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Grundle, D. S.; Löscher, Carolin; Krahmann, G.; Altabet, M. A.; Bange, H. W.; Karstensen, J.; Körtzinger, A.; Fiedler, B.

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Low oxygen eddies in the eastern tropical North Atlantic: Implications for N$_2$O cycling

D. S. Grundle$^{1,2}$, C. R. Löscher$^{1,3}$, G. Krahmann$^1$, M. A. Altabet$^4$, H. W. Bange$^1$, J. Karstensen$^1$, A. Körtzinger$^{1,4}$ & B. Fiedler$^1$

Nitrous oxide (N$_2$O) is a climate-relevant trace gas, and its production in the ocean generally increases under suboxic conditions. The Atlantic Ocean is well ventilated, and unlike the major oxygen minimum zones (OMZ) of the Pacific and Indian Oceans, dissolved oxygen and N$_2$O concentrations in the Atlantic OMZ are relatively high and low, respectively. This study, however, demonstrates that recently discovered low oxygen eddies in the eastern tropical North Atlantic (ETNA) can produce N$_2$O concentrations much higher (up to 115 nmol L$^{-1}$) than those previously reported for the Atlantic Ocean, and which are within the range of the highest concentrations found in the open-ocean OMZs of the Pacific and Indian Oceans. N$_2$O isotope and isotopomer signatures, as well as molecular genetic results, also point towards a major shift in the N$_2$O cycling pathway in the core of the low oxygen eddy discussed here, and we report the first evidence for potential N$_2$O cycling via the denitrification pathway in the open Atlantic Ocean. Finally, we consider the implications of low oxygen eddies for bulk, upper water column N$_2$O at the regional scale, and point out the possible need for a reevaluation of how we view N$_2$O cycling in the ETNA.

Nitrous oxide (N$_2$O) is an important climate-relevant trace gas and the oceans are thought to contribute approximately 35% of all natural sources to the atmosphere. In the troposphere N$_2$O acts as a greenhouse gas and has a global warming potential which is ~300 times that of CO$_2$ over 100 year time-scales. Due to its relative chemical stability, N$_2$O also survives transport to the stratosphere where it undergoes photochemical reactions that destroy ozone. In the oceans, N$_2$O is produced via the nitrification and denitrification pathways. During nitrification, N$_2$O can be produced as a by-product during ammonia oxidation (AO), or through nitrifier-denitrification whereby AO organisms reduce nitrite (NO$_2^-$) to N$_2$O. In oxygenated waters, nitrification-N$_2$O yields (i.e. those arising from either AO or nitrifier-denitrification) are small, however, under low DO concentrations nitrification-N$_2$O yields may increase substantially. As DO concentrations approach anoxic conditions, denitrification can also be 'turned on', and although it can both produce and consume N$_2$O, net denitrification yields up to 2% have been observed.

Due to the sensitivity of N$_2$O production to low oxygen conditions, the greatest oceanic accumulations, and likely the largest fluxes to the atmosphere, occur in the vicinity of suboxic and anoxic oxygen minimum zones (OMZs), such as those found in the Arabian Sea and the eastern tropical Pacific. In comparison, the more ventilated Atlantic Ocean, with higher oxygen concentrations, has lower N$_2$O production and concentrations. Here, we demonstrate for the first time, however, that recently discovered low oxygen mesoscale eddies in the otherwise oxygenated tropical North Atlantic, can induce substantial increases in N$_2$O production and cause shifts in the N$_2$O cycling pathways.

The OMZ in the North Atlantic Ocean is rather well ventilated, and lowest DO concentrations are around 40 µmol kg$^{-1}$. Recently, however, coherent mesoscale cyclonic eddies (CE) and anticyclonic mode water eddies (ACME) in the eastern tropical North Atlantic (ETNA), which form off the coast of west Africa along topographical features such as headlands, and then propagate westwards past the Cape Verde Islands, have been shown to create extremely low DO concentrations (as low as ~2 µmol kg$^{-1}$). The low DO concentrations inside the eddy have the potential to have important implications for biogeochemical processes, including...
N$_2$O cycling. Until recently, however, these potential implications have not been studied, as observations have been opportunistic and most have originated from moored and glider based sensors at the Cape Verde Ocean Observatory (CVOO; Fig. 1). In early 2014, however, a dedicated multi-disciplinary shipboard survey of one of these eddies (hereinafter referred to as 'suboxic eddy') was conducted. This survey allowed us to investigate how N$_2$O cycling may be impacted by low oxygen eddies in the ETNA (sampling parameters and stations are outlined in the Methods section). The results from this work not only demonstrate the potential importance of low oxygen eddies as a source of N$_2$O, they also provide insights into how N$_2$O cycling in the ETNA may respond to future DO decreases.

Results and Discussion
Dissolved oxygen and nitrous oxide concentrations. This study was part of a multi-disciplinary investigation of the suboxic eddy, and here we focus on the implications of the low DO concentrations inside the suboxic eddy for N$_2$O processes. The physical characteristics of the eddy and other biological and biogeochemical processes are discussed elsewhere

Figure 1. Locations of the relevant sampling sites. The Cape Verde Ocean Observatory (CVOO), outside eddy station (OES), eddy station 1 (ES1) and eddy station 2 (ES2) are marked with crosses, while the IFM12 and IFM13 glider surveys are indicated by dashed lines. The outer solid red and blue circles represent the position and area of the suboxic eddy during the ES1 and ES2 sampling events, respectively, while the outer solid green circles represent the location of the suboxic eddy during glider surveys (see description in Methods section). Note: the inner green, red and blue circles represent the area of the low DO core of the eddy. This map was created using Mathworks Matlab version R2014b (http://www.mathworks.com), and the coastline data are from the GSHHS (Global Self-consistent, Hierarchical, High-resolution Shorelines) data set published for free use by NOAA (https://www.ngdc.noaa.gov/mgg/shorlines/gshhs.html).
Similar to the DO results, we observed large perturbations to the N₂O conditions inside vs. outside of the suboxic eddy. The highest N₂O concentration at OES (34.2 nmol L⁻¹; Fig. 2b) was within the range of the highest concentrations previously reported for the North Atlantic 14, 23, but somewhat lower than the highest concentration found in the eastern tropical South Atlantic (49 nmol L⁻¹) 24. Corresponding to the vertical depth range of low DO, N₂O concentrations much higher than those previously reported for the North Atlantic were found inside the suboxic eddy, with values as high as 115 nmol L⁻¹ within the ES1 OMZ (100 m depth; Fig. 2b). The high N₂O concentrations we observed in the core of the suboxic eddy are within the range of many of the highest values reported for the eastern tropical Pacific 25–29 and open Arabian Sea 30, 31, although concentrations as high as up to ~500 nmol N₂O L⁻¹ have been reported for the coastal regions of the eastern tropical South Pacific off of Chile 32. The observations reported here demonstrate that N₂O concentrations within ETNA suboxic eddies can reach levels comparable to those from regions that are characterized by well defined OMZs where DO concentrations

Figure 2. Vertical distributions of dissolved oxygen and N₂O concentrations at the out-of-eddy station (OES), and at eddy stations 1 and 2 (ES1 and ES2, respectively). (a) Discrete depth dissolved oxygen (DO) concentrations measured with the CTD-DO sensor at each of our sampling depths. The error bars represent the average propagation of error associated with our DO measurements (see Methods section). (b) Discrete depth N₂O concentrations. The error bars represent the standard deviation of duplicate N₂O concentration measurements. Due to the loss of duplicated samples, standard deviations are not reported for 10 m depth at ES1, and 250 m depth at OES.

Figure 3. Dissolved oxygen concentrations as measured during the IFM13 glider survey on April 7th, 2014.
Ranges were selected based on the observed $\text{N}_2\text{O}$ vs. DO shifts shown in Fig. 4a. The $\text{N}_2\text{O}$ vs. DO relationships towards a 50-fold increase in the amount of $\text{N}_2\text{O}$ produced vs. the amount of DO consumed below 20 bar is similar to estimates from global oxygenated oceanic water masses (8, 9, 29). In contrast, as 50 km and extended from the surface to 250 m depth, and the bulk amount of $\text{N}_2\text{O}$ within this volume was decreased is omitted). The solid lines are linear regressions and the results from the linear regression analyses are included.

A plot of all $\text{N}_2\text{O}$ and DO concentration data showed these two variables to be inversely correlated down to a DO concentration of 10 µmol L$^{-1}$ (Fig. 4a). Between 10 and 5 µmol O$_2$ L$^{-1}$, however, this trend appears to may have begun to reverse as the $\text{N}_2\text{O}$ concentration decreased from 115 to 92.7 nmol L$^{-1}$ (Fig. 4a). It is important to note that the observation of a decrease in $\text{N}_2\text{O}$ concentration between 10 and 5 µmol O$_2$ L$^{-1}$ was based on sampling conducted almost two weeks apart, and, as such, the decrease may have been due to $\text{N}_2\text{O}$ diffusing out of the DO minimum/$\text{N}_2\text{O}$ maximum in the period between our two sampling events. If $\text{N}_2\text{O}$ was diffusing across a high to low concentration gradient, then DO would have also likely been diffusing from high to low concentrations (i.e., into the DO minimum), and this would have started to erode the extremely low DO concentrations we observed. A glider survey of the eddy on April 7th 2014 (i.e., three weeks after our ES2 sampling date) showed that the low DO eddy core was still stable and intact (Fig. 3). To this end, it seems unlikely that diffusion was a major contributor to the decrease in $\text{N}_2\text{O}$ we observed between 10 and 5 µmol O$_2$ L$^{-1}$. A shift from net $\text{N}_2\text{O}$ production to net $\text{N}_2\text{O}$ consumption is another possible explanation for the decrease in $\text{N}_2\text{O}$ concentrations between 10 and 5 µmol O$_2$ L$^{-1}$. Still, it is not an unreasonable proposition as previous results have also shown evidence for a transition from net production to net consumption below 10 µmol O$_2$ L$^{-1}$.

At DO concentrations $\geq$10 µmol L$^{-1}$, a plot of $\Delta \text{N}_2\text{O}$ ([N$_2$O$_{measured}$] – [N$_2$O$_{saturation}$]) vs. AOU (apparent oxygen utilization; [O$_2$$_{measured}$] – [O$_2$$_{saturation}$]) shows two distinct linear relationships (Fig. 4b). Linear relationship 1 (LR1) and 2 (LR2) correspond to DO concentration ranges of 240 to 22 µmol L$^{-1}$ and 18 to 10 µmol L$^{-1}$, respectively. The slope of LR1 (Fig. 4b) indicates that ~8500 mol of O$_2$ were consumed for every mol of $\text{N}_2\text{O}$ produced, and this is similar to previous estimates from the open tropical Atlantic Ocean (13, 14), including the Mauritanian upwelling region (25); it is also similar to estimates from global oxygenated oceanic water masses (8, 9, 29). In contrast, the slope of LR2 implies that only 170 mol of O$_2$ were consumed for every mol of $\text{N}_2\text{O}$ produced, and this points towards a 50-fold increase in the amount of $\text{N}_2\text{O}$ produced vs. the amount of DO consumed below 20 µmol O$_2$ L$^{-1}$. This result agrees with Codispoti et al. (34) and Nevison et al. (29) who showed that $\text{N}_2\text{O}$ production starts to increase substantially below ~20 µmol O$_2$ L$^{-1}$. Our estimate of a 50-fold increase in $\text{N}_2\text{O}$ production vs. DO consumption is, however, higher than results from earlier work which have shown that nitrification-$\text{N}_2\text{O}$ yields can increase 20-fold (36) and 40-fold (36) under low DO concentrations. One possible explanation for our observation of higher $\text{N}_2\text{O}$ production vs. DO consumption could be the presence of $\text{N}_2\text{O}$ production via reductive pathways (i.e., sources of $\text{N}_2\text{O}$ production which do not also consume DO), and evidence for this is discussed below under ‘Nitrous oxide cycling pathways’.

Finally, simple linear regression analyses were used to quantify the $\text{N}_2\text{O}$ vs. DO relationships shown in Fig. 4a at DO concentrations between 250 and 20 µmol L$^{-1}$, <20 and 10 µmol L$^{-1}$, and <10 µmol L$^{-1}$ (Table 1). These DO ranges were selected based on the observed N$_2$O vs. DO shifts shown in Fig. 4a. The N$_2$O vs. DO relationships for each of these DO ranges were then applied to the DO concentrations observed during the high resolution IFM13 glider survey in order to estimate the bulk amount of N$_2$O inside the suboxic eddy core. Based on the DO concentrations measured during the glider survey (Fig. 3), the diameter of the suboxic eddy core was defined as 50 km and extended from the surface to 250 m depth, and the bulk amount of N$_2$O within this volume was estimated to have been $1.8 \times 10^{7}$ mol N$_2$O, or an average of 9.2 mol N$_2$O km$^{-2}$. In comparison, areal N$_2$O over the same depth range at OES was 5.0 mol N$_2$O km$^{-2}$, or almost half that within the suboxic eddy core. This
again highlights that low oxygen eddies have the potential to be important but previously unrecognized sources of marine N\textsubscript{2}O. Quantifying the overall importance of low oxygen eddies is not trivial, however, and it would depend, for example, on factors such as the frequency of their occurrence, their size, and how long they last\textsuperscript{16, 36}. A recent analysis of a 1.1 $\times$ 10\textsuperscript{6} km\textsuperscript{2} area of the ETNA suggests that at any one time ~20% of this area is covered by suboxic eddy cores\textsuperscript{16}. Assuming all of these suboxic eddy cores are similar to the one described here, which showed an almost 100% increase in N\textsubscript{2}O concentrations inside vs. outside of the eddy, this could require bulk upper water column (in this case upper 250 m) N\textsubscript{2}O estimates to be increased by up to 20%. This is a first order estimate, however, and much more shipboard work is necessary to accurately determine the DO and N\textsubscript{2}O conditions within a range of ETNA eddies, and covering their full lifecycles, so that more robust statistical analyses of their potential importance as a source of marine N\textsubscript{2}O can be calculated. Furthermore, if the prevalence of suboxic eddies are also found to be high outside of the ETNA, these types of low oxygen events may be found to be important at the global scale, rather than just the regional scale.

### Nitrous oxide cycling pathways.

Results from isotope and isotopomer (i.e. the intramolecular distribution of $^{15}$N within the linear NNO molecule; $^{15}$N\textsubscript{N} - $^{15}$N\textsubscript{O}) measurements, as well as molecular genetic analyses, point towards shifts in the N\textsubscript{2}O cycling pathways in the core of the suboxic eddy, relative to the more oxygenated waters inside and outside of the eddy. This complete suite of isotope, isotopomer (15N site-preference; SP) and molecular genetic sampling was not conducted at ES2, so our discussion focuses on ES1 with comparisons to OES. At OES, vertical profiles of $^{15}$N\textsubscript{N}-N\textsubscript{2}O, $^{15}$N\textsubscript{O}-N\textsubscript{2}O and SP (Fig. 5) were characteristic of those from regions of the tropical South Atlantic, indicating that N\textsubscript{2}O was produced by a combination of AO and nitrifier-denitrification\textsuperscript{24}. The $^{15}$N\textsubscript{N}-N\textsubscript{2}O:SP ratios were also within the range of those reported for N\textsubscript{2}O produced via nitrification processes\textsuperscript{28}. Furthermore, gene copy numbers and transcripts, which provide an indication of gene abundance and expression, respectively, of $amoa$ and $nirS$ genes can also provide insight into the potential N\textsubscript{2}O cycling pathways. The $amoa$ gene is the classical functional marker gene encoding for a subunit of the ammonia monoxygenase enzyme which catalyzes AO, and a correlation between N\textsubscript{2}O formation by AO and $amoa$ gene expression has been previously demonstrated\textsuperscript{37, 38}. To this end, we consider it reasonable to connect at least the potential for N\textsubscript{2}O formation to $amoa$ abundance and expression. In contrast, the $nirS$ gene encodes for the enzyme involved in NO\textsubscript{3} form\textsubscript{ion} reduction via the denitrification pathway, and recent results have shown a positive relationship between the abundance of $nirS$ genes and N\textsubscript{2}O production by denitrification\textsuperscript{35}. At OES,

### Table 1.

Results from simple linear regression analyses of N\textsubscript{2}O vs. DO at different DO concentration ranges. The results shown here were obtained using data shown in Fig. 2a. The regression equations were used in conjunction with the DO concentrations measured during the IFM13 glider survey of the eddy (see Methods section) in order to estimate the bulk amount of N\textsubscript{2}O inside the suboxic eddy.

<table>
<thead>
<tr>
<th>Dissolved O\textsubscript{2} Concentration Range</th>
<th>N\textsubscript{2}O (nmol L\textsuperscript{-1}) vs. O\textsubscript{2} (µmol L\textsuperscript{-1}) Linear Regression Equation</th>
<th>p-value</th>
<th>R\textsuperscript{2}-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>250–&gt;20 µmol L\textsuperscript{-1}</td>
<td>$N_{2}O_{\text{conc.}} = -0.162 \times O_{2_{\text{conc.}}} + 45.48$</td>
<td>&lt;0.001</td>
<td>0.97</td>
</tr>
<tr>
<td>&lt;20–10 µmol L\textsuperscript{-1}</td>
<td>$N_{2}O_{\text{conc.}} = -6.67 \times O_{2_{\text{conc.}}} + 179.04$</td>
<td>0.03</td>
<td>0.95</td>
</tr>
<tr>
<td>10–5 µmol L\textsuperscript{-1}</td>
<td>$N_{2}O_{\text{conc.}} = 4.30 \times O_{2_{\text{conc.}}} + 71.28$</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

### Figure 5.

Vertical distributions of (a) $^{15}$N-N\textsubscript{2}O, (b) $^{18}$O-N\textsubscript{2}O and (c) $^{15}$N site preference signatures at ES1 (red circles) and OES (black circles). Error bars represent the standard deviation of duplicate N\textsubscript{2}O isotope measurements. Due to the loss of duplicated samples, standard deviations are not reported for 20 and 450 m depth at OES, and 90 m depth at ES1.
amoA gene abundance and expression (Fig. 6a,b) were considerably higher than that of the nirS gene (Fig. 6c,d). This supports our assertion that nitrification was the major source of N₂O within the oxygenated waters of OES, and it is consistent with previous studies in this region. Similarly, above and below the ES1 DO minimum, nitrification processes also appear to be the predominant source of N₂O, as δ¹⁵Nbulk-N₂O and SP ratios were again within the range expected for N₂O produced by nitrifiers, and amoA gene abundance and expression were high. The SP signatures above and below the ES1 DO minimum were, however, in some cases somewhat lower than those found at OES (Fig. 5c). Both nitrifier-denitrification and denitrification yield N₂O with an SP ≤ 0‰, whereas AO yields N₂O with an SP > 30‰. The SP observations therefore indicate that a reductive pathway (i.e. nitrifier-denitrification or denitrification), rather than an oxidative pathway, was a relatively more important source of N₂O at ES1 vs. OES. Given that amoA gene abundance was high above and below the ES1 DO minimum, whereas nirS gene abundance and expression were either undetectable or very low, we suggest that nitrifier-denitrification was the most probable reductive N₂O production pathway above and below the ES1 DO minimum. The potential increase in N₂O production via nitrifier-denitrification at ES1, when compared to OES,
was likely a result of the lower DO concentrations inside vs. outside of the suboxic eddy, as culture investigations have shown that nitrifier-denitrification increases as DO concentrations decrease\(^4\).

Differences in isotope and molecular genetic results inside vs. outside of the suboxic eddy were most prominent within the ES1 DO minimum at 100 m, and our results provide insights into how \(\text{N}_2\text{O}\) cycling changed as the DO concentration dropped to \(\sim 10\mu\text{mol L}^{-1}\). One of the most prominent differences was the \(\delta^{15}\text{N}_{\text{bulk-N}}\) value, which decreased to \(-1.6\%\) within the ES1 OMZ. This is below the lowest value reported for the Arabian Sea\(^3\), and is one of the lowest values reported for seawater, one exception being the Black Sea where a value of \(-10.8\%\) was observed\(^4\). Westley et al.\(^4\) concluded that this \(\delta^{15}\text{N}_{\text{bulk-N}}\) value of \(-10.8\%\) was too low to have been caused by a reductive \(\text{N}_2\text{O}\) production pathway, and instead they concluded that it must have been caused by \(\text{N}_2\text{O}\) produced via AO. Although very low for seawater, the \(\delta^{15}\text{N}_{\text{bulk-N}}\) value we observed within the ES1 DO minimum was not as extreme as that of Westley et al.\(^4\), and taken alone it cannot be used to narrow down the predominant production pathway. That is, based on the \(\delta^{15}\text{N}\) signatures of dissolved inorganic nitrogen from the eastern tropical Atlantic\(^3\), and the range of measured isotope effects of nitrification and denitrification (summarized by Bange)\(^3\), the \(\delta^{15}\text{N}_{\text{bulk-N}}\) value from the ES1 DO minimum could have been produced by either oxidative or reductive pathways. Our molecular genetic results, however, provide further insight into the potential predominant \(\text{N}_2\text{O}\) production pathway. The abundance and expression of \(\text{nirS}\) genes increased substantially within the ES1 OMZ (Fig. 6c,d), and these results, particularly the increase in \(\text{nirS}\) gene expression, suggest that denitrification was actively occurring at the ES1 DO minimum. In contrast, although \(\text{amoA}\) gene abundance was still relatively high (Fig. 6a), the expression of \(\text{amoA}\) genes became undetectable within the ES1 DO minimum (Fig. 6b), thus indicating a substantial reduction in \(\text{N}_2\text{O}\) production via AO. Ultimately, our molecular genetic results provide evidence that implies that denitrification became an important source of \(\text{N}_2\text{O}\) within the ES1 DO minimum. Our suggestion that denitrification was an important source of \(\text{N}_2\text{O}\) at DO concentrations \(\sim 10\mu\text{mol L}^{-1}\) (i.e. ES1 OMZ) is also supported by a recent modelling study\(^5\) and by \(\delta^{15}\text{N}\) tracer measurements\(^5\) which found that denitrification was an important source of \(\text{N}_2\text{O}\) at similar DO concentrations.

While it appears that denitrification was an important source of \(\text{N}_2\text{O}\) within the ES1 OMZ, two lines of evidence point towards the potential for at least partial \(\text{N}_2\text{O}\) consumption by denitrification. Firstly, in comparison to the more oxygenated waters directly above and below it, the \(\delta^{15}\text{N}_{\text{bulk-N}}\) and \(\delta^{18}\text{O-N}_2\text{O}\) values at the ES1 DO minimum decreased and increased, respectively (Fig. 5a,b). The observation of a concomitant \(\delta^{15}\text{N}_{\text{bulk-N}}\)-\(\delta^{18}\text{O-N}_2\text{O}\) decrease and \(\delta^{18}\text{O-N}_2\text{O}\) increase is extremely rare, however, it has been observed in the Black Sea where it was interpreted as indicating simultaneous \(\text{N}_2\text{O}\) production and consumption (i.e. a production source which decreases \(\delta^{15}\text{N}_{\text{bulk-N}}\) and a consumption sink which increases \(\delta^{18}\text{O-N}_2\text{O}\))\(^4\). Secondly, a notable SP increase was also observed in the ES1 DO minimum (Fig. 5c). Similar to nitrifier-denitrification, production of \(\text{N}_2\text{O}\) by denitrification yields \(\text{N}_2\text{O}\) with an SP \(\geq 0\%\), while AO produces \(\text{N}_2\text{O}\) with an SP \(\geq 30\%\)\(^6\),\(^8\). As such, an initial interpretation of the SP result by itself could point towards a larger contribution of \(\text{N}_2\text{O}\) via the AO route. Given the extremely reduced expression of \(\text{amoA}\) genes in the ES1 DO minimum, however, this seems unlikely. Instead, reduction of \(\text{N}_2\text{O}\) to \(\text{N}_2\) can also result in an SP increase, albeit a highly variable one\(^6\),\(^8\), and results have shown that the reduction of \(\text{N}_2\text{O}\) can cause SP to increase even when there are net \(\text{N}_2\text{O}\) gains\(^4\). Based on the simultaneous \(\delta^{15}\text{N}_{\text{bulk-N}}\)-\(\text{N}_2\text{O}\) decrease and \(\delta^{18}\text{O-N}_2\text{O}\) increase, and the increased SP signature, we therefore propose that some of the \(\text{N}_2\text{O}\) produced within the ES1 DO minimum was subsequently reduced to \(\text{N}_2\). 

Although limited in scope, this is the first study to show evidence which points towards \(\text{N}_2\text{O}\) cycling by denitrification in the open Atlantic Ocean. As outlined earlier, \(\sim 20\%\) of the ETNA is covered by low oxygen eddy cores at any one time; as such, if denitrification is also cycling \(\text{N}_2\text{O}\) within these other low oxygen eddies, we may need to change our classical view that \(\text{N}_2\text{O}\) cycling in the ETNA is restricted to nitrification. It is, however, important to point out that while we have suggested that denitrification played a role in cycling \(\text{N}_2\text{O}\) within the suboxic eddy investigated during this study, it only appears to be important at the nanomolar scale (i.e. the scale at which we measure \(\text{N}_2\text{O}\), as at the micromolar scale there was no evidence for \(\text{NO}_3^-\) reduction\(^9\) or biogenic \(\text{N}_2\) production (Altabet and Grundle, unpublished data) inside the suboxic eddy. Still, some of the low oxygen eddies which have been observed in the ETNA with moored and glider based instruments have been characterized by DO concentrations even lower than those reported here\(^15\), and to this end, it is possible that some of the low oxygen eddies in the ETNA may also be sites of fixed \(\text{N}\) losses at the micromolar scale.

**Summary**

The present study has demonstrated for the first time that low DO eddies in the eastern tropical North Atlantic can cause significant shifts in the \(\text{N}_2\text{O}\) cycling dynamics which are typically found in this region. Furthermore, this work has shown that low DO eddies can serve as ideal ‘natural laboratories’ for investigating the impact of decreasing DO concentrations for marine \(\text{N}_2\text{O}\) conditions. In the case of this study, our results showed that at DO concentrations \(<\sim 20\mu\text{mol L}^{-1}\), \(\text{N}_2\text{O}\) production increased substantially, resulting in concentrations which were within the range of the highest \(\text{N}_2\text{O}\) concentrations reported for major OMZ regions such as the open Arabian Sea and eastern tropical Pacific. This result has demonstrated the magnitude by which \(\text{N}_2\text{O}\) production could increase if open ocean DO concentrations decrease in the tropical Atlantic. Isotope and molecular genetic results also provided evidence for a major shift in the \(\text{N}_2\text{O}\) cycling pathways at \(<\sim 10\mu\text{mol O}_2\) \(\mu\text{mol L}^{-1}\); such that it appeared that denitrification not only started to produce \(\text{N}_2\text{O}\), it also started to partially consume some of the \(\text{N}_2\text{O}\). Finally, as DO concentrations decreased to \(<5\mu\text{mol L}^{-1}\) the \(\text{N}_2\text{O}\) concentration also decreased, possibly indicating a switch from net \(\text{N}_2\text{O}\) production to net \(\text{N}_2\text{O}\) consumption. Ultimately, given that this study has shown the capacity of low DO eddies to be \(\text{N}_2\text{O}\) production ‘hotspots’, and because \(\text{N}_2\text{O}\) cycling pathways (i.e. denitrification) not previously thought to occur in the Atlantic were observed, a reevaluation of \(\text{N}_2\text{O}\) budgets and cycling in the tropical Atlantic Ocean may be necessary.
Methods

Glider Surveys. Two Slocum gliders (IFM12 and IFM13) manufactured by Teledyne Webb Research were used in this study to observe the temperature, salinity, dissolved oxygen and current fields north of the Cape Verde archipelago. Between January and April 2014, these gliders were able to confirm the presence of an anti-cyclonic mode-water eddy, which had formed off the coast of Mauritania and then propagated westward toward the Cape Verde Ocean Observatory (CVOO; Fig. 1). IFM12 was deployed on January 10th, 2014 from the Cape Verdean RV Islandia, and it first entered the eddy reported here on January 23rd, 2014. A first section through the eddy core was completed by IFM12 on February 3rd 2014, and results confirmed that it was a low DO eddy. IFM13 was deployed from RV Meteor on March 17th, 2014 and completed a section through the eddy core on April 7th, 2014. The data collected by the gliders underwent post-processing routines that included a glider-speed dependent thermal lag correction of the conductivity cell46, and a mixed lab/in-situ calibration of the Aanderaa Optode oxygen sensor. Finally, the locations of the eddy during the IFM12 and IFM13 deployments, and the locations of the glider sections are shown in Fig. 1, and DO concentrations from the IFM13 section through the eddy are shown in Fig. 3.

Ship-Based Sampling. Between March 6th and 7th 2014, the RV Islandia was used to conduct sampling for a suite of biological, chemical and physical parameters at a station inside the suboxic eddy (eddy station 1; Fig. 1). In order to allow for comparisons between measurements made inside the eddy with conditions outside the eddy, the same suite of samples collected at eddy station 1 (ES1) were also collected at an outside eddy station (OES) during an RV Islandia cruise on February 14th 2014 (Fig. 1). Here we outline the sampling and measurements of parameters that relate to N2O cycling.

Dissolved oxygen (DO) concentrations were measured using a Seabird SBE43 DO sensor that was attached to our conductivity, temperature and depth (CTD) profiler. The DO sensor was calibrated using DO measurements by Winkler titration on duplicated samples collected across the entire range of DO concentrations observed. The detection limit of these measurements was 3 μmol O₂ L⁻¹, and the average standard deviation of the duplicate measurements was ±0.28 μmol O₂ L⁻¹. It is important to note, however, that in order to preserve Niskin bottle water for our N₂O concentration and isotope samples, bottle samples for DO measurements by Winkler titration were not collected on our N₂O vertical sampling casts. Samples for DO measurements by Winkler titration, for the purpose of calibrating the DO sensor, were instead collected on CTD casts immediately before and after our N₂O sampling casts. The average standard deviation between our discrete Winkler DO measurements and our calibrated CTD-DO sensor measurements was ±1.31 μmol O₂ L⁻¹. Considering the errors involved in both our duplicated Winkler DO measurements (±0.28 μmol O₂ L⁻¹) and our CTD-DO measurements (±1.31 μmol O₂ L⁻¹), the average propagation of error associated with the DO concentrations we report for eddy station 1 is ±1.33 μmol O₂ L⁻¹. Discrete depth water samples were also collected from the surface to 450 m depth for the purpose of measuring N₂O concentrations, isotope and isotopomer signatures of N₂O, and for quantifying the abundance and transcripts of ammonia- monoxygenase genes of nitrifying bacteria and archaea, and nitrite reductase genes of denitrifying bacteria (amoA and nirS, respectively; all protocols described below).

On March 18th 2014, we also conducted a CTD-DO survey and collected water samples for N₂O concentration measurements at an additional inside eddy station (eddy station 2; Fig. 1) on the RV Meteor cruise M105. The CTD-DO sensor was calibrated following the same protocols outlined above, and the standard deviation of duplicate DO measurements by Winkler titration was ±0.35 μmol L⁻¹, while the average standard deviation between our discrete Winkler DO measurements and our CTD-DO sensor measurements was ±1.23 μmol L⁻¹. To this end, the average propagation of error associated with the DO concentrations we report for eddy station 2 is ±1.28 μmol L⁻¹.

N₂O concentration, and isotope/isotopomer measurements. Water samples for N₂O concentration, and isotope and isotopomer measurements were collected in duplicate 60 ml and 120 ml serum bottles, respectively, following standard dissolved gas sampling protocols47. Immediately following collection, the samples were poisoned with 100 μl of a saturated HgCl₂ solution and then stored until analysis ashore.

N₂O concentration samples were stored for ~2 months prior to being measured on a gas chromatograph with an attached electron capture detector using the headspace equilibration method described by Grundle et al.47. Final dissolved N₂O concentrations were calculated using corresponding measurements of in situ temperature and salinity, corrected for temperature and pressure during the headspace equilibration following the solubility tables of Weiss and Price48. The average standard deviation of our duplicate N₂O concentration measurements was ±0.8 mmol L⁻¹.

Isotope (δ¹⁵Nbulk-N₂O vs. AIR and δ¹⁸O-N₂O vs. VSMOW) and isotopomer (δ¹⁵Nv-N₂O and δ¹⁵Nβ-N₂O vs. AIR) analysis began with continuous helium (He) gas stripping of dissolved N₂O out of samples as described in Charoenpong et al.49. Briefly, sample water was pumped in and out of a gas extractor (14 ml min⁻¹) through which He was constantly bubbled (90 ml min⁻¹). Quantitative yield was verified by comparison of N₂O recovery from seawater with known N₂O concentration (established by atmospheric equilibration) and with standard gas injected directly into the He gas flow. Following extraction, the method of McIlvin and Casciotti50 was followed in which a purge/trap system was used to purify and concentrate extracted N₂O. This included two-step cryo-focusing with passage through CO₂ and H₂O traps as well as a 30 m × 0.53 mm GS-Q capillary column. Sample N₂O was introduced via continuous He carrier flow into a multicollector IsoPrime isotope ratio mass spectrometer (IRMS). Masses 44, 45, and 46, and masses 30 and 31 which arise from the NO+ fragment of N₂O which is formed in the ion source, were monitored, and sample N₂O was detected as a well-resolved sharp peak which was bracketed by broader reference N₂O peaks. The 45/44 and 46/44 peak areas were used to derive δ¹⁸O-N₂O and δ¹⁵Nbulk-N₂O, respectively, while the 31/30 peak area was used to derive δ¹⁵Nv-N₂O. The δ¹⁵Nbulk-N₂O and δ¹⁵Nv-N₂O values were used to calculate δ¹⁵Nv-N₂O. Calibration of δ¹⁵Nv-N₂O, δ¹⁵Nbulk-N₂O and δ¹⁸O-N₂O was...
accomplished using 4 certified standard gases (supplied by Joachim Mohn) that ranged widely in these values and encompassed those reported here. Calibration for N₂O site-specific isotopomer composition also needs to account for instrument specific ‘scrambling’ in the mass spectrometer ion source between 15N14NO and 14N15NO. This magnitude is on the order of 10% and is manifested as changes in the 30/44 ratio from the values expected in the absence of scrambling. In order to account for this, we took advantage of new standard materials that vary widely in isotopomer composition to perform an empirical curve-fitting calibration. Finally, based on measurements of duplicate samples from each sampling depth, the errors associated with our isotope measurements were ±0.07, 0.17, 0.36 and 0.18‰ for 15N14N21O5, 15N15N21O5, 14N15N21O5 and 15N21O5, respectively.

**Molecular genetic analyses.** Water samples (~2 L) were filtered through 0.2 μm polycrylonitrile membrane filters, which were immediately stored at −80 °C until nucleic acid purification was performed ashore following Löscher et al. RNA was treated with DNase to remove any residual DNA, and RNA purity was verified by non-template quantitative-PCRs for amoA (ammonia monoxygenase) and nirS (nitrite reductase) genes. Reverse transcription was performed following Löscher et al. Quantitative-PCRs of bacterial and archaeal amoA were performed in technical duplicates with standards obtained from Nitrosomonas oceani NC1 and from an environmental clone for archaeal amoA, while the same was achieved for nirS using a standard obtained from Paracoccus denitrificans (F1 1222). All reactions were performed in a volume of 12.5 μl using a Viia7 quantitative-PCR system following the protocols and PCR conditions outlined by Löscher et al. and Lam et al.

**References**

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Author Contributions
D.S.G., B.F. and A.K. designed the shipboard sampling portion of the study, and G.K. and J.K. designed and executed the glider surveys of the suboxic eddy. D.S.G. and B.F. conducted field sampling onboard RV Islandia. D.S.G. and H.W.B. were responsible for N₂O concentration measurements, while D.S.G. and M.A.A. were responsible for the isotope/isotopomer measurements which were conducted using facilities provided by M.A.A. Sampling and measurements of dissolved oxygen were conducted by B.F. The molecular genetic data was processed by C.R.L., and G.K. and J.K. processed the glider data. D.S.G. wrote the manuscript, and all other authors provided feedback.

Additional Information

Competing Interests: The authors declare that they have no competing interests.

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