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# Short-term Response in Soil Health to Management Practices within Large- and Small-Scale Cropping Systems in Arkansas

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Short-term Response in Soil Health to Management Practices within Large- and Small-Scale Cropping Systems in Arkansas

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**Abstract**

Improving soil health is a growing area of interest within large- and small-scale agricultural systems in Arkansas as producers face increasing input costs, increased awareness of variability and intensity in extreme weather events, decreasing resource availability, and suboptimal productivity related to environmental degradation. Practices aimed at improving soil health such as cover cropping and the use of organic amendments are increasingly recommended and adopted; however, research on optimal management in the Mid-South is lacking. Two studies were conducted to evaluate the effectiveness of soil health management under both small- and large-scale farming operations in Arkansas. The objective of the small-scale study was to evaluate the effect of cover crop (CC) termination and poultry litter (PL) application methods and subsequent crop, which included winter barley (*Hordeum vulgare* L.) and soft red winter wheat (*Triticum aestivum* L.), on enzyme activity, nutrient availability, soil carbon, and aggregate stability within the first year. Soil response to CC termination method and PL application method was minimal after only one year. Small grains had a more immediate effect on soil properties related to nutrient cycling, including acid phosphatase and arylsulfatase activities and nutrient availability. Results of a subsequent greenhouse study confirmed the importance of crop species influence on nutrient cycling. These studies indicate that while differences in soil health management may require more evaluation, crop species contribute within a short-time scale to soil rhizosphere dynamics. The objective of the large-scale study was to evaluate the effect of PL application rate combined with flood or furrow irrigation methods on nutrient cycling enzyme activity, nutrient availability, soil carbon, and soil respiration under recently land-leveled rice (*Oryza sativa* L.) production fields within one year. The results of this study did not consistently demonstrate a benefit in applying higher PL rates within the limited period of the study, regardless of irrigation method. The β-D glucosidase activity was the only

soil enzyme to show greater concentrations under increased PL rates and flooded irrigation; however, all measurements of soil carbon were greater under the lowest PL rate combined with furrow irrigation. Soil respiration and nutrient availability were unaffected by PL rate and irrigation method in the first year. These results indicate that more than one year of PL application, under both flooded and furrow irrigated management, may be required to overcome the spatial variability and disturbance caused by land leveling. Overall, the results of both studies demonstrate that changes in soil health from management may not occur on a short-term basis, given the strong legacy effects of past management. However, these legacy effects are not inherently negative and support the role of soil conservation management in building resilient and healthy soils.

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## Introduction

Agriculture has been a fundamental part of Arkansas' cultural and economic development for many years, from the early settlement era when subsistence farming was common practice to the modern era, where commercial production dominates (Williams, 2020). Today, agriculture remains the largest industry in Arkansas contributing $16 billion to the state economy annually (English and Popp, 2022). The primary crops produced in Arkansas include rice (*Oryza sativa* L.), cotton (*Gossypium hirsutum* L.), soybean [*Glycine max* (L.) Merr.], corn (*Zea mays* L.), and wheat (*Triticum aestivum* L.). As of 2021, there were 42,220 farms located on 5.7 million ha of cropland in Arkansas, with 97% of those farms being family-owned operations (USDA-NASS, 2021). The size and scale of these operations vary greatly; for instance, nearly half of the total number of farms in Arkansas fall under the smallest economic sales class, represented by annual sales less than $10,000 (USDA-NASS, 2019). In contrast, 8% of the total number of Arkansas farms are classified under the largest economic sales class, with annual sales exceeding $1 million. These large-scale farms represent roughly 40% of the total cropland acreage and are responsible for 92% of the state's agriculture production output (USDA-NASS, 2019).

Despite considerable differences in acreage, production, and economics, Arkansas farming operations of all sizes must balance profitable production with stewardship of the land in order to be sustainable. The term "sustainable" as it pertains to agriculture can vary both in definition and in practice; however, some generally agreed upon elements include productivity that meets human and animal needs, profitability that maintains the economic viability of farms, and the use of practices that enhance environmental quality (Food, Agriculture, and Conservation Act, 1990). Maintaining environmental quality has become increasingly important as producers face challenges related to climate change (for example, more frequent extreme weather events

and an increased mean global temperature) and as increasing demands are placed on decreasing acreage. A basic element of environmental quality is soil health; soil is not only a life-sustaining ecosystem for plants, animals, and humans, but is also interrelated with other environmental aspects such as air and water quality. Soil health is defined by the Natural Resource and Conservation Service (NRCS) as "the continued capacity of soil to function as a vital living ecosystem that sustains plants, animals, and humans" (USDA-NRCS, 2018). The introduction of the idea of soil health marked a shift away from a predominantly fertility- and physical structure-based view of soil, instead placing an emphasis on the soil as a living system with complex interconnected relationships that exist between physical, chemical, and biological processes (Karlen et al., 2021).

While Arkansas' highly productive alluvial and loessal soils have allowed the agricultural industry to thrive, some traditional management practices associated with the introduction of agriculture have resulted in a decline in environmental quality and agricultural sustainability (Brye and Pirani, 2005). Prior to European settlement in Arkansas, approximately 364,000 ha of the eastern half of the state was covered by the Grand Prairie, a native undisturbed ecosystem that consisted of tall grass prairie, forests, savannahs, and wetlands (ANHC, 2013). Early settlers converted the native ecosystem to agricultural production, establishing continuous tillage and limited crop diversity as the new standards for the region (ANHC, 2013). Brye and Pirani (2005) endeavored to quantify the effect of tilled agriculture on land previously covered by the Grand Prairie and reported significant losses in soil quality from the agricultural sites compared to undisturbed prairie locations including increased bulk density, decreased soil organic matter and total organic carbon and nitrogen, increased soil pH, and a shift in soil surface particle size toward higher clay content, which the authors identified as a risk for greater erosion potential

(Brye and Pirani, 2005). In addition to declines in soil quality associated with agriculture in Arkansas, increased demands and inefficient use of irrigation water have resulted in an alarming rate of depletion of the Mississippi River Valley Alluvial Aquifer (MRVAA) (ANRC, 2020). Some of the properties associated with soil quality loss such as increased bulk density and loss of organic matter are also associated with decreased soil water holding capacity and irrigation efficiency (Dennis et al., 2010). These environmental changes and production challenges have prompted producers, researchers, and governmental agencies to seek out more sustainable, soil health building practices such as conservation tillage, cover cropping, diverse crop rotations, and the use of organic amendments to rebuild and maintain soil health (Fageria, 2007; Wade et al., 2015).

The research detailed in this thesis examines the short-term effects of soil health management within both a small-scale, organic production system and a large-scale, conventional production system. The wide range in production context explored in this research reflects the wide range in production systems that exists in Arkansas, as well as the variety of motivations for adopting soil health building practices and the site-specific challenges that come with adopting those practices. Within the context of small-scale, organic cereal grain production, growing a summer cover crop between cash crops could fulfill the need for diversified weed management and the use of organic amendments such as poultry litter could contribute to organic-compliant soil fertility. Cover cropping and applying poultry litter also build soil organic matter and contribute to microbial diversity, thus improving soil health over time. Investigation into optimal management of these practices as well as the changes in soil health realized will provide guidance to small-scale producers interested in improving soil health as a means for improving overall production. Within the context of large-scale, conventional rice production

that is common in eastern Arkansas, the use of poultry litter on degraded soils following land-leveling serves to reclaim soil function and quality through the addition of organic matter, plant-available macro- and micro-nutrients, and an active microbial population (Brye et al., 2004). Investigation into soil response to poultry litter rate will inform best management practices that balance economics, soil health and environmental conservation, and crop production.

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# Chapter 1

# Literature Review

## Small Grain Production in Arkansas

### *Wheat*

Wheat (*Triticum aestivum* L.) is ranked the third largest field crop produced in the United States in terms of yield, gross farm receipts, and total land area planted (USDA-ERS, 2022). There are six classes of wheat grown in the United States including durum wheat, hard red spring, hard red winter, hard white, soft white, and soft red winter (USDA-ERS, 2022). In Arkansas, soft red winter wheat is considered an economically important crop; Arkansas ranks in the top ten producers of soft red winter wheat and 29$^{th}$ in total winter wheat production in the United States (USDA-NASS, 2020). Despite an overall decline in wheat production over the last 20 years at both the national and state level, Arkansas wheat production has increased in hectares planted and total yield for the past three years. In 2021, Arkansas producers planted 84,984 hectares (210,000 acres) of winter wheat, representing an increase of 190% from the total hectares planted in 2019 (44,515 hectares, 110,000 acres) (USDA-NASS, 2019; USDA-NASS, 2021).

Wheat in Arkansas is grown primarily in three regions: the Mississippi River Delta, the Arkansas River Valley, and the Red River Valley. Winter wheat planting dates vary by region and climatic conditions; however, Arkansas winter wheat is generally planted in October and harvested in June. There are four common planting methods for wheat including conventionally drilled into a prepared seed bed, broadcast incorporated, no-till drilled, and unincorporated broadcast (Keisling et al., 1997). A common planting rotation in Arkansas is the wheat-soybean double-crop, where soybeans are planted soon after wheat harvest, and wheat is planted again after soybean harvest (Keisling et al., 1997). The double-crop system allows producers to harvest two cash crops in a single year, thus diversifying farm income sources and increasing

profitability. While the wheat-soybean double crop system is the most practiced wheat rotation in Arkansas, wheat is also grown in rotation with other summer annual cash crops such as corn and rice (Anders et al., 2002).

In addition to economic gains, the inclusion of winter wheat in annual crop rotations has decreased erosion and reduced soil water loss from runoff or evaporation (Sanford, 1982). Winter wheat provides cool season vegetative cover, allowing it to provide many of the same benefits of a winter annual cover crop even when harvested as a cash crop. For instance, winter wheat can play a role in nutrient scavenging in the same way that a cover crop would when post-harvest residues are returned to the soil. Research conducted in a Colorado vegetable production system found that winter wheat scavenged nitrogen from a depth of 1.5 m (5 ft) in the soil profile (Delgado et al., 1999). Furthermore, wheat can take up 55 to 69.2 kg $P_2O_5$ and 166 kg $K_2O$ per $ha^{-1}$ by boot stage, around 80% of which is recycled when post-harvest wheat residue remains in the field (SARE, 2007). Winter wheat can also contribute to weed suppression through competition. For example, wheat varieties with vigorous growth traits outcompeted and suppressed Italian ryegrass (*Lolium perenne L. ssp. multiflorum [Lam.] Husnot*) growth (Worthington et al., 2015).

Despite the benefits, winter wheat production in Arkansas is not without challenge. The winter and spring growing seasons see the majority of annual rainfall in Arkansas, which when combined with flat, poorly drained loessal soils in eastern Arkansas, results in extended periods of saturated conditions (Mascagni et al., 1995). These conditions can lead to decreased rooting depth as well as increased rates of denitrification (King and Evans, 1987). One approach to alleviating poor drainage for cereal grain production is the use of drainage furrows to redirect surface water (Tacker, 2022). This is a common and effective practice; nevertheless, practices

aimed at improving soil health through increasing organic matter and improving soil structure should be considered in long-term plans for improving soil drainage.

Winter wheat production in Arkansas typically requires applications of N, P, and K fertilizers and lime to maintain optimal soil pH (Roberts & Slaton, 2014). Recommended N rates for winter wheat range from 100 to 156 kg N $ha^{-1}$, depending on crop rotation history and soil texture, and recommended P and K rates are 112 kg $P_2O_5$ $ha^{-1}$ and 156 kg $K_2O$ $ha^{-1}$ for soils with very low soil test levels (Roberts & Slaton, 2014). The cost of fertilizer applied at these rates represents an estimated 35% of total wheat production costs; thus, practices that contribute to overall soil fertility and nutrient holding capacity can be economically and environmentally beneficial (USDA-ERS, 2022).

*Barley*

Barley (*Hordeum vulgare* L.) is a cool season cereal grain that is grown in a wide range of climatic regions across the world. Barley has been grown in the United States as far back as the early 1600s when it was grown by colonists for livestock feed and brewing purposes (Weaver, 1944). The United States currently ranks 11$^{th}$ in global barley production, making up 2% of the estimated 146 million tons produced globally in 2021 (USDA-FAS, 2022). In the U.S., barley is grown primarily in the North Central and Northwest regions, accounting for approximately 80% of total U.S. barley production (USDA-FAS, 2022). Barley is a principal ingredient in the production of beer and nearly three-quarters of the barley produced in the United States is processed into malt for brewing purposes (USDA-FAS, 2022).

Barley production in Arkansas has been minimal for the last several decades; the last recorded barley harvest in the state occurred in 1972 on 404 hectares (1,000 acres) with a

reported yield of 2353.8 kg $ha^{-1}$ (USDA-NASS, 2022). In contrast, 6,474 hectares (16,000 acres) were harvested in 1960 with a reported yield of 1916.7 kg $ha^{-1}$. The decline in production observed in Arkansas and several other states has been attributed to an increase in the profitability and popularity of crops such as corn, wheat, and soybeans for livestock feed. An increase in the market value for these crops marked a decrease in barley grown for livestock feed as producers shifted acreage that had been used for barley into corn, wheat, and soybean production (ARMC, 2022). The shift away from barley was especially prevalent in regions with warmer, wetter conditions that are less favorable for vigorous barley growth than the cooler, drier conditions of the North Central and Northwest regions (ARMC, 2022).

Despite the decline of barley grown for livestock feed, the consistent demand for malting barley has sustained the U.S. barley industry. The growth of the craft brewing industry, in particular, has led to an increase in the demand for locally sourced barley, as many craft brewers have an interest in locally produced ingredients that meet malting quality standards. In Arkansas, the craft brewing industry has nearly doubled in number of breweries and overall production in the last five years. As of 2020, there were 43 craft breweries in Arkansas with an annual production of 41,746 barrels of craft beer compared to only 24,643 barrels and 26 craft breweries in 2015 (Brewers Association, 2022). The growing craft beer industry in Arkansas represents an opportunity for the economic viability of barley production in Arkansas.

*Rice*

Rice (*Oryza sativa* L.) is a semi-aquatic cereal grain that serves as a staple crop for more than half of the global population (USA Rice, 2020). Rice grows across a range of temperate, subtropical, and tropical regions, but production is generally limited to areas with temperatures ranging from 27 to 32˚C (Aghamolki et al., 2014). In the United States, rice is grown primarily

in Arkansas, Texas, Mississippi, Missouri, Louisiana, and California (USA Rice, 2020). Arkansas is the largest rice producer in the nation, accounting for more than 40% of the rice grown in the United States and contributing more than 1 billion dollars to the Arkansas state economy (USA Rice, 2020).

Rice in Arkansas is grown primarily in the Mississippi River Alluvial Plain region in the eastern half of the state on silt-loam, clay, and sandy-loam soils (Hardke, 2021). Roughly 85% of the rice planted in Arkansas rice is drill-seeded into dry fields, while water-seeded rice accounts for less than 5% of the total acres of rice planted in the state. Flooding for dry-seeded rice typically takes place when the rice has reached the 4 – 5 leaf stage and is maintained until 25-35 days after the crop has reached 50% heading, at which point fields are drained in preparation for harvest (Hardke, 2021).

Regardless of the planting method used, water supply is an essential component of rice production. In 2020, 77.3% of total rice acreage in Arkansas was irrigated with groundwater, while the remaining 22.7% was irrigated using reservoirs, streams, and bayous (Hardke et al., 2021). Irrigation for rice production is managed primarily through the method of water delivery to the field and through levee structure and arrangement. Flooding through the levee and gate system is the most common water delivery method, making up 49.9% of rice acres, followed by the more efficient multiple inlet system, which uses poly-tubing to conserve water and labor (31.1%) (Hardke et al., 2021). Furrow irrigation and intermittent flooding are two less common water delivery methods that can improve water-use efficiency, making up roughly 16 and 2% of Arkansas rice land area, respectively (Hardke et al., 2021). As mentioned above, levee structure and arrangement are also important factors in irrigation water management. In 2020, contour levee systems accounted for roughly 49% of total Arkansas rice land area, followed by precision-

leveled (or straight levee) at 37% and zero-graded at 13.3% (Hardke et al., 2021). When compared to conventional contour levee fields, straight levee fields have been shown to decrease water use by 8%, and zero-graded fields show a decrease in water use of 41% (Reba and Massey, 2020).

Irrigation management practices that conserve water are increasingly adopted and incentivized in Arkansas. Over the last several decades, an over-reliance on groundwater for agricultural irrigation has led to an alarming rate of depletion of the Mississippi River Valley alluvial aquifer (MRVAA) as annual withdrawals consistently exceed the rate of recharge (ANRC, 2020). One method that hydrologists use to determine whether groundwater is being used at a sustainable rate is through an estimate of sustainable yield. The sustainable yield of an aquifer is defined as the rate of groundwater withdrawal that can be maintained without adversely affecting the quality or the quantity of groundwater in the aquifer (ANRC, 2014). The sustainable yield of the MRVAA in 2018 was 147 $m^3 s^{-1}$, while the withdrawal rate from the same year was 287 $m^3 s^{-1}$, meaning that only 51% of the groundwater withdrawn was used sustainably (ANRC, 2020; Czarnecki et al., 2008; Reba et al., 2017). While groundwater from the MRVAA continues to be used at unsustainable rates, the reported use for 2018 was roughly 38 $m^3 s^{-1}$ less than data reported for 2015 (325 $m^3 s^{-1}$) (ANRC, 2020). The slight reduction in the depletion rate of the MRVAA can be attributed in part to the adoption of more efficient irrigation management practices by producers in response to declining groundwater levels (ANRC, 2020; Yaeger et al., 2018).

*Precision-Leveled Rice Production*

Precision leveling, or land leveling, is an approach to water conservation that involves altering the natural topography of a field to achieve a small, uniform slope in a single direction

from the top to bottom of a field, allowing for more rapid and uniform water movement across the field. Land leveling is dual purpose as it allows better efficiency for both furrow irrigation and levee-based flood irrigation while also providing better surface drainage of runoff. Land leveling is accomplished by removing topsoil in higher areas and redistributing it into lower elevation areas of the fields, thus improving water efficiency by filling water-holding depressions. To optimize water movement across a field, land is graded to create a precise slope; in Arkansas, a slope ranging between 0.05 and 0.1 percent is recommended for rice that is rotated with other crops (Hardke et al., 2021). A zero-slope (zero-grade) is also utilized in rice production; however, it is only recommended for continuous rice as zero slope grading does not facilitate furrow irrigation nor lends itself to corn and soybean rotations (Hardke et al., 2021). Land leveling increases water efficiency by an estimated 10 to 20% and increases rice yield by an estimated 10% (Young et al., 2004). In order to encourage groundwater conservation, the Arkansas Department of Agriculture offers producers a tax credit incentive of 25% of the project cost of land leveling (ANRC, 2021). As of 2020, precision-leveled and zero-graded rice made up 37.2% and 13.3% of total Arkansas rice production land area, respectively, marking a slight decrease from 2018 when precision graded land area represented 39.3% of total rice production area and a slight increase in zero-graded area (12.8%) (Hardke et al., 2021).

Land-leveled fields reduce water usage, optimize flooding depth, and reduce labor, resulting in greater economic returns than contour levee rice (Watkins et al., 2007). Despite the economic and water conservation gains obtained under precision-leveled fields, a decline in soil quality following land leveling has been reported as cut areas expose subsoil that is low in organic matter, nutrient deficient, and potentially sodic due to the use of irrigation water on poorly drained soils (Brye et al., 2006; Daniels et al., 2002; Robbins et al., 1997). Brye et al.

(2003) reported that soil surface bulk density and clay content increased following land leveling due to changes in spatial distribution. Brye et al. (2005) also observed that rooting depth was reduced by 25% in a precision-graded silt loam soil in Arkansas as topsoil removal brought subsurface hardpans closer to the surface. Soil surface chemical properties also undergo significant changes following leveling; Sharifi et al. (2014) described a significant decrease in soil OM, pH, and N, P, K, and an increase in electrical conductivity (EC) following the precision-leveling of rice fields in Iran. Decreased N was associated with organic matter loss, while decreased available soil P was determined to be a combined result of P-deficient clay subsoils being brought to the surface as well as a decrease in P mineralizing fungi (Sharifi et al., 2014). Furthermore, Miller (1990) concluded that post-land-leveled soil showed low soil sulfur and magnesium and high sodium, all of which were associated with a decline in rice growth. Sulfur is essential for optimal rice yields, and while adequate S is generally supplied by irrigation water and organic matter decomposition in Arkansas, S deficiency may still occur in sandy soils with low organic matter or under continuously flooded rice production (Hardke, 2021). Additional soil nutrient management considerations may, therefore, become necessary post-precision grading, as loss of organic matter reduces nutrient additions as well as nutrient holding capacity.

Along with the chemical and physical changes that occur under precision-leveled fields, shifts in soil biology also occur. Brye et al. (2003) found that while microbial community structure was not affected significantly by land leveling, bacterial and fungal biomass concentration and content significantly decreased following land leveling. In California, topsoil was removed for land-leveling purposes for road construction, resulting in a drastic decrease in microbial biomass and diversity compared to undisturbed sites (Degrood et al., 2005). This

suggests that topsoil removal, and not disturbance alone, causes the observed decline in soil microbial communities following land leveling. In contrast, Parfitt et al. (2013) reported an overall increase in soil microbial biomass carbon from 297 to 510 mg $kg^{-1}$ following land leveling. The same study found a correlation between microbial populations and spatial distribution and concluded that filled areas exhibit increased microbial biomass while cut areas exhibit decreased microbial biomass (Parfitt et al., 2013). Many of the biological, chemical, and physical changes that occur in silt and loam soils due to land leveling are negative trade-offs that producers must take into consideration when determining the long-term economic and sustainability potential of precision grading.

## Poultry Litter as an Organic Amendment

### *Poultry Litter on Land-Leveled Fields*

Poultry litter has been used in Eastern Arkansas as a soil amendment to restore soil productivity in response to the soil degradation observed under precision land leveling. Poultry litter provides plant available macro- and micronutrients, organic matter, and its own assembly of microbes, allowing the amendment to address several of the observed detrimental soil impacts of land leveling compared to inorganic fertilizers, which solely address nutrient loss (Brye et al., 2004). For this reason, poultry litter is currently the only recommended amendment for land-leveled soils in Arkansas (Hardke et al., 2021). The recommended poultry litter application rate for graded rice production in Arkansas is a minimum of 2.2 Mg $ha^{-1}$ (1 ton $acre^{-1}$) (Hardke et al., 2021).

Poultry litter influences soil microbial communities by changing soil chemical properties such as pH and the rate of nutrient cycling, as well as contributing additional microbial

communities to the native soil microbial community (Ashworth et al., 2017; Blair et al., 2014). Brye et al. (2004) suggests that the biological component of poultry litter contributes to soil restoration following severe disturbance. The application of poultry litter on land-leveled soil has increased rice yields following a single application, and continued improvements in yield have been observed for soils receiving sequential annual applications (Hardke et al., 2021). The effect of poultry litter on land-leveled soil can vary depending on soil texture. For instance, Miller et al. (1990) reported that poultry litter applied on land-leveled silt loam soils resulted in a greater and more consistent yield response in rice compared to inorganic fertilizers. In contrast, Brye et al. (2006) observed that physical, chemical, and biological properties were altered by land leveling on a clay soil; however, poultry litter application at a rate of 2.24 Mg $ha^{-1}$ did not result in a significantly positive crop yield response, leading the authors to conclude that clay soils may require a greater poultry litter application rate to result in yield increases.

*Poultry Litter in Organic Crop Production*

Poultry litter is an organic amendment made up of poultry manure and urine, bedding material, and spilt feed. Poultry litter is readily available in northwest Arkansas, with approximately 1.18 million Megagrams of poultry litter produced in Arkansas annually (Scharbor, 2011). Far from being a worthless byproduct, poultry litter is a valuable fertilizer and is used frequently in both organic and conventional agriculture where litter is available. Organic agriculture, in particular, depends on the use of animal manures and compost to replace soil nutrients that are removed during production (Heckman et al., 2009; Watson et al., 2002). Poultry litter provides all essential plant nutrients including macronutrients N, P, and K, secondary macronutrients Ca, Mg, and S, and micronutrients Cu, Zn, and Mo and is therefore a suitable fertilizer for organic production (Bolan et al., 2010). Unlike inorganic fertilizers, the

nutrient content in poultry litter is highly variable and depends on several factors, including the type of poultry, frequency of litter cleanout, in house litter management, and the feeding ration used during production (Watts et al., 2019).

In Arkansas, the average ratio of N: $P_2O_5$:$K_2O$ in poultry litter is 3.0: 3.0: 2.5, and as plants require a greater amount of nitrogen than phosphorus, poultry litter applied to meet nitrogen needs can result in excessive soil phosphorus (Espinoza et al., 2007; Sharpley et al., 2009). Excess soil phosphorus is an environmental concern as P-laden runoff into waterways and leaching into groundwater threatens the health of aquatic environments (Carpenter et al., 1998; Sharpley et al., 2001). Nitrate contamination in groundwater is also a concern with poultry litter application (Steele & McCalister, 1991). Application methods can be used to address these issues; incorporation and subsurface application both reduce nutrient loss from runoff and volatilization (Espinoza et al., 2007; Moore et al., 1995).

*Poultry litter application method on biogeochemical soil properties*

Poultry litter application can significantly impact soil physical, chemical, and biological properties (Bolan et al., 2010). The impact that poultry litter can have on these properties may be partially dependent on the application method utilized; although, research that directly compares poultry litter application methods is lacking. Poultry litter can be incorporated into the soil following application or, in no-till systems, is broadcast and left on the soil surface. Broadcast poultry litter has been shown to result in greater instances of N volatilization and P runoff (Adeli et al., 2012; Webb et al., 2005). In addition, Adeli et al. (2018) found that broadcast, unincorporated poultry litter contributed to increased soil carbon in the soil surface but a significant decrease in soil carbon as depth increased compared to locations receiving inorganic fertilizer or no fertilizer. Researchers in the same study reported that broadcast poultry litter

reduced bulk density as well as K, Cu, and Zn losses (Adeli et al., 2018). Surface applied litter has increased microbial biomass and β-glucosidase and β-glucosiminadase cycling activity compared to locations receiving commercial fertilizer (Acosta-Martinez and Harmel, 2006).

An alternative approach to broadcast application is the use of a "subsurfer", which is an implement developed by the USDA-ARS that allows poultry litter to be applied below the soil surface without the need for grinding the poultry litter before loading it in the implement (Pote et al., 2011). The use of a subsurfer to apply poultry litter has been shown to reduce the potential for nutrient loss through runoff or volatilization (Pote et al., 2014). While the development of the subsurfer was aimed primarily at improving water quality, soil quality is also improved through the retention of nutrients and sediments, as well as through the maintenance of soil physical structure (Lui et al., 2016). Poultry litter, regardless of application method has shown biological benefits; for instance, Brooks et al. (2018) found greater bacterial diversity under both broadcast and subsurface applied poultry litter compared to sites receiving commercial fertilizer and unfertilized sites. Four years after poultry litter application, when microbial activity should have reverted to baseline levels, phosphatase and urease activities remained greater than the baseline activity under both broadcast and subsurface applications (Brooks et al., 2018).

**Cover Crops**

Cover crops are legumes, grasses, or forbs planted during a fallow period for the purpose of providing seasonal erosion control and general soil improvement (USDA-NRCS, 2014). Soil improvement resulting from the use of cover crops is dependent on cover crop species selection and management, but can include increased organic matter, improved soil structure, increased water infiltration, reduced water and wind erosion, reduction in nutrient loss, as well as increased microbial biomass (Dabney et al., 2001; Fryer et al., 2022; Reicosky & Forcella, 1998). Other

important cover crop uses include playing a role in integrated weed management, water quality, carbon sequestration, and providing an additional source of forage for grazing animals (Dabney et al., 2001; Daniels et al., 2019).

Cover crop species selection depends on the goals of the producer, location-related considerations such as climate and water availability, and crop physiology-related characteristics. Cover crops are generally incorporated into rotations between cash crops during otherwise fallow periods; therefore, the season in which a cover crop is grown will vary depending on the cash crop grown (Roberts et al., 2018). The majority of the cash crops grown in Arkansas are summer annuals, thus winter annual cover crops are most common. Winter annual cover crops are planted after the harvest of the summer cash crop, usually between September and November, and are terminated in the early spring before planting the next cash crop. Summer annual cover crops, while not as common in Arkansas, are planted in the summer following the winter cash crop harvest and are terminated between September and October prior to cash crop planting.

While cover crops are not a new agronomic practice, the use of cover crops has increased in the past decade owing to an increased need for sustainable, multifunctional management techniques that benefit both the environment and the producer (Blanco-Canqui et al., 2015). In the United States, the total land area planted in cover crops increased from 4.17 million ha (10.3 million acres) in 2012 to 6.21 million ha (15.4 million acres) in 2017 (USDA-NASS, 2019b). The use of cover crops is expected to continue to increase as producers are offered more incentives for planting cover crops. One such incentive is the recently announced expansion of the United States Department of Agriculture's Environmental Quality Incentives Program (EQIP) to include a Cover Crop Initiative aimed at reaching 12.15 million ha (30 million acres) planted in cover crops by 2030 (USDA Press, 2022). The Cover Crop Initiative allows producers

in 11 states, including Arkansas, to receive assistance from the Natural Resources Conservation Service (NRCS) in adopting cover crops. Financial and technical assistance with the implementation of cover crops is also available through the Environmental Protection Agency's Hypoxia Task Force (HTF) in partnership with the Arkansas Delta Farmers Advocating Resource Management association. The goal of the HTF is to reduce nitrogen and phosphorus nutrient runoff into the Gulf of Mexico through state subsidized nutrient reduction strategies (Mississippi River/Gulf of Mexico Watershed Nutrient Task Force, 2016).

*Okra as a Cover Crop*

There is increasing interest in using okra (*Abelmoschus esculentus* L.) as a cover crop due to its fast growth rate, high biomass production, and drought tolerance. Okra may also offer unique soil health benefits as a cover crop because of its high mucilage production. Mucilage is a viscous, polysaccharide-rich substance produced by nearly all plants that can be secreted from seeds, roots, stems, fruits, and flowers (Galloway et al., 2020). Plant mucilage serves several purposes within plant-rhizosphere interactions including providing a carbon source for beneficial microorganisms, improving soil aggregation, and improving water availability and uptake in the rhizosphere (Ahmed et al., 2015; Czarnes et al., 2000; Galloway et al., 2020; Nazari, 2021). Okra mucilage is primarily composed of galactose, rhamnose, and galacturonic acid and was recently shown to be capable of flocculating small solid particles in suspension (de Alvarenga Pinto Cotrim et al., 2016; Lee et al., 2015). Okra secretes the greatest amount of mucilage from its seed pods, but mucilage is also produced in green leaves, roots, and cortex cells in the stem (Dantas et al., 2021; Girase et al., 2003). As a cover crop in a row crop production setting, the mucilage produced by okra stems, leaves, and roots would be available to the rhizosphere in the form of residue following cover crop termination. Preliminary trials have shown okra to be well suited as

a summer annual cover crop in the Mid-South; however, much of the research has focused on okra biomass production and adaptability to the climate of the Mid-South (Johnson, 2017; Wang et al., 2010). Understanding how these characteristics contribute to soil quality and health will be beneficial in evaluating the suitability of using okra as a cover crop in Arkansas row crop production.

## Cover Crop Management on Soil Health

### *Management on Chemical and Physical Soil Quality*

The addition of cover crops in annual crop rotations may result in a number of soil health related benefits; however, optimizing the impact of a cover crop depends on species selection, planting rate, termination stage and method, and residue management (Balkcom et al., 2020; Blanco-Canqui et al., 2011). Of these management considerations, Wayman et al. (2015) identified species selection and termination stage and method as the most important factors in optimizing nutrient availability from cover crop residues for the following cash crop, as well as reducing weed competition with the following cash crop. The method of cover crop termination is often based on the machinery available to the producer and production system type (i.e., organic, conventional, no-till, etc.); however, termination method is a key aspect of optimal cover crop management that influences residue cover, degradation rate, soil organic carbon build-up, and the timing of nutrient availability. Therefore, termination method should be considered carefully when determining the cost-to-benefit ratio of a given method (Adetunji et al., 2020).

Within organic systems, producers must rely on mechanical termination methods that make use of equipment such as a roller crimper, hay mower, or a field disk, all of which may be as effective in cover crop termination as herbicide application when carried out properly (Creamer & Dabney, 2002). Mechanical termination methods vary in degree of soil disturbance

and in the amount of residue left on the soil surface. Before the widespread use of herbicides in the United States, tillage was a commonly used termination method that served the dual purpose of preparing the field for planting and returning cover crop residues into the soil as a green manure (Sarrantonio & Gallandt, 2008). This method is still used today with the use of a tractor-mounted disk that cuts the soil, uprooting the cover crop and partially burying it. Disking a cover crop involves high plant and soil disturbance and therefore may negate some cover crops benefits as soil structure is destroyed, aboveground residue is minimized, and organic matter degradation is increased (Paul, 2007). An observed consequence of an increased degradation rate is the quick mineralization and loss of nitrogen from crop organic matter, which results in less plant-available nitrogen for the following crop (Hu et al., 1997).

One termination strategy that employs low plant and soil disturbance is the roller crimper method. The roller crimper is a steel drum with metal slats that is rolled over a cover crop to flatten and critically crease or cut the stem, resulting in a thick mat of residue over the soil. This method leaves the soil and cover crop roots undisturbed and leaves the aboveground biomass largely intact for greater surface coverage and slower decomposition (Mirsky et al., 2011). The mulch left by a roller crimper is particularly advantageous in an organic no-till production system in providing weed control and reducing labor and energy costs (Canali et al., 2013; Navarro-Miro et al., 2019). As a result, roller crimpers build soil organic matter over time; however, the immediate effect of the unincorporated residues is a reduction in available nitrogen due to a decreased mineralization potential (Hefner et al., 2020).

Mowing is another commonly used termination method that involves low soil disturbance. This method utilizes a hay mower to cut the cover crop below the terminal growth point, leaving both the soil and belowground biomass undisturbed (Mirsky et al., 2011). This

method differs slightly from the roller crimper method in that the aboveground residue is generally cut into smaller pieces that may decompose more rapidly than cover crop biomass that is left intact (Creamer et al., 1995). Termination stage and height are important in this method because regrowth may occur if the cover crop is terminated too early or is cut above the apical meristem, which would result in the cover crop competing with the cash crop (Creamer & Dabney, 2002).

Additional studies have shown that termination method has an influence on soil organic matter (SOM) through labile carbon (C) and nitrogen (N) pools (Adetunji, 2019; Adetunji et al., 2021; Dabney et al., 2010). A study conducted by Bloszies et al. (2022) showed that cover crops terminated by disking increased N mineralization when compared to mowing and roller crimper termination methods, but C mineralization was not significantly impacted by termination method. Poffenbarger et al. (2015) reported that soil mineral nitrogen was greater when cover crop residue and poultry litter were incorporated versus broadcast applied. Conversely, Baggs et al. (2000) observed an overall decrease in N mineralization following cover crop residue incorporation, which was attributed to a temporary immobilization. This indicates that nutrient mineralization and immobilization following cover crop termination is not solely dependent on termination method but is a result of multiple factors including residue C:N ratios.

Most termination method studies have focused on C buildup and N mineralization, resulting in limited information on the effect of termination method on the availability of macronutrients such as P, K, Ca, Mg, and S. In one of the few studies conducted on cover crop termination method (mowing, disking, roller crimper, and herbicide application) on available primary and secondary macronutrients, researchers concluded that termination method alone did not significantly influence nutrient availability, but termination in combination with cover crop

species significantly influenced P, Ca, and K soil concentrations (Khan et al., 2021). Despite these results, more research is needed to understand the relationship between cover crop termination method and changes in soil chemical properties.

Cover crop management also influences physical soil properties through soil disturbance. Agricultural practices that limit soil disturbance such as no-till and conservation tillage contribute to long term improvements in soil structure and function (Blanco-Canqui & Ruiz, 2018; Reicosky & Saxton, 2007; Weidhuner et al., 2021). Disturbance-minimizing agricultural practices are modeled off natural soil structure that is achieved in undisturbed perennial systems; these systems benefit from vegetation-fauna-soil-climate interactions that result in bio-pore accumulation and aggregate formation (Or et al., 2021). This was exemplified in a long-term study where soils under limited disturbance (no-till) were compared to soils under high disturbance (conventional tillage); So et al. (2008) reported no-till soils had greater soil microporosity, structural stability, and slower rates of runoff. Furthermore, Lucas et al. (2019) reported a decrease in bio-pore length, density, and connectivity within systems exposed to annual tillage over 24 years when compared to undisturbed systems. Thus, cover crop termination methods that rely on soil disturbance to uproot the cover crop may result in reduced aggregate stability and bio-pore density over time.

**Cover Crops on Microbial Activity**

Soil microorganisms play an essential role in the functioning and productivity of an ecosystem. Microbes convert organically bound nutrients into plant-available mineral forms by digesting the organic matter that is added to the soil (Weil & Brady, 2016). Some of the soil microbes that colonize the rhizosphere provide the plant nutrients by fixing atmospheric $N_2$ into plant-available compounds. The soil microbial community also contributes to aggregate

formation and stability by producing mucilaginous polysaccharides that hold soil particles together (Gupta, 2011). In addition to contributing compounds that are essential for plant growth and soil structure, microbes prevent the accumulation of harmful compounds. Many soil microorganisms are capable of producing enzymes that allow them to digest toxic biological compounds, thereby preventing toxin accumulation and persistence in soils (Weil & Brady, 2016). Microbes also prevent the accumulation of toxic quantities of essential elements such as nitrogen, sulfur, phosphate, iron, and manganese through oxidation and reduction reactions that control the availability of these elements (Weil & Brady, 2016).

Soil biological processes simultaneously influence and are influenced by soil chemical and physical properties. Some of the most influential chemical factors for microbial activity that are dependent on land-use include available organic C, C:N ratio, phosphate level, amount of available nitrogen, moisture, and pH (Coyne, 1999). Kuramae et al. (2012) reported that C:N ratio had a strong influence on bacterial community structure as well as enzyme expression under different management systems. In a study that investigated the effect of both proximal environmental factors and site factors on microbial community structure and activity, soil moisture and organic matter had the strongest influences on microbial community structure (Brockett et al., 2012). Inputs containing N and P have significantly shifted microbial populations, with one report showing a decrease in mycorrhizal fungi and oligotrophic bacteria and an increase in copiotrophic bacteria in response to N and P additions (Leff et al., 2015). Additionally, soil pH can be affected by management decisions through the addition of nutrients or liming agents and has been identified as being at least as important as C:N ratio in microbial community structure and composition (Wang et al., 2019).

Soil enzyme activity serves as an indicator of microbial activity, nutrient cycling, and organic matter decomposition in response to changes in the soil environment (Dick & Burns, 2011). Several enzymes have been identified as soil quality indicators for their role in specific nutrient cycles: β-glucosidase is involved in cellobiose hydrolysis and is therefore associated with C cycling, and β-glucosaminadase is associated with C and N cycling because of its involvement in the breakdown of chitin into easily mineralizable amino sugars, and urease is associated with N cycling because of its role in converting urea into ammonia (Ekenler & Tabatai, 2002; Rao et al., 2014). Phosphorus cycling is associated with alkaline and acid phosphatase activity because of its involvement in the hydrolysis of phosphate esters and phosphoric acid into phosphate (Rao et al., 2014). In addition, arylsulfatase activity is linked to S-cycling as it is involved in the hydrolysis of sulfate esters into sulfate (Rao et al., 2014). Kizilkaya & Dengiz (2010) found that β-glucosidase, alkaline phosphatase, and urease activity and total organic carbon were consistently higher in undisturbed forest and pasture systems when compared to agricultural systems, demonstrating the relationship between organic matter and enzyme activity.

Agricultural practices that build organic matter such as cover cropping, no-till, and the addition of organic amendments have been shown to increase enzyme activities (Mangalassery et al., 2015; Nautiyal et al., 2010). Sürücü et al. (2014) found significantly greater urease activity with the incorporation of aboveground and belowground green manure residues, which was attributed to increased available substrate, when compared to cropping systems that incorporated belowground biomass only and control sites that received no additional residues. Furthermore, Hallama et al. (2019) concluded that increased acid phosphatase activity observed with the introduction of cover crops is the indirect result of enhanced arbuscular mycorrhizal fungi

colonization, as well as an increase in organic matter, which has a net-acidifying effect as organic matter decomposes, resulting in a soil pH that is more conducive to acid phosphatase activity. While a considerable amount of literature supports the idea that agricultural practices that increase soil organic matter will increase enzyme activities, the complex interactions that occur between aboveground management and belowground soil processes are still not well-understood and are highly variable. For instance, Gibbs (2020) conducted a study on the effect of cover crop mixes on the stratification of soil N and nutrient cycling enzyme activities in the soil profile and reported that cover crop treatments did not impact phosphatase, arylsulfatase, urease, or β-D-glucosidase activity. These results were attributed to an abundance of soil P and S as well as a great amount of labile C, which is consistent with observations of repressed phosphatase, arylsulfatase, and glucosidase activities in the presence of available products (Kertesz & Mirleau, 2004; Lidbury et al., 2022; Nannipieri et al., 2002). However, enzyme suppression in the presence of abundant products does not always occur; for instance, Tomlinson et al. (2008) reported an increase in alkaline phosphatase that occurred in the presence of high inorganic phosphorus within a continuous fescue system receiving annual additions of alum-treated and untreated poultry litter since 1995. Thus, more research is needed to understand the effect of management systems on the complex interactions that occur between soil microbial communities and the non-living portion of the soil in which organisms live.

**Justification**

Soil health is a growing area of concern within agricultural systems, and management practices aimed at improving soil health are increasingly recommended and adopted. Soil microbial communities respond readily to changes in the environment and can therefore provide an early indication of changes in soil health. However, the soil microbiome exists in a complex

interactive micro-ecosystem where a core community of microorganisms interact with and influence the soil itself as well as plants and other soil organisms. Research on the impact of agricultural management on the soil microbiome is still relatively new and unexplored (Berg et al., 2020). In particular, there is a lack of knowledge about the effects of management practices on soil organisms in soils typical of agricultural production in the Mid-Southern U.S. Investigation into the effects of cover crops, cover crop management, and poultry litter application on Arkansas soils will be critical to determine their effectiveness in improving soil properties associated with soil health cross small- and large-scale production systems.

## Objectives and Hypotheses

The research described in this thesis represents results from two separate short-term studies investigating soil response to the use of poultry litter and other management strategies aimed at improving soil health in the first year of implementation. The first study examines soil response to summer cover crop termination and poultry litter application approach within an organic cropping system. The second study examines the effects of poultry litter application rate on soil quality in land-leveled rice production.

### *Okra Cover Crop Study*

The first objective of this study was to investigate the short-term soil response to cover crop termination (mowing, disking, or roller crimping) and poultry litter application method (subsurface or surface application) on near-surface (0-10 cm) soil C, N, S, and P cycling and related properties. Cover crop termination methods that incorporate residue and physically disturb soil may increase the rate of organic matter decomposition, while termination methods that leave soil undisturbed and residue on the surface can slow degradation and promote the build-up of organic matter over time. Poultry litter used as a fertilizer also contributes organic

matter and influences soil nutrient fate and availability. Extracellular soil enzymes are associated with the microbially mediated decomposition of organic matter and transformation into inorganic constituents or conversely into stabilized forms and thus can serve as indicators of microbial activity and soil nutrient cycling capability. Therefore, it was hypothesized that management practices such as cover crop termination method and poultry litter application strategy that were more physically disruptive to soil would influence C, N, P, and S cycling enzyme activities differently than methods leaving crop residues on the soil surface and where poultry litter was not added.

The second objective of this study was to examine changes in soil physical, chemical, and biological properties within an annual production system following the use of soil quality management practices in relation to perennial management systems. Practices that emulate perennial systems through minimal disturbance and continual plant cover have shown increased microbial functional diversity, C and N content, aggregate stability, and extracellular enzyme activities. Therefore, it was hypothesized that perennial management systems would exhibit greater soil organic matter content, aggregate stability, and soil quality indicators in comparison to the annual management system. It was also hypothesized that the addition of poultry litter along with soil disturbance that occurs under annual management would result in greater short-term enzyme activity compared to perennial management.

*Poultry Litter on Land-Leveled Soil*

The first objective of this study was to evaluate short-term soil biochemical responses to poultry litter application rate on recently land-leveled rice fields by analyzing activity of enzymes related to P, S, C, and N cycling. Land leveling activities often expose subsoil that is lacking in nutrients, organic matter, and microbial populations. Poultry litter is recommended to

reclaim severely disturbed and degraded soils because it is not only a valuable source of nutrients, but also provides organic matter and its own microbial communities. Soils that are poor in both microbial biomass as well as organic matter would be expected to show an increase in microbial activity following the addition of a bioactive fertilizer such as poultry litter. Therefore, it was hypothesized that increasing poultry litter rates will result in increased enzyme activities.

The second objective of this study was to evaluate soil respiration from land-leveled rice fields under different poultry litter application rates in relation to enzyme activities within both furrow irrigated and flood irrigated fields. Soil respiration is an indicator of microbial activity, and it was therefore hypothesized that $CO_2$ respiration rate would be positively correlated with enzyme activities within each poultry litter rate.

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# Chapter 2:

# Management Practices in an Organic Cereal Cropping System Influence Short-term Soil Health Properties

**Abstract**

Improving soil health has become increasingly important in Arkansas as producers face challenges that affect production sustainability such as loss of organic matter, erosion, and nutrient leaching. The use of cover crops and poultry litter in annual cropping systems can build soil health by providing organic matter, enhancing soil biological activity, and improving physical soil structure. However, soil health response to differences in how these practices are implemented and managed remains largely unstudied. Therefore, the objective of this research was to evaluate the effects of okra (*Abelmoschus esculentus* L.) cover crop termination (roller crimper, disk, or hay mower) and poultry litter application methods (top-dress unincorporated, top-dress incorporated, subsurface applied, or no poultry litter control) and subsequent crop species (certified organic LG334 soft red winter wheat (*Tritcum aestivum* L.) and Violetta winter barley (*Hordeum vulgare* L.)) on soil health response within an organic cereal grain production system in relation to nearby perennially managed soils. Soil health response was measured by examining dynamic soil health properties, including soil pH and electrical conductivity, Mehlich-3 extractable nutrients, total organic matter, active carbon (POXC), and soil enzyme activities. Cover crop termination and poultry litter application method did not result in differences in soil health properties within the time frame of this study; however, soil properties related to nutrient cycling were influenced by subsequent crop. Phosphatase activities under wheat and perennially managed soils decreased over the winter growing season, while phosphatase activities under barley remained unchanged. Sulfatase activities increased under barley over the season, while activity under wheat and perennial locations remained unchanged. Subsequently, a greenhouse study was conducted using the same soil collected from the field trial (Enders silt loam and Leadvale silt loam) to confirm the observed effects of wheat and

barley crops on phosphatase and sulfatase activities. Wheat and barley were grown in 15-cm diameter pots (with unplanted soil serving as the control), and soils were collected from the rhizosphere 30 days after planting. Phosphatase activity was significantly lower in the wheat rhizosphere compared to barley and control soils; however, arylsulfatase activity was greatest in the wheat rhizosphere compared to barley and control soils. These results indicate that, while differences in soil health management may require more evaluation, crop species contribute within a short-time scale to soil nutrient cycling activities in the rhizosphere. Further investigation into the functional ecology of the rhizosphere may improve our understanding of plant-soil interactions and allow for optimized management of soil nutrient cycling and plant nutrient uptake.

**Introduction**

Agricultural practices aimed at building soil organic matter (SOM) are promoted to improve soil health, increase the sustainability of production agriculture, and mitigate climate change by sequestering carbon (C) in the soil. Cover crops are an example of a SOM-building agricultural practice that can serve several purposes including keeping the soil surface covered to prevent erosion and suppress weeds, enhancing nutrient cycling, and improving soil structure when combined with no-till management (Wright et al., 2007; Roberts et al., 2018). However, the extent of cover crop benefits is dependent on a number of factors including species selection, termination stage, and residue management (Balkcom et al., 2020). These factors not only determine the amount of C and nitrogen (N) in SOM, but also determine how quickly residue will mineralize into plant available nutrient forms (Adetunji, 2019; Adetunji et al., 2021; Dabney et al., 2010). For instance, a cover crop termination method that causes greater soil disturbance and incorporates residue into the soil, such as disking, may increase the rate of residue

decomposition compared to methods that leave residue unincorporated and soil undisturbed, such as mowing and roller-crimper termination (Bloszies et al., 2022).

The season in which a cover crop is grown can also influence a cover crop's impact on soil properties and agronomic benefits. The majority of the cash crops grown in Arkansas are summer annuals, thus winter annual cover crops are most common. Summer annual cover crops, while not as common, have the potential to fit into winter cash crop rotations, allowing producers to benefit from living soil cover in the short warm season between cash crops There is increasing interest in using okra (*Abelmoschus esculentus* L.) as a cover crop due to its fast growth rate, high biomass production, and drought tolerance. Preliminary trials have shown okra to be well suited as a summer annual cover crop in the Mid-South; however, much of the research has focused on okra biomass production and adaptability to the climate of the Mid-South (Johnson, 2017; Wang et al., 2010). Understanding how these characteristics contribute to soil quality and health indicators will be beneficial in evaluating the suitability of using okra as a cover crop in Arkansas during row crop production cycles.

Cover crops alone are often not enough to meet the nutrient demands of the subsequent crop and are, therefore, often coupled with commercial fertilizer or animal manure application. Similar to cover crops, the use of animal manures such as poultry litter as a fertilizer can result in several benefits outside of the addition of nutrients including increasing soil surface C and microbial biomass and decreasing soil bulk density over time (Acosta-Martinez & Harmel, 2006; Adeli et al., 2018). The use of poultry litter may be optimized when applied via subsurface application, as it reduces nutrient loss and minimizes soil disturbance (Pote et al., 2014). Poultry litter, regardless of application method, has shown biological benefits; for instance, Brooks et al. (2018) found greater bacterial diversity under both broadcast and subsurface applied poultry

litter compared to sites receiving commercial fertilizer and unfertilized sites. Four years after poultry litter application, when microbial activity should have reverted to baseline levels, phosphatase and urease activities remained greater than the baseline activities under both broadcast and subsurface applications (Brooks et. al., 2018).

Soil health can be measured with a suite of physical, chemical, and biological indicators. Physical soil properties associated with soil health may include bulk density, aggregate stability, and soil water content. Chemical soil properties used to determine soil health often include nutrient concentration, pH, electrical conductivity (EC), and measurements of soil organic matter fractions. A somewhat recent biological soil health indicator is extracellular enzyme activity. Soil extracellular enzymes are considered to be dynamic soil health indicators because enzyme activities readily respond to changes in the soil environment (Dick & Burns, 2011). Microbial communities produce enzymes to break down complex organic compounds into plant available forms and are therefore associated with soil nutrient cycling. Several enzymes have been identified as soil quality indicators for their role in specific nutrient cycles: β-glucosidase is involved in cellobiose hydrolysis and is associated with C-cycling, β-glucosiminadase is associated with C and N cycling because of its involvement in the breakdown of chitin into easily mineralizable amino sugars, and urease is associated with N cycling because of its role in converting urea into ammonia (Ekenler & Tabatai, 2002; Rao et al., 2014). Phosphorus (P) cycling is associated with alkaline and acid phosphatase activities because of involvement in the hydrolysis of phosphate esters into orthophosphate (Rao et al., 2014). In addition, arylsulfatase activity is linked to sulfur (S) cycling as it is involved in the hydrolysis of sulfate esters into sulfate (Rao et al., 2014). Management practices that accumulate OM over time allow enzymes

to stabilize in the soil resulting in increased enzyme activities; thus, enzyme activities can provide insight into the soil biological responses to conservation management.

While several studies have documented the positive impact of SOM-building management practices on soil quality (Dabney et al., 2001; Daniels et al., 2019, Fryer et al., 2022; Reicosky & Forcella, 1998), they are still considered unconventional and many barriers to adoption exist. For instance, in Arkansas, financial and technical assistance with the implementation of cover crops is available through both the NRCS's Cover Crop Initiative and the Environmental Protection Agency's Hypoxia Task Force (HTF) (Mississippi River/Gulf of Mexico Watershed Nutrient Task Force, 2016; USDA Press, 2022). However, less than 5% of Arkansas cropland utilizes cover crops (USDA-NASS, 2017). Financial costs, both direct and indirect, along with lack of information are often cited as barriers to the adoption of conservation management practices, as producers fear a negative return on investment if improvements in soil quality are not seen immediately (Duke et al., 2022). Therefore, studying soil quality indicators that can provide early insight into the effect of management decisions on soil health can provide guidance to producers interested in adopting conservation management practices.

The goal of this research was to evaluate combinations of various cover crop termination and poultry litter application methods on short-term soil physical, chemical, and biological responses in an organic production system. Specific objectives were to i) evaluate soil enzyme activities related to C, N, P, and S cycling under conservation management strategies including cover crop termination by mowing, disking, or roller crimping and poultry litter application by subsurface or surface application, and ii) examine changes in soil physical, chemical, and biological properties within an organic cereal grain production system following the use of soil quality management practices in relation to nearby perennial management systems. It was

hypothesized that management practices such as cover crop termination method and poultry litter application strategy that were more physically disruptive to soil would influence C, N, P, and S cycling enzyme activities differently compared to methods leaving crop residues on the soil surface and where poultry litter was not added. It was also hypothesized that perennial management systems would exhibit greater soil organic matter content, aggregate stability, and soil quality indicators in comparison to the annual management system.

## Materials and Methods

### *Field Study Site Description and Experimental Design*

Experiments related to the objectives of this project began in July 2020. This trial was conducted on a 1.6-ha (4 ac) organically managed field and three perennially managed locations at the USDA-ARS Dale Bumpers Small Farm Research Center in Booneville, Arkansas (35˚ N, -93˚ W). The site is in Major Land Resource Area 118A (Arkansas Valley and Ridges, Eastern Part), with an average annual precipitation of 10.4 to 11.5 cm, and average annual temperatures of 14 to 17˚C (USDA-NRCS, 2022). The organically managed field had been under long-term pasture management until 2018, when it was planted with winter wheat followed by soybean [*Glycine maxx* (L.) Merr.], which was followed by winter rye (*Secale cereale* L.) at a rate of 125 kg $ha^{-1}$ (113 lbs $ac^{-1}$) in December 2019, one season prior to planting the okra cover crop. The organic field had received annual poultry litter applications at a rate of 2.2 Mg $ha^{-1}$ (1-ton $ac^{-1}$) prior to the study and lime had been applied at a rate of 5.6 Mg $ha^{-1}$ (2.5-ton $ac^{-1}$) in 2018. The field was tilled using a Maschio SC300 (Melbourne, Australia) rotary tiller before planting the okra cover crop in July 2020. The organic field is comprised of Enders silt loam (fine, mixed, active, thermic Typic Hapludult) and Leadvale silt loam (fine-silty, siliceous, semiactive, thermic Typic Fragiudult) (USDA-NRCS, 2019). The first perennial pasture (predominately tall fescue

(*Festuca arundinacea* L.)) was also mapped to Leadvale silt loam and was under organic pasture management, with only minimal disturbance from periodic mowing and biomass removal. A second perennial location was unmanaged pasture on a Leadvale silt loam (fine-silty, siliceous, semiactive, thermic Typic Fragiudults), where a mixture of native perennial tallgrass prairie species had been planted in 2017 (USDA-NRCS, 2019). The third perennial location was an unmanaged site bordering an agroforestry ecosystem on an Enders silt loam (fine, mixed, active, thermic Typic Hapludults) with a plant community composed of bermudagrass [*Cynodon dactylon* (L.) Pers.], tall fescue, and a mixture of shrubs and forbs (USDA-NRCS, 2019).

The experiment was conducted as a two-factor, randomized complete block with a split-plot arrangement. The whole plot factor was cover crop termination strategy and poultry litter application method as a combined effect. The type of grain crop planted following cover crop termination and poultry litter application method was the split-plot factor. Sampling time was the split-split-plot factor. The two cereal grain crops used in this trial were Violetta winter barley (*Hordeum vulgare* L.) and certified organic LG334 soft red winter wheat (*Tritcum aestivum* L.). The annual cropping system was tilled to prepare for planting prior to any sampling activities. Baseline soil samples were collected from the top 0-10 cm on July 20, 2020. On July 27, 2020, 1.62 ha (4 ac) of Clemson Spineless 80 okra seed from Green Cover Seed (Bladen, Nebraska) was planted at a rate of 20 kg $ha^{-1}$ (18 lb $ac^{-1}$) using a John Deere (Moline, Illinois) 1590 no-till drill. Plots measuring 12.2 by 18.3 m (40 by 60 ft) were established for each of the eight treatments, with three field replicates. The okra cover crop was terminated on October 8, 2020, at the reproductive stage, approximately 11 weeks after planting, by roller crimper, hay mower, or disc according to the randomized plot design. Roller crimping was completed using a John Deere 6125 M tractor pulling a 2.4-m (8-ft) rear-mounted cover crop roller (I&J Manufacturing,

Gordonville, PA). Hay mowed plots were cut using a 3.05-m (10-ft) 3050 CLAAS hay mower (CLAAS of America, Omaha, Nebraska), and disked plots were terminated using a 3.05-m (10-ft) Taylorway field disc (Taylor Pittsburgh Manufacturing, Inc., Winfield, Alabama) set to a depth of 12.7 cm (5 in) and pulled at a speed of 8 kph (5 mph).

A total of eight treatments with different combinations of termination method and poultry litter application method were implemented (Table 1). Two treatments include roller-crimped okra treatments with either a top-dressing or a sub-surfer applied poultry litter. Three treatments where the okra cover crop terminated using disking and combined with poultry litter applied using one of three different application methods. The poultry litter application methods consisted of an incorporated treatment, an unincorporated treatment, and one sub-surface applied treatment. Two hay-mowed treatments included one treatment of top-dressed poultry litter application and one treatment of sub-surface applied broiler litter. The final treatment was a disked termination method without poultry litter application. Three perennial management sites including organic managed pasture, unmanaged pasture, and unmanaged agroforestry border area were used as perennial comparisons.

Poultry litter was applied one week after okra cover crop termination at a rate of 4.5 Mg $ha^{-1}$ across all application methods. Top dressed poultry litter treatments were applied using a BBI Endurance pull-type broadcast multi-use spreader (Salford Inc., Cornelia, Georgia) and were either incorporated following application or left unincorporated on the surface. Incorporation was completed by using a 2.4-m (8-ft) wide field disc (Taylorway, Winfield, Alabama) pulled by a JD 6150 tractor (John Deere, Moline, Illinois) set at a depth of 12.7 cm (5 in). Subsurface poultry litter was applied at the same rate as surface applied treatments on October 20, 2020, at a depth

of 15.24 cm (6 in) with a band width of 2.54-cm (1 in). Cereal grain crops were planted following cover crop termination and poultry litter application. Certified organic LG334 soft red winter wheat (Welter Seed Company, Onslow, Iowa) and cv. Violetta winter barley (Kitchen Seed Company, Arthur, Illinois) were planted at 126 kg $ha^{-1}$ (113 lb $ac^{-1}$) and 124 kg $ha^{-1}$ (111 lb $ac^{-1}$), respectively.

*Field Study Soil Sampling and Analyses*

Soil samples were collected using sterile sampling techniques and were placed on ice immediately following collection. Baseline samples were collected from the top 0-10 cm in July 2020 before the okra cover crop was planted. A second set of composited soil samples were collected from the top 0-10 cm in November 2020, one month after cover crop termination and PL application, and two weeks after cereal grain establishment. The third and final set of soil samples were collected in May 2021 from the top 0-10 cm following grain crop harvest. Climate information from each sampling time is detailed in Table 2 (NOAA, 2023). In addition to composited soil samples, bulk density cores were collected at the final sampling date in June 2021 from the top 0-10 cm. Bulk density cores were oven dried at 105˚C for 72 hours to calculate gravimetric water content and bulk density at sampling.

Soil samples were processed through a 4.75-mm sieve and divided into two split samples within two days of sampling. One subset was air-dried for standard nutrient analysis, pH, electrical conductivity (EC), POM, POXC, total C (TC) and total soil N (TN), and enzyme activity. The second sample group was frozen at -80 ˚C in a Stirling Ultracold (Athens, Ohio) up-right freezer.

Mehlich-III extractable nutrients (i.e., P, K, Ca, Mg, S, Fe, Na, Mn, Cu, and Zn) were measured by inductively coupled plasma (ICP) atomic emission spectroscopy (SPRECTRO CIROS ICP, Fitchburg, MS). Soil samples were oven-dried at 55˚C and passed through a 2-mm sieve. Soil extractions were prepared by shaking 2 (± 0.05) g of dry soil in 20 mL of Mehlich-III extracting solution for five minutes on an oscillating shaker at low speed. Samples were then filtered through Whatman #42 filter paper (Tucker, 1992).

Soil pH and EC were determined by adding 10 mL of Milli-Q® water to 5 g (± 0.05 g) of oven-dried (55˚C) soil. Samples were mixed by inverting by hand and allowing soil particles to settle for 15 minutes before measuring pH and EC with calibrated electrodes and a meter.

Soil aggregate stability was measured using methods adapted from Franzluebbers & Stuedemann (2005). Soil bulk density samples were sieved through a 4.75-mm sieve and oven dried at 105˚C for 24 hours. After oven-drying, 100 (± 0.01) g of each sample were weighed and placed on top of a nest of 2-, 1-, 0.25-, and 0.053-mm pore-size sieves in the wet-sieve apparatus. The soil sample added to the top sieve was oscillated in a 30-cm-diameter by ~120-cm-tall column volume of tap water at 30 cycles per minute for ten minutes. The wet-sieve apparatus was drained and filled with fresh water after processing three replications from each treatment. After the mechanically imposed disturbance, the nest of sieves was removed, and retained soil aggregates from each sieve were manually washed into pre-weighed containers and left to air dry until pooled water evaporated. After air-drying, visibly obvious coarse fragments were removed from the 2.00-mm class size, weighed, and subtracted from the oven-dry soil mass. Fractions comprising each soil sample were oven dried at 105˚C for 24 hours and weighed to determine the water-stable aggregate (WSA) fraction by dividing the mass of aggregates in each size class by

the mass of the original soil sample. Mean weight diameter (MWD) was calculated for each size fraction by multiplying the WSA by the mean diameter of the particle size of that fraction. Mean diameters for the size fractions measured in this study included 3.38 mm for the 2.00 mm mesh size, 1.50 mm for the 1.00 mm mesh size, 0.625 mm for the 0.25 mm mesh size, and 0.152 mm for the 0.053 mm mesh size. Total water stable aggregates (TWSA) was calculated by dividing the sum of the mass of dry soil from each size fraction by the initial sample weight.

The wet-sieving apparatus contained a collection bucket that was positioned below the nest of sieves in the water-holding column that catches silt and clay fractions that are less than 250 μm. Silt and clay samples from the collection bucket were retained after aggregate stability was performed on all three replicates of each sample. The retained silt and clay samples were analyzed for mineral associated OM using the loss on ignition (LOI) method.

Total OM was measured using LOI where oven-dry soil samples were placed in pre-weighed crucibles and weighed. Crucibles were then placed in a muffle furnace (Thermo Electron Corporation, Ashville, NC) and were incrementally heated to a maximum temperature of 550 ˚C. The crucibles containing samples were reweighed following combustion to obtain a final weight representing the mineral (ash) portion of the soil sample. Total OM was calculated by subtracting the final ash weight from the initial oven-dry soil weight, then dividing the result by the oven dry weight (Nelson & Sommers, 1996). Particulate organic matter (POM) was measured by dispersing 0.25 kg. of oven-dry soil in 100 mL sodium hexametaphosphate ($(NaPO_3)_6$) for 16 hours. The solution was then passed through a 53-μm sieve into a tin, and the retained fraction was oven dried at 55°C for 5 days. After oven drying, retained POM fractions were combusted in a muffle furnace (Thermo Electron Corporation, Ashville, NC), and POM

was calculated by dividing the combusted sample amount (mg) by the original 0.25 kg of soil (Six et al., 1998). Total soil C and N were measured by high temperature combustion with an Elementar vario MAX cube (Elementar Americas Inc., Ronkonkoma, NY) organic elemental analyzer. The C:N ratio was calculated by dividing total C by total N.

Labile soil C was measured by determining permanganate oxidizable C (POXC) in soil samples as described by Weil et al. (2003). Soil samples were prepared by weighing 2.5 (± 0.05) g of air-dry, 2-mm sieved soil into a 50-mL centrifuge tube. Then, 18 mL of deionized water and 2.0 mL of 0.2 *M* $KMnO_4$ stock solution was added to each soil sample. Samples were shaken horizontally at 180 oscillations per minute for 2 minutes. Following shaking, soil samples were allowed to settle for 10 minutes in a dark location. Samples were diluted by dispensing 0.5 mL of supernatant into another centrifuge tube containing 49.5 mL of deionized water. Samples, standards, and method blanks were plated by pipetting 200 µL into a clear 96-well plate. Absorbance was read at 550 nm using spectrophotometer software. The POXC in mg $kg^{-1}$ soil was calculated using the following formula:

POXC (mg $kg^{-1}$ soil) = [0.02 mol/ L - ($a$ + $b$ × Abs)] × (9000 mg C/ mol) × (0.02 L solution/ Wt)

in which 0.02 mol/L = initial solution concentration, $a$ = intercept of the standard curve, $b$ = slope of the standard curve, Abs = absorbance of unknown, 9000 = mg of carbon oxidized by 1 mole of MnO4, 0.02 L = volume of stock solution reacted, and Wt = weight of air-dried soil sample in kg.

Soil samples were analyzed for β-D glucosidase, β -glucosiminadase, arylsulfatase, acid phosphatase, and urease activities using microplate assay methods described by Deng et al. (2013) and Deng et al. (2011). Fluorometric assays utilized the following 4-methylumbelliferyl (MUF) substrates: methylumbelliferyl N-acetyl- β -D-glucosaminide for β-glucosiminadase,

methylumbelliferyl β-D-glucopyranoside for β-D glucosidase, methylumbelliferyl phosphate for phosphomonoesterase, and methylumbelliferyl sulfate for arylsulfatase. Fluorometric enzyme assays were incubated for 1 hour at 37˚C in a Modified Universal Buffer solution (pH 6.0) and were analyzed using a Tecan Infinite M200 microplate reader (Tecan Group Ltd., Zurich, Switzerland) at an excitation wavelength of 360 nm and an emission wavelength of 460 nm. Urease activity was measured as described by Cordero et al. (2019). Dichloroisocyanuric acid sodium salt dihydrate was used as a substrate and microplates were incubated at 18˚C for 2 hours. Colorimetric assays were read using a Tecan Infinite M200 microplate reader at an absorbance of 650 nm.

In addition to soil sample collection, plant biomass was also measured at the final sampling date. Biomass density data were collected for total aboveground biomass in both the treatment plots and the perennially managed locations using a 0.33-$m^2$ frame as described by Daubenmire (1959). Three biomass density measurements were recorded from the southwest corner, middle, and northwest corner of each split plot. Each frame was placed to capture three crop rows. Only vegetative materials rooted within the frame were included in density data. Percent areal cover was estimated for okra residue, weed residue (Johnsongrass (*Sorghum halepense* L.) or other large weeds terminated with okra), grain crop, actively growing weeds, bare ground and other (rocks, poultry litter, etc.) for a total coverage of 100% of the area. For the hay-mowed treatments, frame placement was adjusted to capture variability in coverage such that one frame was placed on a mulch layer, one frame was placed in the windrow gaps, and the last frame placed with half the frame on a mulch layer and half on the gap. Within the treatment plots, total aboveground biomass was further categorized into grain crop biomass, broadleaf

weed biomass, and grass weed biomass. Only total aboveground biomass was measured within the perennially managed locations.

*Field Study Statistical Analysis*

To evaluate the effects of cover crop termination and poultry litter application method, cereal grain type, and sampling time on soil health properties, explanatory variables were compared by analysis of variance (ANOVA) in a split-split-plot random complete block design with 3 field replicates using R Studio 4.2.3 (R Core Team, 2022). Under this model, all three explanatory variables including treatment (cover crop termination method and poultry litter application method), crop (barley and wheat), and sampling time (November 2020 and June 2021) were considered fixed effects. Treatment was the whole-plot factor, cereal grain was the split-plot factor, and sampling time was the split-split-plot factor. Perennial locations were included in comparisons, with perennial ground cover compared as both a crop type and a treatment type. The factor of cereal grain type was not introduced until cereal grains had been planted in October 2020; therefore, analysis of variance where crop was considered a factor only included the second and third sampling time and excluded baseline (July 2020) samples. A two-way repeated measures ANOVA was used to determine the effects of sampling time (including July 2020, November 2020, and June 2021 sampling dates) and treatment. A one-way ANOVA was used to analyze baseline samples for treatment effects on both the treatment plots, as well as the annual and perennial cropping systems. When main effect differences were detected, pairwise post-hoc comparisons were conducted using Tukey's Honest Significant Difference (HSD) test at an alpha value of 0.05 with the "agricolae" package version 1.4.0 (de Mendiburu & Yaseen, 2020) in R Studio 4.2.3.

Pearson's correlation coefficients were calculated for soil properties at the November and June sampling dates using the "Hmisc" package version 5.1-0 (Harrell Jr., 2023) in R studio 4.2.3 and were considered significant at an alpha value of 0.05. For the November sampling date, which represented the early cereal grain emergence stage, linear relationships were determined between all soil enzyme activities measured and soil pH, EC, OM, POXC, M3P, and M3S. For the June sampling date, which represented the post-cereal grain harvest stage, linear relationships were determined between all soil enzyme activities measured and soil pH, EC, M3P, M3S, OM, POXC, TC, TN, C:N, TWSA, bulk density, and gravimetric water content.

*Greenhouse Trial Experimental Design*

The subsequent greenhouse trial took place in January 2023 at the University of Arkansas Altheimer Laboratory in Fayetteville, AR. The trial was arranged as a one-factor random complete block design, with eight replicates (n=8). Crop type was the main factor and included certified organic LG334 soft red winter wheat and Violetta winter barley. Unplanted soil served as the control. Air-dried soils from the field study were utilized for this study and included Enders silt loam and Leadvale silt loam soil series. Soil was mixed prior to planting to achieve homogeneity and mixed soil was placed into 15-cm diameter pots to a volume of roughly 850 $cm^3$. Soil-filled pots were rehydrated prior to planting, and wheat and barley were planted at a rate of 4 seeds per pot. Wheat and barley plants were thinned to 2 plants per pot one week after seedling emergence. Greenhouse temperatures were maintained at 25˚ C for the duration of the study, and all crop and control pots were irrigated once a week.

*Greenhouse Trial Soil Sampling and Analysis*

Soil samples and above- and belowground biomass were collected 30 days after planting. Plant biomass samples were collected when plants were at the V2 stage. Aboveground biomass samples were collected by cutting plant leaves and stems from each pot at the soil surface and recording fresh weights (g). Aboveground biomass samples were then dried in a benchtop oven at 55˚ C for one week, and oven-dried sample weights were recorded and extrapolated to biomass densities at the kg $ha^{-1}$ scale. Oven-dried aboveground biomass samples were ground with a Wiley Mini Mill (Thomas Scientific LLC, Swedesboro, NJ) that was fitted with a 1 mm screen and were analyzed for P and S content by inductively coupled plasma (ICP) atomic emission spectroscopy (SPRECTRO CIROS ICP, Fitchburg, MS). Belowground biomass samples were retained from sieved soil samples and were washed to remove excess soil. Fresh belowground biomass weights were recorded, then samples were dried in a benchtop oven at 55˚ C for one week to extrapolate belowground biomass density on a kg $ha^{-1}$ basis.

Plant roots extended to the perimeter of the pots under both the wheat and barley crops; thus, whole-pot soil was considered to be rhizosphere soil. Rhizosphere soil samples were collected by sieving soil through a 2-mm sieve to separate soil and plant roots. A portion of fresh soil was retained to determine gravimetric moisture, and the remaining soil was air-dried for 2 weeks before analysis of soil pH, EC, Mehlich-3 extractable nutrient concentrations, total OM, POXC, and extracellular enzyme activities. All soil analyses were performed according to the same methods used in the field study portion of this research.

*Greenhouse Trial Statistical Analysis*

A one-way analysis of variance (ANOVA) was used to determine the effect of cereal grain crop type on soil health properties using R Studio 4.2.3. The effect of crop type was

considered significant at an alpha level of 0.05, and when appropriate, means were separated using Tukey's HSD test at an alpha value of 0.05 with the "agricolae" package version 1.4.0 (de Mendiburu & Yaseen, 2020) in R Studio 4.2.3. Pearson's correlations were conducted separately for each crop type and control soils using the "Hmisc" package version 5.1-0 (Harrell Jr., 2023). Linear relationships between all soil enzymes and soil pH, EC, M3P, M3S, OM, POXC, and above- and belowground biomass were considered significant at an alpha value of 0.05.

## Results

### Field Trial Results

*Management Impacts on Soil Health Indicators*

Initial soil properties varied both between the organic crop field and the perennial system (Table 3), and between treatments plots within the organic field (Table 4). When treatment plots within the organic field were averaged to represent the entire annual system, properties that varied between systems included soil pH and EC, β-D glucosidase, β-glucosaminidase, M3Ca, M3Mg, M3S, M3Na, M3Fe, and M3Cu (Table 3). Soil pH, EC, β-D glucosidase, β-glucosaminidase, M3Ca, M3Na, and M3Cu were greater in the annual system compared to the perennial locations. Estimated β-D glucosidase activities were 29% and β-glucosaminidase activities were 26% greater in the annual system compared to the perennial system. Conversely, M3Mg, M3S, M3Fe were greater within the perennial system compared to the annual system.

Several properties were not different between systems including all measurements of soil C and N (OM, POM, POXC, TN, and C:N), acid phosphatase, arylsulfatase, and urease activities (Table 3). Averaged across systems, soil organic matter (OM) was 6.33%, total soil C was 3.14%, soil N was 0.34%, C:N was 9.3. Mean soil POM across systems was 19,300 mg $kg^{-1}$, and mean POXC was 673.7 mg $kg^{-1}$. Initial acid phosphatase activities were 43.6 nmol MUF $kg^{-1}$ $hr^{-1}$

in the annual system and 41.2 nmol MUF $kg^{-1}$ $hr^{-1}$ in the perennial system. Arylsulfatase activities were 1.75 and 1.38 nmol MUF $kg^{-1}$ $hr^{-1}$ in the annual and perennial systems, respectively. Estimated urease activities in the annual system were 0.05 mg $NH_4^+$-N $kg^{-1}$ $hr^{-1}$ and 0.06 mg $NH_4^+$-N $kg^{-1}$ $hr^{-1}$ in the perennial system. Extractable nutrients that were not different between the annual system and the perennial location included M3P, M3K, M3Mn, M3B, and M3Zn (Table 3). Averaged across systems, soil M3K concentration was 142 mg $kg^{-1}$, M3P concentration was 35.2 mg $kg^{-1}$, M3Mn concentration was 78.2 mg $kg^{-1}$, M3Fe was 74.1 mg $kg^{-1}$, M3B concentration was 0.04 mg $kg^{-1}$, and M3Zn was 5.78 mg $kg^{-1}$.

Differences were detected in soil chemical properties when treatment plots within the annual system were examined for pre-existing variability, but differences between treatment plots depended on the nutrient being measured (Table 4). Initial soil M3P in July 2020 was greater in the roller crimper, top dress (RCT) plots than the hay mower, top dress (HMT) and disk, no poultry litter (DN) plots. Soil M3Mg was greater in the HMT and DN plots than the hay mower, subsurface applied poultry litter (HMSS) plots. Soil M3S was greater in the plots assigned to receive the RCT treatment compared to plots assigned to receive DTU and HMSS treatments. Differences in M3Na concentrations were detected between three of the four disc termination treatments, with greater M3Na in the disc, top dress incorporated (DTI) plots compared to the plots assigned to receive DN and disc, subsurface poultry litter (DSS) treatments. Soil M3Cu concentrations were greater in the plots assigned to receive roller crimper, subsurface poultry litter (RCSS) compared to plots that later received HMT treatments. No other properties differed between treatments at the initiation of the study.

When the effect of soil health management treatments on soil properties was analyzed over the course of the sampling period, only M3Ca and M3Na were significantly influenced by a

treatment by sampling time interaction (Table 5). In July 2020, M3Ca was not different between treatments plots, but was greater in the plots that would receive the DTU treatment (2283 mg $kg^{-1}$ soil) compared to the perennial management (PM) (916 mg $kg^{-1}$ soil) (Fig.1a). After cover crop termination and poultry litter application in November, M3Ca in DTU plots significantly decreased, such that no differences in M3Ca among treatment plots and PM were detected in November or June. M3Na concentration was significantly greater in DTU plots (24.4 mg kg $soil^{-1}$) than DSS plots (4.27 mg kg $soil^{-1}$) following cover crop termination and poultry litter addition in November (Fig. 1b). By June, M3Na had decreased below detection in all plots including DTU such that no differences in M3Na concentration were detected between treatment plots and perennial locations.

When sampling times were combined, treatment as a main effect significantly influenced β-D glucosidase activities ($p = 0.007$), and POM ($p = 0.03$; Table 5). Averaged over sampling dates, β-D glucosidase activities were 41.4% greater under the disk, subsurface poultry litter (DSS) treatment (29.95 ± 10.15 nmol MUF $kg^{-1}$ $hr^{-1}$) compared to the PM sites (19.67 ± 6.95 nmol MUF $kg^{-1}$ $hr^{-1}$) (Fig 2A). Other enzymes were not significantly affected by treatment. Similar to β-D glucosidase, treatment effects on POM significantly differed between a single treatment and the PM sites. The HMSS treatment resulted in 29% greater POM than PM; however, HMSS was not different from any other soil health management treatment (Fig. 2B). Other soil physical and chemical properties did not significantly differ by soil health management treatments. Analysis of variance showed that total aboveground biomass collected at the end of the study in June 2021 was different between treatments ($p = 0.022$; Table 6); however, treatment means were not significantly different by Tukey's HSD test (data not shown). While not different, total aboveground biomass ranged between 4,892 kg $ha^{-1}$ in the DN

plots to 6,987 kg $ha^{-1}$ in the HMSS plots (data not shown). Cereal grain biomass, seed head weight, and weed biomass were measured only within the annual system; therefore, results do not include perennial locations. The above-mentioned biomass measurements were not significantly different between treatments, but cereal grain biomass ranged from 1,956 kg $ha^{-1}$ in the DN plots to 3,084 kg $ha^{-1}$ in the HMT plots and seed head dry weight ranged from 1,492 kg $ha^{-1}$ in the HMSS plots to 1,895 kg $ha^{-1}$ in the RCT plots (data not shown). Broadleaf weed biomass ranged from 369 kg $ha^{-1}$ in DTU plots to 1,555 kg $ha^{-1}$ in DSS plots, and grass weed biomass ranged from 83 kg $ha^{-1}$ in DSS plots to 1,607 kg $ha^{-1}$ in DTU plots (data not shown).

*Winter Cash Crop and Management Influence on Soil Health Properties*

When the influence of crop species following the implementation of various cover crop termination and poultry litter application methods was examined, significant interactions were detected between crop species and sampling time (Table 7); but treatment by time, crop by treatment, and crop by treatment by sampling time interactions were not significant (Appendix A). Both soil acid phosphatase and arylsulfatase activities significantly differed between November and June depending on crop species (Table 7). Acid phosphatase activity decreased by 57% and 47% from November to June in perennial systems and under organic wheat management, respectively (Fig. 3A). However, activities under barley did not change from November to June. Thus, in June, acid phosphatase activities under barley were greater than activities under perennial systems and wheat. Arylsulfatase activities in November were not different between wheat, barley, and perennial plots (Fig.4A). By June, arylsulfatase activities had increased within barley plots by 61%, but had not significantly changed within perennial and wheat plots. In June, arylsulfatase activities in barley plots were greater than perennial but not different from under wheat management.

Mehlich-3 nutrients were largely unaffected by crop species and sampling time interactions (Table 7), apart from M3S ($p = 0.031$) and M3Na ($p = 0.028$). Both M3S and M3Na showed a decrease from November to June in plots growing barley, while perennial plots and plots growing wheat did not show a significant decrease from November to June (Fig. 4B, Fig 5). Soil M3S under barley decreased by 38.1% over the course of the cropping season (Fig. 4B), but the greater loss occurred with M3Na, which showed a decrease of 99.8%, largely owing to the number of measurements that fell below the detection limit in June (Fig. 5). A significant crop by sampling time was not detected for M3P in soil growing barley, wheat, or perennial plants and values did not decrease from November to June ($p > 0.05$, Fig. 3B).

The β-glucosidase activities, representing C cycling, did not differ between barley, wheat, and perennial locations at any time ($p = 0.275$, Fig. 6A). The POXC was the only soil C fraction that showed a significant crop by time interaction, while POM was affected by time and treatment main effects and total OM differed by sampling time but not crop or soil health management treatment (Table 7). In November, POXC was greater in the perennial locations compared to the barley crop ($p = 0.001$, Fig. 6B). By June, POXC in the perennial soils was not different between barley, perennial, or wheat.

When all soil health management treatments and sampling times were combined, crop species as a main effect was found to significantly influence soil pH, EC, M3Cu, and β-glucosaminidase (Table 7). Soil β-glucosaminidase activities were 27% and 37% greater in plots growing barley compared to plots growing wheat and perennial plots, respectively (Table 8). The lowest pH was measured in soil growing wheat compared to barley and perennial soils, and soil EC was also greater under barley compared to wheat; however, unlike soil pH, EC in both wheat and barley soils did not differ from perennially managed soil. Similarly, M3Cu was greater under

barley compared to wheat, while M3Cu in perennially managed soil did not differ from barley or wheat (Table 8). Some biomass measurements were also shown to vary depending on crop species (Table 9). For instance, broadleaf weed biomass and total aboveground biomass were greater within wheat plots compared to barley plots; whereas grass weed biomass, cereal grain aboveground biomass, and seed head weight did not differ by crop species (Table 9).

Sampling time was the most consistently influential main effect, as several chemical and biological soil properties were significantly different over the sampling period (Table 5). Soil M3P, M3Mg, and M3S showed no change between baseline sampling in July and okra cover crop termination in November, but then decreased in June when the cereal crop had reached maturity to a value that was not different from the values observed at the baseline sampling time (Table 10). Soil pH followed the same seasonal pattern, with a significant decrease occurring between cover crop termination and cereal grain maturity, when soil pH decreased from 6.88 to 6.40. The smallest concentrations of M3Ca and M3Mn were observed in June at cereal grain maturity, with no change occurring between baseline sampling and cover crop termination for either nutrient. Soil POXC and OM were smaller in June than baseline samples and samples collected following okra cover crop termination. All sampling times were significantly different for M3 K and POM; however, the smallest values were observed in the final sampling date, and the greatest values were observed in November, following cover crop termination and poultry litter application. Temporal differences were detected for all soil enzymes except for urease. Soil β-D glucosidase and β-glucosaminidase activities followed a similar seasonal pattern, with the first two sampling dates showing no difference, followed by a decrease in activity in the final sampling date (Table 10).

Correlations between soil enzyme activities and related soil properties were conducted for barley, wheat, and perennial plots in November 2020 (Table 11) and June 2021 (Table 12). In November, β-D glucosidase and β-glucosaminidase activities under the barley crop showed moderately strong positive correlations with OM, POM, and POXC (Table 11). Under the wheat crop, β-glucosaminidase activities showed the greatest number of significant relationships; with negative relationships detected for soil pH, OM, POXC, M3P, and M3S. In the perennial locations in November, only β-D glucosidase and POM showed a significant positive relationship. Measurements of TC, TN, C:N, bulk density, and gravimetric water content were not measured in November and correlations were therefore not calculated for that time. Under the barley crop in June, moderately strong relationships were detected between β-D glucosidase, POM, POXC, M3P, M3S, TC, TN, and C:N. (Table 12). Furthermore, urease activities under the barley crop in June were positively correlated with OM, POM, POXC, and TC and were negatively correlated with gravimetric water content. Under wheat, moderately strong relationships were detected between β-D glucosidase, soil pH, EC, OM, POXC, M3P, TC, and TN. In addition, acid phosphatase and urease activities under the wheat crop were both positively correlated with OM, POM, POXC, and TN. Few correlations were detected in the perennial plots in June; TC was strongly correlated with β-glucosaminidase, acid phosphatase, and arylsulfatase activities. The perennial locations showed the only significant relationship between enzyme activities and total water stable aggregates (TWSA) and bulk density, with both showing a moderately strong relationship with β-glucosaminidase activities.

## Greenhouse Trial Results

### *Crop Influence on Soil Properties*

Crop species influenced several soil properties in the field trial; however, results under the controlled conditions of the greenhouse did not consistently reflect the same crop effects or

magnitudes of influence observed in the field. In the greenhouse, acid phosphatase activity and arylsulfatase activity were once again influenced by crop species (Table 13). Acid phosphatase activities were lowest in soil growing wheat compared to barley and control soil. Acid phosphatase activity under barley plants was 57.7 nmol MUF $kg^{-1}$ $hr^{-1}$ and was 30.6 nmol MUF $kg^{-1}$ $hr^{-1}$ under wheat in the greenhouse (Fig. 3A; Table 13). Arylsulfatase activity was greatest under wheat (0.57 nmol MUF $kg^{-1}$ $hr^{-1}$) compared to barley (0.31 nmol MUF $kg^{-1}$ $hr^{-1}$) and control soils (0.23 nmol MUF $kg^{-1}$ $hr^{-1}$) (Table 13);whereas, β-D glucosidase, β-glucosaminidase, and urease activities were not different between crop species.

Nutrient concentrations were not different between soils growing barley and wheat in the greenhouse; however, differences were observed between crop and control soils, depending on the nutrient (Table 13). Soil M3Cu concentrations were greatest within the control soil but were not different between the wheat and barley. The M3Mg and M3Zn concentrations were both lower in the soil growing barley compared to the control soil, while M3Mg and M3Zn concentrations in soil growing wheat were not significantly different from barley or the control. Soil pH ranged from 6.9 to 7.2 and was not significantly different between any crop treatments. Soil EC was also not significantly different between greenhouse crop treatments and ranged from 0.43 to 0.51 dS $m^{-1}$. Furthermore, soil organic matter and POXC did not differ between crops or the control.

Aboveground and belowground biomass was measured one month after cereal grains were planted, when crops were at the V2 stage. Belowground biomass was not different between wheat and barley plants, but barley had greater aboveground biomass compared to wheat (Fig. 7A; Fig. 7B). Biomass nutrient content analysis showed no significant difference in P content between wheat and barley plants, with wheat averaging 0.44% biomass P and barley averaging

0.45% biomass P. Biomass S content averaged 0.41% in barley plants, which was significantly greater than biomass S in wheat plants (0.29%) (Table 13).

When linear relationships between soil properties were examined within each crop species grown in the greenhouse, differences were detected between soils under respective cereal grains (Table 14). For instance, in barley soils, strong positive relationships were detected between β-D glucosidase activities and total organic matter and aboveground biomass. In contrast, β-D glucosidase activities in wheat soils were not correlated with total organic matter or aboveground biomass and showed a significant negative relationship with soil EC. β-glucosaminidase activities in barley soils were negatively correlated with M3Ca, while β-glucosaminidase activities in wheat soils were not related to M3Ca but were negatively correlated with soil EC. Acid phosphatase activities under barley soils were similar to barley β-D glucosidase activities, with strong positive correlations between total organic matter and aboveground biomass. Acid phosphatase activities under wheat were not related to total organic matter or aboveground biomass but were related to gravimetric water content. Arylsulfatase activities under barley soils were negatively correlated with aboveground biomass, whereas arylsulfatase activities under wheat were not related to any soil property. Urease activities under barley soils were positively correlated with several Mehlich-3 nutrients including M3P, M3S, M3Mn, M3Na, and M3Fe, and were negatively correlated with POXC. Urease activities under wheat were not significantly related to any property. Within control soils, relationships between soil properties and soil enzyme activities were not detected, apart from β-D glucosidase activities, which were related to total organic matter.

## Discussion

### *Cover Crop Management and Poultry Litter Addition Effect on Seasonal Soil Health Properties*

One objective of this study was to examine changes in soil quality indicators in response to management of a summer cover crop with practices that would be commonly employed in an organic cereal grain production system, including mechanical cover crop termination and application of poultry litter as a pre-plant fertilizer for cash crops (Dick, 2011). It was hypothesized that soil enzyme activities and other dynamic properties would vary in response to the management practices implemented depending on the level of disturbance and incorporation of organic residue achieved (Ndiaye et al., 2000; Sürücü et al., 2014). However, across all treatment and perennial locations, with the exception of β-D glucosidase activities, POM, M3Ca, and M3Na, soil properties were largely unaffected by soil management (cover crop termination method and poultry litter application method). Soil M3Ca in DTU plots decreased following cover crop termination and poultry litter application, which was unexpected, as poultry litter contains Ca and has been shown to increase soil extractable Ca immediately following application (He et al., 2008). However, M3Ca concentration was greatest in DTU plots during baseline sampling, which coincided with unusually high soil pH (7.41) in these plots, indicating that the observed treatment by time interaction may have been a soil management legacy effect.

The increase in M3Na after cover crop termination and poultry litter application also occurred within DTU plots and was likely related to the simultaneous decrease in M3Ca, as a decrease in tightly held divalent cations would allow greater adsorption of monovalent cations (Weil & Brady, 2016). In contrast to the observed pre-existing spatial variability of M3Ca, M3Na concentration was not different between plots during baseline sampling. An increase in extractable M3Na following poultry litter application has been documented previously (Haynes & Judge, 2008; Liebhardt, 1976); however, it is not clear why the combination of cover crop

disking and unincorporated poultry litter would have resulted in significantly greater M3Na, outside of cation exchange dynamics between soil Ca and Na.

Soil β-D glucosidase activities were the only enzymes in this study to indicate a significant treatment main effect, with greater activity observed under DSS treatments compared to perennial locations. This effect may have been the result of a legacy effect, as β-D glucosidase activities were greater in the DSS plots compared to the perennial locations prior to the start of the study. While specific management effects on soil enzyme activities and soil organic matter were not consistent in the first year of implementation, strong associations between β-D glucosidase and β-glucosaminidase activities and total OM and POM within the annual system in the first year suggest that these enzymes may be a better early indicator for changes in organic matter compared to other hydrolytic enzymes. Knight and Dick (2004) reported that the sensitivity of β-D glucosidase enzymes to changes in management were primarily a mechanism of the amount of extracellular β-D glucosidase complexed in the soil matrix under different management systems. The amount of enzymes that are able to form protective complexes is linked directly to the amount of colloidal particles in the soil, which confirms the observed positive association with OM and POM in the annual system in this study.

All other enzymes including β-glucosaminidase, acid phosphatase, arylsulfatase, and urease activities were not influenced by soil health management treatments in this study. Soil enzymes are considered rapid indicators that are sensitive to changes in management including soil disturbance and poultry litter addition (Dick & Burns, 2011); however, one year of soil health management may not have been long enough to detect significant changes in activities. This is in agreement with the findings of Feng et al. (2021) who reported that dynamic soil properties such as active carbon and soil enzyme activity required more than 6 years of annual

cover cropping between cash crops before a consistent increase in enzyme activities and soil carbon. Furthermore, Adetunji et al. (2021) reported that phosphatase and urease activities and associated soil properties were not significantly affected by cover crop termination method in a one-year study. The response time of enzyme activities to changes in management is typically cited as 1-3 years, depending on the management and soil type studied (Dick, 2011; Ndiaye et al., 2000), which provides further evidence that the limited soil response observed in this study was likely due to a limited time scale. Furthermore, it is possible that the management history of the organic field used in this study affected the enzyme activities observed; poultry litter had been applied to the study site for two consecutive years prior to the initiation of the study, and the long-term land management of the site had been pasture before organic row cropping began in 2018. Lupwayi et al. (2018) reported that legacy effects of dairy manure application on soil β-D glucosidase, β-glucosaminidase, acid phosphatase, and arylsulfatase persisted 13 years after manure application had ceased. This is due to the ability of soil enzymes to form complexes with clay particles and humic soil fractions, which protect soil enzymes from degradation and allow accumulation over time (Dick, 2011). Thus, prior land use history and poultry litter additions at this site could have resulted in baseline enzyme activities that resisted immediate response to cover crop termination and poultry litter application method.

A significant soil health management treatment effect was also detected for soil POM, with the greatest concentration measured in HMSS plots compared to perennial locations. Previously, researchers have found that soils that were not disturbed increased POM in the soil surface, as aggregation that occurs under undisturbed conditions protects this OM fraction from microbial decomposition (Lavallee et al., 2019; Poeplau & Don, 2013). Increased POM following poultry litter additions has also been reported (Hoover et al., 2019; Kauer et al., 2019);

although, research on the effect of poultry litter application method on soil organic carbon fractions is lacking. While these practices both occurred within HMSS plots, minimal disturbance and subsurface poultry litter application also occurred under the RCSS plots, and similar practices were also implemented within the perennial locations and the other roller crimper and hay mower treatments, indicating that differences in cover crop residue size and dispersal may have affected organic matter decomposition and POM accumulation (Adetunji et al., 2021). Treatment effects were not observed for any other property related to the soil carbon pool. As a measurement of relatively labile soil carbon, POM has been reported as being more sensitive to changes in soil management (such as tillage or fertilization) than total organic matter, dissolved organic matter, and even POXC at times, which may explain the results of this study (Miller et al., 2018; Plaza-Bonilla et al., 2014). Marshall (2018) also reported that the influence of tillage did not significantly influence soil C pools including total OM, POM, and POXC, which was attributed to the buffering effect of high levels of added biomass against short-term change in labile soil C fractions. The site used in the current study also had elevated total OM content (6.4%) prior to the start of the study compared to the 0.5 – 5.0% OM that is expected in cultivated Arkansas soils, which may have buffered against short-term changes in soil C measurements observed in this study.

The limited soil response to cover cropping and soil health management in the first year observed in this study emphasizes the importance of duration in implementing new management strategies. Incorporating cover crops or applying poultry litter for only one year within a newly transitioned organic system may not show a beneficial impact on soil health; however, the continued use of these practices would likely result in measurable differences that could inform best management practices for improved soil health. This is further evidenced by the current

requirement of a 3-year transition period for convention production systems moving to organic production, which is based on the gradual rate of change and strength of residual management effects on the soil ecosystem (Tu et al., 2006; Zinati, 2002).

*Winter Cash Crop Influence on Soil Health Properties*

In addition to examining the effect of soil health management on soil quality in the field study, soil response to subsequent crop species following cover crop termination and poultry litter application was also examined. Crop influence on soil physical, chemical, and biological properties is known to be at least partially dependent on species due to differences in rhizosphere morphology, root exudate quality and quantity, nutrient needs, and plant growth cycles (Paterson, 2003). In the field study, crop species varied across sampling times for M3S, M3Na, POXC, acid phosphatase, and arylsulfatase activities. Crop species as a main effect was detected for β-D glucosaminidase activities, M3Cu, soil pH, and EC. The significant presence of weeds in the crop plots by the end of the sampling period put the detected influence of wheat and barley into question, leading to the initiation of the greenhouse trial. Under the controlled conditions of the greenhouse, acid phosphatase and arylsulfatase activities again behaved differently under the two cereal crops, and several M3 nutrients were also found to vary by crop species.

Acid-phosphatase activity in the field trial decreased within the perennial locations and the wheat plots from November to June sampling times but did not change over the course of the season within the barley plots. Acid phosphatase enzymes are inducible and typically increase during times of limited phosphorus availability and decrease in times of abundant available phosphorus (Dick, 2011); however, in this study, the significant decreases in acid phosphatase activities within perennial and wheat plots over the study did not correspond with an increase in available phosphorus. Instead, M3P concentrations did not significantly vary across sampling

times for barley, perennial, or wheat, but did exhibit a numerical decrease over the season for all crops. Thus, the expected inverse relationship between enzyme activities and available nutrient was not observed for acid phosphatase activities under any crop. It is possible that available P, while numerically decreased, was not reduced enough to induce acid phosphatase production and activity in June. M3P was within the medium range in both barley and wheat plots in June and was only considered low in the perennial plots (Espinoza et al., 2006). As the perennial plots were dominated by tall fescue (*Festuca arundinacea* L.), a cool season grass, the June sampling date would have been a low-growth stage, and consequently would have required less P, thus reducing the need for acid phosphatase production. The same could apply to wheat plots, as available phosphorus is increasingly taken up from tillering stages through stem elongation stages (Feekes stages 2 to 9) and begins to decrease at grain ripening (Malhi et al., 2006; Schilling et al., 1998). The moderate P availability in June may have therefore been adequate to reduce the need for acid phosphatase production and activities.

While the same principle should apply to the barley plots, the lack of change in acid phosphatase within barley plots indicates a difference in P acquisition between the two crops. This theory is further evidenced by the results of the greenhouse trial, which found increased acid phosphatase activities under barley plants when M3P was not considered deficient (Espinoza et al., 2006) and was not different between crops. Biomass nutrient content analysis showed no difference between biomass P in barley and wheat plants at the end of the greenhouse study; however, it is possible that the increased acid phosphatase activities observed in soil growing barley had not yet resulted in greater P uptake. While differences in soil acid phosphatase activities between wheat and barley have not been previously reported, Malhi et al. (2006) reported that P uptake was not different between wheat and barley cultivars in the early

emergence stage but was greater under barley than wheat at harvest, indicating a difference in barley phosphorus requirements depending on growth stage. In the context of this study, a greater need for phosphorus at mature stages in barley when compared to wheat may explain the unchanging acid phosphatase activities observed in barley plots compared to the decrease observed in wheat plots in the field trial.

Arylsulfatase activities also varied by crop species over the sampling dates. Wheat and perennial plots were similar in arylsulfatase activities over the course of the study, while activities under barley responded differently from November to June. Arylsulfatase activities are inhibited by the availability of $SO_4^{-2}$ and $S^{-2}$ to the organisms producing the enzymes (Whalen & Warman, 1996). M3S concentrations did not change over the course of the study in wheat and perennial plots, and likewise, arylsulfatase activities in these plots did not change. Within barley plots, M3S concentrations decreased from November to June, which was reflected in an increase in arylsulfatase activities under barley. The inverse reaction in M3S concentration and arylsulfatase activities that occurred only under barley may support the theory that the barley and wheat rhizosphere influence microbial communities differently due to differences in nutrient requirements. This is further supported by the fact that M3S was never within the plant-growth limiting range for either crop (Espinoza et al., 2006). Under non-limiting $SO_4^{-2}$ conditions, a lack of change or even decrease in arylsulfatase activities would be expected (Klose et al., 2011).

Field results somewhat contrast with the results of the greenhouse trial, which found increased arylsulfatase activities under wheat plants when M3S was not limited and was not different between crops. Biomass S content was greater in barley plants compared to wheat plants at the end of the greenhouse trial, 30 days after planting, which may have contributed to the soil arylsulfatase activities observed in the greenhouse, as barley plants may have acquired

sufficient S and did not need to induce arylsulfatase production. Differences in the field and greenhouse trial results may be due to differences in plant growth stages at sampling times, as field samples were collected 2 weeks after cereal grain planting and again cereal grain maturity, and greenhouse trial samples were collected one month after cereal grain planting. In a study on nutrient uptake and removal by various cereal grains at different growth stages, wheat and barley were found to take up the same amount of $SO_4^{-2}$ roughly 3 weeks after emergence, but barley took up more $SO_4^{-2}$ than wheat at harvest (Malhi et al., 2006). Although crop species response varied between trials, both the field trial and the greenhouse trial show clear differences in sulfur acquisition between the two crops, even if the mechanism responsible cannot be identified within the scope of this research.

Carbon cycling enzymes did not vary by crop species over the field trial sampling period, despite a significant crop species by sampling time interaction for POXC. POXC is a measurement of active C, and has been positively correlated to microbial biomass, total C, and soluble carbohydrate C (Weil et al., 2003). Although POXC is often reported to be the most responsive C fraction to changes in management (Bongiorno et al., 2019), other studies have found that POXC often behaves similarly to total organic matter and may best represent recently stabilized and protected soil C (Xia & Wander, 2021). In this study, POXC content in November was greater in the perennial plots compared to barley but was not different than the wheat plots. By June, no differences in POXC were detected between wheat, barley, or perennial plots. These results are somewhat unexpected, as POXC has been reported to be greater in undisturbed soils compared to cultivated soils (Duval et al., 2018). However, the organic crop field where barley and wheat plots were located had previously been utilized as a pasture and had only been under row crop cultivation for 2 years prior to the initiation of the study. Considering the recent

transition in land use and the inclusion of stabilized OM in POXC measurements, a lack of difference between perennial and crop plots is less surprising. Furthermore, greenhouse trial results confirmed field trial results, as POXC, total OM, and both C cycling enzymes were not significantly different between barley and wheat crops. While differences in soluble carbohydrate C from root exudates have been reported between crop species, these differences would not be expected to be reflected in total OM and may not reflect in POXC that, as mentioned above, includes various fractions of soil C (Vong et al., 2007).

Different crop species have shown varying influence over chemical soil properties within the rhizosphere, which are associated with differences in nutrient uptake, availability, and shifts in soil pH resulting from nutrient uptake (Jungk, 2002; Nye, 1981). Plant roots influence soil pH in the rhizosphere by releasing $H^+$ or $HCO_3^-$ to maintain electrical equilibrium as cations and anions are absorbed into the root, making the soil either more acid or more alkaline depending on the amount of cations or anions taken up by the plant (Nye, 1981). In the field study, soil pH was higher under the barley crop compared to the wheat crop but was not different from the perennial locations, indicating differences in nutrient uptake and removal between barley and wheat. Barley also showed greater M3Cu concentrations and EC compared to wheat plots. Wheat plants have shown a greater dependence on Cu for optimal growth and yield compared to barley and oat plants, which may account for decreased M3Cu concentration in soil growing wheat, owing to increased uptake (Snowball & Robson, 1984).

As expected, dynamic soil properties in the field study showed significant change over the course of the sampling period. Changes in soil nutrient availability occur temporally as temperature and moisture fluctuate and as nutrients cycle through fixed and mineralized forms (Weil & Brady, 2016). In this study, Mehlich-3 extractable nutrients varied by sampling date

with the exception of M3Fe and M3Zn. Both iron and zinc are immobile in the soil and are taken up by plants in trace amounts, which may partially account for the lack of fluctuation over the growing season. Poultry litter additions in November may have also contributed to stabilized zinc and iron concentrations, as availability of these nutrients would be expected to decrease in November, when temperatures were lowest and soil pH was highest (Wang et al., 2016). Biological activity also fluctuated over the course of the study. Enzyme activities are known to change with shifts in temperature, soil moisture, and the soil organic matter pool (Wallenstein & Burns, 2011). β-D glucosidase and β-glucosaminidase activities are linked to cellulose and chitin decomposition, respectively, and are therefore expected to increase following organic matter additions (Deng & Popova, 2011). β-D glucosidase and β-glucosaminidase activities in the field study did not change between the first two sampling dates, both of which followed a large organic matter input event (baseline samples were collected after the incorporation of a cereal rye crop), then decreased in the final sampling time when no organic additions had been made. Moreover, acid phosphatase activities have been linked with C cycling enzymes and soil carbon pools and have also been reported to increase following organic matter additions (Lui et al., 2022). Acid phosphatase activities in the field study steadily decreased over the three sampling dates, which may have occurred as a function of organic and inorganic phosphorus additions. The first sampling date received crop residue additions alone, which coincided with the greatest phosphatase activities, followed by the second sampling date which received organic and inorganic phosphorus from crop residues and poultry litter, which coincided with moderate activities. The final sampling date received no input and coincided with the lowest activities, possibly due to a lack of available organic-P substrate in soil leading to enzyme decomposition, and thus an overall decrease in stabilized extracellular acid phosphatase enzymes. This is further

evidenced by the seasonal changes observed for POM and total OM, which were significantly decreased in the final sampling date. On the other hand, arylsulfatase activities are also associated with organic matter additions (Dick, 2011; Hai-Ming et al., 2014), yet showed the lowest activities in November following poultry litter addition. This may also reflect inorganic sulfur additions from the poultry litter in November, which may have adequately met plant and microbe $SO_4^-$ needs, resulting in temporarily inhibited arylsulfatase activities (Klose et al., 2011).

Urease was the only enzyme to show no change in activity over the course of the study. Urease is involved in the breakdown of urea in to $NH_3$ and $CO_2$ and has been strongly associated not only with N-mineralization but with labile C pools as well (Kandeler et al., 2011). The results of this study contradict the findings of Adetunji et al. (2021) and Weerasekara et al. (2017), who reported that urease activities decreased over the course of a year following the incorporation of cover crop residue due to reduced substrate availability over time. The stable urease activities in this study also contrast with total C and N, OM, POM, and β-glucosaminidase activities (which are also associated with N mineralization) results that significantly decreased from initial sampling in July to the final sampling date in June. Urease activities from this study were also relatively low compared to the expected range of 1.46 to 200 mg $NH_4$ $kg^{-1}$ $hr^{-1}$ activities for agricultural soils (Nannipieri et al., 2002; Kandeler & Dick, 2006), which may indicate that available $NH_3$ was not limited, and urease was therefore not produced. Minimal urease activity may also indicate that the amount of urea (available substrate) from the poultry litter applied in November may not have been substantial enough to result in measurable increases in urease production.

## Conclusion

Overall, differences in cover crop termination and poultry litter application methods were not consistent for soil enzyme activities, soil carbon pools, wet aggregate stability, bulk density, and most soil chemical properties in the first year utilizing a summer cover crop and different soil health management practices to grow a winter cash crop. Prior applications of poultry litter at the site and previous land use as a pasture likely contributed to the limited response to management differences. Investigations into optimal cover crop management and poultry litter application on soil health response may therefore require multiple seasons to detect differences. Additions of organic matter in the annual cropping system (from cover crop residue and poultry litter application) resulted in C cycling potential and soil carbon pools that were not statistically different from or greater than what was observed under perennial management, regardless of termination or application method used. These results indicate that continual OM additions in an organic annual cropping system could result in soil health that is comparable to perennial soil health.

Subsequent crop species had a more immediate effect on soil quality indicators than cover crop and poultry litter management. Phosphorus and sulfur cycling enzymes in particular were influenced by crop type both in the field and in the greenhouse, indicating that barley and wheat crops acquire and utilize phosphorus and sulfur differently. These results highlight the influence of crop species on soil microbial community function and support the theory that soil functional diversity would increase with diversified crop rotations. Further investigation into the functional ecology of the rhizosphere may improve our understanding of plant-soil interactions and allow for optimized management of soil nutrient cycling and plant nutrient uptake.

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## Tables and Figures

**Table 1.** List of treatment by termination method and corresponding poultry litter application method.

| | Combined Treatments | |
|---|---|---|
| **Abbreviation** | **Termination Method** | **Poultry Litter Application Method** |
| RCT | Roller Crimper | Top Dress |
| RCSS | Roller Crimper | Sub-surface |
| DTU | Disk | Top Dress - Unincorporated |
| DTI | Disk | Top Dress - Incorporated |
| DSS | Disk | Sub-surface |
| HMT | Hay Mower | Top Dress |
| HMSS | Hay Mower | Sub-surface |
| DN | Disk | None |

**Table 2**. Monthly average temperature and precipitation data for each sampling time at the Dale Bumpers Small Farms Research Center in Booneville, AR. Data were obtained from the U.S. NOAA (2023).

| | Sampling Time | | |
|---|---|---|---|
| | **July 2020** | **November 2020** | **June 2021** |
| Average temperature (˚C) | 28.8 | 12.7 | 26.2 |
| Maximum temperature (˚C) | 34.3 | 19.8 | 31.6 |
| Minimum temperature (˚C) | 23.4 | 5.56 | 20.7 |
| Average precipitation (cm) | 9.02 | 4.95 | 7.75 |

**Table 3.** Summary of initial soil properties measured in July. Properties from the annual organic crop field were averaged across plots (n=24), and the perennial systems were averaged across locations (n=9).

| | P value | Annual | Perennial |
|---|---|---|---|
| pH | **<0.001[a]*** ** | 6.88 ± 0.09 a | 6.05 ± 0.14 b |
| EC[b] (dS $m^{-1}$) | **<0.001*** ** | 0.279 ± 0.02 a | 0.155 ± 0.03 b |
| *(nmol MUF $kg^{-1}$ $hr^{-1}$)* | | | |
| β-D Glucosidase | **0.023*** | 29.05 ± 1.8 a | 21.65 ± 1.4 b |
| β-Glucosaminidase | **0.017*** | 14.85 ± 0.76 a | 11.39 ± 0.89 b |
| Acid Phosphatase | 0.706 | 43.59 ± 3.4 | 41.27 ± 3.9 |
| Arylsulfatase | 0.096 | 1.75 ± 0.1 | 1.38 ± 0.2 |
| *(mg $NH_4$ $kg^{-1}$ $hr^{-1}$)* | | | |
| Urease | 0.263 | 0.056 ± 0.0004 | 0.048 ± 0.0005 |
| *(mg $kg^{-1}$ soil)* | | | |
| M3P | 0.206 | 41.77 ± 5.7 | 28.72 ± 6.4 |
| M3K | 0.584 | 137.0 ± 7.3 | 147.9 ± 26.3 |
| M3Ca | **0.002**** | 1545 ± 109.3 a | 916.9 ± 79.9 b |
| M3Mg | **<0.001***** | 64.90 ± 2.9 b | 112.6 ± 13.9 a |
| M3S | **0.025*** | 18.46 ± 0.89 b | 25.86 ± 4.7 a |
| M3Na | **<0.001***** | 12.97 ± 1.7 a | 0.00 ± 0 b |
| M3Fe | **0.003**** | 61.75 ± 4.0 b | 86.46 ± 6.0 a |
| M3Mn | 0.788 | 76.88 ± 4.1 | 79.51 ± 11.6 |
| M3Cu | **<0.001***** | 1.35 ± 0.14 a | 0.508 ± 0.06 b |
| M3Zn | 0.553 | 6.84 ± 2.0 | 4.71 ± 2.4 |
| M3B | 0.508 | 0.08 ± 0.07 | 0.00 ± 0 |
| *Soil Carbon and Nitrogen Fractions* | | | |
| OM (%) | 0.715 | 6.24 ± 0.20 | 6.41 ± 0.50 |
| POM (mg $kg^{-1}$) | 0.194 | 22,000 ± 789 | 19,300 ± 705 |
| POXC (mg $kg^{-1}$ soil) | 0.631 | 689.1 ± 31.5 | 658.3 ± 61.1 |
| Total C (%) | 0.366 | 3.29 ± 0.18 | 2.98 ± 0.30 |
| Total N (%) | 0.506 | 0.35 ± 0.01 | 0.33 ± 0.02 |
| C:N | 0.475 | 9.59 ± 0.52 | 8.96 ± 0.26 |

[a] Means followed by a common letter between Annual and Perennial columns are not significantly different by Tukey's HSD test (*$p < 0.05$; **$p < 0.01$; *** $p < 0.001$). Columns without letters indicate no significant differences.

[b] Electrical conductivity (EC), Mehlich-3 phosphorus (M3P), potassium (M3K), calcium (M3Ca), magnesium (M3Mg), sulfur (M3S), sodium (M3Na), iron (M3Fe), manganese (M3Mn), copper (M3Cu), zinc (M3Zn), boron (M3B), organic matter (OM), particulate organic matter (POM), permanganate oxidizable carbon (POXC), total carbon (TC), total nitrogen (TN), and carbon: nitrogen ratio (C:N).

**Table 4.** Summary of initial soil properties measured within the annual system plots in July, averaged across field replicates (n=3).

| **Property** | ***P value*** | **RCT[a]** | **RCSS** | **DTU** | **DTI** | **DSS** | **HMT** | **HMSS** | **DN** |
|---|---|---|---|---|---|---|---|---|---|
| pH | 0.09 | 6.6 | 7.0 | 7.4 | 6.9 | 6.9 | 6.3 | 6.9 | 7.0 |
| EC[b] (dS $m^{-1}$) | 0.13 | 0.304 | 0.241 | 0.281 | 0.335 | 0.353 | 0.220 | 0.203 | 0.297 |
| *(nmol MUF $kg^{-1}hr^{-1}$)* | | | | | | | | | |
| β-D Glucosidase | 0.28 | 26.0 | 27.2 | 25.1 | 25.4 | 42.5 | 28.4 | 28.3 | 29.4 |
| β-Glucosaminidase | 0.43 | 13.7 | 17.3 | 11.5 | 12.7 | 15.1 | 15.0 | 15.4 | 18.1 |
| Acid Phosphatase | 0.85 | 34.3 | 44.8 | 40.9 | 37.9 | 48.4 | 43.1 | 41.4 | 57.9 |
| Arylsulfatase | 0.33 | 1.55 | 1.70 | 1.40 | 1.73 | 2.47 | 1.66 | 1.65 | 1.83 |
| *(mg $NH_4$ $kg^{-1}$ $hr^{-1}$)* | | | | | | | | | |
| Urease | 0.79 | 0.05 | 0.05 | 0.07 | 0.05 | 0.06 | 0.06 | 0.06 | 0.05 |
| *(mg $kg^{-1}$ soil)* | | | | | | | | | |
| M3P | **0.03[c]*** | 87.7 a | 55.1 ab | 32.2 ab | 35.1 ab | 32.5 ab | 24.12 b | 49.3 ab | 18.0 b |
| M3K | 0.54 | 170.3 | 145.2 | 146.3 | 125.9 | 139.3 | 133.8 | 134.4 | 101.1 |
| M3Ca | 0.17 | 1740.3 | 1583.3 | 2283.8 | 1436.4 | 1483.4 | 1080.7 | 1186.7 | 1568.6 |
| M3Mg | **0.02*** | 73.3 ab | 59.9 ab | 53.0 ab | 64.4 ab | 66.7 ab | 79.1 a | 45.2 b | 77.6 a |
| M3S | **0.02*** | 25.0 a | 19.3 ab | 14.6 b | 19.3 ab | 20.3 ab | 17.3 ab | 13.8 b | 18.2 ab |
| M3Na | **0.01*** | 20.8 ab | 7.4 ab | 16.8 ab | 22.3 a | 4.7 b | 15.0 ab | 11.9 ab | 4.9 b |
| M3Fe | 0.30 | 84.4 | 52.6 | 52.7 | 44.6 | 60.1 | 70.8 | 61.4 | 67.5 |
| M3Mn | 0.14 | 76.0 | 91.6 | 62.1 | 102.0 | 58.3 | 76.3 | 74.1 | 74.7 |
| M3Cu | **0.05*** | 1.6 ab | 2.5 a | 1.2 ab | 1.2 ab | 1.1 ab | 0.88 b | 1.3 ab | 1.1 ab |
| M3Zn | 0.06 | 15.4 | 21.1 | 2.4 | 2.5 | 3.3 | 2.8 | 5.6 | 1.6 |
| M3B | 0.49 | 0.58 | 0.0 | 0.0 | 0.0 | 0.06 | 0.0 | 0.0 | 0.0 |
| *Soil Carbon Fractions* | | | | | | | | | |
| OM (%) | 0.08 | 6.68 | 5.43 | 5.65 | 5.67 | 7.36 | 6.21 | 5.86 | 7.10 |
| POM (mg $kg^{-1}$) | 0.29 | 17,448 | 14,529 | 16,514 | 15,461 | 21,584 | 18,386 | 17,985 | 20,394 |
| POXC (mg $kg^{-1}$) | 0.54 | 626.1 | 600.7 | 678.4 | 609.2 | 849.6 | 662.6 | 732.1 | 753.9 |
| Total C (%) | 0.67 | 3.04 | 2.97 | 3.91 | 2.68 | 3.83 | 3.15 | 3.14 | 3.60 |
| Total N (%) | 0.16 | 0.33 | 0.34 | 0.31 | 0.30 | 0.41 | 0.34 | 0.34 | 0.39 |
| C:N | 0.59 | 9.33 | 8.82 | 12.9 | 8.96 | 9.30 | 9.19 | 9.12 | 9.07 |

[a]Treatments include roller crimper/top dress (RCT), roller crimper/subsurface (RCSS), disk/top-dress unincorporated (DTU), disk/top-dress incorporated (DTI), disk/subsurface (DSS), hay mower/top-dress (HMT), hay mower/subsurface (HMSS), and disk/no poultry litter (DN).

[b] Electrical conductivity (EC), Mehlich-3 phosphorus (M3P), potassium (M3K), calcium (M3Ca), magnesium (M3Mg), sulfur (M3S), sodium (M3Na), iron (M3Fe), manganese (M3Mn), copper (M3Cu), zinc (M3Zn), boron (M3B), organic matter (OM), particulate organic matter (POM), permanganate oxidizable carbon (POXC), total carbon (TC), total nitrogen (TN), and carbon: nitrogen ratio (C:N).

[c]Means followed by a common letter between treatment columns are not significantly different by Tukey's HSD test at the 0.05 significance level (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

**Table 5.** ANOVA p values for the effect of treatment, including all cover crop termination methods, poultry litter additions, and perennial management, and sampling times including July, November, and June on soil properties.

| Property | Treatment | Time | Treatment x Time |
|---|---|---|---|
| pH | 0.189 | **0.001[a]**** | 0.084 |
| EC[b] (dS $m^{-1}$) | 0.482 | 0.410 | 0.137 |
| *(nmol MUF $kg^{-1}$ $hr^{-1}$)* | | | |
| β-D Glucosidase | **< 0.01**** | **< 0.001***** | 0.720 |
| β-Glucosaminidase | 0.213 | **< 0.001***** | 0.324 |
| Acid Phosphatase | 0.388 | **< 0.001***** | 0.783 |
| Arylsulfatase | 0.323 | **< 0.001***** | 0.602 |
| *(mg $NH_4$ $kg^{-1}$ dry soil $hr^{-1}$)* | | | |
| Urease | 0.134 | 0.065 | 0.732 |
| *(mg $kg^{-1}$ soil)* | | | |
| M3P | 0.092 | **< 0.001***** | 0.293 |
| M3K | 0.904 | **< 0.001***** | 0.951 |
| M3Ca | **0.013*** | **< 0.001***** | **0.017*** |
| M3Mg | 0.382 | **0.009**** | 0.315 |
| M3S | 0.744 | **0.002**** | 0.474 |
| M3Na | **0.008**** | **<0.001***** | **0.018*** |
| M3Fe | 0.543 | 0.141 | 0.299 |
| M3Mn | 0.663 | **<0.001***** | 0.374 |
| M3Cu | 0.069 | **<0.001***** | 0.367 |
| M3Zn | **0.015*** | 0.935 | 0.054 |
| M3B | 0.350 | **0.025*** | 0.084 |
| *Soil Carbon Fractions* | | | |
| OM (%) | 0.551 | **< 0.001***** | 0.693 |
| POM (mg $kg^{-1}$) | **0.030*** | **< 0.001***** | 0.427 |
| POXC (mg $kg^{-1}$) | 0.312 | **< 0.001***** | 0.545 |
| TC (%) | 0.280 | **<0.001***** | 0.838 |
| TN (%) | 0.266 | **<0.001***** | 0.505 |
| C:N | 0.075 | **0.015*** | 0.747 |

[a] Means were separated using Tukey's HSD at the 0.05 significance level. (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

[b] Electrical conductivity (EC), Mehlich-3 phosphorus (M3P), potassium (M3K), calcium (M3Ca), magnesium (M3Mg), sulfur (M3S), sodium (M3Na), iron (M3Fe), manganese (M3Mn), copper (M3Cu), zinc (M3Zn), boron (M3B), organic matter (OM), particulate organic matter (POM), permanganate oxidizable carbon (POXC), total carbon (TC), total nitrogen (TN), and carbon: nitrogen ratio (C:N).

**Table 6**. ANOVA p values for the effect of treatment on physical properties and plant biomass at the final sampling time (June 2021). Physical properties and total aboveground biomass are measured across soil health management plots and perennial locations, while cereal grain biomass, seed head weight, and weed biomass only include soil health management plots.

| **Property** | **Treatment** |
|---|---|
| *Physical Soil Properties* | |
| TWSA (kg $kg^{-1}$)[a] | 0.093 |
| Bulk density (kg $m^3$) | 0.328 |
| Gravimetric water content (kg $kg^{-1}$) | **0.01[b]*** |
| *Biomass (kg $ha^{-1}$)* | |
| Total aboveground biomass | **0.022*** |
| Cereal aboveground biomass | 0.227 |
| Broadleaf weed biomass | 0.261 |
| Grass weed biomass | 0.264 |
| Seed head dry weight | 0.994 |

[a] Total water stable aggregates (TWSA)

[b] Means were separated using Tukey's HSD at the 0.05 significance level. (*$p < 0.05$, *$p < 0.01$, ***$p < 0.001$).

**Table 7.** ANOVA p values for the effect of treatment, sampling times (November and June), and crop species on soil health properties.

| Analysis | Crop | Time | Treatment | Crop x Time[c] |
|---|---|---|---|---|
| pH | **0.006[a]**** | < **0.001***** | 0.383 | 0.853 |
| EC[b] (dS $m^{-1}$) | **0.002**** | 0.238 | 0.726 | 0.504 |
| *(nmol MUF $kg^{-1}$ $hr^{-1}$)* | | | | |
| β-D Glucosidase | 0.13 | < **0.001***** | 0.131 | 0.275 |
| β Glucosaminidase | **0.003**** | < **0.001***** | 0.220 | 0.200 |
| Acid Phosphatase | 0.456 | < **0.001***** | 0.430 | < **0.001***** |
| Arylsulfatase | 0.942 | **0.013*** | 0.801 | **0.003**** |
| *(mg $NH_4$ $kg^{-1}$ dry soil $hr^{-1}$)* | | | | |
| Urease | 0.051 | 0.121 | 0.202 | 0.29 |
| *(mg $kg^{-1}$ soil)* | | | | |
| M3 P | 0.203 | < **0.001***** | 0.295 | 0.561 |
| M3 K | 0.130 | < **0.001***** | 0.832 | 0.238 |
| M3Ca | 0.082 | **0.013*** | 0.107 | 0.671 |
| M3Mg | 0.252 | **0.003**** | 0.645 | 0.370 |
| M3 S | 0.133 | < **0.001***** | 0.511 | **0.031*** |
| M3Na | 0.104 | < **0.001***** | 0.081 | **0.028*** |
| M3Fe | 0.086 | 0.556 | 0.693 | 0.225 |
| M3Mn | 0.598 | < **0.001***** | 0.287 | 0.412 |
| M3 Cu | **0.015*** | < **0.001***** | 0.095 | 0.424 |
| M3 Zn | 0.545 | 0.936 | **0.022*** | 0.893 |
| M3 B | 0.204 | **0.019*** | 0.278 | 0.404 |
| *Soil Carbon Fractions* | | | | |
| OM (%) | 0.909 | < **0.001***** | 0.896 | 0.097 |
| POM (mg $kg^{-1}$) | 0.094 | < **0.001***** | **0.024*** | 0.147 |
| POXC (mg $kg^{-1}$) | 0.088 | **0.013*** | 0.523 | **0.001**** |

[a] Means were separated using Tukey's HSD at the 0.05 significance level. (*p < 0.05, *p < 0.01, ***p < 0.001).

[b] Electrical conductivity (EC), Mehlich 3 phosphorus (M3P), potassium (M3K), calcium (M3Ca), magnesium (M3Mg), sulfur (M3S), sodium (M3Na), iron (M3Fe), manganese (M3Mn), copper (M3Cu), zinc (M3Zn), boron (M3B), organic matter (OM), particulate organic matter (POM), permanganate oxidizable carbon (POXC).

[c] No significant treatment by crop, treatment by time, or treatment by crop by time interactions were detected and were therefore omitted from the table. Non-significant interactions can be found in Appendix A.

**Table 8.** Mean soil properties within barley, wheat, and perennial plots, averaged across treatments and November and June sampling dates.

| Analysis | Crop Type | | |
|---|---|---|---|
| | **Barley** *(n = 48)* | **Wheat** *(n = 48)* | **Perennial** *(n = 18)* |
| pH | 6.76[a] a | 6.45 b | 6.84 a |
| EC[b] (dS $m^{-1}$) | 0.288 a | 0.231 b | 0.267 ab |
| *(nmol MUF $kg^{-1}$ $hr^{-1}$)* | | | |
| β-D Glucosidase | 24.9 | 22.4 | 18.7 |
| β-Glucosaminidase | 13.3 a | 10.1 b | 9.07 b |
| Acid Phosphatase | 31.7 | 30 | 29.5 |
| Arylsulfatase | 1.37 | 1.38 | 1.16 |
| *(mg NH4 $kg^{-1}$ dry soil $hr^{-1}$)* | | | |
| Urease | 0.09 | 0.07 | 0.05 |
| *(mg $kg^{-1}$ soil)* | | | |
| M3P | 40.7 | 34.3 | 31.2 |
| M3K | 152.0 | 132.9 | 139.5 |
| M3Ca | 1220 | 1084 | 1168 |
| M3Mg | 71.9 | 79.5 | 76.4 |
| M3S | 22.1 | 19 | 21.4 |
| M3Na | 7.39 | 4.14 | 4.26 |
| M3Fe | 53.6 | 63.2 | 56.6 |
| M3Mn | 69.4 | 72.6 | 63.8 |
| M3Cu | 2.14 a | 1.58 b | 2.13 ab |
| M3Zn | 6.6 | 5.72 | 4.75 |
| M3B | 0.129 | 0.08 | 0.226 |
| *Soil Carbon Fractions*[c] | | | |
| OM (%) | 5.52 | 5.42 | 5.39 |
| POM (mg $kg^{-1}$) | 22,080 | 24,130 | 18,850 |
| POXC (mg $kg^{-1}$) | 644.5 | 629.7 | 549.4 |

[a] Means followed by a common letter between crop columns are not significantly different by Tukey's HSD test at the 0.05 significance level. Columns without letters indicate no significant difference between crop types ($p > 0.05$).

[b] Electrical conductivity (EC), Mehlich-3 phosphorus (M3P), potassium (M3K), calcium (M3Ca), magnesium (M3Mg), sulfur (M3S), sodium (M3Na), iron (M3Fe), manganese (M3Mn), copper (M3Cu), zinc (M3Zn), boron (M3B), organic matter (OM), particulate organic matter (POM), permanganate oxidizable carbon (POXC).

[c] Properties that were not measured at both the November and June sampling dates, including total carbon (TC), total nitrogen (TN), and carbon: nitrogen ratio (C:N) are omitted from the table.

**Table 9.** Mean biomass densities including total aboveground biomass collected from barley, wheat, and perennial plots. Cereal grain biomass, seed head weight, and weed biomass densities were collected from only barley and wheat plots, and do not include perennial locations.

| | ***P value*** | **Barley** | **Wheat** | **Perennial** |
|---|---|---|---|---|
| | | **----------(kg ha$^{-1}$) ----------** | | |
| Total Aboveground Biomass | **0.022[a]*** | 6000 b | 6837 a | 5226 b |
| Cereal Grain Biomass | 0.205 | 3074 | 2698 | - |
| Seed Head Weight | 0.255 | 1606 | 1900 | - |
| Broadleaf Weed Biomass | **< 0.001***** | 461 b | 2154 a | - |
| Grass Weed Biomass | 0.734 | 859 | 984 | - |

[a] Means followed by a common letter between crop type columns are not significantly different by Tukey's HSD test at the 0.05 significance level. Columns without letters indicate no significant difference between crop types ($p > 0.05$).

**Table 10**. Soil property means at each sampling date, averaged across soil health treatments, perennial locations, and crop species.

| Analysis | P value | July | November | June |
|---|---|---|---|---|
| | | *(n = 33)* | *(n = 57)* | *(n = 57)* |
| pH | **<0.001[a]*** ** | 6.65 ± 0.10 ab | 6.88 ± 0.07 a | 6.40 ± 0.07 b |
| EC[b] (dS $m^{-1}$) | 0.410 | 0.246 ± 0.02 | 0.270 ± 0.01 | 0.251 ± 0.08 |
| *(nmol MUF $kg^{-1}$ $hr^{-1}$)* | | | | |
| β-D Glucosidase | **<0.001*** ** | 27.0 ± 1.47 a | 27.3 ± 1.18 a | 18.4 ± 0.90 b |
| β-Glucosaminidase | **<0.001*** ** | 13.9 ± 0.66 a | 13.4 ± 0.89 a | 9.24 ± 0.38 b |
| Acid Phosphatase | **<0.001*** ** | 43.0 ± 2.67 a | 36.2 ± 1.55 b | 25.1 ± 1.36 c |
| Arylsulfatase | **<0.001*** ** | 1.65 ± 0.10 a | 1.19 ± 0.06 b | 1.49 ± 0.10 a |
| *(mg $NH_4$ $kg^{-1}$ dry soil $hr^{-1}$)* | | | | |
| Urease | 0.064 | 0.05 ± 0.003 | 0.09 ± 0.01 | 0.07 ± 0.01 |
| *(mg $kg^{-1}$ soil)* | | | | |
| M3P | **<0.001*** ** | 38.2 ± 4.55 ab | 45.5 ± 3.58 a | 27.5 ± 2.50 b |
| M3K | **<0.001*** ** | 140.0 ± 8.69 b | 194.1 ± 10.51 a | 89.9 ± 5.73 c |
| M3Ca | **<0.001*** ** | 1374 ± 95.5 a | 1243 ± 53.5 a | 1065 ± 42.7 b |
| M3Mg | **0.01*** | 77.9 ± 5.64 ab | 85.1 ± 4.12 a | 66.5 ± 4.31 b |
| M3S | **0.002**** | 20.5 ± 1.49 ab | 24.1 ± 1.89 a | 17.2 ± 0.64 b |
| M3Na | **<0.001*** ** | 9.43 ± 1.60 a | 10.46 ± 1.88 a | 0.59 ± 0.33 b |
| M3Fe | 0.141 | 68.5 ± 3.83 | 59.6 ± 3.13 | 56.6 ± 3.90 |
| M3Mn | **<0.001*** ** | 77.6 ± 4.27 a | 88.9 ± 4.53 a | 50.8 ± 4.00 b |
| M3Cu | **<0.001*** ** | 1.12 ± 0.12 b | 2.31 ± 0.16 a | 1.49 ± 0.13 b |
| M3Zn | 0.935 | 6.26 ± 1.56 | 5.89 ± 0.65 | 5.99 ± 1.17 |
| M3B | **0.025*** | 0.06 ± 0.05 a | 0.08 ± 0.02 a | 0.17 ± 0.03 a |
| *Soil Carbon Fraction* | | | | |
| OM (%) | **<0.001*** ** | 6.29 ± 0.20 a | 6.11 ± 0.16 a | 4.80 ± 0.17 b |
| POM (mg $kg^{-1}$) | **<0.001*** ** | 18,430 ± 803 b | 29,360 ± 1025 a | 15,520 ± 468 c |
| POXC (mg $kg^{-1}$ soil) | **0.009**** | 680.6 ± 27.9 a | 660.5 ± 20.1 a | 585.9 ± 24.0 b |
| TC (%) | **<0.001*** ** | 3.20 ± 0.15 a | NA[c] | 2.44 ± 0.07 b |
| TN (%) | **<0.001*** ** | 0.34 ± 0.01 a | NA | 0.28 ± 0.01 b |
| C:N | **0.019*** | 9.42 ± 0.38 a | NA | 8.57 ± 0.17 b |

[a] Means followed by a common letter between sampling time columns are not significantly different by Tukey's HSD test at the 0.05 significance level. Columns without letters indicate no significant difference between sampling times ($p > 0.05$).

[b] Electrical conductivity (EC), Mehlich-3 phosphorus (M3P), potassium (M3K), calcium (M3Ca), magnesium (M3Mg), sulfur (M3S), sodium (M3Na), iron (M3Fe), manganese (M3Mn), copper (M3Cu), zinc (M3Zn), boron (M3B), organic matter (OM), particulate organic matter (POM), permanganate oxidizable carbon (POXC).

[c] Not applicable (NA) because total carbon (TC), total nitrogen (TN), and carbon: nitrogen (C:N) were not measured at the November sampling date.

**Table 11.** Pearson correlation coefficients (r) by crop at early emergence stage (November) for enzyme activities and selected soil properties. Correlations are separated by crop.

| | pH | EC (dS $m^{-1}$) | OM (%) | POM | POXC | M3P | M3S |
|---|---|---|---|---|---|---|---|
| | | | | (mg $kg^{-1}$) | | | |
| | | | | *Coefficient (r)* | | | |
| **Barley (n=24)** | | | | | | | |
| β-D Glucosidase | 0.07 | -0.1 | **0.75*** ** | **0.67*** ** | **0.53*** ** | **0.68*** ** | 0.3 |
| β-Glucosaminidase | 0.17 | 0.03 | **0.78*** ** | **0.68*** ** | **0.47*** | **0.65*** ** | **0.50*** |
| Acid Phosphatase | -0.26 | -0.2 | 0.29 | 0.26 | 0.13 | 0.32 | -0.004 |
| Arylsulfatase | -0.31 | -0.19 | -0.17 | -0.03 | 0.06 | -0.06 | -0.16 |
| Urease | 0.02 | -0.09 | 0.19 | 0.18 | -0.03 | 0.21 | -0.05 |
| **Wheat (n=24)** | | | | | | | |
| β-D Glucosidase | -0.04 | 0.02 | -0.17 | **0.53** ** | -0.19 | -0.27 | -0.32 |
| β-Glucosaminidase | **-0.49*** | -0.1 | **-0.52** ** | 0.24 | **-0.57** ** | **-0.65*** ** | **-0.42*** |
| Acid Phosphatase | -0.01 | -0.12 | 0.07 | **0.43*** | -0.04 | -0.06 | -0.02 |
| Arylsulfatase | **-0.42*** | -0.35 | -0.3 | **0.48*** | -0.38 | -0.27 | -0.16 |
| Urease | -0.002 | 0.17 | -0.42 | 0.05 | -0.31 | -0.12 | -0.05 |
| **Perennial (n=9)** | | | | | | | |
| β-D Glucosidase | 0.11 | -0.45 | -0.09 | **0.69*** | 0.11 | -0.27 | 0.11 |
| β-Glucosaminidase | -0.09 | -0.25 | 0.008 | 0.24 | 0.13 | 0.08 | 0.17 |
| Acid Phosphatase | -0.25 | -0.38 | -0.41 | 0.53 | 0.2 | -0.09 | -0.55 |
| Arylsulfatase | 0.38 | -0.06 | -0.05 | 0.24 | -0.16 | -0.19 | 0.33 |
| Urease | 0.06 | -0.14 | -0.39 | 0.2 | 0.13 | 0.07 | -0.38 |

[a] Relationships were considered significant at $p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

[b] Electrical conductivity (EC), organic matter (OM), particulate organic matter (POM), permanganate oxidizable carbon (POXC), Mehlich-3 phosphorus (M3P), and sulfur (M3S).

**Table 12.** Pearson correlation coefficients (r) at cereal crop maturity (June) for enzyme activities and selected soil properties, separated by crop.

| | pH | EC[a] (dS $m^{-1}$) | OM (%) | POM | POXC | M3P | M3S | Total C (%) | Total N (%) | C:N | TWSA (kg $kg^{-1}$) | Db (kg $m^3$) | θg (kg $kg^{-1}$) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | ----------(mg $kg^{-1}$)---------- | | | | | | | | | |
| | | | | | | | *Coefficient (r)* | | | | | | |
| **Barley (n=24)** | | | | | | | | | | | | | |
| β-D Glucosidase | 0.12[b] | 0.02 | 0.72 | **0.75*** ** | **0.66*** ** | **0.47*** | **0.41*** | **0.54**** | **0.44*** | **0.41*** | 0.20 | 0.37 | -0.23 |
| β Glucosaminidase | 0.22 | 0.28 | 0.31 | 0.38 | 0.35 | 0.19 | **0.47*** | 0.18 | 0.17 | 0.09 | -0.03 | 0.19 | 0.11 |
| Acid Phosphatase | -0.01 | -0.11 | -0.07 | 0.03 | -0.38 | -0.05 | 0.05 | -0.13 | -0.03 | -0.23 | -0.15 | -0.06 | 0.05 |
| Arylsulfatase | -0.13 | -0.01 | -0.08 | 0.06 | -0.32 | 0.14 | 0.05 | -0.15 | -0.10 | -0.17 | -0.08 | -0.03 | 0.05 |
| Urease | -0.02 | -0.17 | **0.63*** ** | **0.72*** ** | **0.65*** ** | 0.32 | 0.08 | **0.45*** | 0.34 | 0.38 | 0.15 | 0.30 | **-0.45*** |
| **Wheat (n=24)** | | | | | | | | | | | | | |
| β-D Glucosidase | **0.56**** | **0.48*** | **0.55**** | 0.40 | **0.56**** | **0.57**** | 0.02 | **0.49*** | **0.63*** ** | 0.15 | 0.06 | 0.10 | -0.16 |
| β Glucosaminidase | **0.64*** ** | 0.34 | 0.16 | -0.01 | 0.09 | 0.21 | -0.13 | -0.03 | 0.08 | -0.10 | 0.36 | -0.15 | 0.09 |
| Acid Phosphatase | -0.16 | 0.03 | **0.66*** ** | **0.51*** | **0.48*** | 0.19 | -0.03 | 0.46 | **0.75*** ** | 0.04 | -0.14 | -0.20 | -0.29 |
| Arylsulfatase | **0.75*** ** | **0.70*** ** | -0.16 | -0.33 | -0.15 | 0.39 | -0.30 | -0.14 | 0.06 | -0.23 | -0.07 | -0.07 | -0.09 |
| Urease | 0.42* | 0.40 | **0.58**** | **0.41*** | **0.55**** | 0.27 | -0.08 | 0.35 | **0.51*** | 0.07 | 0.26 | -0.09 | -0.04 |
| **Perennial (n=9)** | | | | | | | | | | | | | |
| β-D Glucosidase | -0.54 | -0.33 | -0.03 | **0.88**** | 0.66 | 0.52 | **-0.70*** | 0.63 | **0.89**** | -0.21 | 0.20 | 0.34 | 0.08 |
| β-Glucosaminidase | -0.19 | -0.28 | -0.22 | 0.41 | 0.55 | -0.07 | **-0.70*** | **0.78*** | 0.64 | 0.46 | **0.74*** | **0.73*** | 0.25 |
| Acid Phosphatase | -0.20 | -0.09 | -0.17 | 0.55 | **0.74*** | 0.17 | -0.10 | **0.76*** | 0.66 | 0.35 | 0.29 | 0.29 | 0.03 |
| Arylsulfatase | 0.06 | -0.09 | -0.45 | 0.22 | 0.45 | -0.23 | -0.31 | **0.83**** | 0.48 | **0.82**** | 0.65 | 0.69 | 0.20 |
| Urease | -0.33 | -0.06 | -0.37 | -0.01 | 0.08 | -0.23 | -0.13 | 0.29 | 0.30 | 0.52 | 0.28 | 0.07 | 0.53 |

[a] Electrical conductivity (EC), organic matter (OM), particulate organic matter (POM), permanganate oxidizable carbon (POXC), Mehlich 3 phosphorus (M3P), and sulfur (M3S), total water stable aggregates (TWSA), bulk density (Db), and gravimetric water content (θg).

[b] Relationships were considered significant at $p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

**Table 13.** Means (± standard error) for greenhouse trial soil and crop properties by crop type.

| | | Crop Type | | |
|---|---|---|---|---|
| **Analysis** | ***P value*** | **Barley** | **Wheat** | **Control** |
| pH | 0.213 | 7.18 ± 0.12 | 6.92 ± 0.12 | 7.20 ± 0.13 |
| EC[a] (dS $m^{-1}$) | 0.729 | 0.432 ± 0.07 | 0.501 ± 0.06 | 0.508 ± 0.09 |
| *(nmol MUF $kg^{-1}$ $hr^{-1}$)* | | | | |
| β-D Glucosidase | 1.00 | 9.90 ± 1.32 | 9.88 ± 1.52 | 9.89 ± 1.94 |
| β-Glucosaminidase | 0.729 | 3.92 ± 0.32 | 3.58 ± 0.38 | 3.48 ± 0.51 |
| Acid Phosphatase | **<0.001[b]*** ** | 57.7 ± 1.89 a | 30.6 ± 1.78 b | 53.7 ± 1.67 a |
| Arylsulfatase | **0.008**** | 0.31 ± 0.07 b | 0.57 ± 0.07 a | 0.23 ± 0.07 b |
| *(mg $NH_4$ $kg^{-1}$ $hr^{-1}$)* | | | | |
| Urease | 0.314 | 0.03 ± 0.003 | 0.04 ± 0.004 | 0.05 ± 0.02 |
| *(mg $kg^{-1}$)* | | | | |
| M3P | **<0.001***** | 50.4 ± 1.94 b | 54.6 ± 2.32 b | 68.6 ± 3.28 a |
| M3K | **<0.001***** | 102.3 ± 8.72 b | 132.9 ± 14.4 b | 187.2 ± 14.6 a |
| M3Ca | 0.700 | 2441 ± 53.1 | 2476 ± 44.9 | 2541 ± 129.1 |
| M3Mg | **0.025*** | 87.1 ± 1.97 b | 91.2 ± 5.06 ab | 101.3 ± 2.54 a |
| M3S | 0.070 | 25.4 ± 0.95 a | 30.2 ± 1.67 a | 32.0 ± 2.78 a |
| M3Na | 0.062 | 21.9 ± 10.6 | 26.7 ± 9.50 | 59.7 ± 14.0 |
| M3Fe | **0.004**** | 103.4 ± 5.14 b | 110.1 ± 5.43 b | 137.3 ± 8.76 a |
| M3Mn | **0.045*** | 163.6 ± 14.2 a | 164.1 ± 8.03 a | 209.2 ± 17.4 a |
| M3Cu | **0.005**** | 3.67 ± 0.09 b | 3.82 ± 0.21 b | 4.48 ± 0.17 a |
| M3Zn | **0.042*** | 8.80 ± 0.20 b | 9.26 ± 0.72 ab | 10.9 ± 0.63 a |
| M3B | 0.330 | 1.45 ± 1.00 | 1.97 ± 1.07 | 0.21 ± 0.06 |
| *Soil Carbon Fractions* | | | | |
| OM (%) | 0.168 | 0.05 ± 0.00 | 0.05 ± 0.0 | 0.05 ± 0.001 |
| POXC (mg $kg^{-1}$) | 0.222 | 811.7 ± 21.3 | 784.5 ± 15.0 | 833.0 ± 20.4 |
| *Plant Biomass (kg $ha^{-1}$)* | | | | |
| Aboveground Biomass | **0.008**** | 512.7 ± 37.4 a | 354.6 ± 34.2 b | - |
| Belowground Biomass | 0.857 | 173.5 ± 14.6 | 167.1 ± 31.8 | - |
| *Biomass Nutrients (%)* | | | | |
| Biomass P | 0.676 | 0.45 ± 0.01 | 0.44 ± 0.01 | - |
| Biomass S | **<0.001***** | 0.42 ± 0.02 a | 0.29 ± 0.004 b | |

[a]Electrical conductivity (EC), Mehlich 3 phosphorus (M3P), potassium (M3K), calcium (M3Ca), magnesium (M3Mg), sulfur (M3S), sodium (M3Na), iron (M3Fe), manganese (M3Mn), copper (M3Cu), zinc (M3Zn), boron (M3B), organic matter (OM), permanganate oxidizable carbon (POXC).

[b] Means followed by a common letter between crop columns are not significantly different by Tukey's HSD test at the 0.05 significance level. Columns without letters indicate no significant difference. (*p < 0.05, **p < 0.01, ***p < 0.001).

**Table 14.** Greenhouse trial Pearson correlation coefficients (r) for enzyme activities and selected soil properties, separated by crop.

| | pH | EC[a] (dS $m^{-1}$) | OM (%) | POXC (mg $kg^{-1}$) | M3P (mg $kg^{-1}$) | M3S (mg $kg^{-1}$) | AG Biomass (kg $ha^{-1}$) | BG Biomass (kg $ha^{-1}$) |
|---|---|---|---|---|---|---|---|---|
| | *Coefficient (r)* | | | | | | | |
| **Barley (n=8)** | | | | | | | | |
| β-D Glucosidase | -0.20 | -0.30 | **0.76[b]*** | 0.57 | -0.19 | -0.56 | **0.90***** | 0.55 |
| β-Glucosaminidase | 0.23 | -0.40 | 0.38 | 0.36 | -0.02 | -0.45 | 0.58 | 0.23 |
| Acid Phosphatase | -0.03 | 0.04 | **0.77*** | 0.33 | 0.11 | -0.54 | **0.96***** | 0.38 |
| Arylsulfatase | 0.23 | 0.43 | -0.34 | -0.45 | 0.005 | 0.58 | **-0.78*** | -0.35 |
| Urease | 0.69 | -0.11 | -0.15 | **-0.83*** | **0.73*** | **0.73*** | -0.36 | -0.30 |
| **Wheat (n=8)** | | | | | | | | |
| β-D Glucosidase | 0.14 | **-0.79*** | 0.64 | 0.46 | 0.18 | 0.28 | 0.34 | -0.57 |
| β-Glucosaminidase | 0.24 | **-0.83*** | 0.36 | 0.30 | 0.25 | 0.29 | 0.30 | -0.48 |
| Acid Phosphatase | -0.44 | -0.02 | -0.14 | -0.04 | 0.18 | 0.22 | 0.58 | -0.07 |
| Arylsulfatase | 0.36 | -0.67 | 0.69 | 0.58 | 0.45 | 0.55 | 0.51 | -0.36 |
| Urease | 0.23 | 0.30 | 0.22 | 0.48 | -0.23 | -0.34 | -0.06 | -0.29 |
| **Control (n=8)** | | | | | | | | |
| β-D Glucosidase | -0.39 | 0.27 | 0.75 | 0.33 | 0.23 | -0.52 | - | - |
| β-Glucosaminidase | -0.14 | 0.21 | 0.44 | 0.66 | -0.02 | -0.57 | - | - |
| Acid Phosphatase | -0.28 | 0.12 | 0.02 | 0.56 | -0.19 | -0.08 | - | - |
| Arylsulfatase | -0.01 | -0.27 | -0.36 | 0.49 | -0.23 | -0.04 | - | - |
| Urease | 0.55 | -0.61 | -0.49 | -0.25 | 0.28 | 0.19 | - | - |

[a] Electrical conductivity (EC), organic matter (OM), permanganate oxidizable carbon (POXC), Mehlich-3 phosphorus (M3P), and sulfur (M3S), aboveground biomass (AG), and belowground biomass (BG).

[b] Relationships were considered significant at $p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

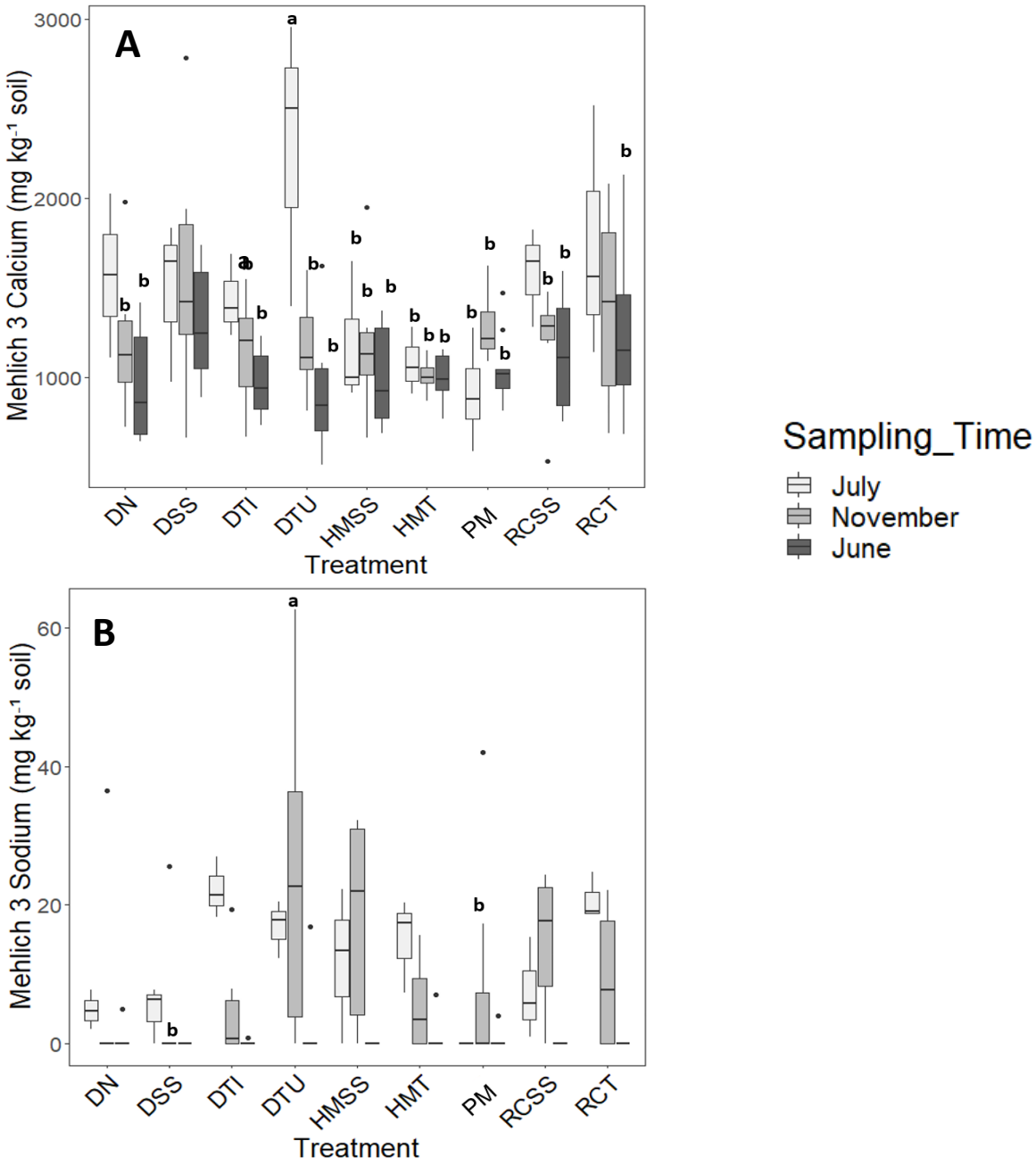


**Fig. 1. (A)** Mehlich-3 calcium (mg $kg^{-1}$ soil) by soil health management treatment across sampling period (n = 147). Boxes without a letter are not different from any treatment at any time (ab). **(B)** Mehlich-3 sodium by soil health management treatments across the sampling period (n = 90). Means at the June sampling time were below detection. Boxes represent the interquartile range, midlines in boxes represent medians, and dots represent outliers. Treatments include RCT (roller crimper, top dress), RCSS (roller crimper, subsurface), DTU (disk, top-dress unincorporated), DTI (disk, top-dress incorporated), DSS (disk, subsurface), HMT (hay mower, top-dress), HMSS (hay mower, subsurface), and DN (disk, no poultry litter), and PM (perennial management). Means followed by a common letter are not significantly different by Tukey's HSD test ($P < 0.05$).

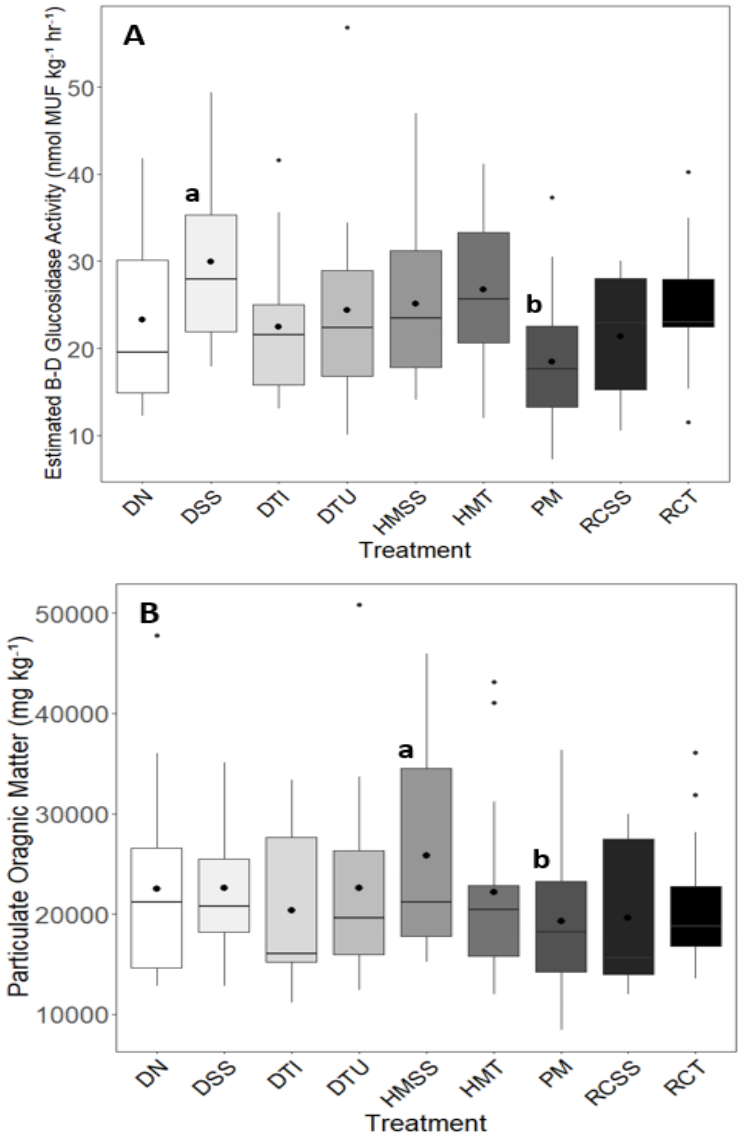


**Fig. 2. (A)** β-D glucosidase (nmol MUF $kg^{-1}$ $hr^{-1}$) by soil health management treatment averaged across crop species and sampling times (n = 147). **(B)** Particulate organic matter (POM) by soil health management treatments averaged across crop species and sampling time (n = 147). Boxes represent the interquartile range, dots within the box represent means, and outer dots represent outliers. Treatments include RCT (roller crimper, top dress), RCSS (roller crimper, subsurface), DTU (disk, top-dress unincorporated), DTI (disk, top-dress incorporated), DSS (disk, subsurface), HMT (hay mower, top-dress), HMSS (hay mower, subsurface), and DN (disk, no poultry litter), and PM (perennial management). Boxes within each graph without a letter are not different from any treatment at any time.

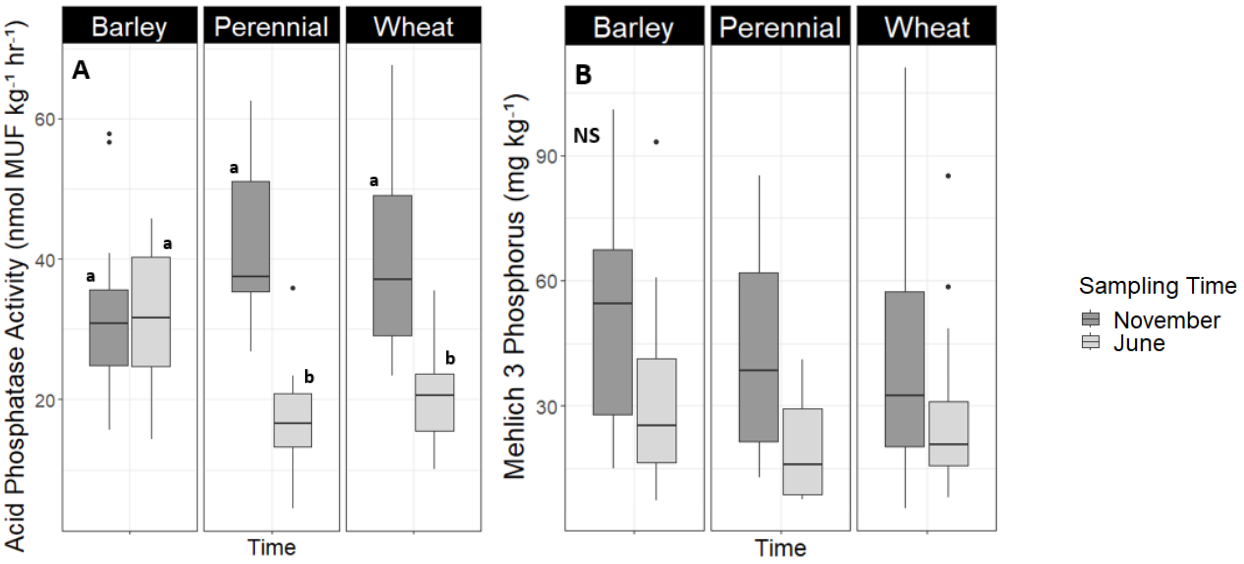


**Fig. 3. (A)** Acid Phosphatase activities (nmol MUF $kg^{-1}$ $hr^{-1}$) by crop from early emergence (November, n = 57) to cereal maturity (June, n = 57). **(B)** Mehlich-3 phosphorus (M3P) (mg $kg^{-1}$) by crop species over the sampling period (not significant, n = 57). Boxes represent the interquartile range, lines within the box represent the median, upper and lower lines represent the maximum and minimum, respectively, and outer dots represent outliers. Means followed by a common letter are not significantly different by Tukey's HSD test ($P < 0.05$).

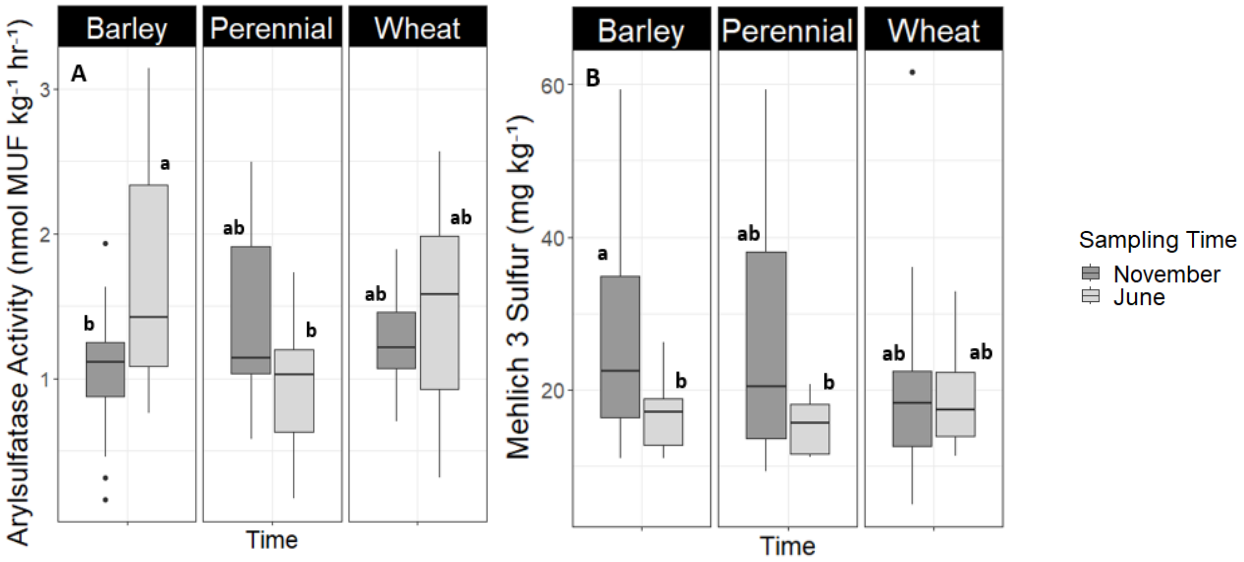


**Fig. 4. (A)** Arylsulfatase activities (nmol MUF $kg^{-1}$ $hr^{-1}$) by crop from early emergence (November, n = 57) to cereal maturity (June, n = 57). **(B)** Mehlich 3 sulfur (M3S) (mg $kg^{-1}$) by crop species over the sampling period (n = 57). Boxes represent the interquartile range, lines within the box represent the median, upper and lower lines represent the maximum and minimum, respectively, and outer dots represent outliers. Means for each property followed by a common letter are not significantly different by Tukey's HSD test ($P < 0.05$).

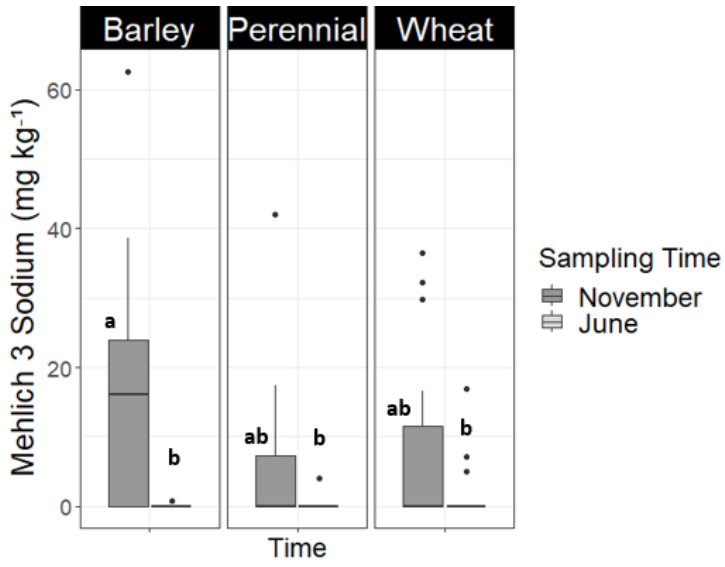


**Fig. 5.** Mehlich-3 sodium (M3Na) (mg $kg^{-1}$ soil) by crop species from early emergence (November, n = 57) to cereal maturity (June, n = 57). Boxes represent the interquartile range, lines within the box represent the median, upper and lower lines represent the maximum and minimum, respectively, and outer dots represent outliers. Means followed by a common letter are not significantly different by Tukey's HSD test ($P < 0.05$).

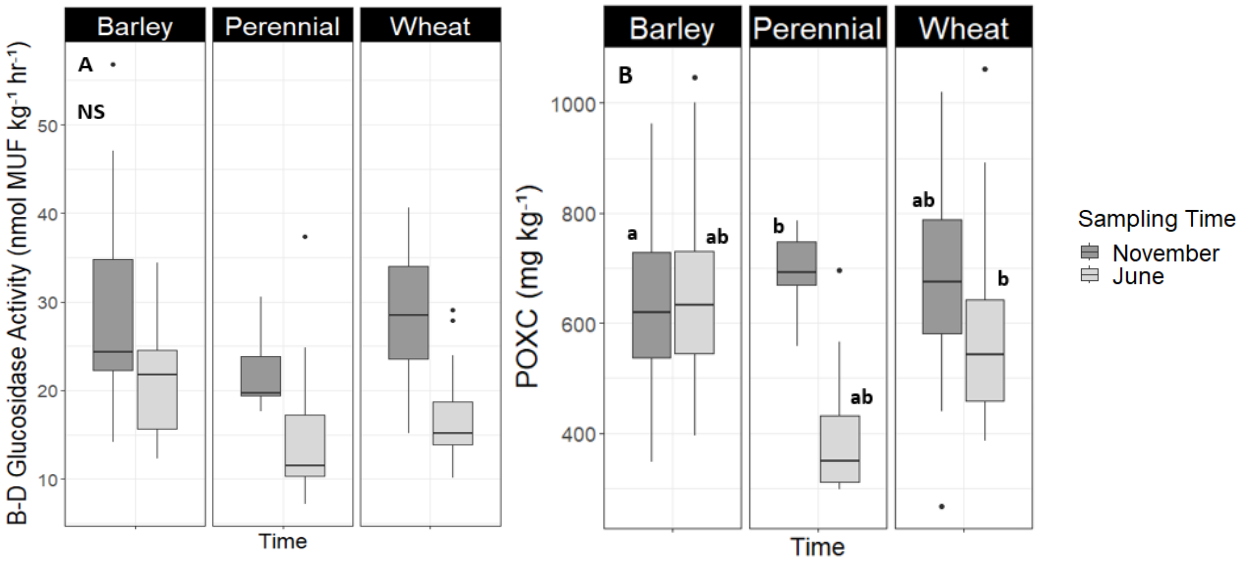


**Fig. 6. (A)** β-D glucosidase activities (nmol MUF $kg^{-1}$ soil $hr^{-1}$) by crop from early emergence (November, n = 57) to cereal maturity (June, n = 57) (not significant). **(B)** Permanganate oxidizable carbon (POXC) (mg $kg^{-1}$) by crop species over the sampling period (n = 57). Boxes represent the interquartile range, lines within the box represent the median, upper and lower lines represent the maximum and minimum, respectively, and outer dots represent outliers. Means followed by a common letter are not significantly different by Tukey's HSD test ($P < 0.05$).

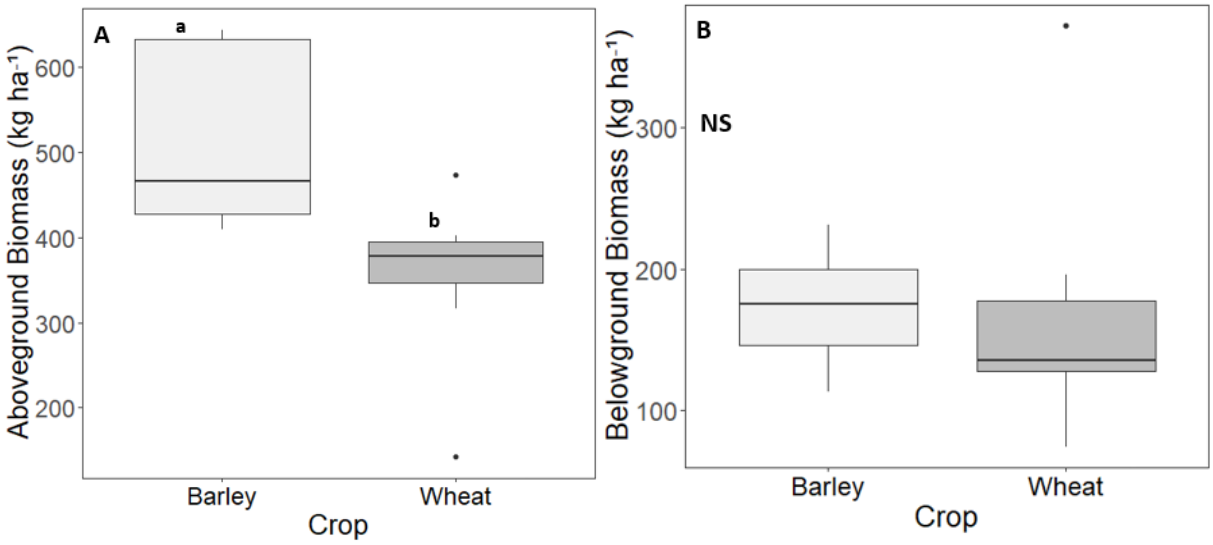


**Fig. 7. (A)** Greenhouse trial aboveground biomass for barley and wheat crops (n = 8). **(B)** Greenhouse trial belowground biomass for barley and wheat crops (n = 8). Boxes represent the interquartile range, lines within the box represent the median, upper and lower lines represent the maximum and minimum, respectively, and outer dots represent outliers. Means followed by a common letter between crop types are not significantly different by Tukey's HSD test ($p < 0.05$).

# Chapter 3:

# Microbial Response to Poultry Litter Rate on Recently Land-Leveled Silt Loam Soil

**Abstract**

Land leveling is a water conservation approach that may increase irrigation efficiency, but potentially degrades soil quality. In Arkansas, the application of poultry litter (PL) to land-leveled soils is promoted to reclaim soil function and quality through the addition of organic matter (OM), plant-available macro- and micro-nutrients, and an active microbial population. In addition to PL application, furrow irrigation is promoted to further improve irrigation efficiency of land leveled soils. While PL applications have been shown to maintain productivity of land-leveled soils it is currently unclear to what extent soil health may be restored under varying PL application rates when used in combination with different irrigation approaches. Therefore, the objective was to evaluate effects of PL application rate combined with either flood or furrow irrigation on soil properties related to biological and biochemical functions, as well as soil chemical and physical properties under recently land-leveled rice (*Oryza sativa* L.) fields. Soil samples were collected from the 0-15 cm depth in fields that had received treatments of 4.5 Mg $ha^{-1}$ PL + furrow irrigation (FUR), 6.7 Mg $ha^{-1}$ PL + flood irrigation (FL), or 9.0 Mg $ha^{-1}$ PL + FL on a privately-owned farm in Newport, AR. Samples were collected three times over the course of a single rice production season (1-, 4-, and 9-months following PL application). Soil β-D glucosidase activities significantly increased over the course of a single rice growing season under the 6.7 Mg $ha^{-1}$ PL + FL and 9.0 Mg $ha^{-1}$ PL + FL treatments. Arylsulfatase activities showed an overall increase under the 6.7 Mg $ha^{-1}$ PL + FL treatment. All other enzyme activities measured did not change over the course of the study, from 1 month after PL application to 9 months after PL application. Soil respiration was unaffected by PL rate and irrigation method and showed consistently low biological activity over the sampling period. All Mehlich-3 extractable soil nutrients showed greater or equal concentrations under the 4.5 Mg ha-1 PL +

FUR treatment compared to the 6.7 Mg ha-1 PL + FL treatment. Overall, PL application rate combined with irrigation method was not a consistently influential factor on short-term soil quality indicators, suggesting that observable differences in PL application rate, regardless of the irrigation method used, require more than one season to take effect.

**Introduction**

Land leveling, also called precision grading, is a water conservation practice that involves the physical redistribution of soil to achieve a flat surface, often with a slight, 0.05 to 0.1 percent slope in a single direction for drainage purposes (Hardke, 2021). The creation of a uniformly flat topography improves irrigation efficiency by filling water-holding depressions, allowing for more rapid and uniform water movement across the field. Land leveling increases water-use efficiency by an estimated 10 to 20% and increases rice yield by an estimated 10% (Young et al., 2004). Irrigation use-efficiency is further increased on land-leveled soils with the use of furrow irrigation in place of conventional flood irrigation, along with reduced input costs (Hardke & Chlapecka, 2020). The practices of land leveling and furrow irrigation are increasingly promoted and adopted in the Arkansas Delta region as a means to slow the depletion of the Mississippi River Valley Alluvial Aquifer (MRVAA), the primary source of irrigation water for cropland in eastern Arkansas (Yaeger et al., 2018). As precision-graded land area in Arkansas continues to increase annually, it is important to consider potential drawbacks to crop production.

Despite the economic and water conservation gains obtained under precision-leveled and furrow irrigated fields, a decline in soil quality following land leveling has been reported, as cut areas expose subsoil that is low in organic matter (OM), nutrient deficient, and potentially sodic (Brye et al., 2006; Daniels et al., 2002; Robbins et al., 1997). Sharifi et al. (2014) described a significant decrease in soil OM, pH, and nitrogen (N), phosphorus (P), potassium (K), and an

increase in electrical conductivity (EC) following the precision-leveling of rice fields in Iran. Decreased N was associated with OM loss, while decreased available soil P was determined to be a combined result of P-deficient clay subsoils being brought to the surface as well as a decrease in P mineralizing fungi (Sharifi et al., 2014).

Poultry litter has been used in eastern Arkansas as a soil amendment to restore soil productivity in response to soil degradation from precision land leveling. Poultry litter provides plant available macro- and micronutrients, OM, and its own assembly of microbes, allowing the amendment to address several of the observed detrimental soil impacts of land leveling compared to inorganic fertilizers, which solely addresses nutrient status (Brye et al., 2004). Previous research on land leveled soils has primarily focused on the effects of PL on rice yield, soil physical and chemical properties, and biological community level abundance and biomass (Brye et al., 2004; Miller et al., 1991). However, less is known about the nutrient cycling response of land-leveled soils to PL application. Nutrient cycling processes are an important component of soil health that influence OM decomposition and nutrient availability, which would contribute to overall restoration of land-leveled soils. Nutrient cycling is driven by microbial communities through the production of extracellular enzymes, which catalyze the decomposition of soil OM (SOM) into inorganic compounds and respond quickly to changes in management (Dick, 2011). Thus, investigation into soil enzyme activities in land-leveled soils would provide information on the general degree of soil health achieved following PL application.

Poultry litter application is promoted for economic and environmental sustainability purposes; however, research into PL application rate on Arkansas land-leveled soils has primarily focused on increasing grain yields (Hardke, 2021). Currently, the University of

Arkansas recommends applying at least 2.25 Mg $ha^{-1}$ to land-leveled fields in order to increase grain yield response (Hardke, 2021); however, little is known about the effect of PL application rate on the restoration of soil biological quality in land-leveled soils. Previous research on PL application rate on soil properties have reported that increased application rates resulted in increased microbial biomass and activity, and increased nutrient-cycling enzyme activities (Acosta-Martinez & Harmel, 2006; Kallenbach & Grandy, 2011). This suggests that applying greater PL rates may stimulate microbial communities and expedite soil health restoration. However, applying greater amounts of PL not only increases the cost of inputs, but also creates the potential for excessive P application, which can lead to water quality concerns. Investigation into poultry litter application rate on Arkansas land-leveled soils is therefore warranted to inform best management practices that balance economic and environmental sustainability.

This study was conducted as a part of a larger study on the use of poultry litter in row crop production on runoff water quality and soil health in the Arkansas Delta. The objective of the present study was to investigate soil physical, chemical, and biological properties related to soil health as affected by various application rates of PL used in combination with different irrigation strategies on recently land-leveled soils. It was hypothesized that increased PL application rates would result in increased nutrient cycling enzyme activities as well as increased biological activity represented by soil respiration. It was also hypothesized that higher PL rates would increase concentrations of soil C and extractable nutrients.

## Materials and Methods

*Site Description*

This study was conducted on a privately-owned farm in Newport, AR that works in cooperation with the University of Arkansas' Discovery Farm Program. This site is in Major Resource Land Area 131A (Southern Mississippi River Alluvium), with an average annual precipitation of 11.3 to 17.2 cm, and an average annual temperature of 14 to 21 ˚C (USDA-NRCS, 2022). Three fields with a soybean cropping history were identified for precision land leveling (Figure 1). Land leveling at this location began and was completed in November 2021, with final grading corrections completed in April 2022. The North field (35°37'21.4" N, 91°19'43.5" W) was approximately 14.16 ha (35 acres) and is mapped to Egam silt loam soil (fine, mixed, active, thermic Cumulic Hapludoll) and Amagon and Forestdale silt loam soils (fine-silty, mixed, active, thermic typic Endoaqualfs) (USDA-NRCS, 2019). The Center field (35°37'09.9" N, 91°19'35.4" W) was 8.09 ha (20 acres) and is also mapped with Egam silt loam and Amagon and Forestdale silt loam soils (USDA-NRCS, 2019). The South field (35°36'58.5" N, 91°19'21.6" W) was approximately 8.09 ha (20 acres) and is mapped as Amagon and Forestdale silt loam, Egam silt loam, and Dexter silt loam soils (fine-silty, mixed, active, thermic Ultic Hapludalfs) (USDA-NRCS, 2019). A control site that measured 0.19 ha (61 m by 30.5 m) was created in the northeast corner of the North field where poultry litter was not applied. The soil is mapped as an Egam silt loam (USDA-NRCS, 2019).

The three rice fields utilized in this study were land leveled in November 2021. The North field was graded with a slope that runs north to south. The maximum depth of cut on this field was 1.1 m, and the maximum fill was 0.76 m. The Central field was graded with a slope

running from west to east, with a maximum cut of 1.5 m and a maximum fill of 0.58 m. The South field was graded with a slope running from south to north, with a maximum cut of 1.2 m and a maximum fill of 0.67 m. Irrigation water was applied in the North field with furrow irrigation, then entered the Central field through a straight levee system before finally entering the South field. The Central and South fields were both flood irrigated for the entirety of the rice growing season in 2022.

*Study Design and Timeline*

The treatments examined in this study consisted of a combination of PL application rate and irrigation method. Poultry litter was broadcast on all three fields in early January 2022 and was left unincorporated on the soil surface. Poultry litter was applied at the following rates: 0 Mg ha-1 (0-ton $ac^{-1}$) in the control plot, 4.5 Mg $ha^{-1}$ (2-ton $ac^{-1}$) in the North field, both of which were combined with furrow irrigation (FUR), and 6.7 Mg $ha^{-1}$ (3-ton $ac^{-1}$) in the Central field, and 9.0 Mg $ha^{-1}$ (4-ton $ac^{-1}$) in the South field, both of which were combined with flood irrigation (FL). Baseline soil samples for this study were collected at the end of January 2022, roughly one month after PL application. Soil samples were collected according to a stratified random sampling scheme, whereby each field was divided into quadrants with four randomized GPS points assigned to each quadrant (Figure 2). Three samples were collected from the top 0-15 cm of soil using a 2.54-cm diameter probe within 2 m of each randomized GPS point and were composited for each quadrant (12 total samples per quadrant for a total of 4 samples per field). Twelve samples were composited into one sample from the top 0-15 cm in the control plot as well.

To prepare for rice planting, straight levees in the Central and South field, and furrows for furrow irrigation in the North field, were constructed in spring 2022. A hybrid long-grain rice

cultivar, RiceTec XP753, was drill seeded in late April 2022 at a rate of 26 kg $ha^{-1}$ in each field. The second sampling date occurred in mid-May 2022, roughly three weeks after rice planting, when rice in the North field was at the one-leaf stage and rice in the Central and South fields was at the two-leaf stage. Urea was applied pre-flood, two weeks after the second sampling date, at a rate of 0.3 Mg $ha^{-1}$. The urea was treated with N(n-butyl) thiophosphoric triamide (NBPT), a urease inhibitor that is commonly marketed under the trade name Agrotain® (Koch Agronomic Services LLC., Wichita, KS). The third and final sampling date occurred on October 11, 2022, two weeks after rice harvest. Climate information from each sampling time is described in Table 1 (NOAA, 2023).

*Poultry Litter Analysis*

Poultry litter samples were collected in January 2022, at the time of application. The litter samples were analyzed for moisture content by drying at 105˚C for 24 hours. Litter samples were also analyzed for pH and EC, and for total N, P, K, and Ca content by inductively coupled plasma (ICP) atomic emission spectroscopy (SPRECTRO CIROS ICP, Fitchburg, MS). The average (n = 2) moisture of the PL was 38.25%, pH was 8.85, and EC was 10.27 dS $m^{-1}$. Analysis showed that the PL contained 27,300 mg $kg^{-1}$ total N, 20,375 mg $kg^{-1}$ $P_2O_5$, 24,500 mg $kg^{-1}$ $K_2O$, and 15,350 mg $kg^{-1}$ Ca.

*Rice Yield Calculation and Soil Sample Analysis*

Rice was harvested in all three fields in late September 2022. Rice grain yields for each field were based on commodity scale weight tickets that were presented on a dry-weight basis. Rice grain yields are expressed in kg $ha^{-1}$.

Soil samples for this study were collected using sterile collection techniques and placed on ice immediately following collection. Once collected, consolidated samples were subsampled and processed according to intended use. Samples were sieved through a 2-mm sieve using a clean sieve and sterilized tools for each new sample. The sieved soil was then divided into two subsamples: roughly 300 g of field-moist soil was placed in the -20˚C freezer, and approximately 300 g was air dried for enzyme activity and $CO_2$ respiration analysis. Mehlich-III P, K, Ca, Mg, S, Fe, Na, Mn, Cu, and Zn were measured after extraction of 1:10 (w:v) soil: Mehlich-III solution and analyzed using inductively coupled plasma (ICP) atomic emission spectroscopy (SPRECTRO CIROS ICP, Fitchburg, MS) (Tucker, 1992). Soil pH and EC were measured with calibrated electrodes and a meter on 1:2 (w:v) soil: water solutions. Total soil C and N were measured by combustion with an Elementar vario MAX cube (Elementar Americas Inc., Ronkonkoma, NY) organic elemental analyzer. Total organic matter (OM) was measured using loss-on-ignition where oven-dry soil samples were placed in pre-weighed crucibles and weighed. Crucibles were then placed in a muffle furnace (Thermo Electron Corporation, Ashville, NC) and were incrementally heated to a maximum temperature of 550˚C. The crucibles containing samples were reweighed following combustion to obtain a final weight representing the mineral (ash) portion of the soil sample. Total OM was calculated by subtracting the final ash weight from the initial oven-dry soil weight, then dividing the result by the oven dry weight (Nelson and Sommers, 1996).

Particle size analysis was completed following the protocol described by Miller & Miller (1987) in which 40 mL of dispersing solution (10 mL of 5% sodium hexametaphosphate, 10 mL of 1.0 M NaOH, 20 mL DI water) was added to 4 g of soil in a 50-mL centrifuge tube. Solutions were placed in an end-over-end shaker overnight at 55 revolutions per minute after which tubes

removed and allowed to settle for 1 hour and 50 min. A 2.5-mL aliquot of suspended clay solution was removed by pipetting 2.5-cm below the surface and depositing in a pre-weighed aluminum tin. Sand was isolated from the remaining contents of the tube with a 53-µm sieve and placed in a separate aluminum tin. All tins were oven-dried overnight at 105℃ and masses were used to determine particle size fraction. The silt portion was calculated by subtracting the sum of the mass of the sand and clay fractions from the mass of the initial soil sample.

Soil samples from the three sampling dates were analyzed for the following enzymes: β-glucosaminadase, β-glucosidase, acid phosphatase, arylsulfatase, and urease. The β-glucosiminadase, β-glucosidase, acid phosphatase, and arylsulfatase assays were measured using fluorometric microplate assays based on 4-methylumbelliferyl substrates as described by Deng et al. (2013) and Deng et al. (2011). Microplates containing soil samples, buffer, and substrate were incubated at 37˚C for one hour before the reaction was terminated using THAM (pH 10). Plates were then read using a Tecan Infinite M200 microplate reader (Tecan Group Ltd., Zurich, Switzerland) at an excitation wavelength of 360 nm and an emission wavelength of 460 nm. Urease activity was measured as described by Cordero et al. (2019). Dichloroisocyanuric acid sodium salt dihydrate was used as a substrate and microplates were incubated at 18˚C for 2 hours. Colorimetric assays were read using a SpectraMax iD3 Multi-Mode Microplate Reader (Molecular Devices, LLC, San Jose, CA, USA) at an absorbance of 650 nm.

In addition to enzyme activity, soil respiration ($CO_2$) was measured using the Solvita® Soil $CO_2$ Respiration kit (Woods End Laboratories Inc., Mt Vernon, ME). Carbon dioxide was evaluated using the burst method wherein 30 cc of air-dry soil (< 3% moisture) was placed in a small beaker, which was then placed into a standardized 475-mL sample jar with an air-tight

gasket lid. Deionized water was carefully added to the surface of the soil (9 mL) so that water evenly dispersed and rapidly equilibrated downward. A $CO_2$ probe containing colorimetric gel was placed into the moist soil and the sample jar lid was closed. Samples were incubated for 24 hours at 21˚C. At 24 hours, $CO_2$ detector probes were read by the Solvita® Digital Color Reader and results were interpreted according to Solvita® Response Guidelines.

*Statistical Analysis*

Analysis of variance (ANOVA) was conducted to evaluate the effects of sampling time and treatments (PL application rate and irrigation method) and their interactions on soil health properties in a split-plot design using R Studio version 4.2.3 (R Core Team, 2022). Irrigation method combined with PL rate was a whole-plot factor and sampling date was a split-plot factor. All explanatory variables were considered fixed effects. Soil samples from the control plot were excluded from statistical analyses because only one composite sample was collected at each time point, thus degrees of freedom in means at each date limited statistical analysis. However, measurements collected from the control are still included in figures and tables for visual comparison. When appropriate, means were separated using Tukey's HSD test at an alpha value of 0.05 with the "agricolae" package version 1.4.0 (de Mendiburu & Yaseen, 2020) in R Studio. Pearson's correlation coefficients were calculated using the "Hmisc" package version 5.1-0 (Harrell Jr., 2023) in R Studio to determine relationships between soil enzyme activities and soil pH, EC, M3P, M3S, OM, POXC, $CO_2$, and particle size fractions at a significance level of $p < 0.05$.

## Results

*Poultry Litter Rate Effects on Rice Yield*

Rice yield for the 2022 season was numerically greater in the fields that received 6.7 and 9.0 Mg $ha^{-1}$ PL application rates (8,873 and 8,583 kg $ha^{-1}$, respectively) compared to yield under the 4.5 Mg $ha^{-1}$ application rate (7,485 kg $ha^{-1}$) (data not shown).

*Poultry Litter Rate Effects on Soil Enzyme Activities and Respiration*

Significant interactions between treatments of PL rate and irrigation method and sampling time were detected for β-D glucosidase ($p < 0.001$), acid phosphatase ($p < 0.001$), and arylsulfatase activities ($p < 0.001$) (Table 2). The β-D glucosidase activities were not different within any treatment from January to May, then significantly increased from May to October for all treatments, with activities under the 4.5 Mg $ha^{-1}$ + FUR and 6.7 Mg $ha^{-1}$ + FL treatments more than doubling, and activities under the 9.0 Mg $ha^{-1}$ + flood irrigation increasing by a factor of 10 (Fig. 3A). Changes in β-D glucosidase activities between treatments over the sampling period differed in May, when β-D glucosidase activities in the 6.7 Mg $ha^{-1}$ + FL treatment were greater than under the 9.0 Mg $ha^{-1}$ + FL treatment in January or May. General trends in enzyme activities in soil receiving no PL + FUR showed β-D glucosidase activities followed the same general seasonal change observed in the PL treatments, regardless of irrigation method, with similar values observed across sampling times, including a numerical increase from May to October.

Although PL + irrigation treatments did not result in significant differences in β-glucosaminidase and urease activities (Table 2), β-glucosaminidase activities showed a similar seasonal pattern to what was observed for β-D glucosidase activities (Fig. 1B) and the inverse (decline in activities in October) for urease activities. β-glucosaminidase activities were greatest in October (3.25 nmol MUF $kg^{-1}$ $hr^{-1}$) at the end of the rice season compared to January and May

(1.18 and 1.00 nmol MUF $kg^{-1}$ $hr^{-1}$, respectively). The control plot showed lower β-glucosaminidase activities compared to the PL + irrigation treatments in January and May, but then increased in October to a level that was similar to the activities observed under the PL + irrigation treatments. In contrast, urease activities measured 1.21 e-05 g $NH_4$ $kg^{-1}$ $hr^{-1}$ October compared to 1.08 e-05 g $NH_4$ $kg^{-1}$ $hr^{-1}$ in January. Urease activities in the control plot were consistently lower than the PL + irrigation treatments at each sampling date and did not follow the trend of decreasing activities measured over time as observed within PL + irrigation treatments (Fig. 3D).

Acid phosphatase activities showed less seasonal change; in May acid phosphatase activities in soil under the 4.5 Mg $ha^{-1}$ + FUR treatment increased 122% from January, then decreased in October to a level that was not different from activities measured in January (Fig. 3C). Acid phosphatase activities in May under the 4.5 PL Mg $ha^{-1}$ + FUR treatment were also more than double the estimated activities observed under the 6.7 Mg $ha^{-1}$ PL + FL and 9.0 PL Mg $ha^{-1}$ + FL treatments at all time points except October in the 6.7 Mg $ha^{-1}$ PL + FL treatment. Activities within the 6.7 Mg $ha^{-1}$ + FL and 9.0 Mg $ha^{-1}$ + FL treatments did not change over the sampling period. Acid phosphatase activities in the control showed consistent numerical decrease over the sampling period, and while they were greater than all PL + irrigation treatments in January, by October, acid phosphatase activities in the control were less than measured in all PL + irrigation treatments.

Arylsulfatase activities did not change over the sampling period within the 4.5 Mg $ha^{-1}$ PL + FUR and 9.0 Mg $ha^{-1}$ PL + FL treatments but more than doubled from January to May in the 6.7 Mg $ha^{-1}$ PL + FL treatment. Arylsulfatase activities in the 6.7 Mg $ha^{-1}$ PL + FL treatment then remained elevated into October, thereby maintaining activities that were greater than initial

activities in January and were greater than activities in January and May soil that received the 4.5 Mg $ha^{-1}$ PL + FUR treatment (Fig. 3E). Similar to acid phosphatase, arylsulfatase activities in the control showed a consistent numerical decrease over the sampling period. When compared to the PL + irrigation treatments, arylsulfatase activities in the control plot were greater in January but had decreased in October to a level that was less than all PL + irrigation treatments.

Respiration at all sampling times typically fell within the low to moderately low biological activities classification designation of Solvita (Mount Vernon, ME) and ranged from 84 to 38.8 $mg^{-1}$ $kg^{-1}$ $d^{-1}$. Soil respiration was influenced by sampling time when all PL + irrigation treatments were averaged ($p = 0.001$, Table 2). Respiration was lowest in May (11.97 ± 0.92 $mg^{-1}kg^{-1}d^{-1}$) during early rice establishment compared to January and October (20.38 ± 2.19 - and 18.93 ± 1.41 $mg^{-1}kg^{-1}d^{-1}$, respectively, Table 4). Soil respiration was not affected by treatment as a main effect or interaction with time (Table 2).

*Poultry Litter Rate and Sampling Time Effects on Soil Physiochemical Properties*

A significant PL application rate + irrigation by sampling time effect was detected for soil EC ($p = 0.005$), M3P ($p = 0.005$), M3K ($p = 0.018$), M3Mn ($p = 0.049$), and M3Zn ($p = 0.029$) (Table 2). The EC was greatest in treatments that received the 4.5 Mg $ha^{-1}$ PL + FUR treatment in January as compared to soil EC under all PL + irrigation treatments and sampling times, except for the 6.7 Mg $ha^{-1}$ PL + FL treatment in January (Fig. 4). Soil EC was not different between PL + irrigation treatments at any other time. In the control plot, soil EC remained consistent over the three sampling dates and ranged from 0.968 to 1.02 dS $m^{-1}$.

Soil M3P concentrations were greater in the 4.5 Mg $ha^{-1}$ PL + FUR compared to the 6.7 Mg $ha^{-1}$ PL + FL and 9.0 Mg $ha^{-1}$ PL + FL treatments in January and May and the 9.0 Mg $ha^{-1}$ PL + FL treatment in October (Fig. 5A). Soil that received the 6.7 Mg $ha^{-1}$ PL + FL treatment

had greater M3P in October as compared to the 6.7 and 9.0 Mg $ha^{-1}$ + FL treatments in May. Soil M3P in the control was not different from the concentrations observed under the 6.7 and 9.0 Mg $ha^{-1}$ PL + FL treatments in January and May and the 9.0 Mg $ha^{-1}$ Pl + FL treatment at the final sampling date in October.

All three PL application rates, regardless of irrigation method, showed a significant decrease in M3K and M3Mn concentration from January to May, then did not change within a PL rate from May to October (Fig. 5B, Fig. 5C). The 4.5 Mg $ha^{-1}$ + FUR treatment resulted in the greatest M3K and M3Mn concentration in January compared to the same rate at other sampling times and both the 6.7 and 9.0 Mg $ha^{-1}$ + FL treatments during all sampling times. No differences in PL rate + irrigation on M3K concentration were detected in October. Soil M3K and M3Mn concentrations in the control plots decreased from January to May and increased from May to October (Fig. 5B, Fig. 5C). Soil M3Zn concentrations were more consistent over the course of the season with few differences except in October when M3Zn in the 6.7 Mg $ha^{-1}$ PL + FL was more than double that under the 9.0 Mg $ha^{-1}$ PL + FL treatment (Fig. 5D).

All soil chemical properties changed over the sampling period, with the exception of soil pH and M3Ca (Table 2). Soil EC, M3Mg, and M3S concentrations were greatest in January compared to the other sampling times (Table 4). Soil M3P and M3Fe concentrations decreased from January to May, then increased again in October to concentrations that were not different from the initial concentration in January. M3Mn also followed the trend of decreasing from January to May, then increasing from May to October; however, the increase observed in October was lower than what was observed in January (Table 4).

Significant treatment by time interactions were not detected for any measurement of soil carbon or nitrogen. However, treatment as a main effect was significant for all soil carbon and

nitrogen measurements, including total OM, POXC, TC, TN, and C:N ratios (Table 3). The 9.0 Mg $ha^{-1}$ PL + FL treatment consistently resulted in the lowest OM, TC, TN, and C:N ratio compared to both of the lesser PL rates and the control plot (which received no PL), regardless of irrigation method. Furthermore, for all measurements of soil carbon and nitrogen, no differences were detected between the 4.5 Mg $ha^{-1}$ PL + FUR and 6.7 Mg $ha^{-1}$ PL + FL treatments, and the control plot showed concentrations that were consistently greater than or equal to that observed under all PL + irrigation treatments (Table 3). The POXC concentration was the exception, as the POXC concentration observed under the 4.5 Mg $ha^{-1}$ PL + FUR and 6.7 Mg $ha^{-1}$ PL + FL treatments were greater than the concentration measured in the control soil. Total OM and C:N ratio began to increase by the end of the rice season in October, and TN began to decrease in May then remained the same into October (Table 4).

Particle size analysis showed that while all fields used in this study fell into the silt loam category, the percentage of sand and silt varied between fields, while the clay percentage was not different across locations. The percentage of sand was greatest in the field where 9.0 Mg $ha^{-1}$ rate of PL was applied (8.69 ± 0.90%) in combination with FL irrigation compared to the field where the 4.5 Mg $ha^{-1}$ PL application rate was applied (4.11 ± 1.20%) in combination with FUR irrigation, which also contained the control plot (1.74 ± 0.48%) (Table 3). In contrast, the percentage of silt was greatest in the field where 4.5 Mg $ha^{-1}$ PL + FUR (93.60 ± 1.20%) had been applied compared to the field that received 9.0 Mg $ha^{-1}$ PL + FL (89.09 ± 0.85%). The percentage of clay ranged from 2.10% to 2.29% in all fields.

*Soil Property Relationships by Application Rate*

Pearson correlations showed relationships between enzyme activities and soil properties based on each PL application rate + irrigation treatment (Table 5). Total OM was positively

correlated with β-D glucosidase activities in soil that received all three PL application rates, regardless of irrigation method, but POXC was positively related to β-D glucosidase only in the 6.7 Mg $ha^{-1}$ PL + FL treatment. Total OM and M3P were positively correlated with β-glucosaminidase activities in soil that received the 4.5 Mg $ha^{-1}$ PL + FUR and 6.7 Mg $ha^{-1}$ PL + FL treatments, but not the 9.0 Mg $ha^{-1}$ PL + FL treatment. Soil $CO_2$ and POXC were positively related to β-glucosaminidase but only under the 6.7 Mg $ha^{-1}$ PL + FL treatment, and soil $CO_2$ was positively correlated with acid phosphatase in the field receiving the 9.0 Mg $ha^{-1}$ PL + FL treatment. Total OM had an inconsistent relationship with acid phosphatase, such that activities were negatively correlated in the field receiving the 4.5 Mg $ha^{-1}$ PL + FUR treatment, not related in the field receiving the 6.7 Mg $ha^{-1}$ PL + FL treatment, and positively related in the field receiving the 9.0 Mg $ha^{-1}$ PL + FL treatment. Acid phosphatase activities under the 4.5 Mg $ha^{-1}$ PL + FUR treatment was also positively related to sand and silt content, whereas clay was positively related to β-glucosaminidase under the 9.0 Mg $ha^{-1}$ PL + FL treatment. Arylsulfatase activities showed a negative relationship with soil EC in the same and under the 6.7 Mg $ha^{-1}$ PL + FL treatment. Overall, there were more positive correlations between enzymes and other soil properties under the 6.7 Mg $ha^{-1}$ PL + FL treatment compared to 4.5 Mg $ha^{-1}$ PL + FUR and 9.0 Mg $ha^{-1}$ PL + FL treatments.

## Discussion

### *Poultry Litter Rate Effects on Rice Yield*

Higher rice yields were observed under the two highest PL application rates combined with flood irrigation compared to the lowest PL rate, which was combined with furrow irrigation. This is consistent with previous studies on PL rate on precision graded rice production, which found that rice yield increased with increasing application rates ranging from 1.1 Mg $ha^{-1}$ to 4.5 Mg $ha^{-}$

[1] (Hardke et al., 2021). The results of this study are also consistent with previous research on furrow irrigated rice compared to flood irrigation; in Arkansas, it is expected that furrow irrigation may result in a roughly 10% yield reduction compared to flooded rice production (Hardke et al., 2020). Differentiating between irrigation method and PL rate as the source of yield difference in this study is not straightforward; however, it is possible that the lower yield observed in the field that received 4.5 Mg $ha^{-1}$ PL + FUR could be a combined result of irrigation water management and lower PL application rate.

*Poultry Litter Rate Effects on Soil Enzyme Activity and Respiration*

β-D glucosidase and β-glucosaminidase activities measured in this study were consistent with the general range in activities observed in silt loam and loam soils under agricultural management with roughly the same organic matter content (between 2 and 3%) (Deng et al., 2013). It was hypothesized that enzyme activities would increase as poultry litter application rates increased; however, this hypothesis did not fit the observed β-D glucosidase and β-glucosaminidase activities at any sampling time. In fact, β-D glucosidase activities were largely not different between poultry litter rates, apart from the May sampling date, which showed lower activity under the greatest poultry litter rate compared to the medium rate (6.7 Mg $ha^{-1}$). This difference was observed under fields receiving the same irrigation method (FL), indicating that irrigation method was not a likely source of variation in β-D glucosidase activities between fields in May. These results suggest that the 6.7 Mg $ha^{-1}$ PL rate may have resulted in a more optimal balance of C and N sources for the microbial population present in May. β-D glucosidase activity is associated with the hydrolysis of cellobiose and cellodextrins into glucose, thus any soil C pool containing cellulosic compounds can serve as a substrate for this enzyme (Deng & Popova, 2011). Increasing organic matter content, therefore, stimulates enzyme production by microbial

communities and promotes the buildup of extracellular enzymes over time as enzymes form complexes with humic colloids (Dick, 2011). Poultry litter is an organic amendment, and increasing the amount of poultry litter added to the soil should be reflected in the amount of labile C in the first year after application. However, in this study all measurements of soil C including OM, POXC, TC, TN, and C:N ratio were lowest under the highest application rate, which may have contributed to the lower β-D glucosidase activity observed in this study under the 9.0 Mg $ha^{-1}$ application rate. Pearson correlations showed that β-D glucosidase activity was positively correlated with OM under each poultry litter application rate but was not consistently related to POXC concentration. Previous studies have shown positive relationships between carbon cycling enzyme activity and POXC, which was generally attributed to the microbial availability of POXC (Liptzin et al., 2020; Margenot et al., 2017). The results of this study may reflect the narrow scope of POXC compared to total OM, as total OM measures labile, intermediate, and stable forms of soil C and is therefore a broader representation of available substrate for enzyme activity. Furthermore, Hurissa et al. (2016) reported that POXC shows stronger associations with the long-term buildup of organic matter compared to the short-term mineralization of organic matter, which may support the inconsistencies in relationships between POXC and C cycling enzyme activities observed in this study, as enzyme activity is a measurement of soil decomposition capability.

β-glucosaminidase activities did not vary by poultry litter rate in this study, regardless of irrigation method. These results support the findings of Acosta-Martinez and Harmel (2006), who reported that β-D glucosaminidase activities were not significantly different between cultivated soils receiving poultry litter rates of 4.5, 6.7, and 9.0 Mg $ha^{-1}$ until the fourth consecutive annual litter application. The lack of immediate response in β-D glucosaminidase

activities to poultry litter rate was attributed to the recalcitrant nature of the substrate, chitin, to decomposition, which may also be the case in this study. Relationships between β-glucosaminidase activity and total OM and POXC were similar to those observed with β-D glucosidase activity, which is expected as both enzymes act on prevalent carbon inputs into soil.

Urease activity in this study was minimal compared to the expected range of 1.46 to 200 mg $NH_4$ $kg^{-1}$ $hr^{-1}$ activities for agricultural soils (Nannipieri et al., 2002; Kandeler & Dick, 2006). The disparity in urease activities from previous research and the activities observed in this study could result from differences in assay methods and differences in soil type (Deng, 2017). It is also possible that low urease activities at this site were influenced by samples collected from areas of exposed subsoil resulting from the land leveling, as enzyme activities are known to decrease with depth (Tabatabai, 1996). In addition to consistently low activity over the sampling period, urease activities did not vary by poultry litter application rate and irrigation method. These results contrasted with Fereidooni et al. (2013), who reported that the highest PL application rate of 0.3 Mg N $ha^{-1}$, which would correspond with the 6.7 Mg $ha^{-1}$ rate in the current study, resulted in the greatest urease activities in low organic matter agricultural soils, which was attributed to the relative abundance of ureolytic microorganisms with increasing PL. This suggests that populations of ureolytic microorganisms in the soils of this study may have been reduced, such that even the addition of biologically active PL did not influence urease production.

Acid phosphatase activities in this study were greater than what was reported by Deng et al. (2013) by a factor of 10. The fertility management of the soils used by Deng et al. (2013) is not stated; however, the addition of poultry litter has been reported to increase phosphatase activities and may account for the elevated activities observed in this study (Acosta-Martinez &

Harmel, 2006; Tomlinson et al., 2008). Furthermore, the consistently low M3P concentrations measured in this study may have contributed to elevated acid phosphatase activities, as production of acid phosphatase is known to increase when inorganic phosphorus is limited (Acosta-Martinez & Tabatabai, 2011). Similar to β-D glucosidase activity, acid phosphatase activities were only different between poultry litter rates in May, when the greatest activities were detected under the lowest poultry litter rate combined with furrow irrigation. This was likely not a result of available inorganic phosphorus, as M3P concentrations were not different between treatments in May, and again, were consistently low across all application rates. Irrigation method may have influenced the observed inconsistencies in acid phosphatase activities between treatments as the two flood-irrigated treatments resulted in lower activity. This may have been a result of variability in microbial community composition under varying soil moisture conditions. Furthermore, while not measured in this study, other studies have reported changes in acid phosphatase activities by microbial community composition, with greater acid phosphatase in soils with high fungal populations (Acosta-Martinez & Tabatabai, 2011; Dighton, 1983).

Arylsulfatase activities in this study were also greater than what has been previously reported for soils under conventional agricultural management (Maharjan et al., 2017). Arylsulfatase is associated with the hydrolysis of ester sulfate into sulfate, and similar to phosphatases, is known to be inhibited by the abundant availability of sulfates in the soil (Klose et al., 2011). However, unlike M3P and acid phosphatase activities in this study, available inorganic sulfur was never below what would be considered deficient (< 10 mg $kg^{-1}$ soil), which suggests that factors outside of sulfate availability could be influencing arylsulfatase activities. Extracellular enzymes are known to stabilize with clay and humic colloids and can persist in the

soil for long periods of time (Dick, 2011); thus, measurements of enzyme activity not only indicate current soil conditions, but also reflect past conditions. In this study, elevated arylsulfatase activities in the presence of sufficient extractable sulfur may be due to the inclusion of accumulated stabilized arylsulfatase enzymes in the measurement of estimated activity. While poultry litter application rate and irrigation method did result in differences in arylsulfatase activities in this study, these differences did not follow a consistent trend by rate or time. Previous studies have shown that arylsulfatase activities will increase with increasing temperature up to 57˚C as microbial activity increases under warmer temperatures (Klose et al., 2011). However, a seasonal increase in arylsulfatase activities from January to May was only observed under the 6.7 Mg $ha^{-1}$ PL + FL treatment, which may reflect differences in microbial community composition between fields, as other contributing factors such as pH, readily available carbon, and available sulfate were not different between PL + irrigation treatments over the sampling period.

Soil enzyme activities reflect the decomposition capability of a soil, and similarly, soil respiration rate reflects the level of activity of soil organisms that are involved in organic matter decomposition (Vargas et al., 2011). In this study, soil $CO_2$ consistently fell within the range of "low biological activity" as defined by the Solvita® soil respiration method across sampling dates and PL + irrigation treatments. These results are unsurprising, as land leveling has been reported to result in decreased microbial biomass due to the sensitivity of bacterial and fungal communities to extensive disturbance and to the exposure of biologically inactive subsoil (Brye et al., 2003). Furthermore, soil respiration is also known to be driven by the availability of labile carbon substrate (Wang et al., 2003). As POXC concentrations were also consistently low across PL + irrigation treatments and sampling dates compared to the expected range (200 – 1000 mg

$kg^{-1}$) for conventional agricultural soils, low soil respiration would be expected (Calderon et al., 2017).

*PL Rate and Sampling Time Effects on Soil Physiochemical Properties*

Soil macronutrients measured in this study fell within the acceptable ranges for crop production with the exception of M3P, which was considered low across all sampling dates and treatments. Moreover, increased PL rate did not result in greater M3P concentration at any time and was even greatest under the lowest application rate (4.5 Mg $ha^{-1}$) combined with FUR irrigation in January. This was unexpected, as PL is considered a valuable source of phosphorus that can result in excessive soil phosphorus accumulation with repeated applications (Bolan et al., 2010). Furthermore, these results contrast with the findings of Brye et al. (2004), who reported that M3P increased on land leveled soils following poultry litter application. While 90 to 100% of the total phosphorus in PL is generally available within in the season of application, it is possible that the observed low biological activity within the recently land leveled soils of this study may have resulted in slower decomposition and therefore hampered the release of inorganic phosphorus from PL (Espinoza et al., 2007). This theory also supports the seasonal M3P results of this study, which showed no change in concentration between the January and October sampling dates under all application rates and irrigation methods. Plant uptake over the course of the growing season would typically be far less than the amount of inorganic P released from PL, which would result in a net increase in P concentration over the season (Sharpley et al., 2010). However, if PL decomposition was slowed due to reduced biological activity, then the amount of inorganic P released from the PL may have been balanced with the amount of P taken up by the rice crop, resulting in no change in soil P over the growing season.

In contrast to M3P, M3K concentrations followed an overall decreasing trend from January to May in the soils receiving the 4.5 Mg $ha^{-1}$ PL + FUR treatment and the 9.0 Mg $ha^{-1}$ PL + FL treatments. This occurred under both irrigation methods, indicating that irrigation method was not likely to have been the cause of seasonal M3K concentration decrease. Instead, these results may have been a function of plant uptake, leaching, or a combination of both, as rice takes up large quantities of K to achieve optimal growth and yield (about 0.22 Mg $ha^{-1}$ over one season), and potassium is mobile in the soil and would be expected fluctuate with flood status (Slaton et al., 2011). Furthermore, soil K primarily originates from mineral sources rather than organic sources, thus M3K would not necessarily be expected to increase with PL decomposition and instead would be greatest following application (Soumare et al., 2023). In concurrence with M3P results, M3K concentrations under the 4.5 Mg $ha^{-1}$ PL + FUR treatment were greater than the concentrations observed under the two higher PL application rates combined with FL irrigation in January. Furthermore, while not affected by treatment by time interactions, M3Ca and M3Mg concentrations were also greatest in soils under the lowest application rate combined with FUR irrigation. The greater macronutrient concentration in soils that received the 4.5 Mg $ha^{-1}$ PL + FUR treatment may be more of a function of spatial variability than PL rate, as greater PL rates should result in greater nutrient concentrations. In addition, the furrow irrigation utilized under this treatment would have resulted in less water moving through the system, potentially leading to less nutrient leaching. Thus, differences in ratios of cut and filled areas, as well as differences in the amount of water moving through the soil could have influenced the nutrient statuses observed in this study.

Most soil micronutrient concentrations were not considered limiting at any time during the study, except for M3Zn, which was suboptimal in October in soils that received the 4.5 Mg

$ha^{-1}$ PL + FUR and 9.0 Mg $ha^{-1}$ PL + FL treatments. Zinc deficiency in precision leveled fields is a common occurrence as Zn-deficient subsoils are brought to the surface (Hardke, 2021). However, the fact that M3Zn concentrations did not reach deficient levels in those fields until October suggests that plant uptake, while trace, likely lead to the reduced M3Zn concentration (Marschner, 1993). Similar to M3K and M3P, M3Mn was greatest under the 4.5 Mg $ha^{-1}$ PL + FUR treatment in January, which again, may be a reflection of differences in M3Mn prior to the start of the study, differences in the distribution of cut and filled soils, or differences in irrigation management.

The use of PL on land-leveled soil in Arkansas is promoted both as a source of essential nutrients and as a biologically active source of organic matter that can restore soil function over time (Hardke, 2021). In a meta-analysis on the effect of organic amendments (including PL) on soil carbon pools, Kallenbach & Grandy (2011) found that increased application rates resulted in increased microbial carbon and nitrogen. Acosta-Martinez and Harmel (2006) also reported that higher rates of PL resulted in greater microbial carbon, and Adeyemo et al. (2018) found greater total organic matter with increased PL application rates compared to no poultry litter on degraded soils. Thus, it was expected that soil carbon, especially labile carbon as represented by POXC, would increase with increasing PL rates. However, the results of this study showed that all measurements of soil carbon including OM, POXC, TC, TN, and C:N ratio were consistently lowest in the soils that received the greatest (9.0 Mg $ha^{-1}$) PL + FL treatment compared to the 4.5 Mg $ha^{-1}$ PL + FUR and 6.7 Mg $ha^{-1}$ PL + FL treatments. Furthermore, all measurements of soil carbon in the control soils (0 Mg $ha^{-1}$ PL + FUR) were numerically greater than what was observed under the 9.0 Mg $ha^{-1}$ PL + FL treatment, which suggests that the preexisting variability from land leveling may have had a greater influence on soil C than PL rate, regardless

of irrigation method, in the first year following application. These results indicate that more than one year of PL application may be required to overcome the reduction in biological activity caused by the significant disturbance of land leveling and to restore full functioning in these highly disturbed soils, which is consistent with the current University of Arkansas Division of Agriculture recommendation of applying sequential annual PL application in order to restore soil quality (Hardke et al., 2021).

## Conclusion

In Arkansas, the application of PL to land-leveled soils is promoted to reclaim soil quality and grain yields following extensive disturbance and degradation. In this study, rice yields showed a response to PL application rate and irrigation method in the first year, with greater PL application rates combined with flood irrigation resulting in greater yields than the lowest PL rate combined with furrow irrigation. Soil quality parameters, on the other hand, did not show a consistent benefit in applying higher PL rates in the first year following application. Dynamic properties, such as enzyme activity and POXC concentration, often showed few differences between soils receiving the highest and lowest PL application rate, regardless of irrigation management. These results suggest that achieving observable differences in application rate on soil properties, even dynamic properties, likely requires consecutive annual applications. However, consecutive application of PL at high rates over the long-term may pose a risk to water quality, as the potential for over application of phosphorus increases. Thus, more research on PL application rate on land-leveled soils receiving varying irrigation methods is necessary to optimize soil restoration while ensuring environmental and economic sustainability.

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**Tables and Figures**

**Table 1.** Monthly average temperature and precipitation data for each sampling time for Jackson County, AR. Data were obtained from the U.S. NOAA (2023).

| | Sampling Time | | |
|---|---|---|---|
| | **January 2022** | **May 2022** | **October 2022** |
| Average temperature (˚C) | 2.33 | 20.8 | 15.5 |
| Maximum temperature (˚C) | 8.11 | 26.1 | 23.4 |
| Minimum temperature (˚C) | -3.44 | 15.5 | 7.61 |
| Average precipitation (cm) | 15.0 | 17.7 | 7.29 |

**Table 2.** ANOVA p values for the effect of irrigation plus poultry litter application rate and sampling time on soil health properties.

| Property | Treatment | Time | Treatment x Time |
|---|---|---|---|
| pH | **<0.001[a]*** ** | 0.682 | 0.858 |
| EC[b] (dS $m^{-1}$) | **<0.001*** ** | **<0.001*** ** | **0.005** ** |
| *(nmol MUF $kg^{-1}$ soil)* | | | |
| β-D Glucosidase | 0.076 | **<0.001*** ** | **<0.001*** ** |
| β-Glucosaminidase | 0.23 | **<0.001*** ** | 0.055 |
| Acid Phosphatase | **0.002** ** | **0.022*** | **<0.001*** ** |
| Arylsulfatase | **0.036*** | **<0.001*** ** | **<0.001*** ** |
| *(mg $NH_4$ $kg^{-1}$ hr)* | | | |
| Urease | 0.272 | **0.008** ** | 0.791 |
| *(mg $kg^{-1}$)* | | | |
| M3P | **<0.001*** ** | **<0.001*** ** | **0.005** ** |
| M3K | **<0.001*** ** | **<0.001*** ** | **0.018*** |
| M3Ca | **<0.001*** ** | 0.070 | 0.257 |
| M3Mg | **<0.001*** ** | **<0.001*** ** | 0.411 |
| M3S | 0.194 | **<0.001*** ** | 0.129 |
| M3Na | ND[c] | ND | ND |
| M3Fe | 0.589 | **<0.001*** ** | 0.111 |
| M3Mn | **<0.001*** ** | **<0.001*** ** | **0.049*** |
| M3Cu | 0.930 | **0.024*** | 0.109 |
| M3Zn | **0.007** ** | 0.404 | **0.029*** |
| M3B | ND | ND | ND |
| *Soil Carbon Fractions* | | | |
| OM (%) | **<0.001*** ** | **<0.001*** ** | 0.19 |
| POXC (mg $kg^{-1}$) | **0.004** ** | 0.485 | 0.057 |
| TC (%) | **<0.001*** ** | 0.303 | 0.120 |
| TN (%) | **<0.001*** ** | **0.002** ** | 0.167 |
| C:N | **<0.001*** ** | **<0.001*** ** | 0.069 |
| *Soil Respiration ($mg^{-1}kg^{-1}d^{-1}$)* | | | |
| $CO_2$ | 0.056 | **0.001** ** | 0.438 |
| *Particle Size Fractions (%)* | | | |
| Sand | **0.006** ** | **0.019*** | 0.844 |
| Silt | **0.009** ** | **0.043*** | 0.841 |
| Clay | 0.882 | **0.008** ** | 0.675 |

[a]Means followed by a common letter between treatment columns are not significantly different by Tukey's HSD test (*$p < 0.05$, **$p < 0.01$, *** $p < 0.001$).

[b]Electrical conductivity (EC), Mehlich-3 phosphorus (M3P), potassium (M3K), calcium (M3Ca), magnesium (M3Mg), sulfur (M3S), sodium (M3Na), iron (M3Fe), manganese (M3Mn), copper (M3Cu), zinc (M3Zn), boron (M3B), organic matter (OM), permanganate oxidizable carbon (POXC), total carbon (TC), total nitrogen (TN), and carbon: nitrogen ratio (C:N).

[c]Not Detected (ND). Limit of Detection for M3Na = 179 mg $kg^{-1}$ and M3B = 33.9 mg $kg^{-1}$

**Table 3.** Mean soil properties (± SE) under each treatment, averaged across January, May, and October sampling dates (n=12). The control plot (0 Mg $ha^{-1}$ + FUR) was not included in statistical analysis but is included in the table for comparison.

| | | Treatment | | | |
|---|---|---|---|---|---|
| **Analysis** | **P value** | **0 Mg $ha^{-1}$ PL + FUR[a]** | **4.5 Mg $ha^{-1}$ PL + FUR** | **6.7 Mg $ha^{-1}$ PL + FL** | **9.0 Mg $ha^{-1}$ PL + FL** |
| pH | **<0.001[b]*** ** | 6.55 ± 0.04 | 5.90± 0.07 a | 5.69 ± 0.10 a | 5.13 ± 0.06 b |
| EC[c] (dS $m^{-1}$) | **<0.001*** ** | 0.100 ± 0.001 | 0.112 ± 0.001 a | 0.107 ± 0.005 a | 0.852 ± 0.004 b |
| *(mg $kg^{-1}$ soil)* | | | | | |
| M3P | **<0.001*** ** | 6.70 ± 0.65 | 10.48 ± 0.68 a | 8.24 ± 1.02 b | 6.62 ± 0.69 b |
| M3K | **<0.001*** ** | 67.74 ± 4.23 | 71.89 ± 5.31 a | 62.81 ± 3.27 b | 56.24 ± 3.86 b |
| M3Ca | **<0.001*** ** | 1306 ± 73.03 | 1065 ± 50.69 a | 863.8 ± 31.51 b | 691.3 ± 48.89 c |
| M3Mg | **<0.001*** ** | 230.1 ± 11.36 | 190.9 ± 8.18 a | 155.9 ± 5.93 b | 169.5 ± 7.16 b |
| M3S | 0.194 | 12.14 ± 0.86 | 13.19 ± 0.68 | 12.88 ± 0.61 | 12.01 ± 0.76 |
| M3Na | ND[d] | ND | ND | ND | ND |
| M3Fe | 0.589 | 62.16 ± 21.00 | 77.92 ± 7.10 | 88.39 ± 13.55 | 78.45 ± 11.94 |
| M3Mn | **<0.001*** ** | 43.02 ± 7.86 | 85.97 ± 8.26 a | 53.51 ± 6.18 b | 64.97 ± 5.97 b |
| M3Cu | 0.930 | 2.58 ± 1.16 | 2.81 ± 0.62 | 2.53 ± 0.46 | 2.73 ± 0.72 |
| M3Zn | **0.007**** | 2.24 ± 0.50 | 3.92 ± 0.40 a | 4.26 ± 0.61 a | 2.36 ± 0.34 b |
| M3B | ND | ND | ND | ND | ND |
| *Soil Carbon Fraction* | | | | | |
| OM (%) | **<0.001*** ** | 2.73 ± 0.11 | 2.47 ± 0.06 a | 2.34 ± 0.10 a | 1.86 ± 0.09 b |
| POXC (mg $kg^{-1}$ soil) | **0.004**** | 61.42 ± 31.39 | 132.8 ± 41.64 a | 148.6 ± 15.74 a | 28.38 ± 13.02 b |
| TC (%) | **<0.001*** ** | 1.05 ± 0.01 | 1.02 ± 0.04 a | 0.94 ± 0.04 a | 0.67 ± 0.05 b |
| TN (%) | **<0.001*** ** | 0.10 ± 0.00 | 0.11 ± 0.00 a | 0.10 ± 0.00 a | 0.07 ± 0.00 b |
| C:N | **<0.001*** ** | 10.85 ± 0.06 | 9.63 ± 0.25 a | 9.46 ± 0.27 a | 8.69 ± 0.32 b |
| *Soil Respiration ($mg^{-1}kg^{-1}d^{-1}$)* | | | | | |
| $CO_2$ | 0.056 | 12.30 ± 2.15 | 19.45 ± 2.08 | 17.66 ± 1.32 | 14.16 ± 1.99 |
| *Particle size (%)* | | | | | |
| Sand | **0.006**** | 1.74 ± 0.48 | 4.11 ± 1.20 b | 7.72 ± 0.97 a | 8.69 ± 0.90 a |
| Silt | **0.009**** | 96.16 ± 0.48 | 93.60 ± 1.20 a | 90.11 ± 1.01 b | 89.09 ± 0.85 b |
| Clay | 0.882 | 2.10 ± 0.03 | 2.29 ± 0.20 | 2.22 ± 0.13 | 2.17 ± 0.25 |

[a] Poultry litter (PL), Furrow irrigated (FUR), flood irrigated (FL)

[b]Means followed by a common letter between treatment columns are not significantly different by Tukey's HSD test at the 0.05 significance level. Columns without letters indicate no significant difference (*p < 0.05, **p < 0.01, *** p < 0.001).

[c]Electrical conductivity (EC), Mehlich-3 phosphorus (M3P), potassium (M3K), calcium (M3Ca), magnesium (M3Mg), sulfur (M3S), sodium (M3Na), iron (M3Fe), manganese (M3Mn), copper (M3Cu), zinc (M3Zn), boron (M3B), organic matter (OM), permanganate oxidizable carbon (POXC), total carbon (TC), total nitrogen (TN), carbon: nitrogen ratio (C:N).

[d]Not Detected (ND). Limit of Detection for M3Na = 179 mg $kg^{-1}$ and M3B = 33.9 mg $kg^{-1}$

**Table 4.** Mean soil properties (± SE) at each sampling date, averaged across treatments of poultry litter application rate and irrigation method (n=12).

| | | Sampling Time | | |
|---|---|---|---|---|
| **Analysis** | **P value** | **January** | **May** | **October** |
| pH | 0.682 | 5.54 ± 0.096 a | 5.63 ± 0.118 a | 5.55 ± 0.149 a |
| EC[b] (dS $m^{-1}$) | **<0.001[a]*** ** | 0.126 ± 0.01 a | 0.931 ± 0.004 b | 0.857 ± 0.004 b |
| *(mg $kg^{-1}$ soil)* | | | | |
| M3P | **<0.001*** ** | 9.16 ± 0.874 a | 6.43 ± 0.810 b | 9.75 ± 0.825 a |
| M3K | **<0.001*** ** | 79.64 ± 3.58 a | 59.04 ± 3.03 b | 52.26 ± 2.48 b |
| M3Ca | 0.070 | 952.8 ± 67.4 a | 850.4 ± 74.4 a | 817.2 ± 36.6 a |
| M3Mg | **<0.001*** ** | 193.1 ± 6.88 a | 164.6 ± 9.42 b | 158.6 ± 3.54 b |
| M3S | **<0.001*** ** | 14.98 ± 0.256 a | 11.13 ± 0.493 b | 11.97 ± 0.657 b |
| M3Na | ND[c] | | | |
| M3Fe | **<0.001*** ** | 90.44 ± 9.52 a | 47.60 ± 5.56 b | 106.72 ± 9.59 a |
| M3Mn | **<0.001*** ** | 89.02 ± 6.83 a | 48.36 ± 7.47 c | 67.06 ± 3.68 b |
| M3Cu | **0.024*** | 3.57 ± 0.55 a | 3.03 ± 0.770 ab | 1.47 ± 0.082 b |
| M3Zn | 0.404 | 3.48 ± 0.36 a | 3.93 ± 0.481 a | 3.13 ± 0.663 a |
| M3B | ND | | | |
| *Soil Carbon Fraction* | | | | |
| OM (%) | **<0.001*** ** | 2.14 ± 0.108 b | 2.02 ± 0.093 b | 2.52 ± 0.105 a |
| POXC (mg $kg^{-1}$ soil) | 0.485 | 126.12 ± 19.0 a | 99.62 ± 40.98 a | 84.07 ± 28.3 a |
| TC (%) | 0.303 | 0.87 ± 0.056 a | 0.83 ± 0.076 a | 0.92 ± 0.05 a |
| TN (%) | **0.002**** | 0.10 ± 0.005 a | 0.09 ± 0.005 b | 0.09 ± 0.004 b |
| C:N | **<0.001*** ** | 8.31 ± 0.158 c | 9.37 ± 0.289 b | 10.10 ± 0.173 a |
| *Soil Respiration ($mg^{-1}kg^{-1}d^{-1}$)* | | | | |
| $CO_2$ | **0.001**** | 20.38 ± 2.19 a | 11.97 ± 0.923 b | 18.93 ± 1.41 a |
| *Particle size fraction (%)* | | | | |
| Sand | **0.019*** | 6.36 ± 1.22 ab | 9.11 ± 1.13 a | 5.04 ± 0.851 b |
| Silt | **0.043*** | 91.76 ± 1.22 a | 88.80 ±1.08 a | 92.24 ± 0.953 a |
| Clay | **0.008**** | 1.88 ± 0.09 b | 2.09 ± 0.111 ab | 2.71 ± 0.258 a |

[a] Means followed by a common letter between treatment columns are not significantly different by Tukey's HSD test (*$p < 0.05$, **$p < 0.01$, *** $p < 0.001$).. Columns without letters indicate no significant difference ($p > 0.05$).

[b]Electrical conductivity (EC), Mehlich-3 phosphorus (M3P), potassium (M3K), calcium (M3Ca), magnesium (M3Mg), sulfur (M3S), sodium (M3Na), iron (M3Fe), manganese (M3Mn), copper (M3Cu), zinc (M3Zn), boron (M3B), organic matter (OM), permanganate oxidizable carbon (POXC), total carbon (TC), total nitrogen (TN), and carbon: nitrogen ratio (C:N).

[c]Not Detected (ND). Limit of Detection for M3Na = 179 mg $kg^{-1}$ and M3B = 33.9 mg $kg^{-1}$

**Table 5.** Pearson correlation coefficients (r) under each poultry litter application rate for enzyme activities and selected soil properties.

| | β-D Glucosidase | β-Glucosaminidase | Acid Phosphatase | Arylsulfatase | Urease |
|---|---|---|---|---|---|
| | ---------- | ---------- | *Coefficient (r)* | ---------- | ---------- |
| **4.5 Mg ha$^{-1}$ + FUR** | | | | | |
| pH | 0.09 | -0.11 | -0.07 | 0.14 | 0.21 |
| EC[b] | -0.41 | -0.41 | 0.03 | **-0.65*** | 0.42 |
| M3P | -0.20 | -0.07 | -0.17 | -0.01 | 0.27 |
| M3S | -0.16 | 0.14 | -0.02 | -0.13 | 0.49 |
| OM | **0.92[a]*** ** | **0.77** ** | **-0.67*** | 0.51 | -0.10 |
| POXC | -0.57 | -0.57 | 0.40 | -0.35 | 0.05 |
| $CO_2$ | 0.44 | 0.44 | -0.49 | 0.02 | 0.57 |
| Sand | -0.36 | -0.19 | **0.66*** | -0.11 | 0.29 |
| Silt | 0.32 | 0.19 | **0.64*** | 0.11 | -0.23 |
| Clay | 0.24 | 0.003 | -0.13 | -0.06 | -0.31 |
| **6.7 Mg ha$^{-1}$ + FL** | | | | | |
| pH | -0.06 | -0.18 | 0.04 | 0.20 | -0.07 |
| EC | -0.52 | -0.25 | -0.06 | **-0.81*** | 0.44 |
| M3P | **0.75** ** | **0.77** ** | **0.62*** | 0.26 | -0.29 |
| M3S | 0.14 | 0.23 | 0.30 | -0.39 | 0.23 |
| OM | **0.85*** ** | **0.93*** ** | 0.56 | 0.53 | -0.36 |
| POXC | **0.69*** | **0.88*** ** | 0.50 | 0.23 | -0.06 |
| $CO_2$ | 0.53 | **0.68*** | 0.45 | 0.04 | -0.04 |
| Sand | -0.02 | -0.22 | 0.11 | 0.09 | 0.46 |
| Silt | -0.07 | 0.14 | -0.17 | -0.13 | -0.39 |
| Clay | **0.64*** | 0.53 | 0.49 | 0.38 | -0.41 |
| **9.0 Mg ha$^{-1}$ + FL** | | | | | |
| pH | -0.16 | -0.15 | 0.52 | 0.30 | 0.47 |
| EC | -0.14 | -0.26 | 0.52 | 0.24 | 0.31 |
| M3P | 0.46 | 0.38 | 0.35 | 0.01 | 0.11 |
| M3S | -0.27 | -0.38 | 0.18 | -0.18 | 0.32 |
| OM | **0.60*** | 0.55 | **0.80** ** | 0.22 | 0.10 |
| POXC | -0.23 | -0.29 | 0.54 | 0.10 | 0.39 |
| $CO_2$ | 0.18 | 0.11 | **0.70*** | -0.01 | 0.27 |
| Sand | -0.50 | -0.51 | 0.22 | **0.63*** | 0.21 |
| Silt | 0.36 | 0.35 | -0.25 | **-0.65*** | -0.14 |
| Clay | 0.55 | **0.64*** | 0.05 | -0.05 | -0.27 |

[a] Means followed by a common letter between treatment columns are not significantly different by Tukey's HSD test (*$p < 0.05$, **$p < 0.01$, *** $p < 0.001$).

[b]Electrical conductivity (EC), Mehlich-3 phosphorus (M3P), sulfur (M3S), organic matter (OM), permanganate oxidizable carbon (POXC), total carbon (TC), total nitrogen (TN), and carbon: nitrogen ratio (C:N).

## Figures

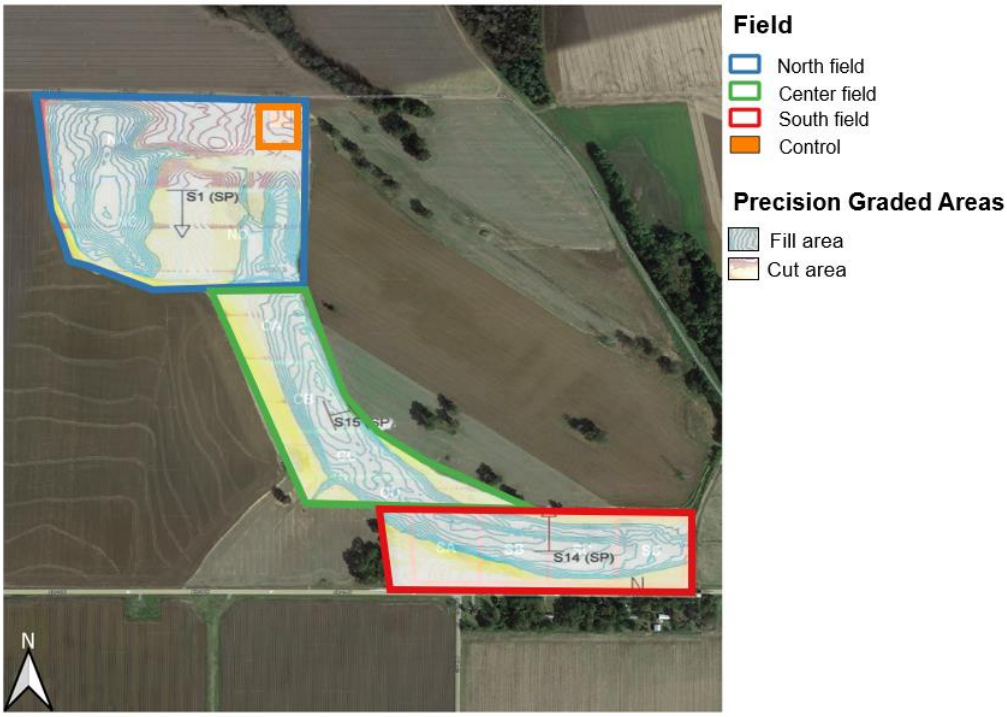


**Figure 1.** Site layout showing the three precision graded fields used in this study with cut and filled areas.

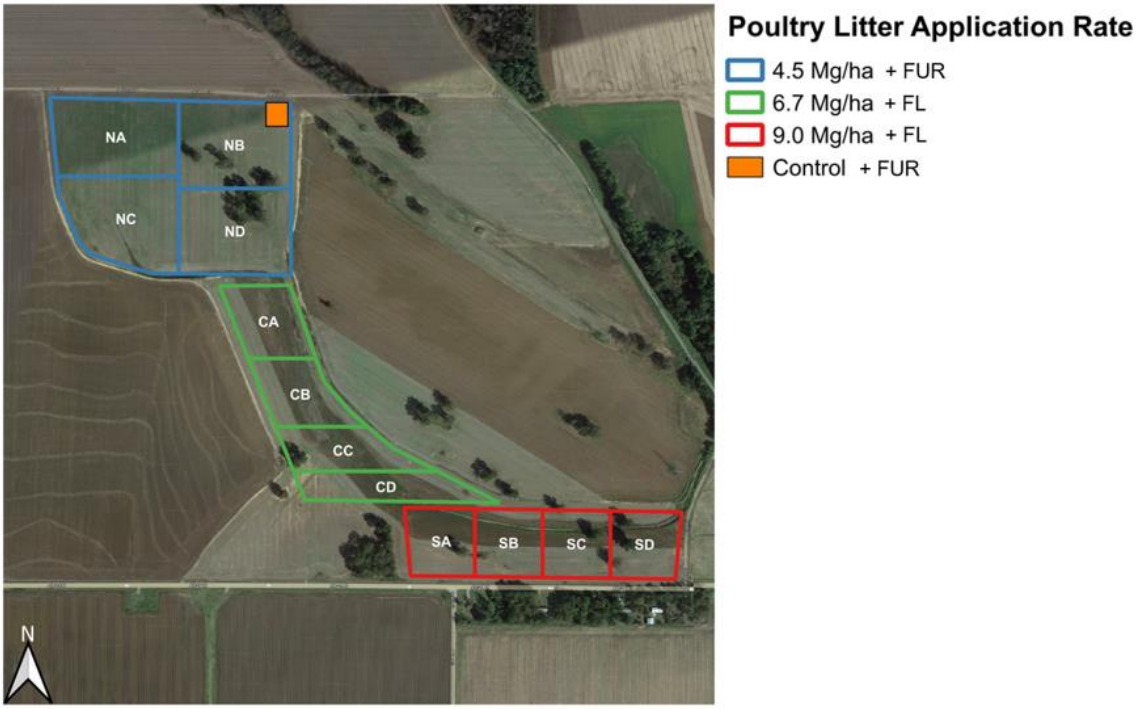


**Figure 2.** Experimental study layout showing sampling quadrants from each field and corresponding poultry litter application rate and irrigation method (FUR = furrow, FL = flood). Quadrants are labeled in each field with North (N), Central (C), and South (S) combined with quadrant A, B, C, or D.

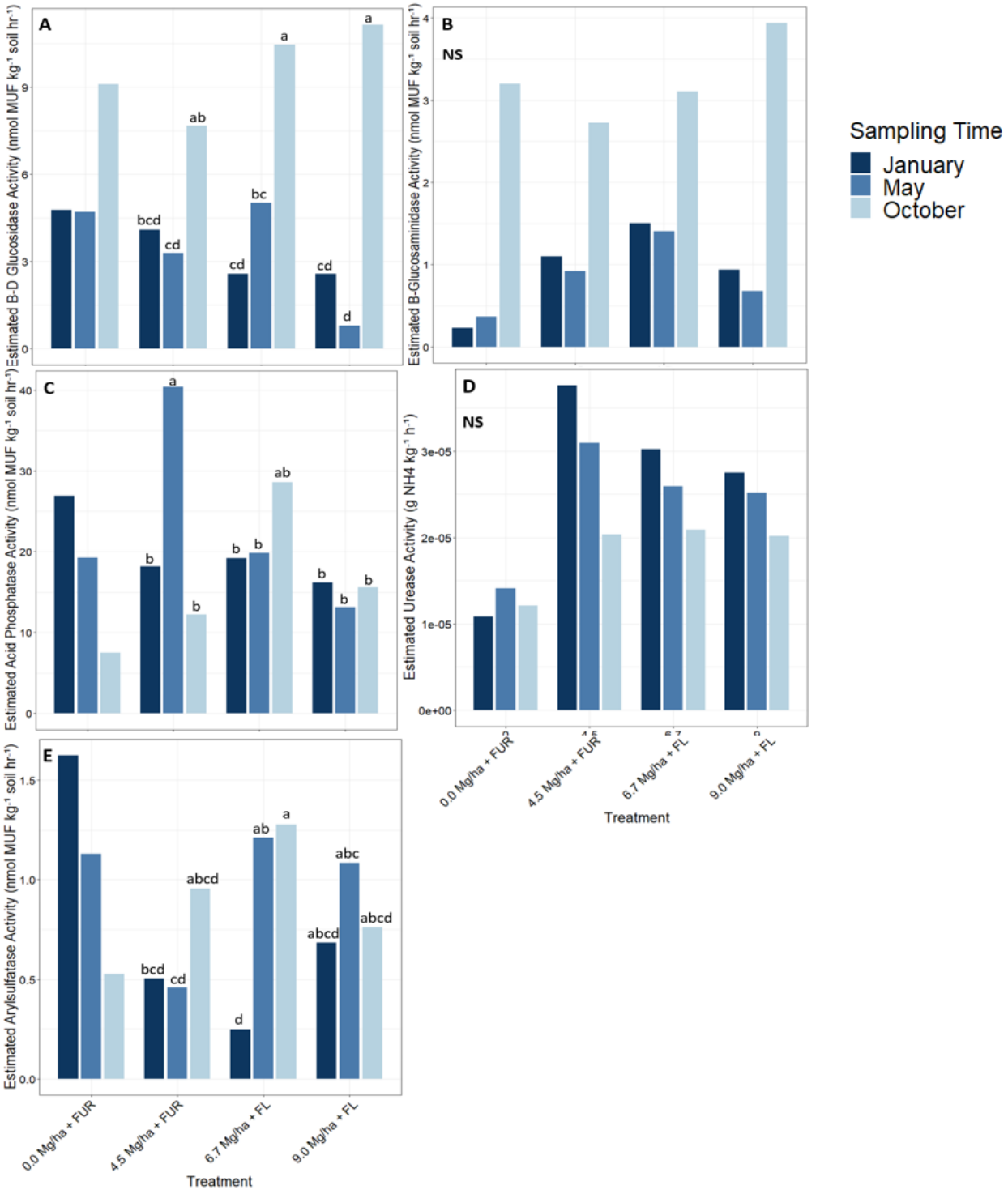


**Figure 3.** Effect of treatment (poultry litter rate (Mg ha$^{-1}$) and irrigation method (FUR = furrow, FL = flood)) by sampling time on soil enzyme activities including: **(A)** β-D glucosidase, **(B)** β-glucosaminidase, **(C)** acid phosphatase, **(D)** urease, and **(E)** arylsulfatase (n=4). Means followed by a common letter are not significantly different by Tukey's HSD test ($P < 0.05$). The 0 Mg ha$^{-1}$ control is included as a visual, and not statistical, reference (n = 1).

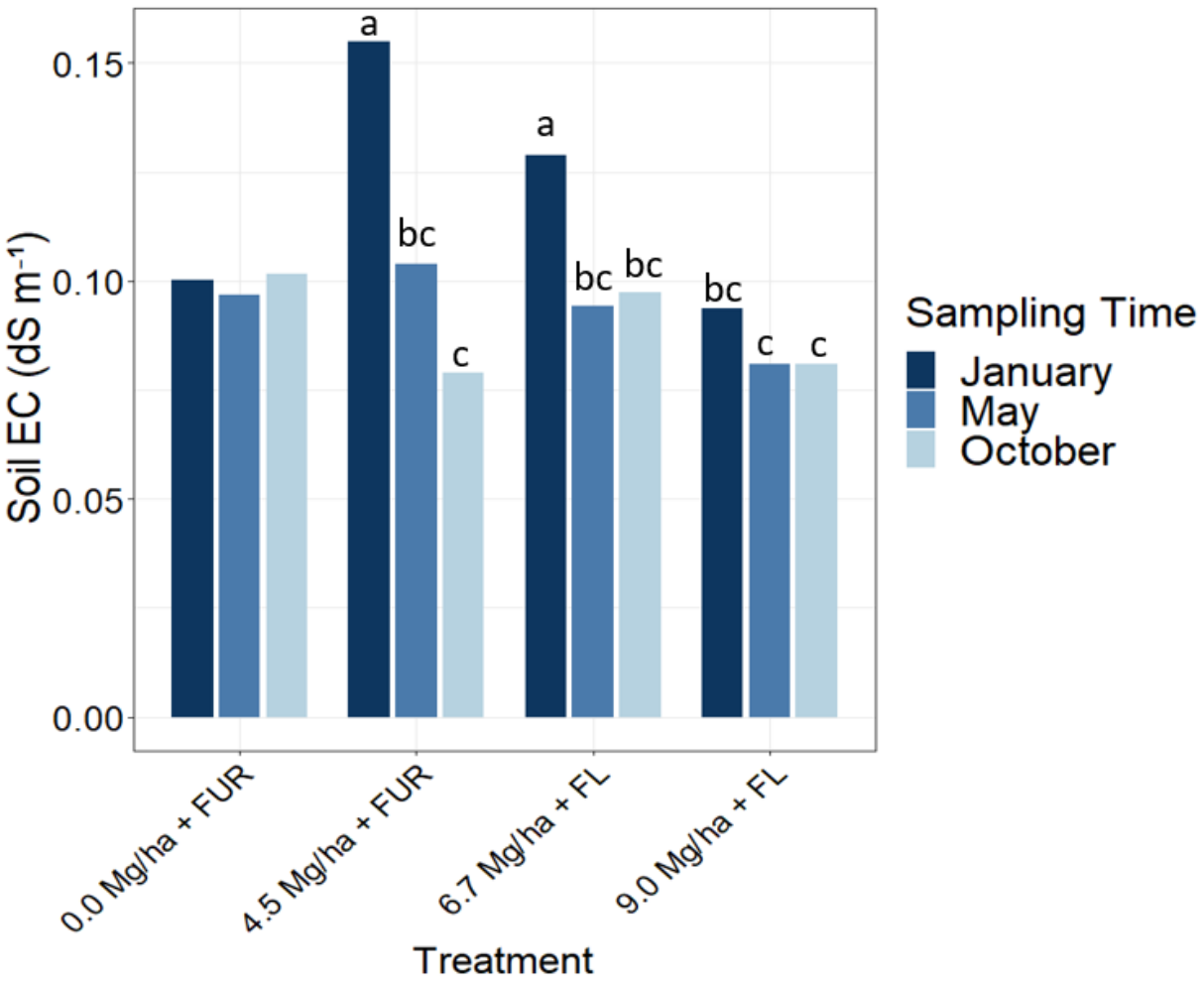


**Figure 4.** Effect of treatment (poultry litter rate (Mg ha$^{-1}$) and irrigation method (FUR = furrow, FL = flood)) by sampling time on soil electrical conductivity (EC) (n = 4). Means followed by a common letter are not significantly different by Tukey's HSD test ($P < 0.05$). The 0 Mg ha$^{-1}$ control is included as a visual, and not statistical, reference (n = 1).

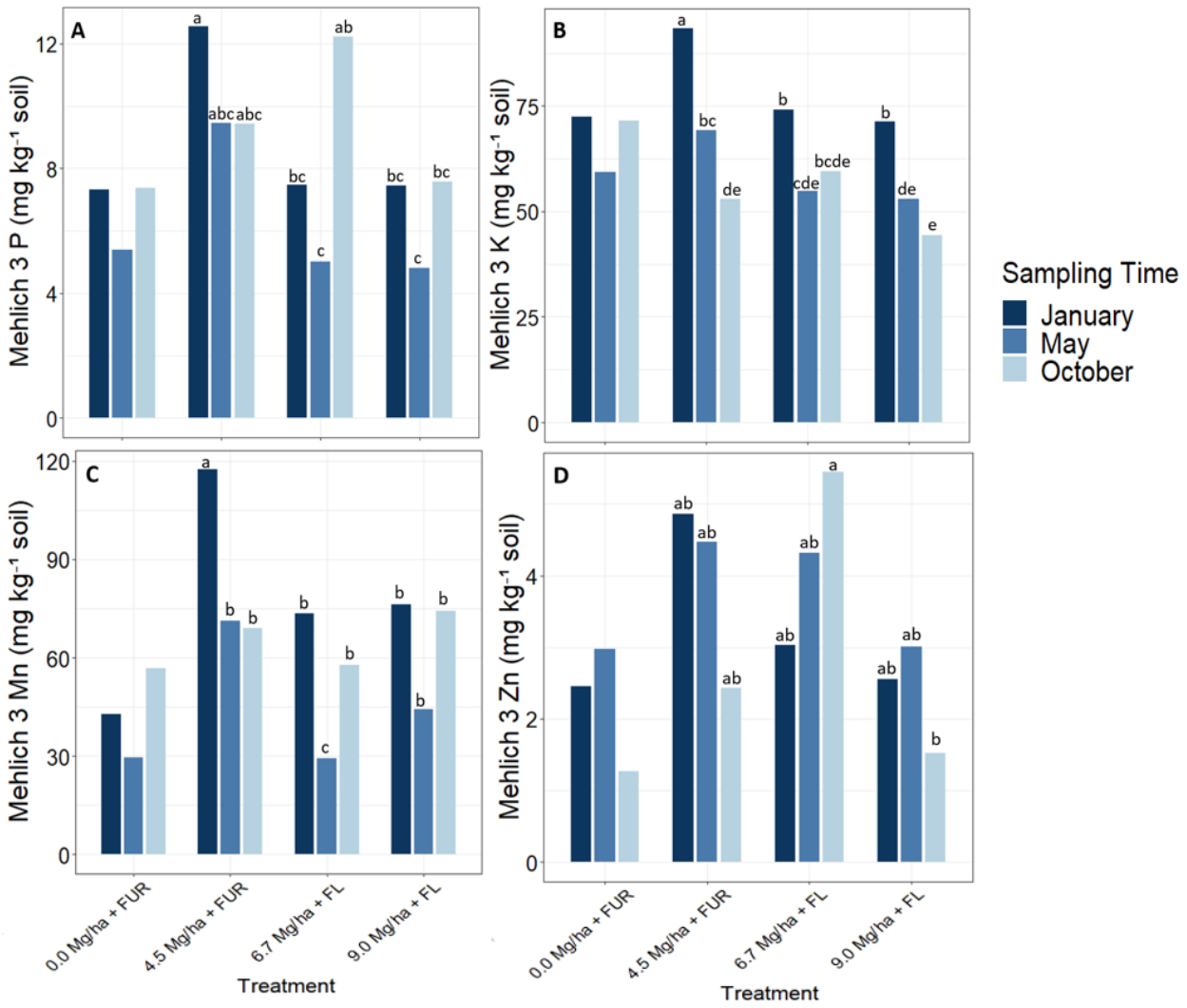


**Figure 5.** Effect of treatment (poultry litter rate (Mg ha$^{-1}$) and irrigation method (FUR = furrow, FL = flood)) by sampling time on Mehlich-3 extractable phosphorus (A), potassium (B), manganese (C), and zinc (D) (n = 4). Means followed by a common letter are not significantly different by Tukey's HSD test ($P < 0.05$). The 0 Mg ha$^{-1}$ control is included as a visual, and not statistical, reference (n = 1).

# Chapter 4: Conclusion

The objective of this research was to evaluate the short-term effectiveness of soil health management practices within small- and large-scale productions systems in Arkansas. The results of the small-scale production study showed that varying cover crop termination methods and poultry litter application methods did not result in differences in soil health within the first year. The legacy of previous soil management can persist for several years and likely buffered against immediate changes in soil health properties. Furthermore, previous additions of poultry litter and the previous perennial ground cover resulted in an annual cropping system that showed similar soil health to what was observed under perennial management, which demonstrates the benefit of continuous organic matter additions to overall soil health. Subsequent winter crops of winter barley and soft red winter wheat had a more immediate influence on dynamic soil properties than soil health management, which may be attributed to differences in nutrient acquisition strategies by crop species. These results highlight the contribution of plant roots to microbial activity, emphasizing the importance of crop species in managing annual production systems for sustained soil health.

The results of the large-scale study showed that increasing poultry litter rate did not consistently result in improved soil health in land-leveled soils within the first year, regardless of irrigation method. The β-D glucosidase was the only enzyme to show increased activities with increasing poultry litter rate, indicating that carbon-cycling enzymes may respond more rapidly to organic matter additions than other nutrient cycling enzymes. Soil carbon measurements and soil respiration did not differ between poultry litter rates and irrigation methods, and soil nutrients showed varied responses to poultry litter rate, with lower poultry litter rates often showing greater nutrient concentrations. The variability in soil responses to poultry litter application rate was likely a reflection of the large amount and extent of spatial variability

imposed by land leveling, as well as the large sampling area represented by each composite sample. Differences in irrigation method also likely influenced soil response to PL rate, as evidenced by greater soil nutrient concentrations in soil receiving the lowest PL rate combined with furrow irrigation, which allowed for overall drier soil conditions. The results of this study indicate that soil biology and biochemistry do not recover in one-year after severe physical disturbance of land-leveling and support the current University of Arkansas System Division of Agriculture recommendation of applying repeated subsequent annual poultry litter applications to land-leveled soils in order to restore soil health to land-leveled fields.

## Appendices

**Appendix A.** Non-significant interactions shown as the ANOVA p values from the okra cover crop study at the November and June sampling dates.

| Property | Treatment x Crop | Treatment x Time | Treatment x Time x Crop |
|---|---|---|---|
| pH | 0.390 | 0.966 | 0.848 |
| EC[a] (dS $m^{-1}$) | 0.566 | 0.755 | 0.338 |
| *(nmol MUF $kg^{-1}$ soil $hr^{-1}$)* | | | |
| β-D Glucosidase | 0.894 | 0.786 | 0.275 |
| β-Glucosaminidase | 0.851 | 0.295 | 0.974 |
| Acid Phosphatase | 0.914 | 0.349 | 0.991 |
| Arylsulfatase | 0.996 | 0.373 | 0.999 |
| *(mg $NH_4$ $kg^{-1}$ $hr^{-1}$)* | | | |
| Urease | 0.454 | 0.944 | 0.212 |
| *(mg $kg^{-1}$ soil)* | | | |
| M3P | 0.403 | 0.996 | 0.832 |
| M3K | 0.238 | 0.687 | 0.850 |
| M3Ca | 0.736 | 0.992 | 0.876 |
| M3Mg | 0.370 | 0.949 | 0.992 |
| M3S | 0.151 | 0.368 | 0.124 |
| M3Na | 0.657 | 0.209 | 0.577 |
| M3Fe | 0.283 | 0.533 | 0.831 |
| M3Mn | 0.412 | 0.294 | 0.164 |
| M3Cu | 0.903 | 0.770 | 0.927 |
| M3Zn | 0.378 | 0.786 | 0.932 |
| M3B | 0.451 | 0.489 | 0.866 |
| *Soil Carbon Fractions* | | | |
| OM (%) | 0.940 | 0.932 | 0.967 |
| POM (mg $kg^{-1}$) | 0.741 | 0.720 | 0.957 |
| POXC (mg $kg^{-1}$) | 0.218 | 0.893 | 0.769 |

[a]Electrical conductivity (EC), Mehlich-3 phosphorus (M3P), potassium (M3K), calcium (M3Ca), magnesium (M3Mg), sulfur (M3S), sodium (M3Na), iron (M3Fe), manganese (M3Mn), copper (M3Cu), zinc (M3Zn), boron (M3B), organic matter (OM), permanganate oxidizable carbon (POXC).

**Appendix B.** Summary of soil properties within the control plot (0 Mg $ha^{-1}$ poultry litter and furrow irrigation) at each sampling date (n = 1).

| | Sampling Date | | |
|---|---|---|---|
| **Analysis** | **January** | **May** | **October** |
| pH | 6.61 | 6.47 | 6.57 |
| EC[a] (dS $m^{-1}$) | 0.10 | 0.97 | 0.10 |
| *(nmol MUF $kg^{-1}$ $hr^{-1}$)* | | | |
| β-D Glucosidase | 4.78 | 4.71 | 9.10 |
| β-Glucosaminidase | 0.23 | 0.37 | 3.20 |
| Acid Phosphatase | 26.9 | 19.2 | 7.53 |
| Arylsulfatase | 1.62 | 1.13 | 0.53 |
| *(mg $NH_4$ $kg^{-1}$ $hr^{-1}$)* | | | |
| Urease | 0.011 | 0.014 | 0.012 |
| *(mg $kg^{-1}$ soil)* | | | |
| M3P | 7.33 | 5.40 | 7.36 |
| M3K | 72.4 | 59.3 | 71.5 |
| M3Ca | 1432 | 1179 | 1307 |
| M3Mg | 238.4 | 207.7 | 244.3 |
| M3S | 11.9 | 10.8 | 13.7 |
| M3Na | ND[b] | ND | ND |
| M3Fe | 103.6 | 35.6 | 47.3 |
| M3Mn | 42.9 | 29.5 | 56.7 |
| M3Cu | 1.57 | 4.89 | 1.28 |
| M3Zn | 2.46 | 2.98 | 1.27 |
| M3B | ND | ND | ND |
| *Soil Carbon Fraction* | | | |
| OM (%) | 2.51 | 2.78 | 2.90 |
| POXC (mg $kg^{-1}$ soil) | 103.4 | 80.9 | 0.00 |
| TC (%) | 1.08 | 1.04 | 1.04 |
| TN (%) | 0.10 | 0.10 | 0.10 |
| C:N | 10.95 | 10.87 | 10.74 |
| *Soil Respiration (mg $kg^{-1}d^{-1}$)* | | | |
| $CO_2$ | 15.80 | 12.70 | 8.40 |
| *Particle size fraction (%)* | | | |
| Sand | 2.63 | 1.62 | 0.99 |
| Silt | 95.25 | 96.35 | 96.87 |
| Clay | 2.12 | 2.04 | 2.15 |

[a]Electrical conductivity (EC), Mehlich-3 phosphorus (M3P), potassium (M3K), calcium (M3Ca), magnesium (M3Mg), sulfur (M3S), sodium (M3Na), iron (M3Fe), manganese (M3Mn), copper (M3Cu), zinc (M3Zn), boron (M3B), organic matter (OM), permanganate oxidizable carbon (POXC), total carbon (TC), total nitrogen (TN), and carbon: nitrogen ratio (C:N).

[b]Not Detected (ND). Limit of Detection for M3Na = 179 mg $kg^{-1}$ and M3B = 33.9 mg $kg^{-1}$