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Manninen, Sirkku

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Deriving nitrogen critical levels and loads based on the responses of acidophytic lichen communities on boreal urban *Pinus sylvestris* trunks

Sirkku Manninen*

Department of Environmental Sciences, University of Helsinki, P.O. Box 65 (Viikinkaari 2a),
00014 Helsinki, Finland

Email: sirkku.manninen@helsinki.fi

Tel. +358 2941 59101

*corresponding author

Abstract

The deposition of reactive nitrogen (N) compounds currently predominates over sulphur (S) deposition in most of the cities in Europe and North America. Acidophytic lichens growing on tree trunks are known to be sensitive to both N and S deposition. Given that tree species and climatic factors affect the composition of epiphytic lichen communities and modify lichen responses to air pollution, this study focused on the impact of urban air pollution on acidophytes growing on boreal conifer trunks. The study was performed in the Helsinki metropolitan area, southern Finland, where annual mean nitrogen dioxide (NO₂) concentrations range from 4--5 µg m⁻³ to >50 µg m⁻³. In addition, background forest sites in southern and northern Finland were included. The results demonstrated elevated N contents (≥0.7%) in *Hypogymnia physodes* and *Platismatia glauca* at all the sites where the species occurred. In the Helsinki metropolitan area, a higher frequency of green algae + *Scoliocosporum chlorococcum* and reduced numerical frequencies of other indicator lichen species (e.g. *Pseudevernia furfuracea*, *Bryoria* spp., *Usnea* spp.) were associated with elevated atmospheric concentrations of NO₂ and particulate matter containing N, as well as elevated concentrations of inorganic N in bark. The N isotope values (δ¹⁵N) of lichens supported the uptake of oxidized N mainly originating from road traffic. Sulphur dioxide (SO₂) also negatively affected the most sensitive species, despite the current low levels (1--4 µg m⁻³ yr⁻¹). Critical levels of 5 µg NO₂ m⁻³ yr⁻¹ and 0.5 µg NH₃ m⁻³ yr⁻¹, and a critical load of 2--3 kg N ha⁻¹ yr⁻¹ are proposed for protecting the diversity of boreal acidophytes. This study calls for measurements of the throughfall of various N fractions in urban forest ecosystems along precipitation and temperature gradients to verify the proposed critical levels and loads.

Keywords: acidophyte frequencies, ammonium, bark N, lichen N, nitrogen dioxide, nitrate

1. Introduction

Lichens consist of nutritionally specialized mycobionts (fungi) living in symbiosis with photobionts (unicellular algae or cyanobacteria or both) (Honegger, 1998). The development of epiphytic lichen communities on tree trunks is determined by various environmental factors, including the degree of illumination and humidity of the environment, age and pH of the bark surface, continuity and age of woodland cover at a particular site and air pollution (Giordani et al., 2014; Hauck, 2011). Rootless, poikilohydric epiphytic lichens with green algae as photobionts are solely dependent on the atmosphere, throughfall and stemflow as sources of nutrients, including nitrogen (N) (Honegger, 1991; Nieboer et al., 1978; Palmqvist et al., 2002). Elements deposited on the lichen surface are transferred as dissolved ions into the fungal cells via the hyphae cortex (Honegger, 1993). The decline of epiphytic lichens in areas affected by anthropogenic emissions of air pollutants has particularly been attributed to the effective uptake of sulphuric acid (H_2SO_4) and sulphate (SO_4^{2-}) derived from sulphur dioxide (SO_2) under humid conditions, leading to morphological and physiological changes in the photosynthesizing algal layers (Rao and Leblanc, 1966). The toxicity of sulphur (S) compounds is dependent on the buffering capacity of both the substrate and the lichen (Skye, 1968).

The cyanobacterial lichen *Lobaria pulmonaria* may only occur in areas where the annual mean SO_2 concentration is $<5 \mu\text{g m}^{-3}$ (Denison et al., 1977). It is generally agreed that *Usnea*, *Bryoria*, *Ramalina* and *Evernia* species are also sensitive to air pollution, especially SO_2 (Gilbert, 1973). However, S pollution is no longer a major threat to lichens in Europe, given that SO_2 emissions, concentrations of SO_2 and aerosol SO_4^{2-} , and S deposition have decreased by 70--90% in Europe since 1980, as a result of the United Nations Economic Commission for Europe (UNECE) Convention on Long Range Transboundary Air Pollution (CLTRAP). For comparison, the concentrations of oxidized and reduced N compounds in the air and precipitation have only decreased by about 25% since 1990 (Tørseth et al., 2012).

Besides SO_2 , nitrogen dioxide (NO_2) has been ranked as a more important factor determining lichen biodiversity than heavy metals in particulate matter (PM) (van Dobben et al., 2001). The current high diversity of epiphytic lichens in cities such as London, UK, with high N emissions from vehicles, is apparently associated with both reduced SO_2 emissions and high concentrations of nitrogen oxides (NO_x) (Davies et al., 2007; Larsen et al., 2007; Purvis et al., 2003). For instance, Davies et al. (2007) attributed the positive relationship between ambient concentrations of NO_x and lichen abundance on *Fraxinus excelsior* trunks to the ubiquitous distribution of N-tolerant (i.e. nitrophytic) species. A significant increase in

nitrophytes has also occurred in all parts of the Netherlands with a high cattle density, especially on deciduous trees with acid bark, such as *Quercus* and *Fagus*. In the same period, several species that prefer or require acid bark, such as *Evernia prunastri*, *Hypogymnia physodes* and *Pseudevernia furfuracea*, have rapidly decreased in abundance (van Herk, 1999). A rapid decrease in the acidophyte *Lecanora conizaeoides* in Europe has also been attributed to its sensitivity to ammonia (NH₃), as well as decreasing SO₂ concentrations. This suggests that some acidophytes are sensitive to both a rise in bark pH and an increase in the ammonium (NH₄⁺) content of the bark (van Herk, 1999, 2001).

In Europe, the empirical critical level (CLE) of NH₃ for epiphytic lichens is 1 µg m⁻³ yr⁻¹ and that of NO₂ for sensitive vegetation and ecosystems 30 µg m⁻³ yr⁻¹ (Cape et al., 2009; CLTRAP, 2017). A critical load (CLO) of 5--10 kg N ha⁻¹ yr⁻¹ is applied for European pine taiga woodland (Bobbink and Hettelingh, 2011; de Vries et al., 2007). However, Giordani et al. (2014) recently determined a CLO of 2.4 kg N ha⁻¹ yr⁻¹ for European forests by means of epiphytic lichens. Supporting this, a CLO of 1--3 kg N ha⁻¹ yr⁻¹ for the protection of the tundra and taiga lichen flora was determined in the United States, based on changes in lichen pigment physiology, abundance and/or community composition (Pardo et al., 2011a). A model used by Geiser et al. (2010) in turn generated a CLE of 0.51 µg m⁻³ for the mean annual concentration of N in ambient-air fine particulates. The lower CLE and CLO values proposed by North American researchers may partly be attributed to the fact that there are still pristine baseline ecosystems in the United States, while in Europe there are none due to considerably higher N deposition over many decades (Pardo et al., 2011b).

Conifers with acid bark, such as *Picea abies* and *Pinus sylvestris* (Kuusinen, 1996), predominate in the boreal zone (Ahti, 1977). Their bark supports the occurrence of acidophytic lichens, which are only able to tolerate low N concentrations or doses (Gaio-Oliveira et al., 2001, 2005). Given the great abundance of larger-sized foliose and fruticose lichens on *P. sylvestris* trunks in relatively sparse northern boreal forests (Bäcklund et al., 2016), the oligotrophic lichen community on *P. sylvestris* trunks may be considered as a good indicator of atmospheric N pollution. Supporting this, Giordani et al. (2014) reported that air pollutant effects on lichens are particularly evident in European nemoral/boreal coniferous forests. Important lichen species on non-eutrophicated conifer bark, even in urban environments in the boreal zone, include *H. physodes*, *Parmeliopsis ambigua*, *Platismatia glauca*, *P. furfuracea* and *Tuckermannopsis chlorophylla* (formerly *Cetraria chlorophylla*) (Ahti, 1977).

Studies on the effects of air pollution on epiphytic lichens in Helsinki, Finland, date back to the 1930s, when Vaarna (1934) reported a lichen desert in the city centre, with only green algae (Protococceae) occurring on the trunks and branches of both deciduous and coniferous trees. Outside the lichen desert, the small thalli of *Xanthoria parietina* on *Acer*, *Tilia* and *Ulmus* were green or greyish green, while the thalli of *Parmelia sulcata* often appeared to become reddish under exposure to elevated NH_3 . Species found on *P. sylvestris* included *Hypocenomyce scalaris*, *H. physodes*, *P. ambigua* and *Parmeliopsis hyperopta*, *P. furfuracea*, *T. chlorophylla* and *Vulpicida pinastri*. For instance, *Usnea hirta* was only occasionally observed. The responses were attributed to road dust, soot, NH_3 and other gaseous emissions from industry, domestic heating and traffic, including railroads and horse faeces (Vaarna, 1934). Recently, negative effects of NO_x on the bryophyte *Pleurozium schreberi*, together with changes in forest vegetation and a reduced soil C:N ratio, have suggested N saturation of the urban forests in the Helsinki metropolitan area (Manninen et al., 2013).

This field study was motivated by the fact that the lichen community on *P. sylvestris* trunks in Helsinki city centre appeared not to have recovered (personal observation), despite a decrease in annual mean SO_2 concentrations from 50--100 $\mu\text{g m}^{-3}$ in the 1950s and 1960s (Taipale, 2006), and still 30--70 $\mu\text{g m}^{-3}$ in 1970, to current concentrations of 1--4 $\mu\text{g m}^{-3}$ (Kaski et al., 2016). While the SO_2 concentrations are below the CLE of 20 $\mu\text{g m}^{-3} \text{ yr}^{-1}$ for forest vegetation and 10 $\mu\text{g m}^{-3} \text{ yr}^{-1}$ for cyanobacterial lichens (Ashmore and Wilson, 1993; CLTRAP, 2017), the CLE of 30 $\mu\text{g NO}_2 \text{ m}^{-3} \text{ yr}^{-1}$ is exceeded in the city centre and close to the main streets and highways (Hannuniemi et al., 2016; Kaski et al. 2016). Moreover, when *H. physodes* occurs on the trunks of *P. sylvestris* in the city centre, the small wrinkled thalli are dark greyish green in colour (personal observation). This suggests eutrophication of the environment due to increased emissions of NH_3 from vehicles with three-way catalysts (Cape et al., 2004; Sutton et al., 2000).

There is little data on N deposition in urban areas, although urban ecosystems may experience atmospheric deposition of reactive N compounds, which is manifold compared to that at regional and national monitoring sites in remote areas (Lovett et al., 2000; Rao et al., 2014; Redling et al., 2013). This is because the pollution monitoring devices located in background areas do not capture the local dry deposition of various gaseous N compounds (Braun et al., 2017), including NO_x from urban automobiles (Elliot et al., 2007, 2009). Moreover, Karl et al. (2017) demonstrated that traffic-derived atmospheric NO_x emissions in Europe apparently are appreciably underestimated. Consequently, the models used to spatially predict estimates of N deposition based on emission data or established deposition collectors

with limited spatial cover underestimate the rates of N deposition in and near cities, especially if the networks do not even measure all of the components that can be deposited (Howarth, 2007; Rao et al., 2014; Redling et al., 2013; Root et al., 2013).

Our understanding of N deposition and its effects on epiphytic lichens under various climatic conditions, and especially in urban ecosystems, may also be biased due to the fact that in Europe, knowledge of N-related changes in epiphytic lichen communities mainly comes from studies on species growing on deciduous trees in areas with high long-term emissions of NH_3 (see e.g. van Herk, 1999 and references therein). Munzi et al. (2014) recently highlighted the need to establish CLEs for NH_3 based on oligotrophic lichen species instead of N-tolerant species (see also Jovan et al., 2012). It has also been suggested in terms of N-deposition effects in boreal forests that the overall quantity of N deposited does not solely determine lichen responses, because the ratio of nitrate (NO_3^-) to NH_4^+ in deposition may change the CLO thresholds and the nature of effects on lichens (Bobbink et al., 2010; Giordani et al., 2014). In addition to changes in species composition, pollutant contents of lichen thalli have been used as indicators for exceedance of N CLOs. For instance, Fenn et al. (2008) suggested that 1.0% of the N in *Letharia vulpina* may indicate exceedance of the N threshold of $3.1 \text{ kg ha}^{-1} \text{ yr}^{-1}$.

This study was performed in the Helsinki metropolitan area, southern Finland, where the daily numbers of cars reach up to 90 000–110 000 on the busiest dual carriageways leading into and out of the city centre and on ring roads (City of Helsinki; Finnish Transport Agency). It was hypothesized that i) air pollution still negatively affects corticolous lichens in the Helsinki metropolitan area and ii) the lichen responses observed are mainly associated with NO_x and/or NH_3 emissions from vehicles (Davies et al., 2007 and references therein). To test the hypotheses, occurrence of selected indicator species on conifer trunks was scored and lichens were collected for analysis of carbon (C) and N contents. Lichens were also analysed for the isotopic composition of N to assess the role of oxidized versus reduced N emissions (Bermejo-Orduna et al., 2014; Pearson et al., 2000). Bark chemical characteristics (Schultz et al., 1997, 1999), and available air quality data were used as anthropogenic environmental drivers in the statistical analyses.

2. Material and methods

2.1 Field sites

The total number of study sites was 44. Thirty-eight of the sites were located at distances of 1.6--30 km from the city centre in the metropolitan area of Helsinki (60°10'N, 24°56'E) (Fig. 1). Three sites in Teijo National Park (60°13'N, 22°57'E; 140 km W from Helsinki) served as southern Finnish background controls. In addition, three sites in Pallas National Park (68°4'N, 24°3'E; 890 km N from Helsinki), from where air quality and deposition data were available, were used as remote background controls. The fieldwork was performed from 13 June to 15 July 2016 in the Helsinki metropolitan area and Teijo, and from 20 to 22 September 2016 in Pallas.

The environmental conditions in terms of climate, air quality and/or deposition for the Helsinki metropolitan area and Pallas are presented in Table 1. The annual means for total NO₂, total SO₂ and total PM_{2.5} (particulate matter, PM < 2.5 µm, includes SO₄²⁻, NO₃⁻ and NH₄⁺ aerosols) concentrations for the sites in the Helsinki metropolitan area were derived from modelled dispersal maps of emissions from road traffic, energy production, shipping and aircraft provided by the Finnish Meteorological Institute (Hannuniemi et al., 2016) and measurements by the Helsinki Region Environmental Services Authority (Kaski et al., 2016). The variation in annual mean concentrations is greatest for total NO₂ due to emissions from road traffic, while the highest total SO₂ concentrations occur close to harbours in the city centre (Hannuniemi et al., 2016; Kaski et al., 2016). The largest coal power plants are also located in the city centre by the sea (Fig. 1). The total PM_{2.5} concentrations are mainly associated with road traffic and domestic wood burning and vary from the local/regional background of 6.5 µg m⁻³ yr⁻¹ to >9 µg m⁻³ yr⁻¹ at the busiest roundabouts (Hannuniemi et al., 2016). While the SO₄-S concentrations in both bulk deposition and throughfall have decreased since the late 1990s in background areas in southern Finland, no such trends have been observed for NO₃⁻-N or NH₄⁺-N (Lindroos et al., 2013; Ruoho-Airola et al., 2015). In fact, N deposition has been increasing in Finnish Lapland since the mid-1990s (Ruoho-Airola et al., 2015).

Fig. 1. Locations of the study sites (yellow dots), the largest coal power plants (purple squares), harbours (blue squares) and Helsinki-Vantaa airport (white square) in the Helsinki metropolitan area (lower map). The main roads are indicated with their national or European numbers. Locations of the background control areas in Teijo and Pallas National Parks are also indicated in the upper map.

Table 1. Temperature, precipitation, air quality, and nitrogen (N) and sulphur (S) deposition in Helsinki city centre and a remote background area in northern Finland (Pallas) (Finnish Meteorological Institute, Air Quality in Finland and Temperature and precipitation statistics from 1961 onwards; Flechard et al., 2011; Hannuniemi et al., 2016; Kaski et al., 2016; Ruoho-Airola et al., 2015).

	Helsinki	Pallas
Temperature (°C)		
Jun-Sep 2016	15.8	11.5
Jun-Sep 1981-2010	15.0	10.7
Annual mean 1981-2010	5.8	-0.6
Precipitation (mm)		
Jun-Sep 2016	83	121
Jun-Sep 1981-2010	64	61
Annual mean 1981-2010	656	506
Air quality ($\mu\text{g m}^{-3} \text{ yr}^{-1}$)		
NO ₂	4-5 to $\geq 50^a$	0.3-1.1
(HNO ₃ +NO ₃ ⁻)-N		0.05
(NH ₃ +NH ₄ ⁺)-N		0.10-0.30
SO ₂	1-4 ^a	0.5
PM _{2.5}	5-11 ^a	2-4
Bulk deposition (kg ha ⁻¹ yr ⁻¹)		
NO ₃ ⁻ -N		1
NH ₄ ⁺ -N		0.5
SO ₄ ²⁻ -S		1
N in precipitation (mg l ⁻¹)		
NO ₃ ⁻ -N		0.10-0.15
NH ₄ ⁺ -N		0.05-0.10

^aTotal concentrations of NO₂, SO₂ and PM_{2.5} derived from modelled dispersal maps of emissions from road traffic, energy production, shipping and aircraft

2.2 Species frequencies

The Finnish standard for mapping air quality with the help of commonly occurring dominant, generalist, epiphytic lichen species on *P. sylvestris* trunks (Suomen standardisoimisliitto, 1990) was used. At each site (approx. 200 m²), with *P. sylvestris* usually being the only tree species, five trees typical of the site (diameter at breast height, DBH ≥ 20 cm; branchless up to at least 3 m) were selected for scoring of the lichens. The average canopy cover of *P. sylvestris* was visually assessed and the average height using a clinometer. DBH was separately measured for each of the five trees used for lichen scoring.

The frequencies of 12 indicator species (0 = absent or 1 = present) were scored at heights of 100–200 cm, i.e. resulting in a frequency of 0–5 for each species per site. The species were *H. physodes*, *P. ambigua*, *P. hyperopta* & *Imshaugia aleurites*, *H. scalaris*, *Bryoria* spp.,

Usnea spp., *P. glauca*, *V. pinastri*, *P. furfuracea*, *T. chlorophylla*, *P. sulcata*, and green algae + *Scoliciosporum chlorococcum*. The frequencies of lichen species were not scored in Pallas, because species such as *P. glauca* and *P. furfuracea*, which are common in the southern subzones of the boreal zone, are rare in the northernmost parts of Fennoscandia (Ahti, 1977; Hale, 1968; Thell and Moberg, 2011).

2.3 Lichen chemistry

Hypogymnia physodes, *P. glauca* and/or *P. furfuracea* were collected for analysis of C and N contents and the isotopic composition of N ($\delta^{15}\text{N}$) from the trunks of trees that had been scored for lichen frequencies. For each species, a pooled sample from the trunks of 2–5 trees was collected, depending on the frequency of thalli. At three sites out of the 38 in the Helsinki metropolitan area, the thalli of *H. physodes* were absent, and at one site they were so few and stunted that samples could not be collected. *Platismatia glauca* could be sampled from six out of eight sites where the species grew, and *P. furfuracea* from 10 sites out of 11. In Teijo, samples of each of the three species were collected from each site, while in Pallas only *H. physodes* was collected, because the two other species did not grow at the sites.

The C and N contents (w/w) of air-dried, ground samples were analysed using high-temperature combustion (Vario MAX CN analyser, Elementar Analysensysteme GmbH, Langenselhold, Germany) at the Department of Forest Sciences, University of Helsinki. The isotopic composition of N was measured on a Thermo Finnigan Delta^{Plus} Advantage isotope-ratio mass spectrometer (ThermoFischer Scientific, Waltham, MA, USA) coupled to an NC 2500 elemental analyser. All samples were analysed in duplicate. Typical reproducibility (1σ), estimated from repeated measurements of in-house reference material and sample replicates, was $\pm 0.3\%$. The isotopic values, normalized using International Atomic Energy Agency (IAEA)-certified isotopic reference materials, are reported in delta (δ) notation, relative to the international standards AIR (N). Analysis of the isotopic composition of N was performed in the Laboratory of Chronology, Finnish Museum of Natural History – LUOMUS, University of Helsinki.

2.4 Bark chemistry

Bark flakes (<3 mm in thickness) were taken at a height of approximately 1.5 m from the trunks of the same trees used for scoring of the lichen frequencies. The samples were cleaned of lichens, air-dried and ground (≤ 1 mm particle size). Five grams of bark was mixed with 50

ml of deionized water and measured after 24 hr for conductivity (Jenway 4010 Conductivity Meter, Cole-Parmer, Stone, Staffordshire, UK) and pH (Inolab Level 1).

For the nitrite + nitrate ($\text{NO}_2^- + \text{NO}_3^-$) and NH_4^+ analyses, 10 ml of deionized water was added to 0.5 g of ground bark. The samples were vigorously shaken by hand, left for 30 min, shaken again, centrifuged for 5 min at 4000 revolutions per minute (rpm) (Wolterbeek et al., 1996) and filtered through a 0.45- μm Millipore filter (Millex HA, cellulose esters; Merck KGaA, Darmstadt, Germany). The $\text{NO}_2^- + \text{NO}_3^-$ -N and NH_4^+ -N concentrations were measured with a Thermo Scientific Gallery Plus Automated Photometric Analyser at the Lammi Biological Station, University of Helsinki, following standard methods (SFS-EN International Organization for Standardization (ISO) 13395 and SFS-EN ISO 11732).

The S content of bark was measured with a Thermo Scientific iCAP 6000 series inductively coupled plasma (ICP) optic emission spectrometer. Sample preparation followed Environmental Protection Agency (EPA) Method 3051 (Microwave Assisted HNO_3 Digestion). The analyses were performed at the Department of Forest Sciences, University of Helsinki.

2.5 Statistical analyses

The data were checked for normality prior to the analyses. Differences in site characteristics, and bark and lichen chemistry between the Helsinki metropolitan area, Teijo and/or Pallas, as well as differences between lichen species were analysed with the Student's t-test or Mann-Whitney U-test. The relationships between the variables were examined with the Spearman rank correlation test or Pearson correlation test and principal component analysis (PCA) (varimax rotation with Kaiser normalization). The lichen and bark elemental data were log-transformed for the analyses. The results were considered significant at $p \leq 0.05$ and as trends at $p \leq 0.1$. Statistic analysis was performed using IBM SPSS Statistics 24.0 for Mac (IBM Corp., Armonk, NY, USA).

3. Results

3.1 Bark chemistry and other site characteristics

In the Helsinki metropolitan area, the canopy cover was negatively correlated with the distance from the city centre and positively with air-pollution variables, such as the atmospheric concentrations of total NO_2 and total $\text{PM}_{2.5}$ (Table 3). The trees close to the city centre also had the greatest DBH, i.e. the correlation between DBH and canopy cover was

statistically significant ($r_s = 0.38$, $p = 0.018$, $n = 38$). The annual mean concentrations of total NO_2 , total $\text{PM}_{2.5}$ and NO_2 derived from road traffic were positively correlated (Table 3), while the annual mean concentration of total SO_2 only correlated with that of the NO_2 derived from shipping.

Table 2. Means $\pm$ standard deviations (SDs) for tree characteristics and *Pinus sylvestris* bark and lichen variables in the Helsinki metropolitan area and Teijo and/or Pallas National Parks in summer 2016. DBH = diameter at breast height. Number of sites: Pallas ($n = 3$), Teijo ($n = 3$) and Helsinki ($n = 38$, except *Hypogymnia physodes* chemistry $n = 34$, *Platismatia glauca* chemistry $n = 6$ and *Pseudevernia furfuracea* chemistry $n = 10$). Letters indicate differences between the study areas at $p < 0.05$ (Student's t-test or Mann-Whitney U-test).

	Pallas	Teijo	Helsinki
Canopy cover (%)	13 $\pm$ 3a	27 $\pm$ 6b	30 $\pm$ 14b
Tree height (m)	15 $\pm$ 5	13 $\pm$ 3	18 $\pm$ 6
DBH (cm)	29 $\pm$ 3	30 $\pm$ 4	30 $\pm$ 4
Bark			
pH	3.29 $\pm$ 0.05a	3.48 $\pm$ 0.06b	3.38 $\pm$ 0.14ab
Conductivity ($\mu\text{S cm}^{-1}$)	168 $\pm$ 19a	123 $\pm$ 9b	155 $\pm$ 39ab
$\text{NO}_2^- + \text{NO}_3^- - \text{N}$ ($\mu\text{g g}^{-1}$)	4.19 $\pm$ 0.68a	2.15 $\pm$ 0.32b	6.30 $\pm$ 6.05a
$\text{NH}_4^+ - \text{N}$ ($\mu\text{g g}^{-1}$)	12.0 $\pm$ 2.7a	15.5 $\pm$ 2.6a	29.6 $\pm$ 19.8b
S ($\mu\text{g g}^{-1}$)	318 $\pm$ 25a	370 $\pm$ 18a	458 $\pm$ 74b
<i>H. physodes</i>			
C (%)	43.1 $\pm$ 0.6a	44.7 $\pm$ 0.2b	44.1 $\pm$ 0.6b
N (%)	0.77 $\pm$ 0.14a	1.00 $\pm$ 0.03ab	1.37 $\pm$ 0.32b
C:N	57.0 $\pm$ 10.5a	44.8 $\pm$ 1.2a	33.9 $\pm$ 8.1b
$\delta^{15}\text{N}$ (‰)	-5.07 $\pm$ 0.34a	-6.45 $\pm$ 0.14b	-5.90 $\pm$ 1.04ab
<i>P. glauca</i>			
C (%)		44.0 $\pm$ 0.5	43.6 $\pm$ 0.2
N (%)		0.97 $\pm$ 0.07	1.16 $\pm$ 0.34
C:N		45.6 $\pm$ 3.1	40.1 $\pm$ 10.1
$\delta^{15}\text{N}$ (‰)		-6.15 $\pm$ 0.44	-5.50 $\pm$ 0.50
<i>P. furfuracea</i>			
C (%)		45.5 $\pm$ 0.2	45.1 $\pm$ 0.5
N (%)		1.32 $\pm$ 0.04	1.55 $\pm$ 0.32
C:N		34.4 $\pm$ 0.9	30.2 $\pm$ 6.3
$\delta^{15}\text{N}$ (‰)		-3.50 $\pm$ 0.06a	-5.25 $\pm$ 0.59b

Table 3. Spearman rank correlation or Pearson correlation coefficients for statistically significant relationships between selected bark chemistry and environmental variables in the Helsinki metropolitan area in summer 2016 (n = 38). The asterisks indicate significances as follows: * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$.

	Canopy cover (%)	Total NO ₂ (µg m ⁻³ yr ⁻¹)	Road NO ₂ (µg m ⁻³ yr ⁻¹)	Shipping NO ₂ (µg m ⁻³ yr ⁻¹)	Total SO ₂ (µg m ⁻³ yr ⁻¹)	Total PM _{2.5} (µg m ⁻³ yr ⁻¹)	Bark pH	Conductivity (µS cm ⁻¹)	log NO ₂ ⁻ +NO ₃ ⁻ -N (µg g ⁻¹)	log NH ₄ ⁺ -N (µg g ⁻¹)
Distance (km) from city centre	-0.42**	-0.65***	-0.49**		-0.59***	-0.48**		-0.39**	-0.47**	-0.57***
sea				-0.75***	-0.62***		0.62***	-0.59***		-0.38*
Canopy cover (%)		0.48**	0.44**			0.50**			0.43**	0.37*
Air quality										
Total NO ₂ (µg m ⁻³ yr ⁻¹)			0.90***		0.92***	0.36*			0.41*	0.51**
Road NO ₂ (µg m ⁻³ yr ⁻¹)					0.92***	0.38*				0.33*
Energy NO ₂ (µg m ⁻³ yr ⁻¹)										0.32*
Shipping NO ₂ (µg m ⁻³ yr ⁻¹)					0.53**		-0.56***	0.39*		
Total SO ₂ (µg m ⁻³ yr ⁻¹)							-0.65***	0.80***	0.35*	0.45**
Total PM _{2.5} (µg m ⁻³ yr ⁻¹)							0.43*			0.43**
Bark pH								-0.69***		
Conductivity (µS cm ⁻¹)									0.47**	0.50***
log NO ₂ ⁻ +NO ₃ ⁻ -N (µg g ⁻¹)										0.65***
log S (µg g ⁻¹)	0.55***	0.51***	0.41*		0.41*			0.46**	0.37*	0.58**

3.2 Frequencies of lichen species

At one site in the Helsinki metropolitan area, 10 out of 12 indicator species were recorded, i.e. all except *P. sulcata* and *T. chlorophylla*. *Hypocenomyce scalaris* was found and green algae + *S. chlorococcum* also grew at the three sites where there was no *H. physodes*. At those sites where *H. physodes* occurred, it was observed on most of the trunks, having an average frequency of 4 across the sites (Fig. 2). The frequency of *V. pinastri* increased as a function of an increasing frequency of green algae + *S. chlorococcum*, while the frequencies of *P. ambigua*, *P. hyperopta* & *I. aleurites*, *Usnea* spp., *P. glauca* and *P. furfuracea* were negatively correlated with the latter (data not shown; all $p < 0.05$). In the Helsinki metropolitan area, *Bryoria* spp. and *P. glauca* were found at inland sites at a distance of ≥ 13 km from the city centre and major point sources, and *Usnea* spp. and *P. furfuracea* at sites ≥ 11 km from the city centre. In Teijo, *P. glauca* and *P. furfuracea* were almost as frequent as *H. physodes* and *P. ambigua* (Figs 2 and 3).

Fig. 2. Average frequencies ($\pm$ SD) of acidophytic indicator lichen species on *Pinus sylvestris* trunks across the sites in the Helsinki metropolitan area or Teijo National Park, southern Finland, in summer 2016. At each site, the frequency (0 = absent, 1 = present) of each species was scored on five trees, i.e. the maximum frequency of each species was 5 per site. The species are arranged from left to right according to the number of sites at which they grew in

the Helsinki metropolitan area, except for the frequency of N-loving green algae + *Scoliciosporum chlorococcum* which is shown on the far right. The number of sites where the species were found in the Helsinki metropolitan area / Teijo are given in parentheses. For instance, *Pseudevernia furfuracea* grew at 11 sites (out of 38) in the Helsinki metropolitan area, it grew at each of the three sites while in Teijo.

Fig. 3. Epiphytic lichens on *Pinus sylvestris* trunks at a) a polluted urban site in the Helsinki metropolitan area, and background sites in b) Teijo and c) Pallas National Parks in southern and northern Finland, respectively.

3.3 C and N contents and $\delta^{15}\text{N}$ values of lichens

The average C content of *H. physodes* was greater in southern Finland, i.e. both the Helsinki metropolitan area and Teijo, than in Pallas. The N contents in *H. physodes* ranged from 0.92% to 2.04% in the Helsinki metropolitan area, with an average N content greater than that in Pallas (Table 2). The highest N contents of *H. physodes* (approx. 2%) were measured at two sites: an island site within 300--800 m from a dual carriageway (52 000 cars per day), Helsinki Zoo and the largest coal power plant, and at a park site approximately 100 m from a four-lane street in the city centre (36 000 cars per day) (City of Helsinki; Finnish Transport Agency). The elevated N contents resulted in a lower average C:N ratio of *H. physodes* in the Helsinki metropolitan area than in both Teijo and Pallas.

The $\delta^{15}\text{N}$ value of *H. physodes* tended to increase as a function of an increasing lichen N content in the Helsinki metropolitan area ($r = 0.30$, $p = 0.082$) (Fig. 4a), while the C content of *H. physodes* was negatively correlated with its N content at $p = 0.1$ ($r = -0.286$). The N contents of *P. glauca* and *P. furfuracea* were positively correlated with that of *H. physodes* ($r = 0.90$, $p = 0.015$ and $r = 0.88$, $p = 0.001$, respectively) in the Helsinki metropolitan area, but

the $\delta^{15}\text{N}$ values of the three species were not correlated (data not shown). Moreover, when *H. physodes*, *P. glauca* and/or *P. furfuracea* occurred at the same sites, the highest N content was recorded in *P. furfuracea*. In the Helsinki metropolitan area, the difference was significant in comparison to *H. physodes* ($t = 2.26$, $p = 0.038$), and in Teijo also in comparison to *P. glauca* ($t = 11.88$, $p < 0.001$ and $t = 7.87$, $p = 0.001$, respectively). The average $\delta^{15}\text{N}$ value of *P. furfuracea* in Teijo was greater than that of *H. physodes* or *P. glauca* ($t = 34.1$, $p < 0.001$ or $t = 10.3$, $p = 0.008$, respectively), and it was also greater than that of *P. furfuracea* in the Helsinki metropolitan area (Table 2).

Fig. 4. a) N content in relation to $\delta^{15}\text{N}$ value in *Hypogymnia physodes*, *Platismatia glauca* or *Pseudevernia furfuracea* in the Helsinki metropolitan area and b) relationship between the N content of *Hypogymnia physodes* and the atmospheric total NO_2 concentration based on combined data from the Helsinki metropolitan area and Pallas (encircled values) in summer 2016.

3.4 Relationships between lichen species and environmental variables

The total and average numbers of indicator lichen species decreased as a function of increasing atmospheric concentrations of total NO_2 and total SO_2 , as well as increasing concentrations of inorganic N fractions and S in bark. *Hypocenomyce scalaris*, *V. pinastri* and green algae + *S. chlorococcum* appeared to be the most tolerant species in terms of N deposition (Table 4). *Hypogymnia physodes* decreased in frequency as a function of increasing concentrations of both atmospheric total NO_2 and bark $\text{NO}_2^- + \text{NO}_3^- - \text{N}$. While the frequencies of *P. ambigua* and *P. furfuracea* responded negatively to both the $\text{NO}_2^- + \text{NO}_3^- - \text{N}$ and $\text{NH}_4^+ - \text{N}$ concentrations in bark, the frequencies of *Bryoria* spp. and *Usnea* spp. were negatively and solely correlated with the bark $\text{NO}_2^- + \text{NO}_3^- - \text{N}$ concentration, and the frequency of *P. glauca* with the bark $\text{NH}_4^+ - \text{N}$ concentration. The frequencies of *P. ambigua* and *P.*

glauca were also negatively correlated with the atmospheric total SO₂ concentration, as was the frequency of *V. pinastri*. *Vulpicida pinastri* was the only species that responded to changes in bark pH, i.e. its frequency increased as a function of increasing bark pH, while *P. ambigua*, *P. hyperopta* & *I. aleurites* and *P. glauca* responded negatively to an increase in the bark S concentration (Table 4). The frequency of green algae + *S. chlorococcum*, in turn, tended to be positively correlated with the bark S concentration ($r_s = 0.32$, $p = 0.053$).

The N content of *H. physodes* was positively correlated with the bark NH₄⁺-N concentrations (Table 4), but it also tended to increase with an increasing atmospheric total NO₂ concentration and bark NO₂⁻+NO₃⁻-N concentration ($r_s = 0.32$, $p = 0.065$ and $r = 0.34$, $p = 0.051$, respectively). When data from the Helsinki metropolitan area and Pallas were combined, the atmospheric total NO₂ concentration explained 45% of the variation in the N content of *H. physodes* (Fig. 4b). Although the C content of *H. physodes* only decreased significantly as a function of increasing bark pH, it also showed negative correlations with the atmospheric total NO₂ and total PM_{2.5} concentrations at $p < 0.1$. The N content of *P. furfuracea* decreased with increasing distance from the city centre ($r = -0.74$, $p = 0.014$), as did that of *H. physodes*, while that of *P. glauca* only tended to weakly to decrease ($r = -0.78$, $p = 0.067$). The N content of *P. furfuracea* actually increased with an increasing NO₂ concentration derived from shipping ($r_s = 0.64$, $p = 0.046$), and the $\delta^{15}\text{N}$ value of *P. glauca* increased with an increasing NO₂ concentration derived from energy production ($r_s = 0.88$, $p = 0.021$).

PCA of air quality and selected lichen and bark variables from the Helsinki metropolitan area resulted in five principal components (PCs) with initial eigenvalues of >1. These PCs explained 74% of the variation in the data. A biplot from the PCA is presented in Fig. 5. PC1 was named the 'N pollution' gradient. The annual mean total NO₂ and road traffic-derived NO₂ concentrations, total PM_{2.5} concentration, the N content of *H. physodes*, bark S concentration and the frequency of green algae + *S. chlorococcum* were positively loaded (>0.50), while the C content of *H. physodes* was negatively loaded on PC1. The annual mean total SO₂ concentration, NO₂ concentration derived from shipping, bark N fractions and conductivity were, in turn, positively loaded on PC2, in contrast to the bark pH. The average number of indicator lichen species was equally strongly negatively loaded on both PC1 and PC2.

Table 4. Spearman rank correlation or Pearson correlation coefficients for statistically significant relationships between lichen variables and environmental factors in the Helsinki metropolitan area in summer 2016 (n = 38, except for *Hypogymnia chemistry* n = 34). The asterisks indicate significances as follows: * p ≤ 0.05, ** p ≤ 0.01, *** p ≤ 0.001.

	Distance from city centre (km)	Total NO ₂ (µg m ⁻³ yr ⁻¹)	Road NO ₂ (µg m ⁻³ yr ⁻¹)	Total SO ₂ (µg m ⁻³ yr ⁻¹)	Total PM _{2.5} (µg m ⁻³ yr ⁻¹)	Bark pH	conductivity (µS cm ⁻¹)	log NO ₂ ⁻ +NO ₃ ⁻ -N (µg g ⁻¹)	log NH ₄ ⁺ -N (µg g ⁻¹)	log S (µg g ⁻¹)
Total number of indicator species	0.60***	-0.36*		-0.41**			-0.40*	-0.45**	-0.45**	-0.43**
Average number of indicator species	0.54***	-0.44*	-0.32*	-0.37*			-0.40**	-0.63***	-0.53**	-0.40*
Frequency										
<i>H. physodes</i>	0.42**	-0.41*						-0.35*		
<i>P. ambigua</i>	0.59***	-0.50**	-0.38*	-0.45**	-0.44**		-0.42**	-0.47**	-0.67***	-0.53***
<i>P. hyperopta</i> & <i>I. alurites</i>	0.32*	-0.37*								-0.54***
<i>H. scalaris</i>							0.34*			
<i>Bryoria</i> spp.								-0.33*		
<i>Usnea</i> spp.	0.46**	-0.41*						-0.36*		
<i>P. glauca</i>	0.60***	-0.40*		-0.35*	-0.36*				-0.49**	-0.37*
<i>V. pinastri</i>		0.35*		-0.33*	0.36*	0.48**	-0.55***			
<i>P. furfuracea</i>	0.46**	-0.59***	-0.46**		-0.51**			-0.40*	-0.45**	
Green algae + <i>S. chlorococcum</i>	-0.41**	0.57***	0.45**		0.52**				0.36*	
<i>H. physodes</i>										
log N (%)	-0.45**				0.48**				0.56***	0.58***
log C (%)						-0.34*				
C:N	0.37*	-0.55***			-0.52**				-0.56***	-0.59***

Fig. 5. Biplot from the principal component analysis (PCA) of the average number of indicator lichen species, frequencies of *Hypogymnia physodes* and green algae + *Scoliciosporum chlorococcum*, *H. physodes* chemistry and environmental drivers (canopy cover, annual means for total concentrations of NO₂, SO₂ and PM_{2.5}, as well as NO₂ concentrations derived from various sources, and selected bark chemistry variables) in the Helsinki metropolitan area in summer 2016. PC1 explained 31% and PC2 19% of the variation in the data.

4. Discussion

4.1 Effects of nitrogen pollution on the epiphytic lichen community

The fact that the frequencies of *P. glauca* and *P. furfuracea* were positively and the frequency of green algae + *S. chlorococcum* negatively correlated with distance from the city centre, but that none of these frequencies correlated with the canopy cover or DBH, provides support for the hypothesis that urban air pollution has a major impact on the acidophytic lichen communities on *P. sylvestris* trunks in the Helsinki metropolitan area. The relationships between the frequencies of indicator lichen species, lichen N contents or $\delta^{15}\text{N}$ values and atmospheric NO₂ and PM_{2.5} concentrations, as well as the concentrations of NO₂⁻+NO₃⁻-N and

NH₄⁺-N in *P. sylvestris* bark provide further support for the negative effects of reactive N compounds derived mainly from road traffic on epiphytic lichens. The results indicate species-specific responses to N forms, however, and some species may have responded to the total N flux rather than to the concentration (Mitchell et al., 2005; Munzi et al., 2010).

Van Herk (1999) classified both *H. physodes* and *P. furfuracea* as acidophytes sensitive to NH₃ as well as the NH₄⁺ concentration of bark, although *H. physodes* has generally been considered as an N-tolerant species (Dahlman et al., 2003; Mitchell et al., 2005). Based on the present results, *P. furfuracea*, *P. ambigua* and *P. glauca*, in particular, responded negatively to an increased bark NH₄⁺ concentration, while green algae + *S. chlorococcum* benefited from the eutrophication of *P. sylvestris* bark. A significant impact of oxidized N compounds (Dahlman et al., 2004) was also found in the Helsinki metropolitan area in terms of the strong negative correlation between the frequency of *P. furfuracea* and atmospheric total NO₂ concentration and the negative correlations between bark NO₂⁻+NO₃⁻-N concentration and the frequencies of *Bryoria* spp., *Usnea* spp. and *P. furfuracea*. The NO₃⁻ sensitivity of fruticose acidophytic lichens is attributed to their low constitutive nitrate reductase activity (Gombert et al., 2003 and references therein).

4.2 Effects of nitrogen pollution on lichen chemistry

The average N contents of *H. physodes* and *P. glauca* have varied from 0.3% to 0.6% (Bruteig, 1993; Dahlman et al., 2003; Geiser and Neitlich, 2007; Geiser et al., 2010; Green et al., 1980; Johansson et al., 2010, 2011; Søbchting, 1995) in background areas such as those receiving 0.5--2.0 kg N ha⁻¹ yr⁻¹ (Dahlman et al., 2003; Johansson et al., 2010, 2011) or 0.02--0.1 mg NH₄⁺-N l⁻¹ in precipitation (Bruteig, 1993; Geiser and Neitlich, 2007). In the Helsinki metropolitan area, the highest N content of *H. physodes* was only twice the lowest content. It was, however, greater than 1.0--1.5% in *H. physodes* that had been exposed to 50--100 kg NH₄NO₃-N ha⁻¹ yr⁻¹ in an irrigation-fertilization experiment in Sweden (Dahlman et al., 2003). The highest N content of *P. glauca* (1.70%) was in turn as high as in the Swedish irrigation experiments (Dahlman et al., 2003; Johansson et al., 2010, 2011, 2012), although the total N deposition in the Helsinki metropolitan area is expected to be lower than in those experiments. The apparently high 'precipitation' explains why the lichen N contents (on *P. abies* branches) were relatively low in the high NH₄NO₃ irrigation-fertilization treatments (Bruteig, 1993; Geiser et al., 2010).

The isotopic composition of rainfall changes when it filters through the *P. sylvestris* canopy, and the δ¹⁵N value of lichens may hence also vary depending on the ratio of dry- to

wet-deposited N-containing gases, aerosol particles and pH (Heaton et al., 1997). Given this, the scatter seen in Fig. 2a may be considered typical for areas with both large point sources and line sources and where the emissions and concentrations of different N forms vary within small spatial scales. Despite the wide variation, the $\delta^{15}\text{N}$ values of *H. physodes* and *P. glauca* suggest an increasing contribution of oxidized N (Ammann et al., 1999; Pearson et al., 2000), with an increasing N content of lichen thalli in the Helsinki metropolitan area. Thus, the $\delta^{15}\text{N}$ values support the important role of NO_3^- in terms of N uptake by epiphytic lichens and the composition of the acidophytic lichen community, including the frequency of *H. physodes* (Hauck and Runge, 2002; Hauck et al., 2002; Lang et al., 1976; Larsen et al., 2007; Schmull et al., 2002). Some studies have actually suggested that at ecologically relevant N concentrations and doses, there is no difference in the uptake of dissolved NO_3^- and NH_4^+ throughout the lichen thallus, especially in boreal and Antarctic lichens (Crittenden, 1998; Johansson et al., 2010). Moreover, the N contents of *P. furfuracea* indicated a higher uptake rate of N by the fruticose species than by the foliose *H. physodes* and *P. glauca*. This was attributed to a high uptake of oxidized N compounds (Dahlman et al., 2004; Lang et al., 1976) given the high average $\delta^{15}\text{N}$ value of *P. furfuracea* in the background area in Teijo.

The N content of *H. physodes* was also elevated in Pallas, where the NH_4^+ -N concentration in precipitation was approximately 0.06 mg l^{-1} and that of NO_3^- -N approximately 0.13 mg l^{-1} (Ruoho-Airola et al., 2015). This was attributed to the overall increase in NO_3^- deposition since the late 1990s (Ruoho-Airola et al., 2015) and the relatively high precipitation in summer 2016, which dissolved the dry-deposited N on the lichen surfaces (Gombert et al., 2003; Lang et al., 1976). Bruteig (1993) reported that the lichen N content was particularly affected by wet NO_3^- deposition in the background areas, because the uptake rate was relatively more efficient at low deposition concentrations and at high altitudes, as in the north, where lichen growth is low (see also Levia, 2002). Moreover, Dahlman et al. (2004) demonstrated that NO_3^- uptake was, to a higher extent, active relative to that of NH_4^+ . Notably, an N content of 0.97% was measured in *Bryoria* spp. in Pallas (Sirkku Manninen, unpublished).

The strong positive correlation between the N content of *H. physodes* and bark NH_4^+ -N concentration was not surprising given the high passive uptake rate of NH_4^+ by green algal lichens (Dahlman et al., 2004; Lang et al., 1976; Palmqvist and Dahlman, 2006) and the 3--7-fold average NH_4^+ -N concentrations versus the $\text{NO}_2^- + \text{NO}_3^-$ -N concentrations of *P. sylvestris* bark in the study areas. The latter is partly attributed to the predominance of NH_4^+ in boreal

forest soils (Bobbink et al., 2010; Högberg et al., 2006), given that soil affects the chemical composition of bark (Gauslaa, 1995; Gustafsson and Eriksson, 1995; Krouse, 1977).

The negative association between the N and C contents of *H. physodes* indicated an impact of elevated atmospheric concentrations and the deposition of reactive N compounds on C assimilation in lichens in the Helsinki metropolitan area. Since there is a seemingly tight regulation of resource investments and metabolic pathways between the symbionts in green algal foliose lichens (Palmqvist and Dahlman, 2006), the low C:N ratios of *H. physodes* thalli in the Helsinki metropolitan area as compared to those in Teijo and Pallas suggested disturbance in C to N stoichiometry between the symbiont partners (Palmqvist, 2000). The N-related decrease in C assimilation and the use of C to reduce NO_3^- to NH_4^+ , as well as to detoxify NH_4^+ , results in reduced growth of thalli (Dahlman et al., 2002; Gaio-Oliveira et al., 2005; Johansson et al., 2011), as observed in the Helsinki metropolitan area.

4.3 Role of SO_2 and other environmental factors

Although the ambient SO_2 and SO_4^{2-} -S aerosol concentrations are currently low in the Helsinki metropolitan area (Hannuniemi et al., 2016; Kaski et al., 2016; Teinilä et al., 2016), the direct deposition of SO_2 and aerosols on lichen surfaces, SO_4 -S in rainwater, and particulate SO_4^{2-} -S deposited on bark (Krouse, 1977) still negatively affect the composition of the acidophytic lichen community. The results thus suggest a need for revision of the CLE for SO_2 and/or CLO for S deposition. In fact, the increase in the frequency of green algae + *S. chlorococcum* towards the city centre may also partly be explained by the positive effect of an elevated concentration of S in the bark on the algal cover (Grandin, 2011). Notably, the SO_2 -tolerant *L. conizaeoides*, which was not included in the list of indicator species, was found at six sites in the Helsinki metropolitan area. An impact of windblown marine SO_4^{2-} compounds and salts on bark chemistry cannot be excluded at some sites, but it was probably minor (Bates and Brown, 1981).

Snow-tolerant species such as *P. ambigua*, *P. hyperopta* and *V. pinastri* (i.e. *Cetraria pinastri*) may be mostly confined to the bases of conifers (Thell and Moberg, 2011), especially in polluted areas (Ahti, 1977). This was also observed in the present study especially in the case of *V. pinastri*, which appeared to be a relatively N-tolerant species. Moreover, it was the only species that clearly responded to the bark pH. Given this and the observed relationships between the frequencies of lichen species and atmospheric and/or bark chemistry, the changes in the lichen community on *P. sylvestris* trunks in the Helsinki metropolitan area can be attributed to the physiological responses of individual species to N

and/or S deposition, rather than to changes in competitive interactions as a result of changes in the bark pH (see e.g. Johansson et al., 2012; van Herk, 1999).

4.4 Nitrogen critical levels and loads

Single small individuals of *Bryoria* spp. and/or *Usnea* spp. (mainly *U. hirta*) were found at sites with atmospheric total NO₂ concentrations of $\leq 5 \mu\text{g m}^{-3} \text{ yr}^{-1}$, except on an island ($12.5 \mu\text{g NO}_2 \text{ m}^{-3} \text{ yr}^{-1}$) and at an inland site ($15 \mu\text{g NO}_2 \text{ m}^{-3} \text{ yr}^{-1}$) in the Helsinki metropolitan area. These results and the elevated N contents in the lichen thalli indicate that the CLE of $30 \mu\text{g m}^{-3} \text{ yr}^{-1}$ (CLTRAP, 2017) does not protect the acidophytic green algal lichens on conifer trunks, at least not when simultaneously occurring with even slightly elevated levels of NH₃ and/or SO₂, and N- or S-containing aerosols (van Herk, 2004). Geiser and Neitlich (2007) attributed the increasing abundance of nitrophytes and the absence of sensitive species to mean wet deposition of $>0.06 \text{ mg NH}_4^+ \text{ l}^{-1}$ and a lichen N content of $>0.6\%$, e.g. in *P. glauca*. While Geiser and Neitlich (2007) were not able to assess the potential role of the high NO_x concentrations typical for the US Pacific Northwest or that of SO₂, Bermejo-Orduna et al. (2014) recorded a strong negative association between the N content and cover of the acidophyte *Letharia vulpina*, with thallus N contents of $<1.0\%$, in the vicinity of a transcontinental highway in the Sierra Nevada Mountains of California. If 1.0% N in *H. physodes* or *P. glauca* was set as a threshold, the CLE of NO₂ would be $\leq 5 \mu\text{g m}^{-3} \text{ yr}^{-1}$, but if the CLE were set based on the N content of *P. furfuracea*, it would be even lower.

No data are available on NH₃ concentrations or PM_{2.5} chemistry in the Helsinki metropolitan area, but an average NH₄⁺ concentration of $0.5 \mu\text{g m}^{-3}$, with 1-hr peaks of up to $2.2 \mu\text{g m}^{-3}$, was measured in PM₁ in the city centre between 1 May 2013 and 30 April 2015. The means for NO₃⁻ and SO₄²⁻ in PM₁ were $0.7 \mu\text{g m}^{-3}$ and $0.9 \mu\text{g m}^{-3}$, respectively, with 1-hr peak concentrations of $6.6 \mu\text{g NO}_3^- \text{ m}^{-3}$ and $2.4 \mu\text{g SO}_4^{2-} \text{ m}^{-3}$ (Teinilä et al., 2016). Using a formula provided by Cape et al. (2004), NH₃ concentrations of up to $10\text{--}12 \mu\text{g m}^{-3} \text{ yr}^{-1}$ were calculated at the edges of traffic lanes with the highest numbers of cars per day (City of Helsinki; Finnish Transport Agency). According to van Herk (2001), *H. physodes* appears to be absent at mean annual concentrations of $>13 \mu\text{g NH}_3 \text{ m}^{-3}$. Pinho et al. (2014) proposed a new CLE of $0.69 \mu\text{g NH}_3 \text{ m}^{-3} \text{ yr}^{-1}$ for Mediterranean evergreen woodlands, based on lichen diversity. Given the low levels of precipitation in both southern and northern Finland, a CLE of $<1 \mu\text{g NH}_3 \text{ m}^{-3} \text{ yr}^{-1}$ and a CLE of $\approx 0.5 \mu\text{g m}^{-3} \text{ yr}^{-1}$ of N in ambient-air fine particulates (Geiser et al., 2010) are proposed for the protection of acidophytic lichens on conifer trunks in boreal forests. Lower CLEs for gaseous and particulate N pollutants are also supported by the

fact that elevated levels of these occur at the same time as elevated levels of SO_2 and SO_4^{2-} in urban areas.

In the USA, the use of passive ion exchange resin-filled collectors (IER) to monitor dissolved inorganic N (DIN) in throughfall has been shown to provide total DIN, NO_3^- -N and NH_4^+ -N rates that correlate well with changes in epiphytic communities and lichen N contents (Fenn et al., 2008; Jovan et al., 2012; McMurray et al., 2013; Root et al., 2013). Based on the regression between the N contents of *P. glauca* and *H. physodes*, the N content of *P. glauca* was calculated for the most polluted sites at which the species did not grow in the Helsinki metropolitan area. The results were further used to predict throughfall rates of total DIN, NO_3^- -N and NH_4^+ -N based on the regressions of Root et al. (2013). The calculations yielded throughfall rates of 2.1--25 kg total DIN $\text{ha}^{-1} \text{yr}^{-1}$, 0.5--19 kg NO_3^- -N $\text{ha}^{-1} \text{yr}^{-1}$ and 0.8--8.1 kg NH_4^+ -N $\text{ha}^{-1} \text{yr}^{-1}$ in the Helsinki metropolitan area. The average total DIN deposition calculated for the sites in Teijo and Pallas were 3 and 1.5 kg $\text{ha}^{-1} \text{yr}^{-1}$, respectively. The latter is equal to the bulk deposition of NO_3^- -N + NH_4^+ -N in Pallas (Ruoho-Airola et al., 2015).

The European CLO of 5--10 kg N $\text{ha}^{-1} \text{yr}^{-1}$ proposed for pine taiga woodland is based on an increase in the occurrence of free-living algae (Bobbink and Hettelingh, 2011; see also Poikolainen et al., 1998). In the USA, McMurray et al. (2013) observed stunted growth of lichen thalli subjected to 4 kg N $\text{ha}^{-1} \text{yr}^{-1}$ in throughfall in the greater Yellowstone area. The CLO of 3.1 kg N $\text{ha}^{-1} \text{yr}^{-1}$ in throughfall has been proposed to protect the integrity of the lichen communities in the *Pinus ponderosa* forests of the Sierra Nevada (Fenn et al., 2008). Geiser et al. (2010) proposed in turn a lichen-based CLO of 2.7 kg N $\text{ha}^{-1} \text{yr}^{-1}$ for atmospheric N deposition in Northwestern North America's maritime forests, with an annual mean precipitation of approximately 450 mm. The N contents of *H. physodes* and/or *P. glauca* in Teijo and Pallas and the frequencies of N-sensitive epiphytes, as well as the frequency of green algae + *S. chlorococcum* in Teijo suggest a CLO of 2--3 kg N $\text{ha}^{-1} \text{yr}^{-1}$ in throughfall for the northernmost conifer forests.

If 10 $\mu\text{g NO}_2 \text{ m}^{-3}$ corresponds to 2.9 kg N $\text{ha}^{-1} \text{yr}^{-1}$ and 1 $\mu\text{g NH}_3 \text{ m}^{-3}$ to 2.6 kg N $\text{ha}^{-1} \text{yr}^{-1}$ on short vegetation (Cape et al., 2004), setting the NO_2 CLE at 5 $\mu\text{g m}^{-3} \text{yr}^{-1}$ and that of NH_3 at 0.5 $\mu\text{g m}^{-3} \text{yr}^{-1}$ would yield a total N deposition of approximately 3 kg N $\text{ha}^{-1} \text{yr}^{-1}$. The total N deposition on epiphytic lichens beneath canopies may be higher (Hanson and Lindberg, 1991), however, and exceed the threshold, partly because organic N compounds are also deposited on and leached from tree canopies (Carlisle et al., 1966, 1967; Piirainen et al., 1998). The uptake of amino acids by lichens may be equal to that of NO_3^- (Dahlman et al., 2004). Thus, it is challenging to assess the contribution of dry, wet and occult deposition of

different N forms to the lichen N content and community composition, especially in urban areas with various N sources, while also taking into account the modifying role of climatic factors.

5. Conclusions

Air pollution in the Helsinki metropolitan area was associated with detrimental effects on lichen community composition. Based on the frequencies of sensitive indicator species and N contents of lichen thalli, CLEs of $5 \mu\text{g NO}_2 \text{ m}^{-3} \text{ yr}^{-1}$ and $0.5 \mu\text{g NH}_3 \text{ m}^{-3} \text{ yr}^{-1}$, and a CLO of $2\text{--}3 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ in throughfall are proposed to protect the biodiversity of acidophytic lichens in boreal forests. Revision of the CLE for SO_2 and the CLO for S deposition is also recommended. The present study calls for monitoring of N throughfall to gain a better understanding of the impact of atmospheric N pollution, especially dry deposition of NO_x and particulate N pollutants, on lichen diversity in both urban and rural areas under different climates.

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References

Ahti, T., 1977. Lichens of the boreal coniferous zone, in: Seaward, M.R.D. (Ed.), Lichen Ecology. Academic Press, London, pp. 145-181.

- Ammann, M., Siegwolf, R., Pichlmayer, F., Suter, M., Saurer, M., Brunold, C., 1999. Estimating the uptake of traffic-derived NO₂ from ¹⁵N abundance in Norway spruce needles. *Oecologia* 118, 124-131.
- Ashmore, M.R., Wilson, R.B., 1993. Critical levels of air pollutants for Europe. Background papers prepared for the ECE Workshop on critical levels, Egham, UK, 23-26 March 1992.
- Bäcklund, S., Jönsson, M., Strengbom, J., Frisch, A., Thor, G., 2016. A pine is a pine and a spruce is a spruce – The effect of tree species and stand age on epiphytic lichen communities. *PLoS ONE* 11(1): e0147004. doi:10.1371/journal.pone.0147004
- Bates, J.W., Brown, D.H., 1981. Epiphyte differentiation between *Quercus petraea* and *Fraxinus excelsior* trees in a maritime area of South West England. *Vegetatio* 48, 61-70.
- Bermejo-Orduna, R., McBride, J.R., Shiraishi, K., Elustondo, D., Lasheras, E., Santamaria, J.M., 2014. Biomonitoring of traffic-related nitrogen pollution using *Letharia vulpina* (L.) Hue in the Sierra Nevada, California. *Science of the Total Environment* 290, 205-212.
- Bobbink, R., Hicks, K., Galloway, J., Spranger, T., Alkemade, R., Ashmore, M., Bustamente, M., Cinderby, S., Davidson, E., Dentener, F., Emmett, B., Erisman, J.-W., Fenn, M., Gilliam, F., Nordin, A., Pardo, L., De Vries, W., 2010. Global assessment of nitrogen deposition effects on terrestrial plant diversity: a synthesis. *Ecological Applications* 20, 30–59.
- Bobbink, R., Hettelingh, J.-P. (Eds.) 2011. Review and revision of empirical critical loads and dose-response relationships. Proceedings of an expert workshop, Noordwijkerhout, 23-25 June 2010.
- Braun, S., Achermann, B., De Marco, A., Pleijel, H., Karlsson, P.E., Rihm, B., Schindler, C., Paoletti, E., 2017. Epidemiological analysis of ozone and nitrogen impacts on vegetation – Critical evaluation and recommendations. *Science of the Total Environment* 603-604, 785-792.
- Bruteig, I.E., 1993. The epiphytic lichen *Hypogymnia physodes* as a biomonitor of atmospheric nitrogen and sulphur deposition in Norway. *Environmental Monitoring and Assessment* 26, 27-47.
- Cape, J.N., Tang, Y.S., van Dijk, N., Love, L., Sutton, M.A., Palmer, S.C.F., 2004. Concentrations of ammonia and nitrogen dioxide at roadside verges, and their contribution to nitrogen deposition. *Environmental Pollution* 132, 469-478.
- Cape, J.N., van der Eerden, L.J., Sheppard, L.J., Leith, I.D., Sutton, M.A., 2009. Reassessment of critical levels for atmospheric ammonia, in: Sutton, M., Reis, S., Baker,

- S.M.H. (Eds.), Atmospheric ammonia. Springer Science + Business Media B.V., Dordrecht, pp. 15-40.
- Carlisle, A., Brown, A.H.F., White, E.J., 1966. The organic matter and nutrient elements in the precipitation beneath a sessile oak (*Quercus petraea*) canopy. *Journal of Ecology* 54, 87-98.
- Carlisle, A., Brown, A.H.F., White, E.J., 1967. The nutrient content of tree stem flow and ground flora litter and leachates in a sessile oak (*Quercus petraea*) woodland. *Journal of Ecology* 55, 615-627.
- City of Helsinki. Traffic volumes in Helsinki. <https://www.avoindata.fi/data/en/dataset/liikennemaarat-helsingissa> (accessed 18.05.2017).
- CLRTAP, 2017. Mapping Critical Levels for Vegetation, Chapter III of Manual on methodologies and criteria for modelling and mapping critical loads and levels and air pollution effects, risks and trends. UNECE Convention on Long-range Transboundary Air Pollution. <http://icpvegetation.ceh.ac.uk/publications/documents/Chapter3-Mappingcriticallevelsforvegetation.pdf> (accessed 14.04.2017).
- Crittenden, P.D., 1998. Nutrient exchange in an Antarctic macrolichen during summer snowfall – snow melt events. *New Phytologist* 139, 697-707.
- Dahlman, L., Näsholm, T., Palmqvist, K., 2002. Growth, nitrogen uptake, and resource allocation in the two tripartite lichens *Nephroma arcticum* and *Peltigera aphthosa* during nitrogen stress. *New Phytologist* 153, 307-315.
- Dahlman, L., Persson, J., Näsholm, T., Palmqvist, K., 2003. Carbon and nitrogen distribution in the green algal lichens *Hypogymnia physodes* and *Platismatia glauca* in relation to nutrient supply. *Planta*, 217, 41–48.
- Dahlman, L., Persson, J., Palmqvist, K., Näsholm, T., 2004. Organic and inorganic nitrogen uptake in lichens. *Planta* 219, 459-467.
- Davies, L., Bates, J.W., Bell, J.N.B., James, P.W., Purvis, O.W., 2007. Diversity and sensitivity of epiphytes to oxides of nitrogen in London. *Environmental Pollution* 146, 299-310.
- Denison, R., Caldwell, B., Bormann, B., Eldred, L., Swanberg, C., Anderson, S., 1977. The effects of acid rain on nitrogen fixation in western Washington coniferous forests. *Water, Air, and Soil Pollution* 8, 21-34.
- de Vries, W., Kros, H., Reinds, G.J., Wamelink, W., Mol, J., van Dobben, H.F., Bobbink, R., Emmett, B., Smart, S., Evans, C., Schlutow, A., Kraft, P., Belyazid, S., Sverdrup, H., van Hinsberg, A., Posch, M., Hettelingh, J.P., 2007. Developments in deriving critical limits

and modelling critical loads of nitrogen for terrestrial ecosystems in Europe. Alterra Report 1382. Wageningen University, The Netherlands, 206 p.

Elliott, E.M., Kendall, C., Wankel, S.D., Burns, D.A., Boyer, E.W., Harlin, K., Bain, D.J., Butler, T.J., 2007. Nitrogen isotopes as indicators of NO_x source contributions to atmospheric nitrate deposition across the midwestern and northeastern United States. *Environmental Science & Technology* 41, 7661–7667.

Elliott, E.M., Kendall, C., Boyer, E.W., Burns, D.A., Lear, G.G., Golden, H.E., Harlin, K., Bytnerowicz, A., Butler, T.J., Glatz, R., 2009. Dual nitrate isotopes in dry deposition: utility for partitioning NO_x source contributions to landscape nitrogen deposition. *Journal of Geophysical Research* 114(G4):G04020.

Fenn, M.E., Jovan S., Yuan, F., Geiser, L., Meixner, T., Gimeno, B.S., 2008. Empirical and simulated critical loads for nitrogen deposition in California mixed conifer forests. *Environmental Pollution* 155, 492–511.

Finnish Meteorological Institute. Air quality in Finland. Finnish Meteorological Institute and Ministry of the Environment. (<http://www.ilmanlaatu.fi/index.php>) accessed on 07.04.2017).

Finnish Meteorological Institute. Temperature and precipitation statistics from 1961 onwards. <http://en.ilmatieteenlaitos.fi/statistics-from-1961-onwards> (accessed on 07.04.2017).

Finnish Transport Agency. Maps and charts. Traffic volumes. <http://www.liikennevirasto.fi/web/en/maps-charts/traffic-volumes#.WSFnBTMRrIE> (accessed 18.05.2017).

Flechard, C.R., Nemitz, E., Smith, R.I., Fowler, D., Vermeulen, A.T., Bleeker, A., Erismann, J.W., Simpson, D., Zhang, L., Tang, Y.S., Sutton, M.A., 2011. Dry deposition of reactive nitrogen to European ecosystems: a comparison of inferential models across the Nitro-Europe network. *Atmospheric Chemistry and Physics* 11, 2703–2728.

Gaio-Oliveira, G., Branquinho, C., Máguas, C., Martins-Loução, M.A., 2001. The concentration of nitrogen in nitrophilous and non-nitrophilous lichen species. *Symbiosis* 31, 187–199.

Gaio-Oliveira, G., Dahlman, L., Palmqvist, K., Martins-Loução, M.A., Máguas, C., 2005. Nitrogen uptake in relation to excess supply and its effects on the lichens *Evernia prunastri* (L.) Ach and *Xanthoria parietina* (L.) Th. Fr.. *Planta* 220, 794–803.

Gauslaa, Y., 1995. The *Lobarion*, an epiphytic community of ancient forests threatened by acid-rain. *Lichenologist* 27, 59–76.

Geiser, L.H., Neitlich, P.N., 2007. Air pollution and climate gradients in western Oregon and

- Washington indicated by epiphytic macrolichens. *Environmental Pollution* 145, 203-218.
- Geiser, L.H., Jovan, S.E., Glavich, D.A., Porter, M.K., 2010. Lichen-based critical loads for atmospheric nitrogen deposition in Western Oregon and Washington Forests, USA. *Environmental Pollution* 158, 2412-2421.
- Gilbert, O.L., 1973. Lichens and air pollution, in: Ahmadjian, V., Hale, M.E. (Eds.), *The Lichens*. Academic Press, New York, pp. 443-472
- Giordani, P., Calatayud, V., Stofer, S., Seidling, W., Granke, O., Fischer, R., 2014. Detecting the nitrogen critical loads on European forests by means of epiphytic lichens. A signal-to-noise evaluation. *Forest Ecology and Management* 311, 29-40.
- Gombert, S., Asta, J., Seaward, M.R.D., 2003. Correlation between the nitrogen concentration of two epiphytic lichens and the traffic density in an urban area. *Environmental Pollution* 123, 281-290.
- Grandin, U., 2011. Epiphytic algae and lichen cover in boreal forests – A long-term study along a N and S deposition gradient in Sweden. *AMBIO* 40, 857-866.
- Green, T.G.A., Horstmann, J., Bonnett, H., Wilkins, A., Silvester, W.B., 1980. Nitrogen fixation by members of the Stictaceae (lichens) of New Zealand. *New Phytologist* 84, 339-348.
- Gustafsson, L., Eriksson, I., 1995. Factors of importance for the epiphytic vegetation of aspen *Populus tremula* with special emphasis on bark chemistry and soil chemistry. *Journal of Applied Ecology* 32, 412-424.
- Hale, M.E. Jr. 1968. A synopsis of the lichen genus *Pseudevernia*. *The Bryologist* 71, 1-11.
- Hannuniemi, H., Salmi, J., Rasila, T., Wernberg, A., Komppula, B., Lovén, K., Pietarila, H., 2016. Pääkaupunkiseudun päästöjen leviämismalliselvitys. Autoliikenteen, energiantuotannon, laivaliikenteen ja lentoliikenteen typenoksidi-, pienhiukkas- ja rikkidioksidi-päästöjen leviämismallinnus. Ilmatieteen laitos.
- Hanson, P.J., Lindberg, S.E., 1991. Dry deposition of reactive nitrogen compounds: A review of leaf, canopy and non-foliar measurements. *Atmospheric Environment* 25, 1615-1634.
- Hauck, M., Runge, M., 2002. Stemflow chemistry and epiphytic lichens diversity in dieback-affected spruce forest of the Harz Mountains, Germany. *Flora* 197, 127-131.
- Hauck, M., Hesse, V., Runge, M., 2002. The significance of stemflow chemistry for epiphytic lichens diversity in dieback-affected spruce forest on Mt. Brocken, northern Germany. *Lichenologist* 34, 415-427.
- Hauck, M., 2011. Site factors controlling epiphytic lichen abundance in northern coniferous forests. *Flora* 206, 81-90.

- Heaton, T.H.E., Spiro, B., Robertson, S.M.C., 1997. Potential canopy influences on the isotopic composition of nitrogen and sulphur in atmospheric deposition. *Oecologia* 109, 600-607.
- Högberg, M., Myrold, D.D., Giesler, R., Högberg, P., 2006. Contrasting patterns of soil N-cycling in model ecosystems of Fennoscandian Boreal Forests. *Oecologia*. 147, 96-107.
- Honegger, R., 1991. Functional aspects of the lichen symbiosis. *Annual Review of Plant Physiology and Plant Molecular Biology* 42, 553-578.
- Honegger, R., 1993. Developmental biology of lichens. *New Phytologist* 125, 659-677.
- Honegger, R. 1998. The lichen symbiosis – What is so spectacular about it? *Lichenologist* 30, 193-212.
- Howarth, R.W., 2007. Atmospheric deposition and nitrogen pollution in coastal marine ecosystems, in: Visgilio, G.R., Whitelaw, D.M. (Eds), *Acid in the Environment*. Springer, Boston, MA. pp. 97-116.
- Johansson, O., Nordin, A., Olofsson, J., Palmqvist, K., 2010. Responses of epiphytic lichens to an experimental whole-tree nitrogen deposition gradient. *New Phytologist* 188, 1075-1084.
- Johansson, O., Olofsson, J., Giesler, R., Palmqvist, K., 2011. Lichen responses to nitrogen and phosphorus addition can be explained by the different symbiont responses. *New Phytologist* 191, 795-805.
- Johansson, O., Palmqvist, K., Olofsson, J., 2012. Nitrogen deposition drives lichen community changes through differential species responses. *Global Change Biology* 18, 2626-2635.
- Jovan, S., Riddell, J., Padgett, P.E., Nash, T.H. III. 2012. Eutrophic lichens respond to multiple forms of N: implications for critical levels and loads research. *Ecological Applications* 22, 1910-1922.
- Karl, T., Graus, M., Stridnig, M., Lamprecht, C., Hammerle, A., Wohlfahrt, G., Held, A., von der Heyden, L., Deventer, M.J., Krismer, A., Haun, C., Feichter, R., Lee, J., 2017. Urban eddy covariance measurements reveal significant missing NO_x emissions in Central Europe. *Nature Scientific Reports* 7: 2536. doi:10.1038/s41598-017-02699-9
- Kaski, N., Aarnio, P., Loukkola, K., Portin, H., 2016. Air quality in the Helsinki metropolitan area in 2015. HSY publications 6/2016. Helsinki Region Environmental Services Authority.
- Krouse, H.R., 1977. Sulphur isotope abundance elucidate uptake of atmospheric sulphur emissions by vegetation. *Nature* 252, 45-46.

- Kuusinen, M., 1996. Epiphytic flora and diversity on basal trunks of six old-growth forest tree species in southern and middle boreal Finland. *Lichenologist* 28, 443-463.
- Lang, G.E., Reiners, W.A., Heier, R.K., 1976. Potential alteration of precipitation chemistry by epiphytic lichens. *Oecologia* 25, 229-241.
- Larsen, R.S., Bell, J.N.B., James, P.W., Chimonides, P.J., Rumsey, F.J., Tremper, A., Purvis, O.W., 2007. Lichen and bryophyte distribution on oak in London in relation to air pollution and bark acidity. *Environmental pollution* 146, 332-340.
- Larsen Vilsholm, R., Wolseley, P.A., Søbchting, U., Chimonides, P.J., 2009. Biomonitoring with lichens on twigs. *The Lichenologist* 41, 189-202.
- Levia Jr., D.F., 2002. Nitrate sequestration by corticulous macrolichens during winter precipitation events. *International Journal of Biometeorology* 46, 60-65.
- Lindroos, A-J., Derome, K. and Nieminen, T. M., 2013. Sulphur and nitrogen deposition in bulk deposition and stand throughfall on intensive monitoring plots in Finland, in: Merilä, P., Jortikka, S. (Eds.), *Forest Condition Monitoring in Finland – National report*. The Finnish Forest Research Institute. <http://urn.fi/URN:NBN:fi:metla-201305087576> (accessed 13.01.2017).
- Lovett, G.M., Traynor, M.M., Pouyat, R.V., Carreiro, M.M., Zhu, W.-X., Baxter, J.W., 2000. Atmospheric deposition to oak forests along an urban rural gradient. *Environmental Science & Technology* 34, 4294-4300.
- Manninen, S., Sassi, M-K., Lovén, K. 2013. Effects of nitrogen oxides on ground vegetation, *Pleurozium schreberi* and the soil beneath it in urban forests. *Ecological Indicators* 24, 485-493.
- McMurray, J.A., Roberts, D.W., Fenn, M.E., Geiser, L.H., Jovan, S., 2013. Using epiphytic lichens to monitor nitrogen deposition near natural gas drilling operations in the Wind River Range, WY, USA. *Water, Air and Soil Pollution* 224: 1487. doi 10.1007/s11270-013-1487-3
- Mitchell, R.J., Truscot, A.M., Leith, I.D., Cape, J.N., van Dijk, N., Tang, Y.S., Fowler, D., Sutton, M.A., 2005. A study of the epiphytic communities of Atlantic oaks woods along an atmospheric nitrogen deposition gradient. *Journal of Ecology* 93, 482-492.
- Munzi, S., Pisani, T., Paoli, L., Loppi, S., 2010. Time- and dose-dependency of the effects of nitrogen pollution on lichens. *Ecotoxicology and Environmental Safety* 73, 1785-1788.
- Munzi, S., Cruz, C., Branquinho, C., Pinho, P., Leith, I.D., Sheppard, L.J., 2014. Can ammonia tolerance amongst lichen functional groups be explained by physiological responses? *Environmental Pollution* 187, 206-209.

941 Nieboer, E., Richarsson, D.H.S., Tomassini, F.D., 1978. Mineral uptake and release by
 942 lichens: an overview. *The Bryologist* 81, 226-246.

943 Palmqvist, K., 2000. Tansley review No. 11. Carbon economy in lichens. *New Phytologist*
 944 148, 11-36.

945 Palmqvist, K., Dahlman, L., Valladares, F., Tehler, A., Sancho, L.G., Mattson J.-E., 2002.
 946 CO₂ exchange and thallus nitrogen across 75 contrasting lichen associations from different
 947 climate zones. *Oecologia* 133, 295-306.

948 Palmqvist, K., Dahlman, L., 2006. Responses of the green algal foliose lichen *Platismatia*
 949 *glauca* to increased nitrogen supply. *New Phytologist* 171, 343-356.

950 Pardo, L.H., Geiser, L.H., Fenn, M.E., Driscoll, C.T., Goodale, C.L., Allen, E.B., Baron, J.S.,
 951 Bobbink, R., Bowman, W.D., Clark, C.M., Emmett, B., Gilliam, F.S., Greaver, T., Hall,
 952 S.J., Lilleskov, E.A., Liu, L., Lynch, J.A., Nadelhoffer, K., Perakis, S.S., Robin-Abbott,
 953 M.-J., Stoddard, J.L., Weathers, K.C., 2011a. Synthesis, in: Pardo, L.H., Robin-Abbott,
 954 M.J., Driscoll, C.T. (Eds.), *Assessment of nitrogen deposition effects and empirical critical*
 955 *loads of nitrogen for ecoregions of the United States. General Technical Report NRS-80.*
 956 *USDA Forest Service, Northern Research Station, Newtown Square, Pennsylvania, USA.*
 957 *pp. 229-284.*

958 Pardo, L.H., Fenn, M.E., Goodale, C.L., Geiser, L.H., Driscoll, C.T., Allen, E.B., Baron, J.S.,
 959 Bobbink, R., Bowman, W.D., Clark, C.M., Emmett, B., Gilliam, F.S., Greaver, T.L., Hall,
 960 S.J., Lilleskov, E.A., Liu, L., Lynch, J.A., Nadelhoffer, K.J., Perakis, S.S., Robin-Abbott,
 961 M.J., Stoddard, J.L., Weathers, K.C., Dennis, R.L., 2011b. Effects of nitrogen deposition
 962 and empirical nitrogen loads for ecoregions of the United States. *Ecological applications*
 963 21, 3049-3082.

964 Pearson, J., Wells, D.M., Seller, K.J., Bennett, A., Soares, A., Woodall, J., Ingrouille, M.J.,
 965 2000. Traffic exposure increases natural ¹⁵N and heavy metal concentrations in mosses.
 966 *New Phytologist* 147, 317-326.

967 Piirainen. S., Finér, L., Starr, M., 1998. Canopy and soil retention of nitrogen deposition in a
 968 mixed boreal forest in Eastern Finland. *Water, Air , and Soil Pollution* 105, 165-174.

969 Pike, L.H., 1978. The importance of epiphytic lichens in mineral cycling. *The Bryologist* 81,
 970 247-267.

971 Pinho, P., Llop, E., Ribeiro, M.C., Cruz, C., Soares, A., Pereira, M.J., Branquinho, C., 2014.
 972 Tools for determining critical levels of atmospheric ammonia under the influence of
 973 multiple disturbances. *Environmental Pollution* 188, 88-93.

- Poikolainen, J., Lippo, H., Hongisto, M., Kubin, E., Mikkola, K., Lindgren, M., 1998. On the abundance of epiphytic green algae in relation to the nitrogen concentrations of biomonitors and nitrogen deposition in Finland. *Environmental Pollution* 102, S1, 85-92.
- Purvis, O.W., Chimonides, P.J., Din, V., Erotokritou, L., Jeffries, T.E., Jones, G.C., Louwhoff, S., Read, H., Spiro, B., 2003. Which factors are responsible for the changing lichen floras of London. *The Science of the Total Environment* 310, 179-189.
- Rao, D.N., LeBlanc, F., 1966. Effects of sulphur dioxide on the lichen alga, with special reference to chlorophyll. *The Bryologist* 69, 69-75.
- Rao, P., Hutyra, L.R., Raciti, S.M., Templer, P.H., 2014. Atmospheric nitrogen deposition inputs and losses along an urbanization gradient from Boston to Harvard Forest, MA. *Biogeochemistry* 121, 229-245.
- Redling, K., Elliott, E., Bain, D., Sherwell, J., 2013. Highway contributions to reactive nitrogen deposition: tracing the fate of vehicular NO_x using stable isotopes and plant biomonitors. *Biogeochemistry* 116, 261-274.
- Root, H.T., Geiser, L.H., Fenn, M.E., Jovan, S., Hutten, M.A., Ahuja, S., Dillman, K., Schirokauer, D., Berryman, S., McMurray, J.A. 2013. A simple tool for estimating throughfall nitrogen deposition in forests of western North America using lichens. *Forest Ecology and Management* 306, 1-8.
- Ruoho-Airola, T., Anttila, P., Hakola, H., Ryyppö, T., Tuovinen, J.-P., 2015. Trends in the bulk deposition and atmospheric concentration of air pollutants in the Finnish Integrated Monitoring catchment Pallas during 1992-2012. *Boreal Environment Research* 20, 553-569.
- Schultz, H., Huhn, G., Shüürmann, G., Niehus, B., Liebergeld, G., 1997. Determination of throughfall rates on the basis of pine bark loads, results of a pilot field study. *Journal of the Air & Waste Management Association* 47, 510-516.
- Schultz, H., Popp, P., Huhn, G., Stärk, H.-J., Schüürmann, G., 1999. Biomonitoring of airborne inorganic and organic pollutants by means of pine tree barks. I. Temporal and spatial variations. *The Science of the Total Environment* 232, 49-58.
- Schmull, M., Hauck, M., Vann, D.R., Johnson, A.H., Runge, M., 2002. Site factors determining epiphytic lichen distribution in a dieback-affected spruce-fir forest on Whiteface Mountain, New York: stemflow chemistry. *Canadian Journal of Botany* 80, 1131-1140.
- SFS-EN ISO 11732. Water quality. Determination of ammonium nitrogen. Method by flow analysis (CFA and FIA) and spectrometric detection (ISO 11732:2005).

- SFS-EN ISO 13395. Water quality. Determination of nitrite nitrogen and nitrate nitrogen and the sum of both by flow analysis (CFA and FIA) and spectrometric detection (ISO 13395:1996)
- Skye, E., 1968. Lichens and air pollution. A study of cryptogamic epiphytes and environment in the Stockholm region. *Acta Phytogeographica Suecica* 52, 1- 123.
- Skye, E., Hallberg, I., 1969. Changes in lichen flora following air pollution. *Oikos* 20, 547-552.
- Søchting, U., 1995. Lichens as monitors of nitrogen deposition. *Cryptogamic Botany* 5, 264-269.
- Suomen standardisoimisliitto 1990. SFS standard 5670. Air quality. Bioindication. Mapping of epiphytic lichens. (in Finnish)
- Sutton, M.A., Dragosits, U., Tang, Y.S., Fowler, D. 2000. Ammonia emissions from non-agricultural sources in the UK. *Atmospheric Environment* 34, 855-869.
- Taipale, L., 2006. Rikkidioksidipitoisuudet alas korkeilla piipuilla – Hanasaari B, in: Huutoniemi, K., Estlander, A., Hahkala, M., Hämekoski, K., Kulmala, A., Lahdes, R., Laukkanen, T. (toim.), Savuntarkastajista päästökauppiaisiin. Suomalaisen ilmansuojelun historia. Gummerus, Jyväskylä, pp.74-75.
- Teinilä, K., Aurela, M., Saarikoski, S., Timonen, H., Hillamo, R., Niemi, J., Kousa, A., 2016. Composition and sources of fine particles at a busy traffic environment in Helsinki city center. Helsinki Region Environmental Services Authority. HSY Publications 7/2016. (https://www.hsy.fi/sites/Esitteet/EsitteetKatalogi/Julkaisusarja/7_2016_pienhiukkasten-koostumus-lahteet.pdf)
- Thell, A., Moberg, R., 2011. Nordic Lichen Flora. Vol. 4. Parmeliaceae. Uppsala, Uppsala University, Nordic Lichen Society.
- Tørseth, K., Aas, W., Breivik, K., Fjæraa, A.M., Fiebig, M., Hjellbrekke, A.G., Lund Myhre, C., Solberg, S., Yttri, K.E., 2012. Introduction to the European Monitoring and Evaluation Programme (EMEP) and observed atmospheric composition change during 1972-2009. *Atmospheric Chemistry and Physics* 12, 5447-5481.
- Vaarna, V.V., 1934. Helsingin kaupungin puiden ja pensaiden jäkäläkasvisto. *Annales Botanici Societatis zoologicæ-botanicæ Fennicæ Vanamo* 5, 1-32.
- van Dobben H.F., Ter Braak, C.J.F., 1998. Effects of atmospheric NH₃ on epiphytic lichens in the Netherlands: the pitfalls of biological monitoring. *Atmospheric Environment* 32, 551-557.

1041 van Herk, C.M., 1999. Mapping ammonia pollution with epiphytic lichens in the Netherlands.
 1042 Lichenologist 31, 9-20.
 1043 van Herk, C.M., 2001. Bark pH and susceptibility to toxic air pollutants as independent
 1044 causes of changes in epiphytic lichen composition in space and time. Lichenologist 33,
 1045 419-441.
 1046 van Herk, K. 2004. A changing flora, the effects of short and long distance nitrogen
 1047 deposition on epiphytic lichens, in: Lambley, P., Wolseley, P. (Eds.), Lichens in a
 1048 Changing Pollution Environment. English Nature, Peterborough, UK. English Nature
 1049 research Reports No. 525. pp. 13-20.
 1050 Wolterbeek, T.Th., Kuik, P., Verburg, T.G., Wamelink, G.W.W., 1996. Relations between
 1051 sulphate, ammonia, nitrate, acidity and trace element concentrations in tree bark in the
 1052 Netherlands. Environmental Monitoring and Assessment 40, 185-201.

Responses of acidophytes growing on *Pinus sylvestris* trunks to reactive N compounds in their environment in the Helsinki metropolitan area and/or Pallas National Park. Damaged *Hypogymnia physodes*, air-pollutant-tolerant *Hypocenomyce scalaris* and green algae + *Scoliciosporum chlorococcum* in the background (photo S. Manninen).