NH₃ concentrations below the current critical level affect the epiphytic macrolichen communities – Evidence from a Northern European City

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HIGHLIGHTS

• NH₃ was measured at roadside and non-roadside sites using passive samplers.
• Changes in epiphytic lichen communities were found at <1 μg NH₃ m⁻³.
• NH₃ decreased the diversity of oligotrophs on Quercus at roadsides.
• NH₃ increased the diversity of eutrophs on Quercus at roadsides.
• The ratio of acidophytes to nitrophytes on Quercus depended on both NH₃ and bark pH.

ABSTRACT

Acidophytic, oligotrophic lichens on tree trunks are widely considered to be the most sensitive biota to elevated concentrations of atmospheric ammonia (NH₃). We studied the relationships between measured NH₃ concentrations and the composition of macrolichen communities on the acidic bark of Pinus sylvestris and Quercus robur and the base-rich bark of Acer platanoides and Ulmus glabra at ten roadside and ten non-roadside sites in Helsinki, Finland. NH₃ and nitrogen dioxide (NO₂) concentrations were higher at the roadside than non-roadside sites indicating trafﬁc as the main source of NH₃ and nitrogen oxides (NOx). The diversity of oligotrophs on Quercus was lower at the roadside than non-roadside sites, while that of eutrophs was higher. The abundance and presence of oligotrophic acidophytes (e.g., Hypogymnia physodes) decreased with increasing NH₃ concentration (2-year means = 0.15-1.03 μg m⁻³) especially on Q. robur, while those of eutrophic/nitrophilous species (e.g., Melanohalea exasperatula, Physcia tenella) increased. The abundance of some nitrophytes seemed to depend only on bark pH, i.e., their abundances were highest on Ulmus, which had the highest average bark pH. Overall, the results of lichen bioindicator studies may depend on tree species (bark pH) and lichen species used in calculating indices describing the air quality impact. Nevertheless, Quercus is recommended to be used to study the impact of NH₃ alone and in combination with NOx on lichen communities, because the responses of both oligotrophic acidophytes and eutrophic species can already be observed at NH₃ concentrations below the current critical level.

1. Introduction

The emissions of sulfur dioxide (SO₂) and nitrogen oxides (NOₓ) have decreased by 90 % and 50 %, respectively, in the European Union over the past 30 years thanks to regulation of emissions from large industrial...
and energy production point sources and traffic. However, ammonia (NH₃) emissions have only been reduced by 20 % over the same period (Sohhi et al., 2022). Urban, traffic-derived NH₃ emissions are low compared to those from agriculture (Reche et al., 2015). However, they may contribute significantly to poor local air quality in terms of concentrations of NH₃ and ammonium (NH₄⁺) in particulate matters <2.5 μm in diameter (PM₂.₅). The increased traffic-related NH₃ emissions arise from the introduction of three-way catalytic converters in gasoline vehicles (Heeb et al., 2012) and, also the adaptation of selective catalytic reduction (SCR) by the addition of urea or NH₃ to diesel exhaust systems to meet nitrogen oxide (NO) emission standards (Carslaw and Rhy's-Tyler, 2013).

Emissions of reactive nitrogen (N) compounds from traffic are implicated in changing the diversity of epiphytic lichens. The high sensitivity of some rootless, poikilohydric epiphytic macrolichens to, e.g., nitrogenous air pollutants is largely due to their physiology and morphology. They also lack guard cells and a waxy epidermis and thus there is an unlimited influx of water-soluble N compounds into the lichen thallus (Nash, 2008). A shift in lichen species that thrive in nutrient-rich environments (a.k.a. eutrophs, eurytrophs, nitrophilous, nitrophytes) has been reported e.g., in Zeeland in the Netherlands (van Herk, 2009) and in London (Davies et al., 2007). In Zeeland, the increased frequency of nitrophytes on deciduous tree trunks close to busy roads was attributed to traffic-derived NH₃ (van Herk, 2009), while in London and its surroundings, transport-related NOx seemed to have had a large impact on lichens (Purvis et al., 2003; Davies et al., 2007; Larsen et al., 2007; Gadsdon et al., 2010). A recent study of epiphytic lichens in the urban area of Munich, Germany, also showed an impact from nitrogen dioxide (NO₂) on species composition, the most frequent species being nitrophilous (Sebald et al., 2022). However, NH₃ was not monitored in the German study.

The critical level of NH₃, i.e., the concentration above which direct adverse effects on epiphytic lichens may occur, is 1 μg NH₃ m⁻³ yr⁻¹ (Cape et al., 2009), while that of NOx for vegetation is 30 μg m⁻³ yr⁻¹ (expressed as NO₂) (UNECE, 2017). The impact of NH₃ and NOx on lichen communities arises mainly from their direct toxicity (Gaio-Oliveira et al., 2005) and effect on bark pH (de Bakker, 1989; van Herk, 2001), while lichen species interactions play a minor role (von Herk, 2001; Carter et al., 2017). Ammonia is considered harmful especially to acidiphilic lichens (a.k.a. oligotrophic, oligotrophs, nitrophilous, nitrophytes) which thrive on acidic bark in environments with lower nutrient supply (Woolsey et al., 2009). Based on laboratory experiments, N-tolerant species can oxidize surplus NH₄⁺ to nitrate (NO₃⁻), which is a non-toxic form of N, and hence nitrophytes can cope with higher tissue total N concentrations than oligotrophs (Gaio-Oliveira et al., 2004, 2005).

Many field studies show, however, that lichen responses to air pollution vary considerably and the results can usually not be attributed to a single abiotic or biotic factor. For example, Llewelyn et al. (2020) found only early-successional communities of epiphytic lichens on oaks (Quercus) in London’s urban parks. This was attributed to the legacy effects of SO₂ emissions, and current N pollution and particulate emissions. Lichen diversity also varied depending on the host tree species and decreased with increasing size (girth) of trees and tree crowding (i.e., decreasing amount of light). The most abundant species across the studied urban parks in London were nitrophilous Physcia adscendens, P. temelia, and Phaeophyscia orbicularis (Llewelyn et al., 2020).

An earlier study of macrolichens on pine (Pinus sylvestris) trunks in Helsinki, located on Finland’s southern coast, showed that the number of oligotrophic macrolichens decreased with increasing concentrations of SO₂ and NO₂ in the air, and those of sulfur (S) and inorganic N in bark (Manninen, 2018). Moreover, the increase in the total N concentration of Hypogymnia physodes with increasing PM₉.₅ and bark NH₄⁺ concentrations pointed to the impact of traffic-derived NH₃. Ammonia data was not available for Manninen (2018), however, and the study was only performed on pines which had an average bark pH of 3.4. In the present study, we wanted to examine trees with different bark pH levels. Consequently, we monitored ambient NH₃ concentrations using passive samplers and investigated the relationships between ambient air NH₃ concentration and the abundance of epiphytic macrolichens on the acidic bark of P. sylvestris and Quercus robur and the alkaline bark of Acer platanoides and Ulmus glabra. Our hypothesis was that the traffic-derived NH₃ emissions were high enough to have a major impact on epiphytic lichen communities especially at the edges of roads. The diversity and abundance of oligotrophic species was hypothesised to decrease and those of eutrophic species to increase with increasing NH₃ concentration. We also hypothesised that the potential NH₃-related increase in bark pH and subsequent changes in lichen community would be seen especially as a decrease in the abundance of acidophytes and as an increase in that of nitrophytes on Quercus. This was because we expected the acidic bark of Quercus to have a naturally higher pH than that of Pinus and hence serve as a substrate for both acidophytes and nitrophytes. We also studied the relationships between epiphytic macrolichen community composition and modelled NO₂ concentration, NH₃-to-NO₂ ratio, bark pH, and tree size (girth).

2. Material and methods

2.1. Study area

The study was performed in Helsinki (60°10′N, 24°56′E), southern Finland, from 2019 to 2021. In the city centre, the annual mean temperature was 6.5–8.2 °C, while the mean temperature of the coldest and the warmest months were −6.6 °C (Feb 2021) and 21.4 °C (Jul 2021), respectively. The annual precipitation ranged between 656 and 741 mm (Kaisaniemi station, 60°18′N, 24°94′E) in 2019–2021 (Finnish Meteorological Institute, 2022). In 2019, the total NOx emissions from energy production in Helsinki were 3226 metric tons while those resulting from vehicular traffic were 1418 metric tons (HSY, 2020). The SO₂ concentration has decreased from >30 μg m⁻³ yr⁻¹ in the 1970s to <1 μg m⁻³ yr⁻¹ (Korhonen et al., 2021).

The locations and the number of sites were decided based on traffic densities (e.g., Helsinki Region InfoShare, n.d.), occurrence of at least three suitable trees in terms of trunk circumference (size) and condition (Finnish Standards Association, 2014), and funding available for the NH₃ monitoring and analyses. Traffic-derived NH₃ concentrations have been shown to fall by 90 % within the first 10 m away from road edges (Cape et al., 2004). In our study area, however, it was not possible to make transects (based on a single tree species) in the urbanised city centre. Moreover, the transects outside the city centre would have been P. sylvestris transects. Pinus sylvestris is a common native tree species in the Helsinki region, but not as common in the city centre as planted deciduous trees native to more southern latitudes. Consequently, we had 20 sites of which ten were located at road sides (mostly >10 m from the road edge) and the other ten non-roadside sites (>100 m from the road edge) (Fig. 1). The roads ranged from the busiest urban dual-lane carriageways (sites 1, 11, 12, 13) to a single-lane carriageway in a small urban forest (site 2). The number of tree species per site varied from one to four. When possible, we chose trees growing at the edge of roads or forests patches to minimize the variation in light conditions.

2.2. Measurement of ambient NH₃ concentration

Ambient NH₃ concentrations were measured from Oct 2019 - Sep 2021 using UKCEH ALPHA® Samplers (Tang et al., 2001). Triplicate samples were used for each measurement and were supplied by the UK Centre for Ecology and Hydrology (UKCEH). These were attached to aerodynamically-shaped shelter at each site and placed at approx. 2.4 m above the ground. Five of the shelters were attached on Acer, two on Pinus, seven on Quercus, four on Ulmus, one on a pole, and one on the wall of an automated air quality monitoring station operated by the Helsinki Region Environmental Services Authority (HSY) next to site 8. The samplers were changed once a month and sent to the UKCEH for NH₃ analysis. The detection limit of the samplers is 0.02 μg m⁻³ (Puchalski et al., 2011). The results were corrected for temperature using the monthly mean temperature obtained from the Kaisaniemi weather station operated by the Finnish Meteorological Institute.
2.3. Modelled NO₂

Nitrogen dioxide concentrations for Oct 2019–Sep 2021 were not measured because they could be taken from a concentration map provided by the Helsinki Region Environmental Services Authority (HSY, 2022a). The concentration map for the Helsinki metropolitan area is compiled from the open data of the ENFUSER model developed by the Finnish Meteorological Institute. The modelling system combines air quality monitoring for NO₂, ozone (O₃), SO₂, PM₂.₅ and PM₁₀ with information about emissions, land use, the weather situation and produces air quality information on an hourly basis with a resolution of 13 × 13 m (Johansson et al., 2022). The highest correlations between modelled and measured monthly averages have been obtained for NO₂ (0.908 for 2017 and 0.927 for 2018). Road traffic dominates over other NO₂ sources, although the concentrations near main roads are often limited by the availability of O₃. The NO₂ contribution of power plants is even less than that of cargo and passenger ships. Some outlier stations were found when model predictions were compared to measured concentrations. For NO₂ modelling the outlier stations were explained by i) underestimated decay rate of NO₂ (regional background), ii) difficulties in estimating the emissions of auxiliary engines of berthing ships and the additional road traffic induced by shipping (shipping terminal), and iii) a significant amount of mostly diesel-driven taxi traffic (airport). For more information on the performance and validation of the ENFUSER model see Johansson et al. (2022). Ratios of NH₃ to NO₂ were calculated using measured NH₃ and modelled NO₂ from Oct 2019–Sep 2021.

2.4. Abundance of lichens

The abundance of lichens was scored on tree trunks at roadside and non-roadside sites from Aug–Oct 2019. The number of tree species per site varied from one to four since we wanted to include as many tree species out of the four as possible at each site. As tree age, i.e., the pollution history of a tree, may affect lichen flora (Llewellyn et al., 2020), tree girth was used as a proxy for that effect.
The scoring of lichens was performed by applying the European Standard EN 16413:2014 (Finnish Standards Association, 2014) as follows. Three trees with a girth of 50–250 cm at breast height were studied per tree species at each site. A plastic ladder quadrate with five 10 cm × 10 cm squares was used for scoring the lichens by placing the top of the ladder at 1.50 m above the ground. Presence of foliose and fruticose macrolichens, Cladonia spp., and green algae + Scoliciosporum chlorococcum was recorded in each of the five 10 cm × 10 cm squares of the quadrate ladder on the cardinal compass points (N, E, S, W), yielding a maximum presence score of 20 for each lichen species, Cladonia spp. or green algae + Scoliosisporum chlorococcum per tree trunk. Data on lichen data was used to calculate the 20 sites, making altogether 111 trees. The lichen data was used to cal-

Scoliciosporum chlorococcum

Bealey, 2011). We also calculated a Lichen Atmospheric Nitrogen

We had Acer and Quercus at 11, Ulmus at eight, and Pineus at seven out of the 20 sites, making altogether 111 trees. The lichen data was used to calculate mean abundances for each lichen species per tree species at each site. Oligotrophic or eutrophic species (Supplement 1) were distinguished based on the Central European classification by Wirth (2010). Species with eutrophication values (N) 1–4 were considered as oligotrophs and those with 5–9 as eutrophs (Wirth, 2010). Total Lichen Diversity Value (LDV) was calculated for each tree species and across deciduous trees at each site (Finnish Standards Association, 2014) as were the corresponding values for oligotrophs (LDV oligo) and eutrophs (LDV eutro). The LDV method can be used to get information on the long-term effects of environmental stress on epiphytic lichens at different geographical scales (Asta et al., 2002; Bealey, 2011). We also calculated a Lichen Atmospheric Nitrogen index (I L AN = I AN − I LN) (Wolseley et al., 2009) for each tree species using Hypogymnia spp., Parmeliopsis ambiguca/Cladonia spp., Platismatia glauca/Tuckermanopsis chlorophylla, Pseudovernia furfuracea, and Usnea spp. as indicator species for acidophytes (I AN), and P. orbitalis/P. nigricans, P. adscendens/P. tenella, Physconia spp., Xanthomendoza fulva, and Xanthoria spp. as indicator species for nitrophytes (I LN). The I AN thus ranged from 5 to − 5 depending on whether there were only acidophytic or nitrophytic indicator species. Calculating I AN was expected to show both direct NH3 impacts as well as the impact of potentially NH4-related increases in bark pH on epiphytic lichen communities especially on Quercus.

2.5. Bark pH and lichen N concentration

Bark flakes (<3 mm in thickness) with no lichens were taken from a height of about 1.5 m for pH measurements. We collected samples from nine sites for Acer, seven sites for Pineus, ten sites for Quercus, and seven sites for Ulmus. The number of replicate samples per site varied from one to three for each tree species. Bark pH readings were taken using a Hanna HI8424 pH meter equipped with a flat-tip electrode (pHC2441–8 probe from Radiometer analytical, HACH Lange Sensors SAS). Three small pieces of bark per replicate sample were placed into a glass petri dish. A drop (0.05–0.1 ml) of deionized water was placed on one piece of bark at a time and the electrode then placed immediately onto the drop and allowed to rest on the moistened bark. The readings were allowed to stabilize for 1–3 min, then recorded (Spier et al., 2010; Kricke, 2002). The probe was then rinsed with deionized water and dried before being used for the next sample.

Lichen total N concentration was used as a proxy for total N deposition (Rooth et al., 2013). Thalli of H. physodes and/or Parmelia sulcata were taken from three trunks per tree species (Pineus and/or Quercus) per site when it was possible to take a representative sample, i.e., the existing thalli did not look badly bleached or otherwise discoloured and damaged. The thalli were pooled to one sample per lichen species per tree species per site. The air-dried samples were cleaned, ground using a ball mill, and analysed for total N (w/w) using high-temperature combustion (LECO Carbon/Nitrogen Analyzer CN828 with Cornerstone Brand Software) at the Department of Forest Sciences, University of Helsinki. LECO’s EDTA standard (502-896-250) was used in calibration. Detection limit for N was 0.02 %.

2.6. Statistical analyses

The relationships between measured NH3 concentrations and modelled NO2 concentrations in Oct 2019 – Sep 2021 at the 20 sites were tested using Pearson correlation and Regression tests. The air quality results from the roadside sites and non-roadside sites were compared using independent samples t-test, while Mann Whitney U-test was used to investigate differences in lichen variables between the roadside and non-roadside sites. Spearman rank correlation test was used to test the relationships between lichen variables and the environmental variables, i.e., measured NH4 concentrations, modelled NO2 concentrations, calculated NH4-to-NO2 ratios, and bark pH. The total N concentration of H. physodes vs. that of P. salzica on Quercus was compared using Mann-Whitney U-test. Given the hypotheses, 1-tailed significances were used for pair-wise comparisons except for NH4-to-NO2 ratio, tree girth, and total LDV. The analyses were performed using SPSS Version 27.

3. Results

3.1. Measured NH3 concentrations and modelled NO2 concentrations

The monthly NH3 concentration ranged from 0 μg m−3 (site 9) to 3.29 μg m−3 (site 5) (Supplement 2) while the 2-year site mean ± SD ranged from 0.15 ± 0.10 μg NH3 m−3 (site 7) to 1.03 ± 0.38 μg NH3 m−3 (site 11) (Fig. 2). The 2-year mean NH3 concentration was higher at the roadside sites (0.62 ± 0.19 μg m−3) than non-roadside sites (0.40 ± 0.15 μg m−3) (p = 0.009). The highest non-roadside NH3 concentration was measured at site 5 which is surrounded by the (experimental) fields of the Faculty of Agriculture and Forestry, University of Helsinki. The NH3 concentrations showed a seasonal cycle being higher in summer than winter (Supplement 2).

The ranges for the monthly and the 2-year mean NO2 concentrations in Oct 2019–Sep 2021 were 4–33 μg m−3 and 7–19 μg m−3, respectively (Fig. 2). The lowest values were found at site 7 and the highest values at site 11. The 2-year mean NO2 concentration was also higher at the roadside (12.5 ± 4.1 μg m−3) than non-roadside sites (8.4 ± 1.1 μg m−3) (p = 0.006).

The modelled NO2 concentrations and the measured NH3 concentrations from Oct 2019–Sep 2021 correlated positively across the sites (r = 0.811, p < 0.001, n = 20) (Fig. 2). The 2-year mean NH4-to-NO2 ratio did not differ between the roadside and non-roadside sites (0.051 ± 0.010 and 0.046 ± 0.016, respectively). This was because the highest ratio of 0.071 was measured at the non-roadside sites 5 and 8, while the lowest ratio of 0.022 was measured at the non-roadside site 7.

When analysed by tree species, the measured NH3 concentration was higher at the roadside than non-roadside sites for Acer and Quercus. The
modelled NO2 concentration was also higher at the roadside than non-roadside sites for Quercus (Supplement 1).

### 3.2. Bark pH and lichen N concentration

Bark pH ranged from 2.8 (Pinus, site 2) to 6.1 (Ulmus, site 8). The mean bark pH for Pinus was 3.3 followed by that of 4.8, 5.4 and 5.7 for Quercus, Acer and Ulmus, respectively. Only the bark pH of Quercus increased from 4.5 to 5.1 with increasing NH3 concentration ($r_S = 0.802$, $p = 0.005$, $n = 10$) (Fig. 3a), while the bark pH of both Quercus and Pinus increased with increasing NH3-to-NO2 ratio ($r_S = 0.673$, $p = 0.033$, $n = 10$ and $r_S = 0.775$, $p = 0.041$, $n = 7$, respectively) (Fig. 3b). No correlations were found between bark pH and modelled NO2 concentration in any of the four tree species and the bark pH values of individual tree species did not differ between the roadside and non-roadside sites (Supplement 1).

The total N concentration of H. physodes varied from 0.65% (Quercus, site 14) to 2.44% (Pinus, site 5) and that of P. sulcata from 1.27% (Quercus, site 10) to 2.30% (Quercus, site 11). The mean N concentration of P. sulcata was higher than that of H. physodes at the five sites from which we had samples of both lichen species from Quercus ($p = 0.008$). However, H. physodes on Pinus seemed to have a higher total N concentration than P. sulcata on Quercus at the two sites where lichens were sampled from pine and oak (site 5: 2.44% vs. 1.92%, site 16: 2.37% vs. 1.29%, respectively). The total N concentration of P. sulcata on Quercus increased with increasing NH3 concentration ($r_S = 0.810$, $p = 0.015$, $n = 8$) (Fig. 4). Each tree species at the roadside vs. non-roadside sites are presented in Supplement 1.

The oligotrophic H. physodes was by far the most abundant lichen species on Pinus with a mean ± SD of 7.6 ± 2.3, while the most abundant species on Quercus was the eutrophic P. sulcata (13.2 ± 1.6) followed by H. physodes. The eutrophic P. tenella was, in turn, the most abundant species on both Acer (13.8 ± 1.7) and Ulmus (14.5 ± 1.9) followed by P. sulcata and P. orbicularis, respectively.

### 3.3. Lichen community

We found 28 foliose or fruticose macrolichen species (Supplement 1). The total number of species was lowest on Pinus (9) and highest on Acer and Quercus (23). The total LDV across the sites was lowest for Pinus (24.3 ± 8.5) and highest for Quercus (49.1 ± 13.5). The lichen community on Pinus was dominated by oligotrophs and acidophytes at each site yielding a mean LDV of 23.9 and a mean LAN of 1.86, while the lichen communities on Acer, Quercus and Ulmus were dominated by eutrophs. The LAN value of Ulmus was negative at each site resulting in the lowest mean value - 3.25. The total LDV, LDV oligo, LDV eutro and LAN values for each tree species at the roadside vs. non-roadside sites are presented in Supplement 1.

### 3.4. Lichen data in relation to environmental variables

#### 3.4.1. Oligotrophs and eutrophs in relation NH3 and NO2

The total LDV did not correlate with environmental factors, whereas the LDV oligo decreased with increasing NH3 concentration when analysed across deciduous tree species. Moreover, the LDV oligo of Acer and Quercus was lower at the roadside than non-roadside sites (Supplement 1). The impact of NH3 on oligotrophs arose especially from the negative response of H. physodes on Quercus (Table 1, Fig. 5a). Pseudovernia furfuracea was the only oligotroph whose abundance correlated with NO2 concentration – the relationship being negative across deciduous trees.

Although the LDV eutro did not correlate significantly with NH3 concentration, the LDV eutro of Quercus was higher at the roadside than non-roadside sites (Supplement 1). At the lichen species level, the abundances of eutrophic Melanohalea exasperatula and P. tenella on Quercus increased with increasing NH3 concentration (Fig. 5a), while the abundance of P. ascendent on deciduous trees increased with increasing NH3-to-NO2 ratio (Table 1). Moreover, the abundances of M. exasperatula and X. fulva on Quercus were higher at the roadside than non-roadside sites (Supplement 1). In contrast to other eutrophs, the abundance of P. sulcata on deciduous trees decreased with increasing NH3 concentration.

#### 3.4.2. Acidophytes and nitrophytes in relation NH3, NO2 and bark pH

The LAD across deciduous trees became more negative with increasing NH3 concentration. However, at the tree species level, only the LAN of Quercus was related to NH3 concentration (Table 1). At the lichen species level, the abundance of nitrophytic P. tenella increased with increasing NH3 concentration. In contrast to H. physodes, the abundance of Cladonia spp., which was used as one of the acidophytic indicators when calculating the LAN, increased on deciduous trees with increasing NH3 concentration. The LAN of Quercus also became more negative with increasing NO2 concentration, and it was negatively correlated with the bark pH of Quercus and that across deciduous trees (Table 1). At the lichen species level, the abundance of H. physodes decreased with the increasing bark pH of Quercus. In contrast, the abundance of H. physodes on Pinus increased with increasing bark pH (Fig. 5b). The abundances of acidophytic P. ambigua and T. chlorophylla, in turn, correlated negatively with bark pH across deciduous trees, while the abundances of nitrophytic P. orbicularis, P. nigricans, P. tenella, and Xanthoria parietina correlated positively (Table 1).
Table 1: Spearman rank correlation coefficients for the relationships (p < 0.05) between lichen indices or species abundances and environmental variables (measured NH3 concentrations, modelled NO2 concentrations, and calculated ratios of NH3 to NO2 in Oct 2019 – Sep 2021, and bark pH) for Quercus robur and across deciduous trees (Acer platanoides, Quercus robur and Ulmus glabra). Data on Cladonia spp. also presented. LDV_Oligo = Lichen Diversity Value for Oligotrophs, LAN = Lichen Atmospheric Nitrogen index. Significances of correlations: *p-values <0.05, **p-values <0.01, and ***p-values <0.001 indicated as *, **, and ***, respectively.

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3.4.3. Other relationships

The abundance of green algae + S. chlorococcum on Pinus correlated positively with both NH3 concentration and NH3-to-NO2 ratio (rS = 0.999, p < 0.001, n = 7 and rS = 0.800, p = 0.031, n = 7, respectively) as did as that on Quercus and NH3 concentration (rS = 0.661, p = 0.027, n = 11).

The abundance of nitrophilous X. fulva only increased with increasing tree girth when studied across deciduous tree species (rS = 0.430, p = 0.018, n = 30), the impact arising mainly from Quercus (rS = 0.650, p = 0.030, n = 11).

4. Discussion

4.1. Measured NH3 concentrations and modelled NO2 concentrations

To our knowledge, this is the first study to report measured NH3 concentrations and to investigate the impact of traffic-derived NH3 emissions on epiphytic lichens in a northern European urban environment. The NH3 (and NO2) concentrations were higher at the roadside than non-roadside sites despite the facts that the roadside sites varied a lot in terms of traffic densities and that we only had two real roadside vs. non-roadside pairs of sites (Acer sites 4 vs. 3 and Quercus sites 11 vs. 10). The strong positive correlation between NH3 and NO2 concentrations points to motor vehicles as the main source of reactive N forms at the roadside sites.

The 2-year mean NH3 concentration was 35 % higher at the roadside sites than non-roadside sites. The 2-year mean NH3 concentrations especially at the roadside sites were apparently slightly lower than under pre-Covid conditions due to the reduced traffic volumes in March 2020 – Sep 2021, an impact also seen as reduced NO2 concentrations at air quality monitoring stations (Korhonen et al., 2021). Overall, the monthly NH3 concentrations in Helsinki were comparable to, e.g., 0.2–1.7 µg m⁻³ (May–July 2011) across south-central Ontario, Canada (Wattmough et al., 2014), but lower than 1.74–3.62 µg m⁻³ in Cromwell Road 2, London, in 2021 (Stephens et al., 2021), or 1.0–4.3 µg m⁻³ (2002 – 2003) along a 520 m forest transect near a highway south of Munich, Germany (Kirchner et al., 2005).

The seasonal variation in NH3 concentration is a well-known phenomenon in natural, agricultural, and urban areas (e.g., Pryor et al., 2001; Scudlark et al., 2005; Walker et al., 2004). The increase in summertime NH3 concentrations is partly attributed to increased rates of microbial activity and volatilization of NH3 from vegetation and soils during warmer months (Asman et al., 1998). On the other hand, the SCR performance is limited under urban-type driving because the engine temperature is too low for efficient injection of urea (Fu et al., 2013). This effect is considered to be especially applicable during the winter in northern climates, resulting in reduced NH3 emissions from traffic. The highest monthly NH3 concentration measured in Oct 2020 at the non-roadside site 5 may relate, e.g., to volatilization of NH3 from the N saturated ecosystem as no fertilization had taken place or cattle kept in the surrounding fields in the autumn 2020 (Tapani Jokiniemi, personal communication 22nd Nov 2021).

The modelled NO2 concentrations (7–19 µg m⁻³ yr⁻¹) at the sites were low compared to those, e.g., in London (28–92 µg m⁻³ yr⁻¹) (Davies et al., 2007) where marked impacts of NO2 on epiphytic lichens on deciduous trees have been reported (Davies et al., 2007; Gadsdon et al., 2010). The monthly mean NO2 concentrations at roadsides still ranged from 39 to 51 µg m⁻³ and those of NO2 were 27–52 µg m⁻³ in 2018 in London (Greater London Authority, 2022). In Helsinki, the highest measured NO2 concentration was 32 µg m⁻³ yr⁻¹ and that of NO2 was 21 µg m⁻³ yr⁻¹ at the continuous monitoring stations in 2018 (HSY, 2022b). The NO2 concentrations in Helsinki are closer to those measured, e.g., in Munich (24–43 µg m⁻³ yr⁻¹), Germany, where NO2 has also been shown to drive the composition of epiphytic lichens on deciduous trees (Sebald et al., 2022).

The 2-year mean NH3-to-NO2 ratio would have been 0.56 at the non-roadside site 5 if the outlier concentration from Oct 2020 had been left out. The value is still slightly higher than the mean NH3-to-NO2 ratio for roadside sites. Overall, the mean NH3-to-NO2 ratio of 0.048 across the 20 sites was higher than, e.g., that of 0.037 (range 0.026–0.051) calculated across the sites of Gadsdon et al. (2010) in Epping Forest in the north-east of London.

4.2. Impact of NH3 on lichen communities

We hypothesised that the diversity of oligotrophs would decrease and that of eutrophs increase with increasing NH3 concentration, and that the effects would be seen especially at roadsides due to traffic-derived NH3 emissions. Despite the low NH3 concentrations, the LDV_Oligo on deciduous trees decreased with increasing NH3 concentration supporting our hypothesis. Moreover, despite the low number of sites per tree species and the low number trees per species at each site, the LDV_Oligo of Acer and Quercus was lower and the LDV_Euto of Quercus higher at the roadside than non-roadside sites. Our limited data hence suggests that diversity indices such as the LDV_Oligo and the LDV_Euto may indicate NH3-induced changes in lichen communities already at low NH3 levels.
Pinho et al. (2011) considered functional diversity variables such as total LDV as accurate and robust indicators of the \( \text{NH}_3 \) effects on ecosystems around both point and diffuse sources of \( \text{NH}_3 \). Llop et al. (2012) showed, however, that foliose, eutrophic, xerophytic (drought tolerant), and basophilous lichen functional groups respond in a different direction under urban disturbance than do \( N \)-sensitive species. Therefore, the impact of \( \text{NH}_3 \) may be masked when total LDV is used as an indicator. Supporting the results of another study by Pinho et al. (2014), the total LDV in the present study did not correlate with \( \text{NH}_3 \) concentration. This can be attributed to the increase in eutrophs offsetting the decrease in oligotrophs as suggested also by our results.

Classifying species into tree functional groups in terms of their environmental requirements has been successfully used, e.g., by Llop et al. (2012) when they studied the responses of epiphytic lichens to an urban environment without any reference to air quality. However, Pinho et al. (2011) found in their study that the diversity of mesotrophic species (LDV\textsubscript{Mes})

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**Fig. 5.** a) The abundances of oligotrophic *Hypogymnia physodes* and eutrophic *Melanohalea exasperatula* and *Physcia tenella* on *Quercus robur* in relation to mean \( \text{NH}_3 \) concentration and b) the abundance of (oligotrophic) acidophyte *Hypogymnia physodes* in relation to bark pH from Oct 2019 – Sep 2021 in Helsinki, Finland. (The trend lines and \( R^2 \) values derived from Excel for illustrative purposes).
grouped according to the eutrophication tolerance index of Nimis and Martellos (2008) responded to NH₃ in a similar way as oligotrophs. The NH₃ concentration ranged from 1.5 to 16.2 μg m⁻³ in the vicinity of an agricultural point source in a cork oak Mediterranean woodland (Pinho et al., 2011). We only classified lichen species as oligotrophs or eutrophs in terms of their nutrient requirements (eutrophication) and deduce that omitting mesotrophs probably does not have a major impact on the interpretation of our results.

In the present study, the Lₐₓ showed a decreased presence of acidophytes in relation to nitrophytes on Quercus as response to elevated deposition of both reduced and oxidized forms of reactive N. Hence, all recent studies have that of its contribution to the LAN with increasing bark pH. The impact arose especially from the response of the dominant acidophyte H. physodes. Overall, the acidophytic lichen species and genera used to calculate the Lₐₓ in the present study have a pH indicator value ≤ 5.2 (class 1–4), while the nitrophytes have that of ≥ 5.3 (class 6–9) according to the classification of Wirth (2010). The abundances of some nitrophytes used to calculate the Lₐₓ increased with increasing bark pH across deciduous trees, i.e., the species were most abundant of Ulmus, which had the highest bark pH, while the abundances of other species were positively correlated with NH₃ concentrations or NH₃-to-NO₂ ratios. Given that bark pH values did not differ between roadside and non-roadside sites, the observed community level responses, especially at the roadside sites, are mainly attributed to direct impact of NH₃ (and NO₂). Below we discuss the responses of dominant lichen species and on those of species which showed clear responses to environmental variables.

4.3. Responses of lichen species - oligotrophic acidophytes

_Hypogymnia physodes_ was the most abundant oligotrophic acidophyte on _Pirus_ and _Quercus_ at both the roadside and non-roadside sites. The negative effects of NH₃ were clearly observable in the decreased abundance of _H. physodes_ on the acidic bark of _Quercus_. De Balcker (1989) did not find any _H. physodes_ on roadside _Quercus_ in an agricultural area and attributed its loss to NH₃. Since the 1980s, _H. physodes_ has further declined in the Netherlands despite reductions in atmospheric NH₃ concentrations (Aptroot and van Herk, 2007; Sparrius, 2007). Although this may partly be due to warming climate (Aptroot and van Herk, 2007), _H. physodes_ has been ranked strongly acidophilous and strictly nitrophilous (Barkman, 1958), and sensitive to NH₃ but not to NO₂ (van Dobben and ter Braak, 1999; Gombert et al., 2006; Davies et al., 2007). The observed decrease in the abundance of _H. physodes_ on _Quercus_ is hence mainly attributed to NH₃.

Our total N concentration results from _H. physodes_ as well as those of Sächtig (1991), cited by Sächtig, 1995) and Manninen (2018) suggest high uptake of NH₃ and NH₃⁺ by the species especially when growing on evergreen conifers with a high leaf-area index. This can lead to accumulation of toxic levels of NH₃⁺. The high sensitivity of _H. physodes_ to urban N deposition has also been attributed to formation of ammonium nitrate (NH₄NO₃) on lichen surfaces when dry deposited N is dissolved by dew or rain. Ammonium nitrate is a salt and causes osmotic problems for the uptake of water through their surface in acidophytes but not in more drought tolerant nitrophytes (Frahm, 2013). Supporting this, lichen communities have been found to be more eutrophic at moisture-stressed sites e.g., in forested mountains of the Pacific Northwest, USA (Root et al., 2015), or in cities with an urban heat island (Munzi et al., 2014). It may be noted that calcium chloride (CaCl₂) is used to de-ice roads in Helsinki. However, the impact of salt spray on lichens is considered minor given that Armstrong (1990) did not find CaCl₂ to reduce the growth of acidophytic _Parmelia saxatilis_ and neither of that of nitrophilous _X. parietina_.

Llop et al. (2012) concluded that oligotrophic, hygrophytic (i.e., species which require abundant moisture), acidophilic, and crustose and frusticose lichen functional groups are the lichens most sensitive to urban disturbance in the same direction. Supporting this, we did not find at any of our sites, e.g., _Bryoria fuscescens_, which has also been rapidly disappearing in Europe (Sparrius, 2007). Supporting our results, Pinho et al. (2014) reported fewer N sensitive lichen species on Quercus suber at <1 μg NH₃ m⁻³. Moreover, the correlation patterns observed by van Herk et al. (2003) strongly suggested that NH₃⁻N in precipitation decreased the probability of occurrence of acidophytes such as _B. fuscescens_ and _U. hirta_ even at 0.3 mg l⁻¹. However, van Herk et al. (2003) could not rule out an additional role of NO₃⁻ in precipitation especially in the case of _U. hirta_. We found only one _U. hirta_ thalli (on _Quercus_) at the non-road sites 14 and 16.

The pH of bark also strongly contributed to the decreased diversity of oligotrops on deciduous trees. For example, Larsen Vilsbolom et al. (2009) reported loss of nitrophobes from _Quercus_ taking place at pH 4.8 on twigs, while the frequency of nitrophytes started to increase above pH 5. In our study area, the abundance of _H. physodes_ on deciduous tree trunks seemed to peak at pH ≤ 4.5. The pH requirement of _H. physodes_ is 4.1–4.8 (class 3) according to Wirth (2010). The bark pH of _Quercus_ at our sites was somewhat lower and that of _Ulmus_ higher than reported, e.g., by Spier et al. (2010) for urban trees.

_Cladosonia_ species are ranked as sensitive to eutrophication (Wirth, 2010). The increase in the abundance of _Cladosonia_ spp. on deciduous trees with increasing NH₃ concentration in the current study may be explained by the existence of _Cladosonia_ species with higher N tolerance. For example, _C. chlorophyta_ has been classified as nitrophyte by Geiser et al. (2010) and Matos et al. (2017).

4.4. Responses of lichen species – eutrophs (nitrophytes)

The most NH₃ tolerant eutrophs/nitrophytes on deciduous trees appeared to be _M. exasperatula_, _P. aipolia_, _P. tenella_, _X. fulva_ and _X. polyarpa_ based on their increasing abundances with increasing NH₃ concentration, NH₃-to-NO₂ ratio and/or higher abundances on _Quercus_ at the roadside than non-roadside sites. Davies et al. (2007) ranked _M. exasperatula_ as highly sensitive to NO₂ (9 in a scale of 1–10), but its abundance did not correlate with NO₂ concentrations in our study area. The result may at least partly be explained by the high NO₂ and NO₃ levels in London (Davies et al., 2007) vs. those in Helsinki. It is notable that the abundance of _M. exasperatula_ was not related to bark pH as was that of, e.g., _P. orbicularis_ which has been shown to have a high N tolerance (Frahm, 2013). Compared to the bark pH of _Quercus_ (4.1–5.1) at urban NO₂-dominated sites in London (Larsen et al., 2007), both the bark minimum and maximum pH values (4.5 and 5.3, respectively) were slightly higher in Helsinki.

_Parmelia saxatilis_ was the most abundant species on _Quercus_ at the roadside sites. The species has been classified both as an eutroph (Geiser et al., 2010; Wirth, 2010) and a mesotroph (i.e., neutrophyte having a moderate N requirement) (Sparrius, 2007; Nimis and Martellos, 2022), and as being indifferent to NO₃ (Gombert et al., 2006). Our results suggested _P. saxatilis_ as being negatively affected by NH₃ and/or bark pH when growing on _Acer_ and across deciduous trees. In comparison, Larsen et al. (2007) reported a decrease in the abundance of _P. saxatilis_ with increasing bark pH on _Quercus_. A direct toxic effect of NH₃ is suggested by the increase in the total N concentration of _P. saxatilis_ on _Quercus_ with increasing NH₃ concentration. The total N concentration of _P. saxatilis_ has been shown to increase linearly with an increasing ratio of NH₃⁻N to NO₂⁻N in both wet and total deposition (Boltersdorf et al., 2014). Overall, differences in the responses of
macrolichens to NH₃ are partly attributed to species-specific uptake rates of NH₄⁺ vs. NO₃⁻ vs. organic N (amino acids) (Dahlman et al., 2004).

4.5. Critical levels

Pinho et al. (2014) recommended a critical level of 0.69 μg NH₃ m⁻³ yr⁻¹ based on epiphytic lichen diversity on Quercus in Mediterranean evergreen woodlands. The range for NH₃ concentration in the area was from 0.15 to 5.12 μg m⁻³. Frati et al. (2006), in turn, did not find 0.5–1.0 μg NH₃ m⁻³ yr⁻¹ (together with 19 μg NO₂ m⁻³ yr⁻¹) to affect the diversity of epiphytic lichens on Q. pubescens across a bark pH range of 5.5–6.8. We observed an approx. 50% decrease in the abundance of H. physodes on Quercus at >0.5 μg NH₃ m⁻³ month⁻¹, with a corresponding bark pH of 4.8, compared with sites <0.5 μg NH₃ m⁻³ month⁻¹. The abundances of nitrophytes M. exasperata and P. tenella, in turn, showed a clear increase already at 0.5 μg NH₃ m⁻³ month⁻¹ at some Quercus sites. Although the NH₃ concentrations from March 2020 onward especially at the roadside sites were apparently lower than the pre-Covid concentrations, a critical level of 0.5 μg NH₃ m⁻³ yr⁻¹ is suggested to protect the oligotrophic/acidophytic lichen communities better than that of 1 μg NH₃ m⁻³ yr⁻¹ (Cape et al., 2009), especially if elevated levels of NOₓ and spikes in NH₃ concentrations occur simultaneously.

Frati et al. (2008) concluded that Pinus species, given their acidic bark, are good indicators for the impacts of NH₃-related increases in bark pH on the occurrence of nitrophytes based on street-side pines (P. pinea) in Italy. The bark pH of P. pinea ranged 3.3–7.7 (mean 5.6) at the Italian sites (Frati et al., 2008) and was clearly higher than that of P. sylvestris at our sites. The average NH₃ concentration at our Pinus sites (0.15–0.57 μg m⁻³ month⁻¹) was clearly too low to promote the occurrence of nitrophytes resulting from NH₃-induced increases in bark pH. Negative correlations between bark pH and the occurrence of acidophytes on P. sylvestris in Helsinki were not found in the earlier study, but the frequencies of P. ambigua, Parmeliopsis hyperopta & Imaugia aleuticae, and P. glauca decreased with increasing NH₃-N concentration (range 16–110 μg g⁻¹) in pine bark (Manninen, 2018). Overall, we recommend using Q. robur which also has an acidic bark, for detecting the indirect NH₃-related pH effects on both acidophytes and nitrophytes in areas with relatively low levels of NH₃.

Watmough et al. (2014) studied the impact of NOₓ and NH₃ emissions from highway traffic on epiphytic lichen richness in forest monitoring plots in south-central Ontario, Canada, where the mean springtime concentrations were 1.3–27 μg NO₂ m⁻³ and 0.2–1.7 μg NH₃ m⁻³. They found only 10 foliose macrolichen species on Acer and only one species (Phaeophyscia rubropulchra) was found at sites with >20 μg NO₂ m⁻³ yr⁻¹ or >1.4 μg NH₃ m⁻³ yr⁻¹. We, in turn, found P. ambigua only on Pinus and/or Quercus at sites with ≤10–11 μg NO₂ m⁻³ yr⁻¹ together with ≤0.57 μg NH₃ m⁻³. Supporting the current results and those of Manninen (2018) regarding epiphytic lichens on P. sylvestris (Vandinther 2019) showed the dry deposition of NO and that of NO₂ as being strong drivers of lichen community structure in boreal Jack pine (Pinus banksiana) forests in northwestern Canada. Given our results, the new, human-health based guideline value of 10 μg NO₂ m⁻³ yr⁻¹ recommended by the World Health Organisation (WHO, 2021), and the conclusions by Greaver et al. (2023), we suggest that the critical level of NO₂ for vegetation, especially epiphytic lichens, should be decreased.

The greater accumulation of N in H. physodes on Pinus vs. P. sylvestris on Quercus at the same sites is attributed to the higher total deposition of inorganic N under evergreen Pinus canopy vs. that under the deciduous canopy of Quercus given the higher surface area of pine needles relative to that of oak leaves (Fenn and Bytnerowicz, 1997; Jovan et al., 2012). The highest total N concentration of P. sylvestris on Quercus was found at the roadside site 11 along with the highest mean concentrations of NH₃ and NO₂. The highest total N concentrations of H. physodes on Pinus were, in turn, found at the non-roadside sites 16 and 5 with mean concentrations of NH₃ and NO₂ about half of those at site 11. Emissions from coal and wood burning power plants and shipping also contribute to the total N deposition in the area. To put the results into a wider spatial scale, it is noted that the lowest total N concentration in H. physodes on Pinus in Helsinki was about two-fold and the highest about five-fold greater than that of 0.45% and 0.53% at the remote background sites of the UNECE Integrated Monitoring network in eastern and northern Finland, respectively, in August 2019 (Supplement 3).

The dispersal capacity of species which are sensitive to acid deposition seems to be poor (Weldon and Grandin, 2021). Moreover, both P. ambigua and Hypogymnia spp. reproduce mainly by soredia (Nimis and Martellos, 2022) and may hence be highly vulnerable in their juvenile stages as their soredia do not easily colonize trunks with established communities of (nitrrophic) crustose and foliose lichens (Mayer et al., 2013). The regional species pool was clearly impoverished by historical acid deposition and is today affected by concentrations of reactive N forms that exceed critical levels for the most sensitive acidophytes. In other words, the total N deposition exceeds the critical load which may initiate a shift from pollution-sensitive to pollution-resistant lichen species at levels as low as 1.5 kg N ha⁻¹ yr⁻¹ (Geiser et al., 2021). There is no N deposition data for the area but based on the total N concentration of H. physodes the total (throughfall) deposition at the most polluted sites is over five-fold greater compared to that of ≤4 kg N ha⁻¹ yr⁻¹ in the background areas in eastern and northern Finland (Marchetto et al., 2021).

Gadsdon et al. (2010) suggested that the proportion of lichen cover comprising nitrophytes (on Quercus) is a useful index where NO₂ dominates over NH₃ such as London. In our study area, a clear dominance of NO₂ did not exist at the most urban sites. Our results suggest marked direct and indirect impacts from low NH₃ concentrations alone and in combination with low NO₂ concentrations on multiple lichen variables. In Helsinki, LDV_Oligo_LAN, and the abundance of H. physodes showed similar patterns across each tree species at roadside vs. non-roadside sites (Supplement 1). However, we cannot attribute all the NH₃-related impacts to the emissions from road traffic because the highest bark pH of Ulmus and that of both Quercus and Pinus were seen at the non-roadside sites 8 and 5, respectively, with the highest mean NH₃-to-NO₂ ratio. Given this, including the ratio of NH₃ to NO₂ to analyses is recommended when studying the impact of oxidized and reduced N compounds on epiphytic lichens in areas where multiple forms of atmospheric N compounds co-occur.

5. Conclusions

The concentrations of gaseous air pollutants are low in our study area making it possible to detect the impact of small increases above background concentrations and current critical levels on the most sensitive biota such as epiphytic lichens. We found NH₃-related changes in the presence and abundance of oligotrophic acidophytes and eutrophs/nitrophytes despite the fact, that we only had (for practical reasons) three individuals per tree species at each site, which often resulted in large within-site variation in lichen variables. However, the responses of lichen species to reactive N forms and changes in bark pH vary, and results based on the responses of lichen functional groups may hence vary depending on the species included in the calculated indices. At any rate, both P. sylvestris and Q. robur support acidophytic lichen communities under low NH₃ and NO₂ pollution and can be used in biodiagnostic studies of N pollution. Despite the small number of sites per tree species, the present results suggest that the critical levels and/or loads for epiphytic lichens on conifers might be lower than those for epiphytic lichens on deciduous trees due to the higher throughfall deposition of N under conifer canopies. This needs to be furthered to assess the critical levels of NH₃ and NO₂ for epiphytic lichens under boreal climate with low precipitation that may lead to high concentrations of NH₄NO₃ when the dry-deposited NH₃, NO₂, and N in PM₂.5 are dissolved in rain or dew.

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CRediT authorship contribution statement

Sirkku Manninen: Conceptualization, Methodology, Validation, Investigation, Formal analysis, Data curation Writing – Original draft preparation, Funding acquisition.

Data availability

Data will be made available on request.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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