

Species and $\delta^{15}\text{N}$ Signatures of

nitrogen TRANSFORMATIONS

*in the Suboxic Zone
of the Black Sea*

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Nitrogen is an essential nutrient for living systems in the ocean; it is a component of proteins, chlorophyll, and nucleic acids. In the oceans, nitrogen exists as a dissolved gas (N_2), dissolved organic matter, or as the dissolved inorganic ions nitrate (NO_3^-), nitrite (NO_2^-), and ammonium (NH_4^+). Nitrogen has a relatively short lifetime in the ocean and we are still striving to understand its sources and sinks. Transformations under oxygen-limited conditions are some of its main sinks. The Black Sea is an ideal location to study nitrogen reactions that occur under low-oxygen conditions because it has a well-defined low-oxygen, or suboxic, zone at the boundary between its oxic surface layer and sulfidic (anoxic) deep water.

The distributions of nitrate and ammonium in the suboxic zone suggest that a form of chemodenitrification called anaerobic ammonium oxidation, or the anammox reaction ($\text{NO}_2^- + \text{NH}_4^+ = \text{N}_2 + 2 \text{H}_2\text{O}$), plays an important role in nitrogen cycling. New analytical results for N_2 support this reaction, but a mass balance on nitrogen suggests that NH_4^+ from particulate organic nitrogen (PON) remineralization in the suboxic zone is just as important as the upward flux of NH_4^+ from the deep water. Analyses of $\delta^{15}\text{N}$ (ratio of the two stable N isotopes, $^{14}\text{N}/^{15}\text{N}$) on NO_3^- , NH_4^+ , and N_2 exhibit significant temporal variability and are difficult to reconcile with a steady-state model involving total consumption of NO_3^- and NH_4^+ in the suboxic zone. A coupled transport-reaction model that accurately simulates ventilation by the Bosphorus plume can explain the distribution of total concentrations, but cannot simultaneously explain $\delta^{15}\text{N}$ of NO_3^- , NH_4^+ and N_2 . Some mechanism is required to remove ^{15}N so that the N_2 produced is light.

$$Flux = -k \frac{\partial C}{\partial z} + wC$$

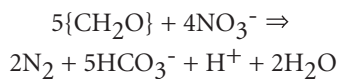
$$\frac{[PON]}{\partial t} = \mu \cdot [PON] \cdot \frac{[O_2]}{[O_2]_{1/2} + [O_2]}$$

$$-k \frac{\partial C}{\partial z}$$

OCEAN NITROGEN CYCLE

Denitrification is the main sink of nitrogen from the ocean. About half of the ocean denitrification occurs in suboxic zones of marine sediments (Brandes and Devol, 2002). The rest occurs in oxygen-minimum zones in the water column of the world's oceans.

At least two pathways are known that convert fixed nitrogen into nitrogen gas. The traditional explanation for the removal of NO_3^- is heterotrophic denitrification according to:

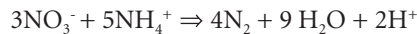


Heterotrophic denitrification in the ocean is a well-known and well-studied process (e.g., Brandes et al., 1998) and has long been considered the main pathway. Bacteria turn to NO_3^- as an electron acceptor to oxidize organic matter after O_2 has been decreased to limiting concentrations (2-10 μM) (e.g., Cline and Richards, 1972).

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However, there is increasing evidence from sediment and water-column studies that chemo-denitrification (also called anaerobic ammonium oxidation or anammox) may be important in suboxic environments where there is a source of NH_4^+ . Anammox has long been proposed to account for simultaneous

removal of NO_3^- and NH_4^+ to produce $\text{N}_2(\text{g})$ (e.g., Richards, 1965). Biological ammonium oxidation coupled to denitrification of NO_3^- to produce N_2 under anaerobic conditions



was first demonstrated in biofilms from bioreactors containing wastewater (Muller et al., 1995). More detailed investigations demonstrated that NO_2^- was the main electron acceptor (van de Graaf et al., 1997; Jetten et al., 1997). More recent ^{15}N studies have demonstrated that this denitrification occurs via a completely novel metabolic pathway involving NH_4^+ as the electron donor, hydroxylamine as the electron acceptor, and hydrazine, which is ultimately converted to N_2 , as an intermediate (van de Graaf et al., 1997). Strous et al. (1999) subsequently discovered that bacteria of the order *Planctomycetales* mediate the anammox reaction and determined its phylogenetic identity using PCR amplification (a technique

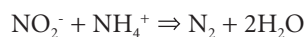
that reduces NO_3^- to NO_2^- or nitrification that makes NO_2^- from NH_4^+ (from PON) in order for anammox to occur.

Some studies have suggested that manganese species play a role in N cycling. Murray et al. (1995) suggested that Mn^{2+} could reduce NO_3^- to N_2 . Luther et al. (1997) proposed that NH_4^+ could be oxidized by MnO_2 . So far, neither of these ideas has been supported by subsequent studies (Thamdrup and Dalsgaard, 2002).

Pore-water distributions of NH_4^+ and NO_3^- in sediments, suggest that anammox is important throughout the world's organic-rich sediments (e.g., Emerson et al., 1980). Direct evidence for anammox has been obtained from numerous sediment locations including the Baltic-North Sea (Thamdrup and Dalsgaard, 2002), Thames River estuary (Trimmer et al., 2003), and the Arctic (Rysgaard et al., 2004). Anammox plays a significant role in the suboxic water column of Golfo Dulce (Dalsgaard et al., 2003). Kuipers et al. (2002) used 16S RNA plus other data

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that facilitates identification of precise segments of DNA) of its 16S rRNA. Note that the actual anammox reaction reduces NO_2^- not NO_3^-



thus there must also be some denitrifica-

and experiments to show that anammox bacteria are indeed present and active in the suboxic zone of the Black Sea.

SUBOXIC ENVIRONMENTS IN THE BLACK SEA

The Black Sea with its oxic, suboxic, and anoxic layers is an excellent site to study these reactions and their impact on nitrogen species distribution. Because of its excellent depth resolution and good temporal stability, the suboxic zone of the Black Sea is the best site for a general study of redox reactions involving nitrogen (Murray et al., 1995). In the Black Sea these reactions are spread out over vertical spatial scales of meters as compared with centimeter and millimeter scales in sediments. Several recent cruises (especially, R/V *Knorr* 2001 and R/V *Knorr* 2003, chief scientists J.W. Murray and G. Luther) have obtained new hydrographic, O₂, H₂S and nutrient data which are available on the web sites (<http://www.ocean.washington.edu/cruises/Knorr2001> and <http://www.ocean.washington.edu/cruises/Knorr2003>).

The vertical redox zonation in the Black Sea results from the strong vertical density gradient in the water column, which exists because there is large input of freshwater from rivers at the surface and input of saline water through the Bosphorus to the deep water (Neuman, 1942; Murray et al., 1991). Deep water is replaced slowly so that O₂ is totally consumed by respiration of sinking organic matter. As a result, the deep water is dominated by sulfate reduction and contains high concentrations of hydrogen sulfide. At the boundary between the oxic surface and sulfidic deep waters

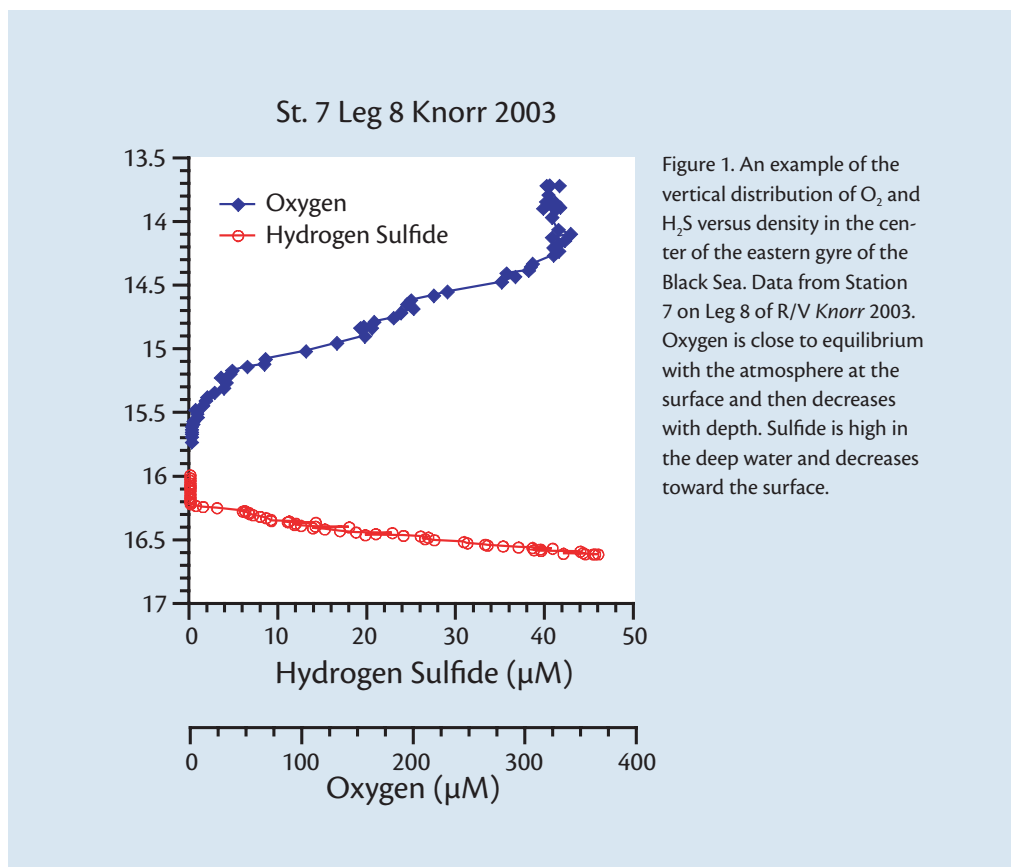
there is a well-developed and well-studied suboxic zone (Murray et al., 1989, 1995) that is a uniquely appropriate place to study oxidation-reduction reac-

(Vinogradov and Nalbandov, 1990; Murray et al., 1995) and they are deeper near the margins and shallower in the central gyres. Thus, plotting data from different

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tions involving nitrogen species. Figure 1 is an example of the vertical distributions of O₂ and H₂S versus depth and density. Density (σ_θ in kg/m³) is usually used as a depth scale because density surfaces dome in the center of the Black Sea. Biogeochemical features are frequently associated with density surfaces

locations versus density removes scatter seen in data plotted versus depth. In this example, dissolved oxygen decreases to concentrations less than 10 μ M by about $\sigma_\theta = 15.40$ (at 75 m) and sulfide does not begin to increase above 10 nM until $\sigma_\theta = 16.20$ (at 130 m). There is no formal definition of suboxic conditions, but these



two isopycnals ($\sigma_\theta = 15.40$ and 16.20) are the boundaries suggested by Murray et al. (1995). There is a density barrier of $\Delta\sigma_\theta \approx 0.50$ to 0.8 (corresponding to a depth separation of ≈ 50 m) that separates the oxygen and sulfide gradients and implies that sulfide is not oxidized directly by the downward flux of oxygen (Brewer and Murray, 1973).

Such O_2 and H_2S distributions as shown in Figure 1 were first observed during the 1988 R/V *Knorr* expedition. These distributions differed from previous data, which showed overlap of O_2 and H_2S (the so-called C-Layer) (e.g., Sorokin, 1972). Thus, there has been debate about whether or not the 1988

(and later) data represent a new feature in the Black Sea, perhaps due to increasing eutrophication or changes in freshwater input (Murray et al., 1989). Recent analyses of historical data (Buesseler et al., 1994; Konovalov and Murray, 2001) suggest that the suboxic zone has existed for at least the past few decades.

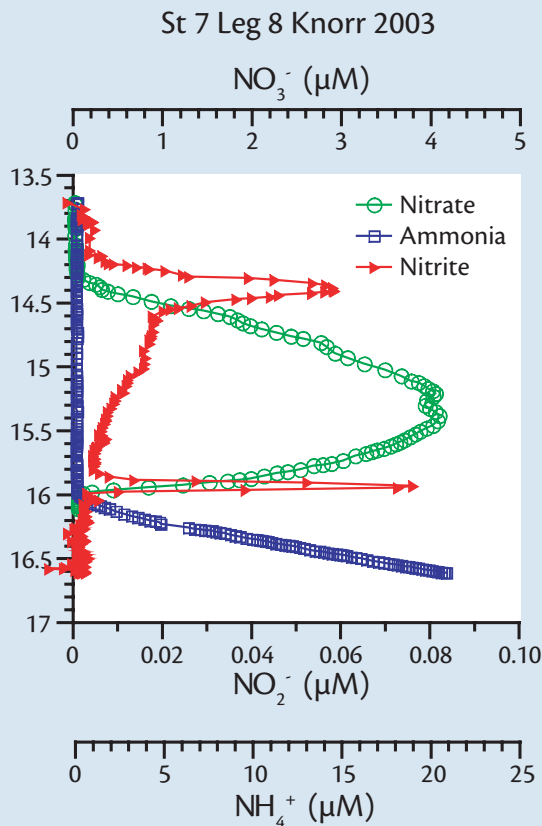
In the southwest region of the Black Sea the O_2 and H_2S distributions are more complicated because the Bosphorus Strait is the only connection of the Black Sea with the Marmara Sea and Mediterranean Sea. As high-saline water enters the Black Sea through the Bosphorus and flows across the continental shelf, it mixes with overlying water characterized by a

temperature minimum, called the cold intermediate layer (CIL). The CIL forms at the surface of the Black Sea in the winter (Gregg and Yakushev, 2005). The resulting mixture of Bosphorus outflow and CIL is called the Bosphorus plume, which injects water into the pycnocline and deep water of the Black Sea. It also injects oxygen, nitrate, and other biogeochemicals. Thus, stations occupied near the Bosphorus plume have very complicated vertical profiles reflecting the interleaving of injected water masses (e.g., Konovalov et al., 2003). This is a region of enhanced biogeochemical reactions. The effects of these reactions are spread rapidly along isopycnal surfaces throughout the rest of the Black Sea. Konovalov and Murray (2001) calculated that a major part of the upward flux of sulfide is consumed by O_2 injected horizontally from this source.

DISTRIBUTION OF NITROGEN SPECIES

Figure 2 shows an example of the distributions of NO_3^- , NO_2^- and NH_4^+ versus density in the central gyre of the eastern Black Sea. From Station 7 on Leg 8 of R/V *Knorr* 2003. Nitrate is low in the euphotic zone due to biological uptake. It increases with depth as O_2 decreases due to nitrification. At the depth where O_2 has been reduced to low concentrations, NO_3^- reaches a maximum and then decreases with depth into the suboxic zone. The linear decrease in NO_3^- with depth suggests that the profiles may be diffusion controlled with all the NO_3^- consumption occurring at the lower boundary (e.g., $\sigma_\theta = 16.0$). There are often two NO_2^- maxima located near the upper and lower boundaries of the NO_3^- maximum corresponding to zones of nitrification (shallow maximum) and denitrification (deep

Figure 2. Vertical distribution of NO_3^- , NO_2^- and NH_4^+ versus density in the central gyre of the eastern Black Sea. From Station 7 on Leg 8 of R/V *Knorr* 2003.



maximum) (Ward and Kilpatrick, 1991); NO_2^- is an intermediate in both reactions. Ammonium is high in the sulfidic deep water due to ammonification of PON. It decreases upward and goes to zero at the same depth (density) as NO_3^- . The disappearance of NO_3^- and NH_4^+ at the same density is consistent with the argument that there is a downward flux of NO_3^- and an upward flux of NH_4^+ that are consumed in a narrow density range at about $\sigma_\theta = 16.0$ by the anammox reaction.

Figure 3 plots N_2/Ar versus density from data collected during three different cruises in 2000, 2001, and 2003 (from Fuchsman and Murray, submitted). The features seen in the data include:

- Most of the water column is supersaturated with N_2 with a maximum centered at $\sigma_\theta \approx 16.0$. This is the density where NO_3^- and NH_4^+ go to zero.
- Except for 2003, N_2 is supersaturated in the shallow layers ($\sigma_\theta < 14.5$) resulting from upward flux of N_2 from the suboxic zone.
- There is significant interannual variability; the maximum was largest in 2000 and decreased to 2003.
- The shallow concentrations can be reset to atmospheric saturation after intense storms as seen by the data from 2003 when the N_2/Ar ratio was in equilibrium with the atmosphere at the surface, which had a density of $\sigma_\theta = 14.5$ at that time.

NITROGEN BALANCE

Figure 4 plots the data for NO_3^- , NH_4^+ , and excess N_2 versus depth in the central western gyre (from R/V *Knorr* 2003). Excess N_2 was calculated from the N_2/Ar ratio by assuming that Ar was at atmo-

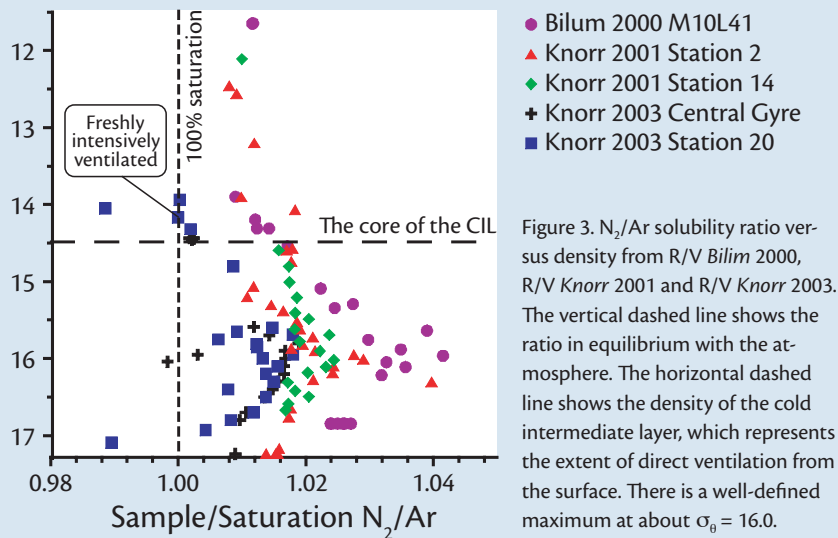


Figure 3. N_2/Ar solubility ratio versus density from R/V *Bilim* 2000, R/V *Knorr* 2001 and R/V *Knorr* 2003. The vertical dashed line shows the ratio in equilibrium with the atmosphere. The horizontal dashed line shows the density of the cold intermediate layer, which represents the extent of direct ventilation from the surface. There is a well-defined maximum at about $\sigma_\theta = 16.0$.

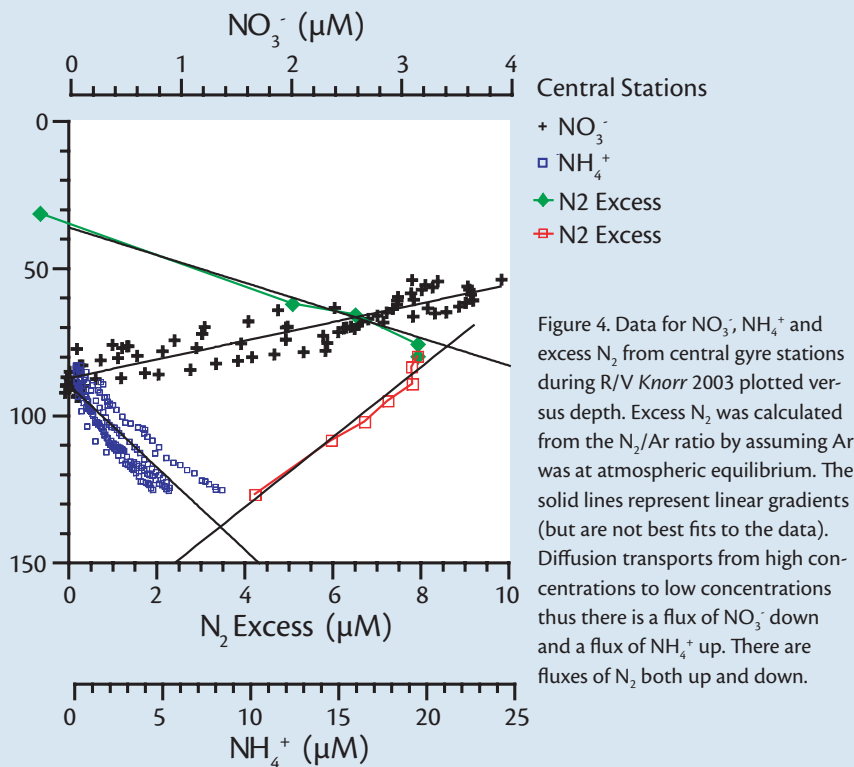


Figure 4. Data for NO_3^- , NH_4^+ and excess N_2 from central gyre stations during R/V *Knorr* 2003 plotted versus depth. Excess N_2 was calculated from the N_2/Ar ratio by assuming Ar was at atmospheric equilibrium. The solid lines represent linear gradients (but are not best fits to the data). Diffusion transports from high concentrations to low concentrations thus there is a flux of NO_3^- down and a flux of NH_4^+ up. There are fluxes of N_2 both up and down.

spheric equilibrium. This is usually an acceptable assumption, but it needs to be tested. Water depth (rather than density) was used to compare the concentration gradients, which are proportional to the diffusive fluxes. We don't need to know the magnitude of the vertical eddy diffusion coefficient for these comparisons, but we do assume that it is the same for all species over this small depth range. The data show that the gradients for NO_3^- down, NH_4^+ up, and N_2 up and down are very close to linear when plotted against depth, which is consistent with the argument that the profiles themselves are controlled by diffusion rather than reaction.

Each mole of N_2 has two nitrogen atoms. Thus, the average gradients for N are:

Species	Gradient (mol N m^{-4})
NO_3^-	0.13
NH_4^+	0.17
Total N_{in}	0.30
$\text{N}_{2\text{up}}$	0.21
$\text{N}_{2\text{down}}$	0.08
Total N_{out}	0.59

There appears to be twice as much flux of N out as N_2 , than flux of N in as NO_3^- and NH_4^+ . The difference probably comes from remineralization of sinking PON. Variability in the flux of organic

matter (export production) may explain the variability in denitrification seen in the N_2/Ar data.

NITROGEN ISOTOPE DISTRIBUTIONS

The distribution of nitrogen isotopes, ^{14}N and ^{15}N , gives clues to the controlling reactions. In the Black Sea, $\delta^{15}\text{N}-\text{NO}_3^-$ (the N isotope ratio, $^{14}\text{N}/^{15}\text{N}$ in NO_3^-) is high in the shallowest samples ($\delta^{15}\text{N} \sim 12\text{‰}$) (Figure 5) (Fuchsman and Murray, submitted). These values are higher than the average values for $\delta^{15}\text{N}-\text{NO}_3^-$ in open ocean seawater (~ 4.5 to 6‰). They decrease to values between 7‰ and 9‰

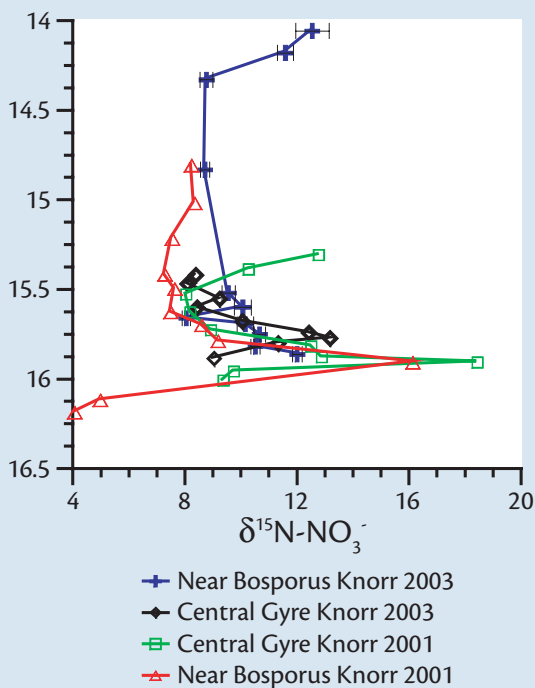


Figure 5. $\delta^{15}\text{N}$ on NO_3^- versus density. Samples were collected during the R/V *Knorr* 2001 and 2003 cruises. There is a prominent maximum for $\delta^{15}\text{N}$ of NO_3^- just above $\sigma_t = 16.0$ where the values reach $+18$.

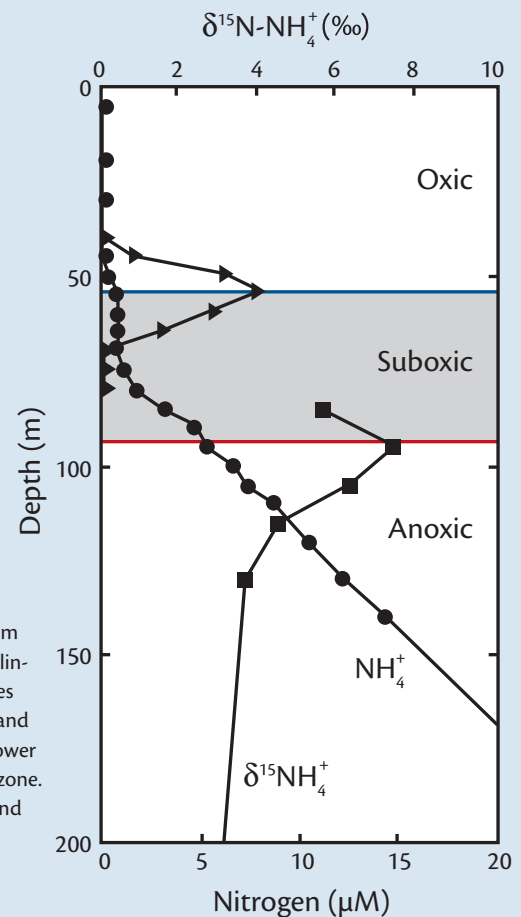


Figure 6. $\delta^{15}\text{N}$ on NH_4^+ from *Knorr* 1988. Data from Velinsky et al. (1991). The values are ~ 3 in the deep water and increase to $10-15$ at the lower boundary of the suboxic zone. Concentrations for NH_4^+ and NO_3^- are also shown.

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in the zone where NO_3^- increases with depth ($\sigma_t = 14.8$ to 15.5). In the suboxic zone, where NO_3^- decreases with depth, $\delta^{15}\text{N-NO}_3^-$ increases to values over 18‰ . In the deepest samples, just before NO_3^- has been reduced to zero, $\delta^{15}\text{N-NO}_3^-$ de-

creases to values less than $5\text{--}6\text{‰}$.

$\delta^{15}\text{N-NH}_4^+$ is very low in the deep water of the Black Sea ($\sim 2\text{‰}$) and increases to a maximum of $\sim 7\text{‰}$ at the base of the suboxic zone, then decreases slightly before NH_4^+ concentrations decrease to

values below detection (Figure 6) (Velinsky et al., 1991).

$\delta^{15}\text{N-PON}$ (Figure 7) (Coban-Yildiz et al., in press) is uniform from the euphotic zone to the suboxic zone in the range of $\delta^{15}\text{N} = 2.5\text{‰}$ to 7.5‰ . It increases to a small maximum of about 9‰ in the suboxic zone and then decreases to $\sim 2.5\text{‰}$ in the deep anoxic zone.

The main feature of the $\delta^{15}\text{N-N}_2$ profile (Figure 8) (Fuchsman and Murray, submitted) is the pronounced minimum in $\delta^{15}\text{N}$ in the suboxic zone coinciding with the N_2 maximum (Figure 3). $\delta^{15}\text{N}$ decreases to values less than 0.0‰ at $\sigma_t = 16.0$. In the surface layer values of

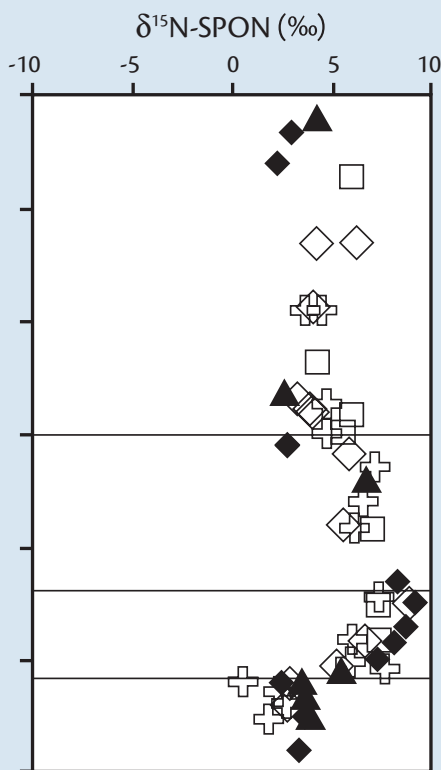


Figure 7. $\delta^{15}\text{N}$ on PON from Knorr 2001. Data from Coban-Yildiz et al. (in press). Values in the suboxic zone are ~ 7.5 .

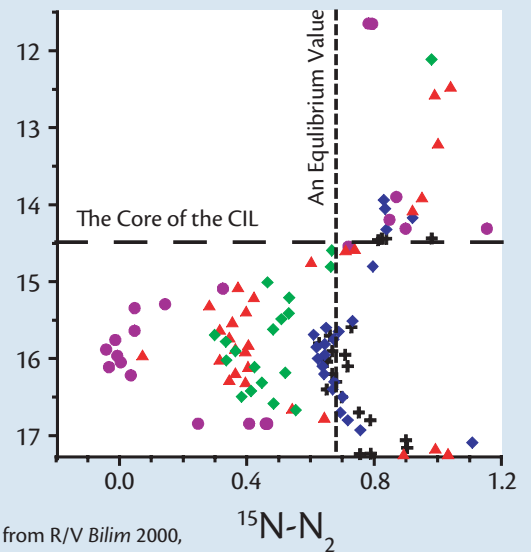


Figure 8. $\delta^{15}\text{N}$ on N_2 from R/V *Bilim* 2000, R/V *Knorr* 2001 and R/V *Knorr* 2003. Data are from Fuchsman and Murray (submitted). The vertical line represents the value in equilibrium with the atmosphere. The horizontal line is the density of the cold intermediate layer. There is a strong minimum in $\delta^{15}\text{N}$ in the suboxic zone at $\sigma_\theta = 16$ where values can be as low as 0.0 .

- Bilum 2000 M10L41
- ▲ Knorr 2001 Station 2
- ◆ Knorr 2001 Station 14
- ⊕ Knorr 2003 Central Gyre
- ◆ Knorr 2003 Station 20

$\delta^{15}\text{N}-\text{N}_2$ are greater than 1.0‰. They increase to similar values in the deep water as well. Both the surface- and deep-water values are enriched in ^{15}N and $\delta^{15}\text{N}$ is greater than the value for atmospheric equilibrium ($\delta^{15}\text{N}-\text{N}_2 \approx 0.7\text{‰}$). The final observation is that there is strong temporal variability from 2000 to 2003.

The distribution of nitrogen isotopes depend on the extent, type, and site of nitrogen fixation, nitrification, and denitrification and whether this is an open or closed system. Nitrogen fixation, which converts N_2 to fixed nitrogen, could result in enriched $\delta^{15}\text{N}-\text{N}_2$ if the isotopic enrichment factor (ϵ) is negative and N_2 is not replaced from the atmosphere by gas exchange. During denitrification, $\delta^{15}\text{N}$ of NO_3^- increases and $\delta^{15}\text{N}$ of N_2 decreases as the ^{14}N isotope in NO_3^- is preferentially utilized during heterotrophic denitrification (Brandes et al., 1998). Thus, at first glance, the increase in $\delta^{15}\text{N}-\text{NO}_3^-$ and the decrease in $\delta^{15}\text{N}-\text{N}_2$ look reasonable and as expected. But this interpretation becomes more complicated when you realize that NO_3^- and NH_4^+ react to completion at $\sigma_t = 16.0$ and when there is total consumption there should be no fractionation. As both source species both have $\delta^{15}\text{N}$ of about 6‰, it is impossible to maintain $\delta^{15}\text{N}-\text{N}_2 = 0$ at steady state.

PHYSICAL BIOGEOCHEMICAL MODEL

The Black Sea biogeochemical structure results from a complicated superposition of vertical fluxes of sinking particulate carbon on the three-dimensional circulation system influenced by ventilation from the Bosphorus plume (Kononov et

al., 2003). To numerically analyze reactions and budgets of nitrogen species we used a physical transport model that includes simulation of the Bosphorus plume and assumes isopycnal distribution of all properties (Ivanov and Samodurov, 2001). The resulting one-dimensional vertical balance equations are written as

$$\text{Flux} \Rightarrow -k \frac{\partial C}{\partial z} + wC$$

$$\left(\frac{\partial}{\partial z} \left(-k \frac{\partial C}{\partial z} \right) + w \frac{\partial C}{\partial z} \right) \Rightarrow R + \frac{\partial w}{\partial z} (C_b - C)$$

where

- k = vertical diffusion coefficient and $-k \frac{\partial C}{\partial z}$ = diffusive flux,
- w = vertical velocity and
- wC = the advective flux occurring due to displacement of the Black Sea deep waters with the waters from the Bosphorus plume,
- R = rate of biogeochemical production-consumption,
- C_b = concentration in the “Bosphorus plume,” and
- C = concentration in ambient water.

The physical parameters in the model have been verified using ^{137}Cs data from 1986 to 2003 that was produced from the Chernobyl nuclear accident. (S.K. Kononov, National Academy of Sciences of Ukraine, unpublished results, 2005).

Transformations of O_2 , PON, DON (dissolved organic nitrogen), NO_3^- , NO_2^- , N_2 , NH_4^+ , H_2S , S° , and dissolved and particulate Mn(II) and Mn(IV) and Fe(II) and Fe(III) are included in the model. Dissolved organic N is split into reactive and unreactive fractions. PON is split into the fraction sinking from the eupho-

tic zone (PON) and the fraction that is bacterially produced (PON[B]). Equations that parameterize biogeochemical transformations are written to follow either chemical or biological uptake (i.e., Michaelis-Menten) kinetics. For example, oxidation of sulfide by oxygen is parameterized as a chemical process.

$$R \Rightarrow k \cdot [\text{H}_2\text{S}] \cdot [\text{O}_2]$$

Microbiologically mediated processes, like oxidation of PON by oxygen, are parameterized by Michaelis-Menten kinetics.

$$\frac{\partial [\text{PON}]}{\partial t} \Rightarrow \mu \cdot [\text{PON}] \cdot \frac{[\text{O}_2]}{[\text{O}_2]_{1/2} + [\text{O}_2]}$$

The specific (k) and maximum (μ) rates were taken from the literature, when available, and adjusted to fit the data. All values of k and μ were assumed constant. Additional information is given in Kononov et al. (in press).

SIMULATION OF CONCENTRATIONS

The physical-biogeochemical model can describe all the data. Model simulated profiles (lines) are compared with observed distributions (individual symbols) and are plotted versus density in Figure 9. Species shown include O_2 , H_2S , S° , NO_3^- , NO_2^- , NH_4^+ , and N_2 . The concentrations are constrained by the mass balance for nitrogen. This constraint limits the range of possible values for k and μ and allows evaluation of the importance of individual processes. A sub-oxic zone is simulated as seen in the data (Figure 1) and NO_3^- and NH_4^+ decrease to 0 at about $\sigma_t = 16.0$ (Figure 2). The N_2 profile has a maximum at $\sigma_t = 16.0$

as well (Figure 3). An important result for the N balance is that PON (~50%) and DON (~40%) are the main sources of N to the box defined by $\sigma_t = 14.5$ to 17.2 (as also implied by the N balance based on Figure 4). The main sinks for N are the upward fluxes of N_2 (~70%) and NO_3^- (~30%).

SIMULATION OF $\delta^{15}N$ DISTRIBUTIONS

To simulate the distributions of $\delta^{15}N$, all reactions involving N were written in terms of both ^{14}N and ^{15}N (Konovalov et al., in press). The rate of ^{15}N transformations are proportional to the rate of ^{14}N transformations, to the ratio of $[^{15}N]/[^{14}N]$, and fractionation factors $\alpha = \epsilon/1000 + 1$, where ϵ is the isotopic enrichment factor.

Thus, the rate of oxidation of ^{15}N -PON is:

$$\frac{\partial [^{15}N - PON]}{\partial t} \Rightarrow$$

$$\frac{\partial [^{14}N - PON]}{\partial t} \cdot \frac{[^{15}N - PON]}{[^{14}N - PON]} \cdot \frac{1}{\alpha} \Rightarrow$$

$$\frac{\mu}{\alpha} \cdot [^{15}N - PON] \cdot \frac{[O_2]}{[O_2]_{1/2} + [O_2]}$$

Published data on fractionation (α) or isotopic enrichment (ϵ) factors are limited. It is often difficult to identify, if the published values are specific for individual reactions or processes that may involve a number of reactions. Still, the published values for the isotopic enrichment factors (ϵ) generally vary from 0 to 40‰. Several model simulations of the $\delta^{15}N$ signatures have been conducted. It was shown above that this model correctly simulated the distribution of N species.

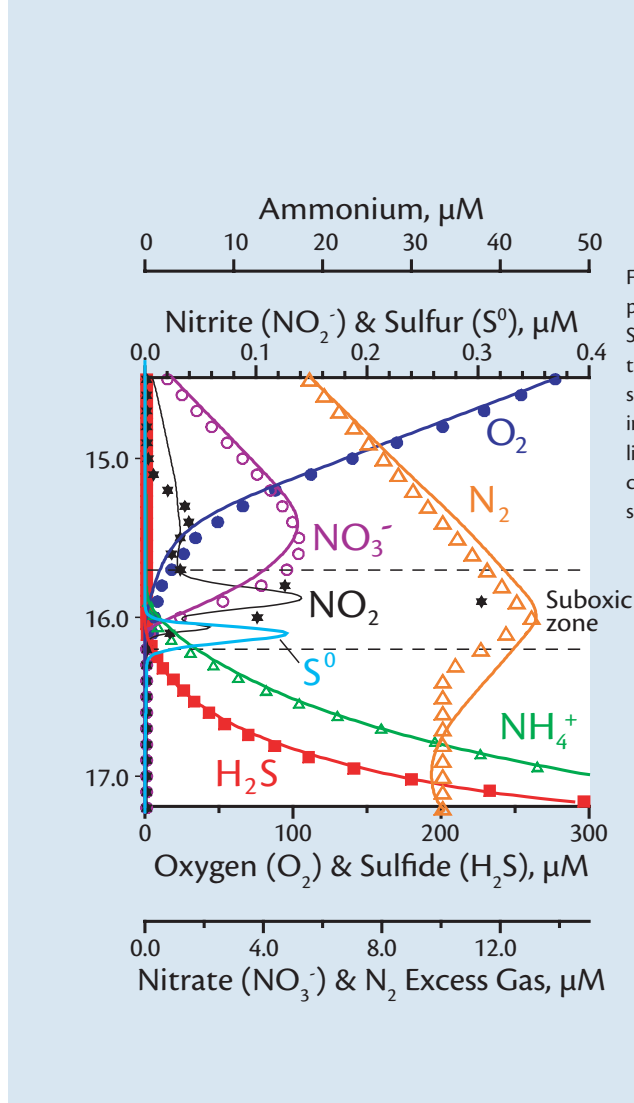


Figure 9. Model Simulations of profiles of species concentrations. Symbols are representations of the data. Solid lines are model simulations. The suboxic zone is indicated by horizontal dashed lines. The model represents the concentrations of the chemical species quite well.

We varied the set of N-involved redox reactions and their values but we always assumed that reduction of NO_3^- and oxidation of NH_4^+ in the suboxic zone ultimately resulted in production of N_2 . The model was tuned to simulate $\delta^{15}N$ profiles in the oxic and anoxic layers (Figure 10). The initial conditions used were derived from the observations of real data and are shown as dashed lines. Model simulations are shown for 90 days, 1 year, and 13 years.

The enrichment factors (ϵ) required to fit the data are given below:

- nitrate (NO_3^-) reduction, for all reductants (e.g., CH_2O , S^0) $\epsilon = 25 - 30\%$
- nitrite (NO_2^-) reduction and oxidation

$\epsilon = 10 - 15\%$

- reactions of particulate organic nitrogen to NH_4^+ $\epsilon = 2 - 5\%$
- ammonium (NH_4^+) consumption or oxidation (e.g., anammox) $\epsilon = 0$ to a few %.

Variations in α (i.e., ϵ) for denitrification and anammox did not change the final result. Complete consumption of NO_3^- (NO_2^-) and NH_4^+ does not result in any fractionation of N isotopes. Steady-state profiles were easily obtained for $\delta^{15}N$ - NO_3^- and $\delta^{15}N$ - NH_4^+ in 90 days, but $\delta^{15}N$ - N_2 decreases initially then increases rapidly. At steady state (presumably after several decades), the $\delta^{15}N$ - N_2 must equal 8‰ because this is the value for $\delta^{15}N$ -


NO_3 , $\delta^{15}\text{N-NH}_4^+$, and $\delta^{15}\text{N-PON}$, the main sources of nitrogen. This is much higher than the observed values of 0.0 to 0.8‰ and it proves that total conversion of NO_3 , NH_4^+ , and PON with $\delta^{15}\text{N}$ in the range of 5 to 7 can not make N_2 with $\delta^{15}\text{N} = 0$ to 0.8‰.

The conclusion is that a steady state model that can correctly simulate the concentrations cannot simultaneously model $\delta^{15}\text{N}$ of NO_3 , NH_4 , and N_2 . This conclusion leads to several alternative explanations that we are exploring, but two possibilities are that (1) there is a process, like adsorption of heavy NH_4 on MnO_2 , that transports heavy NH_4 from the suboxic zone to the anoxic zone, and

(2) the nitrogen profiles are not at steady state, especially with respect to the sinking flux of PON. A time series of observations and a non-stationary model are required to reproduce both concentration and $\delta^{15}\text{N}$ profiles.

ACKNOWLEDGEMENTS

We thank Amy Callahan for analyses of samples from 2000 and 2001. Danny Sigman (Princeton University) trained our group in the use of his $\delta^{15}\text{N-NO}_3$ technique using N_2O . Tolga Uysal and Emre Pamukcu (both at Bogazici University), helped with the nutrient analyses and hydrographic data at sea. George Luther (University of Delaware)

and Brad Tebo (Scripps Institution of Oceanography) helped organize and conduct the research cruises. The lab groups of Steve Emerson and Paul Quay (both at University of Washington) assisted with the N_2 and isotope analyses. Support was provided by NSF OCE 0081118 and MCB 0132101. 

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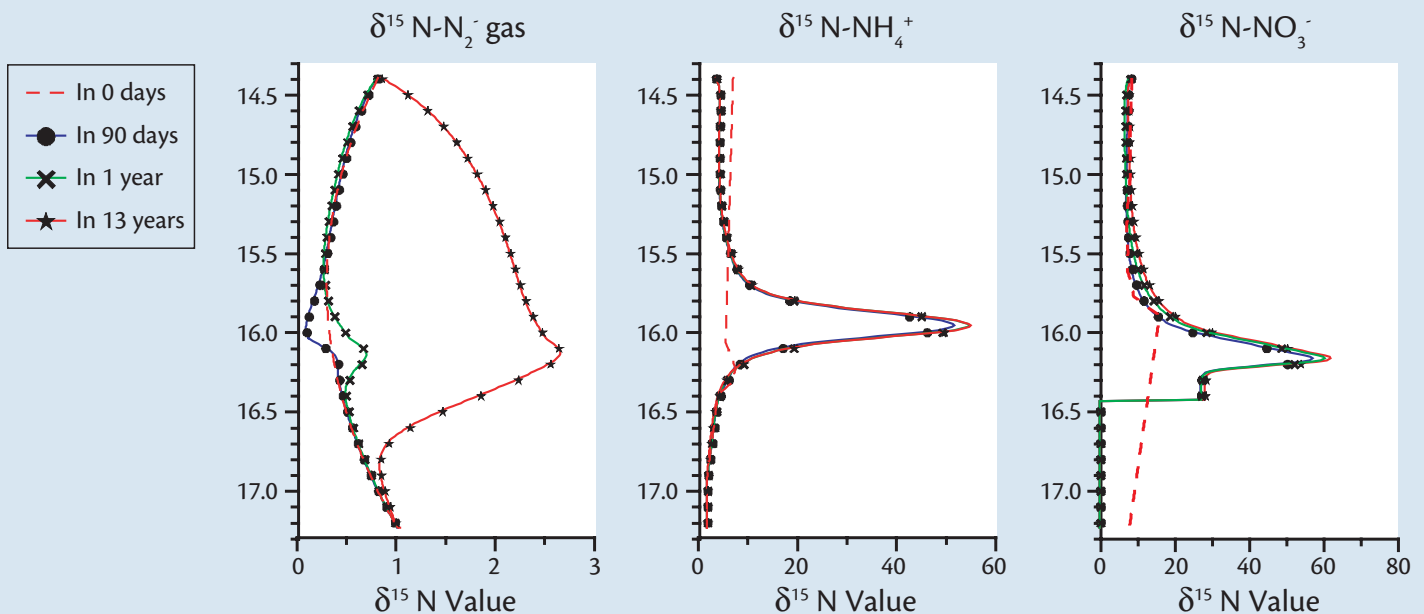


Figure 10. Model simulations of the distributions of $\delta^{15}\text{N}$ for N_2 , NH_4^+ and NO_3^- . Dashed lines represent initial conditions that were based on the observed distributions in the depth ranges where they exist. With these initial conditions, the model was run for 13 years. While the model can describe the steady state profiles of $\delta^{15}\text{N-NH}_4^+$ and $\delta^{15}\text{N-NO}_3^-$, but the $\delta^{15}\text{N}$ for N_2 cannot reach steady state and gets increasingly more positive with time.

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