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# Forms, Importance and Sources of Dissolved Organic Nitrogen (DON) in the Environment: A Review

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**Abstract** Dissolved organic nitrogen (DON) is an integral part of the dissolved organic carbon (DOC) reservoir, encompassing substances with nitrogen content. Within aquatic ecosystems such as lakes and rivers, DON molecules originate from photosynthetic entities like algae and plants, as well as the discharge of nitrogenous waste from mammals. Additional pathways introducing organic nitrogen into water include soil leaching, sewage discharge, and air deposition. Given the predominantly biological origin of most DON molecules, the pool comprehensively encompasses nitrogenous compounds present in living organisms. Notably, prevalent components of freshwater DON comprise proteins, unbound amino acids, amino sugars derived from cell walls, and nucleic acids sourced from RNA and DNA. Additionally, waste products like urea and methylamines are commonly detected in these environments. It is noteworthy that prevailing wastewater treatment methods predominantly target the removal of dissolved inorganic nitrogen (DIN) compounds, often overlooking non-reactive DON. Consequently, untreated DON significantly contributes to the overall nitrogen load. In wastewater treatment facilities (WWTPs) employing biological nitrogen removal (BNR) processes, DON constitutes the majority of the nitrogen present in the effluent. Although dissolved combined amino acids (DCAA) and dissolved free amino acids (DFAA) collectively contribute less than 4% and 1%, respectively, to the DON in the effluent, the main challenge lies in simultaneously maximizing the removal of DIN and the recovery of DON.

**Keywords:** Dissolved Organic Nitrogen (DON); Importance of DON; Atmospheric DON Deposition; Wastewater; Landfill Leachate.

## 1 Introduction

DON designates the nitrogen-enriched segment of the dissolved organic carbon (DOC) pool, and these compounds are capable of passing through filters featuring a pore size of 0.1 µm [1], [2]. Being the nitrogen-containing fraction of dissolved organic matter (DOM), DON is a nitrogen reservoir with biological reactivity that has the potential to degrade water quality in nitrogen-sensitive environments within aquatic ecosystems [3], [4]. Within the aquatic ecosystems, DON, unlike inorganic nitrogen forms such as nitrate, nitrite, and ammonium, is likely made up of a diverse array of compounds with differing reactivity, bioavailability, and concentration [5]. These compounds encompass amino acids, urea, and humic substances, and when combined with inorganic nitrogen, they encourage the proliferation of phytoplankton and bacteria [6]–[8].

The detection of DIN is readily achievable in various aquatic environments, whereas the determination of DON necessitates multiple chemical analyses in order to obtain a singular measurement. Research on the occurrence and treatment of DON in waste streams that are abundant in N,

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such as sewage, landfill leachate, sludge return liquor, agricultural run-off, and similar sources, has only recently started to receive significant attention [9]. The concern arises because most N removal methods are primarily focused on addressing inorganic N, even though certain biological processes also contribute DON to the effluent. Notwithstanding these concerns, the majority of research endeavors concentrate on comprehending the behavior of DON in wastewater, whereas a limited number of studies have investigated the dynamics of DON in landfill leachate.

Wastewater treatment plants are globally implementing advanced treatment units in response to the enforcement of more stringent discharge limits for nitrogen and phosphorus [10]–[12]. Despite a notable reduction in the overall concentration of total dissolved nitrogen (TDN) in the effluent, there has been an observed increase in the relative contribution of DON to the TDN, rising from 10% to 23% in the upgraded WWTPs [30]. The effluent from (WWTPs continues to be the main source of DON in surface water [1], [13]. For instance, the period between 1994 and 2010 witnessed notable improvements in the WWTPs, leading to a significant decrease of 33% in the discharge of TDN into Long Island Sound [14]. However, during the same period, there was an observed increase of 20% in the loads of DON [14]. In comparison to DIN, DON can yield nearly ten times the amount of chlorophyll a/mg N, particularly the low molecular weight portion (LMW-DON, <1 kDa), which has the potential to stimulate the growth of phytoplankton [15]. The number of studies conducted on DON at WWTPs increased annually by 5 to 10% from 2003 to 2022, covering various topics, including its characterization, bioavailability, biological, and physicochemical treatment methods. In a recent investigation, the N composition in landfill leachate was examined across different stabilization phases, revealing that the DON content in landfill leachate varies between 16 mg/L and 218 mg/L.

Limited research has been conducted on the correlation between the discharge of DON and the proliferation of algae in aquatic ecosystems. This is despite the significant potential of diverse DON sources to greatly enhance the growth of indigenous microorganisms and detrimental algal species [8], [16]–[18]. Although Liu et al. (2012) conducted a laboratory-scale study that showed a decrease in the bioavailability of hydrophobic organic nitrogen to the chlorophyte *Selenastrum capricornutum* [19], there is currently a lack of comprehensive field studies examining the response of algal blooms to DON derived from landfills or other anthropogenic waste sources.

The present study undertook a comprehensive analysis of the prevailing scientific literature pertaining to the forms, importance and sources of the DON in the environment.

## 2 Forms and Importance of DON

Common forms of nitrogen that are found in aquatic environments include oxidized and reduced inorganic forms (nitrate, nitrite, ammonium, and ammonia), as well as organic molecules, dissolved and particulate forms, and various combinations of these [20]. Various concentrations of these nitrogen types are present across a broad spectrum. Particulate nitrogen refers to nitrogen particles that are larger than 200 nm, whereas dissolved nitrogen refers to nitrogen particles that are less than 200 nm [21]. This distinction between dissolved and particulate nitrogen is useful for studying and managing nitrogen cycling in a wide range of environments, including aquatic systems and wastewater treatment processes [22]–[24]. Understanding the nitrogen dynamics and reactivity in these systems requires this partitioning. Different nitrogen forms have different size ranges, which are shown in Figure 1. This graphic depiction is helpful because it allows one to visually investigate the size distribution of the various nitrogen species present in a particular environment.

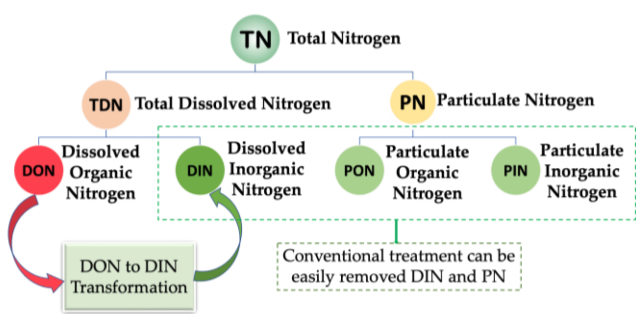


**Fig. 1** The size variation observed among different forms of nitrogen.

In conventional wastewater treatment systems, the particulate nitrogen is typically successfully reclaimed in an effective manner during the primary treatment phase, and the remaining nitrogen is subsequently removed through the use of biological treatment processes [25], [26]. DIN is the species that can be eliminated with the highest degree of effectiveness. Because DON has a lower reactive potential than DIN, recovery techniques frequently target DIN as well. However, due to the fact that DON is recovered in a less effective manner, it has the potential to bypass treatment systems and become a significant component of the TN found in effluent.

## 3 Sources of DON

The DON that is found in marine ecosystems can be broken down into two primary source categories: native sources, which means that it is produced naturally within the ecosystem itself, such as in the water column, and nonnative sources, which means that it is brought in from outside sources, such as wastewater.

### 3.1 Native Sources

Various species, such as phytoplankton, nitrogen ($N_2$)-fixing organisms, bacteria, micro- and macrozooplankton, and viruses, are capable of generating dissolved organic nitrogen (DON) in aquatic environments.

#### 3.1.1 Phytoplankton and $N_2$ Fixers

Phytoplankton have the ability to produce DON through multiple mechanisms, such as active excretion or efflux, passive diffusion of metabolic byproducts across cell membranes, or release facilitated by trophic interactions when influenced by zooplankton or viruses [27]–[29]. Nitrogen-fixing organisms can serve as a substantial source of newly fixed N within marine ecosystems situated in tropical and subtropical regions [30], [31]. *Trichodesmium*, a colonial cyanobacterium that does not have heterocysts, plays a vital function as the main nitrogen fixer in maritime environments [32]–[34].

Both the overflow model and the passive diffusion model were proposed by Fogg in 1966 as separate theoretical frameworks for instant release [35]. Exudation is a phenomenon characterized by the accumulation of an excessive amount of photosynthate, which is primarily attributed to nutrient limitations, as postulated in the overflow model. Autolysis, in which an organism produces enzymes that break down its own cell membranes, frequently resulting in cell disintegration, is another direct release mechanism. Other examples include the reaction to changes in osmotic pressure, the loss of inorganic or organic nitrogen due to prolonged exposure to light, and other similar processes [36]. Based on the passive diffusion concept, the liberation of DON from the cell takes place through the diffusion of LMW molecules from concentrated intracellular reservoirs to the less concentrated extracellular environment facilitated by the cell membrane [37]. According to Hasegawa et al.'s findings, there is evidence indicating that smaller plankton exhibits a higher efficiency in the release of DON compared to larger plankton [37]. In regions of upwelling in the central Atlantic, characterized by the prevalence of phytoplankton larger than 2 µm, the proportion of total nitrogen acquisition released as DON is less than 30% [38]. Conversely, in oligotrophic regions characterized by the prevalence of picophytoplankton, the fraction of total nitrogen released as DON frequently surpasses 50%, albeit with significant variability in this ratio [38].

*Trichodesmium* is recognized for its contribution to nitrogen fluxes in marine environments. Methods like the release of amino acids, DON, and ammonium ($NH_4^+$) facilitate these direct activities [39], [40]. Additionally, *Trichodesmium* indirectly influences nitrogen fluxes by facilitating the transformation of DIN and DON. This process is aided by the presence of bacteria and grazers that coexist within *Trichodesmium* colonies [39], [40]. Marine cyanobacterium *Trichodesmium* is found in the Gulf of Mexico, is responsible for the release of a significant amount of nitrogen. Specifically, it releases approximately 40-51% of the nitrogen it has previously fixed. This release consists of approximately 25% to 50% NH4+, while the remaining portion is likely attributed to DON [41]. *Trichodesmium* exhibits a propensity for dissemination within the Caribbean Sea and the Atlantic Ocean, during which it has the capacity to discharge up to 50% of the nitrogen it had previously assimilated in the form of DON [42]. Researchers looked at how quickly surface waters fix nitrogen and how much DON is released as a result [43]. This investigation was carried out along a transect that spanned the Atlantic Ocean at a latitude of -24.5° N. The analysis focused on two distinct fractions: those smaller than 10 µm and those larger than 10 µm [43]. Distinct size fractions did not show any statistically significant differences in the DON release rates, which varied from 0.001 to 0.09 nmol N/L/h. According to the findings, about 23% of the total nitrogen fixation was released as DON for particles less than 10 µm and 14% for particles bigger than 10 µm [43]. Furthermore, it is anticipated that the subtropical Atlantic Ocean *Trichodesmium* colonies would release around 0.32–15 nmol of nitrogen per colony per hour due to the enzymatic activity of bacterial peptidase and ß-glucosamidase [44].

### 3.1.2 Bacteria, Micro- and Macrozooplankton, and Viruses

In the intricate web of nitrogen dynamics, bacteria significantly contribute to the multifaceted process of DON release through a myriad of sophisticated mechanisms. These mechanisms encompass the sophisticated orchestration of enzymatic secretion, the nuanced phenomenon of passive diffusion across cell membranes, the intricate breakdown of particulate organic matter, and the orchestrated release facilitated by trophic interactions, including but not limited to bacterivory or viral infection [45]. Phytoplankton have the ability to passively obtain DON through the process of bacterial uptake, followed by the excretion of NH4+ during bacterivory [46]. A study presented its findings regarding the discharge of urea in the Gulf of Riga during bioassays [47]. In the scrutiny of urea production within cultures of two marine bacteria, a discernible pattern emerged, highlighting the culmination of highest urea accumulation during the transitional interval between the growth deceleration phase and the commencement of the stationary phase in bacterial growth [48]. Moreover, an additional study conducted an examination of bacterial DON synthesis utilizing D-amino acids and muramic acid as indicators [49]. The empirical results gleaned from the research elucidate that D-amino acids derive from an array of macromolecules extending beyond peptidoglycan [50]. Within the purview of this study, it is contended that bacterial organic matter assumes a consequential role, constituting an estimated 50% contribution to the overall presence of DON in the ocean [50].

Comprising flagellates and ciliates, microzooplankton exhibit the capability to release DON into the surrounding environment through a myriad of mechanisms, including but not limited to secretion and egestion. In stark contrast, macrozooplankton, exemplified by copepods, demonstrate a multifaceted proficiency in generating DON through diverse mechanisms, which incorporate the decomposition of fecal pellets, the secretion of substances, and active involvement in feeding processes characterized by inefficiency, as elucidated in the visual representation provided in Figure 2 [49]. In the context of planktonic ecosystems, the act of grazing and bacterivory involves the processing and distribution of C and N [51], [52]. This process can lead to the release of DON due to inefficient feeding mechanisms [53]. When the components of DON are evacuated as waste or when fecal pellets are broken down or dispersed, this process is known as excretion [52]. In addition, the vertical migration behavior exhibited by zooplankton can play a role in facilitating the active transportation of DON throughout the water column [54]. Research on several microzooplankton species found that around 9 ± 6% (n = 5) of the nitrogen these organisms take in is released as DFAA and/or DCAA [76]. In the context of macrozooplankton, it has been observed that they excrete approximately 13 ± 12% (n = 11) of their nitrogen content in the form of DON, which is commonly quantified using DFAA, urea, or DCAA [76]. According to a supplementary study, it has been documented that approximately 25 ± 12% (n = 6) of the total dissolved nitrogen (TDN) released is present in an organic state [54]. In addition, micrograzers play a pivotal role in the extraction of DON. This phenomenon is observed in the coastal waters of Japan, where bacteria efficiently consume a range of 58-103% of the recently generated DON [37]. In the Monterey Bay and Southern California Bight, the $NH_4^+$ production rate is attributed to the release of DON to the tune of 40% (Bronk and Ward, 1999) [55]. Similarly, it was found that the DON release contributes to 59% of the $NH_4^+$ production rate in Japanese coastal waters [37].

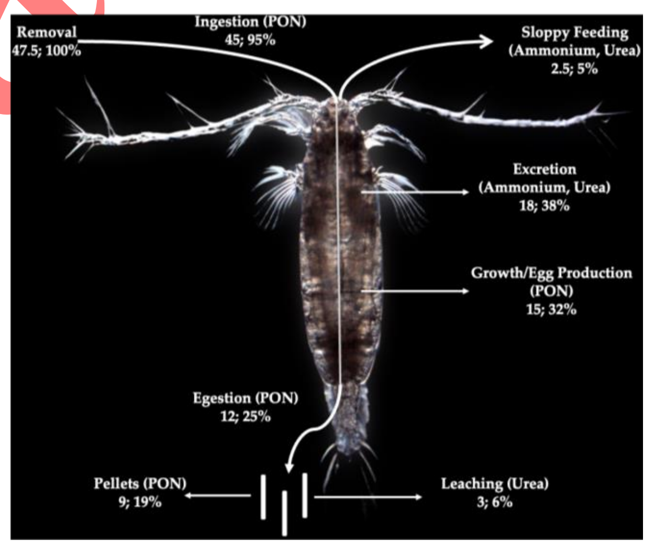


**Fig. 2** As it feeds on *Thalassiosira weissflogii*, the copepod *Acartia tonsa* releases nitrogen into the environment. The average rate of nitrogen release or assimilation is represented by the first value, which is calculated in nanograms per individual per hour, and the proportion of nitrogen eliminated from the suspension is shown by the second value, which is computed from the starting value [52]. Please take note that PON stands for particulate organic nitrogen.

Moreover, viruses could impose significant selection pressures on the composition of microbial communities by selectively targeting certain planktonic species while disregarding others. According to Breitbart (2012), this phenomenon has the potential to result in the formation of microbial communities that exhibit increased susceptibility to grazing or inefficient feeding [56]. Currently, there is a prevailing understanding that the occurrence of significant rates of viral lysis and lytic infections is more probable in situations where both grazing and sinking rates are reduced. In order to carry out their study, Middelboe and Jørgensen (2006) employed a particularly engineered virus to infect a model strain of *Cellulophaga sp* [57]. After viruses lyse bacteria, the researchers measured how much DFAA, DCAA, and substances from bacterial cell walls were released into the cell. The research findings revealed that a significant proportion of the DFAA (approximately 83%) was derived from peptidoglycan. Viral infections also exert an influence on the sinking rates of phytoplankton

### 3.2 Non-native Sources

Rivers, groundwater, atmospheric deposition, wastewater, and landfill leachate are recognized as substantial sources of non-native DON discharge.

#### 3.2.1 Rivers and Groundwater

According to Czerwionka's study on 2016, the mean concentration of DON in river waters is estimated to be around 23.8 ± 18.1 μmol N/L [13]. The carbon-to-nitrogen (C:N) ratio of about 32.5 ± 16.3 is displayed by the DON component, which makes up an average of 57.7 ± 23.7% of the total TDN pool [13]. Based on the calculations conducted by Seitzinger and Harrison (2008), it has been determined that the combined annual N transport to the coastal ocean by the world's 25 largest rivers amounts to 23.81 Tg [58]. This encompasses the entirety of total nitrogen (TN), which comprises DON, DIN, and particulate nitrogen (PN). Approximately 5.02 Tg of N per year is attributed to DON. In another study, it was observed that in nine rivers located in the eastern United States, DON constituted a significant proportion of the TDN pool, varying between 8% and 94% [59]. According to a study by Goolsby et al. (2001), it was found that in the Mississippi-Atchafalaya River Basin, DON accounted for approximately 24% of the overall annual N discharge [60]. These studies collectively highlight the importance of DON as a substantial component of the overall N content in river systems. Therefore, it is imperative to consider DON when evaluating the impact of N loading on coastal aquatic ecosystems.

The existing body of research on the various forms of reduced nitrogen present in groundwater has indicated that DON plays a substantial role. According to a study, it was found that the predominant forms of nitrogen transported from groundwater into Tampa Bay, Florida, are in reduced states, with $NH_4^+$ and DON being the primary constituents [61]. A separate investigation carried out in the northern Gulf of Mexico provided an estimation that DON constituted around 52% of the overall submarine groundwater discharge [62]. Additionally, it was found that DON contributed to approximately 27.7% of the TN load originating from groundwater [62].

Aquifers along the US east coast contain DON concentrations that vary greatly, from 0 to 107 μmol N/L [63]. However, the majority of studies report average concentrations within the 10 to 20 μmol N/L range [63]. Groundwater has the potential to function as a substantial contributor of DON to the marine environment. It was calculated that $1.3 \times 10^5$ mol DON discharged daily from a volcanic island in Hwasun Bay, Jeju, Korea, as an example of subsurface groundwater discharge [64]. All of the above studies add together to show that DON is a major form of N that ends up in groundwater after being introduced to watersheds from humans.

#### 3.2.2 Atmospheric Deposition

The phenomenon of atmospheric nitrogen deposition encompasses the transport and deposition of atmospheric organic nitrogen. Findings from a study in 2013 demonstrate the widespread nature of this feature of nitrogen deposition [65]. However, despite its prevalence, there is still a lack of comprehensive understanding regarding atmospheric organic nitrogen. There is consistent documentation of concentration gradients and indications of long-distance air transport during the land-to-sea transition. This occurrence is often noticed, and that is significant. This is corroborated by the findings of Cornell et al. (2003), who noted that while atmospheric deposition can originate from a variety of local sources, the observed gradients suggest the influence of broader atmospheric processes [66].

The organic nitrogen concentration in rainwater collected in coastal areas is approximately 5 μmol N/L, as reported in a study [66]. The composition of atmospheric organic nitrogen in marine and coastal regions encompasses a wide range of molecules, including urea, amines, amino acids, peptides, amides, nitro-polycyclic aromatic hydrocarbons, humic-like materials, and unidentified compounds [67]–[70]. According to another study, DFAA has the potential to make a substantial contribution, accounting for as much as 50% of the total water-soluble organic nitrogen (WSON) [70]. Glycine, seen below, is an abundant amino acid in marine WSON, making up around 40-60% of the DCAA or DFAA pools [71]. The concentrations of urea in the atmosphere display considerable variability, ranging from 2 to 50% of the total WSON content [70]. Dry deposition has the potential to make a more significant contribution to the deposition of WSON compared to wet deposition, which involves rainfall. According to the findings of a study, the dry deposition concentrations observed in the Eastern Mediterranean region were approximately three times greater (17.4 mmol N/m2) than the corresponding annual wet deposition values (4.8 mmol N/m2) [72].

The proportion of DON in the overall pool also exhibits variability, as evidenced by DON representing 22.7% during the rainy season and 38.6% during dry periods [72]. It is important to highlight that there is no apparent discernible pattern indicating a rise in DON levels in rainwater over the span of one hundred years, as emphasized by the authors who acknowledge the difficulty in comparing data. But rainfall patterns may influence the deposition of DON in the atmosphere, with heightened precipitation periods leading to higher DON deposition, emphasizing the vital role of rainfall in delivering this crucial nitrogen source to ecosystems [72].

### 3.2.3 Wastewater

A report indicates that human activities, and more especially discharges of wastewater, are accountable for around thirty percent of the entire quantity of DON that is discharged into the ecosystem that is found all over the world [65]. Proteins, nucleic acids, amino acids, urea, and micropollutants are among the many chemical components that make up DON that is produced from wastewater. These compounds originate from a wide range of places, including pharmaceuticals and agriculture [6]. The presence of DON in WWTPs can be attributed to both the input from influent sources and the microbial processes occurring within the treatment facility [10]. The concentrations of DON in municipal wastewater are generally moderate, typically ranging from 3 to 7 mg N/L [3]. Nevertheless, it has been observed that wastewater originating from industries such as pesticides, textiles, and pharmaceuticals may contain elevated levels of DON, ranging from 12 to 71 mg N/L [73]. The primary compounds responsible for this phenomenon are carbamates and pyrimidines. A study highlighted the observation that DON has the potential to enhance algal growth in controlled laboratory environments [19]. It was found that around 80% of the DON present in wastewater can be classified as bioavailable. The bioavailability of DON was found to be associated with the hydrophilic characteristics of the organic matter, leading to its classification as bioavailable DON [19]. Conversely, DON, which exhibited hydrophobic properties, was considered resistant. Therefore, the advancement of treatment techniques that enhance the extraction and recuperation of DON from wastewater holds promise in mitigating the discharge of overall TN into aquatic ecosystems and supporting the attainment of sustainable nutrient management goals.

### 3.2.4 Landfill Leachate

As a result of the considerable expenses and practical difficulties involved in implementing on-site treatment, it is common practice to divert leachate originating from landfills to WWTPs. Significant concentrations of nitrogen-containing compounds are frequently observed in aged leachates. Landfill decomposition of municipal solid waste (MSW) entails three separate processes. The terms used to describe these three steps are aerobic, acidogenic, and methanogenic [74]. The leachates originating from the methanogenic phase are predominantly composed of recalcitrant constituents, such as humic substances [75].

It is important to highlight that there is a lack of inclusion of assessments regarding DON content in municipal solid waste leachate analysis plans. This limited reporting hinders comprehensive understanding in this particular area. Most likely, the chemicals that make up leachate-induced DON are of low molecular weight and so cannot be adequately removed by conventional treatment processes [76]–[78]. There exists a concern regarding the potential bioavailability of refractory DON in aquatic environments as a result of its introduction through WWTPs [79]. In aquatic environments, photochemical reactions possess the ability to transform DON into relatively more easily degradable substances, such as primary amines or ammonia-N [80]. Although the practice of discharging landfill leachates to WWTPs is commonly regarded as a cost-effective approach, it is important to recognize that organic nitrogen can make a substantial contribution to the nitrogen content found in WWTP effluents. A thorough comprehension of organic nitrogen in landfill leachates is essential for the purpose of mitigating the introduction of nitrogen into aquatic environments and the subsequent negative consequences it entails.

## 4 Conclusion

In summary, this comprehensive examination highlights the pivotal role of DON in aquatic ecosystems, with a particular emphasis on freshwater environments. The origins of DON are multifaceted, stemming from diverse sources such as photosynthetic organisms, mammalian nitrogenous waste, soil leaching, sewage discharge, and atmospheric deposition. Proteins, free amino acids, amino sugars derived from cell walls, nucleic acids, urea, and methylamines are some of the key components that give DON its characteristic appearance in freshwater. Notably, while wastewater treatment predominantly targets DIN, the untreated DON fraction emerges as a substantial contributor to the overall nitrogen load, particularly in the effluents of wastewater treatment plants employing biological nitrogen removal processes.

The analysis also underscores the challenges associated with concurrently removing DIN and recovering DON. There is a growing need for more effective methods of managing DON due to the fact that DON concentrations in effluents are outpacing TDN levels. This is still the case even though wastewater treatment technology has come a long way. Furthermore, the study illuminates the limited attention given to understanding the dynamics of DON in landfill leachate, emphasizing the importance of considering DON in nitrogen-rich waste streams.

Additionally, the analysis delves into the various forms and significance of DON, highlighting its biological reactivity and potential impact on water quality in nitrogen-sensitive environments. The study categorizes DON sources into native and non-native, detailing contributions from phytoplankton, nitrogen-fixing organisms, bacteria, micro- and macrozooplankton, rivers, groundwater, atmospheric deposition, wastewater, and landfill leachate. The research also explicates DON's role in nitrogen transport through rivers and groundwater, its substantial contribution to wastewater and landfill leachate, and the visual representation of size variation among different nitrogen species. Conclusively, the analysis underscores the intricate dynamics of DON in aquatic ecosystems, advocating for heightened attention, research, and innovative strategies to effectively manage and mitigate its impact on water quality.

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