

Acid Fumigation Method

Removing Carbonates from Soil to Determine Total Organic Carbon

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

(Ramnarine *et al.*, 2011; Premrov *et al.*, 2018)

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 cryogenes.

***Hydrochloric acid (HCl) 12 M: Use in the fume hood at all times. Wear lab coat and use nitrile (blue) gloves at all times.** Very hazardous in case of skin contact (corrosive, irritant, permeator), of eye contact (irritant, corrosive), of ingestion. Slightly hazardous in case of inhalation (lung sensitizer). Non-corrosive for lungs. Liquid or spray mist may produce tissue damage particularly on mucous membranes of eyes, mouth and respiratory tract. Skin contact may produce burns. Inhalation of the spray mist may produce severe irritation of respiratory tract, characterized by coughing, choking, or shortness of breath. Severe over-exposure can result in death.

1. Label 20-mL glass scintillation vials with the black wax crayon (up to 36 vials)
2. Weigh (g) the empty vials without the lids (at least six significant figures)
3. Weigh ~300 mg subsample of air- or oven-dried soil into a vial and record weight of soil + vial without lid (at least six significant figures)
4. Place vials (without lids) in oven at ≥ 40 °C for 48 hours (shorten time if soil was already oven dried); let vials sit out to cool before weighing and record weight of soil + vial without lid
5. Retrieve 2–4 L liquid nitrogen in the 4 L liquid nitrogen storage case
6. Moisten each soil in the vials with 150 μ L of milliQ water
7. 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
8. Place the vials with moistened soil on the porcelain plate
9. Fill a glass beaker with 100 mL of 12 M HCl and several unused boiling stones; place the beaker in the center of the porcelain plate with the vials (for two layers, add another porcelain

- plate on top of the vials and beaker and add the additional vials with moistened soil on top of the porcelain plate and another glass beaker with HCl as previously detailed)
10. 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
 11. Add liquid nitrogen to the container that holds the glass cylinder that traps the HCl 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
 12. Place the glass cylinder trap in the container with the liquid nitrogen and secure with the two semi-circle white lids
 13. 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
 14. Hook up the desiccator to the trap and vacuum pump using the hosing
 15. Switch on the vacuum pump (check oil first and make sure the vacuum belt is not obstructed)
 16. 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
 17. The HCl will start boiling after about 30 seconds; allow the HCl to boil for a total of about 1 minute (be careful not to let the HCl 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)
 18. Screw the desiccator cap plug closed (down) or un-align the hole in the cap and the nozzle to seal the desiccator
 19. Switch off the vacuum pump and remove the hosing
 20. Allow the samples to incubate for between 24 and 56 hours for soils containing ~0.6–2% inorganic carbon, respectively (refer to the sources cited to determine if this incubation time)
 21. After the incubation time, follow steps 5 and 11–12
 22. **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**
 23. **RESEAL the desiccator** (i.e., close the plug)
 24. **Hook up the desiccator to the trap and vacuum pump using the hosing**
 25. **Switch on the vacuum pump** (check oil first and make sure the vacuum belt is not obstructed)
 26. **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 HCl vapor for 1-2 minutes**

27. **RESEAL the desiccator** (i.e., close the plug) **and then turn off the vacuum pump**
28. **Remove the vacuum hosing from the desiccator**
29. Repeat steps 22–28
30. Slide off the desiccator lid under the fume hood and remove the HCl beaker; pour the waste into a properly labeled glass 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)
31. 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
32. Carefully clean the inside of the desiccator and lid (do not clean off the grease) and the porcelain plate in the sink with distilled water and dry with paper towels (wear eye protection and a mask in case of HCl mist)
33. Place the clean and dry desiccator under the fume hood and place the samples back on the porcelain plate inside the desiccator
34. Put the lid on the desiccator and slide it back and forth to create a seal
35. Repeat steps 22–28 for 2 hours to remove all the HCl vapor (**VERY IMPORTANT!**)
36. After subjecting the soil samples to repeated vacuum evacuation for at least 2 hours, remove the vials
37. When the cylinder trap is thawed, empty the HCl waste into the proper waste container
38. Place vials (without lids) in oven at ≥ 60 °C until soil is dry (this may take several days)
39. After oven drying, let vials sit out to cool before weighing and record weight of soil + vial without lid
40. Manually disrupt the soil in the vials with a glass rod to return the soil to a fine powder
41. **Return samples to oven for another 24 hours at 105 °C to remove residual HCl vapor**
42. The soil now can be weighed into tins (~15–40 mg per tin) for total organic carbon analysis
43. **IMPORTANT:** The acidified soil sample will weigh more than the original soil sample that contained carbonates; so that the organic carbon in the soil subsample is not diluted, a conversion factor must be applied to the soil in the tin according to (Ramnarine *et al.*, 2011):

$$\text{Corrected weight (mg)} = [\text{weight (mg) of soil in tin}] \times \text{correction factor}, \quad (1)$$

where

$$\text{Correction factor} = \frac{\text{soil weight (mg) pre fumigation (48 h weight)}}{\text{soil weight (mg) post fumigation (48 h weight)}}. \quad (2)$$

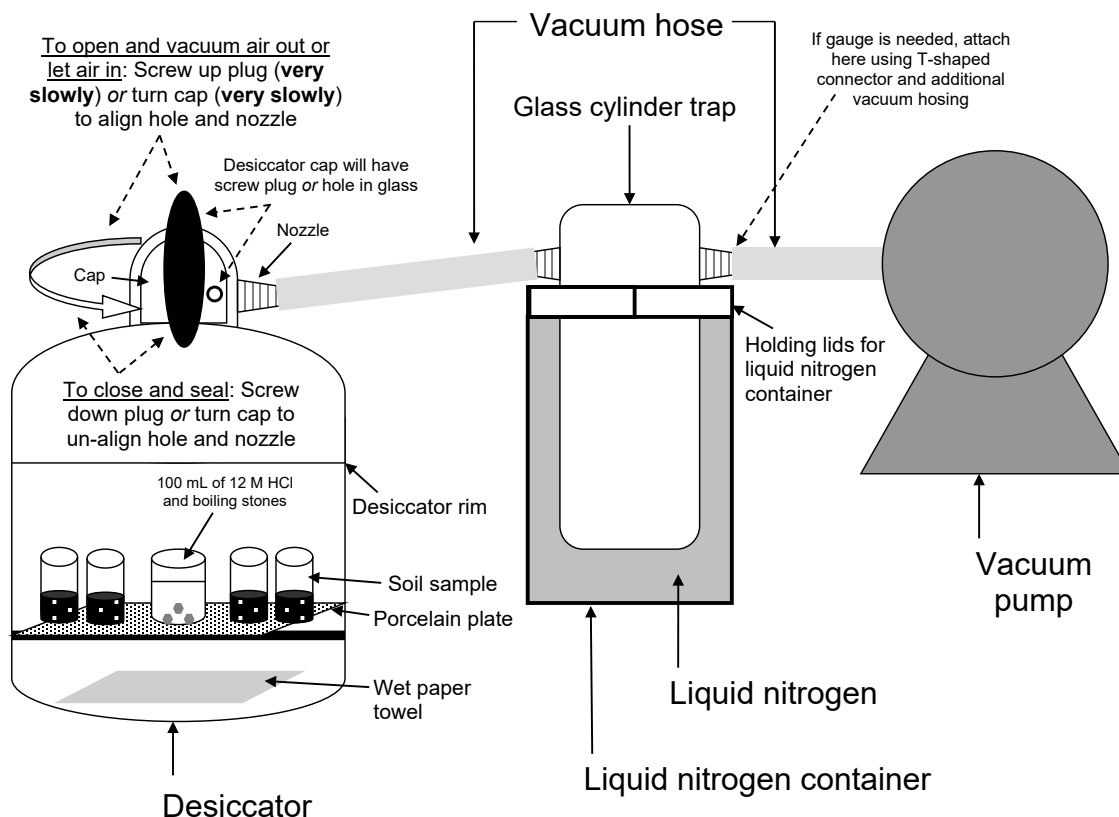


Diagram of desiccator. Modified from Pak Chow (2011), *Chloroform fumigation of soil samples for microbial biomass C and N*.

*Notes: Walthert et al. (2010) recommend a pH of 6.0 as the critical value for removing carbonates; nitrogen content for acid fumigation treated samples was found to change with longer treatments, and therefore the untreated soil nitrogen content determination should be used. Measurements of low amounts of inorganic carbon relative to organic carbon (<10 wt%) are unreliable and should only be measured by direct inorganic carbon analysis (Hedges & Stern, 1984; Walthert *et al.*, 2010).

References

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Date: March 2018

Update record

Update By: Cole Gross

Date: September 2020

Changes: Increased temperature at which to dry soils post fumigation due to potential acid contamination and damage of elemental analyzer; drying at higher temperature helps to remove all residual HCl vapor

Update By:

Date:

Changes: