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*Supplement of*

## **Biodegradability of dissolved organic carbon in permafrost soils and waterways: a meta-analysis**

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## INCUBATION PROTOCOL

### REQUIRED MATERIAL PER INCUBATION

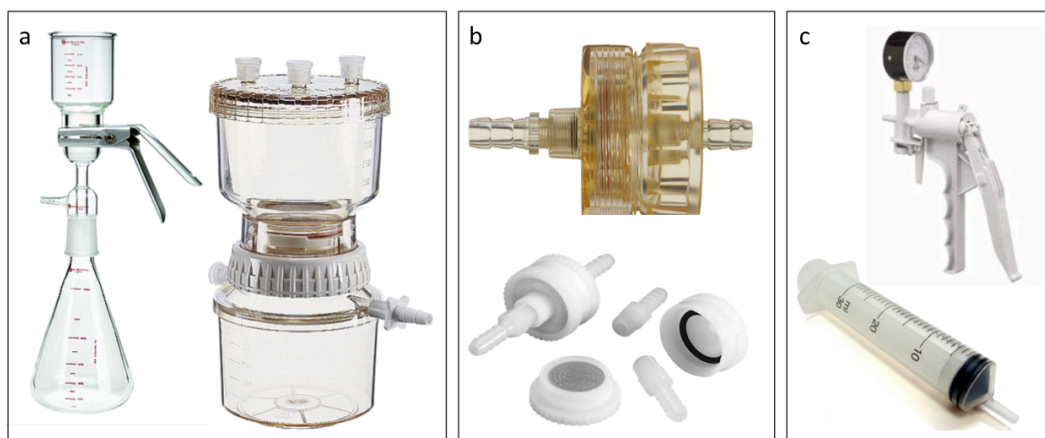
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- Clean 1L sampling flask
- Clean 1L flask to use for filtration
- Nitrile or rubber gloves
- Labeling tape and waterproof pen
- Temperature sensor/thermometer
- 30 pre-ashed (minimal 6h at 450 °C) transparent or amber 40 mL glass vials (15 vials needed for experiment, 15 vials needed for transport)
- 30 vial caps with clean silicone septa
- Pre-ashed (minimal 6h at 450 °C) glass fiber filters (nominal pore size 0.7 µm) with diameter 25 or 47mm depending on your filtration set-up
- Filtration unit (e.g. filter tower with (manual) vacuum pump, in-line filter holder connected to peristaltic pump), see Figure 1
- Concentrated HCl, and pipet to use for HCl
- Material for experimental add-ons or optimization:
  - For nutrient-amended incubations: prepare nutrient solutions from KNO<sub>3</sub>, NH<sub>4</sub>Cl, and K<sub>2</sub>HPO<sub>4</sub>.
  - Oven or incubator to maintain a constant temperature.

### SAMPLE COLLECTION

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- Collect water samples using gloves in clean sample container, pre-rinse with sample three times.
- Measure water temperature and latitude/longitude, and make additional field notes when desired (e.g. pH, conductivity, O<sub>2</sub>, turbidity).
- Transport the sample in chilled (but not frozen) and dark conditions back to the location where the incubation will be performed.
- As soon as possible after collection, we recommend maximally within 12 hours, start with the filtration (see next step). Do not freeze the samples.
- If sampling soil leachate, a collection of soil for determination of dry bulk density and soil moisture is desirable (Lajtha et al. 1999).



**Figure S1.** Examples of (a) filter towers (b) inline filter holders, and (c) vacuum or pressure devices which could be used.

### **FILTRATION AND PREPARATION**

- Filter water samples through ashed and pre-rinsed glass fiber filter (Figure 1), collect the filtrate in the clean 1L flask. Use gloves.
- Pre-label 15 vials (40mL) with sample code, incubation time point and triplicate number (e.g. K-0-a, K-0-b, K-0-c, K-2-a, K-2-b, K-2-c, etc.)
- Pour the filtrate into the glass vials (40mL), and fill each vial with 30 mL filtrate. Use 15 vials in total, consisting of five triplicate sets for each time point: T = 0, T = 2, T = 7, T = 14 and T = 28 days.
- Use caps with silicone septa.

### **INCUBATION AND ANALYSIS**

- Incubate vials in the dark.
- Incubate with loose caps and shake regularly (once a day, temporarily tightening caps) to avoid O<sub>2</sub> depletion.
- Incubate samples at room temperature (ca. 20 °C). Document the temperature throughout the incubation and use an oven or incubator if available.
- At every time point (including T = 0 days): re-filter the incubated samples through ashed and pre-rinsed 0.7 μm filters. Store the filtered samples in pre-ashed 40mL glass vials, acidify to pH 2 with 30μL concentrated HCl (36% or 11.6M). Cap tightly and store dark and chilled until analysis.
- Determine DOC concentration, and calculate BDOC (biodegradable dissolved organic carbon; in %) from the change in DOC concentration during the 28 day incubation, relative to the initial DOC concentration.

In the main text, we suggest and describe a few protocol extensions that could be used to assess further methodological and environmental controls on BDOC.

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**Table S1** Site characteristics and BDOC results from our standardized circumarctic incubation experiments.

Location		Country	Lat	Lon <sup>1</sup>	Time	Site characteristics				BDOC results <sup>5</sup>					Inoculum
Location	Sampling		°N	°E	Date	Water-shed km <sup>2</sup>	Category <sup>3</sup>	Perma-frost zone <sup>4</sup>	DOC at T=0	BDOC loss					
					T=0	JD <sup>2</sup>				T=2	T=7	T=14	T=28		
<i>Aquatic incubations</i>															
Yukon River	Mainstem at Pilot Station	US	61.94	-162.86	4-Jul	185	831386	river	Discont.	4.6	0.7	6.3	11.2	12.2	no inoculum
										4.5	0.4	5.0	6.2	7.9	1% inoculum
										4.5	1.6	6.0	8.0	9.2	10% inoculum
Richardson Creek	Tributary Yukon	US	65.65	-149.08	4-Jul	185	9.8	stream	Cont.	32.5	2.3	2.4	2.4	4.3	no inoculum
										32.5	-0.9	3.8	2.4	3.1	1% inoculum
										32.7	1.5	4.3	3.6	4.6	10% inoculum
Kolyma River	Mainstem at Cherskii	Russia	68.74	161.28	24-Jun	175	652924	river	Cont.	6.5	-1.8	3.5	3.4	24.1	no inoculum
										6.8	1.0	3.1	3.9	26.1	1% inoculum
										6.4	-3.7	-0.5	-2.0	18.9	10% inoculum
					18-Jul	199	3.4	-3.9	-18.6	-15.2	-14.2	no inoculum			
							3.3	0.3	-17.2	-16.7	-14.0	1% inoculum			
							3.4	-0.8	-17.1	-18.4	-15.0	10% inoculum			
							30-Aug	242	7.6	1.3	1.3	-17.8	1.6	no inoculum	
7.6	1.1	2.4	2.2	3.3	1% inoculum										
7.6	3.2	2.4	-6.0	5.0	10% inoculum										
Y3 stream	Tributary Pantaleikha	Russia	68.76	161.45	18-Jun	169	17	stream	Cont.	27.1	2.8	5.5	7.3	9.4	no inoculum
										25.7	5.4	6.1	6.1	9.2	1% inoculum
										25.4	7.0	7.6	6.9	9.3	10% inoculum
					21-Jul	202	19.9	-5.5	-16.0	-16.8	-15.0	no inoculum			
							20.0	-3.1	-17.4	-13.5	-14.7	1% inoculum			
							19.7	-4.3	-16.9	-17.1	-15.7	10% inoculum			
							30-Aug	242	18.1	0.3	0.6	1.1	3.3	no inoculum	
18.1	0.2	0.4	1.2	3.5	1% inoculum										
18.1	0.5	0.0	2.3	2.2	10% inoculum										
Mackenzie River	Mainstem at Tsiigehtchic	Canada	67.45	-133.77	9-Jun	160	175000	river	No perma-frost	6.5	-1.0	-1.9	-0.4	-3.7	no inoculum
										6.5	-0.5	-3.3	-0.8	-1.7	1% inoculum
										6.5	-1.0	-3.3	-0.6	-1.3	10% inoculum
					22-Jul	203	5.0	1.5	0.0	0.0	-0.9	no inoculum			
							5.0	0.2	-0.9	1.0	-0.7	1% inoculum			
									5.0	-0.1	0.1	1.1	-0.7	10% inoculum	

<i>Soil leachate incubations</i>																					
Toolik, near LTER site	core 1	US	68.61	-149.59	27-May	147	n.r.	soil leachate	Cont.	2.5	18.7	22.5	21.3	23.7	no inoculum						
													2.3	11.8	5.6	12.6	30.8	1% inoculum			
															2.3	13.3	7.6	20.0	33.4	10% inoculum	
					15-Sep	258									22.1	18.4	26.7	31.9	34.6	no inoculum	
															22.1	35.3	23.6	42.4	48.4	1% inoculum	
															23.2	36.8	24.8	45.1	39.4	10% inoculum	
Toolik, near LTER site	core 2	US	68.61	-149.59	27-May	147	n.r.	soil leachate	Cont.	3.2	14.4	27.2	33.6	37.4	no inoculum						
															3.0	19.1	10.1	25.4	33.2	1% inoculum	
																2.8	14.3	23.2	9.7	28.2	10% inoculum
					15-Sep	258									16.2	10.4	3.8	0.0	17.5	no inoculum	
															15.9	-20.6	4.2	16.1	13.5	1% inoculum	
															15.4	10.4	14.1	12.4	18.8	10% inoculum	
Toolik, near LTER site 3	core 3	US	68.61	-149.59	27-May	147	n.r.	soil leachate	Cont.	2.1	-3.8	-24.0	-0.3	16.4	no inoculum						
															2.2	12.8	14.0	12.0	31.4	1% inoculum	
																2.1	-30.4	15.8	21.7	29.2	10% inoculum
					15-Sep	258									16.5	17.3	21.3	20.3	28.1	no inoculum	
															15.7	16.0	17.2	22.4	23.3	1% inoculum	
															16.1	11.3	11.8	12.1	22.4	10% inoculum	

1 °W is listed as negative degrees

2 JD is Julian day

3 Categories defined as soil leachates, streams (<250km<sup>2</sup>), large streams (>250km<sup>2</sup> and <25,000km<sup>2</sup>), rivers (>25,000km<sup>2</sup> and <500,000km<sup>2</sup>) and large rivers (>500,000km<sup>2</sup>)

4 Watersheds are categorized according to dominant permafrost zonation, e.g. Mackenzie watershed has 16%, 29%, 55% continuous, discontinuous and no permafrost, respectively, and is here classified as "no permafrost".

5 BDOC is biodegradable dissolved organic carbon; it is calculated as the change in DOC concentration (mg/L) during the incubation, relative to the initial DOC concentration, and is reported in percent for each time step. A negative BDOC loss has been set to 0 in statistical analysis.