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# Detection of transient denitrification during a high organic matter event in the Black Sea

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## Key Points:

N<sub>2</sub> gas maximum was found in hypoxic waters (30 μM O<sub>2</sub>) in the Black Sea

δ<sup>15</sup>N isotopic calculations indicate N<sub>2</sub> production inside particles in hypoxic waters

No anammox DNA was found indicating N<sub>2</sub> production by denitrification in particles in hypoxic waters

Keywords: Denitrification, Anammox, Black Sea, Organic Matter, N<sub>2</sub> gas, Stable N Isotopes

Index: 4802 Anoxic environments, 4870 Stable isotopes, 4840 Microbiology and microbial ecology, 4820 Gases

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## Abstract

$\text{N}_2$  production by denitrification can occur in anoxic water or potentially inside organic particles. Here we compare data from the Black Sea, a permanently anoxic basin, during two organic matter regimes: suspended particulate organic matter concentrations were high in the oxycline after the spring bloom in March 2005 compared to lower organic matter concentrations in June 2005, May and October 2007, July 2008 and May 2001. For all cruises,  $\text{N}_2$  gas had a maximum in the suboxic zone [ $\text{O}_2 < 10 \mu\text{mol L}^{-1}$ ]. During the high organic matter event [March 2005], an additional shallower  $\text{N}_2$  gas and  $\delta^{15}\text{N}-\text{N}_2$  maxima occurred above the suboxic zone in the oxycline where oxygen concentrations were 30-50  $\mu\text{mol L}^{-1}$ . Examination of 16S rRNA indicated that anammox bacteria were not present in the oxycline. The  $\delta^{15}\text{N}$  of biologically produced  $\text{N}_2$  in the oxycline in March 2005 was significantly enriched (+7 to +38‰), not depleted, as would be expected from water column fractionation. A simple diffusion calculation indicated that ammonium produced from remineralization inside particles could be oxidized to nitrate and then completely consumed by denitrification inside the particle. In this calculation, half of denitrified N atoms originated from organic N [ $\delta^{15}\text{N} = 11\text{‰}$ ] and half of N atoms originated from ambient nitrate [ $\delta^{15}\text{N} = 5-7\text{‰}$ ], producing enriched  $\delta^{15}\text{N}-\text{N}_2$  values. We suggest that denitrifiers were active in microzones inside particulates in hypoxic waters above the suboxic zone of the Black Sea. Denitrification in particles may also explain previous data from the oxycline above ocean oxygen deficient zones.

## 1. Introduction

Nitrate, nitrite and ammonia are essential nutrients for microbial life, and limit growth in much of the surface ocean (Moore et al., 2013). This fixed nitrogen is converted to nitrogen gas in oxygen deficient water columns and sediments. Two known processes are involved in  $N_2$  production; denitrification and anammox. Heterotrophic denitrifying bacteria use organic matter to reduce two nitrates to form nitrogen gas, producing nitrite, and nitrous oxide as intermediates. Anammox bacteria reduce nitrite with ammonium to autotrophically produce  $N_2$  with hydrazine as an intermediate. Ammonium is produced from the organic matter remineralization during denitrification, and this ammonium can be used by anammox (Devol, 2003). Autotrophic denitrification can also occur with  $N_2$  production using reduced sulfur instead of organic matter (Batchelor & Lawrence, 1976).

Several lines of evidence indicate that abundant organic matter stimulates  $N_2$  production. Total  $N_2$  production was correlated with organic N concentrations and surface chlorophyll and productivity in the suboxic zone of the Black Sea (Fuchsman et al., 2008; Kirkpatrick et al., 2012; Nezlin, 2008) and with particulate organic matter flux in the Cariaco Basin (Montes et al., 2013). Anammox rates also correlated with particulate matter flux into the oxygen deficient zone of the Eastern Tropical South Pacific (Kalvelage et al., 2013). Addition of sterilized sediment trap material significantly increased denitrification rates in the three major marine oxygen deficient zones: the Arabian Sea, Eastern Tropical South Pacific (ETSP), and Eastern Tropical North Pacific (ETNP) (Babbin et al., 2014; Chang et al., 2014; Ward et al., 2008), and removal of all particles decreased rates of both anammox and denitrification (Ganesh et al., 2015). In the ETSP and ETNP oxygen deficient zones, denitrification genes were enriched in the particulate fraction compared to free-living fraction (Fuchsman et al., 2017; Ganesh et al., 2014, 2015). Thus, there appears to be a link between heterotrophic denitrification and organic matter and more specifically with organic particles.

$\text{N}_2\text{O}$  production and reduction are 50% inhibited at 200 and 300 nM oxygen respectively (Dalsgaard et al., 2014). However, in the marine environment, there are oxygen gradients inside organic matter aggregates (Ploug, 2001; Ploug et al., 1997; Ploug & Bergkvist, 2015). Oxygen utilization in particles is reaction limited rather than transport limited in fully oxygenated seawater, which is why aggregates generally are not anoxic (Ploug, 2001). The potential for transport limitation of oxygen, and therefore anoxia, is greatly dependent on particle size, with aggregates  $>1$  mm being much more susceptible to anoxia (Klawonn et al., 2015). In diatom aggregates, diffusion limitation of oxygen occurred below ambient  $\text{O}_2$  concentrations of  $100 \mu\text{M}$  (Ploug & Bergkvist, 2015; Stief et al., 2016) and the interior of aggregates became 50% anoxic at  $20 \mu\text{M}$   $\text{O}_2$  (Ploug & Bergkvist, 2015). In the lab, nitrite, ammonium,  $\text{N}_2\text{O}$  and  $\text{N}_2$  were all produced inside diatom aggregates under ambient  $\text{O}_2$  concentrations of  $50 \mu\text{M}$ , indicating nitrate reduction and denitrification (Stief et al., 2016). In addition, in similar experiments, nitrate from vacuoles inside diatoms was denitrified under anoxic conditions (Kamp et al., 2016). Experiments with  $>3$  mm size particles of  $\text{N}_2$ -fixing cyanobacteria found  $\text{N}_2\text{O}$  production and denitrification under dark conditions in fully oxic waters though these rates were two orders of magnitude lower than rates of ammonification of nitrate (Klawonn et al., 2015). Nitrate reduction occurred in sinking aggregates in  $135 \mu\text{M}$   $\text{O}_2$  water near the sea floor (Wolgast et al., 1998). However the importance of  $\text{N}_2$  production in natural sinking particles is unknown.

Denitrification in particles may be important in hypoxic waters above open ocean oxygen deficient zones.  $\text{N}_2\text{O}$  production by partial denitrification has been detected in the oxycline above the ETSP oxygen deficient zone (Ji et al., 2015) and  $\text{N}_2\text{O}$  reduction to  $\text{N}_2$  was detected in the oxycline above the ETNP oxygen deficient zone (Babbin et al., 2015). Additionally, a  $30 \mu\text{M}$  maximum in  $\text{N}_2$  in the oxycline above the oxygen deficient zone has also been reported in an eddy in the ETSP under highly productive conditions (Altabet et al.,

2012). The maximum in  $N_2$  above the oxygen deficient zone in this eddy was enriched in  $\delta^{15}N$  compared to equilibrium with air (Altabet et al., 2012), even though  $N_2$  inside oxygen deficient zones is generally depleted compared to equilibrium with air (Fuchsman et al., 2018). Averaged over the ocean, denitrification in particles may be significant. Global modeling of aggregates in the ocean predicted that inclusion of denitrification inside particles in hypoxic waters should double the global water column denitrification rates (Bianchi et al., 2018).

$N_2$  production processes have been studied in the Black Sea suboxic zone.  $N_2$  gas typically has a maximum in the lower suboxic zone, but concentrations vary between cruises (Fuchsman et al., 2008; Kirkpatrick et al., 2012). Rates for anammox but not denitrification have been detected in the suboxic zone using spiked  $^{15}N$  experiments (Jensen et al., 2008).

However, mRNA for proteins involved in both anammox and denitrification have been detected, indicating activity of both processes (Kirkpatrick et al., 2012). Denitrification appears to be present during times of higher productivity of organic matter (Fuchsman et al., 2008; Kirkpatrick et al., 2012). Anammox bacteria are present in the  $N_2$  gas maximum in the lower suboxic zone, where there is an upward flux of ammonium from the sulfide layer (Fuchsman et al., 2012a; Kirkpatrick et al., 2012). Similar to open ocean oxygen deficient zones, anammox bacteria appeared to be free-living in the Black Sea (Fuchsman et al., 2012a). On the other hand, *Sulfurimonas*, a bacterium associated with autotrophic denitrification utilizing reduced sulfur was enriched in particles in the Black Sea suboxic zone (Fuchsman et al., 2012b). Compared to other cruises in the summer and fall, there were high particulate organic matter concentrations in the oxycline above the suboxic zone of the Black Sea after the spring bloom in March 2005. In this paper we use in situ tracers, such as  $\delta^{15}N$  and DNA, to examine  $N_2$  production in waters with 30-50  $\mu M O_2$ , during this event.

## 2. Study site

The Black Sea is a semi-enclosed marginal sea with oxic, suboxic and sulfidic layers (Murray et al., 1995). The hydrological balance of the Black Sea is influenced by freshwater input from rivers such as the Danube, which mix into surface waters, while salty Mediterranean waters flow through the Bosphorus and fill the deep basin (Murray et al., 1991). As a result the Black Sea is permanently stratified with respect to density. Due to its stratification, the Black Sea has a series of water layers well-defined by characteristic densities. Below the surface waters is the Cold Intermediate Layer (CIL) with a characteristic core density of  $\sigma_{\theta} \approx 14.5$ . In most of the Black Sea, the CIL represents the lower boundary of direct communication with the atmosphere in winter, though the CIL is often not fully replenished at that time (Pakhomova et al., 2014b). Below the CIL is the oxycline, which leads to the suboxic zone (~50 m thick) where oxygen is  $< 10 \mu\text{mol L}^{-1}$  and hydrogen sulfide is  $< 10 \text{ nmol L}^{-1}$  (Murray et al., 1995). Below the suboxic zone is the sulfidic layer that extends to the sediments at approximately 2100 m. Water circulates around the perimeter of the Black Sea in the 20-80 km wide Rim Current (Korotaev et al., 2006). The cyclonic rim current separates the coastal regions from the western and eastern central gyres. An autonomous profiling float deployed in the Western Gyre at 200 m was found to take 6 months in the Rim Current to travel to the northeast Black Sea (Korotaev et al., 2006).

The Black Sea is known to have both a spring phytoplankton bloom in March and a fall phytoplankton bloom. The fall bloom, usually found in late October and November, occurs when strong winds cause mixing and entrainment of nutrients into euphotic waters (Mikaelyan et al., 2017). The spring bloom alternates between diatoms and coccolithophores (Mikaelyan et al., 2015), and the fall bloom is composed mostly of diatoms (Stel'makh et al., 2009).

In this paper we focus on a high organic matter event after the spring bloom in March 2005 where there was  $N_2$  production in the oxycline above the suboxic zone in the Western Gyre, and we compare isotope and concentration data to profiles obtained in summer and fall at a repeat station in the northeast Black Sea (Yakushev et al., 2006) in 2005, 2007 and 2008 (Fig. 1) and to previously published data from the Western Gyre in May 2001.

### 3. Methods

Due to the strong density stratification of the Black Sea, characteristic inflections in the water-column profiles (such as nitrate) vary with depth but are associated with specific density values (Murray et al., 1995). Therefore, results presented here are plotted against potential density ( $\sigma_\theta$ ) rather than depth (m). A comparison of depth and density at our two study sites is seen in Figure S1.

#### 3.1 Sampling

Samples for  $N_2$  gas, nutrients, suspended particulate organic matter, total organic nitrogen, and the  $\delta^{15}N$  of these species were collected in the western central gyre of the Black Sea in late March 2005 on cruise 403 of the R/V *Endeavor* (42° 30' N, 30° 45' E). Samples from the Endeavor cruise were obtained using a CTD-Rosette with Sea Bird sensors and 10 L Niskin bottles. On this cruise a Sea Tech Flash Lamp Fluorometer S/N 117S, with excitation at 425 nm and emission at 685 nm, and a C Star Single Channel Transmissometer CST-480DR were attached to the CTD-Rosette and used to collect *in situ* fluorescence and beam attenuation, respectively. The fluorometer had been calibrated for chlorophyll a in November 2004, so fluorescence units are reported as  $\mu g L^{-1}$  chlorophyll a. Samples for microbial DNA were taken at the same station and are published (Fuchsman et al., 2011; Fuchsman et al., 2012b; Fuchsman et al., 2012a).



Samples for N<sub>2</sub> gas, nutrients, suspended particulate organic matter, total organic nitrogen, and the  $\delta^{15}\text{N}$  of these species were also collected at station 2200 (bottom depth 1300 m) in the northeast Black Sea in late June 2005 (44.46° N, 37.95° E) on the R/V *Akvanavt* with a CTD rosette and seabird sensors and 5 L Niskin bottles. However, no DNA samples were obtained on the June 2005 cruise. Similar samples from May 19 – 21 and October 3 – 5, 2007 were also obtained on the R/V *Akvanavt*, while samples from July 2008 were obtained on the RV *Ashamba* with a pump profiling system with an attached CTD probe (Pakhomova et al., 2014a). All samples in 2007 and 2008 were taken at 44.3 – 44.4° N, 37.5 – 37.9° E (water depth >1 km) where the slope drops precipitously allowing easy access to deep water. Care was taken to cross the Rim Current, to minimize the influence of coastal waters. *nirS* mRNA sequences for anammox and denitrification and N<sub>2</sub>:Ar ratios for May and October 2007 and July 2008 were published in Kirkpatrick et al. (2012). Data from the Western Gyre in May 2001 was published in Fuchsman et al. (2008), but is shown here in figures as a comparison.

### 3.2 Nutrients

Nitrate, nitrite and ammonium from the Western Gyre in March 2005 were measured shipboard. Nitrate concentrations for samples obtained in June 2005 in the northeast Black Sea were measured later at the University of Washington on unfiltered acidified samples. However, in June 2005 nitrite and ammonium concentrations were measured the same day as collected at the P.P. Shirshov Institute, Southern Branch, Russia as were all three nutrients in May and October 2007 and July 2008. In all cases nitrate was reduced to nitrite using a cadmium column, and nitrite was measured using sulphanilamide and N(1-naphthyl)-ethylenediamine (Armstrong et al., 1967) using a two channel Technicon Autoanalyzer II system. Ammonia was analyzed using the indophenole blue procedure (Slawky & MacIsaac, 1972).

Oxygen and sulfide samples were measured using Winkler titrations for O<sub>2</sub>, and an iodometric method for sulfide (Cline, 1969). In both cases, dry flasks were filled with argon prior to sampling to avoid contamination by atmospheric oxygen and corrections for oxygen in reagents were made as in Yakushev et al (2012).

### 3.3 Total organic nitrogen

Total organic nitrogen samples from the Western Gyre in March 2005 were frozen immediately. Samples from the northeast Black Sea from June 2005 were acidified to pH 2 upon collection and were adjusted to pH 6-8 prior to analyses. In May 2007, October 2007, and July 2008 dissolved organic nitrogen water samples were prefiltered with a 0.7 µm pore size GF/F and were acidified to pH 2. Both total organic nitrogen (TON) and dissolved organic nitrogen (DON) samples were measured by oxidation to nitrate with peroxodisulphate (Valderrama, 1981). The peroxodisulphate (Merck) was purified by recrystallization to lower the N blank to between 0 to 0.4 µmol L<sup>-1</sup>. Samples were autoclaved in previously pre-combusted glass ampoules for 1 hour, then NO<sub>3</sub><sup>-</sup> was measured using the Technicon Autoanalyzer II system described above. The in situ concentrations of NO<sub>3</sub><sup>-</sup> were subtracted to calculate TON and DON. In deep samples ammonium concentrations were also subtracted. The efficiency of oxidation was tested using urea and EDTA, and the recoveries varied between 95% to 100%. One Black Sea sample was repeated in each run as an internal standard and was within 0.5 µM between runs. Additionally, the repeat of the same 11 µM sample 5x in one run had a standard deviation of 0.4 µM.

### 3.4 δ<sup>15</sup>N-NO<sub>3</sub><sup>-</sup> and δ<sup>15</sup>N-TON

Samples for δ<sup>15</sup>N-NO<sub>3</sub><sup>-</sup> from the Western Gyre in March 2005 were frozen immediately. Samples from the oxic and suboxic water column in the northeast Black Sea from June 2005 were acidified to pH 2 upon collection and were adjusted to pH 6-8 prior to

analyses. In May 2007, October 2007, and July 2008 prefiltered water samples ( $<0.7 \mu\text{m}$ ) were acidified to pH 2 and used for [DON],  $\delta^{15}\text{N}$ -DON and  $\delta^{15}\text{N}$ - $\text{NO}_3^-$  analyses. Frozen  $\delta^{15}\text{N}$ - $\text{NO}_3^-$  samples from October 2007 and July 2008 were also analyzed. Frozen  $\delta^{15}\text{N}$ - $\text{NO}_3^-$  samples from October 2007 are previously published (Kirkpatrick et al., 2018).

In samples with  $>0.5 \mu\text{mol L}^{-1}$  nitrate, nitrate was reduced to nitrous oxide by bacteria. For most samples *Pseudomonas chlororaphis* (Sigman et al., 2001) was used but frozen samples from October 2007 and July 2008 were analyzed for  $\delta^{15}\text{N}$ - $\text{NO}_3^-$  using *Pseudomonas aureofaciens* (Casciotti et al., 2002). In all cases, nitrous oxide was extracted using a fully automated extraction system. The 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. For each run, International Atomic Energy Agency standard IAEA-N3 dissolved in Black Sea nitrate free surface water was measured in triplicate. These standards were adjusted to the established value of 4.7‰ and samples were adjusted simultaneously (Sigman et al., 2001). For samples analyzed with *Pseudomonas aureofaciens* standards IAEA-N3, USGS 34 and USGS 35 were used. A blank, containing only bacteria and media, was analyzed for  $\text{N}_2\text{O}$  with every run and found to be negative. All samples were analyzed in duplicate. Error propagation included the standard deviation of duplicate samples as well as of the triplicate IAEA-N3 standards. Nitrite was not removed from the samples, but was below  $0.1 \mu\text{mol L}^{-1}$  in all depths except for several depths in June 2005 such as  $\sigma_\theta = 15.95$  and  $15.98$  where nitrite concentrations were  $0.15 \mu\text{mol L}^{-1}$ , or 10% of the nitrate concentration, and an upper nitrite maximum of  $0.19 \mu\text{mol L}^{-1}$  was at  $\sigma_\theta = 14.2$ . Typically nitrite is only removed in samples with  $>0.2 \mu\text{mol L}^{-1}$  nitrite (Buchwald et al., 2015; Fuchsman et al., 2018). Reduction of pH to pH 2 causes nitrite to become  $\text{N}_2$  gas, which would not be analyzed with these techniques (Granger & Sigman, 2009). Thus the agreement

between frozen and acidified samples (Figure 2) supports that nitrite does not significantly affect our  $\delta^{15}\text{N-NO}_3^-$  data.

Samples for  $\delta^{15}\text{N-TON}$  were analyzed similarly after being oxidized to nitrate as described above except that values for  $\delta^{15}\text{N-NO}_3^-$  were subtracted post analysis.  $\delta^{15}\text{N-TON}$  is not reported for depths where significant amounts of ammonium were present ( $>\sigma_\theta=15.95$ ) because the  $\delta^{15}\text{N-NH}_4^+$  was not determined for samples on these cruises.

### **3.5 N<sub>2</sub>:Ar ratios, [Ar] and $\delta^{15}\text{N-N}_2$**

Gas samples used for N<sub>2</sub>:Ar ratios and  $\delta^{15}\text{N-N}_2$  were collected in evacuated 185 mL glass flasks sealed with a Louwers-Hapert valve and containing dried mercuric chloride (Emerson et al., 1999). Water flowed through tubing directly from the Niskin bottle to the flask without contact with the air. Usually to prevent air contamination when taking dissolved gas samples, the neck of the closed evacuated glass flask is flushed with CO<sub>2</sub> before being flushed with water from the Niskin and then opened. CO<sub>2</sub> was used in gas sampling in March 2005 and May 2001. However, in June 2005, May and Oct 2007 and July 2008 at the northeast Black Sea, no CO<sub>2</sub> was available, so the neck of the closed flask was flushed with water from the Niskin for an extended period and all bubbles in the neck were removed before opening the flask. Duplicate samples were not obtained. After each cruises, flasks were weighed, and the water in the half full flasks was equilibrated with the headspace by rotating overnight in a water bath at a known (room) temperature. Water was removed from the flask immediately after removal from the water bath. Gas samples were cryogenically processed to completely remove CO<sub>2</sub> and water vapor. For samples from 2005 and after, a known concentration of <sup>36</sup>Ar spike was added to each sample during cryogenic processing. Measuring the <sup>36</sup>Ar:<sup>40</sup>Ar ratios allowed concentrations of <sup>40</sup>Ar to be measured rather than estimated. Gas samples were then measured at the Stable Isotope Lab, School of

Oceanography, University of Washington on a Finnegan Delta XL isotope ratio mass spectrometer. A standard containing zero oxygen was used for samples from the suboxic and anoxic zones. In deep samples  $N_2:Ar$  ratios,  $^{36}Ar:^{40}Ar$  ratios and  $\delta^{15}N-N_2$  were also corrected for methane as described in Fuchsman et al. (2008). Measured  $[Ar]$  allowed  $[N_2]$  to be calculated from  $N_2:Ar$  ratios. Argon and nitrogen saturation was calculated following Hamme and Emerson (Hamme & Emerson, 2004). Precision for standards was  $\pm 0.06\text{‰}$  for  $\delta^{15}N-N_2$  and  $\pm 0.003$  for  $N_2:Ar$  Sample:Saturation values.

The effect of oxygen on the measurement of  $\delta^{15}N$  was determined using a series of five flasks that contain variable amounts of oxygen but the same  $N_2:Ar$  ratio and  $\delta^{15}N$  of nitrogen gas. The correction for  $\delta^{15}N-N_2$  increased as  $[O_2]$  decreased. In the Endeavor 2005  $\delta^{15}N$  data published in Fuchsman et al. (2008), several samples contained oxygen but less oxygen than found in the calibration standards. The calculated slope from the standards was originally extrapolated and used for these data. However, upon further investigation, we established that the oxygen correction remained constant over a range of low oxygen values (the calibration standard containing the least oxygen was in this range). Though the filament in the mass spectrometer had been replaced between March 2005 samples and the more detailed test, we assume the shape of the curve was the same through time. The correction for the lowest oxygen standard obtained concurrent with the March 2005 data was applied to all relevant samples. In short, the original oxygen correction (Fuchsman et al., 2008) for samples with small amounts of oxygen for data from March 2005 was too large, and the  $\delta^{15}N$  data presented here have been corrected accordingly, becoming slightly more depleted (0.1‰ to 0.2‰) in the lower oxycline. The  $\delta^{15}N$  value of the no oxygen standard used in March 2005 was also determined using air containing oxygen and an oxygen correction. After examining all the oxygen deficient zone and Black Sea data from UW from 2005-2012 measured against

the same standard and knowing the present value of the no oxygen standard determined with a copper furnace, the value of the no oxygen standard has been adjusted for this time period.

Thus the  $\delta^{15}\text{N}$  values for anoxic depths in March 2005 are shifted 0.1‰ heavier from previously published values (Fuchsman et al., 2008). Other older data from Fuchsman et al. (2008) were measured with different standards and are assumed to be unaffected.

It should be noted that carbon monoxide has the same mass as nitrogen gas. The use of liquid  $\text{N}_2$  to remove  $\text{CO}_2$  from the samples generally eliminates this problem by preventing  $\text{CO}$  generation in the source of the mass spectrometer (Manning et al., 2010). Natural occurring carbon monoxide is not removed cryogenically, but is usually 4-6 orders of magnitude lower than  $\text{N}_2$  concentrations (Manning et al., 2010). The exception being photochemically produced  $\text{CO}$  in surface waters (Manning et al., 2010). Samples below surface waters are extremely unlikely to be affected by carbon monoxide.

### **3.6 Suspended particulate matter**

In March 2005, two liters of seawater were filtered on deck through previously precombusted GF/F filters (nominal pore size  $0.7 \mu\text{m}$ ) and frozen to make a depth profile from the surface to 275 m ( $\sigma_\theta = 16.8$ ) with 2-5 m resolution in the oxycline/suboxic region. Samples from June 2005, May 2007, October 2007, and July 2008 were processed similarly except that 5 L of seawater was filtered and filters were dried at  $60^\circ\text{C}$ . After returning to the lab, filters sat in  $\text{HCl}$  fumes for 24 hours to remove particulate carbonates. Isotopes, concentrations, and C:N ratios were measured in the SIL with a Finnigan Delta XL isotope ratio mass spectrometer connected to a NC2500 CE Instruments Elemental Analyzer by a Finnigan MAT ConFlo II. Unused precombusted filters were used as a blank. Nicotinic Acid standards produced reproducible and accurate isotopic results down to  $13 \mu\text{g}$  of N. Thus the isotopic values of samples with less than  $13 \mu\text{g}$  of N were discarded. For March 2005

samples, since 2 L of water was filtered rather than 5L, sample sizes were too small to obtain good isotopic values at deeper depths including the suboxic zone. Suspended particulate nitrogen concentrations, but not isotopes, for May and October 2007 have been previously published (Kirkpatrick et al., 2018).

## 4. Results

The concentration and nitrogen isotope ( $^{14}\text{N}$  and  $^{15}\text{N}$ ) values for  $\text{N}_2$ ,  $\text{NO}_3^-$ , total or dissolved organic nitrogen, and suspended particulate organic nitrogen were sampled in the Western Central Gyre in March 2005 and in the northeast Black Sea in June 2005, May and October 2007 and July 2008 (Fig. 1). As will be seen below, the March 2005 data from the end of the spring bloom had both organic matter and  $\text{N}_2$  gas maxima in the hypoxic waters of the oxycline above the suboxic zone. This  $\text{N}_2$  gas maximum in the hypoxic zone was in addition to the large suboxic zone  $\text{N}_2$  gas peak consistently found in the Black Sea (Fuchsman et al., 2008). The other four cruises in the northeastern Black Sea, as well as previously published data from May 2001, are used to represent lower productivity summer and fall conditions in the Black Sea. Concentration and isotope data presented here can be found in the supplementary data.

### 4.1 Nutrients and Hydrographic Conditions

As is typically seen, density surfaces were at deeper depths in the northeastern Black Sea than at the Western Gyre (Oguz, 2002; Fig. S1). Surface densities varied significantly between cruises and were freshest in the northeast Black Sea due to the influence of freshwater input into that region (Table 1; Fig. S1). The July 2008 cruise occurred soon after a large storm.

Oxygen and sulfide profiles were fairly consistent on density surfaces between cruises (Fig 2a). However, there was some variability in the density of the upper boundary of the

suboxic zone ( $\sim 10 \mu\text{mol L}^{-1}$ ). The width of the suboxic zone varied from 17 m in May 2007 to 35 m in May 2001 (Table 1). Sulfide was generally first detected at  $\sigma_\theta = 16.10$  (Table 1) and increased to  $378 \mu\text{mol L}^{-1}$  at 2000 m. Nitrate was generally below detection in surface waters, except in May 2007 when it was  $0.5 \mu\text{mol L}^{-1}$  (Figure 2d). Nitrate concentrations increased to a maximum of  $4.5$  to  $7 \mu\text{mol L}^{-1}$  right above the suboxic zone (Figure 2; Table 1). Nitrite concentrations generally had both a shallower and deeper maximum, which varied between  $0.05$  and  $0.19 \mu\text{mol L}^{-1}$ . The highest nitrite concentrations were seen in June 2005 and May 2001 (Figure 2c; Table 1). Ammonium concentrations were generally  $< 0.1 \mu\text{mol L}^{-1}$  until the lower suboxic zone ( $\sigma_\theta = 15.8$ - $16.0$ ; Table 1) where they increased continuously to  $98 \mu\text{mol L}^{-1}$  at 2000 m. However, in October 2007, ammonium was low but measurable in the oxycline (Figure 2e).

#### **4.2 Concentrations and isotopes of suspended particulate organic matter**

The Black Sea typically has high concentrations of S-POC and S-PON in surface waters, which decrease with depth through the oxycline and upper suboxic zone. A second maximum is found at the chemosynthesis maximum at the boundary with the sulfidic zone (Coban-Yildiz et al., 2006). Variations on this typical profile can be seen in the four NE Black Sea datasets and in May 2001 (Figure 3 and S2). For example, in June 2005 in the northeast Black Sea, concentrations of suspended particulate organic carbon (S-POC) and nitrogen (S-PON) decreased from the surface ( $6.9 \mu\text{mol C L}^{-1}$ ;  $1.1 \mu\text{mol N L}^{-1}$ ) to the middle of the suboxic zone ( $1.1 \mu\text{mol C L}^{-1}$ ;  $0.14 \mu\text{mol N L}^{-1}$ ) ( $\sigma_\theta = 15.8$ ; 143 m) where concentrations then began to increase again (Fig. 3c and S2).  $\delta^{15}\text{N}$  values in the euphotic zone were around or below 0‰. Maximum  $\delta^{15}\text{N}$  values were between 6 and 7‰ at the [S-PON] concentration minima from  $\sigma_\theta = 14.5$  to  $\sigma_\theta = 15.9$ . At the bottom of the suboxic zone,



[S-PON] concentration increased ( $0.4 \mu\text{mol N L}^{-1}$ ), and  $\delta^{15}\text{N}$  values were depleted as much as  $-1\text{‰}$  (Fig. 3d).

In May and October 2007 and July 2008 S-POC and S-PON had similar shaped depth profiles as in June 2005 (Fig. 3c and S2; Kirkpatrick et al., 2018). In May  $\delta^{15}\text{N}$  values in the euphotic zone were between  $-1$  to  $+2\text{‰}$ , but increased to  $5\text{-}6\text{‰}$  in the oxycline and  $3\text{‰}$  in the suboxic zone (Fig. 3d). In October  $\delta^{15}\text{N}$  values in the euphotic zone were  $1.5\text{-}3\text{‰}$ , but increased to  $9\text{‰}$  in the oxycline and  $8\text{‰}$  in the suboxic zone (Fig. 3d). In July 2008  $\delta^{15}\text{N}$  values in the euphotic zone were  $4\text{-}4.5\text{‰}$ , but increased to  $11.5\text{‰}$  in the oxycline and  $8\text{-}10\text{‰}$  in the suboxic zone (Fig. 3d).

The profile of suspended particulate organic matter concentrations, in the Western Gyre in March 2005, was significantly different than seen in any of these other cruises (Fig. 3a,b). In March 2005 S-POC and S-PON were  $9 \mu\text{mol C L}^{-1}$  and  $1.2 \mu\text{mol N L}^{-1}$  in the surface and then significantly increased in the oxycline with a maximum of  $14 \mu\text{mol L}^{-1} \text{ C}$  and  $1.3 \mu\text{mol L}^{-1} \text{ N}$  at  $\sigma_{\theta}=15.2$ . Then S-POM decreased sharply to  $3 \mu\text{mol L}^{-1} \text{ C}$  and  $0.25 \mu\text{mol L}^{-1} \text{ N}$  at  $\sigma_{\theta}=15.6$ . The maximum in particulate organic matter extended for  $\sim 10$  meters, from 60 to 70 meters. The  $\delta^{15}\text{N}$  of PON was around  $3\text{‰}$  at the organic matter maxima ( $\sigma_{\theta}=14.5\text{-}15.2$ ) and then increased to  $11\text{‰}$  (Fig. 3b). The C:N ratios increased from about 6 in the organic matter maximum to 8-10 in and below the suboxic zone (Fig. 3a). Below the suboxic zone [S-PON] concentrations were  $0.4 \mu\text{mol L}^{-1} \text{ N}$  and [S-POC] were  $4 \mu\text{mol L}^{-1} \text{ C}$ .

The maximum in suspended particulate material observed in the oxycline in March 2005 corresponded with a sharp reduction in transmissivity (Fig. 4), which decreased from 84% in surface waters to 73% at 63 meters. Below this thin transmissivity minimum, transmissivity increased to 88%. This minimum in transmissivity was accompanied by a maximum in fluorescence ( $9.9 \mu\text{g L}^{-1}$  chlorophyll a) (Fig. 4). Salinity was uniform until

$\sigma_\theta=14.5$  (60 m) and then increased with depth. Potential temperature decreased from 7.8°C at the surface to 7.1°C at the Cold Intermediate Layer (30-60 meters) and then increased with depth. Thus, the transmissivity minimum and fluorescence maximum were found right below the Cold Intermediate Layer, in the oxycline, at a depth range of sharp density increase. The transmissivity minimum was at the same depth (63 m) on 29 March and on 03 April, suggesting that the bulk of the organic material remained at that depth. However, the magnitude of both transmissivity and fluorescence anomalies were reduced on 03 April (from 73% to 78% transmissivity and from 9.8 to 6  $\mu\text{g L}^{-1}$  chlorophyll a [data not shown]), indicating some organic matter loss.

### 4.3 $\delta^{15}\text{N-NO}_3^-$ , TON and $\delta^{15}\text{N-TON}$

$\delta^{15}\text{N-NO}_3^-$  was fairly variable in the upper water column.  $\delta^{15}\text{N-NO}_3^-$  ranged from 5-6‰ in the oxycline ( $\sigma_\theta=15.2-15.4$ ) on both 2005 cruises (Figure 2b, c). In May 2007,  $\delta^{15}\text{N-NO}_3^-$  was 8-9‰ in the CIL and then decreased to 7‰ in the oxycline (Figure 2d). In July  $\delta^{15}\text{N-NO}_3^-$  had a maximum at 55 m of 12‰ then decreased to 6‰ in the CIL, but was 8‰ in the oxycline (Figure 2f).

$\delta^{15}\text{N-NO}_3^-$  profiles typically have an enriched maximum in the lower suboxic zone (Fuchsman et al., 2008), and similar profiles were seen in most of the sample sets shown here (Fig 2). In March 2005,  $\delta^{15}\text{N-NO}_3^-$  increased to 15‰ at  $\sigma_\theta=15.85$ . In June 2005, the  $\delta^{15}\text{N-NO}_3^-$  only increased to 7‰ at  $\sigma_\theta=15.95$  (Fig. 2). In May 2007,  $\delta^{15}\text{N-NO}_3^-$  increased to 13‰ in the lower suboxic zone (Figure 2d). In July  $\delta^{15}\text{N-NO}_3^-$  increased to 29‰ in the lower suboxic zone (Figure 2f).

In October 2007  $\delta^{15}\text{N-NO}_3^-$  was measured from 2 different types of samples—acidified samples and frozen samples. Both results were similar with between 5-7‰ throughout the oxic water column and then increasing to 10‰ in the suboxic zone (Figure

2e). In July 2008, frozen and acidified samples were also similar, though not necessarily measured at the same depths (Figure 2f). These values are quite similar to those seen in the oxycline and suboxic zone of the Baltic Sea (Frey et al., 2014).

Concentrations of total organic nitrogen (TON) in March 2005 increased from 13-14  $\mu\text{mol L}^{-1}$  in the surface to 17  $\mu\text{mol L}^{-1}$  at  $\sigma_\theta=14.6-14.7$  and then decreased to 10-11  $\mu\text{mol L}^{-1}$  in the suboxic zone (Fig. 5a). TON concentrations are elevated throughout the  $\sigma_\theta=14.6-15.3$  region of the [PON] maximum in March 2005. When PON concentrations are subtracted from TON, these dissolved organic N (DON) concentrations are still elevated in this region (Figure S3). Concentrations were fairly similar between TON/DON samples from March 2005 and DON samples from May and October 2007 (Figure 5a). In May 2007, DON concentrations were 14  $\mu\text{mol L}^{-1}$  from the surface to the upper oxycline and then decreased to 10-12  $\mu\text{mol L}^{-1}$  in the suboxic zone, and in October 2007, DON concentrations were highest at 30 m (21  $\mu\text{mol L}^{-1}$ ) and then decreased to 11  $\mu\text{mol L}^{-1}$  in the oxycline and then further to 8  $\mu\text{mol L}^{-1}$  in the suboxic zone (Figure 5a). However, in July 2008, DON concentrations were the lowest seen, with concentrations highest at 30 m (12  $\mu\text{mol L}^{-1}$ ) and decreasing to 8  $\mu\text{mol L}^{-1}$  in the oxycline and suboxic zone (Figure 5a). In June 2005, TON concentrations were quite high (15-24  $\mu\text{mol L}^{-1}$ ) (Figure 5a). These values are consistent with coastal measurements from 2001 (Ducklow et al., 2007), implying some coastal influence in June 2005.

Surprisingly, isotopic values for TON/DON samples were quite variable between cruises.  $\delta^{15}\text{N}$ -TON from June 2005 was 4‰ in the oxic zone and increased to around 8‰ in the lower suboxic zone (Fig. 5b).  $\delta^{15}\text{N}$ -DON from May 2007 was between 4-6‰ throughout the oxic and suboxic water column (Fig. 5b). Though only collected 5 months later, in October 2007, the  $\delta^{15}\text{N}$ -DON was around 8‰ in the euphotic zone, but was generally

between 10-12‰ at depth, with a maximum of 14‰ in the upper suboxic zone (Fig. 5b). In July 2008  $\delta^{15}\text{N}$ -DON was slightly heavier at around 11‰ in the euphotic zone and increased to 13-15‰ at depth (Fig. 5b). In contrast,  $\delta^{15}\text{N}$ -TON in March 2005 was around 13‰ at  $\sigma_t=15.4$  and decreased to 8‰ in the suboxic zone (Fig. 5b).  $\delta^{15}\text{N}$ -TON in March 2005 was different from all the other cruises in that it was more enriched in the oxycline compared to the suboxic zone.

Though some samples were prefiltered (DON) and some were not (TON), the concentrations of N in particulates is generally quite small compared to the dissolved or total organic N. For example, at  $\sigma_t=15.4$  in March 2005 [TON] is  $11 \mu\text{mol L}^{-1}$  and  $\delta^{15}\text{N}$ -TON is 13‰ while [PON] is  $0.4 \mu\text{mol L}^{-1}$  and  $\delta^{15}\text{N}$ -PON is 11‰. If the PON is subtracted from the TON to calculate DON, the isotope composition of DON is still 13‰. At  $\sigma_t=15.4$  in June 2005, [TON] is  $17.5 \mu\text{mol L}^{-1}$  and  $\delta^{15}\text{N}$ -TON is 6‰ while [PON] is  $0.25 \mu\text{mol L}^{-1}$  and  $\delta^{15}\text{N}$ -PON 6.8‰. If the PON is subtracted from the TON to calculate DON, the isotope composition of DON is still 6‰. The suspended PON pool is generally too small to shift the TON pool and its isotopes, so we feel it is acceptable to examine these sample sets together.

#### 4.4 N<sub>2</sub> and Ar

N<sub>2</sub>:Ar ratios in March 2005 had two maxima. A large maximum in the suboxic zone, similar to those seen in other years (Fuchsman et al., 2008) and a smaller maximum in the oxycline above the suboxic zone (Fig 6a). Recalibrated  $\delta^{15}\text{N}$ -N<sub>2</sub> values for March 2005 had a maximum in the oxycline of 0.86‰ and values of 0.60-0.65‰ in the suboxic zone (Fig. 6b).

In June 2005, only a N<sub>2</sub>:Ar maximum in the suboxic zone was seen. Both N<sub>2</sub> and Ar were unusually supersaturated in all the northeast Black Sea samples when compared with data from March 2005 and previous measurements from the Western Gyre (Fuchsman et al., 2008). In June 2005, N<sub>2</sub>:Ar supersaturation increased from 1.008 in the surface to a

maximum of 1.026 at  $\sigma_t=15.9$  and then decreased 1.012 in the deep water (Fig. 6a). Seawater in the Black Sea below the CIL is formed from the mixing of the high salinity Bosphorus outflow with the Cold Intermediate Layer (CIL) (Murray et al., 1991). The Ar and N<sub>2</sub> supersaturation due to this mixing was calculated in Fuchsman et al. (2008) and is seen in Figures 6c, d as the mixing line. In all cruises, argon concentrations decreased with increasing temperature and depth. However, argon concentrations from the northeast Black Sea were greater than predicted values calculated from mixing of the Bosphorus with the CIL (Fuchsman et al., 2008), (Fig. 6c) and to data from the Western Gyre in March 2005 (Fuchsman et al., 2008) (Figure 6c). As argon is an inert gas, the high argon concentrations seen here could be due to two possibilities: mixing with an unknown end member, or tiny air contamination due to the lack of CO<sub>2</sub> to flush the neck of the sample bottle during sampling (*see Methods*). As the data was reproducible within and between cruises, an air leak would have to have been constant (except for three samples with high Ar concentrations that were removed as definite air contamination). This seems unlikely. The shape of the N<sub>2</sub>:Ar profile (except the oxycline) was extremely similar between the March 2005 and June 2005 cruises.

For June 2005, the  $\delta^{15}\text{N-N}_2$  was 0.60-0.65‰ in the suboxic zone, and values in the oxycline mirrored that of March 2005.  $\delta^{15}\text{N-N}_2$  for May 2007, October 2007 and July 2008 have not been previously published. Values in the upper water column hovered around equilibrium with air, but values became somewhat lighter in the oxycline (0.5-0.68‰) and were between 0.6-0.7‰ in the suboxic zone and upper sulfidic zone in May and October and slightly more enriched in July (up to 0.75‰) (Figure 6b).

## 5. Discussion

$\delta^{15}\text{N}$  values of nitrate, S-PON, and TON/DON are variable in the Black Sea and can shift in time scales less than a year.  $\delta^{15}\text{N}$  of TON/DON and S-PON appear to shift together (Figures 3, 5). More depleted organic N values in May 2007 could be related to the presence of  $0.5\ \mu\text{M}$  nitrate in the surface waters. Larger surface nitrate concentrations allow phytoplankton to fractionate the nitrate, making the organic N more depleted (e.g. Rafter et al., 2013). Consistent with this, the  $\delta^{15}\text{N}\text{-NO}_3^-$  in surface waters was quite enriched in May 2007 (Figure 2d). We suspect a similar situation in June 2005, but nitrate isotope samples were not obtained for surface water samples. The greatly enriched  $\delta^{15}\text{N}$  of S-PON and DON values in July 2008 could be due to the lower DON and S-PON concentrations on this cruise compared to the others, presumably due to consumption (Figures 3, 5). The entire  $\delta^{15}\text{N}\text{-NO}_3^-$  profile was also more enriched in July (Figure 2f) potentially because nitrate at depth was formed from remineralized organic matter. Total or dissolved organic N contains both reactive and unreactive organic N. The variability of TON/DON isotopes with time (Figure 5) indicates that a significant proportion of the organic N was reactive.

In the following sections, we use samples from the northeast Black Sea in June 2005, May and October 2007, and July 2008 as a comparison to data from the Western Gyre in May 2001 (Fuchsman et al., 2008) and March 2005. An autonomous profiling float deployed in the Western Gyre at 200 m took 6 months to travel in the Rim Current from the western to the NE Black Sea (Korotaev et al., 2006). Thus, the two sites are connected in time scale comparable to the sampling intervals investigated here.

## 5.1 Suspended matter comparison

There is evidence that fresh organic matter was exported to the oxycline in March 2005, the typical time of year for a spring bloom (Mikaelyan et al., 2017). A maximum in monthly averaged satellite chlorophyll a concentrations occurred in February 2005 with significant amounts of chlorophyll still present in March (Nezlin, 2008). Chloroplasts from both diatoms and a member of the Cryptophyta phylum were found in pyrosequences of 16S rRNA at  $\sigma_\theta=15.3$  in March 2005 (Fuchsman et al., 2011), indicating the origin of this organic matter. Concentrations of suspended organic matter in the Western Gyre in March 2005 had a large maximum at  $\sigma_\theta=14.5-15.2$ , then decreased sharply with depth (Fig. 3a, b). The  $\delta^{15}\text{N}$  of this maximum was around 3‰ (Figure 3b), and C:N values were  $\sim 6$ , (Figure 3a) indicated fresh, non-degraded, material (Rice & Tenore, 1981). The distributions of suspended particulate organic carbon and nitrogen seen in June 2005, May and October 2007 and July 2008 in the northeast Black Sea (Fig. 3c) and in May 2001 in the Western Gyre (Fig. 3c; Coban-Yildiz et al., 2006; Fuchsman et al., 2008) were simpler and did not include a maximum in the oxycline. Instead concentrations steadily decreased from the surface to the middle of the suboxic zone where they increased (Figure 3c) due to chemoautotrophic production with reduced sulfur (Coban-Yildiz et al., 2006; Yilmaz et al., 2006).  $\delta^{15}\text{N}$ -PON values from the northeast Black Sea (5-11‰; Figure 3d) or the Western Gyre in 2001 (7‰) (Figure 3d; Coban-Yildiz et al., 2006) were also more enriched than those seen in March 2005 indicating more degraded material.

The suspended matter maximum in the oxycline in March 2005 was also recorded as a transmissivity minimum and fluorescence maximum at 63 m ( $\sigma_\theta= 15.1$ ) (Fig. 4). However, the increase in phytoplankton biomass at 60-65 meters was surprising since the euphotic zone, as delineated by 1% light level, was not measured, but is usually at 15-30 meters (Yilmaz et al., 2006). The transmissivity and fluorescence anomalies were at the same depth

(63 m) on 29 March and 03 April but diminished over time, suggesting that the bulk of the particulate organic material remained at that depth though some was either consumed or continued to sink. It should be noted that the 60-70 meters region corresponds to a large density change ( $\sigma_\theta=14.68-15.3$ ; Fig 4). Marine snow has been found to accumulate at density discontinuities when the density change  $\Delta\sigma_\theta > 0.04$  (MacIntyre et al., 1995). The change in density in March 2005 was an order of magnitude larger. We hypothesize that a sinking bloom of phytoplankton got caught at the density discontinuity. In any case, it appears that the bloom injected fresh organic material straight into the oxycline of the Black Sea where oxygen was 30-50  $\mu\text{M}$ .

## 5.2 N<sub>2</sub>:Ar comparison

Data from all cruises showed a N<sub>2</sub>:Ar maximum in the suboxic zone. In March 2005, there was also an additional N<sub>2</sub> and N<sub>2</sub>:Ar maximum in the oxycline above the suboxic zone associated with the fresh organic matter.  $\delta^{15}\text{N-N}_2$  from the maximum in the oxycline in March 2005 was enriched compared to equilibrium with air (Figure 6).  $\delta^{15}\text{N-N}_2$  from the oxycline in March 2005 was similar in magnitude to enriched  $\delta^{15}\text{N-N}_2$  data from a N<sub>2</sub> maximum in the oxycline above the ETSP oxygen deficient zone in a highly productive eddy (Altabet et al., 2012). Both of these oxycline datasets are enriched compared to  $\delta^{15}\text{N-N}_2$  in the Black Sea suboxic zone (Figure 6) or  $\delta^{15}\text{N-N}_2$  in oceanic oxygen deficient zones (Fuchsman et al., 2018). The maximum in  $\delta^{15}\text{N-N}_2$  in March 2005 (Western Gyre) was slightly offset deeper than the organic matter maximum and encompassed the entire depth range where the organic matter was consumed ( $\sigma_\theta=15.2-15.5$ ; Fig. 7). The N<sub>2</sub>:Ar depth profile also had a maximum in this same depth range. There was no maximum in N<sub>2</sub>:Ar in the oxycline depth range ( $\sigma_\theta=15.2-15.5$ ) in June 2005, May 2007, July 2008 or in the western



gyre in May 2001 (Fig 6a) (Fuchsman et al., 2008). In October 2007, which occurred at the beginning of the fall bloom period when productivity was higher than on the other northeastern cruises, N<sub>2</sub>:Ar supersaturation was higher than found in the other NE Black Sea cruises (Figure 6a) and mRNA from denitrifying bacteria were present in the upper suboxic zone (Kirkpatrick et al., 2012).

We assume that the extra N<sub>2</sub> gas from the oxycline in March 2005 is produced in situ. The Western Gyre is in the center of the Black Sea, separated from coastal sediments by the rim current (Figure 1). Thus, the nitrogen gas in the oxycline has three potential sources:

$$[\text{Total N}_2] = [\text{Abiotic N}_2] + [\text{Biogenic N}_2 \text{ diffused from below}] + [\text{In situ biogenic N}_2] \quad [1]$$

N<sub>2</sub> saturation values are determined by equilibration with the atmosphere at specific temperatures and salinities (Fig 6d) (Hamme & Emerson, 2004). However, because seawater in the oxycline forms by mixing of the Bosphorus inflow with the CIL and this mixing results in supersaturation of gases, a mixing line better represents the abiotic component of the N<sub>2</sub> gas in Eq. 1 (Fig 6d) (Fuchsman et al., 2008).

The N<sub>2</sub>:Ar profiles from the NE Black Sea are all shifted to larger concentrations when compared to those in the Western Gyre. We know from mRNA data that both anammox and denitrifying bacteria were consistently active in the lower suboxic zone of the NE Black Sea (Kirkpatrick et al., 2012). However, due to the extra super saturation of both N<sub>2</sub> and the inert gas argon throughout their depths profiles, including deep waters, it appears that the northeast Black Sea has another end member that is mixing N<sub>2</sub> and Ar into the water. The argon supersaturation in the northeast Black Sea increased with depth (Fig S4), which is consistent with a shelf sediment end member source. For example, the northeast Black Sea is home to many methane seeps (Egorov et al., 2008; Marina et al., 2011). Excess mantle-

derived argon is known to be found in hydrothermal vent fluids (Stuart & Turner, 1998; Winckler et al., 2001) and argon is known to degas in water rock interaction (Tolstikhin et al., 1996). Whatever the actual source, we can use the excess argon seen in the northeast Black Sea to quantify the potential magnitude of the third end member and remove it, allowing comparison to the Western Gyre N<sub>2</sub> data. For the northeast Black Sea June 2005 data, if we subtract the argon mixing line from the measured argon concentration, multiply the residual by 50 N<sub>2</sub>:Ar (Figure S4) and then subtract from the measured N<sub>2</sub> concentration, we can make the northeast and Western Gyre profiles overlap (Figure S5). Similarly, the May and October 2007 and July 2008 data from the northeast Black Sea will overlap with the March 2005 data if the residual is multiplied by 60 N<sub>2</sub>:Ar (Figure S4-S5). From this calculation, we estimate the ratio of N<sub>2</sub>:Ar in this third end member to be 50-60. The ratio of N<sub>2</sub>:Ar in air is 83.6. Thus the ratio of 50-60 is less than air and is consistent with the production of argon from water rock interactions or similar sources. After this removal, the difference between N<sub>2</sub> from March 2005 and June 2005 at the oxycline maximum above the suboxic zone were 2-3 μmol/kg. However, given the uncertainty involved in this removal, we do not use this data in the calculations below.

### 5.3 N<sub>2</sub> production in the oxycline

The concentrations of excess N<sub>2</sub> at the March 2005 oxycline maximum relative to the N<sub>2</sub> gas mixing line, ranged from 3.5 to 8 μmol kg<sup>-1</sup> (Figure 8). As seen in equation 1, we may not be able to simply compare N<sub>2</sub> gas in the oxycline in March 2005 to an abiotic mixing line because some biogenic N<sub>2</sub> may be found in the oxycline due to vertical diffusion of biogenic N<sub>2</sub> from the suboxic zone below (Fuchsman et al., 2008; Konovalov et al., 2008). We compare N<sub>2</sub> concentrations from March 2005 to a previously published profile from May 2001 (Fig 6d) (Fuchsman et al., 2008) and an inverse model of the March 2005 (Fig 6d)

(Konovalov et al., 2008). The inverse model replicates the initial conditions before the N<sub>2</sub> production event described here because the calculations did not include N<sub>2</sub> production in the oxycline and failed to fit the N<sub>2</sub> maximum in the oxycline (Konovalov et al., 2008). By subtracting concentrations from May 2001, or modeled data (Konovalov et al., 2008) from measured concentrations in the oxycline in March 2005, we estimated the extra N<sub>2</sub> produced during this event. Values varied from 2-4 μmol kg<sup>-1</sup> (Figure 8), which is very similar to values calculated from the residual to corrected northeast N<sub>2</sub> data (Figure S4). For comparison, biologically produced excess N<sub>2</sub> in the suboxic zone in March 2005 had a maximum of 10 μmol kg<sup>-1</sup> (Fuchsman et al., 2008).

In order to calculate the δ<sup>15</sup>N-N<sub>2</sub> produced during this event, we assumed that the δ<sup>15</sup>N-N<sub>2</sub> at equilibrium with air (0.68‰; Knox et al., 1992) represented the initial condition in the Western Gyre before the N<sub>2</sub> production event in March 2005. An upward flux of N<sub>2</sub> from the suboxic zone would also have similar isotopic values (0.6-0.7‰; Figure 6). We used the extra excess of N<sub>2</sub> calculated using data from May 2001, the inverse model, and the mixing line as the background concentrations, and found the isotopic composition of the extra excess N<sub>2</sub> by mass balance. The calculated δ<sup>15</sup>N-N<sub>2</sub> of the *in situ* biological N<sub>2</sub> in the oxycline in March 2005 ranged from +7 to +38‰ where values calculated with the largest concentrations of extra N<sub>2</sub> had the least enrichment (Fig. 8). Exact numbers should not be taken too literally as a difference in 0.5 μmol kg<sup>-1</sup> extra excess can change the δ<sup>15</sup>N of the produced N<sub>2</sub> by 3-5‰. However, the calculation does indicate that the *in situ* biological N<sub>2</sub> due to this event would be greatly enriched compared to dissolved gas that has been equilibrated with the atmosphere.

The ratio of transformation rates of <sup>14</sup>N to <sup>15</sup>N is defined by an isotope effect (ε), and is often negative as lighter isotopes undergo transformation at a higher rate. The isotope

effect ( $\epsilon$ ) for denitrification from cultures is between -10 and -25‰ (Kritee et al., 2012) and is  $\sim$  -25‰ for  $N_2$  production in marine oxygen deficient zones (Fuchsman et al., 2018). For anammox in wastewater treatment facilities, the isotope effect is -23 to -29‰ for ammonia and -16‰ for nitrite (Brunner et al., 2013). In oxycline of the Black Sea in March 2005, a -25‰ isotope effect with the reactant nitrate at 5-7‰ would produce instantaneous  $N_2$  of -18 to -20‰. However, the calculated  $\delta^{15}N-N_2$  of the *in situ* biological  $N_2$  in the oxycline in March 2005 ranged from +7 to +38‰. Even though these isotope effects are reduced in systems like the Black and Baltic Seas, where reactants are completely consumed in the suboxic zone (Frey et al., 2014; Konovalov et al., 2008), in all normal cases, the reactant,  $NO_x^-$ , should become more enriched and the product,  $N_2$ , more depleted.

The important question is how can enriched  $N_2$  be produced in the oxycline. Nitrate with  $\delta^{15}N-NO_3^-$  at 4-7‰ is more depleted than the enriched  $N_2$  (Fig. 2b). The  $\delta^{15}N$  of suspended particulate organic matter was 3‰ from  $\sigma_\theta=14.6$  to 15.2, but the S-PON sample from  $\sigma_\theta=15.4$  had  $\delta^{15}N-PON$  of 11‰ (Figure 3b). Total organic N is the most isotopically enriched N species measured (Figure 5b).  $\delta^{15}N-TON$  reached a maximum of 13‰ and  $\delta^{15}N-PON$  was 11‰ just below the zone of enriched  $N_2$  (Fig. 3, 5, 6). Thus, in March 2005 TON and S-PON at these depths were greatly enriched compared to ambient nitrate, but were similar to organic N values seen in October 2007 and July 2008 (Fig. 5). We suggest organic N was a potential N source for the excess  $N_2$ .

We examined suspended PON. Sinking and suspended PON have been found to have different  $\delta^{15}N$  values in the euphotic zone in the open ocean due to trophic effects during consumption and defecation by zooplankton (Altabet, 1988). In our case consumption by zooplankton would produce sinking particles at least 3.5‰ more enriched (for one trophic level) or greater (for more than one trophic level). For the phytoplankton in the organic

matter maximum at  $\sigma_{\theta}=15.2$ , consumption by zooplankton would increase the  $\delta^{15}\text{N}$  to at least 6.5‰. However, sinking PON was not collected, so the exact values are unknown.

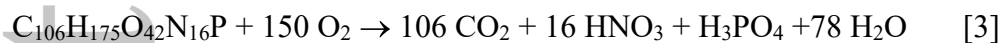
#### 5.4 $\text{N}_2$ production within particulate matter

The maximum suspended PON at  $\sigma_{\theta}=15.2$  was  $2 \mu\text{mol L}^{-1}$  in March 2005 and decreased to  $0.3 \mu\text{mol L}^{-1}$  at the top of the suboxic zone (Figure 3b). Total organic N has much higher concentrations (Figure 5). However, consumption of DON would not allow physical separation of the  $\text{N}_2$  producing bacteria from the high oxygen concentrations. This purported  $\text{N}_2$  production would be occurring in water with  $30\text{-}50 \mu\text{mol L}^{-1}$  oxygen, even though anammox and denitrification are 50% inhibited at 900 nM and 300 nM oxygen respectively (Dalsgaard et al., 2014). In the marine environment, there are oxygen gradients inside organic matter aggregates (Ploug, 2001; Ploug et al., 1997; Ploug & Bergkvist, 2015). In diatom aggregates, which at least partially form the organic matter here (Fuchsman et al., 2011), diffusion limitation of oxygen occurred starting at ambient  $\text{O}_2$  concentrations of  $100 \mu\text{M}$  (Ploug & Bergkvist, 2015; Stief et al., 2016) and aggregates were 50% anoxic at  $20 \mu\text{M}$   $\text{O}_2$  (Ploug & Bergkvist, 2015). It is likely that oxygen concentrations were reduced inside the particles during this high organic matter event in the Black Sea, allowing conditions inside the particles that were conducive to denitrification. The molecular diffusion coefficient ( $k$ ) in seawater ( $8^\circ\text{C}$ ) of oxygen ( $1.55 \times 10^{-5} \text{ cm}^2\text{s}^{-1}$ ) is greater than that of nitrate ( $1.16 \times 10^{-5} \text{ cm}^2\text{s}^{-1}$ ). Thus, nitrate, at concentrations  $<5 \mu\text{M}$ , would also be diffusion limited in this system.

We can test our theory of local N use within the particle with a simple calculation of volumetric respiration rates of both oxygen and nitrate. The balance between the source and sink for oxygen and nitrate inside a particle can be calculated as follows:

$$\text{respiration} = \frac{\text{Gradient}_C \times k \times \text{SurfaceArea}_{\text{aggregate}}}{\text{Volume}_{\text{aggregate}} \times \text{DBL}} \quad [2]$$

where DBL is the diffusive boundary layer or the width of the layer around the particle where diffusion determines fluxes into the particle and  $k$  is the molecular diffusion coefficient (Ploug et al., 1997).  $\text{Gradient}_C$  represents the gradient of oxygen or nitrate between inside and outside of the particle. If we compare  $\text{O}_2$  respiration and nitrate respiration in the same particle, the surface area of the particle ( $\text{cm}^2$ ), volume of the particle ( $\text{cm}^3$ ), and diffusive boundary layer (cm) of the particle cancel in equation 2. In the zone of enriched  $\text{N}_2$  in the oxycline ( $\sigma_\theta=15.2-15.4$ ), ambient nitrate was  $4.5 - 4.8 \mu\text{mol L}^{-1}$  and the oxygen concentration was  $52 - 33 \mu\text{mol L}^{-1}$  in March 2005. As both oxygen and nitrate concentrations are low in the surrounding waters, here we assume that there is total consumption of oxygen and nitrate within the particle. The oxygen flux is larger than the nitrate flux when we assume molecular diffusion coefficients ( $k$ ) in seawater. Using stoichiometry for aerobic respiration (Eq. 3) (Anderson, 1995), we can calculate the amount of ammonia produced and then oxidized to nitrate during organic matter degradation.



If ammonia produced and oxidized to nitrate is compared to the nitrate diffusing in from the ambient water in the zone of enriched  $\text{N}_2$  ( $\sigma_\theta=15.2-15.4$ ), the ratio of N atoms originating from particulate matter to the number of N atoms diffusing from ambient water would be  $1.1 \pm 0.3$  for denitrification. If anammox, which uses ammonia and nitrite, occurred inside the particles instead of denitrification, the ratios would be  $1.6 \pm 0.4$  as more oxygen would be available to oxidize organic matter instead of ammonia. Ammonia and nitrite oxidation can occur even under very low oxygen conditions (Bristow et al., 2016; Lam et al., 2007) and have been previously documented in particles (Klawonn et al., 2015). DNA for a member of the ammonia oxidizing genus *Nitrosospira*, (Teske et al., 1994) was found at

$\sigma_\theta=15.3$  (Fuchsman et al., 2011) and ammonia oxidizing archaea have been previously found at these depth in the Black Sea (Lam et al., 2007). These calculations suggest, assuming ammonium oxidation occurs inside a particle, that in the oxycline, there is the potential for more  $\text{NO}_x^-$  to be produced from organic N inside the particle than arrives by diffusion from ambient seawater. While we cannot know how much PON was consumed before the sampling dates, the extra suspended  $\sim 1.7 \mu\text{mol L}^{-1}$  PON at  $\sigma_\theta=15.2$  in March 2005 could, using the 1.1 ratio of N atoms originating from particulate matter to the number of N atoms diffusing from ambient water, produce  $3.2 \mu\text{mol N L}^{-1}$ , which in the range of  $2\text{-}4 \mu\text{mol N L}^{-1}$  of extra excess  $\text{N}_2$  in the oxycline. Thus the PON maximum was the right magnitude needed to produce the  $\text{N}_2$  in the oxycline.

### 5.5 $\text{N}_2$ producing bacteria

The water column  $\delta^{15}\text{N-N}_2$  values are not useful for clarifying the relative importance of anammox versus denitrification for  $\text{N}_2$  production. However, bulk water DNA samples from the western central gyre in March 2005 ( $\sigma_\theta=14.9\text{-}16.4$ ) along with one particulate sample ( $> 30 \mu\text{m}$ ) at  $\sigma_\theta=15.8$  were analyzed with both TRFLP and pyrosequencing of the V6 variable region of 16S rRNA (Fuchsman et al., 2011; Fuchsman et al., 2012b; Fuchsman et al., 2012a). Though the maximum in anammox normalized TRFLP peak height does correlate with the maximum of  $\text{N}_2$  gas in the suboxic zone (Fuchsman et al., 2012a) (Fig. 7), both datasets indicate that anammox bacteria were not present in the oxycline. Out of 10,000 pyrosequences from  $\sigma_\theta=15.3$  in the oxycline, none were related to known anammox bacteria (Fuchsman et al., 2011). Bacteria that perform the anammox process are monophyletic, or closely related, and all anammox bacteria found in the marine environment are in the genus *Scalindua* (van de Vossenberg et al., 2013), so these data suggest the absence of the

anammox process. Particulate and water samples from the suboxic zone were compared in March 2005, and the anammox bacteria were clearly free-living in the suboxic zone (Fuchsman et al., 2012a). Anammox was also found to be free-living in two open ocean oxygen deficient zones (Ganesh et al., 2014, 2015), indicating that this is its general lifestyle. Thus, we conclude that anammox bacteria were not associated with the particles or the anomalous  $\delta^{15}\text{N-N}_2$  in the oxycline of the Black Sea (30-50  $\mu\text{M O}_2$ ).

In contrast, both V6 pyrosequencing and TRFLP did detect a phylotype of the *Methylophilus* family of betaproteobacteria [BS029; Accession number GU145414] in the oxycline (Fuchsman et al., 2011), and the *Methylophilus* TRFLP depth profile had a maximum peak height from  $\sigma_\theta=15.3$  to 15.6 and then decreased to undetectable at  $\sigma_\theta=15.9$ , correlating with decreasing nitrate concentrations (Fig. 7). In other systems, stable isotope probing techniques have identified *Methylophilus* relatives as potential denitrifiers using methanol (Ginige et al., 2004; Kalyuzhnaya et al., 2009). However, relatives of *Methylophilus* can also use oxygen (Doronina et al., 2005) and *Methylophilus* was not particle-attached in the suboxic zone (Fuchsman et al., 2011). Unlike anammox, denitrification is not monophyletic (Zumft, 1997), so other unknown denitrifying bacteria may also have been present. In fact, most nitrite reductase (*nirS*) sequences from Black Sea samples in 2003 and 2007 did not match known denitrifiers (Kirkpatrick et al., 2012). Additionally, denitrification genes were enriched in the particulate fraction in two oxygen deficient zones (Fuchsman et al., 2017; Ganesh et al., 2014, 2015), implying that at least some denitrifiers live in particles. In summary, anammox bacteria were not found in the oxycline where  $\text{N}_2$  production occurred, but a potential denitrifying bacterium was present and other denitrifying bacteria may have been present as well.

## 5.6 Calculation of $\delta^{15}\text{N}_2$ created from particle N



We can use a simple calculation to see if our data are consistent with denitrification inside sinking aggregates. From the calculation above (Eq. 2 and 3), we use a ratio of 1.1 N atoms from nitrate produced inside the particle for every 1 N atom from nitrate that diffuses in from outside. Assuming complete consumption and using the ratio of 1.1 N atoms from nitrate produced within the particle ( $\delta^{15}\text{NO}_3^-$ ,<sub>produced</sub> (‰)) for every 1 N atom from nitrate diffused from ambient water ( $\delta^{15}\text{NO}_3^-$ ,<sub>ambient</sub> (‰)) and the range of possible values, we use Eq. 4 to calculate the  $\delta^{15}\text{N-N}_2$  of ‘extra’  $\text{N}_2$ .

$$\frac{1 \times \delta^{15}\text{NO}_3^- \text{,Ambient} + 1.1 \times \delta^{15}\text{NO}_3^- \text{,Produced}}{2.1} = \delta^{15}\text{N}_{2,\text{Extra}} \quad [4]$$

The  $\delta^{15}\text{N}$  of nitrate that diffused in from the ambient water was 5-7‰ (Fig 2b). We can compare this to calculations of nitrate produced from organic matter remineralization, where the fractionation factor is -3.5‰ (Macko et al., 1994). At the same depth where  $\delta^{15}\text{N-TON}$  was 13‰ and  $\delta^{15}\text{N-PON}$  was 11‰ (Figures 3b, 5b). In addition, heterotrophic denitrification preferably utilizes proteins (Van Mooy et al., 2002), and in algae proteins are 3‰ more enriched than the whole cell (Macko et al., 1987), implying that the organic N actually consumed by denitrifiers could be more enriched than our bulk values. Nitrate produced from the heavy organic matter would be 7.5-10.5‰. As nitrate was diffusion limited, we assume the nitrate in the particle is a small pool that is completely consumed without fractionation.

Using equation 4, the predicted  $\delta^{15}\text{N-N}_2$  of ‘extra’  $\text{N}_2$  was in the range from 6.3 – 8.8‰. The calculated  $\delta^{15}\text{N-N}_2$  of the *in situ* biological  $\text{N}_2$  from the oxycline in March 2005 from measured  $\text{N}_2$  ranged from 7-38‰. Our calculations indicate that denitrification within particulate matter, where nitrate was diffusion limited and completely consumed, could produce enriched  $\text{N}_2$  under the conditions observed in March 2005 in the Western Gyre. On the other hand, fractionation of nitrate in the water column from denitrification ( $\epsilon = \sim 25\text{‰}$ )

would produce highly depleted  $\delta^{15}\text{N-N}_2$ , which would not be consistent with our measurements. Denitrification in particles is the most parsimonious explanation for enriched  $\text{N}_2$  measurements in the oxycline in March 2005.

## 6. Conclusions

In March 2005, high concentrations of organic matter were found in the oxycline above the suboxic zone of the Western Gyre in the Black Sea. A maximum  $\text{N}_2$  concentration correlated with the consumption of organic matter in the oxycline ( $30\text{-}50 \mu\text{mol L}^{-1} \text{O}_2$ ). DNA of anammox bacteria was not found at these depths (Fuchsman et al., 2011; Fuchsman et al., 2012a). However, a potential denitrifying bacterium was found and other denitrifiers were suspected to be present. The combination of microbial data and  $\text{N}_2$  gas concentrations indicate that denitrification could have occurred in the oxycline of the Black Sea during this high organic flux event in March 2005.  $\delta^{15}\text{N-N}_2$  produced during this event was calculated three ways, all of which indicated a source of significantly enriched  $\text{N}_2$ . Organic matter was enriched while ambient nitrate was not. Enriched  $\delta^{15}\text{N-N}_2$  at these depths could be most logically explained by the occurrence of denitrification inside organic matter aggregates. In this scenario, half of N atoms that formed  $\text{N}_2$  originally came from organic matter, but were immediately oxidized to nitrate or nitrite inside the aggregate. These data suggest that denitrifiers may be adapted to live and be active inside sinking aggregates in low oxygen water columns. Thus, denitrification in hypoxic waters may be temporally variable, with rates linked to organic matter export from the euphotic zone. This event most likely occurred because a sinking spring phytoplankton bloom got stuck at a sharp density gradient in the oxycline of the Black Sea and injected fresh organic matter directly into low oxygen waters, causing enough denitrification to occur to perturb the isotopic composition of the large  $\text{N}_2$  pool.

A  $N_2$  gas maximum and enriched  $\delta^{15}N-N_2$  in an oxycline layer has also been reported above the ETSP Oxygen Deficient Zone under highly productive conditions in an eddy (Altabet et al., 2012). The enrichment of  $\delta^{15}N-N_2$  in the oxycline of the ETSP eddy was of similar magnitude to the anomaly in the Black Sea discussed here. The biological  $N_2$  produced in the oxycline at this station in the Eastern Tropical South Pacific was greater than predicted from nitrate consumption (Altabet et al., 2012), which would be consistent with an additional N source from particulate organic matter. Additionally denitrification rates have been measured in the oxyclines above both the ETNP and ETSP oxygen deficient zones despite the inhibition of denitrification at nanomolar oxygen concentrations (Babbin et al., 2015; Dalsgaard et al., 2014; Ji et al., 2015). We suggest that heterotrophic denitrification in aggregates under hypoxic conditions may be common over oxygen depleted waters but may become more pronounced and more easily detectable during high productivity events. Future studies should provide experimental evidence to support or adjust the claim that half of marine water column denitrification occurs in hypoxic waters on particles (Bianchi et al., 2018).

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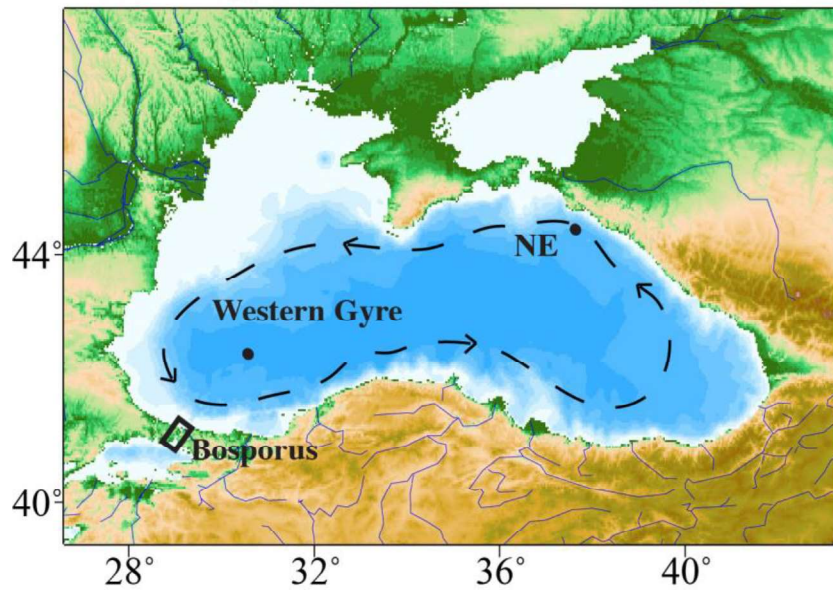
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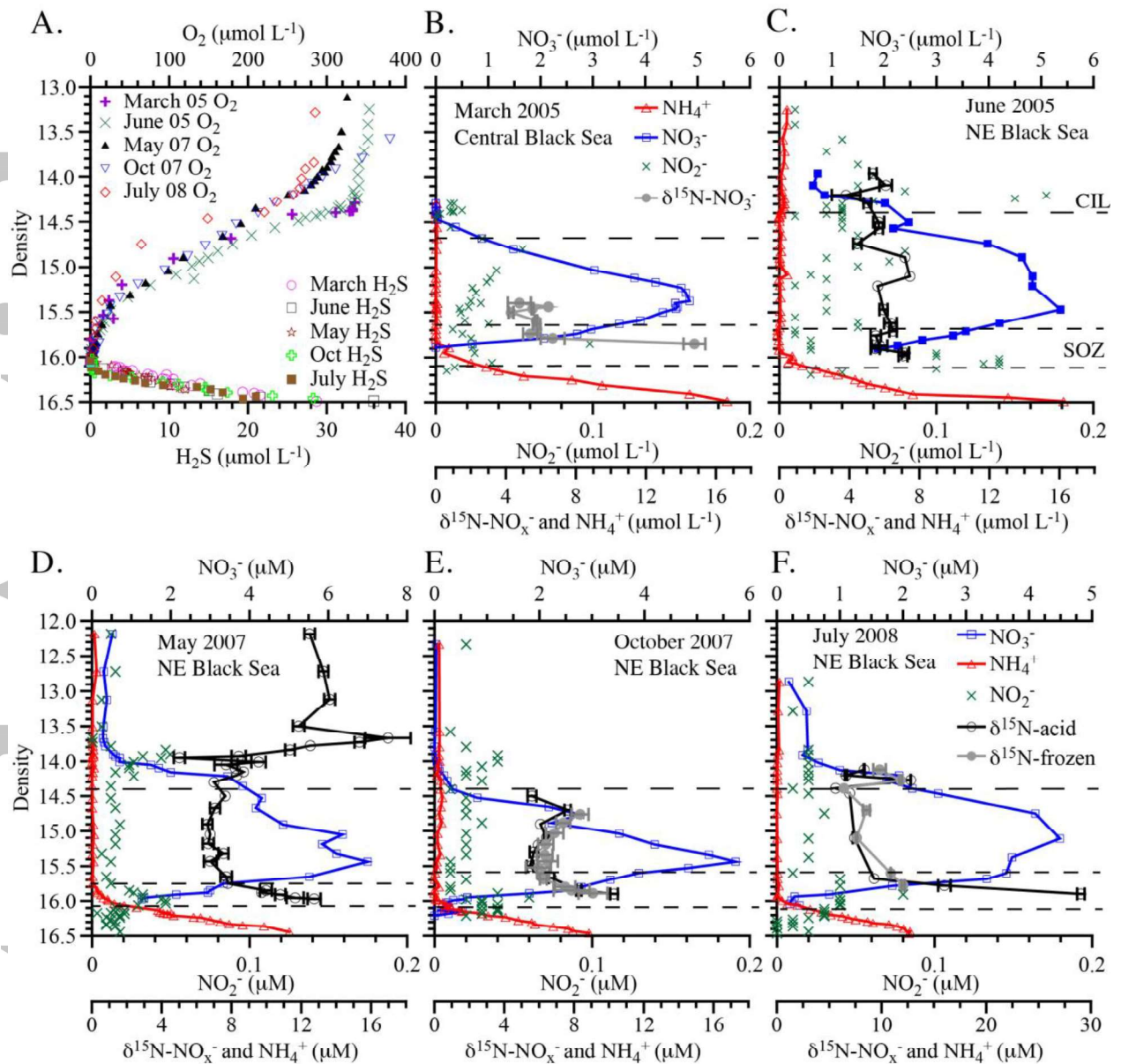
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Table 1. Hydrographic conditions across Black Sea cruises. SOZ stands for suboxic zone, and  $w$  stands for width of the suboxic zone in meters.

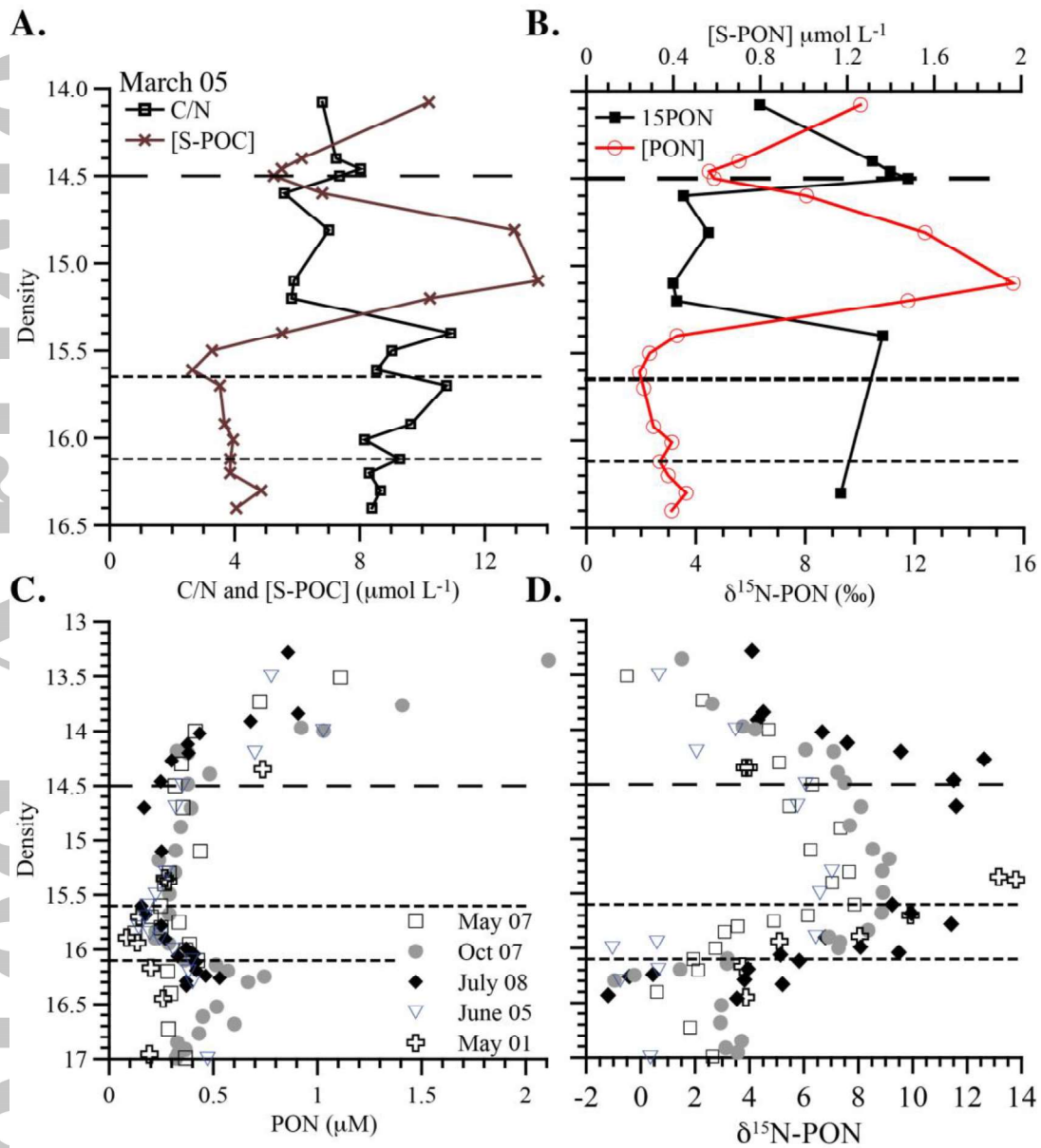
	Surface	Top of SOZ	Top of H <sub>2</sub> S flux	$w$	Nitrate max	Top of NH <sub>4</sub> <sup>+</sup> flux	Upper NO <sub>2</sub> <sup>-</sup> max	Lower NO <sub>2</sub> <sup>-</sup> max
<b>May 01</b>	$\sigma_\theta=12.5$	$\sigma_\theta=15.38$ (74m)	$\sigma_\theta=16.1$ (109m)	35	5.4 $\mu\text{M}$ $\sigma_\theta=15.5$ (77 m)	$\sigma_\theta=16.0$ (98 m)	0.08 $\mu\text{M}$ $\sigma_\theta=14.38$ (50m)	0.12 $\mu\text{M}$ through SOZ
<b>Mar 05</b>	$\sigma_\theta=14.3$	$\sigma_\theta=15.68$ (77m)	$\sigma_\theta=16.1$ (104 m)	27	4.8 $\mu\text{M}$ $\sigma_\theta=15.38$ (71m)	$\sigma_\theta=15.9$ (90 m)	0.05 $\mu\text{M}$ $\sigma_\theta=14.8$ (61 m)	0.08 $\mu\text{M}$ $\sigma_\theta=15.85$ (88m)
<b>Jun 05</b>	$\sigma_\theta=10.6$	$\sigma_\theta=15.74$ (140 m)	$\sigma_\theta=16.12$ (164 m)	24	5.4 $\mu\text{M}$ $\sigma_\theta=15.47$ (125m)	$\sigma_\theta=15.95$ (150m)	0.19 $\mu\text{M}$ $\sigma_\theta=14.2$ (42 m)	0.15 $\mu\text{M}$ $\sigma_\theta=15.98$ (152m)
<b>May 07</b>	$\sigma_\theta=11.6$	$\sigma_\theta=15.75$ (135 m)	$\sigma_\theta=16.04$ (152 m)	17	7 $\mu\text{M}$ $\sigma_\theta=15.45$ (120 m)	$\sigma_\theta=15.8$ (138m)	0.06 $\mu\text{M}$ $\sigma_\theta=13.9$ (45 m)	0.05 $\mu\text{M}$ $\sigma_\theta=16.0$ (148m)
<b>Oct 07</b>	$\sigma_\theta=11.0$	$\sigma_\theta=15.6$ (107 m)	$\sigma_\theta=16.1$ (137 m)	30	5.8 $\mu\text{M}$ $\sigma_\theta=15.44$ (104 m)	$\sigma_\theta=15.98$ (135m)	0.04 $\mu\text{M}$ $\sigma_\theta=14.0$ (40m)	0.05 $\mu\text{M}$ $\sigma_\theta=15.95$ (125m)
<b>July 08</b>	$\sigma_\theta=10.8$	$\sigma_\theta=15.6$ (114 m)	$\sigma_\theta=16.1$ (141 m)	27	4.5 $\mu\text{M}$ $\sigma_\theta=15.1$ (94 m)	$\sigma_\theta=15.95$ (131m)	0.04 $\mu\text{M}$ $\sigma_\theta=14.75$ (84m)	0.08 $\mu\text{M}$ $\sigma_\theta=15.9$ (128m)



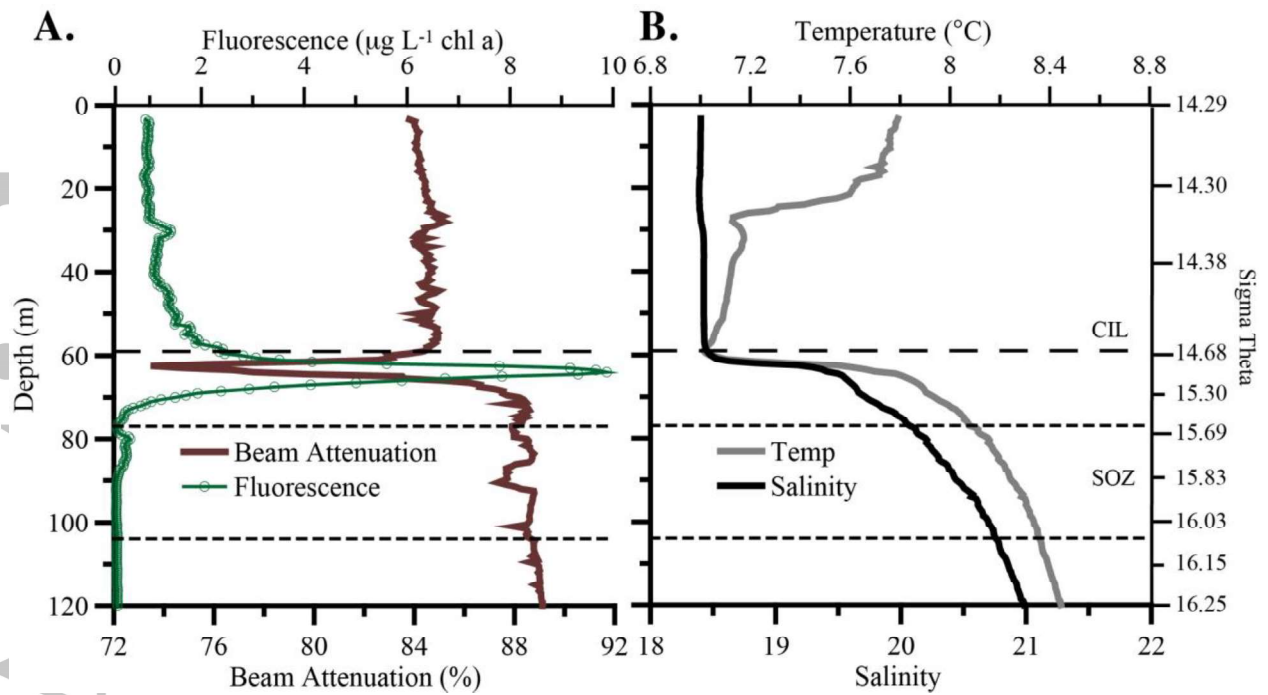
**Figure 1.** Map of the Black Sea indicating the stations described in this study. A schematic of the Rim Current is shown in a dashed line and the current direction is indicated by arrows. The Bosphorus inflow is indicated by a black square. Both the Western Gyre (May 2001 and March 2005) and Northeast Black Sea (June 2005, May and October 2007, July 2008) are repeat stations.



**Figure 2.** Contextual data. (A) Oxygen and sulfide data from all cruises, (B) nutrient and  $\delta^{15}\text{N-NO}_3^-$  profiles from March 2005, (C) from June 2005, (D) from May 2007, (E) from October 2007 and (F) from July 2008.  $\delta^{15}\text{N-NO}_3^-$  from acidified samples are in black and from frozen samples are in grey. Dashed lines indicate the boundaries of the suboxic zone (SOZ) and the bottom of the Cold Intermediate Layer (CIL).

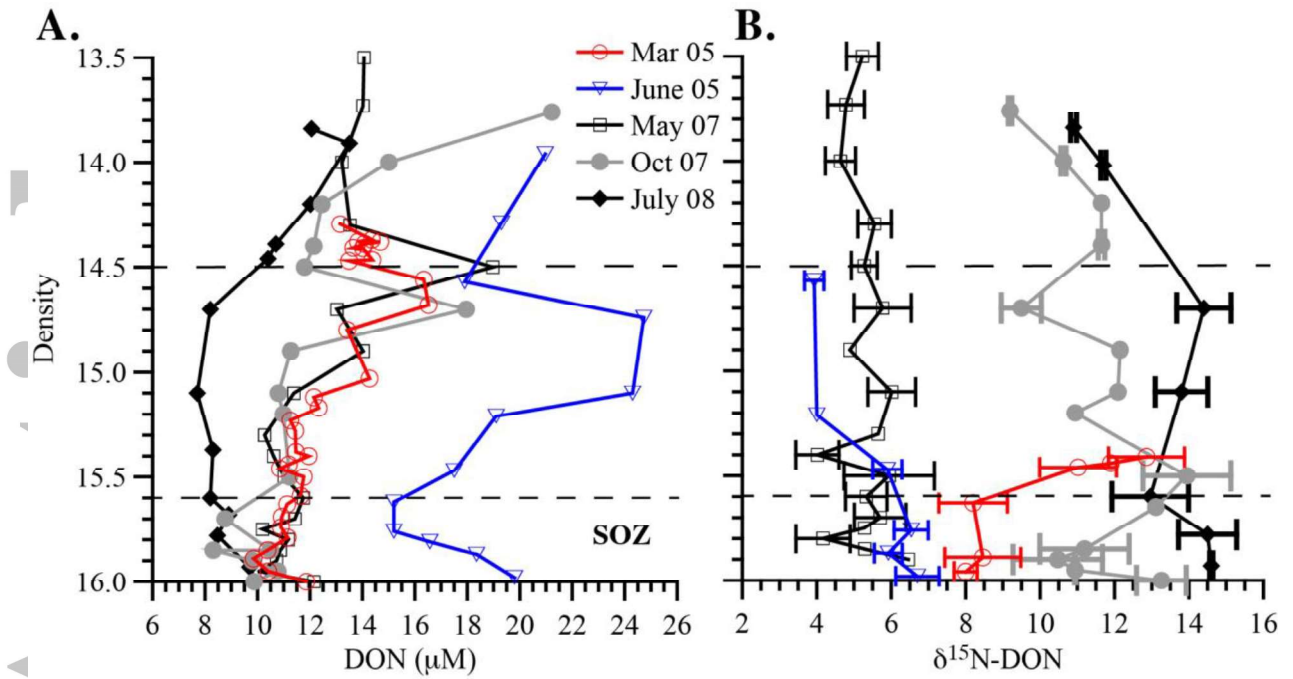


**Figure 3** Suspended particulate matter (S-POC and S-PON) for samples (A) C:N ratios (squares), [S-POC] (x) from the Western Gyre in March 2005 (B) [S-PON] (open diamonds) and  $\delta^{15}\text{N}$ -SPON (filled squares) from the Western Gyre in March 2005, and (C) [S-PON] and (D)  $\delta^{15}\text{N}$ -SPON for the northeast Black Sea in June 2005, May and October 2007, and July 2008. Dotted lines indicate the boundaries of the suboxic zone (SOZ). Dashed line indicates the Cold Intermediate Layer (CIL).



**Figure 4** CTD data from the Western Gyre in March 2005 cast 5 vs. depth. (A) Beam attenuation (brown) and fluorescence (circles) and (B) potential temperature (grey) and salinity (black). The second y-axis indicates the corresponding density to each depth. Dashed lines indicate the top and bottom of the suboxic zone (SOZ) and the Cold Intermediate Layer (CIL).

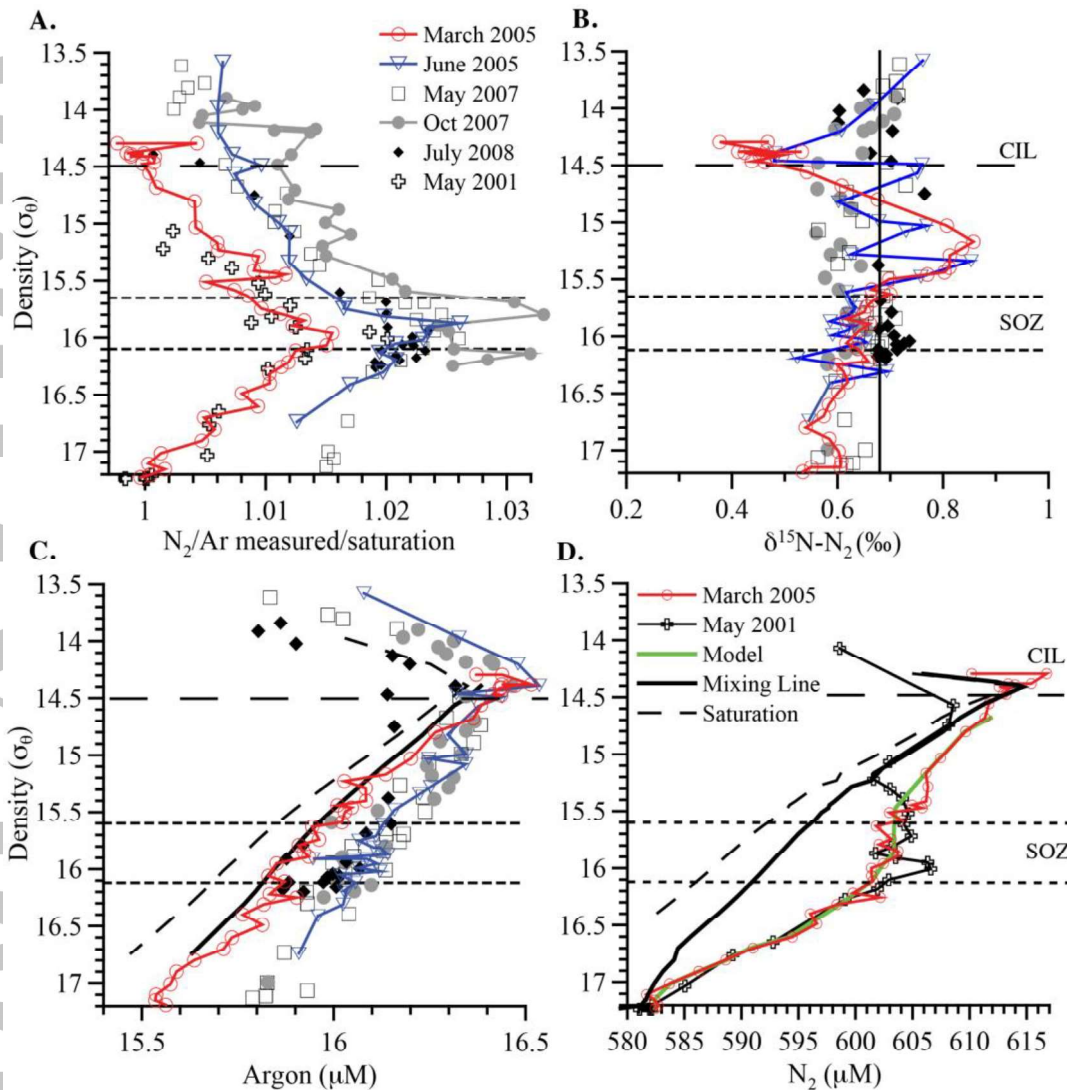




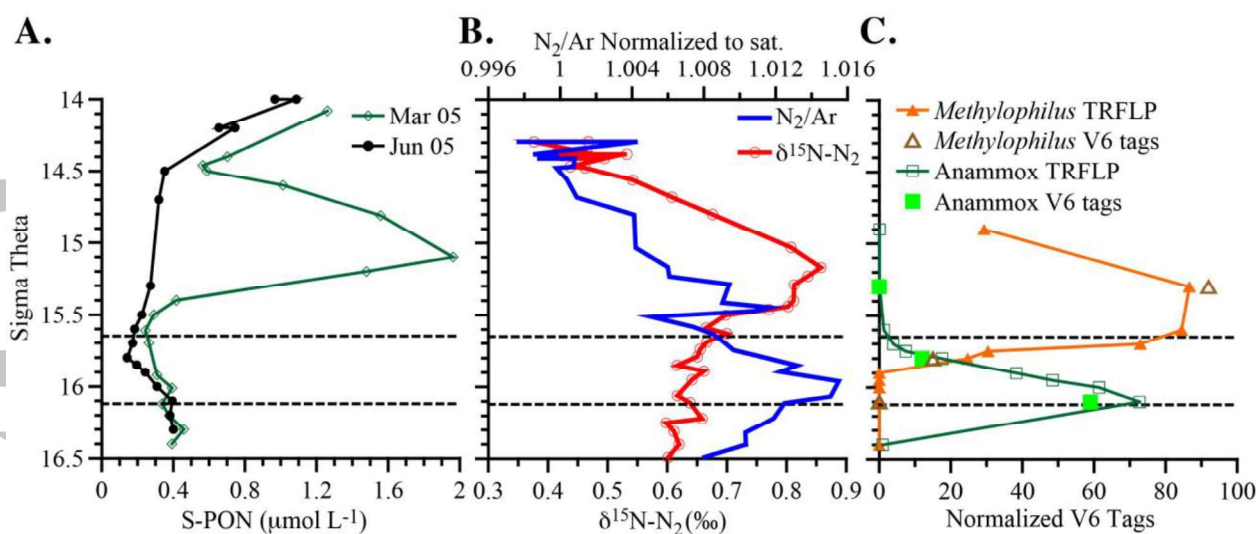
**Figure 5** Organic N comparison (A) Concentration of total organic nitrogen (TON) and (B)  $\delta^{15}\text{N-TON}$ . Dashed lines indicate the boundaries of the suboxic zone (SOZ) and the bottom of the Cold Intermediate Layer (CIL).

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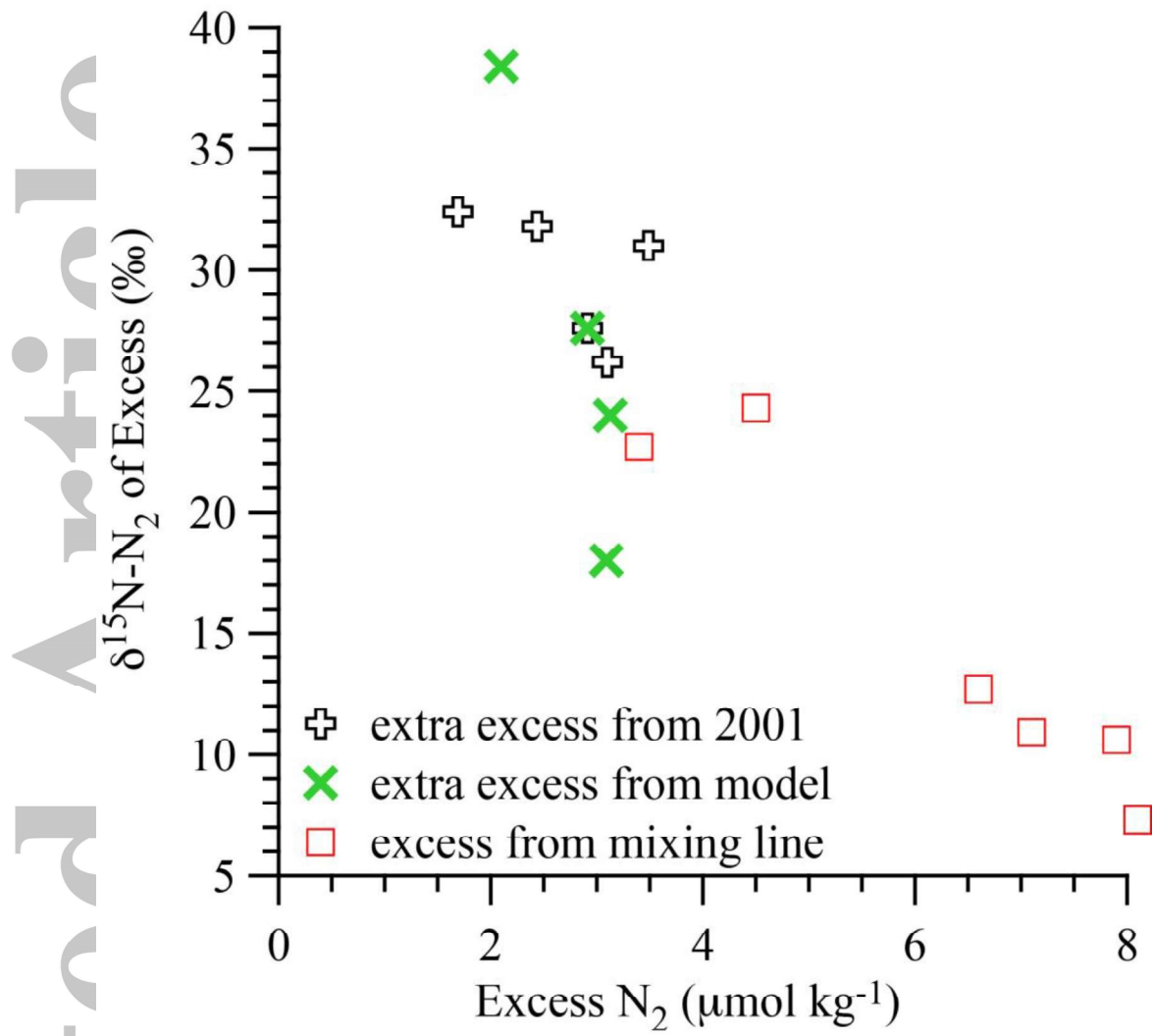




**Figure 6.** Comparison of  $N_2$  and Ar analyses. (A)  $N_2/Ar$  normalized to saturation for March and June 2005, May and October 2007 and July 2008 (Kirkpatrick et al., 2012) and May 2001 (Fuchsman et al., 2008). (B)  $\delta^{15}N-N_2$ . The vertical line indicates equilibrium with air. (C) [Ar]. Argon was not measured in May 2001. The mixing line for argon (bold black line) is calculated from the mixing of the Bosphorus into the Black Sea (Fuchsman et al., 2008). In (D) the concentration of  $N_2$  from March 2005 is compared to data from 2001 (circles), an inverse model of the 2005 data that does not include  $N_2$  production in the oxycline (green; Konovalov et al., 2008). The mixing line for  $N_2$  (bold black line) is calculated from the mixing of the Bosphorus into the Black Sea (Fuchsman et al., 2008). Horizontal dotted lines indicate the boundaries of the suboxic zone (SOZ). Horizontal dashed line indicates the cold intermediate layer (CIL).



**Figure 7.** Synthesis of data. (A) Comparison of suspended PON from March and June 2005 indicates a large maximum in March. (B) Maxima in N<sub>2</sub>/Ar and δ<sup>15</sup>N-N<sub>2</sub> co-exist and occur at the same depths where S-PON concentrations decrease. (C) A depth profile of *Methylophilus* clone BS0129 (triangles: normalized TRFLP peak height; brown triangles: normalized V6 tags), a potential denitrifier, and anammox bacteria phylotype *Candidatus* Scalindua sorokinii (empty squares: normalized TRFLP peak height; squares: normalized V6 tags), at the Western Gyre in March 2005. *Cand. S. sorokinii* was identified in TRFLP profiles obtained with Planctomycetes specific primers (Fuchsman et al., 2012a). All V6 tags (Fuchsman et al., 2011) within 97% similarity to the named sequence are shown. TRFLP peak height is a relative scale so the axis is not shown.



**Figure 8.** The amount and  $\delta^{15}\text{N}$  of  $\text{N}_2$  gas produced during a high organic matter flux event in March 2005. The concentration of  $\text{N}_2$  gas produced was calculated three ways, using the mixing line as initial conditions (squares), May 2001 values as the initial condition (crosses), or an inverse model of the March 2005 data as the initial condition (X).