<u>Supplementary Material for:</u> Understanding ecohydrological responses of aquatic nature-based solutions in urban streams and ponds through an integrative multi-tracer approach

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- Table S1 showing the characteristics of each sample site within subgroups, incl. watershed size, level of urban sealing, urban green space, mean water levels for ponds and mean daily discharge for streams.
- Table S2 showing a summary of basic physicochemical parameters
- Figure S1 showing young water fractions
- Figure S2 showing timeseries of basic physicochemical parameters for each sample subgroup.
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- Table S3 showing a summary of water chemistry parameters
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- Table S4 showing a list of all macrophyte species found in pond and stream sites
- Table S5 showing a summary of the beta diversity parameters; turnover β_{SIM}, nestedness β_{SNE} and overall variation in species composition β_{SOR}.
- Figure S5 showing distance to centroid for bacteria and diatoms/algae
- Table S6 showing a summary of the results from the distance-based redundancy analysis.

Table S1: *Study site characteristics listed by group, including watershed size, mean water level in cm (WL), mean daily discharge in m^3/s(Q), and the distribution of sealed surfaces (%) and urban green space (%). (Source: Geoportal Berlin/ALKIS, Corine Land Cover CLC5 (2018).)*

	Site	Watershed size (km^2)	Mean pond depth (m)	Sealed surface (%)$^+$	Urban Green (%)*	Forest (%)°	nbS function
RESTORED PONDS (n=4)							
	Obersee (OS-01-P)	37.5	1.49	34	16.3	Na	Surface water retention, constructed reedbelts,
	Wuhleteich (WR-09)	4.6	0.75	11.5	36.6	Na	Wetland habitat
	Orankesee (OS-02-P)	37.5	2.62	34	16.3	Na	Swimming pond, recreational use, constructed reedbelts
	Piano See (PT-01)	19.4	0.5	40.5	7.8	Na	Rainwater retention, greywater use, urban cooling
RESTORED STREAM SITES (n = 8)			**Mean Q (m^3/s)**				
Effluent impacted (n = 5)	Panke SP (PR01)	3.9	0.7 (±0.5)	19.6	12.3	Na	Fish steps, flood control, water retention, stillwater and riparian zones
	Südpanke (PR03)	0.95	NA	58.8	<1%	na	Aesthetic benefits, urban cooling
	Panke OL (PR02)	5.5	0.7 (±0.5)	21.5	20.9	na	Streambank vegetation,
	Erpe (ER02)	23.1	0.9 (±0.6)	5.7	8.2	41.1	Riparian zones, stream regulation, flow and erosion control, shading
	Erpe HG (ER01)	23.1	0.9 (±0.6)	5.7	8.2	41.1	Riparian zone, bank vegetation, habitat
Non-effluent impacted (n = 3)	Wuhle (WR08)	7.4	0.05 (±0.06)	11.5	24.7	5%	Removal of weir, mowing of instream/bank vegetation, fish steps, berms
	Neue Wuhle(WR15)	11.2	0.05 (±0.06)	11.0	19.0	Na	Reactivation of riparian zones, natural stream bed, dead wood zones, mowing
	Wuhle OL(WR01)	5.8	0.32 (±0.3)	7.5	37.4	8.1	Natural stream bed, bank vegetation, mowing

* Green space includes urban green spaces such as parks and areas used for sports and leisure (i.e. football field, golf course).
\+ Considering level of sealing >99%.
° Forest cover includes forest (coniferous, broadleaf, mixed), natural grasslands, woodland and shrubland

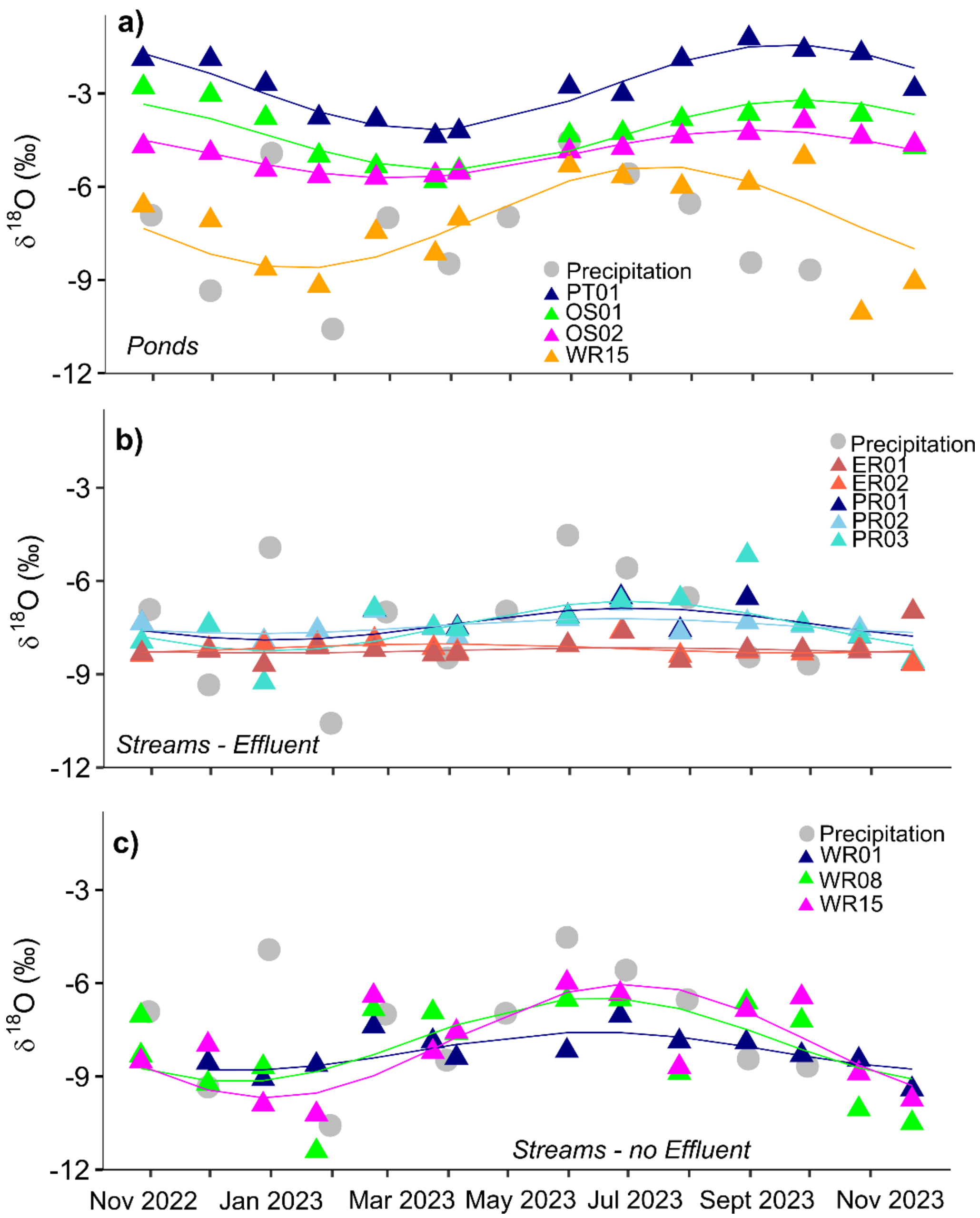

Figure S1: *Isotopic variability of precipitation (grey), stream and pond isotopes for a) ponds, b) effluent impacted streams and c) non-effluent impacted streams. Sinusoidal cycles (in color) were fitted to monthly stream isotope data using IRLS (after von Freyberg et al. 2018) for estimates of young water contributions (< 2-3 months old).*

Table S2: *Summary of basic physicochemical parameters given as mean values and standard deviation (SD) over the sampling period.*

	EC (µS/cm)		**pH (-)**		**Temp (°C)**		**DO (mg/l)**	
	Mean	*SD*	*Mean*	*SD*	*Mean*	*SD*	*Mean*	*SD*
Restored ponds	570	243	8.1	0.6	12.6	7.1	10.3	2.4
Restored stream sites – Effluent	1067	232	8.1	0.5	14.1	4.7	8.5	1.7
Restored stream sites – no Effluent	532	279	7.6	0.4	11.6	5.1	4.9	2.7

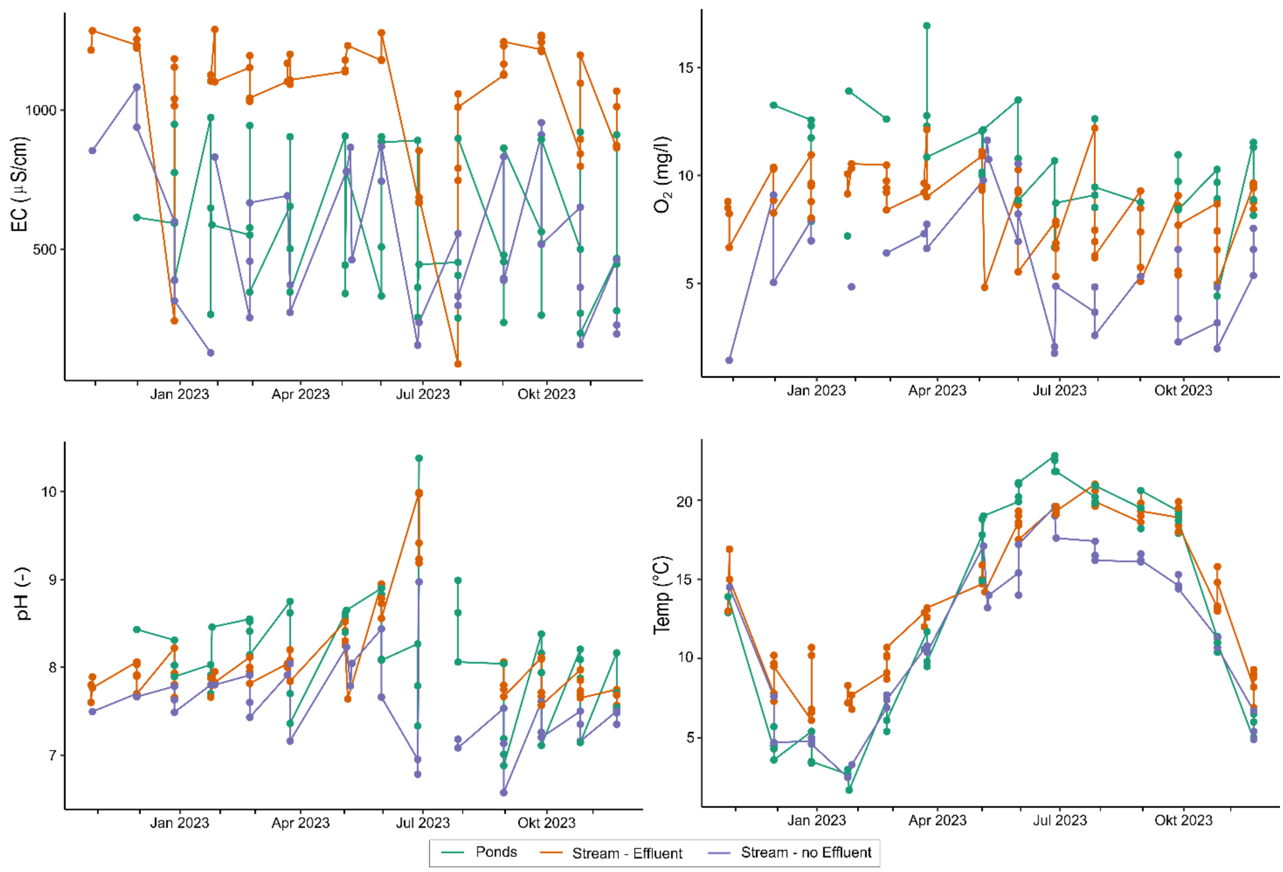

Figure S2: *Timeseries of basic physicochemical parameters of stream and pond sampling sites over the sampling period October 2022 – November 2023. Sampling groups are indicated by color.*

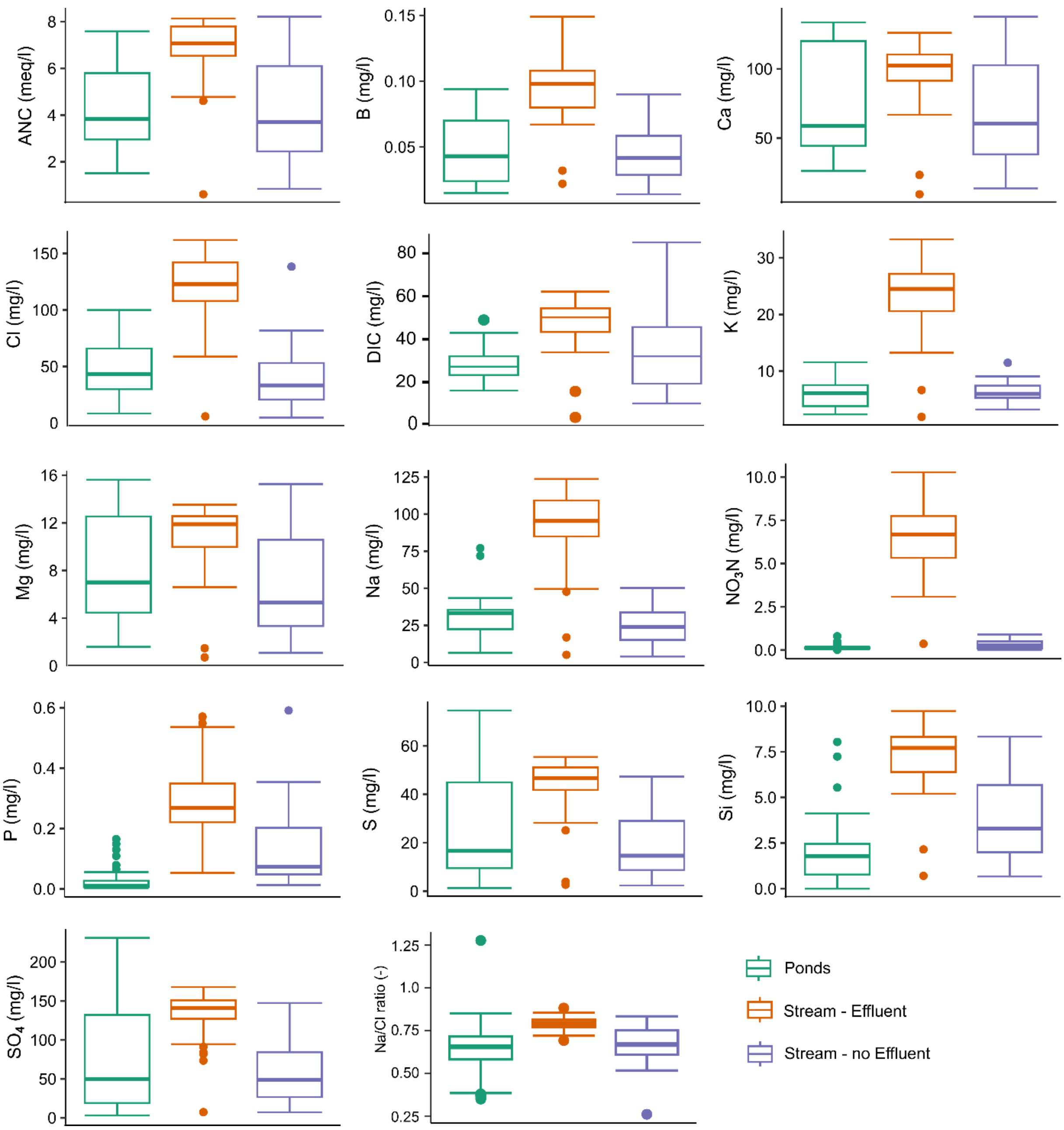

Figure S3: *Boxplots of water chemistry parameters for sample subgroups. Horizontal lines indicate mean values, whiskers indicate minimum and maximum values and outliers are shown as points. Colors correspond to sample subgroups.*

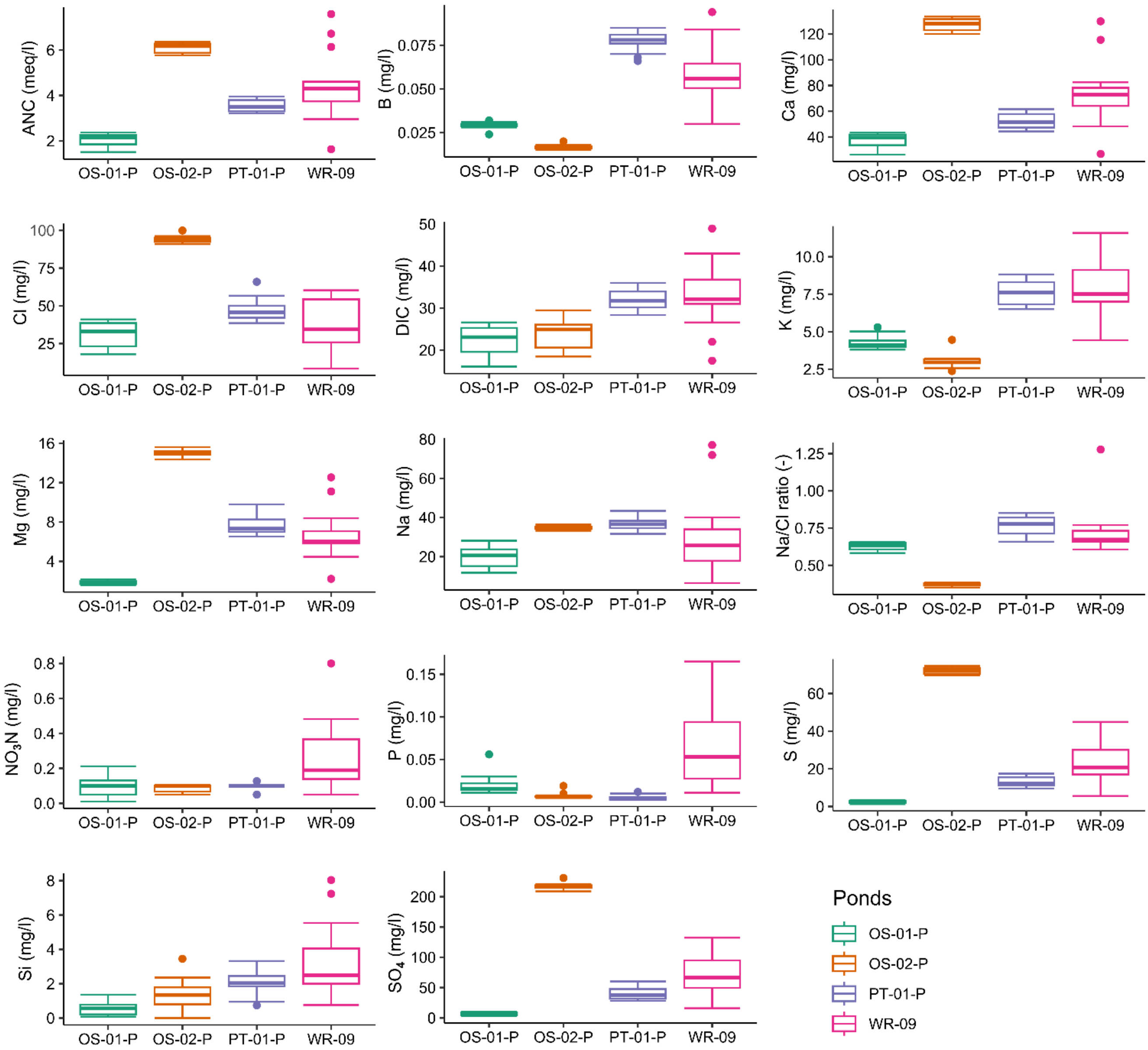

Figure S4: *Bar plots of water chemistry at pond sites. Horizontal lines indicate mean values, whiskers indicate minimum and maximum values and outliers are shown as points. Colors correspond to sample subgroups.*

Table S3: *Summary of hydrochemical parameters in the different subgroups over the sampling period given as arithmetic mean and standard deviation (SD).*

	Restored ponds		Urban restored – Effluent		Urban restored – no Effluent	
Parameter	*Mean*	*SD*	*Mean*	*SD*	*Mean*	*SD*
ANC (meq/l)	4.1	1.7	7.1	0.9	4.1	2.2
DIC	27.8	6.8	49.2	6.2	35.6	19.0
B	0.05	0.03	0.1	0.02	0.04	0.02
Ca	73.5	36.5	104.1	13.8	68.9	38.9
Cl	51.9	26.7	128.4	22.7	37.9	25.6
K	5.8	2.5	26.3	4.4	6.3	1.6
Mg	7.9	4.8	11.4	1.7	6.8	4.5
Na	30.6	12.6	99.4	16.9	23.9	11.8
NO_3N	0.14	0.13	7.2	1.6	0.3	0.2
P	0.02	0.04	0.34	0.12	0.13	0.12
S	28.1	27.1	47.6	7.8	19.5	13.6
Si	1.9	1.6	7.9	1.12	3.9	2.3
SO_4	83.7	81.7	139.4	24.1	60.0	41.2
Cu	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Zn	0.01	0.02	0.01	0.01	0.04	0.1
Fe	0.06	0.07	0.04	<0.01	0.2	0.1

Table S4. *List of macrophyte species found in stream and pond sites.*

Acorus calamus L.	*Lysimachia nummularia L.*
Agrostis stolonifera L.	*Lythrum salicaria L.*
Berula erecta (Huds.) Coville	*Mentha aquatica L.*
Bidens tripartita L.	*Myosotis scorpioides L.*
Calamagrostis sp.	*Myosoton aquaticum (L.) Moench*
Callitriche sp.	*Myriophyllum spicatum L.*
Caltha palustris L.	*Nymphaea alba L.*
Calystegia sepium (L.) R.Br.	*Persicaria amphibia (L.) Delarbre*
Carex acuta Curtis	*Persicaria hydropiper (L.) Delarbre*
Carex acutiformis Ehrh.	*Persicaria maculata (Rafin.) S.F. Gray*
Carex aquatilis Wahlenb.	*Phalaris arundinacea L.*
Carex elata All.	*Phragmites australis (Cav.) Steud.*
Carex elongata L.	*Potamogeton pectinatus L.*
Carex hirta L.	*Potamogeton pusillus L.*
Carex pseudocyperus L.	*Potamogeton rutilus Wolfg.*
Ceratophyllum demersum L.	*Ranunculus repens L.*
Eleocharis palustris (L.) Roem. & Schult.	*Salix sp.*
Elodea canadensis Michx.	*Schoenoplectus lacustris L.*
Epilobium hirsutum L.	*Scirpus sylvaticus L.*
Epilobium palustre L.	*Sparganium emersum Rehmann*
Eupatorium cannabinum L.	*Typha angustifolia L.*
Glyceria maxima (Hartm.) Holmb.	*Typha latifolia L.*
Hedera helix L.	*Urtica dioica L.*
Iris pseudacorus L.	*Veronica beccabunga L.*
Juncus effusus L.	
Lemna minor L.	
Lycopus europaeus L.	
Lysimachia punctata L.	

Table S5: *Beta diversity statistics for each sampling group, including compositional variation (β_{SOR}), turnover (β_{SIM}), and the resulting nestedness (β_{SNE}).*

Sample Subgroup	β_{SIM}		β_{SNE}		β_{SOR}	
	16S	*DIV4*	*16S*	*DIV4*	*16S*	*DIV4*
Ponds	0.37	0.49	0.04	0.06	0.41	0.55
Stream – Effluent	0.25	0.36	0.05	0.02	0.30	0.38
Stream – no Effluent	0.16	0.29	0.13	0.16	0.28	0.45

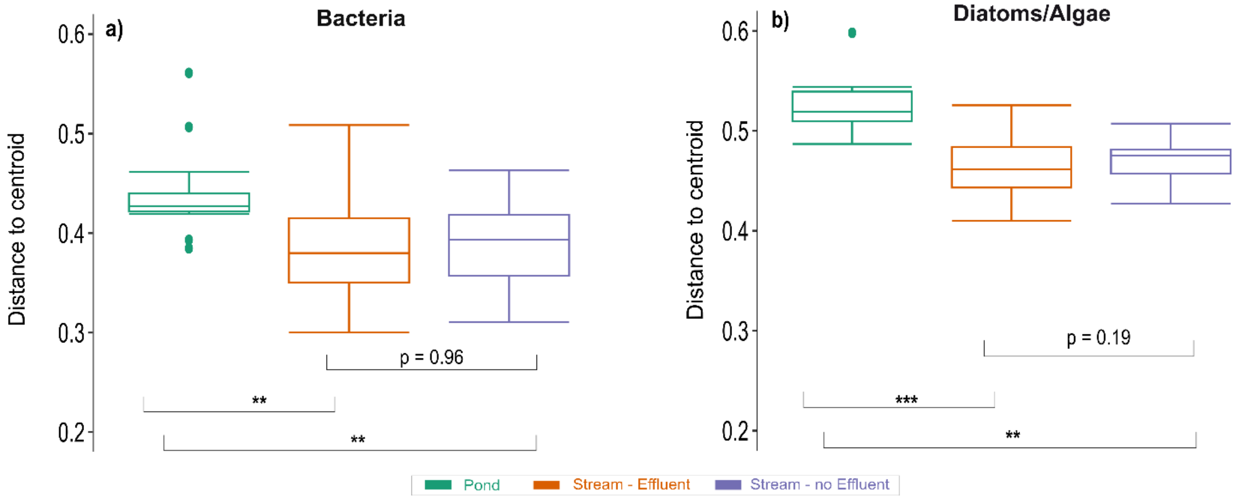

Figure S5: *Distance to centroid for a) bacteria and b) diatoms/algae. Asterisks denote the significance level at 0.05 (*), 0.01 (**) and 0.001 (***).*

Table S6: *Relationships between restored streams and ponds and environmental variables obtained by distance-based redundancy analysis. Asterisks denote the significance level at 0.05 (*), 0.01 (**) and 0.001 (***).*

	Parameters	p-value	F-value	R^2	R^2_{adj}
Bacteria					
Streams	FYW*** Temp** DIC*** Q*** EC* Na* B*** Green** DO**	0.001	2.36	0.69	0.46
Ponds	Water level** Temp* DIC*** FYW** SO_4* Na** lc-excess*	0.004	3.09	0.83	0.53
Diatoms					
Streams	Sealed** Green*** NO_3N* Q** Temp** ANC** FYW*** Si*	0.001	2.1	0.68	0.35
Ponds	Sealed** Water level** FYW** Na* lc-excess**	0.01	1.43	0.66	0.2
Macrophytes					
Streams + Ponds	Water level*** Ca*** pH** FYW* P** DO** ANC** NO_3N***	0.001	2.91	0.73	0.49

Sampling Methods:

Stable Water 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). Standards of Vienna Standard Mean Ocean Water (VSMOW) of International Atomic Energy Agency (IAEA) were used for calibration. The relationship between δ^2H and δ^{18}O of stream water samples, relative to the Global Meteoric Water Line (GMWL) was estimated through deuterium excess (d-excess) and calculated as: d-excess = δ^2H – 8* δ ^{18}O. Variations in d-excess indicate evaporative fractionation prior to sampling (Dansgaard, 2012). The evaporation line was calculated using least-squares regression. The local meteoric water line (LMWL) was derived using weighted daily precipitation isotopes from the Steglitz Urban Ecological Observatory in Berlin (Kuhlemann et al., 2021), for the period February 2019 – September 2023. The LMWL for Berlin was calculated as:

$$\delta^2 H = 7.803 * \delta^{18} O + 7.101 \quad (1)$$

To assess evaporation effects, line-conditioned excess (lc-excess) was calculated as:

$$lcexc = \delta^2 H - 7.8 * \delta^{18} O - 7.1 \qquad (R^2 = 0.98, p < 0.001) \quad (2)$$

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. We used observed precipitation isotopes from Berlin Steglitz and stream and pond water isotopes to estimate fraction of event water that reached the stream or pond within the previous 2-3 months. We compared sine-wave fit amplitudes of monthly isotope samples with amount weighted precipitation δ^{18}O and δ^2H.

Physicochemical parameters and hydrochemistry

The following physicochemical parameters were measured in the field on a monthly basis using a handheld multiprobe (Multi 3630 IDS, WTW, Weilheim, Germany): pH (sensor SenTix940, precision ±0.0004), dissolved oxygen (DO, FDO925, precision ±0.5% DO), water temperature (precision ±0.2°C), and electric conductivity (EC, TetraCon925, precision ±0.5%). Hydrochemical analysis focused on solute tracers, such as major ions and dissolved inorganic carbon (DIC) to determine nutrient levels, water source endmembers and flow paths. Grab water samples were membrane filtered (0.45µm cellulose acetate) in the lab before further analysis.

Dissolved anions (chloride, Cl^-, sulfate, SO_4^{2-}, nitrate NO_3^-) were measured in 0.2µm membrane filtered samples by ion chromatography (Metrohm CompactIC 930, conductivity detection after chemical suppression). Dissolved inorganic carbon (DIC) was determined by thermocatalytic combustion/infrared spectrometry (Shimadzu TOC-L Total Organic Carbon Analyzer). Dissolved metals and other elements (aluminum, Al, boron, B, calcium, Ca, iron, Fe, potassium, K, magnesium, Mg, manganese, Mn, sodium, Na, phosphorus, P, sulfur, S, silicon, Si, zinc, Zn) were determined in acidified samples (0.25M HCl) by inductively coupled plasma-optical emission spectroscopy (ICP-OES, Thermo Scientific iCAP 7600). Analytical precision for dissolved anions and metals was <3%. We also calculated acid neutralizing capacity (ANC), using a mass balance approach of major anions and cations, which were converted to meq/l, as follows:

$$ANC = (Na + K + Ca + Mg) - (Cl + SO_4 + NO_3N) \quad (3)$$

Statistical Analyses

All statistical analyses were done in R (v.4.3.2) (R Core Team, 2021). To further evaluate site-level differences in species dissimilarity, beta diversity was assessed using the betapart package (Baselga and Orme, 2012) in R. We used Hellinger transformed abundance data, and evaluated the explanatory power of temporal and spatial variation in microbial communities in response to environmental variables. Environmental data was normalized by z-score transformation and collinearity between variables was assessed by calculating variance inflation factors (VIF). Through forward selection, the highly correlated variables were dismissed until VIF scores of remaining variables were <10. Through Monte Carlo permutation test based on 1000 randomizations, most significant environmental effects on microbial assemblage were evaluated and explanatory power of RDA model using selected environmental variables was evaluated through ANOVA (α=0.05) and coefficient of determination (R^2, R^2_{adj}).