GLOBAL META-ANALYSIS AND METAGENOMICS APPROACH ON THE SOIL MICROBIOME ASSOCIATED WITH COVER CROPPING

BY

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THESIS

Submitted in partial fulfillment of the requirements
for the degree of Master of Science in Crop Sciences
in the Graduate College of the
University of Illinois at Urbana-Champaign, 2020

Urbana, Illinois

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ABSTRACT

Soil nutrient loss is one of the major causes of soil degradation that threatens future global food security. Cover cropping is a promising sustainable agricultural method with the potential to enhance soil health and mitigate consequences of soil degradation. As one of the agricultural practices that can affect cover cropping, effects of tillage on cover cropping have been widely researched as well. Because cover cropping and tillage can form an agroecosystem distinct from that of bare fallow, the soil microbiome is hypothesized to respond to the altered environmental circumstances. Therefore, studying their impact on the soil microbiome is necessary because the soil microbes are important drivers of soil processes including those relevant to soil health. The objectives of this MS research were i) estimate the baseline effect size of cover cropping on soil microbial abundance, activity, and diversity, ii) identify environmental and agricultural factors that affect the cover crop effects sizes on the soil microbiome, iii) further understand the cover crop effects on the soil microbial diversity by investigating the shifts in the soil microbial compositions, and iv) contribute to understanding how the relationship between cover cropping and the soil microbiome may affect the soil health.

A meta-analysis was conducted to estimate the global average effects of cover cropping on the soil microbiome. This study compiled the results of 60 relevant studies reporting cover cropping effects on soil microbial properties to estimate global effect sizes and explore the current landscape of this topic. Overall, cover cropping significantly increased parameters of soil microbial abundance, activity, and diversity by 27%, 22%, and 2.5% respectively, compared to those of bare fallow. Moreover, cover cropping effect sizes varied by agricultural covariates like cover crop termination or tillage methods. Notably, cover cropping effects were less pronounced under conditions like continental climate, chemical cover crop termination, and conservation tillage. This meta-analysis showed that the soil microbiome can become more robust under cover cropping when properly managed with other agricultural practices. However, more primary research is still needed to control between-study heterogeneity and to more elaborately assess the relationships between cover cropping and the soil microbiome.

This meta-analysis revealed that cover cropping affect the overall soil microbial diversity and that tillage is a major cofactor that affect this relationship. To further investigate the cover

cropping and tillage effects on the soil microbial diversity, a metagenomics study was conducted. This second part of the study was to observe compositional changes in the soil microbiome in response to cover cropping and tillage. Also, this study sought to identify microbial indicators that can be used to gauge responses of microbial guilds with functions relevant to soil health. This study used soil DNA data from a long-term cover cropping and tillage experiment on corn and soybean rotation in Illinois, USA. This study found that copiotrophic bacterial decomposers increased with legume cover crops and tillage, while oligotrophic and stress tolerant bacteria did so with bare fallow and no-till. Fungal groups responded to cover cropping and tillage based on their physiology, interaction with plant hosts, and nutrient strategies. This study also found an ammonia-oxidizing archaea species that increased with bare fallow. The consistent patterns that the microbial groups in this study display make them potential microbial indicators. Also, grass cover crops with no-till showed most potential for soil nutrient loss.

Overall, this MS research found that cover cropping significantly enriches the soil microbiome. However, cover cropping effects may apply differential pressures on microbial groups with different adaptations so that the overall diversity is not changed significantly. This research suggests that timing and other agricultural practices like tillage need to be carefully considered to direct the changes in the soil microbiome to benefit the soil health.

ACKNOWLEDGMENTS

I would like to show my greatest respect and appreciation to my advisor Dr. Maria B. Villamil for guiding me through my program with patience and encouragement. I also thank my committee members Dr. Chance Riggins, Dr. Sandra Rodriguez-Zas, and Dr. Maria C. Zabaloy for supporting me through my program. I would like to extend my appreciation to Dr. Gevan Behnke, Kato Samuel, and Dr. Laibin Huang for aiding in my research.

I also could not have come this far without the support from my fiancée Natsumi Komatsu, my parents, my late grandfather, and friends in Korea. Thank you all for believing in me and supporting me all the way.

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CHAPTER 1: META-ANALYSIS ON THE GLOBAL COVER CROPPING EFFECTS ON THE SOIL MICROBIOME

ABSTRACT

Cover cropping is a promising sustainable agricultural method with the potential to enhance soil health and mitigate consequences of soil degradation. Because cover cropping can form an agroecosystem distinct from that of bare fallow, the soil microbiome is hypothesized to respond to the altered environmental circumstances. Despite the growing number of primary literature sources investigating the relationship between cover cropping and the soil microbiome, there has not been a quantitative research synthesis that is sufficiently comprehensive and specific to this relationship. This study conducted a meta-analysis by compiling the results of 60 relevant studies reporting cover cropping effects on soil microbial properties to estimate global effect sizes and explore the current landscape of this topic. Overall, cover cropping significantly increased parameters of soil microbial abundance, activity, and diversity by 27%, 22%, and 2.5% respectively, compared to those of bare fallow. Moreover, cover cropping effect sizes varied by agricultural covariates like cover crop termination or tillage methods. Notably, cover cropping effects were less pronounced under conditions like continental climate, chemical cover crop termination, and conservation tillage. This meta-analysis showed that the soil microbiome could become more robust under cover cropping when properly managed with other agricultural practices. However, more primary research is still needed to control between-study heterogeneity and to more elaborately assess the relationships between cover cropping and the soil microbiome.

1.1 INTRODUCTION

With the global population expected to reach nine billion by the year 2050, agriculture faces a major predicament of moderating its pressure on the environment while meeting that future food demand (Alexandratos and Bruinsma, 2012). One of the crucial drivers of this impending problem is soil degradation by conventional agriculture (Conacher, 2009; Stavi and Lal, 2015). Much attention has been given to restoring and maintaining soil health, and to exploring and validating alternative practices such as reduced tillage or crop rotations to not only

conserve and restore soil health, but also to address other agricultural side-effects like nutrient leaching, water pollution, and soil erosion (Bengtsson et al., 2005; Kessel et al., 2013; Paustian et al., 2016).

Cover cropping is appreciated as a viable sustainable agricultural practice expected to provide many benefits like preventing soil erosion and nutrient leaching, weed suppression, and carbon sequestration (Daryanto et al., 2018; Poeplau and Don, 2015; Sturm et al., 2018; Thapa et al., 2018). These benefits largely develop from the physically, chemically, and biologically distinct agroecosystem that cover crops shape compared to that under bare fallow (Kaye and Quemada, 2017; Marshall et al., 2016; Reicosky and Forcella, 1998). Considering the extent of changes due to cover cropping, the soil microbiome is expected to respond to such modifications especially to those of the soil environment (Abdollahi et al., 2014; Abdollahi and Munkholm, 2014). Cover cropping may impact soil microbial functionality responsible for important soil ecosystem services, especially as the agricultural soil microbiome is sensitive due to its typically low diversity (Tsiafouli et al., 2015). As a crucial component of soil health, the soil microbiome response to cover cropping needs to be assessed to support its viability as a conservation practice.

Many studies have explored the effects of cover cropping on the soil microbiome, finding evidences of benefits like increased microbial biomass (King and Hofmockel, 2017), microbial enzymatic activities (Surucu et al., 2014), and evenness of relative abundances of bacterial taxa (Li et al., 2012). Yet, recent advancements in genetics and bioinformatics technologies have led to more efficient, precise, and accurate measurements of soil microbial properties (Gao et al., 2018; Lienhard et al., 2014). With an increasing number of studies using these contemporary methods, synthesizing their results is necessary to make general claims about the cover cropping effects on the soil microbiome. As a method of quantitative synthesis, meta-analysis can estimate a global effect from studies with heterogeneous conditions (Koricheva et al., 2013). Indeed, many meta-analyses have reported on the relationships between cover cropping and crop yield (Marcillo and Miguez, 2017), greenhouse gas (GHG) emission (Basche et al., 2014), and weed suppression (Osipitan et al., 2018). However, there has not been an extensive meta-analysis dedicated to cover cropping effects on the soil microbial properties.

A few meta-analyses on similar topics exist, but they were either confounded by studies with non-cover cropping practices, limited in microbial taxa, or confined themselves to

traditional soil microbial properties (Bowles et al., 2017; Daryanto et al., 2018; McDaniel et al., 2014; Venter et al., 2016). McDanniel et al. (2014) included cover cropping studies in their meta-analysis on the effects of crop rotation and management on soil carbon (C) and nitrogen (N) dynamics. Their results showed that cover cropping increased total soil C and N; however, these properties are not the direct measures of the soil microbiome. More pertinent measures would have been microbial biomass C (MBC) and N (MBN). Venter et al. (2016) used Shannon's diversity index to measure the effects of crop rotation on soil microbial diversity, concluding that microbial density is enhanced with crop diversity; but their results were not specific to cover cropping. The meta-analysis by Bowles et al. (2017) reported positive effects of cover cropping on microbial colonization of plant roots but focused only on arbuscular mycorrhizal fungi (AMF). Overall, there is a critical lack of global perspective on cover cropping effects on the soil microbiome despite the accumulating number of relevant studies.

The goal of this study was to conduct a comprehensive meta-analysis to fill this gap of knowledge in cover cropping research. Specifically, this meta-analysis assessed whether i) soil microbial abundance, activity, and diversity differ under cover cropping compared to bare fallow, and whether ii) cover cropping effects on soil microbiome are dependent to environmental or managerial factors.

1.2. MATERIALS AND METHODS

1.2.1. Literature selection and data extraction procedure

From September 2018 to March 2019, relevant peer reviewed articles were searched in Web of Science, SCOPUS, and Google Scholar. Search terms were generated from combinations of: scientific names of cover crop species, known measures of soil microbial properties, and methodology terms (Table B.1). This resulted in an initial collection of 985 studies. This collection was refined for studies that met the criteria for this meta-analysis: i) experimental design allowed pairwise comparison between cover cropping treatments and bare fallow controls, ii) defined cover cropping as crops that are not harvested nor removed, thereby excluding studies with crop residues, iii) field or greenhouse studies, iv) the study reported sample sizes, means, and standard errors; if these statistics were not reported, authors were contacted or the statistics were calculated if possible. After this screening process, 60 studies

reporting 48 soil microbial parameters (Table S2) remained. This process is outlined in Figure 1.9 modified from PRISMA flow diagram by Moher et al. (2009).

The chosen studies were thoroughly examined to extract necessary information like experimental design, environmental conditions, and the soil microbial properties. The soil microbial properties were categorized into soil microbial abundance, activity, and diversity to represent the response variables (Table B.2 and 1.B3). Data only presented in figures were extracted using WebPlotDigitizer (Version 3.9; Rohatgi, 2015). Agricultural conditions and practices were recorded to assess their interactions with cover cropping effects. For fertilizer data, rotation average N input by year was recorded if different amounts of N were applied in each year of a rotation. For experimental site information, the site's Köppen climate classification was recorded; if this information was missing, the region of the site was approximated using Google Earth, then assigned the climate according to the climate classification entry in Wikipedia (Arnfield, 2019; Beck et al., 2018). Soil order was recorded in USDA soil taxonomy; those without USDA soil taxonomy equivalent were recorded as reported ("Soil Taxonomy | NRCS Soils," n.d.). Spring growth suppression methods of the cover crops were also categorized into mechanical and chemical termination methods. Tillage type was categorized into conservation (reduced tillage or no-till) and conventional tillage (any other tillage methods). If cover cropping planting and termination dates varied by year, dates of the sampling years were used. If a study's soil sampling occurred multiple times a year or in multiple years, results from each sampling event were recorded. If the study only reported averages over multiple sampling events, the last sampling date was recorded. If the exact date of such events were not reported, the 15th of the reported month was recorded as an average.

1.2.2 Statistical Analysis

The statistical method of this meta-analysis follows the procedures described in Koricheva et al. (2013) for mixed-effects model with study weights:

$$T_i = \theta_k + e_i \, , e_i \sim \mathrm{N}(0, \sigma_i^2) \quad (1.1)$$

$$\theta_k = \mu + \varepsilon_k \, , \varepsilon_k \sim \mathrm{N}(0, \tau^2) \quad (1.2)$$

This model assumes that the observed effect size of a study (T_i) is distributed around the true study effect size (θ_k) with a within-study variance of σ_k^2 (1.1), which is then distributed around the global true effect size (μ) with a between-study variance of τ^2 (1.2) (Koricheva et al., 2013).

1.2.2.1. Calculating global effect size means and variances

The effect sizes of cover cropping on soil microbial properties were measured as the log response ratio (LRR, T_i), calculated as natural log of the ratio between the mean of a response variable under cover cropping treatment ($\bar{Y}_{CC}$) over that of the control ($\bar{Y}_{NC}$):

$$T_i = LRR = \ln(\frac{\bar{Y}_{CC}}{\bar{Y}_{NC}}) \quad (1.3)$$

Cover cropping treatments and controls with comparable conditions, such as sampling depth and sampling year, were paired to calculate the effect size. Therefore, a study can yield multiple effect sizes if it reported each results from multiple treatments of different cover crop species or mixtures, experimental sites, or sampling years.

Estimate of the study variance ($\hat{\sigma}_k^2$) was calculated from the following formulae:

$$s^2 = \frac{\sum_{i=1}^{n}(Y_i - \bar{Y})^2}{n} \quad (1.4)$$

$$\hat{\sigma}^2{}_k = \frac{s_{CC}^2}{n_{CC} * \bar{Y}_{CC}^2} + \frac{s_{NC}^2}{n_{NC} * \bar{Y}_{NC}^2} \quad (1.5)$$

Here, s^2 is the reported variance of the mean of the response variable ($\bar{Y}_i$), and *n* is the sample size, which is the study's number of replications. The variance s^2 needed to be reported by the literature or be obtained from the authors.

With the study effect sizes and variances calculated, R package metafor and its function rma were used to calculate the global effect sizes, 95% confidence intervals (CI), and total between-study heterogeneity (I^2) (Viechtbauer, 2010). If the CI of a global effect size mean does not include zero, then the cover cropping effect on a soil microbial parameter is statistically significant. I^2 is the proportion of total between-study heterogeneity in total variability among observations. A large I^2 might imply that studies are too different from each other to perform a meta-analysis. However, identifying significant effects from the covariate factors as the sources of heterogeneity can resolve this issue. Function funnel was used to produce the funnel plots for each soil microbial parameters to visually check significant heterogeneity and publication bias (R Core Team, 2019; Viechtbauer, 2010).

1.2.2.2. Selecting response variables

Of the 48 soil microbial parameters reported, statistical analyses were conducted on those with at least 30 observations. Those with fewer observations came from less than three studies,

which is too few for meta-analysis. The 13 soil microbial parameters that met the criteria were grouped into three categories: abundance, activity, and diversity. Soil microbial abundance and activity parameters are common metrics recommended by the U. S. Department of Agriculture (USDA) Natural Resources Conservation Service (NRCS) as soil health indicators particular to soil microbial properties (NRCS, 2018). The units of the parameters in this study are listed in Table B.2.

The selected soil microbial abundance parameters estimate the overall size of the soil microbial community: colony forming units (CFU), MBC, MBN, and phospholipid fatty acid (PLFA). Soil microbial activity parameters included two enzyme activities, β-glucosidase (BG) and phosphatase (Phos), and laboratory soil respiration (CO2-C). Finally, soil microbial diversity parameters that reflect the richness, diversity, or evenness of a soil microbial population included Operational Taxonomic Units (OTU), Chao 1 richness index, Shannon-Wiener Index (H'), genetic richness (S), Pielou's Evenness Index (J), and Simpson's Diversity Index (1-D).

1.2.2.3. Assessing the effects of moderators on cover crop effects on soil microbial properties

This study assessed whether cover cropping effect size means varied by agricultural factors to explain the between-study heterogeneity and infer on the importance of these factors on cover cropping management. Agricultural factors will henceforth be referred to as "moderators", to be consistent with how package *metafor* dubs covariate factors (Viechtbauer, 2010). Table S3 summarized the moderators and their levels. These moderators were chosen based on their prevalence in the database, and relevance to cover cropping management and soil microbial properties. In summary, discrete moderators were climate, soil order, cover crop type, cover crop termination method type, tillage type, N fertilization, and soil sampling timing. Continuous moderators were soil pH, annual N fertilizer rate, cover cropping duration, and soil sample depth.

The function *rma* was used for the statistical analysis on the effects of moderators on cover cropping effect sizes. Also, ANOVA provided the overall significance of each moderator effect. For discrete moderators, an estimate of the effect size means and CIs for each combination of a moderator's levels and soil microbiome parameters were calculated, and then were visually analyzed the significance with forest plots. Combinations of soil microbial

parameters and discrete moderators with at least 30 observations were considered. Combinations were further subset by moderator level if there were at least five observations.

For continuous moderators, *rma* was used and was included the continuous moderators in the function to calculate the estimate of the coefficients, their associated p-values, and R^2. The relationship was considered significant if its *rma* p-value was significant, therefore the coefficient is likely not zero, and if the R^2 was reasonably high (>10%). Combinations of soil microbial parameters and continuous moderators with less than 30 observations were disregarded.

1.3. RESULTS

1.3.1 Overview of cover cropping effects on soil microbial properties

Overall, global cover cropping effect size means were significantly larger than zero for all soil microbial properties, as shown in Figure 1.1 and Table 1.1. Global effect size means of soil microbial abundance parameters (CFU, MBC, MBN, and PLFA) ranged between 0.14 and 0.41, and activity parameters (BG, Phos, and CO2-C) ranged between 0.14 and 0.35. Global effect sizes for diversity parameters (OTU, H', S, J, and 1-D) were also positive but much smaller, ranging from 0.003 to 0.05. As shown in Table 1, total heterogeneity (I^2) for OTU, S, and 1-D were very small, while it was very high for the other ten parameters (46~99.9%), which can be explained by effects from the moderators. Funnel plots also confirmed this result where many observations for parameters except OTU, S, and 1-D were not contained in the funnel, which indicate between-study heterogeneity and possible publication bias (Figure B.6). Indeed, each soil microbial parameters had at least one moderator to explain their between-study heterogeneity.

1.3.2 Moderator effects on the soil microbial abundance

Effects of climate were significant for all abundance parameters except CFU, which only reported one climate category (Figure 1.2). For MBC, effect size means by climate varied significantly in the order of tropical (0.87), temperate (0.30), arid/semi-arid (0.19), and continental (0.08), from highest to lowest. For MBN, continental climate had a significantly lower effect size mean (0.05) than arid (0.29) and temperate (0.28) climates. For PLFA, the temperate climate had a significantly larger effect size mean (0.28) than tropical (0.08) and

continental climates (0.09). Overall, the continental climate had lower effect size means than others.

Soil order also had significant relationships with MBC, MBN, and PLFA (Figure 1.3). For MBC, Oxisols had a significantly larger effect size mean (1.02) than Entisols (0.25), Alfisols (0.13), and Mollisols (0.17); however, Oxisols had much fewer observations (n = 15) than Mollisols (n = 121) and Alfisols (n = 86). For MBN, Mollisols had significantly larger effect size mean (0.27) than Ultisols (0.05). For PLFA, effect size means for Entisols (0.29) and Ultisols (0.36) were significantly larger than those of Alfisols (0.09) and Inceptisols (0.08). Except for MBN, less fertile soils like Oxisols, Ultisols, and Entisols had larger effect size means than those of more fertile soils.

Cover crop termination method had significant effects only on PLFA, where mechanical termination effect size mean (0.16) was significantly larger than that of chemical termination (0.09) (Figure 1.4). Cover crop type had significant but inconsistent effects on CFU and MBC. Grass cover crops had the highest effect size mean (0.82), followed by Others (0.23) and Mixed (0.02) for CFU. Conversely, Mixed (0.34) was significantly larger than Grass (0.17) for MBC. Nitrogen fertilizer input demonstrated no significant effects for PLFA.

Soil sampling timing had significant effects on MBC and PLFA (Figure 1.7). For MBC, sampling after the cash crop harvest (0.30) and during the cover crop (0.38) had larger effect size means than that of sampling during the cash crop (0.18). For PLFA, the opposite was observed where sampling during the cash crop (0.24) had the highest effect size mean than compared to those of sampling during cover crop (0.12), after cover crop termination (0.04) and before cash crop planting (0.05). Overall, while sampling timing had a significant influence on effect size means, the influence was inconsistent. Finally, tillage types were significant for CFU and MBC. Conventional tillage methods had larger effect sizes for CFU (0.67) and MBC (0.38) than no-till and reduced tillage (CFU: 0.27; MBC: 0.21). For continuous moderators, soil sample depth had significant negative correlation with CFU (β_1 = -0.05; p-value < 0.001; R^2 = 0.35; Figure B.2; Table B.4).

1.3.3. Moderator effects on the soil microbial activity

Effects of climate was significant for BG, where arid climates had a larger effect size (0.33) than that of continental (0.12); temperate climates also had a lower effect size mean (0.08) but the CI slightly overlapped with arid climates (Figure 1.2).

Soil order was significant for CO2-C where the Entisols effect size mean (0.54) was significantly larger than that of Ultisols (0.24) (Figure 1.3). Cover crop termination method was only significant for Phos where mechanical termination had a larger effect size mean (0.29) than that of chemical termination (-0.08) (Figure 1.4). Cover crop type was significant for CO2-C only, where effect size mean of Other cover crops (0.62) was significantly larger than that of Legume (0.21) (Figure 1.5). N fertilizer input was not significant for soil microbial activity (Figure B.1).

Soil sampling timing was significant for Phos and CO2-C (Figure 1.7). For Phos, effect size mean of sampling during cover crop (0.37) was significantly larger than that of sampling after cash crop harvest (-0.11). For CO2-C, sampling during cover crop (0.52) was larger than that during cash crop (0.28). Tillage type was not significant for soil microbial activity (Figure 1.6).

Only BG had a significantly positive yet very weak linear relationship with annual N fertilizer amount ($\beta_1 = 0.00154$; p-value < 0.001; $R^2 = 0.11$; Table S4). Visually (Figure B.3), however, these results seem dubious, as effect sizes at higher N input were not significantly larger than that at lower N fertilizer rate, which confirmed that the association is very weak. This was also supported by the overlapping CI for MBC effect sizes between N fertilized and non-fertilized observations (Figure B.1).

1.3.4. Soil microbial diversity

The soil microbial diversity parameters OTU, Chao 1, H', S, J, and 1-D had a wide range of between-study heterogeneity from 0.3% to 92.5%. Despite the high heterogeneity for H' (92.5%) and Chao 1 (46.1%), none of the ANOVA results were significant (Table 2.4). Soil order was significant for Chao 1, where the effect size mean of Mollisols (0.06) was larger than that of Entisols (<0.001) (Figure 1.3). Cover crop termination method had a significant effect on H' and J (Figure 1.4). In both cases, mechanical termination had larger effect size mean (H: 0.025; J: 0.007) than that of chemical termination (H': -0.001; J: -0.006), similar to results of soil

microbial abundance and activity. Tillage type was significant for S and J (Figure 1.6). Like soil microbial abundance and activity, conventional tillage had larger effect size mean (S: 0.044; J: 0.021) than that of conservation practice (S: -0.016; J: -0.006). For Chao 1, effect size means from sampling during cash crop (0.056) and before cash crop planting (0.081) was significantly larger than that of sampling after cash crop harvest (-0.046) (Figure 1.7).

OTU had statistically significant negative correlations with soil pH (β_1 = -0.04; p-value = 0.003; R^2 = 0.65; Figure B.8) and soil sample depth (β_1 = -0.003; p-value = 0.021; R^2 = 0.38; Figure B.2). Soil pH ranged from 6.28 to 8.3, and the negative correlation between OUT and pH was expected, as the soil microbiome generally thrives under neutral pH condition (Fierer and Jackson, 2006; Lauber et al., 2009). However, this relationship had small number of observations and much skewed distribution, requiring careful interpretation of this result. Chao 1 also demonstrated significant negative correlation with N fertilizer rate (β_1 = -0.0007; p-value = 0.0096; R^2 = 0.36; Figure B.3).

1.4. DISCUSSION

1.4.1 Overall positive effects of cover cropping on soil microbial properties

Past meta-analyses have generally suggested positive effects of cover cropping on soil microbial properties (Daryanto et al., 2018; McDaniel et al., 2014; Venter et al., 2016). Indeed, cover cropping increased all 13 soil microbial parameters in this meta-analysis as well. However, heterogeneity between studies was high for most of the soil microbial parameters with the exception of those with fewer observations: OTU, S, and 1-D. According to the significant differences between effect size means by moderator levels, most of the high heterogeneity could be attributed to the effects of agricultural moderators on the soil microbial parameters.

All four soil microbial abundance parameters increased with cover cropping treatments by large ratios (14.5~40.7%). Considering that cover cropping provides above- and belowground plant biomass and root exudates known to boost soil microbial growth and prevent rich topsoil from eroding, the significant cover cropping benefits on soil microbial abundance were indeed expected (Vukicevich et al., 2016). Meta-analysis by Daryanto et al. (2018) reported similar increases in MBC, MBN, and microbial biomass P (MBP), and significantly decreased soil loss under cover cropping treatments. Based on the consistency with past meta-analyses and

significant mean global effect sizes, these results suggest that cover cropping can be expected to increase soil microbial abundance.

BG and Phos are two of the four enzymes accepted by the USDA NRCS as indicators of general microbial activity for soil health assessment along with N-acetyl-β-D-glucosaminidase and arylsulfatase (NRCS, 2018). The positive global effect size means for these enzymes and CO2 respiration rate suggest positive cover cropping effects on soil microbial activity. Since BG reflects the last step in cellulose decomposition, an increase in BG activity is expected with increased cellulose input from cover crop decomposition; likewise, increases in other enzymes responsible for previous processes in cellulose decomposition would be expected (Shewale, 1982). As for Phos, the presence of organic P substrates can promote phosphatase production. Cover crops return the biomass P to the soil during decomposition which could have resulted in increased Phos (Almeida et al., 2018; Hallama et al., 2019; Nannipieri et al., 2011; Sharma et al., 2018). Moreover, a meta-analysis by Hallama et al. (2019) suggested that cover cropping indirectly enhances soil P availability. For example, cover cropping may enhance AMF colonization that improves access to P pool, or change soil pH to levels more favorable for Phos and other enzyme activities. Meanwhile, since some plants are known to produce phosphatase themselves, this result requires careful interpretation to account for plant-originated Phos (Tarafdar and Claassen, 1988).

This meta-analysis is the first to exclusively assess the effects of cover cropping on soil microbial diversity. The most closely related meta-analysis focused on soil microbial diversity and richness and reported positive weighted mean differences of 3.36% for diversity and 15.11% for richness (Venter et al., 2016). However, their analysis focused on the effects of crop rotations that happened to include cover cropping studies. Compared to those of soil microbial abundance and activity, the present study's global effect size means for diversity parameters were also positive but almost ten-fold smaller on average. In fact, the global effect size mean for Simpson's diversity index was negative (-0.009) until 6 outliers with relatively extreme variances (>0.4) or effect sizes (<-0.5) were removed. Nonetheless, such sensitivity may be limited to parameters with smaller number of observations like 1-D. However, without historical references for comparison and with effect sizes small enough to raise doubt on the significance of cover cropping effects on the soil microbial diversity, making a solid and generalized statement on this relationship will require more primary research and meta-analyses.

1.4.2 Significance of agricultural moderators

Statistical results suggested that agricultural moderators can determine how responsive soil microbial properties are to cover cropping effects. The environmental moderators, climate and soil order, had significant effects on soil microbial abundance and activity. Results varied by parameters for observations on tropical, arid, and temperate climates, but continental climates consistently had the smallest effect size means. Interestingly, 46% of the studies on continental climates were on productive soils like Alfisols and Mollisols, primarily from the fertile agricultural regions like the Midwest, USA (NRCS, 2005). Consistently lower effect size means for continental climates may be attributed to the high fertility of these soils on which cover cropping benefits experience diminishing return on already productive soils. Overall, climate results indicate that cover cropping can improve the soil microbiome especially in regions expected to have less robust soil microbiome. However, previous studies warn that cover cropping may put more pressure on dry agroecosystems , highlighting the need for careful irrigation and management decisions (Calderon et al., 2016).

Meanwhile, the main effects from soil order exhibited conflicting results, with less productive soil orders showing larger effect size means for MBC and PLFA and smaller effect size means for MBN and Chao 1. This discrepancy should be further explored with an emphasis on interactions between climates and soil orders. However, the current database has too few observations to make reliable inference on interactions. Together, climate and soil order should be considered when managing cover cropping to maximize the benefits.

Management factors also had significant influences on the cover cropping effects sizes. Tillage type consistently affected cover cropping effects where conservation tillage had smaller effect size means than those of conventional tillage. This result initially seemed contradictory to previous findings which reported the benefits of reduced tillage or no-till on various soil properties (Blanco-Canqui and Ruis, 2018; Bowles et al., 2017; Hussain et al., 1999; Zuber and Villamil, 2016). For example, a meta-analysis on the effects of tillage on soil microbiome by Zuber and Villamil (2016) reported negative effect sizes for soil microbial properties with conventional tillage. Another meta-analysis by Bowles et al. (2016) on the effects of cover cropping and tillage on AMF colonization reported benefits of alternative tillage methods, although they did not find evidence for benefits from interactions between cover cropping and tillage. Considering these past findings, negative effects of conventional tillage on the soil

microbial properties may have been mitigated by cover cropping, thereby pronouncing the cover cropping effects. Another potential explanation is that bare fallow under conservation tillage often allows weed covers that can mimic some cover cropping effects, thereby leading to smaller cover crop effect size compared to that under conventional tillage.

Chemical cover crop termination methods that used herbicide showed smaller cover crop effect size means than mechanical termination methods. This result may be relevant to herbicide effects on plants and soil microbiome. Past studies have found that herbicides may directly impact soil properties and the microbial community. For example, herbicides may decrease soil denitrification (Tenuta and Beauchamp, 1996), promote plants to exudate ammonium, thus stimulating growth of specific microbial functional groups (Damin et al., 2010, 2008; Mijangos et al., 2010; Nyerges et al., 2010; Zabaloy et al., 2017), and temporarily change microbial respiration and biomass (Nguyen et al., 2016). Because both termination method categories included studies with tillage and those without, tillage or other mechanical methods are unlikely to have contributed to the differences. Although further investigation is necessary to verify this result, it suggests that mechanical termination will maximize cover crop benefits.

As expected, soil sampling timing had significant effects on soil microbial properties, where either observations during the cover crop or cash crop phases had larger effect size means. This result emphasizes that soil sampling timing must be accounted for in the analysis of soil microbial properties, as they are time dependent. More than half of the observations were during cash crop phase ($n > 600$), followed by the cover cropping phase with just under 300 observations. For consistent research synthesis without a timing bias, primary research should report the crop phase of soil measurements.

1.4.3. Limitations of this study

While the cover cropping effects on soil microbial activity are clearly positive, this relationship must be interpreted carefully because microbial activity correlates with both abundance and diversity. First, the increase in microbial activity could be attributed to an overall increase in microbial abundance, and their significant positive correlation has been observed by others (Acosta-Martinez et al., 2011). More work is needed to discern whether activity increased because of changes in abundance of active microbes or via an increase in per-capita enzyme production rate. Of course, both may be responsible. Indeed, effect sizes on BG and Phos had

positive linear relationships with MBC, although the number of observations was small for Phos (Figure 1.9). This result also suggests other correlations between enzymes and microbial abundance parameters, such as Phos and PLFA or MBP, are likely. However, more studies reporting both soil microbial activity and abundance are needed perform multivariate analysis and to confirm these results.

Second, soil microbial activity closely intertwine with microbial diversity because extracellular enzyme production varies by soil microbial group and is not universal, especially for soil microbial activities responsible for ecosystem services like nutrient cycling (Wang et al., 2017; Zang et al., 2018). To assess cover cropping effects on these specific soil microbial processes, using soil microbial genes and their products involved in those processes are potentially more informative than the parameters assessed in this study. For example, to understand cover cropping effects on N fixation, abundance changes in genes like *nifH* and their products should be analyzed. Some studies in this study's database included this type of information but the studies were too sparse. Moreover, if the identities of soil microbial groups harboring specific genes are known, assessing cover cropping effects on their relative abundance may strengthen the argument that cover cropping enhances soil microbial processes beneficial for agriculture. However, studies reporting both soil microbial activity and diversity are lacking, and information linking soil microbial groups with specific enzyme productions and genomic data is largely unavailable (Hai et al., 2009; Wang et al., 2017). Therefore, more future cover cropping studies connecting soil microbial diversity and activity are needed.

As a meta-analysis, this study will inevitably share the methodological limitations of its compiled primary research. For example, current enzyme activity assays are optimized for laboratory conditions and may not accurately distinguish soil enzymes that were segregated physically and biologically, therefore overestimating the *in situ* activity. Laboratory enzyme assays require disturbing the soil aggregates , which may release stabilized enzymes that would have been inactive *in situ* (Burns, 1982; Wallenstein and Weintraub, 2008). Also, enzyme activity assays may not accurately demonstrate *in situ* activity because of the *in vitro* conditions of the assays. Current enzyme assay methods are done under ideal conditions for enzyme activity, which can overestimate the actual enzyme activities *in situ* (Tabatabai, 2003). The similar is also true for some microbial abundance parameters like CFU that cultures and counts the microbes in the laboratory condition. In general, the understanding of the role of management

practices on the soil microbial community will be limited by the best available methods, and research will be required to reevaluate the state of knowledge as better methodologies develop.

1.4.4. Current state of cover cropping research on soil microbiome and future needs

Out of 48 soil microbial parameters reported by a total of 60 studies, only 13 had a statistically significant number of observations ($n \geq 30$). MBC was the parameter with the greatest number of observations (403 observations). The most studied soil microbiome property was microbial abundance, and further research seems unnecessary with the clear cover cropping benefits that this study has demonstrated. Soil microbial activity had the second most studies, primarily represented by two enzyme activities. These enzymes alone are insufficient considering the vast complexity of soil microbial activity crucial for agriculture. Therefore, more enzymes and the genes coding them need to be studied to better understand the still largely unknown complexity of soil microbial activity. As for soil microbial diversity, most studies reported diversity indices derived from changes in relative abundances of soil microbial phyla or genera; some derived from a broader classification such as PLFA data (gram +/-, fungi, and eukaryote). Some studies used community catabolic profiles like average well color development (AWCD) which can capture both activity and diversity. However, the number of such studies was small and they are subject to limitations on data integration arising from various methodological considerations like cell culture conditions (Konopka et al., 1998; Preston-Mafham et al., 2002; Weber et al., 2007).

The current landscape of cover cropping research and its effects on soil microbial properties is still unable to answer more complex questions. Making meaningful inferences on such questions like “how much do changes in soil microbial abundance contribute to changes in activity” requires more studies that address comprehensive sets of soil microbial parameters. Nevertheless, this meta-analysis marks a meaningful start in this effort, and the trend seems hopeful as half of the studies in this study’s database were conducted in the last four years (2016-2019), thanks to developing technology, lowering costs, increased interest in sustainable agriculture, and accumulating experience. Meaningful updates on this meta-analysis could be possible with a larger database in the near future that would include analyses that this study could not perform due to insufficient number of observations.

1.5. CONCLUSION

As the first meta-analysis dedicated to evaluating the cover cropping effects on soil microbial properties, this study concludes that cover cropping generally enhances soil microbial abundance, activity, and, to a lesser degree, diversity. With proper implementation considering termination methods, climate, soil order, and tillage, cover cropping will build a more robust soil microbiome. Other than these significant moderators, this study found no strong evidence for dependence on other agricultural factors. This meta-analysis showed that cover cropping still needs more research but also demonstrated that this need is being met with an increasing number of recent relevant studies. Nonetheless, this study urges more researchers to investigate the interactions between microbial properties and cover cropping practices as more important answers surrounding the complex interactions still lie unveiled. With a database large enough to perform more complex analysis, future meta-analyses may reveal specific cover cropping effects on the soil microbiome that are relevant to both agricultural and environmental interests.

TABLES AND FIGURES

Table 1.1. Global results of cover cropping effects on 13 soil microbial parameters with at least 30 observations, reporting global effect size means, its 95% confidence interval (CI), number of observations (n), estimated total heterogeneity (τ^2), and total between-study heterogeneity (I^2). The 13 soil microbial parameters were: colony forming unit (CFU), microbial biomass C (MBC) and N (MBN), phospholipid fatty acid (PLFA), β-glucosidase activity (BG), phosphatase activity (Phos), respiration (CO2-C), operational taxonomic unit (OTU), Chao 1 richness index, Shannon's diversity index (H'), genetic richness (S), Pielou's evenness index (J), and Simpson's diversity index (1-D).

soil microbiome parameter	*Global Mean*	*n*	*CI*	τ^2	I^2
CFU	0.407	54	0.117	0.167	97.461
MBC	0.254	408	0.029	0.060	85.542
MBN	0.256	197	0.051	0.094	84.620
PLFA	0.145	436	0.026	0.046	82.202
BG	0.138	155	0.038	0.042	99.930
Phos	0.181	60	0.106	0.153	99.920
CO2-C	0.349	39	0.088	0.032	89.396
OTU	0.033	32	0.017	0.000	3.504
Chao 1	0.050	78	0.022	0.003	46.088
H'	0.023	199	0.009	0.002	92.475
S	0.030	57	0.019	0.000	0.311
J	0.010	50	0.008	0.001	72.098
1-D	0.003	61	0.002	0.000	20.116

Table 1.2. ANOVA results of effects of agricultural moderators on soil microbial abundance parameters: colony forming unit (CFU), microbial biomass C (MBC) and N (MBN), and phospholipid fatty acid (PLFA). Df is the degrees of freedom and p-values less than threshold 0.05 are in bold. Dashes (-) indicate that that combination of soil microbiome parameter and moderator had less than two levels, therefore unable to perform ANOVA.

	CFU			*MBC*			*MBN*			*PLFA*		
Moderators	Df	Error Df	p-value	Df	Error Df	p-value	Df	Error Df	p-value	Df	Error Df	p-value
Climate	-	-	-	3	404	**0.000**	2	194	**0.015**	2	433	**0.000**
Soil Order	1	50	0.524	5	261	**0.000**	2	66	**0.030**	3	420	**0.000**
cover cropping Termination	1	34	0.152	1	374	**0.042**	1	177	0.889	1	404	0.256
cover cropping Type	2	51	**0.000**	3	404	0.063	3	193	0.135	3	432	0.290
Tillage Type	1	52	**0.044**	1	335	**0.001**	1	166	**0.004**	-	-	-
Sample Timing	1	20	**0.000**	3	404	**0.000**	2	194	0.644	4	431	**0.000**
N Fertilizer	1	20	**0.003**	1	369	0.584	1	193	0.151	1	350	**0.002**
N Fertilizer Rate	1	20	0.297	1	337	0.326	1	172	**0.027**	1	350	0.143
Soil pH	1	34	0.758	1	294	0.899	1	193	0.351	1	76	0.213
cover cropping Duration	1	34	0.134	1	368	0.252	1	176	0.999	1	404	0.458
Sample Depth	1	52	**0.001**	1	406	**0.000**	1	195	0.342	1	434	0.206

Table 1.3. ANOVA results of effects of agricultural moderators on soil microbial activity parameters: β-glucosidase activity (BG), phosphatase activity (Phos), and respiration (CO2-C). Df is the degrees of freedom and p-values less than threshold 0.05 are in bold.

	BG			*Phos*			*CO2-C*		
Moderators	Df	Error Df	p-value	Df	Error Df	p-value	Df	Error Df	p-value
Climate	2	152	**0.000**	2	57	0.144	2	36	**0.044**
Soil Order	1	118	**0.001**	3	50	**0.001**	4	34	0.088
cover cropping Termination	1	153	0.646	1	58	**0.001**	1	31	0.999
cover cropping Type	3	151	**0.007**	3	56	0.267	3	35	0.052
Tillage Type	1	130	0.876	1	34	**0.033**	1	8	0.464
Sample Timing	2	152	**0.047**	2	57	**0.002**	2	36	0.384
N Fertilizer	1	153	**0.003**	1	50	0.462	1	32	**0.021**
N Fertilizer Rate	1	126	**0.001**	1	22	0.522	1	32	0.467
Soil pH	1	107	**0.001**	1	33	0.484	1	14	0.608
cover cropping Duration	1	153	**0.000**	1	51	0.278	1	26	0.541
Sample Depth	1	153	0.905	1	58	0.092	1	37	0.191

Table 1.4. ANOVA results of effects of agricultural moderators on soil microbial diversity parameters: operational taxonomic unit (OTU), Chao 1 richness index, Shannon's diversity index (H'), genetic richness (S), Pielou's evenness index (J), and Simpson's diversity index (1-D). Df is the degrees of freedom and p-values less than threshold 0.05 are in bold. Dashes (-) indicate that that combination of soil microbiome parameter and moderator had less than two levels, therefore unable to perform ANOVA, or the combination had no observations.

	OTU			*Chao 1*			*H'*			*S*			*J*			*1-D*	
Moderators	Df	Error Df	p-value	Df	Error Df	p-value	Df	Error Df	p-value	Df	Error Df	p-value	Df	Error Df	p-value	Df	Error Df	p-value
Climate	2	29	**0.032**	2	75	0.610	2	196	0.366	2	54	0.658	1	48	0.077	2	58	0.084
Soil Order	1	26	**0.000**	2	70	0.463	4	153	0.261	1	16	0.430	-	-	-	1	52	0.073
cover cropping	1	28	0.433	1	73	0.331	1	171	0.520	1	41	0.183	1	34	**0.021**	1	54	0.235
Termination																		
cover cropping	2	29	**0.004**	3	74	0.077	3	195	0.667	2	54	0.423	1	48	0.077	3	57	**0.009**
Type																		
Tillage Type	1	26	**0.010**	1	30	0.938	1	155	0.254	1	47	0.062	1	48	**0.047**	1	29	**0.000**
Sample Timing	1	30	**0.008**	3	74	0.420	3	195	0.293	2	54	0.844	2	47	**0.038**	2	58	**0.008**
N Fertilizer	1	30	0.188	1	76	0.379	1	194	0.969	1	55	0.786	1	48	0.598	1	59	0.485
N Fertilizer Rate	1	30	0.564	1	47	0.247	1	147	0.943	1	12	**0.000**	1	12	**0.009**	1	34	0.253
Soil pH	1	30	**0.001**	1	75	0.412	1	137	0.286	1	5	0.130	-	-	-	1	56	0.656
cover cropping	1	28	**0.000**	1	73	0.286	1	135	0.634	1	5	0.130	-	-	-	1	54	**0.005**
Duration																		
Sample Depth	1	30	**0.028**	1	76	0.367	1	197	0.334	1	55	0.952	1	48	0.650	1	59	0.826

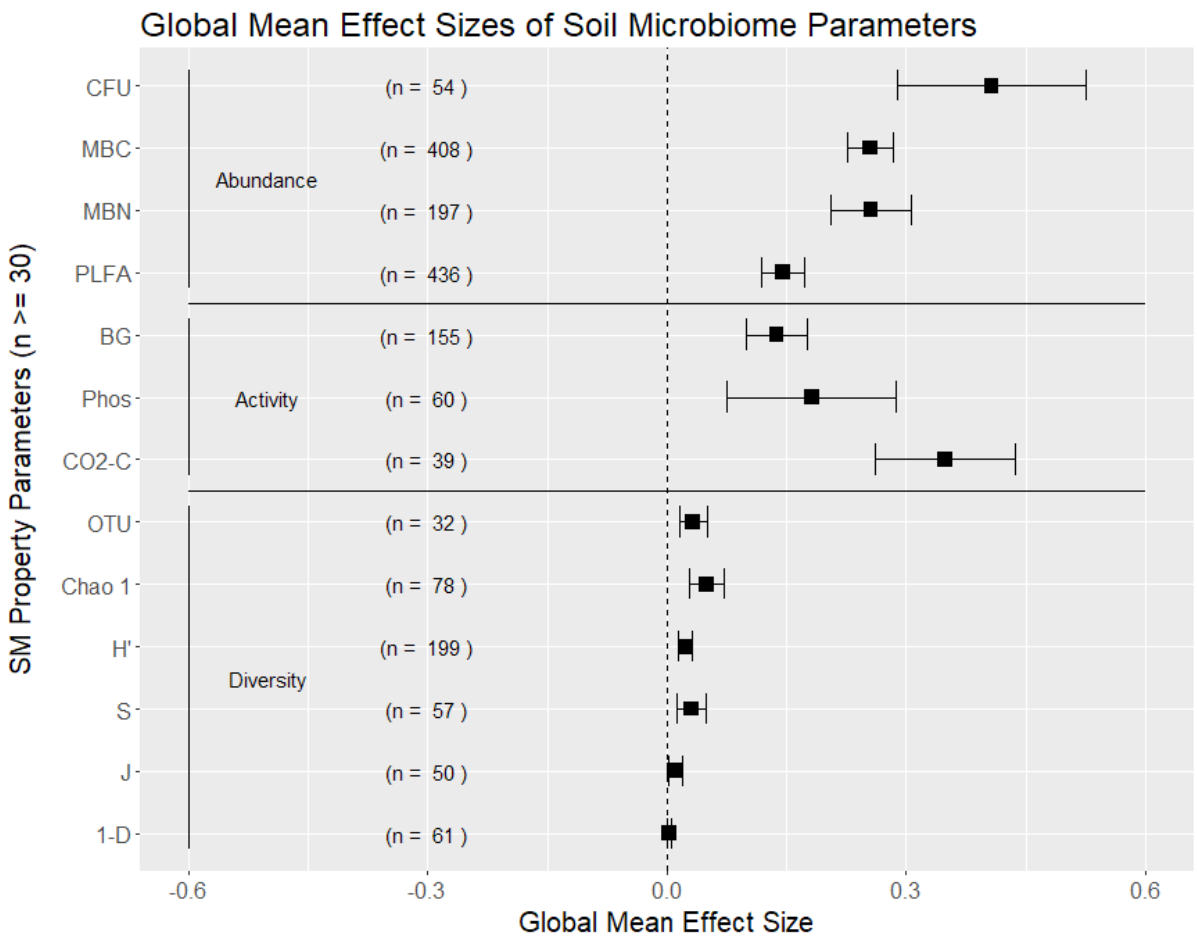

Figure 1.1. Forest plot of global effect size means for 13 soil microbial properties with at least 30 observations: colony forming unit (CFU), microbial biomass C (MBC) and N (MBN), phospholipid fatty acid (PLFA), β-glucosidase activity (BG), phosphatase activity (Phos), respiration (CO2-C), operational taxonomic unit (OTU), Chao 1 richness index, Shannon's diversity index (H'), genetic richness (S), Pielou's evenness index (J), and Simpson's diversity index (1-D). Numbers in the parentheses are the number of observations used to calculate the global effect size mean. Whiskers are 95% CIs. Means larger than zero indicate that soil microbiome parameter was larger with cover cropping than bare fallow.

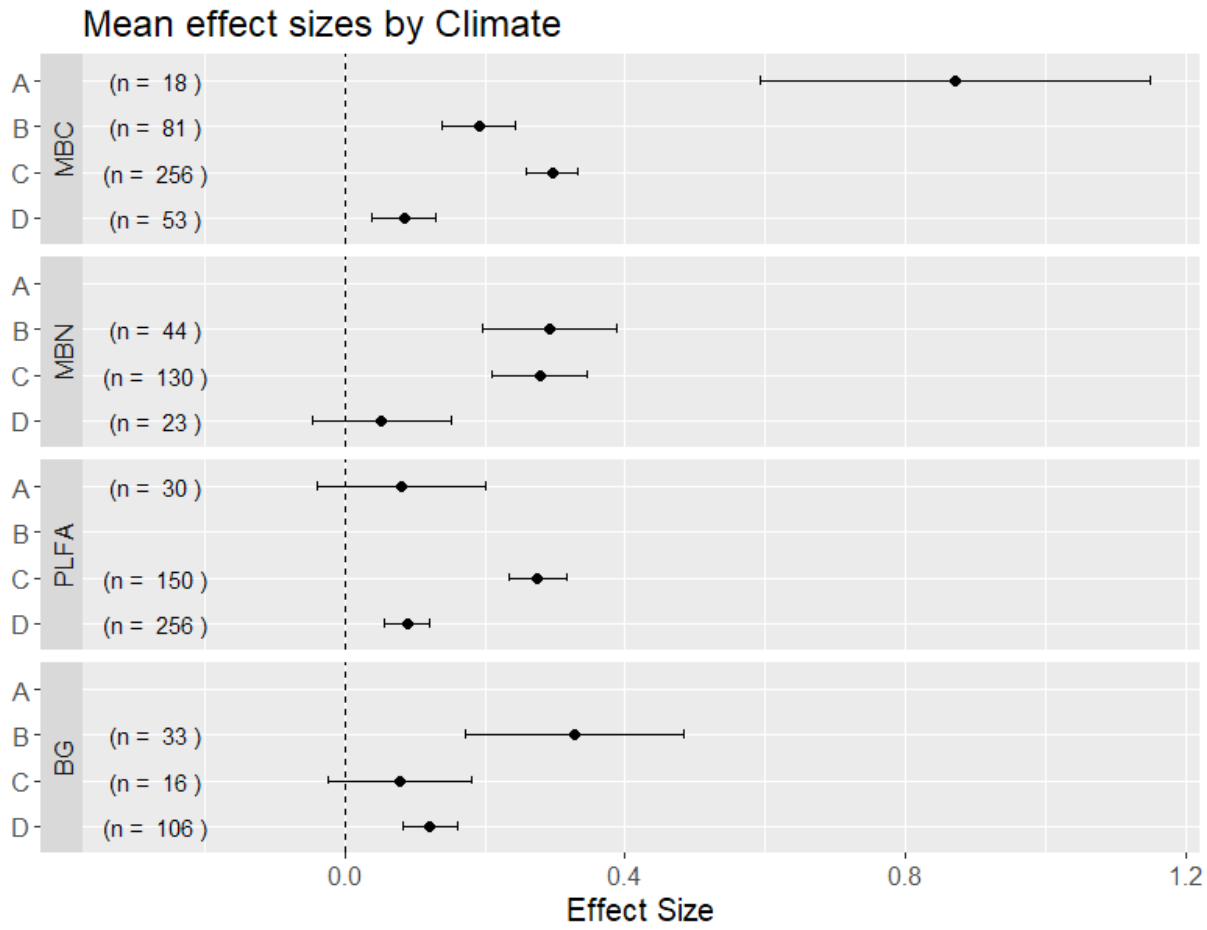

Figure 1.2. Forest plots of interactions between soil microbial parameters and climate that had levels with significant differences between effect size means. Number of observations per level is noted in parentheses. Climate is classified by A (tropical), B (arid/semi-arid), C (temperate), and D (continental). Significant soil microbial parameters were microbial biomass C (MBC) and N (MBN), phospholipid fatty acid (PLFA), and β-glucosidase activity (BG). Levels (y-axis) with means larger than zero indicate that cover cropping increased the soil microbiome parameter at those levels, and decreased if the means smaller than zero. Levels with CIs that do not overlap indicate that their effect size means are significantly different.

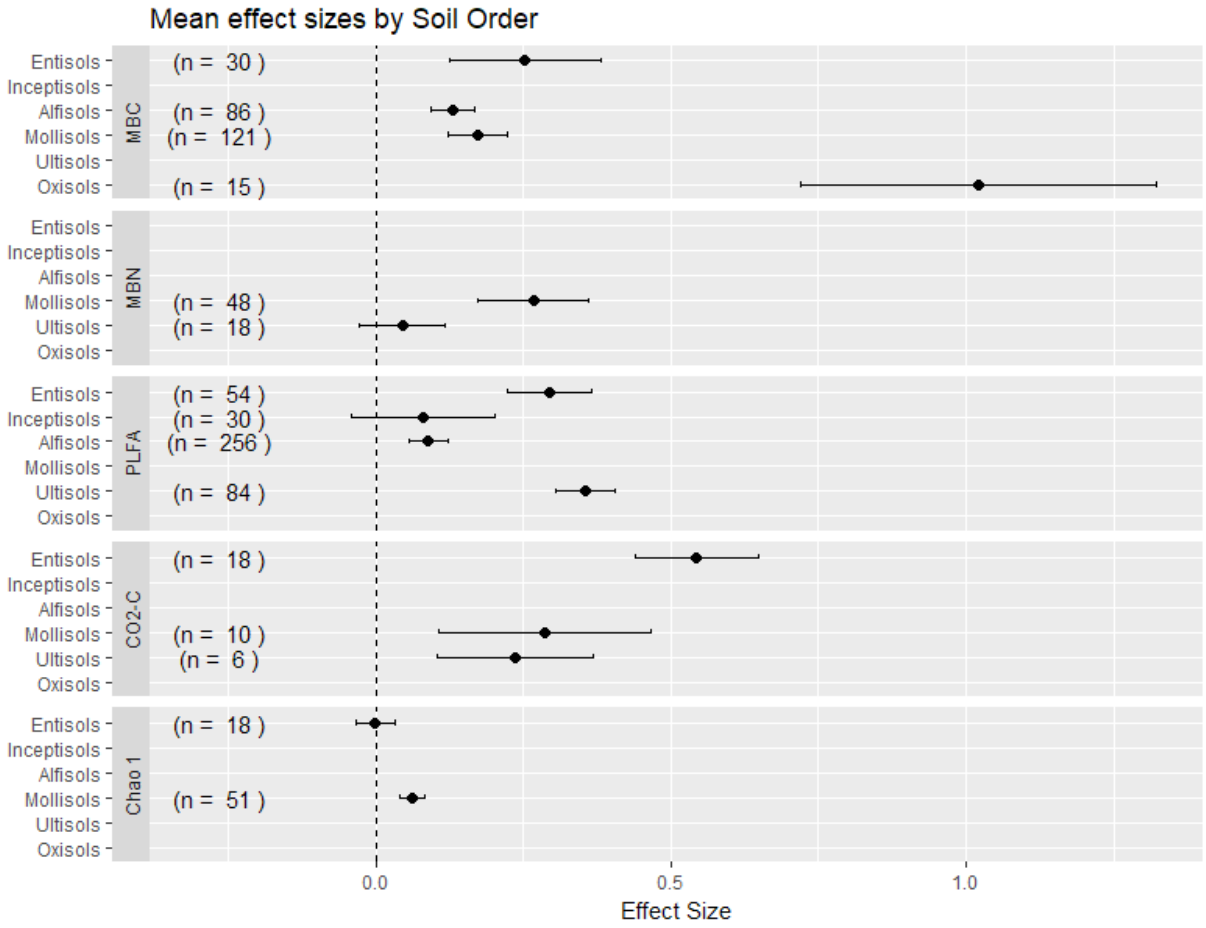

Figure 1.3. Forest plots of interactions between soil microbial parameters and soil order that had levels with significant differences between effect size means. Number of observations per level is noted in parentheses. Significant soil microbial parameters were microbial biomass C (MBC) and N (MBN), phospholipid fatty acid (PLFA), respiration (CO2-C), and Chao 1 richness index. Levels (y-axis) with means larger than zero indicate that cover cropping increased the soil microbiome parameter at those levels, and decreased if the means smaller than zero. Levels with CIs that do not overlap indicate that their effect size means are significantly different.

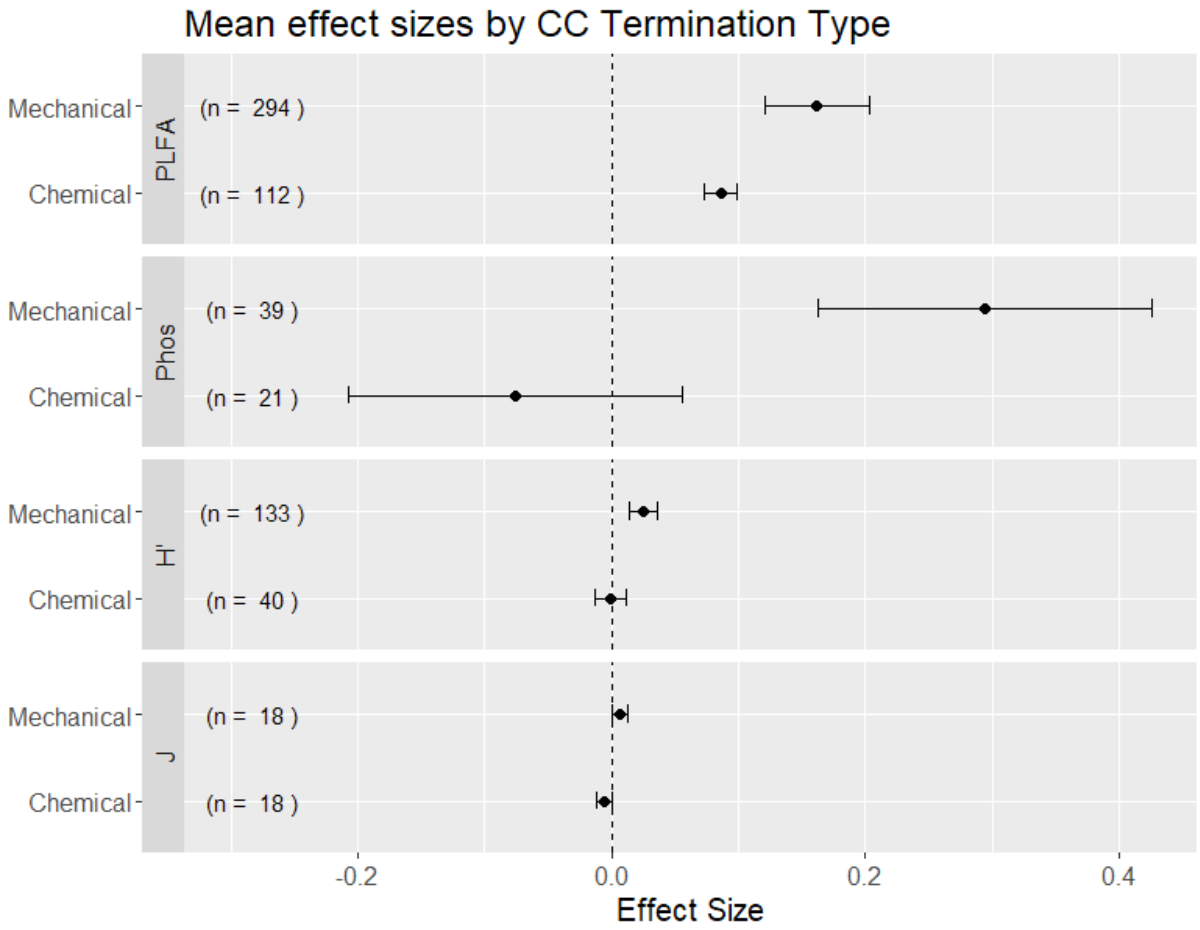

Figure 1.4. Forest plots of interactions between soil microbial parameters and cover cropping termination method type that had levels with significant differences between effect size means. Number of observations per level is noted in parentheses. Significant soil microbial parameters were phospholipid fatty acid (PLFA), phosphatase activity (Phos), Shannon's diversity index (H'), and Pielou's evenness index (J). Levels (y-axis) with means larger than zero indicate that cover cropping increased the soil microbiome parameter at those levels, and decreased if the means smaller than zero. Levels with CIs that do not overlap indicate that their effect size means are significantly different.

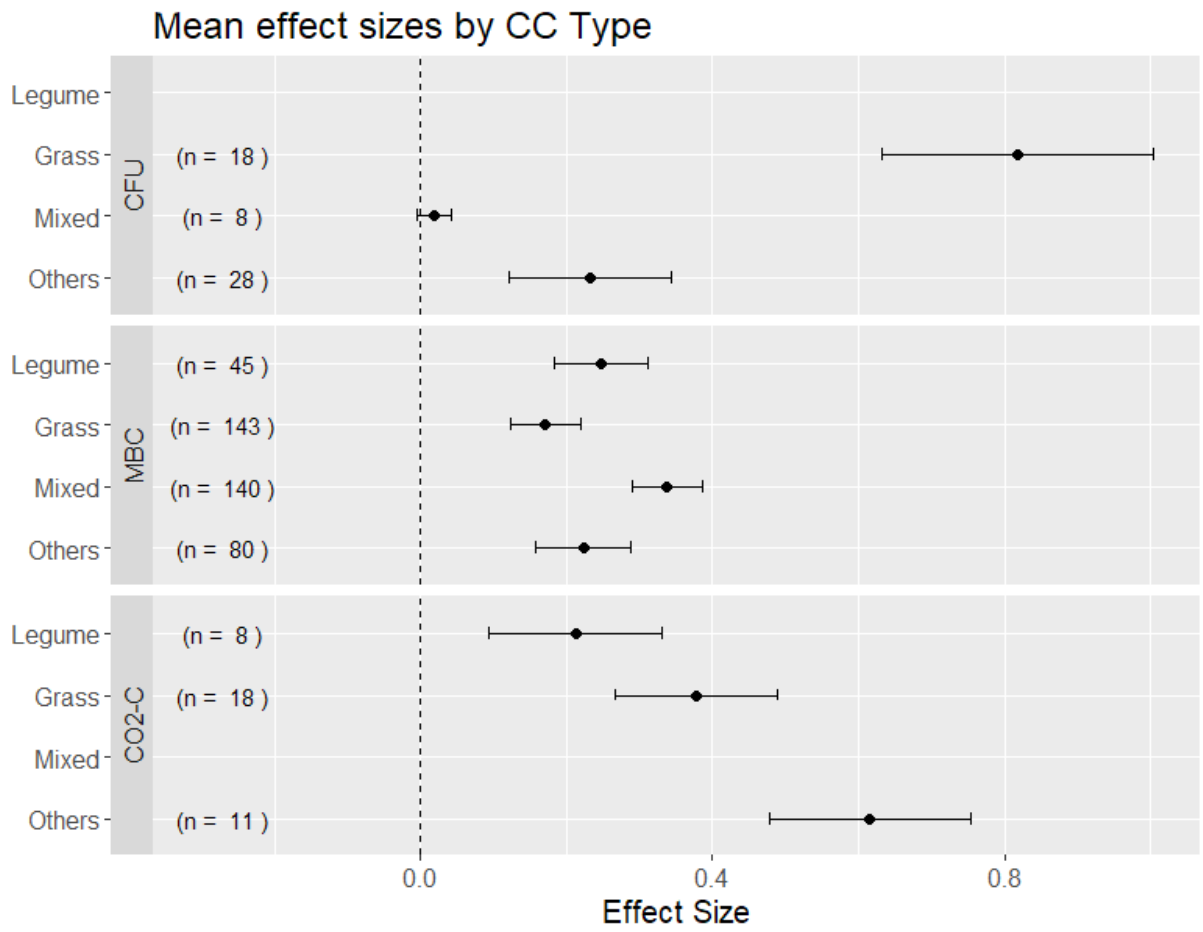

Figure 1.5. Forest plots of interactions between soil microbial parameters and cover cropping type that had levels with significant differences between effect size means. Number of observations per level is noted in parentheses. Significant soil microbial parameters were colony forming unit (CFU), microbial biomass C (MBC), and respiration (CO2-C). Levels (y-axis) with means larger than zero indicate that cover cropping increased the soil microbiome parameter at those levels, and decreased if the means smaller than zero. Levels with CIs that do not overlap indicate that their effect size means are significantly different.

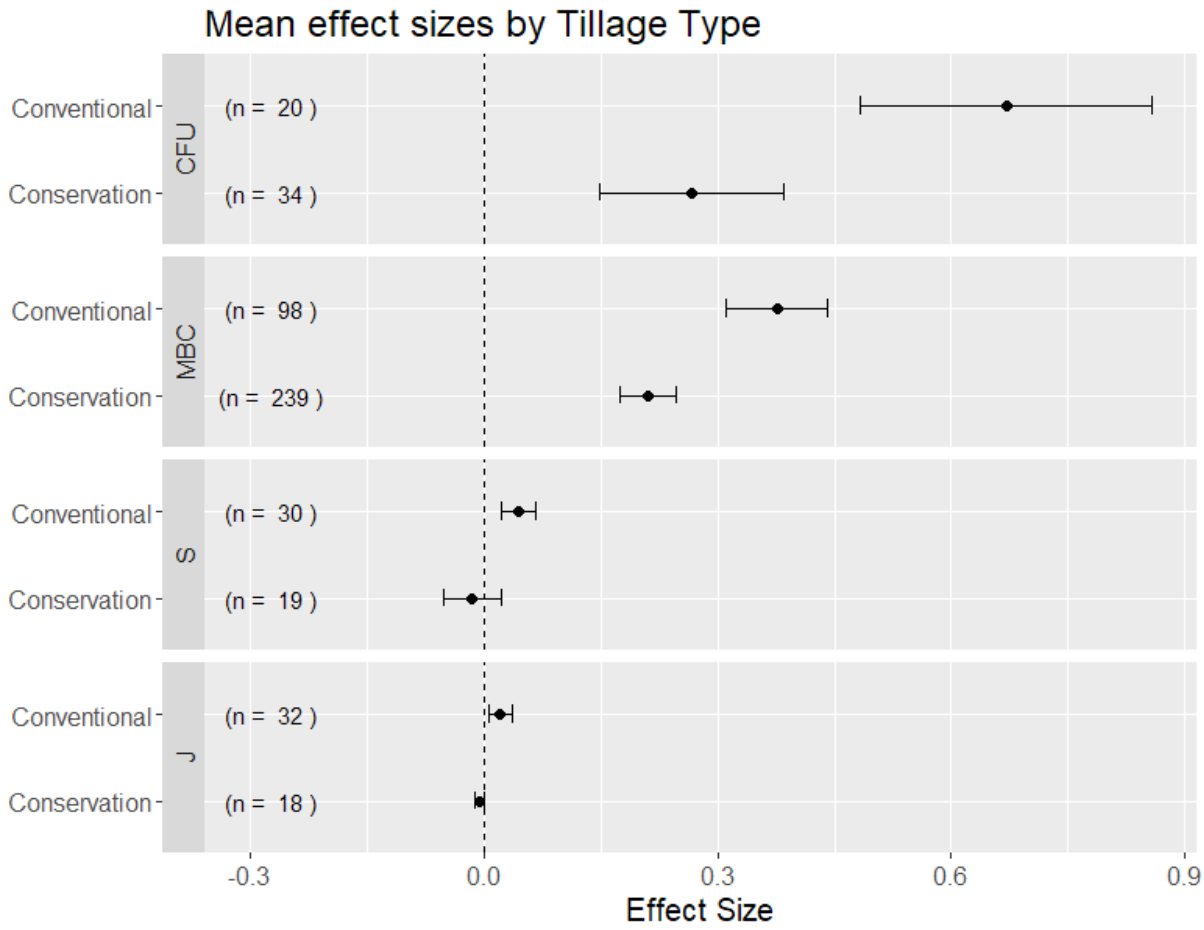

Figure 1.6. Forest plots of interactions between soil microbial parameters and tillage type that had levels with significant differences between effect size means. Number of observations per level is noted in parentheses. Significant soil microbial parameters were colony forming unit (CFU), microbial biomass C (MBC), genetic richness (S), and Pielou's evenness index (J). Levels (y-axis) with means larger than zero indicate that cover cropping increased the soil microbiome parameter at those levels, and decreased if the means smaller than zero. Levels with CIs that do not overlap indicate that their effect size means are significantly different.

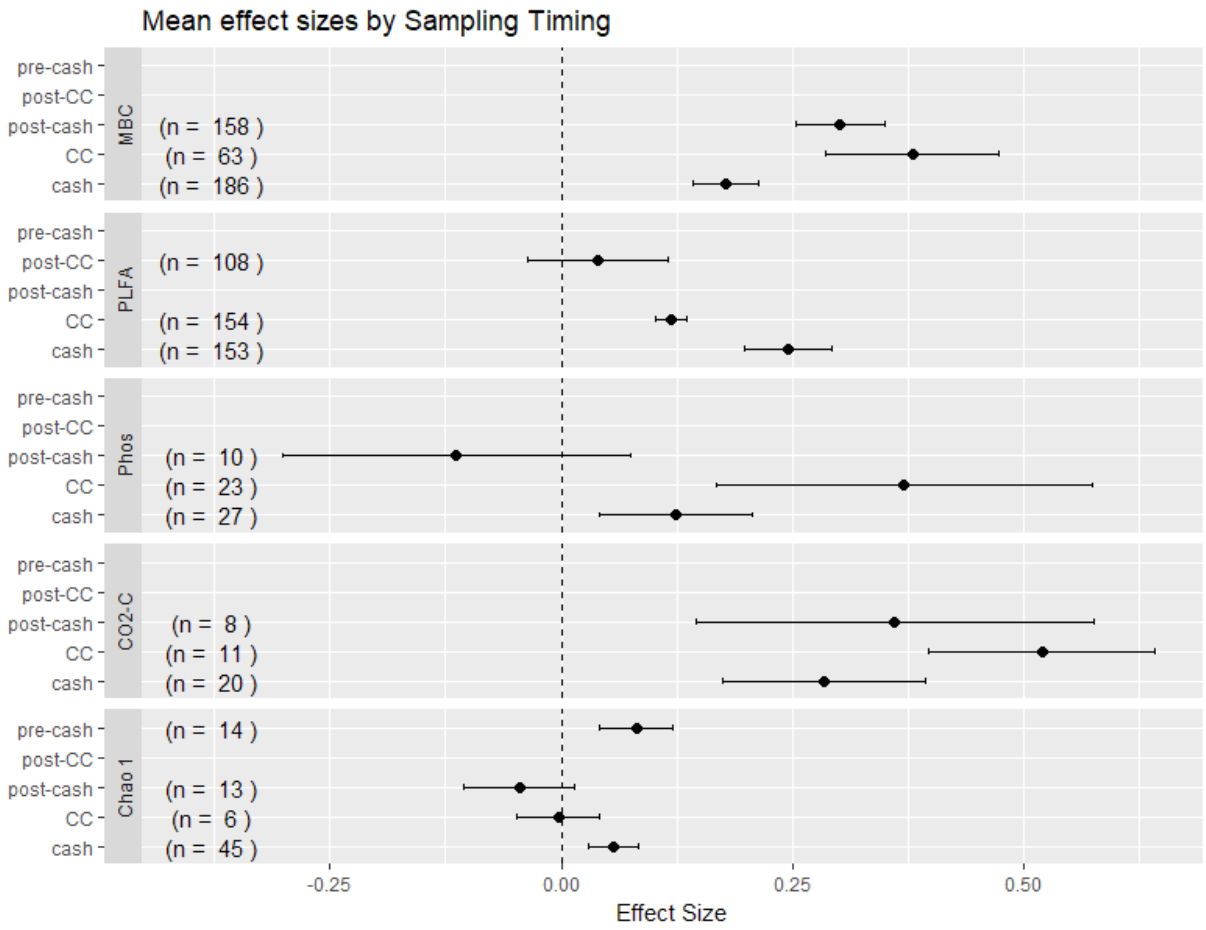

Figure 1.7. Forest plots of interactions between soil microbial parameters and soil sampling timing that had levels with significant differences between effect size means. Number of observations per level is noted in parentheses. Significant soil microbial parameters were microbial biomass C (MBC), phospholipid fatty acid (PLFA), phosphatase activity (Phos), respiration (CO2-C), and Chao 1 richness index. Levels (y-axis) with means larger than zero indicate that cover cropping increased the soil microbiome parameter at those levels, and decreased if the means smaller than zero. Levels with CIs that do not overlap indicate that their effect size means are significantly different.

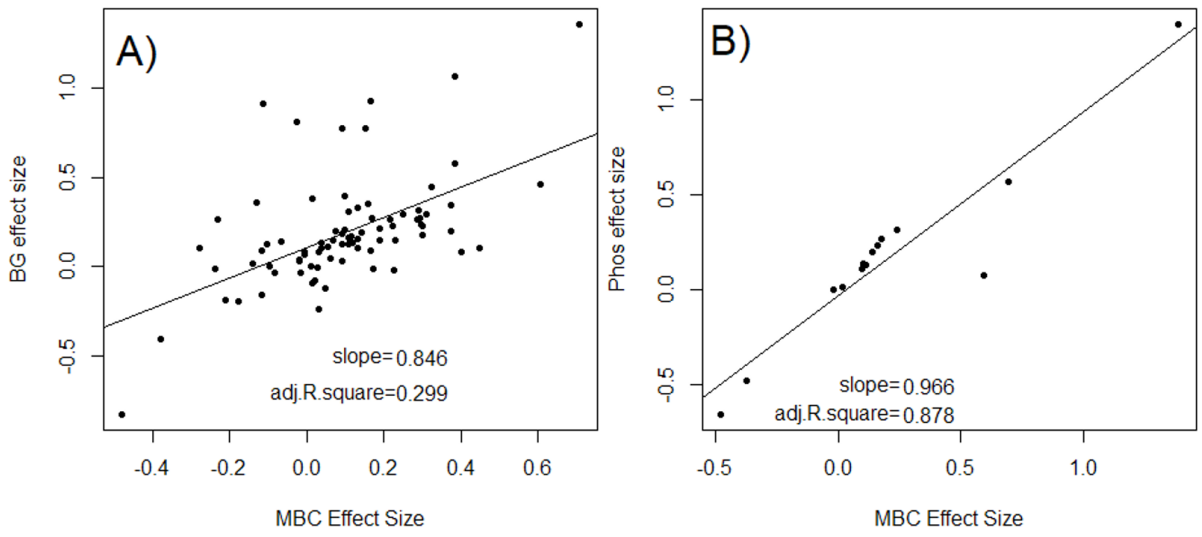

Figure 1.8. Scatter plot and linear regression of cover cropping effect sizes of β-glucosidase (BG; A) and those of phosphatase activity (Phos; B) on those of microbial biomass C (MBC). The linear coefficient of the model (slope) and R2 are noted. Both linear coefficients had significant (p-values). These relationships signify the unit change in soil microbial activity by abundance.

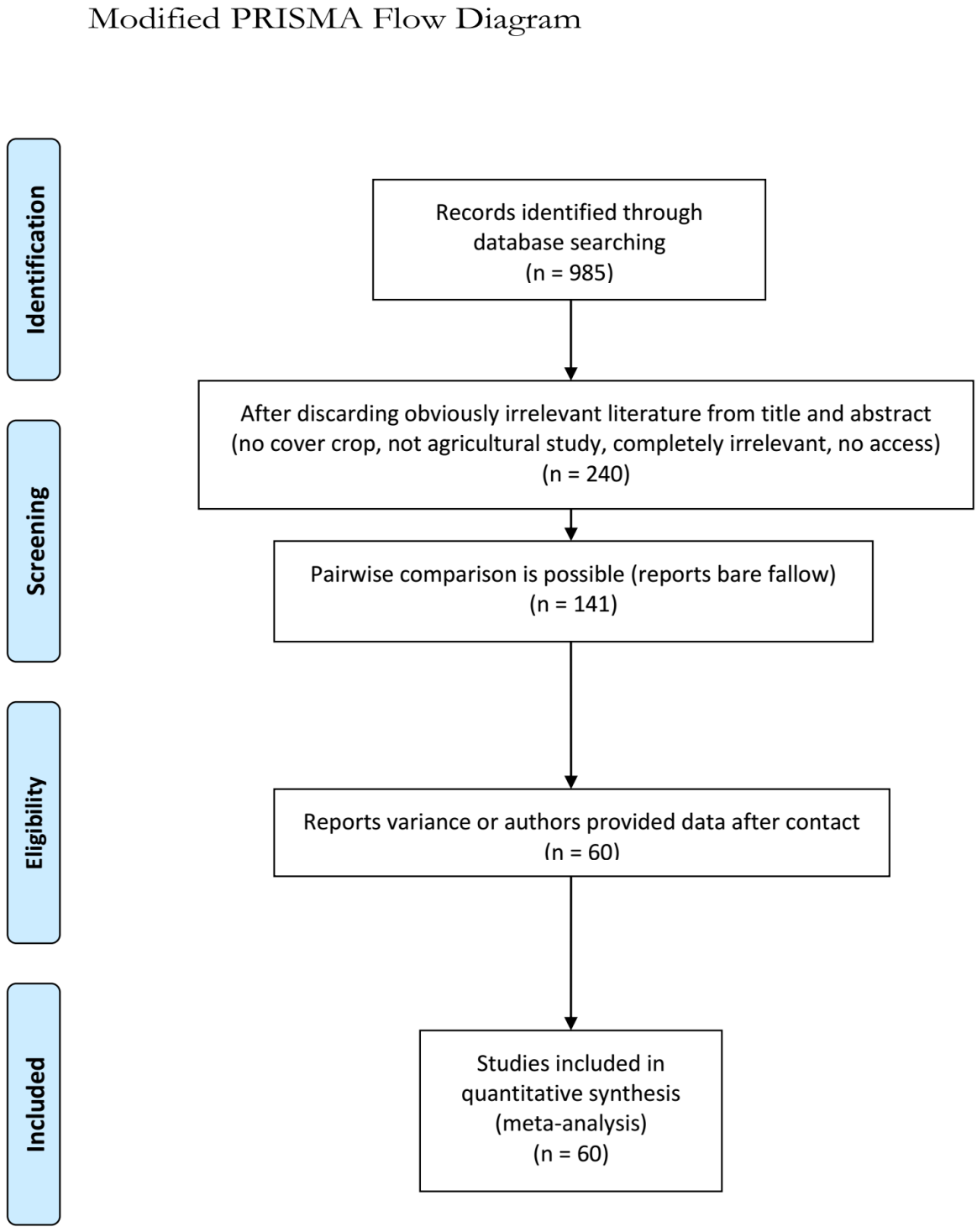

Figure 1.9. PRISMA flow diagram modified from that by Moher et al. (2009). The chart shows what criteria was applied and how many literature remained at each stage (n=).

CHAPTER 2: METAGENOMIC ANALYSIS ON THE RESPONSES OF THE SOIL MICROBIOME TO COVER CROPPING AND TILLAGE

ABSTRACT

Cover cropping (CC) has been promoted as a viable strategy to mitigate soil nutrient loss that threatens future global food security and environmental integrity. However, the research on how the soil microbial community respond to CC and tillage, which may greatly alter this relationship, has only recently begun to employ metagenomics to scrutinize this topic at a finer detail below the whole community level. This metagenomics study measured the responses of absolute abundances of each microbial operational taxonomic units (OTUs) to CC and tillage treatments to identify sensitive microbial indicators that can gauge the responses of specific microbial groups to these practices. This study used soil DNA data from a long-term experiment on corn and soybean rotation in Illinois, USA, that compared grass CC and legume-grass CC rotation to bare fallow, and chisel tillage to no-till. Overall, CC and tillage significantly shifted the microbial composition but not the whole community's richness and diversity. This study identified 18 bacterial, 12 fungal, and 1 archaeal potential indicator species whose responses to CC and tillage were consistent with their known physiological and ecological characteristics, thereby representing important microbial guilds that occupy different soil ecological niches. Legume-grass CC rotation and tillage increased the abundances of copiotrophic microbes while bare fallow and no-till favored oligotrophic/stress-tolerant guilds. Grass CC displayed intermediate results and more than halved the soil nitrate level compared to the other two systems. These results suggested that grass CC and no-till has better capability to reduce soil nutrient loss than legume CC.

2.1. INTRODUCTION

The future of global food security depends on preventing further soil degradation and restoring the affected areas (FAO, 2015). Soil chemical imbalance is a major cause of soil degradation whose detriments extend beyond the soil and into the waters and atmosphere. As one of its major sources, excess fertilizer input has been polluting the water sources (Pennino et al., 2017), disrupting the marine ecosystems (US-EPA, 2017), and emitting greenhouse gas (GHG)

(Fowler et al., 2013). In the USA, federal and state agencies are responding to these threats. For example, Environmental Protection Agency (EPA) developed comprehensive nutrient reduction strategies (US-EPA, 2013).

Cover cropping has been widely researched and promoted as a promising strategy to mitigate nutrient loss (IL-EPA et al., 2015). Cover crops (CC) are grown between harvesting and planting cash crops to provide various benefits to the agroecosystem, including scavenging excess nutrients (Kaspar et al., 2012; Tonitto et al., 2006), preventing soil erosion (Daryanto et al., 2018), improving soil organic matter (SOM) and water retention (Villamil et al., 2006; Villamil et al., 2008), and suppressing weeds (Daryanto et al., 2018; Quemada et al., 2013). Cover cropping provides a physical cover above ground and root structures belowground that protect the soil from water and wind erosion, preventing nutrient losses (Snapp et al., 2005). Also, CC take up soil nutrients and immobilize them into biomass, leaving less nutrients to be lost (Acuña and Villamil, 2014; Behnke and Villamil, 2019).

Yet the benefits potentially achieved with CC depend on environmental factors such as soil fertility (Behnke et al., 2020), weather conditions and length of the growing season (Behnke and Villamil, 2019). Management practices are also crucial factors, such as tillage (Dozier et al., 2017; Villamil et al., 2006), seeding strategies (Haramoto, 2019), fertilization (Wittwer and van der Heijden, 2020), and time and method of suppression of spring growth (Kim et al., 2020; Wayman et al., 2014). Among these practices, the effect of tillage on soil properties has been widely studied, finding effects like decreasing soil aggregate stability and water infiltration (Blanco-Canqui and Ruis, 2018), decreasing soil organic C (SOC) content (Kibet et al., 2016), and reducing soil compaction and soil moisture in the subsoil (Feng et al., 2018). Studies also evaluated tillage effects on soil nutrient loss and found high possibility that tillage can worsen nutrient loss via runoff (Endale et al., 2019) and leaching (Singh et al., 2018). Therefore, many studies investigated whether tillage influences the CC benefits. For example, Singh et al. (2018) found that nitrate (NO_3^-) leaching was greater with legume CC than grass CC and bare fallow when the soil was tilled. Also, short-term studies like Acuña and Villamil (2014) and Dozier et al. (2017) found that tillage affected neither CC capability to scavenge soil N nor soybean and corn yield. Meanwhile, five year-long study by Behnke et al. (2020) on the CC and tillage practice in Illinois found that chisel tillage increased corn yields by 4% but did not affect

soybean yield regardless of CC presence, and that grass-only CC rotation significantly reduced soil NO_3^- compared to bare fallow.

Due to their direct and indirect effects on soil properties and nutrient cycling, CC in combination with tillage practices have the potential to alter the structure and function of the soil microbiome. Soil microbes are the major drivers of the agriculturally and environmentally important soil biogeochemical processes (Frasier et al., 2016; Hallama et al., 2019; Hirsch and Mauchline, 2015; Thomas et al., 2017). For example, much of the soil N cycle is dictated by the soil microbes that involve in the N addition or loss (Coskun et al., 2017). Here, biological N-fixation annually adds a global estimate of about 60 Tg of N in agricultural lands (Fowler et al., 2013). The soil microbiome also controls the amount of soil inorganic N through immobilization and mineralization, which also affects plant nutrient availability (Jacoby et al., 2017). Moreover, nitrifying and denitrifying microbes contribute to nutrient loss by converting soil N into compounds vulnerable to leaching and emission (80 and 13 Tg per year, respectively, globally) (Coskun et al., 2017; Hirsch and Mauchline, 2015). Therefore, soil microbiome is a crucial factor for soil nutrient loss, and understanding the contributions of cover cropping and tillage to the soil microbiome is a necessary task.

A few recent meta-analyses have summarized the past research on this relationship (Daryanto et al., 2018; Kim et al., 2020; Venter et al., 2016; Zuber and Villamil, 2016). A comprehensive meta-analysis on CC effects by Daryanto et al. (2018) reported that soil microbial abundance measured as microbial biomass carbon (MBC), nitrogen (MBN), and phosphorus (MBP) all increased under CC. Another meta-analysis by Kim et al. (2020) reported that cover cropping increased not only abundance, but also activity and overall diversity. They also reported that CC effects depend on other management practices including tillage, where no-till decreased the CC effect size on the soil microbiome (Kim et al., 2020). Likewise, Zuber and Villamil (2016) found that conservation or no-till strategies increased microbial abundance, respiration, and enzyme activities associated with plant residue decomposition.

However, these studies were limited to the responses of the microbial community as a whole. The whole microbiome as a unit is too broad to evaluate microbial guilds or individual microbes that can represent important microbial processes and potentially act as indicators of sustainable management. When scrutinizing a complex system, its parameters that are sensitive to stimuli are used as indicators to represent the effects on the system (Villamil et al., 2008). For

example, soil properties like SOM content that are sensitive to management have been selected as indicators of the soil quality (Villamil et al., 2008), while ecological studies use indicator species to assess changes in the ecosystems (Siddig et al., 2016). Likewise, responsive microbial groups can be used as microbial indicators to gauge the soil microbial responses to management (Schloter et al., 2018). For instance, Wolińska et al. (2018) proposed five bacterial genera including *Nitrospira* and *Burkholderia* as microbial indicators of soil resistance to agriculture. The same can be applied to find microbial indicators to measure effects of CC and tillage practices on the soil microbiome.

With the advancement of metagenomics, taxonomic and functional profiling of the soil microbial community has been widely adopted by cover cropping research. This led to the quantification of the individual responses of each microbial taxa at different scopes (i.e. phyla, classes, order, family, and genera, or functional guilds), and identify sensitive groups as potential microbial indicators (Balota et al., 2014; Schloter et al., 2018). So far, metagenomics studies found that soil microbial groups are primarily sensitive to CC and tillage induced changes in the soil nutrient availability, and respond differentially based on their r/K strategies or substrate preferences (Alahmad et al., 2019; Pascault et al., 2013; Romdhane et al., 2019). For example, a study by Alahmad et al. (2019) on wheat-green pea-maize rotation investigated the effects of legume-grass mix CC and N fertilization on the soil microbial taxonomic and functional compositions. This study found that CC treatments recruited more specialist species, like *Streptomyces grisemus* in phylum Actinobacteria, than bare fallow, which the authors speculated as a result of changes in the soil nutrient from CC-originated C and N compounds like root exudates (Alahmad et al., 2019).

Another study by Romdhane et al. (2019) on the effects of CC termination methods on soil microbial composition found that CC biomass had positive correlation with soil organic C and soil C:N ratio, which the authors attributed to C-rich CC root exudates. They found that soil C:N ratio, SOC, and total soil N differentially affected the relative abundances of microbial genera; for example, two unknown genera of phylum Gemmatimonadetes had positive relationship with soil C:N ratio while *Salinibacterium* of Actinobacteria had a negative relationship (Romdhane et al., 2019).

Pascault et al. (2013) also demonstrated that CC induced changes in the soil nutrient availability are time-dependent and create transitions in the dominant soil microbial groups. This

study described that phyla Firmicutes and Proteobacteria dominated the fresh organic matter (FOM) degrading community at the earlier stages of residue decomposition (Pascault et al., 2013). However, phyla like Acidobacteria and Gemmatimonadetes dominated the SOM degrading community after easily degradable nutrients have been depleted (Pascault et al., 2013). Moreover, Pascault et al. (2013) speculated that lower C:N of alfalfa CC residue promoted initial FOM degrading microbial groups to execrate more exoenzymes, which later degraded more SOM and benefited the succeeding microbial groups, compared to wheat CC. This alluded that C:N of the CC residues is an important factor of soil nutrient dynamics and subsequent microbial responses.

Meanwhile, agricultural metagenomics studies on archaea mostly focused on the ammonia-oxidizing archaea (AOA), which not only is important for the soil microbial N cycling, but also seemingly the dominant archaeal group in agricultural soil based on current methodology (Babin et al., 2019; Segal et al., 2017; Somenahally et al., 2018). Past studies have identified genus *Nitrososphaera* as one of the most abundant and consistently sensitive group to changes in the soil, therefore a likely candidate for an indicator archaea group (Babin et al., 2019; Zhalnina et al., 2013). Study by Schmidt et al. (2018) on the effects of depth, tillage, and CC on the soil microbiome found that archaea responded positively to CC but less so compared to bacteria. This study suggested that, like bacteria, CC impact on soil nutrient availability is an important factor because archaea are less competitive in nutrient-rich environment (Schmidt et al., 2018; Valentine, 2007).

Tillage also affects the soil nutrient availability by breaking and incorporating crop residues into the soil, thereby aiding microbial decomposition. A study by Sharma-Poudyal et al. (2017) investigated the effects of tillage on soil fungal community, comparing no-till to chisel tillage. This study found that saprophytic fungal genera like *Humicola* were more dominant with no-till, while genera like *Cladosporium* did so under tillage (Sharma-Poudyal et al., 2017). The authors attributed these contrasting responses to each microbial group's substrate preferences, explaining that the former group is better adapted to degrading intact biomass under no-till, while the latter prefers more labile C and N sources from tilled crop residues (Sharma-Poudyal et al. 2017). This tillage effect on crop residues also applies to CC residues, amplifying their impact on the soil nutrient availability and microbial composition (Lupwayi et al., 2004; Lynch et al., 2016). This study also emphasized the physical effects of tillage that can increase soil

abundances of plant pathogens and endophytes by incorporating the infected biomass into the soil. The differences in fungal morphology may also be a factor where tillage can disrupt hyphal fungi but less so for conidia producing fungi (Sharma-Poudyal et al., 2017). This tillage physical disturbance also affects soil aeration (Khan, 1996), which soil microbes are also highly sensitive to according to their metabolic adaptations (Degrune et al., 2017; Linn and Doran, 1984). These sensitive microbes include anaerobes that perform processes like denitrification that lead to soil nutrient loss, emphasizing the importance of CC and tillage effects on these soil properties and how the microbial indicators respond to them (Coskun et al., 2017; Hirsch and Mauchline, 2015).

Metagenomics studies introduced so far have investigated how the soil microbial groups respond differentially to the soil properties that CC and tillage alter significantly; these efforts also identified sensitive microbial groups that are potential microbial indicators. Studies like these should be further accumulated to improve the list of microbial groups that are consistently sensitive to effects of CC and tillage, and verify them as the microbial indicators. This is especially true for reports at genus and species level that only started accumulating recently. As a part of this effort, this study analyzed the responses of the bacterial, archaeal, and fungal communities to CC and tillage, using metagenomics data from a long-term cover cropping and tillage experiment in Illinois, USA, under corn (*Zea mays* L.) and soybean (*Glycine max* L.) rotation. The objectives of this study was to i) identify microbial groups whose abundance differed significantly by CC and tillage treatments, ii) determine to which specific factors these microbial groups were sensitive to, based on other microbial, soil, and CC biomass properties and past reports, and iii) evaluate what implications do CC and tillage effects on these factors and their subsequent microbial responses have on soil nutrient loss. Results of this study will help illustrate a more accurate picture of the soil microbiome under cover cropping and tillage, which will lead to better use of these practices to reduce soil nutrient loss.

2.2. MATERIALS AND METHODS

2.2.1. Experimental site description

The experimental site was established in the fall of 2012, at Crop Sciences Research and Education Center at Urbana, IL (40.057N, 88.227W), as part of a larger effort to investigate cover cropping and tillage on soil properties and yields (Dozier et al., 2017; Behnke et al., 2020). The experimental site spanned Drummer–Catlin–Flanagan soil association where 70% of the site

was Drummer silty clay loam (fine-silty, mixed, superactive, mesic, Typic Endoaquoll), 20% Flanagan silt loam (fine, smectitic, mesic, Aquic Argiudoll), and 10% Catlin silt loam (fine-silty, mixed, superactive, mesic, Oxyaquic Argiudoll). These dark-colored soils developed under prairie on mostly level to very gently sloping (0 to 2%) topography in upland positions. Flanagan is somewhat poorly drained, Catlin is moderately to well-drained soil occupying the higher landscape positions, and Drummer is poorly drained soil in the lower positions in the landscape (Soil Survey Staff, 2019).

2.2.2. Treatments and field management practices

The experimental site was arranged in a split-block design with eight blocks total. Four blocks were each assigned to corn and soybean, and the cash crop phase rotated each year. Each block was divided in the N-S direction into tilled (T) and no-till (NT) plots, and subplots of corn soybean rotations with CC were allocated in the W-E direction. The CC treatments included annual ryegrass (*Lolium multiflolum* Lam.) before and after cash crops (CarSar), cereal rye (*Secale cereale* L.) following corn and hairy vetch (*Vicia villoa* Roth.) following soybean (CcrShv), and using corn soybean rotations without CC as unseeded controls (CT). In any year, there were eight subplots for each CT and CarSar, and four under cereal rye phase of CcrShv and four under hairy vetch phase of CcrShv.

Detailed information regarding field management practices during the project period is publicly available (Villamil and Nafziger, 2019). Briefly, the following field management practices were implemented each year from 2012 to 2017. Corn was planted on mid-May except 2012 (mid-April) and 2013 (early-June), and harvested on mid-October to early-November; soybean was planted on mid-May to early-June except 2012 (mid-April) and harvested on mid- to late-October, except in 2017 (mid-June). Pre-plant N fertilizers were applied to corn as urea ammonium nitrate (UAN 28%) at the rate of 190 kg N ha^{-1}. Plots under tillage treatment were tilled by chisel plow down to 20-25 cm deep in the spring following CC suppression before planting the cash crop (mid-May to early-June). The CC seeds were broadcasted by hand on standing cash crops on mid-September, except 2012 (early-October). Seeding rates and growth suppression followed the online decision tool by the Midwest Cover Crop Council (online at: mcccdev.anr.msu.edu/Vertindex.php): 16.8 kg/ha for annual ryegrass, 22.4 kg/ha for hairy vetch, and 100 kg/ha for cereal rye. Cover crops were suppressed with glyphosate [N-

(phosphonomethyl)glycine] at 1.12 kg a.i. ha^{-1} by the end of April. The exact dates of the field practices can be found in Table B.5.

2.2.3. Soil and biomass sampling, DNA extraction, qPCR, and sequencing

Soil samples were collected on the April 21st, 2017 at the end of the project that this study was a part of, following five years since initial CC establishment (Behnke et al., 2020). Eijelkamp grass plot sampler (Eijkelkamp Agrisearch Equipment, Netherlands) was used to take two composited subsamples of 500 g each per subplot to a depth of 10 cm to analyze the soil DNA. Soil samples were kept with ice in the field and stored in the freezer in the laboratory. Three soil core samples with a diameter of 4.3 cm were also taken randomly down to 90 cm depth for each subplot using a tractor-mounted automated soil sampler (Amity Technology, Inc., Fargo, ND, USA). These soil samples were analyzed for soil properties. Soil nitrate-N (NO3-N) and ammonium-N (NH4-N) (mg kg^{-1}) were measured using KCl extraction (1:5 ratio) and analyzed using SmartChem 200 Discrete Analyzer Auto-Spectrophotometer (Westco Scientific Instruments, Inc., Brookfield, CT, USA). Soil phosphorus (P, mg kg^{-1}) was measured by Bray P1 extraction. Soil pH was measured using potentiometry with a Mettler Toledo Ag SevenEasy pH Meter (Schwerzenbach, Switzerland). Soil samples were also air-dried and sieved to 2 mm then sent to commercial laboratory (Brookside Laboratories, Inc., New Bremen, OH, USA). The commercial lab used standard procedures recommended for the U.S. North Central region (Brown, 1998). Here, cation exchange capacity (CEC, cmol kg^{-1}) was determined by summation of exchangeable cations (Ca, Mg, K, Na, H) (Sumner and Miller, 2018). Soil pH was measured with potentiometry by a Mettler Toledo Ag SevenEasy pH Meter (Schwerzenbach, Switzerland), and soil organic matter (SOM, %) by loss on ignition. Cover crop biomass samples were taken in April 11th, 2017 using three random tosses of 0.25m^2 quadrat per subplot and cut at ground level. Biomass samples were oven-dried at 60 °C and recorded their carbon and nitrogen contents (%), C:N ratio, and the dry weight (Mg ha^{-1}). Soil and CC biomass properties are summarized in Table 2.1.

Soil DNA was extracted from 0.25 g of the composited soil samples on June 2019 using PowerSoil® DNA isolation kits (MoBio Inc., Carlsbad, CA, USA), according to the manufacturer's instructions. The quantity and quality of the extracted DNA were tested using Nanodrop 1000 Spectrophotometer according to the manufacturer's protocol (Thermo Fisher

Scientific, USA). Extracted DNA was stored at −20 °C. Illumina HiSeq compatible amplicon library containing individual barcodes for each samples was constructed. For this library, 25 µL PCR reactions were done using a BioRad T100 thermal cycler in 25 µL volumes with 1× buffer (GoTaqfi Flexi buffer; Promega Corp.), with the following composition: 2.5 mM $MgCl_2$, 200 µM dNTPs, 0.4 µM each primer (forward and reverse), 1.0 µL template DNA (pooled amplicons), and 1.0 unit of GoTaq polymerase. PCR parameters were: initial denaturation at 95 ◦C for 10 min, followed by 34 cycles of amplification (45 secs at 95 ◦C; 45 s at 58 ◦C; 45 s at 72 ◦C), and a final extension at 72 ◦C for 10 min. PCR products were visualized on a 1.3% agarose gel containing GreenGlo™ Safe DNA dye (Denville Scientific, Inc. Metuchen, NJ, USA) under UV illumination. Bacterial 16S rRNA gene (V4 region) was amplified using primer set of 515F (GTGYCAGCMGCCGCGGTAA) and 806R (GGACTACVSGGGTWTCTAAT) (Fierer et al., 2005), archaeal 16S using 349F (GTGCASCAGKCGMGAAW) and 806R (GGACTACVSGGGTATCTAAT) (Colman et al., 2015), and fungal ITS region using 3F (GCATCGATGAAGAACGCAGC) and 4R (TCCTCCGCTTATTGATATGC) (Crawford et al., 2012). The primers were designed as 5'-PCR-specific + gene region + 3'-PCR-specific + 10 nt barcode and the Fluidigm platform utilized two primer sets simultaneously to create the final DNA amplicon. Qubit Fluorometer quantified the resulting amplicon libraries, which were then run on Bio-analyzer to evaluate the profile of fragment lengths. The barcoded libraries were pooled in equimolar concentrations and diluted to 10 nM. The diluted libraries were sequenced at the Roy Carver Biotechnology Center Functional Genomics lab at the University of Illinois at Urbana-Champaign (Urbana, IL, USA) using paired-end sequencing on the Illumina MiSeq nano 2 (Illumina, San Diego, CA, USA) yielding 250 nt long reads.

2.2.4. Bioinformatics analysis

Quality check and processing of the sequences were done through QIIME2 (Bolyen et al., 2019; Hall and Beiko, 2018). First, quality of the 16S and ITS marker gene sequences were checked to determine the positions to retain where the average quality score (probability of base calling error) is at least 30 (Li et al., 2015). This resulted in retaining bacterial sequences between base-pair positions 6 to 250, fungal sequences 6 to 200, and archaeal sequences 6 to 136. Next, sequences were denoised by removing chimeric and low-quality sequences with chimera-method consensus option in plugin DADA2 (Callahan et al., 2016). Then, sequences

were aligned to compare them and create the phylogenetic tree. Reference sequences from SILVA ribosomal RNA gene database (silva-132-99-515-806-nb-classifier_2019_4) (Quast et al., 2013) were used to compare bacterial and archaeal 16S rRNA sequences and Fungi_97_classifier_2019_4 for ITS sequences, and clustered them into operational taxonomic units (OTUs) at 97% similarity threshold. The rarefaction curves plateaued at the sampling depths of 5000 sequences per sample for bacteria, 900 for fungi, and 300 for archaea at the cost of losing 2 bacterial, 15 fungal, and 14 archaeal samples (out of 144 total samples for each taxa) because they did not have enough sequences for subsampling. Yet all the subplots were represented by at least one sample. This compromise between sampling depth and sample retention had to be made because lower sampling depths would have underestimated the diversity. At these depths, QIIME2 calculate the observed OTUs (OTUs), Shannon's Diversity Index (H'), and Chao 1 Richness Index (Chao1) for each sample for later α-diversity analysis. Also, weighted UniFrac distance was calculated by QIIME2 to measure β-diversity. The rarefaction curves (Figure B.9) of the microbes are created using package ggplot2 in R, Version 3. 5. 3. (R Core Team, 2019; Wickham, 2016).

2.2.5. Statistical analysis

After processing the DNA sequences and identifying OTUs, the absolute abundances of each OTU were statistically analyzed to identify indicators microbes and gauge treatment effects (Props et al., 2017; Tang, 2019). First, the JMP "Predictor Screening" platform used a bootstrap forest partitioning method to rank most responsive OTUs based on their contribution to predicting CC and tillage treatment effects (SAS Institute Inc., 2019). This led to selection of 42 out of 1832 OTUs for Bacteria, 5 out of 19 OTUs for Archaea, and 36 out of 313 OTUs for Fungi. The selected taxa each contributed a minimum one percent to the variability captured by the model algorithms.

Principal component analyses (PCA) were then used to further select indicator bacterial and fungal taxa from the list of top contributing microbes from the previous procedure. As described above, the previous procedure selected only five archaeal OTUs, therefore, archaea were not included in the PCA procedure because further selection was unnecessary. First, the top contributing bacteria and fungi were each grouped into a smaller set of uncorrelated composite variables, or Principal Components (PCs), to be use as dependent variables in a follow-up

ANOVA. To do this, the FACTOR procedure was used in SAS with option priors=1. Then, top contributing PCs with eigenvalues ≥1 that also explained at least 5% of the variability of each bacterial and fungal data were selected for further analyses; OTUs that consist these top contributing PCs are considered potential microbial indicators. Therefore, a PC is an aggregate of correlated variables, or OTUs included in this procedure, each with unique correlation with the PC represented by the PC loading value (Tabachnick et al., 2007). In other words, responses of a PC to treatments summarize the responses of the PC's OTUs to the treatments, which differ by each OTU's PC loading value. A positive PC loading of an OTU within a PC indicates that this OTU is positively correlated to the PC: an increase in the PC score results in an increase in the OTU. Likewise, negative loadings indicate a negative correlation. OTUs with the same sign indicate a similar direction of the response. Thus, microbial variable loadings greater than |0.5| were considered in the interpretation of each PC as they indicate a strong effect on that PC.

Next, linear mixed models were fitted to the PCs extracted in each set using PROC GLIMMIX in SAS (link=id) to evaluate the response of the selected microbial taxa to the effects of tillage, CC rotations, and their interaction. Blocks were considered random effects. The model used to estimate the effects of CC and tillage treatments was a generalized linear mixed effects model:

$$Y_{ijk} = \mu + B_i + T_j + CC_k + T \cdot CC_{jk} + \varepsilon_1 + \varepsilon_2 \quad (2.1)$$

Here, *Y* is the response variable, μ is the global mean of *Y*, *B* is the random block effect, *T* is the fixed tillage effect, *CC* is the fixed CC effect, *T*CC* is the fixed tillage and CC interaction effect, ε_1 is the plot error, and ε_2 is the subplot error. The response variables analyzed by this model were PC scores of the top contributing PCs from previous PCA procedure, the α-diversity indices calculated by QIIME2, and soil and CC biomass properties. This procedure yielded ANOVA (Type III) results and least-square means (lsmeans) of the response variables separated by treatment levels. Relationships that had at least marginally significant ANOVA results (p-value < 0.1) and significant mean separation results (α=0.05) were considered for further analysis.

Of the PCs that had at least marginally significant ANOVA results (p-value<0.1), the responses of top contributing PCs and their microbial indicator OTUs selected by PCA were visualized by using both the OTUs' PC loadings and mean separation results of the PC scores from previous procedures. As described above, PC loading values of the OTUs represent their

individual relationship to the PCs that they consist. Multiplying the PC loading value of an OTU to the mean PC scores separated by the treatment levels yields this OTU's responses to each of the treatment levels. For example, if PC1 had means PC scores of 10 for NT and -10 for T, they were each multiplied by PC loading of -0.60 of the selected microbial taxon X in PC1. The products, -6 for NT and 6 for T, indicate that the abundance of species X increased under T relative to that of NT. Package ggplot2 in R, Version 3. 5. 3., were used to create figures illustrating relative responses for each significant relationships between PC scores and treatments (R Core Team, 2019; Wickham, 2016). To compare the responses of the selected taxa to the overall patterns of their parent phyla, above PCA and ANOVA procedures were also applied to the relative abundances of microbial phyla.

The α-diversity indices (OTUs, H', and Chao1) were compared between treatments using the same mixed effects model and PROC GLIMMIX procedure as above. The α-diversity data was retrieved from alpha rarefaction data using QIIME2 View. These data were in matrices of α-diversity indices calculated in 10 iterations for each sample. As these iterations did not differ significantly, the last iteration for each sample was used for analysis. The β-diversity measured by weighted UniFrac distance was analyzed with pairwise PERMANOVA by QIIME2 to compare differences between treatment levels using pseudo-F test statistics and their p- and q-values (expected false positive and negative, respectively, rate in multiple hypothesis testing) (Anderson, 2017; Storey, 2003).

2.3. RESULTS

2.3.1. Soil and cover crop biomass properties

Table 2.1 shows the mean separation of the soil and CC biomass properties by CC and tillage treatments. As for soil properties, CEC did not have significant influence from neither tillage ($p = 0.43$) nor CC ($p = 0.63$). Soil pH was also not affected by neither tillage ($p = 0.72$) nor CC ($p = 0.74$). Likewise, SOM did not have significant relationship with tillage ($p = 0.34$) and CC ($p = 0.38$). While tillage did not have significant impact on soil NO3-N ($p = 0.85$), CC did have marginal effects ($p = 0.07$) where it halved under CarSar (0.85 mg kg^{-1}) compared to CcrShv (1.80 mg kg^{-1}) and CT (1.83 mg kg^{-1}). However, NH4-N did not have significant effects from tillage ($p = 0.50$) and CC (0.85). Soil P level also did not differ by tillage ($p = 0.74$) and CC ($p = 0.18$).

As for CC residue properties, the biomass C content did differ significantly by tillage ($p = 0.04$) and marginally by CC ($p = 0.08$). No-till had more residue C content (42.77%) than till (41.46%); CcrShv biomass had more C (42.85%) than CarSar (41.38%). However, CC biomass N content did not differ by tillage ($p = 0.14$) and CC ($p = 0.99$). The CC residue C:N ratio did not differ by tillage ($p = 0.58$) and CC ($p = 0.41$). The CC dry weight also did not vary by tillage ($p = 0.27$) and CC ($p = 0.33$)

2.3.2. Overall characterization of the soil microbiome

2.3.2.1. Bacteria

Overall, the bacterial community had more than 1.2 million 16S V4 region sequences clustered into 1832 different OTUs. The means of observed OTUs, H', and Chao1 are summarized in Table 2.2. Number of OTUs also did not differ by tillage ($p = 0.54$) and CC ($p = 0.38$). Neither tillage ($p = 0.57$) nor CC ($p = 0.27$) had significance on H'. Also, Chao1 was not affected by tillage ($p = 0.54$) and CC ($p = 0.37$).

The β-diversity based on weighted UniFrac distance and pairwise PERMANOVA between treatment levels showed some significant differences between tillage treatments (Table 2.3). Bacterial communities between T and NT showed significant pseudo-F of 4.325 ($p = 0.001$; $q = 0.001$). But they did not differ between CC treatments: CT to CarSar ($p = 0.296$; $q = 0.444$), CT to CcrShv ($p = 0.192$; $q = 0.444$), CarSar to CcrShv ($p = 0.774$; $q = 0.774$).

The most relatively abundant phylum across the samples was Proteobacteria (34.2%), followed by Actinobacteria (20.4%), Chloroflexi (9.7%), Acidobacteria (9.5%), and Bacteriodetes (8.5%). The most relatively abundant classes were Gammaproteobacteria (15.1%), Alphaproteobacteria (14.1%), Thermoleophilia (9.6%), Actinobacteria (8.9%), and Bacteroidia (8.4%). As shown in Figure 2.1, phyla Acidobacteria, Bacteroidetes, Chloroflexi, Gemmatimonadetes, and Proteobacteria responded significantly ($p = 0.0023$) to tillage effects, where Bacteroidetes and Proteobacteria were more abundant under tillage than no-till while the other three responded oppositely. Other microbial phyla, including fungi and archaea, did not have significant PCA and ANOVA results ($p > 0.1$, data not shown)

2.3.2.2. Fungi

The fungal community had 213,860 ITS region sequences clustered into 313 OTUs. Number of OTUs did not show significant relationships with tillage ($p = 0.26$) and CC ($p = 0.89$). Also, H' was not affected by tillage ($p = 0.11$) and CC ($p = 0.30$). Lastly, tillage ($p = 0.28$) and CC ($p = 0.90$) did not have significant effects on Chao1 (Table 2.2).

Fungal communities showed significant β-diversity among both tillage and CC treatments (Table 2.3). Between NT and T showed significant pseudo-F value of 2.782 ($p = 0.019$; $q = 0.019$). Between CC treatments, the pseudo-F value was 5.417 between CT and CarSar ($p = 0.002$; $q = 0.003$), 3.137 between CT and CcrShv ($p = 0.013$; $q = 0.013$), and 4.106 between CarSar and CcrShv ($p = 0.001$; $q = 0.003$).

The most abundant fungal phylum was Ascomycota (54.8%), followed by Basidiomycota (9.5%), and Mortierellomycota (4.6%). The most abundant identified classes were Dothideomycetes (20.3%), Sordariomycetes (14.3%), Leotiomycetes (8.8%), and Tremellomycetes (6.4%).

2.3.2.3. Archaea

The archaeal community had 13,272 archaeal 16S rRNA region sequences clustered into 19 OTUs. The mean of the number of archaeal OTUs did not differ by tillage ($p = 0.58$) and CC ($p = 0.98$). Tillage ($p = 0.73$) and CC ($p = 0.76$) also did not have significant impact on H'. Species richness by Chao1 was also unaffected by tillage (0.58) and CC (0.97) (Table 2.2).

Archaeal communities showed significant β-diversity among CC treatments (Table 2.3). Pseudo-F value between CT and CcrShv was 3.981 ($p = 0.01$; $q = 0.03$) while it was marginally significant between CT and CarSar with 2.901 ($p = 0.054$; $q = 0.081$); it was not significant between CarSar-CcrShv ($p = 0.437$; $q = 0.437$). The β-diversity did not vary by tillage ($p = 0.272$; $q = 0.272$). The archaeal community was dominated by phylum Thaumarchaeota (96.8%) and class Nitrososphaeria (95.8%).

2.3.3. Responses selected microbial taxa to cover crops and tillage treatments

2.3.3.1. Bacteria

Total of six PCs explained 49.4% of the variability in the selected 42 top-contributing bacterial OTUs. The PC1 had eigenvalue of 5.90 and explained 13.4% of the variability,

including positive loadings from seven OTUs belonging to genera *Cellulomonas*, *Solirubrobacter*, *Altererythrobacter*, *Massilia*, and the families *Archangiaceae*, *Burkholderiaceae*, and *AKYH767*; three OTUs in the genera *Gemmatimonadetes* and *Rhodocyclaceae*, and in the family *Gemmatimonadaceae* had negative loadings. The PC2 had eigenvalue of 4.48 and explained 10.2% of the variability, including positive loadings from four OTUs each belonging to the genus *Nocardioides*, the order C0119, the class TK10, and the family *SC-I-84*. The PC3 had eigenvalue of 3.77 and explained 8.6% of the variability, including positive loadings from three OTUs in the class Gitt-GS-136, and the families *Gemmatimonadaceae* and *Geminicoccaceae*. The PC4 had eigenvalue of 2.77 and explained 6.3% of the variability, including positive loadings from three OTUs each in the families *A4b* and *Gemmataceae*, and genus *Haliangium*. The PC5 had eigenvalue of 2.46 and explained 5.6% of the variability, including loadings from two OTUs in the families *SC-I-84* and *Opitutaceae*. The PC6 had eigenvalue of 2.34 and explained 5.3% of the variability, including positive loading from an OTU in the phylum Latescibacteria, and a negative loading from one in family *Gemmatimonadaceae*. This information is summarized in Table B.6.

The ANOVA tests performed on these bacterial PCs detected statistically significant CC and tillage effects (Table 2.4). The PC1 had significant tillage ($p = 0.046$) and CC ($p = 0.012$) main effects where mean scores reflected a contrasting responses of the microbial taxa in that PC1 under tillage (0.303) compared to no-till (-0.303) (Figure 2.2). Likewise, PC1 mean score was statistically different under CcrShv (0.539) compared to bare fallows (-0.526), with CarSar showing intermediate PC score (-0.013) (Figure 2.3). But PC1 did not have significant CC and tillage interaction effect ($p = 0.900$). The PC2 did not have significant tillage ($p = 0.724$), CC ($p = 0.378$), and interaction effects ($p = 0.277$). The PC3 did not have tillage main effect ($p = 0.232$) but had significant main effect from CC ($p < 0.001$), and CC and tillage interaction ($p = 0.026$) where lsmean PC scores were 0.59 for CT, 0.093 for CarSar, and -0.638 for CcrShv, each significantly different from each other (Figure 2.3); lsmean PC scores were higher under NT x CT (0.788), T x CarSar (0.514), and T x CT (0.392) than those of the rest which were negative (Figure 2.4). The PC4 did not have significant tillage effect ($p = 0.849$), but had marginal CC main effect ($p = 0.053$) and interaction effect ($p = 0.053$) where lsmean PC score was higher under CarSar (0.328) than CT (-0.440) (Figure 2.3); lsmean PC score was higher under CC with tillage (T x CarSar, 0.582; T x CcrShv, 0.373) than bare fallow with tillage (-0.866) (Figure 2.4).

The PC5 did not have effects from tillage (p = 0.120), CC (p = 0.137), and their interaction (p = 0.504). The PC6 was not affected by main effects of tillage (p = 0.922) and CC (p = 0.214), but their interaction did have significance (p = 0.006): lsmean PC scores of T x CarSar (0.479), T x CT (0.362), NT x CcrShv (0.287), and NT x CarSar (0.214) were significantly larger than those of NT x CT (-0.543) and T x CcrShv (-0.799) (Figure 2.4). Overall, these results amounted to 18 bacterial OTUs in four PCs responding significantly to CC and tillage treatments.

2.3.3.2. Fungi and Archaea

Total of seven PCs explained 49.1 % of the variability in the 36 selected top-contributing fungal OTUs. The PC1 had eigenvalue of 3.28 and explained 9.1% of the variability, including a positive loading from an OTU in the genus *Tetracladium*, and a negative loading from one in the genus *Penicillium*. The PC2 had eigenvalue of 2.90 and explained 8.1% of the variability, including positive loadings from *Trichoderma spirale*, *Saitozyma podzolica*, and an unknown phylum. The PC3 had eigenvalue of 2.59 and explained 7.2% of the variability, including positive loadings from two OTUs each in the family *Chaetomiaceae* and the class Agaricomycetes. The PC4 had eigenvalue of 2.46 and explained 6.8% of the variability, including positive loadings from two OTUs each in the genus *Ascochyta* and the class Agaricomycetes. The PC5 had eigenvalue of 2.36 and explained 6.6% of the variability, including a positive loading from an OTU in the order Xylariales, and a negative loading from one in the order Agaricales. The PC6 had eigenvalue of 2.2 and explained 6.1% of the variability, including a negative loading from *Minimedusa polyspora*. The PC7 had eigenvalue of 1.88 and explained 5.2% of the variability, including a positive loading from a *Plectosphaerella* OTU. This information is summarized in Table B.7.

The ANOVA tests on these PCs detected significant CC and tillage effects (Table 2.4). The PC1 had significant CC main effects (p = 0.029) where lsmean PC score was higher under CcrShv (0.417) than CarSar (-0.306) (Figure 2.3). The PC1 did not have significant tillage (p = 0.660) and interaction effects (p = 0.220). The PC2 had significant tillage main effect (p = 0.001) where lsmean PC score was higher under tillage (0.463) than no-till (-0.463) (Figure 2.2). There was no significant CC (p = 0.771) and interaction (0.681) effects on PC2. Next, the PC3 had significant tillage main effect (p = 0.008) where lsmean PC score was higher under NT (0.366) than T (-0.366) (Figure 2); CC also had marginal main effect (p = 0.078) where mean PC score

was higher under CT (0.430) than CarSar (-0.273) (Figure 2.3). PC3 did not have significant interaction effect ($p = 0.623$). The PC4 had significant CC main effect ($p = 0.005$) and interaction effect ($p = 0.041$) where lsmean PC score was higher under CarSar (0.727) than the rest which were negative (Figure 2.3); PC score was the highest under NT x CarSar (1.095), and T x CarSar (0.359) was significantly higher than NT x CT (-0.798) (Figure 2.4). The PC5 had significant tillage main effect ($p = 0.024$) where mean PC score was higher under T (0.351) than NT (-0.351) (Figure 2.2). PC5 did not have significant CC ($p = 0.248$) and interaction effects ($p = 0.238$). The PC6 did not have significant tillage ($p = 0.778$) and CC ($p = 0.739$) main effects but did have marginal interaction effects ($p = 0.081$). However, lsmean separation did not show significant differences among CC and tillage interactions. The PC7 did not have significant tillage ($p = 0.582$) and CC ($p = 0.564$) main effects but did have marginal interaction effect ($p = 0.060$), where lsmean PC score of T x CarSar (0.603) was significantly larger than those of T x CcrShv (-0.416) and NT x CarSar (-0.463) (Figure 2.4). These results amounted to 12 fungal OTUs in six PCs responding significantly to CC and tillage.

The five top contributing archaeal OTUs all belonged to the family *Nitrososphaeraceae*: an unidentified *Candidatus Nitrososphaera* OTU, and unidentified archaeal OTUs *SCA1154*, *SCA1158*, *SCA1166*, and *SCA1173*. The OTU belonging to *Ca. Nitrososphaera* did not have significant effects from CC ($p = 0.970$), tillage ($p = 0.970$), and their interaction ($p = 0.997$). The OTU of *SCA1154* was not affected by CC ($p = 0.290$), tillage ($p = 0.461$), and their interaction ($p = 0.164$). The OTU of *SCA1158* did not have significant effects from tillage ($p = 0.221$), CC ($p = 0.620$), and their interactions ($p = 0.240$). That of *SCA1166* also was not affected by tillage ($p = 0.258$), CC ($p = 0.301$), and their interactions ($p = 0.460$). Only the OTU of *SCA1173* had significant main effect from CC treatments ($p < 0.001$) where mean of this OTU was greater under CT (42.52) than CcrShv (17.04) and CarSar (9.77). This OTU did not have significant effects from tillage ($p = 0.188$) and interactions ($p = 0.949$). This information is summarized in Table 2.5.

2.4. DISCUSSION

2.4.1. Long-term tillage and cover crop effects on soil properties

As summarized in Table 2.1, soil properties did not differ significantly by CC and tillage treatments except for NO3-N, which more than halved under CarSar compared to the other CC

treatment and bare fallow control. Likewise, Behnke et al. (2020), which included this study's experimental site, reported that annual ryegrass CC decreased NO3-N by 9% compared to bare fallow, while CcrShv showed intermediate estimates. White et al. (2017) also found that grass CC decreased NO3-N more than legume CC. There are reports that soil NO3-N did not differ by CC types (García-González et al., 2018; Villamil et al., 2008), but meta-analyses on CC reduction of NO_3^- leaching suggest that grass CC generally are more effective at reducing soil NO_3^- (Basche et al., 2014; Tonitto et al., 2006). As for CcrShv, Perrone et al. (2020) estimated that hairy vetch CC can fix up to 136 kg ha^{-1} of N until suppression. This study's legume-grass CC rotation treatment seems to have maintained as much NO3-N as bare fallow partially by increasing the soil inorganic N through N-fixation. This surplus of soil inorganic N under legume CC would have also benefited the soil microbiome. Soil NH4-N may not have differed between treatments because crops preferentially utilize NO_3^- before NH_4^+, which also explains the lower NO3-N under CarSar (Yan et al., 2019).

As for soil pH, there is no evidence that CC affect the soil pH significantly enough to produce visible shifts in the soil microbial composition (Fernandez et al., 2016; Qi et al., 2020; Sharma et al., 2018a; Tiecher et al., 2017). This was consistent with this study's results that saw no significant change in soil pH between CC treatments. Likewise, tillage effect on soil pH is uncertain, as Blevins et al. (1983) found that no-till lowered soil pH of unlimed soil but increased that of limed soil; recent studies found conflicting effects as well (Li et al., 2020; Tiecher et al., 2017). Also, specifically for this study's experimental site, its superactive Mollisols had high CEC that leads to more base saturation, and therefore more buffering capacity against soil pH changes (Lumbanraja and Evangelou, 1991). Although numerous studies emphasized that the soil pH dictates the soil microbial composition, this does not seem to be a factor in this system with no significant CC and tillage effects (Chamberlain et al., 2020; Qi et al., 2020; Xu et al., 2020). The high-CEC soil of the present study may also explain why treatment effects on CEC did not have statistical significance. Like in this study, other reports did not find significant CC and tillage effects on soil CEC (Behnke et al., 2020; Medeiros et al., 2017; Sharma et al., 2018b). Haruna and Nkongolo (2019) reported that CEC increased with cereal rye CC, but the difference was less than 1 cmol kg^{-1}, smaller than the differences in the present study. Indeed, their soil order was Entisols with only little more than half the CEC of that of the present study's Mollisols, whose high CEC makes the soil less sensitive to changes (Soil Survey staff, 1992).

Also, this result is not surprising considering that other properties like soil pH and SOM that are highly associated with CEC did not differ by treatments in this study.

As for SOM, a meta-analysis by Daryanto et al. (2018) on comprehensive effects of CC reported that biomass input from cover cropping generally increases SOC, an important component of SOM. While the means of SOM was indeed larger with CC treatments than bare fallow, this difference was not statistically significant. According to Poeplau and Don (2015), organic C input from CC residues can incite priming effect that compel soil microbes, especially organotrophs, to actively consume SOM for resource. Therefore, priming effect from CC residues could have contributed to insignificant differences between CC treatments and bare fallow despite the SOM input from long-term CC treatments. Moreover, cash crop residues are another sources of SOM, perhaps even more so than from CC. The difference could have been statistically insignificant because cash crop residues provided much of the SOM and dwarfed the differences from CC residues.

Meanwhile, CC biomass properties did not vary significantly between the two CC treatments (Table 2.1). This is likely because the biomass samples were taken during vegetative state of CC when both grass and legume CC have higher N content. The biomass C content was the only exception that differed between both CC and tillage treatments with statistical significance. However, the difference in biomass C was only 3%, which may not have practical significance, and could have been results of minor differences in the C:N ratios of the CCs at the time of sampling.

2.4.2. Cover crop and tillage treatments significantly shifted indicator soil microbes

2.4.2.1 Bacteria

This study showed that bacterial OTUs in PC1, 3, and 4 responded significantly to CC and tillage effects (Figure 2.2 and 2.3). Bacterial PC1 had seven out of ten OTUs that were more abundant under CcrShv than bare fallow, while the remaining three OTUs behaved oppositely. Also, the same seven OTUs were more abundant under tillage while the other three did so under no-till. Interestingly, the seven OTUs that increased with CcrShv have been described as chemoorganotrophs or organic matter decomposers that belong to phyla Actinobacteria, Bacteriodetes, and Proteobacteria (Chen et al., 2017; Garrity et al., 2015; Huntley et al., 2010; Kim et al., 2007; Lang et al., 2015; Li et al., 2014; Whitman, 2015; Yuan et al., 2017; Zheng et

al., 2018). These three phyla have been recognized largely as copiotrophic groups that prefer easily degradable organic matter and adapted to high nutrient environments (Fierer and Jackson, 2006).

The influx of labile sources of C and N from fresh CC residues after suppression is a widely recognized CC benefit (Hubbard et al., 2013; Pascault et al., 2013; Sharma et al., 2018a). Moreover, tillage breaks and incorporates the CC residues into the soil so that microbes have better contact with the biomass, thereby increasing the decomposition rate (Lupwayi et al. 2004; Lynch et al. 2016). Even before suppression, CC exude nutrient rich compounds from their roots that also have been credited to explain the increase in copiotrophic microbial guilds under CC (Coskun et al. 2017; Alahmad et al. 2019; Romdhane et al. 2019). Indeed, studies have found that these three PC1 phyla increase with CC and tillage, and explained their responses as sensitivity to influx of readily available nutrients from CC residues and exudates (Romdhane et al. 2019; Alahmad et al. 2019; Pascault et al. 2013; Sharma-Poudyal et al. 2017). As for the intermediate responses of these seven PC1 decomposers to CarSar, both CC treatments had residues with C:N lower than 24:1 (Table 2.1), which below this ratio the decomposition of these residues compels the microbes to mineralize N and create a surplus of inorganic N (Sainju et al., 2005). In both CarSar and CcrShv, the soil microbes would have utilized the inorganic N to further decompose parts of the CC residues with higher C:N (Sainju et al., 2005). But CcrShv had legume CC phase that fixed N for even more soil inorganic N availability (Perrone et al., 2020), which nitrified into the greater NO3-N content in CcrShv (Table 2.1). This extra soil N availability from legume CC could have further benefited the decomposers, making them most abundant under CcrShv. Overall, the consistency between the past reports on these seven PC1 OTUs as copiotrophic decomposers and this study's results of their sensitivity to soil nutrient availability presents these OTUs as great candidates of microbial indicators representing the copiotrophic decomposer guild. Moreover, the sensitivity of this microbial guild to CC residues and tillage may gauge how actively the soil microbiome can decompose easily degradable components of the CC residues.

On the contrary, three OTUs from PC1 and another three from PC3 that belong to the phyla Chloroflexi, Gemmatimonadetes, and Proteobacteria were significantly more abundant under bare fallow than CcrShv (Figure 2.3). First, PC1 and PC3 each included a different OTUs from family *Gemmatimonadaceae*, in phylum Gemmatimonadetes. As explained above, the lack

of CC residues and exudates in bare fallow makes the soil less rich in C than CC treatments, which compels the organotrophic microbes to use the native SOM to procure the necessary energy sources (Pascault et al., 2013). Pascault et al. (2013) found that Gemmatimonadetes correlated negatively to C inputs to soil and that it dominated the SOM-degrading community, demonstrating that this phylum is oligotrophic and better adapted to scavenging complex C substrates from SOM under low nutrient environment. Considering the consistency with this past finding, these PC1 and PC3 Gemmatimonadetes OTUs may be used as indicators of the oligotrophic microbial guilds that respond conversely to availability of labile C sources than the copiotrophic decomposers.

In addition to soil C availability, all six OTUs in PC1 and PC3 could have responded to the CC and tillage effects on soil aeration and moisture. The family *Gemmatimonadaceae* is reported to be aerobes adapted to lower soil moisture and extreme environments (DeBruyn et al., 2011; Fawaz, 2013; Wang et al., 2014; Zeng et al., 2015). As for the two OTUs in phylum Chloroflexi, class Gitt-GS-136 in PC3 is not well documented, but Anaerolineae in PC1 is a class of strictly anaerobic chemoorganotrophs capable of fermentation (Yamada et al., 2006; Yang et al., 2020). Likewise, reports on the two Proteobacteria OTUs suggest that they either possess fermentative metabolism (PC1 family *A21b*) (McIlroy et al., 2015) or have advantage in anaerobic conditions as facultative anaerobes (PC3 family *Geminicoccaceae*) (Proença et al., 2018). While this study did not measure soil aeration and moisture, there are several reports that no-till and bare fallow make the soil environment more anaerobic and drier. For example, Villamil et al. (2008) found that cereal rye and hairy vetch decreased bulk density, which leads to less porosity for air and water. Also, Demir and Isik (2019) found that no-till reduced soil water availability. Martínez et al. (2016) found that no-till decreased soil gas transport, thereby making the soil more anaerobic. The anaerobic and dry conditions put strong selection pressures on the soil microbiome and increase relative abundances of anaerobic (Degrune et al., 2017; Linn and Doran, 1984) or more stress tolerant microbes (Schmidt et al., 2018). Overall, above reports on these six PC1 and PC3 OTUs represent the oligotrophic microbes that occupy different ecological niche from the seven copiotrophic OTUs from PC1. Therefore, these OTUs have the potential to be used as microbial indicators for stress-tolerant microbial guilds.

The OTUs in bacterial PC4 had statistically significant CC and tillage interaction effects (Figure 2.4). Their abundances increased under CC with tillage compared to bare fallows with

tillage, and no-till showing intermediate results. First, *Haliangium* is an aerobic predatory genus (Huntley et al., 2010; Wang et al., 2020) and members of its parent order Myxococcales can survive in oligotrophic environments through predation (Huntley et al., 2010). As described earlier, CC and tillage enhance the labile soil nutrient availability, and increase the microbial abundance as demonstrated in a meta-analysis by Kim et al. (2020). As a dominant predatory group, Myxococcales and its *Haliangium* OTUs will not directly benefit from the CC residues but indirectly from the increasing prey population that multiply on nutrient-rich soil environment under CC and tillage (Table 2.1). Consistent with this study's results, Jin et al. (2019) found that relative abundances of Myxoccocales and *Haliangium* increased with legume-grass mixed CC. Also, Gao et al. (2017) reported that wheat CC increased the relative abundance of Myxococcales. Likely, *Haliangium* is sensitive to the prey population controlled by CC and tillage effects on the soil nutrients, and is a good candidate as microbial indicator of predatory bacteria groups.

Another PC4 bacterial OTUs, in the family *Gemmataceae,* is a strictly aerobic chemoorganotroph (Kulichevskaya et al., 2017) and its parent phylum Planctomycetes has been found to increase with CC (Alahmad et al., 2019; Verzeaux et al., 2016). The OTUs in family *A4b* belongs to the class Anaerolineae, previously described as anaerobic chemoorganotroph, which Jin et al. (2019) reported to have increased with CC as well. These two OTUs are probably decomposers and one of them in *Gemmataceae* is likely to be sensitive to changes in the soil nutrients and aeration from CC and tillage in similar way as the PC1 copiotrophic decomposers. However, as described, *A4b* is likely an anaerobe, which is expected to be more competitive under no-till and bare fallow like its sister taxa in PC1 (Yamada et al., 2006; Yang et al., 2020). Because not much is known about the family *A4b*, this study can only speculate that this genus has different sensitivity to soil nutrient and aeration as its sister taxa in PC1. Indeed, Romdhane et al. (2019) demonstrated that each genera of Chloroflexi, parent to Anaerolineae, responded contrastingly to soil properties like SOC and soil N.

Overall, this study identified bacterial OTUs that responded to CC and tillage consistently with their known ecological and physiological characteristics. These responses were mostly attributed to bacterial sensitivity to the soil nutrient altered by CC and tillage, which was also suspected by similar past studies (Alahmad et al., 2019; Romdhane et al., 2019; Pascault et al., 2013). In addition to soil nutrients, this study found that CC and tillage effects on soil

aeration and moisture could also have differential selective pressure on the bacterial community. Each of the bacterial groups found in this study well represented various microbial guilds including copiotrophic and oligotrophic decomposers, predators, and stress-tolerant microbes. This study suggests that these bacterial indicators can provide a snapshot of the microbial properties like capability to decompose different types of nutrient sources, or responses to the indicators of soil health. Moreover, meta-analysis by Kim et al. (2020) questioned how much soil microbial abundance contributed to the greater microbial activity under CC. Results of the present study suggest that compositional changes that favored decomposers adapted to fresh CC residues could translate to differential production of enzymes such as β-glucosidase studied by Kim et al. (2020) that is involved in cellulose decomposition. This alludes that compositional changes under CC may be responsible for increased activity, but also raises the possibility that enzymes produced by microbial guilds not favored by CC may respond less or even negatively to the practice.

2.4.2.2. Archaea and Fungi

All five indicator archaeal OTUs identified in this study belonged to the family *Nitrososphaeraceae*, one of the most represented ammonia oxidizing archaea (AOA) and important initiators of nitrification (Kerou and Schleper, 2016; Taylor et al., 2010). Like in this study, the dominance of archaeal population by *Nitrososphaeraceae* was also observed by Somenahally et al. (2018). Also similar to this study's results, Segal et al. (2017) did not find significant tillage effects on archaea. Of these five indicator archaea, only the unidentified archaeon *SCA1173* responded significantly to CC and tillage where it was more abundant under bare fallow than CC. Ammonia-oxidizing archaea like *SCA1173* are known to dominate in acidic soils (He et al., 2012), and Zhalnina et al. (2013) found that OTU *Ca. Nitrososphaera*, parent to one of the OTUs in the present study, is positively correlated with soil NH_3^+ level. As NH_3^+ is the substrate for ammonia oxidation, the same is expected to apply to *SCA1173* as well (Hirsch and Mauchline, 2015). Ammonia oxidizing microbes gain energy primarily from ammonia oxidation, unlike typical organotrophic decomposers like bacteria in PC1 and PC4 whom organic C is the energy source (Prosser and Nicol, 2012). Without CC residues nor root exudates, bare fallow soil is C-limited than those of CC treatments, thereby favoring lithotrophic AOA over organotrophic decomposers. Indeed, nitrifiers including AOA have been found to thrive in lower

energy environment like bare fallow (Valentine, 2007), which explains why *SCA1173* was more abundant in bare fallow. Overall, this result suggests that cover cropping may decrease nitrification from archaea by promoting competitiveness in other microbial groups with fresh organic C sources. Also, considering that *Nitrososphaeraceae* dominates the archaeal population in agricultural soil, *SCA1173* is also a strong candidate for indicator archaea representing both AOA and the archaeal community.

Fungal species in this study responded to CC and tillage by two possible factors: morphology and soil nutrient. First, the most striking consistency found in fungal responses was the relationship between fungal morphology and tillage. Abundances of fungal species in PC2, 3, and 5 differentially responded to tillage according to their morphology (Figure 2.2). Hyphal, or possibly hyphal, fungi including *T. spirale*, and three species each belonging to the family Chaetomiaceae, class Agaricomycetes, and order Agaricales increased under no-till, while those more abundant under tillage were yeasts (*S. podzolica*) or conidia producing species (order Xylariales) (Aliyu et al., 2019; Baiyee et al., 2019; Hibbett et al., 2014; Smith et al., 2003; Walther et al., 2005; Wang et al., 2019). This result is consistent with the findings of Sharma-Poudyal et al. (2017) where hyphal *Humicola* species (Chaetomiaceae) increased with no-till. Besides other explanation that the authors have discussed, they also emphasized that physical disruption of the hyphal structure can be detrimental for fungi (Sharma-Poudyal et al., 2017). The peculiar consistency between fungal morphology and their responses to tillage gives weight to this past speculation that fungal morphology is a major factor in sensitivity to tillage.

Second, similar to bacteria, CC and tillage impact on the soil nutrient may be a sensitive factor for the fungal community. Fungi are mostly organotrophic decomposers, which is especially true for the phyla Ascomycota and Basidiomycota that dominated this study's fungal community (Wang et al., 2016). Indeed, fungal genera in PC1, 3, and 4 include decomposer species, for example, *Penicillium* and *Tetracladium* in PC1 (Klaubauf et al., 2010; Yadav et al., 2018), family Chaetomiaceae in PC3, and *Ascochyta* in PC4 and (Osono, 2003; Sharma-Poudyal et al., 2017). The class Agaricomycetes, in PC3 and PC4, includes species adapted to a wide variety of niches including decomposers, pathogens, and mutualists. Further taxonomic identification is required to determine how its species in this study relate to CC effects (Hibbett et al., 2014). Sharma-Poudyal et al. (2017) suggested that crop residues that are broken and incorporated into soil by tillage promote fungal groups that prefer easily decomposed nutrient

sources, indicating that fungal community share similar sensitivity towards soil nutrients as bacteria. This observation can also be applied to CC residues, which provide easily degraded nutrient sources unlike bare fallow. Of the six fungal species except those of Agaricomycetes, PC1 species were more abundant under CcrShv while those of PC4 were so under CarSar. This is somewhat consistent with Finney et al. (2017) that found higher non-AMF fungi abundance under either hairy vetch or cereal rye CC than bare fallow. These four fungal species may be copiotrophic species adapted to easily degradable nutrient sources, and responded positively to the nutrient influx from CC residues. Likewise, the two fungal species in Chaetomiaceae and Agaricomycetes in PC3 increased under both bare fallow and no-till, much like the oligotrophic bacteria in PC1 (Figure 2.1 and 2.2). Indeed oligotrophic fungi are not rare in the kingdom (Wainwright et al., 1993), and Agaricomycetes is known saprotrophic fungal group with ability to degrade complex lignin, which is a characteristic of an oligotrophic microbe (Fester et al., 2014). Therefore, this contrast between potential copiotrophic and oligotrophic fungal groups to CC and tillage supports sensitivity to soil nutrient as a possible explanation for fungal responses to the treatments.

Overall, this study's analysis demonstrated that the responses of the fungal species to CC and tillage could be affected by two factors: fungal morphology and soil nutrient. Unfortunately, the data of this study alone were not enough to confirm the contributions of these factors, especially because of the vague identities of some of the selected fungal species. Nonetheless, this study present these fungal species as potential fungal indicators and encourage revealing their identities and characteristics with further studies.

2.4.3. Cover crop and tillage shifted β- but not α-diversity

In light of the significant CC and tillage effects on the abundances of the microbial groups and bacterial phyla, α- and β-diversity responded contrastingly to the treatments (Table 2.2 and 2.3). As for β-diversity, bacterial community composition differed significantly by tillage treatments only (Table 2.3). This might be reflecting how only tillage had the significant impact on the relative abundances of bacterial phyla (Figure 2.1). Past works also have shown significant tillage (Wang et al., 2020; Xia et al., 2019) and CC (Frasier et al., 2016) effects on bacterial β-diversity. Meanwhile, fungal β-diversity differed significantly between both tillage and CC treatments. Indeed, studies like Schmidt et al. (2019), Detheridge et al. (2016), and

Piazza et al. (2019) found that fungal community shifted significantly with both CC and tillage. Unlike bacteria, fungal β-diversity may have responded to both tillage and CC because the number of fungal OTUs was only a sixth of that of bacteria, therefore each fungal species has more statistical impact on the β-diversity and responses of indicator fungi selected in this study to treatments were enough to be reflected on the β-diversity. Contrarily, bacterial β-diversity was not consistent with the responses of selected indicator bacteria because the overall bacterial richness was too great for their behaviors to shift the bacterial composition. Likewise, archaeal β-diversity only differed significantly between bare fallow and both CC treatments, comparable to the response of the indicator archaeon *SCA1173* to CC treatments. With only 19 archaeal OTUs identified, perhaps one influential OTU was enough to shift the archaeal β-diversity. These results strongly suggest that indicator OTUs' influence on β-diversity is biased by the microbial richness of the samples, which is determined by a study's capability to detect microbial OTUs. Therefore, like bacteria, significant responses of indicator microbes may not reflect β-diversity in reality, and more complete detection of the fungal and archaeal OTUs may change β-diversity results. Nonetheless, the β-diversity results confirmed that the CC and tillage effects, described in the discussion of each microbial PCs, can shift the soil microbial community composition.

Contrary to β-diversity, α-diversity indices did not respond to any of the treatments. This can be interpreted that CC and tillage changed the composition of the microbial community but not necessarily the richness and evenness of the microbiome. Meta-analysis by Kim et al. (2020) that compiled traditional α-diversity indices reported that CC effect sizes on these measures were statistically positive, but very small (average 2.5% increase) compared to those of microbial abundance (27%) and activity (22%). Considering this small global effect size, the present study's result is not as surprising as an individual study. Moreover, this meta-analysis also found that CC effect size on H' was not significant under chemical CC termination, which is consistent with no difference in α-diversity between CC treatments in the present study's results that also chemically terminated the CC (Kim et al., 2020). Unlike CC effects, there has not been a research synthesis effort for tillage effects on the soil microbial diversity, but individual studies showed that diversity was higher under no-till or reduced tillage (Dorr de Quadros et al., 2012; Legrand et al., 2018; Schmidt et al., 2018). However, the increases in these studies were also small, for example 2.2% in H' by Legrand et al. (2018) and 12% in Schmidt et al. (2018). Overall, the results of α- and β-diversities indicate that certain changes made by CC and tillage

like soil nutrient and aeration may shift the soil microbial composition, but the shift may not affect the community's diversity and richness.

2.4.4. Limitation and future direction

This study's results provided an account of CC and tillage effects on the taxonomic compositions of the soil microbiome. This means that this study only tells half the story which needs to be complemented by functionality data. Therefore, further research is needed to assess the changes in various functional genes or enzymes to confirm that the community compositional changes indeed translate into soil microbial ecological services. Moreover, this study analyzed the absolute abundances of the microbes, which is still valid (Props et al. 2017; Tang 2019), but analyzing the relative abundances may reveal different microbial indicators. Another shortcoming of this study is that many of the microbial indicators were unidentified at lower taxonomic ranks, which obscures their roles and characteristics in the microbiome. This study, however, identified potentially important microbial groups so that future research effort can be directed to them. On that note, this study should also be reproduced to confirm whether this study's indicator microbes and their sensitivities are consistently found in different times or regions.

2.4.5. Implications on nutrient loss reduction

Overall, the soil microbial responses and soil properties results suggest that grass CC have better potential to reduce nutrient loss than legume CC. This study found compelling evidence that legume cover cropping and tillage favor the copiotrophic microbial guilds that are at the first line of decomposition that targets easily degradable parts of the crop residues. This is likely achieved by the legume CC fixing N for themselves and leaving more soil inorganic N for the microbial demands while tillage incorporates and breaks the residues for better microbial access. With the extra N, these microbial groups will rapidly decompose CC and even high C:N cash crop residues. This pushes the soil N cycle towards N mineralization because CC residues suppressed at vegetative state have lower C:N, thereby increasing the soil inorganic N (Sainju et al., 2005). This was evident in this study's results where soil NO3-N was similar between CcrShv and bare fallow, but significantly lower for CarSar with grass-only CC (Table 2.1). While this abundance of soil inorganic N may benefit subsequent cash crop yield (Marcillo and

Miguez, 2017), it also means more soil N subject to loss via nitrification and denitrification, which again is supported by the higher NO3-N under CcrShv (Hirsch and Mauchline, 2015). This sensitivity of microbial decomposers to the synergy between legume CC and tillage tells the microbial side of the story of why grass CC have been found to be more effective in reducing soil nutrient loss (Daryanto et al., 2017; Gonzalez, 2018; Thapa et al., 2018). Considering that CcrShv in this study was a rotation of grass and legume CC, the potential soil N loss under legume-only CC system could be even greater than what is found in this study. Therefore, this study proposes that combining no-till with grass CC is the best cover cropping system for reducing the soil N loss; likewise, legume CC need to be managed with no-till to minimize nutrient loss.

2.5. CONCLUSION

Understanding CC and tillage impact on the soil microbiome and ultimately soil nutrient loss requires unraveling how the complex soil microbiome respond to these practices. This metagenomics study is a part of this effort which sought to identify indicator microbes that can represent important microbial guilds responsible for the soil nutrient dynamics. This study used statistical approaches including bootstrap forest partitioning and PCA to select the sensitive microbes, and used generalized mixed effects model to quantify their responses to CC and tillage. This resulted to identifying 18 bacterial, 12 fungal, and an archaeal indicator OTUs that represented microbial guilds that each responded differently to changes in the soil environment by CC and tillage. These responses translated into shifts in the soil microbial composition but not the community richness and diversity. This study also found evidence that tillage is detrimental for hyphal fungi, and that bare fallow favors nitrifying ammonia-oxidizing archaea. The increase of copiotrophic decomposers under legume-grass rotation CC coincided with higher soil NO3-N than grass-only CC. This may be a combined result of increased soil inorganic N from N-fixation by legume CC and the increased copiotrophic decomposers compelled to mineralize N from CC residues with low C:N ratio. The greater soil inorganic N increases the risk of soil nutrient loss. Therefore, while this study's results need to be confirmed by soil microbial biomass and functionality data, they strongly suggest grass CC and no-till as better option for soil nutrient loss reduction.

TABLES AND FIGURES

Table 2.1. Means and standard errors of the mean (in parentheses) of the six soil properties and four cover crop biomass properties by tillage and cover crop rotation treatment in 2017 sampling. Six soil properties were soil cation exchange capacity (CEC, cmol kg-1), soil pH (pH), soil organic matter content (SOM, %), soil nitrate content (NO3-N, mg kg-1), soil ammonium content (NH4-N, mg kg-1), and soil phosphorus content (P, mg kg-1). The four cover crop biomass properties are biomass carbon (C, %) and nitrogen (N, %) contents, their ratio (C:N), and biomass dry weight per hectare (Dry Weight, Mg ha^{-1}). Factors in bold indicate significant treatments effect ($p<0.1$) and those within the same row with same letters indicate are not statistically different ($\alpha=0.05$).

	Tillage[a]			Cover crop[b]			
Soil properties	NT	T	p-value	CT	CarSar	CcrShv	p-value
CEC (cmol/kg)	21.78 (2.49)	24.04 (2.49)	0.43	24.07 (2.57)	21.59 (2.57)	23.06 (2.57)	0.63
pH	5.95 (0.16)	5.90 (0.16)	0.72	5.96 (0.16)	5.94 (0.16)	5.88 (0.16)	0.74
SOM (%)	3.76 (0.15)	3.83 (0.15)	0.34	3.73 (0.15)	3.81 (0.15)	3.85 (0.15)	0.38
NO3-N (mg/kg)	1.52 (0.23)	1.47 (0.23)	0.85	**1.83 (0.33) a**	**0.85 (0.33) b**	**1.80 (0.33) a**	**0.07**
NH4-N (mg/kg)	12.62 (0.81)	11.90 (0.81)	0.50	11.84 (1.06)	12.68 (1.06)	12.27 (1.06)	0.85
P (mg/kg)	5.42 (1.51)	5.79 (1.51)	0.74	6.38 (1.56)	4.31 (1.56)	6.13 (1.56)	0.18
Cover crop biomass							
C (%)	**42.77 (0.50) a**	**41.46 (0.47) b**	**0.04**		**41.38 (0.53) b**	**42.85 (0.59) a**	**0.08**
N (%)	2.93 (0.16)	2.65 (0.15)	0.14		2.79 (0.16)	2.79 (0.17)	1
C:N	15.43 (0.71)	15.91 (0.66)	0.58		15.24 (0.69)	16.10 (0.78)	0.41
Dry Weight (Mg/ha)	1.80 (0.10)	1.93 (0.09)	0.27		1.81 (0.09)	1.92 (0.10)	0.33

[a]NT, no-till; T, chisel tillage.
[b]CT, bare fallow control; CarSar, annual ryegrass following both corn and soybean; CcrShv, cereal rye following corn, hairy vetch following soybean.

Table 2.2. Means and standard errors of the mean (SEM) of the three α-diversity parameters of taxa bacteria, fungi, and archaea by tillage and cover crop rotation treatment from soil DNA sampling. The α-diversity measures were number of observed OTUs (OTUs), Chao 1 Richness Index (Chao1), and Shannon's Diversity Index (H'). Factors in bold indicate significant treatments effect ($p<0.1$) and those within the same row with same letters indicate are not statistically different ($\alpha=0.05$).

		OTUs			Chao1			H'		
Taxa	Treatment[a]	Mean	SEM	p-value	Mean	SEM	p-value	Mean	SEM	p-value
Bacteria	T	206.04	10.14	0.54	206.35	10.19	0.54	7.30	0.07	0.57
	NT	210.11			210.47			7.33		
	CarSar	202.46			202.72			7.27		
	CcrShv	213.62	10.62	0.38	214.20	10.68	0.37	7.37	0.08	0.27
	CT	208.15			208.31			7.32		
Fungi	T	32.94	1.52	0.26	32.97	1.55	0.28	4.19	0.08	0.11
	NT	30.37			30.45			4.01		
	CarSar	31.95			31.97			4.26		
	CcrShv	31.90	1.56	0.89	31.96	1.58	0.90	4.02	0.12	0.30
	CT	31.11			31.20			4.03		
Archaea	T	11.60	0.65	0.58	11.60	0.65	0.58	3.24	0.08	0.73
	NT	11.16			11.16			3.20		
	CarSar	11.45			11.45			3.24		
	CcrShv	11.41	0.72	0.98	11.43	0.73	0.97	3.24	0.09	0.76
	CT	11.29			11.26			3.17		

[a]NT, no-till; T, chisel tillage; CT, bare fallow control; CarSar, annual ryegrass following both corn and soybean; CcrShv, cereal rye following corn, hairy vetch following soybean.

Table 2.3. The β-diversity of each taxa by tillage and cover crop treatments based on pariwise PERMANOVA on weighted UniFrac distance. Column "Levels" indicates the two treatment levels being compared, and the column "Sample size" indicates the sample size for the particular comparison. The pseudo-F measures the significance of the UniFrac distance between the two treatment levels. The p-value measures the probability of Type I error and the q-value measures the Type II error. Comparisons with significant pseudo-F values (p-value & q-value < 0.10) are in bold.

Taxa	Treatment	Levels[a]	Sample size	pseudo-F	p-value	q-value
Bacteria	Tillage	NT-T	142	4.33	**0.001****	**0.001****
	Cover crop	CT-CarSar	95	1.08	0.296	0.444
		CT-CcrShv	94	1.30	0.192	0.444
		CarSar-CcrShv	95	0.77	0.774	0.774
Fungi	Tillage	NT-T	129	2.78	**0.019****	**0.019****
	Cover crop	CT-CarSar	83	5.42	**0.002****	**0.003****
		CT-CcrShv	87	3.14	**0.013****	**0.013****
		CarSar-CcrShv	88	4.11	**0.001****	**0.003****
Archaea	Tillage	NT-T	130	1.12	0.272	0.272
	Cover crop	CT-CarSar	86	2.90	**0.054***	**0.081***
		CT-CcrShv	87	3.98	**0.010****	**0.030****
		CarSar-CcrShv	87	0.80	0.437	0.437

[a]NT, no-till; T, chisel tillage; CT, bare fallow control; CarSar, annual ryegrass following both corn and soybean; CcrShv, cereal rye following corn, hairy vetch following soybean.
*, p-value<0.1; **, p-value<0.05

Table 2.4. The results of analysis of variance (ANOVA) for the effects of cover cropping (CC), tillage (Till), and their interaction (CC x Till) on top contributing Principal Components (PCs) comprised of bacterial and fungal indicator OTUs selected by JMP®. The provability values for the ANOVA for the effects of treatments and the degrees of freedom (df) are shown on the top rows. The results of mean (lsmeans) separation for CC, Till, and CC x Till and their standard errors of the mean (SEM) are presented at the bottom. Relationships that had both significant ANOVA results (α=0.1) and mean separation results (α=0.05) are bolded. Separated means for in a column with like letters are not significantly different.

		Bacteria						Fungi						
		PC1	PC2	PC3	PC4	PC5	PC6	PC1	PC2	PC3	PC4	PC5	PC6	PC7
Treatments	df	p-value						p-value						
Tillage	1	**0.046**	0.724	0.232	0.849	0.120	0.922	0.660	**0.001**	**0.008**	0.635	**0.024**	0.778	0.582
Cover crop	2	**0.012**	0.378	**<0.001**	**0.053**	0.137	0.214	**0.029**	0.771	**0.078**	**0.005**	0.248	0.739	0.564
CC x Till	2	0.900	0.277	**0.026**	**0.053**	0.504	**0.006**	0.220	0.681	0.623	**0.041**	0.238	0.081	**0.060**
Mean separation[a]														
	NT	**-0.303 b**	0.050	-0.139	-0.030	-0.233	-0.014	0.085	**-0.463 b**	**0.366 a**	0.054	**-0.351 b**	-0.040	-0.074
	T	**0.303 a**	-0.050	0.139	0.030	0.233	0.014	-0.085	**0.463 a**	**-0.366 b**	-0.054	**0.351 a**	0.040	0.074
	SEM	0.192	0.306	0.240	0.215	0.241	0.213	0.300	0.188	0.187	0.190	0.236	0.202	0.224
	CT	**-0.526 b**	-0.183	**0.59 a**	**-0.440 b**	0.312	-0.090	**-0.111 ab**	0.149	**0.430 a**	**-0.528 b**	0.280	-0.131	0.130
	CarSar	**-0.013 ab**	0.024	**0.093 b**	**0.328 a**	0.101	0.346	**-0.306 b**	-0.052	**-0.273 b**	**0.727 a**	-0.178	-0.012	0.070
	CcrShv	**0.539 a**	0.160	**-0.683 c**	**0.112 ab**	-0.413	-0.256	**0.417 a**	-0.097	**-0.157 ab**	**-0.200 b**	-0.102	0.142	-0.200
	SEM	0.225	0.308	0.248	0.235	0.284	0.255	0.276	0.255	0.229	0.252	0.261	0.247	0.260
	NT x CT	-0.759	-0.190	**0.788 a**	**-0.013 ab**	-0.062	**-0.543 bc**	0.001	-0.452	0.753	**-0.798 c**	-0.105	-0.323	**0.224 ab**
	NT x CarSar	-0.317	0.295	**-0.329 b**	**0.073 ab**	0.013	**0.214 ab**	-0.058	-0.488	0.269	**1.095 a**	-0.265	-0.359	**-0.463 b**
	NT x CcrShv	0.167	0.044	**-0.878 b**	**-0.149 ab**	-0.650	**0.287 ab**	0.313	-0.448	0.077	**-0.136 bc**	-0.684	0.560	**0.016 ab**
	T x CT	-0.292	-0.177	**0.392 a**	**-0.866 b**	0.685	**0.362 a**	-0.223	0.750	0.108	**-0.257 bc**	0.665	0.062	**0.035 ab**
	T x CarSar	0.291	-0.248	**0.514 a**	**0.582 a**	0.190	**0.479 a**	-0.553	0.384	-0.815	**0.359 b**	-0.091	0.336	**0.603 a**
	T x CcrShv	0.912	0.275	**-0.488 b**	**0.373 a**	-0.176	**-0.799 c**	0.520	0.254	-0.392	**-0.264 bc**	0.480	-0.276	**-0.416 b**
	SEM	0.315	0.365	0.298	0.332	0.342	0.327	0.350	0.326	0.324	0.302	0.333	0.350	0.348

[a]NT, no-till; T, chisel tillage; CT, bare fallow control; CarSar, annual ryegrass following both corn and soybean; CcrShv, cereal rye following corn, hairy vetch following soybean.

Table 2.5. The results of analysis of variance (ANOVA) for the effects of cover cropping (CC), tillage (Till), and their interaction (CC x Till) on the absolute abundances of top contributing archaeal indicator OTUs selected by JMP®. The provability values for the ANOVA for the effects of treatments and the degrees of freedom (df) are shown on the top rows. The results of mean (lsmeans) separation for CC, Till, and CC x Till and their standard errors of the mean (SEM) are presented at the bottom. Relationships that had both significant ANOVA results (α=0.1) and mean separation results (α=0.05) are bolded. Separated means for in a column with like letters are not significantly different.

		Archaea				
		Ca. Nitrososphaera	SCA1154	SCA1158	SCA1166	SCA1173
Factors	df	p-value				
Tillage	1	0.675	0.461	0.221	0.258	0.188
Cover crop	2	0.237	0.290	0.620	0.315	**<0.001**
CC x Till	2	0.391	0.164	0.240	0.470	0.949
Mean separation[a]						
	NT	9.110	7.972	6.000	33.583	19.264
	T	11.030	5.653	11.861	25.375	26.958
	SEM	3.096	2.439	3.456	6.551	4.975
	CT	4.670	5.792	11.792	34.813	**42.521 a**
	CarSar	13.810	4.063	6.604	30.458	**9.771 b**
	CcrShv	11.730	10.583	8.396	23.167	**17.042 b**
	SEM	3.792	3.058	4.068	7.086	5.740
	NT x CT	4.417	3.292	4.542	44.125	38.042
	NT x CarSar	16.250	5.292	4.750	30.667	5.250
	NT x CcrShv	6.667	15.333	8.708	25.958	14.500
	T x CT	4.917	8.292	19.042	25.500	47.000
	T x CarSar	11.375	2.833	8.458	30.250	14.292
	T x CcrShv	16.792	5.833	8.083	20.375	19.583
	SEM	5.362	3.977	5.261	8.927	7.585

[a]NT, no-till; T, chisel tillage; CT, bare fallow control; CarSar, annual ryegrass following both corn and soybean; CcrShv, cereal rye following corn, hairy vetch following soybean.

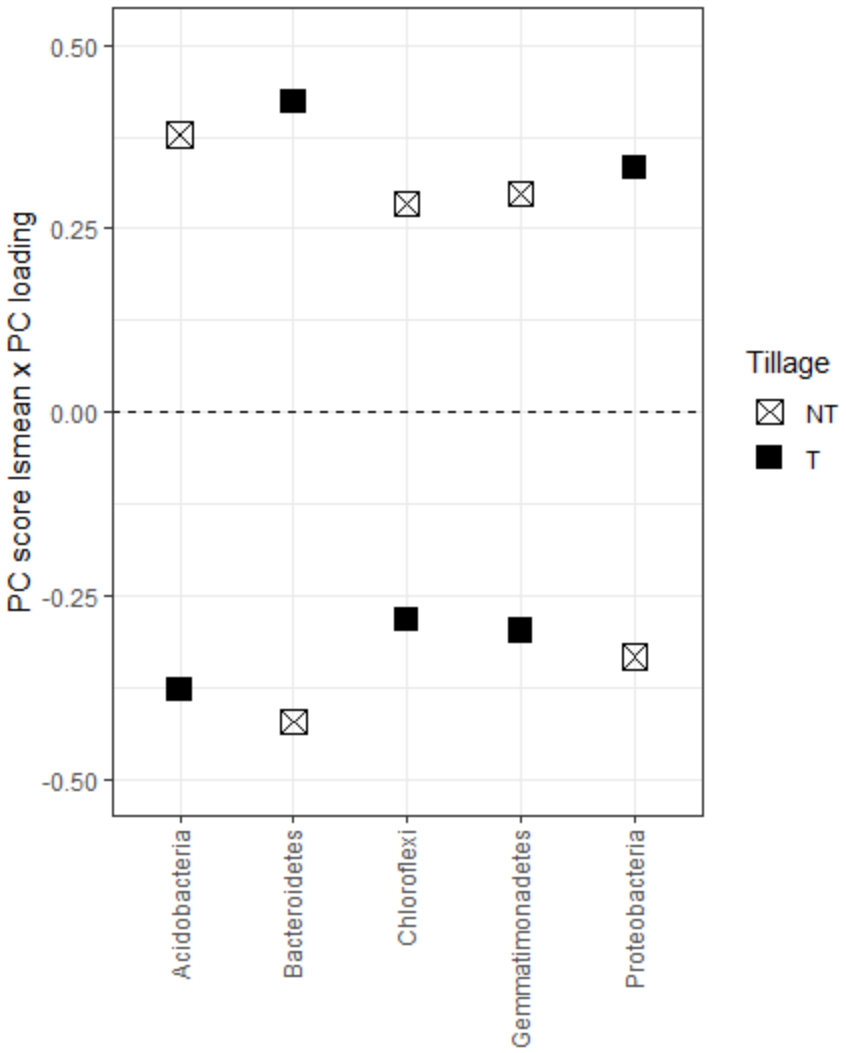

Figure 2.1. Visual representation of the PCA and mean separation on PC scores of the relative abundance of bacterial phyla whose PC scores differed significantly by tillage treatment: no-till (NT) and chisel tillage (T). Filled squares represent the mean of PC score of chisel tillage multiplied by PC loading of each phylum; crossed squares represent those of no-till. Within each phylum, square of higher Y-axis value indicates that the relative abundance of the phylum was larger with the corresponding tillage treatment.

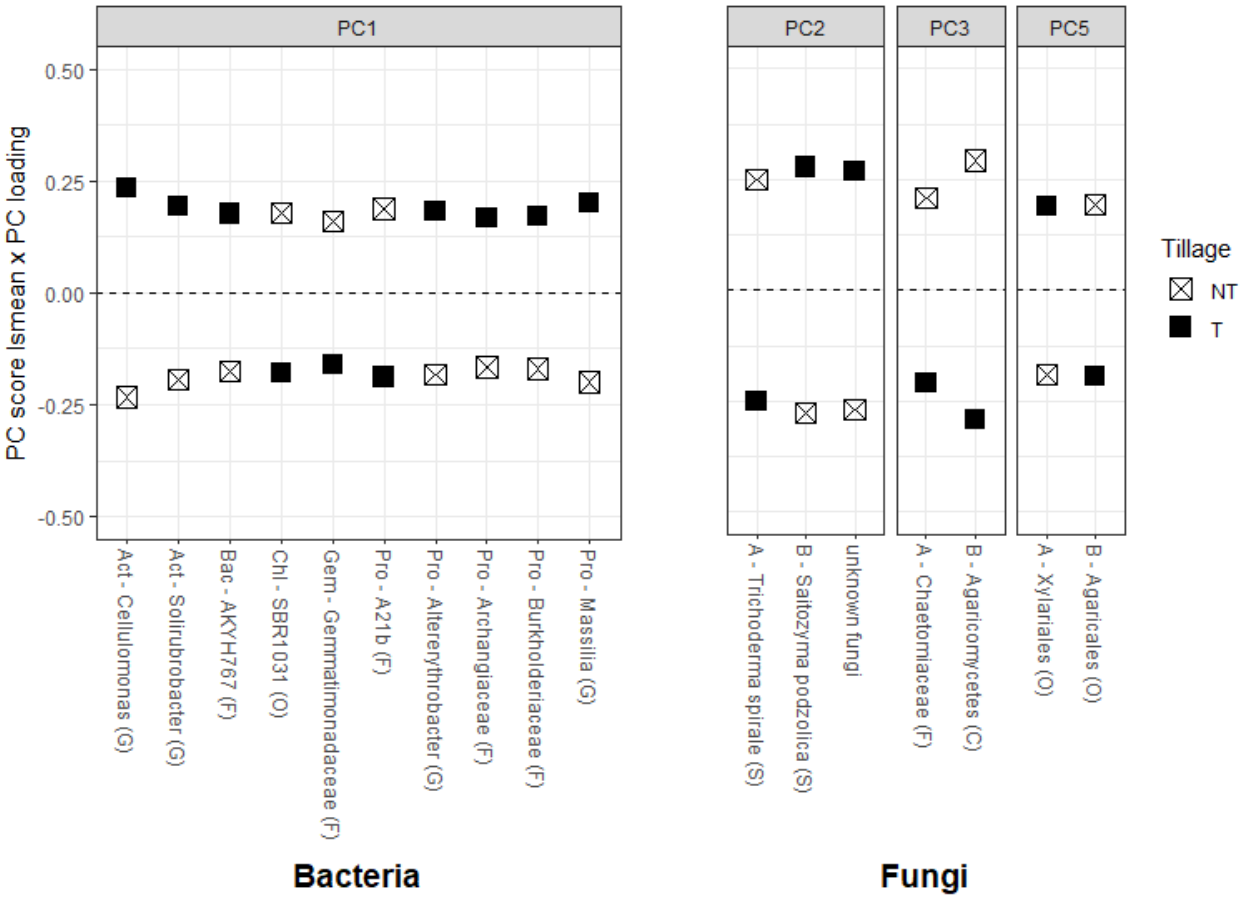

Figure 2.2. Visual representation of the PCA and mean separation on PC scores of the absolute abundance of the indicator bacterial and fungal species by tillage treatment: no-till (NT) and chisel tillage (T). Gray boxes at the top indicates the principal component (PC) that the species are grouped into. Each squares represent the mean of PC score of the corresponding treatment level for that PC multiplied by PC loading of each species; filled square for T, and crossed squares for NT. Within each species, square of higher Y-axis value indicates that the absolute abundance of the species was larger with the corresponding tillage treatment. The taxonomy of the species in X-axis is indicated as the three-letter acronym of the phylum of the species, followed by the name its lowest identified taxonomic rank, and the letter in parentheses indicates that rank. Act, Actinobacteria; Bac, Bacteroidetes; Chl, Chloroflexi; Gem, Gemmatimonadetes; Pro, Proteobacteria; A, Ascomycota; B, Basidiomycota; C, Class; O, Order; F, Family; G, Genus; S, Species.

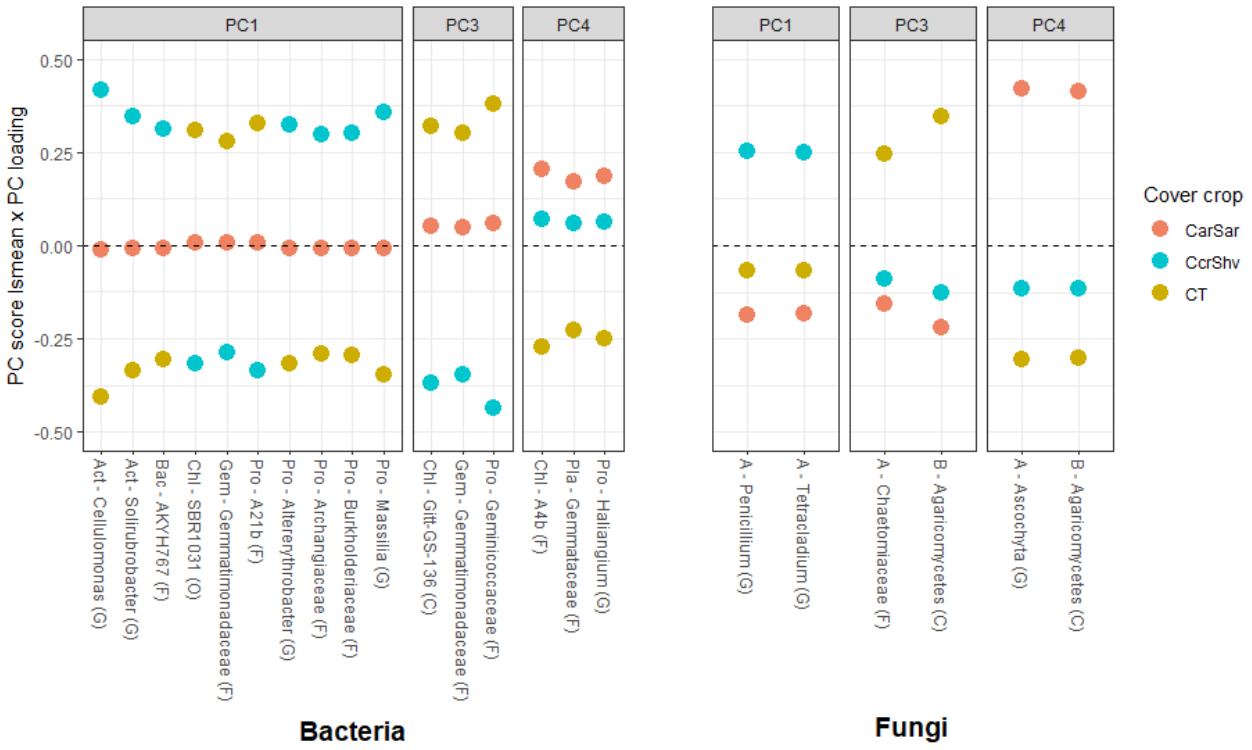

Figure 2.3. Visual representation of the PCA and mean separation on PC scores of the absolute abundance of the indicator bacterial and fungal species by cover crop treatment: bare fallow control (CT), annual ryegrass following both corn and soybean (CarSar), and cereal rye following corn, hairy vetch following soybean (CcrShv). Gray boxes at the top indicates the principal component (PC) that the species are grouped into. Each dots represent the mean of PC score of the corresponding treatment level for that PC multiplied by PC loading of each species; red for CarSar, blue for CcrShv, and yellow for CT. Within each species, dots of higher Y-axis value indicate that the absolute abundance of the species was larger with the corresponding tillage treatment. The taxonomy of the species in X-axis is indicated as the three-letter acronym of the phylum of the species, followed by the name its lowest identified taxonomic rank, and the letter in parentheses indicates that rank. Act, Actinobacteria; Bac, Bacteroidetes; Chl, Chloroflexi; Gem, Gemmatimonadetes; Pro, Proteobacteria; Pla, Planctomycetes; A, Ascomycota; B, Basidiomycota; C, Class; O, Order; F, Family; G, Genus; S, Species.

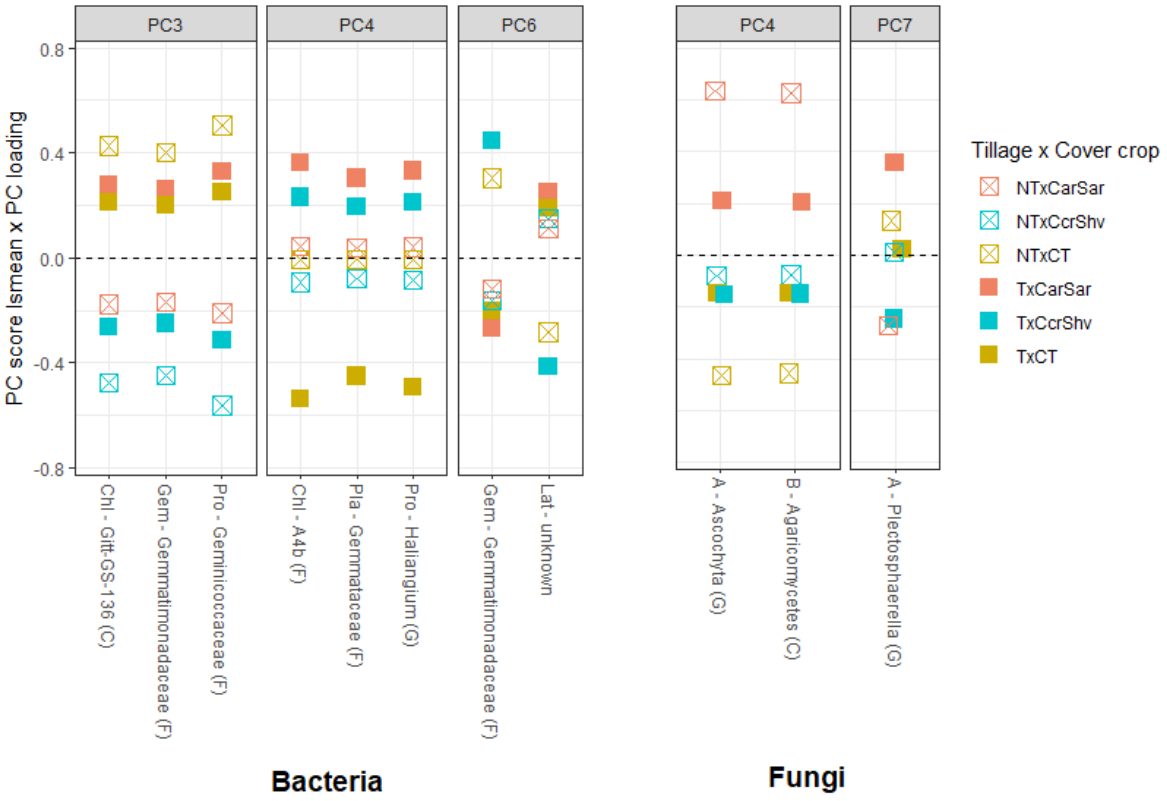

Figure 2.4. Visual representation of the PCA and mean separation on PC scores of the absolute abundance of the indicator bacterial and fungal species by interaction of cover crop and tillage treatments: no-till (NT), chisel tillage (T) interacting (x) with bare fallow control (CT), annual ryegrass following both corn and soybean (CarSar), or cereal rye following corn, hairy vetch following soybean (CcrShv). Gray boxes at the top indicates the principal component (PC) that the species are grouped into. Each squares represent the mean of PC score of interaction level for that PC multiplied by PC loading of each species; crossed box for NT, filled box for T, red for CarSar, blue for CcrShv, and yellow for CT. Within each species, squares of higher Y-axis value indicate that the absolute abundance of the species was larger with the corresponding tillage treatment. The taxonomy of the species in X-axis is indicated as the three-letter acronym of the phylum of the species, followed by the name its lowest identified taxonomic rank, and the letter in parentheses indicates that rank. Act, Actinobacteria; Bac, Bacteroidetes; Chl, Chloroflexi; Gem, Gemmatimonadetes; Pro, Proteobacteria; Pla, Planctomycetes; Lat, Latescibacteria; A, Ascomycota; B, Basidiomycota; C, Class; O, Order; F, Family; G, Genus; S, Species.

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APPENDIX A: META-ANALYSIS DATABASE REFERENCES

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APPENDIX B: SUPPLEMENTARY TABLES AND FIGURES

Table B.1. Lists of terms used to search for relevant primary literature. For each CC species, each column were combined with "AND", and all the terms within each Response Variables, Methods, and Condition were combined with "OR".

Cover Crop Species	Response Variables	Methods	Conditions
("avena sativa" OR oat)	Shannon	qPCR	soil
("brassica juncea" OR "forage turnip" OR "oriental mustard" OR "brown mustard")	CFU or "colony forming unit"	"community level physiological profile*"	microbi*
("brassica napus" OR rape)	Chao1	metabarcoding	
("brassica oleracea" OR kale)	"relative abundance"	DGGE	
("brassica rapa" OR turnip)	OTU or "operational taxonomic unit"	"16S rRNA"	
("Kummerowia striata" OR "common lespedeza")	qCO2 or "metabolic quotient"	16S	
("Lolium multiflorum" OR ryegrass)	MBN	rRNA	
("lotus corniculatus" OR "birdsfoot trefoil")	"substrate untilization"	"internal transcribed spacer" OR ITS	
("medicago sativa" OR alfalfa)	NLFA	ESV	
("melilotus albus" OR "white sweetclover")	"soil enzyme activity"	ASV	
("Melilotus officinalis" OR "yellow sweetclover")	FDA OR "fluorescein diacetate"	"enzyme assay"	
("phleum pratense" OR timothy)	Simpson	pyrosequencing	
("secale cereale" OR rye)	MBC	CLPP	
("securigera varia" OR crownvetch)	PLFA		
("trifolium ambiguum" OR "kura clover")			
("Trifolium hybridum" OR "alsike clover")			
("Trifolium incarnatum" OR "crimson clover")			
("trifolium pratense" OR "red clover")			
("trifolium repens" OR "white clover")			
("Trifolium vesiculosum" OR "arrowleaf clover")			
("triticum aestivum" OR wheat)			

Table B.1. (cont.)

("vicia sativa" OR "common vetch" OR vetch)
("vicia villosa" OR "hairy vetch")
Medicago
Sorghum
"cover crop*" OR "green manure*" OR "living mulch*"

Table B.2. Lists of SM parameters reported by the database and their brief description. The 13 parameters with at least 30 observations are in bold.

SM Parameter	Description
FDA	Fluorescein diacetate (FDA)
CFU	**number of Colony Forming Units (CFU) (count/g soil)**
MBC	**Microbial Biomass Carbon (microgram/g)**
MBN	**Microbial Biomass Nitrogen (microgram/g)**
amoA	number of ammonia-oxidizing organism amoA gene (counts/g soil)
Chao1	**Chao 1 = number of species + (number of singletons)^2/2(number of doubletons)**
H'	**Shannon diversity index H' = $-\sum_{i=1}^{n} p_i ln p_i$ (p_i = relative abundance of species *i*)**
ACE	ACE diversity index
OTU	**Operational Taxonomic Unit (counts)**
S	**Genetic richness based on number of unique amplicons identified (S) (counts)**
J	**Pielou's eveness index ($\frac{H'}{\ln(\text{Genetic Richness})}$)**
1-D	**Simpson's diversity index = $1 - \sum_{i=1}^{k} \frac{n_i^2}{N}$ where N is total number of organisms, n_i is the number of organisms of species *i*, and k is the number of species**
Shannon's H for functional diversity	functional Shannon's index from community level physiological profiling (CLPP) or other measure of diversity based on functional diversity
AWCD	Average Well Color Development
cis11	number of AMF biomarker cis11
FAME	number of fatty acid methylated esters
NAGase	Activity of enzyme NAGase
β-glucosidase	**Activity of enzyme β-glucosidase (BG) (nmol/g/hr; microgram/g/hr)**
β-glucosaminidase	Activity of enzyme β-glucosaminidase

Table B.2. (cont.)

α-galactosidase	Activity of enzyme α-galactosidase
Phosphatase	**Activity of enzyme phosphatase (Phos) (nmol/g/hr; microgram/g/hr)**
CBH	Activity of B-D-1,4-glucosidase
TAP	Activity of tyrosine aminopeptidase
PO	Activity of phenol oxidase
PER	Activity of peroxidase
CO2-C	**soil respiration measured by nanogram of CO2 produced in an hour from gram of soil**
Urease	activity of enzyme Urease
Dehydrogenase	activity of enzyme Dehydogenase
Celullase	activity of enzyme Celullase
Glutaminase	activity of enzyme glutaminase
Arylsulfatase	activity of enzyme arylsurfatase
L-asparaginase	activity of enzyme L-Asparaginase
Invertase	activity of enzyme Invertase
LPS	Labile polysaccharide, measure for activity
TPS	total polysaccharide, measure for activity
EE-GSRP	easily extractable glomalin-related soil protein, AMF activity
T-GRSP	total glomalin-related soil protein, AMF activity
Ala	Activity of enzyme aminolevulinic acid
protease	activity of protease
LAP	activity of leucyl aminopeptidase
AAP	activity of alanine aminopeptidase
BX	activity of b-xylosidase
CB	activity of cellobiohydrolase
NEEA	N acquisition extracellular enzyme activity
PLFA	**Phospholipid Fatty Acid (PLFA) abundance of taxa (microgram PLFA/ g soil?)**
AUC	Area under the curve (AUC) of the C utilization profiles on BIOLOG Ecoplates
Gene copies	number of gene copies of sample microbes relative to the treatments

Table B.3. Lists of agricultural moderators, their brief descriptions, levels for discrete moderators, and their brief descriptions.

Moderators	Description	Levels	Description
Climate	Climate of the experimental site classified by Koppen Classification (Arnfield, 2019). Only the first classification was used because some levels had too few observations for valid comparisons when including secondary classification	A	Tropical
		B	Arid/Semi-arid
		C	Temperate
		D	Continental
Soil Order	Classification of the soil of the experimental site in USDA Soil Taxonomy equivalents.	Alfisols	
		Entisols	
		Inceptisols	
		Mollisols	
		Oxisols	
		Ultisols	
CC Termination	Spring cover crop suppression methods grouped into either mechanical or chemical termination methods	mechanical	cover crop is terminated with mechanical means like undercutting or mulching
		chemical	cover crop is terminated with chemical means like glyphosate
CC Type	Type of cover crops largely grouped into four levels	G	grass cover crops including wheat, rye, and oat
		L	legume cover crops such as soybean and clovers
		M	mixture of cover crops in G and L
		O	any other cover crops species not in G or L; includes Brassicaceae
Tillage Type	Type of tillage largely grouped into conservational and conventional	CONS	Study reports either no-till or reduced-tillage
		CONV	Study reports any tillage methods other than no-till or reduced-tillage

Table B.3. (cont.)

Sample Timing	The timing of soil sampling characterized by which stage in rotation the samples were collected	CC	Soil samples taken during CC growth	
		post-CC	soil samples taken after CC termination but before half-way into cash crop plating	
		pre-cash	soil samples taken after half-way before planting cash crops but before planting cash crop	
		cash	soil samples taken during cash crop growth	
		post-cash	soil samples taken after cash crop harvest	
N Fertilizer	Binary factor indicating whether N fertilizer was applied	Yes	N fertilizer was applied at some point of the rotation	
		No	no N fertilizer was applied at any point of the rotation	
N Fertilizer Rate	Continuous factor with rate at which N fertilizer was applied in annual average of a rotation			kg/ha/yr
Soil pH	Continuous factor of soil pH at the beginning of the experiment			
CC Duration	Continuous factor of number of days between CC planting and termination			days
Sample Depth	Continuous factor of the depth at which soil samples were taken in centimeter			cm

Table B.4. Model coefficients (intercept: β_0, slope: β_1), p-values of the slope, and adjusted R^2 of linear models between continuous moderators and SM parameters, calculated by rma function in metafor package. Significant p-value (<0.05) and R^2 values (>0.10) are in bold.

Factor	Parameter	β0	β1	p-value	n	R^2
N Fertilizer rate (kg/ha/yr)	CFU	0.041	0.006	**0.000**	22	**0.230**
	MBC	0.273	0.000	0.318	339	0.000
	MBN	0.267	-0.002	0.051	174	0.014
	PLFA	0.079	0.000	**0.030**	352	0.014
	BG	0.095	0.002	**0.001**	128	**0.105**
	Phos	0.079	-0.002	0.145	24	1.000
	CO2-C	0.363	0.000	0.975	34	0.000
	OTU	0.031	0.000	0.686	32	0.000
	Chao 1	0.121	-0.001	**0.010**	49	**0.357**
	H'	0.024	0.000	0.336	149	0.002
	S	0.311	0.000	**0.040**	14	**1.000**
	J	0.153	0.000	**0.003**	14	**0.405**
	1-D	0.003	0.000	0.778	36	0.000
Soil pH	CFU	-4.508	0.799	0.061	36	**0.102**
	MBC	0.040	0.032	0.343	296	0.003
	MBN	0.158	0.014	0.796	195	0.000
	PLFA	-0.050	0.022	0.761	78	0.000
	BG	-0.478	0.093	**0.027**	109	0.000
	Phos	-0.188	0.055	0.558	35	0.000
	CO2-C	0.423	-0.027	0.894	16	0.000
	OTU	0.350	-0.042	**0.003**	32	**0.650**
	Chao 1	-0.001	0.008	0.652	77	0.000
	H'	0.054	-0.004	0.635	139	0.000
	1-D	0.045	-0.005	**0.000**	58	0.000
CC Duration (days)	CFU	1.999	-0.008	0.450	36	0.009
	MBC	0.209	0.000	**0.007**	370	0.032

Table B.4. (cont.)

	MBN	0.281	0.000	0.930	178	0.000
	PLFA	0.320	-0.001	0.062	406	0.011
	BG	0.079	0.000	**0.001**	155	0.063
	Phos	-0.048	0.001	0.240	53	0.015
	CO2-C	0.304	0.001	0.116	28	**0.512**
	OTU	0.136	0.000	**0.001**	30	NA
	Chao 1	0.066	0.000	0.401	75	0.000
	H'	0.020	0.000	0.831	137	0.000
	1-D	0.000	0.000	0.755	56	NA
Soil Sample Depth (cm)	CFU	0.801	-0.047	**0.000**	54	**0.348**
	MBC	0.147	0.004	**0.000**	408	**0.128**
	MBN	0.223	0.001	0.509	197	0.000
	PLFA	0.059	0.005	0.052	436	0.007
	BG	0.095	0.005	0.331	155	0.008
	Phos	-0.117	0.029	0.122	60	0.021
	CO2-C	0.368	-0.001	0.881	39	0.000
	OTU	0.081	-0.003	**0.021**	32	**0.374**
	Chao 1	0.081	-0.002	0.063	78	0.000
	H'	0.016	0.001	0.388	199	0.001
	S	-0.003	0.003	0.249	57	0.000
	J	-0.001	0.001	0.372	50	0.000
	1-D	0.001	0.000	0.630	61	**0.306**

Table B.5. Summary of field practices from Savoy, Illinois, throughout the duration of the experiment.

Field Event Type	2012	2013	2014	2015	2016	2017
Broadcast seeding date of cover crop	10/1/2012	9/16/2013	9/17/2014	9/17/2015	9/7/2016	N/A
Biomass sampling	N/A	5/6/2013	4/25/2014	4/27/2015	4/25/2016	4/11/2017
Spring soil sampling	N/A	6/21/2013	5/5/2014	4/30/2015	4/29/2016	4/21/2017
Cover crop suppression	N/A	5/7/2013	5/20/2014	4/29/2015	5/19/2016	4/12/2017
Spring tillage of corn T plots[1]	N/A	6/5/2013	5/20/2014	5/21/2015	5/24/2016	5/17/2017
Planting date of corn[2]	4/12/2012	6/6/2013	5/21/2014	5/22/2015	5/25/2016	5/18/2017
Harvest of corn	N/A	10/29/2013	11/3/2014	10/9/2015	10/28/2016	10/16/2017
Spring tillage of soybean T plots[1]	N/A	6/5/2013	5/20/2014	5/21/2015	5/24/2016	5/17/2017
Planting date of soybean[2]	4/12/2012	6/6/2013	5/21/2014	5/22/2015	5/25/2016	6/6/2017
Harvest of soybean	N/A	10/29/2013	10/29/2014	10/12/2015	10/31/2016	6/17/2017
Fall soil sampling	11/16/2012	12/12/2013	12/15/2014	11/4/2015	11/16/2016	N/A

[1] Tillage was conducted with a chisel plow 20-25 cm deep in plots designated as tilled; no-till received zero tillage.

[2] Pre-plant N fertilizer was applied at a rate of 190 kg N ha^{-1}.

[3] No fertilization

N/A, not applicable.

Table B.6. List of bacterial principal components (PC) comprised by indicator species that contributed at least 5% of the variability in the data and with eigenvalue of at least 1.

Principal Component	Eigenvalue	Proportion (%)	Loadings	Kingdom	Phylum	Class	Order	Family	Genus	Species
PC1	5.90	13.40	0.77	Bacteria	Actinobacteria	Actinobacteria	Micrococcales	*Cellulomonadaceae*	*Cellulomonas*	-
			0.64		Actinobacteria	Thermoleophilia	Solirubrobacterales	*Solirubrobacteraceae*	*Solirubrobacter*	-
			0.58		Bacteroidetes	Bacteroidia	Sphingobacteriales	*AKYH767*	-	-
			-0.59		Chloroflexi	Anaerolineae	SBR1031	-	-	-
			-0.53		Gemmatimonadetes	Gemmatimonadetes	Gemmatimonadales	*Gemmatimonadaceae*	-	-
			0.60		Proteobacteria	Alphaproteobacteria	Sphingomonadales	*Sphingomonadaceae*	*Altererythrobacter*	-
			0.55		Proteobacteria	Deltaproteobacteria	Myxococcales	*Archangiaceae*	-	-
			-0.62		Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	*A21b*	-	-
			0.66		Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	*Burkholderiaceae*	*Massilia*	-
			0.56		Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	*Burkholderiaceae*	-	-
PC2	4.48	10.20	0.53		Actinobacteria	Actinobacteria	Propionibacteriales	*Nocardioidaceae*	*Nocardioides*	-
			0.70		Chloroflexi	Ktedonobacteria	C0119	-	-	-
			0.56		Chloroflexi	TK10	-	-	-	-
			0.54		Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	*SC-I-84*	-	-
PC3	3.77	8.60	0.54		Chloroflexi	Gitt-GS-136	-	-	-	-
			0.51		Gemmatimonadetes	Gemmatimonadetes	Gemmatimonadales	*Gemmatimonadaceae*	-	-
			0.64		Proteobacteria	Alphaproteobacteria	Tistrellales	*Geminicoccaceae*	-	-
PC4	2.77	6.30	0.62		Chloroflexi	Anaerolineae	SBR1031	*A4b*	-	-
			0.52		Planctomycetes	Planctomycetacia	Gemmatales	*Gemmataceae*	-	-
			0.57		Proteobacteria	Deltaproteobacteria	Myxococcales	*Haliangiaceae*	*Haliangium*	-
PC5	2.46	5.60	0.53		Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	*SC-I-84*	-	-
			0.55		Verrucomicrobia	Verrucomicrobiae	Opitutales	*Opitutaceae*	-	-
PC6	2.34	5.30	-0.56		Gemmatimonadetes	Gemmatimonadetes	Gemmatimonadales	*Gemmatimonadaceae*	-	-
			0.52		Latescibacteria	-	-	-	-	-

Table B.7. List of fungal principal components (PC) comprised by indicator species that contributed at least 5% of the variability in the data and with eigenvalue of at least 1. The last row shows the list of archaeal indicator species.

Principal Component	Eigenvalue	Proportion (%)	Loadings	Kingdom	Phylum	Class	Order	Family	Genus	Species
PC1	3.28	9.10	0.61	Fungi	Ascomycota	Eurotiomycetes	Eurotiales	Aspergillaceae	*Penicillium*	-
			0.60		Ascomycota	Leotiomycetes	Helotiales	Helotiaceae	*Tetracladium*	-
PC2	2.90	8.10	-0.54		-	-	-	-	-	-
			0.60		Ascomycota	Sordariomycetes	Hypocreales	Hypocreaceae	*Trichoderma*	*T. spirale*
			0.58		Basidiomycota	Tremellomycetes	Tremellales	Trimorphomycetaceae	*Saitozyma*	*S. podzolica*
PC3	2.59	7.20	0.57		Ascomycota	Sordariomycetes	Sordariales	Chaetomiaceae	-	-
			0.80		Basidiomycota	Agaricomycetes	-	-	-	-
PC4	2.46	6.80	0.58		Ascomycota	Dothideomycetes	Pleosporales	Didymellaceae	*Ascochyta*	-
			0.57		Basidiomycota	Agaricomycetes	-	-	-	-
PC5	2.36	6.60	0.54		Ascomycota	Sordariomycetes	Xylariales	-	-	-
			-0.55		Basidiomycota	Agaricomycetes	Agaricales	-	-	-
PC6	2.20	6.10	-0.54		Basidiomycota	Agaricomycetes	Cantharellales	Cantharellales fam Incertae sedis	*Minimedusa*	*M. polyspora*
PC7	1.88	5.20	0.59		Ascomycota	Sordariomycetes	Glomerellales	Plectosphaerellaceae	*Plectosphaerella*	-
				Archaea	Thaumarchaeota	Nitrososphaeria	Nitrososphaerales	*Nitrososphaeraceae*	*Candidatus Nitrososphaera*	- SCA1154 SCA1158 SCA1166 SCA1173

Table B.8. The forward and reverse sequences of the primers used to amplify sequences.

Gene	Primer name	Sequence (5' to 3')	Length	Reference
Fungal ITS3-4	ITS3F	GCATCGATGAAGAACGCAGC	462	Crawford et al. (2011)
	ITS4R	TCCTCCGCTTATTGATATGC		
Bacteria 16S (V4)	V4-515F	GTGYCAGCMGCCGCGGTAA	252	Fierer et al. (2005)
	V4-806R	GGACTACVSGGGTWTCTAAT		
Archaea 16S	Arch349F	GTGCASCAGKCGMGAAW	528	Colman et al. (2015)
	Arch806R	GGACTACVSGGGTATCTAAT		

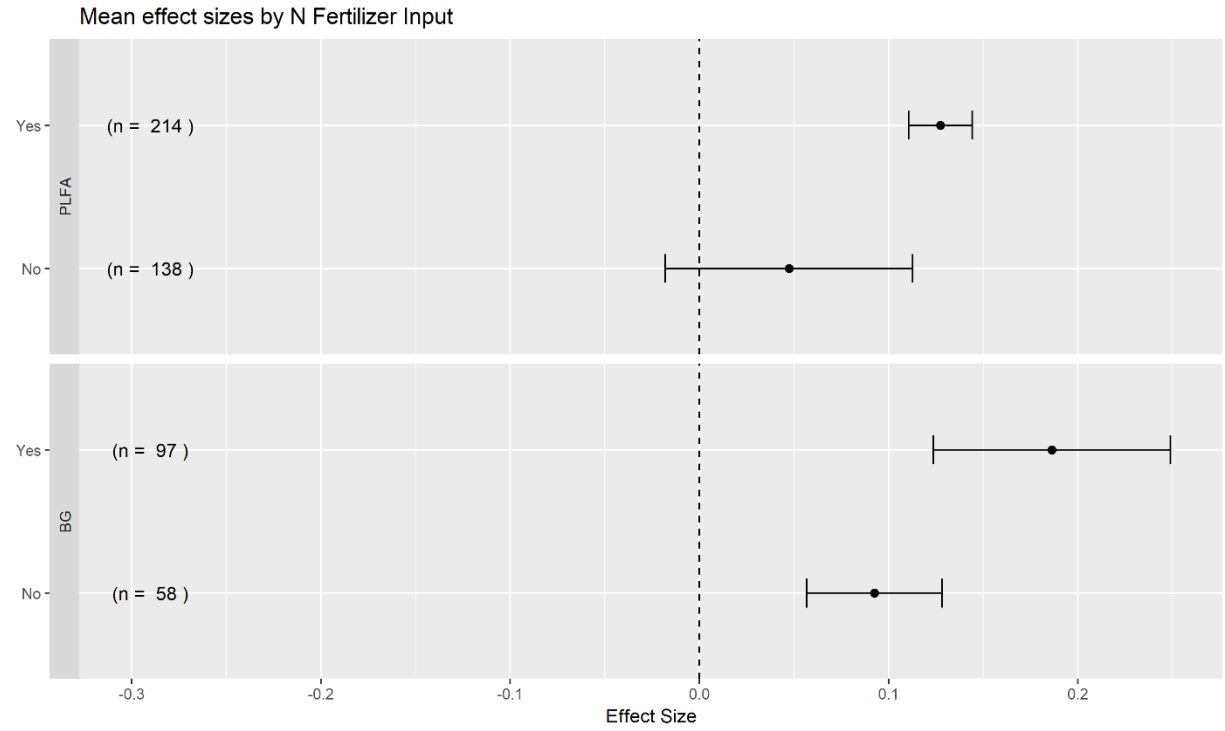

Figure B.1. Forest plots of interactions between phospholipid fatty acid (PLFA) and β-glucosidase activity (BG) and N fertilizer input. Number of observations per level is noted in parentheses. The CIs of two levels in both SM parameters slightly overlapped. Levels (y-axis) with means larger than zero indicate that CC increased the SM parameter at those levels, and decreased if the means smaller than zero.

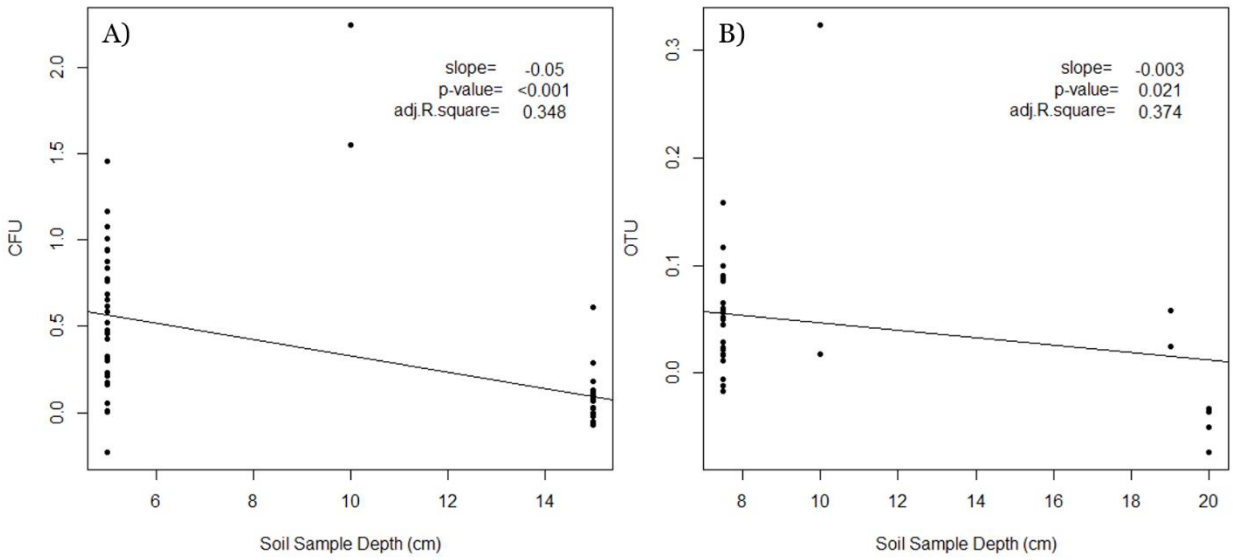

Figure B.2. Scatter plots and linear regressions of CC effect sizes of colony forming unit (CFU; A) and operational taxonomic unit (OTU; B) on soil sample depth (cm) (CFU: n=54; OTU: 32). The linear coefficient of the model (slope), its p-value, and R^2 are noted.

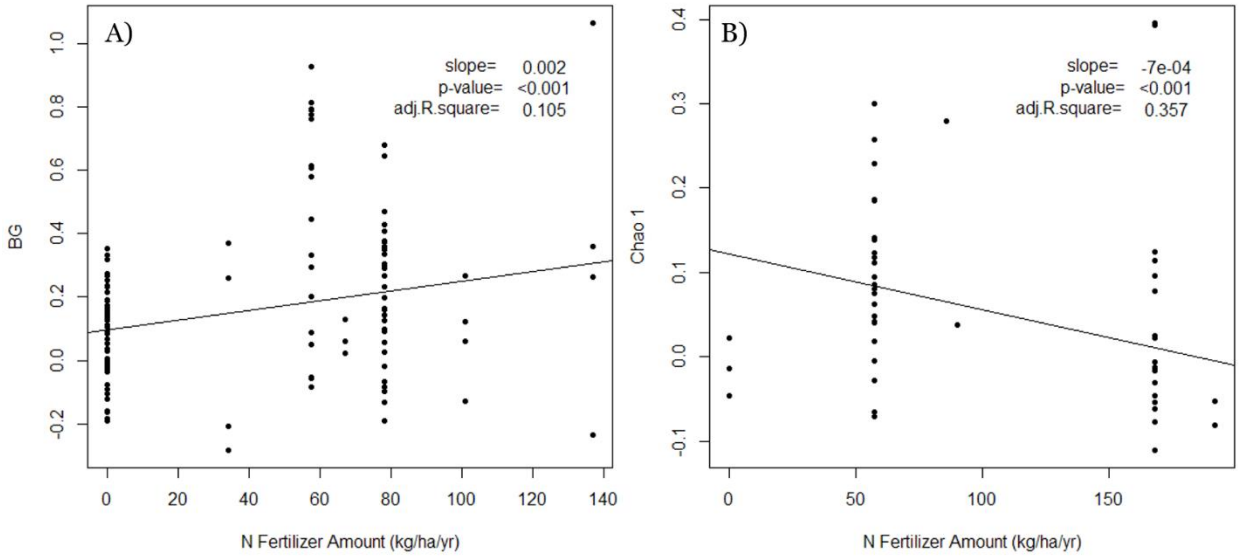

Figure B.3. Scatter plots and linear regressions of CC effect sizes of β-glucosidase (BG; A) and Chao 1 richness index (B) on annual N fertilizer rate (kg/ha/yr) (BG: n=128; Chao 1: n=49). The linear coefficient of the model (slope), its p-value, and R^2 are noted.

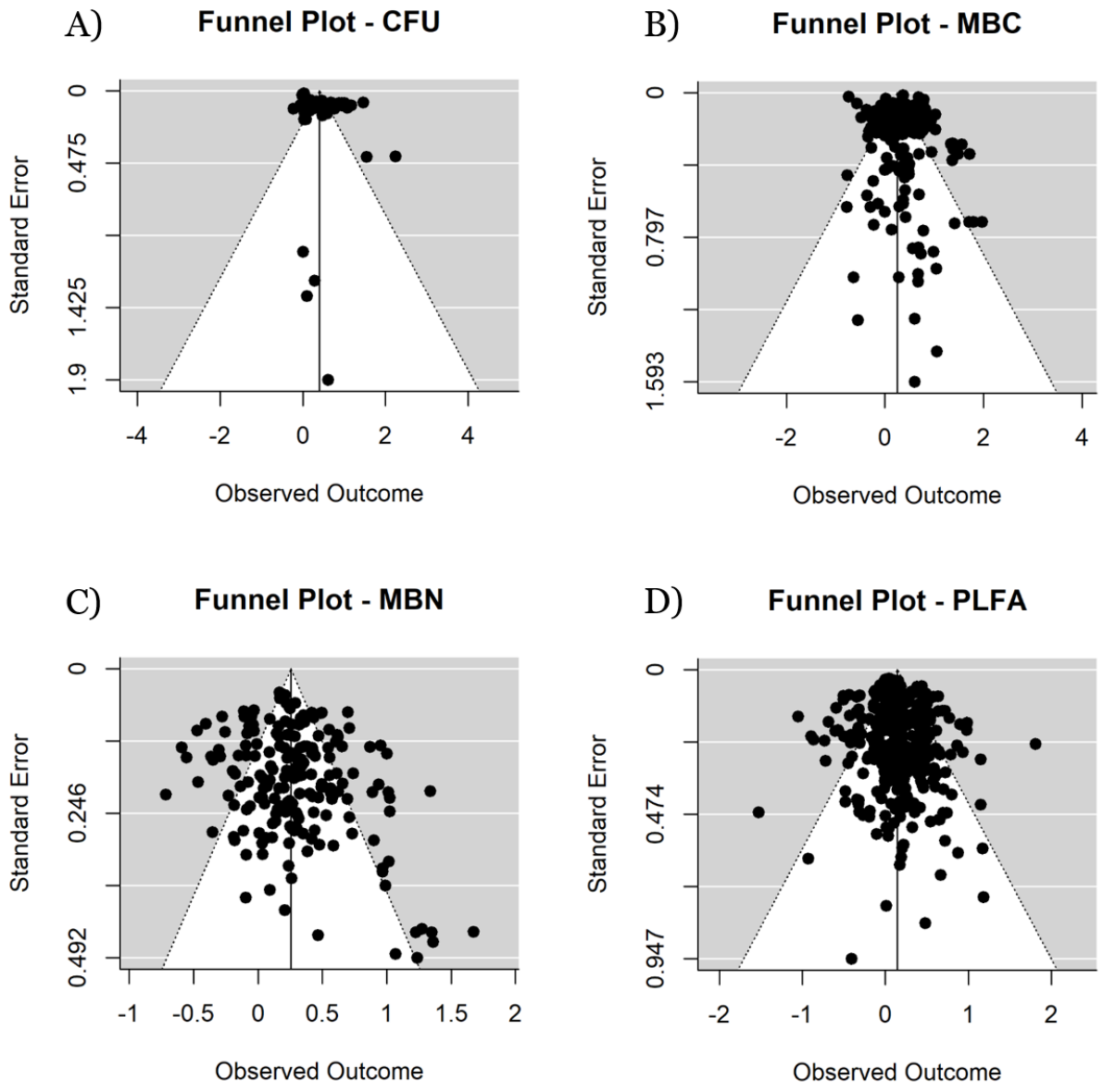

Figure B.4. Funnel plots of SM abundance parameters in the order of colony forming unit (CFU; A), microbial biomass C (MBC; B) and N (MBN; C), and phospholipid fatty acid (PLFA; D). The studies for each SM parameter are homogeneous if most of the points are within the white triangle.

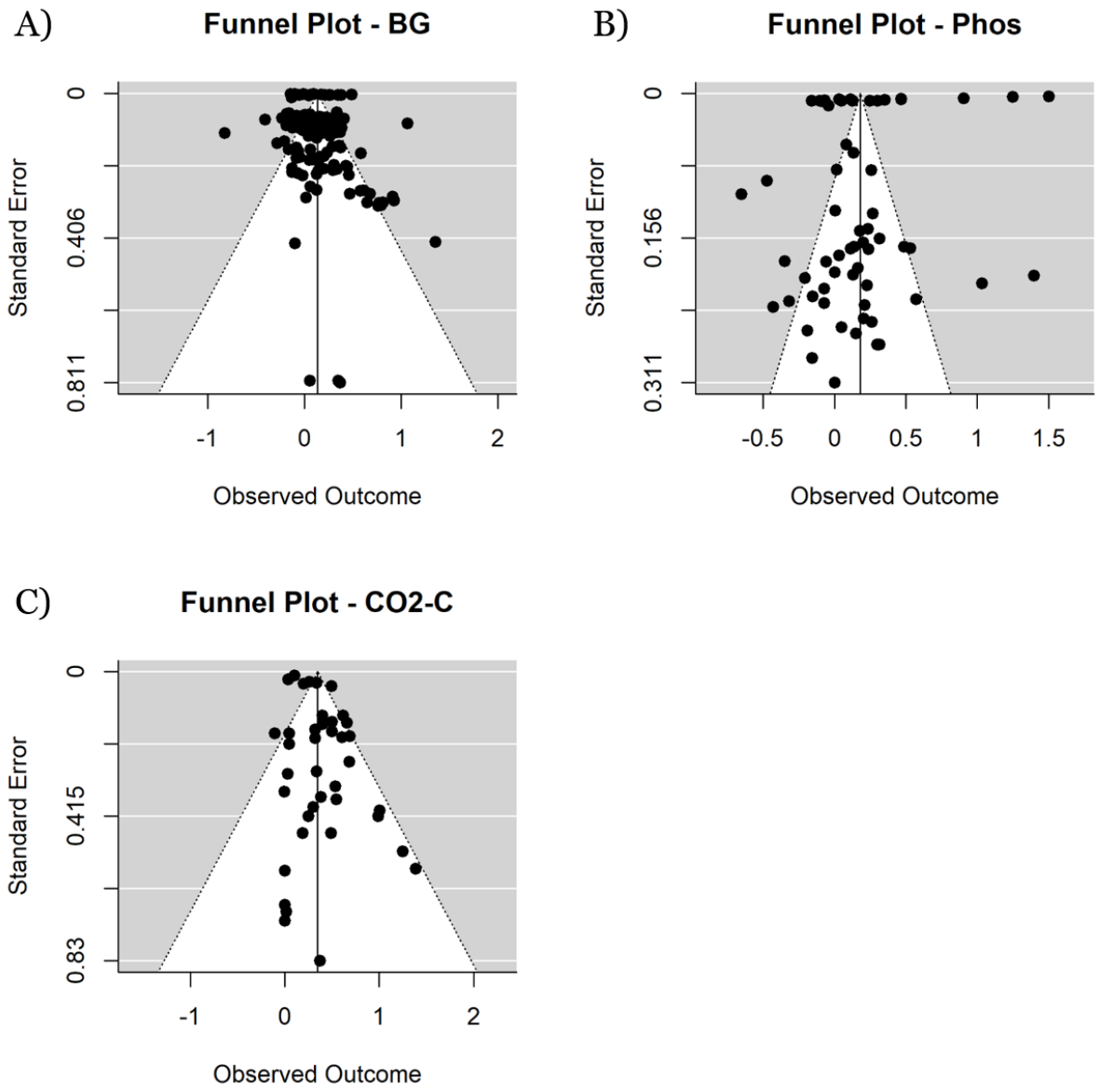

Figure B.5. Funnel plots of SM activity parameters in the order of β-glucosidase activity (BG; A), phosphatase activity (Phos; B), and respiration (CO2-C; C). The studies for each SM parameter are homogeneous if most of the points are within the white triangle.

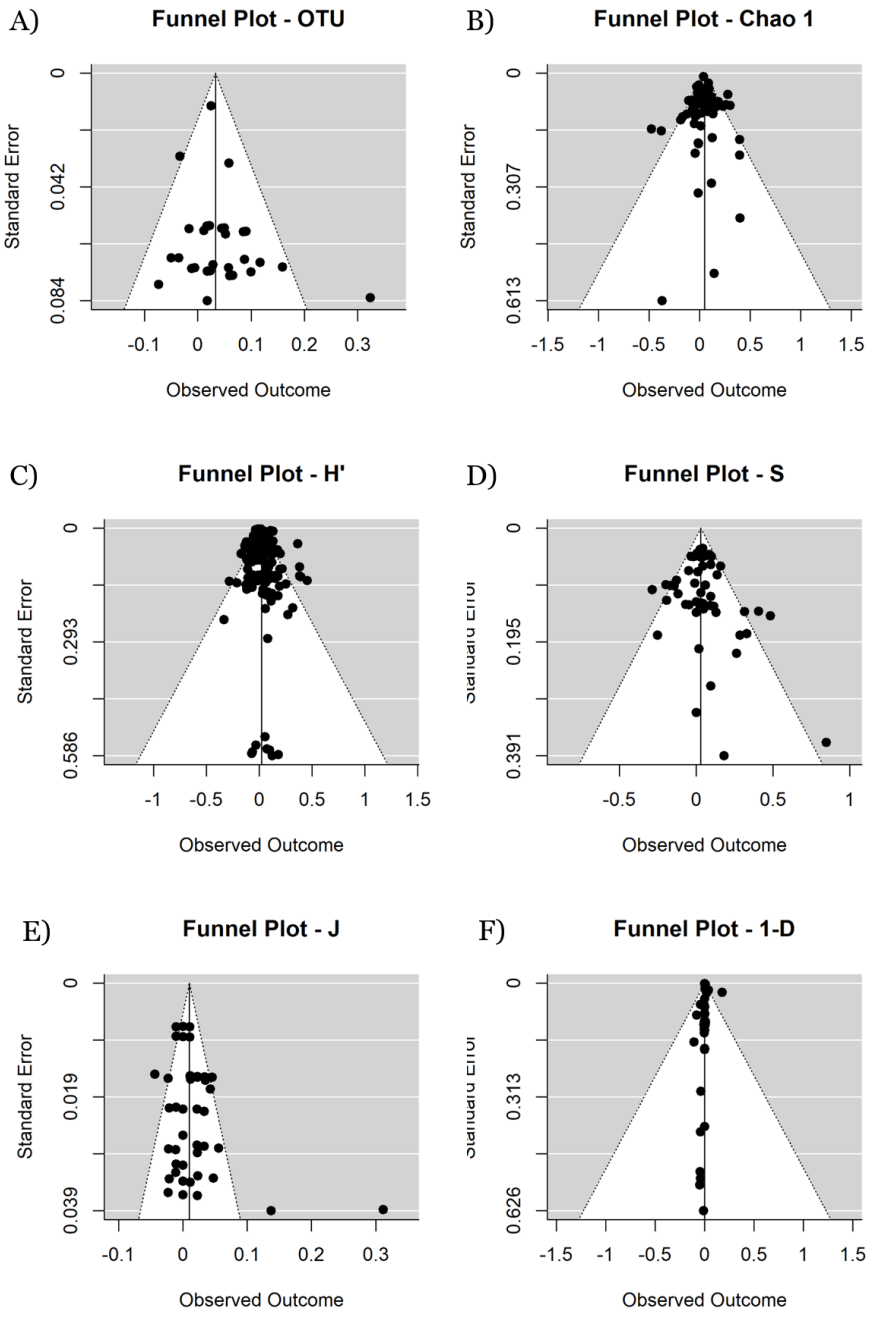

Figure B.6. Funnel plots of SM diversity parameters in the order of operational taxonomic unit (OTU; A), Chao 1 richness index (B), Shannon's diversity index (H'; C), genetic richness (S; D), Pielou's evenness index (J; E), and Simpson's diversity index (1-D; F). The studies for each SM parameter are homogeneous if most of the points are within the white triangle.

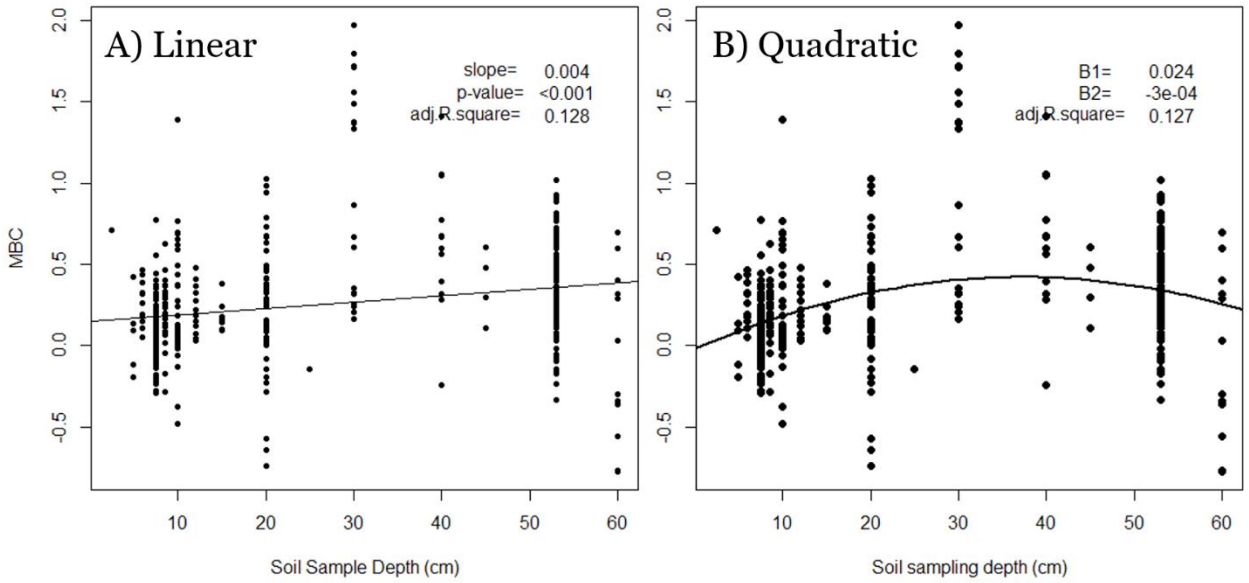

Figure B.7. Scatter plot, linear (A) and quadratic (B) regression of cover cropping effect sizes of microbial biomass C (MBC) and soil sample depth (cm) (n=408). The linear coefficient of the model (slope), its p-value, and R2 are noted. For quadratic model, p-values for both coefficients were <0.001.

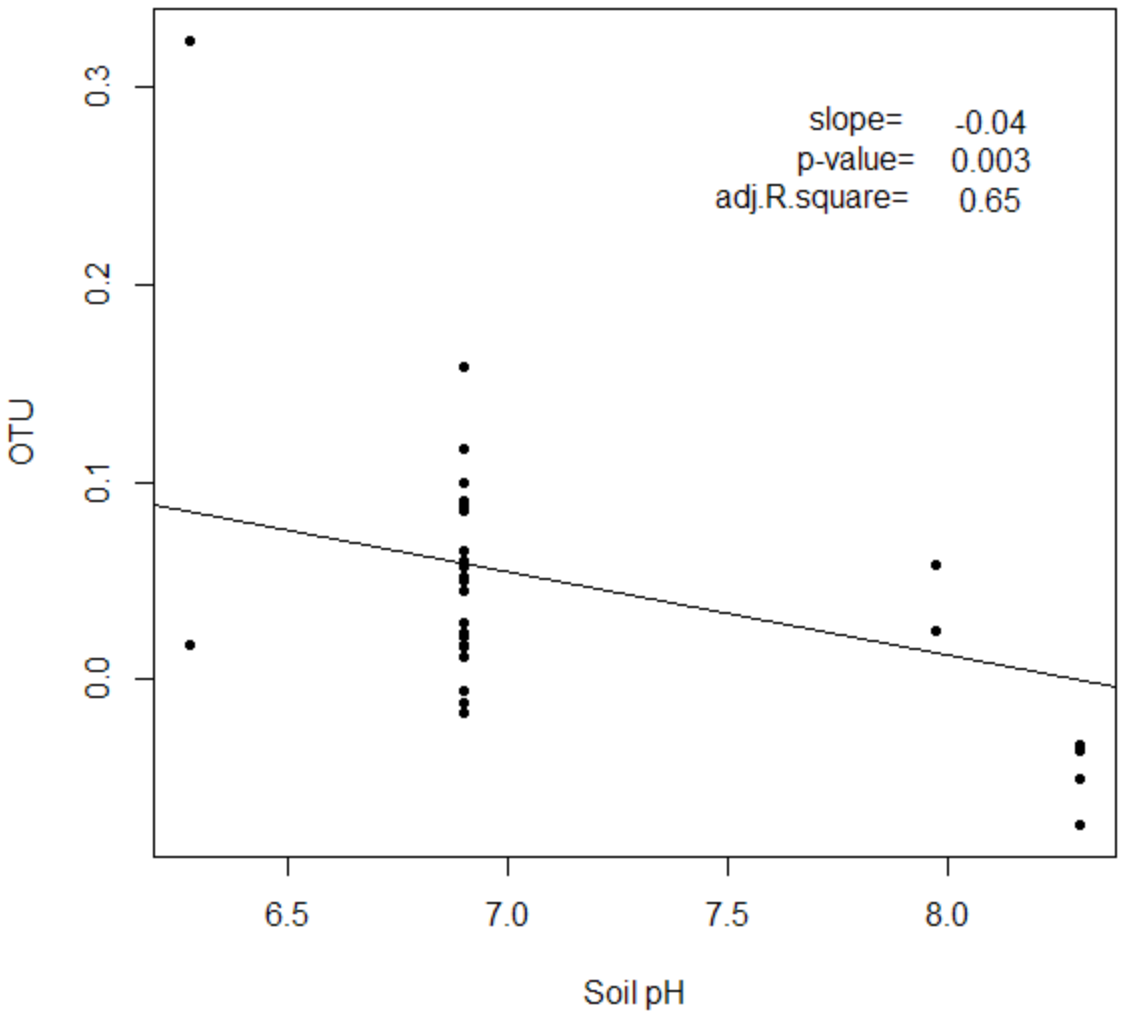

Figure B.8. Scatter plot and linear regression of cover cropping effect sizes of operational taxonomic units (OTU) and soil pH (n=32). The linear coefficient of the model (slope), its p-value, and R2 are noted.

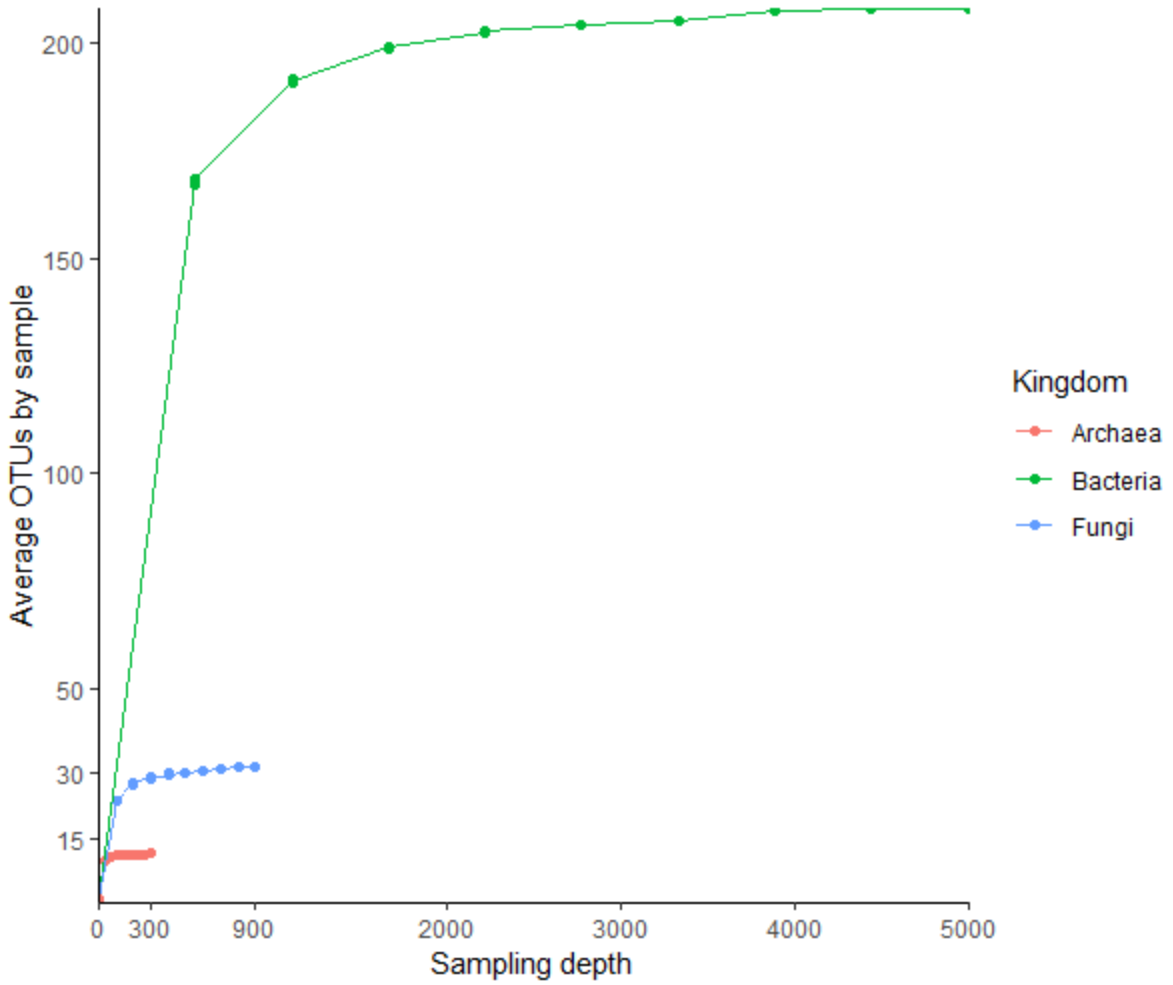

Figure B.9. Rarefaction curves for bacteria (green), fungi (blue), and archaea (red) that show average number of observed OTUs at each sampling depths of sequences to be subsampled for each soil samples.