Reply to report #1

Thank you for your comments.

The authors have incorporated most of the comments of the reviewers, and paper now is greatly improved with the incorporation of salinity, temperature, Chl a and NH4. I commend the authors on their efforts, and find that they have responded appropriately to the reviews. I had just a few minor comments for consideration.

Lines 130-131: I agree with the authors that sample date should be adjusted to a regular spacing. However, I do not agree with the assumption of no not temporal variability between weeks. The authors should use a lineal interpolation (or test other models) to homogenize dates, or in lack of this, demonstrate that the uncertainty introduced with such assumption is not significant.

We used a linear interpolation to homogenize the dates and found an overall error of 4.2%. In this case, we modified the text into “Sampling time varied for every month (usually 20-40 day interval), but for the statistical analysis, data was assumed to be regularly spaced as the uncertainty introduced was not significant (<5%).”

Lines 238-239: The authors should include a reference for the O2 limit used for the inhibition of denitrification.

According to Tiedje (1988), we included the O2 threshold of ~10 µmol L⁻¹ for the inhibition of denitrification.
The following changes have been made in the manuscript:

1. Include the uncertainty introduced from the date shift in line 131.
2. Include the $O_2$ threshold for the inhibition of denitrification in lines 240–241.
3. Add a reference in lines 651–653.
A multi-year observation of nitrous oxide at the Boknis Eck Time-Series Station in the Eckernförde Bay (southwestern Baltic Sea)

Xiao Ma¹, Sinikka T. Lennartz¹,², and Hermann W. Bange¹
¹ GEOMAR Helmholtz Centre for Ocean Research Kiel, Düsternbrooker Weg 20, 24105 Kiel, Germany
² now at ICBM, University of Oldenburg, Oldenburg, Germany

Correspondence to: Xiao Ma (mxiao@geomar.de)

Abstract. Nitrous oxide (N₂O) is a potent greenhouse gas and it is involved in stratospheric ozone depletion. Its oceanic production is mainly influenced by dissolved nutrient and oxygen (O₂) concentrations in the water column. Here we examined the seasonal and annual variations of dissolved N₂O at the Boknis Eck (BE) Time-Series Station located in Eckernförde Bay (southwestern Baltic Sea). Monthly measurements of N₂O started in July 2005. We found a pronounced seasonal pattern for N₂O with high concentrations (supersaturations) in winter/early spring and low concentrations (undersaturations) in autumn when hypoxic/anoxic conditions prevail. Unusually low N₂O concentrations were observed during October 2016–April 2017, which was presumably a result of prolonged anoxia and the subsequent nutrient deficiency. Unusually high N₂O concentrations were found in November 2017 and this event was linked to the occurrence of upwelling which interrupted N₂O consumption via denitrification and potentially promoted ammonium oxidation (nitrification) at the oxic/anoxic interface. Nutrient concentrations (such as nitrate, nitrite and phosphate) at BE are decreasing since 1980s, but oxygen concentrations in the water column are still decreasing. Our results indicate a close coupling of N₂O anomalies to O₂ concentration, nutrients and stratification. Given the long-term trends of declining nutrient and oxygen concentrations at BE, a decrease in N₂O concentration, and thus emissions, seems likely due to an increasing number of events with low N₂O concentrations.

1. Introduction

Long-term observation with regular measurement intervals can be an effective way to monitor seasonal and interannual variabilities as well as to decipher short- and long-term trends of an ecosystem, which are required to make projections of the future ecosystem development (see e.g. Ducklow et al., 2009). Recently, multi-year time-series measurements of nitrous oxide (N₂O), a potent greenhouse gas and a major threat to ozone depletion (IPCC, 2013; Ravishankara et al., 2009), have been reported from the coastal upwelling areas off central Chile (Farias et al., 2015) and off Goa (Naqvi et al., 2010), in the North Pacific Subtropical Gyre (Wilson et al., 2017), and in Saanich Inlet (Capelle et al., 2018).
N\textsubscript{2}O production in the ocean is generally dominated by microbial nitrification ($\text{NH}_4^+ \rightarrow \text{NO}_2^- \rightarrow \text{NO}_3^-$) and denitrification ($\text{NO}_3^- \rightarrow \text{NO}_2^- \rightarrow \text{N}_2\text{O} \rightarrow \text{N}_2$). During bacterial/archaeal nitrification, \text{N}_2\text{O} is produced as a by-product with enhanced \text{N}_2\text{O} production under low oxygen (O\textsubscript{2}) conditions (e.g. Goreau et al., 1980; Löscher et al., 2012). \text{N}_2\text{O} is produced as an intermediate during bacterial denitrification (Codispoti et al., 2005). \text{N}_2\text{O} could be further consumed via denitrification to dinitrogen, however, this process is inhibited with the presence of O\textsubscript{2} because of the low O\textsubscript{2} tolerance of the enzyme involved (Bonin et al. 1989). This incomplete pathway is called partial denitrification and can lead to \text{N}_2\text{O} accumulation (e.g. Naqvi et al., 2000; Fariás et al., 2009).

The oceans including coastal areas contribute ~25\% of the natural and anthropogenic \text{N}_2\text{O} emissions (IPCC, 2013), with disproportionately high emissions from coastal and estuarine areas (Bange, 2006). \text{N}_2\text{O} emissions from coastal areas strongly depend on nitrogen inputs (Seitzinger and Kroeze, 1998; Zhang et al., 2010). The increasing input of nitrogen (i.e. eutrophication) has become a worldwide problem in coastal waters leading to enhanced productivity and severe O\textsubscript{2} depletion caused by enhanced degradation of organic matter (Breitburg et al., 2018; Rabalais et al., 2014). The decline in O\textsubscript{2} concentration (i.e. deoxygenation), either in coastal waters or the open ocean, might result in favorable conditions for \text{N}_2\text{O} production (Codispoti et al., 2001; Nevison et al., 2003). The results of a model study by Kroeze and Seitzinger (1998) indicated a significant increase of \text{N}_2\text{O} in European coastal waters for 2050. Moreover, it has been suggested that \text{N}_2\text{O} production and emissions are very likely to increase in the near future, especially in the shallow suboxic/anoxic coastal systems (Naqvi et al., 2000; Bange, 2006). However, model projections show a net decrease in future global oceanic \text{N}_2\text{O} emission during the 21\textsuperscript{st} century (Martinez-Rey et al., 2015; Landolfi et al., 2017; Battaglia and Joos, 2018).

The Baltic Sea is a nearly enclosed, marginal sea with a very limited access to the open ocean via the North Sea. The restricted water exchange with the North Sea and extensive human activities, such agriculture, industrial production and sewage discharge in the catchment area led to high inputs of nutrients to the Baltic Sea. As a result, the areas affected by anoxia have been expanding in the deep basins of the central Baltic Sea (Carstensen et al., 2014). In order to control this situation, the Helsinki Commission (HELCOM) was established in 1974 and a series of measures have been taken to prevent anthropogenic nutrient input into the Baltic Sea. Consequently, the nutrient inputs (by riverine loads, direct point-sources and, for nitrogen, atmospheric deposition) to the Baltic Sea are declining (HELCOM, 2018a). However, the number of low O\textsubscript{2} (i.e. hypoxic/anoxic) events in coastal waters of the Baltic Sea is increasing and deoxygenation is still going on (Conley et al., 2011; Lennartz et al., 2014). The deoxygenation in the Baltic Sea can affect the production/consumption of \text{N}_2\text{O}. Our group has been monitoring dissolved \text{N}_2\text{O} concentrations at the Boknis Eck Time-Series Station, located in Eckernförde Bay (southwestern Baltic Sea), for more than a decade. In this study, we present monthly measurements of \text{N}_2\text{O} and biogeochemical parameters such as nutrients and O\textsubscript{2} from July 2005 to December 2017. The major objectives of our study were: 1) to decipher the seasonal
2. Material and methods

2.1 Study site

Sampling at the Boknis Eck (BE) Time-Series Station (www.bokniseck.de) started on 30 April 1957 and, therefore, it is one of the oldest continuously operated time-series stations in the world. The BE station is located at the entrance of the Eckernförde Bay (54°31′ N, 10°02′ E, Fig. 1) in the southwestern Baltic Sea. The water depth of the sampling site is 28 m. Various physical, chemical and biological parameters are measured on a monthly basis (Lennartz et al., 2014). There is no significant river runoff to Eckernförde Bay. Hence, the hydrographical conditions are mainly dominated by saline water input from the North Sea and less saline water from the Baltic Proper, which is typical for that region. Seasonal stratification usually starts to develop in April and lasts until October, during which hypoxia or even anoxia (characterized by the presence of hydrogen sulphide, H₂S) sporadically occurs, as a result of restricted vertical water exchange and bacterial decomposition of organic matter in the bottom water (Hansen et al., 1999; Lennartz et al., 2014). Thus, BE is a natural laboratory to study the influence of O₂ variations and anthropogenic nutrient loads on N₂O production/consumption.

2.2 Sample collection and measurement

Monthly sampling of N₂O at the BE Time-Series Station started in July 2005. Triplicate samples were collected from six depths (1, 5, 10, 15, 20 and 25 m). Seawater was drawn from 5 L Niskin bottles into 20 mL brown glass vials after overflow. The vials were sealed with rubber stoppers and aluminum caps. The bubble-free samples were poisoned with 50 µL of a saturated mercury chloride (HgCl₂) solution and then stored in a cool, dark place until measurement. The general storage time before measurements of the N₂O concentrations was less than three months.

The static headspace-equilibrium method was adopted to measure the dissolved N₂O concentrations in the vials. 10 mL helium (99.9999 %, AirLiquide, Düsseldorff, Germany) headspace was created in each vial with a gas-tight glass syringe (VICI Precision Sampling, Baton Rouge, LA). Samples were vibrated with Vortex (G-560E, Scientific Industries Inc., New York, USA) for 20 seconds and then left for at least two hours until equilibrium. 9.5 mL subsample of the headspace was subsequently injected into a GC-ECD (gas chromatograph equipped with the electron capture detector) system (Hewlett-Packard 5890 Series II, Agilent Technologies, Santa Clara, CA, USA), which was calibrated with two standard gas mixtures (N₂O in synthetic air, 320 ppb and 1000 ppb, Deuste-Steininger GmbH, Mühlhausen, Germany and Westfalen AG, Münster, Germany) prior to the measurement. The average precision of the
measurements, calculated as the median standard deviation from triplicate measurements, was 0.4 nM. Triplicates with a standard deviation of >10% were omitted. More details about the N\textsubscript{2}O measurement can be found in Kock et al. (2016). Dissolved oxygen (O\textsubscript{2}) concentrations were measured by Winkler titrations (Grasshoff et al., 1999). Nutrient concentrations were measured by the Segmented Continuous Flow Analysis (SCFA, Grasshoff et al., 1999). A more detailed summary of the parameters measured and methods applied can be found in Lennartz et al. (2014).

### 2.3 Times series analysis

A time-series can be decomposed into three main components, i.e. trend, cycle and residual component (Schlittgen and Streitberg, 2001). We used the Mann–Kendall test and wavelet analysis to detect the trend and periodical cycles in the time-series data, respectively. As for the residual component, we highlight unusual high/low N\textsubscript{2}O concentrations during 2005-2017 and discuss the potential causes for these events.

#### 2.3.1 Wavelet analysis

In order to decipher periodical cycles of the parameters collected at the BE Time-Series Station, a wavelet analysis method was adopted. Wavelet analysis enables the detection of the period and the temporal occurrence of repeated cycles in time-series data. One of the requirements for wavelet analysis is a regular, continuous time-series. Since there is data missing (maximum 2 months in a row) in the BE time-series, due to terrible weather or the ship’s unavailability, missing data was interpolated from the previous and following months. Sampling time varied for every month (usually 20-40 day interval), but for the statistical analysis, data was assumed to be regularly spaced as the uncertainty introduced was not significant (<5%). Considering the band width in both frequency and time domain, a Morlet mother wavelet with a wave number of 6 was chosen (Torrence and Compo, 1998). The mother wavelet was then scaled between the frequency of a half-year cycle and the length of the time-series with a stepsize of 0.25. The wavelet analysis was conducted with the MatLab code by Torrence and Compo [2004]. More information about the method can be found on the website [http://paos.colorado.edu/research/wavelets/](http://paos.colorado.edu/research/wavelets/).

#### 2.3.2 Mann–Kendall test

Mann–Kendall test (MKT) is a non-parametric statistical test to assess the significance of monotonic trends for time-series measurements. It tests the null hypothesis that all variables are randomly distributed against the alternative hypothesis that a monotonic trend, either increase or decrease, exists in the time-series on a given significance level $\alpha$ (here $\alpha=0.05$). MKT is flexible for data with missing values and the results are not impacted by the magnitude of extreme values, which makes it a widely used test in hydrology and climatology (e.g. Xu et al., 2003; Yang et al., 2004). However, MKT is sensitive to serial correlation in the time-series. The presence of positive serial correlation would increase the probability of trend detection even though no such
trend exists (Kulkarni and von Storch, 1995). In order to avoid this situation, data from 12 months were tested individually. It is assumed that there is no residual effect left from the same month last year, considering that the nitrogen species are rapidly biologically cycled. The Matlab function from Simone (2009) was used for the MKT.

2.4 Calculation of saturation and sea–to–air flux density

$N_2O$ saturations ($S_{N2O}$, %) were calculated as:

$$S_{N2O} = 100 \times \frac{N_2O_{obs}}{N_2O_{eq}}$$  

where $N_2O_{obs}$ and $N_2O_{eq}$ (in nM) are the observed and equilibrated $N_2O$ concentrations in seawater, respectively. $N_2O_{eq}$ was computed as a function of surface seawater temperature, in situ salinity (Weiss and Price, 1980) and the dry mole fractions of atmospheric $N_2O$ at the time of the sampling. Since the atmospheric $N_2O$ mole fractions were not measured at the BE Time-Series Station, atmospheric dry mole fractions of $N_2O$ were derived from the monthly average of $N_2O$ data at Mace Head, Ireland (AGAGE, http://agage.mit.edu/), instead.

$N_2O$ flux density ($F_{N2O}$, in µmol m$^{-2}$ d$^{-1}$) was calculated as:

$$F_{N2O} = k_{N2O} \times (N_2O_{obs} - N_2O_{eq})$$

where $k_{N2O}$ (in cm h$^{-1}$) is the gas transfer velocity calculated with the method given by Nightingale et al. (2000), as a function of the wind speed and the Schmidt number ($Sc$). The wind speed data were obtained from the Kiel Lighthouse (see: www.geomar.de/service/wetter/), which is approximately 20 km away from the BE Time-Series Station. The wind speed was normalized to 10 m ($u_{10}$) to calculate $k_{N2O}$ (Hsu et al., 1994). $k_{N2O}$ was adjusted by multiplying with $(Sc/600)^{-0.5}$, and $Sc$ was computed as:

$$Sc = \frac{v}{D_{N2O}}$$

$$D_{N2O} = 3.16 \times 10^{-6} e^{-1837.0/RT}$$

where $v$ is the kinematic viscosity of seawater, which is calculated from the empirical equations given in Siedler and Peters (1986), and $D_{N2O}$ is the diffusion coefficient of $N_2O$ in seawater. $R$ is the universal gas constant and $T$ is the water temperature in K.

3. Result and discussion

3.1 Overview

$N_2O$ concentrations at the BE Time-Series Station showed significant temporal and depth-dependent variations from 2005 to 2017 (Fig. 2). $N_2O$ concentrations fluctuated between 1.2 and 37.8 nM, with an overall average of 13.9±4.2 nM. This value was higher than the results from
the surface water of Station ALOHA (5.9–7.4 nmol kg\(^{-1}\), average 6.5±0.3 nmol kg\(^{-1}\), Wilson et al., 2017), which is reasonable considering the weak anthropogenic impact in the North Pacific Subtropical Gyre. The \(\text{N}_2\text{O}\) concentrations at BE were much lower than those measured at the time-series station in the coastal upwelling area off Chile (2.9–492 nM, average 39.4±29.2 nM in the oxyclines and 37.6±23.3 nM in the bottom waters, Farias et al., 2015) and a quasi-time series station off Goa (Naqvi et al., 2010), where significant \(\text{N}_2\text{O}\) accumulations were observed in subsurface waters at both locations. Our measurements were comparable to the time-series station from Saanich Inlet (~0.5–37.4 nM, average 14.7 nM, Capelle et al., 2018), a seasonally anoxic fjord which has similar hydrographic conditions as BE.

\(\text{NO}_2^-\) concentrations fluctuated between below detection limit of 0.1 \(\mu\text{M}\) and 1.6 \(\mu\text{M}\), with an average of 0.2±0.3 \(\mu\text{M}\). \(\text{NO}_3^-\) concentrations varied from below detection limit of 0.3 \(\mu\text{M}\) to 17.9 \(\mu\text{M}\), with an average of 2.0±2.8 \(\mu\text{M}\). The temporal and spatial distributions of nitrite (\(\text{NO}_2^-\)) and nitrate (\(\text{NO}_3^-\)) were similar during 2005–2017. A clear \(\text{O}_2\) seasonality can be seen with severe \(\text{O}_2\) depletion in the bottom waters during summer and autumn. Anoxia with the presence of \(\text{H}_2\text{S}\) were detected in September/October 2005, September 2007, September/October 2014, and September–November 2016. All of the extremely low \(\text{N}_2\text{O}\) concentrations (<5 nM) were observed in the bottom waters in autumn, coinciding with hypoxia/anoxia, while the high \(\text{N}_2\text{O}\) concentrations (>20 nM) sporadically occurred at different depths either in spring or autumn.

### 3.2 Seasonal cycle

Significant cycles at different frequencies were detected via wavelet analysis at the BE Time-Series Station during 2005–2017 (Fig. 3). A half-year \(\text{NO}_2^-\) cycle sporadically occurred in 2007–2009, 2013 and 2015. There is a seasonal \(\text{NO}_2^-\) variability (at the frequency of 1 year) between 2007 and 2016 (times before 2007 and after 2016 were outside the conic line), except during 2010–2012, when high \(\text{NO}_2^-\) concentrations were not observed in winter (Fig. 2). A biennial cycle of \(\text{NO}_2^-\) could be observed as well during 2008–2015. The \(\text{NO}_3^-\) concentrations were dominated by an annual cycle and a minor half-year cycle. The biennial cycle only occurred in 2008 and 2009. A remarkable seasonal variability of dissolved \(\text{O}_2\) prevailed all the time, which is also obvious from the times series data shown in Fig. 2. The annual \(\text{N}_2\text{O}\) cycle became gradually more and more evident until 2014, then declined and reoccurred less intensely in 2016. The periodical cycle was also present at other frequencies, indicated by the broadening of the red area before 2015 in Fig. 2d. For example, a biennial \(\text{N}_2\text{O}\) cycle occurred during 2013–2015.

The half-year cycles of \(\text{NO}_2^-\) and \(\text{NO}_3^-\) were probably associated with algae blooms which usually occur in each spring and autumn (Fig. S1 and S2). Since the time between the two blooms differed between years, the cycles were weak and thus not present in every year. Due to the fact that there was no half-year \(\text{O}_2\) cycle at all, nutrients apart from \(\text{O}_2\) might be the “drivers” of the sporadic half-year \(\text{N}_2\text{O}\) cycle in 2008 and 2015, because \(\text{N}_2\text{O}\) production depends on the concentration of the bioavailable nitrogen compounds (Codispoti et al., 2001).
Generally the wavelet analysis indicated a strong annual cycle for NO$_2^-$, NO$_3^-$, dissolved O$_2$ and N$_2$O at the BE Time-Series Station, which enabled us to explore the seasonal pattern with annual mean data. Although extreme values were excluded as a result of averaging, the smoothed results generally reflect the seasonality of these parameters. Here, we focus on the annual cycle.

The annual mean vertical distribution of dissolved O$_2$, NO$_2^-$, NO$_3^-$ and N$_2$O are shown in Fig. 4. Due to the development of stratification, the mixed layer was shallow in summer and deep in late autumn/winter. O$_2$ depletion was observed in bottom waters from late spring until late autumn. The seasonal variations of NO$_2^-$ and NO$_3^-$ were significantly correlated with each other ([NO$_3^-$] = 11.59[NO$_2^-$] - 0.51, R$^2$ = 0.80, n=72, p<0.0001) and high concentrations were observed for both in winter. Minimum N$_2$O concentrations were found in the bottom waters during September and October, presumably as a result of consumption during denitrification under anoxic condition (Codispoti et al., 2005). High N$_2$O concentrations were observed in late spring and late autumn, respectively. In late spring N$_2$O accumulated in the bottom waters because the stratification prevented mixing of the water column. In late autumn, however, N$_2$O could be ventilated to the surface and thus emitted to the atmosphere due to the breakdown of the stratification. The high N$_2$O concentrations could be attributed to enhanced N$_2$O production via nitrification and/or denitrification within the oxic/anoxic interface (Goreau et al., 1980; Codispoti et al., 1992). Since there is no clear O$_2$ concentration threshold, N$_2$O production from both nitrification and the onset of denitrification overlap at oxic/anoxic interface. To this end, direct N$_2$O production measurements (i.e. nitrification/denitrification rates) are required to decipher which process dominates the formation of the different N$_2$O maxima.

High N$_2$O concentrations prevailed all over the water column in winter/early spring. NH$_4^+$ is released from the sediment into bottom waters due to the degradation of organic matter, especially after the autumn algae bloom (Fig. S1 and S2). The stratification usually completely breaks down at this time of the year and the water column becomes oxygenated. Denitrification is inhibited by the presence of high concentrations of dissolved O$_2$ (> 20 µmol L$^{-1}$, which is higher than the O$_2$ threshold of about 10 µmol L$^{-1}$, Tiedje, 1988) and thus nitrification is presumably responsible for the high N$_2$O concentrations in winter/early spring.

### 3.3 Trend analysis

The MKTs were conducted for the surface (1m) and bottom (25m) N$_2$O concentrations and saturations of the individual 12 months, respectively. Significant decreasing trends were detected for the concentrations in the bottom waters for February and August (Table 1a), and for the saturations in the surface for September and in the bottom for August and November (Table 1b). These results indicated that some systematical changes in N$_2$O took place at BE. For example, the significant decrease in N$_2$O concentration/saturation in August might be associated with the increasing temperature, which reinforces the stratification and accelerates O$_2$ consumption in the bottom waters (Lennartz et al., 2014). As a result, hypoxia/anoxia starts earlier and thus enables
the onset of denitrification to consume N$_2$O. During most of the months, trends in N$_2$O concentration and saturation were not significant during 2005–2017.

A significant nutrient decline has been observed at the BE Time-Series Station since the mid-1980s, however, Lennartz et al. (2014) found that bottom O$_2$ concentrations were still decreasing over the past 60 years. The ongoing oxygen decline was attributed to the temperature-enhanced O$_2$ consumption in the bottom water (Meier et al., 2018) and a prolongation of the stratification period at the BE Time-Series Station (Lennartz et al., 2014). Please note that the trends in nutrients and O$_2$ concentrations were detected based on the data collection which lasted for approximately 30 and 60 years, respectively, while the N$_2$O observations at BE Time-Series Station has lasted for only 12.5 years. Further MKT analysis for nutrients, temperature and oxygen for months with significant trends in N$_2$O concentrations did not show any significant results (p>0.05). The significant trends in N$_2$O concentrations thus do not seem to be directly related to one of these parameters, and we cannot state a reason for the significant trends of N$_2$O concentration in February and the N$_2$O saturation in September and November at this point. Presumably, a longer monitoring period for N$_2$O is required to detect corresponding trends in N$_2$O and oxygen or nutrients.

3.4 Extreme events

3.4.1 Low N$_2$O concentrations during October 2016-April 2017

Besides the low N$_2$O concentrations occurring in autumn, we observed a band of pronounced low N$_2$O concentrations which started in October 2016 and lasted until April 2017 (Fig. 5). In this period N$_2$O concentrations varied between 5.5–13.9 nM, with an average of 8.4±2.0 nM. This is approximately 40% lower than the average N$_2$O concentration during the entire measurement period 2005–2017. The average N$_2$O saturation during 2005–2017 was 111±30%, while from October 2016 to April 2017, the N$_2$O saturations were as low as 43–93% (average 62±10%).

Undersaturated N$_2$O waters have been previously reported from the Baltic Sea: Rönner (1983) observed a N$_2$O surface saturation of 79% in the central Baltic Sea and attributed the undersaturation to upwelling of N$_2$O-depleted waters. Bange et al. (1998) found a minimum N$_2$O saturation of 91% in the southern Baltic Sea where the hydrographic conditions were significantly influenced by riverine runoff. Walter et al. (2006) reported a mean N$_2$O saturation of 79±11% for shallow stations (<30 m) in the southwestern Baltic Sea in October 2003. The low-N$_2$O event at BE was unusual because the concentrations were much lower than those reported values and it lasted for more than half a year.

Although the observed temperatures and salinities during October 2016–April 2017 were comparable to other years (Fig. S1), it is difficult to evaluate the role of physical mechanism in the low-N$_2$O event because of insufficient data for water mass exchange at the BE Time-Series
Station. Here we mainly focused on the chemical or biological processes. Anoxia events with the presence of H2S were observed in the bottom waters for three months in a row during September–November 2016. This is an unusual long period and is unprecedented at the BE Time-Series Station. In December 2016 the stratification did not completely break down. Although the water column was generally oxygenated, bottom O2 concentrations were the lowest observed during the past ten years. Considering the classical view of N2O consumption via denitrification under hypoxic and anoxic conditions, we inferred that denitrification accounted for low N2O concentrations in the bottom layer. However, the question still remains where the low-N2O-concentration water in the upper layers came from.

In September 2016, low N2O concentrations were only observed in the bottom waters where the anoxia occurred. However, the situation was different in the following months. During October/November 2016, N2O concentrations were homogeneously distributed in the water column. Although the stratification gradually started to break down in late autumn, the density gradient was still strong enough to keep the bottom waters at anoxic conditions and prevented the low-N2O-concentration to reach the surface. Thus we inferred that the unusual low N2O concentrations in the upper layers (above 20 m) were probably resulting from advection of adjacent waters. Due to the fact that the upper layers were well-mixed and oxygenated, in situ N2O consumption in the water column could be neglected. We suggest therefore, that the N2O depleted waters were resulting from consumption of N2O in bottom waters elsewhere and then they were upwelled and transported to BE. Hence, N2O consumption via denitrification might have been, directly or indirectly, responsible for the low N2O concentrations during October–November 2016.

In December 2016, the bottom waters were ventilated with O2. Although N2O consumption by denitrification should have been inhibited by the high concentrations of O2 (Codispoti et al., 2001), the N2O concentrations did not restore to their normal level under suboxic conditions. Since January 2017, the whole water column was well mixed and oxygenated. Usually a significant nutrient supply could be observed starting in November (Fig. 4) as a result of remineralization and vertical mixing, but the average NO2− and NO3− concentrations during November 2016–April 2017 were 0.2 and 1.4 μM, respectively, which was about 50% and 60% lower than in other years. Ammonium (NH4+) and chlorophyll a concentrations during this period were comparable to that of other years (Fig. S1). Secchi depth, a proxy of water transparency, was 3.8 m in March 2017, which is only slightly lower compared to the monthly average value for March (4.5±1.8 m). There is no exceptional spring algae bloom and thus we infer that assimilative uptake of nutrients by phytoplankton was not responsible for the low nutrients concentrations. The nutrient deficiency might be attributed to enhanced nitrogen removal processes like denitrification or anammox (Voss et al., 2005; Hietanen et al., 2007; Hannig et al., 2007) during the prolonged period of anoxia in autumn 2016. During the low N2O event, we found that N2O concentrations were positively correlated with both NO2− ([N2O]=7.02[NO2−]+7.36, R2=0.29, n=24, p<0.01) and NO3− ([N2O]=0.80[NO3−]+7.36, R2=0.51,
n=24, p<0.0001). These results indicate that the development and maintenance of the low-N$_2$O-concentration was closely associated with nutrient deficiency. Especially after the breakdown of the stratification, when denitrification was no longer a significant N$_2$O sink, nutrients might have become a limiting factor for N$_2$O production.

In general, the low-N$_2$O-concentration event during October 2016–April 2017 can be divided into two parts: in the stratified waters during October–November 2016, O$_2$ played a dominant role and N$_2$O was consumed via denitrification under anoxic conditions. In the well-mixed water column during December 2016–April 2017, nutrient deficiency seemed to have constrained N$_2$O production via nitrification under suboxic/oxic conditions.

In recent years a novel biological N$_2$O consumption pathway, called N$_2$O fixation, which transforms N$_2$O into particulate organic nitrogen via its assimilation, has been reported (Farias et al., 2013). This process can take place under extreme environmental conditions even at very low N$_2$O concentrations. Cornejo et al. (2015) reported that N$_2$O fixation might play a major role in the coastal zone off central Chile where seasonally occurring surface N$_2$O undersaturation was observed. The relatively high N$_2$ fixation rates in the Baltic Sea (Sohm et al., 2011) highlight the potential role of N$_2$O fixation (Farias et al., 2013). However, we cannot quantify the role of biological N$_2$O fixation for the N$_2$O depletion in the Baltic Sea due to the absence of N$_2$O assimilation measurements.

### 3.4.2 High N$_2$O concentrations in November 2017

High N$_2$O concentrations were observed at the BE Time-Series Station in November 2017. The average value reached 35.4±1.5 nM, which was the highest concentration measured during the entire sampling period from 2005 to 2017. Dissolved N$_2$O was homogeneously distributed in the water column, but this event did not last long. In December, dissolved N$_2$O returned to normal levels and the average concentration in the water column was comparable to that of other years. Average N$_2$O saturation in November 2017 was 322±10%, which was also the highest for the past 12.5 years. This value was much higher than the maximum surface N$_2$O saturation reported by Rönner (1983) in the central Baltic Sea, but was comparable to the results observed in the southern Baltic Sea (312%, Bange et al., 1998). Bange et al. (1998) linked the enhanced N$_2$O concentrations to riverine runoff because those samples were collected in an estuarine area, however, the riverine influence around the BE Time-Series Station is negligible. As a result, the impact of fresh water input can be excluded.

Dissolved O$_2$ seemed to play a dominant role in the high N$_2$O concentrations. Enhanced N$_2$O production usually occurred at the oxic/anoxic interface, which was closely linked to the development of water column stratification. In general the breakdown of the stratification is faster than its establishment at the BE Time-Series Station. As a result, it took about half a year for bottom O$_2$ saturation to gradually decrease from ~80% to almost 0% (i.e. anoxia), but only two months to restore normal saturation level in 2010 (Fig. 6). In late autumn, surface water
penetrated into the deep layers via vertical mixing and eroded the oxic/anoxic interface. The entire water column quickly became oxygenated and the enhanced N$_2$O production was stopped.

Hypoxia/anoxia at BE is usually observed in the bottom waters in autumn, but in September 2017, hypoxic water (O$_2$ saturation<20 %, which was close to the criterion for hypoxia, see Naqvi et al., 2010) was found in the subsurface layer (10 m) as well. Surface O$_2$ saturation was only ~50%, which was the lowest during the sampling period 2005–2017. The density gradient of the water column in September 2017 was much lower than in other years. These results indicate the occurrence of an upwelling event at BE Time-Series Station in autumn 2017, which might be a result of the saline water inflow from the North Sea considering the change of salinity in the water column (Fig. S1). Strong vertical mixing has interrupted the hypoxia/anoxia and bottom O$_2$ saturation reached ~60% in October 2017. The presence of O$_2$ prevented N$_2$O consumption via denitrification, as a result, we did not observe a significant N$_2$O decline during that period (Fig. 5).

Considering the fact that a significant autumn algae bloom was observed in autumn 2017 (as indicated by high chlorophyll a concentrations, see Fig. S1), severe O$_2$ depletion in the bottom water could be expected. Although the bottom O$_2$ saturation was only slightly lower in November than in October, we speculate that even lower O$_2$ saturation (but not anoxia) might have occurred between October and November. The “W-shaped” O$_2$ saturation curve (see Fig. 6) suggests that the stratification did not completely break down in October and that there might have been a reestablishment of the oxic/anoxic interface providing favorable conditions for enhanced N$_2$O production. Due to the degradation of organic nitrogen, NH$_4^+$ is released from the sediment into bottom waters (Dale et al., 2011), especially in autumn when O$_2$ is low (Fig. S2). NH$_4^+$ concentrations in November 2017 were lower than in other years (Fig. S1), and NO$_2^-$ concentrations were higher (Fig. 5), indicating that nitrification occurred in bottom waters. To this end, we suggest that the reestablishment of the oxic/anoxic interface promoted ammonium oxidation (the first step of nitrification). In this case, N$_2$O could have temporary accumulated because its consumption via denitrification was blocked. Meanwhile, the relatively low density gradient (i.e. low stratification) allowed upward mixing of the excess N$_2$O to the surface. However, we inferred that that this phenomenon would only last for a few days due to the rapid breakdown of stratification at the BE Time-Series Station.

Due to the development of the pronounced stratification, the oxic/anoxic interface prevailed in summer/early autumn as well, but we did not observe N$_2$O accumulation during these months. One of the potential explanations is that enhanced N$_2$O production only took place within particular depths where strong O$_2$ gradient existed, but our vertical sampling resolution was too low to capture this event. Also enhanced N$_2$O production might be covered by the weak mixing which brought low-N$_2$O water from the bottom to the surface.

The upwelling event played different roles in autumn 2016 and 2017. First, upwelling took place somewhere else but at BE because of the strong density and O$_2$ gradient in the water column
during autumn 2016. Second, bottom water remained anoxic in autumn 2016, while the
compensated water for upwelling in 2017 penetrated through stratification and brought O₂ into
bottom water (Fig. 6), which caused enhanced N₂O production. Similarly, autumn upwelling was
detected in 2011 and 2012 when we found relatively low O₂ concentrations in subsurface layers
(10 m) (Fig. 2), but we did not observe an increase in bottom O₂ concentrations and N₂O
concentrations remained low during that time. These upwelling events seem to be driven by
saline water inflow considering the prominent increase in salinity, but the mechanism dominates
O₂ input into bottom water before the stratification break down remains unclear.

3.5 Flux density

During 2005–2017, surface N₂O saturations at the BE Time-Series Station varied from 56 % to
314 % (69–194 % excluding the extreme values discussed in Sect. 3.4), with an average of
111±30 % (111±20 % without the extreme values). Generally the water column at BE was
slightly oversaturated with N₂O. Our results are in good agreement with the estimated mean
surface N₂O saturation for the European shelf (113%, Bange, 2006).

We found a weak seasonal cycle for surface N₂O concentrations, with high N₂O concentrations
occurring in winter/early spring and low concentrations occurring in summer/autumn, but no
such cycle for N₂O saturation (Fig. 4; Fig. 7). The seasonality in concentration but not in
saturation could be largely attributed to the effect of temperature on N₂O solubility: In summer
when surface N₂O concentrations are low, N₂O saturations are increased by the relative high
temperature; and vice versa in winter. Although salinity also affects N₂O solubility, its
contribution is negligible compared to temperature. Temperature alleviated the fluctuation of
surface N₂O saturation and thus affected the sea-to-air N₂O fluxes. We conclude that temperature
plays a modulating role for N₂O emissions.

The wind speed (u₁₀) at the BE Time-Series Station ranged from 1.1 to 14.0 m s⁻¹, with an
average of 7.0±2.7 m s⁻¹. N₂O flux densities varied from -19.0 to 105.7 μmol m⁻² d⁻¹ (-14.1–30.3
μmol m⁻² d⁻¹ without the extreme values), with an average of 3.5±12.4 μmol m⁻² d⁻¹ (3.3±6.5
μmol m⁻² d⁻¹ without the extreme values). However, the true emissions might have been
underestimated because our monthly sampling resolution is insufficient to capture short-term
N₂O accumulation events due to the fast breakdown of stratification in autumn. The uncertainty
introduced in the flux density computation was estimated to be 20% (Wanninkhof, 2014). The
flux densities at the BE Time-Series Station are comparable to those reported by Bange et al.
(1998, 0.4–7.1 μmol m⁻² d⁻¹) from the coastal waters of the southern Baltic Sea, but are slightly
lower than the average N₂O flux density reported by Rönner (1983, 8.9 μmol m⁻² d⁻¹) from the
central Baltic Sea. Please note that the results of Rönner (1983) were obtained only from the
summer season and therefore are probably biased because of missing seasonality.

In December 2014, a strong saline water inflow from the North Sea was observed, which was the
third strongest ever recorded (Mohrholz et al., 2015). Although the salinity in December 2014
was comparable to other years, a remarkable increase in salinity was observed in the following
several months. However, we did not detect a significant N$_2$O anomaly or enhanced emission
during that time. Similarly, Walter et al. (2006) investigated the impact of the North Sea water
inflow on N$_2$O production in the southern and central Baltic Sea in 2003. The oxygenated water
vented the deep Baltic Sea and shifted anoxic to oxic condition which led to enhanced N$_2$O
production, but the accumulated N$_2$O was unlikely to reach the surface due to the presence of a
permanent halocline (Walter et al., 2006).

Although we observed extremely high N$_2$O flux density in November 2017, the low-N$_2$O-
concentration (<10 nM) events have become more and more frequent during the past ten years
(Fig. 2). This phenomenon seldom occurred before 2011, but remarkable low N$_2$O
concentrations can be seen in 2011 and 2013, and to a less extent in 2012 and 2014. Similar
events lasted for several months in 2015 and for even more than half a year during 2016–2017.
The most striking was that the low-N$_2$O-concentration water was not only detected in bottom
waters, but also at surface which would significantly impact the air-sea N$_2$O flux densities.
Although the MKT result did not give a significant trend for the N$_2$O flux densities, the data
presented in Fig. 8 suggest a potential decline of N$_2$O flux densities from the coastal Baltic Sea,
challenging the conventional view that N$_2$O emissions from coastal waters would most probably
increase in the future, which was based on the hypothesis of increasing nutrient loads into coastal
waters. Due to an effective reduction of nutrient inputs, the severe eutrophication condition in the
Baltic Sea has been alleviated (HELCOM, 2018b), but ongoing deoxygenation points to the fact
that it will take a longer time for coastal ecosystems to feedback to reduced nutrient inputs
because other environmental changes such as warming may override decreasing eutrophication
(Lennartz et al., 2014).

4. Conclusions

The seasonal and inter-annual N$_2$O variations at the BE Time-Series Station from July 2005 to
December 2017 were driven by the prevailing O$_2$ regime and nutrients availability. We found a
pronounced seasonal cycle with low N$_2$O concentrations (undersaturations) occurring in
hypoxic/anoxic bottom waters in autumn and enhanced concentrations (supersaturations) all over
the water column in winter/early spring. Significant decreasing trends for N$_2$O concentrations
were found for few months, while most of the year, no significant trend was detectable in the
period of 2005–2017. During 2005–2017, no significant trends were present for O$_2$ and nutrients
either, but these parameters all show significant decreasing trends on longer time scales (~60
years) at BE. Our results show the strong coupling of N$_2$O with O$_2$ and nutrient concentrations,
and suggest similar changes on comparable time scales. Further monitoring of N$_2$O at BE time
series station is thus important to detect changes. Further studies on N$_2$O
production/consumption by nitrification and denitrification and analysis of the characteristic N$_2$O
isotope signature might be very helpful to decipher the potential roles of O$_2$/nutrients for N$_2$O
cycling.
Temperature plays a modulating role for the N$_2$O emission at the BE Time-Series Station. Although the hydrographic condition at BE is generally dominated by the inflow of saline North Sea water, this did not affect N$_2$O production and its emissions to the atmosphere. It seems that events with extremely low N$_2$O concentrations and thus reduced N$_2$O emissions became more frequent in recent years. Our results provide a new perspective onto potential future patterns of N$_2$O distribution and emissions in coastal areas. Continuous measurement at the BE Time-Series Station with a focus on late autumn would be of great importance for monitoring and understanding the future changes of N$_2$O concentrations and emissions in the southwestern Baltic Sea.

Data availability

Data are available from the Boknis Eck Database: www.bokniseck.de

Author contribution

X.M., S.T.L. and H.W.B. designed the study and participated in the fieldwork. N$_2$O measurements and data processing were done by X.M. and S.T.L. X.M. wrote the manuscript with contributions from S.T.L. and H.W.B.

Competing interests

The authors declare that they have no conflict of interest.

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References


Table 1. The results of the Mann-Kendall test for the surface and bottom N\textsubscript{2}O concentrations and saturations of the 12 individual months.

**Table 1a. MKT results for N\textsubscript{2}O concentrations**

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<th>April</th>
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<th>August</th>
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<td>1</td>
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<td>p</td>
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**Table 1b. MKT results for N\textsubscript{2}O saturations**

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<td>1</td>
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<td>0.37</td>
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<tbody>
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<td>Depth/m</td>
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<td>p</td>
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\(p\) indicates the \(p\)-value of the test, which is the probability, under the null hypothesis, of obtaining a value of the test statistic as extreme or more extreme than the value computed from the sample. (~) indicates a rejection of the null hypothesis at \(\alpha\) significance level and a decreasing trend is detected.
Fig. 1 Location of the Boknis Eck Time-Series Station in the Eckernförde Bay, southwestern Baltic Sea. (Map from Hansen et al., 1999)
Fig. 2 Vertical distributions of dissolved \( \text{O}_2 \), \( \text{NO}_2^- \), \( \text{NO}_3^- \), and \( \text{N}_2\text{O} \) from the BE Time-Series Station during 2005–2017.
Fig. 3 Wavelet power spectra of NO$_2^-$ (a), NO$_3^-$ (b), dissolved O$_2$ (c) and N$_2$O (d) from the BE Time-Series Station. Red areas indicate high, blue indicate low power. The black conic line indicates the significant area where boundary effects can be excluded.
Fig. 4 Average vertical distributions of dissolved O$_2$, NO$_2^-$, NO$_3^-$, and N$_2$O from the BE Time-Series Station during 2005–2017. The black line indicates the mixed layer depth, which was calculated based on a potential density anomaly of 0.15 kg m$^{-3}$ from the sea surface (1m).
Fig. 5 Vertical distribution of dissolved O$_2$, NO$_2^-$, NO$_3^-$, and N$_2$O from the BE Time-Series Station during July 2016–December 2017. Please note that the high N$_2$O concentrations in November 2017 were removed for better visualization.
Fig. 6 Variations of bottom O₂ saturation in 2010 (blue) and 2017 (red).
Fig. 7 Wavelet analysis and the variation of surface N₂O concentrations (a, b) and surface N₂O saturations (c, d). The dashed red line in (d) indicates the saturation of 100%.
Fig. 8 Variation of N$_2$O flux density at the BE Time Series-Station during 2005–2017. Negative values indicated N$_2$O influx from the atmosphere and positive values indicated N$_2$O efflux to the atmosphere.