Supplement of Biogeosciences, 12, 6915–6930, 2015
http://www.biogeosciences.net/12/6915/2015/
doi:10.5194/bg-12-6915-2015-supplement

Biogeosciences Open Access EGU

Supplement of

Biodegradability of dissolved organic carbon in permafrost soils and aquatic systems: a meta-analysis

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INCUBATION PROTOCOL

REQUIRED MATERIAL PER INCUBATION

- 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_3, NH_4Cl, and K_2HPO_4.
  - Oven or incubator to maintain a constant temperature.

SAMPLE COLLECTION

- 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_2, 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).

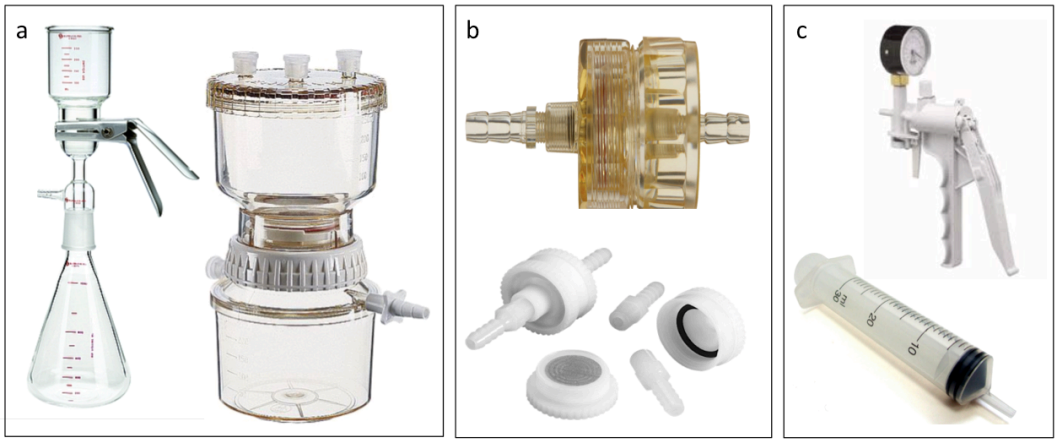

Figure S1. Examples of (a) filter towers (b) inline filter holders, and (c) vacuum or pressure devices which could be used.

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_2 depletion.
- Incubate samples at room temperature (ca. 20 ºC). 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					**Time**		**Site characteristics**			**BDOC results**[5]					
Location	Sampling	Country	Lat	Lon[1]	Date		Water-shed	Category[3]	Perma-frost		BDOC loss				Inoculum
			°N	°E	T=0	JD[2]	km[2]		zone[4]	DOC at T=0	T=2	T=7	T=14	T=28	
Aquatic incubations															
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 inocolum
										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 inocolum
										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 inocolum
										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 inocolum
										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 inocolum
										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 inocolum
										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 inocolum
										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 inocolum
										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	1750000	river	No perma-frost	6.5	-1.0	-1.9	-0.4	-3.7	no inocolum
										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 inocolum
										5.0	0.2	-0.9	1.0	-0.7	1% inoculum
										5.0	-0.1	0.1	1.1	-0.7	10% inoculum

Soil leachate incubations															
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 inocolum
										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 inocolum
										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 inocolum
										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 inocolum
										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 inocolum
										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 inocolum
										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^2), "large streams" (>250km^2 and <25,000km^2), "rivers" (>25,000km^2 and <500,000km^2) and "large rivers" (>500,000km^2)

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.