Functional traits of epiphytic lichens respond to alkaline dust pollution

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ABSTRACT

Dust pollution has a harmful impact on the environment and human health. Lichen trait-based metrics are increasingly used to monitor effects of air pollution, but studies using this technique to monitor the effects of dust pollution are still scarce. Functional traits of lichens along a gradient of long-term alkaline dust pollution were investigated. Species composition was affected along this gradient according to two easily identifiable "soft" traits (growth form and main reproductive strategy) and one expert-assessed "hard" trait (species preference for substrate pH). Particularly, crustose species and lichens with sexual reproduction were related to the most polluted side of the gradient and higher pH, while foliose narrow-lobed species and lichens with asexual reproduction were associated with the opposite side.

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1. Introduction

The concentrations of many air pollutants (e.g., SO_2, CO, C_6H_6) have decreased in Europe during the last decades, but particulate matter (PM) is still problematic, and EU limits of PM continue to be exceeded in large parts of Europe (Guerreiro et al., 2015). PM is a complex heterogeneous mixture of solid particles suspended in the air which differ in size (ca. 0.1–10 μm), origin and chemical composition (Grantz et al., 2003). Dust pollution usually refers to primary and coarse PM (larger dust particles) originating from natural or anthropogenic sources; rock quarrying, combustion processes, kiln grinding or road surfaces are the commonest anthropogenic sources. Dust particles originating from these sources are usually dispersed by wind, and generally deposited near to the emission source (e.g., Farmer, 1993; Branquinho et al.,

2008; Paal et al., 2013). Desert dust, transported over thousands of kilometres by desert storm, is one of the commonest natural sources of dust pollution (Middleton, 2017).

Dust pollution causes several detrimental impacts; for example, cement dust poses harm to human health, provoking respiratory diseases (WHO, 2013; WBCSD, 2015). Its effects also impact on the environment; alkaline dust emissions increase the pH value and change chemical composition of soil and other substrates, thus altering the composition of plant communities and species richness (e.g., Gilbert, 1976; Farmer, 1993). Long-term alkalization of soil, for example, provokes "nemoralization" of pine forest ecosystems, increasing species richness and frequency of uncommon plants which are not naturally occurring in boreal ecosystems (Paal et al., 2013).

Air monitoring stations that directly measure the PM content in ambient air provide real-time data on pollutant concentrations in its surrounding area. However, such information is usually acquired from a restricted number of monitoring stations due to substantial costs and operational constraints (e.g., constant supply of electric

power for its operation), limiting its application in environmental monitoring (Conti and Cecchetti, 2001; Guidotti et al., 2003). Although monitoring stations provide information on pollutant concentrations, they do not directly reflect their effects on the ecosystem. Ecological indicators arise as a more cost-effective approach to monitor air pollution issues. They enable us to have higher spatial resolution (i.e. to have a higher number of sampling sites when compared to the restricted number of air monitoring stations), integrating simultaneously the effects at the ecosystem level.

For many years, taxonomic diversity metrics, such as species richness, were employed to quantify ecosystem change in response to environmental drivers. Though undeniably important to depict the component of biodiversity loss, recent studies have shown that for some important pollutants, such as nitrogen (Pinho et al., 2012a), and other global change drivers, these metrics could be unresponsive (e.g., Dornelas et al., 2014; Vellend et al., 2017). In fact, more than species loss, we seem to be currently observing shifts in communities which taxonomic diversity indices are unable to depict. Trait-based metrics are being increasingly considered as better indicators to quantify ecosystem functionality in response to global change drivers (Díaz and Cabido, 2001; Suding et al., 2008; Mouillot et al., 2012). Functional traits refer to characteristics of the organisms (such as morphological or metabolic attributes) thought to be relevant to ecosystem functioning and/or its response to the environment (Díaz and Cabido, 2001). Simply put, a trait-based approach facilitates the direct comparison between many species regardless of their taxonomic identity, allowing determination of how the environment (biotic and/or abiotic) selects for different traits across large environmental gradients, without taxonomic or geographical constraints. Hence, these metrics represent a more universal approach, which allows detection of the shifts in species composition accounting for species redundancy and abundance, and with the possibility of making a link with ecosystem functioning.

Lichens are extremely dependent on the atmosphere for nutrition due to their physiological and metabolic features (Nash, 2008); they absorb nutrients directly from the atmosphere, including atmospheric pollutants. This is the key reason why they have more than 100 years of history as excellent ecological indicators to track the effects of air pollution and other major global change drivers (e.g., Nimis et al., 2002; Loppi, 2014; Sujetovienė, 2015; Matos et al., 2017). The earlier works were based on taxonomic diversity metrics (Hawksworth and Rose, 1970; Gilbert, 1973). As atmospheric pollutants that exerted an overall deleterious effect on all species (like SO_2) decreased as a result of emission control policies, taxonomic diversity was increasingly replaced by other metrics. In fact, shifts in communities have been observed in response to nitrogen pollution (e.g., Frati et al., 2007; Pinho et al., 2011, 2012a), fires (Giordani et al., 2016), land use intensity (Stofer et al., 2006) and thus lichen trait-based metrics are being increasingly used to track the effects of several global change drivers. Lichen traits related to tolerance to eutrophication are used to track the effects of nitrogen pollution resulting from cattle load or even to establish the European critical levels for ammonia (Pinho et al., 2012b; Giordani et al., 2014). Lichen trait-based approach has been used not only in pollution and environmental monitoring studies (Ellis, 2012; Giordani et al., 2012), but also, for example, in planning conservation activities for lichens, where the analytical scheme 'common species/driver/trait/driver/rare species' was employed, based on which recommendations for conservation management of alvars were proposed (Leppik et al., 2015).

Previous studies concerning dust pollution from anthropogenic activities have demonstrated that long-term dust pollution influences lichen diversity, abundance and community structure,

directly or through effects on lichen substrates, due to pH increase of substrate or its hypertrophication (e.g., Loppi and Pirintsos, 2000; Seaward and Coppins, 2004; Marmor et al., 2010; Degtjarenko et al., 2016a), together with negative impacts on genetic variation of a widespread lichen-forming fungus (Degtjarenko et al., 2016b). Dust emission is expected to increase in future due to expansion of industrialization and urbanization, especially with the synergistic effect of climate change (Fiore et al., 2015). Moreover, increased erosion and dust storm events are expected in the future due to climate change, potentially contributing to an overall increase of dust particles (Middleton, 2017). Hence, it is timely and important to investigate the suitability of trait-based metrics to track dust pollution under a global change perspective so that we can take measures to control and mitigate its effects on human and ecosystem health. The aim of our research was to study the lichen functional traits along a gradient of long-term, alkaline dust pollution released from limestone quarrying, and estimate the applicability of trait-based metrics to track the effects of dust pollution.

2. Material and methods

2.1. Study area

The study area is located in northern Estonia (Harju County) and has a characteristic temperate climate with a mean annual temperature of 6 °C, a mean annual precipitation of 672 mm, and an average wind speed of 3.7 m/s (Estonian Weather Service, 2017). The study took place in forests and urban forest parks, dominated by Scots pine (*Pinus sylvestris*, hereafter "pine"), in the surroundings of four large limestone quarries: Vasalemma (59°14′22″N, 24°18′19″E), Harku (59°23′51″N, 24°34′18″E), Väo (59°26′6″N, 24°53′43″E), and Maardu (59°26′58″N, 25°1′55″E) (Fig. 1; see Marmor and Degtjarenko (2014) and Degtjarenko et al. (2016a) for more detailed descriptions of the study area).

Quarrying and limestone use have a very long tradition in Estonia, dating back to the 13th century (Ministry of the Environment, 2011). The excavation of limestone from these four quarries amounts to half of the total quarried limestone in Estonia per year, amounting to c. 2.6 million m³ (Geoguide Baltoscandia, 2012), mostly used as a compound in civil engineering and for the cement industry (Perens and Kala, 2007). In general, dust emissions from the aforementioned quarries contain a high amount of $CaCO_3$ (>50%) and MgO (c. 14%), small amounts of SiO_2, Al_2O_3, and Fe_2O_3 (Perens and Kala, 2007; Reinsalu, 2008). The pH of pure limestone in water solution is very high, for example the pH of cement dust (using limestone as raw material) in water suspension is c. 12.3–12.6 (Mandre, 2000). Dust from limestone mining is emitted to the atmosphere by drilling–blasting and crushing operations and by transportation of extracted material (Geoguide Baltoscandia, 2012). The actual amount of released dust pollution is unknown, but the maximum allowed quantity of PM reported by the Environmental Board of Estonia varies immensely from 1.2 to 220.0 tonnes per year (in Maardu and Vasalemma, respectively; Environmental Board, 2017).

2.2. Sampling

Sampling was carried out during spring and summer of 2013. Sampling was stratified along the distance to the pollution source. Lichens were sampled in 32 plots of 25 m radius located at different distances (3 m–3340 m) in all possible directions from the perimeter of the nearest limestone quarry (Vasalemma – 10 plots, Harku – 10 plots, Väo – 5 plots, Maardu – 7 plots) along a gradient of dust pollution within four distance ranges (0–500; 501–1000;
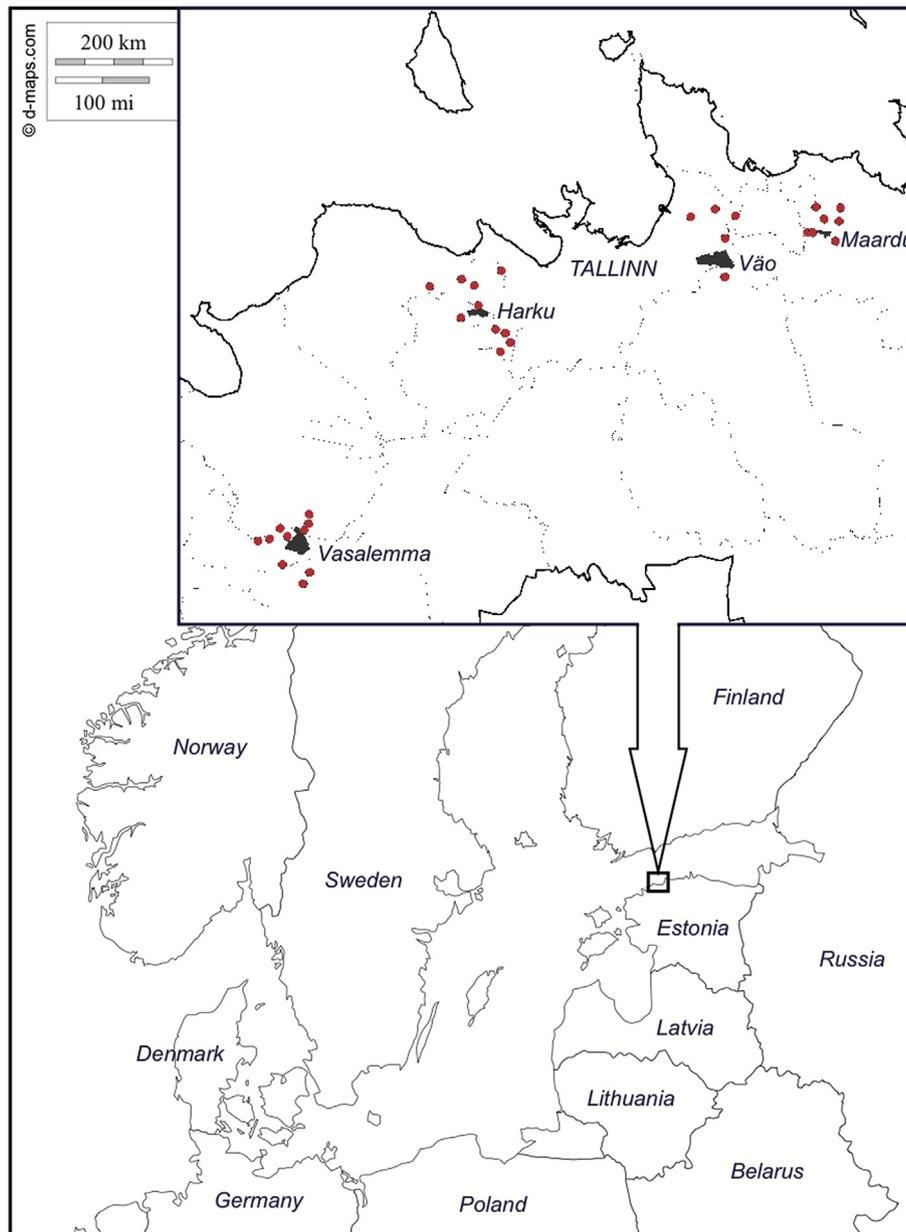
Fig. 1. The study area with sample plots (marked with red circles) in the surroundings of four limestone quarries (Vasalemma, Harku, Väo and Maardu) in northern Estonia, by MapInfo Professional ver 9.5 (Degtjarenko et al., 2016a; the map of Scandinavia was taken from free map resource http://d-maps.com/carte.php?num_car=5977&lang=en).

1001–2000; >2000 m; Fig. 1). Pine forests and urban forest parks were selected, but where it proved impossible to find these habitats, smaller groups of pine trees were studied. To avoid pseudoreplication, the minimum distance between sample plots was c. 400 m. Sampling plots were situated >100 m from the closest paved or gravel road to avoid potential effects of traffic pollution. We calculated distances from the center of each sample plot to the edge of the nearest quarry using orthophotos from the web map server of the Estonian Land Board (2015).

Five random pine trees (with >50 cm circumference) were examined in every plot, totalling 160 pine trees. For each tree, lichen cover was determined using the line cover method: a measuring tape was attached round the sample trunk at the height of 120 cm, and all locations, where any species was intersecting the lower edge of the measuring tape, were recorded. Tree circumference was registered for calculating lichen cover. Lichens that were

hard to determine in the field were collected and identified in the laboratory with the help of stereomicroscope, light microscope, and thin layer chromatography (TLC) with solvent A (Orange et al., 2001). Lichen nomenclature follows Randlane et al. (2016).

2.3. Lichen functional diversity

Five categories of lichen traits were selected to classify the recorded species (61 in total; Table S1). The first two traits sort species according to their maximum ecological tolerance to substrate pH and to poleotolerance (likelihood of a lichen to be found in areas with different levels of human disturbance) (Table 1). The functional groups of tolerance of substrate pH are known to respond to air pollution (e.g., Llop et al., 2012; Paoli et al., 2014; Suija and Liira, 2017). Potentially, the poleotolerance may also be related to the way lichen species respond to dust pollution. These

trait classifications are considered "hard" as they are expert-assessed and may be difficult to apply in regions of the world where lichen biota is poorly known. In addition, three easily identifiable "soft" traits were also tested as a potential tool that can be used universally, even in regions of the world where lichen species identification is hard to perform. The type of primary photobiont was chosen because different photobiont types are known to have different sensitivities to air pollution (Branquinho et al., 2011). The same concept was applied for growth form, as these appear to be related to the way they intercept particles from the atmosphere, reflecting to some extent their sensitivity to air pollution (Branquinho et al., 1999). Finally, the main type of reproduction was also tested, as different reproductive strategies imply different strategies of distribution that relate to different levels of pollution (Giordani et al., 2012). A summary of these five traits and their respective functional groups is presented in Table 1. The classification follows Nimis (2016) and Nimis and Martellos (2017), a database widely used in lichen functional ecology in Southern Europe (e.g., Llop et al., 2012; Matos et al., 2015). Because this is a national database, not all species occurring in Europe are included; for five species found in our work and absent from the database, the ecological tolerance of substrate pH and poleophoby was retrieved from the flora of Estonian microlichens (Randlane and Saag, 2004) and the lichen flora of Great Britain and Ireland (Smith et al., 2009). Species traits were combined with species cover to calculate the community weighted mean (CWM; Lavorel et al., 2008) for each functional group in each plot. This index represents the mean functional group value in the community, weighted by the abundance of species belonging to that trait (Lavorel et al., 2008). This was calculated in CRAN software R (R Core Team, 2013) using the 'dbFD' function of the FD package (Laliberté and Legendre, 2010).

2.4. Environmental data

Pine bark pH was also measured as previous studies have repeatedly recorded strong positive correlations between dust pollution load, pH of the phorophyte bark and distance from the source of alkaline dust pollution (e.g., Gilbert, 1976; Marmor and Randlane, 2007; Paoli et al., 2014). Two pieces of bark were collected from each pine sampled at 120 cm from the ground: one from the side facing the quarry and the other from the opposite side. The bark pH was measured in the laboratory using a flathead pH meter Consort C532. To allow rapid solution of hydrogen ions, 0.5 mL of 0.1 KCL according to Schmidt et al. (2001) was dripped on the bark 1 min before the measurement. The mean bark pH of every tree was calculated based on an arithmetic mean of pH measurements (calculations of mean pH of every tree were based on mean hydrogen ion calculations and then transformed back into pH value). Tree circumference was also used as a possible environmental driver. The temperature and quantity of precipitation as confounding factors were not recorded in this study. According to the available data from the Estonian Weather Service (2017), annual mean temperature (1961–1990) and annual mean precipitation (1961–1990) do not vary between sample plots.

2.5. Data analyses

Non-metric multidimensional scaling (NMS) ordination was performed on a matrix of species abundance per sampling site to extract the most prominent gradient in lichen species composition. This was done with the matrix based on absolute values of cover, and also with the relativized cover matrix (relativizing by row). The general pattern obtained was similar (data not shown), so the relativized matrix to minimize other environmental factors was selected as suggested by Matos et al. (2017). Bray-Curtis distance was used as it is regarded as the most suitable for community analysis (McCune et al., 2002). The best solution was chosen from 500 runs, each with a random start (500 iterations per run) and evaluated with a Monte Carlo test (250 randomizations). The variability represented by the solution was assessed by calculating the coefficients of determination (R^2) between original plot distances and distances in the final ordination (McCune et al., 2002). Environmental (bark pH and distance to pollution source) variables were overlaid in the solution to assess if dust pollution was the main driver of lichen community composition. The site effect was also incorporated in the analysis by including geographical co-ordinates as a possible environmental factor and by including the quarry (four quarries were sampled) as a group factor. Functional variables (CWM of each functional group) were also overlaid in the solution as vectors to understand which traits mediated the response of lichen community. Correlations between these variables and NMS scores were calculated using Spearman correlations (ρ) to account for possible nonlinearity in the relationships (correlations were considered significant for $p < 0.05$). Correlation was determined using CRAN software R (R Core Team, 2013).

Table 1
Description of lichen traits chosen and their respective functional groups (according to Nimis (2016) and Nimis and Martellos (2017)).

Trait	Functional group	Description	Symbol
Preference for substrate pH	Acidophilous	Species with an ecological preference for very acid to acid substrate	Ac
	Basophilous	Species with an ecological preference for sub-neutral to basic substrate	Ba
Poleophoby	Natural	Species occurring in undisturbed to semi-natural habitats	Na
	Disturbed	Species occurring in moderately to heavily disturbed habitats	Di
Primary photobiont	Chloroccocoid	With Chloroccocoid photobiont (Green algae)	Ch
	Trentepohlia	With *Trentepohlia* (Green algae)	Tr
Growth form	Crustose	Species with crust-like thallus, firmly and entirely attached to the substrate by the lower surface	Cr
	Leprose	Species with thallus like crustose but its surface consistsing of a granular mass and is always decorticated	Lp
	Squamulose	Species with thallus composed of small scales	Sq
	Foliose narrow-lobed	Species with leaf-like thallus, partly attached to the substrate, with narrow lobes	Fn
	Foliose broad-lobed	Same as 'Foliose narrow-lobed' but with broad lobes	Fb
	Fruticose	3D-like structure, attached by one point to the substrate with the rest of the thallus standing out from the surface of the substrate	Fr
Type of reproduction	Asexual	Species reproducing mainly with soredia, soredia-like, isidia or isidia-like structures	As
	Sexual	Species reproducing mainly sexually by spores	S

3. Results

The mean pH of tree bark, ranging from 6.23 closer to the source of dust pollution to 3.14 in sites located further away from it, showed a significant negative logarithmic relationship with distance ($R^2_{adj} = 0.62$, p < 0.001; Fig. 2).

A shift in lichen communities was observed along the dust pollution gradient (Fig. 2). The NMS ordination joint plot showed the location of the sampling sites based on lichen community composition. The analysis suggested a two-dimensional solution, as the addition of a third one resulted in only a slight reduction of the minimum stress. Final stress of the ordination was 7.72% and minimum stress was lower than expected by chance (p = 0.004). Axis one explained 58% of the variation in lichens communities, and axis two only 15.7% in a total of 73.7%. The first axis of the ordination clearly represented a gradient of dust pollution as evidenced by the high correlation with distance to pollution source ($\rho = 0.87$, p < 0.001) and with tree bark pH ($\rho = -0.91$, p < 0.001). These variables were overlaid in the solution as vectors (Fig. 2). The second axis was not correlated with distance to pollution source or bark pH, and because these were environmental variables of our interest, this axis was discarded from further analyses. The tree circumference was not related with the first axis of the ordination. Site location did not interfere with first axis of the ordination, i.e. with dust pollution; site related variables (geographical coordinates and "quarry" as a group factor) appeared only in the second axis, which was unrelated to dust pollution. Additionally, neither group nor geographical coordinates were related to dust pollution drivers measured (bark pH or distance to quarry; Table 2).

The functional groups significantly correlated with the ordination solution (Table 2) which were overlaid as vectors in Fig. 2. Regarding growth form, crustose species were related to the side of the gradient closer to the dust pollution source and with higher pH, while foliose narrow-lobed species were associated with the opposite side of the gradient. The main type of reproductive strategy also responded; species having a sexual type of reproduction associated with the most polluted side of the gradient, and

those with asexual reproduction (by soredia and/or isidia) with the opposite side. The "hard" trait regarding species preference for substrate pH followed the pH gradient along the dust pollution gradient, with species preferring sub-neutral, slightly basic or basic substrate associated with the most polluted side of the gradient, and those with a preference for acid substrate correlated with the opposite side. Following the method of Pinho et al. (2011), the ordination to see if species were misclassified was first checked; species distribution along axis 1 of the ordination, and the highly
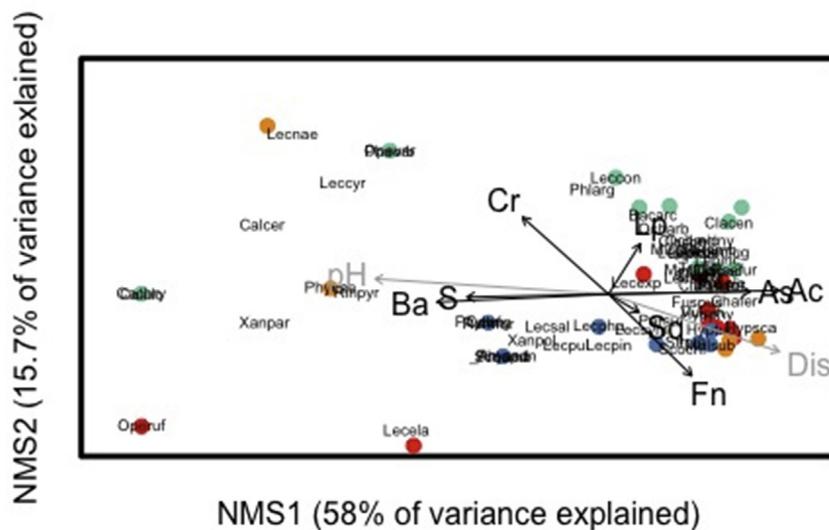
Table 2
Spearman correlations coefficients between biodiversity variables and environmental variables: Dist, distance to pollution source; Circ, tree circumference; pH, bark pH; XX, longitudinal coordinates of the centre of sample plot; YY, latitudinal coordinates of the centre of sample plot; Group, the quarry as group factor; Ch, chloroccocoid; Tr, *Trentepohlia*; Cr, crustose; Fb, foliose broad-lobed; Fn, foliose narrow-lobed; Fr, fruticose; Lp, leprose; Sq, squamulose; As, asexual sorediate; Se, sexual; Ac, acidophilous; Ba, basophilous; Dis, disturbed; Na, natural. Significant correlations are marked:*p < 0.05,**p < 0.01,***p < 0.001.

Variable			Axis 1	Axis 2
Environmental		Dist	0.87***	−0.13
		Circ	0.01	−0.41*
		pH	−0.91***	0.03
		XX	−0.06	−0.61***
		YY	−0.01	−0.68***
		Group	−0.01	0.57***
Trait-based	Main photobiont type	Ch	0.13	−0.20
		Tr	−0.13	0.20
	Growth form	Cr	−0.58***	0.49*
		Fb	−0.30	−0.31
		Fn	0.58***	−0.52*
		Fr	0.21	0.44*
		Lp	0.45*	0.35
		Sq	0.46**	−0.08
	Main reproductive strategy	As	0.89***	0.01
		Se	−0.89***	−0.01
	Substrate pH	Ac	0.81***	−0.04
		Ba	−0.79***	−0.22
	Poleophoby	Dis	−0.815	−0.34
		Na	0.16	0.31

Fig. 2. Non-metric multidimensional scaling (NMS) analysis of species composition. Vectors represent significant correlations between community composition and environmental (grey) and functional variables (community level weighted mean, black). Only vectors with a significant Spearman correlation (p < 0.05) with individual axis 1 site scores (Table 2) are represented to prevent crowding: Dist = distance to pollution source; pH = tree bark pH; Cr = crustose; Fn = foliose narrow-lobed; Lp = leprose; As = Asexual; S = sexual; Ba = basic; Ac = acid. Names indicate species' centroids (i.e. species scores along axes 1 and 2). Coloured circles indicate the location of sampling sites; red circles − Harku quarry; green circles − Vasalemma quarry; blue circles − Maardu quarry; yellow circles − Väo quarry.

significant correlation found between species scores and species classification into basic or acid (Spearman R = −0.73, p < 0.0001; N = 60) confirmed the adequacy of the classification. Overall, of the five traits selected, only the main type of photobiont and poleophoby showed no response to the dust pollution gradient.

4. Discussion

Different approaches have been previously suggested for indicating the effects of dust pollution using lichens and other cryptogamic organisms, such as the presence of a particular lichen on pine bark (e.g., *Phaeophyscia orbicularis*, *Physcia dubia* and *Xanthoria parietina*; Marmor and Randlane, 2007; Marmor et al., 2010; Degtjarenko et al., 2016a), bryophyte species (e.g., *Pylasia polyantha*, *Radula complanata* and *Orthotrichum pallens*; Paal and Degtjarenko, 2015; Degtjarenko et al., 2016a), or free-living green chlorophyte algae *Trentepohlia* growing on pine trees (Marmor and Degtjarenko, 2014). Concerning the taxonomic diversity metrics, the relationship between species richness and dust pollution was not linear and clear, but depends on initial environmental conditions, lichen phorophytes and distance from a source of pollution (e.g., Gilbert, 1976; Marmor and Randlane, 2007; Degtjarenko et al., 2016a), the abundance of lichens being a more reliable metric to indicate the effects of alkaline dust pollution (Degtjarenko et al., 2016a).

The purpose of our research was to study the response of lichen functional traits along a gradient of alkaline dust pollution released from limestone quarrying, and to estimate the applicability of trait-based metrics to track the effects of dust pollution. Trait-based metrics are considered more universal than taxonomic diversity indices, and thus could be applied in other regions of the world (Branquinho et al., 2015). Our study showed that lichen communities shifted along the dust pollution gradient (Fig. 2), and one "hard" and two "soft" functional traits of lichens mediated this shift. Regarding "soft" traits, main reproductive strategy and growth form were associated with the dust pollution gradient (Fig. 2; Table 2). The "hard" trait concerning species preference for substrate pH also followed the pH of pine bark along the pollution gradient (Fig. 2; Table 2); however, the latter functional group is not uniform since it contains species both tolerating and preferring substrates from sub-neutral to basic. The functional groups of crustose lichens, species with sexual reproduction and species which usually grow on sub-neutral or slightly basic substrates, but appear on pine bark, have the potential to be used as ecological indicators of alkaline dust pollution (Fig. 2; Table 2).

Concerning growth form, crustose species were related to intensively dust-impacted sites, whereas foliose narrow-lobed species were associated with the less polluted sites (Fig. 2; Table 2). To date, several studies have investigated the lichen diversity in response to dust pollution (e.g., Martin and Nilson, 1992; Marmor et al., 2010; Degtjarenko et al., 2016a), but there is no detailed study concerning the functional groups of lichen growth forms in response to gradients of dust pollution. According to previously published studies, it could be concluded that crustose species were dominant on pines at closest distances to dust pollution sources, and foliose and fruticose were more sensitive to dust pollution (e.g., Martin and Nilson, 1992; Marmor and Randlane, 2007; Marmor et al., 2010). Crustose lichens seem to be less sensitive to dust pollution probably because they have less surface of thalli exposed to the dust particles, being more tolerant to emissions of air pollution (Giordani et al., 2012). It has been previously demonstrated that fruticose species, e.g., *Bryoria*, growing on pine bark disappeared at closest distances from cement works, and that the fruticose species were being replaced by foliose species (Kortesharju and Kortesharju, 1989). The fruticose species

were also associated with low environmental levels of air pollution in oak forests of West Carpathian (Guttova et al., 2017). In our study, fruticose species were not significantly related to the gradient of dust pollution; probably due to the fact that only a few fruticose species were recorded (Table S1). However, Llop et al. (2012) showed that foliose species were dominant in lichen communities near traffic areas, while crustose and fruticose lichens were more sensitive to traffic pollution in a Mediterranean urban environment. It seems that phorophyte species and its initial (before impact of pollution) bark pH are important in shaping the lichen communities under conditions of alkaline dust pollution (Marmor and Randlane, 2007), and their functional traits respectively. The growth form of lichens, being an easily recognizable characteristic (even when lichen biota is not well known) could have potential for evaluating the effects of dust pollution. Therefore, we suggest that an increase in abundance of crustose and a decrease in narrow-lobed foliose lichens could be used as ecological indicators of alkaline dust pollution in boreal pine forests.

Regarding the reproduction trait, lichens that reproduce mainly sexually were associated with dust-polluted areas, whereas species that mainly reproduce asexually were more abundant in clean air areas (Fig. 2; Table 2). To date, little is known about how reproductive strategies are related to environmental pollution, especially to the dust pollution. Our results are consistent with those of Stofer et al. (2006) who showed that species mainly reproducing sexually occurred more often in open and intensively disturbed landscapes (subjected to higher dust deposition), while species with vegetative symbiotic propagules were more often found in less disturbed landscapes. A similar pattern to ours was also found in relation to soil pH; lichens mostly reproducing sexually were associated with higher pH soils, while species mostly reproducing asexually by soredia occurred on soils of coastal and inland sand dunes with lower pH (Jüriado et al., 2016). Our finding is contrary to that of Giordani et al. (2012) who suggested that an increase in substrate pH caused by atmospheric deposition favoured sorediate species. Currently, this contradictory evidence remains unexplained and further studies should clarify the response of reproductive strategy of lichens under conditions of air pollution. Nonetheless, our results demonstrated that the trait of main reproductive strategy was influenced by alkaline dust pollution, and that sexually reproducing lichens appeared more successful in the polluted conditions than vegetatively reproducing species. Sexual crustose lichens are known as early colonists on young tree bark (Hedenås and Ericson, 2000; Randlane et al., 2017); also under environmental disturbance by alkaline dust pollution, sexually reproducing lichens behave as pioneer species similar to stress-tolerant or/and ruderal species in regard to reproductive strategies (Grime, 1977; Topham, 1977; Rogers, 1990), attempting a more successful dispersal between suitable habitats (Stofer et al., 2006) under environmental disturbance by alkaline dust pollution. Concerning the lichen photobiont, the main type of photobiont did not show any associations with the dust pollution gradient, perhaps due to bias in types of photobiont in our study (only three lichens were with *Trentepohlia*; Table S1).

Only one "hard" trait, concerning tolerance to substrate pH, responded to the dust pollution gradient; species preferring sub-neutral, slightly basic or basic substrate associated with the most polluted side of the gradient and species with a preference for acid tree bark associated with the least polluted side (Fig. 2; Table 2). Several studies have shown that limestone dust pollution has a neutralizing effect for acidic bark, provoking a shift of lichen communities along a limestone pollution gradient from typical acidophilous to neutrophilous and basiphilous species (Loppi and Pirintsos, 2000; Paoli et al., 2014; Degtjarenko et al., 2016a). Enrichment by the functional groups of neutrophilous and basiphilous species and reduction or absence of the functional group of

acidophilous lichens is also relevant near other PM pollution sources, such as near traffic areas (e.g., Marmor and Randlane, 2007; Llop et al., 2012), power plants (Marmor et al., 2010) and cement industries (e.g., Martin and Nilson, 1992; Suija and Liira, 2017). Similar effects on "hard" traits, regarding their ecological preference to substrate pH, could also be observed near sources of atmospheric nitrogen pollution (Frati et al., 2007; Pinho et al., 2011), causing an increase of nitrophilous species and a decrease of acidophilous ones due to an increase of phorophyte bark pH which could also be a result of atmospheric nitrogen pollution (Van Herk, 2001; Llop et al., 2012). Moreover, Van Herk (1999) suggested that abundance of nitrophilous lichens could be used for spatial mapping of nitrogen pollution, while Loppi and Pirintsos (2000) indicated that all epiphytic lichens growing at distances up to 50 m from the limestone quarries could also be regarded as nitrophilous species. Considering these statements, the use of functional groups regarding ecological tolerance to substrate pH in biomonitoring of limestone dust-impacted areas should be interpreted with caution, excluding the potential presence of nitrogen or ammonia pollution in surrounding areas.

The mechanism and possible confounding factors causing the shift of lichen functional groups are debatable. In many studies the changes in lichen communities are basically explained by a remarkable decrease of substrate acidity (e.g., Gilbert, 1976; Paoli et al., 2014). Loppi and Pirintsos (2000) suggested that chemistry or raw material of dust was not important in shaping epiphytic lichen communities, as lichens appeared to be affected more by direct physical effects of deposited dust at the closest distances from quarries. Giordani and Malaspina (2017) demonstrated that bark pH did not drive an abundance of lichen functional groups under high eutrophication levels in northwest Italy. Spier et al. (2010) concluded that lichen communities responded to a complex interaction of tree-mediated factors rather than only bark pH. Several studies also suggested the large amounts of deposited dust particles mechanically impact on lichens by degrading photosynthetic pigments (Zaharopoulou et al., 1993) or causing visible injures (Józwiak and Józwiak, 2009). Such injures of thalli were not visually observed during our fieldwork, and therefore we suggest that the main effect of dust was chemical, and not mechanical in our study. However, theoretically minor mechanical effects, e.g., dust layer closing pores and pseudocyphellae on lichen thalli, cannot be excluded even when the injures are not visible. For that reason, we consider the effect of dust pollution on lichen functional traits as synergistic in our study. Furthermore, different factors, e.g. amount and frequency of precipitation in polluted area, could potentially confound the effects of dust pollution on lichen diversity, but the appropriate studies are lacking at present.

Our work revealed that functional traits metrics of lichens (viz., growth form, main reproductive strategy and ecological tolerance to substrate pH) could serve as potential tools for indicating the effects of dust pollution. Based on the present study, it is obvious that listed functional groups of lichens appear to be useful ecological indicators for tracking the effects of dust pollution in pine forests in boreal and hemiboreal regions. Further research should explore confounding effects (e.g., precipitation) of dust emissions and a geographical range of applicability of proposed trait-based metrics as indicators of alkaline dust pollution, taking account the phorophyte species and initial environmental conditions. In our study, we considered both "soft" (easily determined, even when lichen biota is not well known) and "hard" (those that require a good expert knowledge of the species) traits in trait-based metrics. Our results supported the opinion that "soft" functional traits of lichens were more independent and easily applied ecological indicators for tracking the pollution effects than "hard" traits. Further research on functional traits of lichens in response to

PM pollution should try to explore additional traits, such as size of thalli, and to study performance of functional traits as ecological indicators in a gradient of natural dust pollution.

To conclude, our study highlights once again the importance of functional trait-based metrics showing the potential of lichen functional traits for tracking the effects of dust pollution in biomonitoring studies, as a high-resolution approach to complement data from monitoring stations and other direct measurements.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.funeco.2018.08.006.

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