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## Keynote 1 – 25 April 2018

### Managing the impact of fine sediment on river ecosystems

**John Iwan Jones<sup>1</sup>**

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Sediment plays a pivotal role in determining the physical, chemical and biological integrity of aquatic ecosystems. However, human activities have increased loads of fine sediment delivered to rivers, such that it is now one of the most widespread and detrimental forms of aquatic pollution. Enhanced fine sediment bed loads impact adversely upon aquatic ecosystems by altering hyporheic physicochemical properties, degrading habitat condition and by directly impairing biota. The biological impact of fine sediment is likely to be a consequence of a combination of the source of fine sediment, and both the amount and rate of delivery and retention, as well as the susceptibility of the resident community. Here, flow plays a critical role: there is considerable potential for flow and fine sediment load to interact, with consequences for the benthic and hyporheic environment.

Given its key role in determining water quality, and the spatial scale over which mitigation must be implemented to achieve reductions in inputs, a sound evidence base is critical to understanding the impact of fine sediment on freshwater ecosystems. Here, I present outputs from a programme of research into the impact of fine sediment on aquatic systems involving national-scale field survey identifying sources and impacts of fine sediment, field scale experimental manipulations and modelling of the influence of sediment on hyporheic conditions and fish egg survival. We have developed and tested an empirical index relating invertebrate community structure to pressure from fine sediment, and have coupled biological endpoints (invertebrates and fish) to a landscape-scale modelling framework to explore the impact of land-use management interventions at the national scale. Identifying where fine sediments are causing issues and the management options available to mitigate these impacts is fundamental if we are to achieve water policy goals.

## Keynote 2 – 26 April 2018

**The Resazurin-Resorufin “smart” tracer technique: One decade of hydrologic applications****Julia L.A. Knapp<sup>1</sup>**<sup>1</sup> Center for Applied Geoscience, University of Tübingen, Germany

The reactive compound resazurin is transformed to resorufin in the presence of living microorganisms, for which reason it can be used as proxy of ecosystem respiration. The reactive tracer approach has been applied to assess metabolic activity in flowing surface water systems since it was first presented by Haggerty et al. (2008) one decade ago. These hydrologic applications of the reactive tracer system range from investigations on the cellular scale to river reaches and catchments. Whereas most small-scale investigations aimed at a better understanding of process-related properties and the link between the reaction mechanism and metabolic activity, larger scale approaches used the reactive tracer to estimate metabolic activity in different systems, usually through reactive stream-tracer tests. In order to relate different scales of reactivity, resazurin has also been applied to relate metabolic activity observed in the subsurface to respiration seen by in-stream tracer recordings.

In this talk I will revisit major hydrologic applications and milestones of the reactive tracer system in the past 10 years and highlight the advancements brought by the reactive tracer. I will furthermore discuss the merits and performance of resazurin, also in comparison to other, conventional methods used for the assessment of stream metabolism. Remaining obstacles for its use as a proxy for ecosystem respiration in surface waters as well as potential directions for future work will also be addressed.

## Reference:

Haggerty, R., A. Argerich, and E. Marti (2008), Development of a "smart" tracer for the assessment of microbiological activity and sediment-water interaction in natural waters: The resazurin-resorufin system, *Water Resources Research*, 44.

### Keynote 3 – 27 April 2018

## The turbulence constraint OR How much nitrate can a stream remove?

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The discharge of excess nitrogen – mainly as nitrate – to streams and rivers poses an existential threat to both humans and ecosystems. This threat is partially buffered by stream potential to remove nitrogen delivered to the river network. Nitrate is removed when it comes into contact with algae and bacteria growing on (and within) the sediment on the bottom of a stream. These algae and bacteria need nitrate to grow and therefore store it up or convert it to harmless di-nitrogen gas which is released back to the atmosphere. There is hence a huge interest in understanding how this process works and, in some cases, how it can be made more efficient.

This contribution discusses which are the most important factors that control nitrate removal rates in streams. In addition to streambed-related processes, it is hypothesized that in some streams turbulence can also exert a control on how fast nitrate is removed. This hypothesis is tested by developing a simple framework to calculate how fast nitrate would be removed if only constrained by the rate at which turbulence transfers nitrate to the bottom of the stream. These calculated rates are then compared with the previously published measurements of nitrate removal in 72 streams across the U.S. during the LINX II experiment.

Results show that the importance of turbulence for nitrate removal depends on the degree of nitrogen pollution in the stream. In pristine streams, nitrate removal is constrained by the rate of turbulent transport of nitrate to the stream bottom, while in polluted streams processes within the sediments are the most important controlling factor. The proposed theory provides an upper limit for nitrate removal in streams that is based on turbulent transport considerations. This limit represents a guidance for the design of stream restoration efforts.

## Hydrologic drivers of streamflow correlation and spatial patterns of flow regimes

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A proper characterization of streamflow variability in space and time represents a significant scientific challenge with a wide range of implications for human water uses and ecosystem services conservation. Despite this, relatively few rivers are adequately monitored and improving the density of existing gauging networks is often challenged by technical and economical limitations. Therefore, in most practical settings, observational data about spatial and temporal patterns of flow regimes may be inadequate. Following a stochastic framework, this work provides a physically based analytical characterization of the spatial correlation between daily streamflow time-series at two arbitrary locations along a river network. In particular, the streamflow spatial correlation is expressed as a function of the main geomorphoclimatic properties of the contributing catchments. We prove that the spatial correlation of daily streamflows represents an effective and synthetic index that quantitatively encapsulates the similarity between the hydrographs at two arbitrary outlets, and highly correlated outlets share similar hydrologic signatures across a wide range of geomorphoclimatic conditions. Model predictions can thus be adopted to regionalize streamflow regimes, identify redundancy or gaps in streamflow gauging networks and characterize spatial patterns of flow regimes along stream networks. We also discuss how predicted streamflow correlations can be used to couple river sites having similar flow regimes in absence of discharge data. The possibility to identify river reaches experiencing analogous streamflow characteristic is especially promising for the study of hyporheic exchange, and all those biogeochemical processes controlled by the spatiotemporal variability of flow conditions along river networks.

## Geophysical exploration as support for the spatial interpretation of groundwater-stream water exchanges observed by point and distributed techniques in heterogeneous streambeds

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The spatial and temporal variability of the factors governing groundwater-stream water interactions in the hyporheic zone still challenges our aim to estimate hyporheic exchanges. The application of distributed techniques such as fiber optics distributed temperature sensing (FO-DTS) has improved our capacity to portray the complex natural spatial and temporal variability of hyporheic exchanges. However, the technique is limited for estimating fluxes unless active configurations are applied. Point estimates can estimate fluxes but neglect the spatial variability of the riverbed. The upscaling methods based in the combination of these two types of techniques are still of low reliability due to the lack of integration of the variability of key sediment factors in their estimation processes.

Specific distributed techniques are required to explore the key sediment factors governing hyporheic exchanges such as hydraulic conductivity. Given that dense sediment properties exploration is time consuming and alters the hydraulics, an alternative time-effective, non-invasive and high resolution approach is required to identify the spatial and in depth variability of hydraulic conductivity in the streambed. Electromagnetic mapping (EM) is a geophysical technique with great potential to infer sediment properties related with the hydraulic conductivity.

This study aims to test the capability of electromagnetic conductivity mapping (EM) to characterize the variability of hydraulic conductivity in heterogeneous streambeds. Additionally, the study highlights the potential of the technique to distinguish the component of hyporheic exchange (groundwater, or local hyporheic flow) causing the specific thermal patterns observed in the SWI with fiber-optics distributed temperature sensing.

## The Kolmimeter - a new method for the quantitative measurement of colmation in the hyporheic zone

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In Europe, 80 % of all streams are far away from the so-called “good ecological stage”. By 2027, all streams, which are not classified as “heavily modified”, have to reach the good ecological stage according to the European Water Framework Directive (WFD). Billions of Euros have already been spent and many more are intended for the renaturation of streams and rivers. However, for the European stream policy the implementation of the WFD might become a disappointment.

This is probably the result of the erosion of fine sediments from the catchment due to intensive landuse: Fine sediments clog the interstices of the hyporheic zone – a dynamic process called colmation.

Colmation processes are of particular importance for stream management. On the other hand, no techniques were yet available for a reliable and fast quantitative measurement of colmation. Meanwhile, a newly developed device the “Kolmimeter®”, is available, which allows for a quantitative, time and cost efficient measurement of colmation. The principle is to measure sediment porosity by the injection of water.

The first field study on the applicability of the Kolmimeter® yielded strong correlations between colmation, WFD assessment and fauna, in particular hyporheic meiofauna.

## StygoTracing - a new biological tracing method for underground waters

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Hydrological exchange processes, in particular groundwater-surface water-interactions, are essential for the hyporheic zone as well as for groundwater ecosystems and for the quality insurance of drinking water. Such hydrological dynamics are assessed using various techniques, for example chemical tracers. However, the application is associated with a number of disadvantages, such as potential disturbance of natural habitats, dilution effects (low recovery rate), etc. Furthermore, to record the reoccurrence of the tracers, you have to be in the right spot at the right time.

Here, we present a promising alternative technique to trace hydrological processes using biological tracers. We use a population genetic approach, which allows for estimating the similarity of individuals and populations and thus the connectivity in natural and man-made systems (e.g. water supply networks). The „StygoTracing“ approach was successfully applied first to uncover the origin of surface water leakage into subsurface waters in the framework of a drinking water quality assessment. Currently, we are testing this approach to characterize hydrodynamic processes in catchments and water protection areas. The first, very recent results from the field are promising.

## Effect of regional groundwater flow on local hyporheic fluxes

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Rivers and aquifers are continuously exchanging water driven by processes that occur on different temporal and spatial scales ranging from small stream features to large geological structures. A key zone for the interaction between these two flow components is the hyporheic zone that forms by stream water that in- and exfiltrates in the permeable sediments surrounding the river corridor. Biological activity and contaminant fate and transport strongly depends on the exchange processes in hyporheic zone. Due to the effect of deep groundwater circulation on hyporheic exchange fluxes, a multi scale approach is applied to reach a comprehensive understanding of the effect of groundwater circulation on the intensity and fragmentation of hyporheic zone fluxes. Specifically, my research investigates the effect of catchment scale up-welling groundwater flow on local hyporheic fluxes. In our approach, we have used numerical models and field experiments to quantify this effect.

The model statement is based on the 3D Laplace equation, which has been applied independently on two ranges of topographical scales to obtain a superimposed solution, a description of the heterogeneous subsurface hydraulic conductivity and the ground surface topography. Local and catchment scale models were developed to quantify the local hyporheic fluxes in the stream network and regional groundwater flow field. Superimposing the flow fields of these two distinct scales result in measuring the effect of groundwater flow on hyporheic fluxes. This effect of the large-scale groundwater flow on the hyporheic flow was characterized by changes in the vertical exchange velocity at the stream-bed interface as well as the size and fragmentation of upwelling zones.

Radon ( $Rn^{222}$ ) and temperature have been used as the natural trace elements to find the variation in hyporheic depth and fragmentation of upwelling zone due to the effect of groundwater flow in wet and dry periods. Water level manipulation was implemented at the outlet of lake Stortjärn, Krycklan, during August 2017. The manipulations involved blocking a v-notch weir (completely) at the outlet and creating a flood pulse in the stream with a pump. Continuous temperature measurement was done using temperature lances at eight locations including both up- and down-welling regions. Radon concentration measurement was carried out for surface water, groundwater, and porewater samples. Preliminary results show that the hyporheic depth as well as the location and size of up-welling zones are highly dependent on the surface water level.

## Non-Darcy inertial fluid flow in heterogeneous porous media

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We provide an experimentally-based investigation of main features of inertial effects associated with flow in explicit pore spaces characterising permeable media. We focus on the effect of the heterogeneity of pore space morphology on inertial features of flow and relate these to relevant mechanisms driving momentum transfer. Experimental data that allow identifying the effects of porous structure on flow and transport through porous media in the inertial range are scarce. We illustrate the results of our experimental investigations aimed at documenting and characterising these complex processes at diverse Reynolds numbers in randomly structured porous media. The key objectives of the study are to (a) explore the effects of the randomness of the morphological structure of porous media on the relative importance of inertial and viscous forces and (b) assess the range of Reynolds numbers in which inertial flow predominantly contributes to dispersive processes. Transport characteristics of the randomly structured porous media and the influence of inertial force on longitudinal and transverse dispersion coefficients were studied. Our results display significant increase in solute dispersivity which is calculated when the Reynolds number increases.

## Physical and biogeochemical response of groundwater to flood events

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Hyporheic exchange flows (HEFs) between streams and shallow groundwater in river banks are key drivers of nutrient cycling and pollutant degradation rates (e.g., Boano et al., 2014). Hence, HEFs serve as self-purification mechanisms of freshwater bodies and are of major importance for water quality and ecosystem functioning (e.g., Brunner et al., 2017).

Recent studies have identified transient river discharge and the hydraulic conductivity of streambanks as major controls on HEFs (e.g., Gomez-Velet et al., 2017). However, studies on the spatial and temporal variability of HEFs based on field data remain scarce.

To study the physical and biogeochemical response of shallow groundwater to flood events we used a combination of established methods for hydraulic data (water heads, slug tests) and hydro(geo)chemical data (radon, major ions, nitrate, micropollutants, microbial activities) in combination with novel methods (continuous dissolved (noble) gas measurements). Our experiments were conducted at a restored reach of a losing stream in an urban area in Zurich, Switzerland. Our data show that during normal flow conditions exchange between the stream and shallow groundwater is only marginal. However, during flood events, we observe a rapid increase of enhanced river water infiltration delivering oxygen, nutrients and other solutes to the adjacent groundwater. The rate of enhanced streamwater infiltration depends on the frequency and intensity of the flood event as well as on the hydraulic conductivity of the streambed and -bank. This rapid delivery of streamwater seems to be attributed to the flushing of the clogged riverbed during intense enough flood events, which results in higher hydraulic conductivities and hence increased infiltration rates. In addition to quantifying water exchange rates, our continuous measurements of dissolved (noble) gases enable us to calculate the overall oxygen turnover in the groundwater. Such an estimate can only reliably be given by accounting not only for oxygen consumption but also for excess air (which is the inclusion and dissolution of air bubbles into groundwater during groundwater recharge). Therefore, oxygen turnover can only be quantified if argon (which serves as a conservative proxy for oxygen) and oxygen concentrations are determined synchronously (Mächler et al., 2013a; Mächler et al., 2013b). Our findings show that flood events and the hydraulic conductivity of the streambed and -bank are key controls on hyporheic exchange flows, hence, governing gas exchange, nutrient cycling, and pollutant turnover within the near-stream environment.

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## Differential heat propagation in ripples, dunes and riffle-pool bedforms and its impact on hyporheic exchange flows

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The physical, chemical and biological processes occurring in the hyporheic zones are often temperature-dependent. Thermal shifts have a direct impact on bacterial activity which primarily controls the degradation of organic matter, attenuation of contaminants, and nitrogen cycling. Heat propagation and distribution within streambeds is dependent on the induced pressure gradients along the sediment-water interface, which in turn are caused by interactions between channel flow and bed topography. Hitherto, little attention has been paid to the interplay between different bedform geometries (such as ripples, dunes and alternating bars) and their impact on heat propagation and hyporheic zone behaviors. To understand these interactions, we constructed a numerical model that coupled Darcy flow with heat transport equations. Diurnal temperature fluctuations in the water column were imposed at the sediment-water interface. Our results show the diurnal oscillations in surface water temperature propagate differently for different bedforms. Variations in dynamic viscosity generate significant changes in hyporheic flux, residence time distributions and solute transportation in the streambed. Understanding thermal patterns within the streambed with geomorphological controls facilitates deeper understanding of water chemistry and quality.

## Exploring the role of transient flood pulses on bedform-induced exchange under gaining and losing conditions

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Spatiotemporal variations of bedform-induced hyporheic flows depend significantly on the flow conditions and local geomorphological settings. Understanding transient bedform-driven hyporheic processes requires comprehensive investigation on the interplay of time-varying hydraulic forces, streambed topography, channel geomorphology and ambient groundwater conditions. The aim of the present study is to systematically explore the bedform induced hyporheic responses to flood events with two different intensities, three different bedform topographies, four channel slopes and ten groundwater up/downwelling conditions. By using a reduced-order model, the spatial extent of hporheic zone, the intensity of hyporheic exchange fluxes, the flux-weighted residence time distribution, and the hyporheic efficiency to denitrification process are used to assess the impacts of flood events. we find that HZ is formed and enlarged by increasing local pressure gradient induced by floods and bedforms; and compressed by increasing groundwater gaining or losing intensities. This competing process is further complicated by site-specific streambed topography and channel morphology. Additionally, we notice that increasing flood intensities can reduce the impacts of groundwater flow, but it is hard to overcome the geomorphologic controls. These findings highlight the necessities of evaluating hyporheic behaviors in a more comprehensive framework.

## Effect of bioclogging on hyporheic biogeochemistry in river dunes

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Riverbed sediments are biogeochemical hotspots because they host a number of important reactions that influence the fate of nutrients in streams and groundwater and play a key role in nutrient dynamics. These transformations are mediated by microorganisms attached to the hyporheic sediments in the form of biofilms. The metabolic activity of hyporheic microbes relies on water-borne solutes that are supplied by water exchanged with the nutrient-enriched stream. An important aspect to take into consideration to study microbial processes within the hyporheic zone is the feedback that microbes exert on nutrient fluxes through the process of bioclogging. Bioclogging is the filling of pore space caused by biofilm growth and gas production, with a consequent reduction of streambed porosity and permeability. Unfortunately, the influence of microbial metabolic activity on the hydrochemical conditions within the hyporheic zone is poorly understood since it is limited by the difficulty of data collection within streambed sediments. Furthermore, available models capture the effects of hyporheic exchange flow in microbial metabolism and biogeochemical transformation, but they do not consider the consequences of the bioclogging and larger-scale feedbacks on rates and patterns of hyporheic exchange flow.

In the present work, we introduce a hydrobiogeochemical model to study how the growth of heterotrophic and autotrophic microorganisms affects the transport and transformation of dissolved nitrogen compounds in bed form-induced hyporheic zones. The aim is to investigate how the coupling between hyporheic exchange, nitrogen metabolism, and biomass growth influences the biogeochemical and hydrodynamic processes, leading to an equilibrium between permeability reduction associated with microbial growth and microbial metabolism. The bioclogging process strongly limits the extent of hyporheic exchange producing sharp fronts with significant hyporheic flow confined only in the shallow portion of the domain where high rates of bacterial growth occur. Our findings show that biofilm-induced bioclogging strongly regulates physical and biogeochemical processes in the hyporheic zone since it limits both exchange flux and biogeochemical transformation rates, and controls the distribution of different types of microorganisms in many streams and rivers.



## OECD 308 tests to explore differences in persistence of pharmaceuticals and microbial diversity between two rivers

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Persistent pharmaceuticals in aquatic ecosystems are of particular societal concern and the OECD 308 guideline is often used to obtain the biodegradation half-lives required for risk assessment. The environmental relevance of OECD 308 has been criticized in recent years regarding the difficulty to interpret multiphasic processes (biotic and abiotic) and the lack of compartment-specific half-lives for water or sediment as an outcome. In particular, biodegradation processes in the sediment can vary according to the microbial communities, which may be impacted by the settings chosen for the test (i.e. sediment-water ratio, aerobic-anaerobic conditions and initial concentration levels). In this study, we have investigated the differences in biodegradation of a mixture of 9 pharmaceuticals (acetaminophen, caffeine, carbamazepine, diclofenac, fluconazole, metformin, oxazepam, tramadol, and venlafaxine) using water and sediment collected from rivers Fyris and Gründlach, before and after the discharge of a wastewater treatment plant (WWTP).

Bottle incubations were set following the OECD 308 guideline, spiked with pharmaceuticals and incubated for 40 days at 16°C in the dark with daily aeration. Water samples were taken at 10 time points and analyzed in UHPLC-MS/MS. The microbial community composition in the sediment was analyzed with Illumina sequencing of bacterial 16S rRNA to provide more insight into the biodegradation potential in the different treatments.

The dissipation half-lives obtained for diclofenac, tramadol, oxazepam and venlafaxine are significantly different ( $p < 0.001$ ) between rivers and between locations. This means that the biotic and abiotic processes influencing the dissipation are divergent between rivers GR and FY, but also between sediments taken up- and downstream the WWTP. The sterile controls with sediment sampled from river GR after the WWTP present slight or negligible change in concentrations for all compounds, which implies that the dissipation found in non-sterile sediment can be mostly attributed to biotransformation. The downstream location in river GR has the lowest organic carbon content (0.41% TS) from the four sediments. Sorption cannot be completely excluded for the three other sediments, as the concentrations of the nine compounds decreased in the sterile controls. However, the half-lives of non-sterile treatments are significantly shorter than sterile treatments ( $p < 0.01$ ) for all compounds except carbamazepine, indicating that dissipation reflects a combination of biodegradation and sorption mechanisms. In general, compound concentrations remained constant in river water treatments, with only caffeine and acetaminophen showing signs of degradation. The compounds displayed constant concentrations in the controls with sterile water, and therefore hydrolysis was not a relevant transformation pathway in our experimental set up.

Our results show that dissipation of pharmaceuticals obtained from OECD 308 experiments is divergent for rivers with different characteristics and between the locations upstream and downstream of a WWTP. Biodegradation and sorption in the sediment were key removal mechanisms of pharmaceuticals, in contrast, no degradation was generally observed in water-only incubations. However, differences in the treatments could be better explained by the divergent biodegradation potential in each of the four sediments. Analysis of the bacterial communities' richness, evenness and composition in river sediment and water will aid in the interpretation of the different half-lives obtained.

## The effect of unsteady streamflow on oxygen consumption in a sandy streambed under varying vertical flux conditions

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Wastewater treatment plants which discharge treated effluent into river systems can drastically alter downstream flow regime from steady flow velocities to water velocities which fluctuate on a diurnal cycle. The impacts of flow variations are expected to have complex and non-linear impacts on the temporal and spatial redox dynamics within the hyporheic zone.

The objective of the current study was to qualitatively describe the effect of non-steady flow on oxygen consumption under a range of vertical flux conditions. A novel flow control system was deployed in a purpose-built 260 cm-long by 29 cm-wide flume. Oxygen dynamics were investigated by using planar optodes to measure oxygen concentrations in the hyporheic zone at a high temporal and spatial resolution.

We found that using steady conditions to approximate unsteady conditions will cause systematic underestimation of hyporheic exchange flux and that vertical flux condition can have a stimulatory or suppressive impact on the microbial community. These results will be useful for the calibration of models and provide river managers with important information on how to promote hyporheic exchange and enhance the bioremediative potential of the hyporheic zone.

## What rules a river's self-cleaning dynamics: hydrology or microbiology? – Insights from a mesocosm experiment

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Urban rivers receive high loads of personal care products, pharmaceuticals, and their transformation products from waste water treatment plant outfalls. Continuous exposure to these organic micropollutants represents a considerable threat to river ecosystems, and ultimately restricts how they can be used. Nevertheless, rivers can display self-cleaning capacity for degradable and sorptive substances depending on hydrological dynamics and the presence of certain microbial communities. High diversity of the microbial community and increased hyporheic flow may enhance micropollutant transformation, but few data currently exist on the numerous substances which occur in the aquatic environment.

In the present work, we used mesocosm experiments to investigate the role of sediment microbial diversity, nutrient turnover, hyporheic flow, and redox zonation on pollutant transformation processes. In total, 24 circulating flumes were setup under semi-controlled conditions. The sediments consisted of sand inoculated with three serial dilutions of biologically active sediment. Sediment morphology in the flumes was also modified using bedforms to induce varying extents of hyporheic exchange flow. Nutrients were added resembling the concentrations in an urban river receiving treated waste water. After 12 days of inoculation, a set of micropollutants (mainly pharmaceuticals), was injected to obtain an initial concentration of

approximately 10 µg/L per substance. Unfortified biotic and abiotic controls using sterile sediment were also included.

Surface water and pore-water were sampled from the flumes 10 times over the course of 78 days. Preliminary results indicate that both microbial diversity and hyporheic flow have an effect on the degradation rates of certain pollutants as well as on nutrient turnover.

## Enantiomeric Fractionation – an indicator of biotransformation during a water-sediment flume study

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A large number of environmental organic contaminants features stereogenic structural elements. While abiotic processes usually act identical on enantiomers, biotic processes are often enantioselective due to preferential microbial uptake or enantioselective biotransformation. It is therefore most likely, that a molecule's enantiomeric composition changes throughout its environmental life cycle.

In June 2017, 24 water-sediment test flumes were set up in order to study the fate of 31 organic contaminants. Major variables were hyporheic flow and the amount of river sediment inoculum. Over a period of 56 days, the enantiomeric composition of eight chiral organic contaminants was monitored in surface water, whereby celiprolol, a so far barely studied beta-blocker, revealed an interesting enantioselective behavior.

## Transformation of the antidiabetic drug metformin in mesocosm and river systems

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Metformin is among the most commonly prescribed antidiabetic pharmaceuticals globally. It is not metabolized in humans and enters waste streams largely unchanged. Consequently, high metformin concentrations (up to 47 µg/L) have been reported in wastewater treatment plant effluents. Little is known about the behavior of metformin in the environment, but exposure to concentrations as low as 40 µg/L is known to cause intersex in male fish.

In this 3-part project, the transformation of metformin in two artificial river systems and in the river Erpe was investigated. These data will help to elucidate risks associated with occurrence of metformin in the environment, as well as factors controlling its attenuation. The overall project goals were to assess degradation rates and product formation under environmentally realistic conditions with a specific focus on the effect of sediment morphology and macrophytes on metformin attenuation. We were also interested in studying the effect of metformin exposure on sediment microbial communities.

The first part of this work was conducted at a large scale artificial stream facility at UBA (Germany) using two recirculating outdoor flumes (100 m length each). Both flume systems were equilibrated for several years prior to dosing. The first flume was spiked with 12 µg/L of metformin while the second served as a blank control. Non-reactive tracers (BO33-, LiBr) were added in order to distinguish between various processes (i.e. sorption vs degradation) contributing to metformin attenuation. In a follow-up mesocosm study, the influence of microbiology and hyporheic flow on metformin transformation was investigated using 24 smaller recirculating flumes. Samples of surface and pore water, and sediments were collected in both studies at regular timepoints. Oxygen profiles, along with SO<sub>4</sub>, Fe<sup>2+</sup> and NH<sub>4</sub>, and pore water flow velocity were measured over the duration of the experiments. Finally, the medium sized, eutrophic lowland river Erpe at the eastern edge of Berlin, Germany served as a model to study the fate of metformin and guanylurea in a natural system. Samples of surface water and pore water were taken during two field campaigns in April and June 2016.

For all experiments, instrumental analysis was carried out by UHPLC-MS/MS and GC-MS while microbial communities were characterized using quantitative real-time PCR and time-resolved amplicon Illumina MiSeq sequencing targeting the 16S rRNA genes and transcripts. Results generated from these experiments indicate that Metformin undergoes degradation in both artificial and natural systems. Importantly, high concentrations of the transformation product guanyurea were formed, which explains its frequent observation in the aquatic environment. The impact of sediment morphology and macrophytes on metformin distribution are reported here for the first time.

## Sorption of micropollutants to soil and biofilm

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Sorption is one of the main factors influencing the fate of micropollutants in environment, beside biotransformation and microbial degradation processes. A number of pharmaceuticals of concern originated from insufficient treatment of wastewater are released into the river. Three selected pharmaceuticals were chosen to study the sorption processes into hyporheic river sediment and biofilm using column and batch experiments. Biofilm were cultivated by using column experiments for three weeks duration. As for batch sorption experiments, 6 g of air-dried sediment samples were added into 30 mL of mixed solution for producing ratio of 1:5, while 10 g of biofilm were added into 10 mL of mixed solution for ratio of 1:1. The results of batch kinetic adsorption study revealed that these pharmaceuticals achieve equilibrium within 24 hours. Sorption of the pharmaceuticals onto soil and biofilm were calculated using mass balance. Results obtained were described by Freundlich equations. Carbamazepine recorded the highest sorption onto soil, while sorption onto biofilm was found to be sulfamethoxazole. The results also showed general trend of decreasing soil sorption capacity with increasing pH in solution. Carbamazepine recorded the highest  $K_f$  values ranging from 37.8 to 57.96 L/kg while sulfamethoxazole showed dramatic changes from pH 5 ( $K_f = 15.9$  L/kg) to pH 9 ( $K_f = 1.8$  L/kg). There was no sorption detected for ibuprofen onto biofilm, although this compound has the highest  $\log K_{ow}$  and the lowest water solubility. Organic carbon content, solution pH and charge of compounds were found to be the major factor governing the soil sorption processes of micropollutants.

## Microbial removal of organic micropollutants in the hyporheic zone

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Microbial turnover and degradation of nutrients and pollutants are among the prominent ecological services the hyporheic zone provides. Emerging micropollutants such as pharmaceuticals and personal care products reaching the aquatic environment via urban effluent discharge are hypothetically amenable to biodegradation; a major attenuation mechanism in the hyporheic zone.

Biodegradation potentials of two pharmaceutical residues ubiquitously detected in receiving rivers, i.e. Metoprolol (Beta-blocker) and Ibuprofen (non-steroidal anti-inflammatory drug) by the hyporheic zone of an impacted river has been investigated. Batch microcosms containing hyporheic zone sediment were used to estimate removal of metoprolol in oxic and anoxic conditions while ibuprofen degradation was tested in oxic microcosms only. Biodegradation potentials and associated key degraders were determined using HPLC-MS and amplicon Illumina MiSeq sequencing targeting the 16S rRNA genes and transcripts.

Metoprolol and ibuprofen were transformed in hyporheic zone sediments but not in abiotic controls, suggesting an important role of microbial activity. In refed microcosms, metoprolol biodegradation occurred faster in oxic compared to anoxic setups following initial incubation of 65 days. In oxic microcosms, metoprolol was biotransformed mainly to metoprolol acid while in anoxic conditions metoprolol acid and hydroxy-metoprolol were formed, indicating variable microbial activity under the different incubation conditions. Such metabolites were completely degraded within 30 days under both conditions. Ibuprofen biodegradation occurred in the absence and presence of supplemental acetate within 24 hours in refed setups following initial incubation of 11 and 16 days, respectively. Carboxyibuprofen and 1-, 2-, 3-hydroxy-ibuprofen were ibuprofen metabolites. Based on time resolved Illumina sequence analyses, hitherto unidentified microbial degraders of ibuprofen affiliating with the phyla *Proteobacteria*, *Chloroflexi*, *Acidobacteria*, *Actinobacteria*, *Bacteroidetes* and *Firmicutes* were identified.

Moreover, fate of pharmaceutical mixtures as commonly detected in the aquatic environment is under investigation where variable removal mechanisms depending on physicochemical properties of the individual compounds are involved. Preliminary results, however, indicate biodegradation to be the most robust organic micropollutant removal mechanism in the hyporheic zone for a majority of the compounds.

## Assessing changes in invertebrates traits: the effect of large wood

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Large wood (LW) has become an integral component of many river restoration schemes. Indeed, previous studies have shown that the introduction of wood into a stream induces changes in: i) flow turbulence and diversion ii) channel structural complexity (i.e. pool frequency, depth and sinuosity of the river profile) iii) carbon input and habitat availability. The connection between wood structure and its ecosystem functions (e.g. habitat and food resource), has been extensively described for benthic macroinvertebrates in both lowland and upland rivers but not for hyporheic invertebrates. Also, large wood has been hypothesised to have a major role in enhancing hyporheic exchange flows, which is an essential prerequisite for the presence of a hyporheic invertebrate community, however to date no study has directly investigated this link.

Therefore, this study examined the effects of in-channel wood on the trait profiles of both benthic (macrofauna, retained by 500µm sieve) and hyporheic (meiofauna, retained by 45µm sieve) invertebrate assemblages at the reach scale. We predicted LW to be associated with different community functional traits than no-wood sites, taking into account LW-driving processes: increasing vertical hyporheic exchange, sediment hydraulic conductivity, oxygen availability, and decreasing temporal stability and denitrification. As confirmed by the Within Reach-Campaign Analysis and the Conditional Inference Tree Approach (CIT), functional traits differed between wood and no-wood sites in both benthic and hyporheic assemblages and between macro- and meio-fauna assemblages. In the benthic zone, wood sites are mainly characterized by specific physiological/morphological trait modalities (i.e. high body flexibility, temporarily attached or flying organisms, aquatic passive dispersal, maximum potential size > 0.25-0.5 cm and spiracle respiration) and trophic preferences (i.e. deposit feeding or shredding as feeding habits and detritus, dead plant and microorganisms as food sources) related to flow disturbance and presence of organic matter. In contrast, in no-wood sites, biological (i.e. asexual reproduction, isolated eggs and diapause or dormancy as resistance forms) and morphological/behavioural modalities (i.e. burrowers, maximum potential size < 0.25 cm or > 0.5-1 cm, and no or little body flexibility) indicate an increase of temporal stability and a reduction of physical flow constraints. Similarly, in the hyporheic zone, wood-related traits, especially morphological or physiological (i.e. cylindrical, life span < 1/yr, number of cycles ≤ 1/yr) and ecological (i.e. medium, fast flow velocity and twigs, roots, sand, cobbles preferences), reflect an increase of disturbance due to flow, while in no-wood sites physiological traits (i.e. maximum potential size > 0.25-0.5 cm, interstitial and crawler, no resistance form or diapause) seem more influenced by changes of interstitial space characteristics.

Searching for a common structure between environmental data and taxa traits, did not demonstrate strong association between wood-related traits and environmental variables. This result suggests that LW in the study stream affects functional trait diversity of benthic and hyporheic invertebrates but shared traits between wood and no-wood sites and opposite patterns of trait frequencies between samples do not support the direct correlation with environmental variables.

## Investigating conditions of the rhizosphere in a suburban river in response to WWTP effluent unloading

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In this study we are interested in the responses of macrophytes' rhizosphere with the concentration of nutrients from WWTP effluent loading in a lotic ecosystem located at a lowland suburban river in Berlin, Germany. Unloading of treated wastewater into a stream is known to increase the biomasses of macrophytes in a lotic system (Gücker et al., 2005). A sampling program of surface water with rhizospheric pore water of native macrophytes were performed in a suburban river affected by intermittent WWTP effluent loading. The location of the sampling was categorised to upstream and downstream reaches of a WWTP during the sampling campaigns. Two prominently present macrophytes, *Potamogeton pectinatus* and *Sparganium emersum*, were distributed along the river stretch. Samples were taken in rhizospheric pore waters of these species, coupled with surface water and macrophyte-free pore water. The samples are analysed for nutrient, alkalinity, salinity and pharmaceutical compounds. No increase of nitrate concentration, a commonly observed effect from a WWTP effluent discharge, were noted in habitats permeated with macrophytes. Although both *Potamogeton* sp and *Sparganium* sp indicate similar nutrient concentrations throughout the upstream and downstream reaches, the latter reported higher concentration of sulfate and ammonium after the effluent loading point, indicating a better potential of nutrient retention for *Sparganium* sp. This present study coupled with results of an ongoing pharmaceutical analysis could indicate the responses of how different macrophyte species could create conditions better nutrient cycling.

## Mechanistic understanding of an ecosystem service: Efficiency of emergent pollutant biodegradation in the hyporheic zone

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Water is considered the most indispensable natural resource, yet organic pollution of freshwater sources is widespread. In recent years, there has been increasing concern over the vast array of emerging organic contaminants (EOCs) in the effluent of wastewater treatment plants (WWTPs). However, bacterial biofilms inhabiting the pore-space of streambed sediments may degrade several of these EOCs. Here, we report how dissolved organic carbon (glucose) and predator-prey interactions drive the capacity of streambed bacteria to process a model EOC (ibuprofen). Glucose had a significant positive effect on population density and activity of bacterial ibuprofen degraders. Thus, when glucose was present, ibuprofen removal efficiency increased. Moreover, low and medium levels of predation by a ciliate protozoan, stimulated bacteria population growth and ibuprofen removal whereas high levels of predation resulted in lower bacterial growth and less ibuprofen removal. This hormesis-like effect of predation interacted synergistically with glucose availability resulting in a notable increase in bacterial population growth and EOC removal. Our results suggest that, even under a scenario of increasing nutrient input, bacterial degraders are able to maintain the self-purifying capability of the system. These findings are of considerable interest to fully understand the underlying mechanisms of an important ecosystem services given that eutrophication of freshwater systems is increasing worldwide. In addition, our results emphasize the importance of preserving natural predator-prey interactions in order to maintain and sustain ecosystem services.

## Unveiling biofilm architectural differentiation in sedimentary environments

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Hydrated porous systems with their high surface availability are attractive substrates for microbial colonization. For instance, the hyporheic zone of streams hosts a diverse ensemble of microbial communities, generally organized in biofilms concealed within the sediment interstices. There, topographic heterogeneity and fluid motion constrain biofilm growth by altering solute supply and hydrodynamic stress. Yet, the interplays between hydrodynamics in porous landscapes, biofilm structure and function remain poorly understood. To better appreciate these links, we designed porous landscapes differing in topographic heterogeneity by arranging matrices of pillars within planar and transparent fluidic devices. The landscapes were saturated with stream water, and by means of a peristaltic pump a laminar flow was imposed. Biofilm formation and local hydrodynamics were investigated using time-lapse microscopy and micro-particle image velocimetry. During the experiment two clearly different architectures formed: a biofilm coating the grains, and streamers extending through the pore space. By image processing, we separated and quantified these two architectures. We showed that the architectural differentiation was the result of different biophysical processes: in situ growth of biofilm as well as particle retention. Our experiments shed new light on the biofilm mode of life in the dark of the hyporheic zone.

