Nitrous oxide cycling in the Black Sea inferred from stable isotope and isotopomer distributions

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Abstract

The low-oxygen regions of the world's oceans have been shown to be major sources of nitrous oxide, a trace gas in the atmosphere that contributes to both greenhouse warming and the destruction of stratospheric ozone. Nitrous oxide can be produced as a by-product of nitrification or an intermediate of denitrification; low oxygen conditions enhance the yield of nitrous oxide from both pathways. We measured the concentration and isotopic composition of dissolved nitrous oxide at several stations in the Black Sea, an anoxic basin with a well-defined suboxic layer that separates the ventilated surface waters from the sulfidic deep waters. Our data show that in contrast to other low-oxygen marine regions, nitrous oxide does not accumulate in the Black Sea at significant levels. Moreover, whereas the reduction of nitrous oxide by denitrification usually yields residual gas that is enriched in both stable isotopes, in the Black Sea declining nitrous oxide concentrations are accompanied by enrichment in ^{18}O-N$_2$O but depletion in ^{15}N-N$_2$O. We measured a minimum δ^{15}N-N$_2$O value of -10.8 ± 0.8‰ vs. air N$_2$, by far the lowest measured to date for seawater. Measurements of the distribution of ^{15}N within the linear nitrous oxide molecule reveal that this unusual isotopic signal is most pronounced in the end-position nitrogen, and that site preference, or the tendency for ^{15}N to be found in the center-position nitrogen, co-varies positively with ^{18}O-N$_2$O. We surmise that the highly unusual isotopic composition of Black Sea nitrous oxide is the result of two processes: production of ^{15}N-depleted nitrous oxide by ammonium oxidation followed by its reduction by denitrification, which causes enrichment in ^{18}O and enhancement of ^{15}N-site preference. Bottle incubation experiments with

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[15]N-ammonium and ^{15}N-nitrite reveal that both oxidation and reduction pathways to nitrous oxide are active in the Black Sea suboxic zone.

1. Introduction

Nitrous oxide (N_2O) is a potent greenhouse gas that also contributes to destruction of stratospheric ozone. Nitrous oxide exists in trace amounts in the atmosphere (about 318 ppb) but is increasing at a rate of 0.25% yr^{-1} and may significantly affect global climate over the next 100 yr (Albritton and Meira Filho, 2001). The oceans are a significant source of N_2O to the troposphere (Nevison et al., 1995), contributing about 30% of natural sources and 17% of total identified sources (Albritton and Meira Filho, 2001). Nitrous oxide can be produced in the oceans by nitrification, the conversion of ammonium to nitrite and nitrite to nitrate in the presence of free oxygen, and can be produced and consumed by denitrification, oxidation of organic matter using nitrogen oxides as electron acceptors in the absence or near absence of free oxygen (Zafiriou, 1990; Capone, 2000). In the open ocean, nitrification most likely dominates N_2O production in oxygenated water (Dore et al., 1998; Popp et al., 2002), but accumulation of N_2O in the oxygen minimum zone has been attributed to both nitrification (Yoshinari, 1976; Cohen and Gordon, 1978; Kim and Craig, 1990; Butler and Elkins, 1991) and denitrification (Yoshida et al., 1989; Yamagishi et al., 2005), and the question as to which process is more important remains controversial. Nitrification and denitrification may both be significant sources of N_2O in regions of intense upwelling and oxygen draw-down (e.g., seasonal upwelling off the west coast of India: Naqvi et al., 2000), and nitrifying organisms can produce N_2O from nitrite using a denitrification pathway under low-oxygen conditions (Yoshida et al., 1989; Ostrom et al., 2000; Wrage et al., 2001; Casciotti, 2002). The remaining ambiguities in the marine N_2O cycle make it difficult to constrain its current fluxes from the ocean, or to forecast response of this important greenhouse gas to such forcings as coastal eutrophication (Bange, 2000) or open-ocean iron enrichment (Fuhrman and Capone, 1991; Jin and Gruber, 2003). Recent identification of anaerobic ammonia oxidation (Dalsgaard et al., 2003; Kuypers et al., 2003) as a quantitatively significant process in the marine nitrogen cycle (Devol, 2003) further complicates our understanding of the processes that can produce and consume N_2O.

The highest concentrations of N_2O in the ocean have been found in low-oxygen conditions over the western Indian shelf (533 nM; Naqvi et al., 2000) and above the oxygen minimum zones in the eastern tropical North Pacific (173 nM; Westley et al., 2001) and eastern tropical South Pacific (173 nM, Codispoti et al., 1992). Nitrous oxide production by chemoautrophic ammonia oxidizers has been shown to be enhanced by low oxygen conditions (Goreau et al., 1980). In addition, trace amounts of oxygen promote NO production by denitrifiers as the enzyme N_2O reductase is more sensitive to inhibition by oxygen than are other enzymes in the denitrification pathway (Knowles, 1982). The association of N_2O with low oxygen motivated our participation in the June 2001 R/V *Knorr* research cruise to the Black Sea, an anoxic basin with a well-defined suboxic layer that separates the ventilated surface waters from the sulfidic deep waters (Murray and Izdar, 1989). Murray et al. (1995) described a nitrate maximum at the upper boundary of the suboxic zone with nitrite maxima above and below the nitrate peak in concentration. They identified probable zones of nitrification in the upper nitrite maximum and denitrification in the lower nitrite maximum. Both regions are candidates for N_2O production, and the lower region is a candidate for N_2O consumption. We measured the concentration and stable isotopic compositions of N_2O from three stations in the Black Sea, with additional concentration measurements near the mouth of the Bosporus and over the northwestern shelf (Fig. 1). Our data show that in contrast to other marine low-oxygen regions, N_2O does not accumulate in the Black Sea at significant levels. This work accords well with initial measurements of N_2O concentration performed on water samples collected during the 1988 Black Sea expedition (Butler and Elkins, 1991). Here, we apply the additional tools of stable isotope analysis of ambient N_2O as well as stable isotope tracer
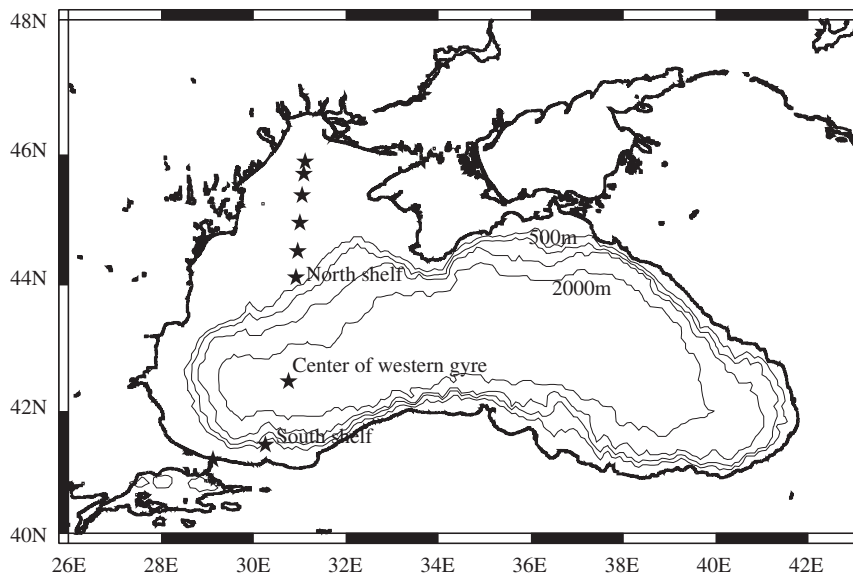
Fig. 1. Map showing sample locations for nitrous oxide analysis on Leg 2 of the R/V *Knorr* Black Sea Expedition (June 1–10, 2001). Data presented in this paper come from the center of the western gyre (42.48°N, 30.76°E), a south shelf-break station (41.46°N, 30.26°E) and a north shelf-break station (44.12°N, 30.92°E). Depth contours are drawn every 500 m from 500 to 2000 m.

addition experiments to understand better the Black Sea N_2O cycle.

Culture studies have shown that bacterial production of N_2O by nitrification and denitrification produces gas depleted in ^{15}N and ^{18}O relative to its source material (Yoshida, 1988; Barford et al., 1999; Casciotti, 2002), whereas consumption of N_2O by denitrification leaves the residual gas isotopically enriched (Barford et al., 1999). Natural abundance stable isotope measurements of dissolved N_2O in seawater have been used to infer rates and pathways of N_2O production (Yoshida et al., 1989; Yoshinari et al., 1997; Dore et al., 1998; Naqvi et al., 1998; Ostrom et al., 2000; Westley et al., 2001; Popp et al., 2002). Recent innovations in mass spectrometry that enable determination of the position of ^{15}N within the linear NNO molecule (Toyoda and Yoshida, 1999) provide an additional dimension for studying mechanisms of N_2O production in the ocean (Popp et al., 2002; Toyoda et al., 2002) and terrestrial ecosystems (Perez et al., 2001). Toyoda and Yoshida (1999) used the term "isotopomers" to distinguish between molecules of the same mass but different mass distributions. We report the first isotope and isotopomer measurements of N_2O from the Black Sea. We also present the results of bottle incubations with ^{15}N tracers performed on water collected from above and within the suboxic zone (defined as that layer with oxygen concentration

below 10 μM and low oxygen gradients but no measurable sulfide) and discuss their implications for N_2O cycling in the Black Sea. The combined natural abundance isotope and isotopomer measurements, and isotope uptake experiments reveal that the N_2O pool is highly dynamic and that nitrous oxide is simultaneously produced and consumed in the suboxic zone of the Black Sea.

2. Methods

Water samples were collected from CTD casts at the mouth of the Bosporus (41.21°N, 29.12°E), the center of the western gyre (42.5°N, 30.75°E), a southern shelf-break station (41.46°N, 30.26°E), a northern shelf-break station (44.12°N, 30.92°E), and at four stations over the northwestern shelf of the Black Sea (Fig. 1). Samples were transferred from Niskin bottles mounted on a 24-bottle CTD-rosette into pre-combusted glass serum bottles using Tygon tubing, allowing each serum bottle to overflow at least twice its volume to minimize gas exchange with the atmosphere during sample collection. Samples were preserved with mercuric chloride and sealed with gas-tight butyl rubber septa, and sent by air freight to the University of Hawaii and the Tokyo Institute of Technology. Oxygen and nutrient concentrations from the same Niskin bottles were measured at sea by colleagues

using standard Winkler titrations (Konovalov et al., 2002) and a shipboard autoanalyzer system (Tugrul and Salihoglu, 2002). All CTD and nutrient data are available on the web at www.ocean.washington. edu/cruises/Knorr2001.

Nitrous oxide concentrations were measured at the University of Hawaii by headspace equilibration in a gas-tight syringe, followed by packed-column gas chromatography using an electron capture detector (Cohen, 1977) calibrated with commercial gas mixtures. Precision of this method is estimated to be $\pm 5\%$ based on replicate sample analyses.

Total or "bulk" $\delta^{15}N$ and $\delta^{18}O$ in N_2O were measured at the University of Hawaii using a modification of the analytical system used to measure $\delta^{13}C$ of dissolved methane in seawater (Sansone et al., 1997) as described in Dore et al. (l998) and Popp et al. (2002). Isotope values are reported using delta notation as per mil versus air N_2 for nitrogen and per mil versus Vienna Standard Mean Ocean Water (VSMOW) for oxygen (e.g. $\delta^{15}N = [(R_{sample} - R_{standard})/R_{standard}] \times 1000$, where $R = {}^{15}N/{}^{14}N$). Analyses of triplicate 240 ml samples of near-surface seawater (~ 6 nM N_2O) yield reproducibilities of better than $\pm 0.5\%$ for $\delta^{15}N$ and $\pm 0.8\%$ for $\delta^{18}O$. The isotopic composition of the laboratory standard gas was characterized using the traditional analytical methods of Yoshida and Matsuo (1983) and Kim and Craig (1993). Calibration of N_2O concentration on the mass spectrometer is achieved using a commercial gas mixture and an appropriate range of sample loops, and corresponds well with those determined using the gas chromatograph.

Isotopomer analyses were performed at the Tokyo Institute of Technology using an MAT 252 mass spectrometer following the method of Toyoda and Yoshida (1999). Nitrous oxide is an asymmetric linear molecule (NNO) with one nitrogen atom in the central or α position and a second nitrogen atom in the end or β position. Given this structure and the stable isotopes of oxygen and nitrogen (${}^{14}N$, ${}^{15}N$, ${}^{16}O$, ${}^{17}O$ and ${}^{18}O$), there are 12 possible isotopomers of N_2O but only five are sufficiently abundant in nature to quantify. Toyoda and Yoshida (1999) devised a method for distinguishing between ${}^{14}N{}^{15}N{}^{16}O$ and ${}^{15}N{}^{14}N{}^{16}O$ by measuring the difference in mass between N_2O ions and fragment NO ions produced in the ionization chamber of the mass spectrometer. Thus $\delta^{15}N^{\alpha}$ is a measure of ${}^{15}N$ enrichment in the central or α position of N_2O, whereas $\delta^{15}N^{\beta}$ is a measure of ${}^{15}N$ enrichment in the

end or β position. Total or "bulk" $\delta^{15}N$ and $\delta^{18}O$ data are also available from these measurements. The reproducibilities of this method reported for triplicate analyses of 125 ml samples of near-surface seawater (6.7 nM dissolved N_2O) are $\pm 0.9\%$ for $\delta^{15}N^{\alpha}$, $\pm 1.5\%$ for $\delta^{15}N^{\beta}$, $\pm 0.6\%$ for bulk $\delta^{15}N$, and $\pm 0.9\%$ for $\delta^{18}O$ (Toyoda et al., 2002). A comparison of 30 pairs of samples collected between the surface and 4000 m in the subtropical North Pacific shows that the University of Hawaii measurements differ from the Tokyo Institute of Technology measurements by an average of -0.13% in "bulk" $\delta^{15}N$ and -1.37% in $\delta^{18}O$ (Popp et al., 2002). We report values from both laboratories without adjustment.

Water samples for two sets of exploratory ${}^{15}N$-tracer incubation experiments were collected from CTD casts at the center of the western gyre (42.5°N, 30.75°E) using acid-washed (10% HCl) silicone tubing and new serum bottles that had been soaked for 24 h in 10% HCl at 60 °C, prior to being rinsed in MilliQ water and combusted overnight at 500 °C. Water was allowed to overflow twice to minimize gas exchange, the collecting tube was carefully withdrawn, chilled ${}^{15}N$ tracer solutions were added by pipette, and the bottles were sealed with non-toxic, gas-impermeable Viton rubber stoppers and aluminum crimps. The tracers added were solutions of 99.9% ${}^{15}N$ ammonium chloride and potassium nitrite, each added to separate bottles at a concentration estimated to make up 10% of the ambient concentration of the relevant ion. Tracer volumes were 200 μl or smaller to minimize the amount of dissolved oxygen introduced with the tracer solution, and sample handling time was kept as short as possible. An ammonium uptake experiment was performed on water collected from $\sigma_t = 15.3$, which had oxygen and ammonium concentrations of 14 μM and 100 nM, respectively, and an ambient temperature of 8 °C. Parallel ammonium and nitrite uptake experiments were performed on water collected from $\sigma_t = 15.6$, which had an oxygen concentration of 4 μM, ammonium and nitrite concentrations of 100 and 40 nM, respectively, and an ambient temperature of 8 °C. Experiments were incubated in the dark at 8 °C for 12, 24 or 48 h before being terminated with an injection of mercuric chloride. Controls were handled in an identical manner as treatments, except no tracer solutions were added. Bottles were sent to the University of Hawaii and the Tokyo Institute of Technology by air freight and were analyzed by

mass spectrometry as described above to determine the uptake of tracer ^{15}N into the dissolved N_2O pool. Many incubations showed increases in mass 46, which we interpret as evidence for doubly labeled N_2O (i.e. $^{15}N^{15}N^{16}O$), since it is unlikely that the ^{18}O content of N_2O in our tracer incubations would vary between treatments and controls. We reallocate excess mass 46 units to mass 45 to account for the total concentration of ^{15}N in the N_2O pool.

3. Results and discussion

3.1. Distribution of the concentration and isotopic compositions N_2O

Nitrous oxide concentrations measured at the center of the western gyre, the southern shelf break, and the northern shelf break are shown in Fig. 2. We show data from each station in two panels: the left panels (Fig. 2(A), (C) and (E)) show N_2O concentration with the calculated saturation value (i.e. the concentration of N_2O that would exist if the water were in equilibrium with air, calculated from CTD values of temperature and salinity following Weiss and Price (1980)) and nitrate concentration (Tugrul and Salihoglu, 2002) plotted for comparison. The right panels (Fig. 2(B), (D) and (F)) show oxygen, sulfide (Konovalov et al., 2002) and ammonium (Tugrul and Salihoglu, 2002) concentrations for the same density interval. At each station, the concentration of N_2O showed a small maximum near $\sigma_t = 15$, directly above the nitrate maximum and coincident with the base of the oxycline, where oxygen concentrations ranged between 30 and $100\,\mu M$. The maximum was most pronounced at the western gyre station (Fig. 2(A)), where N_2O concentration reached 14.4 nM at $\sigma_t = 15.3$ (70 m). This represented 118% saturation relative to water of the same temperature and salinity in equilibrium with air. The flux of N_2O to the mixed layer from this maximum in N_2O concentration calculated from the concentration gradient ($0.184\,nM\,m^{-1}$) assuming an eddy diffusion coefficient of $9.26 \times 10^{-6}\,m^2\,s^{-1}$ (Samodurov and Ivanov, 1998) was $147\,nmol\,m^{-2}\,d^{-1}$. A similar calculation performed on data from the eastern tropical North Pacific (Westley et al., 2001) and assuming an eddy diffusion coefficient of $2 \times 10^{-6}\,m^2\,s^{-1}$ (Lewis et al., 1986) yielded $5950\,nmol\,m^{-2}\,d^{-1}$ making the Black Sea a rela-

tively insignificant source of N_2O to the troposphere.

Our western gyre N_2O profile was almost identical to measurements performed in 1988 from water collected near the center of the Black Sea (43°N, 34°E: see Fig. 1) and analyzed using gas chromatography (Butler and Elkins, 1991). The similarity suggests that the modest maximum in N_2O concentration near the base of the oxycline, above a zone of rapid N_2O decline, is a persistent and reproducible feature in the Black Sea. One characteristic of the N_2O concentration profile that did not appear in the 1988 measurements is the small peak in N_2O concentration at $\sigma_t = 15.9$ (92 m), which also appeared at the southwest shelf-break station at around $\sigma_t = 16.2$ (about 140 m) but did not appear at the northwest shelf-break station. These secondary N_2O concentration maxima are most likely caused by the lateral injection of oxygenated Bosporus water within and below the suboxic zone (Samodurov and Ivanov, 1998; Konovalov et al., 2003) allowing the oxidation of ammonium to N_2O, nitrite and nitrate. In addition, Mediterranean water injected into the Black Sea via the Bosporus plume contains elevated levels of N_2O (about 200% saturation, measured at $\sigma_t = 26.9$, or 60 m, at the mouth of the Bosporus during this study), and this N_2O may influence concentration measurements at the southwest shelf station and western gyre stations. The existence of pronounced N_2O anomalies at the southwest shelf station and their absence at the northwest shelf station are consistent with the entrainment of Mediterranean water in a counterclockwise rim current (Oguz et al., 1994). During June 2001, Bosporus inflow water appeared to exert its strongest influence on the southwest shelf region of the Black Sea (Luther et al., 2002; Konovalov et al., 2003).

The $\delta^{15}N$ and $\delta^{18}O$ values of dissolved N_2O measured at the center of the western gyre (measured at the Tokyo Institute of Technology), the southwestern shelf break and the northwestern shelf break (both measured at the University of Hawaii) are shown in Fig. 3. We consider the central western gyre station in detail: between the surface and $\sigma_t = 14.4$ (about 55 m), the $\delta^{15}N$ and $\delta^{18}O$ values of dissolved N_2O averaged 7.6‰ and 45.7‰, respectively, which were similar to those of clean tropospheric air ($\delta^{15}N = 6.4$‰ vs. air N_2 and $\delta^{18}O = 45.5$‰ vs. VSMOW: Rahn and Wahlen, 1997) taking into account the minimal fractionation (0‰ for ^{15}N and -0.8‰ for ^{18}O) due to air-to-sea
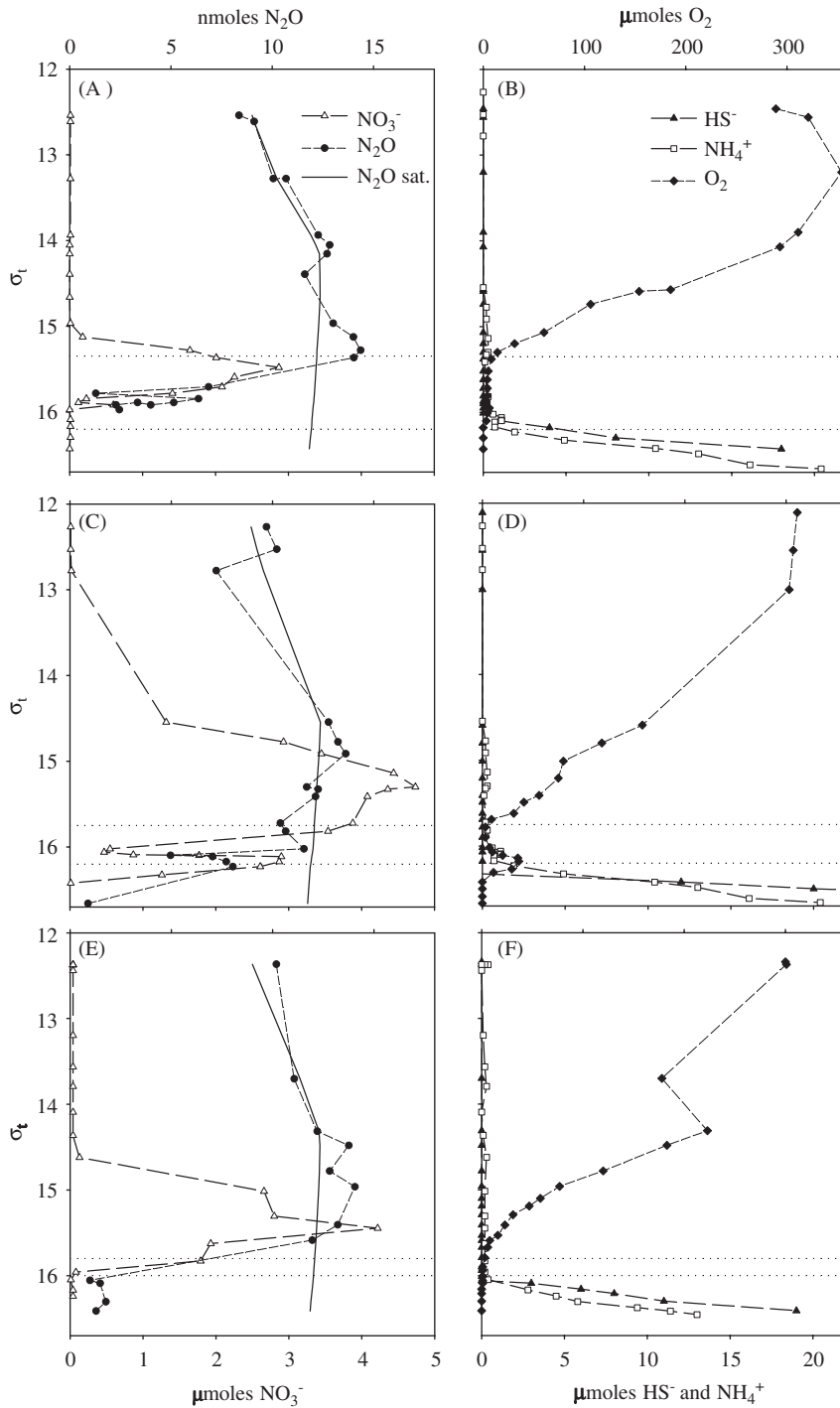
Fig. 2. The left panels show the concentration of nitrate (open triangles) and nitrous oxide (filled circles) measured at (A) the center of the western gyre, (C) the south shelf break and (E) the north shelf break. The solid line shows N_2O saturation calculated following Weiss and Price (1980) using CTD temperature and salinity data for each cast. The right panels show the corresponding concentrations of hydrogen sulfide (filled triangles), ammonium (open squares), and oxygen (filled diamonds) measured in the same water samples from (B) the center of the western gyre, (D) the south shelf break and (E) the north shelf break. The dotted horizontal lines mark the upper and lower boundaries of the suboxic zone defined by oxygen concentrations below $10 \, \mu M$, low oxygen gradients and no measurable sulfide.
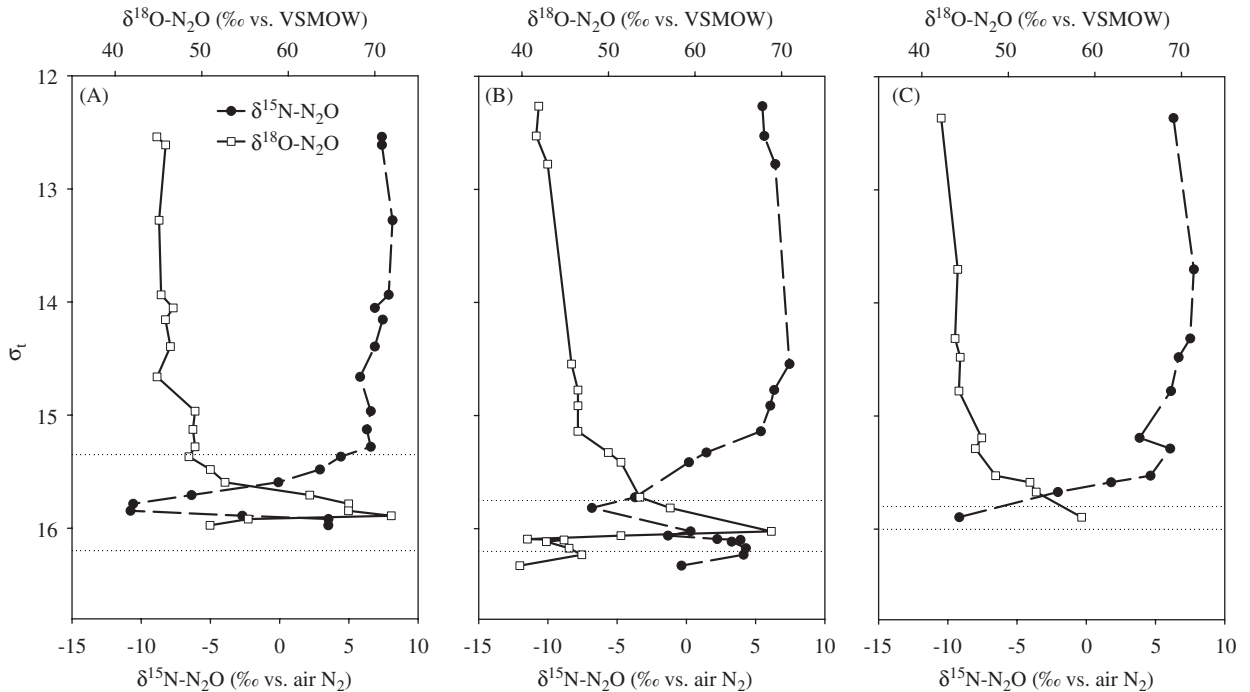
Fig. 3. The dual stable isotopic composition of N_2O measured at (A) the center of the western gyre, (B) the south shelf break and (C) the north shelf break. Values of $\delta^{15}N$-N_2O vs. air N_2 are plotted as filled circles and values of $\delta^{18}O$-N_2O vs. VSMOW are plotted as open squares. The dotted horizontal lines mark the approximate upper and lower boundaries of the suboxic zone.

transfer (Inoue and Mook, 1994). This indicates the strong influence of air-sea gas exchange on the isotopic characteristics of dissolved N_2O at these depths. Between $\sigma_t = 14.4$ and 15.3 (or 70 m), there was a slight depletion in ^{15}N combined with a small enrichment in ^{18}O (at $\sigma_t = 15.3$, $\delta^{15}N = 6.6 \pm 0.3‰$ and $\delta^{18}O = 49.2 \pm 0.6‰$), corresponding to a concentration maximum of 14.4 ± 0.7 nM. Oxygen concentration declined from almost 200 to 14 μM in this interval (Konovalov et al., 2002). Between $\sigma_t = 15.3$ and 15.85 (or 70–90 m), as O_2 declined to 3 μM, N_2O concentration also decreased, and the residual N_2O became increasingly enriched in ^{18}O but depleted in ^{15}N, with a minimum $\delta^{15}N$-N_2O value of $-10.8 \pm 0.8‰$ vs. air N_2 at $\sigma_t = 15.85$. This value was significantly lower than the lowest $\delta^{15}N$-N_2O reported for seawater to date (0.8‰ vs. air N_2 for upwelled waters in the Arabian Sea: Naqvi et al., 1998) and represented a depletion in ^{15}N of 18.2‰ relative to surface values. The $\delta^{18}O$-N_2O value at this location was $67.0 \pm 1.1‰$, representing an enrichment of 22.1‰ relative to surface values.

Below $\sigma_t = 15.85$, the isotopic trends reversed, with an increase in $\delta^{15}N$ and a decrease in $\delta^{18}O$

coincident with an increase in N_2O concentration. This trend reversal also was seen at the south shelf station but not at the north shelf station, which suggests that it is associated with the Bosporus plume. However, since there are no isotopic measurements of N_2O in the Bosporus, it is impossible to determine if the signal is produced by the transport and mixing of Bosporus-derived N_2O into the Black Sea, or by processes induced by the oxygenated Bosporus water creating new N_2O in situ.

3.2. Processes producing and consuming N_2O

The concomitant depletion in ^{15}N and enrichment in ^{18}O with decreasing N_2O concentration observed in the density interval of $\sigma_t = 15.3$ to $\sigma_t = 15.85$ contrasted with previously reported dual isotopic measurements of N_2O from other low-oxygen marine environments: Yoshinari et al. (1997), Naqvi et al. (1998), and Westley et al. (2001) all reported enrichment in both isotopes with decreasing N_2O concentration. The unusual isotopic signature in Black Sea N_2O could simply reflect unusual isotopic

compositions of precursor molecules. Alternatively, it could be derived from production and consumption processes, either an unusual combination of nitrification and denitrification reactions observed in other environments, or a novel biochemical pathway to N_2O. Available isotopic information for nitrate and ammonium, the presumed precursors to N_2O, cannot account for the unusually low $\delta^{15}N$-N_2O values. Dissolved nitrate samples collected from the same hydrocast as N_2O in the center of the western gyre were analysed for $\delta^{15}N$ (see Murray et al., 2005) but showed the opposite trend to $\delta^{15}N$-N_2O. In the density interval between $\sigma_t = 15.5$ and $\sigma_t = 15.85$, $\delta^{15}N$-NO_3^- increased from ~ 8‰ to ~ 19‰ while $\delta^{15}N$-N_2O decreased from 2.9‰ to -10.8‰. Fewer data are available for ammonium: Fry et al. (1991) reported a $\delta^{15}N$ value of 3.8‰ for ammonium collected at a depth of 218 m in the Black Sea, well within the anoxic zone, and Velinsky et al. (1991) found low $\delta^{15}N$-NH_4^+ (around 2‰) in Black Sea anoxic water, a maximum $\delta^{15}N$-NH_4 of ~ 8‰ at the suboxic/anoxic interface (around $\sigma_t = 15.85$), and a somewhat lower value of

~ 6‰ in the suboxic zone. There was no evidence for unusually low values of $\delta^{15}N$-NH_4 in the oxycline. These factors point instead to isotopic fractionations associated with the reactions that produce and consume N_2O. Here, the additional measurements of isotopomers of N_2O can provide valuable insight: since the precursor molecules only contain one atom of nitrogen, the distribution of ^{15}N within the linear NNO molecule should depend largely on reaction processes and not on $\delta^{15}N$ of the precursors (Toyoda et al., 2002).

The left panel of Fig. 4 shows the $\delta^{15}N$ composition of N_2O from the western gyre station, with "bulk" or total $\delta^{15}N$ plotted along with $\delta^{15}N^\alpha$, a measure of $\delta^{15}N$ in the central position nitrogen atom, and $\delta^{15}N^\beta$, $\delta^{15}N$ in the end position (following Toyoda and Yoshida, 1999). The right panel of Fig. 4 shows $\delta^{18}O$ composition of N_2O as well as the ^{15}N-site preference, or $\delta^{15}N^\alpha$–$\delta^{15}N^\beta$, a measure of the distribution of ^{15}N within the N_2O molecule. At all depths $\delta^{15}N^\alpha$ was enriched relative to $\delta^{15}N^\beta$, yielding a positive ^{15}N-site preference. Between the surface and $\sigma_t = 14.4$, while there is considerable
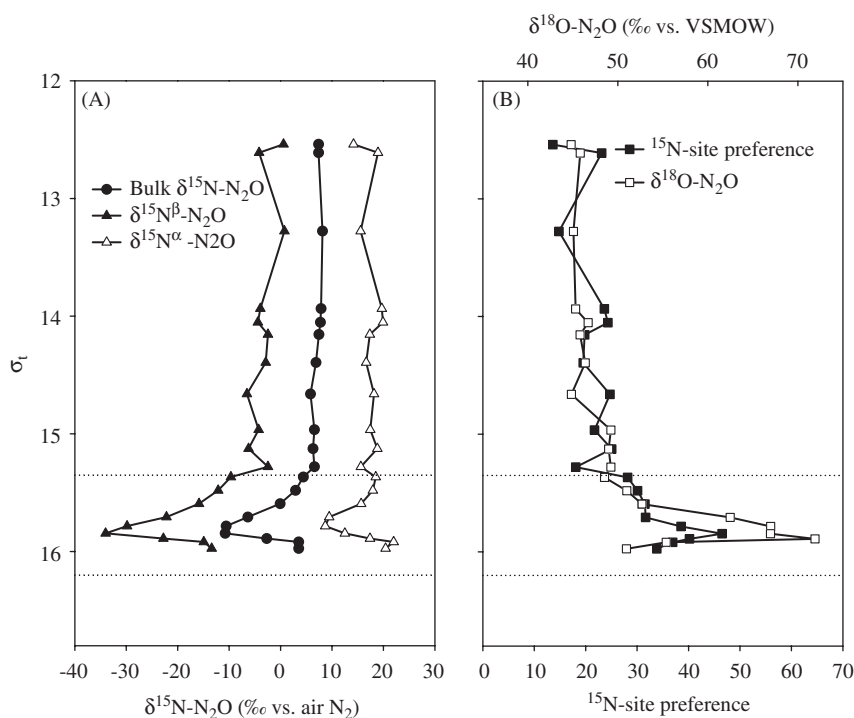

Fig. 4. The isotopomer composition of N_2O measured at the center of the western gyre. In panel (A), the $\delta^{15}N$-N_2O vs. air N_2 (filled circles) is decomposed into $\delta^{15}N^\alpha$ or ^{15}N abundance at the central position in NNO (open triangles) and $\delta^{15}N^\beta$ or ^{15}N abundance at the end position in NNO (filled triangles). Panel (B) shows ^{15}N-site preference, calculated as $\delta^{15}N^\alpha$–$\delta^{15}N^\beta$ (filled squares) plotted with $\delta^{18}O$-N_2O vs. VSMOW (open squares) for comparison. The dotted horizontal lines mark the approximate upper and lower boundaries of the suboxic zone.

noise in the data, the average ^{15}N-site preference was 19.8‰, similar to the tropospheric value of 18.7 ± 2.2‰, measured in air collected near the ground surface in Japan (Yoshida and Toyoda, 2000). The strong δ^{15}N-N$_2$O minimum at $\sigma_t = 15.85$ was present in both δ^{15}N$^\alpha$ and δ^{15}N$^\beta$, although it was considerably more pronounced in δ^{15}N$^\beta$. This yielded a sharp maximum in ^{15}N-site preference at this depth, with a value of 46.5‰, which coincided with the maximum in δ^{18}O . Site preference showed a positive linear correlation with δ^{18}O with a slope close to 1 ($R^2 = 0.72$) suggesting that the process that led to ^{18}O enrichment also led to enrichment in ^{15}N$^\alpha$, relative to ^{15}N$^\beta$. In contrast, the correlations between δ^{18}O and bulk δ^{15}N, δ^{15}Na and δ^{15}N$^\beta$ are all negative, suggesting the simultaneous action of two processes on the N$_2$O pool, one that introduces ^{15}N-depleted nitrogen into N$_2$O, and another that consumes N$_2$O leaving the residual with greater δ^{18}O and site preference.

If consumption were the only process affecting the isotopic composition of N$_2$O in the Black Sea suboxic zone, we could treat the suboxic zone as a closed system and apply the Rayleigh fractionation model to evaluate isotope effects associated with N$_2$O removal (Mariotti et al., 1981). In this case, the isotope effects can be calculated from the slopes formed when each delta value is plotted against $-\ln(f)$, where f is the fraction of N$_2$O remaining. Fig. 5 shows δ^{18}O, bulk δ^{15}N, ^{15}N-site preference, δ^{15}N$^\alpha$ and δ^{15}N$^\beta$ plotted as linear functions of $-\ln(f)$ for the density interval from $\sigma_t = 15.3$, the N$_2$O concentration maximum, to 15.85, the δ^{15}N-N$_2$O minimum. There is a strong, positive correlation between δ^{18}O and $-\ln(f)$ with a slope of 20.9 and an R^2 value close to 0.99. This implies that the reduction of N$_2$O yields a product that is depleted in ^{18}O by 20.9‰ relative to the source N$_2$O, leaving the residual N$_2$O correspondingly enriched in ^{18}O. Bulk δ^{15}N shows nearly the opposite trend, with a negative isotope effect of 18.2‰ ($R^2 = 0.99$), leaving residual N$_2$O depleted in ^{15}N. This trend is enhanced in the end-position nitrogen: the isotope effect for δ^{15}N$^\beta$ is -28‰ ($R^2 = 0.96$) whereas the isotope effect for δ^{15}N$^\alpha$ is only -8.4‰ ($R^2 = 0.72$). This difference results in the ^{15}N-site preference effect of 19.6‰ ($R^2 = 0.77$), similar in magnitude to the δ^{18}O isotope effect. It is difficult to imagine a bacterially mediated process that selectively consumes ^{15}N-enriched N$_2$O causing a negative isotope effect: Barford et al. (1999) reported a positive bulk ^{15}N isotope effect of 12.9 ± 2.6‰ for steady-state
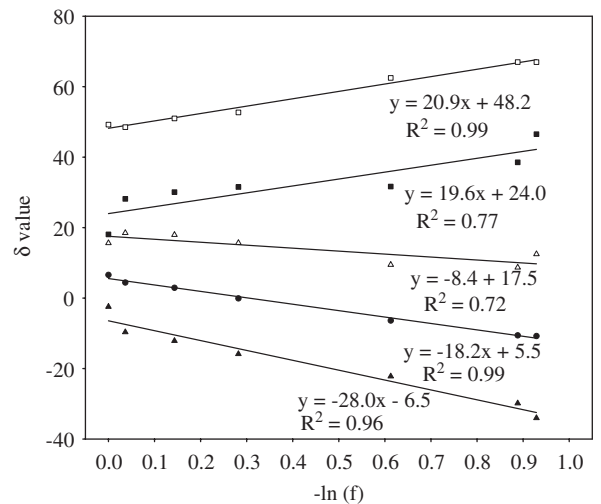

Fig. 5. Isotope values of N$_2$O from the suboxic zone at the center of the western gyre (from $\sigma_t = 15.3$ to 15.85) plotted against $-\ln(f)$, where f is the fraction of N$_2$O remaining. Open squares show δ^{18}O-N$_2$O, filled squares show ^{15}N-site preference, filled circles show bulk δ^{15}N-N$_2$O, open triangles show δ^{15}N$^\alpha$-N$_2$O, and filled triangles show δ^{15}N$^\beta$-N$_2$O. Results of linear regression analysis for each data set are shown as solid lines and linear equations with concomitant R^2 values: according to the Raleigh model, the slopes represent the isotopic fractionations associated with N$_2$O consumption.

reduction of N$_2$O to N$_2$ by a typical denitrifier, *Paracoccus denitrificans*. Thus the assumptions of the Rayleigh model, that the Black Sea suboxic zone behaves like a closed system and that consumption is the only process affecting the isotopomer distributions of N$_2$O, are unlikely to be true. Instead, it is likely that the isotope effects inferred from the Black Sea data are the result of more than one reaction: a production process that yields N$_2$O depleted in ^{15}N combined with a consumption process that selectively enriches the center position nitrogen in ^{15}N and the oxygen in ^{18}O.

Consumption that proceeds by attacking the N–O bond should yield residual N$_2$O with increased δ^{18}O and site preference because the presence of heavier isotopes increases bond strength. Yung and Miller (1997) showed that the relative bond strengths within isotopomers of N$_2$O vary inversely with their zero point vibrational energies, or ZPEs: ^{14}N–^{14}N–^{16}O has the highest ZPE and thus the weakest bonds, followed by ^{15}N–^{14}N–^{18}O, then ^{14}N–^{14}N–^{18}O and then ^{14}N–^{15}N–^{16}O. Thus regardless of the isotopic composition of the starting material, N$_2$O consumption should enhance the δ^{18}O and δ^{15}N of the residual (i.e. ^{14}N–^{14}N–^{16}O is

consumed more readily than any other isotopomer), but this effect will be more pronounced in $\delta^{15}N^{\alpha}$ than $\delta^{15}N^{\beta}$, leading to enhanced site preference. If we superimpose this consumption process onto a source of ^{15}N-depleted N_2O, we can reproduce our measured isotope and isotopomer profiles. Since the net effect of N_2O consumption should be ^{15}N-enrichment of the residual (e.g., Barford et al., l999 reported a bulk ^{15}N isotope effect of $12.9 \pm 2.6‰$ for the steady-state reduction of N_2O), and we estimate an apparent ^{15}N isotope effect of $-18.2‰$, it is likely that the original N_2O produced in the suboxic zone of the Black Sea is more extremely depleted in ^{15}N than our measurements reveal.

Both nitrification and denitrification produce N_2O that is depleted in ^{15}N relative to its source material: Yoshida (1988) measured an isotope effect of more than $60‰$ for N_2O production from ammonium by the soil nitrifier *Nitrosomonas europaea*; Casciotti (2002) estimated an analogous isotope effect of around $13‰$ for the marine nitrifier, *Nitrosomonas marina*; and Barford et al. (1999) reported an isotope effect of $28.6 \pm 1.9‰$ for the steady state production of N_2O from nitrate by the denitrifier, *Paracoccus denitrificans*. Let us first consider denitrification: at $\sigma_t = 15.85$, bulk $\delta^{15}N$-N_2O is $-10.8‰$ while $\delta^{15}NO_3^-$ is $\sim19‰$ (Murray

et al., 2005), a difference of $\sim30‰$. This is within the range of uncertainty of the measured isotope effect for denitrification; however, the bulk $\delta^{15}N$-N_2O value is likely to be an overestimate since it reflects combined isotope effects of production and consumption, and the isotope effect of consumption is strongly positive. Ammonium oxidation is a better candidate: measured $\delta^{15}NH_4^+$ is $\sim6‰$ in the suboxic zone (Velinsky et al., 1991), and known isotope effects of N_2O production from ammonium range from $13‰$ (Casciotti, 2002), which would be too small to yield $\delta^{15}N$-N_2O of less than -10.8, to more than $60‰$ (Yoshida, 1988), which would be adequate. Ammonium may be supplied by upward diffusion from the anoxic zone, where free ammonium is abundant (see Fig. 2), or by the remineralization of organic matter.

3.3. ^{15}N uptake experiments

Results from the two ^{15}N uptake experiments are summarized in Table 1. At $\sigma_t = 15.3$, the uptake of ^{15}N ammonium into N_2O was clearly apparent at 24 and 48 h, with additions that represented 2% and 4% of the ambient ammonium concentration resulting in $\delta^{15}N$-N_2O values ranging from $220‰$ to $620‰$ vs. air N_2. The total concentration of N_2O

Table 1
Results of N tracer incubation experiments performed with water samples collected from the center of the western gyre in the Black Sea

Tracer added	Incubation time (h)	N_2O (nM)	$\delta^{15}N_2O$ (‰)	^{15}N in N_2O (pM)
Water from $\sigma_t = 15.3$				
None	24	11.8	5.2	87.4
2 nM $^{15}NH_4Cl$	24	11.4	221.6	102.3
4 nM $^{15}NH_4Cl$	24	8.1	621.9	96.4
None	48	11.8	4.4	87.3
2 nM $^{15}NH_4Cl$	48	9.5	436.0	100.7
4 nM $^{15}NH_4Cl$	48	9.6	541.1	108.9
Water from $\sigma_t = 15.6$				
None	0	10.8	−0.2	79.4
None	24	9.8	−0.5	72.0
None	48	10.4	0.4	76.5
5 nM K$^{15}NO_2$	12	9.4	24.6	70.8
5 nM K$^{15}NO_2$	24	9.5	3.1	70.1
10 nM K$^{15}NO_2$	24	10.3	3.0	76.0
5 nM K$^{15}NO_2$	48	9.6	1.5	70.7
10 nM K$^{15}NO_2$	48	9.4	3.0	69.3
20 nM $^{15}NH_4Cl$	12	10.9	178.7	94.5
40 nM $^{15}NH_4Cl$	12	9.2	59.4	71.9
20 nM $^{15}NH_4Cl$	24	10.4	54.5	80.6
40 nM $^{15}NH_4Cl$	24	10.4	36.7	79.3
20 nM $^{15}NH_4Cl$	48	10.6	129.5	88.0
40 nM $^{15}NH_4Cl$	48	10.6	183.1	92.2

remained unchanged or declined slightly during the course of the experiment in treatments relative to controls (N_2O concentration was 11.8 nM in both controls and ranged from 8.1 to 11.4 nM in the treatments) but the concentration of ^{15}N in N_2O increased in all four treatments (it is 87 pM in the controls and ranges from 96 to 109 pM in the treatments). If we ignore kinetic isotope effects and assume that the production of ^{15}N-labeled N_2O in our treatments above control levels represents 2% and 4% of the total N_2O production in our treatments, then we can calculate N_2O production rates from each treatment. They range from 110 to 370 pM d^{-1} or 110 to 370 nmol m^{-3} d^{-1}, and are adequate to support the upward vertical flux of 147 nmol m^{-2} d^{-1} calculated from the concentration gradient.

The results of the tracer uptake experiment at $\sigma_t = 15.6$ were less straightforward. The incorporation of ^{15}N-labeled ammonium into N_2O was apparent in all ammonium treatments but did not increase consistently with time, appearing highest at 12 h, and lower at 24 and 48 h. This was likely caused either by natural heterogeneity in the discreet water samples collected for this experiment or an experimental artifact that enhanced growth in the 12-h bottle or inhibited growth in the 24- and 48-h bottles. The nitrite addition experiments also showed ^{15}N uptake after 12 h, but not after 24 or 48 h. Concentration of N_2O declined slightly in all but one treatment relative to the time zero value, yielding net N_2O consumption. While we cannot quantify net production of N_2O from this experiment, the elevated $\delta^{15}N$-N_2O values for all ammonium treatments and the 12-h nitrite treatments demonstrated that N_2O can be produced in this environment by both ammonium oxidation and nitrite reduction, and that N_2O is simultaneously produced and consumed. This finding supports the argument that the anomalous isotopomeric characteristics of N_2O in the suboxic waters of the Black Sea are the result of simultaneous production and consumption processes.

3.4. Comparison with other low-oxygen environments

The low N_2O concentration in the oxygen-depleted waters of the Black Sea is puzzling when compared to the much higher concentrations found in other low-oxygen environments, such as the shelf off western India (Naqvi et al., 2000), the eastern tropical North Pacific (Westley et al., 2001), and the eastern tropical South Pacific (Codispoti et al., 1992). The low N_2O concentration in the Black Sea could be caused by lower production rates, higher consumption rates, or some combination of both. Since N_2O production by nitrification and denitrification is tied to remineralization of organic matter, relatively low fluxes of organic matter from the Black Sea euphotic zone may cause low production of N_2O. Burlakova et al. (2002) calculated particulate organic carbon fluxes from the euphotic zone to the oxycline for data collected on nine cruises from 1988 to 1994. Their June 1989 value is 22 mg C m^2 d^{-1}, which is similar to the average value of 29 mg C m^2 d^{-1} obtained from 5 yr of monthly sediment trap deployments at Station ALOHA in the oligotrophic subtropical North Pacific (Karl et al., 1996), and significantly lower than the organic carbon flux rates of 48–276 mg C m^2 d^{-1} for the eastern tropical South Pacific (Lipschultz et al., 1990 in Voss et al., 2001). Considerations of the stable isotope and isotopomeric characteristics of N_2O at Station ALOHA yield a minimum estimate of 200 ± 100 nmol m^{-2} d^{-1} for the flux due to in situ N_2O production (Dore et al., 1998; Popp et al., 2002). This is essentially indistinguishable from our estimates of 147 nmol m^{-2} d^{-1} for N_2O production in the Black Sea. Our results were obtained in June 2001; particulate organic carbon fluxes from the Black Sea euphotic zone are higher in the spring (e.g., Burlakova et al., 2002, report values of 57 and 47 mg C m^2 d^{-1} for March and April 1988, respectively), and it is possible that measurements performed shortly after a spring bloom would yield higher estimates of N_2O production. This would lend support to the hypothesis that N_2O production in the Black Sea is controlled by the supply of particulate organic matter.

The alternative hypothesis is that low N_2O concentrations are maintained by high rates of N_2O consumption in the Black Sea suboxic zone. Certainly the rapid declines in both nitrate and N_2O concentrations within the suboxic zone are consistent with complete denitrification of all nitrogen oxides to dinitrogen gas. The simultaneous increase in $\delta^{18}O$-N_2O and ^{15}N-site preference with depth and decreasing N_2O concentration provide evidence for a reduction reaction that attacks the N–O bond, since N_2O molecules containing [14]N–[16]O bonds disappear at a greater rate than molecules containing either [15]N–[16]O or [14]N–[18]O bonds. However, denitrification alone cannot account for the full

isotopomeric signal: while ^{15}N-site preference increases, bulk δ^{15}N-N$_2$O decreases, with the decrease more pronounced in δ^{15}N$^{\beta}$-N$_2$O (end position N), but also present in δ^{15}N$^{\alpha}$-N$_2$O (central position N). To generate this signal requires the initial production of N$_2$O that is strongly depleted in δ^{15}N, which can then yield N$_2$O with high δ^{18}O-N$_2$O and ^{15}N-site preference values following partial consumption by denitrification. The production of ^{15}N-depleted N$_2$O appears to occur within the suboxic zone, where δ^{15}N-N$_2$O reaches a minimum, suggesting that its nitrogen source is not the rain of organic matter from the euphotic zone above but the diffusion of free ammonium from the anoxic zone below. This production is balanced by consumption due to denitrification, generating the low concentrations and distinct isotopomer characteristics of N$_2$O observed in the Black Sea suboxic zone. It is interesting to note that the only other species of nitrogen that shows a δ^{15}N minimum in the suboxic zone is nitrogen gas, and this isotopic feature has been attributed to in situ production of nitrogen gas by ammonium oxidation with nitrite as the oxidant (see Murray et al., 2005). ^{15}N uptake experiments performed on water collected from $\sigma_t = 15.6$ confirm the existence of pathways for the conversion of both ammonium and nitrite to N$_2$O, demonstrating the potential for simultaneous ammonium oxidation and denitrification in the middle of the suboxic zone.

4. Conclusions

In terms of N$_2$O cycling, the Black Sea appears significantly different from other marine low oxygen environments: unlike the eastern tropical South Pacific (Codispoti et al., 1992) and North Pacific (Westley et al., 2001) and the Arabian Sea (Naqvi et al., 1998), there is no significant accumulation of N$_2$O in the Black Sea oxycline. Our lowest value of δ^{15}N-N$_2$O (-10.8 ± 0.8‰ vs. air N$_2$) is much lower than the lowest value for a marine environment reported to date (0.8‰ vs. air N$_2$ for upwelled waters in the Arabian Sea; Naqvi et al., 1998) and falls within the range of values reported for soil emissions: 0.1‰ to -25‰ vs. air N$_2$ (Kim and Craig, 1993) and 4.5‰ to -33.9‰ vs. air N$_2$ (Perez et al., 2000). However, the combination of very low δ^{15}N-N$_2$O with very high δ^{18}O-N$_2$O found in the Black Sea suboxic zone is unprecedented. The highest reported value of soil δ^{18}O-N$_2$O is 49‰ vs. VSMOW (Perez et al., 2000), much lower than our

maximum value of 67.0 ± 1.1‰ vs. VSMOW. In general δ^{15}N-N$_2$O and δ^{18}O-N$_2$O covary in a positive manner in marine and terrestrial systems (Rahn and Wahlen, 2000). Here the additional information of ^{15}N-site preference within N$_2$O proved invaluable, enabling us to distinguish between two processes affecting the N$_2$O pool, one that produces severely ^{15}N-depleted N$_2$O and one that subsequently consumes this N$_2$O by attacking the N–O bond, leaving residual N$_2$O with high values of δ^{18}O-N$_2$O and ^{15}N-site preference. Denitrification is the consumption process, and we have argued that the ^{15}N-isotope effect of the production process is consistent with ammonium oxidation by nitrifiers and falls between the range of values measured for marine (Casciotti et al., 2003) and terrestrial (Yoshida, 1988) species of Nitrosomonas bacteria.

The difference in nitrous oxide dynamics between the Black Sea and other low-oxygen marine environments may simply result from the permanent presence of a high concentration of ammonium directly below the suboxic zone, providing a steady supply of substrate for ammonium oxidizing bacteria. The low-oxygen regions of the eastern tropical Pacific do not exhibit high concentrations of free ammonium (Codispoti and Christensen, 1985; Westley et al., 2001), and intense low-oxygen conditions over the South West Indian shelf are driven by seasonal upwelling and are thus transient. However, we cannot rule out the possibility that N$_2$O in the Black Sea is influenced by processes other than conventional nitrification and denitrification, such as the recently discovered activity of anaerobic ammonium oxidizers at the suboxic/anoxic interface (Kuypers et al., 2003), or the proposed coupling between the nitrogen and manganese redox cycles (Luther et al., 1997; Murray et al., 2002). While the Black Sea does not emerge from this research as an important source of N$_2$O to the troposphere, the highly unusual isotopomer distributions reported here challenge our current understanding of the production of N$_2$O by nitrification and denitrification. Future work in the Black Sea suboxic zone should explore other biotic and abiotic pathways of N$_2$O formation.

References

Albritton, D.L., Meira Filho, L.G., 2001. Technical Summary of the Working Group 1 Report, Intergovernmental Panel on Climate Change, Geneva, Switzerland.

Bange, H.W., 2000. It's not a gas. Nature 408, 301–302.

Barford, C.C., Montoya, J.P., Altabet, M.A., Mitchell, R., 1999. Steady-state nitrogen isotope effects of N_2 and N_2O production in *Paracoccus denitrificans*. Applied and Environmental Microbiology 65 (3), 989–994.

Burlakova, Z.P., Eremeeva, L.V., Konovalov, S.K., 2002. Inventory and fluxes of particulate organic carbon and nitrogen in the Black Sea oxic/anoxic water column. In: Yilmaz, A. (Ed.), Oceanography of the Eastern Mediterranean and Black Sea: Similarities and Differences of Two Interconnected Basins. Tubitak Publishers, Ankara, Turkey, pp. 514–522.

Butler, J.H., Elkins, J.W., 1991. An automated technique for the measurement of dissolved N_2O in natural waters. Marine Chemistry 34 (1–2), 47–61.

Capone, D.G., 2000. The marine microbial nitrogen cycle. In: Kirchman, D.L. (Ed.), Microbial Ecology of the Oceans. Wiley-Liss, New York, pp. 455–493.

Casciotti, K.L., 2002. Molecular and stable isotopic characterization of enzymes involved in nitrification and nitrifier-denitrification. Ph.D. Thesis. Princeton University, Princeton, 237pp.

Casciotti, K.L., Sigman, D.M., Ward, B.B., 2003. Linking diversity and stable isotope fractionation in ammonia-oxidizing bacteria. Geomicrobiology Journal 20, 335–353.

Codispoti, L.A., Christensen, J.P., 1985. Nitrification, denitrification and nitrous oxide cycling in the eastern tropical South Pacific Ocean. Marine Chemistry 16 (4), 277–296.

Codispoti, L.A., Elkins, J.W., Yoshinari, T., Friederich, G.E., Sakamoto, CM., Packard, T.T., 1992. On the nitrous oxide flux from productive regions that contain low oxygen waters. In: Desai, B.N. (Ed.), Oceanography of the Indian Ocean. Oxford-IBH, New Delhi (India), pp. 271–284.

Cohen, Y., 1977. Shipboard measurements of dissolved nitrous oxide in seawater by electron capture detector. Analytical Chemistry 49, 1238–1240.

Cohen, Y., Gordon, L.I., 1978. Nitrous oxide in the oxygen minimum of the eastern tropical North Pacific: evidence for its consumption during denitrification and possible mechanisms for its production. Deep-Sea Research 25 (6), 509–524.

Dalsgaard, T., Canfield, D.E., Petersen, J., Thamdrup, B., Acuna-Gonzalez, J., 2003. N_2 production by the anammox reaction in the anoxic water column of Golfo Dulce, Costa Rica. Nature 422, 606–608.

Devol, A., 2003. Nitrogen cycle: Solution to a marine mystery. Nature 422, 575–576.

Dore, J.E., Popp, B.N., Karl, D.M., Sansone, F.J., 1998. A large source of atmospheric nitrous oxide from subtropical North Pacific surface waters. Nature 396 (6706), 63–66.

Fry, B., Jannasch, H.W., Molyneaux, S.J., Wirsen, C.O., Muramoto, J.A., 1991. Stable isotope studies of the carbon, nitrogen and sulfur cycles in the Black Sea and the Cariaco Trench. Deep-Sea Research 38 (2A), S1003–S1019.

Fuhrman, J.A., Capone, D.G., 1991. Possible biogeochemical consequences of ocean fertilization. Limnology and Oceanography 36 (8), 1951–1959.

Goreau, T.J., Kaplan, W.A., Wofsy, S.C., McElroy, M.B., Valois, F.W., Watson, S.W., 1980. Production of NO_2^- and N_2O by nitrifying bacteria at reduced concentrations of oxygen. Applied and Environmental Microbiology 40, 526–532.

Inoue, H.Y., Mook, W.G., 1994. Equilibrium and kinetic nitrogen and oxygen isotope fractionations between dissolved and gaseous N_2O. Chemical Geology 113 (1–2), 135–148.

Jin, X., Gruber, N., 2003. Offsetting the radiative benefit of ocean iron fertilization by enhancing N_2O emissions. Geophysical Research Letters 30 (24), 2249.

Karl, D.M., Christian, J.R., Dore, J.E., Hebel, D.V., Letelier, R.M., Tupas, L.M., Winn, CD., 1996. Seasonal and inter-annual variability in primary production and particle flux at Station ALOHA. Deep-Sea Research II 43, 539–568.

Kim, K.R., Craig, H., 1990. Two-isotope characterization of N_2O in the Pacific Ocean and constraints on its origin in deep water. Nature 347 (6288), 58–61.

Kim, K.-R., Craig, H., 1993. Nitrogen-15 and oxygen-18 characteristics of nitrous oxide: a global perspective. Science 262 (5141), 1855–1857.

Knowles, R., 1982. Denitrification. Microbiological Reviews 46, 43–70.

Konovalov, S.K., Murray, J.W., Luther, G.W., Buesseler, K.O., Friederich, G., Tebo, B.M., Samodurov, A.S., Gregoire, M., Ivanov, L.I., Romenov, A.S., Clement, B., Murray, K., 2002. Oxygen fluxes, redox processes and the suboxic zone in the Black Sea. In: Yilmaz, A. (Ed.), Second International Conference on Oceanography of the Eastern Mediterranean and Black Sea: Similarities and Differences of Two Interconnected Basins. Tubitak Publishers, Ankara, Turkey, pp. 566–577.

Konovalov, S.K., Luther, G.W., Friederich, G.E., Nuzzio, D.B., Tebo, B.M., Murray, J.W., Oguz, T., Glazer, B., Trouwborst, R.E., Clement, B.G., Murray, KJ., Romanov, A.S., 2003. Lateral injection of oxygen with the Bosporus plume—fingers of oxidizing potential in the Black Sea. Limnology and Oceanography 48 (6), 2369–2376.

Kuypers, M.M.M., Sliekers, A.O., Lavik, G., Schmid, M., Jørgensen, B.B., Kuenen, J.G., Damsté, J.S.S., Strous, M., Jetten, M.S.M., 2003. Anaerobic ammonium oxidation by anammox bacteria in the Black Sea. Nature 422, 608–611.

Lewis, M.R., Harrison, W.G., Oakley, N.S., Hebert, D., Piatt, T., 1986. Vertical nitrate fluxes in the oligotrophic ocean. Science 234, 870–873.

Lipschultz, F., Wofsy, S.C., Ward, B.B., Codispoti, L.A., Friederich, G.E., Elkins, J.W., 1990. Bacterial transformations of inorganic nitrogen in the oxygen-deficient waters of the Eastern Tropical South Pacific Ocean. Deep-Sea Research 37 (10), 1513–1541.

Luther III, G.W., Brendel, P.J., Silverberg, N., Sundby, B., Lewis, B.L., 1997. Interactions of manganese with the nitrogen cycle: alternative pathways to dinitrogen. Geochimica et Cosmochimica Acta 61 (19), 4043–4052.

Luther III, G.W., Nuzzio, D.B., Glazer, B., Konovalov, S.K., Friederich, G.E., Trouwborst, R.E., Romanov, A., Tebo, B.M., 2002. Mediterranean inflow through the Bosporus injects massive amounts of O_2 into the suboxic zone of the southwest Black Sea. EOS Transactions AGU 83 (4) (Ocean Sciences Meeting Supplement): Abstract OS41I-03.

Mariotti, A., Germon, J.C., Hubert, P., Kaiser, P., Letolle, R., Tardieux, A., Tardieux, P., 1981. Experimental determination of nitrogen kinetic isotope fractionation: some principals; illustration for the denitrification and nitrification processes. Plant and Soil 62 (3), 413–430.

Murray, J.W., Izdar, E., 1989. The 1988 Black Sea Oceanographic Expedition: Overview and new discoveries. Oceanography 2 (1), 15–21.

Murray, J.W., Codispoti, L., Friederich, G.E., 1995. Oxidation–reduction environments: the suboxic zone in the Black Sea. ACS Advances in Chemistry Series (Aquatic Chemistry: Interfacial and Interspecies Processes) 244, 157–176.

Murray, J.W., Konovalov, S.K., Callahan, A.E., 2002. Nitrogen reactions in the suboxic zone of the Black Sea: new data and models. In: Yilmaz, A. (Ed.), Oceanography of the Eastern Mediterranean and Black Sea: Similarities and Differences of Two Interconnected Basins. Tubitak Publishers, Ankara, Turkey, pp. 591–602.

Murray, J.W., Fuchsman, C., Kirkpatrick, J., Paul, B., Konovalov, S.K., 2005. Species and ^{15}N signatures of nitrogen transformations in the suboxic zone of the Black Sea. Oceanography 18, 24–35.

Naqvi, S.W.A., Yoshinari, T., Jaya Kumar, D.A., Altabet, M.A., Narvekar, P.V., Devol, A.H., Brandes, J.A., Codispoti, L.A., 1998. Budgetary and biogeochemical implications of N_2O isotope signatures in the Arabian Sea. Nature 394 (6692), 462–464.

Naqvi, S.W.A., Jayakumar, D.A., Narvekar, P.V., Naik, H., Sarma, V., D'Souza, W., Joseph, S., George, M.D., 2000. Increased marine production of N_2O due to intensifying anoxia on the Indian continental shelf. Nature 408 (6810), 346–349.

Nevison, CD., Weiss, R.F., Erkinson, D.J.I., 1995. Global emissions of nitrous oxide. Journal of Geophysical Research 100 (15809–15820).

Oguz, T., Aubrey, D.G., Latun, V.S., Demirov, E., Koveshnikov, L., Sur, H.I., Diaconu, V., Besiktepe, S., Duman, M., Limeburner, R., Eremeev, V., 1994. Mesoscale circulation and thermohaline structure of the Black Sea observed during HydroBlack '91. Deep-Sea Research I 41 (4), 603–628.

Ostrom, N.E., Russ, M.E., Popp, B., Rust, T.M., Karl, D.M., 2000. Mechanisms of nitrous oxide production in the subtropical North Pacific based on determinations of the isotopic abundances of nitrous oxide and di-oxygen. Chemosphere: Global Change Science 2 (3), 281–290.

Perez, T., Trumbore, S.E., Tyler, S.C., Davidson, E.A., Keller, M., de Camargo, P.B., 2000. Isotopic variability of N_2O emissions from tropical forest soils. Global Biogeochemical Cycles 14 (2), 525–536.

Perez, T., Trumbore, S.E., Tyler, S.C., Matson, P.A., Ortiz-Monasterio, I., Rahn, T., Griffith, D.W.T., 2001. Identifying the agricultural imprint on the global N_2O budget using stable isotopes. Journal of Geophysical Research 106 (D9), 9869–9878.

Popp, B.N., Westley, M.B., Toyoda, S., Miwa, T., Dore, J.E., Yoshida, N., Rust, T.M., Sansone, F.J., Russ, M.E., Ostrom, N.E., Ostrom, P.H., 2002. Nitrogen and oxygen isotopomeric constraints on the origins and sea-to-air flux of N_2O in the oligotrophic subtropical North Pacific gyre. Global Biogeochemical Cycles 16 (4), 1064.

Rahn, T., Wahlen, M., 1997. Stable isotope enrichment in stratospheric nitrous oxide. Science 278, 1776–1778.

Rahn, T., Wahlen, M., 2000. A reassessment of the global isotopic budget of atmospheric nitrous oxide. Global Biogeochemical Cycles 14 (2), 537–544.

Samodurov, A.S., Ivanov, L.I., 1998. Processes of ventilation of the Black Sea related to water exchange through the Bosporus. In: Ivanov, L., Oguz, T. (Eds.), NATO TU-Black Sea project ecosystem modeling as a management tool for the black sea: symposium on scientific results. NATO ASI Series. Kluwer Academic Publishers, The Netherlands, pp. 221–235.

Sansone, F.J., Popp, B.N., Rust, T.M., 1997. Stable carbon isotopic analysis of low-level methane in water and gas. Analytical Chemistry 69, 40–44.

Toyoda, S., Yoshida, N., 1999. Determination of nitrogen isotopomers of nitrous oxide on a modified isotope ratio mass spectrometer. Analytical Chemistry 71, 4711–4718.

Toyoda, S., Yoshida, N., Miwa, T., Matsui, Y., Yamagishi, H., Tsunogai, U., Nojiri, Y., Tsurushima, N., 2002. Production mechanism and global budget of N_2O inferrred from its isotopomers in the western North Pacific. Geophysical Research Letters 29 (3), 7-1–7-4.

Tugrul, S., Salihoglu, I., 2002. Spatial and temporal variations in the hydro-chemical properties of the Black Sea upper layer. In: Yilmaz, A. (Ed.), Oceanography of the Eastern Mediterranean and Black Sea: Similarities and Differences of Two Interconnected Basins. Tubitak Publishers, Ankara, Turkey, pp. 578–582.

Velinsky, D.J., Fogel, M.L., Todd, J.F., Tebo, B.M., 1991. Isotopic fractionation of dissolved ammonium at the oxygen-hydrogen sulfide interface in anoxic waters. Geophysical Research Letters 18 (4), 649–652.

Voss, M., Dippner, J.W., Montoya, J.P., 2001. Nitrogen isotope patterns in the oxygen-deficient waters of the Eastern Tropical North Pacific Ocean. Deep-Sea Research I 48, 1905–1921.

Weiss, R.F., Price, B.A., 1980. Nitrous oxide solubility in water and seawater. Mar. Chem. 8 (4), 347–359.

Westley, M.B., Popp, B.N., Rust, T.M., Sansone, F.J., 2001. Sources and isotopic compositions of nitrous oxide in the subtropical North Pacific and the eastern tropical North Pacific. In: Yoshida, N. (Ed.), Proceedings of the First International Symposium on Isotopomers. Yokohama, Japan. pp. 169–175.

Wrage, N., Velthof, G.L., Beusichem, M.L.V., Oenema, O., 2001. Role of nitrifier denitrification in the production of nitrous oxide. Soil biology and biochemistry 33, 1723–1732.

Yamagishi, H., Yoshida, N., Toyoda, S., Popp, B.N., Westley, M.B., Watanabe, S., 2005. Contributions of denitrification and mixing on the distribution of nitrous oxide in the North Pacific. Geophysical Research Letters 32 (L04603).

Yoshida, N., 1988. ^{15}N-depleted N_2O as a product of nitrification. Nature 335 (6190), 528–529.

Yoshida, N., Matsuo, S., 1983. Nitrogen isotope ratio of atmospheric N_2O as a key to the global cycle of N_2O. Geochemical Journal 17, 231–239.

Yoshida, N., Toyoda, S., 2000. Constraining the atmospheric N_2O budget from intramolecular site preference in N_2O isotopomers. Nature 405, 330–334.

Yoshida, N., Morimoto, H., Hirano, M., Koike, I., Matsuo, S., Wada, E., Saino, T., Hattori, A., 1989. Nitrification rates and ^{15}N abundances of N_2O and NO_3^- in the western North Pacific. Nature 342 (6252), 895–897.

Yoshinari, T., 1976. Nitrous oxide in the sea. Marine Chemistry 4 (2), 189–202.

Yoshinari, T., Altabet, M.A., Naqvi, S.W.A., Codispoti, L., Jayakumar, A., Kuhland, M., Devol, A., 1997. Nitrogen and oxygen isotopic composition of N_2O from suboxic waters of the eastern tropical North Pacific and the Arabian Sea; measurement by continuous-flow isotope-ratio monitoring. Marine Chemistry 56 (3–4), 253–264.

Yung, Y.L., Miller, C.E., 1997. Isotopic fractionation of stratospheric nitrous oxide. Science 278, 1778–1780.

Zafiriou, O.C., 1990. Laughing gas from leaky pipes. Nature 347, 15–16.