Supplementary Information

Agriculture versus wastewater pollution as drivers of macroinvertebrate community structure in streams

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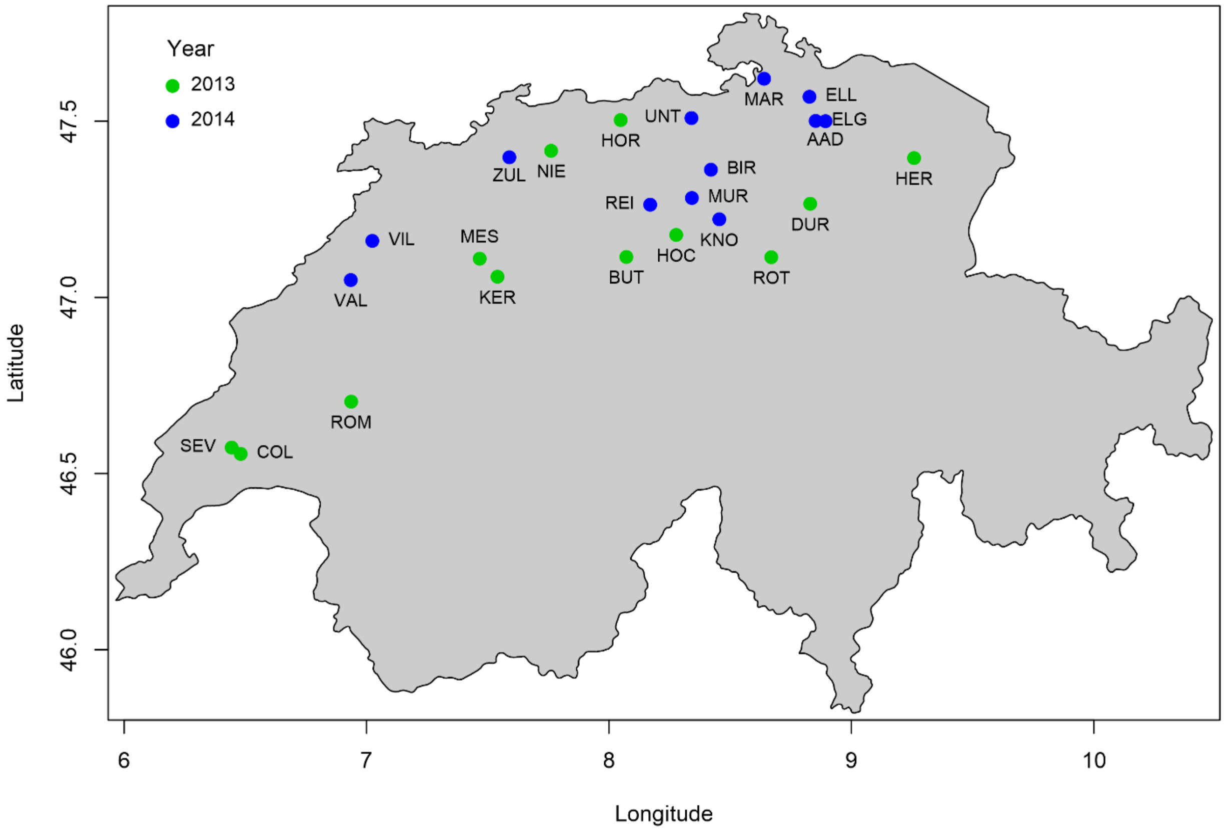


Fig. S1 Map of Switzerland showing the location of the study sites. AAD: Aadorf, BIR: Birmensdorf, BUT: Buttisholz, COL: Colombier, DUR: Dürnten, ELG: Elgg, ELL: Ellikon, HER: Herisau, HOC: Hochdorf, HOR: Hornussen, KER: Kernenried, KNO: Knonau, MAR: Marthalen, MES: Messen, MUR: Muri, NIE: Niederdorf, REI: Reinach, ROM: Romont, SEV: Sévéry, UNT: Unterehrendingen, VAL: Val.de-Ruz, VIL: Villeret, ZUL: Zullwil.

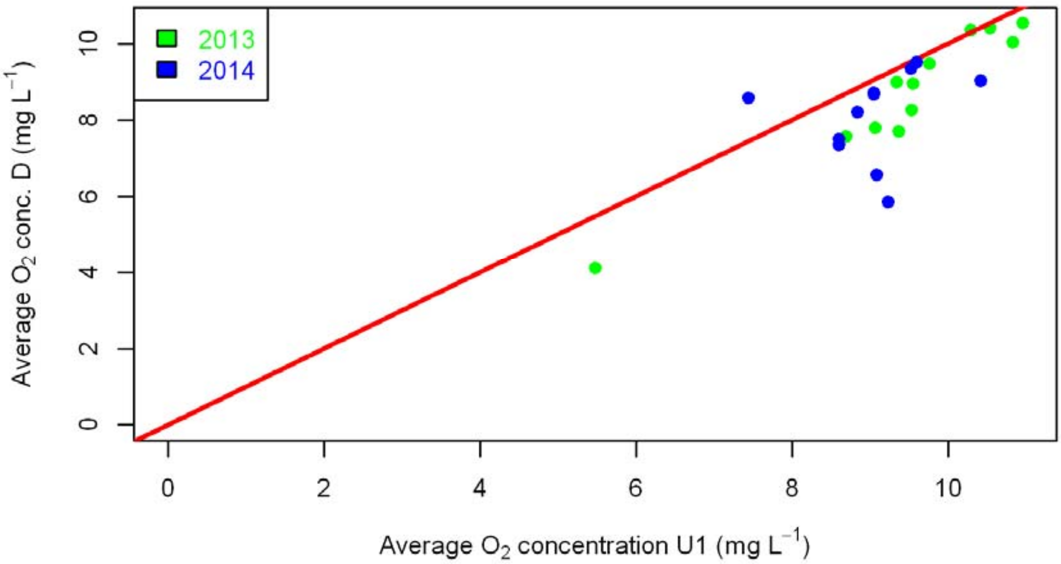


Fig. S2: Comparison of average oxygen concentrations at the upstream (U1) and downstream locations (D) of the 2013 and 2014 sites. The red line indicates the 1:1 line.

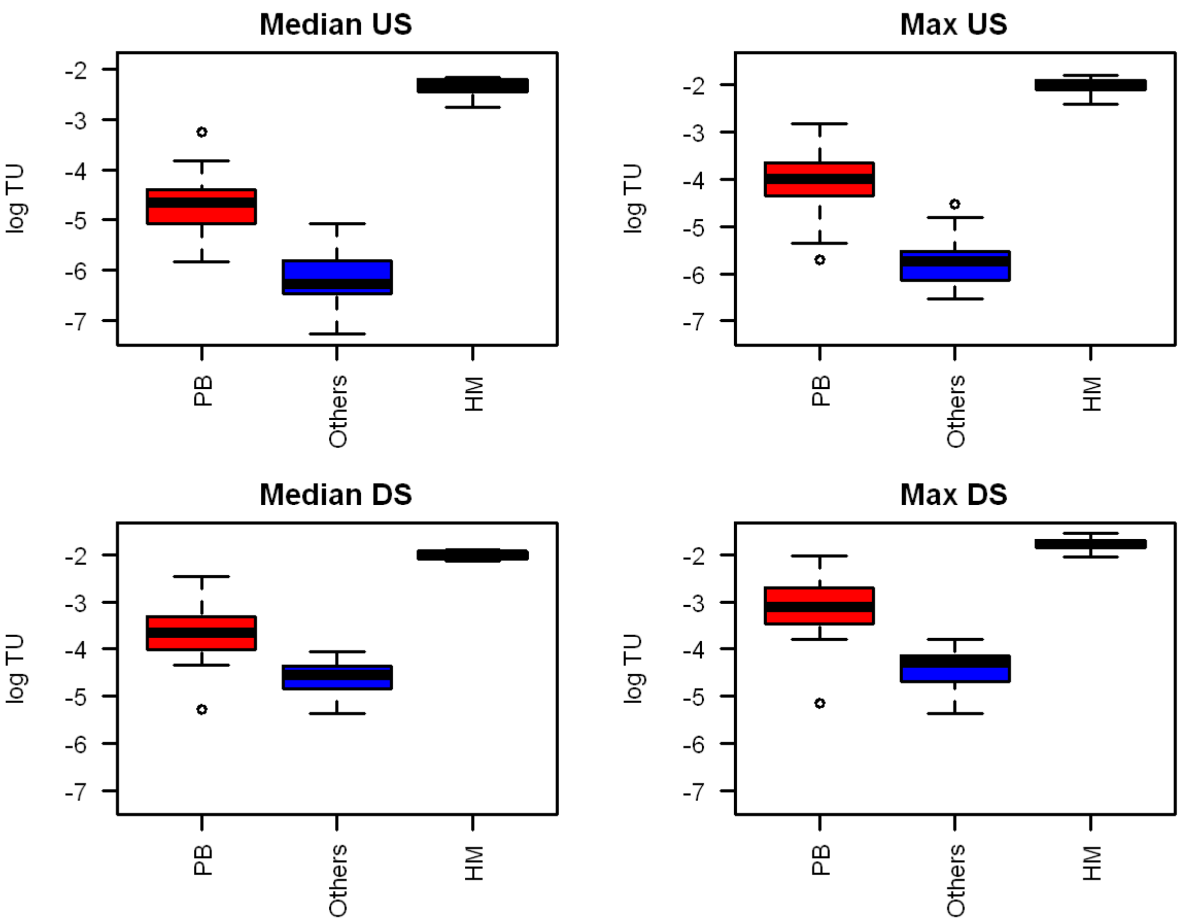


Fig. S3: Comparison of toxic units specifically derived for macroinvertebrates (log values, median (left) and maximum values (right) of TUs sum across all sampling dates per location) for pesticides (PB; organic-synthetic plant protection products and biocides), other organic MPs (Others; pharmaceuticals, household chemicals, sweeteners etc.), and heavy metals (HM) at the U1 and D locations.

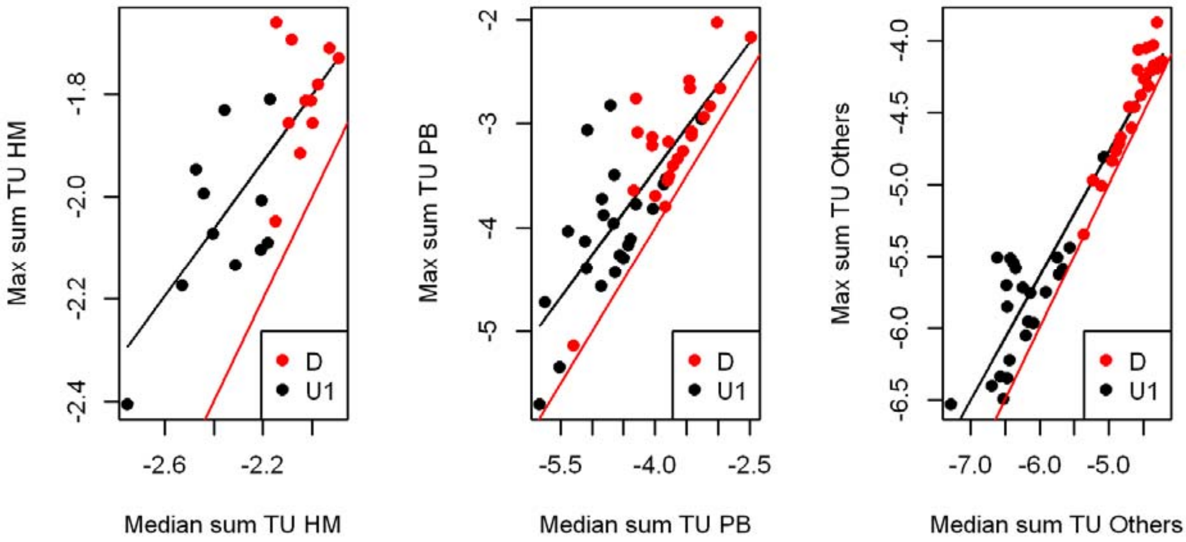


Fig. S4: Comparison of median and maximum TUs calculated for each location across all sampling dates per compound class. Black: upstream locations U1, red: downstream locations D. HM: heavy metals, Others: organic micropollutants other than pesticides and biocides, PB: pesticides and biocides.

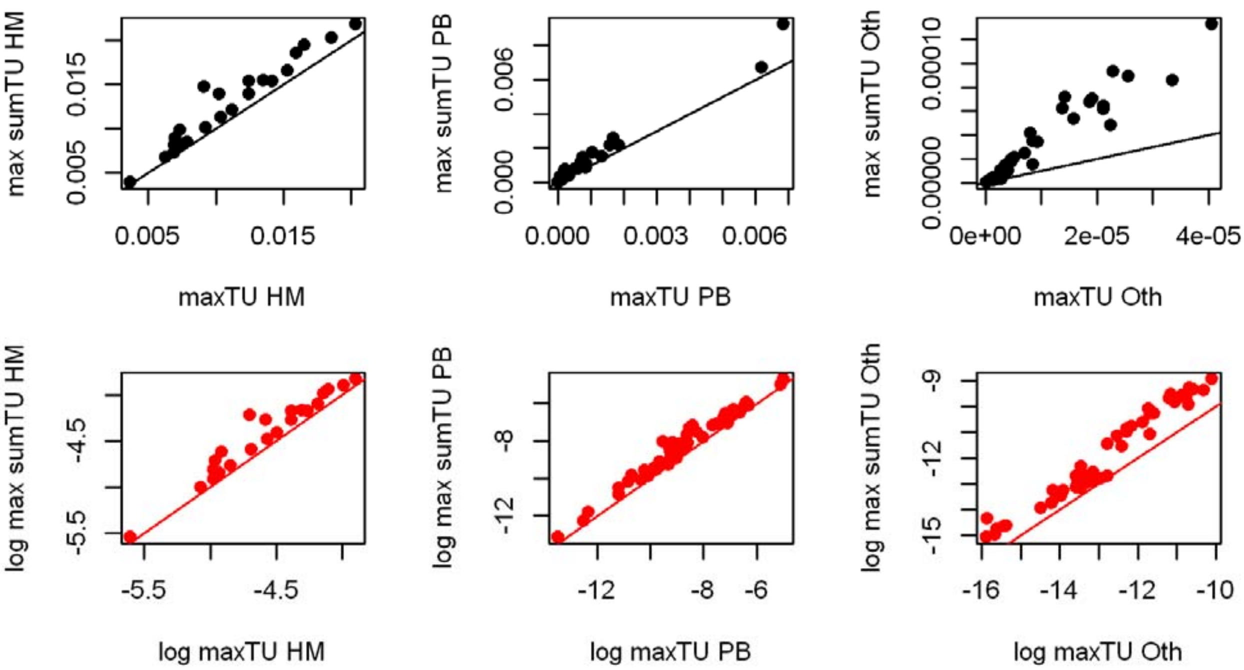


Fig. S5: Comparison of the maximum TUs values across all sampling dates per compound class and location and the max sum of TUs per compound class and location. Black: original values, red: logarithmic values as used in the subsequent statistical analyses. The red and black lines indicate the

1:1 lines. HM: heavy metals, Others: organic micropollutants other than pesticides and biocides, PB: pesticides and biocides.

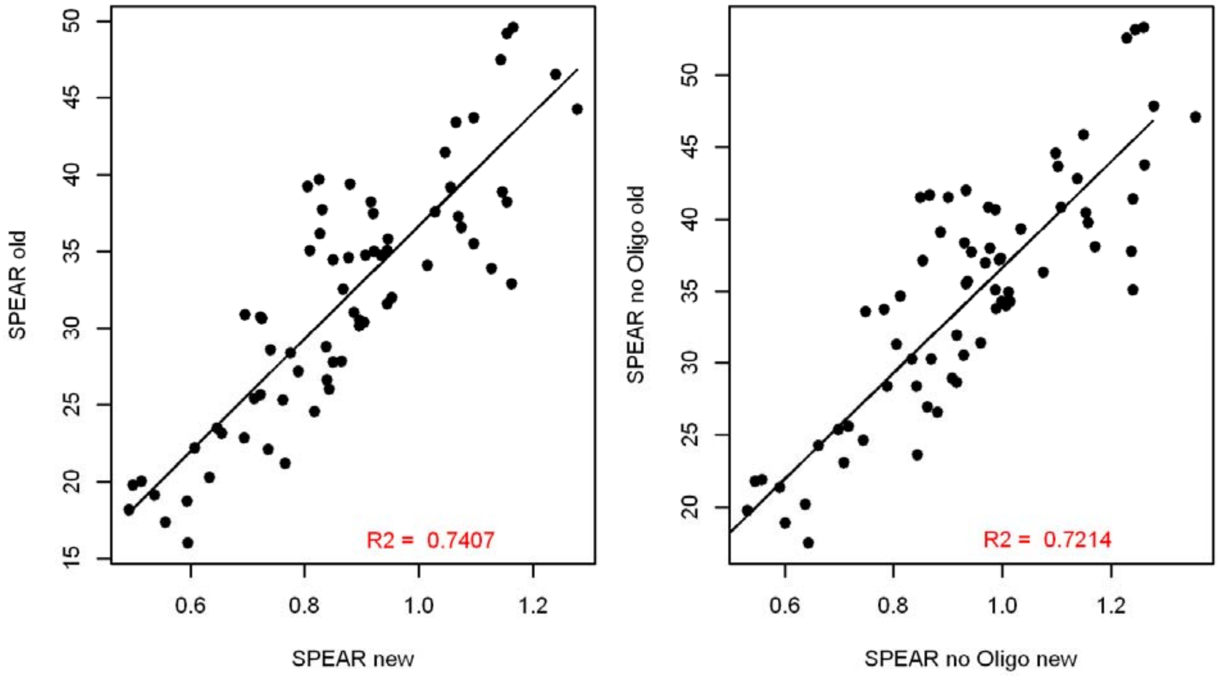


Fig. S6: Comparison of SPEAR values calculated with SPEAR 2018.05 (SPEAR new) and a previous version of the SPEAR calculator (SPEAR Calculator v0.9.0; SPEAR old). Left panel: all taxa included, right panel: oligochaetes excluded.

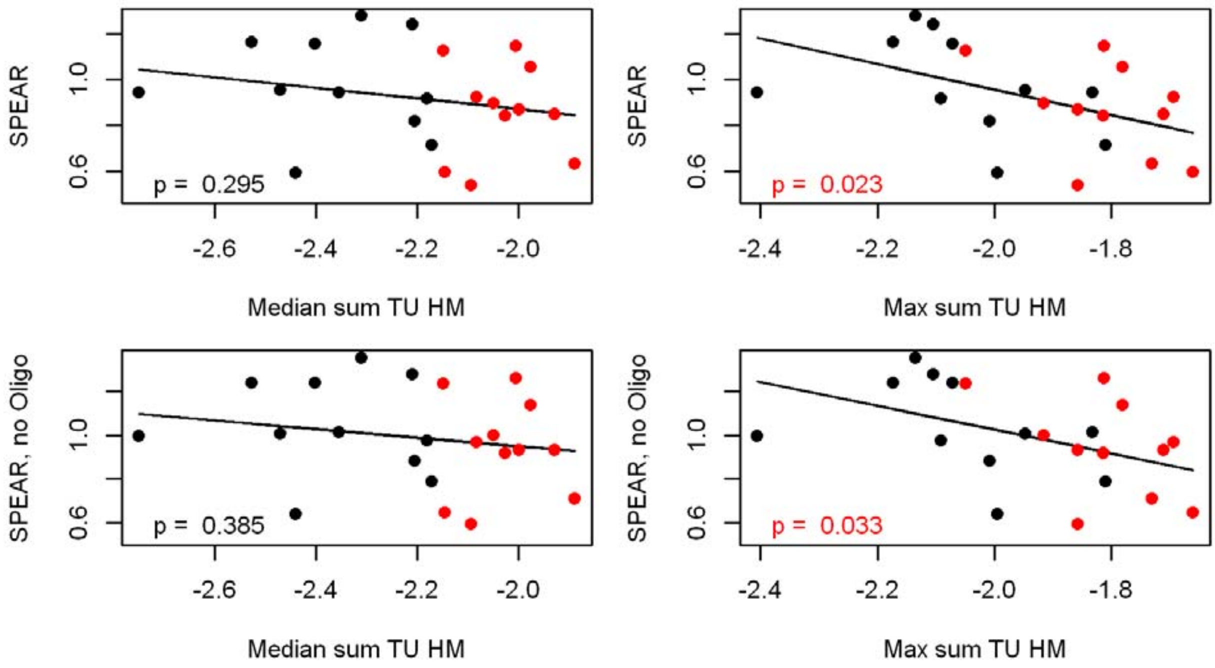


Fig. S7: Comparison of median (left) and maximum (right) TU sums for heavy metals (HM) calculated for each location across all sampling dates and all heavy metals with the SPEAR values (top: all taxa, bottom: oligochaetes excluded) at each location. Black: upstream locations U1, red: downstream locations D.

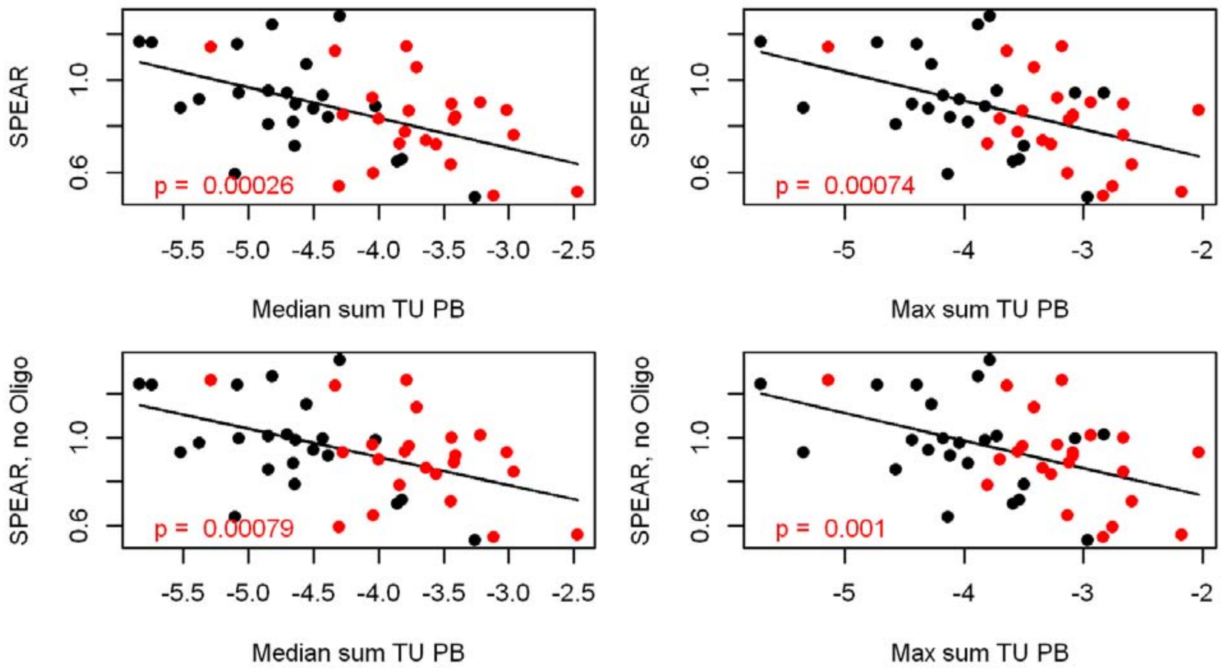
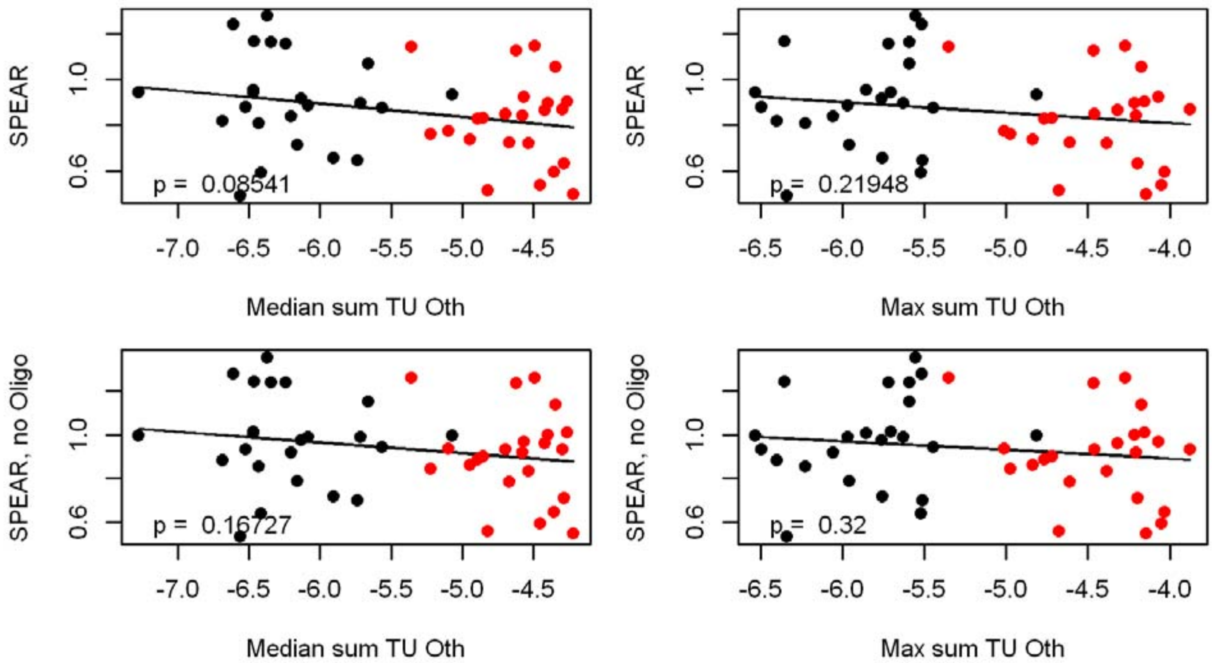


Fig. S8: Comparison of median (left) and maximum (right) TU sums for pesticides (PB) calculated for each location across all sampling dates and all heavy metals with the SPEAR values (top: all taxa, bottom: oligochaetes excluded) at each location. Black: upstream locations U1, red: downstream locations D.



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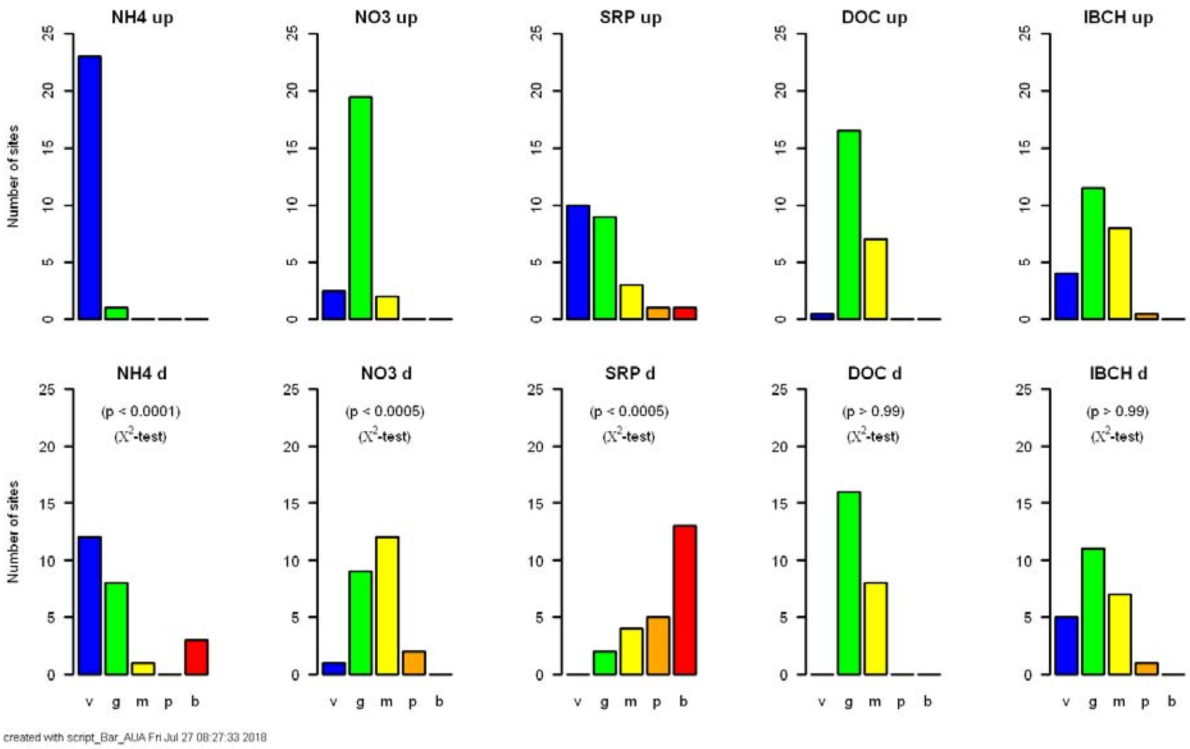

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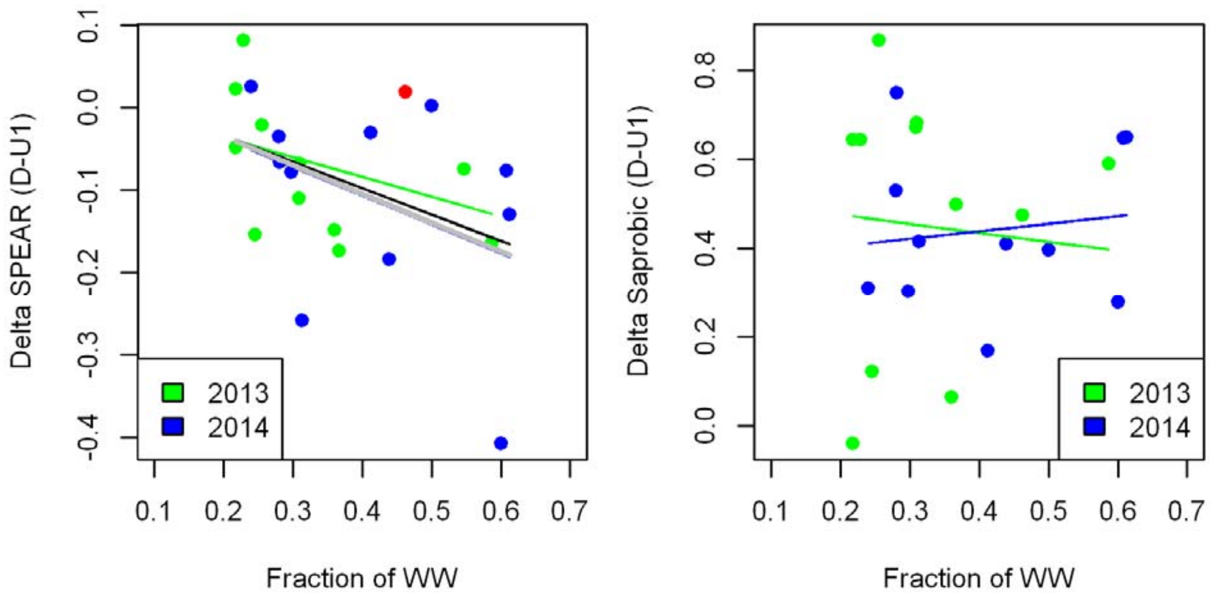

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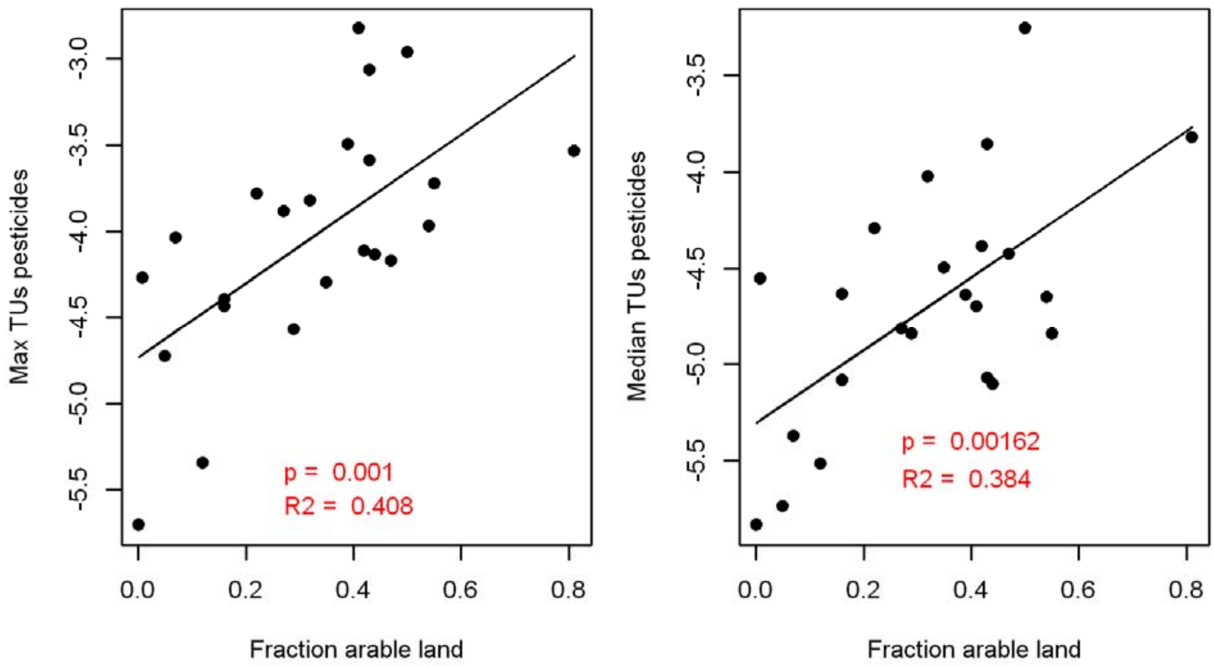

Fig. S12: Relationship between the fraction of arable land and the toxic units resulting from pesticides (log units, maximum (left) and median values (right) of TU sums of all pesticides across all sampling dates per location) upstream (U1).

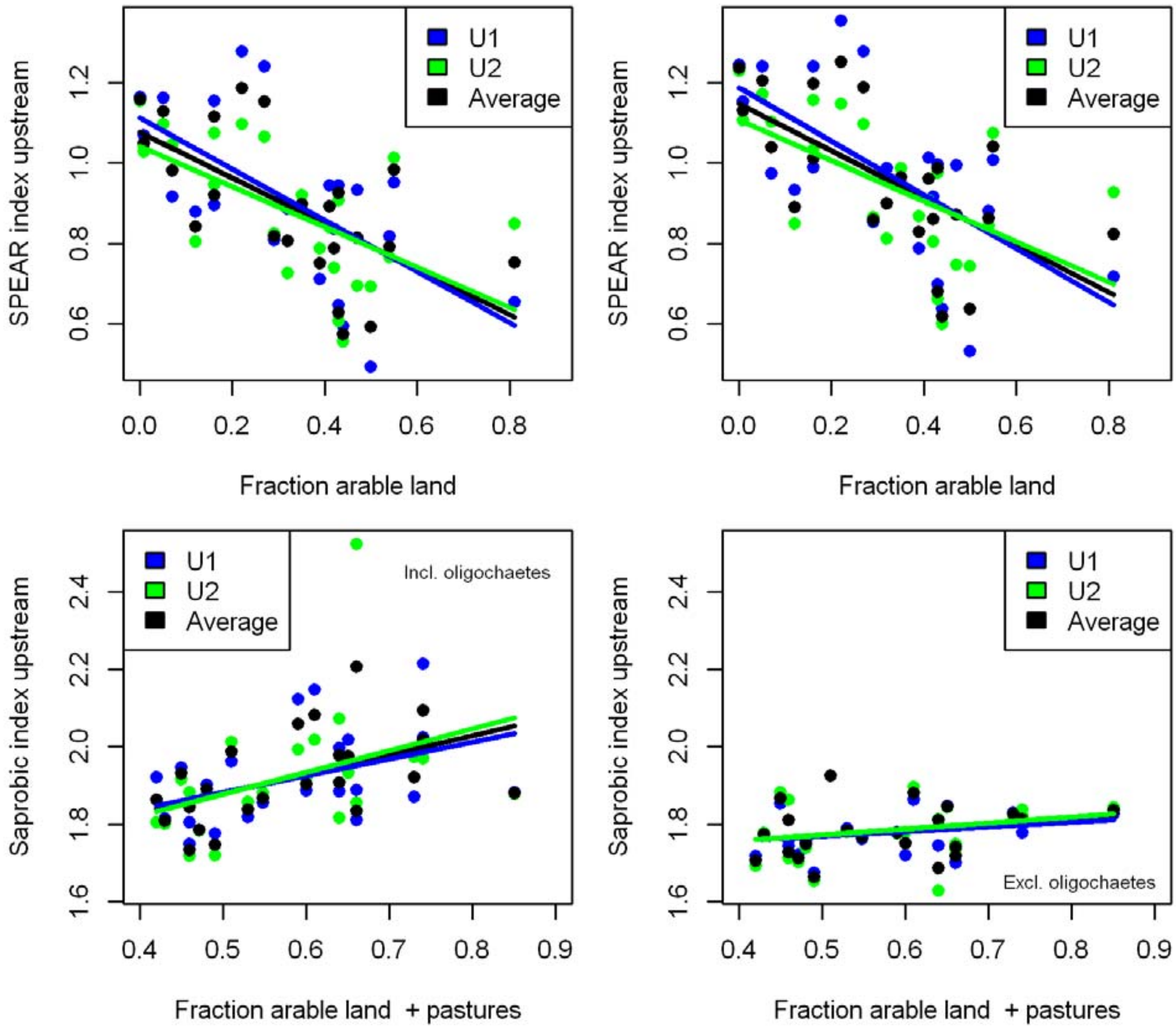

Fig. S13: Relationship between land use and the SPEAR index (top row) and the Saprobic index (bottom row) at the upstream locations with (left column) and without oligochaetes (right column). The coloured lines represent the Pearson correlations (Including oligochaetes: SPEAR index (average between U1 and U2 at each site): adjusted R^2 = 0.376, p = 0.001; Saprobic index (average): adjusted R^2 = 0.214, p = 0.015; excluding oligochaetes: SPEAR index (average): adjusted R^2 = 0.380, p = 0.001; Saprobic index (average): adjusted R^2 = 0.015, p = 0.26).

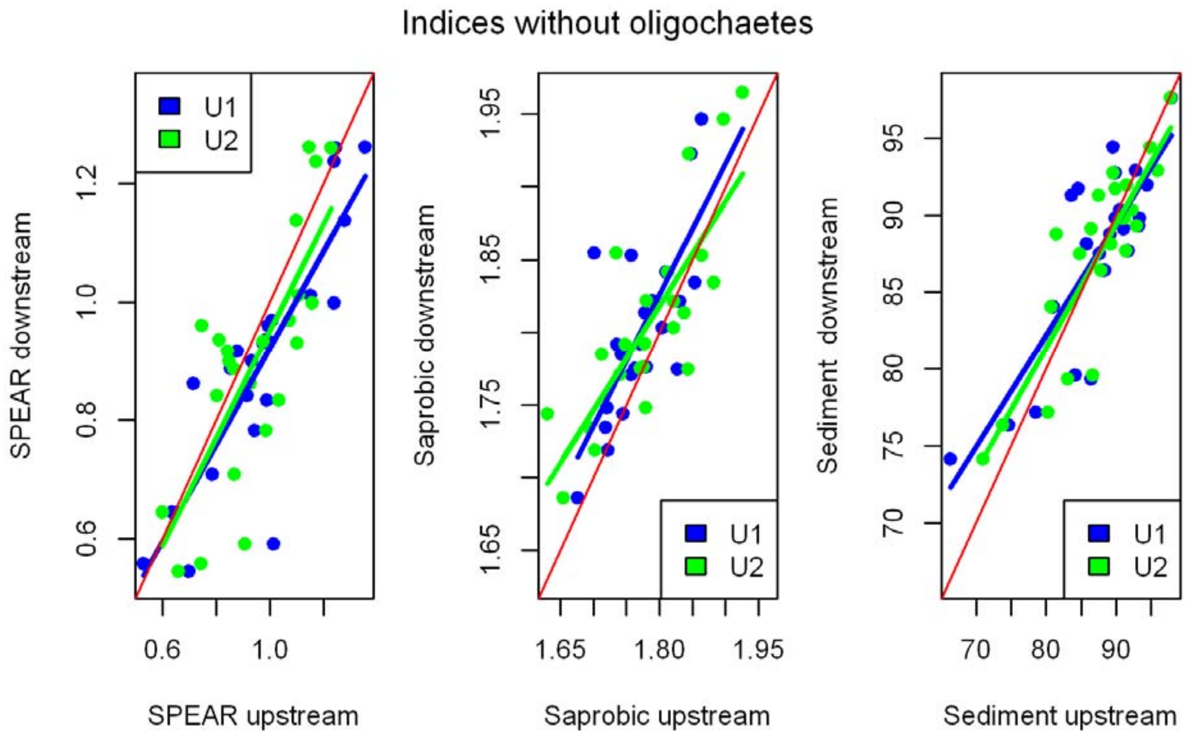

Fig. S14: Relationships between the three indices (SPEAR, Saprobic and Sediment) at the downstream locations as a function of the upstream values excluding the oligochaetes. The blue and green lines represent the Pearson correlations, the red line the 1:1 line (SPEAR index: adjusted R^2: 0.800 and 0.847, p-values = 6 x 10^{-9} and 3 x 10^{-10} for U1 and U2, respectively; Saprobic index: adjusted R^2: 0.592 and 0.617, p-values = 10^{-5} and 5 x 10^{-6} for U1 and U2, respectively; Sediment index: adjusted R^2: 0.664 and 0.742, p-values = 10^{-6} and 8 x 10^{-8} for U1 and U2, respectively).

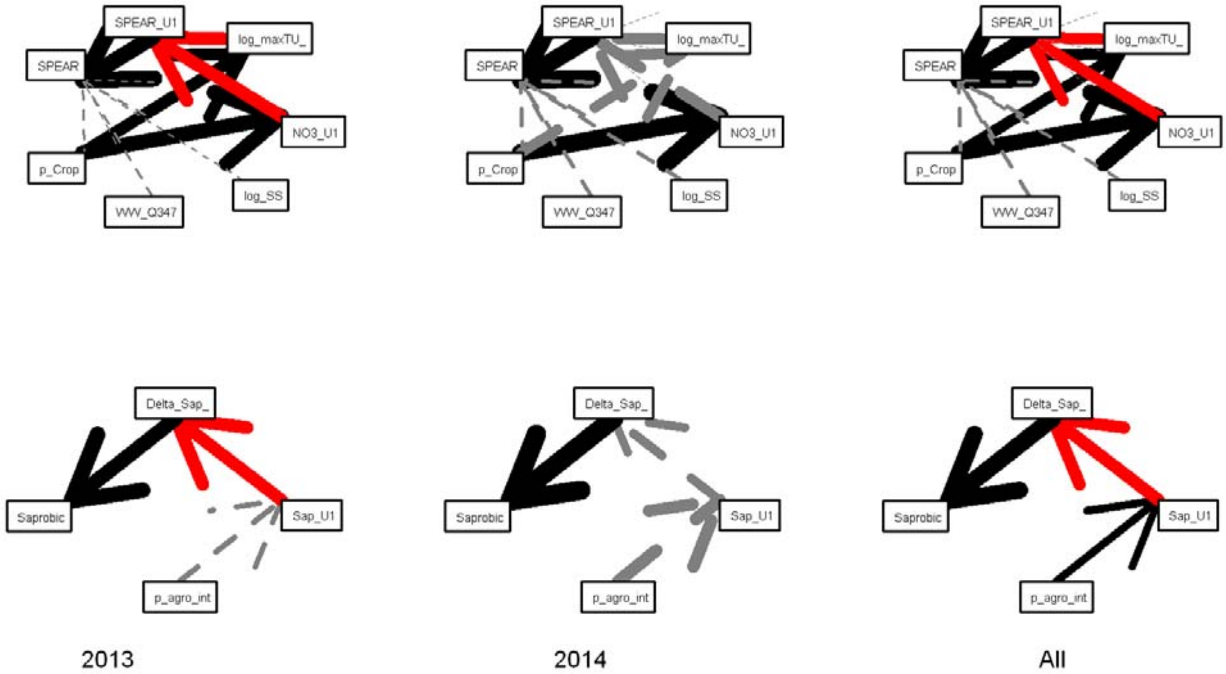

Fig. S15: Structural equation models for the SPEAR index (top row) and the Saprobic index (bottom row) including oligochaetes at the downstream locations D for the 2013 sites, the 2014 sites, and all sites combined. Red arrows indicate a negative linear relationship, black arrows positive linear relationships, grey, dashed lines non-significant relationships. The line thickness represents the statistical significance (see Tab. S5 for more statistical details). Delta_Sap: Difference between saprobic index at D and U1; log_maxTU_: maximum TUs of pesticides (logarithm), log_SS: Total suspendible (benthic) sediment (logarithm), NO3_U1: nitrate concentration at U1, p_agro_int: areal fraction of arable cropping and pastures in the catchment, p_Crop: areal fraction of arable cropping in the catchment Saprobic: saprobic index at D, Sap_U1: saprobix index at U1, SPEAR: SPEAR index at D, SPEAR_U1: SPEAR index at U1, WW_Q347: fraction of WW under low flow conditions.

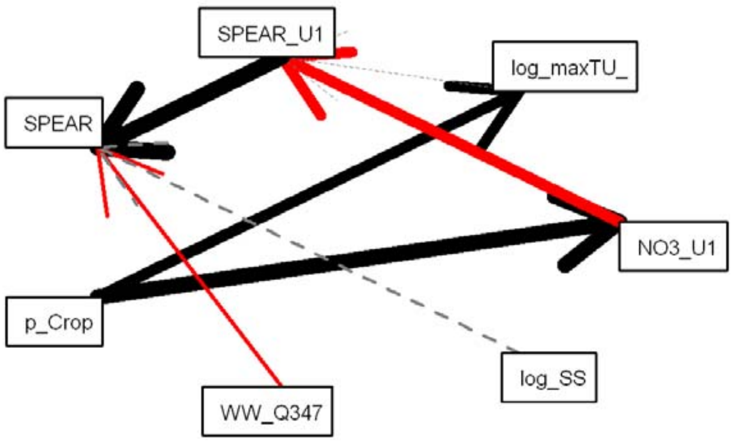

Fig. S16: Structural equation models for the SPEAR index (top row) at the downstream locations D for all sites excluding Hornussen (see also Fig. S11). Red arrows indicate a negative linear relationship, black arrows positive linear relationships, grey, dashed lines non-significant relationships. The line thickness represents the statistical significance (see Tab. S5 for more statistical details). log_maxTU_: maximum TUs of pesticides (logarithm), log_SS: Total suspendible (benthic) sediment (logarithm), NO3_U1: nitrate concentration at U1, p_Crop: areal fraction of arable cropping in the catchment SPEAR: SPEAR index at D, SPEAR_U1: SPEAR index at U1, WW_Q347: fraction of WW under low flow conditions.

Supplementary tables

Table S1: Land use, catchment size and fraction of wastewater downstream of the 24 EcoImpact sites.

Site	ARA_NR	YEAR	Catchment size	Fraction Arable	Fraction Orchard	Fraction Pasture	Fraction Forested	Fraction Urban	Fraction Agri intense	Q_{347}	Q_{mean}	QWW_{dry}	Fraction WW_Q_{mean}	Fraction WW_Q_{347}
			[km^2]	[%]	[%]	[%]	[%]	[%]	[%]	[$m^3 d^{-1}$]	[$m^3 d^{-1}$]	[$m^3 d^{-1}$]	[-]	[-]
Aadorf	455100	2014	35.0	0.16	0.01	0.32	0.36	0.14	0.48	16855	64800	5277	0.081	0.313
Birmensdorf	24201	2014	46.7	0.22	0.01	0.21	0.38	0.16	0.43	13997	84672	8575	0.101	0.613
Buttisholz	108300	2013	6.8	0.42	0.04	0.32	0.11	0.14	0.74	2022	11232	1107	0.099	0.547
Colombier	563000	2013	11.8	0.81	0.01	0.04	0.07	0.08	0.85	1460	15552	334	0.021	0.229
Dürnten	11301	2013	17.4	0.16	0.02	0.44	0.17	0.21	0.60	6471	63072	2372	0.038	0.367
Elgg	21701	2014	13.3	0.27	0.01	0.19	0.41	0.13	0.46	3292	37152	1446	0.039	0.439
Ellikon a. Thur	21801	2014	24.1	0.55	0.02	0.11	0.19	0.14	0.66	6178	27648	2546	0.092	0.412
Herisau	300102	2013	16.3	0.01	0.01	0.54	0.24	0.20	0.55	11007	42336	6462	0.153	0.587
Hochdorf	103100	2013	28.1	0.47	0.04	0.26	0.13	0.14	0.73	14869	50976	4614	0.091	0.310
Hornussen	416700	2013	37.0	0.29	0.01	0.20	0.43	0.08	0.49	2765	48384	1278	0.026	0.462
Kernenried	41100	2013	65.9	0.43	0.02	0.08	0.29	0.18	0.51	31277	119232	11261	0.094	0.360
Knonau	701	2014	16.6	0.41	0.01	0.24	0.20	0.14	0.65	3637	33696	2183	0.065	0.600
Marthalen	3501	2014	26.5	0.54	0.01	0.10	0.21	0.13	0.64	6138	25920	1472	0.057	0.240
Messen	245700	2013	37.4	0.50	0.02	0.11	0.32	0.07	0.61	10480	70848	2284	0.032	0.218
Muri	423600	2014	15.5	0.43	0.02	0.23	0.19	0.15	0.66	5011	32832	3047	0.093	0.608
Niederdorf	289100	2013	25.1	0.12	0.03	0.33	0.46	0.08	0.45	9815	36288	2142	0.059	0.218
Reinach	414100	2014	43.6	0.44	0.02	0.20	0.20	0.15	0.64	25047	74304	12546	0.169	0.501
Romont	209600	2013	47.6	0.35	0.01	0.39	0.16	0.10	0.74	18040	93312	4425	0.047	0.245
Rothenthurm	137000	2013	7.4	0.00	0.00	0.47	0.46	0.06	0.47	3223	23328	825	0.035	0.256
Sévéry-Pampigny	550000	2013	7.2	0.32	0.00	0.10	0.52	0.06	0.42	1339	7776	414	0.053	0.309
Unterehrendingen	404300	2014	30.3	0.39	0.02	0.20	0.27	0.14	0.59	13815	41472	4120	0.099	0.298
Val-de-Ruz	647600	2014	63.7	0.17	0.00	0.34	0.43	0.05	0.51	5064	141696	4379	0.031	0.865
Villeret	44800	2014	63.0	0.05	0.00	0.48	0.40	0.07	0.53	9609	175392	2697	0.015	0.281
Zullwil	262200	2014	7.1	0.07	0.04	0.39	0.45	0.09	0.46	1682	12096	473	0.039	0.281

Table S2. Summary of environmental variables recorded (i.e., physicochemical and catchment properties). Mean values used in analyses unless specified. See Methods for more details.

Category	Group	Description	Measurement units
Water quality	General water chemistry	Electrical conductivity	µS/cm 20 °C
		pH	H^+ mol/L
		Alkalinity	mEq/L
		Hardness	mmol/L
		Na^+, K^+, Ca^{2+}, Mg^{2+}, Cl^-, SO_4^{2-}-S	mg/L
	Nutrients	NH_4^+, NO_2^-, total and soluble reactive phosphorus (TP and SRP), NO_3^-, total nitrogen (TN), SiO_4^{4-}Si, Total and	µg /L
		dissolved organic carbon (TOC and DOC)	mg/L
	Sediment	Total suspended solids (TSS)	mg/L
	Heavy metals	Ag, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, Zn (filtered/unfiltered)	µg/L
	Organic micropollutants	57 compounds including pharmaceuticals, pesticides, household chemicals etc.‡	ng/L
Habitat†	Inorganic	Total and inorganic benthic suspendible sediment ($SS_{benthic}$ and ISS)	kg/m^2
	Organic	Organic benthic suspendible sediment ($OSS_{benthic}$)	kg/m^2 , %
	Hydromorphology	Flow velocity	m/s

		Wetted channel width and water depth	m
Catchment	Area	Total upstream area	ha
	Landuse	‘Urban’, ‘Orchards’, ‘Arable cropping’, ‘Pasture’, ‘Forest’	%

†: further habitat descriptors see Tab. S3.

‡ : further details on the compounds can be found in (Munz et al. 2017).

Tab. S3: Habitat descriptors for the 72 sampling locations 2013 and 2014.

Tab EcoImpact habitat characteristics

Data sources: Field protocols Aquabug (Q:\Querprojekte\EcoImpact\Monitoring\Data\01_Macroinvertebrates\2013\Raw invertebrate data and materials received from AQUABUG); Table created: Christian Stamm, 29.10.2017 (sites 2013); Marta Reyes 7.11.2017 (sites 2014)

Coding (general): qualitative properties are ranked bdtween natural = 1 to artificial = 0
Coding culvert: yes = 1; no = 0
Coding width variability: pronounced = 1; limited = 0.5; no = 0
Coding stream bed modifications: none = 1; 0 < x < 10 % = 0.95; 10 < x < 30% = 0.8; 30 < x < 60% = 0.55; 60 < x < 100% = 0.2; 100% = 0
Coding natural jumps : yes = 1; no = 0
Coding variability depth : pronounced = 1; limited = 0.5; no = 0
Coding material stream bed modification :no quantitative coding
Coding modification river bank: data for each side has a weight of 50%; same coding as for stream bed modifications (for each side separately)
Coding permeability of material: data for each side has a weight of 50%; permeable = 1; impermeable = 0
Coding width river bank: Average between both sides (m)
Coding quality river bank: data for each side has a weight of 50%; natural (=gewässergercht) = 1; impacted (= gewässergfremd) = 0.5; artificial = 0
Coding barriers presence: no quantitative coding (yes, no)
Coding barriers quality: natural = 1; artificial = 0; unknown = NA
Coding mud presence: no quantitative coding (no, moderate, pronounced)
Coding mud origine: natural = 1; artificial = 0; unknown = NA
Coding turbidity presence: no quantitative coding (no, moderate, pronounced)
Coding turbidity origine: natural = 1; artificial = 0; unknown = NA
Coding coloration presence: no quantitative coding (no, moderate, pronounced)
Coding coloration origine: natural = 1; artificial = 0; unknown = NA
Coding FeS: no quantitative coding (no, moderate, pronounced)
Coding FeS origine: natural = 1; artificial = 0; unknown = NA
Coding colmation: no = 1; moderate = 0.5; strong = 0
Coding colmation origine: natural = 1; artificial = 0
Coding heterotrophs: no = 1; rarely = 0.9; little = 0.75; moderate = 0.5; strong = 0
Coding heterotrophs origine: natural = 1; artificial = 0; unknown = NA
Coding autotrophs algae: none = 1; < 10% = 0.9; moderate = 0.5; pronounced = 0.25
Coding autotrophs bryophytes:none = 1; < 10% = 0.9; moderate = 0.5; pronounced = 0.25
Coding autotrophsmacrophytes: none = 1; < 10% = 0.9; moderate = 0.5; pronounced = 0.25
Coding dead wood: absent = 1; scattered = 0.5; pile = 0

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39
Site	Location	Surv	Date	Altitude	Average width (m)	Average (	Culvert	Width var	Stream b	Natural ju	Variability	Material s	Modificat	Permeab	Width riv	Bank qua	Barriers p	Barrier q	Mud pres	Mud origi	Turbidit	Turbidit	Colorat	Colorat	Foam p	Foam o	Smell p	Smell o	FeS pr	FeS ori	Colmati	Colmati	Heterotr	Heterot	Algae	Bryoph	Macrop	Dead wood
Aadorf	D	R.W	####	498	5	NA	1	0	0.8	0	1	other, im	0	1	8	1	NA	NA	no		no	NA	no	NA	no	NA	no	NA	no	NA	0.5	1	0	NA	0.5	0.5	0.9	1
Aadorf	U1	R.W	####	500	5.5	NA	1	0	0.8	0	1	other, im	0	1	6.5	1	NA	NA	moderate	1	no	NA	no	NA	no	NA	no	NA	no	NA	0.5	1	0	NA	0.9	0.5	0.9	1
Aadorf	U2	R.W	####	501	6	NA	1	0	0.8	0	1	other, im	0	1	5	1	NA	NA	moderate	1	no	NA	no	NA	no	NA	no	NA	no	NA	0.5	1	0	NA	0.9	0.5	0.9	0.5
Birmensdorf	D	N.Mc	####	450	6.5	NA	1	0.5	0.95	0	0.5	Steinsch	0.2	1	4	0.5	NA	NA	no	NA	no	NA	no	NA	modera	0	yes	0	no	NA	0	NA	0	NA	0.25	0.9	0.9	1
Birmensdorf	U1	N.Mc	####	450	6.5	NA	1	0.5	0.95	0	0.5	Steinsch	0.2	1	4.5	0.5	NA	NA	no	NA	no	NA	no	NA	no	NA	no	NA	no	NA	0	NA	0	NA	0.5	0.9	0.9	1
Birmensdorf	U2	N.Mc	####	450	6	NA	1	0.5	0.95	0	0.5	Steinsch	0.2	1	4.5	0.5	NA	NA	no	NA	no	NA	yes	NA	modera	NA	no	NA	no	NA	0.5	NA	0	NA	0.5	0.9	0.9	1
Buttisholz	D	P. St	####	548	2	0.2	1	0.5	1	1	0.5	NA	0.2	1	2	0.5	no	NA	no	NA	no	NA	no	NA	yes	NA	no	NA	modera	NA	0.5	NA	0	NA	0.9	0.9	0.9	NA
Buttisholz	U1	P. St	####	548	2	0.2	1	0.5	1	1	0.5	NA	0.2	1	0.5	0.5	no	NA	no	NA	no	NA	no	NA	modera	NA	no	NA	modera	NA	0.5	NA	0	NA	0.9	0.9	0.9	NA
Buttisholz	U2	P. St	####	548	1.5	0.3	1	0	0.95	0	0.5	NA	0.55	1	1.5	0.75	yes	0	no	NA	no	NA	no	NA	modera	NA	no	NA	modera	NA	0	NA	0	NA	0.9	0.9	0.9	NA
Colombier	D	N. M	####	492	3	0.3	1	1	1	1	1	NA	1	1	2	1	no	NA	no	NA	no	NA	modera	NA	no	NA	no	NA	modera	NA	0.5	NA	0	NA	0.9	0.9	0.9	NA
Colombier	U1	N. M	####	492	2	0.2	1	1	1	1	1	NA	1	1	2.5	1	no	NA	no	NA	no	NA	modera	NA	no	NA	no	NA	pronou	NA	0.5	NA	0	NA	0.9	0.9	0.9	NA
Colombier	U2	N. M	####	492	2	0.2	1	1	1	1	1	NA	1	1	2.5	1	no	NA	no	NA	no	NA	modera	NA	no	NA	no	NA	pronou	NA	0.5	NA	0	NA	0.9	0.9	0.9	NA
Dürnten	D	R. W	####	490	3.4	0.7	0	0	0.8	0	0.5	natural st	0	1	20	1	no	NA	moderate	0	no	NA	no	NA	modera	0	modera	0	modera	0	0	1	0	NA	0.25	0.9	1	NA
Dürnten	U1	R. W	####	500	3.2	0.3	1	0	0.8	0	0.5	natural st	0	1	5	1	yes	0	no	NA	no	NA	no	NA	no	NA	no	NA	no	NA	0	1	0	NA	0.9	0.25	0.9	NA
Dürnten	U2	R. W	####	500	3.2	0.3	1	0	0.8	0	0.5	natural st	0	1	5	1	no	NA	no	NA	no	NA	no	NA	no	NA	no	NA	no	NA	0	1	0	NA	0.9	0.25	0.9	NA
Elgg	D	R.W	####	499	3.8	NA	1	0	0.95	0	0.5	other, im	0.55	1	3	0.5	NA	NA	no	NA	no	NA	no	NA	no	NA	no	NA	no	NA	0.5	1	0	NA	0.9	0.9	0.9	1
Elgg	U1	R.W	####	499	3.5	NA	1	0	0.95	0	0.5	other, im	0.55	1	12	0.5	NA	NA	no	NA	no	NA	no	NA	no	NA	no	NA	no	NA	0.5	1	0	NA	0.9	0.9	0.9	1
Elgg	U2	R.W	####	500	3.4	NA	1	0.5	0.95	0	0.5	other, im	0.8	1	3	0.5	NA	NA	no	NA	no	NA	no	NA	no	NA	no	NA	no	NA	0.5	1	0	NA	0.9	0.9	0.9	1
Ellikon	D	R.W	####	378	4.5	NA	1	0.5	1	0	0.5	NA	1	NA	4	1	NA	NA	moderate	1	no	NA	no	NA	no	NA	no	NA	no	NA	0.5	NA	0	NA	0.9	0.5	0.9	1
Ellikon	U1	R.W	####	378	4.5	NA	1	0.5	1	0	0.5	NA	1	NA	4.5	0.75	NA	NA	no	NA	no	NA	no	NA	no	NA	no	NA	no	NA	0.5	NA	0	NA	0.9	0.5	0.9	1
Ellikon	U2	R.W	####	378	6	NA	1	1	1	1	1	NA	0.8	0	3	1	NA	NA	moderate	1	no	NA	no	NA	no	NA	no	NA	no	NA	0.5	NA	0	NA	0.9	0.9	0.9	0.5
Herisau	D	R. W	####	690	6.5	0.3	1	1	1	0	1	NA	NA	NA	2	1	no	NA	yes	0	no	NA	no	NA	modera	0	modera	0	no	NA	0.5	1	0	NA	0.25	0.9	1	NA
Herisau	U1	R. W	####	694	6.5	0.2	1	0	0.2	0	0.5	natural st	0	1	15	1	yes	0	yes	NA	no	NA	no	NA	no	NA	no	NA	no	NA	0.5	1	0	NA	0.25	0.5	1	NA
Herisau	U2	R. W	####	695	7	0.4	1	1	1	0	1	NA	1	1	12.5	1	yes	1	yes	NA	no	NA	no	NA	no	NA	no	NA	no	NA	0.5	1	0	NA	0.25	0.9	1	NA
Hochdorf	D	P. St	####	470	5	0.5	1	0	1	0	0.5	NA	0	1	2	0.5	no	NA	no	NA	no	NA	no	NA	modera	NA	no	NA	modera	NA	0.5	NA	0	NA	0.5	0.9	0.9	NA
Hochdorf	U1	P. St	####	470	5	0.3	1	0	1	1	0.5	NA	0.5	1	8	0.75	no	NA	no	NA	no	NA	no	NA	modera	NA	no	NA	modera	NA	0.5	NA	0	NA	0.9	0.9	1	NA
Hochdorf	U2	P. St	####	470	5	0.4	1	0	1	0	NA	NA	0	1	3	0.5	no	NA	no	NA	no	NA	no	NA	modera	NA	no	NA	modera	NA	0.5	NA	0	NA	0.9	0.9	0.9	NA
Hornussen	D	S. Ki	####	358	6	0.4	1	0	0.95	0	0.5	natural st	0	1	6	1	yes	0	no	NA	no	NA	no	NA	modera	NA	modera	0	no	NA	0	0	0	NA	0.9	1	1	NA
Hornussen	U1	S. Ki	####	358	6	NA	1	0	0.95	0	0.5	natural st	0	1	6	1	yes	0	no	NA	no	NA	no	NA	modera	NA	modera	0	no	NA	0	0	0	NA	0.9	0.9	1	NA
Hornussen	U2	S. Ki	####	NA	6	0.6	1	0	0.95	0	1	natural st	0	1	6	1	yes	0	no	NA	no	NA	no	NA	modera	NA	modera	0	no	NA	0	0	0	NA	0.9	1	1	NA
Kernenried	D	P. St	####	505	5	0.3	1	1	1	1	0.5	NA	1	1	5	1	no	NA	no	NA	no	NA	no	NA	modera	NA	no	NA	yes	NA	0.5	NA	0	NA	0.9	0.5	0.25	NA
Kernenried	U1	P. St	####	505	4	0.3	1	NA	1	1	0.5	NA	0.9	1	3.5	1	no	NA	no	NA	no	NA	no	NA	no	NA	no	NA	yes	NA	0	NA	0	NA	0.9	0.5	0.9	NA
Kernenried	U2	P. St	####	505	4	0.4	1	1	1	1	0.5	NA	1	1	5	1	no	NA	no	NA	no	NA	no	NA	NA	NA	no	NA	yes	NA	0	NA	0	NA	0.9	1	0.5	NA
Knonau	D	N.Mc	####	420	3	NA	1	0.5	1	0	0.5	NA	0.95	1	4	0.5	NA	NA	no	NA	no	NA	no	NA	modera	0	yes	0	modera	NA	0.5	NA	0	NA	0.25	0.9	0.9	1
Knonau	U1	N.Mc	####	420	3	NA	1	0.5	1	0	0.5	NA	0.95	1	4	0.5	NA	NA	no	NA	no	NA	no	NA	no	NA	no	NA	no	NA	0.5	NA	0	NA	0.5	0.9	0.9	1
Knonau	U2	N.Mc	####	420	3.5	NA	1	0.5	1	0	0.5	NA	0.1	1	5	1	NA	NA	no	NA	no	NA	no	NA	no	NA	no	NA	no	NA	0.5	NA	0	NA	0.5	0.9	0.9	1
Marthalen	D	P.Sh	####	374	1.8	NA	1	0.5	1	1	0.5	NA	0.9	1	2.5	1	NA	NA	moderate	NA	no	NA	no	NA	modera	0	no	NA	no	NA	1	0	0	NA	0.5	0.9	0.5	1
Marthalen	U1	P.Sh	####	374	1.6	NA	1	0.5	1	NA	0.5	NA	1	NA	7.5	1	NA	NA	moderate	NA	no	NA	no	NA	no	NA	no	NA	no	NA	0	NA	0	NA	0.9	0.9	0.9	0.5
Marthalen	U2	P.Sh	####	374	2	NA	1	0.5	0.95	0	0.5	Steinsch	0.575	1	3	1	NA	NA	moderate	NA	no	NA	no	NA	no	NA	no	NA	no	NA	0	NA	0	NA	0.5	0.9	0.9	0.5
Messen/Limpach	D	P. St	####	470	3.2	0.4	1	0	0	0	0.5	concrete	0	1	5	0.5	no	NA	no	NA	no	NA	no	NA	modera	NA	no	NA	modera	NA	0.5	NA	0	NA	0.5	0.9	0.25	NA
Messen/Limpach	U1	P. St	####	470	3.2	0.4	1	0	0	0	0.5	concrete	0	1	5	0.5	no	NA	no	NA	no	NA	no	NA	modera	NA	no	NA	no	NA	0.5	NA	0	NA	0.5	0.9	0.25	NA
Messen/Limpach	U2	P. St	####	470	3.2	0.4	1	0	0	0	0	concrete	0	1	5	0.5	no	NA	no	NA	no	NA	no	NA	modera	NA	no	NA	no	NA	0.5	NA	0	NA	0.5	1	0.5	NA
Muri	D	S.Kn	####	450	3	NA	1	0.5	1	0	0.5	NA	0.475	1	6	0.75	NA	NA	no	NA	no	NA	no	NA	modera	NA	no	NA	modera	0	0.5	0	0	NA	0.25	0.9	0.9	1
Muri	U1	S.Kn	####	450	3.5	NA	1	0.5	1	0	0.5	NA	0.75	1	8.5	0.75	NA	NA	no	NA	no	NA	no	NA	modera	NA	no	NA	modera	0	0.5	0	0	NA	0.9	0.9	0.9	1
Muri	U2	S.Kn	####	450	4	NA	1	1	1	0	0.5	NA	0.975	1	8.5	0.75	NA	NA	no	NA	no	NA	no	NA	no	NA	no	NA	modera	0	0.5	0	0	NA	0.9	0.9	0.9	1
Niederdorf	D	S. Ki	####	441	5	0.4	1	1	1	0	0.5	NA	0.6	0	1	1	no	NA	no	NA	yes	1	no	NA	yes	0	no	NA	no	NA	0.5	NA	0	NA	0.5	1	1	NA
Niederdorf	U1	S. Ki	####	457	5	0.3	1	0.5	0.95	0	0.5	other, im	0.55	1	NA	NA	yes	0	no	NA	yes	NA	no	NA	no	NA	no	NA	no	NA	0.5	1	0	NA	0.9	1	1	NA
Niederdorf	U2	S. Ki	####	445 (?)	4.6	0.3	1	0.5	1	0	NA	NA	0.975	1	1.5	1	no	NA	no	NA	yes	0	no	NA	yes	NA	no	NA	no	NA	0.5	1	0	NA	0.9	1	1	NA
Reinach	D	S.Kn	####	510	7	NA	1	0.5	1	0	0.5	NA	0.575	1	3	1	NA	NA	no	NA	no	NA	no	NA	modera	0	no	NA	yes	0	0	0	0	NA	0.25	0.5	0.9	1
Reinach	U1	S.Kn	####	513	5	NA	1	0.5	1	0	0.5	NA	0.6	1	2.5	1	NA	NA	no	NA	no	NA	no	NA	no	NA	no	NA	modera	0	0	0	0	NA	0.25	0.9	0.9	1
Reinach	U2	S.Kn	####	516	6.5	NA	1	0.5	0.95	0	1	Steinsch	0.2	1	2.5	1	NA	NA	no	NA	no	NA	no	NA	modera	NA	no	NA	no	NA	0	0	0	NA	0.9	0.9	0.9	1
Romont	D	N. M	####	686	3	0.35	1	0.5	1	0	0.5	NA	0.95	1	0.5	0.5	no	NA	no	NA	moderat	NA	modera	NA	modera	NA	strong	0	modera	NA	0.5	NA	0	NA	0.5	0.7	0.7	NA
Romont	U1	N. M	####	689	3	0.35	1	1	1	0	1	NA	1	1	5.5	0.5	no	NA	no	NA	moderat	NA	modera	NA	no	NA	no	NA	pronou	NA	0.5	NA	0	NA	0.9	0.9	0.5	NA
Romont	U2	N. M	####	689	3	0.4	1	0.5	1	0	1	NA	1	1	1	0.5	no	NA	no	NA	moderat	NA	modera	NA	no	NA	no	NA	pronou	NA	0.5	NA	0	NA	0.9	0.9	0.5	NA
Rothenthurm	D	R. W	####	910	2.6	0.4	1	0.5	1	0	0.5	NA	0	1	0	0.5	no	NA	no	NA	no	NA	no	NA	no	NA	no	NA	no	NA	1	NA	0	NA	0.9	1	0.9	NA
Rothenthurm	U1	R. W	####	910	2	0.4	1	0.5	1	0	0.5	NA	0	1	0	0.5	no	NA	no	NA	no	NA	no	NA	no	NA	no	NA	no	NA	1	NA	0	NA	1	0.9	1	NA
Rothenthurm	U2	R. W	####	910	2	0.9	1	0.5	0.95	0	0.5	NA	0	1	0	0.5	no	NA	no	NA	no	NA	no	NA	no	NA	no	NA	no	NA	1	NA	0	NA	1	0.9	0.9	NA
Sévéry	D	N. M	####	590	2.5	0.2	1	1	1	1	1	NA	0.9	0.5	10	1	yes	0.5	no	NA	no	NA	modera	NA	modera	NA	modera	0	pronou	NA	0.5	NA	0	NA	0.9	0.9	0.9	NA
Sévéry	U1	N. M	####	598	2.5	0.2	1	1	1	1	1	NA	1	1	8.25	0.75	yes	1	no	NA	no	NA	modera	NA	no	NA	no	NA	pronou	NA	0.5	NA	0	NA	0.9	0.9	0.9	NA
Sévéry	U2	N. M	####	607	2.5	0.2	1	1	0.95	1	1	natural st	0.67	1	2.25	1	yes	1	no	NA	no	NA	modera	NA	no	NA	no	NA	pronou	NA	0.5	NA	0	NA	0.25	0.9	0.9	NA
Unterehrendingen	D	N.Mc	####	430	5.5	NA	1	1	1	0	1	NA	1	NA	5.5	1	NA	NA	no	NA	no	NA	no	NA	modera	0	modera	0	modera	NA	0.5	NA	0	NA	0.25	0.9	0.9	0.5
Unterehrendingen	U1	N.Mc	####	430	5	NA	1	0.5	1	0	0.5	NA	0.6	1	4.5	0.75	NA	NA	no	NA	no	NA	no	NA	modera	NA	no	NA	no	NA	0.5	NA	0	NA	0.5	0.9	0.9	1
Unterehrendingen	U2	N.Mc	####	430	4	NA	1	0.5	0.95	0	0.5	Steinsch	0.975	1	9.5	1	NA	NA	no	NA	no	NA	no	NA	modera	NA	no	NA	modera	NA	1	NA	0	NA	0.5	0.9	0.9	1
Valderuz	D	P.Sh	####	714	4	NA	1	0	1	0	0.5	NA	0.55	0.5	1	0.5	NA	NA	moderate	NA	moderat	NA	no	NA	modera	0	modera	NA	yes	NA	0.5	NA	0.75	NA	0.25	0.5	0.9	1
Valderuz	U1	P.Sh	####	714	5	NA	1	0.5	1	0	0.5	NA	1	NA	4	1	NA	NA	no	NA	no	NA	no	NA	no	NA	no	NA	yes	NA	1	NA	0	NA	0.5	0.9	0.9	0.5
Valderuz	U2	P.Sh	####	714	5	NA	1	0.5	1	1	0.5	NA	1	NA	3	1	NA	NA	no	NA	no	NA	no	NA	modera	NA	no	NA	yes	NA	1	NA	0	NA	0.25	0.5	0.9	0.5
Villeret	D	P.Sh	####	731	4	NA	1	0.5	1	1	0.5	NA	1	NA	4	1	NA	NA	no	NA	no	NA	no	NA	no	NA	no	NA	modera	NA	1	NA	0	NA	0.5	0.5	0.9	1
Villeret	U1	P.Sh	####	733	5	NA	1	0.5	1	NA	0.5	NA	1	NA	6	1	NA	NA	no	NA	no	NA	no	NA	no	NA	no	NA	modera	NA	0	NA	0	NA	0.5	0.5	0.9	1
Villeret	U2	P.Sh	####	735	5	NA	1	0.5	1	0	0.5	NA	0.55	1	1	0.75	NA	NA	no	NA	no	NA	no	NA	no	NA	no	NA	modera	NA	0.5	NA	0	NA	0.5	0.5	0.9	1
Zullwil	D	P.Sh	####	549	2	NA	1	0.5	1	1	0.5	NA	0	1	2.5	1	NA	NA	no	NA	no	NA	no	NA	no	NA	no	NA	no	NA	0.5	1	0	NA	0.25	0.25	0.9	0.5
Zullwil	U1	P.Sh	####	550	3	NA	1	0	1	1	0	NA	0	1	3.5	1	NA	NA	no	NA	no	NA	no	NA	modera	0	no	NA	no	NA	0	1	0	NA	0.5	0.5	0.9	1
Zullwil	U2	P.Sh	####	551	2.7	NA	1	0.5	1	1	0.5	NA	0	1	3.5	1	NA	NA	no	NA	no	NA	no	NA	no	NA	no	NA	no	NA	0	1	0	NA	0.5	0.5	0.9	NA

Table S4. Summary of predictor variables used in the variation partitioning models. See Table 1 for definition of environmental variables. DF: degrees of freedom; F: Fisher's F; P: level of significance; PC: physico-chemical (combining Water quality and habitat as listed in Tab. S1); VP: variation partitioning model. Note that for different VP models, the term *Water Quality* component in the table may represent different sets of variables as listed in Tab. S1: VP1,2: general water chemistry, sediments, HMs, MPs.

Model	Component	With oligochaetes Fraction variance explained (adj. R2)	DF	Residual DF	F	p	Without oligochaetes Fraction variance explained (adj. R2)	DF	Residual DF	F	p
VP1	Natural	**0.140**	7	17	2.50	0.001	**0.206**	7	17	3.18	0.001
	Space	**0.235**	7	17	3.52	0.001	**0.274**	7	17	4.16	0.001
	Human	**0.393**	14	17	3.72	0.001	**0.202**	14	17	2.49	0.001
	Total	**0.681**	28	17	4.43	0.001	**0.687**	28	17	4.61	0.001
VP2	Natural	**0.170**	7	24	2.82	0.001	**0.237**	7	24	3.67	0.001
	Space	**0.255**	7	24	3.73	0.001	**0.321**	7	24	4.61	0.001
	Chemicals	**0.299**	7	24	4.21	0.001	**0.121**	7	24	2.36	0.001
	Total	**0.587**	21	24	4.04	0.001	**0.606**	21	24	4.30	0.001
VP3	Natural	**0.118**	7	27	1.99	0.004	**0.196**	7	27	3.09	0.001
	Space	**0.177**	7	27	2.48	0.001	**0.213**	7	27	3.28	0.001
	Habitat	**0.133**	7	27	2.78	0.001	**0.061**	7	27	2.04	0.002
	Total	**0.420**	18	27	2.81	0.001	**0.546**	18	27	4.00	0.001
VP4	Natural	**0.124**	7	29	1.90	0.004	**0.269**	7	29	3.90	0.001
	Space	**0.168**	7	29	2.22	0.002	**0.280**	7	29	4.01	0.001
	Land use	0.006	7	29	1.12	0.333	**0.037**	7	29	2.21	0.006
	Total	**0.293**	16	29	2.17	0.001	**0.522**	16	29	4.08	0.001
VP5	Natural	**0.171**	7	30	2.89	0.001	**0.256**	7	30	3.62	0.001
	Space	**0.205**	7	30	3.26	0.001	**0.262**	7	30	3.68	0.001
	WW	**0.233**	1	30	16.04	0.001	-0.001	1	30	0.94	0.461
	Total	**0.543**	15	30	4.25	0.001	**0.484**	15	30	3.81	0.001
VP6	Natural	**0.194**	7	28	3.03	0.001	**0.256**	7	28	3.56	0.001
	Space	**0.216**	7	28	3.26	0.001	**0.269**	7	28	3.64	0.001
	Nutrients	**0.235**	3	28	6.07	0.001	0.015	3	28	1.30	0.139
	Total	**0.522**	17	28	3.89	0.001	**0.500**	17	28	3.64	0.001
VP7	Natural	**0.191**	7	29	3.05	0.001	**0.267**	7	29	4.05	0.001
	Space	**0.214**	7	29	3.29	0.001	**0.283**	7	29	4.23	0.001
	MPs	**0.231**	2	29	8.45	0.001	**0.064**	2	29	3.21	0.001
	Total	**0.519**	16	29	4.03	0.001	**0.549**	16	29	4.43	0.001
VP8	Natural	**0.171**	7	30	2.63	0.001	**0.254**	7	30	3.80	0.001
	Space	**0.204**	7	30	2.94	0.001	**0.269**	7	30	3.97	0.001
	PB	**0.158**	1	30	9.80	0.001	**0.037**	1	30	3.38	0.002
	Total	**0.445**	15	30	3.40	0.001	**0.522**	15	30	4.27	0.001
VP9	Natural	**0.186**	7	30	2.86	0.001	**0.256**	7	30	3.64	0.001
	Space	**0.199**	7	30	3.00	0.001	**0.262**	7	30	3.7	0.001
	Others	**0.186**	1	30	11.97	0.001	0.002	1	30	1.15	0.298
	Total	**0.474**	15	30	3.70	0.001	**0.487**	15	30	3.85	0.001
VP10	Natural	**0.186**	7	26	2.97	0.001	**0.251**	7	26	3.64	0.001
	Space	**0.230**	7	26	3.43	0.001	**0.291**	7	26	4.06	0.001
	Nutrients	**0.036**	2	26	2.02	0.033	0.003	2	26	2.63	0.368
	MPs	**0.032**	3	26	1.78	0.017	**0.052**	3	26	1.06	0.001
	Total	**0.519**	19	26	3.95	0.001	**0.552**	19	26	3.92	0.001
VP11	Natural	**0.196**	7	27	3.10	0.001	**0.262**	7	27	3.73	0.001
	Space	**0.226**	7	27	3.42	0.001	**0.283**	7	27	3.95	0.001
	Nutrients	**0.101**	3	27	3.23	0.002	0.012	3	27	1.25	0.181
	Pesticides	**0.024**	1	27	2.48	0.013	**0.034**	1	27	3.03	0.001
	Total	**0.546**	18	27	4.01	0.001	**0.533**	18	27	3.86	0.001
VP12	Natural	0.055	7	3	1.23	0.315	0.076	7	3	1.30	0.278
	Space	0.217	7	3	1.90	0.071	**0.244**	7	3	1.96	0.044
	Nutrients	0.033	3	3	1.19	0.354	-0.020	3	3	0.78	0.557
	Heavy metals	-0.015	1	3	0.83	0.531	-0.020	1	3	0.89	0.582
	Total	**0.657**	18	3	3.23	0.003	**0.638**	18	3	3.06	0.002

Table S5: Statistical properties of the Structural Equation Models for the SPEAR and the Saprobic indices for the individual years and the combined data sets. For the SPEAR index, values are also shown for model runs excluding one site (Hornussen) as outlier. Delta_Sap: Difference between saprobic index at D and U1; log SS: Total suspendible (benthic) sediment (logarithm), NO3 U1: nitrate concentration at U1, p_agro_int: areal fraction of arable cropping and pastures in the catchment, p_Crop: areal fraction of arable cropping in the catchment Saprobic: saprobic index at D, Sap_U1: saprobix index at U1, SPEAR: SPEAR index at D, SPEAR_U1: SPEAR index at U1, WW_Q347: fraction of WW under low flow conditions.

SPEAR index										
Predicted	**Predictor**	**2013 sites**			**2014 sites**			**All sites**		
		Slope	**Std error**	**p-value**	**Slope**	**Std error**	**p-value**	**Slope**	**Std error**	**p-value**
NO3 U1	p_Crop	**6.79**	**0.67**	< 0.001	**6.56**	**0.82**	< 0.001	**6.71**	**0.51**	< 0.001
log_maxTU_PB U1	p_Crop	**2.41**	**0.70**	0.006	1.71	0.86	0.077	**2.16**	**0.57**	0.001
SPEAR U1	NO3 U1	-0.05	0.03	0.158	-0.11	0.05	0.077	**-0.10**	**0.03**	0.003
SPEAR U1	log_maxTU_PB U1	-0.11	0.07	0.120	-0.01	0.12	0.918	0.00	0.06	0.975
SPEAR D	SPEAR U1	**0.90**	**0.15**	< 0.001	**0.89**	**0.19**	0.002	**0.89**	**0.10**	< 0.001
SPEAR D	WW Q347	-0.24	0.22	0.317	-0.34	0.26	0.237	-0.31	0.15	0.054
SPEAR D	log SS	0.08	0.12	0.54	0.15	0.12	0.258	0.13	0.07	0.110
Adjusted R2 (SPEAR index):				**0.753**			**0.669**			**0.766**
Fisher's C		17.76		0.72	31.67		0.083	25.26		0.065
AIC		49.76			63.67			55.26		
AICc		-59.04			-27.00			123.83		
SPEAR index (without the site Hornussen)										
Predicted	**Predictor**	**2013 sites**			**2014 sites**			**All sites**		
		Slope	**Std error**	**p-value**	**Slope**	**Std error**	**p-value**	**Slope**	**Std error**	**p-value**
NO3 U1	p_Crop	**6.77**	**0.70**	< 0.001	**6.56**	**0.82**	< 0.001	**6.71**	**0.52**	< 0.001
log_maxTU_PB U1	p_Crop	**2.39**	**0.73**	0.010	1.71	0.86	0.077	**2.14**	**0.57**	0.001
SPEAR U1	NO3 U1	-0.05	0.03	0.173	-0.11	0.05	0.077	**-0.10**	**0.03**	0.003
SPEAR U1	log_maxTU_PB U1	-0.12	0.07	0.110	-0.01	0.12	0.918	-0.01	0.06	0.862
SPEAR D	SPEAR U1	**0.94**	**0.14**	< 0.001	**0.89**	**0.19**	0.002	**0.91**	**0.10**	< 0.001
SPEAR D	WW Q347	-0.36	0.22	0.142	-0.34	0.26	0.237	**-0.35**	**0.15**	0.031
SPEAR D	log SS	0.10	0.11	0.405	0.15	0.12	0.258	0.13	0.07	0.086
Adjusted R2 (SPEAR index):				**0.811**			**0.669**			**0.789**
Fisher's C		17.65		0.726	31.67		0.083		24.92	0.301
AIC		49.65			63.67				56.92	
AICc		-41.02			-27.00				165.72	
Saprobic index										
Predicted	**Predictor**	**2013 sites**			**2014 sites**			**All sites**		
		Slope	**Std error**	**p-value**	**Slope**	**Std error**	**p-value**	**Slope**	**Std error**	**p-value**
Sap U1	p_agri_int	0.33	0.28	0.2643	0.60	0.35	0.1182	**0.43**	**0.21**	0.0494
Delta_Sap	Sap U1	**-1.72**	**0.45**	0.0032	-0.63	0.50	0.2365	**-1.24**	**0.33**	0.0013
Saprobic	Delta_Sap	**0.65**	**0.09**	< 0.00001	**0.76**	**0.19**	0.0031	**0.68**	**0.09**	< 0.00001
Adjusted R2 (Saprobic index):				**0.824**			**0.600**			**0.737**

S3 Supplementary method descriptions

M1 Estimation of benthic suspendible sediment

The amount of total benthic suspendible sediment ($SS_{benthic}$; kg/m) was estimated using the 'Quorer method' (Quinn et al. 1997). $TSS_{benthic}$ is the sum of suspendible inorganic sediment ($SIS_{benthic}$) and suspendible organic sediment ($SOS_{benthic}$), as defined by (Clapcott et al. 2011). First, grab water samples were taken from the water column to determine the background level of total suspended sediments (TSS_{river}). Then the benthic substrate in an open drum (20 cm diameter, 0.03 m^2) at eight random locations within each sampling reach was vigorously disturbed using a steel rod (duration of 30 s) to collect replicate samples of total suspended sediment ($TSS_{benthic}$; i.e., a 250-mL water column sample taken from inside the drum). $TSS_{benthic}$ is the sum of the background level (TSS_{river}) and the benthic suspendible sediments ($SS_{benthic}$). Samples were placed in a cooling box on ice for transport before storage at 4°C in the laboratory until analysis. They were processed within 48 hours of collection.

To estimate the organic and inorganic fractions of the suspendible sediment, a subsample (50 mL) of the water sample was then filtered through a pre-ashed, pre-weighed filter (GF/C, 47 mm diameter), before being dried for 48 hours at 48° C, weighed, ashed for 4 hours at 400° C, and reweighed. The proportion of organic sediment was calculated by dividing the difference between the dried and ashed filter by the pre-ashed dry mass of the sample minus the mass of the filter. The estimates of benthic suspendible sediment were corrected for the background levels of suspended particles TSS_{river} (Eq. S1):

$$SS_{benthic} = TSS_{benthic} - TSS_{river} \qquad \text{(Eq. S1)}$$

Invertebrate community descriptors

To associate specific environmental stressors with invertebrate communities, we used three trait-based indices (the SPEAR Pesticides, Saprobic, and Sediment Index). We also calculated these trait-based indices minus the oligochaete worms, due to the strong influence of this taxa on community responses to wastewater (Burdon et al. 2016).

M2 SPEAR Index

The SPEAR Pesticides Index (SPEAR Index hereafter) describes the proportion of taxa (%) susceptible to pesticides. Lower relative abundances of SPEAR taxa indicate pesticide stress and it is used extensively as an index of stream health in Europe (Liess and von der Ohe 2005, Beketov et al. 2009). The Species At Risk (SPEAR) index used is designed to detect and quantify the effects of pesticides (e.g., insecticide toxicity) on macroinvertebrate communities (Schäfer et al. 2007, Schriever et al. 2007). This multiple trait-based approach links pesticide stress and community composition by utilizing traits that reflect the ecological requirements of the invertebrates and pesticide effects (Liess and von der Ohe 2005). The traits involved are 1) the physiological sensitivity to organic toxicants, 2) generation time, 3) presence of aquatic life stages during exposure, and 4) recovery potential. The SPEAR pesticides index is calculated as the relative abundance of sensitive taxa "at risk" to be affected by pesticides:

$$\mathrm{SPEAR}_{\mathrm{pesticides}} = \frac{\sum_{i=1}^{n} \log(x_i + 1) \times y}{\sum_{i=1}^{n} \log(x_i + 1)} \qquad \text{(Eq. S2)}$$

where n is the number of taxa, xi is the abundance of taxon i, and y is a binary variable (1 if taxon i is classified as SPEAR, 0 if not). Calculations were performed to the lowest practicable taxonomic levels for abundances; research suggests that the explanatory power of the family-level SPEAR pesticides is not significantly lower than the species-level index (Beketov et al. 2009).

For the calculation of the SPEAR pesticides index, the SPEAR Calculator v0.9.0 was used (Knillmann et al. 2018). In the SI we also report the results obtained with the SPEAR_Calculator 2016 (see Fig. S5. This differs from Burdon et al. (2016) where the SPEAR pesticides index was calculated using the now deprecated R package ‘rSpear’ (Szoecs 2013).

M3 Saprobic Index

The Saprobic Index ranges between 1 and 4, and increases with greater amounts of easily degradable organic material, indicating shifts in the invertebrate community towards taxa that are more tolerant of low oxygen conditions (Bunzel et al. 2013). Despite the extensive technological improvements in wastewater treatment, modern WWTPs can still be an important source of oxygen-depleting organic pollution (Bunzel et al. 2013). Thus, we used the invertebrate community data to calculate an index reflecting the saprobic condition of our sites. This was based on the German Saprobity Index, which is the core metric of organic pollution within the official German Water Framework Directive assessment system for macroinvertebrates (Bunzel et al. 2013).

German saprobic trait values for relevant taxa were obtained from: www.freshwaterecology.info. This online resource is a taxa and autecology database for freshwater organisms (Version 5.0, Date accessed: 26.03.15; for more information see Schmidt-Kloiber & Hering(2015)). Where relevant trait values were not available for taxa using German scores, alternative values from Austria and Slovakia were used. We used mean values for taxa where there were multiple genera within each group. Saprobic Index scores for individual taxa are listed in Burdon et al. (2016).. The Saprobic Index (SI), a number between 1 and 4, is the “weighted mean” of all individual indices calculated as:

$$SI = \sum_{i=1}^{n} (S_i \times a_i) \Bigg/ \sum_{j=1}^{n} a_j \qquad \text{(Eq. S3)}$$

where for any given taxa i the product of abundance ai and saprobic trait score Si expresses the saprobic value for that taxon. Higher SI values indicate shifts in the macroinvertebrate community towards species that are more tolerant of low oxygen conditions (Bunzel et al. 2013) .

The Saprobic index was calculated in Excel (Microsoft Excel 2010, Version 14).

M4 Sediment Index

The Sediment Index (otherwise known as the Empirically-weighted Proportion of Sediment-sensitive Invertebrate index; E-PSI) is a biomonitoring tool designed to identify the degree of sedimentation in rivers and streams (Extence et al. 2011). This calculates the proportion of sediment-sensitive taxa in a community; lower relative abundances of these taxa indicate sediment stress. The E-PSI was calculated using modified values from (Turley et al. 2016). Extence et al. (2011) originally assigned invertebrate sensitivity ratings following an extensive review of the literature and use of expert knowledge regarding ecological and biological traits. Traits that may result in sensitivity to fine sediment include feeding, locomotion, and respiratory attributes. Taxa with PSI (Proportion of Sediment-sensitive Invertebrate index) ratings of “highly sensitive” and “moderately sensitive” were constrained to sensitivity weights between 0.50 and 1.0, and those rated as “moderately insensitive” and “highly insensitive” between 0 and 0.49 (Turley et al. 2016). A modified version of the original PSI index’s equation was used to calculate E-PSI scores:

$$E\text{-}PSI = \sum_{i=1}^{n} log[(W_i \times a_i) + 1] / \sum_{j=1}^{n} log\left[\left(W_j \times a_j\right) + 1\right] \qquad \text{(Eq. S4)}$$

where for any given sediment-sensitive taxa i the product of abundance ai and the sensitivity weights score Wi expresses the sensitivity to fine sediment for that taxon. Similarly, for any given taxa j (sediment-sensitive and insensitive; i.e., all taxa) the product of abundance aj and the sensitivity weights score Wj expresses the sensitivity to fine sediment for that taxon. E-PSI scores range from 0 (heavily sedimented) to 100 (unsedimented).

M5 Calculation of effect sizes

The differences between upstream and downstream sites for the different variables are expressed as effect sizes by Cohen’s d according to the following equation:

$$\mathrm{d} = \frac{\overline{\mu_1} - \overline{\mu_2}}{\sqrt{\frac{(n_1-1)\times s_1^2 + (n_{21}-1)\times s_2^2}{n_1+n_2-2}}} \qquad \text{(Eq.S5)}$$

Because of different sampling strategies, the observations at the upstream and downstream locations were evaluated in slightly different manners (see Table S6).

Table S6: Overview about the evaluated variables.

Variable	**Reference stdev**	**Spatial difference**
General water chemistry (separately for each parameter) Parameters considered: Electr. conductivity, alkalinity, hardness, Na, K, Ca, Mg, Cl, SO4, SiO4, TSS	The standard σ deviations is calculated from the temporal averages at each of the 2 upstream locations	For each site, Cohen's d is calculated according to Eq. S5 (D – U1); Median value across sites is reported
Nutrients, Parameters considered: NH4, nitrite, NO3, TN, SRP, TP, TOC, DOC	The standard σ deviations is calculated from the temporal averages at each of the 2 upstream locations	For each site, Cohen's d is calculated according to Eq. S5 (D – U1); Median value across sites is reported
MP concentrations	The standard σ deviations per compound is calculated from the temporal average at the upstream location at each site (2 data in 2013; 6 in 2014); if concentrations < LOQ; σ is calculated from the variance between zero and the max. LOQ for the respective compound at this site	For each site and each compound, Cohen's d is calculated according to Eq. S5 (D – U1);
Heavy metal concentrations	The standard σ deviations is calculated from the temporal replicates at locations US1	For each site, Cohen's d is calculated as the median difference (D – U1) of the temporal replicates divided by σ. The median is taken across sites.
SPEAR index	The standard σ deviations is calculated from the 2 upstream locations	For each site, Cohen's d is calculated according to Eq. 5 (D – U1); Median value across sites is reported
Saprobic index	The standard σ deviations is calculated from the 2 upstream locations	For each site, Cohen's d is calculated according to Eq. 5 (D – U1); Median value across sites is reported
Rarefied Taxa richness EPT	The standard σ deviations is calculated from the 2 upstream locations	For each site, Cohen's d is calculated according to Eq. 5 (D – U1); Median value across sites is reported

References:

Beketov, M. A., K. Foit, R. B. Schäfer, C. A. Schriever, A. Sacchi, E. Capri, J. Biggs, C. Wells, and M. Liess. 2009. SPEAR indicates pesticide effects in streams - Comparative use of species- and family-level biomonitoring data. Environmental Pollution **157**:1841 - 1848.

Bunzel, K., M. Kattwinkel, and M. Liess. 2013. Effects of organic pollutants from wastewater treatment plants on aquatic invertebrate communities. Water Research **47**:597-606.

Burdon, F. J., M. Reyes, A. C. Alder, A. Joss, C. Ort, K. Räsänen, J. Jokela, R. I. L. Eggen, and C. Stamm. 2016. Environmental context and disturbance influence differing trait-mediated community responses to wastewater pollution in streams. Ecology and Evolution **6**:3923–3939, doi: 3910.1002/ece3923.2165.

Clapcott, J. E., R. G. Young, J. S. Harding, C. D. Matthaei, J. M. Quinn, and R. G. Death. 2011. Sediment assessment methods: protocols and guidelines for assessing the effects of deposited fine sediment on in-stream values. . Cawthron Institute, Nelson, New Zealand.

Extence, C. A., R. P. Chadd, J. England, M. J. Dunbar, P. J. Wood, and E. D. Taylor. 2011. The assessment of fine sediment accumulation in rivers using macroinvertebrate community response. River Research and Applications **29**:17-55.

Knillmann, S., P. Orlinskiy, O. Kaske, K. Foit, and M. Liess. 2018. Indication of pesticide effects and recolonization in streams. Science of the Total Environment **630**:1619-1627.

Liechti, P. 2010. Methoden zur Untersuchung und Beurteilung der Fliessgewässer. Chemisch-physikalische Erhebungen, Nährstoffe., Bundesamt für Umwelt, Bern.

Liess, M., and P. C. von der Ohe. 2005. Analyzing effects of pesticides on invertebrate communities in streams. Environmental Toxicology and Chemistry **24**:954-965.

Munz, N., L. Melo, M. Reyes, U. Schönenberger, H. Singer, B. Spycher, D. de Zwart, M. Junghans, J. Hollender, and C. Stamm. 2017. Pesticides drive risk of micropollutants in wastewater-impacted streams during low flow conditions. Water Research **110**:366–377.

Quinn, J. M., B. A. Cooper, R. J. Davies-Colley, J. C. Rutherford, and R. B. Williamson. 1997. Land use effects on habitat, water quality, periphyton and benthic invertebrates in Waikato hill-county streams. New Zealand Journal of Marine and Freshwater Research **31**.

Schäfer, R. B., T. Caquet, K. Siimes, R. Mueller, L. Lagadic, and M. Liess. 2007. Effects of pesticides on community structure and ecosystem functions in agricultural streams of three biogeographical regions in Europe. Science of the Total Environment **382**:272-285.

Schmidt-Kloiber, A., and D. Hering. 2015. www.freshwaterecology.info – An online tool that unifies, standardises and codifies more than 20,000 European freshwater organisms and their ecological preferences. Ecological Indicators **53**: 271-282.

Schriever, C. A., M. H. Ball, C. Holmes, S. Maund, and M. Liess. 2007. Agricultural intensity and landscape structure: Influences on the macroinvertebrate assemblages of small streams in northern Germany. Environmental Toxicology and Chemistry **26**:346-357.

Szoecs, E. 2013. rSpear: Calculate SPEAR pesticides in R (http://www.systemecology.eu/SPEAR/index.php).http://cran.r-project.org/web/packages/rspear).

Turley, M. D., G. S. Bilotta, R. P. Chadd, C. A. Extence, R. E. Brazier, N. G. Burnside, and A. G. G. Pickwell. 2016. A sediment-specific family-level biomonitoring tool to identify the impacts of fine sediment in temperate rivers and streams. Ecological Indicators **70**:151-165.