Concentration and natural stable isotope profiles of nitrogen species in the Black Sea

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A B S T R A C T
We measured natural abundance profiles of N₂, nitrate, ammonium, suspended particulate organic nitrogen (PON) and total organic nitrogen (TON) concentrations and nitrogen stable isotopes in the Black Sea during five research cruises: R/V Bilim September 1999, R/V Bilim September 2000, Voyage 162, Leg 17 of the R/V Knorr 2001, Voyage 172, Leg 7 of R/V Knorr 2003 and Cruise 403 of R/V Endeavor 2005. Excess N₂ of biogeochemical origin had maximum values that were centered at the lower part of the suboxic zone. The δ¹⁵N of this excess N₂ was highly depleted in 2000 and 2001 (−30‰ to −40‰) and was much less depleted in 1999, 2003 and 2005 (−1‰). The variabilities of N₂/Ar ratios, excess N₂ and δ¹⁵N of excess N₂ cannot be explained by variability in the profiles of nitrate and ammonium. Using a nitrogen mass balance of NO₃⁻,NH₄⁺ and N₂ we determined that a missing N flux was required to complete the nitrogen mass balance in some years, especially 2000 and 2001. We suggest that this missing flux is most likely from remineralization of sinking PON. The δ¹⁵N of the missing nitrogen flux was also highly depleted in 2001 and 2000. The concentration profiles of nitrate, nitrite and ammonium approached zero, indicating total consumption to form nitrogen gas. However, in 2000 and 2001 the δ¹⁵N of excess N₂ increased with increasingly excess N₂, which is consistent with partial consumption.

We propose that there are different states for the nitrogen system in the Black Sea and the system varies between them due to climate oscillations and variations in the major nitrogen budget components. When the system is at a steady state, total consumption of nitrate and ammonium occurs in the suboxic zone and partial denitrification is of minor importance. This results in higher values of δ¹⁵N N₂ and lower values of excess N₂ in the suboxic zone. The balance of nitrate and ammonium fluxes into the suboxic zone with N₂ gas fluxes out of the suboxic zone in 1999, 2003 and 2005 imply that the system allowed total consumption at steady state in those years. Remineralization of depleted organic matter, presumably from nitrogen fixation in the euphotic zone, is necessary to support N₂ production of ~1% during complete consumption of nitrate, nitrite and ammonium. Near surface (oxic region) values of δ¹⁵N N₂ in 2000 and 2001 (0.9‰) are more enriched than for N₂ in equilibrium with the atmosphere (0.68‰). These enriched values can be explained by the consumption of N₂ during nitrogen fixation with a fractionation effect between −1.3 to −2.6‰. When the system is in a transitory state, like that observed in 2000 and 2001, sinking organic matter from a phytoplankton bloom may stimulate both partial denitrification and the anammox reaction which can produce depleted N₂ and support higher values of excess N₂ in the suboxic zone.

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1. Introduction

Fixed inorganic nitrogen, consisting of nitrate, nitrite and ammonium, is converted to nitrogen gas in the suboxic zones of marine sediments and oxygen minimum zones in the ocean. This transformation is the major sink of nitrogen from the ocean (Christensen et al., 1987; Brandes and Devol, 2002). At least two pathways are known for this conversion: heterotrophic denitrification where nitrate is the electron acceptor for oxidation of organic matter (2NO₃⁻ + organic carbon → N₂), and anammox which is the anaerobic oxidation of ammonium by nitrite (NH₄⁺ + NO₂⁻ → N₂ + 2H₂O) (Jetten et al., 1999). Both pathways require very low oxygen environments. Denitrifying bacteria use nitrate as a terminal electron acceptor for organic matter oxidation when oxygen concentrations decrease below threshold values (Devol, 1978). Canonically, denitrification proceeds via the series of bacterially mediated reductions described by:

\[ \text{NO}_3^- \rightarrow \text{NO}_2^- \rightarrow \text{NO} \rightarrow \text{N}_2 \rightarrow N_2 \]  

Both denitrification and anammox have been shown to be present in the Black Sea (Ward and Kilpatrick, 1991; Kuyper et al., 2003; Westley et al., 2006; Kirkpatrick et al., 2006; Oakley et al., 2007), but the temporal variability of their relative importance has not been previously studied.

The main source of nitrogen to the open ocean is thought to be biological nitrogen fixation (e.g. Brandes and Devol, 2002; Deutsch et al., 2001) the enzyme catalyzed reduction of nitrogen gas (N₂) into biomass. Recent work suggests that nitrogen fixation and denitrification are closely linked because water upwelled from ocean areas of denitrification are deficient in fixed nitrogen, which creates conditions that favor nitrogen fixation (Deutsch et al., 2007). Historically it was thought that rivers were the predominate source of nitrogen to the Black Sea (Cociasu et al., 1996), but nitrogen fixation was recently observed in the Black Sea by McCarthy et al. (2007). Calculations with McCarthy et al’s new data suggest that the rate of input from nitrogen fixation can be of the same order of magnitude as river input depending on the depth and temporal scales of nitrogen fixation.

In this paper, we present new data from the Black Sea for natural abundance profiles of dissolved nitrogen gas, nitrate, nitrite, ammonium, suspended particulate organic nitrogen (S-PON) and dissolved organic nitrogen (DON) concentrations and stable nitrogen isotopes to provide insight into processes involving nitrogen. These data give us an opportunity for analyzing the relationship between nitrogen sinks (anammox/denitrification) and sources (N₂-fixation) in this well studied, enclosed basin.

The issues that we will address are:

1) What are the vertical and temporal variations in nitrogen species and their nitrogen isotopic signatures?
2) How do the processes of denitrification, anammox and N₂-fixation control these distributions and their variability?
3) What role does the flux of organic matter play in determining the variability?

1.1. Study site

The Black Sea has become 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). This semi-enclosed marginal sea has a physical and chemical structure that is determined by its hydrological balance (Caspers, 1957; Sorokin, 1983). The water column is strongly stratified with respect to density, which is controlled by the salinity, except in the upper layer of the seasonal thermocline.

The surface layer is well oxygenated, while the deep layer is anoxic and sulfidic. At the boundary between the oxic surface and anoxic deep layers there is a suboxic zone (~50 m thick) where oxygen is <10 μM and hydrogen sulfide is <10 nM (Murray et al., 1995). Above the suboxic zone there is a layer of cold water called the Cold Intermediate Layer (CIL) with a characteristic core density of ρ₀ = 14.5. The temperature and ventilation frequency of the CIL depends on winter weather (Tolmazin, 1985) because the CIL is replenished in the central gyres during occasional periods of intense vertical mixing in the winter (Gregg and Yakushev, 2005) and horizontally by advection from the Northwest Shelf (Tolmazin, 1985). In most of the Black Sea, the CIL represents the lower boundary of direct communication with the surface. Water from the bottom layer outflow of the Bosporus Strait mixes with the overlying CIL forming the Bosporus Plume (Murray et al., 1991; Buessler et al., 1991; Ivanov and Samodurov, 2001). This plume enters the Black Sea as thin intrusions into theoxic, suboxic and anoxic layers below the CIL (Oguz and Rozman, 1991; Konovalov et al., 2003). The depth of this ventilation varies depending on the conditions of mixing in the Bosporus and on the continental shelf in the SW Black Sea (Özsoy et al., 1993). These intrusions inject oxygen, nitrate and other oxidized species into the anoxic layers, where they are reduced. The injection of oxygen is thought to be responsible for oxidation of over half the basin-wide sink of sulfide (Konovalov and Murray, 2001). Therefore, the Bosporus Plume is thought to be important for preserving the origin of the suboxic zone and the separation of the oxic and sulfidic layers (Konovalov et al., 2000; Konovalov and Murray, 2001). In general, anomalies in biogeochemical distributions due to ventilation by the Bosporus Plume are only observed in the SW region near the Bosporus Strait (Konovalov et al., 2003).

In the Black Sea, distributions vary spatially with depth, but less so with density. Characteristic inflections in the water-column profiles of many diverse elements such as nitrate, manganese, cesium isotopes, and mesoplankton are associated with specific density values regardless of when and where they were sampled (Vinogradov and Nalbandov, 1990; Codispoti et al., 1991; Buessler et al., 1991; Lewis and Landing, 1991; Murray et al., 1995). Use of the density scale facilitates comparison of specific features from different locations, times and investigators. Therefore, results presented here will be plotted against density (ρ₀) rather than depth (m).

The Black Sea is an ideal place to study the relationships between the nitrogen transformations. Redox reactions are well resolved in the suboxic zone (Brewer and Murray, 1973; Vinogradov and Nalbandov, 1990; Codispoti et al., 1991). The vertical scale of oxidation–reduction gradient is on the order of meters to tens of meters (Murray et al., 1999; Murray and Yakushev, 2006), allowing researchers to easily sample the sequence of redox reactions. Nitrate, nitrite, and ammonium...
Fig. 1. A map of the Black Sea with stations at which nitrogen species were sampled over the 1999 to 2005 period. The station coordinates and names are given in Table 1. The depth of the Black Sea is color coded where darker blue is deeper. This map of the Black Sea was created by the Remote Sensing Department, MHI.
concentrations all decrease to zero in the lower part of the suboxic zone suggesting that anammox is a major process (Murray et al., 1995; Kuypers et al., 2003; Jensen et al., 2008). Nitrate reduction rates were measured by Ward and Kilpatrick (1991). Jensen et al. (2008) measured no denitrification in the Black Sea, but denitrification has been measured previously (Westley et al., 2006), so the relative importance of denitrification and anammox in the Black Sea suboxic zone is still under debate. Little is known about N$_2$-fixation in the Black Sea (Sorokin, 2002), but recent rate measurements by McCarthy et al. (2007) suggest that it occurs in both the euphotic zone and the suboxic zone.

2. Methods

2.1. Sample collection

Samples for nitrogen species concentrations and isotopes were collected in the Black Sea in September 1999 and 2000 on R/V Bilim, in May–June, 2001 on Voyage 162, Leg 17 of the R/V Knorr; in April 2003 on Voyage 172, Leg 7 of the R/V Knorr and in late March 2005 on Cruise 405 of R/V Endeavor. All stations sampled in this study are shown in Fig. 1. The station coordinates are given in Table 1. Water column samples were collected using CTD-Rosettes with 10 L Niskin bottles and Sea Bird sensors. For the Knorr and Endeavor cruises, charts of cruise tracks, station locations, participants, hydrographic (T, S, density), oxygen/sulfide and nutrient data are given on the websites:

www.ocean.washington.edu/cruises/Knorr2001
www.ocean.washington.edu/cruises/Knorr2003
www.ocean.washington.edu/cruises/Endeavor2005

N$_2$/Ar ratios, $\delta^{15}$N–N$_2$, and concentrations of NO$_3$, NO$_2$, NH$_4$, H$_2$S and sulfide were also determined on samples from the R/V Bilim 1999 cruise in the southwest Black Sea (M30M41) and the R/V Bilim 2000 cruise in the Bosphorus Strait, and in the southwest Black Sea (M10L41 and LS0L20). During the 2001 Knorr cruise, samples were obtained from the Bosphorus (St. 1), near the Bosphorus Plume (St. 14) and the central Western Gyre (St. 2). During the 2003 Knorr cruise, samples were obtained from the Sea of Marmara (St. 21), a station near the Bosphorus Plume (St. 20) and the central Western Gyre (St. 12). During the 2005 Endeavor cruise samples were obtained from the central Western Gyre (St. 2). N$_2$/Ar ratios, $\delta^{15}$N–N$_2$, $\delta^{15}$N–NO$_3$, $\delta^{15}$N–NH$_4$, and TON, NO$_3$, NH$_4$, and NO$_2$ concentrations were all obtained from the same Niskin bottles.

2.2. Oxygen and sulfide concentrations

Oxygen and sulfide samples from Bilim 2000 were analyzed by S. Tugrul, Institute of Marine Science at the Middle Eastern Technical University, Turkey. Winkler titrations were used for O$_2$, and an iodometric method for sulfide. Oxygen and sulfide samples were measured during Bilim 1999, Knorr 2001, 2003 and Endeavor 2005 by S. K. Konovalov and A. Romanov (Marine Hydrophysical Institute, Ukraine) using both wet chemical (Cline, 1969) and polarographic (Luther et al., 2001) techniques. Oxygen and sulfide were analyzed on samples from both the CTD-Rosette and a pump profiling system (Codispoti et al., 1991).

2.3. Nutrient concentrations

During Bilim 1999; 2000 and Knorr 2001, nutrients were analyzed on shipboard by S. Tugrul. In 2001, additional samples were acidified to pH 2 and were reanalyzed for nitrate at the lab of D. M. Sigman (Princeton University), using an oxygen-chemiluminescent NO detector on-line with a reducing zinc vandate solution (Garside, 1982) that allows nitrate to be detected in the presence of sulfide. For Knorr 2003 and Endeavor 2005, nutrients were analyzed using a two channel Technicon Autoanalyzer II system. Deep water samples were diluted with nitrate free Black Sea surface water to reduce sulfide content. In all cases, nitrate was reduced to nitrite using a cadmium column. Nitrite was measured using sulphanilamide and N-(1-naphthyl)-ethylenediamine (Armstrong et al., 1967). Ammonium was analyzed using the indophenol blue procedure (Slayker and MacIsaac, 1972).

2.4. N$_2$/Ar ratios, Ar concentrations and $\delta^{15}$N–N$_2$

Water samples for N$_2$/Ar ratios and $\delta^{15}$N–N$_2$ were collected in evacuated 250 mL glass flasks. In the half-full flasks, the water was equilibrated with the headspace overnight and then removed. Gas samples were cryogenically processed and measured at the Stable Isotope Lab (SIL), School of Oceanography, University of Washington on a Finnegan MAT 251 or a Finnegan Delta XL isotope ratio mass spectrometer as described by Quay et al. (1993) and Emerson et al. (1999). Precision for standards was ±0.010 for 15N–N$_2$, and ±0.003 for N$_2$/Ar Sample/Saturation values. In samples from 1999, 2000, and 2001 a standard containing low concentrations of oxygen was used for samples from the suboxic and anoxic zones while in 2003 and 2005, a standard containing zero oxygen was used. To determine the isotopic composition of a zero oxygen standard by comparison with the universal standard, air, the affect of oxygen on the ionization efficiency of the mass spec must be well known throughout the entire range of oxygen concentrations. Samples for N$_2$/Ar ratios and $\delta^{15}$N–N$_2$ from 2003 and 2005 were also corrected for methane. The affect of methane was measured by the mass spec in each environmental sample. In the deep water of the Black Sea, where methane concentrations are highest, methane corrections reduced $\delta^{15}$N–N$_2$ by 0.25%, and N$_2$/Ar measured/expected ratios decreased by 0.007. In the water column, methane began to affect measurements below $c_m=16.7$ for $\delta^{15}$N–N$_2$, where observed values were decreased by 0.1%. and below $c_m=16.0$ for N$_2$/Ar ratios where decreased by 0.1. As methane in the Black Sea is thought to be at steady state with concentrations that have changed little with time (Kessler et al., 2006), correction factors for methane were applied to the 1999, 2000, and 2001 gas data.

For some calculations, N$_2$/Ar ratios were converted to µmole of N$_2$ using values of dissolved argon at atmospheric saturation. Argon saturation was calculated as done by Hammre and Emerson (2004). In 2005, argon concentrations were measured directly by adding a known concentration of $^{36}$Ar spike to each environmental sample, and measuring the $^{36}$Ar/$^{40}$Ar Sample/Saturation values. The $^{36}$Ar/$^{40}$Ar ratio was used to calculate N$_2$ for other years (see discussion).

2.5. $\delta^{15}$N–NO$_3$–

Samples for $\delta^{15}$N–NO$_3$ were acidified to pH 2 upon collection and were adjusted to pH 6–8 prior to analyses. Nitrate was reduced to nitrous oxide by Pseudomonas chlororaphis and nitrous oxide was extracted using a fully automated extraction system (Sigan et al., 2001). The 2001 samples were analyzed in the lab of D. M. Sigman (Princeton University). Selected samples were run in triplicate. Results were adjusted using an IAEA-N3 standard in Gulf Stream surface water. The 2003 samples were analyzed in the lab of E. Steig (University of Washington, Quaternary Research Center) on a DeltaPlus mass spectrometer with a Finnegan Precon system and Gasbench. Results were adjusted using an IAEA-N3 standard in Black Sea nitrate free surface water. Both media blanks, containing only bacteria and media, and blanks of Black Sea surface water were also analyzed. All samples were analyzed in triplicate. Any stray points were eliminated using a Q-test. Error calculation

Table 1

<table>
<thead>
<tr>
<th>Cruise</th>
<th>Station</th>
<th>Latitude</th>
<th>Longitude</th>
</tr>
</thead>
<tbody>
<tr>
<td>R/V Bilim 1999</td>
<td>M30-M41</td>
<td>41° 31′</td>
<td>30° 59′</td>
</tr>
<tr>
<td>R/V Bilim 2000</td>
<td>M10-L41</td>
<td>42° 10′</td>
<td>29° 41′</td>
</tr>
<tr>
<td>R/V Bilim 2000</td>
<td>LS0-L20</td>
<td>41° 50′</td>
<td>29° 20′</td>
</tr>
<tr>
<td>R/V Bilim 2000</td>
<td>L34-L19</td>
<td>41° 34′</td>
<td>29° 19′</td>
</tr>
<tr>
<td>R/V Bilim 2000</td>
<td>L27-L18</td>
<td>41° 27′</td>
<td>29° 18′</td>
</tr>
<tr>
<td>R/V Bilim 2000</td>
<td>Bosphorus</td>
<td>41° 02.60′</td>
<td>29° 02.60′</td>
</tr>
<tr>
<td>Knorr 162-17 2001</td>
<td>1</td>
<td>41° 12.80′</td>
<td>29° 07.40′</td>
</tr>
<tr>
<td>Knorr 162-17 2001</td>
<td>2</td>
<td>42° 30.00′</td>
<td>30° 46.00′</td>
</tr>
<tr>
<td>Knorr 162-17 2001</td>
<td>4</td>
<td>41° 27.50′</td>
<td>30° 15.75′</td>
</tr>
<tr>
<td>Knorr 172-07 2003</td>
<td>12</td>
<td>42° 30.00′</td>
<td>31° 00.00′</td>
</tr>
<tr>
<td>Knorr 172-07 2003</td>
<td>20</td>
<td>41° 26.11′</td>
<td>29° 34.77′</td>
</tr>
<tr>
<td>Knorr 172-07 2003</td>
<td>21</td>
<td>40° 51.83′</td>
<td>28° 55.33′</td>
</tr>
<tr>
<td>Endeavor 403 2005</td>
<td>2</td>
<td>42°30.38′</td>
<td>30°45.10′</td>
</tr>
</tbody>
</table>
includes error of the triplicates as well as of the quadruplicate standards
analyzed that same day. The precision of the technique, as determined by
triplicate samples, was 0.2%. Nitrite was not removed from the samples but
was ≤0.06 µM at the Central Gyre stations during both 2001 and 2003. In the
2001 dataset, nitrite contributed ≤3% to NO3 and the error is likely within
the error of the measurements. At αNO2 = 15.75 in the 2003 dataset, nitrite con-
tributed 7% to NO3. At other depths in 2003, the effect of nitrite is likely
within the error of the measurements. Elevated nitrite concentrations, at
0.5 µM, in the intrusion of the Bosporus Plume (αNO2 = 16.1–16.4) at St.14 in 2001
cause measurements there to be considered δ15N-NO3.

2.6. δ15N-NH4+

Samples for δ15N-NH4+ were acidified to pH 2 after collection. δ15N-NH4+
determined using distillation to a basic solution followed by adsorption
onto zeolite (Velinsky et al., 1989). The zeolite was removed by filtration and
combusted in a NC2500 CE Instruments Elemental Analyzer. Isotopic ratios
were measured on a DeltaPlus Finnigan connected to the Elemental Analyzer
by a Finnigan MAT ConFlo II in the SIL. Standards made of NH4Cl in Millipure
water were analyzed with the same procedure with a precision of 0.4‰.
Standards were prepared in both pure water and in 22 g/L NaCl. The NH4Cl(5‰)
standard was also directly combusted to determine its true isotopic
composition. The solid and liquid standards compared well with values of
1.60% and 1.68%, respectively, showing that there was no fractionation
during the method. NH4+ concentrations were measured on the liquid re-
maining after distillation, the distillate, and liquid remaining after filtration.
These samples were stored at 4°C and analyzed for ammonium concentra-
tion on the Technicon Autoanalyzer II system.

2.7. Suspended particulate organic matter (S-POM)

At St. 2 in 2001, samples for the concentration and isotopic composition of
suspended particulate organic matter (S-POM) were subsampled from
large volume submersible McLane pump Quartz microfi
ders (nominal
pore size 0.7 µm) and frozen. Concentrations were measured by K. Krogsland
(University of Washington) on a Leaman Labs CHN elemental analyzer.
Isotopes were measured in the SIL with a DeltaPlus Finnigan connected to a
NC2500 CE Instruments Elemental Analyzer by a Finnigan MAT ConFlo II.
Samples for S-POM were also collected in 2005 but sample sizes were too
small, thus the isotopic values were suspect and are not reported here. All
samples were blank corrected.

2.8. Total organic nitrogen

Unfiltered water samples for total organic nitrogen (TON) were collected
in 2001, 2003 and 2005 and acidified to pH 2 to prevent microbial decom-
position. Samples from 2000 were preserved with HgCl2. After adjusting to
pH 7, the concentration of TON was measured by oxidation to nitrate with
peroxodisulphate (Valderrama, 1981). The peroxodisulphate (Merck) was
puri
d by recrystallization (Nydahl, 1978). Samples were autoclaved in glass
ampoules for 1 h, then NO3 was measured using the Technicon Autoanalyzer
II system as described above. The natural concentrations of NO3 and
ammonium were subtracted to calculate TON. The Hg2+ in samples from
2000 affected the efficiency of the cadmium column. A standard was
measured every five samples, and the column was replaced when needed.
The efficiency of oxidation was tested using urea, ammonium, and EDTA and
the recoveries varied between 95% and 100%. One Black Sea sample was
repeated in each run as an internal standard. The peroxodisulphate blank was
generally between 0 and 0.4 µM.

3. Results

3.1. Oxygen and sulfide

Representative profiles for oxygen and sulfide in the middle of the western
gyre are shown in Fig. 2A. Oxygen concentrations in the surface layer (0–30 m)
varied from year to year with sea surface temperature and then decreased to
near zero in the suboxic zone. Sulfide concentrations increased below the
suboxic zone to levels above 400 µM near the bottom (≤2000 m). The density
boundaries of the suboxic zone (≤10 µM O2 and first appearance of detectable
sulfide) for stations sampled in this study varied only slightly between years and
stations (Table 2) and are comparable with previously reported data. Oxygen
and sulfide data did not clearly demarcate a suboxic zone for station L50L20, a
station located in the southwestern shelf region, but northeast of the Bosporus
Plume, occupied during the R/V Bilim 2000 cruise. There was also no typical
suboxic zone at station 14, a station in the SW region influenced by the Bosporus
Plume in 2001, nevertheless, oxygen and sulfide never overlapped. Continuous

Table 2

<table>
<thead>
<tr>
<th>Central St.</th>
<th>M3OM41 1999</th>
<th>M10L41 2000</th>
<th>St 2 2001</th>
<th>St 12 2003</th>
<th>St 2 2005</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;10 µM O2</td>
<td>15.7</td>
<td>15.35</td>
<td>15.38</td>
<td>15.5</td>
<td>15.65</td>
</tr>
<tr>
<td>H2S detected</td>
<td>16.29</td>
<td>16.05</td>
<td>16.1</td>
<td>16.13</td>
<td>16.1</td>
</tr>
<tr>
<td>Mixing site St.</td>
<td>St 14 2001</td>
<td>St 20 2003</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;10 µM O2</td>
<td>NA</td>
<td>15.5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>H2S detected</td>
<td>16.3</td>
<td>16.18</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The edges of the suboxic zone are determined by <10 µM O2 and 10 nM H2S
when polarographic techniques were used, and >2 µM H2S for volumetric
techniques.
pump profile oxygen and sulfide data indicate intrusions from the Bosporus Plume at this station (Konovalov et al., 2003). We refer to these as the Mixing Stations in Discussion as their complicated profiles reflect intrusions from the Bosporus.

3.2. Nutrients

Representative examples of NO$_3$, NO$_2$ and NH$_4$ profiles in the Black Sea are shown in Fig. 2B. The boundaries of the suboxic zone are shown for reference. Nitrate concentrations were below detection in the surface waters, increased to a maximum at the top of the suboxic zone and then decreased to zero within the suboxic zone. Nitrite profiles were variable between stations, but often had two maxima, one corresponding to the nitrate increase and one to the nitrite decrease. The deep maximum was usually slightly above the density where nitrate decreased to zero. Maximum NO$_2$ to the nitrate decrease. The deep maximum was usually slightly above the density where nitrate decreased to zero. Maximum NO$_2$ concentrations were ≤0.3 μM. Ammonium concentrations began to increase in the deeper part of the suboxic zone and continued increasing to levels reaching 100 μM near the bottom. Densities and magnitudes of nitrite and nitrate maxima for each station are shown in Table 3.

3.3. N$_2$/Ar sample/saturation ratios

A unique aspect of this research was the precise measurement of N$_2$ concentrations in the Black Sea. N$_2$ was measured as the N$_2$/Ar ratio and reported as the N$_2$/Ar ratio in the sample relative to atmospheric equilibrium. Doing so eliminates temporal variations due to changes in atmospheric equilibration. N$_2$/Ar sample/saturation ratios in surface water (c$_n$ = 12 to 13) were close to 1.00 corresponding to atmospheric equilibrium (Fig. 3A). In 2003, the surface water had a density (c$_n$ = 14.4), close to that of the CIL, and the gas ratios suggest that the CIL had been recently equilibrated with the atmosphere. Temperature data from Leg 6 of Knorr Voyage 172 in March 2003 indicated that ventilation of the CIL had occurred just before sampling due to intense storms (Gregg and Yakushev, 2005).

N$_2$/Ar sample/saturation ratios had a broad maximum in the suboxic zone centered at about c$_n$ = 16.0 (Fig. 3A). The magnitude of this maximum varied temporally and decreased from 1.025 in 1999 and 1.03 in 2000 to 1.02 in 2001 and 1.015 in 2003 and 2005. The ratios in all cases decreased with depth below the suboxic zone.

Ar was measured on samples from 2005 and the results are shown in Fig. 4. The Ar concentrations vary inversely with temperature, and Ar is always slightly supersaturated. The degree of supersaturation decreased from 1.5% at the surface to 0.25% at c$_n$ = 14.5 and then increased slightly with depth to 1.6% at 2000 m (c$_n$ = 17.22). Interestingly, temperature increases with depth in the Black Sea. Therefore, Ar concentrations decrease with depth, which is opposite of the distributions in the open ocean.

3.4. Nitrogen isotopes

We determined the nitrogen isotope ($^{14}$N and $^{15}$N) values for N$_2$, NO$_3$, NH$_4$ and suspended PON. The presence of $^{14}$N and $^{15}$N species provides additional information for evaluating nitrogen transformations because the rates of these processes are sensitive to the isotope mass. The ratio of $^{14}$N to $^{15}$N transformation rates is defined by a fractionation factor (α) that usually exceeds 1, as lighter isotopes undergo transformation at a higher rate, also written as the isotope effect ($\epsilon$=(α−1)×1000). The isotope effect ($\epsilon$) of denitrification is usually in the range of 28.6±13%. In culture experiments (Barford et al., 1999). Ammonox is expected to also result in isotope

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**Table 3**
The concentrations (μM) and densities, in sigma theta, at the nitrate and nitrite maxima

<table>
<thead>
<tr>
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<tr>
<td>c$_n$ μM</td>
<td>c$_n$ μM</td>
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<td>15.6</td>
<td>14.7</td>
<td>15.5</td>
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<td>15.1</td>
<td>*</td>
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<td>15.85</td>
</tr>
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<td>St 20 2003</td>
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<tr>
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<tr>
<td>NO$_3$ max</td>
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<td>15.5</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NO$_2$ max</td>
<td>16.3</td>
<td>14.17</td>
<td></td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

* Concentration for entire suboxic zone.

---

![Fig. 3](image-url) Temporal variability in A) N$_2$/Ar ratios normalized to saturation and B) $\delta^{15}$N–N$_2$ for the R/V Bilim 1999 (red), R/V Bilim 2000 (blue), R/V Knorr 2001 (purple), R/V Knorr 2003 (black) and R/V Endeavor 2005 (maroon). All data have been corrected for the interference of methane in the measurement. Horizontal dotted lines indicate the suboxic zone (SOZ). Dashed line indicates the core of the Cold Intermediate Layer (CIL). Central Gyre stations are underlined in the legend. Vertical dotted lines indicate nitrogen gas saturation in seawater and an atmospheric equilibrium $\delta^{15}$N value of 0.68‰.

---

fractionation, but this has not yet been measured. The anomalies of $^{15}$N, relative to the reference air, are expressed as

$$\delta^{15}N = \left( \frac{^{15}N/^{14}N_{sample}}{^{15}N/^{14}N_{reference}} - 1 \right) \times 1000,$$

3.5. $\delta^{15}$N–NO$_3$

Results for $\delta^{15}$N–NO$_3$ from 2001 and 2003 are shown in Fig. 5. The boundaries of the suboxic zone are shown for reference. In 2001, $\delta^{15}$N–NO$_3$ values decreased with depth from 12.5 to 8‰ in the shallowest part of the nitricline ($\sigma_\theta$ = 14.2 to 15.0) (St 20 Knorr 2003) (Fig. 5A). The minimum in $\delta^{15}$N–NO$_3$ was in the region of the nitrate maximum ($\sigma_\theta$ = 15.5). As nitrate decreased, $\delta^{15}$N–NO$_3$ increased to values as high as 18‰. In 2003 we observed that as nitrate approached zero, $\delta^{15}$N–NO$_3$ decreased again to values between 8 to 9‰. In 2001 and 2003, $\delta^{15}$N–NO$_3$ increased from ~8‰ in the CIL to 13.5‰ in the upper part of the suboxic zone (Fig. 5B).

3.6. $\delta^{15}$N–N$_2$

In most years $\delta^{15}$N–N$_2$ was enriched relative to seawater in equilibrium with the atmosphere (0.68‰; Knox et al., 1992) in surface waters and depleted in the suboxic zone (Fig. 3B). The $\delta^{15}$N–N$_2$ values were ~1.0‰ in the surface waters in 2001 and 0.85‰ in 2000 and 2003. In 1999 surface waters were in equilibrium with the atmosphere and in 2005 $\delta^{15}$N–N$_2$ values (~0.4‰) were more depleted than equilibrium. $\delta^{15}$N–N$_2$ values at the density of the N$_2$/Ar maximum varied temporally with minimum values of ~0.1‰ in 2000, 0.3‰ in 2001, 0.5% in 2005 and 0.6‰ in 1999 and 2003. In the deep anoxic water, $\delta^{15}$N–N$_2$ was approximately 0.5 ± 0.1‰. 3.7. $\delta^{15}$N–NH$_4$+

$\delta^{15}$N–NH$_4^+$ was only analyzed in deep water samples from St 2 2001 and St 12 2003, in the Western Central Gyre, for the density range $\sigma_\theta$ = 16.8 to 17.24. $\delta^{15}$N–NH$_4^+$ in these samples averaged +1.68 ± 0.28‰. This agrees with measurements of +1.6‰ (Fry et al., 1991) on leg 2 R/V Knorr 1988 cruise and +1.7 ± 0.2‰ (Velinsky et al., 1991) on leg 3 R/V Knorr 1988 cruise. Velinsky et al. (1991) observed that $\delta^{15}$N–NH$_4^+$ increased at shallower depths to a maximum of +7.5‰ at $\sigma_\theta$ = 16.2 and then decreased to +5.5‰ at $\sigma_\theta$ = 16.07 in the lower layer of the suboxic zone.
3.9. Total organic nitrogen (TON)

The concentrations of total organic nitrogen are shown in Fig. 7. In 2000 and 2001, TON concentrations ranged from 13 to 17 μM. In 2003, TON values were between 11 and 14 μM, and in 2005 TON concentrations were between 11 and 13 μM for $\sigma_M = 15.2$ and below, then increased to 15 μM in surface waters. TON samples should consist mainly of dissolved organic nitrogen, but the use of acidified unfiltered samples allows particles to contribute to the scatter in the data. TON values from 2001 had the greatest variability (13–21 μM). Satellite data indicate a phytoplankton bloom just before the 2001 cruise (McCarthy et al., 2007) and high POC-Chl-a ratios suggest an elevated detrital component to S-PON (Çoban-Yıldız et al., 2006).

3.10. The Bosporus Plume

At station 14, in the southwest region of the Black Sea that was occupied during the Knorr 2001 cruise, oxygen, temperature and $\delta^{15}N$ data are consistent with injection by the Bosporus Plume to form ventilated layers (Konovalet al., 2003) (Fig. 8). Both the temperature (not shown) and oxygen data had frequent maxima between density values of $\sigma_M = 15.5$ and a smaller, second maximum at $\sigma_M = 16.2$ (Fig. 8B). Nitrite had only one maximum of 0.53 μM at $\sigma_M = 16.3$. $\delta^{15}N$–NO$_3$ values remained at 8% through the upper nitrate maximum but increased to a maximum of 16% at $\sigma_M = 15.95$, and then decreased to 4.5% at $\sigma_M = 16.1$ (Fig. 8B). Nitrite concentrations were large enough to affect $\delta^{15}N$–NO$_3$ in the Bosporus Plume ($\epsilon_N = 16.1–16.4$). The Bosporus Plume, as indicated by a deep nitrate maxima (3.3 μM), had depleted NO$X$ (4.5%), and enriched nitrogen gas (0.52‰) with a lower amount of excess nitrogen gas ($N_2/Ar = 1.011$).

3.11. Bosporus and Marmara Sea

The NO$X$/Ar and $\delta^{15}N$ values for nitrogen species in the Bosporus and Marmara Sea reflect their waters of origin. The Bosporus can be split into two layers: water in the surface layer flows from the Black Sea to the Sea of Marmara, and the deep water flows from the Sea of Marmara into the Black Sea (Özsoy et al., 1993). The NO$_3$, NO$_2$ and TON concentrations are in Table 4. NO$_3$/Ar ratios in the bottom, high salinity layer of the Bosporus ranged from 1.007 to 1.011 (Table 4). $\delta^{15}N$–NO$_3$ in the bottom layer of the Bosporus was $-0.35\%$ (Table 4). $\delta^{15}N$–NO$_2$ values were $-5\%$, similar to the value for nitrate in open Atlantic seawater (Thunell et al., 2004). The values for $\delta^{15}N$–N$_2$ and NO$_3$/Ar in the surface layer of the Bosporus (Table 4) resembled their source water, the surface water of the Black Sea.

Nitrogen gas and isotope samples were obtained at the surface and at $\sigma_M = 28.5$ in the Marmara Sea. Water with $\sigma_M = 28.5$ in the Marmara Sea is the

![Fig. 7. Temporal variability in the concentration of total organic nitrogen at the Central Western Gyre. Dotted lines indicate the suboxic zone. Insert: Shallow data versus depth. The density at the surface of the Black Sea changes between cruises. The insert highlights TON in the surface layer. Symbols are the same as in Fig. 3.](image)

**Fig. 7.** Temporal variability in the concentration of total organic nitrogen at the Central Western Gyre. Dotted lines indicate the suboxic zone. Insert: Shallow data versus depth. The density at the surface of the Black Sea changes between cruises. The insert highlights TON in the surface layer. Symbols are the same as in Fig. 3.

![Fig. 8. Injection of the Bosporus plume at Station 14 R/V Knorr 2001 A) Oxygen (triangles), and normalized NO$_3$/Ar ratios (filled circles). B) Nitrogen species concentration and $\delta^{15}N$ in the water column: nitrate (empty circles), nitrite (diamonds), and $\delta^{15}N$–NO$_3$ (x).](image)

**Fig. 8.** Injection of the Bosporus plume at Station 14 R/V Knorr 2001 A) Oxygen (triangles), and normalized NO$_3$/Ar ratios (filled circles). B) Nitrogen species concentration and $\delta^{15}N$ in the water column: nitrate (empty circles), nitrite (diamonds), and $\delta^{15}N$–NO$_3$ (x).
source of deep water flowing through the Bosporus to the Black Sea. N2/Ar ratios from $\sigma_\theta=28.5$ were 1.008. $\delta^{15}N–N_2$ was enriched to 1.3‰, and $\delta^{15}N–NO_3^-$ values were ~5‰ (Table 4).

### 4. Discussion

The large interannual variability in N2/Ar and $\delta^{15}N–N_2$ in the suboxic zone was surprising considering the stability seen in hydrographic properties and the profiles of oxygen, sulfide, nitrate and ammonium. In this discussion, we hypothesize that the observed variability is due to variability in organic matter fluxes to the suboxic zone. To support this hypothesis we examine whether nitrogen from organic matter contributes to N2 production by calculating a N-atom and $\delta^{15}N$ mass balance for the suboxic zone. We then discuss possible origins of the excess N2 by examining the concentration and $\delta^{15}N$ of the excess N2 formed by biological processes. We will then explore how linkages between variable organic matter fluxes, partial NO3$^-$ reduction and euphotic zone N2 fixation could produce the variability we observe.

#### 4.1. Excess N2 and $\delta^{15}N_2$

Our N2/Ar data suggest that Black Sea waters are supersaturated with N2 (Fig. 3A). In order to constrain possible origins of the N2, we need to determine the magnitude and variability of the reservoir of excess N2 in the suboxic zone and its value of $\delta^{15}N$.

We independently determined Ar concentrations on samples from 2005 and used these to calculate N2 concentrations. An Ar–potential temperature relationship determined from 2005 data was used to estimate Ar for the other years. The profiles of N2 concentrations are compared with the N2 solubility based on potential temperature (Hamme and Emerson, 2004) in Fig. 9A. The solubility curves diverge in shallower water because the temperature of the CIL, which depends on the severity of recent winters, varied interannually from 6.9 °C in 2003 to 7.7 °C in 2001.

Mixing of two waters results in an apparent supersaturation of gas concentrations because, while the mixing line between two end members for the conservative property potential temperature is straight, the solubility line for N2 curves (see for example, Henning et al., 2006; Ito et al., 2006). Assuming that the deep water in the Black Sea forms by variable mixing of the Bosporus outflow and the Cold Intermediate Water (centered at $\sigma_\theta=14.5$) (Murray et al., 1991) then the solubility line after mixing is the correct reference for calculating biogeochemical anomalies. We calculated the concentration for each depth after mixing of these two end-members based on their salinity values (Lee et al., 2002). As the temperature of the CIL is variable between years and residence times increase with depth (Lee et al., 2002) we back calculated an average CIL temperature for each depth by assuming a constant Bosporus temperature and using the mixing ratio calculated by salinity. This temperature was used to calculate an average saturated N2 concentration for the CIL. The mixing line solubility is shown as a dashed line in Fig. 9A. The apparent supersaturation due to mixing alone varies from 0.7% in the suboxic zone to 1.2% at the bottom. Excess N2 was

![Fig. 9](image-url)

**Table 4**

| Nutrient concentration and $\delta^{15}N$ values for the Bosporus and Marmara Sea |
|---------------------------------|--------|--------|--------|--------|--------|
| Year                           | NO3$^-$(μM) | NO2$^-$(μM) | TON (μM) | $\delta^{15}N–NO_3^-(‰)$ | $\delta^{15}N–N_2-(‰)$ |
| Marmara Sea surface (10 m)     | 2003    | 1.8     | 0.05    | 14.2    | 16.2±0.2 | 0.85 | 1.0007 |
| Marmara Sea ($\sigma_\theta=28.55$) | 2003    | 11.1    | 0.09    | 10.4    | 5.4±0.1  | 1.3  | 1.009  |
| Bosporus surface (15 m)         | 2000    | <1.5    | 0.1     | N/A     | N/A      | 0.70 | 1.006  |
| Bosporus surface (1 m)          | 2000    | 6.0     | 0.2     | 10.6    | N/A      | 0.3  | 1.011  |
| Bosporus bottom ($\sigma_\theta=26.9$) | 2001    | 0.04    | 0.01    | 23.7    | N/A      | 1.01 | 1.003  |
| Bosporus bottom ($\sigma_\theta=26.9$) | 2001    | 5.52    | 0.2     | 16.4    | 5.3±0.2  | 0.5  | 1.007  |

Ammonium values were all below the detection.
calculated relative to the mixing line (Fig. 9B) and the maximum values (ranging from 7.5 to 20 μmol kg\(^{-1}\)) or 2 to 4% of total N\(_2\) are centered in the lower part of the suboxic zone in the density range where NO\(_3\) and NH\(_4\) decrease to zero. The year 2000, which had the most depleted δ\(^{15}\)N–N\(_2\), also had the largest excess N\(_2\).

We calculated the δ\(^{15}\)N values for the excess N\(_2\) by assuming that the measured δ\(^{15}\)N was composed of the sum of the 15N from the mixing line and the 15N for the added N\(_2\). In the following mass balance, where R is the isotopic composition, the only unknown is R\(_{\text{excessN2}}\).

\[
R_{\text{mixing line}} \times [N_2]_{\text{mixing line}} + R_{\text{ExcessN2}} \times [\text{excess N}_2] = R_{\text{measured}} \times [N_2 \text{ measured}]
\]  

(2)

The individual values calculated for δ\(^{15}\)N of excess N\(_2\) are plotted versus density in Fig. 9C. The values in the suboxic zone from different years fall into two different populations. The values for 1999, 2003 and 2005 are relatively uniform at a mean value of −1‰ in the suboxic zone. The values for 2000 and 2001 are depleted from −40‰ to −30‰ respectively. The more depleted δ\(^{15}\)N of the total N\(_2\) in 2005 (0.45‰) compared to 2003 and 1999 (0.6‰) may be explained by more depleted δ\(^{15}\)N–N\(_2\) in the CIL in 2005. δ\(^{15}\)N–N\(_2\) in the CIL is likely affected by N\(_2\) fixation in the euphotic zone (see Section 4.7). Thus the amount of nitrogen fixation in the euphotic zone may affect the δ\(^{15}\)N–N\(_2\) in the suboxic zone by mixing processes.

4.2. Residence times and N\(_2\) production rates

Knowing the residence time of N\(_2\) in the suboxic zone, and comparing the residence time of N\(_2\) with that of water is key to understanding the time scale at which N\(_2\) concentrations are sensitive to variability in fluxes. The residence time of N\(_2\) can be calculated as the inventory divided by the output. The inventory was calculated by integrating the total N\(_2\) in the suboxic zone. The outputs were the advective and diffusive fluxes of total N\(_2\) calculated using constants for advection and diffusion from both Lee et al. (2002) and Ivanov and Samodurov (2001) (Section 4.4). Results using the two parameter sets were almost identical. We did the calculations with and without the nitrogen fixation rates in the suboxic zone measured by McCarthy et al. (2007). Addition of this rate would represent an additional sink for N\(_2\) of 0.8 μmol N m\(^{-2}\) d\(^{-1}\). The average values for the residence time of N for the 5 years studied were 3.2 ± 0.4 year without N\(_2\)-fixation and 0.9 ± 0.1 year with N\(_2\)-fixation.

It is important to understand whether physical forcing can affect the variability of N\(_2\) in the system. There are two estimates of the residence time of water in the suboxic zone. The first was 4.8 ± 3.6 years as calculated from chlorofluorocarbon data from 1988 (Lee et al., 2002) and the second was 8 years using the volume of water in the suboxic zone and the volume of inflow to the suboxic zone from the Bosphorus Plume (Lee et al., 2002). Modeling by Konovalov et al. (2008-this issue) found that if N\(_2\) production was stopped, and N\(_2\) fixation was not accounted for in the model, but advection and diffusion continued, N\(_2\) concentrations could vary over the same time scales as we observed. However, measured δ\(^{15}\)N values could not be replicated in this scenario. If N\(_2\)-fixation is important in the suboxic zone, then the residence time of N\(_2\) is significantly less than that of the water, which implies that interannual and season variations due to variations in biogeochemistry and particulate nitrogen fluxes could be observable.

The steady state rate of N\(_2\) production from the combination of denitrification and anammox can be calculated as the excess N\(_2\) divided by the residence time. These rates ranged from 0.20 to 0.34 mmol m\(^{-2}\) d\(^{-1}\) without N\(_2\)-fixation and from 0.73 to 1.21 mmol m\(^{-2}\) d\(^{-1}\) with N\(_2\)-fixation. Production rates (not including N\(_2\) fixation) from 1999 and 2000 (0.33–0.34 mmol m\(^{-2}\) d\(^{-1}\)) were significantly higher than rates for 2001, 2003 and 2005 (0.20–0.22 mmol m\(^{-2}\) d\(^{-1}\)). N\(_2\) production rates from Jensen et al. (2008), from both isotope pairing experiments and a reaction diffusion model, are consistent with the lower range of nitrogen production calculated here assuming that nitrogen fixation does not occur in the suboxic zone.

4.3. Profiles of concentrations and isotopes of N species and associated processes

The Black Sea provides ideal conditions for studying several aspects of nitrogen cycling. Nitrate is low in the euphotic zone due to biological consumption (1% \(I_0\), typically occurs at 15–30 m) (Yilmaz et al., 2006) (Fig. 2B). Below the euphotic zone NO\(_3\) increases due to aerobic respiration (nitrification). After oxygen decreases to close to zero at about \(\alpha\Phi = 15.5\), nitrate decreases to zero at a density of \(\alpha\Phi \approx 15.95\), near the base of the suboxic zone due to nitrate reduction reactions. δ\(^{15}\)N–NO\(_3\) increases to a maximum as high as 18‰, as the concentration of NO\(_3\) decreases (Fig. 5). The formation of enriched nitrate is consistent with partial denitrification. Ammonium begins to increase below that depth due to ammonification and accumulates to concentrations of about 100 μM in the deep sulfidic layer. There are often two nitrite maxima. The shallow one originates because nitrite is an intermediate in nitrification. The deep maximum reflects nitrite’s role as an intermediate in both denitrification and nitrification (Ward and Kilpatrick., 1991; Lam et al., 2007). Because anammox is a reaction between NO\(_2\) and NH\(_4\), the variability in NO\(_3\) concentrations and δ\(^{15}\)N may provide clues for explaining variability in N\(_2\) production and its value of δ\(^{15}\)N. Yet the NO\(_3\) and NH\(_4\) distributions suggest total consumption by anammox at the bottom of the suboxic zone, and there is a maximum in N\(_2\)/Ar centered in the same depth range (Fig. 3).

Both suspended PON and TON are highest in the euphotic zone and decrease with depth. S-PON concentrations increase near the base of the suboxic zone (Fig. 6 and Çoban-Yıldız et al., 2006). Evidence from mass balance calculations, and microbial rate measurements indicate that chemosynthesis produces new particulate organic nitrogen in the suboxic zone, but the maximum in chemosynthesis occurs below the suboxic zone boundary in the upper part of the sulfide zone (e.g. Sorokin, 1972; Brewer and Murray, 1973; Çoban-Yıldız et al., 2006; Yilmaz et al., 2006). If PON plays an important role in the nitrogen mass balance for the suboxic zone, then depleted N\(_2\) could be produced due to respiration of depleted PON or due to δ\(^{15}\)N fractionation during partial conversion of NO\(_3\), NH\(_4\) and PON to N\(_2\).
4.4. The nitrogen mass balance

We used the concentration profiles of NO$_3^-$, NH$_4^+$, N$_2$ and TON to calculate a mass balance for nitrogen in the suboxic zone and to evaluate the contribution of organic nitrogen (PON and TON) to N$_2$ production. The mass balance was described by vertical advection (w) and vertical diffusion (k) fluxes across the upper and lower boundaries of the suboxic zone and a term representing lateral advection flux term from the Bosphorus Plume. This last term is similar to the ventilation term in the model by Ivanov and Samodurov (2001) and Konovalov et al. (2008-this issue). The steady state mass balance is written as:

\[
 w_{\text{in}} [C]_{\text{bottom}} + k_{\text{bottom}} \frac{\partial [C]_{\text{bottom}}}{\partial Z} + (w_{\text{out}} - w_{\text{in}}) [C]_{\text{plume}} = \frac{\partial [C]_{\text{plume}}}{\partial Z} + J_{\text{BC}}
\]

(3)

where w is the vertical advection velocity at the top (out) and bottom (in) of the suboxic zone, k is the vertical mixing coefficient, C is the concentration and \( J_{\text{BC}} \) is the rate of N$_2$ production balanced by biochemical inputs of other nitrogen species. The concentrations in the lateral intrusions (\( C_{\text{plume}} \)) were calculated as a mixture of values from the CIL and the Bosphorus in the ratio of 7.89:1 as determined by Lee et al. (2002). The output fluxes were calculated from the concentration profiles of total N$_2$ (Fig. 9A). To obtain gradients, straight lines were fit to the nitrate and N$_2$ data. The ammonium data were fit with a natural log function. Either linear or polynomial fits were used to obtain gradients for the total organic nitrogen data, depending on which gave the best fit in a given year. The equations were differentiated to get the gradients at the suboxic zone boundary. Molar concentrations of N$_2$ were multiplied by 2 to get the atom fluxes.

We calculated the mass balance using two different sets of upwelling velocities and mixing coefficients from the models of temperature and salinity by Ivanov and Samodurov (2001) and of CFC data by Lee et al. (2002).

The fluxes of NO$_3^-$ and NH$_4^+$ are compared with the N$_2$ fluxes (as nmol N m$^{-2}$ s$^{-1}$) out for 1999, 2000, 2001, 2003 and 2005 in Table 5. The net N$_2$ fluxes were always out with both sets of physical parameters and were dominated by the upwelling fluxes. With the Lee et al. (2002) parameters there was always a net inorganic nitrogen flux out of the suboxic zone, meaning that an additional source nitrogen was required. The required nitrogen varied from 3.0±2 nmol N m$^{-2}$ s$^{-1}$ in 1999 to 6.3±2 nmol N m$^{-2}$ s$^{-1}$ in 2001. The imbalance in 2003 was less than the error of the calculation. The years 2000 and 2001 required the largest additional flux. When using parameters from Ivanov and Samodurov (2001), the fluxes in 1999, 2003 and 2005 were balanced within the error of the calculation. A significant additional source of nitrogen was required in 2000 and 2001 (4.3±2 and 11.8±2 nmol N m$^{-2}$ s$^{-1}$ respectively). These different sets of parameters, both of which can reasonably describe the physical properties, result in different results for the nitrogen mass balance. But considering the uncertainty of ±2 nmol N m$^{-2}$ s$^{-1}$, only the imbalance for 2001 was significantly different for Lee et al. (2002) and Ivanov and Samodurov (2001). Nitrogen remineralized from organic nitrogen is the most likely additional source for nitrogen. Some of this organic N could be provided by input of TON (composed of both dissolved and suspended organic matter) but TON fluxes were not large enough by themselves to compensate the N imbalance. For example, in 2005 (with the Lee et al. parameters) the net TON flux was 0.59 nmol N m$^{-2}$ s$^{-1}$, which was about 25% of the organic N flux required. Regardless of the model used, the years 2000 and 2001 appear to require a substantial additional flux of nitrogen that we suggest is mostly sinking PON.

For comparison, sediment trap data from a single deployment of 4 days in May 1988 observed a sharp decrease in PON flux from 10 nmol m$^{-2}$ s$^{-1}$ at the top of the suboxic zone to 3.3 nmol m$^{-2}$ s$^{-1}$ at the bottom of the suboxic zone (Karl and Knauer, 1991). The sinking PON flux consumed during this brief period was 7 nmol m$^{-2}$ s$^{-1}$. Presumably, this particulate organic nitrogen was converted to ammonium, which was consumed in anammox fast enough that the dissolved ammonium concentration remained below detection. Kirkpatrick et al. (2006) observed that anammox-type sequences were present throughout the suboxic zone and they dominated the clone library at the density (\( \sigma_\text{k}=15.8 \)) of the NO$_2^-$ maximum. Thamdrup et al. (2006) suggested a similar scenario for the oxygen minimum zone off northern Chile.

Kuyper et al. (2003) calculated a rate for anammox of 7 nM d$^{-1}$ assuming that the rate of anammox equaled the upward

<table>
<thead>
<tr>
<th>Year</th>
<th>Net N$_2$ flux</th>
<th>Net NO$_3^-$ flux</th>
<th>Net NH$_4^+$ flux</th>
<th>Net N$_2$ flux</th>
<th>Net NO$_3^-$ flux</th>
<th>Net NH$_4^+$ flux</th>
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<tbody>
<tr>
<td>1999</td>
<td>2.98 in 3.04 out</td>
<td>1.02 in 0.54 out</td>
<td>3.35 in 0.62 in</td>
<td>Balanced 2.96 in</td>
<td>Balanced Balanced</td>
<td></td>
</tr>
<tr>
<td>2000</td>
<td>8.37 out 4.50 in</td>
<td>1.71 out 0.58 in</td>
<td>2.97 in 0.57 in</td>
<td>4.23 in 4.51 in</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2001</td>
<td>15.76 out 5.94 in</td>
<td>0.90 out 0.97 out</td>
<td>3.05 in 0.56 in</td>
<td>11.80 in 6.35 in</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2003</td>
<td>2.92 out 1.60 out</td>
<td>1.75 out 0.31 out</td>
<td>3.17 in 0.57 in</td>
<td>Balanced Balanced</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2005</td>
<td>2.68 out 3.42 out</td>
<td>1.27 out 0.50 out</td>
<td>3.08 in 0.58 in</td>
<td>Balanced 3.34 in</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
NH₃ flux. However, our mass balance calculations suggest that in some years a significant, but variable, source of N for N₂ production probably comes from sinking particulate organic nitrogen (PON).

4.5. The mass balance for δ¹⁵N

A mass balance for δ¹⁵N was used to determine the δ¹⁵N of the flux required to satisfy the δ¹⁵N mass balance. We calculated the mass balance for δ¹⁵N using the same approach used for the nitrogen mass balance (Section 4.4) (after Quay and Stutsmann, 2003). We determined the δ¹⁵N of the flux that was needed to balance the N-atom mass balance, using transport parameters from both models. Using parameters from Ivanov and Samodurov (2001) values for 2000 and 2001 were highly depleted (−43 ± 18‰ and −7 ± 1‰ respectively). Note that these values are the δ¹⁵N of the flux, not the δ¹⁵N of a species. Using parameters from Lee et al. (2002) the values fall into two groups. For data from 2000, 2001, and 2005, the needed organic N flux was depleted in δ¹⁵N and the specific values were −31 ± 13‰, −0 ± 3‰ and −5 ± 11‰, respectively. For data from 1999, the missing organic N flux was enriched (+8 ± 6‰).

4.6. Temporal variability

The depth profiles of nitrogen species (N₂/Ar, δ¹⁵N−N₂, δ¹⁵N–NO₃⁻, [NO₃⁻], [NO₂⁻] and total organic nitrogen (TON)) displayed interannual variability in the Western Central Gyre (Figs. 3, 5, 7, 9, and Table 3). The variability was not random and there are some clear patterns in the data. The maximum values of the N₂/Ar saturation ratios were highest in 1999 and 2000 and decreased slightly in 2001, 2003 and 2005. The N₂ production rates for the suboxic zone were also highest in 1999 and 2000 and lower in 2001, 2003 and 2005. δ¹⁵N–N₂ showed the greatest variability and was most depleted in 2000, increased slightly in 2001, slightly more in 2003 before decreasing again in 2005. The δ¹⁵N of the excess N₂ and the δ¹⁵N of the missing nitrogen source were both extremely depleted in 2000 and 2001 but were quite constant in 1999, 2003 and 2005. By comparison, the net NH₃ and NO₃ fluxes changed little during this period. A likely conclusion from the N mass balance was that 1999, 2003 and 2005 were the years when the fluxes were balanced or close to balanced (within the uncertainty of the calculations) while 2000 and 2001 appear to require a large additional flux which we hypothesize is organic nitrogen.

Murray et al. (1995) proposed a simple model where N₂ production would occur due to total consumption of the upward flux of NH₃ and the downward flux of NO₃. Because the vertical profiles of NH₃ and NO₃ vary little, the variability we observe in δ¹⁵N of excess N₂ must be due to variability in the fluxes of additional reactants and additional processes. The processes of partial consumption by denitrification/anammox and N₂-fixation may play important roles.

4.7. Evidence for nitrogen fixation

Little is known about nitrogen fixation in the Black Sea, but it has traditionally been assumed to be unimportant because of large inputs of nitrogen from coastal sources. The N₂/Ar in the surface layer (Fig. 3A,B) often approaches the value expected for equilibrium gas exchange between surface ocean and atmosphere, but the values of δ¹⁵N₂ are often higher than atmospheric. These elevated values of δ¹⁵N₂ at depths above the CIL could be explained by nitrogen fixation if N₂ was fixed faster than it could be replaced by gas exchange. The NO₃/PO₄ ratios in the upper layers of the Black Sea (shallower than the CIL) are typically ≤4 (Fig. 10) suggesting favorable conditions for N₂ fixation (e.g. Deutsch et al., 2007; Capone and Knapp, 2007). Though Sorokin (2002) summarized Russian studies in which nitrogen fixing microbes were isolated, the first direct measurements of rates of nitrogen fixation were reported by McCarthy et al. (2007).

We calculated the possible rate and δ¹⁵N fractionation for nitrogen fixation using a box model approach. We constructed a three-box model with a mixed layer box, a subsurface layer box (just under the mixed layer), and a CIL box. The depth of 7m for the mixed layer box was determined by uniform temperature and salinity data. We picked the subsurface layer for this calculation because our data for δ¹⁵N for the S-pon from the euphotic zone just below the mixed layer of the NE Black Sea near Gelendzhik in July 2005 and May 2007 has an isotopic composition around 0‰ (Fuchsman, unpublished data), which is consistent with N₂-fixing organisms (e.g. Carpenter et al., 1997). We calculated the advective and diffusive fluxes of nitrogen and δ¹⁵N into and out of this subsurface layer. The advection velocity (w) was taken from the model of Ivanov and Samodurov (2001). The physical parameters of Lee et al. (2002) were only determined below the CIL (σθ = 14.5). A range of eddy diffusion coefficients (k) were tested, but the results were most consistent with the data with a k between 0.8−1×10⁻⁴ m²s⁻¹ from the subsurface layer to the surface mixed layer, and a k near 5×10⁻⁵ m²s⁻¹ from the CIL to the subsurface layer. Both values are consistent with measured values from Gregg and Yakushev (2005). Equations for mixing of isotopes during eddy diffusion were after Quay and Stutsmann (2003). We first solved for the N₂-
fixation rate. Then, using that rate, and assuming δ\(^{15}\)N of N\(_2\) in the euphotic zone was as measured, we calculated δ\(^{15}\)N\(_{\text{fix}}\).

For 2001, the N\(_2\) fixation rates for the subsurface layer were between 0.12–0.15 mmol N m\(^{-3}\) d\(^{-1}\) when the fractionation factor (ε) for nitrogen fixation was \(-1.3 to -2.6\%\) which is in the range of \(-2.6\pm1.3\%\) reported for nitrogen fixation in Sachs and Repeta (1999). When the diffusion coefficient between the subsurface and mixed layers was decreased, the calculated rate of nitrogen fixation increased and the magnitude of epsilon became less negative. Nitrogen fixation rates in the subsurface layer were comparable to measured nitrogen fixation rates in the Baltic Sea in July (0.09 mmol N m\(^{-3}\) d\(^{-1}\)) (Oehlendieck et al., 2000) and the coastal waters of Tanzania (0.1 mmol N m\(^{-3}\) d\(^{-1}\)) (Lugomela et al., 2002) but much higher at rates at station ALOHA (0.0002–0.004 mmol N m\(^{-3}\) d\(^{-1}\)) (Montoya et al., 2004). Our calculated value of 0.15 mmol m\(^{-2}\) d\(^{-1}\) is equivalent to \(-150 \text{ mmol N kg}^{-1} \text{ d}^{-1}\) which is about twice as large as measured by McCarthy et al. (2007). The significance of this difference is uncertain because we know so little about the rates and variability of nitrogen fixation in the Black Sea. Nitrogen fixation appears to be a plausible explanation for production of depleted δ\(^{15}\)N-PON and enriched δ\(^{15}\)N-N\(_2\) in the near surface layers.

Additional evidence for nitrogen fixation comes from the δ\(^{15}\)N values of suspended and sinking organic matter. The flux of sinking particulate matter has been shown to vary both seasonally (Muramoto et al., 1991) and interannually (Burlakov et al., 2003). The magnitude of the PON flux and its δ\(^{15}\)N appear to also vary seasonally. Çoban-Yıldız (2003) (working with M. Altabet, UMass, Dartmouth) reported PON and δ\(^{15}\)N-PON on Honjo sediment trap samples (BSK2) from 477 m collected in 1988/1989. When the PON flux was large (summer and fall) the δ\(^{15}\)N was more depleted (+1%), while when the PON flux was low (later winter and spring) δ\(^{15}\)N was more enriched (+3% to +5%). If we assume that normal plankton in the surface ocean have δ\(^{15}\)N\(_{\text{fix}}\) = +5%, and that PON made from N\(_2\)-fixation has δ\(^{15}\)N\(_{\text{fix}}\) = 0%, (e.g. Carpenter et al., 1997) the variability in δ\(^{15}\)N of the sinking PON flux suggests that a significant fraction (\(-80\%\)) of the sinking PON flux could be of N\(_2\)-Fixation origin.

If N\(_2\)-fixation is confirmed to be important in the Black Sea it will provide support for the hypothesis that N\(_2\)-fixation and suboxic regions with denitrification/anammox are closely linked. Previous coupling has been described; denitrification and anammox consume fixed N in oxygen deficient ocean areas resulting in low N/P ratios, and water upwelled with low N/P ratios creates conditions that favor N\(_2\) fixation (Deutsch et al., 2007). What we describe in the Black Sea includes an additional feedback where remineralization of PON made by N\(_2\)-fixation can be a factor that results in production of isotopically depleted N\(_2\).

4.8. The evidence for partial consumption

There were 3 years (1999, 2003, 2005) when the δ\(^{15}\)N of the inventory of excess N\(_2\) was constant at \(-1\%\) and 2 years (2000, 2001) when the δ\(^{15}\)N of excess N\(_2\) was highly depleted. The δ\(^{15}\)N of the missing fluxes followed the same temporal pattern with more depleted values in 2000 and 2001. These were the same years when the inventories of excess N\(_2\) were most depleted. The years 2000 and 2001 were also the years when organic nitrogen was needed to balance the nitrogen budget of the suboxic zone (Section 4.4).

To examine this in more detail we looked to see if there was a relationship between δ\(^{15}\)N of excess N\(_2\) and the magnitude of excess N\(_2\) (Fig. 11). The values of δ\(^{15}\)N for 1999, 2003 and 2005, as seen in the depth profiles (Fig. 9C), were fairly uniform at about \(-1\%\), with no significant dependency on the amount of excess N\(_2\). The lack of a correlation between δ\(^{15}\)N and excess N\(_2\) in 1999, 2003 and 2005, along with knowledge that the nitrate and ammonium fluxes into the suboxic zone appear to balance N\(_2\) fluxes, seem to imply that the system was characterized by complete consumption of reactants (NO\(_3\) and NH\(_4\)) in those years.

The δ\(^{15}\)N values for excess N\(_2\) in 2000 and 2001 were much more depleted. The data for these 2 years also show a trend of increasingly enriched δ\(^{15}\)N with increasing excess N\(_2\) and the rates of increases were almost identical for both 2 years (Fig. 11). The forms of these trends is consistent with Raleigh distillation where fractionation varies with the fractional extent of reaction (where f varies from 0 to 1). Thus, the results of calculations of the nitrogen mass balance (Table 5) and data in Fig. 11 reveal both substantial contribution of PON to the nitrogen cycle and partial \(^{14}\)N/\(^{15}\)N fractionation in the suboxic zone in 2000 and 2001. Excess N\(_2\) can be envisioned as a metric of the extent of reaction (f). If we knew the maximum excess N\(_2\) that could be produced, we could calculate the actual values of the extent of reaction. But this maximum N\(_2\) value depends on the N budget in the suboxic zone, which varies from year to year. Instead we can use an inverse approach where we assume the alpha for denitrification, which is equal to 1.030, and calculate a hypothetical Raleigh fractionation curve. Raleigh fractionation curves fit the data when the maximum possible amount of excess N\(_2\) was 35 μmol/kg in 2000 and 18 μmol/kg in 2001. Thus the

Fig. 11. δ\(^{15}\)N of excess N\(_2\) versus Excess N\(_2\). All data are from the suboxic zone; only depths with < 10 μm O\(_2\) are shown here. Note that the upper boundary of the suboxic zone in 2005 was at a deeper density level than in other years. Excess N\(_2\) and δ\(^{15}\)N of the excess N\(_2\) are relative to the two end-member mixing solubility line. The solid horizontal line indicates \(-1\%\). Dashed curved lines indicate Raleigh Distillation curves with ε = -30%, where the maximum N\(_2\) excess used varied between 35 μmol/kg for 2000 and 18 μmol/kg for 2001. Symbols are the same as in Fig. 3.
depleted N₂ gas data from 2000 and 2001 are consistent with partial denitrification.

We hypothesize that a pulse of sinking particulate organic carbon from a phytoplankton bloom may have stimulated partial consumption because it was observed in the years when there were significant contributions of PON to the nitrogen mass balance. Using satellite data, Nezlin (2001) described an unusually large phytoplankton bloom that occurred in 1998–1999, however, a longer term study by Oguz and Ediger (2006) found several similarly sized blooms.

4.9. Variability in the importance of nitrogen transformations

We hypothesize that variability in the flux of organic matter (both PON and TON) can influence the N₂ distribution and its δ¹⁵N signature in several ways. These factors include the relative importance of anammox versus denitrification and the overall rate of N₂ production. They also include the export production of PON and the relative importance of PON production by N₂ fixation. The flux of organic matter changes the nitrogen and δ¹⁵N mass balances depending on its magnitude and δ¹⁵N signature.

Sinking PON is remineralized to NO₃⁻ (nitrification) in the oxic zone and to NH₄⁺ in the anoxic zone. If NO₃⁻ and NH₄⁺ are the only nitrogen forms to fuel N₂ production in the suboxic zone, the δ¹⁵N of the N₂ produced will ultimately be equal to the average of the δ¹⁵N of the upward flux of NH₄⁺ and the downward flux of NO₃⁻/NO₂⁻. This would result in the lowest values of excess N₂ and enriched values of δ¹⁵N-N₂.

Within the suboxic zone PON is remineralized to NH₄⁺ and nitrite that can be anaerobically oxidized to N₂ via anammox (Kirkpatrick et al., 2006; Lam et al., 2007). This results in a higher excess N₂ and more depleted values of δ¹⁵N-N₂, especially when PON has been produced by N₂ fixation.

The flux of POC can also fuel denitrification. Engström et al. (2005), for example, suggested that the relative importance of anammox versus denitrification in marine sediments was inversely correlated with the reactivity of organic matter, i.e., denitrification was more important than anammox when the input of fresh organic matter was largest. We suggest that the importance of anammox versus denitrification in the Black Sea may also depend on the flux of reactive organic matter. There were 2 years (2000 and 2001) when the excess N₂ was of the highest magnitude and when 50–90% of the nitrogen mass balance in the suboxic zone appeared to be supported by respiration of organic matter.

Existing rate measurements from field studies are consistent with these suggestions. Using ¹⁵N–NO₂⁻ enrichment experiments, Jensen et al. (2008) did not find evidence of denitrification in the Black Sea in August 2005. However, Westley et al. (2006) measured denitrification rates in May/June 2001. The coupled model used by Konovolov et al. (2008–this issue) showed that in order to successfully model the concentration profiles at steady state, especially NO₃⁻ and N₂ profiles, it was necessary that anammox support ~90% of the N₂ production.

The δ¹⁵N–N₂, should depend on the relative importance of anammox and denitrification and on δ¹⁵N signatures of the major nitrogen sources (nitrate, ammonium, PON). Partial denitrification is a known process that results in enriched NO₃⁻ and depleted N₂. The δ¹⁵N of the upward flux of NH₄⁺ is probably fairly constant (~±5‰; Velinsky et al., 1991). The δ¹⁵N of sinking PON should depend on the relative importance of ON production by primary production versus N₂-fixation. McCarthy et al. (2007) documented that N₂-fixation occurs in the Black Sea but we know little about its spatial and temporal variability. We do know that the δ¹⁵N of PON produced during N₂-fixation is approximately 0‰. The remineralization of NH₃⁺ from sinking PON and the intensity of partial denitrification can be quite variable depending on seasonal to interannual changes in new production. In 3 years (1999, 2003 and 2005) there was no fractionation and the δ¹⁵N of the excess N₂ was constant at ~−1‰. The nitrogen mass balance was well described by interaction of nitrate and ammonium in and by the flux of N₂ from the suboxic zone. In 2000 and 2001, there was intensive production of depleted N₂ that requires both a substantial contribution of PON to the nitrogen balance in the suboxic zone and partial consumption.

We argue that anammox and denitrification both occur. Anammox is limited by supply of NH₄⁺ from remineralization of PON and the upward flux. Denitrification is limited by supply of fresh organic matter. There are some years (1999, 2003, 2005) when the system might be characterized as a steady state when production of NO₃⁻ and NH₄⁺ equals consumption. This is the system modeled by Konovolov et al. (2008–this issue). There are other times (e.g. 2000, 2001) when production of NO₃⁻/NH₄⁺ was greater than consumption because of input of fresh organic matter. Under these conditions depleted N₂ is produced. What we measured in our time series was a “snap shot” of the transition of the system from a perturbed condition (2000, 2001) to another state as represented by the years before and after (1999, 2003 and 2005).

To understand the timescales and processes causing variability in the N cycle of the Black Sea suboxic zone, it will be important to continue this time series of measurements with seasonal and interannual sampling and with additional molecular level measurements of the presence and activities of bacteria that mediate anammox, denitrification and N₂-fixation.

5. Conclusions

Using new data from the Black Sea from 1999 to 2005, we demonstrated that there is temporal variability in profiles of N₂/Ar, δ¹⁵N–N₂, δ¹⁵N–NO₃⁻, and total organic N, as well as in the maximum concentrations of nitrite and nitrate. A maximum in N₂/Ar and a minimum in δ¹⁵N–N₂ occur in the suboxic zone. Ar measurements allowed us to directly calculate the concentrations of N₂. Excess N₂ was calculated relative to a mixing line derived from the Bosporus and Cold Intermediate Layer end members. The maximum in excess N₂ was located near the bottom of the suboxic zone and varied from 9 to 20 μmol kg⁻¹. The δ¹⁵N of the excess N₂ was calculated to be ~1‰ in 1999, 2003 and 2005 but was highly depleted at ~30% and ~40% in 2001 and 2000. We also found that, in 2000 and 2001, the δ¹⁵N of excess N₂ increased with increasing excess N₂. This trend is consistent with partial consumption by denitrification/anammox, though the concentration profiles suggest total consumption of NO₃⁻ and NH₄⁺ at the bottom of the suboxic zone.

Using a nitrogen mass balance we determined that in some years (especially 2000 and 2001) a missing N flux is required.
to complete the nitrogen mass balance. We suggest that this missing N flux is most likely from remineralization of sinking PON. A similar mass balance for δ15N revealed that the δ15N of the missing N flux was highly depleted in 2000 and 2001.

We propose a transitory system where sinking organic matter from a phytoplankton bloom may stimulate partial consumption by some combination of denitrification and anammox to produce depleted N2. When the system is in one state (possibly steady-state), total consumption occurs, primarily by anammox. Still, remineralization of depleted organic matter, presumably from nitrogen fixation in the euphotic zone, is necessary to support N2 production of 1–1%, during complete consumption of nitrate, nitrite and ammonia. Our measured values of δ15N–N2 in the surface layers support the argument that N2-fixation is occurring in the Black Sea but temporal variations in the rates and consequences of nitrogen fixation and variations in the δ15N of sinking organic N remain unresolved. When the system is in another state (perturbed, non-steady-state), partial 14N/15N fractionation fueled by an influx of fresh PON into the suboxic zone substantially contributes to the mass and δ15N balance. Correlations have been found between the North Atlantic Oscillation (NAO) and physical parameters and biological reservoirs and rates in the Black Sea (Oguz et al., 2006). These climate oscillations may be the driving force of this variability in the Black Sea.

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