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 the loads and composition of nutrients in the coastal zone. In a case study during July 2013, we sampled an 80km transect along the Elbe estuary under 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 were able 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 oxidation are consistent with pure culture assessments. We found that the estuarine nitrogen budget of the Elbe estuary is governed by settling, resuspension, and remineralization of particulate matter, and further used our stable isotope data to quantify sources and sinks of nitrogen in the Elbe estuary. Based on an isotope mass balance calculation, we reproduced internal N-cycling and associated isotope dynamics. We find that the reactivity and concentration of particulate matter in the estuary is the main control of nitrogen isotope dynamics and of remineralization. Moreover, the isotope mass balance underscores this role of the delivery and reactivity of particulate matter, but it also allowed us to pinpoint additional sinks of reactive nitrogen, such as the 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 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 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 loads are only slowly and gradually declining (Pätsch et al., 2010). Nitrate sources are linked to agricultural land-use in the catchment (Johannsen et al., 2008;Radach and Pätsch, 2007). Along with an overall improvement of estuarine oxygen levels in comparison to the early 1990s after the fall of the Iron Curtain

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(Spiekermann, 2021), nitrification gained in importance (Dähnke et al., 2008). Today, nitrification in the estuary at times even doubles the nitrate load of the agriculturally impacted river (Sanders et al., 2018). In consequence, 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 Directive.

Despite improvements in the Elbe over the past decades, estuarine O_2 depletion remains a recurrent phenomenon, especially in summer and under low discharge conditions (Schöl et al., 2014;Kerner, 2007). In summer, oxygen concentration in the Elbe regularly falls below 3 mg L^{-1} in the harbor region (Spieckermann, 2021), leading to an accumulation of ammonium and nitrite especially in the harbor region.

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 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 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 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 -2.5‰ (Möbius, 2013).

In the Elbe estuary, nitrification is 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 over a large stretch of the freshwater section of the estuary. The spatial extent of nitrite and ammonium accumulation thus gave us the unique opportunity to constrain in-situ isotope effects of nitrification in a natural system, based on isotope assessments of N-bearing compounds. We further use this detailed assessment of nutrient and isotope composition in the estuary to determine the factors that control biological nutrient turnover in summer in the estuary.

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2. Material and Methods

2.1 Study site

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 catchment is dominated by agricultural land-use and is inhabited by ~25 million people (Lozán and Kausch, 1996). Most important nitrogen inputs stem from agricultural sources in the upper and middle reaches of the Elbe (Hofmann et al., 2005;Johannsen et al., 2008) and reach the Elbe via diffuse inputs to its tributary rivers. Point-sources play a subordinate role (Hofmann et al., 2005). 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). Riverine nutrients have been reduced by ~50% since the 1990s, 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 third largest port in Europe.

2.2 Sampling

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 via the vessel's water inlet at a depth of 2 m, 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 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).
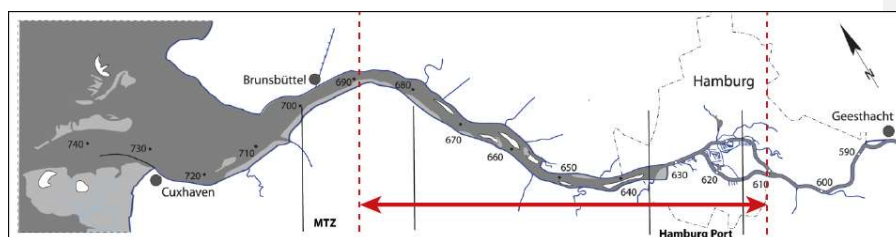
Figure 1: The Elbe estuary. The stretch of the sampling transect is indicated in red.

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 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 an automated continuous flow system (AA3, Seal Analytical, Germany) and standard colorimetric techniques (Hansen and Koroleff, 2007). Detection limits were 1 µmol L^{-1} for nitrate, 0.5 µmol L^{-1} for nitrite and 0.5 µmol L^{-1} for ammonium. 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 in replicate by HPLC (Jasco 980, Jasco, Germany) (Meincke et al., 1992).

2.4 Analyses of suspended particulate matter

Suspended particulate matter (SPM) was analysed for its elemental composition (carbon and nitrogen content), and isotopes thereof. Sensor data of chlorophyll fluorescence were further used as an indicator of suspended matter quality.

We analysed total particulate carbon (POC) and total nitrogen (TN) concentrations (weight%) of SPM using a Thermo Flash EA 1112 (Thermo, Germany). The precision of the measurement was 0.05% for carbon and 0.005% for nitrogen, measurements were calibrated with a certified acetanilide standard (IVA Analysentechnik, Germany). δ^{15}N values of SPM were analysed with a isotope ratio mass spectrometer (Delta Plus XP, Thermo, Germany) after combustion in an elemental analyser (Flash EA 1112, Thermo, Germny). IAEA-N1, IAEA-N2 and an additional certified sediment standard (IVA Analysentechnik, Germany) were measured every eight to ten samples during each run to assure measurement quality and to assess the long-term analytical performance. A single-point calibration was performed with IAEA-N1 with an assigned δ^{15}N value of 0.4‰ (Bahlmann et al., 2010). The standard deviation for IAEA-N1 isotope analyses was less than 0.2‰.

2.5 Nitrification rates

Nitrification rates were measured following a modified version of the ISO/DIN 15685:2001 standard test (DIN15685 2001). The method is described in detail in Sanders and Laanbroek (2018). In short, 50 ml of river water were incubated in 100 mL glass bottles with open caps in the dark at room temperature (22±2°C, i.e., close to the average water temperature of ~24°C) 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, which indicted that remineralisation of easily accessible organic matter was completed. This approach is used to address potential nitrification that is possible fuelled by organic matter in the given water sample. Details are discussed in Sanders and Laanbroek 2018.

In total, we conducted 4 replicate analyses; in 2 bottles 0.5 uM potassium chlorate was added to inhibit nitrite oxidation (Belser and Mays, 1980), and 2 unamended replicates were measured in addition. Process rates were calculated by plotting concentration change over time, with the steepest portion of the slope corresponding to the rate. The rate limiting step of nitrification was ammonia oxidation, and the slope of amended and unamended incubations did not differ significantly between the measurements. Hence, nitrification rates presented are the mean values of all 4 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 (Thermo, Germany), coupled to an isotope ratio mass spectrometer (Delta Plus XP, Thermo, Germany). 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). In samples with elevated nitrite concentration, $\delta^{15}N_{NO2}$ was analysed separately, and we present $\delta^{15}N_{NO3}$ values that were corrected for the contribution of nitrite.

2.6.2 Nitrite isotopes

During the sampling campaign, high nitrite and ammonium levels in some samples allowed for isotope analysis. $\delta^{15}N_{NO2}$ was analyzed in samples with [NO2$^-$] ≥ 2 µmol L^{-1} according to previously described protocols (Böhlke et al., 2007). 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). The minimum ammonium concentration required for isotope analyses in our setup is 2 µmol L-1. 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:

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

$\delta^{15}N_{substrate}$, $\delta^{15}N_{product}$, $\delta^{15}N_{initial}$ 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.

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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. In this section, ammonium concentration is low (maximum of 3.5 µmol L^{-1}), and we assume that ammonium oxidation has only a negligible impact on nitrite isotopes.

We calculated the isotope effect of re-mineralization of particulate nitrogen ($^{15}\varepsilon_{remin}$) 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 (Table S1). 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, and turbidity increased again.

Chlorophyll fluorescence rapidly dropped at the entrance to the harbour region. A transient maximum was then found around stream km 648, slightly after maximum values for turbidity. We saw no increase of chlorophyll fluorescence towards the estuarine turbidity maximum. The molar C / N ratio of particulate matter revealed inverse trends, with values of ~6.6, an increase in the harbour basin, and a decrease towards 6.7 at stream km 648, where we measured the transient chlorophyll maximum. In the outer regions of the estuary. C / N increased to values near 10 (Figure 2 and Table S1).

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 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.

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).

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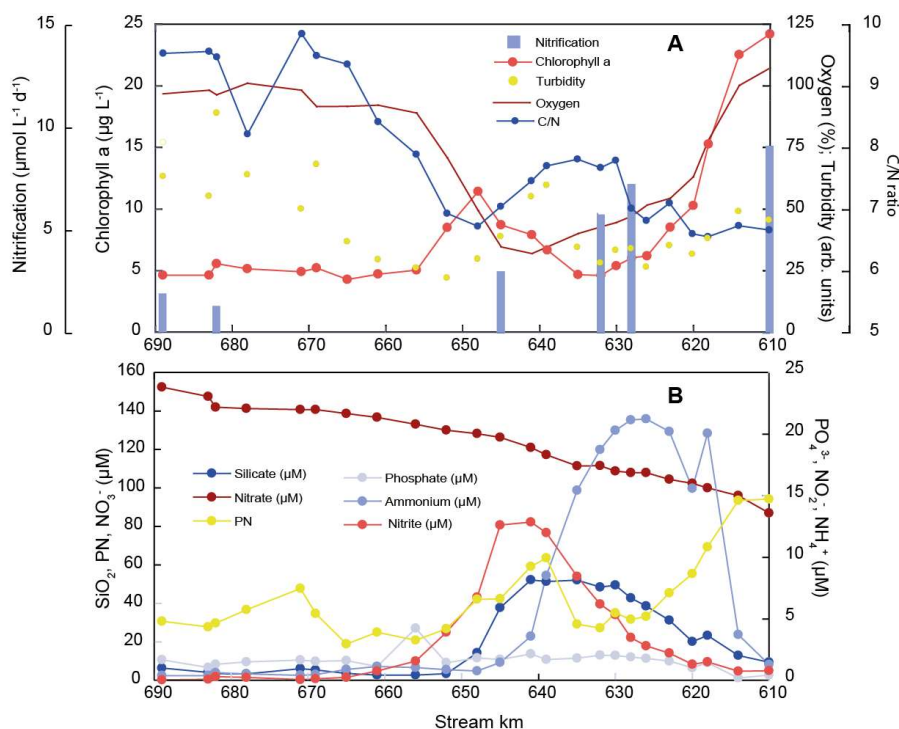
Figure 2: Nutrient concentration, particulate matter composition, biogeochemical trends and nitrification rates along the Elbe Estuary.

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 $\delta^{18}O$ 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).
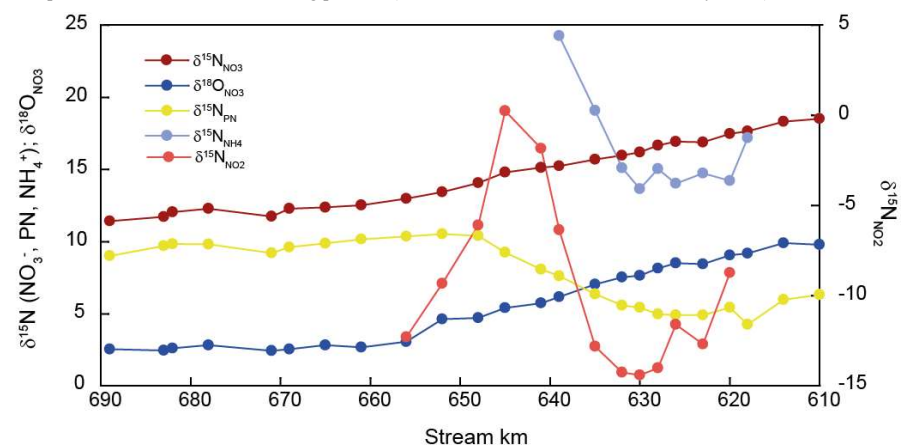
Figure 3: Isotopic changes of DIN and PN along the estuary

Nitrogen isotope composition of the suspended particulate matter ($\delta^{15}N$-SPM) is also most dynamic in the harbour region. $\delta^{15}N$-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, $\delta^{15}N$-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, $\delta^{15}N$-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 $\pm$ 1.3 ‰ (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, $\delta^{15}N$-NH4 was +17.7‰, values then decreased to ~15‰ in the harbor 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 $\pm$ 1.7‰.

To calculate the isotope effect of remineralization, we used an open system approach that was based on $\delta^{15}N$ of suspended particulate matter (ln(N%), Möbius 2013) across the entire transect. This approach yielded an isotope effect of ε_{remin}= 4.4 $\pm$ 0.8‰ (Fig. 4).
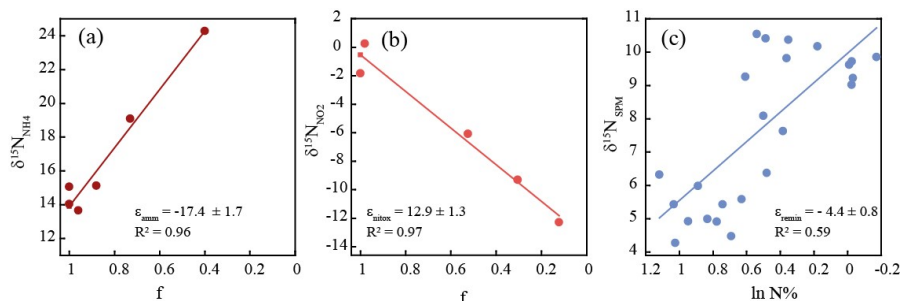
Figure 4: Overview of calculated fractionation factors for turnover of (a), ammonium, (b), nitrite, and (c), particulate nitrogen along the respective Elbe stretch. The initial f denotes the remaining fraction of the respective substrate. See sections 2.7and 3.3 for details of the calculation.

4. Discussion

4.1 Deriving N-cycling from isotope effects

Based on the computation of individual isotope effects of nitrogen cycling processes, we will now investigate the estuarine nitrogen cycle in more detail. While 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), their 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 to assess their isotopic signatures accurately.

In this study, we captured a rare event in the Elbe estuary 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 led to an unusually vast extension of nitrite and ammonium peaks in the estuary. This allowed a unique perspective on associated isotope effects of riverine N cycling processes.

The Elbe estuary is 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).

Currently, nutrient and carbon turnover is most dynamic within the harbor region (Amann et al., 2015;Sanders et al., 2018), where oxygen depletion at present frequently occurs in summer (Schöl et al., 2014). The isotope and concentration changes in our dataset underscore the role of this region. 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 characterize these dominant turnover processes and their driving forces.

Notably, nitrate isotope changes in the estuary only faintly reflect nitrate regeneration dynamics (Fig. 2A, Fig. 3). Sanders et al. (2018) recently evaluated nitrate production in the estuary. They found that changes in oxygen isotopes of nitrate were indicative of intense nitrification, and that newly added nitrate had an oxygen isotope

signature close to that of ambient water. This holds true in our dataset as well, we find a characteristic drop of oxygen isotope values in the harbor region indicating the incorporation of light oxygen from H_2O into nitrate. Nonetheless, in comparison to nitrite and ammonium isotope dynamics, changes of nitrogen isotopes in nitrate 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.

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 that are slightly higher than those measured by Sanders et al (2018) with the same method. Overall, the distribution of nitrification along the estuary matches the succession of ammonium and nitrite accumulation through the harbor basin and the previous assessment by Sanders et al. (2018). The spatial extent of ammonium and nitrite accumulation during our cruise enabled us to compute individual isotope effects for N-turnover processes.

Between stream km 626 and 639, we calculated the isotope effect for ammonium removal as -17.4 ‰, which is towards the lower end of pure 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‰, stream km 641 - 656) 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). The isotope effect thus appears unaffected by additional nitrite sinks or sources in this stretch. We thus assume that nitrite oxidation is the main sink for nitrite in this river section.

In the river section between stream km 626 and 646, ammonium concentration decreases, and ammonium and nitrite isotopes could be 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 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 nitrite. Within the given 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).

4.2 The role of suspended matter

Our results underscore that nitrification is a prominent N-cycling process in the harbor region. We will now investigate in detail the factors that control the magnitude of this key process. We find that 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.

Generally, the concentration and composition of suspended particulate matter, such as chlorophyll or particulate nitrogen concentration, varies along the Elbe Estuary (Fig. 2). Suspended matter in estuaries is very heterogeneous and is affected by remineralization, resuspension, and of course by phytoplankton growth (e.g., Middelburg, 2019;Middelburg and Herman, 2007). It is thus evident that SPM composition should be closely interlinked with nutrient and isotope dynamics. An indication for that is that nitrification is also highest in the upstream part of the stretch, where organic matter is fresh. Throughout the sampling stretch, N content (N%) of SPM and nitrification rates are closely correlated ($r^2 = 0.91$, cf. Table S1).

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

SPM reactivity is an important control of remineralization in the estuary. Based on 'Redfield' stoichiometry, fresh algal material should have a C / N ratio of ~ 7 (Redfield et al., 1963;Martiny et al., 2014;Middelburg, 2019), which is what we find in the uppermost samples. With ongoing degradation of organic material, this ratio increases. Easily degradable, nitrogen-rich compounds (e.g., amino acids or pigments) are remineralized, and carbon-rich organic matter remains (Islam et al., 2019;Middelburg, 2019;Middelburg and Herman, 2007).

Based on C / N ratios and chlorophyll, we find that SPM is not equally reactive throughout the estuary. O_2 saturation, an indicator for heterotrophic 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, 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 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.

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 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 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 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).

Hence, we use the sum of DIN plus particulate nitrogen (PN) to address transformations in the nitrogen pool. For mass balance calculations, please see supplementary material (S1). We find substantial fluctuations in the total nitrogen pool over the reach (as much as 16 μmol L^{-1} between two sampling points, Figure 5). However, these shifts are almost entirely controlled by fluctuations in the concentration of PN (Figure 5 and Table S1), and, in line with our previous assessments, show that PN affects estuarine N processing. Notably, there were no erratic changes to the δ^{15}N of the PN pool, but rather a smooth and continuous transformation over the entire 90 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 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.
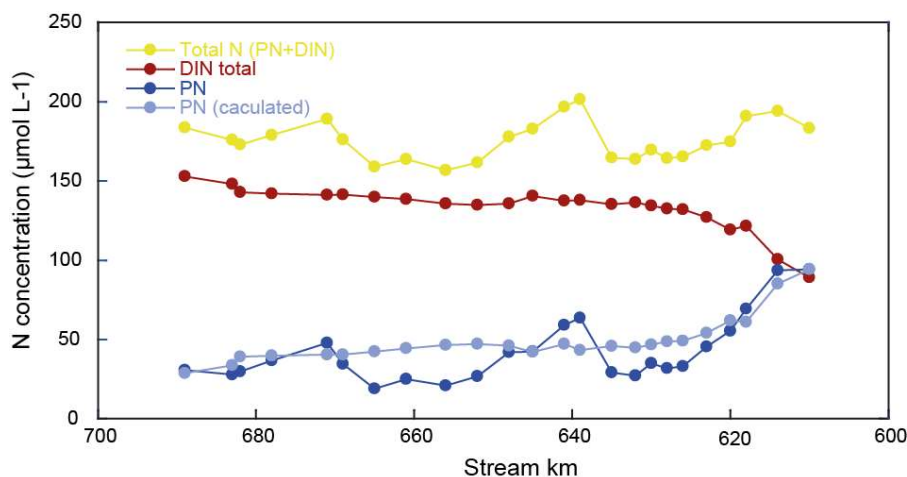
Figure 5: N concentration along the estuary. Total N fluctuations are driven by PN fluctuations. Light blue is the calculated concentration of particulate matter that is assumed to satisfy mass balance.

4.4 Implications of the N isotope mass balance

Finally, we will use our mass balance calculations to investigate whether we can track additional sources or sinks of nitrogen in the estuary that so far remained unrecognized. For this, we will turn to the isotope module of the mass balance calculations. Based on the assumptions of PN settling and resuspension, we were able to satisfy the mass balance for each 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 that our mass balance calculations are based upon. Over large sections of the transect, we find net removal of nitrogen, and that isotope values calculated for the lost 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 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 (Dähnke et al., 2008;Deek et al., 2013).
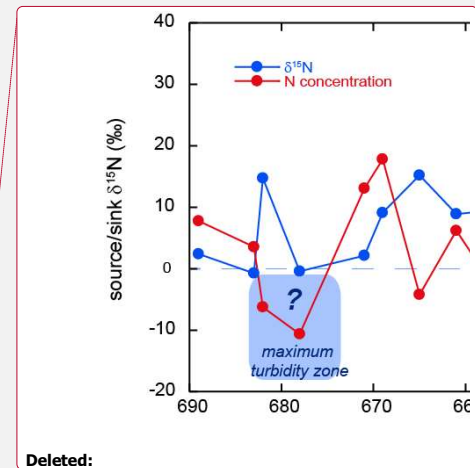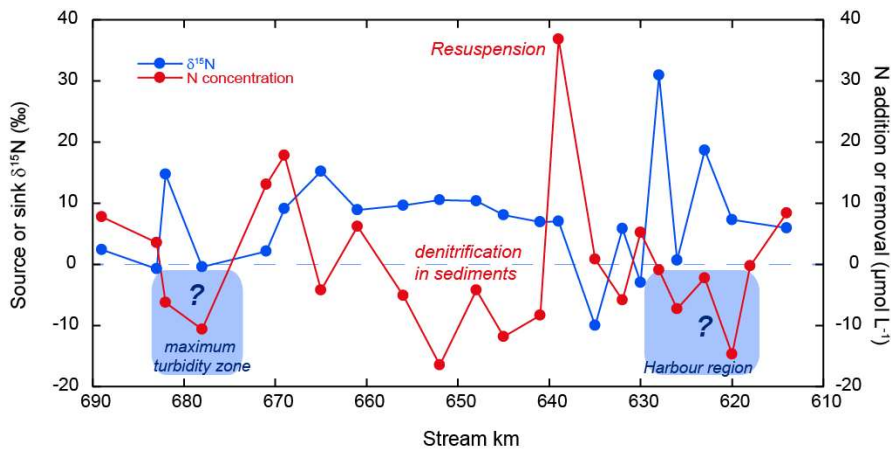
Figure 6: Isotope signature and concentration of N that is removed or added based on the outcome of the isotope mass balance calcualations. Boxes indicate cases in which the isotope composition of missing N rules out isotope-neutral processes like settling or resuspension of particulate matter or sedimentary denitrification

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 as high nitrous oxide production that cannot plausibly be explained by nitrification alone (Brase et al., 2017). Moreover, 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.

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 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 $\delta^{15}N$ 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 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 nitrification. The intensity of nitrification in the estuary is coupled to reactivity of particulate matter. Ammonium assimilation competes with nitrification and produces 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.

Overall, we assume that changes in particulate matter quality and transport 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.

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 suggestions and additions provided by YV.

Competing interests.

The authors declare that they have no conflict of interest.

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