



Understanding ecohydrology and biodiversity in aquatic nature-based solutions in urban streams and ponds through an integrative multi-tracer approach

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Abstract. Rapid urbanization and climate change affects ecohydrology, biodiversity and water quality in urban freshwaters. Aquatic nature-based solutions (aquaNBS) are being widely implemented to address some of the ecological and hydrological challenges that threaten urban biodiversity and water security. However, there is still a lack of process-based evidence of ecohydrological interactions in urban aquaNBS, and their relationship to water quality and quantity issues at the ecosystem level. Through a novel, integrative multi-tracer approach using stable water isotopes, hydrochemistry and environmental DNA we sought to disentangle the effects of urbanization and hydroclimate on ecohydrological dynamics in urban aquaNBS and understand ecohydrological functioning and future resilience of urban freshwaters. Stable isotopes and microbial data reflected a strong influence of urban water sources (i.e. treated effluent, urban surface runoff) across stream NBS. The results show potential limitations of aquaNBS impacts on water quality and biodiversity in effluent-impacted streams, as microbial signatures are biased towards potentially pathogenic bacteria. Urban ponds appear more sensitive to hydroclimate perturbations, resulting in increased microbial turnover and lower microbial diversity than expected. Furthermore, assessment of macrophytes revealed low diversity and richness of aquatic plants in both urban streams and ponds, challenging the effectiveness of NBS in contributing to aquatic diversity. This also demonstrates the need to adequately consider aquatic organisms in planned restoration projects, particularly those implemented in urban ecosystems in terms of habitat requirements. Our findings emphasize the utility of integrating tracer approaches to explore the interface between ecology and hydrology, and provide insights into the ecohydrologic functioning of aquaNBS and their potential limitations. We illustrate the benefit of coupling ecological and hydrological perspectives to support future NBS design and applications, that consider the interactions between water and the ecosystem more effectively.

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1 INTRODUCTION

Urban freshwater systems face rising anthropic and environmental pressures - from high concentrations of nutrients and other pollutants to severe hydromorphological alterations and declining streamflow, resulting in reduced biotic richness and habitat degradation (Oswald et al., 2023; Richardson and Soloviev, 2021). Still, urban waterbodies have major potential in contributing
40 to climate mitigation and adaptation (Bartrons et al., 2024; Hack and Schröter, 2020; van Rees et al., 2023). Rapid urbanization has been a dominant paradigm and source of unintended consequences for water quality. As a result, changing perception in in how urban waterbodies are valued has led to a surge in stream restoration measures and implementation of nature-based solutions (NBS) as a way to address some of the ecological and societal challenges that threaten biodiversity and human well-being in urban environments (Everard and Moggridge, 2012; Fletcher et al., 2024; van Rees et al., 2023).

45 In urban areas, nature-based solutions are often implemented to mitigate floods and increase water retention, enhance groundwater recharge, alleviate the urban heat island effect and provide green space while also delivering major amenity and health benefits for residents (Dorst et al., 2019; Seddon et al., 2020). Aquatic nature-based solutions (aquaNBS) in particular are applied to increase water security and reduce other water-related risks, making them a key tool for urban climate mitigation and adaptation (Pinho et al., 2023). As the need for urban climate resilience continues to rise, so do concerns about the
50 effectiveness and sustainability of NBS approaches to tackle challenges at a low cost, while delivering benefits for nature and society (Nelson et al., 2020; van Rees et al., 2023; dos Reis Oliveira et al., 2020; Seddon et al., 2020). The lack of conclusive evidence and understanding of the role of NBS in improving climate resilience and local biodiversity, and their interaction with biological and hydrological conditions within water bodies, hampers a sustainable implementation of NBS that seek to maximize the synergies while limiting trade-offs between multiple ecosystem services (Pauleit et al., 2017; Raymond et al.,
55 2017).

Safeguarding stream biodiversity remains crucial to sustaining wider ecosystem functioning and building climate resilience under rapid change (Van der Cruysse et al., 2024). Constructed wetlands and floodplain restoration, stream reconnection, urban gardens and re-vegetation of streambanks, as well as bioswales or retention ponds, can help improve impaired aquatic ecosystems, targeting different ecosystem services and functions (Davis and Naumann, 2017; Dorst et al., 2019; Stefanakis,
60 2019). However, in urban systems, the motivation for implementation of small-scale NBS is often guided by simple ecological principles, such as local restoration of terrestrial and aquatic vegetation, habitat improvement, or specific local water management targets such as water quality and flood control, while also delivering societal benefits (Van der Cruysse et al., 2024; Hack and Schröter, 2020; Hale et al., 2023). Such small-scale measures run the risk of not considering the overarching landscape-scale stressors and complex technological realities that define urbanized systems (i.e. hydrology, water chemistry,
65 land use) (dos Reis Oliveira et al., 2020). In severely disturbed stream ecosystems, hydromorphological changes and/or flow management do not automatically guarantee the return and establishment of diverse ecological communities, especially if basic microbial processes, nutrient-biota relationships and aquatic food webs are not considered (Hilderbrand et al., 2023; Leps et al., 2016).



70 Sustaining the benefits that come with biodiverse and resilient natural system requires healthy stream microbiomes, which include bacteria, archaea and microalgae, as these are essential to fundamental ecosystem processes (Sehnal et al., 2021). Similarly, the adequate development of primary producers, such as large aquatic plants (macrophytes), is crucial as they are an important component of the trophic network. As macrophytes drive competition for phytoplankton, habitat, shelter, and food for other organisms, they significantly contribute to the overall biodiversity of aquatic ecosystems (Chambers and Maberly, 2024). The positive impact of macrophytes similarly affects microbial communities, as links have been found
75 between specific microbiomes and different habitat zones (i.e. leaf, roots, sediments, surrounding water), which are directly or indirectly provided by submerged macrophytes (Zhu et al., 2021), or between microbial communities and emergent macrophytes root systems (Huang et al., 2020). Nevertheless, in urban freshwater systems, the multitude of biotic and abiotic stressors have not only led to the propagation of diverse microbial communities (McLellan et al., 2015; Vignale et al., 2023; Warter et al., 2024), but also created conditions that inherently limit macrophyte development (Gebler and Szoszkiewicz,
80 2022). Given that aquatic microbes and plants are thus intrinsically linked to physicochemical conditions, and in particular eutrophication, they are especially useful indicators of urban aquatic ecosystem health and functioning (Lavoie et al., 2018; Smucker et al., 2022).

Given that evidence of increased biodiversity in NBS is still scarce, using richness and diversity of microbial communities, such as planktonic bacteria, benthic algae and diatoms, in conjunction with macrophytes, as integrative tracers of stream
85 restoration and freshwater diversity, strengthens our understanding of nutrient management and restoration effectiveness. The rapid advancement of novel molecular and DNA-based approaches (i.e. environmental DNA, eDNA) now allow for targeted and efficient monitoring and evaluation of broader ecological patterns (Klaus et al., 2015; Smucker et al., 2022). A growing number of studies used DNA-based tracers to link microbial communities to surface and subsurface flow paths (Klaus et al., 2015; Pfister et al., 2017), nutrient levels (Mansfeldt et al., 2020; Smucker et al., 2022; Zeglin, 2015) or urbanization and
90 climate effects (Numberger et al., 2022; Warter et al., 2024). The number of studies using high throughput sequencing of microbial communities is rapidly increasing, particularly in the urban context where higher taxonomic resolution is important to disentangle anthropogenic influences on stream aquatic diversity and the hydrological cycle (Urycki et al., 2022). Even more, in conjunction with other physical and chemical tracers such as stable water isotopes or major ions, information on water sources and flow paths, as well as nutrient levels and pollution sources can provide a robust tracer-based assessment of
95 ecological, hydrological and chemical conditions (Kuhlemann et al., 2022; Marx et al., 2021; Warter et al., 2024).

In this study, we present empirical evidence, including stable water isotopes, microbial and macrophyte richness and diversity, and hydrochemistry, from established aquaNBS in the city of Berlin, Germany. We aim to disentangle the hydrological and physiochemical links with microbial diversity across restored urban streams and ponds. The aim of this study was to understand how urbanization, hydrology and physiochemistry relate to microbial patterns and aquatic biodiversity in urban restored
100 streams and ponds, and evaluate the implications for future NBS approaches and restoration of urban freshwater systems. More specifically, our objectives were: i) to characterize the main water sources and pathways, and microbial patterns in urban aquaNBS, including species richness, alpha and beta diversity; ii) identify the environmental drivers of microbial patterns and



ii) evaluate the implications of catchment scale stressors on aquaNBS efficiency. Our findings contribute to a broader understanding of the role of microbial processes in urban stream restoration and offer critical evidence to support future design and implementation of urban aquaNBS that equally support biodiversity, ecology and societal goals.

2 DATA AND METHODS

The city of Berlin is located in the dry NE of Germany and receives, on average, 580 mm of annual precipitation (1991-2020 mean) (DWD,2023)Characteristic of this drier region is that the majority is lost through evapotranspiration (~56%) (Limberg, 2007), with the remaining water distributed as urban storm drainage (12%), and groundwater recharge (27%). Berlins water supply is provided through bankside filtration involving extraction of surface water and groundwater from underlying aquifers. The majority of water is generated through riverbank filtration from major rivers like the Spree, which receives water from several local tributaries that enter the Spree from the north. Groundwater heads are higher near the Spree river (< 4 mbgl), and generally deeper (>10 mbgl) on the plateaus. Despite the low recharge and comparatively high evapotranspiration, Berlin has numerous wetlands and a large number of surface water bodies, including small to medium-size lakes and ponds (~700) (SenUVK, Senate Department for Environment, 2023) (Fig. 1c).

The central area of Berlin is highly urbanized (~60%), with 35% impervious surfaces (Fig. 1d). There are also large areas of contiguous urban green space (12.1%) and forest (17.7%). Water quality and quantity of the major and smaller tributary rivers are characterized by land use and urban storm water drainage, combined sewer overflows and water abstractions (Möller and Burschweiger, 2008). Catchment characteristics are shown in Table S1.

2.1 Site Description

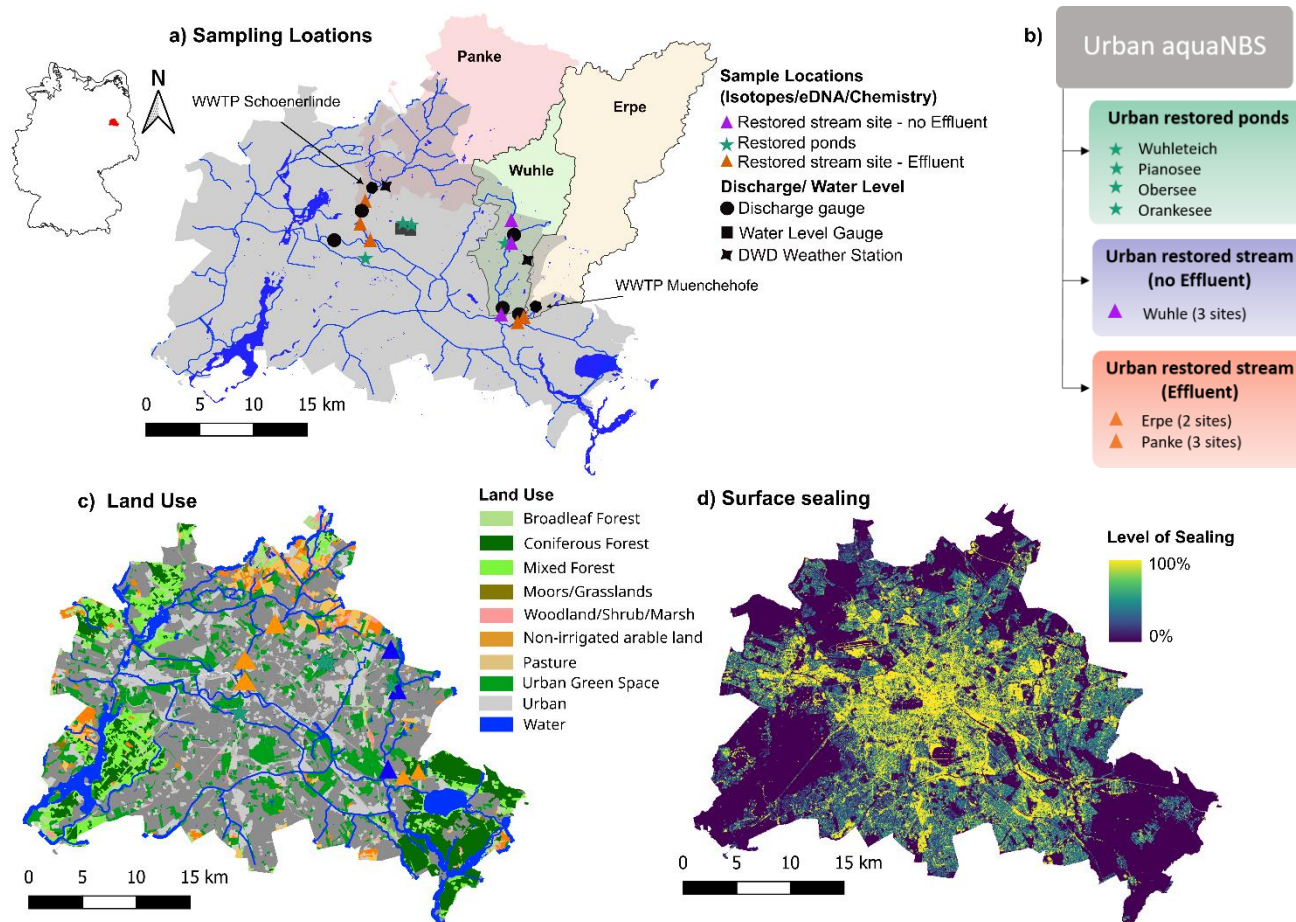
To capture the diversity of urban freshwater systems, a range of stream and pond sites were selected (Fig. 1a). Our goal for stream sites was to choose a representative set of sequential set of sites downstream, experiencing varying flow conditions, urbanization effects and surrounding land use, and where access and logistics allowed for regular sampling. Three local tributaries of the Spree – Erpe, Panke, Wuhle, were selected., Daily discharge for streams and water levels for ponds were obtained from publicly available data (SenUVK, 2024). These streams are characterized by different land uses, discharge and restoration measures. Also, for pond sites regular access was an important criterion for selection, along with similar size, setting (i.e. park) and permanence. The full comparison included 12 study sites: eight in streams and four urban ponds. Sites were further divided into three groups differentiated between streams with a substantial inflow of effluent from nearby wastewater treatment plants (WWTP), and streams which do not receive effluent, in order to separate the effects of water sources on the physical and biological functioning of stream ecosystems (Fig. 1b). These three sample groups are: i) restored streams – with effluent impact, ii) restored streams – without effluent impact and iii) urban restored ponds (Fig. 1b). In the following, we only refer to them as streams or ponds as all of them are restored.



2.2.1 Urban restored streams – with effluent impact

Erpe and Panke are both strongly impacted by treated WWTP effluents discharging from upstream treatment plants (Fig. 1a).
135 Effluent contributions to discharge vary seasonally, ranging between 80% during normal flows and 100% during low flow
periods (Kuhleemann et al., 2021; Marx et al., 2021). Along both streams, successive stream restoration has been completed
within the last 10 years to improve water quality and habitat conditions (SenUVK, Senate Department for Environment, 2009,
2013a). These included the removal of weirs, regular mowing of in-stream and bank vegetation, establishment of still-water
zones and a return to near natural channel structures (SenUVK, 2013b). Along the Erpe (222 km²), two stream sites (Erpe HG
140 and Erpe) in the lower catchment lie within a 1.2 km reach. The river has a peri-urban character, with surrounding land use
including agriculture and extensive wetlands (Fig. 1). Both Erpe sites are surrounded by high-density natural forest and a
comparatively low degree of sealed surface, as they are in a less densely populated part of the catchment. Mean discharge is ~
0.8 m³/s (2001-2024), most comprised of effluent discharge from the WWTP Muenchehofe (mean: 0.5 m³/s).

The Panke catchment (220 km²) is characterized by higher urban density, but also retains large amounts of green
145 space and forest, especially in the upper catchment. The three sites are along a 6 km reach in the lower catchment. The mean
discharge is ~ 0.9 m³/s (2008-2024), again with substantial effluent discharge (mean: 0.8 m³/s) from the WWTP Schoenerlinde.
The most upstream site (Panke SP) features a meandering channel design and fish steps, as well as reestablished riparian zones.
At the second site (Panke OL), located within a highly urban part of the city, stream banks were re-vegetated. and stillwater
zones were implemented. The third site (Südpanke) is a side channel of the main river where an entire stream section (~ 800m)
150 was redesigned as part of an urban green-blue structure to improve channel flow and habitat, runoff retention and provide
green space for residents (SenUVK, Senate Department for Environment, 2009).



155 **Figure 1: a) Map showing study sampling sites. b) Urban freshwater sites were grouped into three groups: urban restored ponds, urban restored streams and urban degraded streams, which are further subdivided based on their dominant water sources. c) Map showing land use and d) surface sealing. Data source for basemaps: Geoportal Berlin (2022).**

2.2.2 Urban restored streams – without effluent impact

The river Wuhle (100 km²) primarily receives urban storm drains and runoff throughout the catchment (SenUVK, Senate Department for Environment, 2013b). Three sites were sampled along a 10km river reach, with the first two (Neue Wuhle and Wuhle) located in the upper catchment within 4km of each other and surrounded by extensive green space. The third (Wuhle OL) is located in the more urbanized lower catchment near the stream outlet. The stream does not receive treated effluent anymore (since 2003) (SenUVK, 2013b). The course of the river and its streambed have been strongly modified through straightening, deepening and the construction of weirs to regulate flows. However, substantial efforts returned the stream to more natural conditions and increase water retention and flood mitigation. Mean discharge is ~0.3 m³/s (2002-2023), with sections of the river regularly drying out during the summer, especially in the upper catchment.

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2.2.3 Urban restored ponds

We included four representative urban ponds; each constructed or restored within the last 25 years. Pianosee (1.2 ha) is an entirely artificial pond within a highly urbanized setting near Potsdamer Platz in central Berlin (~40% sealing, <10% green space). The pond has a maximum depth of 1.8m (mean 0.5m) and is primarily used for rainwater retention, provision of greywater for nearby office buildings, and urban cooling. Excess rain water is stored in underground cisterns, with the majority of the water pumped to the pond. A two-stage mechanic-biologic filtration system cleans incoming rainwater and surface runoff. No water level data was available for this pond, as it is not regularly monitored. Obersee (3.7 ha) is an artificial pond situated in a natural depression. It has a maximum depth of 3m (mean 1.49m) and serves primarily as a retention pond and to filter surface runoff. It is situated in an urban park, albeit in a highly urbanized area and experiences frequent water quality issues, such as phosphorus overload, as well as excessive silt accumulation. The shore is largely lined by concrete walls, along which reed belts were constructed to support natural filtration. Orankesee (4.1 ha) is in close proximity to Obersee, although the lakes are not connected. It is a natural lake fed by groundwater, with a maximum water depth of ~6.5m (mean 2.6m). Half of the pond is closed off for swimming and reed belts and stillwater zones serve as habitat for water fowl. Water quality can be highly variable as the pond also receives urban runoff, but is generally better than in Obersee due to the constant supply of freshwater. Wuhleteich (3 ha) is also an artificial structure located within a nature reserve with a maximum water depth of ~2m (mean 0.75m). The pond is fed by precipitation and water from the nearby Wuhle. The shore is characterized by dense bank vegetation and reed belts, and pond substrate is very silty.

2.3 Stable water isotopes and hydrochemistry

Stable water isotopes, physicochemical parameters (pH, electrical conductivity (EC), dissolved oxygen (DO) and water temperature) and solute tracers (i.e. major ions and anions) were sampled monthly from October 2022 to November 2023. For stable isotopes water was filtered (0.22 μm cellulose acetate) into 1.5 ml vials (LLG Labware). All samples were analyzed using Cavity Ring-Down Spectroscopy with a Picarro L2130i Isotopic Water Analyser (Picarro Inc., Santa Clara, CA). Young water fractions (YWF) were calculated using open access code by Von Freyberg et al. (2018), using an iteratively re-weighted least squares (IRLS) fitted sine-wave approach. For major ions and anions grab water samples were membrane filtered (0.45 μm cellulose acetate) in the lab before further analysis. Further details regarding sample preparation and analysis can be found in the Supplementary Materials.

2.4 Microbial eDNA sequencing and analysis

Environmental DNA was collected seasonally in October 2022, January 2023, May 2024, and July 2024. Water samples (150 – 400 ml) were field-filtered. Filters were preserved in 96% ethanol at -20°C. DNA extraction and community analysis of bacteria and diatoms/algae was carried out on an automated workstation biomek i7 hybrid (Beckman Coulter GmbH, Krefeld, Germany) at the Berlin Center for Genomics in Biodiversity Research as described by Warter et al (2024). Sequences were



analysed using cutadapt v 4.4 (Martin, 2013) and dada2 v 1.21.1 (Callahan et al., 2016) for R (v 4.2.0) (R Core Team, 2021). The resulting amplicon sequence variants (ASVs) were taxonomically identified using the PR2 v. 5.0.0 database (Vaulot et al. 2023) or RDP trainset 18 database (Callahan 2020). Bacteria were identified to genus level and diatoms/algae to species level. 200 Unclassified ASVs (“NA” for species or genus level) and rare ASVs (< 100 total reads or in fewer than 10% of the samples) were excluded from analysis to reduce influence of sequencing artefacts.

2.5 Macrophyte sampling

An aquatic vegetation survey was carried out at each site in September 2023. Each site included two macrophyte survey sites: i) a more natural and vegetated site, characterized by well-developed, undisturbed vegetation along the bank, and 205 ii) a more artificial and less vegetated site, indicating more anthropogenic disturbances. Each site spanned a 10 by 10-metre square and was divided in half into a terrestrial and an aquatic zone, where a qualitative and quantitative inventory of all macrophytes was carried out. See Szoszkiewicz et al. (2024, in review) for a detailed sampling design. The survey included all groups of vascular aquatic plants, including emergent, submerged, free-floating and floating rooted macrophytes identified at the species level. The abundance of each taxon was estimated using a 9-point level cover scale (e.g. Szoszkiewicz et al., 210 2020). Based on field data, species richness (N), relative abundance, Shannon diversity were calculated for each site.

2.6 Statistical Analysis

Spatial and seasonal differences in physiochemical parameters and hydrochemistry were identified using Kruskal-Wallis testing ($\alpha = 0.05$). Assessments of microbial community patterns were performed on relative read-abundance data. Non-metric multidimensional scaling (NMDS) was used to evaluate spatial patterns in microbial diversity using Bray-Curtis distance 215 matrices. Community structures were assessed through pair-wise comparison analysis of similarities (ANOSIM). Genus and species richness (i.e. number of species present) was calculated for bacteria and diatoms/algae respectively. Species diversity was evaluated through Shannon diversity (Shannon H Index), which takes into account the relative abundance of different species rather than just species richness. Differences in alpha diversity for microbial and macrophyte communities were evaluated through nonparametric Kruskal Wallis tests (p-value of <0.05) and mixed two-way analysis of variance (ANOVA). 220 Taxonomic β -diversity was assessed using binary data and Sorensen dissimilarity for all pairwise combinations of assemblages between sample subgroups (n=3). Multivariate dispersion using Jaccard dissimilarity was calculated and tested for homogeneity based on the mean distance of samples to their corresponding group centroid (“distance to centroid”) to summarize variation in community structure among groups (Anderson et al., 2006). Statistical differences in mean centroid distances between sample groups were tested using PERMANOVA through the Adonis function with 999 permutations. 225 Distance-based Redundancy Analysis (dbRDA) was used to explore the relationship between environmental parameters.

3 RESULTS

3.1 Stable water isotopes

Stable water isotopes illustrated the spatial differences in water sources and flow paths between ponds and streams (Fig. 2a). The evaporation line was well below the GMWL and the LMWL. Isotopic signatures from ponds confirmed strong evaporative, which is also reflected in the more depleted Ic-excess values, particularly in summer and autumn. In comparison, effluent impacted streams showed limited variability and more depleted values, with moderately negative Ic-excess as a result of concurrent mixing of multiple water sources, including local groundwater, surface runoff and effluent discharge. Streams with no effluent discharge showed more variability with seasonally more depleted signatures and overall more enriched Ic-excess . Estimates of young water fractions (YWF; % of water younger than 2-3 months) in ponds were high, ranging between 50 to > 90% (Table 1). YWF in the effluent-impacted Panke and Erpe were characteristically lower (~10%), due to the dampening influence of wastewater (see also Figure S1). However, there was some variability in YWF estimates from samples of the Panke river, with estimates of up to 50% in the most downstream restored section, which is highly influenced by weir management and urban surface runoff. YWF from the non-effluent impacted Wuhle ranged from 40% in the lower catchment to > 90% in the upper catchment. This is in line with the variable influence and response of streamflow to seasonal precipitation and surface runoff and groundwater in the upper and lower catchment, respectively.

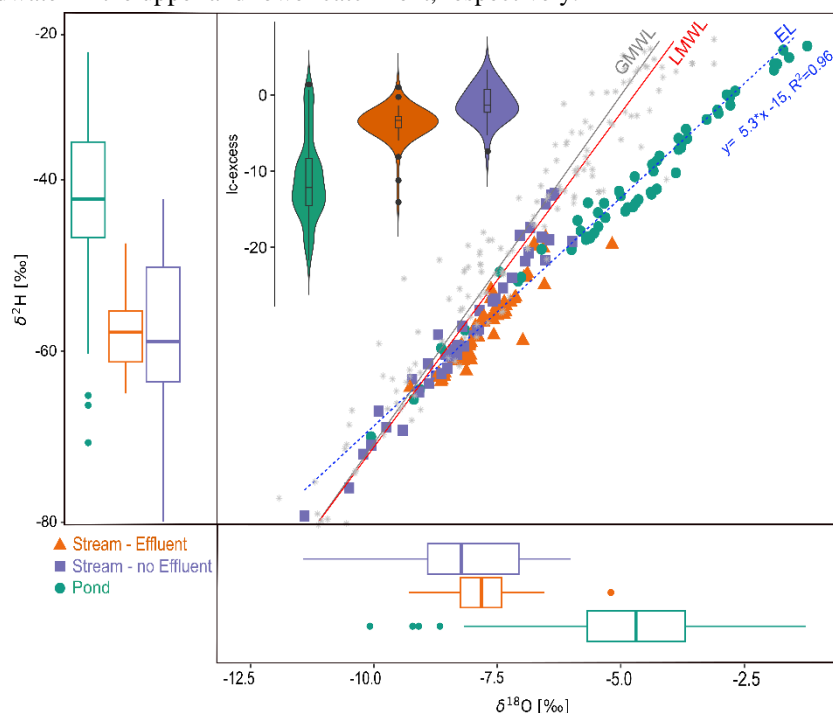


Figure 2: Dual isotope plot (center) and box plots (left, bottom) showing the isotopic composition of the different surface water types as well as Ic-excess (inset). In boxplots, the horizontal line indicates the mean, whiskers indicate the range of values from minimum to maximum. Outliers are indicated as dots. Global meteoric water line (GMWL, grey), amount-weighted local meteoric water line (LMLW, red) from precipitation samples in Berlin-Steglitz and evaporation line (EL, black) are given for reference.



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Table 1: Estimations of young water fractions for stream and pond sites from sine-wave fitting, including coefficient of determination (R^2_{adj}) and residual standard errors (RSE).

	$\delta^{18}\text{O}$ YWF	p-value	R^2_{adj}	RSE	
	Pond				
250	<i>Pianoteich</i>	0.96	<0.001	0.89	0.33
	<i>Orankesee</i>	0.53	<0.001	0.93	0.14
	<i>Obersee</i>	0.77	<0.001	0.76	0.44
	<i>Wuhleteich</i>	~1.0	0.002	0.59	0.88
255	Streams - Effluent				
	<i>Südpanke</i>	0.54	0.01	0.47	0.49
	<i>Panke SP</i>	0.32	0.057	0.29	0.42
	<i>Panke OL</i>	0.17	0.31	0.04	0.36
260	<i>Erpe</i>	0.11	0.13	0.13	0.17
	<i>Erpe HG</i>	0.04	0.83	0.14	0.22
	Streams – no Effluent				
265	<i>Neue Wuhle</i>	0.96	0.03	0.38	0.96
	<i>Wuhle</i>	0.95	0.02	0.37	1.15
	<i>Wuhle OL</i>	0.38	0.03	0.41	0.41

3.2 Hydrochemistry

270 Across all stream and pond sites, pH was slightly alkaline with means around 7.9 (sd = 0.6; Fig S1, Table S2). EC varied distinctly between sample groups ($p < 0.001$), with higher EC in effluent impacted streams. Between ponds, EC also varied significantly, with higher EC observed in Obersee. (Fig S2, Table S2). Highest DIC concentrations were observed in the lower catchment sites of the non-effluent impacted Wuhle (up to 85 mg/l), but on average were highest in effluent impacted Erpe and Panke and lowest in ponds. Dissolved oxygen (DO) in surface water varied significantly between subgroups ($p = 0.02$), reflecting seasonal differences in local biological productivity. DO levels were highest in ponds and lowest in non-effluent impacted Wuhle in autumn and summer. Water temperatures ranged between 19- 23°C in summer and 1.5 – 6.8°C in winter.

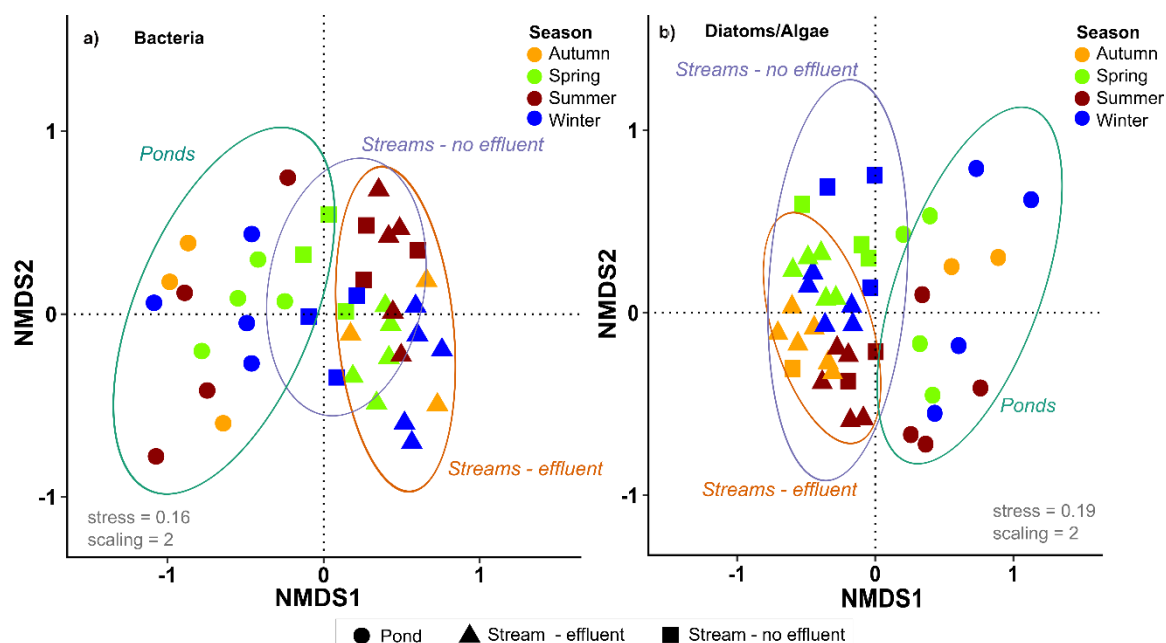
280 Clear differences in major ion sources between stream and pond sites reflected the tributary nutrient loads from effluent discharge into streams and the influence of dominant water sources (e.g. GW, surface runoff, precipitation) in ponds and non-effluent impacted streams also seen in the isotopic signatures (see also Fig. S3). Most notably in the effluent impacted Erpe and Panke, levels of nitrate nitrogen (NO_3N), SO_4 , Ca, K, Mg, P, S, Si were much higher compared to non-effluent impacted streams and ponds (see Table S3). Concentrations of Na and Cl were on average highest in effluent impacted stream, but also in Obersee. Elevated concentrations of Ca were observed in effluent impacted streams as well as in Obersee (see Fig. S3), which is likely a result of the varying GW contributions from local aquifers, which tend to be Ca-rich in Berlin. Of all ponds, Obersee showed also higher levels of S and SO_4 . Other trace metals such as Cu, Fe, Mn and Zn levels were negligible



285 in all samples (< 0.001 mg/l). ANC was highest in effluent impacted streams due to overall increased nutrient loads. ANC showed large variability in the non-effluent impacted Wuhle and ponds, likely as a result of varying source water contributions (i.e. urban runoff, GW), also evident in the more variable stable isotope signatures.

3.3 Microbial community characterization

290 Of the 14034 bacterial ASVs, 6693 could be assigned to a genus, and 5465 of these were present in at least one sample. The final data set of bacteria consisted of 42 samples, with a total of 212 genera and a mean of 83 genera in each sample (range 31-161). For diatoms/algae, 7125 ASVs were obtained, of which 4626 could be assigned to a species and of those 2188 were detected at least in one sample. The final dataset of diatoms/algae consisted of 46 samples, with a mean of 89 genera per sample (max=162, min=5). NMDS ordination of microbial communities shows an acceptable representation (scaling =2, stress < 0.2 for both) (Fig 3). The community structures of bacteria and diatoms/algae were significantly different according to the NMDS (both $p = 0.001$), with communities of ponds being clearly differentiated from streams. Significant differences between stream and pond sites were confirmed by pairwise comparison of similarities (ANOSIM, $r=0.51$, $p = 0.001$) for bacteria and diatoms/algae (ANOSIM, $r= 0.46$, $p=0.001$). Microbial communities in effluent and non-effluent impacted streams appear closely clustered in the ordination plot. However, the pairwise comparison indicated significant differences between effluent and non-effluent impacted streams in both bacteria (ANOSIM: $r=0.53$, $p=0.001$) and diatom/algae communities (ANOSIM: $r=0.35$, $p=0.001$). Seasonal differences in bacterial assemblage were significant in effluent impacted streams ($p = 0.001$), but negligible in other groups. In diatoms/algae, samples of streams visibly converge in all seasons, suggesting similar seasonal community assemblage, although pairwise comparison (ANOSIM, $r= 0.31$, $p=0.001$) indicates some distinction between seasonal samples.





305 **Figure 3: Non-metric multidimensional scaling using Bray-Curtis dissimilarity for a) bacteria (genus level) and b) diatoms/algae (species level) in urban streams and ponds with scaling = 2 and stress of 0.16 and 0.19 for bacteria and diatoms/algae respectively. Seasonal samples are indicated by colors, shapes denote different sample groups. Ellipses indicate the 95% confidence interval for each sample group.**

310 3.4 Alpha diversity

In ponds, about ~50% fewer bacterial genera could be found (mean: 55) compared to stream sites (mean: ~110). Pairwise comparisons of genus richness indicated no difference among streams ($p=0.72$), but between pond and stream groups ($p<0.001$) (Fig. 4a). Most notably, in certain ponds and the non-effluent impacted Wuhle, there was a high relative abundance of Cyanobacteria (up to 35%). In ponds, they were most abundant in spring and lowest in autumn, while in the Wuhle they were most abundant in winter. Shannon diversity was not significantly different between ponds and effluent impacted streams ($p=0.12$), while diversity in the non-effluent impacted Wuhle clearly differed from ponds and the effluent impacted Erpe and Panke (Fig. 4d).

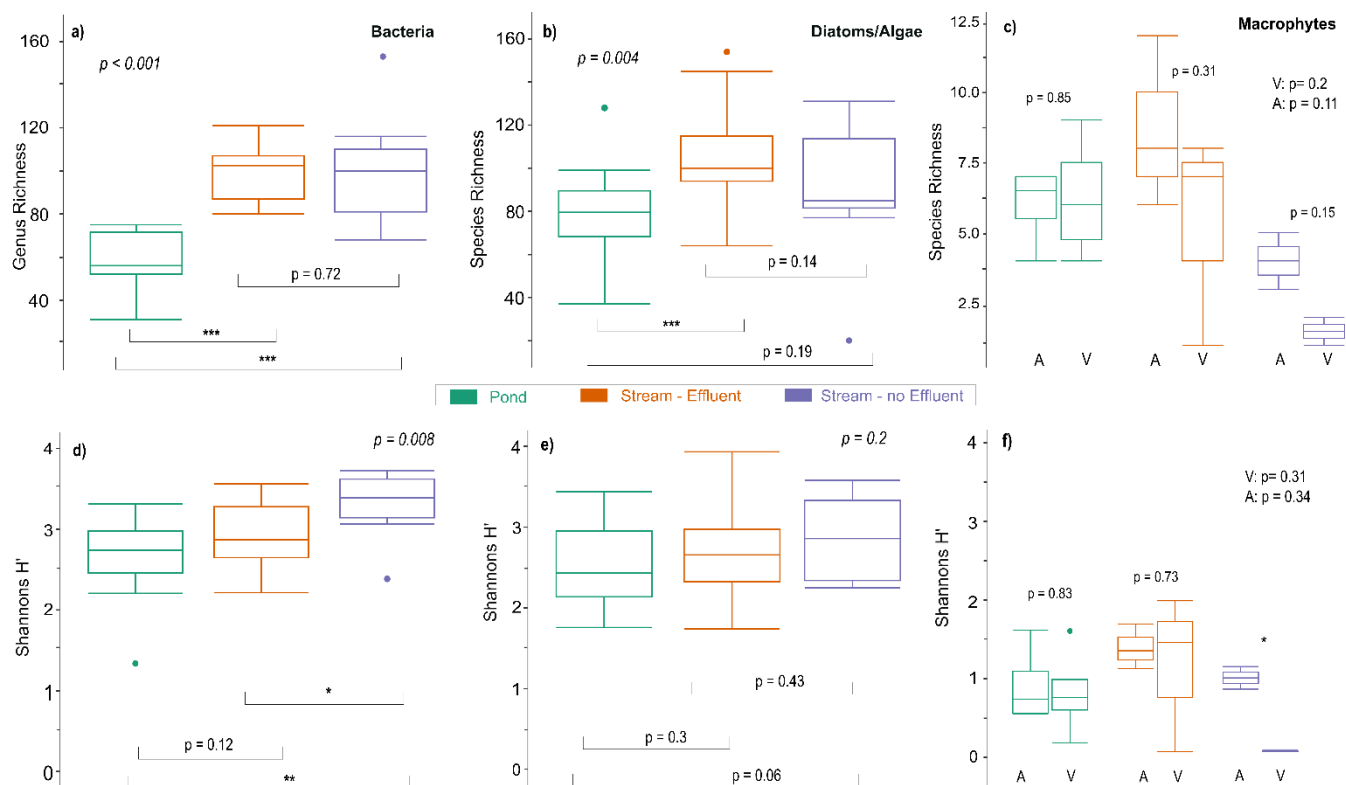
Diatom/algae species richness differed significantly among all sample groups ($p=0.004$) (Fig. 4b), with fewer species in ponds (mean: 75) compared to streams (mean: 103). Post-hoc pairwise comparison revealed significant differences between ponds and the effluent impacted Wuhle ($p<0.001$). Shannon diversity was similar ($p=0.2$) in all sample groups, suggesting no significant divergence in species diversity (Fig. 4e). Although the composition of diatom/algae communities in streams appeared significant according to ANOSIM, the similarities in Shannon diversity suggest limited variation in species composition.

Macrophyte richness was low in all streams (Fig. 4c). Overall species richness did not show significant variability between sample groups across levels of naturalness (all $p>0.1$). Species richness was lowest in the non-effluent impacted Wuhle, with the identified taxa indicative of more eutrophic conditions, such as *Phalaris arundinacea*, *Iris pseudoacorus*, *Persicaria hydropiper* or *Sparganium emersum* (see Table S4 for a complete list of species). These species represent emergent macrophytes (helophyte vegetation), which develop well in shallow zones of stagnant or slow-flowing waters and are also characteristic for nutrient rich water or sediments. The highest species richness was observed in the effluent-impacted Panke (n=12). Also, emergent macrophytes were dominant in the Panke, but beyond them, a greater diversity of submerged vegetation was recorded, with most of the species specific to nutrient-rich waters. Shannon diversity also showed no significant variability between sample groups, except in the non-effluent impacted Wuhle ($p=0.01$) (Fig. 4f). However, this group only consists of 2 sample sites, thus may not be fully representative.

Pond sites exhibited greater stability and comparable levels of macrophyte diversity. The diversity indices obtained from ponds were significantly higher than those from effluent-impacted streams, yet lower than those from the non-effluent impacted Wuhle. Additionally, the variability among individual ponds was comparatively lower. The findings indicated that, regardless of whether the sites were categorized as ponds, effluent, or non-effluent streams, the more modified locations were characterized by increased biodiversity. This phenomenon was less attributable to the anthropogenic alterations of the aquatic



ecosystems and more related to the underdeveloped riparian or terrestrial vegetation linked to the transformation of the bank
 340 zone. Consequently, these modifications resulted in improved light availability (reduced shading), fostering enhanced growth
 and diversity of macrophytes.



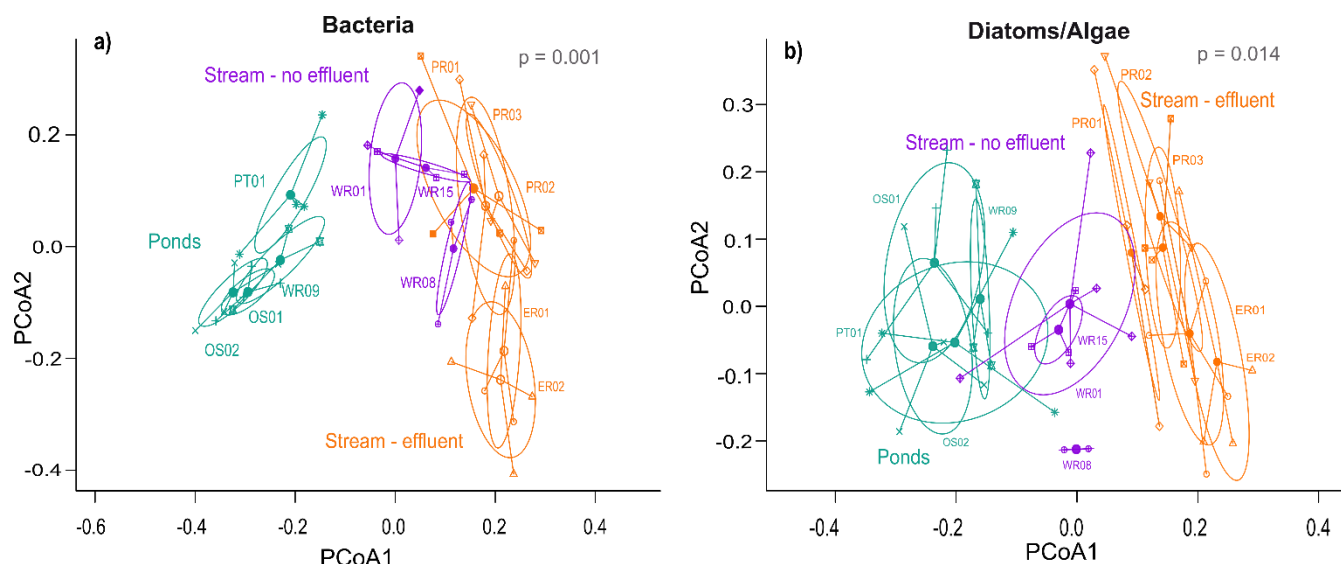
345 **Figure 4: a) Genus richness for bacteria, b) species richness for diatoms/algae and c) species richness for macrophytes. d-f) Shannon diversity for bacteria, diatoms/algae and macrophytes. Horizontal lines in the box plots represent median values, dots indicate outliers. Coloring corresponds to sample subgroups. P-values denote significant differences in alpha diversity measured across all sample groups or between two individual groups. Significance is indicated with asterisks at the 0.05 (*), 0.01 (*), 0.001 (***) level. Macrophyte sampling sites are differentiated between vegetated (V) and artificial (A) sites.**

3.5 Beta diversity

Beta diversity dispersion, considering Bray Curtis dissimilarity, varied between all sample groups for bacteria ($F=2.91$, $p =$
 350 0.001) and diatoms/algae ($F= 3.02$, $p= 0.01$) (Fig. 5). Overall, bacterial community dissimilarity was highest in ponds (0.41),
 compared to effluent and non-effluent impacted streams (0.30 and 0.28 respectively). Similarly, β -diversity of diatoms/algae
 was highest in ponds (0.51) compared to streams (0.45 in effluent impacted, 0.47 in non-effluent impacted) (see also Fig. S5).
 Considering all possible pairs of comparison between stream and pond sites, microbial dissimilarity was significant only
 between ponds and streams, but not between the two stream groups (bacteria: $p = 0.96$; diatoms/algae: 0.19), which contrasts
 355 the dissimilarity observed in the NMDS (Fig. 3). Partitioning total beta diversity into the relevant components, bacterial



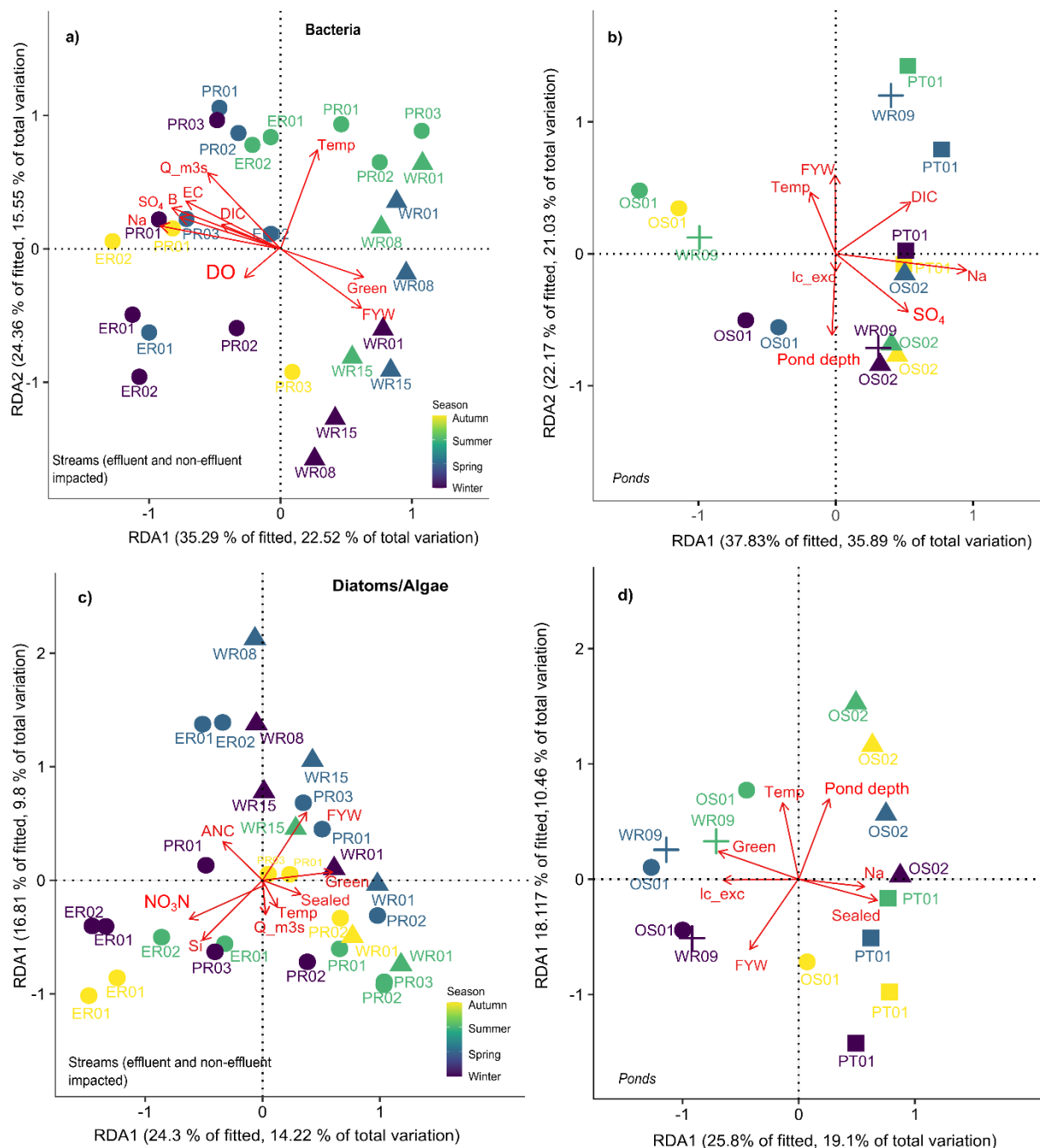
seasonal turnover (β_{SIM}) was highest for ponds (0.37) and lowest in the non-effluent Panke and Erpe (0.16), (see also Table S5). Similarly, diatom seasonal turnover was also highest in restored ponds (0.49) and lowest in the non-effluent impacted Wuhle (0.29).



360 **Figure 5: Multivariate beta diversity dispersion of bacteria and diatom/algae. Beta diversity is measured as the distance of samples to their group centroid (using Jaccard distances), shown here as the first two axes of a PCoA. On the PCoA, a change in site dispersion around the centroid indicates a change in beta diversity, while a change in the centroid location indicates species turnover. Symbols represent each stream or pond site, colors indicate the overall grouping. Significance levels are indicated at the 0.001 (***) or 0.05 (*) level.**

365 **3.6 Influence of environmental variables on microbial and macrophyte communities**

Distance-based RDA analysis revealed that almost 50% ($R^2 = 0.69$, $R^2_{adj} = 0.46$) of the variation of bacteria in streams and ponds ($R^2 = 0.83$, $R^2_{adj} = 0.53$) could be explained by physicochemical and hydrological parameters (see Table S6 for summary of RDA model results). The environmental variables most significant for the bacteria-environment relationship in streams were discharge ($p=0.001$), water temperature ($p=0.001$), DIC ($p=0.007$), DO ($p=0.004$), green space ($p=0.003$), FYW ($p=0.005$), Na ($p=0.04$), B ($p=0.04$), and EC ($p=0.01$) (Fig. 6a). Opposite relationships of bacterial community assemblage with nutrients and discharge variability separated bacterial communities between effluent and non-effluent impacted streams while FYW was positively correlated in non-effluent impacted Wuhle, where FYW were generally higher than in effluent-impacted Panke and Erpe (see also Table 1). Statistically, the first two RDA axis were significant (dbrDA1: $p=0.001$; dbrDA2: $p=0.007$), cumulatively explaining 38% of the total variation, with most of the variation occurring along the first axis (22.52%). Along axis 2, water temperature was a key driver of seasonal changes of bacterial communities, broadly separating summer and spring from winter and autumn samples, especially in the effluent impacted streams.



380 **Figure 6: Distance-based redundancy analysis (dbRDA) plot of DBLM (distance based linear model) of key environmental parameters fitted to the variation in bacteria and diatoms/algae for a) and c) streams (effluent and non-effluent impacted) and b) and d) ponds. Arrows represent direction of parameter effect, colors indicate sampling seasons, symbols denote subgroups for streams and pond sites.**

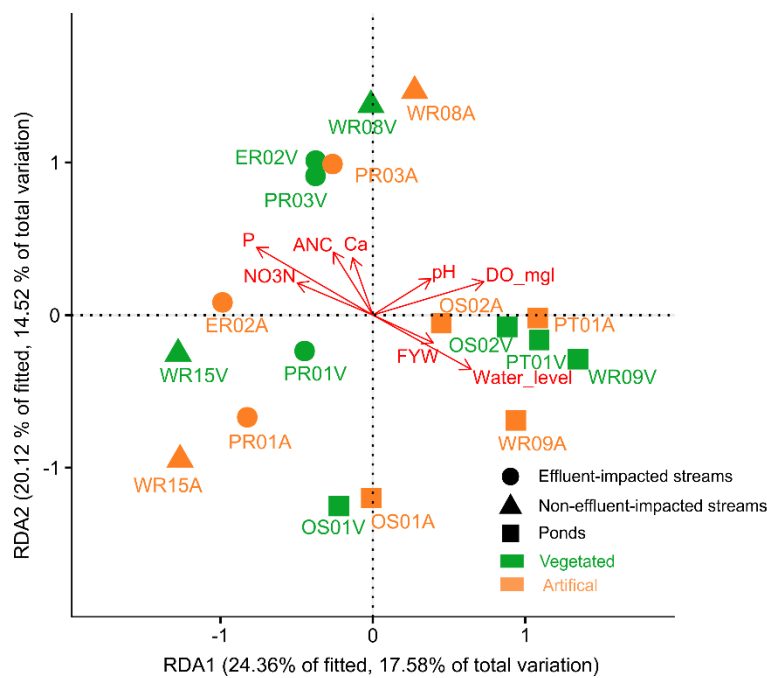


In ponds, pond depth ($p=0.013$), water temperature ($p=0.019$), DIC ($p=0.001$), FYW ($p=0.003$), Na ($p=0.01$), SO_4 ($p=0.03$) and lc-excess ($p=0.01$) were most significant (Fig. 6b). The first two axes were significant (dbRDA1: $p=0.003$; dbRDA2: 385 0.03), with ~ 36% of the variation occurring along the first axis (Fig. 6b). Na, DIC and SO_4 were positively correlated to bacterial assemblage in ponds PT01 and OS02, in line with increased surface runoff reaching these ponds. FYW, water temperature, lc-excess and pond depth broadly separated seasonal samples, indicating a seasonal sensitivity to changing hydroclimate conditions, in line with the highly variable water isotope signatures and evaporative fractionation observed in ponds.

390 For diatoms/algae up to 30% of the variation in diatom/algae communities ($R^2 = 0.61$, $R^2_{\text{adj}} = 0.32$) in streams and ~ 20% in ponds ($R^2 = 0.66$, $R^2_{\text{adj}} = 0.2$) could be constrained through surrounding landuse, hydrologic and physicochemical parameters. In streams, FYW ($p=0.001$), green space ($p=0.001$), sealed surfaces ($p=0.009$), discharge ($p=0.004$), NO_3N ($p=0.01$), water temperature ($p=0.003$), ANC ($p=0.002$), and Si ($p=0.03$) (Fig. 6c) were most significant. The parameters that significantly constrained diatom communities were the % sealed surfaces ($p=0.004$), pond depth ($p=0.005$), Na ($p=0.04$), and FYW 395 ($p=0.002$).

Due to only one sample date, pond and stream macrophytes were assessed together and not separated. Multivariate RDA showed, that up to 50% of macrophyte diversity ($R^2 = 0.73$, $R^2_{\text{adj}} = 0.49$) could be explained by concentrations of NO_3N ($p=0.001$), P ($p=0.004$), Ca ($p=0.001$), alkalinity ($p=0.008$), pH ($p=0.002$), DO ($p=0.007$), FYW ($p=0.01$) and water levels (0.001). The results broadly match the patterns observed in microbial communities, with nutrient-rich waters in effluent- 400 impacted streams accommodating specific species not found in the other sites. The generally higher young water contributions, due to greater precipitation influence, and higher DO levels in ponds, compared to streams, appear to contribute to the similar levels of macrophyte diversity observed across ponds, with the exception of OS-01, which had a high abundance of *Iris pseudoacorus L.* (swamp iris) and dense reedbeds (*Typa angustifolia L.*), which were less dominant or absent in the other ponds. Water level fluctuations appeared positively correlated with species diversity in ponds, but negatively in streams.

405



410 **Figure 7: Distance-based redundancy analysis (dbRDA) plot of DBLM (distance based linear model) of key environmental parameters fitted to the variation in relative abundance of macrophytes for streams and ponds. Symbols denote sample groups, colors denote artificialness (green – vegetated, orange – artificial). Arrows represent direction of parameter effect, colors indicate sampling seasons, symbols denote subgroups for streams and pond sites.**

4. DISCUSSION

4.1 Differences in microbial and macrophyte diversity

415 This study provides an integrated evaluation of the chemical and hydrological functioning of contrasting urban aquatic nature-based solutions. The observed differences in microbial diversity supported the influence of effluent on community composition in the Erpe and Panke, throughout the stream continuum (see also Kuhlemann et al., 2021; Marx et al., 2021; Warter et al., 2024). As the human microbiome is reflected in treated effluent, multiple studies have already tied the effects of rapid urbanization and increasing connectivity between urban water bodies and sewer lines, to changes in natural freshwater bacterial communities across urban streams (Lee et al., 2020; McLellan et al., 2015; Numberger et al., 2022; Vignale et al., 2023). In
420 cases where surface waters are subject to various urban water sources such as urban storm drains and effluent discharge, their good ecological status remains constantly compromised through indirect impacts on habitat or direct toxicity from an influx of nutrients and harmful substances (i.e. antibiotics, pharmaceuticals etc.) (Büttner et al., 2022; Lee et al., 2020; Reid et al., 2019). This is most evident in the abundance of highly specific bacterial communities associated with the human digestive system (i.e. *Romboutsia*, *Intestinibacter*, *Clostridium*), and N and P tolerant diatoms (i.e. *Nitzschia amphibia*, *Polytoma uvella*)



425 throughout the whole downstream environment of the effluent-impacted Erpe and Panke. Similarly, the high abundance of
cyanobacteria during summer and autumn in the non-effluent impacted Wuhle, is likely a result of low water quality and more
eutrophic conditions, propagated by high urban runoff after storm events. In both cases, hydrochemistry, eDNA and stable
water isotopes confirm the overarching fingerprint of urban-specific water sources on ecologic and hydrologic functioning,
which indicates a certain mismatch between the initial restoration goals and restoration measures undertaken. Overall, the
430 findings build on a previous study by Warter al. (2024) in Berlin, confirming that the extensive influence of wastewater limited
the ecological potential, favouring the abundance of more nutrient-tolerant species. The higher richness of macrophyte species
in the effluent-dominated Erpe and Panke suggests that in urban environments, where organisms are affected by multiple
pressures, the effluent input may be an important factor favouring the development of aquatic plants that prefer or tolerate high
concentrations of nutrients (Gebler and Szoszkiewicz, 2022; Silva et al., 2020)). This may create additional beneficial
435 contributions to the purification of water from nutrients (Levi et al., 2015; Toerien and Toerien, 1985). Aquatic plants can also
be an important element in shaping microbial communities, which is not only related to specific species of macrophytes (Wang
et al., 2024), but also to their functional traits and diversity (Özbay, 2018; Zhu et al., 2021). As Kumwimba et al. (2023) show,
specific functional groups of microbes may be associated with hydrophyte litter decomposition, which may further promote
the development of microbial communities associated with reducing nutrient loads (indicated also by (Toerien and Toerien,
440 1985). Still, the higher richness in effluent-impacted streams and the fact that bacteria show a more pronounced influence from
effluent discharge than diatoms/algae may warrant further examination, particularly of sediment communities and composition
as they constitute another reservoir of organic and inorganic compounds over time (Haller et al., 2009; Huang et al., 2020;
Wang et al., 2023).

While nutrient levels and hydrologic variability were effective indicators of microbial and macrophyte communities in streams,
445 pinpointing the key factors of microbial variability in ponds was more difficult. The observed low alpha diversity contained
within urban ponds was at first surprising, but is generally in line with previous studies which noted low biodiversity in urban
ponds, including aquatic plants, macroinvertebrates and amphibians, due to the influence of water quality and urban landscape
structure (Hamer and Parris, 2011; Oertli et al., 2023; Oertli and Parris, 2019). As the number of plant and animal species
inhabiting a pond is strongly impacted by the surrounding landuse, a more urban matrix tends to limit the dispersal or exchange
450 of species, often leading to biologic isolation of certain ponds (Oertli and Parris, 2019). Similarly, the exposure to urban water
sources (i.e. storm runoff) and water level fluctuations, can create variable conditions that can promote greater species turnover
(Siddha and Sahu, 2022), which is line with the observed high beta diversity and turnover. The high evaporative enrichment
also suggests a certain sensitivity to hydroclimate, with purely rainfed ponds such as Pianosee, Wuhleteich and Obersee at risk
of experiencing disproportionate water losses during hot and dry periods.

455 As urban ponds are often designed with predominately social or aesthetic ecosystem services in mind, the promotion and
conservation of local native biodiversity through suitable, if less aesthetic habitat often falls behind (Bartrons et al., 2024;
Cuenca-Cambronero et al., 2023; Oertli and Parris, 2019). The resulting biotic homogenization, due to a desire for uniform
environmental conditions and functional designs thus creates habitat niches that will generally benefit only a limited number



of species (Hassall, 2014; Numberger et al., 2022). Beside physicochemical conditions, an observed lack of natural substrates
460 and suitable plant beds, and the presence of steep concrete shorelines likely limited the establishment of more diverse aquatic
species and macrophytes.

Ultimately, for any type of urban aquaNBS or ecosystem restoration to be successfully implemented and contributions to
biodiversity and water quality ensured, consideration of the ecohydrological dimensions (i. water sources and pathways,
interaction with vegetation and surrounding landscape) is essential, in addition to larger catchment scale stressors. As the
465 movement of water, matter and organisms, and interactions with the surrounding landscape is substantially different in
urbanized systems (Oswald et al., 2023), prioritizing the scales on which stream restoration measures should have measurable
impact (local, regional, or watershed) is critical for the viability of the NBS.

4.2 Future resilience of urban aquaNBS

Our study provides a novel inventory of hydrological and ecological functioning within stream and pond aquaNBS, while
470 simultaneously challenging some of the restoration measures that were undertaken, and their actual contribution to aquatic
diversity. The disparity between the perceived effectiveness of restoration and the actual impact on aquatic diversity and habitat
quality still presents a major issue for restoration efforts especially in urban areas, due to a continued lack of post-hoc
assessment of restoration objectives (Hilderbrand et al., 2023; dos Reis Oliveira et al., 2020). Based on our results, we argue
that to select appropriate restoration measures and avoid ecosystem disservices (Lyytimäki and Sipilä, 2009), it is essential to
475 first identify current and most relevant combinations of stressors that affect ecological conditions of streams – i.e. increased
nutrients, urban runoff, surrounding landuse. For this, the integrated application of environmental tracers, such as stable
isotopes and eDNA have proven useful in quantifying the biophysical interactions needed to characterize ecosystem
functioning, which in turn can help to implement appropriate restoration measures.

Secondly, in riverine ecosystems it is crucial not to disregard natural system complexity in favour of simplistic and static
480 minimum flows, especially considering the effects climate change and urban stormwater (Acreman et al., 2014; Arthington et
al., 2006). In the case of the non-effluent impacted Wuhle, an increasing trend towards intermittency in recent years and more
variable flow regimes, coincide with an observed limited macrophyte diversity, lower water quality and high abundance of
cyanobacteria. This suggests that past restoration measures may not be able to counteract the ongoing loss of hydrological
connectivity, making it unlikely to improve ecological conditions in future. As such, projections of longer droughts followed
485 by intense rainfall is likely to reduce the efficacy of NBS that depend on vegetation-surface water interactions to improve
water quality and streamflow regimes (Fletcher et al., 2024). In drought-prone regions, effluent discharge has already become
an increasingly important water source for sustaining such declining stream flow regimes (Luthy et al., 2015), stabilizing
discharge variability and keeping baseflows high even in times of drought (Lawrence et al., 2014; Plumlee et al., 2012).
However, there are noted caveats to consider for aquatic habitats, with potentially long-term effects on ecology, chemistry and
490 flow dynamics, which are still not well established (Büttner et al., 2022). As an alternative, integrating aquaNBS in the form
of restored wetlands or enhanced river channel restoration to engage hyporheic filtration processes or slow-rate soil infiltration



would support the removal of pollutants in effluents before they are discharged into stream ecosystems (Büttner et al., 2022; Cross et al., 2021; Stefanakis, 2019).

495 Due to the pervasive influence of urbanization on water quality in the studied ponds, the contributions towards aquatic diversity ultimately appears to be quite limited. Indeed, a caveat to this conclusion is that we did not consider terrestrial vegetation or higher-order aquatic organisms, such as amphibians, macroinvertebrates or fish, as this was outside the scope of this study. However, diversity patterns of bacteria and diatom communities already provided an initial assessment of ecological conditions, which could be clearly linked to hydrology, water quality and urban influences. This is not to say that urban waterbodies cannot support freshwater biodiversity at all or provide storm water storage, nutrient processing or recreation opportunities (Fletcher et al., 2024; Hassall, 2014). Indeed, there are many examples of threatened and endangered species
500 finding refuge in urban parks, wetlands and ponds. Examples include populations of macroinvertebrates (Hill et al., 2016), damselfly- and dragonflies (Goertzen and Suhling, 2015) and amphibians (Holtmann et al., 2017). However, the proximity to human activities, limited green space, and increasing exposure to urbanization and climate effects, clearly affected microbial diversity in the studied sites. Here we argue that pond design must consider not only physical requirements but also the
505 physiochemical habitat quality and biological functions in order to reconcile ecologic with aesthetic or social ecosystem services (Bartrons et al., 2024).

In future, to improve the success and longevity of restoration projects, clear expectations need to be set for restoration, provision of ecosystem services and contributions to biodiversity (Bartrons et al., 2024; Oertli and Parris, 2019). The focus on trade-offs between societal and environmental issues, such as flood control, recreation or carbon storage, often leaves
510 ecological and biodiversity aspects behind or considered separately, when in fact they are deeply connected and often share the same drivers (Seddon et al., 2020). More importantly, approaches may vary between protecting relatively unimpacted natural streams and restoring already degraded streams. For example, restoration goals in heavily degraded streams may aim to create ecosystem structure and functions that provide certain services and social benefits, rather than returning ecosystems to natural conditions (Fletcher et al., 2024).

515 Despite certain caveats to urban stream restoration and urban aquatic nature-based solutions, well designed approaches that match relevant biophysical and ecological objectives and restoration goals by considering basic microbial processes, physiochemistry and hydrological functioning of aquatic environments as well as relevant spatio-temporal scales, can greatly support climate change mitigation whilst also provide key benefits to people and the natural environment. Due to the important functions they perform, macrophytes can be a vital element in restoring and shaping the appropriate structure of aquatic
520 ecosystems (Suren, 2009). This can also increase the diversity of all organisms associated with its occurrence and the general diversity of freshwaters (Fu et al., 2024; Paice et al., 2016). However, to achieve positive ecological outcomes, including enhanced water quality and rehabilitation of native populations, site-specific management and restoration strategies tailored to biotic and abiotic conditions (i.e. habitat, streamflow permanence) are crucial.



5 CONCLUSIONS

525 Our analysis of urban aquatic nature-based solution and restoration measures in streams and ponds within the city of Berlin demonstrated the influence of anthropogenic and environmental stressors on hydrologic variability, water chemistry, and microbial diversity. Despite the extensive restoration measures undertaken in all streams within the last decade, urbanization remains a multifaceted driver that impacts aquaNBS. Our use of an integrated multi-tracer approach proved to be highly insightful in qualitatively assessing microbial diversity and relationships with environmental parameters, addressing questions related to the effectiveness of restoration measures and aquaNBS in contributing to biodiversity. Our research has shown that 530 i) microbial diversity is heavily influenced by urban water sources and ecological potential may be severely limited in effluent impacted streams; ii) microbial diversity was lowest in urban ponds, with high abundance of cyanobacteria and low water quality; iii) high abundance of cyanobacteria and low macrophyte richness and diversity in non-effluent impacted streams suggests low contributions to local aquatic diversity despite extensive restoration; iv) microbial processes should be considered 535 in future restoration efforts to create balanced ecosystems and improve ecological status of degraded ecosystems; v) the still limited research on restoration effects on biodiversity warrants further research to enhance climate resilience of urban waterbodies.

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545 CRediT author contribution statement:

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Investigation: MMW, DG

Formal Analysis: MMW, DG

550 Data Curation: MMW, DG

Writing-Original Draft: MMW

Writing – Review & Editing: DT, CS, MM, TG, KV, DG

Visualization: MMW

Supervision: DT, CS

555 Funding acquisition: DT, MM, KV



Data Availability:

Data will be made available on request. Metadata can be found on the Freshwater Research and Ecology Database (FRED) at doi: 10.18728/igb-fred-939.0. Climate data was publicly available from the German Weather Service (DWD, 2023). Stream discharge and water levels are publicly available from the Berlin Senate (SenUVK, 2024).

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The authors declare that they have no conflict of interest.



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