The critical levels of atmospheric ammonia in a Mediterranean holm-oak forest in North-Eastern Spain

Laura Aguillaume[1], Anna Avila[1,2*], Pedro Pinho[3,4], Paula Matos[3], Esteve Llop[5] and Cristina Branquinho[3]

[1]CREAF, Centre for Ecological Research and Forestry Applications, Cerdanyola del Vallès, 08193, Spain.

[2]Universitat Autònoma de Barcelona, Cerdanyola del Vallès, 08193, Spain.

[3]cE3c, Centre for Ecology, Evolution and Environmental Changes, Faculdade de Ciências, Universidade de Lisboa, Portugal.

[4]Cerena, Centre for Natural Resources and the Environment, Instituto Superior Técnico, Universidade de Lisboa, Portugal.

[5] Departament de Biologia Evolutiva, Ecologia i Ciències Ambientals-Botànica i Micologia, Universitat de Barcelona, Avda. Diagonal 643, 08028, Barcelona, Spain.

*Corresponding author, Anna Avila: anna.avila@uab.cat; Tel: +34 93 581 46 69; skype: annaavila1

Laura Aguillaume : aguillaume.laura@gmail.com

Pedro Pinho: ppinho@fc.ul.pt

Paula Matos: psmatos@fc.ul.pt

Esteve Llop: ellop@ub.edu

Cristina Branquinho: cmbranquinho@fc.ul.pt

Aguillaume, L. et al. "The critical levels of atmospheric ammonia in a Mediterranean holm-oak forest in North-Eastern Spain" in Water, air and soil pollution, vol. 228, issue 3 (March 2017), art. 93. DOI 10.1007/s11270-017-3286-8

Abstract

Despite recent regulations, atmospheric ammonia (NH_3) emissions have not changed much over the last decades and excessive nitrogen remains as one of the major drivers for biodiversity changes. To prevent deleterious effects on species and ecosystems it is very important to establish safety thresholds, such as those defined by the Critical Level (CLE) concept, "the concentration above which direct adverse effects on receptors may occur, based on present knowledge". Empirical critical levels of atmospheric NH_3 have mainly been reported for temperate forests and there is a lack of information for Mediterranean forests.

Here, we provide a case study on NH_3 CLEs for a typical Mediterranean ecosystem, the holm-oak (*Quercus ilex*) forest. To derive the CLE value, we measured NH_3 concentrations for 1 year at a distance gradient in the forest surrounding a point source (cattle farm) and used diversity changes of lichen functional groups to indicate the onset of adverse effects. We estimate a NH_3 CLE threshold of 2.6 µg m^{-3}, a value that is higher than that reported in other Mediterranean ecosystems and suggests that the site has been already impacted by NH_3 pollution in the past. In a more general context, this study confirms the validity of lichen functional groups to derive CLEs in Mediterranean forests and woodlands and contribute to the body of knowledge regarding the impacts of NH_3 on ecosystems.

Key-words: Critical levels; ammonia; ecological indicators; lichen functional groups; Mediterranean; *Quercus ilex* forest, N pollution

1. **Introduction**

Anthropic changes in the nitrogen (N) cycle are considered one of the major global threats to the sustainability of our planet (Rockström et al., 2009). Ammonia (NH_3) emissions contribute to this general increase of reactive nitrogen cycling in the biosphere, causing changes in biodiversity and ecosystem functioning (Galloway et al., 2003; Galloway and Cowling, 2002), and affecting human health, mostly due to the NH_4^+ formation of health-impairing fine particles (Bell et al., 2007). Most NH_3 emissions to the atmosphere are due to intensive livestock farming, animal waste storage and fertilizer application to open fields. However, NH_3 gas has a low residence time in the atmosphere and is either quickly deposited in the vicinity of point sources (Asman et al., 1998) or converted into ammonium nitrate and sulfate fine particles that travel for long distances (Finlayson-Pitts and Pitts Jr, 1999).

To protect ecosystems and human health from the impacts of excessive N, several abatement measures have been proposed under the UN framework (Convention on Long-Range Transboundary Air Pollution, CLRTAP). The development of such emissions abatement policies is underpinned by the concept of Critical Level (CLE), which is defined as “the concentrations above which direct adverse effects on receptors, such as plants, ecosystems or materials, may occur according to the present knowledge” (Posthumus, 1988). A recent revision of NH_3 CLEs proposed a value of 3 µg m^{-3} to protect vegetation, and 1 µg m^{-3} to protect lichens and bryophytes (Cape et al., 2009). This was done since some studies demonstrated that the previously accepted value for CLE in CLRTAP (8 µg m^{-3}) would not protect ecosystems functions.

Most studies on the N effects on ecosystems refer to temperate and boreal ecosystems. Mediterranean ecosystems are regarded as hotspots of biodiversity (Myers et al., 2000), and some studies have shown that they are particularly vulnerable to the increase of excessive N (Ochoa-Hueso et al., 2011; Phoenix et al., 2006). Still, studies about the effects of N in Mediterranean ecosystems remain underrepresented.

Evidence of long-term changes in lichen and bryophyte communities exposed to different NH_3 concentrations showed that they are within the most sensitive components of terrestrial ecosystems (Cape et al., 2009). Lichens depend on wet and dry atmospheric deposition for their nutritional requirements and their growth depends on atmospheric nitrogen; however, different species have different nutritional requirements. Under increasing NH_3 levels, N-sensitive lichen species cannot thrive, being replaced by N-tolerant communities. In addition, ammonia deposition on the tree bark raises its pH, accelerating the replacement of lichen species (Van Herk, 1999). For these reasons, several works in Europe and North America have used epiphytic lichens as ecological indicators of the effects of atmospheric NH_3 (Pinho et al., 2008; 2009; Sparrius, 2007; Van Dobben and Ter Braak, 1998; Van Herk, 1999). It is currently accepted that functional groups, rather than total lichen species richness, should be used to interpret NH_3 effects at the ecosystem level (Fenn et al., 2008; Pinho et al., 2012a). This approach is based on species specific sensitivities to N. Lichens species can be classified into functional groups according to their tolerance to eutrophication: oligotrophic (sensitive), mesotrophic (intermediate sensitive) and nitrophytic (tolerant). A similar classification has been used by other authors using the terms acidophyte, neutrophyte and nitrophyte lichens (Fenn et al., 2008; Geiser et al., 2010; Geiser and Neitlich, 2007; Jovan, 2008; Sparrius, 2007; Van Herk, 2001).

CLE thresholds have been proposed for Mediterranean evergreen open woodlands based on functional changes in lichen communities in Portugal (Pinho et al., 2011; 2012; 2014a; 2014b). Still, more research is warranted in this region to characterize the response of other ecosystem types such as dense forests. In Spain, NH_3 emissions are closely linked to agricultural activities. At a national level, Spanish NH_3 emissions were 384 Gg the period 2008-2012, 13% higher than in 1980-1985 (Aguillaume et al., 2016a). Despite this, little research has been carried out to assess the effects of this pollutant in Spanish forest ecosystems.

Our aim in this work is to estimate the empirical CLE of atmospheric NH_3 for a Mediterranean holm-oak (*Quercus ilex* L.) forest in NE Spain, thereby validating the use of lichen functional groups in this type of Mediterranean forests. This was done by sampling atmospheric NH_3 and lichen functional diversity in a closed canopy Mediterranean evergreen holm-oak forest in Catalonia (NE Spain), at increasing distances from a cattle barn point-source of ammonia.

2. Material and methods

2.1. Study area

The study was carried out in a holm-oak forest in NE Spain, 65 km away from Barcelona, and close to the Montseny Mountains. The forest surrounds a barn of ~130 beef cattle, permanently housed in an area of 1500 m^2. Holm-oak forests are the dominant vegetation type in the region, which has a humid continental Mediterranean climate. The 15 sampling sites were located at increasing distances from the point source, from adjacent to the barn up to a maximum distance of 620 m (Fig. 1). The distance between points ranged between 30 and 200 m. A higher density of sampling points was devised near the barn in order to obtain a good coverage at the vicinity of the point source. Ammonia gas has a low residence time in the atmosphere being quickly deposited in the vicinity of point sources, usually in less than 1000 m (Asman et al., 1998), depending on the source intensity, wind direction and land-cover. Here the maximum distance was enough to characterize the gradient of effects, as the NH_3 concentrations did not change much after 300 m. Sampling sites were located downwind of the prevailing winds of the area and their altitude varied between 618 and 690 m. Meteorological data were not available at the sampling sites and were retrieved from the nearest meteorological station, Sant Julià de Vilatorta (www.meteovilatorta.cat), which is located 3.2 km away from the sampling area, and thus is considered representative of the meteorological conditions of the study sites. For the study period (January 2013 to January 2014), total annual rainfall was 868 mm, mean annual temperature was 11ºC, at Sant Julià de Vilatorta. Dominant winds in the area were from the southwest (http://www.idescat.cat/pub/?id=aec&n=214). The study year was characterized by a wet spring and lower than usual rainfall in autumn (Fig. 2). Temperature showed the typical shape of highest values in July and August of the Mediterranean climate (Fig. 2).

Total inorganic nitrogen deposition at La Castanya station (Montseny) which is located at a linear distance of 15 km from the study site was 12.3 kg N ha^{-1} yr^{-1} (Aguillaume et al., 2017).

2.2. Ammonia measurements

From 28 January 2013 to 22 January 2014, NH_3 concentrations were measured using high-sensitivity ALPHA and Radiello© passive diffusion samplers at 15 points downwind of the barn (Fig. 1). ALPHA samplers are user-manufactured devices composed of a polyethylene PTFE tube containing an external membrane through which air flows to a collection filter coated with citric acid (Tang et al., 2001). ALPHA passive samplers were prepared at CREAF following the procedure described in Tang et al. (2001). The Radiello passive samplers are commercially available and their reliability has been tested by the European Reference Laboratory for Air Pollution (ERLAP). Radiello diffusion tubes were deployed at 6 sites in parallel with ALPHA samplers. Samplers were attached to trees approximately 2 m above the ground. Both type of samplers were collected and replaced by a new kit every 2 weeks (occasionally 3 weeks), to a total of 22 sampling periods along the study period. The concentrations measured by the two systems were highly correlated (R^2=0.98; $p<0.0001$, n=133), indicating that concentrations measured with ALPHA samplers were appropriate (as compared with the commercial Radiello samplers) for deriving NH_3 concentrations. The reported NH_3 concentrations were the average of the 2 samplers for the 6 replicated sites and ALPHA concentrations for the remaining 9 sites. Biweekly data were averaged to produce monthly mean concentrations.

To extract NH_3 from Radiello samplers, the specifications from Radiello were followed (Fondazione Salvatore Maugeri, 2006), while for ALPHA samplers the protocol described in Tang et al. (2001) was used. Ammonium in the extracts was analyzed by colorimetry with Flow Injection Analysis (Tecator©). Detection limit was 2 μeq L^{-1} (0.04 mg NH_4^+ L^{-1}). During every exposure period, unexposed samplers were used as travel blanks for each type of passive sampler (n=24) and these data were used to discount contamination during transport (blank concentrations were 1-15% compared to sample concentrations). After collection, all samples were kept refrigerated (4ºC) in darkness until analysis was performed.

2.3. Lichen survey, identification and classification

Holm oak trees adjacent to the air NH_3 sampling tree were selected for the lichen biodiversity survey. An average of 4 trees was inventoried in the vicinity of each NH_3 sampling tree, except for the site closest to the farm where no trees were available for sampling (Fig. 1). The lichen biodiversity measurement followed a standard protocol which was designed to give a lichen diversity value, LDV, that takes into account species richness and frequencies (Asta et al., 2002). Whenever possible, species were identified in the field but doubtful species were determined in the laboratory. Specimens only identified at genus level were not used in this study.

The approach used in our work was based on the classification regarding eutrophication tolerance developed by Nimis and Martellos (2008) for Italy, a procedure that has also been successfully applied for Portugal (Pinho et al., 2012a). Pinho et al. (2011) validated Nimis and Martellos (2008) classification for sensitive and tolerant lichen species by ranking them along a measured long term NH_3 atmospheric concentration gradient, and two lichen species were reclassified. Here, we followed Nimis and Martellos (2008) classification with Pinho et al. (2011) corrections, and applied this classification for the first time in Spain. Species with values from 4 to 5 were classified as nitrophytic (LDVnitro), species with values of 3 were classified as mesotrophic (LDVmeso) and species with a value up to 2 were classified as oligotrophic (LDVoligo). We considered the highest value given in this classification for each lichen species recorded. Several lichen-variables were calculated: (1) species richness corresponded to the total number of species, (2) total lichen diversity value (LDVtotal) corresponded to the sum of all species frequencies in each tree (Asta et al., 2002). The lichen diversity value was also calculated distinguishing for eutrophication tolerance functional group by summing the species frequencies of each functional group. In that manner, oligotrophic, nitrophytic and mesophytic LDV indices (LDVoligo, LDVnitro, and LDVmeso, respectively) were obtained. Functional group values were relativized as percentage of the total diversity value (LDVtotal) for each tree.

Although Nimis and Martellos (2008) classification refers to the Italian flora, it is also suitable for this work. Firstly, species found in our study are relatively ubiquitous, and occur also in Italy. Secondly, both areas share a Mediterranean climate type and the same database classification has been used successfully in other areas with a Mediterranean climate (Pinho et al., 2011). Finally, the accuracy of species classification into functional groups was checked with a principal component analysis applied on a matrix of the species frequencies for each tree, considering only species appearing in more than 3 trees. The relative location of species in the first axis of the ordination agreed well with species classification derived from Nimis and Martellos (2008), confirming the given functional group (data not shown) and assuring a correct classification of species into functional groups in our study area.

2.4. Statistical analysis

Because we had more sampled trees than Alpha samplers, trees nearest to each Alpha sampler were grouped and their lichen variables values were averaged. Annual NH_3 concentrations at each site were obtained by averaging all samplings along the study period. The lichen descriptors were plotted against annual atmospheric NH_3 concentrations of the sampled sites along the gradient (n=15).

CLEs were obtained following the proposal of Cape et al. (2009), with the adaptation for lichen LDVs developed by Pinho et al. (2012). The procedure is based in a linear regression fit between NH_3 concentrations and lichen LDV values. Because the CLE corresponds to the concentration of atmospheric NH_3 above which direct adverse effects may occur according to present knowledge (Cape et al., 2009), the NH_3 concentration at the first point with altered lichen values was considered to estimate the critical level. In order to provide a conservative estimate (i.e. not to underestimate this values), the CLE were determined considering the 95% confidence band of the regression (Cape et al., 2009). In order to ensure a linear fit the NH_3 concentration values were log transformed. Statistical analyses were performed with Statistica (StatSoft 2004) and Sigmaplot 11.0 (Systat Software Inc., San Jose, CA, USA).

3. **Results**

3.1. Ammonia concentrations

3.1.1 Temporal variability

Monthly atmospheric NH_3 concentrations averaged for the 15 sites downwind from the barn varied between 5.6 and 12.7 µg m^{-3}, with the highest NH_3 concentrations occurring at the end of summer and autumn (Table 2), a period in the year of high temperatures and low precipitation (Fig. 2). However, meteorological variables seemed not to have an effect on this pattern, since linear correlation was non-significant for NH_3 concentrations *vs.* precipitation ($r= -0.25$; $p=0.43$) and mean temperature ($r= 0.19$; $p=0.49$).

The highest concentrations corresponded to the site closest to the farm which varied between 27.0 and 72 µgm^{-3} along the year while the lowest were at the furthest site from the farm, ranging from 0.9 to 3.2 µg m^{-3} (Table 3).

3.1.2 Spatial variability

Site averaged NH_3 concentrations for the sampling period plotted against distance to the barn showed an exponential decrease with increasing distance, with values around 2-3 µg m^{-3} at distances greater than 300 m, with a minimum of 1.8 µg m^{-3} at 615 m from the barn (Table 3; Fig. 3). The lowest value was about double than the average NH_3 concentrations reported from La Castanya background rural site in Montseny (0.7 µg m^{-3}; García-Gómez et al., 2016) which is 15 km distant from the study site.

3.2. Lichen functional diversity

A total of 53 species were recorded in this study (Table 2): 13 were nitrophytic, 18 mesotrophic and 22 oligotrophic. Total LDV ranged from 21 to 83, LDVnitro varied from 3 to 75, LDVmeso ranged from 0 to 18 and LDVoligo ranged from 1 to 21. Most of the species observed were crustose lichens (54%), with only a few squamulose and leprose species (6%), while the remaining species were foliose (30%) and fruticose species (10%). On average, the LDV of crustose species comprised about 47% of total LDV, while fruticose species was on average only 1% of total LDV. The most frequent species (>50% occurrence) were *Candelaria concolor, Flavoparmelia caperata, Hyperphyscia adglutinata, Lecanora chlarotera, Pertusaria amara* and *Phlyctis argena.*

Species richness and total LDV were not significantly related with average NH_3 concentrations (Fig. 4). By contrast, lichen functional variables were significantly related to average NH_3 concentrations, especially the nitrophytic functional group (Fig. 5). LDVoligo and LDVmeso were significantly and negatively correlated with NH_3 concentrations, while LDVnitro showed a significant positive relationship (Fig. 5).

The critical levels of atmospheric ammonia were calculated taking into consideration the first point with an altered biodiversity, starting on the lowest concentration of NH_3 (Cape et al., 2009; Pinho et al., 2012). Because there are no background levels for biodiversity in this region, the values obtained were compared to other studies in the Iberian Peninsula (Pinho et al., 2011; 2012; 2014b). For both oligotrophic and nitrophytic functional groups, the first point (at the lowest NH_3 concentration) was considered to represent background biodiversity values (unaltered) and thus the second point was considered altered. This resulted in CLE values of less than 3.1 and 2.6 µg m^{-3} for the oligotrophic and nitrophytic functional groups, respectively (Fig. 6). The difference between the two CLE values was within the confidence bands interval, and for that reason the values were not considered to be different from each other.

4. Discussion

CLE values were calculated based on lichen functional groups related to nitrogen tolerance. The good correlations obtained for oligotrophic and nitrophytic functional groups confirmed the validity of using nitrogen functional groups to derive CLEs for semi-natural holm-oak Mediterranean forests. The NH_3 CLE value was estimated to be 2.6 – 3.1 µg m^{-3} depending on the lichen functional group considered.

Similar to earlier works, total lichen diversity metrics (LDVtotal and species richness) were poorly related to atmospheric NH_3 concentrations (Pinho et al., 2008; Van Dobben and Ter Braak, 1998). Though total diversity has been successfully used for monitoring atmospheric pollutants that similarly affect various species (Asta et al., 2002; Giordani, 2007; Pinho et al., 2008; Svoboda, 2007), these metrics fail to assess the effects of atmospheric NH_3, as seen here. This happens because lichen species have different degrees of tolerance to nitrogen, which, despite N being a nutrient, can become toxic at high concentrations. By contrast, lichen functional groups based on this differential tolerance to eutrophication were significantly related with NH_3 atmospheric concentrations (Fig. 5). This confirms the value of functional group metrics as ecological indicators of nitrogen deposition (Giordani, 2007; Giordani et al., 2014; Pinho et al., 2011; 2014a; 2014b).

Our proposed CLEs were slightly higher than: i) the recommended CLE at the European level (1 µg m^{-3}); ii) the previous value reported for a Portuguese semi-natural area in an open cork oak woodland (1.9 µg m^{-3}; Pinho et al., 2011), iii) the recent values obtained for remote areas in Portugal, 0.6 µg m^{-3} (Pinho et al., 2014a). However, the value is similar to the 2.5 µg m^{-3} NOEC (No Observable Effect Concentration) suggested for an area influenced by a pig farm in Italy (Frati et al., 2007). This may be indicative that we are in an environment with a high pollution background. In fact, the lowest NH_3 concentrations found in our work (1.8 µg m^{-3}) were above the background levels registered for European remote areas (1 µg m^{-3}; Cape et al., 2009), for Portugal (1.4 µg m^{-3}; Pinho et al., 2012) and NE Spain (0.7 µg m^{-3}, García-Gómez et al., 2016) The higher CLE values we obtained, related to Portugal, may be explained by the background high emissions in the region surrounding the sampling area, located in the Plana de Vic region which has a tradition of intense pig and cow farming and extended agricultural activity (Otero et al., 2009). Further, NO_x emissions from traffic and from the industrial activity originated in the nearby city of Vic (7 km; 41.627 inhabitants in 2014), may add to the N pollution load of this region, and to the study site in particular. In fact, NO_2 emissions measured at an air quality station located 11 km up north of the study area amounted to 22 µg m^{-3} in 2013, one of the highest values recorded by the Catalan Air Quality Network (Xarxa de Prevenció i Vigilància de la Contaminació Atmosfèrica, Generalitat de Catalunya 2014). Hence, some past eutrophication might have occurred in the studied forest that together with other pollutants emitted by industries from the region contributed to the high background pollution.

The high background pollution in our study area resulted in similar CLEs based on oligotrophic and nitrophytic functional groups (Fig. 6). This may be the result of the loss of the most sensitive species due to the high background pollution. An exhaustive work on epiphytic lichens in this region's holm oak forests proposed, among other things, a list of species that indicate the conservation status of these Mediterranean forests (Longán, 2006). From the 23 species indictors of high conservation status, only 12 were found in our inventories. Even if we take into consideration that some species may be absent due to geographic location (i.e. located in coastal or inland regions, or on mountains), some sensitive species still seem to have disappeared from what is considered a high quality preserved forest in this region. Due to the uncertainty derived from the probable loss of N-sensitive oligotrophic species in this forest, we considered the nitrophytic lichen functional group as more reliable for the indication of CLEs. Thus, we propose a CLE value of 2.6 µg m^{-3}.

Our results suggest that the CLE may represent, like in the case of the mentioned Italian pig farm (Frati et al., 2007), a CLE for an already impacted area. The CLE value obtained in our study, clearly distinct from other studies, show the importance of assessing CLEs under different environmental conditions.

Climate has also been shown to influence the determination of ammonia CLEs as it determines dry and wet deposition rates, eventually resulting in different N loads and effects on ecosystems (Jovan et al., 2012). Likewise, the type of forest structure may also influence CLEs determination. For example, in closed canopy dense forests, ammonia may take longer to enter the system and its deposition rate may be lower due to the barrier and filter effects of the dense tree cover, resulting in higher CLEs when comparing to ecosystems composed of sparse tree cover (Pinho et al. 2011). Our results suggest that background pollution was the main factor for CLEs determination. Probable high historical background levels at the site produced an impacted environment where the most sensitive species had already been lost, and consequently, high CLE values were found. The role of environmental conditions on CLE determination has been previously highlighted by other authors (Cape et al. 2009), and point to the need of further research, like the one here presented, focusing on different types of ecosystems and different environmental conditions.

Ammonia air concentrations showed a typical exponential decrease with distance from the barn, reaching stable low values in less than 1 km from the point source. This is in agreement with previous works showing that NH_3 is deposited in the vicinity of point sources and has a low residence time in the air (Frati et al., 2007; Pitcairn et al., 1998; Sanz et al., 2007). We did not find any clear seasonal trend for NH_3 atmospheric levels during an annual period, nor close correlations with meteorological variables. Seasonal increases of NH_3 atmospheric concentrations have been reported due to NH_3 volatilization in response to temperature raise during the warm season (Behera et al., 2013; Sommer et al., 1991) and due to higher microbiological activity during rainy periods (Kumar et al., 2004), but such a pattern was not observed in this study.

5. Conclusions

The atmospheric NH_3 CLE for a semi-natural Mediterranean evergreen forest in NE Spain was determined to be 2.6- 3.1 µg m^{-3}, based on lichen functional diversity. Such values are higher than the currently accepted European CLE of 1 µg m^{-3} (Hallsworth et al., 2010) and than previous CLEs reported for open woodlands in western Iberian Peninsula obtained with the same methodology (Pinho et al., 2012; 2014a). The values proposed represent the CLE for an impacted ecosystem due to an historical N exposure from farming and agriculture. Nonetheless, determination of this value is still locally very important to establish a protective threshold to that vegetation type. These results are also of international relevance since they demonstrate that lichen functional groups can be used to derive CLEs at a wide range of ecosystems, independently of its forest structure, and because they contribute to the growing body of knowledge regarding the direct impacts of atmospheric ammonia on the understudied Mediterranean ecosystems.

Acknowledgements

The financial support from the Spanish Government projects CGL2009-13188-C03-01 and MONTES-Consolider CSD-2008-00040 is fully acknowledged. PM would like to thank COST Action FP0903 for financial support of a short term scientific mission trough contract ECOST-STSM-FP0903-120912-019761. PM, PP and CB acknowledge FCT-MEC support by contracts BD/51419/2011, BPD/75425/2010 and Investigador FCT. The comments of one reviewer are greatly appreciated.

References

Asman, W. A., Sutton, M. A. & Schjørring, J. K.: 1998, 'Ammonia: emission, atmospheric transport and deposition', New Phytologist 139, 27-48.

Aguillaume, L., Rodrigo A. & Avila, A.: 2016, 'Long-term effects of changing atmospheric pollution on throughfall, bulk deposition and streamwaters in a Mediterranean forest', Science of the Total Environment 544, 919-928.

Aguillaume, L., García-Gómez, H., Izquieta, S., Alonso, R., Elustondo, D., Santamaría J.M., & Avila, A.: 2017, 'Estimating dry deposition and canopy exchange in Mediterranean holm-oak forests with a canopy budget model: a focus on N deposition', Atmospheric Environment 152, 191-200.

Asta, J., Erhardt, W., Ferretti, M., Fornasier, F., Kirschbaum, U., Nimis, P., Purvis, O., Pirintsos, S., Scheidegger, C. & Van Haluwyn, C.: 2002, 'European guideline for mapping lichen diversity as an indicator of environmental stress', British Lichen Society.

Behera, S. N., Sharma, M., Aneja, V. P. & Balasubramanian, R.: 2013, 'Ammonia in the atmosphere: a review on emission sources, atmospheric chemistry and deposition on terrestrial bodies', Environmental Science and Pollution Research 20, 8092-8131.

Bell, M. L., Dominici, F., Ebisu, K., Zeger, S. L., & Samet, J. M.: 2007, 'Spatial and temporal variation in PM2. 5 chemical composition in the United States for health effects studies', Environmental Health Perspectives 15, 989-995.

Cape, J., Van der Eerden, L., Sheppard, L., Leith, I. & Sutton, M.: 2009, 'Evidence for changing the critical level for ammonia', Environmental Pollution 157, 1033-1037.

Fenn, M., Jovan, S., Yuan, F., Geiser, L., Meixner, T. & Gimeno, B.: 2008, 'Empirical and simulated critical loads for nitrogen deposition in California mixed conifer forests', Environmental Pollution 155, 492-511.

Finlayson-Pitts, B. J. & Pitts Jr, J. N.: 1999, Chemistry of the upper and lower atmosphere: theory, experiments, and applications, Academic Press.

Fondazione Salvatore Maugeri.: 2006, 'Instruction manual for Radiello sampler'. Edition 01/2006. http//www.radiello.com.

Frati, L., Santoni, S., Nicolardi, V., Gaggi, C., Brunialti, G., Guttova, A., Gaudino, S., Pati, A., Pirintsos, S. & Loppi, S.: 2007, 'Lichen biomonitoring of ammonia emission and nitrogen deposition around a pig stockfarm', Environmental Pollution 146, 311-316.

Galloway, J. N., Aber, J. D., Erisman, J. W., Seitzinger, S. P., Howarth, R. W., Cowling, E. B. & Cosby, B. J.: 2003, 'The nitrogen cascade', Bioscience 53, 341-356.

Galloway, J. N. & Cowling, E. B.: 2002, 'Reactive nitrogen and the world: 200 years of change', AMBIO: A Journal of the Human Environment 31, 64-71.

García-Gómez, H., Aguillaume, L., Izquieta, S., Valiño, F., Avila, A., Elustondo, D., Santamaría, J.M., Alastuey, A., Calvete-Sogo, H., González-Fernández I., Alonso, R.: 2016, 'Atmospheric pollutants in peri-urban forests of *Quercus ilex*: evidence of pollution abatement and threats for vegetation ', Environmental Science and Pollution Research 23, 6499-6413.

Geiser, L. H., Jovan, S. E., Glavich, D. A. & Porter, M. K.: 2010, 'Lichen-based critical loads for atmospheric nitrogen deposition in Western Oregon and Washington Forests, USA', Environmental Pollution 158, 2412-2421.

Geiser, L. H. & Neitlich, P. N.: 2007, 'Air pollution and climate gradients in western Oregon and Washington indicated by epiphytic macrolichens', Environmental Pollution 145, 203-218.

Giordani, P.: 2007, 'Is the diversity of epiphytic lichens a reliable indicator of air pollution? A case study from Italy', Environmental Pollution 146, 317-323.

Giordani, P., Calatayud, V., Stofer, S., Seidling, W., Granke, O. & Fischer, R.: 2014, 'Detecting the nitrogen critical loads on European forests by means of epiphytic lichens. A signal-to-noise evaluation', Forest Ecology and Management 311, 29-40.

Hallsworth, S., Dore, A., Bealey, W., Dragosits, U., Vieno, M., Hellsten, S., Tang, Y. & Sutton, M.: 2010, 'The role of indicator choice in quantifying the threat of atmospheric ammonia to the 'Natura 2000'network', Environmental Science & Policy 13, 671-687.

Jovan, S.: 2008, 'Lichen bioindication of biodiversity, air quality, and climate: baseline results from monitoring in Washington, Oregon, and California'.

Jovan, S., Riddell, J., Padgett, P. E., & Nash, T. H.: 2012, 'Eutrophic lichens respond to multiple forms of N: implications for critical levels and critical loads research', Ecological Applications 22, 1910-1922.

Kumar, R., Gupta, A., Kumari, K. M. & Srivastava, S.: 2004, 'Simultaneous measurements of SO 2, NO 2, HNO 3 and NH 3: seasonal and spatial variations', Current Science 87, 1108-1115.

Longán, A.: 2006, Els líquens epífits com a indicadors de l'estat de conservació del bosc mediterrani: proposta metodològica per als alzinars de Catalunya, Institut d'Estudis Catalans.

Myers, N., Mittermeier, R. A., Mittermeier, C. G., Da Fonseca, G. A. & Kent, J.: 2000, 'Biodiversity hotspots for conservation priorities', Nature 403, 853-858.

Nimis, P.L & Martellos S.: 2008, 'The information system on Italian lichens' http://dbiodbs.univ.trieste.it/

Ochoa-Hueso, R., Allen, E. B., Branquinho, C., Cruz, C., Dias, T., Fenn, M. E., Manrique, E., Pérez-Corona, M. E., Sheppard, L. J. & Stock, W. D.: 2011, 'Nitrogen deposition effects on Mediterranean-type ecosystems: an ecological assessment', Environmental Pollution 159, 2265-2279.

Otero, N., Torrentó, C., Soler, A., Menció, A., & Mas-Pla, J.: 2009, 'Monitoring groundwater nitrate attenuation in a regional system coupling hydrogeology with multi-isotopic methods: the case of Plana de Vic (Osona, Spain)', Agriculture, Ecosystems & Environment 133, 103-113.

Phoenix, G. K., Hicks, W. K., Cinderby, S., Kuylenstierna, J. C., Stock, W. D., Dentener, F. J., Giller, K. E., Austin, A. T., Lefroy, R. D. & Gimeno, B. S.: 2006, 'Atmospheric nitrogen deposition in world biodiversity hotspots: the need for a greater global perspective in assessing N deposition impacts', Global Change Biology 12, 470-476.

Pinho, P., Augusto, S., Martins-Loução, M. A., Pereira, M. J., Soares, A., Máguas, C. & Branquinho, C.: 2008, 'Causes of change in nitrophytic and oligotrophic lichen species in a Mediterranean climate: Impact of land cover and atmospheric pollutants', Environmental Pollution 154, 380-389.

Pinho, P., Branquinho, C., Cruz, C., Tang, Y. S., Dias, T., Rosa, A. P., Máguas, C., Martins-Loução, M.-A. & Sutton, M. A.: 2009, 'Assessment of critical levels of atmospheric ammonia for lichen diversity in cork-oak woodland, Portugal', Atmospheric Ammonia, Springer, pp. 109-119.

Pinho, P., Dias, T., Cruz, C., Sim Tang, Y., Sutton, M. A., Martins-Loução, M. A., Maguas, C. & Branquinho, C.: 2011, 'Using lichen functional diversity to assess the effects of atmospheric ammonia in Mediterranean woodlands', Journal of Applied Ecology 48, 1107-1116.

Pinho, P., Llop, E., Ribeiro, M., Cruz, C., Soares, A., Pereira, M. & Branquinho, C.: 2014a, 'Tools for determining critical levels of atmospheric ammonia under the influence of multiple disturbances', Environmental Pollution 188, 88-93.

Pinho, P., Martins-Loução, M.-A., Máguas, C. & Branquinho, C.: 2014b, 'Calibrating Total Nitrogen Concentration in Lichens with Emissions of Reduced Nitrogen at the Regional Scale', Nitrogen Deposition, Critical Loads and Biodiversity, Springer, pp. 217-227.

Pinho, P., Theobald, M., Dias, T., Tang, Y., Cruz, C., Martins-Loução, M., Máguas, C., Sutton, M. & Branquinho, C.: 2012, 'Critical loads of nitrogen deposition and critical levels of atmospheric ammonia for semi-natural Mediterranean evergreen woodlands', Biogeosciences 9, 1205-1215.

Pitcairn, C., Leith, I., Sheppard, L., Sutton, M., Fowler, D., Munro, R., Tang, S. & Wilson, D.: 1998, 'The relationship between nitrogen deposition, species composition and foliar nitrogen concentrations in woodland flora in the vicinity of livestock farms', Environmental Pollution 102, 41-48.

Posthumus, A.: 1988, 'Critical levels for effects of ammonia and ammonium', Proceedings of the Bad Harzburg Workshop, pp. 117-127.

Rockström, J., Steffen, W., Noone, K., Persson, Å., Chapin, F. S., Lambin, E. F., Lenton, T. M., Scheffer, M., Folke, C. & Schellnhuber, H. J.: 2009, 'A safe operating space for humanity', Nature 461, 472-475.

Sanz, M., Montalvo, G., Monter, C., Sanz, F., Illescas, P., Piñeiro, C. & Bigeriego, M.: 2007, 'Ammonia concentration around two poultry farms in the central plateau of spain', Ammonia Emissions in Agriculture, 370.

Sommer, S. G., Olesen, J. E. & Christensen, B. T.: 1991, 'Effects of temperature, wind speed and air humidity on ammonia volatilization from surface applied cattle slurry', The Journal of Agricultural Science 117, 91-100.

Sparrius, L. B.: 2007, 'Response of epiphytic lichen communities to decreasing ammonia air concentrations in a moderately polluted area of The Netherlands', Environmental Pollution 146, 375-379.

Svoboda, D.: 2007, 'Evaluation of the European method for mapping lichen diversity (LDV) as an indicator of environmental stress in the Czech Republic', Biologia 62, 424-431.

Tang, Y. S., Cape, J. N. & Sutton, M. A.: 2001, 'Development and Types of Passive Samplers for Monitoring Atmospheric NO2 and NH3 Concentrations', The Scientific World Journal 1.

Van Dobben, H. & Ter Braak, C.: 1998, 'Effects of atmospheric NH< sub> 3</sub> on epiphytic lichens in the Netherlands: the pitfalls of biological monitoring', Atmospheric Environment 32, 551-557.

Van Herk, C.: 1999, 'Mapping of ammonia pollution with epiphytic lichens in the Netherlands', The Lichenologist 31, 9-20.

Van Herk, C.: 2001, 'Bark pH and susceptibility to toxic air pollutants as independent causes of changes in epiphytic lichen composition in space and time', The Lichenologist 33, 419-442.

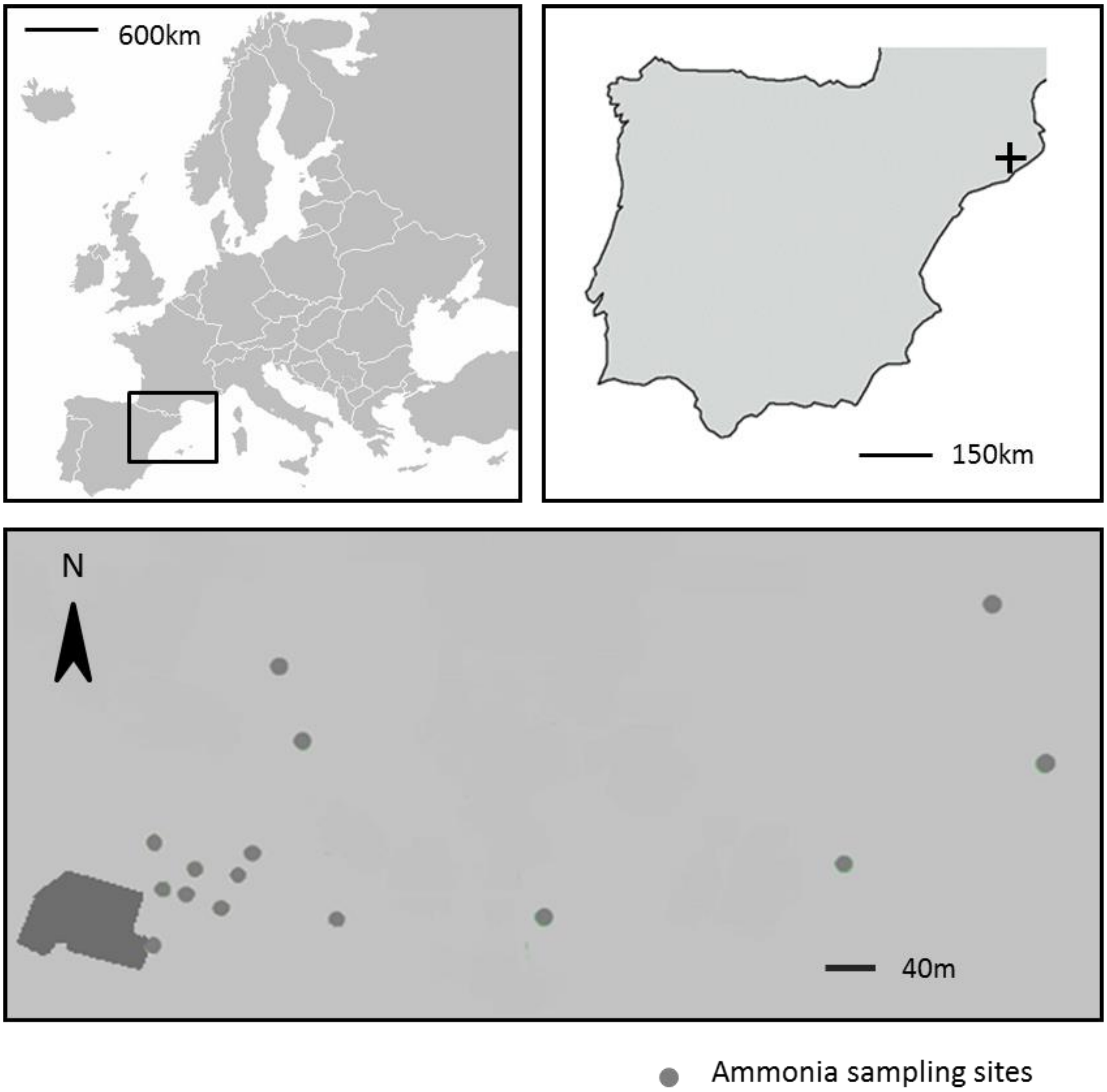


Fig. 1. Above: Location of the study area in Europe and Spain (+). Down: Location of the ammonia sampling sites (dots) in the distance gradient to the barn (shaded area).

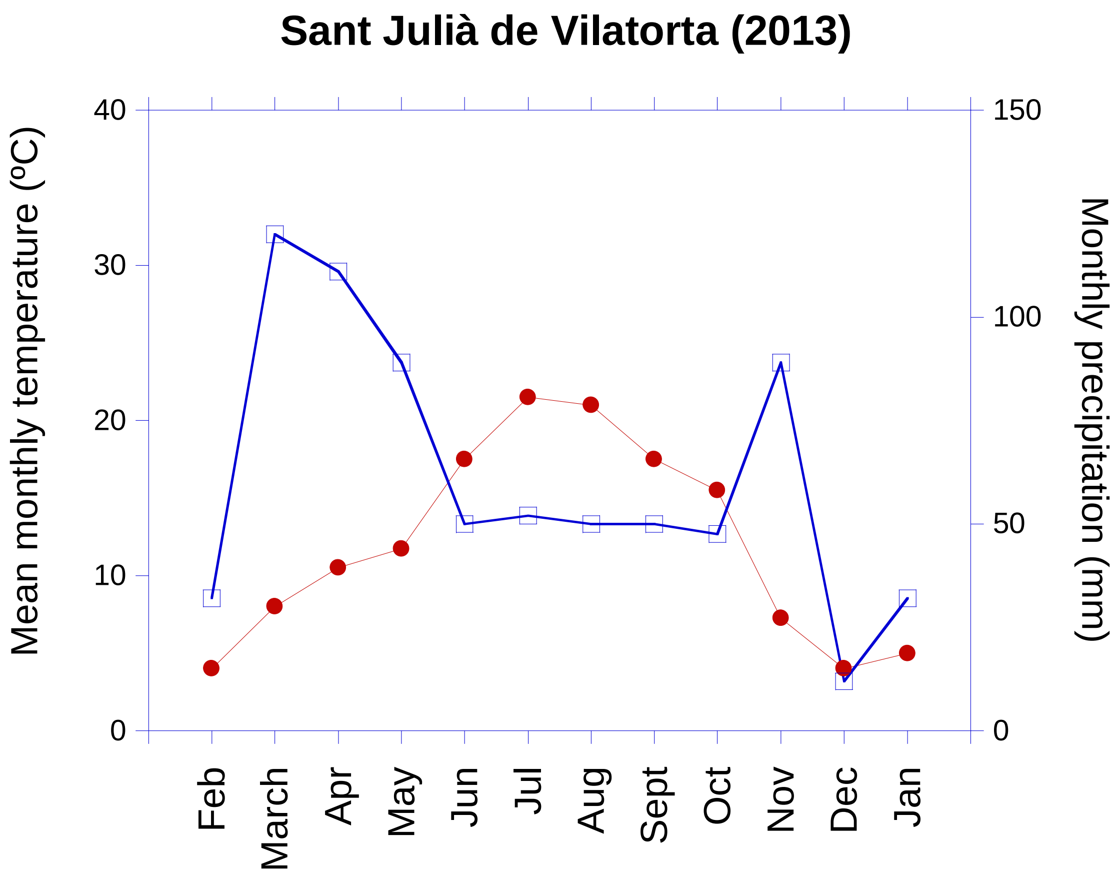


Fig. 2. Mean monthly temperature (ºC) and monthly precipitation (mm) at Sant Julià de Vilatorta Meteorological Station for the study period (February 2013 to January 2014).

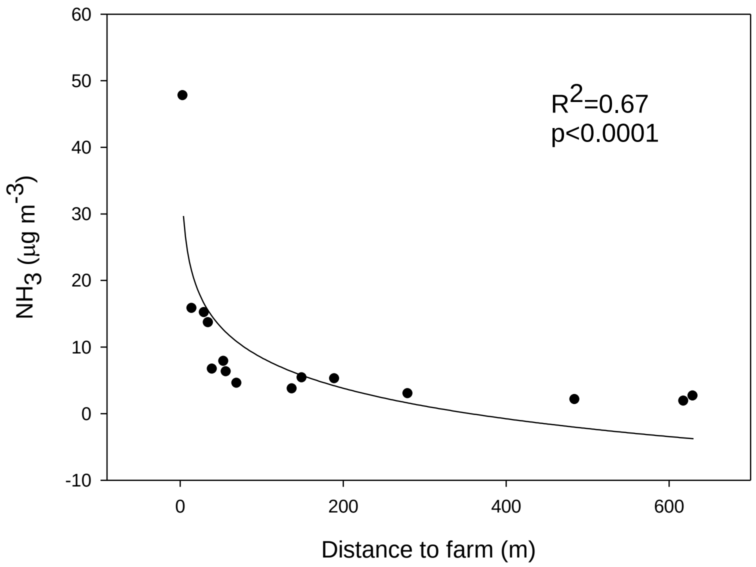
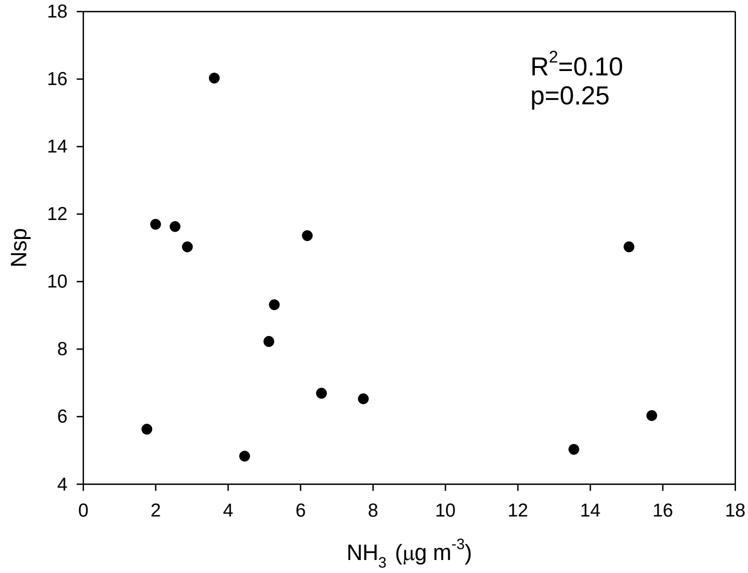


Fig. 3. Relationship between NH_3 air concentrations (µgm^{-3}) and distance to the farm point source (n=15).



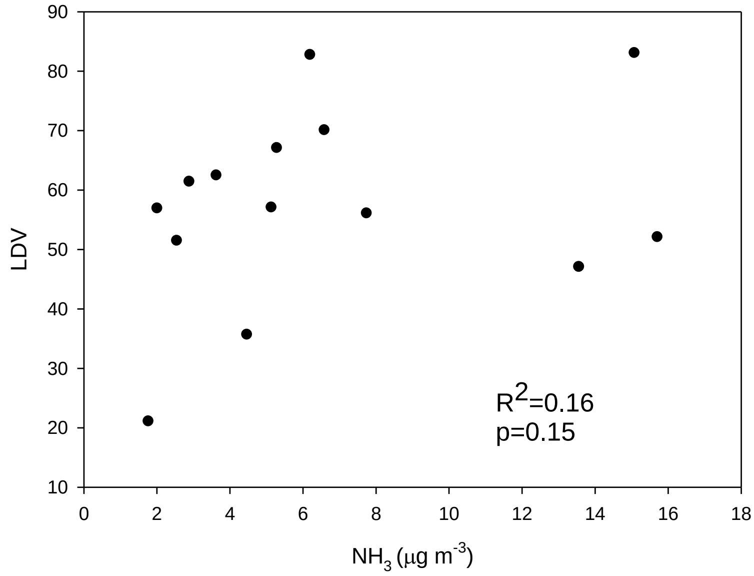


Fig. 4. Relationship between NH_3 air concentrations (µgm^{-3}) and number of species (N sp) and total lichen diversity value (LDV).

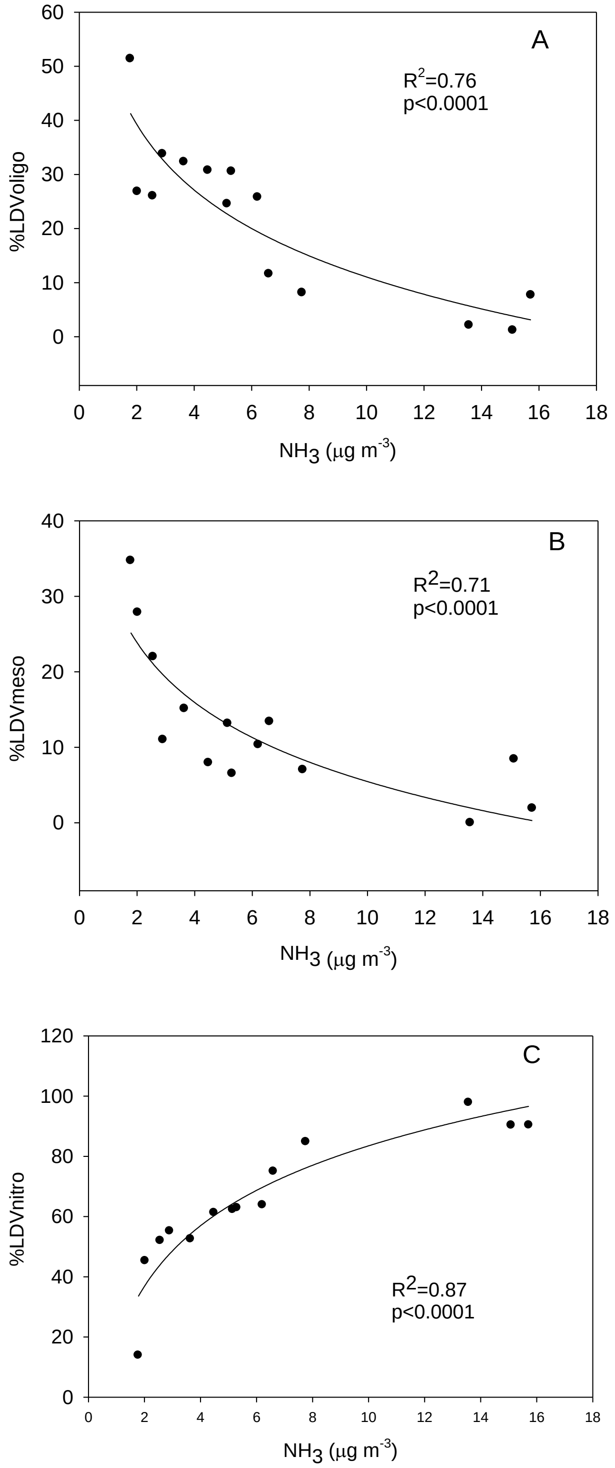


Fig. 5. Relationship between NH_3 air concentration (µgm^{-3}) and relative lichen diversity values for oligotrophic (% LDVoligo, A), mesotrophic (% LDVmeso, B) and nitrophytic (% LDVnitro, C) functional groups (n=14).

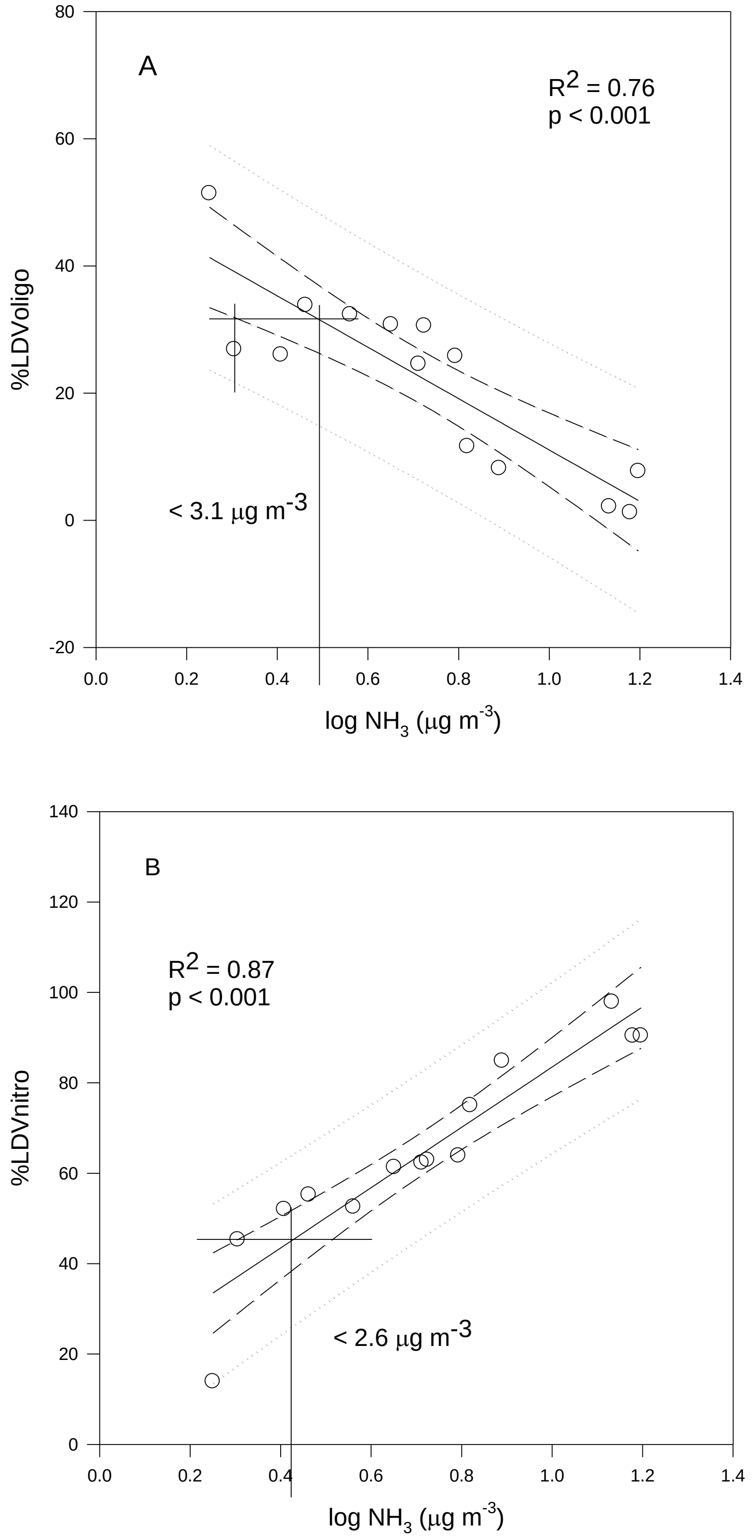


Fig. 6. Determination of the critical levels of atmospheric NH_3 considering the oligotrophic (A) and nitrophytic (B) functional groups. Annual mean NH_3 concentrations (µgm^{-3}) were log transformed (n=14).

Tables

Table Table 1. Lichen species found on *Quercus ilex* with indication of the maximum eutrophication tolerance index (Nimis and Martellos 2008), and if these species are an indicator of high (H) or low (L) conservation forest.

Species name	**Eutrophication tolerance index**	**Indicator of state of conservation forest**
Acrocordia gemmata (Ach.) A. Massal.	1	
Agonimia tristicula (Nyl.) Zahlbr.	1	H
Anaptychia ciliaris (L.) Körb.	3	
Arthonia radiata (Pers.) Ach.	3	
Bacidia polychroa (Th.Fr.) Körb.	1	
Biatoridium monasteriense Körb	2	
Caloplaca ferruginea (Huds.) Th.Fr.	3	
Caloplaca pollinii (A.Massal.) Jatta	3	L
Candelaria concolor (Dicks.) Stein	5	
Candelariella xanthostigma (Ach.) Lettau	3	
Chrysothrix candelaris (L.) Laundon *	3	
Dimerella pineti (Ach.) Vězda	2	
Enterographa crassa (DC.) Fée	2	H
Evernia prunastri (L.) Ach.	3	
Flavoparmelia caperata (L.) Hale	3	
Flavoparmelia soredians (Nyl.) Hale	3	L
Graphis scripta (L.) Ach.	2	H
Gyalecta truncigena (Ach.) Hepp	2	H
Hyperphyscia adglutinata (Flörke) Mayrh. &Poelt	5	L

Hypogymnia physodes (L.) Nyl.	2	
Lecanographa amylacea (Pers.) Egea & Torrente	1	
Lecanora carpinea (L.) Vain.	3	
Lecanora chlarotera Nyl.	5	L
Lecanora conisella Nyl.	2	
Lecanora horiza (Ach.) Linds.	3	
Lecanora hybocarpa (Tuck.) Brodo	2	L
Lecanora strobilina (Spreng.) Kieff.	1	
Lecidella elaeochroma (Ach.) M.Choisy	4	L
Lepraria incana (L.) Ach.	2	H
Lepraria lobificans Nyl.	2	H
Melanelixia fuliginosa subsp. glabratula (Duby) O. Blanco, A. Crespo, Divakar, Essl., D. Hawksw. &Lumbsch	3	
Melanelixia subaurifera (Nyl.) O. Blanco, A. Crespo, Divakar, Essl., D. Hawksw. & Lumbsch	3	
Normandina pulchella (Borrer) Nyl.	3	H
Opegrapha atra Pers.	2	
Opegrapha rufescens Pers.	1	
Opegrapha varia Pers.	2	H
Opegrapha viridis (Ach.) Behlen & Desberger	1	
Parmelia sulcata Taylor	3	
Parmotrema perlatum (Huds.) M.Choisy	2	

Pertusaria albescens (Hudson) M.Choisy & Werner	3	
Pertusaria amara (Ach.) Nyl.	3	
Pertusaria hemisphaerica (Flörke) Erichsen	2	
Pertusaria pertusa (Weigel) Tuck.	2	
Phaeophyscia chloantha (Ach.) Moberg	4	
Phaeophyscia hirsuta (Mereschk.) Essl.	4	
Phaeophyscia orbicularis (Necker) Moberg	5	
Phlyctis agelaea (Ach.) Flot.	2	
Phlyctis argena (Spreng.) Flot.	2	H
Physcia adscendens (Fr.) Oliv.	5	L
Physcia clementei (Turner) Maas Geest.	3	
Physconia distorta (With.) J.R.Laundon	4	
Physconia enteroxantha (Nyl.) Poelt	4	
Physconia grisea (Lam.) Poelt	5	
Physconia perisidiosa (Erichsen) Moberg	3	
Pleurosticta acetabulum (Neck.) Elix & Lumbsch	3	
Porina aenea (Wallr.) Zahlbr.	1	H
Pseudevernia furfuracea (L.) Zopf v. furfuracea	2	
Punctelia subrudecta (Nyl.) Krog	3	
Pyrenula chlorospila Arnold	2	
Ramalina canariensis J.Steiner	4	
Ramalina farinacea (L.) Ach.	2	L
Ramalina fraxinea (L.) Ach.	3	

Ramonia subsphaeroides (Tav.) Vezda	-	H
Schismatomma decolorans (Sm.) Clauzade & Vězda	3	
Strigula ziziphi (A.Massal.) Cl.Roux&Sérus.	3	
Thelopsis rubella Nyl.	1	H
Usnea rubicunda Stirton	2	
Xanthoria parietina (L.) Th.Fr.	5	L

*value updated following Pinho et al., 2011.

Table 2. Basic statistics of monthly NH_3 concentrations of n= 15 sampling sites for the period February 2013 to January 2014. Concentration units = µg m^{-3}.

Month	Mean	St. dev.	Max.	Min.
Feb	10.2	7.7	27.0	1.9
March	9.4	11.7	47.3	1.1
Apr	10.1	9.6	40.3	2.0
May	5.6	9.0	36.7	0.9
Jun	8.2	10.3	42.8	1.5
Jul	7.9	10.0	40.7	1.7
Aug	11.8	14.7	59.6	1.8
Sept	10.6	13.9	56.9	1.9
Oct	12.7	17.8	72.3	1.4
Nov	7.9	9.5	40.8	2.3
Dec	9.9	13.9	57.2	0.6
Jan	7.9	9.9	42.2	2.2

Table 3. Basic statistics of site NH_3 concentrations in a distance gradient from a point source for n=12 months (February 2013 to January 2014). Concentration units = µg m^{-3}.

Distance (m)	Mean	Std. Dev.	Max.	Min.
1	47.0	12.3	72.3	27.0
5	15.7	3.7	21.5	9.5
5.5	14.8	4.0	20.7	8.7
5.6	7.7	2.4	11.6	2.8
5.7	13.3	3.5	18.9	6.6
6	6.5	2.6	11.6	3.1
7	6.1	1.9	8.9	2.0
10	5.3	1.6	8.1	2.9
160	4.4	1.3	6.0	1.9
170	5.1	1.3	7.3	2.7
200	3.6	1.4	5.6	1.5
300	3.0	1.3	5.1	1.3
500	2.6	0.6	3.6	1.8
615	1.8	0.6	2.7	0.6
620	2.1	0.7	3.2	0.9

COMMENTS FOR THE AUTHOR:

Reviewer #3: Manuscript reviewed is very interesting and worthy for publication in WASP journal.

Reviewer #7: WATE-D-16-00066: The critical levels of atmospheric ammonia in a Mediterranean forest in North-Eastern Spain.

Laura Aguillaume, Anna Avila, Pedro Pinho, Paula Matos, Esteve Llop and Cristina Branquinho

The manuscript describes the assessment of lichen functional groups with distance from an ammonia emissions source, and the determination of a critical level of ammonia. The manuscript is well suited to Water Air and Soil Pollution, and will make an important contribution to the literature with respect to the assessment of impacts from ammonia emissions. I recommend that the article be published following major revisions. The revisions are described below should not be too taxing for the authors but they are important.

Overall, the revisions should primarily focus on four aspects: (1) improvement of the written English, I urge the authors to get a native English speaker to edit the manuscript prior to re-submission;

We want to thank the reviewer for very useful comments. Consequently, we have revised the language and rearranged the text , notably in Material and Methods, Results (Ammonia concentration section) and Discussion, to better clarify the arguments.

Below, we provide the answers (ASW) to the various points raised by the reviewer:

ASW - English was revised as requested.

(2) the authors need to provide more details on the ammonia sampling, given that the critical limit is set against observed ammonia, the authors need to show that observations are reliable;

ASW – We fully agree. This section on Ammonia measurements has been rewritten in to give more specific details. New text in L 146-165.

(3) the authors need to provide further details on the determination (method) of the critical limit, why the second altered point? If only two points are needed then why measure 15 sites along the transect?

ASW- The method for determining the critical levels follows Cape et al. (2009). Briefly the method looks for sampling sites that are considered altered and non-altered, and determines the critical level by taking into consideration the confidence interval in the relationship between atmospheric ammonia and a biodiversity variable. Sampling along a gradient is necessary because we do not know a priory which point is altered. Additionally, multiple points are necessary to establish the confidence interval in the relationship. This is further explained in the Methods section (L225-235).

(4) as the authors note, the CLE may represent an already impacted site. This needs further discussion. Are 'typical' oligotrophic lichen species already missing for that habitat? Expand discussion on the lichen species that were observed and the likelihood that the site is already impacted. Additional line-specific comments are provided.

ASW - Further discussion was added as requested. Please see discussion and answers to specific comments.

Specific comments by line (L) number:

L26. In general ammonia emissions have stayed the same during the last few decades (more-or-less) or decreased slightly.

L28. Delete "For that reason".

L34. Delete "To fill this gap".

ASW- Thank you for suggestions. The abstract has been fully rewritten and your above comments have been taken into account.

L61. Are humans impacted by excessive nitrogen? There are direct impacts from elevated nitrogen but excessive suggests nitrogen saturation.

ASW –the impacts in human health are mostly due to the fact that NH_4^+ is part of the particulate matter (PM 2.5). This sentence was altered to reflect this. (L54-55).

L63-64. Reword, meaning is unclear. Perhaps state "The development of emissions abatement policies is underpinned by the concept of 'critical levels' defined…"

ASW – Reworded as requested (L65).

L74. Delete "thus they deserve increased research efforts."

ASW – Deleted and reworded. (L77).

L80. Delete "including the nitrogen supply".

ASW – Deleted as requested. (L81).

L83. You do not need to state this as it is already included in the previous sentence "This shift is caused by an increase of N availability which favours N tolerant species, as well as by".

ASW – This sentence was removed as requested. (L82-83)

L120. Add space "1500 m²".

ASW – The space was added (L120).

L121. The authors need to present data on wind direction (during the study period). The authors only present rainfall and temperature BUT these data are not cited in the manuscript. Wind direction is equally important.

ASW – Precipitation and temperature are commented in the text and Fig. 2 is cited (L140).

Data on wind direction added as requested (L138-140).

L136. State length of transect, provide details on sites. How they were selected. Distance between sampling sites, etc. Justify the transect distance. Is 600 m long enough? 1000 m better?

ASW – Information on maximum distance from the ammonia point source was added and the transect disposition is justified
In general, the influence of a single source of atmospheric ammonia can be detected up to 1000m, but that depends greatly on the wind direction, the source intensity and land-cover. Here sampling reveal that the background levels were observed near 300m from sources, probably due to the fact that this was not a very intensive farm. Thus the 600m transect was sufficient. A sentence was added to the Methods section to support this (L129-130).

L140. Add space "2 m".

ASW – The space was added

L142. The authors have not reported results for the travel blanks. The measured air concentration is typically estimated exposed samplers less travel or laboratory blanks. This should be reported in the results of supporting information.

ASW – Information added (L168-174).

L142. How many samplers were deployed over the entire study? How many laboratory and travel blanks?

ASW – The requested information was added in the rewritten section 2.2. Ammonia measurements.

L143. Reword "One sampler was deployed per…"

ASW – Modified in new rewritten section 2.2. Ammonia measurements

L144. Similarly the authors have not reported the results of the replicate samplers.

Again, this should be stated in results or supporting information. Given that the objective is to set / develop critical levels, the authors need to demonstrate that ambient ammonia results were reliable.

ASW – Correlation between both sampling methods was r2=0.98, indicating a very good replicability. This is stated in the text (L.158). The way the final concentrations for every site is obtained (as average between methods for the replicated sites and the Alpha result for the rest) is stated in L. 160-164.

L147. Further details on the Radiello could be provided in supporting information.

ASW – A reference has been added for the reader to find more information on Radiello samplers and procedures (L167)

L163. Refer to the exact Figure.

ASW –Fig.1 cited (L180)

L203-204. Above you state that ALPHA samplers were exposed for 2-3 weeks. Here you state monthly. Clarify? Was the annual average estimated as a weight (exposure period) average?

ASW – We sampled biweekly and data were averaged to produce averaged monthly values. A new Table (Table 2) has been added to show basic statistics of monthly values and Fig. 3 has been deleted, since the same information appears in Table 2.

L208-214. This section is too brief. Expand and provide more detail on the procedure, especially for L211-213.

ASW – Further information was added to this section (L225-235).

L222. The monthly average would be better shown as box-plots. This would provide details on the variability across the transect each month. Alternatively provide as a table (in supporting information is okay).

ASW – A new Table 2 has been added (see above). Also Table 3 has been included with basic statistics for the gradient sites. See new text in sections 3.1.1 Temporal variability and 3.1.2 Spatial variability.

L225. Reword "The highest NH3 concentrations were observed during the…".

ASW – See new text in section 3.1 Ammonia concentrations

L230. Approximately 620 m from barn! Provide details on transect in the methods section.

ASW – See new text in section 3.1 Ammonia concentrations and more details provided in the Method section 2.1

L250-251. Why the second point. How is an 'altered' point determined? What is the required spacing on observations points? If there were more (or less) points close to the barn then this would influence the critical limit? Do points closer to the barn suffer from forest edge effect?

ASW – Thank you, the sentence was poorly written. It was not the second point automatically; it was the first point with altered biodiversity. Thus we have re-written the text to accommodate this (L279-289). The altered point was determined by taking into consideration other studies where a background biodiversity was available (for example a background value of nitrophytic was found to occur approx. below 15% and for oligotrophic above 40%). Taking these values as general guidelines, the first altered point was considered to be the second. There are no a priory spacing between points because we do not know a priory the concentrations in the field, but a good approach is to place the samplers increasing the distance between samplers as we move away from the source (due to the log deposition of ammonia). This was done in our study, but note that fitting a model to the relationship the importance of the precise placement of the points is decreased. Because the critical levels were observed to occur at the lowest concentration, placing more points far from the barn could influence the calculated CLEs. We cannot estimate how much the values would differ, but probably not very significantly. Yes, there is an edge effect whenever NH_3 flows intercept vegetation (due to higher deposition values there). However, we used the observed NH_3 concentration, which already takes this into consideration (i.e. the measured values are the ones actually experienced by lichens).

L252. Clarify. This suggests that nitrophytic lichen increase before oligotrophic lichens are lost? Is this typical?

ASW –This is right, but because the difference between the two CLE values was within the confidence bands interval, they were not considered to be different from each other. This explanation was added to the Results section (L 288). Additionally, a new point was made in the Discussion, suggesting that we are dealing with an area with high background levels of pollution, and thus the oligotrophic species may have already been impacted before present. As a consequence, we consider the values obtained with nitrophytic functional groups to be more reliable.

L256. Is it important that this is 'For the first time'? If it was the second time would results be less important?

ASW –In fact, as we are talking about CLEs, a second time would be likewise important, as it would represent an update of the values according to present knowledge. Anyway, we have rewritten the paragraph without mentioning this aspect (L292-296).

L269. Reword "the recommended CLE at the European level".

ASW – reworded as requested

L273. This suggests that perhaps the CLE in this study (current study) and that associated with the pig farm may represent CLE for impacted areas, i.e., most sensitive species (the oligotrophic species) are already lost? Provide a discussion on the species that were observed, and if key species for the habitat are already missing.

ASW – we have added the suggestion saying that this may represent a CLE for an impacted area . And we have added a discussion on the species that typically indicate a high quality conservation status in this region forests to complement it as requested (L335-346).

L277. Yes I agree. So what does this CLE represent? The CLE for an impact ecosystem? However, it is worth noting that the CLE from this study is less than the recommended limit.

ASW – The determined CLE represents the value for an already impacted ecosystem. We added further information to the discussion section that supports this view, including numbers about the sensitive species found (L334-339).Please note that the CLEs observed here was higher than the CLE suggested at the European level (1 ug/m3), and comparable to CLEs observed in impacted ecosystem. It was lower than the CLE for vegetation (3 ug/m3), but in our study we are considering lichens, so this comparison is not justified.

L294-295. Yes, expand on this point.

ASW – We have expanded as requested (L353-366), discussing better the changes that environmental conditions may have on CLE determination.

L296. Format of citations, 'and others'?

ASW – citation corrected.

L296. Reword, "…2009); different …".

ASW – the sentence was reworded (L365-366).

L301. What are the background levels for this region? Did you measure at 1 km? Then can you state they reach background in less than 1 km?

ASW – At the La Castanya site, which is considered a rural background forest site and lies aprox 15 km from the study site, the NH_3 mean concentration for the period Feb 2011 to Feb 2013 was 0.7 ug/m3 (García-Gomez et al. 2016, Environmental Science Pollut. Res.). In our study gradient, NH_3 conc. were aprox. constant (1.8 - 2 ug/m3) from 300m on. It seems that for this impacted site, background concentrations are reached at this spatial scale.

L320. They also contribute to growing body of analysis regarding direct impacts from ammonia.

ASW – we have added this information to the conclusions.