Chloroform Fumigation Direct Extraction Procedure

(modified from Vance et al., 1987; Soil Biol. & Biochem., 19(6):703-707)

Theory:

Chloroform is an effective biocide and does not solubilize non-microbial soil organic matter or render it decomposable. A relationship exists between the amount of C rendered extractable by fumigation (Ec; defined as [organic C extracted by 0.5M K_2SO_4 from a fumigated soil]-[organic C extracted from a non-fumigated soil] and biomass C. This modification quantifies the amount of radiolabeled petroleum hydrocarbon that is incoroprated into biomass C in soils contaminated with ^{14}C-labeled compounds.

Materials:

Incubated soil samples of known water content previously contaminated with ^{14}C-labeled compounds.
Geiger counter
0.5 M K_2SO_4 solution
ethanol-free $CHCl_3$
boiling chips
dessicator
paper towels
scintillation cocktail
scintillation counter

Procedure:

(1) Setup Geiger counter in the area that you are working. Handle all materials with gloves and appropriate personal protective equipment. Do all the work in the radioactive hood and label all soil containers with radioactive tape.
(2) Nine portions of air dried soil (3 control samples from soil microcosm, 3 control samples that have been water extracted, and 3 water extracted samples), 50g ODE (can be scaled down to 20g ODE) are weighted into 100mL glass beakers.
(3) The control samples (should be 6) are immediately extracted with 200mL of 0.5 M K_2SO_4.(If using 20g ODE extract with 80mL potassium sulfate). The other three are fumigated in a dessicator lined with wet filter paper to maintain humidity. Inside the dessicator as well place about 25mL ethanol-free $CHCl_3$ in a small beaker with a few boiling chips.
(4) Evacuate the dessicator until the $CHCl_3$ has boiled for 2 minutes and then place in the dark at 25ºC. Fumigate for 24 hours.
(5) After 24 hours, repeatedly evacuate the dessicator in order to remove the resdual $CHCl_3$ vapor in the soil.
(6) For extraction, soil is transferred to a 350-500mL Erlenmyer flask, and 200mL of 0.5mL 0.5M K_2SO_4 added. Place the flasks on an oscillating shaker for 30 minutes.
(7) Filter suspension (Whatman No 42).
(8) Organic C extracted can be determined using acid digestion, $K_2Cr_2O_7$, and ferrous ammonium sulfate back titration (see paper for exact protocol).
(9) The total amount of radiolabeled petroleum hydrocarbon incorporated into microbial biomass C can be determined based on the scintillation counts obtained from the K_2SO_4 extracted C. These values should be expressed as

µg^{14}C / gram OD soil. These values can be used to extrapolate back to microbial biomass ^{14}C using the following equation:

$$\textbf{Biomass } ^{14}\textbf{C} = 2.64 * E_c$$

where E_c is the difference between ^{14}C extracted from the fumigated and non-fumigated (control) treatments. The ^{14}C obtained from the control samples that have been water extracted will be used to estimate the amount of overlap that occurs between the water soluble ^{14}C pool and the directly extracted microbial biomass ^{14}C pool.