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EFFECTS OF DEPTH AND COVER CROP TREATMENT ON THE FUNCTIONING AND DIVERSITY OF SOIL MICROBIAL COMMUNITIES

EMILY HANSEN

40 Pages

Globally, agriculture is strained by the unpredictable effects of climate change, as well as flooding, drought, erosion, and decreases in soil fertility. To meet these challenges, agricultural systems must develop new techniques while promoting sustainable intensification practices. Cover crops can provide farmers with an off-season cash crop, while also positively impacting the soil and reducing reliance on less sustainable conventional farming practices. The objective of this research was to evaluate the impact of different cover crops on soil microbial communities at varying depths. Soils were sampled in the fall and spring from experimental plots (Illinois State University Farm, Lexington, IL) that contained pennycress; cereal rye; pea, clover, radish, oat mix; or fallow soil. EcoPlates™ were used to conduct community-level physiological profiling (CLPP) and test the hypothesis that cover crop type and soil depth are strong determinants of microbial community diversity and functioning in an agricultural field. I predicted: (a) the physiological soil profile of the microbial community, as measured by the rate and ability to metabolize a variety of carbon sources, would change across depths and differ among cover crop types, (b) shallower depths would contain microbial communities with greater functional diversity, (c) the overall functional diversity of the microbial community would be greater under cover crops than in unplanted (fallow) fields, and (d) microbial functional diversity would be greatest in soils where cover crops with higher C:N ratios were grown, as crop residues decompose more slowly and provide more carbon for microbial metabolism. I found that the

CLPP of the microbial community changed both across depths and between cover crop treatments. In both the fall and spring, evenness, richness, and Shannon diversity declined with sampling depth. Although the overall functional diversity of the microbial community did not differ among cover crop treatments, some differences were found in how the communities associated with the different cover crops metabolized the 31 carbon sources. Finally, I did not find a connection between the C:N ratio of the cover crop and the functional diversity of the microbial community. Cover crops are only one potential component to sustainable intensification, and more work is needed to develop agricultural practices that minimize environmental harm.

KEYWORDS: cover crops; soil microbial community; EcoPlate™; functional diversity; agriculture

EFFECTS OF DEPTH AND COVER CROP TREATMENT ON THE FUNCTIONING AND DIVERSITY OF SOIL MICROBIAL COMMUNITIES

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EFFECTS OF DEPTH AND COVER CROP TREATMENT ON THE FUNCTIONING AND DIVERSITY OF SOIL MICROBIAL COMMUNITIES

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E.H.

CONTENTS

CHAPTER I: INTRODUCTION

By 2050 the global population is projected to reach 9.7 billion, and agricultural practices must adapt and increase yields to feed this growing population (FAO, 2019). Currently, >12% of the world's population is malnourished, and the unpredictable effects of climate change, including flooding, drought, erosion, and decreases in soil fertility already strain the capacity of agriculture world-wide (Godfray et al., 2010). To counter these changes, agricultural systems must look to new techniques and technologies, while promoting sustainable intensification practices. The exploitation of plant-soil feedbacks and above-belowground interactions has been identified as one means of addressing global food insecurity (Bommarco et al., 2013; Orrell and Bennett, 2013). Cover crops can be one cost-effective means of exploiting above-belowground interactions and increasing the sustainability of agricultural systems, while also positively impacting the soil and reducing some of the need for conventional farming practices that are harmful to the environment (Gesch et al., 2010; Kirkegaard et al., 2008; Krupinsky et al., 2006; Tscharntke et al., 2012).

Cover crops are grown primarily to preserve agricultural soil, especially during the off-season or between cash crops (Weil and Kremen, 2007). Often they are sown immediately after harvest of the main cash crop, grow throughout the fall before a period of dormancy in the winter, and resume growth in the early spring before soils are warm and dry enough for the next cash crop to be planted (Weil and Kremen, 2007; Wittwer et al., 2017). Cover crops provide many direct benefits to the health of croplands. They prevent soil erosion caused by winter and spring rains, provide organic carbon to slow the decline of soil organic matter, and reduce nitrogen loss to groundwater (Staver and Brinsfield, 1998; Weil and Kremen, 2007). Additionally, legume cover crops can replace a portion of the nitrogen fertilizer required by

nitrogen-intensive crops (such as corn) via biological nitrogen fixation (Frye et al., 1985; Power, 1987). Certain cover crops with deep root systems (e.g., oilseed radish) have also shown the potential to alleviate the problem of subsoil compaction in a no-till cropping system (Williams and Weil, 2004). Brassica cover crops (like pennycress) contain glucosinolates that when broken down control weeds, disease, insects, and nematodes (Brown and Morra, 1996; Kirkegaard et al., 1999; Kirkegaard and Sarwar, 1998; Vukicevich et al., 2016).

Microbes influence plant growth and health through their interactions with plant tissues in the rhizosphere. Microbial communities in the soil regulate plant diversity, composition, and productivity (van der Heijden et al., 2008). Interactions between plants and microbes can occur through symbiotic processes in which microbes live partially or entirely within plant tissues, through associative processes in the rhizosphere (the region of soil surrounding the plant's roots) or live freely within the soil. They enhance plant growth and seed production by increasing the supply or accessibility of nutrients—like nitrogen, phosphorous, and potassium—for the host plant (Dinesh et al., 2013; Jacoby et al., 2017). Endophytic microbes can encourage plant biomass formation through numerous biological processes; most notably, biological nitrogen fixation, phosphate solubilization, and production of antibiotics (Souza et al., 2015). Plants can in turn affect soil microbial communities by producing root exudates that contain carbon-rich compounds (such as amino acids, sugars, and phenolics) that attract AM fungi and nitrogen-fixing bacteria (Akiyama et al., 2005; Badri and Vivanco, 2009; Broeckling et al., 2008). Plants can also increase microbial biomass by producing high quality and quantity litter, and increase soil moisture, which is beneficial to microbes that occupy shallower soil depths (Fanin et al., 2014; Pell et al., 2009).

In agricultural systems, land management decisions—such as tillage intensity, crop identity, and herbicide and fertilizer use—can influence plant-microbe interactions. Typical agricultural inputs degrade soils over time and can discourage plants from forming beneficial associations with soil microbiota. Microbes can influence crop performance, crop-weed competition, and the overall resilience of the system, but negative plant-soil feedbacks can arise from the accumulation of soil-borne pathogens during repeated monocultures (Menalled et al., 2019).

The carbon to nitrogen ratio (C:N) of a cover crop plays an important role in determining its potential effects on soil health and the microbial community. The C:N ratio affects crop residue decomposition and nutrient cycling; the faster the crop residues are consumed by soil microorganisms, the less time those residues will be protecting the soil surface (USDA Natural Resources Conservation Service, 2011). Additionally, the carbon content of a crop directly affects the amount of microbial biomass in soils, with greater soil carbon content associated with greater microbial biomass (Bradford et al., 2013). A crop with a C:N ratio near 25:1 will be consumed by soil microorganisms relatively quickly with little excess carbon or nitrogen left in the soil. A crop with a C:N ratio above 25:1 will result in soil microorganisms tying up excess nitrogen in the soil (immobilization) as they consume the carbon from the crop. While this scenario could create a nitrogen deficit in the soil, a crop with a high C:N ratio has the advantage of decomposing slowly, thus providing more cover. Alternatively, if a crop has a C:N ratio of less than 25:1, soil microorganisms will deposit the excess nitrogen in the soil (mineralization) (Miller, 2000).

The addition of cover crops to existing cropping systems can positively impact microbial communities by breaking up negative plant-soil feedbacks, which, in turn, may benefit the

overall productivity of farmlands. Selection of specific cover crop mixtures could be used by growers to manipulate soil bacteria to the benefit of their cash crops (Vukicevich et al., 2016). For instance, populations of beneficial microbes could potentially be increased by including plants in a cover crop mixture from diverse functional groups; legumes, C4 grasses, C3 grasses, non-leguminous forbs, and brassicas all have the potential to benefit soil microbial communities (Eisenhauer et al., 2011; Milcu et al., 2013). Cover crops have the potential to increase microbial diversity, which is essential to the suppression of soil-borne plant diseases (van Elsas et al., 2002). Cover crops may also facilitate nutrient cycling, increase soil organic matter content and nitrogen fixation, maintain topsoil, and improve weed control (Altieri, 1999; Dean et al., 2016; Reddy et al., 2003; Sainju & Singh, 1997; Williams, 1998). Additionally, cover crops may potentially provide nourishment to obligate mutualists during the winter months, leading to increased crop productivity in the spring (Kabir and Koide, 2002).

The objective of this research was to evaluate the impact of different cover crops on soil microbial community functional diversity at varying depths. The numerous benefits of cover crops to an agricultural system have been established, yet there remains a knowledge gap in how specific cover crops and cover crop mixtures may impact soil microbial communities (Vukicevich et al., 2016). There is the potential to choose specific crops or mixtures based on their effects on not only the incoming plantings, but also on the overall health benefits to soils (Kim et al., 2020). Additionally, depth is an important factor in evaluating the effectiveness of a cover crop to build soil organic carbon (Lal et al., 2015; Olson and Al-Kaisi, 2015). Traditional sampling methods homogenize up to 15 cm of the top layer of the soil, which often does not show significant changes in organic carbon year to year (Tautges et al., 2019). While it often takes many years for shifts in soil carbon content to become apparent, small changes may be

evident from one growing season to the next if soils are sampled at smaller depth increments and across a greater range of depths (Poeplau and Don, 2015).

I conducted community-level physiological profiling (CLPP) at smaller depth increments across a greater portion of the root zone depth to test the hypothesis that, due to strong effects of plant root growth and carbon input on rhizosphere properties, cover crop type and soil depth are strong determinants of microbial community functional diversity in an agricultural field. I predicted: (a) the physiological soil profile of the microbial community, as measured by the rate and ability to metabolize a variety of carbon sources, would change across depths, and differ among cover crop types; (b) shallower depths would contain microbial communities with greater functional diversity; and (c) the overall functional diversity of the microbial community would be greater under cover crops than in unplanted (fallow) fields . Additionally, I predicted (d) microbial functional diversity would be greatest in soils where cover crops with higher C:N ratios were grown, due to more available carbon for microbial consumption and slower decomposition of crop residues. Alternatively, microbial functional diversity may be greatest in soils where a mixed cover crop is grown than a single cover crop, as greater aboveground diversity is associated with greater belowground diversity.

CHAPTER II: MATERIALS AND METHODS

Study area description and field sampling

Soils were sampled from an ongoing experiment that was established in the fall of 2020 at the Illinois State University Research Farm in Lexington, IL (McLean County; 40.674641, -88.783492) to investigate carbon sequestration by cover crops. A randomized block design was used with four blocks (Figure 1), each containing four treatment plots: (1) a fallow control plot; (2) a pea, clover, radish, and oat mix (ProHarvest™, 28 kg/ha); (3) wild-type pennycress (5.6 kg/ha); and (4) cereal rye (65 kg/ha). Cereal rye has the highest C:N ratio of the cover crops used in this study (80:1), the mixed cover crop has the lowest C:N ratio (12:1), and pennycress has an intermediate C:N ratio (26:1). Blocks I, II, and III were located on a Drummer and El Paso silty clay loam soil series with a 0-2 percent slope, and Block II was located on a Catlin silt loam soil series with a 2-5 percent slope (Soil Survey Staff, 2010). Historically, the plots had been part of a corn-soy rotation under conventional tillage. The plots were converted to no-tillage in 2019 and cover crops were first grown in the fall of 2020. Plots were fertilized preceding corn being planted in Spring 2020. Plots were sampled in the fall of 2021 following soy in all plots (November 2021) and the spring of 2022 after the cover crops had established (May 2022).

Vegetative growth varied visibly among cover crops and fields in the spring. To better interpret potential significant effects, the percentage of soil covered by vegetation was measured with the app Canopeo™ in a 15 cm x 15 cm square centered over the point of sampling. Using sterile technique, three soil cores were collected per plot. Six depth sections were collected from each core: 0-2 cm, 2-4 cm, 4-6 cm, 13-15 cm, 28-30 cm, and 43-45 cm and the three cores were pooled by depth. Samples were kept on ice until processed in the lab within six hours.

Soil microbial analyses

Biolog EcoPlates™ were used to evaluate the functional diversity of the soil microbial community. EcoPlate™ is a community-level profiling tool with which the investigator can quantify microbial community functional diversity. EcoPlate™ utilizes 31 different carbon sources and a redox dye indictor in order to indicate microbial activity. The 31 carbon sources can be categorized into six functional groups: polymers, carbohydrates, carboxylic acids, amino acids, amines, and phenolic compounds. Each 96-well microplate contains the 31 carbon sources and control wells in triplicate to ensure sufficient replication. The community-level physiological profile obtained from the EcoPlates™ involves three main components: the rate of color development, the richness and evenness of the response among wells, and the pattern of metabolism among wells (Gryta et al., 2014). These three components can be used to compare different soil microbial communities.

10 g of each homogenized soil sample was blended and diluted with sterile 1X phosphate-buffered saline to a final concentration of 1:100. The EcoPlates™ were inoculated with 120 µL of the diluted sample and incubated at 25°C. The optical density (OD_{590}) of the wells was measured using a multilabel plate reader at 24, 48, 72, 96, and 120-hour time intervals (Gryta et al., 2014). The remaining portion of each soil sample was dried at 60°C for 48 hours and the soil moisture was calculated.

Data analysis

The data analysis was generated using SAS™ 9.4 software (Copyright (c) 2002-2012 by SAS Institute Inc., Cary, NC, USA). Using the optical densities, I calculated the average well color development (AWCD), which is the average of the triplicate readings for each carbon source and indicates microbial metabolism of the carbon. Using these values I calculated the

richness (= number of carbon sources used), evenness (= variation in response among wells), and Shannon diversity (*H*'), using the methods outlined in Garland (1997) and Zak (1994). To determine which of the five days of plate development would yield the clearest differences in these responses I conducted a preliminary MANOVA with day of development as a repeated measure, cover crop and depth as fixed effects, and AWCD as the response variable.

Using PROC MIXED with depth as a repeated measure, crop as a fixed effect, and block as a random effect I examined how these factors affected richness, evenness, and Shannon's *H*. In each analysis, Akaike information criterion (AIC) was used to select among a linear or quadratic model, with or without an interaction of the continuous variable (depth) and crop. In all cases, a linear model without interaction gave the lowest AIC score. To better understand variation in the pattern of carbon usage, the average well color development for the 31 carbon sources was synthesized into fewer axes using a principal components analysis (PCA). Principal components with eigenvalues >1 were analyzed with a mixed model to test the effects of cover crop and depth on community composition (Lagerlöf et al., 2014). Then I analyzed the AWCD for each of the six functional groups individually to determine how the microbial community under different cover crops and at different depths used available carbon. Soil moisture was initially included as a covariate in the analyses of diversity but subsequently removed due to lack of a significant effect. I analyzed % moisture to determine whether cover crops affected soil moisture and how % moisture changes across depths.

CHAPTER III: RESULTS

Fall 2021

In the fall, all experimental plots contained soybeans at full maturity (stage R8) at the time of sampling. The average soil moisture across all experimental plots was 21% (Figure 2a). Across sampling depths soil moisture declined from 0-13 cm ($F_{1,78} = 7.82$, $p = 0.007$, slope = -0.0006±0.0002). Soil moisture did not differ among cover crop treatments ($F_{3,9} = 0.69$, $p = 0.582$; Figure 2b).

The average well color development (AWCD) increased over the 5-day incubation period. Preliminary analysis of AWCD indicated the largest difference occurred between days 3 and 4 and so day 4 readings were used for all subsequent analyses.

The number of carbon sources metabolized by the microbial community declined with depth ($F_{1,79} = 54.35$, $p < 0.0001$, slope = -0.265±0.036). On average, 80% of the 31 carbon sources were metabolized at the soil surface and this average declined to half of the sources used at 43 cm (Figure 3a). While richness changed with depth it did not differ among cover crop treatments ($F_{3,9} = 0.62$, $p = 0.617$; Table 1). Evenness (the similarity in color development among wells) also declined with depth ($F_{1,79} = 39.24$, $p < 0.0001$, slope = -0.003±0.0004; Figure 3c) but did not differ among cover crop treatments ($F_{3,9} = 0.69$, $p = 0.579$; Table 1). Because these two components of diversity exhibited trends for depth but not cover crop, a single measure of diversity, Shannon's *H*, also declined with depth ($F_{1,79} = 44.57$, $p < 0.0001$; Figure 3e). Thus, as depth increased, there were fewer carbon sources being metabolized, more variation in the degree of metabolism, and diminished functional diversity.

The first three principal components (PC) from analysis of the EcoPlate™ AWCD explained 74.8% of the variation in the microbial metabolism of the 31 carbon sources.

Inspection of factor loadings shows high correlation among carbon sources (Supplementary Table 1). Most of the carbon sources loaded on PC 1 and PC 2, with 11 loading heavily on both factors (Table 2). PC 2 was strongly and positively correlated with carbohydrates and polymers, and PC 3 was positively correlated with carboxylic acids (Table 2). Analysis of the factors revealed that the cover crop planted the previous season significantly affected both PC 2 ($F_{3,9}$ = 4.00, p = 0.046) and PC 3 ($F_{3,9}$ = 8.28, p = 0.006), but not PC 1 ($F_{3,9}$ = 0.86, p = 0.497). Control plots scored high for PC 2 and low for PC 3, whereas pennycress plots scored low for PC 2 and high for PC 3 (Figure 4a). This indicates that soil in the control plots included a greater density or diversity of microbes capable of metabolizing a range of carbohydrates and polymers compared to soil from pennycress plots ($t_{3,9}$ = 3.41, p = 0.033; Figure 4a). Pennycress plots ($t_{3,9}$ = -4.63, p = 0.006) and mixed cover crop plots ($t_{3,9}$ = -3.72, p = 0.020) metabolized a greater number of carboxylic acids than did control plots. While the control and pennycress tended to separate out, the cereal rye and the mixed species cover crop clustered together (Figure 4a), indicating more balanced metabolism of carbon sources. Sampling depth had a significant impact on PC 1 ($F_{1,79}$ = 25.46, p < 0.0001, slope = -0.026$\pm$0.005), PC 2 ($F_{1,79}$ = 24.78, p < 0.0001, slope = -0.028$\pm$0.006), and PC 3 ($F_{1,79}$ = 4.17, p = 0.045, slope = -0.012$\pm$0.006) with a decline in metabolism of carbon sources with depth. As depth increased, values for all PCs declined.

A closer look at how the metabolism of each functional group of carbon sources changed across depths and among cover crop treatments showed that usage of all functional groups declined significantly with increasing depth (Figure 5, Table 4). Across all depths, the trend was that phenolic compounds were least metabolized, and at shallower depths that polymers were the most metabolized. Beyond depths of 13 cm there were fewer differences in the metabolism of

each functional group. The most differences in functional group usage were observed from the surface to 4 cm. The cover crop planted the previous season did not have a significant effect on the pattern of functional group usage (Table 3).

Spring 2022

Cover varied among cover crops in the spring and was more heterogeneous than observed for soy in the fall. Because the goal of this research was to identify effects of cover crops on soil microbes, I deliberately chose areas of the experimental plots in the spring where coverage was highest and haphazardly sampled within those areas. Cereal rye provided the greatest coverage (83.5%), followed by pennycress (54.8%) and mixed (48.4%). Control plots had the least amount of plant coverage (4.04%). There was overall poor establishment of mixed cover crop experimental plots. At the time of sampling these plots contained mostly radishes, with few peas, clover, or oats. Control plots contained a mix of weeds, with high overall plot coverage and I sampled locations in control plots that had little plant material. The average soil moisture across all plots in the spring was 15% (Figure 2a). Similar to the fall, soil moisture declined from the surface to 15 cm, then gradually increased ($F_{1,78} = 10.82$, $p = 0.002$, slope = 0.0006±0.0002). Cover crop treatment significantly affected soil moisture, with control plots having greater soil moisture than cover crop plots ($F_{3,9} = 8.43$, $p = 0.006$ (Figure 2b).

As with the fall, AWCD increased over the 5-day incubation period. Preliminary analysis of AWCD indicated the largest difference occurred between days 2 and 3, and no difference was found between days 3 and 4. Day 3 readings were used for subsequent analyses in the spring.

The number of carbon sources metabolized by the microbial community (richness) declined with depth ($F_{1,79} = 116.15$, $p < 0.0001$, slope = -0.228±0.021; Figure 3b). On average,

74% of the 31 carbon sources were metabolized at the soil surface and this declined to 30% of the sources used at 43 cm. Richness did not differ among cover crop treatments ($F_{3,9} = 0.43$, $p = 0.735$; Table 1). Evenness declined with depth, indicating that the community is becoming more variable as depth increases and some microbes are becoming relatively more abundant ($F_{1,79} = 71.85$, $p < 0.0001$, slope = -0.002±0.0002; Figure 2d. Evenness did not differ among cover crop treatments ($F_{3,9} = 1.36$, $p = 0.315$; Table 1). Shannon diversity also declined with depth ($F_{1,79} = 113.36$, $p < 0.0001$; Figure 2f). These results mirrored those seen in the fall, demonstrating that as I sample deeper through the soil profile, measures of diversity (evenness and richness) decline.

The first three principal components from analysis of the EcoPlate™ output explained 62.3% of the variation in the microbial metabolism of the 31 carbon sources. As in the fall, all functional groups loaded heavily on PC 1 (Table 2; Supplementary Table 1). PC 2 was dominated by carbohydrates, and PC 3 had all functional groups represented except for amino acids (Table 2). Analysis of the factors revealed that the cover crop present in the spring significantly affected PC 2 ($F_{3,9} = 4.16$, $p = 0.042$), but not PC 1 ($F_{3,9} = 2.74$, $p = 0.106$) or PC 3 ($F_{3,9} = 0.23$, $p = 0.871$). This significant effect for PC 2 was due to low values for cereal rye relative to the other cover crop treatments (Figure 4b). This indicates that soil in the cereal rye plots included a lower abundance or diversity of microbes capable of metabolizing a range of carbohydrates compared to soil from mixed cover crop plots ($t_{3,9} = -3.04$, $p = 0.057$). Cereal rye tended to separate out, while all other treatments and the control clustered together (Figure 4b). Sampling depth had a significant impact on PC 1 ($F_{1,79} = 108.43$, $p < 0.0001$; slope = -0.043±0.004), PC 2 ($F_{1,79} = 5.93$, $p = 0.017$; slope = -0.015±0.006), and PC 3 ($F_{1,79} = 5.39$, $p =$

0.023; slope = -0.014±0.006) with a decline in metabolism of carbon sources with depth. As seen in the fall, as depth increased, values for all PCs declined.

Usage of all functional groups decreased across depth (Figure 6, Table 4). Similar to the fall, the trend was that phenolic compounds were least metabolized across all depths, but in the spring they were metabolized more by soil microbes present under cereal rye than under pennycress ($t_{3,9}$ = 4.58, p = 0.006), mixed cover crops ($t_{3,9}$ = 3.34, p = 0.036), and the control group ($t_{3,9}$ = 3.41, p = 0.032; Table 3). At shallower depths, amino acids and carboxylic acids tended to be metabolized more than other functional groups. The usage of carbohydrates was also greater at the surface than at all other depths.

CHAPTER IV: DISCUSSION

The aim of this study was to investigate how the ability of sampled microbes to metabolize 31 types of carbon as a measure of community function changes across soil depths and among different cover crop treatments using community level physiological profiling with BIOLOG EcoPlates™. Because plant root growth adds carbon and affects rhizosphere properties, I postulated that soil depth as well as the presence and type of cover crop would significantly affect the soil microbial community (Schmidt et al., 2018; Vukicevich et al., 2016). I predicted: (a) the physiological soil profile of the microbial community, as measured by the rate and ability to metabolize a variety of carbon sources, would change across depths and differ among cover crop types, (b) shallower depths would contain microbial communities with greater functional diversity, (c) the overall functional diversity of the microbial community would be greater under cover crops than in unplanted (fallow) fields, and (d) microbial functional diversity would be greatest in soils where cover crops with higher C:N ratios were grown, as there is more carbon available for microbial consumption and slower decomposition of crop residues. Alternatively to prediction (d), microbial functional diversity may be greatest in soils where a mixed cover crop is grown than a single cover crop, as greater aboveground diversity is associated with greater belowground diversity

I found a consistent trend regarding depth that accorded with predictions, whereas trends associated with cover crop treatment were equivocal. For all measures of the soil microbial community, microbial activity declined linearly with soil depth, in accord with prediction (a). Microbes sampled near the surface metabolized nearly all of the carbon sources. Variation in the degree of metabolism of these carbon sources was also lower near the surface. However, even within the top 15 cm of the soil profile that is normally homogenized for soil analysis, the trend

in reduced activity with depth was evident in metabolism of all carbon sources. As sampling continued deeper into the soil profile, fewer carbon sources were metabolized and the variation in degree of metabolism increased. Both the number of carbon sources used and the evenness in use contribute to diversity as reflected in the Shannon diversity index, which declined as I sampled deeper through the soil profile and further outside of the rhizosphere, in accord with prediction (b). This relationship between depth and functional diversity is likely due to the decline in resource availability and reduction in carbon quality that occurs farther outside the rhizosphere (Fierera et al., 2002). At the surface more crop residues are available for microbes to decompose, and near the top of the soil profile greater plant root growth provides root exudates to nourish the microbial community (Rankoth et al., 2019).

Analysis of principal components as well as analysis of each functional carbon group further supported the conclusion that the soil microbial community changed with depth. While the overall metabolism of all functional groups decreased across depth, there were also shifts in which groups were most metabolized. In the fall, polymers were the most metabolized substrate group closer to the soil surface. Biolog EcoPlates™ contain four polymers, with all being metabolized by the microbial community at all depths, but greater color development at the time of plate reading indicated more thorough metabolism from the soil surface to 4 cm. In the spring, there was a shift to carboxylic acids and amino acids being more highly metabolized at the soil surface. There was also a noticeable spike in the metabolism of carbohydrates at the soil surface that was not observed at other depths. This could potentially be caused by the cover crops cultivating different microbes than soy did in the fall.

Trends in the impact of the cover crops on the soil microbial community did not strongly support prediction (c), (d), or the alternative prediction. In the fall, the cover crop planted the

previous season significantly affected the metabolism of carbohydrates and carboxylic acids, as indicated by the principal components analysis. This demonstrates that the cover crop planted the previous season has some lasting effects on the microbial community, even after the soy cash crop grew through the following summer. However, in the spring, the cover crop planted the previous fall and still in the field primarily affected only carbohydrate-metabolizing bacteria. This reduced impact of cover crops is likely due to the poor establishment and delayed germination observed. Because of this it was also difficult to evaluate the impact of varying C:N ratios or varying crop diversity on the microbial community. Additionally, while functional group analyses may not have shown a significant impact of the cover crop at the level of the functional group, the principal components analysis indicated that the cover crop planted is having an impact across the metabolism of all 31 carbon sources.

In the analysis of spring samples, I found the microbial community metabolized fewer carbon sources and had greater variance in their use, resulting in lower diversity. The six functional groups were also metabolized to different extents in the fall and the spring, with shifts in which groups were most commonly metabolized at each depth increment. Because samples were collected and processed at different times and length of plate development differed between fall and spring, samples from the two times are not directly comparable. However, several trends are notable. In both the fall and the spring phenolic compounds were the least metabolized. One possible explanation is that there are fewer phenolic-metabolizing bacteria present in agricultural soils. Phenolic compounds are commonly produced by plants and have been shown to play a key role in plant-microbe interactions and nodule formation, but application of synthetic nitrogen fertilizers can decrease nodule formation (Ohyama et al., 2017; Peters and Verma, 1990). The principal components analysis revealed a different pattern in the trend of carbon source

metabolism in the fall vs. the spring. EcoPlate™ output explained less variation in the microbial metabolism of the 31 carbon sources in the spring than in the fall (74.8% and 62.3%, respectively). In the fall, pennycress and control plots tended to separate out, with cereal rye and mixed cover clustered together. In the spring, though, cereal rye separated out with all other treatments clustered together. One explanation is that there may be a seasonal component contributing to changes in the microbial community. Other studies have shown that seasonal variation, such as precipitation and temperature fluctuations, can impact the activity of the soil microbial community (Hamel et al., 2006). Additionally, the amount of available carbon changes throughout the growing season, with less being available when there is less vegetative cover at the soil surface (Kaiser and Heinemeyer, 1993).

In the fall, I observed more differences in diversity measures among experimental plots, despite all plots having soybeans planted most recently on them. In the spring, the diversity measures were more consistent across experimental plots, despite significant differences in the amount of plant coverage. This seems to indicate that better establishment of the cover crop (like in the case of cereal rye) does not necessarily lead to increases in measures of diversity (Eisenhauer et al., 2011). Functional diversity in control plots was similar to the diversity of metabolism by microbes in soil under cover crops. Unplanted (fallow) plots contained a wide variety of weeds present throughout the spring, and often had vegetation present before the germination of some cover crops (mixed cover treatment), which contributed to these plots having diversity measures on par or exceeding those in treatment plots. This seems to indicate that the diversity of live plants may be equally important to successful establishment when it comes to microbial diversity (Jordan and Vatovec, 2004; Milcu et al., 2013). Additionally, studies have shown that the functional and phylogenetic diversity of plant communities have

complex interactions with soil organisms that can ultimately impact the diversity of soil microbial communities (Valencia et al., 2022).

Previous studies have shown EcoPlates™ to be an effective method for examining changes in the soil microbial community in agroecosystems (Bonanomi et al., 2020; Gomez et al., 2006; Zhou et al., 2008). EcoPlate™ studies can provide support for further work using molecular techniques that can provide more detailed information about the structure of the microbial community. Microbial biomass and bacteria:fungi ratios provided by molecular techniques, like PLFA and EL-FAME, can be useful for determining the impact of cover crops on the microbial community. As a culture method, EcoPlates™ cannot capture the full extent of what is happening in the soil. Cover crops may be impacting total microbial biomass, but not the overall functional diversity or species diversity of the microbial community (Frasier et al., 2016). Additionally, EcoPlates™ only contain 31 carbon sources, which may not capture all those able to be metabolized by a microbial community.

CHAPTER V: CONCLUSIONS

While I did not find our cover crop treatments had much impact on the soil microbial community, other studies have demonstrated that cover crops affect the composition and diversity of microbial communities (Finney et al., 2017; Frasier et al., 2016; Kim et al., 2020). Our results demonstrate some of the difficulties that farmers face when implementing cover crops, like poor establishment and delayed germination. Cover crops still show considerable promise, though, as a way to make our agricultural practices more sustainable. With consideration for the needs of each specific farmer, cover crops can be chosen that maximize benefits to the overall health of soils while maintaining agricultural production.

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APPENDEIX A: TABLES

Table 1. Least squares means for richness, evenness, and Shannon diversity for each treatment (CR = cereal rye; F = fallow/control; PC = pennycress; PCRO = pea, clover, radish, and oat)**.**

	FALL					
	Richness		Evenness		Shannon diversity	
	Mean	SE	Mean	SE	Mean	SE
CR	19.575	2.313	0.885	0.0222	2.844	0.134
F	21.132	2.313	0.904	0.0222	2.934	0.134
PC	22.413	2.313	0.916	0.0222	3.026	0.134
PCRO	22.329	2.313	0.908	0.0222	2.975	0.134

	SPRING					
	Richness		Evenness		Shannon diversity	
	Mean	SE	Mean	SE	Mean	SE
CR	16.712	1.218	0.884	0.0102	2.748	0.0683
F	16.569	1.218	0.880	0.0102	2.749	0.0683
PC	16.606	1.218	0.880	0.0102	2.742	0.0683
PCRO	17.520	1.218	0.897	0.0102	2.819	0.0683

Table 2. Number of carbon sources from each functional group that loaded heavily on each principal component.

		PC 1		PC 2		PC 3	
	Total	Fall	Spring	Fall	Spring	Fall	Spring
Polymers	4	2	2	4	0	0	1
Carbohydrates	10	6	3	8	9	0	1
Carboxylic Acids	7	5	5	2	2	2	2
Amino Acids	6	3	3	2	0	1	0
Amines	2	2	2	1	0	1	1
Phenolic Compounds	2	1	1	0	0	1	1

Table 3. Least squares means for each of the six functional group for each treatment (CR = cereal rye; F = fallow/control; PC = pennycress; PCRO = pea, clover, radish, and oat)**.** Cover crop treatments differed only in phenolics in the spring. Means with different letters were significantly different at $p < 0.05$.

	FALL											
	Polymers		Carbohydrates		Carboxylic acids		Amino acids		Amines		Phenolics	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
CR	0.988	0.171	0.870	0.149	0.936	0.148	0.973	0.144	0.840	0.171	0.589	0.115
F	1.147	0.171	0.916	0.149	0.962	0.148	0.939	0.144	0.762	0.171	0.409	0.115
PC	1.018	0.171	0.927	0.149	1.073	0.148	1.065	0.144	0.917	0.171	0.635	0.115
PCRO	1.152	0.171	0.951	0.149	1.006	0.148	1.086	0.144	0.971	0.171	0.607	0.115

	SPRING											
	Polymers		Carbohydrates		Carboxylic acids		Amino acids		Amines		Phenolics	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
CR	0.592	0.059	0.466	0.103	0.782	0.059	0.798	0.065	0.638	0.070	0.441^{a}	0.052
F	0.540	0.059	0.628	0.103	0.722	0.059	0.695	0.065	0.517	0.070	0.318^{b}	0.052
PC	0.554	0.059	0.564	0.103	0.734	0.059	0.753	0.065	0.603	0.070	0.276^{b}	0.052
PCRO	0.599	0.059	0.688	0.103	0.803	0.059	0.835	0.065	0.682	0.070	0.321^{b}	0.052

Table 4. Results of PROC MIXED of each functional group.

	FALL				
GROUP		CROP			DEPTH
	d.f.	F Value	Pr > F	Slope	SE
Polymers	1,79	0.36	0.784	-0.022	0.003
Carbohydrates	1,79	0.08	0.967	-0.021	0.002
Carboxylic Acids	1,79	0.62	0.620	-0.017	0.002
Amino Acids	1,79	0.44	0.731	-0.018	0.002
Amines	1,79	1.05	0.417	-0.020	0.002
Phenolic Compounds	1,79	1.61	0.255	-0.012	0.002

	SPRING				
GROUP		CROP			DEPTH
	d.f.	F Value	Pr > F	Slope	SE
Polymers	1,79	0.84	0.504	-0.008	0.001
Carbohydrates	1,79	3.29	0.072	-0.014	0.002
Carboxylic Acids	1,79	1.56	0.267	-0.014	0.001
Amino Acids	1,79	1.61	0.255	-0.013	0.001
Amines	1,79	1.73	0.231	-0.012	0.001
Phenolic Compounds	1,79	7.78	0.007	-0.009	0.001

Supplementary Table 1. Factor loadings from principal components analysis of 31 carbon sources.

	Varimax rotated factor pattern					
	Fall			Spring		
	PC 1 (% of variation = 63.8)	PC 2 (% of variation = 6.0)	PC 3 (% of variation = 5.0)	PC 1 (% of variation = 47.3)	PC 2 (% of variation = 8.5)	PC 3 (% of variation = 6.5)
alpha-Cyclodextrin	29	**86**	22	20	22	25
alpha-D-Lactose	36	**78**	19	17	**77**	-4
alpha-Keto Butyric Acid	19	34	10	-5	2	12
beta-Methyl-D-Glucoside	**43**	**71**	23	35	**70**	37
D-Cellobiose	**48**	**71**	19	28	**68**	**44**
D-Galactonic Acid-gamma-Lactone	**56**	**56**	17	**76**	34	-11
D-Galacturonic Acid	**75**	23	11	**85**	5	-1
D-Glucosaminic Acid	**67**	38	3	**50**	**46**	28
D,L-gamma-Glycerol Phosphate	23	39	-2	12	**57**	9
D-Malic Acid	36	**61**	**53**	25	**50**	**48**
D-Mannitol	**87**	25	14	**75**	**44**	11
D-Xylose	34	**81**	22	30	**80**	31
gamma-Amino Butyric Acid	**79**	39	27	**72**	36	34
Glucose-1-Phosphate	39	**76**	15	29	**78**	28
Glycogen	31	**81**	16	14	14	**64**

(Table Continues)

Supplementary Table 1, Continued

	Varimax rotated factor pattern					
	Fall			Spring		
	PC 1 (% of variation = 63.8)	PC 2 (% of variation = 6.0)	PC 3 (% of variation = 5.0)	PC 1 (% of variation = 47.3)	PC 2 (% of variation = 8.5)	PC 3 (% of variation = 6.5)
Glycyl-L-Glutamic Acid	32	**74**	-11	8	14	3
2-Hydroxy Benzoic Acid	11	3	5	14	9	-10
4-Hydroxy Benzoic Acid	**60**	33	**55**	**47**	27	**59**
i-Erythritol	**41**	**75**	20	30	**74**	-12
Itaconic Acid	**60**	**42**	**45**	**58**	27	24
L-Arginine	**81**	35	25	**79**	20	32
L-Asparagine	**87**	32	13	**78**	24	31
L-Phenylalanine	31	22	**49**	18	30	2
L-Serine	**78**	**47**	16	**78**	32	28
L-Threonine	19	14	2	8	9	4
N-Acetyl-D-Glucosamine	**66**	**51**	16	**46**	**72**	26
Phenyethylamine	**42**	**50**	**64**	**44**	27	**65**
Putrescine	**81**	34	13	**68**	2	28
Pyruvic Acid Methyl Ester	**54**	**50**	19	**62**	34	**47**
Tween 40	**69**	**43**	28	**69**	31	29
Tween 80	**76**	**45**	12	**69**	29	2

APPENDIX B: FIGURES

Figure 1. Experimental design and location at the ISU Farm in Lexington, Illinois. Each block (I-IV) contains four treatments: (1) unplanted control; (2) pea, clover, radish, oat mix; (3) pennycress; and (4) cereal rye.

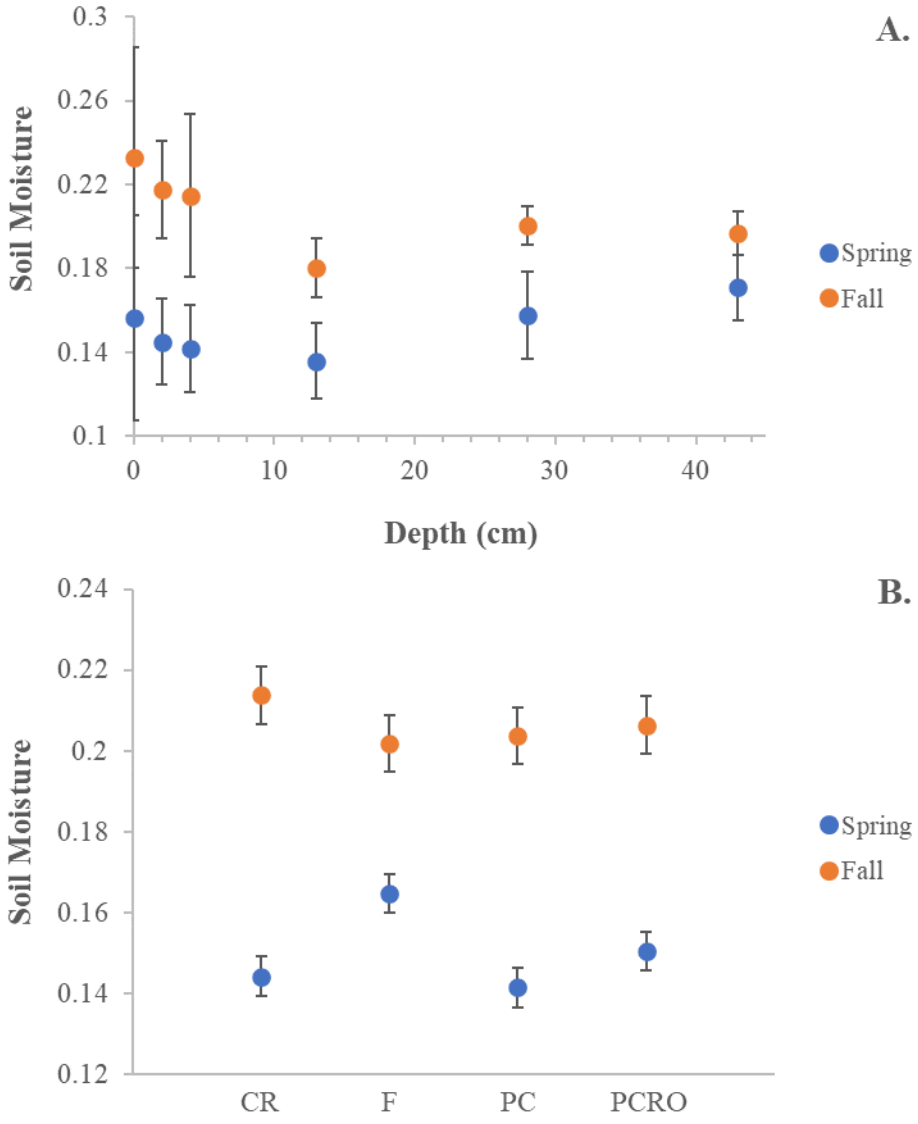

Figure 2. Soil moisture means (±SE) for sampling depth (A) and LSMEANS (±SE) for cover crop treatment (B). CR = cereal rye; F = fallow/control; PC = pennycress; PCRO = pea, clover, radish, and oat. Depths were sampled in increments: 0-2 cm, 2-4 cm, 4-6 cm, 13-15 cm, 28-30 cm, and 43-45 cm.

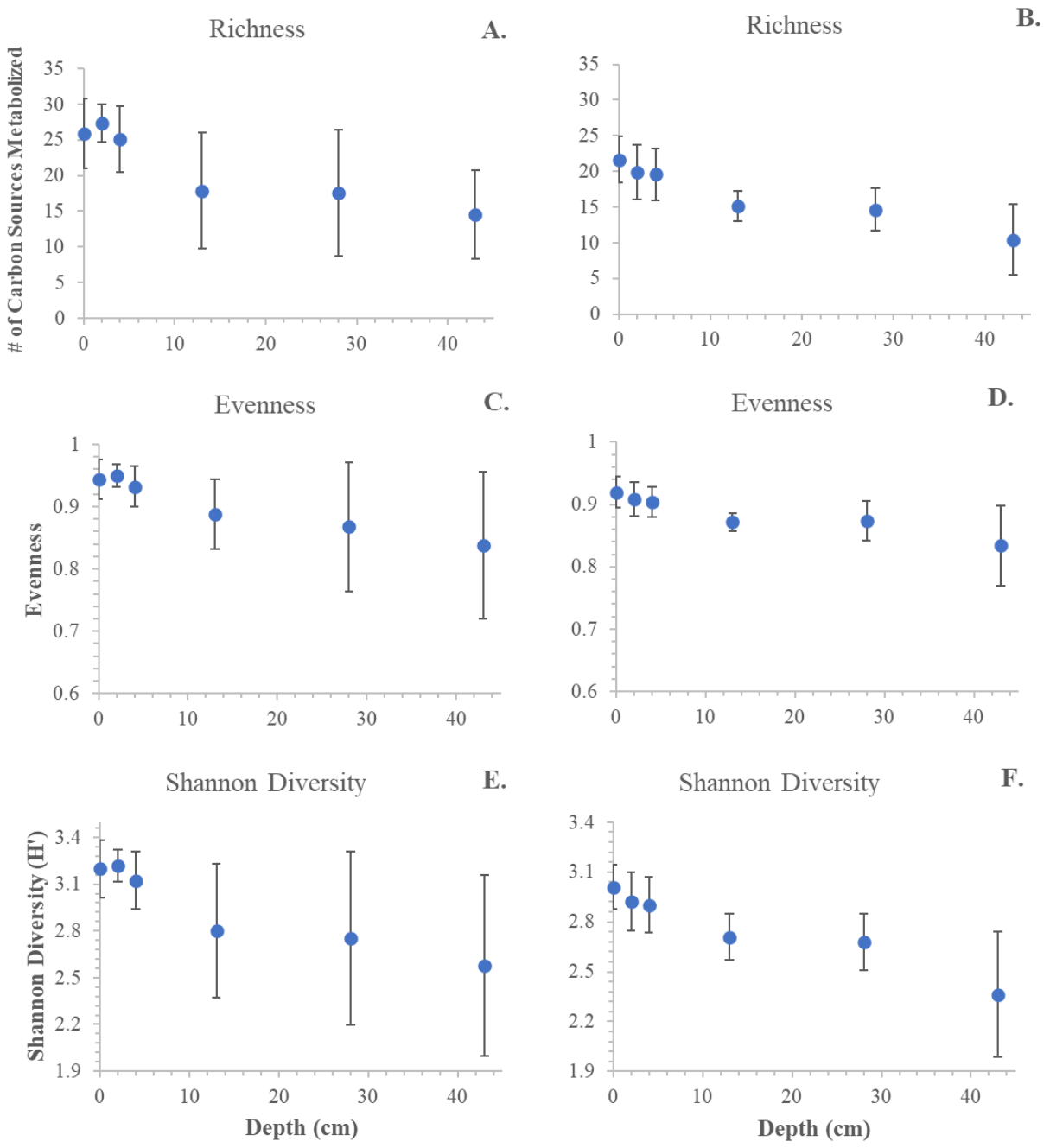

Figure 3. Mean richness, Evenness, and Shannon diversity (±SE) across depths in the fall (A, C, E) and the spring (B, D, F). Depths were sampled in increments: 0-2 cm, 2-4 cm, 4-6 cm, 13-15 cm, 28-30 cm, and 43-45 cm.

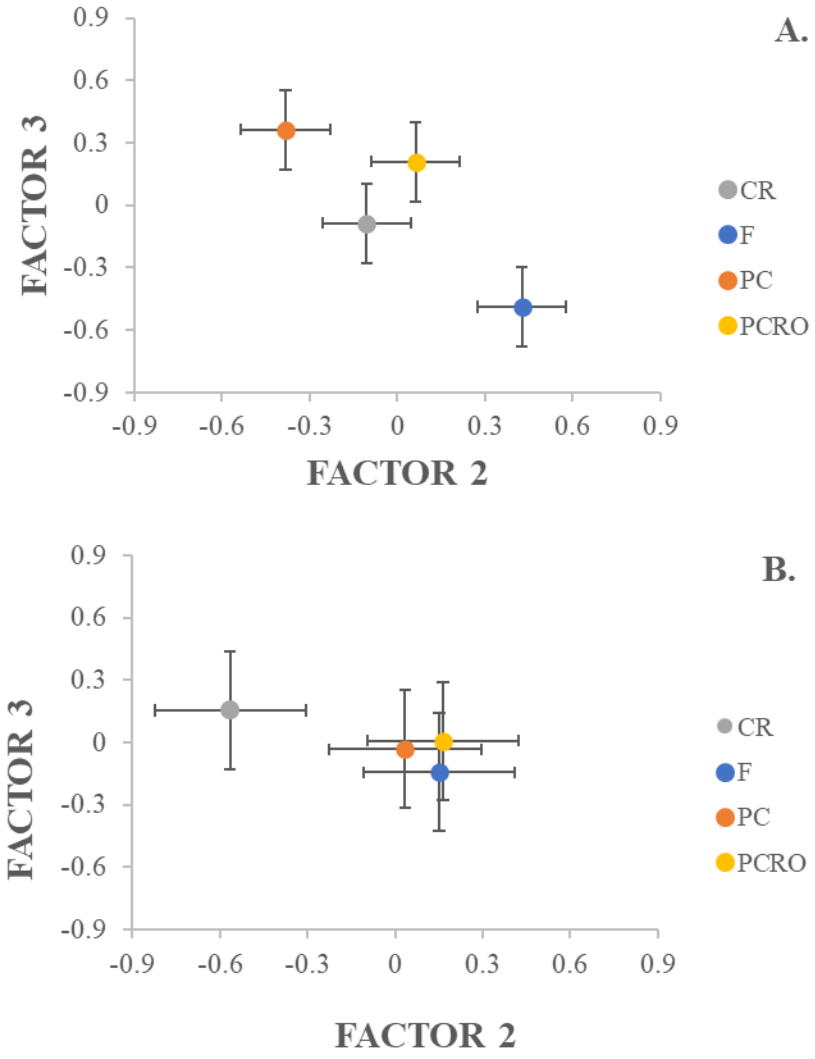

Figure 4. Principal components analysis of metabolism of the 31 carbon sources by each crop for the fall (A) and spring (B). Treatments: CR, cereal rye; F, fallow control; PC, pennycress; PCRO, pea, clover, radish, and oat.

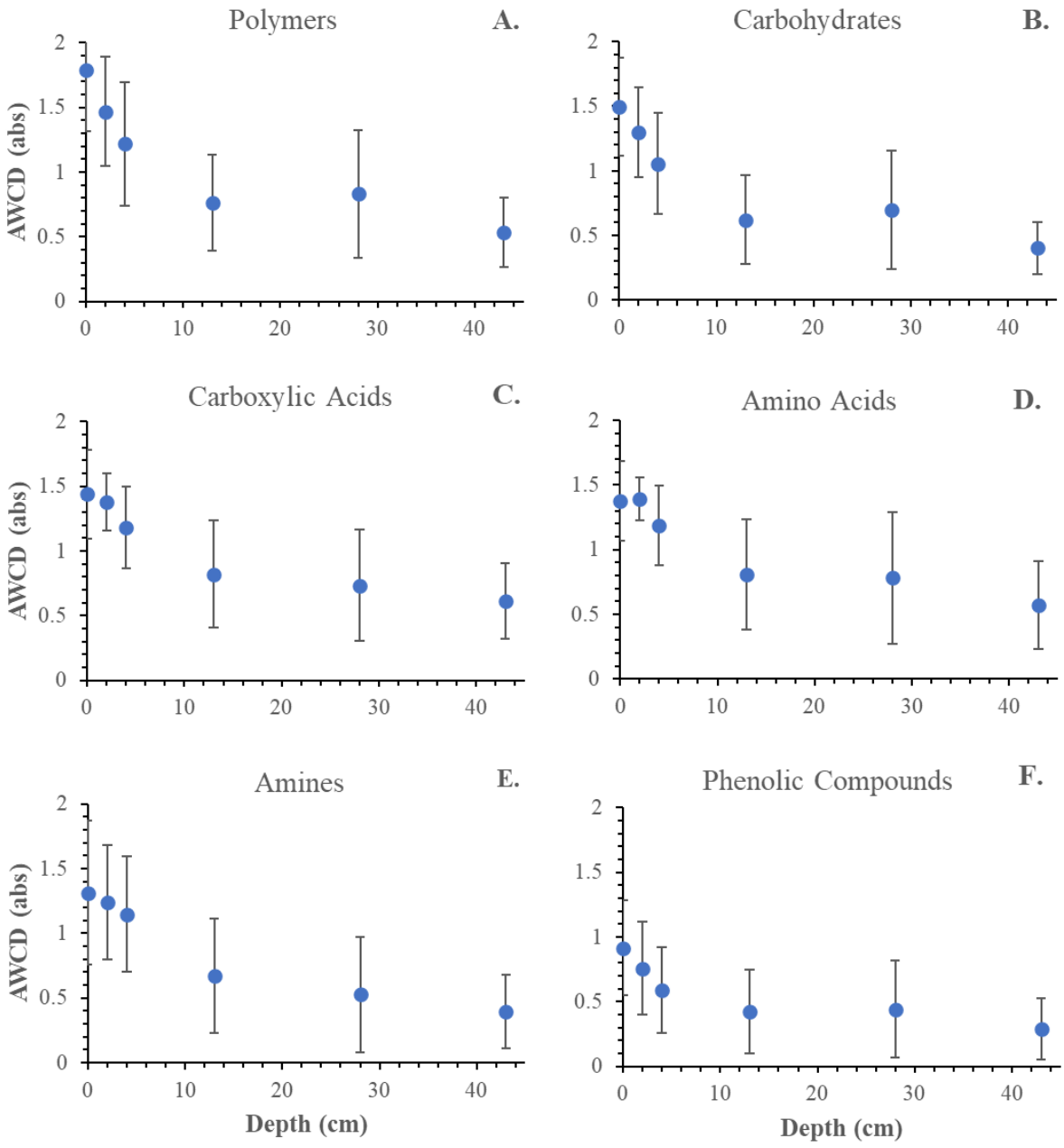

Figure 5. Metabolism of each functional group (mean AWCD ±SE) by depth for the fall. Depths were sampled in increments: 0-2 cm, 2-4 cm, 4-6 cm, 13-15 cm, 28-30 cm, and 43-45 cm.

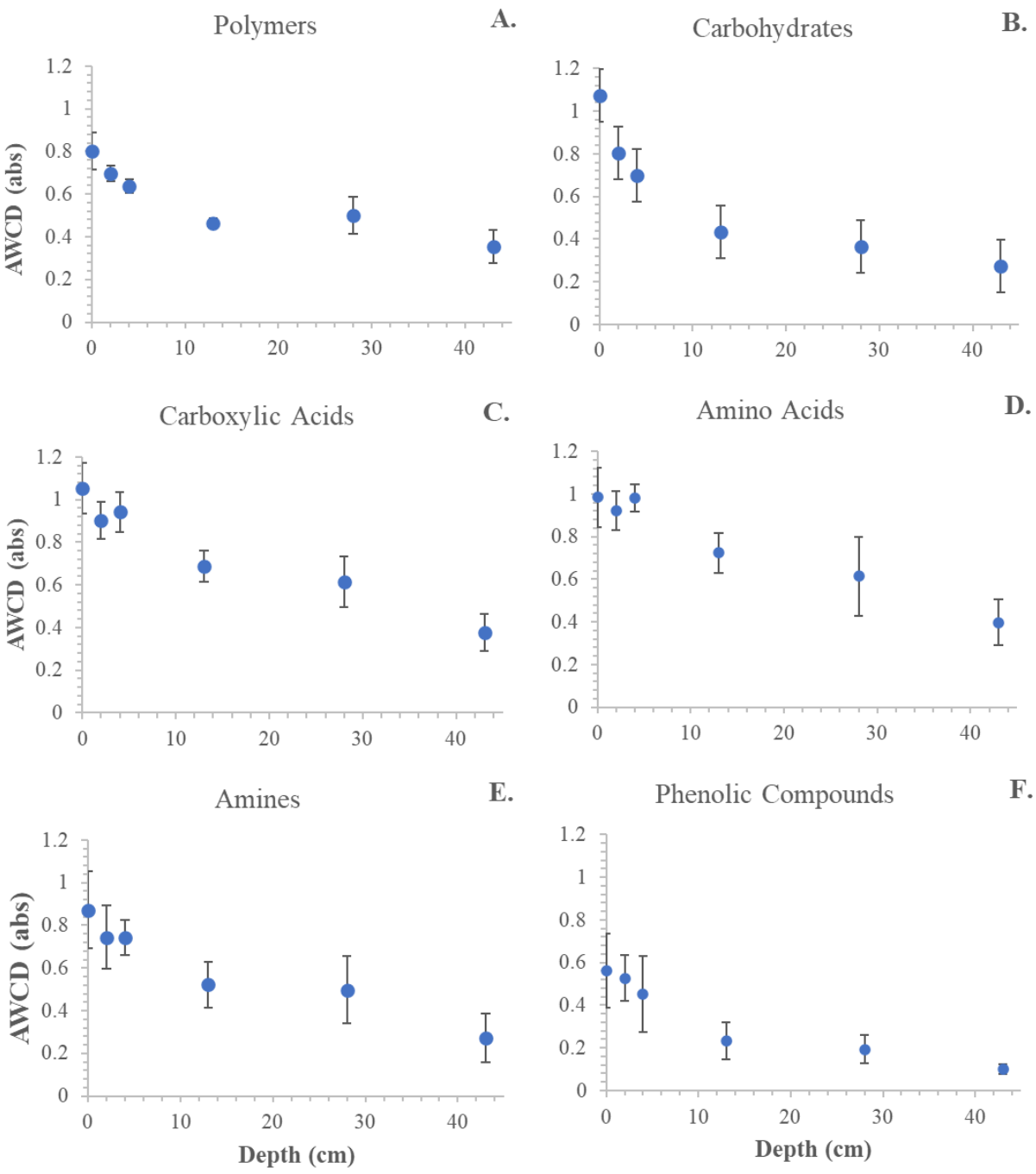

Figure 6. Metabolism of each functional group (mean AWCD ±SE) by depth for the spring. Depths were sampled in increments: 0-2 cm, 2-4 cm, 4-6 cm, 13-15 cm, 28-30 cm, and 43-45 cm.