

## INTRODUCTION

Application of X-ray computed micro-tomography ( $\mu$ -CT) in soil science research has a great potential for studying relationships between soil physical micro-environments and biological soil processes. Most of previous studies concluded that low X-ray dose (<30 Gy) did not impact growing plant roots and microbial populations (Zappala et al., 2013). However, the X-ray dose applied for  $\mu$ -CT scanning can be substantially greater. E.g., in the  $\mu$ -CT scanning conducted at Argonne APS facility for this study, the dose was as high as 260 Gy. In order to use the  $\mu$ -CT tools in analysis of soil biological processes, it is imperative to understand possible artifacts that treatment with X-ray  $\mu$ -CT might have on soil microbial community structures and their activities.

## OBJECTIVES

To investigate whether or not subjecting soil samples to  $\mu$ -CT scanning influences:

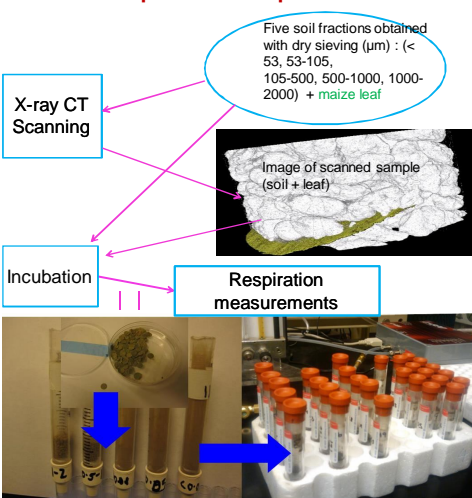
- subsequent microbial activity, as expressed via soil respiration in the samples.
- microbial community composition of the scanned samples.

## MATERIALS AND METHODS

### Soil sampling

Soil samples collected from 0-15 cm depth at conventional and organic corn-soybean-wheat rotation treatments of LTER site, Kellogg Biological Station, MI. The soil type is Typic Hapludalfs with coarse loamy texture.

### Incubation experiment setup



### X-ray $\mu$ -CT scanning

- Scanning was conducted at the Advanced Photon Source of Argonne National Laboratory (station BMD-13).
- Two replications of each soil fraction used in incubations (Fig. 1) scanned with 6.5  $\mu$  resolution.
- Four individual intact aggregates 4000-6000  $\mu$  in size scanned with 13  $\mu$  resolution.

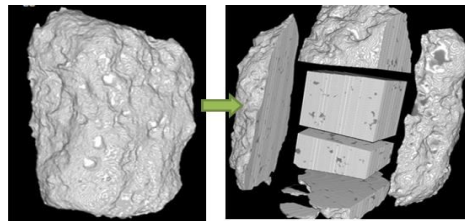


Fig. 2. Cutting of scanned 4000-6000  $\mu$  aggregates used for microbial analysis.

### Microbial community measurements

- The scanned 4000-6000  $\mu$  aggregates were cut into 7 pieces and microbial community analysis was conducted in each cut piece (Fig 2).
- Total genomic DNA was isolated using MoBio's Powersoil Kit.
- The V3-V5 region of the 16S rRNA gene was amplified and sequenced by 454 pyrosequencing.
- Microbial community analyses were performed using Mothur.

Fig. 1. Incubation experiment setup and respiration measurements.

- The mass of soil and maize leaf samples used for respiration study was  $\sim$ 0.6 and 0.0025 g, respectively, in each incubation vial with six replications.
- Soil water was added to 50% of total porosity
- The incubation was run for 120 days at 22°C
- CO<sub>2</sub> emission from soil was measured using LI-820 on day 1, 2, 4, 8, and then every week.

## Results

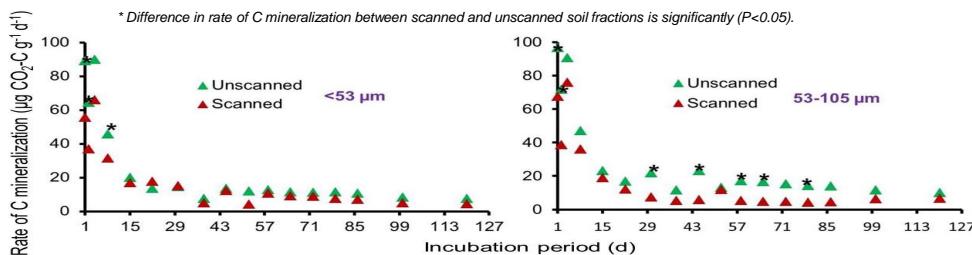


Fig. 3a. Effect of X-ray CT scanning on rate of carbon mineralization in different soil fractions.

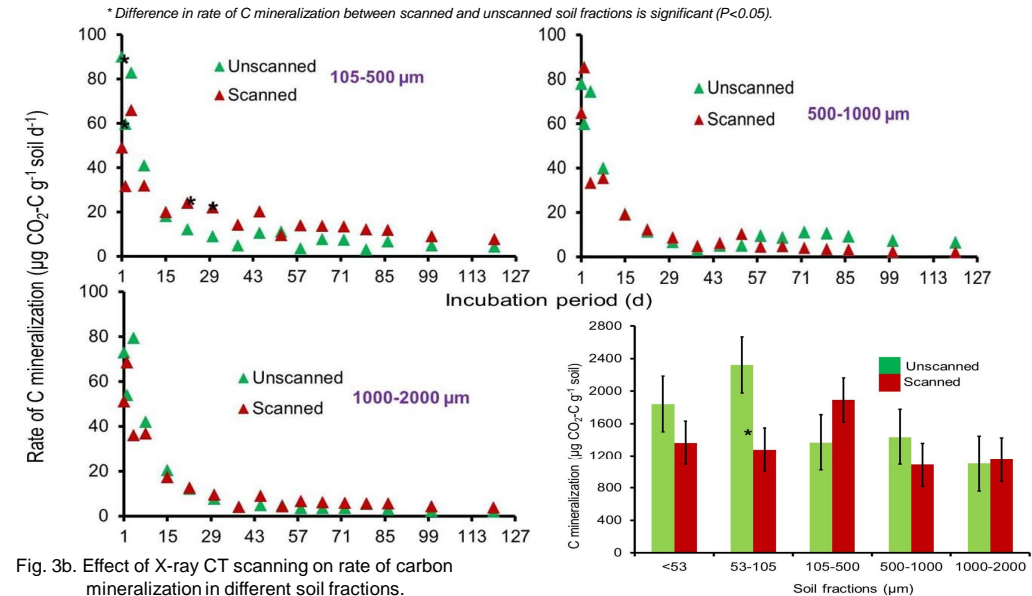


Fig. 3b. Effect of X-ray CT scanning on rate of carbon mineralization in different soil fractions.

Table 1. Mean percentages of different microbial groups in scanned and unscanned 4000-6000  $\mu$  aggregates of the conventional and organic management systems. In bold are cases when scanned and unscanned results were different at ( $p < 0.05$ )

Group	Conventional management		Organic management	
	Scanned	Unscanned	Scanned	Unscanned
Acidobacteria	0.199	0.268	0.127	0.089
Actinobacteria	0.216	0.0162	0.279	0.303
Armatimonadetes	0.0017	0.0011	0.0023	0.0017
Bacteroidetes	0.0029	0.0050	0.032	0.021
Chloroflexi	0.014	0.012	0.010	0.011
CyanobChloroplast	0.009	0.007	0.039	0.023
Firmicutes	0.023	0.026	<b>0.032a</b>	<b>0.047b</b>
Gemmatimonadetes	0.010	0.016	0.022	0.023
Nitrospira	0.008	0.010	0.004	0.005
Planctomycetes	0.005	0.004	<b>0.008a</b>	<b>0.013b</b>
Proteobacteria	0.184	0.211	0.273	0.291
unclassified	0.313	0.255	0.160	0.152
Verrucomicrobia	<b>0.012a</b>	<b>0.020b</b>	0.008	0.011
WS3	<b>0.0007a</b>	<b>0.004b</b>	0.0002	0.0004

\* Difference in cumulative C mineralization between scanned and unscanned soil fractions is significantly at ( $P < 0.05$ )

Fig. 4. Effect of X-ray CT scanning on cumulative carbon mineralization in different soil fractions.

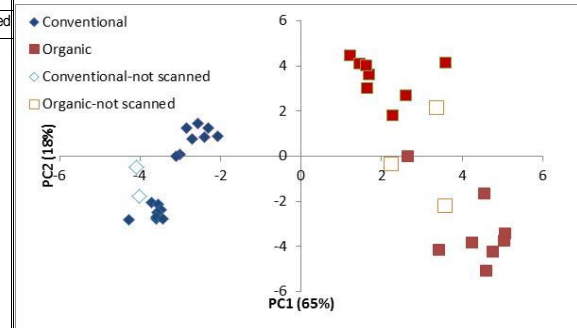


Fig. 5. Principal component analysis of the 4000-6000  $\mu$  aggregate pieces subjected and not subjected to scanning.

## CONCLUSIONS

- Overall soil respiration in scanned aggregates tended to be lower than in unscanned aggregates; however, the magnitude of scanning effect differed depending on the duration of the incubation period and size of soil fractions.
- Effect of scanning on composition of soil microbial community was relatively minor with only four of 14 instances of low abundance groups being significantly different between scanned and unscanned samples.

## ACKNOWLEDGEMENT

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## REFERENCE

Zappala et al. 2013. Effects of X-ray dose on rhizosphere studies using X-ray ray computed tomography. PLOS ONE 8: e67250.