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NO_2 air pollution drives species composition, but tree traits drive species diversity of urban epiphytic lichen communities☆

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A B S T R A C T

Lichens serve as important bioindicators of air pollution in cities. Here, we studied the diversity of epiphytic lichens in the urban area of Munich, Bavaria, southern Germany, to determine which factors influence species composition and diversity. Lichen diversity was quantified in altogether 18 plots and within each, five deciduous trees were investigated belonging to on average three tree species (range 1–5). Of the 18 plots, two were sampled in control areas in remote areas of southern Germany. For each lichen species, frequency of occurrence was determined in 10 quadrats of 100 cm^2 on the tree trunk. Moreover, the cover percentage of bryophytes was determined and used as a variable to represent potential biotic competition. We related our diversity data (species richness, Shannon index, evenness, abundance) to various environmental variables including tree traits, i.e. bark pH levels and species affiliation and air pollution data, i.e. NO_2 and SO_2 concentrations measured in the study plots. The SO_2 levels measured in our study were generally very low, while NO_2 levels were rather high in some plots. We found that the species composition of the epiphytic lichen communities was driven mainly by NO_2 pollution levels and all of the most common species in our study were nitrophilous lichens. Low NO_2 but high SO_2 values were associated with high lichen evenness. Tree-level lichen diversity and abundance were mainly determined by tree traits, not air pollution. These results confirm that ongoing NO_2 air pollution within cities is a major threat to lichen diversity, with non-nitrophilous lichens likely experiencing the greatest risk of local extinctions in urban areas in the future. Our study moreover highlights the importance of large urban green spaces for species diversity. City planners need to include large green spaces when designing urban areas, both to improve biodiversity and to promote human health and wellbeing.

1. Introduction

Air pollution has detrimental negative effects on human health (Chambliss et al., 2014; Hoek et al., 2013; Lelieveld et al., 2015; Shindell et al., 2011; Silva et al., 2016), crop production (Lapina et al., 2016; Shindell et al., 2011), ecosystems (Liu et al., 2013) and biodiversity (Clark and Tilman, 2008; Matson et al., 2002). In Europe, different kinds of outdoor air pollution are particularly relevant for public health: gaseous pollutants including SO_2 and NO_x and particulate air pollutants (Crippa et al., 2016).

Toxic nitrogen oxides (NO_x) are synthesized during high temperature combustion from fuel-bound nitrogen. During combustion, first nitrogen monoxide (NO) is formed, which is subsequently oxidised by ozone to NO_2. This process takes place partly within plant canopies and is often accompanied by substantial NO_2 depositions to plant and soil surfaces (Butterbach-Bahl et al., 2011). The major source of NO_2 is the combustion of fossil fuels by power plants and transportation devices like airplanes and cars (Baumbach, 1994; Butterbach-Bahl et al., 2011). After a peak in the 1980s, NO_2 concentrations in Germany have decreased and, while still high in urban areas, have largely remained

Abbreviations: Nitrogen dioxide (NO_2), sulphur dioxide (SO_2); Detrended Correspondence Analysis (DCA), Principal Component (PC); Principal Component Analysis (PCA), Tukey's Honestly Significant Difference (HSD) test.

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below the NO_x-ceiling since 2011 (https://www.eea.europa.eu/themes/air/country-fact-sheets/2021-country-fact-sheets/germany, date accessed: June 10, 2022) (Melkonyan and Kuttler, 2012; Vorbeck and Windisch, 2001).

Another source of air pollution is sulphur dioxide (SO_2). This pollutant results from combustion processes utilizing sulphurous fuels, such as mineral oils or coal. Exhaust from diesel engines is a major SO_2 source in cities (Vorbeck and Windisch, 2001). By limiting sulphurous fuels in "first world" countries, global SO_2 pollution has decreased over the last 20 years (Baumbach, 1994), whereas nitrogen dioxide pollution has remained constant. This has caused increases in the abundance of nitrophilous species (Gadsdon et al., 2010).

Lichens are terrestrial organisms arising from a symbiosis between a filamentous fungus, the mycobiont, and green algal or cyanobacterial photobionts as well as additional bacterial and fungal species (Grube et al., 2009; Spribille et al., 2016). Many lichens are highly sensitive to environmental conditions yet still have evolved to grow in extreme environments, such as Arctic tundra landscapes (Thomson, 1984), the Antarctic (Castello and Nimis, 1997), deserts, and oligotrophic grassland ecosystems where competition from higher plants is low (Boch et al., 2016). Lichens can only tolerate a narrow environmental spectrum, e.g., with respect to light and humidity (Wirth et al., 2013). They are also sensitive to the physical and chemical conditions of the substrate, e.g., the pH-value of tree bark. Many lichen species also react very sensitively to air pollution (Hawksworth and Rose, 1970; Hawksworth et al., 1973; Munzi et al., 2010; van Herk et al., 2003; Vorbeck and Windisch, 2001). Pollutants can easily infiltrate into a lichen's hyphal network and they accumulate in lichen thalli unless they get washed out by rain. As the metabolism of lichens remains activated at low temperatures just above the freezing point (Dickson, 2000) or even below (Ahmadjian, 1970), pollutants continue to have an impact on lichens in winter, a time when the highest amounts of pollutants are emitted e.g. from heating systems of private households that involve combustion of fossil fuels (Vorbeck and Windisch, 2001). Due to their high sensitivity, epiphytic lichens are excellent biological indicators of air pollution (Chaparro et al., 2013; Hauck, 2010; Hawksworth and Rose, 1970; Sujetovienė, 2015).

In the last 150 years, there has been a dramatic loss of epiphytic lichens in Germany and one of the most important factors responsible for this decline is air pollution by acidifying and fertilizing substances (Hauck, 2010). Air pollution by SO_2 and NO_2 peaked in the 1970s, but SO_2 levels declined subsequently due to technical innovations, which allowed lichen species to recolonize lichen deserts in city centers (Lackovičová et al., 2013; Paoli et al., 2021). Air pollution however doesn't only affect cities: High nitrogen levels in ecosystems cause nitrophilous species to replace other epiphytic lichen communities (Hauck, 2010) by competitive advantage. van Herk (2001) reported large effects of nitrogen pollution (ammonia) on epiphytic lichens growing on *Quercus robur* in the Netherlands, causing nitrophilous species to reach high abundances and acidophilic species to disappear in the most polluted sites. Also, the nitrophilous lichen species were less affected by SO_2 pollution than other lichens species. Studies of epiphytic foliose lichens in Ontario found a decline of lichen richness with increasing nitrogen pollution (McDonough and Watmough, 2015; Watmough et al., 2014). Similarly, a study that sampled lichens in *Quercus robur* canopies in the UK showed that NO_2 concentrations are negatively correlated with total lichen cover (Gadsdon et al., 2010). Nitrogen pollution has a direct negative influence on lichen physiology, influencing nitrophobic species more than nitrogen tolerant species (Munzi et al., 2014).

The species composition and diversity of epiphytic lichen communities is not only influenced by air pollution effects but also by the habitat a lichen is growing in, by characteristics of the trees they grow on, i.e bark properties like waterholding capacity and bark pH (Kubiak and Osyczka, 2020), the tree species (Bäcklund et al., 2016), tree size and tree diameter (Johansson et al., 2007; Johansson et al., 2009). In a direct comparison, habitat type (open vs forested) was more important for the species diversity of epiphytic lichens than tree species (Kubiak and Osyczka, 2020), but it is not clear how air pollution effects factor into this equation. Here, we study the diversity of epiphytic lichens in an urban environment in order to assess the relative importance of air pollution concentrations vs. tree characteristics on lichen diversity. Specifically, the goals of this study were to:

1) Identify the main drivers of lichen diversity at the level of individual trees and at the plot-level;
2) Characterize the strength and direction of NO_2 and SO_2 air pollution effects on lichen diversity;
3) Predict epiphytic lichen diversity by integrating information on air pollution and tree traits (e.g., trunk diameter).

2. Material and methods

2.1. Study area

Munich, the capital of Bavaria, is located in the south of Germany. With an area of 310.71 km^2 and almost 1.5 million inhabitants, Munich is the third largest and most densely populated municipality of Germany; the metropolitan region of Munich is inhabited by 6.12 million people and has a share of 78.9% of housing and 16.9% of traffic area (https://en.wikipedia.org/wiki/Munich, date accessed: October 21, 2021). In the city of Munich, pollution from combustion of fossil fuels is most important, especially road traffic and burning of fossil fuels for heating purposes.

The city lies on the northern edge of the Alpine foothills and is located at 519 m above sea level. The climate of Munich is temperate oceanic to continental with mild summers (Köppen climate classification Cfb-Dfb) (Belda et al., 2014). Winters are cool, with monthly average temperatures between 0 and 5 °C and summer temperatures are moderate to warm (15–20 °C). With a precipitation ranging from 50 to 110 mm, all months are humid, amounting annually to 1000 mm (Belda et al., 2014). The mean annual temperature is 8.8 °C (https://de.climate-data.org, date accessed: October 19, 2021).

2.2. Sampling and identification of lichens and of environmental parameters

Between October 2018 and April 2019, 16 locations (plots) were sampled in Munich. The study sites included parks and green spaces within the city, with some sites being very close to heavy road traffic (e.g. Landsberger Allee) or hubs for railway (e.g. Stachus). In each location, we sampled lichens on five trees, and in all but two locations they were sampled on at least three different tree species. Locations were chosen based on a traffic-related air quality map (Figure 6 in Vorbeck and Windisch, 2001) to maximize variation in air pollution across sites. Thus, we chose five locations within Munich with presumed high air pollution, five with moderate air pollution, and six with low air pollution (Table 1). In addition, we took samples from a site close to the nature reserve "Hemhofer-Eggstätter Seenplatte" in Chiemgau (approx. 60 km southeast of Munich) and a second site located within the nature reserve "Allgäuer Hochalpen" in Hinterstein valley in Allgovia (approx. 100 km southwest of Munich) as controls with minimal air pollution and comparatively low human impact, mainly in the form of forestry and alpine cattle farming, for which pastures are created in the area.

The eight tree species included in our study were chosen from a list in the Verein Deutscher Ingenieure (VDI) guideline 3799 (Verein Deutscher Ingenieure, 1995). Only trees with a minimum diameter of 0.6 m and a minimum height of 4 m were included, as trees of roughly the same age should provide improved comparability. Moreover, skewed, damaged and dead trees were omitted. To be included, trees had to be free-standing, i.e. no trees situated within closed canopy were sampled. An overview over the tree species sampled is given in the Supplement, Table S1. Altogether 90 trees were investigated. Tree

Table 1
Overview of the 18 studied sites (plots), showing locality, geographic coordinates, air pollution levels according to extrapolated air quality data from Vorbeck and Windisch (2001) and NO_2 and SO_2 values measured in 2018 or 2019 as reported in the column "Year". Given is the average, with measured values in parentheses. Also shown are the number of trees sampled with neutral (Neu.), moderately acidic (Mod.) and acidic bark (Aci.), respectively. Plots were sorted by air pollution level. For further information on the sampled trees, see Table S1.

Sampling locality	ID	Latitude	Longitude	Pollution	NO_2		SO_2		Year	Plot	Neu.	Mod.	Aci.
					µg/m^3	ppb	µg/m^3	ppb					
Allgovia Giebelhaus	AG	47.42168	10.41205	very low	1.71 (1.71; 1.71)	0.89 (0.89; 0.89)	3.35 (3.35; 3.35)	1.26 (1.26; 1.26)	2018	10	0	5	0
Chiemgau	CH	47.92237	12.33544	very low	3.55	1.84	2.49	0.93	2019	9	1	2	2
Botanical Gardens	BG	48.16441	11.49825	low	2.21 (1.88; 2.53)	1.15 (0.98; 1.31)	3.15 (3.15; 3.15)	1.18 (1.18; 1.18)	2018	11	3	2	0
Pasing	PA	48.15094	11.46147	low	5.35 (4.80; 5.89)	2.78 (2.49; 3.06)	3.29 (3.29; 3.29)	1.23 (1.23; 1.23)	2018	15	1	4	0
Sendlinger Park	SP	48.10630	11.54080	low	8.38 (5.90; 10.86)	4.36 (3.07; 5.65)	2.99 (2.97; 3.00)	1.12 (1.11; 1.13)	2019	2	3	2	0
Müllberg	MB	48.21281	11.62874	low	8.95 (1.50; 16.40)	4.66 (0.78; 8.53)	3.10 (2.85; 3.35)	1.17 (1.07; 1.26)	2019	8	1	2	2
Denninger Straße	DS	48.14930	11.62392	low	9.60 (3.65; 15.57)	5.00 (1.90; 8.10)	3.18 (3.00; 3.35)	1.20 (1.13; 1.26)	2019	6	3	2	0
Ostfriedhof	OF	48.12022	11.58589	low	10.05 (4.26; 15.85)	5.24 (2.22; 8.24)	2.99 (2.97; 3.00)	1.12 (1.11; 1.12)	2019	4	1	1	3
Tierpark	TP	48.10035	11.54772	moderate	13.66 (11.78; 15.54)	7.11 (6.13; 8.08)	3.15 (3.15; 3.15)	1.18 (1.18; 1.18)	2018	12	2	3	0
Theresienwiese	TW	48.12700	11.54708	moderate	14.10 (6.15; 22.04)	7.33 (3.20; 11.46)	2.91 (2.84; 2.97)	1.09 (1.06; 1.11)	2019	1	2	3	0
Lothstraße	LS	48.15370	11.55440	moderate	14.51 (1.82; 27.19)	7.55 (0.95; 14.14)	3.56 (3.56; 3.56)	1.34 (1.34; 1.34)	2018	14	2	3	0
Stachus	ST	48.14239	11.56419	moderate	14.96 (11.38; 18.54)	7.78 (5.92; 11.38)	3.33 (3.33; 3.33)	1.25 (1.25; 1.25)	2018	18	2	3	0
Freimann	FR	48.19353	11.61451	moderate	14.38 (6.76; 22.00)	7.48 (3.51; 11.44)	3.57 (3.57; 3.57)	1.34 (1.34; 1.34)	2018	17	1	4	0
Olympiapark	OP	48.17150	11.54700	high	17.80 (15.54; 20.05)	9.26 (8.08; 10.43)	3.57 (3.57; 3.57)	1.34 (1.34; 1.34)	2018	16	1	4	0
Riemer Straße	RS	48.13987	11.65289	high	18.57 (13.52; 23.62)	9.66 (7.03; 12.28)	3.08 (2.81; 3.34)	1.15 (1.05; 1.25)	2019	7	3	2	0
Tierheim	TH	48.14010	11.66629	high	20.23 (11.85; 28.61)	10.52 (6.16; 14.88)	3.08 (2.81; 3.34)	1.15 (1.05; 1.25)	2019	3	1	3	1
Effner Platz	EP	48.15160	11.61660	high	23.29 (7.14; 39.43)	12.11 (3.71; 20.50)	3.18 (3.00; 3.35)	1.20 (1.13)	2019	5	2	3	0
Landshuter Allee	LA	48.15592	11.53686	high	42.87 (39.27; 46.46)	22.30 (8.08; 24.16)	3.32 (3.32; 3.32)	1.25 (1.25; 1.25)	2018	13	2	3	0

species included four species with neutral bark: *Acer platanoides* (13 trees investigated), *Fraxinus excelsior* (14), *Juglans regia* (3), *Populus* sp. (2), two species with moderately acidic bark: *Acer pseudoplatanus* (21), and *Tilia* sp. (29) and two species with acidic bark: *Prunus avium* (2), and *Quercus robur* (6). For each tree, the following data were recorded in the field: tree circumference, measured at 1 m height with a measuring tape (later converted to tree diameter assuming a round trunk shape), tree species, bark pH class, the cover of bryophyte competitors (i.e. moss cover) and geographic coordinates obtained from the 'Latitude Longitude' Android application (version 1.34, https://play.google.com/store/apps/details?id=com.mylocation.latitudelongitude&hl=en_US&gl=US), run on a mobile phone.

Vorbeck and Windisch (2001) provide information on bark pH classes for tree species in the area of Munich. These bark pH classes were assigned to each tree investigated and included into our model as a variable on a rank scale, ranging from 1 (acidic, i.e. pH < 4) to 3 (neutral, i.e. pH > 5.5); to be more intuitive, the scale was inverted relative to Vorbeck and Windisch (2001) so that the lowest value would represent the most acidic pH class. For the plot-level analyses, the pH class values of each of the five trees per plot were averaged.

To record the species diversity and abundance of lichens and the moss cover on the tree stems, we used a sampling grid that contained ten 0.1×0.1 m squares, arranged in 2 columns and 5 rows. The grid was placed on the north side of the tree, extending upwards from a height of 1 m above ground. For each grid, the percentage coverage value of bryophytes was recorded for each square of the grid and an average coverage percentage was calculated to provide information about the abundance of competing epiphytic bryophyte communities on each tree. For the plot-level analyses, the moss cover values were averaged across the five trees.

Subsequently, we sampled all lichen species and recorded their frequency (range: 0–10) within each grid. For instance, if a lichen species was found in 5 of 10 squares in the grid, a frequency of 5 was scored for the respective tree. Additionally, lichen species were scored with a frequency of one if they grew somewhere on the lower 2 m of the tree trunk outside the sampling grid to account for those species with very low frequency that were not captured by the grid by chance. To obtain tree-level lichen abundance values, the frequency values determined for all species on a given tree were summed up. To obtain plot-level lichen abundance values, the tree-level abundances were summed up for the five trees of the plot.

Lichen species were identified with routine methods including microscopy, spot-tests with chemicals according to Wirth et al. (2013). If necessary, thin-layer chromatography was performed (Culberson and Kristinsson, 1970). Some specimens could only be identified to genus level. Species lists were compared with the lichen mapping report of Munich and all species that had not been reported previously by Vorbeck and Windisch (2001) were compared with relevant herbarium specimens of the Bavarian State Collection (M). Lichen species were classified as acidophilic if they had reaction indicator values of 1–3 (i.e., extremely to quite acidic) in Wirth's (2010) list of indicator values for Central European lichens. Nitrophobic lichens had eutrophication indicator values from 1 to 3 (i.e., no to weak eutrophication), while nitrophilic lichens had values from 7 to 9 (i.e., quite strong to very strong eutrophication) according to Wirth (2010). These classifications are provided in the Supplement, Table S2 along with the frequency data.

To quantify air pollution by NO_2 and SO_2, at each site, we set up two DIF 500 RTU-RA – Combined nitrogen dioxide (NO_2) and sulphur dioxide (SO_2) diffusion tubes (Gradko International Limited, Winchester, UK). The diffusion tubes were set up at a height of ca. 1.5 m for two to three weeks in September 2018 and April/May 2019 and after exposure, they were analyzed with Ion Chromatography by Gradko. For comparability among the pollution measurements of each year, we measured in autumn in one year and in the following late spring.

2.3. Data analyses

Tree species, tree diameter, moss cover, bark pH, NO_2 and SO_2 concentrations served as predictor variables in statistical models. To investigate the relative effects of air pollution and tree characteristics on lichen diversity, four indices were used: lichen species richness, Shannon index, evenness, and lichen abundance. These indices were the response variables in general linear mixed effects models and in univariate linear models (see below). The Shannon index accounted for both species richness and abundance to describe diversity. Species richness was defined as the number of species found per tree or per plot. The index by Heip et al. (1998) was used to quantify evenness, i.e., how equal species are in terms of their number of individuals. All indices were calculated on the individual tree level and the plot level using the R package 'vegan' (Oksanen et al., 2016). Tree-level lichen abundance was defined as the summed frequency of a given species within the sampled grid (range: 0–10). For the plot-level analyses, species abundances of the five trees sampled were summed up to obtain a plot-level estimate of lichen abundance.

Prior to constructing the statistical models, the predictor variables were standardized by subtracting their mean and dividing by 2 standard deviations to examine relative, comparable effect sizes (Gelman and Hill, 2007). The NO_2 data were $\log_{10}$ transformed to achieve normal distribution prior to standardization. Three types of models were utilized. First, multivariate linear models were used to explore how accurately the combined predictor variables predicted lichen diversity. Second, univariate models were used to understand the effects of SO_2 and NO_2 concentration on lichen diversity. Third, general linear mixed effects models were constructed to quantify relative effect sizes, i.e. the relative importance of the different predictors for explaining variation in the lichen diversity indices. In the general linear mixed effects models, we used tree species as random effect and moss cover, bark pH, NO_2 and SO_2 concentrations, and tree diameter as fixed effects.

To study the effect of the environmental variables on species composition, Detrended Correspondence Analysis (DCA) was conducted using the 'decorana' function in the R package 'vegan' (Oksanen et al., 2016). To see if there were major differences among sites in environmental variables, Principal Components Analysis (PCA) was conducted with the 'prcomp' function of R. Prior to the PCA, NO_2 data were again $\log_{10}$ transformed to achieve normal distribution. All analyses were carried out in R version 4.1.2 (R Core Team, 2021).

3. Results

On the 90 trees investigated, 73 taxa were found; 66 were determined to species level; seven additional taxa could only be determined to genus level (Table S2). On average, 5 species were found per tree (range: 1–10 species). The highest species numbers were found in the control areas, each of which had species not occurring elsewhere (Table S2). NO_2 concentrations ranged from 1.71 to 42.87 (mean 13.57) $\mu g\ m^{-3}$ and SO_2 concentrations from 2.49 to 3.57 (mean 3.18) $\mu g\ m^{-3}$ (Table 1). Plot-level mean moss cover ranged from 3.8 to 42%, plot-level mean tree diameter from 29 to 69 cm (Table S3).

3.1. Tree-level analyses

The multivariate linear regression model including all predictor variables (Fig. S1) predicted 31% of the variance in species richness, 30% of the variance in the Shannon index, 20% of the variance in evenness and 21% of the variance in abundance (see R^2 values in Fig. S1).

The effect size comparison using a general linear mixed effects model (Fig. 1, Table 2) showed that pH explains most of the variation in tree-level lichen species richness, Shannon index, and lichen abundance but not in evenness. SO_2 and NO_2 concentrations explained a significant portion of the variance in evenness, where the relationship with SO_2 was positive and with NO_2 negative, indicating that higher SO_2 or lower NO_2 levels were associated with higher evenness, respectively. None of the other variables (moss cover, tree diameter) had significant effects on tree-level lichen diversity.

In univariate models, NO_2 concentration had no significant effect on species richness, Shannon index, evenness or abundance (Fig. S2). SO_2 concentrations explained 9% of the variation in the evenness, with higher SO_2 levels associated with higher evenness. The univariate models showed that SO_2 concentrations had no significant effect on species richness, abundance, or Shannon index (Fig. 2). Lichen species richness, Shannon index, and lichen abundance differed significantly between trees of 'neutral' pH class and those of 'moderate acidic' or 'acidic' pH class, with significantly lower diversity estimates for the neutral pH class (Tukey's HSD test, N = 90 trees, $p < 0.05$; Fig. 3).

Tree-level PCA (Fig. S3) served to understand the variation among individual trees with respect to environmental variables. The most important factors identified were SO_2 concentration (explaining 38% of the variance of PC1), moss cover (28%) and tree diameter (27%). NO_2 concentration (46%) and average pH level (34%) explained the most variance on PC2 (Fig. S3). Trees from Allgovia (site 10) and Chiemgau (site 9) formed their own clusters at the extremes of PC1 or PC2, indicating distinct environmental conditions.

3.2. Plot-level analyses

The multivariate linear regression explained 59% of the variance in plot-level species richness, 53% of the variance in the Shannon index, 37% of the variance in evenness and 45% of the variance in lichen abundance (see R^2 values in Fig. S4).

The effect size comparison based on general linear mixed effect models (Fig. S5, Table 3) showed that tree diameter explained significant variance in species richness and pH class explained lichen abundance, respectively (Fig. S5). In univariate linear models, there were no significant effects of SO_2 or NO_2 concentrations on lichen diversity (Fig. S6; Fig. S7).

Plot-level PCA served to understand the main differences between the sampled plots. The first three PC axes explained 72.5% of the variance in the data. The most important factors were very similar to the tree-level PCA: SO_2 concentration (explaining 33% of the variance of PC1), moss cover (26%) and tree diameter (26%). NO_2 concentration (39%), average pH level (37%) and tree diameter (22%) explained the most variance on PC2 (Fig. S3).

In a plot-level DCA, species clustered into several groups, with the largest variance in the data (73.8%) occurring along DCA1 (Fig. S8). Plots situated in the city of Munich clustered around the origin, with the exception of site 11 (Botanical Gardens). The plot-level DCA emphasized the importance of NO_2 concentration for species composition; none of the other variables had a significant effect (Fig. S8).

4. Discussion

In this study we analyzed lichen diversity data from several locations in the city of Munich, also measuring NO_2 and SO_2 concentrations, which allowed us to test hypotheses on the effects of air pollution on lichen diversity in Munich.

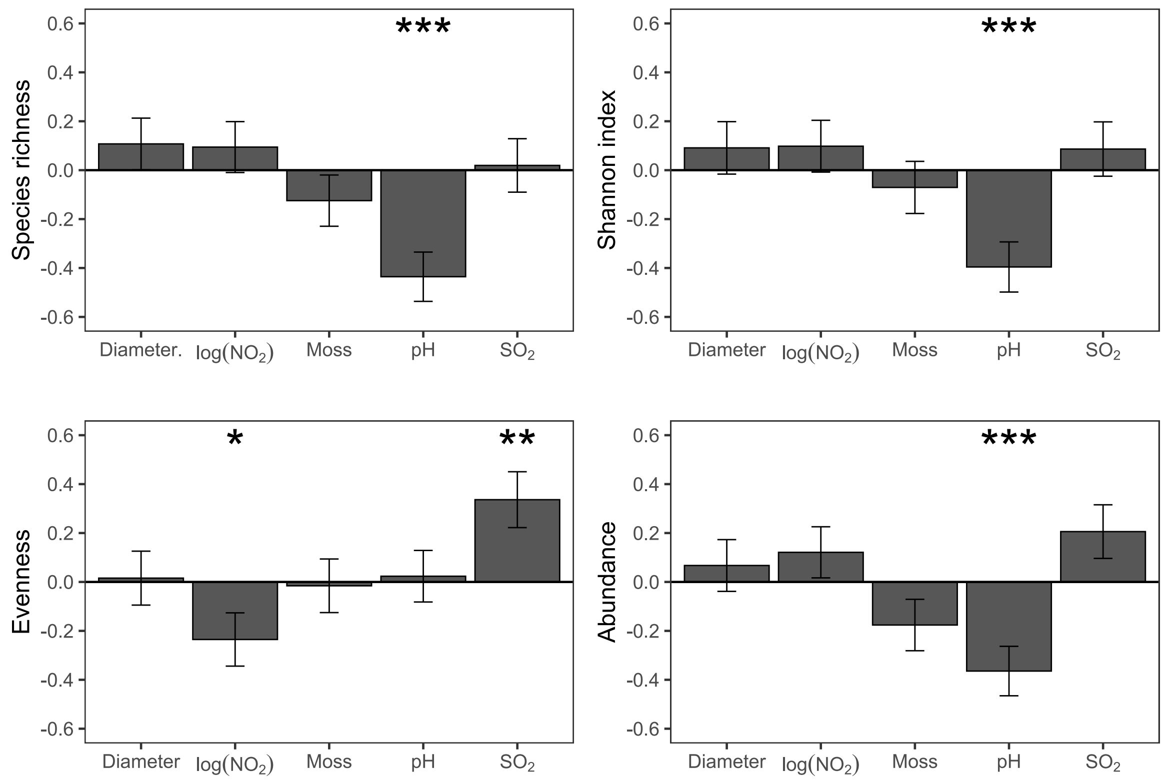

Fig. 1. The relative effect sizes of tree traits and NO_2 and SO_2 concentrations [μg m^{-3}] on tree-level lichen diversity. All predictor and dependent variables were standardized before analysis to allow for direct comparison. Lichen species richness, Shannon-index, evenness, and abundance were predicted with a mixed effects model, using NO_2 and SO_2 concentrations, bark pH, tree diameter, and moss cover as fixed effects and tree species as random effect. NO_2 concentration was log-transformed to obtain normally distributed vectors. N = 90 trees.

Table 2
Results from the generalized linear mixed-effect models predicting lichen species richness, abundance, Shannon index, and evenness, used in tree-level effect size comparisons. The fixed effects log(NO_2), SO_2, pH level, tree diameter, and moss cover were standardized with the function (x - mean(x))/(2*sd(x)). Random effect: tree species. Significant *p*-values are marked with asterisks (* <0.05; ** < 0.01; *** <0.001), borderline significant values were marked with a plus (+: 0.10 > *p* > 0.05). N = 90 trees. Reported are R^2 values, standard errors, *t*-values and *p*-values.

	Species richness				Abundance			
Fixed effects	**Value**	**SE**	***t*-value**	***p*-value**	**Value**	**SE**	***t*-value**	***p*-value**
Intercept	0.000	0.050	0.000	1.000	0.000	0.050	0.000	1.000
log(NO_2)	0.094	0.104	0.907	0.367	0.121	0.104	1.160	0.250
SO_2	0.019	0.109	0.177	0.860	0.206	0.110	1.878	0.064+
pH level	−0.436	0.101	−4.322	<0.001***	−0.364	0.101	−3.603	<0.001***
Tree diam.	0.107	0.105	1.019	0.312	0.067	0.106	0.636	0.527
Moss cover	−0.125	0.105	−1.188	0.239	−0.176	0.105	−1.675	0.098+
	Shannon index				**Evenness**			
Fixed effects	**Value**	**SE**	***t*-value**	***p*-value**	**Value**	**SE**	***t*-value**	***p*-value**
Intercept	0.000	0.050	0.000	1.000	0.000	0.052	0.000	1.000
log(NO_2)	0.098	0.106	0.925	0.358	−0.235	0.109	−2.160	0.034*
SO_2	0.086	0.111	0.778	0.439	0.336	0.114	2.944	0.004**
pH level	−0.396	0.102	−3.862	<0.001***	0.023	0.105	0.222	0.825
Tree diam.	0.091	0.107	0.850	0.398	0.016	0.110	0.142	0.888
Moss cover	−0.071	0.107	−0.662	0.510	−0.016	0.110	−0.144	0.886

4.1. Lichen diversity and its drivers

We found 47 species within in the city of Munich. A previous study reported 57 species for the city of Munich. The larger species number found in the previous study is not surprising, given that the authors investigated a much larger number of trees, thus increasing the likelihood to detect more species (Vorbeck and Windisch, 2001). All the lichen species that occurred with high frequency in our study were also found in the previous study, with one exception (*Lepraria finkii* – common in our sampling sites but not reported in 2001). All remaining common lichen species found by us in Munich were nitrophilic lichens (see Table S2), indicating that the urban epiphytic habitats are considerably influenced by nitrogen depositions.

While we found evidence for an important effect of air pollution in our data from the urban area of Munich, studies from little polluted regions such as northern Norway and Scotland emphasize the

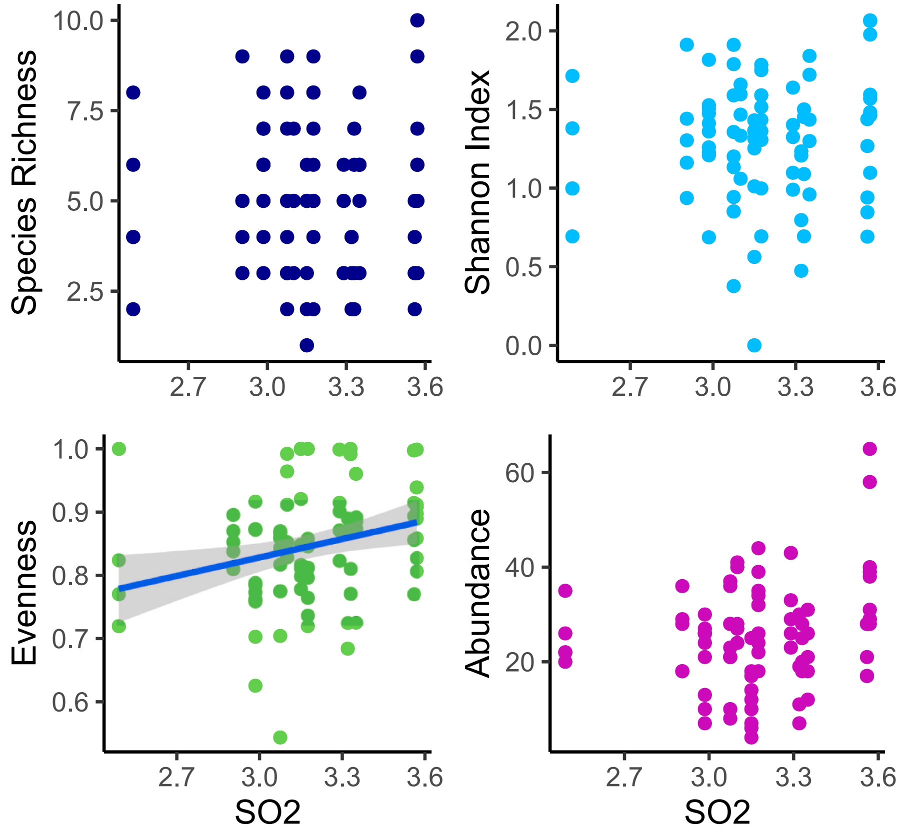

Fig. 2. Univariate linear model quantifying the effect of SO_2 concentration [μg m^{-3}] on lichen diversity, showing tree-level estimates of lichen species richness, Shannon-index, evenness, and abundance in response to SO_2 concentration. N = 90 trees. There was a significant positive relationship of evenness (R^2 = 0.090, p = 0.0012) with SO_2 concentration.

importance of macroclimatic factors such as precipitation or temperature over forest structure and air pollution effects in explaining lichen species composition (Ellis and Coppins, 2010b; Werth et al., 2005), or the importance of forest structure over spatial and macroclimatic variables (Moning et al., 2009). Our present study encompassed a gradient in air pollution. Responses in community composition to climatic variability were not investigated because the majority of plots were proximate within the city of Munich. We therefore expected species composition to reflect either tree traits or the air pollution gradient. The key drivers of lichen diversity were both tree traits and pollution levels (see Fig. 1 and Fig. S5). Not all diversity indices were similarly influenced by tree traits and pollution levels. While variation in species richness, Shannon index and lichen abundance was best explained by tree traits, evenness and species composition (see DCA results) were best explained by pollution levels. This implies a functional decoupling between lichen species richness and abundance from species composition/evenness, because each was driven by different factors. Similar results were reported by Ellis and Coppins (2010a), who found that habitat factors (i.e. the local extent of old-growth woodland) best explained lichen species richness, while pollution best explained species composition, as in our study. In our study, the Allgovia region contained the bulk of both the clean-air-indicating, nitrophobic species and acidophilic species. Additionally, some of the species at this site have also been considered as indicators of old-growth forests such as *Pannaria conoplea, Peltigera collina, Loxospora elatina* and *Normandina pulchella* (Ellis and Coppins, 2019). These old-growth indicators were absent in sites within the city of Munich with the exception of a minute *Normandina pulchella* specimen found at one moderately polluted site. In contrast, the sites studied in the city of Munich were dominated by generalists and by nitrophilic lichens such as *Phaeophyscia orbicularis, Physcia adscendens, Physconia grisea* and *Xanthoria parietina*.

4.2. Effects of NO_2 and SO_2 pollution on lichen diversity and composition

We measured quite considerable NO_2 but low SO_2 concentrations in the study area. Historically, SO_2 concentrations were high, causing the city center of Munich to be largely devoid of lichens in the 1950s (Vorbeck and Windisch, 2001, and references therein). Since the 1970s, however, lichens recovered, and in a survey of the year 2000, nitrophytes were present in the city center, indicating that in the past two decades, nitrogen pollution has gained in importance (Vorbeck and Windisch, 2001). We found that the most frequent species in our survey were nitrophilic lichens. In our study, NO_2 and SO_2 concentrations had no significant effects on lichen species richness, Shannon index and lichen abundance. However, there was a significant relationship between NO_2 and SO_2 concentrations and tree-level evenness, although these results should not be overinterpreted, given that the urban communities studied were not very species-rich. With decreasing NO_2 levels and increasing SO_2 levels, lichen species on individual tree stems were more evenly distributed. Such pollution effects on evenness were not determined in the plot level lichen data. However, NO_2 was borderline significant (p= 0.074), indicating a necessity for more extensive sampling. Some NO_2 concentrations we measured within the city of Munich were quite substantial (maximum 46.46 μg/m^3 or 24.26 ppb, see Table 1, which is above the annual mean value allowed for outside air by the European Union, see https://www.umweltbundesamt.de/themen/unterschied-zwischen-aussenluft, date accessed: 7. January 2022). However, only the highest NO_2 concentration we measured was similar in magnitude to the annual mean concentrations reported for urban areas by Melkonyan et al. (2012). Our remaining NO_2 concentrations

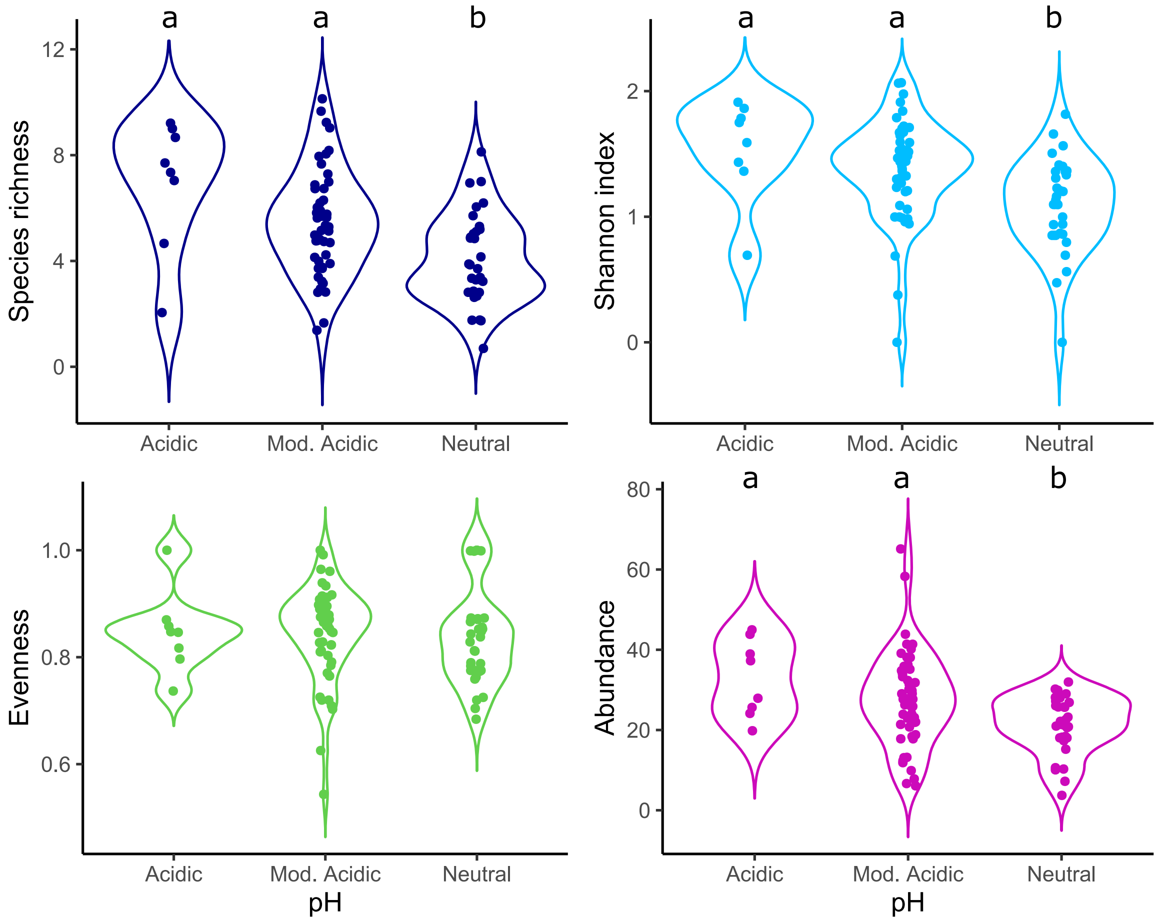

Fig. 3. Violin plot, showing the effect of phorophyte pH level on different estimators of tree-level lichen diversity, i.e. lichen species richness, Shannon-index, evenness, and abundance. N = 90 trees. Letters above plots indicate which groups differ from each other at the 0.05 significance level according to a Tukey-Kramer post-hoc test.

Table 3
Results from the generalized linear mixed-effect models predicting lichen species richness, abundance, Shannon index, and evenness, used in plot-level effect size comparisons. The fixed effects log(NO_2), SO_2, pH level, tree diameter, and moss cover were standardized with the function (x - mean(x))/(2*sd(x)). Random effect: tree species. Significant *p*-values are marked with asterisks (*: <0.05), borderline significant values were marked with a plus (+: 0.10 > *p* > 0.05). N = 18 plots. Reported are R^2 values, standard errors, *t*-values and *p*-values.

	Species richness				Abundance			
Fixed effects	Value	SE	*t*-value	*p*-value	Value	SE	*t*-value	*p*-value
Intercept	$-4.333*10^{-17}$	0.090	0.000	1.000	$9.965*10^{-17}$	0.104	0.000	1.000
log(NO_2)	−0.314	0.196	−1.601	0.135	0.194	0.227	0.852	0.411
SO_2	−0.130	0.219	−0.592	0.565	0.332	0.253	1.311	0.214
pH level	−0.401	0.189	−2.119	0.056+	−0.582	0.219	−2.661	0.021*
Tree diam.	0.556	0.216	2.578	0.024*	−0.050	0.249	−0.201	0.844
Moss cover	0.063	0.204	0.307	0.764	−0.076	0.236	−0.324	0.752
	Shannon index				Evenness			
Fixed effects	Value	SE	*t*-value	*p*-value	Value	SE	*t*-value	*p*-value
Intercept	$1.313*10^{-16}$	0.096	0.000	1.000	$-1.641*10^{-16}$	0.112	0.000	1.000
log(NO_2)	−0.454	0.210	−2.159	0.052+	−0.475	0.243	−1.956	0.074+
SO_2	0.019	0.234	0.083	0.935	0.375	0.271	1.385	0.191
pH level	−0.366	0.203	−1.805	0.096+	0.002	0.234	0.007	0.995
Tree diam.	0.343	0.231	1.485	0.163	−0.423	0.267	−1.588	0.138
Moss cover	0.164	0.219	0.751	0.467	0.355	0.253	1.403	0.186

were more similar to what was reported for rural sites in Germany, or intermediate between ‘urban’ and ‘rural’.

In our study, NO_2 pollution substantially influenced the species composition of sampling plots, as assessed by DCA, and sites with little NO_2 influence, i.e. two pure air control sites and the Botanic Gardens of Munich differed in species composition, with each of these sites hosting species not found elsewhere. These sites were especially rich in acidophilic species which were otherwise less common in the city of Munich. All other epiphytic lichen communities were fairly similar in species composition and were dominated by nitrophilic lichens (see Table S2). It is therefore possible that the nitrophilic lichens may have masked the NO_2 air pollution effects in our data, as previously shown in a study focusing on lichen functional groups (Llop et al., 2012), so that no effects of NO_2 on total species richness, Shannon index and lichen abundance could be detected.

Previous studies found negative effects of SO_2 pollution on lichen diversity during times when SO_2 pollution had reached toxic levels and influenced lichen diversity significantly (Hawksworth and Rose, 1970), e.g. reducing species richness and cover (McCune, 1988). In the 1970s and 1980s, SO_2 air pollution was very high, but has since declined drastically due to more strict regulations of emissions (van Dobben, 1996; Vorbeck and Windisch, 2001). The SO_2 concentrations we measured were low (up to 1.34 ppb), within a range where this type of pollution may have a slightly fertilizing effect, or it may even neutralize the influence of alkaline dust pollution, which was reported as an important type of pollutant in the Munich area (Vorbeck and Windisch, 2001). It is therefore perhaps not surprising that we did not detect significant effects of SO_2 concentrations on species richness, Shannon index and abundance, because current SO_2 levels are too low to be toxic.

Some studies have shown that lichens accumulate particulate matter (Bergamaschi et al., 2007; Giordano et al., 2005) such as magnetic emissions from break wear (Winkler et al., 2020), which may lead to cellular damage. We did not have data on levels of particulate matter in our study sites, but in a study from Chile, an index of lichen diversity that considered lichen abundance responded to particulate matter, i.e. to the number of days per year that the particles exceeded the mean annual level established by legislation (Varela et al., 2018). It would be very interesting for future studies to investigate the effects of air pollution via particulate matter on lichen diversity.

On the tree-level, species richness, Shannon index, and lichen abundance were determined mainly by pH level in our study, so pH is the main driver of lichen diversity of individual trees. The effects of NO_2 pollution on tree-level lichen diversity might have been masked by the high variation among tree individuals, because there was considerable variation in tree species among each plot. Another study found an effect of total N deposition on tree-level lichen diversity, when broad-leaved tree species were investigated (Welden et al., 2018). On the plot-level, because several trees were investigated, variation among individual trees was excluded. Future studies addressing the effects of air pollution on community-level epiphytic lichen diversity should sample multiple tree replicates in a plot design to be able to detect pollution effects.

Plot-level lichen abundance or evenness were not affected by NO_2 concentrations. One possible explanation is that NO_2 concentrations were high enough to homogenize diversity levels throughout the area of Munich, but our measurements of NO_2 pollution discriminate between high-pollution inner city sites and sites with lower pollution on the outskirts of the city. By its fertilizing effect, NO_2 pollution mainly alters the community composition towards nitrophilic species, but it does not seem to lead to reduced lichen abundance or species richness. A possible alternative explanation is that at locations with high NO_2, the increased abundance of nitrophilic species compensates for the decreased abundance of nitrophobic species. Instead, lichen abundance was best explained by bark pH levels, i.e. more acidic bark leading to increased lichen abundance.

Our results on the diversity of epiphytic lichens in the city of Munich show clearly how important green spaces and parks are for the improvement of air quality within urban areas, with lower air pollution measured and higher lichen diversity found in the largest green investigated space, the Botanical Gardens which is part of a several square km large green space surrounding the Nymphenburg castle. Air pollution effects are detrimental for human health and wellbeing (Hoek et al., 2013; Lelieveld et al., 2015; Silva et al., 2016). Including green spaces in city planning is essential because reducing air pollution in cities will lead to improved human health and the avoidance of unnecessary suffering.

4.3. Effects of tree traits on lichen diversity

Plot-level species richness was determined by tree diameter, explaining 55.6% of the variance in the lichen data. Several prior studies have also found relationships between species richness and tree diameter (Nascimbene et al., 2008; Thor et al., 2010; Uliczka and Angelstam, 1999), and larger trees would be expected to host more lichen species for several reasons. First of all, trees of larger diameter have a larger surface for propagules to land on than smaller trees. Larger trees frequently also have different bark properties, as the bark of larger trees tends to be coarser, providing different habitat conditions than the bark of small trees. Lastly, trees of larger diameter tend to be older, so they will have had more opportunities to be colonized, although there is also a considerable turnover of species over time (Löbel et al., 2006).

Among the tree traits, we found that the bark pH level was most important, best explaining tree-level variation in Shannon index and species richness, and tree and plot-level lichen abundance. The importance of substrate pH differences for lichen species composition and the occurrence of individual lichen species is well established (Barkman, 1958; Mezaka et al., 2008; van Herk, 2001). However, in a recent study in an eutrophicated area, there was no relationship of lichen functional groups with bark pH values or other tree traits (Giordani and Malaspina, 2017; Llop et al., 2012). In our data, high lichen abundance was associated with low bark pH levels, similar to what Caceres et al. (2007) found for Atlantic rainforests of northeastern Brazil, although these sites are ecologically quite different from our sites. As we grouped tree species into bark pH classes, in our study, pH effects may be somewhat confounded with other tree traits such as tree species identity, which has been found to be one of the most important factors explaining the distribution of epiphytic cryptogams (Mezaka et al., 2008; Sündhofer et al., 2021). We found no significant effect of bark pH on the plot level, presumably because by combining the pH class data, the pH effect was hidden.

Moreover, one might think that bark pH may differ depending on road traffic intensity leading to variability in bark pH among conspecific trees, which would not have been included by our approach. Bark pH levels within species may vary both with pollution levels (Frahm et al., 2009) and with the amount of rainfall (Kovářová et al., 2022 and references therein). Along with lower pollution levels in the control areas, higher rainfall might explain some of the differences between the control areas and the city of Munich. Some studies have shown a relationship between air pollution and bark pH levels, and whether or not an effect is found may depend on the buffering capacity of the bark of a respective tree species. For example, Frahm et al. (2009) studied the effect of road traffic on bark pH of several deciduous tree species in Germany. He found no relationship except in oaks (*Quercus* sp.), for which bark pH levels increased with traffic intensity, which was attributed to the low buffer capacity of oak bark. Similarly, there was no relationship between bark pH of small-leaf lime (*Tilia cordata*) and road traffic intensity in a study from Estonia (Marmor and Randlane, 2007). Therefore, we would not expect major variation in bark pH among conspecific individual trees in our study area. However, to make a confident conclusion on the effects of pH on lichen diversity vs. potential effects of unmeasured tree traits, additional data are necessary.

4.4. Prediction of epiphytic lichen diversity

Cristofolini et al. (2008) investigated the abundance of epiphytic lichens in northern Italy and reported that substrate and habitat-related factors resulted in non-parametric multiplicative regression models with higher explanatory power than those integrating only pollution-related factors. Also in our study, models based on tree-characteristics resulted in higher R^2 than models based on air pollution. While the study is not directly comparable with ours given the different types and specifications of models, we can point out that future studies characterizing the effects of air pollution should attempt to standardize the investigated epiphytic habitats as far as possible, but this may be difficult to achieve.

By integrating information on tree characteristics and air pollution, we were able to reliably model epiphytic lichen diversity. In our study, multivariate models to predict lichen diversity, evenness, and abundance on the plot level generally had a higher predictive power than models at the individual-tree level (see Figs. S1, S4). This underscores the necessity to aggregate tree-level measurements to plot level to obtain reliable estimates of lichen diversity. The lower explanatory power of the tree-level models is a consequence of the sizable variability in lichen diversity among individual trees. Plot-level models including tree characteristics, NO_2 and SO_2 concentrations as predictor variables performed best in predicting species richness and the Shannon index, whereas evenness and abundances were predicted with slightly less confidence. Future studies could combine the data of several trees to improve the predictive power of models of lichen diversity.

5. Conclusions

Lichens serve an important purpose as bioindicators of long-term air pollution in cities heavily impacted by road traffic. As our case study from parks and green areas within the city of Munich showed, tree-level lichen diversity and abundance were mainly determined by tree traits, not by air pollution. However, the species composition was driven by NO_2 pollution levels and the most common lichen species in our study were nitrophilic. These results confirm that ongoing NO_2 air pollution within cities is a major threat to lichen diversity, with nitrophobic/acidophilic lichens likely experiencing the greatest risk of local extinctions in urban areas in the future. This study represents the first data on lichen diversity and composition in the city of Munich since 2001, two decades after the last study has been published, and it shows the importance of green spaces for the air quality within cities. Future studies could investigate more sites within Munich to provide an even more detailed picture of lichen diversity and composition in this important urban area.

Credit author statement

Veronica Sebald: Investigation, Data curation, Software, Formal analysis, Visualization, Writing – original draft. Andrea Goss: Investigation, Data curation, Software, Formal analysis, Visualization, Writing – review & editing. Elisabeth Ramm: Investigation, Data curation, Writing – review & editing. Julia V. Gerasimova: Investigation, Writing – review & editing. Silke Werth: Conceptualization, Methodology, Writing – original draft, Writing – review & editing, Supervision.

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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.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2022.119678.

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