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

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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 δ$_{15}^N$ values (-5‰ to 5‰, [7]) with very high values of δ$_{18}^O$ (>60‰ [7, 8]), whereas runoff from agricultural soils or manure is isotopically enriched in δ$_{15}^N$ (>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 δ$_{15}^N$ and δ$_{18}^O$ 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 $^{15}N_{NO_3}$ 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\textsubscript{3}\textsuperscript{-} 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 \textsuperscript{15}N-labelled nitrate, is a useful tool to discern nitrification and NO\textsubscript{3}\textsuperscript{-} 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\textsubscript{3}\textsuperscript{-} isotope investigations to discern the effect between external sources vs. internal processes and their joint impact on the water column nitrate (NO\textsubscript{3}\textsuperscript{-}) and ammonium (NH\textsubscript{4}\textsuperscript{+}) concentrations and NO\textsubscript{3}\textsuperscript{-} 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\textsubscript{3}\textsuperscript{-} 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\textsubscript{3}\textsuperscript{-} 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\textsubscript{3}\textsuperscript{-} in comparison to NO\textsubscript{3}\textsuperscript{-} 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 km\textsuperscript{2} and an annual mean water discharge of 1.33 m\textsuperscript{3} s\textsuperscript{-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 3.7 cm; 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$_2$O) produced by denitrifying *Pseudomonas aureofaciens* (ATCC #13985). The N$_2$O 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
the overlying water had a NO$_3^-$ concentration comparable to site water, which had been
determined before. Three unlabelled cores remained and were used as control samples. All cores,
including the control samples, were then incubated for 24 hours in darkness. Samples, and
according control samples, were taken directly after label addition, after 8 and 24 hours by
creating a slurry where the reactive sediment layer (1 cm) was gently mixed into the overlying
water column [29]. At each point, three labelled replicates and one control without label addition
were sampled. To stop microbial activity, the slurry was filtered immediately (Minisart© NML,
0.45 µm, Sartorius) and samples were stored frozen until nutrient concentration and δ$^{15}$N$_{NO3}$
values were measured. All samples were analysed in duplicate and were calibrated similar to
above. The standard deviation was <0.3‰ (n>3) for natural abundance samples and 6‰ (n>3)
for enriched samples.

Rates were then determined by the temporal changes in the pool size and $^{15}$N abundances in the
$^{15}$N-amended NO$_3^-$ pool of the incubated cores. The NO$_3^-$ decrease is assumed to be NO$_3^-$
consumption.

2.4 Calculations

2.4.1 Isotope dilution model

In our assessment, we define NO$_3^-$ consumption as the sum of assimilation and denitrification,
and NO$_3^-$ production is defined as nitrification.

NO$_3^-$ production and consumption in cores were calculated based in the $^{15}$N isotope dilution
model [20, 21]. The following equations were used

\[
p = \frac{\ln(I_t/I_0)}{\ln(P_t/P_0)} (P_0-P_t/t)
\] (1)

where \( p \) is production in µmol L$^{-1}$ h$^{-1}$, \( t \) is incubation time, \( P_0 \) is the initial NO$_3^-$ concentration at
incubation time 0, \( P_t \) is the NO$_3^-$ concentration at time \( t \), and \( I_0 \) and \( I_t \) represent $^{15}$N atom excess.

NO$_3^-$ consumption was calculated by using the decrease of NO$_3^-$ concentration (\( P \)), the content of
$^{15}$N-NO$_3^-$ (\( p \)) and the natural abundance of $^{15}$N-NO$_3^-$ (\( k \)):

\[
c = \ln[(p_0-k_0P_0)/(p_t-k_tP_t)] (P_0-P_t)/\ln(P_0/P_t)/t
\] (2)

where \( c \) is the rate of consumption in µmol L$^{-1}$ h$^{-1}$. 
Both turnover rates were then converted into $\mu$mol 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 analysis

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:

\[
\delta^{15}N_H = f_F \delta^{15}N_F + f_D \delta^{15}N_D + f_A \delta^{15}N_A \\
\delta^{18}O_H = f_F \delta^{18}O_F + f_D \delta^{18}O_D + f_A \delta^{18}O_A \\
1 = f_F + f_D + f_A
\]

(3) (4) (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:

\[
\delta^{15}N_a = \frac{\sum_i \delta^{15}N_i \times concN_i \times flow_i}{\sum_i concN_i \times flow_i}
\]

(6)

$\delta^{15}N_i$ is the isotope value for a certain month and station, conc$N_i$ is the concentration in $\mu$mol 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 1 µM 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 ($306 \pm 133 \, \mu mol \, N \, m^{-2} \, d^{-1}$) and significantly ($p = 0.03$) exceeded $NO_3^-$ consumption ($109 \pm 106 \, \mu mol \, N \, m^{-2} \, d^{-1}$). In autumn, $NO_3^-$ consumption increased ($442 \pm 189 \, \mu mol \, N \, m^{-2} \, d^{-1}$) and at this time of year significantly ($p = 0.01$) exceeded $NO_3^-$ production ($199 \pm 199 \, \mu mol \, N \, m^{-2} \, d^{-1}$). (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 $\mu mol \, N \, m^{-2} \, d^{-1}$, whereas consumption rates in spring and summer clearly exceeded production with rates around 8400 $\mu mol \, m^{-2} \, d^{-1}$ (spring: $8068 \pm 2052 \, \mu mol \, N \, m^{-2} \, d^{-1}$, summer: $8842 \pm 1513 \, \mu mol \, N \, m^{-2} \, d^{-1}$).

[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 NO₃⁻ 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 NO₃⁻ source at the pristine station and almost 90% of the NO₃⁻ from this station can be attributed to pristine terrestrial sources, e.g. soil nitrification (Tab.3). δ¹⁵N(NO₃) (-1.3‰ to 0.6‰) and δ¹⁸O(NO₃) values (4.4‰ to 5.3‰) with corresponding NO₃⁻ 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 δ¹⁵N(NO₃) and enriched δ¹⁸O(NO₃) values are typical for this region [32] and reflect a forest dominated catchment [34] with NO₃⁻ derived from nitrification in pristine soils [7, 9]. The impact of atmospheric deposition is evident in spring, when depleted δ¹⁵N(NO₃) (-2.7‰) and enriched δ¹⁸O(NO₃) (7.6‰) values indicate a dilution with snowmelt [35, 36, 37] (Fig. 3).

Mayer et al. [6] estimated that NO₃⁻ 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 \( \delta^{18}O_{NO_3} \) values over 15‰ are measured at low NO\(_3^-\) concentrations. In contrast, \( \delta^{18}O_{NO_3} \) 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 \( \delta^{18}O_{NO_3} \) 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 \( \delta^{18}O_{NO_3} \) 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 \( \delta^{18}O_{NO_3} \) (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 (\( \delta^{15}N_{NO_3}: 0.1‰ \) and \( \delta^{18}O_{NO_3}: 51.7‰ \)) [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 \( \delta^{18}O_{NO_3} \) 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 \( \delta^{15}N_{NO_3} \) and 2‰ to 3.5‰ for \( \delta^{18}O_{NO_3} \) (Fig. 3) are consistent with values reported for NO\(_3^-\) derived from soil organic N [7]. Winter data values of \( \delta^{15}N_{NO_3} \) 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].
4.2.1. Pristine station

At the pristine station, NO$_3^-$ production and consumption rates were low, and did not exceed a production value of 306 µmol N m$^{-2}$ d$^{-1}$. These lower production rates correspond closely to other studies in forest dominated streams that found rates of 0 to 3570 µmol N m$^{-2}$ d$^{-1}$ [4, 40] while NO$_3^-$ consumption seems to be highly variable in pristine areas, and can be as high as to 51143 µmol N m$^{-2}$ d$^{-1}$ [3, 40, 45]. Our results at the pristine site clearly fall in the lower range of reported values.

In spring, there was no detectable N-turnover (i.e. nitrification or NO$_3^-$ consumption) in sediment cores, probably due to low temperature (3.7°C) in the water column that impeded biological activity. Although the chosen incubation temperature was higher than the in situ temperature, this temperature increase had no measurable effect on the incubation experiment in this season. We assume that the other factors, like organic matter quality or turnover activity of the microbial community, play a more important role than temperature in this case [e.g. 44, 46, 47].

In contrast to spring measurements, turnover rates increased in summer and autumn, showing a strong trend towards NO$_3^-$ consumption in autumn. This trend is somewhat surprising, as we expected assimilation (which, in our assessment, is part of NO$_3^-$ consumption) to be highly active in summer based on higher phytoplankton activity [48, 49]. However, it seems in this case that the role of NO$_3^-$ production increased in significance, which has been found previously in small streams in forested catchments [4]. We assume that NO$_3^-$ production is fuelled by high ammonification providing ample NH$_4^+$ for nitrification [4]. Apparently, the impact of nutrients provided by soil, or by remineralization of planktonic organic matter in the sediment, e.g. NH$_4^+$ from ammonification, fosters nitrification and thus has a stronger impact on NO$_3^-$ concentration in this river section during summer seasons than phytoplankton activity.

In autumn, NO$_3^-$ consumption dominated over production. While we cannot discern the role of denitrification and assimilation, we hypothesize that in this case, denitrification dominates and that phytoplankton activity is lower. This is supported by chlorophyll a measurements in the nearby Bode River (Harz mountains), where primary production activity decreases at this time of the year [50]. Lower primary production and an increase in periphyton in autumn can lower the 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


http://dx.doi.org/10.1016/j.envpol.2014.06.043.


http://dx.doi.org/10.1016/0883-2927(95)00013-A.


http://dx.doi.org/10.1016/j.marchem.2004.02.001.


### 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>
<thead>
<tr>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td>Station</td>
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<td>River km</td>
<td>Temp °C</td>
<td>Temp °C</td>
<td>Temp °C</td>
</tr>
<tr>
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<td>12.6</td>
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<td>2</td>
<td>Transient - Urban</td>
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<td>12.5</td>
<td>13.5</td>
<td>1.2</td>
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<td>3</td>
<td>Transient - Urban</td>
<td>11.8</td>
<td>12.6</td>
<td>14.2</td>
<td>1.3</td>
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<tr>
<td>4</td>
<td>WWTP</td>
<td>16.2</td>
<td>16.7</td>
<td>16.4</td>
<td>3.4</td>
</tr>
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<td>5</td>
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<td>17.1</td>
<td>16.1</td>
<td>3.1</td>
</tr>
<tr>
<td>6</td>
<td>Agriculture</td>
<td>24.4</td>
<td>16.4</td>
<td>15.8</td>
<td>2.7</td>
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<th>Discharge station</th>
<th>Station name</th>
<th>Discharge [m³ s⁻¹]</th>
<th>Discharge [m³ s⁻¹]</th>
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<td>Steinerne Renne</td>
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<td>0.11</td>
<td>0.12</td>
<td>0.41</td>
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<tr>
<td>6</td>
<td>Mahndorf</td>
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<td>1.07</td>
<td>1.48</td>
<td>2.20</td>
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</table>

Weather conditions

- 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>
<thead>
<tr>
<th>Station</th>
<th>Anthropogenic influence</th>
<th>River state</th>
<th>Water depth</th>
<th>Sediment type</th>
<th>Incubation Temp [°C]</th>
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<td>Summer 6/26/2014</td>
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<tr>
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<td>Pristine</td>
<td>natural</td>
<td>0.1-0.2m</td>
<td>gravel, sand, silt</td>
<td>16</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Autumn 9/10/2014</td>
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<tr>
<td></td>
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<td>16</td>
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<td></td>
<td></td>
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<td>Spring 4/16/2015</td>
</tr>
<tr>
<td>6</td>
<td>Agriculture</td>
<td>regulated</td>
<td>0.5-0.7m</td>
<td>clay, silt</td>
<td>16</td>
</tr>
<tr>
<td></td>
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<td>16</td>
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<td></td>
<td></td>
<td>12</td>
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</tbody>
</table>
Table 3. Calculated values of the isotope mixing model (IMM) and the GIS-data based calculations of areas influencing the watershed

Table 3

<table>
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<th>Station</th>
<th>IMM [%]</th>
<th>GIS [%]</th>
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<td>Pristine (forest region)</td>
<td>Atmospheric Deposition</td>
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<td>89.7</td>
<td>6.5</td>
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<td>2+3</td>
<td>63.3</td>
<td>0.8</td>
</tr>
<tr>
<td>4+5+6</td>
<td>17.6</td>
<td>1.2</td>
</tr>
<tr>
<td>total</td>
<td>21</td>
<td>1.1</td>
</tr>
</tbody>
</table>

Source values for IMM

<table>
<thead>
<tr>
<th>Pristine (forest region)</th>
<th>Atmospheric deposition</th>
<th>Agricultural Land – Manure fertilization</th>
</tr>
</thead>
<tbody>
<tr>
<td>δ¹⁵N [%]</td>
<td>-3 (¹)</td>
<td>0.4 (²)</td>
</tr>
<tr>
<td>δ¹⁸O [%]</td>
<td>2 (¹)</td>
<td>75 (*)</td>
</tr>
</tbody>
</table>

(¹) [32] Mueller et al. 2015
(³) [2] Aravena et al. 1993
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 δ^{15}N\textsubscript{NO}_3 vs. δ^{18}O\textsubscript{NO}_3 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).