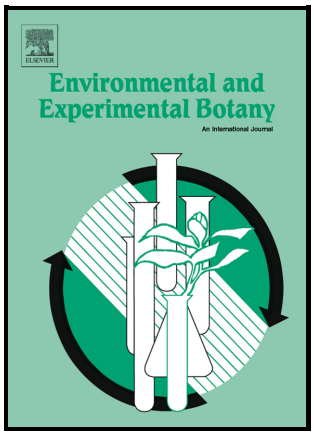
Journal Pre-proof



Lichen Symbiotic Stress as a Precursor to Biodiversity Loss – Rapid Assessment Using *Evernia prunastri* as a Bioindicator for Nitrogen Pollution

Lumbani Mwafulirwa, Ajinkya G. Deshpande, Matthew R. Jones, Mark A. Sutton, Christopher J. Ellis

PII: S0098-8472(26)00025-0

DOI: https://doi.org/10.1016/j.envexpbot.2026.106333

Reference: EEB106333

To appear in: *Environmental and Experimental Botany*

Received date: 29 October 2025
Revised date: 2 February 2026
Accepted date: 20 February 2026

Please cite this article as: Lumbani Mwafulirwa, Ajinkya G. Deshpande, Matthew R. Jones, Mark A. Sutton and Christopher J. Ellis, Lichen Symbiotic Stress as a Precursor to Biodiversity Loss – Rapid Assessment Using *Evernia prunastri* as a Bioindicator for Nitrogen Pollution, *Environmental and Experimental Botany,* (2026) doi:https://doi.org/10.1016/j.envexpbot.2026.106333

Lichen Symbiotic Stress as a Precursor to Biodiversity Loss – Rapid Assessment Using *Evernia prunastri* as a Bioindicator for Nitrogen Pollution

Lumbani Mwafulirwa [a,1,*], Ajinkya G. Deshpande [b], Matthew R. Jones [b], Mark A. Sutton [b], and Christopher J. Ellis [a]

[a] Royal Botanic Garden Edinburgh, Edinburgh EH3 5LR, UK

[b] UK Centre for Ecology & Hydrology, Bush Estate, Penicuik, Midlothian EH26 0QB, UK

[1] Current Address: School of Agri-Food Technology & Manufacturing, University of Lincoln, Riseholme Park, Lincoln LN2 2LG, UK

* Corresponding author: Lumbani Mwafulirwa, University of Lincoln, Riseholme Park, Lincoln LN2 2LG, UK. LMwafulirwa@lincoln.ac.uk

Abstract

Excess reactive nitrogen (N_r) emitted from farming (NH_x) and fossil fuels (NO_x) is a major global threat to biodiversity and ecosystem function. Environmental N_r is often monitored using bioindicators such as lichens, which provide valuable insights in the absence of instrumental monitoring stations. As key bioindicators, lichen responses to N_r have been widely studied using either short-term highly controlled laboratory experiments or field sampling, linking functional aspects of lichen biology with real-world outcomes. However, a missing component in the available evidence base could be provided by field-scale experiments to isolate the response of lichens to contrasting N_r levels over longer time periods. Here, we investigated the response of the bioindicator lichen *Evernia prunastri* to contrasting ammonia (NH_3) concentrations within a novel field-scale experiment and over a 12-week period. We measured fungal cell membrane damage and algal chlorophyll content as markers related to lichen tissue nitrogen accumulation, revealing impacts on the fungal and algal symbionts and explaining net outcomes on lichen relative growth rates. We compared the results of the field-scale experiment to trends observed in the real world. Our results suggest that *E. prunastri* tissue nitrogen content becomes saturated at 1.3% with long-term NH_3 concentrations of c. 2 μg m^{-3}, beyond which the species experiences unmitigated physiological damage. This response is however critically dependent on the exposure duration, which interacts with atmospheric NH_3 to constrain acclimation through increased chlorophyll content, while

causing accumulative damage to fungal cell membranes that compromises growth and leads to eventual mortality.

Keywords biodiversity loss, lichen bioindicators, lichen symbionts, nitrogen pollution, fungal cell membrane integrity, chlorophyll fluorescence ratio F735/F700

1. Introduction

Since the mid-20th Century there has been an accelerating global increase in levels of atmospheric reactive nitrogen (Nr) caused by human activities, including a release of reduced Nr from agricultural sources (Asman et al. 1998; Liu et al. 2022; Fowler et al. 2013) and oxidised Nr from industrial and domestic fossil fuel combustion (Dignon 1992; Larkin et al. 2017). This excess atmospheric Nr is a major driver of biodiversity loss (Bobbink et al. 2010; Dise et al. 2011), causes disruption to ecosystems and the supply of services and goods (Clark et al. 2017), and has negative human health impacts (Wolfe and Patz 2002; Townsend et al. 2003), contributing to an estimated c. 7 million excess deaths annually (Singh and Kumar 2022).

These multiple problems of Nr pollution are widely monitored and managed using bioindicators (Martínez et al. 2021), being exemplified here for lichens as one of the most sensitive Nr bioindicator groups (Bobbink et al. 2003; Pardo et al. 2011). Lichen bioindicators can be used as proxies to reconstruct levels of Nr pollution in the absence of instrumental monitoring (Van Herk 1999; Pinho et al. 2017; Niepsch et al. 2023), thus filling gaps for data poor regions, they evidence the ecological consequences of excess Nr for what is increasingly recognised as a structurally and functionally important guild (Elbert et al. 2012; Porada et al. 2014; Asplund and Wardle 2017), and they are visible features of the environment that engage public monitoring of Nr pollution (Davies et al. 2011; Welden et al. 2018) and environmental education (Zedda 2023). Lichen bioindicators have therefore been used as instruments of Nr policy, such as in setting critical levels and loads as key targets for emissions reduction (Cape et al. 2009a; Cape et al. 2009b; Geiser et al. 2021; Bobbink et al. 2022; Franzaring and Kösler 2023).

Monitoring of the ecological effect of Nr pollution using lichen bioindicators is frequently based on the contrasting sensitivity of different species to the amount and type of atmospheric Nr (Will-Wolf et al. 2018; 2015; Smith et al. 2020), with bioindicators being categorised as either sensitive (oligotrophic/nitrophobes) or tolerant (eutrophic/nitrophiles), and with their abundance, frequency, or occurrence scored within a Nr pollution index (Matos et al. 2017). There is a high level of complexity in the biological sensitivity/tolerance of lichens to Nr, with multiple contrasting responses to reduced compared to oxidised Nr. For example, gaseous ammonia (NH_3) may induce direct toxicity (Paoli et al. 2010; 2015) as well as cause indirect effects where adsorption of NH_3 raises bark pH to cause community-scale loss of 'acidophytes' (De Barker 1989; Van Herk 2001; 1999) that are adapted to achieve homeostasis of macro- and micronutrients (metal ions) at low substratum pH values (Hauck and Jürgens

2008; Hauck, Jürgens, Willenbruch, et al. 2009; Hauck, Jürgens, Huneck, et al. 2009). Among the varied biological responses, experimental evidence has pointed to the role of excess Nr in disrupting the balanced nature of the lichen symbiosis (Johansson et al. 2011; 2012; Wang et al. 2019), with a subsequent regulatory break-down between the heterotrophic mycobiont (fungus) and autotrophic photobiont (alga or cyanobacteria). This focus on symbiotic balance can be coupled with promising techniques for the easy and rapid characterisation of the status of symbiotic partners, including fungal physiological integrity (Munzi et al. 2009) and photobiont population status (Liu et al. 2019). Importantly, the characterisation of an imbalance between the fungus and photobiont offers a route to recognising lichen stress in situations where excess Nr might be causing incipient harm, in advance of species loss and community change. This creates an opportunity to address the problem of excess Nr before species loss occurs and ecosystem harm becomes embedded.

This paper describes a study using the common lichen *Evernia prunastri* to test the impacts of atmospheric Nr on fungal cell membrane damage/physiological integrity (because the thallus is predominantly fungal biomass) and on algal chlorophyll content/photobiont population (as the photosynthetic partner in this species is a green alga, *Trebouxia*), using two scenarios. First, a unique experiment in which ammonia is released into a forest plot (Deshpande et al. 2024), with lichen thalli exposed to controlled time-dose gradients (hereafter referred to as the transplant experiment), and second, for lichen thalli sampled across large-scale biogeographic gradients in atmospheric ammonia concentration, aiming to provide a wider verification of experimental results.

2. Materials and Methods

2.1 Transplant Experiment

Thallus specimens of *E. prunastri* were collected (April 2023) from oak twigs (*Quercus* sp.) located in a clean-air region (c. 0.42 μg m^{-3} NH_3 concentration) of the north-eastern Scottish Highlands (Ardross, Fig. 1). Following collection, thalli were transferred to the laboratory, where they were cleaned of extraneous debris, air-dried for approximately 48 h to stop metabolic activity, and then stored in the dark at room temperature until experimentation (within 3 weeks of collection).

The transplant experiment was conducted within a uniform plot of birch trees (*Betula* sp.) in south-eastern Scotland (Glencorse, Fig. 1), that contained a wind-controlled ammonia enhancement system (Fig. 2a); for full technical details see Deshpande et al. (2024). The enhancement system created a point-source plume of exponentially decreasing ammonia

concentration, with vertical and horizontal ammonia values monitored along a transect that spanned 0 to 44 m distance from the source (Deshpande et al. 2024). Ammonia was only released when the wind direction was oriented down the transect (275–345°) and above wind speeds of 0.3 m s^{-1} (Deshpande et al. 2024). This resulted in ammonia concentrations that are realistic of known point source emissions (e.g., poultry farms (Pitcairn et al. 2002)) and where monthly ammonia concentrations can vary with meteorology. Thalli were transplanted (May 2023) at three distances of approximately 5 m, 13 m, and 32 m downwind from and facing the ammonia source; additionally, control thalli were placed outwith the experiment and subject to background levels of ammonia (Fig. 2b).

For experimentation, the air-dried *E. prunastri* thalli were weighed and then fixed alongside a unique ID code onto four 20 cm × 35 cm nets (30 thalli per net) using pieces of small plastic-coated garden wire. Each net was then fixed onto the main stem of a tree (Fig. 2a) at c. 1.5 m aboveground height for the three downwind distances, and a slug barrier tape was fixed around the edges of each net on the tree. After transplantation, lichens were immediately sprayed with deionised water. Five thalli were randomly sampled from each net after one week, two weeks, one month, and three months of exposure; mean monthly (May–June 2023) rainfall and relative humidity (2 m aboveground) at the experimental plot were c. 50 mm and 79%, respectively (Deshpande et al. 2025). The sampled thalli were immediately transferred to the laboratory where they were cleaned of extraneous debris, air-dried for approximately 48 h, weighed to determine thallus growth, and separated into two portions. One portion was used for analysis of fungal cell membrane damage and estimation of photobiont chlorophyll content, and a second portion was used for analysis of lichen total nitrogen content. Experimental thalli were complemented by equivalent measurements from five thallus samples from the donor site, which remained untreated and represented thallus baseline condition at time-zero. All thallus samples were air-dried in the same laboratory (c. 21°C and 40% relative humidity) to control/reduce potential variation.

2.2 Ammonia Concentrations at the Experimental Site

Atmospheric ammonia concentrations (χ) at the transplant experimental site were measured using passive diffusion samplers (UKCEH Adapted Low-cost Passive High Absorption (ALPHA®) samplers) (Tang et al. 2001). The ALPHA samplers were placed just above the transplanted lichens on tree trunks and facing the same direction as the lichens (Fig. 2a) to obtain measurements that closely represented the concentrations to which the lichens were exposed, including at the background/control location. The ALPHA samplers can measure

concentrations as low as c. 0.03 µg m^{-3} and up to c. 100 µg m^{-3}. The samplers were changed monthly, and 15-min ammonia concentrations for each sampler were modelled using the ammonia release data as:

$$\chi = (Mean\ monthly\ NH_3\ concentration\ -\ mean\ monthly\ background\ NH_3\ concentration) \times \left(\frac{total\ number\ of\ minutes\ in\ the\ month}{number\ of\ minutes\ of\ NH_3\ release\ during\ the\ month}\right) + \ mean\ monthly\ background\ NH_3\ concentration$$

The modelled 15-min concentrations were then used to calculate cumulative ammonia concentrations at the transplant locations during the experimental period (Fig. 2b).

2.3 Biogeographic Sampling

To understand transferability of experimental results across the wider landscape, five *E. prunastri* thalli were sampled from twigs (February and March, 2024) at each of 13 sites distributed across atmospheric Nr pollution gradients (Fig. 1), mostly in Scotland (the study region), but extending into England to secure higher UK values in ammonia concentration. Nr pollution at the sampling sites was determined for the period 2019–2021, having been modelled at a 1 km scale from measurement stations within the UK-CEH CBED-programme (RoTAP 2012; Vieno et al. 2016) (see https://www.apis.ac.uk/cbed-concentration-based-estimated-deposition). The sampled thalli were transferred to the laboratory where they were cleaned of extraneous debris and treated exactly as the experimental thalli (see above), being air-dried for approximately 48 h and separated into two portions used for analysis of fungal cell membrane damage as well as estimation of photobiont chlorophyll content and for analysis of lichen total nitrogen content.

2.4 Thallus Growth

Experimental lichen growth was estimated based on the difference in thallus air dry weight (DW) before transplantation and after their respective periods of exposure in the experiment. A relative growth rate (RGR; µg mg^{-1} d^{-1}) during the exposure period was determined as follows: $RGR = (\ln(DW_{end}) - \ln(DW_{start})) \times 1000/\Delta t$, where ln, DW_{start}, DW_{end}, and Δt denote natural log, lichen air dry weight (mg) before transplantation, lichen air dry weight (mg) after exposure, and exposure duration (number of days), respectively. RGR is an ecologically

relevant measure of the vitality/performance of the whole lichen useful for monitoring the effects of environmental stress (Gauslaa et al. 2021).

2.5 Fungal Physiological Integrity and Chlorophyll Content

Fungal physiological integrity was inferred by placing thallus portions into deionised water and measuring the electrical conductivity of the solution, which is related to electrolyte leakage caused by cell membrane damage (Munzi et al. 2009; Hatsugai and Katagiri 2018). Approximately 200 mg of air-dried lichen thallus (with entire tips and cutting only across a narrow basal portion of the lobe) was placed into a 50 mL plastic tube and gently rinsed with deionised water for approximately 5 s to remove particles that had simply been deposited on the lichen surface as well as those that may result from the cut surface, discarding the rinsing water. Because the thalli were desiccated and lacked turgor pressure, cutting the material at this stage is not expected to significantly influence subsequent electrolyte leakage from intact, hydrated cells. After rinsing, 20 mL of fresh deionised water was added to the tube – corresponding to 100 mg air-dried thalli per 10 mL deionized water – soaking the lichen thalli for 1 h. The sample was then removed from the solution, and the solution was measured for electrical conductivity using a conductivity meter (HI8733; Hanna Instruments Inc., Woonsocket, RI, USA). Comparable studies have measured electrical conductivity before and after boiling to standardise for differences in tissue biomass (Whitlow et al. 1992; Hatsugai and Katagiri 2018), including for lichens (Munzi et al. 2012; Yemets et al. 2015), but by initially controlling for thallus size and dry mass we can assume electrolyte leakage is comparable across our samples (Munzi et al. 2009; Hatsugai and Katagiri 2018).

The chlorophyll content of the same sample was then estimated with the chlorophyll fluorescence ratio (CFR) F735/F700 using a portable chlorophyll content meter (CCM-300; Opti-Sciences Inc., Hudson, NY, USA) set to record an average for five readings taken at different positions of the thallus. On each day of chlorophyll content estimation, the meter was calibrated using a standard. The CFR F735/F700 is an established proxy for the chlorophyll content in plants (Gitelson et al. 1999), and the CCM-300 has been shown to be effective on very small leaves and difficult to measure samples such as lichens (Liu et al. 2019; Vannini et al. 2019; 2020; Bianchi et al. 2020), with significant linear correlations between CCM-300 measurements and total extractable chlorophyll contents reported in lichens (Liu et al. 2019).

2.6 Thallus Total Nitrogen Content

Total nitrogen content for thallus portions was measured with a Thermo Fisher Scientific Flash SMART 2000 elemental analyser, for a 3.5 mg milled air-dried lichen sample. Thallus total nitrogen content is expressed as a percentage on a dry weight basis.

2.7 Statistical Analysis

Statistical analyses were conducted in R-4.4.2 (R Core Team 2024) using $\alpha = 0.05$. ANOVA was used to test the effects of atmospheric ammonia concentration and exposure duration on thallus responses in the transplant experiment: lichen RGR, total nitrogen content, electrolyte conductivity, and chlorophyll content. Prior to analyses, data were checked for significant outlier values, normality, and homogeneity of variance before conducting ANOVA. Tukey's HSD test was used to investigate differences between individual means where statistically significant effects were found. Furthermore, Pearson product-moment correlation analysis was used to assess relationships between lichen RGR (potential dependent variable), and total nitrogen content, electrolyte conductivity, and chlorophyll content (potential independent variables). Where a significant negative correlation with lichen RGR was found, the paired relationship was formally tested using simple linear regression.

Expanding to the wider, biogeographic sampling of lichen thalli, regression was used to test the possible effects of ammonia concentration and total nitrogen deposition on averaged values of lichen total nitrogen content, electrolyte conductivity, and chlorophyll content. Where a relationship was observed, curve fitting used the simplest linear or non-linear fit that standardised residuals.

3. Results

The average atmospheric ammonia concentrations at the three experimental transplant distances (5 m, 13 m, 32 m) and for the control/background were c. 32.57, 11.51, 3.59, and 0.67 μg m^{-3}, respectively (Fig. 2b). Note that lichen data are available for analysis across all transplant distances, and exposure durations, except for the longest duration of three months (83 days) at the closest distance to the ammonia source (5 m), which caused mortality of all experimental thalli.

There was a significant effect of transplant distance (i.e., atmospheric ammonia concentration), exposure duration, and their interaction on lichen RGR, electrolyte conductivity, and total nitrogen content (Table 1; Fig. 3). Lichen RGR tended to be positive (up to 6 days) or decline to be slightly negative (from 13 days) for the control, 13 m, and 32 m distances, though declined dramatically to be strongly negative (from 13 days) for the 5 m

distance (-14.59 µg mg^{-1} d^{-1}, after 32 days). Lichen total nitrogen content was consistently lower at the control location (c. 0.36–0.59%) where it increased slightly with exposure duration, though increased dramatically with exposure duration for other transplant distances, with nitrogen concentration being higher in rank order from 32 m through 13 m to 5 m distance (Fig. 3b). Electrolyte conductivity initially decreased for all thalli (up to 6 days), then increased slightly before dropping again for the control, or showing a consistent increase with exposure duration for other transplant distances in rank order from 32 m through 13 m to 5 m distance (Fig. 3c). Chlorophyll content – estimated with the CFR F735/F700 – was significantly affected by the exposure duration (Table 1), starting to increase (from 32 days), and dramatically so for transplant distances at 13 m and 32 m (Fig. 3d).

In the transplant experiment, lichen RGR was not significantly correlated with chlorophyll content but was significantly and negatively correlated with total nitrogen content (r = -0.39, $p < 0.001$) and electrolyte conductivity (r = -0.63, $p < 0.001$) (Fig. 4a). There was a positive relationship between total nitrogen content and electrolyte conductivity (r = 0.65, $p < 0.001$) and total nitrogen content and chlorophyll content (r = 0.70, $p < 0.001$). There was a weaker correlation between electrolyte conductivity and chlorophyll content (r = 0.23, $p < 0.05$). Linear regression analysis (Fig. 4b & c) confirmed significant negative relationships between lichen RGR and total nitrogen content (R^2 = 0.15, $p < 0.001$) and electrolyte conductivity (R^2 = 0.40, $p < 0.001$).

Analysis of thalli sampled from across the UK demonstrated that as the modelled atmospheric ammonia concentration increased: 1. There was an initial increase in tissue nitrogen content, though apparently saturating above a concentration of 1.5 µg m^{-3} (Fig. 5a; R^2 = 0.42, p = 0.017 with 11 *df*), 2. That there was no clear relationship with electrolyte conductivity (Fig. 5b), but 3. That there was a significant linear relationship with chlorophyll content (Fig 5c; R^2 = 0.51, p = 0.006 with 11 *df*).

4. Discussion

The aim of this study was to investigate dose-exposure effects of ammonia concentration relevant to a widely used bioindicator guild – lichen epiphytes – exemplified here by assessing the response of the lichen *E. prunastri*. This builds on the recognised importance of longer-term experiments that can link the nitrogen level with extended (ecologically realistic) exposure durations (Munzi et al. 2010), in our case over 12 weeks, with a view to identifying easily recoverable responses that can signal incipient harm for lichen bioindicators. The study had a special focus on ammonia because although total emissions of Nr in the UK and

elsewhere are stable or declining, this trend is largely driven by reduction of oxidised Nr, while reduced Nr from ammonia emissions remains persistently high (RoTAP 2012; Sutton et al. 2020). Moreover, the lifetime of ammonia in the atmosphere may increase in the UK due to reduction in atmospheric levels of sulphur dioxide (SO_2), which is one of the major precursors for ammonia transformation to particulate ammonium (Tang et al. 2018). Under real-world conditions ammonia will be converted to ammonium (NH_4) as fine particulate aerosols (e.g., ammonium sulphate [$(NH_4)_2SO_4$] and ammonium nitrate [NH_4NO_3]) (Asman et al. 1998; Krupa 2003; Tang et al. 2018), with both gaseous ammonia and dry and wet deposition of ammonium salts being received onto the surfaces of lichens, and diffused into tissues as a free base (NH_3) or ammonium cations (NH_4^+). The study was conducted using a transplant experiment to control the dose of ammonia (Deshpande et al. 2024), resulting in ammonia concentrations that are realistic of known point source emissions such as close to poultry farms (Pitcairn et al. 2002), for different durations of exposure, combined with a wider sampling of thalli to test transferability to real-world conditions. These components are discussed separately below.

First, in the experiment, there appears to have been an effect of ammonia on the control thalli, including declining lichen RGR, increasing total nitrogen content, an initial increase and then a decline in electrolyte conductivity, and an increase in chlorophyll content. Even though the measured ammonia concentration for the control, being on average 0.67 µg m^{-3}, is below the critical level for lichens, estimated at c. 1 µg m^{-3} (J. Neil Cape et al. 2009; J.N. Cape et al. 2009), or an average of 1.44 µg m^{-3} with upper (97.5%) and lower (2.5%) confidence intervals of 2.08 and 0.88 µg m^{-3}, respectively (Ellis et al. 2022), the modelled data (Fig. 1) create an expectation that the background ammonia concentration at the Glencorse experimental site (c. 1.01 µg m^{-3}) will nevertheless be higher than the values at the Ardross donor site (0.42 µg m^{-3}). Changes to the control thalli can therefore be interpreted as an acclimation of *E. prunastri* to the slightly higher background ammonia concentration (i.e., c. 0.67 µg m^{-3}), albeit below the current set critical level for lichens (1 µg m^{-3}). If correct, then the initial slight increase in electrical conductivity for the control thalli may not be related to cell membrane damage, but represent an adjustment of electrochemical gradients if NH_4^+ accumulation within the cytosol is balanced by efflux of K^+ (Ryan and Walker 1994; Munzi et al. 2009). Likewise, an increase in chlorophyll content is consistent with acclimation towards upregulation of photosynthesis to scavenge excess cytosolic Nr into amino acids (Hauck 2010; Hauck and Wirth 2010), consistent with a shifting balance of mycobiont and photobiont symbionts (Dahlman et al. 2003; Palmqvist and Dahlman 2006).

In contrast to the control, other thallus samples show responses with increasing severity of impact related to transplant distance and exposure duration, though with a difference in the outcome for lichen RGR between thalli at the 5 m distance and those at the 13 m and 32 m distances. From 13 days at the 5 m distance lichen RGR becomes strongly negative, with a loss of thallus integrity, fragmentation, and with total mortality of thalli from 83 days. In contrast, thalli at 13 m and 32 m appear to better survive the exposure to ammonia and to mitigate the loss of thallus mass by day 83. At these distances although consistently increasing electrolyte conductivity indicates recurrent cell membrane damage (without the recovery observed for control thalli), an increasing chlorophyll content suggests there may be partial acclimation through the upregulation of photosynthesis, though the longer-term outcome for *E. prunastri* remains to be determined considering that lichen RGR remained negative from 13 days. In contrast, at 5 m distance, electrolyte conductivity is relatively higher, while chlorophyll content remains low and – rather than being interpreted as absence of an effect on photobionts – it suggests instead that growth of the photobiont population was compromised by the higher values of ammonia, with an inability to contribute to acclimation. This would be consistent with declines in photosynthetic efficiency previously observed for *E. prunastri* with an increasing dose and duration of exposure to Nr (Munzi et al. 2010; 2012).

Correlation analysis confirmed that the responses measured here represent a broad syndrome of incipient harm to *E. prunastri*, in which exposure to higher concentrations of ammonia, over longer time periods, causes accumulation of nitrogen in tissues, cell membrane damage, an increase in chlorophyll content, and a decline in growth. However, the detailed breakdown of the syndrome can explain why there is a weaker negative relationship in the linear regression between lichen RGR and total nitrogen content (because increasing total nitrogen content can be acclimated to some degree), and a stronger negative relationship between lichen RGR and electrolyte conductivity (because fungal cell membrane damage is a precursor to overall decline in physiological integrity). It also explains why there is no relationship between lichen RGR and chlorophyll content, since the CFR F735/F700 increased as an apparent acclimative response at intermediate levels of nitrogen, but remained relatively unaffected at low nitrogen levels, or unresponsive at high nitrogen levels (and therefore non-linear).

Second, the patterns observed for the transplant experiment appear to be supported by the biogeographic sampling, indicating broader transferability. Collection of specimens was limited to sites in which *E. prunastri* was extant, an obvious point that nevertheless explains the lack of a clear relationship between modelled ammonia and thallus electrolyte conductivity,

since consistent increase in electrolyte conductivity was a precursor to mortality in the transplant experiment, the corollary of which is a very low likelihood of locating any surviving mature thalli with high electrolyte conductivity under real-world conditions. Instead, for situations in which *E. prunastri* was able to survive long-term through acclimation, there appeared to be an increase in thallus nitrogen content up to a saturating level (c. 1.3% tissue nitrogen), which is also consistent with an increase in chlorophyll content as a mode of sequestering excess nitrogen into amino acids. *E. prunastri* has been regarded as relatively sensitive to nitrogen pollution, in comparison with *Xanthoria parietina* for example (Gaio-Oliveira et al. 2005), and it is interesting to note that levels of tissue nitrogen for *X. parietina* have been reported at > 3% (Gaio-Oliveira et al. 2001). As an outcome, *E. prunastri* could not be located where modelled ammonia concentration values exceeded c. 2 μg m^{-3}. This is again consistent with the transplant experiment, since fungal physiological integrity appeared to be compromised even at the lowest experimental ammonia concentration (32 m ≈ 3.59 μg m^{-3}), with tissue nitrogen concentrations increasing with exposure duration, and after 83 days approaching the highest levels observed for extant field specimens, therefore resulting in negative lichen RGR.

In conclusion, our study – focusing on aspects of lichen response (fungal cell membrane damage and algal chlorophyll content) that can be easily and rapidly measured – confirmed that the ability of *E. prunastri* to cope with Nr depends on the interaction of dose and exposure duration. Assuming the modelled UK ammonia concentrations represent a long-term average (Fig. 1), then consistent exposure to ammonia levels ≥ 2 μg m^{-3} would appear to cause a pathway towards thallus death (characteristic of the experimental response at 32 m, over 83 days), resulting in species absence under real-world conditions. *E. prunastri* may have an ability to acclimate to ammonia levels ≤ 0.67 μg m^{-3} (experimental control), but with the survival rate at ammonia levels ≥ 2 μg m^{-3} being dependent on the pattern of dose-exposure and the subsequent dynamic time-course of both acclimation and loss of fungus physiological integrity. Further understanding is needed of the underlying mechanisms of the physiological impacts on lichens, including acclimative responses, below the current critical level of ammonia (1 μg m^{-3}). The capacity for acclimation may depend not only on the genotypic identity of the photobiont (within *Trebouxia*), since photobiont identity can be variable within the specificity of a lichenised fungus (Piercey-Normore 2006; Werth and Sork 2014; Dal Grande et al. 2018), conferring ecological fitness differences, but could also depend on the composition and response of the holobiont (Aschenbrenner et al. 2016), representing areas for future investigation.

Acknowledgements

We thank R Hollands, K Nishii, L Forrest, and M Hart for their logistical support towards laboratory work, M Grenier, N van Dijk, D Harvey, and C Johnstone for their support towards field work, and anonymous reviewers for their constructive comments to improve the paper. The work was supported by UK Research and Innovation (UKRI) as a Global Challenges Research Fund grant, the 'GCRF South Asian Nitrogen Hub' (NE/S009019/1). It represents a contribution to the work of the GEF/UNEP International Nitrogen Management System (INMS) and the International Nitrogen Initiative (INI). This Glencorse field facility is supported by NERC, through the 'UKCEH National Capability for UK Challenges Programme' (NE/Y006208/1).

Data Availability

The data that support the findings of this study is held in the RBGE repository and can be made available on request.

References

Aschenbrenner, Ines A., Tomislav Cernava, Gabriele Berg, and Martin Grube. 2016. 'Understanding Microbial Multi-Species Symbioses'. *Frontiers in Microbiology* 7 (February). https://doi.org/10.3389/fmicb.2016.00180.

Asman, Willem A. H., Mark A. Sutton, and Jan K. Schjørring. 1998. 'Ammonia: Emission, Atmospheric Transport and Deposition'. *New Phytologist* 139 (1): 27–48. https://doi.org/10.1046/j.1469-8137.1998.00180.x.

Asplund, Johan, and David A. Wardle. 2017. 'How Lichens Impact on Terrestrial Community and Ecosystem Properties'. *Biological Reviews* 92 (3): 1720–38. https://doi.org/10.1111/brv.12305.

Bianchi, Elisabetta, Renato Benesperi, Giorgio Brunialti, et al. 2020. 'Vitality and Growth of the Threatened Lichen Lobaria Pulmonaria (L.) Hoffm. in Response to Logging and Implications for Its Conservation in Mediterranean Oak Forests'. *Forests* 11 (9): 995. https://doi.org/10.3390/f11090995.

Bobbink, R., M. Ashmore, S. Braun, W Fluckiger, and I.J.J. van den Wyngaert. 2003. 'Empirical Nitrogen Critical Loads for Natural and Semi-Natural Ecosystems: 2002 Update.' In *Empirical Critical Loads for Nitrogen.*, edited by B. Achermann and R. Bobbink. Environmental Documentation. Swiss Agency for Environment, Forest and Landscape SAEFL.

Bobbink, R., K. Hicks, J. Galloway, et al. 2010. 'Global Assessment of Nitrogen Deposition Effects on Terrestrial Plant Diversity: A Synthesis'. *Ecological Applications* 20 (1): 30–59. https://doi.org/10.1890/08-1140.1.

Bobbink, Roland, Christin Loran, and Hilde Tomassen. 2022. *Review and Revision of Empirical Critical Loads of Nitrogen for Europe*. Umweltbundesamt/German Environment Agency. https://www.umweltbundesamt.de/en/publikationen/review-revision-of-empirical-critical-loads-of.

Cape, J. Neil, Ludger J. Van Der Eerden, Lucy J. Sheppard, Ian D. Leith, and Mark A. Sutton. 2009. 'Reassessment of Critical Levels for Atmospheric Ammonia'. In *Atmospheric Ammonia*, edited by Mark A. Sutton, Stefan Reis, and Samantha M.H. Baker. Springer Netherlands. https://doi.org/10.1007/978-1-4020-9121-6_2.

Cape, J.N., L.J. Van Der Eerden, L.J. Sheppard, I.D. Leith, and M.A. Sutton. 2009. 'Evidence for Changing the Critical Level for Ammonia'. *Environmental Pollution* 157 (3): 1033–37. https://doi.org/10.1016/j.envpol.2008.09.049.

Clark, Christopher M., Michael D. Bell, James W. Boyd, et al. 2017. 'Nitrogen-induced Terrestrial Eutrophication: Cascading Effects and Impacts on Ecosystem Services'. *Ecosphere* 8 (7): e01877. https://doi.org/10.1002/ecs2.1877.

Dahlman, Lena, Jörgen Persson, Torgny Näsholm, and Kristin Palmqvist. 2003. 'Carbon and Nitrogen Distribution in the Green Algal Lichens Hypogymnia Physodes and Platismatia Glauca in Relation to Nutrient Supply'. *Planta* 217 (1): 41–48. https://doi.org/10.1007/s00425-003-0977-8.

Dal Grande, Francesco, Gregor Rolshausen, Pradeep K. Divakar, et al. 2018. 'Environment and Host Identity Structure Communities of Green Algal Symbionts in Lichens'. *New Phytologist* 217 (1): 277–89. https://doi.org/10.1111/nph.14770.

Davies, L., J.N.B. Bell, J. Bone, et al. 2011. 'Open Air Laboratories (OPAL): A Community-Driven Research Programme'. *Environmental Pollution* 159 (8–9): 2203–10. https://doi.org/10.1016/j.envpol.2011.02.053.

De Barker, A. J. 1989. 'Effects of Ammonia Emission on Epiphytic Lichen Vegetation'. *Acta Botanica Neerlandica* 38 (3): 337–42. https://doi.org/10.1111/j.1438-8677.1989.tb01357.x.

Deshpande, A.G., M.R. Jones, E. Vandome, et al. 2025. 'Meteorological Data and Ammonia Concentration and Deposition Rates from an Ammonia Enhancement Experiment Site, Glencorse, UK, 2023-2024'. EIDC. https://catalogue.ceh.ac.uk/id/89b48766-ab8d-40f9-b9f1-f50df6af131f.

Deshpande, Ajinkya G., Matthew R. Jones, Netty Van Dijk, et al. 2024. 'Estimation of Ammonia Deposition to Forest Ecosystems in Scotland and Sri Lanka Using Wind-Controlled NH3 Enhancement Experiments'. *Atmospheric Environment* 320 (March): 120325. https://doi.org/10.1016/j.atmosenv.2023.120325.

Dignon, Jane. 1992. 'NOx and SOx Emissions from Fossil Fuels: A Global Distribution'. *Atmospheric Environment. Part A. General Topics* 26 (6): 1157–63. https://doi.org/10.1016/0960-1686(92)90047-O.

Dise, Nancy B., Mike Ashmore, Salim Belyazid, et al. 2011. 'Nitrogen as a Threat to European Terrestrial Biodiversity'. In *The European Nitrogen Assessment: Sources, Effects and Policy Perspectives*, edited by Albert Bleeker, Bruna Grizzetti, Clare M. Howard, et al. Cambridge University Press. https://doi.org/10.1017/CBO9780511976988.023.

Elbert, Wolfgang, Bettina Weber, Susannah Burrows, et al. 2012. 'Contribution of Cryptogamic Covers to the Global Cycles of Carbon and Nitrogen'. *Nature Geoscience* 5 (7): 459–62. https://doi.org/10.1038/ngeo1486.

Ellis, Christopher J., Claudia E. Steadman, Massimo Vieno, et al. 2022. 'Estimating Nitrogen Risk to Himalayan Forests Using Thresholds for Lichen Bioindicators'. *Biological Conservation* 265 (January): 109401. https://doi.org/10.1016/j.biocon.2021.109401.

Fowler, David, Mhairi Coyle, Ute Skiba, et al. 2013. 'The Global Nitrogen Cycle in the Twenty-First Century'. *Philosophical Transactions of the Royal Society B: Biological Sciences* 368 (1621): 20130164. https://doi.org/10.1098/rstb.2013.0164.

Franzaring, Jürgen, and Julia Kösler. 2023. *Review of Internationally Proposed Critical Levels for Ammonia: Proceedings of an Expert Workshop Held in Dessau and Online on 28/29 March 2022*. Umweltbundesamt/German Environment Agency. https://www.umweltbundesamt.de/en/publikationen/review-of-internationally-proposed-critical-levels.

Gaio-Oliveira, G, C Branquinho, C Máguas, and MA Martins-Loução. 2001. 'The Concentration of Nitrogen in Nitrophilous and Non-Nitrophilous Lichen Species'. *Symbiosis* 31 (1–3): 187–99.

Gaio-Oliveira, Gisela, Lena Dahlman, Kristin Palmqvist, Maria Amélia Martins-Loução, and Cristina Máguas. 2005. 'Nitrogen Uptake in Relation to Excess Supply and Its Effects on the Lichens

Evernia Prunastri (L.) Ach and Xanthoria Parietina (L.) Th. Fr.' *Planta* 220 (5): 794–803. https://doi.org/10.1007/s00425-004-1396-1.

Gauslaa, Yngvar, Maja Maslać Mikulec, and Knut Asbjørn Solhaug. 2021. 'Short-Term Growth Experiments – A Tool for Quantifying Lichen Fitness across Different Mineral Settings'. *Flora* 282 (September): 151900. https://doi.org/10.1016/j.flora.2021.151900.

Geiser, Linda H., Heather Root, Robert J. Smith, Sarah E. Jovan, Larry St Clair, and Karen L. Dillman. 2021. 'Lichen-Based Critical Loads for Deposition of Nitrogen and Sulfur in US Forests'. *Environmental Pollution* 291 (December): 118187. https://doi.org/10.1016/j.envpol.2021.118187.

Gitelson, Anatoly A, Claus Buschmann, and Hartmut K Lichtenthaler. 1999. 'The Chlorophyll Fluorescence Ratio F735/F700 as an Accurate Measure of the Chlorophyll Content in Plants'. *Remote Sensing of Environment* 69 (3): 296–302. https://doi.org/10.1016/S0034-4257(99)00023-1.

Hatsugai, Noriyuki, and Fumiaki Katagiri. 2018. 'Quantification of Plant Cell Death by Electrolyte Leakage Assay'. *Bio-Protocol* 8 (5). https://doi.org/10.21769/BioProtoc.2758.

Hauck, Markus. 2010. 'Ammonium and Nitrate Tolerance in Lichens'. *Environmental Pollution* 158 (5): 1127–33. https://doi.org/10.1016/j.envpol.2009.12.036.

Hauck, Markus, and Sascha-René Jürgens. 2008. 'Usnic Acid Controls the Acidity Tolerance of Lichens'. *Environmental Pollution* 156 (1): 115–22. https://doi.org/10.1016/j.envpol.2007.12.033.

Hauck, Markus, Sascha-René Jürgens, Siegfried Huneck, and Christoph Leuschner. 2009. 'High Acidity Tolerance in Lichens with Fumarprotocetraric, Perlatolic or Thamnolic Acids Is Correlated with Low pKa1 Values of These Lichen Substances'. *Environmental Pollution* 157 (10): 2776–80. https://doi.org/10.1016/j.envpol.2009.04.022.

Hauck, Markus, Sascha-René Jürgens, Karen Willenbruch, Siegfried Huneck, and Christoph Leuschner. 2009. 'Dissociation and Metal-Binding Characteristics of Yellow Lichen Substances Suggest a Relationship with Site Preferences of Lichens'. *Annals of Botany* 103 (1): 13–22. https://doi.org/10.1093/aob/mcn202.

Hauck, Markus, and Volkmar Wirth. 2010. 'Preference of Lichens for Shady Habitats Is Correlated with Intolerance to High Nitrogen Levels'. *The Lichenologist* 42 (4): 475–84. https://doi.org/10.1017/S0024282910000046.

Johansson, Otilia, Johan Olofsson, Reiner Giesler, and Kristin Palmqvist. 2011. 'Lichen Responses to Nitrogen and Phosphorus Additions Can Be Explained by the Different Symbiont Responses'. *New Phytologist* 191 (3): 795–805. https://doi.org/10.1111/j.1469-8137.2011.03739.x.

Johansson, Otilia, Kristin Palmqvist, and Johan Olofsson. 2012. 'Nitrogen Deposition Drives Lichen Community Changes through Differential Species Responses'. *Global Change Biology* 18 (8): 2626–35. https://doi.org/10.1111/j.1365-2486.2012.02723.x.

Krupa, S.V. 2003. 'Effects of Atmospheric Ammonia (NH3) on Terrestrial Vegetation: A Review'. *Environmental Pollution* 124 (2): 179–221. https://doi.org/10.1016/S0269-7491(02)00434-7.

Larkin, Andrew, Jeffrey A. Geddes, Randall V. Martin, et al. 2017. 'Global Land Use Regression Model for Nitrogen Dioxide Air Pollution'. *Environmental Science & Technology* 51 (12): 6957–64. https://doi.org/10.1021/acs.est.7b01148.

Liu, Lei, Wen Xu, Xiankai Lu, et al. 2022. 'Exploring Global Changes in Agricultural Ammonia Emissions and Their Contribution to Nitrogen Deposition since 1980'. *Proceedings of the National Academy of Sciences* 119 (14): e2121998119. https://doi.org/10.1073/pnas.2121998119.

Liu, Shuai, Su Li, Xiao-Yang Fan, et al. 2019. 'Comparison of Two Noninvasive Methods for Measuring the Pigment Content in Foliose Macrolichens'. *Photosynthesis Research* 141 (2): 245–57. https://doi.org/10.1007/s11120-019-00624-x.

Martínez, D. Nayeli, Edison A. Díaz-Álvarez, and Erick De La Barrera. 2021. 'Selecting Biomonitors of Atmospheric Nitrogen Deposition: Guidelines for Practitioners and Decision Makers'. *Nitrogen* 2 (3): 308–20. https://doi.org/10.3390/nitrogen2030021.

Matos, Paula, Linda Geiser, Amanda Hardman, et al. 2017. 'Tracking Global Change Using Lichen Diversity: Towards a Global-scale Ecological Indicator'. *Methods in Ecology and Evolution* 8 (7): 788–98. https://doi.org/10.1111/2041-210X.12712.

Munzi, S., L. Paoli, E. Fiorini, and S. Loppi. 2012. 'Physiological Response of the Epiphytic Lichen Evernia Prunastri (L.) Ach. to Ecologically Relevant Nitrogen Concentrations'. *Environmental Pollution* 171 (December): 25–29. https://doi.org/10.1016/j.envpol.2012.07.001.

Munzi, S., T. Pisani, and S. Loppi. 2009. 'The Integrity of Lichen Cell Membrane as a Suitable Parameter for Monitoring Biological Effects of Acute Nitrogen Pollution'. *Ecotoxicology and Environmental Safety* 72 (7): 2009–12. https://doi.org/10.1016/j.ecoenv.2009.05.005.

Munzi, Silvana, Tommaso Pisani, Luca Paoli, and Stefano Loppi. 2010. 'Time- and Dose-Dependency of the Effects of Nitrogen Pollution on Lichens'. *Ecotoxicology and Environmental Safety* 73 (7): 1785–88. https://doi.org/10.1016/j.ecoenv.2010.07.042.

Niepsch, Daniel, Leon J. Clarke, Jason Newton, Konstantinos Tzoulas, and Gina Cavan. 2023. 'High Spatial Resolution Assessment of Air Quality in Urban Centres Using Lichen Carbon, Nitrogen and Sulfur Contents and Stable-Isotope-Ratio Signatures'. *Environmental Science and Pollution Research* 30 (20): 58731–54. https://doi.org/10.1007/s11356-023-26652-8.

Palmqvist, Kristin, and Lena Dahlman. 2006. 'Responses of the Green Algal Foliose Lichen *Platismatia Glauca* to Increased Nitrogen Supply'. *New Phytologist* 171 (2): 343–56. https://doi.org/10.1111/j.1469-8137.2006.01754.x.

Paoli, Luca, Ivana Maslaňáková, Alice Grassi, Martin Bačkor, and Stefano Loppi. 2015. 'Effects of Acute NH3 Air Pollution on N-Sensitive and N-Tolerant Lichen Species'. *Ecotoxicology and Environmental Safety* 122 (December): 377–83. https://doi.org/10.1016/j.ecoenv.2015.08.025.

Paoli, Luca, Stergios Arg. Pirintsos, Kiriakos Kotzabasis, Tommaso Pisani, Eleni Navakoudis, and Stefano Loppi. 2010. 'Effects of Ammonia from Livestock Farming on Lichen Photosynthesis'. *Environmental Pollution* 158 (6): 2258–65. https://doi.org/10.1016/j.envpol.2010.02.008.

Pardo, Linda H., Mark E. Fenn, Christine L. Goodale, et al. 2011. 'Effects of Nitrogen Deposition and Empirical Nitrogen Critical Loads for Ecoregions of the United States'. *Ecological Applications* 21 (8): 3049–82. https://doi.org/10.1890/10-2341.1.

Piercey-Normore, Michele D. 2006. 'The Lichen-forming Ascomycete *Evernia Mesomorpha* Associates with Multiple Genotypes of *Trebouxia Jamesii*'. *New Phytologist* 169 (2): 331–44. https://doi.org/10.1111/j.1469-8137.2005.01576.x.

Pinho, P., C. Barros, S. Augusto, M.J. Pereira, C. Máguas, and C. Branquinho. 2017. 'Using Nitrogen Concentration and Isotopic Composition in Lichens to Spatially Assess the Relative Contribution of Atmospheric Nitrogen Sources in Complex Landscapes'. *Environmental Pollution* 230 (November): 632–38. https://doi.org/10.1016/j.envpol.2017.06.102.

Pitcairn, C.E.R, U.M Skiba, M.A Sutton, D Fowler, R Munro, and V Kennedy. 2002. 'Defining the Spatial Impacts of Poultry Farm Ammonia Emissions on Species Composition of Adjacent Woodland Groundflora Using Ellenberg Nitrogen Index, Nitrous Oxide and Nitric Oxide Emissions and Foliar Nitrogen as Marker Variables'. *Environmental Pollution* 119 (1): 9–21. https://doi.org/10.1016/S0269-7491(01)00148-8.

Porada, Philipp, Bettina Weber, Wolfgang Elbert, Ulrich Pöschl, and Axel Kleidon. 2014. 'Estimating Impacts of Lichens and Bryophytes on Global Biogeochemical Cycles'. *Global Biogeochemical Cycles* 28 (2): 71–85. https://doi.org/10.1002/2013GB004705.

R Core Team. 2024. *R: A Language and Environment for Statistical Computing*. V. 4.4.2. R Foundation for Statistical Computing, released. https://www.R-project.org.

RoTAP. 2012. *A Review of Acidification, Eutrophication, Heavy Metals and Ground-Level Ozone in the UK | Review of Transboundary Air Pollution (RoTAP)*. UKCEH. http://www.rotap.ceh.ac.uk/.

Ryan, P.R., and N.A. Walker. 1994. 'The Regulation of Ammonia Uptake in *Chara Australis*'. *Journal of Experimental Botany* 45 (8): 1057–67. https://doi.org/10.1093/jxb/45.8.1057.

Singh, Shishir, and Rakesh Kumar. 2022. 'Air Pollution and Its Associated Impacts on Atmosphere and Biota Health'. In *Extremes in Atmospheric Processes and Phenomenon: Assessment, Impacts and Mitigation*, edited by Pallavi Saxena, Anuradha Shukla, and Anil Kumar Gupta. Springer Nature Singapore. https://doi.org/10.1007/978-981-16-7727-4_3.

Smith, Robert J., Sarah Jovan, Daniel Stanton, and Susan Will-Wolf. 2020. 'Epiphytic Macrolichen Communities Indicate Climate and Air Quality in the U.S. Midwest'. *The Bryologist* 123 (3). https://doi.org/10.1639/0007-2745-123.3.516.

Sutton, Mark A., Netty Van Dijk, Peter E. Levy, et al. 2020. 'Alkaline Air: Changing Perspectives on Nitrogen and Air Pollution in an Ammonia-Rich World'. *Philosophical Transactions of the Royal Society A: Mathematical, Physical and Engineering Sciences* 378 (2183): 20190315. https://doi.org/10.1098/rsta.2019.0315.

Tang, Y.S., J.N. Cape, and M.A. Sutton. 2001. 'Development and Types of Passive Samplers for Monitoring Atmospheric NO2 and NH3 Concentrations'. *The Scientific World JOURNAL* 1: 513–29. https://doi.org/10.1100/tsw.2001.82.

Tang, Yuk S., Christine F. Braban, Ulrike Dragosits, et al. 2018. 'Drivers for Spatial, Temporal and Long-Term Trends in Atmospheric Ammonia and Ammonium in the UK'. *Atmospheric Chemistry and Physics* 18 (2): 705–33. https://doi.org/10.5194/acp-18-705-2018.

Townsend, Alan R., Robert W. Howarth, Fakhri A. Bazzaz, et al. 2003. 'Human Health Effects of a Changing Global Nitrogen Cycle'. *Frontiers in Ecology and the Environment* 1 (5): 240–46. https://doi.org/10.1890/1540-9295(2003)001[0240:HHEOAC]2.0.CO;2.

Van Herk, C. M. 1999. 'Mapping of Ammonia Pollution with Epiphytic Lichens in the Netherlands'. *The Lichenologist* 31 (1): 9–20. https://doi.org/10.1006/lich.1998.0138.

Van Herk, C. M. 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 (5): 419–42. https://doi.org/10.1006/lich.2001.0337.

Vannini, Andrea, Giulia Canali, Mario Pica, Cristina Nali, and Stefano Loppi. 2020. 'The Water Content Drives the Susceptibility of the Lichen Evernia Prunastri and the Moss Brachythecium Sp. to High Ozone Concentrations'. *Biology* 9 (5): 90. https://doi.org/10.3390/biology9050090.

Vannini, Andrea, Luca Paoli, Alessio Russo, and Stefano Loppi. 2019. 'Contribution of Submicronic (PM1) and Coarse (PM>1) Particulate Matter Deposition to the Heavy Metal Load of Lichens Transplanted along a Busy Road'. *Chemosphere* 231 (September): 121–25. https://doi.org/10.1016/j.chemosphere.2019.05.085.

Vieno, M., M. R. Heal, M. L. Williams, et al. 2016. 'The Sensitivities of Emissions Reductions for the Mitigation of UK $PM_{2.5}$'. *Atmospheric Chemistry and Physics* 16 (1): 265–76. https://doi.org/10.5194/acp-16-265-2016.

Wang, Ming, Chuanhua Wang, Lin Yang, and Hua Guo. 2019. 'Impacts of Short-Term Nitrogen Addition on the Thallus Nitrogen and Phosphorus Balance of the Dominant Epiphytic Lichens in the Shennongjia Mountains, China'. *Journal of Plant Ecology* 12 (4): 751–58. https://doi.org/10.1093/jpe/rtz013.

Welden, N.A., P.A. Wolseley, and M.R. Ashmore. 2018. 'Citizen Science Identifies the Effects of Nitrogen Deposition, Climate and Tree Species on Epiphytic Lichens across the UK'. *Environmental Pollution* 232 (January): 80–89. https://doi.org/10.1016/j.envpol.2017.09.020.

Werth, Silke, and Victoria L. Sork. 2014. 'Ecological Specialization in *Trebouxia* (Trebouxiophyceae) Photobionts of *Ramalina Menziesii* (Ramalinaceae) across Six Range-covering Ecoregions of Western North America'. *American Journal of Botany* 101 (7): 1127–40. https://doi.org/10.3732/ajb.1400025.

Whitlow, Thomas H., Nina L. Bassuk, Thomas G. Ranney, and Deborah L. Reichert. 1992. 'An Improved Method for Using Electrolyte Leakage to Assess Membrane Competence in Plant Tissues'. *Plant Physiology* 98 (1): 198–205. https://doi.org/10.1104/pp.98.1.198.

Will-Wolf, Susan, Sarah Jovan, Peter Neitlich, JeriLynn E. Peck, and Roger Rosentreter. 2015. 'Lichen-Based Indices to Quantify Responses to Climate and Air Pollution across Northeastern U.S.A'. *The Bryologist* 118 (1): 59. https://doi.org/10.1639/0007-2745-118.1.059.

Will-Wolf, Susan, Sarah Jovan, Matthew P. Nelsen, Marie T. Trest, Kathleen M. Rolih, and Ann H. Reis. 2018. 'Lichen Indices Assess Local Climate and Air Quality Status in the Mid-Atlantic Region, U.S.A.' *The Bryologist* 121 (4): 461. https://doi.org/10.1639/0007-2745-121.4.461.

Wolfe, Amir H., and Jonathan A. Patz. 2002. 'Reactive Nitrogen and Human Health:Acute and Long-Term Implications'. *AMBIO: A Journal of the Human Environment* 31 (2): 120–25. https://doi.org/10.1579/0044-7447-31.2.120.

Yemets, Olena, Yngvar Gauslaa, and Knut Asbjørn Solhaug. 2015. 'Monitoring with Lichens – Conductivity Methods Assess Salt and Heavy Metal Damage More Efficiently than Chlorophyll

Fluorescence'. *Ecological Indicators* 55 (August): 59–64. https://doi.org/10.1016/j.ecolind.2015.03.015.

Zedda, Luciana. 2023. 'The Importance of a Transformative Biodiversity Education for Perceiving, Appreciating and Supporting Lichen Diversity in German Urban Environments'. *The Lichenologist* 55 (5): 161–68. https://doi.org/10.1017/S0024282923000312.

Table 1

Variance analysis (*p*-values) of parameters measured in *Evernia prunastri* in the transplant experiment. RGR, relative growth rate (µg mg^{-1} d^{-1}); EC, electrolyte conductivity (µS cm^{-1}); CFR, chlorophyll fluorescence ratio F735/F700; TN, tissue total nitrogen content (%); *df*, degrees of freedom.

Parameters	Effects of transplantation location, exposure duration, and their interaction		
	Transplantation location	Exposure duration	Location × Duration
df	3	4	11
RGR	<0.0001	<0.0001	0.0003
TN	<0.0001	<0.0001	<0.0001
EC	<0.0001	<0.0001	0.0003
CFR	0.0946	<0.0001	0.3763

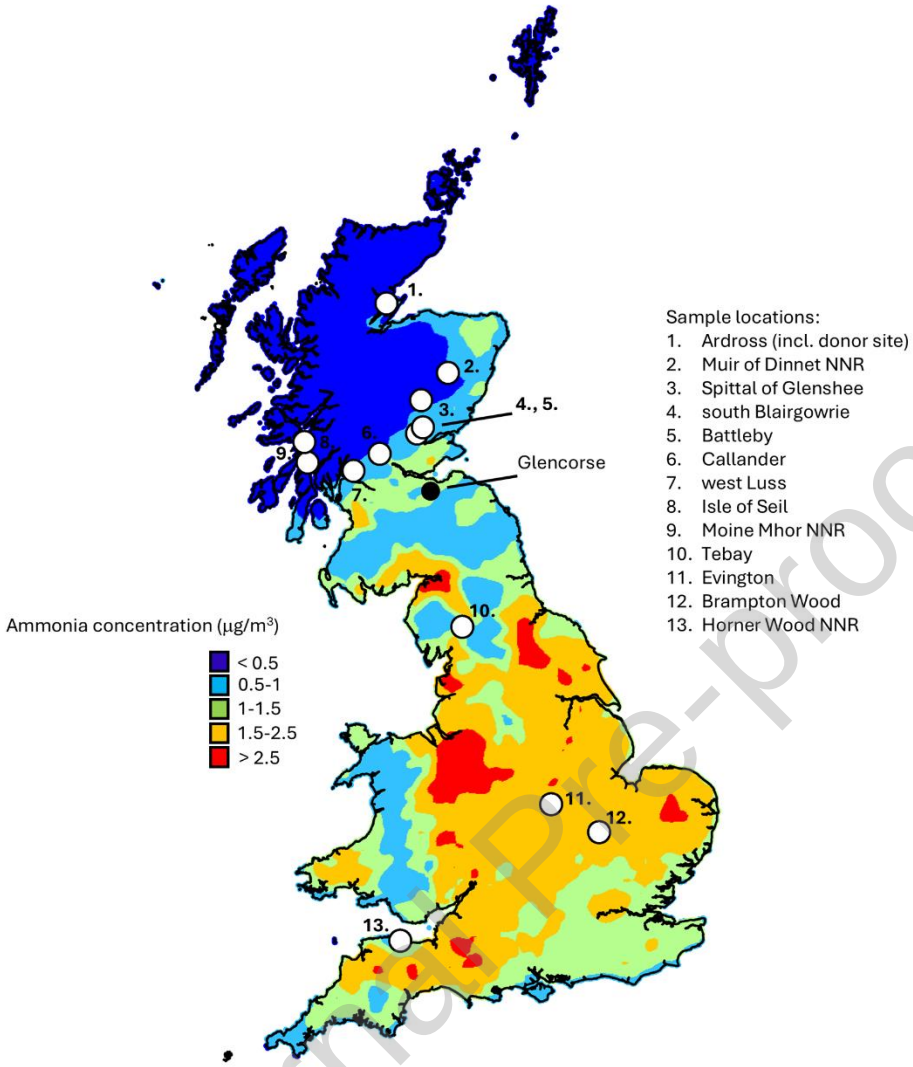


Figure 1. Ammonia concentration modelled at a 1 km resolution, and sampling locations for *Evernia prunastri* thalli across Scotland and England; also showing the location of the Glencorse experimental site and noting that Ardross doubled as the donor site for experimentation. Nr pollution was determined for the period 2019–2021, modelled from measurement stations within the UK-CEH CBED-programme (RoTaP, 2012; Vieno et al., 2016; see https://www.apis.ac.uk/cbed-concentration-based-estimated-deposition).

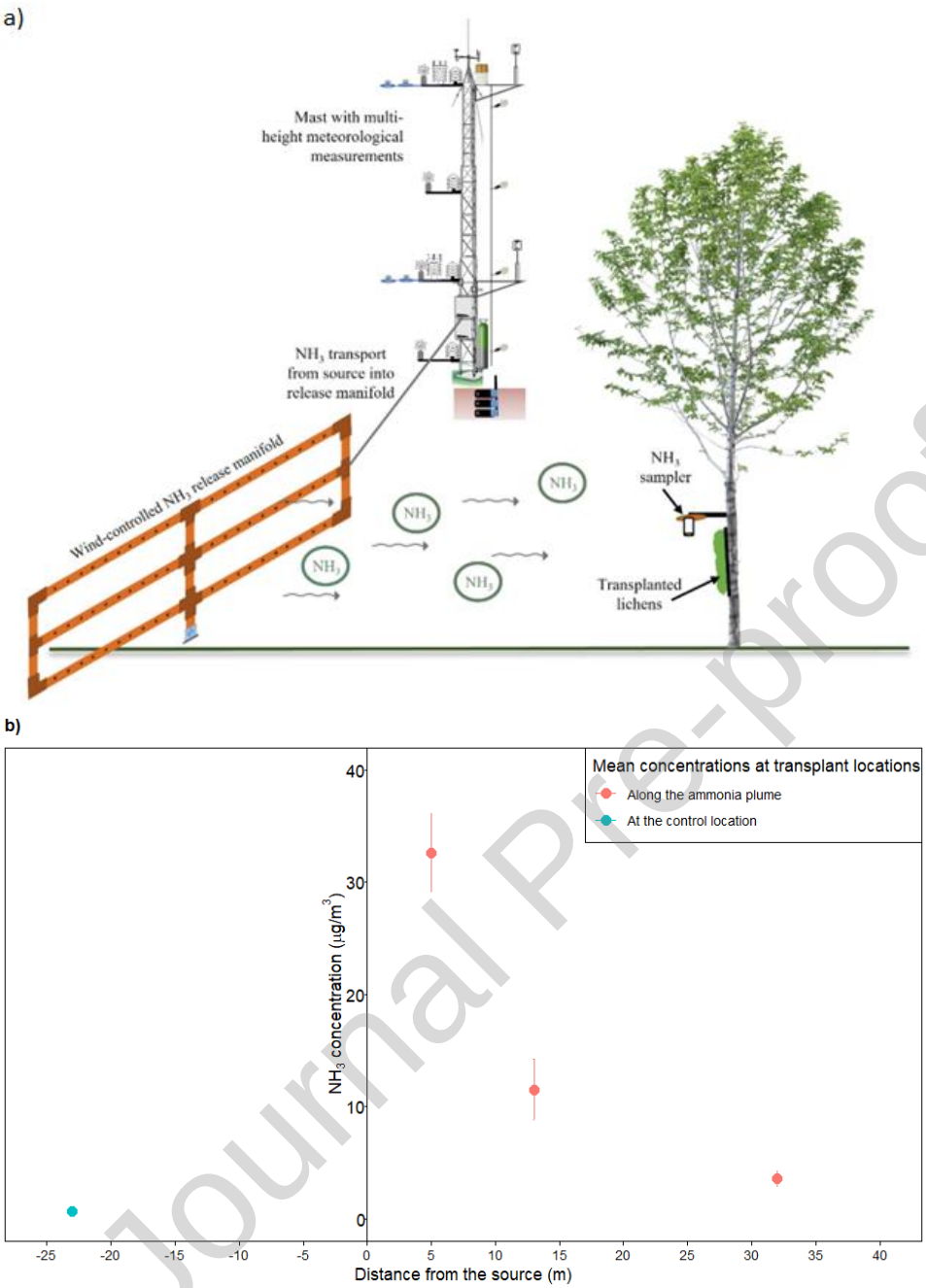


Figure 2. Schematic of the ammonia enhancement system at Glencorse and lichen transplants on a tree (a), and the cumulative mean atmospheric ammonia (NH_3) concentrations (averaged across the sampling time points at 6, 13, 32, and 83 days of thalli exposure) at the transplant locations during the experimental period (b). The transplant locations included three distances (i.e., a transect) within the enhancement system at 5 m, 13 m, and 32 m from the ammonia source and a control that was outwith the enhancement system.

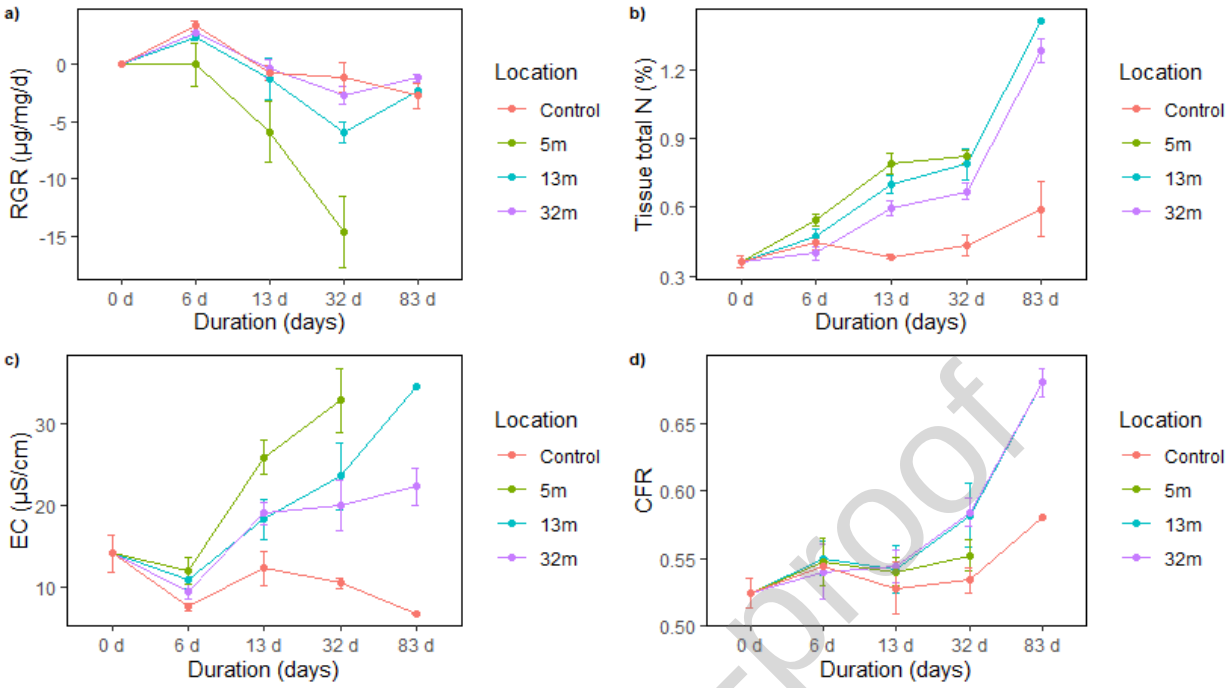


Figure 3. Lichen relative growth rate (RGR) (a), tissue total nitrogen content (b), electrolyte conductivity (EC) (c), and the chlorophyll fluorescence ratio (CFR) F735/F700 as a proxy for chlorophyll content (d), measured in *Evernia prunastri* thallus samples from a control and at three distances within the ammonia enhancement system. Values are mean ± 1 SEM. Missing values at 83 days for the 5 m distance are due to thallus mortality.

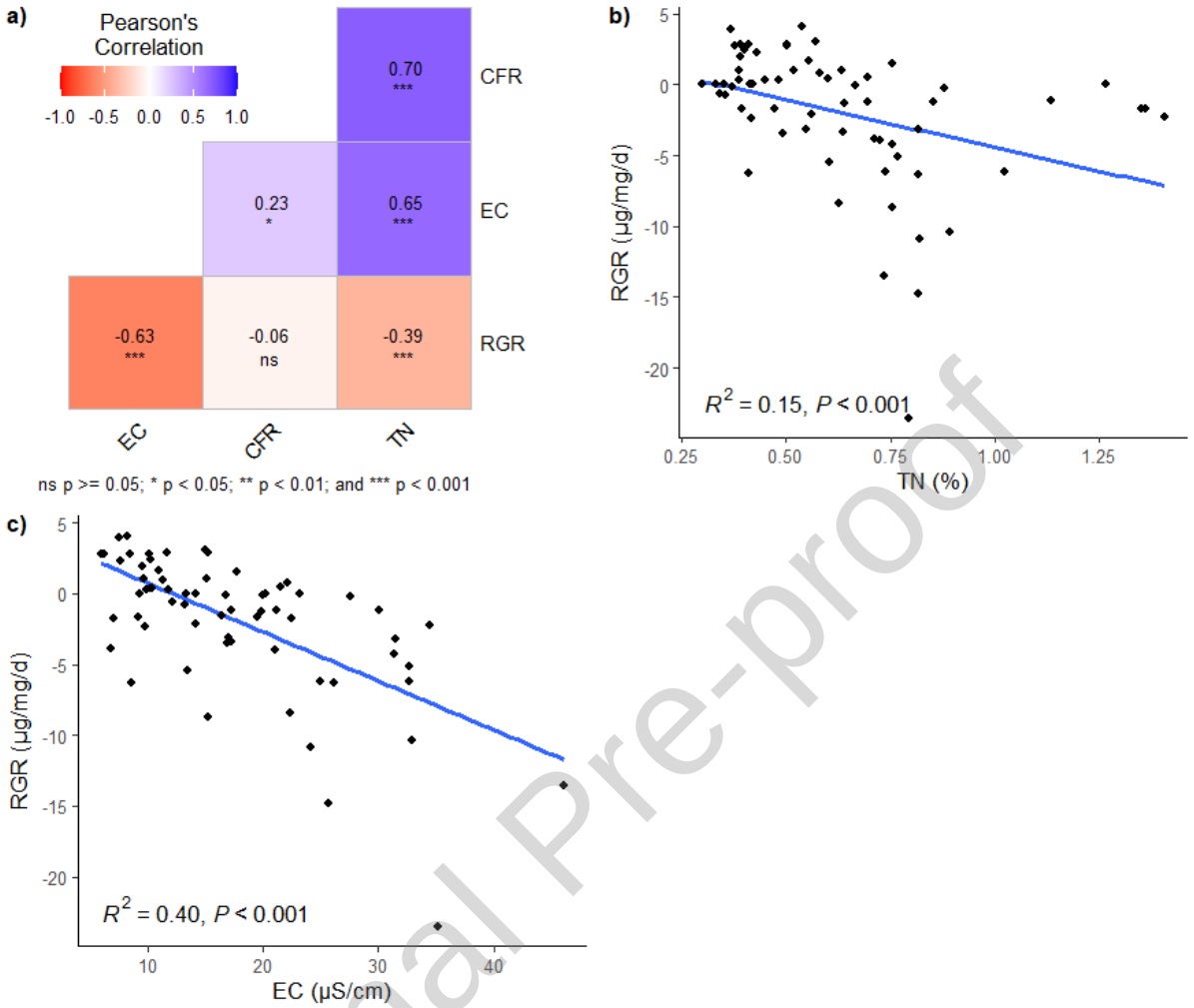


Figure 4. Relationships (Pearson's product moment correlation) between response variables measured for *Evernia prunastri* thalli during the transplant experiment (a): RGR, relative growth rate; EC, electrolyte conductivity; CFR, chlorophyll fluorescence ratio F735/F700 as a proxy for chlorophyll content; TN, tissue total nitrogen content. Significant relationships (linear regression) between RGR and TN (b) and EC (c). Values shown in the correlation matrix plot are correlation coefficients (*r*).

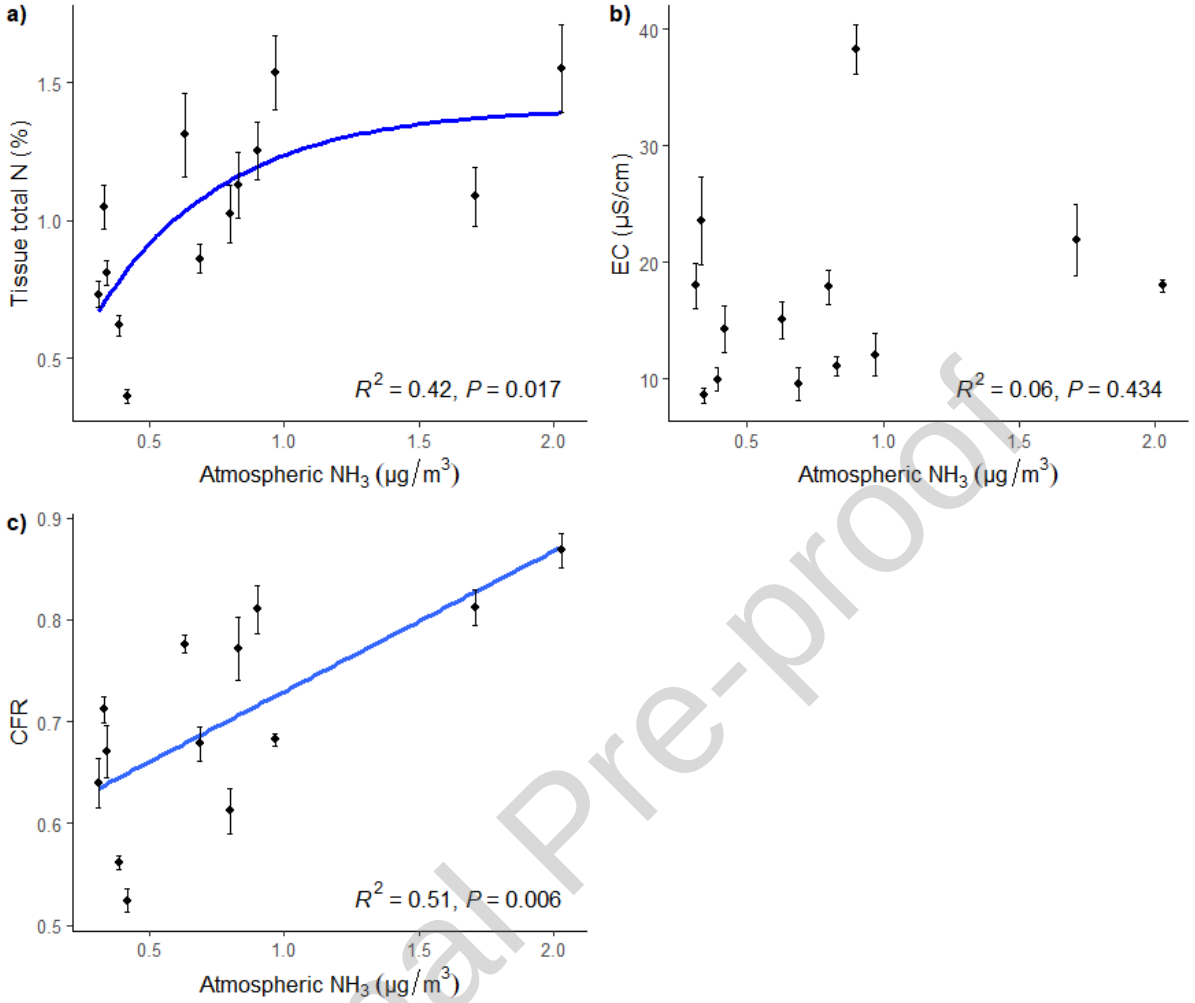
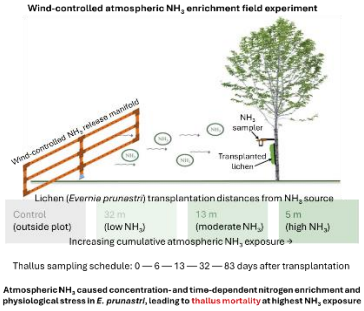


Figure 5. Comparison of modelled ammonia concentration with the response of field sampled *Evernia prunastri* thalli for 13 sites across Scotland and England (cf. Fig. 1). Values for tissue total N, EC, and CFR are mean ± 1 SEM. N, nitrogen content; EC, electrolyte conductivity; CFR, chlorophyll fluorescence ratio F735/F700 as a proxy for chlorophyll content.

Graphical abstract



Authorship Contribution Statement

Lumbani Mwafulirwa: Conceptualization, Investigation, Writing – original draft, Methodology, Validation, Visualization, Writing - review & editing, Formal analysis, Data curation. **Ajinkya G. Deshpande**: Conceptualization, Investigation, Writing – original draft, Methodology, Visualization, Writing - review & editing, Data curation. **Matthew R. Jones**: Conceptualization, Funding acquisition, Methodology, Validation, Writing - review & editing, Supervision, Resources. **Mark A. Sutton**: Conceptualization, Funding acquisition, Writing - review & editing, Project administration, Resources. **Christopher J. Ellis**: Conceptualization, Funding acquisition, Writing - original draft, Methodology, Validation, Writing - review & editing, Visualization, Formal analysis, Project administration, Supervision, Resources, Data curation.

Declaration of Interest

The authors declare no conflicts of interest.

Highlights

- We studied the response of a lichen bioindicator to contrasting NH_3 concentrations.
- *E. prunastri* thalli were transplanted into a field-scale experiment over 12 weeks.
- The results of the experiment were compared to trends observed in the real world.
- The results show that *E. prunastri* tissue nitrogen content became saturated at 1.3%.
- Accumulative damage to fungal cell membranes led to eventual lichen mortality.