

Burn Severity Lab Experiment (BSLE) Laboratory Protocol

Pacific Northwest National Laboratory River Corridor Science Focus Area – Research Campaign 3

Solids

Bulk Chemical Analyses

Before leaching, reserve chars homogenized from a single burn treatment for bulk chemical analysis. Be sure the reserved amount has equal parts of each species in the treatment and type of feedstock material (i.e., even amounts of woody vs. canopy material).

- 1) Weigh out 13-15g of a representative char sample in a cleaned capitol vial
- 2) Thoroughly grind char in ball mill for 5-10min or until finely ground.
- 3) Sub-sample solid chars for various analyses when needed and archive.

Leaching

Make Leaching Solution:

Create ion concentrations representative of rain water/natural waters found in northwestern U.S., *without inorganic N* following rationale in [Smith et al., 2002](#).

- 1) Create Stock Solution (then chill in 4C fridge)

Synthetic rainwater Solution, NO CARBONATES: Obtain salts: MgCl_2 , CaCl_2 , NaCl , KCl , Na_2SO_4 (see table 1)

Table 1. List of chemical salts and their target masses for synthetic rainwater solution

Salt	CAS #	Amount Needed for Stock Solution (mg)
$\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$	7791-18-6	79
$\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$	10035-04-8	48
NaCl	7647-14-5	339
KCl	7447-40-7	83
Na_2SO_4	7757-82-6	32

- a. Make Stock Solution 1:

Combine 79 mg MgCl_2 , 48 mg CaCl_2 , 339 mg NaCl , and 83 mg KCl into 1L of Milli-Q water. This stock is 1000x more concentrated than our final solution.

- b. Make Stock Solution 2:
Add 32 mg Na_2SO_4 into 1L of Milli-Q water. This stock is 1000x more concentrated than our final solution.

Create final concentration for 1L leaching solutions (no earlier than 5 days before leaching char).

- a. Remove each rainwater stock solution from the fridge, bring to room temperature and then shake vigorously for 1-2min.
- b. Add 1mL of each stock solution 1 & 2 into 1L flask, Fill up to 1L with Milli-Q water.
- c. Mix by inverting 1L volumetric flask at least 50 times. Then let sit for 20-30minutes.
- d. With clean pipette tips, remove 1mL of solution and put into a small beaker. Measure pH. (pH should be around ~5)

Leach Dissolved Organic Matter:

- 1) Label **pre-combusted glass 1L pyrex bottles** with appropriate treatment names and record tare weight. Weigh out **25g** of char into the **1L pyrex bottle**. Record canopy, woody and total weight. This should be completed once per biological replicate. (*3x for each individual solid char sample*)
 - a) *if solid char sample didn't have enough weight for 3x 25g replicates, leach less mass in smaller volume of solution while holding ration of solid to solution constant.
- 2) Pour **1L of leaching solution** created above over char filled pyrex bottles. Screw and secure acid washed cap on tightly.
- 3) Agitate at **25°C** for **24h @ 160rpm** in the dark.
- 4) Mesh strain leachate. Record pH (with pH electrode).
 - a) Take unfiltered subsamples
- 2) Filter leachates through 0.7µm (includes colloidal) filter.
 - a) Take 0.7µm subsamples
- 3) Filter leachates through 0.2µm ('truly dissolved') filter.
 - a) Take 0.2µm subsamples
- 4) Dry 0.7µm filter for 24 hours and archive.
- 5) Transition wet leached chars to labelled ziploc bag. Archive in -20°C freezer.
- 6) Be sure all aliquots are taken for each leachate sample
- 7) Archive & store all aliquots in the appropriate locations until time of analyses.

Solid Phase Extraction – Leachates

- 1) Dilute 5mL of DOC sample in 25mL of Milli-Q water. Preserve remaining concentrated volume for CDOM analysis. Don't dilute blank samples.
- 2) Run 6x diluted DOC sample on TOC-L to measure NPOC and TN.
- 3) Load a target of 22mgC onto 1000mg of PPL sorbent from remaining leachate volume.
- 4) Freeze and archive any remaining 0.2 μ m leachate in 50mL centrifuge tubes.
- 5) Archive MeOH extract in -80 freezer. Take aliquots from SPE extracts as detailed below/ in spreadsheet.

Long term storage of samples:

1. MeOH extracts archive at -80C
2. POM filters, dry and archive at room temperature
3. Filtered water must be processed or frozen within <1wk of collection/generation.