Nitrogen reactions in the suboxic zone of the Black Sea: new data and models (291)

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Abstract- New data for N₂ were obtained from the Black Sea. These data show that excess N₂ is close to atmospheric equilibrium at the air-sea interface of the Black Sea. There is a maximum of excess N₂ over atmospheric equilibrium in the suboxic zone that reaches 20 µM. The excess is centered at a density of σₜ=16.0 which is where nitrate and ammonium go to zero. Excess N₂ decreases with depth below the suboxic zone but remains elevated at about 10 µM in the deep water. The transport-reaction model results show that the upward flux of ammonia and the downward flux of nitrate can simulate this maximum in excess N₂. A reaction in the form of NO₃⁻+NH₄⁺=N₂ is required. Such a reaction is similar to the Anammox reaction observed in other suboxic reactor and natural environments. The origin of excess N₂ in the deep water is an interesting problem. The flux of nitrate from the Bosporus plume (original Bosporus inflow plus entrained cold intermediate layer water) cannot support the observed excess of nitrogen gas in the anoxic zone below the depth of 400m. The high values of excess N₂ in the deep water appear to result from the combined lateral flux of both nitrate and nitrogen gas that are entrained to the Bosporus plume from the Cold Intermediate Layer water. The excess nitrogen gas entrained from the CIL ultimately originated from the Anammox reactions in the suboxic zone. The residence time of nitrogen is on the time scale of years in the suboxic zone and increases to hundreds of years in the deeper layers of the sea. Temporal variability could be observed in the pycnocline region. The excess N₂ in the deep water should progressively increase with time reflecting the increased rate of denitrification that has resulted from eutrophication.

Keywords- Black Sea, Suboxic Zone, Ventilation, Nitrogen Gas, Modeling

Introduction

The Black Sea is an ideal model site, or observatory, to study the sequence of reactions occurring over the redox gradient (redoxcline) from aerobic to anaerobic conditions. Such redoxclines are fascinating study sites as they contain communities of
microorganisms that catalyze oxidation-reduction reactions with a resulting change in speciation of the elements involved. In the Black Sea, at the boundary between the oxic surface and anoxic deep layers, there is a suboxic zone (at approximately 100m depth) where \( \text{O}_2 \) and \( \text{HS}^- \) are extremely low and do not exhibit any perceptible vertical or horizontal gradients (Murray et al., 1989). The distributions of nutrients (e.g. \( \text{NO}_3^- \), \( \text{NH}_4^+ \)) and metals (e.g. Mn, Fe) and the absence of \( \text{O}_2 \) and \( \text{HS}^- \) suggest the occurrence of novel metabolic processes within the suboxic zone (e.g., anaerobic Mn(II), Fe(II), \( \text{NH}_4^+ \) oxidation and the possibility for very low light photosynthetic reactions). Nitrogen cycling is an integral part of many of these reactions. Many features of the suboxic zone of the Black Sea make it an ideal location to study some of the poorly studied microbially-catalyzed reactions:

1. This is a **low \( \text{O}_2 \) environment**. The \( \text{O}_2 \) concentrations vary from \(<2 \ \mu\text{M}\) to \(10 \ \mu\text{M}\) (Luther III et al., 1991) and do not exhibit any perceivable gradients (Murray et al., 1989).

2. The chemical distributions suggest a **vertically layered sequence** of nutritional environments and biological communities

3. The redox gradients are vertically **well separated** (on the order of m rather than cm or less) which makes them easier to study than similar gradients in sediments and biofilms

4. Chemical distributions are **stable and predictable** on density surfaces on an interannual time scale. The distributions in this layer have been reproduced repeatedly over the past 10 years, most recently during the 2001 Knorr cruise.

5. Many reactions involve production and consumption of **gases** (e.g. \( \text{N}_2 \), \( \text{N}_2\text{O} \)) and are only carried out microbially which serve as the good tracers of specific metabolic processes.

6. The water column environment lends itself well to experimental studies of the role of bacteria in specific processes. For example, poisoned and inhibited control experiments can be more readily applied to study the relative importance of **biological versus abiological** reactions.

7. This is a **low light environment** (\( I < 0.0005\% \) of surface irradiance (Overmann et al., 1992)) but bacteriochlorophyll-e is present, suggesting a role for light harvesting reactions at very low light levels (Repeta & Simpson, 1991).

8. This site is readily **accessible**.

In this suboxic zone a complete progression of redox reactions appears to be well resolved in the water column, in a predictable and reproducible way, on characteristic density surfaces (Fig. 1), within a suitable depth range for resolution by pump-type sampling (Vinogradov and Nalbandov, 1990; Codispoti et al., 1991; Murray et al.,
Other anoxic interfaces and suboxic environments have been observed, but they are either unpredictable (not at steady state, e.g. Saanich Inlet, (Emerson et al., 1982; Tebo et al., 1984); Orca Basin, (Sheu, 1990; VanCappellen et al., 1998; Wiesenberg et al., 1985); Chesapeake Bay, (Luther III et al., 1988; Millero, 1991; Cariaco Trench, (Hastings & Emerson, 1988; Zhang & Millero, 1993)) or occur in sediments that are intrinsically more difficult to study in a predictable way with large volume samples. Suboxic conditions exist in the oxygen minima of the world's oceans (especially the northern and southern eastern tropical Pacific and the Arabian Sea) but there they do not extend through the entire sequence from aerobic to anoxic conditions (e.g. Cline & Richards, 1972; Rue et al., 1997).

The characteristic features of chemical species in the vertical profiles fall on the same density surfaces generally regardless of the location (Fig. 1)(Table 1; Murray et al., 1995). The main exception is the SW region where the suboxic zone as defined here does not exist because of ventilation by the Bosporus plume (see Murray et al., 2002). Thus, chemical distributions and redox reactions can be discussed in terms of a vertical density scale and can be clearly identified and sampled. The oxygen and sulfide distributions are of particular interest because they are used to define the oxic-anoxic interface. In the central gyre regions, dissolved oxygen decreases to concentrations less than 10 µM by about $\sigma_t = 15.6$ (Fig. 1) and sulfide does not begin to increase (>10 nM) until $\sigma_t = 16.15$ (dashed lines in Fig. 1) (Murray et al., 1995). Oguz et al (2001) reproduced these distributions with a simple 1-D model that linked a sequence of biogeochemical reactions with simple physics.

**Fig. 1** The distributions of oxygen, sulphide, nitrate, ammonium and dissolved manganese. This is a composite Fig. showing oxygen from Knorr 1988 and the rest of the data are from Knorr 2001.
**Temporal Variation**

Temporal variability exists for the thickness of the suboxic zone (Konovalov and Murray, 2001). This is primarily due to changes in the upper boundary where the decrease of oxygen with depth is influenced by oxygen consumption by the downward flux of particulate organic matter, which increased from the 1970s to the 1990s due to eutrophication, and oxygen injected by climate related variability in ventilation of the Cold Intermediate Layer (CIL) (Murray et al., 2002). The lower boundary, or the first appearance of sulfide, remained stable through this period.

The distributions of nitrate and ammonium versus density from the 1969 Atlantis II cruise and 1988 Knorr cruise are shown in Fig. 2. Nitrate data from the 1995 R/V Professor Kolesnikov is also shown. This Fig. demonstrates that the distributions and gradients have varied by several-fold over a time scale of decades. The concentrations of both nitrate and ammonium increased from 1969 to 1988. Nitrate then decreased from 1988 to 1995. The nitrate maximum stayed at about the same density during this time, while the profile of ammonium shoaled slightly. The increase in nitrate probably reflects eutrophication of the Black Sea due to anthropogenic inputs while the increase in ammonium is probably due to an increase in the flux of particulate organic matter resulting from this eutrophication. As a result of the changes in the gradients, the fluxes of nitrate and ammonium into the suboxic zone have also varied. Thus there should be corresponding changes in the rate of denitrification and production of N₂.

![Fig. 2 Temporal variability in nitrate and ammonium versus density](image)

**New data for nitrogen species**

New data for nitrogen species were collected from the 1999 and 2000 R/V Bilim cruises. Additional samples from the Knorr 2001 cruise are still being analyzed. Gas samples were collected according to the method of Emerson et al. (1991) and Quay et al. (1993). Water collected from rosette casts was introduced into evacuated glass “eggs”
containing mercuric chloride poison until half-full, or about 100 ml. Samples were shipped back to the UW lab where they were equilibrated in a temperature controlled water bath for at least 12 hours and then extracted and purified of CO₂ and H₂O. Measurements of N₂/Ar and d¹⁵N₂ were made on each sample by direct injection into a Finnigan MAT 251 isotope ratio mass spectrometer. Measurements were made relative to in-lab standards which are calibrated periodically to outside air.

Data from the Bilim 2000 cruise are shown in Fig. 3. Samples were taken from the western part of the western gyre. N₂ concentrations were calculated from the N₂/Ar ratios assuming atmospheric equilibrium with Ar. Excess N₂ is close to zero at the sea surface as expected for atmospheric equilibrium. Throughout the rest of the Black Sea water column there is excess N₂. The concentrations increase linearly from the surface to a density of about σ₀ ~16.0 and then decrease with depth. The prominent maximum in the suboxic zone (with excess N₂ of 20 µM) corresponds to the depth where NO₃⁻ and NH₄⁺ decrease to zero (Fig. 1). Nitrogen decreases below the suboxic zone but remains elevated with a fairly high value of excess N₂ = ~10 µM in the deep Black Sea from 400m to 1000m.

This profile leads to two immediate questions
1. Can the observed distribution of nitrate and ammonium support the observed maximum of N₂?
2. How can we explain the excess of nitrogen gas observed in the deep water anoxic zone?

These questions can be addressed using model simulations.

Model simulations of the nitrogen species
Modeling studies were used to address the questions posed in the preceding section. A one-dimensional stationary (steady state) model of vertical exchange in the Black Sea (Fig. 4A), based on the solution to the inverse problem for temperature and salinity, with known inputs for salt and heat (Samodurov and Ivanov, 1998; Ivanov and
Samodurov, 2001), was used to calculate the basin-average vertical distribution of turbulent diffusivity, $k(z)$, and vertical velocity, $w(z)$. The source of water to the upper boundary ($q_B$) corresponds to the Bosporus Inflow with mean temperature and salinity values of $T_B$ and $S_B$. The upper water column is divided into two layers. The upper layer from depths $Z_*$ to $Z_o$ is the zone of entrainment. The deeper layer from $Z_o$ to $Z_b$ is the zone of ventilation. The boundary $Z_o$ is set at 100m or $\sigma = 15.75$. The intrusion volume equals the original Bosporus inflow plus entrainment of overlying water (mostly CIL) on the shelf. The ratio of entrainment is adjusted to make steady state $T$, $S$ profiles. Upwelling ($w(z)$) is calculated from the underlying ventilation and then the value of $k(z)$ is adjusted. The calculated profiles of vertical velocity (Fig. 4C) and turbulent diffusivity (Fig. 4D) vary throughout the water column and are essentially non-linear.

Fig. 4  (a) Schematic diagram of vertical model with entrainment; (b) Residence times from model (line) and as calculated from CFC tracers (Lee et al., 2002)(vertical bars),  (c) Model calculated upwelling velocity versus density, (d) Model calculated vertical mixing rate versus density. See Samodurov and Ivanov (1998) and Ivanov and Samodurov (2001) for complete model description.
These physical transport parameters are used to calculate fluxes and to recalculate the vertical profile of chemical species (e.g., nitrogen gas) for a specified rate of production (source term). The residence time of water in layer $Z_o$ to $Z_b$ increases with depth from a few years at $\sigma_t = 15.5$ to $>300$ years at $\sigma_t = 16.9$ because the rate of ventilation decreases with depth. The residence times from the model are shown as a curve in Fig. 4B. These agree well with residence times (bars in Fig. 4B) from the CFC model of Lee et al (2001).

The Input of NO$_3$ with the Bosporus inflow

We first conducted calculations to determine if the flux of NO$_3$ with the Bosporus plume could support the excess N$_2$ in the deep water. Some of the NO$_3$ comes from the input of Mediterranean water through the Bosporus and some comes from entrained water of the CIL. We assume that the NO$_3$ injected reacts with sulfide to produce N$_2$ (possibly by the reaction $2\text{NO}_3^- + \text{HS}^- = \text{N}_2 + \text{SO}_4^{2-}$ or $\text{S}_8^{n_0}$). The lateral flux of nitrate from the upper layer to the Bosporus plume and the lateral flux of nitrate injected into the lower, anoxic zone is shown in Fig. 5a. The right panel (Fig. 5b) shows the equivalent vertical integration of the lateral flux of nitrate converted to nitrogen gas (two moles of nitrate produce one mole of nitrogen gas) that must exist to compensate this calculated flux of nitrate to the anoxic zone. Once the vertical source/sink flux is known, the corresponding profile of nitrogen gas can be calculated using the physical transport model with known $K$ and $W$. The resulting profile of N$_2$ (Fig. 6a versus density; Fig. 6b versus depth) demonstrates that the flux of nitrate with the Bosporus plume is not sufficient to support the observed excess of N$_2$ below the depth of 400 m. Only ~20% of the observed excess of N$_2$ (~2 µM of the ~10 µM required) can be attributed to the flux of nitrate. Another source of N$_2$ is required.

Fig. 5 (a) The vertical flux of NO$_3$ to the Bosporus plume by entrainment (negative above $\sigma_t = 15.75$) and the flux of NO$_3$ injected by the Bosporus Plume into deep water below $\sigma_t = 15.75$ (positive) (b) the equivalent vertically integrated lateral flux of N$_2$
Entrainment of N₂ from the CIL

Another source of nitrogen gas for the anoxic zone is the excess nitrogen gas that exists above the suboxic zone (Fig. 3). This excess N₂ is originally produced in the suboxic zone and then transported to the overlying CIL. The water from the CIL is then entrained into the Bosporus plume and the nitrogen gas is ventilated into the deep water. This source appears to be several fold larger than that from nitrate. The lateral entrainment and ventilation fluxes of N₂ versus density are shown in Fig. 7a. The combined integrated vertical flux due to ventilation of both N₂ and NO₃ is shown in Fig. 7b. Entrained N₂ provides about 11 µM excess over its saturated value compared to about 2 µM of N₂ from the NO₃ in the plume water.

Fig. 6 (a) Vertical profile versus density of excess N₂ resulting from the source/sink terms from Fig. 5 and the physical transport terms. (b) Excess N₂ profile versus depth.

Fig. 7 (a) Entrainment (negative) and ventilation (positive) fluxes of N₂ versus density, (b) the combined vertically integrated lateral flux of N₂ (from NO₃ and recycled N₂) versus depth.

The calculated vertical profile of N₂ versus density (Fig. 8a) and depth (Fig. 8b) demonstrates that the combined fluxes of nitrate and N₂ can support the observed excess of nitrogen in the anoxic zone. Some comes from ventilation of NO₃ but most comes from ventilated N₂ originally produced in the suboxic zone.
**1-D Biogeochemical - Transport Model**

The numerically simulated vertical distributions of the major components of the 1-D BGC model are shown in Fig. 9. Model simulated profiles of $O_2$, $H_2S$, Mn(II), NO$_3$, NH$_4$ and N$_2$ are shown. Model results are shown as dashed lines while the observed data are shown as solid lines. Two model lines are shown for N$_2$ in the deep water. One is the steady state solution and the other is the non steady state profile after 100 years.

**Fig. 8** (a) Predicted profiles of excess N$_2$ versus density; (b) versus depth.

Fig. 9 1-D model distributions of $O_2$, $H_2S$, Mn(II), NO$_3$, NH$_4$ and N$_2$ versus density. The model results are dashed lines while the observed data are solid lines.

Fig. 10 Residence time of N$_2$ as a function of density.
The simulated maximum of about 17 µM in the suboxic zone is close to the observed value of 15 µM (Fig. 3). The upward flux of ammonia and the downward flux of NO$_3$ result in a N$_2$ maximum that agrees well with the data. This supports the argument that some apparent net Anammox like reaction in the form of NO$_3$ + NH$_4$ = N$_2$ occurs. The simulated profile of excess nitrogen gas shows good agreement with the observed linear profile above the suboxic zone. The deep water part of the simulated N$_2$ profile remains far from steady state after a simulated period of 100 years. Concentrations of N$_2$ in the lower part of the anoxic zone show a tendency to increase with time, but the rate of this growth is very small and the residence time of nitrogen gas ($\tau = \text{concentration/flux}$) increases dramatically towards the bottom layer where $\tau \sim 1000$ years (Fig. 10).

Summary and Conclusions
A. Excess N$_2$ is close to atmospheric equilibrium at the air-sea interface of the Black Sea. There is a maximum of excess N$_2$ over atmospheric equilibrium in the suboxic zone that reaches 20 µM. Excess N$_2$ decreases with depth below the suboxic zone but remains elevated at about 10 µM in the deep water.
B. The transport-reaction model results show that the upward flux of ammonia and the downward flux of nitrate can simulate this maximum in excess N$_2$. A reaction in the form of NO$_3$ + NH$_4$ = N$_2$ is required. Such a reaction is similar to the Anammox reaction observed in other suboxic reactor and natural environments.
C. The flux of nitrate with the Bosporus plume (original Bosporus inflow plus entrained cold intermediate layer water) cannot support the observed excess of nitrogen gas in the anoxic zone below the depth of 400m.
D. The high values of excess N$_2$ in the deep water result from the combined lateral flux of both nitrate and nitrogen gas that are entrained to the Bosporus plume. Recycled N$_2$ makes the largest contribution. The excess nitrogen gas entrained from the CIL ultimately originated from Anammox like reactions in the suboxic zone.
E. The residence time of nitrogen is on the time scale of years in the suboxic zone and increases to hundreds of years in the deeper layers of the sea. The rate of denitrification (via Anammox) due to flux of nitrate and ammonium to the suboxic zone varies several-fold on a time scale of years to decades due to eutrophication of the Black Sea. Thus the presently observed excess of nitrogen of 10 µM in the anoxic zone corresponds to a lower rate of denitrification that existed prior to the current conditions. The excess N$_2$ in the deep water should progressively increase with time reflecting the increased rate of denitrification that has resulted from eutrophication.

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References


Konovalov S.K. and 11 others (this volume) Oxygen fluxes, redox processes and the suboxic zone in the Black Sea.


