Spatial and temporal variability of soil biological and chemical parameters following the introduction of cover crops into a conventional corn-cotton rotational system

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A paper from the Proceedings of the
16th International Conference on Precision Agriculture
21-24 July 2024
Manhattan, Kansas, United States

Abstract.

*Methods to characterize soil microbial diversity and abundance are labor intensive and require destructive sampling that incurs a per unit cost. There are advantages to replacing current methods with remote sensing approaches; the most obvious of which is spatially explicit representation of microbes on agricultural landscapes. Such a method will ultimately address open questions related to (1) the spatial scale of variability in soil microbial activity, and (2) the behavior of microbes in cover cropped production systems. To answer these questions, a one-year small plot study was undertaken as part of a larger multi-year investigation into the benefits of cover cropping and alternative nitrogen sources to crop yield and soil health. The broader study utilizes a split-plot experimental design where cash crop is the main factor and cover crop treatment is the sub-factor. Plots are rotated annually between corn (*Zea mays *L.) and cotton (*Gossypium hirsutum *L.). The four plots used in this study were drawn from plots that most immediately had produced cotton. Of the plots utilized, two were planted with a three-way blend of Austrian winter field pea (*Pisum sativum *L.), Daikon radish (*Raphanus sativus *L. subsp.* Longipinnatus*), and cereal rye (*Secale cereale *L. var. Elbon) and two were untreated control plots that had not been planted to cover crops. Intensive soil sampling following a nested hierarchy method with three stages (30, 10, and 3 m) was conducted within each of the four plots at five timings (n = 600). Sample timings followed key points in management, including cover termination, cash crop planting, mid-season, open-boll, and harvest. Sample locations were geotagged to maintain consistency across timings. Soil samples were analyzed for total carbon (TC), total nitrogen (TN), organic matter (OM), and genes (16S, 18S,* ure*C,* pho*A, and*

cbb*LR). A mixed model was applied to evaluate differences in soil parameters. Treatment and sample timing were considered fixed effects, while plot and stage were considered as random effects for temporal and spatial analyses, respectively. Genes were analyzed by the Wilcoxon signed-rank test to compare the change in values from time 1 to time 2, which were the only dates for which samples were yet available. Differences for all tests were separated using least squares means at the 0.05 significance level. Significant interaction was observed for TC (p=.0274), TN (p<.0001), and OM (p=.0274). While the post-harvest sample of the no cover crop treatment was consistently ranked at the top of the results for all variables, it was not significantly different from other sample times. However, there was no clear trend in significance that was observed across all variables with respect to treatment by sample time interaction. Similar trends in behavior were logically observed between TC and OM over the study; this trend extended to temporal variability. The spatial variability in TN was relatively large (>45%), even at shorter distances, while variability for TC and OM was considerably lower (15%) at shorter distances but increased more rapidly than TN with stage. This suggests the future iteration of samples should be drawn from distances smaller than 10 m for all variables, as might be expected given what is known regarding soil microbes. All genes had statistically significant shifts in their abundance between the first and second sample timings (16S, p<0.0001; 18S, p<0.0001,* ure*C, p<0.0001;* pho*A, p<0.0001;* cbb*LR, p=0.0040); data for other timings was not yet available for analyses.*

Keywords.
soil microbes, soil carbon, soil organic matter, conservation agriculture, conservation efficacy.

Background

Methods to characterize soil microbial diversity and abundance are labor intensive and require destructive sampling that incurs a per unit cost. Soil borne microorganisms comprise approximately less than 1% of the volume of soil, but major biogeochemical cycles and activities are dominated by this small volume. However, it is well understood that the soil microbiome is relatively variable, even down to the mm scale, which is often owed to unknowns such as cultivation-dependent ideal physiological conditions (Shade et al. 2012; Van Eunen et al. 2010). Soil spatial microbial variability is also related to micro-environments created by abiotic and biotic characteristics (Ladau and Eloe-Fadrosh 2019) such as crop root structure and canopy, soil structure (e.g., sand vs clay), moisture variability, and nutrient access. There are advantages to replacing current sampling methods with remote sensing approaches; the most obvious of which is spatially explicit representation of microbes on agricultural landscapes. Such a method will ultimately address open questions related to (1) the spatial scale of variability in soil microbial activity, and (2) the behavior of microbes in cover cropped production systems. To answer these questions, a one-year small plot study was undertaken as part of a larger multi-year investigation into the benefits of cover cropping and alternative nitrogen sources for crop yield and soil health.

Materials and Methods

The broader study utilizes a split-plot experimental design where cash crop is the main factor and cover crop treatment is the sub-factor. Plots are rotated annually between corn (*Zea mays* L.) and cotton (*Gossypium hirsutum* L.). This current study was conducted in 2022, following 3 cycles of crops and covers. The four plots used in this study were drawn from plots that most immediately had produced corn (2021) and were rotated to cotton (2022). Of the plots utilized, at the time of sample collection, two were planted with a three-way blend of Austrian winter field pea (*Pisum sativum* L.), Daikon radish (*Raphanus sativus* L. subsp. *Longipinnatus*), and cereal rye (*Secale cereale* L. var. Elbon) and two were untreated control plots that had not been planted to cover crops. Intensive soil sampling following a nested hierarchy method with three stages (30, 10, and 3 m) was conducted within each of the four plots at five timings (n = 600). Sample timings followed key points in management, including cover termination (time 1), cash crop planting (time 2), mid-season (time 3), open-boll (time 4), and harvest (time 5). Sample locations were geotagged to maintain consistency across timings. Each soil sample represented a composite of three individual cores collected in a triangle around the geotagged location; a metal template was used to ensure consistent spacing between these samples. The soil probe was cleaned between samples by inserting the probe into a non-sampled soil site within 1 m of the geotagged location to prevent cross-contamination of samples. Soil samples were submitted to the Mississippi State University soil test lab for analysis of percent total carbon (TC), percent total nitrogen (TN), and percent organic matter (OM). The lab determined TC and TN via combustion and OM using the loss on ignition method. Soil samples underwent gravimetric moisture content, DNA extraction, and quantitative polymerase chain reaction (qPCR) analysis for genes 16SrRNA, 18SrRNA, *ure*C, *pho*A, and *cbb*LR. Analysis of spatial variability in soil parameters followed methods from Webster et al. (2006), effectively using a linear mixed model in which stage was considered a random effect, and estimating the components of variance at each stage. The model was implemented with Proc MIXED (SAS software, SAS Institute, Inc). Temporal differences in soil chemical parameters were evaluated with generalized linear mixed models performed using Proc GLIMMIX. Treatment and sample timing were considered fixed effects, while plot was considered a random effect. Sample location was specified as the subject in the MODEL statement. Differences were separated using the LSMEANS statement with ADJUST = Tukey, which uses maximum likelihood and adjusts for multiple comparisons. At this time of writing, only the first two sample dates were available for soil microbiological parameters, therefore these data were evaluated with a Wilcoxon signed-rank test to compare the change in values from time 1 to time 2. The non-parametric test was selected due to the low sample size as well as the non-normal nature of the sample data, as determined by using Shapiro Wilk.

Results and Discussion

Similar trends in behavior were logically observed between TC and OM over the study. This is unsurprising as a portion of TC exists within the soil as OM. The spatial variability in TN was relatively large (>45%), even at shorter distances, while variability for TC and OM was considerably lower (15%) at shorter distances but increased more rapidly than TN with stage. This suggests that future iterations of samples should be drawn from distances smaller than 10 m for all variables, as might be expected given what is known regarding soil microbes. The spatial variability offered by a biotic force such as TN availability would dictate the spatial variability of associated microbial populations, and more specifically their genetic potential. For example, in areas of high TN, a microbial population's genetic potential may comprise more C acquisition (e.g., *cbb*LR), while in a TN poor area, genes associated with N release (e.g., *ure*C) may be more appropriate. A more recent finding suggests that machine learning can assign a level of scale based solely on microbial composition (Thompson et al. 2017), indicating that microbial diversity is a product of their micro and macro scaled environments.

Significant interaction was observed in the temporal analysis for TC ($p=.0274$), TN ($p<.0001$), and OM ($p=.0274$). While the post-harvest sample of the no cover crop treatment was consistently ranked at the top of the results for all variables, it was not significantly different from other sample times. However, there was no clear trend in significance that was observed across all variables with respect to treatment by sample time interaction (Fig. 1). Regarding genes, all genes had statistically significant shifts in their abundance between the first and second sample timings (16S, $p<0.0001$; 18S, $p<0.0001$, *ure*C, $p<0.0001$; *pho*A, $p<0.0001$; *cbb*LR, $p=0.0040$). The first timing comprised a relatively fallow period; though cover crop activity had been terminated, some volunteer and slowly decaying cover crops remained. The second timing comprised the early part of planting and warming soils, thus changes to the microbial genetic potential were not surprising. Microbial populations ebb and flow throughout the growing season dependent upon warming and soil moisture, available nutrients, and stage of plant growth, thus temporal variations are expected. As seed germination and early plant growth occur, there is an expected shift in the microbial population in the immediate vicinity of the plant as the rhizosphere begins to establish (Bei et al. 2021).

Summary

Overall, this study determined that there is substantial nutrient variability in the soil even at short distances. In addition, the temporal microbial variability in soils is owed to shifts in agronomic management as well as abiotic and biotic factors. When considering most sampling efforts to characterize a modestly sized experimental field (e.g., 0.25 ha) comprise only a few samples, this can introduce misinterpretation of findings, which ultimately could lead to improperly implemented agronomic recommendations.

Acknowledgments

This research was funded by USDA-ARS National Program 216: 6064-21660-001-000D and USDA-ARS Award #58-6064-9-007.

References

Bei, Q., Moser, G., Müller, C., & Liesack, W. (2021). Seasonality affects function and complexity but not diversity of the rhizosphere microbiome in European temperate grassland. *Science of The Total Environment*, *784*, 147036.

Ladau, J., & Eloe-Fadrosh, E. A. (2019). Spatial, temporal, and phylogenetic scales of microbial ecology. *Trends in Microbiology*, *27*(8), 662–669.

Shade, A., Hogan, C. S., Klimowicz, A. K., Linske, M., McManus, P. S., & Handelsman, J. (2012). Culturing captures members of the soil rare biosphere. *Environmental microbiology*, *14*(9), 2247–2252.

Thompson, L. R., Sanders, J. G., McDonald, D., Amir, A., Ladau, J., Locey, K. J., et al. (2017). A communal catalogue reveals Earth's multiscale microbial diversity. *Nature*, *551*(7681), 457–463.

Van Eunen, K., Bouwman, J., Daran-Lapujade, P., Postmus, J., Canelas, A. B., Mensonides, F. I., et al. (2010). Measuring enzyme activities under standardized in vivo-like conditions for systems biology. *The FEBS journal*, *277*(3), 749–760.

Webster, R., Welham, S. J., Potts, J., & Oliver, M. (2006). Estimating the spatial scales of regionalized variables by nested sampling, hierarchical analysis of variance and residual maximum likelihood. *Computers & Geosciences*, *32*(9), 1320–1333.

Figures

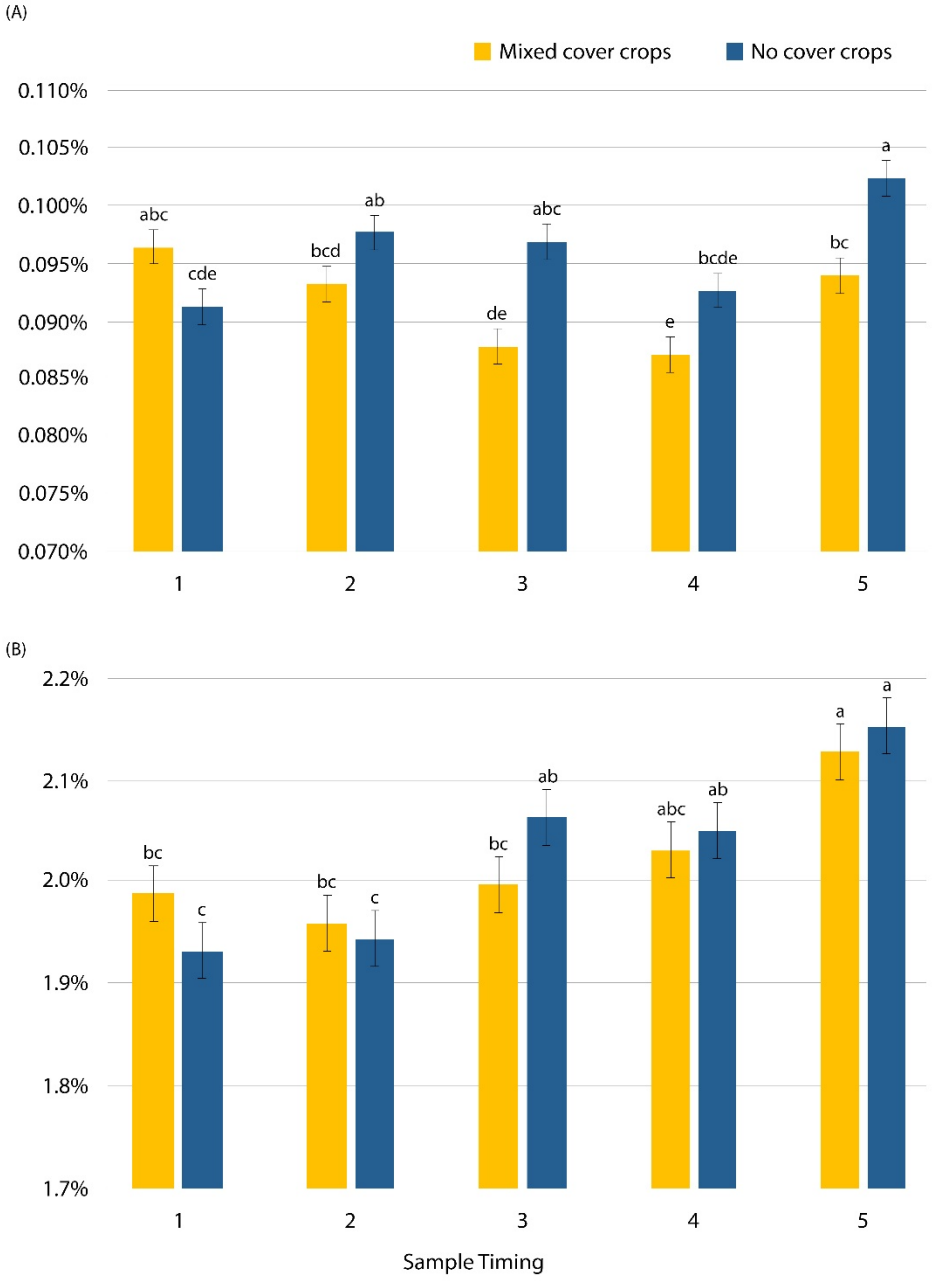

Figure 1. Mean values for total nitrogen (A) and organic matter (B) measured at five sample times corresponding to cover termination (1), cash crop planting (2), mid-season (3), open-boll (4), and harvest (5) in experiment plots either planted to mixed stands of cover crops (mixed cover crops) or without cover (no cover crops). Total carbon presented a similar pattern to organic matter, and is not presented. Letter designations convey statistical differences at the 0.05 level.