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Epiphytic lichen diversity in central European oak forests: Assessment of the effects of natural environmental factors and human influences

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We detected the different responses of lichens to ecological predictors in polluted and unpolluted areas.

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ABSTRACT

We investigated lichen diversity in temperate oak forests using standardized protocols. Forty-eight sites were sampled in the Czech Republic, Slovakia and Hungary. The effects of natural environmental predictors and human influences on lichen diversity (lichen diversity value, species richness) were analysed by means of correlation tests. We found that lichen diversity responded differently to environmental predictors between two regions with different human impact. In the industrial region, air pollution was the strongest factor. In the agricultural to highly forested regions, lichen diversity was strongly influenced by forest age and forest fragmentation. We found that several natural factors can in some cases obscure the effect of human influences. Thus, factors of naturality gradient must be considered (both statistically and interpretively) when studying human impact on lichen diversity.
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1. Introduction

Over the last decade, new screening techniques have been developed that use lichens in biomonitoring. In particular, the European guideline for mapping lichen diversity as an indicator of environmental stress (lichen diversity value method) was published by Asta et al. (2002). This technique is based on the fact that epiphytic lichen diversity is greatly and steadily diminished with increasing air pollution and environmental stress. The frequency of species' occurrences on a defined portion of a tree trunk is used as an assessment of diversity (the lichen diversity value, LDV) which is employed as a parameter to estimate the degree of environmental stress. This technique has been mainly applied to assess and monitor environmental alteration especially in relation to the effect of atmospheric pollution in several European countries (Pinho et al., 2004, 2008; Castello and Skert, 2005; Larsen et al., 2006; Giordani, 2006, 2007; Svoboda, 2007; Cristofolini et al., 2008) or also to study the influence of climate and several other factors on the distribution of lichens (Cristofolini et al., 2008; Giordani and Incerti, 2008).

Loppi et al. (2002) proposed an approach following the LDV technique for estimation of degree of naturality in selected ecosystems or vegetation types (biotopes). Naturality classes were obtained using LD values taken from standard plots in different localities with various degrees of natural versus disturbed (harvested, polluted, etc.) status. But how the term "naturality" is defined is, to some extent, a matter for debate. The term "natural" should be reserved for areas free from heavy human use and from significant pollution deposition, whether from near or distant sources (Loppi et al., 2002). Any deviation from expected health or diversity of organisms from natural/normal status (i.e. not affected by human activity) could be considered as a disruption of naturality. Lichens, being extremely sensitive to disruptions in naturality resulting from air pollution, especially that involving SO_2 (cf. Nimis et al., 2002), or forest fragmentation (e.g. Fritz et al., 2008; Hedenås and Ericson, 2008; Ranius et al., 2008; Moning and Müller, 2009), offer premium utility as indicators of naturality. Other factors influencing lichen diversity are entirely natural, that is, acting independently of any local human influence (especially climatic parameters). However, these ecological factors can distinctly shift the outcomes of anthropogenic factors on LDV, or differences in LDV attributed to anthropogenic impacts may be due solely to natural ecological factors. For example, Giordani (2007) detected various lichen diversity values responding to local rainfall despite a nonvarying regional pollution level. Unfortunately, only a few studies have followed the recommendations of Loppi et al. (2002) by adjusting their protocol to account for natural environmental variables when using LDV to measure naturality (Castello and Skert, 2005; Frati and Brunialti, 2006; Giordani, 2007; Svoboda and Peksa, 2008).

The objective of the present study is to analyse the effects of natural environmental predictors and human influences on lichen

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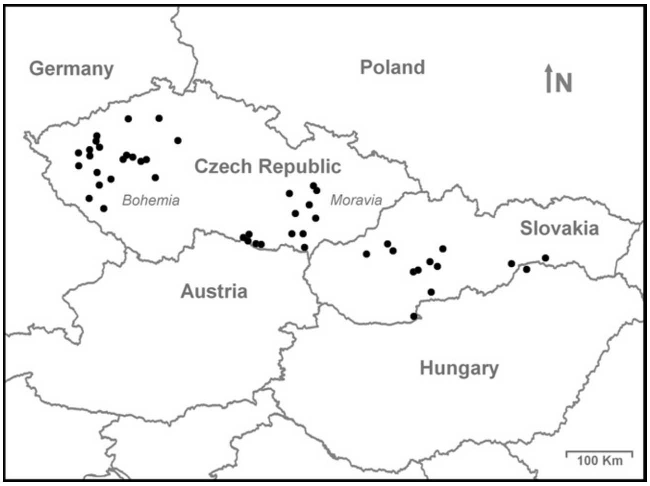

Fig. 1. Map of localities. Black circles – screened localities (Bohemia – north-west; Moravia, Slovakia and Hungary – south-east). Map was constructed using ArcGIS 9.1 and PhotoFiltre studio 9.2.2.

diversity in central European oak forests. We tested and assessed the degree of dependence between the variety of environmental factors and lichen diversity.

2. Material and methods

2.1. Study area

In the temperate climatic zone of central Europe, diverse types of oak forests can naturally occur in lowlands up to ca 600 m a.s.l., especially in semi-dry or sub-xerothermic habitats (Janssen and Seibert, 1991; Neuhäuslová et al., 1997). Remnants of natural (or close to natural) oak forests occur in several site types. From several types or associations of oak forests, it was necessary to choose those suitable for sampling. Associations were defined adopting the classification used in the conservation-listing database NATURA 2000 (www.natura.org). From this classification, the following biotopes were selected (for exact descriptions see, for example Chytrý et al., 2001): thermophilous oak forests (*Quercion pubescenti-petraeae*, *Aceri tatarici quercion*) and acidophilous oak forests, preferably among the latter the dry acidophilous oak forest (*Quercion petraeae*, *Genisto-germaniae quercion*). From a lichenological point of view, we assumed no significant difference among these vegetation types. This hypothesis was supported by screening of additional environmental variables. We did not investigate phytocoenological aspects of these associations more deeply for the present study. In the field, only basic characteristics (oak species, group and subgroup of temperate oak forest) related to NATURA 2000 biotopes were recorded.

In total 48 localities were chosen for this study: 29 in Bohemia (Czech Republic), 6 in Moravia (Czech Republic), 12 in Slovakia and 1 in Hungary (Fig. 1). During the field work we omitted potential sites which had characters that were not appropriate for testing (see sampling design). Selected forests included 42 sites with *Q. petraea* or mixed *Q. petraea* with *Quercus pubescens*, and 6 localities with *Quercus cerris* or *Q. cerris* were mixed with *Q. petraea/pubescens* (tree species were identified using the key of Hejný and Slavík, 1990). Zonal forests of natural or seminatural status were chosen. Much helpful literature is available describing these natural or close to natural areas (e.g. Němec and Ložek, 1996; Mackovčin and Sedláček, 1999, 2002) since many of them are protected as nature reserves or as protected landscape areas within the Czech Republic. In Slovakia, the catalogue of biotopes (Stanová and Valachovič, 2002) and the database of protected areas (www.sazp.sk) were particularly useful to identify appropriate sampling sites and to obtain information about these sites.

Our selection of sampling sites followed several criteria: incline of up to 35°; locality not directly affected by traffic, industry or other type of direct anthropogenic disturbance; minimal understory layer cover; oak canopy cover higher of at least 75%; forest age of at least 40 years. These localities are generally found in rocky escarpments, high slopes and in other relatively inaccessible areas, because more accessible sites are severely impacted during centuries by intensive human use in the central European landscape.

2.2. Sampling design

Forty-eight sampling sites were selected using criteria described above, based on biotope type, and proportionally on the geographical surface and possible anthropogenic influence on forests (industrial impacts, agriculture, air pollution) as far as possible in the three countries. We chose sites no smaller in area 1000 m^2 (100 × 100 m). We sampled within well-developed oak vegetation as near to the centre of the locality as possible, a 50 × 50 m area (sampling plot). The LDV data record frequencies of all lichen species in quadrate segments in a terrain grid positioned on four cardinal points of the selected tree. LDV measurements were taken on ideally 5 oak trees (at least 3) in each locality.

2.3. Lichen sampling

On each tree, the presence of epiphytic lichen species (including microlichens) was recorded using a terrain grid. Following the recommendations of Asta et al. (2002) for tree selection, sampling was limited to solitary trees (in the forest context) not directly influenced by the shrub layer, not leaning, and with diameter greater than 20 cm.

Lichen diversity value per sampling plot was indicated as the mean of the LDV values obtained from individual trees (following protocols in Asta et al., 2002). Beside this, we recorded species richness (independently to LDV measurements) throughout the whole sampling plot on all oak trees (up to 2 m from the ground) including all epiphytic lichens (macrolichens and microlichens).

2.4. Environmental data

Environmental data at tree- and plot-level were collected for each sample. These included oak species; forest age (data from Forest Management Institute – www.uhul.cz, or age of the oldest trees estimated by extrapolation after tree rings based on another cut or damaged trees in the locality; historical maps 1842-52 were also checked for forest continuity, unfortunately older data are not available); forest fragmentation (as % of forestation in 10 × 10 km square in the neighbourhood of the locality); GPS-derived coordinates (north-GPS, east-GPS); altitude; mean annual potential direct radiation (calculated according to Herben (1987) as potential direct solar radiation as a function of slope angle, orientation and latitude of the measured site); mean annual precipitation (Veselský et al., 1958); and air pollution levels in 1996 and 2005 (mean annual concentrations of SO_2 (SO_2-96, SO_2-05), NO_x (NO_x-96, NO_x-05) and particulate matter up to 10 μm in diameter (PM10s-96, PM10s-05) – data obtained from the Czech Hydrological and Meteorological Institute, www.chmi.cz). Air pollution in sampled sites in 1996 (in 2005) varied from 5 to 35 (0 to 10) $\mu g\ m^{-3}$ for SO_2, 10 to 35 (10 to 35) $\mu g\ m^{-3}$ for NO_x and 10 to 65 (10 to 35) $\mu g\ m^{-3}$ for PM10s annual means.

Main characteristics are reported in Appendix.

2.5. Statistical analyses

Descriptive statistics, non-parametric ANOVA (Kruskal–Wallis test) and correlation coefficients were calculated using PAST software ver. 1.74 (Hammer et al., 2001). Correlations between environmental parameters were assessed using Spearman's rank correlation coefficient (non-parametric test). For statistical analyses one parameter was selected from a group of highly correlated parameters (e.g. PM10s-96 from values of air pollution). Simple and partial Mantel tests (Mantel, 1967; Smouse et al., 1986; Legendre and Legendre, 1998) were performed to test for correlations and covariations between distance (dissimilarity) matrices. Matrices were calculated for LDV, species richness, forest age, forest fragmentation, north-GPS, east-GPS, altitude, potential radiation, precipitation, PM10s-96 and air pollution (Euclidean distances between sites based on values of air pollution standardized to standard deviation). The environmental distance matrices were obtained from difference in parameter values between two samples. The significance of correlations was tested by Monte-Carlo permutation test, simulation of 1000 randomizations (software ZT ver. 1.0, Bonnet and Van de Peer, 2002). Owing to the division of our data to north-western (Bohemia) and south-eastern (Moravia, Slovakia, Hungary) geographical clusters (Fig. 1), we divided the dataset into two parts to test influence of particular environmental factors within these areas. Sampling sites and environmental parameters were ordinated using indirect multivariate analysis (principal component analysis) in CANOCO ver. 4.5 (ter Braak and Šmilauer, 1998).

3. Results

3.1. Lichen species composition

In total 111 taxa of epiphytic lichens were determined in all localities.

Identified lichens can be ranged into three groups with respect to frequencies of occurrence in accordance with publications:

1) Common epiphytes of broad-leaves trees, acidophytes to neutrophytes (Purvis et al., 1992; Wirth, 1995; van Herk, 2002) dominate in majority of localities e.g. *Amandinea punctata*, *Candelariella reflexa*, *Candelariella xanthostigma*, *Cladonia coniocraea*,

Table 1
Descriptive statistics of lichen diversity, central European oak forests.

	N	Mean	Median	Min	Max	25° percentile	75° percentile	Standard deviation
LDV	48	42	40	17	88	29	53	±19
Species richness	48	22	23	3	46	13	28	±10

Hypocenomyce scalaris, *Hypogymnia physodes*, *Lecanora chlarotera*, *Lecanora conizaeoides*, *Lecanora expallens*, *Lepraria incana*, *Melanelia fuliginosa*, *Parmelia sulcata*, *Phlyctis argena*, *Physcia adscendens*, *Physconia enteroxantha*, *Punctelia jeckeri*.

2) Lichens typical for oak forests, relatively often occurring in well-developed forest communities (Hilitzer, 1925; Rose, 1974; Wirth, 1995) e.g. *Bacidia rubella*, *Bryoria fuscescens*, *Chaenotheca chryso-cephala*, *Chaenotheca trichialis*, *Flavoparmelia caperata*, *Lecanora albella*, *Lecanora carpinea*, *Melanelia subargentifera*, *Parmelina tiliacea*, *Pertusaria albescens*, *Pertusaria amara*, *Pleurosticta acetabulum*, *Physconia grisea*, *Physconia perisidiosa*, *Ramalina farinacea*, *Ramalina pollinaria*, *Tuckermannopsis chlorophylla*.
3) Rare species (Rose, 1976, 1992; Purvis et al., 1992; Wirth, 1995; Pišút, 1999), for example *Acrocordia gemmata*, *Calicium* spp., *Caloplaca lucifuga*, *Caloplaca ferruginea*, *Cetrelia* cf. *olivetorum*, *Chaenotheca phaeocephala*, *Parmelina quercina*, *Ramalina fraxinea* were found in natural sites, related to predominantly south-eastern localities (pasture-woodlands, forests).

Lichen diversity (both LDV and species richness) did not significantly differ between the three sampled oak species (Kruskal–Wallis test $p > 0.05$). Mean diversity values obtained from particulars trees were 52 (LDV) and 10 (species richness).

Statistical analyses of lichen species composition of studied oak forests are the subject of upcoming article therefore we did not provide more detailed results.

3.2. Lichen diversity value

Following the recommendations of Loppi et al. (2002), we stratified our data according to the range of anthropogenic impacts – from relatively industrial Bohemia, through the agricultural regions of Moravia, to the highly forested regions of Slovakia. From the whole area, we obtained the LD values for particular localities ranging from 17 to 88 (Table 1; Appendix). The highest LD values were recorded inside the seminatural and natural sites in regions with higher forest cover and low air pollution levels (central Slovakia), and the lowest in the intensively human-exploited landscape affected by various pollution sources in Bohemia and Moravia. Species richness varied from 3 to 46 taxa for locality (sampling plot), with again, higher values found in Slovakia, and lower values in Bohemia and Moravia (Table 1; Appendix). Our results related to the naturality assessment are consistent with other findings (Loppi et al., 2002; Giordani, 2007; Svoboda, 2007). However, obtained data are insufficient for construction of naturality classes after the original methodology (Loppi et al., 2002).

3.3. Correlations between variables

The principal component analysis (PCA) based on species composition separated along the first ordination axis (21.9% of the explained variation) most of the north-western sites from south-eastern sites (Fig. 2). Consequently, north- and east-GPS coordinates were found to be highly correlated with first axis. In addition, species richness and LDV were positively correlated with precipitation, east-GPS and with forest fragmentation (i.e. forest cover) negatively with north-GPS and with increasing concentrations of pollutants.

In the Mantel tests we focused on testing the relationships between diversity of lichens (LDV, species richness) and environmental factors (Table 2). In addition, we examined correlations among variables with particular influence on lichen diversity (partial results are shown in Table 3).

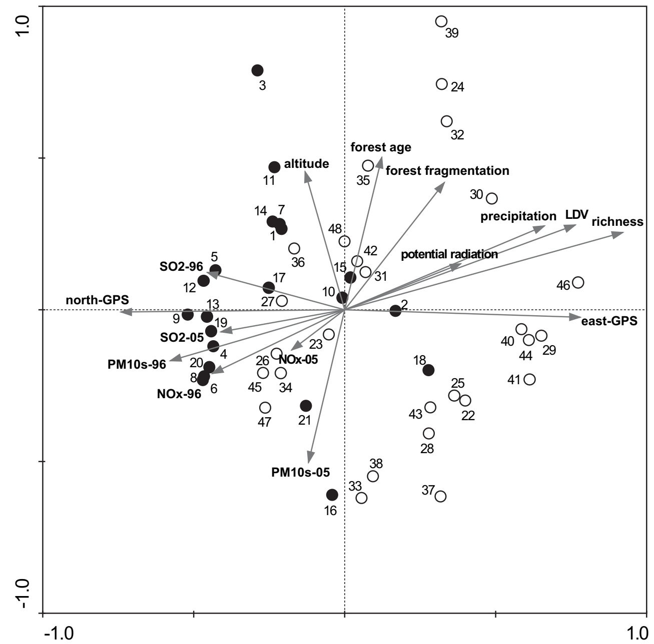

Fig. 2. Ordination plot of principal component analysis (PCA). Distribution of localities and environmental parameters in relation to species data. Black circles – north-west localities, white circles – south-east localities.

As shown in the lower left part of Table 2, the lichen diversity (LDV/species richness) was significantly ($p < 0.05^*$, $p < 0.01^{**}$, $p < 0.001$ without asterisk) positively correlated with precipitation ($r = 0.64/0.50$), forest fragmentation ($r = 0.49/0.25$), altitude ($r = 0.29/0.09^*$), potential radiation ($r = 0.14^{**}/0.36$) and forest age ($r = 0.19/0.17$), negatively with PM10s-96 ($r = 0.34/-0.47$).

Additionally, we detected a distinct relationship among geographical position of investigated localities (GPS-coordinates) and lichen diversity as well as among some environmental variables ($p < 0.001$). LDV/species richness were positively correlated with east-GPS ($r = 0.34/0.51$) and negatively with north-GPS ($r = -0.38/-0.49$). North-GPS showed the positive correlation with air pollution ($r = 0.25$) and altitude ($r = 0.28$), and east-GPS was negatively correlated with these two parameters ($r = -0.15$, $r = -0.33$) (Table 2). These correlations revealed differences in climate conditions and anthropogenic impact between north-west and south-east regions in the investigated area (similarly to PCA, Fig. 2).

We found rather different results for the north-western and south-eastern localities (Table 2 – upper right part). In the north-west, lichen diversity (LDV/species richness) was highly correlated ($p < 0.05^*$, $p < 0.01^{**}$, $p < 0.001$ without asterisk) mainly with PM10s-96 ($r = -0.77/-0.68$), precipitation ($r = 0.48^{**}/0.25^*$) and altitude ($r = 0.43/0.26^{**}$); forest age and fragmentation were correlated only weakly (not significant). In the south-east, the situation was different. Since there were largely only lower, background air pollution levels, pollution only weakly influenced the dataset or the relationship was not significant. Parameters highly correlated with lichen diversity in that region ($p < 0.001$) were forest fragmentation ($r = 0.78/0.61$), precipitation ($r = 0.74/0.56$), forest age ($r = 0.45/0.40$) and altitude ($r = 0.42/0.32$).

To better elucidate the influence of particular environmental factors on lichen diversity, we tested covariations between human-caused and natural variables (partial Mantel tests). Partial results are shown in Table 3. In the whole study area Influence of the forest age on lichen diversity was relatively high ($r = 0.12–0.26$), non-interactively overriding all other factors except for precipitation, altitude in the case of LDV and radiation in the case of species richness. Air pollution

Table 2
Results of Mantel tests with respect to LDV and species richness and significance of the relationships. Lower left part – calculated on the whole dataset, upper right part north-western part (upper numbers) and south-eastern part (lower numbers).

	LDV[a]	Richness[b]	Age[c]	Forest[d]	N-gps[e]	E-gps[f]	Altitude	Radiation[g]	Precip.[h]	PM10s-96[i]	Pollution[j]
LDV[a]		0.61***[k] 0.80***	−0.23* 0.45***	ns[k] 0.78***	−0.47*** −0.62***	−0.55*** 0.61***	0.43*** 0.42***	ns 0.21**	0.48** 0.74***	−0.77*** −0.28**	ns ns
Richness[b]	0.68***		ns 0.40***	−0.24** 0.61***	ns −0.61***	−0.25** 0.62***	0.26** 0.32***	0.31** 0.31***	0.25* 0.56***	−0.68*** ns	ns ns
Age[c]	0.19***	0.17***		ns 0.41***	0.29** −0.32***	ns 0.28***	0.41*** 0.54***	0.27** 0.41***	−0.40** 0.56***	ns ns	ns ns
Forest[d]	0.49***	0.25***	0.17***		0.33*** −0.54***	0.19* 0.47***	−0.19* 0.55***	−0.20* 0.20**	ns 0.69**	ns −0.35***	ns ns
N-gps[e]	−0.38***	−0.49***	ns	−0.13**		0.32** −0.56***	ns ns	0.21* ns	−0.32* −0.34***	0.38*** 0.55***	ns −0.25*
E-gps[f]	0.34***	0.51***	ns	0.24***	−0.78***		−0.67*** 0.18*	ns 0.36***	ns 0.57***	0.40*** ns	ns 0.18*
Altitude	0.29**	0.09*	0.43***	0.15**	0.28***	−0.33***		0.32** 0.26***	ns 0.69***	−0.45*** ns	ns ns
Radiation[g]	0.14**	0.36***	0.30***	ns	−0.13**	0.36***	0.14**		ns 0.46***	−0.28* 0.25**	ns ns
Precip.[h]	0.64***	0.50***	0.27***	0.45***	−0.47***	0.61***	0.22***	0.37***		−0.41** ns	ns ns
PM10s-96[i]	−0.34***	−0.47***	0.13**	−0.12**	0.59***	−0.36***	ns	−0.17***	−0.19***		ns ns
Pollution[j]	−0.11*	−0.17**	ns	ns	0.25***	−0.15**	ns	−0.13**	ns	0.48***	

[a] LDV.
[b] Species richness.
[c] Forest age.
[d] Forest fragmentation.
[e] N-gps – north-GPS.
[f] E-gps – east-GPS.
[g] Annual mean potential direct solar radiation.
[h] Precipitation.
[i] Particulate matter up to 10 μm in diameter in 1996.
[j] Air pollution.
[k] $p > 0.05$ ns, $p < 0.05$*, $p < 0.01$** $p < 0.001$*** (for other definitions see Material and methods).

(PM10s-96) showed strong influence on lichen diversity, having always a significant negative influence ($r = -0.15$ to -0.51). Forest fragmentation also strongly affected lichen diversity ($r = 0.15$–0.50); its correlation with lichen diversity significantly reduced only covariation with precipitation in the case of species richness. Precipitation had the leading effect as a natural ecological predictor of lichen diversity ($r = 0.28$–0.64), and it can override the influences of any human-caused factors except of that of air pollution.

These results prompted us to evaluate the suitability of two predictors – LDV and species richness – for the assessment of biotope naturality. Our results suggest that the better predictor is LDV rather than species richness. LDV was highly correlated ($p < 0.01$) with 13 environmental variables and species richness with 11 of these human-caused and natural parameters.

4. Discussion

The construction of the meaningful naturality (alteration) scales could be rather difficult in climatically homogenous areas with pronounced human impacts (e.g. air pollution), since there is no clear reference of the potential maximum LDV in natural conditions. However, in the diverse landscapes of central Europe it may be also difficult to explicate the background of a naturality gradient. We investigated an area with varying land-use (industrial, agricultural and highly forested landscape), but also with climatic heterogeneity (various altitudes, precipitation levels etc.). We focused in our study especially on the analysis of relationships between lichen diversity and environmental parameters important for naturality evaluation. We found several important factors affecting lichen diversity in oak forests. Among human-caused factors, we detected essential effects of air pollution, forest fragmentation and forest age. However, natural parameters related to climate (precipitation amount, altitude and potential direct solar radiation) were also significantly correlated with lichen diversity. Hence, we tested the covariations between human-caused and natural factors.

4.1. Air pollution

We confirmed the results of many authors that lichen diversity is clearly affected by air pollution in urban and suburban areas (Giordani et al., 2002; Pinho et al., 2004; Frati and Brunialti, 2006; Larsen et al., 2006; Svoboda, 2007). Neither natural nor anthropogenic influence decreased the negative effect of air pollution, even in the less affected and less urbanized south-eastern part of the study area. The negative effects of air pollution were more evident in the more polluted north-west portion of the study area, where air pollution was highly negatively correlated with lichen diversity and covariations with other factors did not significantly decrease the relationship. This result is in accordance with the findings of Giordani (2007), who found that in urban areas, air pollutants are still the main factor limiting lichen diversity, even under any ameliorating conditions.

Air pollution should be tracked by each of its significant component substances. The negative effect of sulphur dioxide on lichens has been thoroughly studied (cf. Hawksworth and Rose, 1970; van Dobben et al., 2001), but these effects are distinct from eutrophication and NO_x which may each have various effects on lichens in different concentrations (van Herk, 2002; Gombert et al., 2004; Larsen et al., 2006; Pinho et al., 2008). Additionally, dust from roads and agricultural fields could favour some species (Loppi et al., 1997; Pinho et al., 2008) and therefore shift LDV measurements. In our project, we surveyed sites minimally influenced by major roadways (a direct source of NO_x) or by other nearby pollution

Table 3
Results of partial Mantel tests with respect to LDV and species richness, significance of relationships and interactions among variables. Example: third column third line (0.22***) shows that age significantly influenced LDV independently from the effect of north-GPS coordinate in the whole study area.

		N-gps		E-gps		Altitude		Radiation		Precipitation	
		Tot[a]	NW[b]/SE[c]	Tot	NW/SE	Tot	NW/SE	Tot	NW/SE	Tot	NW/SE
Age	LDV	0.22***	ns 0.35***	0.19***	−0.36** 0.37***	ns	−0.49*** 0.30***	0.16**	−0.24* 0.41***	ns	ns ns
	Richness	0.21***	ns 0.27***	0.18***	ns 0.30***	0.14**	ns 0.28***	ns	ns 0.31***	ns	0.19* ns
Forest	LDV	0.48***	0.16* 0.67***	0.45***	ns 0.71***	0.47***	ns 0.72***	0.49***	ns 0.77***	0.30***	ns 0.55***
	Richness	0.21***	−0.21* 0.43***	0.15**	−0.21* 0.47***	0.24***	−0.20* 0.55***	0.24***	−0.20* 0.59***	ns	−0.24** 0.37***
PM10s-96	LDV	−0.15**	−0.72*** ns	−0.24***	−0.71*** −0.30**	−0.36***	−0.71*** −0.34***	−0.32***	−0.79*** −0.35***	−0.29***	−0.71*** −0.41***
	Richness	−0.26***	−0.68*** 0.26**	−0.36***	−0.65*** ns	−0.48***	−0.65*** −0.20*	−0.45***	−0.65*** −0.26**	−0.45***	−0.65*** −0.20*

[a] The whole dataset.
[b] North-western part.
[c] South-eastern part of the study area. For explanations of remaining abbreviations see Table 2.

sources. All pollutants (SO_2, NO_x and PM10s) were highly correlated to each other and collectively negatively influenced lichen diversity.

4.2. Forest fragmentation

As the next important factor for naturality assessment we identified forest fragmentation. Lichen species with poor dispersal abilities may be more sensitive to habitat fragmentation and increasing distance among suitable habitats (Hedenås and Ericson, 2008). Reduction of forest cover, often followed by intensive agriculture use, has caused distinct changes of water regime in the landscape (cf. Buchtele et al., 2006). Contrary to humid sites, forest fragmentation could have a major influence on the majority of organisms (including poikilohydric lichens) living in areas with naturally low(er) precipitation levels. This hypothesis is supported by our results: in the interaction tests (correlations between lichen diversity and environmental factors), only precipitation level had higher *r*-values than "forest fragmentation".

The distinct negative effects of habitat fragmentation (decreasing forest size, edge effects and increasing dispersal distances for epiphytic lichens and bryophytes) were demonstrated by Löbel et al. (2006). Giordani (2007), in results consistent with ours, found highly predictive negative influences brought about by tree harvest and forest fires acting on lichen diversity.

4.3. Forest age

Forest age is the third human-influenced factor which effect on lichen diversity we examined (there are almost no windstorms or fires affecting age of oak forests in central Europe). This factor showed the weakest influence on diversity when it was tested after removal of covariation with other ecological variables. This is slightly different result in relation to several studies which found out close positive relationship between forest (tree) age and lichen diversity (Fritz et al., 2008; Moning and Müller, 2009; Ranius et al., 2008; Uliczka and Angelstam, 1999; for a review see Humphrey, 2005). However, these researchers carried out their studies in areas relatively homogenous with respect to other environmental factors (climate, air pollution) therefore the relationship of lichen diversity with forest age may be more definite. In addition, some authors focused their studies only to crustose lichens (Ranius et al., 2008) or to macrolichens (Uliczka and Angelstam, 1999) what may also differentiate results.

4.4. Natural factors

All of the tests showed that mean annual precipitation has the leading effect as an ecological predictor for lichen diversity. It was strongly positively correlated with lichen diversity and its relationship was never significantly decreased by other factors in the interaction tests. Our results confirmed the crucial role performed by precipitation amount in determining epiphytic lichen floras (Hilitzer, 1925; Barkman, 1958; Loppi et al., 1997; Giordani, 2006, 2007; Geiser and Neitlich, 2007; Giordani and Incerti, 2008). In addition to precipitation, we detected significant influence of altitude and radiation on lichen diversity which is in agreement with results of Giordani (2006, 2007), Cristofolini et al. (2008) and Ranius et al. (2008).

5. Conclusions

The effect of environmental factors on lichen diversity results from their power and from the degree of dependence between the variables. Some correlations among human-caused and natural factors may be very high. It may be very difficult to determinate an influence of particular factor on lichens (living organisms): parameter with stronger correlation with lichens could sometimes override the effect of other environmental parameter. According to our results, the difference in precipitation amount may significantly decreased the effect of air pollution in some areas. However, strong air pollution levels may obscure effects of altitude or/and forest age, factors usually regarded as determining for epiphytic lichen diversity. Although these findings are not so surprising, it is necessary to take the covariations between the variables into account and analyse more environmental factors when we evaluate the human impact on the environment using lichens.

In the present study, we tested the correlations between environmental factors and both LDV and species richness. Since these diversity values are strongly correlated and show in almost all cases the same relationship with ecological variables, LDV seems to be very good predictor of lichen diversity in the biomonitoring studies. Detecting species richness of a sampling plot is more time consuming in the field than LDV records (even if LDV needs subsequent calculation of index). For these reasons, the mandated European guideline (as a standardized protocol for mapping lichen diversity) is a good instrument for use in naturality assessment of oak forests in the central Europe.

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Appendix.

Localities under study with their main characteristics including lichen diversity value (LDV) and species richness (number of species).

Number of locality	Locality name	Country	North-West/ South-East	LDV	Number of species	Forest age (year)	Forest fragmentation (%)	GPS coordinate N (°)[a]	GPS coordinate E (°)[a]	Altitude (m)	Potential radiation[b]	Slope inclination (°)	Precipitation (mm/year)	PM10s_1996 ($\mu g\,m^{-3}$)	PM10s_2005 ($\mu g\,m^{-3}$)	SO_2_1996 ($\mu g\,m^{-3}$)	SO_2_2005 ($\mu g\,m^{-3}$)	NO_x_1996 ($\mu g\,m^{-3}$)	NO_x_2005 ($\mu g\,m^{-3}$)
1	Bělýšov	CZ	NW	51	18	100	35	49,44977	13,20115	640	6,382	20	600	15	22	10	4	25	10
2	Bezemín	CZ	NW	61	35	40	45	49,85434	13,02657	460	5,044	0	650	15	22	10	4	25	10
3	Blatenský svah	CZ	NW	25	26	200	40	50,09735	13,37193	500	5,718	25	500	35	22	25	4	10	10
4	Černý Orel	CZ	NW	18	10	150	50	50,19177	14,71919	180	5,044	0	550	65	35	25	4	35	27
5	Červený Kříž	CZ	NW	33	12	120	70	49,99162	13,92978	400	5,044	0	550	35	22	10	4	25	22
6	Červený Újezd	CZ	NW	21	3	80	35	50,50103	13,84256	500	5,009	2	550	65	35	35	10	35	22
7	Dětaňský chlum	CZ	NW	35	20	150	40	50,19116	13,32795	530	5,044	0	550	35	22	35	4	25	10
8	Doubí	CZ	NW	22	5	80	35	49,78596	13,35016	365	5,431	5	550	55	35	25	10	35	27
9	Hádky	CZ	NW	40	10	130	40	49,69333	13,58352	460	5,044	0	550	55	35	25	10	35	35
10	Chlumská hora	CZ	NW	40	27	200	35	50,00746	13,20258	633	5,581	10	550	35	22	10	4	25	10
11	Kružínský vrch	CZ	NW	35	21	200	35	50,18596	13,31653	535	5,044	0	550	35	22	35	4	25	10
12	Malá Pleš	CZ	NW	37	9	100	75	49,99351	13,85685	450	3,917	20	550	35	22	10	10	10	10
13	Osinalice	CZ	NW	17	7	150	60	50,50261	14,37858	370	6,653	30	550	45	35	35	4	25	27
14	Rabštejn	CZ	NW	49	17	150	55	50,05115	13,02608	480	5,061	30	500	35	22	10	4	25	10
15	Slapy	CZ	NW	42	27	120	20	49,70810	14,32426	400	6,679	30	550	15	22	10	4	10	10
16	Tetín	CZ	NW	35	15	100	30	49,93594	14,08703	380	5,068	15	500	45	35	25	4	35	22
17	Týřov	CZ	NW	40	16	150	80	49,96636	13,81818	400	5,581	10	550	35	22	10	4	25	10
18	Velká hora	CZ	NW	33	27	140	25	49,95087	14,15841	400	6,356	20	550	45	35	10	4	35	22
19	Vladař	CZ	NW	36	10	250	30	50,07696	13,21088	690	6,662	30	550	35	22	25	4	25	10
20	Zábělá	CZ	NW	29	5	150	25	49,29216	13,45950	330	3,413	30	550	45	22	25	4	25	10
21	Zlín	CZ	NW	40	18	100	25	49,60658	13,36672	390	5,083	0	550	35	22	10	4	25	10
22	Aggtelek	HU	SE	56	28	90	80	48,48198	20,61385	220	5,942	15	650	15	22	10	4	10	10
23	Andělova zmola	CZ	SE	22	20	90	20	49,54241	17,05455	300	6,353	30	650	40	35	10	4	10	10
24	Boky	SK	SE	46	36	200	75	48,57088	19,01503	563	6,904	30	900	35	35	10	4	25	22
25	Burda	SK	SE	53	28	120	50	47,82695	18,75818	310	5,941	10	600	15	35	10	4	10	10
26	Čížov nahoře	CZ	SE	29	13	80	50	48,88273	15,85010	410	5,122	0	550	15	22	10	4	10	10
27	Čížov skalisko	CZ	SE	29	25	150	50	48,88234	15,84867	380	6,904	35	550	15	22	10	4	10	10
28	Hádecká plan.	CZ	SE	38	27	80	40	49,22425	16,70455	390	4,668	15	550	35	35	25	4	35	35
29	Jankov vršok	SK	SE	88	41	120	90	48,72650	18,35685	450	5,410	5	900	15	22	10	4	10	10
30	Kašivárová	SK	SE	82	34	250	85	48,46325	18,77451	580	5,707	10	900	15	22	10	4	10	10
31	Kňaží stol	SK	SE	54	23	100	75	48,81558	18,28166	500	6,188	25	1000	15	22	10	4	10	10
32	Kojatín	SK	SE	75	31	250	85	48,44825	18,73675	415	6,454	30	900	15	22	10	4	10	10
33	Kolby	CZ	SE	22	23	120	15	48,94979	16,64338	295	5,122	0	550	15	35	10	4	25	10
34	Lebeďák	CZ	SE	23	13	100	35	49,48819	16,60570	350	6,912	35	600	15	22	10	4	10	10
35	Lukov Rambach	CZ	SE	45	24	200	60	48,85478	15,88404	300	5,097	35	600	15	22	10	4	10	10
36	Lukov Uhlíř. st.	CZ	SE	43	20	90	60	48,86296	15,88996	410	5,241	2	600	15	22	10	4	10	10

(*continued on next page*)

37	Malhotky	CZ	SE	39	32	150	35	49,14867	17,05343	320	6,888	30	600	35	35	10	4	10	22
38	Milovická stráň	CZ	SE	21	21	100	25	48,63023	16,69325	230	4,953	3	550	15	35	10	4	25	10
39	Nad lomem	SK	SE	54	38	200	50	48,52240	19,09870	445	6,416	20	800	15	22	25	4	25	22
40	Palanta	SK	SE	70	34	120	35	48,62635	20,95240	310	5,942	15	700	15	22	10	4	10	10
41	Plešivec	SK	SE	46	37	120	70	48,55078	20,39416	395	6,145	20	700	15	22	10	4	10	10
42	Popice	CZ	SE	44	24	120	35	48,81903	16,02285	340	5,122	0	550	15	22	10	4	25	22
43	Príboj	SK	SE	60	27	250	40	48,74608	19,22746	460	6,888	30	900	35	35	10	4	25	22
44	Tematín	SK	SE	81	40	150	65	48,68194	17,92283	360	6,888	30	900	15	22	10	4	10	10
45	Terezské údolí	CZ	SE	27	11	120	25	49,59564	17,02912	325	6,047	20	650	45	35	10	4	25	10
46	Terno	SK	SE	85	46	120	60	48,17118	19,02516	336	6,188	15	800	15	22	10	4	10	10
47	Velký Kuntínov	CZ	SE	21	9	90	15	48,95308	16,83331	300	5,122	0	600	15	35	10	4	25	10
48	VVU Dědice	CZ	SE	53	29	150	80	49,34509	16,94752	330	6,786	30	650	15	22	25	4	10	10

[a] Coordinate system WGS 84.
[b] Mean annual potential direct solar radiation.

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