

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

Modified from: S. E. Hobbie, 5 May 1998, (<http://www.stanford.edu/group/Vitousek/>)

Materials Needed:

- 0.5 M K₂SO₄ (87.13g K₂SO₄ per 1L extractant)
- Chloroform (Fisher # C606-1)
- Alumina-Basic (Fisher # A941-500)
- Bottle-top pipetter
- Squeeze bottle with 0.5 M K₂SO₄
- Funnels (Fisher # 10-500-20)
- Funnel racks
- Whatman No. 1 filter paper (Fisher # 09-805C)
- Specimen cups (Fisher # 14-375-148)
- Scintillation vials (Fisher # 03-341-72C)
- 50 ml glass beakers
- Vacuum dessicator
- Boiling chips (Fisher # S716842)
- Aluminum weigh boats (Fisher # 08-732-101)

For each sample, you will require 3 subsamples:

- One subsample (10 g) for determining gravimetric soil moisture
- One non-fumigated sample (10 g oven-dry equivalent) for immediate extraction with 0.5 M K₂SO₄.
- One fumigated sample (10 g oven-dry equivalent).

Gravimetric Soil Moisture

48 hours @ 105°C, See *Gravimetric Soil Moisture Protocol*

Extraction

Note: extraction is identical to *Soil N Extraction Protocol* but with 0.5M K₂SO₄ instead of 2M KCl

- Place non-fumigated subsample in a specimen cup with 50 ml 0.5 M K₂SO₄.
- Place on shaker for 1 hour at 200 rpm.
- Allow samples to settle for 1 hour after shaking.
- Gravity filter the supernatant through pre-wetted (with 0.5 M K₂SO₄) filter paper, folded to fit funnels.
- Transfer filtered extracts to labeled sample vials, leaving at least 1cm head space to prevent bursting.
- Freeze extract to store.

Fumigation

***** FUMIGATIONS MUST BE DONE IN THE FUME HOOD *****

***** CHLOROFORM IS TOXIC *****

- Remove the ethanol from the chloroform by mixing 500 ml over 25 g of basic grade 1 alumina in a flask and stir for 10 minutes. (or purchase ethanol-free chloroform).
- Place samples to be fumigated in 50 ml glass beakers. Mark beakers with pencil, as sharpie will run in chloroform. Put the beakers with soil into a vacuum dessicator. Beakers can be stacked in the dessicator by layering with shelf.
- Place a 50-ml beaker or scintillation vial containing ~1 spatula boiling chips and 20 ml of chloroform in the dessicator.
- Evacuate until chloroform boils. Vent. Repeat this step three more times, NOT venting the last time.
- Let sit in the dark for 5 days: cover the dessicator with a black garbage bag (darkness prevents the chloroform from breaking down).
- Release the vacuum.
- Draw a vacuum for 1 minute and release to remove the chloroform. Repeat three times.
- Extract the sample as above.

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

See *Kjeldahl* and *PerSulfate Digestion Protocols*.

See *Plate Reader Inorganic N Protocol*.

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.