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Short Communication

Microalgal biofilms on common yew needles in relation to anthropogenic air pollution in urban Prague, Czech Republic

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HIGHLIGHTS

- Biofilms on needles in a city area consist of algae, fungi and particulate matter.
- High short-term NO_2 levels lead to higher abundance of algae and biofilm cover area.
- PM_{10} concentrations were negatively correlated with abundance of algae on needles.
- Yew needle longevity was not correlated with the local air pollution levels.

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ABSTRACT

Excessive occurrence of microalgae on needles of gymnosperms was reported for the first time in the 1980s from the Scandinavian countries. Since then, it has been repeatedly encountered on needles from various European forest habitats. The abundance of these biofilms has been related to the climatic conditions, such as temperature and precipitation, as well as to the air pollution by nitrogen and sulfur oxides. Urban areas typically have relatively homogenous climates and profound variation in levels of air pollution. Therefore, variation in the occurrence of biofilms in localities within an urban area may be related to local anthropogenic air pollution. We investigated the abundance of biofilms occurring on needles of the common yew (*Taxus baccata*) in the city of Prague, Czech Republic. The biofilms were composed of algae, fungi and particulate matter. The cover area of the biofilms was marginally explained by a positive influence of short-term maximum atmospheric levels of nitrogen dioxide (NO_2). The amounts of the microalgae were also positively influenced by short-term maximum NO_2 levels. In addition, high atmospheric levels of particulate matter (PM_{10}) were related to low abundance of algae. The microbial biofilms growing on widely cultivated conifers, such as the common yew, form one of the few commonly occurring natural communities in highly urbanized central areas of temperate European cities. Consequently, we propose that microscopic analysis of biofilms may be used as a rapid and cheap method to collect ecological data. Such data may be used in biomonitoring schemes illustrating the effects of anthropogenic air pollution on natural microcommunities in urban areas.

1. Introduction

Subaerial algae, which typically form phototrophic biofilms, occur in a wide range of natural habitats, such as on plant surfaces (bark, needles, leaves), soil, rock and stones, as well as on various anthropogenic surfaces, such as concrete walls, stone sculptures, old iron constructions, and wooden fences (Freystein et al., 2008; Hoffmann, 1989; Rindi et al., 2009). They have so far attracted less attention from researchers than the freshwater or marine algae, perhaps because of the strikingly low morphological diversity found in subaerial algal communities. The cells of subaerial algae are usually spherical or ovoid, or they form simple filamentous thalli. Cellular shapes with low surface-to-volume ratios may have evolved in response to stressful conditions, such as frequent desiccation (Ettl and Gärtner, 1995). These characteristics of the subaerial microalgae allow them to be easily dispersed by wind (Hoffmann, 1989). Most taxa cannot be unambiguously identified under the light microscope. Consequently, subaerial algal communities are poorly known, and many basic questions regarding their ecological dynamics, including their response to anthropogenic air pollution, remain unanswered. However, research has suggested that community structure and abundance of subaerial phototrophic biofilms may be related to concentrations of the air pollutants, such as NO_x and SO_2 (Bråkenhielm and Qinghong, 1995; Göransson, 1988; Grandin, 2011; Neustupa and Albrechtová, 2003; Poikolainen et al., 1998). Microalgal cells growing on terrestrial substrates are directly exposed to the environment because they are not covered by any protective layer. In

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addition, their generation times are much faster than those of the lichens, bryophytes, or vascular plants. Therefore, they may reflect environmental changes considerably faster than other taxa (Bråkenhielm and Qinghong, 1995; Marmor and Degtjarenko, 2014).

The abundance and community species composition of subaerial algae growing on tree bark seem to be influenced by air quality, typically expressed as NO_x and SO_2 deposition (Brück, 1983; Freystein et al., 2008; Poikolainen et al., 1998). A study of tree bark biofilms growing in the urban area of Köln, Germany, confirmed that the air pollution probably provided additional nutrients to subaerial algae (Brück, 1983). Biofilms were more abundant on trees growing in highly polluted areas in the city center than on those in the peripheries, where the pollution levels were lower. Likewise, a survey of the microalgae on tree bark in differently polluted areas in Leipzig, Germany, showed that some algal taxa were more common in more polluted areas, while other preferred less polluted localities (Freystein et al., 2008). A long-term study in Finland illustrated regional changes in the abundance of microalgae growing on the bark of Norway spruce between 1985 and 1995 (Poikolainen et al., 1998). The authors found a slight positive correlation between the abundance of algae and NO_x, NH_3 and SO_2 deposition. It has been presumed that the growth of algae was promoted by higher deposition of NO_x, whereas the high SO_2 concentrations inhibited their growth (Poikolainen et al., 1998). Since the sulfur deposition in Northern Europe declined between 1985 and 1995, the abundance of bark-inhabiting subaerial algae generally increased.

In contrast to corticolous biofilms, the common occurrence of microalgae on the needles of conifers, such as *Picea abies* L. and *Pinus sylvestris* L., was reported for the first time in the 1980s, mostly in Scandinavia (Bråkenhielm and Qinghong, 1995; Göransson, 1988; Peveling et al., 1992; Søchting, 1997). The microalgae forming these biofilms do not directly parasitize the needles (Peveling et al., 1992), but Neustupa and Albrechtová (2003) suggested that they may contribute to the premature shedding of needles, which results from competition for resources, such as light or CO_2. Göransson (1988) first showed a correlation between nitrogen deposition and the abundance of algae growing on needles. The regional distribution patterns of microalgae on needles resembled those reported by Poikolainen et al. (1998) for corticolous biofilms: as nitrogen deposition diminished towards the north, so did the abundance of microalgae on spruce needles. Bråkenhielm and Qinghong (1995) also showed that two parameters of biofilms on spruce needles in Sweden (thickness and colonization rate) were positively correlated with the length of the growing season, and with nitrogen and sulfur deposition. Subsequently, a long-term survey of the dynamics of microalgal biofilms on spruce needles was established as a part of an Integrated Monitoring Program (UNECE, 2013). This monitoring, conducted between 1997 and 2009, documented a continuous decrease in the algal cover on spruce needles in Sweden that was correlated with the decrease of major air pollutants, such as NH_4 and SO_2 (Grandin, 2011). In regions with continuously very low air pollution, such as Northern Sweden, no biofilms occurred on spruce needles, possibly because of the lack of nutrients.

These large-scale studies proved that the abundance of biofilms on needles may provide useful data for the assessment of the air pollution. However, to the best of our knowledge, there has been no research on the biofilm abundance on a smaller scale, such as within a single city. Cities are spatially relatively circumscribed, and typically have homogenous climatic conditions, but highly variable air pollution loads (City Development Authority Prague, 2013; Masiol et al., 2014). Therefore, the abundance of subaerial biofilms may be directly related to the anthropogenic air pollution. This study was designed to quantify the abundance of biofilms on yew needles in relation to air pollution data within the urban area of Prague, a city of 1.25 million inhabitants. There were several reasons for choosing the common yew as a model organism for monitoring biofilms, as follows: the species is a) native to the region and thrives in the climatic conditions of the area (Cope, 1998); b) evergreen, and individual needles typically survive for more than five seasons, which allows biofilms to develop sufficiently; and c) possibly the most widely grown conifer in urban areas of temperate European cities.

While microbial growths on spruce needles have been found to be almost entirely composed of algae, with few fungal hyphae present (Peveling et al., 1992), the composition of microbial communities on yew needles varies from entirely algal to entirely heterotrophic (personal observation). For this reason, a new parameter 'amount of algae' (AA) was established to describe the abundance of the phototrophs in the biofilm samples. The aim of this study was to find out whether three parameters characterizing microbial growths (cover, thickness, and AA) were correlated with the site-specific concentrations of air pollutants. Our hypothesis was based on the premise that the biofilm abundance is significantly influenced by air pollutants. We hypothesized that the abundance of biofilms will be positively correlated with concentrations of the pollutants that have been considered to be a source of nutrients (NO_2, NO_x). Conversely, we also tested whether high concentrations of potentially toxic pollutants, such as SO_2, were associated with a low abundance of biofilms. In a more general context, we aimed to evaluate the potential of microbial biofilms on needles as bioindicators: as a model group for biomonitoring anthropogenic pollution at smaller scales and in areas with few other suitable natural communities, such as in the central areas of big cities.

2. Materials and methods

2.1. Localities, air pollution data and sampling

The metropolitan area of Prague is situated in the temperate climatic zone, and has relatively humid conditions, relatively severe winters, no dry season, and relatively warm summers (Dfb category of the Köppen-Geiger classification). The mean annual temperature is 8.2 to 9.1 °C and the mean annual precipitation amounts to 530 to 580 mm. The area is characterized by a considerable air pollution gradient from the city center towards the peripheral parts. Within this gradient the mean concentrations of NO_x vary from 20 to 150 μg m^{-3}, SO_2 from 4 to 10 μg m^{-3}, and PM_{10} from 20 to 40 μg m^{-3}. Air pollution gradients are mainly caused by local traffic emissions, the biggest sources of pollutants, as well as by relatively extensive areas of vegetation, separating the central districts from the peripheries (ENVIS, 2013). An emission model based on annual concentrations of pollutants derived from 8647 reference data points was published by the Prague Institute of Planning and Development (City Development Authority Prague, 2013). This publicly available model generates estimations of concentrations of the main air pollutants (PM_{10}, SO_2, NO_2, NO_x, CO) within the city area. The model has been derived from the Industrial Source Complex (ISC2) Gaussian dispersion model of the U.S. Environmental Protection Agency (EPA, 1992).

The 15 study sites were situated in various urban parks planted with stands of the common yew (*Taxus baccata* L.). The sites (50 × 50 m^2) were randomly distributed in different parts of the city with varying anthropogenic air pollution levels and involved most of the suitable common yew stands in the central city parts (Supplementary Geospatial Data S1). The samples were taken in September 2012. At each site, four yew trees were randomly selected. Then, four adjacent branches facing north were selected at height 125–175 cm on each tree and five needles from each branch were sampled; thus each site was represented by 80 needles. The age of individual needles, as well as the age of the oldest needles present on a branch, was calculated by counting the annual growth increments. The age of the oldest needles was used as an indirect measure of the health status of the tree (UNECE, 2013). A hemispheric photograph of each tree sampled was taken with a Canon PowerShot A590 IS camera with a wide-angle objective Soligor DHG 0.19xFish Eye.

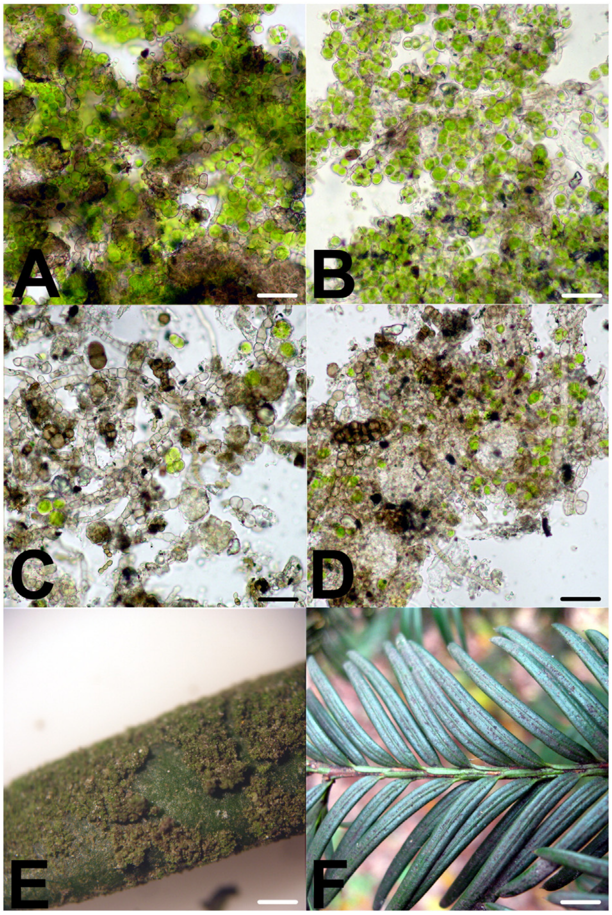

Fig. 1. Contents of the biofilms on yew needles. Microphotographs showing the composition of biofilms with varying proportions of phototrophic microalgae and heterotrophic biomass (A–D), detail of a biofilm growing on a yew needle (E), and a branch of a common yew with weakly developed biofilm cover (F). Bar = 40 μm (A − D), 500 μm (E), 4 mm (F).

2.2. Evaluation of the biofilm data

The needles were inspected immediately after sampling with a binocular magnifier (Olympus SZ61) and a light microscope (Olympus CX31). The abundance of the biofilms on the upper surface of needles was estimated by using two semiquantitative parameters introduced by Bråkenhielm and Qinghong (1995). The cover area (C) was defined as the estimated percentage of the needle surface covered with the microbial growth in needles with the most extensive cover (Bråkenhielm and Qinghong, 1995). The parameter C was assigned values of 1 to 4, as follows: 1 = 0–25%, 2 = 25–50%, 3 = 50–75%, and 4 = 75–100%. The thickness of the biofilms (T) was estimated on a three-level semi-quantitative scale, as follows: 1—very thin, 2—detectable colonies, and 3—a growth rising above the needle's surface, easily detectable when observed from the side. Due to the fact that the biofilms were not composed exclusively of algae, the third parameter 'AA' was introduced for the estimation of the phototrophic component. Typically, the biofilms were formed by a mixture of algae, fungi, other microorganisms and particulate matter. The proportion of the phototrophic component varied considerably among samples. The parameter AA was evaluated under the light microscope (magnification 400×) and was categorized as follows: 0—no algae present, 1—sporadic algal clumps (microcolonies) within predominantly non-phototrophic biofilm mass, 2—scarce to minority algal proportion, less than 50% of the biofilm mass, and 3—algal cells forming more than 50% of the biofilm mass. Estimation for each sample was based on an average of five microscopic slides (20 × 20 mm) inspected.

2.3. Data analysis

The open sky proportion (OSP), used as a proxy for the illumination of sites, was analyzed by using Gap Light Analyzer, ver. 2.0 (Frazer et al., 1999), and the values were expressed as the percentage of open sky above the sampling points on trees. The air pollution data, acquired from the model, were expressed as annual means of PM_{10} (coarse particulate matter less than 10 μm), SO_2, NO_2, NO_x and CO, for each study site in μm m^{-3}. In addition, the maximum 24 hourly levels of PM_{10} and hourly maximum levels of SO_2 and NO_2, detected in the year 2012, were acquired from the air pollution model and used for the analyses, evaluating the relationship between the biofilm and the air pollution levels. The biofilm data were expressed as the mean C, T and AA values for each site (Supplementary Table S1); the air pollution data were converted to ranks corresponding to classes of the air pollution levels for each site. Relationships between the biofilm data, OSP and the age of the oldest needles were evaluated by Spearman's rank-order correlation analyses. Spatial autocorrelation of the biofilm data was evaluated by Mantel tests comparing matrices of geographic distances and differences in biofilm data among sites. Given the ordinal scale of the response variables, relationships between the biofilm data and the air pollution levels at sites were assessed by a set of suitable generalized linear models (GLM), namely the ordered probit regressions, implemented by the *polr* function of the MASS package (Venables and Ripley, 2002) in R, ver. 2.15.3 (R Development Core Team, 2012). The measures of fit of the ordered probit models, such as maximum likelihood pseudo-R^2, McFadden pseudo-R^2 (Long, 1997), were computed by the *pR2* function of the *pscl* package in R (Jackman, 2014). The McFadden pseudo-R^2 values by definition reach lower values than the ML-R^2 and the value higher than 0.2 indicates a very good fit of a model (Long, 1997). In parallel, the mean age of the oldest needles per site (calculated as the site mean from each branch; $n = 16$ branches per site) was also related to the air pollution by the probit regression

Table 1
Results of the ordered probit regression analyses to evaluate the effects of air pollution factors on biofilm parameters estimated on the ordered categorical scale (cover, thickness, and amount of algae). Significant p-values are indicated in bold.

Variable [μg m^{-3}]	Coefficient	Standard error	95% confidence interval		*t*-statistic	*p*-value
Mean cover; AIC = 92.0, ML-R^2 = 0.41, McFadden pseudo-R^2 = 0.11, p-value = 0.048						
PM_{10} (24-hour max)	−0.120	0.799	−1.685	1.446	−0.15	0.881
NO_2 (1-hour max)	1.351	0.614	0.148	2.553	2.20	**0.028**
CO (mean)	−0.589	0.339	−1.255	0.076	−1.74	0.083
Mean thickness; AIC = 103.4, ML-R^2 = 0.19, McFadden pseudo-R^2 = 0.04, p-value = 0.0781						
Mean amount of algae; AIC = 85.6, ML-R^2 = 0.64, McFadden pseudo-R^2 = 0.21, p-value = 0.0015						
PM_{10} (24-hour max)	−0.438	0.932	−2.265	1.388	−0.47	0.639
NO_2 (1-hour max)	2.160	0.767	0.657	3.662	2.82	**0.005**
PM_{10} (mean)	−1.654	0.733	−3.092	−0.217	−2.26	**0.025**

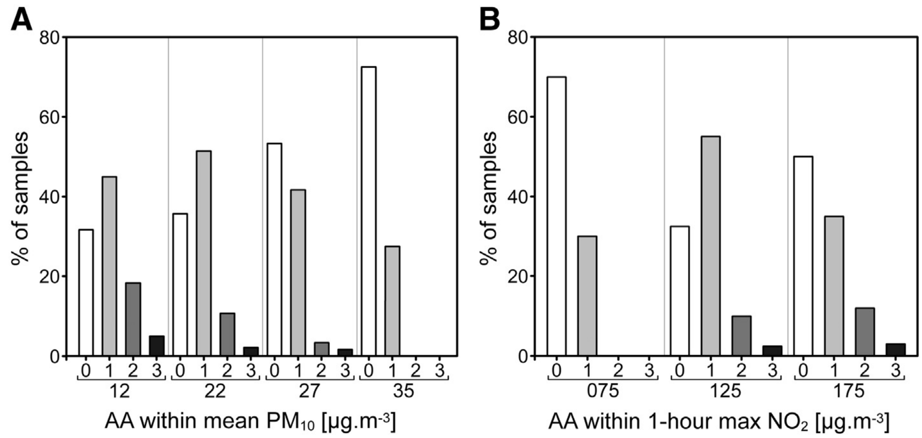

Fig. 2. Relationships between amount of algae (AA) levels and the classes of the mean PM_{10} (A) and hourly maximum NO_2 concentrations (B) as estimated by the Prague air pollution model.

analysis. Optimal models, avoiding collinearity among closely related independent variables, were chosen by the forward stepwise search based on Akaike's information criterion (AIC) values, using the *stepAIC* function of the MASS package (Burnham and Anderson, 2004). After testing all the predictor terms, we also tested the interaction of the significant effects in individual models.

3. Results

The biofilms growing on common yew needles in the urban area of Prague varied considerably in their appearance. Their color varied from dark brown to green, primarily due to varying proportions of the microalgal component (Fig. 1A–D, E–F). Besides algal cells, the biofilms were mostly composed of fungal hyphae and spores (Fig. 1C–D) and particulate matter (Fig. 1D). The microalgae could be morphologically identified as members of the morphologically defined genus *Apatococcus* (Trebouxiophyceae). The globular cells, 6.0 to 13.0 μm in diameter, occurred as unicells, sarcinoid colonies of 4 to 32 cells, or irregular clumps. The cells were surrounded by thin cell walls and lacked any mucilaginous sheaths. They possessed parietal, irregularly shaped plastids without pyrenoids. Members of other genera were not observed, but taxonomic affiliation of individual chlorelloid cells, typical for multiple trebouxiophycean taxa, could not be ascertained.

The semiquantitative parameters used to describe the biofilm community were mutually tightly correlated. The C of the biofilms was positively correlated with their T ($N = 60$; $\rho = 0.75$, $p = 0.0016$). However, these parameters were also positively correlated with the AA, a parameter designed to evaluate the proportion of the phototrophic component (C vs. AA: $\rho = 0.68$, $p = 0.0054$; T vs. AA: $\rho = 0.75$, $p = 0.0014$). The mean age of the oldest needles was significantly correlated with the biofilm amounts (age vs. C: $\rho = 0.34$, $p = 0.0078$; age vs. T: $\rho = 0.44$, $p = 0.0008$) and it was also less strongly related to the amount of algae (age vs. AA: $\rho = 0.29$, $p = 0.0255$). Conversely, the OSP was not correlated with the biofilm data, but there was a moderately significant negative relationship between this parameter and the mean age of the oldest needles ($\rho = -0.28$, $p = 0.0299$). No spatial autocorrelation was detected in any of the biofilm parameters.

Out of the two measures of the total abundance of the biofilms (cover area and thickness), only cover area yielded a marginally significant GLM illustrating its relationship with the air pollution data (Table 1). The cover area proved to be positively related to the hourly maximum NO_2 levels. The stepwise forward selection procedure resulted in a well fitted model (McFadden pseudo-$R^2 = 0.21$) explaining AA in relation to the major air pollutants (Fig. 2; Table 2). The AA values proved to be positively related to the hourly maximum NO_2 levels. In addition, a significant negative relationship between the mean PM_{10} levels and the AA was also demonstrated. Needle longevity, evaluated by the mean age of the oldest needles, could not be significantly explained by the available air pollution data. No significant interactions were detected among individual factors in models evaluating relation of biofilm and air pollution data.

Table 2
Counts of needles in individual categories of the amount of algae (AA) and hourly maximum NO_2 and mean PM_{10} levels as estimated by the Prague air pollution model.

		NO_2 (1-hour max) [μm m^{-3}]			PM_{10} (mean) [μm m^{-3}]			
		75	125	175	12	22	27	35
AA	0	28	52	50	19	50	32	29
	1	12	88	35	27	72	25	11
	2	0	16	12	11	15	2	0
	3	0	4	3	3	3	1	0

4. Discussion

Earlier research on biofilms on the needles in boreal and temperate forests illustrated that they were chiefly composed of phototrophic green microalgae (Grandin, 2011; Peveling et al., 1992). However, we showed that the biofilms growing on yew needles in a Central European urban area also included substantial proportions of heterotrophic biomass, such as fungal hyphae and spores, and particulate matter. The deposition of the fine-grained PM fraction is especially common in urban areas (Langner et al., 2009). Consequently, the high levels of PM_{10} probably lead to an increase in the heterotrophic part of the biofilms, but they simultaneously decrease the abundance of the phototrophic part, possibly by shading the microcolonies of cells that colonize the needle's surface. Cover area of the biofilms, as well as the high amount of algae, was positively related to high levels of deposition of nitrogen oxides. Notably, the hourly maximum NO_2 concentrations were the significantly related factor in the optimal model explaining the abundance of microalgae as well as the total cover area of the biofilms (Table 1). Similar relationships between subaerial microalgal communities and NO_x deposition were previously documented in large-scale studies (Bråkenhielm and Qinghong, 1995; Grandin, 2011; Poikolainen et al., 1998). However, in these studies, NO_x and SO_2 pollution were tightly correlated with latitudinal climatic gradients, such as mean temperature, and the primary causative factors for the increased development of the biofilms could not be ascertained (Bråkenhielm and Qinghong, 1995). Conversely, the present study was conducted at the smaller spatial scale of a single city, with homogenous climate but profound differences in pollution patterns. It was demonstrated that the development of biofilms was primarily related to the increased deposition of NO_2, originating chiefly from car traffic (ENVIS, 2013). Atmospheric emissions of nitrogen oxides probably increase the trophic levels of the needle surface so that relatively rapid development of the biofilms is possible. The hourly maximum concentrations of NO_2 were more closely linked to the biofilm parameters than the annual mean values. We believe that this pattern could be due to the patterns of the microalgal population dynamics. Typically, populations of the subaerial algae spend relatively long periods during their life cycle in inactive states, with very low photosynthetic performance and almost zero cell division rates, in response to adverse abiotic conditions, such as low humidity or temperature (Lüttge and Büdel, 2010); however, they are able to resume population growth rapidly once conditions become more favorable. Similar patterns may also take place in response to changes in nutrient concentrations. The maximum levels of NO_2 deposition may lead to short-term population growth that relates to the total abundances of the biofilms. In contrast to the NO_2 values, the SO_2, evaluated both as hourly maxima and as mean concentrations, did not significantly explain biofilm parameters. This could indicate that subaerial microalgae are not strongly sensitive to the toxic effects of SO_2 air pollution; however, pollution by SO_2 at the study sites was much less pronounced than nitrogen oxides deposition, so the potentially toxic effects of SO_2 may have been overshadowed by the NO_2-related impacts.

Our data do not support the relationship between premature needle shedding and local air pollution loads, previously demonstrated for Scots pine (Lampu and Huttunen, 2001) and Norway spruce (Pfanz et al., 1994). The concentrations of the pollutants were too low to affect the needle shedding or, alternatively, the needles are well adapted to the high air pollution of the inner city area, and to the development of biofilms; thus their longevity was not decreased. Conversely, needle longevity seemed to be slightly decreased by higher irradiation levels on trees with more open canopies, as evaluated by the OSP. This pattern may be explained by the well-known fact that yew is adapted to shaded habitats in the forest understory (Cope, 1998).

We did not analyze the taxonomic structure of the microalgal communities, but they were chiefly composed of green algae. Subaerial green algal assemblages usually consist of chlorelloid and sarcinoid members of the Trebouxiophyceae; these taxa typically dominate biofilms growing on tree bark (Freystein et al., 2008; Neustupa and Škaloud, 2008; Neustupa and Štifterová, 2013; Rindi et al., 2009). Recent molecular phylogenetic studies illustrated that high levels of previously unknown cryptic diversity of chlorelloid algae occur in these microhabitats (Hallmann et al., 2013). Therefore, as well as affecting the abundance of microalgae in biofilms, pollution may also affect the structure of the assemblages. Similar patterns were ascertained in microalgal assemblages growing on tree bark in the Leipzig area, Germany. Analysis of the taxonomic composition of biofilms at three locations differing in their pollution loads showed that the diversity of corticolous biofilms was increased in the more polluted parts of the city (Freystein et al., 2008). This pattern could be related to the eutrophication of the bark surfaces caused by the increased deposition of nitrogen oxides.

5. Conclusions

The present study showed that air pollution in an urban area affected the cover area of the biofilms, as well as the proportion of their phototrophic component. The biofilm abundances proved to be affected by the short-term maximum NO_2 deposition levels, possibly indicating temporally limited eutrophication events supporting growth of the microorganisms on needles. In parallel, the proportion of the microalgae negatively related to deposition of the particulate matter (PM_{10}), possibly by shading the cells on the needle's surface. The needles of conifers, such as the common yew, form one of the few commonly occurring natural microhabitats present in highly urbanized city centers. Microscopic analysis of subaerial microalgae provides a rapid and cheap way to illustrate the response of biofilms to anthropogenic air pollution. Therefore, we propose that this procedure could be used in a biomonitoring scheme, similar to the corresponding parts of the large-scale Integrated Monitoring Program (UNECE, 2013), to detect changes in biotic communities in relation to air pollution in urban areas.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found in the online version, at http://dx.doi.org/10.1016/j.scitotenv.2014.11.031. These data include Google map of the most important areas described in this article.

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