Using lichen functional diversity to assess the effects of atmospheric ammonia in Mediterranean woodlands

Pedro Pinho[1], Teresa Dias[1], Cristina Cruz[1], Y. Sim Tang[2], Mark A. Sutton[2], Maria-Amélia Martins-Loução[1,3], Cristina Máguas[1] and Cristina Branquinho[1]*

[1]*Universidade de Lisboa, Faculdade de Ciências, Centro de Biologia Ambiental, FCUL, Ed. C2, 5° Piso, Campo Grande, 1749-016 Lisboa, Portugal;* [2]*Centre for Ecology and Hydrology, Edinburgh Research Station, Bush Estate, Penicuik, Midlothian, EH26 0QB, Scotland; and* [3]*Universidade de Lisboa, Museu de História Natural, Jardim Botânico, R. Escola Politécnica, 58 1250-102 Lisboa, Portugal*

Summary

1. Atmospheric ammonia (NH_3) is one of the main drivers for ecosystem changes world-wide, including biodiversity loss. Modelling its deposition to evaluate its impact on ecosystems has been the focus of many studies. For that, universal indicators are needed to determine and compare the early effects of NH_3 across ecosystems.

2. We evaluate the effects of atmospheric NH_3 in ecosystems using lichens, which are one of the most sensitive communities at the ecosystem level. Rather than measuring total diversity, we use a functional diversity approach because this is potentially a more universal tool.

3. We evaluated the spatial and temporal patterns of atmospheric NH_3 concentrations ($[NH_3]_{atm}$) emitted from a point-source over a 1-year period in a cork oak Mediterranean woodland. We observed a temporal pattern of $[NH_3]_{atm}$, with maximum concentrations during autumn.

4. The distribution of lichen species was *c.* 90% explained by $[NH_3]_{atm}$. The tolerance of lichen species to atmospheric NH_3, based on expert knowledge from literature, was tested for the first time against direct measurements of atmospheric NH_3. Most species were well classified, with the exception of *Lecanora albella* and *Chrysothrix candelaris*, which were more tolerant than expected. Our updated lichen classification can be used to establish lichen functional groups that respond to atmospheric NH_3, and these can be used in other Mediterranean countries.

5. Increasing $[NH_3]_{atm}$ led to a complete replacement of oligotrophic by nitrophytic species within 65 m of the NH_3 source. The geostatistical analysis of functional diversity variables yielded a spatial model with low non-spatial variance, indicating that these variables can cope robustly with high spatial variation in NH_3.

6. *Synthesis and applications.* Our results support the use of functional diversity variables, such as a lichen diversity value, as accurate and robust indicators of the effects of atmospheric NH_3 on ecosystems. The spatial modelling of these indicators can provide information with high spatial resolution about the effects of atmospheric NH_3 around point- and diffuse sources. As this methodology is based on functional groups, it can be applied to monitor both the impact of atmospheric NH_3 and the success of mitigation strategies.

Key-words: agriculture, atmospheric NH_3, biodiversity loss, cattle, ecological indicators, global change, spatial analysis

Introduction

Global increase in the availability of reduced nitrogen (i.e. NH_3 or NH_4^+) is considered a major threat to global biodiversity, and its impact is expected to increase world-wide (SCBD 2006). The effects of nitrogen (N) enrichment on world-wide ecosys-tem functions are highlighted by Rockstrom *et al.* (2009). They consider that anthropogenic interference with the N cycle has already exceeded a safety boundary, beyond which negative impacts on human activities could occur at continental or global scales. Most reduced N released to the atmosphere is emitted as atmospheric ammonia (NH_3) (Robarge *et al.* 2002). The main anthropogenic sources of atmospheric NH_3 are agri-cultural activities, mainly intensive farming with fertilizers and

*Correspondence author. E-mail: cmbranquinho@fc.ul.pt

intensive animal husbandry (Galloway *et al.* 2003; EPER, 2004). Livestock housing facilities are major point-sources of NH_3. Atmospheric NH_3 concentrations ($[NH_3]_{atm}$) near these facilities are high, decreasing exponentially with distance, reaching background values after a 1–2 km (Pitcairn *et al.* 1998, 2003; Sutton *et al.* 1998). However, little is known about the spatial and temporal patterns of NH_3 dispersion under a Mediterranean climate. Moreover, the effects of atmospheric NH_3 on ecosystems are largely unknown although impacts are being detected world-wide (Erisman, Grennfelt & Sutton 2003; Cape *et al.* 2009a; Bobbink *et al.* 2010), including biodiversity loss (Suding *et al.* 2005). Therefore, there is a strong need for reliable tools to assess the effect of $[NH_3]_{atm}$ on ecosystems.

Ecological indicators are measurable characteristics of the structure, composition or function of ecosystems (Niemi & McDonald 2004), which have been used to assess the effect of natural and anthropogenic disturbances. Ecological indicators can integrate the effects of disturbances and they can provide information with high spatial resolution. Their use is especially important in areas that lack chemical measurements, as in the case of atmospheric NH_3 in most Mediterranean areas (Cape *et al.* 2009b).

Lichens are symbiotic organisms widely used as ecological indicators to monitor the effects of environmental changes (Cislaghi & Nimis 1997; Giordani, Brunialti & Alleteo 2002; Augusto, Maguas & Branquinho 2009). Epiphytic bryophytes are an alternative very sensitive group (Larsen *et al.* 2007) but they are not so widespread, particularly in drier Mediterranean areas. Lichens are more widespread and are reported to be among the most sensitive organisms to atmospheric NH_3 (van Herk 1999; Wolseley *et al.* 2006a; Cape *et al.* 2009a). Previous work has related the presence of different lichen communities to $[NH_3]_{atm}$ (van Dobben & ter Braak 1999; Wolseley *et al.* 2006b) in other parts of the world. These studies have considered either total N deposition (Fenn *et al.* 2008) or have used short-term measurements of $[NH_3]_{atm}$ (over <1 month) and with insufficient samples to allow spatial modelling (Frati *et al.* 2006, 2007).

It has been shown (Pinho *et al.* 2009) that under increasing eutrophication, the richness and abundance of nitrophytic species increases and that of oligotrophic species decreases. This allows species to be grouped according to their eutrophication tolerance (Suding *et al.* 2005). An expert-knowledge classification is available for Mediterranean lichen species (Nimis & Martellos 2008) but it has never been tested against a measured gradient of NH_3.

Spatial modelling of lichen diversity as an ecological indicator requires an average value from *c.* 12 trees around a site (Asta *et al.* 2002; Pinho *et al.* 2008b). This is possible when the main environmental factor under study is relatively homogenous across sampling points at the same site, such as industrial pollution, forest management or climate. In those cases, lichens in nearby trees (e.g. <100 m apart) will be similar (Pinho *et al.* 2008a). However, $[NH_3]_{atm}$ changes over very short distances (tens of metres) with the largest variations occurring within 1 km from the source (Sutton *et al.* 1998). Sampling sites, even if located close to one another can be subject to very different $[NH_3]_{atm}$. Thus, averaging lichen variables from a number of

trees grouped *a priori* may result in very high variability over a short spatial range, leading to poor interpolation capabilities. Therefore, a new approach must be adopted when dealing with spatial information on lichen diversity to address the high variation in the $[NH_3]_{atm}$.

The use of geostatistics, namely calculation of variograms for variables, allows analysis of how variables are spatially distributed. Spatial continuity is the distance up to which the variable presents a spatial structure (Fortin & Dale 2005), indicating the range of the effect of the main environmental factors influencing that variable. The nugget effect is the proportion of the variable without a spatial structure at the spatial scale the measure was made. It can be used to determine uncertainty in variables at short spatial ranges (Fortin & Dale 2005), which is related to the unwanted effects of environmental variables, i.e. those that do not have a spatial structure at that sampling resolution (Soares 2000), such as the possible effects of tree size on lichens. Moreover, calculating variograms for variables allows determination of their anisotropy, i.e. a preferential direction in which spatial continuity is higher (Fortin & Dale 2005), which can be related to the effects of dispersion of the key environmental variables influencing the variables, such as pollutants.

Our objectives were to study the spatial and temporal characteristics of the dispersion of $[NH_3]_{atm}$ in Mediterranean-type ecosystems and also to determine its effects on lichen species and communities to validate the expert-knowledge classification of lichens into functional groups. Our aim was to contribute to the development of a robust and accurate method to map the effects of atmospheric NH_3 on Mediterranean-type ecosystems. We carried out the work in a Mediterranean semi-natural cork oak woodland (a Natura 2000 habitat, ALFA, 2004) growing in the vicinity of cattle-farming activities.

Lichen classification was confirmed for most species except two that were reclassified. The dispersion of $[NH_3]_{atm}$ changed over time and space and explained most of the variance of the lichen variables. Increasing $[NH_3]_{atm}$ leads to a complete replacement of oligotrophic by nitrophytic functional groups. The geostatistical analysis of lichen functional diversity variables confirmed the high robustness of these variables as ecological indicators of the effect of $[NH_3]_{atm}$. Lichen functional diversity can thus be used both to assess the current effects of atmospheric NH_3 and to determine the effectiveness of future mitigation strategies.

Materials and methods

The study area is located in Portugal, 30 km east of Lisbon (Fig. 1). To the northern edge of the cork oak *Quercus suber* L. woodland, *c.* 200 beef cattle are permanently housed in a barn (800 m²), which is roofed, with open sides; the animals can move over a small enclosure of 1000 m². This is the main source of $[NH_3]_{atm}$ in the area. The intensity of land use and the structure of the woodland are homogeneous, thus avoiding the possible interference effects of dust and light reported by other authors in Mediterranean areas (Loppi & Pirintsos 2000). There are areas of extensive pasture to the south-west of the woodland, mainly grazed by sheep. There are two motorways 11 km to the west and a military aviation training facility 2·5 km to the north. Background N deposition from NO_2 is *c.* 3 kg (N) ha^{-1} year^{-1}
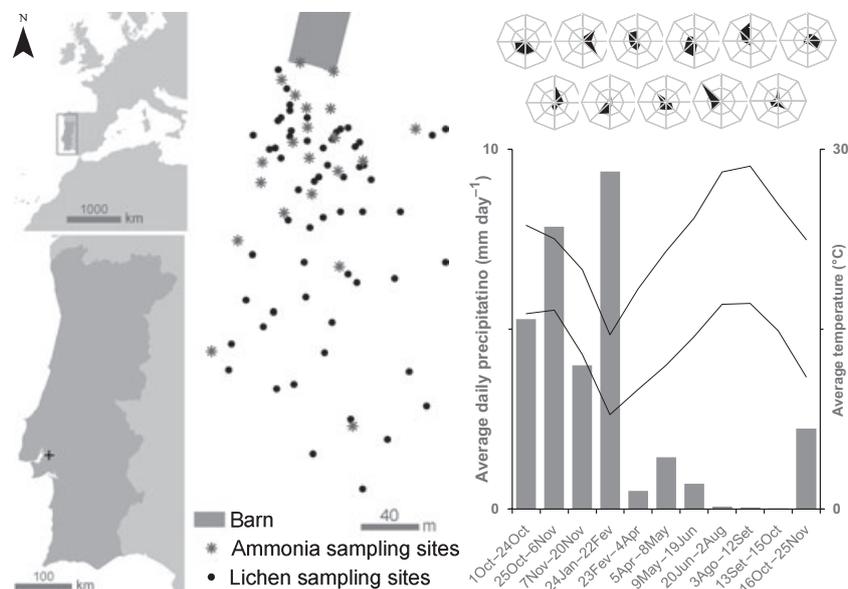
Fig. 1. Left: location of the study area in Europe and in Portugal (+); centre: location of the 21 atmospheric NH₃ and 74 lichen sampling sites; right: climate during the study period, from the nearest station (23 km to the West); climate for each sampling period: average daily precipitation (rectangles), averages of maximum and minimum temperatures (lines), wind direction in % hours with wind coming from each direction (the full octagon is 50%).

(EMEP 2008). Soils in the region are wind-deposited sandy alluvium, the average annual temperature is 17·5 °C, and the average annual precipitation is 600 mm (averages 1931–1960) (IA 2006). The study area represents a typical Mediterranean climate and is located in the thermomediterranean climatic belt (Rivas-Martinez & Rivas-Saenz 2009), characterized by dry, hot summers and rainy, mild winters.

AMMONIA AND LICHEN SAMPLING

Epiphytic lichens and $[NH_3]_{atm}$ were recorded in the cork oak woodland to the south of the cattle barn (Fig. 1). The $[NH_3]_{atm}$ was measured in eleven sampling periods from October 2006 to November 2007 (1–24 October; 25 October–6 November; 7–20 November; 24 January–22 February; 23 February–4 April; 5 April–8 May; 9 May–19 June; 20 June–2 August; 3 August–12 September; 13 September–15 October; 16 October–25 November). High-sensitivity adapted low-cost passive high absorption (ALPHA) passive diffusion samplers were used (Tang, Cape & Sutton 2001). Measurements were carried at 21 sampling locations (two ALPHA samplers at each point) at 2 m height, downwind of the cattle barn (Fig. 1). In some locations, additional field blanks (closed ALPHAs) were used for quality assessment purposes. Ammonia concentrations in these field blanks were always below the detection limit (i.e. not significantly different to zero), ensuring the atmospheric origin of the ammonia detected by the ALPHA samplers, which were exposed (open). Mean distance between NH₃ samplers was 28·1 m. Most sampling points were located within 200 m of the barn, because that is where the largest variation in concentrations was expected (Sutton *et al*. 1998).

The ALPHA samplers containing a citric-acid-impregnated cellulose fibre adsorbent (13% w/v) were colorimetrically analysed (Spectra Rainbow A-5082 spectrophotometer, Tecan, Männedorf, Switzerland) for ammonium (N-NH₄⁺) using a modified Berthelot reaction (Cruz & Martins-Loução 2000) at the University of Lisbon. At four sampling locations, additional ALPHA samplers provided and analysed by the Centre for Ecology and Hydrology in the UK using the Ammonium Flow Injection Analysis system (Sutton *et al*. 2001)

were set up in parallel with those prepared by the University of Lisbon for quality assurance purposes. The ratio of the $[NH_3]_{atm}$ determined by a Delta System (Denuder for Long-Term Atmospheric sampling) to the ALPHAS ranged between 0·87 and 0·85 for the sampling points nearer the ammonia source. The $[NH_3]_{atm}$ values were expressed in µg m⁻³.

Epiphytic lichens on the main trunks of cork oak trees were sampled using a grid according to a standard protocol (Asta *et al*. 2002). Seventy-four trees were selected for the survey (Fig. 1), following several criteria: deviation from vertical < 15º, no branches and un-harvested cork at sampling height, and no visible diseases (Pinho *et al*. 2004). The mean distance between trees was 14·8 m. On each tree, a 50 × 10 cm grid, divided into five 10 × 10 cm subunits, was placed on the four main aspects of the trunk above the cork-harvest zone. Lichen species frequency was taken as the number of squares in which it was observed. A lichen diversity value (LDV) (Asta *et al*. 2002) was calculated for each tree, as the sum of the frequencies of all species. This index therefore accounts both for species number and frequency. Besides individual species frequency and total LDV (using all species), we calculated three functional diversity variables: (i) LDVoligo (considering N-sensitive species only); (ii) LDVnitro (considering N-tolerant species only); and (iii) LDVmeso (considering the intermediate species only). These functional diversity variables were calculated by grouping species according to an eutrophication tolerance index (Nimis & Martellos 2008). The highest classification for each lichen species was considered: species classified as 4 and 5 were considered nitrophytic (LDVnitro), 1 and 2 as oligotrophic (LDVoligo) and 3 as intermediate (LDVmeso) (Table 1). These threshold values of each class (or functional group) were chosen to ensure a good distribution of LDV values in each class.

GEOSTATISTICAL ANALYSIS AND INTERPOLATION OF LICHEN FUNCTIONAL DIVERSITY VARIABLES

The sites of lichen sampling and ALPHAs location were not the same (Fig. 1). To relate lichen variables to $[NH_3]_{atm}$, we interpolated all lichen variables for the entire study area, using each tree as a sample

Table 1. Lichen species observed, short name (used in Fig. 3), eutrophication tolerance classification according to the bibliography (Nimis & Martellos 2008) and the functional group assigned in this work

Species	Short name	Eutrophication tolerance	Functional group
Amandinea punctata (Hoffm.) Coppins & Scheidegger 1993	Aman punc	4	Nitrophytic
Caloplaca ferruginea (Huds.) Th.Fr. 1861	Calo ferr	3	Mesotrophic
Caloplaca holocarpa (Ach.) Wade 1965	Calo holo	5	Nitrophytic
Candelariella reflexa (Nyl.) Lettau 1912	Cand refl	5	Nitrophytic
Chrysothrix candelaris (L.) Laundon 1981	Chry cand	3 (1)	Mesotrophic
Diploicia canescens (Dickson) Massal. 1852	Dipl cane	4	Nitrophytic
Evernia prunastri (L.) Ach. 1810	Ever prun	3	Mesotrophic
Hyperphyscia adglutinata (Flörke) Mayrh. & Poelt 1979	Hype adgl	5	Nitrophytic
Lecanora albella (Pers.) Ach. 1810	Leca albe	3 (1)	Mesotrophic
Flavoparmelia caperata (L.) Hale 1986	Flav cape	3	Mesotrophic
Parmotrema hypoleucinum (J.Steiner) Hale 1974	Parm hypo	2	Oligotrophic
Parmotrema reticulatum (Taylor) M.Choisy 1952	Parm reti	2	Oligotrophic
Flavoparmelia soredians (Nyl.) Hale 1986	Flav sore	3	Mesotrophic
Parmelia sulcata Taylor 1836	Parm sulc	3	Mesotrophic
Parmelina tiliacea (Hoffm.) Hale 1986	Parm tili	3	Mesotrophic
Pertusaria amara (Ach.) Nyl. 1873	Pert amar	3	Mesotrophic
Physcia adscendens (Fr.) Oliv. 1882	Phys adsc	5	Nitrophytic
Physcia tenella (Scop.) DC. v. tenella 1905	Phys tene	4	Nitrophytic
Physconia enteroxantha (Nyl.) Poelt 1966	Phys ente	4	Nitrophytic
Physconia grisea (Lam.) Poelt 1965	Phys gris	5	Nitrophytic
Ramalina canariensis J.Steiner 1904	Rama cana	4	Nitrophytic
Ramalina farinacea (L.) Ach. 1810	Rama fari	2	Oligotrophic
Ramalina fastigiata (Pers.) Ach. 1810	Rama fast	3	Mesotrophic
Ramalina pusilla Duby 1830	Rama pusi	2	Oligotrophic
Teloschistes chrysophthalmus (L.) Th.Fr. 1860	Telo chry	3	Mesotrophic
Usnea ceratina Ach. 1810	Usne cera	1	Oligotrophic
Usnea rubicunda Stirton 1881	Usne rubi	2	Oligotrophic
Xanthoria parietina (L.) Th.Fr. 1860	Xant pari	4	Nitrophytic

Tolerance values are the maxima assigned in bibliography and range from 1 (present in sites with no eutrophication) to 5 (present in sites with very high eutrophication) (Nimis & Martellos 2008). For two species, the values in the bibliography were corrected following this study (original classification is given in brackets).

(n = 74). Using this interpolated map, the values of the lichen variables were determined at the NH_3 sampling locations (n = 18). The inverse procedure (i.e. interpolating $[NH_3]_{atm}$ and estimating its values at the lichen sampling locations) was not advisable because there were more samples of lichen variables, and thus, the degrees of freedom would be artificially inflated for the statistical tests. Furthermore, using lichen variables with more samples allows a more robust geostatistical analysis. A spherical model was fitted to the lichen variables because it was the model best adjusted to the short-range characteristics of those variables. This model included anisotropy and nugget effects and was used for interpolating lichen variables, using ordinary kriging considering the six nearest trees, and for testing the robustness of functional diversity response to $[NH_3]_{atm}$.

DATA ANALYSIS

To analyse lichen community composition within the study area, we performed a principal components analysis (PCA) of the species frequency data at the tree level (N = 74), using species that occurred in two or more trees. We correlated the maximum value given to each species on the eutrophication tolerance index (ranging from 1 to 5) with the species PCA scores, using Spearman rank order correlations to check the accuracy of the classification used (Nimis & Martellos 2008).

We related site scores to $[NH_3]_{atm}$ (N = 18) to test whether $[NH_3]_{atm}$ could be the main driving force for species ordination on the

PCA. The relationship between lichen functional diversity variables (LDVs) and $[NH_3]_{atm}$ was also assessed (N = 18). All correlations were performed using the values of lichen variables (PCA scores, LDVtotal, LDVnitro, LDVmeso and LDVoligo) at the NH_3 sampling locations estimated as explained previously. These estimated lichen variables were plotted against the observed values of the average $[NH_3]_{atm}$ (N = 18, samplers outside the spatial range of the trees were not used, Fig. 1). A curve was fitted to each biplot minimizing the squared residuals, $y = a*\ln(x) + b$ (for positive relations) and $y = a*x - b$ (for negative relations).

The robustness of the lichen functional diversity variables as indicators of the impact of NH_3 was evaluated by the proportion of the nugget effect for each lichen variable, determined from the geostatistical analysis detailed earlier. We interpreted the nugget effect as a reflection of the intra-site variability (because of the sampling of different trees). Thus, variables with a lower nugget effect were interpreted as more robust ecological indicators because it has lower intra-site variability. Accuracy was evaluated by the strength of the relationship between lichen variables and the $[NH_3]_{atm}$. The residuals of the relationships approached a normal distribution, although care must be taken because the number of available samples was low. The homogeneity of variance of the residuals met the necessary assumptions. Geostatistics and mapping were done with ArcMap software (ESRI 2008) and correlations and function fitting with Statistica software (Statsoft 2008).

Results

Average $[NH_3]_{atm}$ values ranged between 34·7 and 1·51 $\mu g \, m^{-3}$ (Fig. 2, right), with the highest values observed near the source (the cattle barn) and decreasing exponentially with distance from the barn. $[NH_3]_{atm}$ varied greatly between sampling periods: the $[NH_3]_{atm}$ measured at the site with the highest mean concentration (34·7 $\mu g \, m^{-3}$) ranged between 8·04 and 74·98 $\mu g \, m^{-3}$. By comparison, at the site with the lowest mean concentration (1·51 $\mu g \, m^{-3}$), monthly values ranged between 0·37 and 2·78 $\mu g \, m^{-3}$. Additionally, by averaging all sampling sites for each sampling period (Fig. 2, left), we could observe large variations between sampling periods, with the highest $[NH_3]_{atm}$ observed in autumn (October–November).

A total of 28 epiphytic species were found in the study area (Table 1). Of these, 11 species were nitrophytic, 11 were mesotrophic and six oligotrophic. In the species ordination with a PCA (Fig. 3), the first factor accounted for 21·5% of species variance. In the PCA diagram (Fig. 3), species considered oligotrophic were grouped in the positive portion of the first axis, while species considered nitrophytic were grouped in the negative portion. This grouping of species according to their functional group along the main PCA axis confirmed the adequacy of the classification used (Nimis & Martellos 2008), although two exceptions were found: *Lecanora albella* and *Chrysothrix candelaris*. The expert classification of Nimis & Martellos (2008) sorted these species in the eutrophication index with the value of 1, indicating that these species should be restricted to sites with no eutrophication. However, both species were located in a mid-position on the PCA ordination (Fig. 3), which was not compatible with the previous classification. Thus, they were reclassified for this study as being mildly tolerant to eutrophication, i.e. mesotrophic (with a maximum value of 3 in the eutrophication tolerance index). All subsequent calculations of LDV considered this reclassification.

The accuracy of the eutrophication index used (Nimis & Martellos 2008) was verified by correlating the index species classification with the respective PCA score. This correlation
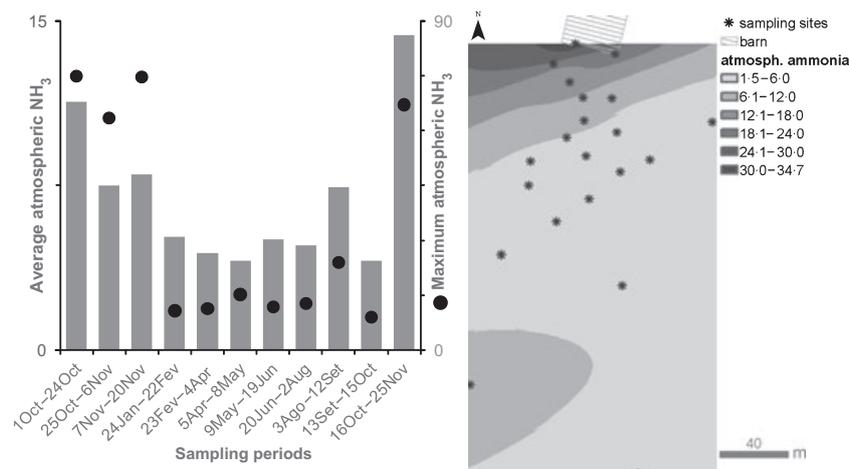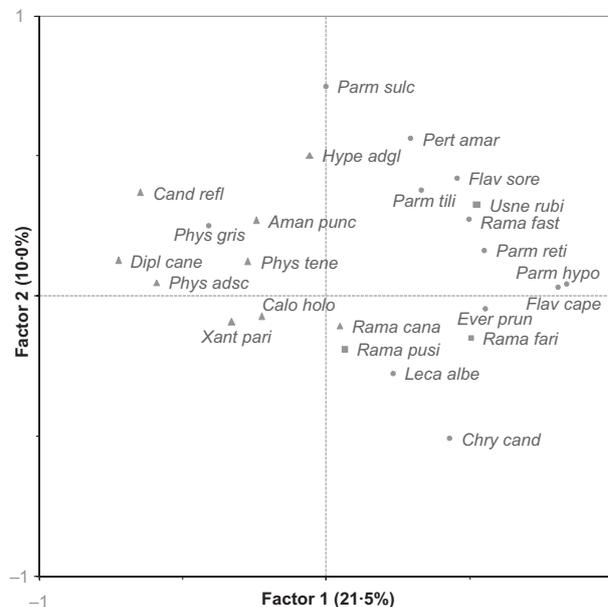

Fig. 3. Representation of the first two factors of a principal components analysis of species frequencies. Species are coded according to their classification: nitrophytic (triangles), mesotrophic (circles) or oligotrophic (squares). Key to species names in Table 1. $N = 74$.

was found to be highly significant (Spearman $R = -0.75$, $P = 0.000029$; $N = 24$).

The correlation between the site scores of the PCA Factor 1 (Fig. 3), with the average $[NH_3]_{atm}$ (Fig. 2 right), was highly significant, as demonstrated in the correlation plot (Fig. 4), in which the positive portion of the first axis of the PCA was associated with lower $[NH_3]_{atm}$ and the negative portion with higher values.

A variogram analysis of each lichen variable allowed modelling of their spatial structure (Table 2), showing that all had similar anisotropy, i.e. the direction in which the spatial continuity is maximal (Table 2), with the main orientation on the north/north-east direction and with the main range *c*. two
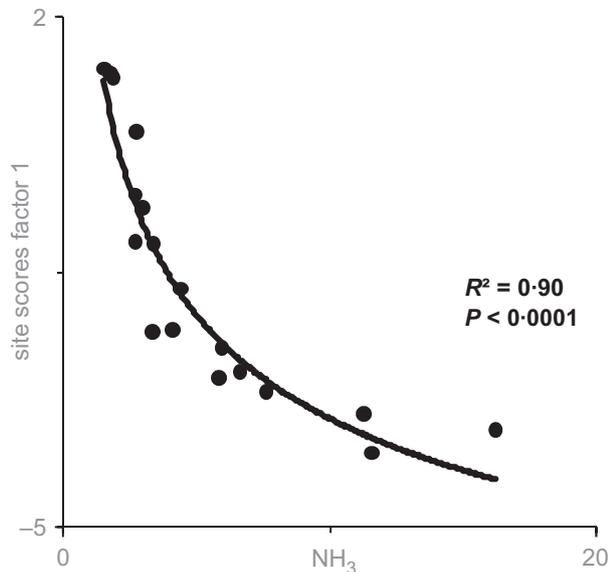

Fig. 2. Left: $[NH_3]_{atm}$ ($\mu g \, m^{-3}$) on each sampling period, showing the average (bars, left axis) and maximum values (black dots, right axis) of all sampling sites; $N = 21$); right: mapping of the interpolation of $[NH_3]_{atm}$ for the study area (average of the 11 time periods).

Fig. 4. Correlation between average $[NH_3]_{atm}$ ($\mu g\ m^{-3}$) and site scores of factor 1 in the PCA analysis (Fig. 3). $N = 18$.

Table 2. Characteristics of the model adapted to each lichen variable after variogram analysis

Variable	Major/minor range	Main direction	Nugget/total variance
LDVtotal	2·63	19·2°	0·54
LDVnitro	2·25	52·2°	0·33
LDVmeso	2·55	19·8°	0·15
LDVoligo	1·99	54·1°	0·14

Major/minor range is an indicator of anisotropy in the variables (1 indicating no anisotropy); main direction is that of higher spatial continuity (0° indicating north); nugget/total variance is the proportion of non-spatially structured variance in the data (0 indicating all variance with a spatial structure, 1 the opposite). $N = 74$ for each lichen variable.

times greater than the minor range. The major difference between the lichen variables was the relative importance of the nugget effect. Higher values of nugget effect indicate a lack of spatial continuity at short range because of the influence of environmental factors such as tree size (Table 2). It was observed that: (i) LDVtotal showed a nugget effect accounting for more than half of the total variance; (ii) LDVnitro an intermediate weight of the nugget effect (*c.* 1/3 of total variance); and (iii) LDVoligo and LDVmeso the smallest nugget effect.

Total LDV varied between 61 and 11, with the lowest values observed 50 m south of the cattle barn (Fig. 5). The lichen functional diversity variable LDVmeso ranged from 0 to 43 and presented a pattern similar to LDVtotal, but the highest values were observed *c.* 150 m south-east of the cattle barn (Fig. 5). The variable LDVoligo ranged from 0 to 21, with the lowest values observed near the barn and the highest ones furthest from it, while LDVnitro presented an opposite distribution and ranged between 0 and 32 (Fig. 5).

The estimated values of the lichen functional diversity variables were determined in the locations where the $[NH_3]_{atm}$ was measured (Fig. 1) and were plotted against the annual averages of $[NH_3]_{atm}$. For total LDV, this correlation resulted in a weak logarithmic relationship ($R^2 = 0.33$), with higher total LDV at the lowest $[NH_3]_{atm}$ (Fig. 6). However, when considering LDV divided according to the corrected eutrophication tolerance index (Nimis & Martellos 2008), highly significant relationships with $[NH_3]_{atm}$ were found (Fig. 6). The variables LDVmeso and LDVoligo were significantly and negatively related to $[NH_3]_{atm}$. The variable LDVnitro was significantly and positively related to annual $[NH_3]_{atm}$ (Fig. 6).

Discussion

Both the average values and the spatial patterns of $[NH_3]_{atm}$ observed in this study agree with other studies around NH_3 point-sources (Sutton *et al.* 1998; Frati *et al.* 2007), with the highest values recorded near the source and an exponential decrease with increasing distances (Fig. 2, right). However, the observed temporal pattern was different, with highest values of $[NH_3]_{atm}$ during the autumn (Fig. 2, left). This pattern seems to be independent of wind direction (Fig. 1): although some high-emission periods coincided with north winds, others did not. NH_3 emissions are usually higher during the warmer season, from natural sources (Blackall *et al.* 2008), agriculture point-sources (Harper, Sharpe & Simmons 2004) and multiple agriculture sources of NH_3 at a landscape scale (Dammgen 2007). Higher $[NH_3]_{atm}$ in summer is explained in the literature by increased NH_3 volatilization because of higher temperatures (Huber & Kreutzer 2002; Robarge *et al.* 2002; Harper, Sharpe & Simmons 2004; Sharma *et al.* 2010). However, all these studies were carried out in temperate climates. The few studies of NH_3 emissions in the Mediterranean region were not designed to assess seasonal patterns or climate effects (Frati *et al.* 2006, 2007). As the Mediterranean summer is very dry (Fig. 1, right), there is very low soil water availability, which reduces microbiological activity (Sommer *et al.* 2003) and atmospheric NH_3 emissions. Higher $[NH_3]_{atm}$ was observed in this study mostly in the autumn (Fig. 2) when rainfall was higher and temperatures milder (Fig. 1). Additional N was probably released as a result of litter decomposition. Another study carried out under a monsoon climate showed similar trends to ours, with higher $[NH_3]_{atm}$ observed during the wet season (Kumar *et al.* 2004). These authors suggested that the wet conditions during monsoon were more favourable for microbial activity (Kumar *et al.* 2004). Nevertheless, in our study, there were also dry periods with high $[NH_3]_{atm}$ (3 August to 12 September, Figs 1 and 2, left), highlighting that the mechanisms that govern NH_3 emission in Mediterranean areas are complex and require further study.

For the first time, we have been able to test the classification of lichen species according to eutrophication tolerance (Nimis & Martellos 2008), using a single source of atmospheric NH_3. In general, the classification performed well, as confirmed by: (i) the very significant correlations between the distribution of species in the PCA and measured $[NH_3]_{atm}$ (Fig. 4); and (ii) the
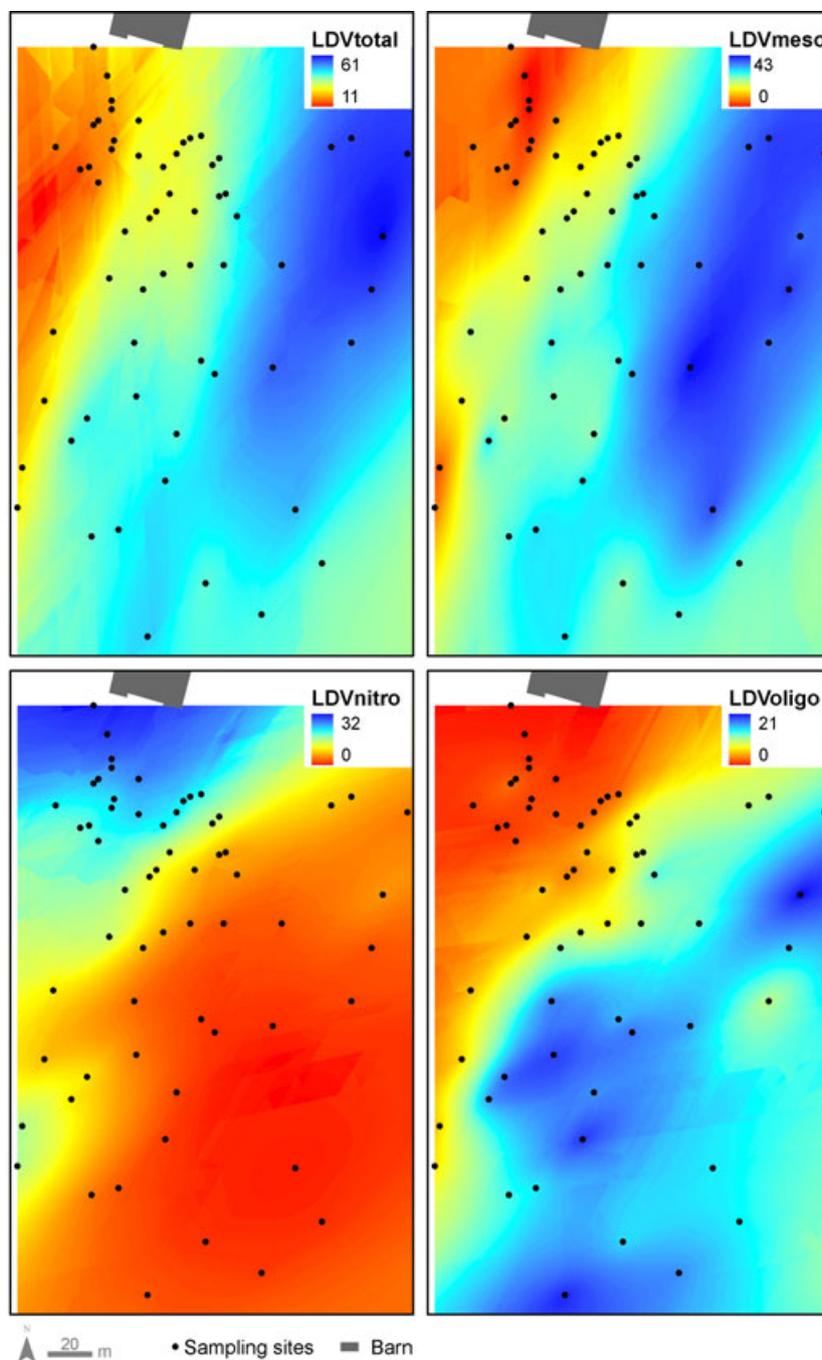
Fig. 5. Interpolation of lichen functional diversity variables, presenting total values (top left) and divided according to functional traits regarding eutrophication tolerance. The dots are the sampling sites (trees), and the grey area to the north is the barn.

highly significant correlation between the species classification on the eutrophication index and the species position as the first factor of the PCA. Nevertheless, of the 28 species tested, two were shown to be misclassified: *L. albella* and *C. candelaris* (Fig. 3). These species were previously considered oligotrophic (1) (Nimis & Martellos 2008) but, based on our results, should be reclassified as mesotrophic (1–3). In a study of Scottish Atlantic woods, Mitchell *et al.* (2005) found that under very low [NH_3]$_{atm}$ (max. 0·2 μg m^{-3}), the species *C. candelaris* was associated with the highest [NH_3]$_{atm}$, which was also positively correlated with N wet deposition and stem-flow (Leith *et al.* 2008). Although [NH_3]$_{atm}$ was much lower than those we observed, it seems that this species can tolerate some level of NH_3 and wet N deposition. This confirms that the eutrophication index, which was developed based on the classification of species in Italy, is likely to perform well in Portugal. We support the use of this index, with correction of the two species referred to above, for further work, although tests of other species in different climates against single sources of NH_3 would be valuable.
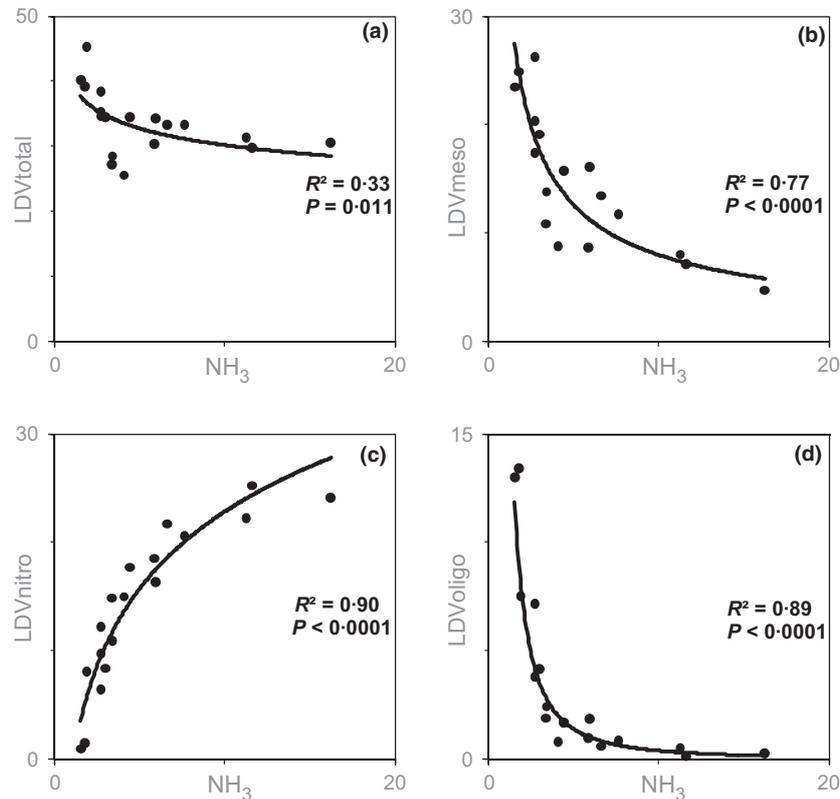
Fig. 6. Relation between average $[NH_3]_{atm}$ ($\mu g\ m^{-3}$) measured for 1-year period and four lichen functional diversity variables: (a) all species (LDVtotal) were divided according to functional groups based on eutrophication tolerance, (b) mesophytic (LDVmeso), (c) nitrophytic (LDVnitro) and (d) oligotrophic (LDVoligo). $N = 18$.

FUNCTIONAL GROUPS AS ECOLOGICAL INDICATORS
OF ATMOSPHERIC AMMONIA EFFECTS

Both LDVtotal and total species richness are sensitive ecological indicators and can be used to monitor the effect of atmospheric pollution (Pinho *et al.* 2004, 2008a; Giordani 2007; Svoboda 2007). However, these global lichen variables are only efficient when the pollution is detrimental for all species, e.g. SO_2 (Hawksworth & Rose 1970) or atmospheric Cu (Branquinho *et al.* 1999). Nitrogen is an essential nutrient to all organisms; therefore, it is not surprising that LDVtotal displayed less significant correlations with $[NH_3]_{atm}$ ($P = 0.011$) (Fig. 6a) and poor spatial structure, with high variability at intra-site level (Table 2). Other authors have found non-significant relations between lichen communities and $[NH_3]_{atm}$ when considering all species together (Van Dobben & Ter Braak 1998; Frati *et al.* 2007). In our study, the low response of LDVtotal to $[NH_3]_{atm}$ was because of the opposing response of some species to NH_3: while some species responded positively, others responded negatively (Figs 4 and 6). Thus, using total lichen diversity measurements with NH_3 gradients can mask the changes in these communities.

We have shown that lichen functional groups, after correcting the two misclassified lichen species, reveal a clear and complete community shift from oligotrophic to nitrophytic species (Figs 5 and 6). Thus, for the first time, here we show that the theoretical model of Sparrius (2007) is supported by empirical data (Fig. 6): oligotrophic species decline under increasing $[NH_3]_{atm}$ and nitrophytic ones increase (Fig. 6c,d). Lichen functional groups have been used as indicators of N pollution at both regional (Fenn *et al.* 2007; Rogers, Moore & Ryel 2009) and local scales (Ruisi *et al.* 2005). However, earlier studies in southern Mediterranean countries found that increases in nitrophytic species could be related to an increase in $[NH_3]_{atm}$, but no pattern could be detected for oligotrophic species (Ruisi *et al.* 2005; Frati *et al.* 2007). Here, we have shown a good correlation for the entire lichen community. Other environmental factors, such as dust (Loppi & Pirintsos 2000), could have contributed to the results from earlier studies. In this area, where other environmental factors are either absent or their effects were homogenous, it was possible to determine clearly the effect of $[NH_3]_{atm}$ alone on lichen functional diversity. The variogram analysis, namely the low importance of the nugget effect (Table 2), demonstrates the robustness of lichen functional diversity as an indicator of the effect of $[NH_3]_{atm}$ (Table 2), implying that two nearby trees are very likely to present similar values of LDVoligo, compared with LDVtotal. Taken together with the very significant correlations obtained between functional diversity variables and $[NH_3]_{atm}$ (Fig. 6), our results reinforce the use of functional diversity as an accurate and robust indicator of the effects of atmospheric NH_3.

It is appropriate to note here that the species classified as mesotrophic in relation to N, as indicated by LDVmeso, were most similar in response to the oligotrophic species, as the LDVmeso value uniformly decreased with increasing $[NH_3]_{atm}$, with no evidence of a maximum score at intermediate $[NH_3]_{atm}$. This indicates that within the range of annual $[NH_3]_{atm}$ of this study ($1.5–16.2$ μg m^{-3}), the mesotrophic species responded as weakly oligotrophic. Further studies at lower $[NH_3]_{atm}$ (i.e. in the range $0–1.5$ μg m^{-3}) would be needed to assess the extent to which mesotrophic species may benefit from low background $[NH_3]_{atm}$.

In summary, considering lichen functional diversity based on eutrophication tolerance revealed that there was a complete community shift under increasing $[NH_3]_{atm}$ (Fig. 2), with oligotrophic species being replaced by nitrophytic ones (Fig. 6c,d), while total abundance was only slightly changed (Fig. 6a). Thus, the lichen functional diversity variables LDVoligo and LDVnitro are highly accurate (Fig. 4) and robust (Fig. 3, Table 2) as indicators of the effects of $[NH_3]_{atm}$. They are potentially broadly applicable, because these variables were calculated using standard methods (Asta *et al.* 2002) and the functional groups were defined according to an international classification (Nimis & Martellos 2008). It may be possible to map the effects of $[NH_3]_{atm}$ on ecosystems with high spatial resolution (Fig. 3) to identify critical levels (Cape *et al.* 2009a). This could help to determine the success of techniques for ameliorating the effects of NH₃, including abatement, ammonia sequestration, planting tree belts near cattle farms (Dragosits *et al.* 2006), N-related land restoration policies such as land retirement (Fraser & Stevens 2008) and determination of a safe distance between a NH₃ source and protected ecosystems. The concentration–response relationships demonstrated here (Fig. 6c,d) need to be further extended with studies at small $[NH_3]_{atm}$ around and below the critical level of 1 μg m^{-3} for ammonia (Cape *et al.* 2009a,b). Lichens are among the most sensitive communities to atmospheric NH₃; thus, they could also be used as early-warning indicators of the effects of NH₃ for other less sensitive components of the ecosystem. It is important to highlight that although lichen species are not the same all over the world, the functional groups of oligophytes and nitrophytes are present and can thus be utilized everywhere.

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