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Michelle L. Gedeon

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THE BIOGEOCHEMISTRY OF DISSOLVED ORGANIC NITROGEN IN THE SUBTROPICAL NORTH PACIFIC

By

Michelle Liane Gedeon

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ABSTRACT

THE BIOGEOCHEMISTRY OF DISSOLVED ORGANIC NITROGEN
IN THE SUBTROPICAL NORTH PACIFIC

By

Michelle Liane Gedeon

In marine systems, dissolved organic nitrogen (DON) can affect the nitrogen budget, primary production, and potentially the production of nitrous oxide. Water samples were collected in October 1998, January 1999 and May 2000 from Stations ALOHA (22°45'N, 158°W) and Station 2 (16°N, 150°W) located in the oligotrophic North Pacific Subtropical Gyre (NPSG). The concentration and isotope value of isolated DON (>100 Da) were measured simultaneously. DON concentrations generally range between 4-7 μM in surface waters, decreasing with depth to approximately 2 μM. As DON decreases in concentration it becomes enriched in ^{15}N. This inverse relationship is consistent with microbial assimilation and degradation of DON. There is also seasonal variation in the $\delta^{15}N$ values of DON. Samples collected in summer are depleted in ^{15}N relative to those collected in winter, suggesting a shift in nitrogen source. During the summer months stratification of the water column favors *Trichodesmium* spp., a cyanobacteria that fixes ^{15}N depleted dinitrogen (0 ‰). DON produced under these conditions can be injected into deeper waters and undergo ammonification, resulting in a large substrate pool for nitrification. Nitrous oxide is a by-product of nitrification and may be vented to the atmosphere via diffusion or deep mixing. Our results support relatively short time scales for DON cycling. Evidence of DON involvement in both primary production and the nitrous oxide pump demonstrates the key role DON can play in the NPSG.

To my extraordinarily loving and supportive husband David and the Faith we share

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INTRODUCTION

Dissolved organic nitrogen (DON) is often the most abundant form of fixed nitrogen in oligotrophic marine ecosystems (Sharp, 1983). In surface waters (≤100m) of the North Pacific subtropical gyre (NPSG) DON is usually hundreds of times more abundant than inorganic nitrogen and frequently comprises more than 90% of the total dissolved nitrogen pool (Karl et al.,1993). Dissolved organic nitrogen is comprised of a number of different compound classes including polysaccharides, nucleic acids, proteins, humic acids and dissolved free amino acids (Keil and Kirchman 1991, Bronk and Glibert 1993, Koike and Tupas 1993). Despite the potential importance of DON, its role in primary production and nitrogen cycling is not well understood. This is in part due to a long-standing perception that the DON pool is predominantly refractory (Williams and Druffel, 1988). A number of studies, however, suggest that there is an important bioavailable component of the DON pool, citing turnover times on the order of days, as opposed to hundreds or thousands of years (Toggweiler, 1989, Williams and Druffel, 1988). Bronk et al. (1994) estimated 10±1 days for complete turnover based on ^{15}N enrichment. Benner et al. (1997) isolated high molecular weight (1-100nm) dissolved organic matter (DOM) and estimated a turnover of 0.3-0.5% DOM per day. Given the large size of the DON pool and the importance of nitrogen as a limiting nutrient, if even a fraction of DON is bioavailable then nitrogen availability has been underestimated (Fuhrman, 1992). This finding has particular relevance in oligotrophic systems.

In addition to its potential effect on the nitrogen budget, DON may be involved in nitrogen cycling reactions which have local, regional and global scale environmental significance. For example, nitrogen fixing organisms can produce new DON (Karl et al.,

1997) which enters the water column via microbial release or the breakdown of particulate organic nitrogen (PON). Mineralization of this new DON to ammonium, a substrate for nitrification, may allow DON to play a role in nitrous oxide production as nitrous oxide is a by-product of nitrification. This is important because nitrous oxide is a gas which contributes more to the greenhouse effect than carbon dioxide, and is increasing at an annual rate of 0.3 % (Prinn et al. 1990). Nitrous oxide production is of particular interest in aerially extensive oceanographic systems due to the potentially significant ocean-atmosphere flux. Our study sites, Station ALOHA (22°45'N, 158°W) and Station 2 (16°N, 150°W), are located in just such a system, the large ($2x10^7$ km^2), oligotrophic, NPSG. Data has been collected from Station ALOHA, our primary sampling site, over an 13 year period. These data were collected as part of the Hawaii Ocean Time-series (HOT) program and reveal that this system, once thought to be homogeneous in space and time, exhibits dramatic change with respect to microbial community structure and nutrient cycling on seasonal, interannual and decadal time scales (Karl, 1999).

Station ALOHA is characterized by high DON concentrations (5-6 μM) and low nitrate concentrations (< 10 nM) in surface waters. There is a persistent, deep chlorophyll maximum layer centered near 115-125 m and a deep oxygen minimum at 800 m (Karl, 1999). The permanent nutricline is located between 200-600 m (Karl et al., 2001). Nutrients needed for primary production, such as nitrate and phosphate, are primarily supplied via recharge from deeper waters due to vertical diffusion and horizontal transport from adjacent systems (Karl, 1999 Short term eddy-driven mixing events are also important nutrient recharge mechanisms (Letelier et al., 2000). Nitrogen

fixation is also an important source of new nitrogen, particularly when stratification of the water column allows for blooms of free living (e.g., *Trichodesmium*) and endosymbiotic (e.g., *Richelia*) nitrogen-fixing cyanobacteria (Karl, 1997).

Measurements of variations in the stable isotopic abundances of nitrogen contained in selected pools (e.g., ^{14}N and ^{15}N) have provided important insight into the origins and transformations of organic and inorganic nitrogenous species (Saino and Hattori, 1987; Altabet et al., 1991; Ostrom et al., 1997). Similarly, temporal shifts in the source of DON, as well as participation in nitrogen cycling reactions, should be evident by measuring the nitrogen isotope ratios of DON. The $\delta^{15}N$ values of high molecular weight marine DON isolated via ultrafiltration suggest a major role for DON in the upper ocean nitrogen cycle (Benner et al., 1997). Feuerstein et al. (1997) demonstrated that 95 % or more of DON can be effectively isolated from freshwater, quantified and measured isotopically and that 99 % of dissolved inorganic nitrogen can be quantitatively removed from salt water samples. Our objective is not only to isolate and quantify DON, but to use nitrogen isotopes to examine the role of DON in nitrogen cycling within the NPSG.

MATERIALS AND METHODS

Samples analyzed for the concentration and isotopic composition of DON were collected from two stations within the NPSG. Water was collected at ALOHA (22°45'N, 158°W) on October 19, 1998, January 13, 1999 and May 25, 2000. Samples were also obtained at a second station (Station 2, 16°N, 150°W) on May 28, 2000. Samples collected in October 1998 and January 1999 were taken aboard the R/V Moana Wave in coordination with the HOT program. Collections made in 2000 were part of a larger Pacific transect cruise on the R/V Roger Revelle. Water (0.4 to 1.0 L) was collected in 14 L (R/V Moana Wave) and 20 L PVC bottles (R/V Roger Revelle), immediately vacuum filtered through acid washed, 45 mm diameter, 0.2 μm Supor polyethersulfone filters and frozen in acid washed Nalgene bottles for subsequent processing and analysis.

In the laboratory, seawater samples were thawed, concentrated approximately 5-fold to 80-200 mL by rotary evaporation (ca.10 mm of Hg and 38 °C), and transferred to 100 Dalton molecular weight cut off (MWCO) dialysis membranes (Spectra/Por CE Membrane). To ensure quantitative recovery, round bottom flasks used during rotary evaporation were rinsed 3 times with 4 mL of ultra-clean deionized water (Epure, Barnstead) and this water was added to the membrane. The samples were suspended in 4 L beakers and initially dialyzed against a solution of 8 % combusted NaCl dissolved in ultra-clean deionized water for up to 10 days (4 °C-6 °C). Beginning on the second day of dialysis, the initial NaCl solution was diluted daily by 1-2 % until the sample was eventually suspended in a salt-free solution. We found that initially suspending the sample in an 8 % saline solution reduced, but did not impede, diffusion across the membrane. Reducing the salt concentration gradually, prevented a steep concentration

gradient that can result in a high diffusion rate, expansion of the membrane pores and potentially the loss and isotopic fractionation of the DON. Following dialysis, the contents of each dialysis bag were removed using a combusted Pasteur pipette and transferred to a combusted round bottom flask. To assist in the quantitative transfer of DON, the dialysis membrane was rinsed 3 times with 2 mL of Epure water and this rinse was added to the flask. Each sample was reduced to a volume of approximately 5-10 mL. The concentrate and subsequent rinses (3, 4 mL rinses) were transferred to a pre-combusted quartz ampoule (ca. 60 mL). Combusted quartz wool was loosely added to the necks of the quartz ampoules to prevent sample loss under vacuum. All glassware, quartz wool and NaCl used in the preparation and analysis of DON samples were combusted at temperatures of at least 450 °C for 1 h, borosilicate pipettes which have a lower heat tolerance were combusted at 375 °C for 1 h and, in accordance with Feuerstein et al., 1997, CuO was combusted at 850 °C for 4 h to ensure complete oxidation. Each vessel was frozen in liquid nitrogen and dried *in vacuo* on a glass high-vacuum line. Once the samples dried, combusted copper oxide (Aldrich, 2 g) and copper (Elemental Microanalysis, 6 g) were added and the samples were re-evacuated. The vessels were then sealed, combusted at 850 °C for 1 h to convert the DON to N_2 and slowly cooled at a rate of 0.4 °C/min. Nitrogen gas was separated cryogenically and the isotope value determined on a PRISM (Micromass) mass spectrometer. Isotope ratios are expressed in per mil (‰) notation:

$$\delta^{I}E = [(R_{sample}/R_{standard}) - 1] * 1000$$

where I is the heavier isotope of element E and R is the abundance ratio of the heavy to light isotope. The standard for $\delta^{15}N$ is atmospheric nitrogen gas. The nitrogen

concentration of a sample is determined based on the mass 28 ion beam within a calibrated volume in the dual-inlet mass spectrometer. Calibration was performed every day that a DON sample was analyzed.

RESULTS and DISCUSSION

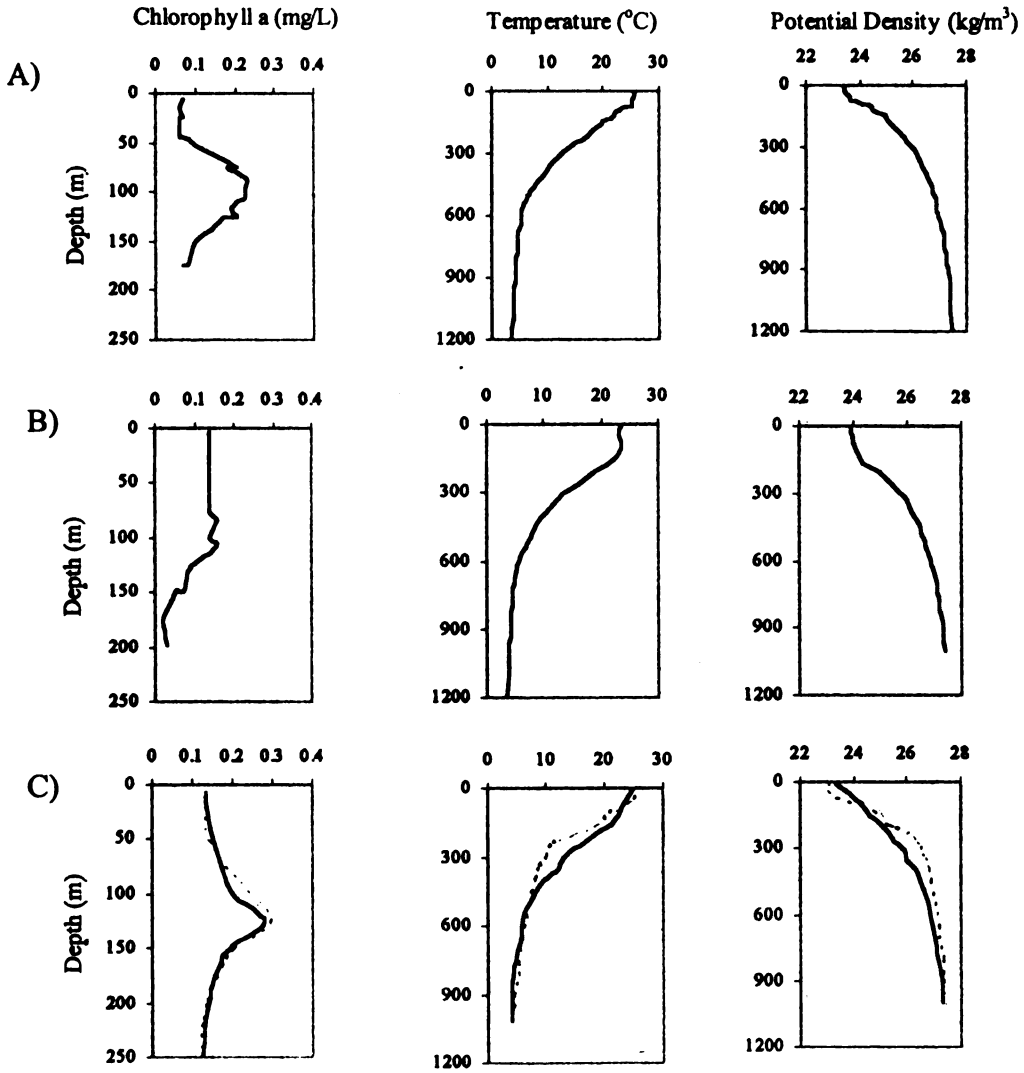
The concentration of DON far exceeds that of dissolved inorganic nitrogen in the upper water column (0-100 m), however our understanding of DON cycling in marine systems is still in its early stages. Here we present the first data set in which all marine DON greater than 100 Da has been isolated and isotopically characterized to further elucidate its ecological role in the oligotrophic NPSG.

We believe our study sites in the NPSG, Station ALOHA and Station 2, are representative of the regional habitat and therefore may contribute to the fundamental understanding of biogeochemical processes in this major biome. The majority of our data comes from the well-characterized Station ALOHA, however Station 2 samples have been incorporated into our May data set due to a limited number of ALOHA samples. There are no distinct differences in the water column data from these two stations (Figure 1C). However, DON and water column samples were collected from these sites over three different seasons (winter, summer and fall) allowing us to explore how temporal changes in physical and biological forcing can influence the composition and cycling of DON.

Density profiles show that there is distinct seasonal variation in the depth of the mixed layer (Figure 1). Historically this layer reaches 40 to 100 m at ALOHA (Karl, 1999). Deepening of the mixed layer is primarily the result of winter cyclones which cross the NPSG on a weekly basis (Karl, 1999). Our water column data show that the mixed layer reaches depths of approximately100 m in January, 50 m in May and 75 m in October. These depths are consistent with previous observations during these months (Karl, 1999). Temperatures, however, remain relatively constant in the upper 50 m (24-

25 °C). The concentration of chlorophyll and the depth of the chlorophyll maximum vary seasonally. In October the chlorophyll concentration reached 0.23 μg/L and extended from 85 to 105 m. The maximum concentration in January was 0.16 μg chlorophyll/L between 85 and 105 m decreasing to a low 0.02 μg/L at 175 m. The chlorophyll concentration in May peaked sharply, reaching 0.29 μg/L at 125 m (Figure 1).

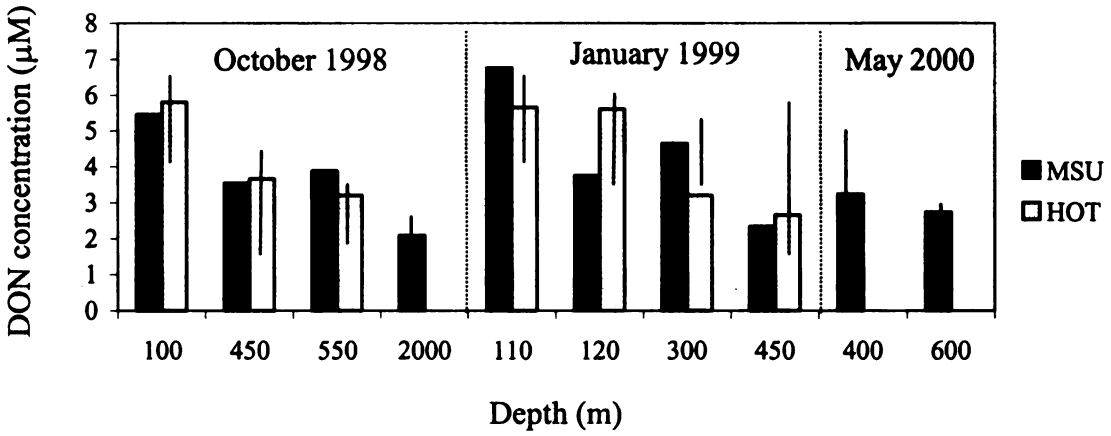
Figure 1



Water column data from Station ALOHA (solid lines) and Station 2 (dashed lines): A) October 1998, B) January 1999 and C) May 2000.

Understanding the trend in DON concentration at our sampling sites is one of the keys to deciphering the reactivity and cycling of DON in the NPSG. The first step in assessing these data was to compare our DON concentration values to those determined by the HOT core measurement program for the same cruise and/or with the range of data for the corresponding month between 1988 and 1999 (Figure 2). Comparative concentration data for our Station 2 samples were not available, as this station is not affiliated with the HOT program.

Figure 2



DON concentration data from ALOHA both determined by our dialysis and sealed tube combustion technique at Michigan State University (dark bars) and reported in the HOT database (light bars) for water samples from the same cast. Lines represent the range of HOT data at that particular month and depth between 1988 and 1999.

Our samples underwent sealed tube combustion and estimates of DON concentration were based on the ion beam produced within a calibrated volume of the mass spectrometer. Dissolved organic nitrogen concentrations available in the HOT database were estimated as the difference between total dissolved nitrogen (TDN) and nitrate + nitrite (N+N) concentrations (Karl et al., 2001). Total dissolved nitrogen concentrations were determined by UV oxidation (Armstrong et al., 1966) followed by measurements of

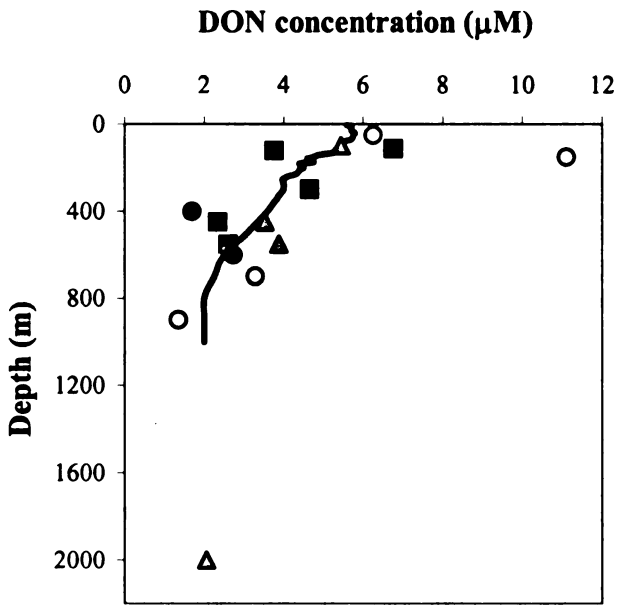
N+N and ammonium as hydrolysis products (Walsh, 1989). Nitrate + nitrite concentrations were determined using a four-channel Technicon Autoanalyzer II continuous flow system. The N+N analysis method is based on recommendations of Technicon Industrial Systems (1979) with modifications from Armstrong et al. (1967). The excellent agreement between our data and those of the HOT database validates both our DON isolation technique and our method for determining DON concentration.

In the development of our dialysis method it became apparent that a salt gradient was imperative in order to achieve adequate diffusion. Dialysis of a concentrated marine sample against ultra-pure deionized water can result in rapid diffusion of water into the 100 Da MWCO membrane, expanded pore size and subsequent fractionation of the DON. Several lots of 100 Da MWCO membrane were used in the course of our method development and sample analysis. According to the manufacturer, while pore size is constant across lots, porosity can vary substantially, causing individual lots to differ in their diffusion rates and consequently in their integrity when exposed to high salt concentrations. We explored using larger pore size membranes. When a Spectra/Por CE 3500 Da MWCO membrane was used to dialyze our salt solution against ultra-pure deionized water no expansion was detected. This indicates that when 3500 Da MWCO or larger pore size membranes are used the membrane is not compromised during dialysis and therefore it is not necessary to dialyze against a saline gradient.

There is a distinct decrease in DON concentration down water column. With the exception of our 150 m sample collected in May 2000, concentrations generally range from 4-7 μM in near surface waters to approximately 2 μM below 800 m (Figure 3). The down water column trend in our data is consistent with that observed for the average

DON concentrations obtained from the HOT database (Karl et al., 2001). There is an inherent variability in DON concentrations particularly near the surface that can be observed in our data but is not obvious from the historical average. Concentrations reaching almost 10 μM have been measured several times over the course of the HOT program. This variability is a result of seasonal and temporal changes in the environmental conditions of the NPSG (Karl, 1999).

Figure 3



DON concentration data from ALOHA (closed symbols) and Station 2 (open symbols). Data collected in October 1998 are indicated by triangles, January 1999 by squares and May 2000 by circles. The line is average HOT DON data 1988-1997 from Karl et al., 2001.

Depth related trends in DON concentration reflect the dynamic nature of this large organic reservoir. The elevated DON concentrations in the upper water column are a result of local production, including passive release from photosynthetic microorganisms as well as lysis of phytoplankton and bacterial cells due to heterotrophic grazing, cell death and virus-induced lysis (Karl et al., 1998; Bronk and Glibert, 1993). In certain

oceanic, coastal and estuarine environments an average of 25-41 % of dissolved inorganic nitrogen taken up by phytoplankton can be released as DON (Bronk et al., 1994).

The decrease in DON concentration down water column is evidence of net assimilation and remineralization to nitrate (Figure 3). According to our data, at depths of only 400 m DON concentrations were reduced by 50 % or more relative to those in the upper 200 m. This relates to the observation that dissolved organic nitrogen is utilized quickly, predominantly by bacteria (Karl et al., 2001). Utilization of low molecular weight DON compounds, such as dissolved combined amino acids and dissolved DNA, can fuel up to 50 % of bacterial growth (Keil and Kirchman 1991; Jorgensen et al., 1994). Bacteria are considered the predominant consumers of DON, however there is evidence that some phytoplankton have cell-surface enzymes that may allow them to utilize more DON than previously thought (Bronk et al., 1994).

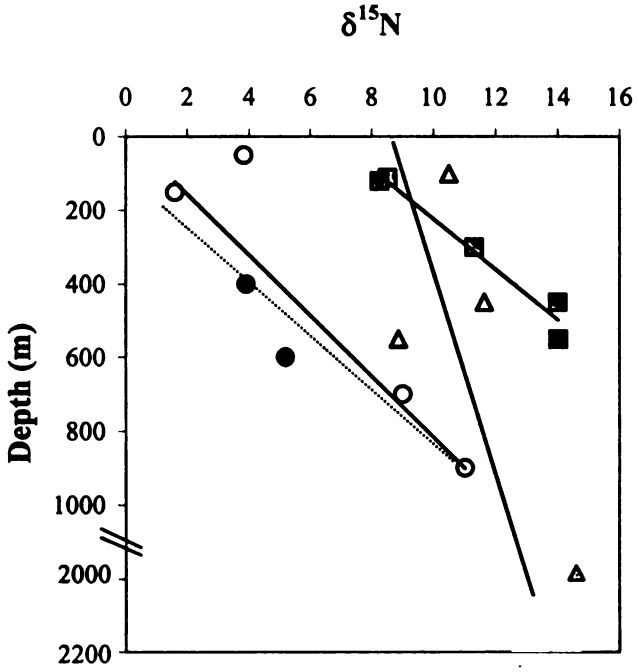
The relative contributions of labile and refractory components to the DON pool is still uncertain, however we do know that DON not assimilated by microbes can aggregate into colloids and particles which can then sink and undergo mineralization (Fenchel et al., 1998). Some of this sinking material is inherently refractory. For example, structural macromolecules such as peptidoglycans, which are important components in bacterial membranes, have been identified as major contributors (~64 %) to the high molecular weight (>1000 Da) fraction of DON (McCarthy et al., 1998). However, it is important to note that this molecular weight fraction only accounts for approximately 25 % of the DOM pool (Benner et al., 1997). Recalcitrant biomolecules likely contribute to a deep ocean pool of oxidation resistant, lower water column DON with a radiocarbon age of

over 4000-6000 years old (Williams and Druffel, 1988). The observation that the reservoir of DON in deep waters retains refractory components may explain why DON concentrations do not drop to zero but rather appear to stabilize at 2 μM. Historically DON concentrations in the NPSG have been reduced to 2 μM at depths as shallow as 350 m, but more commonly this stabilization occurs somewhere between 500-800 m and remains relatively constant to at least 2000 m (Karl et al., 2001).

While our concentration data suggest that DON is actively involved in NPSG nitrogen cycling , isotopic analyses provide more detailed insight into the dominant processes that contribute to the formation and utilization of this abundant nitrogen source. A number of interpretations can be made from the trends found in our DON isotopic data. There is a depth-dependent trend in each data set which demonstrates that DON isolated from surface waters is depleted in ^{15}N relative to samples taken at depth (Figure 4). The data from January (R^2=0.96, p=5.0 x 10^{-4}) and May (R^2=0.80, p=.02) are significantly correlated. When the 50 m May sample is excluded from the regression the data are significant at the 99 % confidence level (R^2 = 0.93, p=7.7 x 10^{-3}). Although the DON concentration of this sample is appropriate to the season and depth from which it was taken, the observation that the isotope value is enriched, rather than depleted, in ^{15}N relative to data from 150 m suggests that the nitrogen transformation processes within the mixed layer may vary from those taking place below the pycnocline. The elevated $\delta^{15}N$ value could result from phytoplankton uptake of a residual pool of ^{15}N enriched nitrate or ammonium (our $\delta^{15}N$ values for nitrate from ALOHA are >4.5 ‰) and subsequent production of DON. This is consistent with the observation that kinetic isotope effects result in mass discrimination when ^{14}N is preferentially incorporated into the product of

the reaction leaving the residual substrate enriched in ^{15}N (Mariotti et al., 1981). Alternatively, this sample may reflect a ^{15}N enriched pool of residual DON that could be achieved by intense recycling of DON in the mixed layer. The October concentration data are not as highly correlated (R^2=0.71, p=0.16) as the other two seasons and may represent a transitional period between seasons.

Figure 4



$\delta^{15}N$ values for DON. Samples collected from Station ALOHA in October 1998 are indicated by triangles, January 1999 by squares and May 2000 by closed circles. Open circles represent data collected in May from Station 2. The solid lines are least-squares regressions for each month. The dashed line is the May least-squares regression excluding the 50m point.

The roughly inverse relationship between concentration and $\delta^{15}N$ is consistent with down water column degradation of DON. However, correlations between the $\delta^{15}N$ values of DON and the natural log of the DON concentration were not significant (F-test, p=0.05) indicating that degradation does not adhere to a Rayleigh model (Mariotti et al.,

1988; Ostrom et al., 2002). This suggests that the isotopic composition of DON is not controlled by a single unidirectional reaction. This is not surprising for an actively cycling pool of organic nitrogen likely comprised of a variety of compounds. In the upper water column where production, and hence concentrations, of DON are high the nitrogen isotope ratios are correspondingly low, reflecting the selective release of ^{15}N depleted DON from microbial cells. As this DON is assimilated and degraded by microbial activity the residual pool becomes progressively more enriched in ^{15}N with depth. Although fractionation during degradation is the most likely cause of the increase in $\delta^{15}N$ values of DON with depth, it is important to stress that there are a number of degradative processes that can result in isotopic enrichment of DON. Enrichment of the DON pool can occur as microbes preferentially assimilate isotopically depleted DON. This occurs either as low molecular weight organics are directly assimilated by bacteria or, in the case of larger proteins and other nitrogenous polymers, when membrane bound hydrolytic enzymes first break down the compounds until they are suitable for assimilation (Fenchel et al., 1998). The microbial process of ammonification which converts DON to NH_4^+ (and eventually to NO_3^- via nitrification) can also result in the degradation and the consequent enrichment of DON with depth. In addition to degradation reactions the high $\delta^{15}N$ values of DON (>12 ‰), collected well below the euphotic zone in both the fall and winter, may be a result of bacterial uptake of regenerated, ^{15}N enriched, ammonium with subsequent production of enriched DON (McCusker et al., 1999). The low concentrations of ammonium in the NPSG (<1 μM) are consistent with a residual pool that has undergone bacterial utilization.

While increasing $\delta^{15}N$ values of DON is a trend seen in all three data sets, the range that these values span varies with season, reflecting the physical conditions and succession of primary producers within the NPSG. The distinct seasonal difference among the three data sets is most evident in the comparison of winter and summer samples (Figure 4). In May, the DON pool has isotope values ranging from 1.6 to 11.0 ‰. The lower end of this range suggests the influence of nitrogen-fixing organisms in upper water column DON production (atmospheric N_2=0 ‰). Samples collected in January have DON that is relatively more enriched in ^{15}N, with values ranging from 8.3 to 14.0 ‰. These values are consistent with DON produced by nitrate utilizing primary producers. Nitrate isotope values from samples collected at Station ALOHA were analyzed in our laboratory and found to be 4.5-9.5 ‰ (Sutka et al., in preparation). Similar to the samples collected in January, the isotope values from the October DON samples span 8.9 to 15.0 ‰, again consistent with DON production predominantly fueled by nitrate or ammonium.

Recent studies provide evidence that nitrogen fixing cyanobacteria are an important seasonal source of new nitrogen and, hence, DON (Karl et al., 1997). There are at least three key groups of nitrogen-fixing cyanobacteria in the NPSG including: (1) the large filamentous, non-heterocystous, bloom-forming *Trichodesmium* spp. (Capone et al., 1997), (2) the background population of small (3-5 μm) unicellular nanocyanobacteria (Zehr et al., 2001), and (3) endosymbiotic associations between the unicellular heterocystous cyanobacterium *Richelia* and several species of diatoms (Villareal,1999) which also form aperiodic blooms.

At Station ALOHA nitrogen fixation is most prevalent during the summer months when stratification of the water column allows for the selection of the bloom-forming, nitrogen fixing microbes (Karl et al., 1997). In the case of *Trichodesmium*, up to 50 % of their fixed nitrogen may be released as dissolved new nitrogen (Glibert and Bronk, 1994). Interestingly, concentrations of DON are slightly, although not statistically, higher during the fall and summer months as compared to winter and spring (Karl et al., 2001). In addition there is evidence that during El Niño Southern Oscillation (ENSO) events, prolonged stratification of the water column allows for extended periods of nitrogen fixation leading to even greater DON production (Karl et al., 1997). During one such episode, a dramatic increase in DON concentration coincided with an increase in *Trichodesmium* spp. at Station ALOHA (Karl et al., 1997). These seasonal and interannual changes in DON production are supported by our work and by the work of Karl et al. (1997). The data suggest a direct relationship between DON and the nitrogen fixing organisms of the microbial loop and further support the labile nature of DON.

The net production of DON that occurs during ENSO episodes would result in local storage and eventual export of DON. Furthermore, increases in NPSG DON over the course of the HOT program have been attributed to the influence of nitrogen fixation (Church et al., in press; Karl et al., in press). In the NPSG, ENSO enhanced nitrogen fixation can result in a supply of up to 51 $mmolNm^{-2}y^{-1}$ (Karl et al., 1997). Fixed nitrogen released from microbes can accumulate as DON in the euphotic zone. This DON is then exported to the lower water column most likely due to mixing events and the sinking of aggregated DON. Mineralization of the exported DON results in a substantial substrate pool for nitrification, the predominant mechanism for the production

of nitrous oxide in this region, according to studies conducted at ALOHA (Dore et al., 1996; Ostrom et al., 2000). Nitrous oxide produced in the deep waters of the NPSG could ultimately be vented to the atmosphere by diffusion and deep mixing events, contributing to the increasing atmospheric concentration of this greenhouse gas. This chain of events, beginning with ENSO induced stratification of the water column and resulting in the production of nitrous oxide, would effectively create a climatically driven nitrous oxide pump and place DON in a key intermediary position amidst the ecosystem changes that occur in the NPSG.

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