Final Draft
of the original manuscript:

Brase, L.; Sanders, T.; Daehnke, K.:

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In: Isotopes in Environmental and Health Studies (2018) Taylor & Francis

DOI: 10.1080/10256016.2018.1428580

Anthropogenic changes of nitrogen loads in a small river: external nutrient sources vs. internal turnover processes

Lisa Brase[1, 2], Tina Sanders[1], Kirstin Dähnke[1]

[1] Institute for Coastal Research, Helmholtz Centre Geesthacht, Geesthacht, Germany

[2]Institute of Geology, University of Hamburg, Hamburg, Germany

Lisa Brase, Max-Planck-Straße 1, Geesthacht, D-21502, phone: +49 (0)4152 87 2831, email: lisa.brase@hzg.de

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Anthropogenic nutrient inputs increase the N-load in many aquatic systems, leading to eutrophication and potential changes of biological N-retention capacity. In this study, nitrate inputs in a small river were investigated along a gradient of anthropogenic influence. We aimed to determine changes in nitrate load and isotope signatures in the water column and to identify the anthropogenic influence on biological nitrogen assimilation and nitrification or denitrification in sediments. In seasonal sampling campaigns, we analysed dissolved inorganic nitrogen concentrations (DIN), and stable isotopes of nitrate. To differentiate rates of nitrate production and consumption in the pristine vs. agricultural river section, intact sediment cores were incubated with ^{15}N-labelled nitrate. $\delta^{15}N$ values of nitrate in the pristine river section were low, reflecting natural sources, but, as expected, increased with nitrate concentration in all seasons along the gradient. In general, nitrate retention and consumption were higher in the anthropogenically impacted than in the pristine river section, and nitrate consumption exceeded production. In addition to our measurements, modelled results also show that even in a small river, the anthropogenically enhanced consumption capacity is overwhelmed by surplus N-inputs, and nitrate consumption cannot increase in turn with external loads.

Keywords: biogeochemistry, nitrification, isotopes, anthropogenic influences, mixing model, isotope dilution

1. Introduction

Excess nitrogen from fertilizer application and wastewater treatment plant (WWTP) discharge has led to wide-spread eutrophication in aquatic ecosystems. This anthropogenic nutrient enrichment has a number of consequences, notably a change in the balance between nitrogen (N)-retention and elimination in rivers and streams: With additional nitrate (NO_3^-) input, denitrification is promoted, which may counteract eutrophication [1]. On the other hand, additional nutrients increase phytoplankton production, and easily accessible carbon sources can promote bacterial growth, so that N-retention in a given system is increased, at the cost of increased biomass production [2, 3, 4].

To assess the anthropogenic impact and the role of internal turnover in rivers, not only concentration measurements of nutrients have proven utility. Stable isotope measurements of dissolved inorganic nitrogen (DIN), mainly dual isotopes in NO_3^- and their specific compositions, can complement nutrient data, e.g. [2, 5, 6]. NO_3^- isotope signatures are source-specific: Atmospheric deposition has relatively low $\delta^{15}N$ values (-5‰ to 5‰, [7]) with very high values of $\delta^{18}O$ (>60‰ [7, 8]) , whereas runoff from agricultural soils or manure is isotopically enriched in $\delta^{15}N$ (>8‰, e.g. [9, 10, 11]). Furthermore, stable isotopes also reflect natural isotopic fractionation by biological processes. For example, during NO_3^- assimilation by phytoplankton, the ratio of $\delta^{15}N$ and $\delta^{18}O$ in the residual NO_3^- increase in parallel to one another [12], whereas during denitrification in freshwater environments, this increase follows a ratio of 1.5:1 to 2:1 [13, 14]. However, in contrast to the water column, the isotope effect of denitrification is often not expressed when it occurs in sediments [15], because nitrate transport is mainly limited by diffusion. Other explanations are either due to bottom water nitrate which entered the sediment and was denitrified before it has the opportunity to escape back into the water column or the denitrification isotope effect being balanced by low $^{15}N_{NO3}$ isotopes from nitrification [16]. Many studies focused on N-turnover in large river systems, [e.g. 6, 10, 17], where nitrification in the catchment and the stream itself regenerates significant amounts of NO_3^-. NO_3^- concentration and isotopes in large rivers can theoretically be affected by diffuse sources, groundwater input, and by internal NO_3^- uptake due to assimilation or denitrification, [e.g. 2, 9, 18]. In small rivers it is possible to separate point sources from diffuse inputs to some extent, as well to separate influencing areas, and thus to determine influences of external nutrient sources and internal

nutrient turnover more precisely. Consequently, investigating small rivers provides an advantage in addressing the relative importance of various sources and turnover processes separately. One possible solution is provided by incubation assays: As biological NO_3^- production and consumption act simultaneously in rivers [1, 19], incubation assays can be used to separate these processes. The isotope dilution technique, which is based on the addition of ^{15}N-labelled nitrate, is a useful tool to discern nitrification and NO_3^-consumption [e.g. 20, 21, 22]. This technique is often applied in sediment incubations, where N-turnover is higher than in the water column.

In this study, we combined sediment incubations with natural abundance NO_3^-isotope investigations to discern the effect between external sources vs. internal processes and their joint impact on the water column nitrate (NO_3^-) and ammonium (NH_4^+) concentrations and NO_3^- isotope inventory. We investigated a small river with a small catchment area along a land-use gradient, ranging from a pristine upper region to an agricultural setting further downstream. To identify nitrate sources, we measured DIN concentrations and the dual NO_3^- isotope values and then applied them to an isotope mixing model which was set in comparison to a GIS-based model. Moreover, we calculated rates of NO_3^- production and consumption in all seasons based on sediment incubations. Our main intention was to (a) quantify the role of nitrification in the river as a source of NO_3^- in comparison to NO_3^- derived from external sources, and (b), to assess the impact of increasing nitrogen loads on nitrification, assimilation and denitrification in the river.

2. Material and Methods

2.1 *Site description*

The Holtemme River in Saxony-Anhalt is ~ 47 km long, with a small catchment area of 278 km² and an annual mean water discharge of 1.33 $m^3 s^{-1}$ (gauge Mahndorf – station 6, Fig. 1). Climate conditions in the catchment area of the Holtemme are typical for Central Europe, with wet summers and cold dry winters [23].

The Holtemme encompasses pristine regions as well as regions that are subject to anthropogenic influences. Its source is in the region of the Harz Mountains, 860 m above sea level. The headwaters lie in a forest dominated national park with steep, small waterfalls, and rapids. The

national park ends at the city Wernigerode, where the regulation of the river begins. Further downstream, the river is influenced by a combination of urban runoff, a WWTP and agricultural fields, until it discharges into the Bode river.

Its water depth undergoes seasonal variations with water depths between 8 cm up to 200 cm, the average in the pristine section was 10 cm to 30 cm and 50 cm to 100 cm in the agricultural section.

[Figure 1 near here]

2.2 ***Sampling***

2.2.1. Water samples

Seasonal sampling campaigns took place in June 2014, September 2014, February 2015 and April 2015. Weather conditions during sampling were mainly sunny; a slight rain event in summer did not show notable effects on discharge (Tab. 1) or nutrient concentration (data not shown).

Water samples for nutrient and isotope analyses were taken along a 20 km section at six stations following a pristine-agricultural gradient (Fig.1, Tab.1). For nutrient and stable isotope analysis of nitrate, surface water was sampled with a bottle (PVDF, 0.5 L) in the middle of the stream. Samples were filtered immediately (Minisart© NML, 0.45 µm, Sartorius), stored cool in PE bottles (100 ml) and were frozen within 10 hours until further analysis in the lab.

[Table 1 near here]

2.2.2. Sediment samples

Sediment cores were taken at stations 1 and 6 in spring, summer and autumn. In winter (February 2015), no cores could be taken because deeper sediment layers were frozen. At each station, twelve cores were taken (PMMA core liners, ID 3.7cm; approximately 10 cm of sediment, 13 cm of overlying water), sealed and stored in a cooling box for transportation. The middle of the river bed was covered with stones and gravel at both stations, hence, cores were taken towards the river bank where the sediment was accessible.

2.3 *Laboratory analysis*

2.3.1. Water samples for nutrient and isotopic composition

DIN concentrations in water samples were measured with a continuous flow auto analyser (AA3, SEAL Analytical) using standard colorimetric techniques [24]. Stable isotopes of NO_3^- were determined using the denitrifier method [25, 26]. This method is based on the analysis of nitrous oxide (N_2O) produced by denitrifying *Pseudomonas aureofaciens* (ATCC #13985). The N_2O was purified, concentrated on a GasBench II and measured on an isotope ratio mass spectrometer (Delta V Advantage, Thermo Scientific). Samples were calibrated against the international standards IAEA-NO_3 ($\delta^{15}N$: +4.7‰, $\delta^{18}O$: +25.6‰) and USGS34 ($\delta^{15}N$: -1.8‰, $\delta^{18}O$: -27.9‰), with a standard deviation of <0.2‰ for $\delta^{15}N_{NO3}$ (n=4) and <0.5‰ for $\delta^{18}O_{NO3}$ (n=4).

If NO_2^- concentration is lower < 1% of NO_3^- concentration, the effect on isotope determinations of NO_3^- is negligible and there is no need to remove NO_2^- before measuring dual stable isotopes of NO_3^- [27].

2.3.2. Intact sediment core incubation – Isotope dilution experiment

The sampled sediment cores were placed in buckets, and stored open under water. Additional water from each sampling site was used for the storage of cores. The cores were submerged in a reservoir filled with the river water of the corresponding sampling site. To avoid anoxia in the sediment cores, river water in the reservoirs was oxygenated with aquarium pumps and constantly stirred [28]. A preliminary test over 48 hours showed that this storage method did not lead to increased anoxia in the sediment cores. Reservoirs containing sediment cores were placed in a water filled tank and pre-incubated at a constant temperature (summer and autumn: 16°C, spring: 12°C, cf. Tab.2) for 36 hours. After pre-incubation, river water in the reservoir was removed until the top of the core liners was above water level, and the remaining water in each core was oxygenated separately using aquarium pumps. Great care was taken to avoid sediment resuspension during oxygenation of cores.

[Table 2 near here]

At the beginning of the experiment, the overlying water (~90 ml) above the sediment of 9 cores was labelled, aiming for a labelling percentage of 1 at% with $Na^{15}NO_3$ (98 atom % ^{15}N, Sigma-Aldrich®). To avoid an increase in rates due to substrate addition, the label solutions added to

148 the overlying water had a NO_3^- concentration comparable to site water, which had been
149 determined before. Three unlabelled cores remained and were used as control samples. All cores,
150 including the control samples, were then incubated for 24 hours in darkness. Samples, and
151 according control samples, were taken directly after label addition, after 8 and 24 hours by
152 creating a slurry where the reactive sediment layer (1 cm) was gently mixed into the overlying
153 water column [29]. At each point, three labelled replicates and one control without label addition
154 were sampled. To stop microbial activity, the slurry was filtered immediately (Minisart© NML,
155 0.45 µm, Sartorius) and samples were stored frozen until nutrient concentration and $\delta^{15}N_{NO3}$
156 values were measured. All samples were analysed in duplicate and were calibrated similar to
157 above. The standard deviation was <0.3‰ (n>3) for natural abundance samples and 6‰ (n>3)
158 for enriched samples.

159 Rates were then determined by the temporal changes in the pool size and ^{15}N abundances in the
160 ^{15}N-amended NO_3^- pool of the incubated cores. The NO_3^- decrease is assumed to be NO_3^-
161 consumption.

162 ## 2.4 *Calculations*

163 ### *2.4.1 Isotope dilution model*

164 In our assessment, we define NO_3^- consumption as the sum of assimilation and denitrification,
165 and NO_3^- production is defined as nitrification.

166 NO_3^- production and consumption in cores were calculated based in the ^{15}N isotope dilution
167 model [20, 21]. The following equations were used

168 $$p = [\ln(I_t/I_0)]/[\ln (P_t/P_0)] (P_0-P_t/t) \quad (1)$$

169 where p is production in µmol L^{-1} h^{-1}, t is incubation time, P_0 is the initial NO_3^- concentration at
170 incubation time 0, P_t is the NO_3^- concentration at time t, and I_0 and I_t represent ^{15}N atom excess.

171 NO_3^- consumption was calculated by using the decrease of NO_3^- concentration (P), the content of
172 ^{15}N-NO_3^- (p) and the natural abundance of ^{15}N-NO_3^- (k):

173 $$c = \ln[(p_0-k_0P_o)/(p_t-k_tP_t)] (P_0-P_t)/\ln(P_0/P_t)/t \quad (2)$$

174 where c is the rate of consumption in µmol L^{-1} h^{-1}.

Both turnover rates were then converted into µmol N m^{-2} d^{-1}, using the ratio of measured volume-to-surface area (of the boundary layer between water column and sediment) of each core [30].

2.4.2 Isotope mixed model – IMM vs. GIS analysation

To compare the output of agriculture distribution of GIS analysis (ArcGIS Desktop, Environmental Systems Research Institute Inc.) to the results of the isotope mixed model, mass balanced equations were used [31] to estimate the contribution of the NO_3^- sources in the Holtemme River:

$$\delta^{15}N_H = \int_F \delta^{15}N_F + \int_D \delta^{15}N_D + \int_A \delta^{15}N_A \quad (3)$$

$$\delta^{18}O_H = \int_F \delta^{18}O_F + \int_D \delta^{18}O_D + \int_A \delta^{18}O_A \quad (4)$$

$$1 = \int_F + \int_D + \int_A \quad (5)$$

Where H represents the NO_3^- isotope values from the Holtemme river and the subscripts F (pristine – forest region), D (atmospheric deposition) and A (agricultural area) the three source values which were used for the IMM.

The NO_3^- isotope values of H ($\delta^{15}N$ and $\delta^{18}O$) are the concentration of annual load weighted mean isotope values:

$$\delta^{15}N_a = \frac{\sum_i \delta^{15}N_i \times concN_i \times flow_i}{\sum_i concN_i \times flow_i} \quad (6)$$

$\delta^{15}N_i$ is the isotope value for a certain month and station, $concN_i$ is the concentration in µmol and $flow_i$ the flow in m^3 $month^{-1}$. Since only flow rates for Station 1 and 6 were available, the flow rates of station 1 were used for 1-4 and for 5 and 6 those of station 6 – according to the observed alteration of the river bed.

To calculate the relative proportion of potential NO_3^- sources we presumed three candidate sources: NO_3^- from a pristine forest region ($\delta^{15}N$ = -3‰, $\delta^{18}O$ = 2‰), typical for the Harz mountains [32], atmospheric deposition in Germany ($\delta^{15}N$ = 0.4‰, $\delta^{18}O$ = 75‰) [8] and runoff from agricultural land (including manure fertilization) ($\delta^{15}N$ = 13.9‰, $\delta^{18}O$ = 3.4‰) [2].

3. Results

3.1 *DIN concentrations*

NO_3^- concentration increased downstream during all seasons (Fig. 2a). Pristine NO_3^- concentrations (station 1) ranged from 15 µM to 55 µM with the highest concentration in spring and lowest in autumn. Along the transect from station 1 to 5, NO_3^-concentration increased up to 160 to 200 µM, with the highest values being recorded in the summer. There was no further increase between stations 5 and 6, and even a slight decrease in concentration between these stations in autumn and winter. In summer, NO_3^- concentration peaked at station 4 downstream the WWTP, with NO_3^- concentrations of > 400 µM.

NH_4^+ concentration was below 1 µM at stations 1 to 3, but showed a peak at station 4 from spring to autumn (Fig.2b), with a maximum value of 70 µM in summer. The NH_4^+ concentration then dropped again to 1 to 4 µM further downstream.

NO_2^- was only detectable at station 4 (3 µM in summer, 2 µM in, autumn and 1µM spring). NO_2^- concentration was always < 1% of NO_3^- concentration and its effect on isotope determinations was therefore negligible.

[Figure 2 near here]

3.2 *Dual stable isotopes of NO_3^- ($\delta^{15}N$ and $\delta^{18}O$)*

$\delta^{15}N_{NO3}$ at the pristine station 1 was approximately 0‰ in summer and autumn, and -1‰ to -2.5‰ in winter and spring. $\delta^{18}O_{NO3}$ values were relatively high and values ranged from 4‰ to 8‰. $\delta^{15}N_{NO3}$ at station 2 and 3 were always similar to each other and relatively enriched (~4‰ to 6.5‰) in comparison to station 1. $\delta^{15}N_{NO3}$ was higher in summer and autumn (6‰ - 7‰) than in winter and spring (1‰ - 5‰). The $\delta^{18}O_{NO3}$ was approximately 2‰ at all seasons, except in spring where it reached 4‰. At station 4, 5 and 6, dual isotope values of nitrate were similar in winter and spring. Relative to the upstream stations 2 and 3, $\delta^{15}N_{NO3}$ values were elevated (up to 10‰) and $\delta^{18}O_{NO3}$ was slightly enriched (3‰ - 4‰). This pattern was evident in all seasons, with the one exception being in summer: While $\delta^{15}N_{NO3}$ at station 4 increased to 15‰, there was no immediate effect on $\delta^{18}O_{NO3}$, which remained stable at 1.5‰ and increased further downstream to ~6‰ (Fig. 3a, b).

Generally, $\delta^{15}N$ values in summer and autumn were elevated relative to winter and spring values. The $\delta^{18}O$ values were highest in spring, but the seasonal variation was less pronounced than for $\delta^{15}N$ (Fig.3).

[Figure 3 near here]

3.3 *NO_3^- turnover in core incubations*

Sediment cores for incubations were taken at the pristine station 1 and at the downstream station 6 in spring, summer and autumn.

In general, NO_3^- consumption (the sum of assimilation and denitrification) and NO_3^- production (i.e. nitrification) were significantly lower at the pristine station than at the agriculturally impacted station.

We did not measure significant biological NO_3^- processing in spring at the pristine river site (Fig.4a). In summer, NO_3^- production (i.e. nitrification) was active (306 ± 133 µmol N m^{-2} d^{-1}) and significantly ($p = 0.03$) exceeded NO_3^- consumption (109 ± 106 µmol N m^{-2} d^{-1}). In autumn, NO_3^- consumption increased (442 ± 189 µmol N m^{-2} d^{-1}) and at this time of year significantly ($p = 0.01$) exceeded NO_3^- production (199 ± 199 µmol N m^{-2} d^{-1}). (Fig.4a)

At the agriculturally impacted station 6 (Fig.4b), NO_3^- consumption was higher than NO_3^- production in all seasons, although only significantly in spring ($p = 0.04$) and summer ($p = 0.04$) (Fig.4b). NO_3^- production ranged from 869 to 1589 µmol N m^{-2} d^{-1}, whereas consumption rates in spring and summer clearly exceeded production with rates around 8400 µmol m^{-2} d^{-1} (spring: 8068 ± 2052 µmol N m^{-2} d^{-1} , summer: 8842 ± 1513 µmol N m^{-2} d^{-1}).

[Figure 4 near here]

3.4 *IMM and GIS*

A principal component analysis (PCA) explains 94.6% of the variance in the first two principle components and reveals that the covariation of ^{18}O and ^{15}N of our samples along the transect can be divided into 3 groups, following a gradient of anthropogenic impact: comprised of the pristine station 1, transient stations 2 & 3 with an intermediate anthropogenic impact, and mainly agricultural stations 4 to 6.

A GIS-based analysis of the catchment of the suite of sampling stations confirms this land-use gradient (Tab.3). There is no anthropogenic influence at the pristine station 1, the catchment of stations 2 and 3 is comprised of 20.6% anthropogenic land use, and at stations 4-6, this portion rises to 47.8%.

The IMM yields a pristine influence of 89.7% at the first station, which decreases up to 63.3% in the transient area and drops to 19% in the agricultural area. The atmospheric influence is 6.5% in the pristine region, decreasing to 0.8% in the transient stations and increasing slightly in the agricultural area (1.4%). The influence of agriculture increases continuously up to 79.6% in the agricultural part (transient area: 35.9%, pristine area: 3.8%).

[Table 3 near here]

4. Discussion

4.1 *NO_3^- source assessment using an isotope mixing model*

Our primary goal was to discern the relationship between external DIN sources vs. internal processing, using a small river as a model system, because we expected that source attribution in this case should be possible.

If our assumptions regarding the N-sources in the catchment are correct, the load weighted mean isotope values should reflect the GIS-based data, even though atmospheric deposition is missing in our GIS data set.

The IMM shows that direct atmospheric deposition is only a relevant NO_3^- source at the pristine station and almost 90% of the NO_3^- from this station can be attributed to pristine terrestrial sources, e.g. soil nitrification (Tab.3). $\delta^{15}N_{NO3}$ (-1.3‰ to 0.6‰) and $\delta^{18}O_{NO3}$ values (4.4‰ to 5.3‰) with corresponding NO_3^- concentrations of 17.5 µM to 38 µM in summer, autumn and winter are in the range for pristine sites [33] (Fig. 2 and 3). Depleted $\delta^{15}N_{NO3}$ and enriched $\delta^{18}O_{NO3}$ values are typical for this region [32] and reflect a forest dominated catchment [34] with NO_3^- derived from nitrification in pristine soils [7, 9]. The impact of atmospheric deposition is evident in spring, when depleted $\delta^{15}N_{NO3}$ (-2.7‰) and enriched $\delta^{18}O_{NO3}$ (7.6‰) values indicate a dilution with snowmelt [35, 36, 37] (Fig. 3).

Mayer et al. [6] estimated that NO_3^- in forested watersheds is almost completely derived from soil nitrification processes, with an additional minor influence of atmospheric deposition. They

find that a direct attribution of atmospheric deposition as the dominant N input can only be determined if $\delta^{18}O_{NO3}$ values over 15‰ are measured at low NO_3^- concentrations. In contrast, $\delta^{18}O_{NO3}$ values lower than 15‰ indicate no direct contribution of atmospheric deposition to riverine NO_3^-. The main reason for that is intensive cycling of NO_3^- derived from atmospheric deposition, which rapidly alters the atmospheric $\delta^{18}O_{NO3}$ signature already after one immobilization/mineralization cycle [6]. This must also be the case at station 1, in the forest dominated area of the Holtemme river, where relatively higher $\delta^{18}O_{NO3}$ values were measured at lowest NO_3^- concentrations, but the influence of direct atmospheric deposition to riverine NO_3^- concentration was barely visible in isotope values of $\delta^{18}O_{NO3}$ (4.5‰ to 6‰) or, consequently, in results of IMM output (atmospheric deposition: 6.5%). Thus, it is highly likely that internal N cycling plays a major role in this forested dominated area, making it difficult to estimate the exact effect of atmospheric deposition because the ^{18}O signal is rapidly removed during nitrate assimilation in catchments.

Nevertheless, Voss et al. [17] determined significant, albeit low, atmospheric influences in rivers receiving >50% N from agricultural runoff. These authors used deviating end-member values for atmospheric deposition ($\delta^{15}N_{NO3}$: 0.1‰ and $\delta^{18}O_{NO3}$: 51.7‰) [9], because different analytical methods (denitrifier vs. silver nitrate) yield substantially different $\delta^{18}O$ signatures [7]. The denitrifier method always showed $\delta^{18}O$ values >60‰ for atmospheric NO_3^-, whereas $\delta^{18}O_{NO3}$ isotope ratios measured with the silver nitrate method can be lower, especially at low NO_3^- concentrations, which is attributed to different reactions with oxygen, e.g. exchange with O in glass vials or a contamination by other O-bearing materials in the silver oxide [38]. To check the IMM sensitivity regarding the isotope signature of atmospheric deposition measured with the silver nitrate method, another IMM was applied using the source signatures applied in Voss et al. [17]. The percentage of atmospheric influence increased slightly to 9.5% at station 1, but remained negligible in the rest of the river, suggesting that atmospheric deposition is only a minor source of direct N-deposition to the Holtemme.

The GIS data indicates that stations 2 and 3 reflect a transient state with medium anthropogenic impact (Tab.3). Isotope signatures of 1‰ to 7‰ for $\delta^{15}N_{NO3}$ and 2‰ to 3.5‰ for $\delta^{18}O_{NO3}$ (Fig. 3) are consistent with values reported for NO_3^- derived from soil organic N [7]. Winter data values of $\delta^{15}N_{NO3}$ are ~6‰, and suggest that, in comparison to station 1, anthropogenic discharge, e.g. manure and septic waste, increase in significance. Overall, this is supported by the

IMM results where agricultural NO_3^- increases from 3.8% to 35.9%, but the pristine influence is still dominant (63.3%).

The agricultural part of the river (stations 4 to 6) showed an increase in NO_3^- concentration in all seasons. NO_3^- increase is mainly caused by diffuse inputs rather than by nitrification in the river, which is indicated by almost no change of NO_3^- isotopes in winter and spring (Fig. 3). $\delta^{15}N_{NO3}$ values >9‰ indicate a dominance of waste water [39], and the $\delta^{18}O_{NO3}$ values fall within a range typical of agricultural sites (2‰ to 5‰) (Fig.3), which we also attribute to an input of fertilizer and soil- or manure-derived NO_3^- [2, 10].

Due to its relatively limited importance (max. 6.5%), we assumed that the omission of atmospheric deposition as a NO_3^- source in the GIS data would not seriously affect the agreement of IMM and land use data. 'Indeed, the model overall agrees with land-use data, but the impact of agriculture at stations 2+3 and stations 4-6 is excessive and differs by up to 30% from GIS data (Tab.3). Such a poor agreement of IMM and land use data sets of pristine sources was also found previously in the Baltic sea catchment [17]. The authors noted that the reliability of GIS data for source attribution was rarely tested and our results confirm this: The IMM output suggest an amount of anthropogenic N that is disproportionate to land-use. In general, the application of the IMM over the whole river (Tab.3 'total') showed that the NO_3^- concentration of the watershed is mainly influenced by agriculture, with a subordinate role of NO_3^- from pristine sources, while atmospheric deposition only played a minor role. This result and the accompanying isotope values are consistent with data from other rivers that are influenced by agriculture, e.g. the Warnow River [9], and thus appears more reliable than the GIS based source attribution.

4.2 *N-turnover in the river*

We investigated NO_3^- production (i.e. nitrification) and consumption (i.e. the sum of assimilation and denitrification) in the river to assess the effect of sedimentary processes on DIN concentration – and potentially, isotope composition - in the water column. The balance of nutrients in a river and the proportion of N-retention and elimination can be altered by external factors. It can be enhanced by additional nutrient input, temperature rise and/or organic matter supply [40, 41, 42] which is a limiting parameter for heterotrophic denitrification [43, 44].

344 *4.2.1. Pristine station*

345 At the pristine station, NO_3^- production and consumption rates were low, and did not exceed a
346 production value of 306 µmol N m^{-2} d^{-1}. These lower production rates correspond closely to
347 other studies in forest dominated streams that found rates of 0 to 3570 µmol N m^{-2} d^{-1} [4, 40]
348 while NO_3^- consumption seems to be highly variable in pristine areas, and can be as high as to
349 51143 µmol N m^{-2} d^{-1} [3, 40, 45]. Our results at the pristine site clearly fall in the lower range of
350 reported values.

351 In spring, there was no detectable N-turnover (i.e. nitrification or NO_3^- consumption) in sediment
352 cores, probably due to low temperature (3.7°C) in the water column that impeded biological
353 activity. Although the chosen incubation temperature was higher than the *in situ* temperature,
354 this temperature increase had no measureable effect on the incubation experiment in this season.
355 We assume that that the other factors, like organic matter quality or turnover activity of the
356 microbial community, play a more important role than temperature in this case [e.g. 44, 46, 47].
357 In contrast to spring measurements, turnover rates increased in summer and autumn, showing a
358 strong trend towards NO_3^- consumption in autumn. This trend is somewhat surprising, as we
359 expected assimilation (which, in our assessment, is part of NO_3^- consumption) to be highly active
360 in summer based on higher phytoplankton activity [48, 49]. However, it seems in this case that
361 the role of NO_3^- production increased in significance, which has been found previously in small
362 streams in forested catchments [4]. We assume that NO_3^- production is fuelled by high
363 ammonification providing ample NH_4^+ for nitrification [4]. Apparently, the impact of nutrients
364 provided by soil, or by remineralization of planktonic organic matter in the sediment, e.g. NH_4^+
365 from ammonification, fosters nitrification and thus has a stronger impact on NO_3^- concentration
366 in this river section during summer seasons than phytoplankton activity.

367 In autumn, NO_3^- consumption dominated over production. While we cannot discern the role of
368 denitrification and assimilation, we hypothesize that in this case, denitrification dominates and
369 that phytoplankton activity is lower. This is supported by chlorophyll a measurements in the
370 nearby Bode River (Harz mountains), where primary production activity decreases at this time of
371 the year [50]. Lower primary production and an increase in periphyton in autumn can lower the
372 amount of oxygen that diffuses into the sediment [51]. Denitrification at this time is likely

stimulated by an increase of benthic organic carbon sources as an electron donor [43, 44, 52], stemming from phytoplankton production in summer.

4.2.2. Agricultural station

Contrary to the pristine river section, NO_3^- production and consumption rates in the agricultural area were high (up to 8842 µmol N m^{-2} d^{-1}), indicating that the additional nutrient input promotes turnover rates [51]. Nevertheless, NO_3^- consumption was the predominant turnover process in every season, suggesting that NO_3^- limitation is released and there is ample organic substrate fuelling denitrification [3, 42, 51]. Highest turnover rates of both, NO_3^- production and consumption, were measured in summer while lowest turnover rates were measured in autumn.

In spring, we found that NO_3^- consumption at the agricultural site clearly exceeded production. This may be due to higher temperatures (Tab.1) compared to the pristine station, promoting microbiological processing, and maybe additional inputs of NO_3^- and organic material due to the human and animal waste [41, 44]. This can release the limitation of denitrification by organic matter and nutrient loads and hence, will lead to an increase in consumption rates.

In autumn, NO_3^- consumption decreases. As with the pristine section, we expect assimilation to decrease at this time of the year, so that the remaining uptake should mainly be due to denitrification.

4.3 *The role of nitrification as an internal nitrate source*

Another focal point of our study was the role of nitrification, along the stream. Nitrification dominated over NO_3^- consumption at the pristine station in summer but gross rates were low in this river section (199 to 306 µmol N m^{-2}). In addition to the overall increase in NO_3^- consumption in the agricultural area, we also saw a rise in NO_3^- production with production rates between 869 to 1589 µmol N m^{-2}. The magnitude appeared to be linked to external inputs: NO_3^- production was highest in summer, and at this time, we also found high DIN concentrations downstream the WWTP. We assume that high NH_4^+ concentrations in the WWTP are a source for intense nitrification in the river [40, 53]. Based on the lower water discharge in summer, it is likely that the additional amount of NH_4^+ is less diluted than in other seasons, increasing the average NH_4^+ concentration in the river so that it is still detectable at the last station of the

transect. Therefore, NO_3^- production rates in the agricultural section are controlled by external nutrient inputs, in this case especially by the WWTP. This additional NO_3^- can then in turn lead to a further increase in NO_3^- consumption. However, the direct effect of this dissolved NO_3^- due to internal production may be minor here, because our rate measurements suggest that consumption exceeds production by a factor of 4 – 5 (Tab. 3) and such additional effect due to coupled nitrification-denitrification appears to be of limited importance.

Like organic matter supply, the additional NO_3^- may stimulate sedimentary NO_3^- consumption [51, 54, 55] which is the case in the agricultural part of the river (Fig. 4). However, increase in consumption does not significantly affect NO_3^- concentration in the water column: Neither a decrease in NO_3^- concentration nor an increase in isotopic composition of NO_3^- was measureable in the surface water (see Fig. 2 and 3). The minor influence of consumption on NO_3^- concentration in the water column is also reflected in the estimated overall consumption in the river. Estimation was done by multiplying the determined consumption rate (µM N m^{-2}) from the incubation experiment with the estimated sediment surface area of our transect (97650m^2) and set to relation to the measured NO_3^- load in the river (NO_3^- concentration in µM multiplied by the water discharge in m^3/s). It showed that only ~ 9% of external NO_3^- inputs were consumed in annual average. Along with the lack of NO_3^- concentration decrease and measureable changes in isotopic composition, the low percentage of NO_3^- consumption additionally demonstrates that the influence of sedimentary consumption on NO_3^- loss in the water column decreases, even though an overall increase in NO_3^- consumption rates was measured in the agricultural section.

Hence, we find that sedimentary NO_3^- consumption only had a minor influence on water column NO_3^- concentration and thus, was inefficient in counteracting excess NO_3^- loads [51, 55]. In addition, even in such a small river and in cases where we found high sedimentary consumption, effects of internal biological turnover on water column isotopic composition were barely detectable and apparently masked by external N inputs.

Summing up, based on the IMM results, isotope measurements in the water column and the incubations, the NO_3^- load along the river is mainly affected by diffuse inputs rather than by biological activities. This assumption is underlined by the measured winter data which also shows an increase in NO_3^- concentration and isotopic enrichment from the pristine to the agricultural section (Fig. 2 and 3). Since almost no biological activity is expected during winter

time [e.g. 56, 57], measured NO_3^- concentration increase, as well as the isotopic enrichment, must be due to anthropogenic discharge rather than by biological nitrogen turnover. Hence, the supplementary input of anthropogenic derived NO_3^- seemed to be the major contributor to the riverine NO_3^- load.

5. Conclusions

As expected, we find that the source of NO_3^- in this small river, changes along the transect. There was no visible effect of internal processing on isotope composition in the water column outside the pristine area, except a minor effect of WWTP discharge in summer. The importance of NO_3^- derived from soil nitrification in forest soils decreases, and the influence of anthropogenic derived NO_3^- gains more weight. The IMM data show that in the pristine river section, NO_3^- derived from forest soil nitrification acts as a major contributor to riverine NO_3^-, but this percentage decreases by about 70% downstream with increasing human land use.

In the pristine area, we find that NO_3^- production at times exceeds NO_3^- consumption, whereas a shift towards dominant NO_3^- consumption occurred in the anthropogenically impacted section during all seasons, independent of seasonal factors. Nevertheless, this caused no visible change in NO_3^- concentration or isotope composition in the water column in the agricultural area. This suggests that consumption only inefficiently removed N in the surface water, and that the filter capacity of the sediment was exhausted.

Thus, even in a small river, the enhanced consumption rate in the sediment cannot cope with anthropogenic derived NO_3^- loads and cannot reduce the potential for eutrophication. Instead, we find that additional anthropogenic DIN inputs not only increase consumption, but also production rates, which at times had a notable effect on water column NO_3^- concentration and isotope composition. This increased NO_3^- input can have additional adverse effects on rivers of higher orders.

6. Acknowledgments

We thank A. Bratek and T. Weinzierl for GIS analysis. M. Ankele, C. Naderipour and R. Lendt are gratefully acknowledged for their help with field work and sample analyses.

7. References

1. Seitzinger SP. Denitrification in freshwater and coastal marine ecosystems: ecological and geochemical significance. Limnol Oceanogr. 1988;33(4part2):702-724.
2. Aravena R, Evans M, Cherry JA. Stable isotopes of oxygen and nitrogen in source identification of nitrate from septic systems. Groundwater. 1993;31(2):180-186.
3. Schiller Dv, Martí E, Riera JL. Nitrate retention and removal in Mediterranean streams bordered by contrasting land uses: a ^{15}N tracer study. Biogeosciences. 2009;6(2):181-196.
4. Starry OS, Valett HM, Schreiber ME. Nitrification rates in a headwater stream: influences of seasonal variation in C and N supply. J N Am Benthol Soc. 2005 2005/12/01;24(4):753-768. doi: 10.1899/05-015.1.
5. Burns DA, Kendall C. Analysis of $\delta^{15}N$ and $\delta^{18}O$ to differentiate NO_3^- sources in runoff at two watersheds in the Catskill Mountains of New York. Water Resour Res. 2002;38(5):9-1-9-11.
6. Mayer B, Boyer EW, Goodale C, et al. Sources of nitrate in rivers draining sixteen watersheds in the northeastern US: Isotopic constraints. Biogeochemistry. 2002;57(1):171-197.
7. Kendall C, Elliott EM, Wankel SD. Tracing anthropogenic inputs of nitrogen to ecosystems. Stable isotopes in ecology and environmental science. 2007;2:375-449.
8. Beyn F, Matthias V, Dähnke K. Changes in atmospheric nitrate deposition in Germany – An isotopic perspective. Environ Pollut. 2014 11//;194:1-10. doi: http://dx.doi.org/10.1016/j.envpol.2014.06.043.
9. Deutsch B, Mewes M, Liskow I, et al. Quantification of diffuse nitrate inputs into a small river system using stable isotopes of oxygen and nitrogen in nitrate. Org Geochem. 2006 10//;37(10):1333-1342. doi: http://dx.doi.org/10.1016/j.orggeochem.2006.04.012.
10. Chang CC, Kendall C, Silva SR, et al. Nitrate stable isotopes: tools for determining nitrate sources among different land uses in the Mississippi River Basin. Can J Fish Aquat Sci. 2002;59(12):1874-1885.
11. Wassenaar LI. 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. 1995;10(4):391-405. doi: http://dx.doi.org/10.1016/0883-2927(95)00013-A.
12. Granger J, Sigman DM, Needoba JA, et al. Coupled nitrogen and oxygen isotope fractionation of nitrate during assimilation by cultures of marine phytoplankton. Limnol Oceanogr. 2004;49:1763-1773.
13. Böttcher J, Strebel O, Voerkelius S, et al. Using isotope fractionation of nitrate-nitrogen and nitrate-oxygen for evaluation of microbial denitrification in a sandy aquifer. J Hydrol. 1990;114(3):413-424.
14. Mengis M, Schif S, Harris M, et al. Multiple geochemical and isotopic approaches for assessing ground water NO_3^- elimination in a riparian zone. Groundwater. 1999;37(3):448-457.
15. Brandes JA, Devol AH. Isotopic fractionation of oxygen and nitrogen in coastal marine sediments. Geochim Cosmochim Acta. 1997;61(9):1793-1801.
16. Lehmann MF, Sigman DM, Berelson WM. Coupling the $^{15}N/^{14}N$ and $^{18}O/^{16}O$ of nitrate as a constraint on benthic nitrogen cycling. Mar Chem. 2004 8//;88(1–2):1-20. doi: http://dx.doi.org/10.1016/j.marchem.2004.02.001.

17. Voss M, Deutsch B, Elmgren R, et al. Source identification of nitrate by means of isotopic tracers in the Baltic Sea catchments. Biogeosciences. 2006;3(4):663-676.
18. Johannsen A, Dähnke K, Emeis K. Isotopic composition of nitrate in five German rivers discharging into the North Sea. Org Geochem. 2008 12//;39(12):1678-1689. doi: http://dx.doi.org/10.1016/j.orggeochem.2008.03.004.
19. Jenkins MC, Kemp WM. The coupling of nitrification and denitrification in two estuarine sediments. Limnol Oceanogr. 1984;29(3):609-619.
20. Nishio T, Komada M, Arao T, et al. Simultaneous determination of transformation rates of nitrate in soil. Jpn Agric Res Q. 2001;35(1):11-17.
21. Koike I, Hattori A. Simultaneous determinations of nitrification and nitrate reduction in coastal sediments by a ^{15}N dilution technique. Appl Environ Microbiol. 1978;35(5):853-857.
22. Nishio T, Koike I, Hattori A. Estimates of denitrification and nitrification in coastal and estuarine sediments. Appl Environ Microbiol. 1983;45(2):444-450.
23. Döring J. Zu den Klimaverhältnissen im östlichen Harzvorland. Hercynia-Ökologie und Umwelt in Mitteleuropa. 2004;37(2):137-154.
24. Hansen HP, Koroleff F. Determination of nutrients. Methods of Seawater Analysis: Wiley-VCH Verlag GmbH; 2007. p. 159-228.
25. Casciotti K, Sigman D, Hastings MG, et al. Measurement of the oxygen isotopic composition of nitrate in seawater and freshwater using the denitrifier method. Anal Chem. 2002;74(19):4905-4912.
26. Sigman D, Casciotti K, Andreani M, et al. A bacterial method for the nitrogen isotopic analysis of nitrate in seawater and freshwater. Anal Chem. 2001;73(17):4145-4153.
27. Casciotti K, McIlvin M. Isotopic analyses of nitrate and nitrite from reference mixtures and application to Eastern Tropical North Pacific waters. Mar Chem. 2007;107(2):184-201.
28. Trimmer M, Risgaard-Petersen N, Nicholls JC, et al. Direct measurement of anaerobic ammonium oxidation (anammox) and denitrification in intact sediment cores. Mar Ecol Prog Ser. 2006;326:37-47.
29. Crowe SA, Canfield DE, Mucci A, et al. Anammox, denitrification and fixed-nitrogen removal in sediments from the Lower St. Lawrence Estuary. Biogeosciences. 2012;9(11):4309.
30. Cowan J, Pennock J, Boynton WR. Seasonal and interannual patterns of sediment-water nutrient and oxygen fluxes in Mobile Bay, Alabama (USA): regulating factors and ecological significance. Mar Ecol Prog Ser. 1996;141:229-245.
31. Phillips DL, Koch PL. Incorporating concentration dependence in stable isotope mixing models. Oecologia. 2002;130(1):114-125.
32. Mueller C, Krieg R, Merz R, et al. Regional nitrogen dynamics in the TERENO Bode River catchment, Germany, as constrained by stable isotope patterns. Isot Environ Health Stud. 2016;52(1-2):61-74.
33. Durka W, Schulze E-D, Gebauer G, et al. Effects of forest decline on uptake and leaching of deposited nitrate determined from ^{15}N and ^{18}O measurements. Nature. 1994;372(6508):765-767.
34. Mayer B, Bollwerk SM, Mansfeldt T, et al. The oxygen isotope composition of nitrate generated by nitrification in acid forest floors. Geochim Cosmochim Acta. 2001 8/15/;65(16):2743-2756. doi: http://dx.doi.org/10.1016/S0016-7037(01)00612-3.

35. Hastings MG, Steig E, Sigman D. Seasonal variations in N and O isotopes of nitrate in snow at Summit, Greenland: Implications for the study of nitrate in snow and ice cores. Journal of Geophysical Research: Atmospheres. 2004;109(D20).
36. Pardo LH, Kendall C, Pett-Ridge J, et al. Evaluating the source of streamwater nitrate using $\delta^{15}N$ and $\delta^{18}O$ in nitrate in two watersheds in New Hampshire, USA. Hydrol Process. 2004;18(14):2699-2712.
37. Piatek KB, Mitchell MJ, Silva SR, et al. Sources of nitrate in snowmelt discharge: evidence from water chemistry and stable isotopes of nitrate. Water Air Soil Pollut. 2005;165(1-4):13-35.
38. Révész K, Böhlke J-K. Comparison of $\delta^{18}O$ measurements in nitrate by different combustion techniques. Anal Chem. 2002;74(20):5410-5413.
39. Burns DA, Boyer EW, Elliott EM, et al. Sources and transformations of nitrate from streams draining varying land uses: evidence from dual isotope analysis. J Environ Qual. 2009;38(3):1149-1159.
40. Bernhardt ES, Hall Jr RO, Likens GE. Whole-system estimates of nitrification and nitrate uptake in streams of the Hubbard Brook Experimental Forest. Ecosystems. 2002;5(5):419-430.
41. Zhu S, Chen S. The impact of temperature on nitrification rate in fixed film biofilters. Aquacult Eng. 2002;26(4):221-237.
42. Richardson WB, Strauss EA, Bartsch LA, et al. Denitrification in the Upper Mississippi River: rates, controls, and contribution to nitrate flux. Can J Fish Aquat Sci. 2004;61(7):1102-1112.
43. Christensen PB, Nielsen LP, Sørensen J, et al. Denitrification in nitrate-rich streams: Diurnal and seasonal variation related to benthic oxygen metabolism. Limnol Oceanogr. 1990;35(3):640-651.
44. Newcomer TA, Kaushal SS, Mayer PM, et al. Influence of natural and novel organic carbon sources on denitrification in forest, degraded urban, and restored streams. Ecol Monogr. 2012;82(4):449-466.
45. Mulholland PJ, Tank JL, Sanzone DM, et al. Nitrogen cycling in a forest stream determined by a ^{15}N tracer addition. Ecol Monogr. 2000;70(3):471-493.
46. Strauss EA, Lamberti GA. Effect of dissolved organic carbon quality on microbial decomposition and nitrification rates in stream sediments. Freshw Biol. 2002;47(1):65-74.
47. Johnson LT, Royer TV, Edgerton JM, et al. Manipulation of the dissolved organic carbon pool in an agricultural stream: responses in microbial community structure, denitrification, and assimilatory nitrogen uptake. Ecosystems. 2012;15(6):1027-1038.
48. Basu B, Pick F. Phytoplankton and zooplankton development in a lowland, temperate river. J Plankton Res. 1997;19(2):237-253.
49. Wehr JD, Descy JP. Use of phytoplankton in large river management. J Phycol. 1998;34(5):741-749.
50. Rode M, Kiwel U. How to use new online monitoring techniques to improve river water quality modeling at the river reach scale. In: R. Seppelt AAV, S. Lange, D. Bankamp, editor. International Congress on Environmental Modelling and Software Managing Resources of a Limited Planet, Sixth Biennial Meeting; July 1-5; Leipzig, Gemany2012. p. 3038-3041.

51. Mulholland PJ, Helton AM, Poole GC, et al. Stream denitrification across biomes and its response to anthropogenic nitrate loading. Nature. 2008;452(7184):202-205.
52. Arango CP, Tank JL, Schaller JL, et al. Benthic organic carbon influences denitrification in streams with high nitrate concentration. Freshw Biol. 2007;52(7):1210-1222.
53. Peterson BJ, Wollheim WM, Mulholland PJ, et al. Control of nitrogen export from watersheds by headwater streams. Science. 2001;292(5514):86-90.
54. Deek A, Emeis K, van Beusekom J. Nitrogen removal in coastal sediments of the German Wadden Sea [journal article]. Biogeochemistry. 2012;108(1):467-483. doi: 10.1007/s10533-011-9611-1.
55. Marti E, Aumatell J, Godé L, et al. Nutrient retention efficiency in streams receiving inputs from wastewater treatment plants. J Environ Qual. 2004;33(1):285-293.
56. Dawson R, Murphy K. The temperature dependency of biological denitrification. Water Res. 1972;6(1):71-83.
57. Pfenning KS, McMahon PB. Effect of nitrate, organic carbon, and temperature on potential denitrification rates in nitrate-rich riverbed sediments. J Hydrol. 1997 1997/01/01;187(3):283-295. doi: http://dx.doi.org/10.1016/S0022-1694(96)03052-1.

8. Tables and figure captions

Table 1. Ambient conditions during transect sampling: anthropogenic influences, river kilometre and water temperatures for each station, discharge of stations 1 and 6 (http://www.hochwasservorhersage.sachsen-anhalt.de/), and according weather conditions

Table 1

Season			Summer	Autumn	Winter	Spring
Date			6/24/2014	9/8/2014	2/6/2015	4/14/2015
Station	Anthropogenic influence	River km	Temp [°C]	Temp [°C]	Temp [°C]	Temp [°C]
1	Pristine	2.7	11.0	12.6	0.3	3.7
2	Transient - Urban	7.4	12.5	13.5	1.2	6.6
3	Transient - Urban	11.8	12.6	14.2	1.3	6.7
4	WWTP	16.2	16.7	16.4	3.4	8.4
5	Agriculture	18.5	17.1	16.1	3.1	9.1
6	Agriculture	24.4	16.4	15.8	2.7	9.4
Discharge station	Station name		Discharge [$m^3 s^{-1}$]	Discharge [$m^3 s^{-1}$]	Discharge [$m^3 s^{-1}$]	Discharge [$m^3 s^{-1}$]
1	Steinerne Renne		0.11	0.11	0.12	0.41
6	Mahndorf		0.36	1.07	1.48	2.20
Weather conditions			Mainly sunny	Mainly sunny	Snow covered	Snow melt

Table 2. Conditions during sediment core sampling and incubation experiments: river regulation, water depth and major sediment type; starting date of incubation experiment and incubation temperature

Table 2

	Anthropogenic influence	River state	Water depth	Sediment type	Incubation Temp [°C]		
Station					Summer 6/26/2014	Autumn 9/10/2014	Spring 4/16/2015
1	Pristine	natural	0.1-0.2m	gravel, sand, silt	16	16	12
6	Agriculture	regulated	0.5-0.7m	clay, silt	16	16	12

630 Table 3. Calculated values of the isotope mixing model (IMM) and the GIS-data based
631 calculations of areas influencing the watershed

632

633 Table 3

	IMM [%]			*GIS [%]*	
Station	**Pristine (forest region)**	**Atmospheric Deposition**	**Agricultural Land - Manure**	**Pristine**	**Agricultural & Urban Land**
1	89.7	6.5	3.8	100	0.0
2+3	63.3	0.8	35.9	79.4	20.6
4+5+6	17.6	1.2	81.2	52.2	47.8
total	21	1.1	77.9		

Source values for IMM

	Pristine (forest region)	**Atmospheric deposition**	**Agricultural Land –Manure fertilization**
$\delta^{15}N$ [‰]	-3 [(1)]	0.4 [(2)]	13.9 [(3)]
$\delta^{18}O$ [‰]	2 [(1)]	75 [(*)]	3.4 [(3)]

[(1)] [32] Mueller et al. 2015

[(2)] [8] Beyn et al. 2014 [(*)] F. Beyn, personal communication, 2014

[(3)] [2] Aravena et al. 1993

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Figure 1. Site map of the Holtemme River created in ArcGIS; different colors indicate land use classes (pristine, urban, agriculture), the sampling stations are located as black points: station 1 - pristine area, station 2 & 3 - urban area, station 4 - WWTP (brown rectangle), station 5 & 6 - agricultural area

Figure 2. DIN concentrations for each sampling point along the river transect over all seasons. (a) Nitrate concentration in µM during summer (rectangle), autumn (circle), winter (triangle) and spring (plus) (b) Ammonium concentration in µM at the according season

Figure 3. Seasonal profiles of $\delta^{15}N_{NO3}$ vs. $\delta^{18}O_{NO3}$ isotope values along the Holtemme River

Figure 4. Nitrate turnover rates at the pristine station 1 (a) and at the agriculturally impacted station 6 (b) from spring to autumn (note: different scales)