Microbial Biomass Carbon and Nitrogen
by the Chloroform Fumigation/Direct Extraction Method (CFDE)

(Silver Lab – cribbed mainly from Sarah Hobbie's and Paul Brooks's protocols)

Equipment:

Extraction:

- Vacuum dessicator without dessicant
- 50 mL beakers, one for each sample
- 250 mL Erlenmeyer flasks, two for each soil sample and two for blanks
- repipettor with 2L bottle, set to dispense 80 mL (for 0.5 M K_2SO_4)
- parafilm
- boiling chips
- aluminum weighing boats
- kimwipes
- spatula
- specimen cups, two for each soil sample and two for blanks

Digestion:

- 40 75 mL Tecator digestion tubes, with silicone stoppers to fit
- Tecator digestion block
- Hengar boiling granules
- tweezers
- 40 small funnels, to fit in the neck of the Tecator digestion tubes
- Pasteur pipets
- 1 L Erlenmeyer flask
- two-place balance
- repipettor with 1 L bottle, set to dispense 3 mL (for sulfuric acid)

Reagents:

- 0.5 M K_2SO_4
- concentrated sulfuric acid
- ethanol-free chloroform (either stabilized with amylene, or distilled)
- 10 mg N/L as alanine in 0.5 M K_2SO_4

Procedure:

Extraction:

1. For each soil, weigh out the following aliquots (wet weight). Weigh 20 g into the 50 mL beaker, 20 g into the 250 mL flask, and 10 g into the aluminum weigh dish. Mark the beakers with a pencil, since marker will run when exposed to the chloroform.
2. Pipet 80 mL 0.5 M K_2SO_4 into each of the 250 mL flasks with soil and the blank. Cover with parafilm and shake for 1 hour.
3. While the initial extraction is shaking, begin the chloroform fumigation. Place a wet paper towel in the bottom of the vacuum dessicator, and put the 50 mL beakers containing soil on top. Add a 50 mL beaker containing boiling chips and about 30 mL chloroform.
4. Attach the vacuum dessicator to the vacuum pump and evacuate until the chloroform boils. **Make sure that the pump is vented into a hood!** Once the chloroform boils, disconnect from the pump and vent. Repeat twice, for a total of three evacuations. On the last evacuation, do not vent. Store in the dark for 5 days.
5. Filter initial extraction through Whatman #1 filter paper (prewashed with 0.5 M K_2SO_4) into a specimen cup. Label and freeze to store until ready to do the digestion or analyze for TOC.
6. Dry the soil in the weigh dish for 48 hours at 105°C. Cool in a dessicator and weigh to determine moisture content.
7. After 5 days, release the vacuum and remove the chloroform from the vacuum dessicator. Evacuate the vacuum dessicator again, and vent to the atmosphere. Repeat twice, for a total of three evacuations.
8. Transfer the fumigated soils to 250 mL flasks, and extract the fumigated soils as in the initial extraction (steps 2 & 5.)

9. Analyze for TOC on the TIC/TOC analyzer in the Sposito lab.
10. Divide the TOC result by 0.45 to convert the chloroform-labile C pool to the microbial biomass C (Beck et al. Soil Biol Biochem. 29(7):1023-1032.) (Although this number is soil specific, 0.45 is a good estimate.)

Digestion:

11. Thaw the K_2SO_4 extracts.
12. Stand a tube in the 1 L Erlenmeyer, and tare the balance. Weigh approximately 24.00 g (which is 22.5 mL) of blank K_2SO_4 into the tube using a pasteur pipet, and label.
13. Weigh 24.00 g of the 10 mg N/L alanine in K_2SO_4 into three labelled tubes.
14. Weigh soil extracts into the remaining 36 tubes.
15. Add 2 – 3 Hengar boiling granules to each tube.
16. In the hood, add 3 mL of sulfuric acid to each tube using the repipettor.
17. Place in the block and heat to 125 and hold for 30 minutes. Then ramp the temperature to 165 in 10° steps. Allow the water to boil off overnight.
18. In the morning, put small funnels in each of the tubes, and elevate the block temperature to 375 in 10° steps every 20 minutes. Digest at 375° until the tubes are clear and colorless (about 3 – 4 hours.)
19. Once the digestion is finished, remove from the digestion block and allow to cool for 20 – 30 minutes on the ceramic tiles.
20. Dilute each tube to the mark with DDI , then stopper with a silicone stopper and shake several times to mix. **Be very careful during this step!** The digestion is 4% sulfuric acid, which can cause significant injury if it ends up in the wrong place. Don't point the opening of the tube toward anyone, and hold the stopper securely with your thumb. Some of the salt will precipitate when the water is added, but will dissolve easily when the tube is shaken.
21. Decant the digested solution into a labelled specimen cup.
22. Pour off 7 – 8 mL of each digested solution into a Lachat tube, cap and freeze until analysis.
23. Analyze for NH4-N using the TKN method on the Lachat. Reagents are the same as for a KCl soil extract except for the following modifications:

    Carrier: Dilute 600 mL 0.5 M K_2SO_4 and 80 mL concentrated sulfuric acid to 2 liters. Use an orange-white pump tube instead of a red tube.
    Buffer: Dissolve 130 g NaOH, 100 g NaK tartrate, and 53.6 g $Na_2HPO_4 \bullet 7H_2O$ in about 1800 mL DDI, then dilute to 2 liters. Use a red tube instead of a black tube.
    EDTA: Instead of the EDTA solution, use deionized water. Use an orange-white tube instead of a black tube.

    Use a 70 cm sample loop. **The buffer should be the first reagent to go on, and the last one to come off the manifold!** If the reaction is not buffered, the acid in the carrier will cause the salicylate to precipitate in the lines, which can be very very very bad.
24. Correct the resulting numbers by subtracting the blank, then multiplying by 3.333 to account for the dilution. Correct for digestion efficiency by multiplying by 10, and dividing by the mean of the measured concentration of the alanine standards.
25. Finally, convert the chloroform-labile N pool to the microbial biomass N by dividing by 0.54 (again soil-specific, but 0.54 is a good estimate) (Brookes et al., Soil Biol. Biochem. 17:837-842.)