Supplement of

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

J. E. Vonk et al.

Correspondence to: J. E. Vonk (j.e.vonk@uu.nl)

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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 KNO₃, NH₄Cl, and K₂HPO₄.
  - 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, O₂, 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).
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 °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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1 °W is listed as negative degrees
2 JD is Julian day
3 Categories defined as "soil leachates", "streams" (<250km²), "large streams" (>250km² and <25,000km²), "rivers" (>25,000km² and <500,000km²) and "large rivers" (>500,000km²)
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.