Isotopic heterogeneity and cycling of organic nitrogen in the oligotrophic ocean

T. B. Meador and L. I. Aluwihare[1]
Geosciences Research Division, Scripps Institution of Oceanography, University of California San Diego, La Jolla, California 92093-0208

C. Mahaffey[2]
Department of Biological Sciences, University of Southern California, Los Angeles, California 90089

Abstract

We measured the nitrogen (N) isotopic composition (δ^{15}N) of a large set ($n = 38$) of high-molecular-weight (HMW) dissolved organic nitrogen (DON) samples isolated from the tropical and subtropical North Atlantic and Pacific Oceans. The δ^{15}N signature of surface HMW DON is relatively invariable in both oligotrophic basins (4.1 $\pm$ 0.6‰ in the Atlantic; 5.4 $\pm$ 0.8‰ in the Pacific) and shows little correlation with sources or concentrations of N supporting new production in the euphotic zone. While large variations in δ^{15}N of bulk HMW DON are not apparent, δ^{15}N of proteins isolated from sites with relatively high rates of N_2 fixation (>80 μmol N m^{-2} d^{-1}) were consistently depleted in ^{15}N relative to bulk HMW DON and to proteins isolated from sites where N_2 fixation does not routinely occur. This small component of HMW DON appears to be cycling more rapidly than bulk HMW DON and may be indicative of fresh DON contributed by organisms in the surface ocean. Furthermore, δ^{15}N of DNA extracted from the bacterial size fraction (0.2–0.5 μm) revealed that free-living bacteria may be an important sink for isotopically depleted N produced during N_2 fixation. We suggest that there exists a tight coupling between the production and uptake of DON contributed by diazotrophs (N_2 fixers) in regions where N_2 fixation provides a major input of new nitrogen.

The dissolved organic nitrogen (DON) pool quantitatively dominates the surface ocean fixed N reservoir, comprising up to 96–99% of total dissolved N in the oligotrophic ocean (excluding N_2; Abell et al. 2000). Concentration profiles for DON are currently not as well quantified as those for dissolved organic carbon (DOC); however, Bronk (2002) reports a surface ocean mean of 5.8 $\pm$ 2.0 μmol L^{-1} and lower concentrations for the deep ocean in the range of 2–5 μmol L^{-1}. Increased DON concentrations at the surface ocean (over deep values) suggest that there is a surface ocean source for DON. This large reservoir of presumably bioavailable DON may have significant consequences for primary and secondary production, particularly in oceanic regions where nitrogen is considered a limiting nutrient.

In order to further understand processes controlling the cycling of N through DON, this study compared spatial variations in the natural stable nitrogen isotopic composition (δ^{15}N) of the high-molecular-weight (HMW) component of DON, proteins precipitated from HMW DON, and suspended particulate organic nitrogen (PN$_{susp}$) in the oligotrophic ocean. We first examined whether δ^{15}N (where δ^{15}N = [((^{15}N/^{14}N)$_{sample}$/(^{15}N/^{14}N)$_{N2}$) $-$ 1] $\times$ 1,000) compositions of each organic N pool were influenced by the extent of N_2 fixation in the region. Spatially resolved differences and similarities in δ^{15}N enabled us to further contrast the surface ocean residence time of HMW DON and dissolved proteins to that of PN$_{susp}$.

The oligotrophic ocean is an ideal location for this study because there are temporally and spatially varying sources of nitrogen to the surface ocean, each with a distinct isotopic composition. Nitrogen demand by new production in the surface oligotrophic ocean is supported by atmospheric N_2 fixation (δ^{15}N $\approx$ 0‰; Carpenter et al. 1999) and/or by nitrate upwelled from depth (δ^{15}N-NO$_3^-$ = 4.8 $\pm$ 0.2‰, deep ocean average (avg) $\pm$ standard deviation (SD); Sigman et al. 2000). Recycled production supported by ammonia is also expected to exhibit a characteristically depleted isotopic signature (δ^{15}N-NH$_4^+$ $\approx$ -2 to 4‰; Checkley and Miller 1989). Variations in these sources of N supporting euphotic zone production were thought to generate gradients in δ^{15}N of surface PN$_{susp}$ observed both with latitude (Mino et al. 2002) and longitude (Waser et al.

2000; Montoya et al. 2002) in mesotrophic and oligotrophic regions of the North Atlantic Ocean. Also, isotopic composition studies at Station ALOHA in the subtropical North Pacific gyre have revealed that sinking particulate nitrogen (PN_{sink}) records the seasonal variability in the intensities of the two sources of new N to this site (i.e., N_2 fixation and nitrate; Karl et al. 1997; Dore et al. 2002). The isotopically light $\delta^{15}N$ value for summer PN export (mean value = 1.83‰) was attributed to relatively higher N inputs via N_2 fixation during this season, particularly due to blooms of *Trichodesmium* spp. (Karl et al. 1997). Previous studies have shown the capacity for *Trichodesmium* spp. to release up to half of recently fixed N as DON, with a presumably depleted $\delta^{15}N$ signature, at rates on the order of 100 pmol N colony^{-1} h^{-1} (Capone et al. 1994; Glibert and Bronk 1994); and so, the $\delta^{15}N$ of DON may also be expected to exhibit seasonal variability at this site. However, Knapp et al. (2005) produced a time series of total organic nitrogen (TON) concentration and isotopic composition ($\delta^{15}N$-TON) at the Bermuda Atlantic Time Series (BATS), a site where seasonal N_2 fixation has also been invoked (e.g., Carpenter and Romans 1991), and concluded that DON, if produced during N_2 fixation, does not accumulate on seasonal or longer time scales.

Radiocarbon ($\Delta^{14}C$) studies have shown that HMW DOC has a shorter residence time in surface waters (i.e., is more reactive) than the bulk DOC pool; $\Delta^{14}C$-HMW DOC was 10‰ at a site near Hawaii (Repeta and Aluwihare 2006) compared with −180‰ for total DOC at a nearby site in the central North Pacific (Druffel et al. 1992). Radiocarbon measurements necessarily target C, but compounds that constitute HMW DON are a subset of the HMW DOC pool, and so labile HMW compounds may be enriched in N, as has been observed for labile particulate organic matter (e.g., Knauer et al. 1979). For example, Loh et al. (2004) showed that the protein-like fraction of HMW dissolved organic matter (DOM) was enriched in radiocarbon (−21‰ in the Pacific Ocean and +2‰ in the Atlantic Ocean) relative to total DOC. In addition, evidence for lower C : N values of surface HMW DOM (15–18 vs. 18–20 at depth; Benner 2002) and results of chemical composition analyses (Aluwihare et al. 2005) indicate that up to ~40% of surface water HMW DON is chemically distinct from deep-water HMW DON and is therefore reactive on time scales of upper-ocean mixing. While HMW DON is only a fraction of bulk DON (~20–30%; Benner 2002), the above characteristics taken together suggest that components of HMW DON in surface waters may be more reactive than bulk DON. Therefore, while a contribution from N_2 fixation to the bulk DON pool has not yet been conclusively identified (Hansell and Carlson 2001; Knapp et al. 2005), HMW DON, if cycling more rapidly than the bulk DON reservoir, may record such a contribution. We hypothesized that HMW DON in the oligotrophic ocean, if replaced on seasonal/subannual time scales, would exhibit isotopic variations similar to PN_{susp} and be diagnostic of a diazotroph-specific contribution.

Results from this study show that $\delta^{15}N$ of the HMW component of DON ($\delta^{15}N$-HMW DON) is remarkably constant across diverse oceanic regions. Given the docu-

mented variations in $\delta^{15}N$-PN_{sink} and $\delta^{15}N$-PN_{susp} and their correlation to the source of N fueling production in surface waters (Karl et al. 1997; Waser et al. 2000; Mino et al. 2002; Montoya et al. 2002), the unvarying nitrogen isotopic composition of HMW DON isolated from sites spanning a range of nutrient regimes is unexpected. Different $\delta^{15}N$ compositions of PN_{susp} and bulk HMW DON suggest that these pools of N integrate either distinct N sources or time periods of formation. If these organic N fractions are formed contemporaneously in surface waters, then it is unlikely that distinct sources of N would be channeled into DON versus PN, and so we interpret our findings to demonstrate that HMW DON has a longer residence time than PN_{susp} in surface waters. Isotopic compositions of proteins isolated from HMW DON, along with evidence provided by culture studies of *Trichodesmium*, support the existence of a component of HMW DON that cycles rapidly enough to record the $\delta^{15}N$ variability of N source, similar to PN_{susp} and unlike bulk HMW DON. Additionally, $\delta^{15}N$ of DNA extracted from microorganisms (0.2–0.5 μm) living in surface waters with high abundances of *Trichodesmium* confirms the utilization of an isotopically depleted N source (relative to regions where *Trichodesmium* abundance was low). This finding is consistent with the hypothesis that N derived from recent N_2 fixation is rapidly consumed in surface waters and provides a mechanism to explain the low accumulation of recently synthesized DON in surface waters. The DNA and protein results further provide in situ evidence consistent with the proposed release of N by *Trichodesmium* (Capone et al. 1994; Glibert and Bronk 1994) and have implications for the means of nitrogen trafficking between diazotrophs and other primary and secondary producers of the oligotrophic ocean.

Materials and methods

Sample collection—Most field samples collected for this study were obtained through invited participation in three research cruises in the subtropical Atlantic and Pacific Oceans: MP8 (spring 2003), MP9 (summer 2003), and En391 (spring 2004) (Fig. 1). Sample locations and parameters are presented in Table 1. The HMW DON samples isolated as part of this study represent diverse environments of the North Atlantic and North Pacific Oceans. Sampling sites of MP8, in the western equatorial Atlantic (Fig. 1), spanned a salinity range from 24 to 36 (S, in practical salinity units; Table 1); total organic carbon (TOC) concentration also showed significant spatial variability (<70 to >100 μmol L^{-1} C). Some sites exhibited high *Trichodesmium*-specific N_2-fixation rates (Capone et al. 2005) and/or high abundances of *Richelia*, an N_2-fixing symbiont of the diatoms *Rhizosolenia cleveii* and *Hemiaulus hauckii* (Foster et al. 2007). MP9, in the subtropical North Pacific, sampled primarily oligotrophic waters, with high rates of *Trichodesmium*-specific N_2 fixation observed around the Hawaiian Islands and no *Trichodesmium* detected at the western stations (D. Capone and J. Sohm pers. comm.). During En391, in the western subtropical North Atlantic, a variety of nutrient regimes
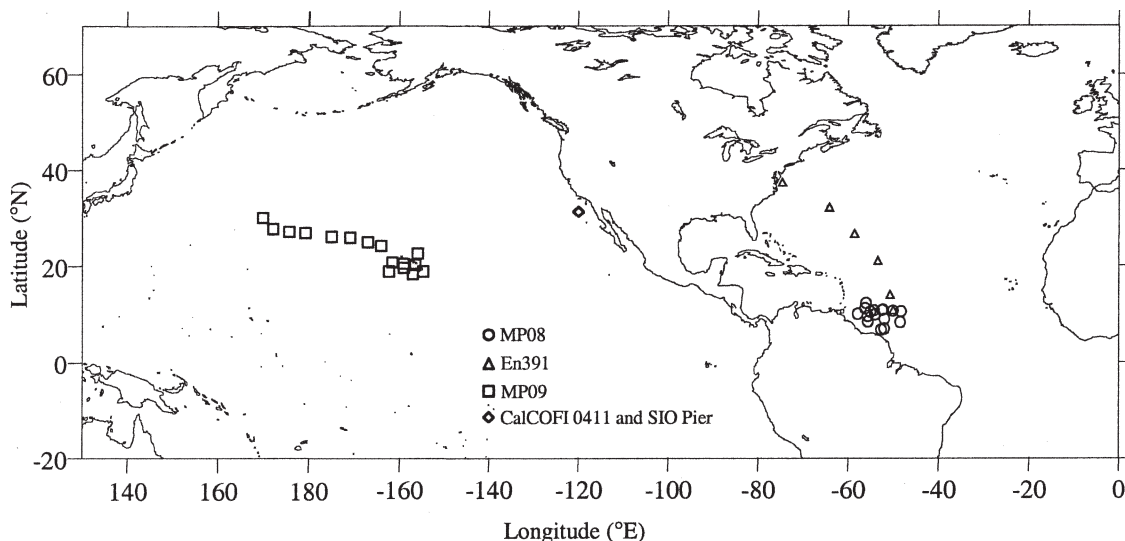
Fig. 1.   Samples were collected from the subtropical North Atlantic Ocean in April–May 2003 (MP08, circles) and April 2004 (En391, triangles). Samples were also collected from the subtropical North Pacific Ocean in July–August 2003 (MP09, squares) and from the Southern California Bight in July 2002, November 2004, and May 2005 (CalCOFI 0411 and the SIO pier, diamond).

from mesotrophic to oligotrophic were sampled, and *Trichodesmium* was present at lower latitude stations (J. Sohm pers. comm.).

HMW DON and PN_{susp} samples were also collected from the Scripps Institution of Oceanography's (SIO) Pier (summer 2002 and spring 2005) and during the California Cooperative Oceanic Fisheries Investigation (CalCOFI) survey cruise in November 2004 (Table 1). The CalCOFI HMW DON sample was a composite of several surface (2 m) open-ocean stations spanning the entire range of the CalCOFI grid ($n = 17$); PN_{susp} samples ($n = 10$) were collected from a subset of the HMW DON sampling stations (DeJesus and Aluwihare pers. comm.).

Isolation of HMW DON and PN_{susp} from seawater— Samples for PN_{susp} were collected by pumping surface seawater with a diaphragm pump through Teflon tubing and through precombusted, 150-mm glass-fiber filters (Whatman GF/F; nominal pore size of 0.7 μm) housed in polycarbonate filter holders. HMW DON was isolated by tangential flow ultrafiltration (UF). Seawater was collected as above from a depth just below the surface (2–3 m) and pumped through either a Millipore, cellulose ester, cartridge prefilter (Cat. No. KW06A10TT1; pore size 0.5 μm; 0.8-m² surface area) and a Pall SuporLife 200, polyethersulfone, cartridge filter (Cat. No. SCS92DP71S; pore size 0.2 μm; 0.5-m² surface area) or a single 0.2-μm prefilter. Filter membranes were enclosed in either stain-less-steel or manufactured polycarbonate housings and were prerinsed with 10% HCl, followed by Milli-Q (Millipore) water to achieve a neutral pH. The pressure within the filtering apparatus was monitored and kept at or below 200 kPa. All hardware was acid rinsed before each cruise; all components used in UF were acid rinsed between samples. Volumes of 200–800 L collected from each site were concentrated by a UF system, designed in collabora-

tion with Separation Engineering Inc., using a 1,000-Da molecular-weight cutoff (MWCO), polysulfone, spiral-wound filter. Ultrafiltered samples were immediately desalted by rinsing with >40 L Milli-Q water to a final volume of ~4 L (Aluwihare et al. 2002; Aluwihare unpubl. data). Frozen, concentrated HMW DON samples were lyophilized under vacuum in the laboratory, and the dry powder was used in a suite of chemical analyses. UF was successful in isolating approximately 20–30% of the total N (TN) in surface seawater samples, as determined by TN concentrations of bulk seawater and dry-weight and elemental composition data of final lyophilized HMW DOM.

TN concentration—Samples of ~40 mL were collected in combusted glass vials by pumping surface seawater through Teflon tubing or sampling from niskin bottles. Samples were acidified with ~40 μL of 12 mol L⁻¹ HCl and stored at room temperature until analysis with a Shimadzu TOC-V fitted with a Shimadzu TNM-1 unit. Acidified seawater was directly oxidized via high temperature catalytic oxidation; CO_2 was quantified by infrared detection, and NO was quantified by chemiluminescence detection. Both inorganic and organic N standards were routinely run to calibrate the instrument. The quality of the data was assessed according to manufacturer's protocol and recom-mendations by Jon Sharp (University of Delaware). For the MP8 data set, the standard deviation for all surface TN concentration analyses was 0.5 μmol L⁻¹ on average, and the average coefficient of variation was 8%.

Elemental and stable isotope composition—The elemental and isotopic compositions of solid, dry HMW DON samples, protein precipitates, and size-fractioned DNA were determined at the SIO's Unified Laboratory Facility using standard elemental analyzer isotope ratio mass

Table 1. Surface concentrations and stable isotope compositions of nitrogen in HMW DON and PN$_{susp}$ isolated during cruises MP8 and En391 in the Atlantic Ocean; MP9, Calcofi 0411, and SIO Pier in the Pacific Ocean; and from *Trichodesmium* cultures. Averages (±SD) are provided for each cruise and for three *Trichodesmium* cultures.

Cruise	Station	Lat. (°N)	Long. (°W)	Salinity (S)	TN (μmol L^{-1})	δ15N-PN$_{susp}$ (‰)	δ15N-HMW DON (‰)	C : N HMW DON
MP8	4	10.78	52.28	36.108	4.2	2.5	3.8	14.0
	5	8.88	52.00	36.278	3.4	ND*	4.9	15.5
	6	6.88	52.03	24.633	5.7	3.9	3.2	16.4
	12	9.81	54.08	34.204	5.6	−1.5	4.6	19.2
	14	11.08	56.25	33.941	6.6	1.5	2.4	17.4
	18	10.35	55.10	33.179	3.7	−0.2	3.8	15.1
	20	8.35	55.67	31.913	5.8	−0.2	3.8	16.5
	26	9.49	55.68	32.769	5.9	−0.3	3.9	17.2
	28	12.18	56.04	34.620	4.1	−0.7	3.8	18.3
	30	10.65	54.28	34.665	4.2	ND	3.9	15.4
	32	10.70	52.53	34.911	3.3	−1.1	4.4	14.8
	34	10.56	50.07	36.075	2.9	−1.3	4.9	15.7
	36	10.44	48.30	36.339	3.9	−0.9	4.1	16.6
	38	8.23	48.49	36.056	6.0	−1.2	4.5	15.9
	41	6.68	52.73	24.267	7.3	3.7	4.4	18.0
	56	8.38	55.61	30.972	8.7	3.7	4.4	16.2
	66	9.93	57.90	32.848	6.3	0.7	5.2	15.4
Avg. ± SD	—	—	—	—	5.2 ± 1.6	0.6 ± 2.0	4.1 ± 0.7	16.3 ± 1.3
En391	1	36.95	75.77	34.596	ND	ND	4.3	16.2
	2 (BATS)	31.83	64.17	36.746	ND	5.3	3.8	12.9
	3	26.32	58.58	37.105	ND	ND	3.2	16.3
	4	20.71	53.46	37.179	ND	ND	3.9	17.6
	5	13.68	50.72	36.329	ND	ND	4.9	15.2
	6	10.50	50.03	36.494	ND	ND	4.2	13.5
Avg. ± SD	—	—	—	—	—	—	4.0 ± 0.6	15.3 ± 1.8
MP9	1 (ALOHA)	22.75	158.00	35.100	5.4	ND	5.5	14.0
	2	26.00	174.98	35.214	6.3	ND	4.8	15.2
	3	30.00	170.01°E	35.612	4.1	ND	5.0	16.2
	4	27.76	172.21°E	35.372	4.4	ND	4.7	14.7
	5	27.38	175.73°E	35.257	4.2	ND	4.5	15.2
	8	25.69	170.94	35.265	4.7	ND	4.5	15.1
	9	24.95	167.01	35.083	4.6	ND	4.8	17.5
	10	24.22	164.01	34.837	4.8	ND	4.9	16.9
	14	18.50	157.00	34.647	ND	−0.4	6.3	14.1
	15	18.94	154.82	34.302	ND	1.7	6.8	13.3
	17	19.54	158.98	34.969	ND	ND	5.8	15.1
	20	20.24	156.50	34.644	ND	1.1	6.2	15.2
	21	20.25	159.19	34.995	ND	−2.5	5.2	11.8
	22	19.11	162.22	34.640	ND	0.5	5.7	14.9
	23	20.50	161.50	34.740	ND	0.3	6.0	16.0
Avg. ± SD	—	—	—	—	4.8 ± 0.7	0.1 ± 1.5	5.4 ± 0.7	15.0 ± 1.4
CalCOFI 0411 (avg. ± SD)	—	~31.00	~122.00	—	3.4 ± 0.6	7.5 ± 1.3	6.1	14.0
SIO Pier	—	32.87	117.25	ND	ND	8.0	5.8	14.2
Trichodesmium culture (avg. ± SD)	—	—	—	—	—	−1.5 ± 0.9	1.5 ± 1.5	11.8 ± 3.6

* ND, no data.

spectrometry (EA-IRMS) techniques (Owens and Rees 1989). The results are reported in the standard δ notation (‰) relative to atmospheric nitrogen (N$_2$). Data were collected using a continuous flow system consisting of a Perkin Elmer CHN analyzer coupled to a ThermoFinnigan Delta Plus IRMS; acetanilide was the standard for both elemental and isotopic measurements and accompanied every run as a check on instrument stability. Samples submitted for isotopic analysis contained >15 μg of nitrogen above the tin cup blank (15 ± 5 μg of C and N), and instrument error ranged from 0.3‰ to 0.5‰ for all samples, unless otherwise noted; typical reproducibility for δ15N measurements was ±0.2‰.

Subsamples (13-mm-diameter discs) for PN$_{susp}$ were randomly selected from freeze-dried 150-mm glass-fiber filters and homogenized using a mortar and pestle and
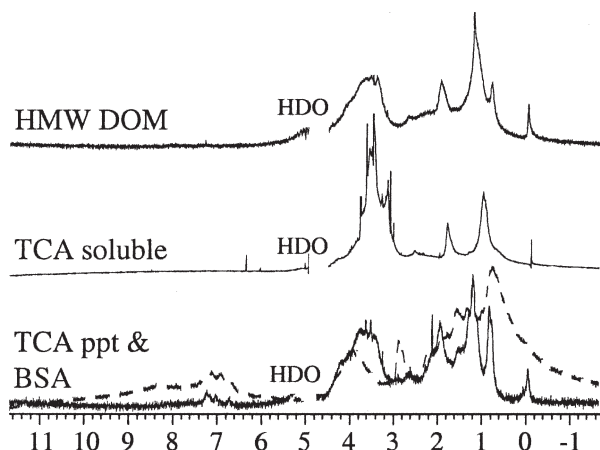
Fig. 2.   ^{1}H-NMR spectrums for HMW DOM and its TCA-soluble and -insoluble (i.e., protein ppt) components. The ^{1}H-NMR spectrum of bovine serum albumin (BSA, dotted line) is shown for reference.

analyzed according to Owens and Rees (1989). The isotopic composition of these samples was analyzed at the University of Southern California using a EuroVector EA coupled to a Micromass Isoprime IRMS. Reproducibility was better than $\pm 0.2\permil$. Comparisons of $\delta^{15}N$ data and other values refer to the probability (p) associated with a two-tailed, homoscedastic Student's t-test.

Isolation of proteins from HMW DON—Proteins were pelleted from HMW DOM by precipitation with trichloroacetic acid (TCA), modified from Yamada and Tanoue (2003). Briefly, dry HMW DOM was resuspended in Milli-Q to a concentration of 10 mg mL^{-1}, and insoluble material was pelleted by centrifugation at 10,000 × g for 10 min. Proteins dissolved in Milli-Q water were then precipitated overnight in 10% TCA and 5% isopropanol at 4°C, followed by centrifugation at 10,000 × g for 20–30 min at 4°C. The pellet was then washed by successive resuspensions in 5% TCA, ethanol, and diethyl ether followed by centrifugation at 10,000 × g for 5–10 min at 4°C for each wash. The protein pellet was then subjected to elemental/isotopic analyses as above for HMW DOM, and proton (^{1}H) nuclear magnetic resonance (NMR) spectroscopy and amino acid analysis were also performed. The ^{1}H-NMR spectrum of the protein pellet (TCA-insoluble fraction) was distinct from that of the TCA-soluble material and shared resonances similar to that of bovine serum albumin (BSA) (Fig. 2). The amino acid composition of the protein pellet was determined by reversed-phase high-pressure liquid chromatography (HPLC) separation and fluorescence detection of *o*-pthalialdehyde and *N*-acetyl-L-cysteine (OPA-NAC) amino acid derivatives following hydrolysis in 6 mol L^{-1} HCl at 110°C for 18–24 h (according to Zhao and Bada 1995). Amino acid analysis revealed that ~60% of the N in the pellet is quantifiable as fluorescent OPA derivatives; this is similar to the amount of N that can be recovered from the amino acid analysis of a pure protein (BSA) and is significantly more than that for bulk HMW DON (~16–30% N; McCarthy et al. 1996;

Aluwihare et al. 2005). Furthermore, major amino acids previously reported to be present in HMW DON were identified as OPA derivatives and showed a similar distribution pattern (with the exception of isoleucine, which was more abundant in the TCA-precipitated pellet; data not shown). In the rest of the manuscript we will refer to the precipitated pellet as protein based on the analyses above.

The radiocarbon content ($\Delta^{14}C$) of one protein pellet and its total HMW DOM counterpart, both collected from 110-m depth (the chlorophyll fluorescence maximum) at the BATS site, were determined following closed tube combustion of the dry pellet (or powder, in the case of HMW DOM) in quartz tubes according to Repeta and Aluwihare (2006) and carbon isotope analysis by accelerator mass spectrometry at Lawrence Livermore National Laboratory.

DNA extraction—Microorganisms for $\delta^{15}N$-DNA analysis were collected from surface waters during En391 by the filtration method described above. Two integrated samples were collected: one from stations with low abundances of *Trichodesmium* (stations 1 and 2) and another at stations with high abundances of *Trichodesmium* (stations 3–6; Table 1). All samples were prefiltered through 0.5 μm to exclude diazotrophs and particle-attached bacteria, and DNA was isolated only from organisms collected on the 0.2-μm filter. Our isolates represent smaller, free-living microorganisms only (between 0.2 and 0.5 μm). Unlike GF/F filters, our 0.5-μm, high-capacity, mixed cellulose filters likely did not retain a significant fraction of bacteria <0.5 μm in size.

DNA was extracted from the 0.2-μm filter using a method similar to Blair et al. (1985), modified by this laboratory (Hansman and Aluwihare unpubl. data). Filters were soaked in ~1.5 L of 1.5 mol L^{-1} NaClO$_4$ for 48 h, and the solution was then concentrated to ~15 mL by ultrafiltration in an Amicon stirred cell with a MWCO of 1,000 Da. The solution containing DNA and other biochemicals was extracted with an equal volume of chloroform:iso-amyl alcohol (24:1, v/v) followed by centrifugation at 10,000 × g. The aqueous, DNA-containing layer was then removed, and the extraction was repeated. Aqueous layers were combined and DNA was precipitated (2×) overnight at −20°C in 70% ethanol and 0.085 mol L^{-1} NaCl and centrifuged for 30 min at 10,000 × g. The supernatant was decanted, and the pellet was washed with 5 mL of cold 95% ethanol and reprecipitated at 10,000 × g for 5 min. The pellet was air dried for 10 min and resuspended in nuclease-free water, and DNA isolation was verified by UV-Vis absorption and agarose gel electrophoresis. In one experiment, 18 mg of material were pelleted after filtering approximately 7,000 L of seawater. The isotopic composition of the pellet was determined by the same method used for HMW DOM above.

Trichodesmium cultures—Two strains of *Trichodesmium* were cultured and harvested to obtain PN and HMW DON for $\delta^{15}N$ comparisons. *Trichodesmium* IMS101 was obtained from the Provasoli-Guillard National Center for

Culturing of Marine Phytoplankton (CCMP) and grown to volumes ranging from 25 mL to 10 L in artificial YBCII medium (Chen et al. 1996). This medium had no nitrate or ammonium but was amended with some N-containing organic molecules (ethylenediamine tetraacetic acid [EDTA], vitamin B12, biotin, and thiamine). Complete consumption of these N-containing substrates and subsequent incorporation of the N atoms into HMW DON could account for no more than 4% (1%, if EDTA is excluded) of the HMW DON isolated from *Trichodesmium* cultures; this estimate was based on typical dry-weight recoveries and elemental compositions of culture HMW DON. The absence of compound-characteristic resonances in ^{1}H-NMR spectra confirmed that these molecules were not significantly concentrated along with HMW DON isolated from *Trichodesmium* cultures. Although the CCMP strain was not axenic, sterile protocols were implemented to avoid an overwhelming bacterial influence. An axenic *Trichodesmium* strain was obtained from J. Waterbury (Woods Hole Oceanographic Institution) and grown to volumes of 1 L in YBCII media. Both *Trichodesmium* strains were maintained in sterile culture flasks and incubated at ~25°C, with a 12:12 light:dark cycle and exposed to 3–6 quanta m^{-2} s^{-1}. Large volume cultures were measured daily for cell counts, TOC, TN, and dissolved inorganic nitrogen (DIN); growth rates were determined by fluorescence on a Turner-Designs fluorometer with excitation/emission wavelengths of 544 and 577 nm.

PN from *Trichodesmium* cultures was filtered onto combusted GF/F filters and subjected to IRMS as described above. Alternatively, *Trichodesmium* cells were pelleted from culture aliquots at 10,000 × g for 5 min and subjected to elemental and isotopic analysis. HMW DON was harvested by filtering *Trichodesmium* cultures through 0.1-μm Whatman Polycap™ TF filters (Cat. No. 3601) and concentrated by UF as described above or with a stirred cell (Millipore Cat. No. 5124; operated according to manufacturer's protocol) using a 1-kDa Millipore membrane (Cat. No. 13342).

PCR amplification of nif*H and DGGE*—Environmental bacterial DNA samples (from organisms that passed through the 0.5-μm filter but were retained on the 0.2 μm-filter, discussed above) were screened for the *nif*H gene by I. Hewson (University of California, Santa Cruz) via amplification with PCR according to Zehr and McReynolds (1989). To ensure that the DNA extraction protocol for isotopic analysis was not biased toward a particular organism, we performed fingerprint analysis via denaturing gradient gel electrophoresis (DGGE), targeting 16S rRNA genes using a universal primer complementary to position 517–534 (5′-ATTACCGC-GGCTGCTGG-3′) and a bacterial primer complementary to position 341–358 with a 40-bp GC clamp (5′-CGCCC-GCCGCGCGCGGCGGGCGGGGCGGGGGCACGG-GGGGCCTACGGGAGGCAGCAG-3′) (Muyzer et al. 1993). PCR amplification and DGGE was performed by F. Malfatti (SIO) according to Don et al. (1991) and Riemann et al. (2000).

PCR amplification and sequencing—To ensure that our filter isolates were representative of the microbial community previously identified to be present in surface waters of the oligotrophic ocean, 16S rRNA gene sequences were amplified from DNA (extracted from 0.2-μm filters) using Takara Ex Taq Polymerase (Takara Mirus Bio Product No. TAK RR001A). The PCR was performed by adding 50–500 ng (as estimated by UV absorption at 260 nm) of the extracted environmental DNA to the PCR mixture as described by the manufacturer's protocol. Universal bacterial 16S primers 27f and 1492r were used at a final concentration of 1 μmol L^{-1}. Products from 6 PCR reactions were combined and purified using the Marligen PCR Purification Kit and concentrated to a volume of 30 μL. Cleaned PCR products were cloned into TA vector PCR 2.1-TOPO® (Invitrogen) and transformed into Top 10 Chemically Competent *E. coli* cells. PCR products of 16S rRNA genes amplified from clone libraries by the M13 primer set (Invitrogen) were digested by the restriction enzymes HhaI and HaeIII at a final concentration of 2 units μL^{-1} for 4 h at 37°C for restriction fragment length polymorphism (RFLP) analysis. Gene fragments were resolved by electrophoresis on a 1.2% agarose gel at 40 V for 2 h in order to compare restriction profiles within the clone library. 16S rRNA clones with unique restriction profiles as determined by RFLP were selected and sequenced to 2–3× coverage, using the universal primers 27f, 338f, 519r, 1055f, 1074r, and 1492r and Big Dye (ABI Technologies, Inc). Of the 25 unique sequences identified by RFLP analysis of a 96-well clone library, 16 were submitted for sequencing. Sequencing products were submitted to Sequegene, and resulting sequence fragments were compiled into contigs using Sequencher (GeneCodes, Inc.). 16S rRNA gene consensus sequences were blasted in Genbank database (http://www.ncbi.nlm.nih.gov) to determine similarity to bacteria previously identified to be present in the oligotrophic North Atlantic.

Results and discussion

The δ^{15}N of bulk HMW DON and PN$_{susp}$—This study has generated δ^{15}N data for 38 HMW DON surface samples (Table 1), producing a substantial data set sufficient for an analysis of the cycling of DON in the oligotrophic ocean. The HMW DON isolated in this study represented 20–30% of the TON in oligotrophic surface waters; this is consistent with previously reported estimates (Benner 2002). The bulk chemical composition of HMW DOM was similar at these sites (Meador unpubl. data), consistent with previous observations that HMW DON is compositionally related at diverse oceanic sites (e.g., McCarthy et al. 1997, 1998; Aluwihare et al. 2005). The average (±SD) δ^{15}N values for HMW DON isolated from the surface subtropical North Atlantic (4.1 ± 0.6‰) and Pacific Oceans (5.4 ± 0.8‰) are lower than those previously reported for similar ocean regions (6.6‰ and 7.8 ± 0.5‰ in the Atlantic and Pacific Oceans, respectively; Benner et al. 1997) but similar to new reports of HWM DON in the Arctic Ocean (4.8 ± 0.6‰; Benner et al. 2005). Also, δ^{15}N-HMW DON values from the subtropical North Pacific are similar to those for HMW
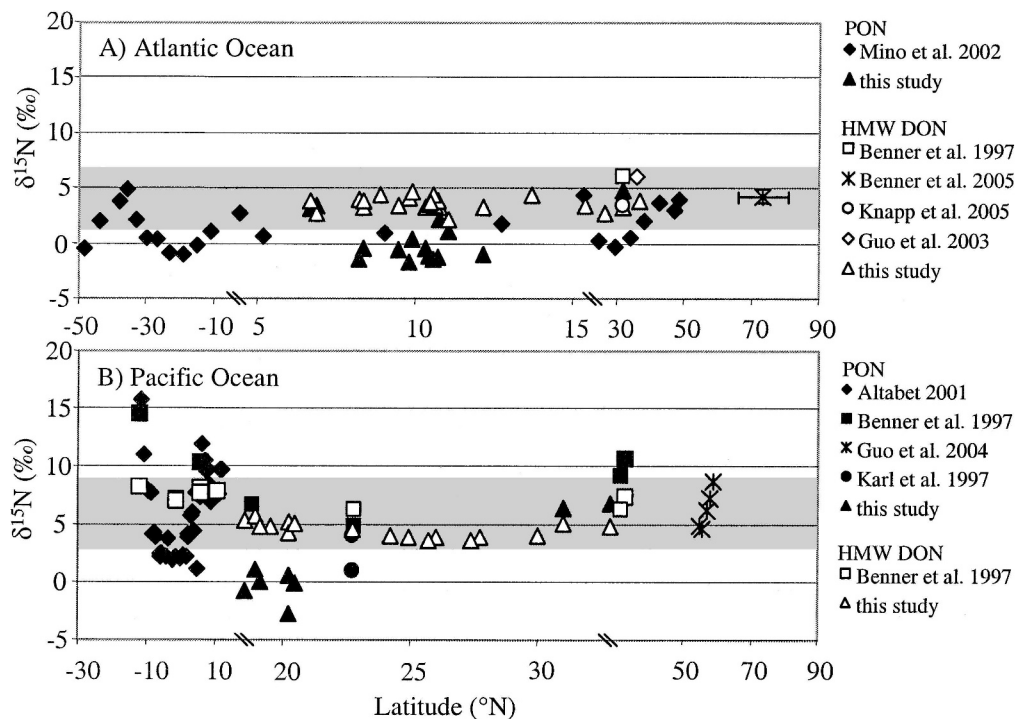
Fig. 3. δ15N values for PON and HMW DON (see legend) along latitudinal gradients in the (A) Atlantic and (B) Pacific Oceans. Values for Knapp et al. (2005) are for total DON. Benner (2005) data are reported as an average (±SD) for a range of latitudes. The gray area indicates the range in δ15N of HMW DON reported in the indicated studies: 2.5–6.6‰ and 4.5–8.3‰ for the Atlantic and Pacific, respectively.

DON from surface waters of the Southern California Bight (5.8‰ and 6.1‰, in the summer and fall, respectively). δ15N-HMW DON isolated from the surface ocean at the BATS station (3.8‰) is similar to the value for TON (3.9‰; Knapp et al. 2005), and this similarity extends to HMW DON and TON samples collected from both the MP8 and MP9 cruises (Knapp pers. comm.). These findings suggest that δ15N values for HMW DON and bulk DON are not distinct.

Several processes likely affect the final value of δ15N-HMW DON, including the δ15N of source N and extent of DON degradation. However, if (1) bulk HMW DON in the oligotrophic ocean has a recent biological source, as shown for bulk DON by incubations of *Trichodesmium* (Capone et al. 1994; Glibert and Bronk 1994) and observed for HMW DOC (Repeta and Aluwihare 2006); (2) HMW DON produced by diazotrophs is depleted in ^{15}N (e.g., Table 1); and (3) HMW DON is cycling on time scales similar to PN_{susp}, then we expect to observe variability in δ15N-HMW DON among sites with different inputs of new N. We anticipate this variability regardless of whether the value of δ15N-HMW DON is conferred by production or degradation processes, analogous to what is observed for δ15N-PN_{susp} (e.g., Mino et al. 2002) and PN_{sink} (e.g., Karl et al. 1997; Altabet 2001).

All the available data for δ15N-DON (primarily δ15N-HMW DON) are compared to δ15N-PN_{susp} from similar latitudes in Fig. 3. Both the greater variability in δ15N-PN_{susp} and the ^{15}N enrichment in HMW DON relative to PN_{susp} in the oligotrophic ocean (10–20°N) are immediately

apparent from this figure. Our findings from a spatial comparison of δ15N-HMW DON (Table 1; Fig. 3) are consistent with Knapp et al. (2005), who demonstrated a lack of seasonal change in δ15N-TON at BATS despite seasonal changes in source N. Here, we extend this observation to several regions of the surface ocean where distinct N sources are quantitatively important. If the accumulating HMW DON reservoir is maintained by biological production in the surface ocean, as has been proposed (e.g., Aluwihare et al. 2005; Repeta and Aluwihare 2006), then our δ15N-HMW DON data require that the seasonal flux of DON through this reservoir must be small, and so bulk HMW DON must have a relatively long (greater than annual) residence time in the upper ocean. Alternatively, biological processes (such as N_2 fixation) that produce isotopically depleted PN in the oligotrophic ocean may not produce similarly depleted DON. To address this possibility, we performed culture studies to investigate the capacity for *Trichodesmium* to produce ^{15}N-deplete HMW DON.

δ15N-HMW DON and the N_2-fixation signal—Tricho-desmium incubation studies indicate that field colonies may release up to half the fixed nitrogen as DON (Capone et al. 1994; Glibert and Bronk 1994), but seasonal or longer-term accumulation of this DON has not been observed (Hansell and Carlson 2001; Knapp et al. 2005). In this study, HMW DON isolated from laboratory cultures of *Trichodesmium* had δ15N signatures between −0.3‰ and 2.5‰ (avg ± SD = 1.5 ± 1.5‰) and was enriched in ^{15}N relative to PN

Table 2. Comparison of station parameters for sites with high and low N_2-fixation rates in the subtropical North Atlantic Gyre (MP8). Values are reported as averages $\pm$ SD for $n = 10$ and 7 (unless otherwise noted) for sites characterized by low or high N_2-fixation rates, respectively. A two-tailed homoscedastic t-test was performed to determine if there was a significant difference between the values for each parameter at the categorized sites. Bold p-values indicate a significant difference between variables measured at low or high N_2-fixation sites.

Parameter	Low N_2 fixation	High N_2 fixation	p-value
N_2 fixation (μmol N m^{-2} d^{-1})*	13.5 $\pm$ 15.1	208.4 $\pm$ 47.9	**0.002**
NO_3^- flux 1 (μmol N m^{-2} d^{-1})*	385.7 $\pm$ 35.7 (3)	306.6 $\pm$ 76.7 (7)	0.134
NO_3^- flux 2 (μmol N m^{-2} d^{-1})*	104.0 $\pm$ 9.6 (3)	83.0 $\pm$ 20.7 (7)	0.139
Sea surface salinity	31.5 $\pm$ 4.6	34.7 $\pm$ 1.7	0.071
Mixed layer depth (m)	30 $\pm$ 24	33 $\pm$ 17	0.768
C:N HMW DOM	17.2 $\pm$ 1.2	15.6 $\pm$ 0.9	**0.008**
Total N (μmol L^{-1})	6.0 $\pm$ 1.6	4.4 $\pm$ 1.2	**0.028**
δ^{13}C HMW DON (‰)	-21.2 ± 2.8	-21.3 ± 0.7	0.966
δ^{15}N PN$_{susp}$ (‰)	1.3 $\pm$ 2.2	-0.2 ± 1.3 (7)	0.136
δ^{15}N HMW DON (‰)	4.0 $\pm$ 0.8	4.2 $\pm$ 0.5	0.624
Δ^{15}N$_{(DON-PN)}$ (‰)	2.7 $\pm$ 2.5	4.4 $\pm$ 1.7 (7)	0.167
δ^{15}N HMW DON$_{reactive}$† (‰)	2.9 $\pm$ 1.4	2.5 $\pm$ 0.8	0.296
δ^{15}N bacterial DNA‡ (‰)	0.0 $\pm$ 0.3	-2.9 ± 0.2	NA§

* Data for N_2-fixation rates and nitrate fluxes were gathered from Capone et al. (2005). Nitrate fluxes 1 and 2 are determined using eddy diffusivity constants of 0.11 and 0.37 cm^2 s^{-1}, respectively.

† δ^{15}N HMW DON$_{reactive}$ = [δ^{15}NHMWDON $- (f_{deep} \times \delta^{15}N_{deep\ HMWDON})$]/$(1 - f_{deep})$, where $f_{deep} = 0.5/(0.25 \times$ [TN]) and $\delta^{15}N_{deep\ HMWDON} = 5.9$‰.

‡ Bacterial DNA samples for isotopic analysis were collected during En391. Low N_2-fixation and high N_2-fixation stations were determined by the abundance of *Trichodesmium* in a net tow.

§ NA, not available.

accumulating in the same cultures (-1.5 ± 0.5‰; Table 1). Macko et al. (1987) reported that proteins are typically enriched in ^{15}N (~ 3‰) relative to whole cells, so a protein-rich HMW DON pool could account for δ^{15}N-HMW DON values observed in our culture studies. Degradation of HMW DON by heterotrophic bacteria present in our *Trichodesmium* cultures may have also caused the relative ^{15}N enrichment of residual HMW DOM by preferential assimilation of isotopically light molecules. However, the δ^{15}N-HMW DON produced in *Trichodesmium* cultures was always ^{15}N depleted relative to δ^{15}N-HMW DON isolated from field sites where both *Trichodesmium* abundance and measured N_2-fixation rates were high (e.g., δ^{15}N-HMW DON = 4.2 $\pm$ 0.5‰ at Atlantic Ocean sites fitting this description) and more similar to PN$_{susp}$ collected at these same sites (Table 2; see also Carpenter et al. 1999; Montoya et al. 2002).

Capone et al. (1994) reported the release of DON by *Trichodesmium* spp. in the form of the free amino acids glutamate and glutamine, which would escape our HMW DON isolation protocol. Glibert and Bronk (1994) reported that the release of LMW DON (defined as <10 kDa) accounted for an average of $\sim$55% of total DON released by *Trichodesmium* colonies. This suggests that the remaining DON is released in the >10-kDa size fraction and that HMW DON isolated in this study (i.e., >1 kDa) should include a significant amount of the DON released by *Trichodesmium*. The present study provides several direct lines of evidence supporting HMW DOM production by *Trichodesmium*: (1) HMW DON accumulated in an axenic *Trichodesmium* culture grown in artificial seawater (4.4 μmol L^{-1}, coincident with the end of exponential phase growth vs. 0.1 μmol L^{-1} in a control

experiment); these concentrations are based on dry weights and elemental composition of HMW DON isolated via UF. (2) HMW DON was harvested from several laboratory cultures with an isotopically depleted δ^{15}N average ($\pm$SD) of 1.5 $\pm$ 1.5‰ ($n = 3$; Table 1). While N-containing organic molecules were added to culture media, the maximum possible contribution of these N atoms to isolated HMW DON results in δ^{15}N-HMW DON variations ± 0.2‰ and within instrument error. (3) *Trichodesmium* IMS101 was labeled with 100% NaH^{13}CO$_3$ and shown to produce HMW DOM with 25% ^{13}C atom enrichment (Meador unpubl. data). Collectively, these findings predict the production of isotopically light HMW DON in association with N_2 fixation at the field sites examined in this study.

There was extensive N_2 fixation during both MP8 and MP9 cruises, with areal rates during MP8 ranging from 0.2 to 532 μmol N m^{-2} d^{-1} (avg $\pm$ standard error = 85 $\pm$ 23 μmol N m^{-2} d^{-1}, $n = 28$; Capone et al. 2005); in addition, measurable abundances of the symbiotic diazotroph *Richelia* were present in the MP8 region (Foster et al. 2007). Based on the culture data above and the measured N_2-fixation rates, the relatively unvarying δ^{15}N-HMW DON along latitudinal gradients (Fig. 3) indicate that N_2 fixation did not significantly affect bulk δ^{15}N-HMW DON in surface waters of the oligotrophic ocean at the time of sampling. The fact that δ^{15}N-HMW DON values are consistent with values for TON at the BATS site (Knapp et al. 2005) and other sites in both the subtropical North Atlantic and Pacific Oceans (Knapp pers. comm.) suggests that DON that is excluded during the isolation of HMW DON does not exhibit a significantly different isotopic composition. Therefore, the observed and reported pro-

duction of isotopically depleted DON by *Trichodesmium* ($\sim$100 pmol N colony^{-1} h^{-1}; Capone et al. 1994; Glibert and Bronk 1994) does not appear to accumulate at the sites examined in this study.

We also investigated the importance of several other parameters related to N cycling in the upper ocean and compared sites with high versus low rates of *Trichodesmium*-specific N$_2$ fixation (Table 2). For those stations experiencing N$_2$-fixation rates higher than 80 μmol N m^{-2} d^{-1} (i.e., high N$_2$-fixation sites), the δ^{15}N-PN$_{susp}$ was $-0.2 \pm 1.3\permil$ (avg $\pm$ SD, $n = 9$; Table 2), and, with the exception of one station, all values were depleted in ^{15}N relative to atmospheric N$_2$. These low values are similar to δ^{15}N of *Trichodesmium* colonies picked from surface waters of the equatorial Atlantic ($-2.15 \pm 0.09\permil$; Carpenter et al. 1999; $-1.08 \pm 0.13\permil$; Montoya et al. 2002). δ^{15}N-PN$_{susp}$ measured at stations with lower *Trichodesmium*-specific N$_2$-fixation rates were more variable, with values ranging from $-1.5\permil$ to $3.9\permil$ (avg $\pm$ SD = $1.3 \pm 2.2\permil$, $n = 8$). Isotopically light PN$_{susp}$ values at oligotrophic sites with low rates of N$_2$ fixation may be a result of several processes, such as (1) N$_2$ fixation by diazotrophs other than *Trichodesmium* and *Richelia*; *Richelia* abundance alone was not significantly correlated with δ^{15}N-PN$_{susp}$; (2) longer residence time of PN$_{susp}$ ($\sim$weeks) relative to measurements of *Trichodesmium* abundance and activity (days) (Montoya et al. 2002); and/or (3) an ecosystem driven by other N sources that confer a ^{15}N-deplete signature to organic N (e.g., extensively recycled NH$_4^+$; Checkley and Miller 1989; or isotopic fractionation of recently injected NO$_3^-$; Altabet 2001). The δ^{15}N of HMW DON showed no significant difference between sites with high or low rates of N$_2$ fixation (avg $\pm$ SD = $4.2 \pm 0.5\permil$ and $4.0 \pm 0.8\permil$, respectively; Table 2) and was consistently enriched relative to δ^{15}N-PN$_{susp}$. While the source of N fueling production in surface waters of the subtropical North Atlantic Ocean appears to provide isotopic constraints for PN$_{susp}$, δ^{15}N of bulk HMW DON is independent of N$_2$-fixation rates or any other combination of N sources that sets the isotopic signature of PN$_{susp}$.

During MP8 there were some variables that did show a significant difference between stations with relatively high and low rates of N$_2$ fixation (see Table 2, bold); for example, C : N of HMW DON was significantly lower at stations with high rates of N$_2$ fixation (15.6 vs. 17.2, respectively; $p < 0.01$; Table 2). This may be evidence for diazotroph exudation of organic matter rich in N; a relatively low C : N ratio was also observed for HMW DON isolated from *Trichodesmium* cultures (avg $\pm$ SD = 11.4 ± 3.0, $n = 4$). However, the lack of variability in δ^{15}N-HMW DON between sites argues against differences in the production of HMW DON and suggests that the observed differences in C : N of bulk HMW DOM at sites with high versus low rates of N$_2$ fixation results from either consumption or production, respectively, of N-poor DOC. Total nitrogen concentrations in surface waters were significantly higher in regions with lower rates of N$_2$ fixation (6.0 μmol L^{-1} vs. 4.4 μmol L^{-1}, respectively; $p = 0.03$; Table 2), but this significance was removed when considering only those stations with surface salinity >33 (p

$= 0.15$), suggesting that there was significant N input from freshwater sources. This was the only parameter that was significantly altered by exclusion of low-salinity stations.

The more stable and heavier δ^{15}N-HMW DON in the MP8 (and MP9) region(s) relative to δ^{15}N-PN$_{susp}$ implies only a small contribution by recent production to the accumulating HMW DON pool. However, the presence of a quantitatively small but fast cycling component cannot be identified based on bulk δ^{15}N-HMW DON alone. For example, isotopically depleted DON added by *Trichodesmium* may be obscured by the presence of a relatively large, background pool of refractory DON. Abell et al. (2000, 2005) proposed that the preferential, rapid consumption of N-rich DOM by biota attempting to alleviate inorganic N limitation in surface waters may be the cause for the observed export of carbon-rich, labile DOM into upper thermocline waters of the North Pacific subtropical gyre. To address the possible existence of a rapidly cycling component of HMW DON, proteins, which are expected to be labile, were isolated from bulk HMW DON for isotopic analysis.

δ^{15}N of HMW DON proteins—Proteins precipitated from one *Trichodesmium* culture was isotopically light and similar to δ^{15}N-HMW DON from the same culture ($-0.7\permil$ vs. $-0.3\permil$; Table 3) but enriched relative to cells (δ^{15}N-proteins $- \delta^{15}$N-whole cell $= \Delta_{proteins-cell} = 1.2\permil$). Thus, as with HMW DON derived from *Trichodesmium* fixing N$_2$, proteins derived from N$_2$ fixation are expected to be isotopically lighter than δ^{15}N-HMW DON measured in field samples (Table 1). Proteins precipitated from HMW DON in oligotrophic environments were significantly depleted in ^{15}N (avg $\pm$ SD = $2.2 \pm 0.9\permil$) relative to bulk HMW DON ($p < 0.01$ at high and $p = 0.015$ at low N$_2$-fixation sites) and to proteins isolated from nonoligotrophic sites experiencing no N$_2$ fixation (avg $\pm$ SD = $5.2 \pm 0.7\permil$; $p < 0.01$) (Table 3; Fig. 4). Isotope effects associated with biosynthesis are expected to produce proteins that are enriched in ^{15}N relative to whole cells (Macko et al. 1987), and so we conclude that the ^{15}N depletion observed for precipitated proteins relative to HMW DON cannot be explained as a biosynthetic isotope effect. As noted previously, the δ^{15}N-PN$_{susp}$ in similar environments is also depleted ($-0.2 \pm 1.3\permil$ and $1.3 \pm 2.2\permil$ at high and low N$_2$-fixation sites, respectively; Table 2; Montoya et al. 2002). The comparatively enriched δ^{15}N-HMW DON in oligotrophic environments and the greater variability in δ^{15}N of HMW proteins relative to δ^{15}N-HMW DON (when all samples in Table 3 are compared) imply that the dynamic δ^{15}N imprint of production/degradation processes is being recorded by proteins but not bulk HMW DOM. Macko et al. (1987) showed that $\Delta_{protein-cell}$ was independent of nitrogen source or source organism. Based on this, we conclude that observed spatial variations in δ^{15}N-protein result from spatial variations in the δ^{15}N of source N. A lack of similar variation in bulk δ^{15}N-HMW DON (except for the distinct isotope signatures of the two ocean basins) leads us to conclude that the protein fraction of HMW DOM is more dynamic. This conclusion is supported by an enriched Δ^{14}C signature of precipitated proteins (77.3‰) relative to bulk
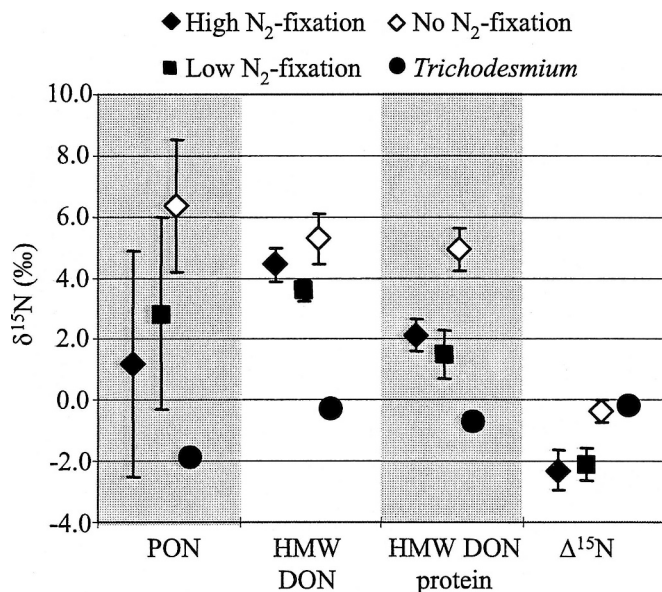
Fig. 4. $\delta^{15}N$ averages of suspended PON and components of the DON pool isolated from sites with high *Trichodesmium*-specific N_2-fixation rates (filled diamonds), low *Trichodesmium*-specific N_2-fixation rates (filled squares), and sites with no N_2 fixation (open diamonds). Error bars represent one standard deviation. $\Delta^{15}N$ refers to the difference in $\delta^{15}N$ of HMW DON from the protein ppt. Values from a *Trichodesmium* IMS101 culture are shown for reference (filled circles).

HMW DOM ($-19.5 \pm 19‰$) observed at the BATS site and is consistent with modern $\Delta^{14}C$ values for the protein-like fraction of HMW DON reported by Loh et al. (2004).

TCA-precipitated proteins typically accounted for an average ($\pm SD$) of $1.9 \pm 0.5\%$ of N within HMW DON; this is far lower than the estimated contribution of total hydrolyzable amino acids to surface ocean HMW DON as determined by HPLC ($\sim 15–30\%$; McCarthy et al. 1996). The minor contribution of precipitated proteins to bulk HMW DON is consistent with the relative invariability of $\delta^{15}N$-HMW DON between sites despite the observed variation in $\delta^{15}N$ of proteins (Table 3). This observation may be explained by either a small diazotrophic contribution to HMW DON or rapid turnover of these isotopically depleted components.

Tight coupling of recently produced DON—Just as $\delta^{15}N$-phytoplankton is influenced by variations in the isotopic composition of inorganic N in surface waters (Karl et al. 1997; Waser et al. 2000; Mino et al. 2002; Montoya et al. 2002), the isotopic composition of bacteria should likewise be affected by the $\delta^{15}N$ variability of their N source. Although culture studies with *Trichodesmium* showed the accumulation of isotopically light HMW DON, only a small fraction of isotopically depleted N was detectable as HMW DON in the field. As a result, we hypothesized that heterotrophic and mixotrophic bacteria in the field serve as a sink for *Trichodesmium*-derived DON. To test this hypothesis, we traced the possible incorporation of isotopically light, dissolved N into DNA extracted from free-living, surface ocean microorganisms isolated onto 0.2-μm filters (0.2 μm < bacteria < 0.5 μm) at sites with high abundances of *Trichodesmium*. For comparison, $\delta^{15}N$-DNA was also obtained from filter samples collected at sites where *Trichodesmium* was either absent or in low abundance. In this study, DNA is assumed to be primarily present in

Table 3. Stable N isotopic composition comparisons of suspended PN and isolated components of the DON pool. Stations are categorized into sites that exhibited high or low rates of N_2-fixation (Capone et al. 2005), or those sites where N_2-fixation has not been shown to occur.

| Station | Lat. (°N) | Long. (°W) | $\delta^{15}N$ (‰) | | | % HMW DOM freshly produced* |
			PN$_{susp}$	HMW DON	TCA ppt	
High N_2 fixation						
MP9.21	20.25	159.19	−2.5	5.2	2.4 ± 1.0	20
MP8.32	10.7	52.53	−1.1	4.4	2.4	43
En391.4	20.71	53.46	ND†	3.9	2.4	57
En391.6	10.5	50.03	ND	4.2	3.6	73
Low N_2 fixation						
BATS	31.93	64.17	5.3	3.8	2.2	58
MP8.28	12.18	56.04	−0.7	3.8	1.7	50
MP8.6	6.88	52.03	3.9	3.2	0.6	51
No N_2 fixation						
MP9.03	30	170.00°E	ND	5	4.7	80
SIO Pier	32.87	117.25	8	5.8	5.7	75
CalCOFI 0411	~31.00‡	~122.00‡	7.5	6.1	5.2	NA§
En391.1	37.38	74.46	ND	4.3	4.1	89
Trichodesmium						
IMS101	NA	NA	−1.9	−0.3	−0.7	NA

* %; HMW DON freshly produced = 100*[$\delta^{15}N_{HMWDON} - \delta^{15}N_{deep\ HMWDON}$)/($\delta^{15}N_{Protein\ ppt} - \delta^{15}N_{deep\ HMWDON}$)], where $\delta^{15}N_{deep\ HMWDON} = 5.9‰$.
† ND, no data.
‡ CalCOFI 0411 represents a mixture of stations within the CalCOFI grid (http://www.calcofi.org).
§ NA, not available.

metabolically active cells, whereas other N-containing biochemicals such as proteins could include a fraction that is absorbed onto filters from the DON reservoir.

The isotopic composition of bacterial DNA isolated during En391 from sites with high abundances of *Trichodesmium* was depleted in ^{15}N ($\delta^{15}N$-DNA = $-2.9\permil$) relative to typical $\delta^{15}N$-PN_{susp} in this region (e.g., MP8; Table 1) and to bacterial DNA isolated from sites where *Trichodesmium* was not abundant ($\delta^{15}N$-DNA = $0.0\permil$). The generally light values for $\delta^{15}N$-DNA ($0.0\permil$ and $-2.9\permil$) are reminiscent of $\delta^{15}N$-PN_{susp} values (e.g., Table 2) that indicated isotopically depleted N sources to primary producers at all sites in the oligotrophic ocean. The observed difference in $\delta^{15}N$-DNA between sites is significant and indicates that site-specific N-cycle processes are important for influencing the $\delta^{15}N$ composition of microorganisms in the isolated size class. We attempt to consider some of these processes below.

The extremely depleted $\delta^{15}N$-DNA of bacteria from *Trichodesmium*-abundant sites ($-2.9\permil$) supports the channeling of *Trichodesmium* derived, ^{15}N-deplete N to heterotrophic and mixotrophic bacteria at sites where *Trichodesmium* was abundant and fixing isotopically depleted N into surface waters. Alternatively, differences in $\delta^{15}N$-DNA could also result from the use of distinct N sources (e.g. nitrate vs. DON) at sites with low versus high rates of N_2 fixation (instead of similar N sources with different isotopic signatures supplying N to the bacterial community at each site). However, even in this case, $\delta^{15}N$-DNA identifies a unique feature in the N supporting bacterial production within the examined size class (0.2–0.5 μm) at sites with high versus low abundances of *Trichodesmium*. The depleted $\delta^{15}N$-DNA of bacteria may also reflect the influence of diazotroph DNA within the sample. *Trichodesmium* colonies and free filaments were likely retained on the 0.5-μm prefilter, but unicellular diazotrophs may have contributed to the bacterial DNA sample (0.2 μm < bacteria < 0.5 μm), and abundances of these organisms could have varied between sites. Molecular analysis of the exact DNA sample used for elemental analysis provides a sufficient means for monitoring the community of organisms that contribute to the isotopic composition of bacterial DNA. Diazotroph contamination of the bacterial DNA samples was ruled out by screening for the *nif*H gene (Hewson pers. comm.).

We also monitored community composition as determined by sequencing 16S rRNA genes from clone libraries and denaturing gradient gel electrophoresis (DGGE) analysis to provide a further check on the possible contamination of our DNA samples by diazotroph-DNA. A variety of different bacterial phylotypes was observed by both DGGE and sequencing of 16S rRNA genes from surface waters with and without *Trichodesmium*. Most bacterial species isolated from sites with high abundances of *Trichodesmium* appear to be of the alpha and gamma proteobacteria clades and are consistent with reported bacterial isolates from similar regions. One clone was most closely related to *Prochlorococcus marinus* MIT9312 and is representative of marine mixotrophic picoplankton being coisolated in the size class that was the focus of the current study. No clones sequenced in this study clustered with 16s

rRNA gene sequences of reported marine diazotrophs (as reported in Karl et al. 2002 and references therein). These results confirm that the $\sim3\permil$ depletion in ^{15}N-DNA of bacteria isolated from sites characterized by high abundances of *Trichodesmium* is not due to fixation of atmospheric N_2.

Dissimilar isotope effects associated with N uptake or different extents of N utilization (e.g., Mariotti et al. 1981) may also contribute to the observed difference in $\delta^{15}N$-DNA. Since the type of N fueling microbial production at each location is difficult to assess, we are unable to successfully address the importance of these factors in the current study. However, both the large difference in $\delta^{15}N$-DNA between sites with high and low abundances of *Trichodesmium* and the strongly depleted $\delta^{15}N$-DNA at sites where *Trichodesmium* were present are entirely consistent with mixotrophic and heterotrophic bacteria playing an important role in the redistribution and trafficking of new, ^{15}N-deplete N introduced to the surface ocean by diazotrophs. Based on these results, we predict that higher heterotrophic and autotrophic bacterial activity may be associated with extensive N_2 fixation. *Trichodesmium*-derived DON or DIN uptake by larger, mixotrophic plankton is also expected; however, the presence of diazotrophs within this same size class of organisms (e.g., Montoya et al. 2002) contaminates $\delta^{15}N$ signals relating to uptake of isotopically light N other than N_2.

Rapidly cycling components of HMW DON—In the preceding discussion, we have stated that $\delta^{15}N$-PN_{susp} exhibits larger spatial variability than $\delta^{15}N$-HMW DON; however, small variations in $\delta^{15}N$-HMW DON are apparent. Concentration of HMW DON in surface waters has been modeled as a mixture of long- and short-lived components (e.g., Hansell and Carlson 2001; Aluwihare et al. 2005). We expect the long-lived component to have a relatively fixed $\delta^{15}N$ composition, while the $\delta^{15}N$ of reactive DON added in surface waters is unknown and likely variable. Variations in the isotopic composition and concentration of reactive DON could lead to small variations in bulk $\delta^{15}N$-HMW DON as is observed in this study. A simple, two endmember mixing model for N isotope ($\delta^{15}N$) and N mass (TN) can be applied to identify the $\delta^{15}N$ of reactive HMW DON at individual sites. The assumptions of the mass balance are that (1) slowly cycling HMW DON has a $\delta^{15}N$ similar to $\delta^{15}N$-HMW DON of the deep ocean, which in this study is taken to be $5.9 \pm 0.9\permil$ (avg $\pm$ SD for seven HMW DON samples isolated from $\geq1,000$ m; Meador unpubl. data), and that (2) vertical gradients in HMW DON concentration can be used to estimate the relative contribution from long-lived and reactive components. The reactive component is defined here as that HMW DON present in surface waters in excess of deep ocean concentrations. Surface HMW DON is taken to be 25% of surface TN (Benner 2002; this study), and deep ocean, refractory HMW DON is estimated to be 0.5 μmol L^{-1} (Benner et al. 1997). Based on these assumptions and the bulk $\delta^{15}N$-HMW DON and TN concentration at each site (Table 1), the $\delta^{15}N$ of reactive HMW DON can be calculated for the site of interest. For

all MP8 sites, the calculated average ($\pm$SD) for $\delta^{15}N$-HMW DON of reactive components was $2.7 \pm 1.1\text{‰}$ (values for high and low N_2-fixation sites are reported in Table 2). This value is significantly depleted in ^{15}N compared to bulk $\delta^{15}N$-HMW DON ($p < 0.01$) and is more similar to HMW proteins isolated from MP8 sites (avg $\pm$ SD $= 1.6 \pm 0.9\text{‰}$, $n = 3$).

Both the concentration and $\delta^{15}N$ of refractory HMW DON (i.e., upwelled HMW DON) are the least constrained variables of this mass balance. However, varying the estimated $\delta^{15}N$ of refractory HMW DON by ± 1 SD in the mass balance calculation reveals that the reactive component of all MP8 stations cannot have an average $\delta^{15}N$ ($\pm$SD) value higher than $3.5 \pm 1.1\text{‰}$. This value remains significantly lower than that of bulk HMW DON isolated from MP8 stations ($p < 0.05$). A similar result is obtained by decreasing the contribution of HMW DON_{deep} to $0.3~\mu mol~L^{-1}$ ($\delta^{15}N$ of reactive HMW DON cannot exceed $3.5 \pm 0.8\text{‰}$). Assuming that the isotopic composition of HMW proteins (Table 3) represents that of the entire reactive HMW DON pool, another, independent mass balance indicates that reactive HMW DON constitutes an average ($\pm$SD) of $47 \pm 13\%$ of bulk HMW DON in the surface ocean. This result is consistent with between 50% and 70% of surface ocean HMW DON being added in the upper ocean (Benner et al. 1997; Aluwihare et al. 2005). Additional measurements for both DON concentration and isotopic composition at several locations and depths are necessary before the mass balance approach utilized above can be comprehensively applied to studies of DON cycling.

At the sites examined in this study, the $\delta^{15}N$ of bulk HMW DON in the surface ocean does not vary similarly to PN_{susp} ($r = -0.31$) and appears to be independent of the source of N fueling new production. While bulk $\delta^{15}N$-HMW DON values are relatively stable, $\delta^{15}N$ of proteins isolated from HMW DON show depleted and spatially variable values. This finding suggests that while a significant fraction of HWM DON is refractory (as demonstrated by spatially invariant $\delta^{15}N$), components of HMW DON may be cycling on shorter time scales and therefore responding to changes in $\delta^{15}N$-source N on time scales similar to PN_{susp}. Our data provide isotopic evidence for the existence of at least two pools of HMW DON in surface waters that cycle on different time scales. This result is consistent with radiocarbon-based studies of DOC that show the presence of both refractory and reactive DOC in surface waters (e.g., Druffel et al. 1992; Repeta and Aluwihare 2006).

Despite an implied increase in the extent of N_2 fixation at Station ALOHA throughout the last decade (Karl et al. 1997; Dore et al. 2002) and the demonstrated depleted isotopic composition of *Trichodesmium*-derived HMW DON (as reported in this study), the $\delta^{15}N$-HMW DON measured at this site ($\delta^{15}N$-HMW DON $= 5.5\text{‰}$) is not significantly different from other sites in the North Pacific where evidence for N_2 fixation has never been documented (Table 3). Based on this result, N_2 fixation does not appear to play a dominant role in imparting the observed N isotopic signature to HMW DON at Station ALOHA. The

observations of Abell et al. (2000, 2005) are able to reconcile this discrepancy. These authors concluded that the documented export of carbon-rich, labile DOM, presumably derived from recent biological production, out of surface waters near Station ALOHA could result from the rapid consumption of labile, recently produced DON in surface waters prior to DOM export into the upper thermocline. The apparent uncoupling of DOC and DON consumption in the oligotrophic surface ocean supports the conclusion that DON derived by diazotrophs is rapidly recycled, a hypothesis also postulated by Knapp et al. (2005) for the BATS site. Direct consumption of this N in surface waters by heterotrophic and mixotrophic bacteria is consistent with the substantially depleted $\delta^{15}N$ signature of bacterial nucleic acids isolated from sites with high abundances of *Trichodesmium*. The potential importance of this N trafficking was identified in the current study as a result of measuring the nitrogen isotopic composition of dissolved proteins and microbial DNA. These results demonstrate the value of compound specific N isotope measurements. This approach together with radiocarbon measurements on the same compounds can provide absolute DON residence time and source information, which is essential for accurately estimating the magnitude of marine C and N fluxes as well as delineating biological processes controlling N cycling in marine systems.

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