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Article

Fitness-for-Purpose of Reactive Nitrogen Monitoring Methods in Ecosystems: A Multi-Faceted Comparative Assessment

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Abstract

Anthropogenic reactive nitrogen (Nr) production now greatly exceeds natural creation, generating cascading environmental impacts on the environment and human health. Effective monitoring of Nr in ecosystems is essential for early warning and policy response, yet the landscape of available monitoring methods is wide and heterogeneous, varying substantially in accuracy, purpose, cost, and ecological scope. This study evaluates nineteen Nr-monitoring methods, grouped into chemistry-based (CM), biodiversity-based (BM), and transplant-based (TM) methods, against 36 fitness-for-purpose (FFP) indicator facets. Facets are organized into intrinsic (direct measurement of nitrogen cycle components), projected (ecological effects the method can indicate), and extrinsic (metaproperties such as cost or precision) types. Scores are derived from 89 core papers, ranked using evidence-normalized weighted-sums (WS) and non-metric multidimensional scaling (NMDS) approaches. CM generally outperform BM and TM, with critical loads, total tissue nitrogen, and nitrogen isotopes leading overall. Lichen diversity, ectomycorrhizal fungi, and Ellenberg's N lead among BM, particularly for projected facets. The NMDS reveals a structural divide and monitoring complementarity between CM and BM, with CM serving as early warning indicators whereas BM integrate cumulative, persistent ecosystem impacts. While no single method seems adequate for comprehensive monitoring, the FFP matrix facilitates selecting optimal monitoring combinations.

Keywords: reactive nitrogen; monitoring; biomonitoring; methodologies; fitness-for-purpose

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1. Introduction

Human activity has fundamentally altered the environment, transitioning the planet into a new geological epoch often referred to as the Anthropocene [1]. In particular, the global nitrogen cycle has been heavily impacted. Global production of reactive nitrogen has risen to approximately 203 Tg N per year, a value that vastly exceeds natural baselines [2]. This surge is largely attributed to two distinct anthropogenic drivers: the Haber-Bosch process, which converts inert atmospheric nitrogen (N_2) into ammonia (NH_3) for agricultural fertilizer, and high-temperature combustion processes in transport and industry that oxidize atmospheric nitrogen into nitrogen oxides (NO_x) [3]. While this anthropogenic intervention has supported population growth through intensified agriculture—sustaining many more people than natural nitrogen availability would likely sustain, estimated at nearly 3.87 billion people ([4])—it has generated environmental challenges that threaten ecosystem stability on a planetary scale.

Climate change is expected to exacerbate these processes further. For example, ammonia (NH_3) emissions, typically associated with agricultural manure and fertilizer application, are highly temperature-dependent [5]; volatilization rates increase exponentially with ambient heat. Consequently, global NH_3 emissions are projected to rise significantly from 65 Tg N yr^{-1} in 2008 to 93 Tg N yr^{-1} by 2100 due solely to projected temperature increases of 5°C [6]. When combined with the increased livestock demand anticipated for a growing global population, these emissions have been projected to reach 135 Tg N yr^{-1}[6].

The environmental consequences of this excess nitrogen are severe and multifaceted, often described as the "nitrogen cascade" [7]. This concept implies that a single atom of reactive nitrogen can cycle through the environment, causing sequential effects from driving ozone formation in the troposphere to interfering with then depositing into soil interfering with biological nitrification processes in the soil, and finally leaching into waterways to drive eutrophication. Ammonium's toxicity may displace sensitive lichens and mosses, while on the other hand, excess nitrogen may also affect biodiversity by competitively promoting nitrophilous plants growth and area expansion, encroaching other plants. A 2011 study by Ariño et al. showed that, in Spain, the ratio of nitrophilous plant occurrence records has steadily increased over the XXth-century, more than doubling their presence in herbaria [8].

In terms of public health, exposure to nitrogen compounds is linked to chronic respiratory diseases, including asthma and premature mortality driven by fine particulate matter ($PM_{2.5}$) formed from atmospheric ammonia and NO_x [9]. Furthermore, groundwater contamination leads to specific acute conditions like methemoglobinemia ("blue baby syndrome"), where nitrate ingestion interferes with the oxygen-carrying capacity of hemoglobin in infants, posing a fatal risk in agricultural regions [10–12]. Keeping NO_2 under the WHO air quality guideline levels would prevent over two thousand new cases of pediatric asthma in Western Europe [13]. In the EU, an increase of 10 $\mu g \cdot m^3$ in air would result in a 5.5% increase in mortality rate, and over fifty thousand premature deaths could be attributed to exposure to NO_2 in Europe [14].

The increase in deposited reactive nitrogen has therefore become an important challenge both in environmental and human health terms, either directly or indirectly through interplay with climate change. The consensus in a recent multi-agency guidance report resulting from an extensive consultation to practitioners highlights the complexity of the nitrogen impacts on human health, environment, and structures due to the variety of N cycle governing factors, reactions, and exposures [15].

To mitigate these risks, effective monitoring is essential. Physical sensors can measure air concentrations and soil deposition, but the ecological impact must be assessed from a variety of additional parameters (often biological) that respond to the upset of the cycle. On the other hand, bioindicators offer a practical, biologically relevant tool to assess nitrogen loads, atmospheric concentrations, and valid biological effects as they integrate the effect of the nitrogen loads in the ecosystem's components. Monitoring reactive nitrogen may offer a way to assess, and might eventually help correct, these loads before irreversible damage occurs.

However, there are several monitoring methods, varying wildly in terms of accuracy, purpose, practicality, costs, and other characteristics. When used in environmental studies, ascertaining these characteristics helps defining their fitness-for-purpose (FFP): whether a reactive nitrogen assessment method can potentially achieve the goal sought, including producing the information relevant to the study and the context to interpret it [16].

A comprehensive literature review successfully evaluated the biomonitoring of atmospheric deposition based on five types of organisms (lichens, bryophytes, vascular epiphytes, herbs, and woody plants) across five methods or parameters (bioindicator value, nitrogen content, stable isotopes, photosynthesis, and enzymatic activity), and advocated for the use of biomonitors as an affordable alternative when other systems or methods might not be adequate or cost-effective [17]. More recently, a very comprehensive global assessment of N impacts on humans and the environment, largely based on critical loads, provided critical guidance for the development of monitoring programmes and strategies at local and global scales [15]. We followed a comparable approach but for the wider spectrum of monitoring methods, cases, and aspects and with a different,

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formally-defined scoring system not restricted to organisms that also considers existing methods not covered in previous reviews.

Our aim is to provide further context to inform about the fitness-for-purpose of Nr assessment methods for environmental monitoring, by pitching them to a variety of characteristics, aspects, features, or parameters (hereinafter “facets”) that could become relevant in their selection, based on a critical review and meta-analysis of a representative sample of published research.

2. Materials and Methods

In 2023 we conducted structured searchers in Web of Science and Google Scholar for highly-cited references dealing with methods to assess and monitor reactive nitrogen in ecosystems over the previous two decades (e.g. [6,18,19]), snowballing them through citations to over 400 unique references. Of these, references that did not provide adequate methodology descriptors or clearly measurable effects were discarded, and a sample of 89 core references positively reporting at least one method in an unambiguous context of reactive nitrogen monitoring remained for full analysis (see Supplementary Material 1). For each selected paper, data was collected on which method(s) were used and in what context. The methods were pooled, and each one was then evaluated for three types of facets:

- **Intrinsic** facets (IF) targeting the actual aspects of the N cycle that the method is designed to measure, assessing specific stocks, flows, or concentrations of various Nr forms (e.g. deposition, fixation, cycling, atmospheric concentration);
- **Projected** facets (PF) informing the biological efficacy and environmental effects of Nr, for example the method's sensitivity to detect subtle changes in species composition and its ability to statistically correlate deposition rates with declines in species richness; and
- **Extrinsic** facets (EF) focusing on the methods’ usability, availability, or popularity (e.g. cost of implementation, availability of historical data baselines, or frequency of updates)—i.e., transversal concepts that can be applicable to any methodology being analyzed.

While intrinsic and projected facets are more directly related to FFP, indicating their suitability for the intended task, extrinsic facets were also taken into account, following Hamilton et al. (2022) criterion of taking into account practicality in addition to accuracy or reliability, as an indirect measure of their fitness through their actual usage scope [16].

For each facet, a three-level score (low-, medium-, high-) was established based on the literature review to assess each method’s suitability to determine that specific facet (Table 1). Levels were established either by analyzing whether and how the methods would cope with, or be able to, assess a facet in detail, often quantitatively, or have actually been used to assess a facet (frequency of use in the literature).

For each facet we proposed specific conditions for a method to be included in each level based on the literature analysis, frequency of use, or type of evidence. A high suitability means that the method is adequate to evaluate that facet, while a low suitability indicates that the method is either unable or generally inadequate to provide a correct evaluation. A medium suitability still enables the method to assess the facet although in a limited way.

Comparable, additional, or more recent cases supporting the suitability were navigated by using Consensus AI for Research (consensus.app, Pro version, April 2026) [20] by issuing the generic prompt “*Find recent references supporting or discussing the use of [method] to measure|assess [intrinsic facet|projected facet] in the context of reactive nitrogen in ecosystems*”. Supplementary Material 2 systematically describes specific details leading to the selection of each facet and provides literature evidence.

Table 1. Facets used to evaluate each method across the literature corpus. Red: intrinsic (IF); blue: projected (PF); gray: extrinsic (EF). See Supplementary File 2 for details.

	Suitability level

Facet	Assessment criterion	Low	Medium	High
atmosphere	Ability to assess reactive nitrogen presence in the atmosphere	generic detection	qualitative identification	quantitative
deposition	Ability to assess the direct transfer of Nr from the atmosphere to the biosphere, soil, and waters	n/a	indirect or ordinal	quantitative
fixation	Ability to assess nitrogen fixation to mobilizable stocks	n/a	either fluxes or stocks measured	fluxes and stocks measured
health	Ability to assess how well the method measures Nr concentrations whose pathological effects have been established	indirect relation with morbidity	qualitative relation with morbidity	correlation with morbidity
limit	Ability to measure exceedance of critical loads	n/a	binary	quantitative
lifetime	Ability to calculate half-lives of nitrogen compounds emitted to the atmosphere	n/a	half-lives ordered	half-lives quantified
reaction	Ability to assess short-lived Nr (NO, NO_2, NO_3^-)	generic detection	qualitative identification	quantitative
recovery	Ability to assess ecosystem recovery after nitrogen deposition decline	n/a	indirect or ordinal	quantitative
reservoir	Ability to assess mobilizable and immobilizable Nr stocks	n/a	quantitative (immobilized) or qualitative	quantitative (mobilizable)
soil	Ability to assess Nr presence in soil	generic detection	qualitative identification	quantitative
source	Ability to measure the inflow of Nr entering the ecosystem	source(s) identified	single source quantified	multiple sources quantified
acidity	References reporting the method as a diagnostic tool to assess ecosystem acidification	<10%	10-50%	>50%
aqu	References using the method as an indicator of air quality reduction	<10%	10-50%	>50%
biomass	References reporting the method for biomass change assessments	<10%	10-50%	>50%
capture	References reporting the method in CO_2 capture assessments	<10%	10-50%	>50%
cycle	References reporting the method in quantified N cycle assessments	<10%	10-50%	>50%
eutrophy	References reporting the method in eutrophication risk assessments	<10%	10-50%	>50%
fire	References using the method as an ecological indicator of wildfire risk	<10%	10-50%	>50%
ghg	Method's relationship with assessment of greenhouse gasses (GHG) changes (other than N_2O)	n/a	indirect	direct
nitrous	References using the method to report atmospheric N_2O (as a GHG)	<10%	10-50%	>50%
ozone	References reporting the method as a factor in tropospheric or stratospheric O_3 assessments	<10%	10-50%	>50%
pests	References reporting the method in pathogen and pest proliferation	<10%	10-50%	>50%
richness	Method's relationship with species richness changes in communities	potential	qualitative	quantitative or ordinal
sequester	References reporting the method in carbon sequestration estimates	<10%	10-50%	>50%

shift	Method's relationship with species composition shifts in communities	potential	qualitative	quantitative or ordinal
toxicity	References reporting the method as a factor in Al, Mn leaching assessments	<10%	10-50%	>50%
data	Instances of use of a method to diagnose nitrogen effects at the ecosystem level*	<50	50-100	>100
update	References using the method within the last 5 years of the sample	<5%	5-10%	>10%
relevant	References citing the method	<5%	5-10%	>10%
cost	Relative resource requirements (field equipment, expertise, laboratory analyses)	heavy	medium	low
precision	Range of possible values a method can express when measuring effects	binary	<30	>30
scale	Type of measurement scale the method can provide	nominal	ordinal	quantitative
climate	Number of the 11 Köppen climate types the method has been used in	<3	3-5	>5
time	How long the method requires to produce reliable, high-precision results	>1 yr	1 mo – 1 yr	<1 mo
location	Breadth of places (from sites to biomes) the method can be applied to	narrow	medium	wide
kingdom	Biological kingdoms where biomonitoring has been identified	1	2 - 3	≥ 4

The resulting matrix of method-facet scores was seriated [21] both for rows and columns in Excel by calculating a evidence-normalized weighted sums [22] with nonevidence penalty as

$$WS_i = \frac{(L_i + 2M_i + 3H_i)(E - N_i)}{3E^2}$$

where for each *i* method (or facet) out of *E*, the number of high (*H*), medium (*M*), low (*L*), and no evidence (*N*) facets (or methods) was computed, both overall (*WS*) and within method and facet groups (WS_f).

Relative differences between methods based on the facet scores were found by non-metric multidimensional scaling (NMDS) [23] based on the Gower distance index [24] using PAST v.4 [25].

3. Results

3.1. Identified Methods

We identified nineteen methods directly relevant to reactive nitrogen monitoring in ecosystems in the literature sample (Table 2). These could be categorized into three distinct functional groups, each offering a different "lens" through which to assess reactive nitrogen in the context of environmental studies:

1. **Chemistry-based Methods (CM),** involving either a direct measurement of nitrogen or nitrogen forms contents in the environment, or analyzing specific physiological responses within organisms to gauge nitrogen stress such as e.g. critical loads, tissue nitrogen, enzymatic activity, isotopic analysis (nine methods);
2. **Biodiversity-based methods (BM),** where shifts in the community structure, species composition, or ecological factors attributable to nitrogen loads are observed over time, such as Ellenberg indicator values or diversity surveys (nine methods); and
3. **Transplant- based methods (TM),** an experimental approach involving moving native or standardized organisms (often sensitive lichens like *Usnea* or mosses like *Sphagnum*) from clean "background" environments to polluted sites and measuring stress indicators (e.g., membrane integrity) over a defined exposure period (two methods).

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Table 2. Methods described in the analyzed core literature sample (n=89). Pr.: Prevalence of the method in the literature sample.

1. Chemistry-based methods (CM)

Abbr.	Method	Description	Target	Ref.	Pr.
AAS	Amino acids	Analysis of free aminoacid contents in tissues	N status	[26]	8%
CFL	Chlorophyll fluorescence	Measurement of photosystem II efficiency	N stress	[27]	7%
CRL	Critical loads	Deposition thresholds below which significant harmful effects on sensitive ecosystem elements do not occur	N deposition	[28]	36%
CNR	C:N ratio	Ratio of carbon to nitrogen in soil or organic matter	N availability	[29]	27%
EZA	Enzyme activity	Measurement of key enzymes	N assimilation	[30]	22%
SGF	Gas fluxes	Measurement of N-containing gas emissions (N_2O, NO_x, NH_3) from soil	N emissions	[31]	8%
NIS	Nitrogen isotopes	Use of stable ($^{15}N/^{14}N$) or radioactive isotopes to trace N	N cycle	[32]	20%
NPR	N:P ratio	Foliar or tissue N:P molar ratio	N limitation	[33]	18%
TNT	Total nitrogen in tissues	Measurement of total N concentration in plant or lichen tissue	N availability	[34]	25%

2. Biodiversity-based methods (BM)

Abbr.	Method	Description	Target	Ref.	Pr.
ELN	Ellenberg's N	Indicator values for Central European vascular plants on a 9-point N scale	N availability	[35]	25%
EMF	Ectomycorrhizal fungi	Changes in diversity and composition of ectomycorrhizal fungal communities	N deposition	[36]	11%
FNS	Functional Nitrogen Index for Species	An index for forest vascular plants	N mineralization	[37]	5%
INR	Invertebrate response	Use of invertebrate community composition (e.g. soil fauna)	N enrichment	[38]	7%
LAN	Landolt	Ecological indicator values for the Swiss flora	N as nutrient	[39]	8%
LDV	Lichen biodiversity	Use of epiphytic lichen diversity and community composition	N exposure	[40]	10%
TDP	Tsyganov, Didukh & Plyuta	Ecological indicator values for the flora of Russia and Ukraine	N as nutrient	[41,42]	3%
ZAR	Zarzycki	Ecological indicator values for vascular plants of Poland	N availability	[43]	1%

3. Transplant-based methods (TM)

Abbr.	Method	Description	Target	Ref.	Pr.
LTR	Lichen transplants	Transplanting lichens from pristine to polluted sites	N exposure	[44]	3%

PTR	Plant transplants	Transplanting either laboratory-standardized or locally collected native material across N gradients	N exposure	[45]	5%

The prevalence in the analyzed literature ranged from 1% (one reference using the Zarzicky ecological indicator values) to 36% for the Critical Loads method. The biochemistry-based methods were used almost twice as often (85%) as the biodiversity-based methods (45%), while six transplant experiments were identified (7%).

N availability was the focus in 60% of papers, while 47% dealt with N deposition. Other targets identified were assimilation (22%), N cycle (21%), limitations (18%), exposure (15%), N as nutrient (11%), N status and emissions (8% each), stress or enrichment (7% each), and N mineralization (5%).

3.2. Classification and Ranking

Figure 1 shows the methods seriated according to their overall fitness as measured by evidence-normalized weighted sum (WS) of facets' suitability scores, separately for each facet type (intrinsic, projected, extrinsic). Unsurprisingly, most chemistry-based methods (CM) scored higher overall than biodiversity- or transplant-based methods, and the facets most strongly associated to them were extrinsic or intrinsic facets. Projected facets drove a more mixed seriation among method types. Critical Loads (CRL) was the CM more consistently ranking high across intrinsic and projected facets, while lichen diversity (LDV) and ectomycorrhizal fungi (EMF) were the fittest BM overall but particularly for projected facets. Ellenberg's N (ELN) was the most extrinsically-faceted fit BM. Transplant-based methods (TMs) scored higher for intrinsic facets than for projected or extrinsic facets.

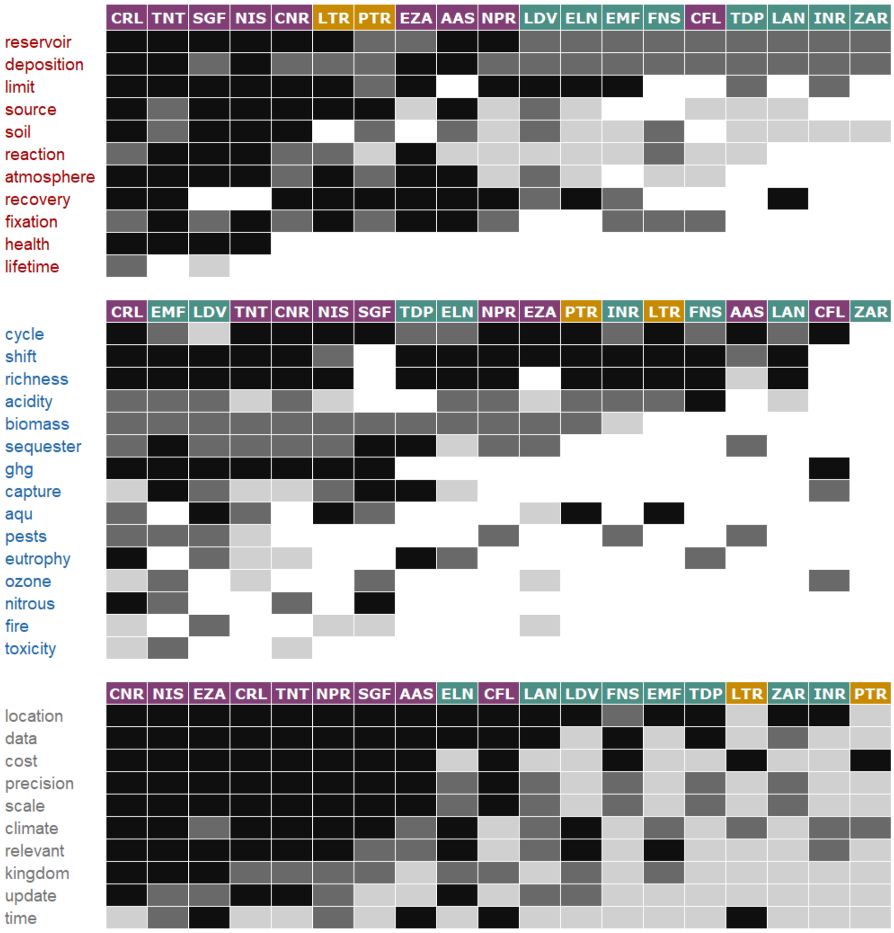

Figure 1. Table of methods (columns) and their facet (rows) scores: high (black), medium (dark gray), low (light gray), seriated by evidence-normalized weighted sums from top to bottom separately by facet type, and left to

right within each facet type. Blank cells: no evidence about the facet for the method across the literature sample. Methods: chemistry-based (purple), biodiversity-based (teal), transplant-based (gold). See abbreviations in Table 2. Facets: top: intrinsic, middle: projected, bottom: extrinsic.

The intrinsic facets *reservoir* and *deposition* were present in all methods, while all other facets were missing from at least three methods each. Among the projected facets, *cycle* was present in all by one method (ZAR) and *shift* and *richness* in all but three; all other facets were more limited.

Being independent from the methods' designs, all extrinsic facets could be evaluated for all methods but the intrinsic facets *reservoir* and *deposition* were the only ones that all methods could assess to at least some degree. Among the projected facets, only *cycle* was common to all methods but one, and *shift* and *richness* were common to more than 80% of methods. The facets *nitrous*, *fire*, and *toxicity* could only be assessed by one BM each.

The group-specific WS_f of methods apportioned among the three types of facets is shown, ordered, in Figure 2. The apportioning procedure attributes WS separately to each of the three facet types, and renormalizes it to the 0-1 interval for the addition of the three, resulting in a slightly different ranking. As compared with the overall seriation, CM scored better than BM or TM. While the top CM trio was identical, CRL scored better than TNT or NIS. Among BM, LDV was also best but EMF and ELN exchanged positions. The main driver of the change in ranking was the set projected facets, with extrinsic facets having a lesser discriminating role.

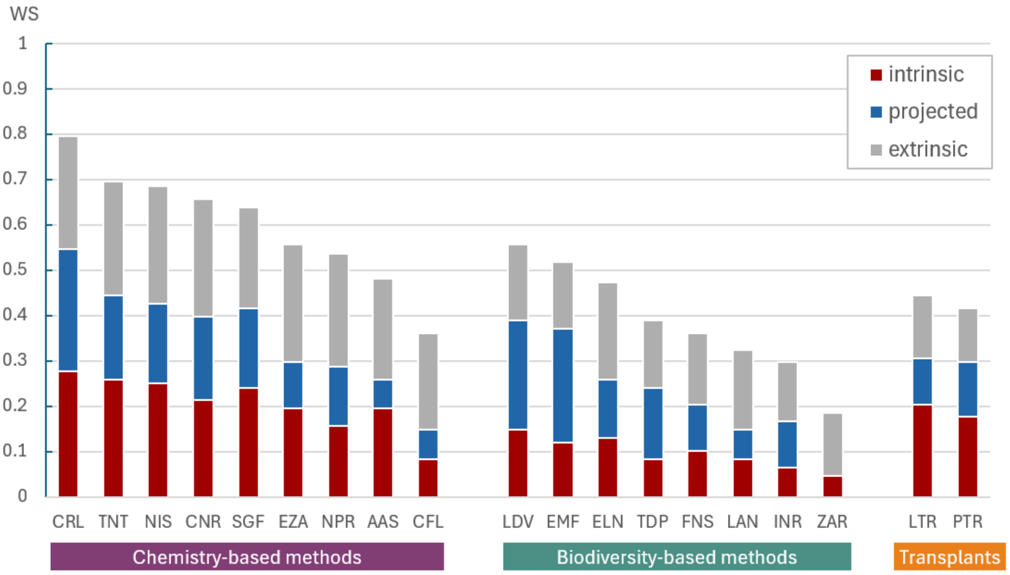

Figure 2. Methods ordered by type and evidence-normalized weighted sum (WS), where WSs have been calculated separately for each group of facets (intrinsic, projected, extrinsic) and apportioned proportionally to the number of facets in each group.

Relationships between methods are represented in Figure 3 as their coordinates in the first two factors of the NMDS based on Gower distances, accounting for 61.1% of the variance in 2D space. There is a clear divide between CMs and BMs mostly along the main variation factor (axis I, which largely reproduces the seriation). TMs occupy an intermediate position, with LTR lining up with CMs and PTR with BMs. The second source of variation (axis II) is the main driver that separates three methods from the rest: CLR, LDV, and EMF, with CFL coming apart at the opposite side. This axis seems more related with the number (as opposed to the value) of the facets that could provide evidence in each method. Ellenberg's N (ELN) is the BM that has the closest relationship with CMs overall.

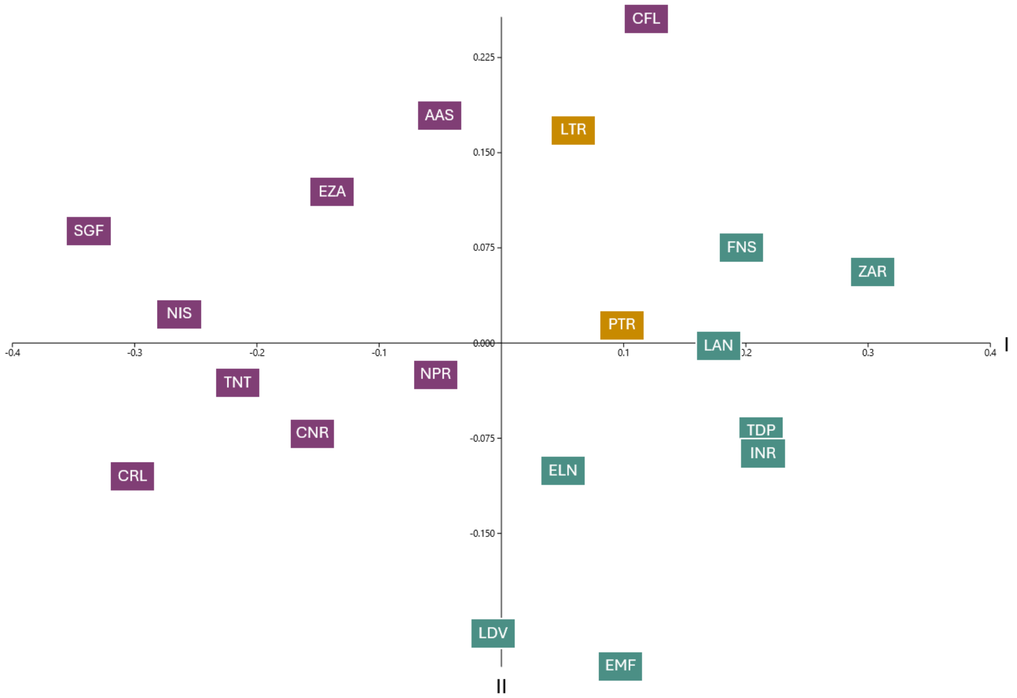

Figure 3. Non-metric multidimensional scaling (NMDS) of the method-facet matrix showing the Gower distance for methods. See abbreviations in Table 2. Stress = 0.2, $R^2 = 0.55$. Purple: CM; teal: BM; gold: TM.

4. Discussion

4.1. Facets

When used as monitoring tools, the nineteen methods we identified in the literature sample had varying degrees of relevance and a variety of limitations, arising from their nature or application fields that offered facets amenable to analysis. While some of these facets thus arose naturally from the literature review, the final roster was the result of extensive discussions, the examination of the nature of the problem at hand (systematize what the methods were good for), and general considerations about the fitness-for-purpose concept. Some facets were relatively straightforward as FFP components, and for them we proposed relatively simple scores (Table 1), even though for some of these we have defined arbitrary thresholds to attribute “good” or “medium” fitness to a method that, nonetheless, strived to maintain a consistent set of cutoffs across all facets. However, scoring tiers for other facets are less amenable to quantification and their selection rationales are more reliant on direct or indirect evidence found across the literature corpus of the use of the method to assess the facet.

The choice to separate facets into intrinsic, projected and extrinsic may also introduce some bias. While we attributed identical weight to each facet, the groups themselves were uneven. We compensated somewhat by apportioning equal weights to the groups for the classification of methods by type of method. Other researchers may want to attribute different weights to facets, and that should certainly cause a different seriation [21]. However, we believe that the separation by facet type does offer additional insight. For example, the intrinsic facets dealt mostly with **components** of Nr and their cycles, while the projected facets focused on the ecosystem **effects**. It is thus natural to pitch both types of facets and observe which intrinsic components seem most associated with what projected effects on the ecosystem. Tabulating all possible pairs between intrinsic and projected facets across the literature sample, some projected facets were more often associated than not with intrinsic facets: *cycle* co-occurred in 85% of the instances with *deposition*, 82% with *reservoir* and 56% with *soil*, while shift co-occurred in 67% of instances with *deposition* and 58% with *reservoir* (Figure 4.)

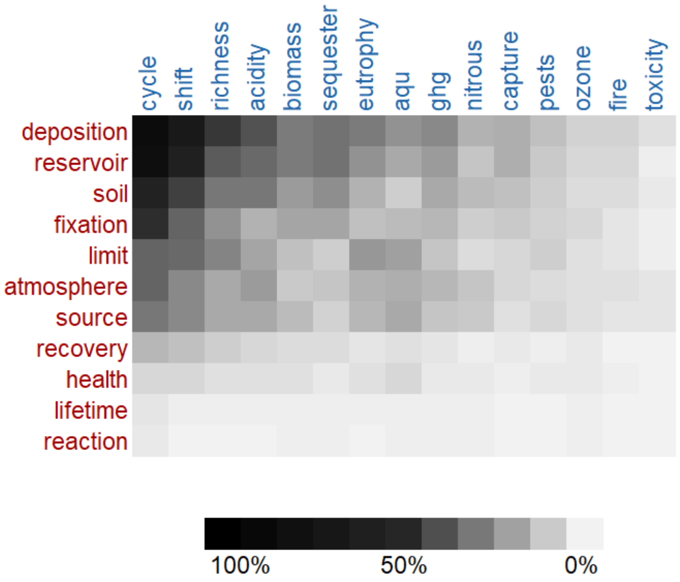

Figure 4. Co-occurrence of projected (columns) and intrinsic (rows) facets across the literature sample, as percentage.

The two decades covered by the literature sample also revealed a shifting pattern (Figure 5). The bulk of the literature centered around 2017-2019 analyzed intrinsic facets, whereas the projected facets were spread further over the period, with a higher fraction of papers covering intrinsic facets than projected facets. Among the projected facets, the shift for the most prevalent facets evolves from *eutrophy* and *acidity*, two sharply delimited and often linked ecosystem issues [46–48], to the more generic facets *cycle* (changes in the nitrogen balance and flow), *shift* (change in the species composition), *richness* (change in the biodiversity), possibly reflecting a corresponding shift in the research priorities and perceived risks in the scientific community (e.g. [49–51]).

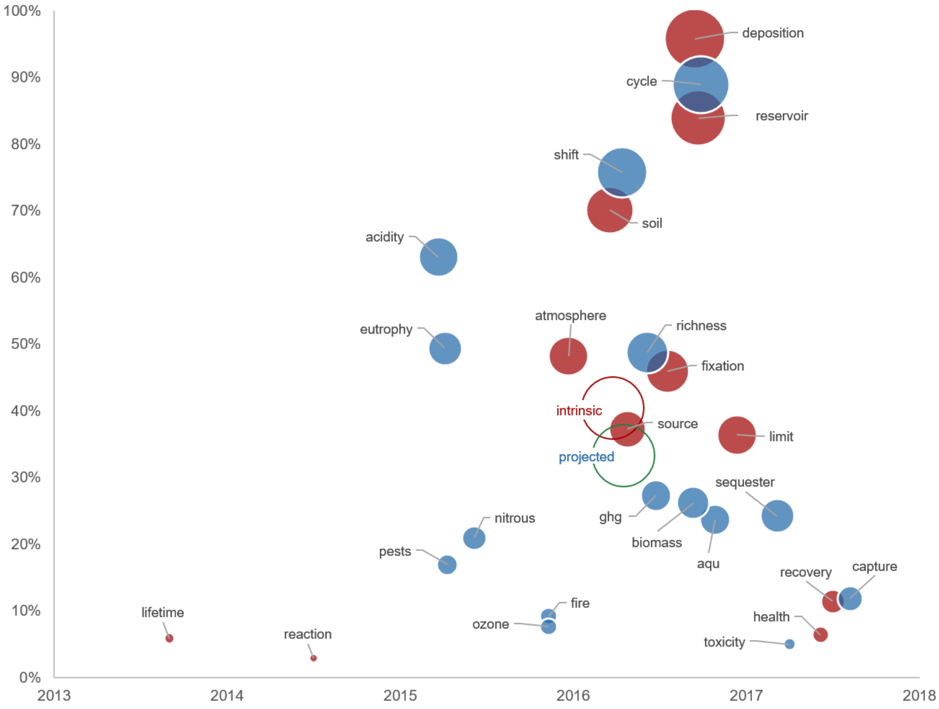

Figure 5. Evolution of facets in the analyzed literature over the two decades 2010-2020. The coordinates of each facet represent the centroid of the publication years of the articles where the facet appeared (horizontal axis) and

the fraction of papers where it appears (vertical axis). Symbol areas correspond to the total number of instances. The hollow circles represent the centroids for the ensemble intrinsic and projected facets.

4.2. FFP

The assessment of stocks, deposition, nitrogen cycle aspects, and changes in the community structure (richness and species composition shifts) are the intrinsic and project facets that most methods seem relatively well-suited to determine. However, these two types of facets are best served by different categories of methods.

CMs perform well for determining the origin of nitrogen, although they vary in their capacity to distinguish specific atmospheric chemical species and therefore their monitoring fitness may be biased. Physical indicators demonstrate that while total tissue nitrogen (TNT) reliably signals general enrichment [52], it responds unevenly to different chemical inputs, often prioritizing nitrate and reduced ammonium in wet precipitation over dry gaseous forms and not distinguishing species [52–54]. The cost of reducing nitrate favors assimilation of already-reduced nitrogen (ammonia, organic compounds) by mosses, which may directly alter EZA and AAS readings. Conversely, SGF can pinpoint fine parameters like the soil compensation point for gaseous ammonia, though ambient temperature variations can alter readings by an order of magnitude [53,55]. Short-lived reactive radicals (NOx) are best captured via tissue concentration kinetics in sensitive lichens [56], which show linear uptake curves within weeks.

On the other hand, BMs monitor the effects of Nr in the ecosystem, but this effect may be affected by multiple factors often working in opposite directions, e.g. the time lag of the ecosystem response (the endpoint, as opposed to the chemically-characterizable midpoint [57]) and the persistence of the effects. While tissue-based CMs (TNT, AAS, NPR) can show the incorporation and washout of compounds quickly and as they happen, usually within weeks to months, some effects induce cascades at the community level that only BMs can observe [58]. ELN and FNS look at the plant floristic profiles, and these can be affected by persistent competitive exclusion by nitrophilous species that can last years or decades [59]. Ectomycorrhizal fungal (EMF) sporocarp production can normalize within 5 years, underlying soil mycelial community profiles remain altered for decades [60]. A Increased plant vulnerability to herbivorous insects and fungal infections due to a past history of high tissue nitrogen no longer detectable through TNT or AAS could be revealed by INR [61]. In that sense, BMs act as accumulators and registers of Nr effects long after the deposition took place and are a best fit to reveal the past history of ecosystem impact.

Several methods can also be used to indirectly monitor other environmental stresses, and their fitness might change by use in combination. For example, tropospheric ozone accelerates leaf aging (a CFL target) [62], which can result in root biomass decline that could be detected through EMF [63]; both methods in combination could be fit as a proxy for ozone impact. Wildfire risk in desertified ecosystems might be predicted through rapid nitrophilous grass growth and subsequent dryout that can be favored by even small CRL exceedances [64–66]. Combining CNR and SGF may assess denitrification rate, which in turn may help monitoring N_2O emissions for global warming potential calculations.

4.3. Limitations

The main strength in this study, but also its main limitation, is the literature selection strategy. Over 11,000 references on reactive nitrogen monitoring in ecosystems can be found in the peer-reviewed literature, which cannot be possibly reviewed manually. While an automated keyword analysis is certainly doable and more so with the aid of a modern LLM (not available at the time we initiated this study), it may offer a partial picture by potentially missing semantics that are crucial here: how to identify and attribute the facets offered by the methods. We observed this prime facie when having to prune the snowball by over 80%, as many papers were not actually establishing the method-purpose facet we were investigating (and that a blind keyword analysis might have taken for good). We therefore decided to do a full, manual review, which would enable us to capture the

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actual facets and measurements contained in the corpus. This necessarily entailed sampling the literature to select which references to analyze through the snowball strategy, but this choice also introduces a known bias toward well-established methodologies rooted in the reference seeds we used and for which FFP uncertainty might be low.

In addition, and despite its use in a tightly controlled workflow, the use of a purpose-specific AI for supplementary literature discovery, retrieval, and facet confirmation might have reinforced bias towards highly cited methods. The AI-assisted search was seeded from the facets set for the 2000-2020 literature sample, but the timeframe was not forward-restricted and the search branches might be overlooking emerging methods or facets such as environmental DNA metabarcoding, remote sensing, or machine-learning models. Future updates to the FFP matrix, once a substantial mass of cases is built over the next decade, will doubtlessly introduce more facets, as exemplified in the preference shift clearly observed in Figure 5.

Our choice to try a well-known but comparatively simple ordination technique for WS might be considered simple. While we created a simple H, M, and L suitability levels as an ordinal score and tried to be consistent along comparable facets, the thresholds for each level were based on very rough rules (such as the 2:1 ratio) that themselves rely on simple lineal or logarithmic assumptions. Other thresholds, more levels, or nonlinear weighting schemes might likely bring about different ordinations, possibly changing our results. Additionally, in our formula we normalized for number of evidences with penalization (facets lacking evidence bumped down the method ranking). While this is a well-established method in the decision-making literature, for some facets the boundary between “no evidence” and “low” is blurry—most prominently for binary-type facets such as *limit* or *lifetime* that effectively lack a “low” score.

Finally, the FFP assessment is inherently context-dependent: a method with a low global WS may be the only adequate tool in a specific context. ZAR, for instance, has the lowest overall WS but provides irreplaceable historical baseline data for Polish and Central European flora. Method rankings should therefore always be interpreted alongside the specific monitoring question rather than applied as absolute quality measures.

5. Conclusions

No single method is adequate for comprehensive nitrogen monitoring. Chemistry-based methods generally outperformed biodiversity- and transplant-based methods across most facets, led by critical loads (CRL), total tissue nitrogen (TNT), and nitrogen isotopes (NIS). CRL was the only method to provide assessable evidence across all 35 facets. Biodiversity-based methods, particularly lichen diversity (LDV), ectomycorrhizal fungi (EMF) and Ellenberg's N (ELN), provided the strongest signals for long-term community-level change, the facets most directly relevant to biodiversity conservation policy. Transplant-based methods occupy a methodologically intermediate position and shift greatly along facet typologies; lichen transplants (LTR) ranked substantially closer to CM than plant transplants (PTR), likely reflecting their greater response precision.

Effective assessment and, eventually, management of reactive nitrogen pollution requires monitoring systems that can simultaneously provide early physiological alerts, quantify deposition fluxes, and track long-term ecological trajectories. A deliberate combination of complementary methods, selected according to the FFP framework proposed here, offers a defensible and policy-relevant approach that can complement existing procedures based on specific methods.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/doi/s1: File 1, Data table and literature corpus list [2,16,18,53,60,67–142]; File 2, Detailed method-by-method and facet-by-facet description, discussion, and additional references [100–266].

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Abbreviations

The following abbreviations are used in this manuscript:

AAS	Amino acids
AI	Artificial Intelligence
BM	Biodiversity-based method(s)
CFL	Chlorophyll fluorescence
CM	Chemistry-based method(s)
CNR	C:N ratio
CRL	Critical loads
E	Number of evidences
ELN	Ellenberg's N
EMF	Ectomycorrhizal fungi
EZA	Enzyme activity
FFP	Fitness-for-purpose
FNS	Functional Nitrogen Index for Species
H	Facet with high suitability
INR	Invertebrate response
L	Facet with low suitability
LAN	Landolt
LDV	Lichen biodiversity
LTR	Lichen transplants
M	Facet with medium suitability
N	Facets with no available evidence | Nitrogen [context-dependent]
NIS	Nitrogen isotopes
NMDS	Non-metric multidimensional scaling
NPR	N:P ratio
Nr	Reactive nitrogen
PTR	Plant transplants
SGF	Gas fluxes
TDP	Tsyganov, Didukh & Plyuta
Tg	Teragram
TM	Transplant-based method(s)
TNT	Total nitrogen in tissues
WS	Evidence-normalized weighted sum
ZAR	Zarzycki

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