Origin and fate of the secondary nitrite maximum in the Arabian Sea

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Abstract. The Arabian Sea harbours one of the three major oxygen minimum zones (OMZs) in the world’s oceans, and it alone is estimated to account for ~10–20 % of global oceanic nitrogen (N) loss. While actual rate measurements have been few, the consistently high accumulation of nitrite (NO$_2^-$) coinciding with suboxic conditions in the central-northeastern part of the Arabian Sea has led to the general belief that this is the region where active N-loss takes place. Most subsequent field studies on N-loss have thus been drawn almost exclusively to the central-NE. However, a recent study measured only low to undetectable N-loss activities in this region, compared to orders of magnitude higher rates measured towards the Omani Shelf where little NO$_2^-$ accumulated (Jensen et al., 2011). In this paper, we further explore this discrepancy by comparing the NO$_2^-$-producing and consuming processes, and examining the relationship between the overall NO$_2^-$ balance and active N-loss in the Arabian Sea. Based on a combination of $^{15}$N-incubation experiments, functional gene expression analyses, nutrient profiling and flux modeling, our results showed that NO$_2^-$ accumulated in the central-NE Arabian Sea due to a net production via primarily active nitrate (NO$_3^-$) reduction and to a certain extent ammonia oxidation. Meanwhile, NO$_2^-$ consumption via anammox, denitrification and dissimilatory nitrate/nitrite reduction to ammonium (NH$_4^+$) were hardly detectable in this region, though some loss to NO$_2^-$ oxidation was predicted from modeled NO$_3^-$ changes. No significant correlation was found between NO$_2^-$ and N-loss rates ($p > 0.05$). This discrepancy between NO$_2^-$ accumulation and lack of active N-loss in the central-NE Arabian Sea is best explained by the deficiency of labile organic matter that is directly needed for further NO$_2^-$ reduction to N$_2$O, N$_2$ and NH$_4^+$, and indirectly for the remineralized NH$_4^+$ required by anammox. Altogether, our data do not support the long-held view that NO$_2^-$ accumulation is a direct activity indicator of N-loss in the Arabian Sea or other OMZs. Instead, NO$_2^-$ accumulation more likely corresponds to long-term integrated N-loss that has passed the prime of high and/or consistent in situ activities.

1 Introduction

In global oceans, NO$_2^-$ is the least abundant of the major inorganic nitrogen ions (NH$_3^+$, NO$_2^-$, NO$_3^-$), representing only < 0.025 % of the 6.6 x 10$^3$ Tg N global oceanic inventory of fixed nitrogen (Gruber, 2008). At the second highest oxidation state (+III) of nitrogen, NO$_2^-$ often occurs as an intermediate in either oxidative or reductive pathways of the N-cycle. It can be produced during the first step of nitrification, when specific groups of archaea or bacteria oxidize ammonia to NO$_2^-$, most of which is then oxidized by a separate group of bacteria to NO$_3^-$. In the reductive pathways, NO$_2^-$ is produced via nitrate reduction, which may further lead to the production of gaseous nitrous oxide (N$_2$O) and dinitrogen (N$_2$), in the stepwise N-loss process known as denitrification (NO$_3^-\rightarrow$NO$_2^-\rightarrow$NO$\rightarrow$N$_2$O$\rightarrow$N$_2$). Denitrification can occur heterotrophically or autotrophically, but the former is presumably more common in seawater. NO$_2^-$...
may also be channeled through another N-loss process called anammox (van de Graaf et al., 1995), in which some autotrophic bacteria use NO$_3^-$ to anaerobically oxidize ammonium (NH$_4^+$) to N$_2$. Alternatively, NO$_3^-$ may be reduced directly to NH$_4^+$ in dissimilatory nitrate/nitrite reduction to ammonium (DNRA).

Though often barely detectable in seawater, NO$_3^-$ can accumulate to micromolar concentrations at the base of the sunlit euphotic zone due to phytoplankton release or nitrification – the so-called “primary nitrite maximum” (Olson, 1981; Dore and Karl, 1996; Lomas and Lipschultz, 2006). “Secondary nitrite maxima” occur deeper down in certain severely oxygen-deficient water columns known as oxygen minimum zones (OMZs). Secondary NO$_3^-$ maxima were first reported in the Arabian Sea in the 1930s (Gilson, 1937), then later also in the eastern tropical north and south Pacific (Brandhorst, 1959; Wooster et al., 1965). Because of the associated low-oxygen conditions, such NO$_3^-$ accumulations are conventionally believed to signify active heterotrophic denitrification, and have since led to a number of denitrification studies particularly targeting NO$_3^-$-laden waters in the past decades, and the obtained point-measurements have frequently been extrapolated to basin-scale N-loss (Fiedeiro and Strickland, 1968; Cline and Richards, 1972; Codispoti and Packard, 1980; Codispoti and Christensen, 1985; Codispoti et al., 1986; Naqvi, 1987; Lipschultz et al., 1990; Devol et al., 2006; Ward et al., 2009). Currently, oceanic OMZs are considered responsible for 30–50% of global oceanic N-loss (Gruber and Sarmiento, 1997; Codispoti et al., 2001; Gruber, 2008).

The Arabian Sea is a semi-enclosed basin with the biogeochemical cycling therein and the surface biological production strongly influenced by seasonal monsoons (Wiggert et al., 2005). During the summer southwest monsoon, anticyclonic circulation in the northern half of the basin induces upwelling of nutrient-rich water along the western boundary and to a lesser extent in the central basin, thus enhancing biological production in those regions. Strong convective mixing caused by winter northeastern monsoonal winds deepens the mixed layer especially in the north, bringing in nutrients from the deep and stimulating surface production. In the two intermonsoonal periods, surface water becomes largely oligotrophic within the basin. Large N-deficits relative to that expected from a constant ratio with phosphate or apparent oxygen utilization (Broecker, 1974; Gruber and Sarmiento, 1997), coincide with prominent secondary NO$_3^-$ maxima (≥0.2 µM), as well as oxygen deficiencies (<5 µM O$_2$) in the subsurface waters of the central-northeastern Arabian Sea (Naqvi et al., 1990; Bange et al., 2000). These contrast with the lack of prominent NO$_3^-$ accumulations or oxygen deficiencies in the more productive waters westwards (Naqvi, 1991). Hence, the majority of denitrification or N-loss has been assumed to occur in the central-NE part of the Arabian Sea (Naqvi, 1991).

Consequently, while direct N-loss rate measurements in the Arabian Sea have been few, those that took place have primarily focused on the zone of prominent secondary NO$_3^-$ maximum in the central-NE basin (Devol et al., 2006; Nicholls et al., 2007; Ward et al., 2009). Only one recent study compared N-loss in the central-NE Arabian Sea with the more productive waters towards the Omani Shelf. Surprisingly, orders of magnitude higher N-loss rates were detected over the shelf (Jensen et al., 2011), versus the very low to undetectable rates in the presumed “active denitrification zone”. While this showed a spatial coupling between N-loss and surface biological production that was in accord with other major OMZs (Kuyers et al., 2005; Thamdrup et al., 2006; Hamersley et al., 2007), the lack of substantial detectable active N-loss in the central-NE Arabian Sea is difficult to reconcile with the prominent secondary NO$_3^-$ maxima and N-deficits therein.

Here we investigate the NO$_3^-$ accumulation in the central-NE Arabian Sea OMZ by examining the active production and consumption mechanisms of NO$_3^-$, including nitrification, NO$_3^-$-reduction, N$_2$O production and DNRA that likely co-occur with N-loss in the OMZs (Lam et al., 2009; Lam and Kuyers, 2011); and compare these findings with those obtained near the Omani Shelf. Activities of these processes were determined via a combination of $^{15}$N-incubation experiments, flux modeling based on nutrient profiles, and expression analyses of biomarker functional genes for respective processes. Lastly, the suitability of NO$_3^-$ accumulations as a conventional active N-loss indicator, and its relationship with N-deficits in the OMZs are further discussed.

2 Methods

2.1 Water sampling and nutrient analyses

Sampling was conducted along a cruise-track encompassing the Omani Shelf and the central-NE Arabian Sea (Fig. 1) at the beginning of the 2007 autumn intermonsoon (Sept/Oct). Samples were collected using a Conductivity-Temperature-Depth (CTD) rosette system equipped with 101 Niskin bottles (Sea-Bird Electronics Inc.) on board the R/V Meteor (M74/1b). Water samples were analysed for NH$_4^+$, NO$_3^-$, NO$_2^-$ and PO$_4^{3-}$ (limits of detection of methods used: 20, 30, 100 and 100 nM respectively) at 10 to 25 m intervals for 12 stations along roughly the cruise-track of the former US Joint Global Ocean Flux Study (Morrison et al., 1999) (Fig. 1). NH$_4^+$ and NO$_3^-$ concentrations were analyzed immediately after sampling with fluorometric and spectrophotometric techniques, respectively (Grasshoff, 1983; Holmes et al., 1999). Samples for NO$_2^-$ and PO$_4^{3-}$ were stored frozen and measured spectrophotometrically (Grasshoff et al., 1999) with an autoanalyzer (TRAACS 800, Bran and Luebbe, Germany) in a shore-based laboratory. N$_2$O in seawater sub-sampled from Niskin bottles was analyzed on...
shipboard by introducing equilibrated headspace samples through a moisture trap and a gas chromatograph at 190 °C with a packed molecular sieve column (1.83 m × 3.175 mm (1/8") SS, 5A, mesh 80/100, Alltech GmbH, Germany), and N_{2}O was subsequently detected with an electron capture detector as previously described (Walter et al., 2006). N-deficits were estimated from the measured total inorganic nitrogen (i.e. NH_{4}^{+} + NO_{3}^{-} + NO_{2}^{-}) and PO_{4}^{3-} concentrations as N* (in units of µM) = [NH_{4}^{+} + NO_{2}^{-} + NO_{3}^{-}] − 16 [PO_{4}^{3-}] + 2.9 µmol kg^{-1} × density (Gruber and Sarmiento, 1997). These N-deficit estimates may be conservative, however, if there is preferential degradation of organic nitrogen as found previously in oxygen-deficient waters (Van Mooy et al., 2002), or remineralization of N-rich organic matter such as potentially resulted from spatially coupled N_{2}-fixation (Capone et al., 1998; Deutsch et al., 2007). Neutral densities were computed from CTD data according to Jackett and McDougall (1997). They are used as reference frames to view nutrient distributions, because water masses preferentially move and mix along neutral density surfaces, or isoneutrals, which are commonly approximated by surfaces of (potential) density, or isopycnals. Nonetheless, isopycnals have to be calculated from some arbitrarily chosen reference pressures and thermobaricity introduces an error when water parcels deviate from the reference pressures. In the Indian Ocean, such discrepancies between the vertical locations of isopycnals and isoneutrals could be substantial (You and McDougall, 1990), and so isoneutrals were used in the current study instead.

2.2 15N-Stable isotope pairing experiments

15N-stable isotope pairing experiments were conducted at six depths throughout the OMZ at each of seven sampling stations (Fig. 1), including two stations near the Omani Shelf and five in the central-NE basin characterized by a prominent secondary nitrite maximum (NO_{2}^{-} ≥ 0.2 µM). Rate determinations for anammox, denitrification and DNRA from these experiments have been reported in Jensen et al. (2011). As these three processes are potential NO_{2}^{-} sinks, some of the main findings are described in the current paper for the evaluation of overall NO_{2}^{-} balance. Using the same set of isotope-pairing experiments, 15NO_{2}^{-} production was additionally measured in incubations with 15NH_{4}^{+} + 14NO_{2}^{-} (5 µM each) and with 15NO_{2}^{-} (20 µM), in order to determine ammonia oxidation and nitrate reduction rates, respectively (McIlvin and Altabet, 2005; Lam et al., 2009). All incubations were conducted at non-detectable O_{2} levels after purging with helium for 15 min (≤ 0.5 µM) (Dalsgaard et al., 2003; Jensen et al., 2008, 2011), except for the oxygen-regulation experiments. In the latter, various amounts of O_{2}-saturated water were injected into the incubation vials which had previously been purged with helium, and the 4 different O_{2} levels achieved within the range of 0–12 µM (Table B1) were verified with an oxygen microsensor. All experiments were time-series incubations of approximately 0, 6, 12, 24, and 48 h conducted at in situ temperature and in the dark. Rates were calculated from the slopes of linear regressions of 15N-production as a function of time (limits of detection for N_{2}, NO_{2}^{-} and NH_{4}^{+} production: 0.15–0.20, 0.5 and 0.5 nM d^{-1}, respectively), and only those with significant production without an initial lag-phase were considered (slope significantly different from zero, t-tests with p < 0.05). Presented are net production rates that have been corrected for the mole fractions of 15N in initial substrate pools. Any isotope dilution in the substrate pools due to the co-occurrence of other concurrent N-cycling processes in the course of incubation, have not been corrected for in these rate calculations.

2.3 Functional gene detection and expression analyses

Water samples (10–15 l) for nucleic acid analyses were filtered through 0.22 µm Sterivex filters (Millipore) and stored at −80 °C until extraction in a shore-based laboratory. RNA and DNA were extracted from the same filters using the Totally RNA Kit (Ambion) with a prior cell lysis (10 mg ml^{-1} lysozyme in 10 mM Tris-EDTA, pH 8; 4 units of SUPERaseIn, Ambion) performed within filter cartridges. Various biomarker functional genes for ammonia oxidation (both archaeal and bacterial), nitrate reduction, anammox, denitrification and DNRA (Table B2) were analyzed using both qualitative and quantitative polymerase chain reactions (PCR). Active expression of these functional genes as transcripts (mRNA), were additionally analyzed via reverse transcription (RT) (Superscript III First-Strand Synthesis Master Mix, Invitrogen) with the respective gene-specific antisense primers, followed by quantification with real-time PCR (Lam et al., 2009; Jensen et al., 2011). The expressed nitric oxide reductase genes (norB), which encode the enzyme

![Fig. 1. Sampling stations (stars and station numbers) along cruise track (yellow) in the Arabian Sea in Sept/Oct 2007. The red dashed line marks the prominent secondary NO_{2}^{-} maximum of ≥ 1 µM in the central-northeastern basin (Naqvi, 1991).](www.biogeosciences.net/8/1565/2011/ Biogeosciences, 8, 1565–1577, 2011)
responsible for the reduction of nitric oxide to N₂O, were RT-PCR amplified and cloned with the TOPO TA Cloning Kit for sequencing (Invitrogen). Positive inserts were sequenced with ABI370XL sequencers (Applied Biosystems) by the GATC Biotech sequencing services, and phylogenetic analyses based on amino acids translated from norB genes were performed with the ARB package (Ludwig et al., 2004). New real-time PCR primers and probes for specific groups of *norB* were designed using the Oligo Design and Analysis Tools (Integrated DNA Technologies), and were further verified with BLAST (Altschul et al., 1997) and own ARB database compiled from *norB* sequences currently available in public databases. All primers and PCR protocols used in this study were listed in Table B2.

Expressions (transcriptions) of key functional genes initiate the production of enzymes that mediate the processes of interest, as opposed to gene presence that merely indicate the genetic potentials of organisms which may never utilize these genes and perform these reactions in situ. Active gene expression in unmanipulated seawater samples can thus serve as independent support for an active process detected, though the relationships between rates and gene expressions are not necessarily straightforward due partly to the vastly different detection limits of various measurement types, and partly to the influence from other transcriptional factors such as stresses, physiological states and post-transcriptional processes. Because a lot is yet to be explored of the immense oceanic microbiome, we do not claim an exhaustive coverage of the functional gene targets by the selected primers.

### 2.4 Reaction-diffusion modeling and statistical analyses

Net production and consumption rates (*R*) of NH₄⁺, NO₂⁻, NO₃⁻, N₂O and N* in the water column of the central-NE Arabian Sea were estimated from their respective measured concentration (*C*) profiles based on a reaction-diffusion model, which is similar to those often applied in sediment porewater studies (Berg et al., 1998):

\[
\frac{d}{dz} \left( K (z) \frac{dC}{dz} \right) + R = 0
\]

where *K*(z) is vertical eddy diffusivity and *z* is depth. *K*(z) is parameterized from Brunt-Väisälä frequencies (Garrett, 1984; Gregg et al., 1986; Fennel and Boss, 2003) computed from CTD data. These input data were interpolated to a computational grid and the differential Eq. (1) was transformed into an inverse linear system. Subsequently, *R* at the various interpolated depth intervals (z) was solved via a numerical method known as Tikhonov regularization. The detailed procedures are described in Lettmann et al. (2011). This model assumes steady states and a lack of significant horizontal advection over the time-spans under consideration, and is thus applied for the central-NE Arabian Sea and not for the western Arabian Sea, due to obvious upwelling and horizontal intrusion of Persian Gulf Water in the latter region. All computations for reaction-diffusion models, as well as statistical analyses (Statistics Toolbox), were performed with MATLAB (Mathworks, Inc.).

### 3 Results and discussion

#### 3.1 Distributions of dissolved inorganic nitrogen and oxygen

Consistent with past observations, nutrient profiling revealed a prominent secondary NO₃⁻ maximum reaching ~5 μM in the central-NE basin (Stations 950–958), centered along the 26.0–26.5 kg m⁻³ neutral-density surfaces, or isoneutrals (Fig. 2b). These coincided with local NO₃⁻ minimum and severe N-deficits represented by the most negative N* values (Gruber and Sarmiento, 1997), where oxygen concentrations fell below 10 μM or apparently anoxic (< 90 nM) as determined by a highly sensitive STOX (Switchable Trace amount OXygen) sensor (Revsbech et al., 2009; Jensen et al., 2011) (Fig. 2 a, e, g). N₂O also accumulated in these oxygen-deficient waters, and was elevated towards the central-NE basin reaching as high as 91 nM (Fig. 2d). Shoaling of isoneutrals (Fig. 2h) and higher surface chlorophyll-a concentrations (Jensen et al., 2011) were observed near the Omani Shelf, indicating residual upwelling and enhanced biological production westward. Surface particulate organic carbon and nitrogen were consequently elevated over the shelf (Jensen et al., 2011), and so were the concentrations of the remineralized NH₄⁺ (≤ 1.6 μM) (Fig. 2c). NH₄⁺ concentrations decreased with depth and were largely close to detection limit (~20 nM) towards the central-NE basin.

#### 3.2 Sources of nitrite

##### 3.2.1 Nitrate reduction to nitrite

Nitrate reduction to nitrite (NO₃⁻+2H⁺+2e⁻ → NO₂⁻+H₂O) is the first step in both denitrification and DNRA, but it is also a standalone process that provides the majority of NO₂⁻ for anammox in the eastern tropical south Pacific (ETSP) OMZ (Lam et al., 2009). In the central-NE Arabian Sea, NO₃⁻ was previously observed to accumulate in the upper OMZ at rates equivalent to the means of 56 % and 14 % of net NO₃⁻ loss near stations 950 and 957, respectively, thus indicating the occurrence NO₃⁻-reduction to NO₂⁻ (Nicholls et al., 2007). In congruence, direct rate measurements of ¹⁵NO₃⁻ reduction to ¹⁵NO₂⁻ in the current study showed readily detectable NO₃⁻-reduction activity within the central-NE OMZ (up to 29.7 ± 4.8 nM d⁻¹) (Fig. 3d), and with rates comparable to those recorded towards the Omani Shelf (up to 24.9 ± 1.8 nM d⁻¹) (Fig. A1). The offshore NO₃⁻-reduction rates fell within the range of those measured in the ETSP OMZ (Lipschultz et al., 1990; Lam et al., 2009), and reached a local maximum at 200 m at St. 957, coinciding with a local
minimum in NO$_3^-$ (Figs. 2 and 3). Although these rates were less than half of those estimated by concentration differences in Nicholls et al. (2007), consistent vertical rate distributions could be observed between the two studies at this station. In addition, the vertical distribution of NO$_3^-$-reduction rates strongly resembled that of NO$_2^-$ concentrations ($r = 0.94$, $p < 0.005$, Pearson correlation), implying a strong influence of the former on the secondary NO$_2^-$ maxima.

The occurrence of NO$_3^-$ reduction was further corroborated by the active expression of the biomarker membrane-bound nitrate reductase gene, narG. The transcript (mRNA) levels determined by RT-qPCR were consistently detectable throughout the OMZ at all stations (Fig. 3, A1–2). Active transcription of another NO$_3^-$ reducing functional gene, napA, encoding the periplasmic nitrate reductase, was however not analyzed in this study. It could also have contributed to NO$_5^-$ reduction in the OMZ but perhaps to a smaller extent (Lam et al., 2009), and thus could partly explain the lack of clear correlation between rates and narG gene expression levels.

3.2.2 Ammonia oxidation

Ammonia oxidation to NO$_2^-$, the first step of nitrification, has been demonstrated to be a significant NO$_2^-$ source in the ETSP OMZ (Lam et al., 2009). However, ammonia oxidation was only measurable in the upper part of the central-NE Arabian Sea OMZ (up to $3.6 \pm 0.04$ nM d$^{-1}$), and only when oxygen concentrations were $\geq 8$ µM based on incubation experiments at various controlled oxygen levels (Table B2). This contrasts with results from the Omani Shelf, where high ammonia oxidation rates (up to $12.5 \pm 3.5$ nM d$^{-1}$) could be detected even in helium-purged incubations for depths deeper into the OMZ (down to 150 m) where oxygen was undetectable ($\leq 0.5$ µM) (Fig. 3). Ammonia oxidation in general seemed to be heavily driven by ammonia-oxidizing archaea, as ammonia oxidation rates were significantly correlated with crenarchaeal cellular abundance determined by 16S rRNA-targeted CARD-FISH (Spearman $R = 0.705$, $p < 0.05$) (data not shown), as well as with the transcript-to-gene ratio of archaeal amoA (Spearman $R = 0.564$, $p < 0.0005$)
Fig. 3. Typical vertical distributions of various chemical and N-cycling functional gene expressions observed in the central-northeastern Arabian Sea OMZ (St. 957, upper panels) and the Omani Shelf OMZ (St. 946, lower panels): (a) oxygen and NO$_2$ \(-$/\)$, (b) anammox rates and the expression of anammox- and denitrifier- nir$S$ genes, (c) DNRA rates and nir$A$ expression, (d) nitrate reduction rates and nar$G$ expression, (e) ammonia oxidation rates, crenarchaeal and bacterial amo$A$ expressions, (f) N$_2$O and the expression of various forms of quinol- and cytochrome-containing nor$B$ genes. The N$_2$O profile for central-NE basin was obtained from St. 950. Please note the different scales used to accommodate the much higher values obtained over the shelf as highlighted in bold. Error bars for rates are standard errors calculated from linear regression, and those for gene expressions represent standard deviations from triplicate real-time PCR runs. Although denitrification rate measurements were made, there was no convincing evidence of its active occurrence.

(Figs. A3 and A4). The amo$A$ gene encodes ammonia monoxygenase subunit A, a key enzyme mediating ammonia oxidation. Ammonia oxidation might also be partly attributed to $\beta$- and $\gamma$- proteobacterial ammonia-oxidizers, as their amo$A$’s were readily expressed throughout the OMZ at levels $\geq$4-fold greater than those of their archaeal counterparts. Unlike the Peruvian OMZ where archaeal amo$A$ generally predominated at the gene level (Lam et al., 2009), combined bacterial amo$A$ gene abundance was comparable to archaeal amo$A$ in the OMZ especially at the central-NE stations (St. 953, 955, 957) (Fig. A3 d, e, f). As oxygen was depleted (< 90 nM) within the central-NE OMZ core where ammonia oxidation rates were not measurable, ammonia-oxidizers could be undertaking alternative anaerobic pathways (Poth and Focht, 1985), such as reducing NO$_2$\(-$/\)$ to N$_2$O in the so-called nitrifier-denitrification. Overall, ammonia oxidation represented only a minor NO$_2$ source (\~{}4–11 % of total depth-integrated NO$_2$ production) relative to NO$_3$\(-$/\)$ reduction in the Arabian Sea OMZs, with the lower values obtained from the central-NE basin (Table 1).
Table 1. (A) Estimated depth-integrated rates of \( \text{NO}_2^- \) sources and sinks in the Arabian Sea OMZ (O\(_2\) < 10 \text{\textmu}M) over the Omani Shelf vs. the central-northeastern basin, based on rates measured via \(^{15}\)N-incubation experiments. (B) Modeled fluxes of \( \text{NH}_4^+ \), \( \text{NO}_2^- \), \( \text{NO}_3^- \), \( \text{N}_2\text{O} \) and net N-loss in the central-northeastern Arabian Sea OMZ. All rates are expressed in units of mmol N m\(^{-2}\) d\(^{-1}\). The net \( \text{NO}_2^- \) balance in the central-NE OMZ estimated from the measured and modeled fluxes are highlighted in italic for comparison. The respective integrated N-loss estimates are indicated in bold.

<table>
<thead>
<tr>
<th>(A) Measured fluxes</th>
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<tr>
<td>Shell** St. 946</td>
</tr>
<tr>
<td>( \text{NO}_2^- ) sources</td>
</tr>
<tr>
<td>( \text{NO}_2^- ) reduction</td>
</tr>
<tr>
<td>( \text{NH}_3 ) oxidation</td>
</tr>
<tr>
<td>Total</td>
</tr>
<tr>
<td>( \text{NO}_2^- ) sinks</td>
</tr>
<tr>
<td>Anammox</td>
</tr>
<tr>
<td>( \text{NO}_2^- ) oxidation(^{d})</td>
</tr>
<tr>
<td>DNRA</td>
</tr>
<tr>
<td>Total</td>
</tr>
<tr>
<td>( \text{NO}_2^- ) Balance</td>
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| Total N-loss        | 4.91 0.12 \(^{c}\) |
| N-loss\(^{f}\)      | 0.15 0.11 ± 0.05  |

\(^{a}\) No data from St. 946; data taken from St. 944, but due to oxygen intrusion in mid-water that might have lowered \( \text{NO}_2^- \) reduction, higher rates for St. 946 are expected. In addition, because anammox and DNRA rates were relatively high at this station, the gross \( \text{NO}_2^- \) reduction rates should be higher than the net rates listed – unlike the lack of significant anammox and DNRA in the central-NE OMZ.

\(^{b}\) Data from St. 953 where in situ \( \text{O}_2 \) concentrations were used in experiments for the upper OMZ, since only anoxic incubations were conducted at St. 957.

\(^{c}\) Potential rates only from experiments with \(^{15}\text{NO}_2^- + ^{15}\text{NH}_4^+\).

\(^{d}\) Rates estimated as the sum of modeled net change in \( \text{NO}_2^- \) (not \( \text{NO}_3^- \)) and the measured \( \text{NO}_2^- \) reduction rates for St. 957. Due to horizontal advection over the Omani Shelf, \( \text{NO}_2^- \) fluxes cannot be estimated with the current models for St. 946. Instead, as \( \text{NH}_3 \) oxidation (first step of nitrification) was detected, we estimate \( \text{NO}_2^- \) oxidation rates to be at least equal to \( \text{NH}_3 \) oxidation rates.

\(^{e}\) From St. 950.

\(^{f}\) Modeled net N-loss rates were calculated as the net production of more negative \( \text{N}^+ \).

* Except for St. 950 where the OMZ started at 155 m.

** Because of the current regimes over/near shelf regions, advective inputs and outputs are likely significant that the calculated balance should not be taken too literally. Besides, as only net \( \text{NO}_2^- \) reduction rates are listed here, the large amounts of \( \text{NO}_2^- \) consumed via anammox and DNRA would imply significantly higher gross \( \text{NO}_2^- \) reduction, whereas the insignificant anammox and DNRA rates in the central-NE render the net rates reasonable estimates therein.

3.3 Sinks of nitrite

3.3.1 Nitrite reduction via anammox, denitrification and DNRA

At the time of our sampling, high rates of \( \text{N}_2 \) production from \( \text{NO}_2^- \) (up to 38.6 nM \( \text{N}_2 \) d\(^{-1}\)), due mostly to anammox as well as in coupling with DNRA, were measured in waters near the Omani Shelf (Jensen et al., 2011), a region previously considered inconducive to active N-loss due to the presence of oxygen (Fig. 3). Oxygen-deficient conditions (\( ≤ \sim 2 \text{\textmu}M \)) were in fact observed below \( \sim 110 \text{ m} \) depth over the shelf, along with very negative N* (down to \( < \sim 10 \text{ \textmu}M \)) but low \( \text{NO}_2^- \) (\( < 0.5 \text{ \textmu}M \)) at OMZ depths (Fig. 2). In contrast, only low sporadic rates of \( \text{N}_2 \) production from \( \text{NO}_2^- \) (0–1.8 nM \( \text{N}_2 \) d\(^{-1}\)) were detected in the central-NE Arabian Sea OMZ (Figs. 3 and A2), which is generally considered the “active denitrification zone” where the prominent secondary \( \text{NO}_2^- \) maximum lay (Naqvi, 1991; Bange et al., 2000). When detected, \( \text{N}_2 \) production was either due to anammox, or the exact pathway could not be fully resolved from the \(^{15}\text{N}\)-isotope pairing experiments (Jensen et al., 2011). There was no clear unambiguous evidence for active denitrification or DNRA in the central-NE Arabian Sea OMZ (Figs. 3 and A2).

Nevertheless, denitrifier-type cd\(_1\)-containing nitrite reductase gene (nir\(S\)) was abundant in the central-NE OMZ and showed relatively consistent expression at two stations (St. 953, 955) (Fig. A2b and d). These results suggested that denitrifiers were potentially active in the central-NE Arabian Sea, but not active enough to confer measurable rates in our study (no significant \(^{30}\text{N}_2 \) production from \(^{15}\text{NO}_2^- \) without time-lag) (Jensen et al., 2011). Although another
recent study reported some moderate denitrification rates at three stations in this region (Ward et al., 2009), detailed timecourse data revealed considerable initial time-lags in the incubations for at least the representative depth shown (Bulow et al., 2010). Should the same criteria be used for rate calculations (e.g. elimination of data with initial time-lags or exponential increase after long incubation hours), results from the two studies might not be so disparate (Jensen et al., 2011). Meanwhile, active denitrification during other times cannot be ruled out, due to the apparently high spatiotemporal variabilities in the Arabian Sea.

3.3.2 N₂O production

Alternatively, some NO₃⁻ might be reduced to N₂O instead of N₂. This was suggested by the active expression of a diverse group of norB genes, which encode nitric oxide (NO) reductases for the conversion of NO to N₂O (Fig. A5). Transcripts of both quinol- and cytochrome-ec-types of norB (pnorB and cnorB, respectively) related to various denitrifying bacteria could be detected in the central-NE Arabian Sea OMZ (Fig. A5). The latter form, cnorB, was more abundant and was present also at near-shelf stations (Figs. 3 and A1–2). It consisted of at least 3 sub-clusters (Fig. A5). The dominant sub-cluster ASc2 was expressed throughout the OMZ, while ASc4 transcripts only occurred at local N₂O minima or OMZ boundaries (Figs. 3 and A1–2).

Despite the lack of detectable production of ¹⁵N-labeled-N₂O from ¹⁵NO₃⁻ incubations in the central-NE OMZ (data not shown), a reaction-diffusion model based on an N₂O concentration profile (St. 950) indicated a net N₂O production of ≤ 6 pM d⁻¹ in the upper part of the OMZ (Fig. 4). This crude rate estimate was indeed below our detection limit (~500 pM d⁻¹), but the depth horizon of modeled N₂O production (~200–300 m) was consistent with the total norB expression observed at the central-NE Station 957 (Figs. 3f and 4f). These findings are also in good agreement with the previous detection of ¹⁵N-labeled N₂O produced from ¹⁵NO₃⁻ via single-end-point incubations (~96 h) for the upper OMZ depths in the same region (Nicholls et al., 2007).

However, N₂O may also be produced by ammonia-oxidizers either via NO₂⁻ or hydroxylamine (Ritchie and Nicholas, 1972), based on active amoA expression yet lack of detectable ¹⁵NO₂⁻-production from ¹⁵NH₄⁺-incubations within the OMZ core. Potential production of ¹⁵N-labeled-N₂O from ¹⁵NH₄⁺-incubations was indeed reported for the upper OMZ depths, despite this being an apparently minor (~2–5 %) contribution of N₂O relative to the reductive pathway (Nicholls et al., 2007). Although no immediate relatives of known ammonia-oxidizer cnorB sequences were recovered in our cnorB clone libraries, it cannot be excluded that all four primer sets used (Table B2) were insufficient to capture the full diversity in the environment such as any unknown norB of potentially archaeal origin, or that the cnorB phylogenies are not equivalent to cell identities based on 16S rRNA genes. Regardless of which microbial players were involved, there was a strong indication of an active albeit likely minor NO₃⁻ sink via N₂O production in the upper OMZ of the central-NE Arabian Sea.

3.3.3 Nitrite oxidation

In spite of the oxygen deficiency within the OMZ, NO₃⁻ may also be consumed by NO₂⁻ oxidation, the second step of nitrification. In this reaction, NO₃⁻ is oxidized to NO₂⁻ by H₂O (NO₂⁻ + H₂O→NO₃⁻ + 2H⁺ + 2e⁻) and the generated electrons are transferred to a terminal electron acceptor, most commonly being O₂ (2H⁺ + 2e⁻ + 0.5 O₂→H₂O) (Kumar et al., 1983; Hollocher, 1984). Anaerobic growths have been documented for a cultured nitrite-oxidizer (Bock et al., 1988; Griffin et al., 2007), though the exact anaerobic metabolic pathways or whether NO₃⁻ is oxidized in such cases remain to be elucidated in environmental settings. In the eastern tropical south Pacific (ETSP), NO₂⁻-oxidizing activities were detected deep into the OMZ, and were found rather insensitive to oxygen deficiency (Lipschultz et al., 1990). Although nitrite oxidation rates were not directly measured in our study, reaction-diffusion modeling on concentration profiles clearly indicated net NO₃⁻ production coinciding with NO₂⁻ consumption, especially in the upper part of the OMZ (Figs. 4 and A6). To date, there have been no known biotic or abiotic processes other than nitrite oxidation that can produce NO₃⁻ in such seawater conditions, except for anammox. In the latter, 0.3 mol of NO₂⁻ is oxidized to NO₃⁻ for every mole of N₂ produced, as a means to replenish the electrons for the acetyl-CoA carbon fixation process within anammox bacteria (van de Graaf et al., 1997; Strous et al., 2006). However, as anammox rates were hardly detectable in the central-NE Arabian Sea, contribution from anammox could not account for the calculated NO₃⁻ production.

Assuming NO₂⁻ oxidation and NO₃⁻ reduction were the only NO₃⁻ producing and consuming processes, respectively. NO₂⁻ oxidation rates could then be estimated as the sum of the measured NO₃⁻ reduction and the modeled net change in NO₃⁻. NO₃⁻ oxidation was thus postulated to occur down to at least 500 m (3 nM d⁻¹, St. 957) in the central-NE OMZ reaching a maximum of 22 nM d⁻¹ at 200 m, which was within the range of those reported for the ETSP (Lipschultz et al., 1990). Although these are only crude estimates, the maximum rate coincided with the maximum NO₃⁻ reduction rate in the upper part of the OMZ at this station (St. 957), and the two rates were often comparable in magnitude. Hence, NO₂⁻ oxidation was most likely the dominant NO₃⁻ sink in the central-NE Arabian Sea, but requires further verification with direct rate measurements, and the use of O₂ or alternative terminal electron acceptors like iodate, manganese (III or IV) or iron (Farrenkopf et al., 1997; Lewis and Luther, 2000; Trouwborst et al., 2006; Moffet et al., 2007) also remain to be determined.
3.4 Nitrite accumulations, N-loss and organic matter in the central-NE OMZ

Taken together, we found ample evidence for NO\textsubscript{2}\textsuperscript{-} production within the prominent secondary NO\textsubscript{2}\textsuperscript{-} maximum in the central-NE Arabian Sea OMZ, predominantly from NO\textsubscript{3}\textsuperscript{-} reduction, and to a certain extent ammonia oxidation in the upper OMZ. There was little evidence for NO\textsubscript{2}\textsuperscript{-} consumption via N-loss as N\textsubscript{2}O or N\textsubscript{2} production with only occasional, low potential rates; whereas NO\textsubscript{2}\textsuperscript{-} oxidation was predicted to be a major NO\textsubscript{2}\textsuperscript{-} sink. When the measured rates of all NO\textsubscript{2}\textsuperscript{-} sources and sinks were integrated over the thickness of the Arabian Sea OMZ, a small net production of NO\textsubscript{2}\textsuperscript{-} (0.06 mmol N m\textsuperscript{-2} d\textsuperscript{-1}) was calculated for the central-NE basin (Table 1A). This low rate is comparable with the net NO\textsubscript{2}\textsuperscript{-} production rates of 0.05 ± 0.03 mmol N m\textsuperscript{-2} d\textsuperscript{-1} (mean ± standard deviation of 4 stations) estimated via reaction-diffusion flux modeling on NO\textsubscript{2}\textsuperscript{-} profiles (Table 1B).

The slow build-up of the secondary NO\textsubscript{2}\textsuperscript{-} maximum corresponded with a general lack of modeled N-loss rates from N\textsuperscript{*} profiles (Figs. 4 and A6d). Only at occasional depths in the central-NE OMZ were more severe N-deficits produced (i.e. N-loss), reaching 2–4 nM N d\textsuperscript{-1} according to modeled results. In fact, those were also the depths where potential N\textsubscript{2} production rates of 1–2 nM N\textsubscript{2} d\textsuperscript{-1} (equivalent to 2–4 nM N d\textsuperscript{-1}; significantly greater than 0.15–0.20 nM d\textsuperscript{-1}, limits of detection) were measured via \textsuperscript{15}N-incubation experiments, along with elevated anammox- and denitrifier nirS expression (Figs. 3, A2, A6).

In contrast, over the Omani shelf where the measured N-loss rates were high, overall NO\textsubscript{2}\textsuperscript{-} production appeared to be exceeded by highly active NO\textsubscript{2}\textsuperscript{-} consumption (Table 1A). Considering the entire dataset for the Arabian Sea OMZ, there was no significant correlation between N-loss rates and NO\textsubscript{2}\textsuperscript{-} concentrations (Spearman rank-test, \( p > 0.05 \)). The most active N-loss (anammox) occurred at low to medium NO\textsubscript{2}\textsuperscript{-} levels, consistent with observations made for the Namibian and Peruvian OMZs (Kuyper et al., 2005; Hamersley et al., 2007; Lam and Kuyper, 2011). Consequently, the aptness of secondary NO\textsubscript{2}\textsuperscript{-} maximum as an indicator for active N-loss in the OMZs becomes questionable.

N-loss via anammox was directly coupled and significantly correlated with nitrate reduction (Spearman \( R = 0.619, \ p < 0.05 \)), DNRA (Spearman \( R = 0.579, \ p < 0.005 \)) and ammonia oxidation (Spearman \( R = 0.556, \ p < 0.0005 \)), whereas the concentration of NO\textsubscript{2}\textsuperscript{-} present in the water column was controlled by the dynamic balance among all these processes. Hence, it is unlikely for NO\textsubscript{2}\textsuperscript{-} to have a simple and direct relationship with N-loss. When examining the regulation of N-loss, controlling factors for these other concurrent N-cycling processes should also be taken into account. A multivariate multidimensional scaling analysis (final stress = 0.0633, 5 non-metric dimensions) on all of the rates, nutrients, gene abundance and expression data, not only confirmed the tight interdependence of these processes, but also revealed the strong associations of active N-loss with NH\textsubscript{4}\textsuperscript{+}, surface particulate organic carbon and nitrogen, as well as total microbial abundance and total RNA concentrations (active signals to generate proteins for various cellular reactions) (Fig. A7, Table B3). These associations
implied an important role of organic matter in controlling microbial processes including N-loss. This is not surprising as both nitrate reduction and DNRA (and also denitrification, if present) are mainly heterotrophic processes that feed on organic matter, whereas ammonia oxidation and anammox, though both being lithoautotrophic processes, require $\text{NH}_4^+$ that needs to be remineralized from organic matter. The dependence of anammox on $\text{NH}_4^+$ was also apparent in the Arabian Sea (Spearman $R = 0.57$, $p < 0.0001$) and other OMZs (Lam and Kuypers, 2011). In other words, N-loss and the coupling processes should be enhanced in more productive water columns. The scarcity of detectable active N-loss in the central-NE Arabian Sea OMZ would then be best explained by the low availability of labile organic matter sinking from the then quasi-oligotrophic surface waters (chlorophyll- $a \leq 0.2 \text{ mg m}^{-2}$) (Fig. A8). Relative to the 10-year record, surface chl- $a$ concentrations at the time of our sampling seemed rather representative for the central-NE basin, implying that our measurements of the subsequently affected N-transformations were likely not far from the typical N-cycling activities in these waters (Fig. A8).

Nonetheless, if there was no consistent N-loss activity in the central-NE OMZ, how did the N-deficit therein become one of the largest in the world’s Ocean? As N-deficit (or N*) is a time-integrated signal, it indicates the cumulative N-loss that has occurred throughout the history of the water mass, and does not provide information on in situ N-loss activities. Large cumulative N-deficits could have resulted from (1) consistently high N-loss in a water mass of short residence time, (2) episodically high N-loss in a water mass of long residence time, or (3) consistently very low N-loss activities in an aged water mass. For the central-NE Arabian Sea OMZ, there is little consensus on the water residence time therein, with estimates ranging widely from 1.6 to 54 years (Sen Gupta et al., 1980; Naqvi, 1987; Somasundar and Naqvi, 1988; Olson et al., 1993). However, our hardly detectable N-loss rates at the time of our sampling could only be explained by scenarios (2) and (3), implying more likely a relatively high water residence time within the central-NE Arabian Sea OMZ. From the measured $\text{NO}_3^-$ inventory and modeled net $\text{NO}_3^-$ production rates through the OMZ ($\sim$100–1000 m; $O_2 < 10 \mu \text{M}$), $\text{NO}_3^-$-turnover times were estimated to be $49 \pm 20$ years (mean $\pm$ standard deviation of 4 stations) in the central-NE basin. Although these are not equivalent to water residence times, and the unaccounted horizontal advection would place the actual turnover times somewhat lower, the comparability between our measured and modeled N-loss rate profiles strongly suggested that horizontal advective fluxes were not exceedingly high to support a much shorter residence time. Hence, high $\text{NO}_3^-$ in the central-NE OMZ is a tenable result of prolonged accumulation of slow net production, which is, in the long run, balanced by slow exchange with water outside the OMZ, where $\text{NO}_3^-$ is eventually oxidized back to $\text{NO}_2^-$. Meanwhile, the most negative N* observed in the central-NE OMZ has likely included some degrees of severe N-loss from basin boundaries, with additional low or episodic N-loss that occurs locally.

Surface primary production in the central-NE Arabian Sea is temporally and spatially patchy in nature, as nutrients are mainly delivered via mesoscale eddies spun off from boundary upwelling (Wiggert et al., 2005). Consequently, spatiotemporal heterogeneities are to be expected in N-cycling activities. Indeed, at the time of our sampling, temperature-salinity plots revealed different vertical structures in the water column at various stations and relative to archived data, with apparent signals of vertical mixing or upwelling only at St. 950 in the central-NE and over Omani shelf (Fig. A9). While most of our sampling times in the central-NE basin seemed representative with respect to the 10-year chl- $a$ record (compared to enhanced chl- $a$ at shelf stations that reflected residual influence of the SW monsoonal upwelling) (Fig. A8), unusually high chl- $a$ was recorded at St. 950 one week prior to our sampling but not two weeks before (Fig. A8c). Vertical stratification at St. 950 also seemed to have weakened during our sampling (Figs. 2h and A9d). These data together strongly suggested the recent passing of an upwelling eddy that has stimulated an episodic algal bloom, and could explain the higher N-loss activity potentials measured at this station (Jensen et al., 2011). How frequent and to what degree these episodic events and mesoscale eddies occur may be deterministic to the overall nitrogen balance in this basin, and should be examined more closely.

4 Conclusions

In summary, the current study showed that at least at the time of our sampling, $\text{NO}_3^-$-reduction was the most consistently active N-cycling process in the central-NE Arabian Sea OMZ. Together with a small degree of ammonia oxidation (upper OMZ), they resulted in a net production and thus accumulation of $\text{NO}_3^-$ in this region. Active $\text{NO}_2^-$ consumption via anammox, denitrification and DNRA were likely hampered directly or indirectly by the deficiency of labile organic matter in the central-NE Arabian Sea, leaving lithoautotrophic $\text{NO}_3^-$-oxidation to $\text{NO}_3^-$ as the plausible major $\text{NO}_3^-$-sink based on modeled calculations. This is not to say that reductive $\text{NO}_3^-$ consumption and active N-loss never occur in the central-NE OMZ at all. Their activities therein are most probably low to undetectable in general, and may be intermitted with occasionally high rates during episodic algal blooms, for instance. A long water residence time could then have enabled the large accumulations of both $\text{NO}_3^-$ and N-deficits (Fig. 5), with the accumulated $\text{NO}_3^-$ eventually dissipated by slow water exchange and oxidized to $\text{NO}_2^-$. Our study has only captured a snapshot of the end of the 2007 SW monsoon, but biological production and subsequent downward fluxes of organic matter are also enhanced.
during the NE monsoon in the Arabian Sea (Honjo et al., 1999; Wiggert et al., 2005). Further investigations are therefore imperative to assess NO$_3^-$ dynamics and N-cycling during the NE monsoon, and to investigate possible seasonal variations throughout the year. However, as our sampling did take place at a time relatively representative of surface production in the central-NE Arabian Sea, our results show that the strong secondary NO$_3^-$ maximum is most likely a signature of an aged water mass with NO$_3^-$-reducing conditions and has experienced past N-loss but fails to result in much further in situ N-loss activity. This may also hold true for other major OMZs in the eastern tropical Pacific and Atlantic. Meanwhile, the near-shelf OMZ waters where in situ N-loss activities are high but NO$_3^-$ hardly accumulates, have rarely been included in global N-budget estimates or model calculations. Consequently, previous N-loss estimates based on few point-measurements extrapolating to large volumes of NO$_3^-$-laden OMZ waters would be erroneous. For an accurate assessment of the N-dynamics in the Arabian Sea and so its true significance in global N-balance, more interdisciplinary field and modeling studies of high spatiotemporal resolution should be extended to coastal regions, and the time and modes of ventilation from the shelves to the central-NE OMZ need to be more fully appraised. Our findings re-emphasize the fact that NO$_3^-$, being a dynamic intermediate, accumulates in the oceans because of a set of ill conditions (e.g. low availability of labile organic matter) that hinders further oxidation/reduction in the N-cycle. In the Arabian Sea OMZ and perhaps others, it is more likely the water upstream rather than right in the heart of secondary NO$_3^-$ maxima where high reductive NO$_2^-$ consumption rates are to be expected, along with most other microbial processes.

**Fig. 5.** A conceptual diagram illustrating that in the central-NE Arabian Sea OMZ, total NO$_2^-$ influx via NO$_3^-$ reduction (NR) and to a lesser extent NH$_3$ oxidation (AO), likely exceeds the combined NO$_3^-$ losses via mainly NO$_2^-$ oxidation (NO), and perhaps some low/intermittent (dashed arrows) anammox (Amx), denitrification (Dn) and dissimilatory nitrite reduction to ammonium (DNRA). The resultant slow accumulation, along with probably a long water residence time ($\tau$), results in high NO$_2^-$ concentrations (C) in the central-NE OMZ. In comparison, despite the apparently higher input rates detected over the shelf (thicker arrows of AO and NR), the similarly high outputs seem to roughly balance the total inputs, thus maintaining a low NO$_3^-$ concentration in the shelf OMZ. Because no convincing evidence has been found to unambiguously confirm denitrification in the current study, this is shown here as dashed arrows to indicate its possible potentials only. Also shown in this diagram are the advective influxes (AV$_i$) and outfluxes (AV$_e$), which are postulated to be greater over shelf regions (thus shorter $\tau$ therein) than in the central-NE OMZ; but they have not been assessed in the current study (as indicated by ‘?’).


