Lichens as spatially transferable bioindicators for monitoring nitrogen pollution☆

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ABSTRACT

Excess nitrogen is a pollutant and global problem that harms ecosystems and can severely affect human health. Pollutant nitrogen is becoming more widespread and intensifying in the tropics. There is thus a requirement to develop nitrogen biomonitoring for spatial mapping and trend analysis of tropical biodiversity and ecosystems. In temperate and boreal zones, multiple bioindicators for nitrogen pollution have been developed, with lichen epiphytes among the most sensitive and widely applied. However, the state of our current knowledge on bioindicators is geographically biased, with extensive research effort focused on bioindicators in the temperate and boreal zones. The development of lichen bioindicators in the tropics is further weakened by incomplete taxonomic and ecological knowledge. In this study we performed a literature review and meta-analysis, attempting to identify characteristics of lichens that offer transferability of bioindication into tropical regions. This transferability must overcome the different species pools between source information – drawing on extensive research effort in the temperate and boreal zone – and tropical ecosystems. Focussing on ammonia concentration as the nitrogen pollutant, we identify a set of morphological traits and taxonomic relationships that cause lichen epiphytes to be more sensitive, or more resistant to this excess nitrogen. We perform an independent test of our bioindicator scheme and offer recommendations for its application and future research in the tropics.

1. Introduction

Nitrogen pollution is a global environmental problem affecting ecosystem function and human health (Erisman et al., 2013; Vitousek et al., 1996). Key sources of pollutant nitrogen are intensive agriculture (Asman et al., 1998; Liu et al., 2022) and burning fossil fuels (Dignon, 1992; Larkin et al., 2017) representing industrial and often urban sources. Ecologically these emissions of pollutant nitrogen can cause a direct toxicity, an excess of nutritional nitrogen (hypertrophication) and/or wider cascades in the soil and water environments. Considering terrestrial ecosystems, nitrogen can be directly toxic to guilds such as lichens (Sutton et al., 2009; Van Herk et al., 2003), which are among the most sensitive bioindicators of nitrogen risk (Bobbink et al., 2002; Cape et al., 2009; Pardo et al., 2011). Additionally, in many ecosystems

nitrogen has a limiting effect on plant productivity, and vegetation is therefore finely structured by individual plant responses to patterns of nitrogen availability (McKane et al., 2002; Miller and Bowman, 2002). Excess nitrogen can disrupt this vegetation structure via multiple non-exclusive pathways, depending on its form and dose, and, for example, soil properties (Bobbink et al., 1998; Maskell et al., 2010; Stevens et al., 2010). Excess nitrogen can lead to hypertrophication and competitive exclusion, or, for soils with reduced buffering capacity deposition of acidic nitrogen (e.g. nitric acid) can cause a build-up of ammonium (limiting the variety of plant available nitrogen), while deposition as ammonium compounds can enhance nitrification, also causing acidification with leaching of cations and increased mobility of metals at toxic levels. These and various other impact pathways may result in a net loss of biodiversity across multiple terrestrial ecosystems (Bobbink et al., 2010; Dise et al., 2011), threatening the collapse of ecosystem function. Furthermore, nitrogen pollution can directly affect human health (Townsend et al., 2003; Wolfe and Patz, 2002), and, in a regional case study, the overall accrued negative impacts of nitrogen pollution were estimated in 2011 to cost the European Union somewhere between €70 billion and €380 billion per annum (Sutton et al., 2011).

The problem of nitrogen pollution, from both agricultural and industrial sources, intensified in Europe and North America during the 20th Century, though release of pollutant nitrogen is now declining or stabilising in these temperate regions owing to emission control policies (Fagerli and Aas, 2008; Lloret and Valiela, 2016; Tørseth et al., 2012). In contrast, nitrogen pollution is spreading and further intensifying across tropical regions that intersect with biodiversity hotpots (cf. Myers et al., 2000; Liu et al., 2022; Van Damme et al., 2018). Indicative figures, based on a combination of measured and modelled data at $1° \times 1°$ gridded resolution, cite high surface ammonia concentrations in Europe at > 8 μg m^{-3} (maxima for reduced N = 19 μgN m^{-3} in southern Europe), annual average concentrations in North America at 0.39–3.74 μg m^{-3} (maxima for reduced N = 7–12 μgN m^{-3} in the eastern United States), with high concentrations in east Asia at > 30 μg m^{-3} (maxima for reduced N = 39 μgN m^{-3} in east China) (Ge et al., 2021, 2022). This drives an urgent need for the assessment and spatial monitoring of nitrogen risk, especially for ecosystems in tropical regions, being comparable to that widely achieved using bioindicators calibrated against critical levels/loads in Europe and North America (Bobbink et al., 2002; Pardo et al., 2011). However, there are challenges to delivery of biomonitoring across tropical regions, exemplified here for lichen epiphytes, which are one of the key bioindicators for managing nitrogen risk (Cape et al., 2009; Geiser et al., 2019). In attempting to use species – such as lichens – as biodindicators (i) the biogeographic pool of species for tropical regions will be different to that of temperate/boreal Europe and North America (Galloway, 2008), where bioindicators have been extensively researched and applied (cf. Ellis et al., 2022), and this prevents the direct transfer of already established bioindicators, while (ii) the biogeographic pool of species for tropical regions will often be incompletely known taxonomically (Lücking et al., 2014; Sipman and Aptroot, 2001), and even more so with respect to the nutrient ecology of the component species. The additional research needed to establish tropical bioindicators *de novo*, especially the underpinning taxonomic clarification, may take decades (estimated at c. 30 years for the single lichen family *Graphidaceae*: Lücking et al., 2014), being inconsistent with the urgent need to verify, monitor and tackle the nitrogen challenge. On that basis, we established hypotheses that could identify tropical bioindicators by extracting potentially transferable elements from European and North American studies. We incorporate two potential routes to transferability. First, we examined taxonomic relatedness: it is arguable that because of shared evolutionary history, and niche conservatism with respect to nutrition (cf. Faĉkovcová et al., 2017; Prinzing et al., 2001), species that are shown to be more or less sensitive to nitrogen in Europe and North America may share this characteristic with related species (at a generic, family or higher level) that occur in

the tropics. Second, we considered traits and especially phenotypic characters: it is arguable that species are rendered more or less sensitive to nitrogen because of a set of recognisable adaptive traits. Convergent evolution would allow transfer of this trait-based framework from Europe and North America to explain the pollution response for species that occur in the tropics, being relevant to a different species pool.

To quantify these hypotheses, this study performed a literature review of published work that had related lichen epiphytes to gradients of nitrogen pollution. Although not geographically restricted in scope, it was recognised *a priori* that a vast majority of such studies would be from Europe and North America. Seeking patterns in how taxonomy and traits relate to nitrogen response, we can provide preliminary recommendations for the use of tropical epiphytic lichen communities as bioindicators where direct knowledge is currently limited.

2. Methods

2.1. Literature review

We performed a literature search of Web of Science (last search January 5, 2022), using the Boolean operators: [lichen* AND nitrog* AND epiphyt* AND (bioindicat* OR indicat*)]. Papers were screened and selected for our analysis where they had reported lichen traits and/or classified lichens into trophic groups, while reporting an unequivocal and significant trait or trophic group response along measured or modelled gradients of nitrogen pollution. Aiming for a quantitative approach, we excluded papers where nitrogen was combined with and inseparable from other pollution types because it formed part of a qualitative index, such as in using a generic gradient of 'air quality'. There was a very low number of papers meeting these criteria for lichen traits, and so in this case the literature search was repeated and also expanded to Google Scholar (last search February 28, 2022), using the Boolean operators: [lichen AND nitrogen AND epiphyte AND (trait* OR phenotype OR growth form)]. Papers from Google Scholar were included for screening when their number of citations ≥50.

2.2. Study weightings

The study context of selected papers was critically examined. Papers were weighted according to how nitrogen had been managed with respect to other covariables, when investigating the lichen response. Other covariables could include spatial location, climate, forest structure and composition, bark characteristics and other forms of pollution such as sulphur dioxide, etc. The weighting was assigned in four categories (i) the sampling design isolated nitrogen and therefore removed the confounding effect of other covariables, e.g. samples within the same region/climate, and collected for the same forest structure/composition, such as along point source nitrogen gradients (weight = 1), (ii) the sampling was broader, but the analysis isolated nitrogen, either as the strongest predictor, or partitioning its unique effect from that of other measured covariables (weight = 0.8), (iii) sampling was broader, while the analysis did not partition the unique effect of nitrogen it was identified as an explanatory factor alongside that of other covariables (weight = 0.5), (iv) sampling was broader, and although there was a relationship with measured or modelled nitrogen there was no consideration of potential covariables (weight = 0.1). The staggered weighting reflected author consensus around the strength of quantitative evidence that linked the lichen response with an effect of nitrogen, ranging from a scenario where a controlled natural experiment isolates the lichen response to the effect of nitrogen (highest weighting = 1), to a scenario where both the lichen response and nitrogen are quantified, but without consideration of other potential covariables (lowest weighting = 0.1).

2.3. Lichen traits

Papers that had reported a significant nitrogen response for lichen

traits were tabulated, taking the weights into consideration, noting the major covariables, as well as any reported threshold for nitrogen impact.

2.4. Trophic groups

Papers that had reported a significant response for lichen trophic groups were found to have adopted three main categories that were summarised as (i) nutritional (nitrophytic, eutrophic, neutrophytic, mesotrophic, oligotrophic), (ii) environmental/substratum pH (acidophytes) and (iii) generic (tolerant, intermediate, sensitive). We considered nitrophytic and eutrophic as being synonymous in responding positively to a nutrient-rich environment (as nitrophytes), and neutrophytic and mesotrophic as being synonymous in having their optima in a moderate nutrient environment (as mesotrophic).

First, we compared the types of nitrogen that papers had used as explanatory variables and examined these according to their respective use of the different trophic groups. Second, all lichen species that had been reported within a trophic group were listed. Nomenclature was standardised to Index Fungorum (as of March 31, 2022: http://www.indexfungorum.org/) and species that had appeared in three or more papers were selected for further analysis. An overall weighting (confidence score) was applied to each of these species as they contributed to a given trophic group. This was simply determined from the weighting of the papers within which the species had been reported (see above) and then accumulating these weighting values according to how nitrogen had been managed. A confidence score for each species could therefore be separated according to its representation across the trophic groups. For example, if species a appeared as a nitrophyte in three papers with weighting 1, it's confidence score = 3; if species b appeared as an oligotroph in two papers with weighting 0.5 and three papers with weight 0.1, its confidence score = 1.3. Species could contribute to multiple trophic groups, depending on how they had been handled by the selected papers. If species c had appeared as a nitrophyte in one paper with a weight of 0.5, and in another paper as mesotrophic with a weight of 0.5, it would be scored equally across both categories. We made a summary of underlying relationships by examining (i) the weightings for papers that had used different trophic groups, (ii) confidence scores for species compared to the number of papers in which they had appeared, and (iii) the number of trophic groups to which a species had been assigned compared to the number of papers in which they had appeared.

The species were then categorised according to their higher-level taxonomy as well as multiple traits derived from the standardised inter-specific trait database LIAS (as of March 31, 2022: http://liaslight.lias.net/) and cross-referenced against regional literature relevant to Europe (Smith et al., 2009) and North America (McCune and Geiser, 2009). These categories were (i) taxonomy at family level, (ii) gross morphology (macrolichens or microlichens), (iii) growth form (fruticose, foliose, squamulose or crustose), (iv) morphological type (hair lichens, other fruticose, *Cladonia*-type, large-foliose (lobe width ≥10 mm), medium-foliose (lobe width 2.5–10 mm), small foliose (lobe width ≤2.5 mm), leprose), (v) gross photobiont category (chlorolichen and trentepohlioid, cephalolichen, cyanolichen), (vi) colour (amalgamated from LIAS to correspond to the 11 colours from McCune & Yang's lichen colour chart, cf. https://lichens.twinferntech.net/pnw/colors.shtml), (vii) cortical integrity (e.g. tomentose, maculate, granular or cracked), (viii) rhizines (with or without, structure as unbranched, furcate, squarrose), (ix) other substructures (e.g. cilia, cyphellae, pseudocyphellae, isidia, soredia), and (x) metabolites, at the resolution of substance-class (Elix, 2014).

Taxonomic families or traits that were represented by fewer than five species were removed. Otherwise, confidence scores were accumulated by considering all the species occurring within a taxonomic family or with a particular trait, and the trophic group to which they contributed. This created a matrix of confidence scores for taxonomy/traits x trophic groups, derived from the initial species scores. The confidence scores were standardised by the maximum for each of the ten taxonomic and trait categories (see above), and the matrix was analysed using detrended correspondence analysis (DCA (Kent, 2012; Kent and Coker, 1992)) in PC-ORD v. 6 (McCune and Mefford, 2011). The DCA ordination axis scores for taxonomic families or traits were adopted as bioindicator weights in response to nitrogen; bioindicator weights were limited to the taxonomic families and traits whose axis scores fell outside the inter-quartile range along a given axis.

2.5. Testing the bioindicator response

We tested the taxonomic family and trait bioindicators against an independent dataset of lichen communities sampled from twenty-one sites in Britain and Ireland (Fig. 1). Sampling used standard ladder transect methodologies (Asta et al., 2002; Matos et al., 2017), with detailed methods provided by Lewis (2012). Samples were collected from the ladder transects with five sub-units positioned downwards from 1.5 m, at the four cardinal aspects of straight grown, mature native oak trees (*Quercus* spp.). Site selection was designed to capture a gradient in atmospheric ammonia. Monthly mean values of atmospheric ammonia concentrations were directly measured at each site for at least a twelve-month period concomitant with the sampling; ammonia was measured using either ALPHA (adapted low-cost passive high-absorption) or DELTA (diffusion denuder systems for long-term atmospheric) samplers (Sutton et al., 2001; Tang et al., 2001).

Sampled lichen species were categorised according to their taxonomic family and traits (see above) and the abundance of these was summed for each ladder transect. Abundances were used to create site bioindicator values using weighted averaging ordination (Kent, 2012; Kent and Coker, 1992), but with the lichen samples treated in two different ways (i) standardising abundance according to the maximum value across samples (i.e. among trunk aspects, trees and sites) with respect to each of the bioindicator taxonomy and trait categories, and (ii) standardising abundance using the maximum value within samples and across the different bioindicator taxonomy and trait categories. In the first case interpretation rests on a comparison among different samples (e.g. from contrasting sites), in the second case interpretation is related to the unique properties of the individual sample. The bioindicator values (responses) were compared, and an appropriate regression curve was plotted to normalise residuals. Furthermore, bioindicator values were partitioned into variance explained at a site, tree and individual quadrat level (Crawley, 2013; Zuur et al., 2009) using a mixed model with Gaussian error structure, implemented using the '*nlme*' package in R (Pinheiro et al., 2020; R Development Core Team, 2020).

3. Results

Our initial Web of Science search identified 174 papers for screening, of which 30 were selected for analysis (see supplementary data, Appendix A), meeting the criteria of having reported lichen traits and/or classified lichens into trophic groups, while identifying an unequivocal and significant trait or trophic group response along measured or modelled gradients of nitrogen pollution. The Google Scholar search identified an additional six papers of which four tested trophic groups and two tested lichen traits. The selected papers encompassed a date range from 1992 to 2019, and spatially all were from the northern Hemisphere, with 47% from Europe and 31% from the United States. A majority of selected papers analysed trophic groups. The five papers that specifically analysed lichen traits (Table 1) highlighted the proportional contribution to the epiphyte community or the abundance of macrolichens, partitioned into fruticose and foliose species, and especially cyanolichens. These studies succeeded in detecting nitrogen, alongside other covariables, as the strongest predictor, but had not necessarily considered correlations between nitrogen and other pollutants. A single study had represented growth form on a continuous scale as specific thallus mass (Hurtado et al., 2020) and had also included $\delta^{15}N$ and $\delta^{13}C$
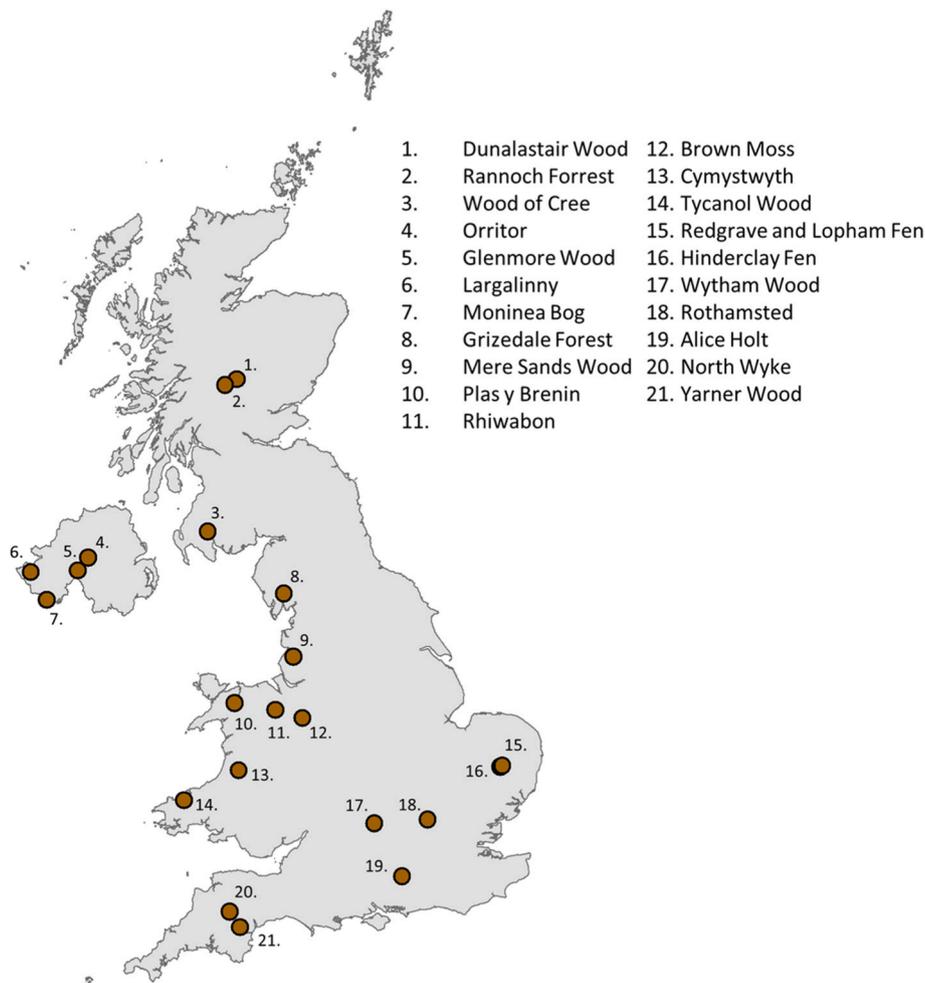
Fig. 1. The distribution of study sites for sampled lichen communities (cf. Lewis, 2012) in comparison with the ambient nitrogen regime.

Table 1

Examples of studies that had specifically examined the response of lichen traits to measured or modelled nitrogen.

Lichen Trait	Significant N Type	Response	Threshold	Covariables	Study
Macrolichens	Total N deposition (incl. throughfall)	Representation in community	$2.4 \text{ kg ha}^{-1}.\text{yr}^{-1}$	Sulphur, altitude, forest structure	Giordani et al. (2014)
Fruticose	Total and cumulative N deposition	Abundance		Sulphur, altitude, forest structure	Cleavitt et al. (2015)
	Total N deposition (incl. throughfall)	Abundance	$2 \text{ kg ha}^{-1}.\text{yr}^{-1}*$	NA	Geiser et al. (2019)
Foliose	Total deposition as NO_3 and NH_4	Richness	$10.2 \text{ kg yr}^{-1}.\text{ha}^{-1}$	Location, climate, sulphur, ozone, edaphic factors	Miller and Watmough (2009)
Cyanolichens	Total and cumulative N deposition	Abundance		Sulphur, forest structure	Cleavitt et al. (2015)
	Total N deposition (incl. throughfall)	Abundance	$1.3 \text{ kg ha}^{-1}.\text{yr}^{-1}*$	NA	Geiser et al. (2019)
Specific Thallus Mass	Total N deposition	NA		Climate	Hurtado et al. (2020)
$\delta^{13}C$	Total N deposition	NA		Climate	Hurtado et al. (2020)

*causing a 20% decline, up to an 80% decline at 10 kg.ha-1.yr-1 (fruticose) or 6 kg.ha-1.yr-1 (cyanolichens).

as proxies for nutrient acquisition.

Of the papers that analysed trophic groups, these appeared divergent in how they identified the relationship between each trophic group category and nitrogen type as predictor (Fig. 2). Papers that had used the generic trophic group category (tolerant, intermediate, sensitive) had also identified the widest spread of nitrogen response types, while papers focussed on a nutritional category (nitrophytic, eutrophic, neutrophytic, mesotrophic, oligotrophic) tended to identify ammonia concentration, but also the compound effect of total nitrogen deposition,

including cumulative, throughfall, and total wet and dry deposition. Papers focussed on the environmental pH (acidophytes) identified the narrowest range of nitrogen response types, with particular representation of nitrogen dioxide.

Considering the different trophic groups, individual papers had incorporated between 9 and 255 species into their analyses, with a total number of 546 species that had been used across all papers as trophic group indicators. Only 24% of papers derived their indicators *de novo*, while the remainder were repeat, confirmatory tests of indicators
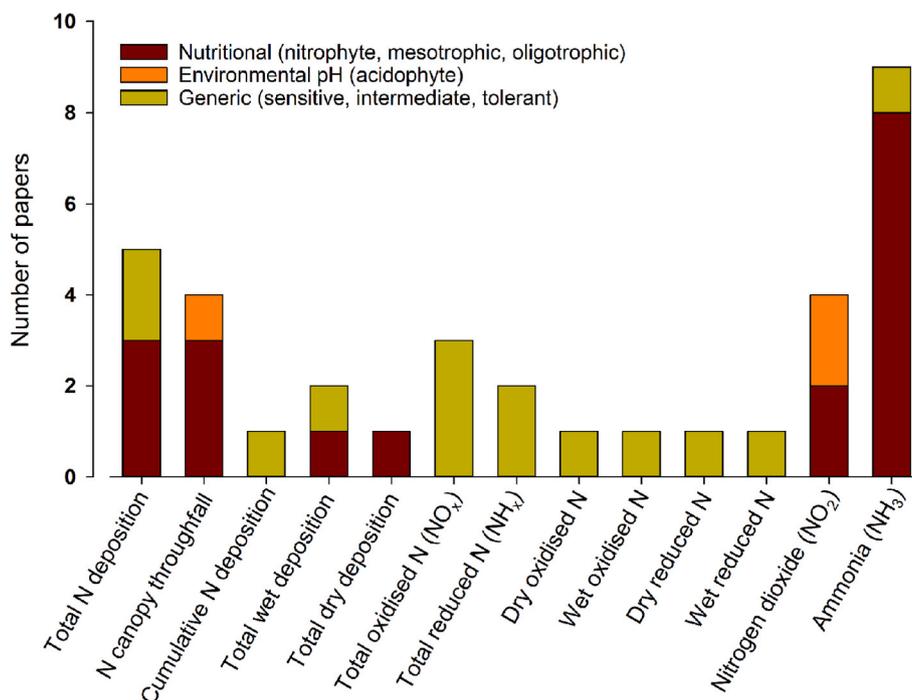
Fig. 2. The number of papers examining different trophic group categories (nutritional, environmental pH and generic), compared to the nitrogen type identified as a significant predictor.

originally borrowed from a smaller number of key regional sources including floras (McCune and Geiser, 2009), web-sites (Nimis and Martellos, 2008), reports (Jovan, 2008; Wolseley et al., 2005) and peer-reviewed papers (Van Herk, 1999). For our analysis, 182 species had been used in three or more papers (see supplementary data Fig. S1, Appendix A).

In building confidence scores for individual species (i) there was a difference in the weightings for papers that had investigated different trophic groups (Fig. 3A), with papers using groups in a nutritional category tending to have isolated nitrogen effects through design, e.g. sampling along point source gradients, while papers using groups in a generic category tended to extract patterns from dispersed landscape samples with a greater number of confounding variables (Fig. 3A), (ii) there was a clear relationship between the number of papers within which a species appeared, and the confidence score for that species (Fig. 3B), with slight skewness around this relationship explained by the trophic group to which a species was preferentially assigned (cf. Fig. 3A), and (iii) the more papers within which a species appeared, the greater the number of trophic groups into which it had been assigned (Fig. 3C). Only 10% of species had been classified consistently into a single trophic group, while 44% appeared in two groups, and 46% appeared in three or more groups. Some species had been classified into multiple groups; for example, *Platismatia glauca* appeared as a trophic group indicator in eleven papers, and had been classified as a nitrophyte, mesotrophic, oligotrophic, and acidophytic, as well as being either intermediate or sensitive. Considering these trends in how papers had been weighted, and the assignment of species into trophic groups, some examples of species with the highest confidence scores were, for nitrophytes, *Physcia adscendens*, *Xanthoria parietina* and *Physconia enteroxantha*, for the oligotrophic group, *Usnea rubicunda*, *Ramalina farinacea*, *Parmotrema reticulatum*, and for acidophytes, *Evernia prunastri*, *Hypogymnia physodes*, and *Hypogymnia tubulosa*.

Having derived confidence scores for species that were assigned to different trophic groups, we could align these to taxonomic families or traits. Certain of the initial taxonomic families or traits were represented by fewer than five species and were dropped from further analysis, including for example tripartite cephalolichens, or lichens with a

trentepohlioid photobiont, leaving a total of fifty-seven taxonomic families or traits for analysis. Ordination by DCA (Fig. 4) resulted in axes describing 86.1% and 7.3% of variation in the original taxonomy/traits x trophic groups matrix, for axes one and two, respectively. There was a clear separation along axis one for traits associated with oligotrophic, acidophytic and sensitive lichen indicators, compared to nitrophytic indicators, with taxonomic families or traits that fall outside the inter-quartile axis one scores as follows. Located at the oligotrophic, acidophytic and sensitive end of the spectrum were *Lobariaceae*, *Nephromataceae* and *Parmeliaceae*, as well as fruticose, including hair-lichens, and large and medium foliose growth forms, cyanolichens, and lichens with cracked upper cortices, and pseudocyphellae, and with suits of metabolites in the substance classes of aliphatic acids, benzyl esthers, orcinol (tri)depsides, and β-orcinol depsidones. Located at the nitrophytic end of the spectrum were *Physiaceae*, *Teloschistaceae*, small foliose growth forms, lichens with pruina, including the community contribution of microlichens, especially crustose species that had a granular structure and/or with a granular cortex, and yellow to orange colours with suits of metabolites in the substance classes of anthraquinones, ergochromes, pulvinic acid and xanthones. There was ambiguity about traits associated with tolerant, intermediate or mesotrophic indicators, which were separated along less informative axis two.

Using the axis one scores for taxonomic families and traits as bioindicator weights and applying a weighted averaging ordination to assign values to the independent lichen community dataset, there was a significant non-linear relationship between bioindicator scores and ammonia concentration, fitted here with a logarithmic curve (Fig. 5), based on both a comparative analysis (standardisation of biodindicator taxonomy and trait categories across samples), and for individual samples (standardisation within samples only). This was for the mean value of ammonia concentration per site, with variability in the bioindicator scores for each of the sites representing measurement error, plus unexplained effects within a tree (e.g. aspect), among trees (e.g. size/age) and among sites (e.g. climate). Variance partitioning suggested that differences among sites accounted for 53.2% and 52.6% of the explained variation in bioindicator scores, for standardisation across and within samples, respectively, with tree and quadrat explaining 15.7% and
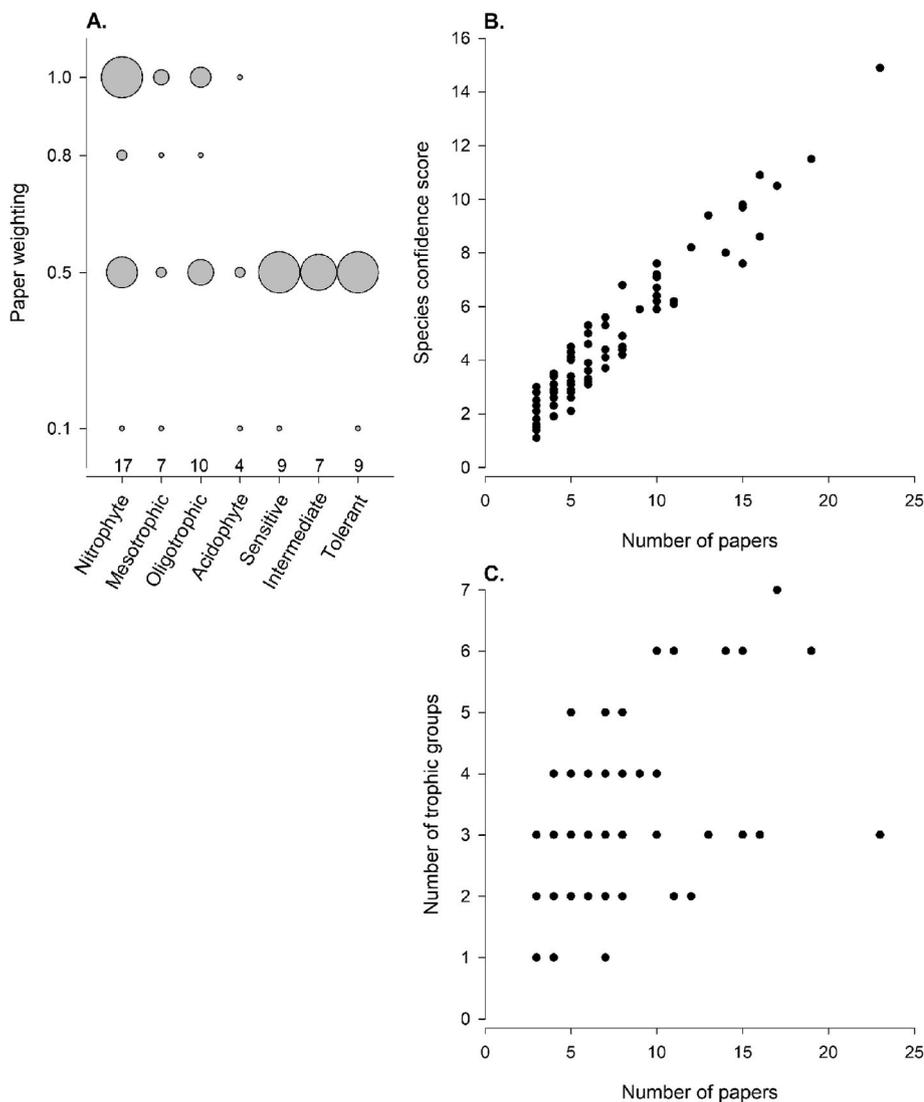
Fig. 3. A. The weightings for papers compared to the trophic groups they had used, the number of papers per trophic group is shown on the lower axis, and the symbols are scaled to the number of papers with a given weighting (largest symbol = 8, smallest symbol = 1), B. Comparison of species confidence scores in relation to the number papers that have used the same species as a trophic group indicator, C. The number of trophic groups into which a species has been assigned in relation to the number papers that have used the same species as a trophic group indicator.
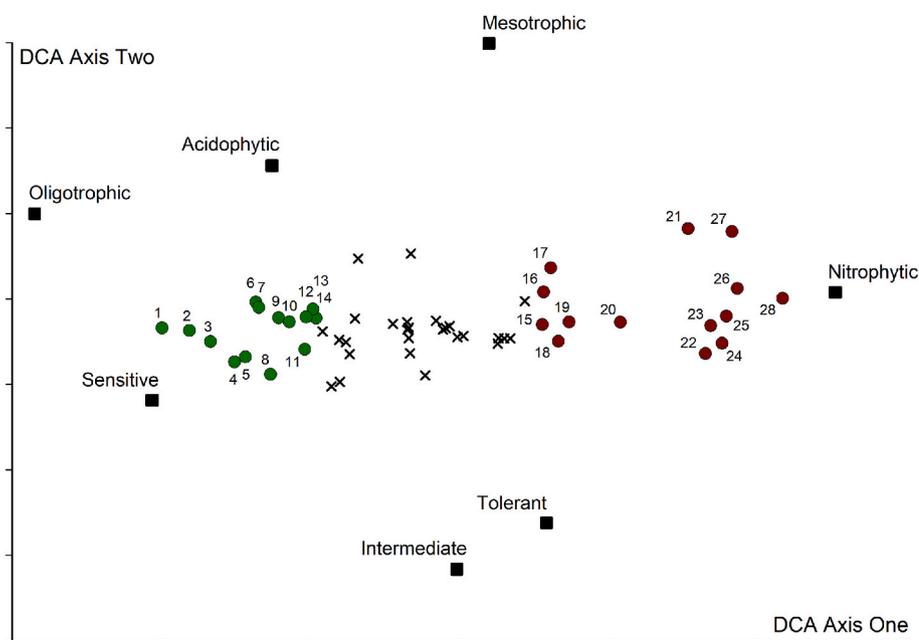
Fig. 4. Ordination by DCA to compare alignment of taxonomy/traits with trophic groups while accounting for the confidence scores of component species; taxonomic families and traits that fall outside the interquartile range of axis one scores are highlighted: 1 = *Lobariaceae*, 2 = hair lichens, 3 = *Nephromataceae*, 4 = benzyl esthers, 5 = large foliose lichens, 6 = fruticose, 7 = aliphatic acid, 8 = cyanolichens, 9 = *Parmeliaceae*, 10 = orcinol depsides, 11 = cracked cortex, 12 = β-orcinol depsidones, 13 = pseudocyphellae, 14 = medium foliose lichens, 15 = yellow colour, 16 = pulvinic acid, 17 = ergochromes, 18 = small foliose lichens, 19 = granular cortex, 20 = pruinose, 21 = microlichens, 22 = *Physciaceae*, 23 = orange colour, 24 = anthraquinones, 25 = *Teloschistaceae*, 26 = crustose, 27 = granular thallus structure, 28 = xanthones. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)
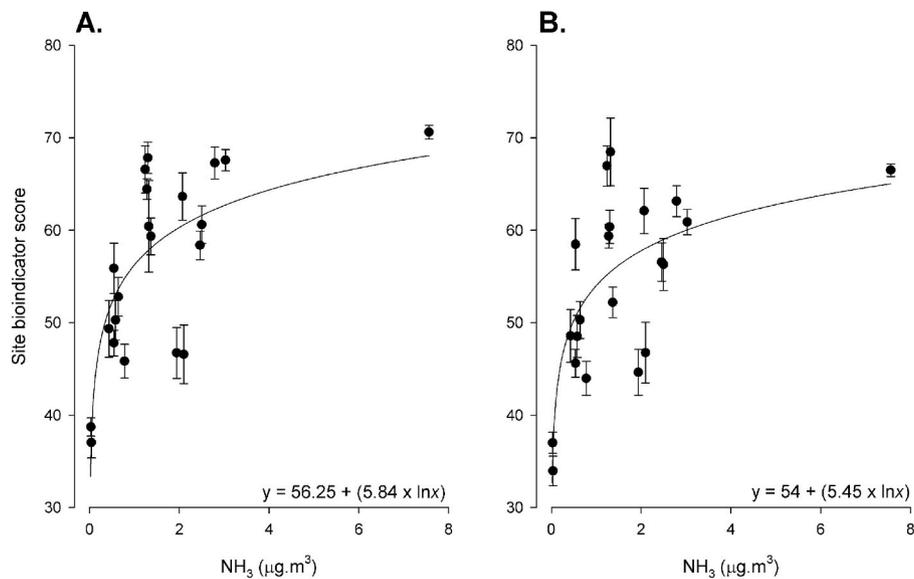
Fig. 5. Measured and modelled ammonia concentration compared to site bioindicator scores based on the weighted averaging of taxonomic families and traits in lichen communities (cf. Fig. 4); A. Comparative analysis with standardisation of biodindicator taxonomy and trait categories across samples (adj-R^2 = 0.5674, $P <$ 0.0001 with 19 df), B. Standardisation within samples (adj-R^2 = 0.5135, $P <$ 0.0001 with 19 df).

19.1%, and 25.8% and 23.2%.

4. Discussion

Nitrogen pollution from agriculture and industry is a major driver of global environmental change (Erisman et al., 2013; Vitousek et al., 1996). Lichens provide one of the key bioindicators for managing nitrogen pollution risk, having been widely used to establish critical levels and loads for different forms of atmospheric nitrogen (Bobbink et al., 2002; Cape et al., 2009; Pardo et al., 2011). Besides the direct consequence of lichen decline for ecosystem function and services (Asplund and Wardle, 2017; Ellis et al., 2021), these levels/loads act as thresholds beyond which there is also a much wider cascade of negative effects on ecosystem health (Bobbink et al., 2010; Dise et al., 2011). The intensity of nitrogen pollution is increasing in the tropics (Liu et al., 2022; Van Damme et al., 2018), in regions of high biodiversity (Myers et al., 2000) and where human livelihoods and well-being are strongly dependent on the sustainable delivery of ecosystem services (Fedele et al., 2021; Kumar and Yahiro, 2014). In a regional case study, recent research has compared established critical levels and loads for lichen bioindicators, to modelled atmospheric nitrogen chemistry, suggesting that up to 90% of Himalayan-forest ecosystems may already be exceeding their nitrogen thresholds for sustained ecosystem health (Ellis et al., 2022). This and other desk-based analyses require direct confirmation through field surveillance of bioindicators. However, direct confirmation is constrained by taxonomic uncertainty and a lack of ecological research for key bioindicators such as lichens, in tropical regions. Our aim was to provide a framework by which patterns in the taxonomy and traits of lichen epiphyte bioindicators, mostly researched for boreal, temperate and Mediterranean Europe and North America (c. 80% of published literature), could be transferred to estimate nitrogen impacts over contrasting biogeographic regions, including the tropics.

The study was based on literature review and meta-analysis of published papers, to recover taxonomy and trait information that has underpinned the use of lichen bioindicators, and we found both consistency and contrast in this existing research. For example, papers that focussed on generic bioindicators (sensitive-tolerant) had used the broadest set of nitrogen forms in explaining the lichen response, as might be expected. Papers focussed on nutritional categories (oligotrophic-nitrophytic) referenced ammonia or total nitrogen as a catch-all,

with an acidification category (acidophytic) tending to reference nitrogen dioxide. However, the identification of a species within a given category appeared to be governed to some degree by the regional and ecological context of the paper, and the baseline from which nitrogen effects were being measured. This type of variability was strongly observed when comparing lichen responses between the eastern and western United States, which have different pollution histories and climates (Cleavitt et al., 2015; Geiser et al., 2019; Will-Wolf et al., 2015). It was nevertheless possible to coalesce the results of multiple papers to identify trends in taxonomy and traits with good potential for transferability. In doing so, two caveats are highlighted here and discussed in more detail below. First, we choose not to account for covariance among the units of investigation (taxonomic identity or particular traits) through a reductive prior analysis, because although these may correlate within the species pools of Europe and North America (e.g. species in the *Teloschistaceae* may tend to be yellow/orange-coloured, small foliose or crustose microlichens, with anthraquinones), the taxonomic and trait categories may associate in contrasting ways for evolutionarily independent species pools located in different biogeographic regions including the tropics, and, when used cautiously, considering them as individual effects offers greater potential transferability. The transferability of individual taxonomic families and traits thus depends on the strength of additional supporting evidence to suggest that each confers a direct sensitivity to nitrogen, rather than appearing to be relevant only through correlation with some other effect. Second, certain taxonomic families important in the tropics are absent from this study (e.g. *Graphidaceae*), and certain traits that are common in tropical ecosystems (e.g. trentepohlioid lichens) were too poorly represented to be robustly analysed based on the European and North American papers. This points to a need for expansion of our framework, with ecological research embedded in the tropics. Notwithstanding these caveats to the interpretation of our results, we were able to identify a taxonomy and trait framework that offers potential transferability in the use of bioindicators. Key features include the following.

1) Nitrogen sensitivity of cyanolichens and associated families (*Lobariaceae*, *Nephromataceae*), as well as fruticose lichens especially hairlichens. This feature of the trophic group analysis (Fig. 4) is also supported by the independent specific analysis of lichen traits (Table 1). These traits offer alternate bioindicators that may be

relevant to different climatic regimes, either wetter with high rainfall (liquid water) for cyanolichens, or drier with humidity and dew-fall for hair-lichens (cf. Esseen et al., 2016; Marini et al., 2011; Phinney et al., 2022). Previous experimental work supports this sensitivity of both cyanolichens and hair-lichens to various forms of nitrogen pollution (Gauslaa et al., 2021; Riddell et al., 2012). A starting point for this sensitivity may be found in the extent to which cyanolichens and hair-lichens scavenge moisture (Gauslaa and Arsenault, 2020; Phinney et al., 2018), either in large amounts over a longer period, or for small amounts rapidly and often, since they occupy opposite ends of a spectrum in moisture supply (Gauslaa, 2014). Either strategy could provide an effective route for uptake of soluble nitrogen into the lichen thallus. This enhanced uptake may partly explain the wider sensitivity of larger/medium-sized foliose lichens beyond members of the *Lobariaceae* and *Nephromataceae*. However, the precise physiological details of the nitrogen response are not fully resolved; for example, lichen sensitivity has been linked to species-specific N:P ratios, and susceptibility to phosphorus limitation, which may in turn destabilise regulatory mechanisms coordinated between the fungus and photobiont (Johansson et al., 2011; Wang et al., 2019). This physiological sensitivity may be patterned according to the variety of symbiotic combinations between fungus, determining lichen gross morphology, and photobiont, such as green-algal, tripartite and cyanobacterial (Palmqvist et al., 2002; Benner & Vitousek, 2007).

2) Tolerance of the families *Physciaceace* and *Teloschistaceae*. These families may be convergently adapted to both high light conditions (for *Teloschistaceae* cf. Gaya et al., 2015; Solhaug et al., 2003) and nitrogen tolerance, with a proposed ability of sun-adapted lichens to up-regulate carbon fixation and absorb excess nitrogen (Hauck, 2010; Hauck and Wirth, 2010). They also encompass covariation with other traits, such as often being small foliose or crustose microlichens, with – again referring to the *Teloschistaceae* – an associated chemistry (anthraquinones, see 4) below) that confers a characteristic yellow/orange colour. As described above, the independence of these covariable traits needs to be critically assessed.

3) In contrast to fruticose and large foliose lichens (see 1) above) small foliose and crustose lichens (as microlichens) appear relatively more tolerant to nitrogen. It is expected that small foliose and microlichens, with a larger surface area per unit internal mass, may be susceptible to supra-saturation (cf. Lange, 2003; Lange and Green, 1996), explaining adaptation towards a more hydrophobic cortex (Lakatos et al., 2006) which could then confer pollution tolerance because of lower uptake of soluble nitrogen (cf. Hauck et al., 2008). This proposition might extend to substructural units of the lichen thallus that increase or decrease moisture absorption, including the presence of pruina (common among genera in the *Physciaceae*), again linking an adaptation to the light environment (Hauck et al., 2007) with nitrogen tolerance (Hauck, 2010; Hauck and Wirth, 2010), while pruina are also strongly hydrophobic (Hauck et al., 2008). In contrast, features that expose the internal lichen thallus, including a cracked cortex or pseudocyphellae, while facilitating gas exchange (Green et al., 1981; Hale, 1981), could logically lead to greater exposure to both dry and wet deposited nitrogen.

4) There also appears to be a role for lichen metabolites in affecting species sensitivity or tolerance to nitrogen pollution. This is again consistent with previous work that has demonstrated the effect of eutrophicating nitrogen on the chemical composition of lichen communities (Hauck, 2011), especially the sensitivity of species with despsides and depsidones, and the tolerance of species with anthraquinones and pulvinic acid. It has been proposed that these chemical classes regulate the uptake of metals at different pH optima (Hauck et al., 2009a; Hauck et al., 2010; Hauck et al., 2009b), leading to physiological fitness differences as the bark pH becomes modified by atmospheric nitrogen deposition. However, there may also be a direct role for certain metabolites in protecting fungal cell membranes (Munzi et al., 2009), with the anthraquinone parietin (found in the *Teloschistaceae*, see 2) above) confirmed experimentally as an antioxidant (for a pyrogallol marker) and upregulated in lichen thalli exposed to excess nitrogen (cf. Paoli et al., 2015; Silberstein et al., 1996).

Although the relevance of taxonomic families and traits in nitrogen bioindication is sometimes equivocal in terms of direct mechanism, being in part an indirect consequence of adaptation to dry and high light environments, shifted substratum pH etc., the effects identified here do coalesce towards a working hypothesis. Hence, we found that the application of taxonomic families and traits, in a bioindicator framework, was able to recover a pattern of ammonia concentration for independent sites in Britain and Ireland. Further testing and expansion of this framework into the tropics is now required. Selection criteria for papers entering the meta-analysis often excluded previously published research in tropical regions, because this had frequently collapsed the direct effect of nitrogen into summary gradients, such as using 'air quality' or 'urbanisation' (e.g. population density, traffic flow) as a proxy for human impact. Nevertheless, existing research in the tropics does appear to support the transferability of the taxonomic families and traits identified here (Käffer et al., 2011; Koch et al., 2019; Saipunkaew et al., 2007; Saipunkaew et al., 2005), for example the distinction between species in the *Parmeliaceae* and *Physiaceae*, or traits including fruticose and cyanolichens (sensitive) or small foliose growth forms and lichens with pruina (tolerant). However, as noted above, certain traits were included in tropical research that were not included here (trentepohlioid lichens, being sensitive), or they had contrasting responses (loosely attached crustose lichens, being sensitive) explained by the more varied morphology for tropical genera (such as *Cryptothecia* and *Herpothallon* spp.) that do not appear in the papers analysed here.

5. Conclusions

In summary, lichens are directly relevant to human well-being in tropical environments, provisioning food, medicine as well as ritual and aesthetic services (Devkota et al., 2017a; Upreti et al., 2005), and they support livelihoods when sustainably harvested for sale into capital markets (Devkota et al., 2017b; Upreti et al., 2005). Furthermore, they provide an ecological warning system, their critical levels/loads being a threshold beyond which there can be a cascade of negative ecosystem consequences (Bobbink et al., 2010; Dise et al., 2011). Our study provides a bioindicator framework on which to build, having potential transferability for equivalent ecosystems in the tropics, e.g. mid-altitude Himalayan forest (Singh and Singh, 1987), where these have comparability to temperate systems in Europe. Applying the biodindicator framework with due caution, it may be possible to infer levels of excess nitrogen through the observed impact on lichen epiphyte communities. Transferability is expected to decrease into the lowland tropics which are bioclimatically, evolutionarily and ecologically more different. Further ecological research on tropical lichen bioindicators would therefore be of immense benefit in the surveillance of ecosystem threat and management of excess nitrogen. This is now being made feasible by the extension of high-resolution atmospheric models into tropical environments (Ge et al., 2021; Ge et al., 2022).

Credit author statement

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2023.121575.

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