



Environmental Analysis Program

Pomona College
Biogeochemistry Laboratory

SOP-L409

Microbial Biomass Carbon – Chloroform Fumigation-Extraction

Standard Operating Procedure

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1. Purpose

This SOP describes the determination of soil microbial biomass carbon (MBC) using the chloroform fumigation-extraction (CFE) method. Chloroform vapor lyses microbial cells, releasing intracellular carbon that is then extracted with 0.5 M K_2SO_4 and quantified as dissolved organic carbon (DOC) (Joergensen, 1996; Vance et al., 1987).

2. Scope

- **Applies to:** Field-moist soil samples collected per [SOP-F400 Soil Sampling and Processing](#) for microbial biomass assessment.
- **Does not apply to:** Air-dried or frozen soils (unless pre-validated for this method); microbial biomass nitrogen (requires additional analytical steps).
- **Location:** EA Program laboratory, SGM 133.

3. Responsibilities

Role	Responsibility
Lab PI	Approve protocol, ensure safety compliance
Trained Personnel	Perform fumigation and extraction, report results
New Students	Complete training and safety orientation before use

4. Definitions

- **Chloroform fumigation-extraction (CFE):** The standard method for measuring soil microbial biomass carbon; soil is fumigated with ethanol-free chloroform to lyse microbial cells, then extracted with K_2SO_4 ; MBC is calculated as the difference in extractable C between fumigated and unfumigated soils divided by the k_{EC} factor (Vance et al., 1987).
- **Extractable organic carbon (EOC):** The organic carbon recovered from soil in K_2SO_4 extract; includes a background pool of soluble organic carbon from non-microbial sources as well as carbon released from lysed microbial cells.
- **k_{EC} factor:** The extraction efficiency correction factor for microbial biomass carbon; commonly 0.45, meaning 45% of lysed microbial carbon is recovered in the extract; must be stated when reporting results (Joergensen, 1996; Vance et al., 1987).
- **Microbial biomass carbon (MBC):** The total carbon contained in the living soil microbial community (bacteria, fungi, protozoa, nematodes); expressed as mg C kg^{-1} oven-dry soil; a key indicator of soil biological health and nutrient cycling potential (Robertson et al., 1999).
- **Potentially mineralizable nitrogen (PMN):** The nitrogen that can be released from soil organic matter by microbial activity under aerobic conditions; correlated with microbial biomass and organic matter quality.
- **Unfumigated control:** Soil extracted with K_2SO_4 without prior chloroform fumigation; provides the background extractable carbon value subtracted from the fumigated extract to calculate MBC (Vance et al., 1987).

5. Safety and Hazards

5.1. Critical Safety Warning

Chloroform (CHCl_3) is a volatile, toxic solvent and a suspected carcinogen. All fumigation steps must be performed inside a fume hood. Do not inhale chloroform vapors. Use ethanol-free chloroform only (Food and Agriculture Organization of the United Nations, 2024).

5.2. Hazards

- **Chemical:** Chloroform (toxic, volatile, suspected carcinogen); 0.5 M K_2SO_4 (mild irritant).
- **Physical:** Vacuum desiccator under reduced pressure; risk of implosion if cracked.

5.3. Required PPE

- ☐ Nitrile gloves (double-gloving recommended when handling chloroform)
- ☐ Safety glasses or goggles
- ☐ Laboratory coat
- ☐ Work inside a fume hood at all times during fumigation

5.4. Emergency Procedures

In case of chloroform spill, refer to the SDS and [SOP-L003 Hazardous Materials Handling](#). Evacuate the area if the spill is large; ventilate the space and notify the supervisor.

6. Equipment and Materials

6.1. Equipment

Item	Notes
Vacuum desiccator	Glass, with stopcock and vacuum line
Vacuum pump	Oil-free preferred
Fume hood	Required for all chloroform handling
Orbital or end-over-end shaker	For extraction step
Centrifuge	Capable of $4,000 \times g$ (optional)
Filtration apparatus	With $0.45 \mu\text{m}$ glass fiber filters
Analytical balance	0.01 g resolution
Incubator or dark cabinet	For 24-hour fumigation at room temperature
TOC analyzer	Shimadzu TOC-L; see SOP-L407

6.2. Reagents and Consumables

Item	Notes
Ethanol-free chloroform	ACS grade; stored in fume hood
0.5 M K ₂ SO ₄ solution	Prepare fresh or within 1 week
Glass fiber filters (0.45 µm)	Pre-combusted at 450 °C for 4 hours
Boiling chips or glass beads	For chloroform evaporation under vacuum
Extraction bottles (100 mL)	Screw-top, HDPE or glass
Filter funnels and flasks	Clean, acid-washed
Sample vials	Acid-washed, for filtered extracts

7. Procedure

7.1. Preparation

1. Prepare 0.5 M K₂SO₄ by dissolving 87.1 g K₂SO₄ per liter of deionized water. The 1:4 soil-to-extractant ratio is standard for CFE (Vance et al., 1987).
2. Pre-combust glass fiber filters at 450 °C for 4 hours and store in a clean container.
3. Determine the gravimetric moisture content of a separate subsample (dry at 105 °C for 24 hours per [SOP-L400](#)) so results can be expressed on an oven-dry-weight basis.

7.2. Fumigation

1. Weigh two paired subsamples of 15 g field-moist soil into labeled extraction bottles.
2. Place one set (fumigated) into the vacuum desiccator inside the fume hood.
3. Place a beaker containing approximately 30 mL of ethanol-free chloroform and a few boiling chips in the center of the desiccator.
4. Evacuate the desiccator using the vacuum pump until the chloroform begins to boil vigorously (approximately 2 minutes) (Vance et al., 1987).
5. Close the stopcock and incubate in the dark at room temperature (approximately 25 °C) for 24 hours (Food and Agriculture Organization of the United Nations, 2024; Vance et al., 1987).
6. The second set (unfumigated controls) is extracted immediately (proceed to §6.3).

7.3. Extraction

1. After 24 hours, release the vacuum inside the fume hood and remove the chloroform beaker.
2. Evacuate the desiccator repeatedly (3–5 times) to remove residual chloroform vapor from the soil.
3. Add 60 mL of 0.5 M K₂SO₄ to each bottle (both fumigated and unfumigated), achieving a 1:4 soil-to-extractant ratio (Vance et al., 1987).
4. Cap bottles and shake on an orbital or end-over-end shaker for 1 hour.
5. Allow the suspension to settle briefly, or centrifuge at 4,000 × g for 10 minutes.
6. Filter the supernatant through a pre-combusted 0.45 µm glass fiber filter into a clean sample vial.
7. Store filtered extracts at 4 °C if not analyzed immediately; analyze within 48 hours.

7.4. DOC Analysis

1. Analyze filtered extracts for dissolved organic carbon using the Shimadzu TOC-L per [SOP-L407 Total Carbon](#).
2. Run 0.5 M K₂SO₄ blanks alongside samples.

7.5. Calculation

Microbial biomass carbon is calculated as (Joergensen, 1996; Vance et al., 1987):

$$\text{MBC} = \frac{C_{\text{fumigated}} - C_{\text{unfumigated}}}{k_{EC}}$$

where:

- $C_{\text{fumigated}}$ = DOC in the fumigated extract (mg C kg⁻¹ oven-dry soil)
- $C_{\text{unfumigated}}$ = DOC in the unfumigated extract (mg C kg⁻¹ oven-dry soil)
- k_{EC} = 0.45, the extraction efficiency coefficient (Joergensen, 1996; Vance et al., 1987)

i Note

The unfumigated extract DOC value represents the background dissolved organic carbon pool and is often reported separately as an indicator of labile soil carbon (Robertson et al., 1999).

8. Quality Control

- Run analytical triplicates for at least 10% of samples; the coefficient of variation among replicates should be less than 15% (Food and Agriculture Organization of the United Nations, 2024).
- Include a reagent blank (0.5 M K₂SO₄ only, no soil) in each batch.
- Include a laboratory reference soil in each batch to monitor method consistency.
- Verify that the TOC calibration $r^2 \geq 0.999$ before reporting results.

9. Waste Disposal

Waste Type	Container	Disposal Method
Chloroform waste	Halogenated waste jug	EH&S hazardous waste pickup
K ₂ SO ₄ extracts	Aqueous waste container	Drain with copious water (if approved) or collect for EH&S
Used soil	Solid waste bag	Standard trash
Used glass fiber filters	Standard trash	Standard trash

10. References

- Vance et al. (1987)
- Joergensen (1996)
- Food and Agriculture Organization of the United Nations (2024)
- Robertson et al. (1999)
- [SOP-L003 Hazardous Materials Handling](#)
- [SOP-L400 Soil Moisture](#)
- [SOP-L407 Total Carbon](#)

Food and Agriculture Organization of the United Nations. (2024). *Standard operating procedure for soil microbial biomass (Carbon): Chloroform fumigation-extraction method*. FAO. <https://www.fao.org/soils-portal/soil-survey/soil-analysis/en/>

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Robertson, G. P., Coleman, D. C., Bledsoe, C. S., & Sollins, P. (1999). *Standard soil methods for long-term ecological research*. Oxford University Press.

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11. Related SOPs

SOP	Title	Relationship
L003	Hazardous Materials	Chloroform handling
L400	Soil Moisture	Required companion analysis
L407	Total Carbon	DOC analysis of extracts
L408	Solvita CO ₂ Burst	Complementary respiration index

12. Training Record

Before performing this procedure independently, personnel must:

1. Read this SOP and [SOP-L003 Hazardous Materials Handling](#) in full
2. Review the SDS for chloroform
3. Observe a trained operator perform the full fumigation-extraction procedure
4. Demonstrate competence with vacuum desiccator operation
5. Perform the procedure under supervision
6. Be signed off by the PI or lab manager