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Short communication

## Can ammonia tolerance amongst lichen functional groups be explained by physiological responses?

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## ABSTRACT

Ammonia (NH<sub>3</sub>) empirical critical levels for Europe were re-evaluated in 2009, based mainly on the ecological responses of lichen communities without acknowledging the physiological differences between oligotrophic and nitrophytic species. Here, we compare a nitrogen sensitive lichen (*Evernia prunastri*) with a nitrogen tolerant one (*Xanthoria parietina*), focussing on their physiological response (Fv/Fm) to short-term NH<sub>3</sub> exposure and their frequency of occurrence along an NH<sub>3</sub> field gradient. Both frequency and Fv/Fm of *E. prunastri* decreased abruptly above 3 μg m<sup>-3</sup> NH<sub>3</sub> suggesting direct adverse effects of NH<sub>3</sub> on its photosynthetic performance. By contrast, *X. parietina* increased its frequency with NH<sub>3</sub>, despite showing decreased capacity of photosystem II above 50 μg m<sup>-3</sup> NH<sub>3</sub>, suggesting that the ecological success of *X. parietina* at ammonia-rich sites might be related to indirect effects of increased nitrogen (NH<sub>3</sub>) availability. These results highlight the need to establish NH<sub>3</sub> critical levels based on oligotrophic lichen species.

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## 1. Introduction

Reactive nitrogen (Nr) in the atmosphere, in the form of particulates such as PM10's, can threaten human health, while increased Nr deposition threatens the existence of many semi-natural ecosystems as we know them (Galloway and Cowling, 2002; Dentener et al., 2006). Ammonia gas (NH<sub>3</sub>), emitted predominantly from agricultural sources, is the main source of reduced Nr and European legislation exists to protect vulnerable ecosystems, for example by establishing empirical critical loads and levels (CLE) for NH<sub>3</sub>.

Ammonia CLE correspond to the NH<sub>3</sub> concentration above which direct adverse effects, i.e. changes in community composition with biodiversity reduction, may occur according to present knowledge on specified sensitive elements of the environment (Cape et al., 2009). Recently, NH<sub>3</sub> CLEs were revised (Cape et al., 2009; Fenn et al., 2008), mainly based on changes in communities and functional groups of lichens, one of the most sensitive components of the ecosystem to Nr excess (Jovan et al., 2012). Presently the CLE for NH<sub>3</sub> for lichens and bryophytes is 1 μg m<sup>-3</sup>

NH<sub>3</sub> where they form a key component of ecosystem integrity (Cape et al., 2009). Pinho et al. (2009, 2012) confirmed the suitability of lichen functional groups for determining the NH<sub>3</sub> CLE, identifying CLE below 1.9 μg m<sup>-3</sup> for European Mediterranean evergreen woodlands. Thresholds were established from changes in functional groups of both oligotrophic and nitrophytic lichen species. Oligotrophic lichens are sensitive to Nr and tend to disappear in ammonia-rich environments, while nitrophytes are tolerant and tend to increase in cover and frequency with ammonia exposure (Pinho et al., 2011). Changes in lichen communities can be due to direct adverse or beneficial effects of the pollutant on the lichen species or to an indirect effect on interspecific relationships (e.g. competition) between them. Although many authors have investigated the complexity of parameters interplaying under field conditions (Jovan et al., 2012; Spier et al., 2010) and the tolerance of lichens to eutrophication (Hauck, 2010), the competitive relationship between species under nitrogen excess and the physiological mechanisms underpinning sensitivity and tolerance are still poorly understood.

A lower extracellular cation exchange capacity in the nitrogen tolerant *Xanthoria parietina* (L.) Th. Fr. than in the nitrogen sensitive lichen *Evernia prunastri* (L.) Ach. was proposed as one of the specific characteristics responsible for the different nitrogen sensitivities in the two species (Gaio-Oliveira et al., 2001). Moreover, detoxification

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mechanisms can work, as known for vascular plants, converting toxic intracellular ammonium in non-toxic forms of nitrogen, like amino acids. Allocation of nitrogen to the photobiont and an increase in chlorophyll concentration with a consequent increase in photosynthetic capacity, needed to provide carbon skeletons for amino acids formation, have been observed in tolerant species (Hauck, 2010 and references therein).

Based on these observations we hypothesized that the ecological success of nitrophytic species under increased availability of N<sub>r</sub> as NH<sub>3</sub> is linked to improved physiological performance of the photobiont, while the disappearing of oligotrophic ones is due to a reduced performance of the algal partner.

To test this hypothesis, we compared the physiological performance, expressed as the maximum photochemical efficiency of the photosystem II (Fv/Fm), under short-term, up to 10 weeks, NH<sub>3</sub> exposure of the sensitive *E. prunastri* and the tolerant *X. parietina* (Nimis and Martellos, 2008), two of the most common species in Mediterranean areas, with their frequency in the field in response to long-term NH<sub>3</sub> exposure.

The interpretation of lichen response can influence environmental policies and management as lichens are commonly used as ecological indicators. Currently, nitrophytic and oligotrophic species equally contribute to the establishment of CLE. Our results show that NH<sub>3</sub> directly affects oligotrophs, indicating a prime indicator role for this functional group and, when not available, nitrophytes, only indirectly affected, should be used instead.

## 2. Material and methods

In cork-oak woodland in south-west Europe (Portugal), annual NH<sub>3</sub> concentrations were determined using passive ALPHA samplers (Tang et al., 2001) exposed at increasing distance from a cattle barn housing c. 200 cows. These samplers trap the ammonia onto filters soaked in citric acid and are replaced at monthly intervals. Concentrations were then interpolated for the study area using ordinary kriging after variogram analysis (CERENA, 2000). In the same area the frequency of epiphytic lichens was scored on 55 trees using the standard “European method” (Asta et al., 2002). After selecting for trees fulfilling the sampling criteria (of the same species-*Quercus suber* L., without visible signs of disease, absence of a secondary branches at the sampling height and small deviation from the vertical), a sampling grid with five 10 × 10 cm was placed on the trunk four main aspects (North, East, South and West) of the trees. The sum on all aspects of the number of grid-squares each species was found on was noted as the species frequency. This value can vary from a minimum of 0 to a maximum of 20 (when the species is found on all the squares on the four aspects). For further details on this study see Pinho et al. (2011, 2012). The frequency of *E. prunastri* and *X. parietina* was then related to the NH<sub>3</sub> concentrations using box plots.

The same species were then collected at sites with low nitrogen availability, with an NH<sub>3</sub> concentration respectively of 1.6 μg m<sup>-3</sup> for *X. parietina* (Penicui,

Midlothian Scotland) and 0.6 μg m<sup>-3</sup> for *E. prunastri* (Peebles, Tweeddale, Scotland). Branches of Elderberry, *Sambucus nigra*, and of English Oak, *Quercus robur*, carrying respectively *X. parietina* and *E. prunastri* were transplanted along an NH<sub>3</sub> gradient at an experimental ammonia release site at Whim (UK), with low ambient total N deposition (8 kg N ha<sup>-1</sup> yr<sup>-1</sup>) (see Leith et al., 2004). All the transplanted branches supported by plastic sticks were inserted facing the NH<sub>3</sub> source at the same height in the open. NH<sub>3</sub> concentrations were measured at the transplant locations, located 12, 30 and 60 m from the NH<sub>3</sub> source, using passive ALPHA samplers 0.1 m above the vegetation (Tang et al., 2001). Transplants were collected after 1, 5 and 10 weeks and the average NH<sub>3</sub> concentration during each exposure period was calculated. Measurements of the Fv/Fm ratio of the transplanted lichens were taken as a stress indicator (Pisani et al., 2009; Strasser et al., 2000). Lichen samples were gently moistened in blotting paper and then dark-adapted for 15 min before fluorescence measurements were taken. The Fv/Fm ratio was measured at room temperature, with the Plant Efficiency Analyzer Handy PEA (Hansatech Instruments LTD, UK). The average Fv/Fm data for each concentration was then related to average NH<sub>3</sub> concentration using non-parametric rank-order (Spearman) correlations.

## 3. Results and discussion

The frequency of *E. prunastri* occurrence along an NH<sub>3</sub> gradient in the field (Fig. 1a) suggests an NH<sub>3</sub> threshold for *E. prunastri* below 3 μg m<sup>-3</sup> since there were hardly any occurrences above this concentration. By contrast the frequency of *X. parietina* increased at NH<sub>3</sub> concentrations greater than 9.1 μg m<sup>-3</sup> (Fig. 1b).

The Fv/Fm values of the two species exposed to NH<sub>3</sub> (Fig. 2) showed that photosystem II in *E. prunastri* was highly sensitive to ammonia concentrations. The fall in Fv/Fm indicates the concentration dependent deleterious effect of ammonia on carbon assimilation in this lichen, confirming its known sensitivity to ammonia (Pirintsos et al., 2009; Munzi et al., 2012). Although not identical, physiological response and field observations suggest an NH<sub>3</sub> threshold for *E. prunastri* of the same order of magnitude and consistent with the NH<sub>3</sub> CLE. It's reasonable that long term exposure induces a lower threshold as already shown in case of cumulative treatments (Sheppard et al., 2011).

These findings suggest that CLE for sensitive lichen species like *E. prunastri* can represent thresholds beyond which the carbon assimilation is so seriously compromised that it prevents the species survival and leads to its disappearance. In other words, excess of NH<sub>3</sub> exerts a direct toxic effect on *E. prunastri*.

*Xanthoria parietina* is a nitrophytic species, with a limited distribution in the presence of low N<sub>r</sub> availability, but becoming dominant with high N<sub>r</sub> availability (van Herk, 1999). In fact, under increasing N<sub>r</sub> availability firstly sensitive species decrease, while tolerant ones can be either promoted or remain indifferent. Based

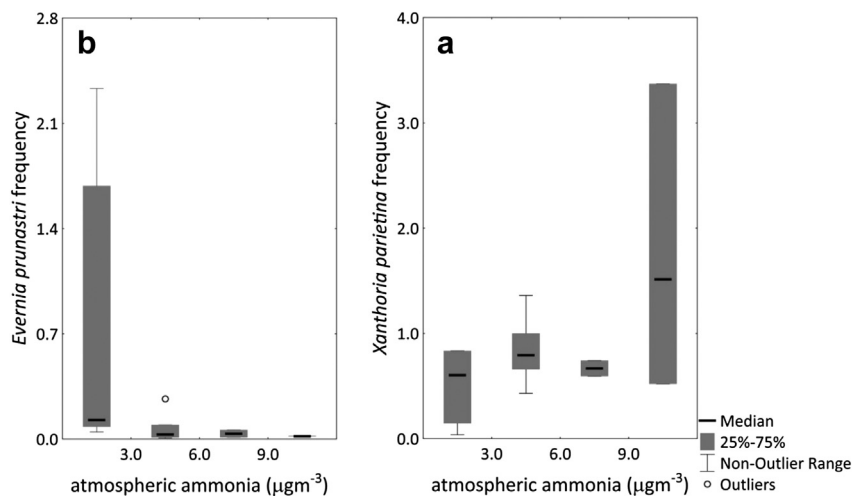
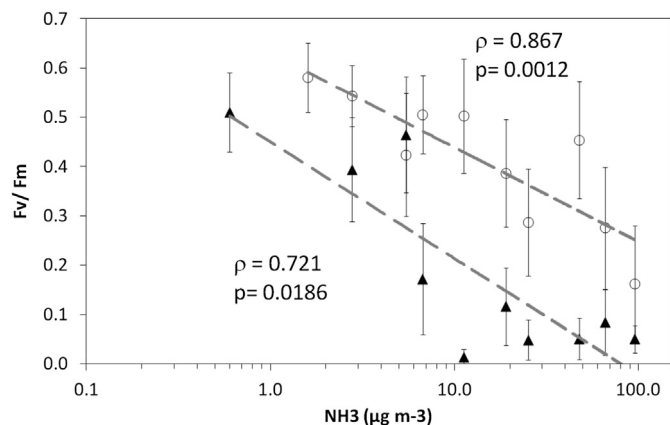


Fig. 1. Frequency of *X. parietina* (a) and *E. prunastri* (b) along a gradient of NH<sub>3</sub> concentrations near a cattle barn in Portugal (n = 55).



**Fig. 2.** Fv/Fm values ( $\pm$ SD) of *X. parietina* (circles) and *E. prunastri* (triangles) at different  $\text{NH}_3$  concentrations. The  $\rho$  is the non-parametric correlation coefficient and the fitted line is the linearly adjusted trend (for illustrative purposes only).

on this, we could have expected a null or even slightly positive effect of  $\text{NH}_3$  availability on carbon assimilation, enabling it to grow and spread. Our findings showed the opposite, with Fv/Fm decreasing with increasing  $\text{NH}_3$  concentration (Fig. 2). This is consistent with Munzi et al. (2010) who showed that high doses and repeated nitrogen supply can be detrimental even for tolerant species like *X. parietina*.

Thus, while our hypothesis is confirmed for *E. prunastri*, where a reduced photosynthetic performance could be associated with its absence from N-rich environments, this is not true for *X. parietina*, whose ecological success in the same environments cannot be explained by an improved photosynthetic performance. However, even if not favoured by or completely indifferent to N effects, the ability to cope with high N concentrations allows *X. parietina* and other nitrophytic species to occupy a wide range of ecological conditions where they can live without competition from sensitive communities. In this case,  $\text{NH}_3$  exerts an indirect positive effect on *X. parietina*.

Honegger et al. (1996), for example, suggested that *X. parietina* has a remarkable regenerative capacity that could provide a significant ecological benefit. Nutrient applications can increase growth rates and competitive ability in *X. parietina* under certain conditions (Welch et al., 2006), enabling colonization of some substrates in transplant experiments (Armstrong, 1993). Finally, Munzi et al. (2013) suggested that *X. parietina* can develop protective mechanisms, initiating rapid repair when growing in the presence of high N availability. The relatively short exposure duration we used in the transplant experiment however, may have been insufficient to assess this possibility.

This work shows how combining physiological responses of two dominant species and ecological impacts of increased availability of  $\text{NH}_3$  in lichens helps us understand why different species are more or less sensitive and how they can be used to indicate  $\text{NH}_3$  enrichment. In particular, oligotrophic species give more accurate indications than nitrophytic species for the establishment of  $\text{NH}_3$  CLE since they are affected directly by increasing availability of this nitrogen form. On the other hand, nitrophytic species can provide an indirect way to determine  $\text{NH}_3$  CLE when other pollutants contribute to the disappearance of sensitive species.

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