ISOTOPE PATTERNS TO TRACE LARGE SCALE NITROGEN FLUXES AND FLOW DYNAMICS IN A EUROPEAN RIVER CATCHMENT

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Für meine Familie

Foreword

This thesis is a cumulative dissertation which includes three accepted publications. The general task of this project was developed at the Department Catchment Hydrology from the Helmholtz Centre for Environmental Research in Halle (Saale). Previous works which also use stable and radioactive isotopes as environmental tracers to recognize matter and solute fluxes as well as turn-over processes were conducted in much smaller river catchments or for totally different topics (e.g. development and chemistry of open-cast mine lakes). With this project, we would like to test the applicability of stable isotopes on meso- to large-scale river catchments on a regional catchment scale. An excellent investigation area was the **TER**restrial **EN**vironmental **O**bservatory (TERENO) in Central Germany. The Bode River catchment is a "playground" for research centres within the Helmholtz Association of German Research Centres. This made it possible to use existing knowledge and (further) develop cooperation. The main part of the work which included field work, laboratory work and interpretation of the results were achieved at the Helmholtz Centre for Environmental Research in Halle (Saale). Important cooperation partners were other departments at the UFZ like the Department of Computational Hydrosystems, Department of River Ecology, Department of Hydrogeology and the Department of Aquatic Ecosystem Analysis.

Now, I would like to say some words of thanks. First of all, I would like to thank the laboratory staff Petra Blümel, Martina Neuber, Silke Köhler, Gudrun Schäfer and Wolfgang Städter who helped me to prepare and analyze the cubic meters of water. Then, I would like to express my gratitude to my mentors and supervisors Prof. Ralf Merz and Dr. Kay Knöller for the continuous support and useful discussions. Also, I would like to thank scientific colleagues from other departments, Prof. Michael Rode, Dr. Andreas Musolff, Dr. Matthias Zink and Dr. Mario Brauns who gave me new stimuli for analysis of the data set and modeling issues.

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Abstract

The nitrate levels in the rivers of our regions have been characterized in recent decades by increasingly and/or constantly higher concentrations. The ever-improving sewage treatment plant technology can clarify settling waters excellently, but the nitrate loads in our waters remain too high in wide regions. High concentrations of nitrate lead to eutrophication, particularly in coastal areas of the marginal seas, such as the Baltic Sea and North Sea, which is reflected by the spread of blue-green algae, the reduction of dissolved oxygen in the water and the resulting dying of many animals and plants living in the water. Therefore, it is of the utmost importance to determine and quantify potential nitrate sources to discuss influencing factors in the nitrogen cycle and to evaluate their environmental relevance.

The challenge in this project was to take a sufficient number of water samples in a large and heterogeneous river basin area on a regular basis to assess the nitrate dynamics of the entire river system. During a period between 2012 and 2015, the river basin area of the Bode in the Harz region in Germany (total size 3200 km²) was investigated more closely. Through monthly and seasonal field campaigns, all relevant inflows (133 measuring points) were regularly sampled and field parameters were recorded. As a result, a previously unique, high-resolution data set for the interpretation of nutrient and flow dynamics could be generated. With the combined evaluation of nutrient loads and isotopic signatures, spatial and temporal source and process dynamics could be estimated. The smaller the sub-basins, the better these processes and source signatures could be determined. The study found that mainly agricultural areas with the use of large quantities of organic fertilizers are the main source of increasing nitrate emissions in the Bode River catchment. In the same way, inflows of purified wastewater from sewage treatment plants play a role in increasing nitrate loads. And the associated isotope signatures can be greatly altered, especially during low-water phases. The hydrological situation thus plays a decisive role for the mobilization and dynamics of nitrate in surface water. A significant natural reduction of the nitrate costs, in particular by denitrification, could not be observed in the surface water stream. Therefore, only preventive activities, such as coordinated land-use management, can contribute to the regulation of nitrate levels in river water.

Zusammenfassung

Die Nitratgehalte in den Flüssen unserer Regionen sind in den letzten Jahrzehnten durch steigende oder gleichbleibend erhöhte Konzentrationen geprägt. Die immer besser werdende Kläranlagentechnik kann Siedlungswässer exzellent klären, allerdings bleiben die Nitratfrachten in unseren Gewässern in weiten Regionen zu hoch. Hohe Konzentrationen an Nitrat führen gerade in den Küstenbereichen der Randmeere wie Ost- und Nordsee zur Eutrophierung, was durch die Verbreitung von Blaualgen, die Verringerung des gelösten Sauerstoffs im Wasser und dem daraus resultierenden Absterben vieler im Gewässer lebender Tiere und Pflanzen deutlich wird. Deshalb ist es von größter Notwendigkeit potentielle Nitratquellen zu bestimmen und zu quantifizieren, als beeinflussende Faktoren im Stickstoffkreislauf zu erörtern und deren Umweltrelevanz zu bewerten.

Die Herausforderung in diesem Projekt war es, in einem großen und heterogenen Flusseinzugsgebiet zeitlich und räumlich eine ausreichende Anzahl an Wasserproben regelmäßig zu nehmen, um Aussagen über die Nitratdynamik des gesamten Flusssystems treffen zu können. Während eines Zeitraumes zwischen 2012 und 2015 wurde das Flusseinzugsgebiet der Bode in der Region des Harzes in Deutschland (Gesamtgröße 3200 km²) näher untersucht. Durch monatliche und saisonale Feldkampagnen wurden alle relevanten Zuflüsse (133 Messstellen) regelmäßig beprobt und Feldparameter aufgenommen. Dadurch konnte ein bisher einmaliger, hochaufgelöster Datensatz zur Interpretation von Stoff- und Fließdynamiken generiert werden. Mit der kombinierten Auswertung von Stofffrachten und Isotopensignaturen konnten räumliche und zeitliche Quell- und Prozessdynamiken abgeschätzt werden. Je kleiner die Teileinzugsgebiete wurden, umso besser sind diese Prozesse und Quellsignaturen bestimmbar gewesen. Im Rahmen der Studie konnte festgestellt werden, dass vor allem landwirtschaftliche Flächen mit der Nutzung großer Mengen organischer Dünger die Hauptquelle für steigende Nitratfrachten im Flusssystem der Bode darstellen. Ebenso spielen gerade im Oberlauf, Zuflüsse gereinigter Abwässer aus Kläranlagen eine Rolle für steigende Nitratfrachten. Stofffrachten und zugehörigen Isotopensignaturen können dort insbesondere während Niedrigwasserphasen stark verändert sein. Die hydrologische Situation spielt demnach eine entscheidende Rolle für die Mobilisierung und Dynamik von Nitrat im Oberflächenwasser. Eine signifikante natürliche Minderung der Nitratfrachten, insbesondere durch Denitrifikation, konnte in den Oberflächenwässern selbst nicht beobachtet werden. Daher können zur Regulierung der Nitratgehalte im Flusswasser einzig Präventivmaßnahmen, wie ein koordiniertes Landnutzungsmanagement, beitragen.

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δ - delta

% - percent

‰ - per mil

List of Abbreviations

AIR - air nitrogen standard

conc. - concentration

e.g. - exempli gratia (for instance)

et al. - et alii (and others)

eq. - equation

EU - European Union

GMWL - Global Meteoric Water Line

IAEA - International Atomic Energy Agency

LHW - State Office of Flood Protection and Water Management, Saxony-Anhalt

LMWL - Local Meteoric Water Line

mHM - mesoscale Hydrological Model

R - isotope ratio

R^2 - coefficient of determination

SRU - German Advisory Council on the Environment

TERENO - Terrestrial Environmental Observatories

TVO - (German) Drinking Water Ordiance

UFZ - Helmholtz-Centre for Environmental Research

USGS - United States Geological Survey

VSMOW - Vienna Standard Mean Ocean Water

WWTP - waste water treatment plant

1 Introduction

1.1 *Nitrate in the environment as a hazard for aquatic systems*

Nitrogen fixed in nitrate is a necessary and harmless nutrient for plants. Although the atmosphere on earth consists of 78 % nitrogen (N_2), this energy source is not useable for plant growth. Therefore, plant-available nitrogen is the limiting factor for a more effective plant production (Ju, Xing et al. 2009, Vitousek, Naylor et al. 2009). In times of a continuous growing world population, increasing agricultural yields can just cover food requirements. With the beginning of industrial nitrogen fertilizer production by the Haber-Bosch process, the terrestrial biological nitrate fixation has doubled (Canfield, Glazer et al. 2010). Three major nitrate sources are mainly responsible for high NO_3^- concentrations in German lakes and rivers (Pattinson, García-Ruiz et al. 1998, Galloway, Aber et al. 2003, Zweimüller, Zessner et al. 2008):

(1) Mineral fertilizers
(2) Organic fertilizers of livestock (manure)
(3) Combustion of fossil fuels

Meanwhile, 26 % of all German groundwater bodies show a bad chemical status and exceed the limit values for the German drinking water Ordinance and the EU Drinking Water Directive, respectively (50 mg L^{-1}) (Völker, Mohaupt et al. 2016). Increasing nitrogen concentrations in surface and groundwater are still an unsolved problem in Germany (SRU 2015). Increasing nitrate concentrations could be responsible for serious health and environmental problems (Spalding and Exner 1993, Burow, Nolan et al. 2010). Nitrogen compounds like ammonia and nitrogen oxides endanger human health, biodiversity, climate as well as the quality of air, soil and water (SRU 2015). Therefore, nitrogen imports contribute to eutrophication (overfertilization) of lakes and seas (Prasuhn and Sieber 2005, Decrem, Spiess et al. 2007). Moreover, nitrogen oxides (NO und NO_2) are known as air pollutants which damage the respiratory tract. Nitrous oxide (N_2O) is a major compound of climate relevant greenhouse gas which damages the ozone layer in the atmosphere. High nitrate concentrations in drinking water affect human health and are suspected of forming carcinogenic nitrosamines.

Thereby, Germany has not managed to improve climate goals in air pollution, water conservation and nature protection, which could have financial consequences (ZEIT-ONLINE 2016). In 2016, the European Commission initiated infringement proceedings because Germany had not arranged sufficient measures to protect water bodies. If they do not start reducing nitrate pollutions effectively within the following months, legal proceedings could be taken to the European Court. If breeches of contract are ascertained, extensive penalty payments will be imposed. Therefore, it is very important that science and politics find effective ways to solve the nitrate pollutions together in the near future. In a report conducted by Germany's Advisory Council on the Environment (SRU) forty proposals for action are stated. Three of them have the highest priority:

(1) Develop an amendment to the Fertilizer Ordinance which regulates output of manure and digestate
(2) Establish an ecological tax for nitrogen surpluses from agriculture
(3) Further develop Europe's air quality policy to reduce high nitrogen oxides in the environment

To achieve these goals, the investigation of regional scale N-dynamics and trends considering hydrochemical and stable isotope observations is useful. Past research related to the quantitative assessment of the nitrogen balance in catchments is mainly focused on small catchments (Mulholland, Helton et al. 2008, Wexler, Goodale et al. 2014). Or they does not include the benefit of stable isotope observations (Musolff, Schmidt et al. 2015). Water stable isotopes were used recently in large European river catchments to investigate hydrological processes but with coarse resolution (Rank, Wyhlidal et al. 2014, Reckerth, Stichler et al. 2017). With the combination of a spatial and temporal monitoring for nitrate stable isotopes, water chemical parameters and subsequent hydrological modeling, we are able to identify different nitrogen sources as well as microbiological turnover processes that consume or produce nitrate. In addition, it can be recognized predominate catchment transformation zones and the impact of different hydrological conditions (base flow and high flow) on the catchment scale nitrogen dynamics. With a combined spatially highly resolved assessment along the river and a long-term monitoring, it is possible to identify hot spots of nitrogen inputs into the stream as well as seasonal N-dynamics in the catchment.

These finding can contribute a tool set that helps predict the nitrate export from large river systems as a precondition for suggesting future safety precautions that guarantee a good ecological status of the water bodies from headwater to coastal waters.

1.2 Thesis objectives and outline

The overall goal of this Ph.D. thesis is to understand nitrogen dynamics caused by climatic and land use change on a meso-scale river catchment. The specific objectives of this thesis are to:

I) Develop a monitoring concept for a biennial investigation in the Terrestrial Environmental Observatory of the riverine Bode system in the Harz mountains, Germany

II) Generate large-scale multi-isotope patterns on the basis of monthly and seasonal stream and precipitation samples to identify sources and turnover processes and interpret matter and flow dynamics

III) Establish a hydrological model (mHM) to regionalize discharge based on available governmental data from the State Office of Flood Protection and Water Management of the Federal State Saxony-Anhalt

IV) Interpretation of nitrate loads in a meso-scale river catchment with seasonal and hydrological variations (extreme events)

V) Evaluate the mobilization and dilution of main nitrate sources from agricultural land use and municipal sewage during an extreme hydrological base flow situation

The thesis is structured as follows:

I) *Chapter 2* gives an overview of the study area and the scientific background especially the nitrate cycle, principles of fractionation and the laboratory analysis.

II) *Chapter 3* characterizes and quantifies large (regional) scale dynamics and trends in water and solute fluxes from the TERENO Bode River catchment by an isotope monitoring approach. During all seasons, more than 130 streams between the first and sixth hydrological order were considered in order to generate spatial and temporal distribution patterns of nitrate sources and shrinks. Precipitation and groundwater were sampled and considered as potential input factors. Regional landscape information like land use, soil, geology, topology and recharge as well as quantitative discharge information is combined with the temporal and spatial isotope distribution patterns.

III) *Chapter 4* focuses on discharge simulations by a mesoscale hydrological model (mhM) which allows a quantitative analysis of nitrate fluxes for all observed tributaries in the Bode river catchment. The investigation of nitrate isotopic signatures for characteristic landscape types allows delineation of dominant NO_3^- sources like nitrified soil nitrogen, fertilizer, and manure. Besides source delineation, the relationship between runoff and nitrate dynamics was analyzed for the entire Bode river catchment and, more detailed, for one major tributary with minor artificial reservoirs (Selke River).

IV) *Chapter 5* compares two sampling campaigns in autumn during base flow conditions at the Holtemme River, one major tributary of the TERENO Bode catchment. Spatially highly resolved measurements of discharge, major tributaries and locations at the main river allow determination of the main actors on the riverine nitrogen dynamics. Based on the presented study, 50 % of the nitrate export from the Holtemme River catchment can be attributed to waste water treatment effluent. The remaining amount is related to agricultural land use.

V) *Chapter 6* completes the thesis with a summary of the main findings and concludes the previous chapters. Recommendations for future research and application of the monitoring approach area are given.

2 Study area and scientific background

2.1 Terrestrial Environmental Observatories - TERENO

TERENO is a long-term network of Terrestrial Environmental Observatories in Germany. From 2008 onwards, fluxes were observed from different environmental compartments: groundwater, vadose zone, surface water and atmospheric deposition. Within this German network, four observatories were established in areas with potentially high climate vulnerability (Figure 2.1). The aim is to investigate social, ecological, and economic impacts of global climate change on a regional catchment scale, and to develop strategies to successfully tackle them (Zacharias, Bogena et al. 2011).

The Central German Lowlands with its Bode river catchment are the investigation area of this thesis. They were mainly selected due to climate vulnerability of summer droughts, decreasing fresh water availability and increasing hydrological extreme events. Since the region is intensively used by agricultural land use, the risk potential for the environment is high. Particularly a leaching of fertilizer is a serious hazard for water quality. Therefore, the challenge of the presented thesis is to characterize and quantify large (regional) scale dynamics and trends in water and solute fluxes which represent changing environmental conditions (e.g. elevated inputs, land use and climate change).

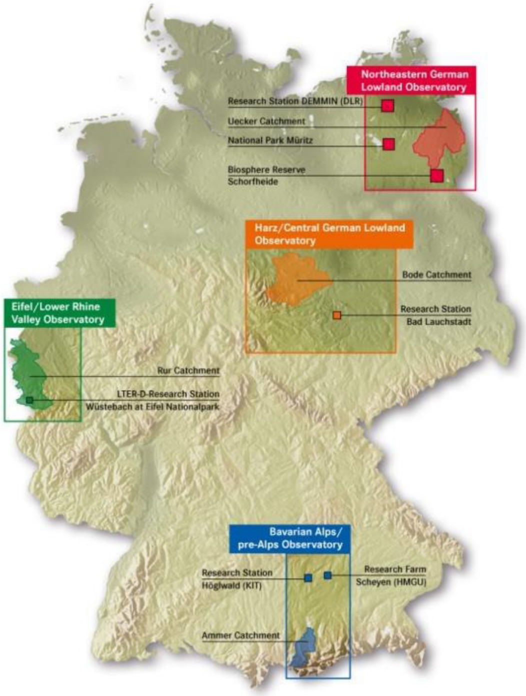

Figure 2.1: Map of Germany with the four selected TERENO observatories including research stations (Zacharias, Bogena et al. 2011). The Bode catchment is the study site of this thesis.

2.2 The nitrogen cycle

In Nature, nitrogen (N) occurs in numerous compounds which are connected in a nitrogen cycle. A schema of the N-cycle in forest ecosystems is shown in Figure 2.2. Basically, three reservoirs of nitrogen exist:

- mainly molecular nitrogen (N_2) within the atmosphere
- nitrogen in living organisms mainly plants
- nitrogen in soil, in dead biomass and in humus

Some of these nitrogen components such as nitrate (NO_3^-) and nitrogen oxide (N_2O) are known as environmental pollutants and are a risk for human health (further details are given in chapter 1.1). The natural abundances of the common ^{14}N and rare ^{15}N stable nitrogen isotopes in a certain compound depend on specific production and consumption processes. For instance, biologically-mediated reactions like assimilation, nitrification and denitrification mainly control nitrogen variations in soil (Kendall and McDonnell 1998). These reactions generally result in increasing $\delta^{15}N\text{-}NO_3^-$ values of the substrate and depleted $\delta^{15}N\text{-}NO_3^-$ values of the product. Because nitrogen is transformed by multiple cycles of mineralization, nitrification, immobilization, plant uptake and denitrification within an ecosystem, it’s a challenge to trace fixed N (Lajtha and Schlesinger 1986). However, transformation processes in aquatic and terrestrial ecosystems including isotopes methods have been observed intensively during the last decade, from laboratory to global scales (Groffman, Altabet et al. 2006, Bai, Houlton et al. 2012, Houlton, Marklein et al. 2015). The intension of the following study is a combined analysis of nitrate concentrations and corresponding isotopic signatures which will provide powerful insights into natural ecosystem processes and the effects of anthropogenic influences.

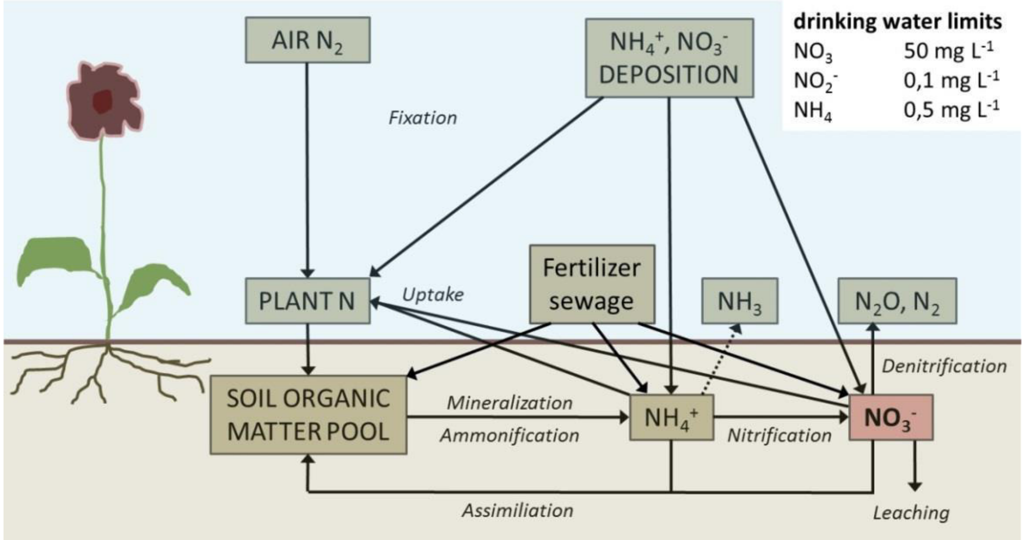

Figure 2.2: Nitrogen transformation processes affecting $\delta^{15}N$ isotopic signatures shown for forested ecosystems (modified from Natelhoffer and Fry (1988) as well as Kendall and McDonnell (1998))

2.3 Nitrate: isotopic signature and fractionation

Nitrate consists of the two elements nitrogen (N) and oxygen (O). The atomic mass of nitrogen is seven, with seven protons and seven electrons. In a natural environment, two stable isotopes for nitrogen exist: ^{14}N, with seven neutrons and ^{15}N with eight neutrons (Table 2.1). The ratio between ^{14}N and ^{15}N in atmospheric air is constant with 1:272 and hence used as international standard (Junk and Svec 1958). Oxygen has three stable isotopes. The most abundant isotope is ^{16}O, with eight protons and eight neutrons (Table 2.1). The other two isotopes ^{17}O and ^{18}O are less abundant whereas ^{18}O, with an occurrence of 0.2 %, is used as isotopic equivalent (Clark and Fritz 1997).

Table 2.1: Occurrence of nitrogen and oxygen stable isotopes after De Laeter, Böhlke et al. (2003)

Isotope	Amount [%]	Relative atomic mass
^{14}N	99.64	14.003074
^{15}N	0.36	15.000109
^{16}O	99.757	15.994915
^{17}O	0.038	16.999132
^{18}O	0.205	17.99916

Nitrogen shows a large range of oxidation states which enables several chemical combinations with numerous isotopic compositions. The oxidation state of nitrogen molecules ranges from -3 (NH_4^+) to +5 (NO_3^-). Isotopic distributions of nitrate are subject to high variations (for example: $^{14}N^{16}O^{16}O^{16}O$, $^{14}N^{16}O^{16}O^{18}O$, $^{15}N^{16}O^{16}O^{16}O$, $^{15}N^{16}O^{18}O^{18}O$).

Generally, the binding energy of heavier isotopes is higher compared to lighter isotopes and the reaction rate is much slower. Lighter isotopes react much faster. In the equilibrium, heavier isotopes prefer the species with higher enthalpy which is the more condensed phase. Organisms prefer using light isotopes (^{14}N) to heavy ones (^{15}N). Therefore, products from organisms are generally isotopically lighter compared to residual substrate (Kendall and McDonnell 1998).

Biological, physical and chemical processes can be noted as irreversible kinetic reaction or reversible equilibrium reactions with distinct isotope fractionation. These processes can be explained as a Rayleigh fractionation with the following eq. (2.1):

$$\delta_{NO3} = \varepsilon \ln f + \delta_{NO3-initial} \qquad (2.1)$$

$\delta_{NO3\text{-initial}}$ is the primary composition of the substrate, f is the remaining fraction of the substrate ($f=c/c_o$), and ε represents the isotope enrichment factor. To explain the principals of fractionation the example of denitrification is used (Figure 2.3). During anaerobic conditions microbiological denitrification can occur. The isotopic composition of the product (N_2) is always lighter during denitrification than the residual substrate (NO_3^-). At the beginning of the reaction (t_0), reactant and product have a certain difference in $\delta^{15}N\text{-}NO_3^-$ values which shows the enrichment factor (ε). During the biological turnover process, the isotopic composition of substrate as well as product increase. When all of the reactant (NO_3^-) is used and the nitrate concentration is at zero, the $\delta^{15}N\text{-}NO_3^-$ signature of the product reservoir (N_2) is equal to the isotopic composition ($\delta^{15}N_{(NO3)\text{-}0}$) of the initial nitrate. The very last residual of nitrate can have a very high $\delta^{15}N\text{-}NO_3^-$ value. That is why isotopic composition depends significantly on the fractionation factor and the remaining reactant reservoir (Kendall and McDonnell 1998).

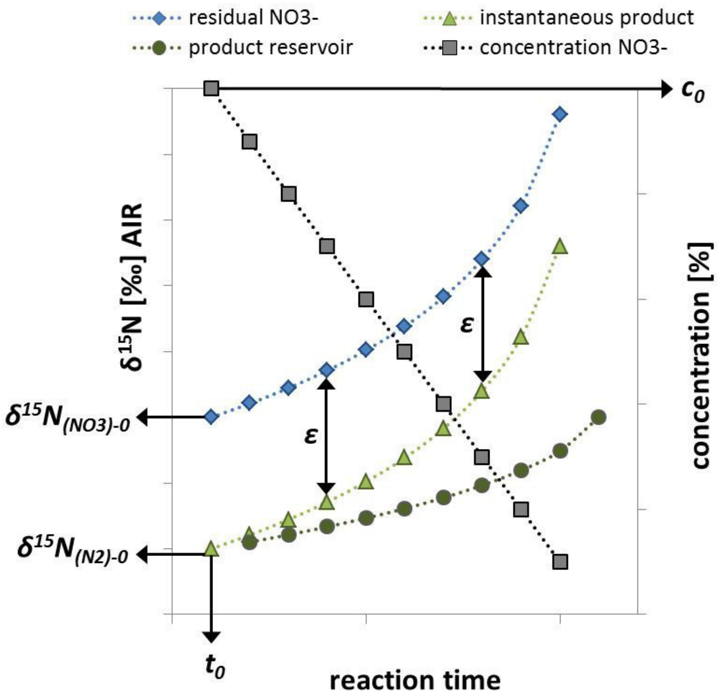

Figure 2.3: Isotopic effects during denitrification

If most of the product (N_2) accumulates in a reservoir, the isotopic differences between nitrate and gaseous nitrogen are not consistent regarding eq. (2.1). Eq. (2.2) considers the isotopic difference between the instantaneous and the accumulated product (N_2) according to Krouse and Tabatabai (1986):

$$\delta_{N2-measured} = \delta_{NO3-measured} - \frac{\varepsilon \ln f}{1-f} \qquad (2.2)$$

Necessary electron donors for the anaerobic denitrification reaction can derive from dissolved organic carbon, organic contaminants (eq. 2.3, toluol) or iron sulfides (eq. 2.4):

$$\boldsymbol{C_7H_8} + NO_3^- + 3H_2O \rightarrow 3N_2 + 7HCO_3^- + 7H^+ \qquad (2.3)$$

$$\boldsymbol{5FeS_2} + 14NO_3^- + 4H^+ \rightarrow 7N_2 + 10SO_4^{2-} + 5Fe^{2+} + 2H_2O \qquad (2.4)$$

Other nitrate consuming processes like assimilation, anaerobic ammonium oxidation or anaerobic nitrification may result in isotope fraction. Additionally, mineralization, sorption and desorption on soil particles can play a significant role in nitrate cycle and isotopic compositions. Detailed information and references are given in Kendall and McDonnell (1998).

2.4 *Nitrate isotope analysis: laboratory denitrifier method*

The isotopic composition of dissolved nitrate in water was analyzed following the so-called denitrifier method according to Sigman, Casciotti et al. (2001) and Casciotti, Sigman et al. (2002). Under this approach, dissolved nitrate is concentrated to gaseous N_2O in headspace vials by a defined bacterial reduction. These denitrifying bacteria lack an active N_2O reductase as shown in eq. (2.5):

$$2NO_3^- \rightarrow 2NO_2^- \rightarrow 2NO \rightarrow \enclose{circle}{N_2O} \rightarrow \cancel{N_2} \qquad (2.5)$$

The nitrous oxide gas product is measured with a stable isotope mass spectrometer to get the mass ratios 45/44 and 46/44 of N_2O for the calculation of δ^{15}N-NO_3^- and δ^{18}O-NO_3^- values of precursor nitrate. Before analysis, a bacterial working culture has to be prepared (Figure 2.4A) using the bacterial strain Pseudomonas chlororaphis (ATCC #13985). The culture medium consists of tryptic soy broth amended with KNO_3, NH_4Cl, and KH_2PO_4. All chemicals incubate for 7 to 16 days at room temperature, shaking the vials at regular intervals. To ensure optimal incubation smell and appearance must be checked frequently. A leavened bakery smell accompanied by a pinkish shimmer coming from the bottle interior mark a successful culture development. Then, the culture is concentrated 3-fold by centrifugation and vented with nitrogen gas stream for two hours. Subsequently, 2 mL of concentrated culture can be added to each sample vial and tightly closed with septum (Figure 2.4B). Before 2 mL of the sample solution can be injected the vials get flushed with helium gas for 15 minutes (Figure 2.4C).

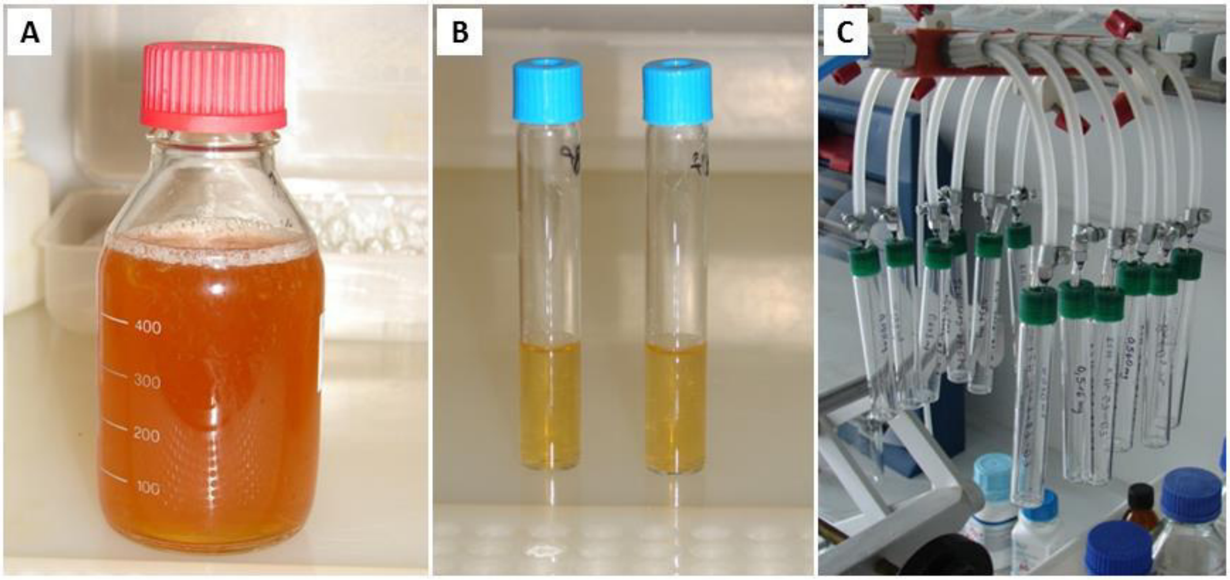

Figure 2.4: Sample preparation for the denitrifier method A: Preparation of bacterial working cultures, B: Concentrated culture in each sample vial tightly closed with septum, C: Helium flushing apparatus

The necessary amount of nitrate ranges between 1 and 2.5 mg L^{-1}. Higher concentrated samples have to be diluted while lower concentrated samples have to be enriched by freeze drying. The incubation time of this culture-sample-solution is two to three days at room temperature. By injecting 0.1 mL of 10N sodium hydroxide (NaOH) and putting all vials into an ultrasonic bath for 5 minutes, incubation stops and all bacteria get killed eventually. The generated N_2O gas in the vial headspace can be measured via mass spectrometer with modified GasBench II system (and 8-port Valco valve and cold trap unit). For calibration and normalization to AIR and VSMOW scale, reference materials are treated as samples (USGS 34, USGS 35, IAEA-NO3). The analytical precision is about 0.2 ‰ for $\delta^{15}N\text{-}NO_3^-$ and 0.5 ‰ for $\delta^{18}O\text{-}NO_3^-$. Around 150 samples can be analyzed per week.

3 Regional nitrogen dynamics in the TERENO Bode River catchment, Germany, as constrained by stable isotope patterns

This chapter is an edited version of Mueller, Krieg et al. (2015):

Mueller, C.; Krieg, R.; Merz, R.; Knöller, K. (2015): Regional nitrogen dynamics in the TERENO Bode River catchment, Germany, as constrained by stable isotope patterns. Isotopes in Environmental Health Studies, 52 (1-2), 16-74, DOI 10.1080/10256016.2015.1019489, accepted 28 January 2015.

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Summary. We used nitrogen and oxygen isotopic signatures of riverine nitrate of main tributary streams from the Bode River in the Harz Mountains, Germany, to delineate nitrate sources and nitrogen transformation processes. Between March 2012 and December 2013 we collected water samples from up to 133 tributary streams several times and determined hydrochemical parameters as well as $\delta^{15}N$ and $\delta^{18}O$ of nitrate. The catchment is strongly influenced by agricultural land use that occupies an area of 2310 km², which is about 70 % of the overall size of the catchment.

Own Contribution. The manuscript was drafted by Christin Müller, who performed the sampling, partly laboratory analysis and interpretation of the data under the direct supervision of Kay Knöller and Ralf Merz. Ronald Krieg was continuously involved in the sample collection and characterization of related parameters.

Abstract. Interactions between hydrological characteristics and microbial activities affect the isotopic composition of dissolved nitrate in surface water. Nitrogen and oxygen isotopic signatures of riverine nitrate in 133 sampling locations distributed over the Bode River catchment in the Harz Mountains, Germany, were used to identify nitrate sources and transformation processes. An annual monitoring program consisting of seasonal sampling campaigns in spring, summer and autumn was conducted. $\delta^{15}N$ and $\delta^{18}O$ of nitrate and corresponding concentrations were measured as well as $\delta^{2}H$ and $\delta^{18}O$ of water to determine the deuterium excess. Additionally, precipitation on 25 sampling stations was sampled and considered as potential input factor. The Bode River catchment is strongly influenced by agricultural land use which is about 70 % of the overall size of the catchment. Different nitrogen sources like ammonia (NH_4) fertilizer, soil-nitrogen, organic fertilizer or nitrate in precipitation show partly clear nitrate isotopic differences. Processes like microbial denitrification result in fractionation and lead to an increase of $\delta^{15}N$ of nitrate. We observed an evident regional and partly temporal variation of nitrate isotope signatures which are clearly different between main landscape types. Spring water sections within the high mountains contain nitrate in low concentrations with low $\delta^{15}N_{NO3}$ values of -3 ‰ and high $\delta^{18}O_{NO3}$ values up to 13 ‰. High mountain stream water sub-catchments dominated by nearly undisturbed forest and grassland contribute nitrate with $\delta^{15}N_{NO3}$ and $\delta^{18}O_{NO3}$ values of -1 ‰ and -3.5 ‰, respectively. In the further flow path, which is affected by an increasing agricultural land use and urban sewage we recognized an increase in $\delta^{15}N_{NO3}$ and $\delta^{18}O_{NO3}$ up to 22 ‰ and 18 ‰, respectively, with high variations during the year. A correlation seems to exist between the percentage of agricultural land use area and the corresponding $\delta^{15}N_{NO3}$ values for sub-catchments. A shift towards heavier isotope values in stream water samples taken in July 2012 is significant (p-value = $6 \cdot 10^{-6}$) compared to samples from March and October 2012. We also see a season-depending impact of microbial denitrification. Denitrification, especially evident in the lowlands, predominantly takes place in the riverbeds. In addition, mixing processes of different nitrate sources and temperature-depending biological processes such as nitrification have to be taken into consideration. Constant-tempered groundwater does not play a noticeably role in the processes of the stream water system. As constrained from oxygen isotope signatures, precipitation associated with low nitrate concentrations does not have an obvious impact on stream water nitrate in the high mountain region.

3.1 Introduction

Nitrate levels are one of the most important quality characteristics for surface and ground water, especially in areas which are strongly affected by domestic sewage or agriculture due to the usage of organic and synthetic fertilizer. 30 % of the total anthropogenic nitrogen input is transported out of the catchments by surface flow and eventually reaches the oceans. The remaining 70 % of human controlled N inputs are stored, denitrified, or volatilized in the basins (Howarth, Billen et al. 1996, Boyer, Goodale et al. 2002). In small agricultural streams in-stream disposal of nitrogen can range between 10 and 70 % of the total N load (Birgand, Skaggs et al. 2007). The denitrification rates and efficiency in temperate climates are highest in summer and lowest in winter controlled by microbial activity and discharge (Hauck 1973, Zweimüller, Zessner et al. 2008). A detailed assessment of nitrate sources and dynamics is crucial for developing preventive measures for the protection of water storage compartments and reservoirs that are important for current and future drinking water supply and to manage nutrient export from river catchments to fragile coastal ecosystems.

Isotopes can be a useful tool to delineate different sources of nitrogen (direct fertiliser input, atmospheric deposition, sewage and wastewater inputs, or leaching of nitrate produced by nitrification of ammonia from agricultural soils) and also to identify (bio)geochemical transformation processes (Hauck 1973, Heaton 1986). Additionally, nitrogen isotope ratios have been proven useful in quantifying the extent of punctuality or diffusivity of a pollution source in rivers (Fogg, Rolston et al. 1998, Harrington, Kennedy et al. 1998). Under closed system conditions, denitrification causes a Rayleigh type isotope fractionation (Mariotti, Mariotti et al. 1982) resulting in a simultaneous enrichment of the heavy nitrogen (^{15}N) and oxygen (^{18}O) isotopes in the remaining nitrate pool as well as a decrease of nitrate concentrations (Delwiche and Steyn 1970, Mariotti, Landreau et al. 1988). On the catchment scale, this straightforward fractionation pattern is often blurred or biased by mixing processes of nitrate from two or more different sources. A further process that clearly determines the fate of nitrogen on catchment scale is nitrification of reduced organic or inorganic nitrogen. Nitrification of fertilizer or sewage derived ammonium is associated with an isotope enrichment factor for nitrate nitrogen between 14 ‰ and 38 ‰ (Casciotti, Sigman et al. 2003). Further nitrification processes related to the mineralization of soil organic nitrogen cause similar isotope fractionation as the ammonium oxidation. They are strongly affected by drainage, topographic position, vegetation, plant litter, land use, temperature, and rain amount (Amundson, Austin et al. 2003, Craine, Elmore et al. 2009). In the study by Boyacioglu, Vetter et al. (2012) stream size, temperature, nutrient concentration as well as discharge were used as denitrification influencing parameters for climate change modeling. Further influenced aspects like river morphology (natural of channelized conditions) or the composition of benthic organic matter was also discussed (Alexander, Böhlke et al. 2009, Boyacioglu, Vetter et al. 2012).

Our approach comprises the analyses of the temporal and spatial variability of isotopic distribution patterns obtained by an isotope monitoring of more than one hundred surface water sites. Due to the well-known nitrogen fractionation characteristics we tested if regional and/or temporal nitrogen isotopic signature differences exist on the catchment scale of the Bode river area in the Harz Mountains. Nitrogen isotopic analyses on different sized tributaries were used to identify sources and process-depending nitrogen isotopic differences depending on climate conditions, river morphology and anthropogenic impact (sewage or rather extremely agricultural land use). The consideration of dominant spatial, temporal and hydrological processes can help to understand the nitrogen cycling in a large-scale river catchment.

3.2 Study area

Our investigation area is the Bode River catchment in the Harz Mountains in Germany (Figure 3.1). It is one location of the Terrestrial Environmental Observatories (TERENO) network (Zacharias, Bogena et al. 2011). The Bode River drains the northern Harz Mountain range and discharges into the Saale River. It has a catchment size of about 3300 km² (David, Borchardt et al. 2013, Halbedel, Büttner et al. 2013) and a mean water discharge of 8.3 m^3s^{-1} on the river outlet (Table 3.1). The Bode River exhibits a moderate to poor ecological status (Ministry of agriculture and environment of State Saxony-Anhalt 2005).

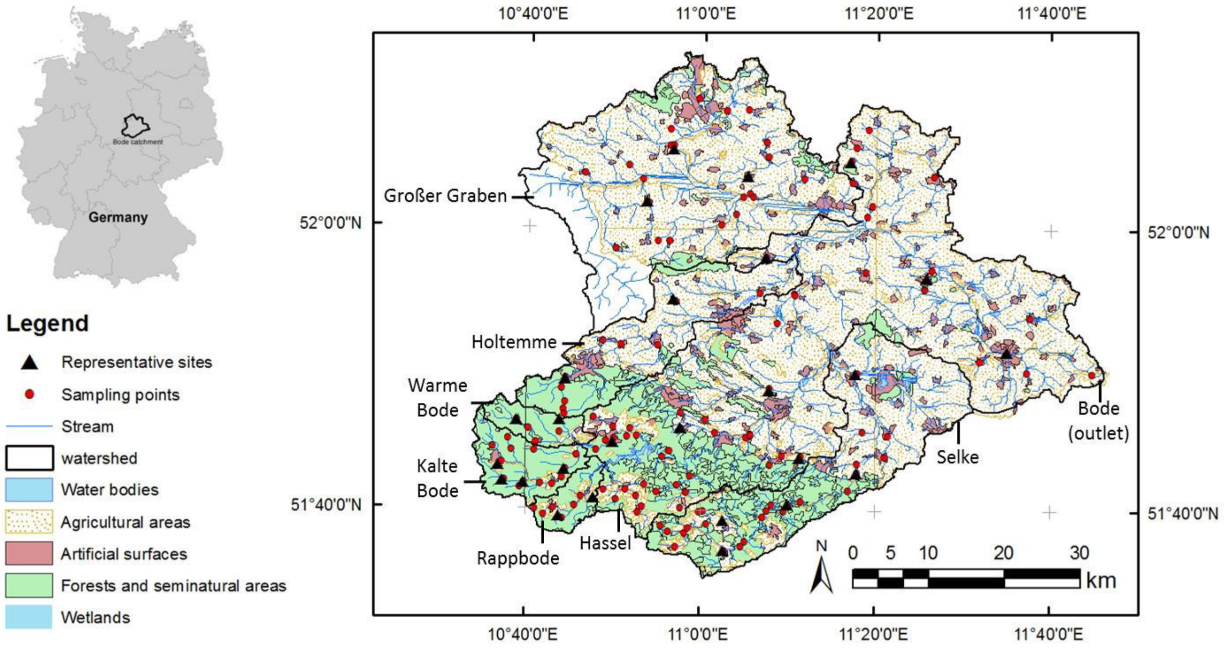


Figure 3.1: Land use of the Bode River catchment in the Harz Mountains, Germany, with main tributary rivers (Kalte Bode, Warme Bode, Hassel, Rappbode, Holtemme, Selke and Großer Graben) as well as all sampling points and 25 representative sampling sites for precipitation sampling. Land use information of the western region (Großer Graben) is not available.

Table 3.1: Stream length, catchment size and recharge of main sub-catchments and the entire Bode catchment

Sub-catchment	Stream length (km)	Catchment size (km²)	Discharge station (LHW)	Mean Q in 2012 (m^3s^{-1})	MNQ [m^3/s], measure time (year)	Number of sampling points	Number of representative sampling sites	Agriculture land use (%)
Warme Bode	23	102	Tanne (579205)	1.63	2.14 (1961-2012)	14	4	7.6
Kalte Bode	17	54	Elend (579305)	0.61	0.72 (1951-2012)	4	1	9.0
Rappbode	11	47	Trautenstein (579405)	0.45	-	6	1	23.3
Hassel	12	44	Hasselfelde (579504)	-	-	7	-	60.3
Holtemme	47	282	Nienhagen (579745)	0.88	1.44 (2006-2012)	12	4	60.9
Selke	64	468	Hausneindorf (579620)	1.14	1.80 (1981-2004, 2012)	23	5	56.4
Großer Graben	46	856	Oschersleben (579810)	1.62	2.36 (2006-2012)	22	3	71.0
Bode	169	3229	Stassfurt (579085)	8.32	12.29 (1989-2006, 2012)	38	7	68.8

3.2.1 Characterization of main stream sub-catchments

General information like stream length, catchment size and mean discharge of main sub-catchment tributaries and the entire Bode region are composed in Table 3.1 and illustrated in Figure 3.1. The discharge is based on government monitored discharge stations from the Landesbetrieb für Hochwasserschutz und Wasserwirtschaft (LHW) in Saxony-Anhalt. Two mountain streams, Kalte and

Warme Bode, arise in about 840 m a.s.l. (above sea level) and flow through integral nature reserves passing some villages and provincial towns. The two rivers join in the pre-river dam forming the Bode River. The Mountain Rivers Hassel and Rappbode feed the Rappbode reservoir, the biggest artificial lake in the Harz Mountains. The reservoir outcome drains into the Bode River. After the reservoir, the stream Holtemme joins the river. It is characterized by steep and small waterfalls and rapids through the Harz foreland. The major tributary Selke is characterized by forestry in the upper river section whereby the lower part is affected by agricultural land use. The major tributary in the lowlands is the Großer Graben, which is an artificial water body. It is characterized by wetlands and strong agricultural land use responsible in poor water quality with high amounts of nutrients and algae. Additionally, the Bode River flows through lowland regions close to confluence with the Saale River which are influenced by brine springs. These salt saturated waters influence ground and surface waters as well as the soil itself characterized by a flora of a littoral type (Currie 1905).

3.2.2 Climatic conditions

The Bode river area is located in middle Germany with characteristically wet summers and cold dry winters (Figure 3.2). The summit of the Harz Mountains is one of the precipitation-richest areas in Germany with up to 1600 mm per year (Ministry of agriculture and environment of State Saxony-Anhalt 2005). In contrast, the Boerde region in the middle and lowlands of the catchment has a mean annual precipitation of 450 mm per year (Ministry of agriculture and environment of State Saxony-Anhalt 2005) with mean annual temperature variations of 9 Kelvin.

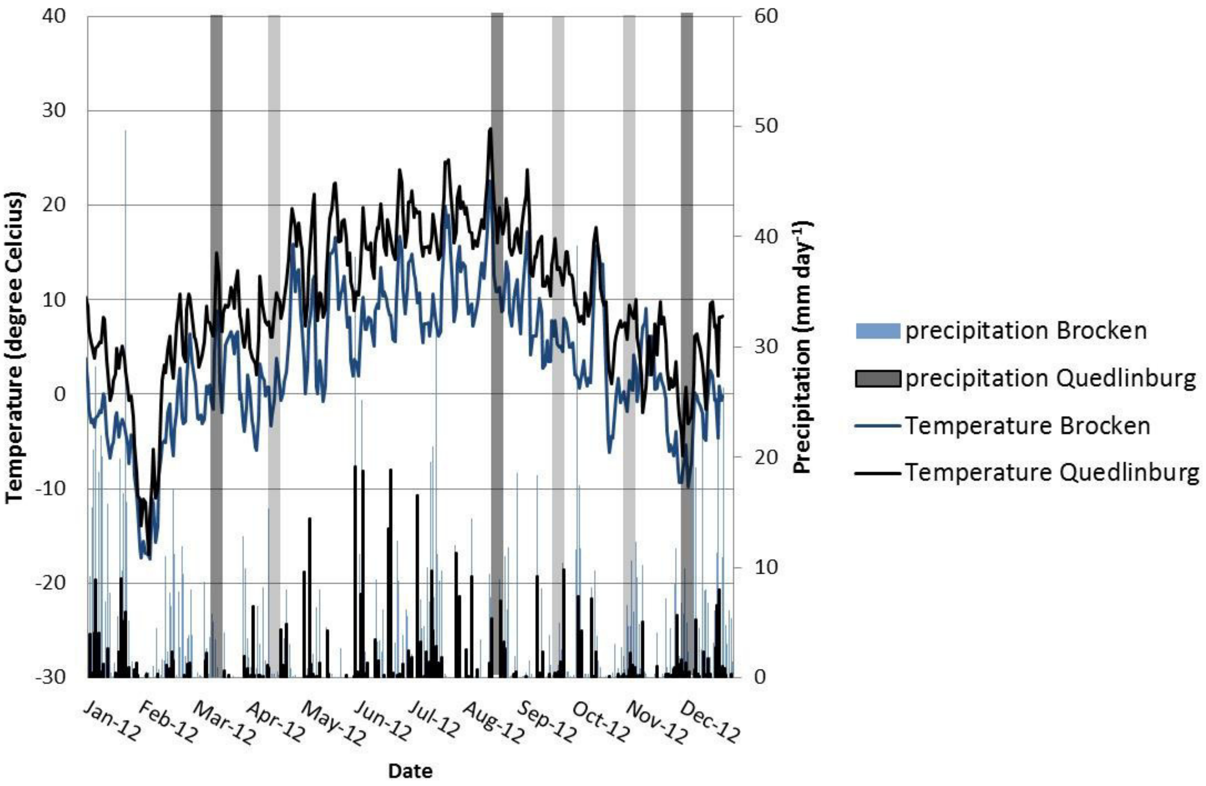


Figure 3.2: Climatic and hydrological situation in the year of sampling. The high mountain region is represented by the Brocken station. The lowlands are typified by the Quedlinburg station. Sampling campaigns (large in dark grey, small in light grey) are shown as columns.

3.2.3 Landscape information

The catchment drains the fault-block Harz Mountains (1141 m a.s.l.) (Jubitz 1964, Bachmann, Ehling et al. 2008), which consists of uplifting Paleozoic rocks (Bozau, Stärk et al. 2013). Parts of the Harz Mountains are impacted by former ore mining activities (Matschullat, Ellminger et al. 1997). Soils with high amounts of heavy metals like arsenic, cadmium, copper and zinc are common and might well be transported with streams during floodwaters (Ernst, Knolle et al. 2009, Osterloh, Bernsdorf et al. 2013). The bedrock of the upper mountains is characterized by schist, and greywacke (flysch) with some granite intrusions on the top of the mountain (Brocken) (Bozau, Stärk et al. 2013) which influence the spring streams Kalte and Warme Bode. In the upper and middle mountain region additionally argillaceous shale occurs. Dominating soil types are podsols and secondary podsols as well as gley and fens which are typical for wet areas (Osterloh, Bernsdorf et al. 2013). The eastern part of the Bode catchment is characterized by Permian salt layers with salt deposits causing brine springs (Reinhardt 1993). The middle and northern region of the catchment are part of the Magdeburger Boerde which is characterized by its fertile black soils. Dominating soil types in the entire Bode catchment are forested and semi-natural areas (23 %), agricultural areas (70 %) and urbanized areas (7 %) (CORINE soil data (EEA 2012)). Forest and grassland are dominating in the southern mountain region. The lowland in the middle and northern part of the catchment is strongly influenced by human impact.

3.3 Methods

3.3.1 Sampling

The stream water monitoring started in March 2012. Three, so called large sampling campaigns, were carried out in March, July and October 2012. During these two-day campaigns 133 stream water samples from different sized streams were taken located all over the Bode catchment. All main tributary rivers flowing into the Bode River were included. As shown in Figure 3.1, the density of sampling points is much higher in the southern part of the catchment due to numerous of mountain streams located there, while less locations were sampled in the middle and northern parts of the catchment. Samples were filtered in the field with 0.45 µm cellulose acetate filters, filled in high density polyethylene (HDPE)-bottles and stored in a refrigerator. A minor number of small river catchments fell dry during summer season and could not be sampled. Additionally, field precipitation water collectors from the company PALMEX, Croatia (Gröning, Lutz et al. 2012) were installed at 25 representative points for the entire Bode catchment (Figure 3.1). The monthly precipitation monitoring program started in November 2012.

3.3.2 Laboratory analysis: nitrate concentrations and isotopic analysis

The nitrate concentrations were measured by ion chromatography with a Dionex ICS-2000 combined with AS50. Before, the pH-value for each stream sample was measured resulting in a range between 7 and 8. The isotopic composition of dissolved nitrate was measured by using the denitrifier method with bacteria strains of *Pseudomonas chlororaphis* (ATCC #13985) (Sigman, Casciotti et al. 2001, Casciotti, Sigman et al. 2002). Isotopic ratios are expressed in delta notation according to eq. (3.1) relative to atmospheric nitrogen (AIR) for $\delta^{15}N$ and relative to Vienna Standard Mean Ocean Water (VSMOW) for $\delta^{18}O$. R describes the ratio of the heavier to the lighter isotope.

$$\delta_{sample}\,[‰] = \left(\frac{R_{sample}}{R_{standard}} - 1\right) \times 1000 \qquad (3.1)$$

A mass spectrometer DELTA V Plus in combination with a GasBench II from Thermo Scientific was used for isotope measurements. The analytical precisions for nitrogen and oxygen isotope measurements of nitrate are 0.4 ‰ and 1.6 ‰, respectively. For calibration, international standards (USGS32, USGS34, USGS35 and IAEA NO3) were applied.

Due to extremely low concentration of nitrate in a number of high mountain streams sampled in August and October 2012 the isotopic signature of nitrate could not be measured in some cases.

Stable isotopes of water (δ^2H, $\delta^{18}O$) were analyzed by laser-based analyzer (L1102-I, Picarro Inc.) with a measurement precision of 1.0 ‰ and 0.3 ‰ for deuterium and oxygen, respectively. δ-values are given relative to VSMOW. Deuterium excess (d, eq. 3.2) was calculated after Dansgaard (1964) as:

$$d\,[‰] = \delta^2H - 8 \times \delta^{18}O \qquad (3.2)$$

3.3.3 Investigation of regional sub-catchment landscape information

Regional landscape information of the CORINE soil data (EEA 2012) were used to calculate the percentage of main soil/landscape types for each sub-catchment by using the “Tabulate Intersection”-analysis in the software ArcGIS 10. This procedure provided information on the relative distribution of main landscape types like agricultural land use, forest, artificial areas, and water bodies.

3.4 Results and discussion

3.4.1 Classification of catchment sources on a spatial and temporal scale

Generally, evident temporal and spatial variances of $\delta^{15}N_{NO3}$ values can be observed along the Bode River. Every tributary releases an amount of nitrate with a characteristic isotope signature and corresponding nitrate concentration into the stream water system. The dual isotope plot (Figure 3.3) for nitrate indicates the variability of $\delta^{15}N$ and $\delta^{18}O$ for the sampling campaigns in March (A), July (B) and October (C), 2012. Characteristic isotope signatures can be recognized for main types of landscape and land use. Different signatures of spring regions, grass and forest dominated regions as well as areas with agricultural land use could be shown.

Spring water shows low $\delta^{15}N_{NO3}$ variations between -4 ‰ and -2 ‰ and higher $\delta^{18}O_{NO3}$ values up to 13 ‰ associated with low nitrate concentrations around 3 mg L^{-1} (Figure 3.3A, exemplarily for March 2012). The highest $\delta^{18}O_{NO3}$ values in spring water can be observed in March 2012, whereby $\delta^{15}N_{NO3}$ increases from -3 ‰ in March to 3 ‰ in October 2012. Streams originating in the east of the Harz Mountains (Hassel, Rappbode, Luppbode) have noticeably higher $\delta^{15}N_{NO3}$-values over the year with a spring water isotope signature of 6 ‰ and corresponding lower $\delta^{18}O_{NO3}$ of -1 ‰. Along the flow path of the four abovementioned major tributaries, $\delta^{15}N_{NO3}$ approaches a value of around 0 ‰ and $\delta^{18}O_{NO3}$ values of -1 ‰ representing spruce dominated, nearly undisturbed regions (Figure 3.3A and Figure 3.4). This $\delta^{15}N_{NO3}$ signal increases to heavier isotope signatures in July and October 2012 (Figure 3.3B, C). The rural areas of the Holtemme and Selke River catchments represent the latter type of high mountain catchment. Small tributaries from the upper Selke River flowing through intact mixed and coniferous forest show the lowest values in $\delta^{18}O_{NO3}$ with -3.5 ‰ and corresponding values in $\delta^{15}N_{NO3}$ of about 1 ‰. Further downstream, the $\delta^{15}N$ and $\delta^{18}O_{NO3}$ values of the stream nitrate rise up to 9 ‰ and 1 ‰, respectively, with generally small variances in $\delta^{15}N_{NO3}$ throughout the year. This is caused by tributaries from sub-catchments in the northern part of the Selke River that are influenced by agricultural land use and show high values of 14 ‰ in $\delta^{15}N_{NO3}$ and 4 ‰ in $\delta^{18}O_{NO3}$.

The highest contribution of nutrients to the Bode River comes from the Großer Graben, a large tributary in the mid-north impacted by urban and agricultural land use. Nitrate with concentrations between 7 mg L^{-1} in October and 50 mg L^{-1} in summer shows $\delta^{15}N_{NO3}$ values between 8 ‰ and 9 ‰ and $\delta^{18}O_{NO3}$ values between 2 ‰ and 5 ‰ in March 2012. In the continued flow path, we recognized an increase in $\delta^{15}N_{NO3}$ and $\delta^{18}O_{NO3}$ up to 11 ‰ and 6 ‰, respectively. The highest impact of industrial farming can be found in the north east of the Bode catchment with nitrate concentrations up to 87 mg L^{-1} (October 2012). Especially in July and October 2012, small tributaries in the lowlands of the tributaries Großer Graben and Selke show extremely enriched $\delta^{18}O_{NO3}$ and $\delta^{15}N_{NO3}$ values compared to the signatures in March 2012 (Figure 3.3A, C). The isotope signature of nitrogen varies up to 5.5 ‰ for theses tributaries which affect clearly the seasonal variation of the Bode outlet.

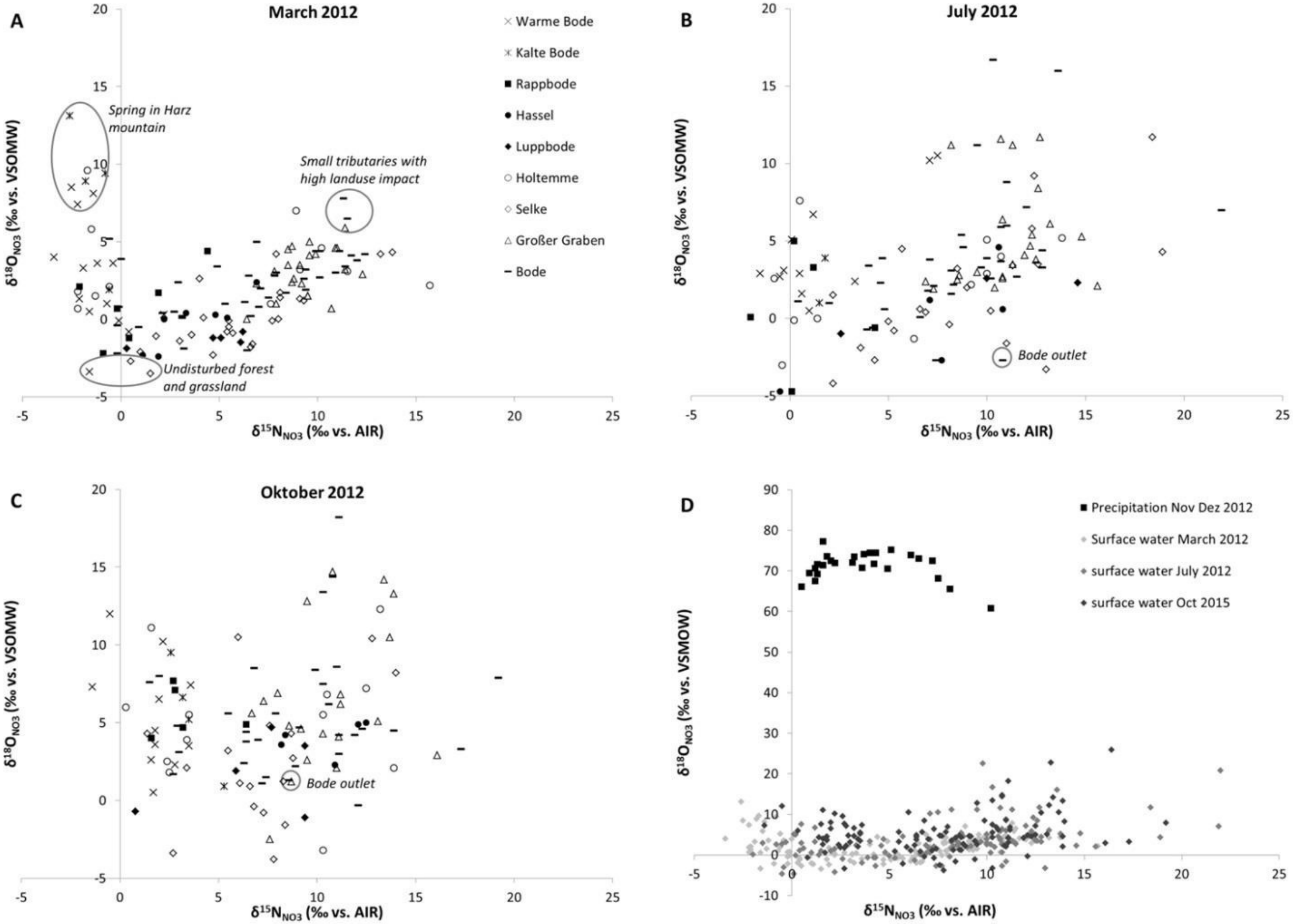


Figure 3.3: Isotopic nitrate dual-plot of surface water collected in March (a), July (b) and October 2012 (c). Figure (d) shows the comparison of all sampling campaigns in correlation with the monthly precipitation from November and December 2012. It indicates typical signatures of spring regions of main tributary rivers, sub-catchments with nearly undisturbed conditions in forest and grassland as well as streams in regions with high influence in agricultural land use. The Bode River outlet is a mixture of the total water income. The variation of nitrate isotopic signatures up to heavier isotope composition is obvious especially from small agricultural tributaries in July and even more in October 2012.

3.4.2 Regional differentiation in sections

With respect to the source of nitrate, the Bode catchment can be differentiated into three major spatial sections as end-members of a conceptual linear mixing model (Figure 3.4) considering the nitrate concentrations and the temporal development:

1) *High mountain section* (representatives: Warme Bode, Kalte Bode): is characterized by low $\delta^{15}N_{NO3}$ values, which slightly increase from March to July and October 2012. These are generally stream waters coming from areas uninfluenced by anthropogenic water management (water reservoirs). The nitrate isotopic signature is dominated by nitrification of soil nitrogen (Kendall and McDonnell 1998) due to continuous mobilization independent from discharge during each sampling campaign. High mountain stream water is strongly influenced by dissolved organic carbon (DOC) which is caused by the input of humic and fulvic acid from the peat bogs of the upper mountains (Bozau, Stärk et al. 2013, Halbedel, Büttner et al. 2013). This is due to biological processes and stream metabolism which may impact the local nitrogen cycling as well (Kendall and McDonnell 1998). Small sub-catchments from the tributaries Hassel, Rappbode and Luppbode dominated by forest and grassland contribute nitrate with depleted $\delta^{15}N_{NO3}$ and $\delta^{18}O_{NO3}$ values of -1 ‰ and 2 ‰, respectively, changing the isotope footprint of the young high-mountain Bode River.
2) *Transfer-section* (representatives: Selke, Holtemme): shows a high variability in $\delta^{15}N_{NO3}$ values throughout the year with increasing agricultural land use downstream and an increasing potential for biological activity (such as nitrification and denitrification processes) due to elevated nutrient loads compared to the high mountain section. Nevertheless, the nitrate load is relatively low with maximum concentrations of 5 mg L^{-1}. Nitrate sources consist of soil-nitrogen in the head water. Subsequently, there is an increased contribution of nitrified ammonium fertilizer during periods of higher discharge.
3) *Intensive agricultural land use-section (*representatives: Großer Graben with tributaries*)*: slowly flowing streams in the lowlands and highly influenced by agricultural land use with high nitrate concentrations (7 to 50 mg L^{-1}), elevated $\delta^{15}N_{NO3}$-values with small variations potentially caused by mixing processes and biological nitrogen transformations. That nitrate originates mainly from nitrification of ammonium fertilizer and is especially mobilized and washed out during rain events.

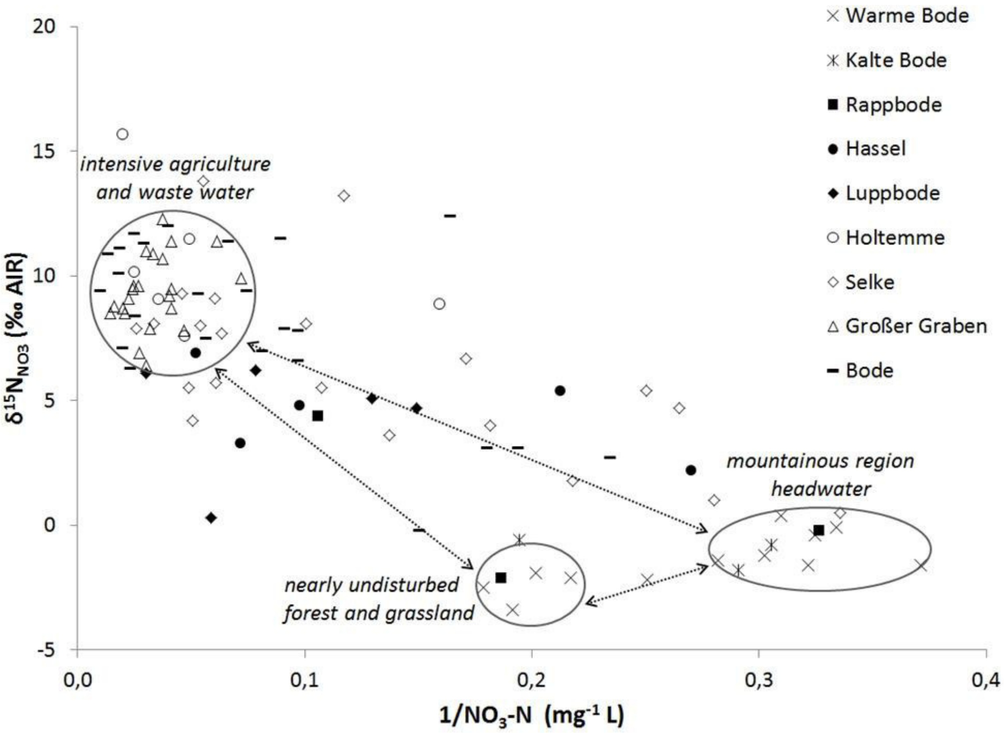


Figure 3.4: Three-component nitrate mixing model. Cross plot of $\delta^{15}N_{NO3}$ vs. nitrate concentration of surface water exemplarily for March 2012. Isotopic signature of $\delta^{15}N_{NO3}$ with corresponding NO_3^- concentrations indicates a three-component mixing system. One source is the mineralized soil organic nitrogen in the high mountains which is activated during low discharge season. Streams in grass and forest dominated regions with slightly increasing nitrate concentrations but still depleted $\delta^{15}N_{NO3}$ signatures. The lowlands indicate heavier $\delta^{15}N_{NO3}$ and high nitrate concentrations resulting from nitrified NH_4^+ fertilizer arousing mainly during high discharge events.

The seasonal variation of major impacting tributaries (Selke, Holtemme, Großer Graben) influences evidently the $\delta^{15}N_{NO3}$ of the Bode outlet up to 4 ‰ (Figure 3.5). However, the seasonal variation of this tributaries itself can be much lower. Generally the highest $\delta^{15}N_{NO3}$-variations emerge in small tributaries of the 1st and 2nd stream order. With increasing hydrological class the variation of nitrogen isotopic signature decreases. The reasons are mixing processes which stabilize the system.

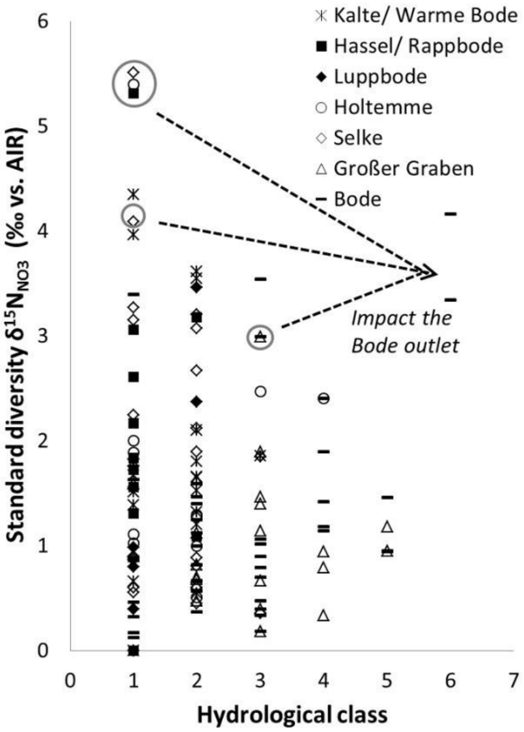


Figure 3.5: Standard diversity of $\delta^{15}N_{NO3}$ compared to the hydrological class of each samples stream. The highest variations are obvious for small tributaries. The standard diversity decreases with increasing hydrological class caused by mixing processes with increasing discharge. The nitrate isotopic signature of the Bode outlet varies clearly which is affected by major tributaries (Großer Graben, Holtemme and Selke).

The varying impact of the abovementioned sections on the spatial development of nitrate loads in the different segments of the Bode River is illustrated in Figure 3.6. The flow of the Bode River starts with light $\delta^{15}N_{NO3}$ values at the regions its origin. Along the further flow path high mountain-section tributaries (Rappbode, Hassel, Luppbode) do not have an obvious impact on the general isotopic signature of the Bode River. With the inflow of major tributaries like Selke and Holtemme the isotopic signature of the Bode River increases clearly. Discharge and concentration of the Bode River rise. The biggest tributary Großer Graben with an enriched ^{15}N content further increases the ^{15}N and nitrate concentration of the Bode River. Agricultural land use with an intensified application of inorganic fertilizer might be the reason for the observed change in isotope signatures (Kendall and McDonnell 1998). The $\delta^{15}N$ variation of the Bode outlet represents the influence of the tributaries Selke, Holtemme and Großer Graben and has nothing in common with the nitrogen isotopic signature of high mountain streams.

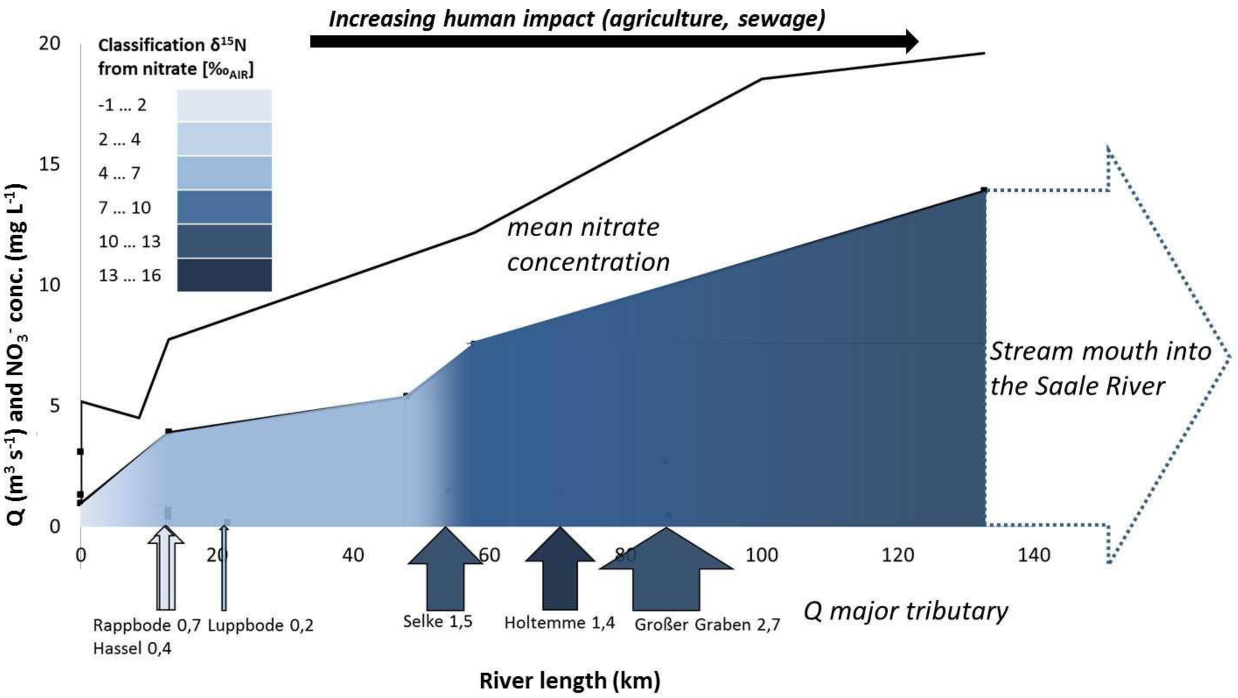


Figure 3.6: Classified $\delta^{15}N_{NO3}$-gradient exemplarily for the sampling campaign in March 2012 with mean nitrate concentrations and the development of the discharge along the Bode River. The dam reservoir of the high mountain streams (Rappbode and Hassel) seems to not have a clear influence on the nitrate isotope signature. Major tributaries such as Selke, Holtemme and Großer Graben with heavy $\delta^{15}N_{NO3}$ composition change the general isotopic signature of the Bode River with increasing impact of agricultural land use and sewage.

3.4.3 Impact of precipitation on the stream water nitrate dynamics

Precipitation water sampled at the 25 representative sampling sites show nitrate concentrations of about 2.8 mg L^{-1} in average and corresponding $\delta^{15}N$ values around 0 ‰ and $\delta^{18}O$ values around 75 ‰ sampled in November and December 2012 (Figure 3.3D). They are dominated by soil emissions and lightning-produced NO_x (Hoering 1957, Freyer, Kley et al. 1993). The nitrogen isotope input information is mainly relevant for stream water in uninfluenced high mountain regions which show also low nitrate concentrations. Strong rain events especially in summer can influence the $\delta^{15}N_{NO3}$ values of mountain streams down to light values. In winter, anthropogenic sources like intensive exhaust fumes (Ammann, Siegwolf et al. 1999) influence the $\delta^{15}N_{NO3}$ of the precipitation up to 21 ‰ and for $\delta^{18}O_{NO3}$ up to 90 ‰. Comparable isotope compositions were measured by Widory (2007) who justify the signal with the effect of natural gas combustions. While the temporal variation of $\delta^{15}N_{NO3}$ in precipitation corresponds with those of the mountain streams Rappbode and Warme Bode, no indication is given from the $\delta^{18}O_{NO3}$ that precipitation nitrate has a direct influence on stream water nitrate. Impact of precipitation nitrate is even less in agricultural land use-sections. The soil zone is known to be an efficient reaction space for nitrogen transformations. Consequently, N-input from precipitation is not directly channeled through the vadose zone under base flow conditions. Instead, this N-input is retained in the soil matrix by different processes such as assimilation/plant uptake and later mobilized by mineralization. Such N transformations in the soil involving nitrification lead to a reset of the ^{18}O variation of nitrate in the pore water so that the original isotope signature of precipitation nitrate cannot be recognized anymore (Casciotti 2009). Beyond that, the residence time of the N-input in the soil zone can reach up to several decades (Sebilo, Mayer et al. 2013).

3.4.4 Impact of denitrification on the stream water nitrate

Data shown in Figure 3.3 generally do not provide strong evidence for a major importance of denitrification on a regional scale. Nevertheless, we evaluated the impact of potential denitrification in relation to the land use (Figure 3.7). Sub-catchments with lower percentages of agricultural land use show visibly lower $\delta^{15}N_{NO3}$ values compared to sub-catchments with a higher percentage of agricultural land use. A shift towards heavier isotope values in stream water samples taken in July 2012 is significant ($p\text{-value} = 6 \cdot 10^{-6}$) compared to samples from the same locations taken in spring and autumn. This indicates a larger impact of microbiological denitrification processes on stream water nitrate with rising temperatures in summer. The temperature dependency implies that most of the denitrification is occurring in the streams, preferably in stream beds. In contrast, groundwater denitrification would not show a seasonal variability in aquifers due to constant groundwater temperatures throughout the year.

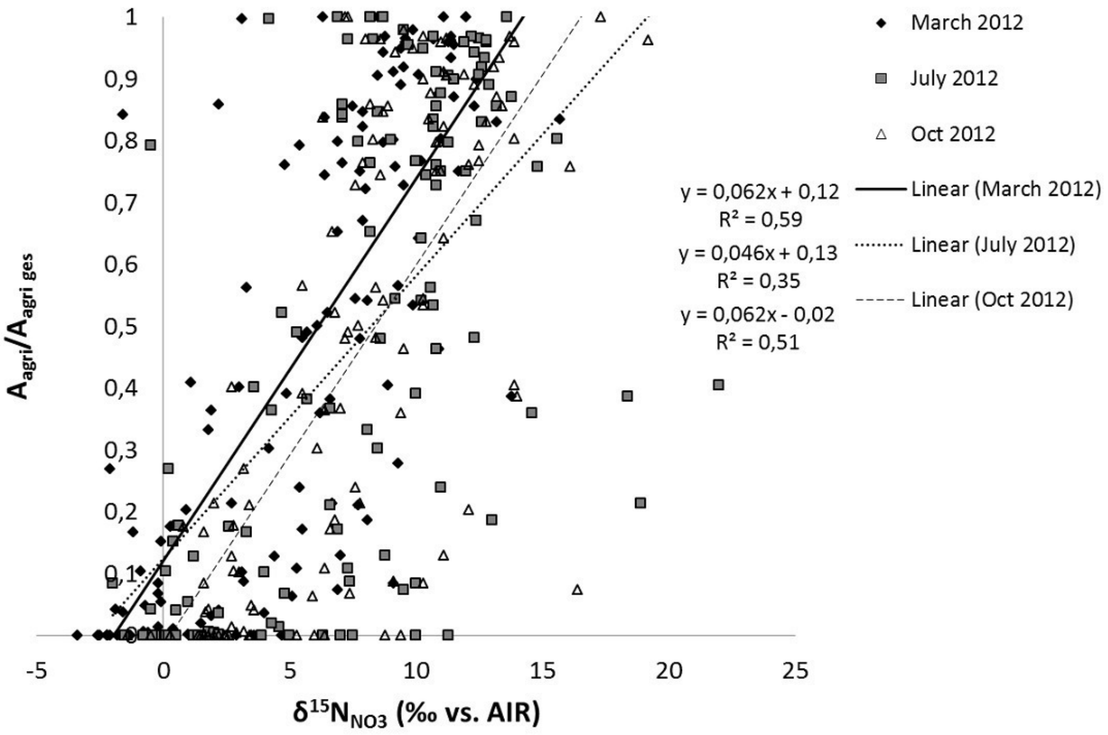


Figure 3.7: The $\delta^{15}N_{NO3}$ signature in the stream water from large sampling campaigns in March, July and October 2012 indicates a correlation between relative percentages of agricultural land use in every single sub-catchment. An increase in heavier $\delta^{15}N_{NO3}$ isotopes in July and decline in autumn suggest the impact of temperature-depending biological microbial nitrification and denitrification processes.

The travel time of the river water correlates with denitrification processes (Boyacioglu, Vetter et al. 2012) which have to be considered on a regional-scale. For this question, the deuterium excess in water can be a useful tool to study the movement at atmosphere-biosphere-lithosphere interfaces and express the influence of evaporation. With a deuterium excess lower than 8 ‰ an impact of evaporation processes in the catchment can be assumed (Gibson, Birks et al. 2008). This is especially evident when small lakes are present in the flow course of the stream (Knöller and Strauch 2002, Hofmann, Knöller et al. 2008). Figure 3.8 indicates that $\delta^{15}N_{NO3}$ increases with increasing evaporation processes or rather traveling time. In this case, microbiological denitrification activities can act much longer and $\delta^{15}N_{NO3}$ rises. Some sub-catchments show extremely depleted deuterium excess values down to -2 ‰ which are influenced by lake effects. This water is stored in lakes and evaporates. However, the highest $\delta^{15}N_{NO3}$ values are not depending on low travel times. Lowland tributaries with high human impact mainly from sewage or fertilizer have the most important effect on the nitrogen dynamic in the Bode catchment.

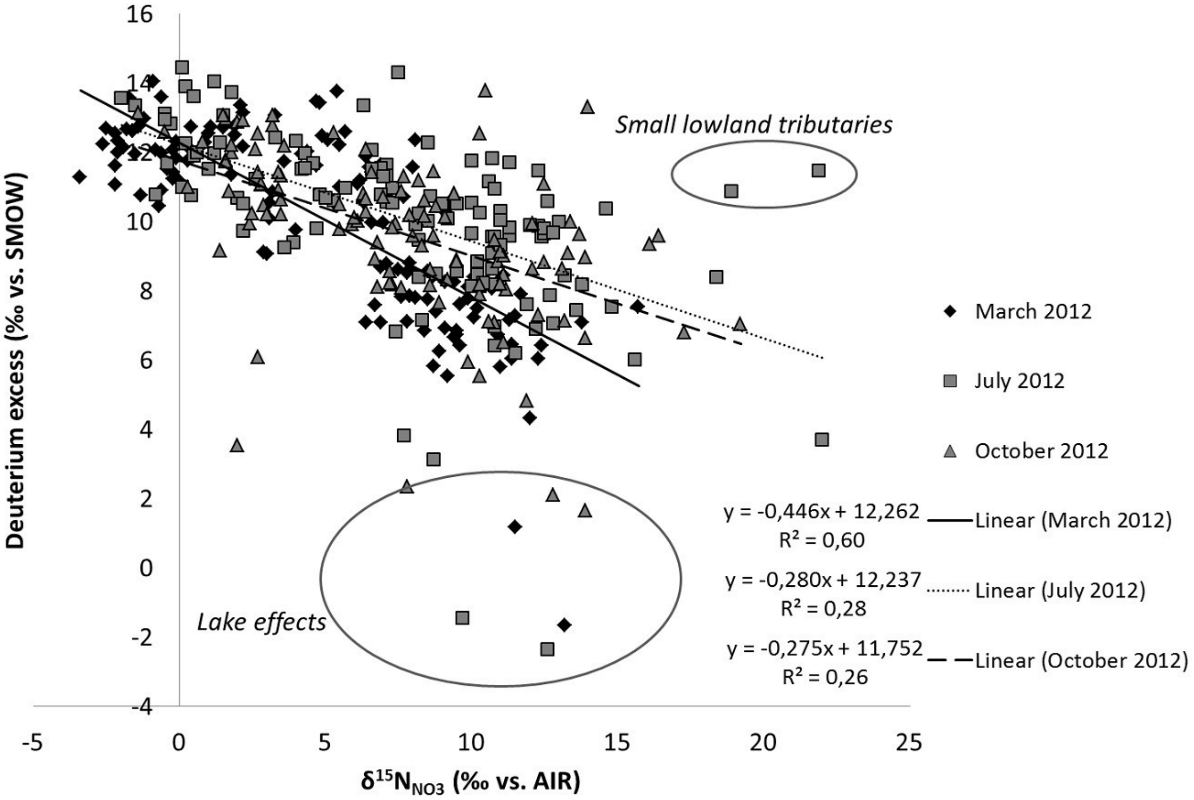


Figure 3.8: Evaporation-depending deuterium excess generally decreases with increasing $\delta^{15}N_{NO3}$ values. Rising evaporation results with longer travelling times of the stream water that correlates with increasing microbial denitrification activity to heavier isotopic nitrogen signatures. Small lowland tributaries which are highly influenced by agricultural land use and extreme high $\delta^{15}N_{NO3}$ values are not affected by evaporation.

3.5 Conclusions

Characteristic nitrogen isotope signatures could be recognized for all main types of landscape on the catchment-scale of the Bode River. The $\delta^{15}N_{NO3}$ values of spring regions, grass and forest dominated regions as well as areas with agricultural land use could be differentiated. High mountain water showed low $\delta^{15}N_{NO3}$ and high $\delta^{18}O_{NO3}$ values associated with low nitrogen loads. High mountain sub-catchments dominated by undisturbed forest and grassland displayed the lowest values in $\delta^{18}O_{NO3}$ and corresponding low values in $\delta^{15}N_{NO3}$. The $\delta^{15}N_{NO3}$ values increase with increasing agricultural land use. The high nitrate concentrations originate mainly from nitrification of ammonium fertilizer. This nitrate is especially mobilized and washed out during rain events. The nitrogen isotopic signature of the Bode River is mainly affected by major tributaries (Selke, Holtemme, Großer Graben) which are influenced by agricultural land use and domestic sewage. The Bode River is a mixture of these isotope signals and varies clearly over the seasonal sampling campaigns. Input compartments like precipitation may mainly influence the N and O isotope signature of nitrate of high mountain streams. The middle and lowlands are predominantly influenced by nitrate coming from anthropogenic sources like fertilizer from agricultural land use that contributes considerably to the stream water nitrate loads and shows high $\delta^{15}N_{NO3}$ values. An evident increase of microbial denitrification processes could be observed in stream water samples from lowland areas dominated by agricultural land use taken in summer. It can be assumed that most of the denitrification observed in the stream system occurs in stream because denitrification would not show a seasonal variability in aquifers that may interact with the stream water. However, highest nitrogen isotope signatures are present for small lowland tributaries with low travel times and therefore small microbial impact. Origin nitrogen signatures of sewage and fertilizer play a dominate role in the Bode river nitrogen dynamics.

4 Discharge driven nitrogen dynamics in a mesoscale river basin as constrained by stable isotope patterns

This chapter is an edited version of Mueller, Zink et al. (2016):

Mueller, C.; Zink, M.; Krieg, R.; Rode, M.; Merz, R.; Samaniego, L.; Knöller, K., Discharge driven nitrogen dynamics in a mesoscale river basin as constrained by stable isotope patterns, Environmental Science and Technology, submitted - accepted. 22 July 2016.

Funding

Data were provided by the German Weather Service (DWD), the Joint Research Center of the European Commission, the European Environmental Agency, the Federal Institute for Geosciences and Natural Resources (BGR), the European Water Archive, and the Global Runoff data Centre. The project is supported by TERENO (Terrestrial Environmental Observatories of the Helmholtz Association). The River Network is supported by ATKIS R DLM 1000 © Bundesamt für Kartographie und Geodäsie, 2003. Topographical information is based on Geobasisinformation on the Vermessungs- und Katasterverwaltung by permission of Landesamt für Landesvermessung und Datenverarbeitung Sachsen-Anhalt Gen.-Nr.I, Verm D/P/086/95.

Summary. The Bode River catchment (total size of 3200 m^2) in the Harz Mountains in Germany was intensively investigated by a monitoring approach with 133 sampling points representing the same number of sub-catchments for a period of two years. The investigation of nitrate isotopic signatures for characteristic landscape types allows the delineation of dominant NO_3^- sources: nitrified soil nitrogen, fertilizer, and manure. Besides source delineation, the relationship between runoff and nitrate dynamics was analyzed for the entire Bode river catchment and, more detailed, for one major tributary with minor artificial reservoirs (Selke River).

Own Contribution. The manuscript was drafted by Christin Müller, who performed the sampling, partly laboratory analysis and interpretation of the data under the direct supervision of Kay Knöller and Ralf

Merz. Michael Rode gave useful information for interpreting the isotope results and a model approach. Ronald Krieg was continuously involved in the sample collection and characterization of related parameters. Luis Samaniego and Matthias Zink developed and performed the mesoscale Hydrological Model.

Abstract. Nitrate loads and corresponding dual-isotope signatures were used to evaluate large scale N dynamics and trends in a river catchment with a strong anthropogenic gradient (forest conservation areas in the mountainous region, and intensively agricultural use in the lowlands). The Bode River catchment with an area of 3200 km^2 in the Harz Mountains and central German lowlands was investigated by a two years monitoring program including 133 water sampling points each representing a sub-catchment. Based on discharge data either observed or simulated by the mesoscale Hydrological Model (mhM) a load based interpretation of hydrochemical and isotope data was conducted. Nitrate isotopic signatures in the entire catchment are controlled by (I) the contribution of different nitrogen sources, (II) by variable environmental conditions during the formation of nitrate, and (III) by a minor impact of denitrification. For major tributaries, a relationship between discharge and nitrate isotopic signatures is observed. This may in part be due to the fact that during periods of higher hydrologic activity a higher wash out of isotopically lighter nitrate formed by bacterial nitrification processes of reduced or organic soil nitrogen occurs. Beyond that, in-stream denitrification seems to be more intense during periods of low flow.

4.1 Introduction

4.1.1 Nitrogen cycling in river systems impacted by human activity

Nitrogen is an essential nutrient for the synthesis of nucleic acids and proteins, for the growth of biomass and thus for agriculture production. On the other hand, it is a major multiplier on eutrophication of rivers, lakes and coastal ecosystems. The emission of nitrate has increased in the last decade triggered by three main causes:

(1) the application of organic or inorganic fertilizer;

(2) the widespread cultivation of rice, legumes, and other crops that are capable of converting atmospheric N_2 to organic nitrogen and

(3) the combustion of fossil fuels which transforms gaseous N_2 and fossil N to reactive nitrogen oxides (Pattinson, García-Ruiz et al. 1998, Galloway, Aber et al. 2003, Zweimüller, Zessner et al. 2008).

To control the impact of nitrate and its sustainable mitigation in aquatic systems, it is crucial to understand and quantify sources as well as biochemical processes which (permanently) remove nitrate (Birgand, Skaggs et al. 2007, Mulholland, Helton et al. 2008, von Schiller, Martí et al. 2008, Wagenschein and Rode 2008, Whitehead, Wilby et al. 2009). Isotope signatures of nitrate can help identify and quantify potential nitrate sources and characterize biogeochemical N-transformation processes (Hauck 1973, Heaton 1986, Durka, Schulze et al. 1994, Kendall and McDonnell 1998, Wankel, Kendall et al. 2007, Kendall, Elliott et al. 2008). Detailed information about relevant biochemical processes of natural and anthropogenic nitrate sources in the nitrogen cycle can be found in the support information part.

4.1.2 Microbiological isotope fractionation in N-compounds

The two major nitrate consuming bacterial processes in aquatic systems, NO_3^- denitrification and assimilation, result in significant isotopic fractionation rates (Table S4.1, supporting information). Nitrate which contains light isotopes (^{14}N and ^{16}O) is generally more rapidly consumed by bacteria than nitrate with heavy isotopes (^{15}N and ^{18}O). Thereby, the residual nitrate pool during microbiological fractionation processes which is measured in the streams is generally more enriched in heavy isotopes.

4.1.3 Impact of environmental conditions on the N-cycle in a river system

Nitrate process dynamics in river catchments are influenced by climate related variables and their changes, especially increasing stream temperature and discharge variability due to changed precipitation patterns (Boyacioglu, Vetter et al. 2012). Similarly, denitrification rates increase with higher water temperatures (Birgand, Skaggs et al. 2007, Zweimüller, Zessner et al. 2008). Boyacioglu, Vetter et al. (2012) found that especially in wet periods and with the canalization of streams, denitrification rates decrease. However, discharge variability has a higher impact on denitrification than seasonal temperature changes (Boyacioglu, Vetter et al. 2012). In contrast, Wexler, Goodale et al. (2014) observed microbial denitrification in temperate forest catchments in groundwater only, whereby surface water showed no evidence for the occurrence of bacterial denitrification. They also found that during small rain events precipitation mobilizes nitrate that makes up about 34 % of NO_3^- in the stream water. This nitrate is obviously derived from the microbial nitrification of soil-nitrogen. Generally, N-inputs by atmospheric deposition exceed the export from catchments. This implies an accumulation in the soil zone or a loss as gaseous N_2 during denitrification (MacDonald, Dise et al. 2002, Aber, Goodale et al. 2003).

4.1.4 Objectives

Past research related to the quantitative assessment of the nitrogen balance in catchments is mainly focused on small catchments (Mulholland, Helton et al. 2008, Wexler, Goodale et al. 2014) or does not include the advantage of stable isotope observations (Musolff, Schmidt et al. 2015). In this study, we clarify potentially occurring nitrogen transformation processes on a meso- to large scale river catchment considering hydrochemical and stable isotope observations. The purpose of this study is to examine spatio and temporal variabilities of nitrate stream loading related to land use and human impact on the N-cycle. This includes the identification of different nitrogen sources and microbiological turnover processes that consume or produce nitrate (chapter 3.5**,** supporting information) using ^{15}N and ^{18}O nitrate isotope data combined with water chemical parameters and hydrological modeling. Furthermore, we intended to recognize and evaluate predominate catchment transformation zones on a large scale and the impact of different hydrological conditions (base flow and high flow) on the catchment scale nitrogen dynamics. Also, we want to investigate to what catchment size (stream order) information on N-sources and N-turnover processes can be reliably differentiated by isotope methods. With our study, we want to contribute a tool set that helps predict the nitrate export from large river systems as a prerequisite for suggesting future safety precautions that guarantee a good ecological status of the water bodies from headwater to coast.

4.2 Study area

The Bode catchment is a hydrologically and hydrochemically intensively studied region in central Germany (Figure 4.1). It is part of the Terrestrial Environmental Observatories (TERENO) network which is hosted within the Helmholtz Association of German Research Centres (Zacharias, Bogena et al. 2011). The Bode catchment is characterized by a spatial gradient of diverse nitrate sources. The southern natural protected headwaters in the Harz Mountains represent the low nitrate concentrated region. With increasing river length the impact of agricultural land use increases. Major tributaries can be differentiated into four main groups after Mueller, Krieg et al. (2015):

- Firstly, the mountainous river *headwaters* with a discharge of about 28 % of total flow which discharge into the Rappbode drinking water reservoir including the Kalte Bode, Warme Bode, Rappbode and Luppbode stream.
- Secondly, *transfer streams* (Hassel, Selke and Holtemme with 27 % of the total discharge) flow between protected areas dominated by forest and areas of agricultural land use. Within in the group of transfer streams, 21 sub-catchments close to the headwater catchments are solely covered by forest. Further downstream, 9 sub-catchments are characterized by an increasing impact of agricultural land use and a mixed forest and agricultural zone (forest and agricultural land use covering each 50 % ± maximal 10 %). The tributaries are dominated mainly by agricultural land use (>60 % of each sub-catchment is covered by arable land) which is the case for 13 sub-catchments.
- Thirdly, the northern *agricultural lowlands* are dominated by arable land (Großer Graben with 21 % of total discharge).
- Fourthly, other minor tributaries which are not considered in the sampling network or diffuse inflow (including groundwater) account for 22 % of the total discharge.

Further characterization of major streams, climatic conditions as well as landscape information can be found in Mueller, Krieg et al. (2015).

The spring water from the Harz Mountains is used for drinking water and electrical power supply which is collected in the Rappbode reservoir. The LHW (Landesbetrieb für Hochwasserschutz und

Wasserwirtschaft) Saxony-Anhalt has 30 observation gauges which continuously register water-levels along the Bode River and major tributaries (Figure 4.1).

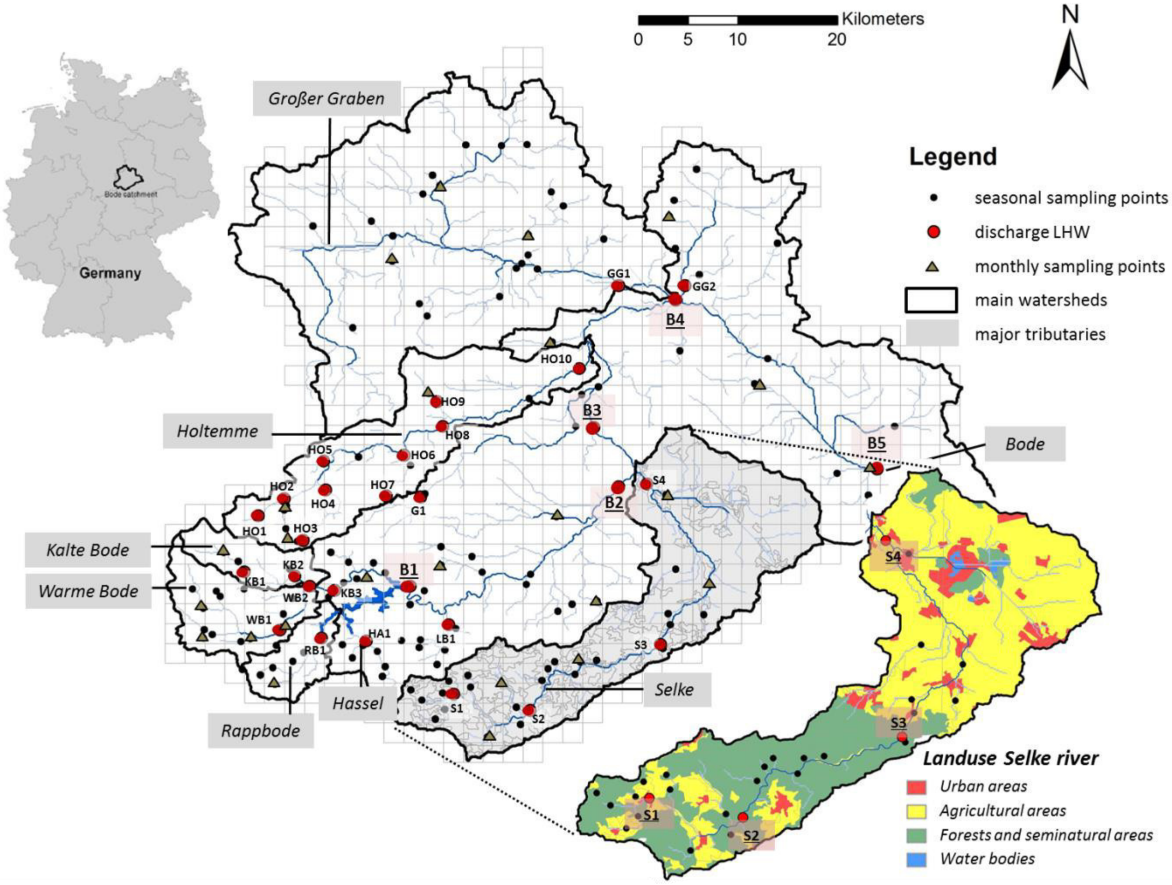


Figure 4.1: Location of the Bode catchment with major sub-catchments and spatial distribution of stream discharge gauges. The Selke tributary is heightened with runoff stations and land use information (EEA 2012).

4.3 Methods

4.3.1 Monitoring approach

In 2012, we started our two-year monitoring campaign in the Bode River catchment. It includes 133 sampling sites (Figure 4.1) which represent nearly undisturbed mountainous regions, forests, settlement areas as well as anthropogenic impacted areas by waste water and agricultural land use. Further information about regional, climatic and hydrological aspects are given in Mueller, Krieg et al. (2015) and Zacharias, Bogena et al. (2011). Spatial high resolution sampling campaigns were performed three times per year. Low resolution sampling campaigns including 25 representative test sites were sampled monthly (starting in 2013), between the high resolution campaigns. All main tributaries flowing into the Bode River were included into the monitoring network. Precipitation was collected monthly at the 25 representative test sites. During high and low resolution samplings campaign water samples were collected at the outlets of the major sub-catchments. Water samples were filtered in the field (0.45 µm cellulose acetate filter) and stored in high density polyethylene (HDPE)-bottles in the refrigerator. Additionally, all water samples were measured for pH-value, temperature and electrical conductivity in the field. The density of sampling spots is much higher in the southern mountainous area due to the high number of small creeks compared to the northern lowland area.

4.3.2 Laboratory analysis: nitrate and isotopic signatures

Laboratory analyses on all water samples were conducted for stable isotope signatures of nitrate ($\delta^{15}N$ and $\delta^{18}O$) and corresponding NO_3 contents within one month after sampling. Concentration of nitrate in water was analyzed by ion chromatography using a Dionex ICS-2000 combined with AS50. Nitrate isotopic signatures were measured with the denitrifier method by using bacteria strains of Pseudomonas chlororaphis (ATCC #13985) (Sigman, Casciotti et al. 2001, Casciotti, Sigman et al. 2002). Isotopic ratios are expressed in delta notation (eq. 4.1). $\delta^{15}N_{NO3}$ is reported relative to atmospheric nitrogen (AIR), whereby $\delta^{18}O_{NO3}$ is expressed relative to Vienna Standard Mean Ocean Water (VSMOW), calculated as:

$$\delta_{sample}\,[‰] = \left(\frac{R_{sample}}{R_{standard}} - 1\right) \times 1000 \qquad (4.1)$$

Ratio R describes the relation between heavier and lighter isotopes. For the analytical measurement a mass DELTA V Plus spectrometer was used in combination with a GasBench II from Thermo Scientific. The standard deviation of the described analytical measurement including sampling and preparation uncertainties is ±1.6 ‰ for $\delta^{18}O$ and ±0.4 ‰ for $\delta^{15}N$. Isotope measurements are conducted in duplicates and are repeated when the standard deviation exceeds the quality control threshold stated above. For calibration of nitrogen and oxygen isotope values, the reference nitrates IAEA-N3 ($\delta^{15}N$: +4.7 ‰ AIR; $\delta^{18}O$: +25.6 ‰ VSMOW), USGS-32 ($\delta^{15}N$: +180 ‰ AIR; $\delta^{18}O$: +25.7 ‰ VSMOW), USGS-34 ($\delta^{15}N$: -1.8 ‰ AIR; $\delta^{18}O$: -27.9 ‰ VSMOW), and USGS-35 ($\delta^{15}N$: +2.7 ‰ AIR; $\delta^{18}O$: +57.5 ‰ VSMOW) were used.

4.3.3 Regionalization of runoff: Hydrological modeling and nitrogen loads

The purpose of simulating the discharge for all investigated sub-catchments of the Bode River basin is to provide information on nitrate export from the respective sub-catchments and to understand the process dynamics across scales. Large stream basins are the sum of its associated sub-catchments and can be regionalized with different rainfall-runoff models, as explained and compared in e.g. Oudin, Andréassian et al. (2008). The mesoscale Hydrologic Model mHM used in this study is based on accepted hydrological conceptualizations and employs a multi-scale parameter regionalization (available at www.ufz.de/mHM). It is predicated on numerical approximations of commanding hydrological processes. Detailed information can be found in Samaniego, Kumar et al. (2010) and Kumar, Livneh et al. (2013). The software mHM is a distributed hydrological model which treats grid cells as hydrological units. It is a conceptual hydrological model similar to HBV (Bergström 1976) or VIC (Liang, Lettenmaier et al. 1994), which considers interception, snow accumulation and melting, infiltration, soil water retention, recharge, groundwater storage and runoff generation as main hydrological processes. The generated discharge consists of direct runoff, slow and fast interflow and baseflow. It is routed through the study domain using the Muskingum method after Tewolde and Smithers (2006).

A key feature of mHM is the estimation of effective parameters called Multiscale Parameter Regionalization technique (MPR (Samaniego, Kumar et al. 2010)). Effective model parameters are estimated using a two-step approach. In a first step the parameters (e.g. porosity) are estimated on the resolution of the morphological input data (i.e. 100 x 100 m). With the help of transfer functions (e.g. pedotransfer function) and a couple of transfer parameters (e.g. coefficients of the pedotransfer function), which are associated with terrain characteristics (e.g. sand and clay content), spatially distributed parameter fields are estimated. In a second step, this parameter fields are upscaled to the resolution of the hydrological simulation (i.e. 1 x 1 km^2). Thus, mHM explicitly accounts for sub grid variabilities and depends only on a couple of transfer or global parameters, which are space and time invariant. They are estimated during an automated calibration. MPR enables mHM to perform satisfying across different locations and scales (Samaniego, Kumar et al. 2010).

The objective of the calibration is to minimize the Nash Sutcliffe Efficiency (NSE) (Nash and Sutcliffe 1970) of discharge and the logarithm of discharge using the Dynamical Dimensioned Search Algorithm (Tolson and Shoemaker 2007). The NSE (eq. 4.2) is calculated as

$$E = 1.0 - \frac{\sum_{i=1}^{N}(Q_{obs}(i) - Q_{sim}(i))^2}{\sum_{i=1}^{N} Q_{obs}(i) - \bar{Q}_{obs})^2} \qquad (4.2)$$

where $\mathrm{Q_{obs}}$ denotes the observed discharge, $\mathrm{Q_{sim}}$ the simulated discharge and $\mathrm{\overline{Q}_{obs}}$ the mean observed discharge for all observations N. The calibration is done on the spatial resolution of 2 km in the time period 2000-2009, whereas discharge simulations for analyzing nitrate loads are conducted at 1 km spatial resolution. The calibration of mHM at a coarser resolution is reasoned in the savings in computational time. Kumar, Livneh et al. (2013) showed that this is a reasonable choice.

Since the Bode catchment is highly influenced by human interaction, the model has been fed by measured discharge time series downstream the area of the biggest reservoir, the Rappbode dam. Thus, the simulated upstream discharge time series has been neglected at this point. The feeding or inflow gauge is located at gauge B1 shown in Figure 4.1. For calibration the gauges B3 and S4 are used (Figure 4.1). B3 is the gauge which is representing the headwaters of the Bode River and advantageously is still not influenced by the hydraulic connection to the neighboring catchment. S4 is the outlet of the Selke River, which is the most natural behaving sub-catchment in the Bode basin. Furthermore, it is used for detailed nitrate dynamics analysis within this study. The estimated discharge at 1 x 1 $\mathrm{km^2}$ is evaluated at 20 out of 30 gauges, which were not or only insignificantly influenced by human activity. All simulations are conducted with a spin up period of 10 years to ensure that the initial conditions are reasonable.

4.4 Results and Discussion

4.4.1 Discharge simulation

The discharge simulations conducted with the hydrological model mHM could not be evaluated at all gauges available in the Bode catchment, because gauges downstream of the Rappbode dam are influenced by upstream water regulations or uptake. The results of the majority of the remaining gauges indicate a sufficient estimation of discharge with an average NSE of 0.65 for all gauges in the validation period 1980-1999 (Table 4.1) according to Moriasi, Arnold et al. (2007). The gauge GG1 reveals low performances because a hydraulic connection from the Großer Graben to the neighboring catchment does exist. This connection leads to the exchange of water between both catchments. At the river Holtemme and its tributaries, many water regulations are located in the upstream areas. In consequence, the downstream gauges are impacted by these regulations. This is the reason for lower NSEs at the gauges HO1, HO3 and HO10. One of the largest summer floods during decades took place in June 2013 in the Mulde, Saale and Elbe catchment, of which the Bode is a tributary (Conradt, Roers et al. 2013). This extreme event is captured by the model simulation, but the peak discharge is usually underestimated. One of the reasons is the magnitude of the event, which has rarely been observed before. This induces errors in the observed discharge time series due to considerable uncertainties in the rating curve. Another reason could be erroneous precipitation data, caused by the high wind speeds during the storm event and insufficient spatial density of rainfall gauges for that event. The underestimation of the flood event in May and June of 2013 can be observed in Figure 4.2 for the Selke tributary and the entire Bode catchment. The underestimation of discharge at the Bode outlet (Figure 4.2) during this period is additionally influenced by supplementary water entering the Bode catchment from the neighboring catchment via the hydraulic connection at the tributary Großer Graben. Finally, the model simulations are sufficient for relating loads observed at the sampling points with simulated discharge.

Table 4.1: Observed and simulated discharge at gauge stations (o - outlet, MQ - mean discharge, NSE - Nash-Sutcliffe efficiency)

Landscape type	ID	River	Time period	MQ $[m^3 s^{-1}]$	NSE		
					2000-2009	1980-1999	2012-2013
	B2	Bode	1961-2013	5.48	0.90	0.91	0.73
	B3	Bode	1893-2013	7.80	0.89	0.85	0.63
Intensive agriculture	B4	Bode	1930-2013	12.97	0.81	0.79	0.68
	B5	Bode (o)	1988-2013	12.64	0.71	0.75	0.67
	GG1	Großer Graben (o)	1986-2013	2.41	0.44^{*1}	0.51^{*1}	0.57^{*1}
Intensive agriculture and forest	HO1	Holtemme	1961-2013	0.28	0.38	0.40	0.38
	HO2	Holtemme	1970-2013	0.34	0.67	0.60	0.58
	HO3	Holtemme	1983-2013	0.13	0.59	0.40	0.45
	HO8	Holtemme	1971-2013	1.34	0.65	0.72	0.59
	HO10	Holtemme (o)	1982-2013	1.55	0.47^{*2}	0.49^{*2}	0.56^{*2}
	S1	Selke	2006-2010	0.33	0.72	-	-
	S2	Selke	1948-2013	1.13	0.64	0.68	0.56
	S3	Selke	1920-2013	1.46	0.70	0.69	0.45
	S4	Selke (o)	1980-2013	1.73	0.79	0.74	0.54
Headwater	KB1	Kalte Bode (o)	1950-2013	0.72	0.57	0.52	0.42
	WB1	Warme Bode	1960-2013	2.06	0.81	0.69	0.75
	WB2	Warme Bode (o)	1950-2013	2.42	0.64	0.67	0.75
	HA1	Hassel (o)	1968-2013	0.54	0.80	0.52	0.69
	RB1	Rappbode (o)	1950-2013	0.75	0.73	0.71	0.76
	LB1	Luppbode (o)	1996-2013	0.35	0.60	0.80	0.47

^{*1} hydraulic connection to the neighboring catchment

^{*2} impact of regulated tributary (Zillierbach dam)

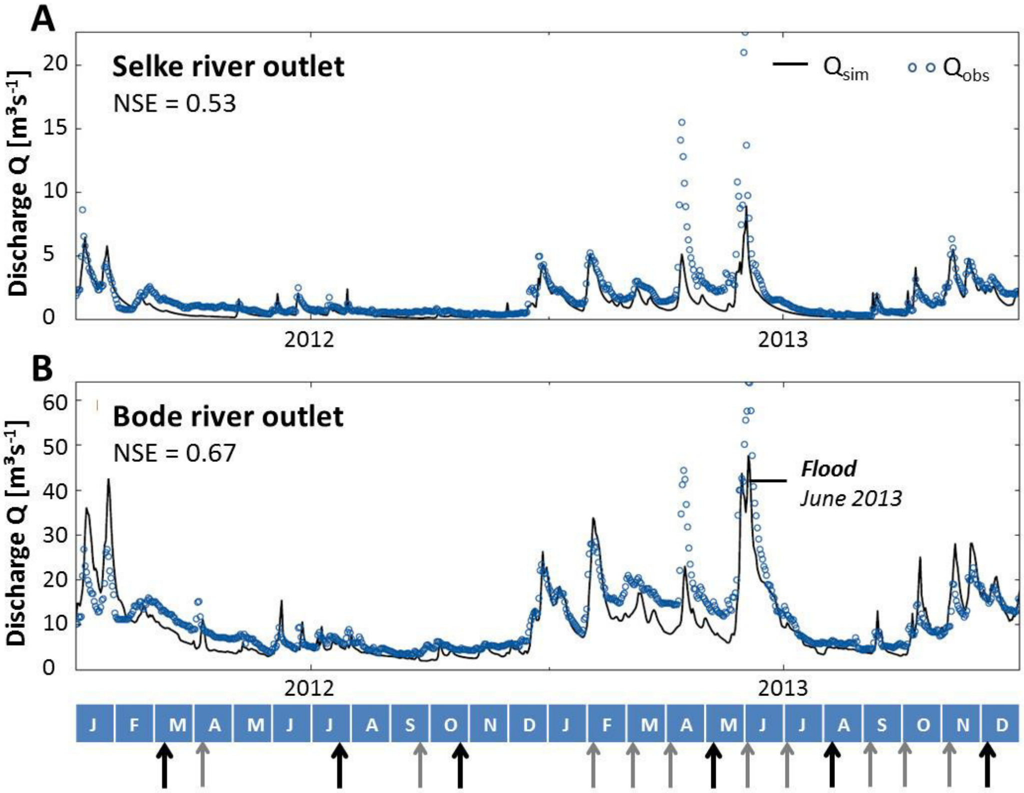


Figure 4.2: Simulated versus observed discharge at the Selke tributary outlet (S4) and the Bode River outlet (B5). Seasonal (↑) and monthly (↑) sampling campaigns are indicated on the temporal scale.

4.4.2 Distribution of discharge and nitrate load on catchment scale

The average annual discharge intensity on the Bode River outlet (B5) is about 8.3 m^3s^{-1} in 2012 and 14.9 m^3s^{-1} in 2013 with a mean nitrate load of about 6.5 t per year. The distribution of mean discharge of major tributaries of the Bode catchment over the years 2012 and 2013 is shown in Figure 4.3A. Discharge at the Bode outlet is composed of four components of comparable size: (I) headwater tributaries, (II) tributaries in mixed land-use catchment with intensive agriculture and forest (transfer streams), (III) tributaries in catchments with agricultural land-use only and (IV) minor tributaries directly to the Bode River or other unspecified inflows such as groundwater.

The corresponding calculated nitrate loads are distributed differently as shown in Figure 4.3B. The lowland agricultural tributaries (Großer Graben) and the transfer streams (Holtemme and Selke River) feature the major nitrate impacts with 73 % of the total nitrate load. Tributaries upstream the Rappbode dam in the high mountains transport low amounts of nitrate (6 % of the total nitrate load). Most of the samples were taken in the agricultural regions with 56 samples representing the major landcover type covering 75 % of the entire Bode catchment area. The forest region and headwaters are characterized by samples from 37 sub-catchments and 24 sub-catchments, respectively. The remaining 16 sub-catchments show a mixed landcover type of intensive agriculture and forest (each 50 % ± maximal 10 %).

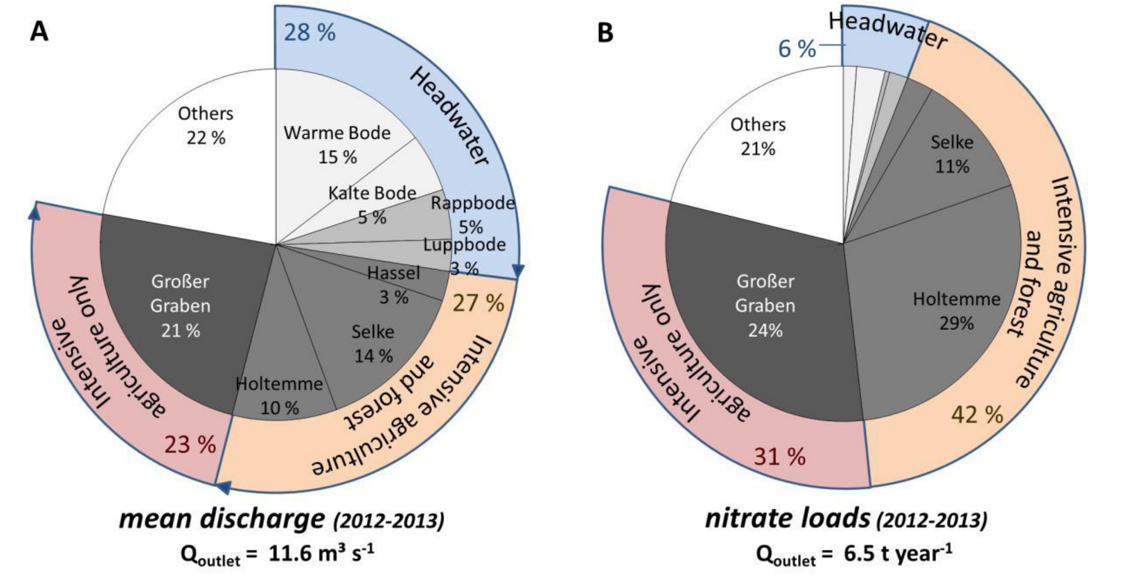


Figure 4.3: A. Annual mean discharge distribution of major Bode River tributaries in 2012 and 2013; B. Distribution of calculated nitrate loads based on measured discharge and nitrate concentration

For further temporal and spatial process dynamics analysis on catchment scale, the discharge and corresponding nitrate loads are weighted to the respective sub-catchment size. The area-weighted or so-called specific discharge ($Q_{specific}$) and nitrate loads ($(NO_3^-)_{specific}$, are calculated using eq. (4.3) and (4.4)), respectively.

$$Q_{specific} = \frac{Q_{sim}}{A_{sub\ basin}} \qquad [\mathrm{mm\ a^{-1}}] \qquad (4.3)$$

$$(NO_3^-)_{specific} = \frac{NO_3^-\ conc.\ *\ Q_{sim}}{A_{sub\ basin}} \qquad [\mathrm{g\ m^{-2}\ a^{-1}}] \qquad (4.4)$$

4.4.3 Temporal and spatial dependency of specific nitrate loads and isotope signatures on specific discharge

To detect possible nitrate sources and sinks, the comparison of specific discharge and nitrate loads between major landcover types along the river system can provide useful information (Mueller, Krieg et al. 2015). In general, the specific nitrate load increases with increasing specific discharge. For agricultural catchments specific nitrate load is much higher than for non-agricultural catchments for the whole range of specific discharges (Figure 4.4A). The specific nitrate loads in agricultural areas are significantly higher compared to non-farming regions with a maximum specific load of 70.3 g m^{-2} a^{-1} and a corresponding specific runoff of 2.2 mm a^{-1}. The specific nitrate loads for headwater, forest and mixed regions do not differ significantly between each other. The strongest correlation between specific discharge and specific nitrate load is obvious for agricultural catchments. For all other land use types, correlations are much weaker. This implies that the nature of the nitrate source is different between agricultural and all other land use types. The temporal variations of nitrate concentrations in (mainly lowland) tributaries are higher than in the main stream itself. The highest coefficients of variation c_v related to temporal nitrate load variability of up to 40 % are obvious for agricultural tributaries in the northern sub-basins (Figure 4.4B). A significant role plays the temporal differentiated release of fertilizer in the agricultural areas as well as fluctuating contribution of urban waste water.

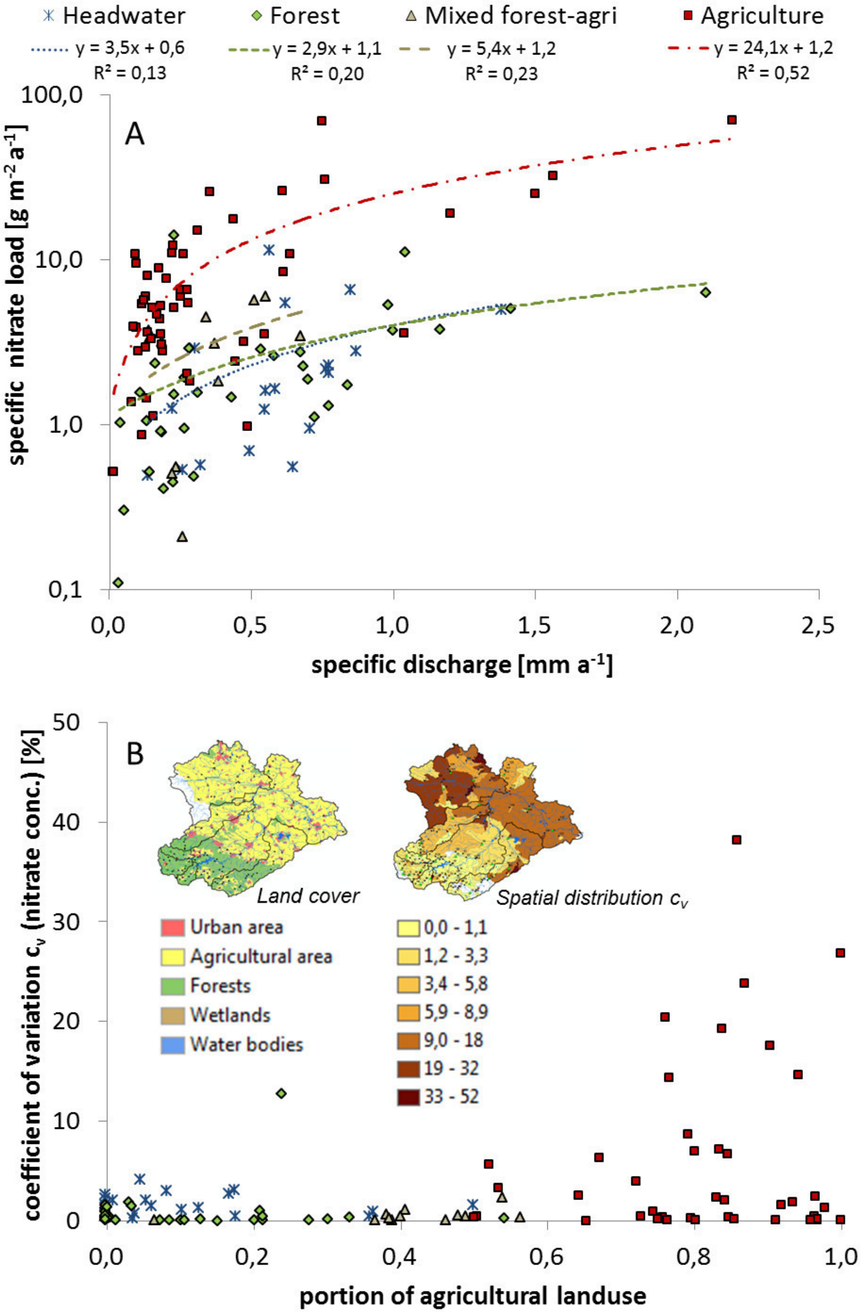


Figure 4.4: A. Trend between specific discharge and specific nitrate load the stream differentiated between major land use types. B. Relationship between calculated portion of agricultural land use for each sub-catchment and the coefficient of variation c_v in the nitrate concentration. Additionally, the spatial distribution of major landcover types and corresponding c_v is shown for the entire Bode River catchment.

The relationship between mean isotopic signatures of nitrate ($\delta^{15}N/\delta^{18}O$) for each sub-catchment and the proportion of agricultural land use is shown in Figure 4.5A and B. The nitrogen isotopic signature generally increases with increasing agricultural portions (Figure 4.5A). The outlet of the main river indicates a $\delta^{15}N_{NO3}$ value of +12 ‰ ± 1 ‰. Headwaters and catchments with forest land-use show a variation range of $\delta^{15}N_{NO3}$ from 0 ‰ to about 8 ‰ with low mean nitrate concentrations of 4.4 mg L^{-1}. These values are slightly higher than in other studies which report $\delta^{15}N_{NO3}$ in forested watersheds between -1 ‰ and +5 ‰ (Nestler, Berglund et al. 2011). For forested catchments with more than 20 % of agricultural land use, a positive shift of the N isotope signatures up to +11 ‰ indicating the impact of N sources related to agricultural practice including waste water. Generally, nitrate sources from agricultural land use are characterized by relatively elevated nitrogen isotopic signatures up to +25 ‰ (Kendall and McDonnell 1998) which are obvious for agricultural dominated tributaries (Figure 4.5C and E). Synthetic fertilizers show only insignificant impact on the isotopic $\delta^{15}N$ signature of the stream network, because they could only lower nitrogen isotopic values towards typical signatures between -3 ‰ and +3 ‰ and corresponding oxygen isotopic signature between +23 ‰ and +24 ‰ (Kendall and McDonnell 1998, Jiang, Jomaa et al. 2014). Increasing inflow of urban or agricultural waste water into the stream network (Figure 4.5E) is assumed to result in a higher pollution especially in the lower Bode region (Rode, Halbedel née Angelstein et al. 2016). On the contrary, the amount of waste water for the major Selke stream just accounts 2.9 % of the entire nitrate loads (Rode, Halbedel née Angelstein et al. 2016).

The oxygen isotopic signature of nitrate at the sub-catchment outlets varies between -0.3 and +9.8 ‰. The main river is a mixture of these signatures and varies between +2.9 and +6.7 ‰. Large $\delta^{18}O_{NO3}$-variations in small catchments can be explained either by reactive isotope fractionation processes during nitrate formation and their dependency on temperature and hydrological conditions or by a minor yet variable direct input of atmospheric nitrate ($\delta^{18}O$-NO_3 +75 ‰ for the Bode region after Mueller, Krieg et al. (2015)) into the stream system. Generally, the oxygen isotopic signatures of nitrate do not show a significant correlation with major land use types (Figure 4.5B). However, it can be noted that, especially in catchments with agricultural land-use, elevated N isotope signatures are accompanied by slightly elevated O isotope signatures (dual nitrate plot in Figure 4.5E). This may indicate a certain impact of bacterial denitrification as a nitrate consuming process occurring in groundwater feeding the springs or in the stream itself (Mariotti, Mariotti et al. 1982). Correlations between isotope signatures and nitrate concentration data do not provide additional evidence for the occurrence of denitrification. This might be due to the fact that the variation of the initial nitrate concentrations exceeds the concentration impact of denitrification.

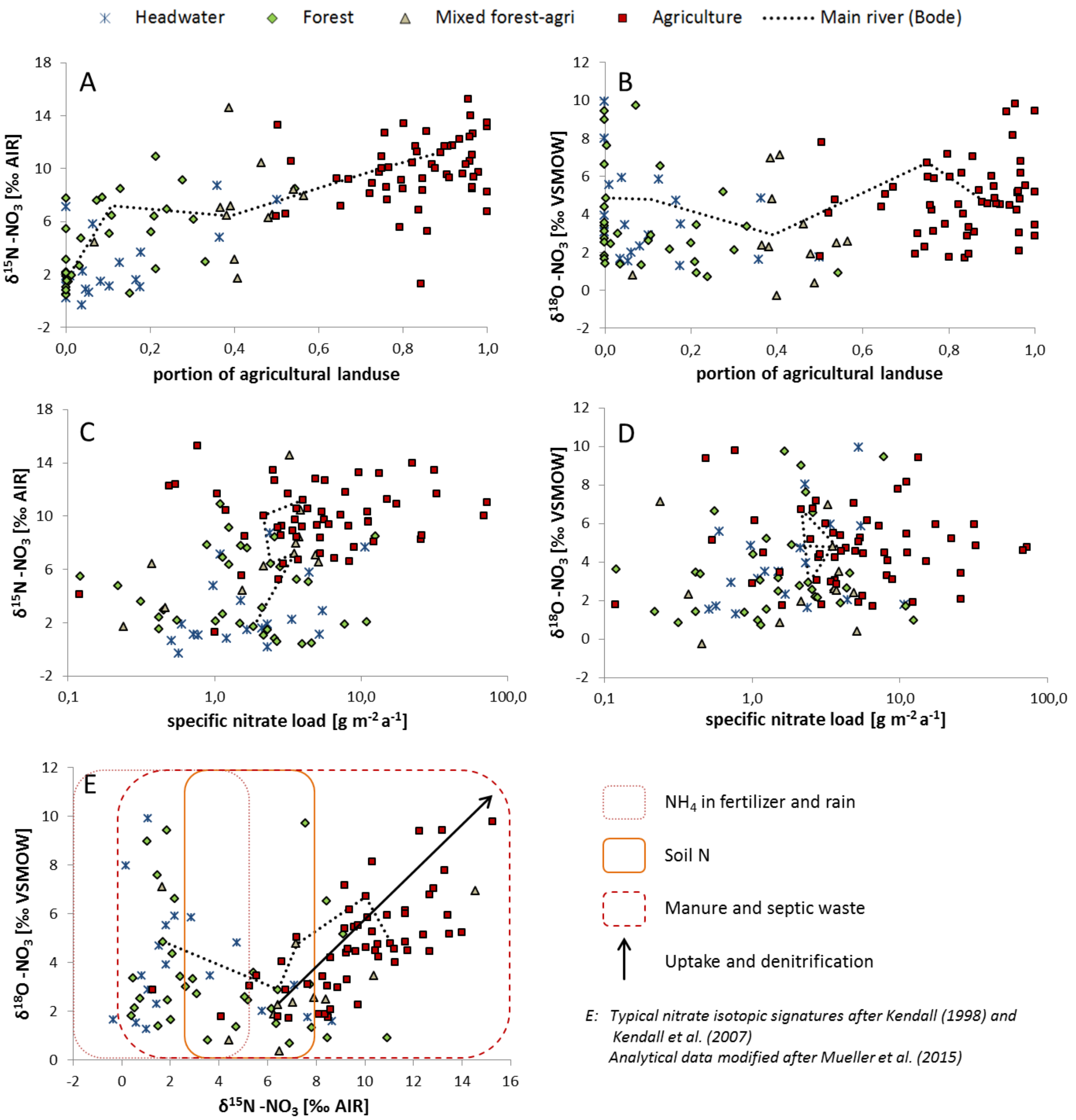


Figure 4.5: Relationship between mean δ^{15}N (A) and δ^{18}O (B) for nitrate and portion of agricultural land use; Relationship between specific nitrate loads and nitrate isotopic signatures δ^{15}N (C) and δ^{18}O (D) as well as mean dual nitrate isotope plot (E) with typical NO_3^- source signatures

4.4.4 Nitrate dynamics related to different hydrological conditions

Slightly elevated oxygen isotope signatures suggest that only a small share of stream water nitrate in headwater catchments directly derives from atmospheric deposition. Groundwater feeding the springs during base flow conditions indicate recycling (immobilization with subsequent mineralization) of atmospheric nitrate within the soil zone. During high flow events (Figure 4.6A), oxygen isotopic signatures of nitrate follow a similar pattern as during base flow conditions (Figure 4.6B). Elevated nitrate concentrations during high flow, however, indicate a more intense mobilization of nitrified soil nitrogen that originates either from the atmosphere or from the decay of organic material.

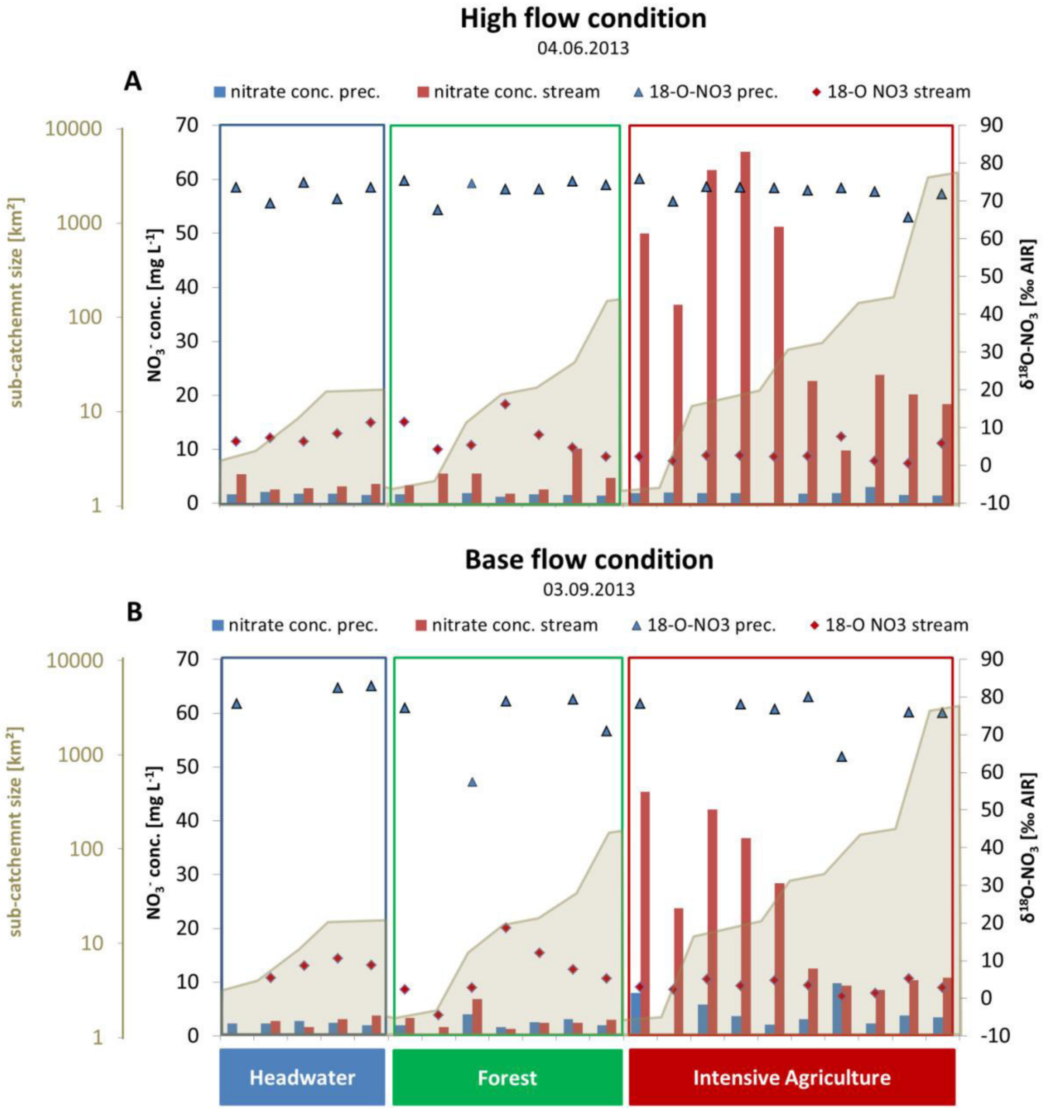


Figure 4.6: Contrasting hydrological conditions (high flow (A) and base flow (B)) with corresponding nitrate concentration and $\delta^{18}O\text{-}NO_3^-$ of stream water and precipitation water related to major land-cover type and sub-catchment size.

4.4.5 Catchment scale assessment of predominant nitrogen transformation zones

To assess nitrate dynamics in a nearly natural environment we selected the Selke River, a tributary of the Bode, as a special study site. Within this catchment we try to identify the influence of changing land use along the river course on nitrate isotopic signatures (Figure 4.1). According to Jiang, Jomaa et al. (2014) and Musolff, Schmidt et al. (2015), the mesoscale Selke River shows increasing inorganic nitrogen (IN as ammonium, nitrate or nitrite) concentrations with rising runoff. Furthermore, the Selke region is affected by high temporal and spatial variations of IN leaching, influenced by hydrological transport processes (Jiang, Jomaa et al. 2014). The mean concentration of inorganic nitrogen along the Selke River increases downstream at the discharge stations (S2 to S3 to S4) from 1.44 to 1.75 to 3.91 mg L^{-1} (Jiang, Jomaa et al. 2014). Corresponding nitrate isotopic signatures show variations from +4 to +13 ‰ for $\delta^{15}N_{NO3}$ (Figure 4.7A) and 0 to +5 ‰ for $\delta^{18}O_{NO3}$ (Figure 4.7B), respectively. Generally, the nitrate isotopic signature increases downstream the Selke River from gauges S2 to S3 to S4. This is due to the increasing impact of agricultural land use. Coefficients of determination (R^2) for the correlation between measured discharge and N-isotope signatures of nitrate range between 0.30 and 0.47 for Selke River stations. Lower R^2 values for Bode River stations (0.16 to 0.20) indicate an even weaker correlation. Even though weak, the observed correlation between discharge and N-isotope signatures may in part be related to an advanced high flow mobilization of N from the nitrification of reduced or organic soil nitrogen (as indicated by a positive correlation between discharge and N-concentrations, see: Jiang, Jomaa et al. (2014) and Musolff, Schmidt et al. (2015)) that relatively less actively emits nitrate during periods of low flow.

Even though the coefficients of determination (R^2) for the correlation between measured discharge and O-isotope signatures of nitrate are very low (0.12 to 0.49 for Selke River stations), a relationship seems obvious in a way that higher discharge is accompanied by decreased $\delta^{18}O_{NO3}$ values. Such trend might in part be due to the enhanced mobilization of isotopically lighter nitrate formed by bacterial nitrification processes of reduced or organic soil nitrogen during periods of higher hydrologic activity and the resulting higher wash out. During nitrification, the oxygen isotope signature of nitrate is controlled by the incorporation of O-H_2O and O-O_2 (+23.5 ‰) into the nitrate molecule. Normally, two O atoms are derived from ambient water while one O atom comes from dissolved oxygen. However, due to variable kinetic and equilibrium fractionation during O incorporation an additional uncertainty of up to 5 ‰ is introduced to the 2:1 ratio (Buchwald and Casciotti 2013). Such different discharge scenarios and the related differences of the residence time in the reactive zone where nitrification is taking place may have significant impact on the isotope fractionation during O-incorporation. Consequently, oxygen isotope signatures of nitrate depend in part on the discharge intensity (Figure 4.7B). The isotopic signature of

NO_3 in precipitation does not affect significantly the isotopic signature of the Selke tributary because it would increase $\delta^{18}O_{NO3}$ values (Mueller, Krieg et al. 2015).

Beyond a different mobilization scheme during periods of higher flow, the observed correlation pattern between isotope signatures, nitrate concentrations and discharge may in part be due to the in-stream turnover of nitrate either by denitrification or by uptake. During low flow conditions, a more intensive denitrification following oxygen depletion of stream water nitrate with biofilms on the stream bed substrate would increase the impact of the nitrate turnover and result in elevated isotope signatures of both nitrogen and oxygen in nitrate (Figure 4.7A, B) and lower nitrate concentrations (Jiang, Jomaa et al. 2014). The slope of the regression line between oxygen and nitrogen isotope signatures measured at three gauging stations of the Selke River is close to 0.5, a value that is considered to be characteristic for denitrification in terrestrial aquatic systems (Kendall and McDonnell 1998). For the two Bode River gauging stations this correlation becomes very weak as mixing processes of nitrate from all tributaries mask the potential isotopic effect of in-stream nitrate turnover.

As a consequence of the general dependency of the variability of isotope signatures on discharge intensity, a reliable resolution of source and process dynamics is only possible for smaller sub-catchments. While an evaluation of the isotope data set with respect to source delineation and certain target processes such as denitrification is still possible for the Selke River catchment, an increasing impact of mixing processes on the isotope signal in the main river does not allow for a differentiated analysis of the N dynamics anymore. After all, our study reveals that the nitrate degradation potential in groundwater or stream water is insufficient to control the nitrate export from the Bode River watershed. Therefore, nitrate loads in the river system can only be regulated by preventive measures especially by an optimized land use management.

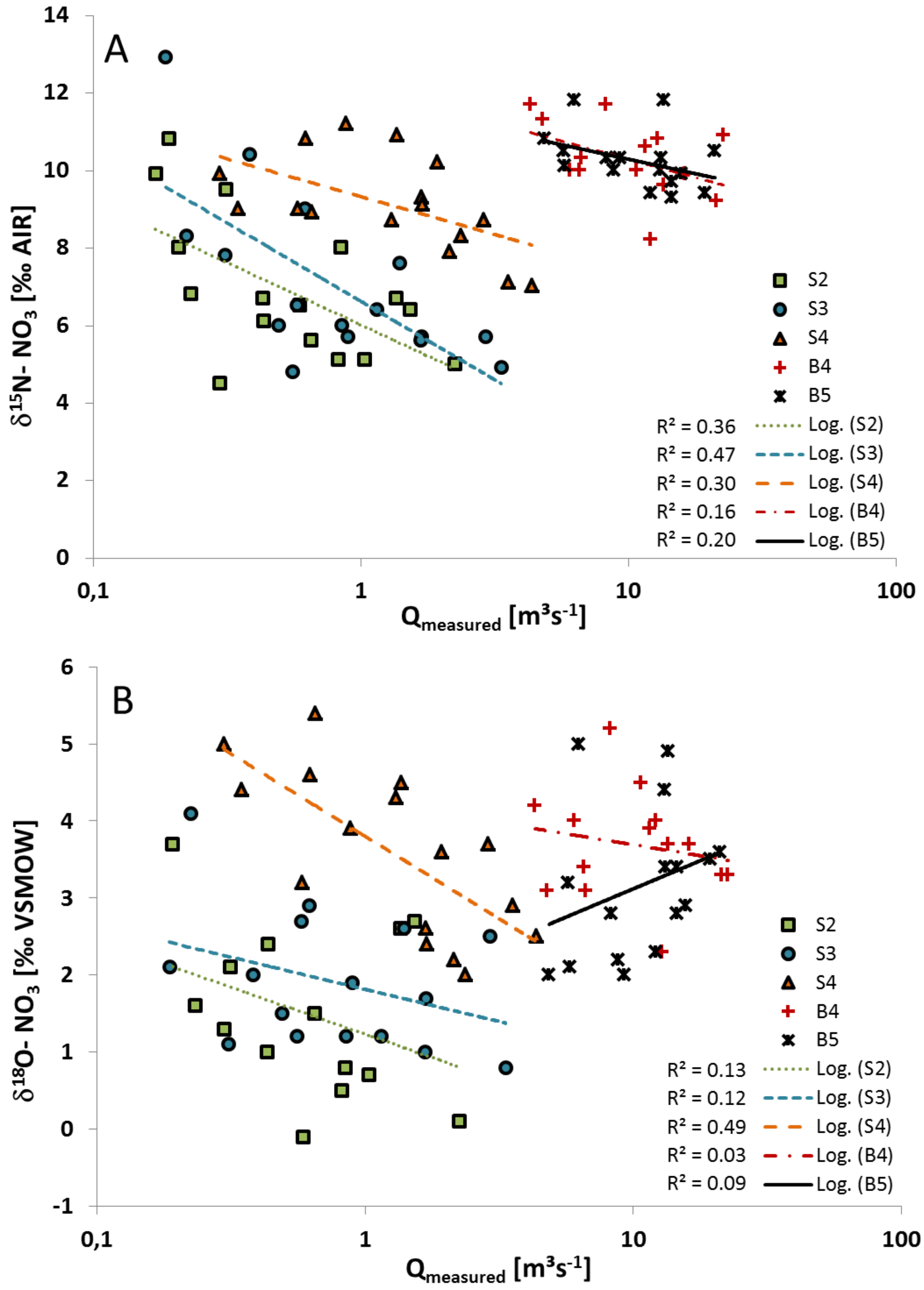


Figure 4.7: Relationship between measured nitrate isotopic signature (δ^{15}N (A) and δ^{18}O (B)) (S2-S3-S4, monthly measurements in 2013) and continuously measured discharge at stations along the tributary Selke River. For comparison, nitrate isotopic signatures and discharge measurements are shown for stations of the Bode River right downstream of the inflow of the Selke River into the Bode River (B4) and on the outlet of the Bode River (B5).

4.5 Supporting information

Text S4.1: Biochemical processes of natural and anthropogenic nitrate sources in the N-cycle

Common nitrate sources for contemporary central European river systems are the atmosphere as dry and wet deposition, industrial fertilizer or manure as well as treated or untreated wastewater. Besides, gaseous nitrogen can be fixed to organic nitrogen by diazotrophic prokaryotes like cyanobacteria or other microorganisms in soil and streams (Raymond, Siefert et al. 2004). In terrestrial systems, biological nitrogen fixation (BNF) rates are nearly balanced with opposed denitrification rates (Canfield, Glazer et al. 2010, Vitousek, Menge et al. 2013) depending on temperature and nutrient supply (Marcarelli and Wurtsbaugh 2006). The nitrogen cycle is influenced by modern N sources like synthetic nitrate from the Haber-Bosch process, nitrogen fixation by agricultural practice as well as the combustion of fossil fuels. These anthropogenic sources double the natural rate of terrestrial BNF (Canfield, Glazer et al. 2010). The formed organic-N can be mineralized to ammonium and furthermore nitrified to nitrate. Subsequently, nitrate can be assimilated by plants and as ammonium by microorganisms in the soil and freshwater and thereby are transferred back to organic-N. Irreversible nitrogen removal is possible by anoxic denitrification processes of soil-NO_3^- into gaseous N_2, or by anaerobic microbial ammonium oxidation (anammox) (Casciotti and Buchwald 2012, Thamdrup 2012).

Table S4.1: Selected nitrogen isotope enrichment factors (ε) and relation to associated nitrate-oxygen ratios for certain forming and removing nitrate processes in stream aquatic systems

Process	$^{15}\varepsilon$ **(‰)**	$^{18}\varepsilon$:$^{15}\varepsilon$
Nitrate sources		
(Aerobic) nitrification	0.0 ± 0.5 (NH_4^+ limited) (Heaton 1986, Kendall, Elliott et al. 2008) -14 to -35 (NH_4^+ abundant) (Feigin, Shearer et al. 1974, Mariotti, Germon et al. 1981)	- (oxygen not contained in substrate)
Nitrate sinks		
(Anaerobic) denitrification	-6 to -25 (Brandes, Devol et al. 1998, Deutsch, Liskow et al. 2005, Kendall, Elliott et al. 2008, Houlton and Bai 2009)	0.4 to 0.7 (field experiment) (Mariotti, Germon et al. 1981, Deutsch, Liskow et al. 2005, Kendall, Elliott et al. 2008, Knöller, Vogt et al. 2011)
Anaerobic ammonium oxidation	23.5 to 29.1 (batch experiment) (Brunner, Contreras et al. 2013)	
Assimilation in organisms	medium ε_{in} = 2.0 ± 0.3 ε_{out} = 1.2 ± 0.4 intercellular ε_{nr} = 26.6 ± 0.2 (Karsh, Granger et al. 2012)	~ 1 (Mariotti, Germon et al. 1981, Deutsch, Liskow et al. 2005, Kendall, Elliott et al. 2008, Karsh, Granger et al. 2012)

5 Tomography of anthropogenic nitrate contribution along a mesoscale river

This chapter is an edited version of Müller, Musolff et al. (2018):

Müller, C.; Musolff, A.; Strachauer, U.; Brauns M.; Tarasova L.; Merz, R.; Knöller, K., Tomography of anthropogenic nitrate contribution along a mesoscale river, Science of the Total Environment, accepted (27/09/2017)

Funding

Data were provided by the German Weather Service (DWD), the Joint Research Center of the European Commission, the European Environmental Agency, the Federal Institute for Geosciences and Natural Resources (BGR), the European Water Archive, and the Global Runoff data Centre. The River Network is supported by ATKIS R DLM 1000 © Bundesamt für Kartographie und Geodäsie, 2003. Topographical information is based on Geobasisinformation on the Vermessungs- und Katasterverwaltung by permission of Landesamt für Landesvermessung und Datenverarbeitung Sachsen-Anhalt Gen.-Nr.I, Verm D/P/086/95. Discharge information is provided by the State Office of Flood Protection and Water Management (LHW).

Summary. The Holtemme River catchment (total size of 282 km^2) in the Harz Mountains in Germany was intensively investigated during base flow conditions within two sampling campaigns in 2014 and 2015. Due to a general low main river discharge (outlet: 1.42 m³ s^{-1} in 2014 and 0.43 m³ s^{-1} in 2015m³ s^{-1}), the impact of agricultural land use and waste water treatment plants on the nitrate export plays the most important role.

Own Contribution. The manuscript was drafted by Christin Müller, partly laboratory analysis and interpretation of the data under the direct supervision of Kay Knöller and Ralf Merz. Ulrike Strachauer and Mario Brauns have organized the sampling campaign and took the samples. Andreas Musolff measured discharge and gave useful information for interpreting the isotope results. Larisa Tarasova performed the discharge separation model.

Abstract. Elevated nitrate concentrations are a thread for water supply and ecological integrity in surface water. Nitrate fluxes obtained by standard monitoring protocols at the catchment outlet strongly integrate spatially and temporally variable processes such as mobilization and turnover. Consequently, inference of dominant nitrate sources is often problematic and challenging in terms of effective river management and prioritization of measures. Here, we combine a spatially highly resolved assessment of nitrate concentration and fluxes along a mesoscale catchment with four years of monitoring data at two representative sites. The catchment is characterized by a strong land use gradient from pristine headwaters to lowland sub-catchments with intense agricultural land use and wastewater sources. We use nitrate concentrations in combination with hydrograph separation and isotopic fingerprinting methods to characterize and quantify nitrate source contribution.

The hydrological analysis revealed a clear dominance of base flow during both campaigns. However, the absolute amounts of discharge differed considerably from one another (outlet: 1.42 $m^3 s^{-1}$ in 2014, 0.43 $m^3 s^{-1}$ in 2015). Nitrate concentrations are generally low in the pristine headwaters (<3 mg L^{-1}) and increase downstream (15 to 16 mg L^{-1}) due to the contribution of agricultural and wastewater sources. While the agricultural contribution did not vary in terms of nitrate concentration and isotopic signature between the years, the wastewater contribution strongly increased with decreasing discharge. Wastewater-borne nitrate load in the entire catchment ranged between 19 % (2014) and 39 % (2015). Long-term monitoring of nitrate concentration and isotopic composition in two sub-catchment exhibits a good agreement with findings from spatially monitoring. In both datasets, isotopic composition indicates that denitrification plays only a minor role. The spatially highly resolved monitoring approach helped to pinpoint hot spots of nitrate inputs into the stream while the long-term information allowed to place results into the context of intra-annual variability.

5.1 Introduction

Nitrate concentrations in surface and groundwater ecosystems have increased in recent decades due to land use change and accompanying application of fertilizer in agriculture as well as from fossil fuel combustion and subsequent atmospheric deposition (Pattinson, García-Ruiz et al. 1998, Galloway, Aber et al. 2003, Zweimüller, Zessner et al. 2008). Although since the 1980s, nitrogen oxide and ammonia emissions in Europe declined by 49 % and 18 %, respectively (Hettelingh, Posch et al. 2014), inputs especially from agricultural fertilizers are still high (Federal Ministry for the Environment and Federal Ministry of Food 2012) resulting in water quality deterioration in groundwater and surface water (Altman and Parizek 1995, Wassenaar 1995, Sebilo, Billen et al. 2003) and are a major control of eutrophication, especially for coastal environments (Prasuhn and Sieber 2005, Decrem, Spiess et al. 2007). Moreover, nitrate increases primary production and has the ability to change food web structures of riverine and coastal ecosystems (Turner and Rabalais 1991, Howarth, Billen et al. 1996). Similarly, elevated nitrate concentrations are the cause for the bad chemical status of 26 % of all groundwater bodies in Germany (Völker, Mohaupt et al. 2016). In 2016, the European Commission filed a law suit against the German Federal Government related to constantly elevated nitrate concentrations in groundwater (ZEIT-ONLINE 2016). Existing and partly legally binding targets failed for river and lake protection, air quality control and natural conservation. In a report from the German Advisory Council on the Environment (SRU 2015) 40 proposed measures coping with nitrate as an environmental pollutant were listed. To draft an amendment for a fertilization ordinance regulating the application of manure and fermentation waste products and to implement a pollution tax for nitrate surplus from agricultural practice are two of the highest priorities. To make effective use of these measures, it is important to characterize and quantify potential nitrate sources and in-stream nitrate processing and its controls in individual catchments. Different sources of nitrate are often characterized by individual isotopic signatures that can be used as fingerprints for source delineation or process mapping in hydrological systems (Rock and Mayer 2004, Xue, Botte et al. 2009). For instance, atmospheric NO_3^-, nitrified soil-nitrogen can be distinguished from synthetic fertilizer by its distinct nitrate isotopic signatures (Aravena, Evans et al. 1993, Wassenaar 1995, Kendall and McDonnell 1998). However, a clear isotope-based distinction between different sources is not always possible. Sometimes N and O isotope signatures overlap as observed for NO_3^- from animal manure and wastewater effluents (Aravena, Evans et al. 1993). Therefore, a combination of stable isotope information with other environmental tracers (i.e. chloride, bromide, manganese, ammonium and iron) (Altman and Parizek 1995, Mengis, Schif et al. 1999) as well as a land use analysis (Nestler, Berglund et al. 2011, Mueller, Zink et al. 2016) can enhance the ability to describe the origin of nitrate. To characterize the mobilization of different nitrate pools, it is also important to investigate discharge and corresponding nutrient loads during different seasonal discharge scenarios. High seasonal and interannual variations in discharge and nutrient flows are associated with changing landuse patterns (Klose, Cooper et al. 2012). Fairbairn, Arnold et al. (2016) investigated micropollutants in a small watershed under different seasonal and hydrological conditions. They found out that agricultural herbicides showed the highest loadings during increased flows. In agriculturally-influenced prairie streams, Kemp and Dodds (2001) found out that nitrate concentrations are negatively correlated with discharge. Therefore, hydrological effects can have various impacts on the water quality of the gaining stream.

The objective of this study is to apply a fingerprint monitoring method to assess spatial and temporal variability of nitrogen sources within a mesoscale river catchment. The studied Holtemme catchment represents a blueprint example of pristine mountainous headwaters and agricultural as well as urban impacts in the downstream parts. The novelty of this study is the combination of spatially highly resolved assessments along the river with a longer-term monitoring in typical land use types: More specifically, two spatially highly resolved snapshot monitoring campaigns were conducted in October 2014 and 2015 during comparable hydrological base flow conditions. Sampling included 27 points within the river, 12 tributaries and two wastewater treatment plants. We measured nitrate isotopic compositions in concert with major ion concentrations and discharge to differentiate the impact of different nitrate sources and to quantify nitrate loads. This data is combined with a multi-annual, monthly monitoring at two stations representing undisturbed and agricultural land use sites. With this concept, we aim at identifying critical spatial areas as well as seasonal variations of nitrate related aspects of the water quality at catchment scale.

5.2 *Study area*

5.2.1 General information

The Holtemme River is a major tributary of the Bode River in the Harz Mountains, Germany (Figure 5.1). The stream is part of the Terrestrial Environmental Observatories' (TERENO) network and therefore one of the best equipped regions for Meteorology and Hydrology in Central Germany (Zacharias, Bogena et al. 2011, Wollschläger, Attinger et al. 2016). The Holtemme basin has a total size of 282 km² (Mueller, Krieg et al. 2015) and a mean annual discharge (MQ) at the outlet of 1.55 $m^3 s^{-1}$ (1982-2013), monitored by the State Office of Flood Protection and Water Management (LHW) Saxony-Anhalt. The long-term mean precipitation between the mountainous region and the lowlands varies between 1951 and 2015 from 1,262 mm to 614 mm (Rauthe, Steiner et al. 2013). The length of the river is 47 km and the altitude range is from 862 m a.s.l. at the headwaters to 85 m a.s.l. at the river mouth. The Holtemme River extends from the Harz Mountains in the south to the beginning of the Central German Lowlands and Magdeburger Börde (one of the most fertile agricultural areas in Germany) in the north (Wollschläger, Attinger et al. 2016). The investigated area is dominated by Mesozoic rocks which are covered by Tertiary and Quaternary sediments (Schuberth 2008). Intensively used agricultural land and non-irrigated arable land is the dominant land use type with a total area of 173.5 km² (61.6 % of the entire Holtemme catchment area) in the middle and northern region of the river basin (Figure 5.1). The mountainous southern region is mainly covered by coniferous forest with an area of 82.1 km² (29.2 % of the total Holtemme catchment). Two waste water treatment plants (WWTP) are located in the catchment collecting the waste water from the major towns (Wernigerode and Halberstadt) and their surroundings; one in Silstedt (WWTP I) and one in Halberstadt (WWTP II) (observation points 16 and 29 in Figure 5.1, respectively). Both WWTP's are connected to a combined and a separated collection system, respectively. During extreme rain events WWTP II has an overflow into the Holtemme River. The capacities of the WWTPs are shown in Table 5.1. The untreated water shows NH_4-N between 30 and 50 mg L^{-1}, N_{ges}-N between 60 and 80 mg L^{-1}. The limits for water discharged from the WWTPs to the Holtemme River are set for ammonium (NH_4-N) to 10 mg L^{-1} and for total nitrogen (N_{ges}-N) to 18 mg L^{-1}. The WWTP II discharges cleaned water with concentrations clearly below those limits (1.3 mg L^{-1} NH_4-N and 3.8 mg L^{-1} N_{ges}-N) (Abwassergesellschaft Halberstadt 2016). The total nitrogen load (TNb) in cleaned water in 2014 was approximately 35.09 kg d^{-1} (WWTP II) or 67.95 kg d^{-1} (WWTP I). Corresponding hydrological and chemical information on the cleaned water from both WWTPs during the sampling campaigns in 2014 and 2015 are shown in Table 5.2.

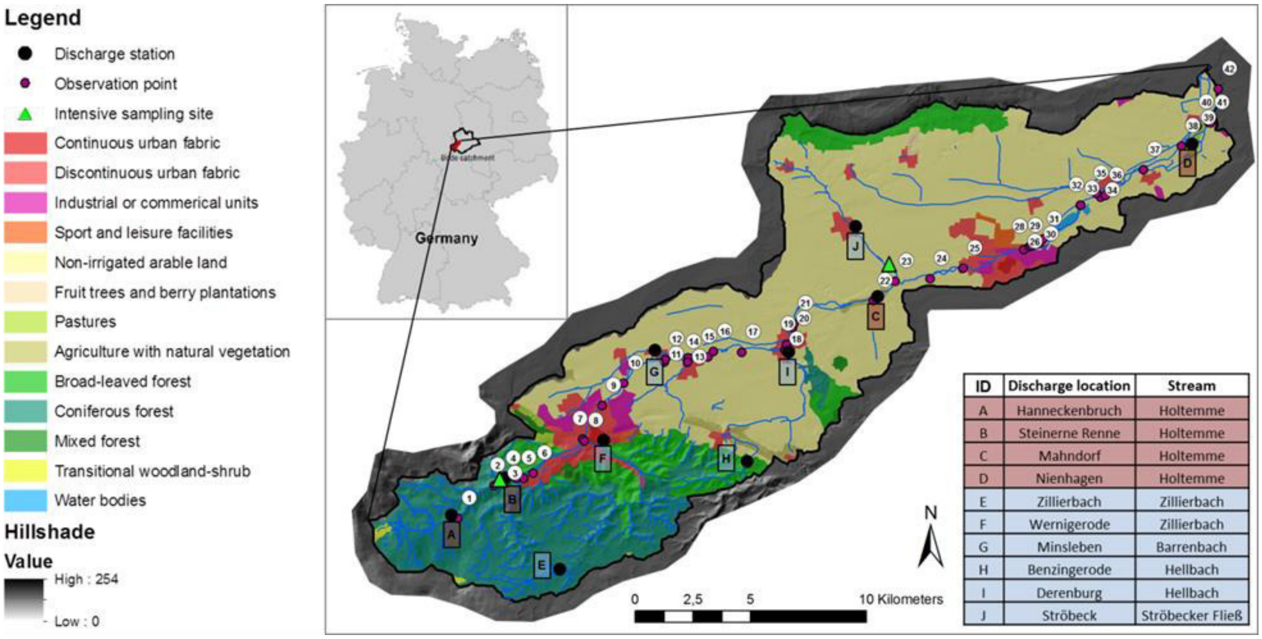
Table 5.1: General information about the WWTPs, exemplarily for 2014 (source: dam operators 'Wasser und Abwasserverband Holtemme-Bode' and 'Abwassergesellschaft Halberstadt GmbH')

waste water treatment plant	**WWTP I**	**WWTP II**
capacity based on inhabitants	80 000	60 000
amount of sewage [L s^{-1}]	115.7	79.3
total nitrogen bound (TNb) – outgoing freight [kg d^{-1}]	67.95	35.09

Table 5.2: Hydrological and chemical information on the cleaned water from the WWTPs on both sampling campaigns measured by the operators of WWTPs (individual samples)

	Date	Q* [$m^3 s^{-1}$]	pH-value	NH_4-N [$mg L^{-1}$]	NO_2-N [$mg L^{-1}$]	NO_3-N [$mg L^{-1}$]	total-N [$mg L^{-1}$]	total-N_{anorg} [$mg L^{-1}$]
WWTP I	06.10.2014	0.144	-	0.036	0.018	4.93	6.55	4.98
WWTP I	05.10.2015	0.112	-	0.018	0.010	4.76	6.67	6.67
WWTP II	07.10.2014	0.191	8.0	2.7**	0.1**	0.5**	4.3**	3.3**
WWTP II	06.10.2015	0.111	7.5	4.9**	0.0**	0.2**	8.0**	5.2**

*daily mean discharge; **mean value of water samples taken one day before and after the 07.10.2014/ 06.10.2015



ID	Discharge location	Stream
A	Hanneckenbruch	Holtemme
B	Steinerne Renne	Holtemme
C	Mahndorf	Holtemme
D	Nienhagen	Holtemme
E	Zillierbach	Zillierbach
F	Wernigerode	Zillierbach
G	Minsleben	Barrenbach
H	Benzingerode	Hellbach
I	Derenburg	Hellbach
J	Ströbeck	Ströbecker Fließ

Figure 5.1: Land use map (CORINE2000 land use dataset (EEA 2012)) with sampling locations and continuously monitored discharge stations (brown label – Holtemme River, blue label – sub-catchments) in the investigation area

5.2.2 Previous research

The investigated Holtemme River catchment (Figure 5.1) is located in an intensively studied area within the Terrestrial Environmental Observatories (TERENO) network (Wollschläger, Attinger et al. 2016). This allows us to interpret our recent findings in relation to already published data sets. For example, in

Mueller, Krieg et al. (2015) and Mueller, Zink et al. (2016), regional and discharge driven nitrogen dynamics for the entire Bode catchment, to which the Holtemme River belongs, were constrained by stable isotope patterns. Dominant nitrate sources (nitrified soil nitrogen, fertilizer, and manure) could be delineated for characteristic landscape types. They showed that streams with runoff larger than 5 m^3s^{-1} nitrogen isotopic signatures converged to a stationary value and could not be assigned to particular nitrogen turnover processes and sources (Mueller, Zink et al. 2016). Additionally, Mueller, Zink et al. (2016) found out that oxygen-nitrate isotopes in surface water may give insights into residence time in the reactive zone during different discharge scenarios. In David, Borchardt et al. (2013) sediment accumulation and element patterns of river bed sediments from stormwater runoff especially from urban regions was examined for the Bode River. They found out that urban regions are important sources of nitrogen pollution in bed sediments. The anthropogenic impact especially from WWTPs on aquatic organisms in the Holtemme catchment was examined by Inostroza, Vera-Escalona et al. (2016). In the course of that investigation, samples from invertebrate species were taken at the same locations and dates as in this study. Numerous environmental pollutants, especially from the WWTPs, were found that may change the composition of the organism population. Besides the contribution from WWTPs, a further major source for riverine nitrate comes from agricultural land use and the respective massive application of fertilizers potentially leading to large nitrate store in catchments (Van Meter and Basu 2015). Positive correlations between nitrogen and phosphate species concentrations and the proportion of total agricultural land are described by Jordan, Correll et al. (1997), Kamjunke, Büttner et al. (2013) and Musolff, Schmidt et al. (2015). Geological and soil characteristics are significantly related to chemical loads in surface water (Sliva and Dudley Williams 2001, Onderka, Wrede et al. 2012). A series of studies could show a typical chemodynamic export pattern for nitrate from agricultural catchments with systematic concentration increases by increasing discharge (Wriedt, Spindler et al. 2007, van der Velde, de Rooij et al. 2009, Musolff, Schmidt et al. 2015). This behavior is typical when nitrate sources are heterogeneously distributed in the catchment and activated under high flow conditions (e.g. activation of tile drains, (Musolff, Schmidt et al. 2015)). Under low flow conditions old water fractions with low nitrate concentrations due to denitrification prevail (van der Velde, de Rooij et al. 2009). All previous findings from Kamjunke, Büttner et al. (2013), Mueller, Krieg et al. (2015), Mueller, Zink et al. (2016) and Musolff, Schmidt et al. (2015) involve the Holtemme River catchment and can therefore be accepted for the investigated area.

In terms of water quality and aquatic ecology, the headwaters of the Holtemme River indicate low human influences. Further downstream, however, several hot spots for nutrient input (N, P) (Jiang, Jomaa et al. 2014, Jiang, Jomaa et al. 2015) and pesticide contamination (Ippolito, Kattwinkel et al. 2015, Münze, Orlinskiy et al. 2015) especially from agriculture contribute to the deterioration of the water quality in the Holtemme River and the subsequent Bode River (Reuter, Krause et al. 2003, Hesse, Krysanova et al. 2012, Musolff, Schmidt et al. 2015). While headwater catchments mainly show a good chemical status, the chemical situation at the outlet has to be considered as poor (Wollschläger, Attinger et al. 2016). The corresponding biological status changed from a bad to poor/moderate (Arle, Mohaupt et al. 2016, Völker, Mohaupt et al. 2016).

5.2.3 Sampling approach

For the spatially highly resolved assessment, water samples were collected from 41 locations in October 2014 and again in 2015 (Table 5.3). In total, 27 sampling points were located along the main river, upstream and downstream of major tributary inflows or in units with major land use changes. Furthermore, 12 water samples were taken from major tributaries (Figure 5.2A) and two sampling sites were located at WWTPs outlet (sampling spot 29 and 16, Figure 5.2B). Discharge was measured on 12 sampling sites along the main Holtemme River and for all sampled tributaries. The sampling concept for spatial assessment applied was a key-date sampling representing the mesoscale Holtemme River in two consecutive years in the same season. All water samples were collected within two (2015) or four (2014) days.

Table 5.3: Distribution and number of sampling sites and available discharge information for both monitoring strategies

	sampling location	number of sampling sites	measured discharge
spatial monitoring	main river	27	12
	tributary	12	12
	WWTP	2	2
temporal monitoring	main river	1	public discharge stations
	tributary	1	

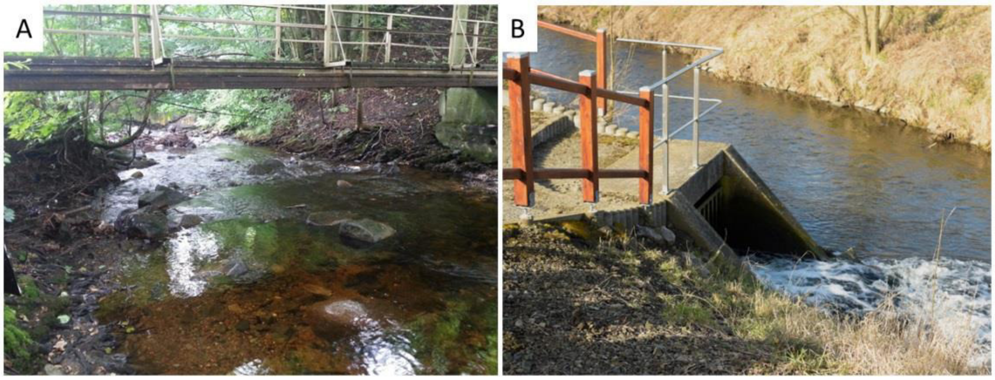


Figure 5.2: During the sampling campaign in 2014: A) Sampling location 5: 'Braunes Wasser' major tributary in the mountainous region, B) 29: inflow from WWTP II c) 33: Reconstruction zone: overflow collecting groundwater from the gravel pit

To assess the temporal variability of the nitrogen dynamics, stream water samples from two intensive investigation sites were taken on a seasonal or monthly basis over a period of four years (2012 to 2015). Note that the time period between 2012 and 2013 of the two sampling sites is part of a larger dataset discussed in Mueller, Zink et al. (2016). One sampling point is located in the headwater region in the Holtemme River before it passes through urban areas (ID 2 'Holtemme', inflow river-km 2.2). The other investigated intensive site is a tributary located in the agricultural region (ID 23 'Ströbecker Fließ', inflow river-km 25.2). Both sampling locations were equipped with runoff gauges operated by LHW (B and J, Figure 5.1).

5.2.4 Hydrological information

The Holtemme catchment is equipped with 10 continuously measuring gauge stations which are operated by the State Office of Flood Protection and Water Management (LHW) Saxony-Anhalt (Figure 5.1). Four discharge stations are located along the main Holtemme River. The other six gauging stations measure the runoff from major tributaries. Precipitation data for the investigation area are available from Rauthe, Steiner et al. (2013). During the sampling campaigns, discharge was also measured directly using handheld devices (OTT ADC, OTT MF Pro) at selected sampling locations which allow us to calculate solute loads.

5.3 Materials and methods

5.3.1 Discharge separation

Discharge may represent a mixture of base flow and quick flow depending on hydrological events such as precipitation or snow melt. The varying mixing ratio is likely to impact nitrate concentrations and isotopic compositions as different pathways for mobilization of nitrogen from natural and anthropogenic sources may be associated with the two flow components. Therefore, we conducted a separation of the total flow into the quick flow and base flow component at all locations where discharge was recorded. Base flow is the flow component representing runoff during non-recharge period and originates from stored sources (WMO 2008). The separation was performed with a simple smoothing method proposed in Institute of Hydrology (1980). The algorithm finds local minima of the total runoff of 5-day periods for the whole time series. In the next step the series of minima are examined to find turning points, which are 1.11 times less than its respective outer values. The turning points are connected by linear interpolation. The resultant line represents the base flow hydrograph, while the flow above base flow is associated with quick component or, in other words, event flow Institute of Hydrology (1980).

5.3.2 Field and laboratory analysis

Temperature (T), pH, oxygen-content (O_2), electrical conductivity (EC) and (partly) discharge were measured in the field using a multiparameter probe (EXO2, YSI Incorporated, Yellow Springs, Ohio, USA). The nitrate and ammonium concentrations were analyzed in the laboratory with a Segmented Flow Analysis according to DIN EN ISO 13395.

Water samples for nitrate isotopic analysis were filtered through 0.22 µm cellulose acetate filters and were stored refrigerated in high-density polyethylene bottles. To measure the isotopic composition of dissolved nitrate, the denitrifier method using a bacteria strain of *Pseudomonas chlororaphis* (ATCC #13985) was applied (Sigman, Casciotti et al. 2001, Casciotti, Sigman et al. 2002). A DELTA V Plus mass spectrometer in combination with a GasBench II from Thermo Scientific was used for nitrate isotope determination. Isotopic ratios are expressed in delta notation according to eq. 5.1 relative to atmospheric nitrogen for $\delta^{15}N$ and relative to Vienna Standard Mean Ocean Water (VSMOW) for $\delta^{18}O$. R characterizes the ratio of the heavier to the lighter isotope.

$$\delta_{sample}\,[‰] = [(R_{sample} - R_{standard})/R_{standard}] \cdot 1000 \qquad (5.1)$$

The standard deviations for nitrogen and oxygen isotope measurements of nitrate are ±0.4 and ±1.6 ‰, respectively. Each sample is measured as a duplicate and represents a mean value. For calibration of nitrogen and oxygen isotopic signatures, the following international standards were used: USGS32, USGS34, USGS35 and IAEA NO3. Stable isotopes of water ($\delta^{2}H_{H2O}$ and $\delta^{18}O_{H2O}$) were analyzed by laser cavity ring-down spectroscopy (L1102-I, Picarro Inc.). The analytical precision of the method is 0.8 ‰ for $\delta^{2}H$ and 0.1 ‰ for $\delta^{18}O$. δ-values are reported relative to the international standard VSMOW.

5.3.3 Landscape information and land use analysis

For each sampling location, we determined the sub-catchment size based on the digital elevation model with a resolution of 25 m and the exact sampling location (source: ASTER). Subsequently, the percentage of main landcover types (forest, agricultural land use and urban region) was calculated for each sub-catchment using the CORINE2000 land use dataset (EEA 2012). The software ArcGIS version 10.4 and the Zonal Statistics Tool were used for the calculation. This tool summarizes values of a raster (land use) within features of another vector dataset (sub-catchment polygons).

5.4 Results and discussion

5.4.1 Hydrological situation and nitrate loads

The amounts of discharge differed substantially between both sampling campaigns (Figure 5.3A). In 2014, we determined higher discharge at all discharge monitoring stations along the main stream. The runoff at the outlet of the catchment was about 0.815 $m^3 s^{-1}$. In contrast, the discharge in 2015 was on average 66 % lower compared to 2014, especially in the headwater region. The outlet of the Holtemme catchment had a runoff in 2014 of about 0.247 $m^3 s^{-1}$ (Figure 5.3A). Nitrate concentrations were similar for both campaigns with 1 to 5 mg L^{-1} in headwater regions and 15 to 16 mg L^{-1} at the outlet (Figure 5.3C). Consequently, mean nitrate loads were higher in 2014 (1847 kg day^{-1}) compared to 2015 (576 kg day^{-1}) as shown in Figure 5.3B. In 2014, the four tributaries closest to the headwater ('Braunes Wasser', 'Zillierbach', 'Barrenbach' and 'Silstedter Bach') located in the mountainous region represented 72 % of the total contribution from inflowing tributaries along the entire reach of the Holtemme River (Table S5.1, Figure S5.1). The sampling locations and the recorded field parameters as well as ion concentrations and respective isotope values were shown in Table S5.1 and Table S5.2. For the single parameters, we calculated the average for data from all locations, the respective standard deviation (SD) and the coefficient of variation (CV) separately for the two campaigns. Samples from 2014 showed higher mean electrical conductivity (1009 µS cm^{-1} ± 1361 µS cm^{-1}) and higher variations compared to samples from 2015 (882 µS cm^{-1} ± 370 µS cm^{-1}).

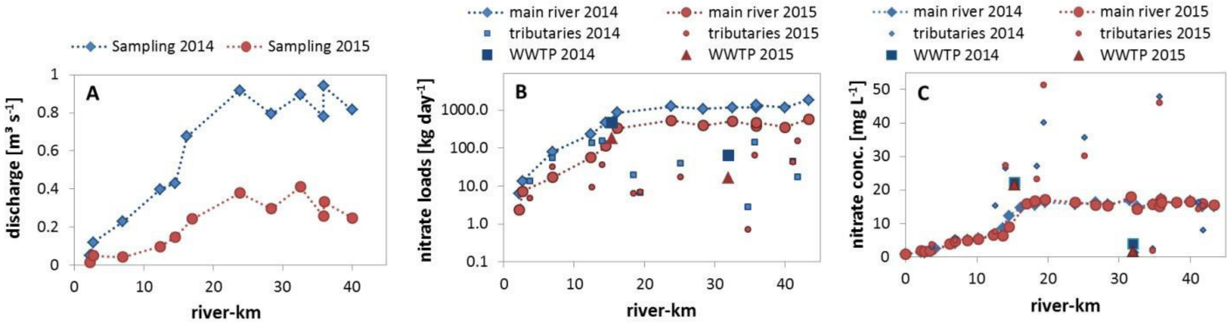


Figure 5.3: Discharge along the main Holtemme River: a comparison of both sampling campaigns; Nitrate loads (B) and concentrations (C) along the main river with major tributaries and locations of WWTPs (Table 5.2)

5.4.2 Discharge separation

Before the sampling campaign in 2014, several rain events occurred that led to an extensive water saturation of the subsoil. In 2015, the amount of rain before the sampling campaign was much lower so that the extent of the water saturation of the subsoil was smaller. Nevertheless, both sampling campaigns represented base flow conditions mainly controlled by water discharge from the subsoil (groundwater). The area-specific base flow along the main stream decreased when the considered catchment-size got larger (Figure 5.4A), which is related to topology and slope. While the shapes of the base flow curves were similar for different locations with peaks of low or high base flow occurring simultaneously, the amplitude of the peaks was strongly dependent on the respective catchment area. Figure 5.4B shows the discharge composition at the outlet of the Holtemme River (discharge station D: Nienhagen) for the time period, 2014 and 2015. In 2014, the average annual base flow at the outlet of the Holtemme with respect to the area of the catchment was 0.27 ± 0.09 mm day^{-1}. In 2015, a quite similar value of 0.25 ± 0.14 mm day^{-1} was determined (Figure 5.4B). Generally, quick flow is closely related to precipitation events but it cannot necessarily be assigned to specific rain events in the case of the two sampling campaigns.

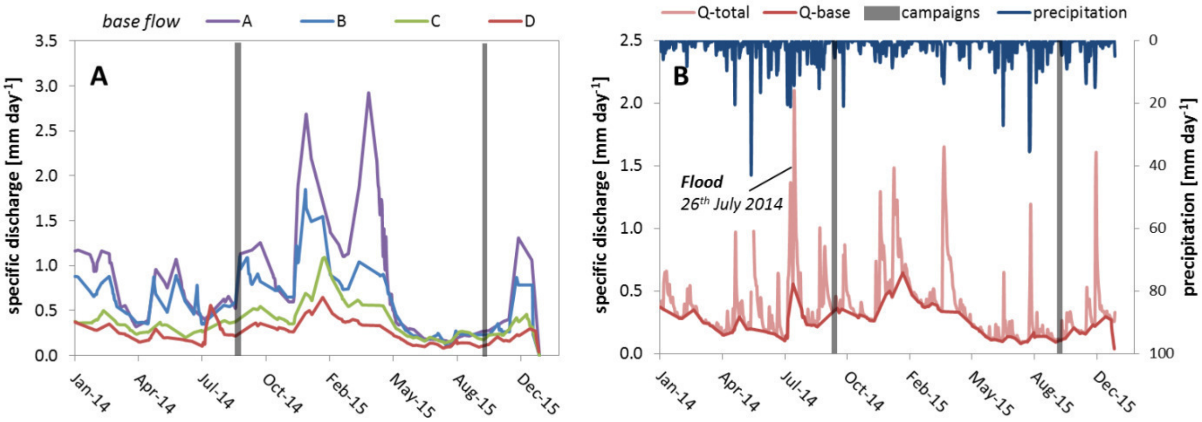


Figure 5.4: Base flow at discharge stations along the main Holtemme River (locations see Figure 5.1) and B: Discharge separation at the outlet of the catchment (discharge station D: Nienhagen)

Table 5.4: Results of the discharge separation with mean specific total discharge and base flow, base flow index (BFI) as well as area-specific precipitation (day of sampling)

LHW discharge station	area [km²]	Q total [mm d^{-1}]	Q base [mm d^{-1}]	BFI	daily prec. [mm d^{-1}]	Q total [mm d^{-1}]	Q base [mm d^{-1}]	BFI	daily prec. [mm d^{-1}]
main stream - Holtemme		**2014**				**2015**			
A) Hanneckenbruch	8.39	1.174	1.174	1.000	0.64	0.268	0.268	1.000	0.72
B) Steinerne Renne	15.41	0.791	0.791	1.000	0.55	0.230	0.230	1.000	0.73
C) Mahndorf	159.81	0.520	0.516	0.993	5.43	0.175	0.175	1.000	0.61
D) Nienhagen	270.89	0.453	0.351	0.776	2.43	0.108	0.108	1.000	3.13
tributaries		**2014**				**2015**			
E) Zillierbach	2.17	0.916	0.916	1.000	0.52	0.159	0.159	1.000	0.60
F) Wernigerode	25.94	0.636	0.636	1.000	0.36	0.306	0.214	0.699	0.64
G) Minsleben	83.27	0.120	0.100	0.828	0.36	0.023	0.023	1.000	0.65
H) Benzingerode	3.28	0.764	0.495	0.648	5.65	0.105	0.105	1.000	0.70
I) Derenburg	25.15	0.134	0.091	0.679	3.78	0.021	0.021	1.000	0.65
J) Ströbeck	14.13	0.061	0.050	0.811	2.04	0.061	0.056	0.920	2.81

The area-specific discharge (Q related to the respective sub-catchment size, [L m^{-2} day^{-1}] = [mm day^{-1}]) along the main stream decreased with increasing total discharge. Generally, the amount of total discharge increased with the flow path of the river (Figure 5.5). Even though the total discharge differed substantially between both sampling campaigns (Figure 5.3), the percentages or rather indexes of the discharge components, base flow and quick flow, were very similar for both years (Figure 5.5). The base flow index (BFI) describes the ratio of base flow to total stream flow and represents the contribution of groundwater (Bloomfield, Allen et al. 2009). In 2015, the total discharge along the main river consisted of BFI 1 whereas two tributaries (F, J) showed an impact of a quick flow component (Table 5.4). In 2014, the BFI along the Holtemme River decreased from 1 (headwater) to 0.78 (outlet) (Table 5.4). Discharge of the tributaries during that sampling period varied between 0.648 and 1 in BFI. While base flow during the campaign in 2014 was above-average, base flow in 2015 was below the average of 0.14 mm day^{-1}. During the sampling campaigns, minor rainfall events occurred that added up to a total amount of 5.6 mm day^{-1} in 2014 and 3.1 mm day^{-1} in 2015 (Table 5.4).

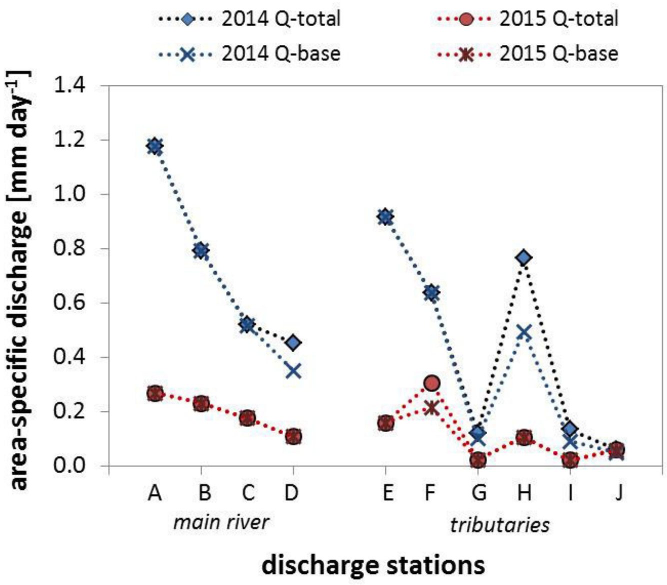


Figure 5.5: Amount of baseflow (q-base) and total discharge (Q-total) at discharge stations along the main river and tributaries

During both campaigns, the impact of quick flow in the total discharge is low to non-existent. Due to a longer residence time and consequently a more intense interaction between water and soil/subsoil matrix, nitrate mobilization from natural and anthropogenic sources is preferably associated with base flow. Quick flow contributions normally result in a dilution of the nitrate concentrations.

5.4.3 Nitrate isotopic signatures

Nitrate isotopic signatures in river catchments were mainly controlled by variable contributions from sources such as nitrified ammonium from precipitation, mineralized and nitrified soil nitrogen, inorganic or organic fertilizers (manure) septic water and effluents from WWTPs (Figure 5.6). Water samples taken from the headwater region showed generally low $\delta^{15}N\text{-}NO_3^-$ values between 0.4 and 4.0 ‰ (AIR) in concert with very low nitrate concentrations between 0.4 and 2.8 mg L^{-1} (Figure 5.7A and B). These concentration and isotopic ranges could be considered as representative for the natural background in headwater catchments for this region (Mueller, Krieg et al. 2015, Mueller, Zink et al. 2016). When nitrate from urban/agricultural regions reached the stream, both nitrogen isotopic signatures and concentrations were substantially changed. Nitrate from septic water and agricultural organic fertilizer increased nitrate concentrations in the main river up to 17.2 mg L^{-1} and $\delta^{15}N_{NO3}$ signatures up to 18.4 ‰. Nitrate concentrations and corresponding nitrogen isotopic signatures behaved similarly for both sampling campaigns (Figure 5.7A).

The overall mean of the nitrogen isotopic signature in nitrate from all sampling locations showed only minor differences between the sampling campaigns in 2014 and 2015 ($\delta^{15}N\text{-}NO_3^-$ 11 ± 5 ‰ AIR) (Table S5.1 and Table S5.2). Also, the general trend of increasing $\delta^{15}N\text{-}NO_3^-$ with increasing nitrate concentration along the river (Figure 5.7B) was similar for both campaigns. When the main river passed urban regions, the nitrogen isotopic composition showed a typical signal from nitrate that came from wastewater with generally $\delta^{15}N\text{-}NO_3^-$ higher than 10 ‰ (AIR) (Heaton 1986, Jordan, Nadelhoffer et al. 1997). This nitrogen isotopic signal remained relatively constant after the inflow from the WWTP I. In contrast, the corresponding oxygen isotopic signature from nitrate showed enriched values in 2015 ($\delta^{18}O\text{-}NO_3^-$ 10 ± 6 ‰ VSMOW) compared to 2014 ($\delta^{18}O\text{-}NO_3^-$ 6 ± 2 ‰ VSMOW) downstream of river-km 15. Generally, oxygen isotope signatures were more variable between both campaigns (Figure 5.7C). In the headwater region, $\delta^{18}O\text{-}NO_3^-$ started from 10.9 ‰ and decreased down to 4.7 ‰ (VSMOW) in 2014. One year later, the $\delta^{18}O\text{-}NO_3^-$ signature in the headwaters was more depleted and varied between 7.6 ‰ close to the spring and 2.0 ‰ further downstream (VSMOW). When the river passed on its course through urban regions the first time, nitrate showed similar oxygen-isotopic signatures during both campaigns (between 3.7 and 7.7 ‰ (VSMOW)). In the 32 river kilometer downstream, the variation of $\delta^{18}O\text{-}NO_3^-$ in the main river as well as in tributaries was very low in 2014 (between 4.3 and 6.5 ‰ (VSMOW)). In contrast, the oxygen signatures of nitrate in 2015 showed a strong increase up to 18.1 ‰ in the main river at river-km 18 downstream of the WWTP I. The oxygen signatures of nitrate further downstream were more variable and generally decreased down to a δ^{18}O- NO$_3^-$-value of 10.9 ‰ (VSMOW) at the outlet.

Elevated isotope values for nitrogen and oxygen were not indicative of a considerable impact of bacterial denitrification because they were accompanied by increased nitrate concentrations (Figure 5.7).

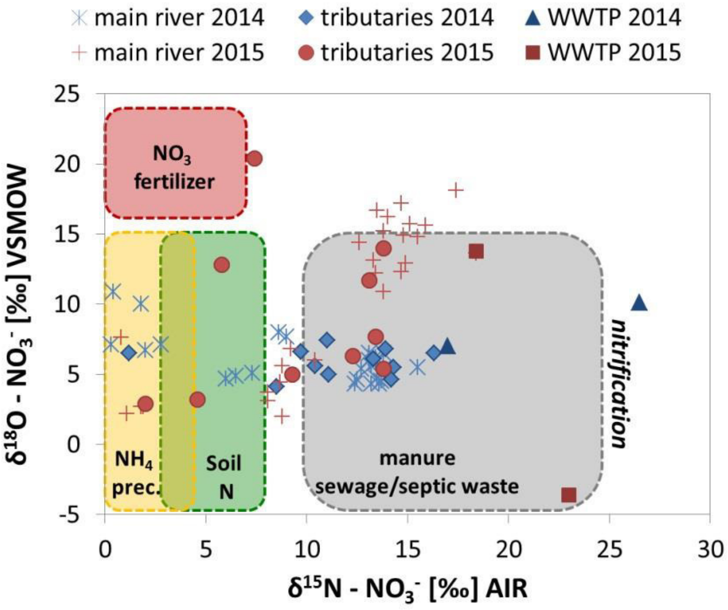


Figure 5.6: Dual nitrate isotope plot and typical isotopic signatures of potential NO$_3$-sources after Kendall, Elliott et al. (2008) and Kendall and McDonnell (1998). Isotopic signature for septic water after Heaton (1986) and Jordan, Nadelhoffer et al. (1997)

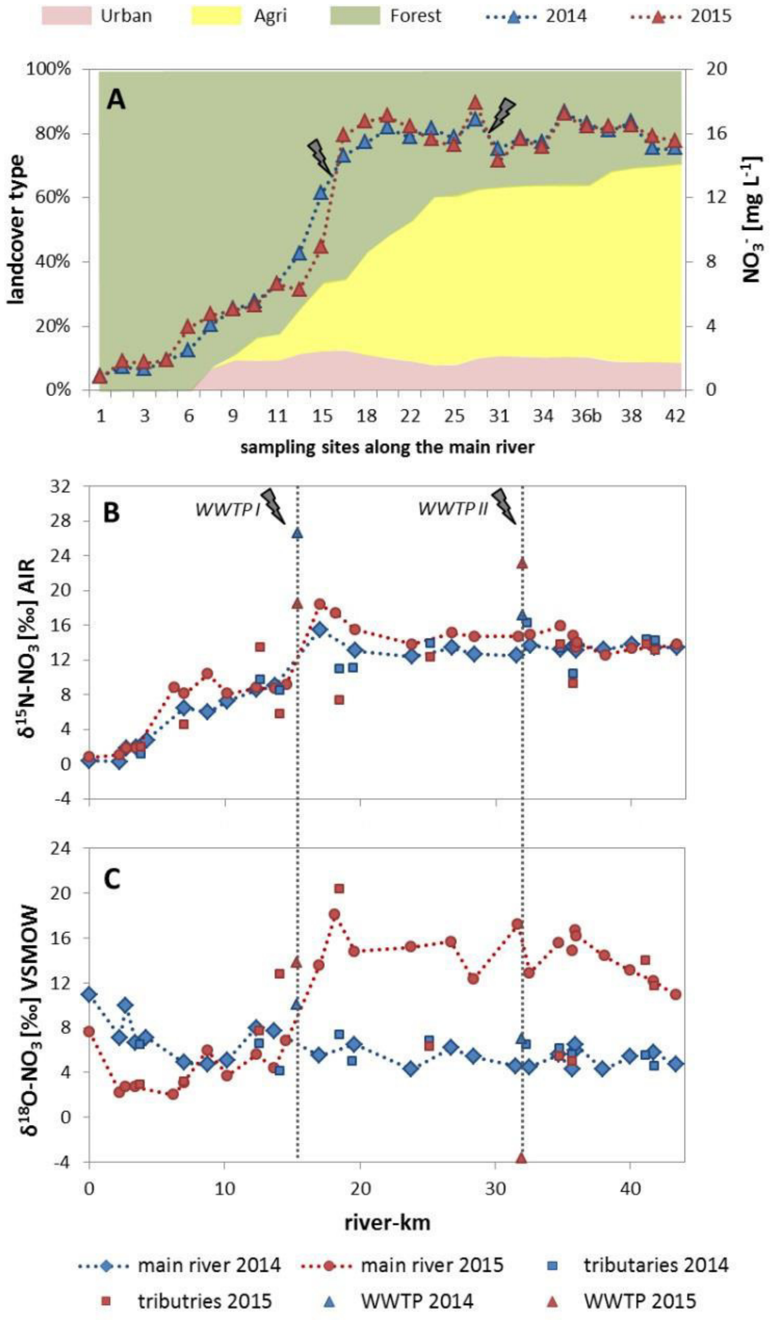


Figure 5.7: Mean NO_3^- concentration with summed major landcover types for both sampling campaigns, B: Nitrogen-nitrate isotope dynamics and C: Corresponding oxygen-nitrate isotope dynamics along the main river for both sampling campaigns

The oxygen-nitrate isotopic signatures in the headwater region could be considered as mainly unaffected by agricultural land use and wastewater effluents. These isotope signatures may provide information on water residence times in the zone where nitrate is formed by nitrification and mobilized by groundwater recharge (Clune and Denver 2012, Mueller, Zink et al. 2016). Differences in the amount of base flow reflect the variability of hydrological conditions that can be associated with different residence time in the upper soil zone for nitrogen turnover. Therefore, the substantial differences between the base flow in 2014 and 2015 may have an effect on the oxygen isotopic signatures of nitrate. The dominant and most straightforward process for the mobilization of nitrate from the soil zone is nitrification. This process predominantly controls the oxygen isotope signature of nitrate by the incorporation of O from the ambient water and O from air (+23.5 ‰) into the nitrate molecule. In the simplest case, two O atoms are derived from H_2O while one O atom comes from dissolved oxygen (e.g. (Hollocher 1984)). Variable kinetic and equilibrium fractionation during O incorporation, however, may add an additional uncertainty of up to +5 ‰ to the 2:1 ratio (Buchwald and Casciotti 2013). Such different discharge scenarios and the related differences of the residence time in the reactive zone, where nitrification is taking place, may have significant impact on the isotope fractionation during O-incorporation. Consequently, oxygen isotope signatures of nitrate are in part related to the discharge intensity. We assume that longer residence times of reactive nitrogen in the unsaturated zone are associated with a more intense recycling involving intermediate nitrogen compounds such as nitrite. Nitrite is known to exchange oxygen isotopes with water rapidly (Casciotti, Sigman et al. 2002, Casciotti, Buchwald et al. 2011). The presence of nitrite from nitrate reduction and its anoxic re-oxidation would incorporate more water derived oxygen in the formed nitrate compared to nitrate that underwent straightforward nitrification without recycling. Therefore, higher residence times in the reactive zone would result in lighter oxygen isotope signatures in nitrate that is transported to the groundwater. For the headwater regions, this hypothesis is confirmed by our observations that during low base flow periods (2015) $\delta^{18}O\text{-}NO_3^-$ is lower compared to high base flow periods in 2014 (Figure 5.7C). The opposed dependency of $\delta^{18}O\text{-}NO_3^-$ on the amount of discharge was observed for the neighboring Selke River catchment (Mueller, Zink et al. 2016). With increasing discharge, lower $\delta^{15}N\text{-}NO_3^-$ und $\delta^{18}O\text{-}NO_3^-$ signatures were measured. The behavior in the Selke River, however, is related to the mobilization of nitrate from different nitrate pools originating in the anthropogenic impact in the catchment. In the headwater region of the Holtemme area, anthropogenic impact played just a minor role. Here, nitrate formation, mobilization and transport are solely controlled by hydrological effects.

Downstream of the headwater region, oxygen isotopic signatures of nitrate showed only minor variations during high base flow conditions in 2014. This implies that nitrate sources with the same isotopic signature are dominating the aquatic system. Any contribution of nitrate with a potentially different isotopic signature was too small to be noticed in the water of the main river. This was different for the hydrological conditions in 2015. During very low base flow in October 2015, nitrate loads were also much lower (Figure 5.3B) and the main river reacted much more sensitively to any nitrate contribution with a different isotopic signature (Figure 5.7). Especially, the effluent from the WWTP and minor tributaries between river-km 14 and 18 changed the nitrate isotopic composition of the main river considerably. Both WWTPs were responsible for a total nitrate load in the main river of 342 kg day^{-1} (19 %) in 2014 and 226 kg day^{-1} (39 %) in 2015. Except for the last two tributaries close to river-km 41, all other major downstream tributaries of the river stretch between 14 and 18 km showed similar oxygen isotopic signatures during both campaigns. Therefore, the elevated $\delta^{18}O\text{-}NO_3^-$ values during the 2015 campaign slowly decreased over the course of the river again.

5.4.4 Temporal variability in different land use types

Nitrate concentrations and corresponding isotopic signatures of nitrate were measured throughout the entire observation period (Figure 5.8A).

The mean nitrate concentrations in the agricultural lowland site were 20 times higher than in the more pristine headwater site (Table 5.5). Area-specific discharge is 8 times higher in the headwater site. Consequently, differences in the exported area-specific nitrate loads between both sites are not as strong as for the concentrations but still more than two times higher at the agricultural site. The coefficient of variation of nitrate concentrations (CV_C) relative to the coefficient of variation of discharge (CV_Q) is higher at the headwater site (CV_C/CV_Q=0.37) compared to the agricultural site (CV_C/CV_Q=0.25). The concentration-discharge relation of nitrate concentration (Figure 5.8B) reveals that concentration variations at the headwater site are independent of the discharge (C-Q slope =-0.005). In contrast, the agricultural site shows a more systematic increase in nitrate concentration with increasing discharge (C-Q-slope = 0.07) according to Fairbairn, Arnold et al. (2016). This is more evident for the $\delta^{15}N\text{-}NO_3$ signature that shows a clear decrease with increasing discharge at the agricultural site (Pearson correlation coefficient PCC=-0.58) which is less significant for the headwater site (PCC=-0.16) (Figure 5.8C). This observation is in line with Musolff, Schmidt et al. (2016) and Mueller, Zink et al. (2016) who reported a groundwater-head and discharge driven mobilization of nitrate pools in lowland catchments. Low groundwater heads and discharge situations (like the two sampling campaigns in 2014 and 2015) lead to the export of older groundwater with slightly lower nitrate concentrations and a stronger enrichment of $\delta^{15}N\text{-}NO_3$. In contrast high groundwater level and discharge exports a larger fraction of younger water with higher nitrate concentrations and lighter isotopic values. Additionally, it can be assumed that a higher impact of denitrification during low base flow conditions (longer residence time) is contrary to the mobilization of different N-pools depending on the saturation status of the saturation status of the aquifer and soil. However, beside its systematic variations in concentration and signature, the agricultural site is less variable in nitrate concentrations (based on CV_C/CV_Q) and isotopic signatures than the headwater site.

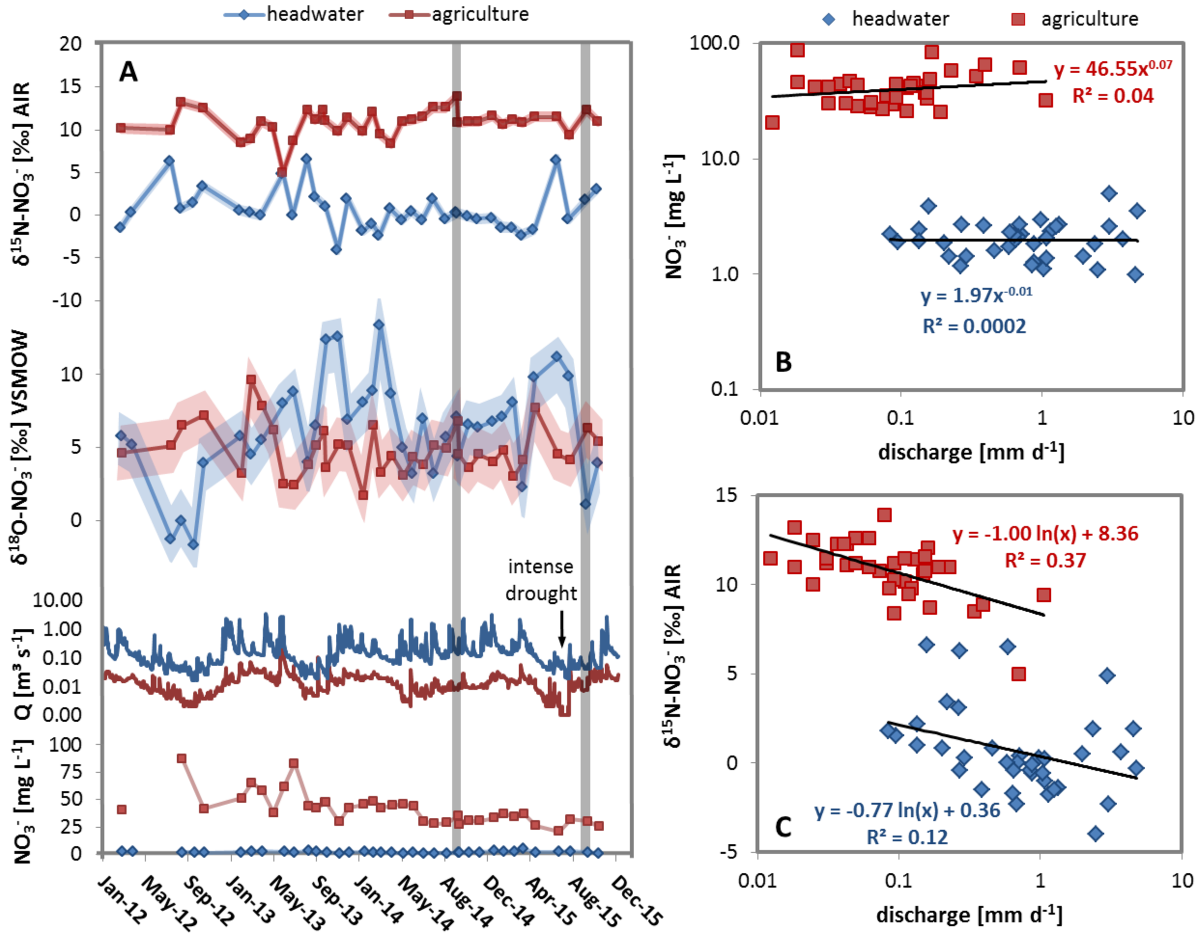


Figure 5.8: Intensive sampling sites within the Holtemme catchment representative for headwater and agricultural regions (shaded area indicates standard deviations for δ^{15}N-NO_3^- and δ^{18}O-NO_3^- ±0.4 ‰ and ±1.6 ‰, respectively). Corresponding relationship between discharge and nitrate concentration (B) or rather δ^{15}N-NO_3^- (C).

Table 5.5: Results from the long term nitrate isotope monitoring (2012-2015) at two intensive investigation sites with average nitrate signatures, nitrate concentrations, discharge and calculated nitrate loads.

location		**δ^{15}N-NO$_3$ [‰] AIR**	**δ^{18}O-NO$_3$ [‰] VSMOW**	**NO$_3$ [mg L^{-1}]**	**Q [m^3 s^{-1}]**	**nitrate load [t a^{-1}]**
Headwater (hw) **(ID 2)**	Mean	0.7	6.2	2.1	0.207	14.9
	StdDiv	2.4	3.5	0.8	0.217	20.5
	CV	3.7	0.6	0.4	1.048	1.4
Agriculture (agri) **(ID 23)**	Mean	10.8	4.9	41.6	0.023	33.3
	StdDiv	1.6	1.6	14.5	0.032	47.6
	CV	0.1	0.3	0.3	1.380	1.4

5.4.5 Comparison of spatially highly resolved and long-time data sets

The mean nitrogen isotope signatures of the intensively investigated headwater and agricultural catchment were in good agreement with the N-isotopic signatures for the respective stretches of the main river during the two key-date sampling campaigns (Figure 5.9). Multi-year averaged nitrate isotopic signatures from long-term data in headwater (hw) and agricultural sites (agri) showed similar magnitudes to the spatially highly resolved monitoring. Nitrogen isotopic signatures were generally depleted in headwater regions whereby agricultural sites showed elevated δ^{15}N-NO$_3^-$ signatures. δ^{15}N-NO$_3^-$ signatures were generally higher during the highly resolved monitoring compared to the long-time mean δ^{15}N-NO$_3^-$ signatures. In contrast, oxygen isotope signatures in headwater were elevated during long-time monitoring and lower in the agricultural sites which resembled with the high-resolution sampling in 2014. The exception was the agricultural stretch of the main river during the extremely low base flow conditions in the 2015 campaign. None of the samples taken from the agricultural catchment over a four-year period showed a δ^{18}O-NO$_3^-$ value higher than 10 ‰ (VSMOW) (Figure 5.8). Consequently, the river nitrate downstream from the stretch between river-km 14 and 18 during the 2015 low base flow campaign with elevated δ^{18}O-NO$_3^-$ values (Figure 5.7C) was dominated by the contribution from the WWTP effluents instead of nitrate derived from agricultural practice. Compared to the long-time data set, nitrogen isotope signatures from the spatial dataset highly resemble low flow conditions. The variations within the representative landscape types are relatively low in relation to the dynamics caused by WWTPs.

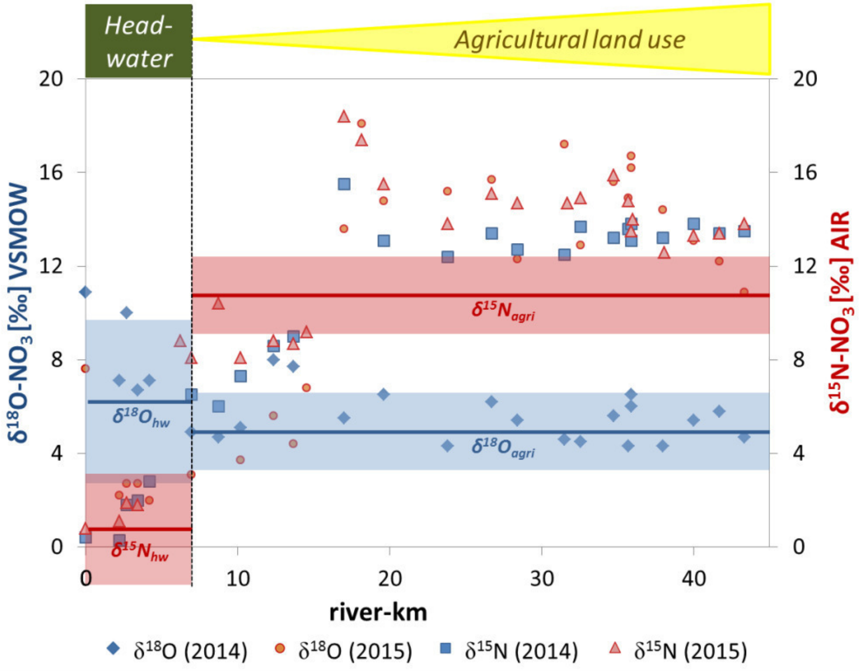


Figure 5.9: Comparison of spatially highly resolved nitrate isotope assessment along the main Holtemme River (transparent graphs) and typical mean nitrate isotopic signatures for headwater (hw) and agricultural areas (agri) from long-term data sets (listed in Table 5.5).

5.5 Conclusion

This study reveals how different sources shape the spatial and temporal variability of nitrate concentration and loads along a mesoscale river system that is characterized by a strong land use gradient from pristine headwater catchments to catchments with intense agricultural land use. The sampling water of headwater catchments offers valuable information on the dependency of nitrate dynamics on variable hydrological conditions. The headwater nitrate concentrations are generally low (between 1 and 5 mg L^{-1}) representing a good chemical status of groundwater that feeds the stream during base flow conditions.

Downstream from the headwater region, two major nitrogen sources become important and raise the nitrate concentration to a level that may substantially disturb ecosystem functioning. Waste water treatment plants are responsible for a total nitrate load in the Holtemme River of 342 kg day^{-1} (19 %) in 2014 and 226 kg day^{-1} (39 %) in 2015. The major nitrate contribution is related to agricultural land use and the associated application of fertilizers. While during normal base flow conditions the isotope fingerprint of river water nitrate is dominated by the inflow from agricultural catchments, during extremely low base flow conditions in 2015 the relatively higher contribution of nitrate from WWTPs overruled the agricultural isotope signature. This was especially evident for oxygen isotope signatures.

Elevated nitrogen and oxygen isotope signatures in combination with decreased nitrate concentrations would be indicative of bacterial attenuation of nitrate. No such combination was observed within this study, neither during the key-date sampling events nor during the long term observation at the intensive investigation sites. This implies that denitrification plays no or a minor role only for controlling nitrate loads in the river. Therefore, nitrate loads should be regulated by preventive measures especially by an optimized land use management and cannot rely on self-attenuation processes in the catchment.

The studied catchment represents a typical situation of a pristine mountainous headwater that is more and more impacted by agriculture and urban land use in the downstream parts. Consequently, the site-specific findings may be transferable to comparable catchment configurations. In light of this, the findings may help to better interpret the results of standard monitoring at catchment outlets that suffer from the strong spatial integration of upstream sources and processes. Key-date monitoring concepts as conducted in this study give valuable insight into the spatial source contribution of nitrate in a mesoscale river catchment. In this context, it helps to interpret longer-term monitoring data that spatially integrates mobilization and turnover processes. Nevertheless, in order to fully understand the river system in terms of the relationship between N-cycling and hydrology, a more intensive monitoring program seems necessary that would provide access to information on a higher temporal resolution at hot spots for N-transformations within the catchment.

5.6 Supporting information

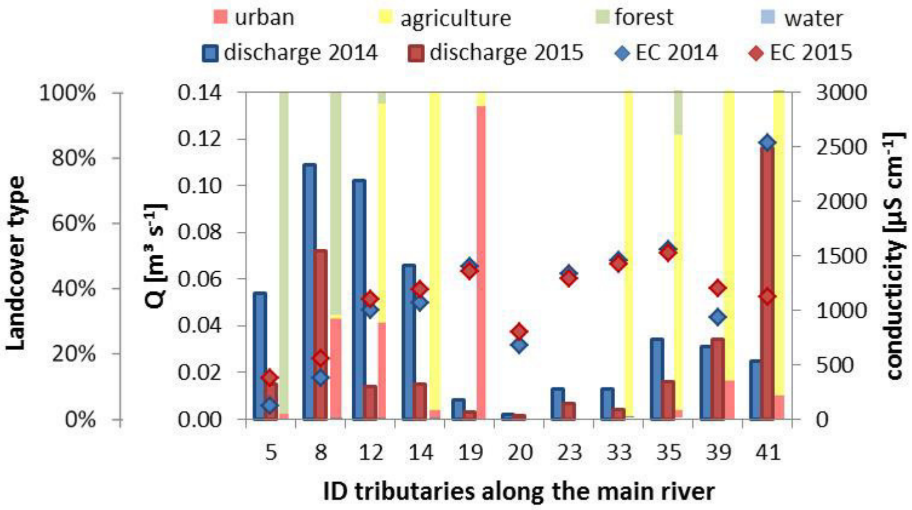


Figure S5.1: Landcover information, discharge and electrical conductivity of sampled tributaries along the main river.

Table S5.1: Measured hydrological, hydrochemical parameters as well as nitrate and water isotopes in stream water, 6th to 9th October 2014

ID	stream	date	river km	pH	EC [µS cm^{-1}]	O_2 [mg/L]	T [°C]	Q [l s^{-1}]	NO_3^- [mg L^{-1}]	NO_3^- load [t a^{-1}]	$\delta^{15}N-NO_3^-$ [‰]	$\delta^{18}O-NO_3^-$ [‰]	$\delta^{18}O-H_2O$	$\delta^{2}H-H_2O$	D-excess
1	Holtemme	06.10.2014	0						0.9		0.4	10.9	-9.2	-60.9	13.0
2	Holtemme	06.10.2014	2.24	6.92	104	13.6	10.6	52	1.4	2.3	0.3	7.1	-9.2	-62.6	11.0
3	Holtemme	06.10.2014	2.7	6.86	99	13.5	10.5	119	1.3	4.9	1.8	10.0	-9.2	-62.2	11.8
4	Holtemme	06.10.2014	3.42	6.89	120	13.4	11.6		1.8		2.0	6.7	-9.2	-62.7	10.8
6	Holtemme	06.10.2014	4.22	7.09	186	13.3	11.7		2.5		2.8	7.1	-9.3	-62.9	11.6
7	Holtemme	06.10.2014	6.98	7.44	321	13.6	12.5	228	4.1	29.2	6.5	4.9	-9.3	-62.9	11.6
9	Holtemme	06.10.2014	8.74	7.79	402	13.3	12.0		5.0		6.0	4.7	-9.3	-63.4	10.8
10	Holtemme	06.10.2014	10.19	7.84	428	13.4	12.3		5.5		7.3	5.1	-9.2	-63.1	10.4
11	Holtemme	06.10.2014	12.35	8.03	457	13.5	12.4	399	6.6	83.0	8.6	8.0	-9.3	-63.1	11.1
13	Holtemme	06.10.2014	13.68	8.05	562	12.9	13.4		8.5		9.0	7.7	-9.0	-61.5	10.4
15	Holtemme	06.10.2014	14.54	8.07	626	13.2	13.6	431	12.3	166.7			-8.9	-61.5	9.7
17	Holtemme	06.10.2014	16.17	7.78	732	13.0	15.1	675	14.6	311.0	15.5	5.5	-8.8	-60.9	9.5
18	Holtemme	07.10.2014	18.15	7.85	752	1.6	13.1		15.4				-8.8	-61.0	9.7
21	Holtemme	07.10.2014	19.61	7.86	779	12.4	13.2		16.3		13.1	6.5	-8.8	-61.5	9.2
22	Holtemme	07.10.2014	23.81	7.92	767	12.3	13.2	918	15.8	456.3	12.4	4.3	-8.8	-61.4	8.9
24	Holtemme	07.10.2014	26.73	8.04	774	12.9	13.3		16.3		13.4	6.2	-8.7	-61.4	8.4
25	Holtemme	07.10.2014	28.39					794	15.7	393.7	12.7	5.4	-8.8	-61.9	8.4
26	Holtemme	07.10.2014	31.52	8.02	806	1.6	13.5		16.8		12.5	4.6	-8.7	-61.0	8.6
31	Holtemme	07.10.2014	32.54	7.94	8777	12.4	14.7	894	15.0	422.9	13.7	4.5	-8.8	-61.3	8.8
32	Holtemme	07.10.2014	34.74	7.98	873	12.0	14.6		15.7		13.2	5.6	-8.8	-61.1	9.3
34	Holtemme	09.10.2014	35.69	8.08	790	10.9	16.2		15.4		13.6	4.3	-8.6	-60.5	8.3
36a	Holtemme	09.10.2014	35.91	8.09	811	10.8	15.9	782	17.3	426.7	13.1	6.0	-8.6	-60.9	7.8
36b	Holtemme	09.10.2014	35.91					941	16.6	492.8	13.8	6.5	-8.6	-61.3	7.5
37	Holtemme	09.10.2014	37.97	8.06	826	10.4	15.6		16.2		13.2	4.3	-8.6	-60.6	7.9
38	Holtemme	09.10.2014	40.01	8.11	825	10.9	16.0	815	16.7	429.0	13.8	5.4	-8.6	-60.4	8.3
40	Holtemme	09.10.2014	41.69	8.16	835	10.7	16.3		15.1		13.4	5.8	-8.6	-59.9	8.6

42	Holtemme	09.10.2014	43.35	8.16	872	11.9	15.8	1420*	15.1	674.3	13.5	4.7	-8.6	-60.1	8.3
5	Braunes Wasser	06.10.2014	3.77	6.86	117	11.9	13.0	54	2.8	4.8	1.2	6.5	-9.3	-63.2	11.6
8	Zillierbach	06.10.2014	6.99	7.92	380	12.9	13.2	109	5.7	19.5			-9.3	-64.3	10.4
12	Barrenbach	06.10.2014	12.61	8.05	1000	13.0	15.0	102	15.4	49.4	9.7	6.6	-7.9	-56.2	7.0
14	Silstedter Bach	06.10.2014	14.07	8.25	1061	12.7	14.0	66	26.5	55.2	8.5	4.1	-8.8	-61.9	8.2
19	Rothe	07.10.2014	18.47	7.76	1404	11.7	12.8	8	27.2	7.0	11.0	7.4	-8.5	-60.8	7.4
20	Hellbach	07.10.2014	19.44	7.17	676	8.9	12.8	2	40.2	2.4	11.1	5.0	-8.4	-60.3	6.6
23	Störbecker Fließ	07.10.2014	25.15	8.24	1334	12.8	12.8	13	35.5	14.4	13.9	6.8	-8.2	-58.5	7.0
30	Alte Holtemme	07.10.2014	32.32	7.70	900	10.8	15.1	0	1.2	0.0	16.3	6.5	-8.3	-59.2	6.8
33	Alte Holtemme	07.10.2014	34.76	7.92	1458	11.0	15.7	13	2.5	1.0	13.3	6.1	-5.1	-42.5	-1.9
35	Asse	09.10.2014	35.71	8.12	1557	9.9	15.8	34	47.8	51.6	10.4	5.6	-8.3	-58.8	7.9
39	Ditch Nienhagen	09.10.2014	41.14	8.01	933	10.0	16.5	31	16.4	16.0	14.3	5.5	-8.6	-60.8	7.8
41	Salzgraben	09.10.2014	41.81	7.89	2530	10.3	16.4	25	7.9	6.2	14.2	4.6	-8.3	-59.2	6.9
16	WWTP	06.10.2014	15.355	7.35	1057	9.3	17.0	144**	22.3	101.2	26.5	10.1	-8.6	-59.8	8.7
29	WWTP	07.10.2014	31.96	7.33	1430	8.9	17.3	191	3.9	23.7	17.0	7.0	-8.5	-60.8	7.2
Average (Mean)				**7.78**	**1010**	**11.4**	**14.1**	**343**	**13.6**	**157.2**	**10.9**	**6.1**	**-8.7**	**-60.7**	**8.8**
Standard deviation (SD)				**0.41**	**1361**	**2.7**	**1.8**	**396**	**10.5**	**201.0**	**5.3**	**1.6**	**0.7**	**3.2**	**2.3**
Relative coefficient of variation (CV)				**5**	**135**	**24**	**13**	**115**	**77**	**128**	**49**	**26**	**8**	**5**	**26**

*measured by the LHW; **measured by the operators of WWTP

Table S5.2: Measured hydrological, hydrochemical parameters as well as nitrate and water isotopes in stream water, 5th to 10th October 2015

ID	stream	date	river km	EC [µS cm^{-1}]	O$_2$ [mg/L]	T [°C]	Q [l s^{-1}]	NO$_3^-$ [mg L^{-1}]	NO$_3^-$ load [t a^{-1}]	δ^{15}N-NO$_3^-$ [‰]	δ^{18}O-NO$_3^-$ [‰]	δ^{18}O-H$_2$O	δ^{2}H-H$_2$O	D-excess
1	Holtemme	5.10.2015	0	57	11.48	7.8		0.8		0.8	7.6	-9.0	-59.5	12.6
2	Holtemme	5.10.2015	2.24	117	11.12	8.7	15.0	1.8	0.9	1.1	2.2	-9.1	-60.8	11.7
3	Holtemme	5.10.2015	2.7	107	11.49	9.1	49.0	1.7	2.6	1.9	2.7	-9.1	-60.2	12.4
4	Holtemme	5.10.2015	3.42	138	11.23	9.3		1.9		1.8	2.7	-9.1	-60.7	12.2
6	Holtemme	5.10.2015	6.24	422	11.14	10.8		3.9		8.8	2.0	-9.1	-61.2	11.7
7	Holtemme	5.10.2015	6.98	495	11.25	11.3	42.0	4.7	6.2	8.1	3.1	-9.1	-61.4	11.3
9	Holtemme	5.10.2015	8.74	548	11.77	11.8		5.0		10.4	6.0	-9.0	-61.5	10.6
10	Holtemme	5.10.2015	10.19	572	12.25	11.9		5.3		8.1	3.7	-9.0	-61.5	10.3
11	Holtemme	5.10.2015	12.35	604	12.28	12.7	98.0	6.6	20.4	8.8	5.6	-8.9	-61.0	10.1
13	Holtemme	5.10.2015	13.68	677	11.71	13		6.2		8.7	4.4	-8.7	-59.7	9.7
15	Holtemme	5.10.2015	14.54	694	11.99	13.3	147.0	8.9	41.3	9.2	6.8	-8.6	-59.8	9.2
17	Holtemme	5.10.2015	17	917	10.35	15.4	242.0	15.9	121.0	18.4	13.6	-8.3	-57.9	8.5
18	Holtemme	5.10.2015	18.15	927	10.55	15.3		16.7		17.4	18.1	-8.2	-57.8	7.4
21	Holtemme	5.10.2015	19.61	930	9.47	14.8		17.1		15.5	14.8	-8.4	-58.8	8.3
22	Holtemme	5.10.2015	23.81	941	9.83	14.3	379.1	16.4	195.9	13.8	15.2	-8.4	-59.2	7.9
24	Holtemme	6.10.2015	26.73	922	9.82	12.4		15.6		15.1	15.7	-8.4	-59.1	7.9
25	Holtemme	6.10.2015	28.39	917	9.85	12.4	296.9	15.2	142.6	14.7	12.3	-8.3	-59.3	6.7
28	Holtemme	6.10.2015	31.67	960	9.42	12.5		17.9		14.7	17.2	-8.3	-59.3	7.2
31	Holtemme	6.10.2015	32.54	1077	9.56	13.9	411.5	14.3	185.1	14.9	12.9	-8.4	-59.3	7.5
32	Holtemme	6.10.2015	34.74	1062	10.47	13.7		15.6		15.9	15.6	-8.3	-59.1	7.5
34	Holtemme	6.10.2015	35.69	1066	10.62	14		15.1		14.8	14.9	-8.2	-58.3	7.2
36a	Holtemme	6.10.2015	35.91	1068	10.43	13.9	256.3	17.2	138.9	13.5	16.7	-8.2	-58.3	7.4
36b	Holtemme	6.10.2015	36.01	1088	10.43	14.1	331.4	16.4	171.3	14.0	16.2	-8.2	-58.2	7.4
37a	Holtemme	6.10.2015	37.97	1058	10.96	13.9				13.2	13.5	-8.1	-57.5	7.2
37b	Holtemme	6.10.2015	38.07	1065	10.69	13.6		16.4		12.6	14.4	-8.0	-57.7	6.2
38	Holtemme	6.10.2015	40.01	1056	11.57	14.3	247.5	16.5	128.6	13.3	13.1	-8.1	-57.4	7.1
40	Holtemme	6.10.2015	41.69	1087	11.79	14.2		15.8		13.4	12.2	-8.0	-57.4	6.9

42	Holtemme	6.10.2015	43.35	1101	11.1	14.3	430.0*	15.5	210.2	13.8	10.9	-8.0	-57.5	6.6
5	Braunes Wasser	5.10.2015	3.77	379	11.16	10		3.6		2.0	2.9	-9.2	-62.0	11.6
8	Zillierbach	5.10.2015	6.99	560	10.98	11.6	72.0	5.0	11.4	4.6	3.2	-9.0	-62.2	9.9
12	Barrenbach	5.10.2015	12.61	1103	10.1	13.4	14.0	7.6	3.3	13.4	7.7	-6.7	-51.1	2.4
14	Silstedter Bach	5.10.2015	14.07	1184	11.53	13.5	15.0	27.3	12.9	5.8	12.8	-8.7	-61.0	8.6
19	Rothe	5.10.2015	18.47	1354	9.96	13.7	32.1	23.3	23.6	7.4	20.4	-8.6	-60.7	7.9
20	Hellbach	5.10.2015	19.44	801	9.75	13.6	15.8	51.4	25.5			-8.9	-62.8	8.3
23	Störbecker Fließ	6.10.2015	25.15	1283	10.01	11.3	66.0	30.2	62.9	12.3	6.3	-8.1	-58.0	6.8
33	Alte Holtemme	6.10.2015	34.76	1423	9.31	14.3	43.2	1.9	2.5	13.8	5.4	-4.2	-39.0	-5.0
35	Asse	6.10.2015	35.71	1521	10.4	12.4	15.9	46.1	23.0	9.3	5.0	-8.4	-59.1	8.5
39	Ditch Nienhagen	6.10.2015	41.14	1199	9.74	13.6	34.4	14.2	15.4	13.8	14.0	-8.1	-58.0	6.8
41	Salzgraben	6.10.2015	41.81	1125	10.52	14	116.3	15.3	56.2	13.1	11.7	-8.0	-57.5	6.8
16	WWTP	5.10.2015	16.17	1150	8.83	17.7	112.0**	21.7	76.6	18.4	13.8	-7.9	-55.7	7.4
29	WWTP	6.10.2015	31.96	1401	7.75	16.8	111.1	1.7	5.9	23.0	-3.6	-8.3	-59.2	7.6
Average (Mean)				**882**	**10.60**	**13.0**	**143.7**	**13.8**	**67.4**	**11.3**	**9.8**	**-8.4**	**-58.7**	**8.1**
Standard deviation (SD)				**370**	**0.97**	**2.0**	**134.7**	**10.9**	**69.6**	**5.1**	**5.7**	**0.8**	**3.7**	**2.9**
Relative coefficient of variation (CV)				**41.97**	**9.10**	**15.55**	**93.70**	**78.58**	**103.25**	**45.28**	**58.12**	**-9.67**	**-6.34**	**35.95**

* measured by the LHW; **measured by the operators of WWTP

6 Synthesis and conclusions

6.1 Summary

6.1.1 Monitoring concept and analysis

Isotope studies conducted over large spatial and/or temporal scales can provide powerful insights into natural ecosystem processes and the effects of anthropogenic influences. During recent decades, nitrate concentrations in surface and groundwater have increased due to land use change and accompanying application of fertilizer in agriculture as well as increased atmospheric deposition. To mitigate nutrient impacts on downstream aquatic ecosystems, it is important to quantify potential nitrate sources, instream nitrate processing and its controls in a river system. The challenge of the overall project is to characterize and quantify large (regional) scale dynamics and trends in water and solute fluxes from a European river catchment representing changing environmental conditions (e.g. elevated inputs, land use and climate change). The investigated area is part of the TERENO (Terrestrial Environmental Observatory) project, the test side of the Bode region in the Harz Mountains, Germany. The catchment is strongly influenced by agricultural land use that occupies an area of 3200 km², which is about 70 % of the overall size of the catchment. During the first field campaign in March 2012, 133 stream water samples were taken in the Bode catchment, most of them in the Harz Mountains. All sampling locations represent the discharge from 133 sub-catchments. Consequently, hydrochemical data and isotopic signatures of the water samples were subsequently assigned to the respective sub-catchments (Figure 6.1).

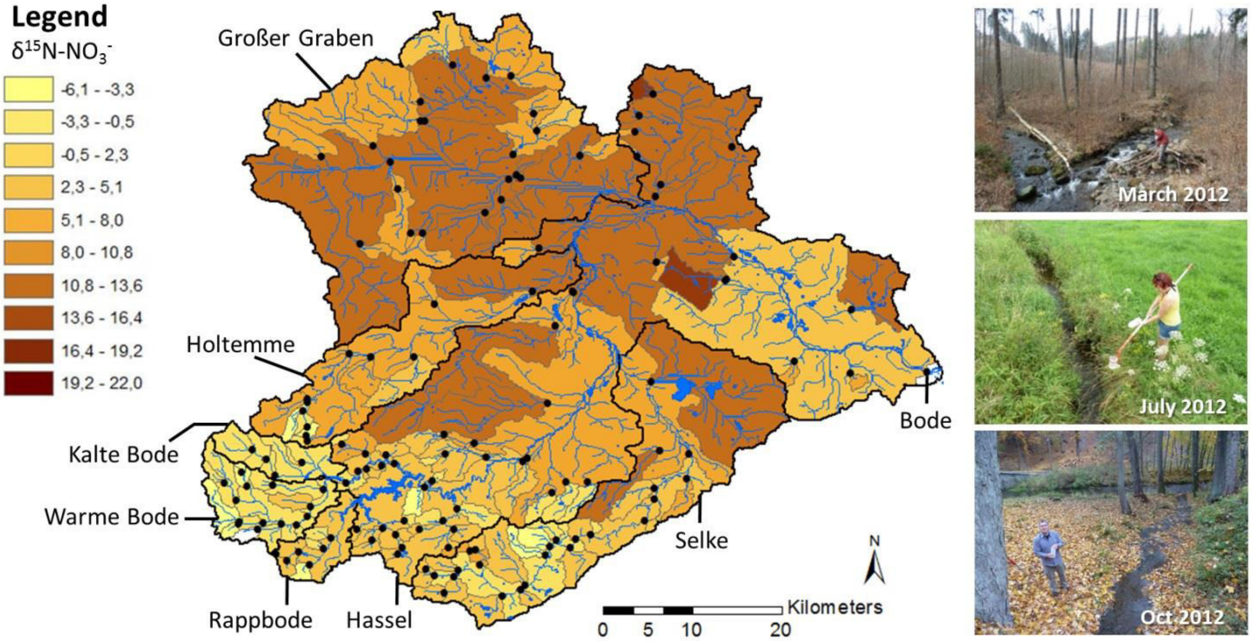


Figure 6.1: Spatial isotope δ^{15}N-NO$_3^-$ pattern of the Bode River catchment (sampling from March 2012)

Laboratory analyses on all water samples were conducted for stable isotope signatures of water (δ^{18}O, δ^{2}H), sulphate (δ^{34}S and δ^{18}O), dissolved inorganic and organic carbon (δ^{13}C), and of nitrate (δ^{15}N and δ^{18}O). The current thesis focuses on the results from nitrate and water isotopic values. The δ^{2}H/δ^{18}O-isotopic signature of water provides an ideal conservative tracer of water sources and mixing processes which is useful for quantifying flow contributions from different tributaries and groundwater as well as active evaporation processes. Isotopic signatures of nitrate yield valuable information on nitrogen transformation processes such as nitrification, denitrification and assimilation and may be used to differentiate between nitrate sources such as fertilizer, wastewater, manure, or atmospheric deposition. Precipitation and groundwater were sampled and considered as potential input factors. Regional isotope patterns of the different isotope systems obtained during the monitoring campaigns are combined with available regional data (e.g. elevation gradient, slope, vegetation cover, land use, soil, geology etc.).

6.1.2 Temporal and spatial nitrate dynamics

Nitrate stemming from different sources such as ammonia (NH_4) fertilizer, soil-nitrogen, organic fertilizer or precipitation partly shows extremely significant δ^{15}N-NO$_3^-$ and δ^{18}O-NO$_3^-$ – differences, which can be used to characterize and quantify the different source contributions (Kendall and McDonnell 1998). We observed in **chapter 3** a significant regional and partially temporal variation of nitrate isotopic signatures throughout the catchment. Stream section in the mountainous region contains nitrate in low concentrations with low $\delta^{15}N\text{-}NO_3^-$ values, whereas streams in the lowlands, affected by an increasing human impact, show a highly regional and seasonal variation in $\delta^{15}N\text{-}NO_3^-$ ranging between 1 ‰ and 14 ‰ (AIR). A clear correlation seems to exist between the percentage of agricultural land use area in the sub-catchments and $\delta^{15}N\text{-}NO_3^-$. Streams within catchments with a higher proportion of agricultural land use show high $\delta^{15}N\text{-}NO_3^-$ signatures of about 8 ‰ to 11 ‰ (AIR) during all seasons. The correlation between land use area and $\delta^{15}N$ is superimposed by a season-dependant impact of microbial denitrification. Denitrification, especially evident in the lowlands, predominantly takes place in the riverbeds. Beyond that, mixing processes of different nitrate sources and temperature-dependant biological processes such as nitrification have to be taken into consideration.

Regional landscape information like land use, soil information, geology, topology, recharge as well as quantitative discharge information is combined with the temporal and spatial nitrate isotope distribution patterns in **chapter 4**. Consecutive discharge simulations using a mesoscale hydrological model (mhM) allow a quantitative analysis of nitrate fluxes for all observed tributaries. The investigation of nitrate isotopic signatures for characteristic landscape types allows the delineation of dominant NO_3^- sources: nitrified soil nitrogen, fertilizer, and manure. Besides source delineation, the relationship between runoff and nitrate dynamics was analyzed for the entire Bode River catchment and, more detailed, for one major tributary with minor artificial reservoirs (Selke River). It becomes apparent thereby that nitrate isotopic variations increase with decreasing discharge. This effect might be due to a local, more intense impact of bacterial denitrification under low discharge conditions (higher residence time) in the anoxic soil zone, in the groundwater that discharges into the river and in the hyporheic zone. $\delta^{15}N\text{-}NO_3^-$ concentrations, and $\delta^{18}O\text{-}NO_3^-$ values decrease with increasing runoff at each sampling point, which can be caused by a preferential wash-out of more readily mobilizable, isotopically lighter fractions of the soil nitrate pool.

For streams with runoff larger than 5 m^3s^{-1} nitrogen isotopic signatures converge to a stable value and cannot be assigned to particular nitrogen turnover processes and sources. Thus, definite conclusions on the origin of nitrate and the overall impact of microbial processes are possible up to a certain watershed size only. This information is relevant for understanding the process dynamics and has to be considered for future monitoring and modeling approaches. Therefore, we focus on the main tributary, the Holtemme River, in **chapter 5** and compare two key date sampling campaigns conducted in 2014 and 2015. Apart from water sampling, we performed spatially highly resolved measurements of discharge at

23 sampling locations including 11 major tributaries and 12 locations at the main river (Figure 5.1). Additionally, there is information available from continuous runoff measurements at 10 sites operated by the local water authorities. Two wastewater treatment plants contribute nitrogen to the Holtemme stream. This contribution impacts nitrate loads and nitrate isotopic signatures depending on the prevailing hydrological conditions. Nitrogen isotopic signatures in the catchment are mainly controlled by different sources (nitrified soil nitrogen in the headwater and manure/ effluents from WWTPs in the lowlands) and increase with rising nitrate concentrations along the main river. Nitrate loads at the outlet of the catchment are different between both sampling campaigns (2014: 1847 kg day^{-1}, 2015: 576 kg day^{-1}) which are associated with various runoffs (at the outlet in 2014: 1.4 m³ s^{-1}, in 2015: 0.4 m³ s^{-1}). The separation of discharge between the base and quick flow indicates baseflow conditions for both sampling campaigns. Especially during the dry period in 2015, the inflow from WWTPs raises the NO_3^- loads and enriches $\delta^{18}O\text{-}NO_3$ values. Oxygen isotope signatures from nitrate are more variable and are controlled by biogeochemical processes in concert with the oxygen isotopic composition of the ambient water. Oxygen isotope signatures of nitrate are in part related to the discharge intensity. We assume that longer residence times of reactive nitrogen in the unsaturated zone are connected with a more intense recycling involving intermediate nitrogen compounds (e.g. nitrite). Stable isotopic compositions of mobile nutrients like nitrate have the potential to serve as a proxy for nutrient transit times. Enriched isotope values for nitrogen and oxygen are not indicative of a significant impact of bacterial denitrification, because they are accompanied by increased nitrate concentrations (from headwater to outlet: 1 to 16 mg L^{-1}). The main reason for the sensitive response from the main river of cleaned waste water is the low discharge at the tributary to the Holtemme River. Therefore, nitrate income from WWTPs with totally different isotopic signatures is minimally diluted and apparent for a long river stretch.

6.2 Sampling approach: concept for meso to large scale monitoring

Within this research, the central task was to answer the question of which nutrient and flow processes on the spatial and temporal scale are relevant for a large-scale river basin like that of the Bode in the Harz Mountains, central Germany. To get an impression of the investigation area, extensive information was gathered at the beginning of the study for land use, geology, soil properties or topography, for example. Since the catchment area of the Bode is part of the so-called TERENO network (see chapter 2.1), it was an ideal platform to use and mix already available scientific results from different disciplines in the environmental field. This allowed an interdisciplinary approach. Due to the large land use gradient (nature reserve at high altitudes and intensively used agricultural areas in the lowland), this catchment area was an ideal test field for studies on large-scale nitrate dynamics in a regional flow system. A great advantage of the investigation area was the very good accessibility by car. There are numerous roads and paths, as well as forest paths which could be used with the appropriate access permit. This allowed many samples to be taken at different main rivers and their tributaries in a relatively short period of time. The time spent at one sampling site for water sampling as well as filtering and measurement of immediate parameters was optimized to 10 minutes. As a result, it was possible to take around 40 samples at different locations in one day. During the planning of the sampling points, good logistical accessibility was ensured. In the case of seasonal sampling campaigns involving up to 133 sampling points, two teams were working on water samples (precipitation and river water) in the high and lowlands during the same period. Care was taken that sampling started at the upper streams and the end of sampling was reached at the catchment outlet. With two teams, it was possible to run the entire river catchment area within two days and to represent the river with a narrow monitoring network of river and rain samples. In the case of monthly sampling campaigns, 25 selected rivers of different sizes were sampled, which could be sampled by a team within two days.

The sampling dates in the years 2012 to 2015 were carried out independently of certain hydrological events. Therefore, a sampling campaign was carried out at the beginning of each month regardless of discharge intensity or rain. By evaluating the nitrate results, it was found that hydrological extreme events (high and low water) played a decisive role in nitrogen dynamics. This was evident both in the second study (chapter 4) and in the results of the third study (chapter 5) on low water events at the Holtemme River catchment. For this reason, monitoring at the Bode was implemented from 2016 using event-related deadline monitoring (which is not yet a subject of this work).

General limitations of monitoring in such a large river basin are, on the one hand, laboratory capacities and, on the other hand, sampling / field work per se. In the following phase, I would like to take a closer look at both of these factors. The number of samples is always related to the possible acquisition of knowledge and should be weighed against the monetary services. Especially the preparation of water samples for the analysis of nitrate isotope (chapter 2.4) requires high personnel and cost-intensive preparation. This must be considered when designing such monitoring and represents a substantial limit of the number of samples both spatially (in terms of the number of samples) and temporally (the number of sampling campaigns). Performing the analysis of the nitrate isotope in the field which has already possible for the water isotope (metcon: module for a continuous injection of water samples for Picarro Analyzers L1102-i/ L1115-i or Picarro A0217 Continuous Water Sampler), is currently unavailable.

Samples must always be collected by personnel on site, filtered and cooled, brought to the laboratory, prepared and then analysed under laboratory conditions. On-site automation is to date not possible.

For monthly monitoring of the catchment area, two to four scientists / technicians are necessary, who take the samples as well as determine on-site parameters. Likewise, the localities of the precipitation collectors must be regularly mown. Vandalism was a major problem, especially among the precipitation collectors. The activity could be countered by the involvement of the surrounding inhabitants, who placed the collectors on their property. In addition, the dedicated residents were able to take rain and / or river water for selected, interesting hydrological events. This kind of citizen science was a valuable contribution to the project. For future work involving such a large area of research, focus should be placed on citizen science.

6.3 Implications and Outlook

The results of the study on nitrate dynamics in flow systems are extremely relevant to current questions in the area of water quality of river systems. Especially in Germany, reports of increased nitrate levels in the groundwater and surface water have been reported over recent months (chapter 1.1). Therefore, such work in this area will continue to be useful and valuable for science and politics in the future. The generated data set provides a great deal of scope for further qualification work, as can be used by the large number of measured chemical parameters and stable isotopes for versatile tasks. The following studies, with regard to the investigated catchment area, may be interesting for future work:

I) Combination of different compartments at an intensive measuring site: sampling of groundwater, hyporheic zone, soil water, surface water, precipitation for the analysis of exchange processes and (microbiological) process dynamics

II) Sampling of extreme events (high and low water) along a main river using high-resolution spatial and temporal monitoring

III) Implementation of these results into nutrient export models: Analysis and modelling of water retention times to obtain information on degradability, degradation and storage of nitrate in different compartments

IV) Relationship of water and nitrate cycle to different hydrological conditions

V) Proof of concept of the preliminary findings in different catchment scales (small, mesoscale and large scale)

This work should be confined to a main tributary of the Bode (for example, Selke or Holtemme) or to an intensive measuring point. Thus mixing effects mainly resulting from the Rappbode dam, can be minimized.

This study is the basis for a comparative assessment with another river catchment at the Helmholtz Centre for Environmental Research - the Erlauf hydrographic basin in the alpine foothills in Austria. Differences between the two regions in terms of precipitation patterns and altitudes have a major impact on the isotopic signatures of sampled surface waters. Previous findings regarding matter fluxes in the nitrogen cycle can be applied in a region with comparable size and discharge intensity.

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Eidesstattliche Erklärung

Declaration under Oath

Ich erkläre an Eides statt, dass ich die Arbeit selbstständig und ohne fremde Hilfe verfasst, keine anderen als die von mir angegebenen Quellen und Hilfsmittel benutzt und die den benutzten Werken wörtlich oder inhaltlich entnommenen Stellen als solche kenntlich gemacht habe.

I declare under penalty of perjury that this thesis is my own work entirely and has been written without any help from other people. I used only the sources mentioned and included all the citations correctly both in word or content.

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Erklärung über bestehende Vorstrafen und anhängige Ermittlungsverfahren

Declaration concerning Criminal Record and Pending Investigations

Hiermit erkläre ich, dass ich weder vorbestraft bin noch dass gegen mich Ermittlungsverfahren anhängig sind. / *I hereby declare that I have no criminal record and that no preliminary investigations are pending against me.*

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Vorträge

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Poster

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European Geosciences Union (EGU) 2017, Wien

Mueller C, Merz R, Zink M, Krieg R, Rode M, Knöller K (11/2015):
Discharge driven nitrogen dynamics in a large river catchment: an isotopic study, Integrates Project "Water and matter flux dynamics in catchments" Assembly 2015, *1st price Best Poster Award*

***Mueller C*, Merz R, Krieg R, Knöller K (12/2012):**
Investigation of Large Scale Hydrological and Hydrochemical Processes by Regional Isotope Patterns, *American Geophysical Union (AGU), San Francisco*

***Mueller C*, Zink M, Krieg R, Rode M, Merz R, Knöller K (09/2015):**
Discharge driven nitrogen dynamics in a large river catchment: an isotopic study, *Jahrestagung der Arbeitsgemeinschaft Stabile Isotope e.V. 2015, Heidelberg*

Fachbereich Wissenschaftliches Tauchen

Brümmer F, Maichl D, Dehmer H, Gutekunst V, *Mueller C*, Pinter S, Rapp L, Russig S, Vetter W, Vetter E, Andres-Brümmer D, Buršić M (05/2016):
Rapid Assessment of the biodiversity of sponges (*Porifera*) and other invertebrates in the coastal waters of Brijuni Islands, northern Adriatic Sea, Croatia, *2nd European Conference on Scientific Diving 2016, University of Gothenburg, The Sven Lovén Centre for Marine Sciences*

***Mueller C*, Sieland R, Merkel B, Knöller K (04/2012):**
Hydrothermal water exhalations at the submarine volcano Panarea, Italy – insights from stable isotope investigations, *European Geosciences Union (EGU), Wien*

***Mueller C*, Barth G, Merkel B (11/2011):**
Geothermal state of shallow submarine geothermal systems of Panarea, Aeolian Islands (Italy), *3rd International Symposium on Occupational Scientific Diving, Porto Cesareo, Lecce (Italien)*

ORGANISATION WISSENSCHAFTLICHER KONFERENZEN

1st European Conference on Scientific Diving, Stuttgart (03/2015): Scientific committee

BETREUUNG WISSENSCHAFTLICHER ARBEITEN

Diplomanden:	**Praktikanten:**
Julia Jäger	Cornelia Menge
Stefanie Kolbe	Denis Bauer
Tiemo Pinkwart	

ZUSATZQUALIFIKATIONEN

Sprachkenntnisse

Englisch	Fließend in Wort und Schrift
Französisch, Italienisch	Basiswissen

EDV-Kenntnisse

Windows Office, ArcGIS	Verhandlungssicher
R, Surfer, ModFlow, PhreeqC	Basiswissen

Führerscheinklasse B

Bootsführerschein See

Tauchqualifikationen:

VDST CMAS Tauchlehrer** (seit 07/2016)
CMAS Scientific Diving Instructor (seit 09/2016)

ENGAGEMENT/ INTERESSEN

seit 10/2017	Landesausbildungsleiterin des Landestauchsportverbandes Sachsen-Anhalt
seit 07/2015	Vorstandmitglied in GAUSS, German Academy of Underwater Sciences
seit 01/2014	Stellvertretende Projektleiterin des Ressorts Scientific Diving im Verband Deutscher Sporttaucher (VDST)
seit 10/2012	Ehrenamtliche Mitarbeit im Ressort Scientific Diving des Verbandes Deutscher Sporttaucher (VDST)

Halle (Saale), den 08.11.2017