

Perspective

Selecting Biomonitors of Atmospheric Nitrogen Deposition: Guidelines for Practitioners and Decision Makers

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Abstract: Environmental pollution is a major threat to public health and is the cause of important economic losses worldwide. Atmospheric nitrogen deposition is one of the most significant components of environmental pollution, which, in addition to being a health risk, is one of the leading drivers of global biodiversity loss. However, monitoring pollution is not possible in many regions of the world because the instrumentation, deployment, operation, and maintenance of automated systems is onerous. An affordable alternative is the use of biomonitors, naturally occurring or transplanted organisms that respond to environmental pollution with a consistent and measurable ecophysiological response. This policy brief advocates for the use of biomonitors of atmospheric nitrogen deposition. Descriptions of the biological and monitoring particularities of commonly utilized biomonitor lichens, bryophytes, vascular epiphytes, herbs, and woody plants, are followed by a discussion of the principal ecophysiological parameters that have been shown to respond to the different nitrogen emissions and their rate of deposition.



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1. Introduction

The purpose of this policy brief is to recommend the use of biomonitors of atmospheric nitrogen deposition. In particular, we focus on lichens and plants, the most common organisms utilized for quantifying deposition. Their importance, the opportunities for use, and the main characteristics of each type of organism are reviewed. A description is also included for the principal analytical techniques utilized for measuring the biomonitor responses to nitrogen deposition.

Biomonitoring of atmospheric deposition is becoming an increasingly accepted practice, although it is still most common in temperate regions of the Northern Hemisphere (Figure 1). While an effort has been made to reduce the utilization of jargon for clarity, the work is based on a systematic review of the academic literature and the authors' experience on biomonitoring. Practitioners looking to implement biomonitoring efforts and readers interested in the biogeochemical, ecophysiological, and analytical aspects of biomonitoring will find the underlying references in the Supplementary Material.

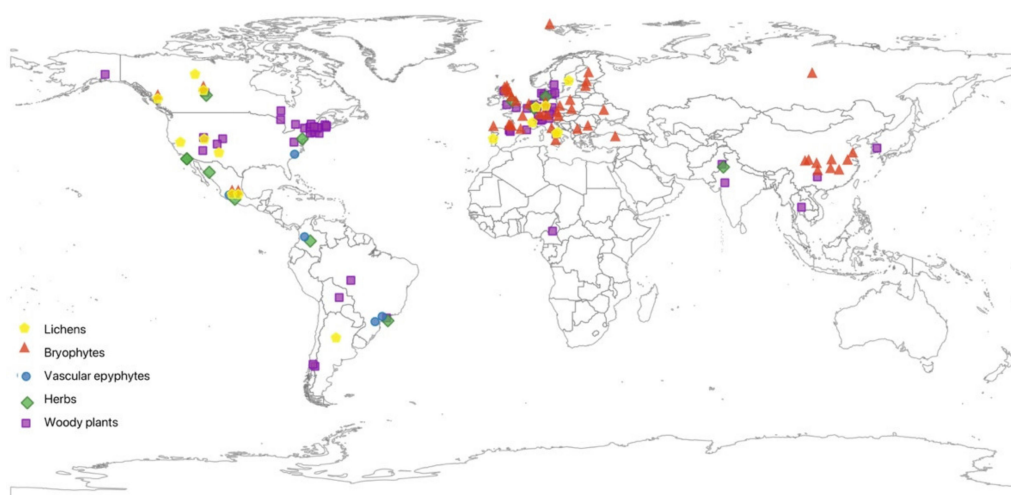


Figure 1. Biomonitoring experiences with lichens, bryophytes, vascular epiphytes, herbs, and woody plants. See underlying studies in the Supplementary Material.

2. Why Should Pollution Be Monitored?

Environmental pollution is a severe threat to public health. In addition to causing significant economic losses due to associated illness and missed days of work, which globally amounted to USD 21 billion in 2015, for example, pollution is responsible for one out of five premature deaths; this is more than the combined mortality of war and other forms of violence, plus that from pandemics such as AIDS, tuberculosis, and malaria [1,2]. Atmospheric pollution is of special concern, as it originates from economic activities such as transportation, industries, and agriculture, which are usually conducted in sites where people are concentrated. A common denominator for these polluting activities is their reliance on fossil fuels, whose combustion releases carbon dioxide and other greenhouse gases that, in addition to their better-known effect of causing climate change [3], have been associated with respiratory disease, stroke, and the accumulation of heavy metals in the internal organs of city dwellers [2,4].

The burden that pollution exerts on public health and general wellbeing is such that access to a clean environment has been elevated to the status of a basic human right [5]. In order to determine whether the public is exposed to safe levels (or not) of various noxious environmental pollutants, governments have issued standards of exposure to substances that comprise atmospheric pollution. Specifically, at least six atmospheric pollutants are usually monitored in cities, namely ozone (O_3) at the ground level, carbon monoxide (CO), sulphur dioxide (SO_2), nitrogen dioxide (NO_2), lead (Pb), and particulate matter, especially suspended particles in the air with diameters below 10 μm (PM_{10}) and below 2.5 μm ($PM_{2.5}$) [6–8].

3. Why Is There a Special Concern about Nitrogen Deposition?

Nitrogen is arguably the most important component of environmental pollution. This may seem counterintuitive considering that nitrogen is an essential element for life and that it is the most abundant gas in the atmosphere, where its molecular form (N_2) is essentially inert. However, ca. 124 Tg of the reactive species of nitrogen, such as NO_x (i.e., NO and NO_2) and NH_x (NH_3 , NH_4^+), are released annually into the environment as a result of human activities, mostly from the use of synthetic fertilizers required for food production and as by-products of internal combustion engines; in contrast, the natural nitrogen fixation by lightning and soil microorganisms does not even reach 30% of such an amount [9]. Although reactive nitrogen compounds can be directly taken up by crops and plants in general, substantial amounts can linger in the environment, with noxious effects on human (and animal) physiology, in addition to being precursors for other forms of pollution, including particulate matter and ozone at the ground level [10,11].

Different maladies have been associated with specific forms of anthropogenic reactive nitrogen. For instance, nitrites (NO_2^-) in the water compete with oxygen in hemoglobin in young children leading to blue-baby syndrome [12]. In addition, direct exposure to gaseous nitrogen oxides has been associated with an increased incidence of respiratory illness in cities [13]. In turn, exposure to NH_3 and NH_4^+ has also been linked with respiratory illness and to various insulin and neurological disorders [14,15]. Nitrogen pollution can also favor the proliferation of vectors and disease agents of mosquito-borne diseases, as well as cholera outbreaks and toxic algal blooms [13].

In addition to the public health and economic consequences of nitrogen pollution, the anthropogenic alteration of the nitrogen biogeochemical cycle is one of the most important causes of biodiversity loss [16–18]. For instance, the deposition of reactive atmospheric nitrogen on the ground leads to soil acidification, which in turn releases ions of aluminum and manganese that are toxic at high concentrations, and to the eutrophication of water bodies that reduces available oxygen for aquatic organisms and can promote the proliferation of noxious organisms [19,20]. Plant herbivory is also stimulated by increased nitrogen deposition, leading, in some cases, to the explosive proliferation of agricultural pests, even in natural environments [21].

Given its biogeochemical importance, monitoring nitrogenous pollution can provide information on the rate and type of reactive nitrogen emissions, as well as on overall ecosystem integrity and air quality.

4. When Is Biomonitoring of Atmospheric Pollution Recommended?

The use of automated or manual stations that utilize electro-physical sensors and chemical analyses is the gold standard for measuring atmospheric concentrations of various compounds of interest. However, the deployment, operation, and maintenance of such systems are onerous and can be out of financial reach in some locations. For example, despite the fact that environmental regulations mandate that atmospheric pollution be monitored in Mexican cities with populations above 500,000, numerous municipalities lack monitoring networks or have stations that are non-operational due to a lack of personnel and other budgetary reasons [22,23].

Biological monitoring of atmospheric pollution has become an increasingly accepted practice for characterizing environmental pollution, including in situations where monitoring stations are lacking [24–26]. Biomonitoring is naturally occurring or transplanted organisms that respond in a quantitative manner to the prevalent levels of environmental pollutants [25,27]. Depending on their responses, they can be classified as accumulation or impact/effect biomonitoring [28]. The former can accumulate pollutants of interest in their tissues with a magnitude that is proportional to the ambient magnitude. In turn impact or effect biomonitoring are able to display specific ecophysiological responses that are also proportional to the prevailing environmental concentrations of the pollutant of interest. Lichens and plants have proved particularly useful for biomonitoring nitrogen deposition, and atmospheric pollution in general, owing to some of their biological characteristics. First, because they are sessile, i.e., they are attached to a substrate in a given site for the duration of their life, which allows them to integrate the environmental conditions over prolonged time periods and they are easily collected, especially when they are abundant in a region of interest [29]. Second, at least part of their nutrient acquisition is conducted directly from the surrounding atmosphere, either by gas exchange or by foliar (or thallus, in the case of lichens) absorption. Third, some of the chemical forms of nitrogen that are deposited as part of atmospheric pollution can actually be assimilated directly by the biomonitor, so that the measurement of ecophysiological processes can help characterize the extent of the surrounding pollution.

5. Guidelines for Biomonitoring Nitrogen Deposition: What, Who, and How

5.1. What: Pathways of Nitrogen Deposition

Anthropogenic reactive nitrogen emissions reach the ground via two pathways of deposition, dry and wet. Depending on the amount of annual rainfall in the region of interest, one or the other can be the most prevalent.

- **Dry deposition** is the process by which atmospheric pollutants, either gases or suspended particles, “fall” on different surfaces at the ground level by the direct action of gravity [30]. Additionally, gaseous compounds can be taken up directly by the vegetation through their leaf stomata [31]. Compounds such as ammonia (NH_3), nitric acid (HNO_3), and nitrogen dioxide (NO_2) make up part of the dry deposition;
- **Wet deposition** occurs when the reactive forms of nitrogen are “washed” from the atmosphere by water. Thus, they are deposited by being dissolved in the rain, fog, snow, or aerosols such as mist spray. It is mainly composed of ammonium (NH_4^+) and nitrate (NO_3^-) ions, but other chemical forms can also be deposited with water, including nitric acid, one of the components of acid rain [31];
- Finally, the sum of all of the nitrogen pollutants that are deposited through the dry and the wet pathways constitutes the **bulk deposition** [32,33].

5.2. Who: Suitable Organisms for Biomonitoring Nitrogen Deposition

Biomonitors have been utilized in various parts of the world to characterize nitrogen deposition, gaining special favor in the Northern Hemisphere (Figure 1; see particular studies in the Supplementary Material). Different species of lichens, bryophytes, and vascular plants, which take up reactive forms of nitrogen, either by their aerial organs or by their root systems, have been utilized to this end (Figure 2). Bryophytic biomonitors are the most frequently utilized in temperate regions, while successful biomonitoring has been conducted with epiphytes in the humid tropics, grasses in drylands, and woody plants in urban areas.

5.2.1. Lichens

Lichens are a symbiotic association between fungus and green algae or cyanobacteria. These organisms grow on rocks, trees, and other surfaces, where they take up nutrients directly from atmospheric deposition because they lack a cuticle [34,35]. Such a direct uptake can result in a deterioration of cell membrane integrity for sensitive species, which in turn leads to the disappearance of some species that have been documented in urban and other polluted areas [36–38].

For tolerant species, the direct uptake and assimilation of nitrogen deposition allow for accurate characterization of the prevalent pollution levels. However, the lack of a cuticle can also permit leaching of the accumulated nitrogen and other nutrients, the proportion depending on the intensity and frequency of precipitation events and the exposure of the lichen [39]. Because this may lead to seasonal fluctuations in the responses to nitrogen deposition, lichen biomonitors are best suited for characterizing dry deposition [25,40].

5.2.2. Bryophytes

Bryophytes, i.e., mosses, liverworts, and hornworts, are the most frequently utilized organisms for biomonitoring atmospheric pollution. Because they also lack a cuticle, they can readily take up and assimilate the prevailing nitrogen pollution, either by direct gas diffusion or when dissolved in water [41]. Similar to the case of lichens, because their tolerance to nitrogen deposition is species-dependent, the presence or absence of certain species can indicate the status of atmospheric pollution in a qualitative manner. Alternatively, the ecophysiological responses of tolerant species can help characterize the sources of reactive nitrogen emissions, the rates of deposition, and the spatial distribution of atmospheric pollution in large areas [42]. In fact, equations have been developed to estimate rates of nitrogen deposition based on the tissue nitrogen content for these plants [43–45]. A weakness of bryophytic biomonitors is that they can become physiologically inactive

during prolonged periods of low environmental humidity, such as the dry season in regions with distinct rainy and dry seasons. As a result, these biomonitors cannot absorb nor record any pollution during such periods of physiological inactivity.

Biomonitor organisms	Ecophysiological parameters				
	Bioindicator	Nitrogen content	Stable isotopes	Photosynthesis	Enzymatic activity
 Lichens					
 Bryophytes					
 Vascular epiphytes					
 Herbs					
 Woody plants					

Figure 2. Commonly utilized biomonitoring organisms and ecophysiological parameters that are responsive to the rate and type of atmospheric nitrogen deposition. The intensity of the cell color for biomonitor and parameter intersections is indicative of its relative frequency based on a review of the academic literature. See underlying studies in the Supplementary Material.

5.2.3. Vascular Epiphytes

Both lichens and bryophytes, which often grow on inert substrates, such as rocks, tree trunks, or built structures, have great biomonitoring potential because their mineral nutrition can be predominantly obtained from atmospheric deposition. However, their lack of a cuticle can seasonally limit their utilization and dampen the pollution signal during the rainy season. This limitation has been overcome by the use of epiphytic vascular plants, which also have predominant (and sometimes exclusive) atmospheric nutrition, but their adaptation to the extremely variable hydric environment of the epiphytic habit, allows them to be physiologically active throughout the year [25,46]. Indeed, the use of atmospheric bromeliads is amply documented in the American continent, especially plants of the genus *Tillandsia*, which occur from the southern United States to Argentina [47]. Two species, *Tillandsia recurvata* (the common name is ball moss) and *T. usneoides* (Spanish moss), are recognized as exceptional biomonitors of dry deposition, as they allow tracking the source and concentration of gaseous nitrogen pollutants throughout the year. In

contrast, their responses to wet deposition are weak, precisely as a consequence of their water-conservation and low-nutrient adaptations [25,48].

For parts of the world where tillandsias are not native, vascular plants with similar characteristics can be considered for biomonitoring, including, for example, epiphytic species in the Polypodiaceae (ferns) or Araceae (arum family) families, which are cosmopolitan [49]. Epiphytic orchids can also be considered for biomonitoring both dry and wet nitrogen deposition [40–52]. In addition to having ecophysiological characteristics similar to those of the tillandsias, some orchids produce pseudobulbs, which are nutrient and water reserve organs that develop every growing season, and are able to record and compartmentalize the prevailing nitrogen deposition over multiple years [52].

5.2.4. Herbs

Grasses and forbs obtain their nitrogen, and nutrients in general, primarily through their roots, which have access to both naturally fixed nitrogen and to nitrogen that reached the soil via wet deposition; in addition, these plants are able to take up gaseous and dry deposited nitrogen through their leaf stomata [53]. As a consequence of their ability to utilize nitrogen from various sources, identifying the effect of a particular signal on these rooted plants can be difficult. However, measurements of the tissue nitrogen concentration and isotopic composition for these plants have been shown to integrate different soil processes in response to increased nitrogen availability and still be able to detect vehicular emissions [54–56]. Herbs can thus make adequate urban biomonitors of nitrogen deposition because they occur spontaneously in disturbed environments and, in some cases, they are actually favored by the increased nitrogen availability that results from pollution [57,58]. Given their tolerance to environmental stress, even in arid regions, herbs have been successfully utilized to biomonitor nitrogen deposition, for instance, to contrast years and identify trends in the emission of N pollutants and urbanization intensity [49,55,56,59,60].

5.2.5. Woody Plants

Shrubs and trees also take up nitrogen from different sources, including from dry and wet deposition. Contrasting with herbs, many of which are ephemeral, the longevity of woody plants allows their utilization to biomonitor nitrogen deposition over many years. Indeed, the study of the nitrogen content and isotopic signals of their growth rings allows for the compilation of time series to determine trends in pollutant emissions [61,62]. Additionally, because these plants are common elements of cities, either because they were “tolerated” in the process of urbanization, because they were deliberately planted as ornamentals, or because their proliferation is favored by the urban environment, trees can provide a somewhat standardized means for biomonitoring nitrogen deposition across different cities [26,59,63,64].

Woody plants can thus be useful biomonitors of dry nitrogen deposition. For example, it has been estimated that these plants are able to assimilate up to 60% of the nitric acid intercepted by their canopies [65], whose source can be tracked by means of isotopic analysis of plant foliar tissues [49,66]. This is particularly important for semiarid regions where the dry deposition represents the largest portion of atmospheric pollution [26,64,67]. The nitrogen content of woody plants also responded to the rate of dry deposition. In particular, their nitrogen content increases with the proximity to highways or industries [59,66,68,69].

Woody plants are also useful biomonitors of wet deposition, with their root uptake of nitrogen from the soil solution. The most straightforward responses are their nitrogen content, which has been found to increase with the rate of atmospheric deposition, and their isotopic status, which can reflect the source of pollution [63]. In addition, the nitrogen isotopic status of trees and shrubs can be utilized for determining nitrogen saturation in different ecosystems [70], the process by which chronic nitrogen deposition alters different ecosystem processes [71,72].

5.3. How: Ecophysiological Parameters That Respond to Nitrogen Deposition

Various ecophysiological parameters can be measured to determine the response of biomonitor species to atmospheric nitrogen deposition (Figure 2). The most adequate suite of techniques to be utilized should be chosen in consideration of the lifeform of the biomonitor, the environmental conditions of the region of interest, and the laboratory and material resources available.

5.3.1. Responses of Bioindicator Species

The most straightforward use of biological organisms for characterizing environmental pollution is based on the presence (or disappearance) of certain species in a region of interest [28]. Lichens have been widely utilized as qualitative bioindicators for identifying the increasing effects of environmental pollution, including those from nitrogen deposition [27,34]. Considering that multiple factors can lead to the local disappearance of species, care should be taken when interpreting the results of bioindicator surveys, as factors such as climate, city greenery maintenance protocols, and the presence of pollution emission sources, need to be considered. For such reasons, it is recommended that bioindicator surveys be conducted periodically, each year, for example, in order to identify changes in the species distribution and abundance as they respond to pollution.

5.3.2. Nitrogen Concentration

Because nitrogen is an essential nutrient and its baseline “natural” availability is rather low, plants readily take up available nitrogen and incorporate it into their tissues. For this reason, when nitrogen deposition increases, so does the total concentration of this element in the tissues of biomonitors. Total nitrogen concentration in plant tissues (measured as the percent of dry weight) can be as low as 0.5%, but it has been measured to be twice as high for plants growing where nitrogen deposition is substantial [44,50,73]. In addition to its ecophysiological usefulness for biomonitoring, measurements of tissue nitrogen concentration are rather inexpensive and are performed by many analytical environmental laboratories internationally.

5.3.3. $\delta^{15}\text{N}$

The proportion of ^{15}N present in biomonitor tissues can reflect the predominant form of pollution in a determined area and the nitrogen saturation status in an ecosystem [25,26,70].

The different sources of nitrogenous compounds have specific $\delta^{15}\text{N}$ values, which are traceable in the vegetation from different environments. In general terms, oxidized nitrogen compounds from emissions in urban environments tend to be positive. For example, NO_x and NO_3^- can reach $\delta^{15}\text{N}$ values of 26‰ and 15‰, respectively [74,75]. These values contrast with those of the natural environments that tend to be negative, but close to zero [76]. In turn, reduced nitrogen compounds, from both urban and rural environments, have very negative values, reaching −56‰ for NH_3 and −15‰ for NH_4^+ [77,78].

With respect to the saturation state of the ecosystems, the $\delta^{15}\text{N}$ values for plants of natural environments are close to zero. However, as the rate of bulk nitrogen deposition increases, the natural nitrogen reactions of soil are subjected to profound alterations, on one hand, owing to a loss of soil microorganisms associated with the nitrogen biogeochemical cycle, and on other hand, because the nitrogen losses increase. As a consequence, the $\delta^{15}\text{N}$ values of terrestrial plants become positive, a value that is a conspicuous indicator of nitrogen saturation [25,70].

5.3.4. Photosynthesis Related Parameters

Pigments are essential components of several steps and processes of photosynthesis and plant metabolism. An effect of increased nitrogen availability is an increased pigment concentration, especially chlorophyll [79]. Measuring chlorophyll content by colorimetry in the laboratory is quite straightforward and various portable optical instruments, which are rather inexpensive, are available for non-destructive measurements of chlorophyll content

in the field [80,81]. Additionally, changes in the chlorophyll *a/b* ratio have been shown to fluctuate in response to increasing nitrogen deposition, and the concentrations of accessory pigments, particularly those from the xanthophyll cycle, can indicate the plant sensitivity to different pollutants [50,79,82]. The development of portable instruments that measure chlorophyll fluorescence, has enabled field measurements of plant photosynthetic activity, which has proven to be most sensitive to nitrogen deposition [50,79,83].

5.3.5. Enzymatic Activity

Measurements of activity for enzymes related to nitrogen metabolism have been useful in quantitative and semiquantitative biomonitoring of atmospheric deposition [48,79]. Nitrate reductase, for instance, is the first enzyme involved in nitrogen assimilation, as it reduces the nitrates taken up from the soil solution into nitrites [84]. For this enzyme, an increase of available nitrogen, such as that resulting from atmospheric deposition, increases its activity [48,85,86]. However, after a certain nitrogen concentration, this enzyme becomes saturated, so a decrease in its activity has been observed in highly polluted sites [85,87].

Phosphomonoesterase is another important enzyme whose activity increases in response to nitrogen deposition, being able to tolerate higher levels of pollution than nitrate reductase [48,79,88]. The activity for this enzyme increases with atmospheric deposition as a plant response to a phosphorus limitation that results from the excess nitrogen in the soil [89]. Enzymatic activity for these enzymes can be measured colorimetrically in the laboratory for fresh plant tissue samples.

6. Policy Perspectives

- Implementation of biomonitoring in localities where automatic stations are not available can help local governments assess whether the human right to a clean environment is being fulfilled.
- The distribution and abundance of conspicuous bioindicator species, especially those with ample geographic distributions, lend themselves to recruit citizen scientist volunteers, who can help create participatory maps of environmental quality in regions of interest.
- Suitable biomonitoring species of different lifeforms can be identified in regions of interest that are relatively abundant and tolerant to the prevailing levels of pollution. The specific parameters to be measured are dependent on the available resources.
- Caution needs to be exercised when selecting biomonitors. Increased rates of nitrogen deposition seem to favor, in some cases, the proliferation of potentially invasive species. While species such as buffelgrass [60] and the castor bean [59] can be excellent biomonitors, they are quite noxious invasive species, so their introduction to new sites is never recommended.
- Because biomonitors can take up various chemical compounds that comprise atmospheric pollution, in addition to those from nitrogen deposition, their adoption can help in an integrated characterization of environmental quality, including responses to various criteria pollutants and other contaminants of concern in each region of interest.

Supplementary Materials: The following are available online at <https://www.mdpi.com/article/10.3390/nitrogen2030021/s1>, Figure S1: PRISMA flow diagram for the systematic literature review conducted for biomonitors of nitrogen deposition, Figure S2: Number of studies identified in the literature review that utilized different biomonitor lifeforms (a) and total number of species per lifeform reported in the literature (b), Table S1: Biomonitors of nitrogen deposition identified in the systematic literature review, which includes references [90–124].

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