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**Key Points:**

- We synthesize a global data set of stream chemistry to examine how the composition of dissolved N and DOC:DON ratios respond to N enrichment
- Under low total dissolved N concentrations, the dominate form of N is highly variable but switches to primarily inorganic forms at high TDN
- With N enrichment, DOM becomes more N-rich (lower DOC:DON ratios) while concentrations of DON are less associated with concentrations of DOC

**Supporting Information:**

Supporting Information may be found in the online version of this article.

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# Gradients of Anthropogenic Nutrient Enrichment Alter N Composition and DOM Stoichiometry in Freshwater Ecosystems

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**Abstract** A comprehensive cross-biome assessment of major nitrogen (N) species that includes dissolved organic N (DON) is central to understanding interactions between inorganic nutrients and organic matter in running waters. Here, we synthesize stream water N chemistry across biomes and find that the composition of the dissolved N pool shifts from highly heterogeneous to primarily comprised of inorganic N, in tandem with dissolved organic matter (DOM) becoming more N-rich, in response to nutrient enrichment from human disturbances. We identify two critical thresholds of total dissolved N (TDN) concentrations where the proportions of organic and inorganic N shift. With low TDN concentrations (0–1.3 mg/L N), the dominant form of N is highly variable, and DON ranges from 0% to 100% of TDN. At TDN concentrations above 2.8 mg/L, inorganic N dominates the N pool and DON rarely exceeds 25% of TDN. This transition to inorganic N dominance coincides with a shift in the stoichiometry of the DOM pool, where DOM becomes progressively enriched in N and DON concentrations are less tightly associated with concentrations of dissolved organic carbon (DOC). This shift in DOM stoichiometry (defined as DOC:DON ratios) suggests that fundamental changes in the biogeochemical cycles of C and N in freshwater ecosystems are occurring across the globe as human activity alters inorganic N and DOM sources and availability. Alterations to DOM stoichiometry are likely to have important implications for both the fate of DOM and its role as a source of N as it is transported downstream to the coastal ocean.

**Plain Language Summary** Ammonium and nitrate in freshwaters have received considerable attention due to their clear ecological and health effects. A comprehensive assessment of N in freshwaters that includes DON is lacking. Including DON in studies of surface water chemistry is important because it can cause eutrophication and certain forms can be rapidly removed by microbial communities. Here, we document how elevated levels of TDN impact the concentrations and relative proportions of all three forms of dissolved N and the stoichiometry of DOM. Our results suggest that human activities fundamentally alter the composition of the dissolved nitrogen pool and the

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stoichiometry of DOM. Results also highlight feedbacks between the C and N cycles in freshwater ecosystems that are poorly studied.

## 1. Introduction

Nitrogen (N) concentrations have increased in freshwaters globally due to anthropogenic activities such as urbanization, widespread agriculture, and fossil fuel combustion (Galloway et al., 2008; Vitousek et al., 2009). N enrichment of freshwater ecosystems has contributed to well-documented, deleterious effects, including harmful algal blooms and hypoxic conditions in inland and coastal waters (Brookfield et al., 2021; Glibert et al., 2006; Seitzinger & Sanders, 1997; Wurtsbaugh et al., 2019). Assessments of the impact of increased N on biogeochemical cycles at both the continental and global scales have primarily focused on inorganic forms (Durand et al., 2011; Helton et al., 2015; Mulholland et al., 2008; Peterson et al., 2001; Rabalais, 2002; Taylor & Townsend, 2010) and their relationships with particulate and dissolved organic carbon (DOC) (Helton et al., 2015; Taylor & Townsend, 2010). Absent from many studies of the freshwater N cycle is the consideration of dissolved organic nitrogen (DON), even though it can represent a significant fraction of the total dissolved nitrogen (TDN) pool (Durand et al., 2011; Heathwaite & Johnes, 1996; Lloyd et al., 2019; Perakis & Hedin, 2002) and can promote harmful algal blooms (Heathwaite & Johnes, 1996; Heisler et al., 2008; Howarth & Marino, 2006; Seitzinger & Sanders, 1997). Biotic uptake of some forms of DON can be rapid (Brailsford et al., 2019a; Brookshire et al., 2005; Mackay et al., 2020), and both the concentration and the stoichiometry of dissolved organic matter (DOM; the molar ratio of DOC:DON) can be highly responsive to changes in inorganic N concentrations (Lutz et al., 2011; Wymore et al., 2015; Yates et al., 2019). Changes in the composition of both TDN and DOM along gradients of N availability will have implications for how C and N interact in freshwater ecosystems, influencing biogeochemical processes and rates of nutrient uptake by aquatic microbial communities (Brailsford et al., 2019b; Del Giorgio & Cole, 1998; Wymore et al., 2019).

One leading hypothesis, based on observations across broad environmental and anthropogenic gradients, is that stream N chemistry changes systematically from DON dominance to nitrate ($NO_3^-$) dominance with increases in anthropogenic activities (Durand et al., 2011; Hedin et al., 1995; Pellerin et al., 2006; Perakis & Hedin, 2002; Vitousek et al., 1998), similar to the theory of N saturation in terrestrial environments where excess inorganic N accumulates in soils (Aber et al., 1989). To date, neither a comprehensive cross-biome test of this hypothesis, nor an assessment of the stoichiometry of DOM in response to increasing TDN concentration in running waters has been undertaken.

A significant attribute of the DOM pool is bulk C:N stoichiometry, which can affect the lability and ecological role of DOM in aquatic ecosystems (Creed et al., 2018; Del Giorgio & Cole, 1998). The stoichiometry of DOM is plastic, responding to watershed conditions, including anthropogenic perturbation, soil C:N ratios, and watershed N inputs (Aitkenhead & McDowell, 2000; Yates et al., 2016, 2019). We use four approaches to examine relationships between the properties of DOM and concentrations of TDN. First, we quantify changes in the central tendencies and the distribution of DOC:DON ratios across the TDN gradient. These two analyses allow us to explore changing N-richness of the DOM pool across systems with differing degrees of anthropogenic disturbance. Next, we explore how scaling properties between concentrations of DON and DOC change with TDN enrichment. Scaling properties, defined here as the log-log slope between concentrations of DON and DOC, describe how DON responds to changes in DOC. A slope near one reflects stoichiometric isometry, which we consider the base expectation for the relationship between concentrations of DON and DOC. Constrained isometric scaling likely reflects interactions and reinforcing feedbacks between microbial communities and their environment, where, despite large variability in nutrient concentration and DOM sources, elemental ratios remain stable within specific ecosystem compartments (Redfield, 1958). Alternatively, departures from isometric scaling reflect the enrichment (>1) or depletion (<1) of N from the pool of DOM and the emergence of novel scaling relationships (Julian et al., 2019). Last, we use coefficients of determination from the log-log regression between DON and DOC to quantify the amount of variation in concentrations of DON that is explained by concentrations of DOC. Reductions in the coefficient of determination, and thus, in the predictability of concentrations of DON by DOC, reflect a greater decoupling of the C and N fractions of DOM. Collectively, we use this suite of analyses to evaluate the sensitivity of DOM stoichiometry to changes in TDN.

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Here, we present the most geographically extensive synthesis of stream water N chemistry and DOM to-date comprising >73,000 water samples from 2,035 sites, spanning temperate, tropical, boreal, desert, and arctic biomes. We use this data set to provide a thorough assessment of how the major forms of TDN (DON, ammonium [$NH_4^+$], and $NO_3^-$) and DOM stoichiometry evolve with increased N enrichment and to gain insight into feedbacks between the biogeochemical cycles of C and N in freshwater ecosystems.

## 2. Methods

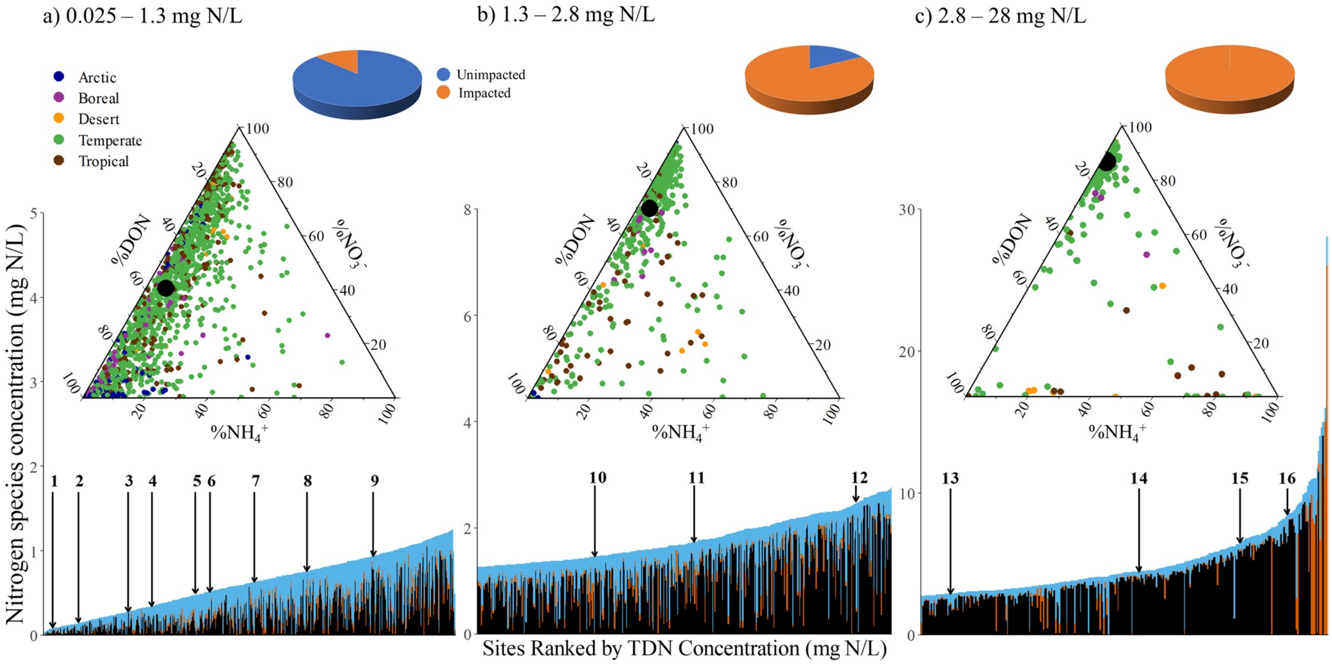
### 2.1. Data Synthesis

Study sites ranged in latitude from −3.66°S to 69.7°N across temperate ($n = 1,531$), tropical ($n = 313$), boreal ($n = 70$), desert ($n = 39$), and arctic ($n = 82$) biomes (Figure S1) and spanned a gradient of watershed land use from unmanaged natural ecosystems lacking significant human disturbance and pollution, to intensive agricultural production and urbanization. Impact at a given site was determined qualitatively based on expert opinions from co-authors familiar with data sets, sites, and associated literature. This assessment was performed on 530 of the 2,035 sites (26%) where information was known. Due to the wide range of anthropogenic disturbance captured within this data set, we used a binary designation of either "impacted" or "unimpacted". We consider impacted systems as those influenced by human activities such as logging, agriculture, settlement and urbanization, and livestock production. We consider systems with relatively small amounts of such disturbances to be unimpacted. We recognize that with global climate change, no ecological systems at the Earth's surface are truly unimpacted. Streams and rivers included in our data set range in size from small first-order headwaters to some of the major waterways on the Earth's surface including the Mississippi, Congo, and the Yukon and Ob Rivers. Data sets were obtained from multiple sources including the NSF-sponsored Long-Term Ecological Research (LTER) program and the Andrews, Bonanza Creek, Hubbard Brook (Campbell et al., 2021), Luquillo (McDowell et al., 2021), Niwot Ridge, and Plum-Island LTERs; the NSF-sponsored Arctic GRO and PARTNERS projects; the United Kingdom NERC DOMAINE program (Yates et al., 2019) and the N speciation data set compiled for the European N Assessment as reported in Durand et al. (2011); the Finnish Environment Institute (SYKE; partly described in Kortelainen et al., 2006; Mattsson et al., 2005); the Oak Ridge National Laboratory (Walker Branch; Griffiths & Mulholland, 2021); the NSF-sponsored Lotic Intersite Nitrogen eXperiment (LINX II; [Mulholland et al., 2008]); the Lamprey River Hydrological Observatory (Wymore et al., 2021); the US Geological Survey (USGS) as presented in Helton et al. (2015); with additional data synthesized from Brookshire et al. (2017), Kaushal and Lewis (2003, 2005) and Spencer et al. (2016). See Supplemental Table S3 for the full citation list.

To be included in our analysis, we required that data sets include measurements of DOC, DON, $NO_3^-$, and $NH_4^+$, or that DON concentrations were calculable from measures of TDN and dissolved inorganic nitrogen (DIN; DON = TDN–DIN, where DIN = $NO_3^- + NH_4^+$). To calculate DOC and TDN from the Finnish data set we multiplied TOC and TN by 0.95 (Kortelainen et al., 2006; Mattsson et al., 2005, 2009). All references to $NO_3^-$ and $NH_4^+$ concentrations and percentages in the main text and figures (as well as for all analyses described below) refer to the atomic portion of N in $NO_3^-$ and $NH_4^+$, respectively ($NO_3$-N, $NH_4$-N). We do not consider the role of nitrite ($NO_2^-$) as it is often either included in measurements of $NO_3^-$ (as $NO_3^- + NO_2^-$) or below detection in most freshwater ecosystems. For consistency across data sets we set minimum detection limits (MDLs) for each solute: DOC (0.1 mg C/L), TDN (0.05 mg N/L), DON (0.01 mg N/L), $NO_3^-$ (0.005 mg $NO_3$-N/L), $NH_4^+$ (0.004 mg $NH_4$-N/L). For TDN, $NO_3$-N, and $NH_4$-N, we used ½ MDL for those samples below MDL. Due to the analytical uncertainties in DON analysis, DON concentrations had to be at least 5% of the TDN pool to be included in our statistical analysis. This 5% threshold removed 2.9% of the samples. Our final data set includes 73,735 observations across 2,035 sites. We then aggregated individual samples from a given site to derive site medians and used those medians in further statistical assessment. All mentions of DOC:DON refer to molar ratios.

### 2.2. Data Analysis

To estimate critical thresholds of TDN concentration at which its composition changes, we applied a change-point analysis of means (Killick & Eckley, 2014) to % DON ordered by TDN concentration. Standard error bounds for change-points were estimated using a Monte Carlo simulation. Iterations ($n = 100,000$)




1) Lookout Creek, Oregon, USA 2) Hubbard Brook, New Hampshire, USA 3) Blackwater stream, Iwokrama Forest, Guyana 4) Lamprey River, New Hampshire, USA 5) Murtopuro, Finland 6) Congo River, Kinshasa, Congo 7) Rio Grande, Texas, USA 8) Caribou Creek, Alaska, USA 9) lowland karst stream, Saiyok National Park, Thailand 10) Kolyma River, Chersky, Russia 11) Bear River, Utah, USA 12) Cuyahoga River, Ohio, USA 13) Loytaneenoja, Finland 14) Mississippi River, Missouri, USA 15) Santa Ana River, California, USA 16) River Ebble, United Kingdom.

**Figure 1.** Compositional shifts within the total dissolved nitrogen (TDN) pool with increasing TDN concentrations. Stacked bar graphs display the range of TDN concentrations with blue, orange, and black colors representing the three major dissolved N species: dissolved organic nitrogen (DON), ammonium ($NH_4^+$), and nitrate ($NO_3^-$), respectively. Panel (a), composition of TDN below first change-point at 1.3 mg N/L; Panel (b), composition of TDN between change-points at 1.3–2.8 mg N/L; Panel (c), composition of TDN above second change-point at 2.8 mg N/L. *Note.* scale of *y*-axis differs by a factor of six across panels (a–c). Inserted ternary plots demonstrate the relative proportions of each dissolved N species within each phase and are colored by biome. Both concentration and proportional data are site medians. The black point within each ternary plot represents the overall median relative abundance for each dissolved N species (see Table 1). Pie charts show how the relative proportion of unimpacted vs. impacted sites changes with each change-point (see supplementary Table 2*). Note.* that pie charts do not include all sites, but only sites where information was available regarding human impacts (*see methods for additional details*). Exemplar sites are numbered to illustrate the broad range of watershed conditions represented. Numbers match footnote below figure.

of the change-point analysis were run with a randomly selected subset of 1,800 sites. Percentage data were arcsine square root transformed prior to analysis. Number of thresholds was determined by visually assessing change in penalty values which were used to assess the maximum log-likelihood that the change-point reflects a real change in medians and not the result of random noise (Figure S2). These statistical change-points were considered a realistic proxy of anthropogenic impact since they conform well to the qualitative assessment of the degree of disturbance for the sites for which this information was available: 87% of the sites below the first change-point were unimpacted and 100% of the sites above the second change-point were impacted (Table S1). Sites that fell between the first and second change-points were primarily designated as impacted (83%).

DOC:DON ratio data were log-transformed prior to analysis (Isles, 2020). Change-point analyses were conducted on median solute percentages or DOC:DON molar ratios with sites ordered by median TDN concentration. We used reduced major axis (RMA) regression to determine scaling relationships and coefficients of determination between concentrations of DON and DOC (Table S2) which is more robust than ordinary least squares as it considers error in both the *x* and *y* dimensions. RMA analyses were performed on log-transformed data with 500 permutations. RMA models were developed using the lmodel2 function in *R*.

## 3. Results

The diversity of streams in our assessment yielded a gradient in TDN concentrations that spanned from 0.025 to 28 mg N/L (Figure 1). Median DON concentrations across streams varied two orders of magnitude from 0.01 to 7.3 mg N/L, while concentrations of the two major inorganic forms of N spanned more than

**Table 1**
*Median Concentration (mg/L), Relative Proportion (%) of Each Dissolved N Species and Median DOC Concentrations and DOM Stoichiometry (DOC:DON Molar Ratio) for Each Group of Sites Based on a Change-Point Analysis of %DON Arrayed by Concentrations of TDN*

| | [TDN] < 1.3 mg/L (*n* = 1,311) | 1.3 mg/L ≤ [TDN] ≤ 2.8 mg/L (*n* = 430) | [TDN] > 2.8 mg/L (*n* = 294) |
|---|---|---|---|
| DON (mg/L) | 0.26 (0.19) 51.0% (29.3) | 0.43 (0.30) 24.8% (16.5) | 0.54 (0.32) 11.5% (6.2) |
| $NO_3^-$ (mg/L) | 0.18 (0.21) 38.7% (31.6) | 1.08 (0.60) 67.9% (22.7) | 3.50 (1.59) 86.1% (7.4) |
| $NH_4^+$ (mg/L) | 0.04 (0.04) 6.1% (4.8) | 0.07 (0.06) 3.9% (3.3) | 0.06 (0.04) 1.4% (1.3) |
| DOC (mg/L) | 4.8 (3.86) | 6.0 (4.15) | 4.95 (2.67) |
| DOC:DON | 21.8 (9.9) | 16.0 (5.8) | 12.2 (4.4) |

*Note*. Parenthetical values are median absolute deviations. Percentages do not add to 100 as they represent median values.

three orders of magnitude: $NH_4^+$ from 0.002 to 26.0 mg $NH_4$-N $L^{-1}$, and $NO_3^-$ from 0.0025 to 14 mg $NO_3$-N $L^{-1}$. Concentrations of DOC ranged from 0.1 to 70 mg C/L.

We identified two TDN concentrations where the composition of TDN significantly changed based on the change point analysis of means: 1.3 (±0.001) and 2.8 (±0.003) mg N $L^{-1}$ (Table 1, Figure S2). To facilitate comparison, we group sites into three categories based on these change points: below 1.3 mg N $L^{-1}$, between 1.3 and 2.8 mg N $L^{-1}$, and above 2.8 mg N $L^{-1}$. At sites below the first change-point (<1.3 mg N $L^{-1}$), the contributions of both DON and $NO_3^-$ to the TDN pool ranged between 0% and 100% (Figures 2a and 2b). Between TDN concentrations of 1.3–2.8 mg N $L^{-1}$, the median relative abundance of DON decreased from 51.0% to 24.9% when compared to sites below the first change-point, and $NO_3^-$ increased from 38.6% to 67.6% (Figures 2a and 2b, Table 1). Although sites within this middle range of TDN concentrations were dominated by $NO_3^-$, they still had variable percentages of organic and inorganic N. Above the second TDN change-point (>2.8 mg N $L^{-1}$), stream N chemistry was dominated by inorganic forms of N. The composition of the inorganic N pool above this second TDN change-point was typically dominated by $NO_3^-$ (Figure 1), which contributed a median of 86.1% (±7.4) to the TDN pool (Table 1). However, 34 sites had a relative abundance of $NH_4^+$ greater than 50% of TDN, with some sites over 90% (Figure 2c). In these high-TDN sites, DON accounted for 11.5% (±6.2) of TDN, demonstrating that the fractional contribution of DON largely decreases with N enrichment, even though the absolute concentration increases.

Increased concentrations of stream water TDN were also associated with stoichiometric changes where DOM became increasingly N-enriched relative to C (Table 1, Figure 2d). At low concentrations of TDN (<1.3 mg N $L^{-1}$), DOM stoichiometry was highly variable and spanned the range of C:N ratios associated with diverse forms of organic matter including woody debris, leaves, and microbes. From below the first change-point to above the second TDN change-point (>2.8 mg N $L^{-1}$), median DOM stoichiometry switched from relatively N-poor (DOC:DON = 22.0), to N-rich (DOC:DON = 12.2) (Figures 2d, Table 1). Moreover, as concentrations of TDN increase, the variability in DOC:DON ratios greatly decreased (Figure 2d), providing direct evidence that N pollution leads to a change in the composition of the stream DOM pool.

Below the first change point (<1.3 mg N $L^{-1}$), we found that concentrations of DOC and DON scale isometrically and that this property persisted between the first and second change points (TDN concentration between 1.3 and 2.8 mg N $L^{-1}$) (Figure 3, Table S2). We detected, however, a pronounced departure from DOC and DON isometry in the most N-polluted streams above the second TDN change point. For this group of sites, the scaling relationship decreased by 20% and had a slope less than 1.0, marking a fundamental shift in the stoichiometry of DOM. We also found a consistent decrease in the amount of explained variation in the relationship between DON and DOC ($r^2$ = 0.66–0.42; Table S2) from below the first change-point to above the second change-point. This provides evidence for the decoupling of DOC and DON concentrations with human impact.

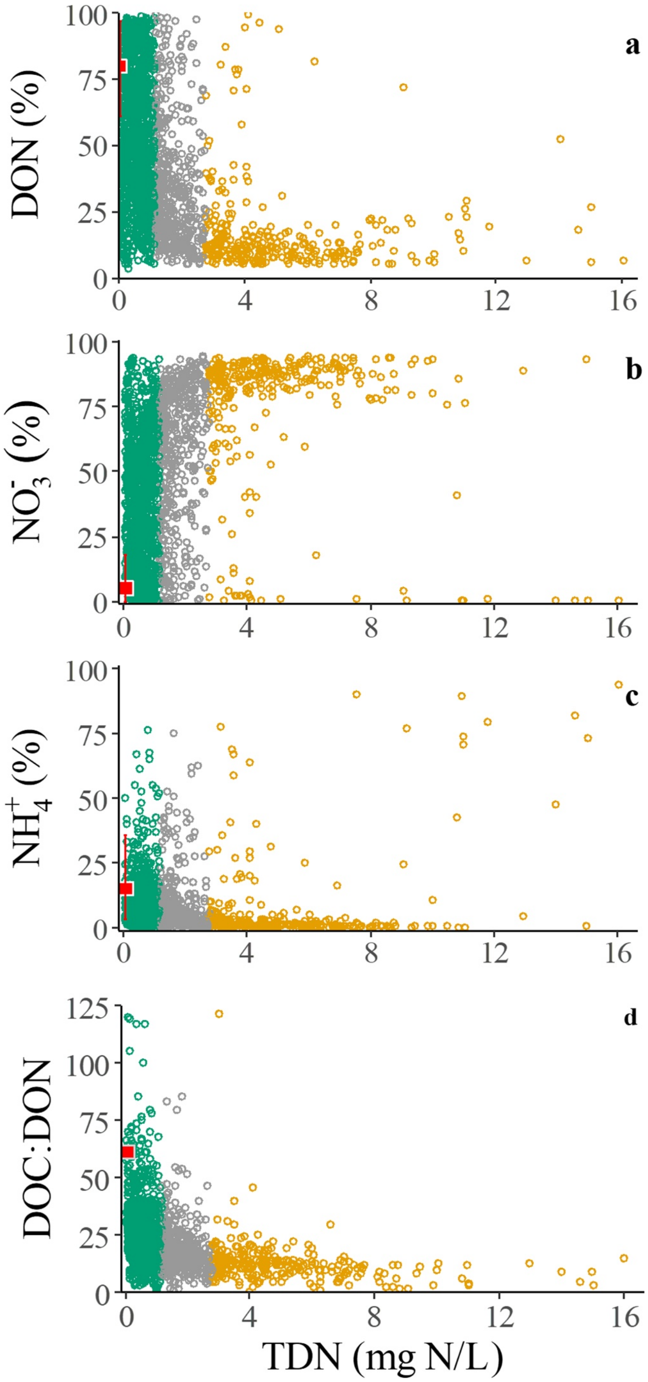



**Figure 2.** Proportions of dissolved nitrogen species and dissolved organic carbon (DOC): dissolved organic nitrogen (DON) stoichiometry vs. total dissolved nitrogen (TDN) concentrations. % DON (a), % nitrate ($NO_3^-$) (b), % ammonium ($NH_4^+$) (c), and DOC:DON molar ratios (d) are plotted against TDN concentrations. Colors represent data above and below critical change-points in TDN: green for TDN < 1.3 mg N/L, gray for 1.3 mg N/L < TDN < 2.8 mg N/L, and orange for TDN > 2.8 mg N/L. Red data point and error bars in panels (a–d) represent means and range of values reported from pristine South American watersheds not included in this study (Perakis & Hedin, 2002). *Note. x*-axes in panels (a–d) have been truncated at 16 mg N/L to better visualize the majority of data points. One data point was omitted at approximately 28 mg N/L.

## 4. Discussion

Although similar patterns and thresholds have been demonstrated at the regional and site-specific scale in freshwater (Durand et al., 2011; Mattsson et al., 2009; Stanley & Maxted, 2008; Vitousek et al., 1998; Yates et al., 2019) and terrestrial ecosystems (Aber et al., 1989), this is the first comprehensive analysis of compositional change of the three major forms of stream dissolved N along a gradient of N enrichment with a broadly distributed data set. We also include a unique examination of DOM stoichiometry across a broad range of nutrient concentrations. Our findings prompt reconsideration of the notion that mid-to high-latitude watersheds exposed to relatively little anthropogenic disturbance are uniformly dominated by DON (Campbell et al., 2000; Goodale et al., 2000; Hedin et al., 1995; Kortelainen et al., 2006; Perakis & Hedin, 2002; van Breeman, 2002). While we acknowledge that our data set is weighted toward temperate sites (North America and Europe), we show striking variation in N composition in streams draining relatively intact watersheds, with patterns consistent across Earth's major biomes (Figure S3).

By grouping our sites by the two identified change-points in TDN concentration, we develop a framework that describes changes in N speciation along a gradient of N enrichment. This empirical framework illustrates that the composition of the TDN pool transitions from highly variable proportions of both DON and DIN to a predominance of $NO_3^-$ that intensifies as systems become N enriched in anthropogenically disturbed watersheds (Figures 1 and S4). Variation in % DON and % $NO_3^-$ below the first change-point of 1.3 mg N $L^{-1}$ (Figures 2a and 2b) suggests a high degree of heterogeneity in the edaphic and aquatic biogeochemical processes that regulate the concentrations of the different forms of N and the export of nutrients to and from streams. This high variability in the relative proportions of reduced and oxidized N species also points to the role of local watershed characteristics (e.g., % wetlands [Hansen et al., 2018], micro-meteorology/climate, state factors [Argerich et al., 2013]), in determining instream N speciation when anthropogenic impacts are relatively small. This result suggests that relatively unaltered stream ecosystems may not necessarily be characterized by high % DON, and conversely, high % $NO_3^-$ may not always be attributed to anthropogenic sources. Some of our data, for example, derive from minimally impacted tropical ecosystems with low concentrations of TDN yet high % $NO_3^-$ due to high rates of nitrification (Brookshire et al., 2012; Peterson et al., 2001).

The range of TDN concentrations between the first and second change-points (1.3–2.8 mg N $L^{-1}$) included watersheds with a significant anthropogenic footprint (e.g., Lloyd et al., 2019; Potter et al., 2014; Rice et al., 2003) (Table S1). In this intermediate region of the N enrichment spectrum, we observed statistically significant shifts in the composition of TDN and in the stoichiometry of DOM, with sites showing a higher proportion of $NO_3^-$, and a decrease in DOC:DON ratios. Moreover, the heterogeneity of TDN composition and DOC:DON stoichiometry started decreasing between the first and second change point, suggesting a decrease in the influence of local watershed characteristics that could result from large landscape-scale modifications such as impervious cover, which alter hydrological pathways and reduce opportunities for retention and transformation within the watershed. The bifurcation of impacted streams above the second change point (>2.8 mg N $L^{-1}$) into either $NH_4^+$

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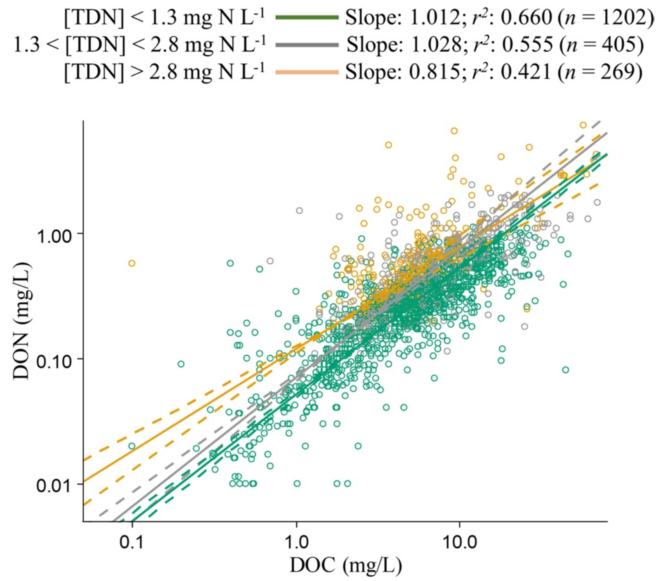


**Figure 3.** Scaling relationships between concentrations of dissolved organic carbon (DOC) and dissolved organic nitrogen (DON) across the identified change-points in concentrations of total dissolved nitrogen (TDN). Data are site medians and dashed lines represent 97.5% confidence intervals. Axes have been log-transformed. Slope values were calculated via reduced main axis (RMA) regressions on logged data. Goodness of fit and sample size is presented for each relationship.

or $NO_3^-$ dominated further emphasizes the loss of heterogeneity from the TDN pool. This pattern likely derives from excess inorganic N inputs from agricultural and urban areas, further sustained where certain reactions are limited due to the lack of terminal electron acceptors (e.g., nitrification rates limited by low $O_2$) or where reaction rates are saturated and unable to efficiently remove inputs (e.g., low carbon availability for denitrification).

The heterogeneity of DOC:DON stoichiometry also decreased with increasing N-enrichment, which was accompanied by a general decrease in DOC:DON ratios. These patterns are consistent with the observation that in less impacted sites, soil C:N ratios provide a strong control on stream DOM stoichiometry (Aitkenhead & McDowell, 2000; Yates et al., 2019), while low molecular weight and N-enriched DOM often dominates in urban and agricultural sites, which often receive direct inputs from human and animal waste including compounds with a low C:N ratio such as urea (Heathwaite & Johnes, 1996; Mattsson et al., 2005, 2009; McDowell et al., 2019; Yates et al., 2019). Increased availability of DIN can also promote switches in the dominant source of N used by aquatic biota. Assimilation of N from organic compounds is more energetically expensive than using inorganic forms, and a switch to reliance on inorganic forms can result in decreases in DOC:DON ratios as DON consumption declines with increasing DIN availability (Lutz et al., 2011; Wymore et al., 2015), while external inputs of N-rich DOM also increase with anthropogenic pollution (Glibert et al., 2006; Lloyd et al., 2019).

We make two central observations across our gradient of TDN concentrations. The first is a shift in the composition of the TDN pool from highly heterogeneous to dominated by DIN with sites characterized as either abundant in $NH_4^+$ or $NO_3^-$. Decreases in the variability of TDN composition (and DOC:DON ratios) in response to increasing TDN concentrations is consistent with the hypothesis that anthropogenic forcing leads to broad landscape-scale homogenization of ecological and biogeochemical processes (Coble et al., 2019; Groffman et al., 2014; Petsch, 2016). This forcing has implications for solute dynamics of freshwater ecosystems (Musolff et al., 2017). The relative rates of multiple N cycling processes occurring across the landscape could mirror changes in the composition of TDN. Specifically, under low levels of perturbation, the landscape and river network could host a mosaic of sources and biogeochemical reactions occurring at different rates, while more disturbed landscapes may be dominated by particular N-enriched DOM sources and certain reactions due to reduced substrate variability. The reduction in variation of N species and DOM stoichiometry in anthropogenically modified landscapes may also reflect the conversion of freshwater ecosystems from transformation-dominated to transport-dominated, where biogeochemical processes saturate and solute export increases (Bernot & Dodds, 2005; Durand et al., 2011; Kumar et al., 2018; Mulholland et al., 2008).

Our second primary observation is the alteration of the stoichiometry of the DOM pool, which becomes N-enriched relative to C, and DOC:DON ratios become less variable as TDN concentrations increase. DOM may serve as a reservoir of N with total N enrichment and from which N can be mineralized depending upon the availability of terminal electron acceptors and changing environmental conditions. DOM as a reservoir of organic N may be similar to soils where DON is retained extensively as mineral associated organic matter (Jilling et al., 2018; Kalbitz et al., 2000; Qualls, 2000) and where C:N ratios of decomposing organic matter determine rates of mineralization or immobilization of inorganic N (Janssen, 1996). Collectively, these results point to a central role of DOM in the N cascade (Galloway et al., 2003) of aquatic ecosystems and in the mechanisms that control the export of DON to downstream receiving waters including estuaries. Our analysis further identifies a coherent shift in the stoichiometric scaling properties of DOC and DON as TDN increases. While concentrations of DON consistently increase across the change points (Table 1), the scaling relationship reveals that under conditions of high TDN, greater availability of DOC results in a disproportionate depletion of organic N per unit increase in DOC. The reduction in the predictability of DOM stoichiometry could result from sources that vary in their DOC:DON ratios and suggests that DON may vary in its molecular composition and play a different biogeochemical role in high TDN sites.

We develop a set of testable hypotheses that describe interactions between the N and C cycles and patterns in stream chemistry and biogeochemical processes associated with the observed changes in the availability of DON and the stoichiometry of DOM. Systems with low DOC:DON ratios may be hot-spots of nitrification, not because C is necessarily limiting (Strauss et al., 2002), but rather because DON provides sufficient N to reduce competition for $NH_4^+$ between heterotrophs and nitrifiers (Wymore et al., 2019). Availability of DON could also reduce rates of assimilatory demand for $NO_3^-$ in systems where the ion is found in low abundance, resulting in a positive effect on rates of denitrification by increasing substrate availability. Alternatively, lower DOC:DON ratios may be indicative of the low availability of multiple terminal electron acceptors (McDowell et al., 2019; Potter et al., 2014), and C-rich forms of DOM are often needed to drive transformations within the N cycle, including $NO_3^-$ uptake (Bernhardt & Likens, 2000; Rodríguez-Cardona et al., 2021). A deeper appreciation of how organic N links C and N cycles across the identified change-points is critical to predicting biogeochemical cycles in Earth's changing freshwater ecosystems. Further, understanding the role of particulate organic matter as a potential source of DOC and DON is needed to integrate organic matter dynamics more fully into the N cycle.

Our analyses do not directly consider the role of specific factors (e.g., % land use, atmospheric deposition) that may drive changes in the composition of TDN and in the stoichiometry of DOM. Such analyses are a logical next step to parse various mechanisms at both the watershed and in-stream scale. Most research examining interactions between land use and stream chemistry focuses on forms of DIN with little consideration of DOM and its stoichiometric balance. One of the striking results from this study is the heterogeneity of % DON, % $NO_3^-$, and DOC: DON ratios below the first change point. Our analysis, however, focused on central tendencies. An examination of site-level variability along similar axes (e.g., Figure 2) would provide insight into the role of hydrology and seasonality to influence the composition of TDN and the energy-nutrient balance of DOM. Investigating how DOC:DON ratios correlate with the application of different forms of nitrogenous fertilizer (e.g., urea vs. anhydrous ammonia) or stocking densities for farm livestock and the storage and handling of manures and slurries could also shed light on how agricultural practices impact this primary compartment of organic matter. Controlled isotopic studies, in particular, could elucidate specific biogeochemical pathways that connect different forms of N to the DOM pool (e.g., Johnson et al., 2013). Last, conducting a similar series of analyses as those presented here that examine how the composition of TDN and DOM stoichiometry change along a series of nested sampling sites could help tease apart the role of watershed area and how in-stream processing contributes to observed patterns.

Human activities are fundamentally altering global biogeochemical cycles (Schlesinger, 2004; Vitousek et al., 1997a; Vitousek et al., 1997b), including in Earth's freshwater ecosystems (Bouwman et al., 2005; Kaushal et al., 2018). Holistic perspectives that simultaneously consider multiple interacting solutes are required to fully understand feedbacks among biogeochemical cycles. Organic nutrients, including organic N, phosphorus, and sulfur, provide a direct link between C and their respective nutrient cycles. Unlike mineral nutrients, however, that often exist in a limited number of ionic forms, organic nutrients present challenges due to their molecular diversity. Considering organic nutrients, and therefore DOM, in the context of nutrient biogeochemical cycles is needed to fully appreciate their ecological and biogeochemical role within ecosystems.

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## Data Availability Statement

The data used in these analyses represent a synthesis of multiple data sets and are openly available through the Environmental Data Initiative at: https://doi.org/10.6073/pasta/50965f9e091ffa833da3c73bce2467fa. The individual data sets and their associated repositories and references can be found in Table S3.

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