

## Introduction

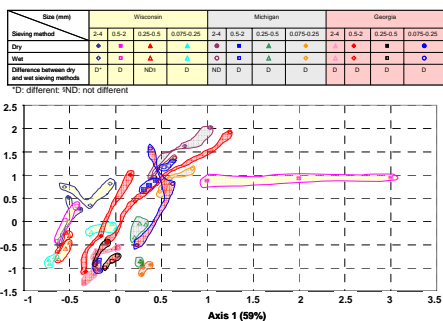
The soil aggregate represents an ecological niche whose chemical and physical properties may contribute to the overall distribution of microorganisms among aggregates of different sizes. Previous studies indicate that the distribution of bacteria and fungi differ among aggregate size fractions and that separation methods using wet sieving techniques results in significant loss of microbial biomass. Fractionation methods used to obtain soil aggregates may also result in changes to other chemical, physical, and biological processes in soil. Understanding the potential impact of fractionation methods on soil aggregates is necessary to predict the occurrence and rate of microbial mediated functions that are of environmental importance such as the degradation of organic contaminants.

## Objectives

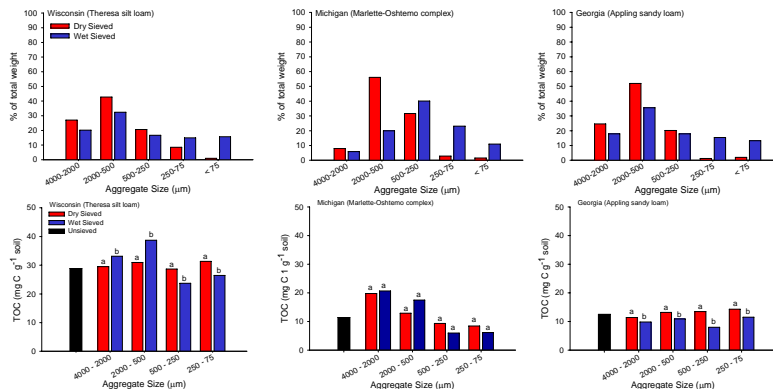
- Determine the impact of soil size-fractionation methods on soil microbial community composition and chemical and physical properties of soil.
- Characterize the microbial community composition in soil aggregates of different sizes using signature fatty acid biomarkers.

## Materials and Methods

- Moist topsoil (0-15 cm) was collected in May, 2003 from Wisconsin (Theresa silt loam (WI), mesic Typic hapludalf, New Berlin), Michigan (Marlette-Oshtemo complex, (MI) mesic Typic hapludalf, Alto), and Georgia (Appling sandy loam (GA), thermic Typic Kanhapludult, Athens).
- The soil was air-dried at 25° C for 24 hours, and gently broken up to pass through a 4 mm sieve to remove large gravel and root pieces, and stored at 5° C until commencement of the experiment.
- The soil samples were sieved to separate four aggregate fractions; 4000 – 2000 μm, 2000 – 500 μm, 500 – 250 μm, and 250 – 75 μm.
- The wet sieving method consisted of suspending three sieves (250 μm, 500 μm, and 2000 μm) in water and “shaking” 200 g of soil at a rate of 30-40 oscillations per minute for 10 minutes. After 10 minutes the water was drained and passed through a 4<sup>th</sup> sieve (75 μm).
- The dry sieving method consisted of using four sieves (75 μm, 250 μm, 500 μm, and 2000 μm) on a Tyler, Ro-Tap® shaker and shaking 200 g of each soil for 3 minutes. A preliminary study showed that there was no significant difference in the distribution of soil aggregates shaken for periods longer than 3 minutes.
- The extraction of fatty acid biomarkers was based on method by Schutter and Dick (2000).
- Total organic carbon, cation exchange capacity, and particle size distribution analysis was conducted on the three soils and the various soil aggregates. All analyses were performed using standard methodology published in either the American Society of Agronomy Monograph No. 9, or USDA-NRCS Soil Survey Investigations Report No. 42, Version 3.0, *Soil Survey Methods Laboratory Manual (1996)*.
- Nonmetric multidimensional scaling multivariate analysis of soil microbial communities was conducted using PC-ORD software (<http://home.centurytel.net/~njm/pcordwin.htm>)



**Figure 1.** Nonmetric multidimensional scaling multivariate analysis of soil microbial communities based on 7 microbial biomarkers listed in Table 1. Axis 1 accounts 59% of the variation in the data, Axis 2 accounts for 37%. The analysis indicates different microbial community composition in soil fractions separated using the two sieving methods. Solid and open symbols in the table above indicate dry sieving method and wet sieving method, respectively. The background color indicating different soils in the table corresponds to that in the figure. The analysis also shows significant differences for soil microbial community among the 3 soils.



**Figure 2.** Weight distribution and total organic carbon (TOC) for aggregates separated using wet and dry sieving methods for the 3 soils (WI, MI, and GA). Different letters above each column indicate significant differences between methods at  $p = 0.10$ .

## Results

**Table 1.** Comparison of dry and wet sieving methods on the amount of 7 significant microbial biomarkers in three distinct soils. Differences between wet and dry sieving methods are significant at  $p = 0.05$ .

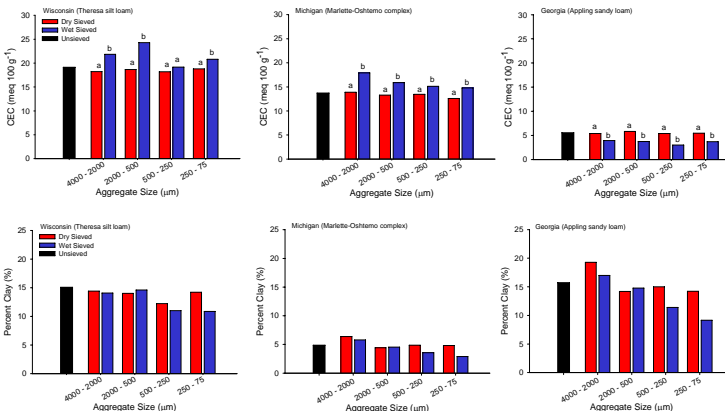
Biomarker	Fatty Acid	Wisconsin			
		4000-2000	2000-500	500-250	250-75
Eubacteria	15:0i	Dry>Wet	Dry>Wet	Dry>Wet	Dry>Wet
Eubacteria	15:0a	Dry>Wet	Dry>Wet	Dry>Wet	Dry>Wet
Eubacteria	16:0i	Dry>Wet	Dry>Wet	Dry>Wet	Dry>Wet
Fungi	16:00	Dry>Wet	Dry>Wet	Dry>Wet	Dry>Wet
Eubacteria	16:1w9	Dry>Wet	Dry>Wet	Dry>Wet	Dry>Wet
Fungi	18:2w	Dry>Wet	Dry>Wet	Dry>Wet	Dry>Wet
Anaerobes	19:0cy	Dry>Wet	Dry>Wet	Dry>Wet	Dry>Wet

Biomarker	Fatty Acid	Michigan			
		4000-2000	2000-500	500-250	250-75
Eubacteria	15:0i	Dry>Wet	Dry>Wet	Dry>Wet	Dry>Wet
Eubacteria	15:0a	Dry>Wet	Dry>Wet	Dry>Wet	Dry>Wet
Eubacteria	16:0i	Wet>Dry	Wet>Dry	Dry>Wet	Dry>Wet
Fungi	16:00	Wet>Dry	Wet>Dry	Dry>Wet	Dry>Wet
Eubacteria	16:1w9	Wet>Dry	Wet>Dry	Dry>Wet	Dry>Wet
Fungi	18:2w	Dry>Wet	Dry>Wet	Dry>Wet	Wet>Dry
Anaerobes	19:0cy	Dry>Wet	Dry>Wet	Dry>Wet	Dry>Wet

Biomarker	Fatty Acid	Georgia			
		4000-2000	2000-500	500-250	250-75
Eubacteria	15:0i	Dry>Wet	Wet>Dry	Dry>Wet	Dry>Wet
Eubacteria	15:0a	Dry>Wet	Wet>Dry	Dry>Wet	Dry>Wet
Eubacteria	16:0i	Wet>Dry	Wet>Dry	Dry>Wet	Dry>Wet
Fungi	16:00	Wet>Dry	Wet>Dry	Dry>Wet	Dry>Wet
Eubacteria	16:1w9	Wet>Dry	Wet>Dry	Dry>Wet	Dry>Wet
Fungi	18:2w	Dry>Wet	Wet>Dry	Dry>Wet	Wet>Dry
Anaerobes	19:0cy	Dry>Wet	Dry>Wet	Dry>Wet	Wet>Dry



**Figure 3.** Cation exchange capacity (CEC) and clay content for aggregates separated using wet and dry sieving methods for the 3 soils (WI, MI, and GA). Different letters above each column indicate significant differences between methods at  $p = 0.10$ .

## Conclusions

- Multivariate analysis of soil microbial communities show significant differences between methods and among the 3 soils (Figure 1).
- Eubacteria, fungal, and anaerobic microbial biomarkers are more sensitive to the wet sieving method than the dry sieving method at the smaller aggregate size fractions (500 – 250 μm and 250 – 75 μm)(Table 1).
- The weight distribution of aggregates tend to be greater in larger aggregate size fractions from the dry sieving method compared to the wet sieving (Figure 2).
- Figure 3 indicates that wet sieved aggregates in the Theresa silt loam (WI) and Marlette-Oshtemo complex (MI) had a larger CEC than dry sieved aggregates due to the presence and expansion of 2:1 smectitic clays which are dominant clay minerals in the two soils. However, in the Appling sandy loam (GA) the CEC was greater in the dry sieved aggregates compared to the wet sieved aggregates. The decrease of CEC in the GA soil was due in part to a decrease in TOC (Figure 2) and clay content (Figure 3) within smaller aggregate size fractions.
- While the distribution of smaller sized (< 500 μm) aggregates is greater in soils which have been wet sieved compared to dry sieved, particle size distribution analysis shows a decrease in the clay content in these same aggregates (Figure 3).
- The fractionation method used to obtain aggregates of various size has a significant impact on properties and processes necessary to predict the occurrence and rate of chemical and microbial mediated functions that are of environmental importance. This study has shown that the use of a wet sieving has a significant impact on the microbial community and that dry sieving methods should be utilized in research focused on the characterization of microbial communities in soil.