Nitrite consumption and associated isotope changes during a river flood event

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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 δ^{15}N-NH$_4^+$ and δ^{15}N-NO$_2^-$ in the Elbe River. In 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 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. 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, 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.

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 fractionation factors 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 very rare inverse fractionation (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 fractionation factor 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 δ^{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).

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 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 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 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 ($\delta^{15}N$-NH_4^+, $\delta^{15}N$-NO_2^-, $\delta^{15}N$-NO_3^-, $\delta^{18}O$-NO_3^-). Filter samples were dried at 50°C and weighed for later determination of C/N ratios, suspended particulate matter (SPM) content, and $\delta^{15}N$-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}, and ammonium was measured fluorometrically with a detection limit of 0.5 µmol L^{-1} based on Holmes et al. (1999).

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 et 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.

$\delta^{15}N$-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,

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

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 $\delta^{15}N$ values of -81.5‰ and -27.5‰, determined via EA/IRMS analysis. All samples were analysed in replicate. Standard deviation of standards and samples was <0.2‰ for $\delta^{15}N$-NO_3^- and <0.5‰ for $\delta^{18}O$-NO_3^-. For nitrite isotope analysis, the standard deviation of $\delta^{15}N$-NO_2^- was <0.3‰, and that of $\delta^{15}N$-NH_4^+ was <0.5‰. The standard deviation 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.

2.4 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, 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).

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

$$\boldsymbol{\varepsilon}_{product} = \frac{\delta\text{-}value_{product} - \delta\text{-}value_{initial}}{f} \tag{3}$$

where δ-value$_{substrate}$, δ-value$_{product}$ and δ-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, and C is the concentration.

3 Results

3.1 General hydrographic properties

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

Dissolved oxygen concentration is correlated to discharge; the concentration is initially about 10 mg L^{-1}, corresponding to an oxygen saturation of >100%. With increasing discharge, the oxygen concentration drops to a minimum of 6.0 mg L^{-1} (corresponding to 63% saturation), before it increases to 7.7 mg L^{-1} (Fig. 1a, 2). After this peak, [O$_2$] decreases, accompanied by a strong increase in water temperature.

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 is ~800 m^3 s^{-1}, nitrate concentration is >200 µmol L^{-1}, nitrite concentration is <1.2 µmol L^{-1}, and ammonium concentration is below the detection limit of 0.5 µmol L^{-1}. DIN concentration increases when discharge rises >3000 m^3 s^{-1} and reaches a distinct maximum shortly after peak discharge (Fig. 1b). Nitrite concentration rises >2.2 µmol L^{-1} and, along with all other nutrients, reaches 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).

Ammonium concentrations rises above the detection limit and reaches a maximum of 3.2 µmol L^{-1} immediately after the peak of SPM, when oxygen concentrations drops <7.7 mg L^{-1}, corresponding to an oxygen saturation <90% (Fig. 1b, Fig. 2). With decreasing discharge, the oxygen concentration rises, ammonium concentration drops below the detection limit, and the overall DIN concentration decreases 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.

3.3 Isotope trends of DIN and particulate nitrogen

During the entire flood (i.e., excluding discharge <3000 m^3 s^{-1}), δ^{15}N-NO$_3^-$ and δ^{18}O-NO$_3^-$ values are negatively correlated with discharge and nitrate concentration. 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). Afterwards, δ-values of nitrate increase 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 $\delta^{15}N\text{-}NO_3^-$ to $\delta^{18}O\text{-}NO_3^-$ is 1.22 (Fig. 3).

Even though nitrite concentration changes gradually over the course of the flood, nitrite isotope values follow a complex pattern (Fig. 1b, 1c). Before the flood, nitrite concentration increases slightly from 1.6 to 1.8 µmol L^{-1}, while $\delta^{15}N\text{-}NO_2^-$ increases 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 is coupled to a clear increase of $\delta^{15}N\text{-}NO_2^-$. This net decrease most likely represents 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. 4, Eq. 2).

At the beginning of the flood event, ammonium concentration rises, so that $\delta^{15}N\text{-}NH_4^+$ could be analysed. Shortly after the SPM peak, $\delta^{15}N\text{-}NH_4^+$ 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, $\delta^{15}N\text{-}NH_4^+$ seems to be only weakly correlated to SPM: The changes in $\delta^{15}N\text{-}SPM$, though ranging from 8.1 to 6.2‰ during the flood event, are minimal at the time of ammonium occurrence. The first $\delta^{15}N\text{-}NH_4^+$ value we measured during the flood is about 4.5‰ lighter than suspended matter.

4 Discussion

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 $\delta^{15}N\text{-}SPM$ from ~8‰ to <6‰ during increasing discharge also indicates the input of terrestrial organic material due to leaching. Terrestrial organic matter has a $\delta^{15}N$-value of about 3.5‰, which is significantly lower than riverine SPM with $\delta^{15}N$ 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 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 <9.3 and 0.8‰, respectively (Johannsen et al., 2008; Schlarbaum et al., 2011). During the flood in June, 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.

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, δ^{15}N-NO$_3^-$ and δ^{18}O-NO$_3^-$ are clearly correlated with [NO$_3^-$] after the nitrate peak (R² 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), during this net 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 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 δ^{15}N-NO$_3^-$ to δ^{18}O-NO$_3^-$ also supports the dominant role of phytoplankton assimilation. 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 along a slope of 1.22 (R² of 0.95, Fig. 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 δ^{18}O-NO$_3^-$ values and thus lead to a slope above 1 (e.g. Wankel et al., 2006).

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 (own unpublished data), 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 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 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. 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 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‰ (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 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 (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. 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 figure 1b, 1c and 4), the apparent isotope effect is $-10.0\pm0.1‰$. 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).

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. 2.4); 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; 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 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.

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. 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 fractionation.

5   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.

10   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 fractionation 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.

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8 Figures

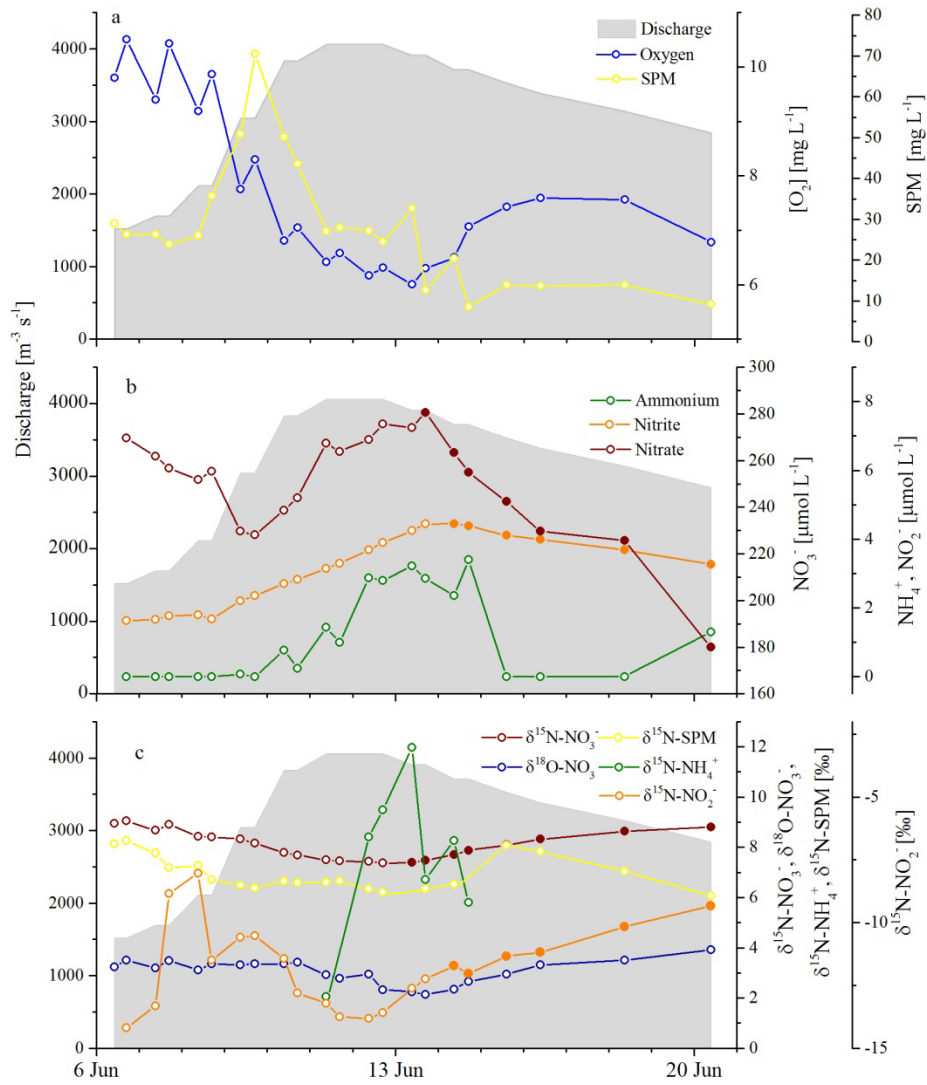



Figure 1a, b, c

Figure 2

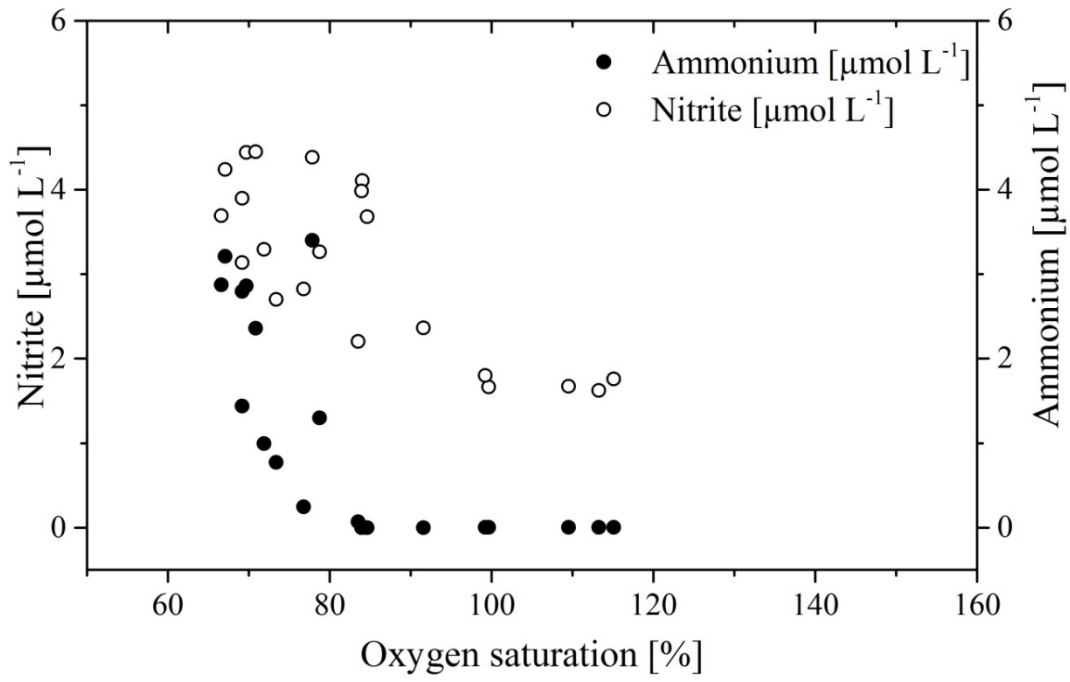
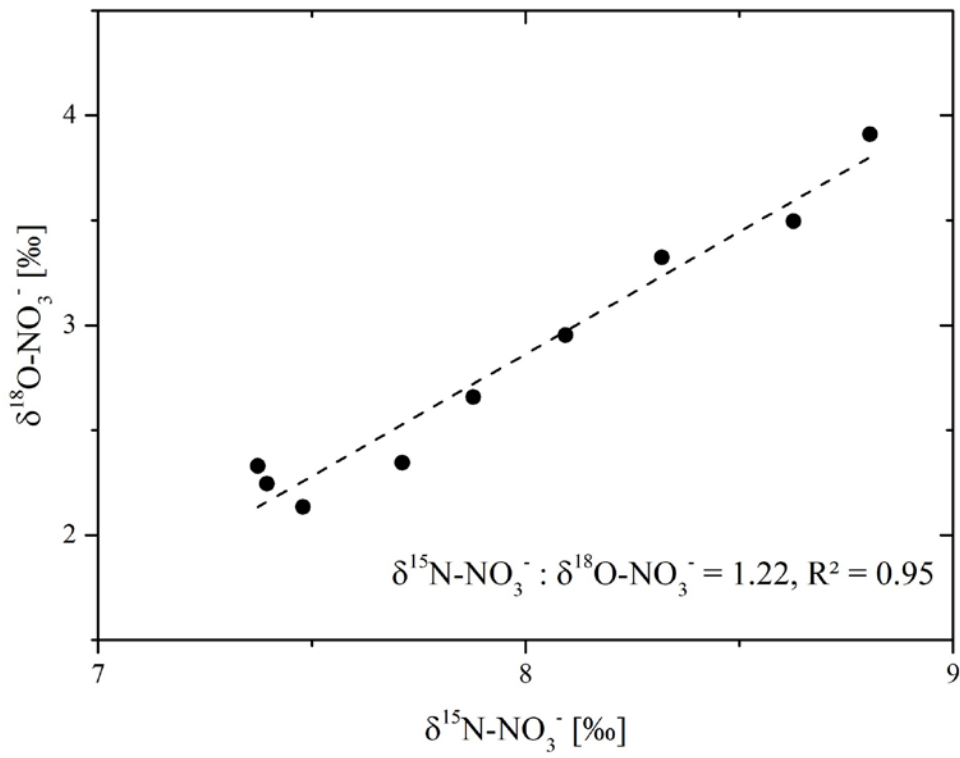
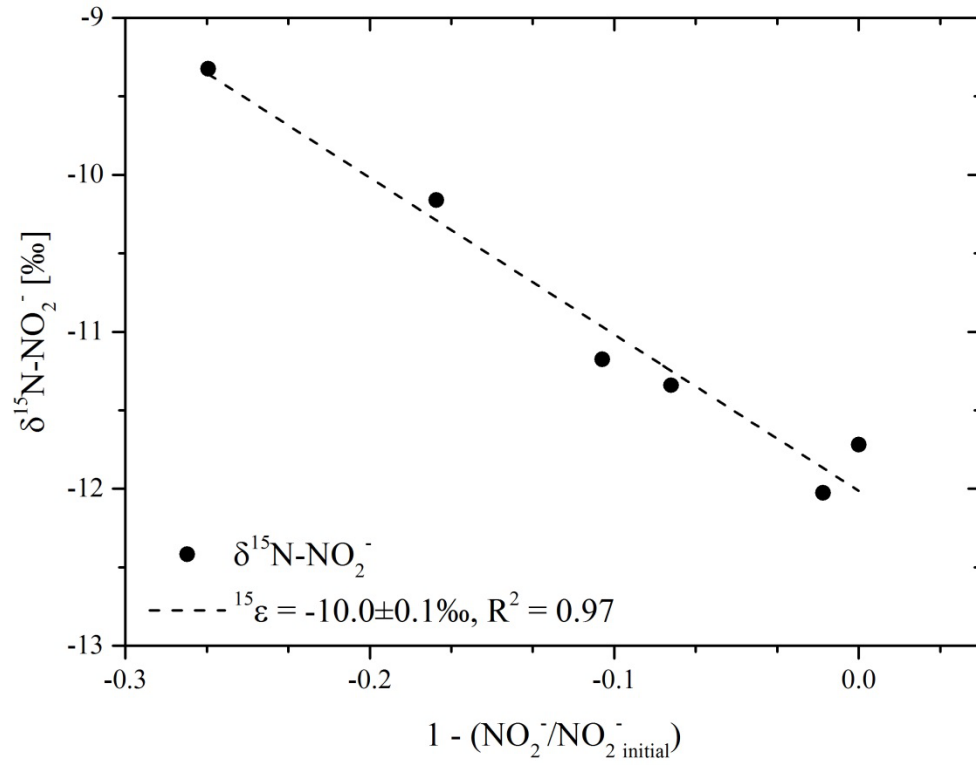
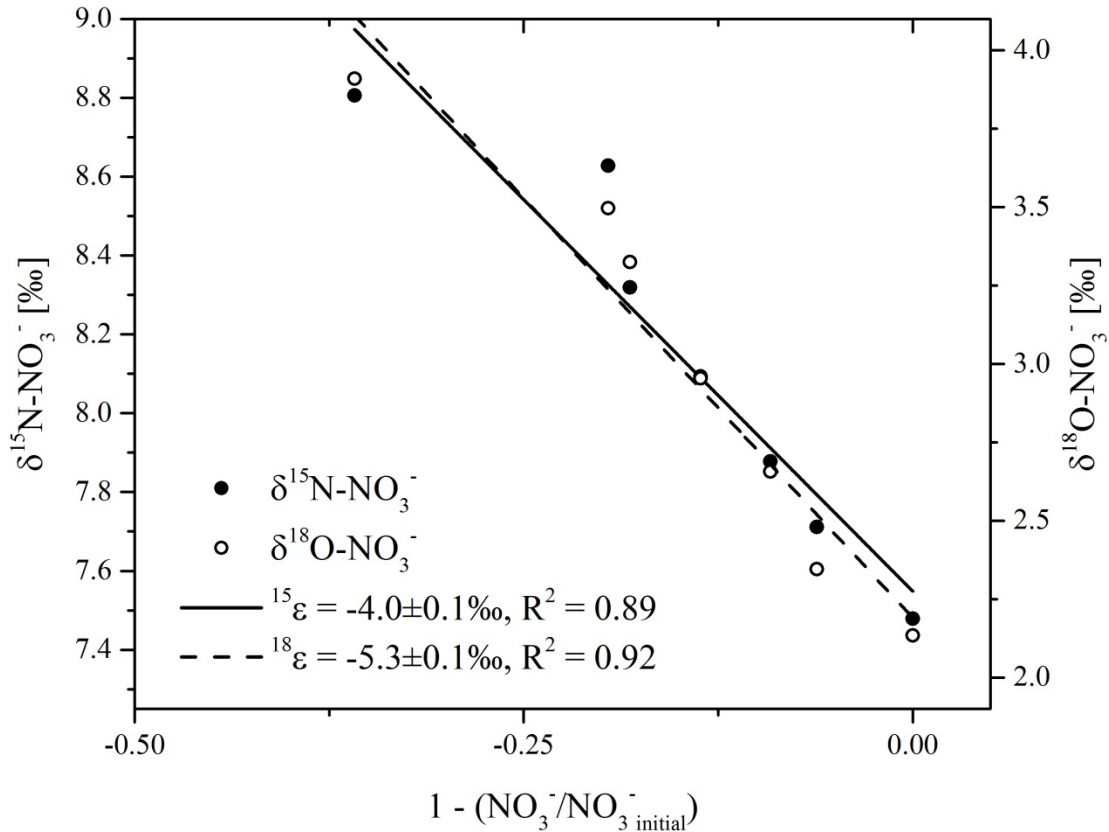
$$\delta^{15}N-NO_3^- : \delta^{18}O-NO_3^- = 1.22, R^2 = 0.95$$

5   **Figure 3**

Figure 4

Figure 5

9 Figure Captions

Figure 1a 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 $m^3 s^{-1}$.

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

Figure 1c Ammonium, nitrite, nitrate, and SPM isotope values in the course of the flood. Calculation of the fractionation factor is based on filled data points.

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

Figure 3 Ratio of $\delta^{15}N$-NO_3^- versus $\delta^{18}O$-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 1.22 with R^2 of 0.95.

Figure 4 Nitrite isotope values versus the remaining fraction of nitrite during the Elbe flood corresponding to the filled data points in figure 1b and 1c. 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 5 Dual nitrate isotope values versus the remaining fraction of nitrate corresponding to the filled data points in figure 1b and 1c. 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.