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Hot Spots of Nitrification in the Elbe Estuary and Their Impact on Nitrate Regeneration

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Abstract Estuaries act as an organic matter and nutrient filter in the transition between the land, rivers and the ocean. In the past, high nutrient and organic carbon load and low oxygen concentration made the Elbe River estuary (NW Europe) a sink for dissolved inorganic nitrogen. A recent reduction in loads and subsequent recovery of the estuary changed its biogeochemical function, so that nitrate is no longer removed on its transition towards the coastal North Sea. Nowadays in the estuary, nitrification appears to be a significant nitrate source. To quantify nitrification and determine actively nitrifying regions in the estuary, we measured the concentrations of ammonium, nitrite and nitrate, the dual stable isotopes of nitrate and net nitrification rates in the estuary on five cruises from August 2012 to August 2013. The nitrate concentration increased markedly downstream of the port of Hamburg in summer and spring, accompanied by a decrease of nitrate isotope values that was clearest in summer exactly at the location where nitrate concentration started to increase. Ammonium and nitrite peaked in the Hamburg port region (up to 18 and 8 μmol L^{-1}, respectively), and nitrification rates in this region were up to 7 μmol L^{-1} day^{-1}. Our data show that coupled remineralization and nitrification are significant internal nitrate sources that almost double the estuary's summer nitrate concentration. Furthermore, we find that the port of Hamburg is a hot spot of nitrification, whereas the maximum turbidity zone (MTZ) only plays a subordinate role in turnover of nitrate.

Keywords Estuary · Nitrification · Stable isotopes

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Introduction

Anthropogenic use of fertilizers over the last 150 years has increased the nitrogen (N) load to the coastal zone via rivers and estuaries (Galloway et al. 2003). Among other consequences, the resulting eutrophication in coastal zones has led to increased oxygen depletion (Diaz and Rosenberg 2008), and supported denitrification in the water column and sediments, which in turn pushed estuaries to act as nitrate sinks (Brockmann et al. 1988; Seitzinger et al. 2006). In the German Bight, high nutrient loads of Rhine, Elbe, and Weser rivers supported the eutrophication of coastal waters (Radach and Pätsch 2007). However, over the last decades, improved waste water management and control of fertilizer application have decreased the riverine load of reactive nitrogen and especially nitrate (Pätsch et al. 2010; Radach and Pätsch 2007). In the Elbe River, the input of ammonium decreased significantly in the early 1990s (Bergemann and Gaumert 2010) after the reunification of Germany led to improved pollution management in the upstream regions. At the same time, higher algae activity resulted in higher oxygen saturation (Hardenbicker et al. 2014; Quiel et al. 2011). Today, nitrogen loads are lower than in the 1990s, and the oxygen concentration in the water column has improved. More recent studies have shown that these changes can modify estuarine processes. Dähnke et al. hypothesised (2008) that the relative importance of nitrification had increased in comparison to denitrification in the Elbe Estuary based on increasing nitrate concentration and the stable isotope signature of the nitrate. However, the quantitative

role of nitrification, its rates and the sites of maximum activity in the estuary were not assessed in detail and are still largely unknown. There are no recent data of nitrification rate measurements in the Elbe Estuary; the latest assessments date back to the 1990s, and thus were done just at the onset of a reduction of pollutants, organic carbon and nutrient inputs to the river and estuary (Kerner and Spitzy 2001).

This study aims to quantify nitrification in the tidal estuary in the view of these changed conditions in the catchment since the 1990s. Beside dissolved inorganic nitrogen (DIN) concentration and net nitrification rates in laboratory incubations, we determined dual stable nitrate isotopes, because nitrate isotopes can provide new insights into biological N turnover and N sources in estuaries (Dähnke et al. 2008; Middelburg and Nieuwenhuize 2001; Sebilo et al. 2006). Specifically, the combined analysis of $\delta^{15}N_{NO3}$ and $\delta^{18}O_{NO3}$ can be used to separate nitrate production (e.g. nitrification) and parallel nitrate consumption (e.g. denitrification and assimilation) (Granger et al. 2004; Wankel et al. 2006).

We identified the Hamburg port region in the freshwater part of the Elbe Estuary as the region of highest nitrification activity. Nitrification varied seasonally and was coupled to remineralization of fresh organic matter, the main source of nitrate regeneration. The occurrence of ammonium in the Hamburg port area was an indicator for nitrification, but clearly not its exclusive N source.

Material and Methods

Study Site

The Elbe River is the largest German river discharging into the North Sea and the largest source of total dissolved nitrogen (TDN) and nitrate for the inner German Bight, which is strongly affected by eutrophication (OSPAR Commission 2008) (Fig. 1). Its estuary extends over 142 km from a weir that separates the tidal from the limnic freshwater part of the river (stream-km 586) to the port of Cuxhaven, where the river enters the German Bight. Stream kilometres are according to the German navigation kilometre; the German-Czech border is defined as stream kilometre 0. Samples were taken in the estuary, between stream kilometre 610, upstream of the City of Hamburg, and 740 in the German Bight. The average river discharge is 704 $m^3 s^{-1}$ (measured at the gauge Neu Darchau, stream-km 536). Nearly 25 million people live within the 148,268 km^2 Elbe River catchment area (Lòzan and Kausch 1996). The water residence time in the estuary ranges from ~10 days during high discharge (~2000 $m^3 s^{-1}$) to ~72 days during low discharge (300 $m^3 s^{-1}$), or ~32 days at mean discharge (Boehlich and Strotmann 2008). The tidal estuary is dredged to enable access to the port of Hamburg, the third largest container port in Europe for large container ships. In consequence, the riverbed is suddenly deepened from ~5 m to up to 15 m, after stream kilometre 618 (Schöl et al. 2014).

Sampling

During five cruises from August 2012 to August 2013, water samples were collected along the Elbe Estuary from the mouth (stream-km 740) to Bunthaus Spitze (stream-km 610) few kilometres upstream of the port of Hamburg (Fig. 1). Cruises were scheduled to meet the onset of the biological activity including the spring bloom (April and May 2013), the middle of the growing season in summer (August 2012 and 2013) and its end (October 2012). The sampling campaigns were conducted with the R/V Ludwig Prandtl, M/S Vogelsand or Störort (Table 1). Samples were taken from 1 m below the water surface with a membrane pump system. Turbidity, salinity, pH and oxygen saturation were measured continuously on-board with the online in situ Ferrybox system (Petersen et al. 2011) in April 2013 and October 2012. During cruises in August 2012, May 2013 and August 2013, these parameters were measured in a flow-through system with a multi probe (YSI 6600). For clarity, we presented 5-min means of these continuous measurements for distinct water sampling stations.

Water samples for nutrient and isotope analysis were immediately filtered through pre-combusted GF/F filters (4 h, 450 °C) and stored in acid-washed (10% HCl overnight) PE-LD (polyethylene low density) bottles. Water samples were frozen on-board at −18 °C for later analysis of nutrients and nitrate isotopes. For determination of nitrification rates, 2 L unfiltered water samples was taken.

Nutrient Analysis

All filtered water samples were analysed in duplicate for concentration 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.

Net Rates of Nitrification

Nitrification rates were derived from produced nitrite and nitrate of incubated water samples without adding substrate like ammonium or nitrite. The rates were calculated by the increase of nitrite and nitrate per time, so that we did not distinguish between ammonium oxidation and nitrite oxidation. During the incubation, the already contained ammonium and the newly produced ammonium by remineralization of organic matter are the substrates for the nitrification. For that approach, 50 mL of freshwater was incubated in four replicates in 100-mL glass bottles on a shaker with open caps in the dark at room temperature. The incubation was started immediately

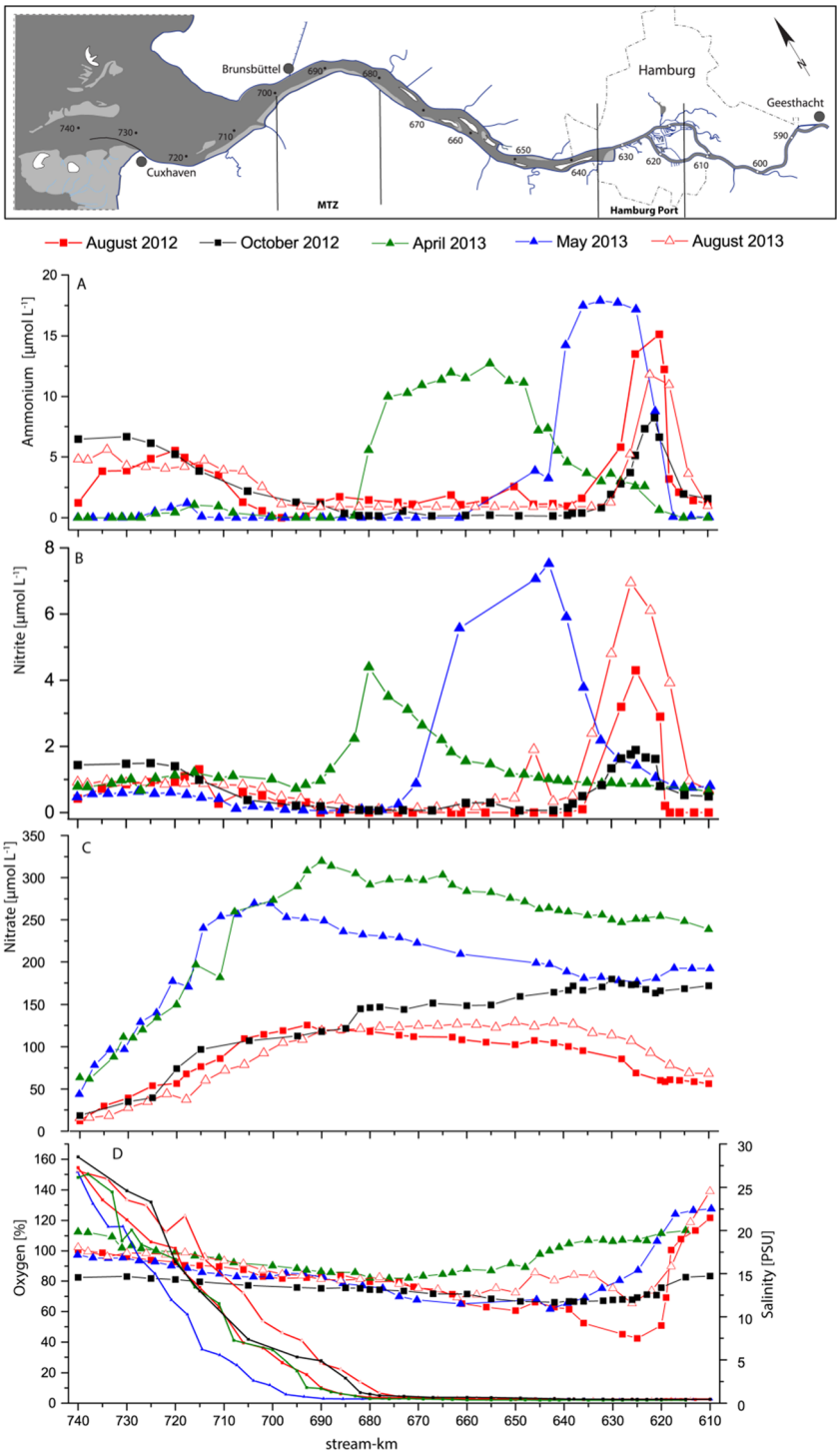

Fig. 1 Dissolved inorganic nitrogen (DIN; ammonium, nitrite and nitrate) and oxygen saturation and salinity in the Elbe Estuary. **a** Ammonium concentration. **b** Nitrite concentration. **c** Nitrate concentration. **d** Salinity (*small symbols*) and oxygen saturation (*large symbols*); stream-km based on the river kilometres starting at the German-Czech border

after sampling on-board during the cruises in October 2012 and April 2013. During the other cruises in August 2012 and 2013, the incubations were started after the transport to the lab, because incubation experiments on the research vessel were not possible. In May 2013, no nitrification measurements were conducted; to avoid heavy changes in the nutrient concentration and metabolism of the nitrifiers, the samples were stored at 4 °C up to 2 days during the storage and transport to the lab. Sub-samples were taken daily or several times a week, 1 mL of sample was centrifuged (15 min, 13,000×*g*) and nitrite and nitrate concentrations in the supernatant were determined immediately by using the HPLC method (Jasco, Germany) (Meincke et al. 1992), because for this methods only a small volume was needed. The incubations were continued (up to 14 days) until nitrite and nitrate concentrations in the samples were stable. The rates were

Table 1 Cruises in the Elbe Estuary

Date	Research vessel	Temperature[a] (°C)	Discharge[b] (m^3/s)
August 2012	MS Vogelsand/MS Störort	20.7 ± 0.9	421
October 2012	RV Ludwig Prandtl	12.5 ± 0.3	373
April 2013	RV Ludwig Prandtl	11.1 ± 1.9	1074
May 2013	MS Vogelsand/MS Störort	15.1 ± 1.8	780
August 2013	MS Vogelsand/MS Störort	20.0 ± 0.7	384

[a] Mean temperature stream-km 610 to 740 and standard derivation

[b] Measurement at gauge Neu Darchau

calculated at the linear and steepest increase of nitrite or nitrite plus nitrate concentration, to exclude lower rates during the lag phase of the incubation.

Stable Isotope Analysis

The dual stable isotope analysis of nitrate was used to distinguish parallel processes that can change the isotope composition and concentration of nitrate in the water column. All water samples were analysed for dual isotopic composition of nitrate plus nitrite ($\delta^{15}N_{NOx}$ and $\delta^{18}O_{NOx}$) using the denitrifier method (Casciotti et al. 2002; Sigman et al. 2001), which is based on the isotopic analysis of nitrous oxide (N_2O). Denitrifying bacteria (*Pseudomonas aureofaciens*, ATCC#13985) that lack N_2O reductase and produce N_2O as final product of denitrification were used for reduction. Nitrate and nitrite of a sample were converted to nitrous oxide. The injected sample volume was adjusted to achieve a sample size of 10 nmol of N_2O. N_2O was extracted from the sample vials 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 two internal standards ($\delta^{15}N$ 1.4 and 7.1‰) were run. The standard deviation of samples and standards was <0.2‰ for $\delta^{15}N_{\mathbf{NOx}}$ (n = 4) and <0.5‰ for $\delta^{18}O_{\mathbf{NOx}}$ (n = 4). In the majority of samples, nitrite was less than 1%, but especially in some samples in the Hamburg port area or in the marine part in the outer estuary with PSU above 20, the percentage was up to 10%. We present the mixed isotope signal of nitrate and nitrite, because the changes of the bulk δ values across the entire estuary were the focus of this study. We will address this issue in the discussion. For the sake of clarity, we refer to isotope signatures as $\delta^{15}N_{NOx}$ and $\delta^{18}O_{NOx}$ hereafter.

Statistical Analysis

All statistical analyses including the test for normal distribution, T tests and Pearson rank correlation analysis were performed with the IBM-SPSS software package version 18 (IBM Corp., Armonk, NY).

Results

DIN Concentration in the Elbe Estuary

The main dissolved inorganic nitrogen (DIN) compound in the Elbe Estuary was nitrate. At the upstream sampling site (stream-km 610), nitrate concentration was ~50 μmol L^{-1} in summer (August 2012/2013), and 150 to 250 μmol L^{-1} in autumn and spring (October 2012, April and May 2013). In summer and spring, nitrate concentration increased from the port of Hamburg (stream-km 615 to 635) to the onset of the salinity gradient in the maximum turbidity zone (MTZ, stream-km 680 to 700) (Fig. 1c). In autumn (cruise October 2012), the nitrate concentration generally decreased along the estuary. In the salinity gradient, a conservative mixing with sea water was observed in all seasons, indicated by a linear decrease in a concentration vs. salinity plot (not shown). One exception was the cruise in October, where we found a loss of nitrate at low salinities and in the freshwater part.

Ammonium and nitrite concentrations were at least an order of magnitude lower than the nitrate concentration, but there was a distinct peak of ammonium maximum closely followed by a peak nitrite maximum in summer and autumn in the port area (stream-km 615 to 635) (Fig. 1a, b). These maxima were shifted downstream during the two spring cruises (May and April 2013), at higher discharge (Table 1). The maximum ammonium concentration of each longitudinal transect ranged from 8.2 μmol L^{-1} in October 2012 to 17.9 μmol L^{-1} in May 2013. We also observed a distinct nitrite maximum in the port region in summer/autumn, which was also shifted downstream in spring, with maximum concentration from 1.9 μmol L^{-1} in October 2012 to 7.5 μmol L^{-1} in May 2013. At the onset of the salinity gradient, a slight increase of ammonium and nitrite was visible, mainly because of remineralization of organic matter stemming from the Wadden Sea area or from SPM in the MTZ.

Net Nitrification Rates

In general, nitrification rates in summer (August 2012/2013) were highest in the Hamburg port region (stream-km 615 to 635) with a nitrate production of up

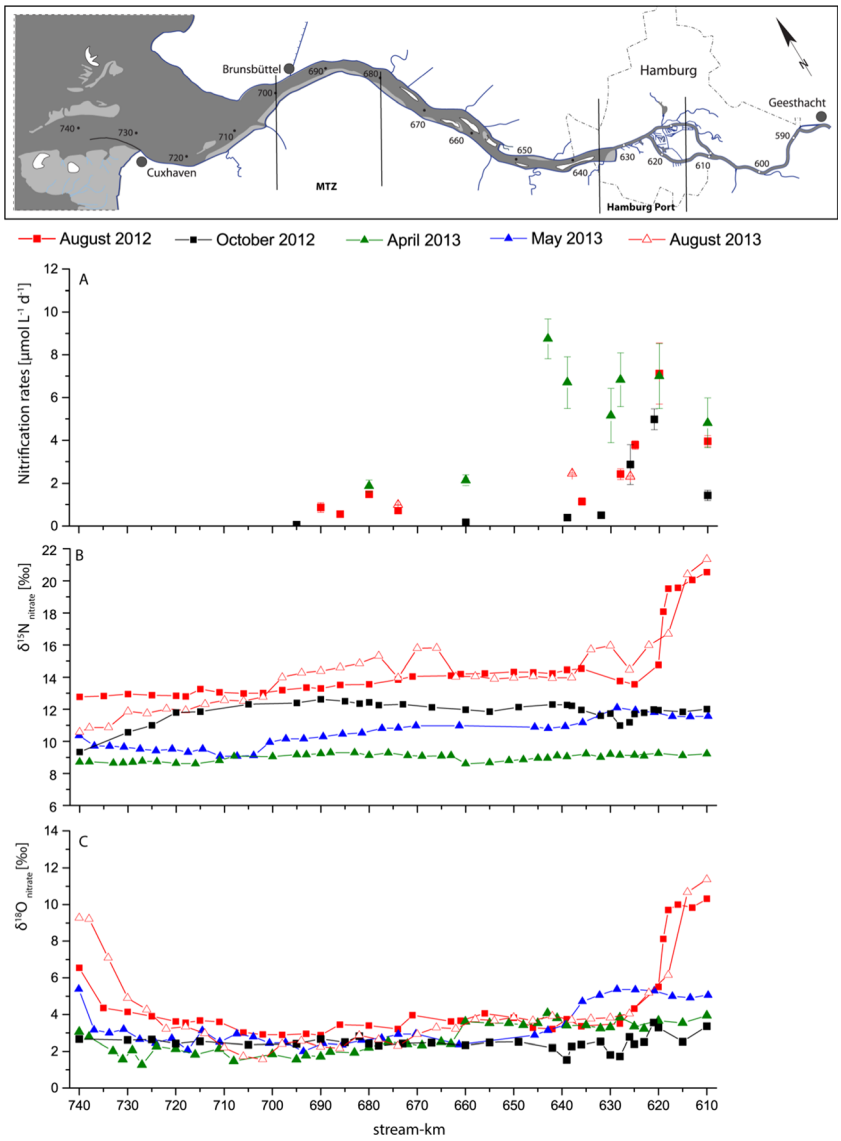

Fig. 2 Nitrification rates and dual stable isotopes of nitrate plus nitrite in the Elbe Estuary. **a** Nitrification rates (µmol L^{-1} day^{-1}) and standard derivation of four replicates. **b** $\delta^{15}N_{NOx}$. **c** $\delta^{18}O_{NOx}$; stream-km based on the river kilometres starting at the German-Czech border

to 7 µmol L^{-1} day^{-1} (Fig. 2a). The nitrification rates in August 2012 in the Hamburg port were significantly higher than the rates in the outer estuary ($p \leq 0.05$). In August 2013, the rates of two samples in the port region were statistically similar, but significantly higher than in the lower estuary at stream-km 675 ($p \leq 0.05$). The rates decreased downstream of the Hamburg port, and were lowest (1–2 µmol L^{-1} day^{-1}) in the outer estuary (from stream-km 670). In April 2013, the zone with increased nitrification activity was wider than in summer (stream-km 610 to 643), and the highest rates (9 µmol L^{-1} day^{-1}) were found further downstream than during summer. Minimum nitrification rates in spring were still as high as 2 µmol L^{-1} day^{-1} and were thus clearly elevated over summer rates. In autumn, October 2012, nitrification rates were lowest, but highest activities in the Hamburg port area were still up to 5 µmol L^{-1} day^{-1}.

Dual Stable Isotope Signatures of Nitrate Plus Nitrite

In the upper estuary, the dual isotope signature of nitrate showed the most pronounced variation with the seasons, leading to enriched values in summer, and more depleted values in winter/spring (Fig. 2b, c). In comparison to winter data (~8‰ for $\delta^{15}N_{NOx}$ and ~2‰ for $\delta^{18}O_{NOx}$, own unpublished data and Johannsen et al. 2008), in summer (August 2012/2013) $\delta^{15}N_{NOx}$ and $\delta^{18}O_{NOx}$ were strongly enriched at stream-km 610 after entering the tidal estuary (>20 and >10‰ for $\delta^{15}N_{NOx}$ and $\delta^{18}O_{NOx}$, respectively). Dual isotope values dropped rapidly to 14‰ for $\delta^{15}N_{NOx}$ and 3‰ for $\delta^{18}O_{NOx}$ in the Hamburg port area. In summer, $\delta^{18}O_{NOx}$ increased in the salinity gradient towards the North Sea downstream stream-km 720.

In spring and autumn, the nitrate in the beginning of the estuary at stream-km 610 was less enriched $\delta^{15}N_{NOx}$ and

$\delta^{18}O_{NOx}$. The $\delta^{18}O_{NOx}$ values were between 2 and 4‰ from stream-km 640 to 700 and thus similar to winter values. In spring and autumn, $\delta^{15}N_{NOx}$ was only slightly enriched above winter values, with maximum isotope values of ~12‰ in October, ~9‰ in April and ~11‰ in May (Fig. 2b, c). $\delta^{15}N_{NOx}$ and $\delta^{18}O_{NOx}$ decreased markedly in the freshwater estuary. In autumn and spring, these changes were less pronounced, and *d* values remained relatively stable (Fig. 2b, c).

Discussion

The Zone of High N Turnover

The main aim of this study was to identify the area of hot spots of nitrification in the Elbe Estuary. Previous studies already identified nitrification as an internal process and therefore as an important nitrate source in the Elbe Estuary (Bergemann et al. 1996; Kerner and Spitzy 2001). Dähnke et al. (2008) and Schlarbaum et al. (2010) concluded, based on isotope investigations in the salinity gradient and the maximum of nitrate in the maximum turbidity zone (MTZ), that this seems to be the main zone of nitrification. These studies, however, did not encompass the Hamburg port area. The MTZ, in general, is likely to support high nitrification rates by particle-bound nitrifiers, because it is the most prominent place of N turnover in other estuaries. In the MTZ, salinity and turbidity increase, so that algae and other plankton organisms that cannot tolerate the increase in light limitation and salinity die off in this region (Muylaert et al. 2000; Telesh et al. 2013). This is the case in other European estuaries, like the Scheldt and Gironde estuaries (Muylaert and Sabbe 1999). According to our assessment, nitrification does occur in the MTZ, but at comparatively low rates. The zone of the most pronounced N turnover and nitrification is the freshwater region of the Elbe Estuary, which includes the Hamburg port region. This decoupling of the MTZ and the zone of highest N turnover can be attributed indirectly to the increase in water depth (from 5 to 15 m) along the longitudinal axis of the Elbe Estuary, due to dredging activities to promote the accessibility of the port of Hamburg. When algal cells reach the port region, the deep water section of the Elbe Estuary, the aphotic zone, dramatically increases and the algae die off, may be due to light limitation, sinking or grazing by zooplankton (Schöl et al. 2014). Consequently, large amounts of fresh, easily degradable organic matter are released into the water column (Kerner and Spitzy 2001). This decoupling is also marked by the production of dissolved organic nitrogen (DON), which is formed during the remineralization of organic matter in this section of the estuary (Schlarbaum et al. 2010). Similar changes concerning the hot spots of nitrification occurred in the Seine Estuary (Garnier et al. 2001).

The decomposition of organic matter creates an optimal habitat for heterotrophic bacteria, as well as for autotrophic nitrifiers, which processes the available ammonium and contributes to the oxygen depletion in the port region of the Elbe Estuary (Amann et al. 2012; Bergemann et al. 1996; Schöl et al. 2014). The zone of maximum nitrification overlaps with the well-studied oxygen-depleted section of the Elbe Estuary and nitrification and therefore contributes to the formation of oxygen depletion. In the 1980s, up to 50% of the dissolved oxygen was consumed by nitrification (Kerner et al. 1995). Since the early 1990s, the NH_4^+ concentration in the estuary has decreased, suggesting that nitrification will not dominate oxygen consumption any longer (Kerner et al. 1995), because ammonium concentration can be the fundamental driver of nitrification. This was the case in the Scheldt in the 1970s (Billen 1975), and while similar assessments for the Elbe are lacking, we presume that the underlying mechanisms were the same. For the contemporary Elbe Estuary, Schöl et al. (2014) used a one-dimensional water quality model to estimate the contribution of nitrification and found that in summer 2006, nitrification was responsible for 16 to 32% of the overall oxygen consumption in the Elbe Estuary.

We did not determine oxygen consumption, but according to monitoring data (River Basin Community, Elbe 2014), the biological oxygen demand over 7 days (BOD_7) in summer months at station Seemanshöft (stream-km 628) was 4.8 mg O_2 L^{-1} in August 2012, or 150 μmol L^{-1} $week^{-1}$. In this month, we measured a mean nitrification rate of 5.3 μmol L^{-1} day^{-1}, or 37.1 μmol L^{-1} per week in the port region. Assuming that 2 mol of O_2 is consumed per mole nitrate that is produced, our maximum nitrification rate is equivalent to an O_2 consumption of 74 μmol O_2 and would thus make up for 25% of the total oxygen consumption, which is in line with the modelling approach by Schöl et al. (2014). We note that our rate measurements are not comprehensive, and in e.g. in April, rates are higher (up to 9 μmol L^{-1} day^{-1}), which then results in a substantially higher share of nitrification in oxygen consumption. Even though our incubation assay doubtlessly is favourable of nitrification, this shows that nitrification can make up for a substantial share of oxygen consumption, although the dominant oxygen consumption process in this part of the Elbe appears to be respiration. This is supported by the fact that nitrification rates were not correlated with oxygen saturation (Table 2).

All these measurements identify the Hamburg port region as an important hot spot of nitrification; nevertheless, we see a clear indication for an increase in nitrate concentration due to nitrification along the entire estuary. In summer, the average increase of nitrate between the Hamburg port region (stream-km 615 to 635) and the maximum turbidity zone (MTZ) (stream-km 680 to 700) was ~60 μmol L^{-1} (Table 2). This nitrate increase reflects the distribution and magnitude of nitrification, and the nitrate concentration and nitrification rates

Table 2 Results of a Pearson rank correlation analysis between nitrification rates and related characteristics determined in the water column and in the sediment of the Elbe Estuary

	Salinity	Temperature	Turbidity	pH	Oxygen saturation	Ammonium	Nitrite	Nitrate	$\delta^{15}N_{N\alpha x}$	$\delta^{18}O_{N\alpha x}$	Nitrification rates
Salinity		0.183	**−0.231**	**0.207**	**0.356**	−0.108	−0.103	**−0.554**	**−0.250**	−0.032	−0.222
Temperature	0.017		**0.299**	**−0.259**	−0.198	0.080	−0.016	−0.512	0.780	0.480	0.580
Turbidity	**0.007**	**0.000**		**−0.265**	−0187	**−0.225**	−0.093	−0.098	**0.271**	0.014	−0.029
pH	**0.007**	**0.001**	**0.002**		**0.869**	0.034	−0152	0.033	−0.095	**0.423**	−0.091
Oxygen saturation	**0.000**	0.010	0.030	**0.000**		−0.142	**−0.220**	0.002	−0.123	**0.365**	−0.180
Ammonium	0.161	0.303	**0.009**	0.659	0.065		**0.486**	0.061	−0.017	**0.246**	**0.581**
Nitrite	0.182	0.835	0.281	0.048	**0.004**	**0.000**		0.125	−0.086	0.003	0.363
Nitrate	**0.001**	**0.000**	0.255	0.669	0.977	0.427	0.104		**−0.589**	**−0.381**	**−0.492**
$\delta^{15}N_{N\alpha x}$	**0.001**	**0.000**	**0.001**	0.220	0.112	0.823	0.263	**0.000**		**0.629**	**0.497**
$\delta^{18}O_{N\alpha x}$	0.681	**0.000**	0.780	**0.000**	**0.000**	**0.001**	0.967	**0.000**	**0.000**		**0.645**
Nitrification rates	0.275	**0.002**	0.887	0.659	0.380	**0.001**	0.063	**0.009**	**0.008**	**0.000**	

The correlation coefficients are presented above the diagonal, the two-tailed significant values below the diagonal. The bold number represented a significant correlation ($p \leq 0.01$)

were significantly correlated ($p \leq 0.01$) (Table 2). Monitoring data of the River Basin Community, Elbe (RBC) confirm that this is a recurrent phenomenon: The average summer concentration of nitrate from 2010 to 2014 increased from approximately 100 μmol L^{-1} at station Zollenspieker (stream-km 598; July and August, $n = 10$) to 140 μmol L^{-1} at stream-km 693 (July and August, $n = 8$) in the MTZ, where the nitrate maximum occurs (River Basin Community, Elbe 2014). The increase depends on processing rates and is therefore coupled to discharge, so that during higher discharge (April and May in our study), the maximum occurs further downstream compared to the summer cruises in August.

Net Nitrification in the Elbe Estuary

Another main aim of the study was to consider the seasonal variability of nitrification and its role in nitrate regeneration. Our results have shown that nitrification rates varied seasonally as well as regionally in the Elbe Estuary. The highest rates were found in the Hamburg port area (stream-km 615 to 635) with up to 7 μmol L^{-1} day^{-1} in summer. The water temperature seems to be an important driver for nitrification rates, indicated by a clear correlation (Table 2). The production of new nitrate and the increase in DIN during the nitrification rate measurements indicates that nitrification was not only fuelled by ammonium present in the water sample, but that another source of ammonium was used. In all incubations, the increase in nitrate concentrations by the end of the incubation exceeded the amount of ammonium that was present in the water sample. In an average of all incubations, the ammonium concentrations were 20% of the produced nitrate with a minimum of 5% and a maximum of 50%.

These measured nitrification rates fit with modelled mean summer nitrification rates in the Elbe Estuary, which range from 7.3 to 11.0 μmol L^{-1} day^{-1} between km 629 and km 660 (Schöl et al. 2014). In general, nitrification rates in estuaries can vary strongly; Damashek et al. (2016) have given an overview on nitrification rates in estuaries worldwide and described a range from near zero to 336 μmol L^{-1} day^{-1}. Compared to the listed maximal rates, our measured rates are relatively low. A comparison with previous assessments of nitrification in the Elbe is problematic. Many studies measured potential nitrification rates, which were measured upon substrate addition and are therefore higher than net nitrification rates. Nevertheless, the resulting nitrification rates in the estuary in the 1950s and 1980s were significantly higher, between 48 and 1176 μmol L^{-1} day^{-1} (Brunhoeber 1990; Rheinheimer 1959; Sowitzki 1992). In a more recent study without ammonium addition, Kerner and Spitzy (2001) found rates from 16 to 122 μmol L^{-1} μM^{-1} day^{-1}, thus lower than previous rate measurements, but still substantially higher than our measurements. In that study, the ammonium concentration in summer had decreased in comparison to previous years and was below 20 μmol L^{-1}, which is comparable to the concentrations we measured. These authors' high nitrification rates can be attributed to the fact that the initial remineralization of easily accessible DON and picoplankton was measured, whereas our lower rate measurements represent nitrification that is coupled to—potentially slower—remineralization of naturally occurring suspended particulate matter (SPM).

So which changes in the Elbe Estuary in the last 50 years may have affected nitrification rates? The most obvious driver was the water column ammonium concentration. Historical ammonium concentrations in the Elbe Estuary were high. When we have a look at the ammonium concentration of the studies presented above, they ranged from 250 to 1200 μmol L^{-1} in 1958/1959 to 15 and 125 μmol L^{-1} in the late 1980s (Sowitzki 1992). But they

were hardly comparable because of different sampling sites and times. At station Seemannshöft (stream-km 628), a long-term sampling station in the Hamburg port, ammonium concentration decreased from 5 mg L^{-1} (~350 μmol L^{-1}) in the beginning of the 1980s to >0.5 mg L^{-1} (35 μmol L^{-1}) at the end of the 1990s (Database of River Basin Community, 2014). In the period 2005 to 2015, the annual mean ammonium concentration was 0.16 mg L^{-1} (~10 μmol L^{-1}) with a maximum of 0.45 mg L^{-1} in winter 2005 (Database of River Basin Community, 2016).

Previous studies suggested that high ammonium concentration was an indicator for high nitrification rates (Böttcher et al. 1995; Schäfer and Harms 1995) and that the ubiquitous decrease in ammonium concentration in the early 1990s caused a decrease in nitrification. Our measurements add further evidence; they show that the nitrification rate has indeed decreased alongside with ammonium concentration, but that it remains an important nitrate source despite decreasing water column ammonium. Furthermore, nitrification rates in the Elbe Estuary are comparatively high where ammonium is detectable (e.g. in the Hamburg port area), but nitrification activity was either detected in all sections of the Elbe Estuary, even when ammonium was below the detection limit of 0.5 μmol L^{-1}. Nevertheless, we found a correlation between ammonium concentration and nitrification rates ($p \leq 0.01$) (Table 2). In spring (April 2013), however, there was a mismatch between ammonium concentration and maximum nitrification rates, the highest rates were found between stream-km 620 and 643 and the ammonium concentration was highest between stream-km 648 and 676. Together with the increase of nitrate above original ammonium levels in the nitrification rate incubations, this suggests that there must be another source for nitrification than ammonium.

An important source of N for nitrification is remineralization of organic matter (Kerner and Spitzy 2001), which explains the high rates we found in the port region. Amann et al. (2012) reported strong particulate organic carbon remineralization in the Hamburg port area, which will also release ammonium as a substrate for nitrification. In the past, nitrification was driven by ammonium feed by external sources, while today ammonium is mainly derived from internal sources, i.e. the remineralization of organic matter. The strong correlation of nitrification with ammonium and SPM concentrations (Damashek et al. 2016) can be seen as additional evidence—high remineralization due to high SPM will generate ammonium, which then is readily nitrified. Thus, remineralization and immediate oxidation of this fresh ammonium fuel nitrate production in the estuary, and the high nitrification rates, prevent a more pronounced accumulation of ammonium or nitrite.

Seasonal Changes of Isotope Values in the Estuary

Nitrate that enters the estuary from upstream was altered concerning concentration and isotope signature dependant on biological nitrate processing in the upstream Elbe River and its catchment. In April 2013, water temperature was low, as were the abundance and activity of phytoplankton, indicated by the low chlorophyll concentration (<15 μg L^{-1}) in comparison to summer (100 μg L^{-1}, data not shown). As a consequence, the isotope values and concentration of nitrate closely resembled typical winter conditions (Johannsen et al. 2008), with $\delta^{15}N_{NOx}$ of 9.2‰ and $\delta^{18}O_{NOx}$ of 3.9‰ and nitrate concentration above 240 μmol L^{-1}. In May 2013, $\delta^{15}N_{NOx}$ and $\delta^{18}O_{NOx}$ were slightly enriched between stream-km 610 and 640 and nitrate concentrations were lower than in April, indicating the onset of nitrate assimilation by phytoplankton. In August 2012 and 2013, $\delta^{15}N_{NOx}$ and $\delta^{18}O_{NOx}$ are clearly enriched, up to 20 and 10‰, respectively, and the nitrate concentration was relatively low (~60 μmol L^{-1}). This was also indicated by a negative correlation between nitrate and its dual stable isotope ($p \leq 0.01$) (Table 2). This nitrate consumption and isotopic enrichment is mainly due to assimilation by phytoplankton in the upstream river (Deutsch et al. 2009; Johannsen et al. 2008; Pusch et al. 2009), a process associated with an equal fractionation of nitrate N and O isotopes (Granger et al. 2004). In October, nitrate concentration increases again, and the dual isotopes are more depleted in comparison to summer. This is indicative of decreasing phytoplankton activity.

If we aim to determine the impact of nitrification on the isotope signal in the estuary, this is obviously best done in a case where phytoplankton activity in the upstream river is dominant, i.e. when we see the strongest isotope enrichment. In summer, the highly enriched $\delta^{15}N$ and $\delta^{18}O$ of nitrate/nitrite dropped quickly in the Hamburg port region, which our rate measurements identified as the region with maximum nitrification.

It should be noted here that we measured the combined nitrate/nitrite pool, and that isotope values of nitrite were not determined separately. The contribution of nitrite was mostly below 1%, but did, especially in the port of Hamburg, sometimes reach 10%, exactly where we found the strongest changes of $\delta^{15}N_{NOx}$ and $\delta^{18}O_{NOx}$. This does introduce a slight bias in the nitrate isotopes, but here, we focus on the absolute decrease of $\delta^{15}N_{NOx}$ and $\delta^{18}O_{NOx}$ values (from 22 to 14 and 10 to 4‰, respectively, between stream-km 610 and 680 due to nitrification). Nitrite is not relevant at these boundary stations, and will not affect our calculations or conclusions.

We calculated the isotope values of the additional nitrate (δ_{ad}) based on $\delta^{15}N_{NOx}$ and $\delta^{18}O_{NOx}$ of nitrate between stream-km 610 and 680 with a simple mixing model:

$$\delta_{ad} = \left(\left(\delta_{680} {}^{*} C_{680}\right) - \left(\delta_{610} {}^{*} C_{610}\right)\right) / \left(C_{680} - C_{610}\right) \quad (1)$$

where C_{610} and C_{680} represent the nitrate concentration at stream-km 610 and 680, respectively, δ_{610} and δ_{680} refer to the isotope signature ($\delta^{15}N_{NOx}$ or $\delta^{18}O_{NOx}$) of nitrate at the corresponding stream-km and δ_{ad} is the isotope signature of

freshly produced nitrate. The respective stream kilometres were chosen as boundaries because stream-km 610 is upstream of the port area and of the ammonium and nitrite peaks, and stream-km 680 represents the onset of the salinity gradient and point of the maximum nitrate concentration.

The calculated $\delta^{15}N_{NOx}$ values of the additional nitrate from stream-km 610 to 680 varied between 5.7 and 8.6‰ (Table 3), which is similar to winter values. The $\delta^{15}N$ value is determined by the isotope signature of the presumed N source. The $\delta^{15}N_{NOx}$ value of particulate organic matter in the estuary is ~5 to 10‰ and thus in the same range as our assumed source of nitrification (Schlarbaum et al. 2011). Isotope fractionation can widely be neglected because (1) the assumed isotope fractionation for remineralization is very low (Möbius 2013) or close to zero (Kendall 1998) and (2) ammonium and nitrite were completely oxidized in the end, so that fractionation does not occur.

The decrease in oxygen isotope values is regarded as an even more sensitive indicator of nitrification. Oxygen that is incorporated into nitrate derives from dissolved oxygen in the water ($\delta^{18}O$ = 23.5‰; Kroopnick and Craig 1972) and, to a large extent, from the water itself (Andersson and Hooper 1983). Elbe water has an average $\delta^{18}O_{H2O}$ value of −8‰ (Johannsen et al. 2008). In one of the few studies that dealt with riverine nitrification, Sebilo et al. (2006) assumed a $\delta^{18}O$ value of 3‰ for newly produced nitrate in the Seine River based on the assumption that one third of the incorporated oxygen derives from O_2 and two thirds stem from H_2O (Fiencke et al. 2005).

Due to various fractionation effects during nitrite oxidation, and equilibrium exchange of NO_2^- and H_2O, the nitrate isotope value can deviate from this theoretical value. Buchwald et al. (2012) presented incubation and environmental data that showed that the $\delta^{18}O_{NO3}$ of newly produced nitrate in the ocean (with $\delta^{18}O_{H2O}$ = 0‰ and $\delta^{18}O_{O2}$ = 24.2‰) most likely lies between −1.5 and +1.3‰. This is close to the value of the ambient water. In our study, we find that the calculated $\delta^{18}O$ value of the newly added nitrate between stream-km 610 to 680 ranges from −2.8 to −9.5‰ (Table 3), which is also close to that of ambient water in the Elbe. The variations in the isotope value of new nitrate may be triggered by the different accumulation times of nitrite—greater nitrite accumulation promotes the exchange with water (Buchwald et al. 2012) and would in our case lead to lower isotope values. The $\delta^{18}O_{NO3}$ value can of course also be elevated due to other processes that occur simultaneously, like nitrate assimilation in summer.

Generally, nitrification leads to a dilution of the existing nitrate pool with fresh nitrate that is always depleted in both $\delta^{15}N$ and $\delta^{18}O$. The decrease of isotope values in the port area is supported by our laboratory-based nitrification rates and identifies the port of Hamburg as the zone of intensive nitrification. In spring and autumn, we did not see such a clear decrease of $\delta^{18}O_{NOx}$, because the incoming nitrate was not enriched in $\delta^{15}N_{NOx}$ or $\delta^{18}O_{NOx}$ due to less phytoplankton activity. Isotope signatures can only be used to identify nitrification if upstream phytoplankton assimilation has already caused a substantial enrichment of nitrate isotope signatures.

Overall, our data clearly demonstrated that nitrification is an important nitrate source in the Elbe Estuary. The similarity of winter background isotope values (which have an isotope signature that resembles soil nitrate; Kendall 1998) and those found in summer downstream the port of Hamburg can be explained, if we assume that nitrification is important across the entire catchment: in the water column, at the sediment-water interface and in soils (Mayer et al. 2002)—and including the estuary itself. Nitrification in all these environments produces nitrate that is isotopically light with respect to $\delta^{18}O$ (Sebilo et al. 2013). Nevertheless, we note that this hypothesis needs to be validated in future investigations.

Table 3 Nitrate and corresponding dual stable isotope changes between stream-km 610 and 680

Date	Nitrate (μmol L^{-1})		Nitrate increase[a]		$\delta^{15}N_{NOx}$ [‰]			$\delta^{18}O_{NOx}$ [‰]		
	Stream-km 610	Stream-km 680	μmol L^{-1}	%	Stream-km 610	Stream-km 680	Added nitrate[b]	Stream-km 610	Stream-km 680	Added nitrate[2]
August 2012	56	118	62	111	20.5	13.5	7.2	10.3	3.4	−2.8
October 2012	172	146	−26	−15	12.0	12.4	n.a.	3.4	2.4	n.a.
April 2013	239	291	52	22	9.2	9.1	8.6	3.9	2.2	−5.6
May 2013	192	232	40	21	11.5	10.5	5.7	5.0	2.5	−9.5
August 2013	68	123	55	81	21.4	15.3	7.8	11.4	2.6	−8.3

n.a. not analysed

[a] Increase of nitrate were calculated based on the data on stream-km 610 and 680

[b] δ values of the newly produced nitrate based on the δ values at stream-km 610 and 680

Conclusion

We found that the Elbe Estuary in summer and spring is a clear source of nitrate, produced by nitrification in the water column of the freshwater part mainly in the Hamburg port area, while the maximum turbidity zone showed comparable low nitrification rates. Intense nitrification during summer times doubles the nitrate concentration, at rates that are much higher than the observed ammonium concentration in the estuary suggests. The nitrate maximum at the onset of the salinity gradient of the Elbe Estuary is thus determined by re-mineralization/nitrification activity in the upper part of the estuary, and hence depends on upstream phytoplankton growth which produces degradable organic matter.

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