Interactions between flow hydrodynamics and biofilm attributes and functioning in stream ecosystems

by

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This dissertation is based on the following research articles (ordered by publication or submission date):

1. **Anlanger, C.**, Noss, C., Risse-Buhl U., Brauns, M., von Schiller D., Weitere, M. and Lorke, A. Linking hydromorphological diversity to biodiversity and functioning in running waters (submitted to *Nat. Comm.*, in revision).

2. **Anlanger, C.**, Risse-Buhl, U., von Schiller, D., Noss, C., Weitere, M. and Lorke, A. (2021), Hydraulic and biological controls of biofilm nitrogen uptake in gravel-bed streams. *Limnol. Oceanogr.*, 66: 3887-3900. https://doi.org/10.1002/lno.11927

3. Risse-Buhl, U., **Anlanger, C.**, Noss, C., Lorke, A., von Schiller, D. and Weitere, M. (2021) Hydromorphologic Sorting of In-Stream Nitrogen Uptake Across Spatial Scales. *Ecosystems* 24, 1184–1202. https://doi.org/10.1007/s10021-020-00576-7

4. Risse-Buhl, U., **Anlanger, C.**, Chatzinotas, A., Noss, C., Lorke, A. and Weitere, M. (2020), Near streambed flow shapes microbial guilds within and across trophic levels in fluvial biofilms. *Limnol. Oceanogr.*, 65: 2261-2277. https://doi.org/10.1002/lno.11451

5. Risse-Buhl, U., **Anlanger, C.**, Kalla, K., Neu, T. R., Noss, C., Lorke, A., & Weitere, M. (2017). The role of hydrodynamics in shaping the composition and architecture of epilithic biofilms in fluvial ecosystems. *Water Res.*, 127: 211–222. https://doi.org/10.1016/j.watres.2017.09.054

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Abstract

Biofilms constitute an integral part of freshwater ecosystems and are central to regulating essential stream biogeochemical functions, such as nutrient uptake and metabolism. Understanding the environmental factors that dictate the composition of biofilm communities and their role in whole-system nutrient cycling remains challenging, given the large spatial and temporal variability of biofilm communities. Pristine mountain streams exhibit a heterogeneous streambed ranging from boulders to sand, provoking high spatiotemporal flow variability. Our current knowledge of the interactions between flow hydrodynamics and biofilm attributes stems from mesocosm studies, which are inherently limited in environmental realism. Moreover, the mechanism linking flow hydrodynamics to microbial biodiversity and ecosystem functioning is currently not studied. My thesis aims to link streambed heterogeneity and the associated development of the flow field to biofilm attributes and nitrogen uptake based on a multidisciplinary field approach. It integrates several spatial and temporal scales ranging from millimeter-sized spots to stream reaches and from milliseconds to minutes (i.e., the hydraulic scale of velocity fluctuations), up to days, months and years (i.e., the hydrological scale of flow fluctuations). I demonstrate that the spatial niche variability of flow hydrodynamics was an essential driver of biofilm community composition, diversity and morphology, in line with the habitat heterogeneity hypothesis initially formulated for terrestrial ecosystems. Furthermore, hydraulic mass transfer associated to flow diversity and biofilm biomass determined biofilm areal nitrogen uptake at scales ranging from spots to the stream reach. At the whole-ecosystem level, flow diversity determined the quantitative role of biofilms compared to other nitrogen uptake compartments by sorting them according to prevailing flow conditions. The magnitude of effects depended on ambient nutrient background and season, suggesting a hierarchy of the environmental controls on biofilms. In summary, my interdisciplinary research provided a mechanistic understanding of how hydromorphological diversity determines the diversity, morphology, and the functional role of biofilms in streams. By improving the understanding of these relationships, my research improves our ability to predict and scale measurements of important stream biogeochemical functions. Moreover, it helps to face the challenges imposed by environmental changes and biodiversity loss.

1 Introduction

1.1 The significance of biofilms in streams

Biofilms are complex microbial communities consisting of different microbial guilds, including bacteria, algae, fungi, and biofilm-dwelling micrograzers (i.e., phagotrophic protists and small metazoans), which cover almost all surfaces in stream ecosystems. They are enclosed in a matrix formed by extracellular polymeric substances (EPS, e.g., Marshall, 1976; Zobell, 1943, Figure 1). Biofilms are an integral part of streams and rivers, constituting primary and secondary producers and representing the main food source for higher trophic levels (Lock et al. 1984; Wetzel 2001; Weitere et al. 2018). Densities of microbial guilds exceed those of planktonic systems by several orders of magnitude (e.g., Costerton & Gessey, 1979). Consequently, biofilms play an essential role in stream ecosystem processes such as the decomposition of organic carbon, nutrient uptake and primary production (Newbold et al. 1982; Sabater et al. 2002). In streams with high light availability, biofilms usually dominate nitrogen uptake compared to other primary uptake compartments (PUC) like fine benthic organic matter FBOM (Tank et al. 2018; Parker et al. 2018).

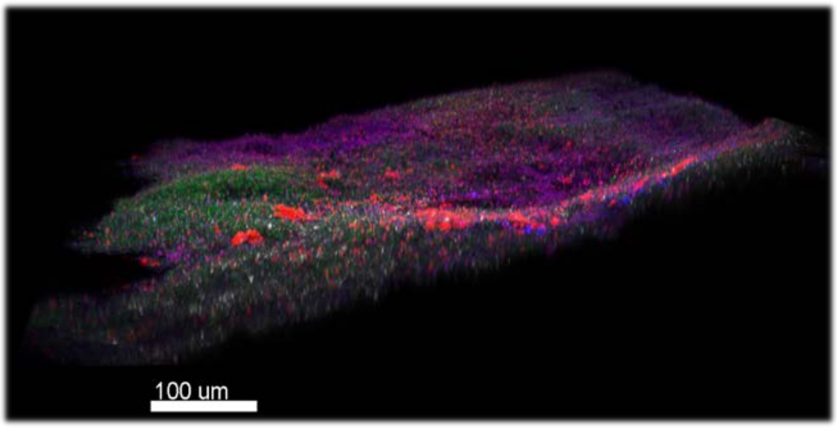


Figure 1: Confocal laser scanning microscopy projection of a stone-associated biofilm sampled in the study streams (3D volume view). Color allocations: green - nucleic acid stain (bacteria), blue - autofluorescence of chlorophyll *a* (algae), cyan - overlay of blue and red channel (phycobilins, cyanobacteria), and red - lectin-specific glycoconjugates of the matrix extracellular polymeric substances (EPS).

The functional significance of stream biofilms depend on their attributes, i.e., community composition, diversity and morphology. A major factor controlling these conditions is the physical environment (i.e., hydromorphology, Battin et al., 2007). Besides the hydromorphological conditions, biofilms depend on nutrient supply from the water column, light conditions, and water temperature (Larned 2010), primarily determined from seasonal conditions in lower-order mountain streams. The quantitative description of factors and processes controlling biofilm attributes and functioning is thus paramount for understanding the regulation of whole-ecosystem processes in streams.

1.2 Hydromorphology is a major environmental driver of stream biofilms

A characteristic feature of pristine streams is the high level of spatiotemporal heterogeneity. Hydromorphological parameters gradually change along the spatial dimensions of the stream flow (longitudinal, lateral and vertical), and biotic communities respond to the inherent environmental conditions as proposed by the River Continuum Concept (Vannote et al. 1980). Moreover, increasing hydromorphological heterogeneity in space and time drives the availability and quantity of habitat niches and resources and promotes species diversity (Habitat heterogeneity hypothesis sensu Tews et al., 2004 and references therein). Empirical support for the relationship between hydromorphological and biological diversity is almost exclusively derived from terrestrial ecosystems, and empirical support from lotic ecosystems is missing so far (Lepori et al. 2005; Palmer et al. 2010; Palmer and Ruhi 2019). Nevertheless, it can be assumed that the large spatiotemporal heterogeneity characteristic of pristine streams is probably a major driver of the attributes and functioning of stream biofilms.

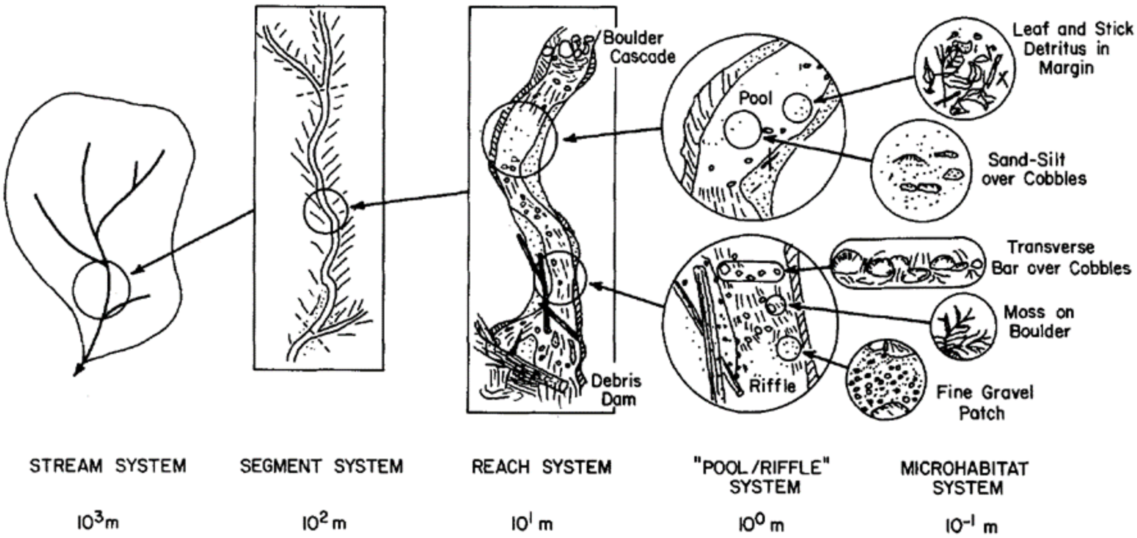


Figure 2: Spatial hierarchy of stream habitats ranging from catchments to microhabitats in streams and rivers. Taken from Frissell et al. (1986).

Habitat heterogeneity of streams and rivers is characterized by spatial and temporal variations of flow velocity, bed topography and channel morphology. Hydraulic processes like wake-like flows, separation zones and vortex shedding provide habitat heterogeneity (Buffin-Bélanger and Roy 1998) and shape benthic habitats for plants and animals in many ways (Statzner et al. 1988; Hart and Finelli 1999). Spatially, streams and rivers are hierarchal organized from microhabitat (~ 10^{-2} - 10^{-1} m, hereafter also referred to as spot) and mesohabitat (10^{0} m) to larger spatial scales including reaches (~ 10^{1} - 10^{2} m), segments (~ 10^{2} m) and catchments (~ 10^{3} m, Frissell et al., 1986, Figure 2). Mesohabitats in gravelbed streams are composed of alternating shallow, fast flowing and turbulent riffle sections and deep, slow flowing pool sections (Montgomery and Buffington 1997). At base flow, pools are sites were fine particulate organic and mineral matter or wood accumulates, whereas riffles are characterized by erosional patterns (Frissell et al., 1986, Figure 2). A framework of the role of habitat heterogeneity for biofilm diversity or the quantification of biogeochemical cycling and water quality in streams needs to include all spatial scales because hydromorphological processes at small scales affect larger scale phenomena and vice versa (Palmer et al. 1997; Biggs et al. 2005; Barton et al. 2013; Peipoch et al. 2016).

Similar to the hierarchical structure of spatial scales, habitat heterogeneity of streams and rivers is also driven by temporal scales. Temporal variations of flow velocity in streams range from milliseconds to minutes (hydraulic scale of velocity fluctuations), up to days, months or years (hydrological scale of flow fluctuations, Figure 3). The temporal component of habitat heterogeneity interacts with the spatial component and the effects of velocity variations over periods of minutes down to seconds are constrained by channel width and depth. In contrast, velocity variations over periods of milliseconds are strongly controlled by viscosity and dissipation of turbulent eddies (Biggs et al., 2005). Previous studies demonstrated that events on the hydrological scale like floods affect the biofilms through physical drag forces and transport of suspended matter which can temporarily reduce biofilm biomass (Biggs 1996, Stevenson et al. 1991), whereas hydraulic-scale flow fluctuations of minutes to seconds control biofilms through mass transfer processes acting at the boundary layer of the streambed (Kim et al. 1992; Larned et al. 2004; Grant and Marusic 2011, Figure 3).

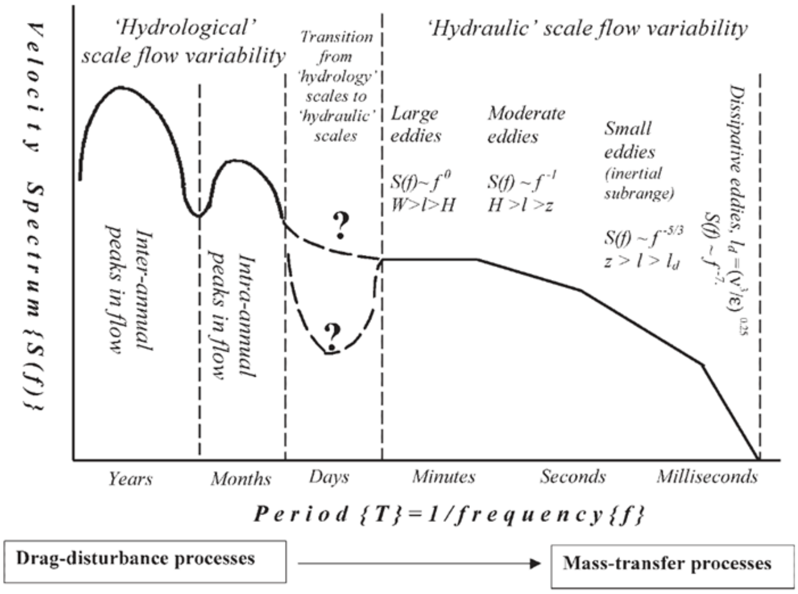


Figure 3: Frequency spectrum for near-bed flow velocity variations (*S(f)*) covering the full range of time scales that are assumed to be relevant for biofilms. W = river width, H= water depth. Taken from Biggs et al. (2005).

1.3 Effects of hydromorphology on biofilms

While there are first studies linking temporal or spatial scales of habitat heterogeneity to macroinvertebrates (e.g., Rempel et al. 2000; Reid and Thoms 2008; Ceola et al. 2014), studies on the effects of hydromorphological habitat heterogeneity on the diversity of biofilms are rare. Singer et al. (2010) showed in streamside flumes with varying bedform height that habitat heterogeneity significantly increased β, but not α or γ diversity of bacterial biofilms. The diversity of early successional stages of biofilms was rather independent of habitat heterogeneity, whereas the diversity of biofilms at equilibrium was determined by habitat heterogeneity (Besemer et al. 2007). Gerbersdorf et al. (2020) found an increase in bacterial diversity at high flow, while the diversity of algal species was correlated with low flow velocity in their flume systems.

Biofilm morphology in streams on smaller timescales is driven by hydrodynamic forces and the mass transfer of nutrients into the biofilms (Stoodley et al. 1998; Biggs et al. 2005). Drag forces acting on the biofilms depend on the organismal size, shape and the shear stress and increase with the square of the flow velocity. Biofilms that are fully submerged within the viscous sublayer (VSL; Vogel, 1994) of the boundary layer (BL) experience reduced drag forces and nutrient supply. To sustain increased drag forces, biofilms adapted to the hydraulic environment and adjusted to the flow conditions (Biggs and Thomsen 1995; Battin et al. 2003a). On the other hand, biofilms can affect drag forces by posing roughness on top of the streambed (Nikora et al. 2002), thereby altering mass transfer (e.g., Taherzadeh et al., 2012). Experiments with monoculture algal mats have shown that mats reduce wake turbulence and form-induced stresses to counteract detachment processes (Larned et al. 2011). Further studies have demonstrated that longer filamentous species prefer areas of lower velocity, where viscous effects dominate, but drag forces are low. Conversely, prostrate algae species grow in high-velocity areas where viscous effects are reduced, but drag forces are higher, e.g., on top of roughness elements (Biggs et al. 1998; Larned et al. 2004). In areas with lower drag forces and nutrient supply, algal species form a stagnant morphology to break through the VSL into the BL, where nutrient supply is enhanced by turbulent transport (Larned et al. 2004). Moreover, biofilm community composition and biomass were more diverse on rough than smooth substrates (Besemer et al. 2009; Schneck et al. 2011). Low drag forces might also support high biomass resulting in nutrient and light limitations for the basal layers of the biofilm (Stevenson et al. 1991) because mean velocities, turbulent stresses and intensities are smaller inside the biofilm resulting in an overall decrease of vertical fluxes.

1.4 Effects of hydraulic conditions on stream functioning

Within the VSL, a concentration gradient may develop, creating a diffusive boundary layer (DBL) where mass transport is controlled by molecular diffusion. Mass transfer across the boundary layer is commonly described as mass transfer velocity, which is controlled by the diffusion coefficients of molecules and the thickness of the DBL. Surface renewal theory, combined with the small eddy model, has been mainly applied to quantify air-water gas transfer (Katul and Liu 2017; Lorke et al. 2019). The surface renewal model states that a fluid parcel is transported to an interface by advection, where it travels along the interface for a certain duration. During this time, the water parcel loses mass and concentration because of the diffusive exchange with the streambed. The small eddy model states that the diffusive exchange is controlled by the smallest isotropic eddies of the Kolmogorov microscale, which can be estimated as a function of the mean turbulent dissipation rate of the turbulent kinetic energy (Lorke and Peeters 2006; O'Connor and Hondzo 2008).

Reidenbach et al. (2010) found that bed roughness enhanced the magnitude and spatial variability of mass flux at the interface between the bed and water. Positive effects of flow velocity on benthic nutrient uptake have been observed in flume experiments (Battin et al. 2003b; Arnon et al. 2013; Roche et al. 2019). Peipoch et al. (2016) were the first to show that higher mean flow velocities positively affected the nitrogen uptake of biofilms in streams. Only recently, Grant et al. (2018) found strong effects of turbulence on reach scale nitrogen uptake by applying the surface renewal model; however, they assumed a homogenous distribution of turbulence within the reaches.

Cardinale et al. (2002) quantified biofilm productivity and respiration in riffles of artificially changed heterogeneity. They showed that simplification of hydraulic habitats generally changes the performance of the biofilm and may lead to an alteration of ecosystem functioning. A later study in flumes with a heterogeneous flow environment demonstrated that nitrogen uptake increased with species richness in algal biofilm communities due to niche partitioning (Cardinale, 2011). These results showed that a mechanistic relationship between diversity and functioning as a central tenet in ecology (for reviews, see Hooper et al., 2005; Loreau et al., 2001) also exists in stream biofilms. Subsequently, Singer et al. (2010) demonstrated that stream bed heterogeneity in flume systems increases the DOC uptake by biofilms, thereby linking stream heterogeneity to biodiversity and ecosystem functioning under natural conditions.

All of these studies increased our knowledge of the dominant role of hydromorphological heterogeneity on biofilm diversity and functioning. However, the studies have mainly focused on specific spatial or temporal scales that do not fully represent stream ecosystems' broad range of hydromorphological characteristics. Moreover, most of the mentioned studies dealt with flume systems lacking natural biofilm inoculum and biofilm functioning or measurements of detailed hydraulic parameters and processes relevant to the scales under consideration. Studies on the effects of hydraulic conditions on biofilm functioning are rare and mostly investigated monodisciplinary, i.e., either by considering the mass transfer into biofilms or the development of biofilm attributes under given hydraulic background conditions. In conclusion, an overall framework that links the broad spatial and temporal range of hydromorphological habitat heterogeneity encountered in pristine streams with the composition and functioning of biofilms is lacking and is, therefore, the aim of this thesis.

2 Objectives and hypotheses

In my thesis, I quantified mechanistic links between hydromorphological habitat heterogeneity, biofilm attributes and nitrogen uptake as a measure of ecosystem functioning in streams. I aimed to reveal process understanding on how attributes of stream biofilms (morphology, community composition and diversity) are related to small- and large-scale variability in streambed morphology and corresponding flow hydrodynamics. The research conducted with this thesis was directed to allow addressing the following goals and interactions (Figure):

1. To evaluate the role of flow and turbulence on biofilm attributes on the microscale.
2. To test how flow and turbulence regulates biofilm nitrogen uptake and how biofilm attributes mediate these effects.
3. To link process-based understanding obtained at the microscale to larger spatiotemporal scales and vice versa.

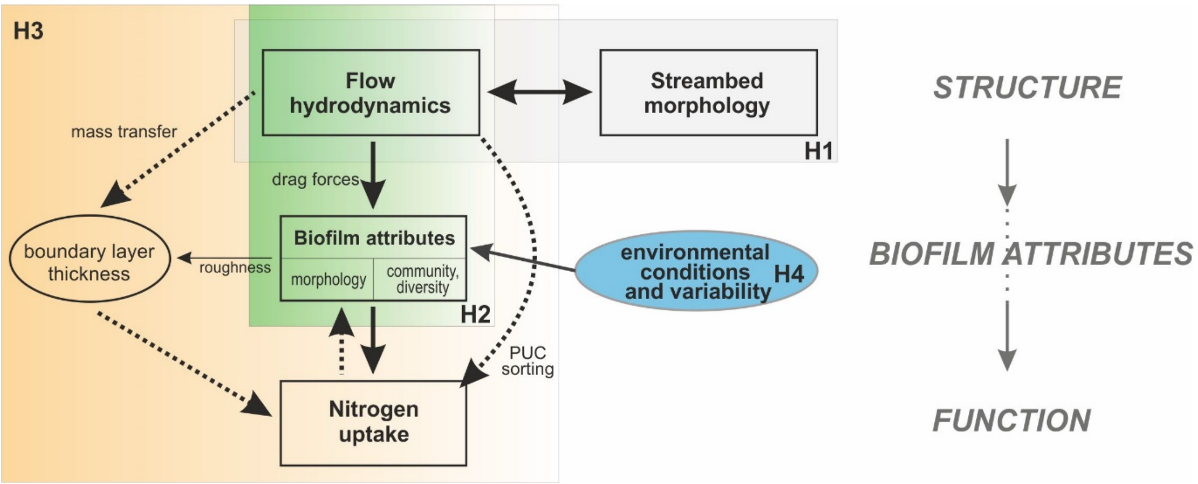
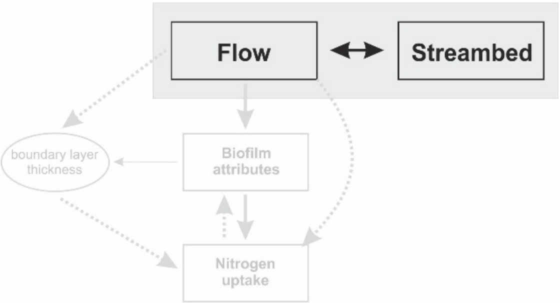


Figure 4: Conceptual diagram that predicts how streambed variability and resulting flow hydrodynamics directly or indirectly control biofilm attributes and nitrogen uptake. The hypotheses of the thesis (H1 – H4) test individual components of the concept and their interrelations are visualized by shaded boxes or circles. PUC… primary uptake compartments.

I established the following four hypotheses to test the individual components of the conceptual interactions. Three hypotheses deal with the interactions between streambed variability and flow hydrodynamics (Hypothesis 1), with the effects of flow hydrodynamics on biofilm attributes (Hypothesis 2) and nitrogen uptake (Hypothesis 3) at varying scales. Finally, the last hypothesis (Hypothesis 4) deals with the effects of environmental variability on the interactions presented in Hypotheses 1 to 3.

Hypothesis 1:

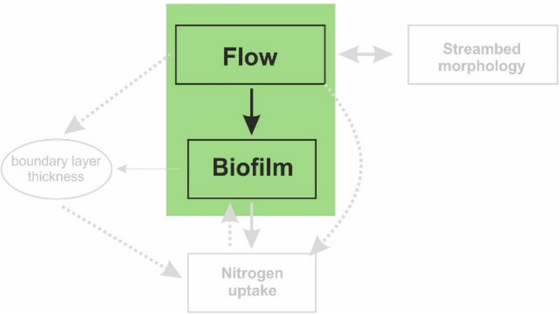
Increased streambed variability leads to higher flow diversity.



Mountain streams are characterized by a heterogeneous streambed morphology inducing highly complex flow fields. I hypothesize that the temporal and spatial flow variability is positively linked to the heterogeneity of the streambed roughness, i.e., higher streambed variability provides a wider range of different hydraulic habitats than homogenous beds.

Hypothesis 2:

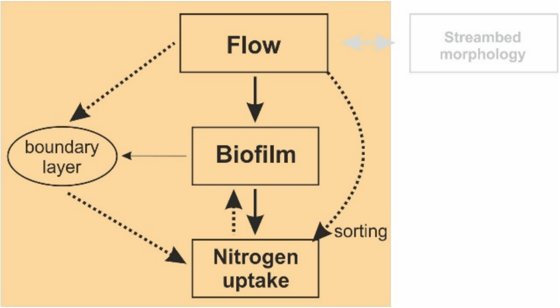
Flow diversity alters the morphology and diversity of biofilms.



Flow diversity effects biofilm diversity depending on the spatiotemporal scale. At the spot scale, the biofilm composition but not the diversity will be affected by flow diversity because species richness can be high over a range of temporal flow variability. With increasing spatial scale, flow variability provides a larger range of niches and thus increases biofilm diversity through niche partitioning. In contrast to biofilm diversity, I hypothesize that biofilm biomass and thickness are negatively related to temporal flow variability at the spot scale. Conversely, spatial flow variability can positively affect total biomass due to the provision of refuges with lower drag forces.

Hypothesis 3:

Flow diversity controls nitrogen uptake directly via altering mass transfer to the streambed and sorting of primary uptake compartments and indirectly via regulating biofilm biomass.

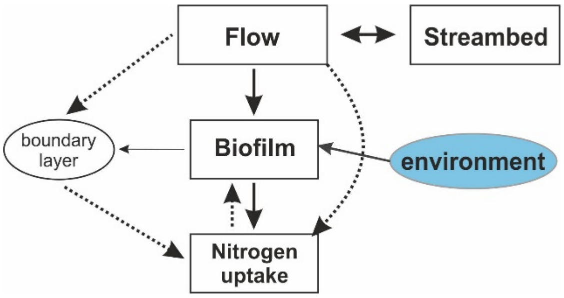


A. Temporal flow variability affects the thickness of the boundary layer and the mass transport from the water column to stream biofilms, thus positively influencing biofilm areal nitrogen uptake. Furthermore, the nitrogen uptake is high for many microorganisms and thus independent of species diversity. Instead, the increase of total areal nitrogen uptake of stream biofilms depends on the increased biomass supported by spatial flow variability (see Hypothesis 2).

B. I hypothesize that areal nitrogen uptake is higher in streams with a large contribution of riffles, where the mass transfer towards the streambed is enhanced by higher mean flow velocity. Moreover, whole-stream nitrogen uptake is higher in riffles due to the hydromorphological favoring of biologically active PUCs, including biofilms.

Hypothesis 4:

The effect size of flow diversity on biofilm attributes and functioning is superimposed by environmental variability.



High nutrient background and light exposition can result in increased biofilm growth, particularly of filamentous autotrophs. More biofilm biomass can assimilate more nitrogen, but biomass-specific nitrogen uptake is reduced due to lower nitrogen mass transfer into the biofilm. Moreover, filamentous autotrophs change biofilm community composition by providing shelter for smaller organisms and thus effect the diversity of biofilms indirectly.

The hypotheses are tested experimentally using data obtained during joint, multidisciplinary field samplings to quantify the complexity of natural stream ecosystems. Five samplings were conducted in each of two mountainous gravel-bed streams in the Bode catchment (Kalte Bode and Selke, Harz Mountains, Saxony-Anhalt, Germany). The streams are comparable in their streambed morphology with typical pool-riffle sequences but have contrasting nutrient concentrations. The samplings were conducted in summer and spring to account for changing environmental conditions. Discharge during samplings ranged from the mean daily discharge to base flow, reflecting the study streams' pre-dominant hydrological regime. There was no bed-forming discharge event during the whole time of the field samplings. Furthermore, discharge was within the same range for a minimum of two weeks before each sampling. I considered spatio-temporal scales of hydromorphological habitat heterogeneity from the microscale to the reach scale and from turbulent to inter-annual scales. Hydromorphological habitat heterogeneity was measured using hundreds of high-resolution 3D measurements of the flow field deploying acoustic Doppler velocimeters (see also Koca et al., 2017 for details). Furthermore, I developed a novel laser-scanning device for measuring streambed morphology with a high spatial resolution (on average 0.01 ± 0.01 cm) and conducted 58 topographic scans of 1 x 1 m patches. Measurements of bulk parameters of the stream morphology, such as streambed slope, widths and depths, complemented the high-resolution measurements. Moreover, I used long-term discharge data from gauging stations and large-scale (i.e., 13 km) topographical data from the local water authorities for extrapolations to larger spatiotemporal scales. Natural biofilms were sampled for detailed laboratory analysis, and their composition and architecture were quantified by confocal laser scanning microscopy. The diversity of microbial guilds (i.e., heterotrophic bacteria, autotrophic cyanobacteria and algae, and phagotrophic protists) were determined by genetic methods (rRNA gene-based terminal restriction fragment length polymorphism T-RFLP), live counting and staining techniques using the light and epifluorescence microscope. Biofilm biomass, chlorophyll *a* and pigment content, carbon, nitrogen and phosphorous content, as well as stable isotope analysis to trace nitrogen uptake, were determined by drying and ashing, high-performance liquid chromatography and mass spectrometry, respectively.

3 Outline

The thesis is divided into five parts, each represented by a published or submitted paper. The hypotheses presented above are tackled in one or more of these papers and are provided in Appendices I to V:

Part 1 (APPENDIX I)

Data from all five samplings are used to evaluate the relationship between flow and morphological variability by deploying a newly developed framework to express hydromorphological diversities (Hypothesis 1). Subsets of this data are used to test the effects of flow variability on the diversity of microbial guilds and the spatial diversity of areal nitrogen uptake (Hypotheses 2, 3 and 4).

> *Anlanger, C., Noss, C., Risse-Buhl U., Brauns, M., von Schiller D., Weitere, M. and Lorke, A. Linking hydromorphological diversity to biodiversity and functioning in running waters (submitted to Nat. Comm., in revision).*

Part 2 (APPENDIX II)

A subset of flow measurements and corresponding biofilm samples from three samplings in each stream was used to test the effects of flow on biofilm composition and architecture (Hypothesis 2 and 4).

> *Risse-Buhl, U., Anlanger, C., Kalla, K., Neu, T. R., Noss, C., Lorke, A., & Weitere, M. (2017). The role of hydrodynamics in shaping the composition and architecture of epilithic biofilms in fluvial ecosystems. Water Res., 127: 211–222. https://doi.org/10.1016/j.watres.2017.09.054*

Part 3 (APPENDIX III)

Another subset of flow measurements and corresponding biofilm samples from the same samplings in each stream was used to test the effects of flow variability on the diversity and abundance of autotrophic cyanobacteria and algae, heterotrophic bacteria as well as phagotrophic protists (Hypothesis 2 and 4).

> *Risse-Buhl, U., Anlanger, C., Chatzinotas, A., Noss, C., Lorke, A. and Weitere, M. (2020), Near streambed flow shapes microbial guilds within and across trophic levels in fluvial biofilms. Limnol. Oceanogr., 65: 2261-2277. https://doi.org/10.1002/lno.11451*

Part 4 (APPENDIX IV)

Two other samplings in each stream were used to test the importance of hydraulic and biological controls on biofilm areal nitrogen uptake at the microscale (Hypothesis 3A and 4), and to extrapolate the findings to larger spatial and temporal scales (Hypothesis 3B).

> *Anlanger, C., Risse-Buhl, U., von Schiller, D., Noss, C., Weitere, M. and Lorke, A. (2021), Hydraulic and biological controls of biofilm nitrogen uptake in gravel-bed streams. Limnol. Oceanogr., 66: 3887-3900. https://doi.org/10.1002/lno.11927*

Part 5 (APPENDIX V)

An extension of the samplings from Part 4 was used to test for secondary effects of hydraulic variability on larger scales (hydromorphological sorting) on whole-stream nitrogen uptake (Hypothesis 3B and 4).

> *Risse-Buhl, U., Anlanger, C., Noss, C., Lorke, A., von Schiller, D. and Weitere, M. (2021) Hydromorphologic Sorting of In-Stream Nitrogen Uptake Across Spatial Scales. Ecosystems 24, 1184–1202. https://doi.org/10.1007/s10021-020-00576-7*

4 Discussion

Existing studies increased our knowledge of the role of hydromorphology on biofilm diversity and functioning. However, an overall framework that links the broad spatial and temporal range of hydromorphological habitat heterogeneity encountered in pristine streams with the composition and functioning of biofilms is lacking so far. In this thesis, I developed a mechanistic framework that links habitat heterogeneity generated by flow hydrodynamics and streambed morphology to biofilm structure and functioning. I established four hypotheses to test the predictions of this overall framework using data derived from extensive field samplings at two mountainous stream reaches with near-natural hydromorphology, but varying nutrient backgrounds, and at two seasons (Figure 5).

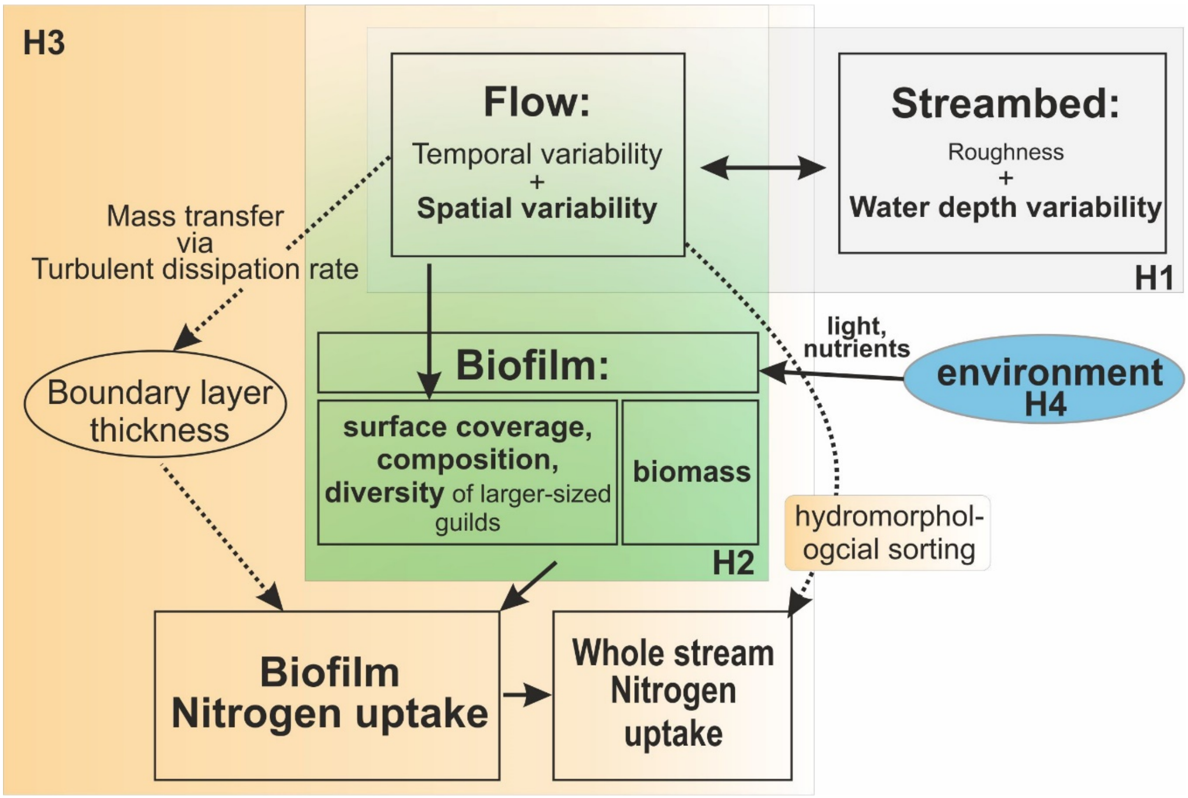


Figure 5: Overall framework illustrating observed mechanisms on how morphological streambed variability and resulting flow diversity directly or indirectly control biofilm attributes and nitrogen uptake. The hypotheses of the thesis (H1 – H4) and their interrelations are visualised by shaded boxes or circles.

Despite the inherent environmental complexity of the studied streams, I could show that all mechanisms previously assumed to affect nitrogen uptake are ultimately connected to the prevailing flow conditions, which are related to streambed properties (grey rectangle in Figure 5, Hypothesis 1, and discussion section 4.1). Flow positively affected nitrogen uptake indirectly via altering biofilm attributes (green rectangle in Figure 5, Hypothesis 2, and discussion section 4.2) and directly via altering the mass transfer processes towards the streambed and via spatial sorting of highly active PUCs (orange rectangle in Figure 5, Hypothesis 3, and discussion section 4.3), while the effect sizes vary with environmental conditions (blue circle in Figure 5, Hypothesis 4, and discussion section 4.4).

4.1 Habitat heterogeneity in stream ecosystems

Hypothesis 1

Main finding: Flow diversity is significantly positively related to the variability of the streambed morphology at spatial scales of riffles and pools.

I used empirical data on flow velocities and streambed morphology to express hydromorphological habitat heterogeneity with a novel diversity framework based on variance partitioning developed within this thesis. The need for this step evolved from the lack of an integrative measure for hydromorphological habitat heterogeneity in fluvial ecosystems. The diversity framework expressed heterogeneity on several spatial and temporal scales and facilitated the direct connection to biodiversity (see section 4.2) and stream functioning (see section 4.3). More importantly, the components of the diversity framework identified common hydraulic and morphological measures as being relevant for niche diversity and physical transport mechanisms. Based on the concept of α, β and γ diversity (Whittaker 1960, 1972), the hydromorphological overall diversity within a specific area or region (γ diversity) is the sum of the mean α and β diversities, which, for the flow diversity, constitute temporal and spatial averages at smaller scales. The morphological diversity was expressed without considering a temporal term. Instead, morphological γ diversity is decomposed into different types of roughness (e.g., grain or streambed roughness, α diversity) and the bed slope or larger-scale topography reflected by the variability of the mean water depths (β diversity, Table 1, Figure 5, APPENDIX I).

The overall flow diversity (flow γ diversity) was dominated by the spatial variability (flow β diversity) induced by differences in the bulk flow characteristics, which were associated with the alternating mesohabitats of riffles and pools. For example, the mean flow velocities were about two-fold higher (Figure 6a), and the turbulent kinetic energy was one order of magnitude higher (Figure 6b) in riffles compared to pools. At the same time, the temporal flow variability (flow α diversity) did not differ between riffles and pools, while streambed roughness (morphological α diversity) was 1.3 times higher in riffles than in pools (APPENDIX I). This supports the notion that not only the streambed roughness induces flow variability (i.e., near-bed turbulence), but mainly the variability of mean near-bed flow velocity in gravelbed streams. Moreover, flow parameters depended on bulk stream characteristics measured within the frame of this thesis which was associated with riffles and pools. For example, the mean flow velocity was positively correlated to water level slope ($R^2 = 0.18$, $p < 0.001$, respectively) and negatively correlated to mean water depth ($R^2 = 0.12$, $p < 0.001$, respectively) and local stream width ($R^2 = 0.11$, $p < 0.001$, respectively). The variability of the mean water depth matches my definition of morphological β diversity which was found to be significantly related to the flow β diversity (APPENDIX I).

Table 1. Overview of the components (α, β and γ diversity) of hydromorphological diversity according to the proposed framework. Hydromorphological diversity is expressed with data on flow velocity and streambed morphology. Modified table from APPENDIX I.

Scale	Diversity	Flow velocity	Physical description	Streambed morphology	Physical description
Spot, Patch	α	Temporal flow variability	Temporal variance of flow velocity $\overline{u'^2}$ normalized by the square of its temporal mean $\bar{u}^2$= (twofold) ratio of turbulent kinetic energy TKE and squared mean flow velocity: $\alpha_{Flow} = \frac{\overline{u'^2}}{\bar{u}^2} = \frac{1/_2 TKE}{\bar{u}^2}$	Streambed roughness	Spatial variance of water depths $\overline{\mathrm{h}'^2}$ normalized by the square of the mean water depth $\bar{\mathrm{h}}^2$= squared relative streambed roughness: $\alpha_{Morpho.} = \frac{\overline{\mathrm{h}'^2}}{\bar{\mathrm{h}}^2}$
Meso, Reach	β	Spatial flow variability	Spatial variance of time-averaged flow velocities $var(\bar{u})$ normalized by the square of their overall mean $\langle\bar{u}\rangle^2$: $\beta_{Flow} = \frac{var(\bar{u})}{\langle\bar{u}\rangle^2}$	(Mean) Water depth variability	Spatial variance of the mean water depths at the spot scale $var(\bar{\mathrm{h}})$ normalized by the square of their overall mean $\langle\bar{\mathrm{h}}\rangle^2$: $\beta_{Morpho.} = \frac{var(\bar{\mathrm{h}})}{\langle\bar{\mathrm{h}}\rangle^2}$
	γ	Overall flow diversity	Total temporal and spatial variance of flow velocity normalized by the square of their overall mean: $\gamma_{Flow} =$ mean $\alpha_{Flow} + \beta_{Flow}$	Overall morphological diversity	Total spatial variance of water depths normalized by the square of their overall mean: $\gamma_{Morpho} =$ mean $\alpha_{Morpho} + \beta_{Morpho}$

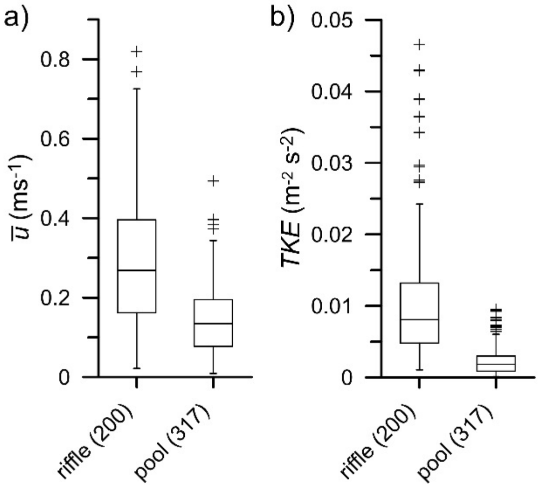


Figure 6: Hydromorphological stream characteristics: mean flow velocities $\overline{u}$ (a) and turbulent kinetic energy *TKE* (b) observed at sampling sites in riffles and pools for both streams. The boxes encompass the 25-75 percentile range with the median value indicated by a horizontal line. Outliers are shown by crosses. Number of flow measurements and roughness measurements for riffles and pools are shown in parenthesis. Summarized data from appendices I to IV.

The importance of mesohabitat-like riffles and pools for flow diversity was also found by Lamarre & Roy (2005), who noticed that turbulent flow structures are more affected by water depth than by streambed elements forming streambed roughness. The variability of the mean streamwise velocity (in terms of high and low-speed streaks close to the bed) mainly depends on relative submergence (i.e., the flow becomes more smooth for larger relative submergence) and less on roughness elements (Cooper and Tait 2008). Furthermore, Buffin-Bélanger et al. (2006) recognized that the spatial heterogeneity of near-bed mean flow increased with decreasing relative submergence, but, also by the simultaneous increase of flow velocity.

Based on the newly developed diversity framework, I demonstrated that flow diversity could be decomposed into a temporal and a spatial component. In summary, the morphological and flow diversity in mountainous streams were closely linked at scales of riffles and pools. Riffles and pools posed contrasting habitat conditions mainly driven by the mean flow velocity and mean water depth. The established description and relationships of flow and morphological diversity can be used to scale hydromorphological diversity up to larger spatiotemporal scales and can be further linked to habitat preferences of biofilm structure and functioning.

4.2 Effects of habitat heterogeneity on biofilm attributes

Hypothesis 2

Main finding: Flow diversity alters the surface coverage and composition of biofilms and, at larger spatial scales, the diversity of larger-sized biofilm guilds.

The results of this thesis provided empirical evidence for the dominant role of near-bed flows in shaping biofilm architecture as well as its community composition (APPENDIX II and APPENDIX IV) and diversity (APPENDIX I and APPENDIX III). On the spot scale, increased turbulent kinetic energy, which is the unscaled temporal flow variability (flow α diversity), favored more compact biofilms, which uniformly covered the stone surfaces. In contrast, at low flow diversity, stagnant and filamentous forms prevailed. At the same time, biofilm thickness and biomass were not affected by flow diversity (APPENDIX II and APPENDIX IV). Temporal flow variability further affected abundances, in particular of larger-sized biofilm guilds (e.g., filamentous autotrophs), causing shifts in communities (APPENDIX III). On larger spatial scales, spatial flow variability (flow β diversity) increased the diversity of autotrophs and phagotrophic protists which highlights the importance of hydromorphological niches (APPENDIX I, Figure 5).

In agreement with studies conducted in the lab (Stevenson 1996; Battin et al. 2003a; Besemer et al. 2007), stream biofilms had stagnant forms at low flow and turbulence conditions (Figure 7a). Here, the strategy for biofilms to get nutrients from the water is to adapt their architecture to protrude out of the DBL into the water column to increase nutrient replenishment (Battin et al. 2003a; Graba et al. 2013) and to enhance streambed roughness and increase turbulent mixing (Nikora et al. 1998, 2002; Larned et al. 2004). Stream biofilms became more compact at high turbulent kinetic energy, and a film of densely packed coccoid cells uniformly covered the stone surfaces (Figure 7b-c). However, the unidirectional growth form observed in the lab (Battin et al. 2003a; Depetris et al. 2022) could not be confirmed in the field, most likely because the flow pattern in rough gravel-bed streams is highly three-dimensional and biofilms grow multi-directional. Biofilm thickness and porosity were not affected or slightly decreased with increasing turbulent kinetic energy, which was contrary to Hypothesis 2.

Also, biofilm biomass was not influenced by flow diversity (APPENDIX IV), supporting the postulated elastic behavior of biofilms while remaining the same biomass at varying flow conditions (Dreszer et al. 2014). Moreover, under field conditions, an equilibrium between growing and sloughing biofilms exists when biofilms reach their carrying capacity (Larned 2010). A high biomass of filamentous thallophytes and algae was found at spots of low and high velocities in the study streams. At low velocities, it can be assumed that the filamentous growth form developed to maximize nitrogen uptake. At high velocities and turbulence, optimum light and nutrient supply can promote the growth of filamentous algae (Steinman and McIntire 1986).

Cardinale (2011) assumed that a large range of hydraulic conditions supports a wide range of niches which a diverse biofilm community can occupy through niche partitioning. The β diversity of autotrophic biofilms and phagotrophic protists increased with spatial flow variability (flow β diversity), which was not the case for bacteria. This contradicts previous findings that bacterial diversity increased with flow variability in flumes with varying bedforms (Besemer et al. 2009). The authors quantified flow variability as the standard deviation of the mean flow velocity and obtained maximum values of 0.07 m s^{-1} in their flumes. In this thesis, the standard deviation of natural flow in the gravelbed streams studied within this thesis was twice as high (0.14 m s^{-1}). This indicates that flume results are not fully applicable to pristine stream ecosystems because there might be thresholds where the effects of flow variability on bacterial diversity might reverse. For example, hydraulic niches created by extreme flow conditions may favor specialized species (Walker 1992; Wild et al. 2022) but reduce overall bacterial diversity. However, this needs confirmation in future studies. Moreover, bacterial communities are characterized by smaller organismic cell sizes, high production of extracellular polymeric substances (Chew et al. 2014; Hou et al. 2018), and a high phenological plasticity (Hall-Stoodley et al. 2004), making them independent from hydromorphological habitat heterogeneity and omnipresent in the stream environment.

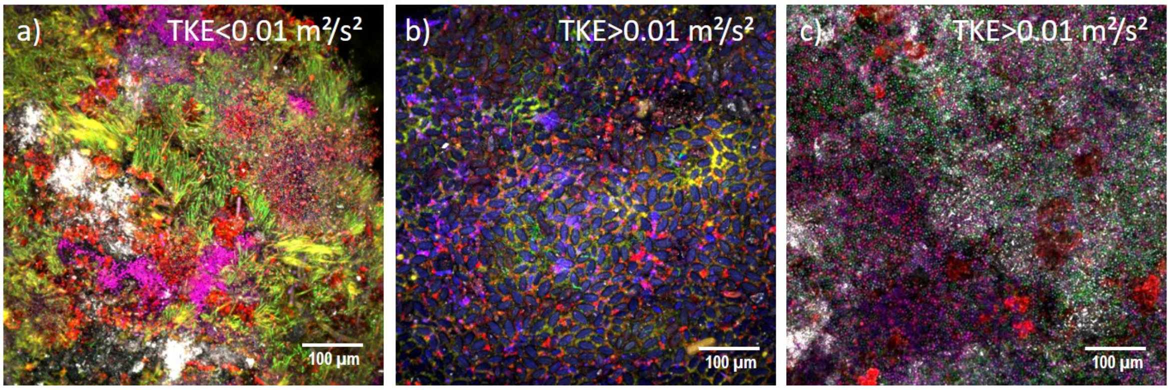


Figure 7: Confocal laser scanning microscopy projection of stone-associated biofilms sampled in the study streams (maximum image projections of image stacks) at low (a) and high turbulent kinetic energy *TKE* (b-c). Color allocations: green - nucleic acid stain (bacteria), blue - autofluorescence of chlorophyll *a* (algae), cyan - overlay of blue and red channel (phycobilins, cyanobacteria), and red - lectin-specific glycoconjugates of the matrix extracellular polymeric substances (EPS).

Most studies dealing with the interactions of flow and biofilms were conducted in flumes where the full range of hydraulic conditions is hard to mimic. For example, mean near-bed flow velocities and turbulent kinetic energy in the studied streams ranged over more than two orders of magnitude (Figure 6), while those ranges in flumes studies are much smaller (Battin et al. 2003a; Singer et al. 2006; Besemer et al. 2009). Moreover, maximum values as measured in my study streams (i.e., mean near-bed velocities of 0.8 m s^{-1} and turbulent kinetic energy of $5x10^{-2}$ m² s^{-2}) are technically hard to achieve in flumes. Nevertheless, it is important to study biofilm attributes across the entire range of flow conditions possible under field conditions to detect peaks and thresholds of, e.g. biomass towards flow and turbulence conditions (Hondzo and Wang 2002). Another advantage of sampling biofilms from natural streambeds is the consideration of their realistic micro-topography. For example, biofilms grown on natural stones have higher biomass than those grown on artificial substrates (Murdock and Dodds 2007) because the latter favor early successional stages. However, flume studies found distinct differences in the effects of flow on biofilms of varying successional stages (Battin et al. 2003a; Besemer et al. 2007), which cannot be transferred to naturally grown biofilms where the successional stage of biofilm communities can hardly be identified.

In a companion study to this thesis (Polst et al. 2018), I aimed to reproduce the near-natural flow gradient in streamside flumes in the mobile aquatic mesocosm facility MOBICOS (Fink et al. 2020, Figure 8) to obtain a near-natural biofilm community. Moreover, I was able to decouple the inherent dependencies between the turbulent kinetic energy and the mean flow velocity in the study stream and could study their individual effects on biofilm attributes. In accordance with the field results (APPENDIX I), I did not find a correlation between turbulent kinetic energy (flow α diversity) and diatom diversity as the dominant group of the studied biofilms. Instead, the diversity of diatoms increased with mean flow velocity underlining the importance of flow β diversity for biofilm diversity. In summary, mesocosm studies with near-natural flow conditions overcome the limitation of traditional flume experiments and help to develop a better process understanding, especially for mechanisms induced by flow variability.

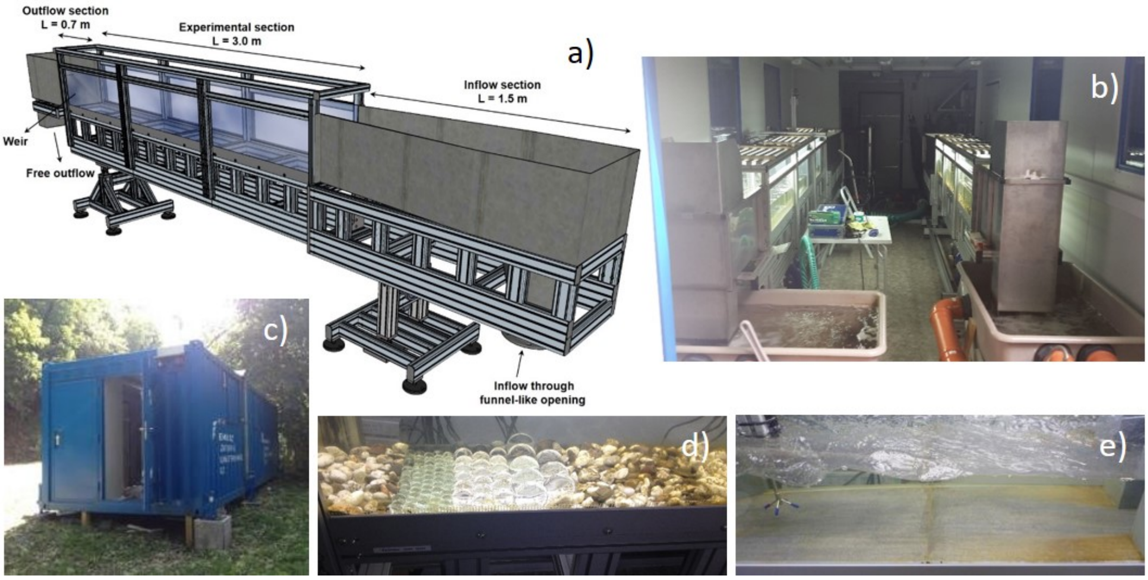


Figure 8. Three-dimensional view of a streamside flume with length x height x width of 5.2 x 0.5 x 0.3 m (a). Two flumes were placed in a mobile aquatic mesocosm facility (MOBICOS, b-c) - one flume with bed roughness created by glass beads of different sizes (d) and one with smooth and fixed bed (unglazed ceramic tiles, e). The setup provided for reproducing the large range of hydraulic conditions in stream ecosystems and a near-natural biofilm community.

4.3 Effects of habitat heterogeneity on ecosystem functioning

Hypothesis 3

Main finding: Higher flow diversity increased areal nitrogen uptake via altering hydraulic mass transfer and affected whole-stream nitrogen uptake via spatial sorting of PUCs.

Despite the inherent environmental complexity of the studied streams, I identified the governing role of hydromorphology on functional hot spots and demonstrated that previously predicted controls on nutrient uptake by biofilms (Jumars et al. 2001; Larned et al. 2004; Grant and Marusic 2011) are connected to the variability of flow. At the spot scale, the two major parameters determining areal nitrogen uptake into biofilms were the turbulent dissipation rate as a function of hydraulic mass transfer and the biofilm biomass as a function of biofilm morphology (APPENDIX IV). At larger spatial scales, the spatial flow variability (flow β diversity) dictated the spatial diversity of nitrogen uptake efficiencies (biomass normalized nitrogen uptake rates, APPENDIX I, Figure 5).

Mass transfer to biofilms was controlled by turbulent dissipations rates, which scaled with the turbulent kinetic energy (unscaled flow α diversity) and the mean flow velocity (unscaled flow β diversity). Consequently, riffle habitats with higher mean flow velocities had higher nitrogen uptake potential than pools (APPENDIX IV). Riffle habitats in gravel-bed streams are therefore hot spots of nitrogen uptake, especially in streams with low nitrogen background concentrations, where the potential of nitrogen uptake is primarily limited by hydraulic mass transfer (Grant et al. 2018). In line with Hypothesis 1, averaging turbulent dissipation rates matched the predictions of mass transfer from hydromorphological bulk properties, i.e., water depth, stream width and slope. The mass transfer velocity measured in the studied streams correlated well with the mean flow velocity and the turbulent kinetic energy near the streambed. The latter can be assumed to scale with the smallest eddies (Biggs et al. 2005), which, according to the energy cascade, gained their energy from large eddies. The size of large eddies is restricted by stream depth and width (Nikora 2010). The process scaling identified in the thesis is essential for the extrapolation of mass transfer processes to larger spatiotemporal scales and transfer results across ecosystems. Data on water depth, stream width, and slope is widely available (e.g., by local water authorities, EU Hydro dataset), facilitating spatial extrapolation. For example, Horgby et al. (2019) used such bulk parameters to extrapolate carbon emission from mountain streams to a global scale deploying scaling relationships derived from small-scale dissipation rates at the water surface (Ulseth et al. 2019). Grant et al. (2018) implicitly assumed but did not test mass fluxes derived from bulk parameters and showed the importance of hydraulically induced mass transfer for nutrient uptake. Similar process scaling could be applied together with many existing datasets on long-term discharge dynamics and stream morphology to quantify the consequences of droughts and intermittency on microbial and macrobial communities at global scales. Scaling of biofilm attributes remains challenging because of the multitude of factors determining biofilm growth. Nevertheless, biofilm biomass may be scaled using a simple statistical approach considering parameters like flow and environmental conditions like light or nutrient background of surface water (Biggs 2000, see also section 4.4).

A central tenet in ecology is the relationship between biodiversity and ecosystem functioning (Tilman et al. 1996), which was also shown for biofilms (Cardinale 2011). In contrast to previous studies, the diversity of biofilm guilds did not explain the diversity or the absolute areal nitrogen uptake rates (APPENDIX I). These seemingly contradicting results are probably due to the study design, as Cardinale (2011a) used a flume system with only 8 biofilm species, while I studied a naturally grown biofilm community with its inherently larger species number and redundancies in functional and performance traits. This argument is supported by my results showing that spatial flow variability is correlated to both, autotroph's β diversity and the β diversity of nitrogen uptake efficiency (biomass normalized nitrogen uptake rates, APPENDIX I) and points towards the direction that the same species assemblage performs differently under varying flow conditions. However, flow diversity did not affect absolute (i.e., mean) areal nitrogen uptake. Instead, biofilm biomass was an important driver of absolute nitrogen uptake (APPENDIX IV) but independent from flow diversity (see Hypothesis 2). The flume study (section 4.2) underlined the importance of biomass, even if the study did not account for other environmental variability, i.e., light, nutrient background and macrograzers (see also section 4.4. for further discussion). Higher biomass positively affected areal nitrogen uptake, whereas those biofilms were less efficient in nitrogen uptake shown by the biomass-specific nitrogen uptake and biofilm biomass and thickness (Figure 9).

Above the important control of flow diversity on areal nitrogen uptake, I could show that the hydromorphological diversity at scales of riffles and pools affected the distribution of PUCs, including biofilms (hydromorphological sorting). For example, the mean flow and water depths in riffles promoted more active primary uptake compartments PUC in whole-stream nitrogen uptake in riffle sections (APPENDIX V). Here, the most active PUC of the studied streams were biofilms and thallophytes compared to wood and fine benthic organic matter FBOM. The latter was favored in pool sections with low flow velocities and larger water depths (see section 4.1). Compared to biofilms or thallophytes, wood and FBOM have a high detrital nitrogen proportion and a low microbial nitrogen proportion making these compartments and pools less productive in terms of nitrogen uptake (Tank et al. 2018). The same results were found in two New Zealand streams where nitrogen uptake of FBOM was higher in pools, and nitrogen uptake of biofilms and thallophytes was higher in riffles (Simon et al. 2004; O'Brien and Dodds 2008). Although the absolute biomass of biofilms was not affected by the hydromorphological differences of pools and riffles (see Hypothesis 2), their relative importance of biofilms at the whole-ecosystem scale compared to other PUCs gained importance.

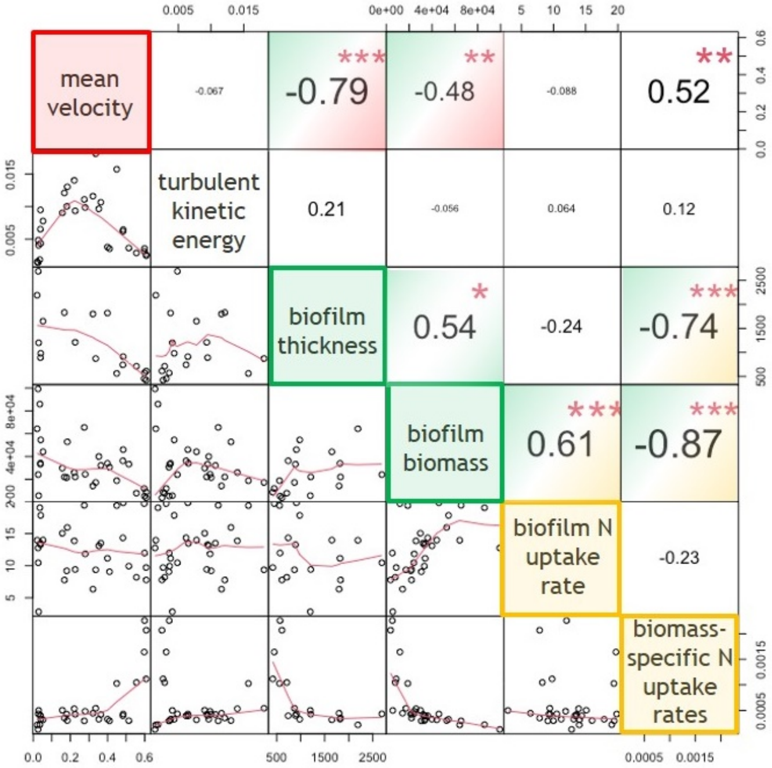


Figure 9. Relationships between flow parameters (red), biofilm morphology (green) and functioning (yellow) obtained in flume experiments (unpublished data). The numbers are Spearman rank correlation coefficient and the red stars the significance level ($p < 0.05$ *, $p < 0.01$ **, $p < 0.001$ ***).

In summary, streams with a large share of riffles have a higher potential to immobilize nitrogen because of increased mass transfer to biofilms and other PUCs and because of a larger share of habitats preferred by active PUCs. It is therefore crucial to sustain the natural configuration of pool and riffle sequences and to restore stream reaches that suffer from hydromorphological degradation following impoundments.

4.4 Effects of environmental variability

Hypothesis 4

Main finding: The effect size of flow diversity on biofilm attributes and functioning varied with environmental variability mainly because of its effects on biofilm biomass.

With my thesis, I aimed to separate larger-scale environmental factors and their variability affecting biofilms. By investigating two seasons and two streams of varying nutrient backgrounds under similar flow conditions, I showed that the effects of flow diversity on biofilm attributes and functioning were nested within season and stream.

The seasonal variation of biofilms may have occurred due to differences in seasonal light availability. For example, daily photosynthetic active radiation (PAR) at the Selke was up to three times lower in the summer than in the spring samplings. Light is known to stimulate biofilm growth (Biggs 1996; Biggs et al. 1998), which peaked during the spring samplings and corresponded with enhanced autotrophic biofilm biomass (Appendices APPENDIX II, APPENDIX III, APPENDIX IV and APPENDIX V), thereby changing community composition (APPENDIX III), increasing areal nitrogen uptake rates and at the same time decreasing biomass-specific uptake rates (Appendices APPENDIX I, APPENDIX IV and APPENDIX V, Figure 9) indicating that higher biomass was less efficient in nitrogen uptake. Excessive growth of filamentous autotrophs (Figure 10) can limit mass transfer into the biofilm matrix (De Beer et al. 1996), and it can be assumed that only a relatively small layer of active biofilm contributed significantly to nitrogen uptake.

Figure 10: Excessive development of filamentous thallophytes and algae in the study streams due to high light availability in spring.

Filamentous thallophytes and algae were found at spots of low and high velocities in the study streams and most likely posed an individual micro-ecosystem providing protected habitats from hydraulic shear forces for smaller-sized or motile biofilm guilds (e.g., bacteria, phagotrophic protists, Willkomm et al. 2007; Risse-Buhl et al. 2009). At the spot scale, the growth of the filamentous autotrophs could have masked the effects of flow variability on biofilm diversity which was also suggested by Battin et al. (2003a). At a larger spatial scale, flow diversity was finally more important for biofilm diversity than environmental variability associated with season and stream (APPENDIX I). Hydromorphological diversity (i.e., β diversities) at the scales of riffles and pools created environmental niches that promoted biofilm diversity. However, the results were obtained during base flow conditions, while temporal extrapolation to scales of decades showed that seasonal discharge dynamics contributed most to flow diversity (APPENDIX I). An increase in the flow variability range may also increase hydraulic niche diversity, with currently unknown effects on biofilm communities. Future studies need to study the interaction of habitat heterogeneity and biofilms up to the threshold where shear stress and bed movement delimit biofilm growth.

Another environmental factor that determined nitrogen uptake efficiencies (sensu Grant et al. 2018) were ambient nutrient concentrations (APPENDIX III). This is in line with previous studies (Grant et al. 2018) and corroborates the existence of a threshold of nitrate concentrations (10^{-3} mol m^{-3}) that delineates the transition where either processes control nitrate uptake at or within the streambed (including biofilm attributes) or physical mass transfer to the streambed. The studied streams, as well as German streams in general, have comparatively high background nitrogen concentrations. According to Ebeling et al. (2021), the average (± SD) nitrate concentration calculated from 759 German streams is 4.1 ± 2.7 mg N L^{-1} nitrate (~10^{-1} mol m^{-3} nitrate), implying that nitrogen uptake is primarily driven by an indirect effect of flow on biofilm attributes rather than mass transfer processes.

4.5 Outlook and future research directions

Turbulent properties measured by Doppler-based methods are known to be affected by the bottom echo at solid boundaries (Brand et al. 2016; Koca et al. 2017). Due to this technical shortcoming, the smallest turbulent eddies, which are affected by water viscosity and the thickness of the DBL, could not be measured directly but 2.3 cm above the streambed. To overcome this shortcoming, promising new techniques making it possible to measure close to solid or biofilm-covered surfaces are available today. For example, Cameron et al. (2013) developed a particle image velocimetry deployment PIV for field application and tested it over macrophyte patches in a lowland stream. A custom-made low-cost PIV system was deployed in a field study by Lorke et al. (2019).

Flow variability has been recognized as an important physical control on the abundance and composition of stream macroinvertebrates and fish (Statzner et al. 1988; Reid and Thoms 2008; Blanckaert et al. 2013). A thorough test on the applicability of the established and tested diversity framework to groups other than biofilms is outside the scope of this thesis. A preliminary test on its applicability to macroinvertebrates collected alongside biofilms (unpublished data) showed that flow β diversity correlated well with the macroinvertebrate β diversity ($R^2 = 0.26$, $p = 0.036$). Although studies suggest a considerable effect of temporal flow variability (flow α diversities) on macroinvertebrates (Statzner et al. 1988), it remains to be studied if the role of the flow α diversity is superimposed by environmental variability similar to the results of my thesis on microbial communities (see Hypothesis 4).

Nitrogen uptake, as studied in this thesis, is an important ecosystem function mediated by biofilms, but biofilms are also involved in the degradation of dissolved organic matter (DOC). It is difficult to predict to which extent the established diversity framework also applies to DOC uptake. Previous flume studies revealed contrasting results on the role of benthic biofilms in DOC cycling. Singer et al. (2010) found that the uptake of labile DOC by benthic bacteria was largely driven by physical processes related to flow diversity. Conversely, whole-stream additions of a natural DOC tracer to mountain streams showed that benthic biofilms played only a minor role in DOC cycling and indicated that hyporheic biofilms are hotspots of DOC uptake (Graeber et al. 2019). The exchange of surface water between the benthic and hyporheic zone and, thus, the supply of DOC to hyporheic biofilms may also depend on hydromorphological diversity. Hence, the framework established here may help to identify relevant hydromorphological drivers for ecosystem functions and ecosystem compartments other than those studied here. This requires field studies that follow and quantify the fate of organic and inorganic matter fluxes across the benthic-hyporheic interface by applying the developed hydromorphological diversity framework.

Another relevant question derived from the research conducted here is if the established concept also holds in other stream types or rivers. For example, lowland rivers are characterized by much larger relative submergence, i.e., lower morphological diversity than streams. Classical ecosystem theory suggests that the role of the water column for whole-ecosystem functioning should increase with increasing water depth (Vannote et al. 1980). Our latest work challenges this contention as we show that nutrient uptake is higher in the benthic than in the pelagic zone (Attermeyer et al. 2023). Given the dominant role of the benthic zone for riverine processes, one may ask if hydromorphological heterogeneity drives biofilm diversity and functions in the same way as in gravel bed, mountain streams studied in this thesis. Future studies have to evaluate if the temporal (α diversity) or the spatial (β diversity) component of flow variability determines the overall diversity (γ diversity) as well as the role of flow diversity stemming from river wood (Schnauder et al. 2022; Anlanger et al. 2022) or large-scale geomorphic features resulting from river meandering.

5 Conclusion

Stream ecosystems exhibiting spatiotemporal heterogeneity are hotspots of biogeochemical cycling (Battin et al. 2023) and biodiversity globally (Meyer et al. 2007). The debate on how habitat heterogeneity affects biological indicators started a few decades ago (Tews et al., 2004). Yet, only recently, the debate was transferred from the terrestrial to freshwater ecosystems, where habitat heterogeneity is characterized by flow velocity and streambed morphology. However, the interactions of hydromorphology, biodiversity and ecosystem functioning remained ambiguous mainly because of a missing process understanding. My thesis advances the understanding of the mechanism of how and to which degree habitat heterogeneity affects the attributes and functioning of biofilms in inherently complex ecosystems. I demonstrated the significance of small temporal (i.e., turbulent) as well as larger spatiotemporal scales. In doing so, I provided scaling frameworks across space and time and encouraged future studies to decipher drivers of biodiversity and global biogeochemical cycles. I clarified how hydromorphological diversity directly (via physical transport processes and spatial sorting of microhabitats) and indirectly (via biological attributes) determines nitrogen uptake. Nitrogen uptake regulates water quality (Alexander et al. 2007; Baker et al. 2012) but is at the same time highly susceptible to human impacts (Brauns et al. 2022). Hence, nitrogen uptake has been identified as an important target of stream restoration (von Schiller et al. 2008). Indeed, the decline in freshwater biodiversity and stream functioning as a result of hydromorphological simplification and modification of streams (Peipoch et al. 2015; Albert et al. 2021; Brauns et al. 2022) calls for an unifying restoration framework (Palmer and Ruhi 2019). However, integrating structural and functional parameters into management practices requires knowledge of the effect of local measures at the microhabitat on larger-scale processes and vice versa and the mechanisms controlling those processes. My thesis significantly advances the understanding of these processes across spatiotemporal scales and has much promise for improving the hydromorphological restoration of stream and river ecosystems.

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Author Contributions

Research articles ordered by publication or submission date:

Anlanger, C., Noss, C., Risse-Buhl U., Brauns, M., von Schiller D., Weitere, M. and Lorke, A. Linking hydromorphological diversity to biodiversity and functioning in running waters (submitted to *Nat. Comm.*, in revision). APPENDIX I

Conception and design: CA, CN, URB, MW, AL; Data acquisition: CA, CN, URB, DvS, MB, ME; Data analysis: all authors; Interpretation of results: all authors; Writing the manuscript: CA, CN, AL with comments from URB, MW and MB; Revising the manuscript: all authors.

Anlanger, C., Risse-Buhl, U., von Schiller, D., Noss, C., Weitere, M. and Lorke, A. Hydraulic and biological controls of biofilm nitrogen uptake in gravel-bed streams. *Limnol. Oceanogr.* 0–3 (2021). APPENDIX IV

Conception and design: all authors; Data acquisition: CA, URB, DvS, CN; Data analysis: CA, DvS, CN, AL; Interpretation of results: all authors; Writing the manuscript: CA, AL with comments from URB, DvS, CN, MW; Revising the manuscript: all authors.

Risse-Buhl, U., **Anlanger, C.**, Noss, C., Lorke, A., von Schiller, D. and Weitere, M. Hydromorphologic Sorting of In-Stream Nitrogen Uptake Across Spatial Scales. *Ecosystems* **24**, 1184–1202 (2021). APPENDIX V

Conception and design: all authors; Data acquisition: URB, CA, DvS; Data analysis: URB, CA, DvS; Interpretation of results: all authors; Writing the manuscript: URB, MW with comments from CA, CN, AL, DvS; Revising the manuscript: all authors.

Risse-Buhl, U., **Anlanger, C.**, Chatzinotas, A., Noss, C., Lorke, A. and Weitere, M. Near streambed flow shapes microbial guilds within and across trophic levels in fluvial biofilms. *Limnol. Oceanogr.* lno.11451 (2020). APPENDIX III

Conception and design: URB, CA, CN, AL, MW; Data acquisition: URB, CA, AC; Data analysis: URB, CA, AC; Interpretation of results: all authors; Writing the manuscript: URB, MW with comments from CA, AC, CN, AL; Revising the manuscript: all authors.

Risse-Buhl, U.**, Anlanger, C.**, Kalla, K., Neu, T.R., Noss, C., Lorke, A. and Weitere, M. The role of hydrodynamics in shaping the composition and architecture of epilithic biofilms in fluvial ecosystems. *Water Res.* **127**, 211–222 (2017). APPENDIX II

Conception and design: URB, CA, CN, AL, MW; Data acquisition: URB, CA, KK, TRN; Data analysis: URB, CA, KK, TRN; Interpretation of results: all authors; Writing the manuscript: URB, MW with comments from CA, KK, TRN, CN, AL; Revising the manuscript: all authors.

Declaration

I hereby declare that the thesis entitled "Interactions between flow hydrodynamics and biofilm attributes and functioning in stream ecosystems" is the result of my own work except where otherwise indicated.

The thesis has not been submitted for any other degree at another university or scientific institution.

Ilsenburg, 23.01.2023 ....

Christine Anlanger

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APPENDICES

APPENDIX I

Linking hydromorphological diversity to biodiversity and functioning in running waters

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Linking hydromorphological diversity to biodiversity and functioning in running waters

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Running head: Hydromorphological effects on biodiversity and functioning

Keywords: flow, turbulence, streambed topography, biofilms, bacteria, autotrophs, phagotrophic protists, nitrogen uptake, ecological niches

Authors' contribution: Conception and design of the work: CA, CN, URB, MW, AL; Data acquisition: CA, CN; Data analysis and interpretation: all authors; Drafted the work and wrote

the manuscript: CA, AL with the help of CN, URB, MW, MB, DvS; all authors helped to revise the work and approved the final submitted manuscript.

Abstract

Hydromorphological diversity is supposed to be an important driver of biodiversity and functioning of running waters. Experimental evidence, however, has been restricted to selected spatial and temporal scales. Here, we present a framework for quantifying hydromorphological diversity based on additive variance partitioning in analogy to established biological concepts based on α, β and γ diversities. By testing this framework with empirical data from streams, we demonstrate that the spatial flow variability (flow β diversity) is the prime driver of the β diversity of autotrophs, phagotrophic protists and nitrogen uptake efficiency thereby underlining the relevance of hydromorphological niches. Our framework facilitates the joint analyses of the interaction of hydromorphology, biodiversity and ecosystem functioning. It can be used to guide hydroecological research by integrating it into a broadened diversity concept and to optimize hydromorphological restoration measures to recover the structure and function of running waters.

Introduction

Environmental heterogeneity induced by physical and biotic factors is a major attribute of ecosystems and can be defined as the variability in processes or patterns over space and time[1,2]. The habitat heterogeneity hypothesis postulates that species diversity increases with environmental heterogeneity because more complex habitats provide more niches and a higher and more diverse supply of resources[3]. Increased habitat heterogeneity should thus increase the ability of ecosystems to maintain their functionality despite temporal variations in environmental conditions[4].

In streams and rivers, habitat heterogeneity is commonly related to the spatial and temporal variability of hydromorphology considered in terms of stream flow velocity and streambed morphology[5,6]. Spatially, habitats are structured hierarchically and extend from microhabitats (~ 10^{-2}–10^{-1}m, hereafter referred to as spots), mesohabitats (10^0 m) to reaches (~ 10^1–10^2 m), segments (~ 10^2 m) and catchments (~ 10^3 m), with mutual interactions among habitats [7,8]. Temporal variations of flow velocities range from milliseconds to minutes (i.e., the hydraulic scale of velocity fluctuations), up to days, months and years (i.e., the hydrologic scale of flow fluctuations[6]).

Most empirical studies in running waters have used bulk measures of hydromorphological parameters (e.g., mean flow velocity, water depth, wetted area, and bed slope) to characterize spatial habitat heterogeneity[9–13], and only a few linked habitat heterogeneity to biological communities at identical scales[14–16]. Moreover, empirical assessments of biogeochemical cycling and water quality in streams are typically conducted at the reach or larger spatial scales[13,17]. Yet, reach-scale properties emerge from strongly varying smaller-scale hydromorphological conditions, which need to be considered for extrapolation to larger spatial scales[2,16,18]. Temporal variation of flow velocity for characterizing heterogeneity at the spot scale has rarely been considered [15,19], even if high-frequency turbulent velocity fluctuations affect the structure and functioning of surface-associated microbial communities (biofilms) in streams[14,20].

In conclusion, the broad range of hydromorphological diversity that potentially affects biodiveristy and functionings of running waters has not been addressed so far. This is urgently needed to improve our understanding of how hydromorphological dynamics across different spatial and temporal scales shape the biodiversity and functioning of these ecosystems[21,22].

Moreover, planning and successful implementation of restoration efforts require a scalable framework to characterize habitat heterogeneity to restore biodiversity and ecosystem functions to natural levels.

Here, we describe a novel framework for characterizing habitat heterogeneity in running waters by a diversity index that combines measures of spatial and temporal variability of hydromorphology across different scales. It allows for analyzing the scale dependence of interactions between hydromorphology, biodiversity and functioning. We adopt this framework to quantify relationships between hydromorphological diversity and biofilm diversity, including bacteria, autotrophs and phagotrophic protists, representing the key guilds of biofilm food webs in running waters[23]. Moreover, we link hydromorphological diversity to stream functioning quantified as areal nitrogen uptake. In doing so, we aim to identify the relevant scales at which flow and morphological diversity of the streambed are interacting and at what scales flow diversity affects biodiversity and the diversity of biogeochemical hot spots.

Results and Discussion

Conceptual framework of hydromorphological diversity

The scale-dependence of biotic diversity is commonly characterized by alpha (α), beta (β) and gamma (γ) diversities[24,25]. The α diversity describes the number of species (i.e., species richness) or species diversity at a particular spot, the β diversity is the change in species richness or diversity between spots, and the γ diversity is the overall species richness or diversity of all spots within a region (Fig. 1a). Partitioning the overall diversity into α and β components should fulfill several basic properties. Among these are the requirements that α and β diversity should vary independently and that γ diversity should be completely determined by α and β diveristy[26]. The latter can be achieved either by an additive or a multiplicative approach between both diversities. The additive approach has the advantage of direct comparability between diversities because the units do not change[27].

We extended the concept of biodiversity partitioning to characterize hydromorphological diversity in running waters (Fig. 1b-d). Hydromorphological diversity at a spot can include temporal fluctuations[2], and we thus define α diversity as the normalized variance of a hydromorphological measure (e.g., flow velocity or water depth) obtained at a particular spot. Similarly, we express γ diversity as the normalized variance of the hydromorphological measure at different spots within a larger spatial scale. Finally, β diversity describes the spatial variance of the mean values and is obtained as $\beta = \gamma$ - mean α, according to the additive definition of diversities. The normalization of the variances avoids inherent dependencies between variance and mean values, which are known to exist for many physical quantities, including flow velocity[28].

Flow α diversity measured at individual spots can be referred to as temporal flow variability and corresponds to the square of the turbulence intensity[29] (i.e., the twofold ratio of turbulent kinetic energy and squared mean flow velocity). Flow β diversity describes the spatial variability and is calculated from time-averaged flow measurements (i.e., the spatial variance of mean flow velocities), normalized by the square of the overall mean velocity. Flow β diversity has been used in several models (e.g., *Mesohabitat Evaluation Model*[30], *Mesohabitat Simulation Model*[31]) or as an index[9] to describe habitat preferences of biotic communities. Finally, flow γ diversity describes the total relative velocity variance as the sum of the diversity due to mean turbulence conditions (i.e., mean α = spatially averaged α diversities) and the diversity due to spatial differences in mean flow velocity (γ = mean $\alpha + \beta$). In combination, the flow mean α and the β diversities integrate temporal and spatial variability, and we refer to it as overall flow diversity (Table 1). Both have been described as important components defining habitat suitability and ecological patterns in running waters across various scales[32,33] but lacked so far a hydromorphological definition.

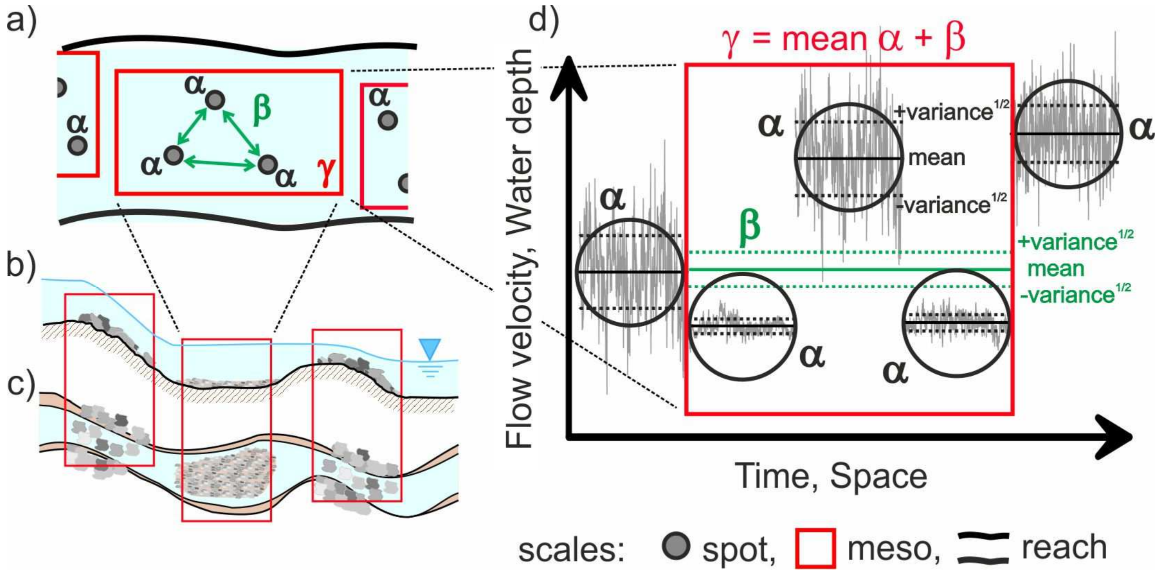

Figure 1. Framework for quantifying hydromorphological diversity and linking it to biodiversity and functioning of streams across spatial and temporal scales. The framework adopts the definitions of biodiversity and describes hydromorphological diversity at individual spots (α diversity), between spots (β diversity, green arrow) and the overall diversity within a larger region (γ diversity (a)). The α diversity describes the variance of flow velocity or water depth measured at individual spots, and γ diversity is the total variance observed at larger scales. Larger scales include riffles and pools at the meso scale or the reach scale (schematic longitudinal transect (b) and plan view (c)). β diversity measures the difference in diversities between spots and, by using an additive approach, represents the variability of mean values at a smaller scale within a larger scale (d). β and γ diversities are shown for the meso scale only. However, the diversities can also be calculated for the reach scale with β diversity expressing the variation between meso habitats and γ diversity expressing the overall diversity of the reach.

Morphological diversity describes spatial variations in streambed elevation, which is commonly decomposed into different types of roughness (e.g., grain roughness) and bed slope, or larger-scale topography[34]. Morphological α diversity describes the variance of water depths normalized by the squared mean water depth at the patch scale (i.e., the smallest scale for morphology, synonym for spot scale, see Fig. 1), which equals the squared relative streambed roughness. The reciprocal value of the morphological α diversity is thus identical to the squared relative submergence[34,35]. At the meso or reach scale, morphological γ diversity describes the variance of streambed elevation normalized by the square of mean water depth, which characterizes the relative roughness at larger scales, and we refer to it as overall morphological diversity. Finally, the morphological β diversity is the variability of the mean water depths at the spot scale normalized by the squared mean water depth (Table 1).

Table 1. Overview of the α, β and γ components of hydromorphological diversity according to the proposed framework based on variance partitioning. Hydromorphological diversity is expressed here based on measurements of flow velocity and streambed morphology. However, the diversity framework is potentially transferable to other environmental factors, such as temperature and light.

Scale	Diver-sity	Flow velocity	Physical description	Streambed morphology	Physical description
Spot	α	Temporal flow variability	Temporal variance of flow velocity normalized by the square of its temporal mean (turbulence intensity squared)	Streambed roughness	Spatial variance of water depths normalized by the square of the mean water depth (squared relative streambed roughness)
Meso, Reach	β	Spatial flow variability	Spatial variance of time-averaged flow velocities normalized by the square of their overall mean	(Mean) Water depth variability	Spatial variance of the mean water depths at the spot scale normalized by the square of their overall mean
	γ	Overall flow diversity	Total temporal and spatial variance of flow velocity normalized by the square of their overall mean (γ= mean $\alpha + \beta$)	Overall morphological diversity	Total spatial variance of water depths normalized by the square of their overall mean (γ= mean $\alpha + \beta$)

Variance partitioning of physical quantities is not new in fluvial hydraulics and flow velocities measured at one particular spot are often decomposed into mean values, which vary with discharge and location, and high-frequency turbulent velocity fluctuations (Reynolds decomposition[6]). The double-averaging approach additionally takes spatial variations of flow properties into account[36–38]. Moreover, variance partitioning has been used in geographical analyses for almost half a century[39]; it has been widely applied in various fields, including landscape ecology[40] and river science[41,42], but has rarely been connected to habitat heterogeneity, biodiversity and ecological functioning.

Application of the diversity framework

Our proposed framework was applied to an existing data set of high-frequency measurements of near-bed flow velocities conducted at two seasons in two gravel bed streams with different nutrient backgrounds[14,20,43]. The data set also included biofilms sampled (quasi) simultaneously at identical spatial scale, which were analyzed for microbial species richness based on both microscopic and molecular assessments[14]. We quantified ecosystem functioning as areal nitrogen uptake of biofilms following the addition of isotopically labeled nitrogen[43,44]. Flow measurements were accompanied by measurements of the streambed topography in 1x1 m patches along the reaches, and were used to quantify morphological diversity. A nested sampling design expanded the spot and patch measurements to the reach scale. The α and γ diversity of each microbial guild was expressed as species richness and the α and γ diversity of areal nitrogen uptake rates and efficiencies were expressed as the coefficient of variation. In accordance with the conceptual framework of hydromorphological diversity, β diversities were calculated by subtracting mean α diversity from γ diversity.

We used linear models to relate the diversities of streambed morphology, microbial guilds and areal nitrogen uptake with flow diversity and found a significant positive relationship in 12 out of 18 linear models (Fig. 2). Increasing β and γ flow diversity was associated with increasing β and γ biodiversity and increasing β and γ diversity of nitrogen uptake efficiencies. In contrast, the mean α diversitiy of microbial guilds and areal nitrogen uptake rates and efficiencies were unrelated to flow diversity and driven by season and nutrient background concentrations (i.e., stream).

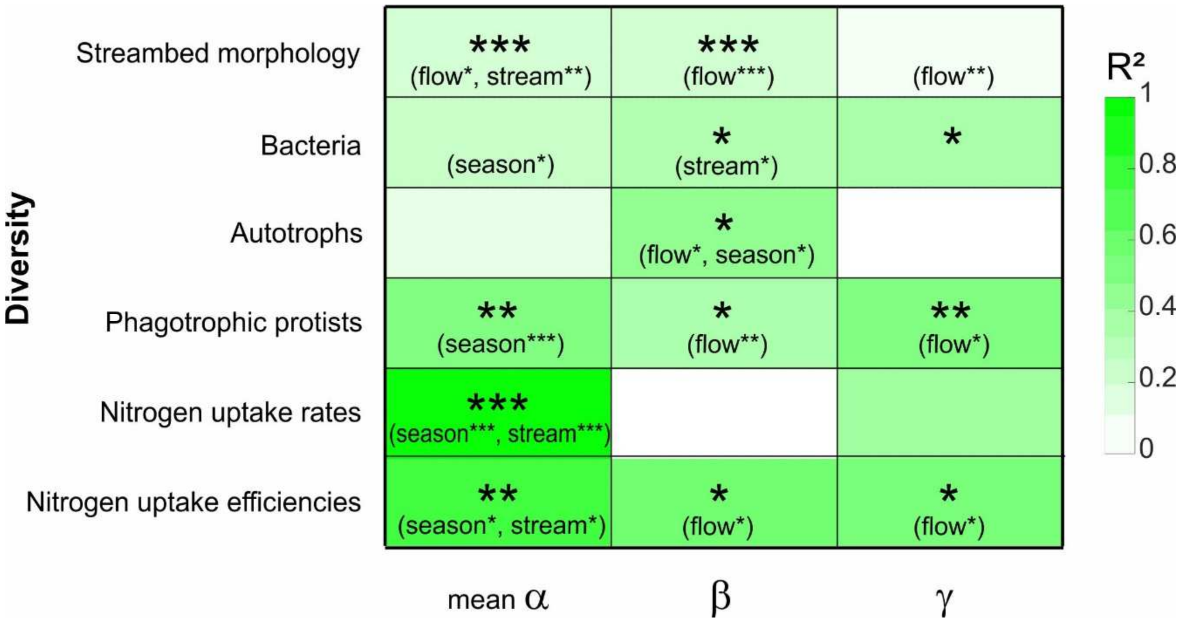

Figure 2. Heatplot visualizing the proportion of variance of different diversities explained by the flow mean α, β and γ diversity (abbreviated as flow), season and stream. The response variables are the morphological mean α, β and γ diversity of the streambed, the mean α, β and γ diversity of microbial guilds (TR-Fs of prokaryotic 16S rRNA genes abbreviated as bacteria, autotrophic morphotypes abbreviated as autotrophs and phagotrophic protist morphotypes abbreviated as phagotrophic protists), and the mean α, β and γ diversity of areal nitrogen uptake rates and efficiencies. Bold stars show the level of significance of the individual models, and the text followed by small stars shows the significance of the explanatory variables ($p < 0.05$ *, $p < 0.01$ **, $p < 0.001$ ***).

Flow and morphological diversities

The mean overall diversities (γ diversities) of flow and streambed morphology increased with increasing spatial scale. This was mainly due to an increase in mean spatial variability (β diversities). In contrast, the mean values of the temporal flow variability (flow α diversity) and streambed roughness (morphological α diversity) increased only slightly or were nearly constant across both scales (Fig. 3a). The mean flow velocity varied stronger between larger-scale features of the stream bed (i.e., pool-riffle structures at the meso scale) than due to small-scale streambed roughness. This result agrees with previous findings that turbulent flow structures are more affected by water depth than by protruding streambed elements[45]. The strong increase in morphological β and γ diversities from the meso to the reach scale in our study was associated with changes in the bulk geometry of the streambed, in addition to the predominant effect of form roughness at smaller scales. Here, the highest relative contributions of β diversity to γ diversity were obvious for morphological diversity and accounted for 77% and 95% at the meso and reach scale, respectively (Fig 3a).

We found a strong relationship between flow and morphological β diversity ($F_{1,69}$ = 21.64, $p < 0.001$, Fig. 2), which was expected given that the mean flow velocity depends strongly on the relative submergence of the streambed. Previous studies have found a wide range of different power law-relationships between relative submergence and mean flow, or vice versa between relative roughness and flow resistance[46]. At high relative submergence, skin friction dominates the resistance force and depends only weakly on the relative roughness (approximately with the power of 1/6). At lower relative submergence, as in the present study, larger contributions from form drag resulted in a nearly linear (power of 1) relationship between flow resistance and relative roughness. Similar results were found in sandy lowland streams[47], highlighting the universality of this relationship for other stream types. The relationship between the relative submergence at the grain scale (morphological mean α diversity) and temporal flow variability (flow mean α diversity) differed among streams (Fig. 2), which may result from differences in bed slope[44] and roughness between stream reaches (Fig. S1 in the Supplement). Seasonal differences were not relevant for any relationships between flow and morphology because of lacking bed-forming discharges during the study.

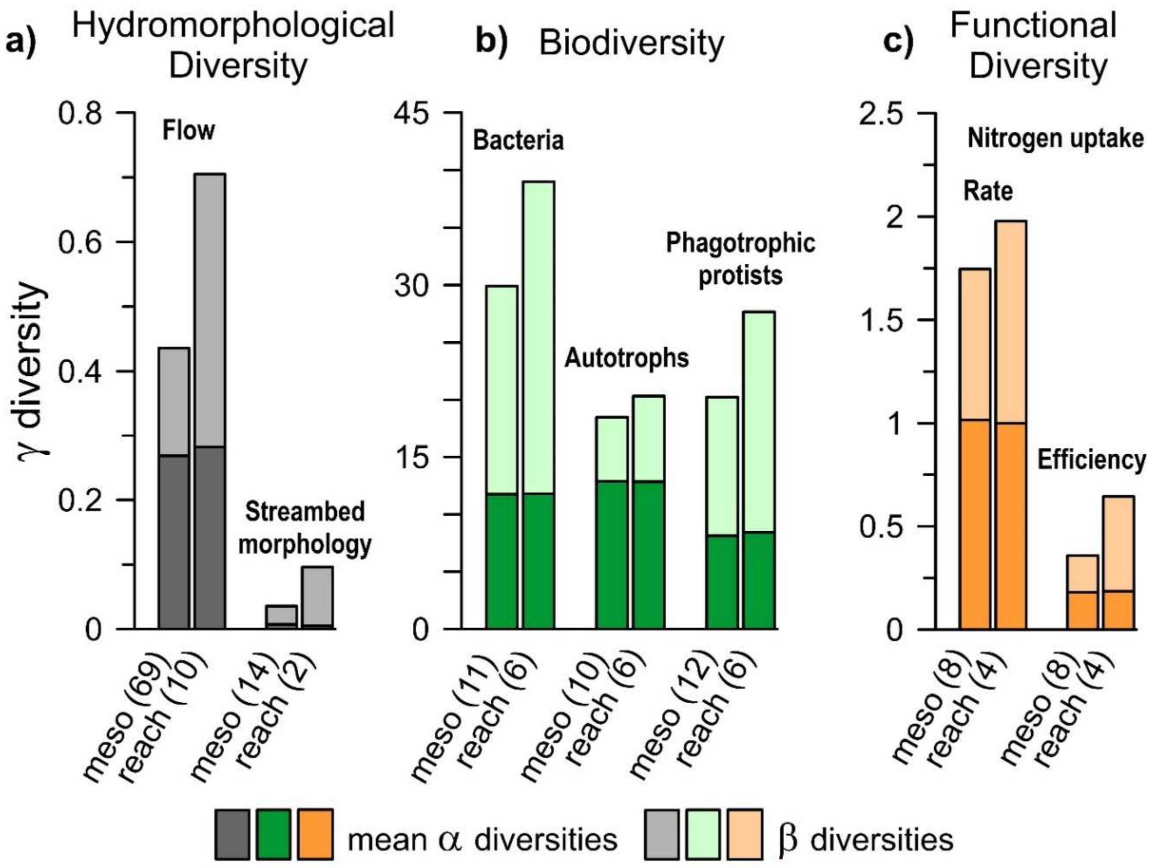

Figure 3. Mean contributions of mean α and β diversities to γ diversity of (a) hydromorphological diversity (flow and streambed morphology), (b) biodiversity including three microbial guilds (T-RFs of prokaryotic 16S rRNA genes abbreviated as bacteria, autotrophic morphotypes abbreviated as autotrophs, phagotrophic protist morphotypes abbreviated as phagotrophic protists), and (c) the diversity of areal nitrogen uptake rates and efficiencies as proxies for ecosystem functioning. Data for each scale and diversity are averaged over all seasons and streams, where the number of data points is shown in parenthesis in the axis labels.

Flow diversity and biodiversity

The temporal flow variability (flow α diversity) did not significantly affect any of the microbial guilds (Fig. 2) demonstrating that species richness can be equally high over a wide range of natural flow variability. However, species identity might still be affected by shifts in species differing in their tolerance towards hydraulic forcing (see Risse-Buhl et al.,[14] for more detailed community analyses). By contrast, spatial (β) flow diversity significantly affected β

diversity of autotrophs ($F_{1,12} = 6.13$, $p = 0.029$) and phagotrophic protists ($F_{1,14} = 11.55$, $p = 0.004$). The latter was also significantly affected by the overall flow diversity, combining both, temporal and spatial variability (flow γdiversity, $F_{1,14} = 16.04$, $p = 0.001$, Fig. 2). In accordance with the hydromorphological diversities, the γdiversity of the studied microbial guilds increased with spatial scale due to an increase in βdiversities. This result followed the prediction of the dual scaling law that states that species richness increases with increasing spatial scale and environmental heterogeneity[48].

On the contrary to bacteria and phagotrophic protists, the overall diversity of autotrophs (γdiversity) showed higher contributions of the mean αdiversity, which was similarly high for both spatial scales (69% and 63% for the meso and reach scale, respectively, Fig. 3b). The overall diversity of autotrophs was high already at the small scales, which implies that the effects imposed by flow variability of riffle-pool sequences are minor for this microbial guild. The autotrophic community that developed during biofilm maturation can act as an ecosystem engineer, resulting in a homogenization of communities at the spot scale by modulating their microenvironment and creating similar biofilm architectures and flow conditions[49]. Moreover, a companion study found that higher turbulent kinetic energy at the spot scale (= 0.5 × unscaled flow αdiversity) favored the growth of larger-sized autotrophs[14]. This might be a reason for the observed effects.

Bacterial diversity did not respond to flow diversity at all, whereas flow diversity at the larger scales affected the diversity of autotrophs and phagotrophic protists. Phagotrophic protists and most autotrophs are relatively large (compared to bacteria) and show a large phenotypic diversity with diverse adaptations to flow and corresponding preference for particular hydraulic niches[50–52]. This makes the sorting of species by hydraulic forces likely. In contrast to phagotrophic protists, the dominant bacterial species occurred irrespective of the turbulent kinetic energy at the spot scale[14]. Here, we confirm this finding also for the flow diversities at larger spatial scales. The lifestyle of bacteria is characterized by smaller organismic size, high production of protecting and fixing extracellular polymeric substances[53,54], and a high phenological plasticity[55]. All these features make them highly ubiquitous and resistant to physical forcing in the stream environment. The high phenotypic plasticity of bacterial genotypes potentially enables the same genotype to occur with adapted phenotypes in different hydraulic niches. However, the high contribution of β diversity to the overall γ diversity suggests a differentiation and the existence of distinct communities at the spot scale for bacteria, which was not affected by flow diversity.

In contrast to our results from stream biofilms[14], a flume experiment found significant correlations between bacterial β diversity and turbulence intensity (flow α diversity) and mean flow velocity (unscaled flow β diversity)[15]. However, the maximum standard deviation of the mean flow velocity in their experiments (0.07 m s^{-1}) was a factor of two lower than the standard deviation in the natural flows at our study sites. This result suggests that bacterial diversity may respond to turbulent velocity fluctuations in the low-velocity range of flumes only but not within the high-velocity range typically found in natural mountain streams. It is important to note that the bacteria were analysed by molecular methods based on 16S rRNA genes, whereas both autotrophs and phagotrophic protists were counted microscopically by phenotypic and morphological features. As the habitat adaptation occurs at the level of the phenotype and as particularly bacteria show an extremely high phenotypic plasticity within particular genotypes, it is possible that the phenotypic bacterial diversity shows different patterns compared to the genotypic diversity analysed here.

In agreement with previous results [14], the mean α diversities of bacteria ($F_{1,13} = 4.90$, $p = 0.045$) and phagotrophic protists ($F_{1,14} = 16.98$, $p = 0.001$) were significantly affected by season, indicating that the variability in environmental conditions (e.g., nutrients, light, temperature, the seasonal succession of predators and prey) constrained biofilms along the whole stream reach (i.e., large-scale effects).

Flow and functional diversity

Mean α and β diversities of the nitrogen uptake efficiency at the meso scale contributed equally to its γ diversity (Fig. 3c), implying that the diversity of nitrogen uptake efficiency within each riffle or pool was comparable to the diversity between riffles or between pools. For areal nitrogen uptake rates, mean α diversity was slightly higher than the β diversity (58% and 42% of the γ diversity, respectively). This was also obvious at the reach scale, where β diversity was 2.5 times larger than the mean α diversity for nitrogen uptake efficiencies but equally high for areal nitrogen uptake rates (Fig. 3c). As the uptake efficiency corresponds to the biomass-specific uptake rate, this finding suggests conditioning of biomass within and between meso-scale structures, making them less diverse in terms of uptake rates.

Temporal flow variability (flow α diversity) had no significant effect on areal nitrogen uptake rate or nitrogen uptake efficiency (Fig. 2). However, we found that the spatial flow variability (flow β diversity) influenced the β diversity of the nitrogen uptake efficiency ($F_{1,8} = 10.69$, $p = 0.011$) and the overall (γ) flow diversity influenced the γ diversity of nitrogen uptake efficiency ($F_{1,8} = 8.78$, $p = 0.018$), but not diversities of the areal nitrogen uptake rate. The maximum rate at which biofilms can take up nitrogen from the stream water can be limited by turbulent mass transfer at the streambed[56], which is related to the temporal variability of the flow and can be high already at the spot scale. This means that flow α diversity may influence functional responses like nitrogen uptake twofold - indirectly via modulation of biofilm attributes (i.e., diversity, architecture) and directly by affecting the mass transfer. As described above, the mean flow α diversity dominated the overall flow (γ) diversity at the meso scale, but did not further increase at the reach scale (Fig. 3a). In summary, our results indicate that, compared to spatial flow variability, mass transfer was an important process for nitrogen uptake already at the meso scale but was finally not limiting nitrogen uptake rates and efficiencies.

To analyze also potential indirect effects of flow diversity on nitrogen uptake, particularly mediated by relationships between biodiversity and functional diversity in the study streams, we additionally related the diversity of each microbial guild to the diversity of the areal nitrogen uptake rate and uptake efficiency, and used stream and season as explanatory variables. We could not find a correlation between the diversities of autotrophs and diversities of nitrogen uptake rates or efficiencies (Fig. S2-S4). However, spatial flow variability was correlated to the autotroph's β diversity and the β diversity of nitrogen uptake efficiency. As described above, autotrophs were characterized by high α and low β diversities (Fig. 3b), which indicates that the functional performance of microbial communities may get adjusted to the prevailing flow diversity by increasing or decreasing abundances of species involved in a particular function.

We found no influence of flow diversity, but of season on absolute mean areal nitrogen uptake rates and efficiencies as measures of the capacity of streams to immobilize nutrients (Fig. S5). Moreover, the mean areal nitrogen uptake rate and efficiency (not their diversity) were not related to α, β or γ diversities of different microbial guilds except for the mean α diversity of bacteria (Fig. S6-S7). This finding contradicts laboratory studies with heterogeneous flows[52], where nitrogen uptake increased with species richness in algal biofilm communities due to niche partitioning. However, contrasting results may due to differences in species richness between laboratory flumes and natural ecosystems, where functional redundancy and domincance effects become important[57,58].

Temporal and spatial upscaling

Scaling relationships – for both space and time – are highly important in ecology and biogeography[18,59]. For example, the species composition, abundance and morphology of biofilms can be influenced by flow conditions during the last days or weeks. Our samplings were conducted at nearly stationary discharge conditions that persisted for at least two weeks before each sampling, and discharge magnitude was comparable between samplings. Thus, the estimated flow α diversities include only the hydraulic but not the hydrological scales of flow variability. The relative importance of the latter was analyzed by the cumulative integral of the composite power spectrum of the temporal flow variability (i.e., flow α diversities) derived from long-term discharge time series. The spectrum revealed that the flow α diversity, as observed during the measurement campaigns, contributed, on average, 20% to the long-term flow α diversity over 16 years (Fig. S8a). This contribution varied between 2% and 70%, depending on the sampling spot.

The cumulative integral of the morphological α diversity of the streambed indicates that also the morphological diversity strongly increased beyond the scales of the study (Fig. S8b). Morphological diversity associated with riffles and pools at the meso scale contributed <10%, while the highest diversity was observed at spatial scales between 100 m and ~2 km. Beyond the reach scale, a further increase in spatial flow variability (flow β diversity) could be expected because of changes in discharge and streambed morphology (i.e., in response to increasing drainage area size and differences in valley topography from headwater to lowland reaches). However, the cumulative mean α spectra of the streambed morphology suggested ‘saturation’ at spatial scales beyond the reach scale, which contradicts a continuous strong increase in flow β diversity. Experimental evidence for a characteristic length scale causing maximal flow β diversity is lacking, and universal relationships might not exist due to different kinds of valley morphology. We can only speculate to what extent biodiversity and functional diversity change beyond the reach scale and for larger temporal scales, including hydrological variability. Based on the findings of this study, it can be assumed that these parameters do not change as long as hydromorphology and environmental conditions do not change. Nonetheless, season was a strong driver of biofilm diversity (Fig. 2) and biomass[43]. Furthermore, increasing drag forces and transport of suspended matter during high-discharge events at hydrological scales can temporarily reduce biofilm biomass[60]. Together, our results point towards a large potential of increasing biofilm diversity and diversity in areal nitrogen uptake with increasing temporal scale.

Conclusions

The importance of hydromorphological habitat heterogeneity for biodiversity and functional diversity in running waters has been repeatedly postulated. However, evidence has been limited to particular spatial and temporal scales of habitat heterogeneity, which were characterized using different metrics. Moreover, the direct link to biodiversity and stream functioning has been missing in many studies and remained on a theoretical approach. Here, we establish a novel diversity framework based on variance partitioning for hydromorphological variables directly relateable to biodiversity and ecosystem functioning across different spatio-temporal scales. The framework thereby considers variables broadly routed in pure hydraulic and morphodynamic research and, at the same time, considered important drivers for biological processes. As a result, we highlight the importance of hydromorphological niches characterized by the spatial variance of the time-averaged flow velocities and mean water depths (i.e., β diversity).

Our framework is potentially transferable to other freshwater ecosystems and ecosystem compartments such as the hyporheic zone, and may include further environmental factors, such as temperature and light. It has been established and tested for microbial communities but has the great potential also to tackle the effect of hydromorphology on the diversity of larger-sized and more motile organism like macroinvertebrates given that flow diversity has been recognized as an important physical control on the composition of stream macroinvertebrate communities[10,61,62].

Hydromorphological simplification and modification of running waters have reduced the complexity and integrity of riverine ecosystems[63], thereby reducing freshwater biodiversity[64] and stream functioning[65]. Biodiversity conservation is one of the most important challenges we face as a society. Our framework facilitates integrative studies on the contributions of biotic and hydromorphological diversity and dynamics to a broadened biodiversity concept in stream ecology[41] and advances the common knowledge on biodiversity–functioning relationships and the governing role of hydromorphological diversiyt .

Methods

Sampling strategy

We established and applied a novel framework for describing diversities using an extensive data set, including flow velocity[14,20,43], streambed topography (unreported measurements), microbial guilds of biofilms[14], and biofilm areal nitrogen uptake[43,44]. Measurements were conducted at identical spatial scales and (quasi) simultaneously, except for biofilm diversity and biofilm nitrogen uptake which were sampled in close vicinity but not at the same spot. The data encompasses five samplings in two mountainous streams with contrasting nutrient background and two seasons.

Flow measurements were conducted at the spot scale (in total 533 sampling spots) and were pooled according to two distinct spatial scales: the mesoscale (spatial extent of hydromorphological habitats, i.e., riffle and pool, in total 8 riffles and 9 pools), and the reach scale (spatial extent of the study reaches, Fig. 1). Streambed topography was mapped at approximately 1x1 m patches along the stream reaches during four campaigns (in total 58 patches) for the analysis of morphological diversity. The streambed surface was stable, and we expected a near bank-full threshold for sediment movement, which was not observed during and between the samplings. We thus pooled measurements of all campaigns to estimate morphologcial meso and reach scale diversities for each stream.

For three out of the five field campaigns, the diversity of three microbial guilds of epilithic biofilms, namely bacteria (T-RFs of prokaryotic 16S rRNA genes), autotrophs (morphotypes of cyanobacteria and eukaryotic algae) and phagotrophic protists (morphotypes of flagellates, amoeba and ciliates) was expressed as species richness at a subset of flow sampling spots. Finally, two field campaigns included measurements of biofilm nitrogen uptake upon ^{15}N labeled tracer addition at a subset of flow sampling spots. We calculated meso and reach scale diversities for areal nitrogen uptake rates and uptake efficiencies (nitrogen uptake rates normalized by nitrogen biomass) for each campaign and stream.

Study sites

The measurements were conducted at two second-order, gravel-bed mountain streams (Selke, N 51°41’11.5’’, E 10°15’34’’, Kalte Bode, N 51°44’33’’, E 10°42’09’’), in Central Germany. Daily discharge data starting from 1921 (Selke) and 1951 (Kalte Bode) and discharge at 15 minutes intervals for more recent timeperiods were available from gauging stations close to the study sites. Long-term mean discharge was 1.52 $m^3 s^{-1}$, and 0.72 $m^3 s^{-1}$ and baseflow was 0.24 $m^3 s^{-1}$ and 0.18 $m^3 s^{-1}$ for Selke and Kalte Bode, respectively. Stream width ranged from seven to eight meters. Soluble reactive phosphorous and dissolved inorganic nitrogen concentrations were up to 3 to 16 times and up to 2 times higher in the stream water of the Selke compared to the Kalte Bode, respectively[14,20,43,44].

Morphological measurements and data analysis

The streambed topography was surveyed with a custom-made laser scanner and, similar to systems already described elsewhere[47,66], the streambed was illuminated with a laser line (Z40M18S-F-643-LP60-V2, Z-Laser, Freiburg, Germany) and the reflected light was observed by two underwater cameras (GoPro Hero3+ Black Edition, 48 fps, 1920 x 1440 px). The bottom elevation along the laser line was reconstructed from the location of the laser line in the field of view of the cameras. Laser and cameras were mounted on a rack (Fig. S9a), which could be moved horizontally at an adjustable height above the bottom. The rack was mounted on a rigid frame deployed at each patch. After leveling the instrument frame, the laser light sheet was moved along several lanes to scan the streambed topography within an area of 0.8 m x 0.6 m. During laser deployment, the frame was covered with lightproof fabric to improve the visibility of the reflected laser line on the bed. The method was restricted to water depths > 10 cm; thus, very shallow areas and areas with emerging stones could not be surveyed. The laser-camera system was calibrated according to an existing study[47]. Individual streambed elevation profiles were merged into a digital elevation model (DEM) of the scanned area with a final horizontal resolution of 0.25 cm (Fig. S9b-c). Although the measurements were obtained at a higher resolution (on average 0.01 cm), we limited the DEM resolution to the smallest measured grain sizes to reduce computational processing time. Data gaps in the DEMs (resulting from, e.g., non-overlapping parts of lanes) were filled using a radial basis function with a multiquadric basis function[67]. Streambed roughness *k* was estimated as the standard deviation of the streambed elevation concerning a planar surface, which we fitted to the observed DEM at each patch. *k* is equivalent to a characteristic vertical roughness height of gravel beds[68]. For each DEM, the distance to the water surface was read from a fixed reference point on the laser-holding vertical unit and added to the depth recorded by the scan.

Streambed roughness at the reach scale and beyond

For spatial extrapolation, longitudinal transects of streambed roughness and water depth were obtained using a remotely controlled laser scan boat (LaSBo)[47]. LaSBo measurements are based on the same laser triangulation method described above but provide longitudinal transects of water depths along the boat trajectory. The measurements were interpolated to a regular longitudinal grid with 0.25 cm resolution. Also, LaSBo operation was restricted to water depths > 10 cm.

Topographical data for a 13 km long stream section comprising the investigated study site at Selke (i.e., 187 geo-referenced cross-sectional surveys) were available from the local water authority. Water depths of the cross-sections were interpolated with nearest neighbors to obtain a resolution of 70 m. The mean daily discharge during the topographical survey of the Selke was 0.26 ± 0.08 m^3 s^{-1} (mean ± standard deviation).

Expression of diversities

Flow and morphological diversity

The flow α diversity (α_u) at each spot was calculated as the normalized temporal variance in all three components (u, v, w) of the measured flow velocity time series:

$$\alpha_u = \frac{1}{\bar{u}^2}\frac{1}{N}\sum_{i=1}^{N}((u_i - \bar{u})^2 + v_i^2 + w_i^2), \tag{1}$$

where $\bar{u} = \frac{1}{N}\sum_{i=1}^{N} u_i$ denotes the mean longitudinal flow velocity and N the number of measurements at each spot (note that the remaining velocity components $\bar{v} = \bar{w} = 0$ after rotation of the measured velocities into the mean flow direction[20]).

Flow γ diversity (γ_u) was calculated by concatenating velocity time series measured at individual spots at the meso or at the reach scale for each measurement campaign as:

$$\gamma_u = \frac{1}{\langle\bar{u}\rangle^2}\frac{1}{n}\sum_{j=1}^{n}\frac{1}{N}\sum_{i=1}^{N}\left((u_{ij} - \langle\bar{u}\rangle)^2 + v_{ij}^2 + w_{ij}^2\right), \tag{2}$$

with $\langle\bar{u}\rangle$ representing the temporally and spatially averaged flow velocities from n sampling spots ($\langle\bar{u}\rangle = \frac{1}{n}\sum_{j=1}^{n}\frac{1}{N}\sum_{i=1}^{N} u_{ij}$). A minimum number of three velocity measurements was chosen to calculate flow γ diversities at each spatial scale.

Finally, β diversity describes the spatial variability obtained from the additive definition of diversities ($\beta = \gamma - \alpha$). Beta flow diversity (β_u) at the meso and reach scale were calculated as:

$$\beta_u = \gamma_u - \overline{\alpha_u}, \tag{3}$$

with $\overline{\alpha_u}$ representing the mean value of all flow α diversities observed at the corresponding scale.

While flow diversities were calculated at all spatial scales based on pooled flow velocity measurements at the spot scale, morphologic diversities were handled slightly differently. The variance of water depths above a DEM normalized by the square of their spatial mean as the smallest morphologic unit is defined to constitute the morphological α diversity (α_h):

$$\alpha_h = \frac{1}{\langle h\rangle^2}\frac{1}{N}\sum_{i=1}^{N}\left(h_{ij} - \langle h\rangle\right)^2, \tag{4}$$

where $\langle h\rangle = \frac{1}{N}\sum_{i=1}^{N} h_i$ denotes the mean water depth and N the number of samples within the DEM. γ_h diversity on the meso and reach scale was calculated by combining all DEMs within the respective spatial scale as:

$$\gamma_h = \frac{1}{\langle\langle h\rangle\rangle^2}\frac{1}{n}\sum_{j=1}^{n}\frac{1}{N}\sum_{i=1}^{N}\left(\left(h_{ij} - \langle\langle h\rangle\rangle\right)^2\right), \tag{5}$$

with $\langle\langle h\rangle\rangle$ representing the spatially averaged mean water depth from n patches ($\langle\langle h\rangle\rangle = \frac{1}{n}\sum_{j=1}^{n}\langle h\rangle_j$).

β_h for the meso and reach scale was calculated from:

$$\beta_h = \gamma_h - \overline{\alpha_h}, \tag{6}$$

with $\overline{\alpha_h}$ representing the mean values of all α_h observed at the corresponding scale.

Temporal and spatial upscaling

For the Selke, power spectral densities of the longitudinal velocity component was estimated for each 20-min measurement using Welch's method[69] with 50% overlap and a Hamming window function. Spectra were normalized by the square of the mean flow velocity. The normalized velocity spectra represent the frequency distribution of components of the flow α diversity (see also equation (1)). The individual spectra from the 20-min flow measurements were log-averaged, and the mean spectrum and the 5% and 95% percentiles were calculated. Next, we constructed a composite spectrum of velocity fluctuations by combining: (1) the log-averaged spectra and their percentiles (frequency range from $3\text{x}10^{1}$ to $4\text{x}10^{-3}$ Hz); (2) the spectra of the mean velocities calculated from 15 min interval discharge data for three months (frequency from $5\text{x}10^{-4}$ to $1\text{x}10^{-7}$ Hz); and (3) the mean velocities calculated from daily mean discharge data for 16 years (frequency from $6x10^{-6}$ to $3x10^{-9}$ Hz). The discharge data were converted to flow velocities using a cross-sectional topographic transect and water level data at the gauging station. The cumulative α diversity for increasing time scales was estimated as the cumulative integral of the composite spectral density from the highest to lowest resolved frequency, i.e., the cumulative variance for increasing time scales.

Similar to flow velocity, a composite power spectrum of water depth variations was estimated by combining the wavenumber spectra of (1) all concatenated LaSBo surveys at the Selke (wavenumber from $2x10^{2}$ to 10^{-2} m^{-1}) and (2) cross-sectional mean water depths calculated from the 13 km survey at the Selke (wavenumber from $7x10^{-3}$ to $1x10^{-4}$ m^{-1}). All spectra were normalized by the corresponding squared mean water depth. The cumulative, normalized variance for increasing length scales was estimated as cumulative integrals of the spectral density function from high to low frequencies. The unresolved wave number range from $7x10^{-3}$ to 10^{-2} m^{-1} was linearly interpolated for integration.

Diversity of microbial guilds

α diversity of microbial guilds, namely bacteria, autotrophs and phagotrophic protists, were represented by species richness at the spot scale[14]. At larger spatial scales, the mean α diversity of all spots within a pre-defined scale (meso or reach scale) was calculated. In addition, γ diversity at the meso and reach scale was calculated by considering all species found in the respective spatial scale. The difference between γ and mean α diversity represents β diversity. At the meso scale, the biofilm-flow subsets of all riffles and all pools within a reach were pooled for each measurement campaign.

Diversity of biofilm nitrogen uptake

Similar to morphological diversity, the variance of spot-scale nitrogen uptake rates and nitrogen uptake efficiencies within each riffle or pool normalized by the mean square was calculated and constitute α diversities (coefficient of variation, CV). To calculate mean α diversities at the meso scale, we averaged all riffle and all pool α diversities separately for each campaign, resulting in a mean α diversity for riffles and a mean α diversity for pools. Next, we calculated the CV for all spots in all riffles and for all spots in all pools along the stream reach for each campaign and considered it as γ diversity: γ(meso scale) $_{\text{riffle}}$ = CV of riffle spots along the reach, and γ(meso scale) $_{\text{pool}}$ = CV of pool spots along the reach. Subtracting the mean α diversity from the γ diversity resulted in the β diversity for riffles and pools of each campaign. At the reach scale, we calculated the mean α diversity from all meso-scale α diversities for each campaign and γ diversity as the CV of all spots within the reach. Finally, we subtracted the mean α diversity from the γ diversity to achieve the β diversity at the reach scale.

Statistical analyses

Linear regression models were used for testing relationships between flow mean α, β and γ diversity and (1) morphological mean α, β and γ diversity, (2) mean α, β and γ diversity of the microbial guilds and (3) mean α, β and γ diversities and mean values of nitrogen uptake rate and efficiency for both streams and all scales and seasons available. The calculation of flow diversities according to the proposed framework was adjusted and calculated for each subset of (2) and (3) so that only identical spatial scales were used. We also tested for relationships between the mean α, β and γ diversity of the microbial guilds and (1) the mean α, β and γ diversities of nitrogen uptake rate and efficiency and (2) the mean values of nitrogen uptake rate and efficiency. We added stream and season as explanatory variables for all models, and the analysis of variance (ANOVA) revealed the component statistics. We refrained from testing for differences between meso- and reach scale because we only sampled one reach per stream. Data were log-transformed if residuals were not normally distributed (Shapiro-Wilk Test). All test results were regarded as significant if $p < 0.05$.

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Supporting Information

Linking hydromorphological variability to biodiversity and functioning in running waters

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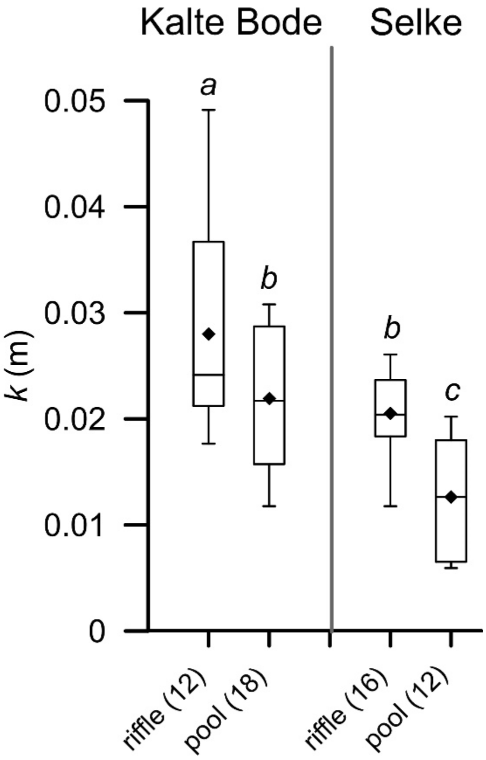


Figure S1. Variability of streambed roughness (*k*) in riffles and pools of the Kalte Bode and Selke. *k* is expressed as the standard deviation around a planar surface area. The boxes encompass the 25-75 percentile range, with the median value indicated by a horizontal line. Mean values are shown by diamonds. Statistical differences (ANOVA, Holm-Sidak test) are indicated by superscripted letters. The number of individual roughness measurements at the patch is shown in parenthesis in the axis labels.

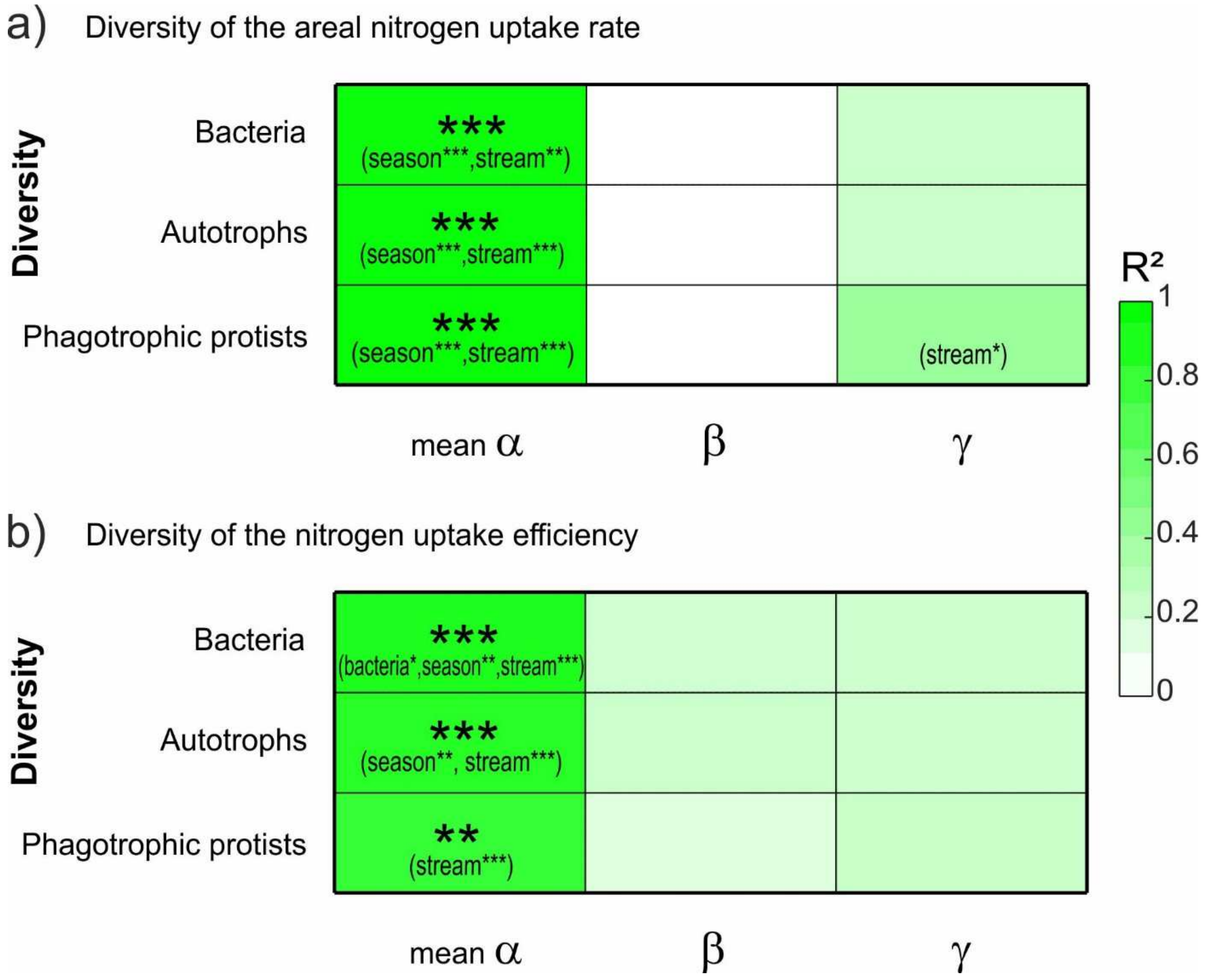


Figure S2. Heatplot visualizing the proportion of variance of (a) the α, β and γ diversities of areal nitrogen uptake rates and (b) the α, β and γ diversities of nitrogen uptake efficiencies that is explained by the mean α, β and γ diversity of microbial guilds, sampling season, and stream. Microbial guilds are TR-Fs of prokaryotic 16S rRNA genes (abbreviated as bacteria), autotrophic morphotypes (abbreviated as autotrophs) and phagotrophic protist morphotypes (abbreviated as phagotrophic protists). The color in the heatplots denotes the coefficient of determination R². Larger bold stars show the significance of the individual models, and the text followed by smaller stars shows the significance of the explanatory variables within the models ($p < 0.05$ *, $p < 0.01$ **, $p < 0.001$ ***).

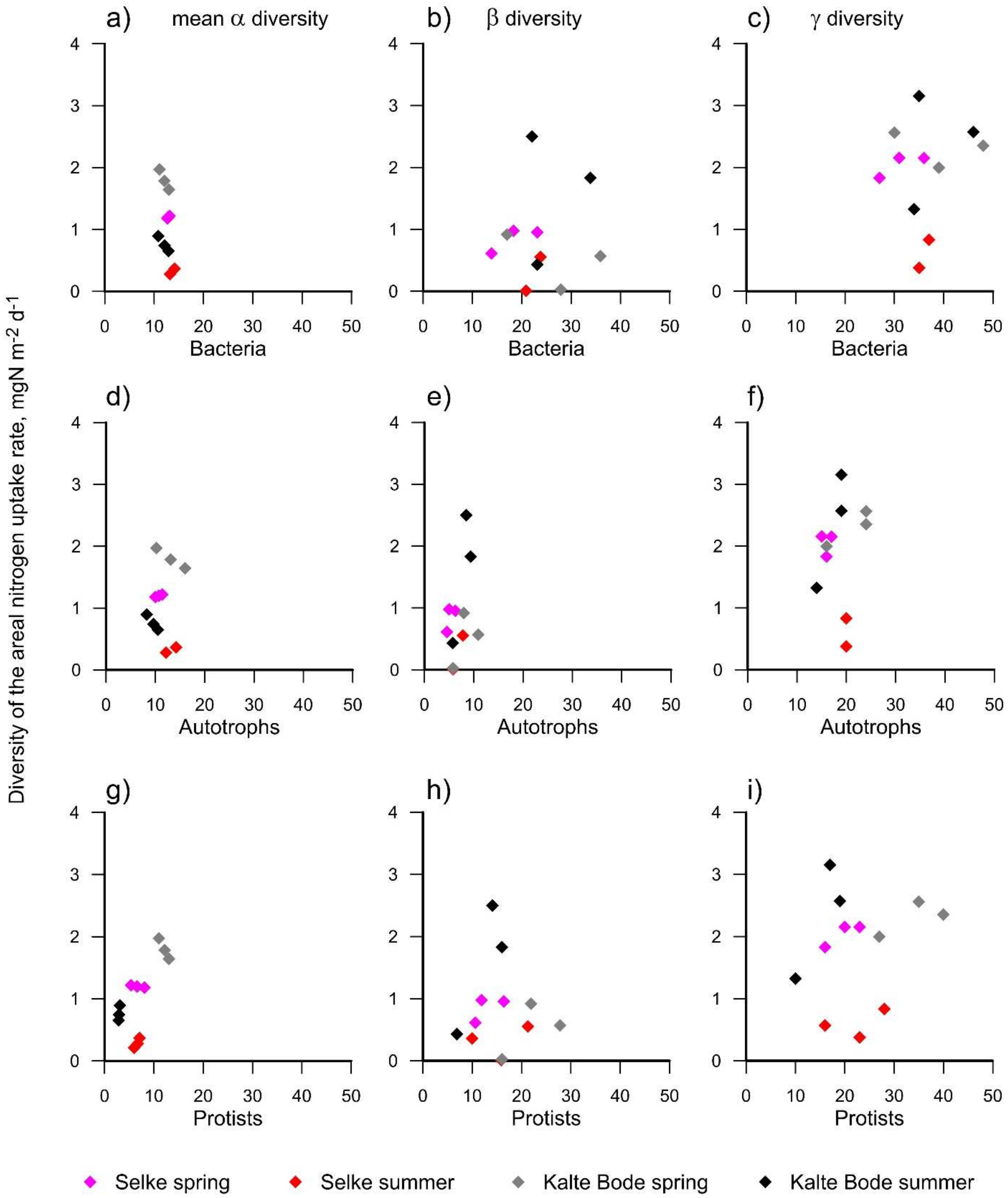


Figure S3. Scatter plots of mean α, β and γ diversities of areal nitrogen uptake rates and mean α, β and γ diversities of microbial guilds (TR-Fs of prokaryotic 16S rRNA genes abbreviated as bacteria (a-c), autotrophic morphotypes abbreviated as autotrophs (d-f) and phagotrophic protist morphotypes abbreviated as protists (g-i)) for both sampling seasons (summer and spring) and streams (Selke and Kalte Bode).

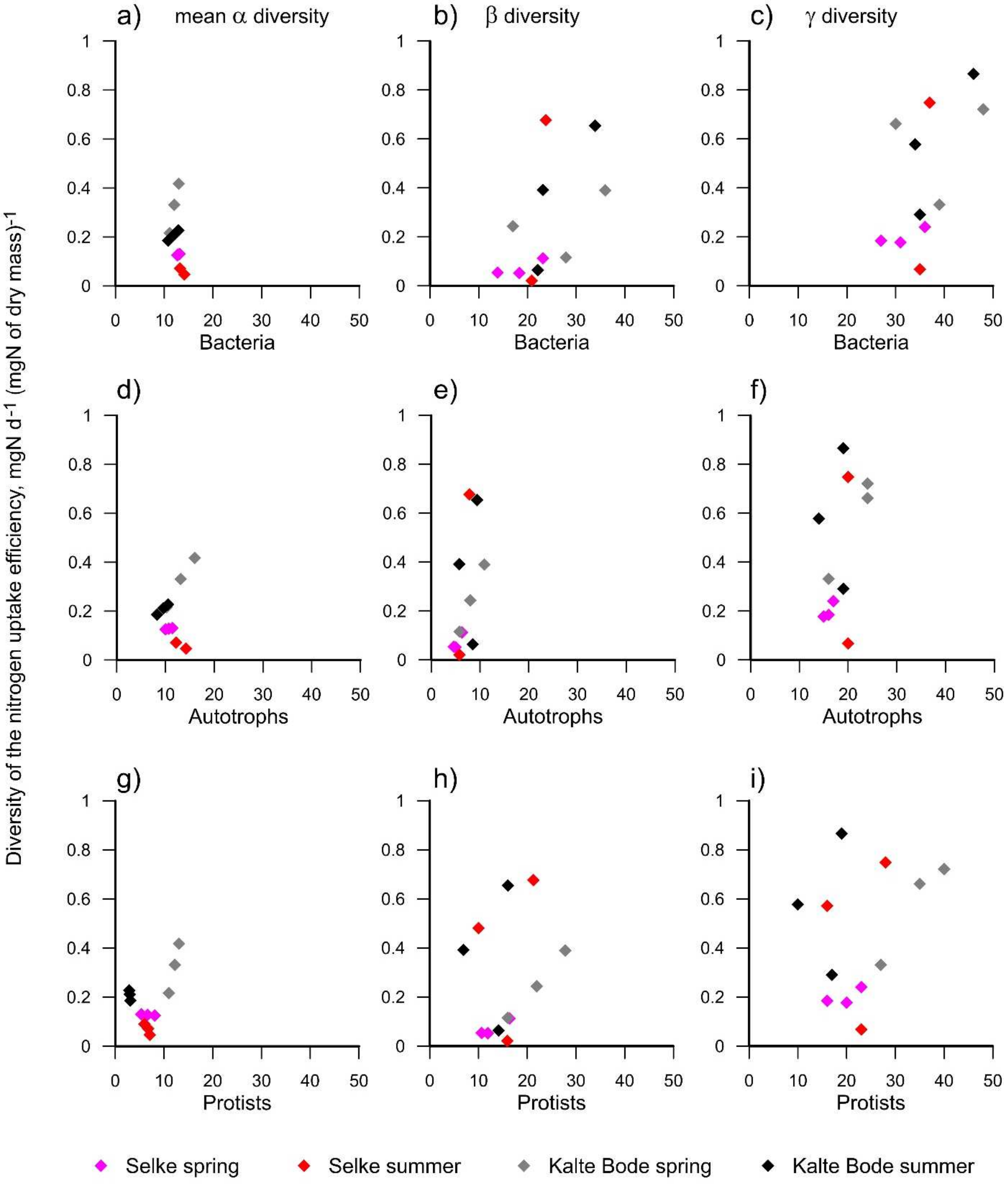


Figure S4. Scatter plots of mean α, β and γ diversities of the nitrogen uptake efficiencies and the mean α, β and γ diversities of microbial guilds (TR-Fs of prokaryotic 16S rRNA genes abbreviated as bacteria (a-c), autotrophic morphotypes abbreviated as autotrophs (d-f) and phagotrophic protist morphotypes abbreviated as protists (g-i)) for both sampling seasons (summer and spring) and streams (Selke and Kalte Bode).

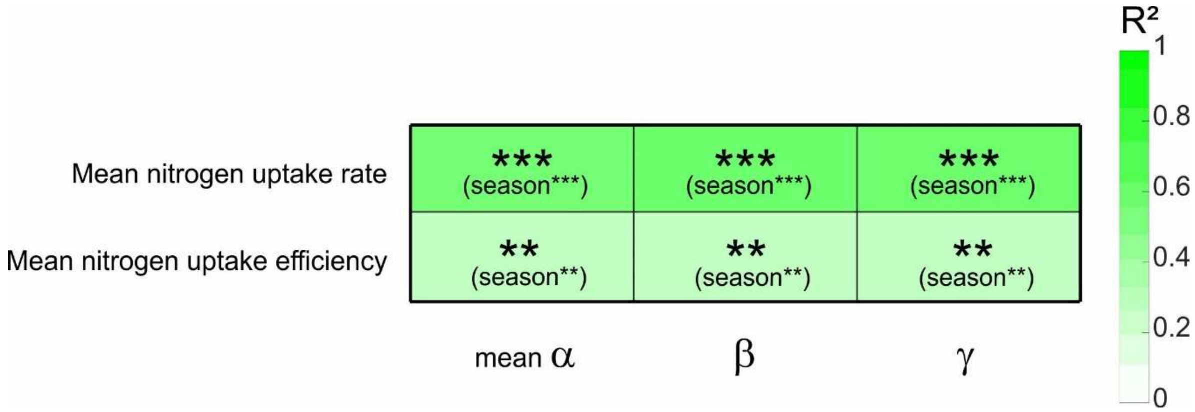
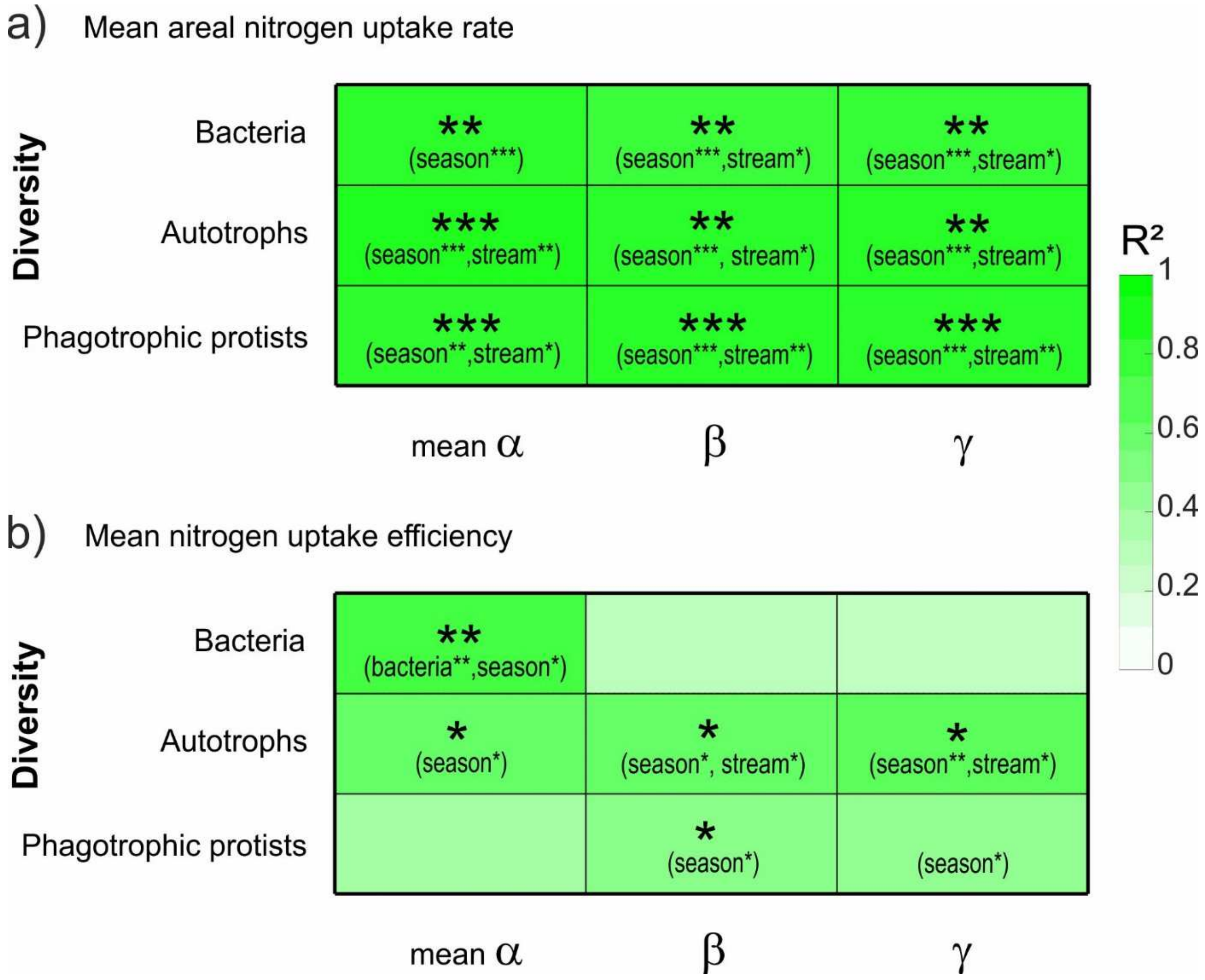


Figure S5. Heatplot visualizing the proportion of variance of mean areal nitrogen uptake rate and mean nitrogen uptake efficiency explained by the mean α, β and γ flow diversity (abbreviated as flow), season and stream (color denotes the coefficient of determination R² adjusted for the number of coefficients). Bold stars in large letters show the significance of the individual models, and the text followed by small stars shows the significance of the explanatory variables within the models ($p < 0.05$ *, $p < 0.01$ **, $p < 0.001$ ***).



a) Mean areal nitrogen uptake rate

b) Mean nitrogen uptake efficiency

Figure S6. Heatplot visualizing the proportion of variance of (a) mean areal nitrogen uptake rate and (b) mean nitrogen uptake efficiency explained by the mean α, β and γ diversity of microbial guilds (TR-Fs of prokaryotic 16S rRNA genes abbreviated as bacteria, autotrophic morphotypes abbreviated as autotrophs and phagotrophic protist morphotypes abbreviated as phagotrophic protists), sampling season, and stream (color denotes the coefficient of determination R^2 adjusted for the number of coefficients). Bold stars in large letters show the significance of the individual models, and the text followed by small stars shows the significance of the explanatory variables within the models ($p < 0.05$ *, $p < 0.01$ **, $p < 0.001$ ***).

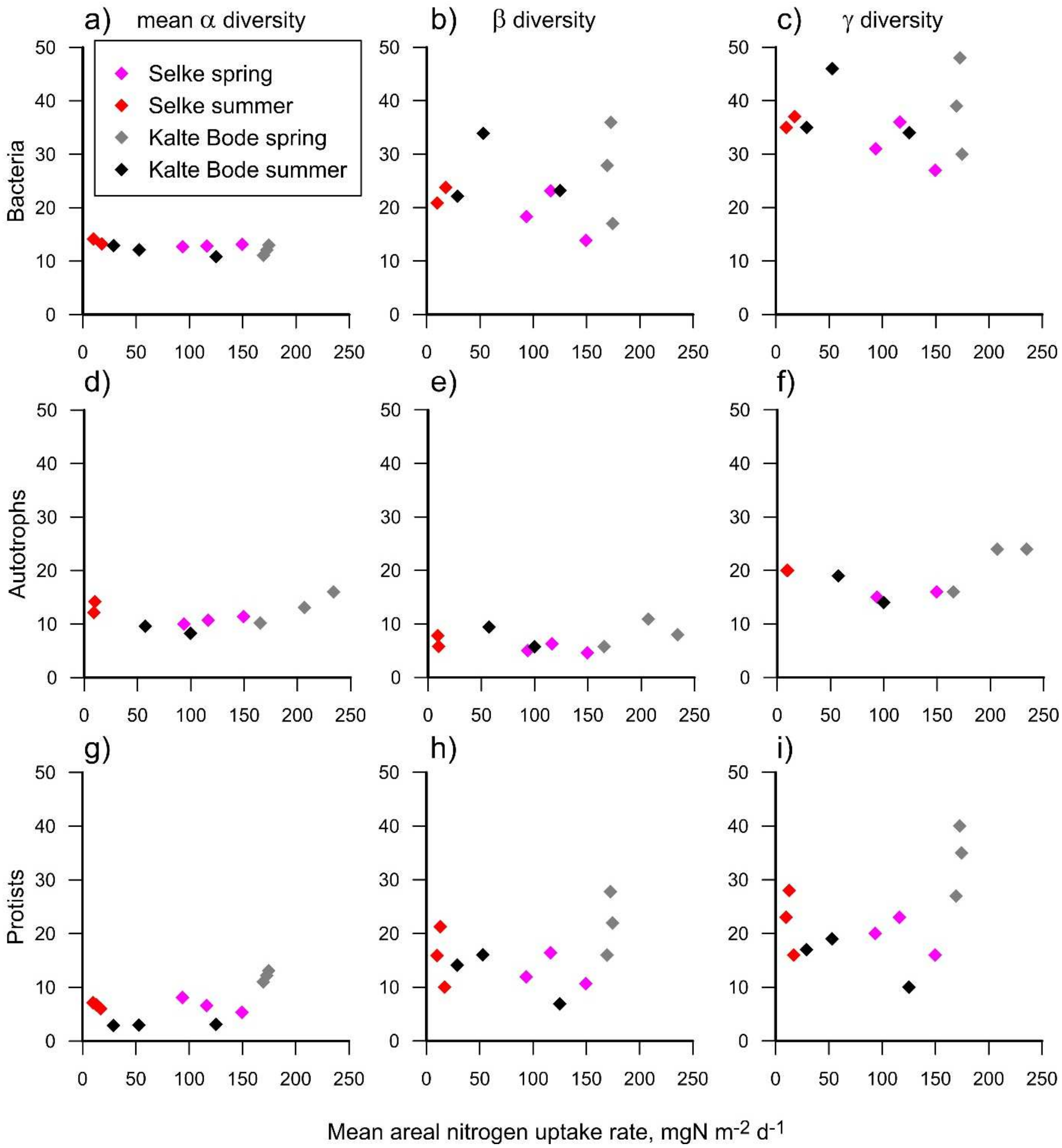


Figure S7. Scatter plots of mean areal nitrogen uptake rates and mean α, β and γ diversities of microbial guilds (TR-Fs of prokaryotic 16S rRNA genes abbreviated as bacteria (a-c), autotrophic morphotypes abbreviated as autotrophs (d-f) and phagotrophic protist morphotypes abbreviated as protists (g-i)) for both sampling seasons (summer and spring) and streams (Selke and Kalte Bode).

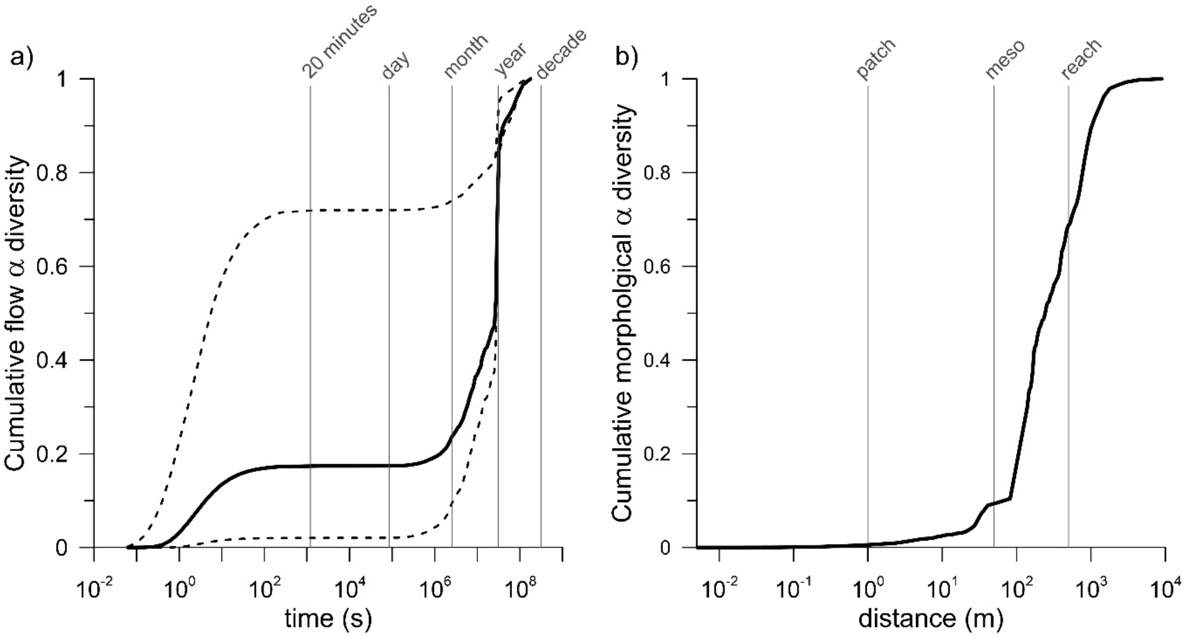


Figure S8. Cumulative integral of the composite power spectrum of (a) the temporal flow variability (i.e., flow α diversity) and (b) the streambed roughness (i.e., morphological α diversity), both normalized with their total variance for the Selke stream. The solid line in (a) shows the cumulative spectrum with the mean spectrum of flow measurements for time scales up to 20 minutes, which is the duration of a single flow measurement in our sampling campaigns and the dashed lines show the cumulative spectra calculated from the 5% and 95% percentiles of the spectra projected to the different sampling locations.

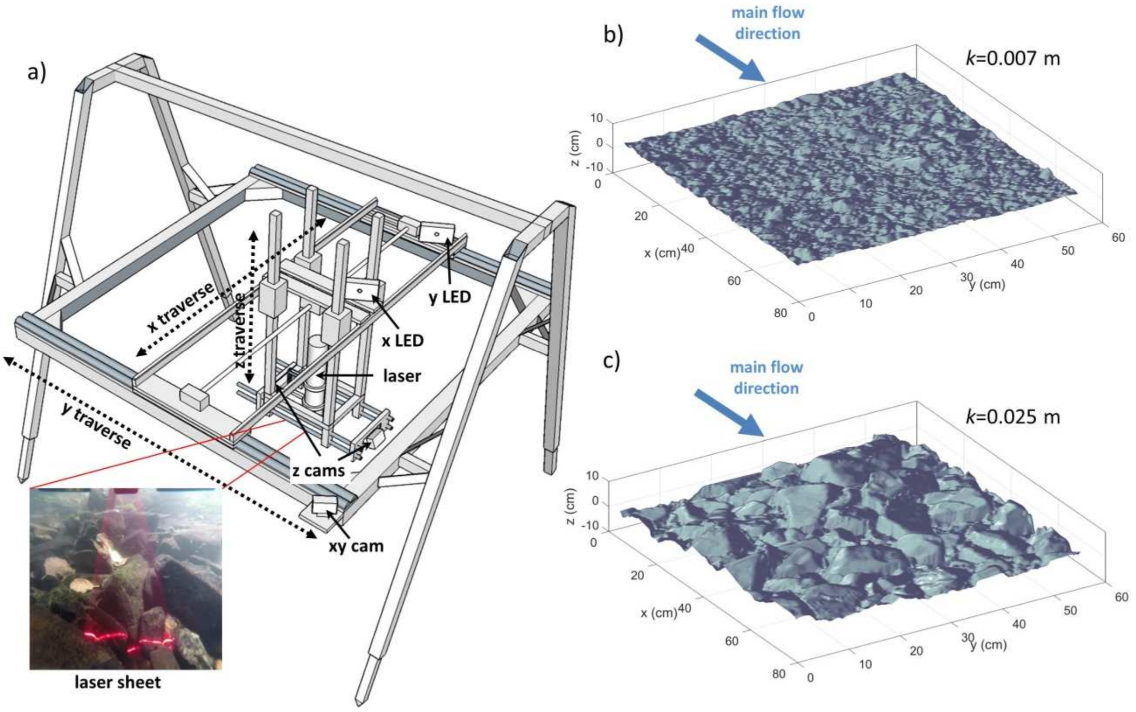


Figure S9. (a) Custom-made instrument frame for high-resolution measurements of streambed topography. Two downward-looking cameras (z cams) were deployed to observe a laser line projected on the stream bottom, while an additional camera (xy cam) was used to locate the horizontal position of the laser within a rectangular frame (approximately 0.8 x 0.6 m). The downward-looking cameras and the laser were mounted on a unit that could be adjusted vertically (z traverse) and moved into x and y directions (x and y traverse). Contour plots of digital elevation models with a horizontal resolution of 0.25 cm measured in a pool (b) and in a riffle (c) section of the Selke, where x, y, z coordinates are aligned with the longitudinal, transversal and vertical flow direction (indicated by the blue arrows), respectively. The estimated streambed roughness k (m) is added for comparison.

APPENDIX II

The role of hydrodynamics in shaping the composition and architecture of epilithic biofilms in fluvial ecosystems

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https://www.sciencedirect.com/science/article/pii/S0043135417308151?via%3Dihub

APPENDIX III

Near streambed flow shapes microbial guilds within and across trophic levels in fluvial biofilms

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APPENDIX IV

Hydraulic and biological controls of biofilm nitrogen uptake in gravel-bed streams

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APPENDIX V

Hydromorphologic Sorting of In-Stream Nitrogen Uptake Across Spatial Scales

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