S0 Contents

The supplement contains additional figures (S1), additional tables (S2), additional methods (S3), detailed results about the hydrologic variables (S4), frequency thresholds for data cleaning (S5, separate file) and a species list (S6, separate file).

S1 Additional Figures

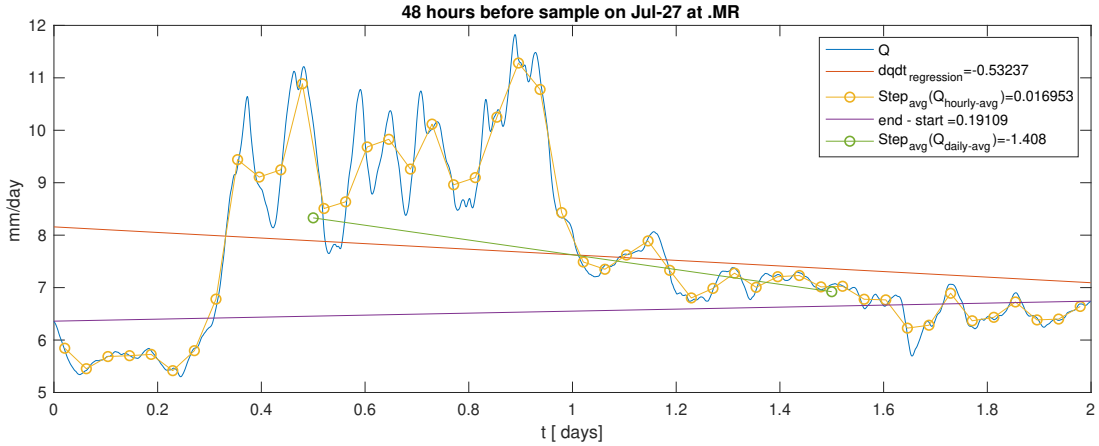

Figure S1. Example determination of *dq/dt* at the morning river "MR" on July 27, 2017, showing four different methods as they relate to discharge. Red line shows the regression of all measurements against time, which was retained. Yellow points show the hourly average of Q used to determine *dq/dt* by hourly time-step. Purple line shows the *dq/dt* as determined by the single, 2-day time step and finally the green dots show the daily average and the *dq/dt* determine by their difference.

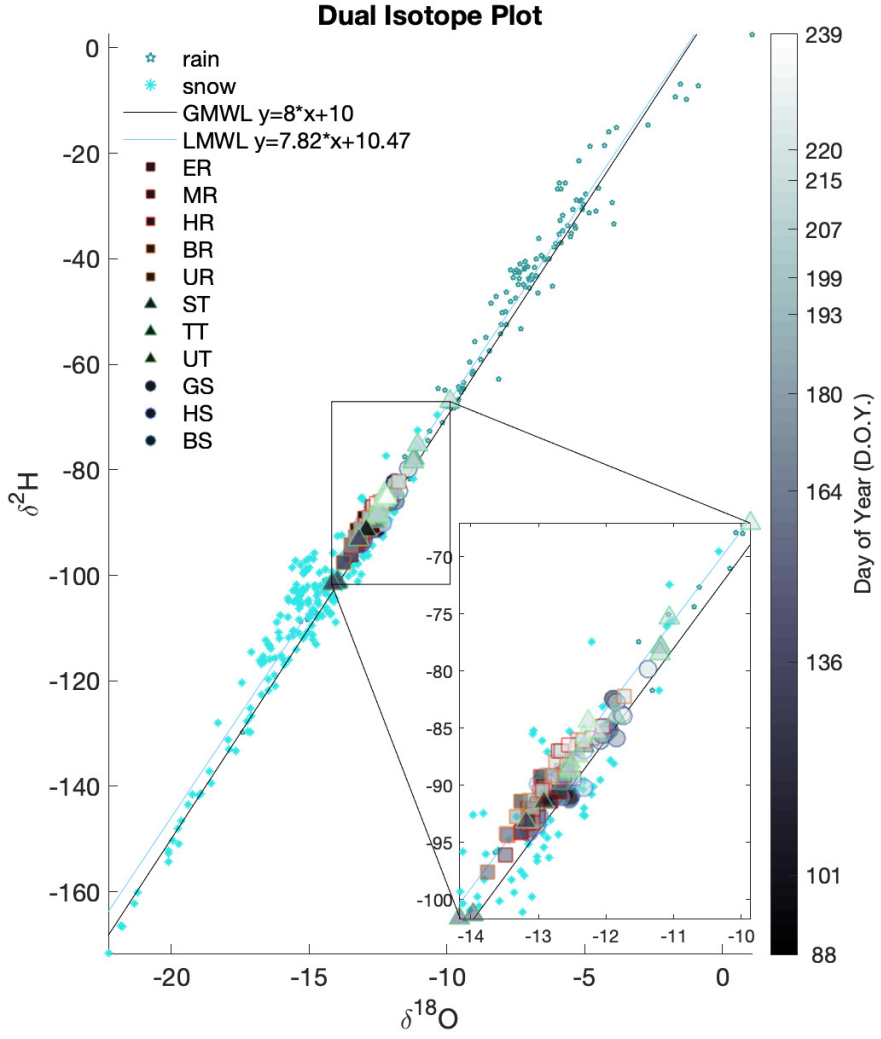

Figure S2. Stable isotopes from precipitation (rain in cyan stars, snow in cyan asterisks) and eDNA sampling sites (red squares are main channel, green triangles are tributaries, and blue circles are springs). Color shading shows date of sample. Local and global meteoric water lines are shown (LMWL, GMWL). The LMWL is calculated with all precipitation samples shown and used to calculate the line conditioned excess, lc-ex. A magnifying inset zooms onto all the samples. Metadata of precipitation isotopes is found in Table S3.

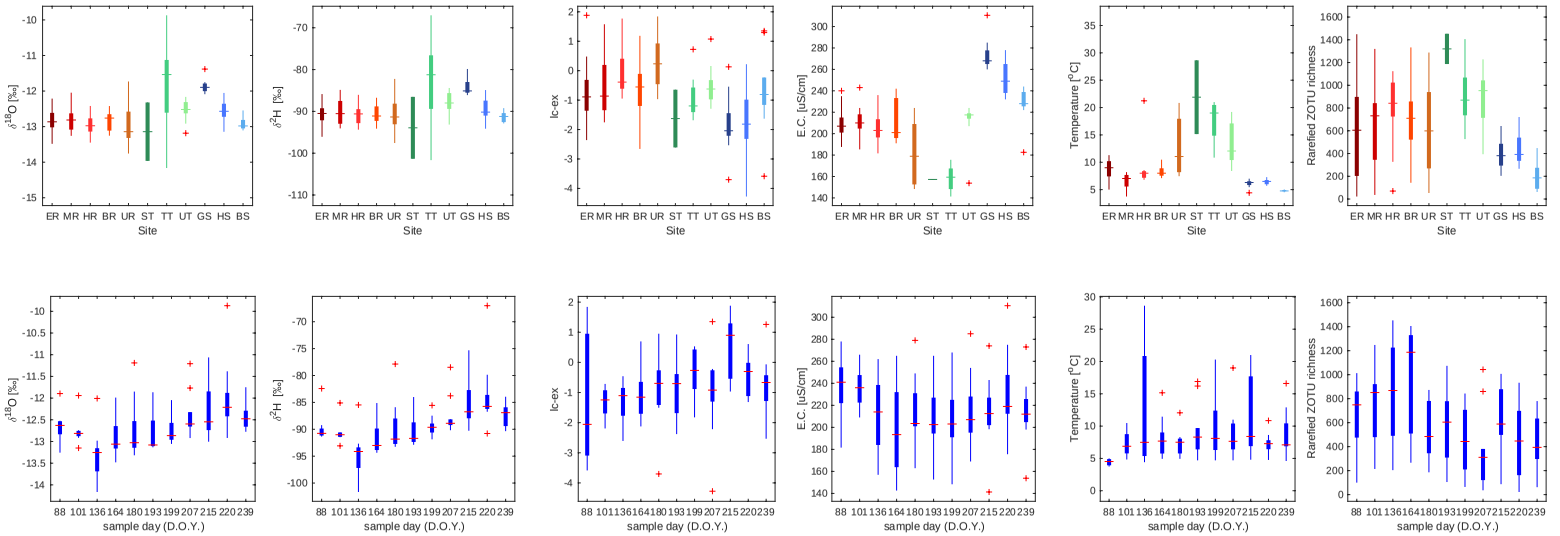

Figure S3. Spatial variability (first row) and temporal variability (second row) is shown as boxplots displaying the median (central box), 25th and 75th percentiles (bottom and top box edges), range (whiskers), and outliers ('+') for $\delta^{18}O$, $\delta^{2}H$, lc-excess, E.C., temperature, and rarefied ZOTU richness over all sampling dates (from left to right).

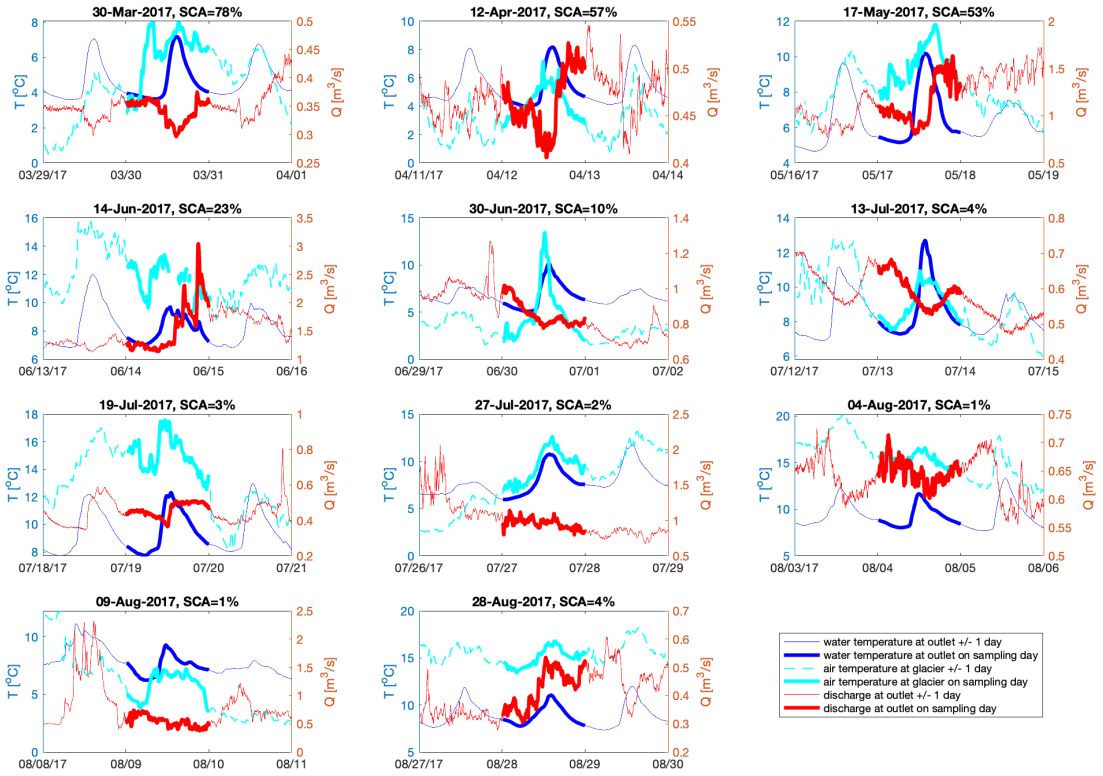

Figure S4. Water temperature at outlet (blue, $[^oC]$), air temperature near glacier (cyan, $[^oC]$), and discharge at outlet (red, $[m^3/s]$) on all sampling days (bold) and the previous and following days (regular weight or dashed). The interpolated daily snow covered area (SCA) is shown as the % of the catchment area in the title.

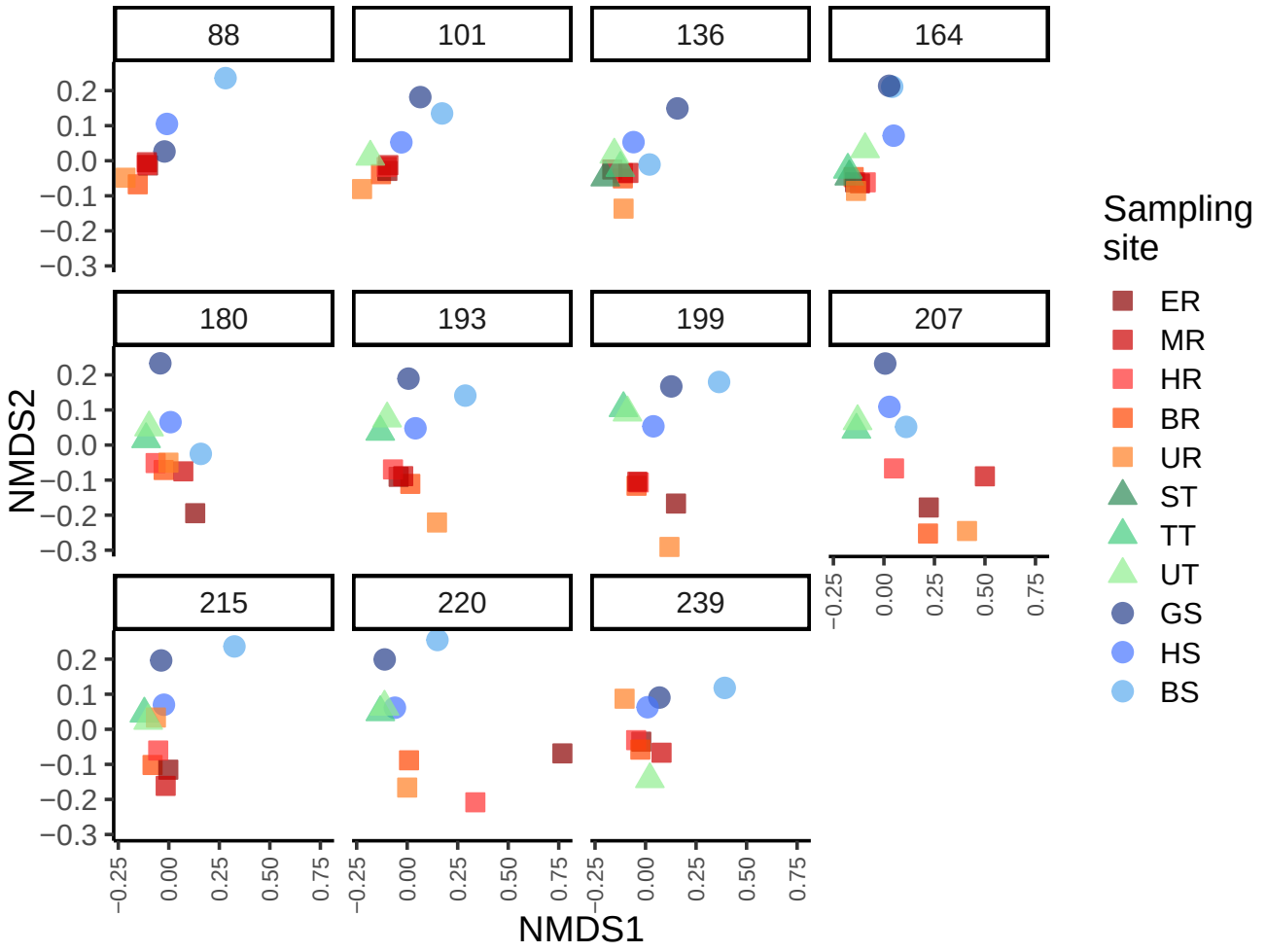

Figure S5. Community composition in the main river channel, tributaries and springs over the sampling season. Facets give the day of the year.

S2 Additional Tables

Table S1. Sampling dates with corresponding day of the year (DOY) and approximate sampling time of the individual sites. NS indicates that the specific site was not sampled at that date.

Sampling day	DOY	ER	MR	HR	BR	UR	ST	TT	UT	GS	HS	BS
March 30	88	18:39	10:34	13:40	15:00	16:50	NS	NS	NS	12:00	13:40	15:00
April 12	101	18:01	09:20	12:30	13:48	16:00	NS	NS	16:00	10:30	12:30	13:18
May 17	136	19:00	09:30	13:30	14:30	15:50	17:30	16:55	15:50	11:20	13:10	14:30
June 14	164	18:00	08:30	13:52	14:21	15:52	17:15	16:22	15:52	12:01	13:52	14:21
June 30	180	18:15	09:30	11:10	13:20	14:25	NS	16:10	14:50	10:20	12:10	13:00
July 13	193	19:20	09:20	11:20	12:10	13:25	NS	17:20	12:55	10:00	11:00	12:30
July 19	199	16:05	08:50	11:05	11:40	12:45	NS	14:05	13:25	09:55	10:40	14:40
July 27	207	16:30	08:40	10:35	15:10	13:20	NS	14:25	14:00	09:30	10:15	14:55
August 4	215	15:50	10:00	11:30	12:40	14:05	NS	14:40	13:45	10:45	11:50	12:55
August 9	220	18:00	09:15	11:30	13:20	14:30	NS	16:20	15:15	10:20	12:00	13:05
August 28	239	18:35	09:23	11:12	12:17	15:12	NS	NS	15:30	17:40	11:42	13:34

Table S2. Table with detailed metadata. Mean and standard deviation of main variables for each sampling site.

Site	**Lat.**	**Long.**	**Elev.** [m. a.s.l.]	**Dist. main** [m]	**Dist. outlet** [km]	**eDNA samp.** [no]	$\delta^{18}O$ [‰]	$\delta^{2}H$ [‰]	**lc-ex**	**E.C.** [$\mu S/cm$]	**Temp.** [^oC]	**ZOTU richness**	**Q** *	**Order** †
ER	46.253	7.109	1248	0	0	11	-12.82 (0.34)	-90.53 (2.79)	-0.75 (1.2)	210 (16)	8.6 (1.9)[1]	637 (484)	m	4
MR	46.253	7.109	1248	0	0	10	-12.81 (0.35)	-90.22 (3.05)	-0.51 (1.13)	212 (14)	6.6 (1.4)[1]	645 (363)	m	4
HR	46.235	7.104	1435	0	2.6	11	-12.95 (0.29)	-90.91 (2.43)	-0.1 (0.81)	205 (14)	9.2 (4.5)[2]	775 (321)	m	4
BR	46.232	7.102	1477	0	3.4	11	-12.86 (0.27)	-90.7 (2.38)	-0.61 (1.04)	211 (20)	8.3 (1.0)[3]	697 (306)	m	4
UR	46.226	7.098	1519	0	4.2	11	-12.96 (0.6)	-90.6 (4.37)	0.25 (0.88)	183 (30)	12.8 (5.4)[4]	623 (416)	m	4
ST	46.240	7.105	1381	78	2.1	2	-13.14 (1.16)	-93.93 (10.47)	-1.63 (1.39)	157 (-)	- (-)	1320 (188)	l	2
TT	46.240	7.104	1518	100	3.2	8	-11.82 (1.3)	-82.89 (10.64)	-0.91 (0.79)	158 (12)	17.5 (4.1)[5]	910 (282)	l	2
UT	46.226	7.097	1538	10	4.2	9	-12.54 (0.32)	-88.09 (2.85)	-0.51 (0.73)	211 (20)	13.1 (3.9)[6]	879 (248)	l	1
GS	46.247	7.106	1351	50	.7	11	-11.87 (0.2)	-84.18 (1.9)	-1.83 (1.01)	274 (14)	6.1 (0.8)[7]	387 (130)	NA	NA
HS	46.235	7.105	1445	40	2.7	11	-12.59 (0.31)	-89.76 (2.85)	-1.8 (1.29)	252 (16)	6.4 (0.5)[8]	443 (141)	NA	NA
BS	46.232	7.103	1469	5	3.3	11	-12.93 (0.17)	-91.31 (1.24)	-0.69 (1.35)	228 (17)	4.8 (0.2)[9]	189 (125)	NA	NA

*Annual Mean Discharge of characteristic type. "m" refers to medium flow, 0.05 - 1 $[m^3/s]$ and "l" refers to low flow, < 0.05 $[m^3/s]$. See: Schaffner et al., 2013.
†Strahler Order. See: Pfaundler, 2005
Note that all variables show the mean value followed by the standard deviation in parentheses. Further detail regarding available temperature measurements. These values were determined using the punctual measurements made with the WTW (multi-3510 with a IDS-tetracon-925, Xylem Analytics, Germany) at the point of sampling whenever possible. Hand measurements were considered preferable to the those from the loggers because they were taken at the exact same location of the sample; however, in the case that those measures were missing, a punctual measurement was used from the temperature logged as follows (instrument, D.O.Y. 2017 start-finish, interval in minutes):

1. HOBO 64K Pendant Temperature/Light, 102 - 187, 15 & WTW TetraCon 325/C, 1 - 365, 1.
2. HOBO 64K Pendant Temperature/Light, 136 - 187, 15.
3. HOBO 64K Pendant Temperature/Light, 102 - 345, 15 & HOBO U24-001 temperature/conductivity, 95 - 299 , 10.
4. HOBO 64K Pendant Temperature/Light, 136 - 194, 15.
5. HOBO 64K Pendant Temperature/Light, 136 - 165, 15.
6. HOBO 64K Pendant Temperature/Light, 125 - 258, 5.
7. HOBO 64K Pendant Temperature/Light, 102 - 365, 15.
8. HOBO 64K Pendant Temperature/Light, 102 - 365, 15.
9. HOBO 64K Pendant Temperature/Light, 1 - 365, 15.

Table S3. Metadata of precipitation samples shown in Figure S2 and used for calculation of lc-ex. Only months with samples are listed. Samples are counted by month and elevation band (low, mid, and high), corresponding to 1240-1500, 1500-2000, and 2000-2456 m a.s.l., respectively. For rain samples, the number of samples is reported. For snow samples, when possible, the number of samples is followed by, in parentheses, the mean sample depth [cm], ± the standard deviation of sample depth [cm], and < the maximum sample depth [cm]. When no information about depth is reported, samples are from the snowpack surface.

Year	Month	Rain Samples			Snow Samples		
		low	mid	high	low	mid	high
2016	Feb				16 (19±17,<60)		
	Mar				12 (47±37,<120)		5 (43±41,<114)
	Jun	3					
	Jul	5				1	2
	Aug	3					
	Sep	6					
	Oct	6					
	Nov	4			23 (11±8,<39)	7 (12±2,<27)	4
2017	Jan				22 (18±11,<43)	3 (18±12,<36)	
	Feb				15 (26±17,<64)		3 (38±29,<79)
	May	1					
	Jun	5	7				
	Jul	3	3				
	Aug	4	5				
	Sep	3	3				1
	Oct	3	1				
	Nov	2	2		21 (7±5,<29)	6 (8±3,<23)	
	Dec				26 (36±21,<97)	5 (20±1,<41)	
2018	Jan				3		
	Feb				9 (62±11,<157)	3 (65±8,<147)	
	Apr				9 (16±7,<50)	3 (16±10,<53)	
	May	5	6				
	Jun	3	3				
	Jul	2	3				
	Aug	4	4				
	Sep	6	15	9			
	Oct	2		2			
	Nov	1	1				

Table S4. Forward and reverse primer sequence for tailed PCR reactions.

Primer name	Primer sequence (5'-3')
mlCOIintF-FS0	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGGGWACWGGWTGAACWGTWTAYCCYC
mlCOIintF-FS1	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGGGGWACWGGWTGAACWGTWTAYCCYCC
mlCOIintF-FS3	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGTAGGGWACWGGWTGAACWGTWTAYCCYCC
jgHCO2198-FS0	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGTAIACYTCIGGRTGICCRAARAAYCA
jgHCO2198-FS1	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGTTAIACYTCIGGRTGICCRAARAAYCA
jgHCO2198-FS2	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGTATAIACYTCIGGRTGICCRAARAAYCA

Table S5. Implemented data filtering steps and the remaining numbers of ZOTUs and reads in the data set.

Filtering step	ZOTUs	Number of reads
Raw data	9,858	12,548,868
Samples only	9,760	9,859,536
Cleaned samples	9,693	9,728,558
Rarefied samples	9,622	2,863,213

Table S6. Pairwise comparison results from a Kruskal Wallis multiple comparison test for 6 variables in Fig. 3 according to 3 water types: main channel (M), spring (S), tributary (T). Significant p-values (< 0.05) are in boldface.

Variable	Compared Groups	Lower Limit 95% Confidence Interval	Est. Group Mean Difference	Upper Limit 95% Confidence Interval	P-value
$\delta^{18}O$	M-S	-39.8517	-23.931	-8.0103	**0.0012**
	M-T	-48.5286	-29.309	-10.0894	**0.001**
	S-T	-26.1283	-5.378	15.3723	0.8161
$\delta^{2}H$	M-S	-32.8618	-16.9411	-1.0204	**0.0338**
	M-T	-45.1718	-25.9522	-6.7327	**0.0044**
	S-T	-29.7615	-9.0112	11.7391	0.5656
lc-ex	M-S	10.9177	26.8384	42.7591	**< 0.001**
	M-T	-6.6348	12.5848	31.8044	0.2747
	S-T	-35.0039	-14.2536	6.4967	0.2414
E.C.	M-S	-60.7102	-44.6414	-28.5726	**< 0.001**
	M-T	-7.4973	11.5389	30.5751	0.3302
	S-T	35.572	56.1803	76.7886	**< 0.001**
Temperature	M-S	19.5197	34.4982	49.4768	**< 0.001**
	M-T	-46.4059	-27.9974	-9.589	**0.0011**
	S-T	-82.4054	-62.4957	-42.5859	**< 0.001**
Rarefied ZOTU Richness	M-S	12.8676	28.9377	45.0079	**< 0.001**
	M-T	-41.4221	-22.3843	-3.3464	**0.0161**
	S-T	-71.9321	-51.322	-30.7119	**< 0.001**

Table S7. ANOVA table for the Kruskal-Wallis test for 6 variables in Fig. 3 according to water type (main channel, tributary, spring) captures the variability in the model by source. The columns refer to the variables, the source of the variability, the sum of squares (SS) due to each source, the degrees of freedom associated to each source (df), the mean squares for each source (MS), χ^2 statistic, and the p-value (Prob> χ^2) which is the probability that the χ^2 statistic can take a value larger than the computer test-statistic value. The rows for each variable refer to the sources of variability which are: variability due to the difference among the group means or between groups (Groups), variability due to difference between the data in each group and the group mean or within groups (Error), and the total variability (Total).

Variable	Source	SS	df	MS	χ^2	Prob>χ^2
$\delta^{18}O$	Groups	18113	2	9057	19	< 0.001
	Error	81129	103	788		
	Total	99243	105			
δ^2H	Groups	11824	2	5912	13	0.0019
	Error	87418	103	849		
	Total	99243	105			
lc-ex	Groups	14844	2	7422	16	< 0.001
	Error	84399	103	819		
	Total	99243	105			
E.C.	Groups	54002	2	27001	56	< 0.001
	Error	48058	104	462		
	Total	102060	106			
Temperature	Groups	11454	2	5727	40	< 0.001
	Error	4795	55	87		
	Total	16249	57			
Rarefied ZOTU richness	Groups	35250	2	17625	37	< 0.001
	Error	66827	104	643		
	Total	102078	106			

Table S8. Results for vector fitting onto NMDS ordination. NMDS1 stands for the first NMDS axis and NMDS2 for the second axis.

Environmental variable	NMDS1	NMDS2	R^2	P-value
Elevation	-0.41804	0.90843	0.0361	0.209
E.C.	0.10819	0.99413	0.3815	0.001
Temperature	-0.84226	-0.53907	0.1325	0.004
δ^{18}O	-0.10514	0.99446	0.1227	0.005
lc-ex	0.02894	-0.99958	0.2125	0.001
Total solar radiation	-0.33603	-0.94185	0.0080	0.745
Baseflow	-0.97878	-0.20491	0.0202	0.435
Snow cover area	0.76542	0.64354	0.0403	0.160
dq/dt	-0.95751	0.28840	0.0463	0.132

Table S9: List of ZOTUs that linked to water types with their specific association- and p-values as provided by a permutation test. We give the Latin and common names (if available) of the taxonomic assignment. Further, as well as their associated habitat (a = aquatic; t = terrestrial; a, t = species that can inhabit both, for example, when they have a larval stage in the water).

Water type	ZOTU identity	Association value	P-value	Order	Family	Species	Common name†	Habitat‡
River	ZOTU13048	0.623	0.001	Diptera	Chironomidae	*Diamesa modesta*	NA	a, t
	ZOTU8411	0.435	0.002	Diptera	Chironomidae	*Micropsectra notescens*	NA	a, t
	ZOTU11229	0.416	0.001	Diptera	Chironomidae	*Diamesa tonsa*	NA	a, t
	ZOTU8853	0.401	0.001	Diptera	Chironomidae	*Diamesa tonsa*	NA	a, t
	ZOTU9647	0.395	0.002	Diptera	Chironomidae	*Diamesa zernyi*	NA	a, t
	ZOTU990	0.386	0.002	Haplotaxida	Lumbricidae	*Aporrectodea sp.*	Earthworms	t
	ZOTU475	0.383	0.002	Haplotaxida	Lumbricidae	*Eiseniella tetraedra*	Squaretail worm	a
	ZOTU1031	0.363	0.003	Lepidoptera	Tortricidae	*Epinotia tedella*	Common Spruce Bell	t
	ZOTU9326	0.363	0.002	Diptera	Chironomidae	*Diamesa zernyi*	NA	a, t
	ZOTU8176	0.322	0.007	Entomobryomorpha	Entomobryidae	*Entomobrya marginata*	Springtail	t
	ZOTU12401	0.322	0.010	Hymenoptera	Formicidae	*Lasius sp.*	NA	t
	ZOTU443	0.306	0.011	Haplotaxida	Lumbricidae	*Eiseniella tetraedra*	Squaretail worm	a
	ZOTU10773	0.281	0.022	Diptera	Chironomidae	*Diamesa steinboecki*	NA	a, t
	ZOTU6952	0.252	0.040	Caudata	Salamandridae	*Salamandra atra*	Alpine salamander	a, t
	ZOTU3700	0.252	0.044	Diptera	Syrphidae	*Eupeodes sp.*	NA	t
Spring	ZOTU6412	0.29	0.012	Passeriformes	Turdidae	*Turdus philomelos*	Song thrush	t
	ZOTU12404	0.25	0.039	Diptera	Simuliidae	*Simulium sp.*	NA	a, t
	ZOTU131	0.25	0.039	Diptera	Chironomidae	*Corynoneura lobata*	NA	a, t
Tributary	ZOTU578	0.500	0.001	Diptera	Chironomidae	*Micropsectra sofiae*	NA	a, t
	ZOTU780	0.471	0.001	Haplotaxida	Lumbricidae	*Octolasion lacteum*	NA	t
	ZOTU3113	0.398	0.001	Diptera	Chironomidae	*Micropsectra sofiae*	NA	a, t
	ZOTU13064	0.397	0.001	Diptera	Simuliidae	*Simulium sp.*	NA	a, t
	ZOTU6150	0.378	0.001	Coleoptera	Psephenidae	*Eubria palustris*	NA	a
	ZOTU7186	0.392	0.002	Diptera	Simuliidae	*Simulium sp.*	NA	a, t
	ZOTU3489	0.375	0.002	Galliformes	Phasianidae	*Lagopus muta*	Rock Ptarmigan	t
	ZOTU4330	0.375	0.002	Anura	Ranidae	*Rana temporaria*	Common frog	a, t
	ZOTU2275	0.367	0.003	Plecoptera	Leuctridae	*Leuctra alpina*	NA	a, t
	ZOTU2186	0.349	0.003	Anura	Ranidae	*Rana temporaria*	Common frog	a, t
	ZOTU9232	0.350	0.004	Diptera	Chironomidae	*Diamesa latitarsis*	NA	a, t
	ZOTU5074	0.346	0.005	Lithobiomorpha	Lithobiidae	*Eupolybothrus tridentinus*	NA	t
	ZOTU6692	0.282	0.008		Cervidae	*Cervus elaphus*	Red Deer	t
	ZOTU7259	0.324	0.009	Diptera	Simuliidae	*Simulium murvanidzei*	NA	a, t
	ZOTU6857	0.323	0.010	Poduromorpha	Hypogastruridae	*Ceratophysella sp.*	NA	t
	ZOTU1478	0.304	0.011	Hemiptera	Aphrophoridae	*Cervus elaphus*	Red Deer	t
	ZOTU3413	0.283	0.015	Haplotaxida	Enchytraeidae	*Henlea perpusilla**	NA	a
	ZOTU3390	0.282	0.015		Bovidae	*Rupicapra rupicapra*	Chamois	t
	ZOTU10316	0.263	0.018	Diptera	Pipunculidae	*Eudorylas longifrons*	NA	t
	ZOTU2393	0.291	0.022	Trichoptera	Limnephilidae	*Halesus rubricollis*	NA	a, t

- continued on next page -

Table S9 – continued from previous page

Water type	ZOTU identity	Association value	P-value	Order	Family	Species	Common name†	Habitat‡
	ZOTU7851	0.288	0.023	Hymenoptera	Tenthredinidae	*Phymatocera aterrima*	NA	t
	ZOTU3345	0.256	0.025	Stylommatophora	Cochlicopidae	*Cochlicopa lubrica*	Glossy Pillar	t
	ZOTU7646	0.260	0.026	Diptera	Chironomidae	*Limnophyes pentaplastus*	NA	a, t
	ZOTU8749	0.263	0.030	Diptera	Limoniidae	*Dicranomyia didyma*	NA	a, t
	ZOTU4677	0.244	0.038	Haplotaxida	Enchytraeidae	*Henlea nasuta**	NA	a
	ZOTU7966	0.263	0.040	Diptera	Hybotidae	*Platypalpus sp.*	NA	a, t
River & tributary	ZOTU2010	0.591	0.001	Diptera	Simuliidae	*Prosimulium latimucro*	NA	a, t
	ZOTU80	0.575	0.001	Haplotaxida	Lumbricidae	*Lumbricus rubellus*	Red Earthworm	t
	ZOTU7461	0.458	0.001	Diptera	Chironomidae	*Diamesa bertrami*	NA	a, t
	ZOTU400	0.433	0.001	Haplotaxida	Lumbricidae	*Eiseniella tetraedra*	Squaretail worm	a
	ZOTU9055	0.397	0.001	Diptera	Chironomidae	*Diamesa zernyi*	NA	a, t
	ZOTU417	0.394	0.001	Haplotaxida	Lumbricidae	*Lumbricus rubellus*	Red Earthworm	t
	ZOTU6858	0.382	0.003	Diptera	Chironomidae	*Diamesa zernyi*	NA	a, t
	ZOTU5294	0.380	0.003	Diptera	Simuliidae	*Prosimulium latimucro*	NA	a, t
	ZOTU8606	0.368	0.006	Diptera	Chironomidae	*Diamesa sp.*	NA	a, t
	ZOTU2447	0.361	0.002	Haplotaxida	Lumbricidae	*Octolasion tyrtaeum*	Woodland white worm	t
	ZOTU9821	0.357	0.002	Diptera	Simuliidae	*Prosimulium latimucro*	NA	a, t
	ZOTU716	0.335	0.005	Lithobiomorpha	Lithobiidae	*Lithobius forficatus*	Common Centipede	t
	ZOTU10370	0.323	0.005	Haplotaxida	Lumbricidae	*Eiseniella tetraedra*	Squaretail worm	a
	ZOTU569	0.313	0.013	Diptera	Simuliidae	*Prosimulium hirtipes*	NA	a, t
	ZOTU10912	0.281	0.020	Coleoptera	Cantharidae	*Ancistronycha abdominalis*	NA	t
	ZOTU8847	0.271	0.026	Diptera	Chironomidae	*Paratrichocladius osellai*	NA	a, t
	ZOTU424	0.243	0.040	Passeriformes	Corvidae	*Pyrrhocorax graculus*	Yellow Billed Chough	t

†Based on Encyclopedia of Life data base (www.eol.org, accessed September 14, 2020).
‡Based on personal knowledge and the Encyclopedia of Life data base (www.eol.org, accessed September 14, 2020). If information was not available we performed or quick internet search.
*Marine species, misidentification potentially due to inaccuracy in the PCR or wrong entry in the data base.

Table S10. Results of the GLMM model selection based on a χ^2-test. DF = degrees of freedom, AIC = Akaike information criterion, BIC = Bayesian's information criterion, logLik = log-likelihood.

Model	DF	AIC	BIC	logLik	Deviance	χ^2	χ^2 DF	P-value
dq/dt + water source + (1|Sampling Site)	5	13308	13322	-6649.1	13298			
dq/dt * water source + (1|Sampling Site)	7	11608	11608	-5787.4	11575	1723.4	2	< 0.001
E.C. + water source + (1|Sampling Site)	5	15713	15727	-7851.6	15703			
E.C. * water source+ (1|Sampling Site)	7	15669	15687	-7827.3	15655	48.629	2	< 0.001

Table S11. Generalized linear mixed effect model results for the random effects. Std. Dev = standard deviation.

Model	Random effect	Variance	Std. Dev.
dq/dt	Sampling site (intercept)	0.043	0.208
E.C.	Sampling site (intercept)	0.040	0.201

Table S12. Results of the generalized linear model selection based on a F-tests. DF = degrees of freedom.

Model	Residual DF	Residual Deviance	DF	Deviance	F	P-value
dq/dt + origin	105	1.00250				
dq/dt * origin	104	0.90183	1	0.10067	11.677	< 0.001
E.C. + origin	105	1.0421				
E.C. * origin	104	1.0359	1	0.0062649	0.6468	0.4231

S3 Metabarcoding laboratory procedure

S3.1 Extraction of eDNA

After the collection of all the eDNA samples, we extracted the samples in a randomized order. We used the DNeasy® Blood and Tissue kit (Qiagen, Hilden, Germany) following the protocol for animal tissue besides a few changes due to the pooling of two filters per site for the extraction. We incubated the first filter with 360 µL ATL buffer and 40 µL Proteinase K for 24 hours. Afterward, we transferred the incubated filter into a new 1.5 mL tube with a hole on the bottom. This tube was then placed into the tube with the remaining incubated buffer mixture. The tube-tube compound was centrifuged for 1 minute at 6000 g in order to remove all the liquid from the filter in the upper tube. The upper tube containing the filter was then discharged. We added the second filter to the lower tube containing the buffer mixture and added another 40 µL of Proteinase K. After an incubation of 24 hours, we used the same method as described above to remove all liquid from the second filter. From this step onwards, we followed the protocol with the only change of using the doubled volumes in order to keep the volume:volume ratios equal until the liquid was pipetted on the spin columns in step 4 of the provided protocol. Finally, the eDNA was eluted in 75 µL AE buffer and stored at –20 °C until further processing.

S3.2 Library preparation

We target a 313 bp fragment of the cytochrome oxidase I (COI, Geller et al. 2013, Leray et al. 2013) for amplicon sequencing. We used an Illumina MiSeq dual-barcoded two-step PCR amplicon sequencing protocol. First, we performed a PCR with modified primers that contained an adaptor-specific tail, a heterogeneity spacer, and the amplicon target site (see Table S4). The extracted eDNA samples were randomized over four 96 well PCR plates. A single PCR reaction consisted of 1X Buffer I (ThermoFisher Scientific, Illkirch Cedex, France), BSA (0.1 mg/µL, GeneON, Ludwigshafen am Rhein, Germany), dNTP (0.2 mM), MgCl2 (1 mM), mICOIintF and jgHCO2198 primer mixes (0.16 µM each, see Table S1), AmpliTaq Gold (1.25 U/µL), and 3 µL of extracted eDNA in a total reaction volume of 30 µL. The PCR regime consisted of 95 °C for ten minutes, followed by 50 cycles of denaturation at 95 °C for 15 seconds, annealing at 62 °C for 30 seconds and elongation at 72 °C for 45 seconds, ending the PCR with a final hold of 72 °C for 5 minutes. Amplification success was verified with the QiAxcel Screening Cartridge by using the AL420 method (Qiagen, Hilden, Germany). Samples that did not amplify were rerun with 1 µL of DNA template instead of 3 µL due to previous indication of inhibited PCR. Per PCR plate we run a positive control (PC) consisting of 2 µL sample and 1 µL artificial DNA (0.01 ng/µL, see Mächler et al., 2019).

For each eDNA sample we run five replicates of the tailed PCR reaction, pooled them and cleaned the pool with the illustra GFX 96 PCR Purification Kit (GE Healthcare, Glattbrugg, Switzerland). Afterwards, we performed a second PCR where we add an index to each of the samples by using the Nextera XT Index kit v2 (Illumina, Zurich, Switzerland). The indexed reactions were cleaned up with SPRI beads (Beckman Coulter, Germany). The indexed reactions were quantified with the Spark® 10M Multimode Microplate Reader (Tecan Group Ltd., Männedorf, Switzerland) and pooled them in equimolar parts into a final pool that we cleaned with SPRI beads. All controls (FC, EC, PC, NC) were run alongside the samples and were pooled according to their concentrations. Controls that were too low to quantify were pooled into the second lowest concentrated pool with 10 μL, equal to the volume of the lowest sample in the respective pool. The libraries were added at 16 pM concentration and PhiX control was added at a 10% concentration. A paired-end (2x300 nt) sequencing was performed on an Illumina MiSeq (MiSeq Reagent kit v3, 300 cycles) following the manufacture's run protocols (Illumina, California, USA).

S3.3 Laboratory conditions and negative controls

We followed the previously described measures for work with eDNA (Deiner and Altermatt, 2014; Deiner et al., 2015; Mächler et al., 2015) in order to minimize contamination. Reused field material like filter housings and syringes were soaked 40 minutes in 2.5 % sodium hypochlorite (i.e. bleach), rinsed with deionized water and treated with UV light prior to the reuse in the field. Further, we implemented three different level of negative controls: First, at the beginning of each field day we implemented a negative filter control (FC) consisting of 1 L MilliQ water that was previously treated with UV-C light and brought to the field to check if reused material was clean. Second, we included for each batch of extractions a negative extraction control (EC) which contained a previously UV treated GF/F filter resulting in 8 extraction controls. Third, we included a negative PCR

control (NC) in each PCR plate containing sigma water instead of DNA. All negative controls were run alongside the eDNA samples during the laboratory workflow.

S3.4 Bioinformatic data processing

The data was demultiplexed and the quality of the reads was checked with FastQC (Andrews et al., 2010). Raw reads were end-trimmed (usearch v10.0.240, R1:30nt, R2:50nt) and merged with an overlap of min 15 bp max 300 bp (Flash, v1.2.11). Next, the primer sites were removed (full length, no mismatch allowed (cutadapt v1.12) and thereafter, the data was quality filtered (prinseq-lite v0.20.4) using the following parameters: size range (100–500), GC range (30–70), mean quality (20), and low complexity filter dust (30). In a next step, UNOISE3 (usearch v10.0.240) was used to determine amplicon sequence variants with a clustering at 97% sequence identity. UNOISE3 has a build-in error-correction to reduce the influence of sequencing errors (Edgar, 2016). An additional clustering at 99% sequence identity was performed to reduce sequence diversity and to account for possible amplification errors in the first PCR resulting in ZOTUs (zero-radius OTUs, thereafter called ZOTUs). This resulted in 12.5 M reads corresponding to 9858 ZOTUS. As a final step, the ZOTUs were assigned to taxa (blast 2.3.0 and usearch v10.0.240, tax filter = 0.9).

S4 Detailed results on hydro-meteorological variables

Annual precipitation in 2017 was 1760 mm, which is close to the long term average of 1920 mm over the period 1961 - 2017 (minimum 1470 mm, maximum 2600 mm, MeteoSwiss 2011). Approximately one third of precipitation measured at the 3 meteorological stations was snow (Fig. **??**, MeteoSwiss 2011). Snow melt peaked in early June, snow cover was reduced to 9% of the watershed area by July 1 (DOY 181), and reached 0% on August 18 (DOY 229). There was a particularly large rain event just before the third sampling date in July (DOY 207) and a second one in mid August (DOY 220) when the stream flooded over bank and changed bed between 1500 and 1600 m a.s.l., abandoning the sampling point UR in early August but was forced back to its original bed by mid August. Average daily air temperature across the catchment was above zero for most days after mid-May. The two tributaries, ST and TT, were dry in early (before DOY 136) and late season (after 164 and 220, respectively). The upper tributary (UT) was under the snow and inaccessible for the first sampling day (DOY 88).

E.C. shows distinct spatial patterns, both in terms of mean observed values at given locations and in terms of the temporal change, although small, of E.C. values for individual locations (Fig. 3, Table S1 and S5). In general, mean values are higher in springs than other observed water types, and generally higher at lower elevations. The main channel shows mid E.C. values and a narrow temporal range of E.C. values, resulting from a mixing of sources (tributaries and springs) throughout the season. Two of the between water type comparisons of E.C. were significant ($p < 0.05$) in mean rank difference according to the Kruskal-Wallis test: main channel to spring (M-S) and spring to tributary (S-T), see Table S4.

Water temperature was the best physico-chemical discriminator of the three types of water (Fig. 3, see Supplementary Material Table S4). Springs had the lowest and most stable water temperature (mean values between 4.7 and 6.5 ^{o}C). Tributaries and additionally the uppermost point on the main channel, UR, showed high water temperature variability (8-21 ^{o}C), and were considerably warmer. Points in the main channel had intermediary values (means between 6.6 and 12.8 ^{o}C). All comparisons of water temperature, M-S, M-T, and S-T, were significant ($p < 0.05$) in mean rank difference according to the Kruskal-Wallis test, making it the best discriminator of the variables besides eDNA (Table S5).

Stable isotopes of oxygen (δ^{18}O) and hydrogen (δ^{2}H) were highly correlated and were well within the range of precipitation (Fig. 3 and S2). The local meteoric water line (LMWL) is close to the standard global meteoric water line (GMWL), see Fig. S2. In general, values of isotopes in the main channel were not consistently intermediary to those in springs and in the tributaries, thus a simple 2 source mixing model using springs and tributaries could not be used on the main channel or outlet, but show clearly the seasonal origin of water (snow fall versus rainfall, Beria et al., 2018). We found significance ($p < 0.05$) in mean rank difference of $\delta^{18}O$ between main channel and spring (M-S) and main channel and tributary (M-T) but not between spring and tributary (S-T). We found significance in mean difference of $\delta^{2}H$ also in those two comparisons, M-S and M-T; and in one comparison of lc-ex, M-S.

The obtained results allow the following conclusions: i) The two ephemeral tributaries TT and ST had the most variation in stable isotopes, suggesting that they were composed mainly of snowmelt, or snowmelt derived groundwater, during part of the season (before June) and mainly of summer precipitation (i.e., rain) later. ii) The lowest spring (GS) most frequently had the highest value of δ^{18}O, indicating its reservoir might be fed mainly by summer precipitation, which has higher concentrations of enriched isotopes. This is confirmed by the deviation of the GS isotope ratios from the LMWL, expressed as a more negative lc-ex. Out of all the samples, the water in the GS had experienced the most phase change (typical for summer rainfall undergoing evaporation or sublimation of snow or ice before melting) since entering the basin. Two points, HS and ST, also had more negative values of lc-ex than most of the sites, however, none of the values of lc-ex are significant according to the definition in the original paper where it was defined (Landwehr and Coplen, 2006). Thus surface evaporation does not dramatically affect the isotopic ratios, in any of the sampling sites.

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