Response letter

We thank the editor and all reviewers for their constructive and helpful comments and suggestions. In the following sections, reviewer comments are written in bold italics, our answers are kept in plain font. Note that we also slightly corrected the revised manuscript for stylistic issues and minor mistakes. These changes do not affect the conclusion of the manuscript and are shown in the marked-up manuscript version.

1. Review comment (RC1) – 26.05.2023

I want to thank the authors for addressing my last round of comments. For example, the N_2O flux and emission have been comprehensively estimated using different parameterizations and wind speeds. However, some of authors' arguments need to be supported by evidence or the authors need to discuss the caveats in the conclusion instead of making assumptions, e.g., the effect of temperature on N_2O production, and N_2O discharge from wastewater treatment plants. Here are my additional comments (line number in the modified manuscript).

We thank the reviewer for their two rounds of in-depth, very helpful and constructive comments and suggestions that really improved our manuscript. In the following, we addressed each issue separately.

23: It is not clear how N_2O production was enhanced by warmer temperature based on the data presented in this study. Is it due to positive correlation between temperature and N_2O saturation shown in Table 4? But not all the datasets had such pattern.

We removed this statement from the abstract and conclusion.

Lines	**Change**
L23	Removed "N_2O production was enhanced by warmer temperatures…"
L476-477	Removed "Biological N_2O production was enhanced by warmer temperatures and…"

25-26: "N_2O saturation did not decrease alongside the decrease in DIN concentration…"

Lines	**Change**
L25-26	Changed "…N_2O saturation did not decrease alongside with DIN concentrations …" to "…N_2O saturation did not decrease alongside the decrease in DIN concentrations…"

26-27: What does it mean for phytoplankton to reestablish? Were phytoplankton limited before 1990 when the water quality was bad?

Yes, that was indeed the case. Before the German reunification in 1990, organic nitrogen mainly emerged from industries and wastewater inputs. High organic matter concentrations, high pollutants levels and low light availability inhibited the developments of algae blooms in the 1980s as shown by the lack of seasonal variability in TON concentration (see Fig. 1 below). Management measures introduced in the 1980s and 1990s have led to an improved water quality, which in turn caused a significant increase of seasonal phytoplankton dynamics (Kerner, 2000; Amann et al., 2012; Hillebrand et al., 2018).

We further elaborate the cause of limited phytoplankton growth in Section 4.5 of our revised manuscript (also see our reply to a comment below).

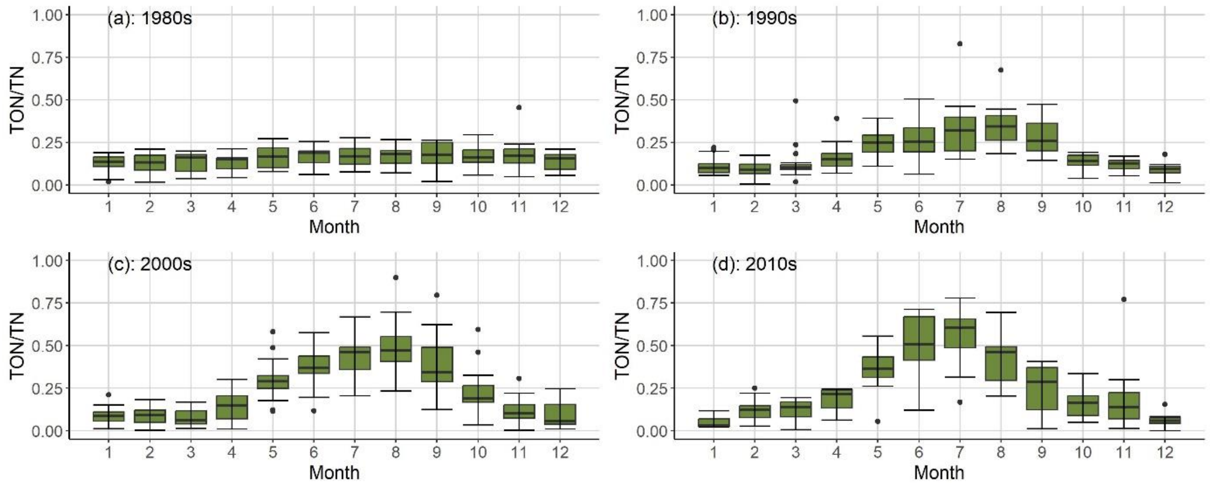

Figure 1: Seasonal variation of TON fraction in TN concentrations in (a) 1980s, (b) 1990s, (c) 2000s and (d) 2010s (Das Fachinfomrationssystem (FIS) der FGG Elbe, 2022; Schulz et al., submitted).

Lines	**Change**
L458-463	Rewrote: “The significant regime change after the 1990s enabled phytoplankton growth to reestablish in the river that had previously been inhibited by high pollutant levels and low light availability (Kerner, 2000; Amann et al., 2012; Hillebrand et al., 2018; Rewrie et al., submitted). The prevailing high nitrification rates in the estuary (Dähnke et al., 2008; Sanders et al., 2018) support an overarching control of organic matter on N_2O production and emissions along the Elbe Estuary.”

61-62: The emission factor (N_2O:DIN) derived from the Elbe River could be compared to different types of riverine systems, not only the rivers with high agricultural nutrient inputs. Such comparison would broaden the results.

Thanks to the reviewer for this comment, in the revised manuscript we also addressed other riverine systems.

Lines	**Change**
L61-62	Changed from "…used the N_2O:DIN ratio for a comparison with other estuaries that receive similar high agricultural nutrient inputs" to "…used the N_2O:DIN ratio for a comparison with other estuaries"
L445-447	Added: "In general, N_2O:DIN ratios vary widely (e.g., Baulch et al., 2012; Maavara et al., 2019; Smith and Böhlke, 2019). Wells et al. (2018) even found a range from -25 % to 7 % of DIN was emitted as N_2O in estuaries with low land-use intensity."

Figure 1: What does the blue color mean in the figure? How is the section separated? Are they the same as shown in Figure 3 for AOU and excess N_2O or for emission calculation?

The light blue colour indicates Wadden Sea areas that are exposed at low tide. We incorporated this into the Figure caption, and corrected the reference. Furthermore, we realised that the black line indicating the Hamburg Port region did not exactly match the separation made in Fig. 3. Thus, we changed the figure so that both regions match.

Lines	**Change**
L87-88	Changed Fig. 1
L89-92	Adapted figure caption: "Map of the Elbe Estuary sampled during our research cruises with stream kilometers (graphic courtesy of FGG Elbe, modified after Amann et al. 2012)). The light blue color indicates Wadden Sea areas that are exposed at low tide."

125: "N_2O saturation was calculated…"

Lines	**Change**
L129	Changed "N_2O saturation were calculated…" to "N_2O saturation was calculated"

155: N_2O_{cw} has already been defined.

Lines	**Change**
L159	Removed "(N_2O_w)" as definition for N_2O concentration in water
L161 – Eq. 4	Changed "N_2O_w" to "N_2O_{cw}" in Equation 4

158: "an indicator for N_2O production from nitrification"

Lines	Change
L162-163	Changed "… an indicator for nitrification (Nevison et al., 2003; Walter et al., 2004)" "… an indicator for N_2O production from nitrification (Nevison et al., 2003; Walter et al., 2004)"

206-209: It may be useful to have a figure to show the spatial distribution of average N_2O flux density as shown in Figure 2 for N_2O saturation and nutrients.

We added the suggested figure to the supplementary material and refer to it in the main text, see Figure 2 below.

Lines	Change
L209-210	Add reference to supplementary material: "…, but also include results using other parametrizations in Table S2 and Fig. S2."
Fig. S2	Added Figure of nitrous oxide flux densities along the Elbe estuary
Fig S4-S14	Changed figure captions and references in the main manuscript

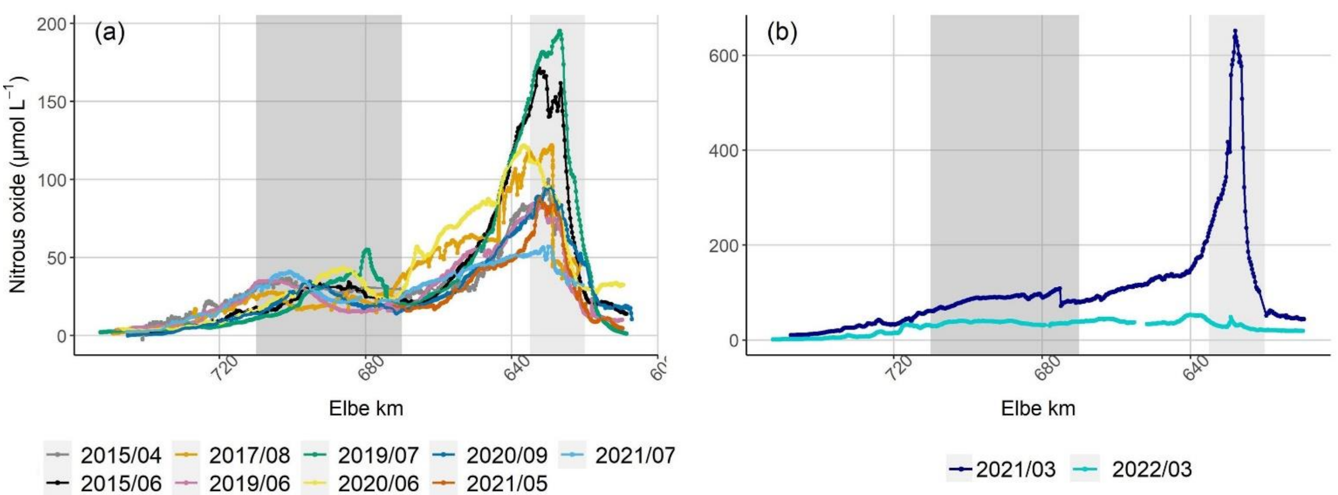

Figure 2: Nitrous oxide flux density along the Elbe estuary calculated after Borges et al. (2004) and in-situ wind speeds (a) in spring and summer, (b) in winter. Light grey shading denotes the Hamburg Port region, dark grey shading the typical position of the maximum turbidity zone (MTZ, Bergemann, 2004). Note the difference in Y-axis scales for the plots.

Section 3.5: It's clear to have a table to show the correlations between N_2O and environmental factors. However, no results were described in this section. How about listing a few key results here such as the positive correlation with nitrite and negative relation to oxygen?

In line with the reviewer, we added a short description of key results to Section 3.5

Lines	**Change**
L250-253	Added: "N_2O saturation showed significant negative correlation with oxygen (Table 4) as well as a consistent negative correlation with pH (Table 4 and 5). Furthermore, nitrite concentrations positively correlated with N_2O saturation in the freshwater section of the estuary (Table 4 and 5)."

278-279: If the N loading decreased, but N_2O concentration or flux kept constant, it may suggest the yield of N_2O production increased or the emission factor increased.

Thanks to the reviewer for this comment. We change the respective sentence in Section 4.1.

Lines	**Change**
L286-288	Changed from "Since N_2O saturation did not decrease in scale with riverine nitrogen input, this suggests that in-situ N_2O production along the estuary is important." to: "As N_2O saturation did not decrease in scale with riverine nitrogen input, this suggests that the yield of N_2O production increased along the estuary."

Figure 4: Colormap of the relative change of SPM concentration is not shown for (a) and (b).

We added a color bar to the plot.

Lines	**Change**
L307-308	Added color bar to Fig. 3

304: (d) and (f)

Lines	**Change**
L314	Changed to "(d) and (f)"

305: What does "This" represent?

Lines	**Change**
L315	Changed "This…" to "This succession of N-bearing substances (Fig. 4, Fig. S4-14) suggests…"

319-320: Is there any evidence to support this argument (e.g., correlation between PN and N_2O)?

In our dataset, we see no clear correlation between PN and nitrous oxide. However, there are several reasons that lead to a distortion of the correlation: (1) High suspended particulate concentrations in the MTZ lead to high PN concentrations - but if this organic matter is highly degraded, it will not add to N_2O generation, leading to distorted correlations. (2) The succession of nitrogen bearing substances during turnover processes like nitrification leads to a spatial offset between fresh organic matter input, ammonium, nitrite and N_2O peaks. In the mesohaline estuary, we identified nitrification as main production pathway for N_2O (section 4.2), which is fueled by fresh organic material from the North Sea providing ammonium as substrate via remineralization. Thus, the amount and quality of organic matter entering the Elbe Estuary has to affect N_2O by controlling nitrification rates. This is in line with findings by Dähnke et al. (2022), who identified the reactivity of particulate matter as the key control of the occurring nitrogen turnover processes along the freshwater Elbe estuary.

334-338: Does the 5% means the fraction of DIN loading from WWTP to the total N load into the Elbe River? Are there any measurements of N_2O associated with WWTP discharge or in the WWTP? Previous studies have shown that discharge of WWTPs may be important source of N_2O to the aquatic system (e.g., Beaulieu et al., 2010; Chun et al., 2020). The authors may need to acknowledge the possibility of N_2O discharge from WWTP instead of rejecting such possibility.

We calculated the wastewater discharge fraction of stream flow according to Büttner et al. (2020), which is based on water masses. For the Elbe Estuary, point sources and WWTP are considered to only play a minor role in nitrogen loadings (Hofmann et al., 2005; IKSE, 2018). During various cruises, we took detours to the outlets of the WWTP in the Southern Elbe, which joins our sampling stretch at stream kilometer 626 and we never observed a significant change in N_2O concentrations. In addition, stable isotope signatures of nitrate did not show signs of a change in source contribution or an increasing importance of wastewater in the Hamburg port region. Thus, we believe the WWTP not responsible for the elevated N_2O concentrations under normal conditions. However, water temperature, riverine nitrogen load and freshwater discharge likely affect the importance wastewater input on N_2O concentrations and emissions. Thus, we acknowledged the possibility of N_2O discharge from the WWTP in the revised manuscript by also addressing previous studies as suggested by the reviewer.

Lines	**Change**
L348-350	Added "However, discharge of WWTPs can potentially be an important sources of N_2O (Beaulieu et al., 2010; Chun et al., 2020; Brown et al., 2022), and the effect of wastewater input on N_2O concentrations and emissions may change with altered river discharge, water temperature and riverine nitrogen loads in the future."

357-358: Why is nitrite favorable for N_2O production by nitrification? Low oxygen does not facilitate nitrification but facilitates N_2O production from nitrification.

We noticed that the statement about nitrite concentrations favouring N_2O production by nitrification and nitrifier-denitrification is misleading and repetitive. N_2O production by nitrifier-denitrification is enhanced by higher nitrite concentrations, which is a sub pathway during nitrification. We revised the sentence by naming only nitrifier-denitrification.

Lines	**Change**
L369	We remove nitrification from this sentence: "High nitrite concentrations are favorable for N_2O production by nitrifier-denitrification (Quick et al., 2019),…"
L370	Changed from "…while low-oxygen conditions facilitate both nitrification and denitrification." to "…while low-oxygen conditions facilitate N_2O production from both nitrification and denitrification."

361-364: Not sure how sedimentary denitrification is proved to be an important source of N_2O. For instance, do you have depth profiles of N_2O to show high N_2O concentration near the bottom water?

We have no depth profiles of N_2O concentrations in the Port of Hamburg, but we performed sediment incubation experiments that showed significant N_2O fluxes from the sediments into the water column. However, these preliminary results are not ready for publication yet.

Brase et al. (2017) elaborated the production process leading to elevated N_2O concentrations in the Hamburg port in detail, which is why we decided against a repetitive elaboration as we made the same observations: Available nitrate in the Port of Hamburg possibly trigger N_2O production via denitrification. Further, at minimum oxygen concentrations, the linear relation between AOU and N_2O_{xs} breaks indicating other processes affecting either oxygen consumption and/or N_2O production. We only measure surface oxygen concentrations and thus, we speculate that lower oxygen levels in deeper water layers and sediments may enable denitrification. Previous studies showed ongoing denitrification and nitrifier-denitrification in the sediments of this region (Deek et al., 2013) and Dähnke et al. (2022) even found signs for possible water column denitrification in the low oxygen zones. Thus, it only seems plausible that denitrification contributes to elevated N_2O concentrations in the Port of Hamburg. However, we currently cannot assess the contribution of sediments and the water column to overall nitrous oxide production and their respective controls.

Lines	**Change**
L374-379	Added: "Overall, our data showed the succession of ammonium, nitrite and N_2O production (Fig. 4b and supplementary material S3-S13) as well as a breakup of the linear relation between AOU and N_2O_{xs} in the Port region (Fig. 3). In combination with previous nitrogen process studies performed in the Elbe Estuary (Deek et al., 2013; Sanders et al., 2018; Dähnke et al., 2022), this supports simultaneous sedimentary denitrification and nitrification in the water column as responsible pathways for N_2O production in the Port of Hamburg (Brase et al. 2017)."

407: This is a hypothesis but not an assumption: elevated ammonium concentration led to higher N_2O production.

Lines	Change
L422	Changed to "We hypothesize that elevated ammonium WWTP loads were rapidly converted to N_2O…"

408-411: Why is the case (your hypothesis) if temperature is an important driver of N_2O production (warmer water likely facilitates N_2O production)?

High (cold) rainwater inflows led to a temperature drop in the WWTP that led to the aggravated operation conditions. In summer, a comparable rain event would not result in a temperature drop in the WWTP and thus, no or at least not the same aggravated operation conditions as in winter would occur. Lower ammonium concentration would exit the treatment plant, which would not trigger intense N_2O production in the estuary. We clarified that the inflow of cold rainwater was the cause for aggravated operation conditions in the revised manuscript.

However, our results indicate that weather events can have drastic effects of on WWTPs and their respective rivers/estuaries. The IPCC (2022) expects more frequent and extensive weather extremes, including both droughts and heavy rainfall. Furthermore, we assume water temperature, riverine nitrogen load and freshwater discharge may influence the importance of input from the wastewater treatment plant on N_2O concentrations and emissions. Therefore, a possible assessment of inputs from WWTPs in light of climate change with the overarching aim of evaluating management measures is an interesting topic for future research.

Lines	Change
L419-421	Added: "This rare event caused a temperature drop in the WWTP due to high inflows of cold rainwater leading to aggravated operation conditions at the time of sampling."
L424-427	Changed from "An important factor for aggravated conditions was a temperature drop in the WWTP caused by cold rain water, we hypothesize that a similar rain event in warmer months would lead to comparable N_2O peaks" to "An important factor for aggravated conditions was a temperature drop in the WWTP caused by cold rain water (HAMBURG WASSER, pers. Comm., Laurich 2022), we therefore hypothesize that a similar rain event in warmer months would not have the same effect."

434: Any ideas about the drivers? temperature and oxygen?

We consider several drivers responsible for seasonal varying N_2O:DIN relation. (1) Temperature: Sanders et al. (2018) measured higher nitrification rates with warmer water temperatures along the Elbe Estuary. Further, warmer temperatures tend to increase microbial processes (Murray et al., 2015; Quick et al., 2019) that could either lead to reduced or enhanced N_2O production depending on the prevailing biogeochemical conditions. (2) Oxygen: Several researchers found oxygen availability a key driver for N_2O production in estuaries (e.g., de Bie et al., 2002; Rosamond et al., 2012; Murray et al., 2015; Yevenes et al., 2017), which also reflects in our data with significant negative correlations between oxygen and N_2O saturation (Table 4 and Table 5). (3) Phytoplankton blooms in the upstream river and North Sea proving substrate for N_2O production in the estuary as well as leading to enhance oxygen depletion in the Hamburg Port further fuelling N_2O production. We will briefly address these possible drivers for the seasonal variation in the revised manuscript.

Lines	**Change**
L452-455	Added "…showing that this relationship even varies seasonally on site due to changing drivers for N_2O production and emissions, e.g., temperature (Murray et al., 2015; Quick et al., 2019) and oxygen levels (de Bie et al., 2002; Rosamond et al., 2012; Yevenes et al., 2017)." The effect of organic matter availability is discussed in the following paragraph (L456-463).

456-454: I am trying to understand the decoupling of historical trend of N_2O and DIN. Is there any historical change in the concentration of organic matter in the river?

Before the German reunification in 1990, organic nitrogen mainly emerged from industries and wastewater inputs. High organic matter concentrations, high pollutants levels and low light availability inhibited the developments of algae blooms in the 1980s as shown by the lack of seasonal variability in TON concentration (see Figure 1 to comment above). Management measures introduced in the 1980s and 1990s have led to an improved water quality, which in turn caused a significant increase of phytoplankton dynamics (Kerner, 2000; Amann et al., 2012; Hillebrand et al., 2018; Rewrie et al., submitted). Furthermore, the improved oxygen conditions led to a shift from dominating denitrification to nitrification (Dähnke et al., 2008). The re-establishment of primary production provides substrate for coupled remineralization and nitrification in the estuary (Sanders et al., 2018; Dähnke et al., 2022) enhancing N_2O production. We elaborated this connection in more detail in the revised manuscript.

Lines	**Change**
L458-463	Rewrote: "The significant regime change after the 1990s enabled phytoplankton growth to reestablish in the river that had previously been inhibited by high pollutant levels and low light availability (Kerner, 2000; Amann et al., 2012; Hillebrand et al., 2018; Rewrie et al., submitted). The prevailing high nitrification rates in the estuary (Dähnke et al., 2008; Sanders et al., 2018) support an overarching control of organic matter on N_2O production and emissions along the Elbe Estuary."

2. Review comment (RC3) – 13.06.2023

The manuscript reports on N_2O concentrations, emission rates and the factors controlling this in the Elbe estuary. Overall, I found this to be a very thorough data set that was well presented and interpreted. The figures were of a high quality and the text was well written and organised. The factors giving rise to N_2O emissions were well considered and discussed. This manuscript will be of interest in the context of global N_2O budgets as well as understanding the factors controlling N_2O emissions. I only have a few minor comments. I congratulate the authors on producing such a well-rounded manuscript.

We thank the reviewer for their nice and helpful comments about our paper and include their suggested changes.

Table 1, final column. DIN concentration (as opposed to load).

Lines	Change
L103 – Table 1	Changed "Average DIN load (µmol L^{-1})" to "Average DIN concentrations (µmol L^{-1})"

Line 360 – a larger fraction of N moving through the estuary is denitrified. This does not mean denitrification is more intense, but rather the products accumulate (or are depleted) within the estuary due to longer residence times (in this case N_2O).

Lines	Change
L372-374	Changed from: "…, because denitrification and nitrification are more intense during longer residence times (e.g. Nixon et al. 1996; Pind et al. 1997; Silvennoinen et al. 2007; Gonçalves et al. 2010)." To "…, because longer residence times lead to the possible accumulation of N_2O produced from either nitrification or denitrification (e.g. Nixon et al. 1996; Pind et al. 1997; Silvennoinen et al. 2007; Gonçalves et al. 2010)"

It is concluded that reducing nitrogen inputs alone would not reduce N_2O emissions. It seems to me that N loads have been reduced, but not enough to stop phytoplankton growth. Wouldn't reducing N loads further reduce phytoplankton growth and eventually reduce N_2O emissions?

This is true. A reduced nitrogen input would reduce phytoplankton growth and thus organic matter availability and N_2O emissions. However, our data adds to the growing number of studies that clearly show a decoupling of the DIN:N_2O ratio and the development of phytoplankton blooms is not solely controlled by nutrient inputs, but also by e.g., temperature, residence time, water depth and grazing. Thus, complex biological and chemical processes control phytoplankton dynamics (Scharfe et al., 2009; Dijkstra et al., 2019; Kamjunke et al., 2021), which will possibly change significantly in the future due to climate change (IPCC, 2022). A holistic approach to water quality mitigation and climate change adaptation is needed to prevent high N_2O emissions. We rewrote this section to highlight the other factors controlling phytoplankton dynamics in the revised manuscript.

Lines	Change
L485-493	Changed from: “High organic matter availability due to phytoplankton blooms driven by river eutrophication fuels nitrification and subsequent N_2O emissions, causing a decoupling of the N_2O:DIN ratio. Therefore, N_2O emissions in heavily managed estuaries with high agricultural loads are clearly linked to eutrophication. Consequently, reducing nitrogen input alone is not sufficient to minimize N_2O emissions from estuaries. Further measures are needed to prevent the developments of intense phytoplankton blooms in rivers and estuaries. Especially considering climate change projections of more frequent and extensive draughts and warmer temperatures (IPCC, 2022), which potentially fuel phytoplankton growth (e.g. Scharfe et al., 2009; Kamjunke et al., 2021; IPCC, 2022).“ to “High organic matter availability due to phytoplankton blooms driven by river eutrophication fuels nitrification and subsequent N_2O emissions, causing a decoupling of the N_2O:DIN ratio. Therefore, N_2O emissions in heavily managed estuaries with high agricultural loads are clearly linked to eutrophication. A reduced nitrogen input would reduce phytoplankton growth and thus also N_2O emissions. However, the development of phytoplankton blooms is not solely controlled by nutrient inputs, but also by e.g., temperature, residence time, water depth and grazing. Thus, complex biological and chemical processes control phytoplankton dynamics (Scharfe et al., 2009; Dijkstra et al., 2019; Kamjunke et al., 2021), which will possibly change in the future due to the effects of climate change (IPCC, 2022). A holistic approach to water quality mitigation and climate change adaptation is needed to prevent high N_2O emissions.”

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