Investigating Reactive Nitrogen Sources that Stimulate Algal Blooms in Baffin Bay

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The views expressed herein are those of the authors and do not necessarily reflect the views of CBBEP or other organizations that may have provided funding for this project.

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Executive Summary

Baffin Bay (TX) has been inundated with reoccurring brown tide, a harmful algal bloom (HAB), and is displaying several symptoms of eutrophication. In order to determine nitrogen sources compounding these water quality issues, Baffin Bay surface water samples were collected at six sites monthly from March 2017 to June 2018 as part of a collaborative effort with the Baffin Bay Volunteer Water Quality Monitoring Group and the Wetz Estuarine & Coastal Ecosystem Dynamics Lab at Texas A & M University – Corpus Christi (TAMU-CC). The isotopic composition of dissolved inorganic nitrogen (DIN) and dissolved organic nitrogen (DON) in samples were measured in order to quantify nitrogen sources to Baffin Bay and infer transformation processes. The overall mean isotopic composition of DIN (δ^{15}N-DIN = 9.8‰) indicates sewage as a primary source but also suggests that remineralization of the DON pool may be a significant source of DIN within the bay. Sharp increases in δ^{15}N-DIN values throughout the study period characterized periods of a DIN uptake and a sharp decline in the summer provides evidence of DIN photoproduction from irradiated DON. The overall mean DON isotopic composition (δ^{15}N-DON = 8.8‰) supports sewage as the primary DON source and relatively lower δ^{15}N-DON in the spring indicates increasing contributions from agricultural sources (e.g. livestock waste and fertilizer). To further investigate DON source contributions to the bay, the IsoSource isotope mixing model was employed and estimates sewage as the primary source of DON (53 ± 4%) followed by atmospheric deposition (18 ± 13%), livestock waste (17 ± 12%), and fertilizer (12 ± 9%).

Fortunately, during the study period, there were no significant HABs but this also negated the opportunity to correlate blooms with nitrogen sources and processing. However, it is well known that HABs require a source of nitrogen to proliferate and since DON represents ~90% of the total dissolved nitrogen in the bay, future nutrient mitigation strategies should focus on reducing DON loading. Results from this study suggest the most effective strategy to achieve this would be to reduce sewage contributions to the bay. Continuous monitoring of the DON concentrations in discharge from wastewater outfalls should be a priority since the removal of organic nitrogen from wastewater treatment plants is often inefficient. If it is found that wastewater outfalls are a significant source of DON, more stringent DON treatment methods should be applied. Currently, the significance of septic system contributions to this sewage source is unknown and should be characterized as should the efficiency of the systems and their associated soil absorption fields (drain fields). Stakeholders should encourage the local community to inspect and perform routine maintenance on septic systems in order to ensure greater nutrient processing efficiency thus preventing groundwater infiltration and subsequent discharge to the bay. The Baffin Bay Volunteer Water Quality Monitoring Group has provided valuable information about the high nutrient levels in Baffin Bay over the last 6 years and this project builds on that work by providing estimates of the significant nitrogen sources to the bay. Ultimately, this work aims to provide a foundation for stakeholders to develop informed nitrogen mitigation strategies.

Acknowledgements

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Introduction

Nitrogen inputs from runoff, riverine input, groundwater and atmospheric deposition are a vital source of nutrients to coastal water bodies. However, excessive nitrogen loading can dramatically alter these ecosystems and lead to various detrimental effects including eutrophication, hypoxia, fish kills and loss of biodiversity (Scuvia and Bicker 2006). Previous nitrogen loading studies have focused on the dissolved inorganic nitrogen (DIN) portion (i.e. nitrite, nitrate, and ammonium) of the total dissolved nitrogen (TDN) pool due to the fact that dissolved organic nitrogen (DON) was historically considered recalcitrant and generally unavailable as a nutrient for organisms in marine environments (Seitzinger at al., 2002; Berman and Bronk, 2003). However, more recent studies have shown that DON is a dynamic participant in the N cycle and contributes a large portion (10% to 80%) of the total dissolved nitrogen (TDN) pool in coastal ecosystems (Schlarbaum et al., 2010; Seitzinger et al., 2002). In addition, 12 to 72% and 35 $\pm$ 13% of DON in freshwater and coastal oceans, respectively, is reported to be bioavailable (Bronk 2002; Longborg and Alvarez-Salgado 2012). While this DON can propagate essential primary production, it has been evidenced that coastal systems with low DIN to DON ratios can be favorable for dinoflagellates and cyanobacteria growth, which can cause the establishment of harmful algal blooms (HABs) (Bronk et al., 2007; Schlarbaum et al., 2010).

Baffin Bay has relatively high DON concentrations and DON to DIN ratios (~9:1) and is regularly inundated with brown tides, consisting of the microalga, *Aureoumbra lagunensis* (Wetz et al., 2017). Brown tides have caused declines in seagrass and benthic invertebrate populations as well as decreases in water quality; all of which can be detrimental to the regional economy and the recreational and commercial fishing industries (Buskey et al., 2001). Brown tide is unique in that it can flourish on DON as a nutrient source, which allows it to persist where other ecologically "healthy" phytoplankton would fail. Due to consistently high DON concentrations in Baffin Bay, it is vital to characterize the sources and processing of DON in order to inform future nitrogen loading and brown tide mitigation strategies.

One approach to determining sources and processing of nutrients is to characterize their stable nitrogen isotopic composition. Nitrogen exists in nature as nitrogen stable isotopes with a mass of 14 atomic mass units (14N) and a mass of 15 amu (15N). Due to this mass difference, different sources of nutrients have differing ratios of 15N:14N, and these different ratios act as a fingerprint for distinct sources (e.g. sewage, livestock waste, fertilizer, atmospheric deposition). Nutrient processing mechanisms also have unique isotope ratio effects associated with them. For instance, organisms such as *Aureoumbra lagunensis* tend to preferentially use the lighter isotope of nitrogen (14N) when assimilating nutrients for growth and energy. This leads to a change in the 15N:14N ratio of the nutrient pool which allows insight to how the nutrients are processed. This approach has been used extensively to investigate inorganic nitrogen (NO_3^-, NH_4^+) sources and processing in estuaries, bays, oceans and rivers (Sigman et al., 2009) and recent advances in isotope instrumentation and analysis methods have allowed for isotopic studies investigating the lesser characterized nitrogen species, DON **(**Knapp et al., 2011; 2018; Tsunogai et al., 2008;

Hadas et al., 2010**).** Since DON is the most abundant form of nitrogen in Baffin Bay, these advanced approaches are ideally suited to investigate nitrogen dynamics in the Bay.

The primary objectives of this study were to 1) characterize the stable isotopic composition of DIN and DON (δ^{15}N-DON, δ^{15}N-DIN) in Baffin Bay samples collected monthly at six stations from March 2017 to June 2018 and 2) utilize stable isotope techniques to investigate DIN and DON sources and processing in Baffin Bay. Additionally, since the elevated concentrations of DON make Baffin Bay uniquely suited to investigate its sources and processing, a goal of this project was to characterize the role of this largely uncharacterized form of nitrogen, and provide insight and change perceptions about the role of DON in nitrogen dynamics as a whole.

Methods

Site Location

Baffin Bay is a shallow (≤ 2 to 3 m depth) south Texas estuary in the north-western portion of the Gulf of Mexico and is an inlet of the larger Laguna Madre system (Figure 1). Baffin Bay is separated from the Gulf of Mexico by the barrier island Padre Island and is isolated from the larger Laguna Madre system due to several shallow reefs located near the mouth of the bay (Simms et al., 2010). Petronila Creek, Los Olmos Creek, and San Fernando Creek are the three creeks that drain into Baffin Bay, however, their freshwater/riverine discharge is ephemeral, and no other major river discharges are received (An and Gardner, 2002; Simms et al., 2010). The precipitation received by Baffin Bay averages between 60 and 80 cm year^{-1}, however, evaporation rates exceed this rate by approximately 60 cm year^{-1}. The combination of the rate of evaporation when compared to precipitation, and the isolated nature of the bay, result in hypersaline conditions with average salinities ranging from about 40 to 50. During approximately seven months of the year strong winds from the southeast continuously blow across Baffin Bay at an average of 15 to 24 km h^{-1} (Simms et al., 2010). As a result, the circulation of water in Baffin Bay is primarily wind driven as it is a microtidal system, and the residence time of the water typically exceeds a year (Smith, 1977).

Sampling

Surface water samples were collected monthly at six sampling sites (Figure 1) located throughout Baffin Bay from March 2017 to June 2018. All samples were collected in 125-mL HDPE bottles that were rinsed with acid, rinsed with type I water as specified by ASTM D1193, ISO 3696, and CLSI-CLRW standards (Resistivity of < 18 (MΩ-cm) at 25 °C and Total Organic Carbon (TOC) < 50 (ppb)), and finally triple rinsed in the surface water sample. Samples were placed on ice until filtered through a 0.2 µm GF/F and frozen.

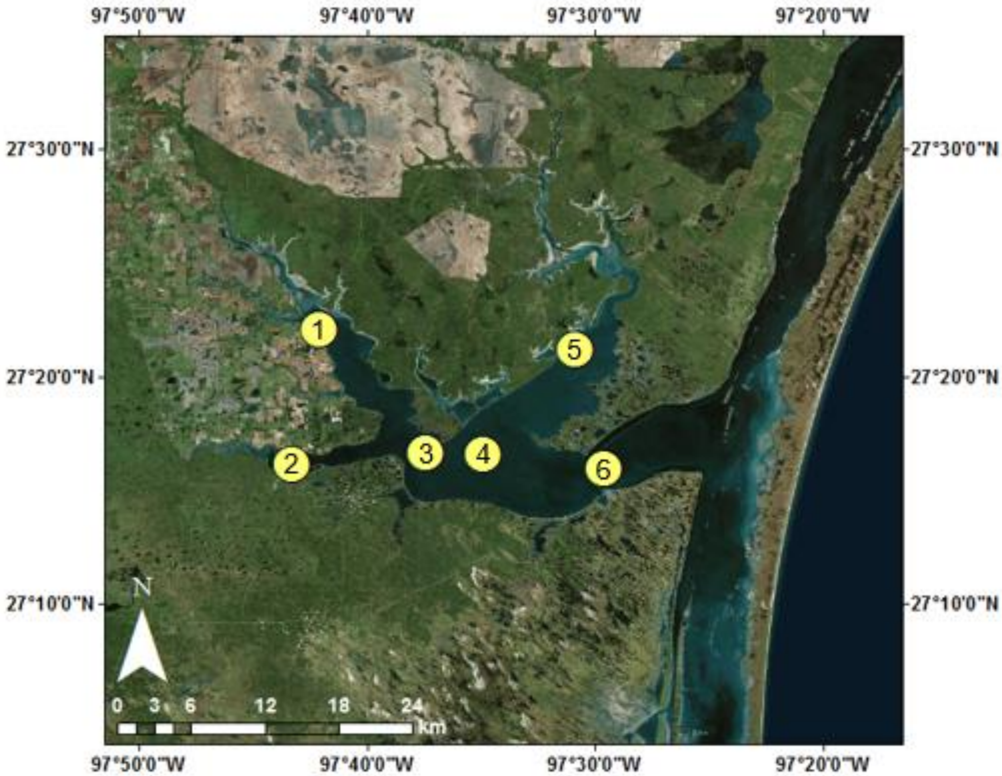

Figure 1. Six sampling site locations (yellow circles) in Baffin Bay, TX, USA.

Isotopic analysis of DIN

DIN (NO_2^- + NO_3^- + NH_4^+) isotopic composition was only measured if DIN concentration were greater than 3 µM. The NO_2^- and NO_3^- portions of DIN are readily measured via the bacterial denitrifier method (Sigman et al., 2001). In order to simultaneously measure the isotopic composition of all DIN components, the NH_4^+ portion of the DIN pool must first be converted to NO_2^-. The NH_4^+ in Baffin Bay samples was oxidized to NO_2^- utilizing the bromate/bromide oxidation method described in Felix et al., 2013 and Zhang et al, 2007**.** Following oxidation, 12 N hydrochloric acid was added to lower the pH of the oxidized samples to a value between the range of three and nine (Felix et al., 2013). Once all DIN was in the form of NO_2^- and/or NO_3^-, the DIN was converted to N_2O via the denitrifying bacteria, *Pseudomonas aureofaciens*. The isotopic composition of NO_3^- and NO_2^- (including converted NH_4^+) was then determined as $\delta^{15}N$-N_2O by injecting the N_2O into a continuous flow isotope ratio mass spectrometer (CF-IRMS) (Sigman et al., 2001). Internationally recognized standards (USGS34, USGS32, IAEA-N3 and USGS35) were measuring during sample analysis to provide a known $\delta^{15}N$-NO_3^- reference for data corrections. Additionally, USGS isotope standards (USGS 25 ammonium sulfate and USGS 26 ammonium sulfate) were oxidized (average of 98.4% conversion efficiency) along with the samples and included as reference samples during isotope analysis in order to check for oxidation efficiency and to correct for any interferences due to reagent blank effects. Values are reported in parts per thousand relative to atmospheric N_2 as follows:

$$\delta^{15}N\ (‰) = [(^{15}N/^{14}N_{sample}) - (^{15}N/^{14}N_{standard})] / (^{15}N/^{14}N_{sample}) * 1000 \qquad (1)$$

Isotopic analysis of TDN

The TDN of the samples was oxidized to NO_3^- using the persulfate method (Tsunogai et al., 2008). The persulfate working reagent was prepared using ultrapure High-Performance Liquid Chromatography (HPLC) Grade water. The average blank concentration (6.7 µM ± 2.8 µM) was mainly attributed to reagent water and since a relatively small amount of persulfate working reagent (0.15 mL) is added to the Baffin Bay samples the overall blank effect is minimal. For instance, since average DON concentrations are high (44.9 ± 15.4 µM), the contribution of the blank is only a small fraction in comparison to the total concentration (average blank percentage: <0.1%), and was not accounted for in δ^{15}N-TDN calculations due to its negligible influence. Representative DON standards (i.e. urea, glycine, EDTA, N-acetyl-D-glucosamine) are oxidized along with the Baffin Bay samples to ensure at least 90% conversion of TDN to NO_3^- from the persulfate oxidation (average oxidation efficiency of 97.8% for all standards). Resulting NO_3^- concentrations were measured via the cadmium reduction colorimetric method (APHA 1992). The standards chosen for the TDN persulfate oxidation method included urea (96.2% recovery average), glycine (103.0% recovery average), EDTA (99.6% recovery average), and N-acetyl-D-glucosamine (93.4% recovery average). Urea was chosen as a standard because it is a form of DON that is a common component used in fertilizers and has been shown to contribute approximately 50% of the N utilized in many coastal regions (Bronk et al., 2002). Glycine was chosen as a standard to represent the dissolved free amino acid (DFAA) portion of the DON pool, which has been found to comprise approximately 1.2 to 12.5% of the total DON pool (Bronk et al., 2002). The N-acetyl-D-glucosamine was chosen as a standard because studies have shown that this biopolymer is representative of the N-acetyl amino polysaccharides (N-AAPs) and are important contributors to the semi-labile pool of DON (N-AAPs can comprise ~40 to 50% of surface ocean high molecular weight dissolved organic matter (HMWDOM) (Aluwihare et al., 2005)). Once the TDN in the sample was converted to the NO_3^- the isotopic composition was measured via the denitrifier method described above (Sigman et al., 2001; Knapp et al., 2005; 2011). The δ^{15}N-DON value was calculated from the measured δ^{15}N-DIN and δ^{15}N-TDN by using the isotope mass balance equation:

$$\delta^{15}N\text{-TDN} = f_{DIN}(\delta^{15}N\text{-DIN}) + f_{DON}(\delta^{15}N\text{-DON}) \qquad (2)$$

where f_{DIN} and f_{DON} stands for the fraction of the concentration of the respective DIN/DON contributing to the TDN concentration of the sample.

Isotope Mixing Model (DON Source Apportionment)

Nitrogen source contributions can be estimated using an isotope mixing model if the isotopic composition of the primary nitrogen sources are known and the isotopic composition of nitrogen in a sample has been measured. For this project a simple four end member isotope mixing model was developed using source signatures of four primary sources (i.e. septic/sewage, livestock waste, fertilizer, and wet atmospheric deposition) and the measured isotopic composition of DON in Baffin Bay samples (equ 3). Table 1 includes the literature δ^{15}N-DON values of DON sources and the δ^{15}N-DON values of local sources used in the mixing model (Campbell 2018). The IsoSource program provided by the EPA was used to employ the mixing model. The IsoSource user inputs the isotopic composition of the DON sources, isotopic composition the DON in the sample, source increment (i.e. 1%) and mass balance tolerance (i.e. 0.1%). IsoSource provides output files which list each feasible solution and descriptive statistics about the distribution of these solutions (e.g. number of solutions, mean, standard deviation, minimum, maximum, 1%ile, median, and 99%ile for each source). The mean of the Monte Carlo iterations performed by IsoSource represent the most frequent feasible source apportionments and these means and standard deviations are presented in the results. However, it is suggested by the EPA for the end user to also consider the full range of possible results and an example of this range is presented in the discussion section (Philips and Gregg 2003).

$$\delta^{15}\text{N-DON}_{bay} = f_{ss}(\delta^{15}\text{N-DON}_{ss}) + f_{wad}(\delta^{15}\text{N-DON}_{wad}) + f_{fert}(\delta^{15}\text{N-DON}_{fert}) + f_{lw}(\delta^{15}\text{N-DON}_{lw}) \quad (3)$$

Where δ^{15}N-DON$_{bay}$ is of the δ^{15}N value of the bay sample, f_{ss} is the contribution of septic/sewage, δ^{15}N-DON$_{ss}$ is the δ^{15}N value of septic/sewage, f_{wad} is the contribution of wet atmospheric deposition, δ^{15}N-DON$_{wad}$ is the δ^{15}N value of wet atmospheric deposition, f_{fert} is the contribution of fertilizer, δ^{15}N-DON$_{fert}$ is the δ^{15}N value of fertilizer, f_{lw} is the contribution of livestock waste and δ^{15}N-DON$_{lw}$ is the δ^{15}N value of livestock waste.

Table 1: δ^{15}N values of reported DON sources and local DON sources (Lee et al., 2012; Russel et al., 1998; Cornell et al., 1995; Choi et al., 2017; Curt et al., 2004, *Campbell 2018).

DON Source	**Literature δ^{15}N value (‰)**	***Local δ^{15}N value (‰)**
Septic/Sewage	+12.8 to +18.6	+14.1
Wet atmospheric deposition	-7.9 to +7.0	+4.4 ± 0.3
Synthetic organic fertilizer	-6 to +2	-0.6 ± 0.3
Livestock waste	+3 to +14	+3.9 ± 0.4

Results

DIN and DON isotopic composition (overall)

The range in δ^{15}N-DIN was 2.5 to 16.8‰ with a simple and concentration weighted mean of 10.3 ± 3.5‰ and 10.0‰, respectively. The range in δ^{15}N-DON was 4.9 to 11.3‰ with a simple and concentration weighted mean of 8.8 ± 1.3‰ and 8.9‰, respectively (Figure 2) (Table 2). The DIN and DON isotopic compositions were correlated versus monthly precipitation and temperature and none of the correlations were significant ($p < 0.5$) (NOAA 2019) (Table 3).

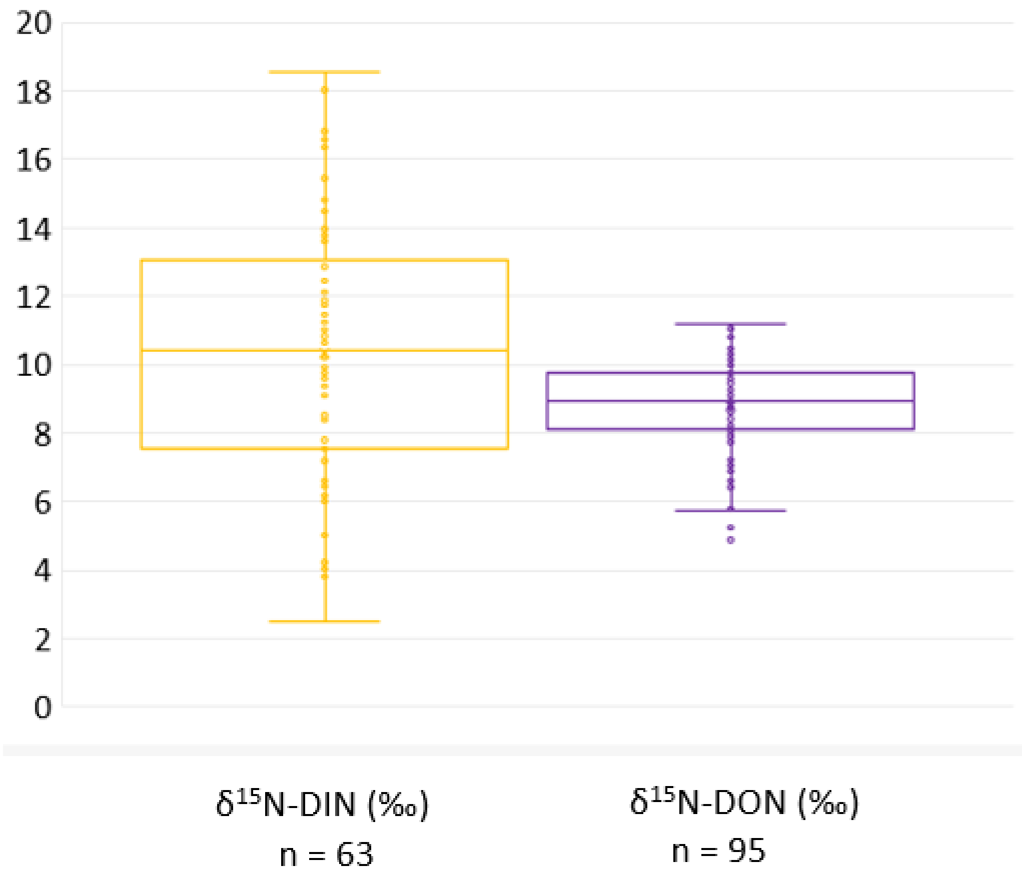

Figure 2. Box and whisker plot summarizing range and mean of all observed DIN and DON isotopic composition.

DIN and DON isotopic composition (temporal)

The concentration-weighted mean δ^{15}N-DIN values in fall, winter, spring and summer were 10.0, 10.4, 10.0, and 7.7‰, respectively (Figure 4). Mean δ^{15}N-DIN values decrease by season from fall/winter to spring/summer, but the only significant difference occurs between winter and summer ($p = 0.02$) (Table 4). The concentration-weighted mean δ^{15}N-DON values in fall, winter, spring and summer were 9.1, 9.8, 8.4, 8.1‰, respectively (Figure 4). δ^{15}N-DON values were only significantly different between winter all other seasons, fall, spring and summer ($p = 0.007$, 0.0001 and 0.01, respectively) (Table 4). Plots of the monthly isotopic composition at are located at the end of this document (Figure 12).

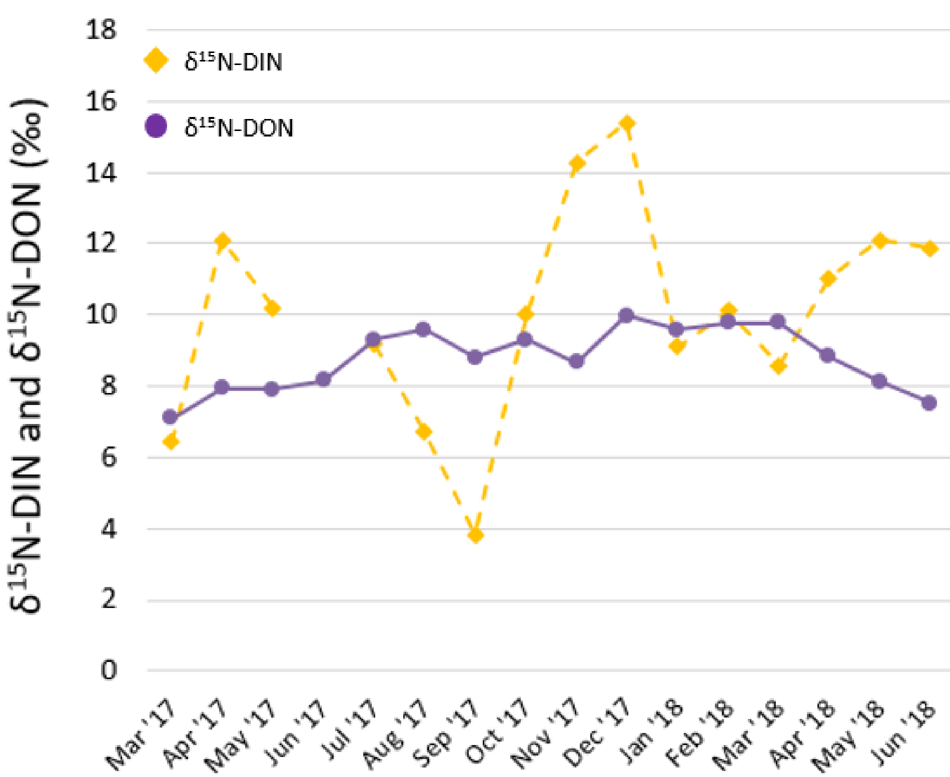

Figure 3. Average monthly DIN and DON isotopic composition at all 6 collection sites in Baffin Bay from March 2017 to June 2018.

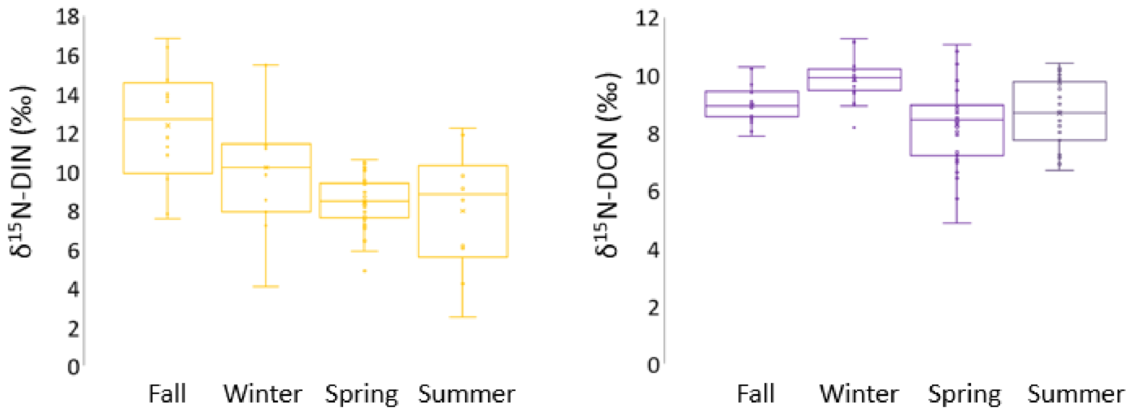

Figure 4. Box and whisker plot summarizing range and mean of observed DIN and DON isotopic composition by season.

DIN and DON isotopic composition (spatial)

The concentration-weighted mean δ^{15}N-DIN values at sites one through six were 8.7, 10.0, 11.6, 8.6, 8.0, 10.8‰, respectively and did not vary significantly among sites ($p > 0.05$) (Table 5, Figure 5). δ^{15}N-DON values at sites one through six were 7.7, 8.3, 8.9, 8.7, 9.0, 9.5‰, respectively and only varied significantly between site 4 and 1 and 2 and between 6 and 2 ($p < 0.05$) (Table 5, Figure 5). Plots of the monthly isotopic composition at each collection site in Baffin Bay from March 2017 to June 2018 are located at the end of this document (Figure 11).

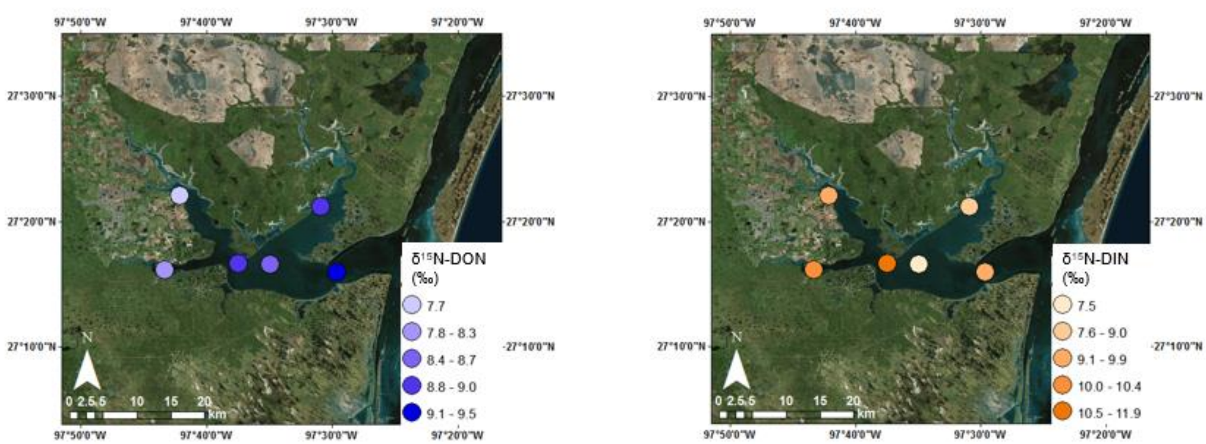

Figure 5. Average DIN and DON isotopic composition at each collection site in Baffin Bay from March 2017 to June 2018.

Isotope mixing model (DON source contributions)

According to the IsoSource mixing model results, the major DON source during the project period was sewage/septic (53 ± 4%) followed by atmospheric deposition (18 ± 13%), livestock waste (17 ± 12%), and fertilizer (12 ± 9%) (Table 6) (Figure 6). The sewage/septic source was the highest contributor in all seasons with the highest seasonal sewage/septic contribution occurring in the winter (61 ± 4%). The percent contribution from sewage/septic was generally higher moving away from the tributaries towards the mouth of the Bay with the lowest contribution at site 1 (43 ± 5%) and the highest at site 6 (59 ± 4%) (Table 6) (Figure 6).

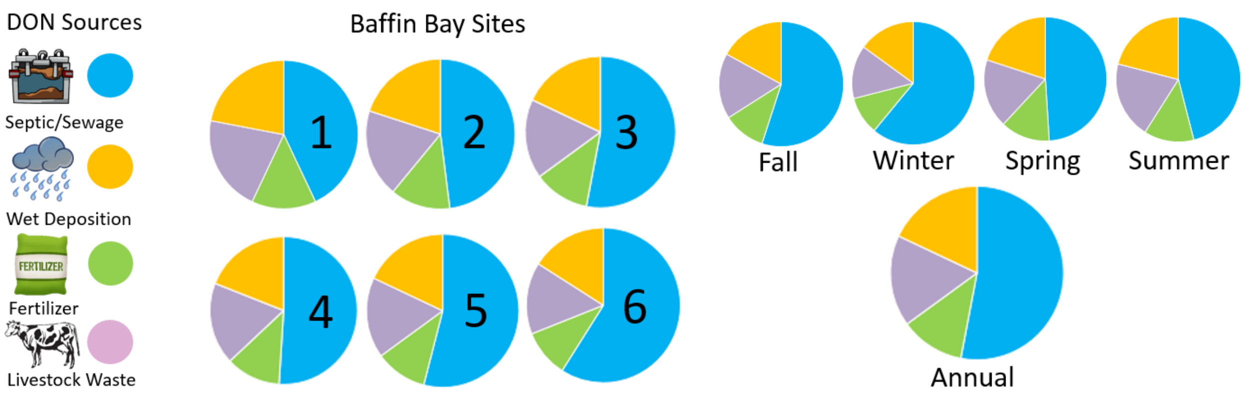

Figure 6. Left) DON source contributions to each Baffin Bay site according to isotope mixing model results. Right) DON source contributions all sites combined during each season and annually according to isotope mixing model results.

Discussion

DIN and DON isotopic composition (overall, temporal, spatial)

For the purposes of this discussion DIN concentration data was obtained from Wetz, 2018 and Wetz, 2019 and DON concentration data was obtained by subtracting DIN concentrations from the TDN concentrations obtained in this study as described in the methods.

Overall

The overall mean DIN isotopic composition (10.0‰) was relatively high and was similar to the literature $\delta^{15}N\text{-}NH_4^+$ values for a sewage/septic source (9.2 ± 2.4‰) (Schmidt et al., 2006, McLaughlin et al., 2012, Liu et al., 2006). The $\delta^{15}N$-DIN may also result from the remineralization of the DON pool in the bay since the isotope effect associated with remineralization is reported to be minimal (±1‰) (Kendall et al., 2007) and the concentration weighted means of $\delta^{15}N$-DIN and $\delta^{15}N$-DON values were within 1.1‰ of each other. However, the $\delta^{15}N$-DIN fluctuates greatly throughout the study period and this speaks to bioavailability of these compounds and various processes effecting DIN species (e.g. nitrification, nitrogen fixation, dissimilatory nitrate reduction to ammonium (DNRA), denitrification). As has been previously reported, the DIN in the bay comprised a small percentage (~10%) of the TDN compared to DON. The DON concentrations in Baffin Bay are high compared to other regional bays but the concentrations are ~40% lower than reported in previous studies (Wetz et al., 2017). Campbell 2018 also reported a 40% lower value for the bay than previous studies, however, this is not a reflection of a decrease in DON but rather how DON is defined in the studies. Samples in this study and the Campbell 2018 study were filtered through 0.2 µm filters and previous studies filtered DON through 0.7 µm filters. The higher pore size allows for particulate organic nitrogen to pass through including most bacteria and some phytoplankton that will then be reported as DON. However, a majority of historical DON studies have filtered samples through 0.7 or 0.45 µm filters so it is often important to use this filtering approach in order to compare to archived concentration data.

The overall DON isotopic composition (8.9‰) in the Bay was similar to the few previous studies investigating lake and estuary systems (5 to 12‰) but was different in both isotopic composition and concentration than the open ocean (5 ± 1‰ and 5 ± 1 µM) (Schlarbaum et al., 2010 and Knapp et al., 2018). When compared to the literature δ^{15}N-DON source values the relatively high δ^{15}N-DON value in the bay indicates a strong contribution from a septic/sewage source (12.8 to 18.6‰) but also implies impacts from mixing with additional significant sources with lower δ^{15}N values (e.g. atmospheric deposition, livestock waste, fertilizer).

Temporal

In order to provide a synopsis of the temporal variations of the DIN and DON isotopic compositions occurring over the 16 month study period, sources and processes are described in five separate periods and are briefly summarized in Figure 7. During this discussion it is important to note that the large majority of DIN is NH_4^+ (~80%) (Wetz, 2018; 2019).

Period I: March 2017 to June 2017

A wet period occurred between March 2017 and June of 2017 (Figure 13) led to increases in DON and DIN concentrations. Previous works have noted increased DIN and DON concentrations following rain events and that these increases coinciding with heavy rain events and have postulated a watershed source (Wetz, 2018). Due to the relatively high δ^{15}N-DIN (~9‰) during this period the primary watershed source was likely sewage (9.2 ± 2.4‰) (Schmidt et al., 2006, McLaughlin et al., 2012, Liu et al., 2006).

The DIN concentration increase was followed by DIN uptake, which is then followed by a decrease in DON. This suggests the population of primary producers were preferentially utilizing DIN but were then able to consume DON as the DIN nutrient source was depleted. Further evidence for the consumption of DIN and DON during this period was the measured increases in Chl a as reported by the Baffin Bay Volunteer Water Quality Monitoring Study (Wetz, 2018). During this period, δ^{15}N-DON values began to increase thus signifying the consumption of the 'lighter' ^{14}DON. While the δ^{15}N-DON was high during this period, suggesting sewage input, the δ^{15}N-DON was relatively lower during the spring than other seasons and indicates inputs from lower δ^{15}N-DON sources (e.g. agricultural runoff (fertilizer and manure) and wet deposition). It is logical that the fertilizer influence would increase during the spring due to agricultural practices and fertilizer use is also evidenced at Baffin Bay during spring periods by significantly increasing atmospheric ammonia (Berner and Felix, *in prep*).

Period II: June 2017 to September 2017

Summer is a biologically active season and it might be expected that δ^{15}N-DIN values would increase as DIN is consumed, however this period also had the highest average temperatures and solar irradiance (Figure 14). Various studies have shown irradiated DON can produce labile DIN photoproducts such as $NH_4^+$and NO_2^- (Bushaw et al., 1996; Kieber et al., 1999; Kitidis et al., 2006; Rain-Franco et al., 2014) with NH_4^+ production reported to be an order of magnitude higher than the other labile photoproducts (Kitidis et al., 2006). Photoproduction rates of NH_4^+ in estuarine systems range from 0 to 220 nmol L^{-1} h^{-1} and have reached as high as 237 µmol N m^{-2} d^{-1} in the Baltic Sea (Kiditis and Uher 2008, Stedmon et al., 2007; Sipler and Bronk 2015). It is expected that the kinetic reaction producing DIN from DON would produce an isotopically 'lighter' DIN product (Thibodeau et al., 2017). The δ^{15}N-DIN during period II decreased from ~+9‰ to ~+4‰ suggesting photochemistry plays a significant role in DIN production during warm months. In addition, Campbell 2018 performed a laboratory study exposing summer Baffin Bay surface water to simulated sunlight and observed a 20% increase in NH_4^+ after six hours of exposure. The δ^{15}N-DON and DON concentration during this period do not change significantly but the photochemical process may not be significant enough to alter such a large pool (i.e DON) of the TDN while significantly changing a much smaller pool (i.e. DIN) of the TDN.

Period III: September 2017 to November 2017

The DIN concentration decreased and remained low during period III and coincided with a large increase in the δ^{15}N-DIN (~+4‰ to ~+13‰). This suggests the lighter ^{14}DIN was consumed, leaving behind a DIN pool that was continually being enriched in ^{15}DIN. Additional evidence for biological consumption was the reported increase in Chl a in the Bay during this period (Wetz, 2018). The δ^{15}N-DON decreased slightly from the previous period and coincided with an increase in DON concentration. This period of DON accumulation is consistent with cell death and phytoplankton exudation of DON towards the end of their growth cycle (Biddanda and Benner, 1997). The exuded DON could have a low δ^{15}N-DON value thus slightly lowering the overall δ^{15}N-DON, however this is just speculation since there are no previous studies reporting the δ^{15}N-DON values of exuded DON.

Period IV: November 2017 to February 2018

This period was marked by high precipitation in December that again led to higher DON concentrations with consistently high δ^{15}N-DON indicating a continued sewage influence. The wet period also led to DIN increases and coincided with δ^{15}N-DIN decreasing from the high observed (~+16‰) at the beginning of this period. This can be attributed to several lower δ^{15}N-DIN sources loading during runoff including fertilizer, livestock waste, and atmospheric deposition. The lack of evidence for DIN consumption during this period could be due to low temperatures slowing biological activity. However, it is important to point out that the significantly higher DIN concentrations observed during lower temperature periods (winter/spring (5.8 µM)), than higher temperature periods (fall/summer (3.3 µM)) may be partially driven by an abiotic process. Gases have greater solubility in cooler waters than warmers water and temperature greatly affects the water-atmosphere exchange of NH_3 (Johnson et al. 2008). This is further supported by observations of a negative NH_3 flux (into the bay) in the winter and positive NH_3 flux (out of the bay) in the summer at Baffin Bay (Dunegan and Felix *in prep*).

Period V: February 2018 to June 2018

The DIN concentration eventually decreased during period V and coincided with a δ^{15}N-DIN increase which indicates consumption by primary producers in the spring period of the growth cycle. The DON increases may be a byproduct of increased organism production in spring and lack of DON being utilized by this organism population. Chl a data is not currently available for this time period to infer biological production. DON concentration peaked in the final month of this study and this again coincided with high rain totals (Figure 13). As observed in the previous spring, relatively lower δ^{15}N-DON values during this spring period may be due to increased agricultural sources (e.g. fertilizer and manure).

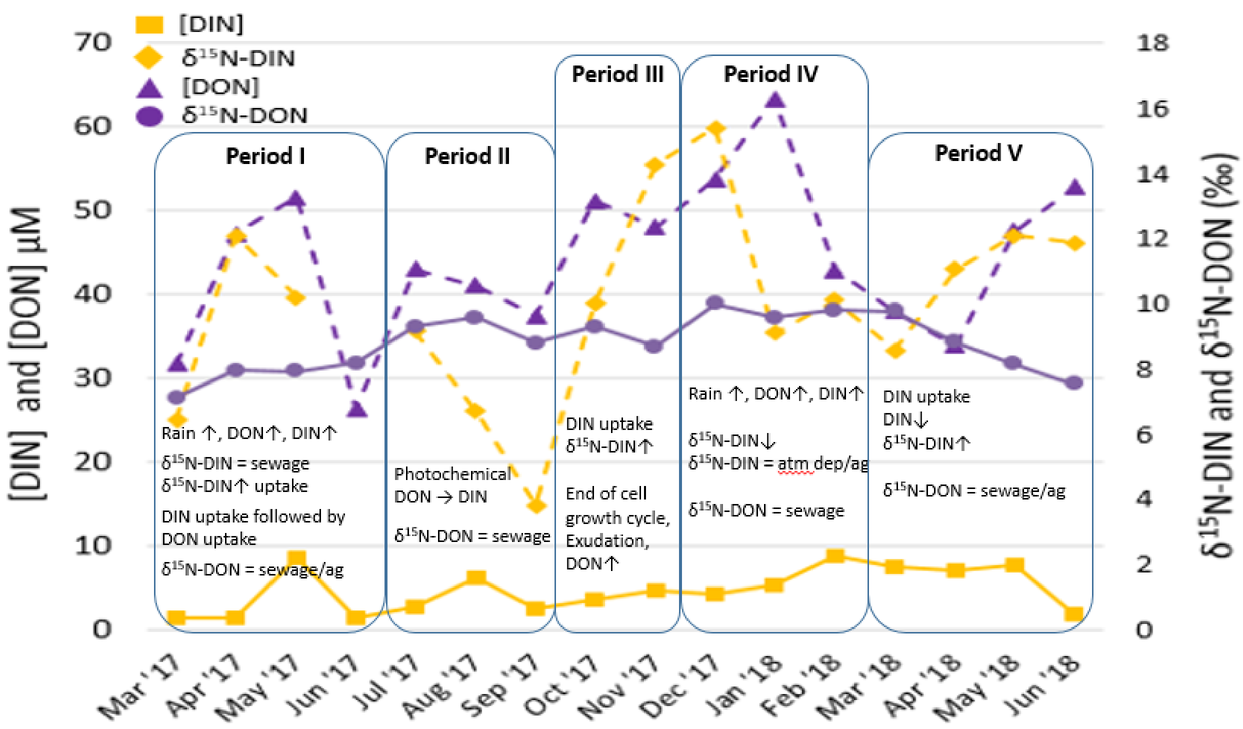

Figure 7. Average monthly DIN and DON concentrations and isotopic composition at all 6 collection sites in Baffin Bay from March 2017 to June 2018. Boxes include summary statements of DIN and DON dynamics during the five periods outlined in the discussion. DIN data was obtained from Wetz, 2018 and Wetz, 2019 and DON concentration data was obtained by subtracting DIN concentrations from the TDN concentrations obtained in this study as described in the methods.

Spatial

DIN concentrations were not significantly different throughout the Bay but were relatively higher in the tributaries possibly due to agricultural and sewage sources associated with terrestrial activities. The δ^{15}N-DIN was also higher at the tributary sites which indicates sewage as a predominant source rather than agricultural sources. Overall, the δ^{15}N-DIN was not significantly different among sites except between sites 1 and 4. This may be because fractionation associated with uptake of DIN is a significant driver of δ^{15}N-DIN at this site as is examined in more detail later in this discussion section. The DON concentrations did not vary significantly spatially and for the most part neither did the DON isotopic compositions. The exceptions were the tributary sites, 1 and 2, which had significantly lower δ^{15}N-DON values than sites 4 and 6 which are furthest from the mainland. The slightly lower δ^{15}N-DON values at the tributary sites likely indicate a higher percent contribution from agricultural sources. The overall spatial homogeneity of DON and DIN concentrations and isotopic concentrations indicate relatively uniform nitrogen source contributions and processing throughout the whole bay system.

Isotope mixing model (DON source contributions)

Results from the isotope mixing model indicate sewage (53%) as the primary DON source to the bay and a portion of the high sewage/septic influence may be attributed to wastewater discharge to creeks feeding the bay. For instance, San Fernando Creek has 12 permitted wastewater facilities that discharge into it and the southern tributary is subjected to Riviera Water Control and Improvement District (WCID) wastewater outfalls (Wetz et al., 2017; TCEQ 2019). Additionally, there are ~63,000 septic systems in the 18 counties of the Texas coastal zone (TCEQ/TSSWCB 2018). A large portion of the Baffin Bay watershed is rural and septic systems provide a cost-effective means of wastewater disposal in rural areas lacking access to a centralized wastewater treatment facility. Due to the anaerobic conditions existing in the septic systems, a majority of the nitrogen in the septic effluent exists as ammonium (NH_4^+) (70 to 90%) and dissolved organic nitrogen (DON) (10 to 30%) (Lusk et al., 2015). However, depending on the physical and chemical properties of the natural soils and the depth of the water table, bioavailable forms of N (e.g. NH_4^+, NO_3^-, DON) can infiltrate the groundwater and subsequently be delivered to coastal waters (Luscz et al., 2007). Sandy soils surrounding Baffin Bay may not provide adequate environments to process the nutrients in septic effluent thus DON and DIN will infiltrate the groundwater. This DON and DIN buildup in groundwater and pore water may be the cause of high DON and DIN associated with high rain amounts. These rain events would cause increased discharges of septic contaminated groundwater to the bay. The highest % contribution from sewage (61%) and highest DON concentrations were in winter which may be driven by high rain amounts in December during the study period. Septic/sewage being the primary nitrogen source to the bay is in contrast to a recent SPARROW nutrient loading model study that predicted that atmospheric deposition and fertilizer were the major nutrient sources (Rebich et al., 2011). However, The SPARROW model did not account for groundwater discharge in the bay, which is the principal delivery mechanism for septic effluent.

The percent contribution from sewage/septic was generally higher moving away from the tributaries towards the mouth of the Bay with the lowest contribution at site 1 (43 ± 5%) and the highest at site 6 (59 ± 4%) (Table 6) (Figure 6). This suggests that sources with lower δ^{15}N-DON values (e.g. atmospheric deposition, fertilizer, and manure) more readily contribute to the DON concentrations at sites in the tributaries that are closer to agricultural activities.

There are pitfalls to be aware of when using mixing models to predict source contributions. For instance, the mixing model results presented represent the most frequent feasible source apportionments. However, it is suggested by the EPA for the end user to also consider the full range of possible results. In order for the reader to visualize this, Figure 8 represents the full complement of possible results. It can easily be seen that the reported means are representative of the most likely contribution scenarios but there are other mixing scenarios for which to be aware.

In addition, a limited set of local source signatures were utilized in this mixing model. It is possible that the δ^{15}N values of these sources may vary and future mixing models would benefit greatly from a more comprehensive source signature inventory. Additionally, the DIN or DON originating from the source can undergo processing (e.g. assimilation, nitrification, photoammonification) that can alter the original source signature through isotope fractionation. The next section more thoroughly investigates the potential of fractionation driving δ^{15}N values in Baffin Bay.

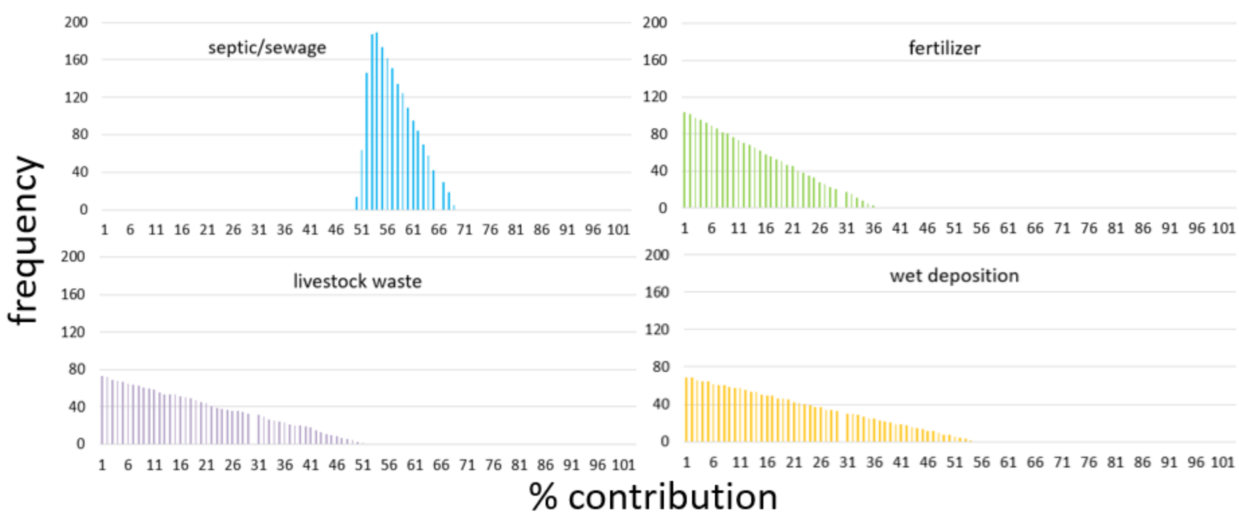

Figure 8. Frequency of percent contributions of each DON source during the fall according to Monte Carlo iteration (n = 1858) output produced by IsoSource program.

Investigating Mixing and Fractionation Processes

Simultaneously investigating the isotopic composition and concentrations of N species allows for determining possible source mixing or processing (fractionation) scenarios resulting in the observed $\delta^{15}N$ value (Kendall et al., 2007; Thibodeau et al., 2017). For instance, plotting $\delta^{15}N$ vs ln(concentration) of a sample set that has been subjected to fractionating transformation processes (e.g. remineralization, photoammonification) will yield straight lines with slopes equal to fractionation effects while plotting this same data as $\delta^{15}N$ vs 1/DON concentration yields curved lines. If the sample concentrations and $\delta^{15}N$ are a result of the mixing of ***two different*** nitrogen sources with ***different*** $\delta^{15}N$ values and concentrations, the $\delta^{15}N$ vs 1/DON concentration plot will yield a straight line (Figure 9). It is important to note that this approach works only in simple scenarios where there is only one major fractionating process affecting the isotopic composition or two major sources with different isotope signatures contributing to the sample $\delta^{15}N$.

The nitrogen isotopic composition of DON and DIN was plotted against corresponding ln concentration and 1/concentration to determine if significant source mixing or fractionation can be inferred as the driver of the $\delta^{15}N$ values in the bay. These were plotted using all data, seasonal data, and site specific data. In all of these plotting scenarios, there was not a significant relationship suggesting a fractionation scenario or mixing scenario except in one instance. The lone scenario showing a significant correlation was plotting lnDIN vs $\delta^{15}N$-DIN at site 4 (Figure 10). The straight line indicates a consistent fractionation process leaving the unprocessed pool of NH_4^+ enriched in ^{15}N. The slope of the line postulates a fractionation effect (ε) of – 8.0‰ which is within the reported range of NH_4^+ uptake (– 9.4 ±6.6‰) (Denk 2017).

This lack of linear relationships seen with all other plots suggests multiple source mixing and/or multiple processes contributing to the $\delta^{15}N$ values in the bay. This is expected in a microtidal estuary with long residence times that is exposed to several known sources and contains various anoxic and aerobic environments for several abiotic and biotic processes to occur.

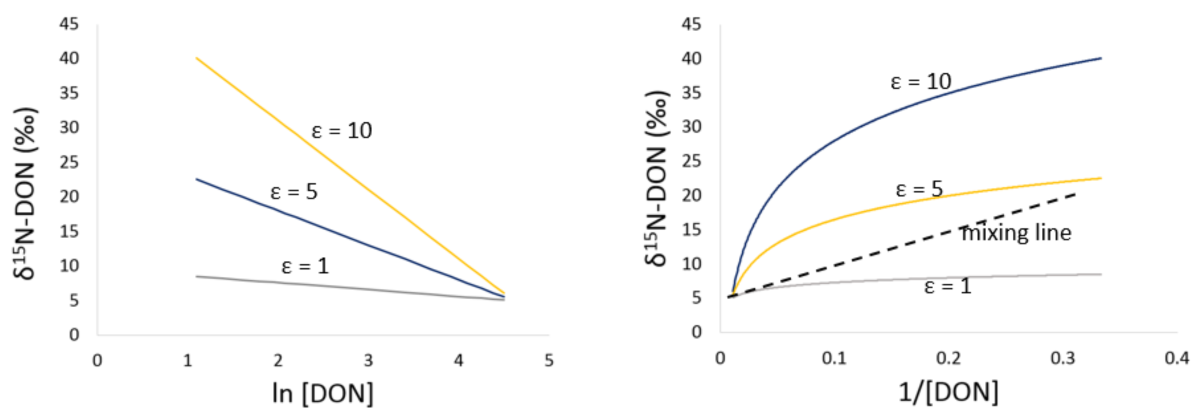

Figure 9. Left: Plotting the $\delta^{15}N$ vs ln DON concentration of a sample set that has been subjected to fractionating transformation processes will yield straight lines with slopes equal to fractionation factors. Right: Plotting this same data as $\delta^{15}N$ vs 1/DON concentration yields curved lines. If the sample concentrations and $\delta^{15}N$ are a result of the mixing of two DON pools with different $\delta^{15}N$ values and concentrations, the data will yield a straight line.

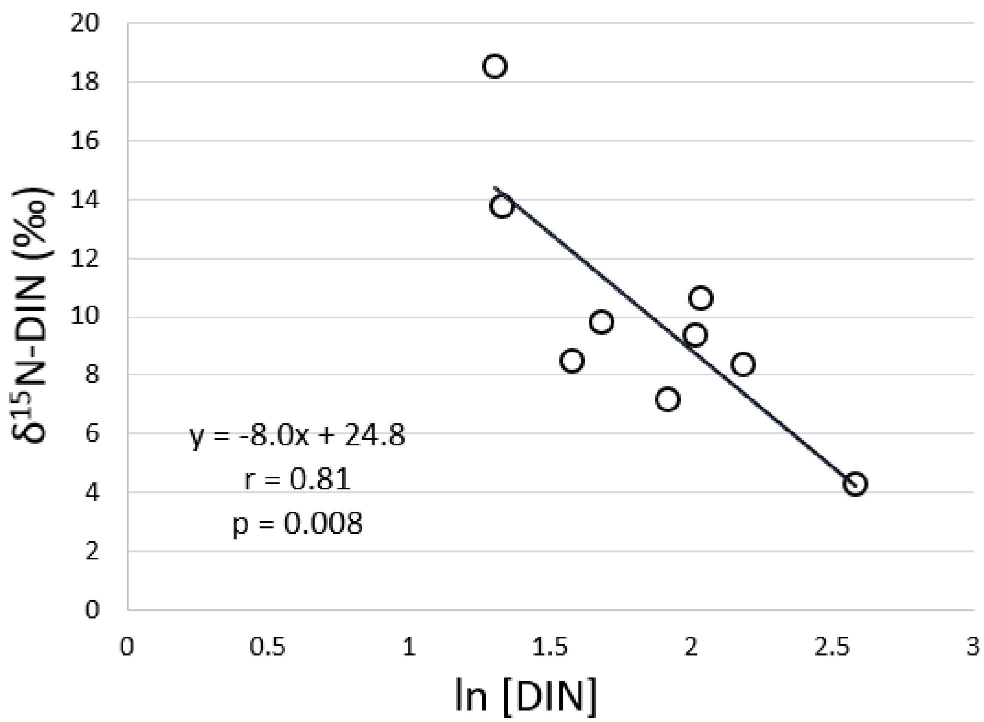

Figure 10. δ^{15}N-DIN vs ln(DIN) concentration at Baffin Bay site 4. The significant correlation and straight line indicate a fractionation process with a ε of -8.0‰. Since 80% of DIN is NH_4^+, this suggests uptake of NH_4^+ controlling the δ^{15}N values.

Conclusion

There has been a diligent ongoing effort to monitor the water quality of Baffin Bay due to the re-occurring HABs and symptoms of eutrophication. Sources of nutrients leading to these issues have been speculated but as stated by Wetz et al. 2017, "*virtually nothing is known about the nutrient source(s) that have allowed for brown tide bloom persistence and/or redevelopment in subsequent years.*" This project aimed to provide DON contribution estimates of significant sources to Baffin Bay in order to enable stakeholders to develop informed nutrient mitigation strategies. The isotope mixing model results suggest that sewage was the primary contributor to DON concentrations in the bay. Sewage input to the Baffin Bay watershed can originate from wastewater outfalls. The location of these outfall point sources are known and continuous monitoring of the DON concentrations discharge should be a priority since the removal of organic nitrogen from wastewater treatment plants is often inefficient, and DON can comprise up to 65% of the dissolved nitrogen in these effluents (Pehlivanoglu-Mantas and Sedlak, 2006). If it is found that wastewater outfalls are a significant source of DON, more stringent DON treatment technology should be applied. Discharge from septic systems are more difficult to monitor as there are hundreds of privately owned septic tanks on the coast of the bay and consistent monitoring of discharge and efficiency would not be possible. In order to understand the significance of septic discharge contribution to the bay, septic effluent from a subset of septic tanks should be characterized as should the efficiency of the septic systems and the soil absorption fields (drain fields). Stakeholders should encourage the local community to inspect and perform routine maintenance on septic systems in order to ensure greater nutrient processing efficiency thus preventing groundwater infiltration and subsequent discharge to the bay.

Additional Figures and Tables

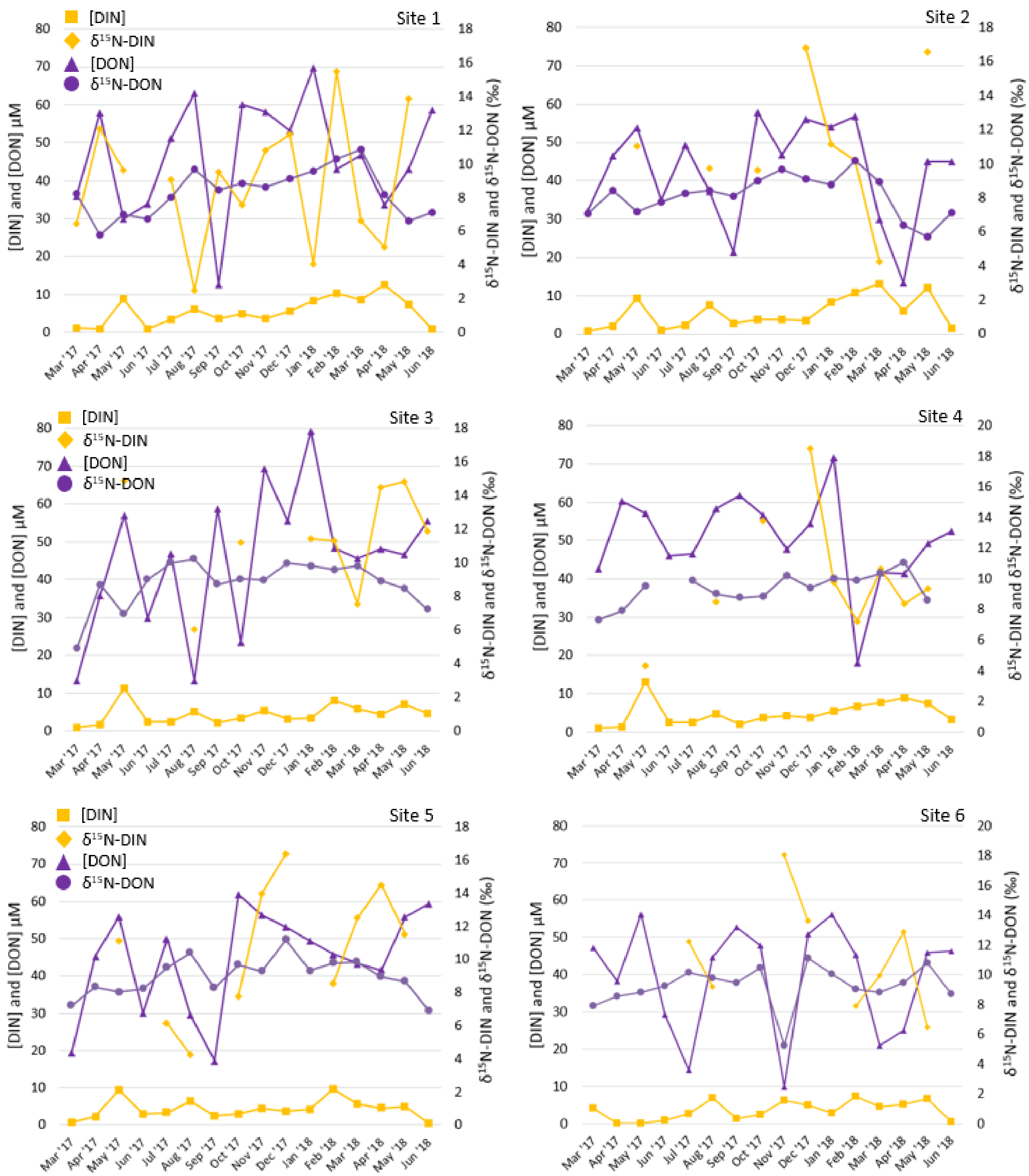

Figure 11. Monthly DIN and DON concentrations and isotopic composition at each collection site in Baffin Bay from March 2017 to June 2018. DIN data was obtained from Wetz, 2018 and Wetz, 2019 and DON concentration data was obtained by subtracting DIN concentrations from the TDN concentrations obtained in this study as described in the methods.

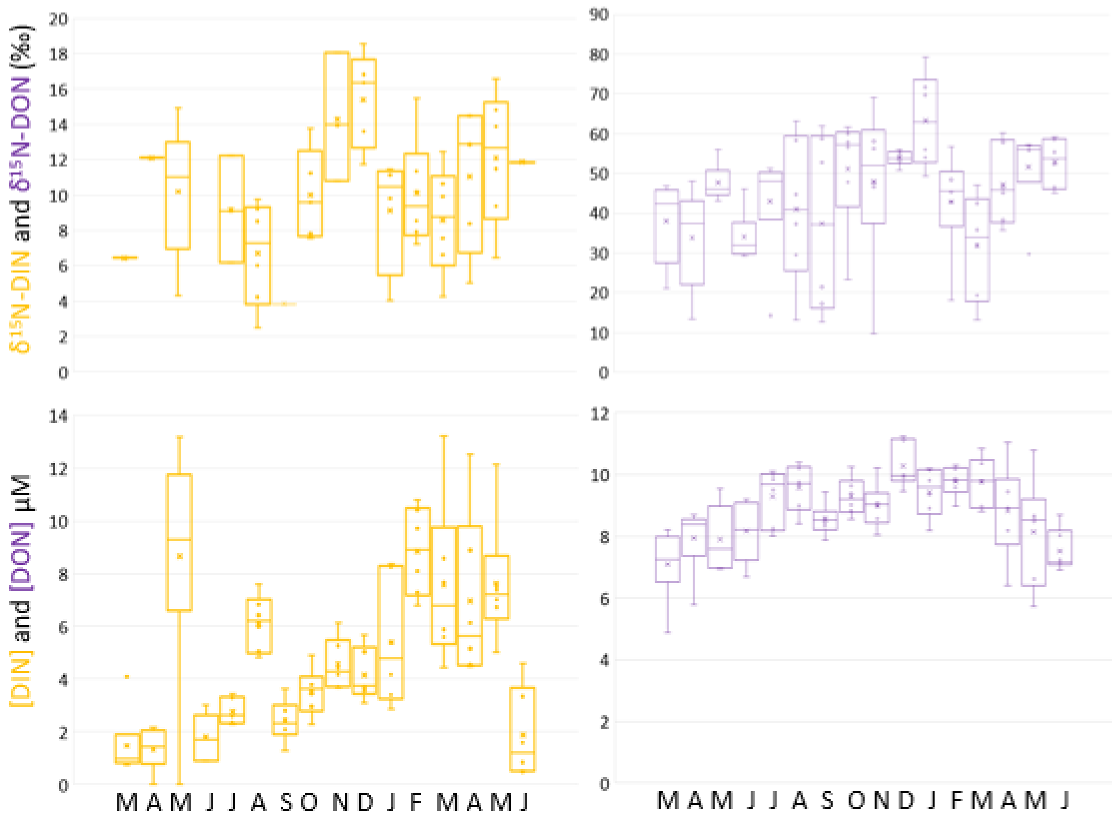

Figure 12. Box and whisker plots summarizing range and mean of monthly DIN and DON concentrations and isotopic composition from March 2017 to June 2018. DIN data was obtained from Wetz, 2018 and Wetz, 2019 and DON concentration data was obtained by subtracting DIN concentrations from the TDN concentrations obtained in this study as described in the methods.

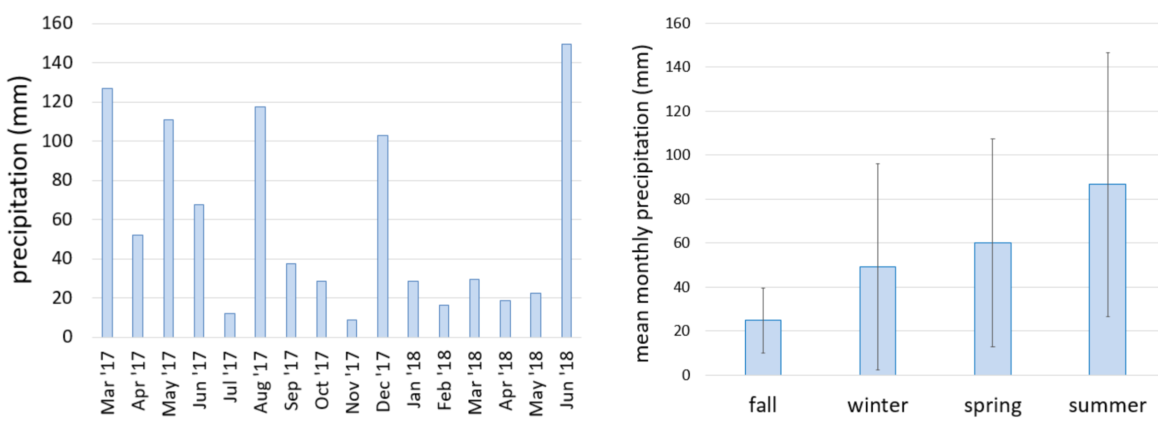

Figure 13. Left) Monthly precipitation (mm) for the Baffin Bay watershed during the project period. Right) Average monthly precipitation (mm) of seasons during the project period.

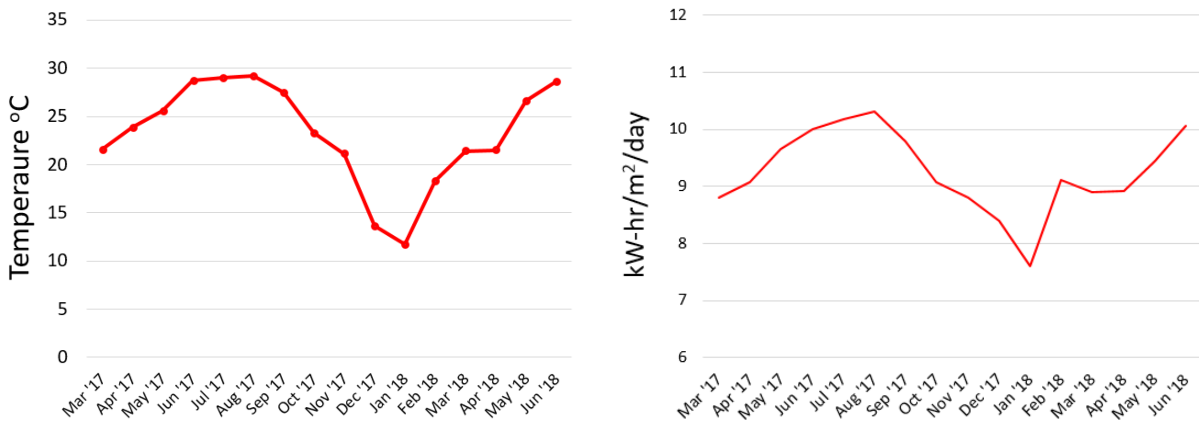

Figure 14. Left) Average monthly air temperature in the Baffin Bay watershed. Right) Average monthly downward thermal infrared (longwave) radiative flux at Baffin Bay (NOAA 2019, NASA 2018).

Table 2: DIN and DON average concentrations and DIN and DON simple and concentrated weighted isotopic composition at each collection site and at all collection sites for each season. DIN and DON concentration date. DIN data was obtained from Wetz, 2018 and Wetz, 2019 and DON concentration data was obtained by subtracting DIN concentrations from the TDN concentrations obtained in this study as described in the methods.

	[DIN] µM	**[DON] µM**	**simple mean δ^{15}N-DIN ‰**	**simple mean δ^{15}N-DON ‰**	**conc. wt. mean δ^{15}N-DIN**	**conc. wt. mean δ^{15}N-DON**
all sites	4.7	44.8	10.3	8.8	9.8	8.9
site 1	5.5	46.9	8.9	8.3	8.7	7.7
site 2	5.6	42.5	11.1	8.2	10.0	8.3
site 3	4.4	45.4	11.5	8.8	11.6	8.9
site 4	4.9	50.4	10.1	9.3	8.6	8.7
site 5	4.3	44.6	10.7	9.0	8.0	9.0
site 6	3.5	39.4	11.3	9.4	10.8	9.5
all sites fall	3.5	45.4	10.7	8.9	10.0	9.1
all sites winter	6.1	53.4	11.6	9.8	10.4	9.8
all sites spring	5.6	41.7	10.4	8.3	10.0	8.4
all sites summer	3.1	42.7	8.0	8.7	7.7	8.1

Table 3. Correlation coefficients (r) between concentration, isotopic composition, precipitation and temperature. Significant correlations are italicized ($p < 0.05$). DIN data was obtained from Wetz., 2018 and Wetz, 2019 and DON concentration data was obtained by subtracting DIN concentrations from the TDN concentrations obtained in this study as described in the methods.

	[DON]	**δ^{15}N-DIN**	**δ^{15}N-DON**	**precipitation**	**temperature**
[DIN]	0.140	0.487	0.413	-0.271	-0.253
[DON]		0.462	0.232	0.029	-0.434
δ^{15}N-DIN			-0.0350	-0.407	-0.070
δ^{15}N-DON				-0.426	-0.473
precipitation					0.206

Table 4. Calculated probability values (p-value) of two-tailed equal variance T-Test for differences between season averages of DIN and DON concentration and isotopic composition for all collection sites. DIN data was obtained from Wetz, 2018 and Wetz, 2019 and DON concentration data was obtained by subtracting DIN concentrations from the TDN concentrations obtained in this study as described in the methods.

[DIN]	**winter**	**spring**	**summer**
fall	*0.000552*	*0.036235*	0.515767
winter		0.61769	*0.000204*
spring			*0.007943*

[DON]	**winter**	**spring**	**summer**
fall	0.161869	0.404651	0.57992
winter		*0.002291*	*0.013892*
spring			0.760524

δ^{15}N-DIN	**winter**	**spring**	**summer**
fall	0.616501	0.37819	0.119453
winter		0.109489	0.023535
spring			0.329789

δ^{15}N-DON	**winter**	**spring**	**summer**
fall	0.00763	0.105539	0.461976
winter		0.000122	0.000758
spring			0.302284

Table 5. Calculated probability values (p-value) of two-tailed equal variance T-Test for differences between site averages for DIN and DON concentration and isotopic composition. DIN data was obtained from Wetz, 2018 and Wetz, 2019 and DON concentration data was obtained by subtracting DIN concentrations from the TDN concentrations obtained in this study as described in the methods.

[DIN]	**Site 1**	**Site 2**	**Site 3**	**Site 4**	**Site 5**
2	0.939				
3	0.369	0.363			
4	0.667	0.631	0.633		
5	0.291	0.292	0.858	0.518	
6	0.090	0.101	0.334	0.177	0.421

[DON]	**Site 1**	**Site 2**	**Site 3**	**Site 4**	**Site 5**
2	0.388				
3	0.800	0.620			
4	0.475	0.089	0.373		
5	0.656	0.666	0.895	0.218	
6	0.167	0.539	0.325	*0.029*	0.316

δ^{15}N-DIN	**1**	**2**	**3**	**4**	**5**
2	0.197				
3	0.095	0.843			
4	0.489	0.581	0.404		
5	0.273	0.791	0.605	0.744	

δ^{15}N-DON	**1**	**2**	**3**	**4**	**5**
2	0.689				
3	0.383	0.182			
4	*0.046*	*0.009*	0.270		
5	0.156	0.049	0.626	0.484	

Table 6: Mean of probable DON source contributions to each site and all sites combined during each season according to isotope mixing model results. DIN data was obtained from Wetz, 2018 and Wetz, 2019 and DON concentration data was obtained by subtracting DIN concentrations from the TDN concentrations obtained in this study as described in the methods.

	δ^{15}DON (‰)	**% septic/ sewage**	**±**	**% fertilizer**	**±**	**% livestock waste**	**±**	**% wet deposition**	**±**
all sites	8.9	53	4	12	9	17	12	18	13
site 1	7.8	43	5	14	11	21	15	22	16
site 2	8.3	48	5	13	10	19	14	20	15
site 3	8.9	53	4	12	9	17	12	18	13
site 4	8.7	51	4	12	9	18	13	19	14
site 5	9.0	54	4	11	9	17	12	18	13
site 6	9.5	59	4	10	8	15	11	16	12
all sites fall	9.1	55	4	11	8	17	12	17	13
all sites winter	9.8	61	4	10	7	14	10	15	11
all sites spring	8.4	49	5	13	10	18	14	20	14
all sites summer	8.1	46	5	13	10	20	14	21	15

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