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Tools for determining critical levels of atmospheric ammonia under the influence of multiple disturbances

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Critical levels (CLEs) of atmospheric ammonia based on biodiversity changes have been mostly calculated using small-scale single-source approaches, to avoid interference by other factors, which also influence biodiversity. Thus, it is questionable whether these CLEs are valid at larger spatial scales, in a multi-disturbances context. To test so, we sampled lichen diversity and ammonia at 80 sites across a region with a complex land-cover including industrial and urban areas. At a regional scale, confounding factors such as industrial pollutants prevailed, masking the CLEs. We propose and use a new tool to calculate CLEs by stratifying ammonia concentrations into classes, and focusing on the highest diversity values. Based on the significant correlations between ammonia and biodiversity, we found the CLE of ammonia for Mediterranean evergreen woodlands to be 0.69 μg m^{-3}, below the previously accepted value of 1.9 μg m^{-3}, and below the currently accepted pan-European CLE of 1.0 μg m^{-3}.

1. Introduction

Agriculture is the source of most atmospheric ammonia (NH_{3atm}) (Galloway et al., 2003), which negatively affects biodiversity and ecosystems functioning (Bobbink et al., 2010; Rockstrom et al., 2009). To protect ecosystems and their services, critical levels (CLEs) of NH_{3atm} have been established. These are the concentrations of atmospheric ammonia ($[NH_3]_{atm}$) above which direct adverse effects may occur to a given ecosystem, according to present knowledge (Cape et al., 2009b; Pinho et al., 2009; Sutton et al., 2009). These effects include impacts on biodiversity (e.g. species and communities composition, such as decreased frequency or abundance of nitrogen-sensitive species or an increase in nitrogen-tolerant species) and effects on physiological performance (e.g. increases in nitrogen content or soil nitrogen leaching). The long-term (annual) pan-European CLE for NH_{3atm} has recently been revised downwards, from 8 to 1 μg m^{-3} (Hallsworth et al., 2010). However, the establishment of CLEs has been mostly based on studies carried out over a small-scale (less than 2 km) using a single NH_{3atm} source (e.g. barns) (Cape et al., 2009b; Pinho et al., 2012b). When CLEs were assessed over large regions, these were dominated by high intensity agriculture activities, with high $[NH_3]_{atm}$ background levels, so that it was not possible to calculate CLEs of NH_{3atm} (Jovan et al., 2012). When regional areas with lower $[NH_3]_{atm}$ background levels were considered, the presence of other disturbance sources did not allowed its calculation (Sutton et al., 2009). To make the calculation of CLEs more universal, allowing inter-regional comparisons under complex land-uses, we need tools to calculate the CLEs across large regions with complex landscapes, in a multi-pollutant context, with lower $[NH_3]_{atm}$ than in high intensity farming areas.

The challenge of considering large regions in the analysis of the effects of NH_{3atm} is the presence of other disturbances, mostly other anthropogenic pollutants. In the European-Mediterranean region, a wide variety of land-cover types co-exist in relatively small areas (Blondel and Aronson, 1999; Farina et al., 2005). The region blends high conservation value areas with low-to-high intensity agriculture and small urban settlements with multiple sources of NH_{3atm} and other pollutants in close proximity. Moreover, Mediterranean ecosystems are strongly underrepresented in studies on the impact of NH_{3atm} on ecosystems to determine ammonia loads and CLEs (Dias et al., 2011; Ochoa-Hueso et al., 2011). Extensive pristine forests can no longer be found in large areas of most European-Mediterranean countries due to historical transformation of

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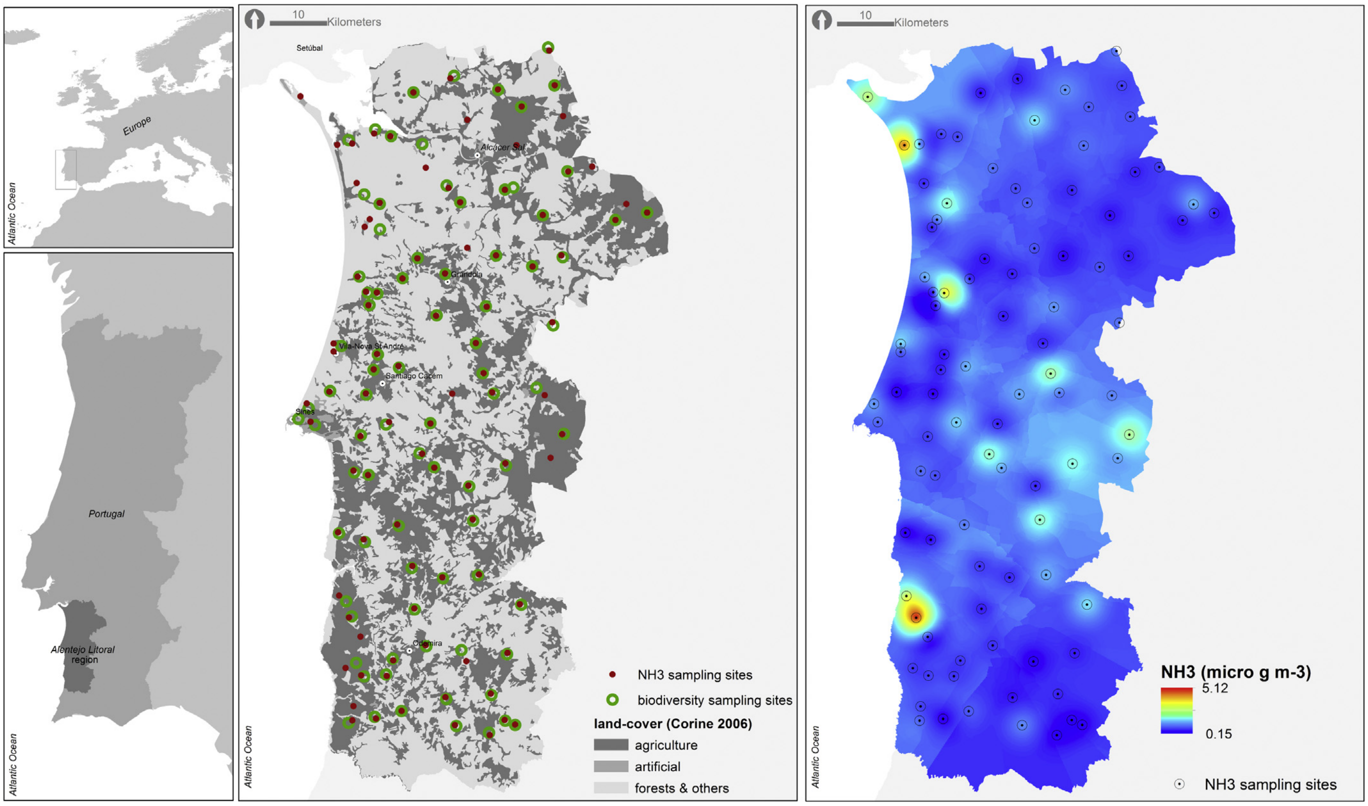

Fig. 1. Left: general location of the study region. Centre: Study area with dominant land-cover classes (Corine 2006) and sampling sites for biodiversity ($n = 84$) and atmospheric ammonia ($n = 96$). Right: Interpolation of average atmospheric ammonia concentrations by ordinary kriging.

ecosystems by man. They have been replaced by Mediterranean evergreen woodlands which are the most widespread forested semi-natural ecosystems in south-western Mediterranean Europe. These savanna-like ecosystems have high biodiversity and provide a large number of other services, depending on low-intensity human management (Bugalho et al., 2011). Thus, it is important to determine the thresholds of NH_{3atm} for these ecosystems, e.g. by looking for changes in their biodiversity.

Lichens are among the ecosystem components most responsive to atmospheric conditions, since they rely entirely on the atmosphere for nutrition and water. For that reason they have been used as indicators of the impacts of pollution (Pinho et al., 2008b), including that caused by agriculture (Pinho et al., 2008a). In fact, bryophytes and lichens are among the most sensitive organisms to nitrogen pollution (Pardo et al., 2011; Cape et al., 2009b), and have been used to set NH_{3atm} CLEs for Europe (Cape et al., 2009a).

Among lichen-based indicators (or variables), total species richness and also variables based on functional response groups (Lavorel and Garnier, 2002) have been considered. The latter are species assemblages with a common response to an environmental factor, and are ideal tools to measure the effect of NH_{3atm} because this pollutant has different effects on species, promoting the tolerant ones and damaging the less tolerant (Gaio-Oliveira et al., 2005; Johansson et al., 2012). More specifically, under increasing NH_{3atm}, nitrophytic species are promoted in abundance and richness while oligotrophic ones decrease (Pinho et al., 2012a, 2009). However confounding factors also play a role: oligotrophic species are sensitive to most pollution types, including industrial pollutants such as SO_2 and NO_x and also to particles from the typical Mediterranean dust (Loppi and Pirintsos, 2000; Pinho et al., 2008b); nitrophytic species are promoted by dust or high light intensity, but resistant to low concentrations of industrial pollutants (Llop et al., 2012). Nevertheless nitrophytic species also disappear in more polluted areas (Pinho et al., 2004).

Our aim was to determine the CLEs of NH_{3atm} for Mediterranean evergreen woodlands, considering a regional level of analysis and the presence of other confounding factors including pollutants. Previously, the calculation of critical levels has been mostly limited to small-scale, single sources of atmospheric ammonia. We considered changes on lichen functional groups, because lichens are among the most sensitive components of ecosystems. We tested the hypothesis that, for a certain atmospheric ammonia class, the highest diversity of lichens can be used to calculate CLE of $[NH_3]_{atm}$.

2. Methods

2.1. Study area and sampling design

The study area is located in south-west Europe, Portugal, comprising the Alentejo Litoral region (Fig. 1). It presents a typical Mediterranean climate and landscape. The annual average temperature is 16.7 °C and the annual rainfall 578 mm (averages 1950–2000) (Hijmans et al., 2005). Most of the region is covered by cork-oak woodlands but significant areas are occupied by agriculture. Small cities are located in the region, which has a total population of 97,925 (INE, 2011) and an area of c. 5303 km^2. Important industrial facilities, including a power plant, industrial harbor, industrial wastewater treatment plant and several petrochemical industries, are located near the coastal city of Sines. The prevailing winds carry pollutants to the south-south-east, which leads to an increase of atmospheric pollutants concentration such as SO_2, NO_x and particles and consequently to a reduction of lichen diversity (Augusto et al., 2012; Pinho et al., 2008a, 2008b).

A total of 96 sampling points were distributed in the region in a random stratified way. Stratification was done by spatial location, dividing the region into a regular 15 km grid and selecting randomly three sampling locations within each grid cell. In each sampling point we characterized $[NH_3]_{atm}$. Epiphytic lichens frequency and richness was also characterized at the nearest cork-oak woodland whenever possible (Fig. 1).

ALPHA (Adapted Low-cost Passive High Absorption) samplers (Tang et al., 2001) were used at 96 sites to measure $[NH_3]_{atm}$. Each sampler containing a citric-acid-impregnated cellulose fiber adsorbent (13% w/v) which was colorimetrically analyzed (Spectra Rainbow A-5082 spectrophotometer, Tecan, Männedorf, Switzerland) for ammonium ($N-NH_4^+$) using a modified Berthelot reaction (Cruz and Martins-Loução, 2000). Two ALPHA samplers were placed at each point and located at 2 m height, away from tree crowns and within a small shelter. $[NH_3]_{atm}$ was measured during three sampling periods (17-June-2009 to 30-June-2009, 08-March-2010 to 22-March-2010 and 15-June-2010 to 29-June 2010). Average daily temperatures were 24.1 °C (max. 24.1 °C, min. 17 °C) during the first period, 13.4 °C (max. 20 °C, min. 5 °C) during the second, and 21.1 °C (max. 21.1 °C, min. 15 °C) during the third. No precipitation occurred during any of the sampling periods (data from Sines meteorological station). Additional field blanks (closed ALPHAs) were used at some locations for quality assessment purposes. The average blank $[NH_3]_{atm}$ values for each date, which were very low, were subtracted from other ALPHA values. The average values of $[NH_3]_{atm}$ were then used in the analysis.

The cork-oak woodlands nearest to the ALPHA points were sampled for biodiversity. Since it was not always possible to find cork-oak woodlands within the defined range (5000 m), 84 sites were sampled. The average distance between $[NH_3]_{atm}$ and biodiversity sampling sites was 509 m (704 m SD, max. 4155 m, min. 12 m). At each site, 5 *Quercus suber* L. trees were sampled for epiphytic lichens using the "European Method" (Asta et al., 2002), placing a 10 × 50 grid on the unharvested portion of the 4 aspects of the tree trunk. This allowed the calculation of species richness (the total number of species at each site) and lichen diversity value (LDV, the sum of the frequency of all species divided by the number of observed trees). We also calculated species richness and LDV of nitrophytic and oligotrophic functional groups, allocating species according to their tolerance to eutrophication. This considered the maximum indicator-value provided for each species by Nimis and Martellos (2008), as already used and adapted from other works (Pinho et al., 2012a, 2011, 2012b). Although this species-classification database considers the Italian territory (Nimis and Martellos, 2008), it can be used to classify the same species elsewhere, including the region studied here. In fact, not only are the species the same (due to geographic proximity and sharing the Mediterranean climate) but also because the accuracy of the classification had previously been tested, and when necessary corrected (Pinho et al., 2011).

2.2. Data analysis

In order to calculate CLEs, biodiversity data was related to $[NH_3]_{atm}$. However, because the sampling sites of $[NH_3]_{atm}$ and biodiversity did not always match, it was necessary to interpolate $[NH_3]_{atm}$ at the biodiversity sampling sites. Interpolation of $[NH_3]_{atm}$ was performed using an ordinary kriging estimator (Goovaerts, 1997). We chose to interpolate $[NH_3]_{atm}$ rather than biodiversity for two reasons: i) to avoid inflating degrees of freedom in posterior statistical analysis ($n = 96$ for ammonia versus $n = 84$ for biodiversity); and ii) because NH_{3atm} presented a stronger spatial continuity than biodiversity, thus providing a more robust model. To do so, we first described the spatial variability of $[NH_3]_{atm}$, estimating a variogram function and fitting a spherical model to variogram estimates. The latter is a well-known theoretical permissible variogram model, frequently applied in earth and environmental sciences (Goovaerts, 1997). For the theoretical model, we estimated an isotropic model with a range (the distance beyond which the covariance remains constant), and a sill (a value of variogram for a distance beyond the range), providing the estimated parameters needed to build the spatial variance-covariance matrix used to solve the ordinary Kriging system and provide the estimates of NH_{3atm}.

A number of biodiversity variables, based on lichen species, were calculated: species richness and LDV, including total and that of functional groups related to eutrophication – oligotrophic (nitrogen-sensitive) and nitrophytic (nitrogen-tolerant). These variables were related to $[NH_3]_{atm}$ using linear regression. However, in contrast to single-sources studies, we expected that a large portion of variance in biodiversity variables would be associated with other variables besides $[NH_3]_{atm}$, such as pollution from industrial or urban areas or dust from bare-lands (Pinho et al., 2008a). In order to take this into account, we stratified the $[NH_3]_{atm}$ variable into 15 classes, each of which included 5 samples, and the sample with the highest biodiversity value in each class was selected. A total of 15 samples were thus selected from each biodiversity variable.

Using these maximum values of the biodiversity variable by class of $[NH_3]_{atm}$, we tested for the possible presence of a no-response concentration, looking for the presence of different slopes on the relationship between the lichen variable and $[NH_3]_{atm}$. This was achieved using Davies' test (Davies, 2002), testing for a non-zero difference in slopes parameters of a segmented linear relationship (but ensuring a near-zero slope for the lower values of $[NH_3]_{atm}$). When this was the case, the break-point was taken as the critical threshold for change. If not, the first point was considered already altered. Finally, CLEs were calculated as in Cape et al. (2009a), taking into account the confidence bands of the significant linear relationships. Piecewise linear regression and Davies' test were performed using the "segmented" R package (R Core Team, 2013), other statistical analysis used Statistica (Statsoft, 2012). Geostatistical analyses were performed using GeoMS (CERENA, 2000) and mapping outputs with ArcMap v.10 (ESRI, 2010).

3. Results

Variogram analysis of $[NH_3]_{atm}$ allowed fitting a spherical isotropic model with a 6000 m range and no nugget effect (i.e. with all variance with a spatial structure at the shortest range), giving us a high confidence in using the interpolated $[NH_3]_{atm}$ at the biodiversity sampling sites. The spatial pattern of $[NH_3]_{atm}$ (Fig. 1 right) showed several areas with high concentration likely caused by annual agriculture (rice to the north-west and centre-east, and vegetables to the south-west). The $[NH_3]_{atm}$ determined at the biodiversity sampling sites ranged from 4.68 μg m^{-3} to 0.17 μg m^{-3} (average = 0.99 μg m^{-3}, SD = 0.76 μg m^{-3}). Nine samples had $[NH_3]_{atm}$ above 2 μg m^{-3} (values ranged from 2.07 to 4.68 μg m^{-3}). These were excluded from further analysis because most changes in lichen communities are already known to occur at much lower values (Pinho et al., 2011) and thus the current pan-European CLEs are established at 1 μg m^{-3} (Hallsworth et al., 2010). The values above 2 μg m^{-3} were also excluded for statistical reasons: due to the low number of samples we would merge into the same class sampling sites with very different $[NH_3]_{atm}$ (because 75 samples were available for concentrations bellow 2 μg m^{-3} and only 9 samples for concentrations from 2 to 4 μg m^{-3}).

As expected, the relationship between lichen variables and $[NH_3]_{atm}$ using all samples was found to be not significant (Table 1, two example plots are shown in Fig. 2 for total number of species and total LDV). However, considering the highest values of lichen richness for each of the 15 classes of $[NH_3]_{atm}$, we found a significant correlation between lichen variables and $[NH_3]_{atm}$, both for the number of oligotrophic and of nitrophytic species (Table 1). These significant relationships were further explored for the calculation of CLEs.

3.1. Critical levels for biodiversity

Taking into consideration the maximum values of lichen species richness by classes of $[NH_3]_{atm}$ (Fig. 3, black circles), we found that the number of oligotrophic and nitrophytic species were significantly related to $[NH_3]_{atm}$. Higher $[NH_3]_{atm}$ was related to a decrease in the number of oligotrophic species and to an increase of the nitrophytic ones. No evidence of a plateau was found for any of those lichen variables (Davies test $p = 0.9431$ for oligotrophic and $p = 0.6362$ for nitrophytic species), so the lowest point was considered to be altered and the CLEs calculated taking into account the confidence bands. The CLEs were found to be 0.77 μg m^{-3} and 0.69 μg m^{-3} for oligotrophic and nitrophytic functional groups. Although these values were very similar, the lower one was chosen

Table 1
Pearson correlation coefficients (R) and p-values between lichen variables (number of species and LDV- Lichen Diversity Value) and atmospheric ammonia. Upper set is for the regional correlation ($n = 75$) and lower set is considering the highest diversity sites for each atmospheric ammonia class ($n = 15$). Significant correlations are shown in bold.

		Total number of species	Total LDV	LDV oligotrophic	LDV nitrophytic	Number of oligotrophic species	Number of nitrophytic species
All sites	R	−0.03	−0.21	−0.01	0.08	−0.22	0.21
	p-Value	0.780	0.071	0.959	0.498	0.059	0.064
"Highest diversity" sites	R	0.39	−0.28	0.19	0.47	**−0.63**	**0.65**
	p-Value	0.154	0.307	0.498	0.077	**0.012**	**0.012**

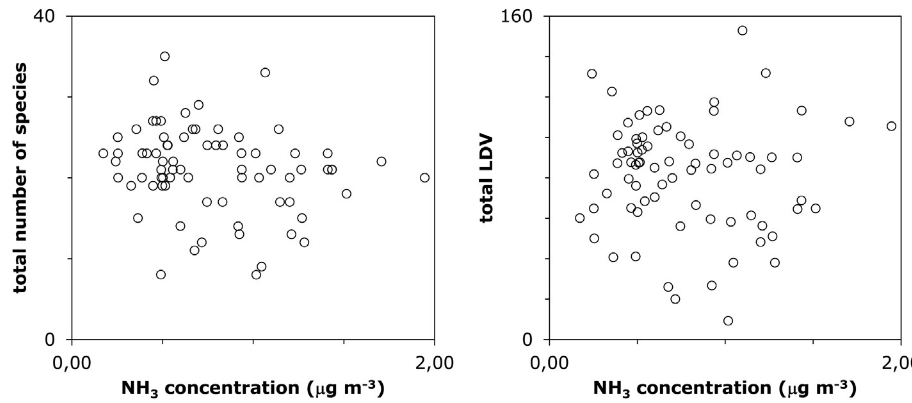

Fig. 2. Relationship between lichen variables using total values (total number of species and total LDV – Lichen Diversity Value – and atmospheric ammonia (NH_3)). The relationships are not-significant ($p > 0.5$). $N = 75$.

as the critical level in order to represent the first signs of change in the lichen community.

4. Discussion

Critical levels (CLEs) of atmospheric ammonia (NH_{3atm}) for Mediterranean evergreen woodlands were found to be 0.69 μg m^{-3} (Fig. 3). Taking into consideration that no plateau for low $[NH_3]_{atm}$ was found, the actual CLEs are likely to be lower than that value and lower than the current pan-European CLEs, set at 1 μg m^{-3} (Cape et al., 2009b). We propose here a tool for calculating CLEs based on biodiversity variables in complex landscapes with several confounding factors. We confirmed our initial hypothesis that if, for each class $[NH_3]_{atm}$ we consider the sites with the highest diversity of lichens, we could determine the CLEs of NH_{3atm} even in the presence of other disturbances.

The $[NH_3]_{atm}$ found in our work were rather low when compared to other studies. In fact, the lowest values of $[NH_3]_{atm}$ we found (0.147 μg m^{-3}) were below the values for agricultural areas: 0.3–106.2 μg m^{-3} (McCulloch et al., 1998); 0.5–60 μg m^{-3} (Fowler et al., 1998); 0.7–266.7 μg m^{-3} (Frati et al., 2007); 1.4–34.0 μg m^{-3} (Pinho et al., 2012b); 1.6–59 μg m^{-3} (Pitcairn et al., 1998); 2–60 μg m^{-3} (Paoli et al., 2010); 7.3–98.2 μg m^{-3} (Rogers et al., 2009). Moreover, the maximum values observed in our study (5.12 μg m^{-3}) were much lower than those studies, reflecting the absence of a nearby high intensity sources of NH_{3atm} in our case. Our observed $[NH_3]_{atm}$ were more comparable with the one observed by Zbieranowski and Aherne (2012) in Ontario, Canada, of 0.1 μg m^{-3} to 3.0 μg m^{-3}, in a study design to characterize only background $[NH_3]_{atm}$. This confirmed that our study area presents low pollution by NH_{3atm}, likely because there are few high-intensity point-sources of NH_{3atm} and most agriculture is of low intensity.

Relating all lichen diversity data with $[NH_3]_{atm}$ at a regional scale did not result in significant correlations. This was very likely due to the prevalent effect of other pollutants, which mask the effects of NH_{3atm}. Pollutants such as SO_2, NO_x and particles are present in the region due to the presence of an important industrial area and have a strong effect on lichen functional groups at a regional and local scale (Pinho et al., 2008a; Ribeiro et al., 2013). This prevalence of the effects of other pollutants commonly prevented the calculation of CLEs of NH_{3atm} at a regional level (Sutton et al., 2009). In this work we showed that considering the sites with highest biodiversity within certain classes of $[NH_3]_{atm}$ resulted in significant correlations between the two variables, allowing the calculation of CLEs.

The correlation between lichen diversity and $[NH_3]_{atm}$ was significant considering the richness of the functional groups, but not considering measures of total diversity (including species richness and LDV). This reinforced the established view (Loppi and DeDominicis, 1996; Van Dobben and Ter Braak, 1998) that the effects of NH_{3atm} on lichens can only be studied considering functional groups (e.g. nitrophytic and oligotrophic).

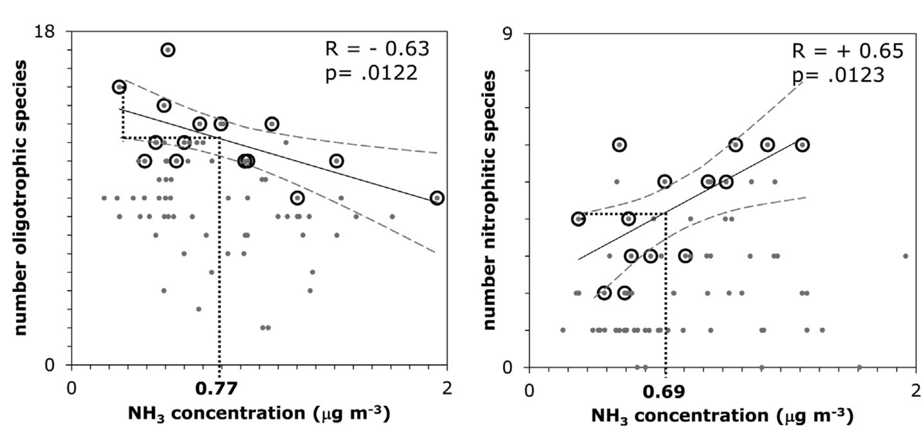

Fig. 3. Calculation of critical levels of atmospheric ammonia (NH_3). All samples (gray dots) were stratified into classes according to $[NH_3]_{atm}$. Within each class, the maximum value of lichen biodiversity variables was chosen (black circles) and related to $[NH_3]_{atm}$. The critical level was established to be 0.69 μg m^{-3}, the lowest of the CLEs for the two functional groups. The dotted black lines refer to the calculation of the critical levels. $N = 75$ for the regional data set, $n = 15$ for the sub-set of maximum diversity by class of atmospheric ammonia.

The method we used looks for the sites with highest biodiversity within each class of $[NH_3]_{atm}$. If that level of biodiversity is present at a certain concentration of ammonia, this is because that biodiversity is able to exist with it. Since we are not sure if that is the maximum possible biodiversity, it is possible that we have underestimated the value of maximum biodiversity for that class of ammonia. Oligotrophic functional groups are sensitive to most disturbances (Pinho et al., 2008b), whereas nitrophytic species will disappear from the most polluted areas (Pinho et al., 2004), and could be promoted by factors such as dust (Pinho et al., 2008b). We highlight that the approach used in this work can be used in other circumstances to detect the influence of a secondary environmental factor. The limitations of this tool are that: i) it requires a sufficient number of samples to contemplate sites only influenced by the secondary environmental factor along a gradient of the main factor; ii) it also requires that any other pollutants are not present in high concentrations at every site (i.e. high background values of pollutants such as SO_2, NO_x and particles).

The CLE of NH_{3atm} for Mediterranean evergreen woodlands determined in this work, 0.69 $\mu g\ m^{-3}$, is lower than the currently accepted CLE of 1 $\mu g\ m^{-3}$ (Hallsworth et al., 2010). Another study found the CLE of the same ecosystem to be 1.9 $\mu g\ m^{-3}$ (Pinho et al., 2012b). That study used a high-intensity point source of NH_{3atm} in a region with multiple agriculture activities, which likely cause a high background value of $[NH_3]_{atm}$. This highlights the importance of considering large areas with low background $[NH_3]_{atm}$ to avoiding overestimating CLEs. Thus, if low background values can be found (as in our study), then lichen diversity is probably a good universal tool to calculate CLEs of NH_{3atm}, even in the presence of other environmental factors. The critical level determined by this study should be considered the threshold concentration for the protection of lichen communities. In fact, since lichens are amongst the most sensitive components of ecosystems, the established critical levels can be regarded as early-warnings of future impacts of NH_{3atm} on other less-sensitive components of the ecosystems.

5. Conclusions

The most important achievement of this work was developing and demonstrating a tool to estimate the critical level of atmospheric ammonia at the regional level, even with the presence of confounding factors such as industrial pollutants. This required considering changes in lichen functional groups and focusing on the sites with the highest lichen diversity for certain classes of the pollutant of interest. Moreover, we found the critical level of atmospheric ammonia for Mediterranean evergreen woodlands to be 0.69 $\mu g\ m^{-3}$, below its previously accepted value of 1.9 $\mu g\ m^{-3}$, and below the currently accepted pan-European critical level (1.0 $\mu g\ m^{-3}$).

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.envpol.2014.01.024.

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