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Do tree-related factors mediate the response of lichen functional groups to eutrophication?

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Abstract

In the last decades, the pollution regime has been drastically changed in most industrialized countries, with a considerable decrease in sulphur dioxide (SO_2) emissions and an increasing relevance of eutrophication compounds, such as nitrogen compounds and particulate matter. This situation hampers the interpretation of data in biomonitoring surveys, as high lichen diversity is not always associated with good air quality. The objective of this study was to test whether the effects of eutrophication on the abundance of different lichen functional groups varies according to some tree-related factors. We analysed the relationships between epiphytic lichen diversity, emissions of main atmospheric pollutants and tree characteristics (circumference and bark pH, light transmitted through the canopy). Hierarchical partitioning of variance and Generalized Linear Mixed Models (GLMM) confirmed that lichen functional groups with different nitrogen tolerances responded to several atmospheric pollutants, with both independent and joint effects, whereas they did not show significant differences depending on main tree-related factors. We demonstrated that, under high eutrophication levels, differences in bark pH did not significantly differentiated the composition of epiphytic lichen communities.

Keywords: *Biomonitoring, air pollution, oligotrophic species, nitrophytic species, nitrogen, hierarchical partitioning*

Introduction

Since the beginning of the industrial age, several major gaseous pollutants (e.g. SO_2 and NO_x) had caused a progressive reduction in the diversity of sensitive organisms, including epiphytic lichens, which are widely used as biomonitors of atmospheric pollution (Giordani et al. 2002; Nimis et al. 2002; Giordani 2007). Over recent decades there has been a strong reduction in acidic pollution as a result of policies to abate sulphur dioxide (SO_2) emissions and an increasing relevance of eutrophication. Eutrophication results from many processes, including both wet and dry depositions of several nitrogen compounds and alkaline dusts. Most of the effects of eutrophication are clearly visible on ecosystems (Vestreng et al. 2007), both within a relatively short distance of the emission source (Pinho et al. 2008; Giordani, Matteucci et al. 2014), and caused by medium to long range depositions (van Dobben & De Bakker 1996; Geiser et al. 2010; Giordani et al. 2012; Giordani, Calatayud et al. 2014).

Under this new pollution scenario, variations in the abundance of lichen functional groups are expected along gradients of atmospheric eutrophication. Particularly, several authors (e.g. van Dobben & ter Braak 1998; van Herk 2001; Sparrius 2007; Geiser et al. 2010; Jovan et al. 2012) proposed a distinction between two main functional groups: nitrophytic species, which are expected to dominated at medium to high ammonia concentrations, vs. oligotrophic species, which are associated to low levels of nutrients (esp. nitrogen compounds) in the surrounding environment. Therefore, the axiom 'the higher the atmospheric pollution, the lower the total lichen diversity' has become questionable (Sparrius 2007).

Apart from direct effects related to their capacity to assimilate and tolerate nitrogen (Hauck 2010), eutrophication increases the pH of the substratum, drastically altering the ion equilibrium at the interface between thalli and tree bark (Van Dobben and De Bakker 1996; van Dobben & ter Braak 1998; Hauck 2010). Moreover, lichen communities respond to a complex interaction of tree-related factors rather than to pH alone (Spier et al. 2010). In fact, other microhabitat conditions (Kermit & Gauslaa 2001; Fritz & Heilmann-Clausen 2010), such as tree age and light regime (Nash 2006) may cause similar responses of lichen communities. For example, both the ammonium concentration and the increased pH of the substratum might affect the functional composition of lichen vegetation at sites

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with high ammonium deposition (van Herk 2001). Moreover, lichen communities should respond more rapidly to recent changes in atmospheric ammonia concentrations on younger substrates, such as twigs and young trees (Wolseley et al. 2006) that have been exposed only to very recent levels of pollutants. In contrast, the relative composition of epiphytic communities on older trees is a function of both past and present contamination levels.

In this study, the hypothesis was that both the interaction between pollutants and tree-related factors mediates the effects of eutrophication substances on the abundance of species in different lichen functional groups classified according to nitrogen tolerance (oligotrophic vs. nitrophytic species). In particular, we aimed to test if the abundances of lichen functional groups on host trees with different circumference (age), light transmitted through the canopy and bark pH were independent of contamination by nitrogen compounds.

Methods

Study area

The Po Plain area in northwest Italy is one of the most altered regions in Europe (Erisman et al. 2011), because of its dense population (approx. 20 million people in approximately 40,000 km^2). This area is a plain surrounded by the Alps, a geomorphology that exacerbates the effects of pollutants. From the beginning of the industrial period to the 1980s, the concentrations of several gaseous pollutants (incl. NO_x and SO_2) increased to very high levels, which largely exceeded European thresholds for atmospheric pollutants. Till 1990 the emissions of SO_2 in the Province of Alessandria (Piedmont), where this study was carried out, exceeded 6000 tons yr^{-1} and NO_x emissions were close to 20,000 tons yr^{-1}. Like in many other industrial areas around the world, the lichen flora of the Po Plain was strongly affected by these pollutants. Lichen communities almost completely disappeared from large areas of the plain (Isocrono et al. 2007), and also showed drastic declines in remote areas. In the last century, most sensitive oligotrophic species (e.g. *Lobaria pulmonaria*, *Nephroma parile*) have disappeared from large areas of the Alps.

For this study, an area in the Po Plain (Piedmont Region, Province of Alessandria) of approximately 90 km^2 was selected. The loads of eutrophication pollution in the area are quite variable, ranging from sites with very intensive agriculture, mainly sweet corn crops near the city of Alessandria, to other sites with low-intensity agriculture, open woodlands and prairies close to the Ligurian Apennines. Kilometric estimated emissions of the main pollutants (ammonia; sulphur dioxide; nitrogen oxides and particulate matter) were obtained from the Piedmont Regional Emission Database (Regione Piemonte 2012). Details of the emissions are shown in Table I. The average yearly precipitation in the study area ranges from 923 to 1074 mm, whereas annual mean temperature ranges from 11.5 to 13°C.

Sampling design

In order to ensure an effective pollution gradient a plot-less sampling design was applied selecting 20 pairs of coordinates by means of a stratified random sampling, in which the survey areas was subdivided into two strata on the basis of the total pollution load.

During the field work, selected points were located using a GPS receiver. At each point, the nearest 4 trees were selected for analysis. In particular, the trees were selected on the basis of their known chemical–physical characteristics of the bark, according to the categorization suggested by Asta et al. (2002): with neutro-basic bark, including that of *Populus spp.*, *Acer spp.* and sub-acid bark, mainly that of deciduous *Quercus spp.* Therefore, at each coordinate two trees for each category were selected, leading to a total of 80 trees analysed.

Lichen sampling

For each tree, the abundance of lichen species was sampled using a sampling grid consisting of a 10 × 50 cm ladder divided into five 10 × 10 cm quadrats. This grid ladder was systematically placed on N, E, S and W sides of the trunk, with the top edge at 1.5 m above the ground, following the standards suggested by Asta et al. (2002). The Lichen Diversity Value (LDV – Asta et al. 2002) was calculated as the sum of the abundance of each species (i.e. number of 10 × 10 quadrats in which the species was recorded) within the subunits on a tree.

To calculate the diversity of functional groups, species were grouped according to nitrogen-tolerance

Table I. Descriptive statistics of response and predictive variables used in this study. Pollutants are reported as estimated kilometric emissions in the study area in 2007 (Regione Piemonte 2012).

Response variables	
LDVnitro	22.1 ± 24.9 (0–113)
LDVoligo	10.0 ± 13.1 (0–42)
Tree-related variables	
Tree circumference (cm)	102.6 ± 64.4 (41–285)
Bark pH	6.03 ± 0.40 (5.28–7.46)
Light (MJ m^{-2} day^{-1})	7.36 ± 4.65(1.65–26.57)
Pollutants	*Total emissions* (*t km^{-2} yr^{-1}*)
NH_3	0.93 ± 0.04 (0.005–3.79)
NOx	1.01 ± 0.04 (0–10.03)
PM10	0.14 ± 0.01 (0–0.94)
SO_2	0.04 ± 0.003 (0–0.42)

Note: Values shown are mean ± standard deviation (minimum–maximum).

(Appendix 1) using a priori classification (Nimis & Martellos 2008). The classification uses a 5-class ordinal scale, where the value 1 is given to lichen species associated to sites with no eutrophication, whereas the value 5 corresponds to species which tolerate a very high eutrophication. For the purpose of this work, the maximum tolerance of each species was considered; that is, the highest value within the range given by the classification. Species classified with 1–2 were considered as oligotrophic and those with 4–5 were considered as nitrophytic. From this data, the LDV (Asta et al. 2002) were calculated based on functional characteristics, obtaining the abundance of 26 oligotrophic (LDVnitro) and 19 nitrophytic species (LDVnitro). Because the target of the paper was on the two contrasting functional groups (nitro vs. oligo), 23 mesophytic species (i.e. those with a maximum value = 3 for nitrogen-tolerance) were excluded by this analysis.

Tree-related environmental predictors

Tree circumference. For each tree, the tree circumference at 130 cm on the soil level was measured with a tape, as a possible predictive variable for the epiphytic lichen diversity (Giordani 2006; Cristofolini et al. 2008).

Bark pH. Bark pH was determined using a modified version of the protocol described by Johnsen and Søchting (1973). A superficial bark sample, 2 mm thick, was selected from each tree, cleared of epiphytes and pulverized using an electric stainless coffee grinder. A portion of the pulverized bark (500 mg) was added to 20 ml distilled water and incubated for 8 h with agitation. The bark pH was measured using a Crison Basic 20 pH meter (Crison Instruments, Alella, Spain) with a combined electrode.

Light. Light was estimated in terms of solar radiation at each tree by analysis of hemispherical photographs with Gap Light Analyzer (GLA) software (Frazer et al. 1999). The hemispherical photograph approach provides an estimate of solar radiation over long periods of time at different sampling units, and takes into account the local variations in site morphology (e.g. slope and aspect) together with canopy coverage.

In the field, the images were obtained using a Canon EOS 350D reflex digital camera with a Sigma 15 mm F. 2.8 fisheye lens. The camera was mounted horizontally on a tripod close to the tree, pointed to the zenith and oriented towards magnetic north. To maximize contrast in low-light conditions, the film speed was set to ISO 400 to ensure high resolution of images. Images were acquired as colour JPEG files and were subsequently converted into greyscale 4272 × 2848-pixel images.

In the laboratory, the files were processed using GLA software. After the registration process, which identified the geographic orientation and the circular extent of the hemispherical image, a threshold value was set to discriminate between canopy and clear sky pixels and to calculate gap light transmission data. For each subplot, estimations of total transmitted solar radiation were calculated in MJ m^{-2} day^{-1}, based on a 2-min solar time step between sunrise and sunset for the full length of the growing season (which was variable, depending on the presence of deciduous or evergreen trees at the sampling unit). These values were computed by calculating the beam fraction as the ratio of direct (beam, H^b) to total (global, H) spectral radiation incident on a horizontal surface at the ground over the specified period (Frazer et al. 1999), as follows:

$$\frac{H_b}{H} = \left[1 - e^{-(3.044\,K_t^{2.436})}\right] \quad (1)$$

where H was obtained as monthly averages of direct measurements from the closest meteorological station (Alessandria) from 1990 to 2005; K^t is the cloudiness index (Iqbal 1983), calculated as $K^t = H/H_0$ and H_0 is the extraterrestrial radiation calculated for each sampling unit with GLA, setting latitude, longitude, elevation, slope, aspect and position of magnetic north at the date of assessment.

To better estimate photosynthetically active radiation at each sampling unit, the spectral fraction (R^p/R^s: the fraction of global solar radiation incident on a horizontal surface at the ground falling within a limited range of the electromagnetic spectrum) was calculated as follows:

$$\frac{R_p}{R_s} = \left[1 - e^{-(0.499\,K_t^{-0.219})}\right] \quad (2)$$

Additionally, as a correction parameter for sky–region brightness, the clear-sky transmission coefficient (T) was set at 0.77, according to bibliographic data reported by Bellocchi et al. (2002) for the study area.

Data analyses

Correlations among variables. In order to explore the patterns of predictive and response variables, Spearman r correlations and biplot graphs were

calculated using the software package Statistica Version 8.0 (StatSoft, Tulsa, OK).

Non-metric multidimensional scaling. A global non-metric multidimensional scaling (NMS) (Kruskal 1964) with Sørensen distance was used, in order to detect the main trends of variability of lichen communities in the survey area. The analysis was performed with PC-ORD version 4.25 (McCune & Mefford 1999), by using the abundances of the lichen species as response matrix. The second matrix included both pollutant and tree-related variables, together with the abundance of the two functional groups (LDVoligo and LDVnitro). Preliminarily to the analysis, some variables (i.e. NOx, SO_2, PM10, NH_3 emissions and the tree circumference) were log transformed.

Hierarchical partitioning. The relative importance of environmental predictors in explaining variations in lichen functional groups (oligotrophic vs. nitrophytic species) was evaluated using a hierarchical partitioning (HP) analysis (Chevan & Sutherland 1991). HP is a statistical method that provides explanatory power, rather than predictive. HP takes into account all possible models in a multiple regression and identifies the most likely causal factors. In the analysis, the variation explained by each variable is split into a joint effect together with the other explanatory variables and into an independent effect not shared with any other variable. The distribution of joint effects shows the relative contribution of each variable to shared variability in the full model. Negative joint effects are possible for variables that act as suppressors of other variables (Chevan & Sutherland 1991). HP is recommended when the predictors are significantly intercorrelated, in order to reduce the indirect effect of high correlation between descriptors on the response variable.

Independent effects of variables operate in two modes, additively or suppressively. The correlations between two variables determine whether the effect of the variable is additive or suppressive. When the majority of variables are additive, the joint effect will be positive, and when the majority are suppressive, it will be negative. The HP was conducted using the Hier.Part package (version 1.0-3; Walsh & McNally 2008) implemented in R version 2.3 (R Development Core Team 2012). The estimated relative importance of each variable was represented by the size of its pure effect.

GLMM model selection. Eighteen possible GLMM models were formulated using the most informative predictive variables (i.e. those with the largest independent effects), as selected by HP, for explaining variation on LDVnitro and LDVoligo. Models were created by considering the single variables and the main interactions between tree circumference and pollutant variables. In each model, the site location was considered as the random effect. The Akaike Information Criterion (AIC) (Akaike 1979) was calculated for each model, using lme4 package in R version 3.0 (R Development Core Team 2012). To rank the models in order from the best to the worst fit, for each of the possible 18 models a ΔAIC was also calculated as the difference in AIC between the given model and the model with the smallest AIC. This procedure was carried out for both response variables (LDVoligo and LDVnitro).

Results

Spearman correlations

Both response variables showed statistically significant Spearman r correlations with pollutants (positive for LDVnitro and negative for LDVoligo) (Table II and Figure 1). The pollutant emissions also showed high and significant correlations among them, whereas no statistically significant correlations were observed among tree-related variables, between tree-related variables and pollutants and between the former and LDVs.

Non-metric multidimensional scaling

A NMS analysis was run in autopilot mode, comparing 1- to 6-dimensional solutions. The best

Table II. Spearman rank order correlations between response and predictive variables collected in the study area.

	LDVoligo	LDVnitro	Light	pH	Circ	NH_3	NOx	PM10
LDVnitro	**−0.66**							
Light	0.01	−0.11						
pH	0.01	0.11	0.14					
Circumference	−0.02	0.14	0.00	0.11				
NH_3	**−0.69**	**0.61**	−0.04	−0.15	0.15			
NOx	**−0.67**	**0.62**	−0.05	−0.15	0.17	**0.92**		
PM10	**−0.68**	**0.64**	−0.10	−0.12	0.16	**0.90**	**0.97**	
SO_2	**−0.70**	**0.65**	−0.08	−0.11	0.14	**0.87**	**0.95**	**0.98**

Note: Values in bold represent statistically significant correlations at $p < 0.05$.

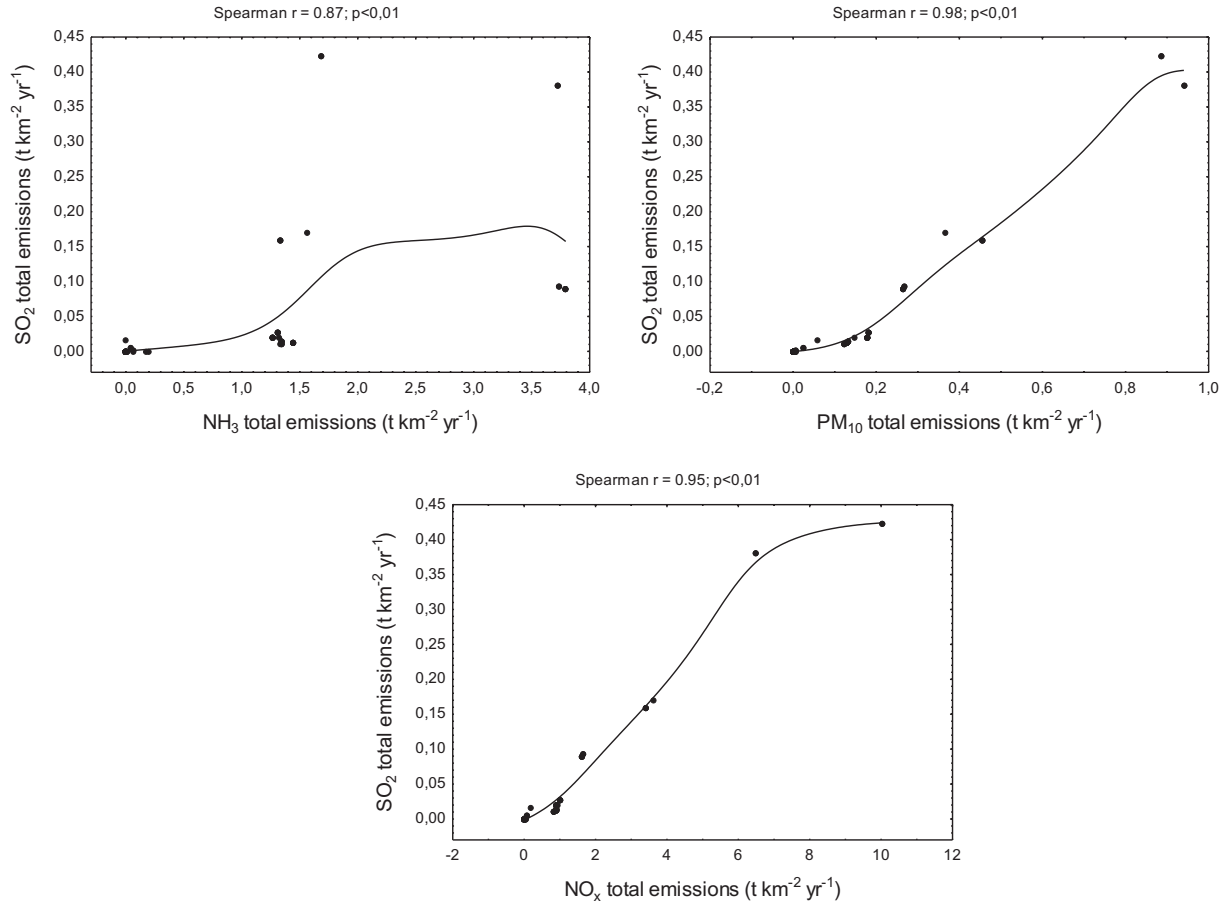

Figure 1. Biplots of the SO_2 emissions and the emissions of the eutrophication compounds in the study area.
Note: The fitted line is a distance weighted least square function with stiffness 0.2.

solution was a three-dimensional configuration (maximized difference between the best of 40 runs of real data and 50 randomized runs, $p < 0.05$ from Monte Carlo test; average stress = 18.5) (Figure 2). Cumulative Pearson r^2 between distances in the original space and distances on the three ordination axes was 0.710. The axis with the highest r^2 (0.275) was labelled Axis 1, followed by Axis 2 (0.254). Axis 3 (not shown) had the lowest r^2 (0.181). LDVoligo decreased along the axis 1 in contrast with a negative gradient of the considered pollutants (NOx, PM10, NH_3 and SO_2) and an increased LDVnitro. The second axis was also partially associated to LDVnitro and to the tree-related predictors (bark pH, light and tree circumference), these latter showing very low correlation with all axes. Similarly, nitrophytic species were mainly associated with positive values of axis 1 and negative values of axis 2, whereas oligotrophic species showed negative scores on axis 1. Only some crustose species laid for positive values of axis 2.

Hierarchical partitioning

Models including the independent effects of all statistically significant predictors on both LDVnitro and LDVoligo had R^2 values of 0.694 and 0.521, respectively. They included the emissions of all considered pollutants (NH_3, NO_x, SO_2 and PM_{10}). Among the tree-related parameters, only circumference was statistically significant, even though it explained a very low amount of variability (ca. 2% for both LDVnitro and LDVoligo). The relative contributions of pollutants to independent effects on LDVnitro and LDVoligo were fairly similar, ranging from 10% (NO_x) to 20% (NH_3 and PM10), although the pollutants had a positive effect on the former and a negative effect on the latter functional group (Figure 3). Ammonia showed positive joint effects with the other predictors (approx. corresponding 21%). A proportion of joint effects (approx. 10%) was associated with both NO_x and PM10, whereas SO_2 accounted for -6% of joint effects, showing antagonistic interactions when other predictors were considered.

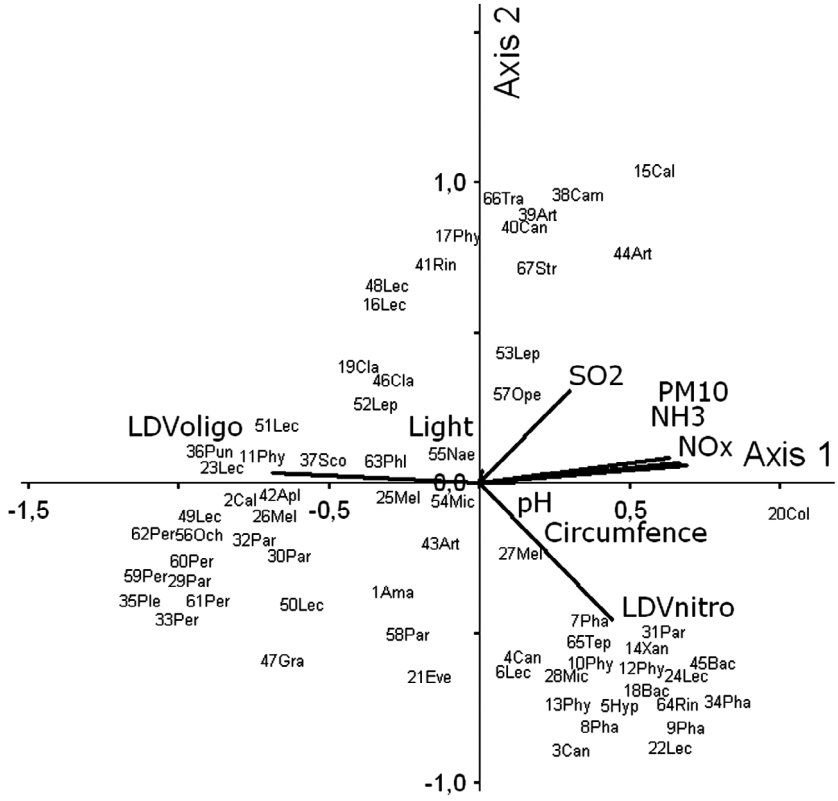

Figure 2. NMS ordination of plots based on lichen species composition, showing the relationship between predictive variables with the abundance of oligotrophic and nitrophytic species (LDVnitro and LDVoligo) along the first two axes. Lengths of arrows for predictive factors represent strength of correlations; directions represent signs. Species codes are reported in Appendix 1.

GLMM

As a support to the results of HP, the model including SO_2 emissions and the interactions between the emissions of eutrophication pollutants ranked highest both for LDVoligo and LDVnitro as response variables (Table III). In both cases, when including also the tree circumference in the model similar results were obtained, the ΔAIC being 0.9 and 7.9 for LDVnitro and LDVoligo, respectively. SO_2 (for LDVnitro) and NH_3 (for LDVoligo) produced the best models with a single variable, but in both cases ΔAIC with respect to the best models were >60.

Discussion

In the present study, the abundances of lichen functional groups were compared by analysing data from an area subjected to gradients of emissions of eutrophication substances.

The questions at the basis of this research were if contrasting lichen functional groups for nitrogen requirement (oligotrophic vs. nitrophytic species) are still effective indicators under a dynamic and co-varying pollution regime of declining SO_2 and increasing N and if their response to eutrophication is robust within a spectrum of microhabitat variability (i.e. tree-related factors). As a result, the effects of tree-related factors were negligible, whereas lichen groups were strongly related to the emissions of main gaseous pollutants, with particular reference to eutrophication compounds, such as NH_3, PM10 and NOx. This is consistent with our previous findings (Giordani, Matteucci et al. 2014) showing that the combined effects of pollutants on the diversity of lichen morpho-functional groups were prevalent, while stand-related factors were more closely associated to overall diversity. In the present study, under high emissions of eutrophication substances, nitrophytic species were generally more abundant than oligotrophs. As a consequence, the responses of lichen communities were highly homogeneous and, within each functional group, there were no significant differences in relation to bark pH, trunk circumference or light availability. That is, the lichen abundance was comparable between sub-acidic and sub-neutral bark, and between small and large trees.

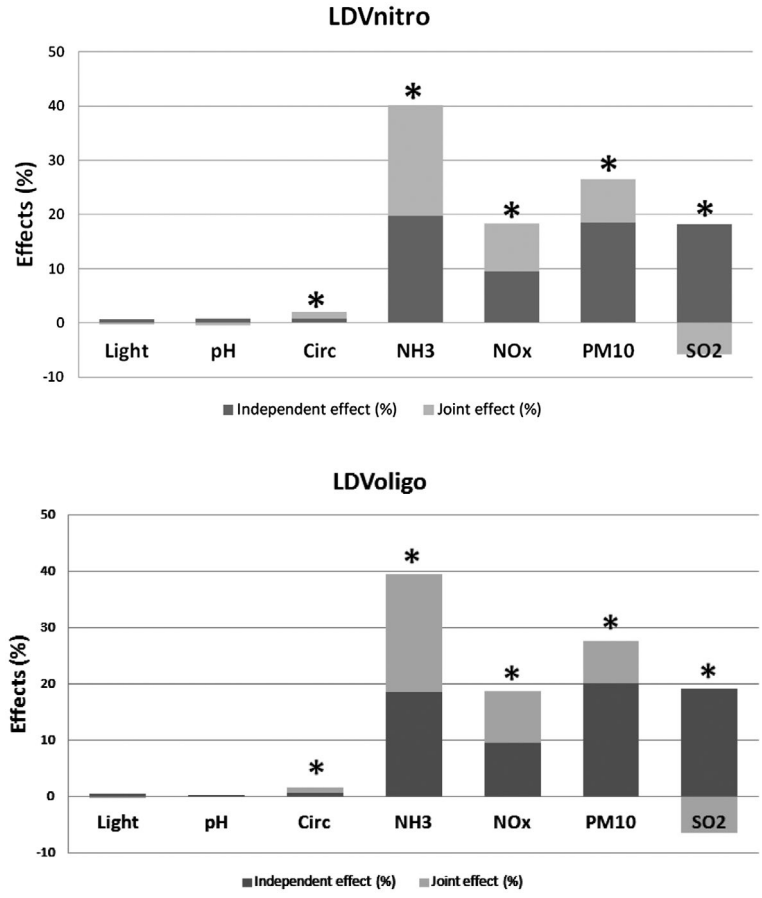

Figure 3. Hierarchical partitioning of independent (dark grey) and joint effects (light grey) of predictors on LDVnitro and LDVoligo. Asterisk (*) marks statistically significant predictors.

Eutrophication as major driver of lichen functional groups

It is still debated whether nitrophytic lichens primarily respond to bark pH, to ammonia, or to other eutrophication compounds (Frati et al. 2007; Pinho et al. 2008; Spier et al. 2010). In their study on a Mediterranean woodland, Pinho et al. (2011) found that atmospheric ammonia concentrations explained approximately 90% of the lichen species distribution. In contrast, Jovan et al. (2012) reported that nitrophytic species did not respond specifically to NH_3, nor did their responses necessarily depend on the pH of the tree substrates. These authors found that nitrophytes reacted to total nitrogen deposition, indicating that they respond to multiple forms of nitrogen. The results of the present study support this latter hypothesis. In fact, there was a high contribution of independent effects of pollutants driving the variations in nitrophytic and oligotrophic lichen species. In quantitative terms, lichens responded similarly to several eutrophication compounds. Moreover, some variability was attributed to joint effects of pollutants. Although the pollutant emissions in the study area were highly inter-correlated, the HP analysis allowed to discriminate among independent effects of each pollutant. Ammonia, PM10 and SO_2 each accounted for approximately 20% of independent effects, while NO_x made a relatively smaller contribution. Thus, despite the considerable decreases in atmospheric SO_2, it still affected the composition of lichen communities in the area. Notably, HP showed that SO_2 was the only predictor showing antagonistic joint effects with other factors, confirming that it partially suppresses the effects of other variables (Chevan & Sutherland 1991). The detectability of independent effects of several pollutants is a critical issue for biomonitoring methods. Its reliability is affected by the close connections among physiological mechanisms of tolerance/sensitivity to different pollutants (Hauck 2010). For example, the low tolerance of some oligotrophic lichens to reduced nitrogen compounds (especially ammonium) is because they have a limited potential to compensate for higher nitrogen-compound levels with higher carbon assimilation. In contrast, nitrophytic species show increased photosynthetic capacity in response to high ammonium levels (see Hauck 2010 for a review). Nitrate assimilation is similarly constrained,

Table III. Rankings of the GLMM models tested for their influence on LDVnitro and LDVoligo in the study area, determined from likelihood measures.

Model	RankAIC	AIC	DeltaAIC	Random effect std. dev.	
				Site	Residual
Response variable: LDVnitro					
NOx * PM10 * NH_3 + SO_2 + (1 | Site)	1	640.8	0.0	11.23	15.56
Circumference + NOx * PM10 * NH_3 + SO_2 + (1 | Site)	2	641.7	0.9	11.35	15.43
Circumference + PM10 + SO_2 + (1 | Site)	3	685.1	44.3	8.29	16.99
Circumference + NOx + SO_2 + (1 | Site)	4	700.5	59.7	17.34	16.14
Circumference + NH_3 + SO_2 + (1 | Site)	5	700.5	59.7	17.23	16.02
SO_2 + (1 | Site)	6	701.6	60.8	19.77	15.93
NH_3 + (1 | Site)	7	702.1	61.3	18.00	15.84
PM10 + (1 | Site)	8	702.5	61.7	19.11	15.98
Circumference + SO_2 + (1 | Site)	9	707.1	66.3	19.18	15.96
NOx + (1 | Site)	10	707.3	66.5	18.95	15.96
Circumference + NH_3 + (1 | Site)	11	707.6	66.8	17.40	15.87
Circumference + PM10 + (1 | Site)	12	707.9	67.1	18.51	16.01
Circumference + SO_2 + interaction + (1 | Site)	13	709.2	68.4	19.28	16.02
Circumference + PM10 + interaction + (1 | Site)	14	711.4	70.6	18.64	16.04
Circumference + NOx + (1 | Site)	15	712.7	71.9	18.95	15.96
Circumference + (1 | Site)	16	714.0	73.2	19.08	15.88
Circumference + NH_3 + interaction + (1 | Site)	17	714.2	73.4	17.31	16.01
Circumference + NOx + interaction + (1 | Site)	18	721.2	80.4	19.01	16.06
Response variable: LDVoligo					
NOx*PM10*NH_3 + SO_2 + (1 | Site)	1	552.7	0.0	0.00	9.74
Circumference + NOx * PM10 * NH_3 + SO_2 + (1 | Site)	2	560.6	7.9	0.00	9.79
Circumference + PM10 + SO_2 + (1 | Site)	3	596	43.3	0.00	10.21
NH_3 + (1 | Site)	4	613.4	60.7	4.60	10.34
Circumference + NH3 + SO_2 + (1 | Site)	5	615.9	63.2	4.71	10.47
PM10 + (1 | Site)	6	617.6	64.9	5.38	10.70
SO_2 + (1 | Site)	7	620.1	67.4	7.20	10.54
Circumference + NH_3 + (1 | Site)	8	621.5	68.8	4.58	10.42
Circumference + NOx + SO_2 + (1 | Site)	9	621.8	69.1	5.48	10.86
NOx + (1 | Site)	10	624.90	72.2	6.54	10.61
Circumference + PM10 + (1 | Site)	11	625.7	73.0	5.36	10.79
Circumference + NH_3 + interaction + (1 | Site)	12	627.2	74.5	4.33	10.42
Circumference + SO_2 + (1 | Site)	13	628.20	75.5	7.24	10.61
Circumference + PM10 + interaction + (1 | Site)	14	630.3	77.6	5.46	10.82
Circumference + SO_2 + interaction + (1 | Site)	15	631.4	78.7	7.24	10.68
Circumference + NOx + (1 | Site)	16	632.9	80.2	6.56	10.69
Circumference + (1 | Site)	17	636.10	83.4	8.28	10.43
Circumference + NOx + interaction + (1 | Site)	18	642.3	89.6	6.60	10.75

because carbon skeletons must be available to bind nitrogen and avoid the excess accumulation of toxic free intracellular ammonium after reduction.

The influence of tree-related factors

Bark pH did not drive the abundance of lichen functional groups in the study area. Although this factor is generally considered as a major driver of epiphytic lichen communities at tree level (e.g. Barkman 1958), recent outcomes suggested that most of variation of the communities at this scale of observation cannot be explained by the pH alone. For example, Spier et al. (2010) found that the tree species more than bark pH alone was more important in determining the composition of acidophytic and nitrophytic communities. These authors suggested that changes in pollution conditions over recent decades could have made epiphytic lichens less sensitive to pH. On the other hand, it could be supposed that bark pH and, more generally, bark-related factors might affect the composition on epiphytic communities at smaller scale, so that abundances of functional groups may vary within the same tree. Even though this paper does not take into account such a small-scale variability, it supports the idea that local pollution regime and tree-related factors interact in driving the relative abundance of lichen functional groups. In fact, the bark pH values observed in this study are generally high (>5), and consistent with an eutrophication effect having caused, in the recent past, an increase in natural values usually observed on sub-acid barks. As a consequence, pH could have been raised up to values higher than optimum for oligotrophic species which are actually rather rare in the study area.

Notably, in this study, the independent effects of tree circumference were considerably low. The weak but significant contribution of trunk circumference (which could be considered as a proxy variable for tree age) on the abundance of lichen functional groups may be related to the dynamics of lichen colonization under a scenario of changing atmospheric pollution. Generally, tree age is expected to have a relevant effect on the diversity and the composition of lichen communities (e.g. Nascimbene et al. 2009; Marmor et al. 2011). Wolseley et al. (2006) suggested

that lichens on tree trunks may have maintained relict lichen communities, either because of previous acidification or ecological continuity of lichens. In contrast, lichen communities on younger substrates, such as twigs, would respond more rapidly to recent changes in ammonia concentrations. In the Po Plain, lichen communities were severely affected by very high concentrations of atmospheric SO_2 that have persisted in this area since the beginning of the industrial period (Isocrono et al. 2007). In recent years there has been a drastic decrease in SO_2, after which lichen communities showed diverging successions depending on the amount of eutrophication pollutants (e.g. PM10 and ammonia) (Giordani et al. 2013; Giordani, Matteucci et al. 2014).

In the study area many young and old trees with both types of bark pH are now subjected to high levels of nitrogen atmospheric emissions. As a result, these trees provide substrates suitable only for highly tolerant nitrophytic species, and consequently the lichen flora encountered was mostly eutrophic. The more sensitive oligotrophic taxa cannot colonize tree trunks in these conditions, in spite of the decreased concentrations of atmospheric SO_2. The high level of atmospheric SO_2 was likely to be the main reason for the disappearance of these communities in the past and, consequently, for the small variation of lichen functional groups in relation to tree age in the area.

The total amount of through-canopy solar radiation had weak effects on the abundances of oligotrophic and nitrophytic species poorly depended on. Similarly, in European forest ecosystems, Giordani et al. (2012), Giordani, Calatayud et al. (2014) observed oligotrophs uncorrelated with predictors describing the light availability under the tree canopy, such as the stem density and basal area. In other cases it was observed that light favoured nitrophytic species and limited oligotrophs (Hauck 2010). In non-eutrophicated areas, lacking sufficient amounts of nitrogen compounds and/or alkaline dusts, nitrophytic lichens are usually observed at sites where sufficient solar radiation is available (Cristofolini et al. 2008). In some cases it was observed a filtering effect of the canopy, thought that the through-fall nitrogen depositions also depend on the canopy surface area, and differences may be observed between hardwood vs. conifer forests (Jovan et al. 2012; Fenn et al. 2013).

Conclusions

From an application perspective, this work provides information to support the development of sampling protocols for lichen biomonitoring of air pollution and to interpret the results in terms of relative contribution of functional groups of nitrogen tolerance. Most sampling protocols recommend a distinction between tree species and/or bark pH categories when selecting trees for sampling in biomonitoring surveys (e.g. Asta et al. 2002; Giordani & Brunialti 2015), based on the assumption that sub-neutral barks might promote lichen colonization even under high atmospheric pollution. According to the results of the present study, this hypothesis should be rejected since differences in bark pH did not significantly differentiated the composition of epiphytic lichen communities. However, more data are needed to investigate if this response is robust under lower level of eutrophication or considering tree species with more acid bark. The HP of variance confirmed that lichen functional groups for nitrogen-tolerance responded to several atmospheric pollutants, with both independent and joint effects. In terms of interpreting biomonitoring results, low diversity data obtained in non-eutrophicated areas should be carefully interpreted, as they could reflect phenomena related to the community succession on new available substrates, rather than the effects of present contamination. Lichen functional groups are confirmed as a robust tool for biomonitoring the effects of atmospheric pollution, but the consistency of their responses throughout environmental gradients, especially at very local scale, seems to be affected by a set of confounding factors, so that the framework of application of this approach has still to be clarified.

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Appendix 1.Taxon list for lichens found in the survey area and categorization into three functional groups for nitrogen tolerance, according to the classification by Nimis & Martellos (2008).

Code	Taxon	Functional group
1Ama	*Amandinea punctata* (Hoffm.) Coppins & Scheid.	nitro
42Apl	*Aplotomma turgida* (A.Massal.) A.Massal.	oligo
39Art	*Arthonia didyma* Körb.	meso
43Art	*Arthonia punctiformis* Ach.	oligo
44Art	*Arthopyrenia grisea* (Schaer.) Körb.	oligo
18Bac	*Bacidia rubella* (Hoffm.) A.Massal.	meso
45Bac	*Bactrospora patellarioides* (Nyl.) Almq.	oligo
15Cal	*Caloplaca cerinella* (Nyl.) Flagey	nitro
2Cal	*Caloplaca pyracea* (Ach.) Th.Fr.	nitro
3Can	*Candelaria concolor* (Dicks.) Stein	nitro
4Can	*Candelariella reflexa* (Nyl.) Lettau	nitro
40Can	*Candelariella xanthostigma* (Ach.) Lettau	meso
19Cla	*Cladonia fimbriata* (L.) Fr.	meso
46Cla	*Cladonia parasitica* (Hoffm.) Hoffm.	oligo
20Col	*Collema nigrescens* (Huds.) DC.	meso
21Eve	*Evernia prunastri* (L.) Ach.	meso
47Gra	*Graphis scripta* (L.) Ach.	oligo
5Hyp	*Hyperphyscia adglutinata* (Flörke) H.Mayrhofer & Poelt	nitro
22Lec	*Lecania cyrtella* (Ach.) Th.Fr.	meso
48Lec	*Lecanora albella* (Pers.) Ach.	oligo
23Lec	*Lecanora allophana* Nyl.	meso
49Lec	*Lecanora argentata* (Ach.) Malme	oligo
53Lec	*Lecanora carpinea* (L.) Vain.	oligo
6Lecc	*Lecanora chlarotera* Nyl.	nitro
50Lec	*Lecanora expallens* Ach.	oligo
24Lec	*Lecanora hagenii* (Ach.) Ach.	nitro
51Lec	*Lecanora pulicaris* (Pers.) Ach.	oligo
16Lec	*Lecidella elaeochroma* (Ach.) M.Choisy	nitro
52Lep	*Lepraria incana* (L.) Ach.	oligo
26Mel	*Melanelixia fuliginosa* (Duby) O. Blanco, A. Crespo, Divakar, Essl., D. Hawksw. & Lumbsch	meso
27Mel	*Melanelixia subaurifera* (Nyl.) O. Blanco, A. Crespo, Divakar, Essl., D. Hawksw. & Lumbsch	meso
25Mel	*Melanohalea elegantula* (Zahlbr.) O.Blanco, A.Crespo, Divakar, Essl., D.Hawksw. & Lumbsch	meso
28Mic	*Micarea denigrata* (Fr.) Hedl.	meso
54Mic	*Micarea prasina* Fr.	oligo
55Nae	*Naetrocymbe punctiformis* (Pers.) R.C.Harris	oligo
56Och	*Ochrolechia subviridis* (Høeg) Erichsen	oligo
57Ope	*Opegrapha celtidicola* (Jatta) Jatta	oligo
29Par	*Parmelia saxatilis* (L.) Ach.	meso
30Par	*Parmelia sulcata* Taylor	meso
31Par	*Parmelina quercina* (Willd.) Hale	meso
32Par	*Parmelina tiliacea* (Hoffm.) Hale	meso
58Par	*Parmotrema perlatum* (Huds.) M.Choisy	oligo
33Per	*Pertusaria amara* (Ach.) Nyl.	meso
59Per	*Pertusaria flavida* (DC.) J.R.Laundon	oligo
60Per	*Pertusaria leioplaca* DC.	oligo
61Per	*Pertusaria pertusa* (Weigel) Tuck.	oligo
62Per	*Pertusaria pustulata* (Ach.) Duby	oligo
7Pha	*Phaeophyscia hirsuta* (Mereschk.) Essl.	nitro
34Pha	*Phaeophyscia insignis* (Mereschk.) Moberg	meso
8Pha	*Phaeophyscia nigricans* (Flörke) Moberg	nitro
9Pha	*Phaeophyscia orbicularis* (Neck.) Moberg	nitro
63Phl	*Phlyctis argena* (Spreng.) Flot.	oligo
10Phy	*Physcia adscendens* (Fr.) H.Olivier	nitro
11Phy	*Physcia stellaris* (L.) Nyl.	nitro
12Phy	*Physcia tenella* (Scop.) DC.	nitro
17Phy	*Physcia vitii* Nádv.	nitro
38Phy	*Physconia distorta* (With.) J.R.Laundon	nitro
13Phy	*Physconia grisea* (Lam.) Poelt ssp. *grisea*	nitro
59Phy	*Physconia perisidiosa* (Erichsen) Moberg	meso
35Ple	*Pleurosticta acetabulum* (Neck.) Elix & Lumbsch	meso
36Pun	*Punctelia subrudecta* (Nyl.) Krog	meso
41Rin	*Rinodina pyrina* (Ach.) Arnold	meso
64Rin	*Rinodina sophodes* (Ach.) A.Massal.	oligo
37Sco	*Scoliciosporum umbrinum* (Ach.) Arnold	meso
67Str	*Strigula stigmatella* (Ach.) R.C.Harris	oligo
65Tep	*Tephromela atra* v. *torulosa* (Flot.) Hafellner	oligo
66Tra	*Trapeliopsis flexuosa* (Fr.) Coppins & P.James	oligo
14Xan	*Xanthoria parietina* (L.) Th.Fr.	nitro