Biogeosciences Discuss., 7, 6051–6088, 2010
www.biogeosciences-discuss.net/7/6051/2010/
doi:10.5194/bgd-7-6051-2010

Biogeosciences
Discussions

Seasonal variations in nitrate isotope composition of three rivers draining into the North Sea

A. Deek[1,2], **K. Emeis**[1,2], **and U. Struck**[3]

[1]GKSS Research Center, Max-Planck-Str. 1, 21502 Geesthacht, Germany
[2]IfBM, University of Hamburg, Bundesstr. 55, 20146 Hamburg, Germany
[3]Museum für Naturkunde, Invalidenstraße 43, 10115 Berlin, Germany

Received: 13 July 2010 – Accepted: 2 August 2010 – Published: 13 August 2010

Correspondence to: A. Deek (astrid.johannsen@gkss.de)

Published by Copernicus Publications on behalf of the European Geosciences Union.

Abstract

Nitrate loading of coastal ecosystems by rivers that drain industrialised catchments continues to be a problem in the South Eastern North Sea, in spite of significant mitigation efforts over the last 2 decades. To identify nitrate sources, sinks, and turnover in three German rivers that discharge into the German Bight, we determined δ^{15}N-NO_3^- and δ^{18}O-NO_3^- in nitrate and δ^{15}N of particulate nitrogen for the period 2006–2009 (biweekly samples). The nitrate loads of Rhine, Weser and Ems varied seasonally in magnitude and δ^{15}N-NO_3^- (6.5–21‰), whereas the δ^{18}O-NO_3^- (–0.3–5.9‰) and δ^{15}N-PN (4–14‰) were less variable. Overall temporal patterns in nitrate mass fluxes and isotopic composition suggest that a combination of nitrate delivery from nitrification of soil ammonia in the catchment and assimilation of nitrate in the rivers control the isotopic composition of nitrate. Nitrification in soils as a source is indicated by low δ^{18}O-NO_3^- in winter, which traces the δ^{18}O of river water. Mean values of δ^{18}O-H_2O were between –9.4‰ and –7.3‰; combined in a ratio of 2:1 with the atmospheric oxygen δ^{18}O of 23.5‰ agrees with the found δ^{18}O of nitrate in the rivers.

Parallel variations of δ^{15}N-NO_3^- and δ^{18}O-NO_3^- within each individual river are caused by isotope effects associated with nitrate assimilation in the water column, the extent of which is determined by residence time in the river. Assimilation is furthermore to some extent mirrored both by the δ^{15}N of nitrate and particulate N. Although δ^{15}N-NO_3^- observed in Rhine, Weser and Ems are reflected in high average δ^{15}N-PN (between 6‰ and 9‰), both are uncorrelated in the time series due to lateral and temporal mixing of PN. That a larger enrichment was consistently seen in δ^{15}N-NO_3^- relative to δ^{18}O-NO_3^- is attributed to constant additional diffuse nitrate inputs deriving from soil nitrification in the catchment area. A statistically significant inverse correlation exists between increasing δ^{15}N-NO_3^- values and decreasing NO_3^- concentrations. This inverse relationship – observed in each seasonal cycle – together with a robust relationship between human dominated land use and δ^{15}N-NO_3^- values

demonstrates a strong influence of human activities and riverine nitrate consumption efficiency on the isotopic composition of riverine nitrate.

1 Introduction

On a global scale, human activity has resulted in a doubling of the reactive nitrogen pool since pre-industrial times (Galloway, 2003) with more localised hot spots at the land-sea interface (Gruber and Galloway, 2008). In the North Sea area, the reactive nitrogen discharge by rivers has increased tenfold since the 1960's over assumed pristine conditions (Behrendt and Opitz, 1999; BMU, 2001). Today, the German Bight in the South Eastern North Sea receives approximately 350 kt of reactive N (mainly in the form of nitrate) per year from rivers draining densely populated and industrialised areas of NW Europe (Johannsen et al., 2008). The increased reactive nitrogen loads and ensuing eutrophication continue to be an environmental pressure on many coastal seas (Howarth and Marino, 2006). The long average water residence time (Weston et al., 2004) permits severe eutrophication to unfold in the German Bight (Hickel et al., 1993). Most nutrients deriving from Germany enter the Southern North Sea via the Rhine and the Elbe rivers and also from medium-sized rivers, such as the Weser River and Ems River (UBA, 2009). Nitrate inputs from point sources have decreased from their peak in the 1980's in response to environmental legislation (Pätsch and Radach, 1997), but the annual average nitrate loads of these rivers are slow in responding to pollution reduction measures, and thus are not solely determined by inputs from manageable sources. There is a continuing need to understand the nature of this apparent buffering. At present, total nitrogen loads in German rivers are thought to derive mainly from diffuse sources, dominated by a reactive nitrogen surplus stemming from agriculture (fertiliser and manure) (Hamm, 1996; Behrendt et al., 2002; Gömann et al., 2005). Previous studies of nitrate isotope properties (Johannsen et al., 2008; Dähnke et al., 2008) and microbial communities (Herfort et al., 2009) in rivers draining into the North Sea suggested that sources and sinks of nitrate within rivers may have been overlooked. Additionally, hydrological characteristics of the rivers highly influence transformation processes of riverborne nitrate, and are controlled by stream size and discharge regime.

The natural abundance of δ^{15}N-NO$_3^-$ and δ^{18}O-NO$_3^-$ has been used to identify sources and to trace transformation processes of riverborne nitrate (Deutsch et al., 2006; Mayer et al., 2002; Sebilo et al., 2006; Wankel et al., 2009; Schlarbaum et al., 2010). Different sources of stream water nitrate (direct fertiliser input, atmospheric deposition, sewage and wastewater inputs, or leaching of nitrate produced by nitrification of ammonia from agricultural soils; Behrendt et al., 2002) are specific in their dual isotopic composition. In precipitation, the δ^{15}N-NO$_3^-$ varies depending on location and season between −4‰ and +9‰ and is similar to the δ^{15}N-NO$_3^-$ of nitrate produced by nitrification (Kendall, 1998). Added attributes of these two sources are distinct ranges of δ^{18}O-NO$_3$ in nitrate, with atmospheric NOx in precipitation having a δ^{18}O value between 20‰ and 60‰ (Kendall, 1998), and nitrate originating from nitrification falling in a range between −10‰ and +16‰ (Kendall, 1998; Mayer et al., 2001; Burns and Kendall, 2002). δ^{15}N-NO$_3^-$ of nitrate from organic fertilizers and from municipal waste water is characterized by high δ^{15}N values between 4‰ and 9‰, sometimes even higher (Grischek et al., 1998).

The isotopic mixture of nitrate sources is processed within rivers by different biological processes that in turn are associated with specific isotopic fractionation. Isotopic fractionation arises from biological processes, such as assimilation and denitrification, and each process is associated with characteristic fractionation factors ε (the isotopic enrichment/depletion of the product relative to the substrate in ‰) that determine the isotopic composition of residual nitrate and of any products. A useful indicator of nitrate assimilation in rivers is δ^{15}N in suspended particulate organic matter (δ^{15}N-PN). Although particulate nitrogen originates from either external sources or from production within the river, analysis of paired δ^{15}N-NO$_3^-$ and δ^{15}N-PN may shed light on the contribution of assimilation.

This study expands on a previous investigation (Johannsen et al., 2008) of river nitrate in five rivers that contribute substantially to eutrophication in the German Bight of the SW North Sea. That study was performed over the course of only one year and did not include particulate matter and δ^{18}O-H_2O. By expanding our time series, we wished to answer the following questions: (1) Can we characterize major nitrate sources by means of specific isotopic fingerprints? (2) How does river discharge and water residence time influence nitrate concentration and its isotopic composition? (3) Do we find evidence for biological processes within the rivers that influence nitrate loads and nitrate isotopic variability?

2 Materials and methods

2.1 Rivers studied

This study was conducted at monitoring sites of three rivers (Rhine, Weser, Ems) that differ substantially in annual loads. The load of Rhine is approximately 220 kt N/year, whereas the rivers Weser and Ems discharge 40 kt and 6 kt N per year, respectively. All three rivers are highly impacted by human activities, the populations in their watersheds are dense, and agricultural land-use is comparatively high (70–90% of urban and arable land-use). More details are given in Johannsen et al. (2008).

2.2 Sampling

Water was sampled by staff of official LAWA (Länderarbeitsgemeinschaft Wasser) monitoring sites located upstream of the estuaries at salinities around 0 (PSU) (Fig. 1). During the first sampling period (2006/2007), surface water samples were filtered through a PVDF filter (0.45 µm). After 2007, water samples were filtered through a pre-combusted (400°C, 6 h) GFF filter (0.45 µm). These were used to determine the concentration and δ^{15}N of particulate N. Filtrate was frozen in acid rinsed PE bottles for analyses of nitrate concentrations and isotopic composition. Aliquots of selected samples were carefully defrosted in February 2010 and sent to the laboratory of the Museum für Naturkunde in Berlin for the analysis of δ^{18}O-H_2O.

2.3 Laboratory analyses

2.3.1 Nitrate concentrations

Nitrate concentrations were measured using a continuous flow analyzer (Bran and Luebbe, Auto Analyzer 3) according a modified method of Armstrong et al. (1967). The method has a detection limit of 6 µmol NO_3^-/L within the expected range of 50–450 µmol NO_3^-/L for anthropogenically influenced rivers.

2.3.2 Isotopic analyses of nitrate

Isotopic analyses of nitrogen and oxygen of NO_3^- were carried out using the denitrifier method (Casciotti et al., 2002; Sigman et al., 2001) which is based on the isotopic analysis of nitrous oxide (N_2O) produced by denitrifying *Pseudomonas aureofaciens* (ATCC#13985) strains. Isotope ratios are expressed in the delta notation (δ^{15}N relative to atmospheric N_2 and δ^{18}O relative to Vienna Standard Mean Ocean Water, both defined as 0‰):

$$\delta\,\text{sample}\,[‰] = (R_{\text{sample}}/R_{\text{standard}} - 1)\cdot 1000\,, \qquad (1)$$

where R is ^{15}N/^{14}N or ^{18}O/^{16}O ratio of sample and standard, respectively. Analyses of δ^{15}N-NO_3^- were corrected using the international nitrate isotope standard IAEA-N3 (δ^{15}N=4.7‰). Analyses of δ^{18}O-NO_3^- were corrected for exchange, fractionation, and blank against IAEA-N3 with an assigned δ^{18}O-NO_3^- of 22.7‰. We applied this value (which is now accepted to be 25.6‰; Böhlke et al., 2003), because we compare results with those of a previous study of river nitrate (Johannsen et al., 2008) that was based on a δ^{18}O-NO_3^- for IAEA-N3 of 22.7‰ (Silva et al., 2000; Révész et al., 2002). Analytical precision (±1σ) based on replicate measurements of samples and standard was generally 0.2‰ for δ^{15}N-NO_3^- and 0.4‰ for δ^{18}O-NO_3^-. It should be noted that the denitrifier method measures the isotopic composition of nitrate+nitrite, but the contribution of nitrite to reactive nitrogen is negligible (<0.3%) in our sample set and has therefore been ignored.

2.3.3 Oxygen isotope measurements of water

Stable isotope ratios of oxygen (^{18}O/^{16}O) in H_2O in water samples were measured with a PICARRO L1102-i isotope analyzer. The L1102-i is based on the WS-CRDS (wavelength-scanned cavity ring down spectroscopy) technique (Gupta et al., 2009). Measurements were calibrated by the application of linear regression of the analyses of IAEA calibration material VSMOW, VSLAP and GISP. The stable isotope ratios of oxygen in H_2O (δ^{18}O-H_2O) are reported in‰ versus VSMOW. For each sample, 6 replicate injections were performed and arithmetic means and standard deviations (±1σ) were used. The reproducibility of replicate measurements is generally better than 0.1‰ for δ^{18}O-H_2O.

2.3.4 Particulate nitrogen

The δ^{15}N of particulate nitrogen on filters (δ^{15}N-PN, expressed in‰ versus air N_2) was determined in duplicate on subsamples of individual GFF-filters with an EA interfaced via a Con-Flo III to an Isotope Mass Spectrometer (Finnigan Delta Plus XP), calibrated using IAEA-N-1 (δ^{15}N=0.4‰) and IAEA-N-2 (δ^{15}N=20.3‰). The reproducibility for δ^{15}N-PN was 0.3‰, but the variability in δ^{15}N-PN for sub-samples of the same filter was higher than 0.3‰ (see error bars in Fig. 2), and was caused by heterogeneities of the material on the filters.

3 Results

The time series of NO_3^- concentrations, δ^{15}N-NO_3^-, δ^{18}O-NO_3^-, δ^{15}N-PN, and δ^{18}O-H_2O of individual rivers are displayed in Fig. 2a–c. Highest nitrate concentrations were measured in January and February of 2007 in all three rivers (Ems>600 µmol/L, Weser 380 µmol/L, Rhine 260 µmol/L), which all displayed a similar seasonal pattern with highest NO_3^- concentrations in the winter seasons (October–March) and lowest in the summer seasons (April–September) over the two years. Mean annual N-NO_3^- discharges and isotopic compositions are listed in Table 1. Rhine River discharged 228 kt N-NO_3^- in 2006/2007 and 195 kt N-NO_3^- in 2007/2008. Weser delivered 28 kt and 58 kt N-NO_3^- to the estuary, respectively, and Ems only 6 and 8 kt N-NO_3^- (Table 1). The hydrological annual course differed and influenced the loads: whereas water discharge of Ems and Weser peaked in spring (between February and April), Rhine has peak discharges during summer months (Fig. 3). N-NO_3^- loads in all rivers co-vary with discharge (Fig. 3), illustrated by the significant positive correlation (>0.9 in all three rivers) of N-NO_3^- loads and discharge (Table 2).

Mean annual (years 2006–2009) δ^{15}N-NO_3^- ranged from 8.1‰ to 8.6‰ in Rhine, from 8.2‰ to 9.2‰ in Weser, and from 11.6‰ to 12.8‰ in Ems with a maximum in the sampling interval 2007/2008. The δ^{15}N-NO_3^- are generally lower in winter than in summer months, and vary inversely with NO_3^- concentrations, reflected in a negative correlation between NO_3^- concentrations and δ^{15}N-NO_3^- (<-0.7; Table 2). Maximum δ^{15}N-NO_3^- was 21‰ in Ems, 13‰ in Weser, and 10.9‰ in Rhine, all in July 2006, and summer maxima were consistently seen in all summers.

The difference in isotopic composition between mean summer and winter varied among the three rivers. Mean load-weighted δ^{15}N-NO_3^- in Rhine and Weser for summer are 0.6‰ – 1.7‰ higher than mean-load weighted winter δ^{15}N-NO_3^-, whereas mean δ^{15}N-NO_3^- in Ems are up to 4.2‰ higher in summer than in winter. A similar (but subdued) seasonality is seen in δ^{18}O-NO_3^- that is usually also higher in summer than in winter. Again, Ems had highest δ^{18}O-NO_3^- of 7.6‰ in July 2006 (Fig. 2a–c), followed by Weser (4.9‰ in May 2007) and Rhine (2.9‰ in August 2007). The pattern of δ^{18}O-NO_3^- in the Rhine River differs from that observed in the Weser and Ems, and both δ^{18}O-NO_3^- and δ^{15}N-NO_3^- had smaller seasonal amplitude through the course of the year in this river.

The highest δ^{15}N-PN in the Ems was 14.5‰, compared to 8‰ in the Weser and Rhine (Fig. 2a–c). δ^{15}N-PN increased systematically towards summer in Ems, whereas a consistent seasonal pattern is not obvious in the Weser and Rhine River. δ^{18}O-H_2O ranged between −10.3‰ and −8.5‰ in Rhine, −8.5‰ and −7.6‰ in Weser, and −8.0‰ and −6.5‰ in Ems (Fig. 2a–c).

4 Discussion

All three rivers under study are impacted by human activity, but the degree of agricultural land use and specializations differ in the catchment areas. The Ems drainage basin is dominated by intensive animal husbandry, because it is more competitive on the prevailing sandy soils, and the livestock production in the Ems catchment is high compared to the German average. Similarly, Weser drains grassland with intensive cattle farming and milk production (Osterkamp et al., 2001; Statistisches Bundesamt, 1990). The Rhine drains forested and urban areas with higher population density (Gömann et al., 2005; Wendland et al., 1993) than the two other rivers; land use is dominated by grassland, cattle production and intensive cash cropping. In a previous study (Johannsen et al., 2008), we found a robust positive correlation (R^2=0.7) between the percentage of urban and agricultural land use in catchment areas and δ^{15}N-NO_3^- values for a range of several rivers. Furthermore, we observed a significant negative correlation between δ^{15}N-NO_3^- values and the nitrate load (R^2<−0.7) for each seasonal cycle. This suggested that mean annual δ^{15}N-NO_3^- over river borne nitrate should generally increase with decreasing annual nitrate loads, as a result of a relatively higher proportion of in-river nitrate consumption. We felt that longer term data sets would substantiate this assumption, and that additional information on the in-river consumption of nitrate should be gleaned from analysis of particulate N. In other studies, significant positive correlations have been observed between δ^{15}N-PN and human population density catchment areas (e.g., Nishikawa et al., 2009), which is directly related to the percentage of residential and agricultural area. We observed the highest average δ^{15}N-PN of 9.2‰ in the river Ems, which is most impacted in terms of agricultural and urban land use. Average δ^{15}N-PN of 6.1‰ in Weser and 6.6‰ in Rhine River are also relatively high and in the same range reported for δ^{15}N-PN in non-pristine rivers (Middelburg and Nieuwenhuize, 1998; Sigleo and Macko, 2002; Voss et al., 2006). δ^{15}N-PN thus appears to be a good indicator for the degree of human impact on watersheds, and together with mean winter δ^{15}N-NO_3^- characterise dominant anthropogenic sources of reactive nitrogen in rivers.

4.1 Nitrate sources

4.1.1 Nitrate from organic and synthetic fertilizers

The high mean annual δ^{15}N-NO_3^- (between 8.1‰ and 12.8‰) in Ems, Rhine and Weser mark them as anthropogenically influenced rivers (Mayer et al., 2002; Johannsen et al., 2008; Deutsch et al., 2006; Voss et al., 2006). In contrast, δ^{18}O-NO_3^- in the range between 0.4‰ and 3.3‰ are low compared to potential direct sources, but compare well with δ^{18}O-NO_3^- determined in non-pristine rivers in Northern Europe (Dähnke et al., 2008; Deutsch et al., 2006; Bristow, 2009).

As stated above, agriculture prevails in all three watersheds, and agricultural soils are known to host large amounts of reactive nitrogen. Nitrate in rivers draining these soils may originate from different sources and processes, such as direct elution of atmospheric deposition, inorganic fertiliser and manure, or recycled nitrate from mineralization of soil organic nitrogen or nitrification of ammonia. All these sources are known to contribute to nitrate stocks in surface and groundwater, and each has more or less specific isotopic compositions (Kendall, 1998). Synthetically produced nitrate in fertilizer has a δ^{18}O-NO_3^- between 18‰ and 22‰, which is close to the atmospheric δ^{18}O value (Kroopnick and Craig, 1972; Amberger and Schmidt, 1987; Wassenaar, 1995). Typical mineral fertiliser applied in Germany is between 19.4‰ and 25.7‰ (Deutsch et al., 2005). Ranges of δ^{15}N-NO_3^- are typically between −5‰ and 8‰ (Kendall, 1998; Macko and Ostrom, 1994); δ^{15}N-NO_3^- between 0.8‰ and 4.4‰ were measured for mineral fertiliser in Germany (Deutsch et al., 2005). The δ^{15}N value of fertilized soils is usually higher (between 0‰ and 14‰; Fogg et al., 1998; Kendall, 1998) than that of fertilizers applied (Kendall, 1998; Macko and Ostrom, 1994), because ^{15}N is enriched by an array of fractionation processes acting on the original isotopic mixture during N-assimilation, harvesting of crops, and denitrification in soils. The relatively low δ^{18}O-NO_3^- in Rhine, Weser and Ems compared to synthetic fertilizers (between 18‰ and 22‰) suggest that direct leaching of mineral fertilizer from soils into rivers is only of minor relevance. This reflects increasing popularity of good (and cheaper) farming practise of targeted synthetic fertilisation immediately near the individual crop plants.

But it is known that agricultural soils in Germany are over-saturated with reactive N from decades of poor fertilising practise (UBA, 2009), and the legacy of excessive fertilisation has undergone massive fractionation when finally reaching stream waters, resulting in high δ^{15}N of this contribution. In contrast to synthetic fertilizers, organic fertilizers are by-products of animal breeding and discharged directly by free range husbandry or are generously applied as organic fertilizer to field crops to remove waste (Renger, 2002). Nitrate originating from this practise (after nitrification of ammonia) has high δ^{15}N values between 8‰ to 25‰ (Kendall, 1998; Macko and Ostrom, 1994) caused by fractionation during ammonia volatilization, which enriches the residual ammonium pool in ^{15}N (Frank et al., 2004). According to Blume (2004), up to 80% of the ammonia in organic fertilizers is volatilized during the first days after application, if not worked into the soil properly. Similar to the residual of mineral fertilizer, subsequent nitrification of the residual ammonia produces NO_3^- enriched in ^{15}N. Together with other processes, such as partial denitrification, selective assimilation by and harvesting of crops, this leads to a progressive enrichment of δ^{15}N-NO_3^- and δ^{18}O-NO_3^- that is then collected by rivers draining intensively farmed watersheds (Ahad et al., 2006; Ostrom et al., 1997; Horrigan et al., 1990).

4.1.2 Nitrate from nitrification

A key process in nitrate generation in soils and a significant source of riverine nitrate pools is nitrification of reduced N species (Hales, 2007). Nitrification is the bacterial oxidation of ammonia to nitrate via nitrite, mediated in two steps by aerobic chemolithoautotrophic bacteria and archaea. The first step, oxidation of ammonia into nitrite, is performed by two groups of organisms, ammonia-oxidizing bacteria (AOB) and ammonia-oxidizing archaea (AOA) dominating both marine and terrestrial environments (Francis et al., 2007). The second step, oxidation of nitrite into nitrate, is known to be performed by four genera of bacteria: *Nitrococcus*, *Nitrospina*, *Nitrospira* and *Nitrobacter* (Casciotti, 2009). *Nitrobacter* was generally considered the dominant or even the sole nitrifier in soils, but evidence has been lately given that *Nitrospira* is as well a common soil bacterium (Bartosch et al., 2002; Attard et al., 2010). Nitrate deriving from nitrification has a δ^{15}N that is a legacy of the ammonia substrate (with reported fractionation factors ε_{AMO} for the ammonium oxidation process that range from 14 to 38‰; Casciotti, 2009 and the references therein). The δ^{18}O of this nitrate is apparently determined by the combined isotope ratios of ambient water and atmospheric (soil) molecular oxygen. Studies in fresh water environments suggest that two of the oxygen atoms originate from ambient water and another one from dissolved oxygen (Hollocher, 1984; Anderson and Hooper, 1983; Kumar et al., 1983), resulting in a δ^{18}O-NO_3^- equal to:

$$\delta^{18}\text{O-NO}_3^- = 2/3\delta^{18}\text{O-H}_2\text{O} + 1/3\delta^{18}\text{O-O}_2\,. \tag{2}$$

Atmospheric oxygen has a known δ^{18}O value of 23.5‰ (Kroopnick and Craig, 1972). δ^{18}O-H_2O ranged between −10.3‰ and −8.5‰ in Rhine, −8.5‰ and −7.6‰ in Weser and −8.0‰ and −6.5‰ in Ems (Fig. 2a–c). Assuming that the δ^{18}O-H_2O of river water is close to the value of ambient soil water used for nitrification, and that δ^{18}O of soil O_2 is approximately equivalent to atmospheric O_2 yields expected δ^{18}O-NO_3^- of 1.6‰, 2.6‰ and 3‰ for nitrate derived from nitrification in Rhine, Weser and Ems, respectively. We note that these are all close to the mean annual δ^{18}O-NO_3^- values measured in the rivers (Table 1). River nitrate in our data set has a slightly lower but similar δ^{18}O-NO_3^- compared with nitrate in tile drain water sampled in winter months from two fields in Northern Germany (2.9–5.1‰) reported in Deutsch et al. (2005) and obtained with a different method. The correspondence of theoretical and found δ^{18}O-NO_3^- in our data set is somewhat surprising, because Eq. (1) makes the assumptions that no isotopic fractionation operates during incorporation of water oxygen or dissolved O_2 into nitrate formed, that the original atmospheric isotopic composition of O_2 used for nitrification is maintained in soils, and that no significant oxygen exchange occurs between H_2O and intermediate compounds involved in NO_3^- formation (Spoelstra et al., 2007; Pardo et al., 2004; Aravena et al., 1993; Kendall et al., 1995; Kendall, 1998; Wassenaar, 1995; Mayer et al., 2001; Kool et al., 2007). Furthermore, recent studies (Wankel et al., 2006; Sigman et al., 2005, 2009, Buchwald and Casciotti, 2010) in marine environments and culture studies have raised uncertainties about the exact source of δ^{18}O in nitrate produced by nitrification and have questioned the premises assumed in Eq. (1). Casciotti et al. (2002) found that the δ^{18}O of recycled nitrate in marine environments is apparently closer to the δ^{18}O-H_2O of ambient water and proposed that δ^{18}O-NO_3^- is set by:

$$\delta^{18}\text{O-NO}_3^- = 5/6\delta^{18}\text{O-H}_2\text{O} + 1/6\delta^{18}\text{O-O}_2\,. \tag{3}$$

We note that our data fit the relationships in Eq. (1) for soil nitrification (Hollocher, 1984; Anderson and Hooper, 1983; Kumar et al., 1983), but that average δ^{18}O-NO_3^- in each river is slightly below the theoretical value. The range of δ^{18}O-NO_3^- found suggests that chemolithoautotrophic nitrification is the dominant source, a process that is used to gain energy and assimilates CO_2. Mayer et al. (2001) and Spoelstra et al. (2007)

suggested that the contribution to δ^{18}O-NO_3^- soil water and dissolved oxygen depends on ammonia concentration and the type of nitrifying organisms present in soils. Heterotrophic nitrifiers (no energy yield) apparently appear in soils with low ammonium concentrations and perform at characteristically low nitrification rates, possibly in a series of oxidation steps of organic-bound amino-groups. In this reaction sequence, two oxygen atoms may originate from the organic nitrogen compound, and only one from water (Mayer et al., 2001). The nitrate thus produced appears to be relatively higher in δ^{18}O-NO_3^- than that produced from the second pathway, nitrification by chemolithoautotrophic consortia (Spoelstra et al., 2007) dominating environments with relatively high nitrification rates (Focht and Verstraete, 1977). As mentioned before, AOB and AOA are the prevailing chemolithoautotrophic ammonia oxidizers. Nitrification by AOA is operative over a wide range of environmental conditions (pH, temperature) in soils, where archaeotal cells far outweigh bacterial cells (Leininger et al., 2006), being potentially important actors within the nitrogen cycle in several ecosystems (Erguder et al., 2009). For the archaea, the isotopic systematics of nitrification is unknown, because they are as yet not cultivated for this experimental purpose. Considering the bacterial ammonia oxidizers, at least the genera *Nitrobacter* is assumed to produce nitrate with a δ^{18}O-NO_3^- according to Eq. (1) (Mayer et al., 2001; Spoelstra et al., 2007).

Under favourable conditions (e.g., availability of ammonia), nitrification in the water column of the river is likely to be an additional source of stream nitrate. During winter months, when ammonium, nitrate and nitrite concentrations were measurable, Herfort et al. (2009) detected genes of *Crenarchaeota* Group 1.1a, the major ammonia oxidizer in marine environments, in Rhine samples and reported that they accounted for 0.3% of the total microbial community in surface waters. Crenarchaeotal nitrification potential in the Rhine River in summer was lower, attributed to higher competition from bacteria and phytoplankton. To our knowledge, nothing is known about the oxygen isotopic composition of nitrate deriving exclusively from archeal nitrification, but it is safe to assume that it constitutes an internal nitrate source for the studied rivers during winter, albeit of unknown magnitude and isotopic fingerprint. In any event, based on the ammonia availability in agricultural soils and in river water, the contribution to nitrate in rivers from internal nitrification should be smaller than the contribution from soil nitrification, although Dähnke et al. (2008) reported significant (>50%) additions to estuarine nitrate pools of the Elbe river from nitrification in the estuarine turbidity maximum.

4.1.3 Atmospheric deposition

The isotopic signature of nitrate deriving from atmospheric deposition is distinct from any other nitrate source. The range of δ^{18}O-NO_3^- in precipitation in Europe is between 55‰ and 75‰ (Durka et al., 1994), whereas the δ^{15}N-NO_3^- of precipitation varies widely due to various sources of oxidized nitrogen and numerous fractionation processes preceding deposition (Heaton, 1986; Freyer, 1991). The δ^{18}O-NO_3^- in river nitrate here are much lower than in atmospheric NO_3^-, so that precipitation cannot be a significant contribution to the nitrate pools of rivers Rhine, Weser and Ems. This agrees with a study by Burns and Kendall (2002), who investigated different nitrate sources in two American watersheds.

4.2 Influence of river discharge

Besides their discharge (Rhine has a seven times higher discharge yield than the Weser and a seventy higher discharge than the Ems; 2500 m^3/s, 370 m^3/s and 36 m^3/s, respectively), the rivers differ in their respective discharge regimes. Whereas the discharge regimes of the rivers Weser and Ems is pluvial, the discharge regime of the Rhine River is glacial-nival upstream (the Alpine Rhine) and pluvial in the lower Rhine region. The Alpine Rhine discharge is controlled by the alpine snow- and glacier melt, which depends on temperature, having a minimum in winter and a maximum in summer. The influence of the pluvial regime increases downstream and the discharge becomes compensated by winter precipitation, but high discharges are maintained during summer (Hirschfeld, 2003; Kempe and Krahe, 2005). This mix of water sources buffers the Rhine hydrograph with regard to discharge minima, so that discharge varies by a factor of three whereas the discharge in Weser and Ems is six times higher during winter than during summer months (Fig. 3). The variability in δ^{18}O-H_2O in Rhine water is a consequence of these different hydrological regimes. As the δ^{18}O-H_2O of precipitation in central Europe decreases southwards (Buhl et al., 1991), the δ^{18}O-H_2O of surface waters in the Rhine catchment is spatially and temporally quite variable. Variations in δ^{18}O-H_2O values reflect the alpine influence in isotopically lighter δ^{18}O-H_2O compared to the precipitation in the lower Rhine area. According to Buhl et al. (1991) and Hirschfeld (2003), discharge of the Alpine Rhine has a δ^{18}O-H_2O between -12‰ an -13.5‰, whereas δ^{18}O-H_2O of lowland precipitation is around -8‰ (Yurtsever, 1975; Bowen and Revenaugh, 2005). We measured lowest δ^{18}O-H_2O (<-10‰) in Rhine at Bimmen-Lobith between April and August when snow melt takes place (Fig. 2a). During winter season (October–March), δ^{18}O-H_2O values are higher (>-9‰) due to higher influence of the pluvial regime. Rivers Weser and Ems are solely rain-fed (de Jonge, 1995), and such seasonal variations in δ^{18}O-H_2O were not observed (Fig. 2b and c).

Nitrate concentrations in rivers depend substantially on the time course of precipitation and its influence on discharge amounts. In seasons of high precipitation and during flood events in winter, when biological activity is low, high amounts of rN are leached and transported into rivers (Hamm, 1996). This is reflected in significant positive correlations ($R^2>0.9$) between NO_3^- loads and water discharge in all 3 rivers (Table 2). It also underscores that diffuse sources dominate the nitrate pools: If more or less constant point sources (waste water inlets, tributaries) were the dominant source, the NO_3^- loads would decrease at times of higher discharges due to dilution (Hamm, 1996). Assuming an ideal dilution, the nitrate concentration of a point source would behave according to:

$$c[NO_3^-]=c_0+A/Q\,, \tag{4}$$

where c_0 is the constant background concentration of NO_3^-, A is the load of a point source and Q the discharge (Hebbel and Steuer, 2006). In Fig. 4 we display a theoretical dilution curve against mean nitrate loads of the river Rhine, Weser and Ems. The actual nitrate discharge clearly differs from the theoretical dilution curve and indicates massive diffuse nitrate inputs instead. Moreover, nitrate concentrations increase with increasing discharge: Low nitrate concentrations in summer months (Fig. 2a–c) increase towards winter accompanied by higher discharges fed by precipitation events by that season. The correlation between NO_3^- concentrations and riverine discharge is best in the river Ems, where precipitation in the catchment is immediately funnelled into the river through permeable sandy soils. The high infiltration rates of the sandy soils favour nitrogen leaching to the groundwater and their discharge into the river (Liersch and Volk, 2009).

But the nitrate concentrations and isotopic mixtures are not solely determined by diffuse groundwater seepage. Seasonally varying biological productivity and nitrate assimilation in the rivers is clearly a major process. Because assimilation is low in winter (as indicated by the indirect, but significant, negative correlation between NO_3^- concentration and water temperature; Table 2), the more intense soil source and low consumption rates result in higher nitrate loads in winter.

4.3 Biological processes in the rivers

Anthropogenic sources of rN external to the rivers determine the base level isotopic composition of NO_3^- in winter, but biological activity alters the initial δ^{15}N-NO_3^- and δ^{18}O-NO_3^- in the course of the year. Two processes that remove nitrate are associated with preferential removal of $\delta^{14}NO_3^-$: denitrification and assimilation of nitrate (Mariotti et al., 1981; Kendall, 1998). Denitrification, a microbial process which is obligate anaerobic supposedly begins below oxygen levels of around 2 mg/L (Painter, 1970) and is unlikely to occur to any significant extent in the well oxygenated water columns of Rhine, Weser or Ems. Denitrification in sediments, according to present knowledge, causes only small fractionation because the rate limiting step is the non-discriminating process of nitrate diffusion (Sebilo et al., 2003; Reinhardt et al., 2006; Lehmann et al., 2007). Although sedimentary denitrification is likely to occur in the river beds and in riparian mud flats, where organic matter substrate is available in high amounts, this nitrate sink would go unnoticed in the isotopic mixture of river nitrate. Assimilation of nitrate, on the other hand, is likely to occur when temperature and light conditions are favourable. During assimilation, microorganisms preferentially incorporate δ^{14}N-NO_3^-. As for N isotopes, δ^{16}O-NO_3^- is favoured relative to δ^{18}O-NO_3^- causing an enrichment of the heavy isotopes δ^{15}N and δ^{18}O in the residual NO_3^- pool. Seasonal variation in both δ^{15}N and δ^{18}O combined with decreasing NO_3^-concentrations in Rhine, Weser and Ems have similar trends with maxima in summer and minima in winter for all sampling periods (Fig. 2a–c), suggesting that NO_3^- is consumed during summer months when biological activity increases. Isotope effects $^{15}\varepsilon$ and $^{18}\varepsilon$ during nitrate assimilation are similar, because the slope of δ^{18}O to δ^{15}N in residual nitrate has been observed to follow a 1:1 line in culture studies (Granger et al., 2004). A plot of δ^{18}O-NO_3^- against δ^{15}N-NO_3^- (Fig. 5) for individual rivers in our study yielded slopes of 0.37, 0.53 and 0.54 for the rivers Rhine, Weser and Ems, respectively. The deviation means that the increases in δ^{18}O and δ^{15}N in nitrate are not solely due to assimilation, and that other processes and nitrate sources interfere with a single phytoplankton sink for nitrate. This is not surprising, because the enrichment slope of 1:1 during assimilation is typically observed in closed systems, where nitrate is consumed without external replenishment. The dominant external input from soil water nitrate seepage obviously represents an open system, where drainage- and ground water supply continuously and diffusely adds nitrate with a more or less invariant isotopic composition over the entire year. In this case, a less steep slope due to incidentally added nitrate is to be expected. Plotting δ^{18}O-NO_3^- versus δ^{15}N-NO_3^- and evaluation of differences in slopes during winter and summer helps to identify a fractionation effect (Fig. 5). Winter δ^{18}O-NO_3^- and δ^{15}N-NO_3^- increase with increasing temperature towards summer in all three rivers (seen also in significant positive correlations in Table 2), but differences emerge for Rhine compared to the patterns observed in the Weser and Ems. The complete lack of correlation between δ^{18}O-NO_3^- and δ^{15}N-NO_3^- in the Rhine and no systematic differences between winter and summer suggest that assimilation is not causing strong fractionation. Rhine River has by far highest discharges of the three (Fig. 3) and we assume that the prevailing high current velocity and low water residence time impede phytoplankton growth (Peterson et al., 2001; Wollheim et al., 2001). Conversely, a decrease in current velocity enhances the removal rate of nitrate from the water column via assimilation and results in δ^{15}N enrichment in the residual nitrate (Trudeau and Rasmussen, 2003). Because of their high surface to volume ratios, smaller streams are more efficient in processing nutrients relative to transport (Alexander et al., 2000). In Ems, the range of NO_3^- concentrations and its isotopic composition is wider than in the rivers Weser and Rhine. Its initial NO_3^- concentration, assumed to be the maximum concentration measured in winter and normalized to 1, declines to 0.1 in the river Ems, whereas the initial NO_3^- concentration declines only to 0.5 in Rhine. Comparing the three rivers, we find a strong relationship between 1) stream size, 2) consumption efficiency and 3) fluctuation of δ^{15}N and δ^{18}O: With higher discharge amounts, the consumption efficiency is lower and summer increase in δ^{15}N and δ^{18}O of nitrate smaller.

Consumption of nitrate produces particulate nitrogen that is locked to the δ^{15}N of nitrate by the fractionation factor ε associated with assimilation. In previous studies, the isotopic fractionation factor (ε) associated with nitrate assimilation was in the range of 4 to 9‰ for field observations (Altabet et al., 1991; Horrigan et al., 1990; Sigman et al., 1999; Wu et al., 1997) and is often assumed as 5‰. Under nitrate-replete conditions, the δ^{15}N-PN produced at any time during assimilation should be δ^{15}N-NO_3^- -5‰ (Mariotti et al., 1981). In theory, we should thus expect a negative correlation of δ^{15}N-PN with NO_3^- concentrations, and is observed in the river Ems ($R^2=-0.45$; $p<0.05$), but is neither observed in the Rhine River ($R^2=-0.18$), nor in the Weser ($R^2=0.098$). The increase of δ^{15}N-PN in the river Ems in April corresponds to the onset of primary productivity and to the decrease of nitrate concentration in river water. Higher rates of N recycling, microbial decomposition of PN and utilization of DIN favour the enrichment of δ^{15}N-PN and higher variability of δ^{15}N-PN in the smaller river Ems. The associated apparent N-isotope effect can be estimated from the difference between the source, δ^{15}N-$NO_3^-$and the product, δ^{15}N-PN ($\Delta\delta^{15}$N). Using the annual δ^{15}N-$NO_3^-$and δ^{15}N-PN values for the three rivers, we estimate an apparent fractionation factor of around 2‰ for the Rhine River, between 2‰ and 3‰ for the Weser and around 3‰ for the Ems. Why only a subdued seasonal amplitude of δ^{15}N-PN has been observed in Rhine River and Weser remains speculation. A possible explanation might be a varying species composition among the rivers and between seasons. Several culture studies (e.g., Montoya and McCarthy, 1995) have shown that fractionation factors are different depending on organisms, e.g. nitrate assimilation by diatoms were associated with higher fractionation than nitrate assimilation by green algae. On the other hand, the Rhine and Weser apparent isotope effects may be lower than expected because external PN-loading masks the effects of autochthonous assimilation.

5 Conclusions

The results presented in this paper demonstrate that the determination of δ^{15}N-NO_3^- and δ^{18}O-NO_3^- values in combination with δ^{18}O-H_2O and δ^{15}N-PN helps to understand the nitrogen cycle in riverine systems. We identified diffuse nitrate inputs from organic fertilizers as major contributors to the riverine nitrogen pools of the rivers Rhine, Ems and Weser. Comparatively high annual mean δ^{15}N-NO_3^- values between 8‰ and 13‰ are indicative for a significant animal waste and manure contribution which is in accordance with the high urban and agricultural land use in the respective catchment areas. A second major diffuse nitrate source is assumed to be nitrate from nitrification in soils. Evidence is given by the combined analysis of δ^{18}O-NO_3^- and δ^{18}O-H_2O. Comparatively low δ^{18}O-NO_3^- between 0.4‰ and 3.3‰ measured in the Rhine, Weser and Ems bear the δ^{18}O signature of ambient water. Measured δ^{18}O-H_2O of riverine water was between −9.4‰ and −7.3‰ which accounts for 2/3 to the δ^{18}O-signal of NO_3^- from nitrification in soils. Besides external nitrate inputs, a potential internal nitrate source during winter when ammonia is abundant was vaguely attributed to water column nitrification. Furthermore, we revealed that assimilative processes are responsible for fractionation of δ^{15}N-NO_3^- and δ^{18}O-NO_3^- values in summer months when biological activity takes place. In all rivers, a decrease in NO_3^- concentrations was inversely correlated with an increase in δ^{15}N-NO_3^- values as consequence of an enrichment of heavy isotopes in the residual NO_3^- pool during assimilation. Variations of isotopic values and fractionation range were attributed to the hydrological characteristics of the respective rivers. The Rhine River is an exception in terms of discharge yield; the nitrate consumption efficiency is lower than in the rivers Weser and Ems due to the fact that a higher current velocity decreases the rate of biogeochemical transformation processes. Strong interdependencies between stream sizes, consumption efficiency and fractionation range were found comparing the three rivers of different sizes. Highest NO_3^- values and highest nitrate consumption rates associated with a high variation of δ^{15}N-NO_3^- values and δ^{18}O-NO_3^- values were observed in the smaller river Ems. We conclude from this that sources of rN and the nitrate consumption efficiency interrelated with the current velocity determines mostly the isotopic composition of riverine nitrate.

Acknowledgements. We thank the GKSS Research Center, Institute for Coastal Research and DFG (Em 37-29) for support. The authors would like to acknowledge N. Lahajnar and F. Langenberg (IfBM, University of Hamburg) providing δ^{15}N-PN data. In particular, we thank T. Gerke (IMBL Bimmen-Lobith), H. Petry (StUA Münster), and B. Freiheit (Senator für Umwelt, Bau, Verkehr und Europa Bremen) for coordination and help with sampling at the river sites. E. Spieck is acknowledged for corrections and helpful comments on an earlier draft.

References

Ahad, J. M. E., Ganeshram, R. S., Spencer, R. G. M., Uher, G., Upstill-Goddard, R. C., and Cowie, G. L.: Evaluating the sources and fate of anthropogenic dissolved inorganic nitrogen (DIN) in two contrasting North Sea estuaries, Sci. Total Environ., 372, 317–333, 2006.

Alexander, R. B., Smith, R. A., and Schwarz, G. E.: Effect of stream channel size on the delivery of nitrogen to the Gulf of Mexico, Nature, 403, 758–761, 2000.

Altabet, M. A., Deuser, W. G., Honjo, S., and Stienen, C.: Seasonal and depthrelated changes in the source of sinking particles in the North Atlantic, Nature, 354, 136–139, 1991.

Amberger, A. and Schmidt, H. L.: Natürliche Isotopengehalte von Nitrat als Indikatoren für dessen Herkunft, Geochim. Cosmochim. Ac., 51, 2699–2705, 1987.

Anderson, K. K., Philson, S. B., and Hooper, A. B.: ^{18}O isotope shift in 15N-NMR analysis of biological N-oxidations: H_2O-NO_2-exchange in the ammonia-oxidizing bacterium Nitrosomonas, Proc. Natl. Acad. Sci. USA, 79, 5871–5875, 1982.

Anderson, K. K. and Hooper, A. B.: O_2 and H_2O are each the source of one O in NO_2 produced from NH_3 by nitrosomonas, 15N-NMR evidence, FEBS Lett., 164, 236–240, 1983.

Aravena, R., Evans, M. L., and Cherry, J. A.: Stable isotopes of oxygen and nitrogen in source identification of nitrate from septic systems, Ground Water, 31, 180–186, 1993.

Armstrong, F. A. J., Sterns, C. R., and Strickland, J. D. H.: The measurement of upwelling and subsequent biological processes by means of the Technicon AutoAnalyzer and associated equipment, Deep-Sea Res., 14, 381–389, 1967.

Attard, E., Poly, F., Commeaux, C., Laurent, F., Terada, A., Smets, B. F., Recous, S., and Le Roux, X.: Shifts between Nitrospira- and Nitrobacter-like nitrite oxidizers underlie the response of soil potential nitrite oxidation to changes in tillage practices, Environ. Microbiol., 12, 315–326, 2010.

Bartosch, S., Hartwig, C., Spieck, E., and Bock, E.: Immunological detection of Nitrospira-like bacteria in various soils, Microb. Ecol., 43, 26–33, 2002.

Behrendt, H. and Opitz, D.: Retention of nutrients in river systems: Dependence on specific runoff and hydraulic load, Hydrobiologia, 410, 111–122, 1999.

Behrendt, H., Kornmilch, M., Opitz, D., Schmoll, O., and Scholz, G.: Estimation of the nutrient inputs into river systems – experiences from German rivers, Reg. Environ. Change, 3, 107–117, 2002.

Blume, H. P.: Handbuch des Bodenschutzes: Bodenökologie und -belastung, vorbeugende und abwehrende Schutzmaßnahmen, Landsberg am Lech, 2004.

BMU: Bundesministerium für Umwelt, Naturschutz und Reaktorsicherheit: Umweltpolitik, Wasserwirtschaft in Deutschland, Teil 1, Grundlagen, 2001.

Böhlke, J. K., Mroczkowski, S. J., and Coplen, T. B.: Oxygen isotopes in nitrate: new reference materials for O-18:O-17:O-16 measurements and observations on nitrate-water equilibration, Rapid Commun. Mass Sp., 17, 1835–1846, 2003.

Bowen, G. J. and Revenaugh, J.: Interpolating the isotopic composition of modern meteoric precipitation, Water Resour. Res., 39, 1299, doi:10.129/2003WR002086, 2003.

Bristow, L. A.: Tracing nitrogen flows across the Southern North Sea: A stable isotope approach, Ph.D thesis, University of East Anglia, School of Environmental Sciences, 2009.

Buchwald, C. and Casciotti, K. L.: Oxygen isotopic fractionation and exchange during bacterial nitrite oxidation, Limnol. Oceanogr., 55, 1064–1074, 2010.

Buhl, D., Neuser, R. D., Richter, D. K., Riedel, D., Roberts, B., Strauss, H., and Veizer, J.: Nature and nurture: Environmental isotope story of the River Rhine, Naturwissenschaften, 78, 337–346, 1991.

Burns, D. A. and Kendall, C.: Analysis of sources of ^{15}N and ^{18}O to differentiate NO_3^- sources in runoff at two watersheds in the Catskill Mountains of New York, Water Resour. Res., 5, 1051, doi:10.1029/2001WR000292, 2002.

Casciotti, K. L., Sigman, D. M., Hastings, M. G., Bohlke, J. K., and Hilkert, A.: Measurement of the oxygen isotopic composition of nitrate in seawater and freshwater using the denitrifier method, Anal. Chem., 74, 4905–4912, 2002.

Casciotti, K. L.: Inverse kinetic isotope fractionation during bacterial nitrite oxidation, Geochim. Cosmochim. Ac., 73, 2061–2076, 2009.

de Jonge, V. N.: The Ems estuary, in: Eutrophic Shallow Estuaries and Lagoons, edited by: McComb, A. J., CRC Press, USA, 81–105, 1995.

Dähnke, K., Bahlmann, E., and Emeis, K.: A nitrate sink in estuaries? An assessment by means of stable nitrate isotopes in the Elbe estuary, Limnol. Oceanogr., 53, 1504–1511, 2008.

Deutsch, B., Liskow, I., Kahle, P., and Voss, M.: Variations in the δ^{15}N and δ^{18}O values of nitrate in drainage water of two fertilized fields in Mecklenburg-Vorpommern (Germany), Aquat. Sci., 67, 156–165, 2005.

Deutsch, B., Mewes, M., Liskow, I., and Voss, M.: Quantification of diffuse nitrate inputs into a small river system using stable isotopes of oxygen and nitrogen in nitrate, Org. Geochem., 37, 1333–1342, 2006.

Dispirito, A. A., Lipscomb, J. D., and Hooper, A. B.: Cytochrome aa3 from *Nitrosomonas europaea*, J. Biol. Chem., 261, 17048–17056, 1986.

Durka, W., Schulze, E.-D., Gebauer, G., and Voerkelius S.: Effects of forest decline on uptake and leaching of deposited nitrate determined from ^{15}N and ^{18}O measurements, Nature, 372, 765–767, 1994.

Erguder, T. H., Boon, N., Wittebolle, L., Marzorati, M., and Verstraete, W.: Environmental factors shaping the ecological niches of ammonia-oxidizing archaea, FEMS Microbiol. Rev., 33, 855–869, 2009.

Focht, D. D. and Verstraete, W.: Biochemical ecology of nitrification and denitrification, Adv. Microb. Ecol., 1, 135–214, 1977.

Fogg, G. E., Rolston, D. E., Decker, D. L., Louie, D. T., and Grismer, M. E.: Spatial variation in nitrogen isotope values beneath nitrate contamination sources, Groundwater, 36, 418–426, 1998.

Francis, C. A., Beman, J. M., and Kuypers, M. M. M.: New processes and players in the nitrogen cycle: the microbial ecology of anaerobic and archaeal ammonia oxidation, ISME J., 1, 19–27, 2007.

Frank, D., Evans, R. D., and Tracy, B.: The role of ammonia volatilization in controlling the natural ^{15}N abundance of a grazed grassland, Biogeochemistry, 68, 169–178, 2004.

Freyer, H. D.: Seasonal variation of ^{15}N/^{14}N ratios in atmospheric nitrate species, Tellus B, 43, 30–44, 1991.

Galloway, J. N., Schlesinger, W. H., Levy, H., Michaels, A., and Schnoor, J. L.: Nitrogen fixation: Anthropogenic enhancement-environmental response, Global Biogeochem. Cy., 9, 235–252, 1995.

Galloway, J. N.: The global nitrogen cycle, in: Treatise on Geochemistry, edited by: Schlesinger, W. H., Elsevier, 557–583, 2003.

Gömann, H., Kreins, P., Kunkel, R., and Wendland, F.: Model based impact analysis of policy options aiming at reducing diffuse pollution by agriculture – a case study for the river Ems and a sub-catchment of the Rhine, Environ. Modell. Softw., 20, 261–271, 2005.

Granger, J., Sigman, D. M., Needoba, J. A., and Harrison, P. J.: Coupled nitrogen and oxygen isotope fractionation of nitrate during assimilation by cultures of marine phytoplankton, Limnol. Oceanogr., 49, 1763–1773, 2004.

Grischek, T., Hiscock, K. M., Metschies, T., Dennis, P. F., and Nestler, W.: Factors affecting denitrification during infiltration of river water into a sand and gravel aquifer in Saxony, Germany, Water Res., 32, 450–460, 1998.

Gruber, N. and Galloway, J. N.: An earth-system perspective of the global nitrogen cycle, Nature, 451, 293–296, 2008.

Gupta, P., Noone, D., Galewsky, J., Sweeney, C., and Vaughn, B. H.: Demonstration of high-precision continuous measurements of water vapor isotopologues in laboratory and remote field deployments using wavelength-scanned cavity ring-down spectroscopy (WS-CRDS) technology, Rapid Commun. Mass Sp., 23, 2534–2542, 2009.

Hales, H., Ross, D., and Lini, A.: Isotopic signature of nitrate in two contrasting watersheds of Brush Brook, Vermont, USA, Biogeochemistry, 84, 51–66, 2007.

Hamm, A.: Wie und woher kommen die Nährstoffe in die Flüsse?, in: Warnsignale aus Flüssen und Ästuaren, edited by: Lozán, J. L. and Kausch, H., Parey Buchverlag Berlin, 105–110, 1996 (in German).

Heaton, T. H. E.: Isotopic studies of nitrogen pollution in the hydrosphere and atmosphere: a review, Chem. Geol., 59, 87–102, 1986.

Hebbel, H. and Steuer, D.: Empirische Untersuchungen zur Berechnung von Frachten in Fließgewässern, in: Discussion Papers in Statistics and Quantitative Economics, edited by: Hauptmann, H. and Krumbholz, W., Hamburg, 2006 (in German).

Herfort, L., Kim, J. H., Coolen, M. J. L., Abbas, B., Schouten, S., Herndl, G. J., and Sinninghe Damsté, J. S.: Diversity of *Archaea* and detection of crenarchaeotal *amoA* genes in the rivers Rhine and Têt, Aquat. Microb. Ecol., 55, 189–201, 2009.

Hickel, W., Mangelsdorf, P., and Berg, J.: The human impact in the German Bight: eutrophication during three decades (1962–1991), Helg. Mar. Res., 47, 243–263, 1993.

Hirschfeld, M.: Isotopen- und Hydrochemie des Rheinsystems: Saisonale Variationen als Konsequenz dynamischer Prozessabläufe des kontinentalen Kohlenstoff- und Wasserkreislaufes, Kölner Forum Geol. Paläontol., 12, 32–39, 2003 (in German).

Hoffman, J. C. and Bronk, D. A.: Interannual variation in stable carbon and nitrogen isotope biogeochemistry of the Mattaponi River, Virginia, Limnol. Oceanogr., 51, 2319–2332, 2006.

Hollocher, T. C.: Source of the oxygen atoms of nitrate in the oxidation of nitrate by Nitorbacter agilis and evidence against a P-O-N anhydride mechanism in oxidative phosphorilization, Arch. Biochem. Biophys., 233, 721–727, 1984.

Horrigan, S. G., Montoya, J. P., Nevins, J. L., and McCarthy, J. J.: Natural isotopic composition of dissolved inorganic nitrogen in the Chesapeake Bay, Estuarine, Coast. Shelf Sci., 30, 393–410, 1990.

Howarth, R. W. and Marino, R.: Nitrogen as the limiting nutrient for euntrophication in coastal marine ecosystems: Evolving views over three decades, Limnol. Oceanogr., 51, 364–376, 2006.

Jickells, T. D.: Nutrient biogeochemistry of the coastal zone, Science, 281, 217–222, 1998.

Johannsen, A., Dähnke, K., and Emeis, K.: Isotopic composition of nitrate in five German rivers discharging into the North Sea, Organ. Geochem., 39, 1678–1689, 2008.

Kempe, S. and Krahe, P.: Water and Biogeochemical fluxes in the River Rhine catchment, Erdkunde, 59, 216–250, 2005.

Kendall, C., Campbell, D. H., Burns, D. A., Shanley, J. B., Silva, S. R., and Chang, C. C. Y.: Tracing sources of nitrate in snowmelt runoff using the oxygen and nitrogen isotopic compositions of nitrate, Biogeochemistry of Seasonally Snow-Covered Catchments, Proceedings of a Boulder Symposium, 339–347, July 1995.

Kendall, C.: Tracing nitrogen sources and cycling in catchments, in: Isotope Tracers in Catchment Hydrology, edited by: Kendall, C. and McDonell, J. J., Elsevier Science, 517–576, 1998.

Komor, S. C. and Anderson, H. W.: Nitrogen isotopes as indicators of nitrate sources in Minnesota Sand-Plain Aquifers, J. Ground Water, 31, 260–270, 1993.

Kool, D. M., Wrage, N., Oenema, O., Dolfing, J., and Van Groenigen, J. W.: Oxygen exchange between (de)nitrification intermediates and H_2O and its implications for source determination of NO_3^-: a review, Rapid Commun. Mass Sp., 21, 3569–3578, 2007.

Kroopnick, P. and Craig, H.: Atmospheric oxygen: isotopic composition and solubility fractionation, Science, 175, 54–55, 1972.

Kumar, S. Nicholas, D. J. D., and Williams, E. H.: Definitive 15N-NMR evidence that water serves as a source of "O" during nitrite oxidation by *Nitrobacter agilis*, FEBS Lett., 152, 71–74, 1983.

Lehmann, M. F., Sigman, D. M., McCorkle, D. C., Granger, J., Hoffmann, S., Cane, G., and Brunelle, B. G.: The distribution of nitrate $^{15}N/^{14}N$ in marine sediments and the impact of benthic nitrogen loss on the isotopic composition of oceanic nitrate, Geochim. Cosmochim. Ac., 71, 5384–5404, 2007.

Leininger, S., Urich, T., Schloter, M., Schwark, L., Qi, J., Nicol, G. W., Prosser, J. I., Schuster, S. C., and Schleppen, C.: Archaea predominate among ammonia oxidizing prokaryotes in soils, Nature, 442, 806–809, 2006.

Liersch, S. and Volk, M.: How realistic is the implementation of the European Water Framework Directive in river basins dominated by agriculture? The example of the Upper Ems River Basin (Germany), International Conference: Megacities: Risk, Vulnerability and Sustainable development, Leipzig, Germany, 2009.

Macko, S. A. and Ostrom, N. E.: Pollution studies using stable isotopes, in: Stable Isotopes in Ecology and Environmental Sciences, edited by: Lajtha, K. and Michener, R. H., Blackwell, 45–62, 1994.

Mariotti, A., Germon, P., Hubert, P., Kaiser, P., Letolle, R., Tardieux, A., and Tardieux, P.: Experimental determination of nitrogen kinetic isotope fractionation: Some principles, illustrations for denitrification and nitrification, Plant Soil Sci., 62, 413–430, 1981.

Mayer, B., Bollwerk, S. M., Mansfeldt, T., Hütter, B., and Veizer, J.: The oxygen isotope composition of nitrate generated by nitrification in acid forest floors, Geochim. Cosmochim. Ac. 65, 2743– 2756, 2001.

Mayer, B., Boyer, E. W., Goodale, C., Jaworski, N. A., van Breemen, N., Howarth, R. W., Seitzinger, S., Billen, G., Lajtha, K., Nadelhoffer, K., van Dam, D., Hetling, L. J., Nosal, M., and Paustian, K.: Sources of nitrate in rivers draining sixteen watersheds in the Northeastern US: isotopic constraints, Biogeochemistry, 57–58, 171–197, 2002.

Mengis, M., Walther, U., Bernasconi, S., and Wehrli, B.: Limitations of using δ^{18}O for the source identification of nitrate in agricultural soils, Environ. Sci. Technol., 35, 1840–1844, 2001.

Middelburg, J. J. and Nieuwenhuize, J.: Carbon and nitrogen stable isotopes in suspended matter and sediments from the Schelde Estuary, Mar. Chem., 60, 217–225, 1998.

Nishikawa, J., Kohzu, A., Boontanon, N., Iwata, T., Tanaka, T., Ogawa, N. O., Ishii, R., and Wada, E.: Isotopic composition of nitrogenous compounds with emphasis on anthropogenic loading in river ecosystems, Isot. Environ. Healt. S., 45, 27–40, 2009.

Osterkamp, S., Kraft, D., and Schirmer, M.: Climate change and the ecology of the Weser estuary region: assessing the impact of an abrupt change in climate, Clim. Res., 18, 97–104, 2001.

Ostrom, N. E., Macko, S. A., Deibel, D., and Thompson, R. J.: Seasonal variation in the stable carbon and nitrogen isotope biogeochemistry of a coastal cold ocean environment, Geochim. Cosmochim. Ac., 61, 2929–2942, 1997.

Pätsch, J. and Radach, G.: Long-term simulation of the eutrophication of the North Sea: temporal development of nutrients, chlorophyll and primary production in comparison to observations, J. Sea Res., 38, 275–310, 1997.

Painter, H. A.: A review of literature on inorganic nitrogen metabolism in microorganisms, Water Res., 4, 393–450, 1970

Pardo, L. H., Kendall, C., Pett-Ridge, J., and Chang, C. C. Y.: Evaluating the source of streamwater nitrate using δ^{15}N and δ^{18}O in nitrate in two watersheds in New Hampshire, USA, Hydrol. Process., 18, 2699–2712, 2004.

Peterson, B. J., Wollheim, W. M., Mulholland, P. J., Webster, J. R., Meyer, J. L., Tank, J. L., Marti, E., Bowden, W. B., Valett, H. M., Hershey, A. E., McDowell, W. H., Dodds, W. K., Hamilton, S. K., Gregory, S., and Morrall, D. D.: Control of nitrogen export from watersheds by headwater streams, Science, 292, 86–90, 2001.

Reinhardt, M., Müller, B., Gächter, R., and Wehrli, B.: Nitrogen removal in a small constructed wetland: An isotope mass balance approach, Environ. Sci. Technol., 40, 3313–3319, 2006.

Renger, A.: Sicker- und Fließzeiten von Nitrat aus dem Wurzelraum ins Grundwasser. Arbeitsbericht Nr. 223/November 2002, Akademie für Technikfolgenabschätzung in Baden-Württemberg, 2002.

Révész, K. and Böhlke, J.-K.: Comparison of δ^{18}O measurements in nitrate by different combustion techniques, Anal. Chem., 74, 5410–5413, 2002.

Schlarbaum, T., Daehnke, K., and Emeis, K.: Turnover of combined dissolved organic nitrogen and ammonium in the Elbe estuary/NW Europe: Results of nitrogen isotope investigations, Mar. Chem., 119, 91–107, 2010.

Sebilo, M., Billen, G., Grably, M., and Mariotti, A.: Isotopic composition of nitrate-nitrogen as a marker of riparian and benthic denitrification at the scale of the whole Seine River system, Biogeochemistry, 63, 35–51, 2003.

Sebilo, M., Billen, G., Mayer, B., Billiou, D., Grably, M., Garnier, J., and Mariotti, A.: Assessing nitrification and denitrification in the Seine river and estuary using chemical and isotopic techniques, Ecosystems, 9, 564–577, 2006.

Sigleo, A. C. and Macko, S. A.: Carbon an d Nitrogen Isotopes in Suspended Particles and Colloids, Chesapeake and San Francisco Estuaries, USA, Estuarine, Coast. Shelf Sci., 54, 701–711, 2002.

Sigman, D. M., Altabet, M. A., McCorkle, D. C., Francois, R., and Fischer, G.: The δ^{15}N of nitrate in the Southern Ocean: Consumption of nitrate in surface waters, Global Biogeochem. Cy., 13, 1149–1166 , 1999.

Sigman, D. M., Casciotti, K. L., Andreani, M., Barford, C., Galanter, M., and Bohlke, J. K.:

A bacterial method for the nitrogen isotopic analysis of nitrate in seawater and freshwater, Anal. Chem., 73, 4145–4153, 2001.

Sigman, D. M., Karsh, K. L., and Casciotti, K. L.: Nitrogen isotopes in the ocean, in: Encyclopedia of Ocean Sciences (update from 2001), edited by: Steele, J. H., Turekian, K. K., and Thorpe, S. A., Academic Press, London, 2009.

Sigman, D. M., Granger, J., DiFiore, P. J., Lehmann, M. F., Ho, R., Cane, G., and Van Geen, A.: Coupled nitrogen and oxygen isotope measurements of nitrate along the Eastern North Pacific margin, Global Biogeochem. Cy., 19, 1–14, 2005.

Silva, S. R., Kendall, C., Wilkison, D. H., Ziegler, A. C., Chang, C. C. Y., and Avanzino, R.: A new method for collection of nitrate from fresh water and the analysis of nitrogen and oxygen isotope ratios, J. Hydrol., 228, 22–36, 2000.

Spoelstra, J., Schiff, S. L., Hazlett, P. W., Jeffries, D. S., and Semkin, R. G.: The isotopic composition of nitrate produced from nitrification in a hardwood forest floor, Geochim. Cosmochim. Ac., 71, 3757–3771, 2007.

Statistisches Bundesamt: Coordination of Information on the Environment CORINE Landcover. Digitale Landnutzungskarten 1:100.000 für die Bundesrepublik Deutschland, Europäische Umweltagentur EUA, Wiesbaden, 1990.

Trudeau, V. and Rasmussen, B.: The effect of water velocity on stable carbon and nitrogen isotope signatures of periphyton, Limnol. Oceanogr., 48, 2194–2199, 2003.

UBA: Umweltbundesamt: UBA Vorhaben 204 63 252. Projekt: Nationale Umsetzung EU-NEC-Richtlinie/CAFE-Strategie, Abschlußbericht 2007

UBA: Umweltbundesamt: Thema: Biologische Vielfalt, Naturhaushalt und Landschaft, Unterthema: Belastung der Umweltmedien und Lebensräume durch Stoffe; Boden, Indikator: Stickstoffüberschuss, available at: http://www.umweltbundesamt-daten-zur-umwelt.de/umweltdaten/public/theme.do?nodeIdent=2879 (last access: 4 April 2010), 2009.

Voss, M., Deutsch, B., Elmgren, R., Humborg, C., Kuuppo, P., Pastuszak, M., Rolff, C., and Schulte, U.: Source identification of nitrate by means of isotopic tracers in the Baltic Sea catchments, Biogeosciences, 3, 663–676, doi:10.5194/bg-3-663-2006, 2006.

Wankel, S. D., Kendall, C., Francis, C. A., and Paytan, A.: Nitrogen sources and cycling in the San Francisco Bay Estuary: A nitrate dual isotopic composition approach, Limnol. Oceanogr., 51, 1654–1664, 2006.

Wankel, S. D., Kendall, C., and Paytan, A.: Using nitrate dual isotopic composition (δ^{15}N and δ^{18}O) as a tool for exploring sources and cycling of nitrate in an estuarine system: Elkhorn

Slough, California, J. Geophys. Res., 114, G01011, doi:10.1029/2008JG000729, 2009.

Wassenaar, L. I.: Evaluation of the origin and fate of nitrate in the Abbotsford Aquifer using the isotopes of ^{15}N and ^{18}O in NO_3^-, Appl. Geochem., 10, 391–405, 1995.

Wendland F., Albert, H., Bach, M., and Schmidt, R.: Atlas zum Nitratstrom in der Bundesrepublik Deutschland – Rasterkarten zu geowissenschaftlichen Grundlagen, Stickstoffbilanzgrößen und Modellergebnissen, Springer, Berlin, Heidelberg, New York, 1993 (in German).

Weston, K., Jickells, T. D., Fernand, L., and Parker, E. R.: Nitrogen cycling in the Southern North Sea: consequences for total nitrogen transport, Estuarine, Coast. Shelf Sci., 59, 559–573, 2004.

Wollheim, W. M., Peterson, B. J., Deegan, L. A., Hobbie, J. E., Hooker, B., Bowden, W. B., Edwardson, K. J., Arscott, D. B., Hershey, A. E., and Finlay, J.: Influence of stream size on ammonium and suspended particulate nitrogen processing, Limnol. Oceanogr., 46, 1, 1–13, 2001.

Wu, J., Calvert, S. E., and Wong, C. S.: Nitrogen isotope variations in the Subarctic Northeast Pacific: relationships to nitrate utilization and trophic structure, Deep-Sea Res. Pt. I, 44, 287–314, 1997.

Yurtsever, Y.: Worldwide survey of stable isotopes in precipitation, Rept. Sect. Isot. Hydrol., IAEA, 1975.

Table 1. N-NO$_3^-$ discharge and load weighted isotope values for the respective sampling periods subdivided into seasons.

River	Period	δ^{15}N-NO$_3^-$ annual	δ^{18}O-NO$_3^-$ annual	N-NO$_3^-$ load (kt) annual	δ^{15}N-NO$_3^-$ summer	δ^{18}O-NO$_3^-$ summer	N-NO$_3^-$ load (kt) summer	δ^{15}N-NO$_3^-$ winter	δ^{18}O-NO$_3^-$ winter	N-NO$_3^-$ load (kt) winter
Rhine	2006/2007	8.1	0.4	**228**	8.5	0.4	**94**	7.9	0.4	**135**
	2007/2008	8.6	1.5	**195**	8.9	1.9	**81**	8.2	1.0	**114**
Weser	2006/2007	8.4	1.2	**37**	9.7	1.9	**10**	8.0	1.0	**27**
	2007/2008	8.2	1.1	**58**	8.7	2.2	**17**	8.0	0.7	**41**
	2008/2009	9.2	1.7	**28**	11.0	2.9	**8**	8.5	1.2	**20**
Ems	2006/2007	11.6	2.3	**6**	15.3	3.3	**1**	11.1	2.2	**5**
	2007/2008	12.8	3.3	**8**	13.8	5.1	**2**	12.2	2.3	**5**

Table 2. Correlation matrix (Pearson's) of biogeochemical parameters of the rivers Rhine, Weser and Ems.

Rhine	T °	$c(NO_3^-)$	NO_3^--load	$\delta^{15}N$-NO_3^-	$\delta^{18}O$-NO_3^-	$\delta^{15}N$-PN	
	-0.453^{**}	0.373^{**}	0.935^{**}	-0.788^{**}	-0.166	-0.422	Discharge
		-0.754^{**}	-0.616^{**}	0.658^{**}	-0.384^{*}	0.138	T °
			0.660^{**}	-0.731^{**}	-0.392^{*}	-0.180	$c(NO_3^-)$
				-0.887^{**}	-0.308^{*}	-0.436	NO_3^--load
					-0.396^{*}	0.324	$\delta^{15}N$-NO_3^-
						0.554^{*}	$\delta^{18}O$-NO_3^-
Weser	T °	$c(NO_3^-)$	NO_3^--load	$\delta^{15}N$-NO_3^-	$\delta^{18}O$-NO_3^-	$\delta^{15}N$-PN	
	-0.574^{**}	0.609^{**}	0.970^{**}	-0.726^{**}	-0.378^{**}	0.05	Discharge
		-0.773^{**}	-0.642^{**}	0.738^{**}	0.732^{**}	0.250	T °
			0.754^{**}	-0.803^{**}	-0.387^{**}	0.098	$c(NO_3^-)$
				-0.764^{**}	-0.401^{**}	0.023	NO_3^--load
					0.446^{**}	0.103	$\delta^{15}N$- NO_3^-
						0.199	$\delta^{18}O$- NO_3^-
Ems	T °	$c(NO_3^-)$	NO_3^--load	$\delta^{15}N$-NO_3^-	$\delta^{18}O$-NO_3^-	$\delta^{15}N$-PN	
	-0.538^{**}	0.671^{**}	0.959^{**}	-0.694^{**}	-0.660^{**}	-0.595^{**}	Discharge
		-0.632^{**}	-0.537^{**}	0.653^{**}	0.929^{**}	0.830^{**}	T °
			0.765^{**}	-0.864^{**}	-0.721^{**}	-0.450^{*}	$c(NO_3^-)$
				-0.699^{**}	-0.681^{**}	-0.589^{**}	NO_3^--load
					0.723^{**}	0.557^{**}	$\delta^{15}N$-NO_3^-
						0.782^{**}	$\delta^{18}O$-NO_3^-

* Correlation is significant ($p<0.05$),

** correlation is significant ($p<0.01$).

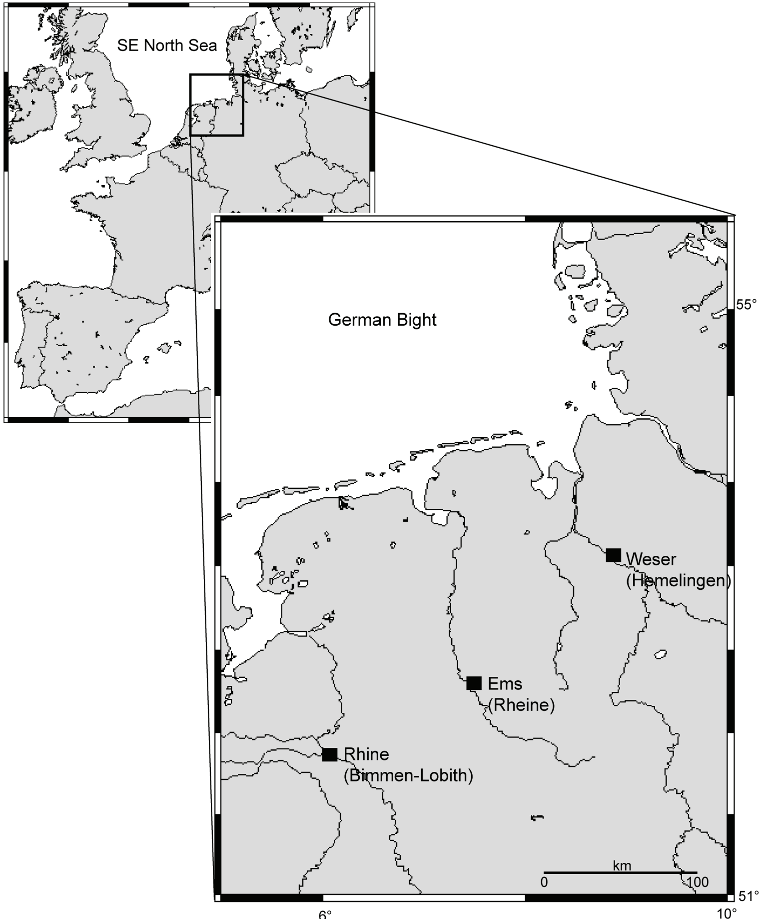

Fig. 1. Sampling locations at the river Rhine, Weser and Ems.

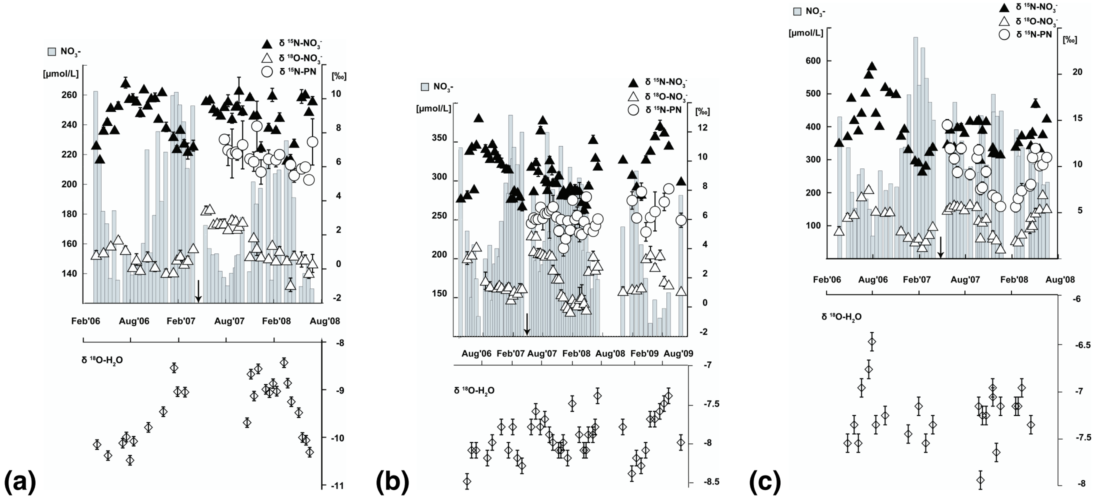

Fig. 2. **(a)** Seasonal cycles of NO_3^- concentrations, δ^{15}N-NO_3^-, δ^{18}O-NO_3^-, δ^{15}N-PN and δ^{18}O-H_2O of the Rhine River. **(b)** Seasonal cycles of NO_3^- concentrations, δ^{15}N-NO_3^-, δ^{18}O-NO_3^-, δ^{15}N-PN and δ^{18}O-H_2O of the river Weser. **(c)** Seasonal cycles of NO_3^- concentrations, δ^{15}N-NO_3^-, δ^{18}O-NO_3^-, δ^{15}N-PN and δ^{18}O-H_2O of the river Ems. The arrow indicates the beginning of the 2nd sampling interval.

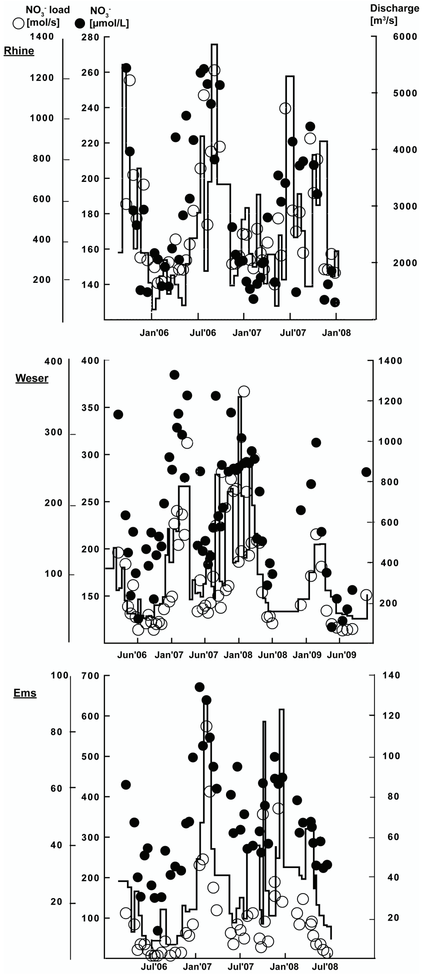

Fig. 3. Temporal variation in discharge in relation to nitrate concentration and nitrate load.

Title Page
Abstract | Introduction
Conclusions | References
Tables | Figures
◀I | ▶I
◀ | ▶
Back | Close
Full Screen / Esc
Printer-friendly Version
Interactive Discussion

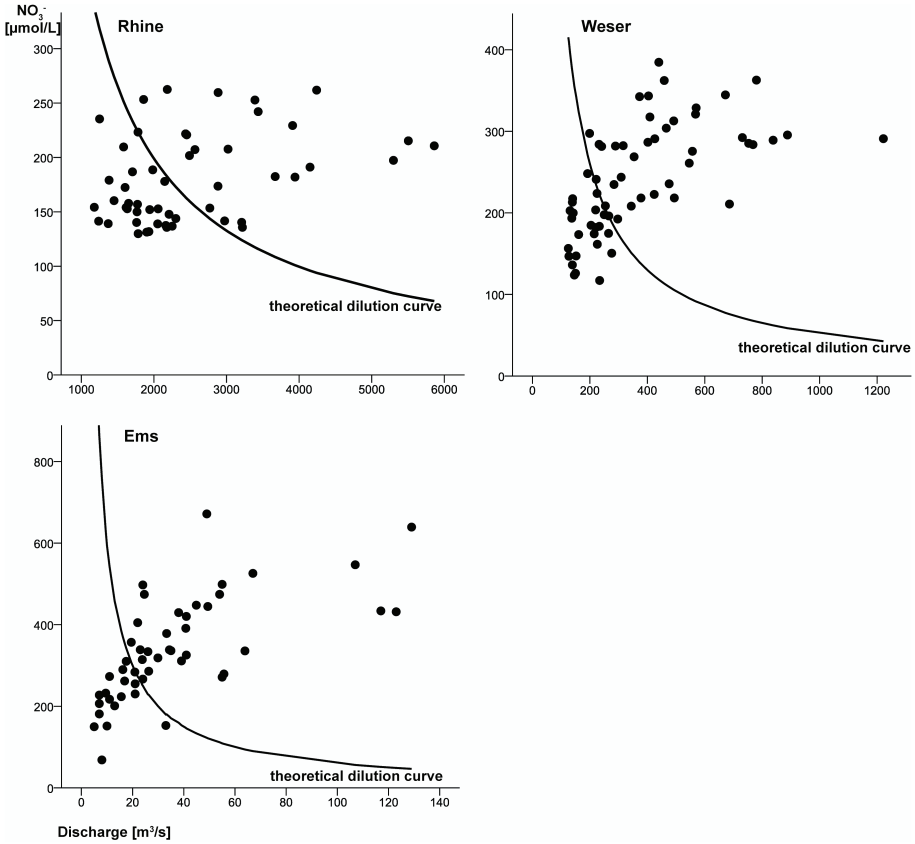

Fig. 4. Nitrate-discharge relationship in comparison with the theoretical dilution curve.

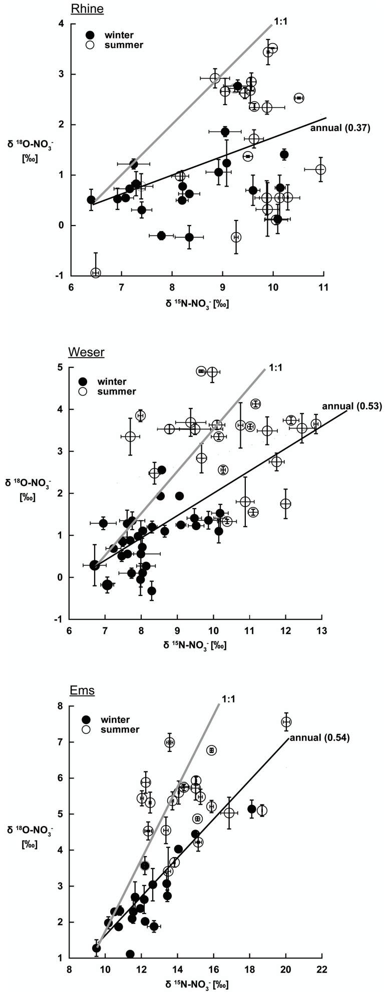

Fig. 5. Variation in δ^{18}O-NO_3^- in relation to δ^{15}N-NO_3^- for the Rhine, Weser and Ems.

Title Page
Abstract
Introduction
Conclusions
References
Tables
Figures
◀I
▶I
◀
▶
Back
Close
Full Screen / Esc
Printer-friendly Version
Interactive Discussion

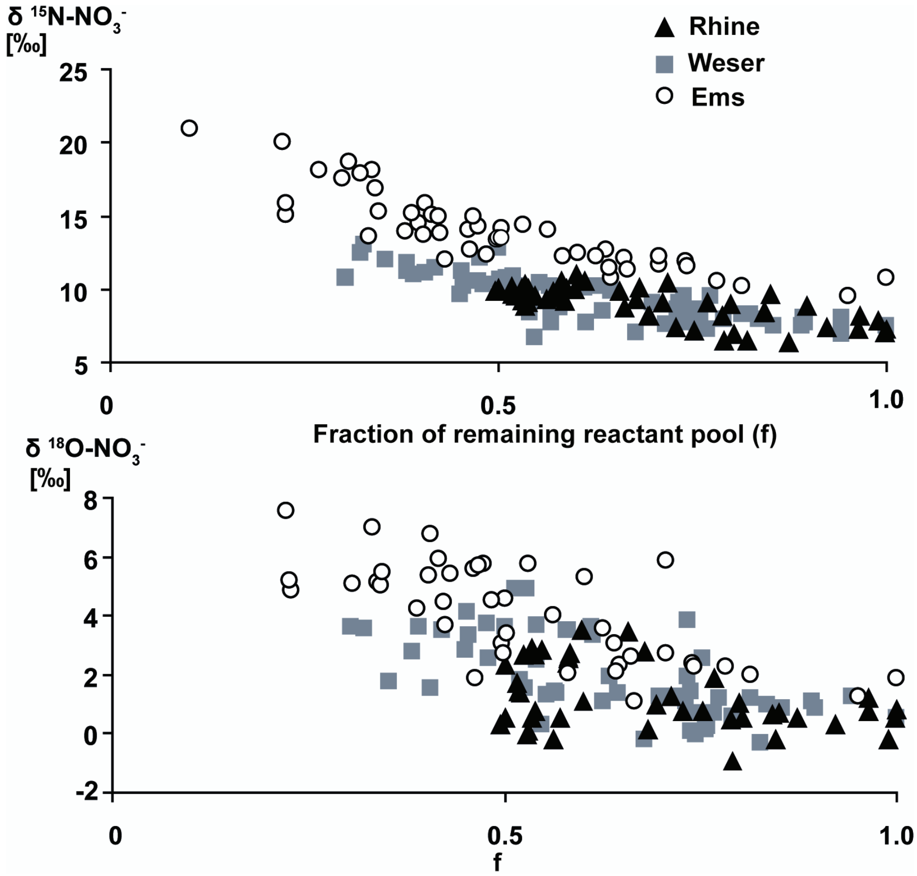

Fig. 6. Change in δ^{15}N-NO$_3^-$ and δ^{18}O-NO$_3^-$ relative to the fraction of the remaining reactant (NO$_3^-$) pool.