

Chloroform Fumigation Method

Determining Microbial Biomass Carbon and Nitrogen

See Gross et al. (2022) [doi:10.1016/j.scitotenv.2021.151337](https://doi.org/10.1016/j.scitotenv.2021.151337)

(Vance *et al.*, 1987; Beck *et al.*, 1997; Jenkinson *et al.*, 2004)

Caution! You must read the Safety Data Sheets (SDSs) before using the chemicals in this experiment. Use gloves and other personal protective equipment when handling chemicals and liquid N. You must also receive training from experienced personnel before performing this experiment. Review lab SOPs for use of corrosives and chloroform.

***Chloroform (>99%): Use in the fume hood at all times. Wear lab coat at all times and use Silver-Shield gloves when working with chloroform. Danger. Harmful if swallowed, toxic if inhaled, causes skin irritation, causes serious eye irritation, causes damage to organs, suspected carcinogen.**

1. Label glass scintillation vials or beakers with the black wax crayon
2. Weigh subsample of field-moist soil (oven-dry equivalent) into vial/beaker (at the same time, collect duplicate soil subsamples for dissolved organic carbon extraction without chloroform fumigation)
3. Retrieve 2–4 L liquid nitrogen in the 4 L liquid nitrogen storage case
4. Desiccator set up:
 - a. Log your fumigation on the log sheet
 - b. Check vacuum pump oil
 - c. Clean the old grease off the desiccator rim and lid
 - d. Add a wet paper towel at the bottom of the desiccator to maintain moisture inside the desiccator
 - e. Clean a porcelain plate and place it in the desiccator
5. Place the vials/beakers with soil on the porcelain plate
6. Fill a glass beaker with 20 mL of ethanol-free chloroform and several unused boiling stones; place the beaker in the center of the porcelain plate with the vials/beakers (for two layers, add another porcelain plate on top of the vials/beakers and add the additional vials/beakers with soil on top of the porcelain plate and another glass beaker with chloroform as previously detailed)
7. Apply new grease thinly and evenly on the rim of the desiccator; put the lid on the desiccator and slide it back and forth until a seal is created and the grease is smoothed out

8. Add liquid nitrogen to the container that holds the glass cylinder that traps the chloroform vapor by quickly freezing it; fill the container about $\frac{1}{2}$ of the way full with liquid nitrogen so that the trap is well immersed in liquid nitrogen
9. Place the glass cylinder trap in the container with the liquid nitrogen and secure with the two semi-circle white lids
10. Make sure that the desiccator cap screw plug is CLOSED (screwed down) or that the hole in the cap is NOT lined up with the nozzle
11. Hook up the desiccator to the trap and vacuum pump using the hosing
12. Switch on the vacuum pump (check oil first and make sure the vacuum belt is not obstructed)
13. VERY SLOWLY screw the desiccator cap plug up or VERY SLOWLY align the hole in the cap with the nozzle by turning the cap to begin evacuating the desiccator
14. The chloroform will start boiling after about 30 seconds; allow the chloroform to boil for a total of about 1 minute (be careful not to let the chloroform boil over the sides of the beaker or splash; if the boiling is too intense, slightly close the desiccator cap plug or slightly un-align the hole in the cap)
15. Screw the desiccator cap plug closed (down) or un-align the hole in the cap and the nozzle to seal the desiccator
16. Switch off the vacuum pump and remove the hosing
17. Allow the samples to incubate for 24–48 hours in the dark (use black opaque trash bags)
18. After the incubation time, follow steps 3 (if more liquid nitrogen is needed) and 8–9
19. **Release the seal by VERY SLOWLY screwing the desiccator cap plug up or VERY SLOWLY aligning the hole in the cap with the nozzle by turning the cap; a long hissing noise should be heard for well-sealed incubations**
20. **RESEAL the desiccator** (i.e., close the plug)
21. **Hook up the desiccator to the trap and vacuum pump using the hosing**
22. **Switch on the vacuum pump** (check oil first and make sure the vacuum belt is not obstructed)
23. **VERY SLOWLY screw the desiccator cap plug up or VERY SLOWLY align the hole in the cap with the nozzle by turning the cap to flush out the chloroform vapor for 1-2 minutes**
24. **RESEAL the desiccator** (i.e., close the plug) **and then turn off the vacuum pump**
25. **Remove the vacuum hosing from the desiccator**
26. Repeat steps 19–25

27. Slide off the desiccator lid under the fume hood and remove the chloroform beaker; pour the waste into a properly labeled container (if the lid is difficult to slide off, blow hot air from a hair blow dryer around the edge of the lid; once the grease is melted, try to slide off the lid)
28. Carefully remove the samples and place them on the counter under the fume hood; remove the porcelain plate and dispose of the waste paper towel at the bottom of the desiccator in a properly labeled waste container
29. Place the samples back on the porcelain plate inside the desiccator
30. Put the lid on the desiccator and slide it back and forth to create a seal
31. Repeat steps 19–25 four times to remove all the chloroform vapor (VERY IMPORTANT!)
32. After subjecting the soil samples to repeated vacuum evacuation, remove the vials
33. When the cylinder trap is thawed, empty the chloroform waste into the proper waste container
34. Use an appropriate extraction method to extract the dissolved organic carbon (use the same method as used for the non-fumigated soil subsamples)
35. Dilute the sample filtrate in 40 mL glass vials with lids (to prevent evaporation) to be run on the TOC machine (in general, 4 mL of filtrate and 16 mL of MilliQ water); store for no more than two days at 4 °C
36. For long-term storage, store filtrate samples in 20-mL plastic vials at –20 °C

$$\text{MBC} = \text{EC}/\text{kEC}$$

$$\text{MBN} = \text{EN}/\text{kEN}$$

EC or EN = the difference between organic carbon or nitrogen, respectively, in fumigated and non-fumigated samples

kEC or kEN = constant estimated to be 0.45

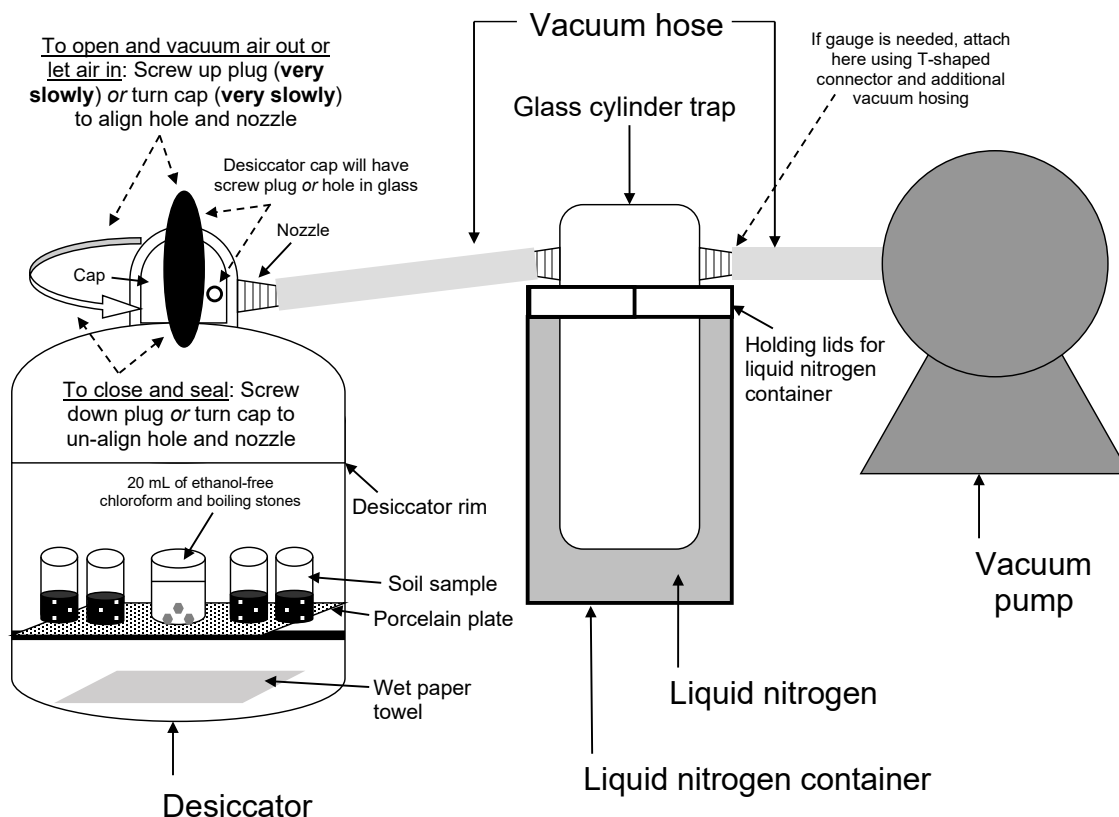


Diagram of desiccator. Modified from Pak Chow (2011), *Chloroform fumigation of soil samples for microbial biomass C and N*.

References

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Update record

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Changes: Updated safety regulations

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