Nitrite removal improves hydroxylamine analysis in aqueous solution by conversion with iron(III)

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Abstract. Dissolved hydroxylamine (NH\textsubscript{2}OH) is a short-lived compound produced in the oceanic environment during nitrification and dissimilatory reduction of nitrate to ammonium (DNRA). The ferric ammonium sulfate (FAS) conversion method is the only method available so far to determine dissolved NH\textsubscript{2}OH in nanomolar concentrations in seawater. We show that side reactions of dissolved nitrite (NO\textsubscript{2}\textsuperscript{−}) can result in a significant bias in the NH\textsubscript{2}OH concentration measurements when applying the FAS conversion method. We propose to scavenge dissolved NO\textsubscript{2}\textsuperscript{−} by addition of sulfanilamide to suppress effectively the undesired side reactions by NO\textsubscript{2}\textsuperscript{−}. This modification of the FAS conversion method will allow a NH\textsubscript{2}OH determination even in oceanic regions with high NO\textsubscript{2}\textsuperscript{−} concentrations. A reliable detection of NH\textsubscript{2}OH in seawater samples can give us a clue about the occurrence of active nitrification or DNRA in the ocean and, therefore, will provide further insights about the oceanic nitrogen cycle.

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Introduction

Hydroxylamine (NH\textsubscript{2}OH) is a short-lived compound of the marine nitrogen cycle.\textsuperscript{[11]} Two microbial pathways that involve NH\textsubscript{2}OH have been identified so far: Ammonium (NH\textsubscript{4}+) oxidation to nitrate (NO\textsubscript{3}−) (i.e. nitrification) and dissimilatory reduction of nitrate to ammonium (DNRA). The idea that NH\textsubscript{2}OH also occurs during the anaerobic ammonium oxidation (anammox) pathway could not be verified.\textsuperscript{[2,3]}

NH\textsubscript{2}OH is formed as an intermediate during the first step of nitrification by ammonia-oxidising bacteria (AOB)\textsuperscript{[4,5]}:

\[
\text{NH}_4^+ \rightarrow \text{NH}_2\text{OH} \rightarrow \text{NO}_2^- 
\]

There is increasing evidence that ammonium oxidation in the ocean is dominated by ammonia-oxidising archaea (AOA) and not AOB.\textsuperscript{[6,7]} Little is known about the pathway of ammonium oxidation by AOA, however, and unlike AOB, a set of genes encoding the NH\textsubscript{2}OH oxidoreductase has not been identified in AOA yet.\textsuperscript{[8]} Thus, potential formation of NH\textsubscript{2}OH during archaean nitrification remains to be proven.

In contrast to nitrification, which occurs in oxic conditions, bacterial DNRA is an anaerobic process that requires anoxic conditions.\textsuperscript{[9–11]} During DNRA, NH\textsubscript{2}OH evolves as an enzyme-bound intermediate in the reduction of nitrite to ammonium. It may be released from the binding site of the enzyme under acidic conditions\textsuperscript{[12]}:

\[
\text{NO}_3^- \rightarrow \text{NO}_2^- \rightarrow (\text{NH}_2\text{OH}) \rightarrow \text{NH}_4^+ 
\]

Dissolved NH\textsubscript{2}OH is not stable in seawater and its turnover times range from 4 h (in artificial seawater) to 8 h (in natural seawater).\textsuperscript{[13,14]} The decomposition of NH\textsubscript{2}OH in aqueous solution is strongly enhanced under alkaline conditions, where NH\textsubscript{2}OH rapidly reacts with ambient oxygen.\textsuperscript{[15]} In addition, several reactions with transition metal ions and complexes\textsuperscript{[16–18]} and the catalytic effect of copper and other heavy metal ions in the decomposition of NH\textsubscript{2}OH\textsuperscript{[17,19]} have been reported. Pronation of NH\textsubscript{2}OH to NH\textsubscript{3}OH\textsuperscript{+} under acidic conditions results in the stabilisation of the molecule.\textsuperscript{[15]}

First attempts to determine NH\textsubscript{2}OH in seawater with a spectro-photochemical method were hampered by a high detection limit.\textsuperscript{[14,20]} On the basis of the work by von Breymann et al.\textsuperscript{[21]} Butler and Gordon\textsuperscript{[13]} developed a method for the determination of NH\textsubscript{2}OH in seawater at nanomolar concentrations. This method is based on the oxidation of NH\textsubscript{2}OH to nitrous oxide (N\textsubscript{2}O) by iron(III) using ferric ammonium sulfate (NH\textsubscript{4}Fe(SO\textsubscript{4})\textsubscript{2}, FAS) as oxidation agent\textsuperscript{[18,22]}:

\[
\text{Fe} (\text{NH}_2\text{OH})^{3+} \rightarrow \text{Fe}^{2+} + \text{H}_2\text{NO} + \text{H}^+ \quad (1)
\]

\[
\text{Fe}^{3+} + \text{H}_2\text{NO} \rightarrow \text{Fe}^{2+} + \text{HNO} + \text{H}^+ \quad (2)
\]

\[
2\text{HNO} \rightarrow \text{N}_2\text{O} + \text{H}_2\text{O} \quad (3)
\]

The resulting N\textsubscript{2}O is subsequently analysed quantitatively using a gas chromatograph equipped with an electron capture detector (GC-ECD). The chemical conversion of NH\textsubscript{2}OH into

Environmental context. Nitrogen is an essential nutrient for marine organisms, and thus an understanding of the marine nitrogen cycle is a crucial factor in predicting the sensitivity of marine life to environmental change. Hydroxylamine is a short-lived intermediate in nitrogen transformation processes, and reliable detection of this compound in seawater can help to identify these processes within the marine nitrogen cycle.
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\[
\text{N}_2\text{O} \rightarrow \text{N}_2\text{O} + \text{H}_2\text{O} \quad (6)
\]

The overall net reaction of Reactions 5 and 6 has a different stoichiometry than the conversion of NH\(_2\text{OH}\) by iron(III) (Reactions 1–3), which requires two molecules of NH\(_2\text{OH}\) to form one molecule of N\(_2\text{O}\). Different conversion efficiencies of these concurring reactions may therefore lead to a further bias.

The aims of this study were (i) to evaluate the potential bias of the side reactions of NO\(_2^+\) in the FAS conversion method and (ii) to find an appropriate treatment to determine NH\(_2\text{OH}\) in seawater samples by avoiding interferences caused by NO\(_2^+\).

**Methods**

For the analysis of dissolved NH\(_2\text{OH}\) we followed the measurement procedure of Schweiger et al., which was slightly modified from the procedure by Butler and Gordon (Fig. 1). The NH\(_2\text{OH}\) concentration in the samples was calculated as follows:

\[
\text{[NH}_2\text{OH]} = \left(\text{[N}_2\text{O}]_{\text{FAS}} - \text{[N}_2\text{O}]_{\text{BG}}\right)/RC \quad (7)
\]

\[
RC = 2 \times m_{\text{std}} \quad (8)
\]

where \(m_{\text{std}}\) is the regression slope of the standard addition and [N\(_2\text{O}\)]\(_{\text{FAS}}\) and [N\(_2\text{O}\)]\(_{\text{BG}}\) are the N\(_2\text{O}\) concentrations of samples with and without FAS conversion. The factor of two in the calculation of the recovery factor (Eqn 8) results from the stoichiometry of the reaction between NH\(_2\text{OH}\) and FAS.

In this manuscript, we investigated the effect of the side reactions of HNO\(_2^+\) on the different stages of the NH\(_2\text{OH}\) analysis according to Schweiger et al. in several laboratory experiments. Therefore, instead of calculating the final NH\(_2\text{OH}\) concentrations, we present the N\(_2\text{O}\) concentrations in the results and discussion section, as the N\(_2\text{O}\) produced from the side reactions may bias the actual NH\(_2\text{OH}\) calculations. Similarly, the side reaction between NH\(_2\text{OH}\) and NO\(_2^+\) involves a different stoichiometry than the reaction between NH\(_2\text{OH}\) and FAS.

In experiments that involve the conversion of NH\(_2\text{OH}\) into N\(_2\text{O}\), we calculated a conversion factor instead of the recovery factor defined above. The conversion factor was calculated in two ways: (a) in experiments with only one standard concentration added it was calculated as the ratio between the difference of N\(_2\text{O}\) concentrations with and without NH\(_2\text{OH}\) addition and the concentration of the NH\(_2\text{OH}\) standard and (b) in experiments with different standard concentrations added it was calculated as the slope of the linear regression between measured N\(_2\text{O}\) concentrations and NH\(_2\text{OH}\) standard additions. Error bars
shown in the figures reflect the standard deviation of triplicate measurements, calculated according to David.[36]

The laboratory experiments were prepared as follows: 10 mL of MilliQ or seawater was placed into opaque vials (20 or 24 mL) that were subsequently sealed with butyl rubber stoppers and crimped before addition of the reactants. In order to remove the background N2O, the vials were purged for 20 min with N2O-free nitrogen gas (99.999 %, AirLiquide, Düsseldorf, Germany) at a flow rate of ~80–100 mL min⁻¹ or the measurements were corrected for background N2O concentrations that were obtained from triplicate control samples. Sodium nitrite solutions (100 μL) were added to the samples, leading to final concentrations between 0.1 and 10 μmol L⁻¹ and thereby covering the range of ambient NO2⁻ concentrations.[31]

Experiments were carried out with three different water types in order to simulate typical matrix effects: We used (i) MilliQ water, (ii) aged filtered surface seawater from the tropical North Atlantic Ocean (~10°N, 30°W, from December 2009) or (iii) unfiltered seawater from the Boknis Eck Time Series Station, located in the Eckernförde Bay in the south-western Baltic Sea (hereafter referred to as BE water; sampling depth 15 m, samples were taken between June 2010 and February 2011).[37,38] Experiments with BE water were carried out within 7 days after sampling. BE water has a lower salinity (typically between 12.5 and 24.5) than the surface seawater from the tropical North Atlantic Ocean (~35).

Sample preparation and general treatments

Stock solutions of NaNO₂ (p.a., Merck KGaA, Darmstadt, Germany, ~20 mg per 100 mL), the exact concentration was calculated from the mass weight) were prepared in MilliQ water a maximum of three days before analysis and stored at 4°C before analysis. If necessary, the stock solution was diluted further to obtain different NO2⁻ concentrations.

FAS solutions (p.a., Merck KGaA, 1.206 g per 100 mL) were prepared in MilliQ water at least three days before the experiments to ensure the complete dissolution of the FAS. The FAS solutions were used for multiple experiments but were renewed at least on a monthly basis to prevent contamination.

Stock solutions of hydroxylammonium chloride (p.a., Merck KGaA, ~20 mg per 100 mL), the exact concentration was calculated from the mass weight) were prepared in an aqueous solution of acetic acid (p.a., Merck KGaA) (3 mL of acetic acid (glacial) per 1 L of MilliQ water, pH ~3) to stabilise the NH₂OH solutions. The stock solutions were diluted further to obtain four different standard concentrations leading to final concentrations in the vials between 0 and 100 nM L⁻¹ at an addition of 100 μmol per vial. All standard solutions were prepared a maximum of 7 days before analysis and stored in the dark at 4°C.

All samples were analysed for their N2O concentrations using a static headspace equilibration method. A 9 to 9.5 mL volume of the headspace was extracted from the equilibrated samples using a gas-tight syringe (VICI Precision Sampling, Baton Rouge, LA, USA). The headspace subsamples were analysed with a GC-ECD system (HP 5890 II, Agilent Technologies, Santa Clara, CA, USA, or Carlo Erba HRGC 5160 Mega Series, ThermoFisher Scientific, Waltham, MA, USA) that was calibrated using at least four different standard gas mixtures (N₂O and synthetic air, Deute Steininger, Mülheim; calibrated against NOAA standard scale at the Max Planck Institute for Biogeochemistry, Jena, Germany) or dilution of the highest standard gas mixture. For details of the analytical method for N2O see Kock et al.[39] and Walter et al.[40]

Table 1. Results from the acidification experiment at the CVOO Time Series Station

<table>
<thead>
<tr>
<th>Treatment</th>
<th>N2O (mean of triplicate measurements) (nmol L⁻¹)</th>
<th>N2O standard deviation (nmol L⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated</td>
<td>174.02</td>
<td>134.46</td>
</tr>
<tr>
<td>HgCl₂</td>
<td>32.46</td>
<td>0.94</td>
</tr>
<tr>
<td>HCl</td>
<td>32.01</td>
<td>2.39</td>
</tr>
<tr>
<td>HgCl₂ + HCl</td>
<td>32.13</td>
<td>1.47</td>
</tr>
</tbody>
</table>

In contrast to Butler and Gordon,[13] samples were not treated with mercuric chloride (HgCl₂) during the experiments, as all samples were analysed within a few days after sampling and acidification of the samples was tested to be efficient to prevent further N₂O production in an earlier experiment.

We tested acidification for conservation with seawater samples from the Cape Verde Ocean Observatory (CVOO) Time Series Station in a comparison experiment with different treatments of the samples. Four triplicates of N₂O samples were taken in 24 mL vials from a depth of 250 m and treated with (a) 50 μL of saturated mercuric chloride solution (p.a., Merck KGaA), (b) 100 μL of hydrochloric acid (2 mol L⁻¹, p.a., Merck KGaA), (c) 50 μL of mercuric chloride solution and 100 μL of hydrochloric acid and (d) were left untreated. The samples were stored over a period of three weeks and analysed using a static equilibration method.[39] Samples treated with mercuric chloride or hydrochloric acid did not show significant differences in N₂O concentrations within the standard deviation of the measurements. The measurements of the untreated samples showed a large variability and strongly differing values (Table 1). Although nitrite measurements were not available for this experiment, no nitrite is usually found at this depth in the North Atlantic Ocean.[51] Therefore, any interference by N₂O production from nitrite can be excluded for this experiment.

Experiments

N₂O production from HNO₂

Background production of N₂O from acidification of NO₂⁻-containing samples was tested in MilliQ and BE waters. In the experiments with BE water the vials were purged with nitrogen gas to remove background N₂O from the vials whereas MilliQ water samples were corrected for background N₂O concentrations measured from a control sample at t₀. Samples were treated with 100 μL of two different stock solutions of NO₂⁻ with concentrations of 0.504 and 9.54 μmol L⁻¹ and acidiﬁed with 100 μL of acetic acid (glacial). A potential inﬂuence of N₂O production from background NO₂⁻ in the BE water was taken into account by measurements of a control run without addition of NO₂⁻. The nitrite concentration of the sampled water at the Boknis Eck Time Series Station was 0.02 μmol L⁻¹. Samples were incubated in the dark at 30°C with the N₂O concentration being measured between 0 and 350 h.

Reaction of NH₂OH with NO₂⁻ to form N₂O

The conversion of NH₂OH by different concentrations of NO₂⁻ was tested in MilliQ water, as background N₂O production from HNO₂ became significant in BE water within the time of the conversion reaction (Fig. 2). Five different NO₂⁻ stock
solutions (100 μL) were added to the sample vials, resulting in concentrations between 0.23 and 3.45 μmol L⁻¹. Subsequently, the samples were treated with 100 μL of acetic acid and 100 μL of hydroxylammonium chloride solution (5.0 μmol L⁻¹). A set of control samples without addition of NH₂OH was measured for each NO₂ concentration, thereby accounting for the background production of NO₂, and the final N₂O concentrations were background corrected from a sample without nitrite addition. Vials were analysed for N₂O concentrations after 24 h.

However, the influence of this reaction on the NH₂OH analysis depends on the kinetics of the concurring reactions of NH₂OH with NO₂ or FAS. Therefore, the conversion reaction of NH₂OH with FAS in the presence of different NO₂ concentrations was also tested: three sets of samples with NO₂ concentrations of 0.162, 0.539 and 4.27 μmol L⁻¹ were treated with 100 μL of glacial acetic acid and four different NH₂OH standards and 100 μL of sodium hydroxide solution (8 M) were added, resulting in a pH close to 10 to stop the background NO₂ production. The pH was measured in five random samples for control of NH₂OH conversion by FAS in the presence of different NO₂ concentrations between 0.23 and 3.45 μmol L⁻¹. All samples were subsequently treated with 100 μL of an acidic solution of sulfanilamide (10 mmol L⁻¹) to ensure a large excess over ambient nitrite concentrations and to enable a rapid and complete decomposition of nitrite.

Removal of NO₂

The removal of NO₂ before acidification of the samples is the simplest way to eliminate negative side reactions during NH₂OH conversion. Appropriate scavengers for NO₂ need to selectively react with NO₂ without affecting the conversion reaction between NH₂OH and FAS.

An acidic solution of sulfanilamide (p.a. VWR International, Darmstadt, Germany, 100 μmol L⁻¹) was therefore tested as a scavenger for NO₂, as sulfanilamide is widely used as a reagent in the detection of NO₂ and NO₃. It reacts selectively with NO₂ with formation of a diazonium salt that is coupled to 1-naphthylamine and forms a spectrometrically detectable dye. Without addition of 1-naphthylamine, the diazonium salt is decomposed with formation of nitrogen gas.

To test the efficiency of sulfanilamide as a NO₂ scavenger, the NH₂OH conversion by FAS was tested during two experiments in filtered seawater from the tropical North Atlantic Ocean with and without addition of sulfanilamide in the presence of NO₂ at two different concentrations (final concentration: 0.873 and 4.37 μmol L⁻¹). All samples were purged with nitrogen before the experiments. 100 μL of an acidic solution of sulfanilamide (10 mmol L⁻¹) were added to half of the vials directly after acidification and before the addition of NO₂. The final sulfanilamide concentration was chosen as 100 μmol L⁻¹ to ensure a large excess over ambient nitrite concentrations and to enable a rapid and complete decomposition of nitrite. The vials were subsequently treated with 100 μL of four different NH₂OH standards and 100 μL of FAS solution and were analysed for N₂O after 20 to 24 h.

Results and discussion

N₂O production from HNO₂

In both media, N₂O concentrations in samples that were treated with NO₂ significantly increased over time, with a much higher increase in N₂O in BE water than in MilliQ water (Fig. 2). Samples with higher NO₂ concentrations showed significantly
higher N₂O production in both experiments. A much larger influence on N₂O production by the medium was found, however. Although N₂O production in MilliQ water stayed moderate and levelled off after 168 h even for high NO₂⁻ concentrations, concentrations continued to increase in BE water until the end of the incubations. The N₂O production exceeded the statistical uncertainty of the N₂O measurements even at concentrations as low as 0.5 µmol L⁻¹ in less than 24 h. The much higher N₂O production in BE water can be explained by an increased number of side reactions of NO₂⁻ with organic compounds or trace metal ions that favour N₂O production in the BE water.³⁰

Reaction of NH₂OH with NO₂⁻ to form N₂O
Samples without NH₂OH addition showed little N₂O production with only a slight increase with increasing NO₂⁻ concentrations, which is in reasonable agreement with the results from the previous experiment (Fig. 3). Elevated N₂O concentrations of ~50 nmol L⁻¹ were found in all samples with NH₂OH addition. No significant difference between the samples with different NO₂⁻ concentrations was found and the conversion factors, calculated as the ratio between the difference of N₂O concentrations with and without NH₂OH addition and the concentration of the NH₂OH standard, were close to one, showing that N₂O is produced almost quantitatively from the comproportionation of NO₂⁻ and NH₂OH even at NO₂⁻ concentrations as low as ~0.2 µmol L⁻¹. This indicates that under acidic conditions only low concentrations of ambient NO₂⁻ are necessary to convert NH₂OH into N₂O.

In contrast to the experiment without FAS addition, the conversion factors in the experiment with FAS addition in the presence of nitrite were significantly lower, ranging from 0.19 (+0.03) to 0.35 (+0.05) (Fig. 4) and were therefore in the range of conversion factors obtained in the reaction between FAS and NH₂OH. Due to the different stoichiometry this reaction yields conversion factors <0.5. A significant change in the conversion factors from ~0.2 to 0.35 occurred between samples with low (<0.54 µmol L⁻¹) and high (2.695 µmol L⁻¹) NO₂⁻ additions.

This indicates that the conversion of NH₂OH is likely dominated by the reaction with FAS, but concentrations of NO₂⁻ in the micromolar range may have the potential to bias the conversion factors towards higher values. Therefore, this effect has to be considered as an additional source of uncertainty within the NH₂OH analysis.

Removal of NO₂⁻
Samples without sulfanilamide addition showed a significant influence of NO₂⁻ on the N₂O production, which is in good agreement with the results of the previous experiments. A significant background production of N₂O from nitrous acid was observed for both NO₂⁻ concentrations, with higher background production from samples with high NO₂⁻ content (Fig. 5). This agrees well with the results from the N₂O production from HNO₂ experiment (see above). These show a significant increase in N₂O concentrations 24 h after acidification of NO₂⁻-containing samples due to the background production of N₂O from HNO₂ (Fig. 2). Furthermore, a significant change in the conversion factor could be observed with increasing NO₂⁻ concentrations, which is the result of an increased influence of the reaction between NO₂⁻ and NH₂OH with increasing NO₂⁻ concentrations, as seen in the experiment involving the reaction of NH₂OH with NO₂ to form N₂O (see above) (Fig. 4).

In contrast, N₂O production from samples with sulfanilamide addition did not change with increasing NO₂⁻ concentration. No change in the background NO₂⁻ concentration or the conversion factor was observed, which leads to the conclusion that NO₂⁻ was successfully removed from the samples.

Conclusions
All experiments show that the NH₂OH determination by FAS conversion into N₂O is significantly affected by the presence of NO₂⁻. On the one hand, the addition of acid to natural waters that
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contain only low amounts of NO$_2^-$ can already lead to a significant production of N$_2$O through the decomposition of HNO$_2$. The importance of this background production strongly depends on the composition of the sample matrix and the storage time of the samples under acidic conditions. For seawater samples with NO$_2^-$ concentrations at a micromolar level a significant bias in N$_2$O within the time of the NH$_2$OH conversion by FAS cannot be excluded.

On the other hand, it could be shown that N$_2$O is also produced almost quantitatively by the reaction of NH$_2$OH with HNO$_2$ even at very low NO$_2^-$ concentrations, thus showing a much higher conversion factor than in the reaction with FAS. A significant effect of this reaction on the efficiency of the NH$_2$OH conversion with the FAS method is only observed at NO$_2$ concentrations in the micromolar range, however.

Owing to these side reactions, we caution that NH$_2$OH concentration measurements without NO$_2^-$ scavenging can lead to an overestimation of the true NH$_2$OH concentrations when NO$_2$ is present in large amounts or (acidified) samples are stored over longer periods. Due to the large number of side reactions and their different behaviour in different reaction media it is difficult to determine a threshold NO$_2$ concentration that can be tolerated during NH$_2$OH analysis. This demands the removal of NO$_2$ from the reaction medium before NH$_2$OH analysis.

We could show that sulfanilamide successfully removed NO$_2^-$ from the samples without affecting the FAS conversion, and the reaction of sulfanilamide with NO$_2$ is sufficiently fast and quantitative\cite{44,45} which means that no extra time is required for the removal of NO$_2^-$. Sulfanilamide thus acts as a suitable NO$_2$ scavenger in NH$_2$OH analysis.

Based on our results, we suggest a modification of the original method\cite{22} by the addition of 100 $\mu$mol L$^{-1}$ acidic sulfanilamide solution to the reaction medium before acidification of the samples to inhibit potential N$_2$O production from side reactions with NO$_2$.

The proposed modification of the FAS conversion method will allow NH$_2$OH determination even in oceanic regions with high NO$_2$ concentrations such as found in the suboxic zones of the north-western Indian (see e.g. Lam et al.\cite{46}) and eastern tropical North and South Pacific Oceans (see e.g. Codispoti et al.\cite{47}). A detection of NH$_2$OH in seawater samples could indicate the occurrence of active nitrification or DNRA. Moreover, as nitrification and DNRA take place at different oxygen concentrations, the detection of NH$_2$OH in seawater samples could be interpreted as a specific indicator for nitrification in oxic and suboxic environments on the one hand and DNRA in anoxic environments on the other hand.

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**Fig. 5.** NH$_2$OH standard addition with ferric ammonium sulfate (FAS) conversion in presence of NO$_2$ without sulfanilamide addition (a) and with sulfanilamide addition (b). Regression parameters are: (a) $[NO_2^-] = 0.882 \mu$mol L$^{-1}$; $y = 0.41 ( \pm 0.03)x + 4.6 ( \pm 1.2)$; $R^2 = 0.99$; $[NO_2^-] = 4.42 \mu$mol L$^{-1}$; $y = 0.66 ( \pm 0.07)x + 32.5 ( \pm 2.8)$; $R^2 = 0.97$; (b) $[NO_2^-] = 0.882 \mu$mol L$^{-1}$; $y = 0.34 ( \pm 0.04)x + 0.70 ( \pm 1.7)$; $R^2 = 0.96$; $[NO_2^-] = 4.42 \mu$mol L$^{-1}$; $y = 0.36 ( \pm 0.03)x + 0.86 ( \pm 1.4)$; $R^2 = 0.98$. 

\[ [\text{NO}_2^-] = 0.882 \mu\text{mol L}^{-1} \]

\[ [\text{NO}_2^-] = 4.42 \mu\text{mol L}^{-1} \]
time series station. The authors also thank Professor Peter Croot and three anonymous reviewers for their very helpful comments on their manuscript. This work was funded by the Chemical Oceanography Research Unit of GEOMAR.

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