Nitrogen isotopes reveal a particulate-matter driven biogeochemical reactor in a temperate estuary

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Abstract. Estuaries and rivers are important biogeochemical reactors that act to modify nutrient loads and composition in the intensively used coastal zone. In a case study during July 2013, we sampled an 80km transect

10 along the Elbe estuary under unusually low-oxygen conditions. To better elucidate specific mechanisms of estuarine nitrogen processing, we tracked the evolution of the stable isotopic composition of nitrate, nitrite, particulate matter, and ammonium through the water column.

We used this exceptional summer situation to constrain the in-situ isotope effects of ammonium and nitrite oxidation and of remineralization at the reach scale. The isotope effects of nitrite oxidation and ammonium

15 oxidation are consistent with pure culture assessments. We found that estuarine biogeochemistry is governed by settling, resuspension, and remineralization of particulate matter. We used the stable isotope data to quantify sources and sinks of nitrogen in the Elbe estuary. An isotope mass balance box-model was developed to reproduce internal N-cycling and associated isotope dynamics. The model underscores the role of the delivery and reactivity of particulate matter, but it also allowed us to pinpoint additional sinks of reactive nitrogen, such as the

20 denitrification of water column nitrate in the intensively dredged and deep Hamburg harbour basin.

Introduction

Estuaries are biogeochemical hotspots of elemental cycling and reduction of contaminant and nutrient loads, playing major roles in the transfer of terrestrial nutrients and organic matter to the coastal zone. With growing use

25 of synthetic fertilizers over the last 150 years, river-borne loads of nitrogen (N) to the coastal zone (Galloway et al., 2003) have greatly increased. Much research over the past several decades has focused on understanding the role of estuaries in mitigating these surplus nutrient inputs (Bonaglia et al., 2014;Eyre and Balls, 1999;Garnier et al., 2010;Pastuszak et al., 2005;van Beusekom and de Jonge, 1998).

As an example, the estuary of the Elbe River, one of the largest rivers discharging into the North Sea has served

30 to remove substantial inputs of surplus reactive nitrogen (Schröder et al., 1996). With improved wastewater management and control of fertilizer application within the watershed, however, the overall riverine load of reactive nitrogen has decreased. While direct ammonium loading was reduced in the early 1990s (Bergemann and Gaumert, 2010), nitrate loading, stemming from more diffuse sources, has shown a much slower and more gradual decrease (Pätsch et al., 2010). In the estuary, dissolved oxygen levels throughout the water column have improved

35 in comparison to the early 1990s, at times even doubling the nitrate load of the agriculturally impacted river (Sanders et al., 2018).

Despite such improvements in the near coastal zone, nitrate (or N in general) pollution remains problematic. Agricultural input of N leads to eutrophication of rivers, surface water bodies, and groundwater, and must be managed and mitigated to protect ecosystem health and meet the requirements of the European Water Framework

40 Directive.

The nitrogen load and composition in rivers and estuaries is largely governed by microbial transformation processes in aquatic systems, including those involving net inputs or outputs (N fixation, denitrification) as well as those that involve cycling transformations (e.g., assimilation, nitrification, remineralization). However, constraining this microbial turnover and recycling of nitrogen can be especially difficult using concentration

45 measurements alone. As a complementary and powerful tool, changes in the isotope composition of reactive N species have been widely leveraged to provide new insights into biological N turnover in estuarine (Dähnke et al., 2008;Middelburg and Nieuwenhuize, 2001;Sebilo et al., 2006), coastal (Dähnke et al., 2010;Wankel et al., 2007) and open ocean systems (Stephens et al., 2020).

This utility has been especially powerful for combined analyses of $\delta^{15}N_{NO3}$ and $\delta^{18}O_{NO3}$ in evaluation of biological

50 N- turnover across a variety of natural systems (Buchwald et al., 2015;Dähnke et al., 2010;Sigman et al., 2009;Wankel et al., 2006). Biological N transformations give rise to isotopic fractionation, leading to measurable shifts in the isotopic ratios of substrate and product pools (Kendall, 1998;Mariotti et al., 1982;Sebilo et al., 2006). These isotopic shifts can be compared with fractionation factors determined from pure culture studies (e.g Buchwald et al., 2012;Casciotti et al., 2003;Granger et al., 2004;Jacob et al., 2018). For example, the ^{15}N

55 fractionation factor for ammonia oxidation in bacterial and archaeal pure cultures ranges from -13 to -41‰ (Casciotti et al., 2003;Mariotti et al., 1981;Santoro and Casciotti, 2011), whereas nitrite oxidation has a fractionation factor from +10 to +12‰, reflecting a unique inverse isotope effect (Casciotti, 2009;Jacob et al., 2018). The fractionation factor imparted during remineralization / ammonification of organic nitrogen (into ammonia) is generally small, with estimates ranging from ~0‰ (Brandes and Devol, 2002;Kendall, 1998) to -

60 2.5‰ (Möbius, 2013).

In the Elbe estuary, nitrification has been shown to be an important source of nitrate generation (Sanders et al., 2018). Here in this study, we evaluate the conditions in the estuary under an intense summer oxygen depletion that led to the accumulation of ammonium and nitrite in the freshwater section of the estuary. We thus leverage a unique opportunity in the same system to constrain in-situ isotope effects of nitrification in a natural system, based

65 on isotope assessments of N-bearing compounds. We further use the nutrient and isotope composition in the estuary to evaluate the control mechanisms of nutrient regeneration, nutrient sources and sinks in the estuary using a box-model.

2. Material and Methods

2.1 Study site

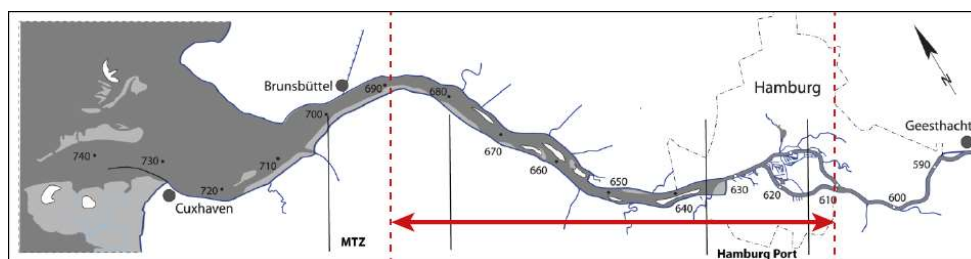
70 The Elbe Estuary extends from the weir in Geesthacht at stream km 586 (measured from the German-Czech border) to Cuxhaven at stream km 714, where the estuary opens to the Southern North Sea. The Elbe River drains an agricultural catchment area that is inhabited by ~25 million people (Lozán and Kausch, 1996). The Elbe River is the most important source of reactive nitrogen to the Southern North Sea, with an average annual load of ~ 75 kt between 2012 and 2016 (Umweltbundesamt, 2021). Nutrient loads have been reduced by ~50% since the 1990s,

75 but the Southern North Sea is still regarded as a problem area of eutrophication (OSPARCommission 2008).

The tidal part of the Elbe estuary also serves as an important shipping fairway. Continuous maintenance dredging enables access for large container ships to the port of Hamburg, the second largest port in Europe.

2.2 Sampling

80 In July 2013, we sampled the water column of the estuary from the mouth to stream km 610, upstream of the city of Hamburg (Figure 1) with the R/V *Ludwig Prandtl*. Surface water samples were collected with an on-board diaphragm pump, and turbidity, salinity, pH and dissolved oxygen were measured continuously using an in-situ FerryBox system (Petersen et al., 2011). Chlorophyll is reported based on fluorescence measurements from a SCUFA Fluorometer (Turner Designs, San Jose, CA, USA), integrated in the Ferrybox. No final calibration was

85 performed, and chlorophyll fluorescence data thus can only be used to assess relative changes along the transect. This SCUFA instrument also reported turbidity, and was calibrated against FNU standards in the laboratory prior to the cruise. Dissolved oxygen in the FerryBox was measured with an optode (Aanderaa Data Instruments AS, Bergen, Norway).

90 **Figure 1: The Elbe estuary. The stretch of the sampling transect is indicated in red.**

Water samples were immediately filtered through pre-combusted (4 hrs, 450°C) GF/F filters (0.7 μm) and stored in acid-washed (10% HCl overnight) PE bottles before they were frozen at −18°C for later analyses of nutrient concentration and dissolved inorganic nitrogen (DIN) stable isotope composition. Filter samples for suspended

95 matter were dried at 50°C directly after sampling and were then stored frozen. Nitrification rates were immediately determined on board using fresh and unfiltered water samples (see section 2.5 below).

2.3 Nutrient analysis

Duplicates of all filtered water samples were analysed for concentrations of ammonium, nitrite und nitrate, using

100 an automated continuous flow system (AA3, Seal Analytical, Germany) and standard colorimetric techniques (Hansen and Koroleff, 2007). In subsamples of nitrification rate measurements, ammonium was analysed manually based on the same colorimetric techniques. Due to restrictions in sample volume, nitrite and nitrate concentrations in these incubation samples were analysed by HPLC (Meincke et al., 1992).

2.4 Analyses of suspended particulate matter

105 Suspended particulate matter (SPM) was analysed for its elemental composition (carbon and nitrogen content), isotopes thereof, and chlorophyll fluorescence. We analysed total particulate carbon (POC) and total nitrogen (TN)

concentrations (weight%) of SPM using a Thermo Flash EA 1112. The precision of the measurement was 0.05% for carbon and 0.005% for nitrogen. $\delta^{15}N$ values of SPM were analysed with a Thermo Delta XP mass spectrometer after combustion in a Thermo Flash EA 1112. IAEA-N1, IAEA-N2 and IVA sediment standards were measured every eight to ten samples during each run to assure measurement quality and to assess the long-term analytical performance. The standard deviation for IAEA-N1 analyses was less than 0.2‰. A single-point calibration was performed with IAEA-N1 with an assigned $\delta^{15}N$ value of 0.4‰(Bahlmann et al., 2010).

2.5 Nitrification rates

Nitrification rates were measured following a modified version of the ISO/DIN 15685:2001 standard test (DIN15685 2001). In short, 50 ml of river water were incubated in 100 mL glass bottles with open caps in the dark at room temperature for up to 14 days (Sanders and Laanbroek, 2018). Up to 5 sub-samples per week were taken, centrifuged (15 min, 13.000 g), and nitrite/nitrate concentrations were determined immediately by HPLC. Incubations were continued until the nitrite and nitrate concentrations in the samples were stable. In total, we conducted 4 replicate analyses; 2 replicates with addition of 0.5 uM potassium chlorate to inhibit nitrite oxidation (Belser and Mays, 1980) and 2 unamended replicates. Nitrification rates were calculated by plotting the change in concentration with time, from which the steepest portion of the slope corresponds to the rate. Ammonia oxidation rates were taken as the increase in nitrite for the samples incubated with chlorate, and nitrite oxidation rates from difference of the increase of the sum of nitrite and nitrate for samples without chlorate. Nitrification rates presented are the mean values of all incubations.

2.6 Stable Isotope analysis

2.6.1 Nitrate isotopes

All water samples were analyzed for isotopic composition of nitrate ($\delta^{15}N_{NO3}$ and $\delta^{18}O_{NO3}$) using the denitrifier method(Casciotti et al., 2002;Sigman et al., 2001), wherein nitrate and nitrite are quantitatively converted to nitrous oxide (N_2O) by denitrifying bacteria (*Pseudomonas aureofaciens* , ATCC#13985) lacking N_2O reductase. Injected sample volumes were adjusted to achieve a sample size of 20 nmol of generated N_2O. N_2O was extracted from the sample vials by a flow of ultra-high purity helium and measured with a GasBench II, coupled to an isotope ratio mass spectrometer (Delta Plus XP, Thermo Fisher Scientific). With each batch of samples, two international standards (USGS34: $\delta^{15}N$: -1.8‰, $\delta^{18}O$: -27.9‰; IAEA-NO$_3^-$: $\delta^{15}N$: +4.7‰, $\delta^{18}O$: +25.6‰) and an internal standard were also measured for standardization. Standard deviation of samples and standards was <0.2‰ for $\delta^{15}N_{NO3}$ (n = 4) and <0.5‰ for $\delta^{18}O_{NO3}$ (n=4).

2.6.2 Nitrite isotopes

During the sampling campaign, high nitrite and ammonium levels in some samples allowed for isotope analysis. The $\delta^{15}N_{NO2}$ was analyzed according to previously described protocols (Böhlke et al., 2007) whereby a culture of *Stenotrophomonas nitritireducens* is used to selectively reduce nitrite to N_2O. For calibration, we used two in-house standards with known $\delta^{15}N_{NO2}$ values of -83.3 ±0.2‰ and +27.6 ±0.2‰ vs. Air-N_2, determined independently via EA-IRMS analysis. For $\delta^{15}N_{NO2,}$ the standard deviation of our measurements was <0.3‰ (n=4).

2.6.3 Ammonium isotopes

$\delta^{15}N$ in ammonium was ($\delta^{15}N_{NH4}$) analyzed based on the method by Zhang et al. (2007). In brief, any sample nitrite was removed by addition of sulfamic acid, followed by conversion of ammonium to nitrite by hypobromite oxidation. Nitrite was then chemically converted to N_2O via addition of acetic acid buffered sodium azide solution and was measured like nitrite and nitrate isotope samples (McIlvin and Altabet, 2005). With each batch of samples, three international ammonium standards (USGS25: $\delta^{15}N$: -30.4‰, USGS26: $\delta^{15}N$ +53.8‰, IAEA N1: $\delta^{15}N$ +0.4) were run for calibration, and an internal standard was used for quality control. The standard deviation of samples and standards was <0.5‰ for $\delta^{15}N_{NH4}$.

2.7 Calculation of isotope effects for nitrite oxidation and ammonium oxidation

To determine isotope effects in the Elbe Estuary, we applied an open-system approach, where the substrate is continuously supplied and partially consumed. This results in a linear relationship of f (the fraction of the remaining substrate, $f = ([C]/[C_{initial}])$) and the isotope values of the substrate, where the slope of the regression line represents the isotope effect (Sigman et al., 2009), calculated following Eq. 1:

$$\varepsilon_{substrate} = \frac{\delta^{15}N_{substrate} - \delta^{15}N_{initial}}{(1-f)}$$

(1)

$\delta^{15}N_{substrate}$, $\delta^{15}N_{product}$, $\delta^{15}N_{initial}$ denote $\delta^{15}N$ values of the substrate and product at the time of sampling and the initial value, and f is the remaining fraction of substrate.

For ammonium and nitrite concentration, we calculated isotope effects along flow sections in which substrate concentration decreased. For ammonium, this corresponds to stream km 626 to 639, for nitrite, we used isotope and concentration values between stream km 641 and 656.

The isotope effect of re-mineralization of particulate nitrogen ($^{15}\varepsilon_{remin}$) was calculated based on changes in the $\delta^{15}N$ and in the N-content of particulate matter (Möbius, 2013) over the entire transect.

3. Results

3.1 Biogeochemistry and nutrients along the estuary

To unravel estuarine biogeochemical cycling, we regard hydrological parameters and nutrient trends in the freshwater section of the estuary, starting in the shallow freshwater section, through the dredged port area, up to the onset of the salinity gradient, when mixing with low concentration seawater occurs.

Water temperature in the estuary was exceptionally elevated for this time of year, with almost 24°C at the uppermost sampling site, and oxygen saturation dropped from ~100% saturation at stream km 610 to minimum values of 32 – 34% in the Hamburg port region. Downstream of the port region, oxygen saturation increased again to final values near 100% in the lower estuary (Fig. 2).

Turbidity and suspended particulate matter concentration were quite variable along the sampling section. However, both show relatively high values at the uppermost stations that drop at the entrance to the harbour region, followed by a peak around stream km 639. Further downstream, in the estuarine turbidity maximum, SPM and turbidity increased again.

Interestingly, chlorophyll fluorescence showed trends similar to SPM, with concentration rapidly dropping at the entrance to the harbour region. A transient maximum was then found around stream km 648, slightly after

180   maximum values for SPM or turbidity. We saw no increase of fluorescence towards the estuarine turbidity maximum.

Generally, nutrient concentrations in the freshwater estuary were dynamic, suggesting active uptake and/or recycling (Fig. 2). As the dominant DIN species, concentrations of nitrate ranged from 87 µmol L^{-1} at stream km 610, upstream of the port region, and increased to a maximum of 152 µmol L^{-1} just before the onset of the salinity

185   gradient at stream km 690. Ammonium and nitrite were present in lower concentrations. The ammonium concentration at the upstream station entering the port region was 2 µmol L^{-1}. Ammonium then peaked in the port region (stream km 615 – 641) with a concentration of 21 µmol L^{-1} at stream km 626. This ammonium peak was followed by a nitrite peak just downstream between stream km 620 and 656, with a maximum concentration of 13 µmol L^{-1} at stream km 641.

190   Silicate concentrations also increased from the upstream station (10 µmol L^{-1}) to a peak at stream km 641 (53 µmol L^{-1}). Further downstream, silicate concentrations then decreased to less than 5 µmol L^{-1}, with a small peak in the maximum turbidity zone of the estuary (stream km 665 to 678, Figure 2).

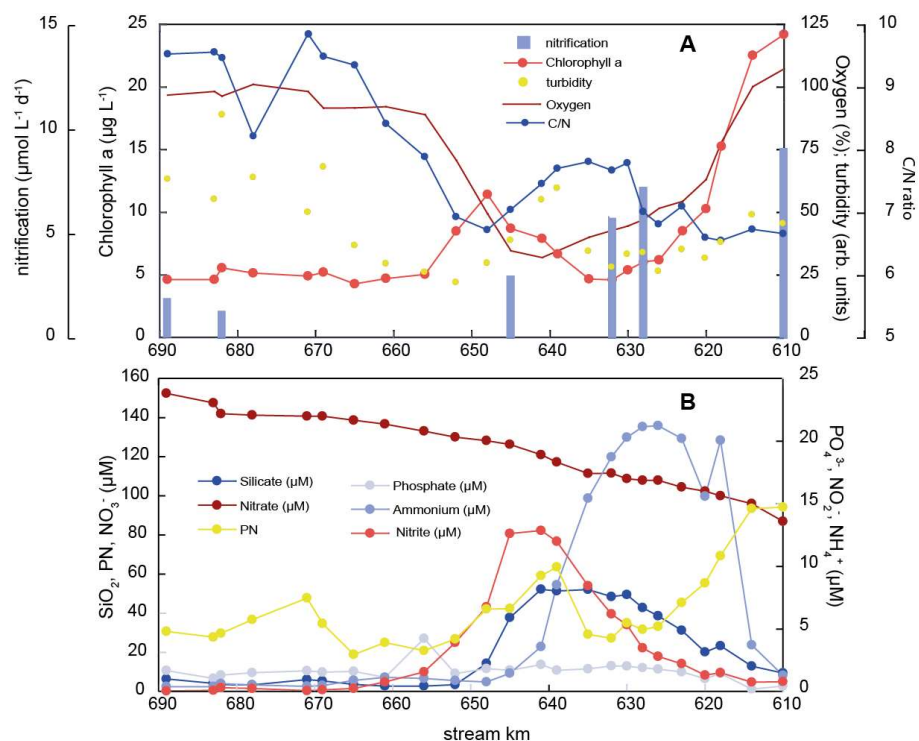
Phosphate concentration is at its minimum upstream of the port region, where the water is shallow. From 0.2 µmol L^{-1} at stream km 618, concentrations decreased to < 2 µmol L^{-1} (1.7 +/- 0.6) and remains relatively stable along

195   the estuary.

Figure 2: Nutrient concentration, particulate matter composition, biogeochemical trends and nitrification rates along the Elbe Estuary.

3.2 Nitrification

Nitrification rates in July 2013 were highest in the upper part of the estuary, including the Hamburg port region (Elbe-km 615 to 635) with a nitrate production of up to 10 µmol L^{-1} d^{-1} (Fig. 2A). Nitrification rate decreased downstream of the port region, reaching minimum values of ~2 µmol L^{-1} d^{-1}. Rates were strongly positively correlated to %N and %C in suspended matter (r^2 = 0.91 and 0.95, respectively), but were not significantly correlated to dissolved oxygen concentration or SPM content. They were, however, negatively correlated to δ^{15}N of SPM, and lower δ^{15}N values coincided with high nitrification rates (not shown).

3.3 Isotopic changes along the estuary

We measured stable isotope signatures of nitrate and of suspended particulate matter along the entire estuary (Fig. 3). Nitrate δ^{15}N and δ^{18}O values are elevated entering the estuary (+19‰ and +10‰ for δ^{15}N and δ^{18}O, respectively), decreasing along the estuary to final values of +11 and +3‰, respectively. The strongest decrease in δ^{18}O occurs in the harbour region (stream km 618 – 656), Fig. 3. Downstream from this point, nitrate stable isotopes exhibited conservative mixing patterns (data not shown, Dähnke et al., 2008;Fry, 2002).

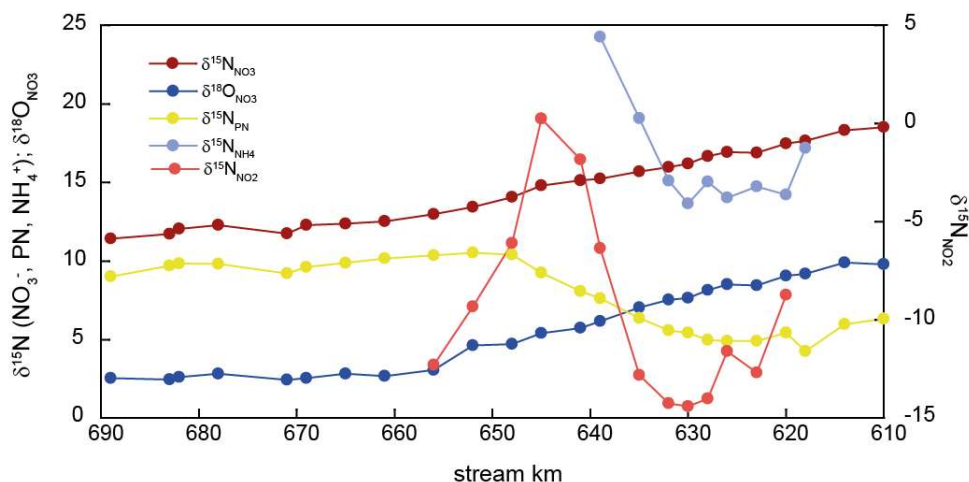



Figure 3: Isotopic changes of DIN and PN along the estuary

Nitrogen isotope composition of the suspended particulate matter (δ^{15}N-SPM) is also most dynamic in the harbour region. δ^{15}N-SPM at the entrance of the sampling transect is +6.3‰, the isotope value drops to a minimum of +4.9‰ as water depth increases from 6 to 18 metres. Between stream km 628 and 648, δ^{15}N-SPM swiftly increases to +10.4‰, and then remains relatively stable throughout the rest of the estuary, with a slight decrease to +9.0‰ towards the onset of the salinity gradient.

Nitrite isotopes were measured between stream km 620 and 656, where concentrations were high enough to analyze for isotopic composition, and exhibited large variations. At the entrance to the harbor, δ^{15}N-NO$_2$ was -9‰, dropped to a minimum of ~-15‰, and then increased, in parallel to increasing concentration, to a maximum of ~0‰ at stream km 641. The subsequent decrease in [NO$_2^-$] was accompanied by decreasing isotope vales that reached a minimum of -12‰ at stream km 656. Based on Eq. 1, we calculated an inverse isotope effect ε$_{nitox}$ of 12.9‰ (Fig. 4)

We measured ammonium isotope values between stream km 618 and 639, in a section where ammonium concentration peaked to maximum values of 21 µmol L^{-1}. Isotope values were anti-correlated with ammonium concentration. At the entrance of the harbor, δ^{15}N-NH4 was +17.7‰, values then decreased to ~15‰ in the harbor

230 region and increased to +24‰ as ammonium concentration decreased (stream km 635 to 639). The isotope effect associated with this ammonium removal was estimated to be ε_{amm} = -17.4‰.

To calculate the isotope effect of remineralization, we used an open system approach that was based on δ^{15}N of suspended particulate matter (ln(N%), Möbius 2013) across the entire transect. This approach yielded an isotope effect of ε_{remin}= 4.4‰ (Fig. 4).

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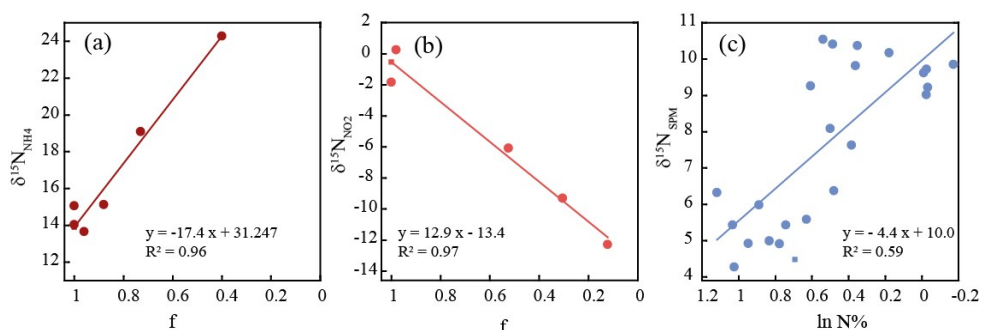


Figure 4: Overview of calculated fractionation factors for turnover of (a), ammonium, (b), nitrite, and (c), particulate nitrogen along the respective Elbe stretch. See sections 2.7and 3.3 for details of the calculation.

240 **4. Discussion**

4.1 Deriving N-cycling from isotope effects

Stable nitrogen isotope dynamics are frequently used to elucidate N-cycling in marine environments (e.g. Gaye et al., 2013;Granger et al., 2013;Sigman et al., 2009). However, the use is often restricted to individual N species, such as nitrate or particulate nitrogen, simply because the concentration of other N–bearing compounds is too low

245 to assess their isotopic signatures accurately.

In this study, we captured a unique situation in which N-cycle intermediates accumulated and could be measured contemporaneously. After the flood in June 2013(Jacob et al., 2016;Voynova et al., 2017), river discharge returned to moderate levels again for only a few days prior to our sampling campaign. Intense cycling processes caused an unusually high accumulation of nitrite, ammonium and particulate nitrogen - allowing a unique perspective on

250 associated isotope effects of riverine N cycling processes.

The Elbe estuary is well-known to be a site of very active N-processing and has undergone dramatic changes over the past several decades: The estuary previously suffered from high nitrogen loads and exhibited intense sedimentary denitrification in the 1980s (Schröder et al., 1996), whereas its importance in N-removal apparently diminished more recently (Dähnke et al., 2008;Deek et al., 2013).

255 Currently, nutrient and carbon turnover is most dynamic within the harbor region (Amann et al., 2015;Sanders et al., 2018), where oxygen depletion frequently occurs in summer (Schöl et al., 2014). Anoxic and oxic processes are known to be closely coupled, with nitrification and denitrification likely to co-occur in close proximity. In

addition, there has been previous demonstration of both nitrification as well as denitrification in the harbor region (Brase et al., 2017;Sanders et al., 2018). With our assessment of isotope effects here, we aim to more fully

260 characterize these dominant turnover processes and their driving forces.

Notably, nitrate isotope changes only faintly reflect nitrate regeneration dynamics. The drop of oxygen isotope values in the harbor region is an indication for nitrification and incorporation of light oxygen from H_2O into nitrate, but overall, isotope changes are dampened due to the large pre-existing nitrate pool. We will therefore elaborate indications for nitrate production based on nitrite and ammonium concentration and isotope changes.

265 If the sequential remineralization of organic N to NH_4^+ and oxidation of NH_4^+ to NO_3^- by nitrification serve as the primary source of new nitrate, a link between the nitrogen content of particulate matter and the isotope signature of freshly produced nitrate should be evident.

We find high nitrification rates, which is in line with the succession of ammonium and nitrite accumulation moving through the harbor basin. The isotope effect for ammonium removal, -17.4 ‰, is towards the lower end of pure

270 culture assessments of the isotope effects of ammonia oxidizing bacteria (-14 to -38, Casciotti et al., 2003). The inverse isotope effect for nitrite oxidation (+12.9‰) in our study corresponds well the isotope effect that has been identified in lab experiments (Casciotti, 2009) as well as under natural conditions in the Elbe catchment (Jacob et al., 2016).

In the river section between stream km 626 and 646, ammonium concentration decreases, and ammonium and

275 nitrite isotopes were measured in parallel. If no other ammonium uptake mechanism occurs, the difference between the isotope signature of ammonium and nitrite, $\delta^{15}N_{NH4}$ - $\delta^{15}N_{NO2}$, should equal the isotope effect calculated based on ammonium concentration. This is not the case, $\delta^{15}N_{NH4}$ - $\delta^{15}N_{NO2}$ is 28.8 ± 2.3‰, larger than the computed isotope effect of ammonium oxidation, suggesting that another ammonium removal process with a lower isotope effect occurs in parallel. A likely candidate is ammonium assimilation by phytoplankton, which would fit with

280 increasing chlorophyll fluorescence along the stretch (Figure 2).

The isotope effect of ammonium assimilation is highly variable, (e.g., Hoch et al., 1992;Pennock et al., 1996;Waser et al., 1998), but usually below 10‰ in estuaries (York et al., 2007) and references therein. For a rough calculation, if we assume a moderate isotope effect of 8 ‰ (cf. Wada and Hattori, 1978;York et al., 2007), approximately 55% of ammonium in the water column are subject to assimilation, 45% are oxidized to nitrate. Within the given

285 uncertainty for the isotope effect of ammonium assimilation, this fits relatively well with the ratio of the ammonium and nitrite peaks (2 to 1) in the estuary. While we cannot safely determine the exact proportion of both ammonium sinks, it seems evident that ammonium assimilation is a relevant ammonium sink in addition to nitrification. This seems plausible, since phytoplankton prefers ammonium over nitrate as a nitrogen source (Dortch et al., 1991).

290 **4.2 The role of suspended matter**

Clearly nitrification is a prominent N-cycling process in the harbor region. However, the rate of nitrification does not appear to be regulated by ammonium concentration, or by the amount of SPM in the water column. Instead, we see that nitrification rates scale with indicators of organic matter quality, such as the N- and C-content of organic matter.

295 Generally, the concentration and composition of suspended particulate matter vary along the estuary. Suspended matter is very heterogeneous and is affected by remineralization, resuspension, and of course by phytoplankton

growth. SPM is thus closely interlinked with nutrient and isotope dynamics, which is also evident in the correlation of N content (N%) of SPM and nitrification rates (Fig. 4).

The calculated the isotope effect of remineralization along the transect was ε^{15}_{remin} = 4.4‰. This isotope effect is

300 a bit higher than the usual assumption for remineralization in sediments, which ranges from insignificant to ~2‰, (Brandes and Devol, 1997;Mobius et al., 2010), but fits well with a previous estimate by Sanders and colleagues in the Elbe estuary (Sanders et al., 2018). Remineralization in the Elbe thus is prominent, and is an important source of N for generation of nitrate.

We find that SPM is not equally reactive throughout the estuary. O_2 saturation, an indicator for heterotrophic

305 metabolism, reaches its minimum in the harbor region, after chlorophyll fluorescence decreased, suggesting active decomposition of limnic algal material (Fig. 2a). The low C/N ratio of SPM, and high silicate concentration in the water column (Fig. 2b) corroborate that comparatively fresh and labile suspended matter is actively remineralized in this section of the Elbe Estuary.

Downstream of the harbor region, SPM reactivity decreases, with lower nitrification rates, increasing C/N ratio,

310 and only faint signs of biological activity at the maximum SPM concentration found in the maximum turbidity zone of the estuary (~stream km 670).

Overall, SPM reactivity shows a strong gradient along the estuary, some sites of high SPM are actively nitrifying or potentially even denitrifying, whereas others reveal very little active N-turnover. We find that downstream of stream km 650, little N- turnover, such as nitrification, takes place, and SPM reactivity decreases, shown by

315 increasing C/N ratios (Fig. 2). Despite the freshwater regime, SPM of marine origin is introduced and dominates in this section of the estuary (Kappenberg and Fanger, 2007). This marine SPM supports only little N-turnover in our setting. In contrast, the increased depth and residence time in the harbor zone effectively promote its behavior as a bioreactor in which marine and limnic SPM are mixed. This mixing supports the co-occurrence of nitrification, denitrification, and remineralization, resulting in dramatic changes in DIN composition.

320 **4.3 Constructing an estuarine N-Budget**

Based on isotope changes in the water column, and on trends in isotopic composition of the respective pools, we were able to describe isotope effects and N-dynamics in the estuary. Next, we construct a total nitrogen isotope mass balance to more thoroughly explore potential processes and controls on the evolution of riverine nitrogen along the estuarine reach. For this approach several key assumptions were made (the validity of which is discussed

325 below).

First, we regard the transect data as representative of a 'snapshot' in time, over which steady state can be assumed. The sampling area, while generally influenced by tidal action, lies entirely within the freshwater portion of the lower Elbe. Furthermore, over this section of the river there are no significant tributaries with respect to the water budget. Thus, our first key assumption is that changes observed in N pool size and isotopic composition are not

330 influenced by in-mixing of other external sources, and that the primary N source is the nitrogen that enters at the top of the reach.

We explicitly disregard dissolved organic nitrogen (DON) in our approach, because previous assessments were equivocal regarding the role of DON in the Elbe estuary, especially in the port region. A previous study (Schlarbaum et al., 2010) found a removal of ~20 µmol L-1 from the combined (DON + ammonium) reduced

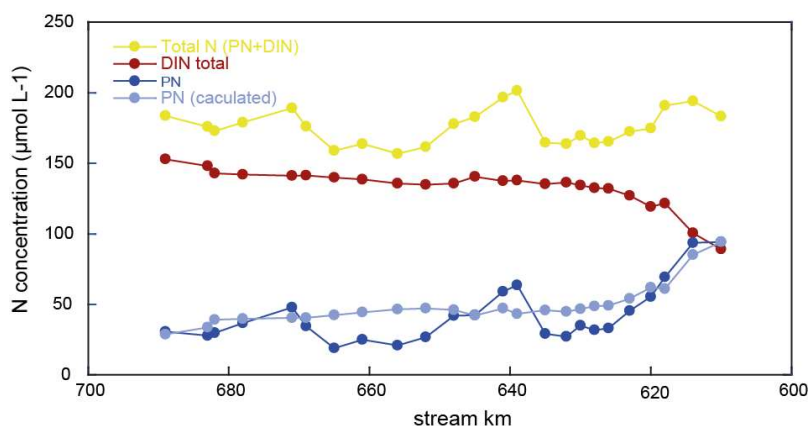
335 nitrogen pool, with an explicitly high contribution of ammonium in the port region. This is removal fits well with ammonium removal in our study, and we therefore assume that the fraction of reactive DON in the combined pool

of reduced nitrogen pool was small. Generally, invoking a large and isotopically dynamic DON pool contrasts with the observed continuum of geochemical parameters otherwise routinely documented along this stretch of the Elbe (Amann et al., 2014;Dähnke et al., 2008;Sanders et al., 2018).

340 Hence, we use the sum of DIN plus particulate nitrogen (PN) to address transformations in the nitrogen pool. We find substantial fluctuations in the total nitrogen pool over the reach (as much as 16 uM between two sampling points). However, these shifts are almost entirely controlled by fluctuations in the concentration of PN (Figure 5), and, in line with our previous assessments, show that PN affects estuarine N processing. Notably, there were no erratic changes to the $\delta^{15}N$ of the PN pool, but rather a smooth and continuous transformation over the entire 90

345 km reach (Figure 2). This is in line with previous measurements that suggest notable changes in turbidity over this section of the Elbe, including the turbidity maximum zone toward the lower end of the reach (Burchard et al., 2018). Thus, we assume that the large fluctuations in PN are due to settling and resuspension of fresh PN and/or patchiness in PN distribution at the surface, which was not captured during our sampling of surface water.

Resuspension and/or particle settling is not considered to be an isotopically discriminating process, so we explicitly

350 invoke resuspension or settling only to satisfy mass balance, without any impact on the total N isotope mass balance. Thus, in practice, the PN concentration has been adjusted at each sampling point to reflect the mass balance complement of the total DIN pool – normalized to the total N entering the river at Km 610. The adjusted PN data are shown in Figure 5.

355 **Figure 5: N concentration along the estuary. Total N fluctuations are driven by PN fluctuations. Light blue is the calculated concentration of particulate matter that is assumed to satisfy mass balance.**

4.4 Implications of the N isotope mass balance

Based on the assumptions of PN settling and resuspension, we were able to satisfy the mass balance for each

360 discrete sampling point, but the N isotopic composition of the total N pool (Figure 5) exhibits distinct variations over the entire reach, which remain unexplainable due to variations of particulate matter resuspension and/or settling alone. Indeed these isotopic variations may therefore reflect isotopically fractionating processes or N inputs that are not addressed by the assumptions underlying the modeling approach.

Over large sections of the transect, we find net removal of nitrogen, and that isotope values calculated for the lost

365 N closely resemble those of the remaining riverine N. Thus, we assume that sedimentary denitrification may be responsible (e.g., net N loss, with little impact on isotopic composition). However, in the upstream zone (km 618

to km 635), the $\delta^{15}N$ of the total N pool becomes too high at many of the sampling points, suggesting a loss of low $\delta^{15}N$ nitrogen (Figure 6). Here we suggest that water column denitrification might be a plausible loss mechanism. A loss of isotopically low $\delta^{15}N$ nitrogen could arise from denitrification of the standing NO_2^- pool, which is

370   consistently low (average of -11.8‰; Figure 3). Even a small removal flux of this very low $\delta^{15}N$ pool, could have an appreciable impact on the evolution of the $\delta^{15}N$ of the total N pool. Adopting mid-range values for the isotope effect of nitrite reduction ($^{15}\varepsilon_{NIR} \sim$ -13‰ to -16‰,(Jacob et al., 2016;Martin et al., 2019), a loss flux of less than 2% of the total N pool would be sufficient to account for the loss of low $\delta^{15}N$ nitrogen. Such a flux is plausible and in the lower range of independent estimates of present day-losses due to denitrification in the Elbe estuary

375   (Dähnke et al., 2008;Deek et al., 2013).

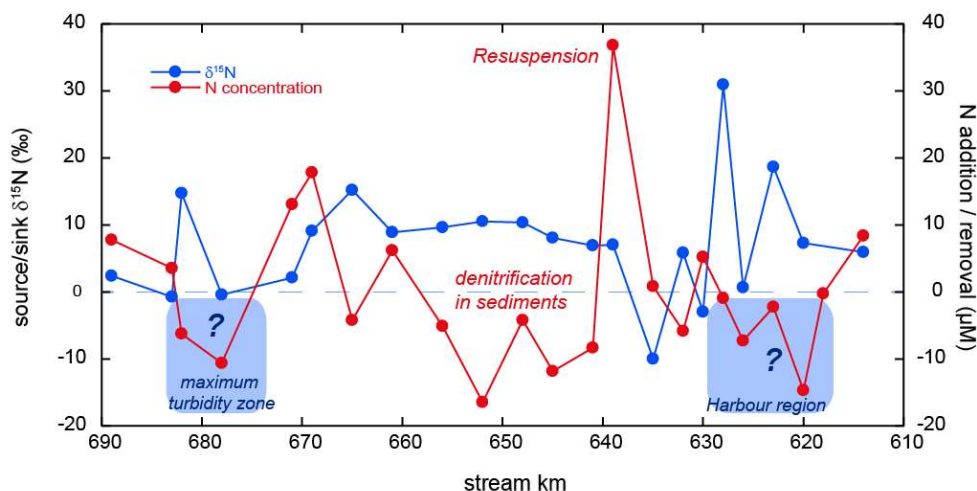



Figure 6: Isotope signature and concentration of N that is removed or added using the box model approach. Boxes indicate cases in which the isotope composition of missing N rules out isotope-neutral processes like settling / resuspension of particulate matter or sedimentary denitrification

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Notably, this argument requires the removal of NO_2^- under apparently oxic conditions (O_2 saturation of $40 – 63\%$) and therefore relies on the existence of low oxygen conditions inside of suspended particulate or flocculate material. However, there is growing evidence for active denitrification in the port region of the Elbe Estuary, such

385   as high nitrous oxide production that cannot be plausibly explained by nitrification alone (Brase et al., 2017). Denitrification and nitrous oxide production in oxic waters with high suspended matter loads has been demonstrated elsewhere (Liu et al., 2013;Quick et al., 2019). Thus, we conclude that a contribution of denitrification in the oxic water column is at least possible in this system – and would satisfy the isotope mass balance as introduced.

390   We find a second region hosting an isotopic imbalance downstream (stream km 671 – 689). In this region, either the removal of a high $\delta^{15}N$ pool, or the addition of N with a low $\delta^{15}N$ value is required to satisfy the isotope mass balance. There are two possible explanations for this scenario. The first one is that in this region, a small loss of nitrate occurs, which we do not account for in our mass balance approach. Since the $\delta^{15}N$ of the NO_3^- pool in this region is relatively high (~+12-13‰), a loss of NO_3^- through diffusion-limited sedimentary denitrification (in

395   which the intrinsic isotope effect is not expressed) would be sufficient to satisfy the N isotope mass balance. The

loss required would not be more than 0.5 to 8.0% of the total N flux, which is in accordance with previous assessments for the Elbe.

Alternatively, it is possible that a minor addition of low δ^{15}N to the total pool is responsible for the isotopic imbalance. A potential source for this may be nitrogen fixation by azotrophic bacteria. However, we acknowledge
400   that this scenario is somewhat unlikely in a eutrophic, N-loaded estuary like the Elbe River. Thus, we assume that a small loss of nitrate via denitrification might occur in the sediments along this stretch of the estuary.

Conclusions

Our data underscore the importance of particulate matter cycling in estuarine biogeochemistry. We confirmed lab-based determinations of nitrogen isotope effects in the estuary and evaluated the role of nitrate regeneration by
405   nitrification. The intensity of nitrification is coupled to reactivity of particulate matter. Ammonium assimilation can limit the magnitude of nitrification, while reactive organic matter is produced that can in turn increase reactivity in the estuary. Jointly with high N loads, this appears to fuel denitrification. We find that the harbor region hosts nitrification as well as denitrification, and speculate that even denitrification in the water column may occur in low-oxygen environments with a high load of suspended particulate matter.

410   Overall, we assume that changes in particulate matter transport patterns can have profound effects on nitrogen biogeochemistry in the estuary, and can alter the balance of nitrification and denitrification. While the overall nitrogen loads of the Elbe River are decreasing, changes in river biogeochemistry can have local effects in the harbor region, with high rates of local nutrient turnover, and increased oxygen consumption resulting in hypoxic zones.

415   **Data availability**

The data are available from the corresponding author upon request.

Author contribution

Author contributions. KD, TS and SDW designed the research. TS carried out the fieldwork, and TS and YV performed the analyses. All authors jointly interpreted the data. KD, TS and SDW wrote the paper, with
420   suggestions and additions provided by YV.

Competing interests.

The authors declare that they have no conflict of interest.

Acknowledegments

The authors would like to thank the captain and crew of the R/V Ludwig Prandtl for their support during the cruise.
425   Markus Ankele is acknowledged for nutrient measurements, and we thank Justus van Beusekom for helpful comments that greatly improved this manuscript.

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