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QUANTITATIVE ASSESSMENT OF NITROGEN DYNAMICS IN ANTHROPOGENICALLY MODIFIED RIVERS AND HYPORHEIC ZONES

DISSERTATION

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DECLARATION OF CONFORMITY

I hereby confirm that this copy conforms with the original dissertation on the topic

“Quantitative assessment of nitrogen dynamics in anthropogenically modified rivers and hyporheic zones”.

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LIST OF ABBREVIATIONS AND ACRONYMS

AC	Activated carbon	
CH ₄	Methane	
CO ₂	Carbon dioxide	
<i>d</i>	depths	m
<i>D_A</i>	assimilatory demand for nitrogen	mg NO ₃ ⁻ -N m ⁻² d ⁻¹
DO	Dissolved oxygen	
DOC	Dissolved organic carbon	
DS	Downstream	
<i>ER</i>	Ecosystem respiration	g-O ₂ m ⁻² d ⁻¹
EU	European Union	
<i>GPP</i>	Gross primary production	g-O ₂ m ⁻² d ⁻¹
h	Hours	
HPFM	Hyporheic passive flux meter	
HZ	Hyporheic zone	
<i>J_N</i>	Horizontal nutrient flux	mg m ² d ⁻¹
KCl	Potassium chloride	
<i>L</i>	length	m
<i>LQ</i>	Limit of quantification	
MDL	Method detection limit	
M	mass flux	mg NO ₃ ⁻ -N s ⁻¹
MLS	Multilevel sampler	
N	Nitrogen	
N ₂	Gaseous nitrogen	
N ₂ O	Nitrous oxide	
<i>NEP</i>	Net ecosystem production	g-O ₂ m ⁻² d ⁻¹
NH ₄	Ammonium	
NO ₂	Nitrite	
NO ₃	Nitrate	
O ₂	Oxygen	
Oct	October	

P	Phosphorous	
PET	Polyethylene	
PFM	Passive flux meter	
PO ₄	phosphate	
Q	discharge	$\text{m}^3 \text{s}^{-1}$
q_x	Horizontal Darcy velocity	cm day^{-1}
q_y	Vertical Darcy velocity	cm day^{-1}
R_d	Retardation factor	
R_N	Removal activity	%
Sept	September	
SO ₄	Sulfate	
SpC	Specific conductivity	$\mu\text{S cm}^{-1}$
SRP	Soluble reactive phosphorous	
SW	Surface water	
T	Tortuosity	-
Temp	Temperature	°C
TN	Total nitrogen	
u	Mean velocity	
U_A	Assimilatory uptake	$\text{mg NO}_3\text{-N m}^{-2} \text{d}^{-1}$
US	Upstream	
U_T	Uptake rate	$\text{mg NO}_3\text{-N m}^{-2} \text{d}^{-1}$
w	Width	m
WWTP	Waste water treatment plant	
τ	Travel time	h

SUMMARY

Matter retention in streams and rivers is an ecosystem service of outstanding ecological as well as economic importance. Studying and monitoring instream nitrate dynamics is essential to reduce the tremendous consequences of eutrophication of freshwater systems and coastal zones. Moreover, the cycling of nitrate in lotic systems is a paradigm for the instream transport and transformation behavior of any other reactive substance subjected to human perturbation of its natural cycle. Identifying instream processes and drivers that dictate nitrate transport in rivers and quantifying the capacity of rivers to retain nitrate is therefore of scientific as well as political interest and was the motivation for this thesis.

Even though understanding and monitoring of instream nitrate dynamics is advanced compared to most other emerging substances of concern (e.g. pharmaceuticals, synthetic and natural hormones), methodologies to directly assess nitrate dynamics are still limited, leaving a high degree of uncertainty to descriptive and predictive models. One major problem of common data acquisition is that the temporal and spatial variability of nitrate processing rates arising from the complex interactions of hydrological and biogeochemical drivers cannot be captured with traditional methods. For technical reasons, most studies have been conducted in small (and rather pristine) streams. Thus, particularly the functional behavior of larger rivers and anthropogenically modified systems is widely uncharacterized.

In this work, two methodologies were developed which allow quantitatively assessing nitrate dynamics on two relevant scales: The reach scale (1), which is of particular interest for monitoring strategies and local hyporheic nutrient fluxes (2), with the hyporheic zone being a key compartment in instream solute cycling. In order to assess the seasonal fluctuation in nitrate dynamics (3), a primary demand on the methods was that they operate continuous or over longer time spans.

On the reach scale, a combined two-station time-series and longitudinal profiling approach based on measurements from automated sensors, provided novel insights into seasonal variations of nitrogen processing and allowed quantitative comparison of the dynamics in a natural versus a heavily modified reach. Uptake was lower and the influence of season on uptake rates more marked in the modified reach. Continuous implementation of the proposed approach, fully covering the annual variations, can essentially improve existing monitoring practices by quantifying the effect of altered morphology and water chemistry on retention rates.

Hyporheic passive flux meters are an efficient tool to quantify time integrative hyporheic nutrient fluxes. In combination with other measurements, the results from a field application unraveled an unexpected hyporheic source-sink behavior for nitrate at the study reach. Different to common observations, not the upper most layer of the hyporheic zone but the layer between 15 and 30 cm was most efficient in removing nitrate, assumedly because substrate limitation is irrelevant in agricultural (nutrient and DOC enriched) streams. Further, higher discharge did not increase hyporheic exchange, because the monotonous morphology and absence of bedforms reduced the usually dominating effect of increased drag resistance with higher flow.

The studies presented here deliver empirical evidence that, on both investigated scales, anthropogenic modifications substantially impact instream nitrate dynamics. Alterations to channel morphology, riparian vegetation, hydrology and water quality change principal ecosystem functions relevant for solute retention in streams and rivers. The presented results show that anthropogenically modified systems may therefore behave unexpectedly if predictions are built on the driver-response correlations observed in natural systems.

Worldwide a large proportion of rivers and streams are modified by humans. However, altered systems are not adequately represented in studies focusing on solute dynamics. Efficient management of such systems, including evaluation of measures to reduce the nitrogen burden on receiving water bodies, requires quantitative knowledge on instream processes and governing drivers. Continuous or time integrative observations are more representative for the solute cycling characteristics of a system than “snapshot”-like assessments. The new methodologies thereby also facilitate extrapolation of local measurements and linking the resulting data with catchment scale models. Overall synthesis of the presented results suggests that such measurements of nitrate dynamics in streams may be used as an indicator for the ecosystem integrity.

1. INTRODUCTION

The ecologic and economic problems caused by eutrophication of marine, coastal and inland waterbodies have been recognized years ago: Anoxia (Diaz and Rosenberg, 2008), fish kills (Carpenter et al., 1998), algae blooms (Hallegraeff, 1993), loss of biodiversity (Seehausen et al., 1997), impairments for drinking water (Qin et al., 2010) or recreational use (Anderson et al., 2002) to name the most widespread impacts. Costs resulting from eutrophication of freshwaters have been estimated to 105-160 million US\$ per year only for England and Wales (Pretty et al., 2003). In the US, economic losses from the recreational sector that were related to nutrient pollution of freshwater bodies could amount up to 1.16 billion US\$ per year (Dodds et al., 2009). While phosphorous is usually the limiting element for primary production in freshwater bodies, nitrogen is the principle cause for eutrophication of marine and coastal waters (Howarth, 1988; Nixon et al., 1996; Blomqvist et al., 2004). The production of synthetic nitrogen fertilizers via the Haber-Bosch process in 1913 did not only allow to boost food production feeding a growing world's population, but has also lead to an increase of reactive nitrogen in the environment (Galloway et al., 2003).

Different to phosphorous, fixed nitrogen (in general ammonium and nitrate) is highly soluble and instantly leached from drained soils, resulting in severe nitrogen burdens on many aquatic ecosystems (Gruber and Galloway, 2008). In western countries, the majority of nitrogen emissions to water bodies originate from diffuse agricultural sources with nitrate as the dominant form (Dupas et al., 2016; Mellander et al., 2012; European Environment Agency, 1991a). Of the 200 billion kg-nitrogen applied worldwide every year for agricultural purposes, roughly one quarter finally runs off into rivers (Galloway et al., 2003; Gruber and Galloway, 2008). Various national and international directives and conventions as for example the EU Nitrate Directive (European Environment Agency, 1991a), the Baltic Sea Action Plan (HELCOM, 2007) or the Urban Waste Water Treatment Directive (European Environment Agency, 1991b) were adopted in order to reduce the pollution of water bodies by nitrate and the problems related to eutrophication. Nevertheless, 26 years after the implementation of the Nitrate Directive, excessive nutrients were still one of the main reasons for the bad ecological status of German and other European surface and groundwater bodies in the last assessment under the Water Framework Directive (Voelker et al., 2016). Rivers and streams are the principal connection pathways between the terrestrial nutrient sources and vulnerable receiving (marine) systems (Dodds, 2007; Nixon et al., 1996). Far from being just transport channels, rivers exhibit a large capacity to process nutrients as well as other substances and to diminish

exports to the receiving water bodies (Seitzinger et al., 2002; Wollheim et al., 2008; Garcia-Ruiz et al., 1998a).

With several relevant compounds, dominantly nitrate, ammonium and nitrite, as well as complex assimilatory and dissimilatory pathways, instream nitrogen cycling also received a lot of scientific attention in the recent decades. However, to this day, methodological restrictions in directly measuring instream cycling rates result in a knowledge gap on quantitative uptake rates, involved processes and influencing factors (Böhlke et al., 2009; Trentman et al., 2015). Despite increasing evidence that human impacts lead to diminished ecosystem services, including the self-cleaning capacity of rivers (Elosegi et al., 2010; Hancock, 2002) particularly the distinct effects anthropogenic alterations to morphological, hydrological and chemical properties of rivers exhibit on instream transformation and retention rates remain vague (Arrigoni et al., 2010; Grimm et al., 2005). As for other important instream processes, profound and quantitative knowledge of nutrient transport, transformation and retention in rivers and responses to changing drivers is essential to prevent further derogation of these important ecosystem services (Malmqvist et al., 2002; Steffen et al., 2015). To this purpose, advanced methods are needed to deliver data for conceptual and computerized models reaching beyond a generalistic and qualitative description of instream processes (Rode et al., 2016b; Mulholland and Webster, 2010a; Boyer et al., 2006). Quantitative reach scale measurements of transformation and retention characteristics are especially important for developing management strategies, because monitoring programs and mediation measures usually operate on this scale. At the same time, distinct cycling patterns at specific local hotspots and the mechanisms of different processes have to be characterized to fully understand cause-effect relationships (Wollheim et al., 2008; Grant et al., 2014).

1.1. GOALS AND QUESTIONS

This thesis addresses the research questions how (1) nitrate is processed during downstream transport and (2) how much of the nitrate entering a stream or river is retained from being exported to receiving water bodies by instream processes. More specifically, different removal processes (e.g. assimilatory versus dissimilatory), their share in total removal and the identification of factors which negatively and positively impact the retention capacity of a river were assessed. Anthropogenic alterations to the water chemistry and hydromorphology were considered as a pivotal influencing factor. It was therefore a further aim to evaluate ecosystem functioning of anthropogenically modified systems, using nitrate cycling as an exemplary function. Hereby, nitrate can be regarded as paradigm for any reactive bioavailable substance subjected to substantial human perturbations of its natural cycle.

Reviewing previous studies on instream nitrate dynamics revealed that knowledge on quantitative instream nitrate removal is still limited because of methodological constraints in measuring nitrate processing rates in situ. These constraints exist on several spatial and local scales and are primarily related to the reactive nature of nitrogen and the variety of often interconnected factors influencing transport and transformation processes of nitrate in streams (Trentman et al., 2015; Ford et al., 2015; Boano et al., 2014). The objective of this study was therefore to develop new methodologies for quantifying instream nitrate dynamics. Even though nitrate was the main focus of this work, other nitrogen species and phosphate were also assessed. First, because processing of various nutrients is often coupled and second because the prospect to later extend the developed methods to further high-priority substances is seen an asset. Besides contributing to instream nutrient research, it was also anticipated that the developed methodologies can improve current monitoring practices. Monitoring groundwater and surface water quality and assessing the impact of mitigation measures is an integral part of the EU Nitrate Directive and the EU Water Framework Directive (Voelker et al., 2016; Arle et al., 2014; European Commission, 2002). Nevertheless, measuring concentrations alone will not be sufficient to improve management strategies. Conceptual and computational models on instream dynamics as well as quantitative evidence from field data are needed to implement efficient programs. Commonly, investigations rely on low frequency grab sampling during working hours, excluding night-times and storm event conditions from monitoring records (Fovet et al., 2016; Halliday et al., 2015). Paradoxically, it has been realized that exactly those time spans can have the main share in overall retention (night time denitrification) or export quantity (elevated loads during storm events) (Bieroza et al., 2014; McKnight et al., 2004). Hence, sampling frequency and

collection time may significantly impact water quality assessments. The principal demand on new methodology was therefore that it yields continuous nitrate processing rates over time spans of at least several days.

Depending on the spatial scale of an assessment, different aspects are important. This work covers two pivotal spatial scales of the instream nitrate cycle:

- (1) Quantitative reach scale dynamics of nitrate and
- (2) Local hyporheic nitrate fluxes and processing rates

Temporal dynamics were an essential aspect on both scales. Thereby, the focus in this thesis was on

- (3) the effect of river morphology on nitrate uptake at seasonal time scales

1.1.1. REACH SCALE QUANTIFICATION OF NITRATE DYNAMICS

The aim of this method development was to continuously measure quantitative instream nitrate removal and transformation rates over a specific reach. As previously existing methods were mainly restricted to small streams, the primary criterion was that the new method is suitable for rivers of all sizes and stream orders.

Further demands were that the method allows to

- distinguish between distinct uptake processes, of which some remove nitrate eternally from the system (denitrification) while others retain it only temporally (assimilation);
- detect hotspots and hot moments of nitrate processing;
- identify and quantify the main factors influencing removal quantity and quality;

The following hypothesis formed the basis for the methodological framework and connected research questions:

- High frequency, high resolution measurements from automated sensors can be used to meet the above mentioned demands (A1);

- Methodological and conceptional adaptations allow the extension of existing methods from chemo-static, hydrologically stable spring-fed rivers to rivers with complex input signals, tributaries and multiple nitrogen species (A2);
- Mass balances between two station time-series serve to quantify uptake rates over a specific reach (A3);
- Longitudinal profiling can be used to detect local uptake hotspots and describe uptake characteristics of the specific reach (A4);
- Assimilatory and dissimilatory shares of total removal can be parted via analysis of diurnal nitrate amplitudes (A5);
- Additional measurements of hydrological and chemico-physical parameters can support the results. Temperature and oxygen profiles can be used to calculate metabolic rates, from which assimilatory nitrogen demand can be derived. Naturally abundant isotopes deliver qualitative evidence on concurring nitrate cycling processes (A6);
- Season and river morphology have a marked effect on uptake quality and quantity. This effect can be quantified at the reach scale (A7);

1.1.2. QUANTIFYING HYPORHEIC NITRATE FLUXES

The aim of this second part was to develop a device for quantifying horizontal nutrient fluxes through the hyporheic zone. Requirements on the method were that

- it delivers time-averaged hyporheic nitrate and phosphate fluxes for a time span of several days;
- it operates during this period without the need of electric power supply or maintenance;
- nutrients and water flow are captured simultaneously within the same device in order to avoid the uncertainties arising from the high local heterogeneity of hyporheic solute transport;
- vertical gradients in nutrient fluxes over depths can be assessed.

The hyporheic passive flux meter (HPFM) was developed based on the following hypothesis:

- PFMs which have originally been designed for the use in groundwater studies can be adapted for the application in the hyporheic zone; the requirement for specific adaptations can be identified based on the existing expertise in groundwater PFMs and additional laboratory experiments (B1)
- an installation and retrieval procedure which prevents contact of the HPFM with the surface water can be implemented (B2);
- the advantages of time integrative measurements of nutrient fluxes through the hyporheic zone from HPFMs can be demonstrated in a comparative field experiment (B3).

1.1.3. HYPORHEIC NITRATE DYNAMICS IN AN ANTHROPOGENICALLY MODIFIED STREAM

As time integrative nutrient flux measurements through the hyporheic zone are novel, it is also relevant to explore how the generated data can be interpreted and which new insights can be gained from the results. This part describes the application of HPFMs in an exemplary field study with the goal to test topical conceptual frameworks on hyporheic nutrient dynamics.

The current scientific knowledge on hyporheic nutrient processing is derived from general theoretical or data driven models or from field studies (most of which in pristine to near natural streams). Since rivers are quite divers and often unique in their assemblage of environmental conditions, it is questionable if general or site specific findings from other systems hold for the site of interest. Especially for anthropogenically modified streams and rivers, direct measurements on hyporheic processes such as nutrient turnover are rare, so that estimates of hyporheic dynamics based on consisting correlations are particularly uncertain in those systems. In this case study, we investigated if established driver-response relationships are valid for an anthropogenically modified stream, using in situ measurements of hyporheic nitrate dynamics as an example. The prevalent hypothesis tested were that

- higher discharge will increase hyporheic exchange, enhancing nitrate transformation in the hyporheic zone (C1);
- the function of the hyporheic zone in cycling nitrate (as source or sink) can be explained by hyporheic oxygen concentration and residence time of water and solutes in the hyporheic zone (C2);

- biotic activity in the hyporheic zone and with it nitrate processing rates are higher during the warmer than during the colder season (C3).
- human activities, specifically channelization and Waste water treatment plant (WWTP) effluent, have a detectable impact on hyporheic nutrient cycling (C4).

2. HIGH FREQUENCY MEASUREMENTS OF REACH SCALE NITROGEN UPTAKE IN A 4TH ORDER RIVER WITH CONTRASTING HYDROMORPHOLOGY AND VARIABLE WATER CHEMISTRY (WEIßE ELSTER, GERMANY)

2.1. ABSTRACT

River networks exhibit a globally important capacity to retain and process nitrogen. However, direct measurement of in-stream removal in higher order streams and rivers has been extremely limited. The recent advent of automated sensors has allowed high frequency measurements, and the development of new passive methods of quantifying nitrogen uptake which are scalable across river size. Here, we extend these methods to higher order streams with anthropogenically elevated nitrogen levels, substantial tributaries, complex input signals, and multiple N species. We use a combination of two station time-series and longitudinal profiling of nitrate to assess differences in nitrogen processing dynamics in a natural versus a channelized impounded reach with WWTP effluent impacted water chemistry. Our results suggest that net mass removal rates of nitrate were markedly higher in the unmodified reach. Additionally, seasonal variations in temperature and insolation affected the relative contribution of assimilatory versus dissimilatory uptake processes, with the latter exhibiting a stronger positive dependence on temperature. From a methodological perspective, we demonstrate that a mass balance approach based on high frequency data can be useful in deriving quantitative uptake estimates, even under dynamic inputs and lateral tributary inflow. However, uncertainty in diffuse groundwater inputs and more importantly the effects of alternative nitrogen species, in this case ammonium, pose considerable challenges to this method.

2.2. INTRODUCTION

Nearly 200 Tg-N (200 billion kg) of reactive nitrogen (N) is added worldwide to the land surface every year for agricultural purposes, with only a small fraction being assimilated by crops (Böhlke et al., 2009; Galloway et al., 2003). While the majority returns to the atmosphere via soil denitrification, a substantial fraction of terrestrial N loads, roughly 25%, ultimately enter into river drainage networks (Galloway et al., 2003; Gruber and Galloway, 2008), resulting in widespread cultural eutrophication of downstream water bodies (Böhlke et al., 2009; Smith et al., 2006). However, far from being non-reactive conduits, river networks exhibit a remarkable capacity to process and remove N in-transit (Galloway et al., 2003). Models suggest that up to 70% of N inputs may be removed during transport through river networks (Garcia-Ruiz et al., 1998; Seitzinger, 1988; Seitzinger et al., 2002; Wollheim et al., 2008).

Despite their importance in global N cycling, direct methods of quantifying in-stream removal in defined reaches have largely been limited to small streams (Ensign and Doyle, 2006; Hall et al., 2013; Tank et al., 2008; Wollheim et al., 2006). For rivers, active methods such as isotope addition (Dodds et al., 2000; Ruehl et al., 2007), plateau enrichment (Mulholland et al., 2002; Wollheim et al., 2008) or even pulse enrichment (Tank et al., 2008; Covino et al., 2010) are commonly impracticable due to the large masses needed to achieve detectable signals. Prior to the advent of high-resolution, high frequency sensors, inferring reliable uptake rates from a passive mass balance approach was typically not feasible because analytical precision was too low and effects of errors in discrete samples too high (Rode and Suhr, 2007). Other passive methods, like naturally abundant isotope fractionation may identify presence of removal processes, but not quantitative estimates of uptake rates (Bartoli et al., 2012; Cohen et al., 2012). Thus, until recently, uptake rates in larger rivers have largely been estimated using models which scale up observations from smaller streams (Wollheim et al., 2006). The resulting high degree of uncertainties represented a substantial knowledge gap on real uptake quantities in higher order streams (Böhlke et al., 2009).

The expanding availability of high-frequency, high-resolution stream solute signals from automated sensors (Kirchner et al., 2004; Pellerin et al., 2009; Saraceno et al., 2009; Rode et al., 2016), has allowed development of new passive methods of inferring uptake rates which scale across river size. Heffernan and Cohen (2010) demonstrated how uptake rates could be calculated from high temporal resolution time-series profiles. This method allows partitioning of N removal into assimilatory and dissimilatory pathways. Hensley et al. (2014) demonstrated an alternative approach using highly temporally and

spatially resolved longitudinal profiling which allows for the detection of uptake “hotspots”. This method also enables the identification of potential sources of dilution or enrichment from lateral inputs which might be misinterpreted as biogeochemical uptake or release when taking a black box approach to reach-scale processing. However, these initial studies took place within very specific study sites, spring-fed rivers, which are fundamentally different from most other river reaches in several ways. The goal of this study was to extend existing methods to higher order streams with anthropogenically elevated N levels, substantial tributaries, complex input signals, and multiple N species.

Springs integrate catchment dynamics over long time scales, producing temporal stability in stream hydraulic parameters such as discharge, stage and residence time, as well as water chemistry (Heffernan et al., 2010). Adaptations to the methodology (Hensley et al., 2015) allowed its application to tidal-spring run, where these properties varied over a well-defined frequency. However, in most river systems, hydraulics and stream water chemistry, two primary drivers of N retention rates (Dodds et al., 2002; Hall et al., 2009; Mulholland et al., 2002), are much more dynamic. Another major challenge, new in the application presented here was the presence of multiple N-species. In rivers where these methods have previously been applied, ammonium (NH_4^+) was below detection limits (Heffernan et al., 2010), such that N uptake estimates could be derived from measurement of a single species: nitrate (NO_3^-). Furthermore, because rates of denitrification and assimilatory uptake exceeded rates of nitrification by several orders of magnitude (Cohen et al., 2012), assumptions made about the time-varying nature of nitrification (Grimm and Petrone, 1997; Gucker and Pusch, 2006; Strauss et al., 2004) were not as critical. We expected that additional NH_4^+ loading might confound quantification of N uptake using NO_3^- alone, and that production of NO_3^- through nitrification might bias estimates of denitrification rates. In this study we evaluated how far NO_3^- sensor signals can be used to interpret overall N dynamics when other N species are also present. We hypothesized that a combination of above mentioned methods and concurrent assessment of autotrophic primary production, ecosystem respiration and estimation of autotrophic N demand from concurrent dissolved oxygen time series would enable differentiation between different N processing pathways.

The morphology and vegetative characteristics of rivers where the method has previously been applied were also relatively constant (Hensley and Cohen, 2012), and spatial variability in processing rates relatively small (Hensley et al., 2014). In other river systems, particularly those subject to anthropogenic modifications, these properties are expected to vary substantially. One extreme example of anthropogenic modifications is river channelization, which may substantially impact nutrient processing dynamics (Laub and Palmer, 2009; O'Connor and Hondzo, 2008). Natural rivers are assumed to have a

higher NO_3^- removal capacity than modified systems (Kemp and Dodds, 2002; Opdyke et al., 2006; Boyacioglu et al. 2012) and restoration efforts can have a positive effect on nitrogen uptake (Bukaveckas, 2007; Stanley and Doyle, 2002). Channelization shortens water residence time, one of the primary predictors of N retention (Seitzinger et al., 2002; Wollheim et al., 2006). Concrete filling of river beds inhibits the rooting of vegetation. In addition to direct assimilation, vegetation promotes sediments favorable for denitrification (Pinardi et al., 2009) as do other natural structures such as gravel bars or downed trees (Groffman et al., 2005). The limitation or absence of hyporheic flow in channelized rivers excludes the main compartment of denitrification (Alexander et al., 2009; Fischer et al., 2005; Rode et al., 2015; Ward et al., 2016; Zarnetske et al., 2011). Choosing two reaches contrasting in morphology and NH_4^+ as study sites guarantees the presence of differences in N cycling dynamics and is therefore an effective way of testing if a combination of longitudinal profiling and two-station time-series is sensitive enough to quantify N cycling rates and inter-reach differences on the reach scale. We expected that a natural study reach would exhibit higher rates of both assimilatory and dissimilatory N uptake than a channelized one. Finally, as seasonal variation in day length and its effect on photic and temperature conditions strongly control rates of nutrient removal and prevalence of different uptake processes (Worrall et al., 2015), we evaluated the method under different temperature and light conditions. While Heffernan and Cohen (2010) did note light driven seasonal variation in processing rates, the proximity of their study sites to the tropics means that their study sites experience less seasonal variation in insolation than streams at higher latitudes. Additionally, as spring-fed rivers there was negligible seasonal variation in temperature.

This study presents the extension of existing methods for deriving N uptake parameters from time series under new conditions. We developed conceptual and methodological supplements and adaptations based on the expected challenges connected to tributary inflow, diurnal variations and additional NH_4^+ loading. We hypothesize that a combination of existing methods allows quantifying N-uptake on the reach scale and distinguishing different removal processes. This hypothesis was tested under contrasting conditions to identify prospects and limitations in the application of presented methods.

2.3. METHODS AND MATERIALS

2.3.1. STUDY SITE

The Weiße Elster is a 250 km long river, originating in the northern Czech Republic and discharging into the Saale River near Halle, Germany. The 4th order river drains a watershed of 5300 km². Land use within the basin varies along a longitudinal gradient, with the headwaters dominated by forests while the lower reaches are dominated by agriculture. Because of agricultural runoff as well as waste water treatment plant effluent, the water quality in the middle to lower reaches is marked by high N concentrations (Wagenschein and Rode, 2008). For our study sites we selected two morphologically contrasting reaches (**Figure 2.1**), later referred to as “NATURE” and “CHANNEL”, within a previously studied (Wagenschein and Rode, 2008; Boyacioglu et al., 2012) section of the middle Weiße Elster. We performed two deployments on each reach, in July and September of 2015. Parameters for each reach are provided in **Table 2.1**.



FIGURE 2.1. STUDY REACHES IN JULY 2015. NATURE (LEFT) WAS UN-ALTERED, AND FEATURED A MEANDERING PATTERN WITH POOLS AND RIFFLES. CHANNEL (RIGHT) WAS STRAIGHTENED AND CONCRETE LINED.

TABLE 2.1. MAIN FEATURES OF THE TWO STUDY REACHES. L- REACH LENGTH, T- TORTUOSITY, Q- DISCHARGE, d-MEAN DEPTH, w- WIDTH, τ - MEAN TRAVEL TIME.

Parameter	NATURE	CHANNEL	Units
L	7100	7600	m
T	2.5	1.4	-
Q	6.2-9.3	6.3-8.3	m ³ s ⁻¹
d	1.7	0.7	m
w	23	23	m
τ	7	3.5	h

The NATURE reach was 7.1 km long (starting point 51°06'11.6"N 12°12'19.0"E), and retains an un-altered, meandering pattern, with varying widths and depths, debris islands, pools and riffles. Riparian vegetation is expansive, and large parts of the reach may be shaded when leaves are present. Allochthonous inputs from litterfall are likely extensive. Due to mining activity in the surrounding area, the groundwater table in the area is unnaturally low. The responsible mining company MIBRAG is continuously monitoring the groundwater level in the Weiße Elster basin. The 6-hour time step readings from 16 groundwater wells in the area of the NATURE reach showed that groundwater table was at minimum 1.5 meters below water level in the Weiße Elster during summer and autumn 2015. Groundwater inflow to the reach is therefore not expected. The only inflow is a mining drainage, consisting of groundwater that is collected in the close by mine, pumped to the surface and flows then via gravity in an open ditch until it discharges into the main stem in the last fifth of the NATURE reach. The discharge of this artificial tributary is continuously measured by the mining company MIBRAG and the assumedly stable (personal communication) water chemistry is analyzed once per week.

The CHANNEL reach started 12 km downstream of the end of NATURE (51°12'27.8"N 12°18'02.4"E). It was 7.6 km long, straightened and completely sealed within a concrete channel. In 1971 a 10 km long stretch of the Weiße Elster was relocated and transferred into a concrete bed in order to prevent it from draining into open pit lignite mines in the area or refilling the artificially lowered groundwater body. These conditions remain valid today (Sächsisches Landesamt für Umwelt, 2013). The cross-sectional profile of the CHANNEL reach is trapezoidal with uniform width and depths. Both banks are unvegetated; shading and litterfall are essentially zero. Because of the concrete lining, there is no groundwater exchange. The only inflow is the Elstermühlgraben, a mill ditch which was extracted from the Weiße Elster just upstream and reenters within the first 0.5 km of the study reach. Its water chemistry is nearly identical to the main stem of the Weiße Elster. There is a waste water treatment plant upstream of the study reach. While NH_4^+ concentrations in NATURE were small and negligible relative to NO_3^- , within CHANNEL NH_4^+ contributed between 10-15 % to the total N load.

2.3.2. TWO STATIONS TIME-SERIES

We installed water chemistry sensor stations at the upstream and downstream ends of each reach. Each deployment lasted 3-4 days. All sensors were synchronized before the start of the experiment by running them simultaneously in a bucket filled with Weiße Elster water. Discrete samples from the same bucket, which were analyzed in the laboratory as stated below, were used as benchmark samples. At each station we installed automated ultra-violet spectrophotometers (ProPS WW, TriOS) to measure NO_3^- . In longtime monitoring, these sensors have shown high stability and concordance to frequent routine laboratory analysis (e.g. Jiang et al., 2015; Rode et al., 2016). The sensors used in this study utilized a path length of 10 mm and measured adsorption over wavelengths from 190-360 nm. Manufacturer stated precision was $0.03 \text{ mg NO}_3^- \text{ N L}^{-1}$ and accuracy was $\pm 2 \%$. Measurement frequency was set to 10 min.

Additionally each station was equipped with a multi-parameter probe YSI 6600 V2/4 (YSI Environmental, Yellow Springs, Ohio) recording the following parameters, accuracy and precision as stated by the manufacturer: pH (precision 0.01 units, accuracy ± 0.2 units), specific conductivity (precision 0.001 mS cm^{-1} , accuracy $\pm 0.5 \%$), dissolved oxygen (precision 0.01 mg L^{-1} , accuracy $\pm 1 \%$), temperature (precision $0.01 \text{ }^\circ\text{C}$, accuracy $\pm 0.15 \text{ }^\circ\text{C}$), turbidity (precision 0.1 NTU, accuracy $\pm 2 \%$) and chlorophyll-a (precision $0.1 \text{ } \mu\text{g L}^{-1}$, linearity: $R^2 > 0.9999$ relative to dilution of rhodamine WT solution of 0 to $400 \text{ } \mu\text{g L}^{-1}$). Measurement frequency was set to 5 min.

Discharge (Q) measurements were obtained at 15 min intervals from gauging stations operated by the local authorities. For NATURE we used the gauging station at Zeitz which is run by the Federal State Authority for Flood Protection and Water Management of Saxony-Anhalt (Landesamt für Hochwasserschutz und Wasserwirtschaft Sachsen-Anhalt) and located 10 km upstream. For CHANNEL we used the gauge at Kleindalzig, situated in the first 300 m of the study reach. This station is managed by the Saxony Federal state authority (Sächsisches Landesamt für Umwelt). Discharge of the mining drainage into NATURE was obtained from the mining company MIBRAG, which provided 20 min time step records from the company run gauging station. The Elstermühlgraben has a constant discharge of $0.5 \text{ m}^3 \text{ s}^{-1}$, we assumed that its water chemistry did not differ from the main channel as it was fed from Weiße Elster water, subtracted 1 km further upstream.

Stream widths (w), depths (d) and bed slopes (s) were obtained from the hydraulic model DYNHYD (Wagenschein, 2006) which is based on 876 cross sectional profiles on a 70 km long stretch of the Weiße Elster during low flow conditions (calibrated Q ranging from 5.6 to $7.0 \text{ m}^3 \text{ s}^{-1}$), including both study reaches. The conductivity signal between upstream and downstream stations was used as a natural tracer

to estimate mean velocity (u) and mean travel time (τ). Mean travel time for NATURE was estimated as 7 hours, while mean travel time for CHANNEL was 3.5 hours.

We utilized a two-station method to calculate within-reach uptake of NO_3^- . Notably Heffernan and Cohen (2010) used a single station, however in their case the spring vent served as a constant upstream station. For rivers without a constant upstream boundary, a two station approach is critical for extracting the in-stream processing signal for non-gaseous solutes such as NO_3^- (Hensley and Cohen 2016). For each time step (t) we calculated the change in NO_3^- mass flux between upstream ($Q_{US_{t-\tau/2}}[\text{NO}_3^-]_{US_{t-\tau/2}}$) and downstream stations ($Q_{DS_{t+\tau/2}}[\text{NO}_3^-]_{DS_{t+\tau/2}}$). We also accounted for the lateral inflows $Q_L[\text{NO}_3^-]_L$, from the mine drainage and the Elstermühlgraben for NATURE and CHANNEL respectively. For the mine drainage we used NO_3^- concentrations from manual grab samples and discharge records provided by the mining company, while for the Elstermühlgraben we assumed equal concentrations as in the main stem and a constant discharge of $0.5 \text{ m}^3 \text{ s}^{-1}$. This net change in NO_3^- load was then divided by the benthic surface area ($w \times L$) of the reach to calculate total net uptake (U_T) in units of $\text{mg NO}_3\text{-N m}^{-2} \text{ d}^{-1}$.

$$U_{T_t} = \frac{Q_{DS_{t+\tau/2}}[\text{NO}_3^-]_{DS_{t+\tau/2}} - (Q_{US_{t-\tau/2}}[\text{NO}_3^-]_{US_{t-\tau/2}} + Q_L[\text{NO}_3^-]_L)}{w \times L} \quad (2.1)$$

We initially assumed that concentrations in the mine drainage stayed constant over each 4 days of the experiment. A derivation of $\pm 10 \%$ was projected on the data to analyze the uncertainty in net uptake (U_T) calculation deriving from variation in drainage water concentration. Though we consider groundwater inflow extremely unlikely because of the groundwater table relative to the stream bed, we similarly estimated the uncertainty in U_T arising from unaccounted inflows by generating an imaginary inflow equal to 5 % the discharge of the study reaches (the maximum we reasoned could be plausible over the reach length) and NO_3^- concentrations of zero (completely denitrified groundwater) and twice the observed concentration (waste water effluent). The effect of sensor accuracy on deduced U_T was also evaluated by adding 2 % to the actual NO_3^- signal at the downstream station.

Using the method of Heffernan and Cohen (2010) we then estimated the amount of U_T composed of autotrophic uptake (U_A) by interpolating a baseline across nighttime values of U_T . Increases in uptake during the day relative to this nighttime baseline were ascribed to U_A . This makes several critical assumptions: First, that nighttime U_T values reflect no U_A . Secondly, it assumes that other NO_3^- processing

pathways such as heterotrophic uptake of NO_3^- by bacteria and fungi, denitrification and nitrification vary linearly between nighttime values.

Net ecosystem production (NEP) was calculated for each time step from the DO profiles using a two station method similar to NO_3^- but also including a re-aeration term. Whereas the single station metabolism method integrates over a limited upstream area, roughly $3u/k$ where u is velocity (m s^{-1}) and k (s^{-1}) is the re-aeration coefficient (Chapra and Toro, 1991), the two station method integrates over the entire reach, making direct comparisons with non-gaseous NO_3^- processing more tenable (Hensley and Cohen, 2016).

$$NEP_t = \frac{Q_{DS,t+\tau/2}[DO]_{DS,t+\tau/2} - (Q_{US,t-\tau/2}[DO]_{US,t-\tau/2} + Q_L[DO]_L)}{w \times L} + k(DO)_{def,t} \times d \quad (2.2)$$

The value of k was estimated from the energy dissipation model (Bott, 2006; Zappa et al., 2007) and was $2.5 \times 10^{-5} \text{ s}^{-1}$ for NATURE and $2.13 \times 10^{-4} \text{ s}^{-1}$ for CHANNEL. The DO saturation deficit $(DO)_{def}$ for each time step was calculated as the mean difference between the saturation DO concentration based on measured water temperature and barometric pressure (German Weather Service), and observed DO over the reach over that time step (i.e. average of US at $t-\tau$ d^{-1} and DS at $t+\tau$ d^{-1}). We assumed no primary production occurred at night, and that nighttime NEP values were equal to ecosystem respiration (ER) (Bott, 2006). We then assumed that ER was constant over 24 hours, and ascribed daytime increases in NEP to gross primary production (GPP) in units of $\text{g-O}_2 \text{ m}^{-2} \text{ d}^{-1}$ (Roberts et al., 2007). We assumed that primary productivity (NPP) was equal to half of GPP (Hall and Tank, 2003; Hall and Beaulieu, 2013; Rode et al., 2016). We then estimated the assimilatory demand for nitrogen (D_A) by converting NPP to molar units, assuming 1 mol C fixed per mol O_2 produced, and multiplying by the ecosystem autotrophic C/N ratio. The molar C/N ratio used was 7 for July and 9 for September, corresponding to data collected during a previous study in the Weiße Elster middle reach (Junge et al., 2005). These estimates of D_A were then compared to U_A calculated from the observed NO_3^- signals. A U_A value substantially less than D_A demand may indicate that D_A is being partially met by an additional source of assimilatory N, potentially NH_4^+ , or that autotrophic uptake of NO_3^- from the water column is being partially compensated by NO_3^- production through nitrification (underestimating actual U_A).

2.3.3. LONGITUDINAL PROFILING

A third set of sensors was used for longitudinal profiling. The longitudinal profiling was conducted in an inflatable rubber dinghy, drifting with the thalweg water velocity with sensors suspended over the side, submersed 15-20 cm. Drifting took 3.5 h in NATURE and 2.25 h in CHANNEL. While Hensley (et al. 2014) showed that sampling speed can be critical to accurately estimating reaction kinetics (i.e. linear vs. exponential decline), estimates of reaction rates (overall profile slope) in reaches of this length are typically much less influenced by sampling speed (in this case $\sim 2u$) because biogeochemical processes generally vary over the time scale of hours. Measurement frequency was set to 5 min. Sensor clocks were synchronized with a handheld GPS unit, providing a location of each measurement. Rates of U_{T-prof} were calculated based on the slopes ($\delta C/\delta x$) of the longitudinal profiles (equation 2.3). For NATURE this was done over two sub-reaches separated by the mine drainage input, while in CHANNEL the profile was fit in its entirety. Similar to the time series analysis, a negative value (negative slope or decreasing concentration with downstream distance) indicates net removal.

$$U_{T-prof} = \frac{Q}{w} \frac{\partial C}{\partial x} \quad (2.3)$$

Statistical uncertainty in the line slope was used to estimate uncertainty in inferred uptake rate. During each deployment we performed both a day time profile and night time profile, typically on the second or third day of the deployment. Day profiles started at noon, night profiling 1 to 2 hours after sunset. By assuming that U_A is negligible at night, we estimated the magnitude of U_{A-prof} by subtracting night profile U_{T-prof} from day profile U_{T-prof} .

Additionally, manual samples for NO_3^- , nitrite (NO_2^-), NH_4^+ , total nitrogen (TN), soluble reactive phosphorus (SRP), total phosphorus (TP), and naturally abundant $\delta^{15}\text{N}_{\text{NO}_3}$ and $\delta^{18}\text{O}_{\text{NO}_3}$ isotopes were taken every 20-30 min. Grab samples for NO_3^- served as benchmark samples for calibration of the sensors as described above. Estimates of NH_4^+ uptake were derived using equation 2.3 for NATURE upstream of the mine drainage input and the entirety of CHANNEL. Downstream of the mine drainage in NATURE there were too few grab samples to fit a slope.

Nutrient species were analyzed in the analytical laboratory of the UFZ on a Segmented Flow Analyser Photometer (DR 5000, Hach Lange): $\text{NO}_3\text{-N}$ and $\text{NO}_2\text{-N}$ at 540 nm (precision of 0.042 mg L^{-1} and 0.012 mg L^{-1} respectively), $\text{NH}_4\text{-N}$ at 660 nm (precision 0.011 mg L^{-1}), SRP at 880 nm (precision 0.003 mg L^{-1}). TP was

oxidatively decomposed and then photometrically measured as SRP following colorant reaction. Stable isotopes of NO_3^- were determined using the denitrifier method at the Helmholtz-Centre Geesthacht (Sigman et al., 2001). Measurements were performed with an isotope ratio mass spectrometer (Delta V Advantage, Software Isodat 3.0, Thermo Scientific) with an analytical precision of $< 0.2 \text{ ‰}$ for $\delta^{15}\text{N}_{\text{NO}_3}$ and $< 0.5 \text{ ‰}$ for $\delta^{18}\text{O}_{\text{NO}_3}$. The isotopic ratios were calculated as delta notations, referring to the ratio of the heavier to the lighter isotopes.

2.4. RESULTS

2.4.1. TWO STATIONS TIME-SERIES

Average NO_3^- concentration during the deployments were 30 % lower in CHANNEL versus NATURE, due to the inflow of low NO_3^- mining water at the end of NATURE (**Table 2.2**).

TABLE 2.2. AVERAGE, MINIMAL (MIN) AND MAXIMAL (MAX) NO_3^- CONCENTRATION (IN $\text{mgNO}_3^- \cdot \text{N L}^{-1}$) IN THE STUDY REACHES AND THE MINE DRAINAGE INFLOW TO NATURE DURING THE TWO MEASUREMENT PHASES.

$\text{NO}_3^- \cdot \text{N}$	NATURE		CHANNEL		Mining water	
	July	September	July	September	July	September
average	2.76	2.84	2.05	2.18	0.047	0.054
min	2.58	2.59	1.81	1.95		
max	2.90	3.06	2.27	2.53		

After accounting for dilution, rates of net NO_3^- removal (U_T) inferred from the flux balance between station signals (**Figure 2.2**) were nearly always negative in NATURE, indicating net removal. In CHANNEL, U_T was frequently positive portending net enrichment (**Table 2.3**).

TABLE 2.3. SUMMARY OF TIME-SERIES MEANS. U_T -NET NO_3^- UPTAKE (NEGATIVE VALUES) OR RELEASE (POSITIVE VALUES), U_{Tday} -AVERAGED DAYTIME NO_3^- UPTAKE/RELEASE, U_{Tnight} -AVERAGED NIGHTTIME NO_3^- UPTAKE/RELEASE, GPP-GROSS PRIMARY PRODUCTION, ER-ECOSYSTEM RESPIRATION, P/R-RATIO OF GPP TO ER, D_A -ASSIMILATORY N DEMAND INFERRED FROM NPP AND C/N.

Parameter	NATURE		CHANNEL		Units
	July	September	July	September	
U_T	-331	-147	-193	-47	$\text{mgNO}_3^- \cdot \text{N m}^{-2} \text{d}^{-1}$
U_{Tday}	-346	-325	-188	-125	$\text{mgNO}_3^- \cdot \text{N m}^{-2} \text{d}^{-1}$
U_{Tnight}	-316	-27	-197	+4	$\text{mgNO}_3^- \cdot \text{N m}^{-2} \text{d}^{-1}$
GPP	1.7	1.6	12.8	7.2	$\text{gO}_2 \text{ m}^{-2} \text{d}^{-1}$
ER	-1.7	-2.1	-8.5	-5.2	$\text{gO}_2 \text{ m}^{-2} \text{d}^{-1}$
P/R	1.0	0.8	1.6	1.4	
C/N	7	9	7	9	mol-C/mol-N
D_A	-51	-39	-399	-176	$\text{mg-N m}^{-2} \text{d}^{-1}$

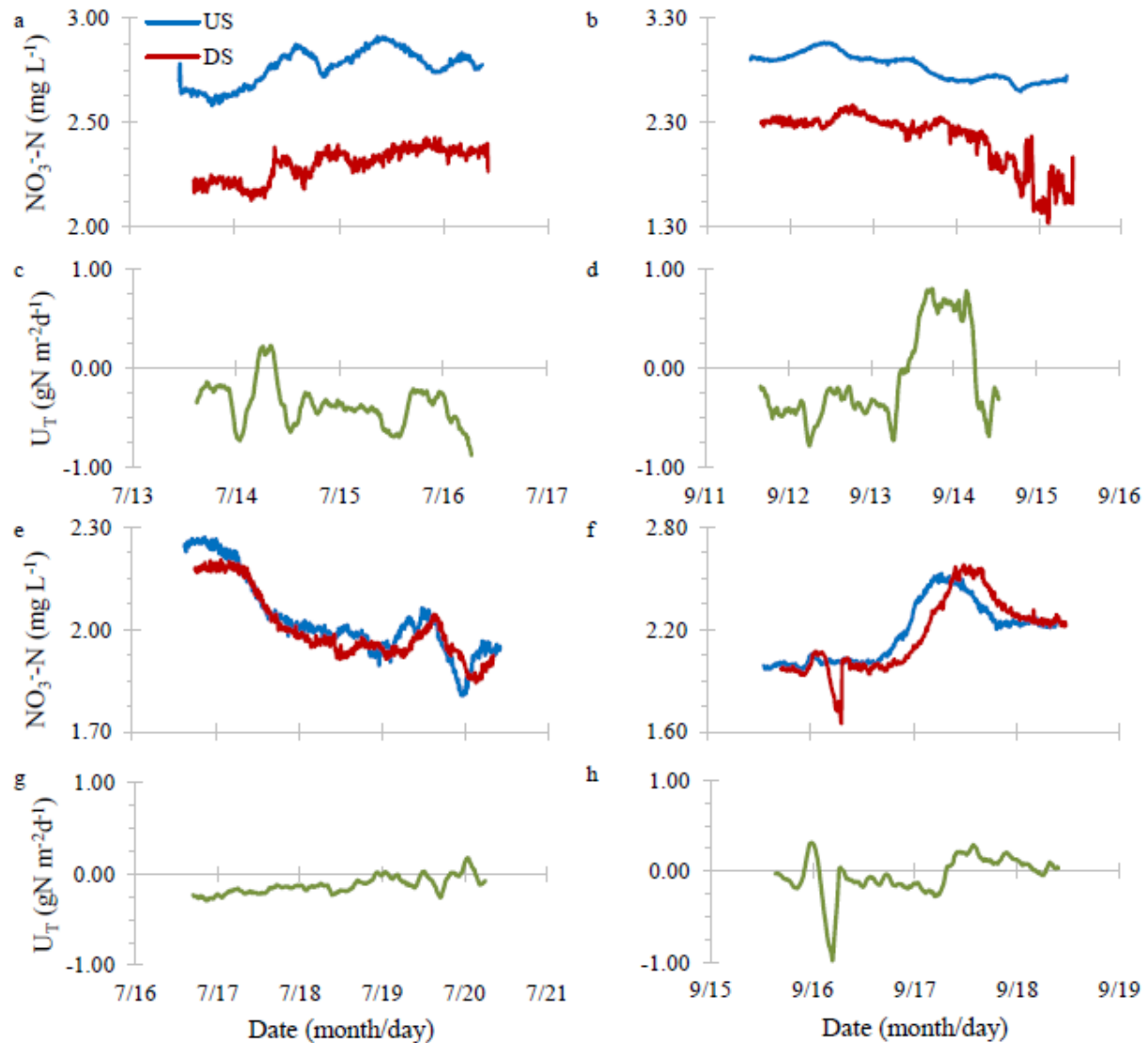


FIGURE 2.2. TIME SERIES NO_3^- PROFILES FOR NATURE (a AND b) AND CHANNEL (e AND f) DURING THE MEASUREMENTS IN JULY (LEFT) AND SEPTEMBER (RIGHT) 2015. THE DECLINE IN NO_3^- IN NATURE IS LARGELY THE RESULT OF DILUTION BY THE INFLOW OF LOW NO_3^- MINE DRAINAGE WATER. U_T IN NATURE (c AND d) TYPICALLY EXHIBITS MORE NEGATIVE VALUES, INDICATIVE OF GREATER NET REMOVAL RELATIVE TO CHANNEL (g AND h).

The resulting uncertainty in U_T arising from potential 10 % variation in NO_3^- concentration of lateral inflows was only 1 % in July and 0.4 % in September. A 5 % unaccounted inflow with twice the NO_3^- than the stream could result in a very large (3 to 10 times) underestimation of real U_T . However, we find this scenario extremely unlikely for two reasons: First, such a high concentration would likely only be found in point-source sewage. Second, the groundwater gradient is out of the stream, and in the case of CHANNEL

the stream bed is completely impervious. Errors related to 2 % instrumental derivation of sensors were 35 % of U_T in NATURE and 400 % of U_T in CHANNEL. This difference arises from the lower actual values in CHANNEL.

The upstream station of CHANNEL consistently exhibited a diel signal in the raw NO_3^- signal, suggesting assimilatory processing further upstream. Using a two-station approach within the study reaches themselves, however we did not observe a coherent diel U_T signal during any of the deployments. Without true nighttime minima to interpolate across, the Heffernan and Cohen (2010) method could not be used to make justifiable estimates of U_A . Instead we compared averaged U_T values for daytime versus nighttime measurement intervals (**Figure 2.3**). Despite the autocorrelation inherent to time-series data and the high variance in these averaged values, general trends are clearly visible: In NATURE average uptake during day was only slightly (9 %) higher than during night in July, whereas in September average uptake was 12 times higher at day than at night. In CHANNEL, U_T during night was even slightly (4 %) higher than during day in July, in September nighttime uptake was negative, signifying net NO_3^- production.

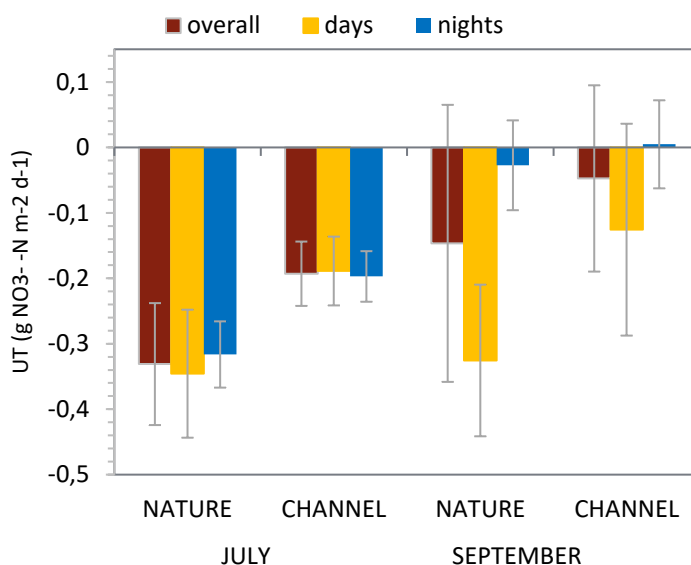


FIGURE 2.3. AVERAGE NO_3^- UPTAKE (NEGATIVE VALUES) OR RELEASE (POSITIVE VALUES) RATES U_T FOR THE TWO SAMPLING PERIODS, AND DIFFERENTIATED BETWEEN DAY AND NIGHT HOURS FOR BOTH REACHES IN JULY (LEFT) AND SEPTEMBER (RIGHT), ERROR BARS INDICATE STANDARD DEVIATION BETWEEN AVERAGED 15 MIN-INTERVAL MEASUREMENTS.

In both study reaches, we observed strong diel signals in DO at both the upstream and downstream stations in July and September (**Figure 2.4, Appendix 2A**). Calculated rates of GPP were generally an order of magnitude larger in CHANNEL versus NATURE, consistent with greater solar forcing due to less riparian shading. We note in NATURE P/R was close to 1 or even slightly net heterotrophic ($GPP < ER$), while CHANNEL was consistently net autotrophic ($GPP > ER$) (**Table 2.3**). A lower P/R is consistent with greater shading and more allochthonous carbon from litterfall in NATURE. With higher rates of GPP, inferred rates of D_A were also higher in CHANNEL (**Table 2.3**).

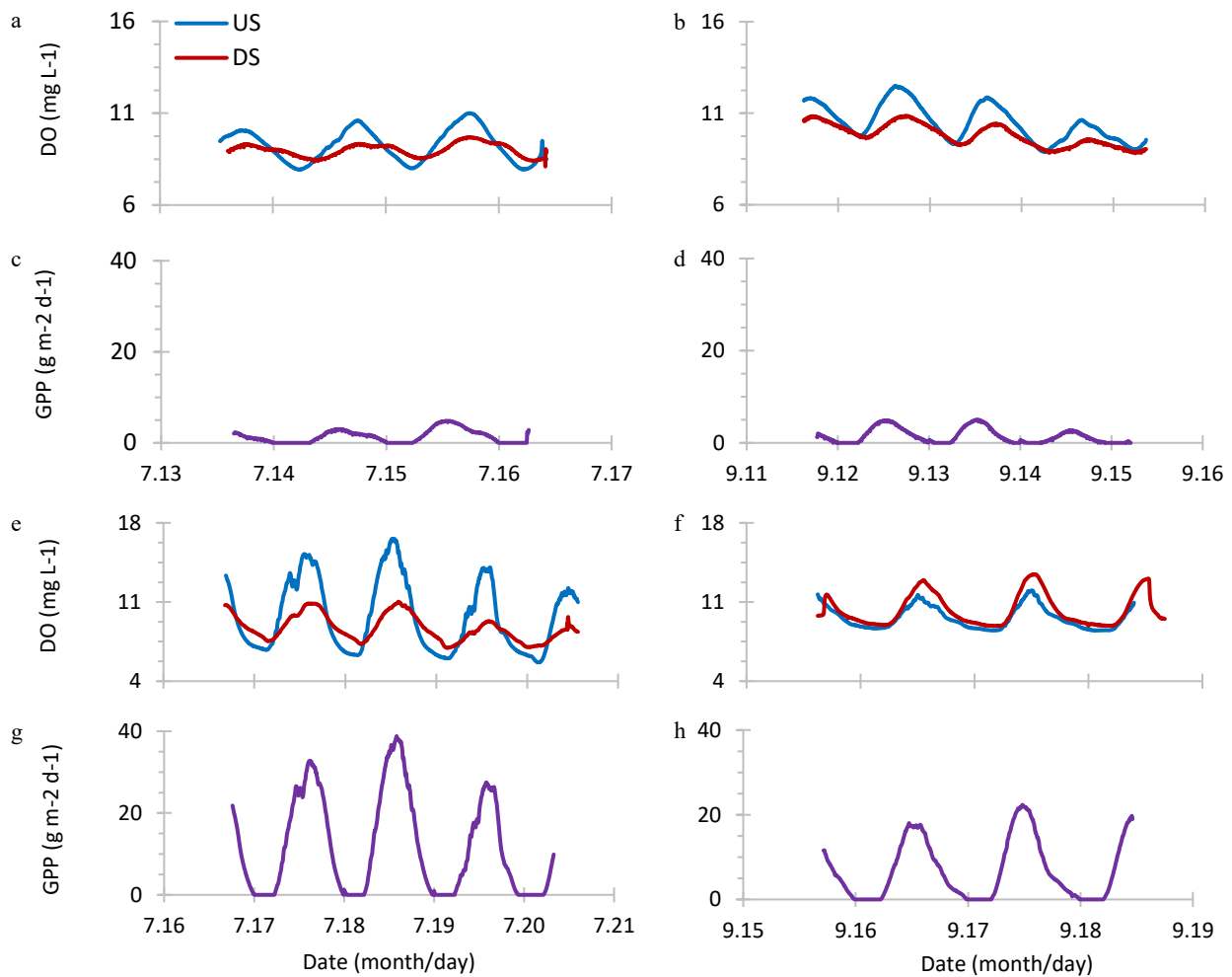


FIGURE 2.4 TIME SERIES DO PROFILES FOR NATURE (a AND b) AND CHANNEL (e AND f) DURING THE MEASUREMENTS IN JULY (LEFT) AND SEPTEMBER (RIGHT). GPP IN NATURE (c AND d) IS ROUGHLY AN ORDER OF MAGNITUDE LOWER THAN IN CHANNEL (g AND h), CONSISTENT WITH THE DEGREE OF RIPARIAN SHADING.

2.4.2. LONGITUDINAL PROFILING

Longitudinal profiling revealed several consistent spatial trends. Most apparent was the dilution effect of the mining water inflow in NATURE (**Figure 2.5**). The majority of the July daytime and both September profiles in the NATURE reach exhibited longitudinal declines, indicating net NO_3^- removal. The July nighttime profile from NATURE and all profiles from CHANNEL slope upward, indicating net NO_3^- production. In most cases, slopes of daytime profiles (**Table 2.4**) were steeper than nighttime profile slopes, suggesting greater removal (or less enrichment) during the day. The slopes of all profiles were relatively uniform, showing a lack of local NO_3^- processing hotspots with one major exception: In NATURE profiles generally sloped downward along the entire reach length (net removal), until the mine drainage, at which point the slopes flipped to sloping upward (net production).

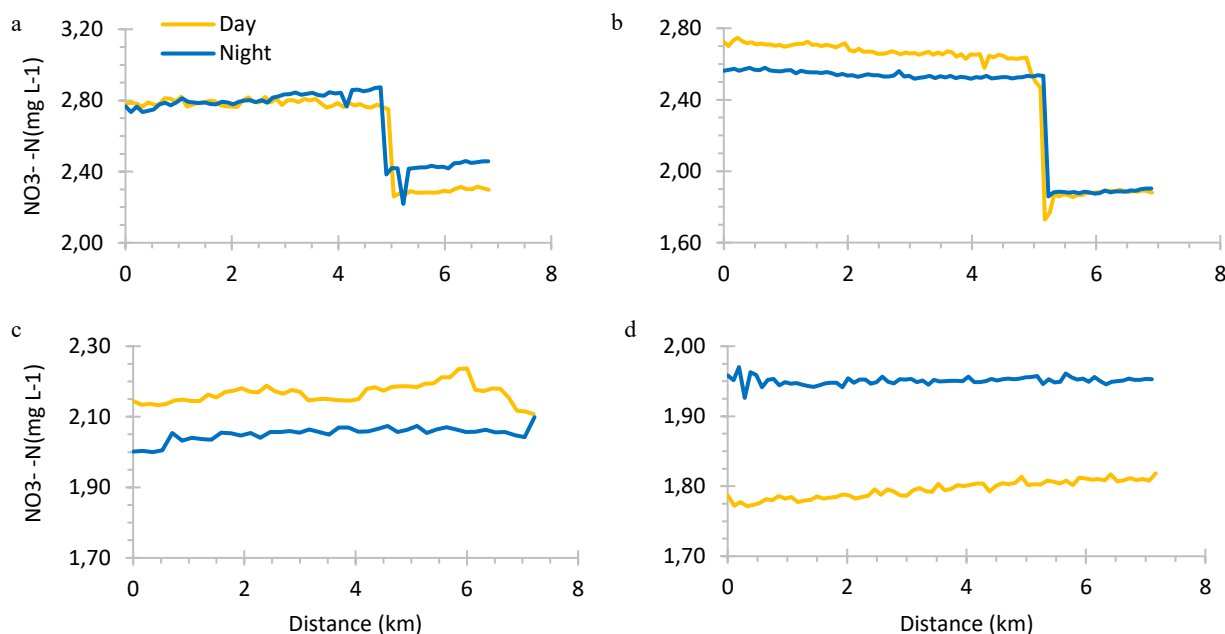


FIGURE 2.5. LONGITUDINAL NO_3^- -N PROFILES FOR NATURE (TOP) AND CHANNEL (BOTTOM) IN JULY (LEFT) AND SEPTEMBER (RIGHT). NOTE THE LARGE DILUTION EFFECT OF LOW NO_3^- MINE DRAINAGE ENTERING NATURE AT 5.2 KM.

TABLE 2.4 SUMMARY OF TOTAL (U_T) AND ASSIMILATORY (U_A) UPTAKE (NEGATIVE VALUES) OR RELEASE (POSITIVE VALUES) INFERRED FROM NO_3^- LONGITUDINAL PROFILING.

	Slope ($\text{mg L}^{-1} \text{m}^{-1}$)	U_T ($\text{mg-N m}^{-2} \text{d}^{-1}$)	U_A ($\text{mg-N m}^{-2} \text{d}^{-1}$)
NATURE			
Upstream of mine drainage inflow			
7/14 Day	$-3.17 \pm 1.74 \times 10^{-6}$	-102 ± 56	-825
7/14 Night	$2.25 \pm 0.19 \times 10^{-5}$	723 ± 60	
9/14 Day	$-2.09 \pm 0.12 \times 10^{-5}$	-533 ± 30	-275
9/14 Night	$-1.01 \pm 0.08 \times 10^{-5}$	-258 ± 19	
Downstream of mine drainage inflow			
7/14 Day	$2.39 \pm 0.35 \times 10^{-5}$	735 ± 111	
7/14 Night	$3.09 \pm 0.41 \times 10^{-5}$	995 ± 133	-260
9/14 Day	$2.84 \pm 0.33 \times 10^{-5}$	467 ± 84	
9/14 Night	$-1.22 \pm 0.26 \times 10^{-5}$	311 ± 66	157
CHANNEL			
7/17 Day	$4.06 \pm 1.77 \times 10^{-6}$	104 ± 45	-56
7/17 Night	$6.23 \pm 1.09 \times 10^{-6}$	159 ± 28	
9/16 Day	$5.44 \pm 0.24 \times 10^{-6}$	139 ± 6	126
9/16 Night	$5.13 \pm 3.09 \times 10^{-7}$	13 ± 8	

The inflow of mining water with 4 to 5 times higher NH_4^+ concentrations than the main stem at the end of NATURE constitutes a major point source of NH_4^+ so that at this point the change in water chemistry may cause a shift in the balance of processing pathways. Overall, NH_4^+ exhibited longitudinal declines in both NATURE and CHANNEL. We believe that this decline was predominantly the result of assimilatory uptake and nitrification rather than volatilization, as the pH never rises above 9 ($\text{pK}_a = 9.24$). NH_4^+ was mainly higher at night than during the day (**Figure 2.6**).

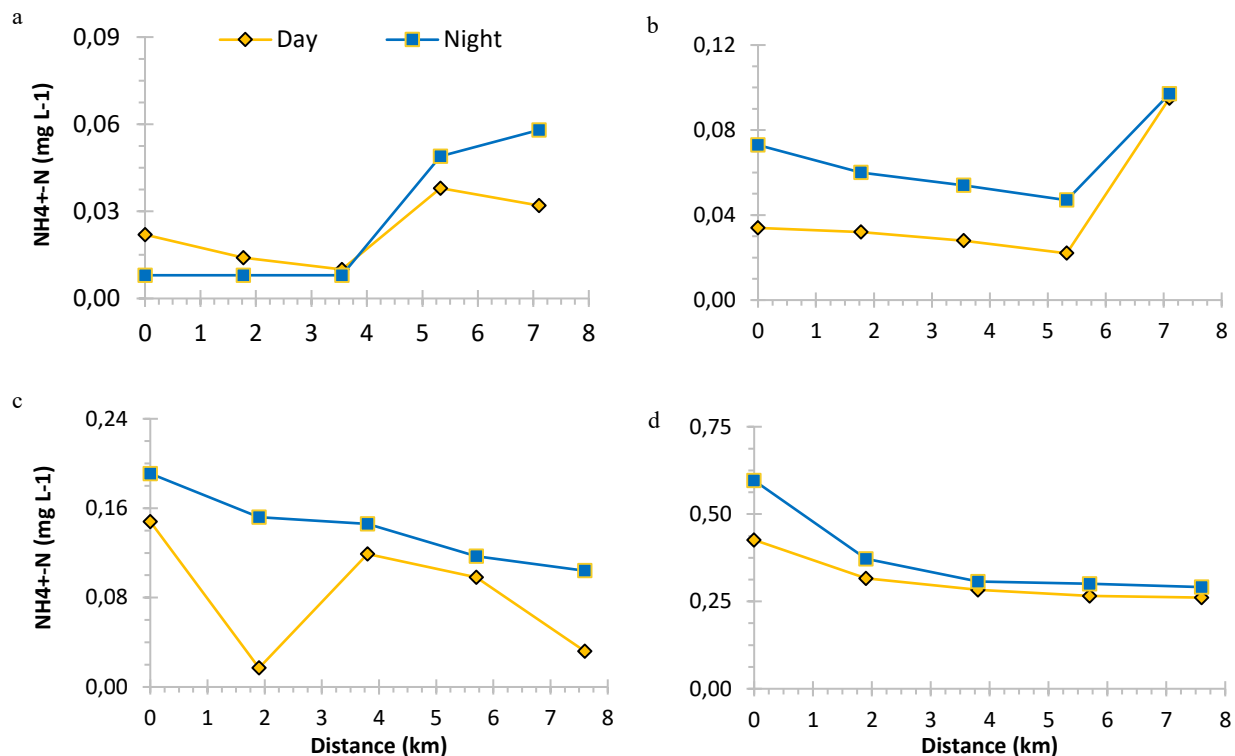


FIGURE 2.6. LONGITUDINAL NH_4^+ PROFILES FOR NATURE (TOP) AND CHANNEL (BOTTOM) IN JULY (LEFT) AND SEPTEMBER (RIGHT). WHILE DILUTING NO_3^- CONCENTRATIONS, THE MINE DRAINAGE ENTERING NATURE AT 5.2 KM IS A MAJOR POINT SOURCE OF NH_4^+ .

Additional to the effect of the mine drainage inflow, concentrations of NH_4^+ become even more elevated in the downstream CHANNEL versus NATURE due to the WWTP situated between the two study reaches so that initial NH_4^+ concentrations are nearly an order of magnitude larger in CHANNEL. NH_4^+ uptake was greater in CHANNEL than in NATURE, day and night behaved conversely in September than July (**Table 2.5**). NO_2^- decreased with downstream distance in NATURE by 70 % (range 0.012 to 0.007 mg NO_2^- -N L⁻¹) in July and by 44 % (range 0.013 to 0.009 mg NO_2^- -N L⁻¹) in September. In contrast, NO_2^- in CHANNEL increased with distance, by 80 % (range 0.010 to 0.017 mg NO_2^- -N L⁻¹) in July and by 60 % (range 0.011 to 0.018 mg NO_2^- -N L⁻¹) in September. Day and night dynamics were very similar.

TABLE 2.5. SUMMARY OF NH_4^+ UPTAKE (NEGATIVE VALUES) AND RELEASE (POSITIVE VALUES) DERIVED FROM MANUAL SAMPLES TAKEN DURING THE LONGITUDINAL PROFILINGS

	Slope ($\text{mg L}^{-1}\text{m}^{-1}$)	$U_{T-\text{NH}_4}$ ($\text{mg-N m}^{-2}\text{d}^{-1}$)	$U_{A-\text{NH}_4}$ ($\text{mg-N m}^{-2}\text{d}^{-1}$)
NATURE			
Upstream of mine drainage inflow			
7/14 Day	$-3.43 \pm 0.66 \times 10^{-6}$	-110 ± 21	-110
7/14 Night	0.00	0	
9/14 Day	$-2.29 \pm 0.36 \times 10^{-6}$	-58 ± 9	64
9/14 Night	$-4.80 \pm 0.63 \times 10^{-6}$	-122 ± 16	
CHANNEL			
7/17 Day	$-1.52 \pm 0.45 \times 10^{-6}$	-389 ± 114	-84
7/17 Night	$-1.19 \pm 0.15 \times 10^{-5}$	-305 ± 39	
9/16 Day	$-2.13 \pm 0.70 \times 10^{-5}$	-545 ± 178	450
9/16 Night	$-3.89 \pm 1.46 \times 10^{-5}$	-995 ± 373	

Discrete longitudinal sampling did not reveal any longitudinal trends in TP, which in both reaches averaged 0.15 mg L^{-1} in July and 0.12 mg L^{-1} in September (**Appendix 2B**).

All deltas in NO_3^- isotopes were smaller than the range of instrumental accuracy (0.2 ‰), so that reliable conclusions could not be made based on these measurements. Still, different trends between the two reaches and between July and September were visible. In July $\delta^{15}\text{N}$ values of NO_3^- were heavier during day in NATURE and indicated a trend of a slight enrichment downstream. In contrast to July, isotope measurements from September showed a relative depletion of $\delta^{15}\text{N}_{\text{NO}_3}$ at day compared to night, with a higher concentration of NO_3^- . In CHANNEL, lighter values of $\delta^{15}\text{N}_{\text{NO}_3}$ were measured in July during day compared to night sampling, whereas in September no differences could be estimated in $\delta^{15}\text{N}_{\text{NO}_3}$. Isotopic fractionation was only detected during day time profiling (**Appendix 2B**).

2.5. DISCUSSION

2.5.1. DIEL VARIATION

While average values of U_T were generally greater during the day (and longitudinal profiles also suggested more removal during the day), diel variation in U_T was not nearly as coherent as in the systems where this method has been used before (Heffernan and Cohen, 2010; Rode et al., 2016). In NATURE in September, a consistent pattern seemed to appear, however the timing was asynchronous with solar forcing and the

GPP signal, with maximum apparent uptake occurring around 6:00 am rather than the expected 12:00 am. Furthermore, there were no consistent nighttime minima in any of the data sets and therefore no clear baseline for partitioning pathways. In NATURE, assumedly due to heavy shading, GPP was low indicating that assimilatory demand may have been small enough that U_A induced variation in NO_3^- was beyond the sampling precision of the sensors. However, this is not likely. Also the effect of uncertainty introduced by measurement accuracy of the temporally varying influx from the lateral inflows on NO_3^- uptake was below 1 % in NATURE as well as in CHANNEL. We suggest that the lack of a diel NO_3^- uptake signal in both reaches was occurring for two reasons: First, the autotrophic demand for N might have been met by a different N species. CHANNEL in particular was enriched in NH_4^+ , which energetically represents a preferential source of metabolic N (Marti and Sabater, 1996; Stanley and Hobbie, 1981). Secondly, NO_3^- uptake might have been confounded by temporally varying production of NO_3^- through nitrification. Rates of nitrification are generally expected to be greater during day because of its dependence on temperature, pH and oxygen saturation (Bernot and Dodds, 2005; Christensen and Sorensen, 1986; Laursen and Seitzinger, 2004; Strauss et al., 2004). Applying the equations derived by Warwick (1986) to the maximal diurnal variation in pH and temperature observed in CHANNEL, these factors might explain a change of 20 % (September) to 30 % (July) in stream nitrification rates, with higher values occurring under higher temperatures and higher pH during day. The modified Arrhenius equation suggested by Shammass (1986) for calculating temperature dependent nitrification rates yields slightly lower (10 to 20 %) variation in nitrification related to temperature. However, the same studies found that an increase of NH_4^+ concentration by 5 % already resulted in 40 % higher nitrification rates. In the Weiße Elster NH_4^+ concentrations were lower during the day than during the night during all longitudinal profilings. The correlation provided by Zhu and Chen (2002) indicates that NH_4^+ concentrations could have caused 50 % higher nitrification rates during night in September and 20 % higher in July. Differences in NH_4^+ concentration between September and July could thereafter result in 4times higher nitrification rates in September than July. Accordingly, the slopes in NH_4^+ during the profile suggest that NH_4^+ uptake was greater during night than during day and that it was overall higher in September than July. Conclusively, NH_4^+ uptake rates in the Weiße Elster were determined more by input concentration than by temperature or pH. Assuming that all the decline of NH_4^+ was due to nitrification (which is unlikely, but allows estimating dimensions) instream NO_3^- production could certainly have counterbalanced denitrification in CHANNEL. This implies, that assuming constant dissimilatory uptake rates might be problematic and that inferring U_A from NO_3^- alone likely underestimates actual uptake of N in reaches like CHANNEL which are enriched in NH_4^+ . The importance of considering the effects of NH_4^+ on inferred

processing rates is further illustrated by the longitudinal profiles of NATURE. In most cases, the majority of the NO_3^- profiles sloped downward indicating net removal. The inflow of the mine drainage in the last 1.5 km changed the water chemistry dramatically, diluting the NO_3^- concentration by about 20 %, but enriching the NH_4^+ concentration by a factor of 2-3. The NO_3^- profiles instantaneously switched to sloping upwards, suggesting the balance of N processing pathways has shifted to net production of NO_3^- . This supports the assumption that NH_4^+ concentration dominantly influenced nitrification rates.

There were several assumptions inherent to the Heffernan and Cohen (2010) method for estimating U_A , which may make it unsuitable in the Weiße Elster and other systems. For one, the method assumes that nighttime maxima reflect no assimilatory uptake, an assumption which is likely for CHANNEL ($\tau = 3.5$ h), however not in NATURE ($\tau = 7$ h) in July, where darkness at this latitude lasts for only 8 h. Second, the method assumes non-assimilatory pathways (heterotrophic uptake, nitrification and denitrification) are constant, or vary linearly between observed nighttime values. As discussed in detail above, we strongly suggest that this might not be the case. Particularly if NH_4^+ nitrification rates follow NH_4^+ concentration which vary on an irregular basis due to WWTP operation, this assumption might not be tenable. Distinguishing averaged night- and daytime intervals of U_T as presented in the present study can be used to detect general trends in net NO_3^- removal. Still, it likewise failed to distinguish denitrification from nitrification for the same reasons mentioned above: time varying NH_4^+ transformation to NO_3^- could not be quantified and stoichiometric calculations did not allow distinguishing assimilatory NO_3^- uptake from assimilatory NH_4^+ uptake.

2.5.2. COMPARISON BETWEEN SAMPLING PERIODS

We recognize that it is speculative to draw broad seasonal inferences from our limited data set. However, time-series and longitudinal profiling both indicate higher net NO_3^- retention in July versus September for both study reaches. We assume that differences between the two periods would mainly relate to irradiation and temperature. In addition to sunlight driven variation in assimilatory uptake, other processing pathways which positively (denitrification) or negatively (nitrification) contribute to net uptake vary in response to temperature (Heffernan and Cohen, 2010; Gücker and Pusch, 2006; Pribyl et al., 2005) Wagenschein and Rode (2008) concluded from their model assessment of NO_3^- uptake in the Weiße Elster that denitrification exceeded assimilation by more than three times during summer, whereas in autumn the amount of uptake allocated to denitrification declined to less than half of total uptake. Similarly, we measured in July in both reaches only slightly lower uptake during night than during day. In September

night time uptake declined to one tenths of the July rates in NATURE and was not measurable anymore in CHANNEL. Likewise, inferred demand for assimilatory N decreased from July to September as a result of decreased GPP and increased C/N. However, this decline was not as large as the measured decrease in net uptake. As a result, in both reaches D_A represented a larger fraction of U_T in September than in July. While we cannot exclude that in CHANNEL this effect might have been induced by higher nitrification rates in September, it must have been attributed to changes in denitrification in NATURE, because NH_4^+ was of minor importance there. For NATURE this shift between prevalent uptake processes can also be seen in the different $\delta^{15}N_{NO_3}$ pattern in July versus September. In July, isotopic data indicated a predomination of NO_3^- uptake, presumably due to assimilation, whereas in September NO_3^- production seems to be more dominant during day time. Contrary in CHANNEL, in July NO_3^- uptake was of minor importance and a predomination of nitrification could be estimated during the day. In September, no differences could be measured in $\delta^{15}N_{NO_3}$ between day and night making an interpretation of microbial processes based on isotopes impossible.

2.5.3. INTER-REACH COMPARISON

Net removal of NO_3^- was substantially (on average 1.8 times in July and 3 times in September) higher in NATURE than in CHANNEL. In fact, at many times during the deployments CHANNEL was a net source of NO_3^- , suggesting nitrification exceeded denitrification and assimilation.

The higher net uptake rates measured in NATURE can be attributed to several factors: First, assuming denitrification follows efficiency-loss kinetics (Mulholland et al., 2009), higher concentrations in NATURE would have caused slightly higher denitrification rates. Alternatively, the second reactant in the denitrification equation, labile carbon, might also have influenced denitrification rates (Heffernan and Cohen, 2010). The lack of allochthonous carbon due to the absence of riparian vegetation, likely contributed to low denitrification rates in CHANNEL (Cooke and White, 1987; Knowles, 1982). Thirdly, the bulk part of denitrification is generally attributed to microbial activity in the hyporheic zone and thus dependent on advective solute exchange between surface and subsurface water (Alexander et al., 2009; Fellows et al., 2001; Harvey et al., 2013). In CHANNEL the sealing of river bed and walls with concrete restricted water exchange with the subsurface in CHANNEL and reduced denitrification. Previous model based studies estimated that denitrification in a natural reach of the Weiße Elster was 1.2 (Birgand et al., 2007) to 2.4 (Rode et al., 2008) times higher than in a channelized one, due to those factors.

However, even though river morphology may contribute to the differences between the reaches, results suggest that NH_4^+ concentrations may be the determinant factor for the reaches examined here. NH_4^+ was much higher in CHANNEL than in NATURE and NH_4^+ mass balances from the profiling suggest that nitrification rates might have counterbalanced NO_3^- removal through denitrification, resulting in lower net removal of NO_3^- in this reach. Additionally, the higher dissolved oxygen and temperature observed in CHANNEL may also result in greater nitrification. It is worth noting that net uptake of total N (sum of NO_3^- and NH_4^+ uptake) was much more similar across the reaches. Based on the longitudinal profiles, net uptake of NO_3^- in CHANNEL was lower (**Table 2.4**), but uptake of NH_4^+ was higher (**Table 2.5**). In conclusion, it was not possible to definitively determine whether the inter-reach differences are related to morphology or water chemistry.

2.5.4. COMPARISON OF THE WEIßE ELSTER AND OTHER RIVERS

Net NO_3^- uptake rates measured in the Weiße Elster (mean 239 and 120 $\text{mg-N m}^{-2} \text{ d}^{-1}$ in NATURE and CHANNEL respectively) are comparable to those measured in other studies in rivers of similar size. For example, Hensley et al. (2014) reported a median of 292 $\text{mg-N m}^{-2} \text{ d}^{-1}$ across 6 subtropical streams of similar discharge, and Ensign and Doyle (2006) found a mean uptake rate of 249 $\text{mg-N m}^{-2} \text{ d}^{-1}$ across 14 4th order streams. The two reaches fall neatly along the regression between NO_3^- concentration and uptake rate for the 72 streams in the LINXII dataset (Hall et al., 2009). For NH_4^+ , Ensign and Doyle (2006) report a mean uptake rate of only 28 $\text{mg-N m}^{-2} \text{ d}^{-1}$ for 4th order streams, but 59 $\text{mg-N m}^{-2} \text{ d}^{-1}$ across all stream orders. This is comparable to rates estimated for NATURE but substantially lower than CHANNEL (mean 73 and 559 $\text{mg-N m}^{-2} \text{ d}^{-1}$ respectively). This may be because CHANNEL is heavily enriched in NH_4^+ . As uptake is influenced by a multitude of factors, for instance NO_3^- and NH_4^+ concentration, primary production, other water chemistry parameters and river morphology, direct comparison across systems is quite difficult. Additionally, different studies utilize distinct measures (e.g. gross versus net uptake) and methods which account for these factors to varying degrees. Thus, the methodology used would also determine the comparability of results to a certain degree, highlighting the need for the establishment of a universally applicable methodology.

2.6. CONCLUSIONS

Methodologies based on high-frequency data from sensors have great potentials to deliver the long needed quantitative data for instream nutrient cycling studies. The presented work is the first application of this methodology in higher order streams with complex input signals, tributaries and anthropogenically impacted water chemistry. The intricacy of natural systems, in this case primarily the composition of the N pool and the presence of inflowing tributaries pose mayor challenges which we assessed in our study. Uncertainty analysis showed that unaccounted inflows can bias the inferred results. While the longitudinal profiling is effective in identifying point sources, it is less suitable for detecting diffuse inflows from groundwater seepage. Due to the special conditions in the Weiße Elster basin related to the mining activity in the region (artificially lowered groundwater table and routine control of groundwater flow by the mining company and an impervious channel bottom in CHANNEL), we could confidently exclude groundwater inflow in this study. When transferring the concept to other systems however, groundwater might be a challenge to the method.

The lack of contemporary high frequency measurements of NH_4^+ and the resulting failure to quantify nitrification, presents the greatest uncertainty on NO_3^- and total N processing rates in this and likely other future studies. Potential nitrification rates derived from NH_4^+ mass balances during the longitudinal profiling deliver rough dimensions of potential nitrification rates in the reaches. They are limited in number (though statistical uncertainty in profile slope in most cases is relatively small) and only represent conditions at the specific sampling time. To our knowledge no portable and fully automated NH_4^+ sensor currently exists. In the future collection of contemporaneous, high-frequency time series of other N species, specifically NH_4^+ but potentially also NO_2^- , and organic nitrogen will substantially improve the ability to quantify the multitude of N cycling pathways.

We did not observe the strong diel variation in assimilatory uptake observed in many other sites, which we believe was due to the overlapping of several time varying processes, some of them natural (temperature related oscillation in denitrification and nitrification) but others potentially artificial and irregular (variation in WWTP inflow). Commonly used assumptions on constancy in dissimilatory processes proved to be invalid under the conditions examined in this study. Thus, the Heffernan and Cohen (2010) method of partitioning N-removal processes based on diurnal variations in NO_3^- concentration, fail in systems where non-assimilatory processes are time varying, or where other N species are of importance.

Our results highlight some of the important controls of temporal and spatial variation in processing rates. Temporally, sunlight and temperature can influence N processing rates over diel as well as seasonal time scales. We only assessed short periods, with the primary goal to pave the way for the future use of high frequency measurements in instream nutrient studies or monitoring. However, continuous application of the presented method will finally allow to quantitatively describe seasonal patterns in NO_3^- cycling and at best to identify hot moments in in-stream N retention.

Despite the limitations, the current study extends previous techniques to higher order fluvial systems with anthropogenically elevated N-level, substantial tributaries, complex input signals, and multiple species of N. We believe that the permanent implementation of automated sensors will become more common in the coming years. The long term observation of variation in nutrient behavior will provide valuable insights into in-stream nutrient dynamics of all rivers. A better understanding of the drivers of variability will allow for better predictions of system dynamics under future conditions such as increased loading, channel modification and climate change. This knowledge will permit better long term management strategies for mitigating the effects of anthropogenic N loading and cultural eutrophication of aquatic ecosystems.

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3. QUANTIFYING NUTRIENT FLUXES WITH A NEW HYPORHEIC PASSIVE FLUX METER (HPFM)

3.1. ABSTRACT

The hyporheic zone is a hotspot of biogeochemical turnover and nutrient removal in running waters. However, nutrient fluxes through the hyporheic zone are highly variable in time and locally heterogeneous. Resulting from the lack of adequate methodologies to obtain representative long-term measurements, our quantitative knowledge on transport and turnover in this important transition zone is still limited.

In groundwater systems, passive flux meters, devices which simultaneously detect horizontal water and solute flow through a screen well in the subsurface, are valuable tools for measuring fluxes of target solutes and water through those ecosystems. Their functioning is based on accumulation of target substances on a sorbent and concurrent displacement of a resident tracer which is previously loaded on the sorbent.

Here, we evaluate the applicability of this methodology for investigating water and nutrient fluxes in hyporheic zones. Based on laboratory experiments we developed hyporheic passive flux meters (HPFM) with a length of 50 cm which were separated in 5-7 segments allowing for vertical resolution of horizontal nutrient and water transport. The HPFM were tested in a 7-day field campaign including simultaneous measurements of oxygen and temperature profiles and manual sampling of pore water. The results highlighted the advantages of the novel method: With HPFM cumulative values for the average N and P flux during the complete deployment time could be captured. Thereby the two major deficits of existing methods are overcome: First, flux rates are measured within one device instead of being calculated from separate measurements of water flow and pore-water concentrations. Second, time integrated measurements are insensitive to short term fluctuations and therefore deliver more representable values for overall hyporheic nutrient fluxes at the sampling site than snapshots from grab sampling. A remaining limitation to the HPFM is the potential susceptibility to biofilm growth on the resin, an issue which was not considered in previous passive flux meter applications. Potential techniques to inhibit biofouling are discussed based on the results of the presented work. Finally, we exemplarily demonstrate how HPFM measurements can be used to explore hyporheic nutrient dynamics, specifically nitrate uptake rates,

based on the measurements from our field test. Being low in costs and labor effective, many flux meters can be installed in order to capture larger areas of river beds. This novel technique has therefore the potential to deliver quantitative data which is required to answer unsolved questions about transport and turnover of nutrients in hyporheic zones.

3.2. INTRODUCTION

Rivers export high loads of nitrogen from inland catchments to the marine environment. The ecological and economic problems caused by eutrophication of coastal and riverine ecosystems have been recognized years ago (Patsch and Radach, 1997; Artioli et al., 2008; Skogen et al., 2014). Decades of nutrient studies have unveiled, that rivers cycle rather than only transport nutrients (Garcia-Ruiz et al., 1998a; Seitzinger et al., 2002; Galloway et al., 2003). In agriculturally dominated areas, in-stream processes may for example retain up to 38 % of nitrate (NO_3^-) and 48 % of soluble reactive phosphate (SRP) inputs (Mortensen et al. 2016). The hyporheic zone, the subsurface region of streams and rivers that exchanges water, solutes and particles with the surface (Valett et al., 1993) and may mix stream-water during the transport through the sediments with underlying groundwater (Triska et al., 1989; Fleckenstein et al., 2010; Trauth et al., 2014), is one key compartment for instream nutrient cycling (Fischer et al., 2005; Zarnetske et al., 2011b; Basu et al., 2011; Stewart et al., 2011). For instance, denitrification, the anaerobic reduction of NO_3^- to gaseous N_2 and in most river systems the dominant dissimilatory process which removes N out of the system (Laursen and Seitzinger, 2002; Bernot and Dodds, 2005; Lansdown et al., 2012; Kunz et al., 2017), often exclusively happens at “reactive sites” in the hyporheic zone (Duff and Triska, 1990; Rode et al., 2015). In addition to biological nutrient uptake, intermediate physical storage in the hyporheic zone disperses the propagation of pollutant and nutrient spikes which could be harmful for receiving water bodies (Runkel, 2007; Brookshire et al., 2009; Covino et al., 2010; Findlay et al., 2011). For those reasons, it is of interest to quantify the amount of nutrients actually reaching the reactive sites in the subsurface collateral to the processes they undergo there (Seitzinger et al., 2006; Zarnetske et al., 2012). Transport rates of water and nutrients from the surface to the subsurface could be attributed to water level, sediment properties and various other hydrological, biological, chemical and physical factors (Böhlke et al., 2009; Boano et al., 2014; Trauth et al., 2015). The complex interactions between these influencing factors and the temporal variability and local heterogeneity of hyporheic processes often cause high uncertainties in quantitative models. However, due to methodological restrictions,

experimental investigations of nutrient dynamics in the hyporheic zone are rare and commonly exclusively of qualitative nature (Mulholland et al., 1997; Grant et al., 2014).

Attempts to quantify hyporheic nutrient processing rates have primarily been based on benthic chamber and incubation experiments (Findlay et al., 2011; Kessler et al., 2012). Those laboratory (mesocosm and flume) experiments can estimate the denitrification potential of the substrates, usually via denitrification enzyme assays. However, the realized denitrification rates depend equally on environmental and hydrological conditions rather than on substrate type or denitrification potential alone (Findlay et al., 2011). Thus, owing to the high variability and complexity of natural systems, hyporheic transport of nutrients cannot satisfactorily be mimicked in artificial set-ups (Cook et al., 2006; Grant et al., 2014).

Direct instream measurements of nutrient dynamics based on whole stream tracer injections, mass balances (McKnight et al., 2004; Böhlke et al., 2009) and more recently high resolution time series from automated sensors (Pellerin et al., 2009; Hensley et al., 2014; Rode et al., 2016a; Rode et al., 2016b) can be used for determining general uptake rates on the reach scale, but do not allow to identify the reaction sites (hyporheic versus in channel or algal canopies) or specific local uptake processes (Ensign and Doyle, 2006; Ruehl et al., 2007). Further, in-stream measurements do exclusively account for water which is re-infiltrating into the main stem after passage through the hyporheic zone. Under losing conditions, where most of the nutrient-influx is flowing towards the groundwater, processing rates in the subsurface cannot be observed in the surface water. Likewise, if groundwater is contributing significantly to surface water chemistry, surface water mass balances do not characterize nutrient cycling in the hyporheic zone realistically (Trauth et al., 2014).

Conclusively, in situ assessments of hyporheic nutrient fluxes are indispensable. Hyporheic nutrient fluxes are commonly calculated from separate measurements of infiltration rates and pore-water concentrations (Kalbus et al., 2006; Ingendahl et al., 2009). The exchange of water between the surface and subsurface is traditionally derived from hydraulic head differences or tracer injections (Fleckenstein et al., 2010; USEP, 2013). Time series of high-resolution vertical temperature profiles have efficiently been used to derive vertical Darcy velocity (q_v) (m d^{-1}) in the streambed. While measurements of vertical Darcy velocities are a valuable asset, primarily horizontal fluxes are needed to assess hyporheic transport and residence time (Binley et al., 2013; Munz et al., 2016). Active heat-pulse tracing enables highly resolved in situ measurements of direction and velocity of hyporheic flow (Lewandowski et al., 2011; Angermann

et al., 2012). These methods are profitable in shallow sediments (max. 15-20 cm) and rivers with fine sediments, but may not be implementable in streams with coarser sediments.

Pore-water solute concentrations are typically determined from analysis of grab samples extracted with drive points (Saenger and Zanke, 2009; USEP, 2013). Alternatively, dialysis cells - so called peepers (Hesslein, 1976; Teasdale et al., 1995) - or gels (Krom et al., 1994), based on the diffusive equilibrium between the solute concentration in the pore-water and the receiver solution in the peeper or the gel, have been used to measure small scale solute distribution over highly resolved profiles on the mm to cm scale. These techniques provide valuable insights into the time specific conditions at the target site. However, they deliver effectively a snap shot of the highly temporally variable hyporheic zone processes, (Cooke and White, 1987) that may not be representative for the overall conditions in the system. Only repeated sampling at high frequencies and over longer timespans as conducted for example by Duff et al. (1998) can account for the short term variability. Attempting to characterize larger areas with these methods is laborious and costly.

In sum, direct quantitative methods for hyporheic flux measurements have two major deficiencies: First, separate measurements of water flow velocities and pore-water concentrations are necessary to calculate mass fluxes. This is not only labor intensive but can, due to the high temporal and spatial variability of hyporheic flow, also lead to incorrect estimates of real flux rates (Kalbus et al., 2006). Second, most techniques for measuring pore-water concentrations exclusively capture the concentration at the sampling time, which may not reflect the overall conditions at the sampling location. New, affordable and efficient methods for the long-term measurement of nutrient fluxes through the hyporheic zone are therefore required to validate and improve models (Boyer et al., 2006; Wagenschein and Rode, 2008; Alexander et al., 2009) and to determine the site specific extent of nutrient processing in the hyporheic zone (Fischer et al., 2009).

In groundwater studies, passive flux meters (PFM) have successfully been used to quantify fluxes of contaminants (Hatfield et al., 2004; Annable et al., 2005; Verreydt et al., 2013) through screened groundwater monitoring wells, integrating time spans ranging from days to weeks. PFM consist of a cylindrical, screened PVC casing filled with activated carbon (AC) as a porous sorbent. As long as the PFM is residing in the monitoring well, dissolved contaminants in the groundwater flowing passively through the meter, are retained on the AC. Furthermore, the AC is preloaded with water soluble resident tracers. The horizontal water flux through the screened media can then be determined from the displacement of these resident tracers, while simultaneously the contaminant flux is quantified based on the solute mass

captured on the AC. First bench-scale and column experiments for PO_4^- have been conducted in the laboratory (Cho et al., 2007). As AC did not prove efficient in capturing nutrients, an anion absorbing resin, a granular matrix originally manufactured for purification processes, was used as sorbent for PO_4^- instead. In theory, the PFM principle should be extendable to other nutrients or other environments. For example, development of Sediment passive flux meters (SBPFM) for the measurement of vertical contaminant seepage has been initiated (Layton, 2015). However, specific tests for NO_3^- as well as assessments under the complexities associated with hyporheic zone processes have not been conducted yet.

In this study, we evaluate the applicability of PFM for the measurement of horizontal nutrient fluxes in hyporheic zones, focusing on NO_3^- . We hypothesized that, while the principal concept of PFM can be maintained, several adaptations will still be necessary: Most importantly, a suitable sorbent for NO_3^- as target nutrient is required. The market of anion absorbing resins is large and offers a wide range of products with varying characteristics (Annable et al., 2005; Clark et al., 2005). Various criteria, like possible interference of resin compounds with the resident tracer analysis or pre-existing background nutrient loads on the resin have to be considered. As experience on resin behavior under field conditions are so far rare, we also expected unforeseen associated challenges, including for example biofouling of resin and /or nutrients. Additionally, a new deployment and retrieval procedure had to be developed. Existing groundwater PFM have been installed into land-based wells. In hyporheic studies, under-water installation requires a technique which impedes contamination with surface water. Corrections for convergence and divergence of flowlines into or around the flux meter have been established in earlier studies (Klammler et al., 2004). However, accounting for an impermeable outer casing of a flux meter is much more complicated and requires additional factors which have to be determined experimentally for each specific application (Hatfield et al., 2004; Klammler et al., 2004; Annable et al., 2005). We therefore intended to deploy the PFM in a way that allows direct contact with the surrounding sediments and minimal manipulation of the natural flow pattern.

Considering these requirements, we developed a modification of the PFM for the application in the hyporheic zone (Hyporheic Passive Flux Meter, HPFM). Based on the results from laboratory analysis and a first field test in a nutrient rich 3rd order stream (Holtemme, Germany), we demonstrate prospects and remaining limitations for hyporheic nutrient studies with HPFM.

3.3. METHODS

3.3.1. CONSTRUCTION AND MATERIALS

The Hyporheic Passive Flux Meters (HPFM) consisted of a nylon mesh which was filled with a mixture of a macroporous anion exchange resin as a nutrient absorber and alcohol tracer loaded activated carbon (AC) for the water flow quantification. In the present study HPFM were constructed 50 cm long and 5 cm in diameter. A stainless steel rod in the middle assured the stability of the device (**Figure 3.1**). To measure vertical profiles of horizontal fluxes of both nutrient and water in the hyporheic zone, the HPFM was divided into several segments using rubber washers. Steel tube clamps were used to attach the nylon mesh to the steel rod placed in the center of the HPFM. The nylon mesh was purchased from Hydro-Bios (Hydro-Bios Apparatebau GmbH, Kiel-Holtenau, Germany) and is available in a wide range of mesh size and thicknesses. We used a mesh size of 0.3 mm. In general, meshes should be as wide as possible because very fine mesh may act as a barrier to water flow limiting infiltration of water and solutes into the HPFM (Ward et al., 2011). However, the mesh should be smaller than the finest sediments, AC or resin granules. As final step, a rope was connected to the tube clamp on the upper end of the HPFM in order to facilitate retrieval.

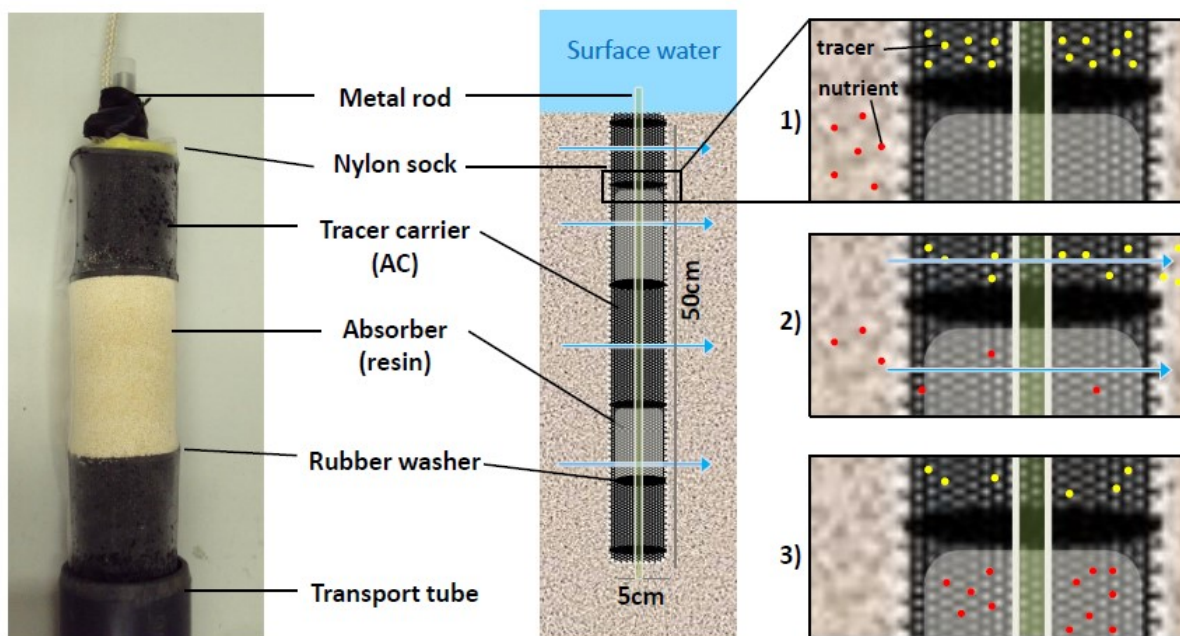


FIGURE 3.1. PHOTOGRAPH OF AN HPFM WITH ALTERNATING SEGEMENTS BEFORE DEPLOYMENT (LEFT), SCHEMATIC PROFILE OF A DEPLOYED HPFM (MIDDLE) AND SCHEMATIC STEPS OF HPFM FUNCTIONING (RIGHT): 1) DIRECTLY AFTER INSTALLATION, TRACER RESIDES ON ACTIVATED CARBON (AC), 2) INFILTRATING WATER WASHES OUT THE TRACER, NUTRIENTS ENTER THE HPFM AND ARE ABSORBED ON THE RESIN, 3) AFTER RETRIEVAL NUTRIENTS ARE FIXED ON THE RESIN, TRACER CONCENTRATION IS DILUTED.

3.3.2. SELECTION AND CHARACTERIZATION OF RESIN

The nutrient sorbent had to meet the following criteria:

- Have a high loading capacity for NO_3^- , PO_4^- and competing anions,
- be free of compounds which could interfere with the alcohol tracer measurements (e.g. organic substances) and
- have a low background of NO_3^- and PO_4^- .

A pre-selection for anion-absorbing resins which were free of organic compounds was made based on information provided by the manufacturers (Purolite®, Lewatit®, Dowex®).

3.3.2.1. NUTRIENT BACKGROUND

Nutrient background on the resins was determined by extracting and analyzing NO_3^- and PO_4^- from each resin ($n = 3$). Therefor 30 mL of 2M KCl was added to 5 g of each pure resin and rotated for 24 hours. The solution was then analyzed on a Segmented Flow Analyser Photometer (DR 5000, Hach Lange) for NO_3^- at 540 nm (detection limit $0.042 \text{ mg NO}_3^- \cdot \text{N L}^{-1}$) and for SRP at 880 nm (detection limit $0.003 \text{ mg P L}^{-1}$). In order to estimate the effect of background concentrations on final results in the actual field application of HPFM, the extractable background concentrations were then converted to nutrient fluxes using a Darcy flux of $q_x = 4 \text{ m d}^{-1}$, an estimate based on hyporheic flow velocities which were measured previously with salt tracer tests at the study site. Likewise, the expected hyporheic nutrient flux was computed from previously examined concentrations in pore water samples and the Darcy flux. The only resin with nutrient background below 5 % of expected concentrations was Purolite® A500 MB Plus (Purolite GmbH, Ratingen, Germany), which had extractable background NO_3^- of $8 \text{ } \mu\text{g NO}_3^- \cdot \text{N g}^{-1}$ wetted resin ($\pm 1.6 \text{ } \mu\text{g g}^{-1}$, $n = 3$) and $0.08 \text{ } \mu\text{g PO}_4^- \cdot \text{P g}^{-1}$ resin ($\pm 1.7 \times 10^{-3} \text{ } \mu\text{g g}^{-1}$, $n = 3$). Purolite® A500 MB Plus was then considered for testing the loading capacity. The limit of quantification LQ for the nutrient extraction resulting from this background was calculated according to the EPA Norm 1020B (Greenberg et al., 1992) as the sum of background concentration and 10 times the standard deviation and amounted to $24 \text{ } \mu\text{g NO}_3^- \cdot \text{N g}^{-1}$ resin and $0.097 \text{ } \mu\text{g PO}_4^- \cdot \text{P g}^{-1}$ resin.

3.3.2.2. LOADING CAPACITY AND BIOFOULING

Purolite® A500 MB Plus is a macroporous anion exchanger on the basis of polyvinylbenzyltrimethylammonium with a typical granular size of 0.88 mm diameter, an average density of 685 g L^{-1} and an effective porosity of 63 %. The theoretical absorbing capacity is indicated in the product sheet as 1.15 eq L^{-1} (molar weight equivalences per liter of resin), corresponding to $71.3 \text{ g NO}_3^- \cdot \text{N L}^{-1}$. Assuming hyporheic flow velocities of $q_x = 4 \text{ m d}^{-1}$ and a concentration of $10 \text{ mg NO}_3^- \cdot \text{N L}^{-1}$, the volume of one HPFM could adsorb NO_3^- for 89 days. However, if multiple anions are present, real loading capacities for NO_3^- are expectedly lower.

For the determination of a realistic loading capacity, three 5 cm diameter columns were filled to a height of 5 cm with wetted Purolite® A500 MB Plus resin, placed in a vertical position and infiltrated with water collected from the study reach. The columns were covered with tin foil to keep them dark and ensure stable temperature. A constant supernatant of 1 cm was kept on all three columns to ensure uniform infiltration at the surface of the column. Water was continuously pumped (peristaltic pump, ISMATEC®

BVP Standard, ISM444) through the columns from top to bottom for 22 days at a speed of 20 mL h^{-1} , which also equals the expected Darcy velocity of $q_x = 4 \text{ m d}^{-1}$. River water was supplied from a 22 L HDPE canister (Rotilabo® EPK0.1). SRP and NO_3^- concentrations in this reservoir were revised daily. The draining water at the bottom outlet of the columns was sampled twice a day and analyzed for SRP and NO_3^- .

Biofilm growth on the resin was assessed by repeating the same experiment in smaller columns ($n = 3$) and extending it for several days after break-through occurred. That way, nutrient consumption by biofilm after the exhaustion of the loading capacity could be monitored. After the experiment we colored samples ($n = 3$) of resin granules from the columns with SybrGreen ($\text{C}_{32}\text{H}_{37}\text{N}_4\text{S}^+$) on nucleic acid and examined them under a confocal laser scanning microscope to depict the degree of bacterial fouling on the granular surface.

3.3.3. PREPARATION OF ACTIVATED CARBON WITH ALCOHOL TRACERS

As designed for the groundwater PFMs, silver impregnated activated carbon (AC) was used as sorbent for the resident alcohol tracers. The same AC as in previous PFM applications (Annable et al., 2005) was used for the HPFM in this study and was provided by the University of Florida, Gainesville. The AC had a bulk density of 550 g L^{-1} , a grain size ranging from 0.42 to 1.68 mm and a hydraulic conductivity $k = 300 \text{ m d}^{-1}$.

Since the magnitude of water flow through the flux meter is unknown a priori, multiple resident tracers with a wide range of tracer elution rates were needed. The retardation factor of a substance R_d is a measure for the rate of elution of the substance from a particular carrier. Alcohols offer a wide range of retardation factors and can easily be mixed and sorbed to the AC (Hatfield et al., 2004; Cho et al., 2007). By choosing the same manufacturer for the AC and the same alcohol mixture as used in the above mentioned studies, we could rely on physical and chemical characterizations and calculated R_d for alcohol partitioning behavior which have been established by Hatfield et al. (2004), Annable et al. (2005) and Cho et al. (2007) (**Table 3.1**).

TABLE 3.1. RESIDENT TRACERS PER LITER OF AQUEOUS SOLUTION AND THEIR PARTITIONING CHARACTERISTICS. RETARDATION FACTORS (R_d) FOR THE SPECIFIC SET OF TRACERS AND AC USED IN THIS STUDY HAD PREVIOUSLY BEEN DETERMINED BY CHO ET AL. (2007)

Resident tracers	Aqueous concentration (g L ⁻¹)	R_d
methanol	1.2	4.9
ethanol	1.2	20
Isopropyl alcohol (IPA)	2.3	109
tert-butyl alcohol (TBA)	2.3	309
2,4-dimethyl-3-pentanol (DMP)	1.2	>1000

An alcohol tracer mixture for approximately 10 HPFM was prepared by combining 100 mL of methanol, 100 mL of ethanol, 200 mL of isopropanol (IPA), 200 mL of tert-butanol (TBA) and 66 mL of 2, 4-dimethyl-3-pentanol (2,4 DMP).

In order to prepare the resident alcohol tracers on the AC, the AC was soaked in an aqueous solution containing the resident alcohol tracers. A standard ratio of 13 mL tracer mixture was added to 1 L of water in a Teflon sealed container and was then shaken by an automated shaker over a period of several hours. Subsequently, 1.5 L of dry activated carbon was added to the aqueous tracer solution and rotated for 12 h to homogenize the AC tracer mixture. After mixing, the supernatant water was discarded and the AC tracer mixture was stored in a sealed container and refrigerated, preventing the evaporation of the alcohol tracers

Similarly to the resins, the AC was tested for background nutrients by extraction with 30 ml KCl per 5 g AC. The activated carbon contained 0.01 mg PO₄⁻P g⁻¹AC ($\pm 7.5 \times 10^{-4}$ mg g⁻¹, n = 3) and 0.08 mg NO₃⁻-N g⁻¹ AC ($\pm 5 \times 10^{-3}$ mg g⁻¹, n = 3), which amounts to 75 % of the expected concentration for NO₃⁻ and 320 % for PO₄⁻. To investigate whether the AC could be cleaned by washing, we repeatedly treated AC samples with distilled water or KCl as depicted in the extraction description above. Nutrients did not leach off under water treatment and neither did KCl treatment satisfactorily reduce extractable background concentration on the AC. After the third washing of AC with KCl, 0.02 mg PO₄⁻P ($\pm 3.3 \times 10^{-4}$ mg g⁻¹, n = 3) and 0.04 mg NO₃⁻-N ($\pm 2.3 \times 10^{-3}$ mg g⁻¹, n = 3) could still be extracted per g AC. Further, it was unclear to which degree replacing absorbed nutrients by KCl would alter the alcohol tracer retardation and extraction on the AC. For those reasons, we decided to keep the nutrient absorbing resin separated from the AC. As AC did not release background nutrients under water treatment, water flowing first through AC and afterwards resin layers was not considered problematic.

3.3.4. DEPLOYMENT AND RETRIEVAL PROCEDURE

HPFM were built, stored dry and transported in 70 cm long standard polyethylene (PET) tubes (58 x 5.3 SDR 11) purchased from a local hardware store (Handelshof Bitterfeld GmbH, Bitterfeld, Germany). To avoid resident alcohol tracer loss, the transport tubes with the HPFMs were sealed with rubber caps and cooled during storage and transport. In the field, prior to installation, the HPFMs were transferred to a stainless steel tube, 5.3 cm inner diameter with a loose steel drive point tip on the lower end. The diameter of the steel tube for installation tightly fitted with the rubber washers at the top and bottom end of the HPFM, so that vertical water flow through tube and HPFM during installation was inhibited. The steel casing and HPFM were driven into the river bed using a 2 kg hammer until the upper end of the HPFM was at the same level as the surface-subsurface interface. The metal casing was retrieved while the HPFM was held in place using a steel rod.

After 7 days of exposure, the HPFMs were retrieved by holding the transport tube in place and quickly drawing the HPFM into the tube using the rope fixed to the upper end of the HPFM. The required length of the transport tube, steel drive casing and retrieval rope was determined by the depth of the water level in the stream. After retrieval, the HPFM were transported to the laboratory.

3.3.5. ANALYSIS AND DATA TREATMENT

In the laboratory, the retrieved HPFM were instantly (after maximal 12 hours) sampled for analysis. Therefor one segment after the other was cut open and the sorbent was segment-wise recovered, homogenized and a subsample transferred to 40 mL glass vials. The subsamples from resin segments were then analyzed for nutrient content, the subsamples from AC segments were analyzed for the remaining alcohol tracers as described in the following paragraphs.

3.3.5.1. WATER FLUX q_x

The AC samples were shipped to the University of Florida for analysis. In the laboratory, the mass of the previously applied mixture of alcohol tracers in standard AC samples and the tracer mass remaining in the final AC samples were extracted with iso-butyl alcohol (IBA). About 10 g of AC samples were transferred into pre-weighed 40 mL vials containing 20 mL IBA. Vials were rotated on a Glas-Col Rotator, set at 20 %

rotation speed, for 24 h. Then, subsamples were collected in 2 mL GC vials for alcohol tracer analysis. The samples were analyzed with a GC-FID (Perkin Elmer Autosystem) (Cho et al., 2007).

The relationship between time averaged specific horizontal discharge q_x (m s^{-1}) through the device and tracer elution is given by equation (1) (Hatfield et al., 2004)

$$q_x = \frac{1.67 r \theta (1 - M_R) R_d}{t} \quad (3.1)$$

where r (m) is the radius of the HPFM, θ is the volumetric water content in the HPFM ($\text{m}^3 \text{m}^{-3}$), M_R (-) is the relative mass of tracer remaining in the HPFM sorbent, t (s) is the sampling duration and R_d (-) is the retardation factor of the resident tracer on the sorbent.

3.3.5.2. NUTRIENT FLUX J_N

NO_3^- and PO_4^- were extracted and analyzed in the laboratory at UFZ in Magdeburg, Germany, similarly to the analysis of background concentrations on the resin: subsamples of 5g resin were treated with 30 mL of 2 M KCl each and rotated for 24h for extraction. The solution was then analyzed as described above.

The time-averaged advective horizontal nutrient flux J_N ($\text{mg m}^2 \text{d}^{-1}$) can be calculated using the following relationship (Hatfield et al., 2004):

$$J_N = \frac{q_x M_N}{2\alpha r L t} \quad (3.2)$$

where M_N (kg) is the mass of nutrient adsorbed, L (m) is the length of the vertical thickness of the segment and α (-) is a factor ranging from 0 to 2 that characterizes the convergence ($\alpha > 1$) or divergence ($\alpha < 1$) of flow around the HPFM. If, like in the case presented here, the hydraulic conductivity of the HPFM sorbent (resin or AC) is much higher than that of the surrounding medium and the HPFM is in direct contact with the sediments (i.e. in absence of an impermeable outer casing or well wall), α can be estimated after Strack and Haitjema (1981)

$$\alpha = \left(\frac{2}{1 + \frac{1}{K_D}} \right) \quad (3.3)$$

where $K_D = k_D / k_0$ is the dimensionless ratio of the uniform hydraulic conductivity of the HPFM sorptive matrix k_D (L T^{-1}) to the uniform local hydraulic conductivity of the surrounding sediment k_0 (L T^{-1}). For more details on the correction factor α and applications where a solid casing is required or the permeability of the surrounding sediments is higher than of the device see Klammler et al. (2004) and Hatfield et al. (2004).

3.3.6. FIELD TESTING OF HYPORHEIC PASSIVE FLUX METERS (HPFMS)

3.3.6.1. STUDY SITE

A 30 m long stretch of the Holtemme River, a 3rd order stream in the Bode catchment, TERENO Harz/Central German Lowland Observatory, served as study site (51°56'30.1"N, 11°09'31.8"E). The testing reach is located in the lowest part of the river, where the water chemistry is highly impacted by urban effluent and agriculture (Kamjunke et al., 2013). Long stretches have been subjected to changes in the natural river morphology by canalization (Landesbetrieb für Hochwasserschutz und Wasserwirtschaft Sachsen-Anhalt, 2009).

The sediments at the selected site are sandy with gravel and small cobbles. Sieving of sediment samples delivered the effective grain size $d_{10} = 0.8$ mm and a coefficient of uniformity $C_u = 3.13$. The effective porosity n_{ef} is 13 %. After Fetter (2001) the intrinsic permeability was estimated to be $K_i = 96$ m² and the hydraulic conductivity $k = 81$ m d⁻¹. Clay lenses are present in the deeper sediments below 35 cm.

Mean discharge in the stream is 1.35 m³ s⁻¹ with highest peaks around 5-6 m³ s⁻¹. Discharge is continuously recorded by the local authorities at the gauge Mahndorf, 15 km upstream of the testing site. In the course of the year, NO₃⁻ concentrations in the lower Holtemme vary between 2 and 8 mg NO₃⁻ -N L⁻¹ (LHW, 2016).

The equipment was installed for a period of 7 days from 4th to 11th June 2015 as illustrated in **Figure 3.2**.

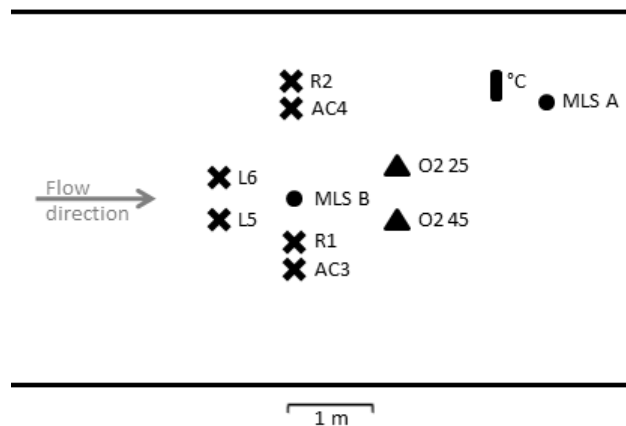


FIGURE 3.2. OVERVIEW OF THE INSTRUMENTAL SETUP AT THE HOLTEMME FOR THE TESTING PHASE IN JUNE 2015.

R1, R2 RESIN ONLY HPFM; AC3, AC4 ACTIVATED CARBON ONLY HPFM; L5, L6 ALTERNATING LAYERED HPFMS; MLSA, MLSB MULTI-LEVEL SAMPLER; O2 25, O2 45 SUBSURFACE OXYGEN LOGGER; °C VERTICAL TEMPERATURE PROFILE.

3.3.6.2. HPFM TESTING

Based on the laboratory results for the nutrient backgrounds and the consequent necessity to keep resin and AC separated two approaches for constructing and deploying HPFM were tested in the field:

Resin only and AC only HPFMs

4 HPFMs were constructed of which 2 contained only resin (R1 and R2) and the other two contained only AC (AC3 and AC4). The HPFMs were then installed in pairs: AC only and resin only next to each other with a separation distance of 30 cm. Those 4 HPFMs were sectioned in 5 horizontal flow segments, each with a vertical length of 10 cm.

For the calculation of the nutrient flux through each segment of R1 and R2, we used the corresponding water flux through the respective segment of AC3 and AC4.

Alternating segments of AC and resin HPFMs

HPFMs L5 and L6 consisted of 7 segments starting and ending with an AC segment and adjacent segments altering between resin and AC (also see **Figure 3.1**). Each segment had a length of 7 cm.

For the calculation of the nutrient flux through the resin segments, we used the interpolated water flow measured in the two adjacent AC segments.

One additional HPFM with alternating layers was used as a control HPFM, in order to assess potential tracer loss or nutrient contamination during storage, transport and deployment/retrieval. This control was stored and transported together with the other HPFMs. After deploying the control HPFM, it was immediately retrieved, transported back to the laboratory and stored until it was sampled and analyzed along with the other HPFMs. The results from the control HPFM also include uncertainties arising from sample storage, analytical processing and the background concentration of nutrients on the resin. Measurements of the other HPFMs were corrected by subtracting the transport, storage and deployment related tracer loss and nutrient accumulation detected in the control.

3.3.6.3. ADDITIONAL MEASUREMENTS

Vertical Darcy velocity (q_v)

The vertical vector of hyporheic Darcy velocities q_v were measured supplementary to the horizontal fluxes assessed with the HPFM in order to estimate the general direction of flow (upwards or downwards) and to calculate the angle of hyporheic flow.

The vertical Darcy velocity q_v (m d^{-1}) in the streambed was calculated using temperature profiles measured between January 2015 and October 2015. According to Keery et al. (2007) and Schmidt et al. (2014), vertical flow velocities can be computed from the temporal shift of the daily temperature signal in the subsurface water relative to the surface water. A multi-level temperature sensor (Umwelt- und Ingenieurtechnik GmbH, Dresden, Germany) was installed at the test site in January 2015. Temperature was recorded at the surface-subsurface interface and at depths of 0.10, 0.125, 0.15, 0.2, 0.3 and 0.5 m in the sediment at a 10 min interval (accuracy of 0.07 °C over a range from 5 to 45 °C and a resolution of 0.04 °C). A numerical solution of the heat flow equation was then used in conjunction with Dynamic Harmonic Regression signal processing techniques for the analysis of these temperature time series. The coded model was provided by Schmidt et al. (2014).

Oxygen profiles

We monitored the subsurface oxygen concentration as a primary indication on the redox status of the hyporheic zone in order to evaluate the potential for NO_3^- reduction and PO_4^- mobilization. Therefore two oxygen loggers (miniDO₂T, Precision measurement engineering Inc.) incorporated into steel tubes acuminated at the lower end were installed in the river bed. The tubes had filter-screens at the measuring depths of 25 and 45 cm below surface-subsurface boundary. Installation was carried out 4 weeks prior to the experiments, allowing enough time for re-equilibration of the surrounding media. The measurement time step was 5 min.

Multi-level samplers (MLS)

Pore-water nutrient concentrations were measured to substantiate the HPFM results. Multi-level samplers as described in detail by Saenger and Zanke (2009) are devices for the manual extraction of hyporheic pore water from several distinct depths. The two samplers A and B used in these experiments were manufactured by the institutional workshop of the UFZ. Like the oxygen loggers both MLS were installed 4 weeks prior to the experiment. They consisted of an outer stainless steel tube with a length of 50 cm and a diameter of 5 cm. Ceramic filters were inserted in this outer steel mantle marking the extraction depths at 5, 15, 25 and 45 cm. The inner sides of the filters were attached to steel pipes that ran to the top of the sampler so that Teflon tubes could be attached. A protective hood was threaded on the upper end of the sampler to preclude particles and sediment entrance. Per sampler and depths 10 mL of pore water was manually extracted by connecting a syringe to the open end of the Teflon tube and slowly sucking up water at a rate of 2 mL min^{-1} . The 4 extraction depths were sampled successively, always

starting with the shallowest depths and continuing with ascendant depths. Manual pore water samples were taken on the 4th and 11th of June 2015, both times between 1 pm and 4 pm local time.

The samples were filtered in the field through a 0.45 µm membrane filter and placed in boro-silica glass vials for transport to the laboratory. Analysis for NO₃⁻, SRP, sulphate (SO₄²⁻) and boron (B) were conducted in the central analytical laboratory of the UFZ, Magdeburg, Germany. Analytical procedure for NO₃⁻ and SRP was done according to the description above.

SO₄²⁻ and B were used as natural tracers for groundwater and surface water respectively. SO₄²⁻ was analyzed on an ion chromatograph (ICS 3000, ThermoFisher, former DIONEX), B was analyzed on an inductively coupled plasma mass spectrometer (ICP-MS 7500c, Agilent). As NO₃⁻ and SRP concentrations in the pore water samples taken on June 4th and 11th 2015 were unexpected and inconsistent with results from the HPFMs, the sampling was repeated on the 8th of October. The aim of this repeated sampling was to investigate whether diurnal variations in subsurface NO₃⁻ and SRP concentrations could explain the discrepancies between MLS and HPFM results. We assumed that the HPFM measurements integrated temporal oscillations, while MLS samples represented the specific concentrations around noon. In order to test this hypothesis, both MLS were sampled twice, the first time in the early morning before sunrise and again in the early afternoon (around 2 pm) during the sampling in October. Those samples were analyzed for NO₃⁻, SRP and SO₄²⁻. Due to technical issues, boron could not be measured in October.

Surface water chemistry

Surface water concentrations of SRP and NO₃⁻ were monitored in order to compare surface and subsurface water chemistry. Therefor we installed an automated UV absorption sensor for NO₃⁻ (ProPS WW, TriOS) at the beginning of the testing reach for the duration of the experiments. The pathway-length of the optical sensor was 10 mm, measuring at wavelengths 190-360 nm with a precision of 0.03 mg NO₃⁻-N L⁻¹ and an accuracy of ± 2 %. The measurement time step was set to 15 min. SRP, SO₄²⁻ and B concentrations in the surface water were assessed with grab samples taken simultaneously to the MLS measurements.

The UV sensor was supplemented with a multi-parameter probe YSI 6600 V2/4 (YSI Environmental, Yellow Springs, Ohio) recording the following parameters: pH (precision 0.01 units, accuracy ± 0.2 units), specific conductivity (precision 0.001 mS cm⁻¹, accuracy ± 0.5 %), dissolved oxygen (precision 0.01 mg L⁻¹, accuracy ± 1 %), temperature (precision 0.01 °C, accuracy ± 0.15 °C) and turbidity (precision 0.1 NTU, accuracy ± 2 %).

3.3.6.4. ESTIMATES OF NITRATE TURNOVER RATES BASED ON HPFM MEASUREMENTS

Estimates for hyporheic removal activity R_N for the specific conditions at the study site during the HPFM testing phase were calculated using the morphological and hydrological parameters summarized in **Table 3.2**.

TABLE 3.2. SELECTED MORPHOLOGICAL AND HYDROLOGICAL PARAMETERS OF THE TESTING SITE FOR THE DURATION OF THE TESTING PHASE FROM 04.06.2015-11.06.2015. RANGES ARE INDICATED FOR DIRECTLY MEASURED PARAMETERS, THE REMAINING PARAMETERS HAVE BEEN CALCULATED FROM LISTED MEANS. HZ= HYPORHEIC ZONE

Surface water	acronym	unit	mean	range
cross sectional area	A_{SW}	m^2	3.41	
depth	h	m	0.565	0.54 - 0.61
width	w	m	6.03	5.57 - 6.29
mean velocity	v	$m\ s^{-1}$	0.097	
discharge	Q_{SW}	$m^3\ s^{-1}$	0.32	0.30 - 0.34
NO_3^- concentration	$C_{NO3\ SW}$	$mg\ NO_3^- - N\ L^{-1}$	2.86	2.16 - 3.26
NO_3^- mass flux	$M_{NO3\ SW}$	$mg\ NO_3^- - N\ s^{-1}$	896	
PO_4^- concentration	$C_{PO4\ SW}$	$mg\ P\ L^{-1}$	0.165	0.111 - 0.231
PO_4^- mass flux	$M_{PO4\ SW}$	$mg\ P\ s^{-1}$	51	
Hyporheic zone upper 50cm				
Assessed depth of HZ	h_{HZ}	m	0.5	
cross sectional area	A_{HZ}	m^2	3.02	

The absolute amount of water passing the screened area of the hyporheic zone Q_{HZ} ($m^3\ s^{-1}$) is the product of the average horizontal vector of the Darcy velocity q_x ($m\ s^{-1}$) measured in the HPFM and the cross sectional area of the upper 50 cm of the hyporheic zone A_{HZ} (m^2). The proportion of water infiltrating the hyporheic zone $\%Q_{HZ}$ (%) was then calculated from the ratio $\frac{Q_{HZ}}{Q_{SW}}$, where Q_{SW} ($m^3\ s^{-1}$) is the average discharge at the study site during the days of measurements, derived from continuous records at the gauche Mahndorf, which were provided by the local authority Landesbetrieb für Hochwasserschutz und Wasserwirtschaft Sachsen-Anhalt.

The NO_3^- removal activity of the hyporheic zone R_N (%) was calculated from the difference in average surface water concentration C_{NO3-SW} ($mg\ NO_3^- - N\ L^{-1}$) and the average concentrations measured with the HPFM C_{NO3-HZ} ($mg\ NO_3^- - N\ L^{-1}$), where C_{NO3-HZ} is the quotient $\frac{J_N}{q_x}$.

3.4. RESULTS

3.4.1. LABORATORY EXPERIMENTS

3.4.1.1. LOADING CAPACITY AND BIOFOULING

Break-through in the sorbent column experiments occurred after 300 pore volumes or 21 days at selected drainage for both NO_3^- and SRP.

In the biofouling experiment, the NO_3^- concentration in the draining water gradually decreased again after break-through. SRP in the draining water was completely depleted 6 h after the break-through. The calculated amount of retained nutrient in comparison to manufacturer value loading capacities of Purolite® A 500MB Plus indicate that the absorbing capacity of the resin in this small column experiment was exhausted after 25.5 hours (**APPENDIX 3A**). We attributed the decrease of nutrients in the draining solution after breakthrough to biotic consumption of SRP (limiting nutrient) and NO_3^- . Under the laser scanning microscope growth of biofilm could be observed on obviously brown stained Purolite® beads of the columns from the biofouling experiment and to a very low degree on beads from the same column which appeared still clean (**APPENDIX 3A**). Browning of Purolite® beads was not observed on Purolite® beads from the loading experiment (bigger columns, experiment not extended after break through) but on the top 2 cm of the HPFM R2 after exposure at the study site.

3.4.2. FIELD TESTING

3.4.2.1. HPFMS AND ADDITIONAL MEASUREMENTS

HPFMs

Deployment required approximately 15 min per HPFM and could be conducted by two persons. The water depth during the installation was 40 to 100 cm, depending on the specific location in the stream.

The average horizontal water flow q_x and nutrient flux J_N measured in the HPFM during the 7-day field testing are illustrated in **Figure 3.3**.

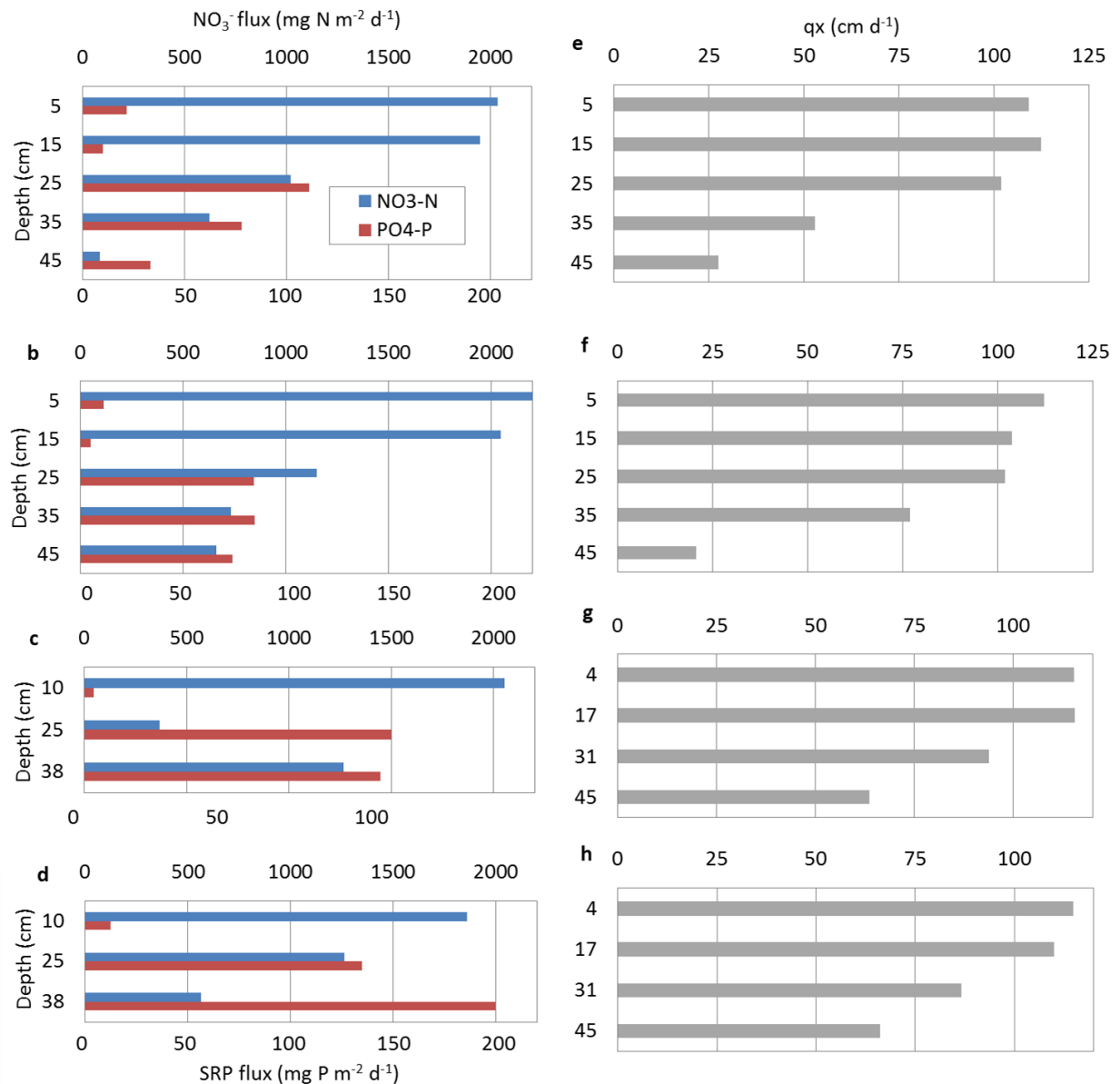


FIGURE 3.3. TIME INTEGRATIVE MEASUREMENTS FOR THE 04.-11.06.2015. LEFT SIDE: HORIZONTAL NO₃⁻-N AND SRP-P FLUX IN mg m⁻² d⁻¹ THROUGH THE RESIN HPFM R1 (a), R2 (b) AND THE LAYERED HPFM L5 (c) AND L6 (d). RIGHT SIDE: CORRESPONDING DARCY VELOCITIES q_x IN CM D⁻¹ THROUGH THE ACTIVATED CARBON HPFM AC3 (e) AND AC4 (f) AND THE LAYERED HPFMS 5L (g) AND 6L (h)

All flux meter except 5L showed declining J_N and q_x with depth. Average horizontal q_x was 76 cm d⁻¹, ranging from 115 cm d⁻¹ in the shallowest layer of 5L to 20 cm d⁻¹ in the deepest layer of AC4. Over the 7-days duration of the experiment, accumulated horizontal flow velocities of $q_x = 8.4$ cm 7 d⁻¹ (± 0.02 cm 7 d⁻¹, $n = 3$) and nutrient fluxes of 29.4 mg NO₃⁻-N m² 7 d⁻¹ (± 0.7 mg m² d⁻¹, $n = 3$) and 36.4 mg SRP m² 7 d⁻¹ (± 6.3 mg m² d⁻¹, $n = 3$) were detected in the control HPFM. Breaking these results down to dial values,

yields $q_x = 1.2 \text{ cm d}^{-1} (\pm 0.003 \text{ cm d}^{-1}, n = 3)$ and nutrient fluxes of $4.2 \text{ mg NO}_3^- \text{ N m}^2 \text{ d}^{-1} (\pm 0.1 \text{ mg m}^2 \text{ d}^{-1}, n = 3)$ and $5.2 \text{ mg SRP m}^2 \text{ d}^{-1} (\pm 0.9 \text{ mg m}^2 \text{ d}^{-1}, n = 3)$. Comparing these fluxes to the J_N values measured with the other HPFM, an average 0.3 % of the uncorrected NO_3^- flux and 5 % of the uncorrected SRP flux were attributed to tracer loss or nutrient accumulation resulting from transport, deployment, retrieval, analytical processing of samples and the background concentrations on the resin.

Vertical Darcy velocity (q_v)

Vertical water flow q_v in the stream bed was predominantly downward from January to October 2015. It was exclusively downward during the HPFM testing phase, ranging from 40 to 55 cm d^{-1} . With this, vertical flow q_v was slightly lower than average horizontal flow q_x . Resulting from the relationship between q_v and q_x the angle of hyporheic flow ($\tan \alpha = \frac{q_v}{q_x}$) was 32° downwards.

Oxygen profiles

We observed strong diel variations in oxygen concentration in the hyporheic zone. During several nights, oxygen was nearly depleted (**Figure 3.4**). The minima and maxima oxygen concentrations in the subsurface occurred contemporarily with the respective extremes in the surface water. Interestingly the amplitude in O_2 oscillation was higher at 45 cm depths than at 25 cm depths.

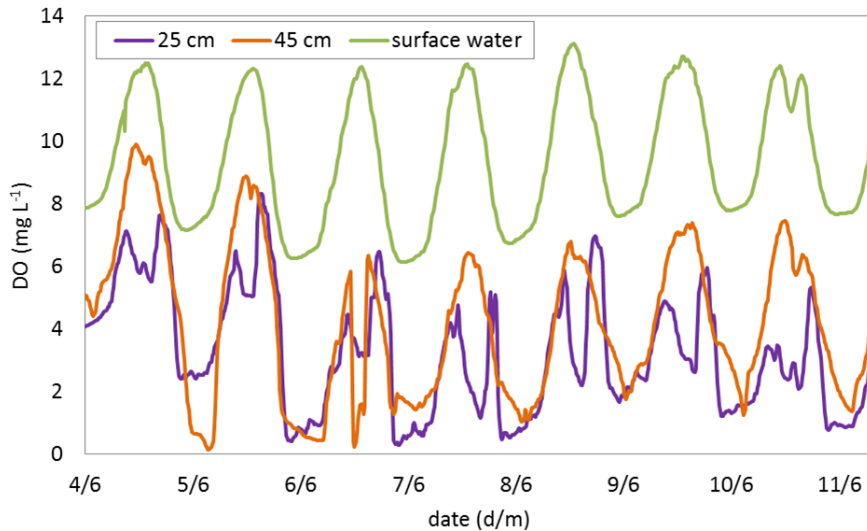


FIGURE 3.4. TIME SERIES OF DISSOLVED OXYGEN CONCENTRATIONS IN THE SURFACE WATER (GREEN) AND THE SUBSURFACE WATER (DEPTH 25 CM, PURPLE AND DEPTH 45 CM ORANGE) AT THE STUDY SITE FROM 04.-11.06.2015

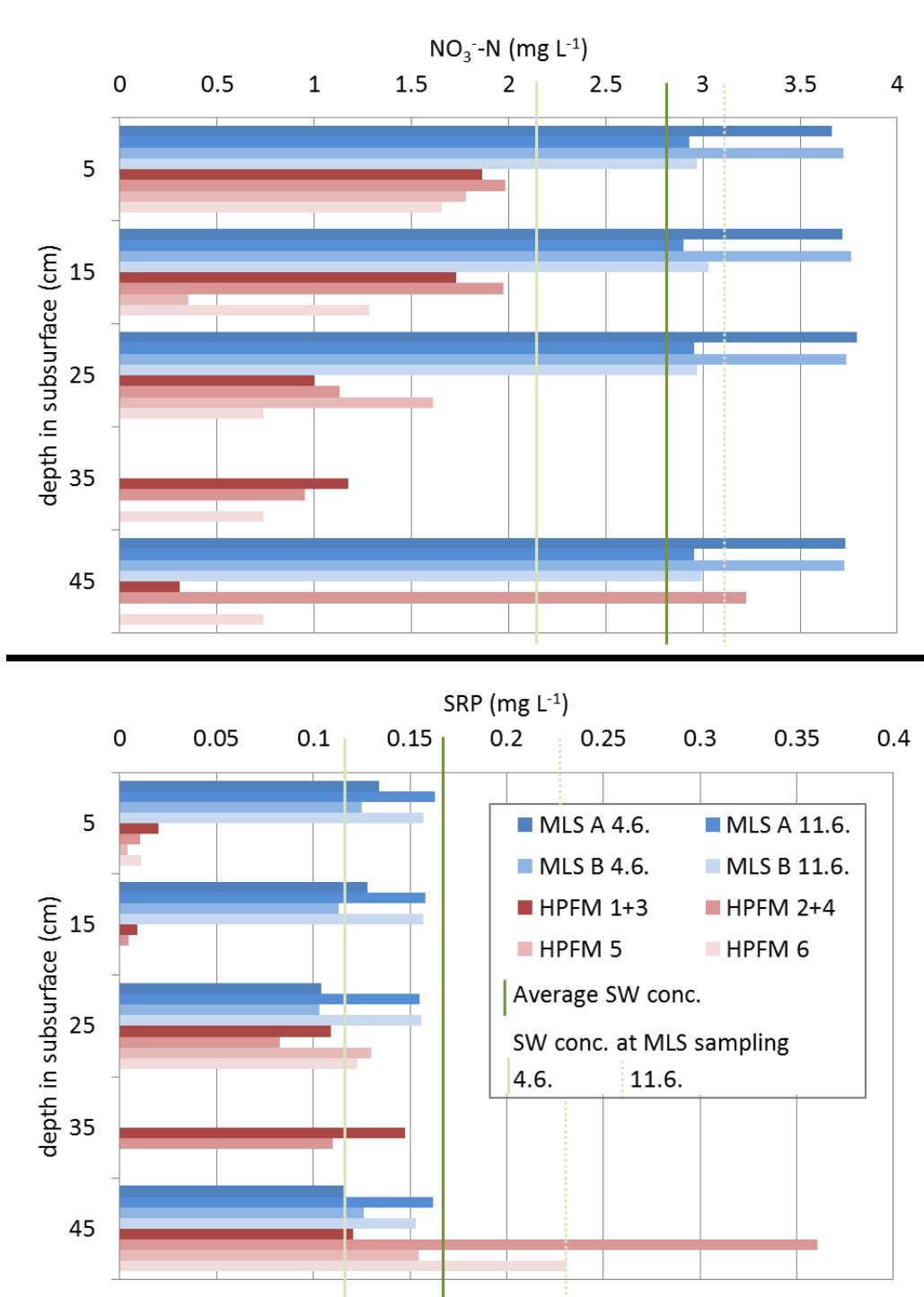


FIGURE 3.5. COMPARISON BETWEEN MANUALLY SAMPLED PORE WATER FROM MLS (RED) AND HPFM (BLUE) FOR NO₃⁻-N (TOP) AND SRP (BOTTOM). EACH MLS WAS SAMPLED ON THE 04. AND 11.06.2015. AVERAGE SURFACE WATER CONCENTRATION DURING THE DEPLOYMENT TIME AS WELL AS THE CONCENTRATIONS AT THE TIME POINT OF MLS SAMPLING ARE MARKED IN GREEN.

Multi-level samplers (MLS)

In order to facilitate direct comparison, nutrient fluxes as measured in the HPFM were converted to flux averaged concentrations which are the quotient of J_N and the respective q_x (**Figure 3.5**).

Overall, nutrient concentrations in the manually sampled pore water taken in June 2015 were higher than the average concentration derived from the HPFM. While the expected increase of SRP and decrease of NO_3^- and water flow with depths was observed in the HPFM, pore water extracted with the MLS showed no change over depth neither for NO_3^- nor SRP. In the repeated manual pore water samples taken in October (**Figure 3.6**) NO_3^- concentrations were uniformly lower in the early morning than in the afternoon, whereas SRP behaved the other way round. This trend was consistent in both samplers even though the average concentration and distribution over depths differed between the samplers A and B.

On both sampling dates in June (04.06. and 11.06.2015) neither SO_4^{2-} nor boron showed a vertical gradient in concentrations in the pore water samples. SO_4^{2-} concentrations of 170 mg L^{-1} on the 4th June and 190 mg L^{-1} on the 11th June were in the same range as surface water concentrations. Likewise, were boron concentrations with $50 \text{ to } 60 \mu\text{g L}^{-1}$ consistence with the concentrations in the surface water, indicating no or only minor groundwater influence. Also in October SO_4^{2-} concentrations in the pore water samples were in the range of surface water concentrations, slightly declining with depth.

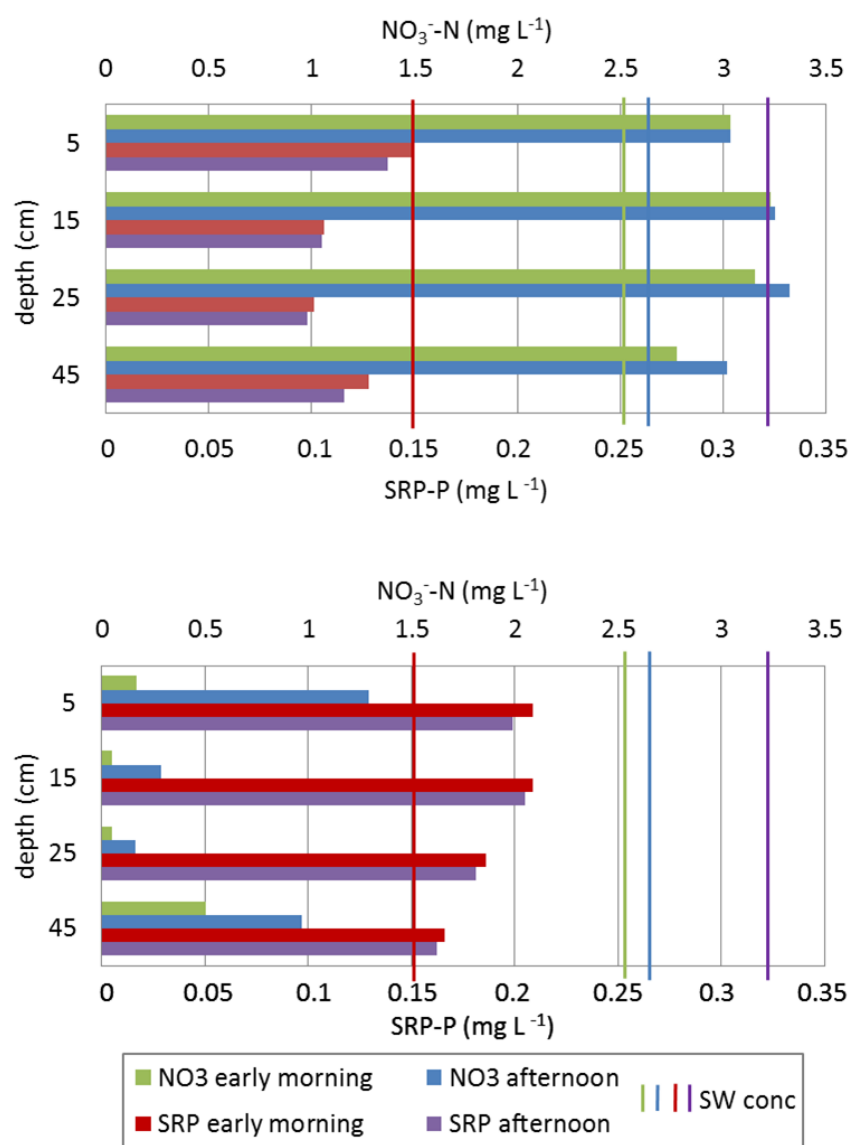


FIGURE 3.6. CONCENTRATIONS OF NO_3^- -N AND SRP IN TIME DIFFERENTIATING MANUALLY TAKEN PORE WATER SAMPLES FROM MLS A (BOTTOM) AND MLS B (TOP) ON 8TH OCTOBER 2015. CORRESPONDING SURFACE WATER CONCENTRATIONS ARE MARKED AS VERTICAL LINES.

Surface water chemistry

Temperature, O₂ and pH showed the expected diurnal amplitudes whereas specific conductivity and NO₃⁻ did not display a distinct diurnal pattern (**Table 3.3.**).

TABLE 3.3 BENCHMARK SURFACE WATER PARAMETERS DERIVED FROM THE CONTINUOUS SENSOR RECORDS FROM 04.06.-11.06.2015 AND 08.10. – 11.10.2015: Temp= TEMPERATURE, SpC=SPECIFIC CONDUCTIVITY, O₂ =DISSOLVED OXYGEN

		Temp °C	SpC μS cm ⁻¹	pH -	O ₂ mg L ⁻¹	NO ₃ ⁻ mg NO ₃ ⁻ -N L ⁻¹
04.-11. June 2015	mean	17.81	1063	8.42	9.37	2.86
	STD	2.57	46	0.27	2.01	0.32
	min	13.38	886	7.75	6.13	2.16
	max	23.79	1224	8.84	13.12	3.26
08.-11. Oct 2015	mean	11.22	951	8.21	10.48	2.75
	STD	2.75	59	0.10	0.91	0.28
	min	6.02	818	7.99	9.09	1.95
	max	15.32	1056	8.44	12.44	3.40

3.4.2.2. ESTIMATES OF NITRATE TURNOVER RATES BASED ON HPFM MEASUREMENTS

With an average water flow of $Q_{HZ} = 2.65 \times 10^{-5} \text{ m}^3 \text{ s}^{-1}$ through the assessed upper 50 cm of the hyporheic zone and across the 6 m width of the stream, 0.008 % of water transported in the river entered the hyporheic zone (**Table 3.4**).

TABLE 3.4 SUMMARIZED PARAMETERS OF NO_3^- TRANSPORT AND REMOVAL THROUGH THE UPPER 50 CM OF THE HYPORHEIC ZONE AT THE TEST SITE FOR THE TESTING PHASE FROM 04.06.-11.06.2015. RANGES ARE INDICATED FOR DIRECTLY MEASURED PARAMETERS, THE REMAINING PARAMETERS HAVE BEEN CALCULATED FROM LISTED MEANS.

	acronym	unit	mean	range
water flow through HZ	Q_{HZ}	L s^{-1}	0.0265	
% of river water entering HZ	$\%Q_{HZ}$	%	0.008	
Horizontal Darcy velocity	q_x	cm d^{-1}	76	20 - 116
average NO_3^- concentration in the HZ	$C_{\text{NO}_3 \text{ HZ}}$	$\text{mg NO}_3^- \text{-N L}^{-1}$	1.39	0.31 - 2.86
% NO_3^- entering the HZ which is removed	R_N	%	52	
potential NO_3^- load entering HZ	$M_{\text{HZ theory}}$	$\text{mg NO}_3^- \text{-N s}^{-1}$	0.08	
NO_3^- load measured in HZ	$M_{\text{HZ measured}}$	$\text{mg NO}_3^- \text{-N s}^{-1}$	0.037	

While the average surface water concentration was $2.86 \text{ mg NO}_3^- \text{-N L}^{-1}$, the average concentration in the subsurface measured with the HPFM was only $1.39 \text{ mg NO}_3^- \text{-N L}^{-1}$. Assuming that the difference between surface and subsurface concentration arose from hyporheic consumption of infiltrating NO_3^- , the average removal rate R_N was 52 %. For SRP the average surface water concentration from 4th to 11th June 2015 was $0.165 \text{ mg P L}^{-1}$, the average concentration in the hyporheic zone was 0.11 mg P L^{-1} .

3.5. DISCUSSION

The application of the HPFM for quantitative in situ measurement of horizontal NO_3^- and SRP fluxes through the hyporheic zone is novel. An earlier study on passive flux meter (SBPFM) in river beds (Layton, 2015) only assessed vertical flow of contaminants and is therefore not comparable to the application presented here. In the current work, adaptations were developed, tested and improved. Those include the choice of an appropriate resin, assessment of biofilm growth on the instruments and an approach that avoids challenges with contamination of the sorbent with nutrients. The results from the control HPFM showed that the uncertainty in measurement related to handling of the HPFM and processing of samples

as conducted in this study is acceptable. Finally, the minimum and maximum deployment time will depend on the Darcy velocity and nutrient concentrations at a study site. Since the values derived from the control incorporate all the processing steps of HPFM and samples, they can be regarded as the method detection limit *MDL* (Greenberg et al., 1992). The *MDL* defines the lower limit for the use of HPFM in cases where nutrient fluxes are very low and deployment time cannot be extended. Based on the accumulated values detected in the control, the minimum deployment time can be estimated. In systems with high nutrient concentrations, usually the flow velocity q_x will be the limiting factor. In our application the *MDL* for q_x derived from the control was 8.4 cm for the complete deployment time (7 days). If the method inherited uncertainty should not be more than 5 % of the total measurement, the product of duration (in days) and velocity (in cm) should be at least 168 (20 times 8.4). As an example: if measured q_x is around 200 cm d⁻¹, one day (24 h) of deployment is sufficient. The lowest q_x detected in our assessment was 21 cm d⁻¹ (in HPFM AC4, see figure 3f), so that actually a deployment duration of 8 days would have been optimal. The same estimation can additionally be derived for expected nutrient fluxes. In systems with low nutrient concentrations, it would be preferable to start estimating the minimum deployment time based on the nutrient fluxes. We recommend that a control HPFM is incorporated in each field application of HPFM in order to determine the specific *MDL*. The upper limit is given by the loading capacity of the resin or complete displacement of all resident alcohol tracers.

The high nutrient background on the AC required the separation of resin and AC in the HPFMs. We tested two different HPFM designs in this study, of which each inherits designated characteristics being more or less beneficial for different specifications: The first approach, pairs of two HPFM where one is used to assess the water flux and the second to capture nutrients is preferable if a highly resolved depth profile is needed (a heterogeneous horizontal flux in the vertical direction). Since this approach assumes that local horizontal heterogeneity is negligible in the range of 20-30 cm, we recommend this type only for the use in uniform systems such as channelized river reaches. Even in those systems however, small scale variability in stream bed and sediment characteristics can cause spatially heterogeneous flow distributions (Lewandowski et al., 2011; Mendoza-Lera and Mutz, 2013). The second approach with alternating nutrient sorbents and water flux measuring segments is therefore preferable in most other cases as long as a high resolution over the vertical profile is not required. In general, several HPFM should be grouped together in order to obtain representative results.

Further improvements of the HPFM for nutrient studies in the subsurface of rivers could be achieved by identifying a nutrient free carrier for the tracers. First, because this would allow measuring nutrient and

water flux at the same location within the device and thereby increase spatial resolution. Second, because in a mixed texture of nutrient absorber and tracer carrier the antibacterial nature of the activated carbon would suppress biofouling on the absorbent. We observed substantial biofilm growth on the resin in the laboratory and on the top 2 cm of the field-deployed HPFM R2. The results of the column experiments suggest that biofilm growth on the resin porous media did not affect its loading capacity. Further, biofilm growth was only visible on columns which were run beyond breakthrough, suggesting that considerable biofouling only started after the loading capacity of the tracer was exhausted. R2 detected higher NO_3^- fluxes in the top layer than the other HPFM. This could be due to contamination of the top layer of this HPFM with surface water (if the HPFM was not introduced sufficiently deep into the sediments). The further implication would be, that this layer was exposed to much higher water and nutrient infiltration, so that the loading capacity was exhausted before the end of the experiment allowing biofilm accumulation. At the current state it is unclear, to what extent the biofilm bound nutrients can be extracted by the procedure used here. Further experiments would also be needed to clarify under which conditions biofilm growth can occur and if bacterial uptake, transformation and release of nutrients influence the concentrations of nutrients inside the HPFM. HPFM segments on which biofilm is visible should be interpreted with caution. Finally, identifying a procedure or materials which completely inhibit biofouling will be an important step in the further development of HPFM.

In addition to instrumental adaptations we presented an installation procedure, which allows for smooth deployment with minimal disturbance of the system. Unlike typical well screen deployments where PFM (Annable et al., 2005; Verreydt et al., 2013) or SBPFM (Layton, 2015) have been inserted into a screened plastic or steel casing, our technique enabled the direct contact of the HPFM with the surrounding river sediments. Disturbing the natural structure of the sediment, potentially resulting in artificial flow paths is intrinsic to all intrusive techniques, including HPFM. Still, dispensing of a solid wall improves the integration of the HPFM in the natural system and prevents the generation of preferential flow paths along the wall of the device. Additionally, the HPFM include a measurement time that is long relative to the duration of the installation, suggesting that the presented method causes lower disturbance compared to other intrusive measurements. While the installation of mini-drive points or heat pulse sensors in sediments coarser than sand may be difficult or even impossible and also proved unfeasible at our field site, installation of the HPFM with the presented technique was successful. The correction for convergence of flowlines into the device or divergence around it is relatively simple and already incorporated in the equation for the flux calculation. Heterogeneous permeability of the hyporheic zone around the HPFM does not distort the correction term as long as the permeability of the surrounding is

coherently higher or coherently lower than the permeability of the HPFM matrix. Pre-measurements are therefore necessary for the selection of a suitable resin and tracer carrier. We believe that the presented approach and equations are applicable for a wide range of field conditions. However, for very coarse sediments, a protection of the HPFM with a solid screen might still be preferred. If fine particles are observed to bypass the mesh and enter the HPFM, a finer mesh should be chosen. We did not observe clogging of the mesh or intrusion of particles at our study, though in highly permeable systems with fine particle transport this might have to be considered.

A mayor advantage of the HPFM method is highlighted by the findings of the 7-day long field testing: In June, we found discrepancies between the average concentrations measured in the HPFM and the concentration found using the MLS. From our measurements it is not possible to prove that the HPFM results are correct and the MLS results biased. Nevertheless, the HPFM showed the expected decline in J_N with depths, whereas the MLS pore water concentrations were similar at all depths. This can be related to two reasons: First, we might have sampled surface water which bypassed along the wall of the MLS. The question would then be why that happened in June but not in October. Second, we might have sampled the MLS at a time point when the hyporheic zone was inactive in respect to nutrient processing. Considering the high diurnal amplitudes in hyporheic oxygen concentration, we assumed that the discrepancy between HPFM and MLS arose from oscillations in hyporheic nutrient concentrations similar to the oxygen pattern. Microbial consumption of O_2 in the sediments can, depending on nutrient concentration in surface water and transfer of these nutrients to the sediments, result in O_2 depletion in the subsurface. Especially in nutrient rich streams the related diurnal oscillations in O_2 concentration favor night time denitrification in the hyporheic zone (Christensen et al., 1990; Laursen and Seitzinger, 2004; Harrison et al., 2005; O'Connor and Hondzo, 2008; Nimick et al., 2011). The redox conditions in the subsurface may also regulate the mobilization/demobilization of phosphate (Smith et al., 2011). The repeated manual sampling of pore water from MLS in October showed diurnal variations of SRP and NO_3^- in the subsurface of the testing reach, supporting the hypothesis that diurnal cycles in benthic metabolism caused temporal variations in hyporheic SRP and NO_3^- concentrations at our study site. As the majority of sampling is commonly conducted during daylight hours, night time conditions are underrepresented in studies relying on single manual sampling events. Flux average concentrations can deviate by more than 50 % from estimates based on single event sampling, as was illustrated by comparison between our manual samples and the average pore water concentrations calculated from the HPFM data. Repeated pore water sampling at high frequencies can be used to determine diurnal dynamics. However, continuing this over a longer time span is laborious, whereas if only few single time specific snap shot samplings are

conducted, the results may not realistically represent the overall conditions at the target site. Our comparison between MLS and HPFM reinforce the need for long term recording of nutrient transport through the hyporheic zone. In general, most of our knowledge on hyporheic nutrient dynamics is based on measured surface water dynamics and models which project these dynamics on hyporheic processing. Theoretically, we could measure nutrient fluxes in the hyporheic zone and estimate whole stream uptake rates from these measurements. However, the substantially higher effort to obtain subsurface data is not justified in most cases. As long as the overall in-stream retention is the focus, surface water monitoring will remain the method of choice. Innovative tracer experiments may even allow quantifying hyporheic exchange in streams. Haggerty et al. (2009) proposed a “smart” tracer approach, where the injected substance resazurin converts irreversibly to resofurin under metabolic activity. While a promising tool for detecting metabolic activity at the sediment-water interface in streams, first, uncertainties about sorption and transformation characteristics of these tracers remain (Lemke et al., 2013) and second, those methods give no evidence about nutrient transport to those reactive sites.

Thus, whenever the nutrient processing function of the hyporheic zone and its quantitative contribution to stream nutrient retention is of interest, for example in the evaluation of restoration measures including a rehabilitation of the river bed, direct measurements of hyporheic fluxes are indispensable. The HPFMs are a valuable approach that can be efficiently used to characterize and quantify nutrient dynamics in a sediment system. We consider that a combination of HPFM, MLS and concurrent measurements of pore water oxygen concentrations, as presented in this study, provide a practical set-up to interpret hyporheic nutrient dynamics.

Like solute concentrations and water flow patterns, the vertical extension of the hyporheic zone varies in time and space and between different rivers and reaches. Our set-up assessed exclusively the upper 50 cm of the hyporheic zone. We found continuously decreasing NO_3^- concentrations with depths, suggesting that this entire area (and potentially deeper) of the subsurface contained active sites for nitrate removal. While it was stated that denitrification is limited to the upper few cm of the hyporheic zone close to the sediment-water interface (Hill et al., 1998; Harvey et al., 2013), our results are in accordance to findings by Zarnetske et al. (2011b) and Kessler et al. (2012) who also report extended active hyporheic zones. Conducting collateral tracer tests, as suggested for example by Abbott et al. (2016), could deliver further evidence and characterize distinct flow paths. Nevertheless, since vertical water movement was overall downward and the lowest concentrations of NO_3^- were observed in the deepest segments of the HPFM,

it is very likely that the hyporheic zone at our study site extends deeper than the 50 cm evaluated. The length of an HPFM can easily be increased, depending on the individual site conditions.

Considering the high spatial heterogeneity of the hyporheic zone, a larger number of HPFM would be needed to derive reliable and statistically supportable rates of hyporheic nutrient dynamics. The following example aims to display further possibilities of interpreting HPFM measurements. At our study site, the hyporheic removal potential R_N of more than 50 % of infiltrating NO_3^- and 30 % of SRP suggests an active hyporheus. Evaluation of the effect of hyporheic removal activity on overall NO_3^- removal in the stream or the normalization of hyporheic uptake to a benthic area requires a flow path length. In the presented example, this length refers to the horizontal vector of the distance the water travels in the subsurface before infiltrating the HPFM. The horizontal vector can be derived from the residence time of water and solutes in the hyporheic zone τ_{HZ} and the horizontal Darcy velocity q_x . Assuming a downward flow direction, τ_{HZ} could be inferred from the vertical Darcy velocity q_y as assessed from the temperature profiling and the hyporheic zone depths of 50 cm. Thereafter, τ_{HZ} conceptually corresponds to the time the water travels through the hyporheic zone before exiting to groundwater and s_{HZ} to the horizontal vector of the flow paths. The nitrate uptake rate $U_{\text{NO}_3\text{-HZ}}$ ($\text{mg NO}_3^-\text{-N m}^{-2} \text{ d}^{-1}$) is then the difference between the theoretically transported NO_3^- mass $M_{\text{NO}_3\text{-HZ theor}}$, which is the product of Q_{HZ} and $C_{\text{NO}_3\text{-SW}}$ and the measured mass flux $M_{\text{NO}_3\text{-HZ real}}$. During the testing phase $U_{\text{NO}_3\text{-HZ}}$ was calculated as $693 \text{ mg NO}_3^-\text{-N m}^{-2} \text{ d}^{-1}$. The same procedure yields a removal (uptake or adsorption) rate for SRP of $U_{\text{PO}_4\text{-HZ}} = 24 \text{ mg PO}_4^-\text{ m}^{-2} \text{ d}^{-1}$. Calculating $U_{\text{NO}_3\text{-HZ}}$ in the same way for each single depth assessed with the HPFM can deliver additional information about vertical gradients on nutrient processing rates and help to identify the most active depth in hyporheic zone. $U_{\text{NO}_3\text{-HZ}i}$ of a particular layer in the hyporheic zone can be derived by the differences in uptake rate between the regarded layer and the overlying layer. For instance the removal rates attributed to the different layers of HPFM L6 would be $U_{\text{NO}_3\text{-HZ}15} = 567 \text{ mg NO}_3^-\text{-N m}^{-2} \text{ d}^{-1}$ in the shallow layer (0 to 15 cm depths), $U_{\text{NO}_3\text{-HZ}30} = 174 \text{ mg NO}_3^-\text{-N m}^{-2} \text{ d}^{-1}$ in the layer from 15 to 30 cm depths and $U_{\text{NO}_3\text{-HZ}45} = 256 \text{ mg NO}_3^-\text{-N m}^{-2} \text{ d}^{-1}$ in the deepest layer from 30 to 45 cm depths. From this example one could conclude that the shallowest sediments are the most efficient ones in term of nitrate removal. While removal activity is first declining with depths it later increases again. This finding is consistent with the higher amplitudes of oxygen concentration in 45cm depths compared to 25 cm depths, also suggesting higher biotic activity at the deepest layer. Potential reasons for this pattern could be decreasing NO_3^- penetration with depth (lower uptake at the middle layer than the shallowest one) which is in the deepest parts counter balanced by increased residence time and stronger reducing conditions.

3.6. CONCLUSIONS AND OUTLOOK

The role of the hyporheic zone as a hotspot for instream nutrient cycling is indisputable (Mulholland et al., 1997; Fellows et al., 2001; Fischer et al., 2005; Rode et al., 2015). Quantitative and qualitative knowledge about the influence of mass transfer on hyporheic nutrient removal is crucial to manage streams and river, especially in the light of increasing worldwide morphological alterations (Borchardt and Pusch, 2009), eutrophication (Ingendahl et al., 2009) and sediment loading (Hartwig and Borchardt, 2015). Despite decades of research on hyporheic nutrient cycling, robust quantitative data on nutrient fluxes through the hyporheic zone are limited, which is mainly due to methodological constraints in measuring nutrient concentrations and water flux in the subsurface of streams (O'Connor et al., 2010; Boano et al., 2014; Gonzalez-Pinzon et al., 2015). Passive flux meters have the potential to fill the gap in measured quantitative nutrient fluxes to the reactive sites in the sediments of rivers. Up to date, HPFM are virtually the only method which can simultaneously capture nutrient and water flux through hyporheic zone within the same device and at the same spatial location. The field testing of several devices proved the general applicability of passive flux meters for quantifying NO_3^- and PO_4^- flux to reactive sites in the hyporheic zone. The hyporheic flux rates of nutrients and nitrate uptake rates, measured in an agricultural 3rd order stream, were generally in agreement with rates reported in the literature. Our results clearly highlight the advantages of HPFM compared to commonly used methods (i.e. grab sampling of pore water and separate measurements of hyporheic exchange and Darcy velocities), first of all the capacity to integrate over longer time periods.

Quantifying nutrient flux to the potentially reactive sites in the hyporheic zone is an essential step to further improve our process based knowledge on hyporheic nutrient cycling. In the future, long-term measurements of nutrient fluxes as obtained from HPFM can feed into and advance the transport part of nutrient cycling models.

We anticipate further improvement and increased use of passive flux meter approaches in order to advance conceptual models of nutrient cycling in the hyporheic zone. We demonstrated modifications which extended PFM application from groundwater to hyporheic zones. Current limitations related to the potential bias of results due to biofilm growth on sorbents require further analysis for the identification of more suitable sorbents. While we focused on nutrients, PFM may also be used for a wide range of other substances like contaminants or trace elements.

Being labor efficient and attractive with respect to relatively low costs, numerous HPFM can be efficiently used to cover larger areas and assess the degree of local heterogeneity. Further, neither advanced technology, maintenance, or power supply are needed which can be extremely advantageous for the use in remote areas or study sites without infrastructure.

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4. HYPORHEIC PASSIVE FLUX METERS REVEAL UNEXPECTED SOURCE-SINK BEHAVIOR OF NITROGEN IN AN ANTHROPOGENICALLY MODIFIED STREAM (HOLTEMME, GERMANY)

4.1. ABSTRACT

Transformation and retention of nitrogen and other biologically reactive solutes in the hyporheic zones of running water is an essential ecosystem service. However, the source-sink behavior of hyporheic zones in anthropogenically modified streams and rivers impacted by intense agricultural or urban land-use, elevated nutrient loading, flow alterations, riparian clear-cutting and channelization remains largely uncharacterized. Therefore, we studied nutrient dynamics in a hydro-morphologically and chemically modified stream reach using a new monitoring approach (Hyporheic Passive Flux Meter). The sampling setup comprised time-integrated, direct measurements of lateral hyporheic nitrate fluxes during two campaigns in early spring and mid-summer covering different temperature and discharge regimes.

Contrary to our expectations, higher stream discharge coincided with substantially lower hyporheic exchange rates. While in streams featuring a natural morphology, bed-form induced hyporheic exchange increases with surface flow velocities, the influence of groundwater level was dominant in this reach with monotonous bed structure. Furthermore, in contrast to low-impact environments, where progressive substrate depletion with depths reduces metabolic rates in the subsurface, we identified not the upper layers, but the intermediate depths (15-35 cm) of the hyporheic zone as the hotspot for nutrient turnover. Overall, the hyporheic zone at the study site functioned partly as nitrate source, but partly also as a sink. However, neither of the commonly used determinants redox state and residence time could explain the source or sink function. Instead, anthropogenically induced fluctuations of water quality constituents were the governing factor. Our results have implications to carefully transfer the knowledge of hyporheic zone processes from “natural” systems to anthropologically modified streams.

4.2. INTRODUCTION

The hyporheic zone, the subsurface region of streams that is permeated with stream water (Valett et al., 1993) also encompasses a zone where stream-water mixes with underlying groundwater (Fleckenstein et al., 2010; Trauth et al., 2014; Triska et al., 1989) and is a key compartment for stream ecosystem functioning (Baker et al., 2000a; Fellows et al., 2001; Findlay et al., 2011; Boulton et al., 2010). The interactions between hydrological, physical, biological and chemical processes in this dynamic ecotone create conditions for intense biogeochemical processing, such as nutrient cycling (Rode et al., 2015; Fischer et al., 2005; Mulholland and Webster, 2010). This reactivity of hyporheic zones may enhance the in stream nutrient retention significantly and therefore provide an important regulating ecosystem service on water quality. Maintaining this ecological function has consequently become a focus of recent management strategies and river restoration projects (Schirmer et al., 2014; Boulton et al., 2010). Therefore, quantifying reach specific rates and short-term as well as inter-seasonal variations of hyporheic nutrient retention capacities and identifying governing factors is essential for integrated management strategies.

In recent decades, numerical and empirical models have enhanced our understanding of the factors which influence hyporheic nutrient dynamics (Trauth et al., 2015; Boano et al., 2014; Fennel et al., 2009). Flow path length and residence time are the most determinant physical factors (Briggs et al., 2014; Zarnetske et al., 2011b; Hill et al., 1998). Both variables are directly coupled with surface-subsurface exchange rates of water and solutes and Darcy velocities in porous sediments. Exchange and transport rates are attributed to stream discharge (Böhlke et al., 2009; Harvey et al., 2013), groundwater level (Trauth et al., 2014; Voltz et al., 2013), sediment characteristics (Pinardi et al., 2009; Hartwig and Borchardt, 2015) and streambed morphology. Superposed on the resulting complex hyporheic transport patterns are short and long term fluctuations in biogeochemical processing rates. In this regard, the redox state is besides the availability of dissolved organic carbon (DOC), nutrient composition, pH and temperature, one of the most important governing factors (Zarnetske et al., 2011a; Arango et al., 2007; Strauss et al., 2002).

In particular with respect to nitrate (NO_3), the role of the hyporheic zone is multifaceted: Under low O_2 conditions, NO_3 may be consumed by denitrifying bacteria residing in “reactive sites” in the hyporheic zone (Nimick et al., 2011; Rode et al., 2015; Laursen and Seitzinger, 2002), whereas when O_2 concentrations are higher, nitrification of Ammonium (NH_4^+) in the hyporheic zones can as well result in net hyporheic NO_3 production (Hedin et al., 1998; Bartoli et al., 2012; O'Connor and Hondzo, 2008; Strauss

et al., 2004). This complex NO_3 source-sink function of hyporheic zones and its local and temporal variability has received increasing scientific interest. Overall, the residence time of water and solutes in the hyporheic zone in relation to the O_2 consumption rate are the predominant determinants on hyporheic nitrate production or reduction (Briggs et al., 2014; Zarnetske et al., 2011). Resupply of nutrients, substrates and O_2 are controlled by the highly variable hyporheic exchange. While light-induced primary production and concurrently the corresponding O_2 concentration and redox conditions in the pore-water may vary on a diurnal basis, temperature regulates the metabolic activity and inherent nutrient transformation rates on a seasonal basis. Thus, both nitrification as well as denitrification increase under higher temperatures (Demars et al., 2011; Berounsky and Nixon, 1990; Garcia-Ruiz et al., 1998) whereas nitrogen (N) assimilation rates are additionally influenced by solar insolation. Overall, instream NO_3 consumption is presumably highest in the warm months. However, not only the overall N processing rate but also the dominance of different processes can vary greatly throughout the year (Kunz et al., 2017b), resulting in a time varying importance of hyporheic zone processes in total instream NO_3 dynamics.

From the foregoing discussion, it is evident that anthropogenic alterations in hydrology and riparian conditions (e.g. Mendoza-Lera and Mutz, 2013; Boano et al., 2010; Packman et al., 2004; Hill et al., 1998; Clifford, 1993) can have wide ranging impacts on hyporheic functions, including nutrient processing (Hester and Gooseff, 2010; Hancock, 2002). Even though, river networks with anthropologically modified characteristics (intense agricultural and urban land-use, elevated nutrient loading, flow alterations and channelization) are widespread worldwide, the functioning of hyporheic zones and specifically the source-sink behavior of NO_3 and other biologically reactive solutes in altered systems is mostly still uncharacterized (Borchardt and Pusch, 2009)

Empirical evidence is needed to test conceptual frameworks and hypothesis regarding conditions which might enhance or reduce the functioning of the hyporheic zone (Boulton et al., 2010). Common techniques for in situ measurements of hyporheic solute dynamics usually have two major deficiencies: First, instantaneous point measurements only display the conditions at a specific location for a specific sampling time, which cannot account for the high short-term variability associated with hyporheic fluxes. Due to the uncertainty arising from this short-term fluctuation, instantaneous measurements are unsuitable to compare distinct systems or seasonal effects within one single location. Also, routine monitoring based on grab samples commonly omits night periods and could miss important extremes such as storm events (Fovet et al., 2016; Bieroza et al., 2014). Second, if Darcy flow velocities and nutrient

concentrations are measured using separate devices, the local heterogeneity of sediment and bed-form properties and the resulting range in fluxes can lead to increased errors.

Kunz et al. (2017a) demonstrated the feasibility of deploying a new hyporheic passive flux meter (HPFM) to assess time-integrated hyporheic P and N fluxes in an agricultural stream in Central Germany. HPFM allow for estimating nutrient fluxes, averaged over several days of continuous recording.

Here we used a combined set-up of several HPFM, repeated manual multilevel pore-water sampling and high resolution data from interstitial temperature and oxygen loggers to directly assess seasonal and diurnal characteristics of hyporheic nutrient transport and processing in a stream subjected to anthropogenic alterations of bed-structure, channel morphology, water quality and hydrology. The aim of this study was to test whether the current framework on influencing factors holds for an anthropogenically modified stream. Specifically, we addressed the NO_3 source-sink function of the hyporheic zone and evaluated the impact of human activities on hyporheic nutrient retention capacities. To support this goal, we developed a conceptual model for generating hyporheic NO_3 removal or production rates from directly measured nutrient fluxes.

4.3. METHODS

We conducted two field campaigns each lasting several days, one in summer and one in early spring, expecting that with higher temperatures and increased insulation the productivity would increase biotic turn-over rates in summer, whereas higher stream discharge would result in enhanced hyporheic exchange and nutrient transport to the reactive sites in the hyporheic zone. We hypothesized that nutrient resupply to the hyporheic zone would be the limiting factor for nutrient turnover during low flow in summer. In early spring, reduced biotic activity together with shorter residence times due to higher discharge and higher hyporheic flow velocities would result in a diminished nutrient processing capacity. Finally, we examined whether diurnal fluctuations in pore-water oxygen concentration were reflected in variations of hyporheic NO_3 uptake or production.

4.3.1. STUDY SITE

A 30 m long stretch of the Holtemme River, a 3rd order stream in the Bode catchment, TERENO Harz/Central German Lowland Observatory, served as study site (51°56'30.1"N, 11°09'31.8"E). In the recent years this stream received increased scientific attention due to its strong gradient in water and structural quality. The 46 km long Holtemme originates in the Harz Nature Reserve, draining a catchment of 278 km². While the upper reaches feature a natural morphology with small cascades and waterfalls, good ecological status and water quality, anthropogenic influence increasingly impairs the stream during its course until it discharges into the Bode River (Kamjunke et al., 2013). Long stretches have been subjected to changes in the natural river morphology by canalization. Even though most of the 23 small hydropower stations have been decommissioned, the weirs still impact the hydrology and ecological connectivity (Wasserwirtschaft, 2009). The study reach is located in the lowest part of the Holtemme, where the water chemistry is highly impacted by urban effluent and agriculture. In these last kilometers the Holtemme is straightened (sinuosity = 1.05) with dammed banks and absent riparian vegetation. The cross sectional profile has a rectangular form with a low width/depth variation.

The sediment at the study site is mainly sandy with gravel and cobbles. Sieving of sediment samples yielded an effective grain size d_{10} = 0.8 mm and a coefficient of uniformity C_u = 3.13. The effective porosity n_{ef} is 13 %. After Fetter (2001) the intrinsic permeability can be estimated at k_i = 96 m² and the hydraulic conductivity K = 81 m day⁻¹. Mean discharge is 1.35 m³ s⁻¹ with highest peaks around 56 m³ s⁻¹ (LHW, 2016). Discharge is continuously recorded by the local authorities at the gauge Mahndorf, 15 km upstream of the testing site. In the course of the year, NO₃ concentrations in the lower Holtemme vary between 2 and 8 mg NO₃-N L⁻¹.

Channel width, depths and velocity were measured on 7.10.2014 and 5.10.2015. Parameters for the relevant periods were derived from regression to the continuous discharge readings and data provided by the local authorities (**Table 4.1**).

TABLE 4.1 SELECTED MORPHOLOGICAL AND HYDROLOGICAL PARAMETERS FOR THE STUDY SITE DURING THE FIELD CAMPAIGNS. Q_{SW} AND $C(NO_3^-)_{SW}$ ARE AVERAGES OF THE CONTINUOUS RECORDS FOR THE ASSESSMENT DURATION.

Parameter	acronym	June '15	April '16	unit
Cross sectional area	A_{SW}	3.41	5.76	m ²
Mean depth	h	0.50	0.86	m
Mean width	w	6.03	6.70	m
mean velocity	V_{SW}	0.10	0.22	m s ⁻¹
Average discharge	Q_{SW}	0.32	0.89	m ³ s ⁻¹
NO ₃ ⁻ -N concentration	$C(NO_3^-)_{SW}$	2.86	2.80	mg L ⁻¹

4.3.2. INSTRUMENTAL SET-UP

Two field campaigns were conducted from the 4th to 11th of June 2015 and from the 1st to 4th of April 2016. Pore-water sampling was additionally repeated on the 8th of October 2015, due to inconsistent results from the first experiment. Surface water temperature during the sampling days in June 2015 ranged from 17 to 25°C, in October 2015 ranged from 14 to 18°C and in April 2016 ranged from 6 to 14°C. During the campaigns, instruments were installed as illustrated in **Figure 4.1**.

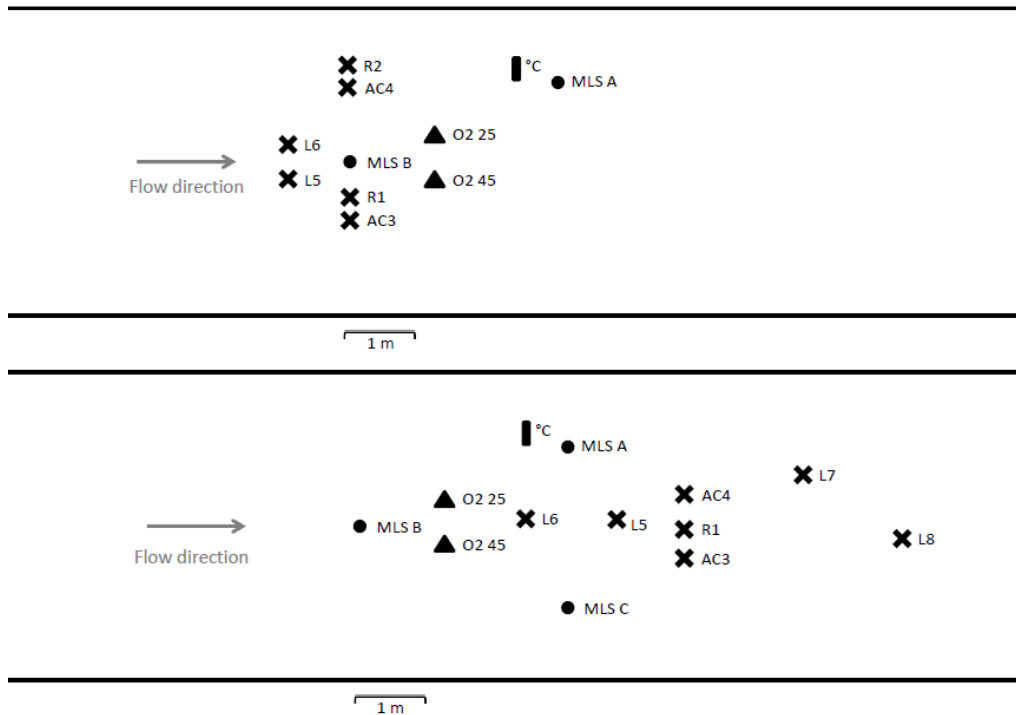


FIGURE 4.1: INSTRUMENTAL SET UP AT THE TESTING REACH IN HOLTEMME IN JUNE 2015 (TOP) AND APRIL 2016 (BOTTOM). MLS: MANUAL MULTILEVEL PORE WATER SAMPLER; °C: TEMPERATURE PROFILING; O2: OXYGEN TIME SERIES AT 25 AND 45 CM BELOW SURFACE WATER –SEDIMENT INTERFACE; R1, R2: RESIN ONLY HPFM; AC3, AC4: ACTIVATED CARBON ONLY HPFM; L5, L6,L7,L8: ALTERNATING LAYERS HPFM.

4.3.2.1. HYPORHEIC PASSIVE FLUX METER (HPFM)

General device properties

Six Hyporheic passive flux meters (HPFM) in June 2015 and seven in April 2016 constitute the core of the present work. HPFM were constructed using a sorbent filled nylon mesh which is deployed in the river bed (**Figure 4.2**) (Kunz et al., 2017a). During the time the HPFM resided in the hyporheic zone, it absorbs infiltrating nutrients, which can be extracted and analyzed after retrieval of the HPFM. The corresponding amount of water passing through the HPFM is assessed using the elution curves of resident alcohol tracers which are loaded on the sorbent before deployment.

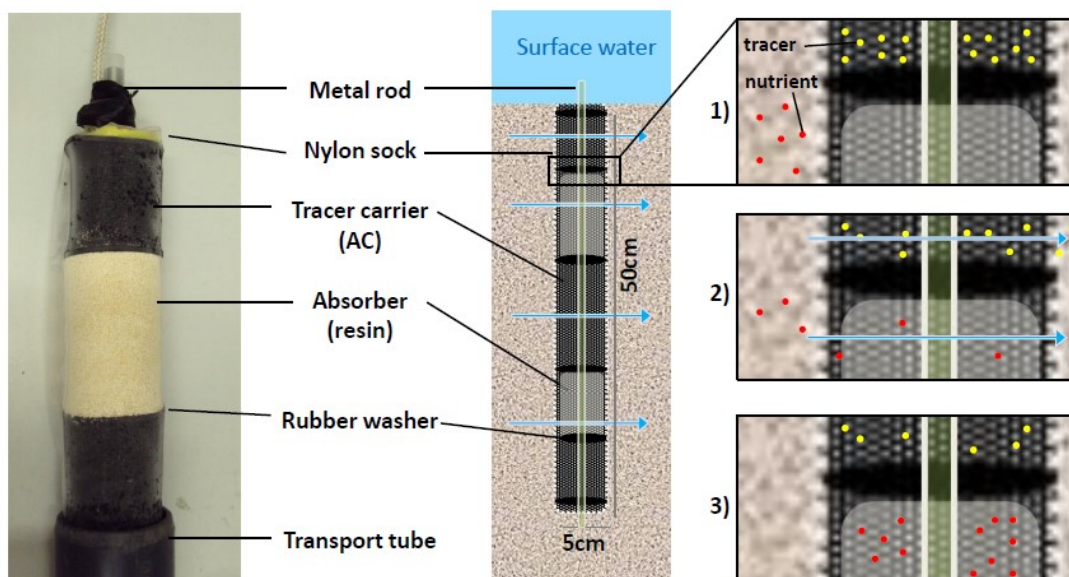


FIGURE 4.2 PHOTOGRAPH OF AN HPFM WITH ALTERNATING SEGMENTS BEFORE DEPLOYMENT (LEFT), SCHEMATIC PROFILE OF A DEPLOYED HPFM (MIDDLE) AND SCHEMATIC STEPS OF HPFM FUNCTIONING (RIGHT): 1) DIRECTLY AFTER INSTALLATION, TRACER RESIDES ON ACTIVATED CARBON (AC), 2) INFILTRATING WATER WASHES OUT THE TRACER, NUTRIENTS ENTER THE HPFM AND ARE ABSORBED ON THE RESIN, 3) AFTER RETRIEVAL NUTRIENTS ARE FIXED ON THE RESIN, TRACER CONCENTRATION IS DILUTED (KUNZ ET AL., 2017a).

Silver impregnated activated carbon (AC) served as sorbent for the resident alcohol tracers. The anion exchange resin Purolite® A500 MB Plus was used for sorption of nutrients. Specific sorption and extraction behavior, physical characteristics and loading capacity have been reported recently by (Kunz et al., 2017a). For more details on the sorbents, tracers and the equations to calculate the horizontal Darcy velocity q_x and the nutrient flux J_N through the screened layer of the hyporheic zone please refer to **APPENDIX 4A**. After retrieval NO_3 , total nitrogen (TN) and soluble reactive phosphorous (SRP) were extracted from the HPFMs and analyzed in the laboratory of the UFZ analytical department in Magdeburg, Germany (for details on the extraction procedure see **APPENDIX 4A** or Kunz et al. (2017a))

The HPFMs for this study were constructed 50 cm long and 5 cm in diameter. Rubber washers divided the HPFM into 5 to 7 segments so that a vertical profile of horizontal water and nutrient fluxes in the hyporheic zone could be measured.

HPFMs were kept in sealed transport PET tubes and cooled in order to avoid tracer loss during storage or transport.

Installation for the presented campaigns

We used two types of HPFMs (For details see **APPENDIX 4A**):

- A) Pairs of resin only and AC only HPFMs
- B) Alternating segments of AC and resin

As quality control we used one additional HPFM with alternating layers assessing potential tracer loss or nutrient uptake during storage, transport, deployment/retrieval, storage and analytical processing of samples. This control was stored and transported together with the other HPFMs. After deploying the control HPFM, it was immediately retrieved, transported back to the laboratory and stored until it was sampled and analyzed along with the other HPFMs. Measurements of the other HPFMs were corrected by subtracting the values detected in the blank.

Installation of HPFMs in the river bed and retrieval after the measuring phase was conducted according to Kunz et al. (2017a) (also see **APPENDIX 4A**). After retrieval, HPFMs transported to the laboratory, where each HPFM was segment wise cut open, homogenized and subsampled into glass vials for further analysis.

4.3.2.2. MANUAL MULTILEVEL PORE WATER SAMPLING

Manual pore water samples were collected from three multilevel samplers (MLS) at depths of 5, 15, 25 and 45 cm below sediment-surface water interface. MLS consist of an outer steel tube of 50 cm length and 5 cm diameter, which is introduced in the sediment (Saenger and Zanke, 2009). The 4 extraction depths were sampled successively as described in detail in Kunz et al. (2017a). Each sample was filtered into a separate glass vial and later analyzed in the laboratory at UFZ. Analysis for NO_3 , SRP, sulphate (SO_4), DOC and Boron (B) were conducted. Analytical procedure for NO_3 and SRP was according to the description in **APPENDIX 4A**. SO_4 and B were used as natural tracers for groundwater and surface water respectively. SO_4 was analyzed on an ion chromatograph (ICS 3000, ThermoFisher), B was analyzed on an inductively coupled plasma mass spectrometer (ICP-MS 7500s, Agilent).

For the DOC analysis, water samples were filtered through 0.45 μm pre-glowed Millipore Filter and adjusted to a pH below 4 with 20 % hydrochlorid acid. DOC was then analyzed by thermic-catalytic oxidation to CO_2 and subsequent IR spectroscopy on a DIMATOC 2000 (DIMATEC Analysentechnik GmbH) (detection limit 0.349 mg C L^{-1}).

Manual pore-water samples were taken on the 4th and 11th of June 2015, both times between 1 pm and 4 pm local time. On the 8th of October 2015 and the 1st and 4th April 2016 each MLS was sampled twice, the first time in the early morning before sun rise and again in the early afternoon (around 2 pm).

4.3.2.3. SURFACE WATER CHEMISTRY

Surface water was monitored with a multi-parameter probe YSI 6600 V2/4 (YSI Environmental, Yellow Springs, Ohio) recording the following parameters: pH (precision 0.01 units, accuracy ± 0.2 units), specific conductivity (precision 0.001mS cm^{-1} , accuracy ± 0.5 %), dissolved O_2 (precision 0.01 mg L^{-1} , accuracy $\pm 1\%$), temperature (precision $0.01\text{ }^\circ\text{C}$, accuracy $\pm 0.15\text{ }^\circ\text{C}$). NO_3 time series were recorded with a UV absorption sensor (ProPS WW, TriOS). The pathway-length of the optical sensor was 10 mm, measuring at wavelengths 190-360 nm with a detection limit of $0.03\text{ mg NO}_3\text{-N L}^{-1}$ and an accuracy of ± 2 %. The measurement time step for both probes was set to 15 min.

4.3.2.4. SUBSURFACE O₂ TIME-SERIES

Time-series for subsurface O₂ concentrations were obtained from two O₂ loggers (miniDO₂T, Precision measurement engineering Inc.) which were installed in the river bed in screened steel casings at depths of 25 and 45 cm below sediment-water interface. The measurement time step was 5 min. Installation of the loggers was carried out 4 weeks prior to each experiment, allowing enough time for re-equilibration of the surrounding media. In April, due to technical issues we could only measure oxygen during the week before the campaign.

4.3.2.5. VERTICAL DARCY FLOW Q_y

A multi-level temperature sensor (Umwelt und Ingenieurtechnik GmbH, Dresden, Germany) was installed at the test site from January 2015 to April 2016. Temperature was recorded at the sediment-water interface and at depths of 0.10, 0.125, 0.15, 0.2, 0.3 and 0.5 m in the sediment at a 10 min interval (accuracy of 0.07 °C over a range from 5 to 45 °C, and a resolution of 0.04 °C). According to Schmidt et al. (2014) vertical Darcy flow velocities q_y were computed from the temporal shift of the daily temperature signal in the subsurface water relative to the surface water. Therefor a numerical solution of the heat flow equation was used in conjunction with Dynamic Harmonic Regression signal processing techniques for the analysis of the temperature time series. The model code was provided by Schmidt et al. (2014).

4.3.3. DERIVING HYPORHEIC DENITRIFICATION RATES

Flux and denitrification rates for the specific conditions at the study site during the HPFM testing phase were calculated using the channel morphological and hydrological parameters summarized in **Table 4.1** and averaged J_N and q_x values measured in the HPFM during the field campaigns.

The downstream angle γ of hyporheic flow can be derived from

$$\tan \gamma = \frac{q_y}{q_x} \quad (4.1)$$

The average NO₃ mass flow per unit depth in the surface water $M(NO_3)_{SW}$ (mg NO₃-N m⁻¹ d⁻¹) during deployment time was calculated as the product of average NO₃⁻ concentration in the surface water $C(NO_3)_{SW}$ (mg NO₃-N L⁻¹) and average river discharge Q_{SW} (m³ s⁻¹), divided by river depths h (m).

$$M(NO_3)_{SW} = \frac{C(NO_3)_{SW} \times Q_{SW}}{h} \quad (4.2)$$

Similarly, the NO_3 mass flow per unit depth through the hyporheic zone $M(\text{NO}_3)_{\text{HZi real}}$ ($\text{mg NO}_3\text{-N m}^{-1} \text{d}^{-1}$) was calculated for 3 different layers i defined by the central depths of the HPFM segments d_i (m), with $d_{0-15} = 0.10$, $d_{15-35} = 0.25$ and $d_{35-50} = 40$. For each campaign we averaged the values from all HPFM for the different depths.

$$M(\text{NO}_3)_{\text{HZi real}} = \frac{C(\text{NO}_3)_{\text{HZi}} \times Q_{\text{HZi}}}{d_i} \quad (4.3)$$

$$\text{with } Q_{\text{HZi}} = q_{xi} \times A_{\text{HZi}} \quad (4.4)$$

$$\text{and } A_{\text{HZi}} = w \times d_i \quad (4.5)$$

$C(\text{NO}_3)_{\text{HZi}}$ ($\text{mg NO}_3\text{-N L}^{-1}$) is the average NO_3 concentration at the corresponding HPFM segment depth obtained by normalizing the mass flux J_{Ni} for water flow v_{xi} by dividing J_{Ni} by q_{xi} , q_{xi} (m s^{-1}) is the average horizontal Darcy velocity measured in the HPFMs, w (m) is channel width and A_{HZi} (m^2) is the assessed hyporheic cross sectional area.

The denitrification rate U_{HZi} ($\text{mg NO}_3\text{-N m}^{-2} \text{d}^{-1}$) is the difference between the theoretically transported NO_3 mass flux $M(\text{NO}_3)_{\text{HZi theor}}$ and the measured mass flux $M(\text{NO}_3)_{\text{HZi real}}$.

$$U_{\text{HZi}} = \frac{M(\text{NO}_3)_{\text{HZi theor}} - M(\text{NO}_3)_{\text{HZi real}}}{S_{\text{HZ}}} \quad (4.6)$$

$$\text{with } M(\text{NO}_3)_{\text{HZi theor}} = C(\text{NO}_3)_{\text{SW}} \times Q_{\text{HZi}} \quad (4.7)$$

The normalization of U_{HZi} to a benthic area requires the length of the hyporheic flow path s_{HZ} (m) (**Figure 4.3**).

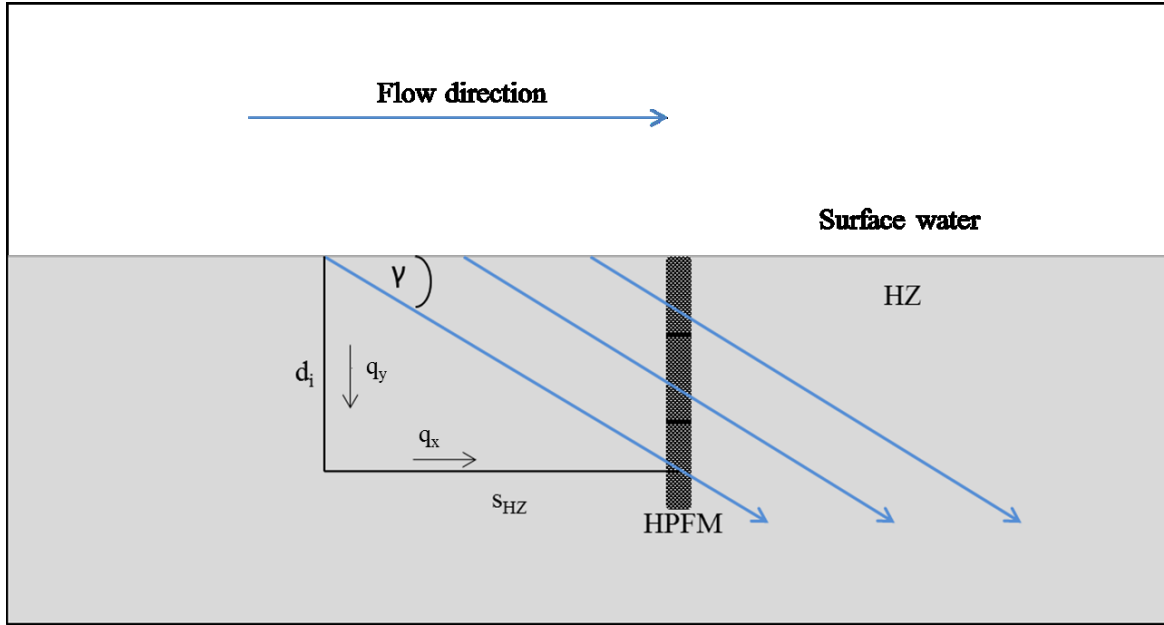


FIGURE 4.3. CONCEPTUAL MODEL OF HYPORHEIC FLOW VECTORS. THE HPFM SCREEN AREA IS MARKED AS A BLACK GRID. γ ANGLE OF THE HYPORHEIC FLOW LINES, q_x HORIZONTAL VECTOR OF THE DARCY VELOCITY, s_{HZ} HORIZONTAL VECTOR OF HYPORHEIC FLOW PATH, q_y VERTICAL VECTOR OF THE DARCY VELOCITY, d_i CORRESPONDING DEPTH IN THE HYPORHEIC ZONE.

Assuming physical flux does not vary along a single flow path, the vertical vector of this flow path is given as the Darcy velocity q_y (m s^{-1}) as assessed with the temperature profiling. Accordingly, τ_{HZi} (h) conceptually corresponds to the time the water travels through the hyporheic zone before it passes segment i of the HPFM. The vertical vector of the flow paths s_{HZi} can thereafter be calculated as

$$s_{HZi} = \tau_{HZi} \times q_{yi} \quad (4.8)$$

$$\text{with } \tau_{HZi} = \frac{d_i}{q_{yi}} \quad (4.9)$$

The NO_3^- removal activity R_{Ni} (%) for each of the three layers d_{0-15} , d_{15-35} and d_{35-50} was calculated from the difference in surface water concentration $C(\text{NO}_3)_{SW}$ and the observed average concentration $C(\text{NO}_3)_{HZi}$.

$$R_{Ni} = \frac{C(\text{NO}_3)_{SW} - C(\text{NO}_3)_{HZi}}{(C(\text{NO}_3)_{SW} + C(\text{NO}_3)_{SW})/2} \times 100 \quad (4.10)$$

4.2. RESULTS

4.2.1. HYPORHEIC NUTRIENT FLUXES AND DARCY VELOCITIES

During both campaigns all HPFMs showed a consistent vertical gradient in lateral Darcy velocity q_x . In April, the HPFM R2 was destroyed during installation and is therefore not represented in the data and graphs. On average, q_x was more than twice as high in June 2015 compared to April 2016 (**Figure 4.4.**). The seasonal difference in vertical Darcy velocities q_y was even more marked: q_y computed from the temperature profiles were with averages of 47 versus 7 cm d⁻¹ 7 times higher in June 2015 than in April 2016. The local angle γ resulting from the relationship between q_x and q_y was 32° in June and 12° in April.

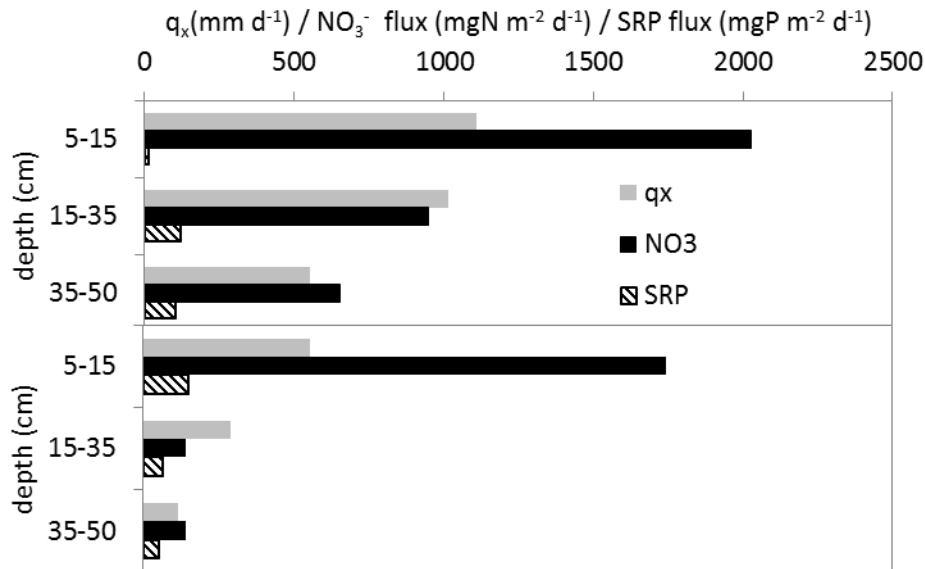


FIGURE 4.4. AVERAGE HORIZONTAL DARCY VELOCITY q_x AND CORRESPONDING NO_3 AND SRP FLUXES IN JUNE 2015 (TOP) AND APRIL 2016 (BOTTOM).

A gradual decline in NO_3 flux over depth was detected in all except one HPFM in June 2015. The pattern in April 2016 was more scattered: At most locations high NO_3 fluxes were observed in the 5-15 cm layer, lowest fluxes in the middle layers and a slight increase in the lowest layers. In June NO_3 comprised between 66 to 70 % of TN over all layers. In April NO_3 made 84 % of TN in the 5-15 cm layer, but only 44 and 48 % of TN in the deeper layers respectively. While SRP in general tended to increase with depths in June 2015 it showed contrary behavior in April 2016, although deviations for SRP between the single HPFM were high in April. When normalized for water flow (J_N divided by q_x), the average NO_3

concentrations were uniformly lowest in the 15-35 cm layer in June as well as in April. Remarkably, in April the NO_3^- concentration in the 5-15 cm layer of L5 and L6 was 5.6 and 8.8 mg N L^{-1} significantly higher than average surface water concentration (2.8 mg N L^{-1}). In the 15-35 and 35-50 cm layers, concentrations were below 1 mg N L^{-1} in those samplers. However, in R2 in June 2015 and R1 in April concentrations around or slightly above surface water concentrations were detected in the deepest layer. Observations during retrieval of the samplers in June suggest that we hit a clay lens with R2. All other HPFMs constantly detected NO_3^- concentrations below surface water concentrations in June (**Figure 4.5.**). SRP concentrations in June consistently increased with depth in all samplers. In April, the same pattern was observed in R1, L6 and L7 whereas L5 and L8 showed decreasing SRP concentration with depths.

q_x , horizontal J_N and flux averaged concentrations for each HPFM are displayed in **APPENDIX 4B.**

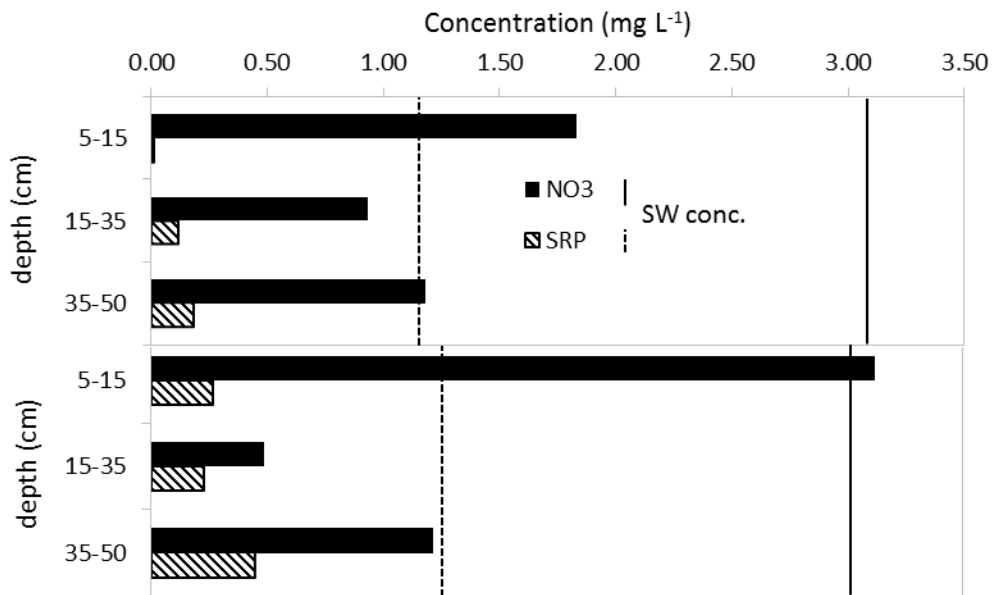


FIGURE 4.5. FLUX AVERAGED CONCENTRATIONS OF NO_3^- -N AND SRP IN JUNE 2015 (TOP) AND APRIL 2016 (BOTTOM) ASSESSED IN THE HPFM. AVERAGE SURFACE WATER CONCENTRATIONS DURING THE CAMPAIGNS ARE MARKED AS LINES.

4.2.2. NUTRIENT CONCENTRATIONS IN PORE WATER

Manual pore water samples didn't show a distinct vertical gradient or reproducible pattern in NO_3 or SRP, neither in June, nor in October 2015 or April 2016 (**APPENDIX 4C**).

In October 2015 NO_3 concentrations in the pore-water were consistently lower in the early morning than in the afternoon. A similar trend was observed in the surface water. Pore-water concentrations of SRP showed the contrary behavior with higher concentrations in the early morning than in the afternoon. Surface water concentrations in the surface water did not change significantly over the day. In April 2016 adverse patterns were observed between the two sampling dates: While on the 1st of April NO_3 concentrations in the pore water slightly increased from dawn until afternoon, they decreased during the same time span on the 4th of April. On both dates surface water concentrations were slightly decreasing from morning to afternoon. SRP in the pore-water generally increased from early morning until afternoon on both dates. Dissolved organic carbon (DOC) displayed the same pattern as NO_3 in MLS B and C. In MLS A no correlation could be detected (**APPENDIX 4C**)

SO_4 and B concentrations were in all depths similar to surface water concentrations during all pore water samplings.

4.2.3. O_2 AND TEMPERATURE

In June 2015 O_2 exhibited strong diurnal amplitudes in surface (6 to 13 mg L^{-1}) and subsurface water (0 to 8 mg L^{-1}) (**Figure 4.6**). Peaks consistently occurred around noon, lowest values shortly after midnight. Subsurface water at 25 and 45 cm below surface water sediment interface became anoxic during several nights during the deployment in June. In the 25 cm profile a sharp drop in O_2 concentration and subsequent recover after 2 to 3 h was frequently observed in the hours around noon. O_2 profiles for April 2016 were markedly distinct. Diurnal amplitudes were much smoother, ranging from 10.5 to 12.5 mg L^{-1} in the surface water and from 6.5 to 9 mg L^{-1} at 25 cm below surface water sediment interface. Maxima in the surface water occurred in the late morning hours and minima in the hours before midnight. Extrema at 25 cm depths were observed with a time offset of 10 to 13 h compared to surface water dynamics. At 45 cm depths concentrations ranged from 2 to 6 mg L^{-1} . The 45 cm profile was completely decoupled from surface water dynamics and did not show any diurnal pattern.

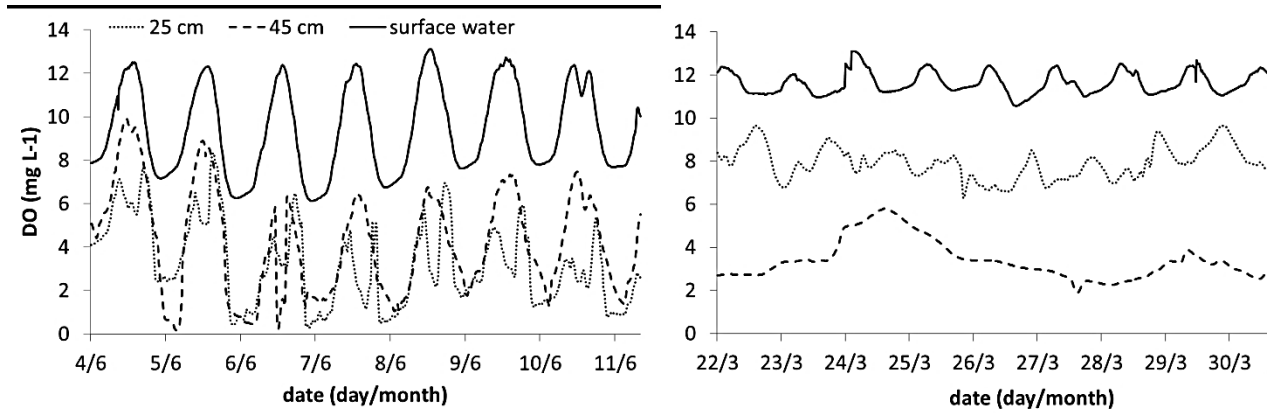


FIGURE 4.6. OXYGEN CONCENTRATIONS IN SUBSURFACE AND SURFACE WATER DURING THE CAMPAIGNS IN JUNE 2015 (LEFT) AND DIRECTLY BEFORE THE CAMPAIGN IN APRIL 2016 (RIGHT). DUE TO TECHNICAL PROBLEMS, OXYGEN COULD ONLY BE ASSESSED IN THE WEEK BEFORE BUT NOT DIRECTLY DURING THE CAMPAIGN IN APRIL 2016.

Temperature in June 2015 varied from 13.5 to 24°C in the surface water and from 14 to 24°C at 25 cm and 15 to 23 °C at 45 cm below surface water – sediment interface. As expected, highest temperatures were detected in the evening (around 8 pm UTC). In April 2016 temperatures in the surface water ranged from 6 to 13.5 °C with highest values around 4 pm which is consistent with lowest O₂ concentrations. In the subsurface water, temperature varied from 8 to 10.5 °C at 25 cm (maxima around 8 am) and from 8 to 9°C at 45 cm (maxima around 1 am). Thereby, subsurface temperature and O₂ concentrations were not coupled in April

4.2.4. HYPORHEIC RESIDENCE TIME AND DENITRIFICATION

NO₃ mass flow per unit depths in the surface water at the study site was $M(NO_3)_{sw} = 137 \text{ kgN m}^{-1} \text{ d}^{-1}$ in June 2015 and $250 \text{ kgN m}^{-1} \text{ d}^{-1}$ in April 2016. NO₃ concentrations in the surface water were similar during both campaigns (**Table 4.1**). The difference in $M(NO_3)_{sw}$ arises from the higher flow velocity and depth of the water column resulting from higher discharge in April 2016 versus June 2015 (**APPENDIX 4D**). Remarkably, the higher stream discharge did not lead to enhanced hyporheic exchange. On the contrary: The lower vertical Darcy velocities q_y in April 2016 produced a 7 times longer hyporheic residence time τ_{HZ} in April than in June. The hyporheic flow paths s_{HZ} were only twice as long in April than in June (**Table 4.2**). The coefficient $q_x q_y^{-1}$ and consequently the angle γ of the flow was around 3 times higher in April,

indicating that the decline in Darcy flow in April versus June was more pronounced in the vertical than in the horizontal component. While average denitrification activity R_N was at a similar range for both campaigns, U_{HZ} was substantially (8.7 times) lower in April than in June. Negative values for R_N and U_{HZ} in the 5-15 cm layer in April signify net NO_3 production. During both campaigns, the 15-35 cm deep layer was the most active one in NO_3 removal.

TABLE 4.2. CHARACTERISTICS OF HYPORHEIC FLOW AND DENITRIFICATION ACTIVITY FOR THREE DEPTHS OF THE HYPORHEIC ZONE AT THE STUDY SITE IN JUNE 2015 (TOP) AND APRIL 2016 (BOTTOM): τ_{HZ} HYPORHEIC RESIDENCE TIME, s_{HZ} LENGTH OF THE HORIZONTAL COMPONENT OF THE HYPORHEIC FLOW PATH, $q_x q_y^{-1}$ COEFFICIENT OF HORIZONTAL TO VERTICAL DARCY FLOW, R_N HYPORHEIC DENITRIFICATION ACTIVITY, U_{HZ} DENITRIFICATION RATE

unit	depth cm	τ_{HZ} hrs	s_{HZ} m	$q_x q_y^{-1}$ -	R_N %	U_{HZ} $\text{mgN m}^{-2}\text{d}^{-1}$
June '15	5-15	5	0.24	2.36	36	486
	15-35	13	0.54	2.16	67	906
	35-50	20	0.47	1.18	59	790
	mean	12.8	0.42	1.90	54	727
April '16	5-15	34	0.80	7.98	-11	-23
	15-35	86	1.03	4.14	83	162
	35-50	137	0.67	1.67	56	111
	mean	85.7	0.83	4.60	43	83

4.5. DISCUSSION

We observed strong differences in hyporheic nitrate removal U_{HZ} between the two sampling campaigns in our study site with U_{HZ} almost 9 times higher in June versus April. Remarkably the reaction rate R_N was in a similar range during both campaigns and in the deepest layer even higher in April than in June. This phenomenon can be explained by the following observations:

4.5.1. DARCY VELOCITIES AND ANGLE OF HYPORHEIC FLOW

Hyporheic flow was not only much slower in April versus June, also the angle of hyporheic flow, resulting from a higher $q_x q_y^{-1}$ ratio, was only half as steep in April. It is usually stated that higher stream discharge and associated increase in river depths and flow velocity enhance hyporheic exchange and accelerate hyporheic flow (Trauth et al., 2013; Cardenas and Wilson, 2007; Packman et al., 2004). Contrarily, we found that at the lower Holtemme horizontal and even more vertical hyporheic flow was much lower in April when average discharge was $Q_{SW} = 0.89 \text{ m}^3 \text{ s}^{-1}$ than in June at $Q_{SW} = 0.32 \text{ m}^3 \text{ s}^{-1}$. Both discharge rates are still in the range of low flow for this stream (LHW, 2016) but while the experiments in June 2015 took place at the end of a longer low flow period, high flows had occurred in the months preceding the sampling in April (**APPENDIX 4D**). The effects of discharge history on sediment permeability can be diverse, depends on many factors (Hartwig and Borchardt, 2015; Siergieiev et al., 2015). Higher flow often enhances hydraulic conductivity of the sediments (Pholkern et al., 2015; Battin and Sengschmitt, 1999), but fine particles mobilized during high flows may also lead to colmation of the hyporheic zone (Schälchli, 1992; Hartwig and Borchardt, 2015).

Considering that several small weirs are located upstream of the study location (LHW, 2009), we assume that sediments are trapped there and that colmation is of negligible importance in the lower Holtemme. However, the annual discharge context also influences the groundwater level. Routine monitoring conducted by the local authorities at a well situated in the same basin (52°02'35.2"N 11°05'38.4"E) recorded groundwater levels of 143 cm below terrain rim in April, but 214 cm below terrain rim in June (LHW, 2016). This suggests that also at our study site the groundwater level was considerably higher in April versus June. In loosing streams like the lower Holtemme, the hydraulic gradient between stream and groundwater, which is directly coupled to the groundwater level, is likely the main driver for surface-subsurface exchange and hyporheic Darcy velocities (Voltz et al., 2013; Trauth et al., 2013). As the decrease of hyporheic flow in April versus June was more marked in vertical than in horizontal direction,

we assume that the difference in hydraulic gradient between groundwater and stream was the major reason for the observed seasonal differences in Darcy velocities and nutrient fluxes.

4.5.2. BIOTIC ACTIVITY

Even though R_N was not higher in June versus April, comparing the magnitudes of the diurnal O_2 amplitudes indicates that both primary production and heterotrophic respiration were remarkably (around 4 times) higher in June than in April. In June O_2 profiles in the subsurface water were clearly dominated by benthic metabolism, so that extrema appeared simultaneously in surface water and at 25 and 45 cm depth. Night time anoxia in the sediment was detected during several nights, probably triggering hyporheic denitrification so that we detected lower NO_3 in the early morning hours than in the afternoon in the bi-dial MLS sampling. The redox state may also regulate the mobility, transformation and retention of PO_4 (Smith et al., 2011; McDaniel et al., 2009; Gabriel et al., 2006). Under reducing conditions reduction of Fe^{3+} mobilizes PO_4 from Fe-complexes while under elevated O_2 concentrations PO_4 remains demobilized (Miao et al., 2006). Nighttime anoxia and coherent phosphate mobilization could therefore as well explain the higher SRP concentrations in the early morning versus the afternoon measurements.

In April the O_2 curve at 25 cm depth closely resembles the surface water O_2 curve with a time offset of 10 to 13 h. Different to June subsurface O_2 dynamics were apparently dominated by transport processes. However, a q_y of around 50 cm d^{-1} would be needed to exclusively attribute the observed offset to advective transport, but actually q_y was only 7 cm d^{-1} . We therefore assume that due to the long residence time and low metabolic activity, both diffusive and advective transport had a visible effect on oxygen dynamics.

4.5.3. EXTENT OF ACTIVE HYPORHEIC ZONE

Contrary to June, when at 45 cm depths hyporheic biogeochemical processes were synchronous with surface water dynamics, biogeochemical activity at this depth was decoupled from surface water dynamics in April. Also considering the high $q_x q_y^{-1}$ ratio (shallow local angle of hyporheic flow γ) in April, we conclude that the extent of the active hyporheic zone declined from a minimum of 50 cm depth in June to approximately 35 to 40 cm depths in April.

4.5.4. THE EFFECT OF REDOX CONDITIONS AND RESIDENCE TIME ON THE NO₃ SOURCE-SINK FUNCTION OF THE HYPORHEIC ZONE

In summer, night time denitrification and PO₄ mobilization (lower NO₃ but higher PO₄ pore-water concentrations in the early morning than in the afternoon) can be attributed to temporal anoxia as discussed above. Though O₂ was also consumed in the subsurface in April, zero O₂ was not detected during that campaign. Different oxygen thresholds for denitrification have been reported in the literature: Marzadri et al. (2012) proposed a value of maximal 4 mg L⁻¹, Briggs et al. (2014) found that hyporheic NO₃ reduction already occurred at O₂ concentrations of 5 mg L⁻¹. In April the 25 cm depth at our study site had O₂ minima over 6 mg L⁻¹ constantly above both thresholds. Still, we detected consistently higher NO₃ levels and lower SRP concentrations in the early morning than in the afternoon on the 4th of April. Keeping in mind that, due to the time offset of approximately 12 h in the subsurface O₂ curve the O₂ minima in April occurred around noon and the maxima during the night, hyporheic NO₃ denitrification and phosphate mobilization assumingly also occurred on the 4th of April. This suggests that hyporheic denitrification may also appear under O₂ concentrations above 6 mg L⁻¹. However, compared to June, night time denitrification was not dominant in April. While in June surface water concentrations of NO₃⁻ were in accordance to the subsurface samples, decreasing during night, they were slightly increasing over night in April. Assumedly, night time denitrification (in the hyporheic zone) was dominating overall NO₃⁻ dynamics in June, whereas in April, due to low hyporheic exchange rates (and low metabolic activities), hyporheic processes had no detectable effect on surface water NO₃⁻ dynamics.

Despite substantial differences in redox conditions and biotic activity, the hyporheic NO₃ removal activity R_N was similar during both seasons. Most likely, longer residence times compensated for lower biotic activity in April. Water residence time in relation to O₂ reaction rate time scales was also suggested as a good predictor for the NO₃ source-sink function of the hyporheic zone by Zarnetske et al. (2012). In their Monod kinetics model simulation the Damköhler number for O₂ was defined as $Da_{O_2} = \tau \times V_{O_2}$, where τ is the water residence time and V_{O_2} is the reaction rate for O₂. Thereafter $Da_{O_2} > 1$ represent systems where O₂ demand is higher than supply and anaerobic conditions would favor denitrification. As a conclusion hyporheic zones with shorter residence times would tend towards net nitrification whereas those with longer residence time would likely display net denitrification.

Similarly, Briggs et al. (2014) showed that the temporally dynamic hyporheic residence time patterns dominated over hydrodynamic conditions and other environmental factors known to affect NO₃ cycling, resulting in a strong correlation ($r = 0.86$) of R_N to residence time. The authors defined a maximal residence time below which the hyporheic zone of their testing site was a net NO₃ source (there < 1.3 h) and a

threshold residence time above which the hyporheic zone was a net NO_3 sink (there > 2.3 h). For the intermediate residence time the hyporheic zone could be either sink or source or oscillate between those two stages. Our campaign in June would clearly belong to the first case: above sink threshold. In April we found the intermediate stage where the location of measurement devices which were between 20 cm to few meters apart of each other, was dependent on whether we measured net denitrification or nitrification. Even in one single location, depending on the depths, we observed net removal or production of NO_3 . Conclusively, accounting for vertical gradients in the hyporheic zone is essential to correctly characterize its source-sink function.

At our study site, at several locations the shallowest layer (5 - 15 cm), which exhibited shortest residence times, acted as a net NO_3 source, whereas the deeper layers were net sinks. While this is in accordance to the above stated R_N to τ_{HZ} relationship, our results clearly outline that neither residence time nor O_2 reaction rates can universally explain hyporheic NO_3 source-sink functions: Highest R_N values were detected in the 15-35 cm layer, even though longer residence times as well as lower O_2 concentrations were detected in the deeper layer. With this, the hyporheic zone at our study site behaved different to the most commonly observed conditions where hyporheic denitrification exponentially decreases with depths, because both electron donors (NO_3) and acceptors (OC) become progressively depleted (Lansdown et al., 2012; Baker et al., 2000b). However, in OC rich sediments, typical for impacted streams in agricultural landscapes, substrate limitation is assumedly only of minor importance (Trauth et al., 2015; Basu et al., 2011; Arango et al., 2007).

Indeed, Trauth et al. (2014) showed in a reactive transport model that under losing conditions the zone where O_2 influx from the surface has already been consumed during the passage through the overlying layer while essential solutes like DOC and NO_3 are still available was mainly determined by q_y . Interestingly, for a similarly sized stream in the same catchment, this reactive zone was located at a depth of around 30 to 40 cm below sediment–surface water interface and a q_y of 0.5 m d^{-1} was identified to create best conditions for denitrification (Trauth et al., 2014). Both factors are in the same range as values found at our study site. This is especially remarkably because the modelled study stream featured a natural morphology, dynamic bed-forms and much coarser sediments (Trauth et al., 2013) which is quite different to the Holtemme reach examined in our study.

Finally, comparison between single locations during our campaigns could not confirm the R_N to τ_{HZ} relationship found by others (Briggs et al., 2014; Zarnetske et al., 2012; Marzadri et al., 2012) as stated above: In April the 5-15 cm layer of L6 and L7 displayed similar residence times, but while net uptake was observed in this layer of L7, it was a net NO_3 source in the 5-15 cm layer of L6.

4.5.5. ANTHROPOGENIC INFLUENCE ON WATER QUALITY

In the systems where R_N thresholds had been defined based on hyporheic residence time and oxygen reaction rates, NO_3 concentrations were more than an order of magnitude lower than in the Holtemme ($0.06 - 0.1 \text{ mg L}^{-1}$ versus 2.8 to 3.1 mg L^{-1}). Established relationships might not work for highly anthropogenically impacted streams. The Da_{O_2} principle for example assumes stable stream-water chemistry on the time scales of hours. In the Holtemme, however, elevated NO_3 levels and the absence of shading riparian vegetation caused strong diurnal oxygen amplitudes. Additionally, pulse outlets from WWTP can cause high short-term variations in solute concentrations, especially NH_4 , in urban effluent influenced streams like the Holtemme. At our study site specific conductivity time-series (**APPENDIX 4D**) showed irregularly occurring peaks during both seasons, which we attribute to NH_4 releases from the WWTPs upstream of the study site. Depending on locally heterogenic sediment characteristics, those NH_4 peaks can reach the different sampling locations and depths in the hyporheic zone more or less attenuated and with variable time delay (Marzadri et al., 2012; Hill et al., 1998; Packman et al., 2004). In June, when the elevated benthic O_2 demand arising from high nutrient level and insulation lead to temporal anaerobia in the hyporheic zones, denitrification was the dominating process. Denitrification assumedly outbalanced nitrification so that the oscillations in surface water chemistry were not obvious in the averaged turnover rates in June. In April however, redox conditions and nutrient flux rates were in the range of the threshold between nitrification and denitrification. In this intermediate stage, oscillations in surface water chemistry were evidently determining the function of the hyporheic zone.

Remarkably, the manual pore-water samples did not as clearly show the inter-seasonal differences neither in vertical gradients nor NO_3 source–sink function of the HZ as the time integrative measurements from the HPFM. We adjusted manual sampling times of pore-water to the daytimes when we expected to find maxima (few hours past noon) and minima (before sunrise) in hyporheic NO_3 concentrations. Those expectations were built on the assumption that primary production would modulate O_2 dynamics which would then define the denitrification potential. For June, this expectation was tenable, whereas in April hyporheic O_2 dynamics were asynchronous to solar forcing. In addition, the randomly occurring NH_4 peaks related to WWTP operation might have biased punctual sampling events.

4.6. CONCLUSIONS

Mechanistic models greatly advanced our understanding of hyporheic flow patterns and can support conceptual frameworks for hyporheic processes. However, the results presented here highlight that assumptions on hyporheic nutrient transformation might not generally hold. Especially in streams where human activities like the operation cycles of Wastewater Treatment Plants and modified morphology superimpose or disturb the natural dynamics, new characteristic patterns may occur. In situ measurements are therefore still indispensable for the site specific characterization of hyporheic nutrient transport and transformation. Especially in systems where human activities have led to changes in morphology, hydrology and water chemistry and thus may invalidate generally accepted concepts, limited research has been completed (Grimm et al., 2005; Groffman et al., 2005). Due to the lack of suitable measurement techniques (Grant et al., 2014; USEP, 2013), quantitative assessments of hyporheic NO_3 fluxes in general are very rare (Zarnetske et al., 2011b; Ward et al., 2016). HPFM measurements integrate the temporal fluctuations of hyporheic nutrient dynamics over several days into one time averaged flux. Thus, the problem of commonly practiced snap shot sampling (Grant et al., 2014; Wollheim et al., 2008) subject to short term and diurnal variations, is negated. In this study, the application of HPFMs, time integrative measurements of hyporheic nutrient fluxes and supplementary parameters in an anthropogenically impacted stream, revealed unexpected features of the NO_3 source-sink function of the hyporheic zone and the hydrological and chemical factors influencing hyporheic NO_3 turnover rates: In June, the intense benthic metabolism arising from excessive nutrient loading and absence of shading vegetation resulted in pronounced subsurface dynamics in oxygen concentration. The effect of temporal anoxia on hyporheic NO_3 processing rates dominated over other influencing factors, resulting in an overall NO_3 sink function of the hyporheic zone. In April however, neither redox state nor hyporheic residence time could satisfactorily explain nutrient cycling patterns. In periods of lower biotic activity, solute concentrations and physical transport patterns were more crucial. At this critical stage, where redox conditions and residence times in the hyporheic zone are close to the threshold between NO_3 consumption or production, short term fluctuations in NH_4 dynamics and small scale local variety in stream bed morphology and sediment characteristics finally determined whether nitrification or denitrification prevailed. This resulted in small scale heterogeneity of net NO_3 source and net NO_3 sink layers or spots of the hyporheic zone in April.

Unexpectedly, we detected partly higher R_N in April than in June despite the lower biotic activity. Nevertheless, even though up to 83 % of the infiltrating NO_3 was removed in the hyporheic zone in April, the absolute retention rate U_{HZ} was almost one order of magnitude lower than in June. This constellation was due to longer residence times arising from surprisingly low exchange rates in April. Hyporheic flow and with it solute flux at our study site was predominantly driven by the hydraulic gradient between stream and groundwater and was, contrary to general assumptions, decreasing with increased discharge. Naturally, higher discharge enhances the infiltration of water and solutes especially by increasing the drag forces of bed forms and meanders (Packman et al., 2004; Boulton et al., 2010; Wörman et al., 2002). If, due to channelization, those morphological features are absent, discharge induced near-bed pressure distributions are only of minor importance (Buffington and Tonina, 2009).

Despite the differences in hydraulic and biogeochemical conditions, horizontal hyporheic flow velocities q_x showed clear declining gradients with depth during both campaigns. Nevertheless, the intermediate depth of 15 to 35 cm below sediment-surface water interface was most effective in NO_3 removal, which is different to the commonly reported exponential decline of hyporheic denitrification rates over depths but in accordance with model results for a similarly sized, though morphological and hydrological distinct stream in the same catchment (Trauth et al., 2014). Assumedly, in agriculturally dominated landscapes, OC availability is entirely high enough to support denitrification also deeper in the subsurface, where denitrifying bacteria benefit from low O_2 .

We conducted the two campaigns during periods which we consider typical for summer and early spring. More repetitions would be needed to derive robust statements about the complete seasonal pattern. However, the down-welling q_y we measured in June were in the range identified by the above mentioned model as ideal for hyporheic denitrification. We therefore assume that the NO_3 removal rates detected in June were close to the maximal capacity of the study reach, outbalancing hyporheic nitrification of WWTP born NH_4 . In April however, low biotic activity fell together with low hyporheic exchange rates, resulted in 9 times higher (727 versus 83 $\text{mg N m}^{-2} \text{d}^{-1}$) U_{HZ} in June compared to April. Considering in addition that, due to higher discharge along with similar NO_3 concentrations in the surface water, the amount of nitrate transported in the channel of the Holtemme was almost twice as high in April compared to June, the relative efficiency of hyporheic NO_3 removal in April declines to roughly 5 % of the values measured in June. We believe that the strong seasonal differences observed in our study stream are further fostered by human activity. First, high nutrient loads and abundant insulation induced hyporheic anaerobia increasing denitrification in June. Second, the unnatural morphology impeded bed-form induced

hyporheic exchange, so that in April, when the hydraulic potential between stream and groundwater was low, exchange rates of water and nutrients declined.

While an increase of bed-form topography and the restoration to a natural morphology would in general enhance hyporheic exchange and with it NO_3 processing, our results also highlight the need to consider seasonal dynamics. In temperate climate regions higher groundwater levels as well as discharge rates in early spring will often exist. Agricultural management should take in account that major fertilizer applications in early spring coincide with climatologically driven increase of run-off and discharge. The resulting high nutrient loads in streams will occur during a period when (1) biotic nutrient uptake is very low due to temperature dependence and (2) hyporheic exchange rates may be small because of recharged groundwater bodies.

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5. DISCUSSION

5.1. EVALUATION OF THE NEW METHODOLOGY

For both, reach scale as well as local hyporheic nitrate dynamics, there is ample evidence that assessments based on low frequency sampling omit important features and can cause inaccurate estimates of key parameters and total processing rates. The new methods presented in this thesis are quantitative and operate continuously over longer time periods. Thereby, they overcome these major problems of traditional methodologies. Further advantages, limitations and prospects beyond the aspects already discussed in the preceding chapters are depicted in detail in this section.

5.1.1. HIGH FREQUENCY MEASUREMENTS OF NITROGEN REMOVAL IN RIVERS

Mass balances, the difference in solute concentration between a selected point in the river and a reference point further upstream, appears to be the most straight forward way to determine nutrient uptake rates on the reach scale. However, as already discussed in detail in Chapter 2.5, analytical precision and temporal resolution of available methods were until recently too low to derive nitrate uptake rates from concentration measurements (Rode and Suhr, 2007). Emerging automated nitrate sensors open new possibilities for nutrient studies and water quality monitoring (Rode et al., 2016a; Rode et al., 2016b; Fovet et al., 2016). In the current study at the Weiße Elster, existing methodologies were extended. The combined approach of two station time-series and longitudinal profiling allowed for the first time to quantify nitrate uptake rates in higher order fluvial systems with substantial tributaries, complex input signals and multiple nitrogen species. Thereby, the primary requirements on the new methodology were satisfied, confirming hypothesis A1 to A3:

Currently available sensors proved sensitive enough to measure nitrate uptake rates on the reach scale (7 km) and to quantify inter-reach differences in nitrate cycling parameters. As anticipated, the main advantage to previously existing methods is the ability for continuous measurements in rivers. Unlike isotope additions which are, owing to the enormous dosage required to achieve detectable signals in larger streams, restricted to small streams, the present method can basically be applied in rivers of all sizes. Assessments as demonstrated in the Weiße Elster will therefore contribute missing knowledge on actual uptake rates in bigger rivers. Nevertheless, we had to constrain the initial goal from quantifying N

turnover rates to quantifying net nitrate turnover rates, because separating the different transformation processes was not completely possible (hypothesis A5). Additional measurements of chemico-physical parameters could partly compensate for these deficits. For instance, assimilatory N demand could be derived from GPP values which base on oxygen and temperature profiles. On the other hand, naturally abundant isotopes did not deliver the anticipated support, so that hypothesis A6 was only partly filled. Even though inter-reach differences as well as seasonal variations in uptake characteristics could be quantified, these differences could not exclusively be attributed to morphology and season alone (hypothesis A7), because of the substantial but indistinct effects of water quality (ammonium concentration) on nitrate dynamics.

A future improvement to the method could be supplementary, higher frequency ammonium measurements at the stations with automated samplers. The adequate sampling frequency will be a tradeoff between resolution and feasibility, depending on the research question or management demands. As ammonium is an important nutrient in many aquatic systems and can substantially contribute to the eutrophication of water bodies (Dodds, 2007; Anderson et al., 2002) the development of automated sensors for highly resolved ammonium measurements is already in progress. Standard Ion Selective Electrodes (ISE) have so far been unsuited due to interference with other naturally occurring ions, large non-linear signal drifts, extensive calibration requirements (daily with 3 to 5 buffer standards) and most of all low accuracy ($\pm 10\%$) and high detection limits (2 mg N L^{-1}) (Korostynska et al., 2013; Winkler et al., 2004). Potentiometric sensors or multi-ISE-sensor arrays coupled with signal processing tools such as artificial neural networks recently delivered promising results (Mueller and Hemond, 2016; Gutiérrez et al., 2008). Even though matrix effects and aging of electrodes remain major challenges, it is assumedly only a question of time until fully deployable, robust and accurate ammonium sensors are available (Mueller and Hemond, 2016; Korostynska et al., 2013).

Despite remaining limitations, the presented sensor data based approach is a promising methodology for assessing reach scale nitrate turnover rates. Studies of several days, as presented here, deliver quantitative values for nitrate processing rates during the period of measurement, from which the qualitative behavior can be deduced. The continuous implementation of such assessments will allow quantitative characterization of system and event specific variations in nitrate processing rates on short terms (for example diurnal or storm event induced) as well as seasonal scales. Thereby, besides delivering new opportunities for research, the use of nitrate sensors will also optimize monitoring practices of surface water quality. Nighttime or storm-event dynamics will for example not be omitted any more from

the data sets on which models and management practices are built. Operation of the sensors is user friendly and labor effective once they are installed. So that, despite high purchasing costs of sensors and dependency on electricity supply, in Germany and many other industrial countries, authorities increasingly use sensors for flux estimates and early warning systems (Rode et al., 2016b). In principal, the presented method can be extended to any solute for which automated high resolution sensors are available. For many other substances, at least qualitative statements can be drawn from the observed nitrate dynamics. In the Weiße Elster, we identified periods and reaches where either denitrification or nitrification was dominant. From this type of observations, conclusions about the local and temporal dynamics of other redox sensitive substances and their transformation can be drawn. However, the presented results from the Weiße Elster highlight that realistic assessments of turnover rates based on sensor records may be confounded by many factors. Several important points have to be considered in the application of such methods: Selecting suitable locations for the sensor installations and determining an appropriate distance between the two stations is the first step in allowing successful performance. To avoid artefacts originating from point sources, it is for example important that water masses are well mixed over the cross sectional profile. The study reaches at the Weiße Elster were selected based on morphological and hydrological parameters, which were obtained during earlier studies (Rode et al., 2008; Wagenschein and Rode, 2008; Wagenschein, 2006). For a correct interpretation of the observed signals prior characterization of a reach is vital. This includes surveilling the hydrology, quantifying tributaries and groundwater contribution and identifying important side parameters which have to be measured simultaneously. In Germany and many other industrial countries, discharge data is widely available from authority operated gauging stations. For the exemplary study at the Weiße Elster additional measurements of an inflowing mine drainage were provided by the mining company. The longitudinal profiling enabled us to exclude the influence of further unknown point sources and to detect processing hotspots in the reaches. Thereby, the methods developed in this study offer great opportunities to intensely investigate solute dynamics in high target streams or critical events such as storm run-off. However, due to the complexity of instream nitrogen processing patterns, as also observed in the Weiße Elster, advanced expertise is essential to process data and to interpret the results. A further challenge will therefore be, to assure the quality of the measurements (e.g. appropriate calibration of the sensors) and to develop automated, computer based programs and software that cope with the demands of real-time records (Rode et al., 2016b; Campbell et al., 2013). Beyond facilitating scientific use of the data, these programs have to be accessible to stakeholders and decision makers which may also require previously training or supplementary stuff.

5.1.2. HYPORHEIC PASSIVE FLUX METER FOR THE QUANTIFICATION OF NUTRIENT FLUXES

While automated high-resolution nitrate sensors have recently enabled new prospects to advance quantification of nitrate dynamics in surface water, equivalent techniques are not available for subsurface studies. By implication, continuous measurements with qualities comparable to the presented reach scale assessment in the Weiße Elster are up to date unfeasible in the hyporheic zone. Here, time integrative measurements from HPFM represent an attractive way to obtain subsurface nutrient fluxes over longer time spans. Passive flux meters have already been established in groundwater surveys. Extending their application to hyporheic nutrient studies was made possible by the modifications presented in this work. The expertise of Michael Annable, Jaehyun Cho, Kirk Hattfield and Suresh Rao on the original passive flux meter formed the basis for this methodological advancement. Additionally, new aspects like biofouling and background nutrient concentrations on HPFM materials were assessed in laboratory and field experiments during this study. Overall, HPFM complied with the methodological demands during the test deployment, confirming hypothesis B1 and B2.

HPFMs operate without complex, sensitive technology or power supply and maintenance during deployment time is not needed. This methodology is therefore ideal for measurements at remote, hardly accessible sites. However, one should consider that well equipped laboratories and experienced personnel are needed for the preparation and analysis. The effect of longer (more than 48 h) transport and storage duration was not assessed in this study and should be tested via a control HPFM.

The comparative experiments at the Holtemme River highlighted the major advantage of HPFMs compared to punctual techniques: Manual grab samples overestimated average concentrations when they were exclusively conducted during daytime, disregarding lower concentration during night. These comparisons approve hypothesis B3. Nevertheless, if diurnal amplitudes in solutes are of interest, repeated manual sampling is still indispensable. The best practice is therefore to combine several measurement techniques. Oxygen time-series for redox state estimations and temperature as a natural tracer for seepage characterization are relatively simple methods and added important findings to this study. Analysis of the two sampling campaigns demonstrated how such a combined set up allows quantifying specific nutrient cycling patterns and the nitrate source-sink function of the hyporheic zone in the target reach. The results could be used to identify natural and anthropogenic factors influencing nitrate processing rates. However, to disentangle the effects and quantify the impacts of influencing factors, more information on bedform, sediment and aquifer structure would be needed. In a perialpine

river in Switzerland, 3D electro resistance tomography was successfully used to characterize effective porosity of the aquifer as well as groundwater flow paths and velocities (Doetsch et al., 2012; Coscia et al., 2011). Such measurements however, were definitely not in the scope of this PhD. Considering the limited expense of the presented methodology, the information gained from the HPFM and supplementary measurements are eminent.

As already depicted in Chapter 3.5., the next step in improving HPFMs should be the identification of sorbents with no or acceptably low nutrient background. The activated carbon used in this study was selected based on previous experiences from colleagues at the University of Florida. This decision was mainly taken to keep the task in the time frame of a PhD project. The market of resins and activated carbon is vast, and the detailed characterization of physical (hydraulic conductivity) and chemical (absorption, tracer retardation, extraction, loading capacity) behavior is time intensive. As an interim solution, we kept the nutrient absorbing resin and the tracer loaded activated carbon separated by either installing two respective HPFMs next to each other or by alternating separated layers of resin and activated carbon. However, it would be preferable to mix both components or to identify one single material which can cover both functions: tracer carrier and nutrient absorber. Thereby, vertical resolution of the measurement could be increased and errors arising from interpolation between two devices or two layers could be minimized. A further emerging challenge was potential biofouling of the resin granules. Biofilm growth was observed on laboratory columns but also on the upper most layer of one field-deployed HPFM in June 2015. Earlier groundwater studies did not assess this potential problem (because it was not of importance). Even though the resin manufacturer asserted that absorbing and extraction behavior of the resin would not be altered by biofilm growth (personal communication with Uwe Wagnitz, Purolite Germany GmbH, Sandersdorf-Brehna, 2015), experimental proof for this statement was not available. Nevertheless, instead of characterizing the effect of biofouling on resin performance and flux measurements or searching for biologically inhibiting techniques, future studies should rather be dedicated to determining an alternative sorbent. The antibacterial nature of silver impregnated activated carbon probably suppresses biofilm growth on the resin if both components were mixed as originally intended. Using a sterile resin (or similar compound) as absorber and tracer carrier may be an attractive solution. Anti-bacterial ion-exchange resins do exist (Patil, 2003), but their applicability in HPFMs has still to be tested. Alternatively, disinfecting the resin with peracetic acid ($C_2H_4O_3$) is suggested in the general resin application guide for Purolite® resins (Purolite GmbH, 2015). As the proposed procedure originally aims at maintaining the water cleaning capacity of the resin, effects on nutrient absorption and extraction or interference with alcohol tracers would have to be evaluated in specific experiments.

Apart of the remaining uncertainties concerning biofouling, the adaptation of (groundwater) PFMs for hyporheic zone studies was successful, suggesting that this methodology can be further extended to other environments. The riparian zone, for example plays a central role in the stream nutrient cycle (Ranalli and Macalady, 2010; Boulton et al., 2010). PFMs could potentially be used to quantify the exchange of solutes between the river and its lateral corridor. Likewise, future applications could consider other target solutes such as contaminants and pharmaceuticals. PFMs have been used to trace toxicants or volatile organic compounds in groundwater (Verreydt et al., 2013; Annable et al., 2005). In hyporheic (nutrient) studies, iron and ammonium may deliver valuable insights because of their importance in redox reactions and the nitrogen cycle (Miao et al., 2006).

5.2. RESULTS FROM THE FIELD STUDIES AND SCIENTIFIC IMPLICATIONS

In this chapter, implications of the results which are beyond the aspects already outlined in chapters 2 to 4 are discussed. The dynamics of nitrate in lotic ecosystems are the primary subject. Further, the overall significance of anthropogenic impacts on riverine ecosystem functioning is addressed, using nitrate transformation as an example.

For this purpose, it is helpful to start with an overview of relevant nitrogen processing pathways.

5.2.1. NITROGEN PROCESSING IN AQUATIC ECOSYSTEMS

Nitrogen which enters an aquatic ecosystem is subjected to various biological processes:

- Assimilation, the autotrophic consumption of dissolved nitrogen by phytoplankton, biofilms and macrophytes, is bound to photosynthetic active phases (Giordano and Raven, 2014; Xu et al., 2012). Especially in eutrophic systems, enhanced assimilatory daytime uptake can create large diurnal amplitudes in nitrate and oxygen concentrations (Arango et al., 2008; Grace and Imberger, 2006; Hall and Tank, 2003). If available, ammonium represents an energetically more advantageous nitrogen source and is therefore preferentially assimilated (Marti and Sabater, 1996; Stanley and Hobbie, 1981). However, if plants are not harvested, most of the incorporated nitrogen is released again into the water column after the plants die (Birgand et al., 2007; Howard-Williams et al., 1983).

Phytoplankton uptake transfers dissolved nitrogen into the particulate phase, which is exported downstream slower than if dissolved (Webster and Patten, 1979). In sum, assimilatory uptake is a mechanism of temporal nitrogen retention, which can essentially retard downstream transport, buffering peaks and the resulting harm on receiving water bodies (Arango et al., 2008; Cooper and Cooke, 1984).

- Denitrification, the microbial reduction of nitrate to gaseous nitrogen (N_2) is the dominant dissimilatory in-stream removal pathway for nitrate (Knowles, 1982; Garcia-Ruiz et al., 1998a). It is mostly restricted to anaerobic sites and microsites in the subsurface of the river bed (Boano et al., 2014; Fellows et al., 2001; Duff and Triska, 1990; Grimm and Fisher, 1984), biofilm mats and connected riverine wetlands (Racchetti et al., 2011). Further, denitrification depends on the availability of an electron acceptor, usually dissolved organic carbon (DOC) (Arango et al., 2007; Zarnetske et al., 2011). Like most biological processes, denitrification increases with higher temperatures (Knowles, 1982; Christensen and Sorensen, 1986). Usually, first order uptake kinetics are attributed to denitrification (Dodds et al., 2002), meaning that higher concentrations induce higher removal rates (Mulholland et al., 2008). At the same time, biotic nitrogen demand gets saturated at a certain level and therefore denitrification efficiency is decreasing with higher nitrate concentration (Covino et al., 2010; Mulholland et al., 2008; Bernot and Dodds, 2005; Christensen et al., 1990).

Incomplete denitrification can result in elevated nitrous oxide (N_2O) levels. N_2O mainly received attention due to its relevance as a greenhouse gas, but it is of less importance for the aquatic nitrogen cycle (Beaulieu et al., 2012; Mulholland et al., 2009).

Different to the assimilatory uptake of nitrate, denitrification eternally removes nitrate from the aquatic system (Bernot and Dodds, 2005; Laursen and Seitzinger, 2002).

- Anammox, the anaerobic oxidation of ammonium with nitrite (NO_2^-) to gaseous nitrogen is a further sink for fixed nitrogen. In the recent years, the use of anammox for efficient sewage treatment has been investigated (Hu et al., 2013; Van der Star et al., 2007). It is assumed that anammox is responsible for up to 50 % of marine N_2 production (Arrigo, 2005; Thamdrup and Dalsgaard, 2002). The significance of anammox in freshwater systems remains unclear, and thus it is often neglected in instream nitrogen studies (Marzadri et al., 2012; Lewandowski and Nützmann, 2010; Kuypers et al., 2003), as well in the presented one. Nevertheless, there is evidence that it may be of similar importance as denitrification in riparian and estuarine sediments (Zhu et al., 2013; Crowe et al., 2012), groundwater (Smith et al., 2015) and hyporheic zones (Wang et al., 2012).

- Nitrification, the dissimilatory oxidation of ammonium to nitrate can be an important in-stream source of nitrate. Besides being temperature dependent, nitrification is also influenced by the oxygen concentration and pH (Strauss et al., 2002). These dependencies can result in large variation of nitrification rates on a diurnal as well as seasonal basis. Commonly, nitrification rates are greater during the warmer season and increase over day versus the night (Warwick, 1986; Shammass, 1986). Several studies reported a strong correlation between nitrification rates and ammonium concentration. However, a general relationship was not identified (Harris and Smith, 2009; Zhu and Chen, 2002; Anthonisen et al., 1976).

Far from being independent, isolated processes, those different pathways of nitrogen cycling are tightly linked to each other. Stream metabolism and the inherent assimilatory nitrogen consumption modulate pH and oxygen concentration in surface and pore-water, which then influence the dissimilatory pathways (O'Connor and Hondzo, 2008; Laursen and Seitzinger, 2004; Christensen et al., 1990; Garcia-Ruiz et al., 1998b). Coupling of nitrification with denitrification and anammox is another example for the interaction of nitrate transformation pathways. Hereby, sediment-bound nitrate production through nitrification can locally fuel denitrification and anammox (Crowe et al., 2012; Seitzinger et al., 2006; Lorenzen et al., 1998). These multiple interconnections together with complex transport and storage patterns inherent to lotic systems are the major reasons, why quantitative characterizations of in-stream nitrate processing rates are a challenging task.

5.2.2. REACH SCALE NITROGEN DYNAMICS IN THE WEIßE ELSTER

The Weiße Elster study can be seen as an exemplary demonstration on how different (natural and anthropogenic) factors can influence nitrogen dynamics in a river reach.

Turnover processes are usually not homogeneously distributed over a reach, but occur at variable patchiness, from large to microscale extension (Lorenzen et al., 1998; Cooke and White, 1987).

In lotic systems, downstream transport and intermediate storage of solutes merge local transformation processes into one accumulated signal (Covino et al., 2010; Doyle, 2005). Accordingly, observed nitrate concentrations at any downstream point are an integrated signal of processes at the observation site, in upstream reaches and in the catchment (Runkel, 2007). Besides biogeochemical processing rates, hydrological and morphological factors such as discharge, tributary or groundwater inflow, connectivity

of storage compartments (in channel or subsurface) and the resulting residence times in the system exhibit a pivotal influence on nutrient turnover in a stream (Becker et al., 2013; Hall et al., 2009). One of the first successes in describing the complex transport and transformation behavior in lotic systems was the spiraling model (Webster and Patten, 1979; Newbold et al., 1981), in which a nutrient atom is transported downstream in a dissolved form, removed from the water column through biotic uptake and is then later released again, closing the loop of the spiral. This conceptual framework relied on steady state data collection and linear regression and failed to differentiate between distinct processes as well as between main channel and storage zone uptake. Advances were achieved by the implementation of transport based approaches derived from nutrient and tracer additions and time series analysis (Runkel, 2007; Payn et al., 2005; Doyle, 2005).

Those reach scale (in general several kilometers) studies are of particular interest for decision makers, because they cover the relevant scale for restoration measures (O'Connor and Hondzo, 2008). Altered channel morphology is amongst the most apparent anthropogenic modifications to natural river systems. The impact of river channelization on ecosystem functions, including instream nutrient dynamics, is substantial in large parts of the world (Laub and Palmer, 2009; Rode et al., 2008; Opdyke et al., 2006; Kemp and Dodds, 2002). Restoration efforts may have a positive effect, if they are well designed (Bukaveckas, 2007; Stanley and Doyle, 2002). However, also adverse impacts of river renaturation on water quality have been observed (Schirmer et al., 2014). In order to ensure that restoration projects meet their goals and to prevent negative outcomes, it is therefore important to gain profound understanding of cycling pathways and influencing parameters and to provide realistic models on which policymakers can rely. Additionally, it is desired to identify hot moments (e.g. certain seasons or singular events) or hotspots (habitats or locations in the stream) of transformation and retention capacity. For instance, determining periods of low retention rates is crucial for adjusting periods when fertilizer application is prohibited (Dupas et al., 2016; European Environmental Agency, 1991a). Identifying the local distribution of environmental functions is essential to protect, enhance or reestablish designated ecosystem services (Hester and Gooseff, 2010). Thus, continuous, quantitative reach scale measurements are needed in order to characterize present and to predict future solute transformation characteristics of rivers. Thereby the complex interconnections of various physical, chemical, and biological processes influencing the cycling of nitrate and other substances make it difficult to quantitatively relate causes and effects (Schirmer et al., 2014; Wohl et al., 2005).

The two morphologically contrasting reaches at the Weiße Elster provided paramount conditions to study the effects of channel modification on nitrogen transport and transformation. Hereby, the channelized reach of the Weiße Elster served as an extreme example of a completely modified river reach, displaying besides the straightened course other typical features such as increased flow velocities, negligible surface storage, a monotonous and sealed river bed and absence of riparian vegetation.

Indeed, channel morphology proved an important determinant of instream nitrogen cycling patterns. The natural reach exhibited a remarkably (1.8 - 3 times) higher capacity to remove nitrate than the channelized one. However, changes in water quality also affected nitrate processing rates so that observed inter-reach differences could not exclusively be attributed to morphology. The impact of the mine drainage inflow on nitrate dynamics illustrated the role of N-species composition on turnover characteristics. Not accounting for an ammonium point source can lead to false conclusions on nitrate dynamics in several ways. As an example, it is commonly assumed that dissimilatory pathways are, as opposed to assimilatory ones, constant over the day. The results presented in this study indicate that this is not true in heavily modified reaches where high nutrient loads and abundant insolation result in extreme diurnal oscillations of temperature and dissolved oxygen. Deriving assimilation rates from the differences between night- and daytime nitrate uptake might therefore be error prone in such systems. Further, monitoring stream nitrate loads alone may not be diagnostic if ammonium imports are neglected. Conclusively, reducing nitrate emissions to streams does not lead to a declining trend in nitrate concentrations if ammonium import remains. From an applied point of view, that means that the positive effect of programs such as improved fertilization plans on water quality cannot be realistically evaluated. This, in turn, may result in inefficient prioritization of measures and demotivate to implement further programs.

While the existence and effects of a point source are still relatively easily assessable, quantifying the impacts of human activities in the catchment on instream processes via changes in water quality or unnatural variations of solute concentrations is much more difficult (Halliday et al., 2015; Bieroza et al., 2014; Birgand et al., 2007). Hereby, reach scale investigations may allow detecting local instream processes and disentangle these from the overlying catchment or basin scale dynamics. When defining the longitudinal extension of a reach, it is crucial to consider that the input signal at the start of a reach is an assemblage of catchment, local and upstream processes which may be similar or different to the instream processes on the target reach.

Climate is the overarching determinant governing hydrology and inherently runoff, transport and turnover of nutrients in a catchment (Dupas et al., 2016; Boyacioglu et al., 2012; Wagener et al., 2010). Additionally,

temperature influences the kinetics of most biological and chemical (instream) processes (Demars et al., 2011; Garcia-Ruiz et al., 1998a). Compiling sound scenarios on ecosystem functioning under a changing climate is challenging, due to the multifaceted driving factors and resulting ecological responses (Steffen et al., 2015; Boyacioglu et al., 2012; Arrigoni et al., 2010; Wagener et al., 2010).

The measurements in the Weiße Elster showed for example that climatic conditions, more than significantly influencing overall nitrate dynamics (supporting hypothesis A7), also provoked a change in dominance of distinct pathways. While assimilation stayed rather constant, denitrification was up to 10 times higher in July than in September. As a consequence, higher future temperatures, as predicted by climate change scenarios, will increase instream nitrate removal mainly via enhanced denitrification rates. Regarding denitrification as an example for microbial processes stimulated by reducing conditions, suggests that incorporating such a possible change in dominant turnover process into model simulations may be important to evaluate the changing behavior of other (redox-sensitive) substances as well.

High frequency solute sensors do not only deliver valuable data sets which can effectively be used to describe and quantify nutrient dynamics in a reach. The data generated today, especially if continuous, may be of even more value in some decades and have the potential to deliver important facts on long-term trends. Continuous, quantitative assessments will eminently improve models and future scenarios, which will hopefully lead to better mitigation of climate and land use change effects on water quality.

5.2.3. HYPORHEIC NUTRIENT FLUXES AT THE HOLTEMME

As in the Weiße Elster study, fundamental principles commonly forming the basis for estimating nitrate cycling patterns proved invalid under the conditions in the Holtemme River testing reach. Disproving hypothesis C1, higher discharge did not lead to higher hyporheic exchange rates. As discussed in detail in chapter 4.5, surface flow driven surface-subsurface exchange as observed in natural systems were trivial in the channelized study reach with monotonous morphology and riverbed structure. Instead, hyporheic exchange rates were primarily determined by the hydraulic gradient between stream and groundwater level. Especially in agricultural streams, this relationship between groundwater level and hyporheic exchange rates can be of significant consequences. Saturated groundwater tables and low temperatures are common for early spring in temperate regions (e.g. northern and middle Europe). There, the main season of fertilizer application may coincide with low biological (nutrient uptake) activities and in addition

low hyporheic exchange. For the EU, periods when fertilization is prohibited have been defined in the amendment of the fertilizer regulation. In Germany, ratification is planned for the first quarter of 2017 (BMEL, 2016). So far, those prohibitions only involve the autumn and winter season. However, as also suggested by previous studies (Dupas et al., 2016), intense fertilization in early spring may be similarly or even more critical.

Alarmingly, estimating hyporheic exchange and inherently hyporheic nutrient retention capacities using surface water discharge (commonly high in spring during snowmelt) as major driver would lead to the opposite assumption: Low biotic uptake is expected to be compensated by enhanced exchange rates. If so, the basin topography and groundwater dynamics are used to predict hyporheic exchange flows on larger scales, e.g. the river basin or valley (Magliozzi et al., 2017; Caruso et al., 2016). Streams or reaches are accordingly classified into losing, neutral or gaining in respect to the dominant direction of stream-groundwater exchange (Trauth et al., 2013; Cardenas and Wilson, 2007; Malzone et al., 2016). It is acknowledged that surface-subsurface exchange patterns differ between those states and that locally and seasonally one system can change from one to the other. On the local scale, usually riffle-pool sequences, channel morphology (for instance meanders) or sediment properties are considered as superimposed, governing drivers of hyporheic exchange rates. In poorly structured streams like the Holtemme, these local drivers are absent so that the larger hydrogeological and topographical setting of the area were the remaining dominant factors inducing hyporheic flow. As a consequence, relationships derived from observed or modelled dynamics at pool-riffle sequences may not be transferable to (anthropogenically modified) systems, where these structures are vastly absent. Further, residence time and oxygen could not satisfactorily explain nitrate turnover rates in the Lower Holtemme (hypothesis C2). Assumedly, the effects of irregularly fluctuating ammonium concentrations caused by WWTP runoff and inherent nitrification rates were dominating at the testing site during the assessments in April. In June, the strong diurnal amplitudes in oxygen concentrations arising from the anthropogenically elevated nutrient levels and abundant insulation were masking the flux mediated turnover patterns. Even though remarkable differences in absolute processing rates and patterns between summer and early spring were detected, the nitrate reaction rate (percentage of infiltrating nitrate which is removed by hyporheic processes) was similar during both seasons. Assumedly, lower biotic activity during the colder months was compensated by longer residence times. Still, the oxygen profiles indicate that biotic activity was several-fold higher in June than in April, supporting hypothesis C3. Seasonal patterns in biogeochemic processes are natural in temperate regions. Nevertheless, the presented results suggest that seasonal as well as diurnal variability in nutrient transformation may substantially be fostered by human modifications to hydromorphology,

riparian vegetation and water chemistry. As already discussed, hyporheic processes are often estimated using empirical or numerical models, partly based on observed surface water concentrations and hydraulics. The hydromorphology and catchment hydrology can deliver important insights into the solute dynamics in a stream. However, it should be kept in mind that models and equations are based on general relationships and that many exceptions to those assumptions exist. This implies that estimating subsurface flow and solute fluxes from surface hydraulics may fail under specific settings. The results presented here indicate that especially systems subjected to anthropogenic modification behave different to natural streams.

Most studies addressing anthropogenic impacts on hyporheic zone functioning focused on fine sediment deposition in agriculturally and urban landscapes, the subsequent colmation of the interstices and the resulting ecological implications (Pholkern et al., 2015; Hartwig and Borchardt, 2015; Ranalli and Macalady, 2010; Boulton, 2007; Grimm et al., 2005; Battin and Sengschmitt, 1999). This process can lead to a complete disconnection of the hyporheic zone and inherently a diminished self-cleaning capacity of the river or stream. At the Lower Holtemme, clogging was not observed (probably because fine particles precipitate above the weir upstream of the study reach). The study reach displayed an, at least temporally, active and well connected hyporheic zone, indicating that also in agricultural stream, the hyporheic zone may be of outstanding importance regarding the water quality. However, at the same time our study underpins that direct and indirect impacts of human activities on hyporheic functions are extremely multifaceted and that more research on hyporheic zones in modified rivers and streams is needed to understand the functions of those peculiar systems.

5.3. MOVING BEYOND AND OUTLOOK

Distinct processing pathways are specific for nitrate. Still, the behavior of a reactive solute in a lotic ecosystem subjected to biological and chemical transformation as well as physical transport and storage is transferable to other substances of anthropogenic origin threatening ecosystem and human health. For instance, pharmaceutical compounds enter rivers, like nitrate, via point sources with WWTP effluent, but also via diffuse inputs from agricultural application of manure (Voelker et al., 2016; Arle et al., 2014).

Advanced investigations of instream nitrate processing pathways in streams and rivers do therefore not only contribute to understand nutrient dynamics, but can also deliver relevant information on the overall functioning of rivers and streams in respect to solute transport, transformation and retention.

Thereby, nitrate can be used as an indicator for evaluating how human activities alter important ecosystem functions which are essential for overall matter retention and the self-cleaning capacity of streams and rivers. Detailed understanding of processes and factors determining instream retention and degradation rates of (harmful) substances are the key to protecting these indispensable ecosystem services. Scientific knowledge and methodology for investigating and monitoring instream nitrate dynamics, though not complete, are advanced and can therefore serve as basis for many other (emerging) substances. Further improvement of methodologies is a fundamental prerequisite to identify and reduce negative impacts on ecosystem services. Measurements have then to be followed by correct interpretation and extrapolation of the results and finally the knowledge has to be transferred into effective measures.

Regarding the first step, the methodological advancements presented here have the potential to improve scientific assessments as well as official monitoring practices. Continuous and quantitative measurements are particularly important to assess and predict the susceptibility of stream ecosystem functions to (changing) drivers. It is for example impossible to define the effect of river restoration programs, to prioritize measures and prevent adverse, unwanted outcomes (Schirmer et al., 2014) without quantitative evidence. In general, there is not one universally best method for the investigation of instream nitrate dynamics. Usually, the choice depends on the site specific conditions at the study site and the research question or management goal. Finally, it will be a question of effort (and costs) in relation to requirements. For instance, compared to manual methods for assessing hyporheic nutrient fluxes over longer time periods, HPFMs are labor effective. However, if diurnal or short term amplitudes are important, repeated manual sampling may still be indispensable. In other cases, it may be sufficient to measure surface water

dynamics and estimate overall processing rates from those observations using a model. Often, a combination of several methodologies delivers the most informative results (Fleckenstein et al., 2010; Groffman et al., 2006).

5.3.1. SCALING AND REPRESENTABILITY

Continuous measurements facilitate conclusions about long term behavior of systems. In the frame of the studies presented here, the total amount of nitrate inputs that is retained from downstream transport could be determined for the examined stream sections and periods. To evaluate the importance of instream processes in total matter retention, it is anticipated to assess retention rates (as total amounts or percentage of inputs) of complete river networks. For nitrate Seitzinger et al. (2006) estimated that globally, river networks could remove 13 % (as opposed to 46 % in terrestrial soils) of terrestrial nitrogen sources by denitrification. Values vary widely between watersheds and regions and also between years. A comprehensive review (Birgand et al., 2007) summarized that instream retention of nitrogen can range from 1 % and 76 % of the inputs, with hydrological residence time, climatic region and geomorphology being the predictor. There is agreement that overall removal is more efficient on the watershed scale than on the reach scale, because of the cumulative effect of continued removal, whereas it is debated whether small streams are more important in nitrate removal (high ratios of stream bed area compared to water volume) or larger rivers (larger transport distance, longer residence times and higher loads) (Mulholland et al., 2008; Wollheim et al., 2006). Indeed, already in 1999, Behrendt and Opitz could show that the hydraulic load (the quotient between mean depths and residence time) could explain 61 % of instream nitrogen removal rates for a large range of river basins in Europe. Integrating the “flashyness” (variability in discharge) of a river network into the analysis improved the results (Basu et al., 2011). Nevertheless, up to today, river stage and residence time are used as main predictors to estimate nitrogen retention rates on basin or catchment scales (Botter et al., 2010; Mulholland and Webster, 2010; Seitzinger et al., 2006). Such basin scale estimates are commonly derived from estimated inputs, for example calculated nitrogen surplus and measured or estimated nitrogen export at the basin outlet. Uncertainties mainly arise from difficulties to relate these large scale water-shed models to experimental data (Alexander et al., 2009; Boyer et al., 2006). First, as different processes are dominant depending on the contemplated spatial extension (Magliozzi et al., 2017; Choi et al., 2000) and because extrapolation of local observations to larger scales remains challenging (Basu et al., 2011; Tank et al., 2008). Second, local heterogeneity may

lead to false estimates if a location specific behavior or property is considered to be representable and extrapolated to the whole system (Fovet et al., 2016; Dent and Grimm, 1999). Finally, modelled uptake rates commonly correspond to annual values, but most direct measurements are conducted during low flow. A synthesis of percentage removal of nitrate by river networks from more than 300 measurements (Alexander et al., 2009) showed that seasonal variations in nitrate removal may be more marked than stream size related variation. The results from the Weiße Elster confirmed that seasonal effects can be large and further indicated that seasonal differences may be more pronounced in modified than in natural rivers. Although this hypothesis still has to be proven by whole year assessments, these findings suggest that insufficient characterization of seasonal dynamics will lead to particularly high errors in estimates for modified systems. Nevertheless, with the presented approach, those seasonal differences could be quantified. It is now of interest if such novel direct measurements can be related to catchment models and improve reliability of large-scale estimates.

Under the conditions in July 2015, around 2 % of the nitrate load was removed in the natural reach of the Weiße Elster and around 1 % in the channelized reach (remark that ammonia retention is not considered in this approximation). Extrapolating the July uptake as measured in the natural reach to the total length of the Weiße Elster (245 km) would mean that all of the nitrate could be removed. This rough approximation is of course utopic, because it assumes that the entire river features the characteristics of the natural reach, that no further inputs occur and that uptake is river size independent. In September, only one third of the July value could be retained and assumedly retention capacity in Winter would be even substantially lower. Remark that with a continuous implementation of the two station approach, all the seasonal dynamics could be characterized and integrated into one exact annual value. For now, considering seasonal and spatial variations in nitrogen uptake, the 18 % annual uptake estimated with the model MONERIS for the whole Weiße Elster catchment (Fuchs et al., 2010; Behrendt et al., 2001) seems realistic. Even more, as instream nitrogen retention in comparable regions, as for example 18 % of inputs for French river networks (Dupas et al., 2013) and 16 % for US rivers (van Breemen et al., 2002) are in the same range. An example for the difficulties in transferring parameters between scales can be illustrated by calculating the hydraulic load for the two assessed Weiße Elster reaches: The hydraulic load is (despite shorter residence times) lower for the channelized than for the natural reach which would lead to higher predicted uptake rates in the channelized reach. The main reason is that it is several times shallower than the natural reach. Shallower and wider rivers may in general be more efficient in nutrient removal owing to larger river bed surface and inherently more contact between the water and the reactive substrate. However, if the river bottom is concrete sealed, this effect is irrelevant. Integrating highly resolved

measurements, which specifically allow quantifying inter-reach differences and seasonal variations into large scale models, will improve predictability by accounting for the specific conditions in the river networks. Quantitative assessments will help to evaluate the ecologic and economic value of rivers. Recently, Siewert (2010) calculated the costs for a further reduction of the annual nitrogen-emissions from WWTPs in Germany to 6700 euro per ton N. Using an annual N load of 2111 tN a⁻¹ in the Weiße Elster (Wagenschein, 2006) as a basis, can give an overview of the monetary value of ecosystem services: 1 % retention (the difference between the natural and the channelized reach) would cost 140 000 euro per year if removed by a WWTP.

To quantify the efficiency of stream networks in retaining solutes, it is conclusively essential to integrate seasonal dynamics and to select representable locations for realistic upscaling of punctual observations. This requires defining the spatial entity for which observed dynamics are valid.

The extension of a catchment or river network is given by topography and the inherent hydrological connections (Birgand et al., 2007; Wagener et al., 2007). Extrapolation of reach scale observations to catchments requires hierarchically relating geomorphic units, hydrological patterns and biologic communities (Seitzinger et al., 2002; Stream Solute Workshop, 1990). All three reflect the interplay of climate and landscape parameters (Botter et al., 2010). On the reach scale, and of direct relevance for this thesis, it is critical to appropriately define the longitudinal extension of a specific reach, which might per se not be related to geographic units.

In Germany and many other states, rivers or river sections are officially classified into different “types”, based on the order in the river network, principal geologic and geographic settings and the degree of anthropogenic modification (Arle et al., 2014; Umweltbundesamt, 2012). The Lower Holtemme would for example be classified as a heavily modified 3rd order stream in the low mountain range, dominated by coarse carbonatic material (LHW, 2015). However, this classification is not necessarily informative regarding complex processes like instream solute transformation and retention capacity of a specific reach. Traditionally, environmental parameters are used to predict instream solute retention capacities. Recent improvements in assessing reach scale solute dynamics, including the methodologies developed in this work, suggest that in future, solute dynamics are used as an indicator for ecosystem integrity. The presented two-station and longitudinal approaches based on sensor measurements can hereby serve for a fast but already detailed analysis of instream processing characteristics. The results from the longitudinal profiling display the local heterogeneity of a single reach. Besides identifying processing hotspots, which may be of interest for more detailed examinations (e.g. locations of outstanding high retention rates), the

profile can also be used to select representable locations. At these locations, key processes, such as hyporheic dynamics, can be further investigated. Thereby, the longitudinal approach serves as a tool to define the representability of single point measurements for a whole reach. Combined with the two station assessment, the overall retention capacities of different reaches can be compared. A realistic comparison is thereby only possible because, different to short term assessments, the temporal fluctuation of processes is entirely captured. It is obvious that the presented experiment at the Weiße Elster mainly served to demonstrate the possibilities of the method. Ideally, common monitoring practices will be replaced by continuous two-station set-ups as presented here. Continuous measurements would further allow placing single short-term investigations into the seasonal context. This will help to decide if a single observation is representable for the longer term behavior of a reach regarding solute transformation or to define periods of outstanding high or low retention capacities. As an example: the HPFM experiments at the Holtemme allowed comparing hyporheic nutrient dynamics between two single periods. We assumed that one week in June and one week in early April can representatively stand for summer and early spring. Yet, there is no proof for this assumption. A collateral two station assessment of nitrate dynamics over the whole year would explicitly place the single observations in the annual context. Longitudinal profiling over several kilometers of the Holtemme could additionally reveal if the described hyporheic processes are representative for the complete lower reach or if local heterogeneity in nutrient processing is too high to extrapolate single point observations. The study reaches at the Weiße Elster and Holtemme were chosen, because they offered optimal conditions to test the respective methodologies. A combination of both assessments was at the time of planning not considered. However, future studies should attempt to integrate punctual investigations, such as hyporheic flux measurements, into longer term, larger temporal and spatial scale characterizations of nutrient dynamics. The example above highlights how much more value such a combination of several methodologies may add to each single investigation.

Ultimately, without misprizing the importance of instream retention and transformation processes, the only way to realistically reduce harmful nutrient exports to sensitive receiving water bodies and the negative consequences of eutrophication of aquatic systems is to reduce nutrient emissions. Even though anchored in European legislations, success of measures is still limited. It is still not decided if established regulations (and controlling their application) are not strict enough (BMEL, 2016), or if the success is only visible with a time lag, owing to the large storages in nutrient saturated soils and waters (Dupas et al., 2016; Kasahara and Hill, 2008). In any case, monitoring and managing nutrient dynamics in the environment will remain an ongoing duty of society.

5.3.2. MODIFIED ECOSYSTEMS

Anthropogenic impacts on ecosystems and their functioning are omnipresent. It has even been proposed to define the current epoch, whose most characteristic trait is the human pressure on the planet, as “Anthropocene” (Waters et al., 2016; Certini and Scalenghe, 2015). While initially greenhouse gas emissions were the focus of the scientific and political discussions, it was recently estimated that the anthropogenic enrichment of biologically available nitrogen in the environment may have similarly serious consequences as carbon dioxide emissions (Steffen et al., 2015; Galloway et al., 2003). Since the mid-20th century, anthropogenic perturbation of global biogeochemical flows, mainly those of nitrogen and phosphorus, have dramatically increased. Today, they vastly exceed the planetary boundaries identified as tolerable for the maintenance of ecosystem functioning and the earth capacity to sustain human life (Steffen et al., 2015; Vitousek et al., 1997). Still, on the background of rising population and increasing food demand, it is almost certain that fertilizer application and nutrient export from soils will keep rising in the decades ahead (de Vries et al., 2013).

Moreover, excessive nutrient emissions are not the only human pressures on aquatic ecosystems. By contaminating water resources with various organic or synthetic compounds, changing landscapes, soil properties, vegetation or hydrology, humans exhibit drastic effects on ecosystems and the services they provide (Voelker et al., 2016; Steffen et al., 2015; Malmqvist et al., 2002). For rivers and streams, direct modifications to the morphology and hydrology are one primary reason for the bad ecological status (Voelker et al., 2016; Arle et al., 2014; Umweltbundesamt, 2012). In Germany, 79 % of all streams are classified as markedly to completely modified (LAWA classification, classes 4 to 7 (Umweltbundesamt, 2012)). Of the world’s 177 biggest rivers (all rivers above 1000 km length) less than 40 % are still free flowing, the remaining majority is regulated by bank stabilization, concrete lining and dams (Bangs and Gods, 2006). While it is evident that human alterations to riverine ecosystems induce negative consequences which may also lead to the decline of essential ecosystem services, many cause-response correlations remain unclear (Wagener et al., 2010; Malmqvist et al., 2002). As a result, while restoration projects may have positive effects on instream matter retention, predictions on the quantitative outcome of such measures are hardly possible (Schirmer et al., 2014; O’Connor et al., 2010; Kasahara and Hill, 2008). Further, adverse effects of restoration measures on distinct ecological functions often have to be tolerated. For example, anoxic conditions, as observed in our study streams, are a primary requirement for denitrification and therefore positively affect instream nitrate removal. In total, anoxia presents a major danger to the health of aquatic ecosystems. First of all, it is lethal to many aquatic organisms.

Additionally, inherent reduction processes may also lead to deliberation of harmful compounds such as heavy metals and increase greenhouse gas emissions from water bodies by partial denitrification of nitrate to N_2O or reduction of carbon dioxide (CO_2) to methane (CH_4). To prevent unforeseen negative outcomes and to effectively prioritize management goals, detailed knowledge and reliable prediction of ecosystem functions under changing drivers is indispensable.

Most studies on instream processes, including those addressing nutrient cycling, have been conducted in rather small pristine or near-natural streams. Besides the already discussed challenges to extrapolate local observations to larger scales, transferring findings from natural study streams to systems where anthropogenic forcing added complexity and changed relevant ecological functions is critical. This thesis delivers further evidence that direct (e.g. river channelization, inlet of point sources) and indirect (urbanization, land-use) anthropogenic modifications to river systems substantially impact instream nitrate processing patterns. The presented results clearly demonstrate that anthropogenically modified rivers and streams respond unexpectedly if hypothesis are built on “textbook wisdom”. Anthropogenically modified systems should therefore not only be acknowledged as such, but also be evaluated considering the altered laws governing the processes in those systems.

Amplified investigations, specifically addressing the ecosystem functioning and interaction of processes in anthropogenically modified rivers and streams, are necessary in order to understand and characterize the behavior of such ecosystems and predict their response to changing drivers. As drivers also will keep changing in the future, permanent revision of conceptual and mathematical models is inevitably. The methodological advancements presented in this thesis may contribute to effectively characterize and monitor matter transport and transformation in lotic systems.

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