Effects of Broiler Litter Application on Crop Yield, Soil Fertility, and Organic Carbon Dynamics in No-Till Corn-Soybean Rotations

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Abstract

The Southeastern United States faces challenges in managing poultry waste sustainably. Utilizing broiler litter (BL) as a soil amendment offers a solution by enhancing crop nutrients and increasing soil organic carbon (SOC). This study assessed the impact of BL rates on soil fertility, crop yield, and SOC in a no-till corn-soybean rotation system. Initiated in 2019 at the University of Tennessee's Milan AgResearch Center, the study evaluated a corn-soybean rotation previously managed with synthetic fertilizers. Treatments included four BL rates (2, 3, 4, and 7 tons acre^{-1}) applied before corn planting, plus controls with and without N, both receiving P and K based on soil test recommendations. No fertilizer was used during soybean years. In 2023, soil samples from four depths (0-5, 5-15, 15-30, and 30-60 cm) were analyzed for bulk density (BD), soil organic carbon (SOC), and microbial biomass carbon (MBC). Pre-planting soil samples from 2022 (soybean) and 2023 (corn) were assessed for macro- and micronutrients, with nitrate (NO_3^-) levels measured at the V4 stage of corn and prior to soybean planting. The 7 tons acre^{-1} BL treatment had the highest pre-plant NO_3^- levels during corn growth and significantly increased corn yields (14.98 Mg ha^{-1}), matching the positive control and 162% above the negative control. Soybean yields (4.27–4.49 Mg ha^{-1}) were unaffected by BL or inorganic N. Mehlich-1 P and K levels increased with BL rates, peaking at 246% and 93% above controls at 7 tons acre^{-1}. Micronutrients like boron (B) and zinc (Zn) also increased significantly, while iron (Fe) and copper (Cu) were unaffected. BL reduced BD at 0-5 cm, with the lowest (1.16 g cm^{-3}) at 7 tons acre^{-1}, improving soil structure. Microbial biomass carbon (MBC) and SOC also increased, with maximum MBC (470 mg kg^{-1}) and SOC concentrations (17.45 g kg^{-1}) at higher BL rates. But the benefits of BL on SOC, BD, and MBC were limited to topsoil in no-till systems, with no significant changes at deeper depths. Overall, this study showed that higher

BL rates (4–7 tons acre^{-1}) in no-till corn-soybean systems improved soil fertility and corn yield, with 7 tons acre^{-1} yielding the best results. However, this rate also caused nutrient buildup, particularly P, requiring careful management to mitigate environmental risks.

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1. GENERAL INTRODUCTION

1.1 Corn-Soybean Rotation

Corn (*Zea mays* L.) is a fundamental crop in the agricultural history of Tennessee, initially serving as a staple food for Native American tribes and continuing to be vital today. It thrives in southern conditions, producing substantial carbohydrates for human consumption, cattle feed, and yields more than any other cereal crop. Corn's significant N requirements, often ranging between 120-250 lbs acre^{-1} depending on soil conditions and yield goals—make it particularly dependent on external N inputs for optimal growth and productivity. This dependency highlights the suitability of corn for rotation with N-fixing crops, such as soybeans, which contribute to soil N replenishment through biological fixation. This "rotation effect," described by Pierce and Rice (1988), demonstrates improved crop yields in rotation systems rather than continuous monocropping. Bhowmik and Doll (1982) found that soybean yields were 10-15% higher when rotated with corn compared to monocropping. Similarly, Sundquist et al. (1982) noted a 10-15% decrease in corn yields when grown continuously, highlighting the benefits of crop rotation.

1.2 Nitrogen Fertilizers in Corn-Soybean Rotation

Nitrogen fertilization is crucial for maximizing corn yield, as corn is an N-intensive crop (Paponov et al., 2005; Uribelarrea et al., 2009). The rate and timing of N application significantly impacts crop N uptake and ultimately grain yield (Kuo & Jellum, 2002). Effective N management minimizes leaching beyond the root zone and reduces surface runoff and gaseous loss of N (Raun & Johnson, 1999; Subedi & Ma, 2005). Understanding how climate, soil factors, and fertilizer management practices interact is essential for optimizing N application rates and timing. These factors influence yield potential and grain quality (Tsai et al., 1992; Sabata &

Mason, 1992; Asghari & Hanson, 1984; Ahmadi et al., 1993; Sogbedji et al., 2001). Proper N management ensures sufficient N availability for maximizing crop productivity in corn while minimizing environmental impacts. A conventional practice to maximize yields is to apply inorganic fertilizers, however, intensive cultivation and continuous use of inorganic fertilizers can deteriorate soil properties and negatively affect air and water quality, necessitating less dependance on synthetic fertilizers to build and maintain soil fertility (Tilman et al., 2002).

The fertility status of a soil depends largely on the soil organic matter content, thus the addition of organic fertilizers can be an ecologically sound way to replenish soil nutrients (Aboudrare, 2009). Organic fertilizers, derived from living organisms or their byproducts, release nutrients more slowly than chemical fertilizers, promoting soil health and reducing leaching losses. However, their decomposition depends on external conditions like temperature and moisture, leading to uneven nutrient availability. Additionally, applying organic fertilizers to meet the N requirement often results in over-application of P and K, and adjusting for P and K can leave N requirements unmet. There is also the fact that, often times large quantities of organic fertilizers are applied to meet crop nutrient requirements, which can be a potential reason for initial nutrient immobilization and temporary shortages (Agbede & Kalu, 1995; Okigbo, 2000; Adekiya et al., 2012).

1.3 Broiler Litter as an Organic Fertilizer

Broiler meat is a popular protein source in the U.S. due to its affordability, versatility, and health benefits. Due to technological advancements, the broiler industry has grown rapidly and became a major agro-industry with an annual business of $35.5 billion (USDA, 2020). This increase in broiler production has led to a significant rise in the generation of manure, litter, and other byproducts. Broiler litter (BL), which includes a mix of manure, bedding materials, feathers, spilled feed, and other organic components, is particularly rich in organic matter and essential nutrients (Watts et al., 2010). When applied to land, BL can enhance nutrient availability, regulate pH, increase organic matter content, improve cation exchange capacity, boost water-holding capacity, and promote soil aggregation. These benefits contribute to improved soil and environmental quality, ultimately leading to increased agricultural productivity (Fallah et al., 2013; Ghosh et al., 2004; Kingery et al., 1994).

Broiler litter is an effective alternative to conventional fertilizers, offering a sustainable solution for disposing of the large quantities of BL produced on poultry farms, particularly in the southeastern United States (Reddy et al., 2004). Utilizing BL not only mitigates the environmental impact of conventional fertilizer use but also provides a more economical option, as the cost of traditional fertilizers continues to rise (Wiatrak et al., 2004; Reddy et al., 2004; Mitchell & Tu, 2005; Hirzel et al., 2007; Tewolde et al., 2009). A study by Bosch and Napit (1992) demonstrated the economic feasibility of transporting BL from regions with surplus to those with a deficit. Their findings indicated that the value of BL as a fertilizer outweighs the transportation costs, even over distances of up to 50 miles.

1.3.1 Broiler litter on Soil Nitrogen

Optimizing N management is widely recognized as a crucial factor in improving crop productivity without compromising environmental stewardship, with applying the correct rate of N at the appropriate time being particularly significant (Magdoff, 1991). Application of organic fertilizers including BL influences soil N dynamics in several ways. When comparing the effects of organic and inorganic fertilizers on soil N content, contradictory results were reported. Studies by Kaur et al. (2005) and Adeniyan et al. (2011) found that organic amendments, such as BL, can significantly improve soil N content in agricultural systems, Kaur et al. (2005) reported that

BL application to maize at 5- and 10-tons hectare^{-1} significantly improved soil N content, driven by both immediate inorganic N release and organic N mineralization. Similarly, Adeniyan et al. (2011) found that BL applied at 4- and 8-tons hectare^{-1} to maize-soybean intercrops in Nigeria achieved soil NO_3^-N levels comparable to inorganic fertilizers like urea, relative to untreated controls. Also, it was reported by Sims et al. (1987) and Cooperband et al. (2002) that inorganic fertilizers consistently resulted in higher soil NO_3-N levels compared to BL, particularly in the first year of application. Sims et al. (1987), in a study on corn (*Zea mays*) in the United States, applied BL at rates of 4.5, 9, and 18 tons per hectare and observed lower NO_3-N availability compared to synthetic N fertilizers due to the slower release of N from BL. Similarly, Cooperband et al. (2002) evaluated BL applied at 5 and 10 tons per hectare to forage crops in Wisconsin and noted a lag in N availability during the first growing season, as BL-derived N relied on mineralization processes, unlike the immediate availability provided by inorganic fertilizers. However, Adeli et al. (2005) studied the effects of BL on a no-till cotton system in Mississippi, applying BL at rates of 4.5, 9-, and 13.5-tons hectare^{-1}, and found no significant difference in residual NO_3^-N levels between soils treated with BL and those treated with commercial N fertilizers. Similarly, Bitzer and Sims (1987) evaluated BL applied at rates of 5 and 10 tons per hectare in corn fields in Delaware and reported that approximately 67% of the organic N in BL was mineralized within one year, while the remainder was released gradually. This gradual release provided a sustained supply of N, potentially reducing the frequency of fertilizer applications. These findings highlight that while BL may initially release N more slowly than inorganic fertilizers, its long-term effects on soil N levels can be comparable or even more advantageous for maintaining soil fertility and crop productivity. Numerous studies have reported BL to be an effective fertilizer for crop production (Adeli et al., 2019; Endale et al., 2008; Hirzel et al., 2007; Tewolde et al., 2013) with similar or greater crop yields compared with conventional synthetic fertilizers (Sistani et al., 2008; Watts & Torbert, 2011). However, the agronomic efficiency of BL as a N source for crops (defined as crop yield response per unit of N supplied) also depends on the application rate. Applying more N than the crop requires does not necessarily lead to proportional increases in yield (Donner & Kucharik, 2003). This underscores the importance of optimizing N application rates to balance yield with positive environmental impacts.

1.3.2 Broiler litter on Soil P, K and other Macronutrients

Broiler litter has been extensively studied for its impact on soil P, K, calcium (Ca), and magnesium (Mg) contents. Broiler litter contains a significant amount of P, with a substantial proportion (60-80%) present in plant-available inorganic forms (e.g., orthophosphate). The remaining P exists in organic or less soluble forms, which can mineralize over time and contribute to plant-available P under suitable conditions (Robinson & Sharpley, 1995). Compared to inorganic fertilizers, BL application has been shown to result in significantly higher soil P levels. For example, in soybean and corn cropping systems, P levels were found to be 78% and 175% greater in BL-treated plots than in those treated with inorganic fertilizers (Reddy, 2009). These findings align with other research that has documented increased extractable P in surface soils following manure applications (Chang et al., 1991; Eghball, 2002).

Repeated BL application can further amplify soil P levels, especially in the upper soil layers. Adeli et al. (2008) demonstrated that repeated BL application for 3 years led to a substantial increase in soil P concentration at the 0-15 cm depth and this increase was proportional to the rate of BL addition. Nyakatawa et al., 2001, observed that continuous BL application resulted in more than a sixfold increase in extractable soil P compared to soils that did not receive litter amendments, similar improvements in soil P were also observed by Kingery et al., 1994 and Mitchell et al., 2007. These findings collectively underscore the significant role of BL in enhancing soil P levels, particularly in the surface layers, which is crucial for crop nutrition and soil fertility

Similar to P, BL applications have a pronounced effect on soil K levels. Potassium is a critical nutrient for plant growth but is often heavily removed from the soil through crop harvest, especially under intensive cultivation without adequate K inputs. Studies have shown that repeated BL application can significantly increase soil K concentrations. Adeli et al. (2008) reported substantial increases in soil K levels after three years of BL application, particularly in the surface soil layers. Watts et al. (2010) found that soil from BL-treated plots contained 32% more K in soybean cropping systems and 110% more K in corn cropping systems compared to plots treated with inorganic fertilizers. These findings are corroborated by Schlegel (1992), who observed an increase in soil K with animal manure compared to the same inorganic fertilizer applied at the same K input rate. The cumulative effects of repeated BL application on soil K were also evident in studies by Kingery et al. (1994) and Stephenson et al. (1990), which reported that long-term BL application significantly increased soil concentrations of extractable K compared to soils without litter amendments. This increase in soil K helps counteract the depletion caused by intensive farming practices and improves soil fertility.

Calcium and magnesium are essential secondary nutrients that are often overlooked in fertilization programs. However, BL application has been shown to have a positive impact on the soil levels of these nutrients. Research indicates that Ca levels at the 0-5 cm depth can be 30% to 58% higher in soils treated with BL compared to those receiving inorganic fertilizers (Edwards et al., 1992; Lund & Doss, 1980). Similarly, Mg concentrations can be up to 40% greater in BL-treated soils compared to those treated with inorganic fertilizers (Chang et al., 1991). Sistani et al. (2008) found that in the later years following BL application, soils treated with BL had higher levels of Mehlich-3 extractable Ca and Mg compared to soils treated with inorganic fertilizers. This increase in Ca and Mg enhances soil structure, improves cation exchange capacity, and boosts overall soil health, which can subsequently improve crop productivity.

1.3.3 Broiler litter on Soil Micronutrients

Broiler litter has traditionally been valued for its macronutrient content, particularly N, P, and K. However, BL also contains significant levels of micronutrients, such as iron (Fe), copper (Cu), manganese (Mn), and zinc (Zn). These micronutrients primarily originate from the birds' diet, where they are added to feed to promote growth, improve feed efficiency, and prevent diseases (Sims & Wolf, 1994). The quantities of Cu, Zn, and Mn added often exceed the birds' nutritional needs, resulting in their excretion and subsequent enrichment in BL. This enrichment makes BL a notable source of soil-applied micronutrients (Moore et al., 1998; Codling et al., 2008).

Kunkle et al. (1981) reported that the concentrations of Cu and Zn in BL were 1-5 times higher than those in the broiler feed. This increase is due to the accumulation of these elements in the litter from multiple feed cycles and their retention in the manure. Similarly, Edwards and Daniel (1992) found that poultry manures are enriched with trace metals, with average concentrations of 304 mg kg^{-1} Mn, 320 mg kg^{-1} Fe, 53 mg kg^{-1} Cu, and 354 mg kg^{-1} Zn. These findings underscore the role of BL as a significant source of trace elements, which can accumulate in soils with repeated applications.

Repeated applications of BL to soil have been shown to elevate levels of micronutrients, particularly in the surface horizons. For instance, Franzluebbers et al. (2004a) found that applying broiler litter at 5- and 10-tons hectare^{-1} significantly increased soil Cu, Zn, and Mn concentrations under bermudagrass in Georgia, highlighting the potential of BL to enhance soil micronutrient availability in forage systems. Similarly, Adeli et al. (2008) observed that BL application led to higher Cu and Zn levels at the 15-cm soil depth and these micronutrients accumulated over time with repeated BL applications. This study also found that unlike P, the Mehlich-3 extractable Cu and Zn did not decline after ceasing BL application, indicating a more sustained presence in the soil.

Repeated applications of BL as a soil amendment can enhance soil macro and micronutrient levels, but such applications have several detrimental effects due to nutrient imbalances and environmental risks. Also, the effectiveness of BL as a nutrient source is compromised by runoff and leaching, particularly in no-till systems. These losses reduce nutrient efficacy and contribute to environmental pollution (Westerman et al., 1983; Edwards and Daniel, 1993; Shreve et al., 1995; Nathan and Malzer, 1994).

1.3.4 Detrimental Effects of Broiler litter Application

When BL is applied to meet crop N requirements, it can lead to the build-up of other nutrients such as P and K. Studies have shown that high rates of BL application can reduce soil's capacity to adsorb P, resulting in increased P mobility and potential contamination of water bodies (Field et al., 1985; Reddy et al., 1980; Wood et al., 1996). Another significant issue is the excessive accumulation of NO_3 in the soil from repeated BL applications that exceed crop uptake. These elevated NO_3 levels pose substantial environmental risks, as they can leach into groundwater and reach surface water bodies through run-off, contributing to eutrophication (Sharpley et al., 1993; Sims, 1995; Adeli et al., 2003). The application of BL also impacts soil K levels. While increased K can benefit K-deficient soils, excessive K levels may cause luxury consumption of K by crops and other nutrient imbalances, affecting the uptake of Ca and Mg by plants (Marchuk & Marchuk, 2018; Rhodes et al., 2018). Over-application of BL can also cause excessive accumulation of micronutrients, which can be problematic. Evidence suggests that Cu, Zn, As, and Mn are detected in surface runoff from fields treated with BL, contributing to the contamination of nearby water bodies. Elevated concentrations of these trace elements can disrupt aquatic life and compromise water quality (Moore, 1998).

1.4 Effects of Broiler Litter on Crop Yield

1.4.1 Broiler litter on Corn Yield

Broiler litter has gained considerable attention as an effective nutrient source for corn production. Extensive research has shown that BL can be as effective as, or even superior to, conventional synthetic fertilizers for crop yield. The integration of BL in crop rotations, such as corn-soybean rotations, consistently produces higher yields compared to using BL or other fertilizers in continuous corn cropping systems. For example, Sistani et al. (2008), in a four-year study on corn in Kentucky, reported that BL applied at rates of 5.6 and 11.2 tons hectare^{-1} produced significantly higher grain yields compared to chemical fertilizers in two of the four years. This yield improvement is due to the additional macro- and micronutrients, along with the residual N provided by BL. This does not imply that inorganic fertilizers were insufficient in providing micronutrients but highlights that BL offers a broader range of nutrients. BL enhances soil organic matter and structure, contributing to improved crop performance and underscoring its role in sustainable agriculture (Adeli et al., 2012; Endale et al., 2008; Hirzel et al., 2007; Tewolde et al., 2013). Endale et al. (2008) demonstrated that application of BL at the rate of 6.7 and 13.4 tons hectare^{-1} in a corn cropping system resulted in an 18% higher grain yield compared to plots treated with inorganic fertilizers. This increase was attributed to enhanced soil water retention and greater nutrient availability provided by the BL amendment. In a similar study, Jn-Baptiste et al. (2013) found that a medium rate of BL application increased corn grain yield by 13% compared to conventional fertilization. However, they did not observe any yield improvement with lower rates of BL compared to yields achieved with inorganic fertilizers. These results underscore that BL, particularly at higher application rates, can significantly improve yields and contribute to long-term soil fertility. This sustained fertility is attributed to BL's slow nutrient release and its ability to enhance soil organic matter and structure. However, the effectiveness of BL varies with application rates, suggesting that optimal rates are crucial for maximizing its benefits while achieving sustained agricultural productivity.

This key advantage of BL in providing sustained fertility is further highlighted in a study done by Evers (1999) who estimated that approximately 60% of N from BL will be available in the first year, with the remainder being carried over to the subsequent years. This finding was further supported by Mitchell and Tu (2005). They studied long-term BL applications in a cotton-corn rotation system in Alabama, applying BL at rates of 4.5 and 9 tons per hectare annually. They observed sustained crop yields over multiple years, attributing this to BL's gradual nutrient release and its positive effects on soil organic matter and nutrient cycling. Similarly, Sims (1987) found no significant difference in corn grain yield between fields treated with raw BL and those treated with ammonium nitrate during the first year. However, in subsequent years, fields applied with BL showed significant yield improvements, highlighting its residual benefits.

1.4.2 Broiler litter on Soybean Yield

While the benefits of BL application for crops like corn, wheat and forages are well-documented, its effects on legume crops like soybeans have been less extensively studied.

Soybeans fix N biologically through their symbiotic relationship with *Bradyrhizobium* bacteria, however there are concerns that animal and poultry manure might negatively affect this N_2-fixation (Mohammadi et al., 2012; Li et al., 2019). Research on this topic has produced mixed results. For instance, Lory et al. (1992) from long-term study of topdressing manure on alfalfa found that it did not have an effect N fixation in, but at the same time Sharpley et al. (1993) through their assessment of BL application to pastures suggested that the N, P, and K contents in BL could enhance the symbiotic relationship in soybeans, potentially improving N fixation and yields.

Soybeans have high nutrient demands, particularly for P and K, especially during early growth stages and the reproductive phase when N demand peaks (Brevedan et al.,1978; Varco, 1999; Wesley et al., 1998). Some studies have shown that the application of a booster dose of synthetic N fertilizers can increase soybean grain yield, particularly when soil NO_3^- N levels are low (Lamb et al., 1990). For example, Brevedan et al. (1978) reported a 22-32% increase in soybean yield with the application of N fertilizers, a response strongly related to soil NO_3^- N content. Given that BL can enhance soil NO_3^- N levels, it is likely to have a similar positive impact on soybean yields. Furthermore, the gradual mineralization of organic N in BL provides a slow-release nutrient source that aligns well with soybean's nutrient uptake patterns, particularly during critical growth stages like flowering and grain filling (Wesley et al., 1998). This slow release of N from BL is particularly beneficial during the R5 growth stage, when soybean's N-fixing capacity begins to decline and the plant's demand for N peaks for protein synthesis (Shibles, 1998). Several studies support this positive impact BL has on soybean yield. McAndrews et al. (2006) observed an increase of 0.2–0.5 Mg ha^{-1} in soybean grain yield in BL-treated plots compared to control or urea-fertilized plots. Similarly, Adeli et al. (2005) observed a 10% increase in soybean yield when BL was applied at rates of 6.7- and 13.4-tons hectare^{-1} in a no-till system in Mississippi, compared to plots treated with inorganic fertilizers. They attributed the yield improvement to BL's gradual nutrient release and its positive effects on soil structure and fertility. In contrast, Singh et al. (2021), in a study on maize-soybean rotation systems in India, found no yield improvements with BL applied at 5- and 10-tons hectare^{-1} in the first year compared to control plots. However, in the second year, they reported a significant 43% yield increase in BL-treated plots, highlighting the cumulative benefits of BL over time due to enhanced nutrient availability.

2. BROILER LITTER AND SOC

2.1 Total Soil Organic Carbon

The increasing atmospheric CO_2 concentration, driven by land use changes and conventional agricultural practices, presents a significant challenge to environmental sustainability. Conventional agriculture, characterized by intensive tillage and heavy reliance on inorganic inputs which lack organic matter, has exacerbated soil carbon depletion. Soil organic carbon (SOC) is a critical indicator of soil's vitality, impacting food production, greenhouse gas emissions, and climate change mitigation (Lorenz & Lal, 2016). Therefore, exploring sustainable soil management strategies to restore SOC is crucial (Lal, 2004) especially in the southeastern USA, where SOC levels tend to be lower than in other regions due to the warm and humid climate, which accelerates the decomposition of organic carbon inputs (Kern and Johnson, 1993).

In arable systems, in addition to supporting soil fertility and crop production, application of organic amendments such as animal manure, and BL support accumulation of SOC content (Turmel et al., 2015; Warren Raffa et al., 2015). Sistani et al. (2008) reported that applying BL to bermudagrass at a rate of 16 Mg ha^{-1} yr^{-1} increased SOC from 11.7 to 17.4 g kg^{-1}. Similarly, Adeli (2007) found that BL application increased SOC and microbial biomass carbon (MBC) by 38% and 26%, respectively, compared to commercial fertilizers applied at the same rate. (Adeli et al., 2009). Watts et al. (2010) found that soil amended with BL retained 33% more C at the 0 to 5 cm depth under 2 different cropping systems compared to inorganic fertilizers. Also, Kingery et al. (1994) reported increased SOC concentration with BL application in tall fescue compared to inorganic fertilizer treatments.

However, several studies reported that BL application has no effect on SOC. For example, Franzluebbers et al. (2001) found no significant increase in SOC concentration with BL in Bermudagrass compared to control. Similarly, Wood et al. (1996) also observed no difference in SOC concentration after 3 years of BL application to corn compared to control. Nonetheless, several studies consistently demonstrate that organic fertilizers, including BL, significantly enhance the active forms of SOC, which are more responsive to management changes compared to total SOC (Wander et al., 1994; Eghball et al., 2004; Jha et al., 2014). Research by Nyakatawa et al. 2001 revealed that BL application can increase SOC in the topsoil by up to 36% compared to inorganic fertilizers. Additionally, Watts et al., 2012 reported that BL increased SOC in the top 0-5 cm of soil by 33% more than inorganic fertilizers in a no-till system.

2.2 Fractional Distribution of Soil Organic Carbon:

Organic inputs like BL affect SOC dynamics based on their decomposition rates and the associated microbial processes (Almeida et al., 2021; Han-Bing et al., 2021; Bonanomi et al., 2021). The quality and type of these inputs influence the distribution of C in different soil pools, which are broadly categorized into three: active, slow, and passive pools (Parton et al., 1988). The active pool consists of recently added organic matter that decomposes quickly, while the passive pool is stable and decomposes over centuries. The slow pool represents a fraction with intermediate decomposition rates and stability.

To understand how different types of organic inputs affect SOC dynamics and their responsiveness to various management practices, it is essential to study different SOC fractions of varying turnover rates. The popular method to separate total SOC into active and stable fractions is to isolate SOC into Particulate Organic Matter (POM) and Mineral-Associated Organic Matter (MAOM) following a physical fractionation method (Elliott and Cambardella., 1991). Microbial biomass carbon serves as an important indicator of microbial activity and soil health, as it reflects the active pool of SOC directly involved in organic matter decomposition and nutrient cycling (Jenkinson and Ladd, 1981). Studies have shown that organic inputs, such as BL, enhance microbial biomass and activity, which in turn can lead to greater SOC accumulation through increased microbial processing of organic matter (Flie ßbach et al., 2007). Broiler litter application has been reported to significantly increase MBC, particularly at higher rates of application, due to the availability of both nutrients and organic substrates for microbial growth (Giacometti et al., 2013). Moreover, repeated applications of organic amendments can sustain elevated levels of MBC, which is crucial for maintaining soil fertility and SOC stabilization (Fraser et al., 2015). The effect of BL on MBC has been linked to improvements in SOC fractions, particularly POM, due to enhanced microbial activity, which helps to break down organic inputs and incorporate carbon into more stable fractions like MAOM over time (Jackson et al., 2003).

3. RATIONALE

The southeastern region of the country plays a pivotal role in broiler production, contributing more than 50% of the nation's output. As the population continues to grow, so does the demand for broiler meat, leading to a significant increase in the production of BL generated by this industry. Utilizing this litter as an agricultural amendment presents an excellent opportunity for not only improving the sustainability of production systems but also for mitigating climate change.

Broiler litter is an excellent organic fertilizer due to the higher content of both macro and micronutrients compared to most other organic nutrient sources. Its utilization as an agricultural amendment offers significant advantages, including enhanced soil fertility and improved crop yield. In addition, the slow mineralization of nutrients from BL reduces leaching and runoff losses of nutrients, making nutrients more available for crop uptake.

However, effective utilization of BL requires careful management to prevent nutrient imbalances. Specifically, excessive application of BL can lead to overloading of P and luxury consumption of K. This mainly happens when BL is applied to meet the N requirement of crops. However, when BL is applied to meet P and K requirements, N becomes insufficient for the crop needs. So, a judicial and targeted application is critical for ensuring crop productivity without compromising environmental stewardship.

Soil organic carbon sequestration in agricultural soils is an important strategy for mitigating climate change. Continuous application of BL has shown promise in enhancing topsoil as well as subsoil C sequestration. Despite its potential, the extent of SOC sequestration by BL amendment, especially in the subsoil, is poorly understood in the southeastern US, a region that favors more C decomposition than accumulation due to its unique climatic and soil characteristics. Recent studies by Nyakatawa et al. (2001), Samson et al. (2020), and Mikha et al. (2017) suggest that continuous BL application can boost SOC stocks in the topsoil and subsoil, not only in the POM fraction but also in the MAOM fraction. However, understanding the interactions between different rates of BL application, total SOC build-up and its distribution in MAOM-POM fractions, and at various depths is needed to assess the climate-mitigation potential of BL.

So, BL which is an excellent soil amendment not only offers agronomic benefits but also supports environmental stewardship and climate change mitigation. However, efficient use of this valuable resource is critical to maximize its multi-faceted benefits. There remains an important knowledge gap to assess the rate of BL needed to optimize the benefits in the no-till cropping systems of the southeastern US. This research will address this knowledge gap by leveraging a field experiment in western Tennessee on corn-soybean rotational system.

The specific objectives of this study:

Objective 1: Determine the changes in soil fertility by the application of different rates of BL in comparison to the recommended rates of inorganic fertilizers in a no-till corn-soybean rotation system.

Hypothesis: 1) Applying higher rates of BL will fulfill the N requirement of corn but may lead to the accumulation of P and K in the soil, and **2)** The residual nutrients from the BL applied to the corn will sufficiently meet the macro and micronutrient needs of the subsequent crops.

Objective 2: Quantify the depth-wise changes in total SOC and its functional fractions with the application of different rates of BL in a no-till corn-soybean rotation system.

Hypothesis: Applying higher rates of BL in a no-till system will significantly increase SOC levels in the entire soil profile compared to synthetic fertilizer application. The organic inputs from BL enhance MBC and reduces soil bulk density leading to improved soil quality and soil structure compared to synthetic N fertilizers.

Objective 3: Evaluate the corn and soybean yields in response to different rates of BL application compared to inorganic fertilizers.

Hypothesis: Corn yields will increase with increasing rates of BL application and the highest rate of BL will result in corn yields comparable to those obtained with inorganic fertilizers, and **2)** Soybean yields will improve in later years due to the carryover of macro and micronutrients from the BL applied in previous years.

4. MATERIALS AND METHODS

4.1 Study Site and Experimental Design

This study was conducted at the University of Tennessee's AgResearch and Education Center in Milan, TN (35.9334701, -88.7221380) on an ongoing corn-soybean rotation field trial initiated in 2019 under no-till management. Prior to 2019, this site was under continuous corn-soybean rotation managed with inorganic fertilizers. The experimental field consists of 4 blocks divided into 24 plots, each measuring 4.572 m × 9.144 m, with 6 rows per block. Corn was planted in the years 2019, 2021, and 2023, while soybean was planted in the years 2020, and 2022. The soil at the site is classified as Providence Silt Loam (fine-silty, mixed, active, thermic Oxyaquic Fragiudalfs) with a 2% slope. The experiment followed a randomized complete block design with four blocks and six treatments. The treatments included four rates of BL applied before planting corn (2 tons $acre^{-1}$, 3 tons $acre^{-1}$, 4 tons $acre^{-1}$, and 7 tons $acre^{-1}$) (T1, T2, and T3 in (**Fig. 1**) and two control treatments: a positive control, which received synthetic N at planting (32 lbs $acre^{-1}$) and at the V6 growth stage (52 lbs $acre^{-1}$), along with P, K, and sulfur (S) based on soil test recommendations; and a negative control, which did not receive synthetic N fertilizer or BL but was amended with P, K, and S according to soil test results. During the soybean phase of the rotation, no fertilizers were applied.

4.2 Soil Sampling and Analysis:

Soil sample collection times varied depending on the measurement. For macro- and micronutrient analysis, samples were collected in 2022 (S_1) and 2023 (S_2), 1 to 3 weeks before soybean and corn planting, from a depth of 0-15 cm. Soil NO_3^-N samples were collected from a depth of 0-30 cm prior to soybean planting in 2022 (S_1) and at the V4 stage of corn in 2023 (S_2). For these analyses, approximately 10 random samples were taken per plot and composited into a single sample per

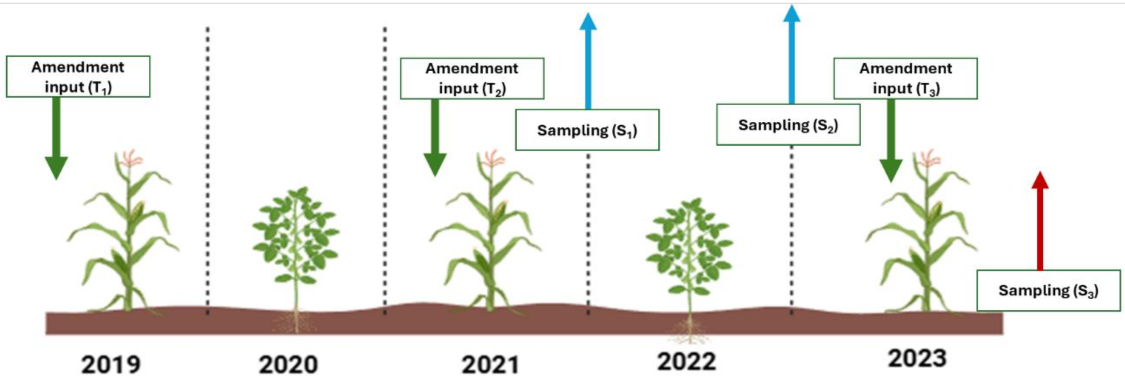

Figure 1. Timeline of broiler litter application and soil sampling in a no-till corn-soybean rotation (2019-2023).

plot. The amendment application and soil sampling timing were such that the treatment effects measured in 2022 (S_1) reflect amendments applied in 2021 (T_2) (one year post-application), while those in 2023 reflect the residual effects of amendments applied in 2021 (two years post-application). The undisturbed soil cores (3 per plot) for bulk density (BD), SOC, and MBC were collected at the end of 2023 growing season in October (S_3).

For the analysis of macro and micronutrients, 5 g of air-dried soils were extracted using Mehlich-1 solution. The soil-solution mixture was shaken on a Thermo Scientific MaxQ 2000 shaker at 180 rpm for 5 minutes. After shaking, the suspension was filtered, and the filtrate was analyzed using Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES), according to the procedure outlined by Sikora and Moore (2014). Soil NO_3^- N was determined by extracting 2 g of soil with 50 mL of 2 N KCl following the method described by Mulvaney (1996). The soil-KCl mixture was shaken for 1 hour, and the resulting extract was filtered. The concentration of NO_3^- in the extract was then quantified using the Timberline Instruments TL2800, an automated analyzer employing a colorimetric method.

For the determination of SOC, MBC, and BD, three random soil cores from each of the 24 experimental units were collected using a truck-mounted hydraulic probe 8 cm diameter to a depth of 60 cm. The cores were sectioned into depth increments of 0-5 cm, 5-15 cm, 15-30 cm, and 30-60 cm. The three cores from each depth increment were composited to a single sample per plot. To measure soil bulk density (BD), the dry weight of the soil and the soil volume were determined following the method of Grossman and Reinsch (2018). After collecting the wet weight of the soil samples, a representative subsample was taken to determine the moisture content by oven-drying these subsamples at 105°C until a constant weight was achieved. The moisture content was calculated as the difference between the wet and dry weight of the subsample. The dry mass of the entire soil sample was then estimated based on the moisture content and the wet weight of the entire soil sample. Bulk density was calculated using the formula:

$BD = Ws / Vs$, where Ws is the weight of dry soil (g), and Vs is the soil volume (cm^3).

A portion of the composited fresh sample was stored at 4°C for microbial biomass carbon analyses, while the remainder was air-dried and passed through a 2 mm sieve for the determination of total SOC. A sub-sample of the air-dried samples was then pulverized using a vial rotator (SampleTek, Model 200), and SOC was determined by dry combustion at 950°C using a CN analyzer (Elementar vario MAX cube, Hanau, Germany). Soil organic carbon was assumed to be equal to total soil C as soil pH was < 7.1 (Al-Kaisi et al., 2005).

Soil microbial biomass carbon (MBC) was determined using the chloroform fumigation-extraction method (Vance et al., 1987). Five grams of field-moist soil were weighed and transferred into a 50 mL centrifuge tube, then fumigated with 2 mL of chloroform for 24 hours. Following fumigation, the soils were extracted with 0.5 M K_2SO_4. Non-fumigated soils were also extracted in the same manner. The MBC in the K_2SO_4 extracts was quantified using a Synergy™ multimode plate reader (BioTek) through colorimetry. Specifically, the absorbance values from the plate reader were converted to C concentrations using calibrated standard curves prepared with K_2SO_4 and oxalic acid. The MBC (mg C kg^{-1} soil) was calculated using the following equation:

$$\mathrm{MBC} = 0.45 \times \left(\frac{C_f \times 0.045}{Weight\ of\ dry\ soil} - \frac{C0 \times 0.045}{Weight\ of\ dry\ soil}\right)$$

where, C_f denotes C concentration of fumigated samples and C0 denotes C concentration of un-fumigated samples. 0.45 is a constant used to represent the efficiency of extraction (Beck et al., 1997).

4.3 Crop Yield

To quantify yield, both corn and soybean were harvested at maturity when the grains reached their optimal moisture levels (15.5% for corn and 13% for soybean). To minimize edge effects, only the two center rows of corn in each plot were harvested for yield calculations. The total weight of the harvested material was recorded, and the yield in Mg ha^{-1} was calculated.

4.4 Statistical Analysis

The study was conducted using a Randomized Complete Block Design (RCBD) with six treatments and four replications. The response variables included soil BD, SOC concentration, MBC, NO_3^- N, macro- and micronutrients, and corn and soybean yields. All statistical analyses were performed using SAS 9.4 (SAS Institute, Cary, NC). A one-way ANOVA was conducted to identify statistically significant differences in the response variables across the six treatments. The effects were considered significant at $p \leq 0.05$. Tukey's Honestly Significant Difference (HSD) test was then used for pairwise comparisons to further analyze differences among treatments. Descriptive statistics, including the mean and standard error of the mean (SEM), were calculated for each response variable within each treatment group using the PROC MEANS procedure in SAS. These statistics provided a comprehensive overview of the central tendency and variability of data.

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5. RESULTS

Note: All comparisons discussed below were evaluated at a significance level of $P \leq 0.05$.

5.1 Effect of Broiler Litter Application on Soil Nitrate Levels

For measuring soil NO_3^- levels, samples were collected during the V4 stage of corn and pre-planting of soybean. Soil NO_3^- levels measured before soybean planting, showed a marked increase with both higher rates of BL application (7 tons acre^{-1} and 4 tons acre^{-1}) compared to the negative control (13.58 lbs acre^{-1}) (**Fig. 2**). However, there were no significant differences in NO_3^- levels among the lower BL rates when compared to both the positive and negative controls. During the V4 corn phase, soil NO_3^- levels varied among the treatments, the treatment receiving 7 tons acre^{-1} of BL was higher than the negative control (25.25 lbs acre^{-1}) and all other BL treatments (2 tons acre^{-1}, 3 tons acre^{-1}, and 4 tons acre^{-1}), which ranged between 29 and 34.75 lbs acre^{-1}. However, the NO_3^- levels with the 7 tons acre^{-1} BL treatment were similar to the positive control, which recorded 62 lbs acre^{-1} and differed by only ~1 lbs acre.

5.2 Effect of Broiler Litter Application on Soil Macronutrient Levels

The two highest BL application rates (4 tons acre^{-1} and 7 tons acre^{-1}) increased soil extractable P levels compared to the positive and negative controls in soils sampled before soybean planting (**Fig. 4**). In particular, 7 tons acre^{-1} increased soil P levels by more than 190% compared to the control treatments. Soil K levels were higher with the 7 tons acre^{-1} BL treatment (116 lbs acre^{-1}), which was 35% higher compared to the positive control and 45% higher than the negative control. Soil Ca and Mg showed no response to synthetic N fertilizer or BL application at soybean phase.

Mehlich-1 extractable P was highest in plots receiving 7 tons acre^{-1} of BL (98 lbs acre^{-1}) before corn planting, which was 246% and 166% higher than positive and negative controls, respectively (**Fig. 3**). The lower BL application rates resulted in extractable P levels that were comparable to both the positive and negative controls, both of which had received synthetic P fertilizer. Extractable soil K levels followed a similar pattern, with the highest levels observed at 7 tons acre^{-1} of BL (140 lbs acre^{-1}) and an increasing trend of extractable K with increased BL rates. The K levels at 7 tons acre^{-1} were higher than that at 4 tons acre^{-1} (96 lbs acre^{-1}) and both these treatments accumulated greater K than the negative control and positive controls. No differences in soil K levels were observed between the two lower BL rates (2 tons acre^{-1} and 3 tons acre^{-1}) and the control treatments. Soil extractable Ca levels before corn planting followed a similar trend to those observed before soybean planting, with no significant differences among treatments. However, unlike the pattern seen for Mg during the soybean phase, during corn phase the 7 tons acre^{-1} BL treatment resulted in higher extractable Mg levels (159.25 lbs acre^{-1}) compared to all other treatments. The 4 tons acre^{-1} BL treatment also showed higher Mg levels (129.25 lbs acre^{-1}) than the positive control. However, no differences in Mg levels were observed between the 4 tons acre^{-1} BL treatment and the other lower BL treatments or the negative control.

5.3 Effect of Broiler Litter Application on Soil Micronutrient Levels

Before the soybean planting, Mehlich-1 extractable micronutrient levels in the soil remained largely unaffected by treatments, except for Zn (**Table 2**). Plots receiving 7 tons acre^{-1}, and 4 tons acre^{-1} of BL exhibited higher Zn levels compared to the controls and the 2 tons acre^{-1} BL treatment. Micronutrients such as Mn, Cu, and Na showed no response to either synthetic fertilizer application or varying rates of BL. Similarly, B and Fe in the soil also remained unaffected by the different treatment.

Except for soil Fe and Cu levels, all other micronutrients responded positively to BL application before corn planting and the concentrations showed a general increase with increasing rates of BL (**Table 1**). Higher BL application rates (4 tons acre^{-1} and 7 tons acre^{-1}) increased the soil B levels compared to control treatments, but the lower BL treatment rates (2 tons acre^{-1} and 3 tons acre^{-1}) was similar B to both positive and negative control treatments. Soil Mn was the highest at the 7 tons acre^{-1} BL rate (27.75 lbs acre^{-1}) compared to the negative (19.5 lbs acre^{-1}) and positive (20.5 lbs acre^{-1}) controls. Similarly, significant increase in soil Na levels were observed only with the higher BL rate of 4 tons acre^{-1} (12 lbs acre^{-1}) and 7 tons acre^{-1} (14.25 lbs acre^{-1}) compared to positive and negative controls (8.25 lbs acre^{-1} to 8.65 lbs acre^{-1}). Extractable sodium (Na) levels at the lower BL rates were comparable to those of the control treatments. Soil zinc (Zn) levels exhibited a different pattern, with the highest BL rate (7 tons acre^{-1}) resulting in a 130% increase in Zn levels compared to the negative control. In contrast, extractable iron (Fe) and copper (Cu) levels showed no significant differences among treatments

5.4 Effect of Broiler Litter on Yield

Soybean yield did not show a statistically significant response to the treatments (**Fig. 5**), with mean yields ranging from 4.27 Mg ha^{-1} to 4.49 Mg ha^{-1} across all treatments. It is important to note that BL applications were made in 2021, a year prior to soybean planting. In contrast, corn yields increased significantly with all BL treatments compared to the negative control, with yields progressively rising as the rate of BL application increased. The 7 tons acre^{-1} BL treatment achieved the highest yield of 14.98 Mg ha^{-1}, which was similar to the yield from the positive control (14.13 Mg ha^{-1}) (**Fig. 5**). Compared to the negative control, BL treatments increased corn grain yield by 40% to 162%, demonstrating the effectiveness of BL in enhancing corn productivity

5.5 Effect of Broiler Litter on Active and Total Soil Organic Carbon and Bulk Density

The treatment effect on SOC concentration was only significant at 0-5 cm depth. At this depth, the two highest BL rates showed the highest SOC (16 g kg^{-1} to 17.4 g kg^{-1}) (**Fig. 6**). There were no differences in SOC concentration observed among other treatments which ranged from 13.5 g kg^{-1} to 14.4 g kg^{-1}. The SOC concentrations in lower depths were progressively lower than the depth above (mean values were 6.7 g kg^{-1} at 5-15 cm, 4.1 g kg^{-1} at 15-30 cm, and 2.7 g kg^{-1} at 30-60 cm), but remained similar among treatments in each depth, indicating minimal impact of surface applied BL or synthetic fertilizers on SOC build-up in lower depths.

The active fraction of C, MBC, also differed by treatments at the top 5 cm soil layer only. At this depth, the highest mean MBC concentration (469.9 mg kg^{-1}) was observed in the treatment receiving 7 tons $acre^{-1}$ of BL, but this was found to be similar to MBC levels in all other BL treatments, which ranged from 373.57 mg kg^{-1} to 467.74 mg kg^{-1} (**Fig. 7**). No differences in MBC were observed when comparing the negative control (428.9 mg kg^{-1}) to the BL treatments. At the 5-15 cm depth, the negative control had lower MBC (146.08 mg kg^{-1}) compared to all other treatments. In the 15-30 cm and 30-60 cm layers, MBC levels were generally lower than in the upper depths, but no differences were found across the treatments.

A significant reduction in soil BD was observed for three out of the four rates of BL application at the 0-5 cm depth compared to negative control (**Fig. 8**). The BD among the BL rates was similar, with the mean value of 1.15 g cm^{-3}, which was similar to the positive control (1.2 g cm^{-3}). At depth sections below 5 cm, BD remained similar across all the treatments (mean value of 1.25 g cm^{-3} at 5-15 cm, 1.26 g cm^{-3} at 15-30 cm and 1.29 g cm^{-3} at 30-60 cm), indicating that the effects of surface applied BL or inorganic fertilizers on soil BD do not extend deeper into the soil profile, particularly in a no-till system, where organic matter and nutrients have limited movement into deeper layers.

6. Appendix I

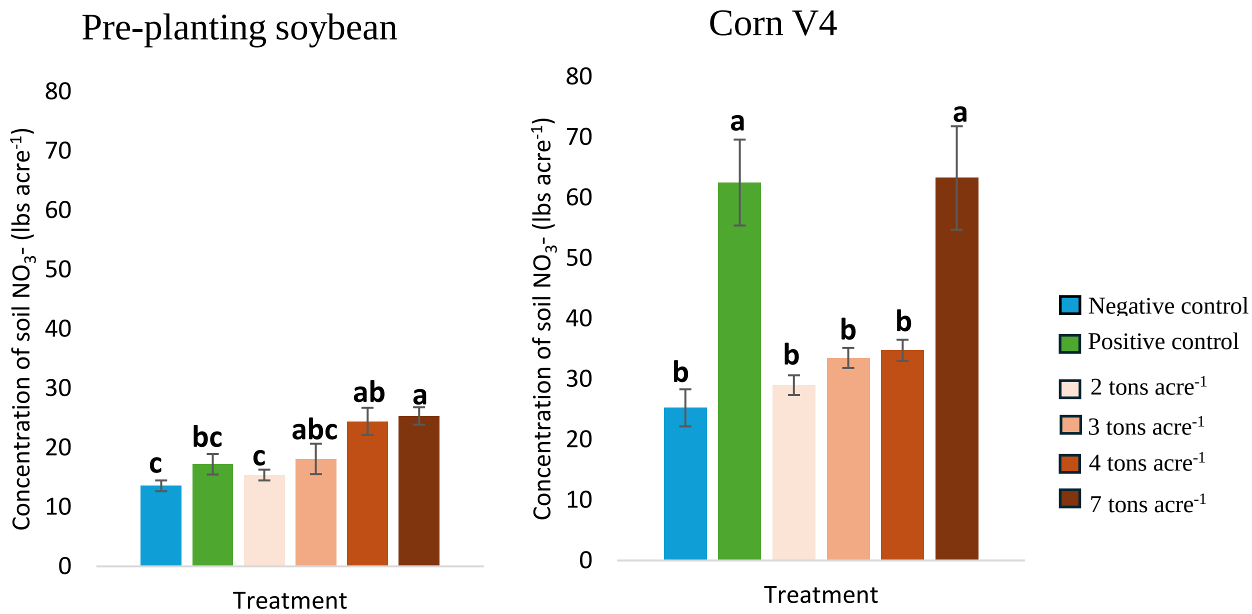

Figure 2. Response of soil NO_3^- to BL and inorganic fertilizer treatments.
Error bars represent the standard error of the mean, and different lowercase letters denote statistically different mean values based on Tukey's HSD test ($P < 0.05$).

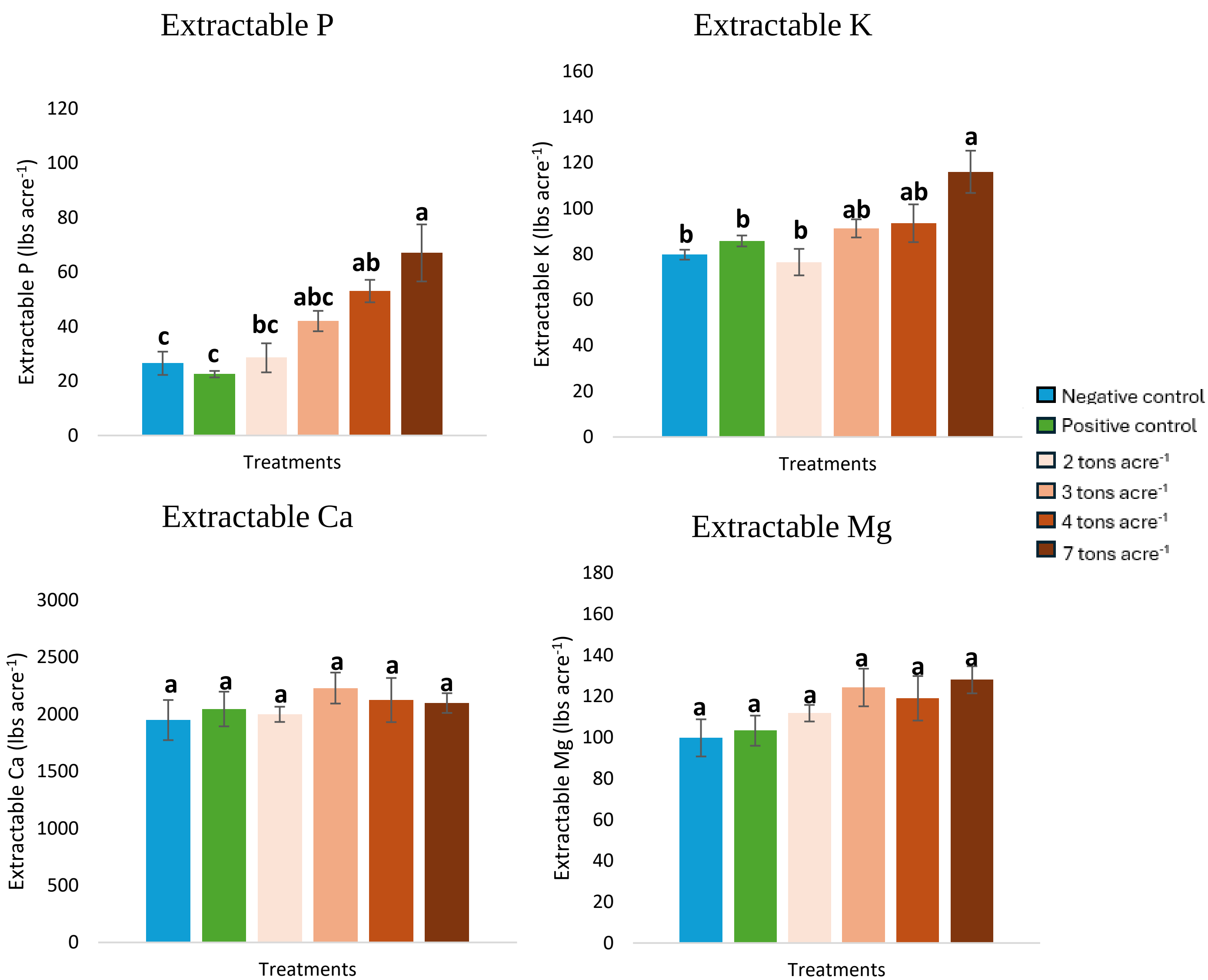

Figure 3. Mehlich-1 extractable macronutrient concentrations at soybean pre-planting. Error bars represent the standard error of the mean, and different lowercase letters denote statistically different mean values based on Tukey's HSD test ($P < 0.05$).

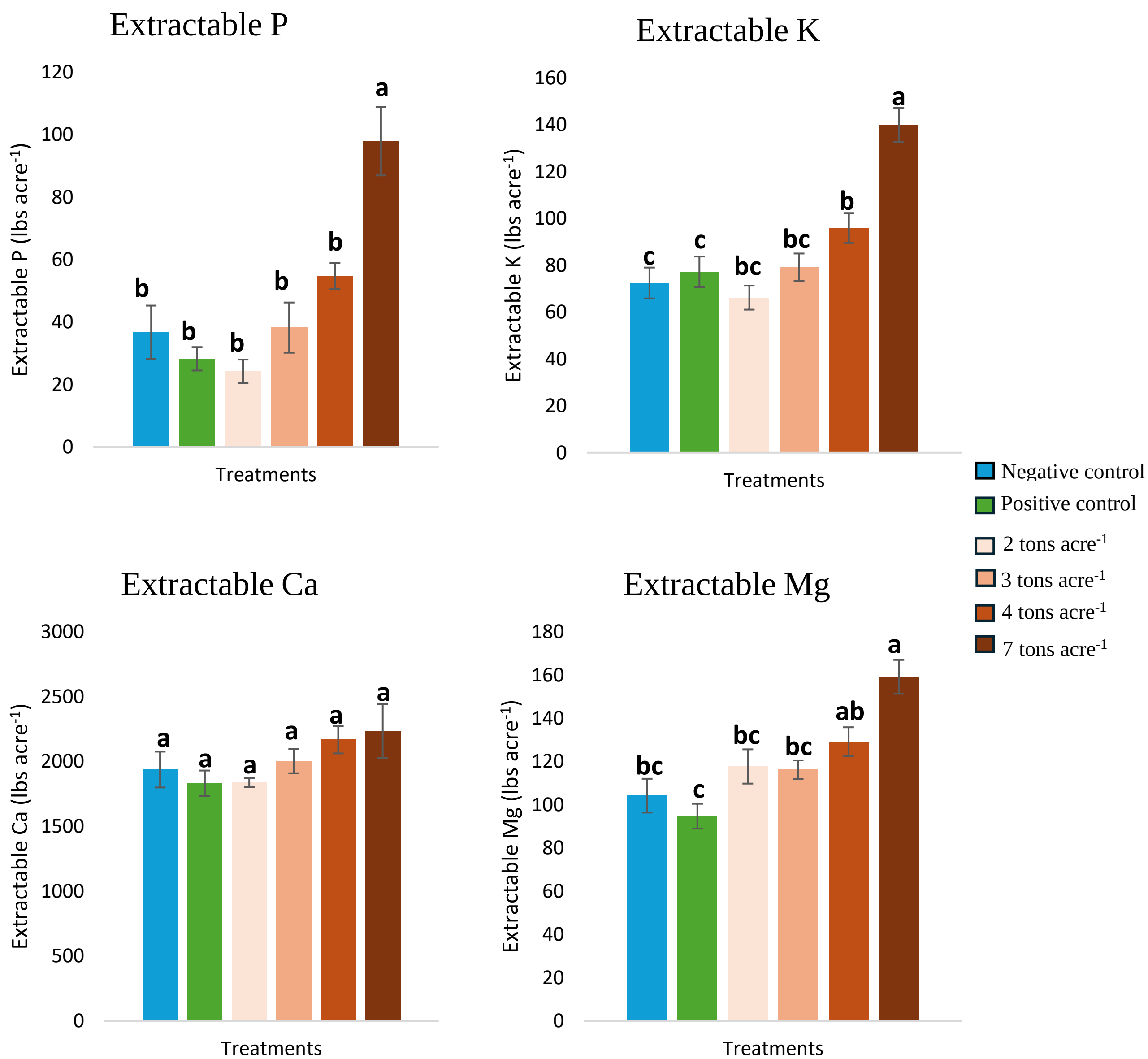

Figure 4. Mehlich-1 extractable macronutrient concentrations a corn pre-planting. Error bars represent the standard error of the mean, and different lowercase letters denote statistically different mean values based on Tukey's HSD test ($P < 0.05$).

Table 1. Mehlich-1 extractable micronutrient concentrations at corn pre-planting.

Treatment	B (lbs acre^{-1})	Fe (lbs acre^{-1})	Mn (lbs acre^{-1})	Na (lbs acre^{-1})	Zn (lbs acre^{-1})	Cu (lbs acre^{-1})
Negative control	0.6 (0.04) c	14.25 (1.03)	19.5 (0.65) b	8.65 (0.23) c	3.03 (0.27) b	1.03 (0.09)
Positive control	0.58 (0.03) c	14.25 (0.75)	20.5 (1.50) b	8.25 (0.48) c	5.07 (1.96) ab	0.98 (0.04)
BL 2 tons acre^{-1}	0.63 (0.03) c	14.5 (0.64)	21.5 (1.32) ab	10.25 (0.48) bc	3.6 (0.14) ab	1.2 (0.07)
BL 3 tons acre^{-1}	0.7 (0.04) bc	14 (0.40)	23.75 (2.78) ab	11.25 (0.86) abc	5.03 (0.74) ab	1.4 (0.05)
BL 4 tons acre^{-1}	0.78 (0.02) ab	14.25 (0.25)	25.5 (0.86) ab	12 (0.70) ab	5.03 (0.17) ab	1.38 (0.04)
BL 7 tons acre^{-1}	0.85 (0.02) a	15 (0.91)	27.75 (0.62) a	14.25 (1.10) a	7.18 (0.51) a	1.7 (0.13)

Means followed by the same lowercase letter are not significantly different at $P \leq 0.05$. Absence of lowercase letters indicates fixed effects were not significantly different. Numbers in parentheses represent the standard error of the mean (SEM).

Table 2. Mehlich-1 extractable micronutrient concentrations at soybean pre-planting.

Treatment	B (lbs acre^{-1})	Fe (lbs acre^{-1})	Mn (lbs acre^{-1})	Na (lbs acre^{-1})	Zn (lbs acre^{-1})	Cu (lbs acre^{-1})
Negative control	0.6 (0.04)	14.25 (1.03)	19.25 (3.42)	16.25 (0.60)	2.78 (0.19) c	1.1 (0.09)
Positive control	0.63 (0.02)	13.5 (0.64)	18.5 (2.02)	7.5 (0.29)	2.93 (0.17) bc	1.15 (0.06)
BL 2 tons acre^{-1}	0.65 (0.03)	15 (0.40)	22 (1.91)	11.5 (0.50)	3.2 (0.09) bc	1.28 (0.06)
BL 3 tons acre^{-1}	0.7 (0.07)	15 (0.91)	28.5 (2.84)	12.75 (0.62)	4.1 (0.25) ab	1.43 (0.06)
BL 4 tons acre^{-1}	0.65 (0.02)	14.75 (0.25)	22.5 (3.80)	12.25 (0.47)	4.4 (0.29) a	1.38 (0.06)
BL 7 tons acre^{-1}	0.75 (0.05)	16.25 (0.25)	29.25 (4.29)	15.75 (0.25)	4.95 (0.53) a	1.65 (0.10)

Means followed by the same lowercase letter are not significantly different at $P \leq 0.05$. Absence of lowercase letters indicates fixed effects were not significantly different. Numbers in parentheses represent the standard error of the mean (SEM).

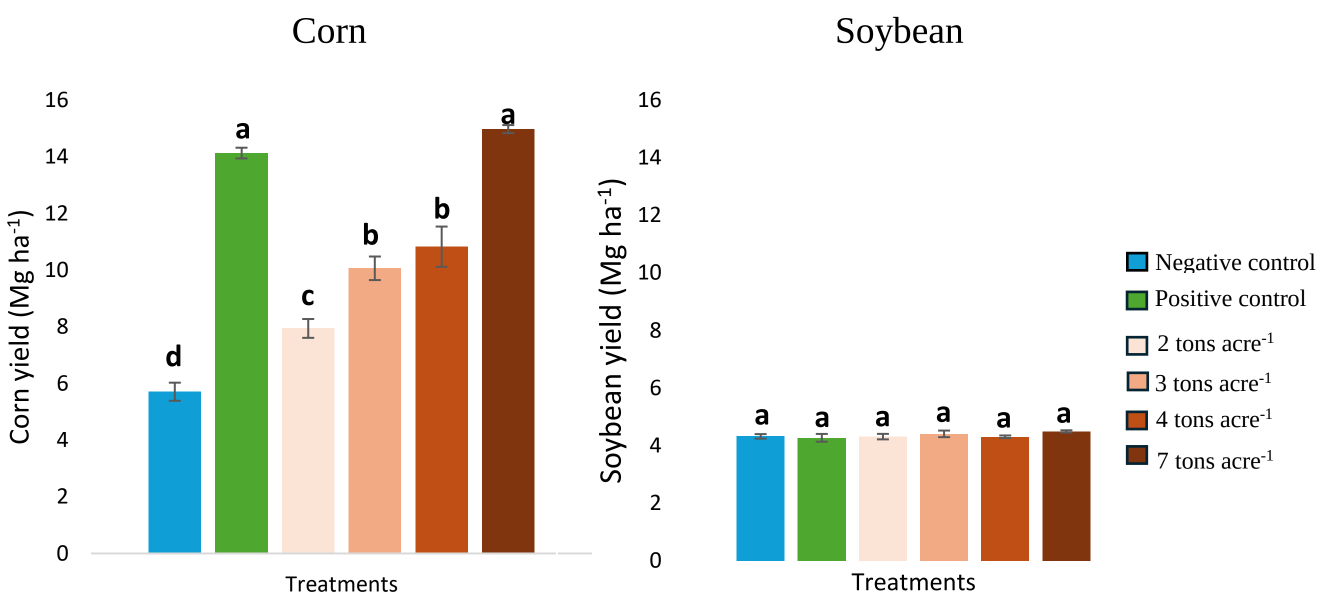

Figure 5. Corn and soybean yield response to BL and inorganic N fertilizer treatments. Error bars represent the standard error of the mean, and different lowercase letters denote statistically different mean values based on Tukey's HSD test ($P < 0.05$).

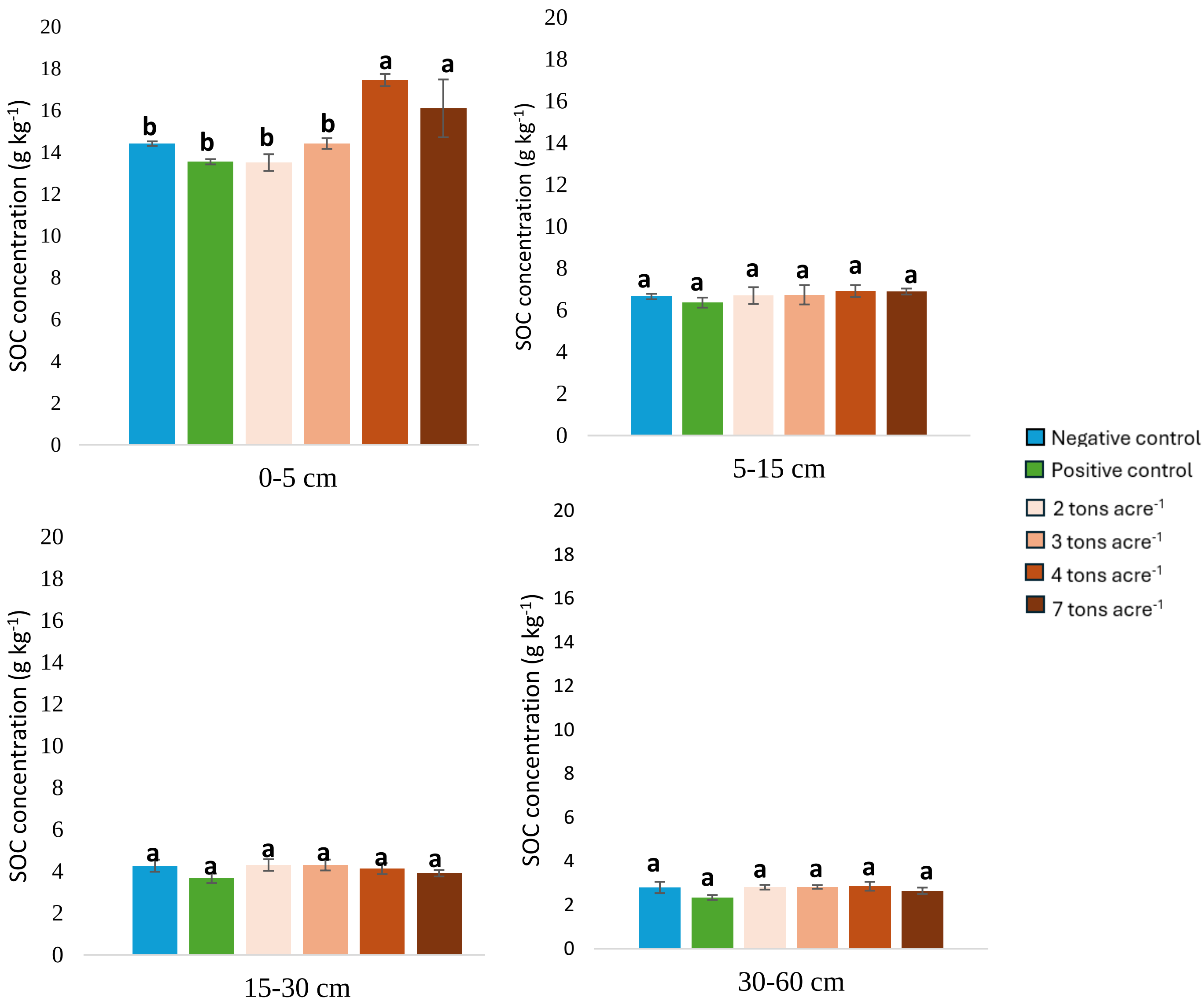

Figure 6. Effect of broiler litter and inorganic fertilizer application on soil organic carbon concentration in different soil depths.
Error bars represent the standard error of the mean, and different lowercase letters denote statistically different mean values based on Tukey's HSD test ($P < 0.05$).

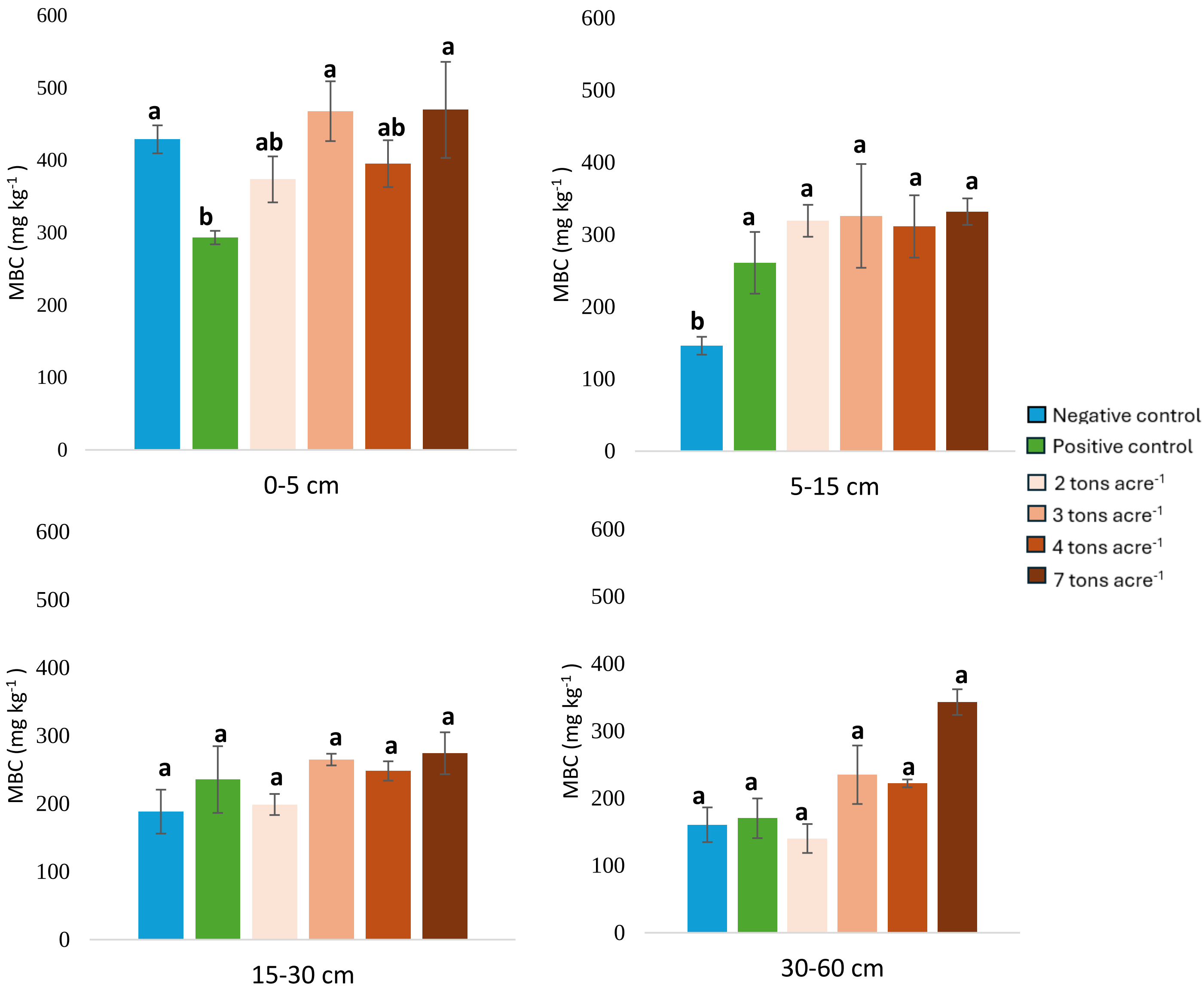

Figure 7. Effect of broiler litter and inorganic fertilizer application on soil MBC in different soil depths.

Error bars represent the standard error of the mean, and different lowercase letters denote statistically different mean values based on Tukey's HSD test ($P < 0.05$).

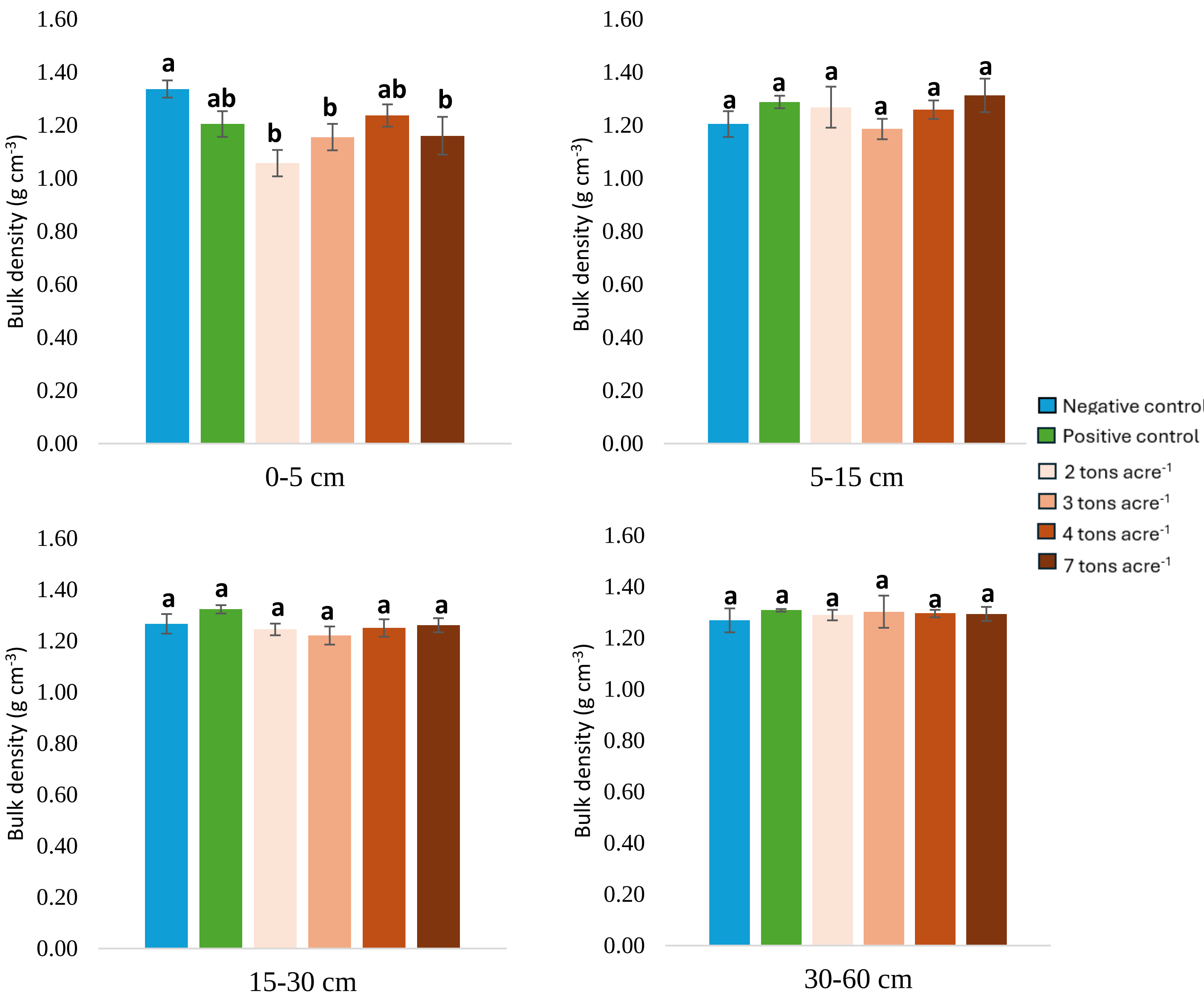

Figure 8. Effect of broiler litter and inorganic fertilizer application on soil bulk density collected at different soil depths.

Error bars represent the standard error of the mean, and different lowercase letters denote statistically different mean values based on Tukey's HSD test ($P < 0.05$).

7. DISCUSSIONS

7.1 Effect of Broiler Litter Application on Soil Fertility

7.1.1 Soil Nitrate-N

The soil NO_3^- concentrations observed pre-planting soybean in 2022 indicate a significant residual effect of the BL amendments applied in 2021. Treatments with higher BL application rates (4 tons acre^{-1} and & tons acre^{-1}) exhibited higher NO_3^- concentrations compared to the lower BL rates and the negative control. This is consistent with the findings of Kingery et al. (1994), who found that the slow mineralization of organic N in BL can contribute to sustained increases in soil NO_3^- over time. The higher NO_3^- levels with higher BL rates reflect the gradual release of N through organic matter mineralization, which persisted into the subsequent cropping season. In contrast, the lower BL rates did not result in significant increases in soil NO_3^- levels compared to the negative control, this could be because the quantity of N supplied at these rates were insufficient to improve NO_3^- concentrations beyond what was supplied by synthetic fertilizers or inherent soil fertility. As noted by Eghball and Power (1999), BL at lower application rates tends to primarily support P and K availability, with limited immediate effects on soil N due to the slow mineralization of organic N.

Soil NO_3^- levels pre-planting corn showed an improvement compared to the soybean phase, likely due to N fixation by soybean and the slower mineralization of BL. The gradual release of N from BL over time resulted in greater NO_3^- availability during the corn phase (2023), which occurred two years after the BL application in 2021, compared to the soybean phase (2022). The significantly higher NO_3^- concentrations observed at the highest BL rate (7 tons acre^{-1}) compared to other BL rates and negative control underscores the role of high BL application rates in maintaining long-term N availability. In contrast, the lack of differences found among the lower BL rates and the negative control, suggests that N mineralization from lower BL rates may not have been sufficient to produce measurable differences in NO_3^- levels. This observation is consistent with Sistani et al. (2008), who reported that the magnitude of residual N effects depends on the rate of organic amendment application. The trend of increasing soil NO_3^- concentrations with higher BL application rates at the V4 growth stage of corn (**Fig. 2**) reflects the sustained residual effects of BL on soil N availability even two years after application. This aligns with previous studies (e.g., Eghball et al., 2002) that highlighted the ability of BL to provide a slow and steady supply of organic N, which undergoes mineralization and nitrification, contributing to elevated soil NO_3^- levels over multiple growing seasons.

Interestingly, there was no difference between the highest BL rate (7 tons acre^{-1}) and the positive control. This can be attributed to the ability of synthetic N fertilizers to enhance vegetative growth in corn, leading to increased crop residue deposition in the soil. Over time, these residues decompose, mineralizing and releasing NO_3^-, which becomes more available during the corn phase. In contrast, soybean relies less on external N due to its ability to fix atmospheric N, resulting in lower utilization of soil NO_3^- during the soybean phase leaving more NO_3^- for the next crop. This effect aligns with findings by Eghball et al. (2002) and Sistani et al. (2008), who demonstrated that synthetic N fertilizers enhance biomass production, which, upon decomposition, contributes to elevated and sustained soil NO_3^- concentrations over subsequent seasons by providing both immediate and residual nutrient availability. This can also be attributed to findings by Franzluebbers (2002), who reported that no-till systems often exhibit delayed N mineralization due to reduced soil disturbance. This slower release likely contributed to the gradual availability of N over time, resulting in the residual NO_3^- levels observed two years after the initial BL application. Achieving soil NO_3^- concentrations comparable to those provided by synthetic N fertilizers required the highest BL application rate (7 tons acre^{-1}). These findings underscores the importance of applying adequate rates of BL to meet the N demands of subsequent crops and maintain soil fertility in the long term. While BL amendments are effective at enhancing soil N availability, their timing and rate of N release may not always align with crop N uptake, potentially leading to inefficiencies.

7.1.2 Soil Macronutrients

The application of BL before corn planting in 2021 influenced soil macronutrient levels in the subsequent year. Before soybean planting, soil extractable P levels remained elevated at higher BL rates, with the 7 tons acre^{-1} treatment still showing a "high" mean soil P concentration of 67 lbs acre^{-1} (Duncan et al., 2015; Savoy and Joines, 2009), potentially contributing to P runoff risks (Fixen, 2009). Soil K levels showed a significant response to BL application prior to soybean planting, (**Fig. 4**), K is taken up in large quantities by crops such as corn; however, a significant portion remains in plant residues. In our system, when these residues are returned to the soil, K is gradually released as they decompose (Havlin et al., 2014; Roberts, 2008). Unlike N and P, K does not form stable organic complexes in the soil. As a result, its availability is closely linked to the breakdown of plant residues, making K readily accessible for future crop uptake (Mengel & Kirkby, 2001). The effects of BL on other macronutrients (Ca and Mg) were less pronounced. For extractable Ca levels, no significant differences were observed during both the corn and soybean phases. In contrast, extractable Mg levels increased with higher BL application rates during the corn phase, whereas no differences were observed during the soybean phase. This discrepancy could be attributed to the increased ability of Ca to form stable complexes in soil, making it less extractable, particularly when using weaker extractants like Mehlich-1. Kingery et al. (1994) suggested that the solubility of Mg is generally higher than that of Ca, which may explain why Mg availability increased during the corn phase. The observed trends may also be linked to the slow mineralization of organic forms of these cations in BL, which releases nutrients gradually.

The application of BL significantly affected Mehlich-1 extractable macronutrient concentrations in the soil at 0-15 cm depth prior to corn planting in 2023, especially for P and K. Both nutrients displayed an increasing trend with higher BL rates. The highest BL application rate (7 tons acre^{-1}) led to a 246% and 166% increase in extractable P compared to the positive and negative controls, respectively. (**Fig. 3**). This increase is likely due to enhanced organic matter content and microbial activity from BL, which promotes the mineralization of organic P into extractable P (Kingery et al., 1994; Sistani et al., 2008). Lower BL rates (2-4 tons acre^{-1}) showed no significant increase in P levels compared to controls, suggesting that a threshold level of organic input is required to improve P availability (Sharpley et al., 1994). At the highest BL rate, soil P concentrations during the corn phase reached 98 lbs acre^{-1}, categorized as "high" according to Mehlich-1 extraction standards where minimal crop response is observed, while soil K levels significantly increased with BL rates of 4 tons acre^{-1} and 7 tons acre^{-1}, with the highest rate resulting in "medium" K levels where there is some response from the crop (Duncan et al., 2015; Savoy and Joines, 2009). The lower BL treatments resulted in "low" K levels, insufficient to meet crop demands. Although the increases in P and K with higher BL rates improve soil fertility, they underscore the need for careful management to avoid nutrient overloading and insufficiency. Elevated P levels, in particular, can lead to environmental risks like runoff and eutrophication in nearby water bodies (Slaton et al., 2013; Sharpley et al., 2001). Therefore, balancing soil fertility benefits with environmental stewardship is crucial (Mallarino et al., 2011).

7.1.3 Soil Micronutrients

Before soybean planting, the response of Mehlich-1 extractable micronutrients to BL was limited (**Table 2**), this muted response could be due to vigorous uptake of nutrients by the preceding corn crop as they become available from the organic form in BL (Mullins et al., 2002). However soil Zn levels increased significantly in plots that received higher BL application rates compared to the negative control, which could be attributed to the capacity of Zn to bind to soil particles and become less available over time, especially in soils with high clay content or neutral to alkaline pH (Kabata-Pendias, 2010).

The application of BL significantly influenced Mehlich-1 extractable micronutrient concentrations in the soil prior to corn planting, particularly for B, Mn, Na, and Zn (**Table 1**). Micronutrient availability generally increased with higher BL application rates, especially at 4 tons acre^{-1} and 7 tons acre^{-1}, highlighting BL's role in enhancing soil fertility through slow mineralization of nutrients from organic to inorganic forms (Kingery et al., 1994; Sistani et al., 2008). Studies by Gaskin et al. (2003) and Mullins et al. (2002) also demonstrated that organic amendments like BL can significantly boost the availability of micronutrients, particularly B and Mn, in soils with low native levels of these nutrients. Zinc availability also improved with higher BL applications, with the 7 tons acre^{-1} rate resulting in a 130% increase compared to the negative control. The absence of significant increases in B, Mn, Na, and Zn at lower BL rates (2-4 tons acre^{-1}) compared to the controls suggests that higher rates of BL application is required to have sustained micronutrient availability (Sistani et al., 2008; Kingery et al., 1994). In contrast, no significant treatment differences were observed for Fe and Cu, though BL-treated soils did show numerically higher Cu levels than the control treatments. The lack of significant changes in Fe and Cu may be due to their high background levels in the soil or their low mobility and strong binding to soil organic matter, especially in the case of Cu, which is tightly bound to organic matter and less extractable by Mehlich-1 (Alloway, 2008). Fe availability, on the other hand, is often influenced by soil pH and redox conditions rather than management practices (Marschner, 1995).

7.2 Effect of Broiler Litter Application on Grain Yield

Despite the residual NO_3^- available in the soil at higher BL rates (4 tons acre^{-1} and 7 tons acre^{-1}), soybean yields showed no significant response to either BL or synthetic N fertilizer treatments. Yields remained consistent across all treatments, ranging from 4.3 to 4.5 Mg ha^{-1} (**Fig. 5**). While N is typically the most yield-limiting nutrient for many crops, soybeans do not rely heavily on external N inputs due to their symbiotic relationship with N-fixing rhizobia (Salvagiotti et al., 2008). The absence of yield differences in soybean can likely be attributed to their N-fixing ability, which allows them to meet their N requirements through biological fixation, reducing the effectiveness of additional N inputs from BL or synthetic fertilizers (Harper, 1987; Ciampitti & Salvagiotti, 2018). Legumes like soybean can also downregulate symbiotic N fixation when increased NO_3^- is available in the soil, which may also be a possible explanation to the lack of yield response in soybeans (Rochester et al., 2001). Additionally, soybeans often prioritize N from biological fixation over soil N, particularly when nodulation is robust, further reducing their reliance on external N sources (Hungria & Vargas, 2000). Additionally, micronutrient availability prior to soybean planting did not exhibit significant changes, which may have further contributed to the lack of yield response across treatments (Havlin et al., 2014).

At the same time, the application of BL in 2021 had a significant impact on corn yields. Corn yields increased consistently with BL application rates compared to the negative control, with the highest yields observed at the maximum BL rate of 7 tons acre^{-1}. This yield increase is comparable to the positive control, which received synthetic N fertilization, suggesting that BL application can sustain NO_3^-N levels in soil to meet corn-N demands. (**Fig. 5**). The strong yield response to BL application can be attributed to the steady mineralization of mineral N from the organic N in the BL, providing a consistent N supply throughout the growing season (Adeli et al., 2022; Moore et al., 2018). This slow-release pattern aligns well with corn's critical growth stages, particularly during early vegetative stages when N demand is high. The increased soil NO_3^- levels observed at higher BL rates at V4 stage of corn planting support this explanation, as the N made available through BL mineralization enhanced corn growth and ultimately yield (Eghball et al., 2002; Franzluebbers, 2002). Corn also has a relatively high demand for micronutrients, and the enhanced availability of these nutrients, especially with higher rates of BL likely contributed to improved yield.

7.3 Effect of Broiler Litter Application on Active and Total Soil Organic Carbon and Bulk Density

The application of BL in 2021 had significant impact on SOC, MBC, and BD at the 0-5 cm soil depth, with higher BL rates (4- and 7-tons acre^{-1}) showing notable improvements in these soil properties. This response was predominantly observed in the topsoil, which could be attributed to the no-till systems practiced, where organic inputs tend to remain concentrated in the upper layers due to minimal soil disturbance (Franzluebbers, 2002). Total SOC concentrations increased significantly at the 0-5 cm depth with the two highest BL rates (**Fig. 6**), aligning with previous studies that have demonstrated the positive impact of organic amendments, like BL, on SOC sequestration (Mikha and Rice, 2004). The addition of carbon and nutrient-rich BL not only provides a direct source of organic matter but also stimulates microbial activity, promoting SOC accrual through the formation of organo-mineral complexes. The absence of significant differences in SOC between the lower BL rates (0.8-1.21 tons acre^{-1}) and control treatments suggests that a minimum level of organic input is required to achieve measurable increases in SOC. Similar findings were reported by studies showing that only higher rates of organic amendments significantly increased SOC, while lower rates had minimal impact. This is likely because higher application rates provide sufficient organic matter inputs to enhance microbial activity and promote the formation of stable organo-mineral complexes, which are key mechanisms for SOC sequestration (Kingery et al., 1994; Franzluebbers et al., 2000; Sistani et al., 2008; Adeli et al., 2008).

Soil BD was significantly reduced at 0-5 cm depth in response to BL application, indicating improved soil structure and reduced compaction due to organic matter input. The average BD across BL treatments was lower than both the positive and negative control treatments, highlighting the role of BL in improving soil physical properties (Blanco-Canqui & Lal, 2009) (**Fig. 8**). While all BL treatments resulted in significantly lower BD compared to the negative control, there were no significant differences between BL treatments and the positive control. This could be attributed to the high levels of crop residue produced with synthetic N fertilizers, which were returned to the soil, reducing the density of the soil. (Albaladejo et al., 2013; Blanco-Canqui & Lal, 2009).

The MBC, one of the active fractions of total SOC, followed a similar trend to SOC and BD, with notable increases in response to BL application at the 0-5 cm depth (**Fig. 7**). This increase is attributed to the additional organic C provided by BL, which serves as a vital substrate for microbial growth (Zhao et al., 2021; Li et al., 2020). The stratification of MBC with higher concentrations near the surface reflects the trends observed for SOC and BD, highlighting that surface-applied organic amendments tend to enhance microbial activity in the upper soil layers (Blevins & Frye, 1993). However, the lack of substantial increases in MBC with lower BL rates compared to the negative control suggests that a significant amount of organic matter inputs is required in enhancing microbial growth under the hot and humid climatic conditions of the study region. This is consistent with findings by Insam and Domsch (1998), who observed that MBC levels recovered rapidly after reclamation on mining sites, with microbial biomass reaching over 70% of their 50-year levels within just 15 years. They noted that the relationship between SOC and microbial biomass weakens over time, with MBC becoming a smaller proportion of SOC as the system matures, indicating a saturation point where further inputs of organic matter no longer proportionally increase microbial biomass.

Interestingly, MBC levels at the 5-15 cm depth were higher in the positive control compared to the negative control, while at the 0-5 cm depth, MBC was higher in the negative control. This pattern could be attributed to the effects of synthetic N fertilizers on root growth and biomass production. Synthetic N fertilizers promote deeper root growth, resulting in greater root exudates at the 5-15 cm depth, which serve as substrates for microbial activity, thereby increasing MBC at this depth (Lu et al., 2011). While at the 0-5 cm depth, higher MBC in the negative control compared to the positive control could be due to the absence of synthetic N inputs, which can sometimes inhibit microbial activity by altering the soil pH or microbial community composition (Ramirez et al., 2010). Furthermore, without synthetic fertilizers, the negative control may rely more on organic matter mineralization as a nutrient source, resulting in increased microbial activity at the surface layer where organic residues accumulate. This highlights the contrasting effects of synthetic N on microbial dynamics at different soil depths.

The reduction in BD with higher rates of BL application at the surface layer likely improved soil porosity and aeration, creating conditions more conducive to microbial growth (Franzluebbers, 2002). Additionally, the increase in organic matter with higher BL application at the surface provided a sustained source of organic C for microbial metabolism. Blevins & Frye (1993) also found that in no-till systems, where surface-applied amendments concentrate in the upper layers, microbial growth and activity typically peak at the surface, corresponding with higher organic matter content and reduced BD. This interconnected improvements in BD, SOC, and MBC underscore the crucial role of organic amendments like BL in enhancing soil health at the surface layer in no-till systems and contribute to the long-term sustainable productivity and climate resiliency. At deeper soil depths (5-15 cm, 15-30 cm, and 30-60 cm), there were no significant changes in SOC, MBC, or BD across treatments. SOC concentrations were markedly lower in the subsoil, with SOC at 0-5 cm being 122%, 264%, and 451% higher than at the 5-15 cm, 15-30 cm, and 30-60 cm depths, respectively across treatments. This stratification is well-documented in no-till systems, where surface-applied amendments remain concentrated in the top layers due to limited soil disturbance and vertical movement (Blevins & Frye, 1993; Jin et al., 2020).

8. CONCLUSIONS

This results from the study highlights the potential of BL as a sustainable nutrient source for no-till systems, with residual benefits extending across multiple cropping seasons. The findings underscore the importance of application rates and soil management practices in optimizing the effectiveness of BL as an organic amendment. Higher BL application rates (4- and 7-tons acre^{-1}) demonstrated significant improvements in soil NO_3^- availability, which persisted into subsequent growing seasons, emphasizing its role as a slow-release N source.

However, the delayed mineralization of BL and the lack of immediate synchrony between N release and crop demand remain key limitations, necessitating further refinement in application timing to maximize nutrient efficiency.

Residual effects of BL application were more pronounced for corn yields, as the slow-release nature of N from BL effectively supported corn productivity, achieving yields comparable to synthetic N fertilizers. In contrast, soybeans, which primarily rely on atmospheric N fixation, showed no response to additional soil N from either BL or synthetic fertilizers. Notably, higher BL rates sustained soil NO_3^- levels comparable to synthetic N fertilizers, demonstrating BL's potential to meet the long-term N needs of corn. However, these residual effects were less impactful for soybeans, further emphasizing the need for crop-specific nutrient management practices when incorporating BL into rotational systems. Future research incorporating multiple soil sampling points across the growing season could better elucidate the temporal dynamics of nutrient release from BL and its interaction with crop uptake patterns. Such insights would help refine BL management practices to balance nutrient availability, minimize inefficiencies, and reduce environmental risks.

Broiler litter application significantly enhanced the availability of macronutrients, particularly P and K, as well as key micronutrients such as Zn, B, and Mn, through its gradual nutrient release. The highest BL application rate (7 tons $acre^{-1}$) notably increased extractable P and K levels, underlining its effectiveness in improving soil fertility. However, the elevated P levels at higher BL rates also emphasized the need for careful nutrient management to mitigate potential runoff risks and environmental impacts. BL application also demonstrated substantial benefits for soil health by enhancing SOC, MBC, and reducing BD in the topsoil (0-5 cm), thereby improving soil structure and organic matter content. However, these benefits were largely confined to the surface layer, as minimal effects were observed at deeper depths (5-15 cm, 15-30 cm, and 30-60 cm), consistent with the limited vertical movement of nutrients in no-till systems. Overall, BL proves to be a sustainable alternative to synthetic fertilizers for nutrient management in no-till corn-based systems. It delivers long-term benefits for soil fertility, SOC accrual, and crop productivity, particularly at higher application rates. However, maximizing these benefits while minimizing environmental risks requires strategic management of BL application rates to balance nutrient availability, soil health, and ecological sustainability.

9. FUTURE WORK

Future research should investigate the potential of BL as a nutrient source to enhance grain and seed quality in corn and soybean, specifically focusing on parameters such as protein content, oil composition, and nutrient density. This would help determine whether BL improves the nutritional value of crops beyond just yield enhancements, as compared to synthetic fertilizers. Additionally, the effects of climatic variability, including temperature and moisture fluctuations, on BL mineralization, nutrient release, and subsequent changes in SOC warrant further exploration. Understanding these interactions will help optimize BL application timing and rates, particularly in no-till systems, where soil microclimatic conditions may vary. Furthermore, it is essential to conduct a detailed benefit-cost analysis to evaluate the economic viability of using BL, considering both the agronomic benefits and the potential environmental risks, such as P runoff or nutrient overloading. Another critical area for future research is the evaluation of greenhouse gas (GHG) emissions associated with BL application, particularly carbon dioxide (CO_2) and nitrous oxide (N_2O), to assess its environmental footprint. This is especially important as some BL rates in our study did not result in significant increases in SOC, which could be partly due to microbial respiration releasing CO_2, thus limiting SOC accrual.

Therefore, it is crucial to study the evolution of CO_2 during BL decomposition to better understand carbon cycling and the potential for long-term carbon sequestration in BL-amended soils. Lastly, the long-term effects of BL on soil microbial communities need to be studied in more detail. Investigating how continuous BL application influences the populations of beneficial microbes involved in nutrient cycling, as well as the potential proliferation of pathogenic microorganisms associated with poultry waste, will provide a more comprehensive understanding of BL's impact on soil health and sustainability.

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VITA

Rahul Chandra was born into a family deeply rooted in the agricultural industry, with both parents working as professionals in the field. Growing up, he was immersed in agricultural practices, which nurtured his passion for plants and the environment. His early exposure to the trade cultivated a lifelong interest in sustainable agriculture and soil health. Rahul earned his BS in Agriculture from Tamil Nadu Agricultural University, India, where he developed a strong foundation in agronomy and crop sciences. He then pursued an MS in Crop Physiology from Acharya N.G. Ranga Agricultural University, Andhra Pradesh, India, focusing on plant responses to abiotic stresses and sustainable farming practices. To further his expertise, Rahul joined the University of Tennessee, Knoxville, where he completed a second MS in Environmental and Soil Sciences. His work there emphasized the role of soil amendments, such as broiler litter, enhancing soil health, nutrient cycling, and carbon sequestration within no-till systems. Rahul's academic journey reflects a commitment to sustainable agriculture and environmental stewardship. During his time at the University of Tennessee, Rahul served as a Graduate Teaching Assistant, mentoring students and conducting practical lab sessions in soil and plant sciences. His dedication to leadership and outreach was evident in his role as a Graduate Student Senator, where he advocated for graduate student interests at the university level. Rahul’s academic and professional accomplishments demonstrate his unwavering commitment to advancing sustainable agricultural practices, promoting soil health, and addressing global challenges in food security and environmental conservation.