

Soil Microbial Biomass Determination

Basic Idea:

The quantity of microbial biomass in a given soil sample is inherently difficult to measure. To some degree, any measurement of microbial biomass is relative – different methodologies or variations in methodologies will yield microbial biomass estimates that are not directly comparable. For this reason, if you want to measure microbial biomass – choose a method and stick with it exactly. Here, I'll discuss 2 methods to measure microbial biomass: substrate induced respiration (SIR) and simultaneous chloroform fumigation-extraction (sCFE), a modification of the traditional chloroform-fumigation extraction method.

SIR estimates microbial biomass based on the short-term respiration rates (CO₂ production) when soil is given an excess supply of labile C. The higher the respiration rate, the more microbial biomass in a given soil. The SIR method is fairly straightforward, consistent, and reasonably quick, however, the biomass estimates are relative – the method will not give direct estimates of microbial biomass C (or N) in soil.

sCFE measures microbial biomass based on the amount of microbial C or N extractable from soil after treatment with chloroform, a cell membrane disruptor. There is a lot of confusion about general chloroform fumigation-extraction techniques, and, (in my opinion) the technique is often carried out in a manner that makes cross-comparison of biomass estimates difficult. For these reasons, I have included a lengthy treatise on the technique (see pages 2 – 4) along with a full description of my modifications to the traditional chloroform-fumigation extraction method. The sCFE technique combines the soil extraction and chloroform exposure steps, increasing the simplicity and efficacy of the normal fumigation-extraction method. The modified method yields estimates of microbial C and N that are well correlated with those obtained by the normal fumigation-extraction method across a range of soil types. The simultaneous chloroform slurry-extraction method is particularly useful for studying soils with a range of porosities and/or water contents where differences in chloroform diffusion rates through soils may affect biomass extraction efficiencies when using the normal fumigation-extraction method.

SIR:

- Weigh 5- 10g soil (fresh wt.) of soil into a 50mL centrifuge tubes equipped with gas-tight lids that have rubber septa for gas sampling.
- Add 10mL yeast solution to each tube. Yeast solution should deliver 20mg yeast/g dry soil. (3g autolyzed yeast extract to 250mL ddH₂O). Other SIR protocols use glucose but I've found that yeast gives higher respiration rates and the goal of the method is to maximize CO₂ production.
- Cap tubes and shake horizontally in 20⁰C room for the duration of the 4 h assay. 10 –20 minutes after sealing the tubes measure the initial (the T0 time point) headspace CO₂ concentrations. This is the T0 time point. Measure

headspace CO₂ concentrations two more times, approx. 2h and 4h after the T0 time point.

- • Measure headspace CO₂ concentrations by injecting 5mL lab air into tube, pull out 5mL air from tube and measure cumulative CO₂ concentrations on an IRGA equipped for static CO₂ analysis. Make sure to record the time that the gas sample was taken.
- • Calculate the slope of the line relating CO₂ concentrations to time. The average respiration rate (µg C-CO₂/gsoil/h) over the 4 h incubation period is an index of the SIR-responsive microbial biomass. Calculate an r² value for the line describing CO₂ concentrations over time to make sure the relationship is roughly linear.
- • This technique is modified from that described in: West and Sparling. 1986. *Journal of Microbiological Methods*. 5: 177-189.

sCFE:

Why modify the traditional CFE method?

- The chloroform fumigation-extraction (CFE) method that is “traditionally” used to measure microbial biomass in soil involves fumigating soils with chloroform for 24 h to 4 days to lyse microbial cells. At the end of the fumigation, both the fumigated sample and an unfumigated “control” sample are extracted with a salt solution. Extractable microbial biomass C or N is then measured as the difference in the amount of C or N extracted from the fumigated and the control samples.

There are several potential sources of error when estimating soil microbial biomass C and N with this method. One problem is that the fumigation-extraction technique relies on the gaseous diffusion of chloroform through soil, so fumigation efficiencies can be reduced in soils that are wet or have low porosities. This may pose particular problems when estimating annual variation in the size of the microbial biomass pool where seasonal differences in soil water content may dramatically alter extraction efficiencies.

Another possible problem is the potential for microbial activity or enzymes to cause changes in extractable C and N during the relatively long fumigation period. One assumption of the method is that the extractable C and N levels in the unfumigated “control” samples are the same as those in the fumigated sample at the end of the fumigation, minus the “flush” of microbial biomass C and N released by the chloroform. However, chloroform fumigation does not stop all microbial or enzymatic activity and a substantial portion of the microbial biomass may survive fumigation. We have measured respiration rates during a 24 h fumigation period that are 64% and 90% of the respiration rates measured in the equivalent unfumigated controls. Significant mineralization of soil organic matter, or added substrate, during the fumigation period may cause changes in non-biomass extractable C and N levels that would not be reflected in the unfumigated “control”. This may be particularly evident in short-term soil labeling experiments with ¹⁴C, ¹⁵N, or ³H labeled substrates where a prolonged fumigation period may lead to a significant disparity between the unfumigated and fumigated samples.

The solution?

We addressed both of these problems by combining the chloroform-exposure and extraction steps while reducing the amount of time samples are exposed to chloroform. Treating the soils with chloroform in a slurry extract reduces any effects of chloroform diffusivity on fumigation efficiencies and allows the control and fumigated (chloroform-exposed) subsamples to be extracted simultaneously. Shortening the chloroform exposure period reduces the chance that extractable C and N levels will change substantially during chloroform exposure.

The traditional CFE and sCFE methods gave very comparable estimates for microbial biomass C and N over a wide range of soil types (see Figure 1 below). On average, the sCFE method rendered approximately 30% less biomass C and N extractable than the traditional CFE method. The two techniques had similar levels of variability between sample replicates in extractable microbial biomass C and N (average coefficients of variation = 0.25% for C and 0.48% for N). The lower recovery of biomass C and N with the simultaneous CSE method is not surprising considering that chloroform exposure times were only 4 h compared to 24 h with the traditional CFE method. Further description of the method and a comparison of the sCFE method to the traditional CFE method is provided in [Appendix 1](#) of my Ph.D. thesis (Fierer, 2003. *Stress Ecology and the Dynamics of Microbial Communities and Processes in Soil*. University of California, 226 pages).

Method Description:

- • For each soil sample, weigh two soil subsamples (chloroform-exposed and control) of 3 to 10 g each into 70 mL glass tubes (Pyrex No. 9825).
- • Add 40 mL of 0.5 M K_2SO_4 to each of the glass tubes containing the soil subsamples. To one subsample add 0.5 mL of EtOH-free chloroform. Seal both the chloroform-exposed and the control samples with chloroform resistant screw caps and shake simultaneously at approximately 150 rev min^{-1} for 4 h.
- • After the shaking period, let the tubes settle for 10 minutes. Decant the top 20-30 mL of the soil extracts and gravity filter through Whatman No. 1 paper folded into a glass funnel. Collect the filtrate in 50mL centrifuge tubes. Filtering only the top portion of the soil extract reduces chloroform contamination in the filtered extract since the liquid chloroform settles to the bottom of the tube.
- • Immediately bubble the filtered extracts vigorously with air for 20-30 min., the most effective method for removing chloroform from extracts. We use house air bubbled through long spinal tap needles. Bubble both the chloroform-exposed and control extracts.
- • You need a blank for both the control and chloroform exposed extracts. (40mL of 0.5M K_2SO_4 with and without chloroform). Treat the blanks in the same manner as the samples. In general, the K_2SO_4 blanks with chloroform added should have levels of dissolved carbon only 25% greater than the very low C concentrations (1-2 ppm C) in the blanks without chloroform.

- • Analyze the control and chloroform-exposed extracts for total dissolved C and N using a persulfate digestion technique. Dissolved C and N concentrations in the K_2SO_4 blanks (no soil added) with and without chloroform added are subtracted from the extract concentrations of the chloroform-exposed and the control samples, respectively. Extractable microbial biomass C and N are calculated as the difference in total dissolved C and N (both organic and inorganic) between the chloroform-exposed subsample and the corresponding control subsample. Express results as μg chloroform extractable biomass C/g dry soil. In order to calculate total microbial biomass C or N (extractable + non-extractable biomass) you need to use a conversion factor. In general, 20 – 40% of the total biomass is extracted by this technique but the percentage extractable will vary between different soils. A further discussion of conversion factors is available in Tate et al. 1988 (*Soil Biology & Biochemistry* 20:329-335) and Dictor et al. 1998 (*Soil Biology & Biochemistry* 30:119-127).