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Stable nitrogen isotope dynamics of dissolved nitrate in a transect from the North Pacific Subtropical Gyre to the Eastern Tropical North Pacific

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Abstract—The stable nitrogen isotopic composition of nitrate, concentrations of inorganic nitrogen and phosphorus, dissolved oxygen and nitrification rates were determined at six stations ranging from the oligotrophic North Pacific Subtropical Gyre (NPSG) to the more productive Eastern Tropical North Pacific (ETNP). Nitrification rates increased along the transect from a maximum rate of 1 nmol L^{-1} d^{-1} at station ALOHA to 23.7 nmol L^{-1} d^{-1} at station 6. In oxic surface waters, nitrate isotopically enriched in ^{15}N (maximum $\delta^{15}N\text{-}NO_3^-$ value of 12.5‰) was most likely the result of assimilatory nitrate reduction. In contrast, high $\delta^{15}N\text{-}NO_3^-$ values (maximum of 12.3‰) in association with high nitrate deficits and anoxic conditions supported the interpretation of isotopic fractionation due to denitrification. A one-dimensional vertical advection and diffusion model was used to estimate the fractionation factor for denitrification at two stations in the ETNP. A comparison of modeled to observed $\delta^{15}N\text{-}NO_3^-$ data indicated an isotopic enrichment factor (ε) of 30‰ at station 4 and 30 to 35‰ at station 5. Isotopically light nitrate (1.1 and 3.2‰) was observed in the upper 200 m of the water column at stations in the ETNP. Tracer studies of $^{15}NH_4$ and biogeochemical indicators of nitrogen fixation supported the interpretation of nitrification as the most plausible explanation for low $\delta^{15}N\text{-}NO_3^-$ values observed in water column samples. Our results are consistent with the occurrence of nitrification within the euphotic zone and for the first time provide corroborating stable nitrogen isotopic evidence for this process. *Copyright © 2004 Elsevier Ltd*

1. INTRODUCTION

Oceans account for approximately half of the world's primary production (Karl, 1999). This productivity is fueled by recharge of nutrients from deep waters and in most of the world's oceans, the availability of fixed nitrogen limits productivity (Howarth, 1988). The availability of nitrogen is not homogeneous and this results in variations in ecosystem productivity. Two systems with contrasting nitrogen dynamics include subtropical/tropical gyres and coastal upwelling zones. Subtropical/tropical gyre ecosystems are characterized by oligotrophic conditions and were once thought to be marine deserts (Karl, 1999). However, given their large size (40% of the Earth's surface area), gyre ecosystems can contribute substantively to global primary productivity (Karl, 1999). In contrast, the fraction of the world's oceans characterized by coastal upwelling zones is small, but eutrophic environments support a disproportionately large amount of primary productivity.

The North Pacific Subtropical Gyre (NPSG) is the largest open ocean gyre (area of 2×10^7 km^2) (Karl, 1999). In this ecosystem, nitrogen and phosphorus are supplied to the surface by diffusion across the thermocline and horizontal transport from adjacent waters (Reid et al., 1978; Karl, 1999). However, the observation that the amount of primary productivity in the NPSG cannot be accounted for by the supply of nutrients by diffusion from deep waters led to a reevaluation of nitrogen cycling (Letelier and Karl, 1998). The periodic appearance of *Trichodesmium* blooms at the long-term monitoring station in the NPSG, ALOHA, and an increase in the N/P ratio of dissolved and particulate matter provided evidence for a significant role of nitrogen fixation in the NPSG (Karl et al., 1997). Quantitative estimates indicate that diazotrophic microbes could fuel up to half of the new production in the NPSG (Karl et al., 1997; Dore et al., 2002).

In the ETNP, nutrients are supplied by upwelling off the Central American coast. The large flux of sinking organic carbon from primary production increases oxygen demand. The result of this increased respiration is a large lens of oxygen deficient water at intermediate depths within the ETNP. The suboxic conditions and availability of nitrate from upwelling provide the necessary environmental conditions for water column denitrification. The associated loss of fixed nitrogen is significant in the region (10×10^{12} to 30×10^{12} g N per year, Cline and Kaplan, 1975) and variations in denitrification rates may contribute to glacial-interglacial changes in the atmospheric concentration of carbon dioxide (Farrell et al., 1995; Ganeshram et al., 1995). In addition to effects on past climate change, the production of nitric oxide and nitrous oxide at the oxic/anoxic interface in these waters has important consequences on future climate change (Ward, 2000).

Studies of ecosystems that differ with respect to nutrient supply provide an important framework for understanding nitrogen cycle controls on primary productivity and climate change (Yoshida et al., 1984; Ganeshram et al., 1995; Dore et al., 1998; Karl, 1999). The current study contributes additional information on nitrogen cycling through the analysis of samples across a west to east transect extending from the NPSG to the ETNP. The stable nitrogen isotopic composition of dissolved nitrate and $^{15}N\text{-}NH_4$ oxidation rates provide a basis to evaluate the predominance of distinct microbial processes

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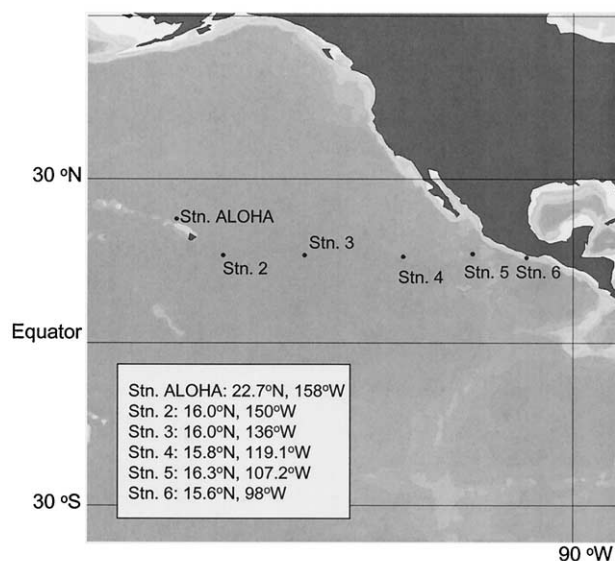
Fig. 1. Locations of sampled stations in a transect from west to east.

along the transect. This suite of data allows the evaluation of nitrogen cycling in two contrasting systems, the NPSG and ETNP.

2. MATERIALS AND METHODS

Water column samples were collected during a May–June 2000 sampling cruise that was part of the Eastern Pacific Redox Experiment (EPREX) aboard the R/V *Roger Revelle*. Six stations were sampled during the cruise along a west to east transect from the oligotrophic North Pacific gyre to the eastern tropical North Pacific (ETNP) (Fig. 1). The water column was characterized at each station by determination of temperature, salinity, dissolved oxygen and chlorophyll fluorescence. Water samples were collected to determine nitrate concentrations and stable nitrogen isotope values.

Nitrate was extracted from the seawater samples by a distillation method (Ostrom et al., 1997). During the distillation NH_4^+ was converted to NH_3 by addition of 4 mL of distilled 5 N NaOH to adjust the pH above 10. Finely ground Devarda's alloy (0.3 g) was added to the sample to reduce NO_3^- to NH_3 for a second distillation. Approximately 250 mL of condensate was collected in a flask containing 30 mL of 0.084 N HCl. The sample was bound to 100 mg of zeolite molecular sieve in two successive bindings and dried at 40°C. Zeolite bound samples were placed in quartz tubes to which excess copper and copper oxide were added, evacuated, sealed and heated to 850°C for 1 h and slowly cooled (Macko, 1981). Nitrogen (N_2) was purified cryogenically on a vacuum line and analyzed on a Prism isotope ratio mass spectrometer (GV Instruments, Manchester, UK). Samples were corrected for background associated with addition of Devarda's alloy using an isotope mass balance equation (Ostrom et al., 1998a). The precision of replicate samples for stable nitrogen isotopic analysis of dissolved nitrate was better than 1‰ (Ostrom et al., 1998a). Stable nitrogen isotopic compositions are expressed in per mil notation (‰):

$$\delta^{15}N = \left[\left(\frac{\left(\frac{^{15}N}{^{14}N} \right)_{Sample}}{\left(\frac{^{15}N}{^{14}N} \right)_{Standard}} \right) - 1 \right] \times 1000 \quad (1)$$

The isotopic standard for $\delta^{15}N$ is atmospheric N_2.

The concentration of dissolved ammonium in samples was determined during shipboard analyses using a modification of the fluores-

cence method (Jones, 1991). The modification to the method involved the removal of residual ammonium from the NaOH-sodium citrate solution. A channeled Plexiglas block with NaOH-sodium citrate and a 10% solution of H_2SO_4 on opposing sides separated by a Teflon membrane with flow rates of 0.32 mL/min for both solutions. Dissolved oxygen concentrations were determined by Winkler titration (Carpenter, 1965). Analyses of dissolved nitrate, nitrite and phosphate concentrations were determined at the University of Washington (Valderrama, 1981; UNESCO, 1994). The detection limits for nitrate and phosphate are 0.02 and 0.1 μM respectively with a precision better than 1%.

For determination of nitrification rates via ^{15}N-ammonia oxidation, seawater samples were collected at the depth of intended in situ incubation. Typically, samples were taken at the nitracline, chlorophyll maximum, upper edge of the chlorophyll maximum and at a depth 150 m below the chlorophyll maximum. To determine nitrification rates, 4 L of seawater was collected at selected depths in the water column. Isotopically enriched (99.9% ^{15}N-NH_4Cl) was added to the seawater that was transferred into glass bottles. The 4 L bottles were lowered back to the depth from which they were taken and incubated in situ for 24 or 48 h. At the end of the incubation period, the bottles were brought to the surface, filtered, transferred to Nalgene bottles and frozen until analysis. For determination of the δ^{15}N value of the dissolved nitrate in the incubation samples, the nitrate was first extracted by distillation. A sample volume of 1 L was distilled following a modification of the technique described by Ostrom et al. (1997) that was necessary to ensure complete removal of residual ^{15}N-NH_4 from the seawater and distillation glassware. To remove ^{15}N-NH_4, the sample was distilled three times with addition of E-Pure water to restore the sample to its initial volume. To remove trace NH_4^+ from the distillation apparatus, the glassware was washed in 1 N HCl and heated to 500°C for 2 h before a fourth distillation to remove residual NH_4 in the sample. Tests of the effectiveness of the modified technique involved analysis of solutions of isotopically characterized NO_3^- in the presence of 5 μM $^{15}NH_4^+$ (99%). The procedure effectively removed the residual NH_4^+ enriched in ^{15}N and yielded the expected δ^{15}N value for the NO_3^- standard. The calculation of nitrification rates from the oxidation of $^{15}NH_4$ was obtained using the equations described in Glibert and Capone (1993). Dilution of labeled ammonium with that contributed from mineralization was not monitored during the short-term duration of the experiments. Consequently, $^{15}NH_4^+$ oxidation rates in this study should be considered conservative estimates.

Nitrate deficits were calculated using equations of Cline and Richards (1972) and Voss et al. (2001). The amount of nitrate used as a substrate during denitrification is the difference between the expected nitrate concentration (Eqn. 2) and the observed concentration of nitrate plus nitrite (Eqn. 3). Using the approach of Voss et al. (2001), the expected nitrate concentration was based on a ratio of 13.8 for the preformed nutrients as determined from the data below 900 m.

$$NO_3^-{}_{(expected)} = 13.8 \times PO_4^{3-}{}_{(measured)} \quad (2)$$

$$NO_3^-\text{ deficit} = NO_3^-{}_{(expected)} - (NO_3^-{}_{(measured)} + NO_2^-{}_{(measured)}) \quad (3)$$

Nitrogen to phosphorus ratios of inorganic dissolved nutrients were calculated according to Fanning (1992).

$$N/P = ([NO_2^-] + [NO_3^-] + [NH_4^+])/[PO_{43}^-] \quad (4)$$

3.

RESULTS AND DISCUSSION

3.1. General Water Column Characteristics

Stations along the transect (Figs. 2–7) capture the gradual broadening, both horizontally and vertically, of anoxic water from west to east and concomitant change from oligotrophic conditions in the NPSG to the more productive conditions in the ETNP (Cline and Kaplan, 1975; Ward and Zafiriou, 1988; Karl, 1999). The oxygen minimum zone occurs at an increas-
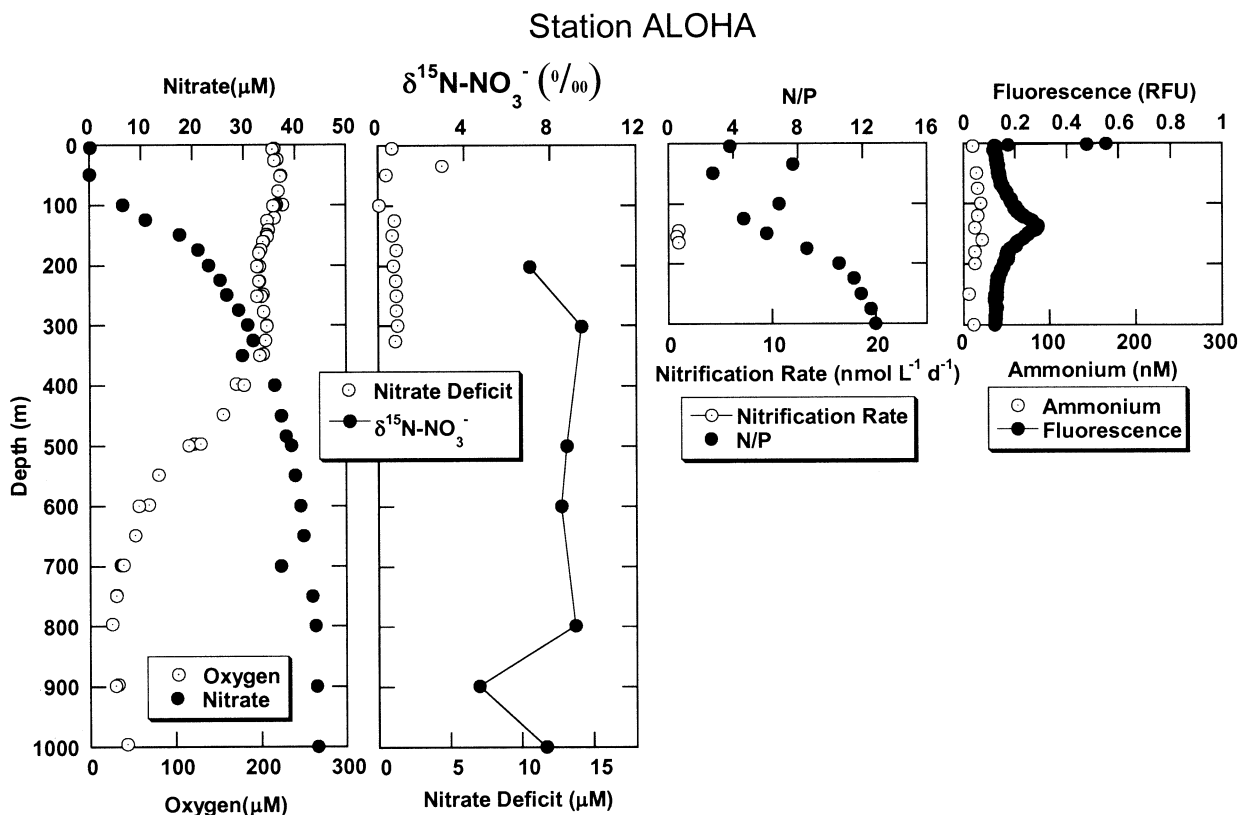
Station ALOHA

Fig. 2. Water column profiles of dissolved oxygen and nitrate, $\delta^{15}N\text{-}NO_3^-$, nitrate deficit, N/P, nitrification rate, fluorescence and ammonium at station ALOHA.

ingly shallow depth from west to east with a change from 700 m at station ALOHA to 65 m at station 6 (Figs. 2 and 7). Furthermore, minimum oxygen concentrations at station ALOHA are 30 μM and decrease to <1 μM at station 6. The decreasing depth of the oxygen minimum zone is associated with a change in depth of the chlorophyll fluorescence maximum from 140 m at station ALOHA to 40 m at station 6 (Figs. 2 and 7). Fluorescence increases from west to east with a maximum of 0.25 relative fluorescence units (RFU) at station ALOHA to 0.95 RFU at station 6 (Figs. 2 and 7). Ammonium concentrations in surface waters at station ALOHA are between 0 and 20 nM and at stations 4, 5 and 6 increase to 100 to 300 nM (Figs. 2, 5, 6 and 7). This is expected due to the enhanced productivity and subsequent mineralization of organic matter in the eastern portion of the transect.

3.2. Nitrogen Isotope Biogeochemistry

The primary microbial processes that influence the nitrogen isotopic composition of nitrate include denitrification, assimilatory nitrate reduction, nitrogen fixation and nitrification (Cline and Kaplan, 1975; Mariotti et al., 1981; Liu et al., 1996; Ostrom et al., 2002). During these microbial-driven nitrogen transformations, isotopic discrimination can result due to a slight difference in the enzymatic reaction rate between ^{15}N and ^{14}N containing compounds (Mariotti et al., 1981). The result is an accumulation of ^{14}N in the products of reactions and ^{15}N in the residual substrates (Mariotti et al., 1981).

Nitrification and nitrogen fixation can result in nitrate that is depleted in ^{15}N. The fractionation factor associated with nitrogen fixation is small and produces fixed nitrogen with a mean $\delta^{15}N$ value of $-2.6 \pm 1.3\permil$ (Sachs and Repeta, 1999). Isotopically light nitrate can be produced by ammonification of fixed nitrogen and subsequent nitrification. Liu et al. (1996) supported this possibility in a study within the western Pacific. Within the Kuroshio current, ^{14}N-enriched nitrate (1 to 3‰) was attributed to the activity of unusually high numbers of a nitrogen fixer, *Trichodesmium* (Liu et al., 1996). However, nitrification can also lead to ^{15}N-depleted nitrate. Laboratory studies reveal that microbial nitrification has a relatively large isotopic enrichment factor (ε of 35‰) (Mariotti et al., 1981; Yoshida, 1988), where ε is defined by:

$$\varepsilon_{p/s} = 1000\,(\alpha - 1) \tag{5}$$

where α is the fractionation defined as:

$$\alpha = \frac{(k^{14}N)}{(k^{15}N)} \tag{6}$$

and $k^{14}N$ and $k^{15}N$ are the instantaneous reaction rates for the molecules containing ^{14}N and ^{15}N respectively. In Conception Bay, Newfoundland and in Lake Superior, nitrification was proposed as the likely cause of low $\delta^{15}N$ values of dissolved nitrate ($\delta^{15}N\text{-}NO_3^-$, average 0.2‰) (Ostrom et al., 1997; Ostrom et al., 1998b).
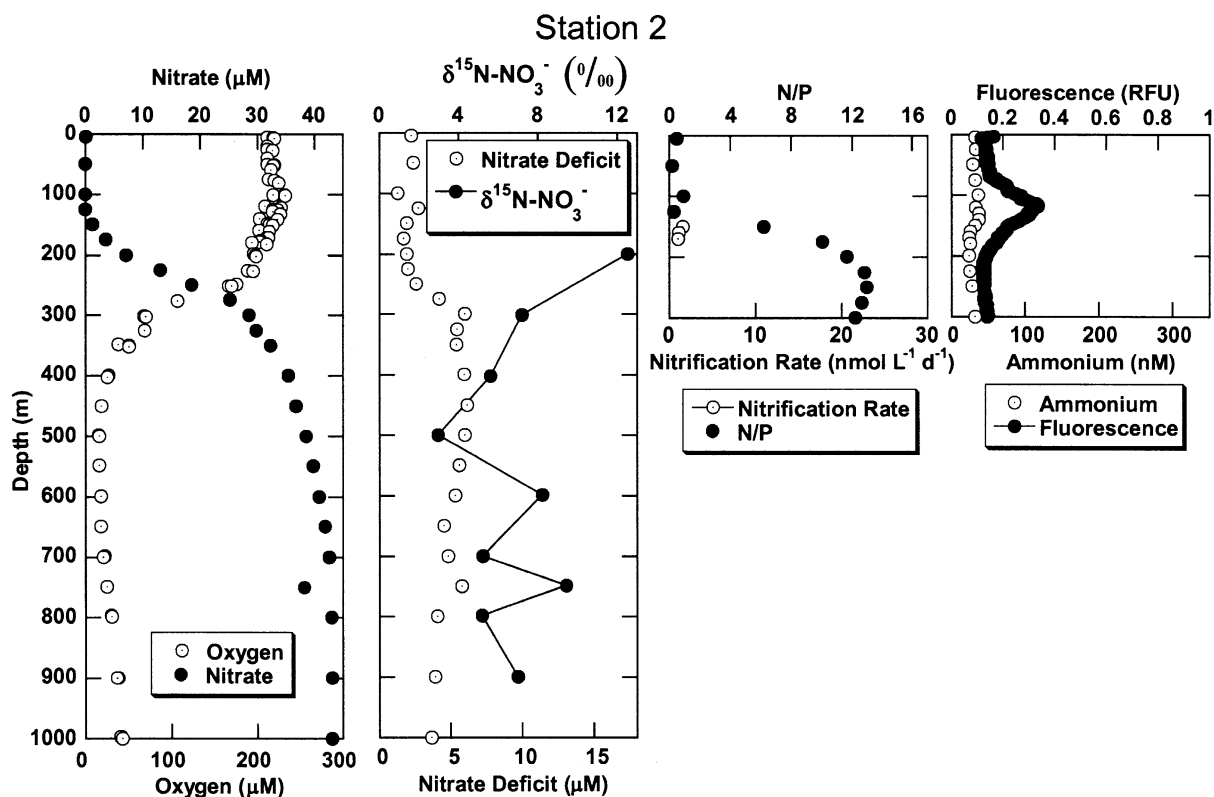
Fig. 3. Water column profiles of dissolved oxygen and nitrate, δ^{15}N-NO$_3^-$, nitrate deficit, N/P, nitrification rate, fluorescence and ammonium at station 2.

Nitrate enriched in ^{15}N has been associated with the processes of denitrification and assimilatory nitrate reduction by phytoplankton (Cline and Kaplan, 1975; Brandes et al., 1998; Voss et al., 2001). During the reduction of NO$_3^-$ to N$_2$, the residual pool of NO$_3^-$ becomes increasingly enriched in ^{15}N. This can be substantive due to a large ε associated with denitrification of approximately 30‰ in previous studies in the ETNP (Voss et al., 2001; $\varepsilon = 27 \pm 3$‰, Brandes et al., 1998). In anoxic regions of the ocean, denitrification results in $\delta^{15}N$-NO$_3^-$ values as high as 18.7‰ (Voss et al., 2001). In oxic waters, preferential assimilation of ^{14}NO$_3^-$ by phytoplankton can also result in high $\delta^{15}N$-NO$_3^-$ (Cline and Kaplan, 1975). However, the fractionation due to assimilatory nitrate reduction, ε of 0 to 19‰, is not as large as that associated with denitrification (Wada and Hattori, 1978; Wu et al., 1997; Altabet et al., 1999; Sigman et al., 1999).

High $\delta^{15}N$-NO$_3^-$ values observed in samples from suboxic (oxygen concentrations equal to or less than 1 μM) waters at our stations suggest denitrification (Figs. 5 to 7). Maximum $\delta^{15}N$-NO$_3^-$ values of 11.8, 12.3 and 10.6‰ were observed in samples taken from stations 4, 5 and 6 respectively. Nitrate deficits within anoxic waters are generally high with maximum values of 14.0, 16.1 and 16.0 μM at stations 4, 5 and 6 and indicate active denitrification (Fig. 5, 6 and 7). In the ETNP, enrichment in ^{15}N of nitrate in association with suboxic waters and high nitrate deficits has previously been attributed to denitrification (Cline and Kaplan, 1975; Brandes et al., 1998; Voss et al., 2001).

3.2.1. Modeling of the fractionation factor for denitrification

The isotopic enrichment factor for denitrification was estimated using a model that incorporated the vertical advection and diffusion of waters in and out of the suboxic waters at stations 4 and 5. The typical model for estimating the fractionation factor for a single step reaction is derived from the Rayleigh equation (Mariotti et al., 1981; Macko et al., 1986). An important assumption in the Rayleigh model is that after the reaction is initiated, there are no additional inputs of substrate and that the reaction is unidirectional. Thus, estimating the fractionation factor for denitrification from a Rayleigh model in open ocean systems is inappropriate because there is an influx of nitrate from water bodies below the zone of denitrification. In open-systems, studies have employed a one-dimensional vertical advection and diffusion model to describe the concentration and isotopic composition of nitrate as a function of depth in the water column (Cline and Kaplan, 1975). The current study applied a vertical advection-diffusion model to estimate ε for denitrification using water column data at stations 4 and 5 (Cline and Kaplan, 1975; Velinsky et al., 1991). Boundary conditions included a depth interval (150–700 m) with a linear temperature and salinity relationship and apparent absence of microbial processes other than denitrification on the nitrogen isotopic composition of dissolved nitrate. The first step is to find an analytical solution of the following differential Eqn. 7 subject to the boundary conditions (Eqn. 8).
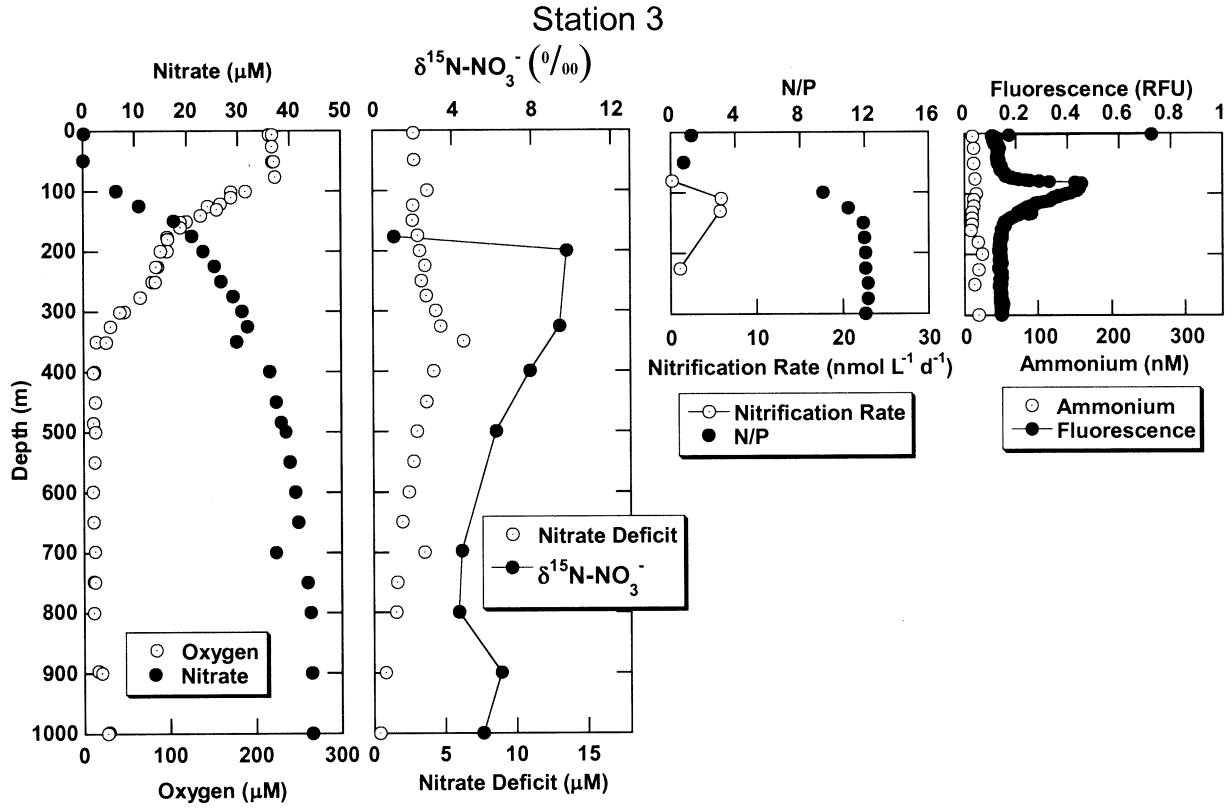
Station 3

Fig. 4. Water column profiles of dissolved oxygen and nitrate, $\delta^{15}N\text{-}NO_3^-$, nitrate deficit, N/P, nitrification rate, fluorescence and ammonium at station 3.

$$\frac{d^2N}{dz^2} + \omega \frac{dN}{dz} - \frac{R_0 \exp(-\mu_0 z)}{K} = 0 \qquad (7)$$

$$N|_{z_1} = N_1; \quad N|_{z_2} = N_2 \qquad (8)$$

where:

z = depth (m)

N = concentration of nitrate (μM)

K = vertical eddy diffusion coefficient (m^2 sec^{-1})

R_0 = rate of denitrification (μmol L^{-1} sec^{-1})

ω = mixing parameter (V_z/K) (m^{-1})

V_z = Vertical velocity (m sec^{-1})

μ_0 = describes the attenuation of microbial activity with depth (m^{-1})

The solution of Eqns. 7, 8 is used to determine the values for the parameters that provide the best comparison of modeled to observed nitrate concentrations. The parameters evaluated in the first step include K, R_0, ω and μ. The second step is to obtain a numerical solution of the isotope portion of our model. Upon substitution of the concentrations of ^{14}N and ^{15}N into Eqn. 7 and by using the definitions of α and δ from Eqns. 6 and 1, Eqn. 9 is obtained:

$$\frac{d^2C}{dz^2} + \omega \frac{dC}{dz} - \left[\left(\frac{1}{\alpha} \right) \left(\frac{C}{^{14}N} \right) - \left(\frac{\alpha - 1}{\alpha} \right) \times 1000 \right] \frac{k_{14N}}{K} = 0 \qquad (9)$$

where $C = {}^{14}N \times \delta$, $\delta = \delta^{15}N-NO_3^-$ (‰) is the stable

nitrogen isotopic composition (Eqn. 1) and α is the isotopic fractionation factor (Eqn. 6). Upon the assumption that the concentration of ^{14}N and the reaction rate are equal to those for the total quantities (Cline and Kaplan, 1975), the following expression is obtained:

$$\frac{d^2C}{dz^2} + \omega \frac{dC}{dz} - \left[\left(\frac{1}{\alpha} \right) \left(\frac{C}{N} \right) - \left(\frac{\alpha - 1}{\alpha} \right) \times 1000 \right]$$
$$\cdot \frac{R_0 \exp(-\mu_0 z)}{K} = 0 \quad (10)$$

Boundary conditions at the two depths z_1 and z_2 are:

$$(N \cdot \delta)_{z_1} = C_1; \quad (N \cdot \delta)_{z_2} = C_2 \qquad (11)$$

We have solved Eqns. 10 and 11 numerically using an implicit finite-difference method. The finite-difference approximations of Eqn. 10 combined with the boundary conditions produced a tridiagonal matrix. This system of equations was solved using a special case of gaussian elimination known as the Thomas algorithm (Roache, 1998). We increased the number of grid points until successive refinements produced no additional change in the results. A typical step size used in our computations is 0.5 m.

A comparison of modeled to observed nitrate concentrations at stations ALOHA to 3 indicated that denitrification was not active at the time that we sampled the water column. The parameters used at station 4 that provided a reasonable com-
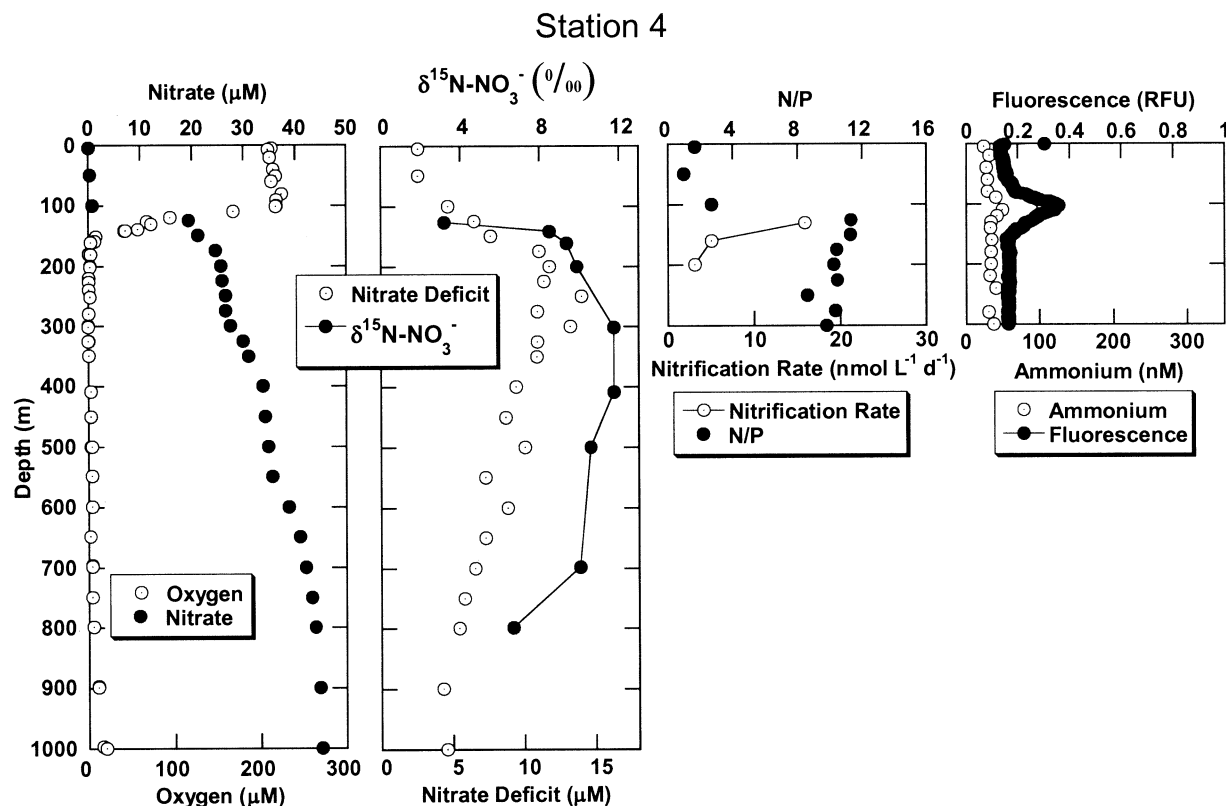
Station 4

Fig. 5. Water column profiles of dissolved oxygen and nitrate, $\delta^{15}N-NO_3^-$, nitrate deficit, N/P, nitrification rate, fluorescence and ammonium at station 4.

parison of modeled to observed nitrate concentrations include $K = 7.65 \times 10^{-5}$ m^2 s^{-1}, $R_0 = 0.85 \times 10^{-7}$ μmol L^{-1} s^{-1}, $\omega = 1.0 \times 10^{-3}$ m^{-1} and $\mu = 0.76 \times 10^{-2}$ m^{-1} (Fig. 8). At station 5, the values for K, ω and μ were the same, but a R_0 value of 1.35×10^{-7} μmol L^{-1} s^{-1} was used (Fig. 9). Despite a good comparison of modeled to observed nitrate concentrations at station 6, ε was not estimated due to insufficient isotopic data in the modeled depth interval. At station 4, the model estimate was an ε of 30‰. Since estimates of ε were very sensitive to changes in $\delta^{15}N-NO_3^-$ near the boundary, we define our estimates of ε based on data near the center of the modeled depth interval. A comparison of modeled to observed $\delta^{15}N-NO_3^-$ at station 5 suggest an ε of 25 to 35‰ (Fig. 9). The non-ideal fit of the modeled to observed data at station 5 is likely due to the low number of observed $\delta^{15}N-NO_3^-$ values or the influence of another biologic process on the concentration and isotopic composition of NO_3^-. A comparison of the results from station 4 to those at station 5 suggest that the isotopic fractionation factor is similar despite a change in the rate of denitrification (R_0) from 0.85×10^{-7} μmol L^{-1} s^{-1} at station 4 to 1.35×10^{-7} μmol L^{-1} s^{-1} at station 5. Our estimates of the isotopic enrichment factor are within the range of previous studies in the ETNP ($\varepsilon = 27$ to 60‰) (Cline and Kaplan, 1975; Brandes et al., 1998; Voss et al., 2001) and closely resemble the value for the majority of the estimates (ε of 30‰).

There is mounting evidence that the majority of mixing in the open ocean occurs along isopycnal surfaces (Jenkins, 1980; McDougall, 1987; Ledwell et al., 1993; Gnanadesikan, 1999).

However, the current study and similar models found a reasonably good fit of modeled to observed dissolved nitrate concentrations in the ETNP (Cline and Kaplan, 1975; Brandes et al., 1998; Voss et al., 2001). In addition, Codispoti and Richards (1976) investigated two different estimates of nitrate deficit in the ETNP with models that incorporated vertical distributions vs. isentropic analysis and found that the two models agreed reasonably well. One possible explanation is that horizontal mixing in the regions where water masses sink could produce vertical gradients (Codispoti and Richards, 1976). Brandes et al. (1998) similarly acknowledged the possible importance of isopycnal mixing and speculated that a model that incorporated both horizontal and vertical processes would estimate a fractionation factor between that of the advection-reaction ($\varepsilon = 25 \pm 2$‰) and diffusion-reaction model ($\varepsilon = 30 \pm 3$‰) in the ETNP.

3.2.2. High $\delta^{15}N-NO_3$ in Oxic and Productive Waters

In the upper water column (<200 m depth), multiple processes influence the distribution of inorganic nitrogen including assimilatory nitrate reduction, nitrogen fixation and nitrification. This leads to the potential for overlap of active processes within similar depth intervals. For example, in an incubation study using ^{15}N-enriched substrates, Ward et al. (1989) demonstrated that nitrification occurs simultaneously with the assimilation of nitrate by phytoplankton. Interactions between microbial-driven nitrogen transformations are further compli-
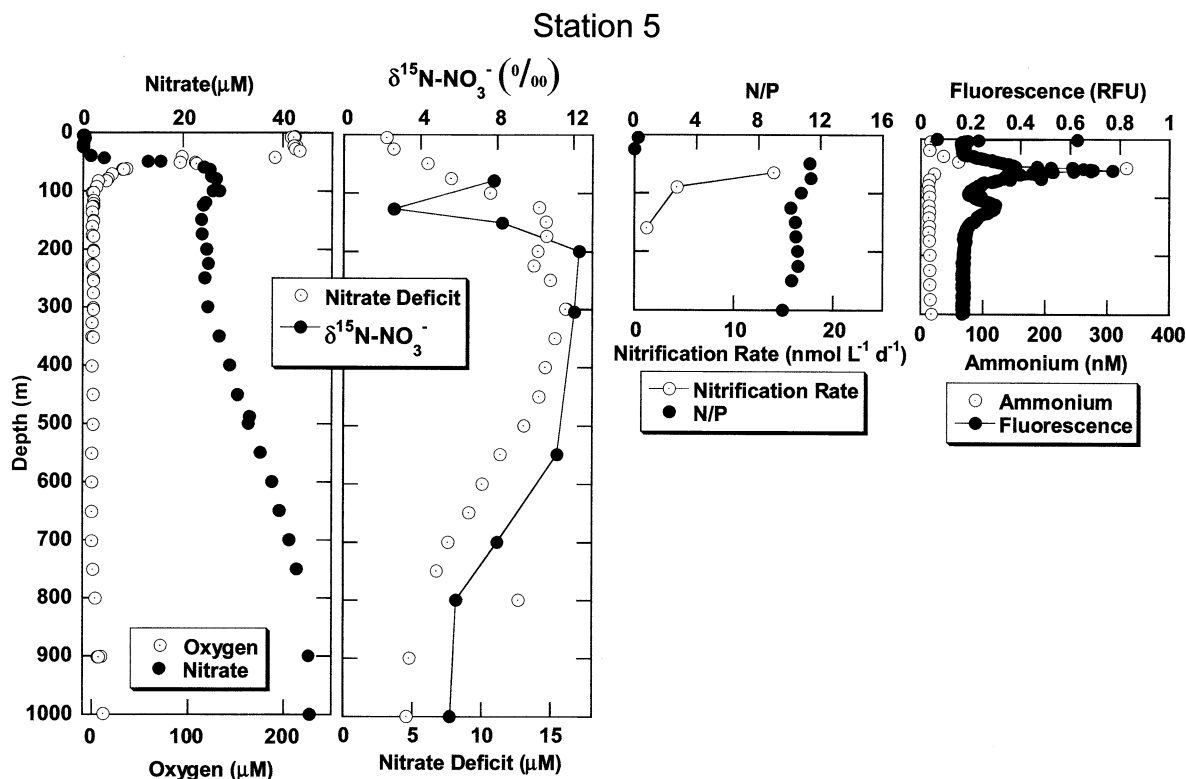
Station 5

Fig. 6. Water column profiles of dissolved oxygen and nitrate, $\delta^{15}N\text{-}NO_3^-$, nitrate deficit, N/P, nitrification rate, fluorescence and ammonium at station 5.

cated in the ETNP where a shallow oxygen minimum zone promotes denitrification at the base of the euphotic zone. Ward (1996) has noted the potential for interaction between nitrification and denitrification at oxic/anoxic interfaces in the environment. At the boundary of the interface, a flux of substrates and products can promote a coupling of the oxidation and reduction of different inorganic nitrogen species (Ward, 1996). As a consequence of the coupling and spatial overlap of processes, interpretations of the isotopic composition of inorganic nitrogen in the upper water column often requires additional geochemical information such as nitrogen to phosphorus ratios, chlorophyll fluorescence and dissolved oxygen concentrations.

In oxic near-surface waters, high $\delta^{15}N\text{-}NO_3^-$ values that occur at stations 2, 5 and 6 are suggestive of assimilatory nitrate reduction (Figs. 3, 6 and 7). In our data, this is most evident at station 6 where high $\delta^{15}N\text{-}NO_3^-$ values of 8.3 and 10.0‰ at 28 and 44 m, respectively, are coincident with the chlorophyll fluorescence maximum (Fig. 7). In addition, localized isotopic maxima in oxic surface waters at stations 2 and 5 likely reflect an influence of assimilatory nitrate reduction (Figs. 3 and 6). This is consistent with previous interpretations of high $\delta^{15}N\text{-}NO_3^-$ found in near-surface waters of the ETNP (Cline and Kaplan, 1975).

3.2.3. Isotopically light nitrate

Low $\delta^{15}N\text{-}NO_3^-$ values (1.1 to 3.2‰) in the upper 200 m depth are observed in our samples from stations 3, 4, 5 and 6 (Figs. 4 to 7). The processes of nitrogen fixation or nitrification

could be invoked as the plausible cause of this isotopically light nitrate. In near-surface waters of the ETNP, low $\delta^{15}N\text{-}NO_3^-$ values (5‰) were attributed to an influence of nitrogen fixation (Brandes et al., 1998). Estimates from an isotope mass balance model indicated that as much as 20% of nitrate was derived from nitrogen fixation (Brandes et al., 1998). However, isotopic data alone cannot distinguish nitrate derived from nitrification and nitrogen fixation. Other lines of evidence indicative of nitrogen fixation include the $\delta^{15}N$ value of particulate organic nitrogen (PON), nitrogen to phosphorus ratios and the abundance of nitrogen fixing organisms (Saino and Hattori, 1987; Liu et al., 1996; Karl, 1999).

Studies in the Western Pacific and Sargasso Sea have suggested that isotopically light PON ($\delta^{15}N$ of −3 to 2‰) is the result of nitrogen fixation activity (Saino and Hattori, 1987; Altabet et al., 1991). These low $\delta^{15}N$ values of PON occur in waters where *Trichodesmium*, a nitrogen fixing organism, are present (Saino and Hattori, 1987; Altabet et al., 1991). The observation that a culture of *Trichodesmium* had a $\delta^{15}N$ value of −1.6‰ provides further evidence that low $\delta^{15}N$ values of PON ($\delta^{15}N\text{-}PON$) can result from nitrogen fixation (Carpenter et al., 1997). Within the same environment (ETNP) and depth interval where we observed isotopically light nitrate, Voss et al. (2001) reported $\delta^{15}N\text{-}PON$ values for samples collected in November to December 1997 between 4.4 and 8.2‰ that are not indicative of nitrogen fixation.

In addition to the nitrogen isotopic composition of PON, the nitrogen to phosphorus ratio of dissolved and particulate matter
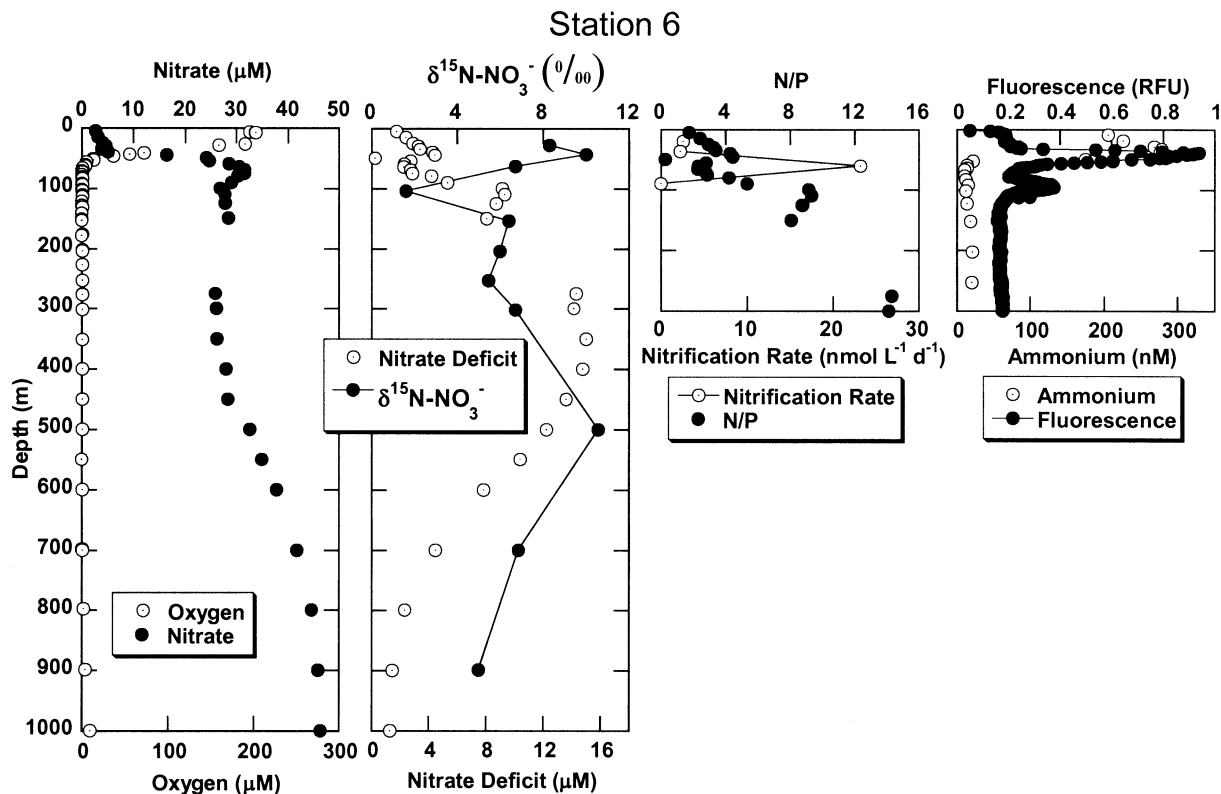
Station 6

Fig. 7. Water column profiles of dissolved oxygen and nitrate, $\delta^{15}N\text{-}NO_3{}^-$, nitrate deficit, N/P, nitrification rate, fluorescence and ammonium at station 6.

is also used to indicate nitrogen fixation. This is based on the observation that cells of nitrogen fixing organisms are characterized by nitrogen to phosphorus ratios in excess of Redfield (16:1 and 44:1 for Redfield and nitrogen fixing cells, respec-

tively) (Carpenter, 1983). Nitrogen to phosphorus ratios of dissolved inorganic nitrogen and phosphorus were demonstrated to be significantly higher than Redfield (40:1) in the Kuroshio current northeast of Taiwan where unusually abundant populations of nitrogen fixers were found (Liu et al.,
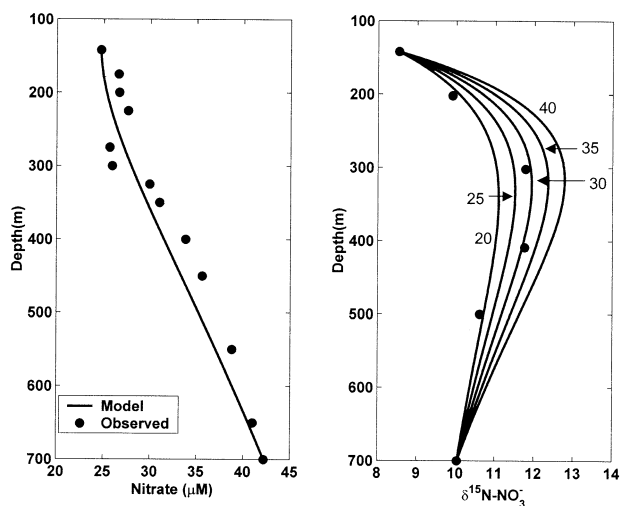

Fig. 8. Comparison of modeled to observed nitrate concentrations and $\delta^{15}N$ values of dissolved nitrate at station 4. Parameters used at station 4 include a K of 7.65E-5 $m^2\ s^{-1}$, R_0 of 0.85E-7 $\mu mol\ L^{-1}\ s^{-1}$, ω of 1.0E-3 m^{-1} and μ of 0.76E-2 m^{-1}. Epsilon values modeled between 20 and 40‰ in 5‰ increments.
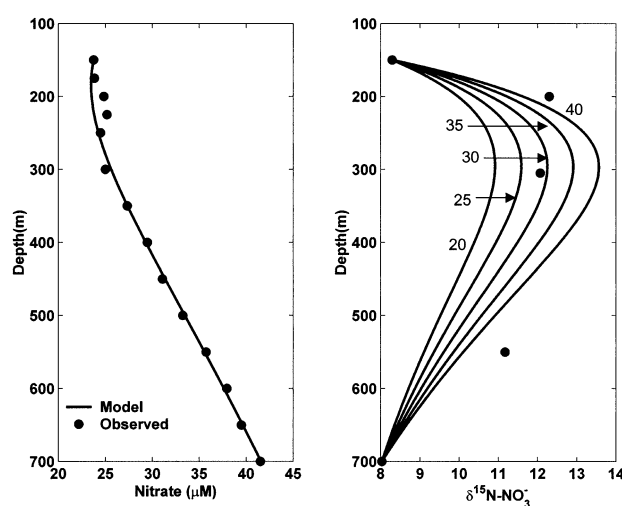
Fig. 9. Comparison of modeled to observed nitrate concentrations and $\delta^{15}N$ values of dissolved nitrate at station 5. Parameters used at station 5 include a K = 7.65E-5 $m^2\ s^{-1}$, R_0 = 1.35E-7 $\mu mol\ L^{-1}\ s^{-1}$, ω = 1.0E-3 m^{-1} and m = 0.76E-2 m^{-1}. Epsilon values modeled between 20 and 40‰ in 5‰ increments.

1996). In previous studies at station ALOHA, an increase above the Redfield ratio to 20:1 or higher in the nitrogen to phosphorus ratio of the dissolved and particulate pools was interpreted by Karl et al. (1997) as evidence for the importance of nitrogen fixation. Our data indicate that maximum nitrogen to phosphorus ratios of total dissolved inorganic nitrogen to total phosphorus were never higher than 12.1:1 in the upper 200 m of all stations (Figs. 2 to 7). In the region of our transect where we observed isotopically light nitrate, the nitrogen to phosphorus ratios are sub-Redfield and not consistent with a significant input of nitrogen via nitrogen fixation. Station ALOHA was demonstrated by Karl et al. (1997) to have seasonal (July to October) and ENSO period increases in the significance of nitrogen fixation. However, we sampled station ALOHA in May and an El Niño event did not take place in 2000.

The abundance of *Trichodesmium* is potentially indicative of the significance of nitrogen fixation in marine environments. In the western Pacific, isotopically light nitrate (1 to 3‰) was observed in waters characterized by a cell density of *Trichodesmium* as high as 4.9×10^6 trichomes m^{-3} (Liu et al., 1996). At station ALOHA, Karl et al. (1997) noted increases in *Trichodesmium* abundance in association with seasonal increases in N to P ratios of particulate and dissolved matter. East of 130°W in the North Pacific *Trichodesmium* has not been observed (Carpenter, 1983). Thus, the locations where isotopically light nitrate were observed in our current study fall outside the easterly range of *Trichodesmium*. Although the influence of nitrogen fixation from other genera (e.g., *Synechococcus*, *Cyanothece* and *Erythrosphaera*) in these waters cannot be discounted; *Trichodesmium* is the dominant diazotroph in tropical and subtropical oceans (Carpenter et al., 1997; Zehr et al., 2000). Therefore, we conclude that aside from isotopically light nitrate, $\delta^{15}N$ values of PON from Voss et al. (2001), nitrogen to phosphorus ratios and *Trichodesmium* abundance from Carpenter (1983) do not suggest nitrogen fixation as a source of isotopically light nitrate. Thus, we consider nitrification as a likely mechanism.

3.2.4. *^{15}N-NH_4 Oxidation Studies*

Previous incubation studies have found nitrification rates of 1.0 to 137.4 nmol L^{-1} d^{-1} at ALOHA and a maximum of 20 nmol L^{-1} d^{-1} in the ETNP (Ward and Zafiriou, 1988; Dore and Karl, 1996). Our incubation studies document that nitrification occurs at every station in this study (Figs. 2 to 7). At station ALOHA, maximum nitrification rates of 1 nmol L^{-1} d^{-1} are at the low range of values from a previous study (1.0–137.4 nmol L^{-1} d^{-1}) (Dore and Karl, 1996) (Fig. 2). The variation in nitrification rates in the current study and the rates determined by Dore and Karl (1996) at station ALOHA could be due to differences in sampling depth or possible temporal dynamics of nitrification rates. The data for station 6 (maximum of 23.7 nmol L^{-1} d^{-1}) are similar to the rate of 20 nmol L^{-1} d^{-1} from a study in the ETNP by Ward and Zafiriou (1988) (Fig. 7). There was an increase in nitrification rates from west to east in the transect with a maximum rate at station 6 ~20 times greater than station ALOHA. High ammonium concentrations (100 to 300 nM) at stations 5 and 6 indicate ample substrate for nitrification. The data also indicate that the

highest rates of nitrification generally occur at the base of the chlorophyll maximum (Figs. 2 to 7). The depth distribution of rate measurements could be explained by light inhibition of nitrifiers and competition for ammonium with phytoplankton. Culture studies have indicated that nitrifiers are inhibited by high light levels (Horrigan et al., 1981). Consequently, at the depths in which phytoplankton are typically most active high light levels are inhibitory to nitrification. With increasing depth in the euphotic zone, phytoplankton activity is eventually inhibited by a lack of light. However, the suppression of nitrification by light is reduced. Our data demonstrate that minima in $\delta^{15}N$-NO_3^- occurs where nitrification was active. Most notably at station 4, the maximum nitrification rate of 15.8 nmol L^{-1} d^{-1} and the minimum $\delta^{15}N$-NO_3^- of 3.2‰ coincide (Fig. 5). However, maximum nitrification rates do not always coincide with isotopic minima. For example at station 5, the nitrification rate is highest at a depth of 65 m, but the $\delta^{15}N$-NO_3^- of approximately 9‰ at a depth of 75 m suggests a predominance of assimilatory nitrate reduction (Fig. 6). This is consistent with a study by Ward et al. (1989) that demonstrated that nitrification and assimilatory nitrate reduction activity at the same depths. The influence of assimilatory nitrate reduction can obscure the isotopic signature of nitrification. The decrease in the $\delta^{15}N$-NO_3^- with depth at stations 5 and 6 above 150 m suggests that the influence of assimilatory nitrate reduction on the isotopic composition of nitrate decreases with depth and the importance of nitrification increases. Thus, isotopically light nitrate observed in the data at stations 5 and 6 (128 and 104 m, respectively) reflects the predominant influence of nitrification processes despite maximum nitrification rates at a depth of 65 m (Figs. 6 and 7). In combination with knowledge of isotopic fractionation during nitrification, the incubation studies support the interpretation that low $\delta^{15}N$-NO_3 (1.1 to 3.2‰) in the data reflect the influence of nitrification rather than nitrogen fixation.

4. CONCLUSIONS

Variations in the relative importance of microbial processes of the nitrogen cycle as a function of ecosystem conditions have implications for trace gas production and primary productivity. Low $\delta^{15}N$-NO_3^- values in the ETNP have been interpreted as indicative of nitrogen fixation (Brandes et al., 1998). In the past, inputs of nitrogen via fixation and the abundance of diazotrophic organisms in the open ocean were considered insignificant. More recently, studies of the NPSG have revealed a significant contribution of nitrogen fixation to primary production (Capone et al., 1997; Karl et al., 1997). The ETNP is a major site for the loss of fixed nitrogen via denitrification and a recent study suggests that nitrogen fixation has the potential to balance the nitrogen losses (Brandes et al., 1998). However, our results indicate that nitrification is an alternative explanation for isotopically light nitrate in the euphotic zone of the NPSG and ETNP during the period of our study. In contrast to nitrogen fixation, nitrification does not increase the pool size of available nitrogen. Furthermore, the oxidation of ammonia to nitrate may result in coupling of nitrification and denitrification that contributes to a loss of fixed nitrogen to the system. If the loss of nitrogen via coupled nitrification/denitrification in the euphotic zone is found to be significant, it has the potential to

impact levels of primary productivity and organic matter export.

The distribution of nitrification activity in the water column is inconsistent with the new production paradigm of Dugdale and Goering (1967). A central component of this paradigm is that increases in the productivity result from delivery of new sources of nitrogen such as nitrate from deep water or nitrogen fixation (Dugdale and Goering, 1967). Thus, nitrate delivered to the euphotic zone via upwelling from deep waters and nitrogen derived from fixation is termed "new nitrogen" (Dugdale and Goering, 1967; Eppley and Peterson, 1979). In contrast, ammonia is termed "regenerated nitrogen" since it is the product of short-term recycling processes within the euphotic zone (Dugdale and Goering, 1967). To maintain steady state levels in productivity, imports of new nitrogen and exports from the system in the form of sinking particles and fisheries harvest must be balanced (Eppley and Peterson, 1979). An important assumption is that nitrification occurs solely in deep waters. It is now recognized that nitrification in the euphotic zone is significant and contributes to the phytoplankton nitrate demand (Ward, et al., 1989; Dore and Karl, 1996). Our results support the interpretation of these studies (Ward, et al., 1989; Dore and Karl, 1996) and for the first time provides corroborating stable nitrogen isotope evidence for euphotic zone nitrification. It is clear that euphotic zone nitrification must be accounted for in models of primary productivity and studies of nitrogen isotope biogeochemistry.

The production of isotopically light nitrate in the euphotic zone has implications for interpretation of nitrogen isotope signals in modern and ancient environments. Variations in the $\delta^{15}N$ of sedimentary organic matter are thought to be indicative of past changes in nutrient inventories or utilization (Farrell et al., 1995; Ganeshram et al., 1995). Ganeshram et al. (1995) suggested that nitrate concentrations in oxygen deficient waters are regulated by variations in the rate of denitrification. They assumed that the isotopic composition of organic matter produced in near-surface waters and deposited in sediments is a function of fractionation during phytoplankton uptake and the isotopic composition of the source nitrate (Ganeshram et al., 1995). A second assumption is that nutrients in near-surface waters of the ETNP are completely utilized by phytoplankton. Thus, the nitrogen isotopic composition of the organic matter should be identical to that of its source (nitrate) and denitrification controls the $\delta^{15}N$ of nitrate (Ganeshram et al., 1995). Increases in denitrification would be reflected as an increase in the nitrogen isotopic composition of nitrate and associated organic matter. However, euphotic zone nitrification could obscure the isotopic signature of denitrification by contributing ^{14}N-enriched nitrate to the nutrient pool. Therefore, the expectation that the nitrogen isotopic composition of organic matter is a function of the extent of denitrification may not be entirely correct. An alternative explanation for fluctuation in the nitrogen isotopic composition of sedimentary organic matter is variation in the importance of euphotic zone nitrification. This could result from increases in upwelling, which would stimulate primary productivity and subsequently increase the supply of regenerated NH_4 to support euphotic zone nitrification. At the present time, the relative importance of increases in the rate of denitrification versus euphotic zone nitrification as mechanisms to influence the isotopic composition of nitrate during glacial periods is unknown. However, our results definitively demonstrate that it is not appropriate to conclude that changes in the rate of denitrification and fractionation during assimilatory nitrate reduction are the only processes driving sedimentary nitrogen isotopic signals.

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