing, pp. 136–142.

Kalapos, T. and Mázsa, K. 2001. Juniper shade enables terricolous lichens and mosses to maintain high photochemical efficiency in a semiarid temperate sand grassland. – Photosynthetica 39: 263–268.

Kukk, T. and Sammul, M. 2006. Area of seminatural Natura 2000 habitat types in Estonia. – In: Sammul, M. (ed.), Year-book of the Estonian Naturalists' Society. Estonian Naturalists' Society, pp. 114–158.

Laasimer, L. 1965. Eesti NSV taimkate. – Valgus.

Leppik, E. et al. 2011. Changes in stand structure due to the cessation of traditional land use in wooded meadows impoverish epiphytic lichen communities. – Lichenologist 43: 257–274.

Leppik, E. et al. 2013. The conservation of ground layer lichen communities in alvar grasslands and the relevance of substitution habitats. – Biodivers. Conserv. 22: 591–614.

Leppik, E. et al. 2015. Functional ecology of rare and common epigeic lichens in alvar grasslands. – Fungal Ecol. 13: 66–76.

Lõhmus, P. 2003. Composition and substrata of forest lichens in Estonia: a meta-analysis. – Folia Cryptog. Estonica 40: 19–38.

Martin, L. et al. 2000. Lichens of Vormsi Island. – Folia Cryptog. Estonica 36: 65–81.

McCune, B. and Mefford, M. J. 1999. PC-ORD. Multivariate analysis of ecological data, ver. 4. – MjM Software Design, Gleneden Beach.

Mielke, P. W. 1984. Meteorological applications of permutation techniques based on distance functions. – In: Krishnaiah, P. R. and Sen, P. K. (eds), Handbook of statistics. Vol. 4. Elsevier Sci. Publ., pp. 813–830.

Orange, A. et al. 2001. Microchemical methods for the identification of lichens. – Brit. Lichen Soc.

Ott, S. et al. 1996. Vegetation of the rock-alvar of Gotland I. Microhabitats and succession. – Nova Hedw. 63: 433–470.

Ott, S. et al. 1997. Vegetation of the rock-alvar of Gotland II. Microclimate of lichen-rich habitats. – Nova Hedw. 64: 87–101.

Pärtel, M. and Helm, A. 2007. Invasion of woody species into temperate grasslands: relationship with abiotic and biotic soil resource heterogeneity. – J. Veg. Sci. 18: 63–70.

Pärtel, M. et al. 1999a. Alvar grasslands in Estonia: variation in species composition and community structure. – J. Veg. Sci. 10: 561–570.

Pärtel, M. et al. 1999b. Landscape history of a calcareous (alvar) grassland in Hanila, western Estonia, during the last three hundred years. – Landscape Ecol. 14: 187–196.

Pärtel, M. et al. 2005. Biodiversity in temperate European grasslands: origin and conservation. – In: Lillak, R. et al. (eds), Integrating efficient grassland farming and biodiversity. Estonian Grassland Soc., pp. 1–14.

Pärtel, M. et al. 2007. Grassland diversity related to the Late Iron Age human population density. – J. Ecol. 95: 574–582.

Poschlod, P. and WallisDeVries, M. F. 2002. The historical and socioeconomic perspective of calcareous grasslands – lessons from the distant and recent past. – Biol. Conserv. 104: 361–376.

Randlane, T. and Saag, A. 1999. Second checklist of lichenized, lichenicolous and allied fungi of Estonia. – Folia Cryptog. Estonica 35: 1–132.

Randlane, T. et al. 2008. Lichens in the new red list of Estonia. – Folia Cryptog. Estonica 44: 113–120.

Randlane, T. et al. 2013. Lichenized, lichenicolous and allied fungi of Estonia. Ver. Dec 31, 2013. – <http://esamba.bo.bg.ut.ee/checklist/est/home.php>, accessed 30 Jan 2014.

Reitalu, T. et al. 2014. Determinants of fine-scale plant diversity in dry calcareous grasslands within the Baltic Sea region. – Agricult. Ecosyst. Environ. 182: 59–68.

Rose, F. 1992. Temperate forest management, its effect on bryophyte and lichen floras and habitats. – In: Bates, J. W. and Farmer, A. M. (eds), Bryophytes and lichens in a changing environment. Clarendon Press, pp. 211–233.

Rose, F. 1974. The epiphytes of oak. – In: Morris, M. G. and Perring, F. H. (eds), British Oak Classey, pp. 250–273.

Rosén, E. 1982. Vegetation development and sheep grazing in limestone grasslands of south Öland, Sweden. – Acta Phytogeogr. Suec. 72: 1–104.

Rosén, E. 1995. Periodic droughts and long-term dynamics of alvar grassland vegetation on Öland, Sweden. – Folia Geobot. Phytotax. 30: 131–140.

Rosén, E. and van der Maarel, E. 2000. Restoration of alvar vegetation on Öland, Sweden. – Appl. Veg. Sci. 3: 65–72.

Sanderson, N. and Wolseley, P. 2001. Management of pasture woodlands for lichens. – In: Fletcher, A. (ed.), Lichen habitat management. Brit. Lichen Soc., pp. 05-1–05-25.

Schei, F. H. et al. 2013. Conservation of epiphytes: single large or several small host trees? – Biol. Conserv. 168: 144–151.

ter Braak, C. J. F. 1986. Canonical correspondence analysis: a new eigenvector technique for multivariate direct gradient analysis. – Ecology 67: 1167–1179.

ter Braak, C. J. F. and Šmilauer, P. 2002. CANOCO reference manual and CanoDraw for Windows user's guide: software for canonical community ordination, ver. 4.5. – Microcomputer Power, Ithaca.

Valladares, F. 2003. Light heterogeneity and plants: from ecophysiology to species coexistence and biodiversity. – In: Esser, K. et al. (eds), Progress in botany. Springer-Verlag, pp. 439–471.

Van der Maarel, E. 1988. Floristic diversity and guild structure in the grasslands of Öland's Stora Alvar. – Acta Phytogeogr. Suec. 76: 53–65.

WallisDeVries, M. F. et al. 2002. Challenges for the conservation of calcareous grasslands in northwestern Europe: integrating the requirements of flora and fauna. – Biol. Conserv. 104: 265–273.

Zobel, M. 1987. The classification of Estonian alvars and their plant communities. – In: Laasimer, L. and Kull, T. (eds), The plant cover of the Estonian SSR. Flora, vegetation and ecology. Valgus, pp. 28–45.