



Review article

Measurements and analysis of nitrogen and phosphorus in oceans: Practice, frontiers, and insights

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ABSTRACT

Nitrogen and phosphorus concentrations in oceans have been extensively studied, and advancements in associated disciplines have rapidly progressed, enabling the exploration of novel and previously challenging questions. A keyword analysis was conducted using the Scopus database to examine chronological trends and hotspots, offering comprehensive insights into the evolution of marine nitrogen and phosphorus research. For this purpose, author keyword networks were developed for the periods before 1990, 1990 to 2000, 2001 to 2011, and 2012 to 2022. Furthermore, analytical techniques employed in the recent decade to determine nitrogen and phosphorus concentrations in seawater were assessed for their applicability and limitations through a critical review of more than 50 journal articles. Taxonomy and nitrogen biogeochemistry were the prominent research interests for the first two periods, respectively, while stable isotopic tracking of nitrogen and phosphorus processes emerged as the dominant research focus for the last two decades. The integration of macroeconomic factors in research development and the chronological rise of interdisciplinary research were identified. Conventional analytical techniques such as spectrophotometry, colorimetry, fluorometry, and elemental analysis were noted, along with emerging techniques like remote sensing and microfluidic sensors.

1. Introduction

Both nitrogen (N) and phosphorus (P) are essential nutrients in seawater, playing a crucial role in primary productivity [1,2]. Excessive amounts of N and P contribute to nutrient pollution in coastal waters in numerous countries [3,4]. Regions heavily affected by excessive N and P inputs include the Northwest Pacific Ocean [5], the Gulf of Mexico [6], the Baltic Sea [7], the North Sea [8], and the Chesapeake Bay [9]. One of the largest ocean dead zones globally is in the Gulf of Mexico, recording maximum surface total nitrogen (TN) and total phosphorus (TP) concentrations of 70 $\mu\text{mol N L}^{-1}$ and 7 $\mu\text{mol P L}^{-1}$, respectively, leading to substantial ecosystem damage [10,11]. The Baltic Sea, with approximate maximum recorded TN and TP concentrations of 70 $\mu\text{mol N L}^{-1}$ and 4 $\mu\text{mol P L}^{-1}$, experiences harmful algal blooms, loss of benthic biodiversity, and decreased fish catches [12]. Similarly, Chesapeake Bay faces seagrass declines due to nutrient pollution, impacting fisheries and the ecosystem [13]. TN includes both inorganic and organic N and is commonly used as a comprehensive indicator of environmental status. Total dissolved nitrogen (TDN) encompasses dissolved

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inorganic nitrogen (DIN) and organic nitrogen (DON). Various methods have been developed to measure particulate N, DON, and DIN. Similarly, methods have been developed and applied to measure P [14]. A diverse range of disciplines conducts analyses of N and P in oceans, with the concepts of measurements and analysis continually evolving to address current concerns and respond to new issues. This evolution is particularly evident in technological advancements, such as Artificial Intelligence (AI), Unmanned Aerial Vehicles (UAV), and quantum computing.

While N and P are essential macronutrients for the growth of oceanic biota, anthropogenic over-enrichment of these macronutrients in coastal regions—resulting from the excessive disposal of waste (land-based sewage discharge and wastewater from coastal aquaculture) and surface runoff enriched with agricultural fertilizers—renders coastal waters unsuitable for human use. This situation leads to issues such as eutrophication and phytoplankton (photosynthetic microbes) blooms, causing hypoxic (oxygen-depleted) or anoxic (oxygen-less) zones that endanger marine life and diminish the visual aesthetics of the coast [15]. Globally, there are approximately 500 such oceanic dead zones [16]. Toxic algal blooms may also develop, acting as biotoxins and posing risks to public health [17]. Furthermore, significant amounts of CO₂ are released into the water when these blooms decay, making a substantial contribution to ocean acidification and the deterioration of coral reefs [18,19].

Research on the measurement and analysis of N and P spans various disciplines within the natural sciences [20–23]. These disciplines encompass marine biology and biotechnology, marine chemistry, ecology, climate science, and paleoceanography. Researchers from these fields, including marine biogeochemists, biologists, ecologists, oceanographers, limnologists, and environmental engineers, investigate nutrient concentrations and cycling to achieve their respective goals. The earliest research papers related to marine N and P research in the Scopus database date back to the 1950s [24]. Since then, various research trends and hot topics have emerged and evolved over time, influenced by contemporary environmental concerns and technological capabilities and requirements. For instance, recent applications of artificial intelligence (AI) and remote sensing have been instrumental in accurately assessing nutrient concentrations, managing them, and restoring large-scale and complex coastal environments [25–28]. However, whether these technologies have become a prevailing trend, characterized by a higher frequency of occurrence compared to other research topics in this area, remains to be determined. Furthermore, as of the present, no scientific analysis has been conducted to identify the chronological trends and hot topics in this area of research. Consequently, the reasons for such trends and hot topics remain unresolved.

Research on N and P in marine environments has been significantly conducted by various disciplines at different locations, parallel to advancements in their measurement and analysis over time. The accurate measurement and analysis of N and P have progressed with the aim of achieving sustainable management of ocean nutrients. Elucidating the measurement and analysis of N and P with efficient strategies is crucial for mitigating the damage caused by excess nutrients. However, systematic reviews on N and P measurement and analysis methods are limited, lacking correlation in the merits of each method for future investigations. Many studies have demonstrated that bibliometric analyses are effective in analyzing research trends, allowing the elucidation of chronological trends, mapping key knowledge landscapes, and identifying relationships between different research areas [29,30]. Furthermore, no systematic reviews compare the measurement methods of N and P in seawater in the context of their merits and frontiers. Therefore, this review aims to identify and present insights into the methods of measuring N and P in seawater and their practices and frontiers. The initial part focuses on insights into N and P research trends in oceans globally, and the later part concentrates on insights into the specific methods of N and P measurement and analysis. The measurement and analysis techniques are presented in the context of chronological, spatial, and methodological trends with merits and demerits to facilitate better environmental management and conservation.

2. Materials and methods

2.1. Data sources, search strategy, and data extraction

This study employed keyword analysis and critically reviewed peer-reviewed journal articles on N and P measurement and analysis in oceanic environments. Initially, three databases (Scopus, Web of Science, and Dimensions) were considered for retrieving scientific papers for this study. However, the Scopus database was finally selected and explored in December 2022 because it is a comprehensive abstract and citation database covering over 77 million records from various sources, including peer-reviewed journals, trade journals, book series, books, and conference materials, dating back to 1788 [31]. While Web of Science has fewer journals compared to Scopus and Dimensions, approximately 99.11% of the journals indexed in the Web of Science database are also indexed in the Scopus database [32]. Although Dimensions features the most extensive journal coverage, unlike Web of Science and Scopus, it does not regulate the content entered into the database through editorial decisions [33]. Consequently, owing to its extensive journal coverage and reliability, we opted to include only peer-reviewed journal articles from various journals across different disciplines in the Scopus database for this review.

The records in the Scopus database underwent initial screening using “ocean,” “N,” “nitrogen,” “P,” and “phosphorous” as search queries to browse through article titles, abstracts, and keywords. Leveraging the Boolean syntax support provided by the Scopus Application Program Interface (API), search queries were structured as a Boolean syntax for more effective initial screening: “Ocean AND ({N} OR nitrogen OR {P} OR Phosphorus).” Built-in filters within the Scopus database were employed to further refine the search records by excluding documents other than journal articles, non-English articles, and articles from non-relevant disciplines such as social sciences, economic science, and business management. A total of 46,503 journal articles published from 1874 to December 2022 successfully passed the initial screening. As the Scopus database permits viewing only the first 2000 search records, articles published each year were sorted by relevance. Consequently, citation and bibliographical information, along with author keywords, were

extracted from the first 2000 articles published annually. Scopus calculates the relevance of a specific article (search record) using a complex statistical relevance model that takes into account multiple factors associated with terms in search queries. These factors include the number of occurrences in the article, significance (ratio: Term frequency/Frequency of documents containing the term), the section containing the term, the position of the first occurrence, proximity of different terms in search queries within the article, and completeness of terms in a search query within the same field of the article [34].

2.2. Method of analysis

2.2.1. Analysis of chronological and spatial trends in research interests

A particular phenomenon or concept must be studied for a considerable period, and a few research articles must be published to establish it as a driving research interest in the scientific community [35]. Therefore, we divided the selected papers into four decades to identify chronological trends in prominent research interests or hotspots. The designated periods were (a) Before 1990, (b) 1990–2000, (c) 2001–2011, and (d) 2012–2022. The period before 1990 (period a) was considered a single period since the number of journal articles published before 1990 on N and P research in oceans was more limited (only 2738).

The present study focused exclusively on the author-defined keywords in publications for trend analysis, disregarding indexed keywords suggested by the Scopus database. This decision was based on the assumption that author-defined keywords offer a fundamental description of the research content in publications [36]. The occurrence of author keywords was analyzed using VOSviewer [37]. In the analysis, author keywords were listed in descending order of occurrence, and the top 100 keywords of each decade were considered. Where two keywords had the same number of occurrences, priority was given to the keyword with the highest number of co-occurrences with other keywords for analysis. Keyword networks were represented in network diagrams, with each bubble symbolizing a keyword, and the diameter of the bubble corresponding to the frequency of occurrence of the keyword. A link or line connecting two bubbles denoted a co-occurrence of the respective keywords within the same journal article. The thickness of the line, i.e., the link strength, indicated the number of such co-occurrences found across the selected range of literature [37]. A minimum link strength of 5 was applied, meaning each link indicated at least five co-occurrences or five journal articles in which the two keywords co-occurred as author-defined keywords. Additionally, VOSviewer was employed to calculate keyword similarity and cluster keywords accordingly, as depicted in Equation (1).

$$S_{ij} = C_{ij} / (O_i \times O_j) \tag{1}$$

where S_{ij} is the similarity between i and j keywords when C_{ij} is the number of co-occurrences of i and j and O_i and O_j are the number of occurrences of i and j, respectively.

The distance between two bubbles (keywords) is inversely proportional to their similarity, and keywords with higher similarities are clustered using similar colors [38,39]. Finally, the research hotspots of each decade were identified and visualized, depicting how those research interests were linked to each other.

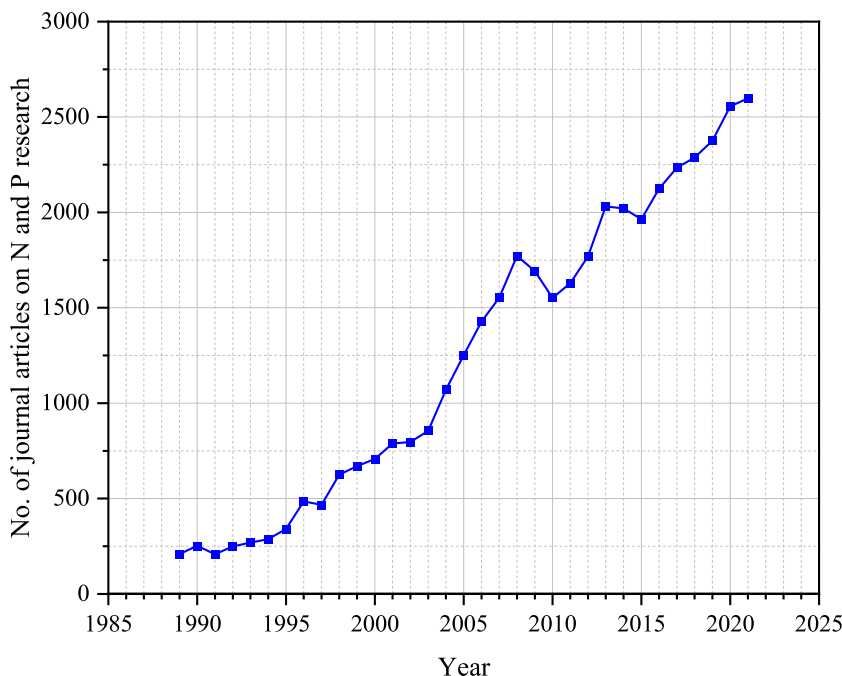


Fig. 1. Yearly distribution of No. of journal articles relevant to N and P research in ocean (based on articles indexed in Scopus database).

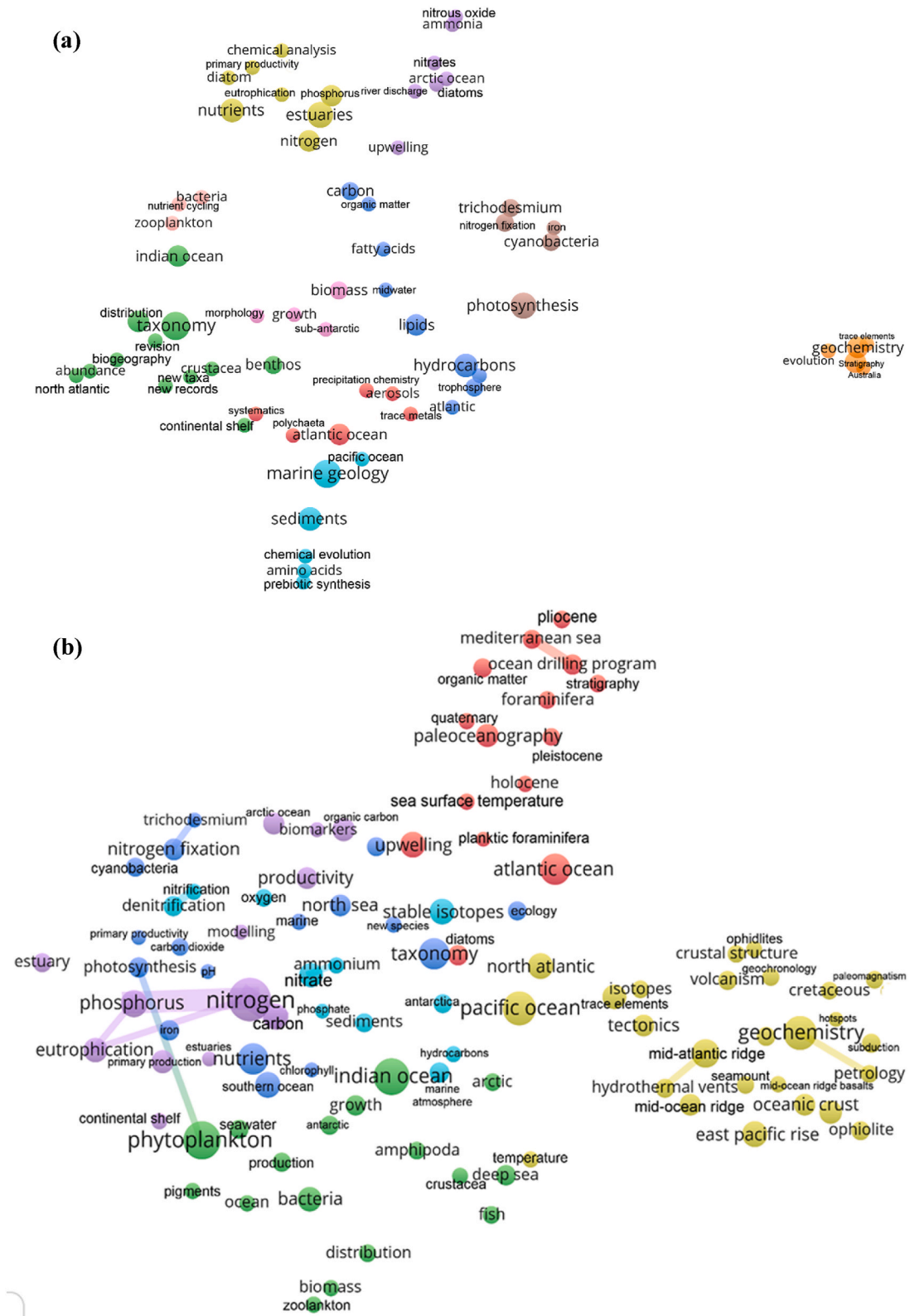


Fig. 2. Network maps of author keywords for period (a) 1990>, (b) 1990–2000, (c) 2001–2011 and (d) 2012–2022.

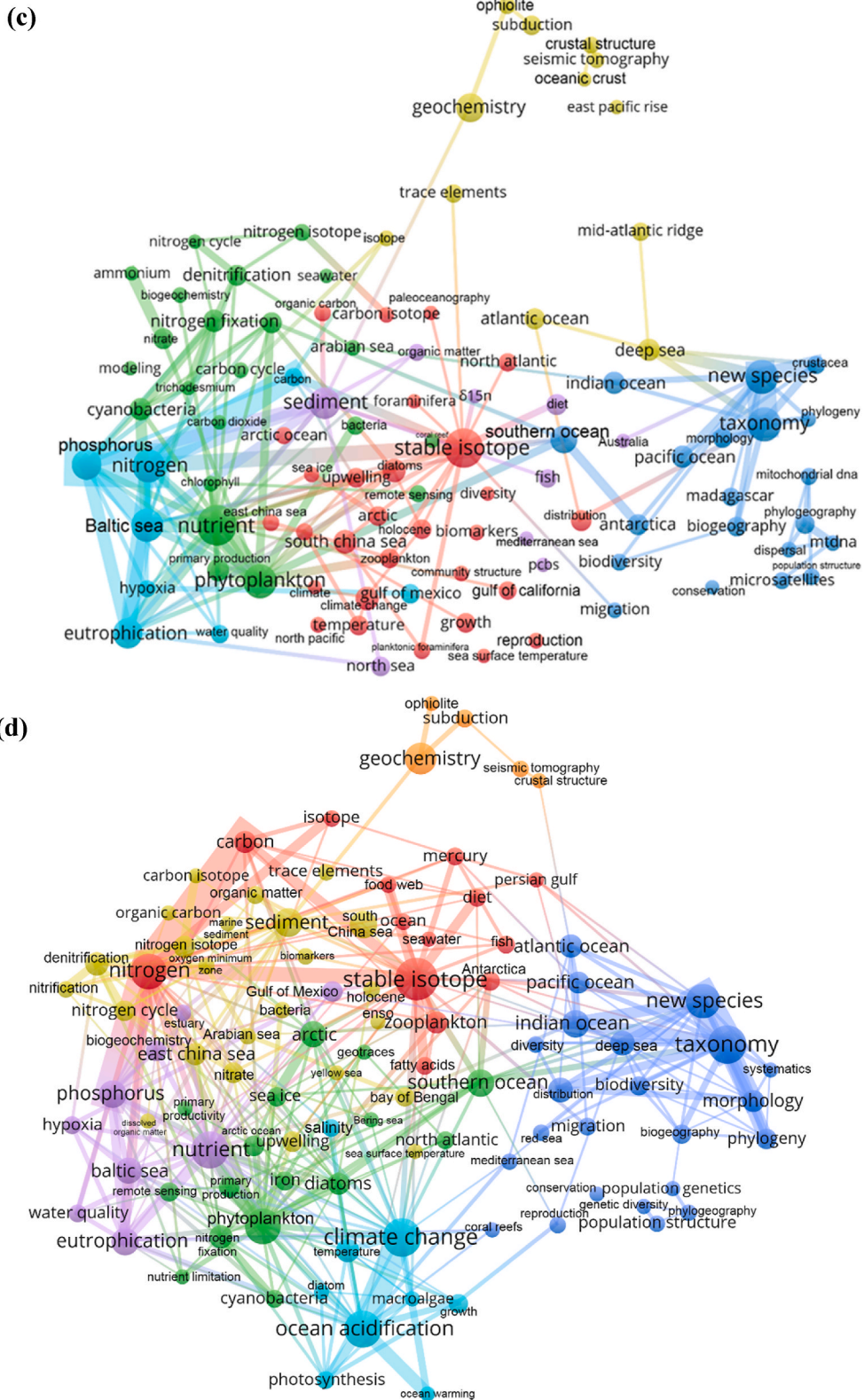


Fig. 2. (continued).

2.2.2. Critical analysis of research articles that measured N and P forms in seawater

A critical review [33,40] was conducted to identify the methods employed in recent research studies to quantify (or analyze) N and P forms in seawater. For this purpose, we reviewed more than 50 journal articles published in the most recent decade, i.e., period (d). To select these articles, we assessed the relevance of titles to the measurement and analysis of N and P in seawater. The measured parameters, methods of measurement, and methods of analysis from the selected journal articles were summarized. Merits and demerits were reviewed in the context of different measurement and analysis methods of N and P in marine environments. Some other reviews have been published on different methods or sensors used for N and P measurement in water samples [41–43]. In contrast, this review explicitly focuses on both chemical analytical methods and emerging non-chemical sensing methods used for N and P measurement in seawater.

3. Results and discussion

3.1. Chronological and spatial trends

Research related to N and P in the ocean, in general, has exhibited increasing popularity among researchers, with minor declines of 12.4% and 3.3% in 2008–2010 and 2013–2015, respectively, as shown in Fig. 1. The significant drop in the number of journal articles in 2008–2010 was attributed to the hindrance of funding for research caused by the Great Recession, which impacted the world economy from 2007 to 2010 [44]. The slight decline in publications in 2013–2015 was a result of the lesser global economic downturn and economic stagnation in Europe in 2012–2013 [45]. Approximately 500 and 1500 articles were published per year in this context in the (b) and (c) periods, respectively, and this value increased to 2000 articles per year in the recent decade. The distribution of articles over the decades was further analyzed through keyword maps, identifying prominent author keywords and their co-occurrence.

Author keyword maps for each period (a) Before 1990, (b) 1990–2000, (c) 2001–2011, and (d) 2012–2022 are depicted in Fig. 2. “Taxonomy” and “Nitrogen” were the most frequently occurring keywords for periods (a) and (b), respectively, while “stable isotopes” was the most frequently occurring keyword for both (c) and (d) periods. Considering the cumulative frequency of occurrence (f) of prominent keywords (Table 1), “Stable isotopes” and “Taxonomy” were identified as the most frequently occurring keywords. Furthermore, climate change and ocean acidification suddenly emerged as research hotspots in the last decade. As anticipated, “Nitrogen” was the keyword with the highest total link strength for all periods since it was a primary search query. “Phytoplankton” was the keyword with the second-highest total link strength for all periods, indicating that the role of phytoplankton was extensively correlated with different research areas associated with N and P in oceans throughout all periods. Additionally, climate change and ocean acidification, the two research hot topics that emerged in the recent decade, also exhibited considerably high total link strength, signifying that the impacts of those phenomena on diverse research areas were studied.

Remote sensing entered the list of prominent 100 author keywords for the first time in decade (c) and doubled its number of occurrences by decade (d), highlighting its growing popularity. In the recent decade, a new cluster emerged, focusing on the emerging research hotspots of “ocean acidification” and “climate change” (i.e., the light blue-colored cluster in network diagram d).

The number of links and similarities between keywords increased in chronological order in network diagrams (a to d). Consequently, the closely packed nature of bubbles (or keywords) also increased in the same order. Furthermore, keywords with high similarity are grouped into 10, 6, 6, and 7 clusters in the a, b, c, and d keyword networks, respectively. The reduction in the number of clusters and the increase in links in chronological order indicate the growing trend of interdisciplinary research.

The most frequently occurring keywords identified in each period are associated with various disciplines related to oceanography, such as chemistry (water chemistry and geochemistry), ecology, marine biology, marine geology, climatology, and Geo-Information Science. The keyword analysis elucidated an increasing trend in interdisciplinary research. This interdisciplinary trend resulted from the gradual broadening of the scope of long-term hot topics such as Nitrogen biogeochemistry, taxonomy, and phytoplankton due to the intersection of knowledge from other disciplines and the emergence of new hot topics like ocean acidification and climate change, which have altered the direction of other disciplines. For instance, Nitrogen fixation was newly linked to climate change and ocean acidification during the recent decade (i.e., period (d)), unlike the previous decades. The keyword network (a) with a significantly higher number of clusters (i.e., 10) compared to (b), (c), and (d), with almost no links, can be explained by the change in research

Table 1

Frequency of occurrence (f) of most frequent author-keywords for (a) 1990 >, (b) 1990–2000, (c) 2001–2011, and (d) 2012–2022 periods.

No	(a) 1990>		(b) 1990–2000		(c) 2001–2011		(d) 2012–2022	
	Keyword	f	Keyword	f	Keyword	f	Keyword	f
1	Taxonomy	8	Nitrogen	52	Stable isotopes	270	Stable isotopes	496
2	Marine geology	7	Phytoplankton	41	Nutrients	251	Taxonomy	419
3	Estuaries	7	Indian Ocean	38	New species	195	Climate change	402
4	Photosynthesis	7	Pacific Ocean	32	Taxonomy	195	Ocean acidification	377
5	Nutrients	7	Geochemistry	32	Phytoplankton	193	Nitrogen	363
6	Sediments	6	Atlantic Ocean	28	Baltic sea	186	Phytoplankton	359
7	Hydrocarbons	5	Taxonomy	28	Nitrogen	178	Nutrients	315
8	Nitrogen	5	Nutrients	27	Phosphorus	153	New species	325
9	Phosphorus	5	Mid-Atlantic ridge	23	Eutrophication	148	Geochemistry	284
10	Atlantic Ocean	5	Phosphorus	22	Geochemistry	137	Phosphorus	218

interests over its relatively longer period and the lack of interdisciplinary research.

Furthermore, “Stable isotopes” remained the most frequent keyword in the last two consecutive decades, respectively, owing to the wide range of applications of stable isotope-based methodologies in N research. These applications include studies on biogeochemical processes and cycles, source tracking [46], food web tracking, and paleoecology, which would be difficult through other means. However, stable isotope-based methodologies in P research studies are rare since P does not have a convenient stable isotope similar to ^{15}N for N [47]. Nevertheless, isotopic ratios of ^{18}O in PO_4^{3-} ions have been used to track the P cycle in the ocean [48]. A notable feature is that climate change and ocean acidification have suddenly emerged as the third and fourth most frequent keywords in the last decade, indicating a rapid increase in research interest in these phenomena. Recent studies have shown that climate change effects, especially ocean warming, changes in precipitation patterns, and salinity changes, act as important drivers that could alter marine nutrient cycles. Studies have indicated that ocean warming alters the microbial community structure [49], the productivity of N fixers, and the ability of phytoplankton to assimilate N in different patterns. For example, two Cyanobacterium genera, *Trichodesmium* and *Crocospaera*, account for nearly half of global biological N fixation and are abundant in tropical and subtropical oceans [50]. Yang et al. (2021) [51] showed that the *Crocospaera* genus could increase N fixation and growth rates under iron-limited conditions from 22 °C to 27 °C. However, these rates declined from 27 °C to 32 °C, temperatures predicted for most of the tropical oceans in the future. In contrast, the *Trichodesmium* genus could continuously increase N fixation and growth rates from 22 °C to 32 °C under iron-limited conditions [52]. This demonstrates diverse responses to the nutrient cycle by microbial communities caused by ocean temperature rise, and their synergistic effects need further study. Furthermore, changes in rainfall patterns can alter the N and P loads received by coastal waters through surface runoff [53,54], and salinity reductions in coastal regions caused by excessive rainfall could reduce the N uptake ability of phytoplankton [55].

Taxonomy has been a prominent research interest in N and P studies in the ocean for an extended period. N and P are essential nutrients for marine food webs, where primary producers primarily rely on them for their nutritional requirements. Moreover, ocean microorganisms play a vital role in ocean nutrient cycles, especially in the N cycle [56]. Much research on taxonomy focused on the interdependence of microbial community structure and N and P stoichiometry in seawater [57–59], N and P uptake rates of different primary producer species, and the factors governing them [60]. Additionally, research has delved into the nutrient acquisition potential of saltwater microbes [61], nutritional grouping [62], microbial nitrification and denitrification [63,64], N fixation rates of different taxonomical groups [65], and metagenomic studies on microorganisms involved in nutrient cycles [66,67]. These investigations serve as a driving force behind the enduring prominence of taxonomy as a research hotspot in the context of N and P studies in the ocean.

Many recent studies report a significant decrease in the pH level of seawater, indicating increasing ocean acidification [68]. Ocean pH levels have currently reached an average of 8.1 (compared to around 8.2 during preindustrial times), reflecting a 25–30% increase in acidity compared to preindustrial times. This increase is primarily caused by the rise in atmospheric CO_2 due to anthropogenic activities and excess nutrients in coastal waters [69,70]. Excess nutrients, primarily N and P, in coastal waters—resulting from anthropogenic inputs such as the disposal of sewage, wastewater, and industrial effluents or atmospheric deposition—cause algal and plankton blooms. Upon the fall of these blooms, large amounts of CO_2 are released into coastal waters due to their decomposition [71]. Furthermore, acidification could trigger microbial community responses, perturbing oceanic nutrient cycles. For example, this could affect the ocean N cycle by considerably reducing ocean nitrification rates, leading to a shift in the microbial community structure of Ammonia oxidizers [72,73]. Different N fixer species have shown contrasting responses to ocean acidification concerning their growth rates and productivity [74,75], necessitating further research to understand the synergic response of N fixers to ocean acidification. On the other hand, acidification could intensify the eutrophication of phosphorus-limited seas by reducing P mineralization rates and releasing P in sediments into seawater [76].

Eutrophication has been a significant focus of research for the past three decades. In the last two decades (c and d), the Baltic Sea has been a hotspot for eutrophication studies. In the most recent decade (d), the East China Sea, South China Sea, Yellow Sea, Bay of Bengal, and Arabian Sea have also emerged as hotspots for eutrophication research. Despite various actions taken to reduce anthropogenic nutrient inputs to the region through the Baltic Sea Action Plan [77], recent studies demonstrate that the Baltic Sea still suffers from severe eutrophication conditions [78,79]. Furthermore, remote sensing is gaining popularity as a preferred medium for observing the eutrophication status and associated factors such as nutrient concentrations. Conversely, Artificial Neural Networks (ANN) did not appear among the prominent 100 keywords due to the scarcity of ANN-based N and P studies compared to other

Table 2

Top 10 countries of N and P research in the ocean in the recent decade and their prominent author-keywords.

Country	Keywords in descending order of their occurrence
USA	Climate change, Stable isotopes, Ocean acidification, Phytoplankton, Denitrification
China	Geochemistry, Phytoplankton, Ocean acidification, Sediments, Eutrophication
Germany	Stable isotopes, Ocean acidification, Taxonomy, Climate change, new species
United Kingdom	Climate change, Ocean acidification, Stable isotopes, Taxonomy, Phytoplankton
France	Stable isotopes, new species, Phytoplankton, Climate change, Taxonomy
Japan	Taxonomy, Seismic tomography, new species, Ocean acidification, morphology
Australia	Climate change, Ocean acidification, Taxonomy, New species, Geochemistry
Canada	Stable isotopes, Climate change, Nitrogen, Phytoplankton, Sea ice
Spain	Stable isotopes, new species, Climate change, Taxonomy, Ocean acidification
Russia	Taxonomy, Morphology, Stable isotopes, new species, Organic matter

Table 3

Methods used by researchers for measuring N and P concentrations in seawater, their usage and example studies.

Analytical method	Description (chemical process/subcategory)	N and P forms measured	Example studies	
Spectrophotometry or Colorimetry (Manual or automated flow analysis)	Azo dye method: NO_2^- react with an aromatic amine producing an azo dye which is quantified by the absorption of light	NO_2^-	[86–111]	
	Hypobromite oxidation (NH_3 and NH_4^+ are oxidized to NO_2^- by BrO^-) and then subject to the azo dye method (detection limit $\sim 0.02 \mu\text{mol L}^{-1}$) [99,112]	NH_3 NH_4^+	[91,92,99,107]	
	NO_3^- reduced to NO_2^- by a Cu-coated Cd granular reductor followed by the azo dye method (detection limit $0.7\text{--}70 \mu\text{mol L}^{-1}$) [113]	$\text{NO}_2^- + \text{NO}_3^-$	[86–106,108,110,111, 114–118]	
	NO_3^- reduced to NO_2^- by a Zn-coated Cd granular reductor followed by the azo dye method	$\text{NO}_2^- + \text{NO}_3^-$	[107,119]	
	Vanadium (III) reduction (V^{3+} in dilute acid solution reduce NO_3^- to NO_2^- and/or NO) followed by the azo dye method	$\text{NO}_2^- + \text{NO}_3^-$	[109]	
	Indophenol blue method (Berthelot's method): NH_3 , excess NaClO and phenol (or sodium salicylate) catalyzed by $[\text{Fe}(\text{CN})_5\text{NO}]^{2-}$ react to form indophenol blue and quantified	NH_3 NH_4^+	[86–88,93,94,96–98,100, 102–104,108,110,116,117, 120]	
	Molybdenum blue method (Phosphomolybdic acid method): $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24}\cdot 4\text{H}_2\text{O}$ and $\text{K}(\text{SbO})\text{C}_4\text{H}_4\text{O}_6$ react in an acidic medium with PO_4^{3-} to form an Antimony-phospho-molybdate complex which is reduced to a blue colour complex by $\text{C}_6\text{H}_8\text{O}_6$ (detection limit $0.03 \mu\text{mol L}^{-1}$) [121]	PO_4^{3-}	[86–91,94–98,101–103, 105–107,109,111,114,115, 117–119,122,123]	
	For ultra-low DIP concentrations	Long flow cell (Liquid Waveguide Capillary Flow Cell (LWCC)) (detection limit $0.002 \mu\text{mol L}^{-1}$) [124]	PO_4^{3-}	[116,124]
		Magnesium Induced Co-precipitation (MAGIC) technique (detection limit $0.0005 \mu\text{mol L}^{-1}$) [124]	PO_4^{3-}	[124,125]
	Alkaline Persulfate digestion method: Oxidative reagent ($\text{H}_3\text{BO}_3 + \text{NaOH} + \text{K}_2\text{S}_2\text{O}_8$) is added and heated to oxidize DON and DOP into DIN and DIP and measured with Molybdenum blue and Cu–Cd reduction methods respectively.	TDP TDN TP	[86,90,93,102,103,106, 111,116,123,126–128]	
	Acid Persulfate digestion method: Particulate fraction filtered and dried. Digested with HCl to extract PIP and PIN. Residue digest with $\text{K}_2\text{S}_2\text{O}_8$ and heat to extract POP and PON. PIP and PIN are measured with Molybdenum blue and Cd reduction methods. POP and PON are measured separately	POP PON PIP PIN	[129]	
	Diacetyl monoxime method: Diacetyl monoxime breaks down into diacetyl when heated. Diacetyl and urea then condense in the same strong acidic medium to form the yellow-colored diazine product. Diazine is stabilized by Thiosemicarbazide and converted to a pink-colored complex in the presence of Fe^{3+} ions	Urea	[130]	
	Sequential Extraction method (SEDEX): TPP is chemically fractionated into 5 operationally defined PP species. PP species are sequentially extracted and measured.	TPP	[126,131,132]	
Fluorometry	OPA method: Oxidative reagent ($\text{Na}_2\text{SO}_3 + \text{Sodium Borate} + \text{Ortho-Phthalaldehyde (OPA)}$) added and fluorescence measured (detection limit $\sim 0.007 \mu\text{mol L}^{-1}$) [133,134]	NH_3 NH_4^+	[90,101,109,116]	
	pH-induced Ammonia gas diffusion method: NH_4^+ diffusion as NH_3 across a microporous hydrophobic Teflon membrane into a flowing stream of OPA reagent to produce a fluorescent adduct	NH_3 NH_4^+	[118]	
	O-Phthalaldehyde fluorometric method: O-Phthalaldehyde reacts with primary amines in the presence of 2-mercaptoethanol to form a highly fluorescent compound	Amino acids	[130]	
High-performance liquid Chromatography (HPLC)	Reverse Phase HPLC (RP-HPLC) (detection limit $7 \mu\text{mol L}^{-1}$) [113]	Amino acids	[93,104,135,136]	
High-temperature catalytic oxidation (HTCO) method	A thermal analyzer is coupled to a chemiluminescence detector. The detector measures NO_x gases produced by combustion of all N species in the presence of a catalyst	TDN	[100,137]	
Elemental analysis	Particulate sample separated by filtration. The residue burned at high temperature. Gaseous sample sent to a chromatography column with a noble gas as the carrier	TPN PON	[86,100,103,126]	
Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES)	PP digestion with reverse aqua regia and dilution. Filtration through $25\text{-}\mu\text{m}$ filter paper and measurement of TP in an ICP-OES apparatus	TPP	[86,122]	

(continued on next page)

Table 3 (continued)

Analytical method	Description (chemical process/subcategory)	N and P forms measured	Example studies
Microfluidic Nutrient Sensors	A chip with miniaturized colorimetric sensors, actuators, and control systems, capable of automated laboratory chemical processes by manipulating fluids through submillimeter scale channels	NO_3^- PO_4^{3-}	[138,139]
Microfiber Mode Interferometric Sensor	Obtain ultra-high Refractive Index sensitivity by manipulating the physical parameters of the microfiber to achieve the Dispersion Turning Point (DTP) of the interference spectrum and determining the concentration by analyzing the output spectrum	NO_3^-	[140]
Electrochemical sensors	Detecting changes in electrical current or voltage produced when specific nutrient molecules undergo chemical reactions at the sensor's surface (detection limit $10\text{--}1000 \mu\text{mol L}^{-1}$) [113]	NO_3^- PO_4^{3-}	[113]
Remote sensing	Hyper-spectral image sensor: Having many narrow bands	TN DIN	[92]
	Multispectral image sensor: Having a limited number of bands	TN TP DIN	[27,141–143]

Note: TN = Total Nitrogen, TP = Total Phosphorus, DIN = Dissolved Inorganic Nitrogen, DIP = Dissolved Inorganic Phosphorus, TPN = Total Particulate Nitrogen, TPP = Total Particulate Phosphorus, PON=Particulate Organic Nitrogen, POP=Particulate Organic Phosphorus, TDN = Total Dissolved Nitrogen, and TDP = Total Dissolved Phosphorus.

analytical methods.

For the spatial analysis of research interests, we selected the top 20 countries that have published research articles on N and P-related studies in the ocean during the recent decade (i.e., period (d)). These countries, listed in descending order based on their number of publications, include the USA, China, Germany, United Kingdom, France, Japan, Australia, Canada, Spain, Russian Federation, India, Brazil, Norway, Italy, Netherlands, South Korea, Mexico, Sweden, Denmark, and Portugal. The prominent author keywords of the top 10 countries are presented in Table 2 in descending order of occurrence. A notable feature is that, despite taxonomy being the second most prominent keyword in the recent decade (Table 1), it did not rank among the top two keywords for most of the top 10 countries, except for Japan and Russia, as indicated in Table 2. Conversely, climate change and ocean acidification, within the context of N and P research in oceans, were studied by most of the top 10 countries. This suggests that these countries were exploring new dimensions in N and P research in addition to the long-standing research hotspots. The G20 intergovernmental forum comprises the world's largest and most advanced economies, representing more than 80% of the world GDP [80]. Among the top 20 countries contributing to N and P research in oceans, only Norway and Switzerland, both high GDP economies, are not part of the G20. Therefore, both high budgetary allocations and special efforts of the G20 to achieve a sustainable marine economy and reduce unsustainable pressures on the marine environment, such as the G20 Action Plan on Marine Litter, may have driven many publications [81]. This indicates that macroeconomic factors played a crucial role in the scientific advancement in the context of N and P research in oceans. Being highly industrialized countries already sensing the impacts of climate change, the USA, United Kingdom, and Australia have focused much on climate change impacts associated with the N and P cycles in the ocean. On the other hand, although China was the second-largest publisher in the context of N and P research in the ocean, they did not considerably focus on the climate change impacts on the topic. Furthermore, Japan and Russia, followed by Germany, the United Kingdom, and Australia, gave more attention to taxonomy due to high biodiversity [82] and oceanic resource exploitation in those regions.

Table 4

Methods used by government monitoring programs for measuring N and P concentrations in seawater.

Country and the institution	N and P forms measured	Analytical method
US EPA	$\text{NO}_2^- + \text{NO}_3^-$	Reduction by Cu-coated Cd granular column and colorimetric analysis by azo dye method
	NH_3	Indophenol blue method (Berthelot's method)
	PO_4^{3-}	Molybdenum blue method (Phosphomolybdic acid method)
	TN	Persulphate digestion
	TP	Persulphate digestion
Australian Coastal Water Quality Monitoring Program	$\text{NO}_2^- + \text{NO}_3^-$	Reduction by Cu-coated Cd granular column and colorimetric analysis by azo dye method
	NH_3	Titrimetric method: raising the pH of a water sample to convert NH_3 to NH_4^+ ions, followed by titration with a standardized acid solution using a pH indicator to determine the endpoint.
	Total Kjeldahl Nitrogen	Ion Selective Electrode Method Macro-Kjeldahl Method or Semi-Micro-Kjeldahl Method
	PO_4^{3-}	Molybdenum blue method (Phosphomolybdic acid method)
	TP	Persulphate digestion method

3.2. Different methodologies used in measuring and analyzing N and P in marine environments

Various methods for measuring different N and P species in seawater in the recent decade are summarized in Table 3. The studies published during this period, which were critically reviewed to identify the usage of these methods, are provided as examples in the “Example studies” column. Additionally, to identify discrepancies between the methods employed by these researchers and those used in regular government monitoring programs, we present the methods utilized by the United States Environmental Protection Agency (US EPA) and the Australian coastal water quality monitoring program in Table 4 [83,84]. While numerous other government monitoring programs exist, such as the water quality monitoring program in China, their data and employed methods are not publicly available and are challenging to access [85].

Chemical analytical methods are more commonly practiced at present compared to novel sensing methods like microfluidic sensors or remote sensing. Nevertheless, it has been observed that sensing methods are emerging as easy-to-use and efficient alternatives. However, government-initiated monitoring programs and the industry still rely on conventional chemical analytical methods. The frequency of use, accuracy, reliability, cost, and drawbacks of the commonly used methods for measuring N and P species are summarized in Table 5. Most of the commonly used methods are economical, with the exception of RP-HPLC and elemental analysis.

Seawater chemistry is more complex compared to freshwater. Seawater contains numerous macro elements or major ions, such as Na^+ , Br^- , Cl^- , SO_4^{2-} and HCO_3^- . In contrast, N and P species are present in much lower concentrations [144]. Hence, measuring N and P in seawater is challenging due to low analyte concentrations, matrix complexity (interferences from major anions), and variable salinity [14,145]. As an example, the nitrate electrode method is frequently used for nitrate measurement in freshwater, but it cannot be applied to seawater due to the interferences from Cl^- , Br^- and HCO_3^- [146]. Therefore, methods used for freshwater analysis are not always applicable to seawater.

Dissolved Inorganic N and P forms (DIN and DIP) in seawater are usually measured by forming a colored compound through manual or automated chemical processes, as outlined in Table 3. The concentration of the developed colored compound is subsequently measured by colorimetry or spectrophotometry, and the concentration of the considered N and P species is determined accordingly. However, the accuracy of such methods depends on both chemical interferences and the sensitivity of the colorimetric or spectrophotometric detectors [14]. While there are other methods available for NO_3^- measurement, the Cu–Cd reduction spectrophotometry method, presented in Tables 3, is highly preferred by many researchers for seawater analysis. This preference is due to its cost-effectiveness, sensitivity, specificity, and higher reduction efficiency compared to other reduction methods [147,148]. For instance, methods such as direct UV spectrophotometry screening, HPLC, Electrochemical sensors, and Automated hydrazine reduction can be used in NO_3^- analysis in water samples. However, the direct UV spectrophotometry screening method is strongly interfered by the presence of organic matter, posing significant challenges in seawater analysis [149]. While HPLC is a complex and expensive technique with a detection limit higher than the Cu–Cd reduction method (due to the higher detection limit, HPLC would also not be suitable for oligotrophic seawaters), it would not be effective in measuring minor ions present in the seawater. However, HPLC is effective in measuring major ions in seawater like Mg^{2+} , Ca^{2+} , and Cl^- [113,150]. Since the Nitrate range in the ocean is generally less than $2 \mu\text{mol L}^{-1}$, Cu–Cd reduction spectrophotometry offers a better detection range compared to other methods [113, 151]. The automated hydrazine reduction method is inapplicable to seawater analysis due to interference by Mg^{2+} ions [152].

As presented in Table 3, several methods have been used for NH_3 measurement, including the indophenol blue method,

Table 5
Summary of usage, accuracy, reliability, cost, and drawbacks of commonly used methods for N and P measurement.

Method	N and P form	Frequency of use	Accuracy	Reliability	Cost	Drawbacks
Azo dye method	NO_2^-	High	High	High	Economical	H_2S may interfere if present in the sample
Cu–Cd reduction azo dye method	$\text{NO}_2^- + \text{NO}_3^-$	High	High	High	Economical	Column maintenance needed, difficult to automate, and time-consuming
Indophenol blue method	NH_3	High	High	Medium	Economical	Presence of high and variable blanks, insensitivity to sub-micromolar concentrations, the toxicity of reagents used (phenol), and time-consuming.
Hypobromite oxidation azo dye method	NH_3	Low	High	Medium	Economical	Narrow detection range and need for ambient air conditions
OPA method	NH_3	Low	High	High	Economical	Only applicable to nanomolar levels of NH_3 concentration, Interfered by Amino acids
Molybdenum blue method	PO_4^{3-}	High	Medium	Medium	Economical	Poor reagent stability, interfered by NO_2^- , AsO_4^{3-} , & F^-
Persulphate digestion method	TDP TDN	Medium	Medium	High	Economical	Some N–O & N–N bonds may not be converted completely, not suitable for extreme levels of N
RP-HPLC	Amino acids	Low	High	High	Expensive	Accuracy affected at very low concentrations
Elemental analysis	TPN PON	Low	High	Medium	Expensive	Samples need to be completely dry & in powder form & Sample size needs to be large enough for the vial

Note: “Expensive” denotes methods that typically require expensive instrumentation, consumables, or maintenance costs. Conversely, “economical” indicates methods that are relatively cost-effective, requiring fewer resources or less sophisticated instrumentation for implementation.

fluorometric method, and hypobromite oxidation pink azo dye method. However, the indophenol blue method remains the most common method due to its analytical simplicity. Nevertheless, it has certain drawbacks such as the presence of high and variable blanks, insensitivity to sub-micromolar concentrations, and the use of toxic reagents (phenol). In contrast, the OPA fluorometric method can measure many sub-micromolar concentrations without using hazardous reagents [133,134]. However, the OPA method is not suitable for NH_3 concentrations greater than micromolar levels since the water sample needs to be extremely diluted to fit the linearity range of the method, and such dilutions may lead to severe errors [153]. Moreover, the Molybdenum blue spectrophotometry method (Phosphomolybdic acid method) was extensively used for Phosphate measurement. However, when the Phosphate concentration of seawater is lower than $0.03 \mu\text{mol l}^{-1}$, Long flow cell (Liquid Waveguide Capillary Flow Cell (LWCC)) technique or Magnesium-Induced Coprecipitation (MAGIC) procedure can be used together with the Molybdenum blue method.

Many studies examining nitrogen and phosphorus concentrations in oceans primarily concentrated on assessing organic nitrogen forms like urea and amino acids, collectively referred to as dissolved organic nitrogen (DON), instead of individually quantifying each species by subtracting dissolved inorganic nitrogen (DIN) from total dissolved nitrogen (TDN) ($\text{DON} = \text{TDN} - \text{DIN}$). A similar method is also employed for DOP ($\text{DOP} = \text{TDP} - \text{DIP}$) [90,123,154]. However, certain authors have measured urea and amino acids as individual components of DON using RP-HPLC, fluorometric, and spectrophotometric methods [93,130,135]. Liquid chromatography was not commonly used for other analytes, such as nitrates and Phosphates, due to the interferences and damage to analytical columns by the salt matrix [155]. Nevertheless, a desalter membrane column can effectively remove this salt matrix, allowing the filtrate to be subjected to ion chromatography for the determination of desired trace analytes [156]. Recent developments showed that Ion chromatography coupled with High-resolution Electrospray Ionization Mass Spectrometry (ESI-MS) with ^{18}O labeled phosphate can be used to measure bioavailable P (i.e., phosphate) in surface water, without bias from the effects of adsorption and uptake by microorganisms [157]. However, the applicability of this method, coupled with a desalination column, for seawater analysis should be further investigated.

Particulate N and P species include PIN, PON, PIP, and POP. The persulfate digestion method is employed to oxidize DON and DOP forms into DIN and DIP. Subsequently, these DIN and DIP forms are measured using the methods mentioned previously. For particulate fractions, an acidic persulphate digestion can be performed, where PIN and PIP are first digested using HCl or H_2SO_4 and measured. Following this, DON and DOP are oxidized into DIN and DIP using persulphate oxidative reagent. Finally, concentrations of DIN, DIP, DON, and DOP in the particulate fraction can be separately measured [129]. Elemental analysis and Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES) were used for direct analysis of TPN and TPP, respectively. If the particulate fraction is extracted and dried, the Persulfate digestion method can be used for both TPN and TPP measurement upon digestion by HCl.

Microfluidic Nutrient Sensors, Microfiber Mode Interferometric Sensors, and Remote sensing can be identified as promising technologies emerging as efficient alternatives to conventional laboratory measurements. Microfluidic sensors do the same chemical reactions as in conventional laboratory tests but precisely control fluids on a micro-scale with very low reagent usage, enabling real-time, high-quality, and high-resolution data acquisition for different N and P species [158,159]. However, commercial applications are still scarce except for a few rare occasions [160]. The lack of commercial applications is attributed to drawbacks such as the use of conventional hydraulic equipment for operation and electronics for sensing, which increases the size and cost of the ultimate platforms, hindering the possibility of commercialization [161]. Nevertheless, this technology is rapidly evolving with promising results. Beaton et al. (2022) [139] recently demonstrated that Microfluidic sensors can even be used in deep-sea environments by developing a low-cost interflow mixer. Moreover, intelligent microfluidic sensor systems developed with the Internet of Things (IoT) and machine learning can potentially facilitate the collaboration of local microfluidic elements located across the globe into a coordinated global network of a microfluidic system for nutrient monitoring in the future [162]. On the other hand, Microfiber Mode Interferometric Sensors are an accurate optical detection method suitable for in-situ monitoring of seawater. They are economical, easy to construct, highly precise and sensitive [140].

The usage of Remote sensing techniques for nutrient detection in seawater is still at an experimental stage. Studies have been done using both Hyper-spectral and Multispectral image sensors to determine the applicability of remote sensing techniques for N and P species detection in the ocean. Hyperspectral image sensors have a high spectral resolution with low spatial resolution, while multispectral image sensors have a low spectral resolution with a high spatial resolution [163]. The applicability of Multispectral image sensors has been studied extensively for N and P spatial distribution analysis due to their high spatial resolution.

Nevertheless, studies have been conducted to identify both optically active variables, such as turbidity, and optically inactive variables, such as DIN. Neural network and machine learning approaches [143], or Multiple Stepwise Regression (MLSR) models, are commonly used to correlate spectral band reflectance parameters with concentrations of N and P species [141]. Yu et al. (2016) [92] identified DIN concentrations in the Bohai Sea using hyperspectral remote sensing data by correlating the reflectance of remote sensing to DIN in-situ concentration measurements using Multiple Stepwise Regression (MLSR) analysis for certain band combinations and observed a high accuracy in results. Soomets et al. (2022) [141] estimated TP and TN concentrations in the North-Eastern Baltic Sea using MLSR on atmospherically corrected angular dependent water-leaving reflectance data for regions that were more than 1 km away from the shoreline (to eliminate the effects of coast or seafloor) obtained from multispectral remote sensing and yielded accurate results for TN estimations while TP estimations were weaker. Nevertheless, Arango and Nairn (2020) [27] estimated both TN and TP in both oligotrophic and eutrophic systems with reflectance data obtained from multispectral remote sensing using MLSR to a considerable accuracy. This difference in results can be rationalized by the slight difference in bands used in the two studies. Arango and Nairn (2020) [27] used a small Unarmed Aerial Vehicle (UAV) instead of satellite-derived data, eliminating the requirement for atmospheric corrections for reflectance values. Arango and Nairn (2020) [27] also used a rather sophisticated methodology, whereas Soomets et al. (2022) [141] used the point extraction method for reflectance extraction, considering each station as its georeferenced

position. Arango and Nairn (2020) [27] used two additional extraction methods called buffer extraction (a buffer zone was defined based on the offset distance of the GPS units around the georeferenced position of each sampling station to extract the average reflectance) and Kriging extraction (kriged surfaces were developed for each analyte) except for point extraction.

Wang et al. (2018) [143] estimated the concentrations of nitrate and phosphate in seawater using both multispectral image reflectance data and satellite-retrieved sea surface salinity data as inputs to a three-layered Artificial Neural Networks (ANN)-based model, getting better results compared to other ANN models that used only the reflectance data [164]. Wang et al. (2018) [143] estimated the nitrate and phosphate concentrations with a mean relative error of 18.2% and 17.2%, respectively, compared to field measurements. It can be said that these error values are on par with the error values obtained in previously mentioned multispectral image data studies. However, an absolute comparison is challenging between the mentioned neural network-based and MLSR-based studies due to the difference in demographics, used spectral bands, and considered analytes.

4. Conclusions

Nitrogen and Phosphorus research has played a starring role in the advancement of knowledge in modern oceanography, with various research hot topics emerging in different periods and regions. The number of journal articles published per year has been consistently increasing, and about 2000 articles are published annually. Taxonomy and nitrogen biogeochemistry were the most prominent research hotspots before 2000, respectively. Stable isotope-based tracking of N and P sources, marine food webs, and biogeochemical processes have become the most prominent research hot topic in the most recent decades. Climate change and ocean acidification suddenly emerged as research hotspots in the recent decade and were linked to many other research hotspots, indicating the trend to investigate climate change effects on oceanographic processes involving N and P biogeochemistry. It was observed that the trend of interdisciplinary research has been remarkably increasing in chronological order due to the broadening of knowledge in various long-term hot topics.

Measuring N and P species in seawater is challenging due to the complexity of seawater chemistry. The most common methods used for measuring N and P species included spectrophotometric or colorimetric azo dye methods for NO_2^- , Cu–Cd reduction azo dye method for NO_3^- , indophenol blue method, hypobromite oxidation azo dye method, and OPA method for NH_3 , Molybdenum blue method for PO_4^{3-} , persulphate digestion method for TDN and TDP, RP-HPLC for amino acids, and elemental analysis for TPN and PON. However, this review identified numerous other methods available for the measurement of various N and P species. When selecting an appropriate measurement method, considerations such as detection ranges, interferences by other ions or compounds, required accuracy, reliability, cost, and time constraints should be taken into account.

Microfluidic nutrient Sensors, Microfiber Mode Interferometric Sensors, and remote sensing were identified as emerging novel methods for N and P monitoring in seawater. Microfluidic Nutrient sensors were rarely used on a commercial basis due to practical technological constraints, but they are advancing at a fast pace with promising results. Remote sensing techniques were also utilized as cost-effective continuous monitoring systems, and their usage is still at an experimental stage but with promising results. Both hyperspectral and multispectral image sensors have been used for this purpose, while the latter is preferred over the former due to its high spatial resolution. Neural networks and MLSR models are used for remote sensing data analysis, and both optically active and inactive N and P species have been determined with considerable accuracy. These emerging technologies show great potential for revolutionizing N and P monitoring in seawater and have the capacity to study and manage nutrient dynamics in the world's oceans. As research continues to evolve, interdisciplinary collaboration and the adoption of cutting-edge technologies will be instrumental in addressing the complex and pressing issues related to marine N and P biogeochemistry in the face of a changing climate and ocean acidification.

Ethical approval

No ethical problems are associated with our work.

Availability of data and materials

Data and materials will be made available on request.

CRedit authorship contribution statement

Hasitha Siriwardana: Writing – original draft, Visualization, Formal analysis, Data curation. **R.S.M. Samarasekara:** Supervision, Project administration, Funding acquisition, Conceptualization. **Damsara Anthony:** Visualization, Software. **Meththika Vithanage:** Writing – review & editing, Supervision, Resources.

Declaration of competing interest

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