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

Adapted from:
http://www.stanford.edu/group/Vitousek
5 May 1998, S. E. Hobbie

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.

Fumigation and Extraction

Important if measuring microbial biomass carbon: before doing CFDE, remove the ethanol from the chloroform by running 500 ml over 25 g of basic grade 1 alumina in a syringe or column. (or purchase ethanol-free chloroform).

1. For each sample, you will require 3 subsamples:
-one subsample for determining gravimetric soil moisture (48 hours @ 100°C)
-one non-fumigated sample (10 g oven-dry equivalent) for immediate extraction with 0.5 M K_2SO_4.
-one fumigated sample (10 g oven-dry equivalent).

2. Place non-fumigated subsample in a specimen cup (e.g., Fisher 14-375-148) with 50 ml 0.5 M K2SO4. Place on shaker for 1 hour. After shaking, filter through pre-leached (with 0.5 M K2SO4) Whatman No. 1 filter paper. Freeze extract to store.

3. Place samples to be fumigated in 50 ml glass beakers. Mark beakers with pencil, as sharpee will run in chloroform. Put the beakers with soil into a vacuum dessicator. Beakers can be stacked in the dessicator by layering with a vented plate.

4. Place a 50-ml beaker or scintillation vial containing boiling chips and 20 ml of chloroform in the dessicator.

5. Evacuate until chloroform boils. Vent.

6. Repeat (5) three more times, not venting the last time. (you may have to replace the boiling chips each time to get the chloroform to boil).

7. Let sit in the dark for 3 days (darkness prevents the chloroform from breaking down; you can cover the dessicator with a garbage bag).

8. Release the vacuum and remove the chloroform.

9. Draw a vacuum and release to remove excess chloroform. Repeat 5-10 times. To ensure that you've vented all the chloroform, you may want to do the famous "sniff test" invented by Eldor Paul (it's probably not that good for your health).

10. Extract the sample in 50 ml K_2SO_4. Shake for 1 hour. Filter through pre-leached (with 0.5 M K_2SO_4) Whatman No. 1 filter paper. Freeze extract to store.

For Microbial Biomass Carbon:

1. 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).

For Microbial Biomass Nitrogen:

1. Digest 20 ml of extract using Kjeldahl digestion. Run digest on autoanlyzer for total N. 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).

Allison Lab Protocol: Microbial Biomass by Fumigation, 1/2008, Steve Allison

Kjeldahl Digests: Vitousek Lab method for acid digests by Doug Turner

Digest solution for a rack of 40;

190ml Sulfuric Acid
100g Potassium Sulfate
20g Cupric Sulfate (5H2O)

Note: Digest solution formulated specifically to run on the Vitousek Lab Alpkem.

Please wear a lab coat, gloves and eye protection.

Start the technicon block heating up (manual button and set on 410°C), it takes 2 hours. If you are digesting solutions, start the block at 210°C.

Place 500ml Erlenmeyer flask on hot plate with a stir magnet.

Using the 250ml graduated cylinder (usually found on the drying rack) measure and pour sulfuric acid into the Erlenmeyer flask. Turn the heat knob to about 4 and mix at slow to medium speed. Rinse the graduated cylinder several times with DI water and replace on the drying rack.

Using a weigh boat, measure out the potassium sulfate. With a powder funnel, slowly add it to the flask. Likewise, measure out and add the cupric sulfate. Let the digest solution stir while you weigh out your samples. It will have a pale blue and milky appearance.

Take off gloves at this point (static interferes with weighing the samples).
Put digest tubes on cardboard next to the balance. Be careful with digest tubes- they are fragile! Tubes can crack or chip very easily. Sample material should be oven dried and held in a dessicator or redried prior to digestion. For leaf tissue use 50 mg. ground to 20 mesh or finer (you can use less if you know your samples have a high N or P content). For soil use 150 mg [if organic use less]. When digesting solutions, you can use up to 20ml. Each rack of 40 samples should have at least 2 tubes for standards (see pine recoveries).

Put gloves on.

Next, add 1 or 2 boiling chips (Hengar Granules) to each tube.
Under the fume hood, dispense 5 ml of digest solution (still stirring) into each tube. Once they are all filled they go on the block for 30 seconds. After 30 seconds lift rack off the block and let the tubes cool down (about 5 minutes).

While the tubes are cooling, get the 5ml pipet out and get the Hydrogen peroxide out of the refrigerator.

Add 1 ml H_2O_2 to each tube and mix (you can mix by hand or with the vortex mixer on low).

Set rack on heating block. If samples boil more than half way up the tube remove the rack (the blanks can go higher). Once they settle down put them on again. When they are all boiling evenly and low, start the two hour time period. (With solutions, gradually increase the temperature until 410°C can be reached; about an hour, and then begin timing.)

After two hours take the tubes off the block. Let them cool under the fume hood (~ 15 min.). Push block off button.

Fill tubes 1/2 way with DI water and mix well with the vortex mixer set at about 9. This is an important step; make sure you mix each tube at least 8-10 seconds. Because the tubes should cool down before diluting to the mark, now is a good time to label and setup the 4ml sample cups in a board.

Bring each tube to volume with DI water, stopper and invert 8 times. Pour samples directly into the corresponding numbered cups. Promptly wipe drips off of gloves and tubes with a paper towel. Rinse stopper with DI water between each tube. Cap the sample cups. Samples are now ready for the Alpkem.

Blank solution: With a 50 or 100ml graduated cylinder, measure out 33.3ml (as best you can) of the digest solution that still remains in the flask. Add this to a 500ml volumetric flask containing about 400ml of DI water. Mix well and bring to the mark. Let this cool while you do clean-up, and then recheck the mark. You'll probably need to add about 2ml of water; then remix. This solution is used for making your standards for the Alpkem, blanks and the Alpkem wash solution. Note: if there is less than 500ml of space in the blank solution bottle, you need not make more. Add the digest solution to the waste container.

CLEAN UP:
Place the Sulfuric acid hazardous waste container in the sink with a filter funnel. Empty 1 or 2 tubes at a time. Then rinse each tube 3-5 times with DI water inside and out (rinse water may go down sink). Rinse boiling chips with DI water and put them in the dirty chips beaker. Clean off counter and all the space you worked at.