HOFMOCKEL LAB
CHLOROFORM FUMIGATION DIRECT EXTRACTION (CFDE) PROTOCOL FOR MICROBIAL BIOMASS CARBON AND NITROGEN

Modified from: Suding Lab protocol, modified from S. E. Hobbie, 5 May 1998

Materials Needed:

- 0.5 M K_2SO_4 (87.13g K_2SO_4 per 1L extractant made in **ultrapure water**)
    - Always test background concentrations of new lots of K_2SO_4!
- Ethanol-free chloroform (for analysis of carbon) (VWR # BJ049-1L)
- Pasteur pipettes and bulbs for pre-leaching filter papers
- Funnels (acid washed)
- Funnel racks (Isenhart lab)
- Whatman No.42 filter paper, **pre-leached 3x with 0.5 M K_2SO_4 + 1x with ultrapure water**; folded (VWR # 28480-106)
- 50mL beakers (acid washed); 1 x # samples + 1 x # of desiccators; label with wax pencil with #'s not sample code
- 50mL conical tubes with flat bottom (acid washed) (Fisher # 0553868) → for storage; label with sample code, date, initials; 2 x # of samples + blanks
- 50mL conical tubes with V-bottom (acid washed) → for centrifuging; label with #'s not sample code; 2 x # of samples + blanks
- Vacuum desiccators (25mm desiccator holds 18 bottles + beaker for chloroform)
- Boiling chips (Fisher # S716842)
- Aluminum weigh boats (Fisher # 08-732-101) or pre-weighed coin envelopes
- Data sheets
- Phosphoric acid for preservation
- Repeat pipettor (clean and rinse well)

Subsample soil

- One subsample for determining gravimetric soil moisture
- One non-fumigated sample (~15 g) for immediate extraction with 0.5 M K_2SO_4; place in V-bottomed conical tube labeled "# T=0".
- One fumigated sample (~ 15 g) in V-bottomed conical tube labeled "# T=24".
- Record weight of soil and # for each sample

Extraction

Note: extraction is identical to Soil N Extraction Protocol but with 0.5M K_2SO_4 instead of 2M KCl

1. Work in blocks (i.e. statistical blocks of samples)
2. Add 45 ml 0.5 M K_2SO_4 to non-fumigated subsample using repeat pipettor to dispense. Cap well.
3. Place on shaker for 1 hour at 200 rpm/low.
4. Balance samples and centrifuge @ 2500 rpm for 2 minutes.
5. Gravity filter the supernatant through pre-leached filter paper.
6. Transfer filtered extracts to 50mL flat-bottom conical tubes, leaving at least 1cm head space to prevent bursting.
7. Add 3 drops of phosphoric acid to the extract (for preservation for TOC analysis).
8. Freeze extract to store.
9. Wash out V-bottom conical tubes, acid wash, and reuse.
10. Rinse and acid wash funnels for T=24 extractions.

FUMIGATIONS MUST BE DONE IN THE FUME HOOD
CHLOROFORM IS A KNOWN CARCINOGEN

1. Empty samples to be fumigated in 50mL beakers. Mark beakers with wax pencil, as sharpie will run when exposed to chloroform. Beakers can be stacked in the desiccators by layering with hardware cloth.
2. Keep conical tubes (do not clean) and use for extraction in 24 hours.
3. Place a 50mL beaker with no soil as blank.
4. Place a 50mL beaker containing ~1 spatula boiling chips and 40 ml of chloroform in the desiccator.
5. Evacuate until chloroform boils, ~ 2-6 minutes. Vent INTO HOOD. Repeat this step three more times, NOT venting the last time. You may have to replace the boiling chips each time to get the chloroform to boil!
6. Let sit in the dark for 24 hours: cover the desiccator with a black garbage bag (darkness prevents the chloroform from breaking down).
7. Pre-fill 50 ml V-bottom tubes emptied in step 1 with 45 ml 0.5M K2SO4
8. After 24 hours, release the vacuum INTO THE HOOD. Remove chloroform beaker.
9. Pull a vacuum on the desiccator for 1 minute; repeat 5 times.
10. In the hood, extract the sample as above:
    a. Carefully transfer soil to matching V-bottomed conical tube, which is pre-filled with 0.5M K2SO4
    b. Transfer soil and extractant mixture back and forth while swirling the beaker until all the soil is in the tube.
    c. Follow extraction protocol above, starting at step 2.

Microbial Biomass Carbon
Determine total dissolved carbon on a TIC/TOC analyzer. The difference between C in the fumigated and non-fumigated samples is the chloroform-labile C pool (EC), and is proportional to microbial biomass C (C):
C=EC/kEC; where kEC is soil-specific, but is often estimated as 0.45 (Beck et al. 1997).

Digestion to determine Microbial Biomass Nitrogen
Digest T=0 and T=24 samples; see Total N Perfulate Oxidation and Nutrient Analysis of Inorganic N protocols.
Analyze digested samples for nitrate only (digestion oxidizes NH_4^+, NO_2^-, and DON to NO_3^-)

The difference between N in the fumigated and non-fumigated samples is the chloroform-labile N pool, and is proportional to microbial biomass N (N):
N=EN/kEN; where kEN is soil-specific, but is often estimated as 0.54 (Brookes et al. 1985).

References
Beck, T., R. G. Joergensen, E. Kandeler, F. Makeschin, E. Nuss, H. R. Oberholzer, and S. Scheu. 1997. An inter-laboratory comparison of ten different ways of measuring soil microbial biomass C. Soil Biol. Biochem. 29 (7):1023-1032.

Brookes, P. C., A. Landman, G. Pruden, and D. S. Jenkinson. 1985. Chloroform fumigation and the release of soil nitrogen: a rapid direct extraction method to measure microbial biomass nitrogen in soil. Soil Biol. Biochem. 17:837-842.