Stable isotope investigations of nitrification in bacterial cultures and in nature

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Juliane Jacob

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Dr. Kirstin Dähnke

und

Prof. Dr. Kay-Christian Emeis

Zusammenfassung

Seit der Industrialisierung sind die Produktion und Nutzung von reaktivem Stickstoff erheblich gestiegen. Der Eintrag von Ammonium und Nitrat aus intensiver Landwirtschaft und Massentierhaltung führt zu Eutrophierung. Hierdurch wird der Stickstoffkreislauf in Oberflächengewässern stark beeinflusst, was u.a. Auswirkungen auf die Lebensgemeinschaft und Wasserqualität hat.

In der vorliegenden Arbeit werden Reinkulturen von Nitrit-oxidierenden Bakterien (NOB) und der Stickstoffkreislauf in der Elbe bei Geesthacht untersucht, um insbesondere die Bedeutung der Nitrifikation zu untersuchen.

Das zweite Kapitel befasst sich mit der Elbeflut im Juni 2013. Starkregenereignisse im Einzugsgebiet der Elbe führten u.a. zu erhöhten Abflussraten und Nitrateinträgen. Gleichzeitig war das Phytoplankton gehemmt und die Nährstoffaufnahme stark reduziert. Nitrifikation fand weiterhin statt, was zu einem außergewöhnlichen Anstieg der Ammonium- und Nitrit-Konzentrationen führte und Isotopenanalysen ermöglichte. Bei anschließender Nitrit-Abnahme wurde auf Grundlage des berechneten Isotopeneffekts und eines Box-Modells eine Kombination aus Nitrifikation (22 bis 36%) und Denitrifikation (64 bis 78%) abgeschätzt. Mit abnehmendem Abfluss sanken alle Nährstoffkonzentrationen und Nitratisotope wiesen auf Phytoplankton-Assimilation hin.

Im dritten Kapitel wird die Nitrit-Oxidation, die von bestimmten NOB ausgeführt wird, untersucht. Charakteristisch für die Aktivität der NOB sind Nitrit-Oxidationskinetiken (Substrataffinität und maximale Nitrit-Oxidationsaktivität). Die Nitrit-Oxidationskinetiken von vier marinen NOB (*Nitrospina watsonii 347, Nitrospira* Ecomares 2.1*, Nitrobacter sp. 311* und *Nitrococcus mobilis 231*) wurden analysiert und mit nicht-marinen verglichen. Die Substrataffinitäten und maximalen Nitrit-Oxidationsaktivitäten der marinen NOB haben eine relativ kleine Variationsbreite, was auf eine Spezialisierung auf niedrige Substratkonzentrationen und geringe Fluktuationen der Umweltbedingungen in marinen Habitaten hinweisen kann. Außerdem wurden die Isotopeneffekte für die Nitrit-Oxidation durch *Nitrospina watsonii 347* und *Nitrospira* Ecomares 2.1 gemessen. Sie zeigten einen seltenen inversen Isotopeneffekt von ca. 10‰, d.h. abnehmende Isotopenwerte des Substrates bei abnehmender Substratkonzentration. Dies ist vergleichbar mit einer früheren Studie mit *Nitrospina marina*, aber signifikant kleiner als *Nitrobacter* sp. 355 und *Nitrococcus mobilis 231*. Mögliche Gründe sind der unterschiedliche Zellaufbau und die Lokalisation des verantwortlichen Enzymes in *Nitrospina* und *Nitrospira* im Gegensatz zu *Nitrobacter* und *Nitrococcus*. Die hier untersuchten NOB sind insbesondere in Sauerstoffminimumzonen vertreten.

Sauerstoffminimumzonen sind Hotspots für Nitrifikation und Denitrifikation und häufig im Fokus von biogeochemischen Modellrechnungen. Die Nitrit-Oxidationskinetiken für marine NOB und die Isotopeneffekte sind daher eine wichtige Erweiterung der Datenbasis.

Im vierten Kapitel wurde ebenfalls die Elbe bei Geesthacht untersucht. Von Juli 2011 bis Mai 2013 wurden regelmäßig Wasserproben u.a. zur Bestimmung der Nährstoffkonzentrationen und der Isotope ($\delta^{15}N\text{-}NO_3^-$, $\delta^{18}O\text{-}NO_3^-$, $\delta^{15}N$-SPM) genommen, sowie die hydrologischen Bedingungen untersucht. Außerdem wurde die Bedeutung der Nitrifikation in der Elbe mit Ratenmessungen bestimmt. Es konnten eine deutliche Saisonalität, aber auch kurzfristige Ereignisse wie Phytoplanktonblüten innerhalb einer Saison identifiziert werden. Im Winter stammte Nitrat hauptsächlich aus Nitrifikation im Einzugsgebiet und Nitrifikationsraten im Fluss waren gering. Im Frühling sanken der Abfluss und der Nitrateintrag aus dem Einzugsgebiet. Gleichzeitig stieg die Primärproduktion und hohe SPM („suspended particulate matter“) Konzentrationen führten zu steigenden Nitrifikationsraten in der Elbe durch gekoppelte Remineralisierung-Nitrifikation, sodass Nitrifikation im Fluss eine wichtige Nitratquelle war. Die Nährstoffkonzentrationen und Nitratisotope zeigten außerdem, dass im Frühling mehr Nitrat assimiliert als nitrifiziert wurde und im Sommer, sowie Herbst eine Kombination aus Assimilation, Nitrifikation und Denitrifikation wahrscheinlich war.

Die vorliegende Arbeit zeigt, dass Nitrifikation in der Elbe eine wichtige Nitratquelle ist und dass Extremereignisse wie eine Flut den sensiblen Stickstoffkreislauf erheblich beeinflussen. In der Elbe treten zeitgleich verschiedene Quellen und Senken auf, wodurch der inverse Isotopeneffekt der Nitrit-Oxidation nicht nachgewiesen werden kann. Um die Nitrit-Oxidation detaillierter zu bestimmen, wurden die Oxidationskinetiken und der inverse Isotopeneffekt und Reinkulturen untersucht.

Abstract

Since industrialization, the production and use of reactive nitrogen have increased considerably. The input of ammonium and nitrate from agriculture and intensive animal husbandry leads to eutrophication. This strongly influences the nitrogen cycle in surface waters, which affects for example the symbiotic community and water quality.

In this thesis, pure cultures of nitrite-oxidizing bacteria (NOB) and the nitrogen cycle in the Elbe River near Geesthacht were investigated to determine the importance of nitrification, especially.

The second chapter deals with the Elbe River flood in June 2013. Heavy rainfalls in the catchment area of the Elbe River led to increased discharge and input of nitrate. Simultaneously, phytoplankton was inhibited and nutrient uptake was strongly reduced. Nitrification continued, which resulted in an exceptional increase of ammonium and nitrite, and isotope analysis was possible. Subsequently, a combined nitrification (22 to 36%) and denitrification (64 to 78%) regime was determined with a box model approach and based on the calculated isotope effect during nitrite decrease. With decreasing discharge, all nutrient concentrations decreased and nitrate isotopes indicated assimilation by recovered phytoplankton.

In the third chapter, the nitrite oxidation performed by distinct NOB is investigated. The activity of NOB is characterized by nitrite oxidation kinetics (substrate affinity and maximum nitrite oxidation activity). The nitrite oxidation kinetics of four marine NOB (*Nitrospina watsonii 347, Nitrospira* Ecomares 2.1, *Nitrobacter sp. 311,* and *Nitrococcus mobilis 231*) were analysed and compared to non-marine NOB. The substrate affinity and the maximum nitrite oxidation activity of the individual species have a narrow range which can point to a specialization to low substrate concentrations and low fluctuations of the environmental conditions in marine habitats. Furthermore, isotope effects of two marine NOB (*Nitrospina watsonii 347* and *Nitrospira* Ecomares 2.1) were analysed. They showed a rare inverse isotope effect of about 10‰, i.e., decreasing isotope values of the substrate with decreasing substrate concentrations. This is comparable to a previous study with *Nitrospina marina* but significantly smaller than *Nitrobacter sp. 355* and *Nitrococcus mobilis 231*. Possible reasons are a different cell structure and the location of the responsible enzyme in *Nitrospina* and *Nitrospira* compared to *Nitrobacter* and *Nitrococcus*. The investigated NOB are especially represented in oxygen minimum zones. Oxygen minimum zones are hotspots of nitrification and denitrification, and are often in the focus of biogeochemical model calculations. The nitrite oxidation kinetics and isotope effects thus are an important extension of the database.

The fourth chapter also deals with the Elbe River at Geesthacht weir. From July 2011 to May 2013, nutrient concentrations and isotopes ($\delta^{15}N\text{-}NO_3^-$, $\delta^{18}O\text{-}NO_3^-$, $\delta^{15}N$-SPM) of water samples were analysed bimonthly and hydrological conditions investigated. In addition, the importance of nitrification in the Elbe River was determined with rate measurements. A pronounced seasonality, but also short-term events like phytoplankton blooms within a season, was observed. In winter, nitrate in the Elbe River was mainly from soil nitrification in the catchment area and the nitrification rates in the river were low. In spring, discharge and nitrate input from the catchment area decreased. Simultaneously, increasing primary production and SPM concentrations fuelled nitrification in the Elbe River due to coupled remineralisation-nitrification, whereas nitrification was an important nitrate source. Furthermore, nutrient concentrations and isotope ratios showed that in spring assimilation exceeded nitrification and a combined assimilation-nitrification-denitrification regime is most likely in summer and autumn.

One of the main outcomes of the research is nitrification being an important nitrate source of the Elbe River. Additionally events like floods interrupt the sensitive nitrogen cycle. In the Elbe River different sources and sinks simultaneously occur, whereas the inverse isotope effect of nitrite-oxidation could not be determined. However, to investigate nitrite oxidation in detail, nitrite oxidation kinetics and the inverse isotope effect in pure cultures were analysed.

Contents

List of Publications

The thesis is composed of three individual scientific publications. The second chapter is published in a peer-reviewed journal, the third chapter is in revision, and the fourth chapter is in preparation for submission.

- Juliane Jacob, Tina Sanders, Kirstin Dähnke: Nitrite consumption and associated isotope changes during a river flood event. *Biogeosciences* (2016), doi:10.5194/bg-13-5649-2016.
- Juliane Jacob, Boris Nowka, Véronique Merten, Tina Sanders, Eva Spieck, Kirstin Dähnke: Oxidation kinetics and inverse isotope effect of marine nitrite-oxidizing bacteria. In revision. *Aquatic Microbial Ecology* (2017).
- Juliane Jacob, Tina Sanders, Kirstin Dähnke: Seasonality of nutrients, N isotopes, and nitrification rates in the Elbe River. In preparation.

1. Introduction

1.1. Eutrophication

Nitrogen is the major constituent of all organisms as a basic element of proteins and DNA and thus a nutrient for primary production. 78% of the atmosphere is dinitrogen (N_2), but this is chemically inert due to its triple bond. Only a few species of prokaryotes are able to reduce N_2 to ammonium (NH_4^+) and thus make it bioavailable. Bioavailable reactive nitrogen (N_r) in aquatic systems are nitrate (NO_3^-), nitrite (NO_2^-), and ammonium (NH_4^+, Canfield et al., 2005), summarized as dissolved inorganic nitrogen (DIN). DIN is taken up and assimilated for algal and bacterial growth and incorporated into organic matter, and thus is a limiting factor for organic matter production (Fowler et al., 2013; Galloway et al., 2003). In the early 20th century, the industrial N_2 fixation via the Haber-Bosch synthesis revolutionized the fertilizer production and led to a severe accumulation of N_r in ecosystems (Galloway, 1998; Gruber and Galloway, 2008). Since 1860, the input of N_r has increased 20-fold to about 150 Tg N yr^{-1} (Galloway and Cowling, 2002). The inputs can be divided into point-sources and diffuse sources. Point sources are fixed stations with direct nitrogen inputs, e.g. waste water treatment plants (WWTP) and power plants. Diffuse sources are inputs from agriculture (e.g. fertilized crops or animal husbandry) or traffic and atmospheric deposition. The relative proportion of the two sources varies in accordance to population density and land use (Smith et al., 1999). Because nitrogen from atmospheric depositions can overcome wide distances, pristine watersheds become rare. The opposite of pristine is eutrophic, which means the accumulation of nutrients in a water body that stimulate the growth of aquatic plant life usually resulting in the depletion of dissolved oxygen. Eutrophication and its impacts have been discussed extensively (e.g. Galloway et al., 2003; Rabalais, 2002; Smith et al., 2006), because of its various negative impacts like loss of biodiversity, shifts in food webs, disruption of ecosystem functioning, loss of habitat, noxious and toxic algal blooms, oxygen deficiency, and therefore, fish kills and loss of harvestable fisheries (Carpenter et al., 1998; Gruber and Galloway, 2008; Rabalais, 2002; Vitousek et al., 1997). Furthermore, rivers play a crucial role in the nitrogen delivery to the ocean, because these inputs far exceed other sources of nitrogen input like nitrogen fixation, atmospheric deposition, and coastal point sources (Rabalais, 2002).

From a historical perspective the Elbe River had a very bad ecological status at the time of the reunification of Germany (water quality class IV), because of severe exposure to pollutants and

nutrients, and very bad oxygen conditions. The algae and bacterial communities were affected, and fish stock was diminished in size and diversity. Based on national and international laws (e.g. “EG-WRRL”) the overall water quality improved, and DIN loads decreased, while the oxygen saturation improved markedly (Pätsch et al., 2010). From 1986 to 2008, annual ammonium loads decreased by 93%, and nitrate loads decreased by 48% in the Elbe River at Seemannshöft (Bergemann and Gaumert, 2008), because of an improved waste water and organic carbon management. Today, the riverine DIN load consists mainly of nitrate, which stems from diffuse sources like urban waste water, surface runoff, and leachate from agriculture soils (Brion et al., 2004; Van Breemen et al., 2002). However, along the river-estuary-continuum, nitrogen processing has the potential to alter river loads significantly.

1.2. Nitrogen cycle

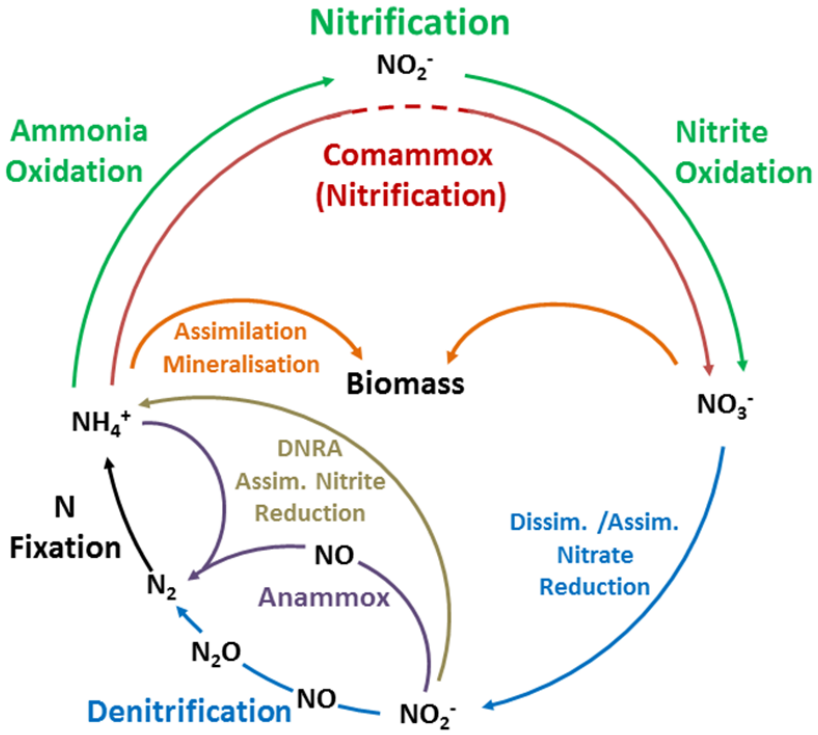

Figure 1.1 Schematic illustration of the key processes of the nitrogen cycle including nitrification, and nitrite oxidation to nitrate, especially. Ammonium, nitrite, and nitrate are stable, whereas nitric oxide, nitrous oxide, and dinitrogen are atmospheric gases, which are released to the atmosphere. Abbreviations: anaerobic ammonium oxidation (anammox), comammox (complete ammonia oxidation), dissimilatory nitrite reduction to ammonia (DNRA), assimilatory (assim.), dissimilatory (dissim.).

In rivers, nitrogen occurs as dissolved or particulate organic (DON; PON) or inorganic nitrogen (DIN as nitrate, nitrite and ammonium). Nitrogen loads can be modified in the river itself (Middelburg and Nieuwenhuize, 2001), which changes the concentration of the nitrogen compounds, but the total amount remains stable (Deutsch et al., 2009). Only denitrification permanently decreases the nitrogen pool by dinitrogen release (Böttcher et al., 1990; Mariotti et al., 1981). Nitrate regeneration via remineralization of organic matter and subsequent nitrification occurs in major rivers throughout Europe, and contributes to nitrate loads in, for example, the Seine, Scheldt and Elbe River (de Bie et al., 2002; de Wilde and de Bie, 2000; Johannsen et al., 2008; Sebilo et al., 2006). Nitrification is ecologically important, because it provides nitrate, which is the largest DIN-compound and substrate for denitrification.

Recently, the complete oxidation of ammonia via nitrite as an intermediate to nitrate, so called comammox, in the NOB genus *Nitrospira* has been discovered (Daims et al., 2016; Kuypers, 2015), which emphasizes the importance of *Nitrospira* organisms.

1.2.1. Nitrification

Nitrification is a two-steps oxidation of ammonia via nitrite to nitrate (cf. Fig. 1.1), which is performed by two different functional groups of chemolithoautotrophic bacteria and archaea, respectively. The first step is mediated by ammonia-oxidizing bacteria (AOB) and ammonia-oxidizing archaea (AOA), while the second step is performed by nitrite-oxidizing bacteria (NOB, Eq. 1.1). The known NOB belong to the genera *Nitrospina* (Watson and Waterbury, 1971), *Nitrospira* (Watson et al., 1986), *Nitrobacter* (Winogradsky, 1892; Woese et al., 1984), *Nitrococcus* (Watson and Waterbury, 1971; Woese et al., 1985), *Nitrotoga* (Alawi et al., 2007; Spieck et al., 2006), and *Nitrolancea* (Sorokin et al., 2012). Furthermore, *Candidatus* Nitromaritima was identified based on metagenomic data (Ngugi et al., 2016).

Nitrification is determined in various oxic environments regardless of temperature (permafrost soils in Siberian Arctic, Alawi et al., 2007, geothermal springs, Lebedeva et al., 2005; Marteinsson et al., 2001) or substrate concentration (WWTP, Juretschko et al., 1998), as well as in OMZ (oxygen minimum zone, Beman et al., 2013; Fuchsman et al., 2011; Labrenz et al., 2007). NOB are phylogenetically heterogeneous (Teske et al., 1994), which is reflected in a wide range of preferences regarding environmental conditions like temperature or substrate concentration (De Boer et al., 1991). NOB activity prevents the accumulation of potential toxic nitrite in the environment (Philips et al., 2002), builds new nitrate for organisms and plants and is respiratory substrate in oxygen-limited environments. The product, nitrate, then is a link to anaerobic denitrification, which decreases nitrate concentration.

Nitrite oxidation can be described as the following equation:

$$NO_2^- + H_2O \rightarrow NO_3^- + 2\,H^+ + 2e^-$$

$$2\,H^+ + 2\,e^- + ½\,O_2 \rightarrow H_2O$$

$$NO_2^- + ½\,O_2 \rightarrow NO_3^- \qquad \Delta G_0 = -74.05\ kJ\ mol^{-1} \qquad (1.1)$$

Nitrification is potentially important in oxic rivers like the Elbe, where additional nitrate fuels phytoplankton growth and thus influences the water quality. Nitrification not only changes nutrient concentrations, but also stable isotope signatures.

1.3. Stable Isotopes

In 1946, Harold Urey described light stable isotopes at first. Since then, many studies that address biological turnover processes were investigated based on isotopes. Elements have natural differences in their stable isotope composition, whereby hydrogen, carbon, nitrogen, oxygen and sulphur are the so called light isotopes. Regarding nitrogen, 99.63% is ^{14}N and only 0.37% is ^{15}N, and the ratio of $^{15}N/^{14}N$ varies. Oxygen composes of three isotopes, 99.759% ^{16}O, 0.037% ^{17}O, and 0.204% ^{18}O. Indicating these differences, the “delta” notation was introduced using a comparison to a standard (cf. Eq. 1.2, McKinney et al., 1950).

$$\delta^{15}\mathrm{N}\ [‰\ \mathrm{vs.\ standard}] = \left(\frac{\left(\frac{^{15}\mathrm{N}}{^{14}\mathrm{N}}\right)_{\mathrm{sample}}}{\left(\frac{^{15}\mathrm{N}}{^{14}\mathrm{N}}\right)_{\mathrm{standard}}} - 1 \right) * 1000 \qquad (1.2)$$

The standard for $\delta^{15}N$ is atmospheric N_2 (called AIR) and for $\delta^{18}O$ is Vienna Standard Mean Ocean Water (VSMOW), which both by definition have a δ-value of 0‰.

Sources and sinks have distinct isotope signatures, whereas stable isotopes have often been used in environmental studies. This thesis is based on nitrogen ($^{15}N/^{14}N$) and oxygen isotope ratios ($^{18}O/^{16}O$) of different compounds like nitrate, nitrite, ammonium, and SPM.

During turnover processes isotope ratios change with specific isotope effects. Pristine nitrate has usually lower isotope values than nitrate from anthropogenic sources (Amberger and Schmidt, 1987; Aravena et al., 1993; Deutsch et al., 2006). However, if large amounts of fertilizer are applied on crops, isotopes are fractionated: ammonia volatilization (Wassenaar, 1995), harvesting of ^{14}N-enriched crops, and nitrification/denitrification in soils (Grischek et al., 1998) and aquifers (Amberger and Schmidt, 1987) leads to ^{15}N enriched nitrogen in the residual compounds (Wassenaar, 1995). The isotope effect ε defines the extent of discrimination, and is usually negative. However, during nitrite oxidation ^{15}N is preferentially oxidised having a positive isotope effect (Tab. 1.1). The database of nitrogen and oxygen isotope effects is large, because isotope effects strongly depend on environmental conditions, biological pathways, and bacterial species.

As illustrated (Fig. 1.1, Tab. 1.1), nitrogen has various pathways and easily connects atmospheric-terrestrial-aquatic ecosystems. Since the massive input of anthropogenic nitrogen due to eutrophication, the pool size and quantitative turnover processes has changed.

Table 1.1 Isotope effects $^{15}\varepsilon$ for relevant transformation processes in aquatic systems as illustrated in Fig. 1.1. ^{14}N preference is expressed by negative isotope effects, and ^{15}N preference is called inverse isotope effect. Note that isotope effects are much lower or absent if substrate limitation occurs.

Turnover process	Reaction	Isotope effects ε	References
Ammonia oxidation (nitrification)	$NH_4^+ \rightarrow NO_2^-$	-14 to -41‰	(Casciotti et al., 2003; Mariotti et al., 1981; Santoro and Casciotti, 2011)
Nitrite oxidation (nitrification)	$NO_2^- \rightarrow NO_3^-$	+9 to +20‰	(Buchwald and Casciotti, 2010; Casciotti, 2009; Jacob et al., in revision)
Remineralization	$N_{org} \rightarrow NH_4^+$	~0 to -2.3‰	(Kendall, 1998; Möbius, 2013)
Ammonium assimilation	$NH_4^+ \rightarrow N_{org}$	-11 to -27‰	(Hoch et al., 1992; Voß et al., 1997; Waser et al., 1998)
Nitrite assimilation	$NO_2^- \rightarrow N_{org}$	-1‰	(Waser et al., 1998)
Nitrate assimilation	$NO_3^- \rightarrow N_{org}$	-5 to -20‰	(Granger et al., 2004; Needoba and Harrison, 2004; Waser et al., 1998)
Sediment denitrification	$NO_3^- \rightarrow N_2$	0 to -3‰	(Brandes and Devol, 1997; Lehmann et al., 2004)
Water column denitrification	$NO_3^- \rightarrow N_2$	-22 to -30‰	(Brandes et al., 1998)
Riparian denitrification	$NO_3^- \rightarrow N_2$	-16‰	(Deutsch et al., 2005; Houlton and Bai, 2009; Kendall et al., 2007)

1.3.1. Calculation of isotope effects

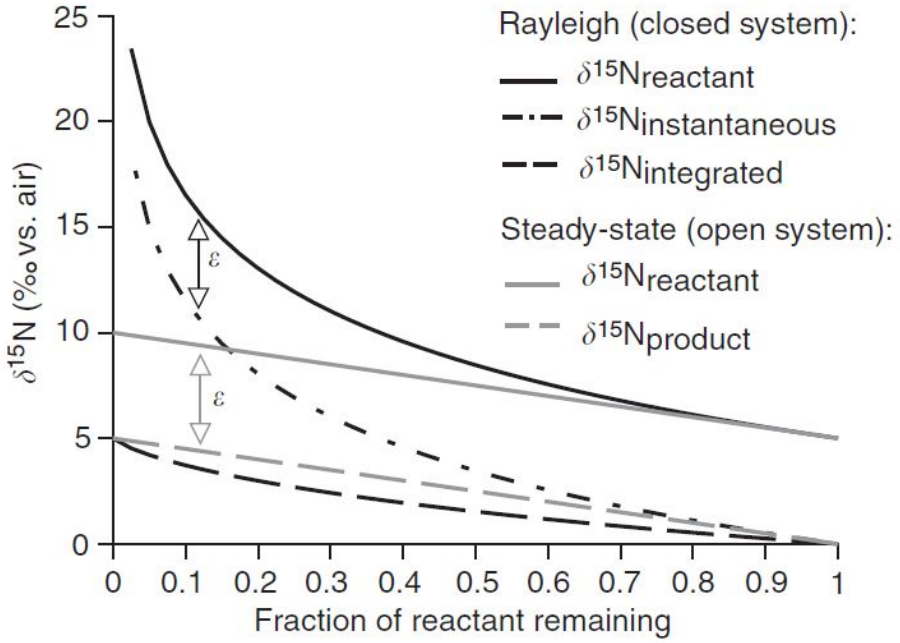

Figure 1.2 Comparison of isotope effects in a closed system (Rayleigh, black lines), and an open-system (steady-state, grey lines). The same isotopic parameters - an isotope effect of 5‰, and a $\delta^{15}N$ of 5‰ of the initial reactant supply – are used for both models (Sigman et al., 2009).

The isotope effects ε can be calculated for closed-system and open-system conditions. Besides the degree of consumption of the substrate pool (*f*), two isotopic parameters (initial isotope value of the substrate, kinetic isotope effect ε) play a central role. If a reaction proceeds with a constant isotope effect and if the product and substrate pools are neither lost nor replenished during the reaction, the process can be described in terms of Rayleigh fractionation (Broecker and Oversby, 1971; Mariotti et al., 1981). At the end of the reaction, substrate is completely consumed, and the isotope value of the product equals the initial isotope value of the substrate (Fig. 1.2). The closed-system model is applied in pure culture experiments (Eq. 1.3, c.f. chapter 3, Buchwald and Casciotti, 2010).

$$\varepsilon_{\text{substrate}} = \frac{\delta\ \text{value}_{\text{substrate}} - \delta\ \text{value}_{\text{initial}}}{\ln f} \quad (1.3)$$

The open-system approach is often used in environmental studies (Eq. 1.4, c.f. chapter 2, e.g. Kendall et al., 2007; Sigman et al., 2009), where substrate is continuously supplied and partially consumed (Mariotti et al., 1981). The residual pool is exported at a steady-state rate such that the gross supply of the pool equals the sum of the product and the residual pool exported. This leads to a linear relation between isotope values and the remaining fraction *f.* The slope of the regression line corresponds to the isotope effect ε. Figure 1.2 illustrates the $\delta^{15}N$ values of the reactant and the product as a function of the remaining fraction, which is left unconsumed. The function is described as

$$\varepsilon_{\text{substrate}} = -\frac{\delta\ \text{value}_{\text{substrate}} - \delta\ \text{value}_{\text{initial}}}{(1-\text{f})} \quad (1.4)$$

Where $\delta\ \text{value}_{\text{substrate}}$ and $\delta\ \text{value}_{\text{initial}}$ are the $\delta^{15}N$ values of the substrate at the time of sampling and the initial value, and *f* is the remaining fraction of substrate at the time of sampling.

1.4. Study site

The Elbe River has a length of 1,092 km and is, after the Rhine River, the second largest river entering the North Sea. The average discharge is about 738 $m^3\ s^{-1}$ (Lozán and Bernhart, 1996), and the catchment area of about 148,000 km² houses almost 25 million people (Behrendt et al., 2004a). The land use in the catchment area is dominated by agriculture (60.6%) and forests (28.6%). 80% of the agriculture are crops, followed by animal breeding. Nitrogen mainly derives from diffuse sources (73% of total nitrogen inputs), which are dominated by groundwater nitrogen inputs to the small tributaries (Behrendt et al., 2004b). From 1986 to 2008, however, annual ammonium loads decreased by 93% to 3,400 t y^{-1}, and nitrate loads decreased by 42% to 64,000 t y^{-1} at Seemannshöft (Bergemann and Gaumert, 2010). At Geesthacht weir ammonium loads were less than 5% of that of nitrate, and nitrite was less than 2%. The relatively high nutrient inputs from the upper part of the catchment, combined with long water residence times in the upstream impounded sections, support high concentrations of planktonic algae. The high productivity leads to conspicuous changes in inorganic nutrient concentrations along the river (Guhr et al., 2003), leading to assumptions whether nitrate is also built in the river via nitrification.

1.5. Focus and thesis outline

1.5.1. Focus and motivation

The nitrogen cycle and in particular the second step of nitrification, the nitrite oxidation, were investigated. An essential part of the thesis was the analysis of nitrification in the Elbe river (near Geesthacht), because it was rarely investigated before. Previous studies focussed on another river section like the Elbe estuary (Dähnke et al., 2008; Schlarbaum et al., 2010) or other processes like combined assimilation-denitrification (Deutsch et al., 2009; Johannsen et al., 2008; Schlarbaum et al., 2011).

In the Elbe River near Geesthacht, it was assumed that nitrate mainly derived from soil nitrification in the catchment, sewage, and/or fertilizer (Johannsen et al., 2008). In a study of the Warnow River, a mixing model determined 86% nitrate from soil drainage, 11% from groundwater, and 3% from atmospheric deposition (Deutsch et al., 2006), and a study based on sixteen American watersheds assumed nitrate almost entirely from soil nitrification (Mayer et al., 2002). However, in-stream built nitrate was not recognised, which raised the question, if nitrification in the Elbe River is quantitatively important. Therefore, seasonal variations of nitrification rates of Elbe River samples were investigated to quantify additional nitrate from nitrification (chapter 4). Furthermore, stable isotopes ($\delta^{15}N\text{-}NO_3^-$, $\delta^{18}O\text{-}NO_3^-$ and $\delta^{15}N$-SPM) were analysed over two years to determine seasonal varying turnover processes and calculated simple box-models.

However, an exceptional summer flood in June 2013 disrupted the seasonal cycling (chapter 2). The motivation was to figure out, how the nitrogen cycle is affected by increasing discharge and potentially decreasing light irradiation due to high turbidity, particularly. Former studies reported a characteristic SPM peak prior to the discharge peak (Baborowski et al., 2004), and $\delta^{15}N$-SPM values were analysed to determine the origin. Furthermore, the succession of nutrient concentrations and isotopes were investigated in order to identify changes in nitrogen cycling, and nitrification, especially.

The third chapter focuses on pure culture incubations. Nitrite oxidation kinetics are important characteristics for the growth of NOB, but data for marine NOB were missing, and potential links between environmental conditions and nitrite oxidation kinetics were not observed. Marine environments provide low substrate concentrations compared to terrestrial environments. In order to find out whether this influences nitrite oxidation kinetics, four marine NOB (*Nitrospina watsonii 347, Nitrospira* Ecomares 2.1, *Nitrococcus mobilis 231,* and *Nitrobacter sp. 311*) were investigated. Furthermore, isotope effects of nitrite oxidation for two NOB (*Nitrospina watsonii 347* and

Nitrospira Ecomares 2.1) were determined, because the data base was scarce, and contradictory in concern of intra- and interspecies aspects (Buchwald and Casciotti, 2010; Casciotti, 2009). First, the maintenance of *Nitrospina watsonii 347* and *Nitrospira* Ecomares 2.1 was ensured, afterwards experiments were performed with various initial concentrations in the optimum range of the substrate. The motivation was to analyse the isotope effect of environmentally important NOB, and to figure out where the significant differences in the published literature were originated.

Both, the determination of nitrification in the Elbe River, and the calculation of isotope effects during nitrification in pure cultures conduce to improve model approaches, especially. For example, a change of the isotope effect of nitrite oxidation, even by 3‰ only, will certainly alter the computed isotope values of nitrite in ocean models (T. Rixen, personal communication, 2016). However, the isotope effects of nitrite oxidation are in the range of about 9 to 20‰ (Buchwald and Casciotti, 2010; Casciotti, 2009; Jacob et al., in revision), and same modelling approaches require even higher isotope effects (Buchwald et al., 2015; Casciotti et al., 2013).

1.5.2. Thesis Outline

The thesis consists of three sections and publications, respectively (chapter 2, 3, and 4).

Chapter 2

Nitrite consumption and associated isotope changes during a river flood event

Juliane Jacob, Tina Sanders, and Kirstin Dähnke

Biogeosciences (2016), doi:10.5194/bg-13-5649-2016

In the Elbe River, nitrate concentrations are enhanced due to intense fertilization and soil nitrification in the catchment area (Johannsen et al., 2008). In summer, discharge is low, nitrate concentration decreases, and ammonium and nitrite concentrations are below the detection limit. However, an exceptional summer flood in June 2013 disrupted the seasonal cycling. We found a unique co-occurrence of ammonium, nitrite, and nitrate and analysed their isotope values. In concert with changes in SPM concentrations and δ^{15}N SPM, as well as nitrate concentration, δ^{15}N-NO_3^- and δ^{18}O-NO_3^-, we calculated apparent isotope effects during net nitrite and nitrate consumption.

During the flood, nitrate as the main DIN-component was leached from the catchment, and nitrate assimilation was reduced, because high turbidity and light irradiance inhibited phytoplankton activity. SPM was probably re-suspended from groyne fields. Usually, ammonium and nitrite accumulation is not observed in summer. However, during the flood, the concentrations increased likely due to remineralisation and nitrification in the water column. δ^{15}N NH_4^+ values increased to 12‰, δ^{15}N-NO_2^- ranged from -8.0 to -14.2‰, and δ^{15}N-NO_3^- and δ^{18}O-NO_3^- were in the relatively narrow range of 7.4 to 9‰ and 2.1 to 3.9‰, respectively. Based on this, an apparent isotope effect $^{15}\varepsilon$ of -10.0±0.1‰ was calculated during net nitrite consumption. The nitrite decrease was expected to be due to nitrite oxidation, which is usually associated with inverse isotope effects. On the basis of these isotope effects, we evaluated the contribution of different uptake and production processes with a simple box-model. We found that a combination of 22 to 36% nitrification and riparian denitrification fits best with measured data for nitrite concentration decrease and isotope increase, and this combination most likely covered the inverse isotope effect.

At the end of the flood, net nitrate consumption occurred, which was associated with isotope effects $^{15}\varepsilon$ of -4.0±0.1‰, and $^{18}\varepsilon$ -5.3±0.1‰. These isotope effects, chlorophyll and oxygen concentration reflect recovering phytoplankton, and a normal summer situation.

My contribution to this publication was sampling and generation of the data, establishing a concept, structuring and writing of the manuscript, as well as being the corresponding author. The contribution of co-authors was structuring and proofreading of the manuscript (Tina Sanders), and establishing a concept, structuring and proofreading the manuscript (Kirstin Dähnke).

Chapter 3

Oxidation kinetics and inverse isotope effect of marine nitrite-oxidizing bacteria

Juliane Jacob, Boris Nowka, Véronique Merten, Tina Sanders, Eva Spieck, Kirstin Dähnke, In revision. *Aquatic Microbial Ecology* (2017)

This manuscript focuses on pure culture incubations to determine the nitrite oxidation kinetics and the isotope effects of marine NOB.

Nitrite oxidation kinetics are important characteristics for the growth of NOB, but data for marine NOB were missing, and potential links between environmental conditions and nitrite oxidation kinetics were not investigated before. We closed that gap by determining the nitrite oxidation kinetics of four marine NOB strains (*Nitrospina watsonii 347, Nitrospira* Ecomares 2.1, *Nitrococcus mobilis 231* and *Nitrobacter sp. 311*), including NOB with periplasmic and cytoplasmic NXR, and compared them to non-marine NOB strains. Substrate affinities of all NOB vary over two orders of magnitude (Nowka et al., 2015 and references therein), and the investigated marine NOB fall within the low end with a rather narrow range. In nature, NOB compete for nitrite, and inter- and intraspecific niche differentiation in regard to nitrite oxidation kinetics is likely (Blackburne et al., 2007; Kim and Kim, 2006; Nogueira and Melo, 2006; Schramm et al., 1999). In marine NOB, small ranges of substrate affinities reflect little niche differentiations, but a general adaption to low substrate concentrations in marine habitats (Wada and Hatton, 1971).

Furthermore, isotope effects of *Nitrospina watsonii 347* and *Nitrospira* Ecomares 2.1 during nitrite oxidation were determined. In accordance to previous studies (Buchwald and Casciotti, 2010; Casciotti, 2009), we observed a rare inverse isotope effect, which reflects a successive depletion of ^{15}N during substrate consumption. The isotope effect of *Nitrospina watsonii 347* was 9.7±0.8‰ and 10.2±0.8‰ for *Nitrospira* Ecomares 2.1, which matches *Nitrospira marina* (9.1±1.8‰, Buchwald & Casciotti 2010), but is significantly different to *Nitrococcus mobilis* (12.8±1.5‰, Casciotti 2009, and 20.2±2.8‰, Buchwald & Casciotti 2010) and *Nitrobacter* sp. 355 (20.6±3.2‰, Buchwald & Casciotti 2010).

Varying initial substrate concentrations have no influence, but different nitrite oxidation kinetics of *Nitrospina* and *Nitrospira* with periplasmic NXR in contrast to *Nitrobacter* and *Nitrococcus* with cytoplasmic NXR probably influence isotope effects. Our measurements provide not only the first assessment of kinetics of four marine NOB, but also present new isotope effects of environmentally important species. Herewith, necessary database for e.g. model calculations in marine environments can be markedly improved.

My contribution to this publication was maintaining the *Nitrospina Watsonii 347,* and *Nitrospira* Ecomares 2.1 cultures, measuring the isotope effect of *Nitrospina Watsonii 347,* calculating the nitrite oxidation kinetics, establishing a concept, structuring and writing of the manuscript, as well as being the corresponding author. The contribution of co-authors was maintaining the NOB cultures, measuring the nitrite oxidation kinetics and proofreading the manuscript (Boris Nowka), measuring the isotope effect of *Nitrospira* Ecomares 2.1 (Véronique Merten), assisting in incubation experiments, structuring and proofreading the manuscript (Tina Sanders), proofreading the manuscript (Eva Spieck), and structuring and proofreading the manuscript (Kirstin Dähnke).

Chapter 4

Seasonality of nutrients, N isotopes, and nitrification rates in the Elbe River

Juliane Jacob, Tina Sanders, Kirstin Dähnke. In preparation for submission

This chapter is based on bimonthly water sampling of the Elbe River at Geesthacht weir from July 2011 to May 2013. From 1986 to 2006, annual ammonium and nitrate loads have decreased by 93% and 48%, respectively (Bergemann and Gaumert, 2008). However, nitrate loads were still enhanced. Therefore, seasonal changing nitrogen sources and sinks, and the contribution of in-stream nitrification as a nitrate source, especially, were investigated. Hydrological parameters (e.g. discharge, water temperature), nutrient concentrations, dual nitrate isotopes, SPM-isotopes were analysed, and nitrification rates were calculated.

We observed a pronounced seasonality with high nutrient concentrations, high δ^{15}N-SPM values, and low dual nitrate isotopes in winter. We assume elevated nutrient inputs from the catchment due to high discharge, and soil nitrification in the catchment was determined as a nitrate source based on dual nitrate isotopes (c.f. Johannsen et al., 2008). Simultaneously, nutrient consumption was low due to limited biological activity. From spring on, decreasing discharge and nutrient concentrations, low δ^{15}N-SPM values, as well as elevated isotope values occurred. SPM and chlorophyll concentrations increased accompanied by decreasing nitrate concentrations and increasing dual nitrate isotopes, which indicated high primary production and nitrate consumption. In spring 2012, nitrate decreased with an isotope effect of -11.4‰, which matches with 75% nitrate assimilation and 25% riparian denitrification. Nitrification in the river can barely be identified based on isotopes due to simultaneous sinks. Therefore, the incubations were performed. Nitrification rates were about 1 µmol L^{-1} d^{-1} in winter and up to 13.4 µmol L^{-1} d^{-1} in summer. We estimated nitrate inputs from nitrification for a period in spring 2012 using a back-of-the-envelope calculation. Nitrate from soil nitrification in the catchment was about 184 µmol L^{-1}, and nitrate from in-stream nitrification was about 346.5 µmol L^{-1}, while the average measured nitrate concentration is 115 µmol L^{-1} due to intense assimilation and denitrification.

My contribution to this publication was sampling and generation of the data, maintenance of the incubation experiments, establishing a concept, structuring and writing of the manuscript. The contribution of co-authors was assisting with incubation experiments, establishing a concept and proofreading the manuscript (Tina Sanders) and establishing a concept, structuring and proofreading the manuscript (Kirstin Dähnke).

2. Nitrite consumption and associated isotope changes during a river flood

Juliane Jacob, Tina Sanders and Kirstin Dähnke

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Abstract

In oceans, estuaries, and rivers, nitrification is an important nitrate source, and stable isotopes of nitrate are often used to investigate recycling processes (e.g. remineralization, nitrification) in the water column. Nitrification is a two-steps-process, where ammonia is oxidized via nitrite to nitrate. Nitrite usually does not accumulate in natural environments, which makes it difficult to study the single isotope effect of ammonia oxidation or nitrite oxidation in natural systems.

However, during an exceptional flood in the Elbe River in June 2013, we found a unique co-occurrence of ammonium, nitrite and nitrate in the water column, returning towards normal summer conditions within one week. Over the course of the flood, we analysed the evolution of $\delta^{15}N\text{-}NH_4^+$ and $\delta^{15}N\text{-}NO_2^-$ in the Elbe River. In concert with changes in suspended particulate matter (SPM) and $\delta^{15}N$-SPM, as well as nitrate concentration, $\delta^{15}N\text{-}NO_3^-$ and $\delta^{18}O\text{-}NO_3^-$, we calculated apparent isotope effects during net nitrite and nitrate consumption.

During the flood event, >97% of total reactive nitrogen was nitrate, which was leached from the catchment area and appeared to be subject to assimilation. Ammonium and nitrite concentrations increased to 3.4 µmol L^{-1} and 4.4 µmol L^{-1}, respectively, likely due to remineralization, nitrification and denitrification in the water column. $\delta^{15}N\text{-}NH_4^+$ values increased up to 12‰, and $\delta^{15}N\text{-}NO_2^-$ ranged from -8.0‰ to -14.2‰. Based on this, we calculated an apparent isotope effect $^{15}\varepsilon$ of -10.0±0.1‰ during net nitrite consumption, as well as an isotope effect $^{15}\varepsilon$ of -4.0±0.1‰ and $^{18}\varepsilon$ of -5.3±0.1‰ during net nitrate consumption. On the basis of the observed nitrite isotope changes, we evaluated different nitrite uptake processes in a simple box-model. We found that a regime of combined riparian denitrification and 22 to 36% nitrification fits best with measured data for the nitrite concentration decrease and isotope increase.

2.1. Introduction

Today's nutrient input to aquatic systems is significantly elevated over pristine background values in rivers and estuaries all over Europe. Since 1860, the input of reactive nitrogen (N_r) has increased 20-fold to about 150 Tg N yr^{-1} (Galloway and Cowling, 2002). The resulting eutrophication and its impacts have been discussed extensively (e.g. Galloway et al., 2003; Rabalais, 2002). In 1985, North Sea bordering countries decided to reduce nutrient inputs by 50%. As a result, the overall water quality improved, and dissolved inorganic nitrogen (DIN) loads decreased, while the oxygen saturation improved markedly (Pätsch et al., 2010). From 1986 to 2006, ammonium inputs to the Elbe River decreased by 93%, and nitrate inputs decreased by 48% (Bergemann and Gaumert, 2008) because of an improved waste water and organic carbon management. Today, the riverine DIN load consists mainly of nitrate, which stems from urban waste water, surface runoff, and leachate from agriculture soils (Van Breemen et al., 2002). However, nitrate regeneration in rivers can also modify DIN loads (Middelburg and Nieuwenhuize, 2001): Remineralization of organic material and subsequent nitrification (Mayer et al., 2001) regenerates nitrate, which then again enters the nitrogen cascade (Galloway et al., 2003) and can either be denitrified (Mariotti et al., 1981) or assimilated by bacteria and phytoplankton (Middelburg and Nieuwenhuize, 2000; Wada and Hattori, 1978). Nitrate regeneration via nitrification occurs in major rivers throughout Europe, and contributes to nitrate loads in, for example, the Seine, Scheldt and Elbe Rivers (Johannsen et al., 2008; Sebilo et al., 2006). A previous study by Johannsen et al. (2008) suggested that in the contemporary Elbe River, nitrate derived from nitrification in soils was the main constituent of the water column nitrate load in winter.

During enzymatically catalysed nitrogen transformation processes, lighter isotopes usually are processed faster than the heavy isotope species, which changes the isotope composition of the source and product (Mariotti et al., 1981).

Nitrification in this context is unique, because it is a two-step-reaction with divergent isotope effects. Wide ranging isotope effects of -14 to -41‰ occur during the first step, ammonia oxidation to nitrite, in pure cultures (Casciotti et al., 2003; Mariotti et al., 1981; Santoro and Casciotti, 2011). The second step, the oxidation of nitrite to nitrate, exhibits a very rare inverse isotope effect (Casciotti, 2009): The newly produced nitrate is heavier than the source nitrite, and the remaining nitrite in turn gets subsequently depleted in ^{15}N during nitrite oxidation.

The interpretation of isotope changes in natural environments during nitrification is complex, and studies addressing the combined isotope effect of ammonia and nitrite oxidation together even in culture are scarce. Moreover, investigations of nitrite oxidation and its isotope effect in natural environments are hampered by the fact that nitrite concentration in actively nitrifying environments usually is too low to analyse isotope values.

This is also the case in the Elbe River: Under normal flow conditions, nitrite is not abundant; the main DIN species is nitrate, which shows a distinct seasonal cycle. Nitrate concentration in winter is >300 µmol L^{-1}; summer values are <<100 µmol L^{-1} due to biological nitrate uptake (Johannsen et al., 2008; Schlarbaum et al., 2011). The interplay of isotopically distinct nitrogen sources and fractionation processes also leads to characteristic summer and winter nitrate isotope values in the water column. Isotope values are highest in summer due to biological uptake and phytoplankton production (Van Beusekom and De Jonge, 1998), and lowest in winter (Johannsen et al., 2008; Schlarbaum et al., 2011). The annual mean $\delta^{15}N\text{-}NO_3^-$ value is 8.5‰ (Johannsen et al., 2008), which is typical for catchment areas with more than 60% of agricultural and urban land use (Grischek et al., 1998).

The normal hydrological conditions were disrupted by an unusual summer flood in the Elbe River in June 2013. Runoff and turbidity increased drastically, and ammonium and nitrite accumulated in the water column, which was a unique opportunity to analyse isotope changes. Phytoplankton is light dependent and should be adversely affected by turbidity, but nitrifiers are not. We thus expected high turbidity and temperature to provide optimum conditions for nitrifiers. The flood may increase nitrification rates due to ample substrate, intense water column mixing, and inhibition of phytoplankton (Karrasch et al., 2001). In this study, we evaluate the role of the river flood on nitrogen cycling and nitrification as a sink of nitrite and ammonium, especially, using stable isotopes. Based on isotope changes of nitrite and nitrate, we calculated the apparent isotope effects during net nitrite and nitrate consumption. Using these apparent isotope effects, we constructed a simple box-model to estimate the contribution of nitrification and denitrification on nitrite consumption. To the best of our knowledge, this is the first investigation of apparent isotope effects during net nitrite consumption in a natural, actively nitrifying river system.

2.2. Materials and Methods

2.2.1. Study site

Nearly 25 million people live in the catchment area of about 148,000 km^2 of the Elbe River. After the Rhine River, the Elbe is the second largest river discharging into the North Sea and the largest source of nitrate and DIN for the inner German Bight (Brockmann and Pfeiffer, 1990). The average discharge is about 738 $m^3\ s^{-1}$ with an annual discharge of 23 km^3 (Lozán and Bernhart, 1996) and a nitrate load of about 76 kt yr^{-1} (Bergemann and Gaumert, 2008). Ammonium is of minor importance and is <5% of the nitrate load, and nitrite is usually <2%.

Our study site at stream kilometre 585 is located upstream of a weir that separates the river from the tidal estuary (53°25´31´´N, 10°20´10´´E). Discharge was measured upstream at the nearest gauge at Neu Darchau, stream kilometre 536.5.

2.2.2. Sampling and concentration analyses

During the flood event in June 2013, surface water samples were taken twice a day from 6 to 14 June from a quay wall at the shore and, with decreasing discharge, once a day on 15, 16, 18, and 20 June. Water temperature was measured immediately after sampling, and samples were transferred into 2 L PE bottles for immediate processing. Water samples were filtered within an hour (preweighed GF/F, precombusted at 450°C, 4.5 hrs), and aliquots of filtered water samples were frozen for later nutrient concentration analyses, and stable isotope composition ($\delta^{15}N\text{-}NH_4^+$, $\delta^{15}N\text{-}NO_2^-$, $\delta^{15}N\text{-}NO_3^-$, $\delta^{18}O\text{-}NO_3^-$). Filter samples were dried at 50°C and weighed for later determination of C/N ratios, suspended particulate matter (SPM) content, and $\delta^{15}N$-SPM analysis. C/N ratios were determined with an Elemental Analyser (Thermo Flash EA 1112) calibrated against a certified acetanilide standard (IVA Analysentechnik, Germany). The standard deviation of C/N analysis was 0.05% for carbon and 0.005% for nitrogen.

Nutrient concentrations were analysed with a continuous flow analyser (AA3, Seal Analytics, Germany). For nitrite and nitrate analyses, standard photometric techniques were used (Grasshoff et al., 2009) with detection limits of 0.1 and 1.0 µmol L^{-1}, respectively, and ammonium was measured fluorometrically with a detection limit of 0.5 µmol L^{-1} based on (Holmes et al., 1999).

2.2.3. Isotope analyses

Dual nitrate isotopes (including nitrite) were analysed using the denitrifier method (Casciotti et al., 2002; Sigman et al., 2001). In brief, water samples were injected into a concentrated *Pseudomonas aureofaciens* (ATCC#13985) suspension to analyse nitrate and nitrite. Nitrite concentration was always <2% of nitrate in water samples. For separate analysis of the nitrogen isotopic signature of nitrite, *Stenotrophomonas nitrireducens* bacteria were used to selectively reduce nitrite (Böhlke et al., 2007). Both bacteria denitrify the substrate to N_2O gas, which was then analysed on a GasBench II, coupled to a Delta V isotope ratio mass spectrometer (Thermo Fisher Scientific). The sample volume was always adjusted to achieve the same gas amount in the samples (final gas amount of 10 nmol in case of nitrate, 5 nmol for nitrite analysis).

For analysis of the ammonium isotopic composition, nitrite was removed by reduction with sulfamic acid (Granger and Sigman, 2009). Afterwards, ammonium was chemically converted to nitrite with hypobromite and ammonium then was reduced to N_2O using sodium azide (Zhang t al., 2007). Ammonium isotopes were analysed in all samples with $[NH_4^+]$ >1 µmol L^{-1}. Sample gas extraction and purification was equivalent to nitrite and nitrate isotope samples.

δ^{15}N-SPM was analysed with an element analyser (Carlo Erba NA 2500) coupled with an isotope ratio mass spectrometer (Finnigan MAT 252).

Isotope values are reported using the common “delta” notation,

$$\delta^{15}\mathrm{N}\ [‰\ \mathrm{vs.\ std}] = \left(\frac{\left(\frac{^{15}\mathrm{N}}{^{14}\mathrm{N}}\right)_{\mathrm{sample}}}{\left(\frac{^{15}\mathrm{N}}{^{14}\mathrm{N}}\right)_{\mathrm{std}}} - 1 \right) * 1000 \qquad (2.1)$$

where the standards for nitrogen and oxygen are atmospheric N_2 and Vienna Standard Mean Ocean Water (VSMOW), respectively.

International isotope standards with known δ-values were used for calibration. IAEA N3 and USGS 34 were used for nitrate isotope calibration; IAEA N1, IAEA N2, and a certified sediment standard (IVA Analysentechnik, Germany) for suspended matter isotope values; and IAEA N1, USGS 25, and USGS 26 were used to calibrate ammonium isotope values. For nitrite isotope analysis, we used in-house potassium nitrite and sodium nitrite standards with known δ^{15}N values of -81.5‰ and -27.5‰, determined via EA/IRMS analysis. All samples were analysed in replicate. Analytical error of triplicate standards and duplicate samples was <0.2‰ for δ^{15}N-NO_3^-

and <0.5‰ for $\delta^{18}O\text{-}NO_3^-$. For nitrite isotope analysis, the analytical error of $\delta^{15}N\text{-}NO_2^-$ was <0.3‰, and that of $\delta^{15}N\text{-}NH_4^+$ was <0.5‰. The analytical error of $\delta^{15}N$-SPM was <0.1‰. For quality assurance, additional internal standards (KNO_3, KNO_2, $NaNO_2$ salts) were analysed in every run.

2.2.4. Calculation of isotope effects

Based on an open-system approach, the isotope effects for the substrate and product pool can be calculated (Sigman et al., 2009). In the case of the flood, conditions are inherently dynamic and new substrate is continuously supplied and partially consumed. The sum of the product nitrogen and the continuously consumed residual nitrogen equals the total supply of reactant nitrogen, because the residual nitrogen is consumed at a steady-state rate (Eq. 2.2, 2.3). In an open-system, this leads to a linear relation between δ-values and *f*, with $f = ([C]/[C_{initial}])$, and the slope of the regression line corresponds to the isotope effect ε (Sigman et al., 2009).

$$\boldsymbol{\varepsilon}_{\text{substrate}} = -\frac{\delta\text{-value}_{\text{substrate}} - \delta\text{-value}_{\text{initial}}}{(1-f)} \tag{2.2}$$

$$\boldsymbol{\varepsilon}_{\text{product}} = \frac{\delta\text{-value}_{\text{product}} - \delta\text{-value}_{\text{initial}}}{f} \tag{2.3}$$

where $\delta\text{-value}_{substrate}$, $\delta\text{-value}_{product}$ and $\delta\text{-value}_{initial}$ are the $\delta^{15}N$ values of the substrate and product at the time of sampling and the initial value, *f* is the remaining fraction of substrate at the time of sampling.

2.3. Results

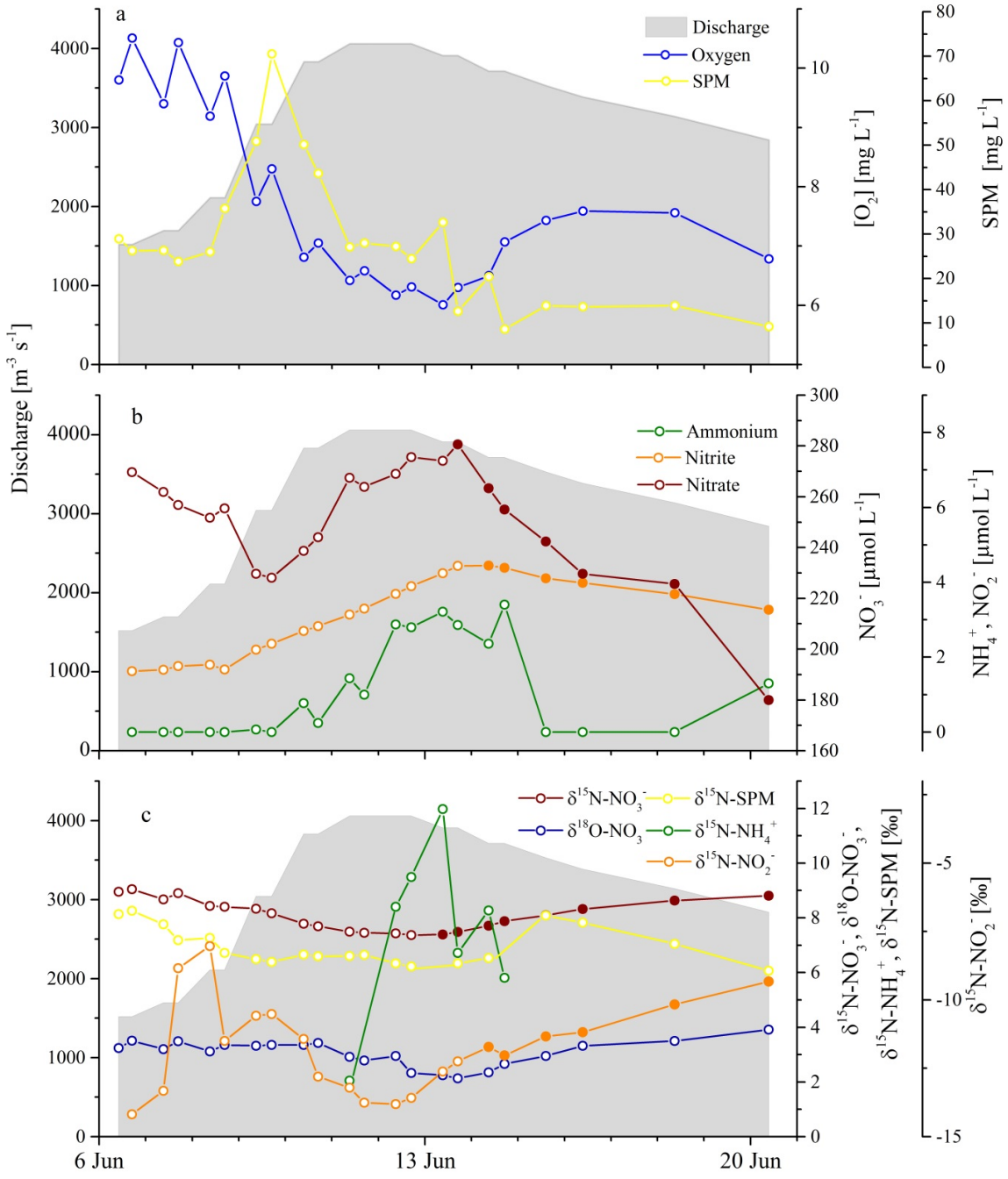

Figure 2.1 (a) Discharge, dissolved oxygen concentration, and SPM concentration of the Elbe River water samples from 6 to 20 June 2013. Flood conditions occur with discharge >3000 m^3 s^{-1}. (b) Ammonium, nitrite, and nitrate concentrations in the Elbe River in the course of the flood. Calculations of the isotope effects are based on filled data points. (c) Ammonium, nitrite, nitrate, and SPM isotope values in the course of the flood. Calculations of the isotope effect are based on filled data points.

2.3.1. General hydrographic properties

Flood conditions (defined by discharge values >3000 m^3 s^{-1} at gauge Neu Darchau, J. Kappenberg, personal communication, 2014) lasted from 9 to 18 June due to extremely high precipitation and resulting runoff in the catchment area. On 11 and 12 June, maximum SPM values of 70 mg L^{-1} were eluted shortly before peak discharge (4060 m^3 s^{-1}) and decreased afterwards to 8.6 mg L^{-1} (Fig. 2.1a). C/N ratios showed the same pattern with a maximum ratio of 10.0, decreasing to 7.6. Throughout the entire flood, the water temperature was high and increased from 16.2 to 21.5°C.

Dissolved oxygen concentration was correlated to discharge; the concentration was initially about 10 mg L^{-1}, corresponding to an oxygen saturation of >100%. With increasing discharge, the oxygen concentration dropped to a minimum of 6.0 mg L^{-1} (corresponding to 63% saturation), before it increased to 7.7 mg L^{-1} (Figs. 2.1a, 2.2). After this peak, [O_2] decreased, accompanied by a strong increase in water temperature.

2.3.2. Nutrient concentrations

Previous studies (Johannsen et al., 2008; Schlarbaum et al., 2011) found high nutrient concentrations in winter and low concentrations in summer. Based on this, our data appear more representative of spring than of summer conditions, because winter and spring 2013 were unusually cold (Van Oldenborgh et al., 2015), so that phytoplankton activity may be delayed. Before the flood, the discharge was ~800 m^3 s^{-1}, nitrate concentration was >200 µmol L^{-1}, nitrite concentration was <1.2 µmol L^{-1}, and ammonium concentration was below the detection limit of 0.5 µmol L^{-1}. DIN concentration increased when discharge rose >3000 m^3 s^{-1} and reached a distinct maximum shortly after peak discharge (Fig. 2.1b). Nitrite concentration rose >2.2 µmol L^{-1} and, along with all other nutrients, reached a maximum of 4.4 µmol L^{-1} on 14 June, followed by a decrease to 3.3 µmol L^{-1} towards the end of the flood event (Fig. 2.1b). Elevated nitrite concentration >2.2 µmol L^{-1} coincided with decreasing oxygen saturation (from 115 to 63%, Figs. 2.1b, 2.2).

Ammonium concentrations rose above the detection limit and reached a maximum of 3.2 µmol L-$^{-1}$ immediately after the peak of SPM, when oxygen concentrations dropped <7.7 mg L^{-1}, corresponding to an oxygen saturation <90% (Figs. 2.1b, 2.2). With decreasing discharge, the oxygen concentration rose, ammonium concentration dropped below the detection limit, and the overall DIN concentration decreased again (Fig. 2.1a, b).

On 9 June, lowest nitrate concentration (228.1 µmol L^{-1}) coincided with increasing discharge to 3000 m^3 s^{-1}. On 14 June and with further increasing discharge, nitrate concentration increased to 280.6 µmol L^{-1}, followed by a decreasing trend towards 180.0 µmol L^{-1} on 20 June.

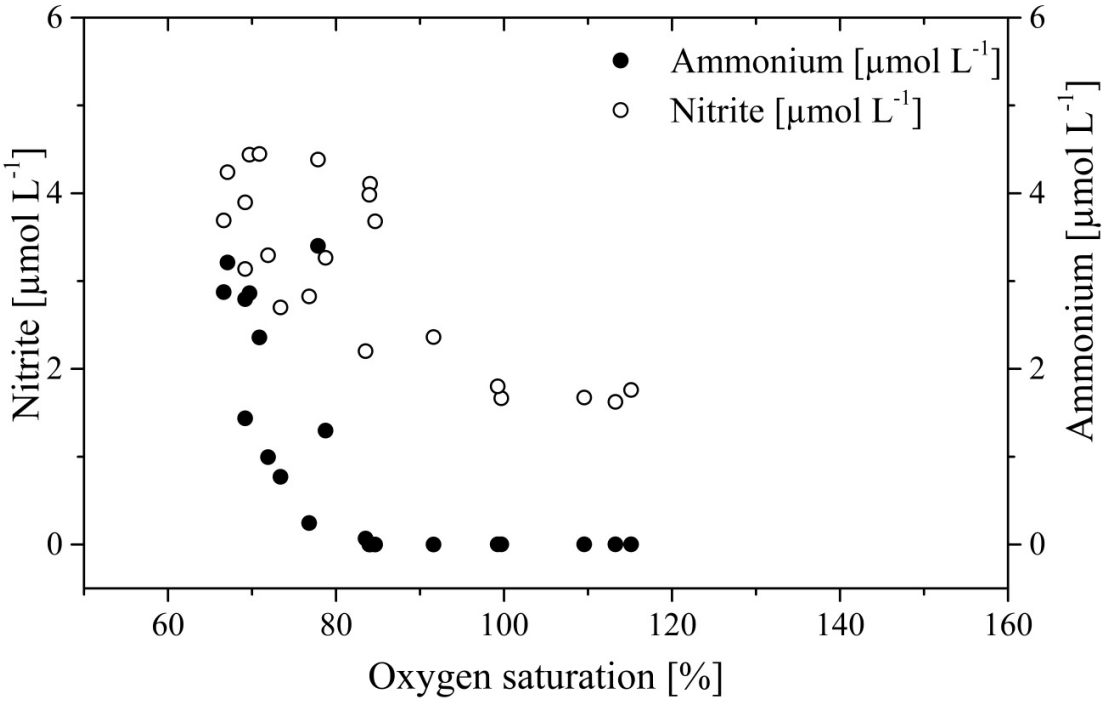

Figure 2.2 Ammonium and nitrite concentrations increase with decreasing dissolved oxygen saturation.

2.3.3. Isotope trends of DIN and particulate nitrogen

During the entire flood (i.e., excluding discharge <3000 m^3 s^{-1}), $\delta^{15}N\text{-}NO_3^-$ and $\delta^{18}O\text{-}NO_3^-$ values were negatively correlated with discharge and nitrate concentration. The range of δ-values of nitrate during the flood was relatively narrow: Initial values of $\delta^{15}N\text{-}NO_3^-$ and $\delta^{18}O\text{-}NO_3^-$ were 9.0 and 3.5‰, respectively, dropping to 7.4 and 2.1‰ when nitrate concentration was highest (Fig. 2.1b, c). Afterwards, δ-values of nitrate increased again, alongside with dropping concentration, reaching values of 8.8 and 3.9‰ for $\delta^{15}N\text{-}NO_3^-$ and $\delta^{18}O\text{-}NO_3^-$, respectively. The ratio of $\delta^{18}O\text{-}NO_3^-$ to $\delta^{15}N\text{-}NO_3^-$ was 1.22 (Fig. 2.3).

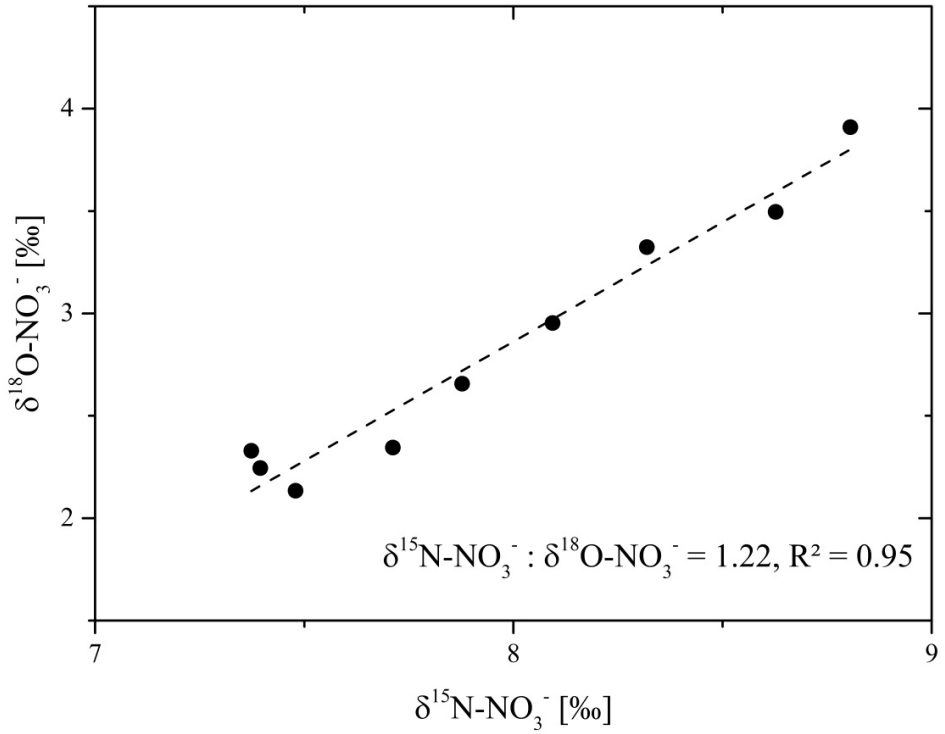

Figure 2.3 Ratio of $\delta^{18}O\text{-}NO_3^-$ versus $\delta^{15}N\text{-}NO_3^-$ values corresponding to decreasing nitrate concentrations from 13 to 20 June and filled data points of figure 2.1b and c. The calculated linear regression has a slope of 1.22 with R^2 of 0.95.

Even though nitrite concentration changed gradually over the course of the flood, nitrite isotope values followed a complex pattern (Fig. 2.1b, c). Before the flood, nitrite concentration increased slightly from 1.6 to 1.8 µmol L^{-1}, while $\delta^{15}N\text{-}NO_2^-$ increased from -14.2 to -8.0‰. At higher discharge (>2000 m^3 s^{-1}), nitrite concentration gradually rose to a maximum of 4.4 µmol L^{-1}, while $\delta^{15}N\text{-}NO_2^-$ decreased from -8.0 to -13.8‰. When discharge decreased, nitrite consumption was coupled to a clear increase of $\delta^{15}N\text{-}NO_2^-$. This net decrease most likely represented co-occurring consumption and production processes, but we were able to calculate an apparent isotope effect ε of -10.0±0.1‰ with R^2 of 0.97 (Fig. 2.4, Eq. 2.2).

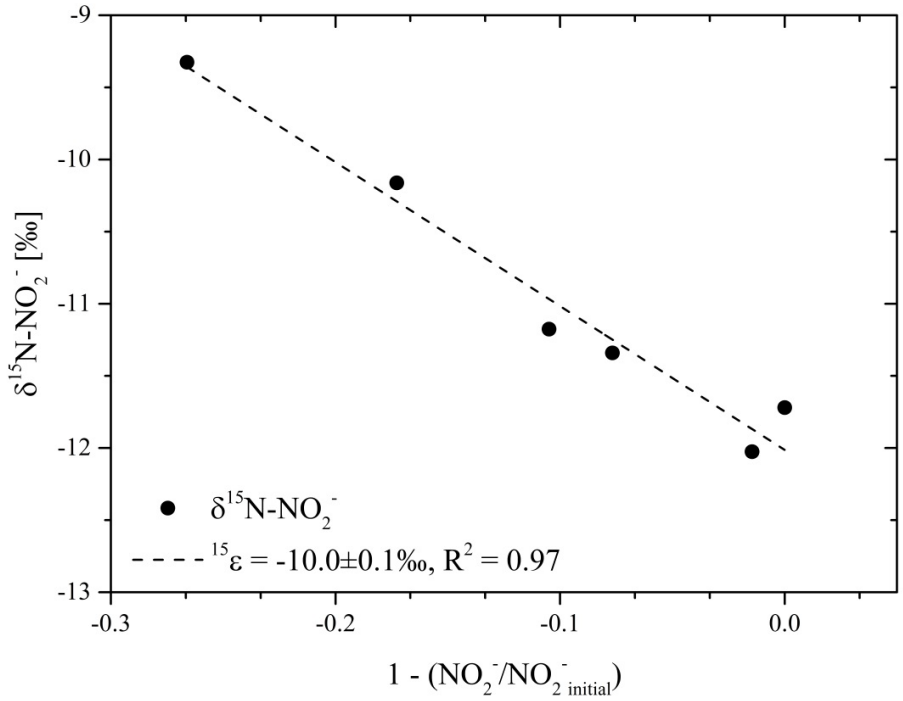

Figure 2.4 Nitrite isotope values versus the remaining fraction of nitrite during the Elbe flood corresponding to the filled data points in figure 2.1b and c. The dashed line indicates the apparent isotope effect during net nitrite consumption with a slope of -10.0±0.1‰ and R^2 of 0.97.

At the beginning of the flood event, ammonium concentration rose, so that $\delta^{15}N\text{-}NH_4^+$ could be analysed. Shortly after the SPM peak, $\delta^{15}N\text{-}NH_4^+$ was about 2‰ and then increased with time to a maximum of 12‰ shortly after peak discharge, followed by a decrease to about 6‰. Although the lowest isotope value coincided with minimal ammonium concentration, there is no distinct correlation of ammonium concentration and its isotope composition. Overall, $\delta^{15}N\text{-}NH_4^+$ seemed to be only weakly correlated to SPM: The changes in $\delta^{15}N$-SPM, though ranging from 8.1 to 6.2‰ during the flood event, were minimal at the time of ammonium occurrence. The first $\delta^{15}N\text{-}NH_4^+$ value we measured during the flood was about 4.5‰ lighter than suspended matter.

2.4. Discussion

2.4.1. Nitrate dynamics and isotope changes during the flood

Nitrate is the primary DIN component in the water column. It is a substrate for phytoplankton assimilation or denitrification, but it is also clearly correlated to discharge, dilution, and to leaching from agricultural soils. This is reflected in the complex changes of nitrate concentration over the course of the flood event, which is in this context comparable to previous river floods (Baborowski et al., 2004).

During the flood, nitrate concentration first decreases with rising discharge, then rises and peaks with peak discharge, decreasing again with lower discharge until the end of the flood event. We assume that up to peak discharge on 14 June, nitrate is mainly determined by hydrographic properties, such as dilution and input from tributaries.

Nitrate concentration decreased from 269.6 to 228.1 µmol L^{-1}, due to an initial dilution of the river nitrate load with high amounts of precipitation and terrestrial runoff. After this minimum, i.e., after 10 June, the input from tributaries and upstream regions gained in importance (Baborowski et al., 2004). Nitrate concentration increased with discharge, which can be attributed to terrestrial soil nitrate that is leached from the catchment area. This soil nitrate stems from nitrification and is an important nitrate source to the river system at this time of the year (Johannsen et al., 2008).

This scenario is supported by SPM values: The high runoff initially results in a peak of SPM from groyne fields, which is eluted directly before the discharge peak (Baborowski et al., 2004). The decrease of δ^{15}N-SPM from ~8‰ to <6‰ during increasing discharge also indicates the input of terrestrial organic material due to leaching. Terrestrial organic matter has a δ^{15}N-value of about 3.5‰, which is significantly lower than riverine SPM with δ^{15}N about 8 – 9‰ (Middelburg and Nieuwenhuize, 1998, and this study). The high C/N ratio during the SPM peak and minimum of nitrate (10 compared to 7.5 before the peak) further suggests that terrestrial organic matter contributes to the riverine signal at this time. Afterwards, the C/N ratio decreases, probably because water masses from tributaries and upstream regions contribute to the pool, as it has been observed during a previous flood event in the Elbe River (Baborowski et al., 2004). At the same time, assimilation by phytoplankton is low, probably due to high turbidity, short residence times, dilution of active cells, and decreased light availability (Deutsch et al., 2009; Voß et al., 2006). After 14 June dropping discharge allows a recovery of phytoplankton, which is also visible in rising oxygen concentration.

The effect of biological processing and assimilation on the nitrate pool can be inferred from concentration and isotope changes. In the Elbe River, summer nitrate concentrations are <<100 µmol L^{-1} and in winter it is >300 µmol L^{-1}. Mean summer $\delta^{15}N\text{-}NO_3^-$ and $\delta^{18}O\text{-}NO_3^-$ values are <18.0 and 7.6‰, respectively, and mean winter values for $\delta^{15}N\text{-}NO_3^-$ and $\delta^{18}O\text{-}NO_3^-$ are <9.3 and 0.8‰, respectively (Johannsen et al., 2008; Schlarbaum et al., 2011). During the flood in June, $\delta^{15}N\text{-}NO_3^-$ is 7.4 – 9.0‰ and $\delta^{18}O\text{-}NO_3^-$ is 2.1 – 3.9‰ (Fig. 2.1c), which is close to winter values and suggests only little biological processing.

In summer and under normal flow conditions, nitrate concentration decreases due to assimilation and biomass production. As a consequence, dual isotope values are negatively correlated with nitrate concentration (Deutsch et al., 2009; Johannsen et al., 2008). During the flood event, $\delta^{15}N\text{-}NO_3^-$ and $\delta^{18}O\text{-}NO_3^-$ are clearly correlated with $[NO_3^-]$ after the nitrate peak (R^2 of 0.90 and 0.93, respectively), which, together with rising $[O_2]$ concentration, pinpoints the onset of biological nitrate assimilation. Accordingly, we calculated the isotope effect using an open-system approach (Eq. 2.2), during this net decrease in nitrate concentration. The isotope effect $^{15}\varepsilon$ is -4.0±0.1‰ (R^2 of 0.89) and $^{18}\varepsilon$ is -5.3±0.1‰, R^2 of 0.92 (Fig. 2.5). This is on the low end of isotope effects reported for nitrate assimilation (Granger et al., 2004; Waser et al., 1998), but fractionation can be affected by residence times, such that the isotope effect is lower when residence times are low (Kendall, 1998). Moreover, we cannot exclude co-occurring nitrate production, which may also contribute isotopically depleted nitrate to the total pool. However, regarding the amount of pre-existing nitrate, we assume that this effect is of lesser importance.

The ratio of $\delta^{15}N\text{-}NO_3^-$ to $\delta^{18}O\text{-}NO_3^-$ also supports the dominant role of phytoplankton assimilation. At the beginning of the flood, $\delta^{15}N\text{-}NO_3^-$ is not correlated with $\delta^{18}O\text{-}NO_3^-$, but when nitrate decreases, the ratio of $\delta^{18}O\text{-}NO_3^-$ to $\delta^{15}N\text{-}NO_3^-$ changes along a slope of 1.22 (R^2 of 0.95, Fig. 2.3). It differs slightly from unity, which is associated with phytoplankton assimilation only (Deutsch et al., 2009; Granger et al., 2004), but this might be due to nitrification, which would lower the $\delta^{18}O\text{-}NO_3^-$ values and thus lead to a slope above 1 (e.g. Wankel et al., 2006).

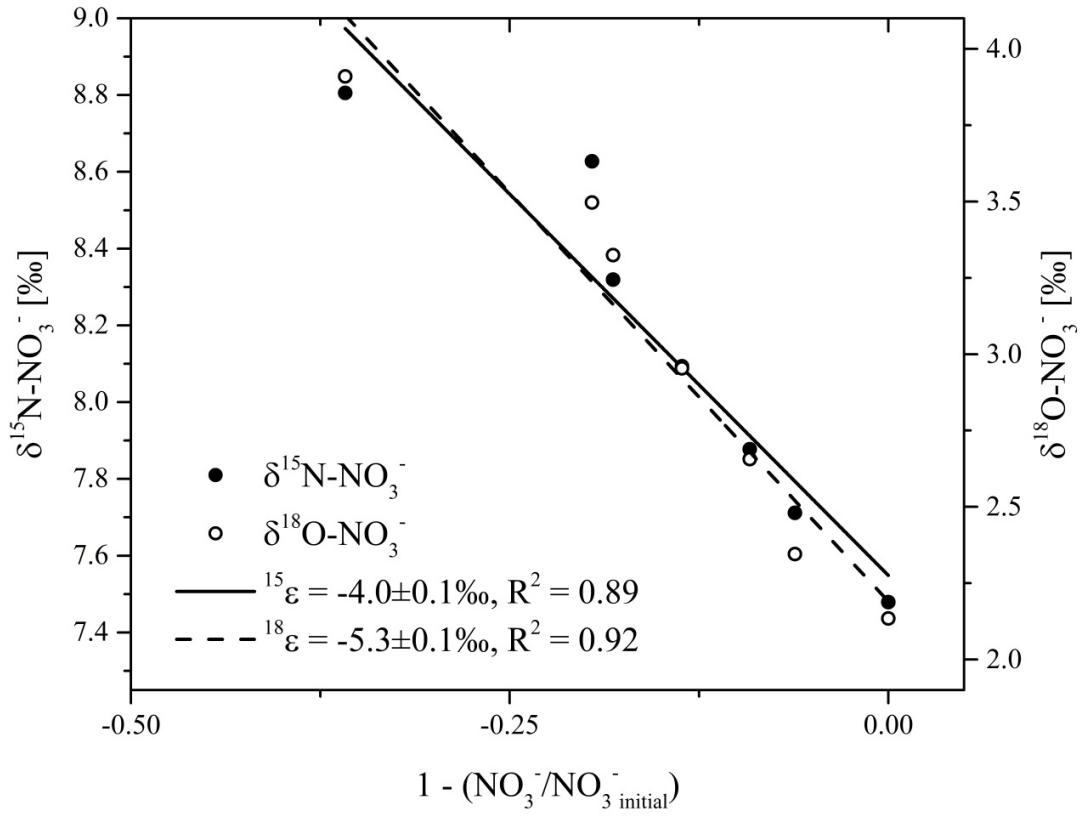

Figure 2.5 Dual nitrate isotope values versus the remaining fraction of nitrate corresponding to the filled data points in figure 2.1b and c. The solid line indicates the apparent isotope effect during net nitrate consumption with a slope of $^{15}\varepsilon$ -4.0±0.1‰ with R^2 of 0.89 and the dashed line is $^{18}\varepsilon$ -5.3±0.1‰ with R^2 of 0.92.

2.4.2. Sources of nitrite and ammonium

Nitrate concentration during the flood is high, but an unexpected and rare event during the flood is the intermediate accumulation of ammonium and nitrite. Generally, these nutrients do not accumulate in the water column in spring and summer (Jacob et al., unpublished), but during the flood, they are present in unusually high concentrations. This indicates that the normal biological turnover processes during the flood are disrupted, probably because discharge and turbidity are high. In the following, we will evaluate sources of ammonium and nitrite, and then discuss those potential sources based on isotope changes.

Both nutrients accumulate at low [O_2], and we speculate that this is due to reduced phytoplankton assimilation. It is unlikely that ammonium in the water column derives from external agricultural sources, because ammonium molecules are positively charged and thus tightly bound to clay particles in soil, and elution with discharge generally does not occur (Mancino, 1983). We

regard remineralization of SPM as the main source of ammonium, which, in turn is then usually immediately assimilated (Dortch et al., 1991) or oxidized to nitrite (Mayer et al., 2001). The first ammonium isotope value we were able to measure in the river was ~2‰, approximately 4.5‰ lighter than the SPM pool (Fig. 2.1c). If ammonium stems from remineralization, this suggests a -4.5‰ fractionation during remineralization. Remineralization is usually associated with a slightly lower isotope effect, but our data are in accordance with Schlarbaum et al. (2011), who found differences of up to -4.5‰ between $\delta^{15}N$ of suspended matter and dissolved organic nitrogen in the Elbe River. A breakdown in assimilation, as indicated by low oxygen concentrations, can then lead to an accumulation of remineralised ammonium. Potential sinks for ammonium are assimilation, when phytoplankton recovers, or nitrification.

Based on isotope changes in ammonium, it remains difficult to distinguish its sinks. The subsequent enrichment of the ammonium pool suggests that light ammonium is removed from the pool. Ammonia oxidation has a strong isotope effect of -14 to -41‰ (Casciotti et al., 2003; Mariotti et al., 1981; Santoro and Casciotti, 2011), and the initial isotopic difference of ammonium and nitrite is 15‰ and thus in the range expected for the isotope effect of ammonium oxidation; this suggests that ammonium is a relevant nitrite source. However, we cannot compute an isotope effect for ammonium consumption over the course of the flood, the concentration remains high for several days, and once it decreases, ammonia immediately falls below the detection limit.

For nitrite accumulation, we also regard external sources, such as an effect of mixing of different water masses as unlikely, because nitrite is generally not abundant in the catchment and is immediately oxidized. Neither is nitrite present in atmospheric deposition (Beyn et al., 2014), which leaves internal sources or a disruption of normal biological processing as a reason for accumulation.

Equivalently to the accumulation of ammonium, the breakdown in phytoplankton activity can lead to the increase in nitrite concentration. In stress situations, phytoplankton can release nitrite from the cells into the water (Lomas and Lipschultz, 2006). The nitrite accumulation may thus be analogous to the primary nitrite maximum (PNM) in the oceans (Lam et al., 2011; Lomas and Lipschultz, 2006; Santoro et al., 2013).

On the sink side, we assume that nitrite assimilation by phytoplankton is of minor importance. Even though the possibility of nitrite assimilation by phytoplankton is commonly accepted (Collos, 1998), it is energetically expensive (Lomas and Lipschultz, 2006). Furthermore, nitrate

and nitrite reduction happens within the cell and an active transport of nitrite through the chloroplast membrane would require additional energy (Lomas and Lipschultz, 2006), making this process unfavourable in the presence of nitrate. Other nitrite sinks are denitrification or nitrification, i.e., nitrite oxidation. In the oxic water column, denitrification is negligible, but it can be quantitatively important, when it occurs in sediments or the riparian zone (Brandes and Devol, 1997; Sebilo et al., 2003).

Nitrification hence may be a sink for both ammonium and nitrite, and one of the goals of our study was to evaluate the role of nitrification during the flood. When ammonium drops below the detection limit with decreasing discharge, nitrite remains above 3 µmol L^{-1} for a few days (Fig. 2.1b). This succession of nitrite and ammonium concentration maxima can indicate successive nitrification acting as ammonium and nitrite sink, respectively (Meeder et al., 2012). Nitrification will, however, need to compete for ammonium with phytoplankton (Ward et al., 1984), and the resulting nitrite may be subject to various consumption pathways.

While we cannot trace any newly produced nitrate into the large pre-existing nitrate pool, the gradual change of nitrite concentration and isotope values provides the unique opportunity to calculate the apparent isotope effect of net nitrite consumption in the river system. When nitrite concentration decreases (see filled symbols in Figs. 2.1b, c and 2.4), the apparent isotope effect is -10.0±0.1‰. This negative isotope effect suggests conventional fractionation during nitrite consumption (R^2 of 0.97). In the light of our hypothesis that nitrification should be promoted during flood conditions, this is surprising, because nitrite oxidation is associated with an inverse isotope effect (Casciotti, 2009).

2.4.3. Nitrite uptake scenarios

As discussed above, potential sinks for nitrite in the river are assimilation, denitrification, and nitrite oxidation. The isotope effect we calculated indicates that nitrite oxidation cannot solely be responsible for nitrite consumption; other processes must occur that cause an increase in the nitrite isotope signal.

One candidate process is nitrite assimilation. As we evaluated above, we assume that it does not play a significant role in the river during the flood, because nitrate and partly ammonium, are present and more favourable substrates. Furthermore, nitrite assimilation would not significantly affect our calculations of the isotope effect, because it is associated with a small isotope effect of -0.7 to +1.6‰ (Wada and Hattori, 1978).

Denitrification, on the other hand, is potentially quantitatively important in the Elbe River (Deutsch et al., 2009). Sedimentary denitrification has little to no impact on isotope values of the water column nitrate pool (Brandes and Devol, 1997; Mariotti et al., 1988) and cannot lead to enriched nitrite isotopes. Denitrification will not occur in the water column, but riparian denitrification may be a nitrite sink with a notable apparent isotope effect (Mengis et al., 1999; Sebilo et al., 2003). If this isotope effect was expressed, it might be an explanation for the measured enrichment in nitrite isotopes. Another explanation may be that the nitrite isotope signature to some extent is coupled to that of ammonium. If nitrite stems from increasingly enriched ammonium, this may lead to an increase in the isotope signature of nitrite.

On the basis of these assumptions, we can calculate different scenarios to constrain the role of nitrite oxidation in the river. In each scenario, we assume that nitrite consumption exceeds nitrite production. Using the open system equations (see sect. 2.2.4); we then aimed to reproduce the nitrite isotope effect of -10.0‰ (cf. Fig. 2.4).

2.4.3.1. Scenario 1 – consumption scenario

For an initial evaluation of nitrite oxidation, we assumed that nitrite is consumed by two nitrite sinks, riparian denitrification and nitrite oxidation, for which we assumed average isotope effects of -16‰ (Deutsch et al., 2005; Houlton and Bai, 2009; Kendall et al., 2007), and +13‰ (Casciotti, 2009), respectively. If these are the only processes that influence nitrite isotopes, the isotope effect in this scenario then basically is the average isotope effect of these two sinks.

In our case, this yields a 22% contribution of nitrite oxidation, whereas denitrification would make up for 78% of nitrite consumption. However, in this case we assume that no ammonium is remineralised, and that no new nitrite is formed via ammonium oxidation, which seems somewhat unlikely.

2.4.3.2. Scenario 2 – constant source scenario

In a second approach, we include ammonium remineralization and nitrite formation from ammonium. The underlying assumption is that ammonium is produced from SPM, and that this new ammonium has an isotope signature that is 2‰ lower than that of SPM (cf. Möbius, 2013), i.e., ~4.5‰. Under these circumstances, the nitrite pool permanently is diluted with nitrite of a constant isotope signature of 4.5‰, assuming that no fractionation occurs, because ammonium turnover is complete.

This newly produced nitrite is isotopically enriched relative to the depleted existing pool (Fig. 2.1c). Our measurements make it impossible to define absolute rates, but to best match our

data, we tried to reproduce the fraction of nitrite removed from the system (now including new production) as well as the slope of nitrite isotope values.

The fraction of nitrite removed (*f* in Eq. 2.2) depends on the ratio of ammonium oxidation (i.e., nitrite production) to nitrite consumption. Nitrite consumption must exceed ammonium oxidation, because nitrite concentration decreases. The nitrite consumption we measured in the Elbe River is best reproduced if assume that 25% of the total nitrite pool are removed, and that the ratio of ammonium oxidation to nitrite consumption is 0.8.

We then changed the ratio of nitrite oxidation to denitrification to match the isotope data, assuming isotope effects of +13‰ and -16‰, respectively, as described for the previous scenario. In this case, the contribution of nitrite oxidation rises to 31%, and denitrification accordingly makes up for 69% of nitrite consumption.

2.4.3.3. Scenario 3 – enriched source scenario

As an upper limit for the contribution of nitrite oxidation, we also addressed the option of changing ammonium source signatures. Ammonium concentration is low during almost the entire time of nitrite consumption. As phytoplankton recovers (evidenced by increased [O_2]), it might well contribute to ammonium consumption. Phytoplankton assimilation of ammonium can have an isotope effect of ~-19‰ (Waser et al., 1998). If ammonium is fractionated during uptake, but also permanently supplied from remineralization, a moderate enrichment of the pool is at least possible. An enrichment to 12‰ during processing seems realistic, we see ammonium isotope values reach 12‰ over the course of the flood. In case the nitrite pool was diluted with increasingly heavy ammonium, the best fit to our data is achieved if we assume a high ratio of ammonium oxidation to nitrite consumption of 0.98 and a contribution of nitrite oxidation of 36%, which seems to represent the upper limit of nitrite oxidation.

All these scenarios are of course sensitive to the input variables, especially the isotope effects assigned to nitrite oxidation and denitrification. It is of course also possible that the entire regime is based on denitrification only, with a moderate isotope effect of -10‰, but this seems improbable. Nitrification is an important process regenerating nitrate in the Elbe River (Johannsen et al., 2008). Therefore, a scenario that includes both consumption processes is plausible, and nitrite isotopes reveal the substantial role of nitrification and remineralisation.

2.5. Conclusions

During an exceptional flood in the Elbe River in June 2013, an intermediate accumulation of ammonium and nitrite in the water column indicates a disruption of normal nitrogen processing. A suppression of nitrate assimilation is reflected in high water column concentration and a very moderate isotope effect of nitrate uptake. Our data suggest that the main source of ammonium is remineralisation of organic material, whereas the changing nitrite concentration and isotopes are influenced by several sources and sinks. Net nitrite consumption in the water column has an apparent isotope effect of -10.0±0.1‰, which clearly cannot be explained by nitrification only, which is associated with inverse isotope effect.

To disentangle nitrite consumption pathways, we constructed a simple box-model with riparian denitrification and nitrite oxidation as potential nitrite sinks. We find that during the flood, the contribution of nitrite oxidation contributes ranges from 22 – 36%, whereas riparian denitrification makes up for 64 – 78% of nitrite consumption. Our nitrite isotope data reveal the substantial role of nitrification and remineralisation during an extreme flood event, but also demonstrate that other sinks, like denitrification in the riparian zone, contribute to nitrite turnover.

While the inverse isotope effect of nitrite oxidation adds more complexity to the isotope budget of the aquatic nitrogen cycle, our data suggest that co-occurring processes disguise this inverse isotope effect in natural environments, which might not only be important in estuarine settings, but also in other environments that show nitrite accumulation in the water column, like oceanic OMZs, where nitrate and nitrite isotopes are frequently used to assess nitrogen dynamics.

Data availability

The dataset is available at Pangaea (doi:10.1594/PANGAEA.865348). Discharge values were obtained from http://koflux1.hzg.de/staff/kappenberg/runoff_data/elbe.abfluss (Wasser- und Schifffahrtsamt Lauenburg, 2016).

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3. Oxidation kinetics and inverse isotope effect of marine nitrite-oxidizing bacteria

Juliane Jacob, Boris Nowka, Véronique Merten, Tina Sanders, Eva Spieck, Kirstin Dähnke

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Abstract

Nitrification, the step-wise oxidation of ammonium to nitrite and nitrate, is important in the marine environment, because it produces nitrate, the most abundant marine DIN component and N-source for phytoplankton and microbes. This study focuses on the second step of the nitrification, which is carried out by a distinct group of organisms (nitrite-oxidizing bacteria, NOB). Nitrite oxidation kinetics are important characteristics for the growth of NOB. We investigate the nitrite oxidation kinetics of marine NOB (*Nitrospina watsonii 347, Nitrospira* Ecomares 2.1, *Nitrococcus mobilis 231,* and *Nitrobacter sp. 311*) and compare the kinetics to those of non-marine species. We also determine the isotope effect during nitrite oxidation of two strains (*Nitrospina watsonii 347* and *Nitrospira* Ecomares 2.1).

The enzyme kinetic of nitrite oxidation is described by Michaelis-Menten kinetics. *Nitrospira* and *Nitrospina* have low half-saturation constants K_m (54 and 37 µM NO_2^-) and low specific activities V_{max} (21 and 37 µmol NO_2^- mg protein^{-1} h^{-1}), whereas *Nitrococcus* is characterized by high K_m values (120 µM NO_2^-) and high specific activities (141 µmol NO_2^- mg protein^{-1} h^{-1}). *Nitrobacter* showed the lowest K_m value (28 µM NO_2^-) of all NOB investigated in this study and had an intermediate maximum specific activity (95 µmol NO_2^- mg protein^{-1} h^{-1}).

The isotope effect during nitrite oxidation by *Nitrospira watsonii 347* and *Nitrospina* Ecomares 2.1 is 10.2±0.9‰ and 9.7±0.8‰, respectively. This confirms the inverse isotope effect described previously; however, it is at the lower end of reported isotope effects. We speculate that low isotope effects reflect differences in NXR location.

3.1. Introduction

In the global ocean, nitrate is the dominant DIN component; its sinks are denitrification and phytoplankton assimilation. Nitrite concentrations are generally low; however, there are regions where nitrite does accumulate to micromolar concentrations. On the one hand, there is the primary nitrite maximum (PNM), which occurs at the base of the euphotic zone due to nitrification or phytoplankton release of nitrite (Dore and Karl, 1996; Lomas and Lipschultz, 2006; Olson, 1981). On the other hand, nitrite also occurs in the secondary nitrite maximum (SNM), which is in the oxygen-depleted deeper water column, where it probably stems from heterotrophic denitrification and ammonia oxidation (Lam et al., 2011).

Important oxygen minimum zones (OMZ) with significant nitrite accumulation are located in the Arabian Sea, the eastern Tropical North Pacific, and the Eastern Tropical South Pacific (c.f. overview in Wright et al., 2012). Even though only 1% of the total ocean volume is defined as OMZs (Lam and Kuypers, 2011), they are responsible for 30 to 50% of the global marine nitrogen loss (Codispoti et al., 2001; Gruber and Sarmiento, 1997), which is evidence for high rates of nitrification and denitrification (Lam and Kuypers, 2011). Although nitrification is an aerobic process, it is also a key process in OMZs.

In this manuscript, we focus on the marine environment and on the second step of nitrification - the oxidation of nitrite by chemolithoautotrophic bacteria. These gain energy from the chemical conversion of nitrite and use CO_2 as a carbon source (Fiencke et al., 2005; Watson et al., 1989). NOB are taxonomically classified based on cell shape and ultrastructural criteria. The known NOB belong to the genera *Nitrospina* (Watson and Waterbury, 1971), *Nitrospira* (Watson et al., 1986), *Nitrobacter* (Winogradsky, 1892; Woese et al., 1984), *Nitrococcus* (Watson and Waterbury, 1971; Woese et al., 1985), *Nitrotoga* (Alawi et al., 2007), and *Nitrolancea* (Sorokin et al., 2012). Furthermore, *Candidatus* Nitromaritima was identified based on metagenomic data (Ngugi et al., 2016). NOB are phylogenetically heterogeneous (Teske et al., 1994), which is reflected in a wide range of preferences regarding environmental conditions like temperature or substrate concentration (De Boer et al., 1991). In the marine realm, *Nitrobacter, Nitrococcus, Nitrospina, Nitrospira, Candidatus* Nitromaritima, and on rare occasions *Nitrotoga* have been found, but only few strains were isolated, and there is limited knowledge on their overall distribution and abundance (Ngugi et al., 2016; Ward, 2011; Watson et al., 1986; Watson and Waterbury, 1971). Profound data for marine NOB are scarce and mainly available for *Nitrobacter* (Both et al., 1992; Laanbroek et al., 1994; Prosser, 1989), including *Nitrobacter 355* isolated from Black Sea surface water, and for *Nitrospira* (Blackburne et al., 2007; Schramm et al.,

1999), including *Nitrospira marina* from the Gulf of Maine*,* the first marine isolate of this genus (Ehrich et al., 1995; Watson et al., 1986).

Nitrite oxidation is catalysed by nitrite oxidoreductase (NXR) enzymes, and two distinct intracellular locations are known. NXR enzymes in *Nitrobacter* and *Nitrococcus* are located on the inner surface of the cytoplasmic and intracytoplasmic membranes (Spieck and Bock, 2005; Sundermeyer-Klinger et al., 1984; Tsien et al., 1968). *Nitrospira* and *Nitrospina* lack intracytoplasmic membranes, and NXR is oriented toward the periplasm (Lücker et al., 2010; Spieck et al., 1998). NXR in different genera of NOB varies in its molecular mass, its location, and in the presence or absence of cytochromes in the electron transport chain.

The activity of NXR can be described based on Michaelis-Menten kinetics, i.e., the half-saturation constant (K_m) and the maximum nitrite oxidation activity (V_{max}). Based on these parameters and the idea of K- and r-selection (Andrews and Harris, 1986; MacArthur and Wilson, 1967), Schramm et al. (1999) classified *Nitrospira* as K-strategist and *Nitrobacter* as r-strategist. K-strategy originally meant selection for competitive ability in crowded populations, and r-strategy referred to selection for high population growth un uncrowded populations (MacArthur and Wilson, 1967). K-strategists among microbes have high substrate affinities at low substrate concentrations, and r-strategists have high maximum specific growth and substrate utilization rates at high substrate concentrations (Andrews and Harris, 1986). So far, nitrite oxidation kinetics for the major marine NOB, *Nitrospina* and *Nitrococcus*, have not been investigated (Füssel et al., 2012).

The isolation of NOB is difficult, so that various approaches have been taken to assess their activity and environmental relevance in nature. One is based on stable isotope investigations of nitrite, which in aquatic environments can shed light on the origin and fate of nitrite (Füssel et al., 2012; Jacob et al., 2016). Nitrite oxidation is coupled to a rare inverse isotope effect, so that nitrite gets subsequently depleted during oxidation (Casciotti, 2009). However, this isotope effect has only been investigated in *Nitrococcus mobilis 231, Nitrobacter* sp. 355, and *Nitrospira marina.* It is known that the isotope effect of a specific reaction can vary depending on enzyme properties and genetic diversity (Casciotti et al., 2003), but so far, the data base has been too sparse to assess the variability of the isotope effect of nitrite oxidation and its link to enzyme variability and Michaelis-Menten kinetics.

In this study we investigated four marine species (*Nitrospira* Ecomares 2.1*, Nitrospina watsonii 347, Nitrobacter* sp. 311*,* and *Nitrococcus mobilis* 231) for their nitrite oxidation kinetics and two species (*Nitrospira* Ecomares 2.1, *Nitrospina watsonii 347*) for their specific kinetic isotope ef-

fects, and compare those to non-marine species. We hypothesize that (1) the environmental conditions in the marine realm (e.g., low substrate concentration in comparison to terrestrial habitats) should be reflected in enzyme kinetics, and (2) aimed to investigate a potential link between enzyme kinetics and the isotope effects of nitrite oxidation.

3.2. Materials and Methods

3.2.1. Bacterial strains and cultivation

Four different genera of NOB were investigated: *Nitrospira* Ecomares 2.1, *Nitrospina watsonii 347, Nitrobacter sp. 311,* and *Nitrococcus mobilis 231*.

Nitrospira Ecomares 2.1 was isolated from a moving-bed filter of an aquaculture system in Büsum, Germany, and has optimal growth conditions in 28 – 30°C seawater (70%, Keuter et al., 2011). *Nitrospina watsonii 347* was originally sampled in 100 m depth of the Black Sea (Spieck et al., 2014). Its temperature optimum is 28°C in 70% seawater (Watson and Waterbury, 1971). *Nitrospira* and *Nitrospina* have substrate optima from 0.5 to 3 mM, and the highest substrate concentration tolerated is 30 mM (Keuter et al., 2011; Spieck et al., 2014). To date, *Nitrospina* has been found exclusively in marine habitats.

Nitrobacter sp. 311 was isolated from surface waters of the tropical Eastern Atlantic Ocean near the west coast of central Africa (J. Waterbury personal communication in Starkenburg et al. 2008). Some species of *Nitrobacter* tolerate up to 45 mM nitrite (Starkenburg et al., 2011). *Nitrococcus mobilis 231* so far is the only isolate of this genus and has to date been found exclusively in marine habitats. It was isolated from surface water of the South Pacific Ocean near the Galapagos Archipelago. Optimal growth conditions are 25 – 30°C in 70 – 100% seawater, and the highest substrate concentration tolerated was 60 mM (Watson and Waterbury, 1971).

3.2.2. Chemical analyses

For nitrite oxidation kinetics experiments, nitrite and nitrate concentrations of *Nitrospira, Nitrospina, Nitrobacter and Nitrococcus* cultures were analysed by high-performance liquid chromatography (HPLC) coupled to an ion pair chromatograph (LiChrospher RP-18 column, Merck; Meincke et al., 1992), and UV detection in an automated system (LaChrom Elite HPLC system; VWR). The cell protein concentration was analysed based on the bicinchoninic acid method (Smith et al., 1985) after cell lysis in 0.15 M NaOH and incubation at 90°C for 30 min.

For nitrite isotope fractionation experiments, *Nitrospina watsonii 347* samples were analysed photometrically using standard colorimetric techniques based on a reaction of Sulfanilamide with N-(1-naphthyl)-ethylenediaminedihydrochloride (NEDA) at wavelength of 542 (Grasshoff et al., 2009). Nitrite and nitrate concentrations of *Nitrospira* Ecomares 2.1 were analysed with an HPLC system (Jasco) (Meincke et al., 1992).

3.2.3. Activity measurements and calculation of nitrite oxidation kinetics

Nitrite oxidation kinetics were calculated based on activity measurements performed after Nowka et al. (2015). 1 L flasks each with 500 mL marine NOB-medium were inoculated with 1% of bacteria suspension. At least three NOB cultures were independently grown with initial nitrite concentrations of 5 mM for *Nitrospira* Ecomares 2.1*,* 3 mM for *Nitrospina watsonii 347,* 5 mM for *Nitrobacter sp. 311, and* 5 mM for *Nitrococcus mobilis 231.* Nitrite-oxidation dependent oxygen consumption was analysed in a microrespiration system (Unisense AS, Denmark), which is constructed with a one-channel oxygen sensor amplifier (OXY-Meter), a Clark-type oxygen microsensor (OX-MR; polarized for at least 48 h before use), a stirring system with glass-coated magnets, 2 mL glass chambers with glass stoppers, a rack for eight chambers, and the data acquisition software MicOx 3.0. The response time (90%) of the oxygen microsensor was below 15 s, and the oxygen uptake of the microsensor was below 1 nM day^{-1} (Gundersen et al., 1998). All measurements were stirred at 200 rpm in a recirculated water bath in thermostat-regulated rooms. 12 to 48 hours after complete nitrite consumption (early stationary phase), sub-samples were transferred to 2 ml glass chambers, sealed with glass stoppers, and submerged in a recirculated water bath. Through a capillary hole in the glass stopper, the microrespiration sensor was inserted, and equilibrated for 15 to 30 min until the signal was stable. Nitrite from stock solutions was added with a syringe through a second capillary hole (Nowka et al. 2015. The measurements started with nitrite concentrations of up to 150 µM for *Nitrospira* Ecomares 2.1*,* 230 µM for *Nitrospina watsonii 347,* 472 µM for *Nitrobacter sp. 311,* and 1250 µM for *Nitrococcus mobilis 231* (Fig. 3.1).

Oxygen consumption rate profiles based on minimum five different concentrations and at least in triplicates of each NOB strain to calculate specific oxidation kinetics. Nitrite consumption was calculated from oxygen consumption based on a ratio of 2:1. These consumption data were used to calculate oxidation kinetics based on Michaelis-Menten equations (Eq. 3.1).

$$V = \left(\frac{V_{max} * [S]}{K_m + [S]}\right) \quad (3.1)$$

where V is the activity, V_{max} is the maximum specific activity (µmol / mg protein / h), K_m is the half-saturation constant for nitrite oxidation (µM), and [S] is the nitrite concentration in µM. We note that we did not work with purified enzyme, but as growth can be neglected due to short-term incubations of a few hours, the use of the terms K_m and V_{max} is justified.

3.2.4. Setup for nitrite fractionation experiments

The batch experiments of *Nitrospina watsonii 347* and *Nitrospira* Ecomares 2.1 represent closed systems, where any nitrite loss is due to nitrite oxidation. Sterile control flasks were incubated in duplicate to exclude abiotic nitrite turnover.

Both NOB strains were cultured in marine NOB-medium with varying amounts of nitrite (modified from Spieck and Lipski, 2011; Watson and Waterbury, 1971) in the dark at 28°C. 250 mL flasks with 100 mL fresh growth medium were inoculated with 10 mL active stock culture. The medium was then amended with nitrite of a known isotope value ($\delta^{15}N$ = -27.5‰).

We performed six *Nitrospira* Ecomares 2.1 and seven *Nitrospina watsonii 347* experiments, which had nitrite concentration between ~100 µM and ~1600 µM (Tab. 3.1), i.e., within the substrate optima for the respective species (Keuter et al., 2011; Spieck et al., 2014). Samples were taken daily until nitrite was consumed. Nitrite was stoichiometrically converted to nitrate (not shown in plots). At each sampling time, 6 mL of culture were sampled with a sterile pipette and centrifuged (20´; 16,000g). The supernatant was analysed for nutrient and isotope composition. Isotope effects of *Nitrospira* Ecomares 2.1 and *Nitrospina watsonii 347* were based on three to eleven samples that showed a linear decrease in substrate concentration over time (Tab. 3.1).

Table 3.1 Summary of nitrite oxidation experiments calculation of isotope effects of nitrite oxidation.

No.	Bacteria species	Initial nitrite concentration [µM L^{-1}]	Number of samples used for ε calculation
1	*Nitrospira* Ecomares 2.1	921	5
2		831	5
3		849	5
4		564	4
5		563	5
6		560	4
7	*Nitrospina watsonii 347*	1624	5
8		1613	5
9		1533	5
10		1600	5
11		123	3
12		114	3
13		600	11

3.2.5. Isotope analyses

Nitrite isotopes were analysed using the denitrifier method (Böhlke et al., 2007; Casciotti et al., 2002; Sigman et al., 2001). In brief, media samples were injected into a *Stenotrophomonas nitrireducens* bacteria suspension. These bacteria selectively reduce nitrite to N_2O gas, which was then analysed on a GasBench II, coupled to a Delta V isotope ratio mass spectrometer (Thermo Fisher Scientific). The sample volume was always adjusted to a final N_2O gas amount of 5 nmol.

Isotope values are reported using the “delta” notation (Eq. 3.2),

$$\delta^{15}\mathrm{N}\ [‰\ \mathrm{vs.\ standard}] = \left(\frac{\left(\frac{^{15}\mathrm{N}}{^{14}\mathrm{N}}\right)_{\mathrm{sample}}}{\left(\frac{^{15}\mathrm{N}}{^{14}\mathrm{N}}\right)_{\mathrm{standard}}} - 1 \right) * 1000 \qquad (3.2)$$

where the standard for nitrogen is atmospheric N_2.

All samples were analysed in replicate and against in-house potassium nitrite and sodium nitrite standards with known $\delta^{15}N$ values of -81.5‰ and -27.5‰, determined independently via EA-IRMS analysis. The standard deviation of standards and samples was <0.3‰ (n>3).

3.2.6. Calculation of isotope effects

The kinetic isotope effect ε can be calculated based on the Rayleigh closed-system equation (Mariotti et al. 1981, Eq. 3.3).

$$\varepsilon = \frac{\delta^{15}\mathrm{N}_{\mathrm{substrate}} - \delta^{15}\mathrm{N}_{\mathrm{initial}}}{\ln(f)} \quad (3.3)$$

where *f* is the remaining fraction of substrate at the time of sampling, and $\delta^{15}N_{initial}$ and $\delta^{15}N_{substrate}$ represent the initial isotope value of nitrite and the isotope value at the time of sampling, respectively.

3.3. Results

3.3.1. Nitrite oxidation kinetics

Key parameters of nitrite oxidation kinetics are the maximum specific activity and the half-saturation constant. They were assessed based on nitrite-dependent oxygen consumption. In nitrite-depleted, early-stationary-phase cultures oxygen consumption initially was small, and increased within a few minutes after substrate addition. The O_2 consumption rates depended on the added nitrite concentration and followed Michaelis-Menten kinetics (Fig. 3.1, Tab. 3.2). The V_{max} values of the investigated species revealed a clear differentiation of specific activities: *Nitrococcus mobilis 231* had the highest maximum oxidation activities (141 µmol NO_2^- mg protein^{-1} h^{-1}), followed by *Nitrobacter sp. 311* (95 µmol NO_2^- mg protein^{-1} h^{-1}). In contrast, the maximum specific activities in *Nitrospina watsonii 347*, and *Nitrospira* Ecomares 2.1 were much lower, 37 µmol NO_2^- mg protein^{-1} h^{-1} and 21 µmol NO_2^- mg protein^{-1} h^{-1}, respectively. *Nitrobacter sp. 311, Nitrospina watsonii 347*, and *Nitrospira* Ecomares 2.1 had low K_m values (28 µM NO_2^-, 37 µM NO_2^- and 54 µM NO_2^-, respectively), whereas *Nitrococcus mobilis 231* had a comparatively high value (120 µM NO_2^-; Fig. 3.1, Tab. 3.2).

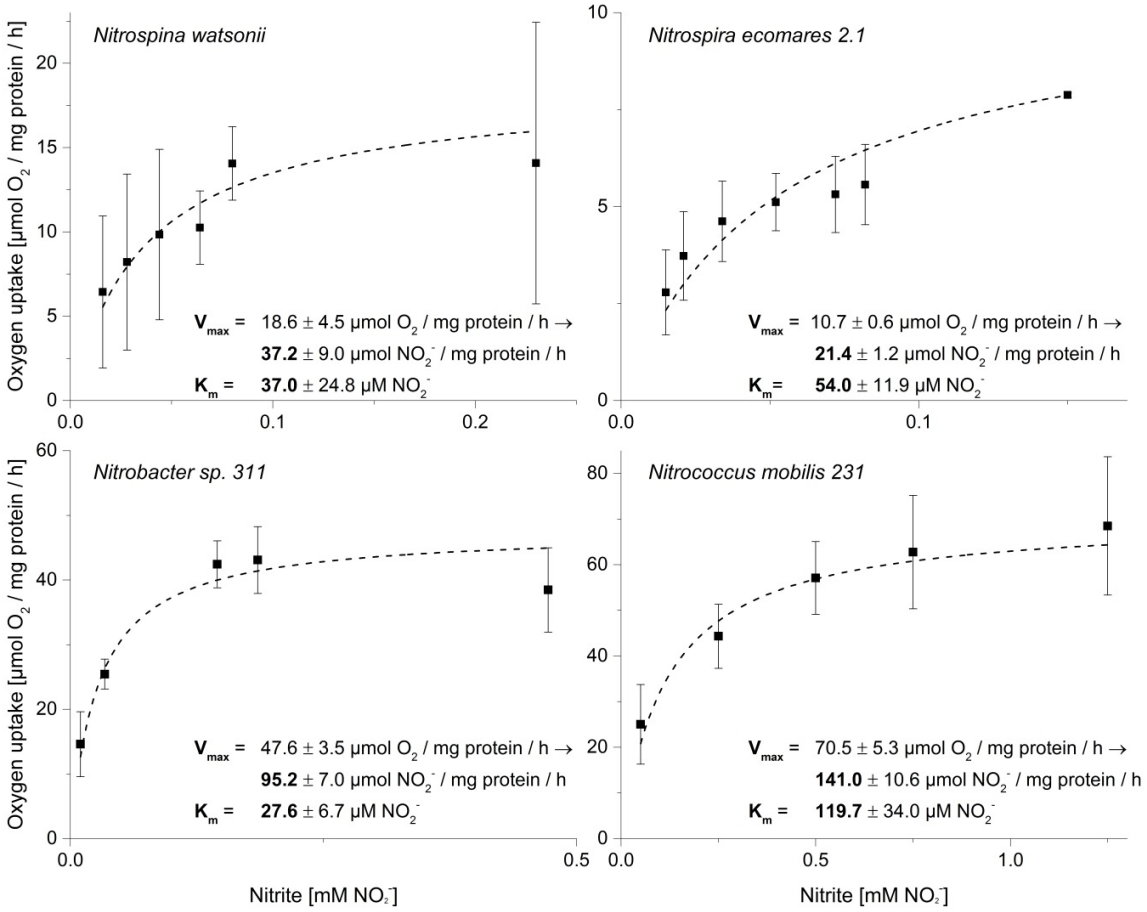

Figure 3.1 Nitrite oxidation kinetics of *Nitrospina watsonii 347, Nitrospira* Ecomares 2.1, *Nitrobacter sp. 311,* and *Nitrococcus mobilis 231*. Michaelis-Menten plots of oxygen uptake versus nitrite concentration of early-stationary-phase cells at 28°C. Mean values and standard deviations resulted from triplicates. The kinetic parameters were calculated by non-linear fitting of Michaelis-Menten equation to the data.

Table 3.2 Results of nitrite oxidation experiments: maximum specific activities and half-saturation constants of four marine NOB. Number of replicates in parentheses.

Bacteria species	Mean max. specific activity V_{max} ± SD	Mean half-saturation constant for activity K_m ± SD
Nitrospina watsonii 347 (3)	37.2 ± 9.0	37.0 ± 24.8
Nitrospira Ecomares 2.1 (4)	21.4 ± 1.2	54.0 ± 11.9
Nitrobacter sp. 311 (3)	95.2 ± 7.0	27.6 ± 6.7
Nitrococcus mobilis 231 (3)	141.0 ± 10.6	119.7 ± 34.0

3.3.2. Isotope analyses and isotope effects

The initial nitrite concentrations are summarized in table 3.1, and the initial nitrite $\delta^{15}N$ was -27.5‰. For up to five days, substrate consumption and isotope changes in each assay were small, but after this lag phase, concentrations and isotope values of nitrite changed rapidly. The isotope values of nitrite decreased during nitrite oxidation in all experiments. At the end of the experiment, $\delta^{15}N\text{-}NO_2^-$ values of up to -50‰ for *Nitrospina watsonii 347* and -65‰ for *Nitrospira* Ecomares 2.1 were reached. Isotope effects were calculated based on Eq. 3.3. In *Nitrospina watsonii 347* cultures, isotope effects were 8.2 to 10.5‰. The replicate regression lines had standard deviations of up to 2.3‰ and were not significantly different from each other ($p>0.05$). We thus calculated a mean isotope effect of 9.7±0.8‰ (Fig. 3.2, Tab. 3.3). The isotope effect $^{15}\varepsilon$ in *Nitrospira* Ecomares 2.1 ranged from 9.0 to 11.5‰. The standard deviation of each regression was smaller than 0.3‰, and replicates were statistically similar ($p>0.05$). Therefore, we calculated the mean value of $^{15}\varepsilon$ based on all experiments, resulting in a mean isotope effect of 10.2±0.9‰ (Fig. 3.3, Tab. 3.3). Overall, we found that the isotope effect $^{15}\varepsilon$ of *Nitrospira* Ecomares 2.1 was not significantly different from that determined in *Nitrospina watsonii 347* ($p>0.05$).

Table 3.3 Results of fractionation experiments: isotope effects and standard deviations.

No.	Bacteria species	Isotope effect [‰]	Standard deviation [‰]
1	*Nitrospira* Ecomares 2.1	9.0	0.2
2		10.0	0.3
3		10.8	0.2
4		11.5	0.3
5		9.9	0.2
6		9.8	0.2
7	*Nitrospina watsonii 347*	8.2	0.7
8		10.5	0.7
9		10.4	0.2
10		9.1	1.9
11		9.7	1.7
12		10.1	2.3
13		9.7	1.8

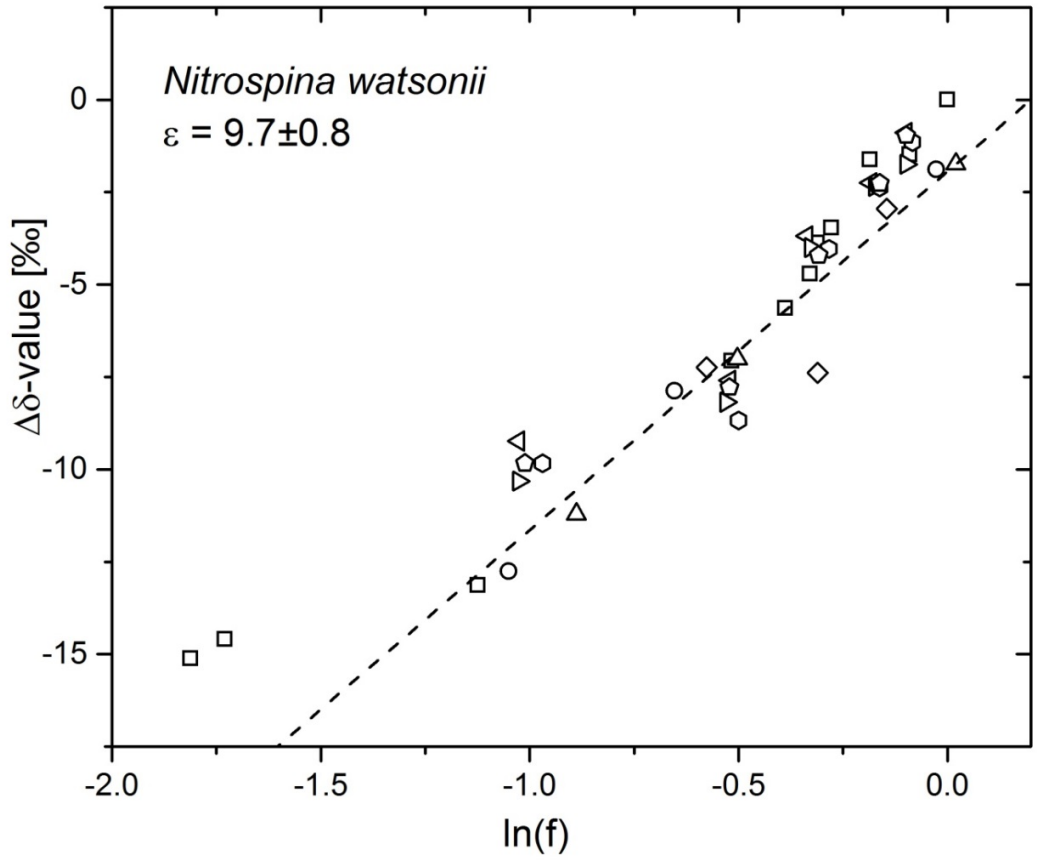

Figure 3.2 Rayleigh-plot for seven *Nitrospina watsonii 347* experiments. Decrease of $\delta^{15}N\text{-}NO_2^-$ relative to the initial $\delta^{15}N\text{-}NO_2^-$ is plotted as a function of $\ln(NO_2^-/NO_{2\,initial}^-)$ during nitrite oxidation (cf. Eq. 3.3 and Granger et al. 2004). Every experiment has its own symbols. Standard deviation of a sample is <0.3‰ and smaller than the symbol. The average of all regressions defines the dashed line and the corresponding isotope effect with an average ε of 9.7±0.8‰.

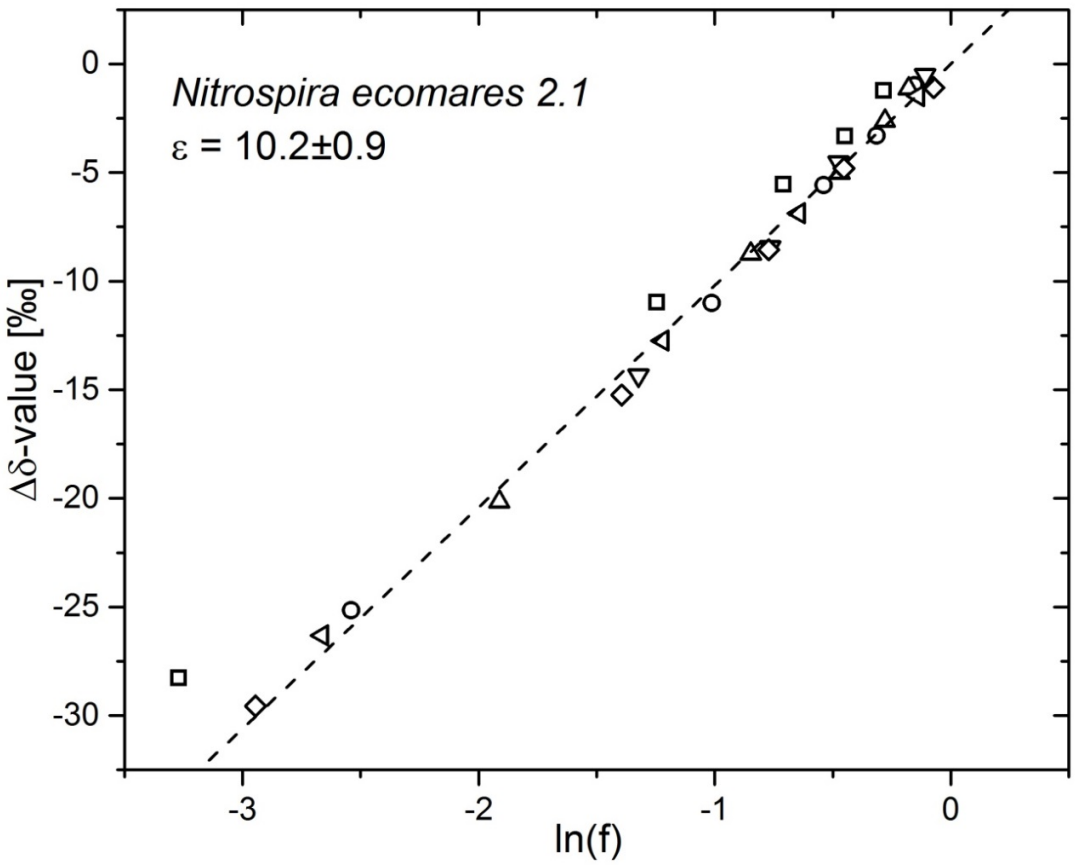

Figure 3.3 Rayleigh-Plot for six *Nitrospira* Ecomares 2.1 experiments. Decrease of $\delta^{15}N\text{-}NO_2^-$ relative to the initial $\delta^{15}N\text{-}NO_2^-$ plotted as a function of $\ln(NO_2^-/NO_{2\,initial}^-)$ during nitrite oxidation (cf. Eq. 3.3 and Granger et al. 2004). Every experiment has its own symbol and standard deviation of a sample is <0.3‰ and smaller than the symbol. The average of all regressions defines the dashed line and the corresponding isotope effect with an average ε of 10.2±0.9‰.

3.4. Discussion

3.4.1. Nitrite oxidation kinetics

So far, an assessment of links between environmental conditions and nitrite oxidation kinetics was difficult, because data for the substrate affinity of marine NOB had not yet been determined. To close this gap, we investigated the nitrite oxidation kinetics of four marine NOB genera, including NOB with periplasmic and cytoplasmic NXR, and compared them with non-marine NOB strains (Fig. 3.4). K_m values for all NOB varied over two orders of magnitude between 9 and >1000 µM NO_2^- (Nowka et al. 2015 and references therein), and the marine NOB we studied fall within the range of K_m values determined to date (Figs. 3.1, 3.4, Tab. 3.2). They do, however, all fall in the low end of K_m values reported so far, and the range of K_m values was rather narrow (28 to 120 µM NO_2^-). In nature, NOB compete for nitrite, and this competition should be reflected in inter- and intraspecific niche differentiation concerning nitrite oxidation kinetics (Blackburne et al., 2007; Kim and Kim, 2006; Nogueira and Melo, 2006; Schramm et al., 1999). In the light of relatively similar K_m values, these niche differentiations seem to be less pronounced among marine NOB, probably because the availability of nitrite as substrate is normally low (Wada and Hatton, 1971). Insofar, the K_m values of all marine NOB we investigated seem to represent an adaptation to these low nitrite concentrations.

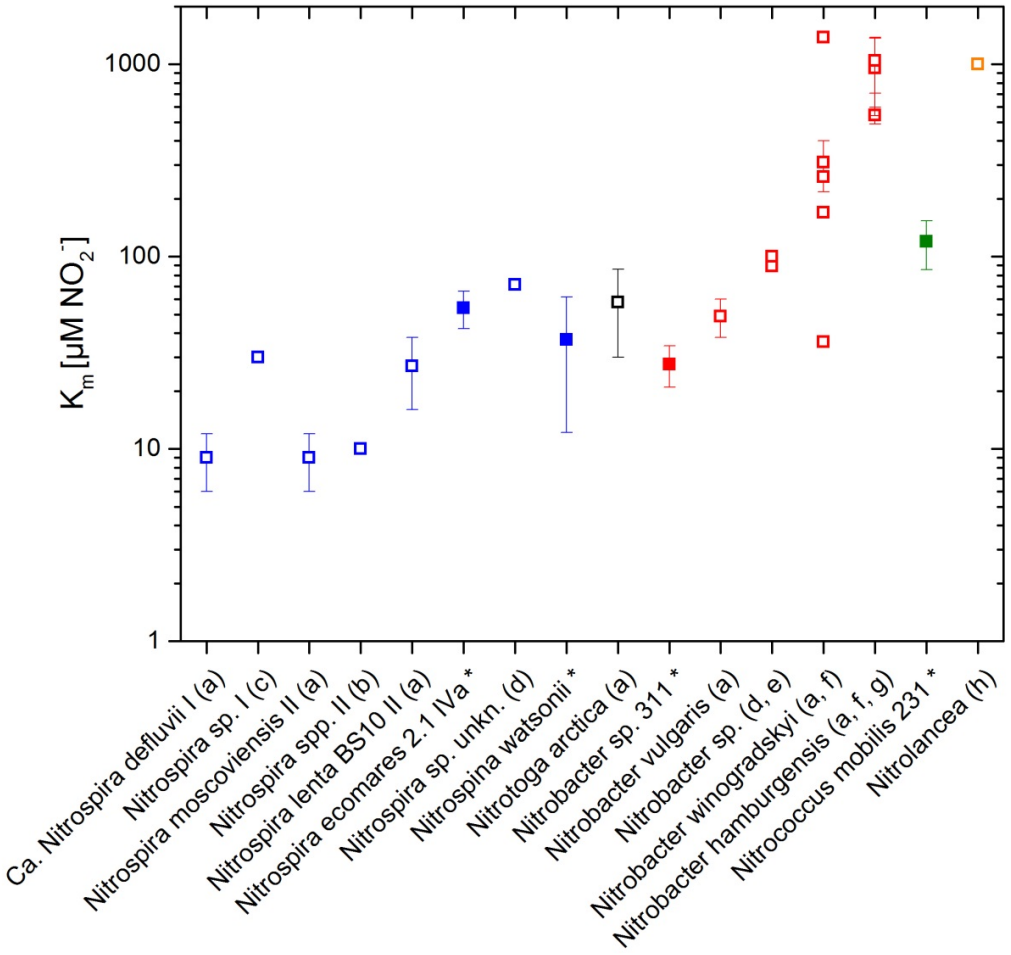

Figure 3.4 Nitrite affinities of *Nitrospira* (blue), *Nitrospina* (black), *Nitrobacter* (red), *Nitrococcus* (green), and *Nitrolancea* (orange). Marine strains from this study are indicated with filled symbols and (*) in the axis caption. Further K_m values are from references (a) Nowka et al. 2015, (b) Schramm et al. 1999, (c) Maixner et al. 2006, (d) Blackburne et al. 2007, (e) Vadivelu et al. 2006, (f) Both et al. 1992, (g) Laanbroek et al. 1994, and (h) Sorokin et al. 2012. Modified after Nowka et al. (2015).

The K_m values of *Nitrospira* Ecomares 2.1 (54 µM NO_2^-) are high compared to other *Nitrospira.* The lowest K_m values in this genus were measured in *Nitrospira moscoviensis* (isolate from heating systems, 9 µM NO_2^-) and *Ca.* Nitrospira defluvii (activated sludge, 9 µM NO_2^-, Figs. 3.1, 3.4, Nowka et al., 2015). One possible explanation for the relatively high K_m values of *Nitrospira* Ecomares 2.1 may be its origin, the strain was isolated from a moving-bed reactor of a fish farm, where high nitrite concentrations are commonly measured (Keuter et al., 2011). Nevertheless, its K_m value is relatively similar to that of the marine *Nitrospina watsonii 347*.

All the other species we investigated were isolated from natural marine environments. Interestingly, *Nitrobacter* sp. 311 had the lowest K_m value of all marine species we investigated, and of all *Nitrobacter* species investigated to date (Fig. 3.4). Isolates from soils, like *Nitrobacter hamburgensis* and *Nitrobacter winogradskyi,* have significantly higher K_m values of up to 1370 µM (Both et al., 1992; Laanbroek et al., 1994). This indicates low substrate affinity, which could be due to low affinities of the nitrite transporter proteins that shuttle nitrite across the cytoplasmic membrane (Nowka et al., 2015).

Overall, our results are somewhat ambiguous. We expected K_m values to fall in the lower range, indicating high substrate affinities, because marine nitrite concentrations are usually low. This is, however, not entirely the case – while K_m values indeed generally are in the lower end of those reported so far, some genera like *Nitrospira*, exhibit even higher substrate affinities and lower K_m values. We speculate that the ecological niche specialization of all marine NOB may be represented in the similarity of their K_m values. This may reflect the fact that variations in substrate concentration in the ocean may be much less pronounced than in diverse terrestrial habitats.

3.4.2. Inverse kinetic isotope effects during nitrite oxidation

In accordance with previous studies (Buchwald and Casciotti, 2010; Casciotti, 2009) we found that the isotope effect of nitrite oxidation is inverse, so that nitrite is successively depleted in ^{15}N. During nitrite oxidation, NXR enzymes form a transition state complex with water molecules (Friedman et al., 1986), from which $^{15}NO_2^-$ is preferentially oxidized to nitrate, whereas the complex with $^{14}NO_2^-$ is more likely decomposed before further oxidation.

In our experiments, we found isotope effects of 9.7±0.8‰ for *Nitrospina watsonii 347* (Fig. 3.2) and 10.2±0.8‰ for *Nitrospira* Ecomares 2.1 (Fig. 3.3, Tab. 3.3). This is in line with the previously determined isotope effect of nitrite oxidation by *Nitrospira marina* (9.1±1.8‰, Buchwald & Casciotti 2010), but clearly lower than that of the marine species *Nitrococcus mobilis* (12.8±1.5‰, Casciotti 2009, to 20.2±2.8‰, Buchwald & Casciotti 2010) or *Nitrobacter* sp. 355 (20.6±3.2‰, Buchwald & Casciotti 2010, Fig. 3.5).

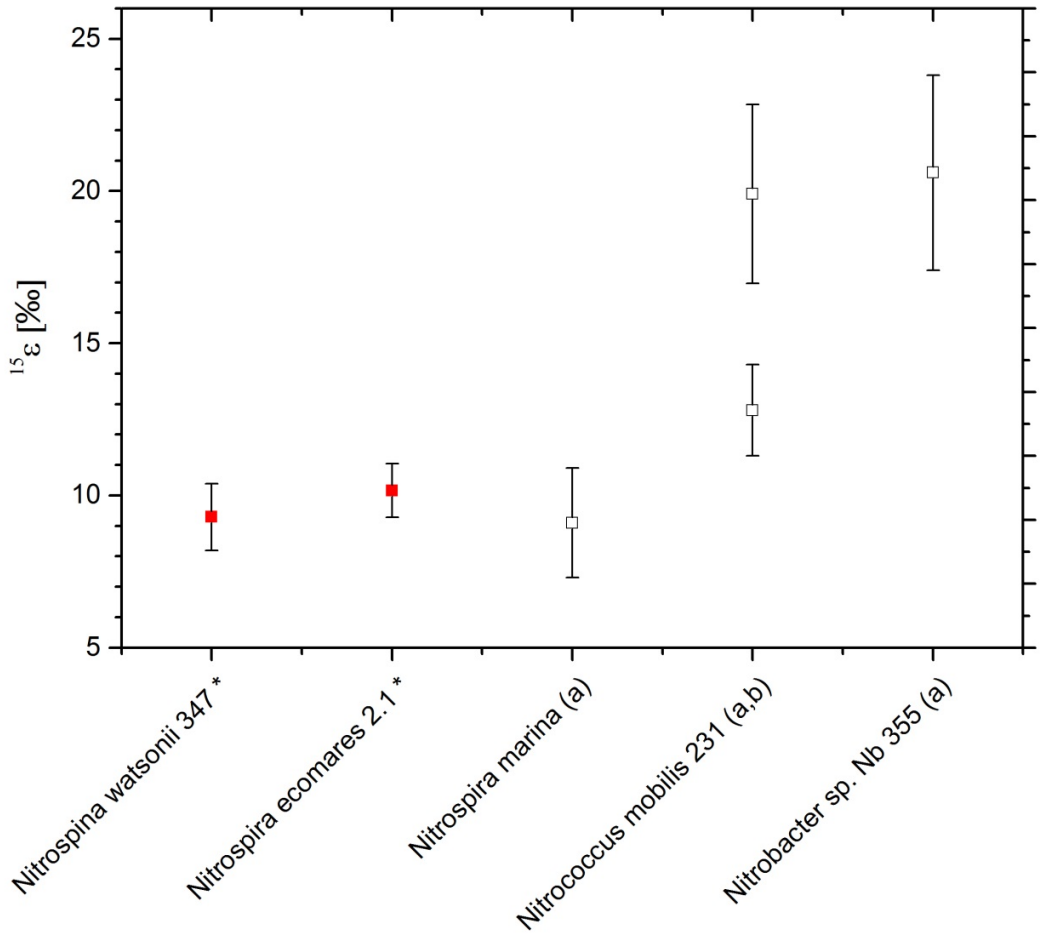

Figure 3.5 Overview of isotope effects during nitrite oxidation of this study (*) and references (a) Buchwald and Casciotti 2010 and (b) Casciotti 2009.

Interestingly, it seems that these differences in isotope effects can be linked to variability in K_m values. Except for the marine strain of *Nitrobacter* in this study, *Nitrobacter* and *Nitrococcus* have high K_m values and thus low substrate affinities, whereas *Nitrospina* and *Nitrospira* have high substrate affinities. We speculate that these high affinities might be reflected in a more efficient turnover of nitrite bearing the light ^{14}N species, possibly due to a better stabilization of the transition state. This would then result in a lower bulk isotope effect. The contrary might thus apply for *Nitrobacter* and *Nitrococcus,* which have significantly lower substrate affinities and larger bulk isotope effects.

Another reason for the range in isotope effects may be the location of NXR. *Nitrospina* and *Nitrospira* have periplasmic NXR, so that neither nitrite nor nitrate needs to be transported through the cytoplasmic membrane. In *Nitrobacter* and *Nitrococcus,* NXR is in the cytoplasm, and ni-

trite/nitrate transporters shuttle nitrite into and nitrate out of the cell (Sorokin et al., 2012; Spieck et al., 1996; Starkenburg et al., 2006). *Nitrospina* and *Nitrospira,* which have been investigated in previous studies (Buchwald and Casciotti, 2010; Casciotti, 2009), have periplasmic NXR and show a significantly smaller bulk isotope effect.

In *Nitrobacter* and *Nitrococcus* with cytoplasmic NXR, nitrite has to be actively transported across the cytoplasmic membrane, and nitrate has to be transported out of the cell to avoid toxicity. This transport can potentially be a bottleneck for oxidation, depending on the substrate affinity and activity of the transporter (Daims et al., 2016). If nitrite is transported into the cell, this can result in an enrichment above the environmental concentration outside the cell. In this case, ample nitrite is present for oxidation, and a more pronounced isotope effect might simply reflect the substrate availability inside the cell. In *Nitrospina* and *Nitrospira,* NXR enzymes are located in the cytoplasmic membrane and the substrate-binding subunit NxrA (Lücker et al., 2010; Sundermeyer-Klinger et al., 1984) is in the periplasm (Koch et al., 2015; Lücker et al., 2013; Spieck et al., 1998). No transport through the cytoplasmic membrane occurs, and this may be reflected in lower nitrite availability, so that a more efficient turnover of nitrite is an advantage. This is also reflected in the energy budget of the strains: In *Nitrobacter* and *Nitrococcus,* protons for nitrite oxidation do not contribute to the proton motive force and little energy is obtained during nitrite oxidation, whereas in *Nitrospina* and *Nitrospira,* protons for nitrite oxidation derive from the surrounding water and contribute to proton motive force, contributing to the cell´s energy gain (Lücker et al., 2010). The orientation of the NXR therefore distinguishes between a highly economical pathway (NXR towards periplasm, e.g. *Nitrospina* and *Nitrospira*), which is reflected in lower isotope effects, and a less energy efficient pathway (NXR towards cytoplasm, e.g. *Nitrobacter* and *Nitrococcus*), which is accompanied by higher isotope effects.

3.4.3. Environmental relevance and model application

The marine NOB strains we used for the assessment of their isotope effect are environmentally relevant and widespread in OMZs, where 30 – 50% of the global oceanic nitrogen loss takes place (Codispoti et al., 2001; Gruber and Galloway, 2008; Gruber and Sarmiento, 1997). In the Arabian Sea OMZ, *Nitrospira* (Lam and Kuypers, 2011), *Nitrospina spp*., and a putative novel nitrite oxidizer clustering between anammox and *Nitrospina* sequences (Lüke et al., 2016) were found. *Nitrospina* was further found near Costa Rica in the eastern tropical North Pacific (Buchwald et al., 2015), and *Nitrospina* and *Nitrococcus* occur in the Namibian OMZ (Füssel et al., 2012).

Our measurements not only represent the first assessment of kinetics of four marine NOB, they also suggest that all marine NOB tend to be K-strategists, probably because of the low substrate availability and relatively little environmental fluctuations in marine habitats. Furthermore, the isotope effects for *Nitrospina watsonii 347* and *Nitrospira* Ecomares 2.1 are significantly different from the previously determined higher isotope effects of *Nitrococcus* and *Nitrobacter*. These differences have important consequences for the interpretation of nitrification and model calculations. Biogeochemical models often are applied in marine systems, where nitrite accumulates, such as the secondary nitrite maximum (SNM) of OMZs (Gaye et al., 2013; Lam et al., 2011), and the isotope effects of nitrite generation and removal are important constraints for such models.

Generally, a wide range of isotope effects has been applied in different models: In the OMZ of the eastern tropical North Pacific off Costa Rica, an isotope effect of 30‰ was calculated for nitrite oxidation (Buchwald et al., 2015). In another model study focusing on the eastern tropical South Pacific, measurements of $\delta^{15}N\text{-}NO_2^-$ could not be reproduced using so far published isotope effects, an isotope effect of 32‰ would have been required to reproduce the data (Casciotti et al., 2013). Contrastingly, in a recent study on isotopic overprinting during combined nitrification-denitrification (Granger and Wankel, 2016), the authors used 16.0±4.5‰ as a model parameter for nitrite oxidation, and, also on the lower end of the applied isotope effects, Gaye at al. (2013) assumed an isotope effect of 13‰ for nitrite oxidation in the Arabian Sea (Casciotti et al., 2009). A change of the isotope effect of nitrite oxidation, even by only 3‰, will certainly alter the computed isotope values of nitrite in ocean models (T. Rixen, personal communication, 2016). The use of such a lower isotope effect may be appropriate, because other OMZ regions (like the eastern tropical North Pacific off Costa Rica OMZ, the Namibian OMZ, the Black Sea OMZ, and the Baltic Sea, Labrenz et al. 2007, Fuchsman et al. 2011, Füssel et al. 2012, Spieck et al. 2014, Buchwald et al. 2015) host predominantly *Nitrospina,* for which we in this study find a low isotope effect. This shows that a more accurate assessment of isotope effects and the kinetic parameters that determine these effects is urgently needed to better constrain biogeochemical models.

3.5. Conclusion

We investigated the nitrite oxidation kinetics of four marine NOB (*Nitrospina watsonii 347, Nitrospira* Ecomares 2.1*, Nitrobacter sp. 311,* and *Nitrococcus mobilis 231*). The range of the K_m values was comparatively narrow, which might reflect a niche specialization towards low substrate concentration and little fluctuation of environmental conditions in marine habitats and places all marine NOB in vicinity to clear (terrestrial) K-strategists. For *Nitrospina watsonii 347* and *Nitrospira* Ecomares 2.1, we also determined the isotope effect of nitrite oxidation, which was 9.7±0.8‰, and 10.2±0.9‰, respectively. This is in line with the former investigations of *Nitrospira marina,* but significantly different from *Nitrobacter* sp. nd *Nitrococcus mobilis 231,* which both have significantly higher isotope effects.

Based on our data, we speculate that the significant differences between *Nitrospina watsonii 347, Nitrospira* Ecomares 2.1, and *Nitrospira marina* on the one side, and *Nitrobacter* sp. 355 and *Nitrococcus mobilis 231* on the other side may be due to differences in enzyme locations. Thus, our data set the basis for an assessment of variances in isotope effects based on cell morphology, which should in the future be pursued with an assessment including non-marine NOB.

Furthermore, our investigation of nitrite oxidation kinetic data and isotope effects of *Nitrospira* and *Nitrospina* has important implications for the use of specific isotope effects in biogeochemical models. The strains we investigated are especially important in OMZs. OMZs are hotspots of nitrification and denitrification, and our data add to the existing data base of the isotope effect of nitrite oxidation. There is a certain mismatch between measured isotope effects and those assumed in models, some modelling approaches require isotope effects significantly above those measured to date, including this study. This suggests that a re-evaluation of modelling approaches may be required to match the isotope effects, because there is no indication for higher isotope effects in NOB from marine OMZs.

Acknowledgements

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4. Seasonality of nutrients, N isotopes, and nitrification rates in the Elbe River

Juliane Jacob, Tina Sanders and Kirstin Dähnke

In preparation for submission

Abstract

From July 2011 to May 2013, a free-flowing station of the Elbe River has been sampled to study the nitrogen cycle, and nitrification, especially. Samples were analysed regarding hydrological conditions, nutrient concentrations, and stable isotopes ($\delta^{15}N\text{-}NO_3^-$, $\delta^{18}O\text{-}NO_3^-$, $\delta^{15}N$-SPM). The aim was to disentangle seasonal changing processes like combined remineralisation and nitrification as nitrate sources, and assimilation and denitrification as sinks. Nitrification rate measurements were performed monthly to determine in-stream nitrification, which is potentially important, but disguised by the multitude of contributing processes.

Distinct seasonal trends with high nutrient concentrations, high $\delta^{15}N$-SPM values, and low dual nitrate isotopes were observed in winter. We assume elevated nutrient inputs from the catchment due to high discharge, and nitrate from soil nitrification, especially. Simultaneously, biological activity and nutrient consumption were low. From spring on, in-stream processes became more important. Decreasing nutrient concentrations and increasing chlorophyll concentrations indicated two phytoplankton blooms in summer. High primary production and SPM concentrations fuelled in-stream nitrification via combined remineralisation-nitrification, which was indicated by a significant correlation of elevated SPM concentrations and highest nitrification rates (13.4 µmol L^{-1} d^{-1}) in summer.

Furthermore, dual nitrate isotopes indicated assimilation as the main nitrate sink in spring, and suggest that assimilation, denitrification and nitrification co-occurred in summer. A back-of-the-envelope calculation highlighted that in-stream and soil nitrification could contribute as much as 531 µmol L^{-1} in spring, while the average measured nitrate concentration is 115 µmol L^{-1} due to intense assimilation and denitrification.

4.1. Introduction

Since the industrial production of synthetic fertilizer, the reactive nitrogen pool has doubled in comparison to pre-industrial times (Galloway et al., 2003). Rates of natural fixation of N_2 to bioavailable nitrogen compounds are exceeded by fertilizer input (Galloway et al., 1995). Most fertilizer is for food production and a lot is widely spread on the crops where it enters natural ecosystems through hydrological or atmospherical pathways uncontrolled (Howarth et al., 1996).

From 1986 to 2006, annual ammonium and nitrate loads decreased by 93% and 48% at Seemannshöft, respectively (Bergemann and Gaumert, 2008). However, nitrate loads in rivers are still enhanced, and in-stream processes can influence the nitrate concentration significantly. Beside assimilation by phytoplankton, DIN loads can be modified by nitrate regeneration in rivers (Middelburg and Nieuwenhuize, 2001): organic matter is remineralised, and subsequently nitrified (Mayer et al., 2001). Nitrate re-enters the nitrogen cascade (Galloway et al., 2003) and can either be assimilated by bacteria and phytoplankton (Middelburg and Nieuwenhuize, 2000; Wada and Hattori, 1978) or denitrified (Mariotti et al., 1981). Assimilation and nitrification change the ratio of DIN-compounds. Denitrification, however, microbially reduces nitrate to dinitrogen gas, which is a quantitatively important sink in the Elbe River (Deutsch et al., 2009).

The role of assimilation in rivers was underestimated in general (Thorp and Delong, 2002), which is connected to the amount of produced nitrate from nitrification. Nitrate regeneration via nitrification occurs in major rivers throughout Europe, and contributes to nitrate loads in, for example, the Seine, Scheldt, and Elbe River (Johannsen et al., 2008; Sebilo et al., 2006). In case of the Elbe River, improved oxygen conditions led to a regime shift from a nitrate sink due to denitrification to a nitrate source due to nitrification (Dähnke et al., 2008). The strongly decreased ammonium input in the Elbe River (Bergemann and Gaumert, 2008) was assumed to decrease nitrification rates (Böttcher et al., 1990; Schäfer and Harms, 1995). Nevertheless, nitrification has been identified as an important nitrate source by nitrification rate measurements in the tidal Elbe estuary (Bergemann et al., 1996; Sanders et al., in revision). In 1997, when ammonium concentrations in summer were below 20 µmol L^{-1}, nitrification rates in the Elbe estuary were 16.8 – 122.4 µmol L^{-1} d^{-1} (Kerner and Spitzy, 2001).

The contribution of every process depends on environmental parameters like temperature, discharge/turbidity, oxygen concentration, organic carbon content, light availability or depth of the euphotic zone, and DIN-concentration (e.g. Gücker and Pusch, 2006; Hedin et al., 1998).

Stable isotope analyses of dual nitrate isotopes can be used in order to disentangle these sources, sinks, and turnover processes (e.g. Battaglin et al., 2001; Mayer et al., 2002;

Sebilo et al., 2006), because biological processes usually favour the light isotope over the heavy one (Kendall, 1998; Mariotti et al., 1981). Every process (assimilation, nitrification, harvesting of crops, and denitrification in soils) has a specific isotope effect representing the degree of isotope enrichment with substrate depletion (Broecker and Oversby, 1971; Rayleigh, 1896; Sigman et al., 2009). Nitrification is a special case, because it is a two-steps-reaction with divergent isotope effects. The first step, ammonia oxidation to nitrite, is associated with an isotope effect $^{15}\varepsilon$ of -14 to -41‰ in pure culture experiments (Casciotti et al., 2003; Mariotti et al., 1981; Santoro and Casciotti, 2011). During the second step, nitrite oxidation, produced nitrate is heavier than the substrate, which results in a rare inverse isotope effect $^{15}\varepsilon$ of +9.1 to +20.6‰ (Buchwald and Casciotti, 2010; Casciotti, 2009; Jacob et al., in revision).

We investigated the free-flowing part of the Elbe River at Geesthacht weir (Elbe-km 585) to encompass the major nitrogen transformation processes, and nitrification, especially. Therefore, we focused on seasonal variations of hydrological parameters (water temperature, discharge, oxygen concentration), particular matter (SPM, C/N, chlorophyll), nutrient concentrations (ammonium, nitrite, nitrate, and silicate), and stable isotopes (δ^{15}N-SPM, δ^{15}N-NO_3^-, and δ^{18}O-NO_3^-). Furthermore, we determined nitrification rates in order to estimate whether nitrification underlies seasonality and to quantify nitrate built in the river by nitrification.

We hypothesise that (1) nitrification is an important nitrate source in the Elbe River and (2) nitrification influences dual nitrate isotopes.

4.2. Materials and Methods

4.2.1. Study site

The Elbe River is a central European river that drains the Czech Republic and Germany and enters the North Sea at Cuxhaven. The river has a total length of 1,094 km and a catchment area of 148,268 km^2. Almost 25 million people live within that area (Lozán et al., 1996). The Elbe River is the second largest stream discharging into the North Sea (Brockmann and Pfeiffer, 1990), and is the biggest nitrate source having a nitrate load of approximately 76 kt NO_3^- yr^{-1} (Bergemann and Gaumert, 2010). Other DIN compounds like ammonium and nitrite are of minor importance, representing <5% and <2%, respectively, of the DIN load. The study site in Geesthacht is located upstream a weir at stream kilometre 585 (53°25´31´´N, 10°20´10´´E). The weir separates the river from the tidal estuary. Discharge was measured daily at gauge Neu Darchau (stream kilometre 536.3, Wasser- und Schifffahrtsamt Lauenburg, 2016). Water

temperature, oxygen, and chlorophyll concentrations were daily measured by the Institute for Hygiene and Environment at Bunthaus Spitze (stream kilometre 610). At Geesthacht weir, water temperature was statistically similar to data from Bunthaus Spitze. Phytoplankton abundance was estimated based on chlorophyll concentrations.

4.2.2. Sampling

Surface water samples were collected with a bucket from the quay wall every two weeks from July 2011 to May 2013. Water was immediately transferred to 2 L acid-washed polyethylene bottles for temperature measurement and immediate processing in the laboratory. Here, water samples were filtered with pre-weighted, pre-combusted (450°C, 4.5 hrs) GF/F filters. Filtered aliquots were frozen for subsequent analyses of nutrient concentrations and isotope values. GF/F filters were dried at 50°C and stored frozen for later determination of SPM concentration, as well as δ^{15}N-SPM analysis.

4.2.3. Nutrient analyses

Nutrient concentrations were analysed by an automated continuous flow analyser (AA3, Seal Analytical, Germany) and standard colorimetric techniques (Grasshoff et al., 2009). The detection limit for nitrite and nitrate was 0.1 and 1 µmol L^{-1}, respectively. Ammonium was analysed fluorometrically with a detection limit of 0.5 µmol L^{-1} (Holmes et al., 1999).

4.2.4. Maintenance of cultures and calculation of nitrification rates

Nitrification rates were measured monthly from August 2011 to January 2013. The experimental setup was modified after ISO/DIN 15685:2001 standard test procedures (DIN15685, 2001). Immediately after sampling, four times 50 mL unfiltered water samples were incubated in 100 ml open glass bottles on a shaker at 18 °C in the dark. Duplicates of AOB activities were amended with $NaClO_3$ to inhibit nitrite oxidizers (Belser and Mays, 1980). Samples were taken daily until a constant nitrite concentration was reached in case of the AOB incubations, and until complete turnover of ammonium and nitrite in case of the combined AOB-NOB incubations.

Samples were centrifuged (15 min, 13.200 rpm, Eppendorf Centrifuge 5415 R) and nitrite concentrations in the supernatant were immediately analysed photometrically using standard colorimetric techniques based on a reaction of Sulfanilamide with N-(1-naphthyl)-ethylenediaminedihydrochloride (NEDA) at wavelength of 542 (Grasshoff et al., 2009) until July 2012, and afterwards nitrite and nitrate concentrations in the supernatant were immediately analysed with a high-performance liquid chromatography system (HPLC, Jasco,

Multiwavelength-detector MD-915, Meincke et al., 1992). Nitrification rates were calculated based on the steepest nitrite or nitrate increase in the incubations (Fig. A1).

4.2.5. Isotope analyses

$\delta^{15}N\text{-}NO_3^-$, and $\delta^{18}O\text{-}NO_3^-$ isotopes were analysed with the denitrifier method (Casciotti et al., 2002; Sigman et al., 2001). This method is based on the bacterial conversion of substrate to nitrous oxide. *Pseudomonas aureofaciens* (ATCC® 13985) denitrify nitrate and nitrite to N_2O (Böhlke et al., 2007), which is then analysed on a GasBench II, coupled to a Delta V isotope ratio mass spectrometer (Thermo Fisher Scientific). To avoid concentration-dependent isotope effects, the sample volume was adjusted to a final N_2O content of 10 nmol for nitrate analysis. The standards for nitrogen and oxygen are atmospheric N_2 and Vienna Standard Mean Ocean Water (VSMOW), respectively.

$\delta^{15}N$-SPM was analysed with a Carlo Erba NA 2500 elemental analyser coupled to an IRMS isotope ratio mass spectrometer (Finnigan MAT 252).

For calibration, international isotope standards (IAEA N3 with $\delta^{15}N\text{-}NO_3^-$ of 4.7‰ and $\delta^{18}O\text{-}NO_3^-$ of 25.6‰, and USGS 34 with $\delta^{15}N\text{-}NO_3^-$ of -1.8‰ and $\delta^{18}O\text{-}NO_3^-$ of -27.9‰), as well as two internal standards were analysed ($\delta^{15}N\text{-}NO_3^-$ of 1.4 and 7.1‰). Analytical error of quadruplicate standards and duplicate samples were <0.2‰ for $\delta^{15}N\text{-}NO_3^-$ and <0.5‰ for $\delta^{18}O\text{-}NO_3^-$. For $\delta^{15}N$ analysis of suspended matter, IAEA N1, IAEA N2 and a certified sediment standard (IVA Analysentechnik, Germany) were used for calibration. The analytical error of $\delta^{15}N$-SPM was <0.1‰.

4.3. Results

4.3.1. General hydrological properties

Discharge values were measured at the gauge Neu Darchau (Wasser- und Schifffahrtsamt Lauenburg, 2016). In line with general climatic properties, discharge was lowest in summer (minimum of 280 m^3 s^{-1}) and highest in winter (~2000 m^3 s^{-1}, Fig. 4.1a). In July 2012, discharge increased from about 380 to 600 m^3 s^{-1} with a concomitant decrease of chlorophyll and oxygen concentration (Fig. 4.1a, b).

Water temperature varied from 0.2°C in winter to 23.4°C in summer, and oxygen concentrations were between 6.2 mg L^{-1} and 14.8 mg L^{-1} at Bunthaus Spitze in April 2012 and 2013 (Fig. 4.1a, Institut für Hygiene und Umwelt Hamburg, 2016).

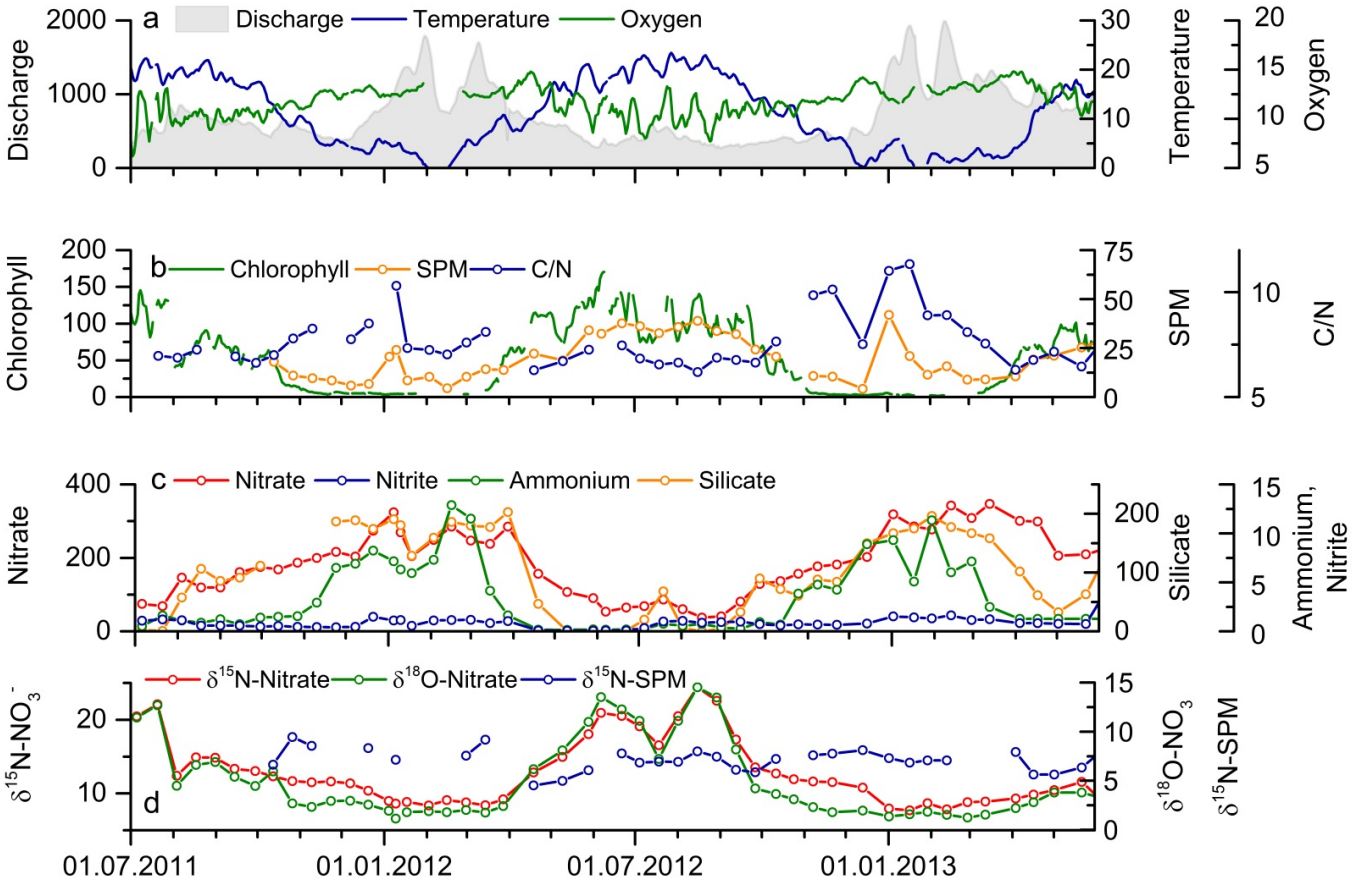

Figure 4.1 (a) Daily measured discharge at Neu Darchau and daily measured temperature and oxygen concentrations at Bunthaus Spitze, (b) chlorophyll concentration daily measured at Bunthaus Spitze, SPM concentration and C/N ratios at Geesthacht weir, (c) nitrate, nitrite, ammonium, and silicate concentrations at Geesthact weir, (d) N- and O-isotope values of nitrate, and SPM.

4.3.2. Particulate matter and chlorophyll concentrations

SPM concentrations were inversely correlated with discharge, with highest SPM concentration in summer (42.0 mg L^{-1}), and lowest in winter (4.2 mg L^{-1}). Furthermore, SPM was elevated prior to discharge peaks in February 2012 and January 2013 (Fig. 4.1a, b).

Generally, C/N ratios were lowest in summer and highest in winter, with a minimum of 6.2 in August 2012, and a maximum of 11.3 in January 2013. Chlorophyll concentration was a good proxy for phytoplankton abundance. In spring, chlorophyll concentrations increased, and were up to 145 µg L^{-1} in late June and early July 2011, 170 µg L^{-1} in early June 2012, and 137 µg L^{-1} in late July 2012. In autumn, concentrations decreased and were below 5 µg L^{-1} in winter at Bunthaus Spitze (Fig. 4.1b, Institut für Hygiene und Umwelt Hamburg, 2016).

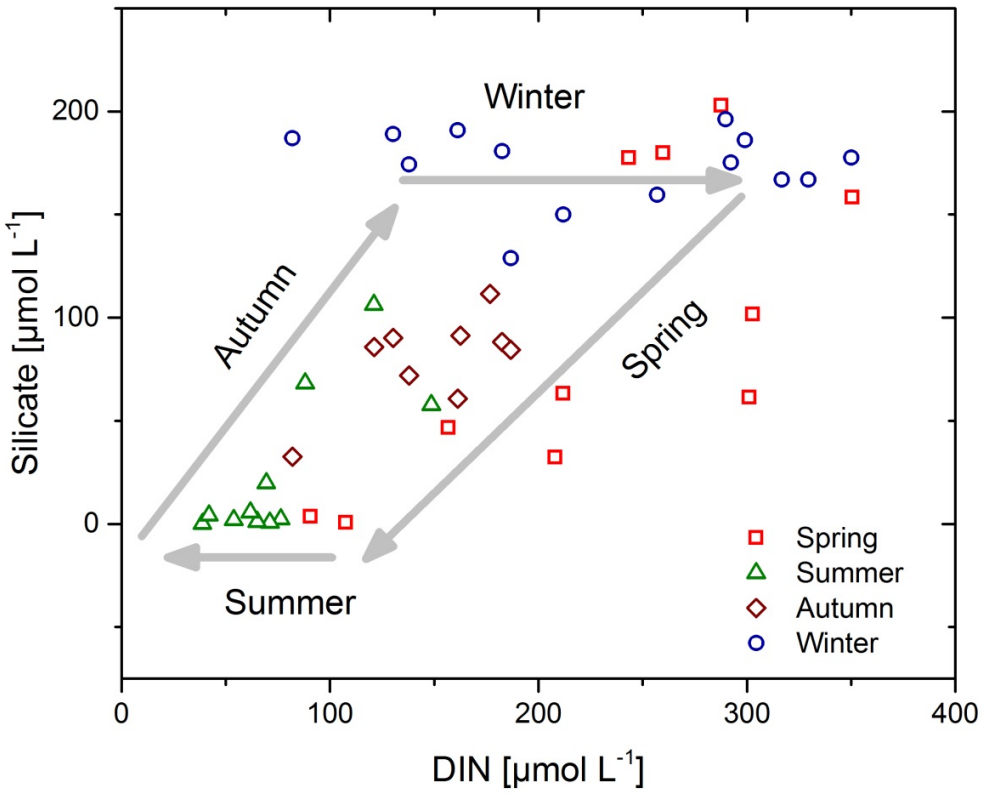

Figure 4.2 Si:DIN ratios with indicated seasonal changes. The Si:DIN ratio followed a slope of 1.12 (R^2=0.97) in spring 2012, a slope of 0.66 (R^2=0.62) in spring 2013, a slope of 0.86 (R^2=0.57) in summer, a slope of 0.44 (R^2=0.38) in autumn, and a slope of -0.02 (R^2= -0.08) in winter.

4.3.3. Nutrient concentrations

All dissolved nutrients exhibited highest concentrations in winter and lowest in summer (Fig. 4.1c). Nitrate was the main DIN component in the Elbe River throughout the year and was always abundant, whereas nitrite and ammonium concentrations approached the detection limit in summer.

In winter, nitrate reached 342.5 µmol L^{-1}, nitrite 1.5 µmol L^{-1}, and ammonium 12.9 µmol L^{-1} (Fig. 4.1c). From March to late August, nitrate concentration decreased to a minimum value of 37.2 µmol L^{-1}, corresponding to a loss of 85%. Nitrite and ammonium were below the detection limit (Fig. 4.1c). In July 2012, ammonium, nitrite, and nitrate were elevated simultaneously to a discharge peak to 600 m^3 s^{-1}.

In winter, silicate was up to 200 µmol L^{-1}, and in summer it approached the detection limit (Fig. 4.1c). There were short-term increases of silicate concentration up to 100 µmol L^{-1} in late summer 2011 and 2012. Silicate was clearly correlated to DIN (Fig. 4.2, Tab. A5). In winter, silicate concentrations stagnated at about 180 µmol L^{-1}, and only DIN increased to 350 µmol L^{-1}. In spring 2012, Si:DIN decreased almost in parallel according to a slope of 1.12 (R^2=0.97) to silicate concentrations below 6 µmol L^{-1}. In autumn, Si:DIN increased according to a slope of 0.44 (R^2=0.37).

4.3.4. Nitrification rates

Nitrification rates varied seasonally with highest rates of 13.4 µmol L^{-1} d^{-1} in summer, and lowest of about 1 µmol L^{-1} d^{-1} in winter (Fig. 4.3, Tab. 4.1). Nitrification rates were correlated with SPM and chlorophyll concentrations (R^2 of 0.88 and 0.76, respectively, *p*-values <0.05, Fig. 4.4, Tab. A5). Standard deviation of quadruplicates was between 0.08 µmol L^{-1} d^{-1} in November 2012, and 1.71 µmol L^{-1} d^{-1} in June 2012 (Tab. 4.1). Large standard deviations originated from different rates of AOB and combined AOB-NOB incubations.

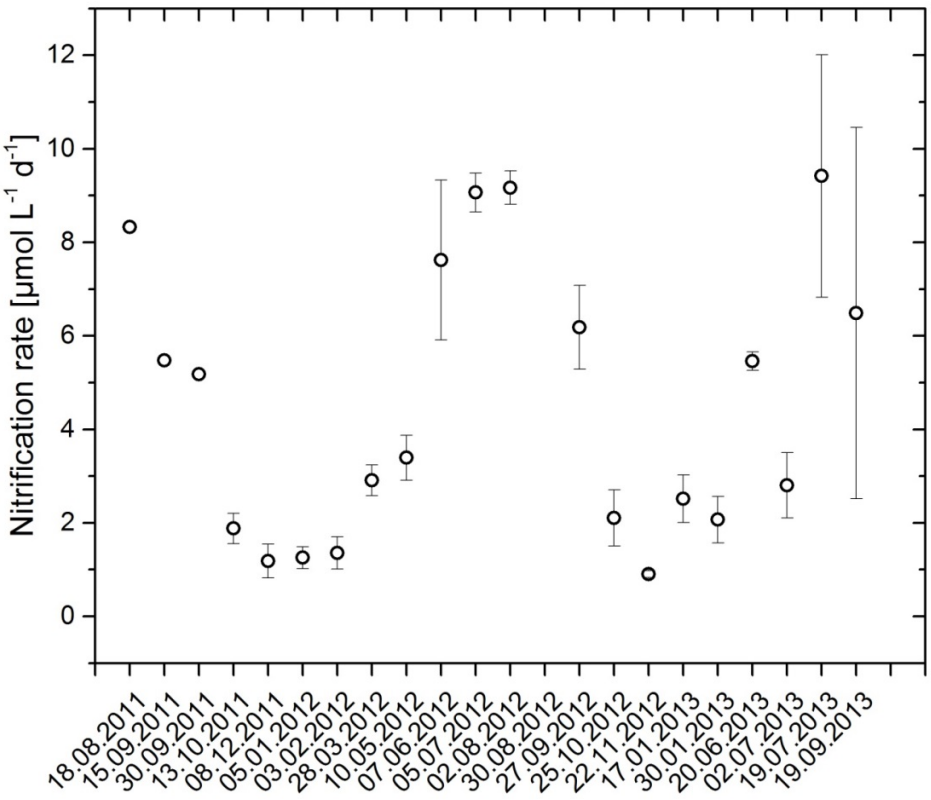

Figure 4.3 Seasonal varying nitrification rates with standard deviations of water samples from the Elbe River at Geesthacht weir.

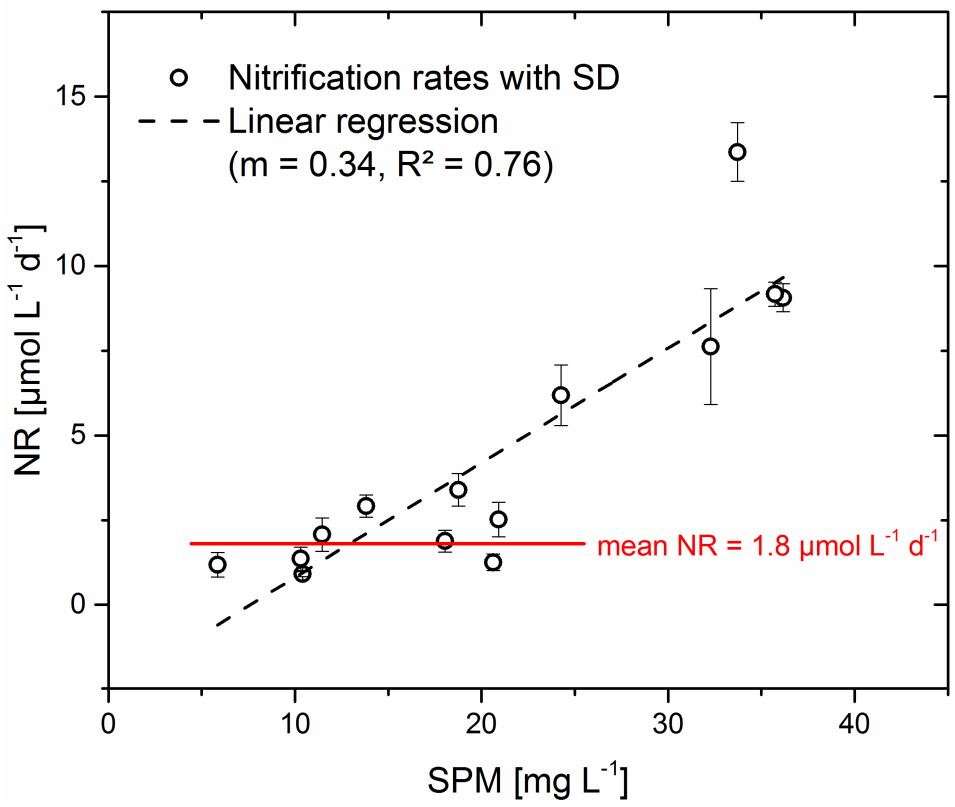

Figure 4.4 Nitrification rates with standard deviation (Tab. 4.1) versus SPM concentrations. Both were correlated with an increasing slope of 0.34 and a pronounced R^2 of 0.76 (Tab. A5).

Table 4.1 Nitrification rates with standard deviations at Geesthacht weir.

Date	Nitrification rate [µmol L^{-1} d^{-1}]	SD [µmol L^{-1} d^{-1}]
18.08.2011	8.3	
15.09.2011	5.5	
30.09.2011	5.2	
13.10.2011	1.9	0.3
08.12.2011	1.2	0.4
05.01.2012	1.3	0.2
03.02.2012	1.4	0.3
28.03.2012	2.9	0.3
10.05.2012	3.4	0.5
07.06.2012	7.6	1.7
05.07.2012	9.1	0.4
02.08.2012	9.2	0.4
30.08.2012	13.4	0.9
27.09.2012	6.2	0.9
25.10.2012	2.1	0.6
22.11.2012	0.9	0.1
17.01.2013	2.5	0.5
30.01.2013	2.1	0.5

4.3.5. Isotope trends of DIN and particulate nitrogen

The isotope values of nitrate were negatively correlated to nitrate concentrations. $\delta^{15}N\text{-}NO_3^-$ and $\delta^{18}O\text{-}NO_3^-$ were highest in summer (24.4‰ and 14.5‰, respectively), and lowest in winter (7.7 and 1.1‰, respectively, Fig. 4.1d). In spring and autumn, the isotopes of nitrate changed according to at a ratio of ~ 1:1 (R^2 of 0.99 and 0.78, respectively). In summer, the slope of $\delta^{18}O\text{-}NO_3^-$ to $\delta^{15}N\text{-}NO_3^-$ was 0.89 (R^2=0.95), and 0.37 in winter (R^2=0.69, Fig. 4.5). The dual nitrate isotope values were also correlated with nitrification rates (R^2 of 0.90 and 0.88, *p*-values <0.05, Tab. A5).

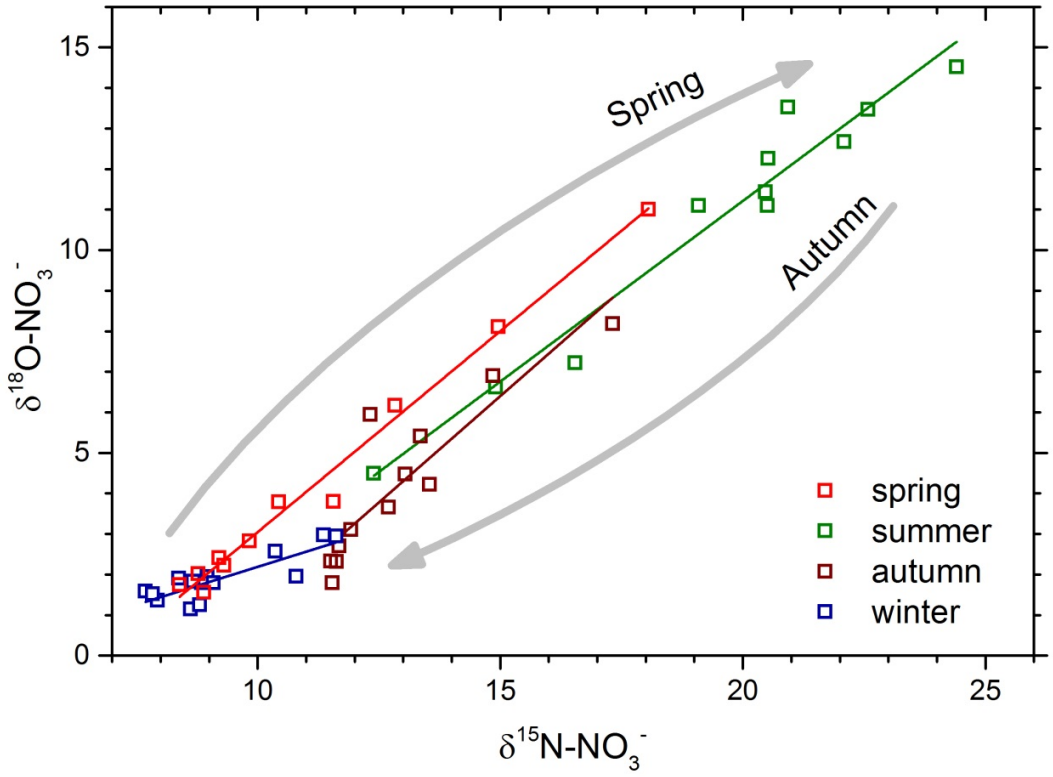

Figure 4.5 Seasonal $\delta^{18}O\text{-}NO_3^-$ to $\delta^{15}N\text{-}NO_3^-$ ratios. The slope is 0.99 (R^2=0.99) in spring, 0.89 (R^2=0.95) in summer, 1.05 (R^2=0.78) in autumn, and 0.37 (R^2=0.69) in winter.

$\delta^{15}N$-SPM values resulted in a complex pattern throughout the observations. In autumn 2011 and winter 2011/2012, isotope values were highest reaching 9.5‰. In spring 2012, $\delta^{15}N$-SPM values had a minimum of about 5‰ and increased to 8‰ towards summer 2012. In late September, a short decrease to 5.9‰ was then followed by an increase to 8.2‰ in December 2012 (Fig. 4.1d), before $\delta^{15}N$-SPM decreased again to <6‰ in late spring 2013.

4.4. Discussion

4.4.1. Nutrient sources and the effect of hydrology

Discharge is a factor that significantly affects the concentration of all dissolved nutrients in the Elbe River. In winter, high discharge correlates with high nutrient concentrations. This influence is most evident for nitrate (R^2 of 0.74, *p*-value <0.05), reflecting seasonal changes in the composition of sources and sinks. In winter, highest discharge is correlated with highest nitrate concentrations, which is mainly due to soil nitrification in the catchment (Johannsen et al., 2008) or is leached from the catchment and enters the Elbe River in various ways (urban waste water, surface runoff, leachate from mineral, and synthetic fertilized agriculture soils; Bleifuss et al., 1998; Kendall, 1998; Van Breemen et al., 2002). In winter, biological turnover in the water column is probably of little importance. In contrast, we found that low discharge, i.e., longer residence time, and higher water temperature promoted uptake significantly in summer. This interplay of less terrestrial nitrate input due to less runoff and intense uptake led to a substantial decrease in nitrate concentration in convert with discharge.

The effect of discharge on ammonium is less pronounced, because ammonium did probably not result from external agricultural sources in the water column: Ammonium molecules are positively charged and thus tightly bound to clay particles in soil, and elution with discharge generally does not occur (Mancino, 1983). We regarded in-stream remineralisation of SPM as the main source of ammonium, which in turn was usually immediately assimilated (Dortch et al., 1991) or oxidized to nitrite and, subsequently, to nitrate (Mayer et al., 2001). In consequence, both ammonium and nitrite were below the detection limit in summer due to rapid turnover.

Silicate derived from small rock fragments and minerals produced in weathering reactions such as clays, from fertilizers, and from release of silica from the sediment during decay of diatoms. Silicate concentration reflected the activity of diatoms, because they need silicate to build their shells. This is evident in the abrupt decrease in spring, so that silicate is below the detection limit in June and August 2012, when chlorophyll concentrations were highest and reflect a phytoplankton bloom (Fig. 4.1a, b, c).

4.4.2. Nutrient sinks and general trends

In winter, nitrate input from the catchment is high, and potential sinks (phytoplankton assimilation, denitrification) are reduced due to discharge effects, low temperatures, low light irradiance, and high turbidity (Fig. 4.1a, c). In spring, discharge and thus input of nitrate and silicate decrease. With residence time, the reaction time in a water parcel increases, along with

increasing temperature, which fuels the biological activity. In consequence, nutrient concentrations decrease. A typical succession of nutrient uptake can be observed in spring 2012 and 2013: The assimilation of ammonium, followed by nitrate uptake reflects phytoplankton preferences at the onset of a bloom (Dortch, 1990, Fig. 4.1c). This affects δ^{15}N-SPM values. Highest values (9.5‰) reflect degraded, refractory material in winter, but the recurring decrease to values <6‰ reflects a change in the SPM composition towards a high contribution of phytoplankton (indicated by high chlorophyll concentrations and low C/N ratios), which preferentially assimilates the light ^{14}N in spring/summer.

Nitrate is never depleted in the water column and is apparently not limiting for phytoplankton growth. Diatoms, the major component of the phytoplankton spring bloom in the Elbe, however, need not only carbon dioxide, light, and nitrogen, but also silicate for their outer shell (Pusch, 2006). In the Elbe River, diatom growth is clearly limited by dissolved silica availability (Schöl et al., 2014). In spring, decreasing concentration of nitrogen and silicate and high chlorophyll concentrations (up to 170 µg L^{-1} in early June 2012), indicate a typical diatom bloom (Fig. 4.1b, c). The bloom declines, when silicate decreases below 4 µmol L^{-1}. However, chlorophyll concentration indicates a secondary bloom in late July 2012 (136 µg L^{-1}), after an increase of silicate to 70 µmol L^{-1} (Fig. 4.1b, c).

This close coupling of nutrient removal is reflected in significant correlation coefficients of silicate to nitrate, nitrite, and ammonium (R^2 of 0.84, 0.49, and 0.79 with *p*-values<0.05, Tab. A5) and a coupled change in Si:DIN ratios in spring 2012, especially (corresponding to Redfield, Fig. 4.2). Si:DIN is 1.12 in spring 2012, 0.66 in spring 2013, and 0.86 in summer 2012. 1:1 is a typical ratio for diatoms in freshwater systems. A ratio below 1:1, as we find it in spring 2013, can represent a reduced proportion of diatoms in the phytoplankton community (Turner et al., 1998; Turner et al., 2003).

In summer, phytoplankton is reduced by grazing and sedimentation, and because the nutrients (other than DIN) are increasingly limiting growth. In autumn, chlorophyll concentration (and phytoplankton abundance) decrease (Fig. 4.1b). Phytoplankton is decomposed, which leads to dissociation of silicate and release of ammonium, so that nutrient concentrations in the water column increase. This reflects the importance of remineralisation, and nitrification, especially.

But is it possible to evaluate the role of nitrification on an annual basis?

4.4.3. The coupling of nitrification, remineralisation and primary production

In winter 2012, ammonium concentration was up to 12.9 µmol L^{-1} at Geesthacht weir, and nitrification rates were 3.4 µmol L^{-1} d^{-1}. In summer, ammonium limitation occurred simultaneously

to highest nitrification rates of 13.9 µmol L^{-1} d^{-1} (Figs. 4.1c, 4.3, Tab. 4.1). These measurements, though lower than previous rate assessments, fit with the range of modelled rates of 7.3 to 11.8 µmol L^{-1} d^{-1} in the Elbe estuary from May to October 2006 (Elbe-km 629 to 660, Schöl et al., 2014). Furthermore, these rates are comparable to a study from 2012/2013 in the Elbe estuary, where rates of up to 10 µmol L^{-1} d^{-1} were observed in the port region of the Elbe Estuary (Sanders et al., in revision). It is somewhat surprising that rates in the port region are lower than at Geesthacht weir, because light limitation and high SPM concentration in the port region should provide favourable conditions for nitrifiers. However, nitrifiers are sensitive to environmental changes, which occur frequently in the tidal estuary. The more stable conditions at our site may be advantageous for nitrifiers.

The pronounced positive correlation of nitrification rates with SPM, C-SPM, and N-SPM, respectively, (R^2 of 0.88, 0.77, and 0.77, respectively, *p*-values <0.05, Fig. 4.4, Tab. A5) suggests that not ammonium, but suspended matter drives nitrification activity. Ammonium is less correlated to nitrification rates (R^2 of -0.65, *p*-value of 0.01, Tab. A5), because ammonium concentrations are below 1 µmol L^{-1} in summer, when nitrification is highest. Especially in summer, we thus assume that remineralised phytoplankton is the main source for nitrification, and remineralisation rates are assumed to be as high as nitrification rates. This is also obvious in late July 2012, when silicate is no longer limiting, which leads to high chlorophyll concentrations and a secondary bloom, a recurring phenomenon in the Elbe River (Scharfe et al., 2009). This high primary production correlates to highest remineralisation/nitrification rates of 13.9 µmol L^{-1} d^{-1} (chlorophyll to nitrification rate with R^2 of 0.76, *p*–value <0.05, Tab. A5). Moreover, we assume that elevated SPM concentrations coincide with high nitrification rates (Fig. 4.4) not only because SPM is substrate for remineralisation, but also because nitrifiers are preferentially associated to particles or flocks (Abril et al., 2000; Owens, 1986).

4.4.4. Nitrate source identification based on stable isotope changes

Isotope values have been traditionally used to identify sources, sinks, and turnover processes (Middelburg and Nieuwenhuize, 2001). The Elbe River catchment is exposed to large amounts of reactive nitrogen. Potential nitrate sources are mineral fertilizer ($\delta^{15}N\text{-}NO_3^-$ of 0.8 to 4.4‰, Deutsch et al., 2005), animal and sewage waste ($\delta^{15}N\text{-}NO_3^-$ of >10‰, Grischek et al., 1998) and nitrified soil nitrate ($\delta^{15}N\text{-}NO_3^-$ of 4 to 9‰, Grischek et al., 1998). The $\delta^{18}O\text{-}NO_3^-$ value of fertilizer is between 18‰ and 22‰, which is close to the atmospheric $\delta^{18}O$ value (Amberger and

Schmidt, 1987; Kroopnick and Craig, 1972; Wassenaar, 1995), and typical mineral fertiliser applied in Germany range between 19.4‰ and 25.7‰ (Deutsch et al., 2005).

The mean annual $\delta^{15}N\text{-}NO_3^-$ value in the Elbe River is ~13‰, which indicates anthropogenic influence (Deutsch et al., 2006; Johannsen et al., 2008; Mayer et al., 2002; Voß et al., 2006). The mean annual $\delta^{18}O\text{-}NO_3^-$ value is 5‰, which is significantly lower than the potential source signature of mineral fertilizer. As our data also suggest, it has been hypothesized before (Johannsen et al., 2008), this reflects the role of nitrified soil nitrogen that quantitatively determines the nitrate isotope value throughout the year. Nitrate built via nitrification has a $\delta^{18}O\text{-}NO_3^-$ value based on the ambient water (-9‰ in case of the Elbe River, S. Bernasconi, personal communication) and the dissolved atmospheric oxygen (23.5‰ Kroopnick and Craig, 1972). In fresh water environments, two oxygen atoms stem from ambient water and one from dissolved oxygen (Aleem et al., 1965; Andersson and Hooper, 1983) resulting in $\delta^{18}O\text{-}NO_3^-$ values equal to

$$\delta^{18}\mathrm{O} - \mathrm{NO_3^-} = \frac{2}{3}\delta^{18}\mathrm{H}_2\mathrm{O} + \frac{1}{3}\delta^{18}\mathrm{O} - \mathrm{O_2} \qquad (4.1)$$

In the Seine River, newly produced nitrate from nitrification has a $\delta^{18}O\text{-}NO_3^-$ value between 0 to 3‰ (Sebilo et al., 2006). For the Elbe River, the calculated isotope value of nitrate from nitrification is 1.8‰, and thus is comparable to winter and load-weighted annual mean $\delta^{18}O\text{-}NO_3^-$ values. Based on the clear correlation to discharge, we thus assume that nitrate from soil nitrification in the catchment area is an important nitrate source.

The interplay of discharge and nitrate concentrations complicates the identification of individual sources and sinks of nitrate. Nevertheless, we will try to identify individual turnover mechanisms of nitrate during the spring bloom 2012 with isotope effects. Isotope effects are calculated based on changing nitrate concentrations and dual nitrate isotopes (Sigman et al., 2009). The sum of the product and the continuously consumed residual equals the total supply, because the residual is consumed at a steady-state rate (Eq. 4.2). In an open system, this leads to a linear relation between δ values and the remaining fraction *f*. The slope of the regression line corresponds to the isotope effect ε (Sigman et al., 2009).

$$\varepsilon_{\mathrm{substrate}} = -\frac{\delta\ \mathrm{value}_{\mathrm{substrate}} - \delta\ \mathrm{value}_{\mathrm{initial}}}{(1-f)} \qquad (4.2)$$

where $\delta\ \mathrm{value}_{\mathrm{substrate}}$, and $\delta\ \mathrm{value}_{\mathrm{initial}}$ are the $\delta^{15}N$ values of the substrate at the time of sampling and the initial value, and *f* is the remaining fraction of substrate at the time of sampling.

In spring 2012, a simple box-model was constructed during the period of nitrate decrease (247 to 90 µmol L^{-1}) and isotope increase ($\delta^{15}N\text{-}NO_3^-$ of 8.8 to 18.1‰, and $\delta^{18}O\text{-}NO_3^-$ of 2.0 to 11.0‰). Assuming that the relevant sinks are riparian denitrification and phytoplankton assimilation, nitrate loss would be 75% nitrate assimilation, and 25% denitrification. This scenario, with an isotope effect $^{15}\varepsilon$ of -11.5‰ most closely matches the measured $^{15}\varepsilon$ of -11.4‰. This assumption agrees with a calculation based on an O:N ratio of 0.89:1, where total nitrate uptake were dominated by assimilation (>75%) in comparison to denitrification (<25%, Deutsch et al., 2009).

However, nitrate is not only consumed, but simultaneously produced by combined remineralisation-nitrification. We estimated the potentially produced nitrate from nitrification. Discharge is correlated with nitrate (R^2 of 0.74, *p*-value <0.05), and in winter, an average of 900 m^3 s^{-1} transports on average 250 µmol L^{-1} nitrate from soil nitrification. Under the assumption of a logarithmic correlation, we expect in spring 184 µmol L^{-1} nitrate from soil nitrification (average discharge of 770 m^3 s^{-1}). In spring 2012 (92 days), nitrification rates were about 3.2 µmol L^{-1} d^{-1}, which would add approximately 294.4 µmol L^{-1} nitrate. However, during this period nitrate decreased from 285 to 90 µmol L^{-1}, which results in overall 673.4 µmol L^{-1} nitrate loss due to assimilation and denitrification.

From mid-July to the end of August 2012, nitrate concentrations further decreased (86 to 37 µmol L^{-1}) with $^{15}\varepsilon$ and $^{18}\varepsilon$ of -12.7 and -12.4‰, respectively. Based on O:N ratios, three potential processes were identified: assimilation, nitrification, and denitrification. Assimilation has an isotope effect $^{15}\varepsilon$ of about -10‰ (Granger et al., 2004; Montoya and McCarthy, 1995). For nitrification, we assume a bulk isotope effect of -20‰ (measured in the Seine river, Sebilo et al. 2006) and for riparian denitrification, we use an estimated isotope effect $^{15}\varepsilon$ of -16‰ (Deutsch et al., 2005; Houlton and Bai, 2009; Kendall et al., 2007). The underlying assumption is that measured isotope effects in our system are most probably a mixture of these three individual isotope effects, and $\delta^{15}N$-SPM is an important substrate.

4.5. Conclusion

From April 2011 to May 2013 a free-flowing station in the Elbe River was investigated concerning sources, sinks, and turnover processes. Overall, a distinct seasonality occurred with highest values in winter, and a pronounced positive correlation of discharge and nutrient concentrations. In winter, in-stream biological processes are negligible because of low ambient temperature. The isotope signature of nitrate and clear correlation to discharge suggest that at this time, nitrate in the Elbe River mainly stems from soil nitrification in the catchment. From spring on, in-stream processes become more important. Based on nutrient concentrations and chlorophyll concentrations, two phytoplankton blooms are identified in early June and the end of July 2012. The high primary production and SPM concentration fuels nitrification in the river, via coupled remineralisation-nitrification, which is mirrored in high nitrification rates that increase with increasing SPM. In summer, nitrification rates peak with up to 13.4 µmol L^{-1} d^{-1}. Apparent isotope effects and nitrate isotope ratios endorse assimilation as the main sink in spring. In summer, however, denitrification contributes as a sink, and nitrification is an important nitrate source. We conclude that dissolved nutrients show a pronounced seasonality. We find clear indications for significant effects of soil nitrification in the catchment and for in-stream nitrification as important nitrate sources. A back-of-the-envelope calculation suggests that these sources could contribute as much as 478.4 µmol L^{1} in spring, while the average measured nitrate concentration is 115 µmol L^{-1} due to intense assimilation and denitrification.

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5. Conclusion and Outlook

5.1. Conclusion

This thesis provides new insights into the nitrogen cycle based on environmental samples from the Elbe River near Geesthacht weir, and NOB pure cultures.

Based on the Elbe River water sampling from July 2011 to May 2013 (chapter 4), a pronounced seasonality was observed. In winter, high discharge was correlated with high nutrient concentrations and very low biological activities including nitrification rates. In this season, nitrate was from soil nitrification in the catchment, which was identified based on relatively low dual nitrate isotopes. From spring on, discharge decreased and water temperature increased, which fuelled biological activity and nutrient concentrations decreased. Increasing chlorophyll concentrations indicated increasing phytoplankton abundance, and nitrate assimilation was represented by increasing dual nitrate isotopes, which have a characteristic O:N isotope ratio of about 1:1. In summer, the O:N ratio changed to 0.89, which indicate a contribution of denitrification, which can decrease the ratio to 0.5:1. However, nitrification rates were up to 13 µmol L^{-1} d^{-1}, which identified nitrification as an important in-stream nitrate source, and would increase the ratio above 1:1. Therefore, in summer a combined nitrification-denitrification-assimilation regime is most likely. Further, a pronounced positive correlation of chlorophyll concentrations to nitrification rates occurred, because SPM can be remineralised to ammonium, which is substrate, and nitrifiers are preferentially associated to particles or flocks instead of free-living.

In June 2013, however, an exceptional summer flood occurred and increasing discharge interrupted the sensitive nitrogen cycle in the Elbe River. Ammonium, nitrite, and nitrate accumulated. High nitrate concentrations and decreasing dual nitrate isotopes reflected inhibited assimilation. Ammonium most likely stemmed from remineralisation of organic material, and nitrite from nitrification in the river, which highlights the importance of nitrification. The apparent isotope effect of net nitrite consumption was -10.0±0.1‰, which deviates from an excepted inverse isotope effect for nitrite oxidation, and clearly indicates other contributing sources or sinks. To clarify this, simple box model calculations were run with nitrification and simultaneous riparian denitrification as potential nitrite sinks. During the flood, the contribution of nitrite oxidation ranged from 22 to 36%, whereas riparian denitrification ranged from 64 to 78% of nitrite consumption. It has been shown that even a single station can be used to unravel sinks. The unique

accumulation of nitrite and possibility of isotope analyses clearly indicate the substantial role of nitrification and remineralisation in the Elbe River, but also show that other sinks like denitrification in the riparian zone contribute to nitrite turnover.

The Elbe River investigation indicates the complex aquatic nitrogen cycle, and that co-occurring processes disguise inverse isotope effects during nitrite oxidation, as during the Elbe flood. This might be important not only in estuarine settings, but also in other environments that show nitrite accumulation in the water column, like oceanic oxygen minimum zones (OMZs), where nitrate and nitrite isotopes are frequently used to assess nitrogen dynamics.

Therefore, environmentally important nitrite-oxidizing bacteria were investigated concerning their nitrite-oxidation kinetics and isotope effects. Nitrite-oxidation kinetics are important growth characteristics, and the four investigated marine NOB (*Nitrospina watsonii 347, Nitrospira* Ecomares 2.1*, Nitrobacter sp. 311,* and *Nitrococcus mobilis 231*) showed relatively narrow substrate affinities compared to non-marine NOB. This could be a niche differentiation to low substrate concentrations in marine habitats, and range the investigated NOB in vicinity to clear (terrestrial) K-strategists. We also determined the isotope effects of nitrite oxidation of *Nitrospina watsonii 347* and *Nitrospira* Ecomares 2.1, which were inverse (9.7±0.8‰, and 10.2±0.9‰, respectively). This is in line with the former investigations of *Nitrospira marina,* but significantly different from *Nitrobacter* sp. 355 and *Nitrococcus mobilis 231*. We speculate that these significant differences may be due to differences in enzyme locations. Thus, our data set the basis for an assessment of variances in isotope effects based on cell morphology. The selection of suitable isotope effects for box models is necessary for reasonable calculations. Box models are often investigated in OMZs, which are hotspots of nitrification and denitrification, and where *Nitrospina* and *Nitrospira* strains are widely distributed. Until now, there is a certain mismatch between measured isotope effects, including this study, and assumptions of higher isotope effects in modelling approaches of OMZs.

5.2. Outlook and future work

This thesis gives new insights into the nitrogen cycle and nitrification, especially, in the Elbe River and nitrite oxidation in pure cultures, but there arises some future work.

In general, ammonium and nitrate input in the Elbe River decreased and water quality improved, but in winter, nitrate concentration was still up to 350 µmol L^{-1} (chapter 4), and input of nitrate from the catchment was an important source (chapter 2). This highlights that processes in the catchment have to be investigated to reduce inputs to the Elbe River. On one side, this includes the amount of fertilizer, whereas in Germany, fertilization is regulated by law ("Düngegesetz", "Wasserrahmenrichtlinie", "Nitratrichtlinie", Bundesrepublik Deutschland, 2009). However, the monitoring approaches do not include small temporal and spatial scales (Sachverständigenrat für Umweltfragen, 2015). Small water bodies are not recognised, and organic nitrogen compounds are often not analysed, monitoring concepts for the biology of groundwater bodies are missing, and indicators and reference values for the status of groundwater ecosystems are needed (Sachverständigenrat für Umweltfragen, 2015). On the other hand, the transportation route from the catchment to the Elbe River is under discussion, because it could be surface runoff, through hydrogeological boundary layers, or through groundwater. The groundwater influence on large rivers like the Elbe is suggested to be relatively small (Alexander Gröngröft, personal communication, 2014), but this is different in smaller rivers and tributaries of the Elbe River, and interdisciplinary work with hydrogeologists from different institutions is needed. A Lagrangian sampling approach could be applied to determine the influence of different tributaries.

The incubations of the Elbe River water samples for nitrification rates were also used for the calculation of isotope effects during nitrification. However, further experiments are needed for resilient database and statistics. Then, the DNA of the bacteria community can be analysed for a better comparison to other environments.

Isotope effects of nitrite oxidation for five marine nitrite-oxidizing bacteria are available; however, they are significantly different (chapter 3). Isotope effects are often used in model calculations, particularly in regions where nitrite accumulates, and even small changes have a big impact. Existing box models can be improved and re-evaluated with the novel nitrite-oxidation kinetics and isotope effects. The contributing bacteria community can be analysed to properly select isotope effects for box models. Further, the data base for freshwater and terrestrial environments should be extended. For example, *Nitrotoga* and *Nitrospira* have been identified as main organisms per-

forming the second step of nitrification (E. Spieck, personal communication, 2016), and data for isotope effects are needed. Isotope effects for combined ammonia- and nitrite-oxidation can be analysed, which would further improve model approaches of the Elbe River where nitrite seldom accumulates.

Until recently, it was considered that nitrification is a two-step process with ammonia-oxidizing bacteria or archaea and nitrite-oxidizing bacteria. However, it surprisingly was discovered that in the genus *Nitrospira* organisms perform complete ammonia oxidation to nitrate, which is called comammox. It is hypothesized that cells not release nitrite at low ammonium concentrations, but immediately oxidize nitrite (Daims et al., 2015). Comammox organisms are absent in marine habitats, but widespread in man-made and natural environments (Daims et al., 2015; Kuypers, 2015). But are they relevant in the Elbe River? Furthermore, comammox of *Nitrospira* is performed with nitrite oxidoreductase (NXR), which is responsible for the inverse isotope effect during nitrite oxidation (chapter 3). In contrast, ammonia oxidation is associated with a negative isotope effects. This raised the question, which isotope effect is pronounced.

Figure captions

Figure 1.1 Schematic illustration of the key processes of the nitrogen cycle including nitrification, and nitrite oxidation to nitrate, especially. Ammonium, nitrite, and nitrate are stable, whereas nitric oxide, nitrous oxide, and dinitrogen are atmospheric gases, which are released to the atmosphere. Abbreviations: anaerobic ammonium oxidation (anammox), comammox (complete ammonia oxidation), dissimilatory nitrite reduction to ammonia (DNRA), assimilatory (assim.), dissimilatory (dissim.).

Figure 1.2 Comparison of isotope effects in a closed system (Rayleigh, black lines), and an open-system (steady-state, grey lines). The same isotopic parameters - an isotope effect of 5‰, and a $\delta^{15}N$ of 5‰ of the initial reactant supply – are used for both models (Sigman et al., 2009).

Figure 2.1 (a) Discharge, dissolved oxygen concentration, and SPM concentration of the Elbe River water samples from 6 to 20 June 2013. Flood conditions occur with discharge >3000 $m^3\ s^{-1}$. (b) Ammonium, nitrite, and nitrate concentrations in the Elbe River in the course of the flood. Calculations of the isotope effects are based on filled data points. (c) Ammonium, nitrite, nitrate, and SPM isotope values in the course of the flood. Calculations of the isotope effect are based on filled data points.

Figure 2.2 Ammonium and nitrite concentrations increase with decreasing dissolved oxygen saturation.

Figure 2.3 Ratio of $\delta^{18}O\text{-}NO_3^-$ versus $\delta^{15}N\text{-}NO_3^-$ values corresponding to decreasing nitrate concentrations from 13 to 20 June and filled data points of figure 2.1b and c. The calculated linear regression has a slope of 1.22 with R^2 of 0.95.

Figure 2.4 Nitrite isotope values versus the remaining fraction of nitrite during the Elbe flood corresponding to the filled data points in figure 2.1b and c. The dashed line indicates the apparent isotope effect during net nitrite consumption with a slope of -10.0±0.1‰ and R^2 of 0.97.

Figure 2.5 Dual nitrate isotope values versus the remaining fraction of nitrate corresponding to the filled data points in figure 2.1b and c. The solid line indicates the apparent isotope effect during net nitrate consumption with a slope of $^{15}\varepsilon$ -4.0±0.1‰ with R^2 of 0.89 and the dashed line is $^{18}\varepsilon$ -5.3±0.1‰ with R^2 of 0.92.

Figure 3.1 Nitrite oxidation kinetics of *Nitrospina watsonii 347, Nitrospira* Ecomares 2.1*, Nitrobacter sp. 311,* and *Nitrococcus mobilis 231*. Michaelis-Menten plots of oxygen uptake versus nitrite concentration of early-stationary-phase cells at 28°C. Mean values and standard deviations resulted from triplicates. The kinetic parameters were calculated by non-linear fitting of Michaelis-Menten equation to the data.

Figure 3.2 Rayleigh-plot for seven *Nitrospina watsonii 347* experiments. Decrease of $\delta^{15}N\text{-}NO_2^-$ relative to the initial $\delta^{15}N\text{-}NO_2^-$ is plotted as a function of $\ln(NO_2^-/NO_{2\,initial}^-)$ during nitrite oxidation (cf. Eq. 3.3 and Granger et al. 2004). Every experiment has its own symbols. Standard deviation of a sample is <0.3‰ and smaller than the symbol. The average of all regressions defines the dashed line and the corresponding isotope effect with an average ε of 9.7±0.8‰.

Figure 3.3 Rayleigh-Plot for six *Nitrospira* Ecomares 2.1 experiments. Decrease of $\delta^{15}N\text{-}NO_2^-$ relative to the initial $\delta^{15}N\text{-}NO_2^-$ plotted as a function of $\ln(NO_2^-/NO_{2\,initial}^-)$ during nitrite oxidation (cf. Eq. 3.3 and Granger et al. 2004). Every experiment has its own symbol and standard deviation of a sample is <0.3‰ and smaller than the symbol. The average of all regressions defines the dashed line and the corresponding isotope effect with an average ε of 10.2±0.9‰.

Figure 3.4 Nitrite affinities of *Nitrospira* (blue), *Nitrospina* (black), *Nitrobacter* (red), *Nitrococcus* (green), and *Nitrolancea* (orange). Marine strains from this study are indicated with filled symbols and (*) in the axis caption. Further K_m values are from references (a) Nowka et al. 2015, (b) Schramm et al. 1999, (c) Maixner et al. 2006, (d) Blackburne et al. 2007, (e) Vadivelu et al. 2006, (f) Both et al. 1992, (g) Laanbroek et al. 1994, and (h) Sorokin et al. 2012. Modified after Nowka et al. (2015).

Figure 3.5 Overview of isotope effects during nitrite oxidation of this study (*) and references (a) Buchwald and Casciotti 2010 and (b) Casciotti 2009.

Figure 4.1 (a) Daily measured discharge at Neu Darchau and daily measured temperature and oxygen concentrations at Bunthaus Spitze, (b) chlorophyll concentration daily measured at Bunthaus Spitze, SPM concentration and C/N ratios at Geesthacht weir, (c) nitrate, nitrite, ammonium, and silicate concentrations at Geesthact weir, (d) N- and O-isotope values of nitrate, and SPM.

Figure 4.2 Si:DIN ratios with indicated seasonal changes. The Si:DIN ratio followed a slope of 1.12 (R^2=0.97) in spring 2012, a slope of 0.66 (R^2=0.62) in spring 2013, a slope of 0.86

(R^2=0.57) in summer, a slope of 0.44 (R^2=0.38) in autumn, and a slope of -0.02 (R^2=-0.08) in winter.

Figure 4.3 Seasonal varying nitrification rates with standard deviations of water samples from the Elbe River at Geesthacht weir.

Figure 4.4 Nitrification rates with standard deviation (Tab. 4.1) versus SPM concentrations. Both were correlated with an increasing slope of 0.34 and a pronounced R^2 of 0.76 (Tab. A5).

Figure 4.5 Seasonal $\delta^{18}O\text{-}NO_3^-$ to $\delta^{15}N\text{-}NO_3^-$ ratios. The slope is 0.99 (R^2=0.99) in spring, 0.89 (R^2=0.95) in summer, 1.05 (R^2=0.78) in autumn, and 0.37 (R^2=0.69) in winter.

Figure A1 Example illustrations of incubation experiments in August 2012. (a) Incubation with $NaClO_3$ for AOB activities, (b) incubation for combined AOB-NOB-activities. Nitrification rates were calculated based on filled circles (chapter 3).

Table captions

List of abbreviations

Anammox	anaerobic ammonia oxidation
AOB	ammonia-oxidizing bacteria
Comammox	complete ammonia oxidation
C_{org}	organic carbon
δ	delta
DIN	dissolved inorganic nitrogen
DNRA	dissimilatory nitrate reduction to ammonia
DON	dissolved organic nitrogen
ε	isotope effect
EA	element analyzer
GF/F	glass fibre filter, grad F
HPLC	high-performance liquid chromatography
IAEA	International Atomic Energy Agency
NOB	nitrite-oxidizing bacteria
NO_x	atmospheric NO and NO_2
N_r	reactive nitrogen
NXR	nitrite oxidoreductase
OMZ	oxygen minimum zone
PNM	primary nitrite maximum
PON	particulate organic nitrogen
R	isotope ratio
SNM	secondary nitrite maximum
SPM	suspended particulate matter
USGS	United States Geological Survey
WWTP	wastewater treatment plant

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Appendix

Following data were used for the three publications:

Table A1 Elbe flood data (chapter 2).

Date	Time [UTC]	Temperature [°C]	pH	Oxygen [mg L^{-1}]	Salinity [psu]	SPM [mg L^{-1}]	NO_3^- [µmol L^{-1}]	NO_2^- [µmol L^{-1}]	NH_4^+ [µmol L^{-1}]
06.06.2013	10:08	16.2	7.9	9.8	0.2	28.9	225.8	4.2	1.3
	17:00	17.5	7.9	10.5	0.2	26.3	269.6	1.6	0.0
07.06.2013	09:10	16.7	7.5	9.4	0.2	26.3	261.8	1.7	0.0
	16:50	18.7	8.2	10.4	0.2	23.8	256.7	1.8	0.0
08.06.2013	09:10	17.6	7.9	9.2	0.2	26.0	251.7	1.8	0.0
	16:45	19.0	8.0	9.9	0.2	35.7	255.4	1.7	0.0
09.06.2013	08:55	17.5	7.7	7.8	0.2	50.8	229.6	2.2	0.1
	16:55	18.7	7.7	8.3	0.2	70.5	228.1	2.4	0.0
10.06.2013	09:25	17.5	7.6	6.8	0.1	50.1	238.7	2.7	0.8
	16:55	18.0	7.6	7.1	0.1	43.7	244.1	2.8	0.2
11.06.2013	09:12	17.5	7.6	6.4	0.1	27.1	267.4	3.1	1.4
	16:48	18.2	7.5	6.6	0.1	28.0	263.9	3.3	1.0
12.06.2013	09:00	17.6	7.6	6.2	0.1	27.2	269.0	3.7	2.9
	17:00	18.4	7.5	6.3	0.1	24.5	275.6	3.9	2.8
13.06.2013	09:15	18.3	7.5	6.0	0.1	32.6	274.2	4.2	3.2
	17:00	18.8	7.5	6.3	0.1	12.6	280.6	4.4	2.9
14.06.2013	08:55	18.1	7.6	6.5	0.1	20.4	263.3	4.4	2.4
	17:05	18.6	7.6	7.1	0.1	8.6	254.9	4.4	3.4
15.06.2013	14:20	20.0	7.7	7.4	0.1	13.9	242.4	4.1	0.0
16.06.2013	09:20	18.8	7.7	7.6	0.1	13.7	229.6	4.0	0.0
18.06.2013	08:55	19.5	7.7	7.6	0.2	13.9	225.7	3.7	0.0
20.06.2013	09:25	21.5	7.7	6.8	0.2	9.2	180.0	3.3	1.3

Table A1 Elbe flood (chapter 2, continued).

Date	Time [UTC]	$\delta^{15}N\text{-}NO_3^-$ [‰]	$\delta^{18}O\text{-}NO_3^-$ [‰]	$\delta^{15}N\text{-}NO_2^-$ [‰]	$\delta^{15}N\text{-}NH_4^+$ [‰]	$\delta^{15}N$-SPM [‰]	Nitrogen [mg L^{-1}]	Carbon [mg L^{-1}]
06.06.2013	10:08	9.0	3.2			8.1	1.7	12.8
	17:00	9.0	3.5	-14.2		8.3	1.6	11.8
07.06.2013	09:10	8.7	3.2	-13.3		7.8	1.5	11.2
	16:50	8.9	3.5	-8.8		7.2	2.1	15.2
08.06.2013	09:10	8.4	3.1	-8.0		7.3	1.9	13.3
	16:45	8.4	3.4	-11.5		6.7	1.4	11.9
09.06.2013	08:55	8.3	3.3	-10.6		6.5	1.3	11.5
	16:55	8.2	3.4	-10.5		6.4	1.1	11.3
10.06.2013	09:25	7.8	3.4	-11.4		6.7	1.2	11.3
	16:55	7.7	3.4	-12.8		6.6	1.0	10.0
11.06.2013	09:12	7.5	2.9	-13.2	2.0	6.6	1.2	12.0
	16:48	7.5	2.8	-13.8		6.7	1.4	12.2
12.06.2013	09:00	7.4	2.9	-13.8	8.4	6.3	1.4	12.0
	17:00	7.4	2.3	-13.6	9.5	6.2	1.1	9.8
13.06.2013	09:15	7.4	2.2	-12.6	12.0		1.3	10.9
	17:00	7.5	2.1	-12.2	6.7	6.3	1.2	9.8
14.06.2013	08:55	7.7	2.3	-11.7	8.3	6.5	1.3	10.5
	17:05	7.9	2.7	-12.0	5.8		1.6	11.9
15.06.2013	14:20	8.1	3.0	-11.3		8.1	2.8	24.0
16.06.2013	09:20	8.3	3.3	-11.2		7.8	2.8	26.8
18.06.2013	08:55	8.6	3.5	-10.2		7.1	1.9	15.1
20.06.2013	09:25	8.8	3.9	-9.3		6.1	2.3	17.1

Table A2 Incubation experiments of *Nitrospira* Ecomares 2.1. Highlighted data were used to calculate isotope effects (chapter 3).

1

Time [d]	Nitrate [µmol L^{-1}]	Nitrite [µmol L^{-1}]	δ^{15}N-Nitrite [‰]
0.2	79.5	921.3	-27.5
1.1	87.1	835.8	-27.7
2.1	88.9	775.4	-27.9
3.1	106.0	745.5	-28.5
4.4	116.2	693.4	-28.7
7.1	291.1	588.7	-30.8
8.1	395.1	453.2	-33.0
9.1	593.5	265.5	-38.5
10.2	805.1	35.0	-55.8
11.1	850.8	11.8	-45.9

2

Time [d]	Nitrate [µmol L^{-1}]	Nitrite [µmol L^{-1}]	δ^{15}N-Nitrite [‰]
0.2	81.6	831.2	-27.8
1.1	90.9	838.3	-27.7
2.1	95.7	787.0	-28.0
3.1	146.0	793.3	-28.3
4.4	113.8	712.2	-28.8
7.1	275.3	606.8	-31.1
8.1	393.3	484.9	-33.4
9.1	591.7	301.9	-38.8
10.2	797.4	65.6	-52.9
11.1	862.8	10.7	-62.4

3

Time [d]	Nitrite [µmol L^{-1}]	Nitrate [µmol L^{-1}]	δ^{15}N-Nitrite [‰]
0.2	849.2	75.1	-27.8
1.1	860.8	85.9	-28.1
2.1	825.0	94.5	-28.4
3.1	789.5	100.2	-28.0
4.4	709.8	110.8	-29.0
7.1	642.3	269.8	-30.5
8.1	530.3	364.9	-32.9
9.1	364.1	550.7	-36.6
10.2	125.4	769.0	-48.0
11.1	17.1	891.6	-62.9

4

Time [d]	Nitrate [µmol L^{-1}]	Nitrite [µmol L^{-1}]	δ^{15}N-Nitrite [‰]
0.2	126.1	563.6	-28.4
1.1	120.0	552.8	-28.8
2.1	130.7	536.8	-29.0
3.2	153.4	515.4	-29.7
4.1	177.2	505.4	-28.9
7.1	336.8	350.3	-33.0
8.2	435.1	262.3	-36.9
9.2	564.4	150.1	-42.8
10.1	655.1	32.2	-58.0
11.2	684.2	5.4	-45.4

Table A2 Incubation experiments of *Nitrospira* Ecomares 2.1. Highlighted data were used to calculate isotope effects (chapter 3, continued).

5			
Time [d]	**Nitrate** [µmol L^{-1}]	**Nitrite** [µmol L^{-1}]	**δ^{15}N-Nitrite** [‰]
0.2	122.9	562.8	-28.1
1.1	127.1	554.1	-28.1
2.1	129.4	549.9	-28.5
3.2	149.0	517.8	-28.7
4.1	185.8	523.7	-29.2
7.1	335.2	358.0	-32.9
8.2	436.9	260.2	-36.7
9.2	559.2	139.7	-43.4
10.1	657.5	29.6	-57.7
11.2	683.0	5.1	

6			
Time [d]	**Nitrate** [µmol L^{-1}]	**Nitrite** [µmol L^{-1}]	**δ^{15}N-Nitrite** [‰]
0.2	109.3	559.5	-27.9
1.1	116.0	541.6	-28.0
2.1	133.8	544.3	-28.3
3.2	159.2	510.4	-29.2
4.1	192.1	487.6	-29.3
7.1	396.1	293.8	-34.7
8.2	537.1	165.1	-40.6
9.2	656.0	39.0	-54.2
10.1	680.4	15.1	-18.2
11.2	681.5	5.1	-34.7

Table A3 Incubation experiments of *Nitrospina Watsonii*. Highlighted data were used to calculate isotope effects (chapter 3).

7

Time [h]	Nitrite [µmol L^{-1}]	$\delta^{15}N$ [‰]
0.0	1.0	
1.0	599.2	-27.9
18.5	547.5	-29.4
25.5	497.6	-29.5
43.0	453.7	-31.4
48.5	438.6	-31.8
66.5	431.2	-32.6
73.5	406.3	-33.5
90.0	357.6	-35.0
171.0	194.7	-41.0
187.0	106.3	-42.5
195.0	97.8	-43.0
234.5	89.0	-43.9
235.5	84.9	-45.0
266.0	72.8	-41.9
258.5	54.2	-50.4

8

Time [h]	Nitrite [µmol L^{-1}]	$\delta^{15}N$ [‰]
0.0	123.4	-27.7
7.8	120.1	-29.6
23.5	64.2	-35.6
32.3	43.1	-40.5

9

Time [h]	Nitrite [µmol L^{-1}]	$\delta^{15}N$ [‰]
0.0	111.9	-27.5
7.8	114.2	-29.2
23.5	67.7	-34.5
32.3	46.0	-38.7

10

Time [h]	Nitrite [µmol L^{-1}]	$\delta^{15}N$ [‰]
0.0	1624.6	-27.1
42.0	1471.8	-28.0
84.0	1352.1	-29.3
120.0	1160.6	-30.8
162.0	964.9	-34.7
204.0	582.3	-36.3

11

Time [h]	Nitrite [µmol L^{-1}]	$\delta^{15}N$ [‰]
0.0	1613.9	-26.6
42.0	1461.1	-28.3
84.0	1355.3	-28.9
120.0	1163.5	-30.6
162.0	949.7	-34.7
204.0	578.2	-36.9

12

Time [h]	Nitrite [µmol L^{-1}]	$\delta^{15}N$ [‰]
0.0	1533.8	-26.4
42.0	1410.9	-27.5
84.0	1304.1	-28.7
120.0	1154.7	-30.4
162.0	930.5	-35.0
204.0	582.0	-36.2

13

Time [h]	Nitrite [µmol L^{-1}]	$\delta^{15}N$ [‰]
0.0	1601.1	-26.5
42.0	1450.4	-27.5
84.0	1361.8	-28.8
120.0	1176.1	-30.7
162.0	950.3	-34.3
204.0	582.2	-36.4

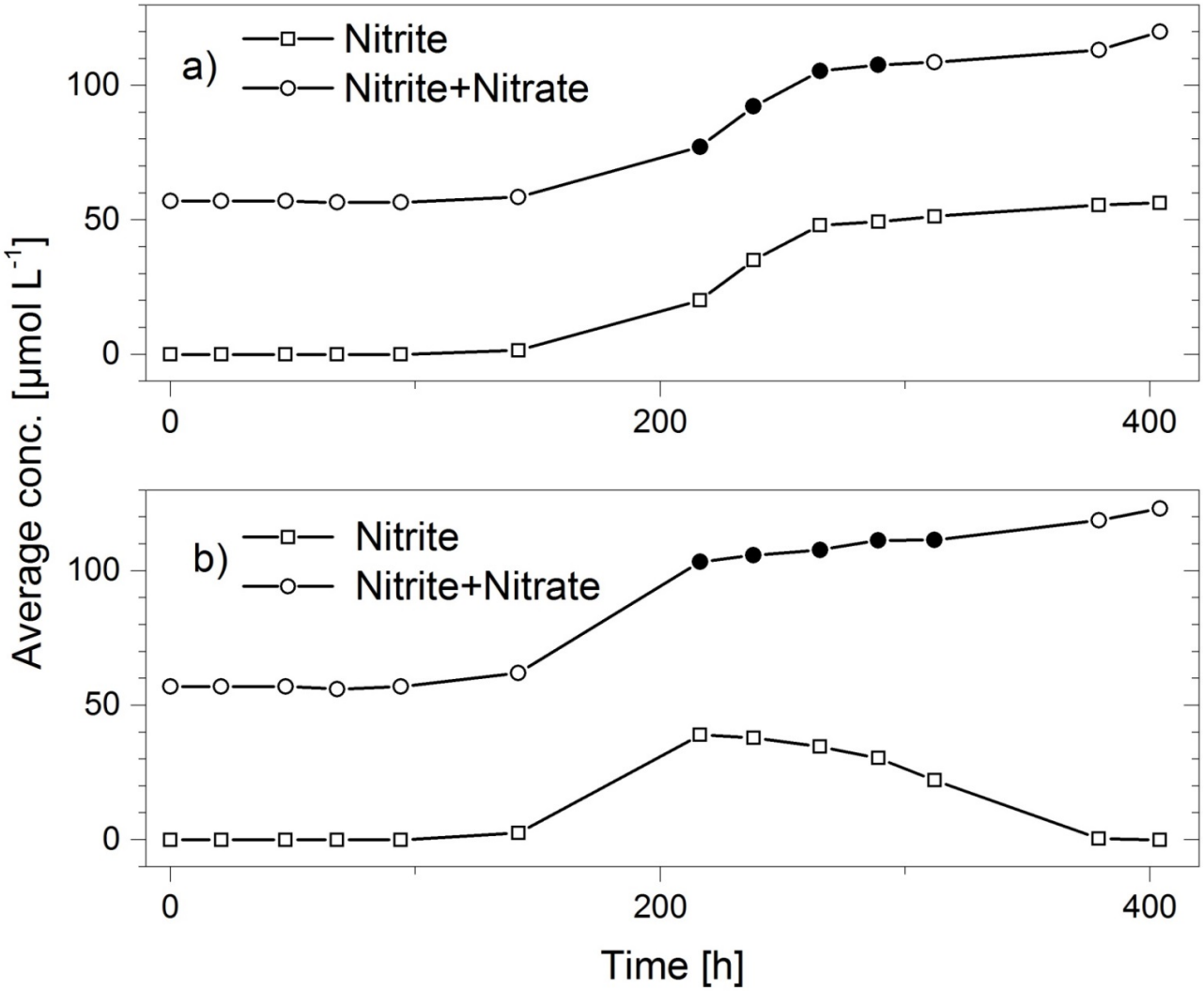

Figure A1 Example illustrations of incubation experiments in August 2012. (a) incubation with $NaClO_3$ for AOB activities, (b) incubation for combined AOB-NOB-activities. Nitrification rates were calculated based on filled circles (chapter 3).

Table A4 Data of the Elbe 2-years study (chapter 4). Abbreviations: Temperature (Temp.), Nitrification rate (NR), Nitrogen (N), and Carbon (C).

Date	Temp.	SPM	NO_3^-	NO_2^-	SiO_4	NH_4^+	NR	$\delta^{15}N$-NO_3^-	$\delta^{18}O$-NO_3^-	$\delta^{15}N$-SPM	N	C
	[°C]	[mg L^{-1}]	[µmol L^{-1}]	[µmol L^{-1}]	[µmol L^{-1}]	[µmol L^{-1}]	[µmol L^{-1} d^{-1}]	[‰]	[‰]	[‰]	[mg L^{-1}]	[mg L^{-1}]
06.07.2011			74.8	1.1	2.3	0.5		20.5	11.4			
21.07.2011			68.4	1.2	0.7	1.6		22.1	12.7		6.1	42.1
04.08.2011			146.4	1.1	57.7	1.1		12.4	4.5		4.4	30.6
18.08.2011			119.5	0.6	106.2	0.9		14.9	6.6		3.3	23.8
01.09.2011	18.0		119.4	0.6	85.8	1.2		14.9	6.9			
15.09.2011	17.0		161.3	0.6	91.3	0.7		13.4	5.4		2.8	19.7
30.09.2011			174.9	0.5	111.6	1.4		13.0	4.5		4.1	27.2
13.10.2011	13.0	18.0	168.7	0.5		1.5	1.9	12.3	5.9	6.7	3.2	22.3
27.10.2011	9.0	10.9	186.7	0.5		1.5		11.7	2.7	9.5	2.2	17.3
10.11.2011	7.0	9.7	199.7	0.4		2.9		11.5	2.3	8.6	1.9	15.4
24.11.2011	5.0	8.5	216.4	0.4	187.0	6.5		11.6	2.9			
08.12.2011	4.0	5.8	203.4	0.5	189.0	6.9	1.2	11.4	3.0		2.0	15.2
21.12.2011	3.0	6.7	274.2	1.5	174.2	8.2		10.4	2.6	8.4	1.9	16.2
05.01.2012	5.0	20.6	324.2	1.1	190.7	7.2	1.3	9.0	1.9			
10.01.2012	4.0	24.1	269.3	1.2	180.7	6.3		8.6	1.1	7.2	1.2	12.6
18.01.2012	3.0	8.4	204.7	0.6	128.8	5.9		8.8	1.8		1.5	11.1
03.02.2012	-0.5	10.3	248.6	1.1	159.5	7.3	1.4	8.4	1.9		1.3	9.6
16.02.2012	0.0	4.5	285.1	1.1	186.1	12.9		9.1	1.8		2.6	18.5
01.03.2012	6.0	10.3	247.1	1.2	179.9	11.5		8.8	2.0	7.6	1.8	13.5
15.03.2012	7.0	14.2	238.3	0.8	177.4	4.2		8.4	1.7	9.2	1.5	12.4
28.03.2012	11.5	13.8	285.0	1.0	202.9	1.6	2.9	9.2	2.4			
19.04.2012	11.5	22.2	156.5	0.1	46.6	0.2		12.8	6.2	4.6	5.4	33.7
10.05.2012	17.0	18.8	107.3	0.1	0.7	0.2	3.4	15.0	8.1	5.0	3.7	24.6
29.05.2012	20.0	34.1	90.4	0.1	3.7	0.2		18.1	11.0	6.1	3.5	25.1

Table A4 Elbe 2-years study (chapter 4, continued). Abbreviations: Temperature (Temp.), Nitrification rate (NR), Nitrogen (N), and Carbon (C).

Date	Temp.	SPM	NO_3^-	NO_2^-	SiO_4	NH_4^+	NR	$\delta^{15}N$-NO_3^-	$\delta^{18}O$-NO_3^-	$\delta^{15}N$-SPM	N	C
	[°C]	[mg L^{-1}]	[µmol L^{-1}]	[µmol L^{-1}]	[µmol L^{-1}]	[µmol L^{-1}]	[µmol L^{-1} d^{-1}]	[‰]	[‰]	[‰]	[mg L^{-1}]	[mg L^{-1}]
07.06.2012	17.0	32.3	53.7	0.1	2.0	0.2	7.6	20.9	13.5			
22.06.2012	21.0	37.6	64.8	0.1	1.0	0.2		20.5	12.3	7.8	4.5	33.5
05.07.2012	22.0	36.2	68.9	0.3	19.8	0.2	9.1	19.1	11.1	6.9	3.8	25.8
19.07.2012	19.0	32.7	86.4	1.0	68.1	0.8		16.5	7.2	7.0	3.6	23.4
02.08.2012	21.0	35.7	60.2	1.1	5.6	0.6	9.2	20.5	11.1	7.0	3.0	20.1
16.08.2012	21.0	39.0	37.2	0.9	0.1	0.8		24.4	14.5	8.0	4.3	26.9
30.08.2012	21.0	33.7	40.6	0.9	4.1	0.4	13.4	22.6	13.5	7.5	4.6	31.5
13.09.2012	18.0	32.1	80.8	1.0	32.7	0.3		17.3	8.2	6.2	3.6	24.4
27.09.2012	14.0	24.3	128.6	0.7	90.1	1.0	6.2	13.5	4.2	5.9	3.9	25.8
12.10.2012	11.0	20.6	136.7	0.6	71.9	0.7		12.7	3.7	7.3	2.9	22.4
25.10.2012	12.0		156.8	0.7	60.7	3.9	2.1	11.9	3.1			
08.11.2012	8.0	10.7	177.1	0.7	88.2	4.8		11.6	2.3	7.6	1.6	15.4
22.11.2012	6.0	10.4	181.8	0.7	84.4	4.3	0.9	11.5	1.8	7.8	1.5	14.8
14.12.2012	-0.5	4.2	202.3	0.8	150.0	8.9		10.8	2.0	8.2	2.0	15.2
02.01.2013	5.0	42.0	318.7	1.5	166.9	9.3		7.9	1.4	7.4	1.0	11.5
17.01.2013	1.0	20.9	285.7	1.4	175.1	5.1	2.5	7.7	1.6	6.9	1.1	12.0
30.01.2013	2.0	11.5	277.1	1.3	196.2	11.3	2.1	8.7	1.8	7.1	1.4	12.0
13.02.2013	1.0	15.7	342.5	1.6	177.6	6.0		7.8	1.5	7.1	1.3	11.7
28.02.2013	2.0	8.9	308.6	1.1	166.9	7.1		8.8	1.2		1.5	12.3
13.03.2013	3.0	9.0	346.8	1.2	158.4	2.5		8.9	1.6		2.1	15.8
04.04.2013	5.0	10.5	300.8	0.8	101.6	1.3		9.3	2.2	8.0	3.6	22.4
17.04.2013	11.0	19.2	299.1	0.8	61.4	1.3		9.8	2.8	5.7	3.4	23.1
02.05.2013	15.0	21.1	206.0	0.8	32.3	1.3		10.4	3.8	5.7	3.2	23.1
22.05.2013	15.0	25.3	209.8	0.8	63.2	1.3		11.6	3.8	6.4	2.8	18.0

Table A5 Correlation coefficients of all measured parameters in the Elbe River at Geesthacht weir, July 2011 to May 2013 (chapter 4). R^2 in bold; underlined coefficients represent a level of significance <0.05. R^2 in bold; underlined coefficients represent a level of significance <0.05 (Pearson correlation).

	Discharge	Temp GW	SPM	NO_3^-	NO_2^-	SiO_4	PO_4^+	NH_4^+	Nitrification	$\delta^{15}N\text{-}NO_3^-$	$\delta^{18}O\text{-}NO_3^-$	$\delta^{15}N\text{-}SPM$	N-SPM	C-SPM	CN-SPM	Temp BH	Oxygen	Chlorophyll	pH	Oxygen sat.
Discharge		0.00	0.10	0.00	0.00	0.00	0.01	0.00	0.10	0.00	0.00	0.82	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.01
Temp GW	**0.62**		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.06	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
SPM	**-0.27**	**0.79**		0.00	0.37	0.00	0.00	0.00	0.00	0.00	0.00	0.09	0.00	0.00	0.41	0.00	0.00	0.00	0.00	0.01
NO_3^-	**0.74**	**-0.84**	**-0.61**		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.39	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
NO_2^-	**0.57**	**-0.51**	**-0.15**	**0.52**		0.00	0.03	0.00	0.53	0.01	0.01	0.20	0.01	0.01	0.01	0.01	0.13	0.01	0.01	0.02
SiO_4	**0.06**	**-0.87**	**-0.68**	**0.84**	**0.49**		0.00	0.00	0.00	0.00	0.00	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
PO_4^+	**0.44**	**-0.78**	**-0.52**	**0.57**	**0.37**	**0.82**		0.00	0.01	0.00	0.00	0.05	0.00	0.00	0.00	0.00	0.07	0.00	0.00	0.00
NH_4^+	**0.45**	**-0.80**	**-0.55**	**0.65**	**0.52**	**0.79**	**0.86**		0.01	0.00	0.00	0.06	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Nitrification	**-0.44**	**0.81**	**0.88**	**-0.79**	**-0.18**	**-0.72**	**-0.77**	**-0.65**		0.00	0.00	0.71	0.01	0.01	0.12	0.00	0.01	0.00	0.01	0.03
$\delta^{15}N\text{-}NO_3^-$	**-0.69**	**0.86**	**0.71**	**-0.91**	**-0.36**	**-0.84**	**-0.64**	**-0.63**	**0.90**		0.00	0.59	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
$\delta^{18}O\text{-}NO_3^-$	**-0.58**	**0.86**	**0.75**	**-0.87**	**-0.36**	**-0.84**	**-0.68**	**-0.63**	**0.88**	**0.98**		0.26	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
$\delta^{15}N\text{-}SPM$	**0.04**	**-0.35**	**-0.31**	**0.16**	**0.24**	**0.43**	**0.41**	**0.35**	**0.14**	**-0.10**	**-0.21**		0.01	0.01	0.11	0.07	0.76	0.01	0.00	0.02
N-SPM	**-0.59**	**0.78**	**0.53**	**-0.70**	**-0.43**	**-0.79**	**-0.79**	**-0.69**	**0.77**	**0.75**	**0.77**	**-0.47**		0.00	0.00	0.00	0.05	0.00	0.00	0.00
C-SPM	**-0.61**	**0.80**	**0.57**	**-0.71**	**-0.41**	**-0.80**	**-0.73**	**-0.68**	**0.77**	**0.76**	**0.77**	**-0.45**	**0.98**		0.00	0.00	0.04	0.00	0.00	0.00
CN-SPM	**0.45**	**-0.53**	**-0.14**	**0.49**	**0.42**	**0.54**	**0.82**	**0.50**	**-0.49**	**-0.48**	**-0.50**	**0.30**	**-0.71**	**-0.59**		0.00	0.13	0.00	0.00	0.00
Temp BH	**-0.59**	**1.00**	**0.77**	**-0.85**	**-0.38**	**-0.83**	**-0.74**	**-0.79**	**0.80**	**0.83**	**0.83**	**-0.34**	**0.78**	**0.78**	**-0.61**		0.00	0.00	0.00	0.00
Oxygen	**0.46**	**-0.76**	**-0.67**	**0.69**	**0.23**	**0.56**	**0.31**	**0.51**	**-0.65**	**-0.60**	**-0.56**	**0.06**	**-0.31**	**-0.32**	**0.25**	**-0.77**		0.08	0.12	0.99
Chlorophyll	**-0.50**	**0.86**	**0.72**	**-0.76**	**-0.40**	**-0.85**	**-0.84**	**-0.75**	**0.76**	**0.85**	**0.90**	**-0.47**	**0.87**	**0.86**	**-0.64**	**0.82**	**-0.39**		0.00	0.00
pH	**-0.41**	**0.76**	**0.54**	**-0.57**	**-0.37**	**-0.77**	**-0.89**	**-0.77**	**0.70**	**0.60**	**0.65**	**-0.68**	**0.78**	**0.72**	**-0.70**	**0.70**	**-0.23**	**0.84**		0.00
Oxygen sat.	**-0.38**	**0.65**	**0.44**	**-0.52**	**-0.34**	**-0.63**	**-0.72**	**-0.61**	**0.58**	**0.61**	**0.65**	**-0.43**	**0.87**	**0.88**	**-0.59**	**0.63**	**0.00**	**0.81**	**0.78**	

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Curriculum Vitae

Der Lebenslauf entfällt aus datenschutzrechtlichen Gründen.

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Hiermit erkläre ich an Eides statt, dass ich die vorliegende Dissertationsschrift selbst verfasst und keine anderen als die angegebenen Quellen und Hilfsmittel benutzt habe.

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Juliane Jacob