



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



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### ABSTRACT

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  $0.69 \mu\text{g m}^{-3}$ , below the previously accepted value of  $1.9 \mu\text{g m}^{-3}$ , and below the currently accepted pan-European CLE of  $1.0 \mu\text{g m}^{-3}$ .

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### 1. Introduction

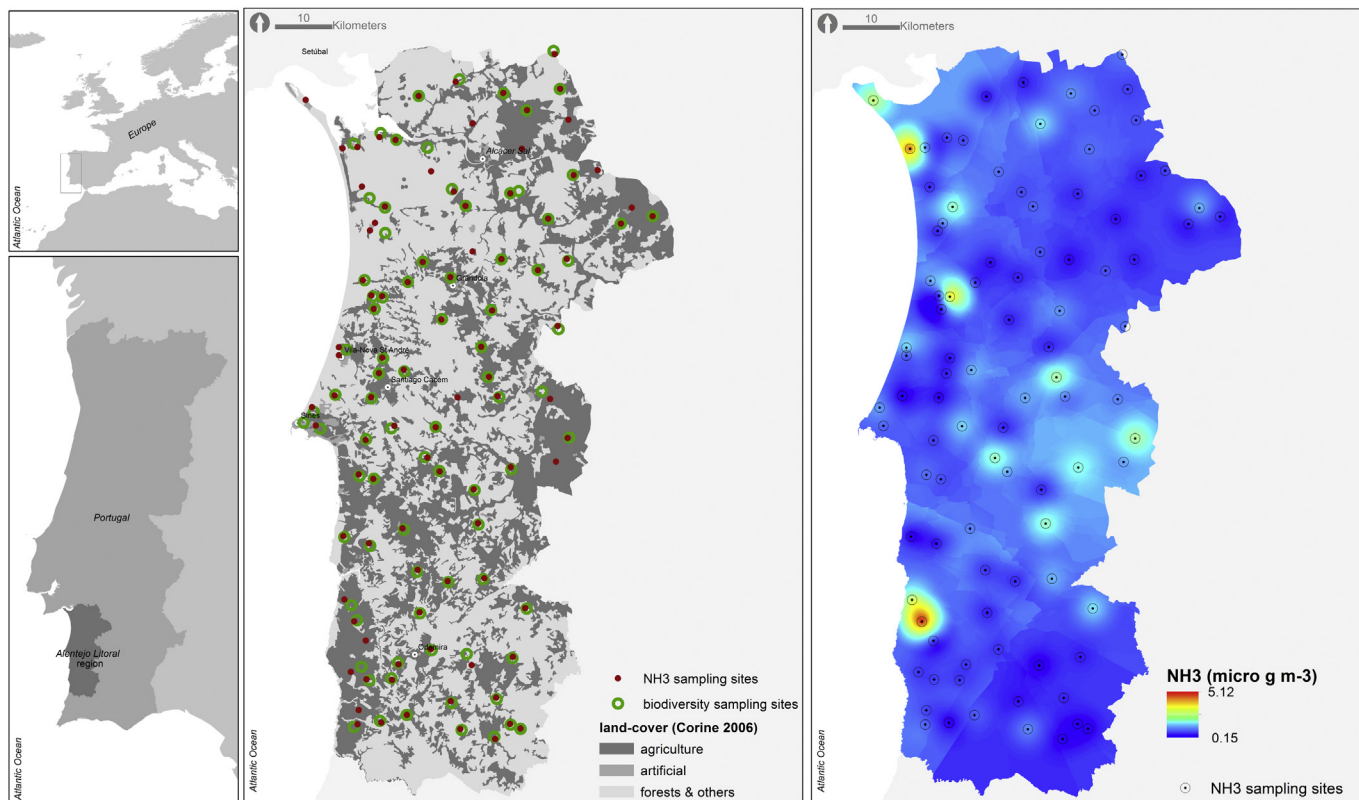
Agriculture is the source of most atmospheric ammonia ( $\text{NH}_3$ ) (Galloway et al., 2003), which negatively affects biodiversity and ecosystems functioning (Bobbink et al., 2010; Rockstrom et al., 2009). To protect ecosystems and their services, critical levels (CLEs) of  $\text{NH}_3$  have been established. These are the concentrations of atmospheric ammonia ( $[\text{NH}_3]_{\text{atm}}$ ) 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  $\text{NH}_3$  has recently been revised downwards, from 8 to  $1 \mu\text{g m}^{-3}$  (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  $\text{NH}_3$  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  $[\text{NH}_3]_{\text{atm}}$  background levels, so that it was not possible to calculate CLEs of  $\text{NH}_3$  (Jovan et al., 2012). When regional areas with lower  $[\text{NH}_3]_{\text{atm}}$  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  $[\text{NH}_3]_{\text{atm}}$  than in high intensity farming areas.

The challenge of considering large regions in the analysis of the effects of  $\text{NH}_3$  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  $\text{NH}_3$  and other pollutants in close proximity. Moreover, Mediterranean ecosystems are strongly underrepresented in studies on the impact of  $\text{NH}_3$  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  $\text{NH}_3$  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  $\text{NH}_3$  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  $\text{NH}_3$  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  $\text{NH}_3$ , 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  $\text{SO}_2$  and  $\text{NO}_x$  and also to particles from the typical Mediterranean dust (Loppi and Pirintso, 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  $\text{NH}_3$  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  $[\text{NH}_3]_{\text{atm}}$ .

## 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  $16.7^\circ\text{C}$  and the annual rainfall  $578\text{ mm}$  (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.  $5303\text{ km}^2$ . 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  $\text{SO}_2$ ,  $\text{NO}_x$  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  $15\text{ km}$  grid and selecting randomly three sampling locations within each grid cell. In each sampling point we characterized  $[\text{NH}_3]_{\text{atm}}$ , 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  $[\text{NH}_3]_{\text{atm}}$ . Each sampler containing a citric-acid-impregnated cellulose fiber adsorbent (13% w/v) which was colorimetrically analyzed (Spectra Rainbow A-5082 spectrophotometer, Tecan, Männedorf,

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