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Short communication

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

S. Munzi [a,*], C. Cruz [a], C. Branquinho [a], P. Pinho [a,b], I.D. Leith [c], L.J. Sheppard [c]

[a] Universidade de Lisboa, Faculdade de Ciências, Centro de Biologia Ambiental, Campo Grande, Bloco C2, 1749-016 Lisboa, Portugal
[b] Centre for Natural Resources and the Environment, Instituto Superior Técnico, Universidade de Lisboa (CERENA-IST-UL), Av. Rovisco Pais, 1049-001 Lisboa, Portugal
[c] Centre for Ecology & Hydrology, Edinburgh, Bush Estate, Penicuik EH26 0QB, UK

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ABSTRACT

Ammonia (NH_3) 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_3 exposure and their frequency of occurrence along an NH_3 field gradient. Both frequency and Fv/Fm of *E. prunastri* decreased abruptly above 3 μg m^{-3} NH_3 suggesting direct adverse effects of NH_3 on its photosynthetic performance. By contrast, *X. parietina* increased its frequency with NH_3, despite showing decreased capacity of photosystem II above 50 μg m^{-3} NH_3, suggesting that the ecological success of *X. parietina* at ammonia-rich sites might be related to indirect effects of increased nitrogen (NH_3) availability. These results highlight the need to establish NH_3 critical levels based on oligotrophic lichen species.

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_3), 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_3.

Ammonia CLE correspond to the NH_3 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_3 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_3 for lichens and bryophytes is 1 μg m^{-3} NH_3 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_3 CLE, identifying CLE below 1.9 μg m^{-3} 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

* Corresponding author.
E-mail addresses: silvana.munzi@gmail.com, ssmunzi@fc.ul.pt (S. Munzi).

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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 Nr as NH_3 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_3 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_3 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_3 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_3 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_3 concentrations using box plots.

The same species were then collected at sites with low nitrogen availability, with an NH_3 concentration respectively of 1.6 μg m^{-3} for *X. parietina* (Penicuik, Midlothian Scotland) and 0.6 μg m^{-3} 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_3 gradient at an experimental ammonia release site at Whim (UK), with low ambient total N deposition (8 kg N ha^{-1} yr^{-1}) (see Leith et al., 2004). All the transplanted branches supported by plastic sticks were inserted facing the NH_3 source at the same height in the open. NH_3 concentrations were measured at the transplant locations, located 12, 30 and 60 m from the NH_3 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_3 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_3 concentration using non-parametric rank-order (Spearman) correlations.

3. Results and discussion

The frequency of *E. prunastri* occurrence along an NH_3 gradient in the field (Fig. 1a) suggests an NH_3 threshold for *E. prunastri* below 3 μg m^{-3} since there were hardly any occurrences above this concentration. By contrast the frequency of *X. parietina* increased at NH_3 concentrations greater than 9.1 μg m^{-3} (Fig. 1b).

The Fv/Fm values of the two species exposed to NH_3 (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_3 threshold for *E. prunastri* of the same order of magnitude and consistent with the NH_3 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_3 exerts a direct toxic effect on *E. prunastri*.

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

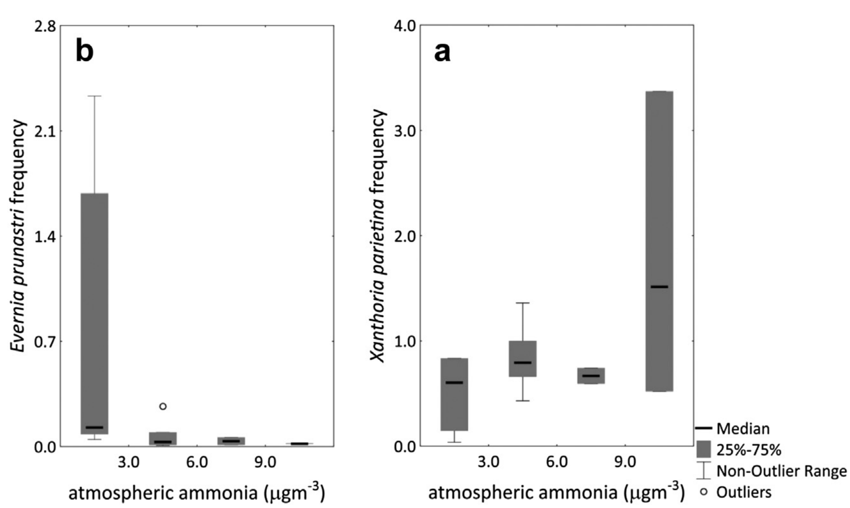

Fig. 1. Frequency of *X. parietina* (a) and *E. prunastri* (b) along a gradient of NH_3 concentrations near a cattle barn in Portugal ($n = 55$).