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The influence of dissolved organic matter composition on microbial degradation and carbon dioxide production in pristine subarctic rivers

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Table S1. Initial chemical characteristics of river water samples (median and range) used in the incubation

		June 2018		October 2018	
		Brown-water river	Clearwater river	Brown-water river	Clearwater river
pH	median	6.9	6.9	7.5	7.5
	range	6.5-7.4	6.4-7.3	7.4-7.8	7.3-7.5
$SUVA_{254}$ (l mg C^{-1} m^{-1})	median	5.7	2.3	6.1	1.6
	range	3.6-6.2	2.1-5.2	2.9-7.1	1.0-5.2
DOC (µmol l^{-1})	median	399	311	455	431
	range	220-455	152-368	334-740	125-642
TN (µmol l^{-1})	median	7.4	5.6	9.3	8.2
	range	5.7-17.0	4.1-6.7	8.8-12.6	5.2-11.2
$NO_3^- + NO_2^-$ (µmol l^{-1})	median	0.5	0.9	1.3	0.8
	range	0.1-2.1	0.8-1.1	0.9-1.7	0.6-1.0
NH_4^+ (µmol l^{-1})	median	1.5	1.0	0.8	1.2
	range	1.3-2.5	0.7-1.5	0.6-2.0	1.1-1.6

Table S2. 16S qPCR Mastermix

Mastermix	x1
Maxima SYBR-Green	12.50 µl
Forward primer (F338)	1.25 µl
Reverse primer (R518)	1.25 µl
NF H_2O	9 µl
Sample DNA	1 µl
Total	25 µl

Table S3. 16S qPCR protocol

Step	T (°C)	time (min)
1.	95	3
2.	95	0:35
3.	53	0:35
4.	72	0:25 + plate read
5.	Go to step 2.	x45
6.	72	1:00
7.	Melt curve 65-95	1:00 + plate read
8.	4	∞

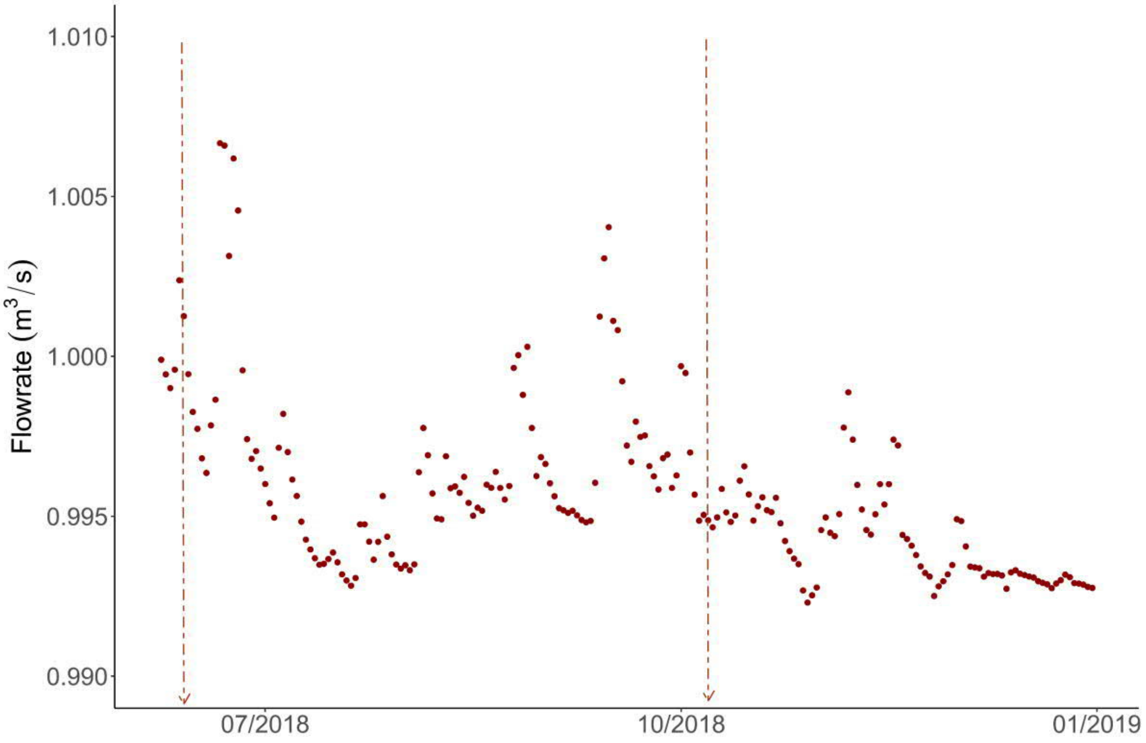
Table S4. Summary of molecular formulas derived from the FT-ICR MS analysis of river water samples before (day 0) and after the incubation (day 21). Values are presented as averages ± standard deviations (n = 6 in the brown-water river and n = 5 in the clearwater river). H/C > 1.5 (%) refers to the proportion of molecular formulas above the molecular lability boundary (MLB) H/C > 1.5.

Sampling	Sample	Total assigned formulas	Mean m/z ratio	Mean H/C ratio	Mean O/C ratio	Mean AI_{mod}	H/C > 1.5 (%)
June 2018	Brown-water river day 0	294 ± 263	352 ± 75	1.16 ± 0.38	0.39 ± 0.18	0.34 ± 0.28	19.2%
June 2018	Brown-water river day 21	251 ± 49	369 ± 83	1.15 ± 0.42	0.40 ± 0.20	0.33 ± 0.32	21.5%
June 2018	Clearwater river day 0	265 ± 19	374 ± 76	1.21 ± 0.39	0.36 ± 0.20	0.30 ± 0.29	22.0%
June 2018	Clearwater river day 21	255 ± 9	376 ± 79	1.20 ± 0.39	0.37 ± 0.21	0.29 ± 0.36	22.8%
October 2018	Brown-water river day 0	145 ± 36	390 ± 82	1.15 ± 0.40	0.42 ± 0.20	0.33 ± 0.30	17.5%
October 2018	Brown-water river day 21	119 ± 45	347 ± 79	1.18 ± 0.39	0.44 ± 0.18	0.31 ± 0.26	20.0%
October 2018	Clearwater river day 0	150 ± 91	372 ± 73	1.31 ± 0.39	0.36 ± 0.20	0.25 ± 0.29	29.7%
October 2018	Clearwater river day 21	206 ± 169	352 ± 67	1.18 ± 0.39	0.42 ± 0.17	0.32 ± 0.26	20.8%

Table S5. Results of multiple linear regression analysis for predicting the cumulative CO_2 production (model 1) and cumulative CO_2/DOC ratio (model 2) from environmental variables. n = 22. VIF = Variance Inflation Factor, AIC = Akaike information criterion. Compound groups refer to the number of m/z peaks in each compound group (FT-ICR MS data). Results of multiple regression analysis for predicting the cumulative CO2 production (model 1) and cumulative CO2/DOC ratio (model 2) from environmental variables. VIF = Variance Inflation Factor, AIC = Akaike information criterion.

Variable	Coefficient	STD error	t value	p value	VIF
Model 1 - dependent variable = CO_2 production (Multiple R^2 = 0.75, Adjusted R^2 = 0.64, F = 7.068 on 5 and 12 DF, p = 0.003, AIC = 213.00)					
DOC concentration day 0	0.353	0.159	2.218	0.047	3.106
DOC concentration day 21	-0.610	0.236	-2.583	0.024	2.530
$SUVA_{254}$ day 0	39.579	9.997	3.959	0.002	2.291
Peptide-like compounds day 0	5.491	1.547	3.549	0.004	1.151
Condensed aromatics day 0	1.860	0.788	2.360	0.036	1.325
Intercept	54.037	76.740	0.704	0.495	
Variable	Coefficient	STD error	t value	p value	VIF
Model 2 - dependent variable = CO_2/DOC ratio (Multiple R^2 = 0.76, Adjusted R^2 = 0.69, F = 10.51 on 4 and 13 DF, $p < 0.001$, AIC = 15.16)					
DOC concentration day 21	-0.003	0.001	-4.256	<0.001	1.160
TN concentration day 21	-0.016	0.032	-0.514	0.616	1.558
Aliphatic compounds day 0	0.003	0.002	1.528	0.150	1.314
Peptide-like compounds day 0	0.024	0.008	3.197	0.007	1.307
Intercept	1.511	0.209	7.212	<0.001	

(a) Brown−water river



(b) Clearwater river

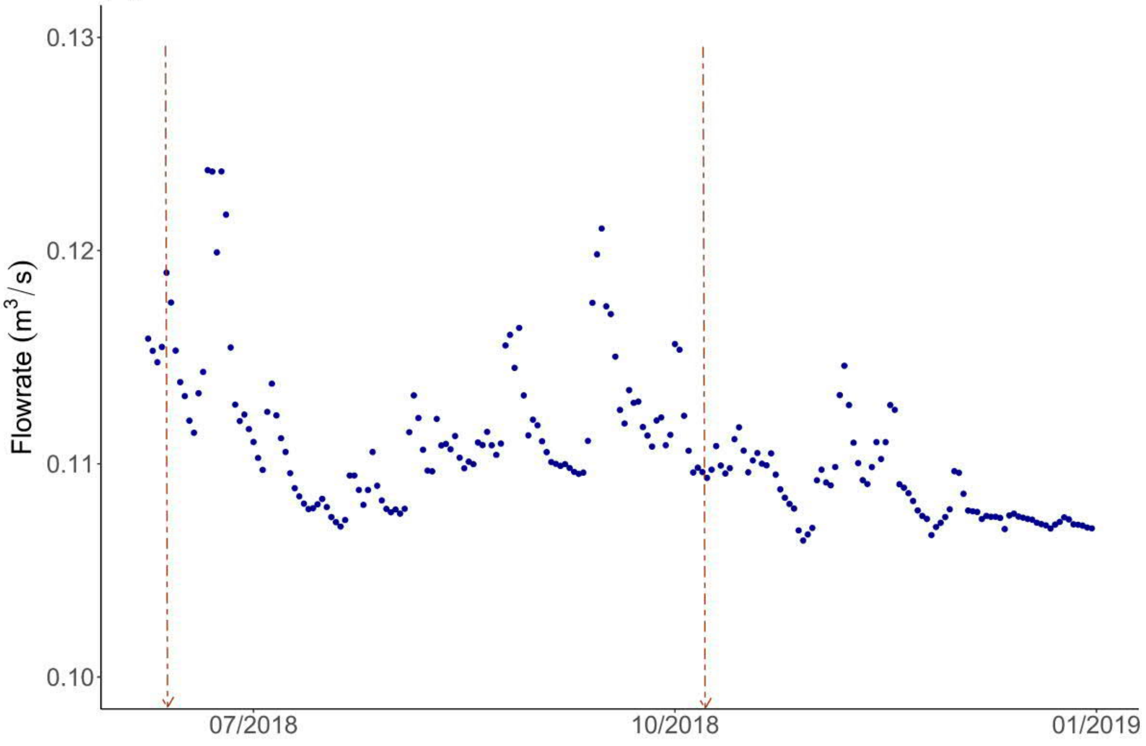
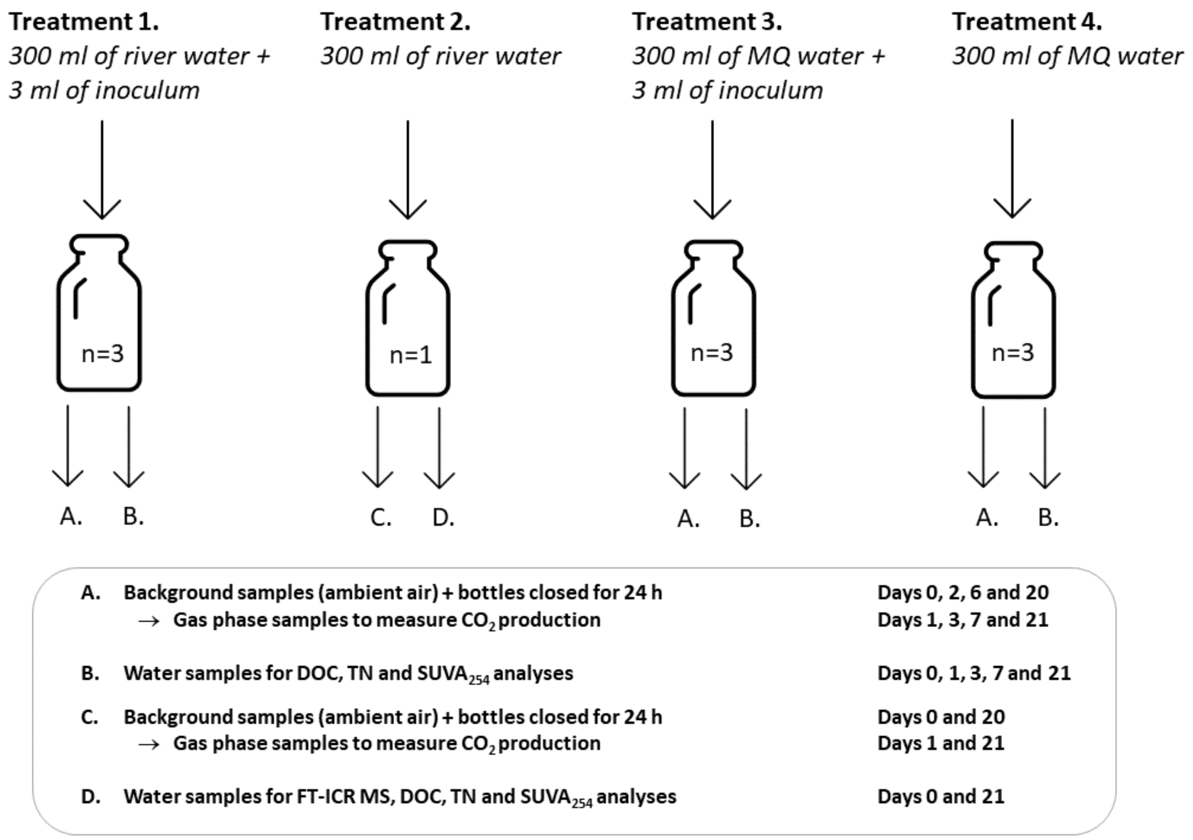


Fig. S1 Estimated water discharge in (a) brown-water and (b) clearwater river determined based on the continuous pressure sensor data and measurements of the flow rate, cross section and water depth in June and October 2018. Arrows represent the sampling occasions.



A.	**Background samples (ambient air) + bottles closed for 24 h** **→ Gas phase samples to measure CO_2 production**	**Days 0, 2, 6 and 20** **Days 1, 3, 7 and 21**
B.	**Water samples for DOC, TN and $SUVA_{254}$ analyses**	**Days 0, 1, 3, 7 and 21**
C.	**Background samples (ambient air) + bottles closed for 24 h** **→ Gas phase samples to measure CO_2 production**	**Days 0 and 20** **Days 1 and 21**
D.	**Water samples for FT-ICR MS, DOC, TN and $SUVA_{254}$ analyses**	**Days 0 and 21**

Fig. S2 Incubation set-up

Supplementary methods

The calculation of the CO_2 concentration

The solubility of CO_2 in water ($K_H(T)$ as mol kg^{-1} bar^{-1}) was calculated according to Henry's law as follows:

$$K_H(T) = K^{\circ}{}_H \, e^{\left[C \cdot \left(\frac{1}{T} - \frac{1}{298.15}\right)\right]} \qquad \text{(S1)}$$

where K°_H is Henry's law constant (0.034 mol kg^{-1} bar^{-1}) for solubility of CO_2 in water at 298.15 K (mol kg^{-1} bar^{-1}), C is temperature dependence constant (2400), and *T* is water temperature (K) in the bottle.

The amount of CO_2 dissolved in water (CO_{2W} mol l^{-1}) was calculated as follows:

$$CO_{2W} = (K_H(T) \cdot 10^{-5}) \cdot (PCO_2 \cdot 10^{-6}) \cdot P_{atm} \cdot V \qquad \text{(S2)}$$

where $K_H(T)$ is the solubility of CO_2 in water converted to mol l^{-1} Pa^{-1}, PCO_2 is the measured CO_2 concentration as ppm converted to percentage, P_{atm} is the gas partial pressure under 1 atm (1.0135 * 10^5) above the boundary of a solution, and *V* is the water volume (l) in the bottle.

The amount of CO_2 released in the headspace of the bottle (CO_{2A} mol l^{-1}) was calculated based on the ideal gas law as follows:

$$CO_{2A} = \frac{0.001 \cdot P_{atm} \cdot (PCO_2 \cdot 10^{-6}) \cdot V}{R \cdot T} \qquad \text{(S3)}$$

where 0.001 is the coefficient for converting gas volume from m^3 to l, P_{atm} is the partial pressure of the CO_2 under 1 atm (1.0135 * 10^5), PCO_2 is the measured CO_2 concentration as ppm converted to percentage (PCO_2 * 10^{-6}), *V* is the air volume (l) in the bottle, *R* is the universal gas constant (8.3145 J mol^{-1} K^{-1}), and *T* is the temperature (K).

The CO_2 production rate (mol l^{-1} d^{-1}) in the bottle during the incubations is the combination of CO_2 dissolved in water (CO_{2W}) and released in the air (CO_{2A}):

$$CO_2 = CO_{2W} + CO_{2A} \qquad \text{(S4)}$$

FT-ICR MS analysis

The molecular composition of DOM was analyzed from the samples without the inoculum before and after 21 days incubation using electrospray ionization (ESI) coupled to ultrahigh-resolution Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR MS). Samples filtered through glass microfiber filters with a nominal pore size of 0.7 µm (Whatman GF/F Glass Microfiber Filters) were prepared using the solid phase extraction (SPE) cartridge (Bond Elut® PPL SPE cartridges, Agilent, CA, USA) to remove inorganic salts (Kim et al. 2003, Dittmar et al. 2008). The SPE samples were diluted with deionized water and methanol to yield a final sample composition of 50/50 (v/v) of water to methanol, and injected into the FT-ICR MS (solariX 7.0T, Bruker Daltonics Inc., MA, USA) using a syringe pump with an infusion rate of 100 µl h^{-1}. The samples were analyzed in negative ion mode. Ions were accumulated in a hexapole for 0.01 s before they were transferred to the ICR cell, and the 100 transients collected using a 2 M Word time domain were co-added. All spectra were externally calibrated using the Tuning Mix standard (Bruker Daltonics Inc., MA, USA) and internally calibrated using the mixture of 10 fatty acids ($C_{15}H_{29}O_2$, $C_{16}H_{29}O_2$, $C_{16}H_{31}O_2$, $C_{18}H_{35}O_2$, $C_{19}H_{37}O_2$, $C_{20}H_{39}O_2$, $C_{22}H_{43}O_2$, $C_{24}H_{47}O_2$, $C_{26}H_{51}O_2$, $C_{30}H_{59}O_2$). The samples were analyzed three times per sample, and the peak list of mass-to-charge ratio (m/z) shared among the three analytical replicates was extracted. Mass lists were produced using a signal-to-noise ratio (S/N) cut-off of 5. Isotope peaks were removed from the list. The molecular formula calculator (Molecular Formula Calculator ver. 1.0; ©NHMFL, 1998) was used to assign an expected molecular formula for each m/z value with a mass accuracy ≤ 0.5 ppm. The m/z values in the range of 150-500 were inserted into the molecular formula calculator. The mass range was selected based on the previous studies reporting intensity maxima of natural organic matter (NOM) occurring in the range of m/z 350-500 (Reemtsma 2009). The following conditions were used for molecular formula assignment: $C = 1 - \infty$; $H = 1 - \infty$; $O = 1 - \infty$; $N = 0 - 2$; $S = 0$; $P = 0$; double bond equivalence (DBE) ≥ 0 (modified from Ide et al. 2020). Since high errors can be associated with the assignments containing S and P (Mostovaya et al. 2017), these formulae were excluded from further analysis. After the molecular formula assignment, molecular formulas not likely to be observed in natural water were eliminated based on rules described in Kujawinski and Behn (2006) and Wozniak et al. (2008). In addition, the peaks with unusually high peak intensity were excluded from further analysis.

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