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Modeling the distribution of nitrogen species and isotopes in the water column of the Black Sea

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Abstract

Using new data for the concentrations and $\delta^{15}N$ of the main nitrogen species of NO₃, NH₄, PON (particulate organic nitrogen) and N₂ in the Black Sea, we studied the distributions of concentrations and δ^{15} N using a coupled physical-biogeochemical model including known biogeochemical processes and nitrogen isotopic fractionation. In the deep anoxic water N2 is supersaturated. This supersaturation results from physical exchange with the Bosporus inflow that includes entrainment of N₂ supersaturated water from above the suboxic zone and its injection into the deep water of the Black Sea. In the suboxic zone there is a N2 maximum and a $\delta^{15}N_2$ minimum at the depth where anammox has been observed. We first used a version of the model that included both denitrification and anammox. Anammox in this case was fueled by the downward flux of NO_3^- and the upward flux of NH_4^+ from the deep water. This steady state model was able to successfully simulate the concentrations of all species and $\delta^{15}N$ of NO₃⁻ and NH_{4}^{+} . Anammox was required to be much more important than denitrification in order to have the maximum of excess N_{2} be at the correct depth and to have a NO₃ profile with the correct shape. This model can not simulate the depleted δ^{15} N values of N₂ observed in the suboxic zone in 2000, 2001 and 2003, unless we assume that PON leaving the suboxic zone is ¹⁵N enriched, as compared to observational data, or that there is a presently unaccounted process to preferably remove ¹⁵NH₄⁺ from the suboxic zone. Our models support the hypothesis that ¹⁵N depleted PON, which could be made in the euphotic zone as the result of N₂fixation, may support production of ¹⁵N depleted N₂ in the suboxic zone. This ¹⁵N depleted PON (with $\delta^{15}N = -2.0\%$) sinks and is remineralized to produce ^{15}N depleted NH₄⁺, which is rapidly consumed by anammox in the suboxic zone to produce ^{15}N depleted N₂. The nitrogen system in the suboxic zone is variable and periods of high excess N₂ correspond to periods with depleted δ^{15} N₂. We hypothesize that the system oscillates between two states. The first occurs when anammox is driven by remineralization of ^{15}N depleted PON produced by N_2 -fixation. This results in ¹⁵N depleted N_2 . The second occurs when anammox is driven by the downward flux of NO₃⁻ and the upward flux of NH₄⁺. This results in ¹⁵N enriched N₂. © 2008 Elsevier B.V. All rights reserved.

Keywords: Di-nitrogen; Anammox; N2-fixation; Stable nitrogen isotopes; Modeling; Black Sea

1. Introduction

* Corresponding author. *E-mail addresses:* sergey@alpha.mhi.iuf.net, sergey_konovalov@yahoo.com (S.K. Konovalov). Di-nitrogen (N_2) production due to respiratory oxidation of organic matter by nitrate and nitrite (denitrification) and anaerobic oxidation of ammonium (anammox)

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is a major process reducing the extent of eutrophication of aquatic systems with low oxygen content (Hulth et al., 2005). These processes are important sinks for nitrate on the global scale (e.g., Brandes and Devol, 2002), and are vitally important for nitrogen cycling in enclosed and semi-enclosed marine systems (e.g., Kim et al., 1997; Brandes et al., 1998; Thunell et al., 2004; Voss et al., 2005). Deutsch et al. (2007) argued that the processes responsible for nitrogen removal (e.g. denitrification/anammox) are linked to processes responsible for adding nitrogen compounds to the sea (e.g. nitrogen fixation). Nitrogen cycling is very dynamic in the oxicsulfidic interface region of the Black Sea, (Brewer and Murray, 1973; Murray et al., 1995). Both N₂ production due to anammox (Kuypers et al., 2003; Lam et al., 2007) and N₂ fixation (McCarthy et al., 2007) have been recently demonstrated for the Black Sea. Thus, the Black Sea is an excellent site to study this relationship.

The goal of this paper is to study the distributions of N species and N isotopes using known biogeochemical processes coupled to a model of physical exchange. Our approach will be to briefly describe the necessary background aspects of the Black Sea in order to set the stage for our modeling work. Then the models for physical exchange, biogeochemical transformation and ¹⁴N/¹⁵N fractionation will be described. The discussion of the results will focus on the ability of the model to reproduce the data with emphasis on N₂ production and cycling and the isotopic fractionation and isotopic budget. Both steady state and temporally variable models will be considered.

2. Background

The Black Sea is a natural laboratory for studying various physical, chemical and biological processes in oxic/suboxic/sulfidic marine systems because the basic hydrographic and biogeochemical characteristics of this marine system are well known (see volumes edited by Izdar and Murray, 1991; Gregoire et al., 2001; Murray, 1991, 2005, 2006). It is an enclosed basin with limited water exchange with the world's oceans through the Marmara Sea and Bosporus Strait (Fig. 1a). The water balance consists of input into the Black Sea of $\sim 300 \text{ km}^3 \text{ year}^{-1}$ of Mediterranean waters (salinity \sim 36) through the Bosporus Strait and export of surface water out of the Black Sea of ~ 600 km³ year⁻¹ (salinity \sim 18). The water and salinity budgets are balanced on decadal time scales. These features of the water and salinity budgets result in the well-known strong permanent pycnocline, which is mainly controlled by the vertical gradient in salinity. The depth and vertical

stratification in the permanent pycnocline vary over the sea but all biogeochemical properties vary precisely with density. An excellent example of how, in vertical profiles of O_2 and H_2S from different locations, the variability versus depth is removed by plotting versus density is shown in Fig. 1b,c. Therefore, the density (sigma-t or sigma-theta) scale is more effective for analysis of biogeochemical properties (Fig. 1b,c), than the depth scale (Murray et al., 1995). Use of the density scale also facilitates comparison of specific features from different locations, times and investigators. For this study, the strong correlation of biogeochemical properties with density simplified our modeling effort (Ivanov and Samodurov, 2001; Konovalov et al., 2006).

A consequence of the vertical stratification is that the surface layer (about 0 to ~50 m) is well oxygenated while the deep layer (~150 m to 2000 m) is anoxic and contains high concentrations of hydrogen sulfide. Between the oxic surface and anoxic deep layers, there is a suboxic zone with boundaries corresponding to densities of σ_t ~15.6 and ~16.2 (Fig. 1c). The concentrations of both O₂ and H₂S are extremely low (<3 µM) in the suboxic zone and do not exhibit significant vertical or horizontal gradients in the central gyre regions on a given density surface in any given year. The suboxic zone in the Black Sea is an important biogeochemical transition zone and is the site of redox reactions involving N, Mn and Fe (Murray et al., 1995).

To a first approximation, the deep water of the Black Sea forms from two end-member mixing of the Bosporus inflow with the cold intermediate layer (CIL), which is centered at a density of about $\sigma_t = 14.5$ (Murray et al., 1991). The CIL is characterized by a temporally variable temperature minimum and is the deepest layer that directly contacts the surface in winter (Gregg and Yakushev, 2005). The extent of ventilation of the CIL depends on winter severity. The average residence time of the CIL is ~ 2 years (Lee et al., 2002). If weak or no ventilation occurs on warm winters, the concentration of oxygen in the CIL decreases (Konovalov and Murray, 2001), while the concentrations of the end products of organic matter and O₂ utilization increase. Most of the mixing between the Bosporus outflow and the CIL occurs on the continental shelf just north of the Bosporus (Latif et al., 1991). The resulting Bosporus plume ventilates the interior of the Black Sea at the depth represented by its density when the water reaches the shelf break. Ventilation of shallower depths is more vigorous than deeper depths (Lee et al., 2002). In general, anomalies in biogeochemical distributions due to ventilation by the Bosporus Plume are only observed in the SW region near the Bosporus



Fig. 1. The Black Sea (a) and the vertical distribution of the major biogeochemical properties vs. depth (b) and sigma-t (c). The data used in b and c are from the extensive ocean data library at the Marine Hydrophysical Institute, Sevastopol, Ukraine. The results discussed and modeled in this paper come from the location indicated (Δ) in the center of the western gyre.

(Konovalov et al., 2003). Distributions on density surfaces throughout the rest of the Black Sea (including the center of the western gyre) are extremely uniform (Fig. 1c).

The suboxic zone provides ideal conditions for studying all aspects of nitrogen cycling including N_2

production. Nitrate is low in the euphotic zone due to biological consumption (1% I_o typically occurs at 15–30 m) (Yilmaz et al., 2006) (Fig. 1c). Low N/P ratios through the euphotic zone suggest that N₂ fixation may be favorable (e.g. Deutsch et al., 2007; Capone and Knapp, 2007; Fuchsman et al., 2008-this issue) and this

process has been recently measured by McCarthy et al. (2007). Below the euphotic zone NO_3^- increases due to aerobic respiration (nitrification). After oxygen decreases to zero, nitrate decreases to zero at a density of \sim 15.95, near the base of the suboxic zone. Ammonium begins to increase below the same depth due to ammonification and accumulates to high concentrations in the deep sulfidic layer. Nitrous oxide (N₂O) decreases to very low concentrations near the bottom of the suboxic zone indicating net consumption (Westley et al., 2006). There is evidence that chemosynthesis produces new particulate organic nitrogen in the suboxic zone (e.g. Sorokin, 1972; Brewer and Murray, 1973; Coban-Yildiz et al., 2006) but the magnitude of this rate is still uncertain. The vertical gradients of nitrate and ammonium (Fig. 1b,c) suggest fluxes of these nitrogen species to the suboxic zone where they are converted to N_2 (Murray et al., 1995; Kuypers et al., 2003) (Fig. 2a). Both denitrification and anammox occur and produce N2 under suboxic and anoxic/non-sulfidic conditions (Ward and Kilpatrick, 1991; Strous et al., 1999; Kuypers et al., 2003), but their relative importance in the Black Sea is unresolved.

New data demonstrate that the Black Sea waters are supersaturated with N₂ (Fuchsman et al., 2008-this issue) (Fig. 2a). A maximum supersaturation in N₂ and a minimum of δ^{15} N₂ are centered in the lower part of the suboxic zone, where denitrification and anammox have been reported (Ward and Kilpatrick, 1991; Kuypers et al., 2003). The maximum supersaturation in N₂ and a minimum of $\delta^{15}N_2$ vary from year-to-year, but both N_2/Ar and $\delta^{15}N_2$ in the surface layer approach the values expected for equilibrium gas exchange between surface ocean and atmosphere. Yet, the high level of N_2 in the deep water of the Black Sea raises questions about the processes that could support supersaturation in the ano-xic water column.

Another unresolved question is the nitrogen isotope (¹⁴N and ¹⁵N) budget for the suboxic zone. The 2000 Bilim set of δ^{15} N data (Fig. 2b), in particular, suggested intensive isotopic fractionation (Murray et al., 2005; Fuchsman et al., 2008-this issue). A deep minimum in $\delta^{15}N_2$ coincided with a maximum supersaturation of N₂ within the suboxic zone. However, the complete consumption of nitrate and ammonium in the suboxic zone, as suggested by their concentration profiles (Fig. 1c), should not result in any isotope fractionation (Brandes and Devol, 2002), but should produce $\delta^{15}N_2$ similar to the $\delta^{15}NH_4^+$ and $\delta^{15}NO_3^-$ at the boundaries of the suboxic zone (+5 to +10%). To make the overall picture even more complicated, δ^{15} N of suspended PON decreases, rather than increases, through the suboxic zone (Coban-Yildiz et al., 2006). Thus, two major known nitrogen species (N2 and PON) leaving the suboxic zone become much lighter than the three major nitrogen species (PON, nitrate, ammonium) entering the suboxic zone.

Three main issues will be addressed in this study: (i) what keeps the deep anoxic waters supersaturated with N_2 , (ii) can we successfully model both the



Fig. 2. The distribution of the N₂/Ar ratio in samples normalized to the N₂/Ar in water equilibrated with the air (a); $\delta^{15}N_2$ values versus σ_{θ} depth scale in the Black Sea water column from 2000–2005 (b), and vertical distributions of $\delta^{15}N$ values for the major nitrogen species (c). Horizontal lines are shown for the boundaries of the suboxic zone (SOZ) and the core of the cold intermediate layer (CIL). Vertical dashed lines are for water equilibrated with the air that is 1.0 for normalized N₂/Ar ratio (a) and 0.68‰ for $\delta^{15}N_2$ (b). Individual symbols are observational data. The solid lines in (c) are the profiles used as references for numerical experiments. The 2000 and 2001 $\delta^{15}N_2$ data are used to draw its reference profile. The $\delta^{15}NO_3^-$ data are from 2001 (Fuchsman et al., 2008-this issue). The $\delta^{15}NH_4^+$ data are from Velinsky et al. (1991). The $\delta^{15}N-PON$ profile is from Coban-Yildiz et al., 2006 and Fuchsman et al. (2008-this issue). Observational data for $\delta^{15}N-DON$ are not available, so its reference profile was assumed equal to $\delta^{15}N-PON$.

concentrations and $\delta^{15}N$ values of nitrogen species in the suboxic zone of the Black Sea using known processes and (iii) what controls the variability of N₂ and $\delta^{15}N_2$ in the suboxic zone?

3. Data and methods

3.1. Data

Data on nitrogen species utilized in this study consist of vertical profiles of the concentrations and δ^{15} N profiles of NO₃⁻, NH₄⁺, PON and N₂. New data have recently become available for the concentrations and isotopic signatures of NO_3^- , NH_4^+ , PON and N_2 (Fig. 2) (Murray et al., 2005, Fuchsman et al., 2008this issue). The distinguishing feature is a maximum in excess N2, located in the suboxic zone that probably originates from denitrification and anammox. We used either average values for specific depths, such as the boundaries of the suboxic zone (Table 1), or we used smoothed high resolution profiles that were fit with numerical simulations (Figs. 2 and 3b) and used in budget calculations. Average profiles of the concentrations of biogeochemical solutes and particulate organic matter were adopted from Konovalov et al. (2000, 2001, 2006). Some of those profiles are shown as symbols in Fig. 3b. The N2 was calculated from separate determinations of N2/Ar and Ar (Fuchsman et al., 2008-this issue).

The presence of ¹⁴N and ¹⁵N species provides additional information on nitrogen transformations because the rates of these processes are sensitive to the isotope mass. The ratio of ¹⁴N to ¹⁵N transformation rates is defined by a fractionation factor (α) that usually exceeds 1, as lighter isotopes undergo transformation at a higher rate. The isotope effect ($\varepsilon = (\alpha - 1) \times 1000$) usually varies from 0 to 35‰ and depends on the nitrogen species and processes (Sigman and Casciotti, 2001). Isotopic fractionation in nature results in anomalies in isotopic ratios for different N species. The anomalies of ¹⁵N, relative to the reference air, are expressed as

$$\delta^{15} N = \begin{pmatrix} \frac{(^{15}N/^{14}N)_{\text{sample}} - (^{15}N/^{14}N)_{\text{reference}}}{(^{15}N/^{14}N)_{\text{reference}}} \\ \times 1000. \tag{1}$$

The isotope effect of denitrification is usually in the range of 25 to 30% (Brandes et al., 1998). Anammox is expected to also result in isotope fractionation, but this has not yet been measured. Data on $\delta^{15}NO_3^-$ (Fuchsman et al., 2008-this issue) reveals an increase in $\delta^{15}NO_3^-$ in the suboxic zone (Fig. 2c) as ${}^{14}NO_3^-$ is preferably used to support production of light N2. Available data on $\delta^{15}N_2$ do suggest intensive nitrogen isotope fractionation in the suboxic zone that results in accumulation of light di-nitrogen (Fig. 2c), as compared to nitrogen of nitrate (Fuchsman et al., 2008-this issue), ammonium (Velinsky et al., 1991), and PON (Coban-Yildiz et al., 2006). Di-nitrogen produced and accumulated in the suboxic zone is more ${}^{15}N$ depleted than N_2 in water equilibrated with the atmosphere (0.68%) (Knox et al., 1992). The major discrepancy originates from the fact that complete consumption of nitrate and ammonium in the suboxic zone should result in $\delta^{15}N_2$ of +5 to +10‰. Another feature of nitrogen cycling in the Black Sea is that the values of N₂ and $\delta^{15}N_2$ were surprisingly variable from 2000 to 2005 (Fig. 2) suggesting that dinitrogen production and potentially nitrogen isotope fractionation should vary from year-to-year.

3.2. Model of physical exchange

We used a model of physical exchange which resolves processes of entrainment of the Black Sea waters to the Bosporus Plume in the upper layer (Z_* to Z_0) and processes of intrusion of the plume waters to the ambient Black Sea waters in the deeper layers (Z_0 to Z_b) (Fig. 3a) (Ivanov and Samodurov, 2001). The

Table 1

The calculated average $\delta^{15}N_2$ value in the suboxic zone based on the adopted $\delta^{15}N$ values of all other nitrogen species and their fluxes to/from this layer

Nitrogen species	δ^{15} N values, ‰	Flux, $\mu M \ m^{-2} \ day^{-1}$	Flux, %
Particulate organic nitrogen, PON	7.0	151.1	33.3
Particulate organic nitrogen (bacterial), PON(B)	3.0	-124.8	-27.4
Dissolved organic nitrogen (labile), DON(L)	7.0	21.7	4.8
Ammonium, (NH_4^+)	3.0	263.2	57.9
Nitrate, (NO_3^-)	9.0	18.2	4.0
Di-nitrogen, (N ₂)	5.4	-165.3	72.6



Fig. 3. The modeled scheme (a) of nitrate and N_2 entrainment in the upper part of the water column and intrusion into deeper layers due to the Bosporus Plume formation and ventilation in the Black Sea and (b) numerically simulated profiles of the concentrations of several major biogeochemical species. The average data (NO₃⁻, O₂, NH₄⁺, H₂S) and observational data (N₂) profiles are shown by symbols and the model results as lines.

assumption of uniform isopycnal distribution of all solutes, which is based on observations (Fig. 1c), has become a common paradigm for the distribution of biogeochemical solutes in this marine basin. This assumption simplifies a 3-D to a 1-D model. It is also assumed that the Mediterranean waters flowing into the Black Sea at depth Z_* (Fig. 3a) entrain ambient Black Sea water in the layer from Z_* to Z_0 forming the Bosporus Plume, which is a stratified mass of water sinking deeper in the water column. The plume entrains both physical and biogeochemical properties from the layer of Z_* to Z_0 . Entrainment is changed for intrusions below the depth Z_0 , which provide heat and salt to the permanent pycnocline and deep waters. A minor fraction of the initial volume of the plume sinks to the bottom layer below Z_b . The choice of Z_* , Z_0 , Z_b , and other characteristics of the model of physical exchange are discussed in detail and justified in Ivanov and Samodurov (2001).

By solving the system of equations for the long-term data (climatology) of the stratification of the Black Sea, Ivanov and Samodurov (2001) obtained vertical profiles of w (the rate of vertical advection) and k (the coefficient of vertical diffusivity) that support the observed average profile of salinity at steady state. These profiles of w and k enable calculation of the flux (Eq. 2) of biogeochem-

ical solutes in the water column (Konovalov et al., 2000, 2001).

$$Flux = -k\frac{\partial C}{\partial z} + wC,$$
(2)

where C is the concentration of a solute at depth z in the Black Sea water column.

For the known steady state distribution of a reactive solute, Eq. 3 allows calculation of the profile of its net production (*R*), if it exists, and the profile of its exchange between the plume and ambient waters $\left(\frac{\partial w}{\partial z}(C_{\rm b}-C)\right)$.

$$\left(\frac{\partial}{\partial z}\left(-k\frac{\partial C}{\partial z}\right)+w\frac{\partial C}{\partial z}\right)=R+\frac{\partial w}{\partial z}(C_{\rm b}-C),\qquad(3)$$

where, in addition to Eq. 2, $C_{\rm b}$ is the concentration of the solute in the Bosporus Plume water.

If the system is not at a steady state, then

$$\frac{\partial C}{\partial t} = R + \frac{\partial w}{\partial z} (C_{\rm b} - C) + \frac{\partial}{\partial z} \left(k \frac{\partial C}{\partial z} \right) - w \frac{\partial C}{\partial z} \tag{4}$$

and we can trace the evolution of the profiles of concentrations for known production/consumption functions (R) and boundary conditions or verify the ability of the model to numerically reproduce the

known evolution of distribution of considered solutes. This verification has been done for time series of 137 Cs data collected by K. O. Buesseler (personal communication, Woods Hole Oceanographic Institution) for the period from 1986 (the year of the accident at the Chernobyl nuclear power station) to 2003. Results of these numerical experiments have verified the ability of this physical model to precisely reproduce the observed evolution in the vertical distribution of 137 Cs (and thus other conservative properties) in the water column.

The last term $\left(\frac{\partial w}{\partial z}(C_b - C)\right)$ in Eq. 3 provides the ability to quantify cycling of solutes in the Black Sea due to both advection-diffusion and entrainment-intrusion, thus to restore steady state profiles of solutes for the known entrainment-intrusion values. Assuming that the solute injected to the anoxic zone must be compensated by its equivalent upward flux to keep the system at steady state, the profile of the flux provides the possibility to reconstruct the profile of its concentrations. Eq. 2 can be written in the form

$$F_n = w_n \cdot C_n - k_n \cdot \frac{C_{n+1} - C_n}{z_{n+1} - z_n},$$
(5)

where F_n is the flux of a solute from the *n*th layer, w_n and k_n are the rate of advection and the diffusion coefficient at the depth of the *n*th layer and C_{n+1} and C_n are the concentrations of the solutes at the deeper and shallower boundary of the *n*th layer.

Eq. 5 can be solved to reconstruct the vertical profile

$$C_{n+1} = C_n - \frac{z_{n+1} - z_n}{k_n} \cdot (F_n - w_n \cdot C_n)$$
(6)

for the known profiles of F, w and k and the concentration at the upper boundary of the reconstructed profile.

We calculated the profile of the vertical flux (F)assuming that it must compensate the input of nitrogen from the plume intrusions (Fig. 3a) maintaining the profile of di-nitrogen at a steady state. We also assumed that nitrate is reduced by sulfide to N2, rather than ammonium (Eqs. 15 and 16) (Kelly-Gerreyn et al., 2001; Hulth et al., 2005). This gives an upper estimate of N₂ contributed to the anoxic zone due to intrusion of nitrate entrained in the Bosporus Plume. We choose the concentration of 600 μ M for N₂ at σ_t =16.0 from the observational data (Fig. 3b) and produced two profiles. One profile (dashed line in Fig. 4) suggests that only entrainment/intrusion of nitrate and physical exchange maintain the profile of di-nitrogen, while another reconstructed profile (solid line in Fig. 4) suggests that di-nitrogen is also entrained in the plume, as are all other



Fig. 4. Observational data (dots) and the reconstructed vertical profiles of N_2 . The dashed line shows the predicted profile maintained by the processes of vertical exchange and entrainment/intrusion of nitrate. The solid line shows the predicted profile for entrainment/intrusion of NO_3^- and N_2 produced in the suboxic zone.

solutes, from the aerobic layer above Z_0 in Fig. 3a ($\sigma_t \sim 15.5$) and it is delivered to the deeper waters with the plume intrusions.

3.3. Models of biogeochemical transformations

A model of biogeochemical processes (Konovalov et al., 2006) was combined with the model of physical exchange (Ivanov and Samodurov, 2001) to allow numerical simulation of the distribution of various species (Eq. 4) for the known set of parameterized processes (R in Eqs. 3 and 4). Almost the entire oxicsuboxic-anoxic water column of the Black Sea (50 m below the sea surface (Z_*) to the bottom) was numerically simulated. All biogeochemical processes within the water column were stoichiometrically coupled. All profiles of biogeochemical species were numerically generated. Neither the structure nor the position of any specific layers were prescribed, but were generated by physical exchange and biogeochemical processes. The choice of both the average profiles and boundary conditions is discussed in detail in Konovalov et al. (2006).

The rates of biogeochemical processes were parameterized as, for example, either in the form of chemical rate laws

$$\frac{\partial [\mathrm{H}_2 \mathrm{S}]}{\partial t} = -k' \cdot [\mathrm{H}_2 \mathrm{S}] \cdot [\mathrm{O}_2], \tag{7}$$

where $[H_2S]$ and $[O_2]$ are concentrations of sulfide and oxygen and k' is the specific rate of this process for

oxidation of sulfide or biological type rate equations (e.g., Michaelis–Menten type)

$$\frac{\partial [\text{PON}]}{\partial t} = -\mu \cdot [\text{PON}] \cdot \frac{[\text{O}_2]}{[\text{O}_2]_{1/2} + [\text{O}_2]},\tag{8}$$

where [PON] and $[O_2]$ are concentrations of particulate organic nitrogen and oxygen, $[O_2]_{1/2}$ is the semisaturation constant, and μ is the maximum specific rate for oxidation of PON. These equations were discussed in detail in Konovalov et al. (2006).

PON is one of the most important parameters because it is the primary source of nitrogen for the water column. Unlike the other biogeochemical solutes, PON is the only biogeochemical parameter in the model that sinks in the water column. Though sinking and suspended PON has been discussed in several publications, including those on the Black Sea (Coban-Yildiz et al., 2000, 2006), transformations of sinking PON to its suspended form and back again have never been parameterized. Thus, we considered that all particulate PON is slow sinking and that this slow sinking PON determines the stock and vertical distribution of particulate organic nitrogen. The sinking velocity was derived from data on the concentrations and flux of PON in the Black Sea published by Karl and Knauer (1991). Those are the only known data for the flux and concentration measured at the same location and at the same time in the Black Sea. The ratio of flux to concentration gave an effective sinking velocity, assuming that the observed concentration was built up by sinking PON. Individual points were fit with a polynomial to create a vertical profile. This profile was used in the numerical model and in all calculations.

Initially, redox transformations and cycling of N₂ in the Black Sea water column were assumed to mainly consist of (i) nitrate and ammonium interaction in the suboxic zone to produce N₂, (ii) fluxes of N₂ from the suboxic zone, and (iii) intrusion of nitrate into the anoxic zone with the Bosporus Plume (Fig. 3a) which resulted in N₂ supersaturation due to an assumed reaction of nitrate with sulfide (Eq. 9). Observational data (Fig. 2) generally support these assumptions (Murray et al., 2005) because nitrate and ammonium are consumed and N₂ supersaturation reached its maximum at a similar density. Nitrate should be entrained to the Bosporus Plume and injected into the anoxic zone, as happens for oxygen (Konovalov et al., 2003). Nitrate could potentially be reduced by sulfide to N_2 that could support additional supersaturation in the anoxic zone (Eq. 9).

$$8NO_3^- + 5HS^- = 4N_2 + 5SO_4^{2-} + H_2O + 3OH^-$$
(9)

The major difference of the biogeochemical model utilized in this work from Konovalov et al. (2006) is that the processes of nitrite (NO₂⁻) production and consumption have been added to the set of biogeochemical parameterizations because nitrite, rather than nitrate, reacts with ammonium in the anammox process (e.g., Kuypers et al., 2003). In addition, we initially assumed that processes of nitrite production and consumption might be important to correctly simulate nitrogen isotopic fractionation. All ammonium and nitrate redox reactions have been split to parameterize NH₄⁺ \Leftrightarrow NO₂⁻ \Leftrightarrow NO₃⁻ \Leftrightarrow NO₂⁻ \Leftrightarrow 2N₂ transformations (Eqs. 10–18).

$$(CH_2O)_{106}(NH_4)_{16}(PO_4) + 212NO_3^-$$

= 106CO₂ + 106H₂O + (PO₄) + 16(NH₄)
+ 212NO_2^- (10)

$$\begin{array}{l} 3(\mathrm{CH}_{2}\mathrm{O})_{106}(\mathrm{NH}_{4})_{16}(\mathrm{PO}_{4}) + 424\mathrm{NO}_{2}^{-} + 424\mathrm{H}^{+} \\ = 318\mathrm{CO}_{2} + 3(\mathrm{PO}_{4}) + 48(\mathrm{NH}_{4}) + 212\mathrm{N}_{2} \\ + 530\mathrm{H}_{2}\mathrm{O} \end{array} \tag{11}$$

$$2NH_4^+ + 3O_2 = 2NO_2^- + 2H_2O + 4H^+$$
(12)

$$2NO_2^- + O_2 = 2NO_3^-$$
(13)

$$NO_2^- + NH_4^+ = N_2 + 2H_2O$$
(14)

$$4NO_3^- + H_2S = 4NO_2^- + SO_4^{2-} + 2H^+$$
(15)

$$4NO_{2}^{-} + 3H_{2}S + 4H_{2}O + 2H^{+}$$

= $4NH_{4}^{+} + 3SO_{4}^{2-}$ (16)

$$3NO_3^- + S^0 + H_2O = 3NO_2^- + SO_4^{2-} + 2H^+$$
(17)

$$2NO_2^- + S^0 = N_2 + SO_4^{2-}$$
(18)

We have also taken into account that nitrite reacts to elemental sulfur producing N_2 (Eq. 18), while nitrite reacts to sulfide producing ammonium (Eq. 16) (Kelly-Gerreyn et al., 2001; Oh et al., 2002; Hulth et al., 2005). This model resulted in steady state profiles for all compounds, including N_2 , and these are shown as solid lines in Fig. 3b for O_2 , H_2S , NH_4^+ , NO_3^- and N_2 .

Anammox was required to be much more important than denitrification in order to have the maximum of excess N₂ be at the correct depth and to have a NO₃ profile with the correct shape. Comparison of biogeochemical processes showed that 96% of N₂ was produced by anammox (Eq. 14), 3% by denitrification (Eq. 11), and 1% by thiodenitrification (Eq. 18). We can make denitrification more important by increasing the specific rate for denitrification and decreasing that for anammox. The problem is that the maximum rate of anammox is close to σ_t =16.0, which is where the maximum supersaturation of N_2 was observed. When we increased the rate of denitrification up to 10% this resulted in increased N_2 supersaturation in the upper part of the suboxic zone, greater depletion of NO₃ than observed and an upward shift of the NH₄⁺ profile. When the rate of denitrification was increased further the overall chemical structure of nitrogen species in the suboxic zone become unrealistic, compared with observations.

3.4. Models of ${}^{14}N/{}^{15}N$ fractionation

Our ability to correctly simulate the distribution of concentrations of biogeochemical species in the water column (Fig. 3b) led us to assume that we could numerically simulate the observed distributions of δ^{15} N values by introducing separate production/consumption functions (*R*) for ¹⁴N and ¹⁵N species. Reference data for the concentrations of all major nitrogen species and their δ^{15} N values were considered to be known (Fig. 2). Dashed lines, representing the data, are shown with the model results in Figs. 5, 6, 7 and 8.

Generally, biogeochemical transformations involving nitrogen, should lead to an enriched $\delta^{15}N$ value in the initial nitrogen specie and a depleted $\delta^{15}N$ value in the product, if the rate of the process is faster for ¹⁴N species, as compared to ¹⁵N species, and if the process results in partial consumption. Following Lehmann et al. (2003), isotopic fractionation was simulated by splitting all nitrogen reactions into parallel processes for ¹⁴N and ¹⁵N species. The rate of a ¹⁴N involved process is α times the rate of its ¹⁵N homologue. Thus, for example, for interaction of nitrate with sulfide (Eq. 15)

$$\frac{\partial \left[{}^{15}\mathrm{NO}_{2}^{-}\right]}{\partial t} = -\frac{\partial \left[{}^{15}\mathrm{NO}_{3}^{-}\right]}{\partial t} = k \left[{}^{15}\mathrm{NO}_{3}^{-}\right] \cdot \left[\mathrm{H}_{2}\mathrm{S}\right] \quad (19)$$

$$\frac{\partial \begin{bmatrix} {}^{14}\text{NO}_2^- \end{bmatrix}}{\partial t} = -\frac{\partial \begin{bmatrix} {}^{14}\text{NO}_3^- \end{bmatrix}}{\partial t}$$
$$= \alpha \cdot k \begin{bmatrix} {}^{14}\text{NO}_3^- \end{bmatrix} \cdot [\text{H}_2\text{S}]$$
(20)

This results in individual ¹⁴N and ¹⁵N profiles for all nitrogen species. The sum of these profiles gives the



Fig. 5. Results of experiment #1 (Table 2). Numerical simulations (solid lines) vs. reference data (dashed lines) of the vertical distribution and temporal evolution of δ^{15} N for N₂, PON, ammonium, and nitrate in the water column.



Fig. 6. Results of experiment #2 (Table 2). Numerical simulations (solid lines) vs. reference data (dashed lines) for the model that underestimates nitrogen isotope fractionation for the process of bacterial production of PON in the suboxic zone. This results in depleted $\delta^{15}N-N_2$ values in exchange for unrealistically enriched $\delta^{15}N-PON$ below the suboxic zone (the difference is shown by the two-headed arrow).

bulk profiles, and Eq. 1 makes it possible to calculate δ^{15} N values at every step of the numerical integration.

Initial profiles of all nitrogen species were split in individual profiles of ¹⁴N and ¹⁵N species following the total concentration profiles and the profiles of δ^{15} N values (Fig. 2c). We considered only one sinking form of PON, thus δ^{15} N–PON values by Coban-Yildiz et al. (2006) and Fuchsman et al. (2008-this issue) were applied to the bulk PON profile and only one type of PON was considered at the upper boundary of the modeled water column.

Information on fractionation factors (α) is limited to very few processes (Brandes et al., 1998; Sutka et al., 2004; Sigman and Casciotti, 2001), but these include nitrification, denitrification, ammonium and nitrate assimilation to organic matter, and N₂ fixation. The major difficulty is that even the known fractionation factors are usually for overall processes, rather than individual redox reactions. Such values have been shown to work successfully (Brandes et al., 1998; Sutka et al., 2004) to numerically simulate incomplete isotope fractionation. For example, nitrate becomes ¹⁵N enriched in the oxygen minimum of the Arabian Sea during partial denitrification (Brandes et al., 1998).

In order to vertically resolve features of nitrogen isotope fractionation in the oxic–suboxic–anoxic water column of the Black Sea, fractionation factors for all individual processes that affect the budget of ¹⁴N and ¹⁵N were identified. These values, when available, were taken from publications, otherwise they were adjusted to reproduce the observed δ^{15} N profiles of all nitrogen species (Table 2).

We found that isotope fractionation factors (α) depend on the type of nitrogen transformation, rather than on individual biogeochemical process. Fractionation factors (α) for nitrate reduction to nitrite or nitrite reduction to N₂, for example, are different but these fractionation factors usually agree within 0.005 from one specific reducer to



Fig. 7. Results of experiment #3 (Table 2). Numerical simulations (solid lines) vs. reference data (dashed lines) for the model that incorporates parameterizations for the hypothesized process of ammonium adsorption/desorption by MnO₂.

another. This difference seems reasonable because bacterially mediated redox processes are usually split into individual oxidation and reduction sub-processes. Thus the nitrogen redox transformations depend mostly on the flow of electrons, rather than to the nature of the supplementary reagent, which can be separated from the nitrogen species by the bacterial cell membrane or simply bound to another reactive center of the cell (Strous et al., 1999; Kuypers et al., 2003). The α values that gave the best fit (Experiment #1 in Table 2) were generally small for ammonium production during aerobic (#1) and anaerobic (#2) respiration of organic matter and for ammonium oxidation (0-2%) (#5), but they increased to 10% for nitrite reduction (#3) and reached 25-30% for nitrate reduction (#2, #7). We were unable to establish α values for ammonium oxidation to nitrite and for nitrite oxidation to nitrate because the concentrations of ammonium and nitrite under oxic conditions were small and $\delta^{15}NH_4^+$ and $\delta^{15}NO_2^-$ data were not available. Generally, the best fit α values were consistent with published data for specific fractionation factors (Brandes et al., 1998; Sutka et al., 2004; Sigman and Casciotti, 2001).

The initial numerical experiment (Experiment #1 in Table 2) demonstrated, that these fractionation factors (α) allowed successful simulation of the profiles of $\delta^{15}NO_3^-$ in the oxic and suboxic layers and $\delta^{15}NH_4^+$ below the onset of sulfide (Fig. 5c,d). The values of $\delta^{15}NO_3^-$ remained almost unchanged with depth above the suboxic zone and started to increase under suboxic conditions towards the onset of sulfide. The values of $\delta^{15}NH_4^+$ increased upward towards the onset of sulfide as lighter fractions of ammonium were consumed to produce ¹⁵N depleted bacterial PON.

The major discovery from these initial numerical experiments was that fluxes of NO_3^- and NH_4^+ into the suboxic zone cannot support production of ¹⁵N depleted N₂ at steady state (Fig. 5a). The profile of $\delta^{15}N_2$ changed over the simulated period of a decade (the



Fig. 8. Results of experiment #4 (Table 2). Model simulations (solid lines with symbols) of δ^{15} N for N₂, PON, NH⁺₄ and NO⁻₃ vs. references (dashed lines) for the situation where anammox is fueled by remineralization of NH⁺₄ from sinking ¹⁵N depleted PON (δ^{15} N–PON at the upper boundary is –2.0% presumably due to N-fixation). The initial state (solid lines with no symbols) was similar to Experiment 1 and the model was run for 20 years.

average residence time of the suboxic zone) to reflect the production of much heavier N₂ than observed. The initially generated $\delta^{15}N_2$ profile (at 90 days) was sensitive to changes in fractionation factors for anammox and denitrification, but the steady state result remained the same with production of ¹⁵N enriched, rather than depleted N₂ (Fig. 5a).

4. Discussion

4.1. Deep water N_2 supersaturation

The entire water column of the Black Sea below the core of the cold intermediate layer (CIL is centered at $\sigma_t \sim 14.5$) is supersaturated with N₂ (Fuchsman et al., 2008-this issue). The maximum of N₂ in the sub-oxic zone is expected because both denitrification and anammox are active there and produce N₂. The

distribution of N₂ above the suboxic zone ($\sigma_t < 15.7$, Fig. 2a) suggests no major biogeochemical sources or sinks because the profile is almost linear vs. σ_t . Upward transport of N₂ from its site of production in the suboxic zone is sufficient to explain the vertical distribution of N₂ above the suboxic zone. Our numerical experiments (Fig. 3b) confirm this conclusion.

Vertical transport processes are not sufficient to explain the supersaturation of N_2 in the deep anoxic layers. The upward advective flux of N_2 compensates the downward diffusive flux in the upper part of the anoxic zone (above ~500 m) making it impossible to achieve supersaturation in the deep anoxic layers by vertical transport alone.

Two other possible sources of N_2 to the deep anoxic layers are related to inputs from the Bosporus Plume (Fig. 3a). One is injection of nitrate which may be reduced to N_2 by reaction with reduced sulfur (reactions

Table 2				
Fractionation factors for individual	processes in numerical	experiments 1 to	4 and published α v	alues

#	Process	α values in Experiment 1 (δ^{15} N-PON=7‰)	α values in Experiment 2 $(\delta^{15}N-PON=7\%)$	α values in Experiment 3 (δ^{15} N-PON=7‰)	α values in Experiment 4 $(\delta^{15}N-PON=-2\%)$	Published α values
1	Oxidation of PON by O2	1.005	1.005	1.006	1.006	<1.005*
	$(CH_2O)_{106}(NH_4)_{16}(PO_4) + 106O_2 = 106CO_2 + 106H_2O + (PO_4) + 16(NH_4)$					
2	Oxidation of PON by NO_3^- (denitrification)	1.025	1.025	1.025	1.025	1.025 *
	5(CH ₂ O) ₁₀₆ (NH ₄) ₁₆ (PO ₄)+424NO ₃ ⁻ +					1.020 -
	$424H^{+}=530CO_{2}+5(PO_{4})+80(NH_{4})+212N_{2}+742H_{2}O$ (Experiment 1 and 2)					1.030 **
	Eq. 10 in Experiment 3 and 4					
3	Oxidation of PON by NO ₂ (denitrification) Eq. 11 in Experiment 3 and 4	_	-	1.010	1.010	
4	NH ₄ ⁺ and DON aerobic assimilation to PON	$1.004 \text{ for } \text{NH}_4^+$	$1.004 \text{ for } \text{NH}_4^+$	$1.004 \text{ for } \text{NH}_4^+$	$1.004 \text{ for } \text{NH}_4^+$	
	$53(CH_2O)_{376}(NH_4)_{16}(PO_4) + 2160(NH_4) + 135(PO_4) = 188(CH_2O)_{106}(NH_4)_{16}(PO_4)$	1.000 for DON	1.000 for DON	1.000 for DON	1.000 for DON	
5	Aerobic nitrification	1.001	1.001	1.000	1.000	<1.005 *
	$NH_4^++2O_2=NO_3^-+H_2O+2H^+$ (Experiment 1 and 2) Eq. 12 in Experiment 3 and 4					
6	Oxidation of nitrite by oxygen Eq. 13 in Experiment 3 and 4	_	-	1.000		<1.005 *
7	NO ₃ thio-denitrification by sulfide	1.030	1.030	1.030	1.030	
	$5H_2S + 8NO_3^- = 5SO_4^{2-} + 4N_2 + 4H_2O + 2H^+$ (Experiment 1)					
	$H_2S + NO_3^- + H_2O = SO_4^{2-} + NH_4^+$ (Experiment 2) Eq. 15 in Experiment 3 and 4					
8	NO_2^- thio-denitrification by sulfide Eq. 16 in Experiment 3 and 4	-	-	1.010	1.010	
9	NH ₄ ⁺ and DON anaerobic assimilation to PON	$1.004 \text{ for } \text{NH}_4^+$	$1.000 \text{ for } \text{NH}_4^+$	$1.005 \text{ for } \text{NH}_4^+$	$1.005 \text{ for } \text{NH}_4^+$	
	$53(CH_2O)_{376}(NH_4)_{16}(PO_4) + 2160(NH_4) + 135(PO_4) = 188(CH_2O)_{106}(NH_4)_{16}(PO_4)$					
10	Anammox	$1.000 \text{ for } \text{NH}_4^+$	1.005 for NH ₄ ⁺	$1.000 \text{ for } \text{NH}_4^+$	$1.000 \text{ for } \text{NH}_4^+ \ 1.030 \text{ for}$	
	$5NH_4^++3NO_3^-=4N_2+9H_2O+2H^+$ (Experiment 1 and 2) Eq. 14 in Experiment 3 and 4	1.030 for NO ₃	1.030 for NO ₃ ⁻	1.030 for NO ₂ ⁻	NO_2^-	
11	NH_4^+ adsorption on $MnO_2 NH_4^+$: $MnO_2=0.04$	-	-	0.970	_	

Notes: * Sigman and Casciotti (2001). ** Brandes et al. (1998).

9). Our model shows that nitrate and vertical exchange alone (dashed line in Fig. 4) cannot explain the observed level of nitrogen gas in the deep anoxic layers. The second possibility is entrainment of N_2 into the Bosporus Plume from the CIL and its injection into the anoxic zone. This process can easily support the observed N_2 profile (solid line in Fig. 4). Thus, "internal" recycling of N_2 , which includes entrainment from the oxic layer and intrusion in the deeper layers (Fig. 3a), is an important control on the distribution of N_2 in the water column of the Black Sea (Fig. 4). Our numerical experiments (solid lines in Fig. 3b) suggest that this ventilation model with "internal" cycling of N_2 can generate a vertical profile of nitrogen similar to that observed (individual empty triangles in Fig. 3b).

Interestingly, the reconstructed profile of N_2 (solid line in Fig. 4) suggests that the level of N₂ supersaturation in the deepest waters should be about 3 µM higher than observed. This difference may be related to temporal variations in the input of anthropogenic nutrients to the sea (Cociasu et al., 1996) and in the concentration of nitrate in the water column (Konovalov and Murray, 2001), which should ultimately result in temporal variations in the intensity of N₂ production in the suboxic zone. Less intensive production of N₂ in the suboxic zone, which may have occurred during times of lower nutrient input to the Black Sea, would result in a lower level of N₂ supersaturation in the suboxic and oxic layers. This would have resulted in a smaller flux of N₂ to the plume and in a lower level of N₂ supersaturation in the deep anoxic zone. This suggests that the presently observed profile of N₂ (individual solid dots in Fig. 4) may not be at steady state, and that the level of N₂ supersaturation in deep waters may increase with time. Such changes would occur on the time scale of millennia which is the residence time of the deepest layers of the Black Sea.

4.2. Nitrogen isotope fractionation and mass balance

Our steady state model (Experiment #1 in Table 2) can numerically reproduce the δ^{15} N profiles for nitrate and ammonium (Fig. 5c,d). However, it was impossible to simulate either the observed values of δ^{15} N₂ or even the shape of δ^{15} N₂ profile (Fig. 5a). Instead of producing a minimum in δ^{15} N₂ profile within the suboxic zone, the model produces a maximum that grows in magnitude over the 13 year duration studied. The simulated PON was also ¹⁵N enriched in the anoxic zone (Fig. 5b), compared with published data (Coban-Yildiz et al., 2006) and all attempts to simulate ¹⁵N values.

We first used a box model or mass balance approach for the suboxic layer. Assuming that the major nitrogen species are known and limited to nitrate, nitrite, ammonium, N₂, and particulate and dissolved organic nitrogen, it is possible to calculate the $\delta^{15}N_2$ value that would balance the budget of nitrogen isotopes for the suboxic zone. For this calculation we used Eq. 3, observed $\delta^{15}N$ values and concentrations for nitrate (Fuchsman et al., 2008-this issue), ammonium (Velinsky et al., 1991) and PON (Coban-Yildiz et al., 2006). We assumed that $\delta^{15}N$ for DON (dissolved organic nitrogen) and PON had equal values (Table 1). The value of $\delta^{15}NO_2^-$ is not needed for these calculations because nitrite is produced and consumed entirely within the suboxic layer. The value of $\delta^{15}N_2$ required to balance this steady state system is 5.4‰, which is much larger than observed (Fig. 2b). It is important to note that these mass balance calculations depend only on the fluxes and δ^{15} N signatures of nitrogen species into and out of the suboxic layer and do not depend on fractionation factors or parameterizations of biogeochemical processes. This calculated value of $\delta^{15}N_2$ also does not account for exchange of N2 between the suboxic zone and the shallower layers, which are in balance with the atmosphere. This would result in lower $\delta^{15}N_2$ values in the suboxic zone due to influx of $\delta^{15}N_2$ depleted nitrogen, as shown by the results of Experiment 1 (Fig. 5a). If the Black Sea were a closed system, there would be only two ways to produce δ^{15} N depleted N₂ at steady state. Either δ^{15} N depleted nitrogen species fuel N₂ production or reactions of δ^{15} N enriched nitrogen species produce both δ^{15} N depleted N₂ and some δ^{15} N enriched unmeasured residual nitrogen product that is removed from the suboxic zone.

We first investigated two hypothetical conditions to explore what would be required to simulate low values of $\delta^{15}N_2$. The first approach suggests redistribution of nitrogen isotopes between the nitrogen species by varying fractionation factors. If we increased the fractionation factor (α) for ammonium in anammox (#10 in Table 2) from 1.000 to 1.005, while α for ammonium in DON assimilation to PON (#9 in Table 2) was decreased from 1.004 to 1.000 (Experiment #2 in Table 2) we could simulate a steady state minimum in the $\delta^{15}N_2$ profile in the suboxic zone as low as 0.26‰ (Fig. 6a). This result is close to the observed 0.3‰ in 2001, but this experiment also resulted in an increase of $\delta^{15}N$ –PON in the anoxic zone to 11–13‰ (Fig. 6b) which is much greater than observed (~3‰).

Another way to balance the steady state nitrogen isotope budget would be to allow there to be a sink of ¹⁵N enriched nitrogen from the suboxic zone for which we have not accounted. For example, we could

hypothesize a process of ammonium adsorption on manganese dioxide in the deeper part of the suboxic zone. Adsorption/desorption is an important process influencing the distribution of ammonium in sediments (Berner, 1976; Boatman and Murray, 1982; Fitzsimons et al., 2006). In order to parameterize this hypothetical process, we assumed that ammonium adsorption on $MnO_2(s)$ (#11 in Table 2) would result in a molar ratio of NH_4^+ to Mn of 0.04 (Experiment #3 in Table 2). Our numerical experiments demonstrate that this process could work if ammonium is adsorbed and transported out of the suboxic zone with an isotopic fractionation factor of 0.97 (Fig. 7). However, while ammonium does adsorb on $MnO_2(s)$ the energetics are primarily electrostatic and such a fractionation factor is unlikely.

4.3. Input of ^{15}N depleted sinking PON produced by nitrogen fixation

We suggest that the most likely explanation for production of light N₂ is input of sinking PON with depleted values of δ^{15} N. This could occur if nitrogen fixation was an important source for the sinking PON.

There are indications that PON may provide a major source of N for anammox. Kirkpatrick et al. (2006) studied the diversity and vertical distribution of Planctomycetes and anammox bacteria in the suboxic zone. An interesting result was that the marine anammox bacterium "Candidatus Scalindua sorokinii" was found at many depths in the suboxic zone, not just where NO₃⁻ and NH₄⁺ decreased to zero ($\sigma_t > 15.90$). "Candidatus Scalindua sorokinii" dominated the clone libraries at $\sigma_t > 15.80$, a depth shallower than the upward flux of ammonia from deep water but which corresponded to the depth of the NO_2^- maximum. Kirkpatrick et al. (2006) suggested that anammox was occurring at this density level utilizing the remineralization of sinking organic matter as the main source of NH₄⁺. Fuchsman et al. (2008-this issue) concluded, using a nitrogen mass balance approach, that the only way to balance the nitrogen budget for the suboxic zone was to have sinking PON be an essential, but variable source of nitrogen.

¹⁵N depleted PON can be produced by two mechanisms: dissimilatory bacterial growth on sinking PON and nitrogen fixation. Lehmann et al. (2002) demonstrated the possibility of generating of ¹⁵N depleted PON due to anaerobic bacterial respiration of detritus. This process may affect PON in the lower suboxic and upper anoxic layers (Fig. 5b), but depleting ¹⁵N in PON from bacterial growth in the suboxic zone would result in ¹⁵N enriched NH₄⁺ that is respired to ¹⁵N enriched N₂. Nitrogen fixing organisms can support a source of ¹⁵N depleted PON to the suboxic zone in exchange for ¹⁵N enriched N₂ above the suboxic zone. Fuchsman et al. (2008-this issue) observed enriched values of $\delta^{15}N_2$ at depths above the CIL and low values of the N:P ratio and suggested this was evidence for N₂ fixation. McCarthy et al. (2007) recently reported direct measurements of N₂-fixation in both the oxic and suboxic layers of the Black Sea. We hypothesize that nitrogen fixation may be a source of $\delta^{15}N$ depleted sinking PON for the Black Sea suboxic zone.

We tested this hypothesis using mass balance calculations and a modeling approach. For the mass balance approach, we used the flux of sinking PON at the upper boundary of the modeled water column as 255 µmol $m^{-2} day^{-1}$, which was determined by Karl and Knauer (1991) using drifting sediment traps. If that sinking PON had a $\delta^{15}N$ of 0.0‰ the resulting steady state value for $\delta^{15}N_2$ would be 2.2‰. That value is not as low as we observed. We could reproduce the lowest values of $\delta^{15}N_2$ of -0.1% if the $\delta^{15}N$ of sinking PON was -5.0%. This value is unreasonably low. The limitation of the mass balance approach is that the calculations assume a single value for each of nitrogen species at the boundaries of the box, rather than a profile.

For the numerical experiments we used the profiles produced by Experiment #1 (Table 2) as initial conditions. The parameters for this run are shown as Experiment #4 in Table 2. We prescribe the δ^{15} N-PON at the upper boundary of the suboxic zone as -2.0%, close to the value of -2.6% for PON made by N₂-fixation recommended by Sachs and Repeta (1999). The simulations were run for 20 years and the model generated profiles are shown in Fig. 8. Sinking ¹⁵N depleted PON is respired in the oxic zone as it is produced from ¹⁵N depleted PON. NH₄⁺ becomes ¹⁵N depleted in the suboxic zone. Most importantly, the values of δ^{15} N₂ become depleted and after 20 years approximate the low values observed in 2000.

Thus, ¹⁵N depleted PON could support production of ¹⁵N depleted N₂ at steady state. We argue that this ¹⁵N depleted PON must be produced by nitrogen fixation in the Black Sea. At present this is only a hypothesis as there are few measurements of N₂-fixation rates, but observational data on the δ^{15} N of sinking PON in the Black Sea remain of great importance for future field work.

4.4. Steady state versus non-steady state models

The variability in both N₂/Ar and δ^{15} N₂ from 2000 to 2005 (individual symbols in Fig. 2) (Fuchsman et al.,

2008-this issue) imply that non-steady state models may be required. The range of inter-annual changes in N₂ profiles was small (Fig. 2a) but larger changes were seen for $\delta^{15}N_2$, which had a minimum close to 0‰ in 2000 then increased in the suboxic zone progressively up to 0.7‰ and up to 1.0‰ just above the suboxic zone in 2005 (Fig. 2b). During this time period the profiles of NO_3^- and NH_4^+ revealed only minor changes and were similar to the example in Fig. 1c. There was a statistically highly significant, inverse linear relationship between N2 and $\delta^{15}N_2$ in the suboxic zone (Fig. 9), with higher concentrations of N2 corresponding to lower values of $\delta^{15}N_2$. The linear trend suggests two end-members. One end member is ¹⁵N depleted with a $\delta^{15}N_2$ of at least ~0.0‰. The second end member is 15 N enriched with a $\delta^{15}N_2$ above atmospheric equilibrium ($\delta^{15}N_2=0.68\%$). We believe that the data in Fig. 9 reveal only part of the story. The linear trend should not be extended beyond the range of N₂ supersaturation, unless atmospheric N₂ is taken into account as the third end member. When N₂ supersaturation decreases to the level governed by atmospheric equilibrium, $\delta^{15}N_2$ should approach 0.68‰ (Knox et al., 1992), rather than $\sim 2.1\%$ which is the intercept of the linear trend with the $\delta^{15}N_2$ axis.

There could be variability of N2-fixation and export production of ¹⁵N depleted PON on both seasonal and inter-annual time scales. Unpublished data by Coban-Yildiz (2003) for the Honjo moored sediment trap BSK2 at 477 m in 1988-1989 showed that in the fall there were high fluxes of isotopically light PON followed in the winter/spring by low fluxes of heavier PON. Variability on longer time scales may be driven by North Atlantic Oscillation (NAO) type decadal climate patterns. For example, Oguz et al. (2006) described strong correlations of the NAO with many physical and biogeochemical properties of the Black Sea, including stratification, surface nutrient concentrations and biological biomass. Along these lines, Nezlin (2001) used remote sensing data to describe an unusually large phytoplankton bloom in the Black Sea during 1998-1999. If N₂-fixation made a significant contribution to the sinking organic matter at this time, this may explain the especially low $\delta^{15}N_2$ in observed in 2000 (Fig. 2).

To test this we envisioned a hypothetical scenario where production of ¹⁵N depleted N₂ was triggered by a pulse of PON produced by N₂-fixation in or before the year 2000, and was then shut down. This scenario might have occurred in response to changing conditions in the suboxic zone. For example, there might have been changes in the magnitude and $\delta^{15}N$ of the sinking PON flux and in the overall intensity of N₂ production.

Fig. 9. Excess N₂ (the observed concentration minus solubility) vs. δ^{15} N–N₂ for the period of 2000 to 2005 (individual symbols) and the linear fit to the data from the suboxic zone (sigma-t from 15.6 to 16.2) (solid line). The equation for the regression is Excess $N_2 = -9.64 * \delta^{15} N_2 +$ 18.54 ($r^2=0.53$). The probability that these two variables are uncorrelated is <0.05%.

We used numerical experiments to test whether physical processes alone could explain the observed interannual variations in N₂ supersaturation and δ^{15} N₂ after an initial perturbation making light $\delta^{15}N_2$. In this case we tested whether the physical processes of advection and diffusion act to smooth out the maximum in N_2 supersaturation and the $\delta^{15}N_2$ minimum in the suboxic zone. In these numerical experiments, all biogeochemical processes were turned off and changes were traced with the model over several years (Fig. 10).

The solid lines in Fig. 10 show that the changes in concentration of N2 due to physical transport are similar to the range to the observed changes. At the same time $\delta^{15}N_2$ would get progressively heavier. The observed changes, however, in $\delta^{15}N_2$ are far greater than can be explained by physical exchange alone (Fig. 10b). Thus, the observed changes in N2 supersaturation can be explained by inter-annual variations in the intensity of N₂ production and physical exchange but the $\delta^{15}N$ of produced N2 must also vary on an inter-annual time scale from depleted $\delta^{15}N_2$ values during times of intensive N₂ production to enriched $\delta^{15}N_2$ values at other times.

We did one final non-steady state experiment to test the response of the system to variability in δ^{15} N–PON at the upper boundary of the modeled water column while all other conditions and the rates of individual processes including N₂ production remained unchanged. In this experiment we allowed the system to be driven by light sinking PON (with $\delta^{15}N = -2.0\%$) for 180 days and then δ^{15} N–PON at the upper boundary was set back to 7.0‰. The resulting impact of fueling the system with light

10 5 1.2 0.0 0.4 0.8 δ15N2, %0





Fig. 10. Numerically simulated year-to-year changes in the distribution of N₂ (a) and δ^{15} N–N₂ (b) exclusively by the processes of physical exchange.

PON for only 180 days was very small. This suggests that in order to fit the observed inter-annual variations in N₂ supersaturation and $\delta^{15}N_2$ and to support fast turnover of N₂ in the suboxic zone (Fig. 9) we require that $\delta^{15}N$ – PON should become more depleted and the rate of its respiration to light ammonium and further to N₂ should increase.

The system may oscillate between two end-members that might depend on the relative importance of two sources of NH_4^+ . One is the upward transport of NH_4^+ from the deep water (with heavy $\delta^{15}N=+5$ to +7%). The second is NH₄⁺ remineralized from sinking PON produced by N₂-fixation (with depleted $\delta^{15}N = -2.0\%$). When N₂ becomes more ¹⁵N depleted, due to remineralization of ¹⁵N depleted PON, N₂ production and concentration also increases. New data are needed to test this hypothesis that δ^{15} N depleted sinking PON produced by N₂-fixation determines the variability in δ^{15} N depleted N₂ produced by anammox/denitrification in the suboxic zone. The most important requirements are seasonal and inter-annual data for N2-fixation rates and the δ^{15} N of PON sinking from the euphotic zone to the suboxic zone.

5. Conclusions

We applied a coupled physical-biogeochemical steady state model that includes simulation of the process of ventilation by the Bosporus Plume and biogeochemical transformations to simulate the concentrations of all the major nitrogen species in the oxic, suboxic and anoxic layers in the Black Sea. In order to correctly simulate the concentrations of N₂ and NO₃⁻ it is necessary for anammox to be much more important than denitrification for production of N₂.

 N_2 is supersaturated in the anoxic deep water and its profile is primarily maintained by physical recycling within the water column that includes entrainment of N_2 to the Bosporus Plume from the upper layer and its transport to the deeper layers with the plume intrusions. The flux of nitrate injected with the plume waters (and its reduction to N_2 by reduced sulfur) and the downward diffusive flux of N_2 from the suboxic zone to the anoxic layers are of minor importance to explain the supersaturation of N_2 in the deep water.

The profiles of N₂ and $\delta^{15}N_2$ have distinctive interannual variations, which were unexpected because the NO₃ and NH₄⁺ concentration profiles vary little from year-to-year. Within the suboxic zone there is a statistically highly significant inverse correlation between excess N₂ (N₂ greater than solubility) vs. $\delta^{15}N_2$. When excess N₂ is high, $\delta^{15}N_2$ is depleted (as low as 0.0‰).

The model that includes an ammox driven by the downward flux of NO₃⁻ and the upward flux of NH₄⁺ can correctly simulate the distributions of $\delta^{15}N-NO_3^-$ and $\delta^{15}N-NO_3^-$ and $\delta^{15}N-NH_4^+$ at a steady state, but the simulated and mass balance derived values of $\delta^{15}N_2$ substantially exceed the observed values. We can explain the low values observed for $\delta^{15}N_2$ with a hypothesis that includes ^{15}N depleted sinking PON produced by N₂-fixation. In this hypothesis ^{15}N depleted sinking PON is remineralized in the suboxic zone producing ^{15}N depleted NH₄⁺ that is rapidly consumed by an ammox.

The inverse linear relationship between excess N_2 and $\delta^{15}N_2$ suggests that the system oscillates between two states that occur because of different sources of NH_4^+ . In one anammox is driven by the upward flux of NH_4^+ from the deep water. This NH_4^+ has $\delta^{15}N\approx 5\%$ thus the N_2 produced is ^{15}N enriched. The second state is when the NH_4^+ is intensively produced by

remineralization of ¹⁵N depleted sinking PON due to N₂-fixation. This PON has $\delta^{15}N \approx -2.0\%$, thus N₂ produced at these times is ¹⁵N depleted.

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