CARBON AND NITROGEN CYCLING AND SOIL QUALITY UNDER LONG-TERM CROP ROTATION AND TILLAGE

BY

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DISSERTATION

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ABSTRACT

Crop rotation and tillage can have substantial impact on the soil environment, the microbial community, and the cycling of C and N. Understanding the relationship between soil organic matter dynamics and the agronomic practices of crop rotation and tillage is key to identifying management that has the potential to enhance the production of the soils of the Midwest. In order to evaluate how tillage influences soil biology, a global meta-analysis was conducted to compare the effect of conventional tillage on microbial properties to that of no-till. Overall, greater microbial biomass and enzyme activities were found under no-till compared to conventional tillage; however, the metabolic quotient was greater in conventional tillage. This indicates that individual microbes in tilled soil are more active compared to those in no-till soils. Despite these results, a large amount of variability remained that was not able to be explained, possibly as a result of the highly variable nature of the biological measures. While a meta-analysis provides a greater inference space, field research can provide greater understanding of the impact of agronomic practices on soil processes and soil quality within a region. Long-term crop rotation and tillage experimental sites were evaluated to assess the influence of both crop rotation and tillage on highly fertile Illinois soils. Using univariate analysis, the second chapter assesses the impact of these agronomic practices on C and N within soil organic matter and microbial biomass. Crop rotations with higher C:N residues and no-till led to greater soil organic carbon and total nitrogen; however, despite the role of microbes in C and N cycling, microbial biomass was not affected by these agronomic practices as expected based on the results of the meta-analysis. The final chapter evaluates how effective these and several other properties are to serve as indicators of soil quality. The results of a principal component analysis indicated that for both crop rotation and tillage, soil parameters related to C and N cycling were very influential

and have great potential as soil quality indicators. Different usage of nitrogenous fertilizers among crop rotations and the stratification of these fertilizers under no-till are other significant aspects of these agronomic practices that were highlighted by the multivariate analysis.

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CHAPTER 1. Meta-Analysis Approach to Assess Effect of Tillage on Microbial Biomass and Enzyme Activities

ABSTRACT

Measures of soil biology are critical for the assessment of soil quality under different agricultural management practices. By modifying soil microclimate, tillage exerts the most important control on soil microbial communities. The objective of this study is to assess the effect of tillage on soil microbial biomass and enzyme activities. A meta-analysis was conducted utilizing 139 observations from 62 studies from around the world; the selected effect size (ES) was $\log_n$ of the response ratio (RR), the mean of the tilled treatment divided by the mean of the no-till control. This ES was calculated for seven different microbial properties—microbial biomass carbon (MBC) and nitrogen (MBN), metabolic quotient (qCO_2), fluorescein diacetate (FDA), dehydrogenase (DHA), β-glucosidase, and urease. Microbial biomass, metabolic quotient and enzyme activities were evaluated due their prevalent usage in evaluation of soil quality and use in soil quality indices. Overall, microbial biomass and all of the enzyme activities were greater under no-till compared to tillage. One exception to this was that under chisel tillage, there was no difference in MBC between the tilled plots and no-till. The qCO_2 was greater under tillage than under no-till indicating more active microbes in tilled soil, perhaps compensating for the reduced quantity. In contrast, when looking at only long-term experiments, qCO_2 was similar under both tillage and no-till, which may indicate that eventually microbes in no-till plots become as active as those in tilled plots even with the larger microbial community. The findings

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of this study illustrate that no-till and even reduced tillage, such as chisel tillage, promote larger microbial communities and greater enzymatic activity.

INTRODUCTION

The primary purpose of tillage in agricultural systems is to enhance crop production through weed control and seedbed preparation. Tillage systems are described based on the degree of soil inversion and percentage of residues remaining on the soil surface following tillage operations. The use of moldboard plow fully inverts the soil, while less intensive tillage vertically disrupts soil without inversion. Conventional intensive tillage practices leave less than15% residue on the surface while conservation tillage practices leave more than 30% of residue as a soil cover at the time of planting of the next cash crop (CTIC, 2015). The negative effects of tillage on soil erosion, degradation of soil structure, soil macro organisms and loss of nutrients and soil organic matter have led to increasing usage of conservation practices. Currently, conservation agricultural practices, such as non-tillage, are practiced on nearly 155 million hectares worldwide, which comprise 11% of the arable cropland in the world (Kassam et al., 2014). North and South America are the greatest adopters of conservation practices with no-tillage adoption rates of nearly 32% and 45%, respectively (Friedrich et al., 2012).

Management practices influence the soil environment and therefore the habitat of soil microorganisms in various ways. Soil organic matter (SOM) dynamics are highly dependent on the microbial community (Acosta-Martinez et al., 2003; Alvaro-Fuentes et al., 2013). The final product of decomposition that remain in the soil, microbial residues, may be resistant to further degradation thereby protecting SOM through biochemical stability or physical protection within soil aggregates (Six et al., 2006; Jastrow et al., 2007; Schimel and Schaeffer, 2012). Improving

the understanding on the rates of decomposition as influenced by management is also fundamental to improve SOM management in cropping systems (Scow, 1997; West and Post, 2002). Crop rotations influence the type and quantity of crop residues being returned to the soils (Karlen et al., 1994; McDaniel et al., 2014); N fertilization increases plant growth and subsequent organic matter inputs and N availability for soil microorganisms and as well as influencing the pH of the soil near the application zone (Geisseler and Scow, 2014). In contrast, tillage can influence microbes by changing both the soil microclimate as well as access to organic matter inputs. The soil microclimate is typically cooler and moister in no-till soils compared to the drier and warmer soils under more intensive tillage (Johnson and Hoyt, 1999; Martens, 2001). Access to organic matter is greater with tillage as organic residues are broken into smaller pieces, which increases the available surface area for microbial colonization, (Johnson and Hoyt, 1999; Balesdent et al., 2000). Changes in the soil environment and soil microbial communities as a result of tillage then influences soil quality. The soil biological parameters investigated in this meta-analysis (microbial biomass, metabolic quotient, and enzymatic activities) were selected as they are commonly utilized in assessments of soil quality and as components of soil quality indices (Bastida et al., 2008).

Reviews by Johnson and Hoyt (1999) and Martens (2001) have both reported greater microbial abundance under no-till soils with a more favorable microclimate compared to soils under conventional tillage; similar results were reported by Kaschuk et al. (2010), Das et al. (2014), and (Balota et al., 2004). The degree to which microbial biomass increased under no-till compared to conventional tillage differed greatly, however, with a 17% increase reported by Das et al. (2014) and a 98% increase reported by Balota et al. (2004). While microbial biomass is often reported to be greater under no-tillage systems, no differences due to tillage have also been

reported in de Gennaro et al. (2014). Despite a relative consensus of greater amounts of microbial biomass C under no-till, measures of microbial activity vary much more widely. Microbial activity measured through the metabolic quotient (microbial respiration/microbial biomass or qCO2) was reported smaller under no-tillage compared to conventionally tilled systems (Balota et al., 2004) suggesting that microbes are more active under conventional tillage. On the other hand, Babujia et al. (2010) found no differences between conventional tillage and no-till practices. Other approaches used to understand microbial activity is through quantification of the functional role microbes play in the cycle of nutrients. Typically, this has been quantified through the rates of enzymatic activity. However, understanding of their dynamics in soil systems as influenced by tillage is less clear and contradictory (Gil-Sotres et al., 2005; Laudicina et al., 2012).

Evaluating the effect of tillage on microbial biomass and activity with a meta-analysis approach will provide a quantitative analysis of the global response of microbial soil characteristics to different tillage practices. A meta-analysis is a statistical method of combining results from multiple data sets to evaluate the magnitude of the effect size as well as patterns of response and sources of heterogeneity (Gurevitch and Hedges, 1999; Borenstein et al., 2011; Koricheva et al., 2013). With this approach, we can also evaluate other possible sources of variability simultaneously influencing microbial properties. We expect minimally disturbed or no-tillage soils to have a larger microbial community as evidenced by greater microbial biomass C (Johnson and Hoyt, 1999; Balota et al., 2004; Das et al., 2014). Further, the reduced rates of soil disturbance are expected to reduce microbial enzymatic activity likely linked to slow rates of C and N mineralization from SOM. Specifically, the objectives of this study were to use a meta-analysis approach to 1) determine the effect size of tillage compared to no-till on microbial

biomass and enzyme activities involved in the C and N cycles and 2) evaluate the influence of other sources of variability on the magnitude and direction of the effect size.

MATERIALS AND METHODS

Data Collection and Database

Data was collected through a process of data mining of the scientific literature using Thomas Reuters Web of Science v.5.16.1. We looked for peer-reviewed articles evaluating the effect of soil management practices on microbial biomass and activity. Keywords used for the initial search included “microbial biomass”, “microbial activity”, and “soil management”. This initial search produced 1242 articles, which were further refined by including “tillage” as another keyword to 380 articles. The literature search was restricted to peer-reviewed papers that were published between January 2000 and December 2014. The reference list from review papers on soil organic matter dynamics and soil microbial properties were further scrutinized to select additional peer-reviewed articles that had not been picked up by the initial search. Figure 1.1 shows a flow chart illustrating the steps in data collection.

To construct the database, results from a publication were included if it met the following criteria for quality control and to ensure appropriate data collection: 1) Studies reported results on a minimum of one of the following soil biological parameters: microbial biomass (measured through chloroform-fumigation extraction (CFE), chloroform-fumigation incubation (CFI) or phospholipid fatty acids (PLFA) and microbial activity (measured through metabolic quotient and enzymatic activity of fluorescein diacetate (FDA), dehydrogenase, β-glucosidase, and urease) as affected by at least a no-till control and an alternative type of tillage used as treatments, 2) Articles reported data collected from field trials in grain-based studies, 3) Articles

had clear specifications of experimental design and a minimum of two replications, 4) Articles included details on the length of the study, 5) Information on the location of the experimental site was provided so that additional environmental characteristics such as mean annual temperature and precipitation and soil texture could be obtained either from the same article or from additional secondary sources. A total of 62 peer-reviewed journal articles were included in the database. Multiple treatment pairs from the same study were included as separate observations when they could be categorized within separate subgroups for one or more moderating variables. A total of 137 treatment pairs were extracted; however, each treatment pair provided data for only a few microbial parameters evaluated within this study. The number of observations for each microbial property differed with 89 for microbial biomass C (MBC), 46 for microbial biomass N (MBN), 29 for metabolic quotient (qCO_2), 19 for fluorescein diacetate (FDA), 43 for dehydrogenase (DHA), 53 for β-glucosidase (β-glu), and 19 for urease. The locations for the studies included were far-ranging, and there were a minimum of three studies on every continent excluding Antarctica (Figure A.1).

Mean estimates for the response variables were extracted from tables, figures, and text. The PlotDigitizer software (http://plotdigitizer.sourceforge.net/) was utilized to extract data from figures. The tillage treatments were further characterized by tillage implements and depth of tillage. Additional classification variables that can help explain the variability in the data set and were included in the database were related to crop management practices including length of rotation and number of species within crop rotation, legume or cover crop use, and average annual nitrogen fertilizer rate. Study parameters such as duration of the experimental plots and depth of sampling were also recorded. When measurements for multiple sampling depths were available, a weighted average of the mean was reported. Environmental factors included in the

database consisted of mean annual temperature and precipitation, soil organic carbon content, and soil texture. Many studies also reported data on multiple sampling dates; in such cases, the spring sampling date was included in the database where possible. If no spring sampling was reported, a fall sampling date was included in the database; the date of sampling was also recorded in the database. Measures of variability were recorded and converted to standard deviations as much as possible; a minimum of 50% and up to 89% of the SDs of treatment pairs for the seven microbial properties were available from the articles. Where missing, authors were contacted and as a last resort, standard deviations were estimated based on the average CV for the known data.

Data Analysis

The statistical analysis was performed using methods for meta-analysis (Hedges et al., 1999) in the SAS statistical software (version 9.4, SAS Institute Inc., Cary, NC). Meta-analysis use in agronomy and soil sciences has increased in the recent years (Miguez and Bollero, 2005; Gardner and Drinkwater, 2009; Kallenbach and Grandy, 2011; Geisseler and Scow, 2014; McDaniel et al., 2014) and has helped to make quantitative inferences on the effect of management practices on soil properties studied across multiple experimental sites, locations and climatic regimes. In this meta-analysis, we quantified the magnitude of the effect of tillage on the dependent variables identified as the response ratios (RR) between microbial properties (microbial biomass C, qCO2, and soil enzymatic activity) in tillage treatments and a control or non-tillage treatment with the following equation:

$$RR = \bar{Y}_{till} / \bar{Y}_{no-till} \qquad [1].$$

A RR greater > 1 suggests a stronger effect of the tillage than non-tillage treatments on microbial soil properties. To normalize the data set, we then used a natural logarithmic transformation

$$LRR = \ln(RR) \qquad [2].$$

The variance (v_i) for each study was calculated using the following equation from Hedges et al. (1999)

$$v_i = \frac{SD_{till}^2}{n_{till} * \bar{Y}_{till}^2} + \frac{SD_{no-till}^2}{n_{no-till} * \bar{Y}_{no-till}^2} \qquad [3]$$

using the squared standard deviation(SD^2), sample size (n) and squared means ($\bar{Y}^2$) of the tillage and no-tillage treatment pairs.

The first step in this meta-analysis was the evaluation of total group heterogeneity in which the null hypothesis of no heterogeneity is tested using the Q_t statistic (Hedges et al., 1999). The LRRs were weighted using the inverse of the variance ($w = 1/v_i$) [4]. If the test for Q_t is significant ($\alpha \leq 0.05$), it suggests that the effect of tillage varied among observations and that the introduction of additional moderator variables may help explain such variability (Miguez and Bollero, 2005). To allow for inferences beyond the only the studies included in this meta-analysis, a mixed model was used with study as a random effect and the moderator variables as fixed. For a mixed model, the means are weighted using the inverse of the total variance where the total variance is the sum of the with-in studies variance (v_i) calculated in equation 3 and the between-studies variance ($\hat{\sigma}_\theta^2$)

$$w^* = 1/(v_i + \hat{\sigma}_\theta^2) \qquad [5]$$

The calculation of $\hat{\sigma}_\theta^2$ followed the steps outline in Cooper and Hedges (1994), whereby the data was first analyzed in a fixed model using weights based only on the inverse of with-in studies variance from equation 4. The results of the fixed model were used to estimate between studies variance using the following equation

$$\hat{\sigma}_\theta^2 = \left[\frac{RSS}{k-p-1}\right] - \sum \frac{v_i}{k} \quad [5]$$

where RSS is the residual sum of squares from the fixed model analysis, k is the number of studies used in the analysis for a particular moderator variable, p is the number of regression parameters, and $\sum \frac{v_i}{k}$ is the average of the with-in study variances. The final step is to run the analysis as a mixed model using weights based on the total variance from equation 5. Because not all observations included data on each moderator variable and the sums of squares were partitioned differently, an estimate of total variance was calculated separately for each moderator variable. When the test of the moderator variable yielded P values ≤ 0.05 for categorical variables, we generated mean effect sizes and 95% confidence intervals. Means with confidence intervals not overlapping zero were considered significantly different.

The distribution of LRR for each microbial property was visually examined using the funnel plots to ensure that data points belonged to the same population distribution and to avoid publication bias. The funnel plot is a scatter plot of the LRR against the sample size or the variance. The general assumption is that effect sizes belonging to the same population have comparable magnitudes. In the effect size vs variance relationship, the former tends to be more variable in experiments with small sample sizes, but variability is reduced as sample sizes increase rendering to a funnel-shaped plot (Borenstein et al., 2009). Funnel plots for each soil microbial parameter are included in the Appendix (Figure A.2). Asymmetrical funnel plots indicate that there may be publication bias. Most of the plots were fairly balanced around the mean, but MBN and qCO_2 funnel plots both show some asymmetry at higher variance levels; therefore, there may be some publication bias for these two variables.

A sensitivity analysis was also conducted to evaluate the effect of changes to the analysis on the results. Different approaches to the analysis were conducted, such as study treated as fixed vs. random, unweighted vs. weighted, removal of influential data. These were compared to

evaluate the robustness of the conclusions drawn from the results. The sensitivity analysis indicated that results from the weighted analysis of a mixed model were different from the simpler models, which indicates that the more complex models are necessary.

RESULTS

The forest plots in Figure 1.2 show the mean log response ratio and the 95% confidence intervals for each of the investigated microbial properties. The mean log response ratios were negative for all of the microbial properties with the exception of qCO_2. The negative mean LRRs ranged from -0.28 for MBN to -0.14 for FDA, while the lone positive mean LRR of qCO_2 was 0.26. The positive LRR for qCO_2 indicates that qCO_2 was greater under tillage than no-till. When the LRR is negative as for microbial biomass and the enzymatic activity, the measured property was greater under the no-till control than the tillage treatment.

Microbial Biomass

The test for heterogeneity for MBC was significant (Q_t=959, df=89, $p<0.0001$), which indicates that the response ratios were not homogenous across all observations and other factors may be influencing the effect of tillage. Further analysis tested the influence of the moderator variables in Table 1.1, which revealed tillage implement and tillage depth significant at $\alpha = 0.05$ for MBC. The forest plot presented in Figure 1.3 shows the means and 95% confidence interval for the different levels of tillage implements. A LRR with a value of 0 indicates that there was no difference between the tillage treatment and no-till for MBC. When the LRR is negative, MBC was greater for the no-till control than the tillage treatment. When the LRR is positive, MBC was greater under tillage than no-till.

For MBC, the four subgroups of tillage implements tested were chisel, disk, moldboard, and moldboard+. The level moldboard+ refers to the use of moldboard plow plus another implement, either chisel or disk tillage. There were a minimum of 12 observations for each of these subgroups. For the more intensive levels of tillage (moldboard and moldboard+), MBC was reduced under tillage compared to no-till. Microbial biomass carbon under chisel tillage did not differ from MBC under no-till; in contrast, the results for disking are very similar to those of the more intensive tillage with less MBC under tillage than no-till. While both chisel and disk tillage are typically considered to be less intensive than moldboard tillage, in some cases, disk tillage sometimes inverts the soil in a similar manner to moldboard plow, especially when set to deeper tillage depths and/or with multiple passes. Like tillage implement type, the depth of tillage was significant and was able to explain some of the variation (Table 1.1). The slope statistically differed from zero with the 95% confidence interval of -0.02 to -0.002. Figure 1.4A shows the scatter plot and regression line. As the depth of tillage increased, the LRR decreased; therefore, deeper tillage reduced MBC compared to no-till more so than shallower tillage.

The test for heterogeneity for MBN was also significant (Q_t=852, df=45, $p<0.0001$). When moderator variables were evaluated for their contribution to the variation, none of the moderator variables tested were significant (Table 1.1). The mean effect size for MBN was -0.19 with a 95% confidence interval of -0.42 to -0.14, indicating like MBC, a reduction in MBN under tillage compared to no-till. However, figure 1.5 shows that although no moderator variable was significant, there is still a great deal of variability within the observations. The forest plot in figure 1.3 shows the LRRs for tillage implements for MBN and all of the 95% confidence limits for the different tillage implement subgroups are below zero, indicating that MBN under tillage is less than no-till for all tillage implements. However, it is important to note that for MBN,

chisel and disk tillage were combined together into one subgroup. Since disk tillage differed from chisel tillage for MBC, it is possible that this is possible that this is also true for MBN; however, because of the low number of observations for disk tillage, these two subgroups were combined together so this could not be further tested.

Metabolic Quotient

Metabolic quotient is the ratio of basal respiration to microbial biomass and is an indicator of how active the microbial community is. The test for heterogeneity was significant for qCO_2 (Q_t=451, df=28, $p<0.0001$). Both study duration and the percentage of clay and silt in the soil were significant and may be able to explain some of the variation (Table 1.1). The metabolic quotient in experiments that had been in place for more than 10 years were similar under both tillage and no-tillage systems as the LRR was near zero (Figure 1.7). In contrast, shorter experiments in place for less than 10 years showed a marked difference between tillage and no-till. With a mean LRR of 0.62, qCO_2 was greater under no-till than tillage in the first 10 years of experiments. Soil texture was also an important contributor to variation within the dataset as the percentage of clay and silt was significant with a slope with a 95% confidence interval of -0.04 to -0.002 (Figure 1.4B). Under sandier soils, the qCO_2 was greater under no-till compared to tilled, but as the percentage of fine particles increased, the smaller the difference between till and no-till became. While not significant at α=0.05, forest plots for both rotation and tillage implement are shown in figure 1.7. All three of the rotations showed a fairly large amount of variability, but the key difference is that under the extended rotation, qCO_2 under no-till was greater than under till as the confidence interval does not cross zero; the extended rotation consisted of at least a three year rotation with a minimum of three crop species. Likewise for

tillage implement, qCO_2 under moldboard tillage was less than no-till, but for chisel or disk tillage there was little difference between tillage and no-till.

Total Microbial Activity

Both FDA and DHA are enzymes that are typically used as indicators of total microbial activity. The test of heterogeneity was significant for both FDA (Q_t=138, df=18, p<0.0001) and DHA (Q_t=2862, df=42, p<0.0001). For FDA, none of the moderator variable were significant (Table 1.2); the log response ratios for FDA can be seen in Figure 1.5. This graph shows that there is some variability, but none of the moderator variables were able to explain it sufficiently. The mean effect size for FDA was -0.14, but the 95% confidence interval encompassed zero (-0.37 to 0.08).

Variation in DHA, in contrast to FDA, had a number of different moderator variables that were significant: sampling depth, precipitation, and percentage clay and silt (Table 1.2). However, the high amount of variability and a number of influential data points make interpretation of the results complicated. For both sampling depth and percentage clay and silt, sensitivity analysis revealed that although slope was significant for both (0.017 and 0.011, respectively), the removal of a single influential point made the slope no longer significant. Therefore, it is doubtful that this is a true explanation of the variation in the data. For annual precipitation, the mean of the slope is negative indicating that at locations with greater precipitation, DHA activity is greater under no-till than till, but in drier conditions, DHA activity is similar regardless of tillage practice. However, it is important to note that the 95% confidence interval for the slope was from -3.73 to 0.0013 (Figure 1.6A). This confidence interval is very wide and does include zero indicating that there is a high amount of variability remaining in the data.

Carbon and Nitrogen Cycling

Two enzymes were included in the meta-analysis to provide insight to how the cycling of carbon and nitrogen may differ under no-till and tilled systems. β-glucosidase is an important enzyme in the carbon cycle as a cellulose. Urease was included to show the nitrogen cycle. The test for heterogeneity for β-glucosidase was, significant (Q_t=4467, df=52, p<0.0001). For β-glucosidase, the moderator variable of sampling depth was significant (Table 1.2) with the 95% confidence interval for the slope as 2.26 to 0.0314 (Figure 1.6B). For soil sampled to greater depths, the difference between tillage and no-till decreased as the LRR became closer to zero. Near the surface, β-glucosidase activity was greater under no-till than tillage.

Like FDA and MBN, the test for heterogeneity was significant for urease (Q_t=202, df=18, p<0.0001), but none of the moderator variables were significant (Table 1.2). The LRR for all of the urease observations are in Figure 1.4 and the majority of the points are just below zero indicating that urease activity was slightly greater in no-till than tillage in most of the observations. This is in agreement with the mean LRR for urease as it was -0.264 with a 95% confidence interval of -0.53 to 0.01.

DISCUSSION

Impact of Tillage on Microbial Properties

One of the key benefits of utilizing soil biological properties is their purported sensitivity of these measures to management changes (Gianfreda and Ruggiero, 2006; Joergensen and Emmerling, 2006) while also being considered to be useful indicators of soil quality (Bastida et al., 2008). Based on the overall mean LRRs for each of the parameters (Figure 1.2), it is evident that all of the soil microbial parameters included in this meta-analysis have the potential to

provide valuable information about the impact of tillage on soil microbial communities, but none can be considered to be more sensitive to management than the others. When selecting which of these microbial properties to measure, the decision should be based primarily on which aspect of microbial community is of interest. Microbial biomass carbon and nitrogen can only indirectly indicate the size of the microbial community, while metabolic quotient and certain enzymatic activities, such as FDA and DHA, can provide insight into the activity of the microbial community. Enzymes related to specific nutrient cycles, such as β-glu in the carbon cycle and urease in the nitrogen cycle, can show how the functional diversity of the microbial community is impacted.

Microbial biomass was reduced under tillage compared to no-till as measured by both MBC and MBN. The greater microbial biomass under no-till soils has been previously reported (Balota et al., 2004; Kaschuk et al., 2010) as there are more favorable environmental conditions under no-till for microbes leading to larger microbial biomass. An important difference from this pattern is that chisel tillage was exception to this trend, as the mean LRR for this subgroup was close to zero indicating that MBC under tillage did not differ from no-till (Figure 1.3). While this does not provide information about the diversity of the microbial community, this result indicates that microbial community size is not suppressed by chisel tillage. In contrast, the impact of disk tillage on microbial biomass was not different from that of a moldboard plow, which is typically considered to disturb the soil much more than other types of tillage implements. If set to a deeper tillage depth, disk tillage can invert the soil and this may lead to similar effects on the soil microbial community as the soil inversion due to a moldboard plow. While the categorization of tillage implement is helpful, the depth of tillage is also important, especially as an explanation for the seeming discrepancy with disk tillage. As expected, the LRR for shallow tillage is near

zero and the LRR becomes more negative for deeper tillage (Figure 1.4A). This matches up with tillage implement analysis as most of the shallow tillage is chisel tillage. As can be seen in figure 1.4A, disk tillage was done to a wide variety of depths with some at very shallow depths and some to as deep as 50-60 cm.

None of the remaining microbial properties differed based on tillage implement or tillage depth. Metabolic quotient was the only microbial property that was greater under tillage than no-till (Figure 1.2). This indicates that the microbes are more active under tillage; it may be possible that the greater access to crop residues due to tillage may lead to an increase in microbial activity; however, this is likely to be a short-term effect and could not fully explain the results we found as only a small number of the observations were measured shortly after tillage. Timing of the sampling was tested as a moderator variable and was not significant so the more likely explanation is that there is greater activity per unit of microorganisms to compensate for the reduced size of the microbial community. It is possible that both explanations are partially responsible for this result, but we cannot tell from this data.

All of the enzyme activities were greater under no-till compared to tilled systems as has been previously reported (Gianfreda and Ruggiero, 2006; van Capelle et al., 2012). The degree to which each enzyme was reduced under tillage compared to no-till was very similar. The greater enzyme activity under no-till indicates there is also greater functional diversity. While outside the scope of this analysis, this may be due to more microbial diversity as well. As with microbial biomass, the greater enzyme activity and potentially microbial diversity under no-till is likely a result of favorable microclimate. Another important aspect is that with less frequent disturbance of the soil, fungal hyphae are less disturbed and fungi play an important role in the cycling of carbon and nitrogen and the enzymes measured (van Capelle et al., 2012).

Experimental Procedure Factors

A number of different experimental artifacts were evaluated as sources of variation, including study duration, sampling timing and depth. Surprisingly, sampling timing was not significant for any of the microbial properties. Others have reported that enzymes, in particular, can differ seasonally (Gianfreda and Ruggiero, 2006) so it was unexpected that sampling timing was not significant for any of the enzymes. This may be related to the manner in which the timing was recorded and analyzed. Each observation was categorized as one of three timing subgroups. The first subgroup was early timing—following tillage and either before or directly following planting of the main crop in the rotation. The second subgroup was during the growing season and the final subgroup was any time after harvest before tillage operations were completed. This categorization was chosen because of the difficulty of using dates or seasons because of the global scale of the data. Ideally, the timing would have been based on how many days or months following tillage, but this was the best compromise for data collection.

Study duration was significant for qCO_2 with long-term experiments having similar microbial activity under no-till as tillage systems; in shorter studies, qCO_2 was greater in no-till than under tillage. Since many of these studies were started on previously cultivated soils, we cannot make any conclusions about how qCO_2 has changed from undisturbed systems. In fact, it is more likely that all of the soils were tilled prior to the initiation of the study. From this we can assume that the effect of tilling the soil on qCO_2 is unlikely to change over time and rather, it is the microbial activity under no-till that has changed over time. The results suggest that after 10 years of no-till the microbial activity has increased to be similar to the activity under tilled soils.

Sampling depth was significant for β-glucosidase with an increase in LRR at greater soil depths, which means that when sampled to greater depths, there was little difference between till

and no-till while when sampled only to shallow depth, β-glu was greater for tillage compared to no-till. This is not too surprising since a common characteristics of no-till soils is stratification of nutrients and therefore microbial communities. Tillage mixes crop residues and nutrients into the soil as well as aerates the soil facilitating microbial growth at deeper depths than possible under no-till soils. For instance, if the depth of tillage in a soil were 30 cm, the LRR would be different depending on if β-glu were measured for only the top 20 cm or the top 30 cm. Microbial activity would be concentrated in the portion of the soil under no-till, but distributed down to at least the depth of 30 cm under tillage. Like we found, the LRR would be negative if measured at 20 cm, but if measured to the greater depth of 30 cm, the LRR would be closer to zero. This pattern is expected to be true for the other enzyme activities as well as microbial biomass, so it is somewhat surprising that sampling depth was not able to explain variation for the other microbial properties.

Environmental Factors

Environmental factors that were assessed as possible sources of variation included climatic factors, such as temperature and precipitation and soil characteristics, such as soil organic carbon and soil texture. In sandier soils, qCO_2 was greater in tilled soils than no-till, but as the percent of fine particles increased, the closer the LRR became to zero. This indicates that with finer soil, microbial activity under no-till was similar to that of tillage. Finer particles, especially clay, play an important role in cation exchange capacity and water holding capacity; soils that have higher levels of those properties may lead to increases in microbial activity as there is more water and nutrients available for microbes under no-till so that qCO_2 is similar to that under tillage.

The LRRs for dehydrogenase were more negative with greater annual precipitation, while under drier conditions the LRRs were close to zero. Under dry conditions, there was little difference between till and no-till, perhaps as microbial activity was suppressed under both systems as soil moisture was scarce. When plenty of precipitation was available, DHA under no-till was greater than that under tillage. This results is somewhat surprising as it is under dry conditions, when no-till soils retain more moisture due to the residue mulch on the surface that we might expect to see greater microbial activity under no-till soils compared to tilled soils. This may be a function of using the mean annual precipitation, which does not necessarily the soil moisture at the time of sampling, nor the amount of precipitation received in the particular year of sampling.

Microbial Property Variability

Despite the significant test for heterogeneity for each, it was not possible to explain the source of variation using moderator variables for three of the microbial properties—MBN, FDA, and urease. This illustrates some of the difficulty in evaluating the effect of tillage (and other management practices) on microbial properties. These measurements are highly variable and even using the meta-analysis approach, it was difficult to determine the sources of that variability. It is possible that other moderator variables that were not provided in the articles could be important and unfortunately will be difficult to assess. Another possibility is that there is not a clear source of variability and the measurements themselves are just highly variable.

CONCLUSIONS

Meta-analysis allows us to draw conclusions from a much larger source of data to determine how microbial properties respond to tillage compared to no-tillage and to determine

how other sources of variation may impact those results. Our analysis compile the results of more than 60 experiments from across the globe for seven different microbial properties. Based on the results of this meta-analysis, microbial biomass and enzyme activities, in general, are greater under no-till than under tillage. There are some exceptions to this; one of the most important is that microbial biomass was not diminished under chisel tillage. Classifying chisel tillage as conservation tillage is in fact accurate from the perspective of the size of the microbial community as it was similar to no-till. Unfortunately, there were no difference among tillage implements for other microbial properties, indicating that for other microbial properties, chisel tillage reduced enzyme activities and microbial biomass nitrogen similarly to other tillage practices. Metabolic quotient was, in general, greater under no-till than tillage; however, for long-term experiments qCO_2 was similar for till and no-till, perhaps indicating an increase in microbial activity under no-till after at least 10 years without soil disturbance. Environmental conditions, such as precipitation and soil texture can also impact the effect of tillage on microbial properties.

Ultimately, evaluating microbial properties is often difficult due to the high variability within the measurements due to a number of factors including seasonal differences and environmental differences. In this analysis, we attempted to test for those sources of variability, but they were surprisingly not as important to the variability as expected. This means that there are other causes for the variability that we were not able to assess. Further use of the meta-analysis approach in soil biology is needed to help to fill in those gaps, especially as these measures are increasingly utilized to assess soil quality and health.

FIGURES

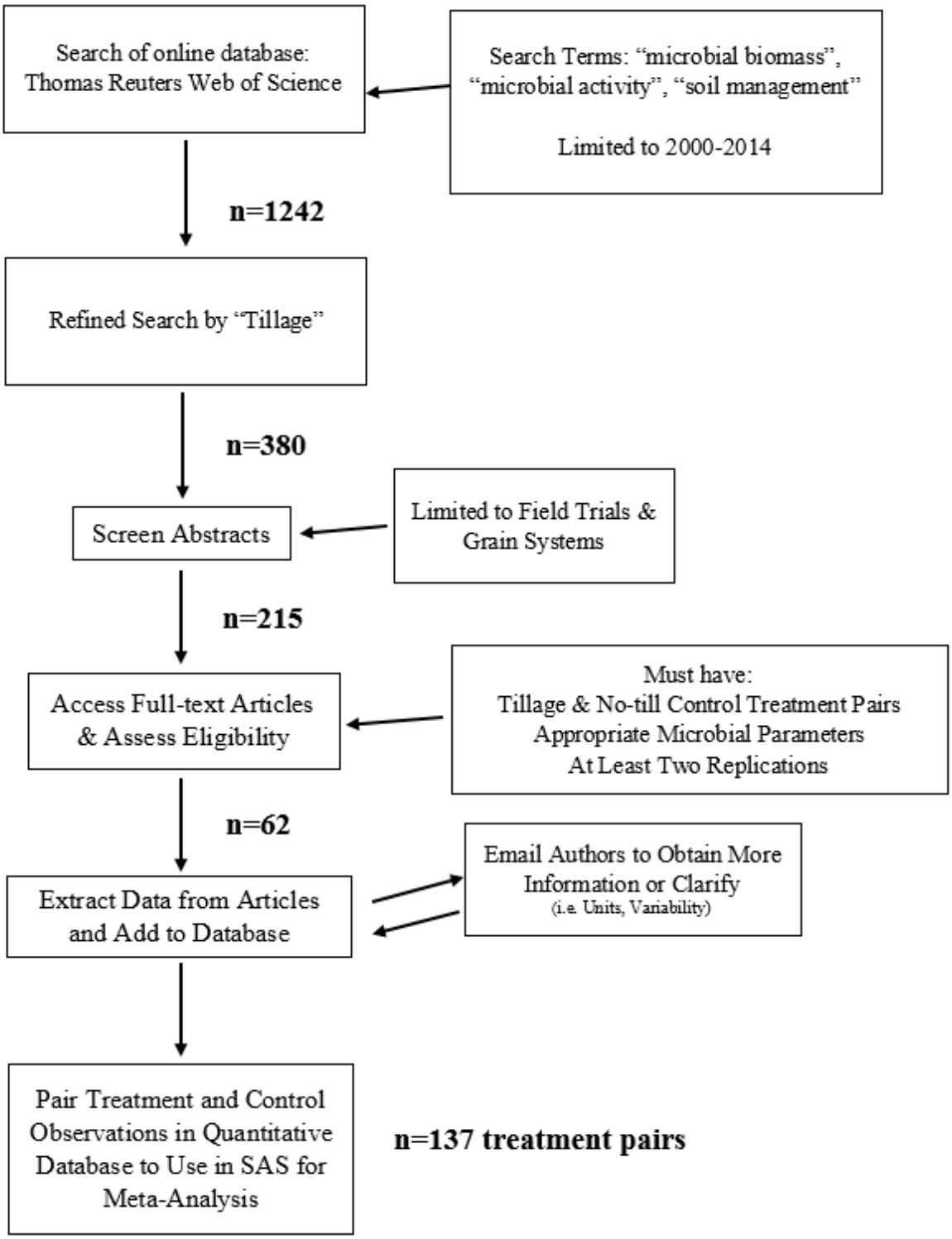

Figure 1.1 Meta-analysis data collection flow chart. Outline of the steps in the data collection process as well as the number of journal articles included at each step.

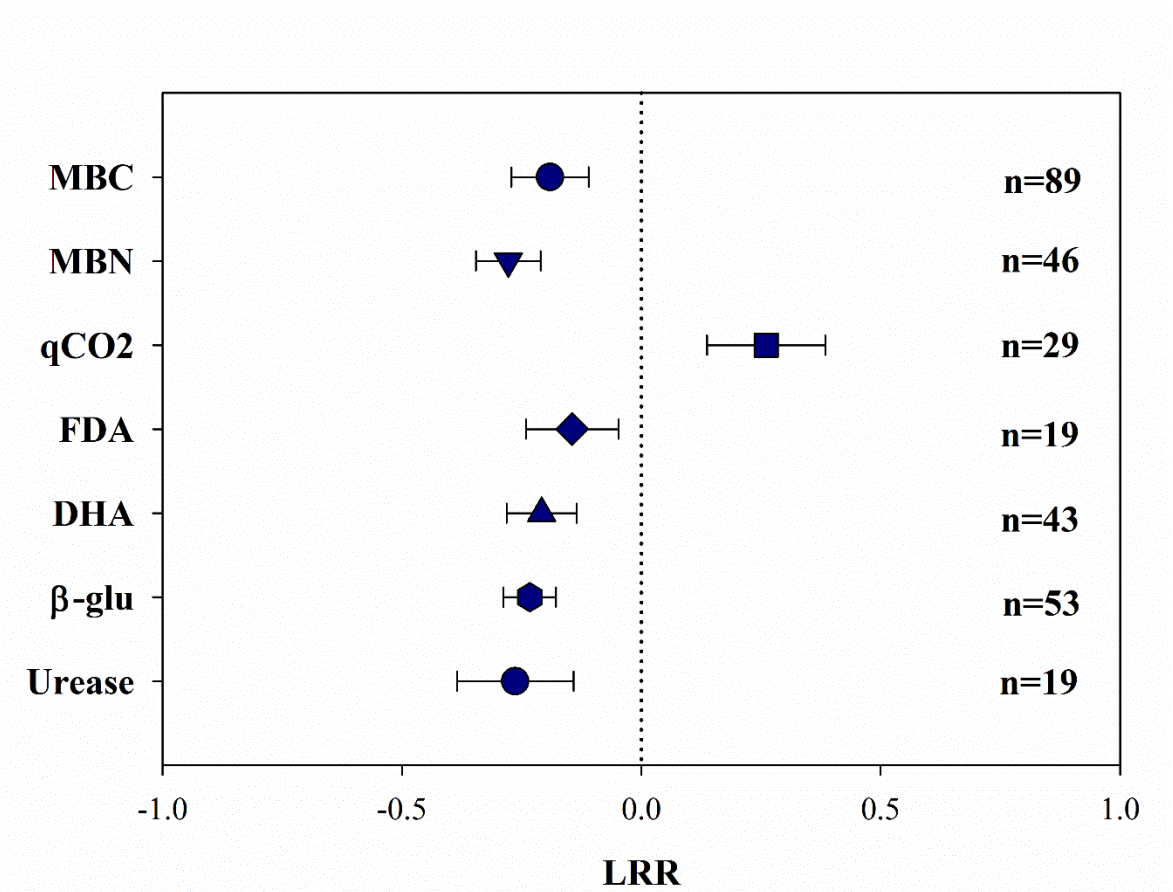

Figure 1.2 Overall mean log response ratios for the seven microbial properties included in the meta-analysis: microbial biomass carbon (MBC), microbial biomass nitrogen (MBN), metabolic quotient (qCO_2), fluorescein diacetate (FDA), dehydrogenase (DHA), β-glucosidase (β-glu), and urease. Values less than zero indicate a decrease in microbial biomass due to treatment and values more than zero indicate an increase in microbial biomass with tillage treatment compared to no-till control.

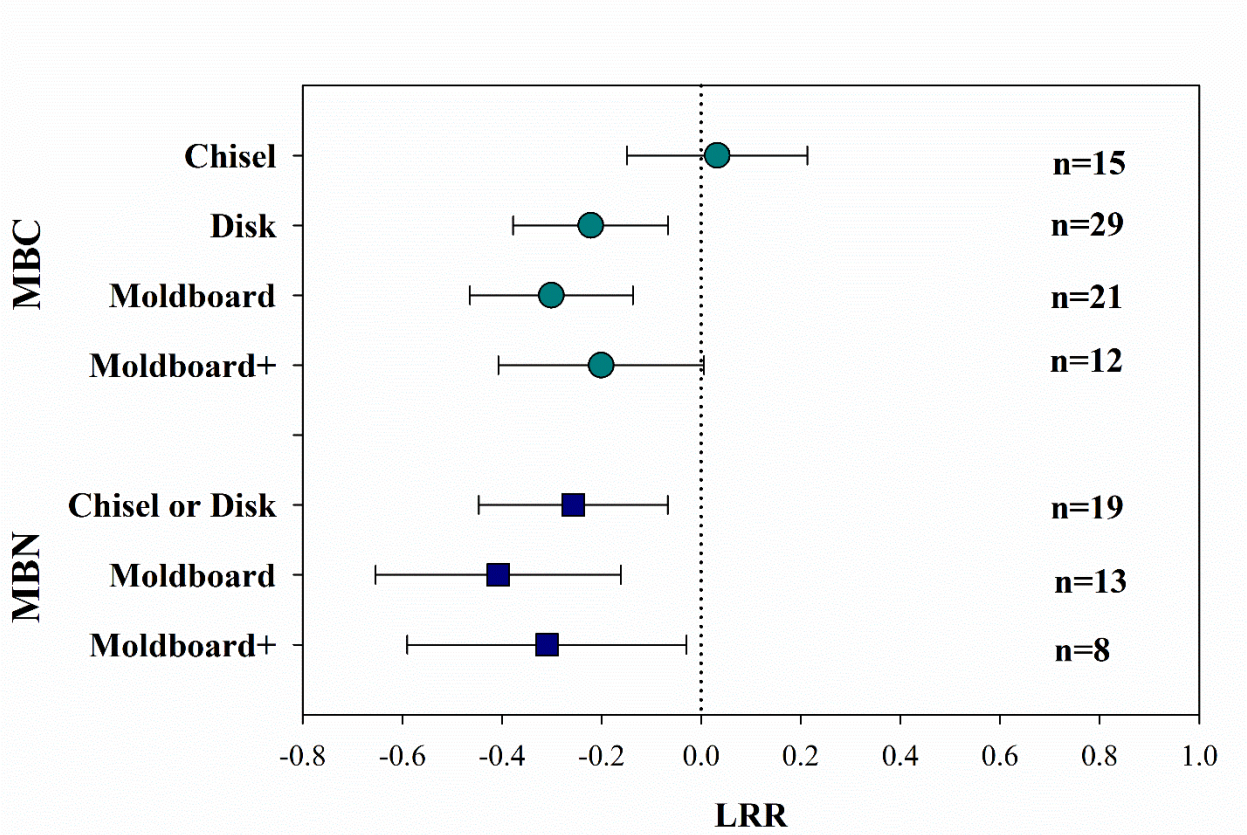

Figure 1.3 Mean log response ratios (LRR) and 95% confidence intervals for tillage implements for microbial biomass carbon (MBC) and nitrogen (MBN). Values less than zero indicate a decrease in microbial biomass due to treatment and values more than zero indicate an increase in microbial biomass with tillage treatment compared to no-till control.

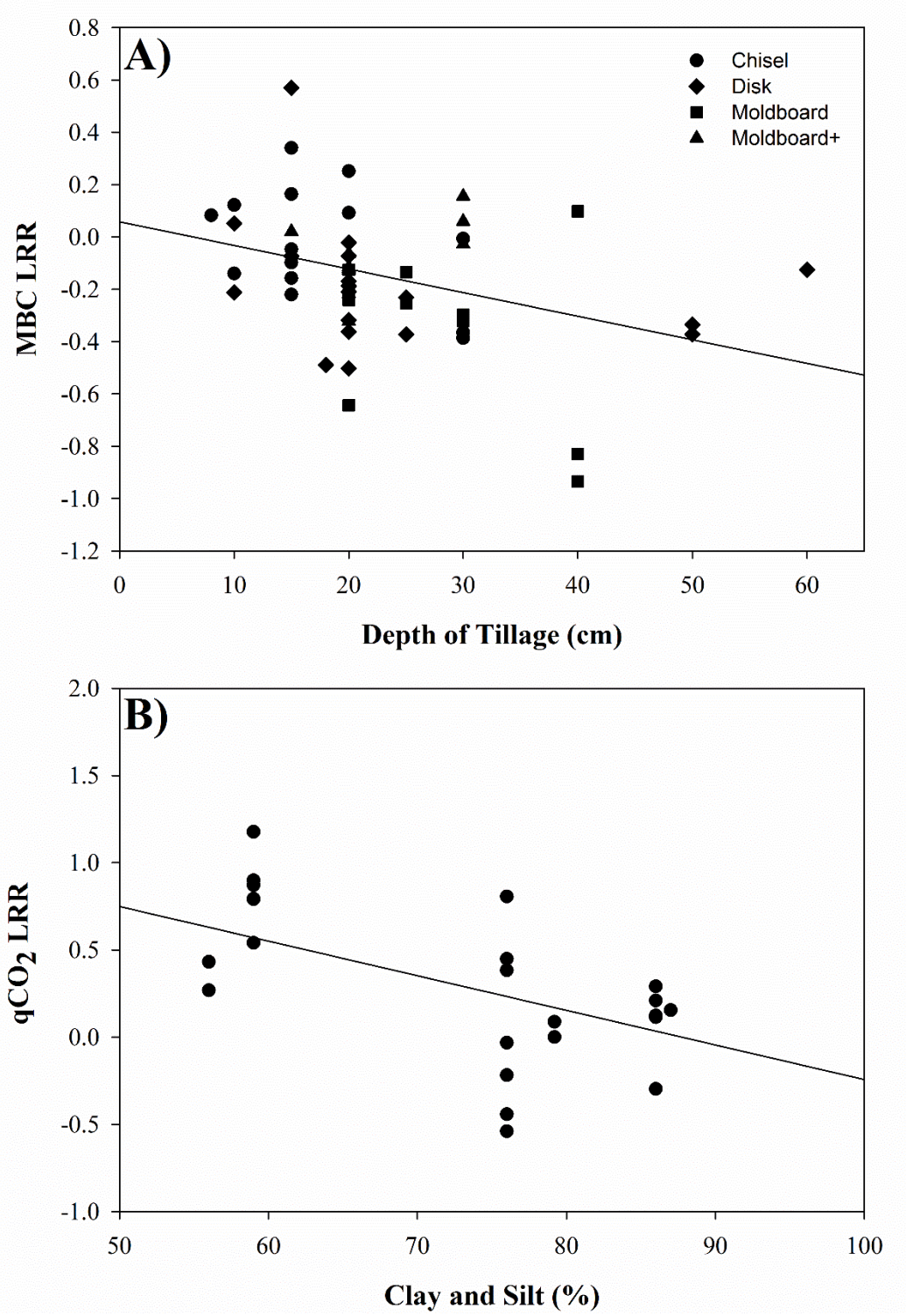

Figure 1.4 Scatter plot with linear regression graph of the natural logarithm of response ratio [tillage mean/no-tillage mean (LRR)] for a) microbial biomass carbon (MBC) versus depth of tillage operations (n=54) and b) metabolic quotient (qCO_2) versus percentage of clay and silt (n=25).

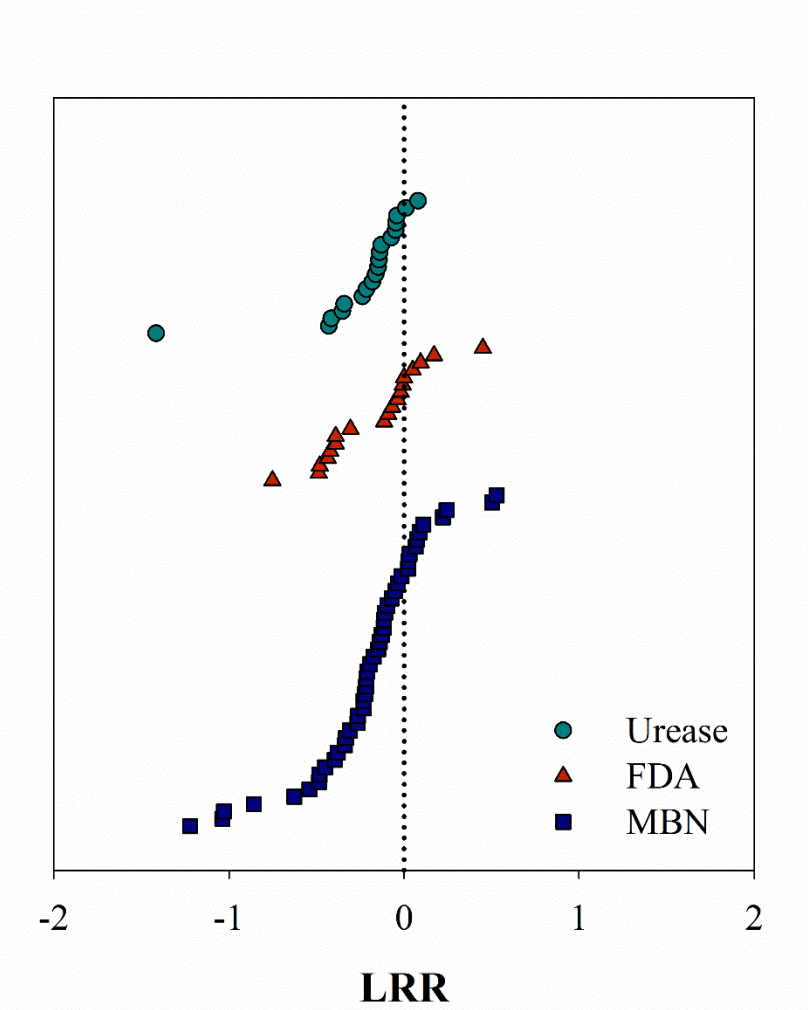

Figure 1.5 Natural log response ratios (LRR) of 46 microbial biomass nitrogen (MBN), 19 fluorescein diacetate (FDA), and 19 urease observations. Values less than zero indicate a decrease in microbial biomass due to tillage and values more than zero indicate an increase in microbial biomass with tillage treatment compared to no-till control.

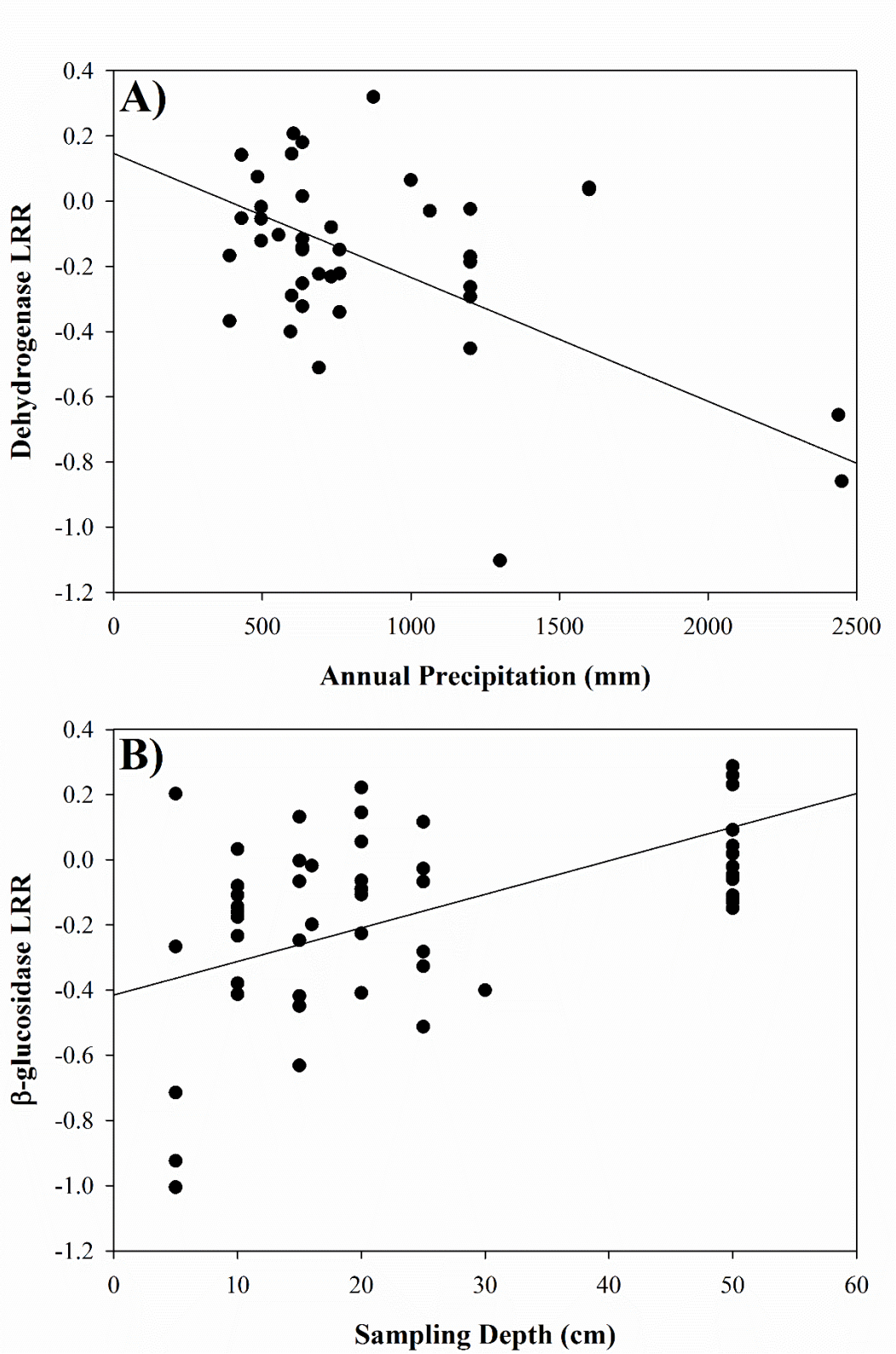

Figure 1.6 Scatter plot with linear regression graph of the natural logarithm of response ratio [tillage mean/no-tillage mean (LRR)] for a) dehydrogenase (DHA) versus mean annual precipitation (mm) (n=43) and b) β-glucosidase (β-glu) versus sampling depth (cm) (n=53).

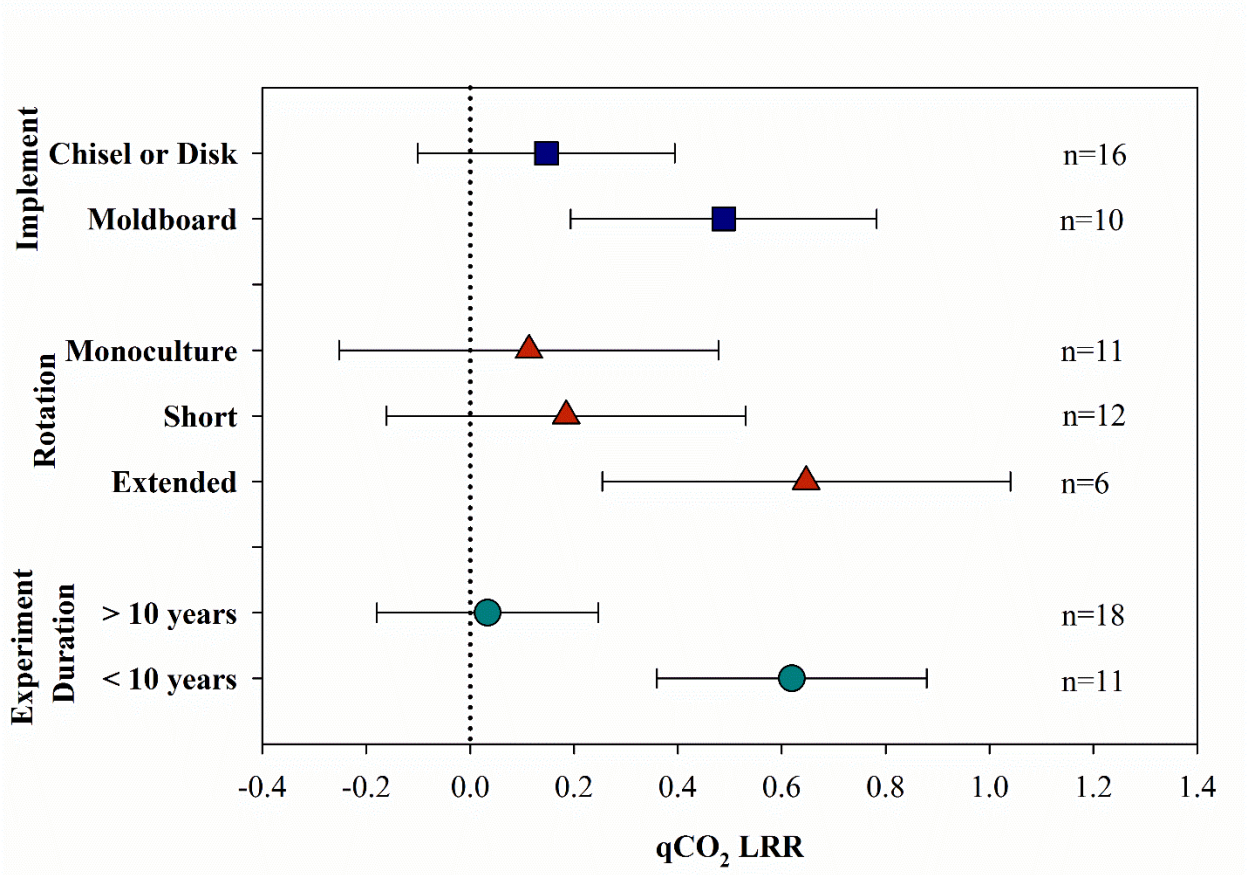

Figure 1.7 Mean log response ratios (LRR) of metabolic quotient (qCO_2) and 95% confidence intervals for three moderator variables—tillage implement, rotation length, and experiment duration. Values less than zero indicate a decrease in microbial biomass due to treatment and values more than zero indicate an increase in microbial biomass with tillage treatment compared to no-till control.

TABLES

Table 1.1 Significance of the tests for management factors impacting the effect of tillage in the mixed model analysis for microbial biomass carbon, microbial biomass nitrogen, and metabolic quotient. DF is degrees of freedom. Probability values less than 0.05 are italicized and in bold.

	Microbial Biomass Carbon			Microbial Biomass Nitrogen			Metabolic Quotient		
Source	DF	Error DF	*p* Value	DF	Error DF	*p* Value	DF	Error DF	*p* Value
Tillage Implement	3	37	***0.024***	2	16	0.574	2	17	0.088
Depth of Tillage	1	22	***0.013***	1	9	0.694	1	6	0.119
Rotation Type	4	37	0.876	3	18	0.228	2	16	0.0587
Legume	1	43	0.741	1	21	0.338	1	18	0.400
Cover Crop	1	42	0.941	1	19	0.666	1	17	0.110
N Fertilizer Rate	1	17	0.560	1	11	0.616	1	5	0.142
Sampling Timing	2	34	0.939	2	20	0.521	2	13	0.765
Sampling Depth	1	43	0.298	1	20	0.297	1	17	0.608
Study Duration	2	40	0.471	2	20	0.687	1	18	***0.003***
Temperature	1	43	0.173	1	21	0.908	1	18	0.305
Precipitation	1	43	0.104	1	21	0.344	1	18	0.678
Soil Organic Carbon	1	39	0.745	1	21	0.387	1	18	0.061
Percentage Clay & Silt	1	32	0.130	1	16	0.295	1	18	***0.031***

Table 1.2 Significance of the tests for management factors impacting the effect of tillage in the mixed model analysis for the enzymatic activities of fluorescein diacetate (FDA), dehydrogenase (DHA), β-glucosidase, and urease. DF is degrees of freedom. Probability values less than 0.05 are italicized and in bold.

Source[†]	Fluorescein Diacetate			Dehydrogenase			β-glucosidase			Urease		
	DF	Error DF	*p* Value	DF	Error DF	*p* Value	DF	Error DF	*p* Value	DF	Error DF	*p* Value
Tillage Implement	1	10	0.618	2	15	0.581	2	28	0.321	–	–	–
Depth of Tillage	–	–	–	1	13	0.290	1	9	0.057	1	3	0.709
Rotation Type	2	10	0.180	1	20	0.957	2	24	0.952	–	–	–
Legume	1	11	0.590	1	20	0.594	1	30	0.791	–	–	–
Cover Crop	1	11	0.548	1	20	0.629	1	31	0.944	–	–	–
N Fertilizer Rate	–	–	–	1	8	0.487	1	20	0.925	–	–	–
Sampling Timing	2	10	0.751	2	19	0.887	2	28	0.653	–	–	–
Sampling Depth	1	10	0.935	1	20	***0.021***	1	30	***0.031***	1	6	0.717
Study Duration	1	11	0.447	1	19	0.155	2	30	0.141	–	–	–
Temperature	1	10	0.648	1	20	0.117	1	31	0.804	1	8	0.724
Precipitation	1	10	0.265	1	20	***0.001***	1	31	0.364	1	8	0.610
Soil Organic Carbon	1	8	0.476	1	20	0.316	1	29	0.264	1	7	0.400
Percentage Clay & Silt	1	8	0.180	1	14	***0.013***	1	24	0.263	1	5	0.185

[†]Some moderator variables were not analyzed for FDA or urease due to too few observations ($k < 7$).

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CHAPTER 2. Soil Organic Matter and Microbial Biomass C and N under Long-term Crop Rotation and Tillage

ABSTRACT

Crop rotation and tillage alter soil organic matter (SOM) dynamics by influencing the soil environment and microorganisms carrying out C and N cycling. The objective of this study is to evaluate the effect of long-term crop rotation and tillage on the quantity of C and N stored in SOM and the size of the microbial community. Experimental sites at two Illinois locations were used to evaluate four rotations—continuous corn (*Zea mays* L.) (CCC), corn-soybean (*Glycine max* [L.] Merr.) (CS), corn-soybean-wheat (*Triticum aestivum* L.) (CSW), and continuous soybean (SSS), each split into chisel tillage (CT) and no-till (NT) subplots. The CSW rotation increased soil organic carbon (SOC) compared to SSS; SSS also reduced total nitrogen (TN) compared to other rotations. No differences among corn-based rotations were found, probably due to seasonal reduction in SOC and TN following corn. Levels of SOC and TN were 7% and 9% greater under NT than CT, respectively. Despite the effect of crop rotation and tillage on C and N within SOM, their influence was less discernable on microbial biomass. Both microbial biomass C and N (MBC, MBN) were unaffected by crop rotation, while tillage affected only MBN at 10-20 cm, likely related to dispersion of N fertilizers throughout the soil. Despite the apparent lack of sensitivity of microbial biomass, changes in SOC and TN illustrate the role of rotation and tillage in SOM dynamics as the inclusion of crops with high C:N residues and use of no-till support soil storage of C and N.

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INTRODUCTION

Crop rotation and tillage practices have a substantial influence on the soil as they alter the soil environment, influencing the habitat and energy source of the microbial community, the primary driver of SOM dynamics (Schimel and Schaeffer, 2012). Microorganisms are responsible for the decomposition of organic matter inputs, in the form of crop residues; as microbes break down residues, C becomes incorporated in to the microbial biomass, which becomes a key component of SOM (Miltner et al., 2012). The formation of soil aggregates are also directly related to microbial activity as microbial products are a binding agent within aggregates (Amézketa, 1999; Blanco-Canqui and Lal, 2004). The microbial residues along with physically protected SOM within soil aggregates are resistant to degradation and contribute to the stability of SOM (Schimel and Schaeffer, 2012). Agronomic practices play a substantial role in influencing the cycling of C through the soil and microbial community, yet environmental factors inherent to the soil and location, such as initial SOM levels, soil texture, and climate, will also affect the degree to which crop rotation and tillage influence SOM dynamics (Needelman et al., 1999; Kumar et al., 2012).

Rotations impact the soil and microbial community through the quantity and quality of residues returned, affecting the microbial process and the final stable product, SOM. As these residues form the bulk of the microbes' energy source, the type of crop and complexity of the crop rotation has a direct influence on the size and structure of the microbial community. A meta-analysis by McDaniel et al. (2014) found that the addition of one or more crops to a continuous cropping of a single crop increased the size of the microbial community as measured by both MBC and MBN, which are also related to the availability of resources and the favorability of the soil habitat.

Beyond affecting soil microbes, the quantity and quality of residues returned to soil from a crop rotation can determine the amount of C and N stored within the SOM. Soil organic matter typically consists of 58% carbon, typically referred to as soil organic carbon (Stockmann et al., 2013). The meta-analysis by McDaniel et al. (2014) reported adding one or more crops into a crop rotation increases both SOC and soil total nitrogen (TN). In a global analysis of 67 studies, West and Post (2002) also found that more complex rotations led to an accumulation of SOC across environments; however, switching from continuous corn to a corn-soybean rotation did not increase C. Higher biomass producing crops, such as corn, provide a larger pool of C to return to the soil, but the rate of decomposition may be slower due to higher C:N ratio of those residues (Karlen et al., 1994; McDaniel et al., 2014). The combination of greater residue production and slower decomposing residues of corn can lead to an accumulation of SOM in rotations that feature those crops more frequently compared to the reduced residue accumulation from soybean every other year from a corn-soybean rotation. Beyond just the residue input to the soil, the use of N fertilizers for crops such as corn and wheat affects the N within the soil as well as the soil pH (Divito et al., 2011). Crop rotations that include legumes have lower N inputs returned to the soil with lower biomass production and, without N fertilization, crop rotations that included soybeans more frequently have been found to lead to lower TN and higher pH (Zuber et al., 2015).

The role of tillage in the relationship between microbes and SOM is primarily through the impact of disturbance of the soil microclimate and the rate of decomposition of crop residues. The use of tillage increases aeration of the soil, reduces soil moisture content and leads to warmer soils in general (Johnson and Hoyt, 1999; Martens, 2001). Crop residues are fragmented into smaller pieces, increasing microbial access to residues accelerating the rate of

decomposition (Johnson and Hoyt, 1999). In no-till (NT) soils, microbes are concentrated near the surface due to the proximity to crop residues; while in tilled soils, microbes and their activities are spread out to a greater depth as a result of the distribution of C inputs throughout all of the disturbed soil layer (Govaerts et al., 2007). A global meta-analysis of the effect of tillage to NT for microbial biomass reported reduced MBC and MBN under tillage compared to NT; however, this difference for MBC was found only for more intensive tillage practices as MBC under chisel tillage was similar to NT (Zuber and Villamil, 2016). Reduced tillage not only leads to a larger microbial community, but can also lead to greater SOM compared to conventional tillage (CT), due to the slower decomposition rate of crop residues under NT. Yet the exposure of SOM from within soil aggregates as the soil is disturbed by tillage may have a greater impact in the SOM differences reported for these systems. This physically protected SOM decomposes when aggregates are disrupted by tillage, and these SOM losses are not offset by the C addition from crop residues (Balesdent et al., 2000). Zuber et al. (2015) reported greater SOC and TN under NT compared to CT on two Illinois soils after 15 years since start of experiments. The global analysis of West and Post (2002) also reported that NT increased C sequestration substantially compared to tillage, and that this difference occurred primarily near the soil surface with 85% of the difference between tillage and no-till found in the top seven centimeters of the soil.

The high agricultural production of Illinois and the Midwestern U.S. relies on the fertile soils of the region; maintaining or improving productivity is a priority that will require maximizing the potential of these soils by selecting highly productive agronomic practices that also improve soil quality. The impact of SOC on numerous other soil properties, including water holding capacity and infiltration, nutrient availability, and soil structure, compel us to understand

how SOM dynamics are influenced by agronomic practices. In Zuber et al. (2015), we examined the effect of crop rotation and tillage on soil physical and chemical properties. In that study, crop rotation, specifically the frequency of soybean within the rotation, affected the TN and aggregate stability of the soil, but did not significantly influence the SOC. Due to the strong relationship between SOC and aggregate formation (Amézketa, 1999), it was surprising that the effect of rotations was greater on aggregate stability, but was not as evident for SOC. This discrepancy indicates that other aspects of the cycling of C may be factors in the formation of aggregates and should be further investigated. Soil microbes are a vital component in SOM dynamics and microbial properties are considered to be more sensitive to environmental changes as shifts in microbial communities can occur much more rapidly than changes to chemical or physical soil properties (Nannipieri et al., 2003).

Further work is needed to evaluate the influence of these agronomic practices on SOM dynamics, specifically their effect on microbial properties, such as MBC and MBN, and the relationship to SOC and TN content of the soils. We hypothesize that crop rotations with high C:N residues or greater biomass production such as continuous corn (CCC) and corn-soybean-wheat (CSW) will lead to greater SOM and TN as well as microbial biomass as both MBC and MBN. Although a short corn-soybean (CS) rotation is more complex than continuous corn, we do not expect greater soil organic matter or microbial biomass, due to the reduced level of crop residues from soybean. It is expected that SOC, TN and both MBC and MBN biomass will be greater for all rotations under no-till than conventional tillage.

The objectives of this study are to 1) evaluate effect of long-term crop rotation and tillage practices on SOC, TN, MBC, and MBN, and 2) determine the sensitivity of these soil properties to management practices and their ability to differentiate among different management practices

after long-term use on two different highly productive Illinois soils. Understanding the effect of crop rotation and tillage can benefit farmers throughout Illinois as they strive to maintain and protect their valuable soil resource and the high productivity of the region.

MATERIALS AND METHODS

Experimental Sites

This study was conducted on two long-term crop rotation and tillage experimental studies initiated in 1996 1) at the Northwestern Illinois Agricultural Research and Demonstration Center (40°55’50” N, 90°43’38” W), approximately 8 km northwest of Monmouth, Illinois; and 2) at the Orr Agricultural Research and Demonstration Center (39°48’4” N, 90°49’16” W), approximately 8 km northwest of Perry, Illinois. At both locations, the experimental design was a split-plot arrangement of treatments rotation and tillage in a randomized complete block design with four replications. The main plot of crop rotation consisted of continuous corn (CCC), corn-soybean (CS), corn-soybean-wheat (CSW), and continuous soybean (SSS) with all phases of each rotation included. Each rotation was split into two levels of tillage: no-till (NT) and chisel tillage (CT). Each main plot was 22 m long by 12 m wide, and sub-plots were 22 m long by 6 m wide. Further descriptions of the management practices are reported in Zuber et al. (2015).

Soils at Monmouth are characterized as Sable silty clay loam (Fine-silty, mixed, mesic Typic Endoaquolls) and Muscatune silt loam (Fine-silty, mixed, mesic Aquic Argiudolls), with about 10% of the study area on Osco silt loam (Fine-silty, mixed, superactive, mesic Typic Argiudolls). These soils are dark colored and very deep with a slope of less than 2%, developed in loess 2-3 m thick over till under prairie vegetation. All three soils are moderately permeable. While Sable and Muscatune are poorly drained and somewhat poorly drained, respectively, Osco

soils are well drained (Soil Survey Staff, 2014). Experimental plots at Perry are primarily located on Downsouth silt loam (Fine-silty, mixed, mesic Mollic Oxyaquic Hapludalfs) and Caseyville silt loam (Fine-silty, mixed, mesic Aeric Endoaqualfs) soils with slope of less than 2%. Both consist of very deep, moderately well drained soils with moderate permeability formed in 1-3 m loess over till under mixed prairie and forest vegetation (Soil Survey Staff, 2014).

Soil Analyses

Soil samples were collected in May 2014 at Monmouth and in May 2015 at Perry; experimental plots were established in 1996 at both locations. Samples were collected using an Amity 4804 tractor mounted hydraulic probe (Amity Technology, Fargo, ND) to take three soil cores 4.3 cm in diameter in each subplot. Soil cores were cut to 0-10 and 10-20 cm depths. The soil samples were air-dried and sieved through 2-mm sieve, and the three subsamples from each plot were composited to provide one sample per plot. Soil organic carbon and TN were analyzed using dry combustion using an automated CHN analyzer (McGeehan and Naylor, 1988; Nelson et al., 1996) at a commercial laboratory (Brookside Laboratories, Inc., New Bremen, OH). Microbial biomass C (MBC) and N (MBN) were determined using a modified version of chloroform fumigation extraction protocol from Rice et al. (1996). Due to the large number of soil samples and the time required for processing, the protocol was modified to use air-dried soil samples. Both fumigated and unfumigated soil samples were rewet to approximately 50% of field capacity for 24 hours prior to fumigation or extraction, respectively (Sparling and West, 1989). Soil samples were fumigated for 48 hours with chloroform before extraction with 0.5 M K_2SO_4. Microbial biomass extracts were analyzed for organic C and total N on Shimadzu TOC-L and TNM-L analyzer (Shimadzu Corporation, Kyoto, Japan). Microbial biomass was calculated as the difference in C or N between the fumigated and unfumigated samples according to

standard protocol. Due to the use of air-dried samples, MBC and MBN values will only be used to as comparative values rather than as actual size of the microbial biomass; therefore, a conversion factor was not used to scale microbial biomass as is recommended in the literature (Rice et al., 1996).

Statistical Analyses

Data analysis was conducted using GLIMMIX procedure of SAS version 9.4 (SAS Institute Inc., 2012). Rotation, tillage, crop phase nested within rotation, and depth were considered fixed effects, and blocks and sites were considered random. Depth was analyzed using a repeated measures approach with the variance-covariance structure of ar(1), autoregressive, or arh(1), heterogeneous autoregressive, selected for each variable based on the Akaike's Information Criterion (Littell et al., 2006). Random interactions with site were tested for significance using -2log likelihood values, and terms that were not significant were removed from the model. A lognormal distribution (dist = logn) was used for MBC and MBN due to the lack of normality of the model residuals. Least square means were separated using LSMEANS and LINES option within GLIMMIX using a Tukey adjustment with α=0.10. When the crop phase was found to be significant, estimate statements were used to test for differences among crops within a specific rotation.

RESULTS AND DISCUSSION

Soil organic carbon was significantly affected by rotation (Table 2.1). The CSW rotation led to greater SOC levels compared to SSS; however, CS and CCC were intermediate and not significantly different from either CSW or SSS (Table 2.2). The greater SOC under CSW is probably related to the slower decomposition rate of the high C:N ratio corn and wheat residues compared to the soybean residues. Similarly, Van Eerd et al. (2014) reported that the inclusion of

winter wheat in a corn-soybean rotation led to greater SOC in two long-term (>10 years) experiments in Ontario, Canada under both NT and CT. Van Eerd et al. (2014) attributed the greater SOC with high frequency wheat crop rotations in part to the greater lignin found in wheat residues, which is recalcitrant and would slow decomposition.. The discrepancy in biomass decomposition among crops also leads to the expectation of greater SOC under CCC compared to CS as reported in a large-scale data analysis by West and Post (2002); however, no differences were found between CCC and CS within this study. Similar results were reported by Kumar et al. (2012) who compared CCC and CS under different tillage practices on two contrasting soils in Ohio.

While no differences in SOC were found between CCC and CS, SOC differed by the crop phases within the short CS rotation with greater SOC in the corn phase (19.8 g kg^{-1}) compared to soybean (17.9 g kg^{-1}) (Figure 2.1). These soils were sampled in late spring; therefore, nitrogen fertilizer had been applied and the current crop had already been planted at the time of sampling. The greater SOC under corn cannot be due to greater biomass from the soybean of the previous year, but is probably related to the relatively rapid decomposition of the soybean residues from the previous year that were already incorporated into the SOC. In contrast, the soils from the soybean phase had greater biomass from the previous year's corn crop, but it is likely those were only partially decomposed due to the slow decomposition of high C:N ratio corn residues (Melillo et al., 1989; Ajwa and Tabatabai, 1994). As any pieces of litter were removed as much as possible during the sieving process (<2 mm) prior to analysis for SOC, these corn residues would have not contributed much to the amount of SOC measured. Beyond this, it is possible that the corn residues induced positive priming, wherein the addition of fresh residues stimulates microbial activity and increases SOM decomposition rates (Blagodatskaya and Kuzyakov, 2008;

Xiao et al., 2015; Shahbaz et al., 2016) with high C:N ratio residues, such as corn, have a stronger effect than lower C:N ratio residues (Moreno-Cornejo et al., 2015). These possible explanations are further supported by the differences among crop phases of the CSW rotation. In contrast to CS rotation, the SOC of the corn (20.8 g kg^{-1}) and soybean phases (19.1 g kg^{-1}) did not differ within CSW; however, the SOC was greater during the wheat phase (21.5 g kg^{-1}) compared to the soybean phase. In both CS and CSW, the SOC of the crop phase immediately following soybean was greater than the SOC measured following corn. This difference between crop phases likely indicates that SOC levels are not as steady as expected from year to year under long-term management due to either or both a flush of SOC following soybean or a decrease in SOC following corn due to the priming effect. This fluctuation may serve as a partial explanation of the failure to detect differences between CCC and CS.

As with SOC, rotation had a statistically significant effect on TN (Table 2.1) with 16% less soil TN under SSS than the corn-based rotations (Table 2.2). Soybean within a rotation has been found to lead to decrease TN (Havlin et al., 1990; Varvel, 1994; Zuber et al., 2015). During the corn and wheat phases, N fertilizer is applied to supplement the plant available N from the soil. Of the N taken up by the plant, approximately 64% for corn (Ciampitti and Vyn, 2012) and 70% for wheat (Delogu et al., 1998) is removed with the grain; the remainder of the N is returned to the soil but is often immobilized within SOC due to the high C:N ratios of those residues (Gentry et al., 2001). This would probably lead to a subsequent increase in TN. However, the difference between the corn-based rotations and SSS may be more complex as it could be also related to a decrease in TN from the continuous soybean cropping. Soybean is a net N user leading to depletion of soil N after continuous usage (Salvagiotti et al., 2008). Ultimately, it is likely that TN decreased under SSS, but it is difficult to determine if TN increased in the corn-

based rotations as a result of N fertilizer inputs or rather was maintained to previous levels. As there were no baseline levels measured on these experimental plots at the time of establishment, the answer to that query is beyond the scope of this study. Despite no differences among the three corn-based rotations, there were significant difference between the crop phases within CS and within CSW (Figure 2.1). For each of these rotations, TN was greater during the crop phase following soybean than following corn, similar to the pattern measured for SOC.

Greater levels of both SOC and TN were found under NT than CT in the surface 10 cm, with no differences in the 10-20 cm soil depth. As residues are added to the soil surface while the soil is undisturbed, NT practices can lead to greater SOC and TN than in tilled soils as has been often reported (Havlin et al., 1990; West and Post, 2002; Varvel and Willhelm, 2011; Van Eerd et al., 2014). As in this study, the difference between NT and CT for SOC and other nutrients has often been found only in the surface soils (Franzluebbers and Hons, 1996; Needelman et al., 1999). Our previous work, Zuber et al. (2015), examined both soil physical and chemical properties, including SOC and TN, and found that C and N stocks to a depth of 60 cm were greater in NT compared to CT when measured two years prior to this study.

Soil organic matter dynamics are highly dependent on microbial communities as the stable form of SOM is a product of microbial decomposition (Fontaine and Barot, 2005; Chabbi and Rumpel, 2009). Microbial biomass can provide an indirect indication about how the size of a microbial community is affected by agronomic practices. Within our study, there were no differences among crop rotations for either MBC or MBN (Table 2.1). In contrast, McDaniel et al. (2014) conducted a global meta-analysis evaluating the effect of crop rotation on microbial biomass and reported large increases in both MBC (20.7%) and MBN (26.1%) with a rotation compared to the use of a single crop regardless of the number of crops within the rotation.

Within the Midwest, studies by Russell et al. (2006) and Ekenler and Tabatabai (2002), both in Iowa, reported greater MBC within a crop rotation compared to a no rotation of crops, which is likely related to the greater diversity of organic matter inputs from a rotation that would stimulate microbial community growth. However, the crop rotation that resulted in increased MBC was more complex than those in our study and included oats and alfalfa. When comparing CCC and CS, Russell et al. (2006) reported greater MBC under CCC than under CS, indicating that the short CS rotation did not augment microbial community size. The lack of differences among rotation for MBC and MBN in our study may indicate that the increase in diversity of residues by including other crops within the rotation is offset by the decrease in biomass available to microbes or differences in the residue quality, especially following the soybean phase of the rotation.

Tillage had a greater influence on microbial biomass than crop rotation as the interaction effect of tillage by depth was significant for both MBC and MBN at α=0.10 (Table 2.1). There was a trend of greater MBC under NT compared to CT at the surface 10 cm (Table 2.3 and 2.4); however, this should be considered with caution as when adjusted with Tukey's for multiple comparisons, this difference was not significant. For MBN, there was no difference between tillage practices at the surface, but from 10-20 cm MBN was greater in NT than CT. These results are rather unexpected as the meta-analysis by Zuber and Villamil (2016) reported greater MBC and MBN with NT compared to tillage. However, the difference between NT and tillage was minimal for MBC when the tillage implement was a chisel plow, such as was used in this study. For MBN, as with SOM and TN, we would expect that any difference between tillage practices would occur at the surface. In this case, the opposite result was found as MBN was greater with NT than with CT at the 10-20 cm depth. However, for most of the crop rotations,

MBN was similar between CT and NT at both 0-10 cm and 10-20 cm depths. The exception to this was CCC, which had MBN at 10-20 cm depth of 1.85 mg kg^{-1} under CT while the other three rotations averaged 5.75 mg kg^{-1}. While the high C:N ratio corn residues in this soil would be expected to lead to a net immobilization as N is incorporated by microbes, the addition of N fertilizer would likely have increased mineralization. The N would have been incorporated into microbial cells as the microbial extracellular and intracellular enzyme broke down the residues. Following the augmentation of microbial biomass, microbe predation would subsequently increase, releasing plant-available N and reducing MBN; this is similar to the microbial loop process following the release of root exudates into the rhizosphere (Bonkowski, 2004; Nannipieri and Eldor, 2009). Microbial metabolism as well as microbial stress and death would also release immobilized N (Schimel and Bennett, 2004; Nannipieri and Eldor, 2009) and could have led to the reduction in MBN found in our study under tilled CCC. Another component of this result may be related to soil pH as the yearly N fertilization likely increased the acidity of the soil as lower pH has been found in rotations with more frequent N fertilization (Divito et al., 2011; Zuber et al., 2015). A decrease in microbial biomass as a result of N fertilization was reported in a meta-analysis by Treseder (2008). The shift in soil pH affects availability of nutrients such as calcium and magnesium within the soil, which in turn can affect the microbial community (Vitousek et al., 1997).

The lack of significant differences among rotations and the relatively low significance of tillage by depth both indicate a surprising lack of sensitivity of microbial biomass to crop rotation and tillage. Microbial biomass, like most biological properties, is highly variable and as a result of sensitivity to environmental conditions, fluctuates seasonally (Carter et al., 1999). It is possible that at the time of sampling the MB was similar regardless of rotation or tillage, but later

changed as a result of root exudates, temperature, or precipitation so a different timing of sampling could have led to more expected results.

CONCLUSIONS

The impact of crop rotation on SOC and TN in two Illinois soils was relatively minor as differences in SOC were found only between CSW and SSS while SSS also led to less TN compared to the corn-based rotations. This indicates that the inclusion of high C:N ratio residues (corn or wheat) within a rotation help to maintain both C and N storage within SOM, but the frequency of these crops within the rotation only minimally affects the levels of SOC and TN. Rather, it is the specific phase within the rotation that is an important factor in our failure to detect differences among the corn-based rotations. The reduced SOC and TN measured during the growing season following corn indicates that even 17 years after establishment, there are seasonal fluctuations within SOM that affects our ability to detect differences among rotations. In contrast to the effect of rotation, tillage had a clear impact on SOM as the use of no-till led to 7% and 9% greater SOC and TN, respectively, compared to tillage. While tillage and, to a lesser degree, rotation influenced the storage of C and N within SOM, their effect on the microbial community was not as clear. Despite the purported sensitivity of biological properties such as microbial biomass, these soil parameters were relatively stable among crop rotation and tillage practices on highly productive Illinois soils. High variability in these measures is likely an important component of the lack of differences. While microbial biomass provides a snapshot of the community size, more in-depth evaluation of the structure and diversity of the microbes may reveal how crop rotation and tillage influence the community in other ways.

FIGURE

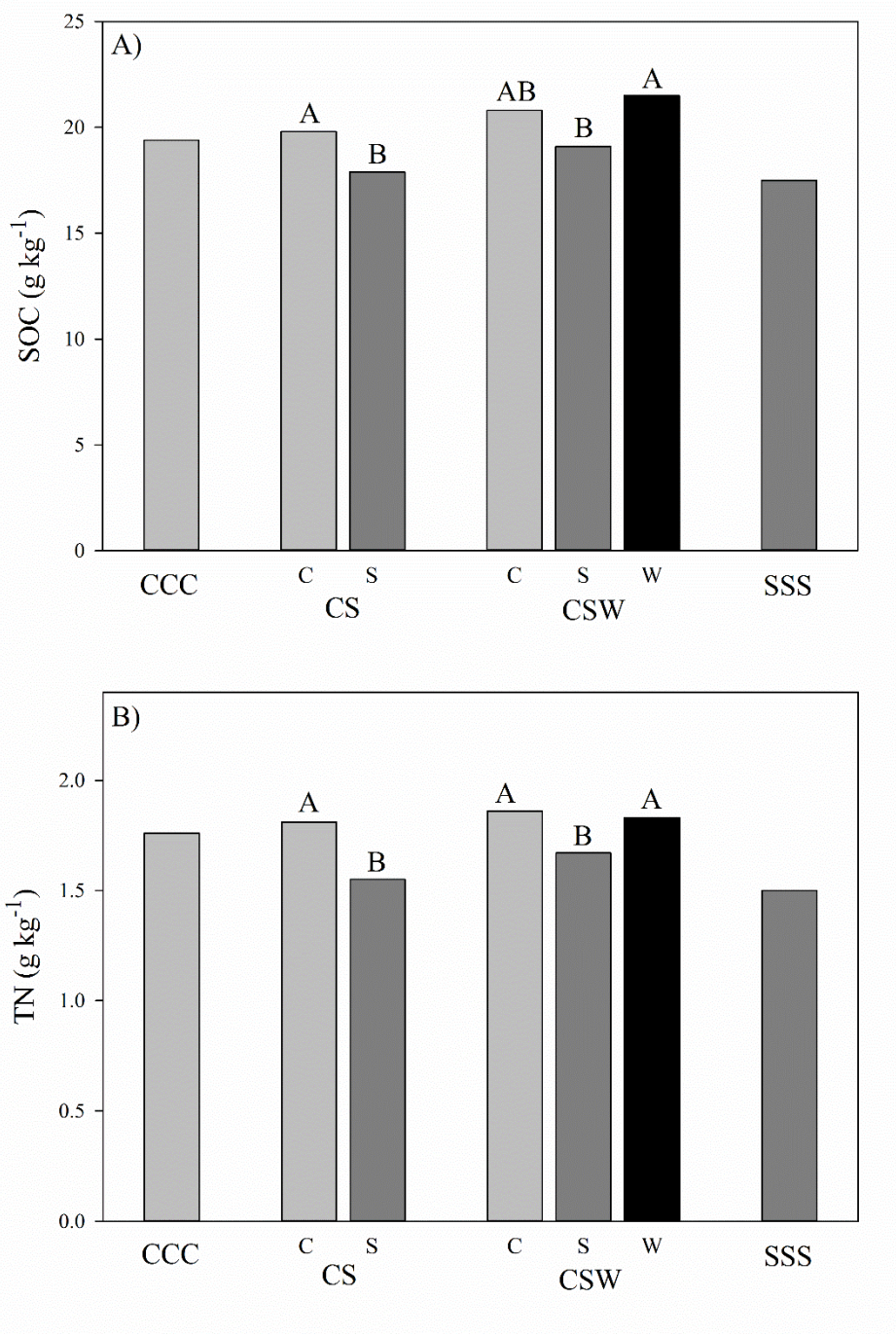

Figure 2.1 Effect of crop phase (Corn, C; Soybean, S; Wheat, W) within rotations of continuous corn (CCC), corn-soybean (CS), corn-soybean-wheat (CSW) and continuous soybean (SSS) on A) soil organic carbon (SOC) and B) total nitrogen (TN) across two sites for 0-20 cm soil depth. Within a crop rotation, bars with the same letter are not significantly different at α=0.10.

TABLES

Table 2.1 Probability values associated with the analysis of variance of the effects of rotation, tillage, and depth and their interactions on the variables—soil organic carbon (SOC), total nitrogen (TN), microbial biomass C (MBC), and microbial biomass N (MBN).

Source	SOC	TN	MBC	MBN
Rotation (R)	**0.057**	**0.001**	0.557	0.507
Tillage (T)	**0.072**	**0.021**	0.249	0.112
R x T	0.550	0.319	0.819	0.358
Depth (D)	**0.001**	0.352	**<0.0001**	**<0.0001**
R x D	0.590	0.117	0.600	0.363
T x D	**0.074**	**0.001**	**0.098**	**0.086**
R x T x D	0.901	0.669	0.267	0.339

Table 2.2 Mean values and standard errors (SE) of soil organic carbon (SOC) and total nitrogen (TN) across two sites with rotation and tillage.

		Depth (cm)						Depth (cm)					
		0-10		10-20		0-20		0-10		10-20		0-20	
		SOC (g kg^{-1})						TN (g kg^{-1})					
Rotation	Tillage	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
CCC[*]	CT	19.8	6.4	17.3	6.3	18.5	6.3	1.83	0.31	1.57	0.13	1.70	0.17
	NT	22.2		18.3		20.2		2.03		1.62		1.82	
CS	CT	19.2		17.3		18.3		1.81		1.50		1.65	
	NT	21.7		17.1		19.4		2.37		1.50		1.94	
CSW	CT	21.4		19.2		20.3		2.04		1.65		1.84	
	NT	23.2		18.2		20.7		2.05		1.55		1.80	
SSS	CT	16.8		16.2		16.5		1.39		1.38		1.38	
	NT	20.1		17.7		18.6		1.78		1.46		1.62	
CCC		21.0	6.4	17.8	6.4	19.4AB[§]	6.3	1.93	0.23	1.60	0.13	1.76 A	0.14
CS		20.5		17.2		18.8AB		2.09		1.50		1.80 A	
CSW		22.3		18.7		20.5 A		2.04		1.60		1.82 A	
SSS		18.4		16.6		17.5 B		1.58		1.42		1.50 B	
	CT	19.3 b[†‡]	6.3	17.5 b	6.3	18.4 B	6.3	1.76 b	0.17	1.52 ab	0.12	1.64 B	0.11
	NT	21.8 a		17.7 b		19.7 A		2.06 a		1.53 ab		1.79 A	

[*]CCC, continuous corn; CS, corn-soybean; CSW, corn-soybean-wheat; SSS, continuous soybean; NT, no-till; CT, conventional tillage.

[†]Letters indicating significant differences are shown only for significant effects (α=0.10).

[‡]Within a combination of factors, means for a specific variable followed by the same lowercase letter are not significantly different (α=0.10).

[§]Within a column and factor, means averaged over depths followed by the same uppercase letter are not significantly different (α=0.10).

Table 2.3 Mean values and standard errors (SE) of natural logarithm transformed microbial biomass C (MBC) across two sites as affected by rotation and tillage. Backtransformed means are shown in parentheses in units of mg kg^{-1}.

		Depth (cm)					
		0-10		10-20		0-20	
Rotation	Tillage	Mean	SE	Mean	SE	Mean	SE
CCC*	CT	3.75 (42.7)	0.31	3.32 (27.6)	0.31	3.53 (34.3)	0.28
	NT	4.12 (61.5)	0.31	3.43 (30.8)	0.31	3.77 (43.5)	0.28
CS	CT	4.02 (55.5)	0.28	3.57 (35.4)	0.28	3.79 (44.3)	0.26
	NT	4.37 (79.3)	0.28	3.61 (36.8)	0.28	3.99 (54.0)	0.26
CSW	CT	4.12 (61.6)	0.27	3.29 (26.9)	0.27	3.71 (40.7)	0.26
	NT	4.23(68.6)	0.27	3.52 (33.7)	0.27	3.87 (48.1)	0.26
SSS	CT	4.13 (61.9)	0.31	3.66 (39.0)	0.31	3.89 (49.1)	0.28
	NT	4.31 (74.7)	0.31	3.32 (27.8)	0.31	3.82 (45.6)	0.28
CCC		3.94 (51.2)	0.27	3.37 (29.1)	0.27	3.65 (38.6)	0.25
CS		4.19 (66.3)	0.25	3.59 (36.1)	0.25	3.89 (48.9)	0.24
CSW		4.17 (65.0)	0.24	3.40 (30.1)	0.24	3.79 (44.3)	0.24
SSS		4.22 (68.0)	0.27	3.49 (32.9)	0.27	3.86 (47.3)	0.25
	CT	4.00 (54.8) a†‡	0.24	3.46 (31.8) b	0.24	3.73 (41.8)	0.23
	NT	4.26 (70.7) a	0.24	3.47 (32.1) b	0.24	3.86 (47.6)	0.23

*CCC, continuous corn; CS, corn-soybean; CSW, corn-soybean-wheat; SSS, continuous soybean; NT, no-till; CT, conventional tillage.

†Letters indicating significant differences are shown only for significant effects (α=0.10).

‡Within a combination of factors, means followed by the same lowercase letter are not significantly different (α=0.10).

Table 2.4 Mean values and standard errors (SE) of natural logarithm transformed microbial biomass N (MBN) across two sites as affected by rotation and tillage. Backtransformed means are shown in parentheses in units of mg kg^{-1}.

		Depth (cm)					
		0-10		10-20		0-20	
Rotation	Tillage	Mean	SE	Mean	SE	Mean	SE
CCC*	CT	1.80 (6.04)	0.36	0.61 (1.85)	0.29	1.21 (3.34)	0.28
	NT	1.86 (6.43)	0.36	1.68 (5.37)	0.28	1.77 (5.87)	0.27
CS	CT	2.19 (8.94)	0.27	1.54 (4.67)	0.22	1.87 (6.46)	0.22
	NT	2.06 (7.86)	0.27	1.51 (4.52)	0.21	1.79 (5.96)	0.21
CSW	CT	2.10 (8.14)	0.23	1.37 (3.94)	0.19	1.73 (5.66)	0.19
	NT	2.04 (7.66)	0.23	1.61 (4.98)	0.19	1.82 (6.18)	0.19
SSS	CT	2.17 (8.73)	0.36	1.13 (3.11)	0.28	1.65 (5.21)	0.27
	NT	2.66 (14.28)	0.36	1.60 (4.97)	0.28	2.13 (8.43)	0.27
CCC		1.83 (6.23)	0.27	1.15 (3.15)	0.22	1.49 (4.43)	0.21
CS		2.13 (8.38)	0.21	1.52 (4.59)	0.18	1.83 (6.21)	0.17
CSW		2.07 (7.90)	0.19	1.49 (4.43)	0.16	1.78 (5.92)	0.16
SSS		2.41 (11.17)	0.27	1.37 (3.93)	0.21	1.89 (6.62)	0.21
	CT	2.06 (7.87) a†‡	0.17	1.16 (3.20) c	0.14	1.61 (5.02)	0.14
	NT	2.15 (8.62) a	0.17	1.60 (4.95) b	0.14	1.88 (6.53)	0.14

*CCC, continuous corn; CS, corn-soybean; CSW, corn-soybean-wheat; SSS, continuous soybean; NT, no-till; CT, conventional tillage.

†Letters indicating significant differences are shown only for significant effects (α=0.10).

‡Within a combination of factors, means followed by the same lowercase letter are not significantly different (α=0.10).

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CHAPTER 3. Multivariate Assessment of Soil Quality Indicators for Crop Rotation and Tillage in Illinois

ABSTRACT

The long-term implementation of crop rotation and tillage influence the soil environment through inputs and disturbance of the soil, which in turn, impact soil quality (SQ). The first step in evaluating SQ is to identify soil parameters that are sensitive to changes in the soil and indicative of soil functions. Soil samples were collected from two Illinois sites with cropping systems and tillage treatments in place for more than 16 years. Crop rotation and tillage were evaluated with separate principal component analyses (PCA) of 20 soil parameters. Six principal components accounted for 74% of variability among rotations. The soil parameters loaded within these components highlighted the strong influence on carbon and nitrogen cycling indicated by greater soil organic carbon, total nitrogen, microbial biomass, and aggregate stability under crop rotations with high C:N residues and biomass production. Other strongly loaded parameters, such as soil pH and nutrient contents, are likely related to the use of nitrogenous fertilizers in grass species. The PCA for tillage explained 73% of variability with six principal components; of those, three were able to separate no-till from conventional tillage. As with rotation, the choice of tillage practice can have a large influence on the cycling of carbon and nitrogen, as decomposition of residues and soil organic matter are accelerated by tillage. No-till was also associated with stratification of pH and other nutrients. For crop rotation and tillage, soil parameters related to carbon and nitrogen cycling and those affected by nitrogen fertilizers have the greater potential for use in comparing SQ under different agronomic practices in Illinois.

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INTRODUCTION

Maintaining or improving soil fertility and productivity is central to developing sustainable agricultural practices. Soil quality is defined as the capacity of a soil to function within ecosystem boundaries to sustain biological productivity, maintain environmental quality and promote plant and animal health (Doran and Parkin, 1994). Soil physical, chemical and biological properties all provide information about different aspects of the soil as a system. Many researchers have developed soil quality indices (SQI) that allow incorporation of many different soil properties into a single value with the purpose of comparing agronomic practices in relation to the productivity of those soils.

The first step in evaluating SQ as an index is to determine the small set of soil properties that will be utilized as soil quality indicators for a given region (Andrews et al., 2004). A suitable indicator should be sensitive to management and should convey information about the soil functions and processes occurring within the soil (Doran and Zeiss, 2000). While several of the SQIs in the literature have utilized soil chemical and physical properties, the sensitivity of biological properties to changing management has increased their use in SQIs (Aziz et al., 2013). Management practices as well as soil type, climate and other environmental characteristics should also be taken into consideration during indicator selection as an individual indicator is not equally useful or sensitive in all locations or situations (Cardoso et al., 2013).

A variety of contrasting methods have been used for selection of sensitive indicators since the concept of SQ arose. As a first approach, Karlen et al. (1994) used expert opinion to weight scores representing different functions of the soil. Later on, quantitative methods to select indicators were based on multivariate analysis of soil properties (Brejda et al., 2000; Shukla et al., 2006; Nosrati, 2013). For example, Wander and Bollero (1999) used principal component analysis (PCA) to evaluate the effect of tillage on SQ in Illinois. To use a PCA as a selection

tool, only those variables that are strongly loaded into the PCs are kept to be used as indicators in a SQI. Wander and Bollero (1999) selected bulk density (BD), aggregate stability, penetration resistance, organic C, total N (TN), K, soil pH, particulate organic matter, basal respiration, and microbial biomass carbon (MBC) as the indicators to separate tillage practices. Andrews et al. (2002) compared several methods of developing an SQI, specifically expert opinion based indicators versus indicators selected using PCA. While both methods provided similar representative SQIs as related to the measurement of environmental and production goals, the strength of a quantitative approach is the avoidance of subjectivity. However, statistical methods of indicator selection require a large data set and may prove more difficult to interpret than using expert opinion to select indicators (Bastida et al., 2008).

While each SQI uses a different set of indicators, certain soil parameters are frequently selected when evaluating agricultural systems. Soil organic carbon (SOC) has often been considered a reliable indicator of SQ as it is so closely related to other soil properties, including soil structure, nutrient availability, water holding capacity, and erosion resistance (Doran and Parkin, 1994; Islam and Weil, 2000; West and Post, 2002) as well as influencing microbial activity (Schimel and Schaeffer, 2012). Other chemical properties commonly selected as indicators include soil pH, cation exchange capacity (CEC), and nutrient availability, connected to the ability of a soil to provide adequate nutrients and support plant growth (Bastida et al., 2008; Cardoso et al., 2013). Physical soil properties such BD, porosity and aggregate stability are often included as they are simple, inexpensive measurements that are related to the aeration of the soil, infiltration capacity as well as the ability to resist erosion processes (Schoenholtz et al., 2000). Properties that are inherent to the soil, such as texture, might not work as indicators. While soil texture impacts many other facets of the soil environment from water holding capacity

to CEC, it is a relatively stable measurement that is unlikely to change as a result of agricultural practices so is not particularly useful as an indicator to differentiate between management practices (Cardoso et al., 2013). Biological properties are receiving increased attention in SQIs as these properties are more sensitive to alterations in the soil environment than physical and chemical soil properties (Jimenez et al., 2002; Nannipieri et al., 2003). In some cases, SQIs have been developed that only include the biological component based on the assumption that changes in chemical and physical properties will be related to the changes in the microbial community (Puglisi et al., 2006; Romaniuk et al., 2011). Biological properties often included as indicators are microbial biomass, metabolic quotient, and enzyme activities (Gil-Sotres et al., 2005; Cardoso et al., 2013). While these properties are very sensitive to changes in agronomic practices, they also have high levels of variability as well as significant temporal fluctuations that need to be considered when using them as indicators in an SQI (Bastida et al., 2008; Cardoso et al., 2013; Zuber and Villamil, 2016).

The goal of the SQI and the agronomic practices that we wish to assess will also influence which indicators are most suitable. If the goal is to reduce environmental impact, the indicators selected may be different from those selected when trying to maximize a soil's productivity. In creating a SQI evaluating different crop rotations, indicators selected may vary from those included in a SQI for tillage practices. Crop rotations influence the soil environment and microbial communities primarily through differences in the quantity and quality of crop residues returned to the soil (McDaniel et al., 2014a). Tillage increases the rate of decomposition of those residues by breaking up the tissues, thus increasing microbial access to the substrates. The soil environment also changes as a mulch layer develops in no-till soils, retaining moisture and lowering the temperature compared to conventionally tilled soils (Johnson and Hoyt, 1999).

Crop rotations that include high C:N residue producing crops like corn (*Zea mays* L.) and wheat (*Triticum aestivum* L.) more frequently as well as the use of no-till have been found to lead to higher SOC, TN, and aggregate stability (Benjamin et al., 2010; Zuber et al., 2015). The sensitivity of these measures to agronomic practices demonstrates their potential as SQI indicators. Karlen et al. (2006) used bulk density, soil pH, aggregate stability, SOC, TN, microbial biomass C, extractable P and K, and penetration resistance in a SQI to assess crop rotations in Iowa and Wisconsin; SOC was found to be the most sensitive indicator to the effects of rotation. Similar SQI indicators used by Jokela et al. (2011) included aggregate stability, BD, SOC, potentially mineralizable N, MBC, pH and soil P to compare grain rotations with forage and pasture systems. Aziz et al. (2013) used MBC, basal respiration, metabolic quotient, SOC, TN, active C, aggregate stability, porosity, and particulate organic matter as components of the SQI to compare three crop rotations under both no-till and conventional tillage. However, these studies included indicators based on available measures and methodologies rather than using a multivariate approach to select indicators. Shukla et al. (2006) used factor analysis to identify SOC as the most sensitive measurement for SQ for comparing five different tillage and crop rotation cropping systems. Fuentes et al. (2009) also reported SOC as a significant indicator for different tillage practices with monoculture and rotations; in addition TN, aggregate stability, penetration resistance, pH, and electrical conductivity were selected as indicators using PCA.

The high productivity of corn and soybean (*Glycine max* [L.] Merr.) in Illinois is directly related to the fertility and quality of the soils. The determination of suitable SQ indicators for this region will help to maintain those high productivity levels as it is vital to protect this key factor in the productivity of the state. We expect that for differentiating among crop rotations soil properties closely related to the crop residue quantity and quality, such as SOC and aggregate

stability will be more sensitive indicators. For tillage, those properties will also be important, as will the physical properties related to the structure and compaction of the soil. Within this study, the objective is to determine which soil properties are most sensitive to crop rotations and tillage practices after long-term management at two Illinois sites with contrasting soils as well as to evaluate how the interaction of crop rotation and tillage practice affects soil quality and potential indicators.

MATERIALS AND METHODS

Experimental Sites

Experimental sites were initiated in 1996 at the Northwestern Illinois Agricultural Research and Demonstration Center (40°55’50” N, 90°43’38” W), approximately 8 km northwest of Monmouth, Illinois and at the Orr Agricultural Research and Demonstration Center (39°48’4” N, 90°49’16” W), approximately 8 km northwest of Perry, Illinois. The experimental layout at both sites was a split-plot arrangement of rotation (four levels) and tillage (two levels), in a randomized complete block design with four replications. Rotation was assigned to the main plot and consisted of continuous corn (CCC), corn-soybean (CS), corn-soybean-wheat (CSW), and continuous soybean (SSS) with all phases of each rotation present each year (seven main plots). Each rotation main plot was split into two levels of tillage: no-till (NT) and chisel tillage (CT). Each main plot was 22 m long by 12 m wide, and sub-plots were 22 m long by 6 m wide. Agronomic management practices are described in more detail in Zuber et al. (2015).

Soils at Monmouth are as Sable silty clay loam (Fine-silty, mixed, mesic Typic Endoaquolls) and Muscatune silt loam (Fine-silty, mixed, mesic Aquic Argiudolls), with about 10% of the study area on Osco silt loam (Fine-silty, mixed, superactive, mesic Typic Argiudolls). These soils are dark colored and very deep with a slope of less than 2%, developed under prairie

vegetation in loess 2-3 m thick over till. All three soils are moderately permeable. Sable and Muscatune are poorly drained and somewhat poorly drained, respectively, and Osco well drained (Soil Survey Staff, 2014). Experimental plots at Perry were primarily located on Downsouth silt loam (Fine-silty, mixed, mesic Mollic Oxyaquic Hapludalfs) and Caseyville silt loam (Fine-silty, mixed, mesic Aeric Endoaqualfs) soils with slope of less than 2%. Both consist of very deep, moderately well drained soils with moderate permeability formed under mixed prairie and forest vegetation in 1-3 m loess over till (Soil Survey Staff, 2014).

Soil Analyses

Soil samples were collected in May 2014 at Monmouth and in May 2015 at Perry; this followed at least six full cycle of each rotation at each site. Samples were collected using an Amity 4804 tractor mounted hydraulic probe (Amity Technology, Fargo, ND) to take three soil cores 4.3 cm in diameter in each subplot. Soil cores were cut to 0-10 and 10-20 cm depths and stored refrigerated at 4°C in plastic bags until analysis. Soil bulk density was determined by the core method (Blake and Hartge, 1986). The remaining soil samples were air-dried and sieved through 2-mm sieve, and the three subsamples from each plot were composited to provide one sample per plot. Three subsamples of the 1- to 2-mm soil fraction were used to determine water aggregate stability (WAS) with an Eijkelkamp wet sieving apparatus (Eijkelkamp Agrisearch Equipment, Netherlands) using the procedure developed by Kemper and Rosenau (1986). Microbial biomass C (MBC) and N (MBN) were determined with chloroform fumigation extraction protocol from Rice et al. (1996) which was modified for air-dried soil samples. Soil samples were rewet to approximately 50% of field capacity 24 hours prior to fumigation or extraction for unfumigated samples (Sparling and West, 1989). Fumigation with chloroform occurred for 48 hours prior to extraction with 0.5 M K_2SO_4. The analysis for organic C and total

N was conducted on a Shimadzu TOC-L and TNM-L analyzer (Shimadzu Corporation, Kyoto, Japan). Air-dried soil samples were sent to a commercial laboratory for the determination of the remainder of the soil properties (Brookside Laboratories, Inc., New Bremen, OH). Soil pH was determined with 1:1 soil:H_2O solution. Soil organic carbon (SOC) and TN were analyzed using dry combustion using an automated CHN analyzer (McGeehan and Naylor, 1988; Nelson et al., 1996). Available P was analyzed following Bray I extraction (Bray and Kurtz, 1945). Other macronutrients (K, Ca, Mg, S) and micronutrients (B, Fe, Mn, Cu, Zn) as well as Na and Al were determined following Mehlich III extraction (Mehlich, 1984). Cation exchange capacity (CEC) was determined by the summation method of exchangeable cations (Ca, Mg, K, Na, H) (Sumner and Miller, 1996).

Statistical Analyses

The data set included 20 variables including the physical properties of BD and WAS, chemical properties of SOC, TN, CEC, pH, P, K, S, Ca, Mg, Na, B, Fe, Mn, Cu, Zn, Al and the biological properties MBC and MBN. Means for 0-10 and 10-20 cm soil depths and the units for each of the variables are shown in Table 1. Soil samples were taken at two depths within each subplot from four blocks at each location for a total of 224 observations.

Statistical analysis was conducted with SAS version 9.4 (SAS Institute Inc., 2012). The first step in analysis was to perform a multivariate analysis of variance (MANOVA) using PROC GLM. Since the purpose of this study was to determine sensitive SQ indicators over the entire region, site was considered random. In order to treat site as a random effect in the next steps of analysis, the data was detrended (Khattree and Naik, 2000); an initial MANOVA was performed modeling only site and residuals were outputted for each of the variables to be used in all

subsequent analyses. A second MANOVA was performed to evaluate if rotation, tillage, depth, and their interactions significantly affected at least one of the variables.

The next step was to conduct a PCA using PROC PRINCOMP; PCA creates new uncorrelated variables called principal components (PCs) that are linear combinations of the original raw variables. These PCs maximize the variability explained by the set of variables (Johnson, 1998). The variables within this data set have differing variances and units; to avoid the dominance of the variable with the highest variance in the first PC, the PCA was conducted on standardized data. Eigenvalues and eigenvectors were extracted from the correlation matrix rather than the covariance matrix. The principal components (PC) that were kept were selected based on the eigenvalues greater than 1 and the proportion of total variability explained by the PCs. Multiple PCAs were performed; one PCA was conducted using all 224 observations while another was analyzed by depth with 112 observation for each depth. Eigenvalues and eigenvectors were compared among the results of the PCAs. For each PC, PC scores for each observation were extracted and a mixed model ANOVA was run for each of the PCs to evaluation the effect of rotation and tillage on soil quality. Loadings greater than 0.40 were included in the interpretation of the PC. The ANOVA was run in PROC MIXED with block as a random effect and rotation, tillage, and their interaction as fixed effects. Least square means were separated using LSMEANS and the PDIFF option within PROC MIXED using a Tukey adjustment with α=0.05.

RESULTS

The results from the MANOVA are shown in Table 2. The effect of site was detrended from the data set prior to this analysis so these results indicate if rotation, tillage, depth or their

interactions significantly affected at least one of the 20 variables evaluated within this analysis across both locations. Rotation, tillage and depth were significant with tillage x depth interaction also being significant. One key finding is the lack of significant difference of the rotation x tillage interaction, which indicates that the effect of rotations on the soil properties does not differ based on the tillage practice being used. The significance of tillage x depth interaction is indicative of the stratification that occurs under NT as residues are accumulated on the soil surfaces while in CT, residues are distributed throughout the entire layer of soil that is disturbed by the tillage action (Franzluebbers and Hons, 1996; Kladivko, 2001). The stratification of NT often leads to differences between NT and CT found only near the soil's surface (Needelman et al., 1999; Zuber et al., 2015). In contrast, the rotation x depth interaction was not significant which shows that any effect of rotation is consistent between the two soil depths evaluated within this data.

Since the effect of tillage differs with depth, but rotation does not and the interaction of rotation and tillage was not significant, we decided to analyze rotation and tillage separately. For rotation, the PCA included observations from both depths and essentially treated the two depths as replicates. To analyze tillage, a separate PCA was conducted for each depth. While this reduced the number of observations utilized in the PCA, it also allowed for the interpretation of which indicators were more useful within the top 10 cm where differences between tillage treatments are more likely to be found. Since separate PCAs were conducted for rotation and tillage, the rotation PCs will be referred to as R-PC and tillage as T-PC throughout the remainder of the text.

Rotation

In the PCA used to evaluate rotation, six PCs were selected as they each had eigenvalues greater than 1 (R-PC6 had an eigenvalue of 0.99). These six PCs (R-PC1 to R-PC6) together explain 74% of the total variability within the dataset (Table 3). The eigenvalue of R-PC1 was 4.82 and this PC explained 24% of the variability. The loading of this PC consisted of positive loadings of TN, K, Fe, S, SOC, CEC, P, and Zn and the negative loading of pH. The next PC had an eigenvalue of 3.02 and explained 15% of the variability; loadings for R-PC2 consisted of positive loadings of Zn, B, SOC, MBC, Ca, Cu, Mg, P and the negative loading of Al. R-PC3, with an eigenvalue of 2.93, explained 15% of the variability, with positive loadings of Mg, Cu, CEC, Ca, WAS, Al and negative loading of Mn. The eigenvalue of the fourth PC was 1.73 and R-PC4 explains 9% of the variability. Four soil parameters were positively loaded into this PC—Na, BD, S, and MBN. MBC was also strongly positively loaded with a value just below the threshold used in this analysis. R-PC5 had an eigenvalue of 1.25 and explained 6% of the variability; this PC was a contrast between Mn and MBN. The final PC had an eigenvalue of 0.99, just below the threshold of 1, but as it explains 5% of the variability and is very close to the threshold, it was retained as it still may provide valuable information. R-PC6 consisted of a contrast between BD and P.

Each of these six R-PCs was evaluated as to their ability to differentiate among crop rotations, and for all six R-PCs, there were significant differences among the mean PC scores of the four different crop rotations. The first three R-PCs primarily were able to separate the two monocultures (CCC and SSS) from one another with the crop rotations (CS and CSW) intermediate between the two. For R-PC1, PC scores were greatest for CCC, followed by CSW and CS, and the smallest PC scores for SSS. In contrast, for R-PC2 the highest PC scores were

for SSS followed by CSW and CS, and the smallest (most negative) score was for CCC. The trend for R-PC3 was similar to R-PC1 with the greatest PC scores for CCC, smallest for SSS, and CS and CSW intermediate. For the last three R-PCs, each was able to separate one particular rotation from the other three, which can help us to separate these rotations beyond the two extreme single crop rotations. The value for R-PC4 of the CSW rotation was much lower than the PC scores of the other three rotations. R-PC5 was able to separate CCC from the other three with a positive PC score, while the scores for the other three rotations were all negative. The final R-PC separated SSS with a much smaller value for R-PC6 compared to the other three rotations.

Tillage

Based on the significance of the tillage x depth interaction from the MANOVA, only the PCA for the surface 10 cm will be shown; no significant differences between tillage practices were found in the 10-20 cm soil depth. For the T-PCA, six PCs were retained as they each had eigenvalues greater than 1, and the six PCs (T-PC1 to T-PC6) explained 72% of the total variability in the observations (Table 4). The eigenvalue for T-PC1 was the largest (4.73) and this PC explained 24% of the variability. T-PC1 was a contrast of CEC, Fe, Al, S, and TN with positive loadings against the negative loadings of pH, B, Ca, and Mg. T-PC2 explained 17% of the variability with mostly positive loadings of SOC, WAS, TN, Cu, and Zn and the negative loading of Mn. T-PC3 explained another 11% of the variability and consisted of only positive loadings for BD, Na, Mg, and Cu. T-PC4 explained 8% of the variability as a contrast between P, K , and Mn versus MBN and WAS. Another 7% of the variability was explained by T-PC5, which had high loadings for MBC, MBN and P. The final PC for the 0-10 cm depth explained 6% of the variability; T-PC 6 was a contrast between BD and P. Principal component scores

were extracted for each of these six T-PCs to evaluate their ability to separate tillage practice. Three of the T-PCs were significantly different for tillage practice –T-PC1, T-PC2, and T-PC5 with all three having positive values for NT and negative values for CT (Table 4).

DISCUSSION

Rotation

Rotation was significant in the ANOVA for each of the six R-PCs. The first three R-PCs essentially provided a separation of the two continuous-cropping sequences, with the two-year CS and three-year CSW rotations intermediate (Table 3). This is illustrated in Figure 3.1A as the PC scores of CCC are primarily in the lower right quadrant while SSS data points are located mostly in the upper right quadrant. The continuous cropping of corn would be expected to alter the soil parameters differently than SSS so it makes sense that the three PCs that together explain 54% of the variability would be able to differentiate between these monocultures. It is, however, very useful that the final three PCs each separate one of the rotations from the other three in order to identify one or more particular soil property that differs in that rotation from the others.

The ability of a crop rotation to influence the soil quality is primarily through the specific inputs related to the crop species included within the rotation (McDaniel et al., 2014). Crops differ in the quantity and quality of residues returned to the soil and the amount of fertilizers, especially N, applied to the soil. Many of the soil parameters related to C and N cycling were found to be strong indicators of differences among crop rotation. In R-PC1, there was a strong relationship between SOC, TN, and CEC, which were all positively loaded; R-PC1 scores for the rotations ranked CCC > CSW ≥ CS > SSS. The greater mean PC scores for CCC compared to the other rotations and SSS is likely related to the higher biomass production of corn compared

to soybean as well as the more frequent application of N fertilizer to corn and wheat. Greater SOC has often been reported for CCC compared to other corn based rotations and SSS (Benjamin et al., 2010; Kumar et al., 2012; Zuber et al., 2015). We (Zuber et al., 2015) also reported lower TN with SSS than CCC and corn-based rotations due to the lack of N fertilization in soybean. Although soybean is able to use biological N fixation to supply N, it must still obtain N from the soil and is a net N user (Salvagiotti et al., 2008).

There is a strong relationship between SOC and CEC as clay minerals and soil organic matter are the two sources of the negatively charged sites that account for CEC, but much of the CEC results from soil organic matter (Parfitt et al., 1995). Cation exchange capacity also had a positive relationship with WAS as can be seen in R-PC3, which followed a similar pattern for the rotations with greatest PC scores for CCC and lowest for SSS. Like CEC, WAS has been found to be highly correlated to SOC (Martens, 2000; Zuber et al., 2015), and both CEC and WAS are dependent on the clay minerals and SOC within the soil (Amézketa, 1999). Another important aspect of C and N cycling is the microbial community as it is microbial activities that are the primary drivers of crop residue decomposition and formation of SOC (Fontaine and Barot, 2005); this is highlighted by the loading of SOC and MBC into R-PC2. The PC scores of R-PC2 contradict those of R-PC1 with the greatest values for SSS indicating that other stronger loaded properties have a greater impact on this PC.

The differing application of N fertilizers among crops affects not only N cycling within the soil, but also likely altered several other chemical properties of the soil. Soil pH is very strongly negatively loaded in R-PC1, while the nutrients Ca, Mg, and Al, whose behavior in soil that is closely related to pH are all loaded into both R-PC2 and R-PC3. The basic cations, Ca and Mg, are able to leach through the soil more easily at lower pH as they are replaced on exchange

sites with H^+ ions while the concentration of Al increases in more acidic soils (Brady and Weil, 1996). The N fertilizer applications in the CCC, CSW, and CS rotations during the corn and wheat phases lead to acidification of the soil (Divito et al., 2011); Zuber et al. (2015) reported soil pH of 5.6 under the corn-based rotations compared to 6.4 under SSS. As expected, crop rotations that include more corn and wheat have larger R-PC1 scores into which pH is negatively loaded. In R-PC2, Ca and Mg are positively loaded while Al is negatively loaded which lead to higher values for the less acidic SSS than the other rotations and CCC which have N fertilizer applied.

Different crop species have varying nutrient requirements and removal rates, so a continuous cropping sequence may lead to different concentrations for specific macro- and micronutrients compared to a rotation or a continuous cropping with a different crop. The macronutrients P, K, and S are all positively loaded into R-PC1, with much stronger loading for K and S compared to P. Based on the nutrient removal rates reported by International Plant Nutrition Institute (2012) and 3-year average for Illinois corn, soybean, and wheat yields from USDA/NASS (2016), the removal of P and S per hectare is greater for corn than soybean or wheat, while K removal is greatest for soybean. The mean scores for CCC were greater for R-PC1 than SSS with other rotations intermediate, which align with what would be expected for K. The nutrient content of K within the soil would be expected to be less with SSS as more K would be removed with the soybean grain than with CCC. Corn removes more P and S than soybean, but this does not align with the loading of R-PC1. This discrepancy may be related to the several other properties being loaded strongly into the PC scores which may have a strong impact on the score than these nutrient contents. In contrast, the loading of P into R-PC2 and the PC scores of the rotations does match with the expectations based on nutrient removal rates.

The micronutrients that are essential for crop growth include B, Cu, Fe, Mn, and Zn. While removal rate of these nutrients may partially account for these results, other factors may also affect the solubility and availability of the micronutrients. Soil pH and the amount of SOC can both affect availability of these nutrients; higher pH tends to reduce their availability, while organic matter within the soil can complex with certain elements to render them less available for plant uptake (Fageria et al., 2002). Two nutrients that are removed in greater amounts with corn than with soybean or wheat are Fe and Zn (Martens and Westermann, 1991); these two micronutrients are both positively loaded into R-PC1. However, the higher PC scores for CCC compared to SSS indicate that the lower pH in CCC may have slightly increased their availability in CCC compared to SSS. The micronutrients B and Cu are both positively loaded into R-PC2 which ranked the rotations with SSS having the largest PC scores to the lowest PC scores for CCC. This may indicate that the removal of these micronutrients is greater for CCC as reported by Martens and Westermann (1991) for B. The removal rates for Cu were reported as similar for both corn and soybean, but this is an older publication and newer hybrid removal rates may have changed. Both corn and wheat are reported to be more sensitive to Cu fertilization (Gupta et al., 2008), which indicates greater plant requirements and may also indicate that removal is greater for these crops. In contrast, Cu is also positively loaded into R-PC3, which ranked the rotations in the reverse order. In the same PC, Mn is negatively loaded and since it is removed at slightly greater amounts in soybean grain (Martens and Westermann, 1991), it is likely that the other components of the PC loading are more important to the final PC score.

For the three final R-PCs, each was able to separate a single crop rotation from the other three. R-PC4 ranked the extended CSW rotation much lower than the other three rotations and as

seen in Figure 3.1B, where CSW observations are clustered mostly in the lower quadrants. The loadings for this PC indicate that BD and surprisingly, Na content, may be helpful in separating this rotation. Bulk density has previously reported to have been reduced with rotations that produced greater amounts of residue, such as CSW and CCC (Coulter et al., 2009). The residues and resulting organic matter are less dense and lead to reduced bulk density over time as they accumulate. By itself, BD is unlikely to be able to separate from the other rotations, especially CCC. While both MBN and S are also loaded into R-PC4, the strongest loading is for Na. This is unexpected as sodium is not an essential nutrient, and there is no reported relationship between these crop species and sensitivity to Na (Gupta et al., 2008). Differences in Na within CSW rotation compared to others may be related to the crop root systems carrying Na upward with water uptake. Continuous corn is the segregated crop rotation for R-PC5 with a greater PC score than the other rotations. R-PC5 positively loads Mn against the negatively loaded MBN. As mentioned above, Mn removal is greatest for soybean so we would expect greater PC scores for CCC. For MBN, the use of a rotation has been found to increase MBN compared to a monoculture (McDaniel et al., 2014b) which would lead to greater PC scores for CCC compared to the other rotations as a result of the negative loading. The final R-PC ranks SSS lower than the other rotations and is a contrast between BD and P. The reduced biomass produced by soybean has been found to lead to greater BD in rotations with more frequent soybean (Coulter et al., 2009; Zuber et al., 2015). As mentioned above, corn removal of P is greater than soybean so with the negative loading of P, it leads to a smaller PC score for SSS compared to the corn-based rotations.

Tillage

Three of the T-PCs were significant for tillage, T-PC1, 2, and 5 with larger PC scores for NT compared to CT for each of them (Table 4). As with crop rotation, tillage affects the cycling of C and N within the soil. This can be seen in T-PC2 where SOC, TN, and WAS are all positively loaded. The use of NT has been reported to increase both SOC and TN compared to CT (Varvel and Wilhelm, 2011; Van Eerd et al., 2014). With NT, residues accumulate on the soil surface and decompose more slowly while tillage incorporates the residues increasing microbial access and the rate of decomposition. Previously protected SOC from within soil aggregates is also exposed to microbial activity, decreasing SOC under CT (Balesdent et al., 2000). As mentioned above, WAS is closely related to SOC, and greater WAS has been reported under NT compared to CT (Zuber et al., 2015). Cation exchange capacity is another soil parameter related to SOC and was positively loaded into T-PC1. As CEC is related to clay content and SOC, the greater SOC under NT may also be correlated with increases in CEC similar to the effect of crop rotations mentioned above. The cycling of C and N are carried out by microbes within the soil; MBC and MBN are measures of the size of the microbial community and are both positively loading into T-PC5. This corresponds with the expectation of a greater MBC and MBN under NT that was reported in a global meta-analysis (Zuber and Villamil, 2016).

Stratification of many soil properties occurs in NT soils as fertilizer inputs and crop residue returns are added to the surface. Within NT, movement of the nutrients relies on moisture content as downward movement occurs with leaching for some elements and upward movement occurs through plant uptake, which is augmented when soil water is available. Soil pH is also stratified as the acidification of the soil as a result of N fertilizer is concentrated near the soil surface (Crozier et al., 1999; Divito et al., 2011). Soil pH and the nutrients that are directly

related to pH—Ca, Mg, and Al are all loaded into T-PC1 with positive loading of Al and negative loading for pH, Ca, Mg. The greater PC score for NT is related to the stratification of pH as the surface soil of NT is more acidic than that of CT in the surface 10 cm. The lower pH also reduces Ca and Mg content while increasing Al. Both S in T-PC1 and P in T-PC3 are positively loaded related to higher PC scores for NT, which is likely related to stratification of the nutrients near the soil surface as phosphorus in particular is immobile in soil (Brady and Weil, 1996). The reduced pH under NT at 0-10 cm can also increase the availability of micronutrients compared to the higher pH under CT (Fageria et al., 2002) as can be seen with the positive loading of Fe in T-PC1 and Cu and Zn in T-PC2 related to the higher PC scores for NT. Two micronutrients are negatively loaded, B into T-PC1 and Mn into T-PC2, suggesting greater levels in CT than NT. Manganese is only weakly loaded into T-PC2 compared to the others already discussed; it is likely that they have a stronger influence on the PC scores than Mn. However, B is strongly negatively loaded into T-PC1 so this may indicate that B has leached more from NT than CT, likely as a result of the greater moisture content of NT soils (Johnson and Hoyt, 1999).

Despite the significant differences between the scores of NT and CT in three of the PCs, it is important to note that there was a great deal of overlap between the two tillage practices (Figure 3.2). There are clear trends in these observations that align with the mean separations, but the high variability in the PC scores suggests that we are not able to clearly separate CT from NT. These results and the difficulty in clearly separating the multi-crop rotations from one another indicate that discriminating among agronomic practices in these highly resilient soils is difficult. Only R-PC4 was able to separate CSW from the other rotations (Figure 3.1B) and CS was not able to be clearly separated at all; in contrast, CCC and SSS rotations were distinct

enough to be able to be completely separated from one another through multiple PCs (Figure 3.1A).

While many of the sensitive soil properties were as expected, there were some surprising absences from the list of indicators. It was somewhat unexpected that BD was not useful in separating tillage practices, but the accumulation of SOC under NT may offset any settling of soils associated with greater BD. Measures of biological properties were not very sensitive to rotations, but this may be related to the high variability of these measures rather than reduced sensitivity to agronomic practices.

CONCLUSIONS

The results of this study show that there are numerous soil properties that are suitable for use as SQ indicators when assessing crop rotation and tillage practices on the highly productive soils of Illinois. Continuous corn and continuous soybean were separated using soil parameters related to the cycling of C and N as well as differences in soil pH and nutrient content likely related to applications of N fertilizers to corn and wheat, but not soybean. Likewise, the two tillage practices presented a contrast of C and N cycling as well as stratification of nutrients and fertilizers within NT that was not present in the mechanically disturbed CT soils. The next step in SQ assessment is to develop a SQI using this subset of variables in conjunction with crop yields as a measure of soil productivity. The continued progress in evaluating soil quality will play a vital role in providing information to farmers as they make agronomic decisions while trying to protect their primary resource—soil.

FIGURES

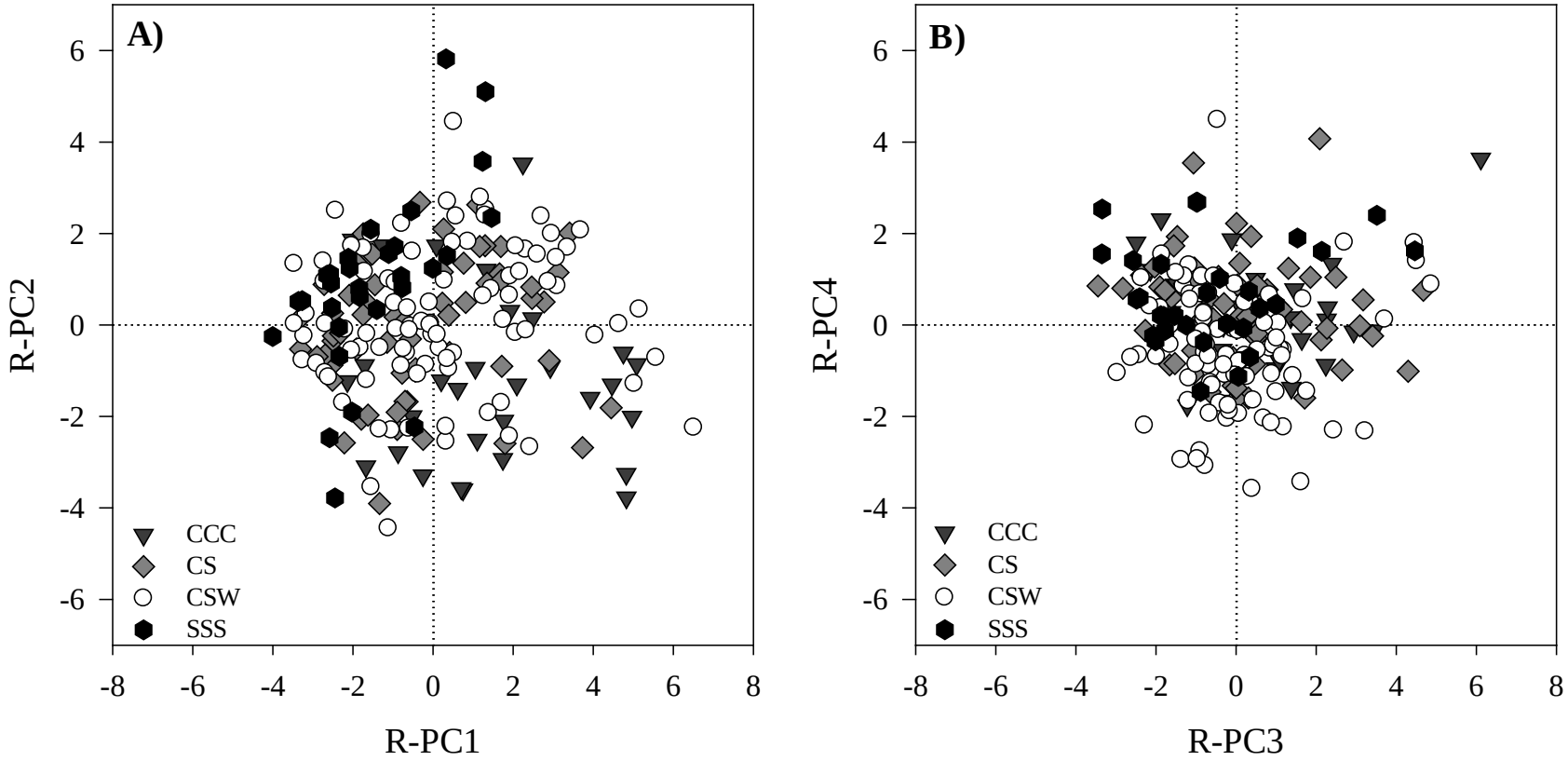

Figure 3.1 Rotation effect on the A) first and second principal components (R-PC1, R-PC2) and B) third and fourth principal components (R-PC3, R-PC4) of the data set for 0-20 cm soil depths. Symbols represent the principal component scores for experimental plots at two Illinois sites with crop rotations of continuous corn (CCC), corn-soybean (CS), corn-soybean-wheat (CSW) or continuous soybean (SSS).

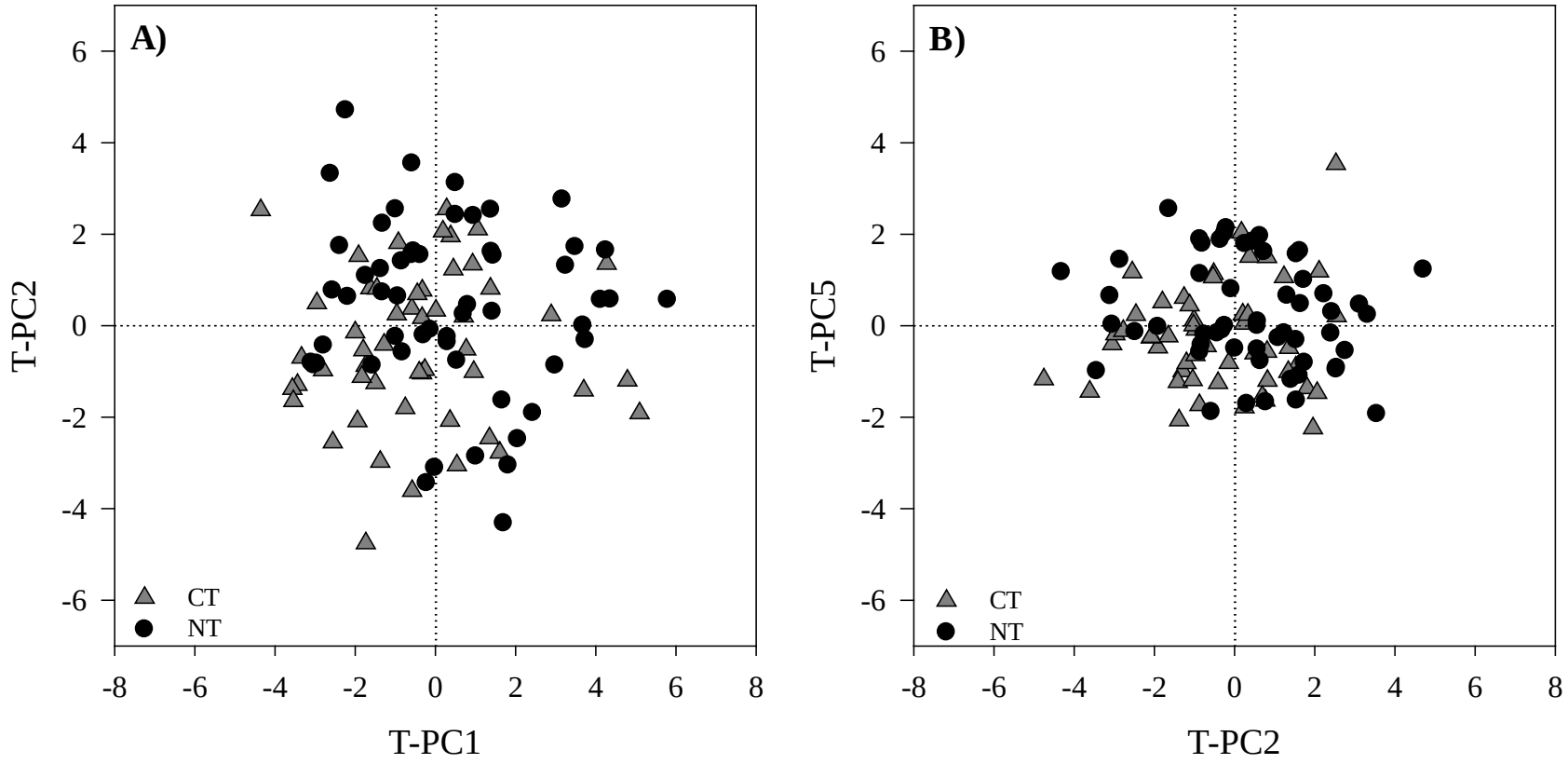

Figure 3.2 Tillage effect on the A) first and second principal components (T-PC1, T-PC2) and B) second and fifth principal components (T-PC2, T-PC5) of the 0-10 cm soil depths. Symbols represent the principal component scores for experimental plots at two Illinois sites with either conventional tillage (CT) or no-till (NT).

TABLES

Table 3.1 Descriptive statistics across crop rotation and tillage treatments for the 20 measured variables included in the multivariate analysis with means and standard error (SE) for 0-10 cm and 10-20 cm soil depths across two sites in Illinois.

	Units	0-10 cm	10-20 cm	SE*
Physical Properties				
BD	$Mg\ m^{-3}$	1.30	1.34	0.01
WAS	$g\ g^{-1}$	0.64	0.68	0.01
Chemical Properties				
SOC	$g\ kg^{-1}$	20.6	17.6	0.31
TN	$g\ kg^{-1}$	1.83	1.52	0.03
pH		5.54	5.86	0.05
CEC	$cmol\ kg^{-1}$	24.8	23.1	0.60
P	$mg\ kg^{-1}$	35.0	11.6	0.93
K	$mg\ kg^{-1}$	232	107	4.54
S	$mg\ kg^{-1}$	8.25	6.95	0.09
Ca	$mg\ kg^{-1}$	2334	2623	31.7
Mg	$mg\ kg^{-1}$	308	315	4.77
Na	$mg\ kg^{-1}$	16.6	17.3	0.30
B	$mg\ kg^{-1}$	0.47	0.48	0.01
Fe	$mg\ kg^{-1}$	165	143	2.05
Mn	$mg\ kg^{-1}$	109.4	94.8	2.05
Cu	$mg\ kg^{-1}$	2.30	2.49	0.03
Zn	$mg\ kg^{-1}$	2.05	1.72	0.03
Al	$mg\ kg^{-1}$	639	655	6.87
Biological Properties				
MBC	$\mu g\ g^{-1}$	71.3	39.5	3.24
MBN	$\mu g\ g^{-1}$	11.2	5.4	0.60

*SE, standard error; BD, bulk density; WAS, water aggregate stability; SOC, soil organic carbon; TN, total nitrogen; CEC, cation exchange capacity; MBC, microbial biomass carbon; MBN, microbial biomass nitrogen.

Table 3.2 Multivariate analysis of variance (MANOVA) results to assess the effect of rotation, tillage, depth and their interactions over 20 measured soil variables across two Illinois sites.

Factors	Wilk's λ	P-Value
Rotation	0.184	<.0001
Tillage	0.647	<.0001
Rotation x Tillage	0.708	0.4509
Depth	0.177	<.0001
Rotation x Depth	0.762	0.872
Tillage x Depth	0.829	0.036
Rotation x Tillage x Depth	0.823	0.997

Table 3.3 Principal component analysis of 20 soil variables for 0-20 cm soil depth with eigenvalues and proportion of variability explained for the first six principal components (PC) with eigenvalues >1. Component correlation scores are shown multiplied by 100; those loadings greater than 0.4 are bolded. Probability values for the analysis of variance (ANOVA) of effect of rotation and the mean PC scores for rotation for the first six PCs.

	PC 1	PC 2	PC 3	PC 4	PC 5	PC 6
Eigenvalue	4.82	3.01	2.92	1.73	1.25	0.99
Proportion	0.24	0.15	0.15	0.09	0.06	0.05
Variable	Component Correlation Scores					
BD*	-25	-24	22	**50**	26	**53**
WAS	14	-4	**57**	-26	-33	7
SOC	**61**	**50**	3	-31	6	32
TN	**75**	30	5	-31	1	31
pH	**-78**	35	-28	10	-5	-7
CEC	**60**	-12	**63**	-13	16	7
P	**56**	**44**	-33	20	2	**-41**
K	**73**	25	-21	11	8	-12
S	**71**	-29	-3	**42**	12	0
Ca	-39	**47**	**59**	-9	21	9
Mg	-27	**45**	**66**	13	-5	-15
Na	-7	-32	38	**60**	0	-3
B	-39	**52**	-2	35	9	-10
Fe	**71**	-7	36	20	0	-25
Mn	27	-12	**-44**	20	**59**	10
Cu	-27	**45**	**65**	15	24	-6
Zn	**47**	**61**	12	9	11	-15
Al	35	**-66**	**44**	12	-19	-14
MBC	24	**50**	-19	39	-28	35
MBN	23	25	-12	**42**	**-65**	16
	Probability Values					
Rotation	<.0001	<.0001	0.0009	<.0001	0.0005	0.0004
	Mean PC Scores					
CCC	1.39 a†	-1.16 c	0.63 a	0.25 a	0.72 a	0.20 a
CS	-0.31 b	-0.11 b	0.14ab	0.36 a	-0.04 b	0.24 a
CSW	0.21 b	0.18ab	-0.11bc	-0.55 b	-0.18 b	-0.02 a
SSS	-1.43 c	0.91 a	-0.59 c	0.68 a	-0.09 b	-0.63 b

*BD, bulk density; WAS, water aggregate stability; SOC, soil organic carbon; TN, total nitrogen; CEC, cation exchange capacity; MBC, microbial biomass carbon; MBN, microbial biomass nitrogen; CCC, continuous corn; CS, corn-soybean; CSW, corn-soybean-wheat; SSS, continuous soybean.

†Means for a specific principal component followed by the same lowercase letter are not significantly different (α=0.05).

Table 3.4 Principal component analysis of 20 soil variables for 0-10 cm soil depth with eigenvalues and proportion of variability explained for the first six principal components (PC) with eigenvalues >1. Component correlation scores are shown multiplied by 100; those loadings greater than 0.4 are bolded. Probability values for the analysis of variance (ANOVA) of effect of tillage and the mean PC scores for of the tillage practices for the first six PCs.

	PC 1	PC 2	PC 3	PC 4	PC 5	PC 6
Eigenvalue	4.73	3.31	2.13	1.61	1.39	1.19
Proportion	0.24	0.17	0.11	0.08	0.07	0.06
Variable	Component Correlation Scores					
BD	8	-26	**57**	21	-11	**52**
WAS	25	**50**	-1	**-45**	-21	-16
SOC	27	**69**	-35	-5	5	39
TN	**48**	**60**	-33	-14	-4	37
pH	**-91**	-13	1	-2	-6	-1
CEC	**74**	39	15	8	-14	3
P	-6	20	-29	**47**	**48**	**-47**
K	33	20	-22	**45**	-18	-13
S	**72**	-22	32	21	8	7
Ca	**-49**	**61**	25	21	-21	9
Mg	**-48**	53	**46**	0	-35	-13
Na	17	-16	**71**	3	15	-4
B	**-69**	13	14	25	8	-2
Fe	**70**	27	31	4	29	-19
Mn	17	**-49**	-12	**52**	9	31
Cu	-31	**59**	**48**	20	1	-4
Zn	3	**69**	0	29	38	1
Al	**77**	-11	36	-18	-1	-23
MBC	-36	16	11	-13	**56**	38
MBN	-24	2	20	**-56**	**54**	1
	Probability Values					
Tillage	0.035	0.007	0.7071	0.139	0.014	0.533
Factor	Mean PC Scores					
Tillage						
CT	-0.39 b[†‡]	-0.41 b	-0.03	0.17	-0.24 b	-0.07
NT	0.37 a	0.41 a	0.06	-0.16	0.25 a	0.06

[*]BD, bulk density; WAS, water aggregate stability; SOC, soil organic carbon; TN, total nitrogen; CEC, cation exchange capacity; MBC, microbial biomass carbon; MBN, microbial biomass nitrogen; NT, no-till; CT, conventional tillage.

[†]Letters indicating significant differences are shown only for significant effects (α=0.05).

[‡]Means for a specific principal component followed by the same lowercase letter are not significantly different (α=0.05).

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APPENDIX. Supplemental Meta-Analysis Figures

Figure A.1 Map of locations of 62 experimental studies included in meta-analysis of the effect of tillage on soil biological properties, including microbial biomass and enzyme activities.



Figure A.2 Funnel plots for microbial biomass carbon (MBC), microbial biomass nitrogen (MBN), fluorescein diacetate (FDA), dehydrogenase (DHA), β-glucosidase (β-glu), and urease. Each graph has the variance of the observation (v_i) plotted against the natural logarithm of the response ratio (LRR); the vertical line is the weighted mean effect size.

