Point-by-Point-Reply to „Nitrification and Nitrite Isotope Fractionation as a Case Study in a major European River” by Juliane Jacob et al. (2016)

All three reviewers shared main concerns with regards to our manuscript, which is that we have only focused on nitrification, that we should use an open-system instead of a Rayleigh approach to calculate isotope effects and that we should use a simple box-model to evaluate nitrite sinks. We addressed these issues thoroughly, to the extent that large sections of the discussion have been changed. In detail, we included the following changes:

- Most importantly, we re-focused the overall discussion from assuming nitrite oxidation as a sole nitrite sink to an evaluation of nitrite consumption as a whole. We agree that our focus might have been too narrow to account for potential nitrite sinks. Accordingly, we have rephrased “nitrite oxidation” to “nitrite consumption”, and added a section about other potential sinks, like riparian denitrification, nitrite assimilation by phytoplankton, dilution and source-mixing (e.g. page 8, line 27; page 9, line 6). Based on isotope dynamics and due to limited evidence for nitrite assimilation by phytoplankton, we conclude, that of these four potential processes, nitrite assimilation is a sink of lesser importance (e.g. page 8, line 23). In contrast, riparian denitrification is a potential nitrite sink, that can have a notable apparent isotope effect (Mengis et al., 1999; Sebilo et al., 2003), page 9, line 17), and we included its role in the discussion (e.g. chapter 4.3).
- We address mixing and dilution effects now in more detail in the manuscript, mainly based on SPM and nitrate dynamics (chapter 4.1). Regarding nitrite, though, we regard dilution and source mixing as unlikely, because nitrite is generally not abundant in the catchment and is immediately removed due to its toxicity (see also original response letter, and manuscript page 8, line 17).
- As suggested by reviewer #1 and #2, we investigated a box-model. In three scenarios, we evaluate the sinks based on isotope effects of coupled riparian denitrification, nitrite oxidation and ammonium assimilation (chapter 4.3):
    - Scenario 1 takes only nitrite consumption due to nitrification and riparian denitrification with divergent isotope effects into account, which results in 22% nitrification. This is somewhat unlikely, because ammonium remineralization and ammonium oxidation thus would not occur (page 9, line 24 et. seq.).
    - Scenario 2 considers constant supply of ammonium with an isotope value of about 4.5‰ from remineralization of SPM and nitrite formation from ammonium. This results in 31% nitrification and 69% denitrification (page 9, line 33 et. seq.).
    - Scenario 3 takes the theory of “cryptic” ammonium cycling” into account. Ammonium is consumed during phytoplankton assimilation and successively gets

enriched in ^{15}N. As a result, the contribution of nitrite oxidation increases to 36% versus 64% denitrification (page 10, line 14 et. seq.).

- Another concern was the calculation of the fractionation factor of nitrite consumption. We replaced the Rayleigh calculation with an open-system assumption (Sigman et al., 2009). The apparent isotope effect during nitrite consumption is -10.0±0.1‰ (page 6, line 8). Furthermore, isotope effects during nitrate consumption after phytoplankton recovery were calculated, with $^{15}\varepsilon$ of -4.0±0.1 and R^2 of 0.89, as well as $^{18}\varepsilon$ -5.3±0.1 and R^2 of 0.92 (page 7, line 18 – 19) and discussed in chapter 4.1.
- We suggest ammonium and nitrite concentrations and isotopes are mainly influenced by biology and minor by hydrology. AOB and NOB have a different behavior/sensitivity to surface irradiance (Horrigan et al., 1981). NOB are more light sensitive (Olson, 1981) and poorly recover from photoinhibition (Guerrero and Jones, 1996). This could be a reason why nitrite can accumulate and the variations in concentrations and isotope values are less pronounced.
- Reviewer #2 asked for nitrification rates. We did indeed not present rate measurements, however, we conducted incubation experiments to determine ammonium oxidation and nitrite oxidation rates over an annual cycle in 2012, which we serve as a proof for nitrification.
- Another point was the ratio of δ^{15}N to δ^{18}O of nitrate, which is 0.82 and deviates from the 1:1 slope associated with assimilation (Granger et al., 2004). (Deutsch et al., 2009) have calculated a comparable enrichment ratio of 0.89, which is attributed to at least 75% nitrate assimilation. Our deviation could be a hint for nitrification in the water column and addition of depleted N. However, nitrite dynamics are rather complex indeed, and isotope changes are more subtle than for nitrite.
- The term “calculated fractionation factor” has been changed to “apparent isotope effect”.
- The title has been changed into “Isotope Effects of coupled Nitrification and Denitrification during a River Flood Event”
- Figure 1 is deleted
- Figure 2a – c → Figure 1 a – c has a different color and legend
- Figure 3 → Figure 2 has a changed x-axis (now oxygen saturation [%])
- New figure 3 shows δ^{15}N vs. δ^{18}O of nitrate with a slope of 0.82 and R^2 of 0.96.
- Figure 4 has a changed x-axis (now (1 - *f*) following an open-system approach) with a slope of -10.0±0.1‰ and R^2 of 0.97.
- New figure 5 shows δ^{15}N and δ^{18}O of nitrate versus (1 – *f*) and indicates the isotope effects during nitrate consumption.

All specific comments are approved and we refer to the original response letter including very detailed comments.

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Isotope Effects of coupled Nitrification and Denitrification during a River Flood Event

~~Nitrification and Nitrite Isotope Fractionation as a Case Study in a major European River~~

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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~~The bulk isotope effect of nitrification is hard to predict: It~~ is a two-steps~~step~~-process, where ammonia is oxidized via nitrite to nitrate. Nitrite usually does not accumulate in natural environments, which makes it ~~even more~~ difficult to study~~unravel~~ the single~~divergent~~ isotope effect~~effects~~ of ammonia oxidation or nitrite oxidation in natural systems.~~both processes.~~

However, during an exceptional flood in the Elbe River in June 2013, we found a unique co-occurrence of ammonium,~~ and~~ nitrite and nitrate~~accumulated~~ in the water column~~ for a short period~~, returning towards normal summer conditions within one week. Over~~Concentrations were sufficient for~~ the course of the flood, we analysed the evolution~~analysis~~ of δ^{15}N-NH$_4^+$ and δ^{15}N-NO$_2^-$ ~~evolution, which has not been studied before~~ in ~~a major European river like~~ the Elbe River. In ~~the~~ concert with changes in suspended particulate matter (SPM) and δ^{15}N-SPM, as well as nitrate concentration, δ^{15}N-NO$_3^-$ and δ^{18}O-NO$_3^-$, we calculated apparent~~the~~ isotope effects ~~fractionation effect~~ during ~~nitrification. We found that in the water column, ammonium and~~ nitrite and~~derived from internal recycling processes, whereas~~ nitrate consumption.

During the flood event, >97% of total reactive nitrogen was nitrate, which was~~mainly~~ 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~~5~~ µmol L^{-1}, respectively, likely due to remineralization, nitrification and denitrification~~ammonium oxidation~~ in the water column. δ^{15}N-NH$_4^+$ values increased up to 12‰, and δ^{15}N-NO$_2^-$ ranged from -8.0‰ to -14.2‰. Based on this~~As water column nitrite concentration decreased~~, we calculated an apparent isotope effect $^{15}\varepsilon$ of -10.0±0.1‰ during~~9.3‰ for~~ nitrite consumption, as well as an~~oxidation. This~~ isotope effect $^{15}\varepsilon$ of -4.0±0.1‰ and $^{18}\varepsilon$ of -5.3±0.1‰ during nitrate consumption. In a simple box-model, we evaluated different nitrite uptake processes and found that a regime with riparian denitrification and 22 to 36% nitrification fits best with measured data for the nitrite concentration decrease and isotope increase. ~~does not correspond to the inverse isotope fractionation with a positive $^{15}\varepsilon$ proposed by pure culture studies. We hypothesize that the molecular mechanisms that lead to inverse fractionation also apply in natural environments, but that the resulting trend in δ^{15}N-NO$_2^-$ in this natural environment is masked by dilution with fresh nitrite stemming from ammonium oxidation.~~

~~Our data are a first approximation of the isotope effect of nitrite oxidation in natural environments and highlight that pure culture results cannot readily be extrapolated to natural microbial assemblages or water bodies.~~

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. (Rabalais, 2002; Galloway et al., 2003).~~; Smith et al., 2006).~~ In 1985, North Sea bordering countries decided to reduce nutrient inputs by 50%. As a result, the overall water quality improved, and ~~especially DIN (~~dissolved inorganic nitrogen (DIN) loads decreased, while the~~as well as~~ oxygen saturation ~~have~~ improved markedly (Pätsch et al., 2010). From 1986 to 2006, ammonium~~Ammonium~~ inputs to the Elbe River decreased by 93%, and nitrate inputs decreased by 48%~~% from 1986 to 2006~~ (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~~ stemming~~ from urban waste water, surface runoff, and leachate from agriculture soils (Van~~van~~ Breemen et al., 2002). However~~; Brion et al., 2004).~~

~~Nevertheless~~, 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~~; Böttcher et al., 1990; de Wilde and de Bie, 2000~~) or assimilated by bacteria and phytoplankton (Wada and Hattori, 1978; Middelburg and Nieuwenhuize, 2000). Nitrate regeneration via nitrification occurs in major rivers throughout Europe, and contributes to nitrate loads in, for example, the Seine, Scheldt and Elbe Rivers (~~de Wilde and de Bie, 2000;~~ Sebilo et al., 2006; Johannsen et al., 2008). 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~~biological~~ nitrogen transformation processes, lighter isotopes usually are processed faster than the heavy isotope species, which changes ~~uptake,~~ the isotope composition of the source and product~~nitrogen changes because biological processes usually favour the light isotope over the heavy ones~~ (Mariotti et al., 1981). ~~; Kendall, 1998). Based on a closed-system Rayleigh distillation model, the fractionation factor ^{15}ε for nitrogen uptake can be calculated; individual uptake processes have specific fractionation factors (Rayleigh, 1896; Broecker and Oversby, 1971).~~

Nitrification in this context is unique, because~~During nitrification, a further obstacle is that~~ it is a two-step-reaction with divergent isotope effects. Wide ranging fractionation factors of -14 to -41~~34~~‰ occur during the first step, ammonia oxidation to nitrite, in ~~different~~ pure cultures (~~Delwiche and Steyn, 1970;~~ Mariotti et al., 1981; Casciotti et al., 2003; Santoro and Casciotti, 2011). The second step, the oxidation of nitrite to nitrate, exhibits very rare inverse fractionation (Casciotti, 2009~~; Buchwald and Casciotti, 2010~~): 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. ~~After complete consumption, the isotope value of the substrate is equal to the product.~~

The~~This illustrates that the~~ interpretation of isotope changes in natural environments during nitrification is complex, and studies addressing the combined fractionation factor of ammonia~~ammonium~~ 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~~concentrations~~ in ~~relatively healthy and~~ actively nitrifying environments usually is~~are~~ too low to analyse~~analyze~~ 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 >~~rises above~~ 300 µmol L^{-1}; summer values are <<100~~in the range of ~80~~ µmol L^{-1} due to ~~intense~~ 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~~distinct~~ 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 δ^{15}N~~d^{15}N~~-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).~~; Johannsen et al., 2008).~~

The~~These~~ normal hydrological conditions were disrupted by an unusual summer flood in the Elbe River in June 2013. Runoff~~ (Fig. 1). Hydrological conditions changed and 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 thus 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 ~~, so that ammonium and nitrite concentration changes can be attributed to~~ nitrification rates due to ample substrate, intense water column mixing, and inhibition~~. Indeed, concentration~~ 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~~nutrients~~, especially, using stable isotopes. We calculated the apparent isotope effects during nitrite and nitrate consumption and constructed a simple box-model~~, rose quickly, which was a unique opportunity~~ to estimate the contribution of nitrification and denitrification on nitrite consumption~~analyze isotope changes~~. To the best of our knowledge, this is the first investigation of isotope fractionation during nitrite consumption~~oxidation~~ in a natural, actively nitrifying~~,~~ river system.

2 Materials and Methods

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~~stream~~ 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,~~et al.,~~ 1996) and a nitrate load of about 76 kt yr^{-1} (Bergemann and Gaumert, 2008~~2010~~). Ammonium is of minor importance and is <5% of the nitrate load, and~~whereas~~ 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 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~~daily~~ on 15, 16, 18, and 20 June. Water temperature was measured immediately after sampling, and samples were transferred into 2 L~~2L~~ 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~~analyzes~~, and stable isotope composition (δ^{15}N-NH_4^+, δ^{15}N-NO_2^-, δ^{15}N-NO_3^-, δ^{18}O-NO_3^-). Filter samples were dried at 50°C and weighed for later determination of C/N ratios, suspended particulate matter (SPM) content, and δ^{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~~SPM content, and δ^{15}N-SPM analysis~~.

Nutrient concentrations were analysed~~analyzed~~ with a continuous flow analyser~~analyzer~~ (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},~~),~~ and ammonium was measured fluorometrically with a detection limit of 0.5 µmol L^{-1}~~µM~~ based on Holmes et al. (1999).

2.3 Isotope analyses~~analyzes~~

Dual nitrate ~~and nitrite~~ isotopes (including nitrite) were analysed~~analyzed~~ using the denitrifier method (Sigman et al., 2001; Casciotti et al., 2002). In brief, water samples were injected into a concentrated *Pseudomonas aureofaciens* (ATCC#13985) suspension to analyse~~analyze~~ 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~~is~~ then analysed~~analyzed~~ 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~~identical~~ gas amount in the samples (final gas amount of 10 nmol in case of nitrate, 5 nmol for nitrite analysis).~~) to avoid concentration dependent fractionation effects.~~

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 et al., 2007). Ammonium isotopes were analysed~~analyzed~~ 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 ~~of suspended matter~~ was analysed~~analyzed~~ with an element analyser (~~analyzer~~ Carlo Erba NA 2500) coupled with an isotope ratio mass spectrometer (Finnigan MAT 252).~~.~~

Isotope values are reported using the common “delta” notation~~ (cf. ~~Eq.,~~1) (McKinney et al., 1950),~~

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

where the standards for nitrogen and oxygen are atmospheric N_2 and Vienna Standard Mean Ocean Water (VSMOW), respectively.~~, which both by definition have a δ-value of 0‰.~~

International isotope~~solid secondary~~ standards with known δ~~δ^{15}N~~-values were used for calibration. IAEA N3 and~~,~~ USGS 34 ~~and an internal potassium nitrate standard~~ were used for nitrate isotope calibration~~analysis~~; IAEA N1, IAEA N2, and a certified sediment standard (IVA Analysentechnik~~Analyzentechnik~~, 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~~55~~‰ and -27.5~~46~~‰, determined via EA/IRMS analysis. All samples were analysed~~analyzed~~ in replicate.~~duplicate to calculate standard deviations.~~ Standard deviation of standards and samples~~reference material~~ was <0.2‰ for δ^{15}N-NO_3^- and <0.5‰ for δ^{18}O-NO_3^-. For nitrite isotope analysis, the standard deviation of δ^{15}N-NO_2^- was <0.3‰, and that of δ^{15}N-NH_4^+ was <0.5‰. The standard deviation of δ^{15}N-SPM was <0.1‰. For quality assurance, additional internal standards (KNO_3, KNO_2, $NaNO_2$ salts) were analysed~~analyzed~~ in every run.

2.4 Calculation of isotope effects

Based on an open-system approach the isotope effects for the substrate and product pool can be calculated ~~The fractionation factor ε can be calculated based on the Rayleigh distillation equation of a closed-system model (Broecker and Oversby, 1971; Mariotti et al., 1981) as~~

~~$\varepsilon_{p/s} = \frac{\delta_s - \delta_{s,0}}{\ln f}$ (2)~~

~~Where p is the product, s is the substrate, δ_s and $\delta_{s,0}$ is the delta value of substrate~~ (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, 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 apparent isotope effect ε (Sigman et al., 2009).

$$\boldsymbol{\varepsilon}_{substrate} = -\frac{\delta\text{−}value_{substrate} - \delta\text{−}value_{initial}}{(1-f)} \qquad (2)$$

$$\boldsymbol{\varepsilon}_{product} = \frac{\delta\text{−}value_{product} - \delta\text{−}value_{initial}}{f} \qquad (3)$$

where δ-value$_{substrate}$, δ-value$_{product}$ and δ-value$_{initial}$ are the δ^{15}N values of the substrate and product at the time of sampling and the initial value, ~~respectively.~~ *f* is the remaining fraction of substrate at the time of sampling, and C is the concentration.

3 Results

3.1 General hydrographic properties

Flood conditions (defined by~~with~~ discharge values >3000 $m^3 s^{-1}$ at gauge Neu Darchau, ~~(~~J. Kappenberg, pers. comm.) last~~occurred~~ 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} are~~were~~ eluted shortly before peak discharge ~~(with~~ 4060 $m^3 s^{-1}$) and decrease~~decreased~~ afterwards to 8.6~~9.2~~ mg L^{-1} (Fig. 1a). C/N ratios show the same pattern with a maximum ratio of 10.0, decreasing to 7.6.~~2a).~~ Throughout the entire flood, the water temperature is~~was~~ high and increases~~increased~~ from 16.2 to 21.5°C.

Dissolved oxygen concentration is~~was clearly~~ correlated to discharge; the concentration is~~was~~ initially about 10 mg L^{-1}, corresponding to an oxygen saturation of >~~about~~ 100%.~~% and more, depending on the time of day of sampling.~~ With increasing discharge, the oxygen concentration drops~~droped~~ to a minimum of 6.0 mg L^{-1} (corresponding to 63% saturation), before it increases~~increasing again~~ to ~~an intermediate maximum of~~ 7.7~~6~~ mg L^{-1} (Fig. 1a, 2).~~.~~ After this peak, [O$_2$] decreases~~decreased~~, accompanied by a strong increase in water temperature.~~ (Fig. 2a).~~

3.2 Nutrient concentrations

Previous studies (Johannsen et al., 2008; Schlarbaum, et al., 2011) found high~~higher~~ nutrient concentrations in winter and low concentrations ~~lower~~ in summer. Based on this, our data~~ seasons. Our data generally are in line with their findings, but~~ appear more representative of spring than of summer conditions, because winter and spring 2013~~2015~~ were unusually cold (Van Oldenborgh et al., 2015), so that phytoplankton activity may~~a slight seasonal offset must~~ be delayed. Before the flood, the discharge is ~ ~~taken into account. Discharge was >~~800 m^3 s^{-1},~~ and~~ nitrate concentration is~~concentrations was~~ >200 µmol L^{-1}, nitrite~~ before the flood. Nitrite~~ concentration is~~was~~ <1.2 µmol L^{-1}, and ammonium concentration is~~was~~ below the detection limit of 0.5 µmol L^{-1}.~~µM.~~

~~The~~ DIN concentration increases~~increased~~ when discharge rises~~rose to~~ >3000 m^3 s^{-1} and reaches~~reached~~ a distinct maximum shortly after peak discharge (Fig. 1b). Nitrite~~2b). At high discharge (>3000 m^3 s^{-1}) nitrite~~ concentration rises~~rose above~~ >2.2 µmol L^{-1} and, along with all other nutrients, reaches~~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. 1b). Elevated nitrite concentration >2.2 µmol L^{-1} coincides with decreasing oxygen saturation (from 115 to 63%, Fig. 1b, 2).~~2b).~~

Ammonium concentrations rises~~concentration rose~~ above the detection limit and reaches~~reached~~ a maximum of 3.2 µmol L^{-1} immediately after the peak of SPM, when~~ and~~ oxygen concentrations drops <7.~~below~~ 7 mg L^{-1}, corresponding~~ (Fig. 2b). The elevation of nitrite concentration above 2.7 µmol L^{-1} is coupled~~ to an~~a decrease in~~ oxygen saturation <90% (Fig. 1b, Fig. 2). ~~(<7.6 mg L^{-1}).~~

With decreasing discharge, the oxygen concentration rises~~rose~~, ammonium concentration drops~~dropped~~ below the detection limit, and the overall DIN concentration decreases~~decreased~~ again (Fig. 1a, b).

On 9 June, lowest nitrate concentration (228.1 µmol L^{-1}) coincides with increasing discharge to 3000 m^3 s^{-1}. On 14 June and with further increasing discharge, nitrate concentration increases to 280.6 µmol L^{-1}, followed by a decreasing trend towards 180.0 µmol L^{-1} on 20 June.~~2).~~

3.3 Isotope trends of DIN and ~~of~~ particulate nitrogen

During the entire flood (i.e., excluding discharge <3000~~below 2000~~ m^3 s^{-1}), δ^{15}N-NO$_3^-$~~-~~ and δ^{18}O-NO$_3^-$~~-~~-values are negatively correlated with discharge and nitrate concentration.~~ (R^2 = 0.8 and 0.5, respectively, not shown in plots).~~ The range of δ-values of nitrate during the flood is relatively narrow: Initial values of δ^{15}N-NO$_3^-$ and δ^{18}O-NO$_3^-$ are 9.0~~‰~~ and 3.5‰, respectively, dropping to 7.4 and 2.1‰ when nitrate concentration is highest (Fig. 1b, c~~2c~~). Afterwards, δ-values of nitrate increase again, alongside with dropping concentration, reaching values of 8.8 and 3.9‰ for δ^{15}N-NO$_3^-$ and δ^{18}O-NO$_3^-$, respectively. The ratio of δ^{15}N-NO$_3^-$ to δ^{18}O-NO$_3^-$ is 0.82 (Fig. 3).

Even though nitrite concentration changes gradually over the course of the flood,~~The~~ nitrite isotope values follow a complex pattern ~~when compared to nitrite concentrations~~ (Fig. 1b, 1c~~2b, Fig. 2c~~). Before the flood, nitrite concentration increases~~increased~~ slightly from 1.6 to 1.8 µmol L^{-1}, while $\delta^{15}\text{N-NO}_2^-$ increases~~increased~~ from -14.2 to -8.0‰. At higher discharge (>2000 $m^3 s^{-1}$), nitrite concentration gradually~~quickly~~ rose to a maximum of 4.4 µmol L^{-1}, while $\delta^{15}\text{N-NO}_2^-$ decreased from -8.0 to -13.8‰. When discharge decreased, nitrite concentration also decreased, coupled to a clear increase of $\delta^{15}\text{N-NO}_2^-$, which corresponds to an apparent isotope effect~~a calculated fractionation factor~~ ε of -10.0±0.1~~9.3~~‰ with R² of 0.97~~98377~~ (Fig. 4, Eq. 2).

At the beginning of the flood event, ammonium concentration rises~~rose~~, so that $\delta^{15}\text{N-NH}_4^+$ could be analysed~~analyzed~~. Shortly after the SPM peak, $\delta^{15}\text{N-NH}_4^+$ is about 2‰ and then increases with time to a maximum of 12‰ shortly after peak discharge, followed by a decrease to about 6‰. Although the lowest isotope value coincides with minimal ammonium concentration, there is no distinct correlation of ammonium concentration and its isotope composition. Overall, $\delta^{15}\text{N-NH}_4^+$ seems~~seemed~~ to be only weakly correlated to ~~the SPM load, but not so much to δ^{15}N~~ SPM: The changes in δ^{15}N-SPM, though ranging from 8.1 to 6.2‰ during the flood event, are~~were~~ minimal at the time of ammonium occurrence. The first δ^{15}N-~~It is interesting, however, that initial δ^{15}N~~ NH_4^+ value we measured during the flood is ~~values were~~ about 4.5‰ lighter than suspended matter.

4 Discussion

4.1 Nitrate~~Nutrient~~ dynamics and ~~evidence in~~ 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 ~~The high terrestrial runoff from the catchment area results in an SPM peak from terrestrial sources, which is eluted directly before the discharge peak. This succession is typical for flood events and has been observed in the Elbe River before (Baborowski et al., 2004; Pepelnik et al., 2005).~~

~~Nitrate concentration initially~~ decreased from 269.6 to 228.1 µmol L^{-1}, due to an initial dilution of ~~with increasing discharge, because~~ the river nitrate load ~~was diluted~~ 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~~nitrate~~ concentration increased with discharge, which~~along with the SPM peak in the river. Both~~ can be attributed to ~~leachate from agricultural soils:~~ terrestrial soil nitrate that is~~,~~ leached from~~due to high precipitation in~~ 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).~~), and during the flood, leached SPM and~~

~~nitrate are extraordinarily high and clearly shown in the water mass.~~ The decrease of δ^{15}N-SPM ~~values~~ from ~~~7.~~8‰ to < 6~~.2~~‰ during increasing~~highest~~ discharge also indicates the input of terrestrial~~fresh~~ 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~~leachate of unfractionated material from the catchment area~~ 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 (Voss et al., 2006; Deutsch et al., 2009). 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. ~~after the SPM peak and maximal concomitant nitrate input. Under normal conditions, biological activity is low in winter, nitrate concentration is high and isotope values are low, because no uptake and hence no fractionation occur.~~ In the Elbe River, summer nitrate concentrations are <<100 µmol L^{-1} and in winter it is >300 µmol L^{-1}. Mean summer ~~typical winter~~ δ^{15}N-NO$_3^-$ and δ^{18}O-NO$_3^-$ values are <18.0 and 7.6‰, respectively, and mean winter values for δ^{15}N-NO$_3^-$ and δ^{18}O-NO$_3^-$ are <~~7.8 –~~9.3 and 0.8~~-~~‰, respectively (Johannsen et al., 2008; Schlarbaum, ~~et al.,~~ 2011). During the flood in June, ~~we see similar values: δ^{15}N-NO$_3^-$ ranges between 7.4 – 9‰ and δ^{18}O-NO$_3^-$ between 2.1 – 3.9‰ (Fig. 2c). The narrow ranges and low values of~~ δ^{15}N-NO$_3^-$ is 7.4 – 9.0‰ and δ^{18}O-NO$_3^-$ is 2.1 – 3.9‰ (Fig. 1c), which is close to winter values and suggests only little biological processing. ~~indicate reduced biological activity (Johannsen et al., 2008).~~

In summer and under ~~Under~~ normal flow conditions, nitrate concentration ~~then~~ decreases due to assimilation and biomass production. ~~(Fig. 1).~~ As a consequence, dual isotope values are~~then~~ negatively correlated~~correlate~~ with nitrate concentration (~~Montoya and McCarthy, 1995; Voss et al., 2006;~~ Johannsen et al., 2008; Deutsch et al., 2009). During the flood event~~). In our study~~, δ^{15}N-NO$_3^-$ and δ^{18}O-NO$_3^-$ are clearly~~negatively~~ correlated with [NO$_3^-$] ~~only~~ after the nitrate~~SPM~~ peak (R² of 0.90~~897~~ and 0.93~~816~~, respectively), which, together with rising [O$_2$] concentration, pinpoints the onset of ~~also pinpointing reduced~~ biological nitrate assimilation. Accordingly, we calculated the isotope effect using an open-system approach (Eq. 2), during this decrease in nitrate concentration. The fractionation factor $^{15}\varepsilon$ is -4.0±0.1‰ (R^2 of 0.89) and $^{18}\varepsilon$ is -5.3±0.1‰, R^2 of 0.92 (Fig. 5). This is on the low end of isotope effects reported for nitrate ~~showing that during flood conditions,~~ assimilation (Waser et al., 1998; Granger et al., 2004), but fractionation can be affected by ~~phytoplankton diminishes, probably due to high turbidity, short residence times, and decreased light availability (Voss et al., 2006; Deutsch et al., 2009~~residence times, such that the isotope effect is lower when residence times are low (Kendall, 1998).

The ratio of δ^{15}N-NO$_3^-$ to δ^{18}O-NO$_3^-$ also supports the importance of phytoplankton assimilation.~~From 14 June on, dropping discharge allows a recovery of phytoplankton, and rising oxygen concentrations indeed suggest that phytoplankton is recovering. In addition to the clear anticorrelation to [NO$_3^-$], dual nitrate isotope values further indicate rising phytoplankton activity.~~ At the beginning of the flood, δ^{15}N-NO$_3^-$ is not correlated with δ^{18}O-NO$_3^-$, but when nitrate decreases, the ratio of δ^{15}N-NO$_3^-$ to δ^{18}O-NO$_3^-$ changes~~after the SPM peak, both isotopes change almost~~

~~in parallel~~ along a slope of 0.82 (R^2 of 0.96, Fig. 3). It differs slightly from ~~960). This is close to~~ unity, which is associated with~~typical of~~ phytoplankton assimilation only (Granger et al., 2004; Deutsch et al., 2009), but this might be due to nitrification, which would lower the $\delta^{18}O\text{-}NO_3^-$ values and thus lead to a slope below 1 (e.g. (Wankel et al., 2006). ~~).~~

4.2 Sources~~The role~~ of ~~nitrification~~

~~As outlined above, nitrate trends can be explained by assimilation and hydrographic properties. This is not quite the case for~~ 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. ~~concentrations during the flood.~~ Generally, these nutrients do not accumulate in the water column in~~during~~ spring and summer (own unpublished data), but during~~time. During~~ the flood, they~~ammonium and nitrite, the substrates for nitrification,~~ 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~~but then decrease again, indicating active nitrification~~.

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 the positive charge of ammonium molecules tightly binds them to clay particles in soil, and elution with discharge generally does not occur (Mancino, 1983). We regard remineralization of ~~We regard~~ SPM as the main source of ~~remineralized~~ ammonium, which, in turn is then usually immediately assimilated (Dortch et al., 1991) or oxidized to nitrite (Mayer et al., 2001). The first ammonium ~~), which then is rapidly detoxified by oxidation to nitrate (Alonso and Camargo, 2006; Philips et al., 2002). Ammonium in the river initially has an~~ isotope value we were able to measure in the river was ~~of~~ ~2‰, approximately 4.5‰ lighter than the SPM pool (Fig. 1c~~2c~~). 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, 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, ~~4‰ fractionation during remineralization,~~ as indicated by low oxygen concentrations, can then lead to an accumulation of remineralized 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‰ (Mariotti et al., 1981; Casciotti et al., 2003; 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 removed due to its toxicity.

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 (Lomas and Lipschultz, 2006; Lam et al., 2011).

On the sink side, we assume that nitrite assimilation by phytoplankton is of minor importance. Even though the possibility of nitrite assimilation~~was recently put forward~~ 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~~(Möbius, 2013). The author found that during remineralization in marine~~ sediments or the riparian zone (Brandes and Devol, 1997; Sebilo~~, isotope enrichment with an isotope effect of about 2‰ occurs. Our value is slightly higher, but it seems plausible that organic material in the Elbe River most likely is more easily accessible than that of marine sediments and~~ 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. ~~subject to more intense fractionation. This fractionation during remineralization also explains the depleted initial δ^{15}N-NH$_4^+$ values we find, and it is in accordance with (Schlarbaum et al., 2011), who found differences of up to 4‰ between dissolved organic nitrogen and suspended matter in the Elbe River. It is unlikely that ammonium in the water column derives from agricultural sources, because the positive charge of ammonium molecules tightly binds them to clay particles in soil, and elution with discharge generally does not occur (Mancino, 1983; Mancino and Troll, 1990).~~

When ammonium drops below the detection limit with decreasing discharge, nitrite remains above 3 µmol L^{-1} for a few days (Fig. 1b~~2b~~). This succession of nitrite and ammonium concentration maxima can indicate~~be taken as an indicator of~~ 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 ~~Ammonium and nitrite accumulate at oxygen concentrations below 7 mg L^{-1} (Fig. 3). As outlined above, phytoplankton activity at this time is low, and nitrification is the main ammonium sink. Ammonium-oxidizing bacteria (AOB) are active under low [O$_2$], but ammonium oxidation obviously cannot keep pace with remineralization, so that ammonium accumulates. δ^{15}N-NH$_4^+$ of the residual increases, because ammonium oxidation has a strong isotope effect of -14 to -38‰ (Delwiche and Steyn, 1970; Mariotti et al., 1981; Yoshida, 1988; Casciotti et al., 2003). Simultaneously, nitrite concentration peaks at low oxygen concentrations and decrease again, when oxygen concentration rise above 7 mg L^{-1} (Bernet et al., 2001; Jianlong and Ning, 2004). δ^{15}N-NO$_2^-$ are negatively correlated to nitrite concentration (fig. 2b, c). Altogether, we see a signal of coupled SPM remineralization and concomitant nitrification to~~ newly produced nitrate into the large pre-existing~~in the Elbe River at lowered [O$_2$].~~

~~Within 7 days, SPM is <14 mg L^{-1}, and ammonium concentration again falls below the detection limit due to complete oxidation of ammonium by AOB. If ammonium were mainly removed via nitrification, this decrease corresponds to an ammonium oxidation rate of about 0.5 µmol L^{-1} d^{-1}, which is well within the range of water column nitrification rates for temperate river systems (Bianchi et al., 1994; Daims et al., 2015; van Kessel et al., 2015). Nitrite concentration drops to typical spring/summer values (i.e. <1 µmol L^{-1}) after the flood event.~~

~~While we cannot trace the newly produced nitrate from nitrification into the much larger~~ nitrate pool, the gradual change of nitrite concentration and isotope values provides the unique opportunity to calculate the isotope effect during nitrite consumption in the river system. When nitrite concentration decreases (see filled symbols in figure 1b, 1c and 4), the apparent isotope effect is -10.0±0.1‰. This fractionation factor is negative, suggesting conventional fractionation during nitrite consumption (R² 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). ~~for the first time calculate the isotope effect during nitrite oxidation in the Elbe River.~~

4.3 Nitrite uptake scenarios

As discussed above, potential sinks for nitrite in the river are assimilation, denitrification, and ~~No inverse fractionation during~~ 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.

~~During the period with decreasing nitrite concentrations, we calculated the isotope effect of nitrite oxidation assuming closed system Rayleigh fractionation (Eq. 2), because ammonium is not abundant in the water column during most of the sampling period, and hence input of new nitrite from ammonium oxidation is hypothetical. In a closed system, δ^{15}N NO$_2^-$ values should behave linear to ln([NO$_2^-$]/[NO$_2^-$ $_{initial}$]), and the slope of the regression line corresponds to the isotope effect (Mariotti et al., 1981; Scott et al., 2004). For the time span with decreasing nitrite concentration, we calculated an isotope effect of -9.3‰±0.6‰. (Fig. 4). To validate our results, we compared them to an open system approach and calculated an isotope fractionation factor based on an open system steady state model (Sigman et al., 2009). Here, the fractionation factor is -10.6±1.0‰, not significantly different from the closed-system approach, also suggesting conventional negative fractionation during nitrite oxidation. This means that during processing, i.e. nitrite oxidation in the water column, the remaining nitrite pool gets subsequently enriched in 15N. This result is surprising, because pure culture experiments with the marine nitrite oxidizing bacteria *Nitrococcus mobilis* (Casciotti, 2009; Buchwald and Casciotti, 2010), *Nitrobacter sp. Nb 355* and *Nitrospira marina* (Buchwald and Casciotti, 2010) suggested that nitrite oxidation exhibited rare inverse isotopic fractionation.~~

~~While some uncertainty naturally arises from the fact that we did not investigate pure cultures, but a biologically diverse setting like the Elbe River, we would like to investigate whether it is plausible that bacterial nitrite oxidation in natural environments rather follows “conventional” than inverse isotope fractionation. This would imply molecular differences on the enzyme level between naturally occurring and isolated nitrite oxidizing bacteria.~~

~~Fractionation factors can indeed vary greatly between different species (Casciotti et al., 2003).~~ 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 (Mariotti et al., 1988; Brandes and Devol, 1997) 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. 2.4); we then aimed to reproduce the nitrite isotope effect of -10.0‰ (cf. Fig. 4).

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; Kendall et al., 2007; Houlton and Bai, 2009), 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 remineralized, and that no new nitrite is formed via ammonium oxidation, which seems somewhat unlikely.

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 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) 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.

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 remineralization.

~~Nevertheless, the inverse fractionation during nitrite oxidation is assumed to be based not on enzyme geometry, but on the stability of an intermediate during the oxidation process: Greater stability of – otherwise labile – intermediates containing ^{15}N facilitates further reaction and thus, nitrite oxidation. This transition state should be identical among nitrite oxidizing bacteria and should not depend on specific (or species specific) enzyme equipment. Hence, we assume that an alternative, conventional isotope effect in natural assemblages of nitrite oxidizers is unlikely; the divergent isotope effect in our study must have another reason.~~

~~The changing nitrite isotope concentration and isotope values are not caused by water column denitrification, because oxygen concentration is above 6 mg L^{-1} (Lehmann et al., 2004) and sedimentary denitrification has little to no impact on water column nitrate isotopes (Brandes and Devol, 1997) and much less so for nitrite, there is no evidence of nitrite release to the water column. Sedimentary denitrification in the Elbe River generally is of minor importance (25%) and nitrate assimilation by phytoplankton is the major sink (Deutsch et al., 2009). Nitrite is not present in atmospheric deposition (Beyn et al., 2014), so that we also exclude an effect of atmospheric deposition on nitrite concentration or isotope changes. Overall, changing nitrite isotope values must be due to nitrification.~~

~~During the flood event, discharge and turbidity are high, and remineralization of suspended matter is intense, as is evidenced by high ammonium and low dissolved oxygen concentrations during the flood. Shortly after the highest discharge, δ^{15}N-NH$_4^+$ is 2‰ (see above), but this value increases during the flood, reaching a maximum of 12‰. Over the course of the flood, oxidation of ammonium proceeds. Several studies suggest a high isotope fractionation factor during the oxidation of ammonium to nitrite, between -14 and -38‰ (Delwiche and Steyn, 1970; Mariotti et al., 1981; Casciotti et al., 2003). Such high fractionation explains the increase of δ^{15}N-NH$_4^+$ to 12‰ (Fig. 2c).~~

~~This ammonium recycling also offers an alternative explanation for the aberrant isotope fractionation effect we calculated for nitrite oxidation. Ammonium concentrations are below the detection limit at the time of decreasing nitrite concentrations, but we speculate that rapid remineralization and oxidation of ammonium proceed. In this case, newly generated, isotopically enriched nitrite from ammonium oxidation would (a) prevent ammonium accumulation and (b) camouflage the inverse isotope effect of nitrite oxidation, because the generation of new, of enriched nitrite exceeds nitrite turnover by nitrite oxidation.~~

~~However, the ammonium concentration is under the detection limit (0.5 µmol L^{-1}) when nitrite removal occurs - can we thus assume that ammonium oxidation is still active at such low concentrations?~~

~~Cryptic element cycling, with *in situ* substrate concentrations near the detection limit, has indeed been reported on various occasions. In the OMZ off the Chilean coast (Canfield et al., 2010) propose that sulfate reduction and sulfide oxidation may long have been overlooked, due to the close coupling of these processes, that leaves no chemical evidence of their activity. Similarly, rate measurements also revealed the activity of anammox in OMZ water at no more than nanomolar concentration of ammonium (De Brabandere et al., 2013). If ammonium recycling is relevant even at such low concentrations, it seems plausible to assume that ammonium oxidation by nitrifying bacteria also occurs in our setting, where concentrations below the detection limit may be as high as 0.5 µmol L^{-1}.~~

~~In such a case, ammonium will rapidly be recycled, and isotope signature of ammonium will be transferred directly to the nitrite pool, because limiting ammonium concentrations prevent any significant fractionation during oxidation (Mariotti et al., 1981). As a result, even if nitrite oxidation is coupled with inverse fractionation, the resulting bulk $\delta^{15}N$-NO_2^- values will show an apparent enrichment due to "dilution" with nitrite derived from relatively heavy ammonium.~~

~~In a back-of-the-envelope calculation, we tested this hypothesis. We assumed inverse fractionation with an isotope effect of +10‰ that acted on nitrite, assuming standard Rayleigh fractionation, and that nitrite oxidation and ammonium oxidation occurred at comparable rates. We found that the resulting isotope value of nitrite indeed showed conventional negative isotope fractionation of ~-8‰, close to our measurements, but only if we assumed that the isotope value of ammonium increased with an isotope effect for ammonium oxidation of -35‰. This, barring considerable uncertainties regarding reaction rates and enrichment factors, this calculation nevertheless suggests that ammonium recycling can change the observed isotope effect of nitrite.~~

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 remineralization of organic material, whereas the changing nitrite concentration and isotopes are influenced by several sources and sinks. 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 fractionation.

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 31 – 36%, whereas riparian denitrification makes up for 64 – 69% of nitrite consumption. Our nitrite isotope data reveal the substantial role of nitrification and remineralization during an extreme flood event, but also demonstrate that other sinks, like denitrification in the riparian zone, contribute to nitrite turnover.

While the~~Our data suggest that nitrification is the main sink of ammonium and nitrite, respectively, in the Elbe River during the flood, where we find intermediate ammonium and nitrite accumulation in the water column. While the main source of ammonium is remineralization of organic material, increasing isotope values over time indicate active microbial ammonium turnover and oxidation of ammonium to nitrite. The subsequent decrease of the nitrite concentration in the water column indicates nitrite oxidation, which surprisingly does not exhibit inverse fractionation, but follows conventional fractionation, with subsequent isotope enrichment of the nitrite substrate during processing. For this removal, we calculated an isotope effect of -9.3‰. We hypothesize that the reason for this surprising isotope effect is a cryptic ammonium cycle, which quickly channels newly generated, isotopically heavy nitrite into the nitrite pool. This continuous dilution masks the inverse enzymatic isotope effect of nitrite oxidation.~~

~~Our data demonstrate that results from pure culture experiments cannot easily be extrapolated to natural systems, because computed isotope effects depend not only on substrate concentration, but also are subject to a complex interplay of biogeochemical processes. While nitrogen isotopes are a powerful tool to unravel individual processes, divergent isotope effects and individual process rates need to be taken into account. The~~ inverse isotope effect of nitrite oxidation adds more complexity to the isotope budget of the aquatic nitrogen cycle, ~~but~~ our data suggest that co-occurring processes disguise this inverse fractionation~~it might not be expressed~~ in natural environments, which might not ~~at all. If this is the case, it holds true not~~ only be important in~~for~~ estuarine settings, but also in other environments~~probably for any environment~~ that show~~shows~~ nitrite accumulation in the water column, like oceanic OMZs, where nitrate and nitrite isotopes are frequently used to assess nitrogen dynamics.

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8 Figures

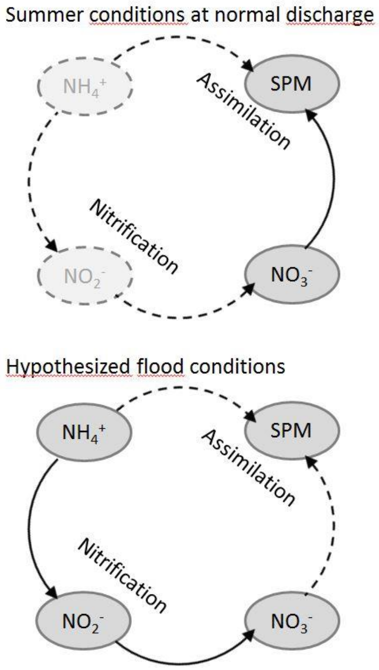


Fig. 1 →

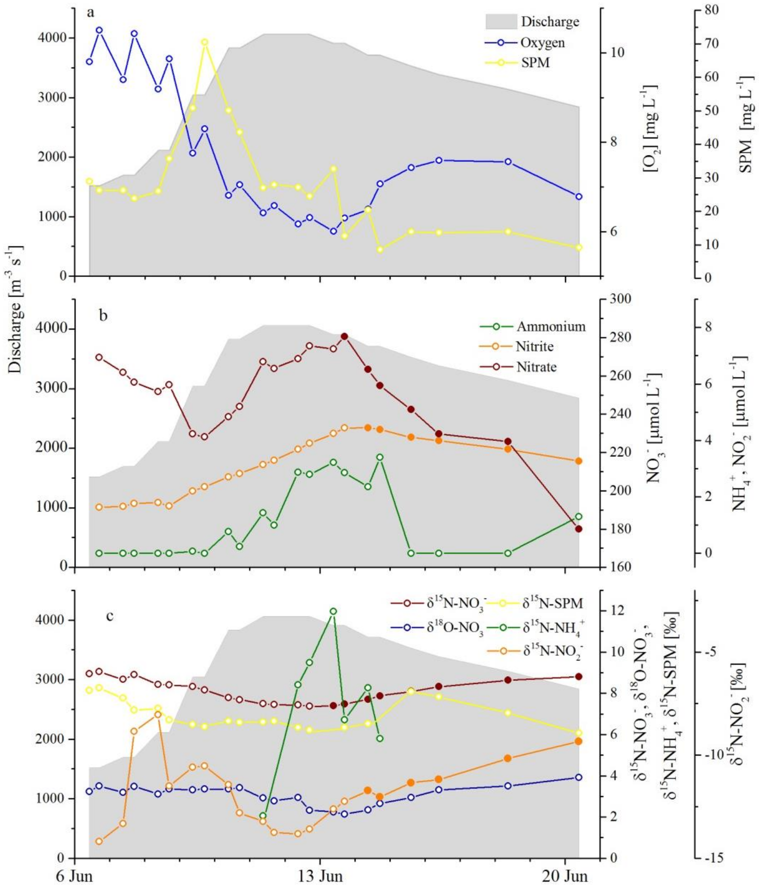
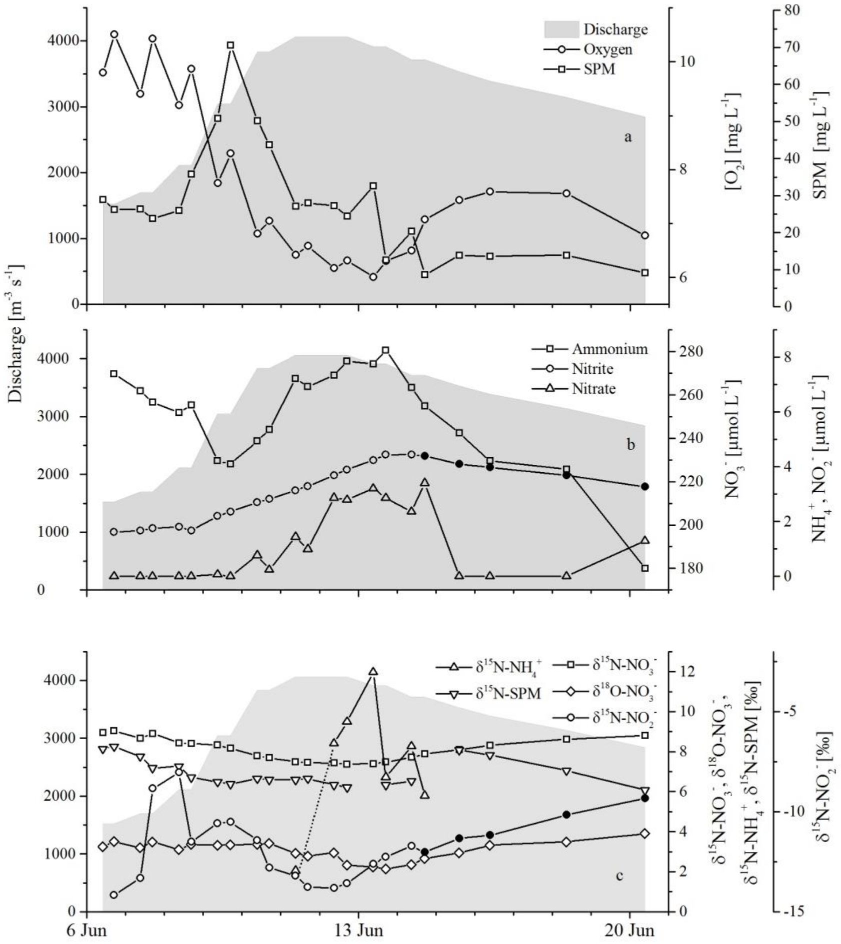
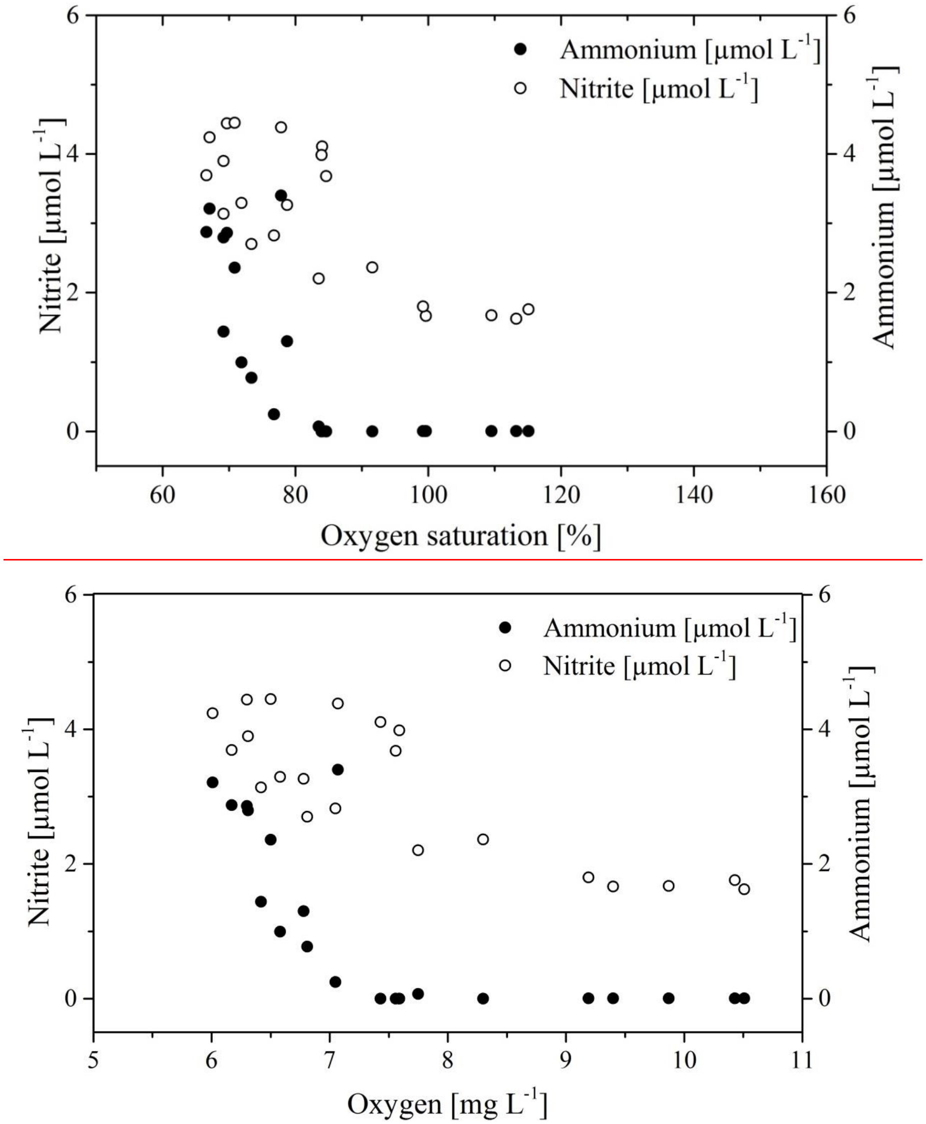


Figure 1a, b, c~~1~~

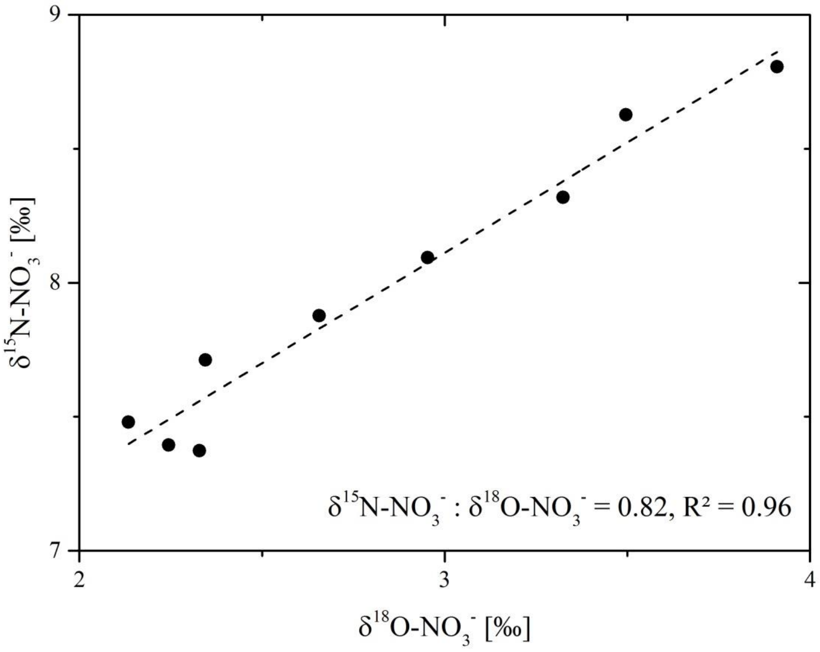


~~Figure 2a, 2b, 2c~~



● Ammonium [µmol L^{-1}]
○ Nitrite [µmol L^{-1}]
Nitrite [µmol L^{-1}]
Ammonium [µmol L^{-1}]
Oxygen saturation [%]
0
2
4
6
60
80
100
120
140
160
● Ammonium [µmol L^{-1}]
○ Nitrite [µmol L^{-1}]
Nitrite [µmol L^{-1}]
Ammonium [µmol L^{-1}]
Oxygen [mg L^{-1}]
5
6
7
8
9
10
11

New figure 2


9
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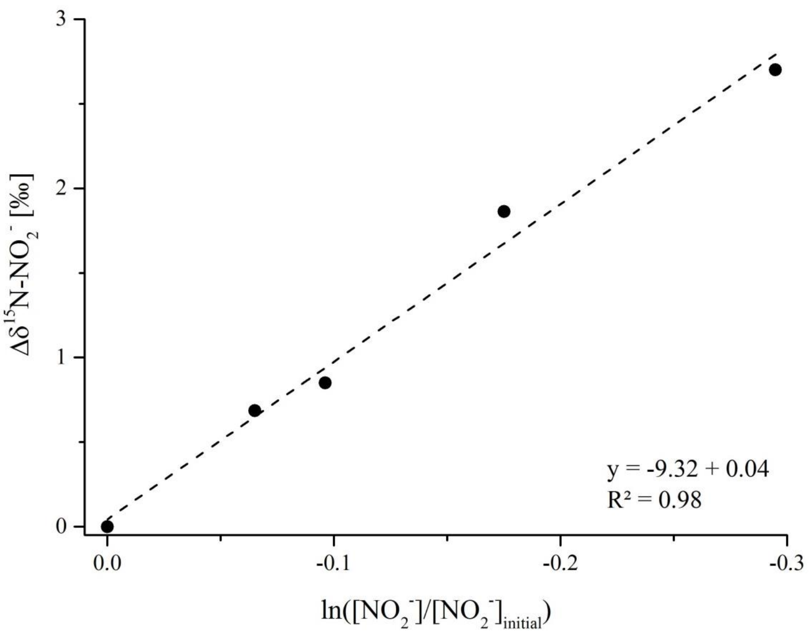
δ^{15}N-NO$_3^-$ [‰]
δ^{18}O-NO$_3^-$ [‰]
δ^{15}N-NO$_3^-$: δ^{18}O-NO$_3^-$ = 0.82, R² = 0.96

- ➔ New figure 3



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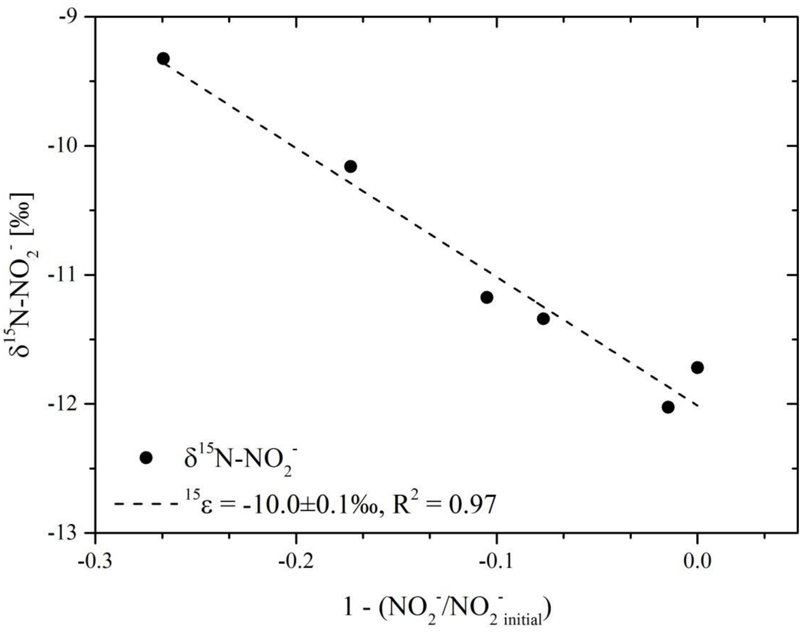

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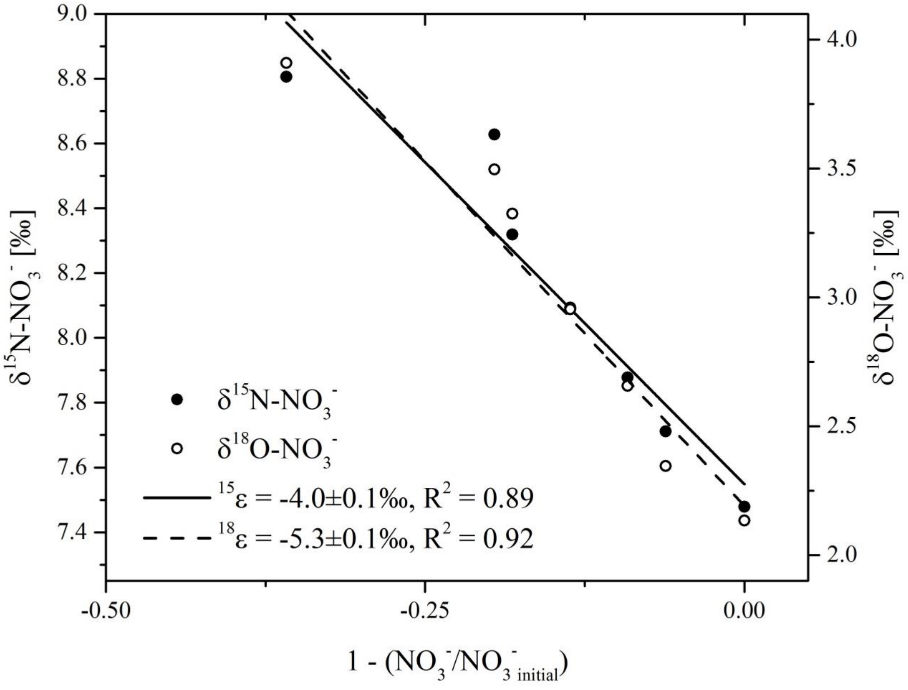

➔ **New figure 4**

9 Figure Captions

Figure 1a~~1 Sketch of summer condition at normal discharge where light colouring indicates concentration below the detection limit compared to the flood condition. Active nitrification is observed during the flood, but assimilation diminished shown as dashed lines.~~

~~Figure 2a~~ Discharge, dissolved oxygen concentration, and SPM concentration of the Elbe River water samples ~~during the flood~~ from 6 to 20 June 2013. Flood conditions occur with discharge >3000 m^3 s^{-1}.

Figure 1b~~2b~~ Ammonium, nitrite, and nitrate concentrations in the Elbe River in the course of the flood. Calculation of the fractionation factor is based on filled ~~dots~~ data points.

Figure 1c~~2c~~ Ammonium, nitrite, nitrate, and SPM isotope values in the course of the flood. Calculation of the fractionation factor is based on filled ~~Filled symbols indicate~~ data points~~that were used for calculation of the fractionation factor~~.

Figure 2~~3~~ Ammonium and nitrite concentrations increase with decreasing dissolved oxygen saturation~~concentration~~.

Figure 3 Ratio of δ^{15}N-NO_3^- versus δ^{18}O-NO_3^- values corresponding to decreasing nitrate concentrations from 13 to 20 June and filled data points of figure 1b and 1c. The calculated linear regression has a slope of 0.82 with R^2 of 0.96.

Figure 4 Nitrite isotope values versus the remaining fraction of~~Rayleigh plot for~~ nitrite ~~oxidation~~ during the Elbe flood corresponding to the filled data points in figure 1b and 1c. The dashed line indicates the apparent isotope effect during nitrite consumption with a slope of -10.0±0.1‰ and R^2 of 0.97.

Figure 5 Dual nitrate isotope values versus the remaining fraction of nitrate corresponding~~. Data points correspond~~ to the filled data points~~symbols~~ in figure 1b~~2b~~ and 1c. The solid~~2c. Dotted~~ line indicates the apparent isotope effect during nitrate consumption~~least square fit~~ with a slope of ~~-9.32 indicating~~ $^{15}\varepsilon$ -4.0±0.1‰ with R^2 of 0.89~~,~~ and the dashed line ~~R^2~~ is $^{18}\varepsilon$ -5.3±0.1‰ with R^2 of 0.92.

~~0.98.~~

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