Soil samples can be tested immediately or stored at 4C, 20C, or 37C for 10 days before testing by microBIOMETER® for microbial biomass (MB) and fungal to bacterial ratio (F:B)

Background: microBIOMETER® test requires field moist fresh samples for accurate analysis. The primary aim of this analysis was to determine storage conditions for sample analysis by microBIOMETER®. Analysis of microbial biomass by carbon fumigation (CF), phospholipid fatty acid analysis (PLFA) and respiration use dried soils for analysis: the secondary aim was to gain information on the changes to the microbial biomass under drying conditions so as to better understand discrepancies between microBIOMETER® and assays calculating MB and F:B in dried soils.

Summary: Analysis of sample stability for testing by microBIOMETER® showed that field moist samples stored at 4C RT, or 37C for 10 days delivered the same results as samples analyzed upon the same days as sampling, and remained suitable for estimation of microbial biomass (MB) and Fungal to Bacterial Ratio (F:B). Air drying of samples caused up to 80% loss of MB over 4 days and a sharp decrease in the F:B ratio indicating that fungi were preferentially lost during drying. Reconstitution of dried samples by rehydrating to 20% did not reliably restore the MB or F:B and cannot be used for testing. Frozen samples analysis by microBIOMETER® did not consistently correlate with microscopy and so placing in a 20C freezer for storage is not recommended.

Methods: Soils from 3 locations were sampled and analyzed by both the microBIOMETER® app and digital microscopy. The first was from an area of cropland that is alternatively planted with soybean or corn (denoted as "field" herein). The second is from a residential lawn that has been covered by fescue for over 35 years (denoted as "grass" herein). The third is from a wooded area that surrounds the wetland formed by runoff of the surrounding sloping property (denoted as "wood" herein).

Storage conditions: Soils were collected 3/30/2020, stored in Ziploc bags and subjected to varying treatments for 24 hours to several months.

- 4 weeks and ongoing
  - Freeze/thaw – store in freezer, thaw entire collected sample, analyze, refreeze
- 24 days
  - Refrigeration (4 C)– minimal air flow — rose to room temp each day analyzed before returning to refrigeration (< 2 hrs. at room temp during analysis).
  - Room Temperature (RT)– sealed bag at 65 F
  - Drying out – open tray at 65 F. Sample was probably not entirely dry for days.
- 14 days
  - Reconstituted (rehydrated) -- dried soils (10 days dried) were reconstituted according to Haney method (20% water –> 8 g soil + 2 ml H_20) and
- 7 days
  - 37 C in sealed bags to prevent evaporation
- 24 hours
  - 70 C in sealed bags to prevent evaporation

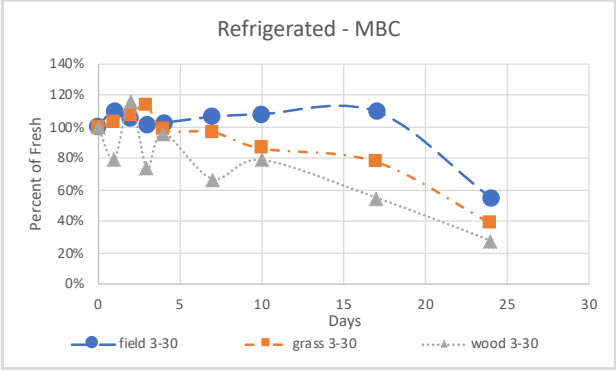
ANALYSIS

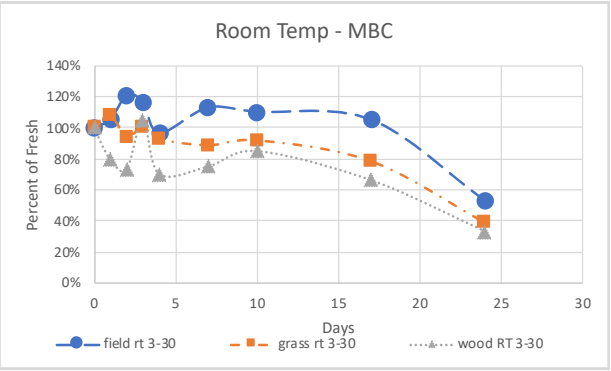
The microBIOMETER® extraction procedure was used to isolate soil microbes. Briefly ½ cc of sifted field moist soil was added to 9.5 ml of microBIOMETER extraction fluid and whisked for 30 seconds with the microBIOMETER® whisker. After settling for 20 minutes, the extract was analyzed by placing 6 drops on the microBIOMETER® test card and soil microbial biomass carbon (MBC, in units μg C g^{-1} soil) and fungal:bacterial ratios (F:B) were estimated using microBIOMETER® smartphone application. With the microBIOMETER® app, F:B is determined colorimetrically.

RESULTS

4C and RT CHANGES IN MICROBIAL BIOMASS BY microBIOMETER® "MBC" OVER TIME

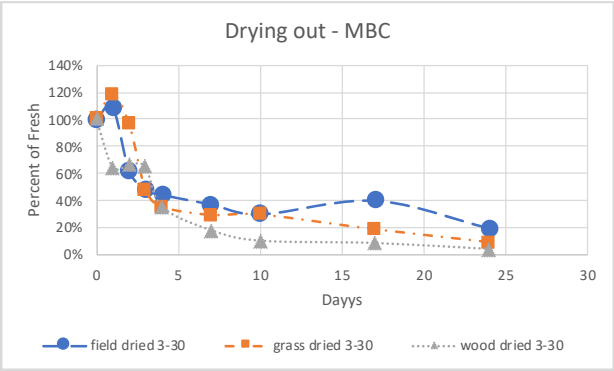
Below are the results of soils measured with microBIOMETER® as microbial biomass carbon " MBC". Day zero is when the soils were collected and percent change over 24 days is displayed. The graphs illustrate that the soil sample reads consistently with microBIOMETER® for 10 days at 4C and RT. We conclude that samples may be assayed for MB in samples stored at RT and 4C for 10 days.





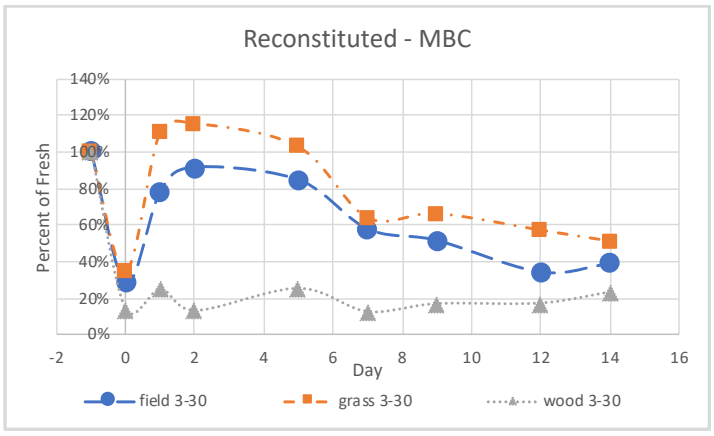
DRIED SOIL CHANGES IN MICROBIAL BIOMASS BY microBIOMETER® "MBC" OVER TIME

Soil was placed in plastic weigh boats and allowed to dry at 20 C over the indicated time. The % water of the samples was never measured, but as there was much rain at the time of collection all samples were moist and presumably they dried at various rates over 5-7 days. microBIOMETER® shows a steep drop in MB over the first 4 days and both record a MB loss of 40% or greater. We conclude that microBIOMETER® testing should not be performed on dried out samples.



RECONSTITUTION OF DRIED SOILS DOES NOT RESTORE THE ORIGINAL POPULATION

Because respiration assays claim that respiration over 24 hours significantly correlates with MB, on day 10 we reconstituted the dried soils to 20% water. As shown below this resulted in a highly variable return of microbial biomass as assayed microBIOMETER®. Below is depicted the recovery after reconstitution. Day -1 represents the MBC measured on the day the soils were sampled. Day 0 is the value after 10 days of drying at room temperature.

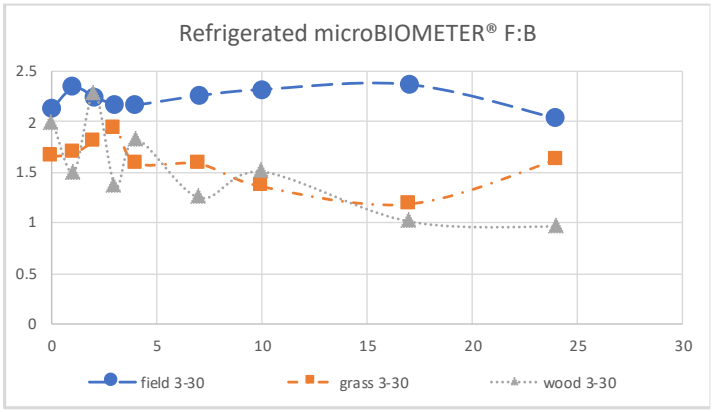


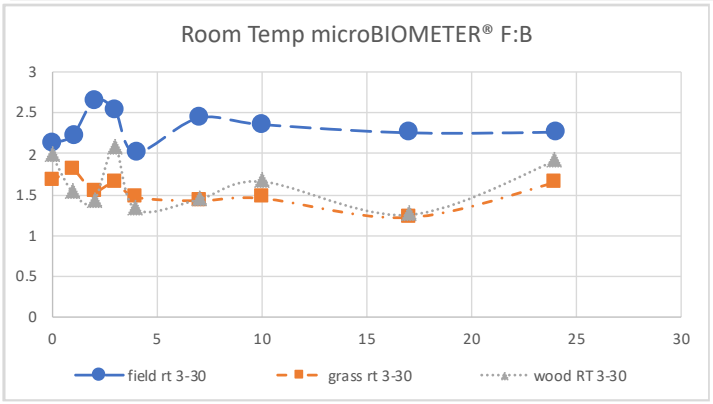
Recovery of grass soil was the greatest, with MBC overshooting the value for fresh soil within one day of rehydration. Field soil recovered to 80% of the fresh value within one day, the usual time allowed for soil reconstitution when used in the Haney test. Soil from the wooded area did not recover at all. We conclude that drying significantly affects the ability to accurately measure microbial biomass. Because both the carbon fumigation and phospholipid fatty acid methods of estimating MB use dried soil, we question whether these methods are measuring the actual MB present in field moist conditons.

4C, and RT CHANGES IN FUNGAL:BACTERIAL RATIOS BY microBIOMETER® "F:B" AND DIGITAL MICROSCOPY OVER TIME

Fungal:bacterial ratios (F:B) were determined using the microBIOMETER® app. With the microBIOMETER® app, F:B is determined colorimetrically.

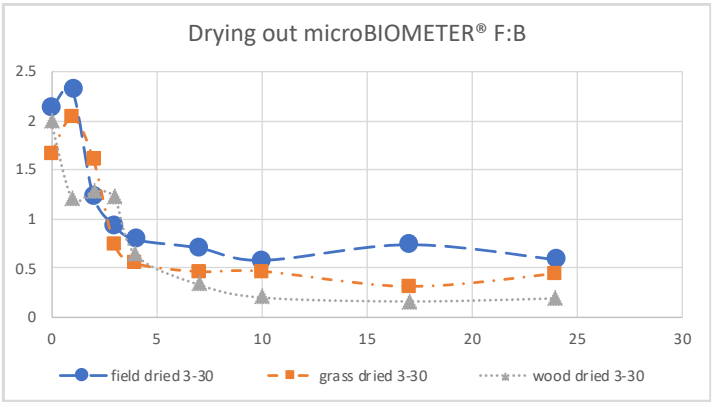
As shown below, microBIOMETER® estimates for F:B stayed near the value for fresh soil for both refrigerated and room temperature treatments for 10 days. There is ±0.5 variability in the microBIOMETER® F:B estimate.

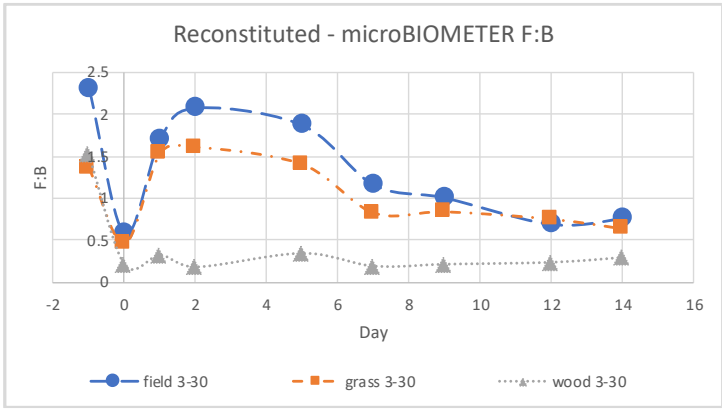




Dried and Reconstituted CHANGES IN FUNGAL:BACTERIAL RATIOS BY microBIOMETER® "F:B" OVER TIME

The loss of 80% of MBC as measured by microBIOMETER® appears to change the microbial population that can recover upon reconstitution to 20% wetness. Shown below, the woods sample did not recover to fresh fungal levels after reconstitution.

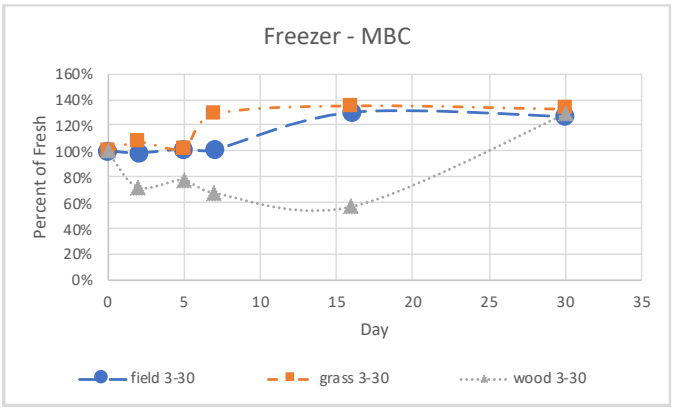


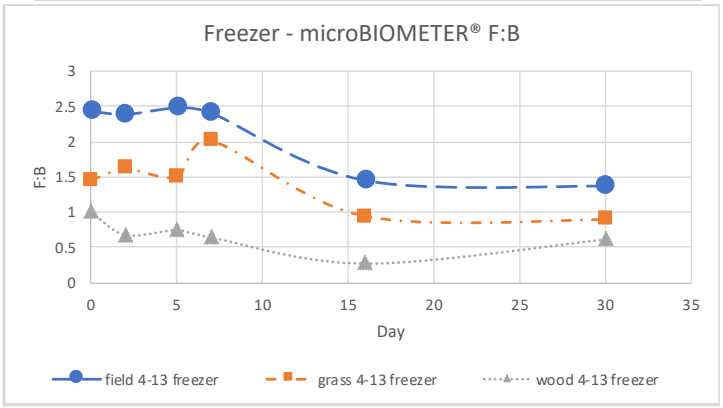


We conclude that fungi are more sensitive to storage conditions than bacteria and, due to great variation in MB recovery during rewetting, that dried samples do not report field soil microbial mass or composition.

FREEZE THAW CHANGES IN MICROBIAL BIOMASS AND FUNGAL:BACTERIAL RATIOS BY microBIOMETER® OVER TIME

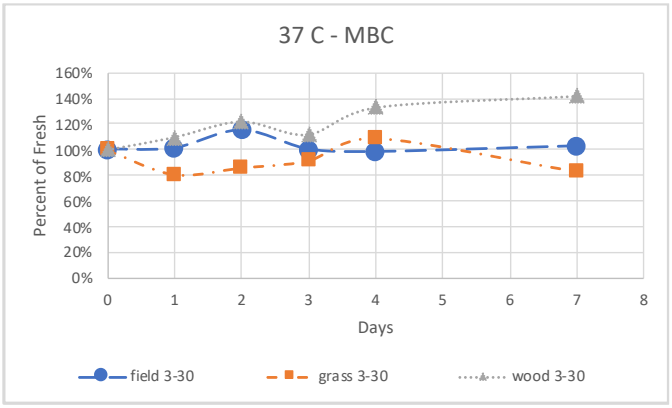
Effect of freezing and thawing on sample integrity for microBIOMETER® assay of soils. The freezer samples showed a decline in F:B ratio by microBIOMETER® after day 7. Since refrigerated, RT and 37C samples were stable for 10 or more days for both MB and F:B, we conclude that intermitant freezing and thawing is not a viable solution for storing soil samples for microBIOMETER®. A study where samples remain frozen but not thawed is underway.

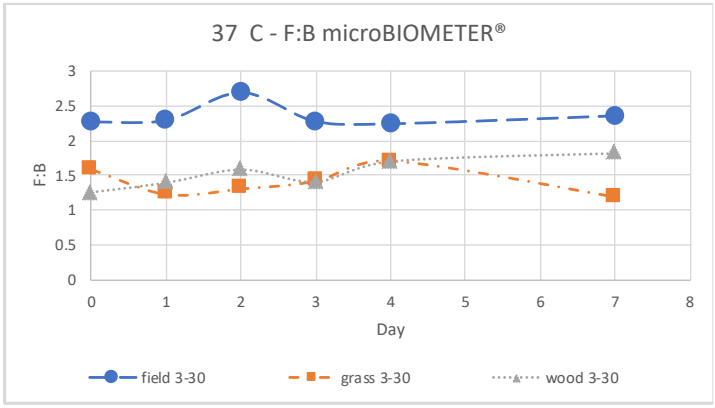




AT 37C, MICROBIAL BIOMASS AND FUNGAL:BACTERIAL RATIOS WERE STABLE FOR 7 DAYS

Samples were placed in bags at 37C for 7 days. Within the limits of the assays, both MB and F:B ratio read consistently over the 7 days. This is probably not surprising since this a common temperature for these soils and microbes.





CONCLUSION:

Soil samples can be tested immediately or stored at 4C, 20C, or 37C for 7-10 days before testing by microBIOMETER® for microbial biomass (MB) and fungal to bacterial ratio (F:B). For accurate estimation we recommend running the assay in triplicate, which is the usual method for soil MBC and F:B analysis.