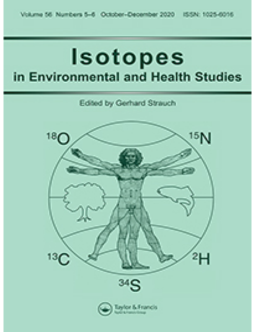

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REVIEW ARTICLE

Strengths and challenges of $\delta^{15}N$ to identify anthropogenic nutrient loading in coastal systems

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ABSTRACT
Coastal ecosystems are under increasing stress from anthropogenic nutrient loading; which is most often assessed through water quality measurements. Here, 136 published studies on the use of $\delta^{15}N$ to identify nutrient loading in coastal systems were analyzed to identify key strengths and challenges when using this isotope technique. $\delta^{15}N$ has been used successfully for this purpose around the globe for over 40 years. Studies have mainly used benthic macroalgae and sediment samples in estuaries and coral reefs of North America and Oceania. Strengths of this technique include timely identification of nutrient loading and its sources, even when inputs are pulsed or assimilated by biota, the benefits of varying isotope turnover rates in different types of samples, sporadic sampling efforts, simple collection and preparation of samples, and relatively low analysis costs. The shortcomings of this technique have led to a loss in popularity in recent times, mainly from isotopic overlap of potential sources and the effects of other confounding factors on isotopic compositions. These challenges can be compensated by simultaneous measurement of other key variables including additional isotopes ($\delta^{13}C$, $\delta^{34}S$), water column nutrient concentrations, and fecal coliforms, highlighting great potential to use this tool.

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1. Introduction

Coastal systems are under ever increasing pressure from a multitude of anthropogenic stressors. Excessive nutrients, mainly nitrogen and phosphorus, entering coastal systems are a key stressor for coastal habitats and have led to habitat deterioration and loss throughout the coastal regions of the world [1,2]. Increased nutrient inputs are the result of population growth and associated coastal development without adequate waste water treatments in place. Nutrient loading on coastal systems is also linked to land use change from natural forests to farming and cattle endeavors along the catchments, leading to increased fertilizer and excrement outputs. Anthropogenic nutrient loading on coastal systems can lead to increased algal productivity, both benthic and pelagic, which can lead to diminished water quality and hypoxia [3,4]. Excess nutrients can also interact synergistically with other anthropogenic stressors, such as food web

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imbalances and increased water temperatures, to further deteriorate coastal habitats [5,6]. Prompt identification of nutrient loading and the specific sources of the nutrient inputs is needed to adequately manage this major coastal stressor.

Physical, chemical, and biological indicators of water quality are commonly used to assess nutrient loading in coastal systems. Standard water quality analyses usually measure nutrient concentrations, salinity and dissolved oxygen in the water column, while biological indicators can include chlorophyll-a and fecal coliform concentrations [7–9]. However, early detection of nutrient loading or loading at pulsed intervals is likely to be missed using only traditional physical, chemical and biological indicators. Water quality measurements in particular tend to require prolonged field and laboratory work, making them in many cases expensive and time-consuming. In addition, these techniques have a limited ability to detect infrequent nutrient pulses that may be easily diluted in the water column, or nutrient inputs that are efficiently assimilated by the biota. The nitrogen isotope ^{15}N has been commonly used in aquatic food web studies, given the fractionation between trophic levels [10]. δ^{15}N has also been used to identify nutrient loading in aquatic systems, highlighting variations in δ^{15}N of aquatic organisms when exposed to anthropogenic nutrient loading [10,11].

Developed nations have the capacity to carry out extensive and intensive water quality monitoring, such as those deriving from the Water Framework Directive established in 2000 in Europe and moving to the Marine Strategy Framework Directive in 2008. Within these large programs, the use of δ^{15}N is not commonly included as a characterization or monitoring tool of nutrient loading in coastal systems. In regions without the capacity to carry out vast and expensive water quality monitoring programs, there is a heightened need for timely identifying nutrient loading in coastal systems.

This study aims to carry out a literature review on the use of δ^{15}N to identify anthropogenic nutrient loading in coastal systems in order to identify the strengths and considerations when using this technique.

2. Methods

Studies on the use of δ^{15}N to identify anthropogenic nutrient loading in coastal systems were located based on a search for published peer-reviewed literature on the search engine google.scholar.com. Articles from the first ten search result pages of each search were carefully examined, composing of ten results per page sorted by relevance. Theses, conference abstracts, and other gray literature were excluded from the analysis, as were experimental studies. Only studies in coastal and marine locations were included, excluding freshwater and terrestrial studies. The following keywords were searched independently and in multiple combinations: ‘nitrogen isotopes’, ‘δ^{15}N’, ‘nutrient loading’, ‘sewage’, ‘effluent’, ‘isotopic identification’, ‘coastal’, ‘marine’, and ‘eutrophication’. An additional search was carried out using the same keywords in combination with ‘Mediterranean’ due to a limited number of studies from this region found in the first search in comparison to the number of research institutes in the region. Published literature was searched up to April 2020. The reference list of each study included was thoroughly examined to identify other key studies on the topic. A total of 136 publications fit the selection criteria spanning 40 years; nonetheless, there are likely other studies that may have been inadvertently not included. Each publication was carefully assessed and the following fields were registered per study: the country in which the study was conducted, the ecosystem studied, type of sample used, species if noted, whether the nutrient source was sampled, and whether the isotopic response was of enrichment or depletion. The studies were mapped globally using the field collection GPS coordinates directly from the studies when provided or using Google Earth to obtain coordinates from presented maps or location names where needed. The tendencies regarding the spatial distribution of sampling effort, type of samples collected, and habitat of the studies were analyzed.

3. Results

The first studies on the use of δ^{15}N to identify anthropogenic nutrient loading in coastal systems within the analyzed literature were published in 1980 and the number of papers on the topic increased over the years (Figure 1). The isotope values of the nitrogen sources were only directly measured in 17% of the studies, and water column nutrient concentrations were simultaneously measured in 37% of the studies [9,12,13]. Some of the studies that did not directly measure isotope values of the nutrient sources or water column nutrient concentrations used values from previous studies. In some of the studies bacteria such as *Enterococci*, human population density or distance from populated locations were used as proxies to quantify sewage input [14–16]. δ^{15}N was measured directly from water samples in only 1% of the studies. Most of the studies carried out sporadic sample collections, many of them on one or only several samplings.

The study locations spanned a total of 48 different countries, highlighting that δ^{15}N is a widely used tool to identify anthropogenic nutrient loading in coastal habitats around the globe (Figure 2). The studies were carried out mainly within estuaries, coral reefs, and

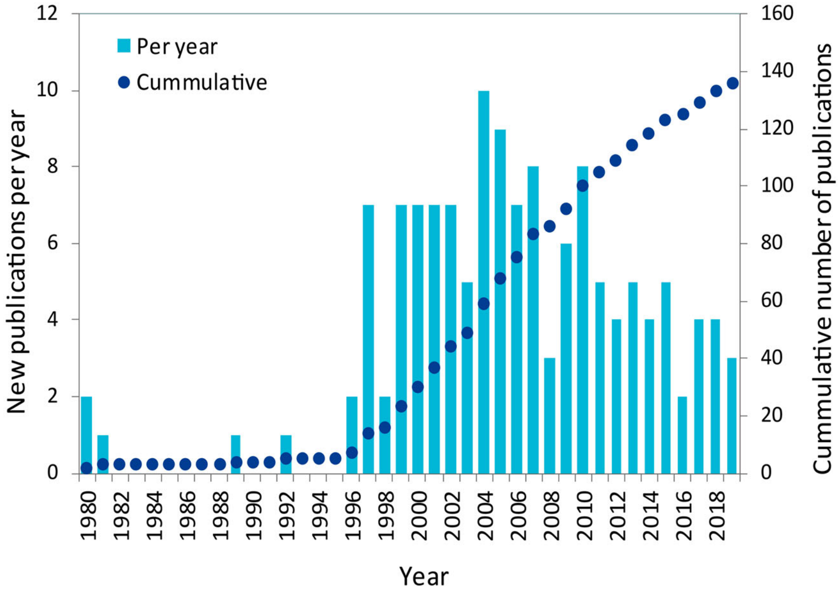

Figure 1. Number of peer-reviewed articles published per year and cumulatively over time which used δ^{15}N of biotic and abiotic samples to identify anthropogenic nutrient loading and nitrogen sources in coastal systems.

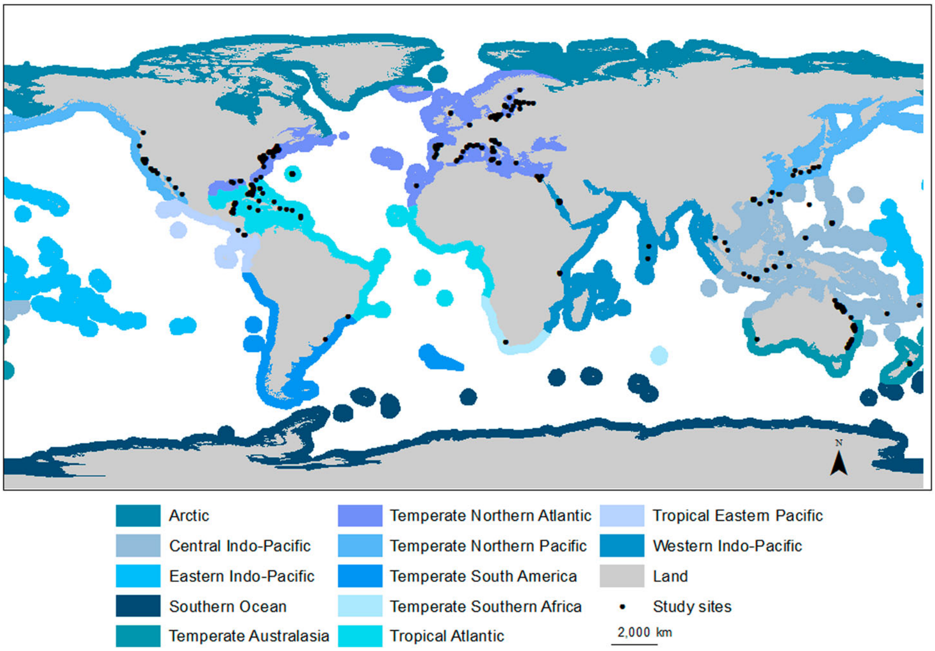

Figure 2. Locations of studies which used $\delta^{15}N$ of biotic and abiotic samples to identify anthropogenic nutrient loading and nitrogen sources in coastal systems. Marine ecoregions of the world are depicted [60].

nearshore marine and coastal areas (Figure 3). Samples were collected from exposed coastal shores or shallow locations all the way down to 300 m depths [17]. The most common type of organisms used in the studies to identify nutrient loading using $\delta^{15}N$ have been macroalgae and bivalves, with sediment and particulate organic matter (POM) common abiotic samples used (Figure 3). A total of 496 species were sampled in the studies, along with other types of samples such as sediment, water, and POM. The number of species sampled in decreasing order was: macroalgae (124 species), fishes (151), polychaetes (28), clams (23), seagrasses (22), shrimp (18), octocorals (12), amphipods (11), stony corals (10), and snails (10). Groups with less than 10 species sampled were: barnacles (8 species), crabs (7), sea cucumbers (6), mangroves (6), salt marsh (5), and mussels (5). Other types of organisms with less than five species represented in the studies included sponges (2 species), anemones (2), solitary (1), leather (2) and black (3) corals, chaetognaths (1), nematodes (1), isopods (4), copepods (2), water fleas (1), tanaids (1), mysids (1), stomatopods (2), lobsters (2), marine worms (4), limpets (3), oysters (4), starfish (2), urchins (3), ascidians (1), shark (1), manatees (1), and birds (4) (a full list of species in the studies is included in the Supplementary Table S1).

Anthropogenic nutrient loading had various effects on $\delta^{15}N$. In the majority of the studies (78%), the reported effect was isotope enrichment, i.e. higher $\delta^{15}N$ values. In contrast, a small number of the studies (11%) reported a $\delta^{15}N$ isotope depletion effect. This depletion was linked to depleted nitrogen sources in the studies, mainly from sewage treatment level or synthetic fertilizers. A small percentage of studies (7%) reported multiple simultaneous effects, some with variations among the response of different types

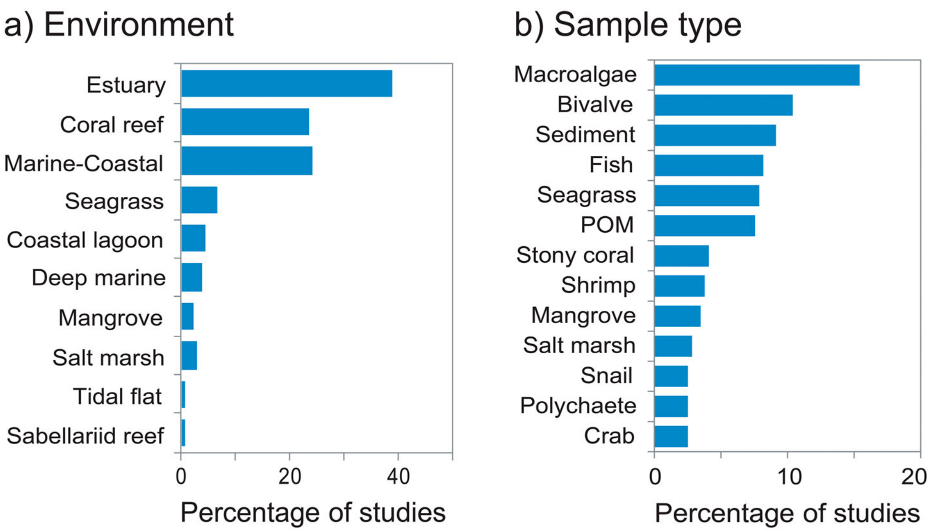

Figure 3. Percentage of studies using $\delta^{15}N$ to identify anthropogenic nutrient loading per environment (a) and type of sample (b). Only samples representing 3% or more are depicted.

of samples, species or spatial variations contrasting temporal trends. Only a limited number of studies (4%) reported a lack of isotopic effect on $\delta^{15}N$, potentially due to the mixing of multiple nutrient sources with contrasting $\delta^{15}N$ values, selection of sampling sites or other factors unaccounted for in the studies.

4. Discussion

Nitrogen isotopes have been widely used to identify anthropogenic nutrient loading in coastal systems in a multitude of habitats worldwide, using biotic and abiotic samples. In contrast to other techniques used to identify anthropogenic nutrient loading, this type of isotope analysis generally consists of sporadic sampling frequency, sample handling and processing are simple, and analysis costs are relatively low, though costs may still surpass available funds in some developing regions. While measuring the total concentration of nutrients in the water column can be useful to identify excessive nutrient concentrations, one of the reasons that $\delta^{15}N$ is such a useful indicator for nutrient loading is that isotope values can show nutrient loading even before nutrient loading is measurable in water column nutrient concentrations or the effects on the ecosystem are noticeable. While $\delta^{15}N$ has been used to identify nutrient loading in many locations around the globe, many habitats and organisms sampled have only been sporadically studied.

4.1. Identification of sources of nutrient loading

Most of the studies reported an enrichment effect on $\delta^{15}N$ from anthropogenic nutrient loading. One of the reasons why $\delta^{15}N$ is useful to identify the flow of nitrogen from anthropogenic activity into coastal systems is that the various nitrogen sources also have particular $\delta^{15}N$ values. Marine algae and plants are particularly useful for identifying nutrient loading, given their baseline position in the trophic web. This leads to naturally lower

δ^{15}N values in photosynthetic organisms, which can be close to atmospheric values. At increasing trophic levels δ^{15}N becomes more enriched, from selective elimination of the lighter ^{14}N isotope [18]. This leads to enriched isotope values in cow, pig and human excrement compared to lower trophic levels. Atmospheric deposition through rain has δ^{15}N values close to or below zero [19]. Most synthetic fertilizers have values around zero, though it depends on the type of fertilizer used with some just above zero and others depleted to almost −5 ‰ [19]. Meanwhile, animal waste and sewage have enriched values averaging > 10 ‰ [19]. Natural values of δ^{15}N vary greatly ranging from −20 to +20 ‰, including those found in plants and animals, soil, rain, air, volcanic, and petroleum [10,18,20]. The available nitrogen in a system can come from multiple sources, with other potential natural sources including nitrogen fixation by cyanobacteria, which has a signal ~ 0 ‰ and upwelling nutrient sources, which have enriched values. For instance, δ^{15}N values on coral skeletons can be enriched when exposed to untreated sewage, while excess fertilizers entering reefs during flood events can lead to a δ^{15}N depletion [21].

Nitrogen sources can become even more enriched depending on the type of waste water treatment applied. Untreated sewage can be enriched to values > 10 ‰, yet the type of treatment can enrich sewage δ^{15}N even further [22]. Active removal of nitrogen in secondary and tertiary treatment leads to higher removal of lighter nitrogen isotopes, with a subsequent δ^{15}N enrichment in sewage effluent as high as ~ 90 ‰ [23]. Therefore, the level of sewage treatment needs to be noted when studying nutrient loading as this can alter the isotope effect on coastal systems.

In contrast, a limited number of the studies identified a depletion effect on δ^{15}N as a response to anthropogenic nutrient loading. Reefs with minimal sewage input should exhibit relatively low δ^{15}N values at each trophic level, reflecting oligotrophic conditions which are dominated by algal fixation of atmospheric nitrogen (~ 0 ‰) [15]. Air (~ 0 ‰) is actually used as the international standard for nitrogen isotope studies [18]. The depletion effect on δ^{15}N due to anthropogenic nutrient loading is a response to the type of nutrient sources. Many fertilizers that incorporate animal excrement are δ^{15}N-enriched. In contrast, synthetic fertilizers rely on atmospheric N fixation and have depleted δ^{15}N values, most often around 0 ‰. In the Caribbean, for instance, by analyzing octocoral δ^{15}N values along subsequent skeletal growth bands, a depletion of δ^{15}N values was found over time which was linked to increased use of synthetic fertilizers in the region [24]. This provides a unique opportunity to identify variations over time not only in the amount of nitrogen but variation in the sources of nitrogen inputs.

While δ^{15}N has been widely used to identify nutrient loading to date, its potential usage to identify the specific nutrient sources has been underused. Several studies sampled δ^{15}N in the suspended POM or in primary producers and relate that to higher trophic levels or consumers, thereby using POM or primary producers as the isotope signal sources. Only a limited number of studies directly measured δ^{15}N from the water bodies or potential nitrogen sources [25–27]. The direct measurement of δ^{15}N of potential nitrogen sources needs to be included in future studies.

4.2. Isotope clock

One of the strengths of using δ^{15}N to identify anthropogenic nutrient loading is that there are varying isotope turnover rates in the different types of organisms. While some organisms have very fast isotope turnover rates, others have very slow turnover rates and some even become historical archives of isotopic information. Macroalgae for instance are the organisms most usually used to represent current isotope composition in coastal environments given that their isotope turnover rates are only a couple of days, particularly for fast-growing species [11,28]. Algal species that grow slower, such as perennial brown algae, can provide integration over longer time periods [27]. Other organisms such as benthic filter feeders or snails, usually have isotope turnover rates of weeks up to months, though some have tissue turnover rates of years [28,29]. Slower growing plants such as mangroves can incorporate isotope data over years, making them ideal for integrating pulsed nutrient inputs that are not evident in water column concentrations [9].

Coral skeletons and animal bones incorporate isotopic information easily over decades to centuries. Manatee bones have been recently used to analyze nutrient loading over a time period of three and a half decades [30]. Coral growth bands from living coral or fossils can be used in combination with other stable and unstable isotopes to date the coral portions and identify potential variations in $\delta^{15}N$ over longer time periods [17,24,31]. Some extremely long-lived organisms provide millenary archives of isotopic information. For example, analysis of the black coral *Leiopathes* sp. from a deep region in the Gulf of Mexico, provided information from the last 2000 years and indicated nutrient enrichment in the last 200 years compared to the previous time period [17]. Undisturbed sedimentary deposits in turn store isotopic information in subsequent deposited layers for up to thousands of years, with the time frame covered by the depth of the sediment sample depending on the vertical accretion rate at each location [32]. Some studies rely on punctual sampling of long-term sediment archives, where nutrient loading or diminishment over time can be identified, such as in sediment profiles from the Baltic Sea [33,34].

The understanding of the various isotope turnover rates in different types of samples is an additional benefit of using $\delta^{15}N$ to identify nutrient loading. Sample collection in almost all of the studies was sporadic, with field sampling occurring only a limited number of times. This technique allows for an experimental design that can sample on one occasion but obtain information of multiple time frames. For example, simultaneously sampling macroalgae, bivalves and mangroves allows integration of days, weeks and years' worth of isotope data on nutrient loading [9]; as opposed to continued sampling over years with water nutrient concentration techniques. Other studies can rely on variations in isotope turnover rates among different tissues even within the same species, with some tissues better suited to identify nutrient loading than others [35].

Some studies do use repeated short-term isotope data, sampling organisms with fast turnover rates at great spatial scales. This allows monitoring of variations over time in the isotope values, i.e. isotope monitoring. This method is useful for instance to identify improved water treatment efforts which lead to diminished nutrient loading [27,36].

4.3. Main factors influencing $\delta^{15}N$ data interpretation

As with all techniques there are considerations in the use of $\delta^{15}N$ to identify anthropogenic nutrient loading. The number of studies per year using $\delta^{15}N$ to identify nutrient loading in coastal habitats appears to have diminished in recent times, a fact that may be linked to particular challenges that need to be taken into account when applying the technique. The literature search was based on relevance, and the potential time lag between time of publication and increased referencing of a study is a potential factor to consider. A key aspect for successful nutrient loading and source identification using δ^{15}N is that sources have isotopically distinct δ^{15}N values, differing among sources and from unimpacted coastal waters; leading to a measurable change in the δ^{15}N of the biotic or abiotic samples analyzed in the field [10]. However, aspects such as fractionation, potential source mixing and overlap of isotope values are key issues to be considered. Nitrogen isotope fractionation may be occurring at various rates from biological processes, such as nitrification, denitrification, nitrogen fixation, volatilization or assimilation, anaerobic oxidation of ammonium (anammox), among others [37–40]. Bayesian mixing models may prove useful in some instances, where incorporating more sources requires measurement of additional isotopes, such as δ^{13}C or δ^{34}S [10]. In some instances, active isotopic tracing by marking potential sources and analyzing their flow into the coast may be feasible [10]. Given indistinguishable isotope values among some sources, another technique to consider would be environmental DNA, which has been used with greater success than stable isotopes (δ^{15}N, δ^{13}C) to identify and estimate the contribution of coastal vegetation sources to seagrass sediments [41]. These approaches, particularly when carried out simultaneously, may unfortunately lead to an increase in the overall cost, which may already be constrained in developing regions.

Natural seasonal and spatial variation in the coastal conditions such as variations in temperature, precipitation, salinity, and circulation patterns may further confound the δ^{15}N values. The enrichment effects on algae for instance may be only evident in the dry season or can vary seasonally overall [42,43]. Increased precipitation can have a δ^{15}N enrichment effect, as seen for soft corals [44]. Nonetheless, the effect of precipitation and associated effluent on coastal δ^{15}N would be linked to the δ^{15}N values of the nutrient sources transported by the effluent, i.e. synthetic fertilizers may lead to a depletion effect. Increased rain may also stimulate phytoplankton productivity, with a possible corresponding fractionation effect on δ^{15}N from biological assimilation to be considered [45]. The type of algal productivity from increased nutrient input should also be taken into account, as cyanobacterial atmospheric nitrogen fixation has δ^{15}N values ~ 0 ‰ [39,46]. In particular, studies that analyze δ^{15}N variation in estuarine environments spanning the salinity gradients have challenges in clearly identifying anthropogenic nutrient loading using δ^{15}N, with unclear or no effects found in some studies [47,48]. In the Venice lagoon, there are marked seasonal variations in δ^{15}N from up to ~ 18 ‰ in the spring down to ~ -6 ‰ in winter, linked to seasonal fluctuation in sewage input [49]. Variations in baseline isotope values and natural variability, particularly at varying salinities, should be understood prior to using δ^{15}N to identify the effects of anthropogenic nutrients on the system.

In the selection of samples to be analyzed, benthic organisms are better suited for using this technique as mobile organisms may move among locations or immigrate from outside the study site [50]. However, a key challenge is that when sampling spatially along nutrient loading gradients, the organisms at the impacted sites are only rarely also found at non-nutrient loaded locations. Given that there are isotope variations among different types of organisms and among species, this can make comparing the same species at multiple locations challenging. Many studies assess species abundance at each location prior to sampling or isotope analysis, while others deploy organisms to the different sites for a specific amount of time. Prior to deployment, a period to equalize the isotope composition of all the organisms is needed if they came from multiple sampling sites. This deployment technique has been used successfully for organisms with faster turnover rates such as macroalgae, bivalves, and fish [11,35,51]. The prevailing currents should be taken into consideration when setting up the sampling design [52].

Long-term sediment cores and coral skeletons should be dated when studying variations over time. Sediments in coastal systems, particularly surface sediments, are highly vulnerable to bioturbation and may therefore not be integrating isotope variations over continuous time frames [32]. Corals present a particular challenge, as it has been shown that coral $\delta^{15}N$ is depleted at greater water depths, which has been linked to diminished light availability [38,53], while some soft corals can also show $\delta^{15}N$ enrichment at greater depths but from POM enrichment [54].

Another aspect to consider is that particularly for animal samples the sex, age, and health of the animal may lead to variations in isotope composition which are not linked to anthropogenic nutrient loading. To avoid additional noise in the $\delta^{15}N$ analyses, animal samples should be allowed a 24-hour time period to clear their guts prior to preparing for stable isotope analysis whenever they are used whole [29,55]. Furthermore, $\delta^{15}N$ can be affected by acidification of samples prior to isotope analysis [56], and unacidified samples should be used whenever possible. Therefore, these factors need to be assessed and controlled whenever possible to diminish confounding factors in the analyses.

4.4. Other parameters to measure simultaneously

Anthropogenic nutrient loading can alter other aspects of coastal ecosystems, and to successfully identify nutrient loading and its sources a multiple tool approach may sometimes be useful. This approach may be particularly useful when multiple potential sources of nutrient loading may have contrasting or overlapping values [19], thereby potentially confounding $\delta^{15}N$ values. In some instances, along with $\delta^{15}N$ values, the total nitrogen concentration in the samples or in the water column is also quantified. For instance, high algal nitrogen content and enriched $\delta^{15}N$ values were found in samples from locations with primary nutrient loading, while contrasting high nitrogen content but depleted $\delta^{15}N$ values were found in agricultural locations due to the use of synthetic fertilizers [57]. Carbon isotopes ($^{12}C/^{13}C$) have also been noted to provide useful information related to nutrient loading. Isotopic $\delta^{13}C$ is indicative of the carbon sources of different organisms, with variations in $\delta^{13}C$ depending on photosynthetic pathways [58]. As such, variation in $\delta^{13}C$ can identify increased terrigenous input from increased effluent which leads to more depleted terrestrial $\delta^{13}C$ values, or changes towards more enriched $\delta^{13}C$ values from increased nutrient availability and associated algal productivity [34,59]. Furthermore, to identify sewage input some studies simultaneously quantify fecal coliforms such as *Enterococcus*, along with isotope analysis [42]. Simultaneous measurements of traditional water quality, such as water column nutrient and phytoplankton chlorophyll-a concentrations, salinity, temperature and dissolved oxygen, along with isotope composition of biotic and abiotic samples should also be incorporated whenever feasible. A systemic approach in which isotopic nitrogen compositions are measured alongside simultaneous measurement of these additional variables will undoubtedly provide a more comprehensive understanding of the flow of anthropogenic nitrogen into coastal systems and the point and non-point sources.

5. Conclusion

This literature review revealed that $\delta^{15}N$ has been successfully used as a tool to identify nutrient loading around the globe in many different habitats. This technique has many strengths, highlighting its usefulness to identify nutrient sources, timely identification of pulsed nutrient loading or at low concentrations in the water column from biotic assimilation, the benefits of varying isotope turnover rates in different types of samples, sporadic sampling efforts, simple sample collection and preparation, and relatively low analysis costs. The shortcomings of this technique have led to a loss in popularity in recent times, mainly from isotopic overlap of potential sources and the effects of other confounding factors on isotope compositions. Despite these challenges, $\delta^{15}N$ continues to provide key information on the input of anthropogenic nitrogen sources to coastal systems. The use of this tool can be enhanced by simultaneous measurement of other key variables, such as additional isotopes ($\delta^{13}C$, $\delta^{34}S$), water column nutrient and chlorophyll-a concentrations, fecal coliforms, and potentially other novel techniques such as environmental DNA. The identified shortcomings of this technique can be adequately compensated, therefore highlighting the potential for use of this tool.

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Disclosure statement

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