Arbuscular mycorrhizal fungi inoculation and phosphorus management: Implications for crop production and soil health

by

Endy Lopes Kailer

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Abstract

Phosphorus (P) is essential for crop production, yet its limited availability in soils creates challenges for sustainable nutrient management. Arbuscular mycorrhizal fungi (AMF) form symbiotic associations with approximately 80% of terrestrial plants, improving P acquisition, influencing crop productivity, microbial communities, soil aggregation, and carbon dynamics. The potential benefits of these associations have increased interest in commercial AMF bioinoculants as a strategy to improve crop production, nutrient use efficiency and soil health. However, the performance of AMF bioinoculants depends on complex interactions not yet fully understood, and their effectiveness under field conditions remains unclear. The overall goal of this dissertation was to evaluate the effects of AMF inoculation and P fertilization on crop performance and soil health under irrigated and dryland field conditions, and to determine how wheat genetic background influences AMF inoculation responsiveness and rhizosphere dynamics under contrasting soil P levels.

A four-year field study was conducted from 2022 to 2025 in an irrigated corn–soybean rotation in soils with very low initial P levels in Scandia, KS. The findings from this study indicate that P fertilization was a stronger regulator of plant–soil–microbial responses than AMF inoculation, as increasing P fertilization consistently increased plant P uptake, grain yield, and Mehlich-3 soil P, while consistently decreasing AMF root colonization and soil AMF abundance. AMF inoculation provided its greatest benefits under low P availability, improving plant growth, P uptake, and P recovery efficiency during the first growing seasons, although these responses became less pronounced as soil P increased over time. Inoculation also increased water-stable macroaggregates, particularly under low to moderate P availability, while microbial communities, extracellular enzyme activities, soil organic carbon stocks, and total soil N were primarily

influenced by sampling year, with no consistent response to treatments. Overall, our results demonstrated the importance of balancing P inputs with AMF function and suggest that the benefits of AMF inoculation may be greater in P-deficient soils, particularly for P uptake, plant growth, and soil structure.

A second four-year field study was conducted from 2022 to 2025 under dryland continuous corn in a soil with medium initial P levels in Manhattan, KS. The findings of this study indicate that P fertilization was a stronger and more consistent regulator of AMF root colonization and soil abundance than inoculation, as both decreased as soil P levels increased. Repeated P fertilization generally increased P uptake and decreased P recovery efficiency, while responses in plant biomass and grain yield were less consistent among years. In contrast to the irrigated, low-P site, AMF inoculation had very limited and inconsistent effects on crop productivity and soil health, with responses varying among years, P rates, and growth stages. The greatest benefits of inoculation occurred in 2023, when increases in AMF root colonization at V6, P uptake, and grain yield coincided with the highest precipitation between V6 and R1, suggesting that precipitation timing may influence inoculation effectiveness under dryland conditions. At 0-5 cm, inoculation also significantly increased Mehlich-3 soil P, while water-stable macroaggregates were positively associated with soil AMF abundance, regardless of inoculation. Overall, soil P availability near sufficiency and established indigenous AMF communities likely limited the additional benefits of introduced AMF, while environmental variability may have further influenced inoculation responses, suggesting that complex interactions shape AMF bioinoculant effectiveness under dryland field conditions.

A greenhouse study was conducted to investigate AMF inoculation responsiveness in six wheat genotypes (Bob Dole, Byrd, KanMark, KS Providence, SY Monument, and Turkey Red)

with distinct root traits and the potential to influence the soil microbiome under low and high P availability. Our results indicate that wheat genotypes differed substantially in their responses to AMF inoculation and P fertilization, with significant genotype × AMF × P interactions for several traits. Inoculation improved plant growth and P uptake in select genotypes, particularly Byrd, Bob Dole, and KS Providence, while producing minimal or inconsistent responses in others. Increasing P availability generally reduced AMF root colonization, although the magnitude of suppression varied among genotypes. Soil microbial community composition and extracellular enzyme activities were primarily influenced by genotype rather than inoculation or P fertilization, with KS Providence and the landrace Turkey Red supporting greater microbial biomass, while Byrd, KS Providence, and Turkey Red generally exhibited greater extracellular enzyme activities. The findings from this study demonstrate that plant genetics strongly regulate mycorrhizal responsiveness and rhizosphere processes and emphasize the potential to integrate mycorrhizal compatibility and rhizosphere traits into breeding programs to improve nutrient use efficiency and soil health in wheat production systems.

Overall, this dissertation provides practical evidence that the benefits of AMF inoculation are strongly context-dependent and heavily influenced by complex interactions between soil P, indigenous microbial communities, plant genotype, and potentially environmental conditions. Understanding these interactions is essential for identifying conditions that favor successful inoculation and determining how AMF bioinoculants can complement agricultural management practices and crop improvement strategies. Future research should build on this evidence to develop practical guidance on when and where AMF inoculation is most likely to be beneficial, improving the reliability and agronomic value of AMF bioinoculants in sustainable agricultural systems.

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Dedication

This dissertation is dedicated to the memory of my grandfather José Milton.



Nobody prepares you for the pain of not being able to say goodbye. For being too busy and too far away chasing your dreams. Somehow, I always thought there would be more time.

If I had known that you would be taken from me so soon, I would have written down your rice pudding recipe and joined you for one more drive in your old car. You were the one who taught me how to drive, but you never got to see the joy on my face when I got my first driver's license. You drove me to my first day of college, and somehow, I always believed you would also be here on my last one.

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Chapter 1 - Introduction and Literature Review

Introduction

Feeding a growing global population with limited resources is one of the most pressing challenges facing modern agriculture. According to the United Nations (UN), the global population is projected to reach approximately 8.5 billion by 2030, placing substantial pressure on food production systems (Gerland et al., 2022). For instance, global crop demand for food, livestock feed, and biofuels is estimated to double by 2050 (Tilman et al., 2011). At the same time, intensive management practices such as frequent tillage, high fertilizer inputs, and simplified cropping systems can disrupt soil biological communities, reduce soil biodiversity, and impair soil function (Beckmann et al., 2019; Graaff et al., 2019; Kehoe et al., 2017; Tsiafouli et al., 2015).

Soil health is the continued capacity of the soil to function as a vital living ecosystem that sustains plants, animals, and humans (Doran & Zeiss, 2000). In the rhizosphere, beneficial and deleterious organisms continuously interact with one another and with plants, producing a wide range of biological responses that influence both soil processes and plant performance. As a result, crop production cannot be fully understood without considering the role of biological activity in plant metabolism and soil health (Trivedi et al., 2016). Although many of these complex relationships remain poorly understood, it is well established that fungi, protozoa, bacteria, nematodes, arthropods, and other soil organisms are closely connected through interactions that can ultimately determine the success or failure of cultivated crops (Fitter & Garbaye, 1994).

Arbuscular mycorrhizal fungi (AMF), broadly known as “ecosystem’s invisible engineers” and symbiont with around 80% of all living plants, are of particular interest due to their ability to regulate both plant nutrition and soil function (Fig. 1.1) (Smith & Read, 2008). Their ability to enhance phosphorus (P) acquisition, influence microbial communities, and alter soil structure

positions AMF as key components of sustainable agroecosystems (Fall et al., 2022; Fitter & Garbaye, 1994; Wilson et al., 2009; Zhang et al., 2024). Consequently, there is growing interest in the use of AMF-based bioinoculants to improve nutrient use efficiency and soil health (Basiru & Hijri, 2022).

Arbuscular Mycorrhizal Fungi as Drivers of Soil Function

Naturally present in most soils, AMF are microorganisms that form symbiotic associations with many plant species, in which the fungi facilitate nutrient uptake by the plant while receiving fatty acids and approximately 20% of the host plant's photosynthetically derived C in return (Bago et al., 2000; Choi et al., 2018; Hodge et al., 2010; Jakobsen & Rosendahl, 1990). The symbiosis increases plant nutrient uptake (Huang et al., 2020; Xie et al., 2020), plant growth (Cobb et al., 2016; Cofré et al., 2020; Han et al., 2025), pathogen resistance (Boyno et al., 2025; Delavaux et al., 2025; Linderman, 2000), drought resistance (Augé, 2001; Birhanu & Negussie, 2024; Malhi et al., 2021), salinity resistance (Romero-Munar et al., 2019; Santander et al., 2020), among other benefits. Beyond plant benefits, AMF also contribute to soil functioning by improving soil aggregation and soil carbon (C) storage, as well as influencing nutrient cycling, microbial activity, and overall soil health (Al-Maliki & Ebreesum, 2020; de Goede et al., 2025; Shukla et al., 2025; Wilson et al., 2009; Zhang et al., 2024).

The benefits of AMF to plant growth and nutrient cycling are well established (Smith & Read, 2008), but their synergistic and competitive interactions with other soil microorganisms remain largely unexplored, especially in cultivated fields (Fall et al., 2022). For instance, recent research shows that the type of nutrients that AMF find along their path dictates the composition of the "hyphosphere" microbial communities (Faghihinia et al., 2024). Once the roots are colonized, fungi hyphae provide access to nutrient-rich areas farther from the depletion zone,

enabling the uptake of less mobile soil nutrients such as P and zinc (Zn), as well as other nutrients such as N, K, Ca, Mg, and micronutrients (Marschner & Dell, 1994).

All interactions between the plant and the fungus begin with a complex communication process, involving a series of signals exchanged between the two organisms. The plant initiates the contact by releasing signals indicative of low P levels, which are regulated by a gene signaling network linked to hormonal responses, such as auxins and ethylene. The plant also releases a range of molecular signals, including sugars, flavonoids, amino acids, vitamins, and others, to convey its nutrient status and invite microbial interaction (Chiou & Lin, 2011; Lei et al., 2011; Rubio et al., 2009).

Studies have revealed that plants respond to P deficiency by releasing strigolactones, such as orobanchol, solanacol, and didehydro-orobanchol, into the rhizosphere (Siddiqi & Husen, 2017; Visentin et al., 2016). These signaling molecules serve as initiators of symbiosis, attracting AMF and triggering spore germination. In response to plant metabolites, AMF produce a suite of chemical signals, collectively known as "myc-factors," which initiate the colonization process (Parniske, 2008). Once the symbiosis is established, the fungal hyphae branches and arbuscules, specialized structures that facilitate nutrient exchange between the plant and the fungus, begin to be formed inside root cells to deliver inorganic P in polyphosphate granules from the extraradical mycelium to the arbuscules (Madrid-Delgado et al., 2021; Oldroyd, 2013).

There is growing interest in soil health, with AMF playing a central role in its maintenance and enhancement. Management practices that support AMF abundance and activity are therefore closely aligned with improvements in soil health. These practices include minimizing soil disturbance (e.g., no-till or reduced tillage), reducing reliance on synthetic fertilizers and pesticides, incorporating cover crops, diversifying crop rotations, selecting or breeding AMF-

responsive cultivars, and applying microbial bioinoculants, among others (Fall et al., 2022; Zhang et al., 2024).

Arbuscular Mycorrhizal Fungi and Soil Aggregation

Soil structure critically influences several important soil properties, including porosity, erosion susceptibility, gas exchange, water and nutrient transport, and C sequestration (Barthès & Roose, 2002; Oades, 1984; Rillig & Mummey, 2006; Six et al., 2000). Soil aggregates are a major component of soil structure and consist of groups of soil particles that are more strongly bound to one another than to their surroundings (Kemper & Rosenau, 1986). Soil aggregates are driven by physical, chemical, and biological forces that directly influence water-holding capacity, porosity, and infiltration (Bronick & Lal, 2005; Rillig & Mummey, 2006). Microaggregates (<0.25 mm) are formed by the adhesive properties of bacteria and fungi metabolic products, whereas macroaggregates (>0.25 mm) are formed as a by-product of the complex interactions between plant roots, soil fauna, and microorganisms and their exudates (Tisdall & Oades, 1982).

Soil aggregation is a dynamic process in which aggregates are continually formed, stabilized, and disrupted through chemical, physical, and biological mechanisms, with strong influence from soil fungi (de Goede et al., 2025). Importantly, AMF are among the most important organisms that control the formation of soil macroaggregates (Bronick & Lal, 2005; Miller & Jastrow, 2000; Rillig & Mummey, 2006). According to Morris et al. (2019), AMF promote macroaggregate formation, slow macroaggregate disintegration, and increase microaggregate turnover. For instance, Ji et al. (2019) reported that AMF inoculation increased soil aggregate stability by elevating the aggregate bond energy under both well-watered and dry conditions.

Mycorrhizal fungi stabilize soil aggregates through extraradical hyphal networks that entangle soil particles (Morris et al., 2019; Rillig et al., 2015; Tisdall et al., 1997). Fungal by-

products are also known to function as biological glues, keeping the building blocks of aggregates together, and as surface sealers (Oades & Waters, 1991; Rillig et al., 2015). In fact, Rillig et al. (2010) demonstrated that AMF mycelia increased water-stable aggregates and soil water repellency even in the absence of other soil biota. Glomalin has been heavily investigated for over three decades as an AMF-derived product that improves soil aggregation and C sequestration by acting as a persistent binding agent (Wright & Upadhyaya, 1996). However, recent studies have shown a lack of a full biochemical description, origin, and defined function of glomalin, as it contains a wide array of non-mycorrhizal compounds of presumably variable origins (Holátko et al., 2021; Irving et al., 2021). Consequently, researchers now generally refer to the extracted material as glomalin-related soil protein (GRSP), an operationally defined fraction of soil organic matter rather than a chemically characterized AMF-derived product (Holátko et al., 2021). Future research is needed to properly determine its true composition, origin, and ecological function.

Wilson et al. (2009) reported that AMF abundance was the dominant factor influencing soil aggregation in native tallgrass prairie, with a strong positive correlation between AMF hyphal abundance and soil aggregation. Similarly, AMF was found to significantly increase macroaggregate formation and slow disintegration rates, while increasing microaggregate turnover (Morris et al., 2019).

Importantly, soil aggregates are widely recognized as important regulators of SOM turnover and early indicators of environmental change, especially changes driven by management practices and soil disturbance (Lavallee et al., 2019; Six & Paustian, 2014). Soil C storage is strongly influenced by how long aggregates persist, since C protected within aggregates is protected from rapid microbial decomposition, as microbial activity is limited inside aggregates

(Six et al., 2002). AMF can also indirectly affect soil aggregation by influencing and regulating root growth, morphology, and rhizodeposition (Rillig & Mummey, 2006).

Arbuscular Mycorrhizal Fungi and Carbon Sequestration

One of the most important ecosystem services provided by soils is the capacity to store organic C derived from photosynthesis. Globally, soil systems contain approximately 2,400 Pg C, representing the largest terrestrial C pool and directly influencing climate regulation (Jobbágy & Jackson, 2000; Wiesmeier et al., 2019). For instance, soils play a major role in the global C cycle, once soil C losses increase CO_2 atmospheric levels, thereby contributing to global warming (Batjes, 1996). The amount of C sequestered in soils depends on several climate, soil, and management factors, including microbial biomass activity, edaphic properties, crop management practices, precipitation, temperature, and substrate quality, among others (Lal, 2003; Rice et al., 2024). Global temperatures are projected to increase in the next decades and understanding how C accumulates in soils is essential for soil management decisions (IPCC, 2021; Maenhout et al., 2024; Nazir et al., 2024).

Interactions between AMF and terrestrial plants directly affect soil C sequestration (Antoninka et al., 2011; Parihar et al., 2020; Rillig et al., 2001). Therefore, understanding the role of AMF in C sequestration and the global C cycle is essential, as AMF regulate the flow of approximately 5 billion tons of C every year, and represent a significant component of the global C cycle (Bago et al., 2000). The annual amount of C allocated to mycorrhizal mycelia has been estimated to approximately 36% of current fossil fuel carbon dioxide (CO_2) emissions (Hawkins et al., 2023).

Mycorrhizae directly enhance soil C sequestration by increasing net primary productivity (NPP) through improved nutrient availability, especially P and nitrogen (N), and by promoting the

formation of stable soil aggregates that protect soil organic C from microbial respiration (Hoeksema et al., 2010; Ma et al., 2025; Rillig, 2004; Six et al., 2000). Additionally, AMF builds an extensive hyphal network, contributing to 20-30% of soil microbial biomass and around 15% of the soil organic C pool (Leake et al., 2004). Ren et al. (2021) have recently reported that AMF significantly increased particulate and light fraction organic C as a direct response to well-developed external hyphae networks, glomalin, and root biomass in maize. Orwin et al. (2011) reported that AMF increased soil C storage and reduced the decomposition rates of saprotrophs due to the lack of nutrients used by the AMF.

Averill et al. (2014) found that mycorrhizal functional traits are as important as soil chemical properties and the physical protection of organic matter in regulating decomposition and soil C storage. Since AMF lack the genetic capability to produce enzymes that directly decompose organic materials for nutrient acquisition, they rely on an indirect strategy of stimulating saprotrophic microorganisms in the rhizosphere through the intentional release of plant-derived C (i.e., priming), which promotes microbial growth and extracellular enzyme activity (Frey, 2019). AMF-derived exudates include low-molecular-weight sugars and organic acids (Hawkins et al., 2023; Toljander et al., 2007). Interestingly, AMF retain plant-derived C and strategically release a portion of this C over time to recruit other soil microorganisms, especially bacteria (Olsson & Johnson, 2005; Qin et al., 2016). Hyphal networks can also serve as ‘microbial highways’ (i.e., a medium for microbial dispersal through the hyphal system) for bacteria to reach organic P pools (Jansa & Hodge, 2021; Jiang et al., 2021), while hyphal exudates, such as fructose, stimulate the activity of phosphatases by hyphosphere (i.e., the soil volume under strong influence by AMF hyphae) bacteria (Zhang et al., 2018). AMF hyphae also move C from highly respiring rhizosphere

zones into soil aggregates, facilitating C sequestration (Rillig & Mummey, 2006; Shukla et al., 2025).

The full scope of AMF influence on soil C pools remains poorly understood. Cheng et al. (2012) reported that AMF decreases soil C under elevated CO_2, likely by stimulating SOC decomposition in AMF-active zones. Staddon et al. (2003) proposed that AMF-derived C may be quickly released back to the atmosphere via respiration, since C transported to the hyphal network was quickly recycled, and most extraradical hyphae turned over within 5 to 6 days. On the other hand, Olsson & Johnson, (2005) reported that most AMF-assimilated C remained up to 32 days after ^{13}C labeling. Hyphal networks likely vary in architecture and recalcitrance, with distinct hyphal pools that vary from fast (days) to slower (weeks) turnover (Friese & Allen, 1991; Zhu & Miller, 2003). Despite the possible rapid turnover, AMF are still considered a vital C sink to the soils. In a 30 cm soil profile, the amount of SOC derived directly from AMF's external hyphal network ranges from 54 to 900 kg ha^{1-} (Zhu & Miller, 2003). Hyphae cells are composed of recalcitrant C compounds (chitin) that stay in the soil matrix for extended periods, varying from years to decades (Treseder & Allen, 2000). Therefore, it is crucial to consider the residence time of AMF-stored C to better understand its contribution to long-term soil C sequestration and its net effect on soil C dynamics.

Phosphorus and Soil Microorganisms

Phosphorus (P) is a vital nutrient that plays a central role in plant growth and development, contributing significantly to various biological processes, such as the formation of DNA, cell membrane components (phospholipids), adenosine triphosphate (ATP), glycolysis, enzyme activation/inactivation, redox reactions, signaling, carbohydrate metabolism, nitrogen fixation, respiration, and photosynthesis (Kafle et al., 2019; Vance et al., 2003). Phosphate ore, a non-

renewable resource, is facing a critical depletion crisis, with forecasts indicating that half of the world's current P reserves will be exhausted within 50 to 100 years (Zhu et al., 2018). Although soils contain significant amounts of P, most of it is in a form that is inaccessible to plants due to its slow diffusion rate and the soil's tendency to adsorb and fix P, resulting in a very low efficiency of P fertilizer utilization (Fig. 1.2) (Roberts & Johnston, 2015; Zhu et al., 2018).

A ubiquitous element found in all soils globally, P occurs in both inorganic and organic forms. Inorganic forms originate from the weathering of primary minerals, including various types of apatite (Rodríguez & Fraga, 1999). Inorganic P typically occurs as soluble and insoluble forms of phosphates. Examples of insoluble P include aluminum, magnesium, calcium, and iron phosphates (Aliyat et al., 2022), while soluble P mostly exists as hydrogen and dihydrogen phosphate ions HPO_4^{2-} and $H_2PO_4^-$ (Yadav & Verma, 2012; Zhu et al., 2018). Approximately 20–80% of the P present in soils is composed of organic P. Inositol phosphate is the most prevalent form of organic P, accounting for more than 50% of the total organic P in soil (Dalai, 1977). Other forms of organic P include glycerol phosphates, phospholipids, nucleic acids, and sugar phosphates, which are primarily found in soil microbial biomass. Together, these forms collectively account for less than 2% of the total organic P in soil. Importantly, organic P in soils is not readily available for plant uptake due to its high molecular weight. To be bioavailable, it must be broken down through hydrolysis into soluble inorganic forms, such as orthophosphate, which can be easily absorbed by plants (Stewart & Tiessen, 1987).

The limited mobility of inorganic P in soil is attributed to the high reactivity of phosphate ions, which readily bind to various soil components, resulting in strong retention (Zhu et al., 2018). As a consequence, only a small fraction of soil P exists as freely available phosphate ions in the soil solution. In acidic soils, orthophosphates bind to iron and aluminum oxides and clay minerals,

whereas in alkaline soils, they primarily bind to calcium carbonate (Hinsinger, 2001). P adsorbed on clays and Al/Fe oxides can be released through desorption reactions, and these various P forms coexist in a dynamic equilibrium, ranging from highly stable and poorly available to readily plant-available pools, including labile P and solution P (Shen et al., 2011). Given the complex interactions and limited P availability in soil, improving P use efficiency is crucial for promoting plant growth, reducing environmental pollution, and improving resource management. Therefore, enhancing P efficiency in soil is essential to optimize plant growth and ensure sustainable resource management.

Phosphate-Solubilizing Microorganisms

Microorganisms play a vital role in soil P cycling, producing organic acids and phosphatases that transform and increase the availability of both inorganic and organic P forms, thereby supporting soil fertility and plant nutrition (Duarte et al., 2022; Prabhu et al., 2019; Rasul et al., 2021). A wide range of microorganisms, including bacteria, fungi, actinomycetes, and algae, can solubilize P and are commonly referred to as phosphate-solubilizing microorganisms (PSMs). These microorganisms transform soil P into plant-available forms, enhancing uptake and utilization by plants, a vital strategy for improving soil fertility and promoting sustainable plant growth (Duarte et al., 2022). The release of immobilized P by PSMs tends to happen when cells die in response to changes in environmental factors, starvation, or predation (Sharma et al., 2013). Interestingly, environmental changes such as drying-rewetting or freezing-thawing cycles, can trigger significant increases in available P in solution and are known as "flush events." These events occur when a large proportion of microbial cells undergo lysis, releasing stored P into the environment, and making it available for uptake by plants and other organisms (Butterly et al., 2009). PSMs use three major strategies to dissolve phosphates: secretion of organic acids,

production of extracellular enzymes, and degradation of organic matter through mineralization (Rawat et al., 2021).

Secretion of Organic Acids

PSMs make insoluble inorganic P available to plants by secreting organic acids such as gluconic, lactic, citric, acetic, malic, and oxalic acids that dissolve phosphate into plant-usable forms and break down organic P forms (Kalayu, 2019; Pang et al., 2024). To dissolve insoluble inorganic P, organic acids compete with phosphate ions for binding sites on soil particles, reduce soil adsorption of phosphate ions, chelate with cations such as Ca^{2+}, Fe^{3+}, Al^{3+}, and Mg^{2+} through hydroxyl and carboxyl groups in soil to release bound phosphate ions, and reduce the pH of the medium promoting the dissolution of insoluble inorganic P such as tricalcium phosphate, dicalcium phosphate, phosphate rock, and hydroxyapatite (Bhattacharyya et al., 2016; Otieno et al., 2015; Rawat et al., 2021). For instance, PSMs excrete essential organic acids such as 2-ketogluconic acid and gluconic acid (Deubel et al., 2000). Inorganic acids produced by PSMs, such as hydrochloric, sulfuric, nitric, and carbonic acids, can also solubilize phosphate, but much less efficiently (Rawat et al., 2021). Interestingly, P-solubilizing fungi tend to produce more acids than bacteria, having greater P-solubilizing activity (Venkateswarlu et al., 1984).

Production of Extracellular Enzymes and Mineralization

Immobilization is a process in which P is bound to organic compounds, while mineralization is the release of P from these compounds (Basílio et al., 2022). Plants cannot directly absorb organic P, as it first needs to be converted to inorganic P through the process of mineralization. When P availability is limited, mineralization occurs through enzymatic hydrolysis (Basílio et al., 2022). Soil microorganisms can facilitate this process by producing enzymes such

as phosphatases, phytases, and C-P lyases that break down organic P into inorganic forms that plants can utilize (Prabhu et al., 2019; Stefanoni Rubio et al., 2016).

Phosphatases are a key group of enzymes involved in mineralizing organic P in soils (Alori et al., 2017; Chen & Arai, 2023). This enzyme group comprises two primary types: acid phosphatase, predominantly secreted by plants and fungi, and found mainly in acidic soils, and alkaline phosphatase, mainly produced by bacteria and some fungi, and found primarily in alkaline soils (Cheng et al., 2023; Fraser et al., 2017). Phosphatases catalyze dephosphorylation by breaking phosphoester or phosphoanhydride bonds in organic compounds, releasing inorganic phosphate and converting organic P into a form readily available for plant uptake (Rawat et al., 2021). Interestingly, research suggests that phosphatases of microbial origin have a higher affinity for organic P compounds compared to those derived from plant roots (Tarafdar et al., 2001), which implies that microorganisms may play a more significant role in P mineralization in soils, as their phosphatases are more efficient at breaking down and releasing P from organic compounds. Phytases are enzymes that catalyze the release of P from phytate, an abundant organic P compound in soil. By breaking down phytate, phytases make P available to plants, increasing its bioavailability and supporting plant growth (Sharma et al., 2013). Finally, C-P lyases are responsible for breaking the C–P bond of organophosphonates (Rodríguez et al., 2006; Sharma et al., 2013).

Fungi play a crucial role in P mineralization by producing a wide range of enzymes that facilitate this process. Specifically, phosphatases or phosphohydrolases catalyze the hydrolysis of esters and phosphoric acid anhydrides, breaking down organic P compounds into inorganic forms that plants can use (Cheng et al., 2023). Furthermore, fungi in soils can explore longer distances

than bacteria, making them potentially more significant contributors to P solubilization in soils (Kucey, 1983).

Mycorrhizal Associations and Soil Phosphorus

Mycorrhizal fungi form symbiotic relationships with plant roots, exchanging C for enhanced water and nutrient uptake (Genre et al., 2020). Forming symbiotic associations with the vast majority of terrestrial plants, AMF create an effective pathway for plants to acquire more P (Shi et al., 2021). Once fungal hyphae absorb inorganic P, it is transported to the arbuscules, where it is released into the interfacial apoplast space (Smith et al., 2003). For instance, a great number of inorganic P-transporters have been identified in the AMF mycelium, and they have been reported to be more active under P-deficient conditions (Garcia et al., 2016).

Two main strategies govern P acquisition in soils: the Plant Direct Absorption Pathway and the Mycorrhizal Uptake Pathway. In the Plant Direct Absorption Pathway, inorganic P gets absorbed into the root's epidermis and root hairs through specialized P transporters located in root hairs (Smith et al., 2003). Plants can employ a wide range of morphological adaptations to increase P uptake under low-P stress, such as increasing root hair length and number, root length and branch density, and lateral root production, among others (Zhang et al., 2021).

Once AMF symbiosis is established, P absorption through the Mycorrhizal Uptake Pathway becomes the primary mode of uptake due to the extensive network of external hyphae that explores a larger soil volume, increasing access to P sources, and the release of organic acids by AMF that enhance P solubility and make it more readily available for absorption (Madrid-Delgado et al., 2021).

To acquire P across a wide range of soil pH levels, AMF have evolved efficient mechanisms for P uptake, utilizing proton and sodium-coupled transporters (H^+/Pi and Na^+/Pi),

but the molecular processes by which the fungus secretes P into plant root cells remain unclear (Johri et al., 2015). Importantly, regardless of the absorption pathway, P uptake occurs against an electrochemical potential, requiring energy in the form of adenosine triphosphate (ATP). Through an ATPase and a generated proton (H^+) gradient, inorganic P can be transported through Pht1 phosphate transporters (Karandashov & Bucher, 2005). The Pht1 family of phosphate transporters is mainly located in the root cell membrane, and its expression is dynamically regulated in response to P availability (Preuss et al., 2010). For instance, phosphate transporters tend to be expressed when inorganic P levels are low (Liu et al., 1998).

Interestingly, the amount of inorganic P that is allocated by AMF can be regulated by either the fungus or the plant, depending on the plant species (Walder et al., 2016). AMF can also reduce nutrient transfer to their symbiotic partner, suggesting a potential mechanism for reciprocal benefit regulation that implies AMF may adjust their nutrient exchange based on the quality of their symbiotic relationship, optimizing their mutualistic interaction (Kiers et al., 2011). It is very well established in the literature that AMF increases P uptake, but several recent studies have been reporting the benefits of the co-inoculation of AMF with phosphate-solubilizing bacteria to increase soil enzyme activity, microbial biomass, and to promote P uptake (Cozzolino et al., 2021; Duan et al., 2023; El Maaloum et al., 2020; Sharma et al., 2020; Varinderpal-Singh et al., 2020; Wahid et al., 2020). For instance, research has shown that AMF excretes carbohydrates from the host plant to stimulate the expression of P-solubilizing genes of PSB that encode enzyme production, increasing P mineralization (Zhang et al., 2018).

Efficient P utilization is crucial for sustainable agriculture and to meet the ever-growing food production demand. However, limited soil P availability and inefficient use of P fertilizers remain major constraints for plant growth and yield. PSMs such as AMF might offer an

ecologically safe solution to enhance soil P availability, improve P fertilizer efficiency, and promote plant growth. While research has identified numerous P-solubilizing microorganisms, more work is needed to understand the complex interactions between PSMs, AMF, and plants to optimize P efficiency in soil and promote sustainable plant growth. Further research is also needed to explore the molecular mechanisms underlying P solubilization and uptake to develop effective strategies to improve P utilization and to reduce environmental impacts.

Bioinoculants in Agricultural Systems

Microbial communities that enhance plant nutrient acquisition and soil health naturally occur in soils, but intensive agricultural practices (such as the heavy use of fertilizers, pesticides, and tillage) can negatively impact these communities by reducing their natural abundance, altering their composition, and disrupting metabolic pathways (Basílio et al., 2022). Bioinoculants are formulations containing living microorganisms with beneficial properties that are applied to seeds, plant surfaces, or soil to colonize the rhizosphere or plant tissues to enhance plant growth, crop yields, and/or soil health (Kumar et al., 2025; Vessey, 2003). Recent data reveal that the market for AMF bioinoculants has been rapidly growing over the last decade, reaching a market size of around 1.39 billion USD globally (Mordor Intelligence Research & Advisory, 2026). According to Kaminsky et al. (2019), an ideal bioinoculant should not spread beyond the application zone, should persist for a limited time, and should not negatively impact humans and the environment.

Numerous studies have demonstrated that AMF inoculation can improve plant growth, nutrient uptake, soil aggregation, and drought tolerance under certain environmental conditions, although the magnitude of these responses varies considerably among cropping systems, genotypes, soil conditions, and AMF species (Al-Maliki & Ebreesum, 2020; Basiru & Hijri, 2022; Cozzolino et al., 2021; Han et al., 2025). Despite their potential, AMF bioinoculant performance

remains highly variable (Koziol et al., 2025). This inconsistency emphasizes the need to better understand how different inoculation strategies affect AMF establishment and persistence in agricultural fields. There is also a critical knowledge gap on the ecological and long-term effects of bioinoculants under field conditions, since most AMF research has been conducted in short-term controlled environments (e.g., greenhouses and microcosms) with sterile soils and inert substrates (Basiru & Hijri, 2022). Field studies are essential to determine how different inoculation strategies influence AMF establishment, persistence, and interactions with native microbial communities over time.

Interestingly, AMF inoculation studies report that bioinoculants significantly affect, in many cases negatively, native AMF communities (Janoušková et al., 2017). Many commercial bioinoculants are selected based on greenhouse study performance, which often ignores ecological traits that could influence their likelihood of establishment under natural conditions (Kaminsky et al., 2019). A recent review by Basiru & Hijri, (2022) suggests that the failure of commercial inoculants might be related to poor product quality due to the lack of proper regulation and quality control, but also due to the lack of information regarding the ecology of the strains. To advance the bioinoculant industry, it is essential to understand the factors and genetic traits that influence the establishment and persistence of the introduced microorganism in a natural ecosystem.

For over two decades, manufacturers have been recommended to submit their products to rigorous standard quality tests (Gianinazzi & Vosátka, 2004), but the lack of regulation and harmonized standards for inoculum quality is still a challenge. For instance, a recent study Koziol et al. (2024) reported that when evaluating twenty-three AMF commercial inoculants, only one had the amount of propagules that was reported by the manufacturer, contrasting with discrepancies that varied from 72-100% on the remaining twenty-two tested products, which

represents less than 2% of the amount of propagules reported by the manufacturer. A new meta-analysis by Koziol et al. (2025) that evaluated 302 AMF inoculant trials has shown that most commercial inoculants showed zero to negligible root colonization, when compared to high standard laboratory-grown inoculants, highlighting the need for the industry to apply rigorous quality control protocols, as research laboratories do.

After decades of bioinoculant research and advances in molecular biology, we must acknowledge that the specific traits driving the successful establishment and long-term impacts of introduced microorganisms in cultivated fields remain poorly understood. Special attention must be given to the study of ecological dynamics by using a combination of metagenomics, metaproteomics, and metabolomics on field studies to better understand what shapes interspecies interactions at a molecular level and how these dynamics influence soil health and crop productivity. Additionally, the lasting impacts of commercial inoculants on native soil communities remain a key concern, as introduced strains may outcompete or alter the functional roles of native microbes, which can enhance or disrupt key processes over time. Addressing these knowledge gaps is essential for developing more sustainable and effective microbial inoculant strategies.

Although significant progress has been made in understanding AMF ecology in the rhizosphere, relatively few long-term field studies have evaluated the persistence of introduced bioinoculants, their interactions with native microbial communities, and their influence on soil health and crop production across varying soil P levels and cropping systems.

Study Objectives

The main objective of this study was to evaluate the effects of AMF inoculation and P fertilization on crop performance and soil health under irrigated and dryland field conditions, and

to determine how wheat genetic background influences AMF inoculation responsiveness under contrasting soil P levels. The objectives of each chapter were:

Chapter 2 - Understanding the Impacts of Arbuscular Mycorrhizal Fungi Inoculation and Phosphorus Fertilization in Irrigated Corn-Soybean Rotation. The objective of this study was to evaluate the effects of AMF inoculation and P fertilization rates on plant growth, P uptake, and soil health in a four-year irrigated corn-soybean rotation.

Chapter 3 - Understanding the Impacts of Arbuscular Mycorrhizal Fungi Inoculation and Phosphorus Fertilization in Dryland Continuous Corn. The objective of this study was to evaluate the effects of AMF inoculation and P fertilization rates on plant growth, P uptake, and soil health indicators in a four-year dryland continuous corn system.

Chapter 4 - Wheat Genotypes Modulate Arbuscular Mycorrhizal Fungi Responsiveness, Soil Enzyme Activity, and Rhizosphere Microbial Composition across Phosphorus Levels. The objective of this study was to (i) compare the responsiveness of six wheat genotypes with distinct root traits and potential to influence the soil microbiome to AMF inoculation under contrasting P fertilization levels in terms of plant growth, P uptake, and AMF colonization dynamics, and to (ii) evaluate how the treatments influence soil health, as soil microbial community composition and soil extracellular enzyme activity.

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Figures



Figure 1.1. Conceptual illustration of the role of arbuscular mycorrhizal fungi (AMF) in wheat phosphorus (P) nutrition. Without AMF (left), P uptake is limited to the root zone, while AMF-colonized plants (right) benefit from extensive fungal hyphal networks that enhance P acquisition and plant growth. Insets display root tissues without AMF colonization (upper left), AMF-colonized root tissues (upper right), poorly structured soil without hyphal networks (lower left), and well-structured soil containing extraradical AMF hyphae (lower right). Illustration by Endy Kailer.

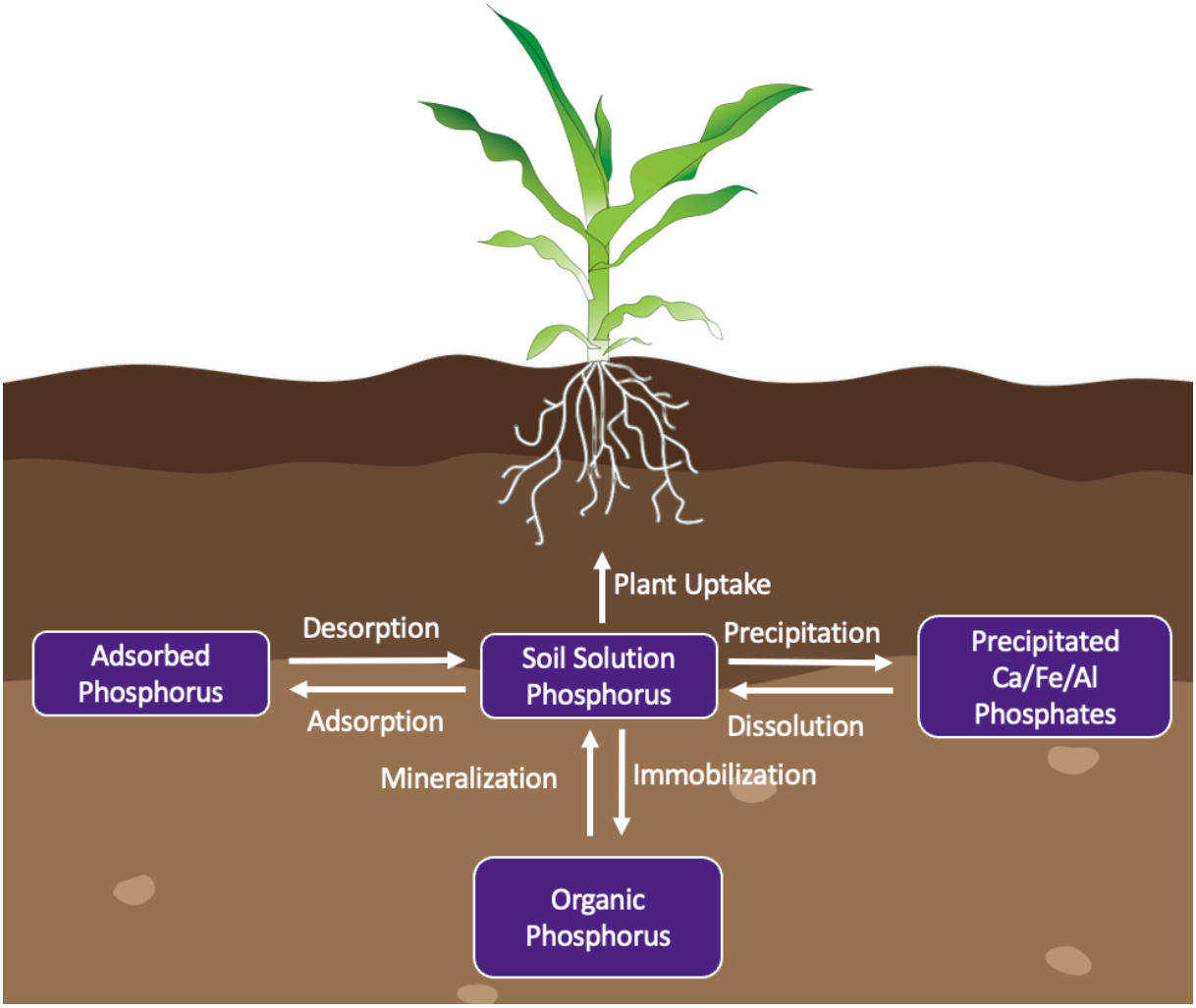


Figure 1.2. Conceptual illustration of the soil phosphorus (P) cycle in the rhizosphere, illustrating the dynamic equilibria between orthophosphate in the soil solution and surrounding inorganic and organic soil pools. Illustration by Endy Kailer.

Chapter 2 - Understanding the Impacts of Arbuscular Mycorrhizal Fungi Inoculation and Phosphorus Fertilization in Irrigated Corn-Soybean Rotation

Abstract

Arbuscular mycorrhizal fungi (AMF) associate with about 80% of terrestrial plants, improving phosphorus (P) acquisition and contributing to soil health. Although AMF naturally occur in many soils, intensive agricultural practices, such as conventional tillage and P fertilizer, can reduce their abundance. AMF bioinoculants may increase AMF abundance in cultivated soils, but their long-term effectiveness under field conditions remains largely unexplored. This study evaluated the effects of AMF inoculation and P fertilization on corn growth, P uptake, and soil health over four years in two irrigated corn–soybean rotations in Scandia, KS. Treatments consisted of AMF inoculation with a commercial multi-species inoculant and four P rates (0, 15, 30, and 60 kg P ha^{-1}), applied during corn years. Measurements included plant growth, grain yield, P uptake, apparent P recovery efficiency (APRE), AMF root colonization, and multiple soil biological, physical, and chemical properties. Fertilization with P regulated plant–soil–microbial responses more strongly than AMF inoculation. Root colonization by AMF increased as Mehlich-3 soil P levels decreased below critical levels of 19.4, 24.0, and 28.0 mg kg^{-1} at V6, R1, and R6, respectively, while colonization remained relatively stable at or above these thresholds. Similarly, soil AMF abundance decreased with repeated P fertilization across multiple years and soil depths. Inoculation increased AMF abundance by 46% at 0–5 cm under no P fertilization in the Corn–Soybean rotation. Inoculation increased plant biomass and P uptake at multiple growth stages and substantially increased APRE at 15 kg P ha^{-1} during the first corn year of each rotation, but these

benefits were not consistently maintained during the second corn year, as soil P levels increased in the soil. Grain yield responded strongly to P fertilization but was not significantly affected by AMF inoculation. However, inoculated corn reached its grain yield plateau at a lower Mehlich-3 soil P level (11.4 mg kg^{-1}) than non-inoculated corn (17.6 mg kg^{-1}), while reaching similar maximum yields. Inoculation also increased the >2 mm water-stable macroaggregate fraction at multiple soil depths and rotations, particularly under low to moderate P levels. In contrast, soil microbial groups, extracellular enzyme activities, soil organic carbon stocks, and total soil N had limited and inconsistent responses to treatments and were mostly influenced by sampling year at each soil depth. Overall, AMF inoculation provided its greatest benefits under low P availability, particularly for P uptake, plant growth, AMF soil abundance, and soil aggregation, while increasing soil P levels progressively reduced AMF colonization and AMF soil abundance. Overall, these findings highlight the importance of balancing P inputs with AMF function and suggest that AMF inoculation might provide greater benefits in P-deficient soils, particularly for P uptake, plant growth, and soil structure.

Introduction

Among the most ecologically and functionally important soil microorganisms worldwide, arbuscular mycorrhizal fungi (AMF) form symbiotic associations with around 80% of terrestrial plants (Shukla et al., 2025; Smith & Read, 2008). Occupying a unique position at the interface between plant roots and the surrounding soil, AMF influence both plant and soil processes, including nutrient uptake, crop productivity, microbial communities, soil aggregation, carbon storage, and nutrient cycling (Bhantana et al., 2021; Cobb et al., 2016; Fall et al., 2022; Wilson et al., 2009; Wu et al., 2024; Zhang et al., 2024). These multiple functions have generated considerable interest in the contribution of AMF to soil health and sustainable agricultural production (Brito et al., 2021; Shukla et al., 2025).

Among the benefits of AMF symbiosis, contributions to phosphorus (P) acquisition are particularly important in agricultural systems (Wu et al., 2024). Although soils may contain considerable amounts of P, only a small fraction is readily available to plants because of its low mobility and strong interactions with soil minerals (Roberts & Johnston, 2015; Zhu et al., 2018). Through extensive extraradical hyphal networks, AMF can explore soil beyond the root depletion zone and increase plant access to P and other nutrients (Smith & Read, 2008; Wu et al., 2024).

Despite their natural occurrence in soils and the well-established benefits of AMF associations, AMF abundance and diversity in agricultural soils are often reduced under intensive management practices, such as conventional tillage and the heavy use of fertilizers (Basílio et al., 2022; Lekberg & Koide, 2005). Soil P availability also strongly regulates AMF, and understanding this interaction is particularly relevant for developing nutrient management strategies that maintain crop productivity while improving P management and soil health.

There is a growing interest in AMF bioinoculants as a strategy to enhance beneficial plant–fungal associations in agricultural soils (Basiru & Hijri, 2022). Bioinoculants are formulations containing beneficial living microorganisms that can be applied to seeds, plants, or soil to enhance plant productivity and soil health (Kumar et al., 2025; Vessey, 2003). Over the years, AMF inoculation has shown potential to improve crop performance and soil health, although the magnitude and consistency of these responses vary considerably among cropping systems, plant genotypes, soil conditions, nutrient availability, and AMF species (Al-Maliki & Ebreesum, 2020; Basiru & Hijri, 2022; Cozzolino et al., 2021; Han et al., 2025).

The effectiveness of AMF inoculation under field conditions may depend on interactions among the introduced fungi, indigenous AMF communities, soil nutrient availability, and agricultural management (Bender et al., 2019; Lekberg & Koide, 2005). In soils with abundant and active indigenous AMF communities, introduced AMF must establish and function within an already complex soil microbial community, which may limit or modify their effects on crop and soil processes (Bender et al., 2019; Fall et al., 2022). At the same time, P fertilization may directly influence both native and introduced AMF, potentially altering the benefits provided by inoculation. Therefore, responses observed in controlled environments may not translate directly to cultivated fields, where soil properties, environmental conditions, management history, and microbial communities add considerable complexity.

Despite the growing interest in AMF bioinoculants, relatively few studies have evaluated their effects across multiple growing seasons under field conditions, as much of the existing research has been conducted in short-term greenhouse experiments, where substrates, soil properties, environmental conditions, and microbial communities may differ substantially from agricultural fields (Basiru & Hijri, 2022). Therefore, multi-year field studies are particularly

important because repeated P fertilization can alter soil P availability over time, while the establishment and persistence of introduced AMF and their effects on soil biological and physical processes may develop across growing seasons.

This chapter evaluated the effects of AMF inoculation and P fertilization rates on plant growth, P uptake, and soil health in a four-year irrigated corn-soybean rotation. We hypothesized that AMF inoculation would improve plant growth, P uptake, and soil health, with the strongest effects observed under low to moderate P fertilization rates.

Materials and Methods

Site Characteristics and Experimental Design

This experiment was conducted at the North Central Experiment Field in Scandia, KS (39.8330038 N, -97.8391954 W) from 2022 to 2025. Soil at the experimental site was characterized as a Crete silt loam (Fine, smectitic, mesic *Pachic Udertic Argiustolls*) with very low soil P levels (Table A.1). Precipitation data during the growing seasons were obtained from the Kansas Mesonet weather station in Scandia, KS, located approximately 6 km from the experimental field (Patrignani et al., 2020) (Table A.2). Supplemental irrigation was applied using a Reinke Maxigator lateral-move irrigation system (Reinke Manufacturing, Deshler, NE, USA) equipped with Nelson Accelerator sprinklers (Nelson Irrigation, Walla Walla, WA, USA).

The experiment was established as a randomized complete block design (RCBD), with a split-plot arrangement, in which AMF inoculation was assigned to the main plots, and P fertilization to subplots, with four replications (Fig. A.1). The plots were 6 m x 12 m, with 3 m clean buffer areas between plots to avoid inoculum drift. Corn (*Zea mays* L.) and soybean (*Glycine max*) were planted in two alternating crop rotations and maintained under an irrigated no-till system. In the Corn–Soybean rotation field, corn was grown in 2022 and 2024 and soybean in

2023 and 2025, whereas in the Soybean–Corn rotation field, soybean was grown in 2022 and 2024 and corn in 2023 and 2025 (Fig. A.2; Table A.3; Table A.4). Wheat was planted every year between growing seasons as a cover crop in both rotations and terminated before planting corn or soybean (Table A.5).

Treatments were applied exclusively during corn years in both rotations and were not reapplied to soybean, resulting in a total of two fertilizations and inoculations in each field over the course of the experiment (Fig. A.2). The inoculation treatment was the presence or absence of Symvado® SC (Mycorrhizal Applications LLC., Grants Pass, OR, USA), containing spores of *Rhizophagus irregularis, Claroidoeglomus etunicatum, Funneliformis mossea*, and *Claroideoglomus claroideum* at a concentration of 1625 propagules mL^{-1}, applied in furrow at planting with an application rate of approximately 146 mL ha^{-1} (2 fl oz ac^{-1}). Fertilizer treatments were four rates of P fertilizer (0, 15, 30, and 60 kg P ha^{-1}), as mono-ammonium phosphate (MAP) (11-52-0). All fertilizer treatments were broadcast by hand within one week before planting. Nitrogen fertilizer was applied before planting each year at a rate of 179 kg N ha^{-1} as anhydrous ammonia (NH_3) (82-0-0).

Plant Sampling

Aboveground plant and root samples were collected at the V6 (sixth leaf), R1 (silking), and R6 (maturity) corn developmental stages, representing key vegetative, reproductive, and maturity stages that capture critical periods of P acquisition and utilization in corn (Ampong et al., 2024). At each sampling time, four randomly selected plants were collected from each plot. Plant biomass samples were dried in a forced-air oven at 50 °C until constant weight, and the dry weight was recorded.

To determine grain yield, corn ears were manually harvested at R6 from a 1.5-m section of two adjacent rows in the center of each plot and shelled to remove the grain. Soybean was harvested using a plot combine. Grain weight and moisture were recorded at the time of shelling. All yield data were corrected to standard moisture contents of 15.5% for corn and 13% for soybean. Aboveground biomass and grain samples were finely ground in a Wiley mill, sieved to pass a 2mm mesh, and digested in a nitropercloric solution (Gieseking et al., 1935), and P concentration was determined by an Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES) (Agilent 5800, Agilent Technologies, CA, USA). Harvest dates were determined annually based on physiological maturity (Table A.3; Table A.4).

Roots from each of the four plants were collected with the soil from their surroundings (up to 30 cm depth) at each sampling time and immediately stored in 70% ethanol for subsequent assessment of AMF colonization. Root colonization by AMF was assessed using a modified staining protocol, substituting phenol with lactoglycerol (Phillips & Hayman, 1970). Root samples were first cleared in a 10% potassium hydroxide (KOH) solution for 40 min in a 70°C water bath, rinsed thoroughly under running water, and acidified in a 2% hydrochloric acid (HCl) solution for 10 min. Roots were then stained in a 0.05% trypan blue solution for 60 min at 60°C and stored in lactoglycerol. AMF root colonization was quantified using the gridline intersect method under a stereoscopic microscope (Giovannetti & Mosse, 1980).

Apparent Phosphorus Recovery Efficiency

Apparent P recovery efficiency (APRE) was calculated to quantify the proportion of applied P fertilizer recovered by the crop. Total aboveground P uptake at R6 was determined as the sum of P uptake in aboveground biomass and grain. APRE was calculated as:

$$APRE(\%) = \frac{(Total\ P\ Uptake\ at\ R6\ Fertilized\ - Total\ P\ Uptake\ at\ R6\ Control)}{Fertilizer\ Applied} x\ 100$$

where total P uptake represents the combined P uptake in aboveground biomass and grain (kg P ha^{-1}), and P fertilizer applied represents the corresponding P application rate (kg P ha^{-1}). The non-inoculated, unfertilized treatment (0 kg P ha^{-1}) within each block served as the reference for calculating APRE for each P fertilization rate (Assefa et al., 2021; Fixen et al., 2015).

Soil Sampling

Soil samples for biological and chemical analyses were collected from each plot of the Corn-Soybean rotation field in the Spring of 2022, 2024, and 2026, and for the Soybean-Corn rotation field in the Spring of 2024 and 2026, and divided into depth increments of 0-5, 5-15, and 15-30 cm. A minimum of 12 cores were collected from each plot, combined, and homogenized into a composite sample for each depth in polyethylene Ziplock® Freezer Bags. Samples were then transported from the field in a cooler with ice and stored in the laboratory freezer at -20°C until analysis. Soils for chemical analysis and enzyme activity were air-dried at room temperature, finely ground, and submitted for pH and soil P analysis at the Kansas State University Soil Testing Laboratory. Soil pH was determined by 1:1 soil slurry (Watson & Brown, 1998) and soil P was extracted by Mehlich-3 (Mehlich, 1984).

Soil samples to measure soil aggregate stability were collected with a shovel and divided into depth increments of 0-5, 5-15, and 15-30 cm with minimum disturbance to preserve the natural structure of aggregates. Samples were then air dried, cleared of coarse roots, and carefully passed through an 8-mm sieve.

Soil bulk density samples were collected using a Giddings probe (Giddings Machine Company, Windsor, Colorado, USA) with a 5 cm-diameter metal tube in the Spring of 2023 and

2026 for both rotations (1 core per plot) and used to compute soil carbon stocks at each depth. Soil organic carbon (SOC) and total nitrogen (TN) concentrations were measured by dry combustion using a LECO CN828 Carbon /Nitrogen analyzer (LECO Corporation, St. Joseph, MI, USA), reporting total levels (inorganic and organic) of C and N on a weight percent basis, following a method from Nelson & Sommers, (1983) for SOC and Bremner & Mulvaney, (1982) for TN. Total carbon is considered equivalent to SOC in soils with pH < 7.2.

Water-Stable Aggregates

Sand-free water-stable aggregates were measured using a Yoder wet-sieving method based on Kemper & Rosenau, (1986) and modified by Mikha & Rice, (2004). Briefly, 100 g of air-dried soil was placed in a Yoder wet-sieving apparatus modified to recover all particle fractions and separated into four size classes: 8–2, 0.25–2, 0.053–0.25, and 0.020–0.053 mm. The soil was placed on a 2 mm sieve, above the 0.25 mm sieve, submerged in 1 L of distilled water for 10 min, then oscillated for 10 min at a stroke length of 4 cm and 30 cycles min^{-1}. Material recovered from each sieve was collected, dried at 50°C for 48 h, and weighed. Fractions >2 and 0.25–2 mm were classified as macroaggregates, whereas 0.053–0.25 and 0.020–0.053 mm were classified as microaggregates. Floating organic matter was removed from the >2-mm fraction. Aggregate fractions were corrected for sand content to obtain sand-free aggregate proportions. For this correction, 5 g of material from each fraction was dispersed overnight (>12 h) in sodium hexametaphosphate solution at a fivefold volume, shaken at 325 rpm for 5 h, and passed through a 0.053-mm sieve. The retained material was oven-dried and weighed to determine sand content, which was used to calculate sand-free aggregate mass.

Phospholipid and Neutral Lipid Fatty Acid Analysis

To monitor changes in microbial community populations in response to our treatments, phospholipid fatty acid (PLFA) and neutral lipid fatty acid (NLFA) analyses were conducted following a modified Bligh & Dyer, (1959) extraction procedure, as described by White & Rice, (2009). Soil samples were lyophilized and finely ground using a mortar and pestle, and total lipids were extracted from 5 g of soil. PLFAs were extracted by incubating the soils in a solution of methanol, chloroform, and phosphate buffer (2:1:0.8 v/v/v). Following extraction, neutral and phospholipid fatty acids were separated from total lipids using preconditioned disposable silicic acid chromatography columns (BAKERBOND SPE Columns). Fatty acids were subsequently released from their glycerol backbones, and methylation was performed to produce fatty acid methyl esters (FAMEs). Then, FAMEs were analyzed on a Thermo Scientific Trace GC-ISQ mass spectrometer (Thermo Fisher Scientific, Waltham, MA, USA) fitted with a DB5-MS analytical column (30 m × 250 μm internal diameter × 0.25 μm film thickness; Agilent Technologies, Santa Clara, CA, USA). Concentrations of fatty acid peaks were quantified relative to an internal standard, methyl nonadecanoate (19:0). Fatty acids were categorized into microbial groups as follows: Gram-Positive Bacteria (i15:0, a15:0, i16:0, i17:0, and a17:0), Gram-Negative Bacteria (2-OH 10:0, 2-OH 12:0, 3-OH 12:0, 2-OH 14:0, 3-OH 14:0, 16:1ω7c, cy17:0, cy19:0), Actinomycetes (10-Me 16:0 and 10-Me 18:0), Arbuscular Mycorrhizal Fungi (AMF; 16:1ω5), and Saprophytic Fungi (18:2ω6,9c). Total microbial biomass was obtained by summing microbial groups and commonly measured fatty acids (11:0, 12:0, 13:0, 14:0, 15:0, 16:0, 17:0, 18:0, and 20:0) (Bonini Pires et al., 2020; Lin et al., 2023).

Extracellular Enzyme Activity

Four soil enzymes were analyzed colorimetrically using p-nitrophenyl-associated substrates, including β-glucosidase (Eivazi & Tabatabai, 1988) associated with the C cycle, acid phosphatase (Eivazi & Tabatabai, 1977; Tabatabai & Bremner, 1969) involved in the P cycle, arylsulfatase (Tabatabai & Bremner, 1970) involved in the sulfur (S) cycle, and N-acetyl-β-glucosaminidase (Parham & Deng, 2000) involved in the C and N cycling. Briefly, triplicates of 0.5 g of air-dried soil, sieved to 2 mm, were placed into individual 20-mL glass vials, with 2 mL of buffer. Two vials received 0.5 mL of a substrate linked to p-nitrophenyl, while the third vial served as a control without the initial addition of the substrate. Vials were capped and incubated at 37 ºC for 1 hour. After incubation, 0.5 mL of 0.5 M $CaCl_2$ solution and 2 mL of a stopping buffer were added to each vial. The control vial then received 0.5 mL of the p-nitrophenyl substrate. The solutions were filtered through Whatman 2V filter paper (9.0-cm diameter), and absorbance was measured at 400 nm using a Genesys 20 Visible Spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA).

Statistical Analysis

Treatment effects on plant and soil responses were analyzed by analysis of variance (ANOVA) after fitting linear mixed-effects models. Plant responses were analyzed separately for each sampling year, with AMF inoculation, P fertilization, and their interaction included as fixed effects. Block and AMF whole plots nested within block were included as random effects. When a significant P effect was detected, regression analyses were conducted to characterize responses to increasing P rates. For significant AMF × P interactions, regressions were fitted separately for each AMF treatment. Linear, quadratic, linear-plateau, and quadratic-plateau models were evaluated for P responses, and model fit was compared using the Akaike information criterion

(AIC), root mean square error (RMSE), and coefficient of determination (R^2). Soil responses were analyzed by depth across sampling years with AMF inoculation, P fertilization, sampling year, and their interactions included as fixed effects. Block, AMF nested within block, and plot were included as random effects to account for the split-plot design and repeated measurements of the same plots across years. For significant P effects, regression analyses were conducted to characterize responses to increasing P rates. For both plant and soil responses, significant effects involving categorical factors were further evaluated using estimated marginal means (EMMs) and pairwise contrasts using the *emmeans* package. Simple effects of AMF inoculation were evaluated within each P rate and, for soil responses, within each P rate and year when appropriate. Year effects for soil responses were also evaluated within each P rate when appropriate. Statistical significance was determined at $P \leq 0.05$. The normality of model residuals was assessed using the Shapiro–Wilk test and Q–Q plots, and model assumptions were further evaluated using residual diagnostics. Data were log or square-root transformed when necessary to satisfy assumptions of normality and homogeneity of variance. Bivariate regression analyses were conducted to evaluate relationships among selected plant and soil variables. Linear mixed-effects models were fitted using the *lmer* function in the *lme4* package, and graphs were generated using the *ggplot2* package. All statistical analyses were conducted in R version 4.1.2 (R Core Team, 2026).

Results

AMF Root Colonization

Root colonization consistently increased with decreasing Mehlich-3 soil P below a critical threshold at all growth stages (Fig. 2.1a). Critical soil P levels increased as the growing season progressed, with linear-plateau models identifying critical levels of 19.4, 24.0, and 28.0 mg kg^{-1} at V6, R1, and R6, respectively, indicating a lower critical P level during early-season root

colonization. Above the critical levels, AMF root colonization plateaued at approximately 63%, 52%, and 31% at V6, R1, and R6, respectively. Colonization also progressively decreased from V6 to R1 and R6, showing an overall decline in AMF root colonization as the crop developed (Fig. 2.1a). Across years, rotations, and growth stages, observations from inoculated and non-inoculated treatments largely overlapped across the soil P gradient, with similar linear-plateau responses between treatments (Fig. 2.1b). Critical soil P levels were 22.3 and 20.7 mg kg^{-1} in the absence and presence of AMF inoculation, respectively. Above these critical levels, root colonization stabilized at approximately 48% without inoculation and 51% with inoculation, indicating that AMF inoculation had little influence on the soil P level at which colonization plateaued (Fig. 2.1b).

Mycorrhizal root colonization had limited treatment response during the first year of corn in the rotation but significant P × AMF inoculation interactions at both V6 and R1 in the second year (Table A.6). In 2022, treatments did not significantly affect colonization at V6 (Fig. 2.2a). In 2023, colonization was not affected by P fertilization (Fig. 2.2b), but inoculation increased colonization by 6% (Fig. A.3). In 2024, colonization increased quadratically with P fertilization without inoculation, reaching a maximum of 81.2% at 33.6 kg P ha^{-1}, while the inoculated treatment did not respond to P fertilization but increased colonization from 45 to 75% at 0 kg P ha^{-1} (Fig. 2.2c). In 2025, colonization responded quadratically to P fertilization only with inoculation, while the non-inoculated treatment had no significant P response (Fig. 2.2d). Inoculation increased colonization from 59 to 79%, and from 52 to 67% at 0 and 60 kg P ha^{-1}, respectively (Table A.7).

At R1, treatments did not significantly affect root colonization in 2022 (Fig. 2.3a). In 2023, colonization decreased linearly with increasing P fertilization, regardless of inoculation (Fig. 2.3b). In 2024, colonization decreased linearly with P fertilization without inoculation but did not affect

the inoculated treatment (Fig. 2.3c). Inoculation increased colonization from 49 to 71%, and from 44 to 63%, at 30 and 60 kg P ha^{-1}, respectively. In 2025, colonization responded quadratically to P fertilization only with inoculation, reaching its lowest value at the highest P rate, while the non-inoculated treatment had no significant response. Inoculation increased colonization from 52 to 63% at 30 kg P ha^{-1} (Fig. 2.3d). At R6, colonization was unaffected by P fertilization in 2022 and 2023 but decreased linearly with increasing P fertilization in 2024 and 2025 (Fig. 2.4a-d).

Plant Growth

Aboveground biomass was primarily affected by P fertilization, while AMF inoculation effects varied by growth stage and year (Table A.6). At V6, biomass increased linearly with P fertilization in 2022 and 2023, had no significant response in 2024, and increased to a plateau at approximately 18.6 kg P ha^{-1} in 2025 (Fig. 2.5a-d). AMF inoculation did not significantly affect V6 biomass except in 2025, when it increased biomass by 13% (Fig. A.4a–d). At R1, AMF inoculation increased aboveground biomass by 29% in 2022 and 9% in 2023, with no significant effects in 2024 or 2025 (Fig. A.5a–d). Biomass responded significantly to P fertilization in all years, increasing quadratically in 2022, with the greatest biomass at 60 kg P ha^{-1}, and linearly in 2023 (Fig. 2.6a, b). In 2024 and 2025, biomass plateaued at critical P rates of 17.9 and 25.6 kg P ha^{-1}, respectively (Fig. 2.6c, d). At R6, biomass increased quadratically with P fertilization in both inoculation treatments in 2022, with AMF inoculation increasing biomass by 41% at 0 kg P ha^{-1} (Fig. 2.7a). In 2023, biomass increased linearly with P fertilization only under inoculation, while the non-inoculated treatment had no significant response (Fig. 2.7b). Inoculation increased biomass by 15% and 21% at 30 and 60 kg P ha^{-1}, respectively. In 2024, biomass increased with P fertilization until reaching a plateau at 18.5 kg P ha^{-1} (Fig. 2.7c). In 2025, biomass did not respond significantly to P fertilization in either inoculation treatment (Fig. 2.7d).

Grain yield was not significantly affected by AMF inoculation but responded to P fertilization in all years (Table A.6), increasing to a plateau at critical P rates of 19.6, 16.5, 14.2, and 15.7 kg P ha^{-1} in 2022, 2023, 2024, and 2025, respectively (Fig. 2.8a–d). Similarly, inoculation did not affect soybean grain yield and P uptake in any of the three growing seasons (Fig. A.7). Despite the lack of a significant yield response, inoculated corn reached the yield plateau at a lower critical soil P level (11.4 mg kg^{-1}) than non-inoculated corn (17.6 mg kg^{-1}; Fig. 2.9a).

Phosphorus Uptake

Phosphorus uptake was predominantly affected by P fertilization, while effects involving AMF inoculation were limited to 2022 and 2023 (Table A.6). At V6, P uptake increased linearly with P fertilization in all years, while inoculation increased uptake by 31% only in 2022 (Fig. 2.10a-d and A.6). At R1, P uptake increased with P fertilization in all years, with significant P × AMF interactions in 2022 and 2023 (Fig. 2.11a, b). In 2022, uptake increased linearly with P fertilization in both inoculation treatments, with inoculation increasing uptake by 65% and 53% at 30 and 60 kg P ha^{-1}, respectively. In 2023, uptake increased linearly with P fertilization in the inoculated treatment but responded quadratically without inoculation, reaching a maximum at 47 kg P ha^{-1}. Inoculation increased uptake by 38% at 60 kg P ha^{-1}. In 2024 and 2025, P uptake increased linearly with P fertilization, regardless of inoculation (Fig. 2.11c, d).

Total P uptake increased significantly with P fertilization in all years (Table A.6), showing linear responses in 2022 and 2023 and quadratic increases in 2024 and 2025 (Fig. 2.12a-d). Across years, total P uptake increased with soil P until it plateaued at similar critical levels with and without AMF inoculation (37 and 34 mg kg^{-1}, respectively; Fig. 2.9b). AMF inoculation increased APRE by 162% in 2022 and 45% in 2023 at 15 kg P ha^{-1}, with no significant differences at 30 or 60 kg P ha^{-1} in either year (Table A.6; Fig. 2.13a, c). In 2024 and 2025, inoculation did not affect

APRE at any P rate (Fig. 2.13b, d). In both rotations, APRE mostly decreased at the highest P rate, by 41% in 2024 and 50% in 2025.

Soil Phosphorus

Soil P had significant P × Year interactions and no responses to AMF inoculation at all depths in both rotations (Table A.8; Table A.9). In the Corn-Soybean rotation at 0–5 and 5–15 cm, soil P increased quadratically with P fertilization in 2024 and 2026 (Fig. 2.14a, b). At 15–30 cm, P fertilization increased linearly with soil P in 2026 (Fig. 2.14c). In the Soybean-Corn rotation, soil P increased quadratically with P fertilization at 0–5 cm in both 2024 and 2026 (Fig. 2.15a). At 5–15 cm, soil P increased linearly with fertilization in 2024 and quadratically in 2026 (Fig. 2.15b). At 15–30 cm, P fertilization had no effect in 2024 but increased soil P quadratically in 2026 (Fig. 2.15c). Differences between sampling years across P rates and soil depths are presented in Tables A.10 and A.11.

Arbuscular Mycorrhizal Fungi Soil Abundance

In the Corn–Soybean rotation, soil AMF abundance was affected by P × Year × AMF interactions at 0–5 cm and P × Year interactions at 5–15 and 15–30 cm (Table A.8). At 0–5 cm, AMF abundance decreased linearly with P fertilization without inoculation in 2024, while the inoculated treatment remained unaffected (Fig. 2.16a). In 2026, AMF abundance decreased linearly with P fertilization in the inoculated treatment and quadratically without inoculation, by approximately 63% and 54%, respectively, from 0 to 60 kg P ha^{-1}. Inoculation also increased AMF abundance by 46% at 0 kg P ha^{-1} (Table A.13; Fig. 2.16a). At 5–15 and 15–30 cm, AMF abundance decreased linearly with P fertilization in both 2024 and 2026 (Fig. 2.16b, c). Soil AMF levels initially increased over the years at both 5–15 and 15–30 cm, but in 2026 decreased to near baseline levels at the highest P rate at 15–30 cm (Tables A.10 and A.12).

In the Soybean–Corn rotation, soil AMF abundance was unaffected by inoculation at all depths, but significant P × Year interactions occurred across depths (Table A.9). At 0–5 cm, AMF abundance decreased linearly with P fertilization in 2024 and 2026, with a 71% reduction from 0 to 60 kg P ha^{-1} in 2026 (Fig. 2.17a). At 5–15 and 15–30 cm, AMF abundance was unaffected by P fertilization in 2024 but decreased linearly in 2026 (Fig. 2.17b, c). Overall, AMF increased from 2024 to 2026 primarily at lower P rates but did not differ between years at the highest P rate (Table A.11).

Water-Stable Macroaggregates

Water-stable macroaggregates >2 mm generally responded positively to AMF inoculation, although responses varied by year and soil depth within each crop rotation (Table A.8; Table A.9). At 0–5 cm in the Corn-Soybean rotation, AMF inoculation significantly increased macroaggregates by approximately 50% at 60 kg P ha^{-1} in 2024 and by 100% at 15 kg P ha^{-1} in 2026 (Table A.13; Fig. 2.18a). In 2024, P fertilization had no effect, while in 2026, macroaggregates decreased linearly with increasing P rates in the inoculated treatment but did not respond without inoculation (Fig. 2.18a). At 5–15 cm, inoculation increased macroaggregates by 107% and 163% at 0 and 15 kg P ha^{-1}, respectively (Fig. 2.18b). Macroaggregates also decreased linearly with increasing P rates with inoculation, with no P response without inoculation. Across soil depths, macroaggregates were highest in 2024 (Fig. 2.18a, c; Fig. A.8a). In the Soybean–Corn rotation, inoculation increased macroaggregates >2 mm by 35% at 0–5 cm and by 64% at 15–30 cm, regardless of P fertilization in 2024 (Fig. 2.19a, d). At 0–5 cm, macroaggregates decreased quadratically with increasing P rates (Fig. 2.19b) and decreased by 19% from 2024 to 2026 (Fig. A.8b). At 5–15 cm, macroaggregates responded quadratically to P fertilization in 2024, while in 2026 they decreased linearly up to a critical P level of 15.9 kg P ha^{-1} and then plateaued (Fig.

2.19c). At 15–30 cm, macroaggregates were higher in 2024 than in 2026, and inoculation increased macroaggregates in 2024 but not in 2026 (Fig. 2.19d).

Water-stable aggregates (0.25–2 mm) responded differently to treatments in each rotation across years and soil depths (Table A.8; Table A.9). At 0–5 cm in the Corn–Soybean rotation, inoculation decreased macroaggregates by approximately 28% at 0 kg P ha^{-1} in 2024 and by 29% at 15 kg P ha^{-1} in 2026 (Fig. 2.20a). In 2026, macroaggregates increased quadratically with P fertilization under AMF inoculation but did not respond without inoculation. At 5–15 cm in 2026, macroaggregates increased linearly up to a critical P level of 29.1 kg P ha^{-1} and then plateaued (Fig. 2.20b). No significant responses to treatments were observed at 15–30 cm (Table A.8). In the Soybean–Corn rotation, water-stable aggregates (0.25–2 mm) increased linearly with P fertilization at 0–5 cm (Fig. 2.21a). At 5–15 cm, macroaggregates increased linearly up to a critical P level of 21.3 kg P ha^{-1} and then plateaued (Fig. 2.21b), with greater macroaggregate abundance in 2026 than in 2024 (Fig. A.8c). No significant responses occurred at 15–30 cm (Table A.9).

Soil Microbial Community Abundance

Soil microbial community abundance varied substantially by year and depth, with limited and inconsistent treatment responses (Tables A.8 and A.9). In the Corn–Soybean rotation, microbial groups generally increased from 2022 to 2026 across depths (Tables A.10 and A.14). P fertilization affected actinomycetes (0-5 cm), total microbial biomass (5-15 cm), and Gram-positive bacteria (5-15 cm), while inoculation reduced total microbial biomass and Gram-positive bacteria at 5–15 cm in 2026 by 11 and 17%, respectively (Fig. A.9a–c; Table A.14). In the Soybean–Corn rotation, temporal changes varied among microbial groups and depths, and inoculation reduced total microbial biomass by 7% at 15-30 cm (Table A.15; Fig. A.9d).

Extracellular enzyme activity

Soil enzyme activities varied among years and depths in both rotations, with very limited responses to treatments (Tables A.8, A.9, A.16, and A.17). In the Corn–Soybean rotation, acid phosphatase activity increased linearly with P fertilization in 2026 (Fig. A.10). In the Soybean–Corn rotation, inoculation reduced acid phosphatase activity by 8 and 10% at 5–15 and 15–30 cm, respectively, and NAG activity by 16% at 15–30 cm (Fig. A.11a–c).

Soil Chemical Properties

Soil chemical properties varied over time, with SOC stocks and TN being mainly affected by year and showing limited and inconsistent responses to treatments (Tables A.8, A.9, A.18, and A.19; Fig. A.12 and A.13). Soil pH decreased linearly with P fertilization at 0–5 cm in 2024 and 2026 in both rotations, while AMF inoculation reduced pH at 15–30 cm only in the Soybean–Corn rotation (Fig. A.14 and A.15).

Discussion

The plant and soil responses from this study advance our knowledge of the complex interactions between AMF inoculation and P management on corn performance and soil health under field conditions. Across multiple years of field sampling, P fertilization regulated plant-soil-microbial processes more strongly than AMF inoculation, while AMF inoculation provided its greatest benefits under low P availability. Collectively, these results partially support our hypothesis that AMF inoculation would improve plant growth, P acquisition, and soil health, with the strongest responses under low to moderate P availability.

Root colonization by AMF consistently increased as soil P levels declined across all sampled stages, up to critical levels of approximately 19, 24, and 28 mg kg^{-1} at V6, R1, and R6, respectively, beyond which colonization plateaued and had little further response to increasing soil

P. Interestingly, these critical levels fall within a similar range to Kansas soil test recommendations for corn, where P fertilizer is generally not necessary when Mehlich-3 soil P exceeds 20 mg kg^{-1}, suggesting that the soil P range associated with reduced responsiveness of AMF colonization also corresponds closely with levels considered sufficient for corn production in Kansas (Ruiz Diaz et al., 2024). The negative relationship between P availability and AMF root colonization is well established and consistent with previous studies in corn showing reduced mycorrhizal colonization under increasing P availability (Liu et al., 2016; Sheng et al., 2012; Wang et al., 2017).

The stage-specific critical P levels identified in this study demonstrated that AMF root colonization was more sensitive to changes in soil P under low to moderate P levels, while colonization stabilized at lower rates above these critical levels. Interestingly, the progressive increase in the critical P level and decrease in AMF root colonization from V6 to R6 suggest that the P threshold associated with AMF colonization suppression shifted as the crop developed. A recent study by Ampong et al. (2024) demonstrated that corn P demand becomes particularly important after V6, and that adequate P availability between V6 and R1 is critical for optimum yield, while sufficient P availability before V6 or from R1 to R6 is less critical. The lower critical P level observed at V6 might be a result of the greater importance of the AMF symbiosis during early crop development, when root systems are still developing, and mycorrhizal hyphae can extend beyond the P depletion zone surrounding roots to increase the volume of soil explored for P acquisition (Basílio et al., 2022; Smith et al., 2003). As the crop develops and root systems expand, the level at which colonization plateaus shifts toward higher soil P levels, suggesting a crop stage-dependent relationship between plant P acquisition and maintenance of the AMF symbiosis. These findings are consistent with the well-established regulation of the mycorrhizal symbiosis by the host plant, once plants supply photosynthetically derived C to AMF in exchange

for nutrients, and as P availability increases, the relative benefit of maintaining this symbiosis may decline, leading to reduced C allocation to the fungal partner and, consequently, lower AMF colonization (Fitter, 2006; Konvalinková et al., 2017; Ven et al., 2019).

Substantial root colonization occurred in non-inoculated plants across all sampled years, indicating a well-established indigenous AMF community at the site. The considerable overlap between inoculated and non-inoculated treatments across the soil P gradient further suggests that inoculation may have supplemented this existing AMF community rather than substantially altering overall mycorrhizal response to soil P. In nonsterile agricultural soils, inoculation introduces fungi into an existing microbial community, and their interactions can influence the establishment and effectiveness of the inoculant (Basiru & Hijri, 2022; Verbruggen et al., 2013). For instance, Lekberg & Koide, (2005) reported that AMF inoculation increased root colonization, but plant responses became less likely when indigenous inoculum potential or soil P was already high. Verbruggen et al. (2013) reported that the establishment of introduced AMF also depends on compatibility with the host and environment, competition with indigenous fungi, and the ecological carrying capacity of the soil.

The most consistent agronomic responses to AMF inoculation were P uptake and plant growth, not grain yield. Inoculation increased P uptake and plant growth at several sampled stages, including significant increases in APRE at the lowest fertilizer rate during the first year of each rotation, consistent with a two-year field study with P fertilization and AMF inoculation in corn that found that AMF inoculation improved crop performance only during the first year, with no detectable benefits in the subsequent year (de Souza Buzo et al., 2023). Importantly, inoculation did not influence APRE at higher P rates and during the second corn year of each rotation, indicating that the inoculant was most effective when P was limiting, as at higher P rates, direct

root acquisition and increasing soil P availability may have reduced the relative contribution of the fungal pathway (Madrid-Delgado et al., 2021). The overall decrease in APRE at the highest P rate suggests the crop recovers less additional fertilizer P, consistent with the greater accumulation of residual soil P observed over time. Also, the greater P recovery observed in the second corn year of each rotation may partly reflect residual P from the previous fertilizer application. While fertilizer P recovery is generally low within a single growing season, long-term studies report greater recovery as P accumulates (Schlegel & Havlin, 2017).

Our results are also consistent with a recent meta-analysis of 187 studies, which reported that AMF inoculation increased plant biomass by an average of 47% and whole-plant P uptake by 105%, while the magnitude of inoculation benefits was significantly smaller under field experiments than under controlled conditions and was greater with single-species inoculants than with multi-species AMF inoculants (Wu et al., 2024). Similarly, a 54-site field trial in Switzerland demonstrated substantial variability in maize responses to AMF inoculation, with growth responses ranging from a 12% decrease to a 40% increase across sites, highlighting the context-dependent performance of inoculants under field conditions (Lutz et al., 2023).

Although AMF inoculation did not significantly increase corn grain yield in the present study, this result is consistent with the substantial variability reported in field evaluations of AMF inoculants (Lahijanian et al., 2026). In a meta-analysis of 70 field inoculation studies of major cereal crops, Zhang et al. (2019) reported an average grain-yield increase of 16% following AMF inoculation, but emphasized that yield responses can vary with crop species, soil pH, and cultivar release date, and that AMF effects were smaller under field than controlled conditions. Therefore, we provide practical evidence that improved P acquisition does not necessarily translate directly into proportional increases in grain yield. It is also worth mentioning that the inoculated treatment

reached the yield plateau at 11.4 mg kg^{-1} Mehlich-3 soil P compared with 17.6 mg kg^{-1} without inoculation, while achieving similar maximum yields, suggesting that inoculation may have enabled corn to reach near-maximum yield at lower soil P availability, potentially reducing the soil P level required to sustain yield.

Previous research has shown that soil P availability can strongly influence the abundance and community composition of native AMF, which may, in turn, affect the establishment of introduced inoculants (Bender et al., 2019). Consequently, positive crop responses to inoculation are more likely when indigenous AMF communities are less developed and root colonization has not yet reached potential levels (Bender et al., 2019).

Repeated P fertilization during corn years progressively increased soil P in both rotations, with P accumulating beyond the surface layer (0–5 cm) and significant responses extending to 30 cm depth by 2026. Similar to AMF root colonization, soil AMF abundance, measured by NLFA, consistently decreased as soil P levels increased across multiple depths and years. Interestingly, AMF inoculation increased soil AMF abundance by 46% at 0–5 cm in the unfertilized treatment of the Corn–Soybean rotation in 2026, highlighting the potential of inoculation to increase AMF biomass in the soil, primarily under low P availability. At lower P rates, soil AMF abundance often increased over time, while at the highest P rate, these temporal increases were absent or decreased to baseline levels. The lack of an inoculation response with increasing P availability further supports the strong regulation of AMF by soil P, as greater P availability can reduce plant mycorrhizal dependence and C allocation to the fungal partner when the benefits of AMF-mediated P acquisition decline (Konvalinková et al., 2017; Madrid-Delgado et al., 2021; Ven et al., 2019). The increase in soil AMF abundance over time may also be partly associated with the introduction of cover crops at the beginning of the experiment, as both fields had already been under corn–

soybean rotations for almost a decade. Despite their potential contribution to AMF abundance, cover crop biomass and P uptake were generally unaffected by AMF inoculation, with significant responses observed only during the 2023–2024 growing season in the Soybean–Corn rotation (Fig. A.16–A.18).

Importantly, the high levels of root colonization and soil AMF biomass without inoculation highlight the importance of managing indigenous AMF communities for crop production and soil health. Practices such as cover cropping, crop diversification, and no-till can naturally promote indigenous AMF by maintaining host plants year-round and reducing disruption of existing hyphal networks (Bowles et al., 2017; Brito et al., 2021). Thus, management practices that maintain active indigenous AMF communities may provide many of the ecosystem functions targeted by commercial bioinoculants without requiring repeated applications of external propagules. Cover crops can increase AMF abundance by maintaining living hosts between cash crops, with a recent meta-analysis reporting greater AMF root colonization, spore abundance, hyphal length density, and extraradical biomass under cover crops compared with fallow (Ge et al., 2025). The consistent responses of AMF root colonization and soil AMF abundance provide important context for interpreting the outcomes of inoculation in both plant and soil responses, suggesting that increasing the supply of AMF propagules may not overcome the strong regulation of the symbiosis by P availability, as when P becomes more abundant, the host may have had progressively less need to allocate C to either native or introduced AMF, reducing the positive effects of inoculation during the second corn year.

Soil aggregates regulate SOM turnover and can respond rapidly to management practices and soil disturbance (Lavallee et al., 2019; Six & Paustian, 2014). Despite limited and highly variable effects on crop performance, AMF inoculation consistently increased the >2mm water-

stable macroaggregate fraction at multiple depths and in both rotations under low P, consistent with the recognized role of AMF in soil structure, as AMF hyphae can physically enmesh soil particles, and contribute to organic binding agents that promote the formation and stabilization of aggregates (de Goede et al., 2025; Miller & Jastrow, 1990; Rillig et al., 2015; Rillig & Mummey, 2006; Wilson et al., 2009). Similarly, Gómez-Leyva et al. (2023) reported increases in soil aggregate stability after AMF inoculation in maize, although the response magnitude depended on soil type and AMF inoculum. Similarly, Morris et al. (2019) found that AMF inoculation significantly increased large macroaggregate formation and slowed disintegration rates, while also increasing microaggregate turnover. Studies have also shown that aggregation responses also depend strongly on the identity of both plant and fungal partners (Piotrowski et al., 2004). A meta-analysis of 93 studies found that AMF significantly improved soil aggregation, with effects comparable in magnitude to their positive effects on plant N and P uptake (Delavaux et al., 2017).

The relationship between AMF and soil aggregation may also contribute to soil C stabilization, as AMF hyphae and aggregate formation can physically protect soil C, although this study found no significant differences in SOC stocks with inoculation (Rillig & Mummey, 2006). The aggregation response to P also tracked changes in AMF abundance. The positive inoculation effects on >2-mm aggregates occurred at low to moderate P rates, while higher P reduced this fraction in inoculated treatments. As increasing P also suppressed AMF abundance, reduced AMF biomass may have weakened their contribution to aggregate stability. However, opposite responses observed between the >2-mm and 0.25–2-mm macroaggregate fractions suggest that treatments may also have shifted aggregates between size classes rather than simply reducing overall aggregation, consistent with the dynamic formation and turnover of soil aggregates (Six et al., 2000; Tisdall & Oades, 1982).

In contrast to the relatively clear and consistent responses of soil AMF abundance and soil aggregation, extracellular enzyme activity, total microbial biomass, and microbial groups did not consistently respond to inoculation or P fertilization. Instead, enzyme activity and microbial abundance changed mainly by sampling year, suggesting that AMF inoculation did not cause a major restructuring of microbial biomass detectable under these field conditions. Differences in treatment responses between rotations were also expected because the Corn–Soybean rotation was sampled two years after P fertilization and AMF inoculation, whereas the Soybean–Corn rotation was sampled one year after treatment application, allowing different amounts of time for treatment effects to develop. In addition, the crop preceding soil sampling differed between rotations, with soybean residue present in the Corn–Soybean rotation field and corn residue in the Soybean–Corn rotation field. Corn and soybean differ substantially in the amount and quality of residue returned to soil, including their C:N ratios, which can influence residue decomposition, nutrient cycling, and soil microbial activity (Broder & Wagner, 1988; Hsiao et al., 2019).

Mycorrhizae can alter rhizosphere microbial communities through changes in C inputs, nutrient acquisition, and hyphosphere interactions, but these responses are context dependent. Therefore, the limited responses of Gram-positive and Gram-negative bacteria, actinomycetes, and saprophytic fungi in our study do not necessarily mean that microbial interactions did not occur, as community composition or function may change without large shifts in the overall abundance of these groups (Frostegård et al., 2011). The limited effects of AMF inoculation on microbial communities in our study may also reflect the relatively favorable environmental conditions at the irrigated site. The AMF influence on microbial communities may become more pronounced under environmental stress, as AMF inoculation under drought has been shown to increase microbial

biomass and drive stronger shifts in rhizosphere microbial community structure and composition than under well-watered conditions (Li et al., 2025).

Similarly, extracellular enzyme activity also had strong temporal and depth-dependent variation and limited responses to AMF inoculation. Acid phosphatase is commonly produced under low P levels to mineralize organic P and is generally suppressed by P fertilization (Gou et al., 2024; Marklein & Houlton, 2012; Touhami et al., 2023). Interestingly, acid phosphatase activity increased at the highest P rate at 0-5 cm in the Corn-Soybean rotation, contrasting with the general expectation that acid phosphatase activity is suppressed by increasing inorganic P availability (Bünemann et al., 2012; Gou et al., 2024; Touhami et al., 2023). Research has shown that some acid phosphatases are P-irrepressible (Rodríguez & Fraga, 1999). For instance, Zheng et al. (2021) reported higher acid phosphatase activity under long-term N and P fertilization treatments compared with the unfertilized control. Janes-Bassett et al., (2022) found that monoesterase activity decreased with inorganic P availability but increased with organic P availability, suggesting that organic P supply can be more important than P deficiency in regulating phosphatase activity. However, the increase in acid phosphatase activity with P fertilization observed in our study may reflect indirect treatment effects, such as greater plant growth and residue with P fertilization and associated rhizosphere activity or decreases in soil pH toward conditions more favorable for acid phosphatase activity.

As soil P accumulated with repeated fertilizations in corn years, soil pH decreased with increasing P rates at 0-5 cm in both rotations, probably because of the use of monoammonium phosphate, which provides a source of ammonium for nitrification, a process known to release protons and decrease soil pH (Chirchir et al., 2026). Similarly, Liu et al. (2016) also reported that soil pH decreased at the highest applied P rate. Importantly, soil pH strongly influences fungal

community composition, nutrient availability, and inoculation response (Gou et al., 2024; Pellegrino et al., 2015; Schütz et al., 2018). Consequently, the progressive changes in both soil P and pH may have jointly contributed to the observed AMF inoculation responses over time (Xu et al., 2025).

Conclusion

Our study brings new evidence of the complex interactions between AMF inoculation and P fertilization under field conditions over multiple years and fills an important knowledge gap left by short-term field and controlled-environment inoculation studies. Across multiple years of field sampling, P fertilization more strongly regulated plant–soil–microbial processes than AMF inoculation, increasing crop growth, P uptake, and grain yield while progressively increasing soil P and reducing AMF root colonization and soil abundance. In contrast, the strongest and most consistent inoculation benefits were associated with P uptake, biomass production, P recovery, and the formation of large water-stable aggregates, while grain yield, soil microbial groups, extracellular enzyme activities, SOC stocks, and total N responded weakly or inconsistently to inoculation. Both fields had very low initial soil P, and inoculation provided measurable benefits primarily under low P availability and during the first year of inoculation, with these benefits becoming less evident as soil P accumulated over time. Overall, these findings highlight the importance of balancing P inputs with AMF function and suggest that AMF inoculation may provide greater benefits in P-deficient soils, particularly for P uptake, plant growth, and soil structure. Our results also demonstrate the importance of evaluating AMF inoculation in the field across multiple growing seasons, as treatment effects varied over time and were strongly influenced by soil P availability, crop rotation, and existing native AMF communities, as well as seasonal variabilities that cannot be captured in controlled-environment studies. Therefore,

responses observed shortly after a single inoculation may not fully capture inoculant performance or its long-term effects on plant and soil processes. Further field research, particularly focused on soil microbial ecology, is needed to better understand interactions between introduced AMF and native microbial communities and how these interactions influence inoculant establishment, persistence, and effectiveness over time.

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Figures

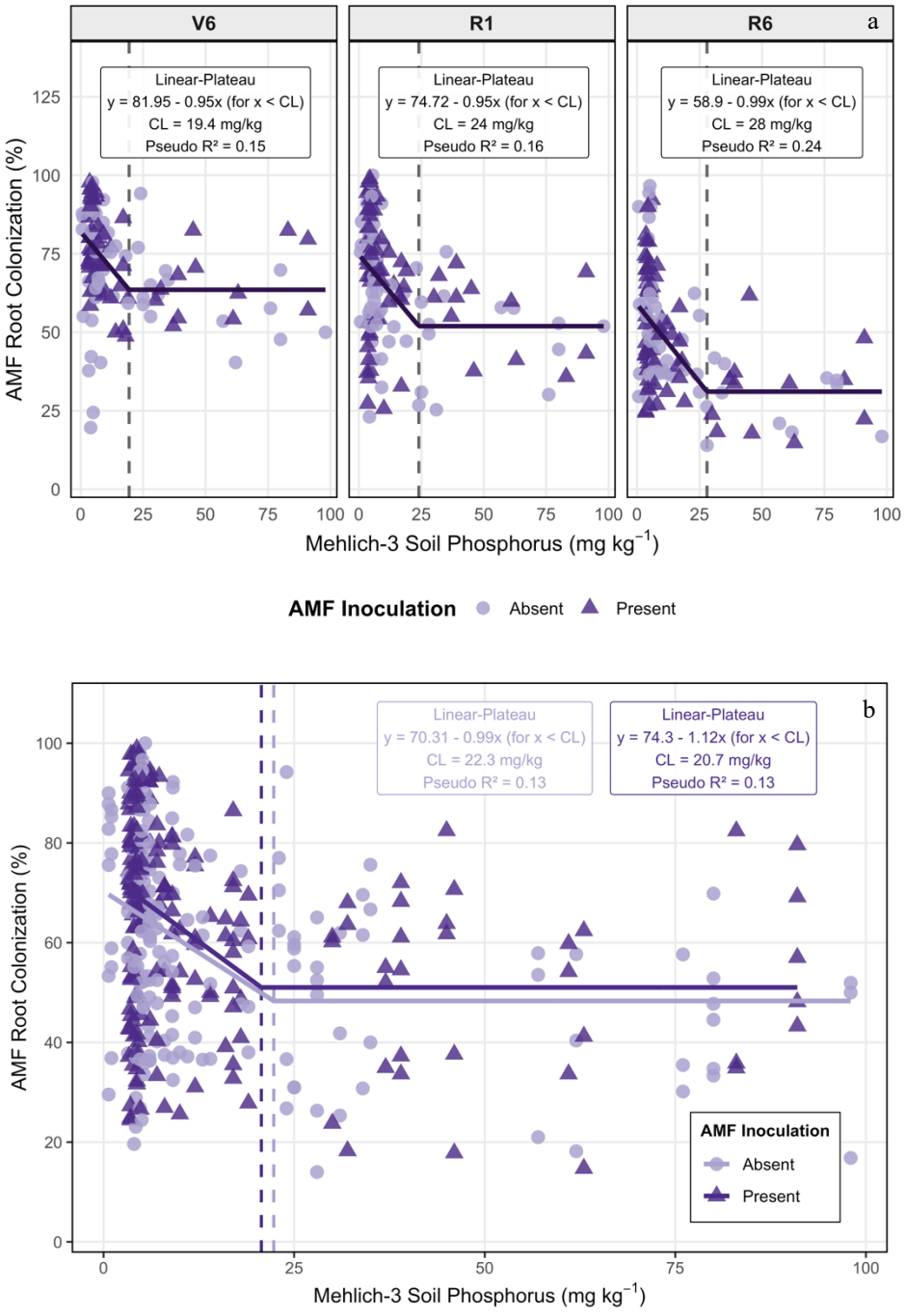


Figure 2.1. Relationship between Mehlich-3 soil phosphorus (P) (0-5 cm) and arbuscular mycorrhizal fungi (AMF) root colonization in the Corn–Soybean and Soybean–Corn rotations from 2022 to 2025 in Scandia, KS. (a) Relationships at the V6, R1, and R6 growth stages across years and AMF treatments and (b) relationships in the absence and presence of AMF inoculation across years, rotations, and growth stages. Circles represent raw observations in the absence of AMF inoculation, and triangles represent raw observations with AMF inoculation.

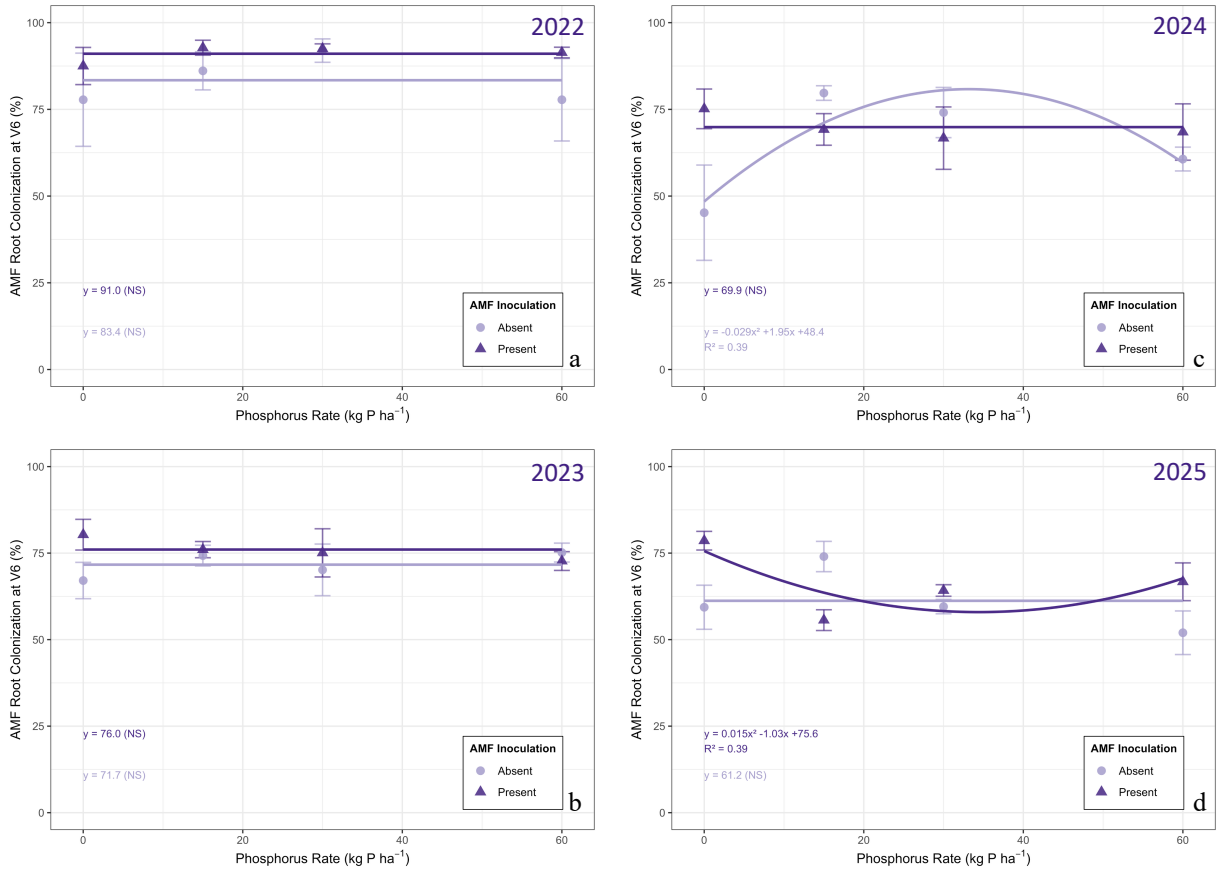


Figure 2.2. Effects of phosphorus fertilization and arbuscular mycorrhizal fungi (AMF) inoculation on AMF root colonization (%) at V6 across years and crop rotations in Scandia, KS. Points represent means ± standard error. The years 2022 and 2023 represent the first corn year of each rotation, while 2024 and 2025 represent the second corn year.

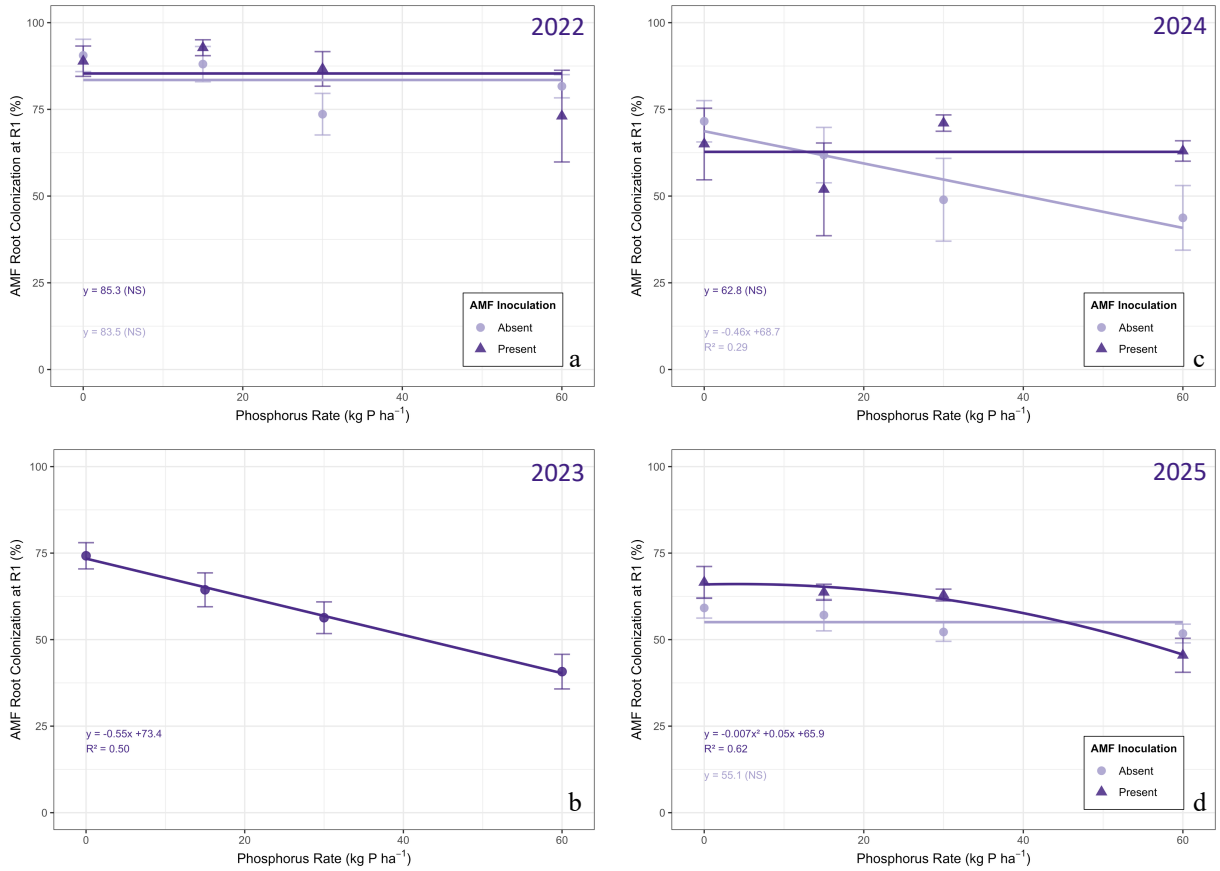


Figure 2.3. Effects of phosphorus fertilization and arbuscular mycorrhizal fungi (AMF) inoculation on AMF root colonization (%) at R1 across years and crop rotations in Scandia, KS. Points represent means ± standard error. The years 2022 and 2023 represent the first corn year of each rotation, while 2024 and 2025 represent the second corn year.

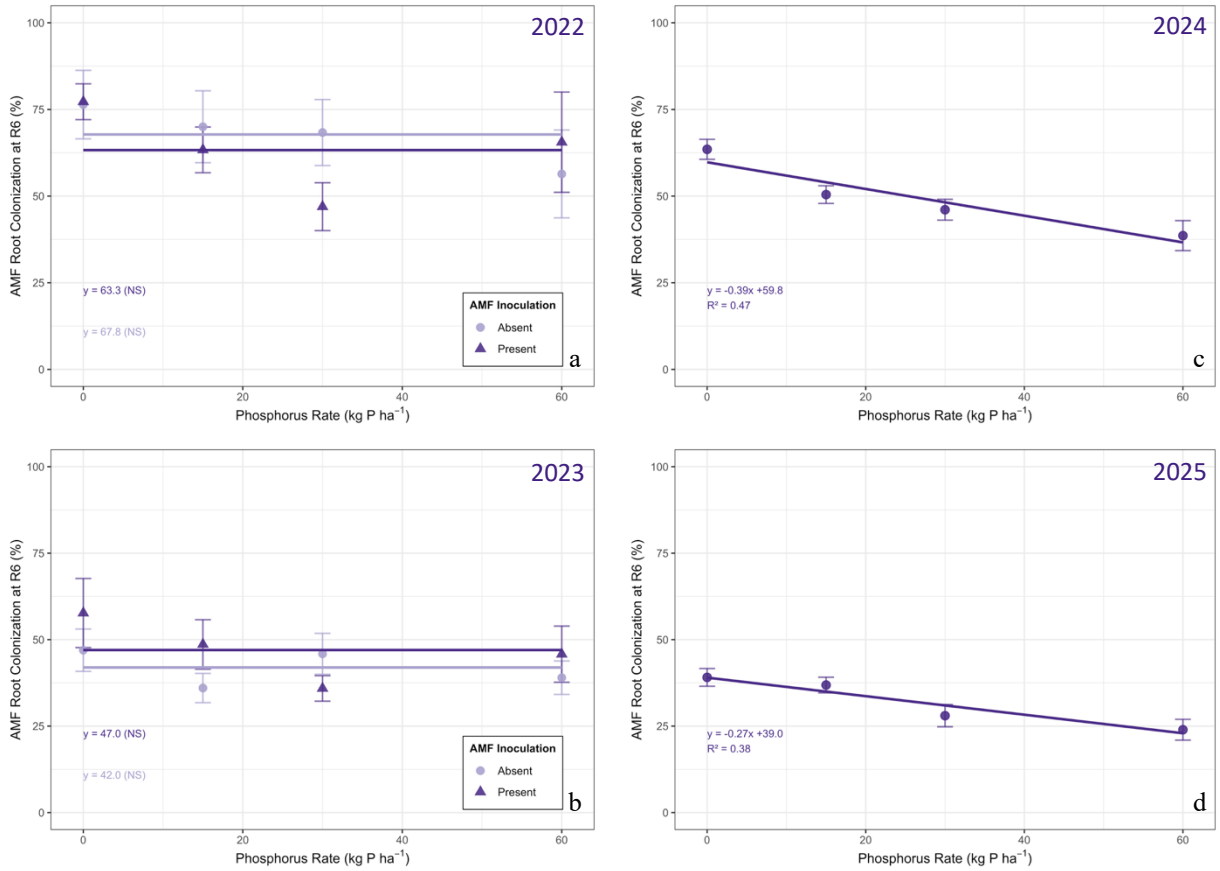


Figure 2.4. Effects of phosphorus fertilization and arbuscular mycorrhizal fungi (AMF) inoculation on AMF root colonization (%) at R6 across years and crop rotations in Scandia, KS. Points represent means ± standard error. The years 2022 and 2023 represent the first corn year of each rotation, while 2024 and 2025 represent the second corn year.

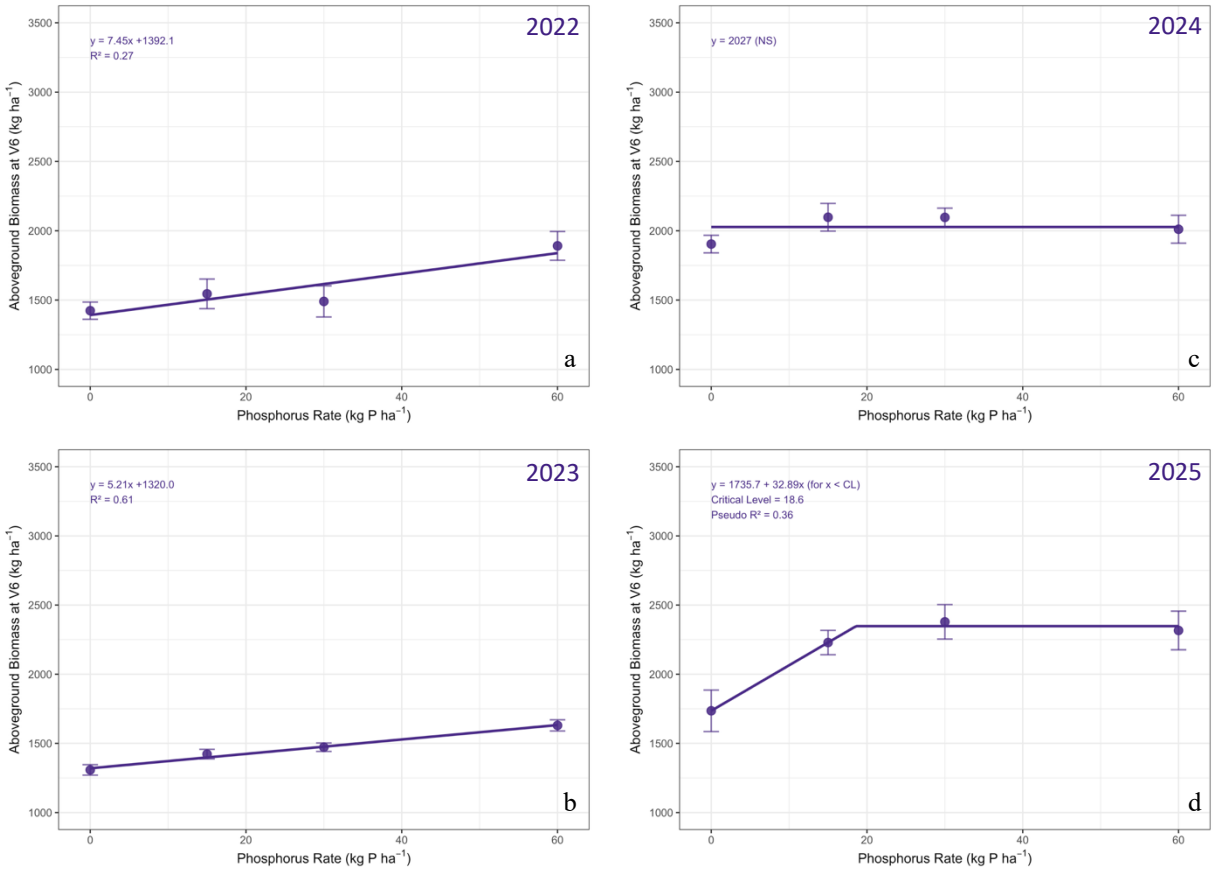


Figure 2.5. Effects of phosphorus fertilization on aboveground biomass (kg ha^{-1}) at V6 across years and crop rotations in Scandia, KS. Points represent means ± standard error. The years 2022 and 2023 represent the first corn year of each rotation, while 2024 and 2025 represent the second corn year.

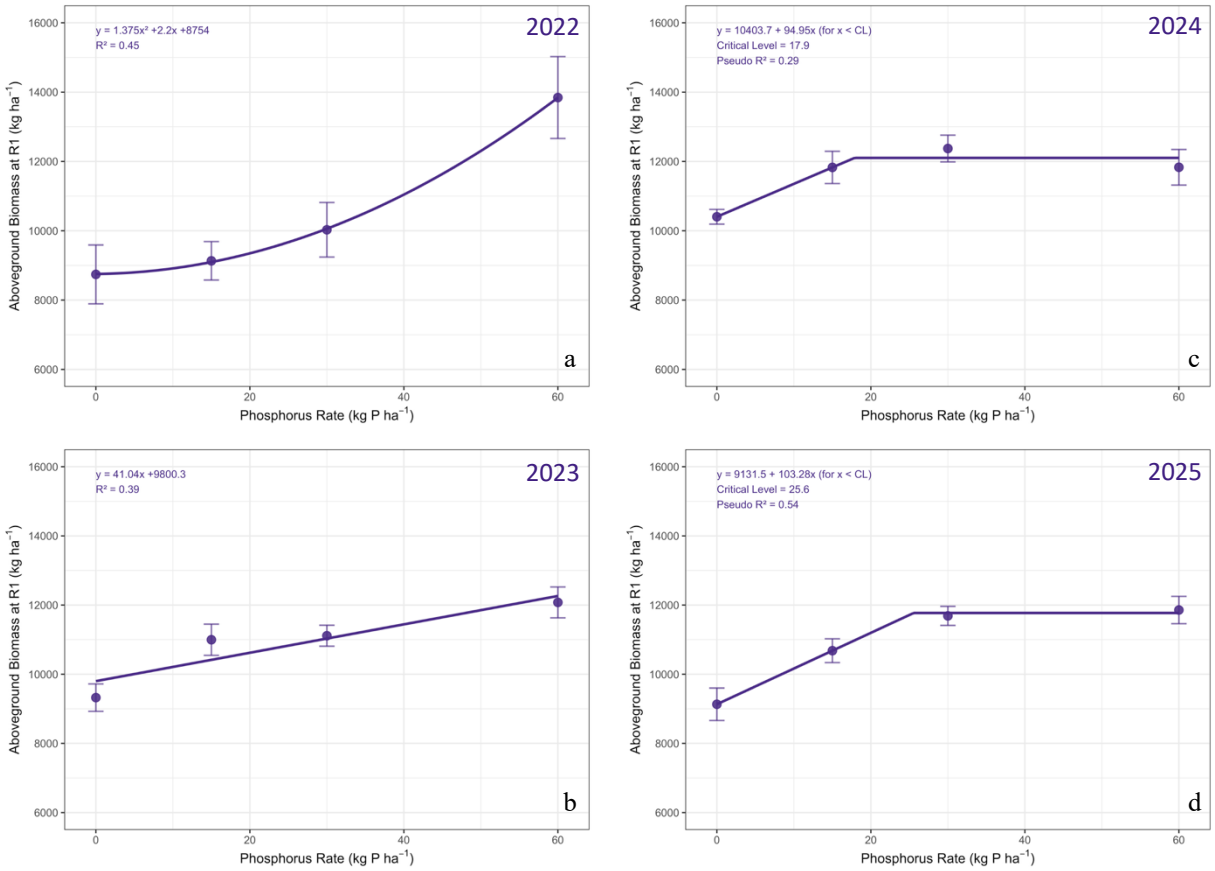


Figure 2.6. Effects of phosphorus fertilization on aboveground biomass (kg ha^{-1}) at R1 across years and crop rotations in Scandia, KS. Points represent means ± standard error. The years 2022 and 2023 represent the first corn year of each rotation, while 2024 and 2025 represent the second corn year.

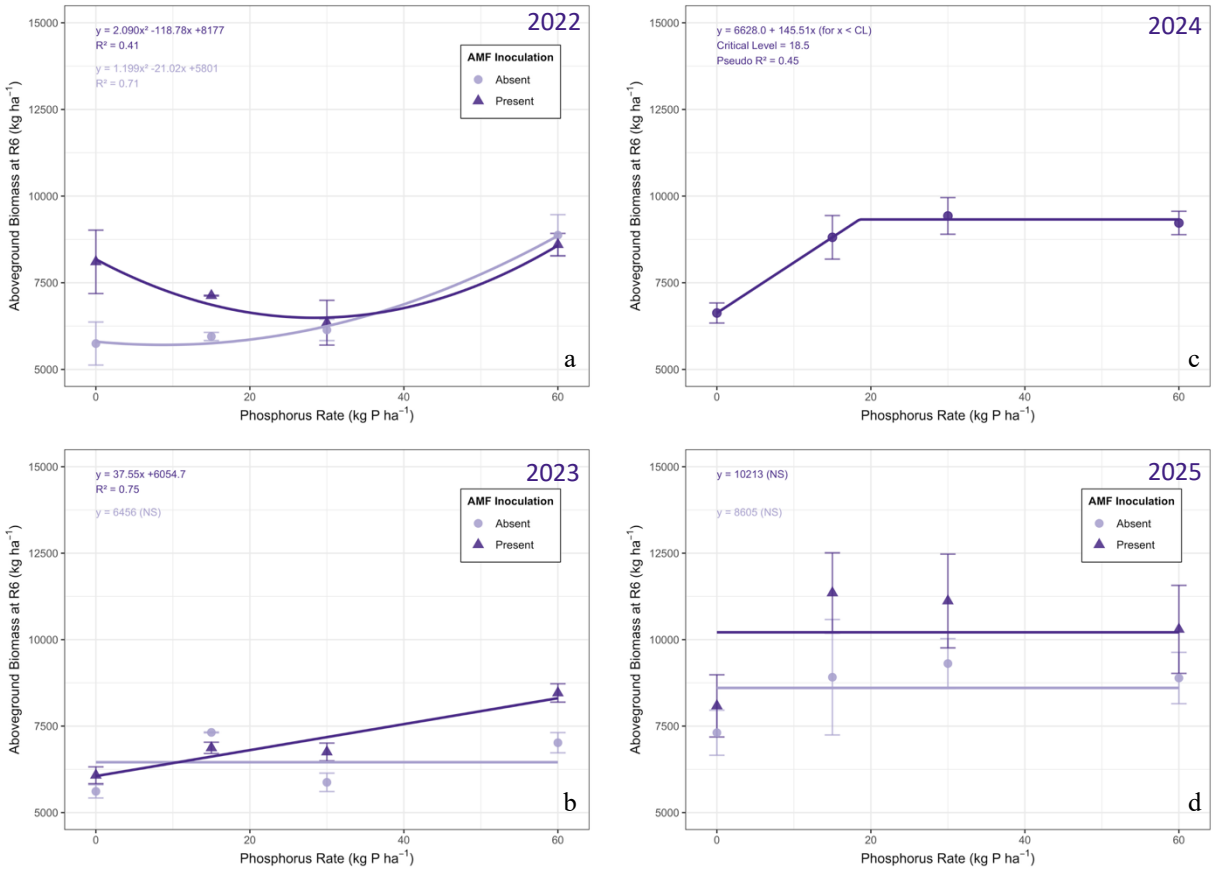


Figure 2.7. Effects of phosphorus fertilization and arbuscular mycorrhizal fungi (AMF) inoculation on aboveground biomass (kg ha^{-1}) at R6 across years and crop rotations in Scandia, KS. Points represent means ± standard error. The years 2022 and 2023 represent the first corn year of each rotation, while 2024 and 2025 represent the second corn year.

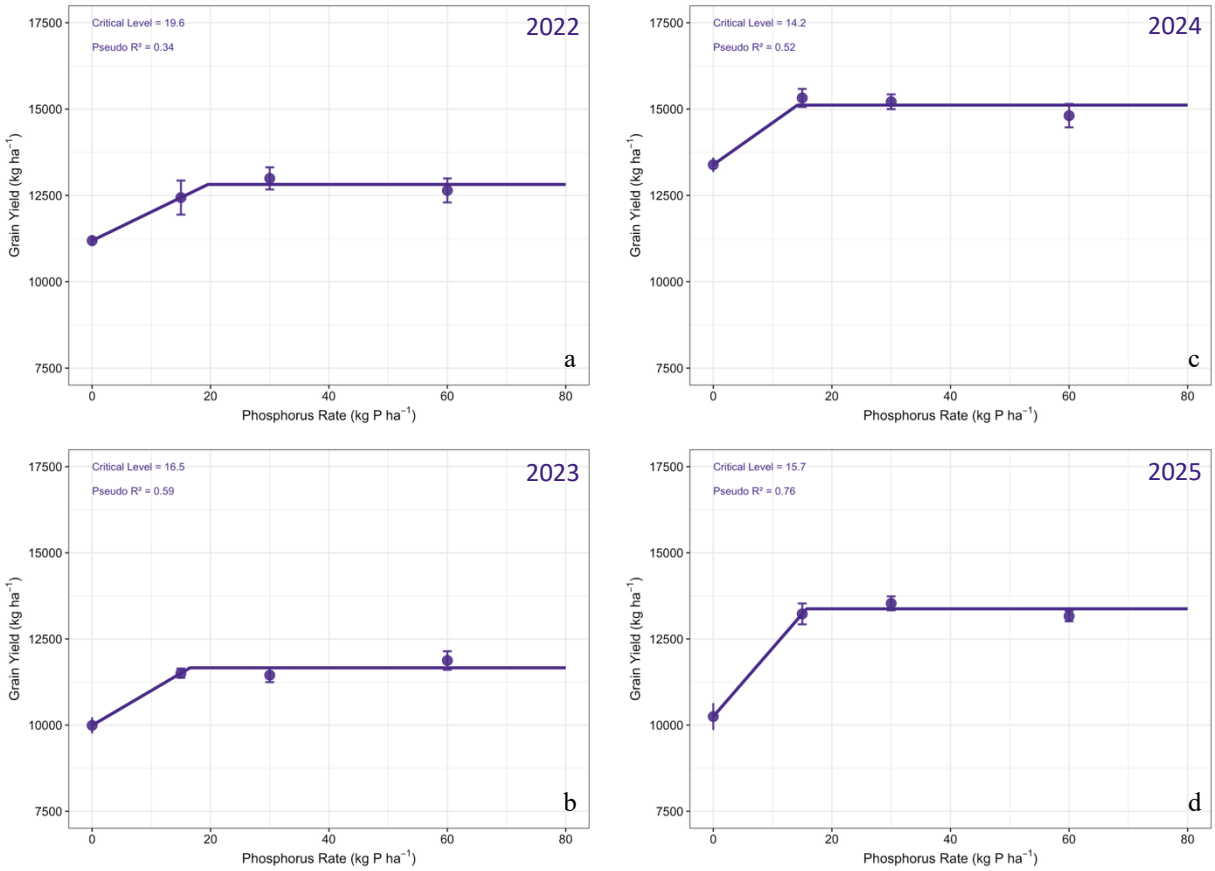


Figure 2.8. Effects of phosphorus (P) fertilization on grain yield (kg ha^{-1}) across years and crop rotations in Scandia, KS. The years 2022 and 2023 represent the first corn year of each rotation, while 2024 and 2025 represent the second corn year. Points represent means with standard error bars.

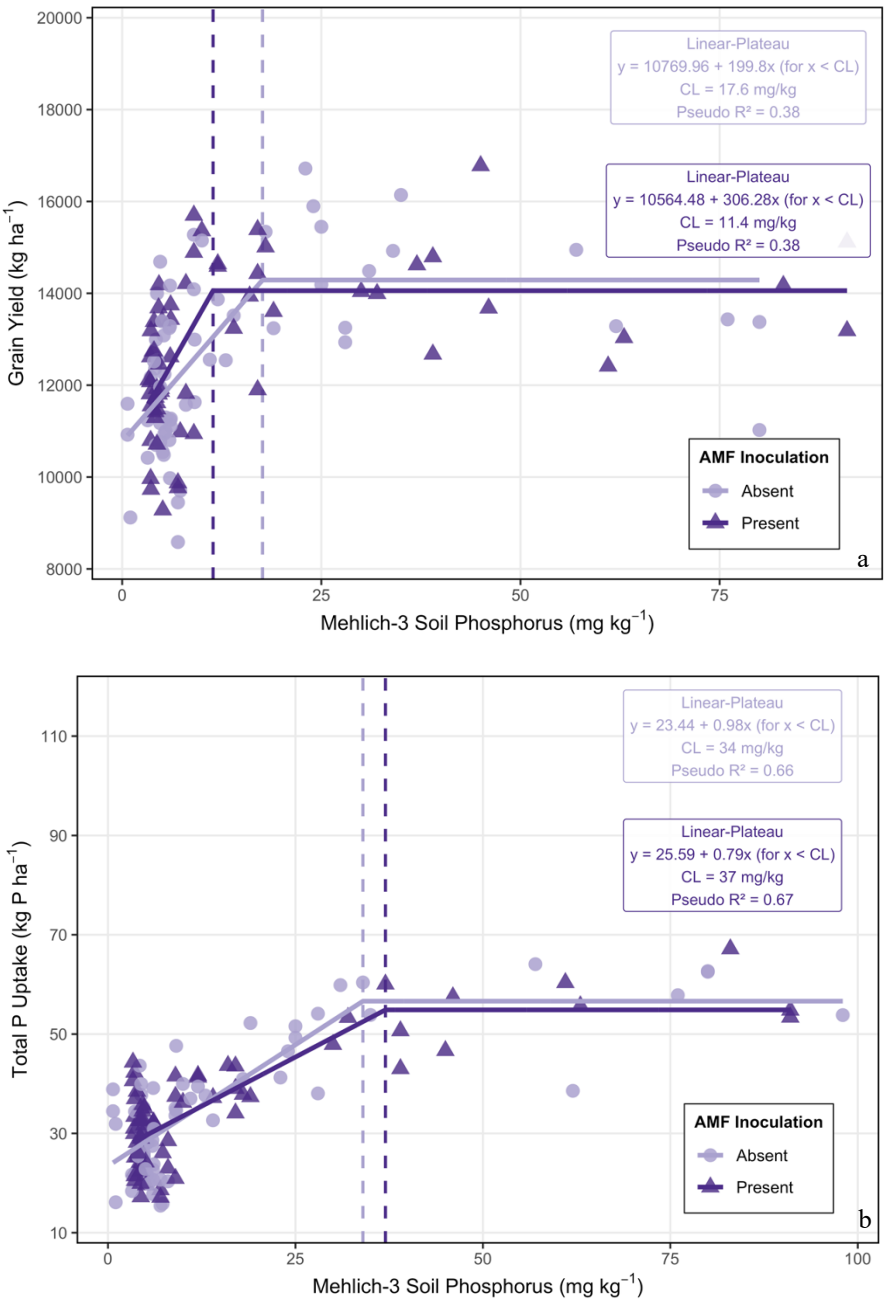


Figure 2.9. Relationship between Mehlich-3 soil phosphorus (P) (0-5 cm) and (a) grain yield and (b) total P uptake at R6 in the Corn–Soybean and Soybean–Corn rotations from 2022 to 2025 in Scandia, KS. Circles represent raw observations without AMF inoculation, and triangles represent raw observations with AMF inoculation.

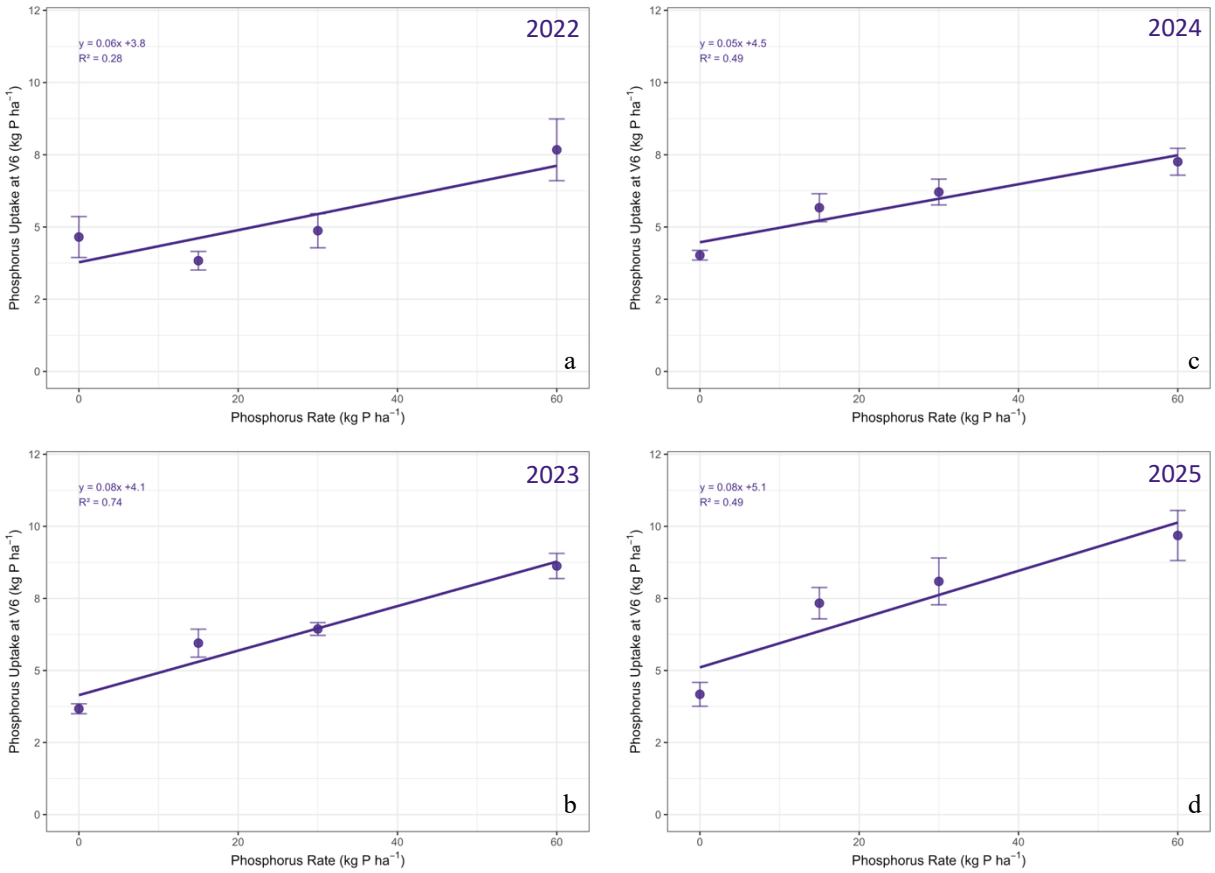


Figure 2.10. Effects of phosphorus fertilization on phosphorus uptake (kg P ha^{-1}) at V6 across years and crop rotations in Scandia, KS. Points represent means ± standard error. The years 2022 and 2023 represent the first corn year of each rotation, while 2024 and 2025 represent the second corn year.

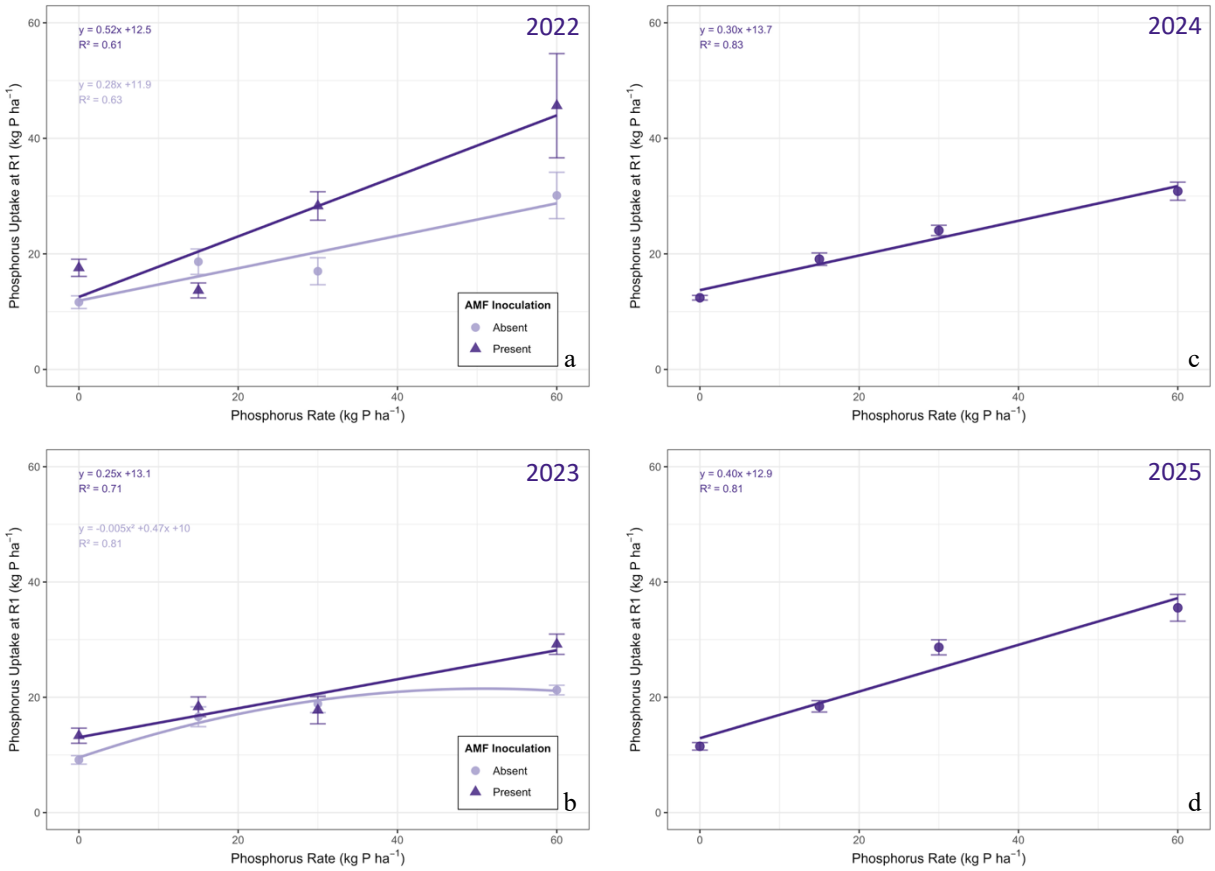


Figure 2.11. Effects of phosphorus fertilization and arbuscular mycorrhizal fungi (AMF) inoculation on phosphorus uptake (kg P ha^{-1}) at R1 across years and crop rotations in Scandia, KS. Points represent means ± standard error. The years 2022 and 2023 represent the first corn year of each rotation, while 2024 and 2025 represent the second corn year.

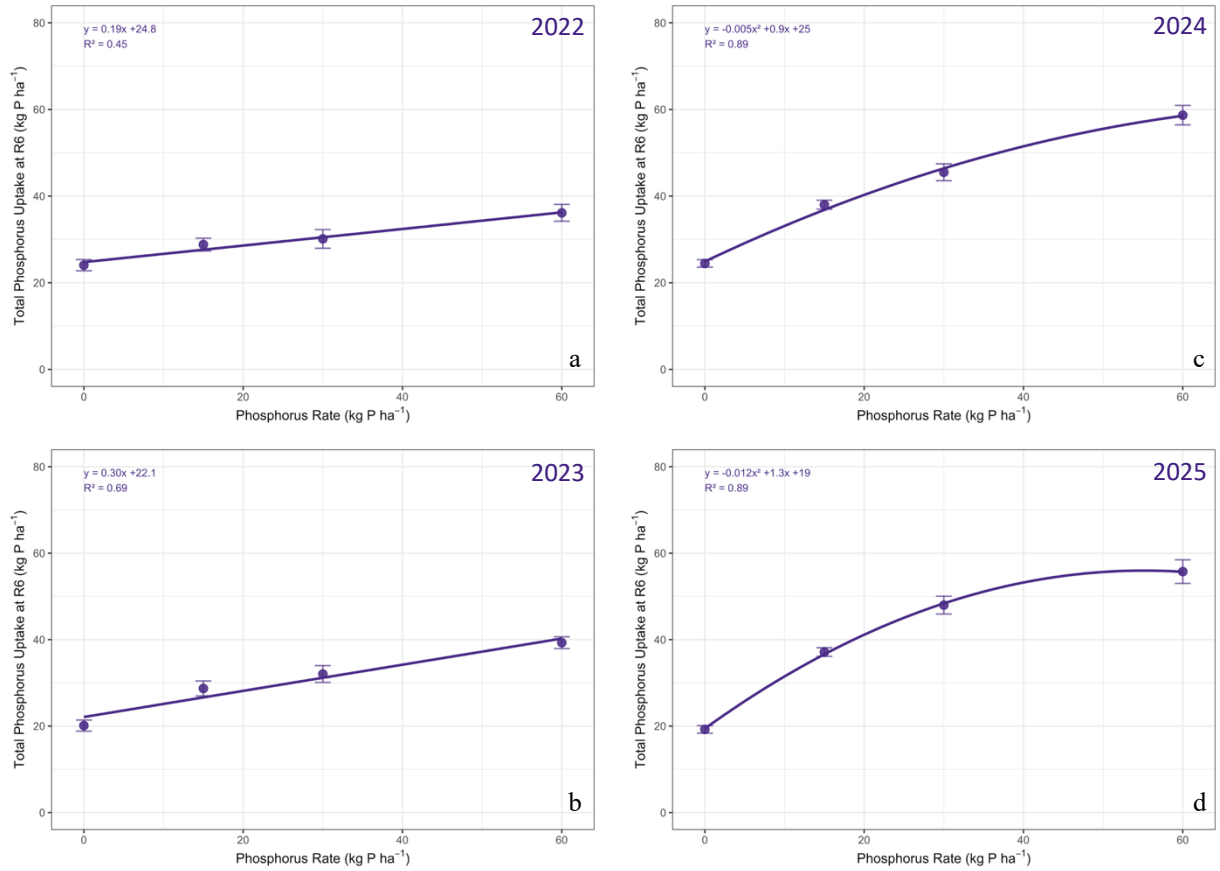


Figure 2.12. Effects of phosphorus fertilization on total phosphorus uptake (kg P ha^{-1}) at R6 across years and crop rotations in Scandia, KS. Points represent means ± standard error. The years 2022 and 2023 represent the first corn year of each rotation, while 2024 and 2025 represent the second corn year.

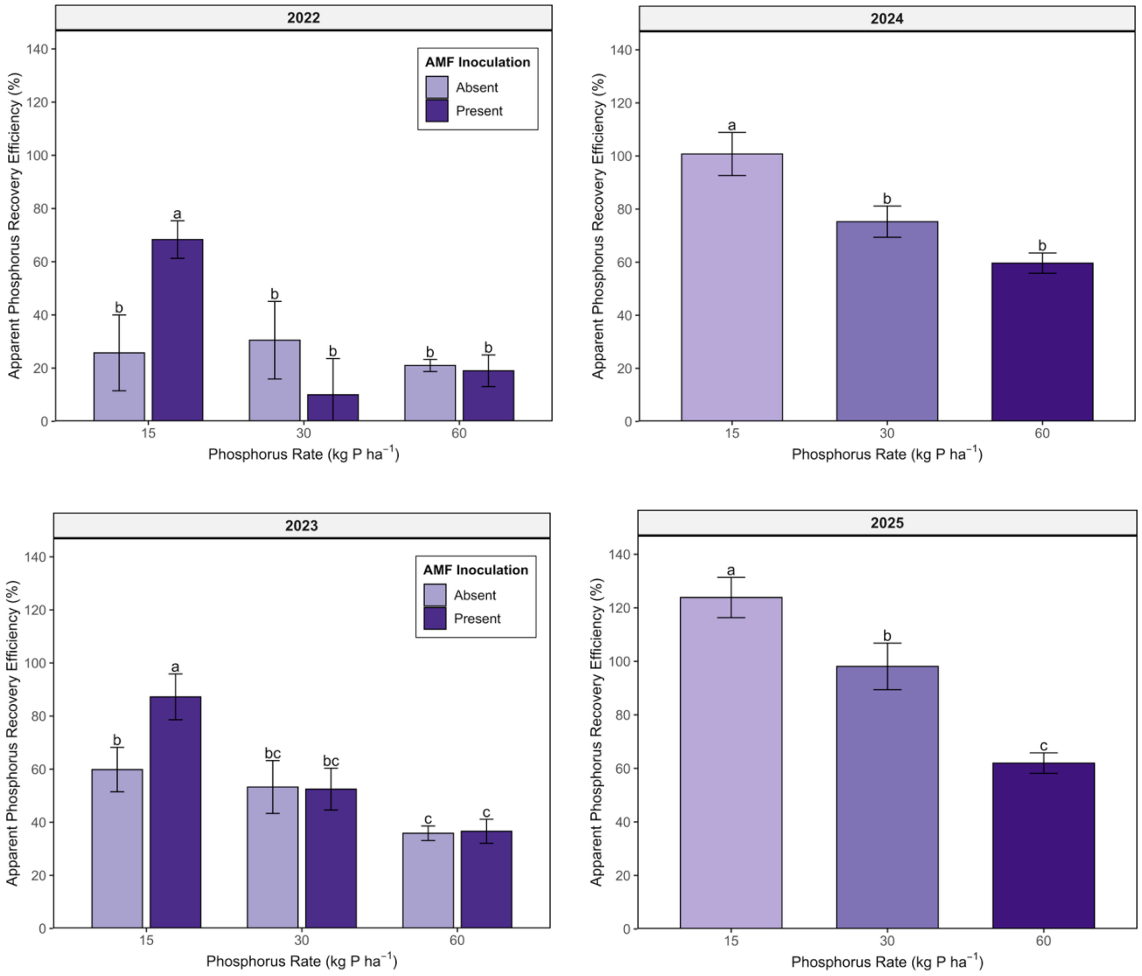


Figure 2.13. Apparent phosphorus recovery efficiency (%) response to phosphorus (P) fertilization and arbuscular mycorrhizal fungi (AMF) inoculation across years and crop rotations in Scandia, KS. The years 2022 and 2023 represent the first corn year of each rotation, while 2024 and 2025 represent the second corn year. Different lowercase letters indicate significant differences among treatments within each year ($P < 0.05$).

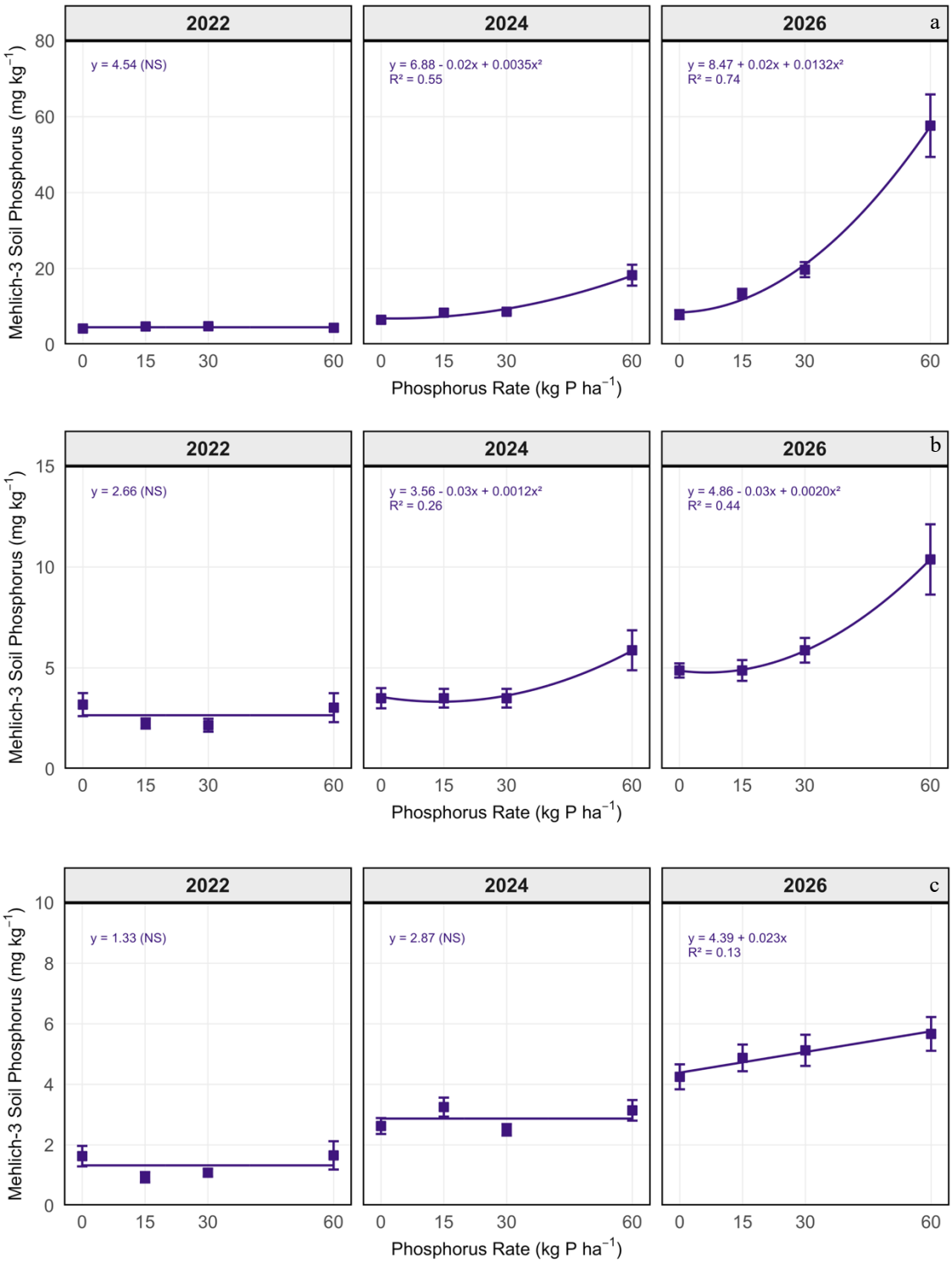


Figure 2.14. Effects of phosphorus fertilization on Mehlich-3 soil phosphorus (mg kg^{-1}) across sampling years (2022, 2024, and 2026) at (a) 0–5, (b) 5–15, and (c) 15–30 cm soil depths of the Corn–Soybean rotation at Scandia, KS. Points represent means with standard error bars.

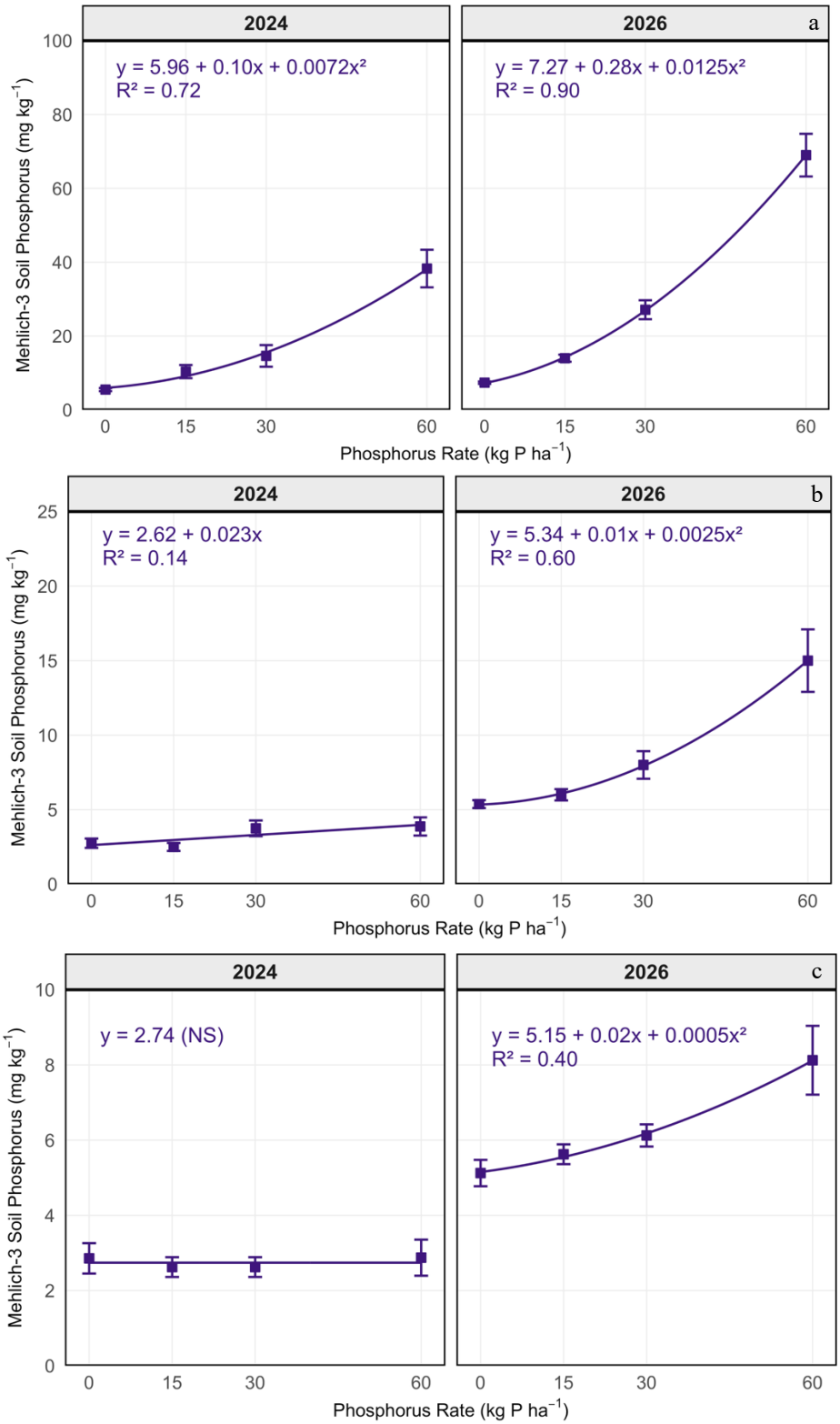


Figure 2.15. Effects of phosphorus fertilization on Mehlich-3 soil phosphorus (mg kg⁻¹) across sampling years (2024, and 2026) at (a) 0–5, (b) 5–15, and (c) 15–30 cm soil depths of the Soybean-Corn rotation at Scandia, KS. Points represent means with standard error bars.

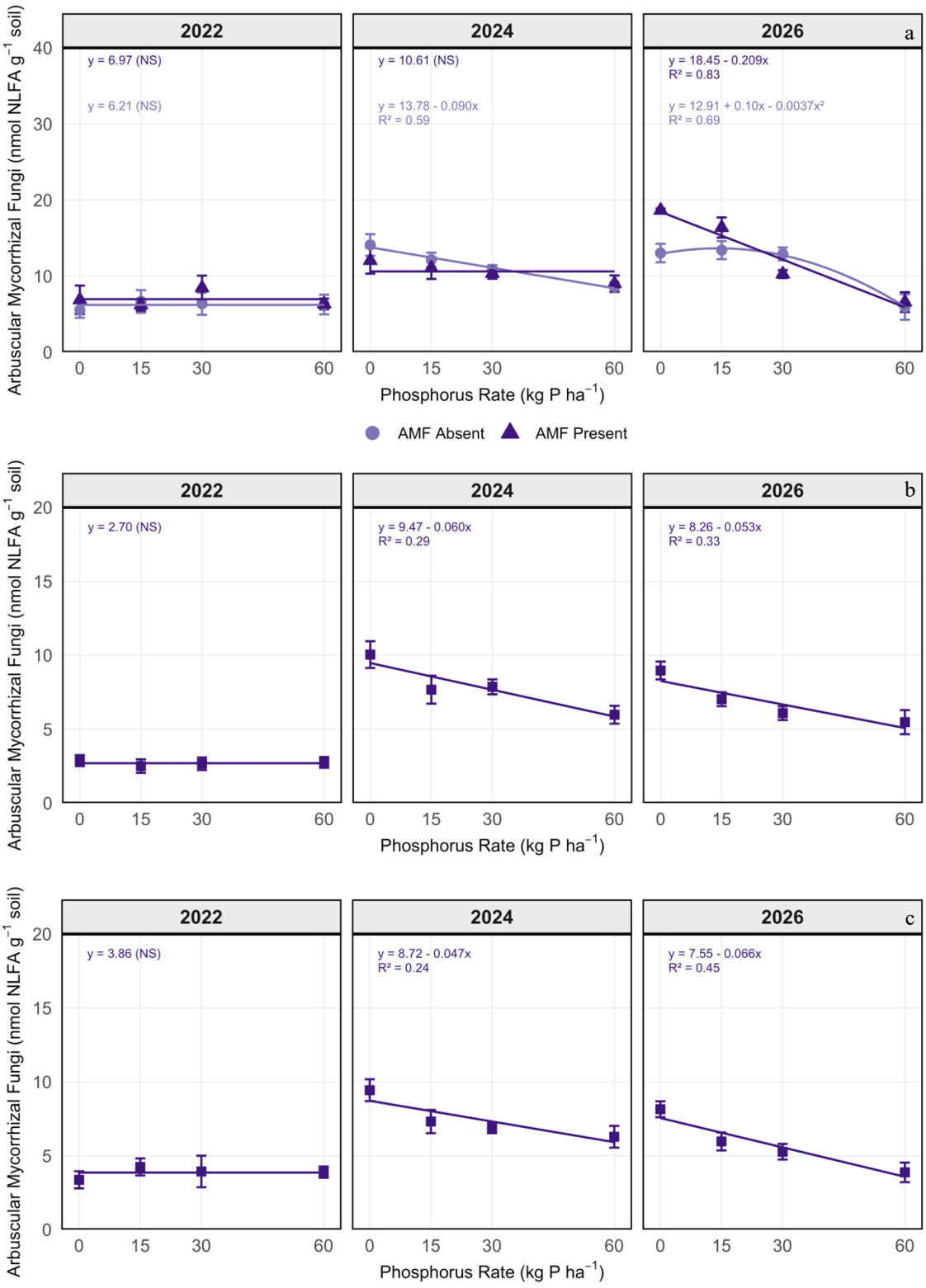


Figure 2.16. Effects of phosphorus (P) fertilization and arbuscular mycorrhizal fungi (AMF) inoculation on soil AMF biomass (nmol NLFA g^{-1} soil) across sampling years (2022, 2024, and 2026) at (a) 0–5, (b) 5–15, and (c) 15–30 cm soil depths of the Corn–Soybean rotation at Scandia, KS. Points represent means with standard error bars.

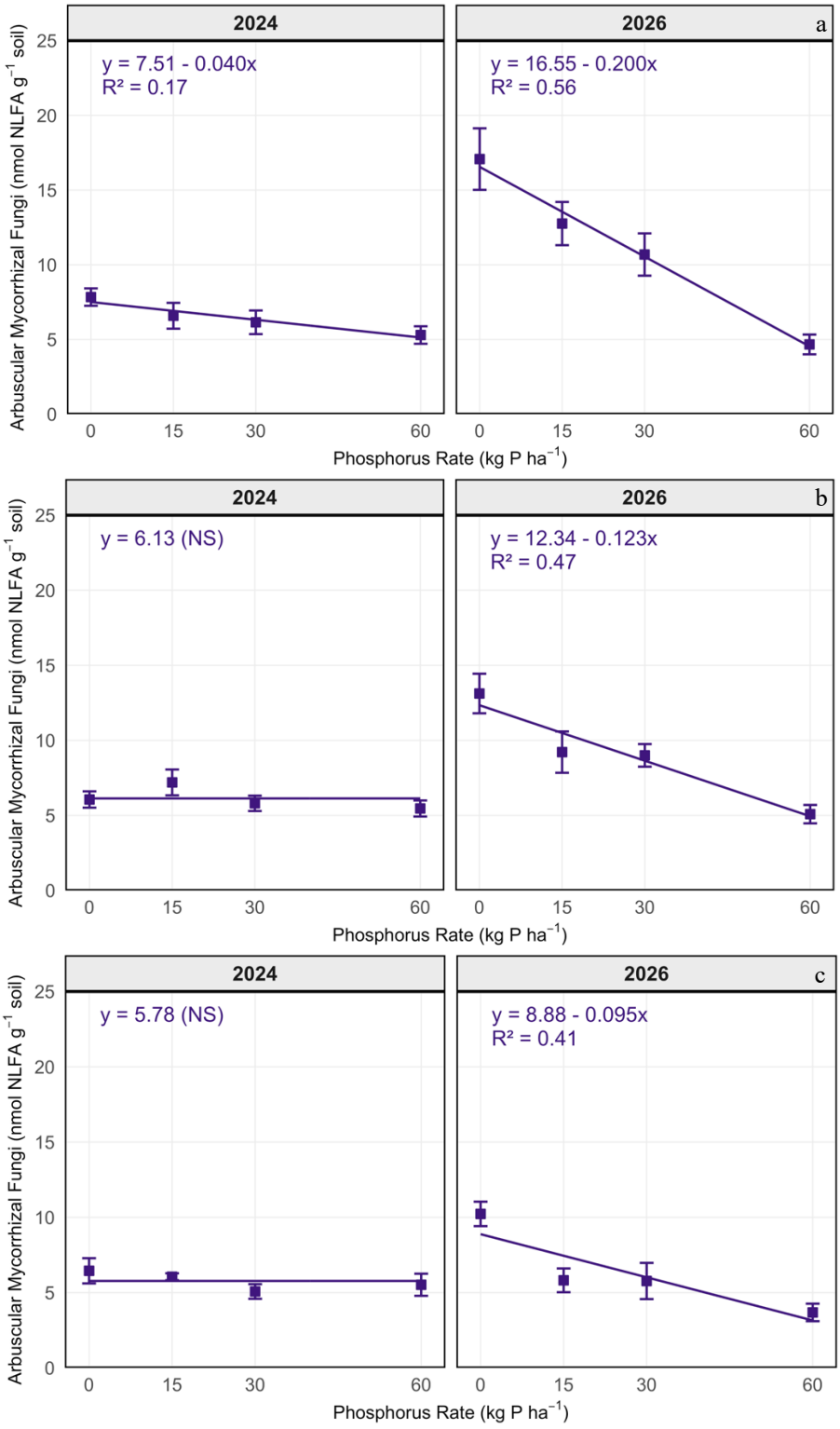


Figure 2.17. Effects of phosphorus (P) fertilization on soil AMF biomass (nmol NLFA g^{-1} soil) across sampling years (2022, 2024, and 2026) at (a) 0–5, (b) 5–15, and (c) 15–30 cm soil depths of the Soybean-Corn rotation at Scandia, KS. Points represent means with standard error bars.

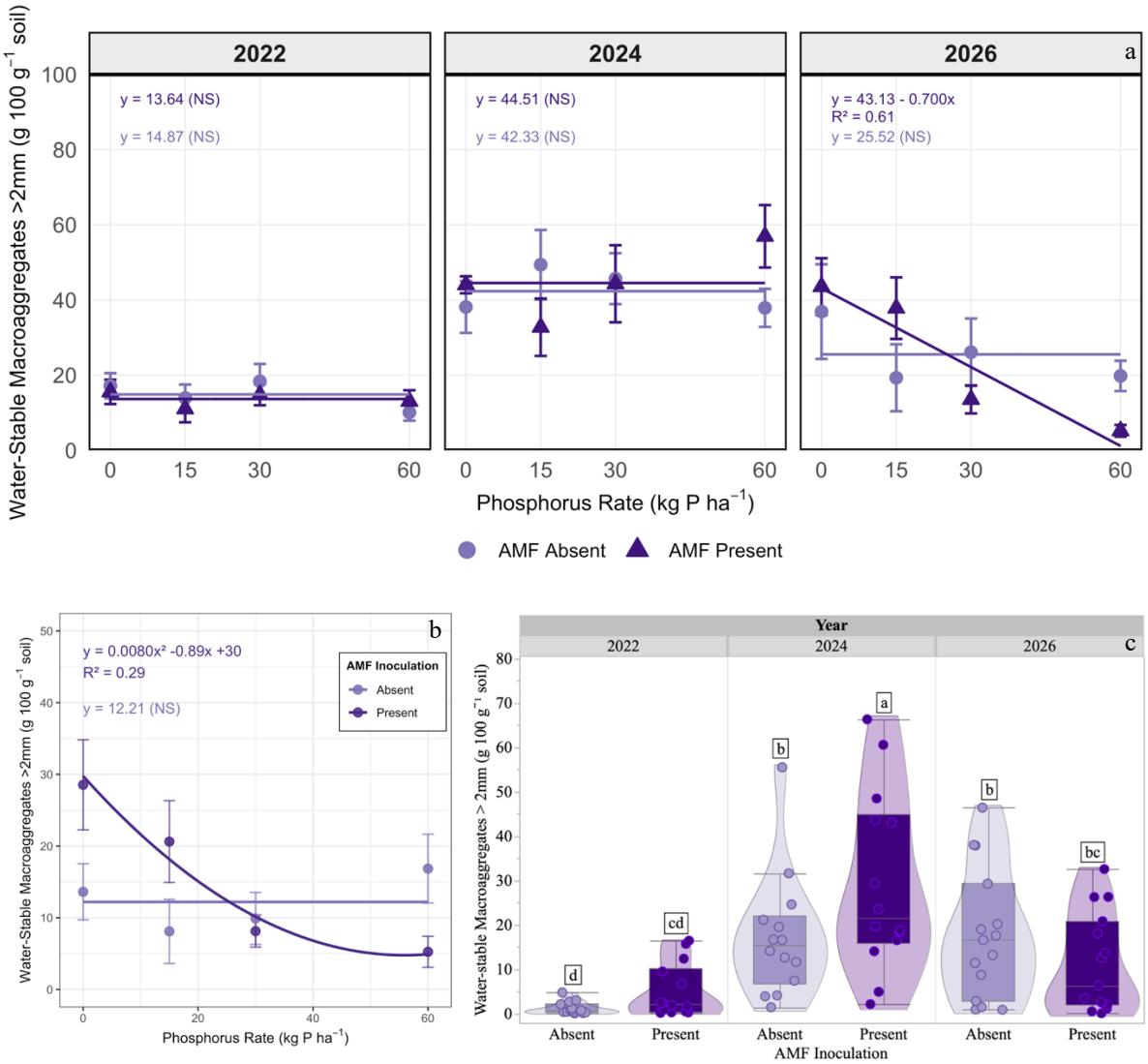


Figure 2.18. Effects of phosphorus fertilization and arbuscular mycorrhizal fungi (AMF) inoculation on water-stable macroaggregates >2 mm (g 100 g^{-1} soil) across sampling years (2022, 2024, and 2026) and soil depths in the Corn–Soybean rotation at Scandia, KS: (a) 0–5 cm, (b, c) 5–15 cm. In panels a and b, points represent means with standard error bars. In panel c, violin plots illustrate the density distribution of the data, with overlaid box plots representing the median (horizontal line) and interquartile range; individual points represent raw field observations. Different lowercase letters indicate significant differences among treatment combinations or years ($P < 0.05$).

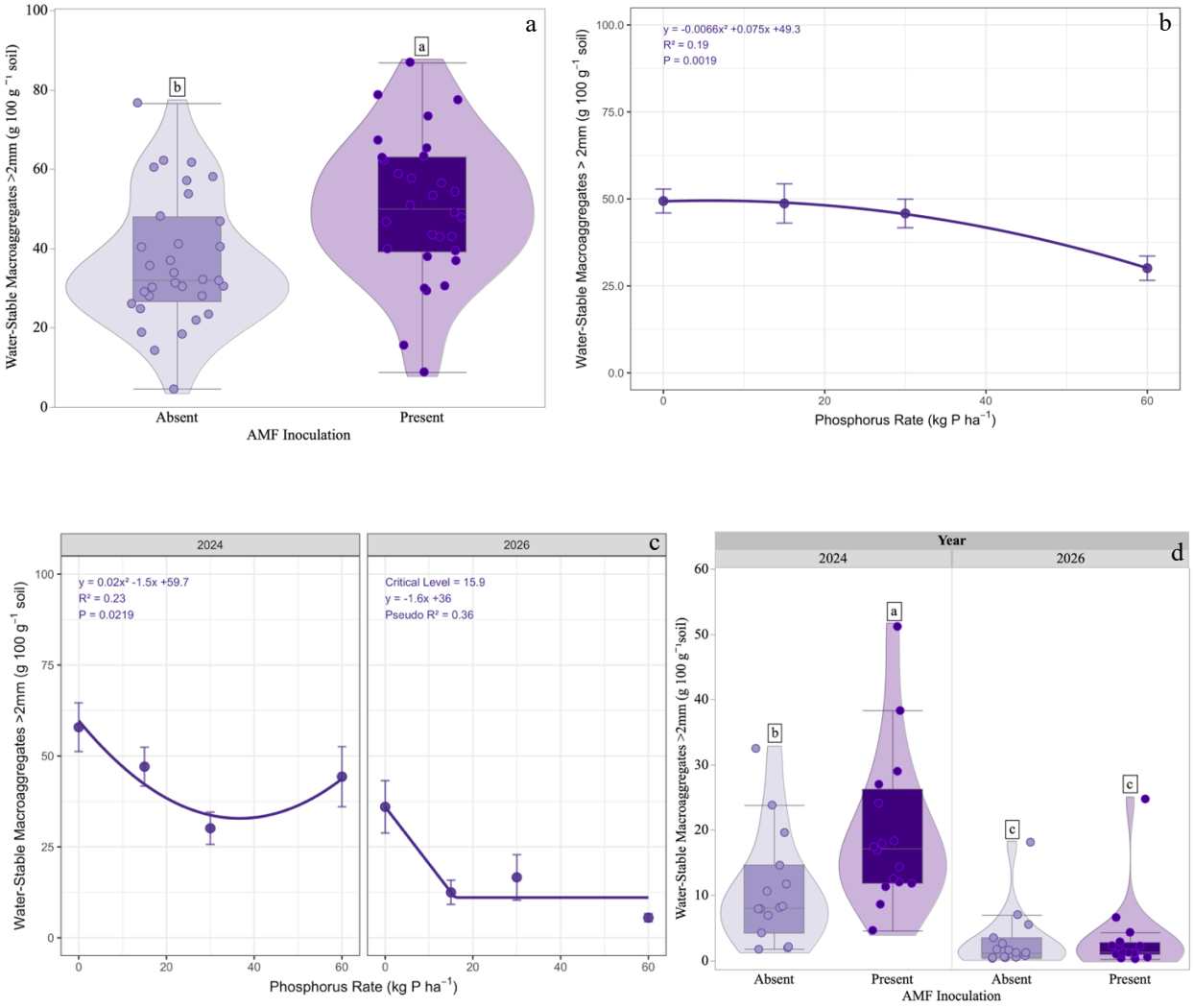


Figure 2.19. Effects of phosphorus fertilization and arbuscular mycorrhizal fungi (AMF) inoculation on water-stable macroaggregates >2 mm (g 100 g^{-1} soil) across sampling years (2024 and 2026) and soil depths in the Soybean–Corn rotation at Scandia, KS: (a, b) 0–5 cm, (c) 5–15 cm, and (d) 15–30 cm. Points in regression panels represent treatment means with standard error bars. Violin plots illustrate the density distribution of the data, with overlaid box plots representing the median and interquartile range; individual points represent raw field observations. Different lowercase letters indicate significant differences among treatments or treatment combinations ($P < 0.05$).

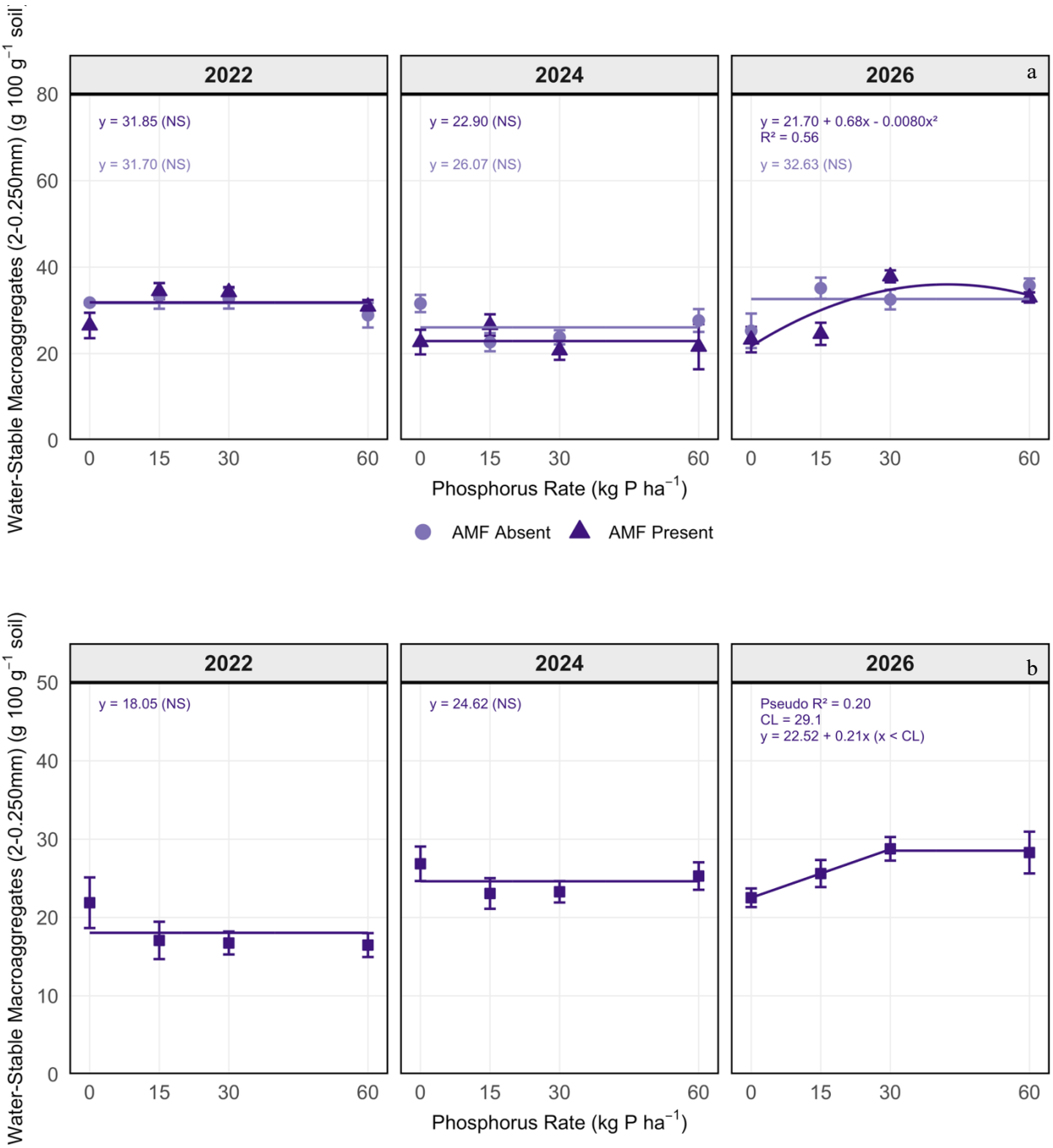


Figure 2.20. Effects of phosphorus fertilization and arbuscular mycorrhizal fungi (AMF) inoculation on water-stable macroaggregates (0.25–2 mm; g 100 g^{-1} soil) across sampling years (2022, 2024, and 2026) in the Corn–Soybean rotation at Scandia, KS at (a) 0–5 cm and (b) 5–15 cm soil depths. Points represent treatment means with standard error bars.

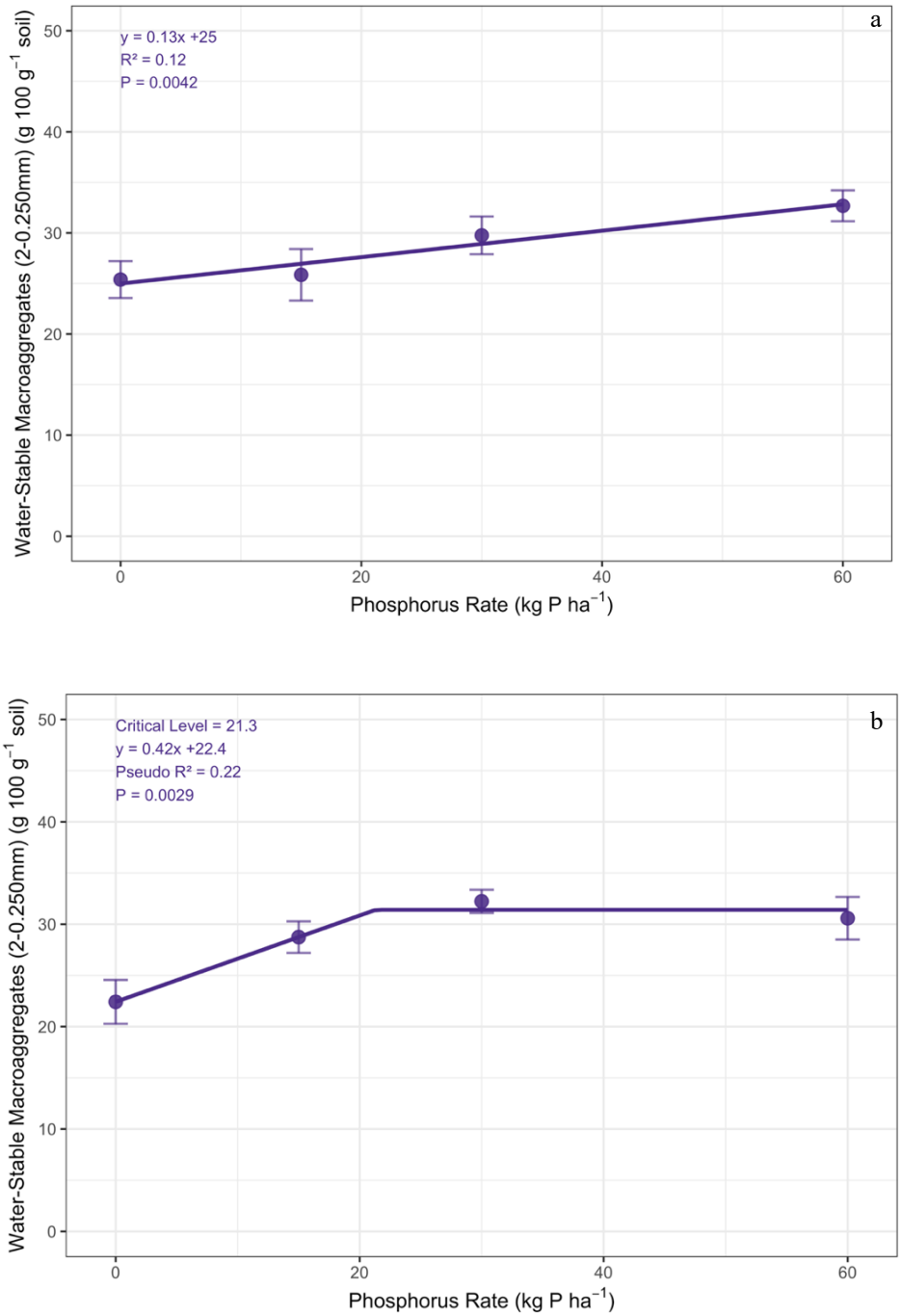


Figure 2.21. Effects of phosphorus (P) fertilization on water-stable macroaggregates (0.25–2 mm; g 100 g^{-1} soil) in the Soybean–Corn rotation at Scandia, KS. (a) Response to P fertilization at the 0–5 cm soil depth, and (b) response to P fertilization at the 5–15 cm soil depth. Points in regression panels represent treatment means with standard error bars.

Chapter 3 - Understanding the Impacts of Arbuscular Mycorrhizal Fungi Inoculation and Phosphorus Fertilization in Dryland Continuous Corn

Abstract

Phosphorus (P) is an essential nutrient involved in key processes that mediate plant growth and development. Arbuscular mycorrhizal fungi (AMF) associate with approximately 80% of terrestrial plants, improving P acquisition and contributing to soil health. AMF bioinoculants have been proposed as a strategy to improve P-use efficiency and soil health, but their effectiveness under field conditions may depend on soil P availability, indigenous AMF communities, and environmental conditions, particularly water availability in dryland systems. This study evaluated the effects of AMF inoculation and P fertilization on corn growth, P uptake, and soil health over four years in a dryland continuous corn system in Manhattan, KS. Treatments consisted of AMF inoculation with a commercial multi-species inoculant and four P rates (0, 15, 30, and 60 kg P ha^{-1}), applied annually. Plant growth, grain yield, P uptake, apparent P recovery efficiency (APRE), AMF root colonization, and multiple soil biological, physical, and chemical soil health indicators were evaluated. AMF inoculation responses varied among years, P rates, and growth stages, with no consistent improvements in plant growth, P uptake, or soil health indicators. The greatest benefits of AMF inoculation were observed in 2023, when inoculation increased root colonization from approximately 65% to over 90% at V6 across fertilized treatments, increased P uptake by 15% at 60 kg P ha^{-1}, and increased grain yield from 7.90 to 11.8 Mg ha^{-1} and from 8.92 to 11.5 Mg ha^{-1} at 30 and 60 kg P ha^{-1}, respectively. Importantly, 2023 also had the highest precipitation between V6 and R1, a critical period for corn P demand. However, inoculation

reduced grain yield by 11% in 2022 and had no significant effect in 2024 or 2025. By 2025, repeated P fertilization reduced root colonization at all growth stages, while across years, colonization decreased with increasing Mehlich-3 soil P at V6 and R1, reaching a plateau of approximately 50% at 25 mg kg^{-1} at R1. Repeated P fertilization generally increased plant P uptake, although responses to AMF inoculation were inconsistent, while APRE was unaffected by inoculation and decreased with increasing P fertilization. Soil P increased with fertilization across depths, and notably, AMF inoculation increased Mehlich-3 P from 110 to 139 mg kg^{-1} at 60 kg P ha^{-1} in 2024, from 21 to 50 mg kg^{-1} without P fertilization and from 159 to 372 mg kg^{-1} at 60 kg P ha^{-1} in 2026 at 0–5 cm. Soil AMF biomass generally decreased with increasing P availability, while water-stable macroaggregates (0.25–2 mm) were positively associated with soil AMF biomass at 0–5 cm across all sampling years. Inoculation also reduced total microbial biomass, Gram-positive bacteria, and actinomycetes by 11%, 11%, and 9%, respectively, at 0–5 cm, while effects on other soil biological, chemical, and physical properties were generally limited and varied among years and depths. Overall, our findings demonstrate that P fertilization was a stronger and more consistent regulator of AMF root colonization and soil abundance than inoculation, while the benefits of inoculation were limited and context dependent. Near-sufficient soil P availability and established indigenous AMF communities likely limited inoculation effectiveness, while environmental variability may have further influenced treatment responses. Promoting indigenous AMF communities through soil health management practices, such as no-till and cover cropping, might be a more reliable strategy for increasing and maintaining mycorrhizal networks, while identifying conditions under which AMF bioinoculants provide additional benefits remains essential for improving the agronomic value of commercial bioinoculants.

Introduction

Phosphorus (P) is an essential nutrient involved in key processes that mediate plant growth and development, including energy transfer (ATP), photosynthesis, respiration, the formation of DNA and cell membranes (Kafle et al., 2019; Vance et al., 2003). Phosphate rock, the primary source of P fertilizers, is a non-renewable resource, raising concerns about its long-term availability (Zhu et al., 2018). Although soils may contain substantial amounts of P, much of it remains unavailable to plants due to strong binding to soil particles, fixation in poorly soluble forms, and slow movement through soil, which collectively limit plant P availability and reduce the efficiency of P fertilizers (Roberts & Johnston, 2015; Zhu et al., 2018). Therefore, proper P management is essential for sustaining crop production while improving fertilizer-use efficiency.

Arbuscular mycorrhizal fungi (AMF) form symbiotic associations with approximately 80% of terrestrial plants, exchanging nutrients and water for plant-derived C (Bago et al., 2000; Choi et al., 2018; Smith & Read, 2008). Once colonization is established, extraradical hyphae extend the effective soil volume explored by plants and can increase access to relatively immobile nutrients, particularly P (Marschner & Dell, 1994; Wu et al., 2024). Beyond nutrient acquisition, AMF can influence crop productivity, microbial communities, soil aggregation, C dynamics, and nutrient cycling, providing potential benefits that extend beyond crop P nutrition alone (Bhantana et al., 2021; Cobb et al., 2016; Fall et al., 2022; Wilson et al., 2009; Zhang et al., 2024).

The potential of AMF to improve nutrient acquisition and provide broader ecosystem services has motivated growing interest in AMF bioinoculants as a strategy to enhance sustainable P management and soil health in cultivated fields (Fall et al., 2022; Zhang et al., 2024). However, nutrient availability strongly regulates AMF symbiosis, with soil P influencing inoculum establishment and crop responses to AMF inoculation (Pan et al., 2025; Smith & Smith, 2012).

The benefits of inoculation may therefore diminish as soil P availability meets crop demand (Sheng et al., 2012; Ven et al., 2019; Wang et al., 2017). However, this is an important agronomic scenario, as farmers considering investing in AMF bioinoculants may be managing fields with substantial soil P levels from previous fertilizer applications rather than the severely P-deficient soils commonly used in short-term, controlled bioinoculant studies that do not reflect conditions in cultivated agricultural fields (Basiru & Hijri, 2022). Therefore, evaluating AMF bioinoculants across a range of P fertilization rates is important for determining whether their effects on crop productivity and soil health are consistent and agronomically meaningful under field conditions.

The performance of AMF bioinoculants in dryland agricultural soils remains poorly understood, particularly where introduced fungi must establish alongside indigenous AMF that may already provide the functions targeted by inoculation (Bender et al., 2019). Inoculation performance may also depend on climatic conditions, management history, soil properties, and the broader native microbial community (Basiru & Hijri, 2022). For instance, in dryland systems, water availability can strongly influence AMF symbiosis development, with moisture limitations potentially reducing spore germination, hyphal development, root colonization, and spore production (Bahadur et al., 2019). Consequently, inoculation responses can vary considerably based on initial soil fertility, indigenous AMF abundance, fungal species, crop genotype, and management practices (Basiru & Hijri, 2022; Bender et al., 2019; Choi et al., 2018; Zhang et al., 2024) Therefore, multi-year field experiments are particularly important for capturing these interactions, as environmental conditions may determine whether introduced AMF can establish and function effectively, particularly in dryland cropping systems, where environmental conditions can strongly influence both crop productivity and the biological processes targeted by inoculation.

Understanding these interactions is essential to identify when AMF inoculation complements P management rather than adding an expense with limited agronomic benefits.

This chapter evaluated the effects of AMF inoculation and P fertilization rates on plant growth, P uptake, and soil health indicators in a four-year dryland continuous corn system. We hypothesized that AMF inoculation would improve plant growth, P uptake, and soil health, primarily under no or low-to-moderate P fertilization.

Materials and Methods

Site Characteristics and Experimental Design

This experiment was conducted in a dryland site at the Agronomy North Farm, in Manhattan, KS (39.212372 N, -96.597744 W) from 2022 to 2025. The soil type at the experimental site was characterized as a Kennebec silt loam (fine-silty, mixed, superactive mesic Cumulic Hapludolls) with medium soil P levels (Table B.1). Precipitation data during the growing seasons were obtained from the Kansas Mesonet weather station in Manhattan, KS, located approximately 4 km from the experimental field (Patrignani et al., 2020) (Table B.2).

The experiment was established as a randomized complete block design (RCBD), with a split-plot arrangement, in which AMF inoculation was assigned to the main plots, and P fertilization to subplots, with four replications (Fig. B.1). The plots were 6 m x 12 m, with 3 m clean buffer areas between plots to avoid inoculum drift. Corn (*Zea mays* L.) was planted and maintained in a no-till system (Table B.3). Wheat was planted every year between growing seasons as a cover crop and terminated before planting corn (Table B.4).

Treatments were applied annually, resulting in four field fertilizations and inoculations over the course of the experiment. The inoculation treatment was the presence or absence of Symvado® SC (Mycorrhizal Applications LLC., Grants Pass, OR, USA), containing spores of

Rhizophagus irregularis, Claroidoeglomus etunicatum, Funneliformis mossea, and *Claroideoglomus claroideum* at a concentration of 1625 propagules mL^{-1}, applied in furrow at planting with an application rate of approximately 146 mL ha^{-1} (2 fl oz ac^{-1}). Fertilizer treatments were four rates of P fertilizer (0, 15, 30, and 60 kg P ha^{-1}), as mono-ammonium phosphate (MAP) (11-52-0). All fertilizer treatments were broadcast by hand within one week before planting. Nitrogen fertilizer was applied before planting each year at 168 kg N ha^{-1} as urea ammonium nitrate (UAN) (28-0-0).

Plant Sampling

Aboveground plant biomass and fine root samples were collected at the V6 (sixth leaf), R1 (silking), and R6 (maturity) corn developmental stages, representing key vegetative, reproductive, and maturity stages that capture critical periods of P acquisition and utilization in corn (Ampong et al., 2024). At each sampling time, four randomly selected plants were collected from each plot. Cover crop biomass was manually collected immediately before termination from a 1-m^2 area at the center of each plot using a handheld electric clipper. Plant biomass samples were dried in a forced-air oven at 50 ◦C until constant weight and the dry weight was recorded. To determine grain yield, corn ears were manually harvested at R6 from a 1.5-m section of two adjacent rows in the center of each plot and threshed to remove the grain. Grain weight and moisture were recorded at the time of shelling. All yield data were corrected to standard moisture contents of 15.5%. Aboveground biomass and grains were finely ground in a Wiley mill, sieved to pass through a 2mm mesh, and digested in a nitropercloric solution (Gieseking et al., 1935), and P concentration was determined by an Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES) (Agilent 5800, Agilent Technologies, CA, USA). Harvest dates were determined annually based on physiological maturity (Table B.3).

At each sampling time, roots from each of the four plants were collected with surrounding soil (up to 30 cm depth) and immediately stored in 70% ethanol for subsequent assessment of AMF colonization. Root colonization by AMF was assessed using a modified staining protocol, substituting phenol with lactoglycerol (Phillips & Hayman, 1970). Root samples were first cleared in a 10% potassium hydroxide (KOH) solution for 40 min in a 70°C water bath, rinsed thoroughly under running water, and acidified in a 2% hydrochloric acid (HCl) solution for 10 min. Roots were then stained in a 0.05% trypan blue solution for 60 min at 60°C and stored in lactoglycerol. AMF root colonization was quantified using the gridline intersect method under a stereoscopic microscope (Giovannetti & Mosse, 1980).

Apparent Phosphorus Recovery Efficiency

Apparent P recovery efficiency (APRE) was calculated to quantify the proportion of applied P fertilizer recovered by the crop. Total aboveground P uptake at R6 was determined as the sum of P uptake in aboveground biomass and grain. APRE was calculated as:

$$APRE(\%) = \frac{(Total\ P\ Uptake\ at\ R6\ Fertilized\ - Total\ P\ Uptake\ at\ R6\ Control)}{Fertilizer\ Applied} x\ 100$$

where total P uptake represents the combined P uptake in aboveground biomass and grain (kg P ha^{-1}), and P fertilizer applied represents the corresponding P application rate (kg P ha^{-1}). The non-inoculated, unfertilized treatment (0 kg P ha^{-1}) within each block served as the reference for calculating APRE for each P fertilization rate (Assefa et al., 2021; Fixen et al., 2015).

Soil Sampling

Soil samples for biological and chemical analyses were collected from each plot in the Spring of 2022, 2024, and 2026, and divided into depth increments of 0-5, 5-15, and 15-30 cm. A

minimum of 12 cores were collected in each plot, combined, and homogenized into a composite sample for each depth in polyethylene Ziplock® Freezer Bags. Samples were then transported from the field in a cooler with ice and stored in the laboratory freezer at -20°C until analysis. Soils for chemical analysis and enzyme activity were air-dried at room temperature, finely ground, and submitted for pH and soil P analysis at the Kansas State University Soil Testing Laboratory. Soil pH was determined using a 1:1 soil slurry (Watson & Brown, 1998) and soil P was extracted by Mehlich-3 (Mehlich, 1984).

Soil samples to measure soil aggregate stability were collected with a shovel and divided into depth increments of 0-5, 5-15, 15-30 cm with minimum disturbance to preserve the natural structure of aggregates. Samples were then air-dried, cleared of coarse roots, and carefully passed through an 8-mm sieve.

Soil bulk density samples were collected using a Giddings probe (Giddings Machine Company, Windsor, Colorado, USA) with a 5 cm-diameter metal tube in the Spring of 2023 and 2026 (1 core per plot) and used to compute soil carbon stocks at each depth. Soil organic carbon (SOC) and total nitrogen (TN) concentrations were measured by dry combustion using a LECO CN828 Carbon/Nitrogen analyzer (LECO Corporation, St. Joseph, MI, USA), reporting total levels (inorganic and organic) of C and N on a weight percent basis, following a method from Nelson & Sommers, (1983) for SOC and Bremner & Mulvaney, (1982) for TN. Total carbon is considered equivalent to SOC in soils with pH < 7.2.

Water-Stable Aggregates

Sand-free water-stable aggregates were measured using a Yoder wet-sieving method based on Kemper & Rosenau, (1986) and modified by Mikha & Rice, (2004). Briefly, a 100 g of air-dried soil was placed in a Yoder wet-sieving apparatus modified for the recovery of all particle

fractions, and separated into four size classes: 8–2, 0.25–2, 0.053–0.25, and 0.020–0.053 mm. The soil was placed on a 2 mm sieve, above the 0.25 mm sieve, submerged in 1 L of distilled water for 10 min, then oscillated for 10 min at a stroke length of 4 cm and 30 cycles min^{-1}. Material recovered from each sieve was collected, dried at 50°C for 48 h, and weighed. Fractions >2 and 0.25–2 mm were classified as macroaggregates, whereas 0.053–0.25 and 0.020–0.053 mm were classified as microaggregates. Floating organic matter was removed from the >2-mm fraction. Aggregate fractions were corrected for sand content to obtain sand-free aggregate proportions. For this correction, 5 g of material from each fraction was dispersed overnight (>12 h) in sodium hexametaphosphate solution at a fivefold volume, shaken at 325 rpm for 5 h, and passed through a 0.053-mm sieve. The retained material was oven-dried and weighed to determine sand content, which was used to calculate sand-free aggregate mass.

Phospholipid and Neutral Lipid Fatty Acid Analysis

To monitor changes in microbial community populations in response to our treatments, phospholipid fatty acid (PLFA) and neutral lipid fatty acid (NLFA) analyses were conducted following a modified Bligh & Dyer, (1959) extraction procedure, as described by White & Rice, (2009). Briefly, soil samples were lyophilized and finely ground using a mortar and pestle, and total lipids were extracted from 5 g of soil. PLFAs were extracted by incubating the soils in a solution of methanol, chloroform, and phosphate buffer (2:1:0.8 v/v/v). Following extraction, neutral and phospholipid fatty acids were separated from total lipids using preconditioned disposable silicic acid chromatography columns (BAKERBOND SPE Columns). Fatty acids were subsequently released from their glycerol backbones, and methylation was performed to produce fatty acid methyl esters (FAMEs). Then, FAMEs were analyzed on a Thermo Scientific Trace GC-ISQ mass spectrometer (Thermo Fisher Scientific, Waltham, MA, USA) fitted with a DB5-MS

analytical column (30 m × 250 μm internal diameter × 0.25 μm film thickness; Agilent Technologies, Santa Clara, CA, USA). Concentrations of fatty acid peaks were quantified relative to an internal standard, methyl nonadecanoate (19:0). Fatty acids were categorized into microbial groups as follows: Gram-Positive Bacteria (i15:0, a15:0, i16:0, i17:0, and a17:0), Gram-Negative Bacteria (2-OH 10:0, 2-OH 12:0, 3-OH 12:0, 2-OH 14:0, 3-OH 14:0, 16:1ω7c, cy17:0, cy19:0), Actinomycetes (10-Me 16:0 and 10-Me 18:0), Arbuscular Mycorrhizal Fungi (AMF; 16:1ω5), and Saprophytic Fungi (18:2ω6,9c). Total microbial biomass was obtained by summing microbial groups and commonly measured fatty acids (11:0, 12:0, 13:0, 14:0, 15:0, 16:0, 17:0, 18:0, and 20:0) (Bonini Pires et al., 2020; Lin et al., 2023).

Extracellular Enzyme Activity

Four soil enzymes were analyzed colorimetrically using p-nitrophenyl-associated substrates, including β-glucosidase (Eivazi & Tabatabai, 1988) associated with the C cycle, acid phosphatase (Eivazi & Tabatabai, 1977; Tabatabai & Bremner, 1969) involved in the P cycle, arylsulfatase (Tabatabai & Bremner, 1970) involved in the sulfur (S) cycle, and N-acetyl-β-glucosaminidase (Parham & Deng, 2000) involved in the C and N cycling. Briefly, triplicates of 0.5 g of air-dried soil, sieved to 2 mm, were placed into individual 20-mL glass vials, with 2 mL of buffer. Two of the vials received 0.5 mL of a substrate linked to p-nitrophenyl, while the third vial acted as a control without the initial addition of the substrate. Vials were capped and incubated at 37 ºC for 1 hour. After incubation, 0.5 mL of 0.5 M $CaCl_2$ solution and 2 mL of a stopping buffer were added to each vial. The control vial then received 0.5 mL of the p-nitrophenyl substrate. The solutions were filtered through Whatman 2V filter paper (9.0-cm diameter), and absorbance was measured at 400 nm using a Genesys 20 Visible Spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA).

Statistical Analysis

Treatment effects on plant and soil responses were analyzed by analysis of variance (ANOVA) after fitting linear mixed-effects models. Plant responses were analyzed separately for each sampling year, with AMF inoculation, P fertilization, and their interaction included as fixed effects. Block and AMF whole plots nested within block were included as random effects. When a significant P effect was detected, regression analyses were conducted to characterize responses to increasing P rates. For significant AMF × P interactions, regressions were fitted separately for each AMF treatment. Linear, quadratic, linear-plateau, and quadratic-plateau models were evaluated for P responses, and model fit was compared using the Akaike information criterion (AIC), root mean square error (RMSE), and coefficient of determination (R^2). Soil responses were analyzed by depth across sampling years with AMF inoculation, P fertilization, sampling year, and their interactions included as fixed effects. Block, AMF nested within block, and plot were included as random effects to account for the split-plot design and repeated measurements of the same plots across years. For significant P effects, regression analyses were conducted to characterize responses to increasing P rates. For both plant and soil responses, significant effects involving categorical factors were further evaluated using estimated marginal means (EMMs) and pairwise contrasts using the *emmeans* package. Simple effects of AMF inoculation were evaluated within each P rate and, for soil responses, within each P rate and year when appropriate. Year effects for soil responses were also evaluated within each P rate when appropriate. Statistical significance was determined at $P \leq 0.05$. The normality of model residuals was assessed using the Shapiro–Wilk test and Q–Q plots, and model assumptions were further evaluated using residual diagnostics. Data were log or square-root transformed when necessary to satisfy assumptions of normality and homogeneity of variance. Bivariate regression analyses were conducted to evaluate

relationships among selected plant and soil variables. Linear mixed-effects models were fitted using the *lmer* function in the *lme4* package, and graphs were generated using the *ggplot2* package. All statistical analyses were conducted in R version 4.1.2 (R Core Team, 2026).

Results

AMF Colonization

AMF root colonization responses to P fertilization and AMF inoculation varied by year and growth stage (Table 3.1). In 2022, AMF root colonization was not significantly affected by either treatment at V6 or R6, but inoculation reduced colonization from 74% to 61% at R1 (Fig. B.2a). In 2023, AMF inoculation increased root colonization at V6 from approximately 65% to over 90% across fertilized treatments (Fig. 3.1a; Table B.5). Colonization remained relatively stable across P rates with inoculation but decreased following a linear-plateau response without inoculation, reaching a plateau at approximately 16 kg P ha^{-1}. In 2024, colonization decreased with increasing P in both inoculated and non-inoculated treatments, but at 60 kg P ha^{-1}, colonization was higher with AMF inoculation (48%) than without inoculation (32%). In 2025, root colonization decreased linearly with increasing P fertilization, from approximately 49% without P fertilization to around 26% at 60 kg P ha^{-1}. At R1, P fertilization did not affect root colonization in 2022 or 2024 (Fig. 3.1b). In contrast, colonization decreased with increasing P fertilization in 2023 and 2025, reaching plateaus at 18.8 and 18.1 kg P ha^{-1}, respectively (Fig. 3.1b). Similarly, in 2025, colonization decreased from approximately 52% without P to approximately 28% at 60 kg P ha^{-1}. At R6, root colonization did not respond to P fertilization from 2022 through 2024 (Fig. 3.1c) but increased with inoculation from 44 to 52% in 2023 (Fig. B.2b). In 2025, colonization decreased linearly with increasing P fertilization, decreasing from approximately 24% without P

to 11% at 60 kg P ha^{-1}. By the final year, increasing P fertilization reduced AMF root colonization at all three growth stages.

There was a negative relationship between root colonization and Mehlich-3 soil P at the V6 and R1 growth stages (Fig. 3.2). At V6, root colonization decreased linearly with increasing soil P, while at R1 followed a linear-plateau response, decreasing until reaching a critical soil P concentration of 25 mg kg^{-1}, after which it stabilized at approximately 50%. In contrast, no significant relationship between root colonization and soil P was observed at R6.

Plant Growth

Aboveground biomass had variable responses to treatments depending on the year and growth stage (Table 3.1; Fig. 3.3). At V6, no treatment effects were observed in 2022, while P fertilization affected biomass from 2023 to 2025 (Fig. 3.3a). Biomass increased linearly with P in 2023 and 2025, but in 2024 it decreased and then plateaued at 16.5 kg P ha^{-1}. At R1, P fertilization affected biomass only in 2023, when biomass increased linearly with increasing P (Fig. 3.3b). At R6, a P × AMF inoculation interaction was observed in 2022 and 2024 (Fig. 3.3c). In 2022, biomass increased quadratically with P fertilization in inoculated plants but was unaffected by P without inoculation, and inoculation decreased biomass by 53% and 21% at 0 and 60 kg P ha^{-1}, respectively. In 2024, biomass decreased linearly with P in inoculated plants and had a quadratic response without inoculation, with inoculation decreasing biomass by 16% at 60 kg P ha^{-1}. No treatment effects were observed in 2023 or 2025.

Grain yield varied between years, with no consistent response to P fertilization or AMF inoculation across the experiment (Table 3.1; Fig. 3.4). In 2022, AMF inoculation reduced grain yield by 11%, with no effect of P fertilization (Fig. 3.4 and B.2c). In 2023, a P × AMF inoculation interaction resulted in grain yield increasing with P with inoculation until reaching a plateau at

31.7 kg P ha^{-1}, while remaining unaffected by P fertilization without inoculation. Also, inoculation increased grain yield from 7.90 to 11.8 Mg ha^{-1} at 30 kg P ha^{-1} and from 8.92 to 11.5 Mg ha^{-1} at 60 kg P ha^{-1}. In 2024, grain yield responded quadratically to increasing P fertilization, regardless of inoculation, reaching its maximum at 60 kg P ha^{-1}, followed by no treatment responses in 2025.

Across years and treatments, the relationship between Mehlich-3 soil P and plant growth varied by growth stage and AMF inoculation treatment (Fig. 3.5). At V6, aboveground biomass increased with soil P regardless of inoculation, reaching similar maximum biomass levels (Fig. 3.5a). However, non-inoculated plants reached a plateau at a lower Mehlich-3 soil P concentration (22.4 mg kg^{-1}) than inoculated plants (37.8 mg kg^{-1}). At R1, biomass increased with soil P without inoculation until reaching a plateau at 50.2 mg kg^{-1}, while no significant relationship was observed with inoculation (Fig. 3.5b). At R6, neither aboveground biomass nor grain yield was significantly related to Mehlich-3 soil P, regardless of inoculation treatment (Fig. 3.5c-d).

Phosphorus Uptake

Response of P uptake to fertilization and inoculation varied among years and growth stages (Table 3.1; Fig. 3.6). Overall, P uptake generally increased with P fertilization, although the magnitude and pattern of the response varied among years and growth stages. At V6, no treatment effects were observed in 2022. In 2023, there was a P × AMF inoculation interaction, with P uptake increasing linearly with P fertilization in both treatments and inoculation increasing P uptake by 17% at 60 kg P ha^{-1} (Fig. 3.6a). In 2024, There was also a P × AMF inoculation interaction in 2024, with P uptake remaining relatively stable across P rates with inoculation, while non-inoculated plants had a quadratic increase with P fertilization. At 60 kg P ha^{-1}, P uptake was 23% lower with inoculation. In 2025, P uptake increased linearly with P fertilization regardless of inoculation. At R1, treatments did not affect P uptake in 2022, but P uptake increased linearly with

P fertilization from 2023 through 2025, regardless of inoculation (Fig. 3.6b). At R6, total P uptake increased linearly with P fertilization, except in 2023 (Fig. 3.6c). In 2023, there was a P × AMF inoculation interaction, with total P uptake increasing linearly with P fertilization in inoculated plants, while remaining unaffected by P fertilization in non-inoculated plants. There was a positive linear relationship between soil P availability and total P uptake across years, regardless of inoculation (Fig. 3.7a).

Apparent P recovery efficiency was generally highest at the lowest P rate and decreased with increasing P fertilization (Fig. 3.7b). In 2022, there were no differences in APRE were observed among P rates, while in 2023, 2024, and 2025, APRE was greatest at 15 kg P ha^{-1}, with differences among the higher P rates varying by year.

Soil Phosphorus

Phosphorus fertilization consistently increased Mehlich-3 soil P across soil depths, with responses becoming more pronounced over time (Table 3.2; Fig. 3.8). At 0–5 cm, P fertilization increased soil P in 2024 and 2026, with responses differing between AMF treatments (Fig. 3.8a). In 2024, AMF inoculation increased Mehlich-3 soil P from 110 to 139 mg kg^{-1} at 60 kg P ha^{-1}. In 2026, inoculation increased soil P from 21 to 50 mg kg^{-1} at 0 kg P ha^{-1} and from 159 to 372 mg kg^{-1} at 60 kg P ha^{-1} (Table B.6). At 5–15 cm, soil P increased with P fertilization in 2024 and 2026 (Fig. 3.8b), whereas at 15–30 cm, a significant increase with P fertilization was only observed in 2026 (Fig. 3.8c).

Soil AMF Biomass

Soil AMF biomass initially increased over time, with higher levels in 2024 than the 2022 baseline, but generally decreased with increasing P fertilization (Table B.7; Fig. 3.9). At 0–5 cm, AMF biomass had a quadratic response to P fertilization in 2024 and decreased linearly with

increasing P rates in 2026 (Fig. 3.9a). At 5–15 and 15–30 cm, AMF biomass decreased linearly with increasing P fertilization in both 2024 and 2026 (Fig. 3.9b–c).

There was a negative relationship between soil AMF abundance and soil P, although statistical significance varied by year and soil depth (Fig. 3.10a). At 0–5 and 5–15 cm, soil AMF abundance decreased significantly as soil P increased in 2022, 2024, and 2026. At 15–30 cm, the negative relationship was significant in 2022 and 2024, but not in 2026. The abundance of water-stable macroaggregates (0.25–2 mm) also increased with soil AMF abundance at the 0–5 cm depth in 2022, 2024, and 2026 (Fig. 3.10b). No significant relationships were detected at 5–15 or 15–30 cm in any year.

Soil Chemical and Physical Properties

Soil chemical and physical properties generally varied over time and across soil depths, with limited treatment effects (Tables 3.2 and 3.3). Total soil N and soil C stock did not respond to treatments and generally increased over time at 0–5 cm, while at deeper depths, total soil N decreased, and soil C stock remained unchanged over time. At 0–5 cm, soil pH varied with P fertilization depending on year and AMF inoculation (Fig. B.3). In 2024, pH decreased with increasing P for both inoculation treatments, while in 2026, this decrease occurred only without inoculation. Despite these differences in P response, soil pH did not differ between inoculated and non-inoculated treatments at any P rate within any year (Table B.6). At 5–15 and 15–30 cm, soil pH also decreased from 2024 to 2026 (Table 3.3). Across years, soil pH was consistently lower in 2026 across P rates and AMF treatments. Water-stable macroaggregates >2 mm varied by year, AMF inoculation, and soil depth (Table 3.3). At 0–5 cm, macroaggregates >2 mm increased from 2022 to 2024 without inoculation, with significantly higher values without inoculation (15.5 g 100 g^{-1} soil) than with inoculation (5.64 g 100 g^{-1} soil) in 2024. By 2026, increases were observed for

both inoculation treatments, with no significant differences between them. At 5–15 and 15–30 cm, macroaggregates >2 mm were highest in 2024. Water-stable macroaggregates of 0.25–2 mm increased over time at all depths, with no significant treatment responses.

Soil Biological Properties

Soil microbial groups generally increased over time across soil depths, often reaching their maximum in 2024, with no consistent treatment effects (Tables 3.2 and 3.4). At 0–5 cm, AMF inoculation reduced total microbial biomass, Gram-positive bacteria, and actinomycetes by 11%, 11%, and 9%, respectively (Fig. B.4a-c). At 5–15 cm, AMF inoculation reduced total microbial biomass and Gram-positive bacteria by 22% and 24%, respectively, in 2024 (Table 3.4). At 15–30 cm, inoculation reduced Gram-positive bacteria by 19% in 2024. Gram-negative bacteria and saprophytic fungi varied by sampling year and soil depth, with no significant response to AMF inoculation or P fertilization (Table 3.4).

Similarly, soil enzyme activity also had limited treatment effects and generally increased over time, with responses varying by soil depth (Tables 3.2 and 3.5). At 0–5 cm, β-glucosidase, acid phosphatase, and NAG activities increased over time, while arylsulfatase remained unchanged (Table 3.5). Acid phosphatase had a P × AMF interaction, with a quadratic response to increasing P without inoculation and no P response with inoculation, and higher activity without inoculation at 15 kg P ha^{-1} across years (Fig. B.5). AMF inoculation also reduced β-glucosidase activity by 28% in 2024 at 0-5 cm, but not at 5-15 or 15-30 cm depths.

Discussion

This study provides field-based evidence that addresses a key knowledge gap in understanding AMF bioinoculant performance under agronomic realistic scenarios. Under these experimental conditions, however, AMF inoculation did not consistently improve plant growth, P

uptake, or soil health indicators, contrary to our hypothesis. The benefits of AMF inoculation were highly variable and context-dependent under dryland field conditions, particularly when soil P levels were sufficient for crop production. For farmers considering investment in AMF bioinoculants, such conditions are common, as many agricultural fields contain substantial soil P accumulated from previous fertilizer applications and therefore have a relatively low probability of responding to additional P inputs (Culman et al., 2023).

In our study, crop performance had limited responses to additional P fertilization, while the effects of AMF inoculation varied by year. Importantly, the experimental site began with an average Mehlich-3 P concentration of approximately 19 mg kg^{-1}, near the critical level of 20 mg kg^{-1} considered for corn production in Kansas, above which only starter P is generally recommended (Ruiz Diaz et al., 2024). Soil P availability was a key regulator of AMF symbiosis. Root colonization decreased with increasing P fertilization at all growth stages by 2025, and bivariate regression analyses showed negative relationships between colonization and Mehlich-3 soil P at V6 and R1, consistent with previous studies in corn reporting reduced mycorrhizal colonization with increasing P availability (Liu et al., 2016; Sheng et al., 2012; Wang et al., 2017). At low P availability, plants increase their investment in the AMF symbiosis, while greater P availability reduces the relative benefit of the symbiosis and can decrease plant C allocation to the fungal partner, consequently reducing AMF colonization (Balzergue et al., 2013; Fitter, 2006; Konvalinková et al., 2017; Ven et al., 2019).

Interestingly, AMF root colonization decreased linearly with increasing soil P at V6, whereas at R1 it plateaued at approximately 50% at a soil P concentration of 25 mg kg^{-1}. The proximity of this breakpoint to soil P levels considered sufficient for corn production suggests that colonization becomes less responsive to additional P as soil P approached agronomic sufficiency.

Nevertheless, substantial colonization persisted above the breakpoint, indicating that P sufficiency did not eliminate the symbiosis. The stronger responses at V6 and R1 than at R6 may be a result of the greater importance of AMF for P acquisition earlier in plant development, when less developed root systems benefit more from fungal hyphae extending beyond root P depletion zones (Basílio et al., 2022; Smith et al., 2003; Zhang et al., 2021). As roots expand and plants approach maturity, their reliance on AMF for P uptake may decrease, weakening the regulatory influence of soil P on root colonization.

The relatively high colonization observed in non-inoculated plants in this study indicates that indigenous AMF were already well established at the site, and inoculation supplemented an existing mycorrhizal network rather than introducing AMF into an otherwise non-mycorrhizal or mycorrhizal-deficient soil. Lekberg & Koide, (2005) have reported that the benefits of AMF inoculation to plant performance tend to decrease when soils already have substantial indigenous AMF inoculum potential or high P availability, even when inoculation successfully increases root colonization. Previous studies have also shown that competition between introduced and indigenous AMF, as well as differences in fungal compatibility with the host plant and local soil conditions, can limit the establishment and persistence of commercial inoculants under field conditions (Basiru & Hijri, 2022; Bender et al., 2019; Hart et al., 2018; Niwa et al., 2018; Verbruggen et al., 2013).

Interestingly, the most consistent positive responses to AMF inoculation in this study occurred at the highest P rate, including increases in root colonization, grain yield, P uptake, and Mehlich-3 soil P. Bender et al. (2019) suggested that inoculation provides negligible benefits when soils have reached their ecological carrying capacity with active native AMF, while the suppression of the native community may reduce competition and increase the niche space

available for introduced AMF. Therefore, the positive responses at high P levels with inoculation may reflect P-induced suppression of indigenous AMF, which could reduce competition and facilitate establishment of the introduced fungi (Bender et al., 2019; Niwa et al., 2018).

Precipitation can also be particularly important for establishing AMF symbiosis in dryland systems, as soil moisture regulates both plant and fungal activity, and can restrict spore germination, hyphal growth, root colonization, and spore production (Bahadur et al., 2019). In dryland soils already near P sufficiency, adequate moisture during inoculum establishment may therefore be necessary to generate detectable benefits from inoculation. In 2023, inoculation increased root colonization at V6 at fertilized P rates, coinciding with the only year in which positive effects from inoculation on P uptake and grain yield were observed. Interestingly, although total precipitation was similar across the four growing seasons, precipitation between V6 and R1 was greatest in 2023, a critical period for corn P nutrition that may coincide with increased demand for AMF symbiosis (Ampong et al., 2024). Therefore, our findings suggest that precipitation timing, rather than total seasonal precipitation, may be an important determinant of inoculation success in dryland production systems, as it may create more favorable conditions for AMF establishment and function when colonization demand is highest.

Plant growth and grain yield responses to P fertilization and AMF inoculation also varied widely by year, which is not unexpected given the site's initial soil P levels and the inherent environmental variability of dryland fields. The initial soil P levels likely limited crop responses to additional P, consistent with a 45-year analysis of 457 P trials in corn, soybean, and wheat in Ohio, where the probability of a yield response decreased with increasing soil-test P. Only 12–14% of trials responded to P fertilization at Mehlich-3 P concentrations above 20 mg kg^{-1}, compared with 67% when soil P was below 10 mg kg^{-1} (Culman et al., 2023). Therefore, the

inconsistent plant biomass and grain yield responses observed in the present study were not unexpected for a field beginning near the soil P concentration generally considered sufficient for corn production, as P availability was likely not the primary constraint on crop growth and yield during much of the experiment. Furthermore, at V6, inoculated plants required a higher Mehlich-3 soil P concentration (37.8 mg kg^{-1}) to reach maximum aboveground biomass than non-inoculated plants (22.4 mg kg^{-1}), despite achieving similar maximum biomass levels, which suggests that inoculation did not improve early plant growth at lower soil P concentrations and instead increased the estimated soil P requirement for maximum biomass production.

Plant P uptake responded more consistently to P fertilization than biomass or grain yield. The decreasing APRE with increasing P rates further supports lower returns from additional P fertilizations at this site. Recovery was generally greatest at 15 kg P ha^{-1} and decreased at greater application rates, indicating that the crop recovered a lower proportion of the applied fertilizer P, consistent with greater accumulation of soil P over time. Zhang et al. (2021) demonstrated that the contribution of AMF to maize P uptake was greatest under suboptimal soil P availability, suggesting that maintaining soil P at suboptimal to sufficiency levels through balanced fertilization may increase the indigenous AMF contributions to crop P acquisition while reducing unnecessary fertilizer inputs. Liu et al. (2016) also reported that while high P inhibits root colonization, moderate P fertilization can increase it.

Successive P fertilizations progressively increased Mehlich-3 soil P in all sampled depths by 2026, while simultaneously suppressing soil AMF abundance, measured by NLFA, and root colonization. Interestingly, AMF inoculation more than doubled Mehlich-3 soil P at 0–5 cm in 2026 at both 0 and 60 kg P ha^{-1}, consistent with other studies reporting increased extractable soil P following AMF inoculation (Ning et al., 2026; Peng et al., 2025). Although AMF are primarily

recognized for enhancing plant P acquisition, they can also influence the redistribution and transformation of soil P pools (Jiang et al., 2021; Liu et al., 2025). Through their extensive hyphal networks, AMF can access soil microsites beyond the reach of roots, promote organic P mineralization through organic acid release, and stimulate other P-solubilizing microorganisms (Madrid-Delgado et al., 2021; Wang et al., 2023). Repeated inoculation over four growing seasons may therefore have modified P cycling sufficiently for differences in extractable P to become detectable by 2026.

Similar to AMF root colonization, soil AMF abundance consistently decreased as soil P levels increased, across multiple depths and years, further demonstrating the strong regulation of AMF by P availability. The reduction in AMF abundance with increasing soil P may reflect reduced plant C allocation to the fungal partner and downregulation of plant signals and genes that promote fungal recruitment, root entry, and symbiosis development, potentially limiting the development and maintenance of extraradical mycelium (Konvalinková et al., 2017; Madrid-Delgado et al., 2021; Smith & Read, 2008; Ven et al., 2019). The limited benefits of inoculation over the course of the experiment may reflect reduced host investment in the symbiosis as P availability increased. Under these conditions, plants may have allocated less C to both indigenous and introduced AMF, suggesting that additional propagules alone were insufficient to counteract the strong P-mediated regulation of the association.

In our study, the high levels of root colonization and soil AMF biomass without inoculation exemplify the importance of managing indigenous AMF communities for crop production and soil health. Practices such as cover cropping, crop diversification, and no-till can naturally promote indigenous AMF by maintaining host plants year-round and reducing disruption of existing hyphal networks (Bowles et al., 2017; Brito et al., 2021). Therefore, management practices that maintain

active indigenous AMF communities may provide many of the ecosystem functions targeted by commercial bioinoculants without requiring repeated applications of external propagules. Importantly, the initial increase in soil AMF abundance in 2024 at all depths may be a direct response to the introduction of cover crops at the beginning of the experiment. A recent meta-analysis showed that maintaining cover crops can support AMF communities by extending host availability, increasing root colonization, spore abundance, hyphal density, and extraradical biomass compared with fallow (Ge et al., 2025). The return of AMF biomass at the highest P rate to levels comparable to the 2022 baseline suggests that repeated high P fertilization has prevented the increase in AMF biomass observed over time at lower P rates.

Unlike the relatively clear and consistent responses of soil AMF abundance and soil P, soil physicochemical properties, extracellular enzyme activity, and major microbial groups showed few consistent responses to AMF inoculation and P fertilization, changing predominantly between depths and sampling years under the field conditions evaluated. Importantly, several response variables showed depth-dependent patterns, with the strongest responses generally occurring at 0–5 cm, highlighting the importance of depth-specific assessment in no-till systems, where biological activity, nutrient accumulation, fertilizer effects, and organic matter inputs can create substantial vertical stratification, potentially obscuring important responses when soil layers are combined (Howard et al., 1999).

Most microbial groups and extracellular enzyme activities increased from baseline and frequently reached their greatest abundance in 2024, possibly as a result of the substantially higher wheat cover-crop biomass produced at the time of the 2024 soil sampling (Fig. B.6), consistent with findings by Thapa et al. (2021), who found that cover crops increased total microbial biomass by 31–41% and combined extracellular enzyme activity when compared with fallow. Higher

cover-crop biomass production increases belowground C inputs through greater root biomass, rhizodeposition, and subsequent decomposition of plant residues, which stimulates microbial growth and activity in the rhizosphere (Buyer et al., 2010; Finney et al., 2017).

Repeated AMF inoculation did not stimulate the broader microbial community. Instead, inoculation reduced total microbial biomass, Gram-positive bacteria, and actinomycetes at 0–5 cm across sampling years. At deeper depths, these effects were limited to 2024, when inoculation reduced total microbial biomass and Gram-positive bacteria at 5–15 cm and only Gram-positive bacteria at 15–30 cm, consistent with findings by Gui et al. (2017) and Welc et al. (2010). Duan et al. (2025) also found that suppressing AMF increased Actinobacteria and Gram-positive bacteria, while having limited effects on Gram-negative bacteria. Because Gram-positive bacteria represented a substantial portion of the total microbial biomass, their reduction after inoculation may have contributed to the observed decrease in total microbial biomass, although the mechanisms underlying this response remain unclear. Importantly, these effects of inoculation on microbial groups varied with sampling year and soil depth rather than representing a persistent response.

Although AMF inoculation did not directly influence macroaggregate formation, water-stable macroaggregates of 0.25–2 mm consistently increased as soil AMF abundance increased at 0-5 cm for all sampled years, highlighting the important role of AMF in soil aggregation. Mycorrhizal hyphae are known to physically enmesh soil particles and smaller aggregates, while fungal-derived compounds and interactions with other rhizosphere microorganisms can promote stabilization of newly formed aggregates (Morris et al., 2019; Rillig et al., 2010; Rillig & Mummey, 2006). Our findings suggest that the abundance and activity of the overall AMF

community, indigenous and inoculated, were more important for aggregate formation than the inoculant alone.

Soil pH generally decreased with P fertilization at 0-5 cm, consistent with Liu et al. (2016), possibly in direct response to repeated monoammonium phosphate applications. The nitrification of ammonium releases protons, contributing to soil acidification and reductions in soil pH (Chirchir et al., 2026). Importantly, soil acidification can negatively affect AMF by limiting root colonization, spore production, and hyphal growth (Gou et al., 2024). Previous studies have also demonstrated that soil pH can influence fungal community composition, nutrient availability, and even responses to AMF inoculation (Gou et al., 2024; Pellegrino et al., 2015; Schütz et al., 2018). Therefore, changes in soil P availability and pH over time may have jointly influenced the observed responses to AMF inoculation (Xu et al., 2025).

Conclusions

This study provides novel evidence of the complex long-term interactions between AMF inoculation and P fertilization under dryland field conditions, addressing important knowledge gaps left by short-term and controlled-environment studies. After evaluating plant and soil responses across multiple growing seasons, our findings demonstrate that AMF inoculation benefits are highly context-dependent in agricultural soils with P availability near levels considered sufficient for crop production, and where established indigenous AMF communities and environmental variability may further limit the effectiveness of introduced fungi. Fertilization was a stronger and more consistent regulator of AMF root colonization and soil abundance than inoculation, while improvements in crop productivity and soil health following inoculation were limited and inconsistent. Importantly, our results suggest that precipitation timing may critically regulate inoculation success in dryland systems, adding another layer of complexity to predicting

bioinoculant performance under field conditions. The relatively high abundance of indigenous AMF and the increase in soil AMF biomass over time also emphasize the potential of management practices, particularly cover cropping, to support mycorrhizal communities already adapted to local soil and environmental conditions. Therefore, promoting indigenous AMF through sustainable management practices may provide a more reliable strategy for enhancing and maintaining mycorrhizal networks than relying exclusively on commercial inoculants. Overall, identifying the soil and environmental conditions under which introduced AMF provide additional benefits beyond those of indigenous communities is essential for improving the effectiveness and agronomic value of commercial bioinoculants. Further long-term field research across contrasting soil types, cropping systems, and precipitation regimes is needed to better understand the factors regulating inoculant establishment, persistence, and performance.

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Tables

Table 3.1. ANOVA P-value summary table of the effects of phosphorus fertilization (P), arbuscular mycorrhizal fungi inoculation (F), and their interactions on plant response variables.

2022			
	P	**F**	**P x F**
Aboveground Biomass at V6	0.693	0.386	0.072
Aboveground Biomass at R1	0.316	0.722	0.579
Aboveground Biomass at R6	**<0.001**	**0.021**	**<0.001**
Phosphorus Uptake at V6	0.938	0.417	0.216
Phosphorus Uptake at R1	0.053	0.705	0.174
Total Phosphorus Uptake at R6	**0.006**	0.621	0.609
Apparent Phosphorus Recovery Efficiency	0.197	0.841	0.899
Grain Yield	0.308	**0.035**	0.429
AMF Root Colonization at V6	0.917	0.893	0.653
AMF Root Colonization at R1	0.505	**0.045**	0.816
AMF Root Colonization at R6	0.105	0.400	0.103
2023			
Aboveground Biomass at V6	**0.037**	0.132	0.498
Aboveground Biomass at R1	**0.011**	0.082	0.488
Aboveground Biomass at R6	0.076	0.908	0.106
Phosphorus Uptake at V6	**<0.001**	0.410	**0.014**
Phosphorus Uptake at R1	**<0.001**	0.284	0.408
Total Phosphorus Uptake at R6	**0.001**	0.560	**0.004**
Apparent Phosphorus Recovery Efficiency	**0.023**	0.897	0.329
Grain Yield	**0.003**	**0.009**	**<0.001**
AMF Root Colonization at V6	**<0.001**	**<0.001**	**<0.001**
AMF Root Colonization at R1	**0.003**	0.259	0.075
AMF Root Colonization at R6	0.588	**0.039**	0.377
2024			
Aboveground Biomass at V6	**0.012**	0.477	0.323
Aboveground Biomass at R1	0.208	0.849	0.267
Aboveground Biomass at R6	**<0.001**	0.309	**<0.001**
Phosphorus Uptake at V6	**0.030**	0.929	**0.005**
Phosphorus Uptake at R1	**<0.001**	0.345	0.988
Total Phosphorus Uptake at R6	**<0.001**	0.817	0.444
Apparent Phosphorus Recovery Efficiency	**0.018**	0.612	0.764
Grain Yield	**<0.001**	0.643	0.950

AMF Root Colonization at V6	**<0.001**	0.397	**<0.001**
AMF Root Colonization at R1	0.129	0.780	0.947
AMF Root Colonization at R6	0.314	0.614	0.053
2025			
Aboveground Biomass at V6	**0.045**	0.842	0.981
Aboveground Biomass at R1	0.476	0.991	0.233
Aboveground Biomass at R6	0.133	0.525	0.268
Phosphorus Uptake at V6	**<0.001**	0.636	0.977
Phosphorus Uptake at R1	**<0.001**	0.595	0.332
Total Phosphorus Uptake at R6	**<0.001**	0.318	0.295
Apparent Phosphorus Recovery Efficiency	**0.025**	0.142	0.992
Grain Yield	0.888	0.487	0.645
AMF Root Colonization at V6	**<0.001**	0.298	0.859
AMF Root Colonization at R1	**<0.001**	0.994	0.900
AMF Root Colonization at R6	**0.029**	0.094	0.706
Cover Crop			
Wheat Biomass (2023-2024)	**<0.001**	0.918	0.584
Wheat Phosphorus Uptake (2023-2024)	**<0.001**	0.773	0.957
Wheat Biomass (2024-2025)	**<0.001**	0.691	0.062
Wheat Phosphorus Uptake (2024-2025)	**<0.001**	0.889	0.099
Wheat Biomass (2025-2026)	**<0.001**	0.373	0.666
Wheat Phosphorus Uptake (2025-2026)	**<0.001**	0.491	0.585

Table 3.2. ANOVA *P*-value summary table of the effects of phosphorus fertilization (P), arbuscular mycorrhizal fungi inoculation (F), over the years (Y), and their interactions on all response variables at the North Farm in Manhattan, KS.

0-5 cm							
	P	**F**	**Y**	**P x F**	**P x Y**	**F x Y**	**P x F x Y**
Soil Carbon Stock	0.859	0.125	**<0.001**	0.932	0.999	0.325	0.831
Soil Total Nitrogen	0.892	0.378	**<0.001**	0.830	0.999	0.596	0.872
Soil pH	0.075	0.900	**<0.001**	0.770	**<0.001**	0.266	**0.006**
Soil Phosphorus	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**0.001**	**<0.001**	**<0.001**
Soil Macroaggregates (>2mm)	0.161	0.226	**<0.001**	0.630	0.114	**0.006**	0.099
Soil Macroaggregates (2-0.250mm)	0.665	0.986	**0.017**	0.323	0.182	0.206	0.930
Betaglucosidase	0.541	**0.011**	**<0.001**	0.495	0.602	**0.007**	0.423
Acid Phosphatase	**0.041**	0.152	**<0.001**	**0.030**	0.728	0.060	0.317
Arylsulfatase	0.105	0.191	0.205	0.255	0.459	0.133	0.942
N-acetyl-β-glucosaminidase	0.694	0.613	**<0.001**	0.062	0.254	0.629	0.240
Total Microbial Biomass	0.408	**0.013**	**<0.001**	0.308	0.080	0.162	0.534
Gram-Positive Bacteria	0.730	**<0.001**	**<0.001**	0.666	0.101	0.308	0.851
Gram-Negative Bacteria	0.143	0.989	**<0.001**	0.893	0.587	0.729	0.814
Actinomycetes	0.251	**0.025**	**<0.001**	0.088	0.379	0.203	0.450
Saprophytic Fungi	0.241	0.360	**<0.001**	0.147	0.212	0.820	0.879
Arbuscular Mycorrhizal Fungi	**0.005**	0.589	**<0.001**	0.826	**0.011**	0.266	0.104
5-15 cm							
	P	**F**	**Y**	**P x F**	**P x Y**	**F x Y**	**P x F x Y**
Soil Carbon Stock	0.979	0.080	0.245	0.812	0.772	0.584	0.614
Soil Total Nitrogen	0.890	0.294	**<0.001**	0.936	0.911	0.948	0.799
Soil pH	0.995	0.261	**0.001**	0.479	0.528	0.382	0.548
Soil Phosphorus	**<0.001**	0.949	**<0.001**	0.753	**<0.001**	0.293	0.163
Soil Macroaggregates (>2mm)	0.567	0.518	**<0.001**	0.839	0.207	0.073	0.946
Soil Macroaggregates (2-0.250mm)	0.822	0.684	**<0.001**	0.612	0.630	0.451	0.963

Betaglucosidase	0.575	0.866	**0.023**	0.600	0.752	**0.018**	0.402
Acid Phosphatase	0.127	0.940	0.707	0.748	0.887	0.180	0.547
Arylsulfatase	0.695	0.926	**<0.001**	0.587	0.629	0.078	0.753
N-acetyl-β-glucosaminidase	0.943	0.719	**<0.001**	0.896	0.340	0.174	0.154
Total Microbial Biomass	0.342	0.053	**<0.001**	0.267	0.506	**0.042**	0.468
Gram-Positive Bacteria	0.226	**0.004**	**<0.001**	0.085	0.456	**0.001**	0.769
Gram-Negative Bacteria	0.164	0.203	**<0.001**	0.853	0.337	0.099	0.468
Actinomycetes	0.162	0.619	**<0.001**	0.462	0.405	0.598	0.469
Saprophytic Fungi	0.531	0.259	**<0.001**	0.069	0.119	0.054	0.513
Arbuscular Mycorrhizal Fungi	**0.007**	0.973	**<0.001**	0.548	**0.004**	0.775	0.900

15-30 cm

	P	**F**	**Y**	**P x F**	**P x Y**	**F x Y**	**P x F x Y**
Soil Carbon Stock	0.189	0.165	0.123	0.124	0.903	0.284	0.342
Soil Total Nitrogen	0.403	0.300	**0.008**	0.482	0.987	0.899	0.909
Soil pH	0.851	0.386	**<0.001**	0.846	0.685	0.338	0.416
Soil Phosphorus	0.076	0.933	**<0.001**	0.554	**0.033**	0.713	0.802
Soil Macroaggregates (>2mm)	0.115	0.302	**<0.001**	0.530	0.601	0.970	0.391
Soil Macroaggregates (2-0.250mm)	0.866	0.991	**<0.001**	0.737	0.722	0.337	0.338
Betaglucosidase	0.226	0.697	**<0.001**	0.671	0.798	0.591	0.470
Acid Phosphatase	0.356	0.942	**0.012**	0.559	0.153	0.220	0.310
Arylsulfatase	0.733	0.906	**<0.001**	0.367	0.591	0.521	0.434
N-acetyl-β-glucosaminidase	0.451	0.794	**<0.001**	0.519	0.350	0.351	0.707
Total Microbial Biomass	0.345	0.615	**<0.001**	0.159	0.766	0.096	0.716
Gram-Positive Bacteria	0.473	0.241	**<0.001**	0.349	0.835	**0.001**	0.620
Gram-Negative Bacteria	0.886	0.856	**<0.001**	0.524	0.362	0.660	0.190
Actinomycetes	0.192	0.807	**<0.001**	0.301	0.804	0.230	0.703
Saprophytic Fungi	0.191	0.640	**<0.001**	0.772	0.075	0.125	0.472
Arbuscular Mycorrhizal Fungi	0.145	0.738	**<0.001**	0.986	**<0.001**	0.201	0.867

Table 3.3. Changes in total soil nitrogen (Mg N ha^{-1}), soil carbon stock (Mg C ha^{-1}), soil pH, water-stable macroaggregates >2 mm (g 100 g^{-1} soil), and water-stable macroaggregates (0.25–2 mm; g 100 g^{-1} soil) across years and soil depths. Values are means ± standard error. Different lowercase letters within each soil depth indicate significant differences ($P < 0.05$); Asterisks (*) indicate significant interactions presented as figures.

AMF Inoculation	Year	Total Soil Nitrogen	Soil Carbon Stock	Soil pH	Water-Stable Macroaggregates (>2 mm)	Water-Stable Macroaggregates (0.25–2 mm)
				0-5 cm		
Absent	2022	0.82 b ± 0.03	8.85 c ± 0.34		3.64 bc ± 0.77	30.2 b ± 1.35
Present					3.57 c ± 0.80	
Absent	2024	0.85 b ± 0.02	10.4 b ± 0.30	*	15.5 a ± 2.24	30.5 b ± 1.78
Present					5.64 b ± 0.83	
Absent	2026	1.14 a ± 0.04	14.9 a ± 0.45		17.2 a ± 3.60	34.3 a ± 1.68
Present					18.6 a ± 4.28	
				5-15 cm		
Absent	2022	1.86 a ± 0.04	20.1 a ± 0.49	6.17 b ± 0.09	1.54 c ± 0.46	27.3 c ± 1.58
Present						
Absent	2024	1.73 b ± 0.04	21.1 a ± 0.60	6.52 a ± 0.07	21.9 a ± 3.40	32.3 b ± 1.63
Present						
Absent	2026	1.62 c ± 0.05	20.6 a ± 0.49	6.06 c ± 0.09	8.62 b ± 1.66	39.9 a ± 1.83
Present						
				15-30 cm		
Absent	2022	2.64 a ± 0.06	29.9 a ± 0.87	6.49 b ± 0.14	0.52 c ± 0.16	20.7 b ± 1.35
Present						
Absent	2024	2.38 b ± 0.04	29.6 a ± 0.61	6.93 a ± 0.10	5.73 a ± 1.23	36.0 a ± 2.02
Present						
Absent	2026	2.09 c ± 0.07	27.4 a ± 0.64	6.48 b ± 0.10	1.84 b ± 0.63	34.0 a ± 1.55
Present						

Table 3.4. Changes in soil microbial community abundance (nmol PLFA g^{-1} soil) across years and soil depths. Values are means ± standard error. Different lowercase letters within each soil depth indicate significant differences ($P < 0.05$). Asterisks (*) indicate significant interactions presented as figures.

AMF Inoculation	Year	Total Microbial Biomass	Gram-Positive Bacteria	Gram-Negative Bacteria	Actinomycetes	Saprophytic Fungi
0-5 cm						
Absent	2022	33.9 c ± 1.24	12.1 c ± 0.44	6.71 c ± 0.30	2.78 c ± 0.11	0.44 b ± 0.02
Present						
Absent	2024	61.9 a ± 3.90	21.1 a ± 0.95	12.1 a ± 1.08	3.92 a ± 0.26	2.09 a ± 0.14
Present						
Absent	2026	50.6 b ± 2.02	17.6 b ± 0.64	9.56 b ± 0.44	3.39 b ± 0.15	1.91 a ± 0.09
Present						
5-15 cm						
Absent	2022	17.1 c ± 1.10	6.31 c ± 0.27	2.98 b ± 0.16	1.68 c ± 0.09	0.07 c ± 0.01
Present		15.8 c ± 1.15	6.23 c ± 0.46			
Absent	2024	35.5 a ± 2.50	14.8 a ± 0.73	6.87 a ± 0.62	3.11 a ± 0.15	0.24 b ± 0.02
Present		27.8 b ± 1.76	11.3 b ± 0.81			
Absent	2026	29.9 b ± 1.26	11.1 b ± 0.42	6.21 a ± 0.44	2.71 b ± 0.10	0.41 a ± 0.02
Present		27.7 b ± 1.92	10.3 b ± 0.67			
15-30 cm						
Absent	2022	9.39 c ± 0.34	3.30 d ± 0.18	1.63 c ± 0.07	1.03 b ± 0.04	0.03 c ± 0.00
Present			4.29 d ± 0.22			
Absent	2024	18.8 a ± 1.09	9.04 a ± 0.55	2.84 b ± 0.24	1.92 a ± 0.11	0.13 b ± 0.01
Present			7.31 b ± 0.52			
Absent	2026	16.0 b ± 0.62	6.26 c ± 0.27	3.19 a ± 0.15	1.91 a ± 0.10	0.17 a ± 0.01
Present			5.98 c ± 0.36			

Table 3.5. Changes in enzyme activity (μg PNP $soil^{-1}$ h^{-1}) each year, across depths. Values are means ± standard error. Different lowercase letters within each soil depth indicate significant differences ($P < 0.05$). Asterisks (*) indicate significant interactions presented as figures.

AMF Inoculation	Year	β-glucosidase	Acid Phosphatase	Arylsulfatase	NAG
			0-5 cm		
Absent	2022	166 b ± 8.66	541 b ± 20.2	56.2 a ± 3.10	31.1 b ± 1.19
Present		171 b ± 8.32			
Absent	2024	264 a ± 22.2	682 a ± 33.0	59.5 a ± 4.29	66.9 a ± 2.80
Present		190 b ± 14.4			
Absent	2026	284 a ± 16.1	651 a ± 29.0	54.9 a ± 4.04	67.2 a ± 3.27
Present		257 a ± 18.7			
			5-15 cm		
Absent	2022	71.5 b ± 6.81	382 a ± 22.4	38.5 c ± 2.00	14.3 b ± 1.08
Present		80.6 ab ± 5.71			
Absent	2024	88.6 a ± 7.31	381 a ± 14.9	48.4 a ± 2.87	21.6 a ± 0.85
Present		75.8 ab ± 6.14			
Absent	2026	86.6 a ± 4.69	398 a ± 15.2	42.5 b ± 2.65	19.5 a ± 1.04
Present		83.7 ab ± 6.28			
			15-30 cm		
Absent	2022	31.0 b ± 1.61	214 a ± 11.2	30.3 b ± 1.40	10.1 b ± 0.90
Present					
Absent	2024	38.0 a ± 2.51	186 b ± 9.85	35.3 a ± 1.62	14.2 a ± 0.78
Present					
Absent	2026	40.7 a ± 2.98	220 a ± 10.7	32.2 b ± 1.96	12.8 a ± 0.51
Present					

Figures

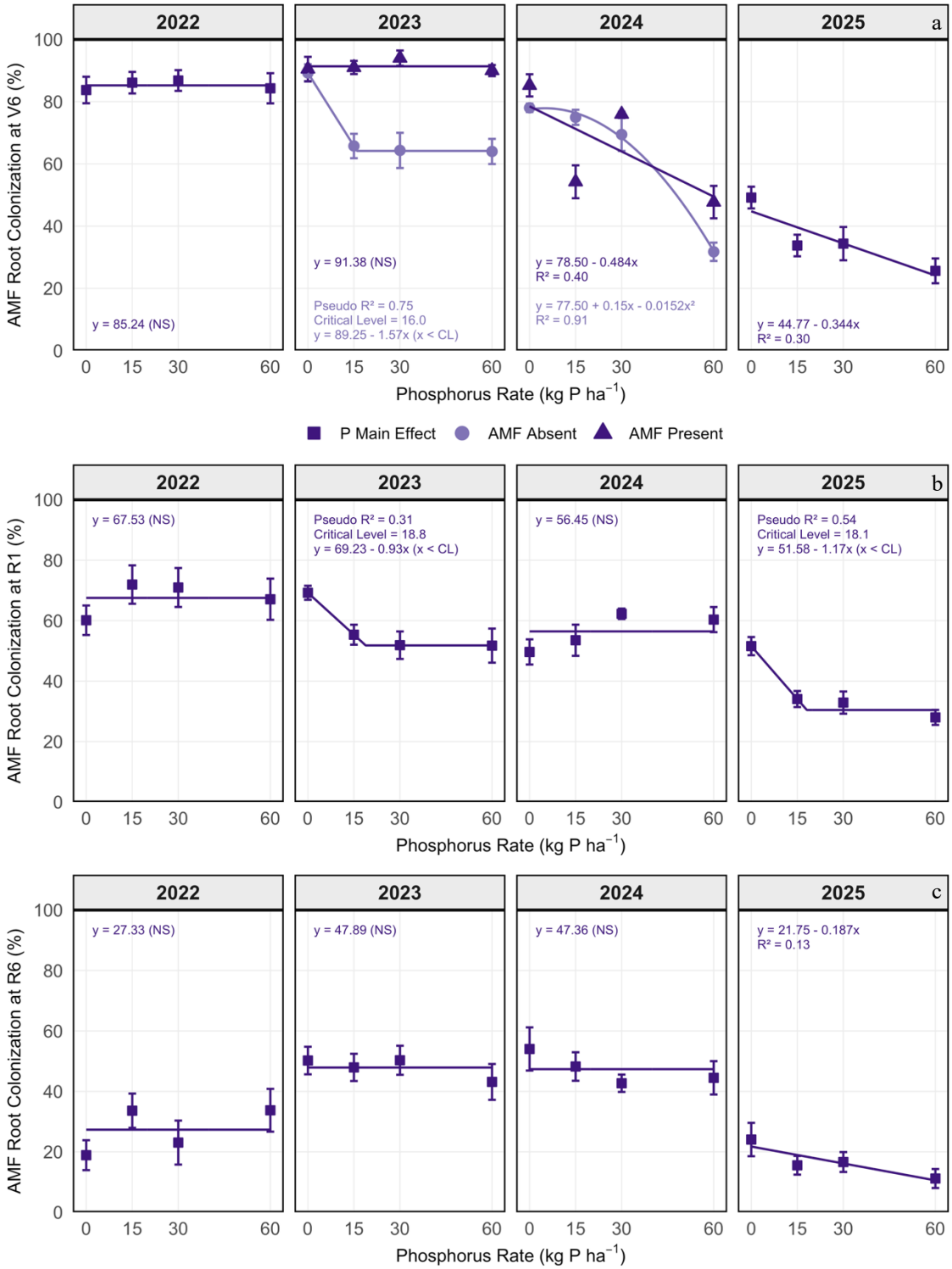


Figure 3.1. Arbuscular mycorrhizal fungi (AMF) root colonization (%) at (a) V6, (b) R1, and (c) R6 in response to phosphorus (P) fertilization and AMF inoculation. Points represent means ± standard error. Squares represent P main effect across AMF inoculation treatments, while circles and triangles represent non-inoculated and inoculated treatments, respectively.

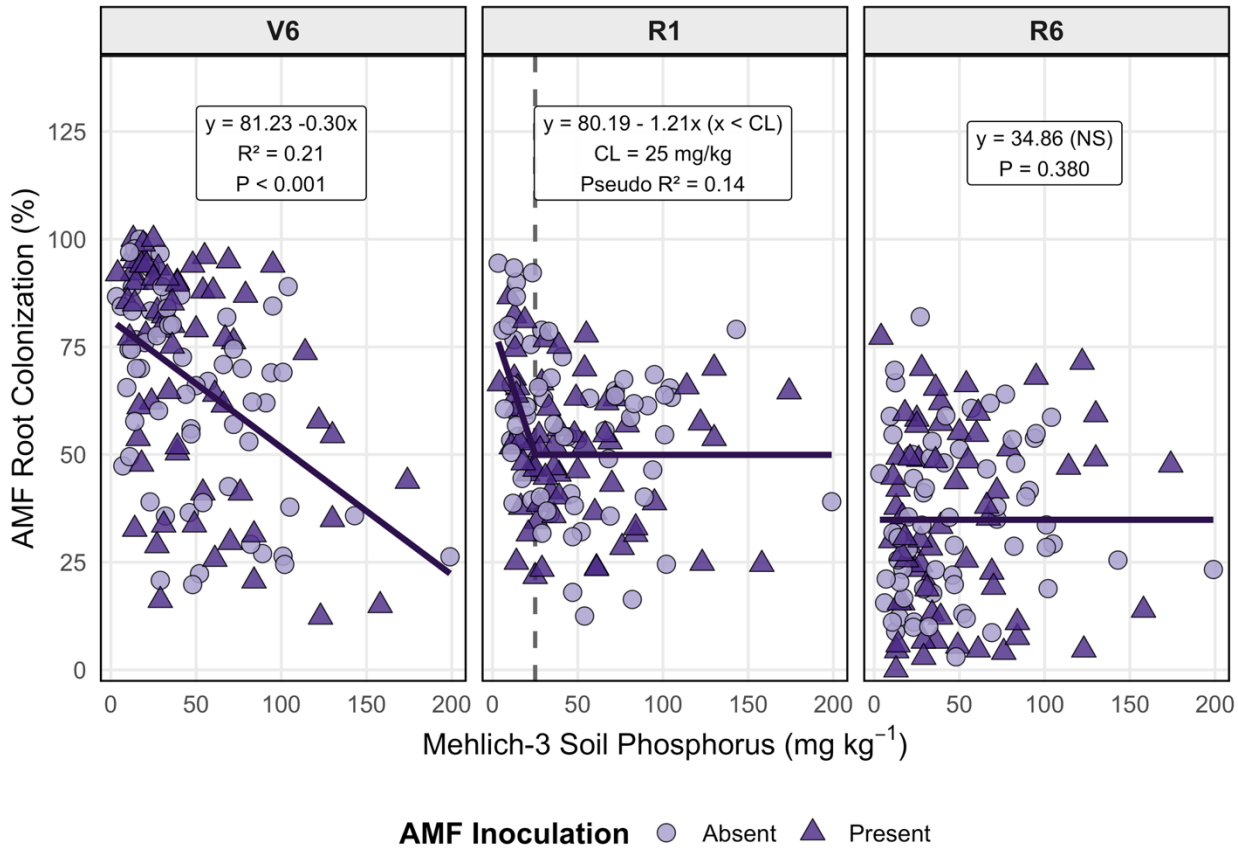


Figure 3.2. Relationship between Mehlich-3 soil phosphorus (P) (0-5 cm) and arbuscular mycorrhizal fungi (AMF) root colonization at the V6, R1, and R6 growth stages across years and AMF treatments. The vertical dashed line indicates the estimated Mehlich-3 P critical level (CL) for the linear-plateau response. Circles represent raw observations without AMF inoculation, and triangles represent raw observations with AMF inoculation.

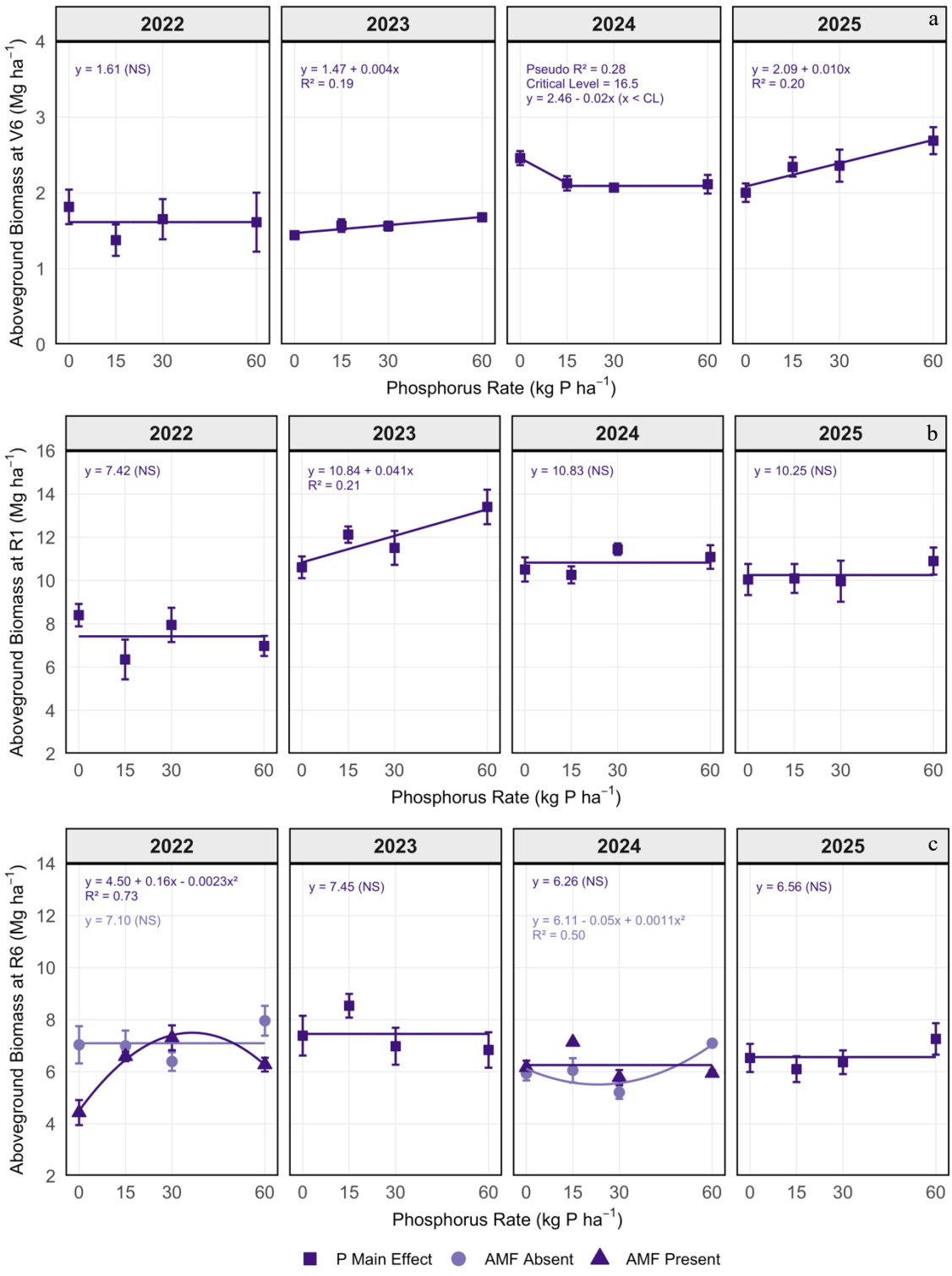


Figure 3.3. Aboveground biomass (Mg ha^{-1}) at (a) V6, (b) R1, and (c) R6 in response to phosphorus (P) fertilization and AMF inoculation. Points represent means ± standard error. Squares represent P main effect across AMF inoculation treatments, while circles and triangles represent non-inoculated and inoculated treatments, respectively.

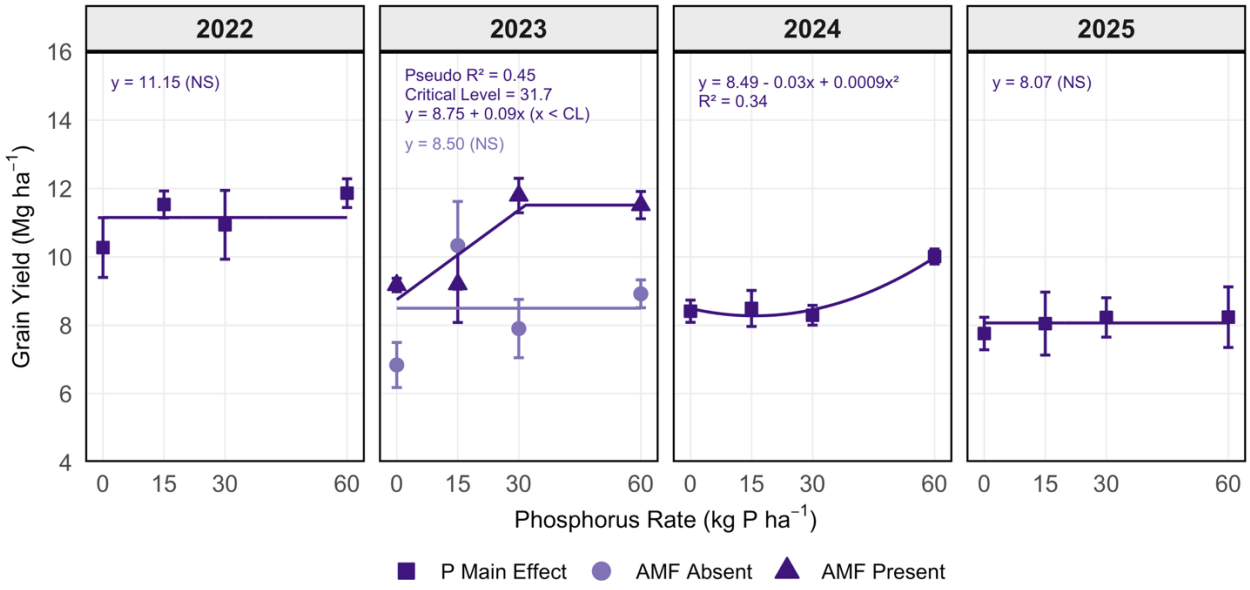


Figure 3.4. Grain yield (Mg ha^{-1}) at R6 in response to phosphorus (P) fertilization and AMF inoculation. Points represent means ± standard error. Squares represent P main effect across AMF inoculation treatments, while circles and triangles represent non-inoculated and inoculated treatments, respectively.

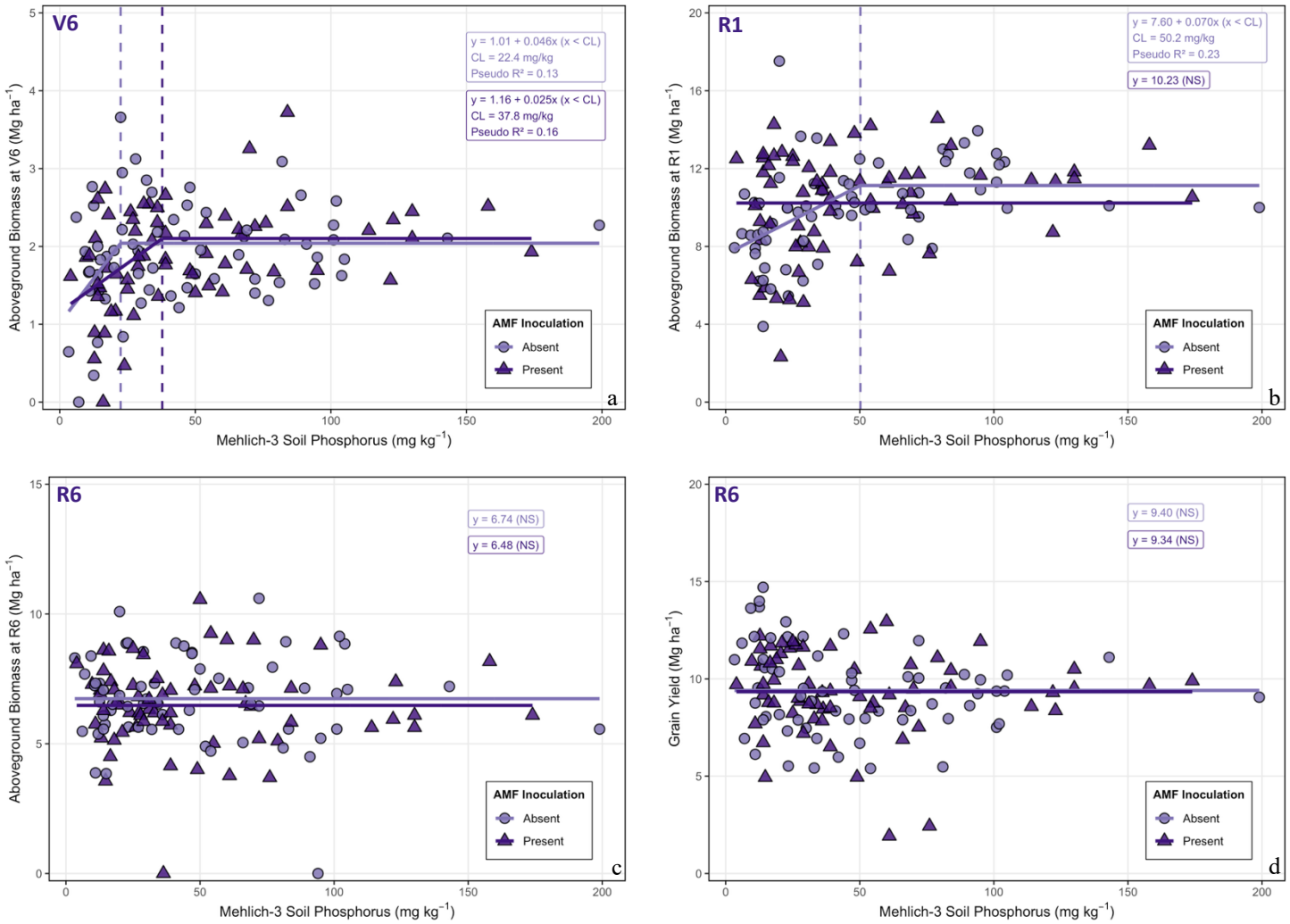


Figure 3.5. Relationships between Mehlich-3 soil phosphorus (P) (0-5 cm) and aboveground biomass at the V6 (a), R1 (b), and R6 (c) growth stages and grain yield at R6 (d) across years and arbuscular mycorrhizal fungi (AMF) inoculation treatments. Circles represent raw observations without AMF inoculation, and triangles represent raw observations with AMF inoculation.

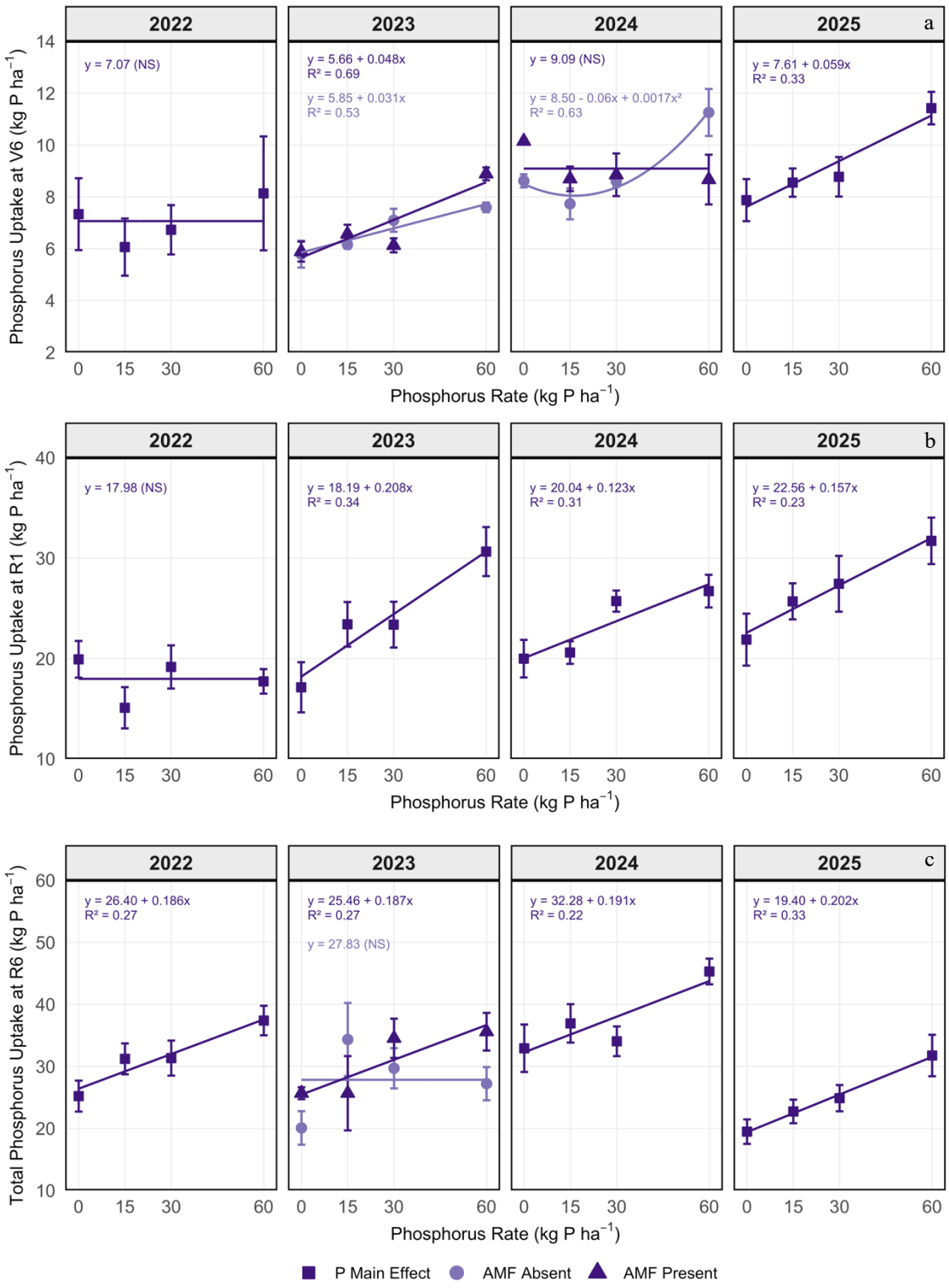


Figure 3.6. Phosphorus (P) uptake (kg P ha⁻¹) at (a) V6, (b) R1, and (c) total P uptake at R6 in response to P fertilization and AMF inoculation. Points represent means ± standard error. Squares represent P main effect across AMF inoculation treatments, while circles and triangles represent non-inoculated and inoculated treatments, respectively.

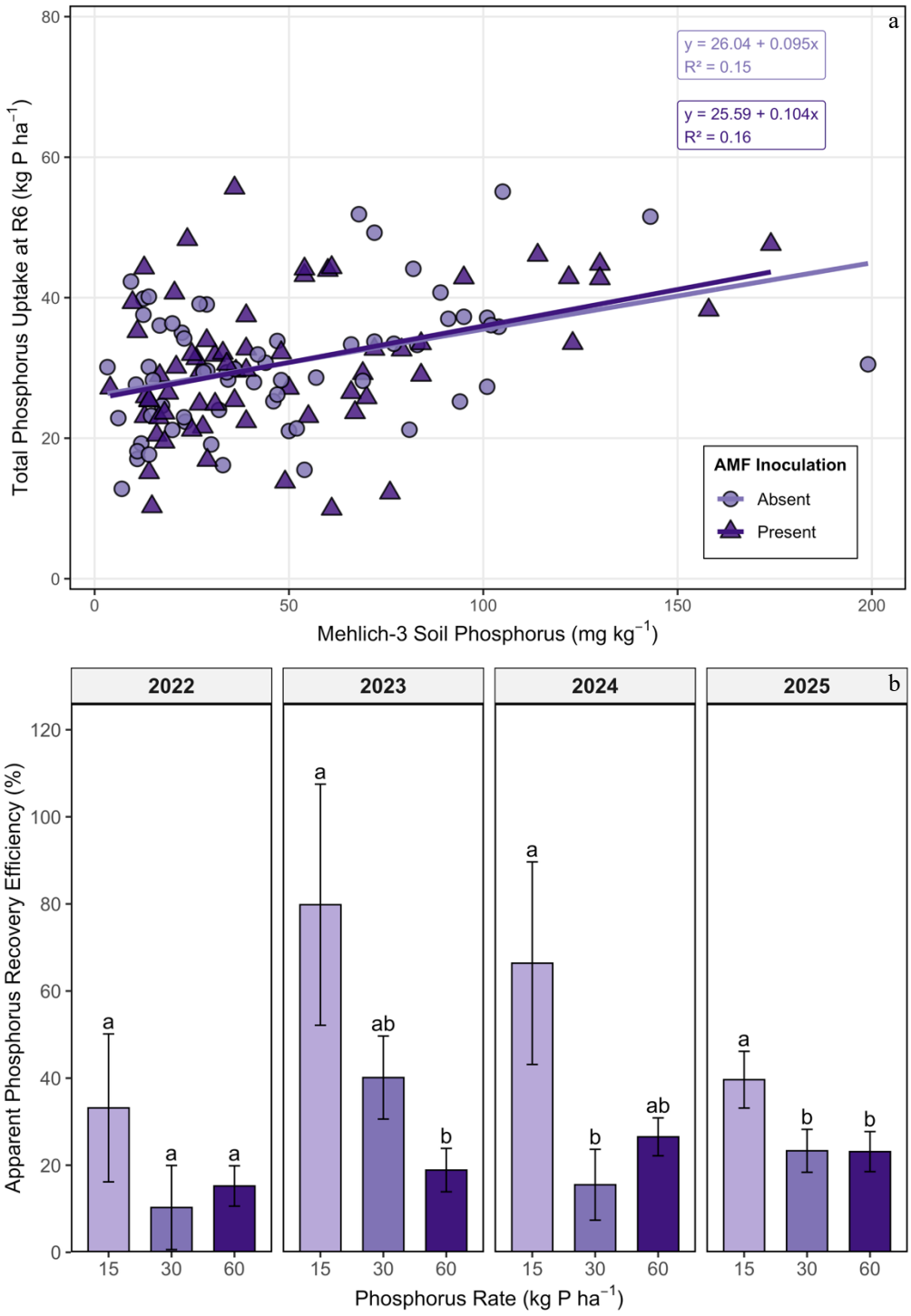


Figure 3.7. Relationship between Mehlich-3 soil phosphorus (P) and total P uptake at R6 (a), and apparent phosphorus recovery efficiency (%) in response to P fertilization from 2022 to 2025 (b). In (a), lines represent fitted linear regressions across years for inoculated and non-inoculated treatments. In (b), bars represent treatment means, and error bars indicate standard errors. Different lowercase letters indicate significant differences among P rates within each year ($P < 0.05$).

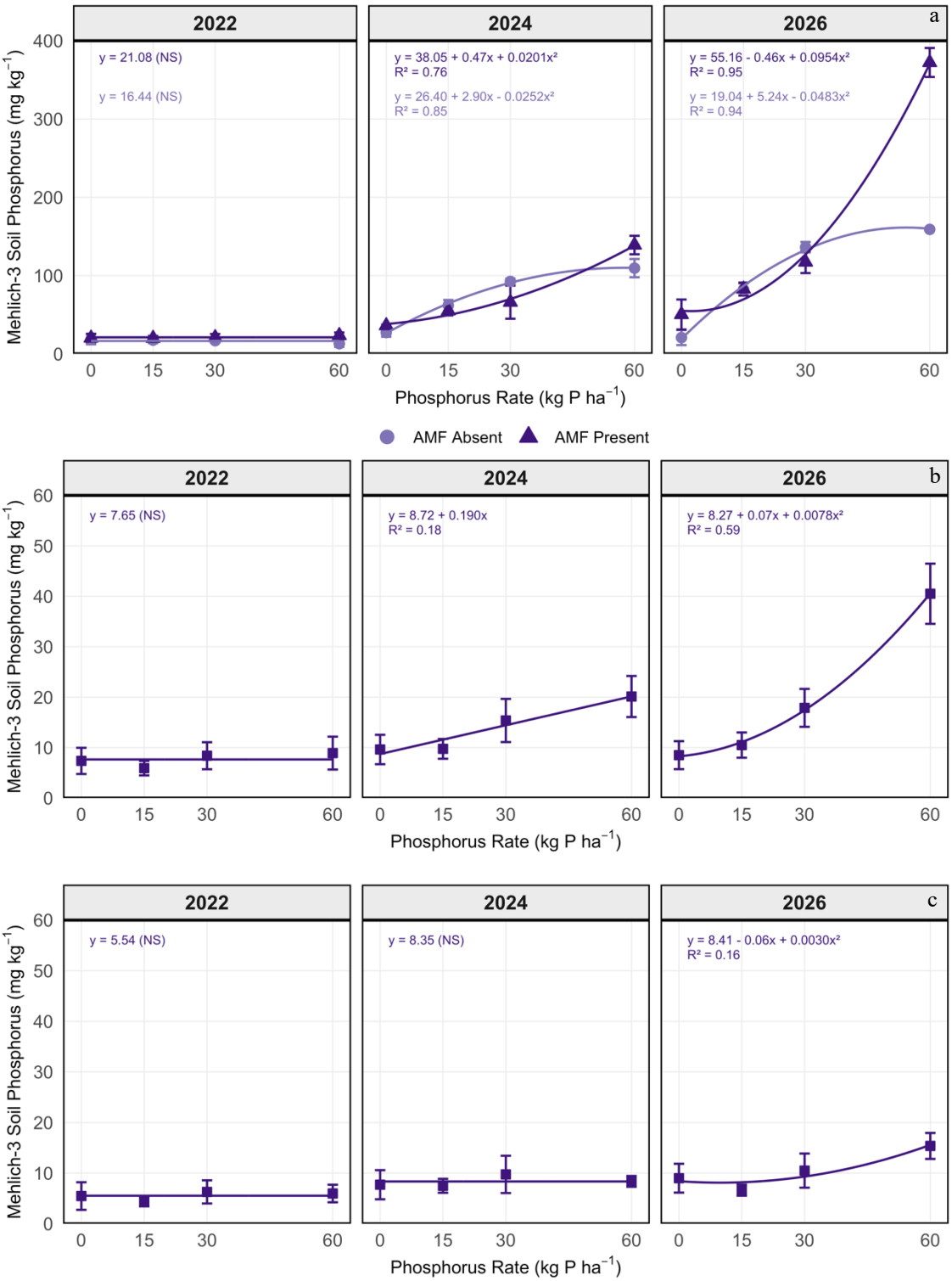


Figure 3.8. Effects of phosphorus fertilization and arbuscular mycorrhizal fungi (AMF) inoculation on Mehlich-3 soil phosphorus (mg kg^{-1}) across sampling years (2022, 2024, and 2026) at (a) 0–5, (b) 5–15, and (c) 15–30 cm soil depths. Points represent means with standard error bars.

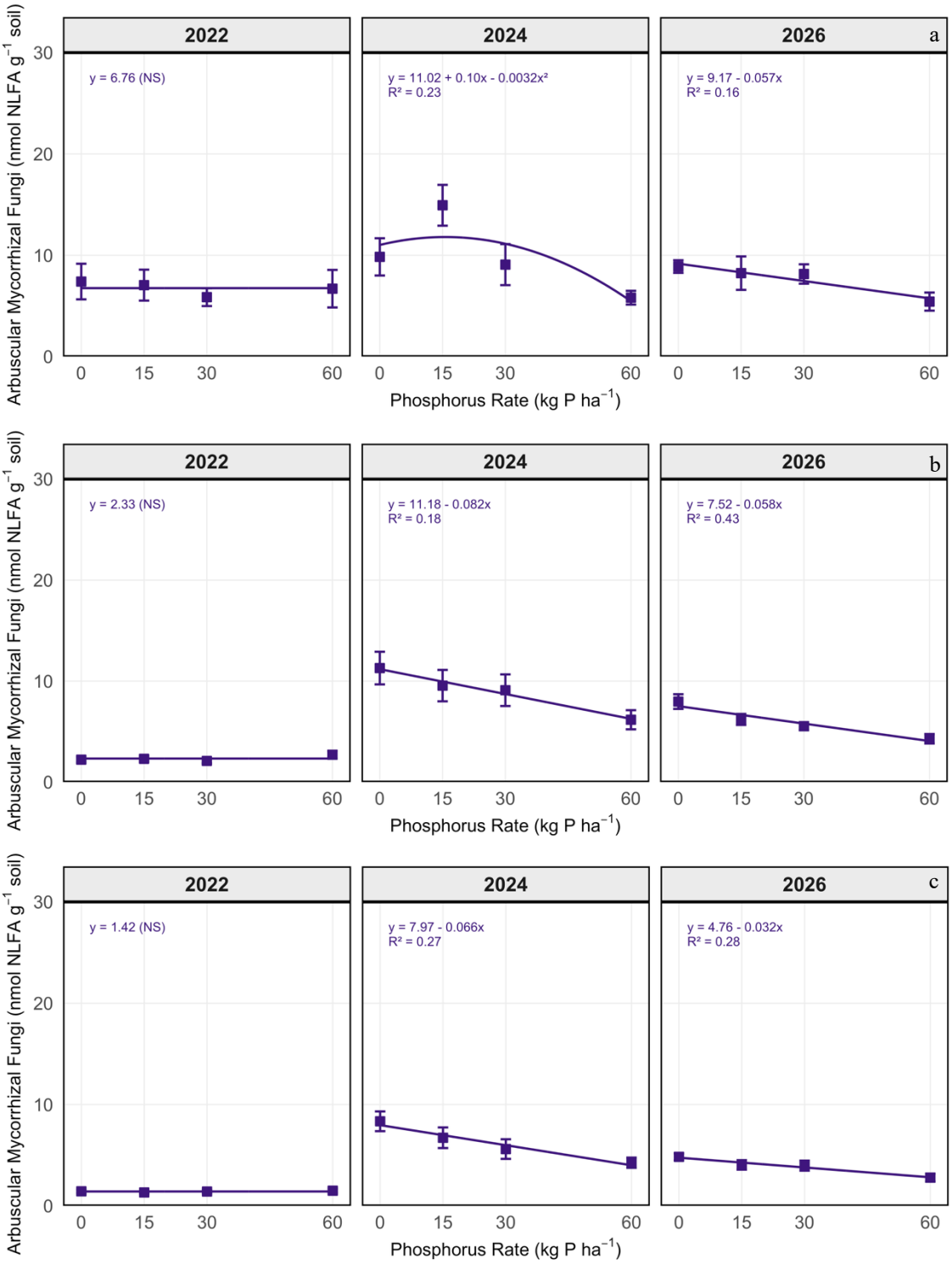


Figure 3.9. Effects of phosphorus (P) fertilization on soil AMF biomass (nmol NLFA g^{-1} soil) across sampling years (2022, 2024, and 2026) at (a) 0–5, (b) 5–15, and (c) 15–30 cm soil depths at the North Farm in Manhattan, KS. Points represent means with standard error bars.

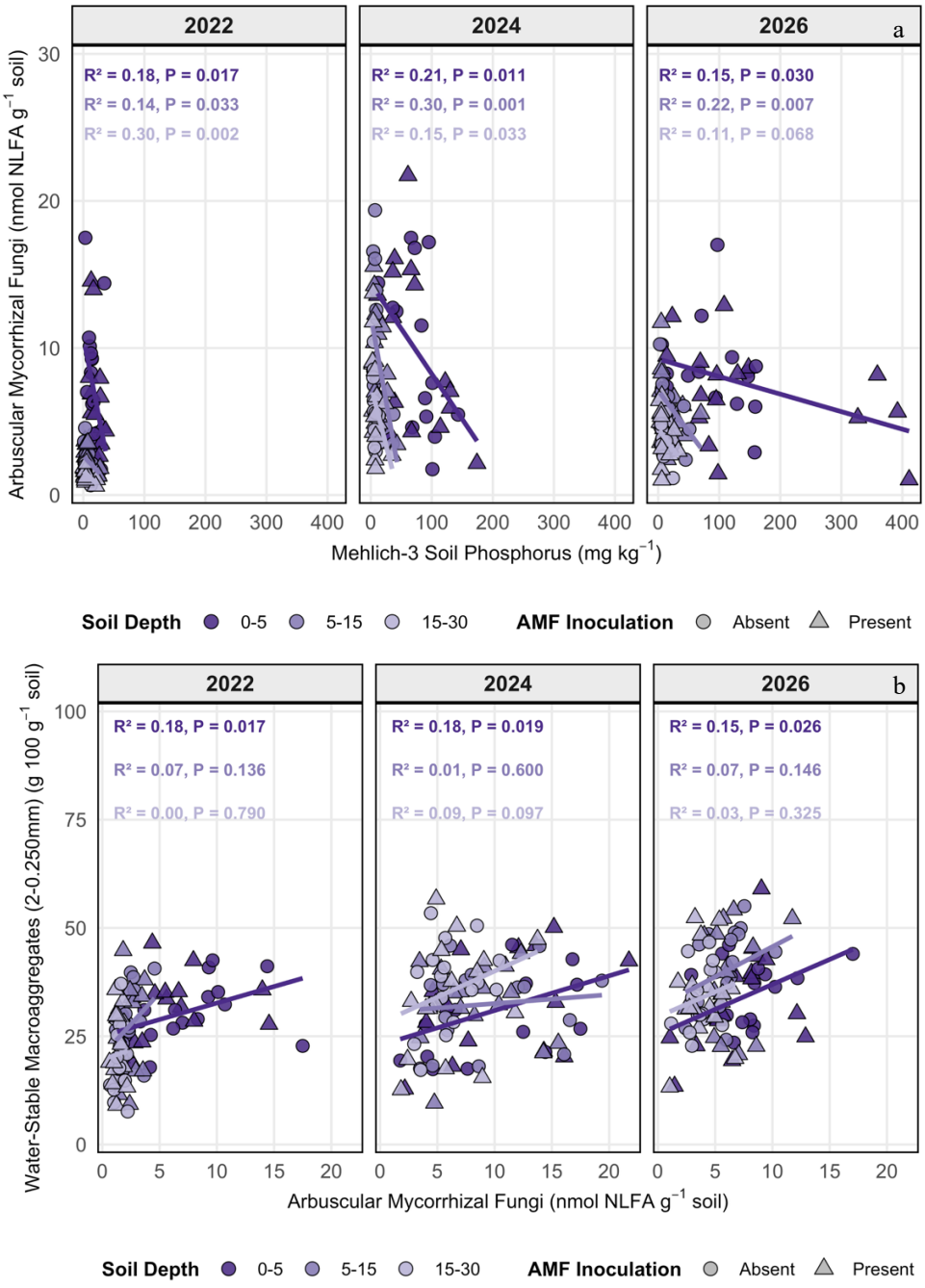


Figure 3.10. Relationships between soil phosphorus (P), soil arbuscular mycorrhizal fungi (AMF) abundance, and water-stable macroaggregates in 2022, 2024, and 2026. (a) Relationship between Mehlich-3 soil P and soil AMF abundance (nmol NLFA g^{-1} soil) at 0–5, 5–15, and 15–30 cm soil depths. (b) Relationship between soil AMF abundance and water-stable macroaggregates (0.25–2 mm; g 100 g^{-1} soil) at 0–5, 5–15, and 15–30 cm soil depths. Circles represent non-inoculated and triangles inoculated treatments. Colors indicate soil depth.

Chapter 4 - Wheat Genotypes Modulate Arbuscular Mycorrhizal Fungi Responsiveness, Soil Enzyme Activity, and Rhizosphere Microbial Composition across Phosphorus Levels

Abstract

Optimizing phosphorus (P) use efficiency, soil health, and plant resilience is essential for sustainable wheat (*Triticum aestivum* L.) production. Arbuscular mycorrhizal fungi (AMF) can enhance P acquisition, influence rhizosphere microbial communities, and increase plant biomass, but benefits often depend on plant genotype, soil fertility, and environmental conditions. A greenhouse study compared the responsiveness of six wheat cultivars with distinct root traits and potential to influence the soil microbiome, Bob Dole, Byrd, KanMark, KS Providence, SY Monument, and Turkey Red, to AMF inoculation under low and high soil P conditions. A full factorial design included two P fertilization levels (0 and 50 g P m^{-3}), and two AMF inoculation treatments (presence and absence of *Rhizophagus irregularis*), with four replicates per treatment. Plant biomass, P uptake, and AMF root colonization were measured at flowering and maturity, while grain yield, soil microbial community composition (PLFA/NLFA), and extracellular enzyme activities were measured at maturity. Significant genotype × AMF × P interactions occurred for several traits. Overall, differences in genotypes modulated the magnitude and direction of responses to AMF inoculation and P fertilization, influencing AMF root colonization dynamics, plant growth, rhizosphere composition, and enzyme activity. Inoculation improved plant growth and P uptake in select cultivars, particularly Byrd, Bob Dole, and KS Providence, whereas other genotypes produced minimal or inconsistent responses. Fertilization with P reduced AMF colonization in most genotypes, but the magnitude of suppression was genotype dependent. Soil

microbial community composition and extracellular enzyme activities were primarily influenced by genotype rather than inoculation or P fertilization, with KS Providence, Turkey Red, and Byrd consistently supporting higher extracellular enzyme activity and abundance of several microbial groups, including total microbial biomass (PLFA) and soil AMF biomass (NLFA). These findings demonstrate that wheat genotypes are a strong determinant of AMF responsiveness and rhizosphere dynamics in soils and highlight the potential to integrate genotype selection with mycorrhizal compatibility and rhizosphere traits into breeding programs to improve nutrient use efficiency and soil health in wheat production systems.

Introduction

Wheat (*Triticum aestivum* L.) is one of the most important staple crops worldwide, providing a significant source of carbohydrates, protein, and fiber in human diets (FAO, 2023; Shewry & Hey, 2015). Meeting the growing global demand for wheat while minimizing environmental impacts requires improving nutrient use efficiency through more sustainable management practices (Simão et al., 2024). Phosphorus (P) is an essential macronutrient for plant growth and development but is often poorly available in soils due to its slow diffusion rate and the soil's tendency to precipitate, adsorb, and “fix” P, resulting in the widespread use of P fertilizers to maintain productivity and low use efficiency (Roberts & Johnston, 2015; Vance et al., 2003). Phosphate ore, a non-renewable resource, is facing a critical depletion crisis, with projections suggesting that half of the world's current P reserves will be exhausted within 50 to 100 years (Zhu et al., 2018).

Soil health is defined as the capacity of soil to function as a vital living system that sustains biological productivity, promotes environmental quality, and maintains plant and animal health (Doran & Zeiss, 2000). Improving soil health is essential to enhance the resilience and productivity of agricultural systems under the challenges posed by climate change (Lal, 2016). Developing production systems that improve P acquisition and use efficiency, and soil health, is therefore a key challenge for sustainable wheat production.

Arbuscular mycorrhizal fungi (AMF) form symbiotic associations with plant roots, enhancing nutrient uptake, especially P, by extending the root’s absorptive capacity through external hyphae expansion. In return, the plant supplies AMF with photosynthetically derived carbon (C) (Smith & Read, 2008). In addition, AMF also influence plant-water relations, soil aggregation, and plant tolerance to biotic and abiotic stresses (Berruti et al., 2016; Smith & Read, 2008; Wilson et al.,

2009). However, AMF benefits vary considerably depending on host plant genotype, soil nutrient status, and environmental conditions, and can even shift toward parasitism under high nutrient availability (Johnson, 2010). Given this variability, incorporating AMF responsiveness as a selection trait in plant breeding programs may enhance the effectiveness of mycorrhizal associations under diverse field conditions.

Historically, wheat breeding programs have primarily focused on the selection and improvement of aboveground traits, often overlooking the equally important 'hidden half' (i.e., the root system) of wheat plants (Bishopp & Lynch, 2015; Ober et al., 2021). Wheat genotypes differ in traits such as root system architecture, exudation patterns, and genetic background, which can influence AMF compatibility, mycorrhizal dependency, and nutrient uptake (Campos et al., 2018; Tawaraya, 2003).

Certain modern cultivars carry genes that affect plant stature (e.g., dwarfing genes), root morphology, or tolerance to soil constraints (e.g., aluminum (Al) toxicity), which may indirectly alter their rhizosphere microbial communities (Alaux et al., 2024; Ryan et al., 2001). Understanding genotype-specific interactions with AMF, particularly under contrasting P availability, is therefore essential for optimizing nutrient acquisition strategies, enhancing crop performance, improving soil health, and developing more sustainable and resilient wheat production systems.

Despite extensive research on AMF and nutrient management in wheat, there is still limited understanding of how wheat genotypes with contrasting genetic backgrounds and selective root traits respond to AMF inoculation under varying soil fertility conditions. In particular, the combined effects on plant growth, P uptake, AMF colonization patterns, rhizosphere microbial community structure, and soil enzyme activity are not well characterized. Clarifying this

interaction could guide farmers and researchers toward genotype-specific mycorrhizal management strategies that improve P use efficiency and soil health without sacrificing wheat yield.

This study was designed to (i) compare the responsiveness of six wheat genotypes with distinct root traits and potential to influence the soil microbiome to AMF inoculation under contrasting P fertilization levels in terms of plant growth, P uptake, and AMF colonization dynamics, and to (ii) evaluate how the treatments influence soil health, as soil microbial community composition and soil extracellular enzyme activity. We hypothesized that (i) AMF inoculation would enhance plant growth and P uptake, particularly under low P levels, with the magnitude of the response varying by genotype; (ii) P fertilization would generally reduce AMF colonization, with genotype-specific responses; (iii) microbial community structure and soil enzyme activity would be influenced by different genotypes, AMF inoculation, and P fertilization. Our main findings improve our understanding of genotype-specific responses to AMF inoculation in the soil–plant system and provide novel evidence on the interactive effects of these factors on plant growth, P uptake, AMF root colonization, and rhizosphere dynamics.

Materials and Methods

Location and Experimental Design

The greenhouse study conducted at Kansas State University used unfertilized soil (0-30 cm) collected from the Agronomy North Farm in Manhattan, KS, USA (39.212372 N, -96.597744 W). The soil was classified as a Kennebec silt loam (fine-silty, mixed, superactive mesic Cumulic Hapludolls) (Table 4.1). The freshly collected soil was air-dried inside the greenhouse and passed through a 2 mm sieve to remove coarse fragments. Sealed 3.78-liter polyethylene pots were filled with 2.5 kg of the sieved unsterilized soil. Throughout the experiment, the average daily minimum

and maximum temperatures inside the greenhouse were 16°C and 27°C, respectively. The experiment was a randomized block design with treatments arranged in a full factorial (2 × 2 × 6), including two P fertilization treatments (0 and 50 g P m^{-3}), two mycorrhizal inoculation treatments (presence and absence of AMF inoculation), and six wheat genotypes (Bob Dole, Byrd, KanMark, KS Providence, SY Monument, and Turkey Red). Each treatment combination had four replicates.

Wheat Genotypes

Wheat genotypes were selected for their contrasting genetic backgrounds, distinct root characteristics, and relevance to modern wheat cultivation. Turkey Red was a foundational landrace introduced by Mennonite settlers in 1873, serving as a historical control due to its tall stature, extensive root system, and genetic legacy in U.S. hard winter wheat (Ball, 1930; Paulsen & Shroyer, 2008; Quisenberry, 1974). Bob Dole (PVP 201900177), a widely grown modern cultivar released in 2018, lacks the classic Green Revolution genes (*Rht-B1b* and *Rht-D1b*) but carries *Rht24*, a height-reducing gene that maintains gibberellic acid (GA) sensitivity, and *ALMT1*, an Al tolerance gene that confers resistance through malate efflux at the root tips, traits that may influence root exudation patterns and shape the rhizosphere microbiome (Sasaki et al., 2004). KanMark (PVP 201500379), released in 2014, carries both *Rht-B1b* and *Rht8*, a dwarfing gene that does not rely on GA insensitivity, representing a unique dwarfing gene combination that may influence microbiome composition. Released in 2022, KS Providence (PVP 202300377) includes *Rht-B1b* and the 1B.1R wheat-rye chromosomal translocation, implicated in increasing drought tolerance and root architecture. SY Monument (PVP 201400332), one of the most widely planted commercial cultivars in Kansas, also carries *Rht-B1b* and *ALMT1*, with a distinct genetic background and the potential to modify the rhizosphere microenvironment. Lastly, released in 2011, Byrd (PVP 201200432) has a notable genetic contribution from *Aegilops tauschii*, a wild

progenitor of common wheat, and is recognized for its allelopathic potential and strong drought resilience, attributed to its distinctive root architecture (Haley et al., 2012; Rudd et al., 2014).

Fertilization and AMF Inoculation

The soil used in this study had a low initial Mehlich-3 phosphorus (P) concentration of 13 mg P kg^{-1} soil, indicating a low-P soil with a high potential to respond to P fertilization. The P fertilization treatment consisted of 50 g P m^{-3}, equivalent to 100 kg P ha^{-1}, applied as ammonium dihydrogen phosphate ($NH_4H_2PO_4$), and incorporated into the soil. To ensure nitrogen (N) would not limit growth, all pots received a total of 63.5 g N m^{-3}, a rate equivalent to 127 kg N ha^{-1}, supplied entirely as ammonium nitrate (NH_4NO_3) in the P-unfertilized treatment, and as a combination of NH_4NO_3 and the N present in the P-fertilized treatment. All fertilizer sources were thoroughly incorporated into each pot before planting. Inoculated pots received 1000 spores of *Rhizophagus irregularis* (VBSF-268), provided by Valent BioSciences Corp., IL, USA, suspended in 1 mL of water, while non-inoculated pots received 1 mL of distilled water at seedling transplant.

Plant Growth, Harvest, and Measurements

Seeds from all wheat genotypes were sown in trays filled with a mix of vermiculite and perlite (1:1) and placed in a vernalization chamber at 4 °C for eight weeks. During this period, trays were watered as needed before transplanting. Eight seedlings were transplanted into each pot and placed in a greenhouse, where natural light was supplemented with growth lights to provide 12 hours of light per day for the first two weeks and 16 hours per day from the third week until harvest, after approximately 14 weeks. To avoid nutrient deficiencies, plants were fertilized with 10 mL of a modified Hoagland's solution lacking P, a week after transplanting and at flowering (Hoagland & Arnon, 1938). All pots were irrigated daily and received the same amount of water. Irrigation frequency was adjusted based on the plant growth stage. Pots were supervised daily, and manual

weeding was conducted as needed. At both flowering (35 to 53 days after transplanting, varying by genotype) and physiological maturity (88 to 100 days after transplanting, varying by genotype), four plants per pot were randomly selected, harvested by cutting at the soil surface, and dried at 50 °C until constant weight. Dry aboveground biomass weight was recorded, samples were finely ground in a Wiley mill, digested in a nitropercloric solution (Gieseking et al., 1935), and P concentration was determined by an Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES) (Agilent 5800, Agilent Technologies, CA, USA).

Mycorrhizal Root Colonization Assessment

Roots from each plant were carefully removed at each harvest, washed, and preserved in 70% ethanol for subsequent assessment of AMF colonization. Root colonization by AMF was assessed using a modified staining protocol, substituting phenol with lactoglycerol (Phillips & Hayman, 1970). Root samples were first cleared in a 10% potassium hydroxide (KOH) solution for 12 h at room temperature, rinsed thoroughly under running water, and acidified in a 2% hydrochloric acid (HCl) solution for 10 min. Roots were then stained in a 0.05% trypan blue solution for 60 min at 60°C and stored in lactoglycerol. Hyphal and arbuscular AMF colonization were quantified using the gridline intersect method under a stereoscopic microscope (Giovannetti & Mosse, 1980).

Soil Processing

After harvest at maturity, all soil from the pots was thoroughly homogenized and separated for subsequent analysis. Soils for chemical analysis and enzyme activity were air-dried at room temperature, finely ground, and submitted for chemical analysis at the Kansas State University Soil Testing Laboratory. Soils for phospholipid and neutral lipid fatty acid analysis were homogenized and immediately stored at -20°C. Initial soil chemical characteristics were measured and are presented in Table 4.1.

Phospholipid and Neutral Lipid Fatty Acid Analysis

Phospholipid fatty acid (PLFA) and neutral lipid fatty acid (NLFA) analyses were conducted to detect shifts in microbial community populations in response to our treatments. Total lipids were extracted from 5 g of soil following a modified Bligh & Dyer, (1959) extraction procedure, as described by White & Rice, (2009). Briefly, soil samples were collected from each pot, immediately frozen, lyophilized, and finely ground using a mortar and pestle. The extraction of PLFAs was performed by incubating the soils in a solution of methanol, chloroform, and phosphate buffer (2:1:0.8 v/v/v). Following extraction, neutral and phospholipid fatty acids were separated from total lipids via preconditioned disposable silicic acid chromatography columns (BAKERBOND SPE Columns). Fatty acids were subsequently released from their glycerol backbones, and methylation was performed to produce fatty acid methyl esters (FAMEs). Analysis of the resulting FAMEs was carried out using a Thermo Scientific Trace GC-ISQ mass spectrometer (Thermo Fisher Scientific, Waltham, MA, USA) fitted with a DB5-MS analytical column (30 m × 250 μm internal diameter × 0.25 μm film thickness; Agilent Technologies, Santa Clara, CA, USA). Concentrations of fatty acid peaks were quantified relative to an internal standard, methyl nonadecanoate (19:0). Fatty acids were categorized into microbial groups as follows: Gram-Positive Bacteria (i15:0, a15:0, i16:0, i17:0, and a17:0), Gram-Negative Bacteria (2-OH 10:0, 2-OH 12:0, 3-OH 12:0, 2-OH 14:0, 3-OH 14:0, 16:1ω7c, cy17:0, cy19:0), Actinomycetes (10-Me 16:0 and 10-Me 18:0), Arbuscular Mycorrhizal Fungi (AMF; 16:1ω5), and Saprophytic Fungi (18:2ω6,9c). Total PLFA biomass was obtained by summing microbial groups and commonly measured fatty acids (11:0, 12:0, 13:0, 14:0, 15:0, 16:0, 17:0, 18:0, and 20:0) (Bonini Pires et al., 2020; Lin et al., 2023). Changes in microbial community composition can be

linked to shifts in soil functional potential, which can be further assessed through extracellular enzyme activities.

Extracellular Enzyme Activity

Three soil enzymes were analyzed colorimetrically using p-nitrophenyl-associated substrates, including β-glucosidase (Eivazi & Tabatabai, 1988) associated with the C cycle, acid phosphatase (Eivazi & Tabatabai, 1977; Tabatabai & Bremner, 1969) involved in the P cycle, and arylsulfatase (Tabatabai & Bremner, 1970) involved in the sulfur (S) cycle. Briefly, triplicates of 0.5 g of air-dried soil, sieved to 2 mm, were placed into separate 20-mL glass vials, each containing 2 mL of buffer. Two of the vials received 0.5 mL of a substrate linked to p-nitrophenyl, while the third vial acted as a control without the initial addition of the substrate. Capped vials were incubated at 37 ºC for 1 hour. After incubation, 0.5 mL of 0.5 M $CaCl_2$ solution and 2 mL of a stopping buffer were added to each vial. The control vial then received 0.5 mL of the p-nitrophenyl substrate. The solutions were filtered through Whatman 2V filter paper (9.0-cm diameter), and absorbance was measured at 400 nm using a Genesys 20 Visible Spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA).

Statistical Analysis

Treatment effects on plant and soil responses were analyzed using a linear mixed-effects model. A three-way ANOVA was conducted with AMF inoculation, P fertilization, and wheat genotype as fixed effects, including all interactions (AMF x Genotype x Fertilization, AMF × Genotype, Fertilization × Genotype, and AMF × Fertilization). Block was included as a random effect to account for spatial variability within the experimental design. Data were log or square root transformed when necessary to satisfy the assumptions of normality and variance. Individual treatment differences were examined using Tukey's honestly significant difference (HSD) multiple

comparisons test ($P \leq 0.05$). To improve clarity and interpretation, significant three-way interactions (AMF × Genotype × Fertilization) and main effects were presented in figures. Two-way interactions (AMF × Genotype, Fertilization × Genotype, and AMF × Fertilization) were presented in tables. All statistical analyses were conducted using R version 4.1.2 (R Core Team, 2026).

Results

AMF Root Colonization

Microscopic analysis of stained roots confirmed AMF root colonization, displaying classic mycorrhizal structures such as hyphae, arbuscules, and vesicles (Fig. 4.1). Hyphal colonization at flowering was significantly affected by the three-way interaction between P fertilization, AMF inoculation, and wheat genotype (Table 4.2). Byrd had a strong positive response to AMF inoculation under both fertilization treatments, with hyphal colonization increasing by 278% in fertilized soil and 30% in unfertilized soil (Fig. 4.2A). In contrast, SY Monument had a 127% increase in hyphal colonization with inoculation under fertilized conditions, but colonization decreased by 36% in unfertilized soil. KS Providence responded significantly to AMF inoculation only under unfertilized conditions, with an 81% increase in colonization. No other genotypes had significant responses to AMF inoculation at the flowering stage. Across genotypes, P fertilization significantly decreased AMF hyphal colonization regardless of inoculation, except in inoculated SY Monument, where P fertilization had no significant effect (Fig. 4.2A).

At maturity, hyphal colonization was significantly affected by the three-way interaction between P fertilization, AMF inoculation, and wheat genotype (Table 4.2). Bob Dole had a 42% increase in hyphal colonization with AMF inoculation under fertilized conditions (Fig. 4.2B). Similarly, Byrd responded positively to inoculation under fertilized conditions, increasing by

104%. In contrast, KS Providence had a significant reduction in colonization with inoculation under fertilized conditions, decreasing by 40%. No other genotypes had significant inoculation effects at maturity. Across genotypes, P fertilization decreased AMF hyphal colonization in the absence of inoculation for Bob Dole, Byrd, KanMark, and SY Monument. However, KS Providence and Turkey Red had an opposite trend, where AMF inoculation under high P levels reduced colonization compared to their uninoculated controls (Fig. 4.2B).

At flowering, arbuscular colonization was significantly affected by the three-way interaction between P fertilization, AMF inoculation, and wheat genotype (Table 4.2). Byrd responded positively to AMF inoculation under fertilized conditions, with colonization increasing by 276% (Fig. 4.2C). KS Providence responded to inoculation only under unfertilized conditions, with a 75% increase in arbuscular colonization. SY Monument had contrasting responses: under unfertilized conditions, AMF inoculation reduced arbuscular colonization by 47%, whereas under fertilized conditions, colonization increased by 85%. No other genotypes had significant inoculation effects at flowering. Across genotypes, P fertilization significantly reduced arbuscular colonization regardless of inoculation, except for SY Monument, where P fertilization did not decrease colonization in the inoculated treatment (Fig. 4.2C).

At maturity, arbuscular colonization was significantly affected by the interaction between P fertilization and wheat genotype (Table 4.2). Byrd had the highest overall colonization but decreased by 57% with fertilization (Table 4.4). SY Monument had a similar trend, with a 36% reduction in arbuscular colonization, while KS Providence decreased by 53% with fertilization. In contrast, Bob Dole, KanMark, and Turkey Red maintained consistently low arbuscular colonization (<10%) across both fertilization levels and did not exhibit significant differences.

Overall, P fertilization reduced arbuscule colonization for most genotypes, with the greatest absolute decrease observed in Byrd (Table 4.4).

Plant Growth and Grain Yield

Significant two-way interactions were detected between AMF inoculation and genotype, and between P fertilization and genotype, for aboveground biomass at flowering (Table 4.2). AMF inoculation increased flowering biomass of Bob Dole by 20%, of Byrd by 26%, and in Turkey Red by 27% compared with uninoculated plants (Table 4.3). In contrast, KanMark, KS Providence, and SY Monument were not significantly affected by AMF inoculation. Fertilization significantly increased aboveground biomass for all genotypes except Byrd. The largest relative increase occurred with Turkey Red, where biomass increased by 84%, producing more biomass than all other genotypes. KS Providence increased by 48%, KanMark by 79%, SY Monument by 25%, and Bob Dole by 28% compared with their unfertilized controls (Table 4.3).

A significant two-way interaction between AMF inoculation and genotype was also observed for aboveground biomass at maturity (Table 4.2). Mycorrhizal inoculation increased maturity biomass in Bob Dole by 54% compared with uninoculated plants, without significant responses in the other genotypes (Table 4.3). Turkey Red produced the highest biomass overall, but AMF inoculation did not significantly affect growth (Table 4.3).

Significant two-way interactions were observed for grain yield between AMF inoculation and genotype, and between P fertilization and genotype (Table 4.2). For the AMF and genotype interaction, inoculation increased yield for Bob Dole by 75% compared with uninoculated plants, while the other cultivars had no significant changes (Table 4.3). For the fertilization and genotype interaction, P fertilization increased yield for Bob Dole (34% increase), KanMark (46% increase), and Byrd (164% increase), with no significant differences for the remaining genotypes (Table 4.3).

Root biomass at maturity was significantly affected by the three-way interaction between P fertilization, AMF inoculation, and genotype (Table 4.2). Bob Dole had a 10-fold increase with AMF inoculation only when P fertilizer was present (Fig. 4.3A). Byrd was only significantly affected by inoculation under unfertilized conditions (4-fold increase), while inoculated KanMark pots had significantly higher root biomass under P fertilization (4-fold increase). In KS Providence, AMF inoculation increased root biomass in both fertilized and unfertilized soils (8 and 12-fold increase, respectively). SY Monument was not significantly affected by AMF inoculation. Turkey Red had a significant increase in root biomass without inoculation under unfertilized conditions (2-fold increase) and had the highest root biomass overall among all genotypes, averaging 2.49 g pot^{-1} in unfertilized soil and 3.84 g pot^{-1} in fertilized soil (Fig. 4.3A).

Root:shoot ratio at flowering was significantly influenced by the three-way interaction among P fertilization, AMF inoculation, and wheat genotype (Table 4.2). Bob Dole exhibited a significant response to AMF inoculation under fertilized conditions, with a 10-fold increase, while Byrd responded significantly under unfertilized conditions, with a 5-fold increase (Fig. 4.3B). KanMark also responded positively under fertilized conditions, with a 3-fold increase. KS Providence increased under both fertilization regimes, with a 12-fold increase under unfertilized conditions and a 9-fold increase under fertilized conditions. In contrast, the root:shoot ratio of Turkey Red was significantly lower under unfertilized conditions, with a 2-fold decrease. No other genotypes had a significant response to AMF inoculation (Fig. 4.3B).

Phosphorus Uptake

Uptake of P at flowering was significantly influenced by the three-way interaction between P fertilization, AMF inoculation, and genotype (Table 4.2). Mycorrhizal inoculation increased P uptake under unfertilized conditions by 61% for Bob Dole, 80% for Byrd, and 33% for Turkey

Red (Fig. 4.4A). No significant inoculation effects were observed for the remaining genotypes at flowering. Regardless of inoculation, P fertilization increased P uptake in all genotypes (Fig. 4.4A).

Uptake of P at maturity was significantly affected by the three-way interaction among P fertilization, AMF inoculation, and genotype (Table 4.2). For Bob Dole, AMF inoculation increased P uptake by 80% under unfertilized conditions, and by 65% under fertilized conditions (Fig. 4.4B). Byrd was significantly affected by inoculation only in unfertilized soil, with a 106% increase. Under fertilized conditions, AMF inoculation increased in P uptake for KS Providence by 123%, and by 102% in SY Monument. Turkey Red consistently had the highest P uptake across treatments. Regardless of inoculation, P fertilization increased P uptake in all genotypes (Fig. 4.4B).

Grain P uptake at maturity was significantly influenced by the two-way interaction between AMF inoculation and wheat genotype (Table 4.2). Inoculation increased grain P uptake by 69% for Bob Dole, and 30% for Byrd and KS Providence. In contrast, inoculation decreased grain P uptake by 22% for Turkey Red. No significant responses to inoculation were observed for KanMark or SY Monument (Table 4.4).

Soil Enzyme Activity and Rhizosphere Microbial Composition

All enzymes were significantly affected by wheat genotypes (Table 4.2). For acid phosphatase, KS Providence, Turkey Red, and Byrd had the highest activities and were not significantly different from each other (Fig. 4.5A). On the other hand, SY Monument and Bob Dole had the lowest acid phosphatase activity (Fig. 4.5A). For arylsulfatase, KS Providence had the highest activity and was significantly greater than all other genotypes, while SY Monument had the lowest activity (Fig. 4.5B). For betaglucosidase, KS Providence had the highest activity

and was significantly greater than all other genotypes, while SY Monument and Bob Dole had the lowest activities (Fig. 4.5C).

Total microbial biomass was significantly affected by wheat genotypes (Table 4.2). Similar to soil enzyme activity, KS Providence had the highest microbial biomass, followed by Turkey Red, while Bob Dole had the lowest value among all genotypes (Fig. 4.6A). Gram-positive bacteria, gram-negative bacteria, and actinomycete biomass were significantly influenced by wheat genotype (Table 4.2). Among the genotypes, KS Providence presented the highest biomass, followed by Turkey Red, while Bob Dole had the lowest biomass across all three microbial groups (Fig. 4.6B-D). Saprophytic fungal biomass was also significantly affected by wheat genotype (Table 4.2). KS Providence supported the highest fungal biomass, while all the other genotypes had lower and statistically similar values (Fig. 4.6E). Arbuscular mycorrhizal fungal biomass was significantly affected by wheat genotype (Table 4.2). In contrast to the other microbial groups, Turkey Red had the highest AMF biomass, which was substantially greater than all other genotypes (Fig. 4.6F). Byrd ranked second, while Bob Dole had the lowest AMF biomass (Fig. 4.6F).

Discussion

The plant and soil responses observed in this study advance our understanding of the soil–plant system and provide practical evidence on the potential for genotype-specific management strategies to optimize mycorrhizal associations and rhizosphere dynamics. Importantly, the soil used in this study is naturally abundant in native AMF propagules, as evidenced by high AMF root colonization in the uninoculated plants. Using non-sterile soils allows us to observe critical interactions between the inoculum and the native microbial community that cannot be observed in studies using sterilized soils or artificial media (Al-Khaliel, 2010). For instance, earlier studies

confirmed that wheat forms symbiotic associations with *Rhizophagus irregularis* in soils with extremely low initial AMF spore densities (Mohammad et al., 2004); however, far less information is available on inoculation performance in soils already rich in native AMF communities. Importantly, competition between indigenous and introduced species can negatively affect the host plant, as AMF tend to allocate resources toward competition for space and C, such as increasing intraradical mycelium and vesicle formation, rather than investing in nutrient-acquiring structures that benefit the plant, such as arbuscules and extraradical mycelium (Bennett & Bever, 2009; Janousková et al., 2013). Although AMF inoculation can enhance plant growth in inoculum-limited soils, these benefits cannot be readily extrapolated to soils where well-established native AMF communities are already present (Hart et al., 2018).

All genotypes were well colonized by AMF in this study, with root colonization responses differing markedly among wheat cultivars, indicating that host genetic background significantly influences colonization success. Large variation in AMF colonization among wheat cultivars has been previously reported (Elliott, 2019; Lehnert et al., 2017; Zhu et al., 2001), indicating genotype–fungus specificity. Supporting our hypothesis, P fertilization significantly reduced AMF hyphal and arbuscular colonization in most genotypes at flowering, with a lower effect at maturity, consistent with previous reports of reduced AMF colonization in wheat under high P (Nguyen et al., 2026). This P-induced suppression likely reflects a host-controlled reduction in C investment to fungal structures once direct P uptake meets nutritional demands (Konvalinková et al., 2017; Smith & Read, 2008).

The magnitude and direction of inoculation effects at each P level were highly genotype dependent, with Byrd maintaining higher colonization rates even under fertilization. This pattern indicates that plant genetic background modulates how P downregulates mycorrhizal associations

(Hetrick et al., 1996) and suggests that certain genetic combinations allow for sustained fungal associations regardless of nutrient context, an important trait for breeding cultivars that can consistently benefit from AMF under variable soil fertility conditions. Importantly, limited root colonization does not necessarily indicate weak mycorrhizal function, as the symbiosis may still effectively transfer nutrients to the host plant (Smith et al., 2004). Plant performance is also not simply a function of colonization intensity but depends on ecological context, including nutrient status, the native AMF community, and specific plant–fungus combinations (Fitter, 2006). Importantly, early-season assessment of mycorrhizal colonization is critical, since initial treatment effects may fade over time (Lekberg & Koide, 2005).

Our results demonstrate that wheat genotypes differ strongly in their responsiveness to AMF inoculation in terms of plant growth and nutrient uptake. While AMF inoculation enhanced plant growth and P uptake in select cultivars, such as Byrd, Bob Dole, and KS Providence, other genotypes showed minimal or even negative responses, demonstrating the genotype-specific nature of mycorrhizal inoculation responsiveness. Across genotypes, Byrd and Bob Dole were consistently the most responsive cultivars to AMF inoculation for plant growth and P uptake. Byrd carries a strong genetic contribution from *Aegilops tauschii*, a species previously associated with well-structured root systems and enhanced compatibility with AMF (Mohammedali et al., 2025; Wang et al., 2018). Well-structured root systems can potentially enhance chemical signaling that favors AMF recruitment, as wild relatives often retain mycorrhiza-favoring traits that have been reduced in modern genotypes (Boyno et al., 2023; Hetrick et al., 1993). Importantly, the D genome of *Aegilops tauschii* has been identified as the key driver of AMF colonization of its progeny hexaploid wheat lines (Hetrick et al., 1993). Previous studies have also reported that by integrating *Aegilops tauschii* into the genome, wheat cultivars may promote a microbiome rich in beneficial

Glomeromycetes fungi (Tkacz et al., 2020). Although higher root colonization was observed in Byrd under both P fertilization levels at flowering, increased colonization resulted in stronger gains in root biomass and phosphorus uptake, mainly under low-P conditions, supporting our hypothesis that AMF inoculation enhances plant growth and P uptake, particularly with limited P availability. These findings suggest that wild-derived genetic material may confer adaptive belowground traits that might have been eroded with breeding and disrupted plant-microbe interactions.

The cultivar Bob Dole, which carries both the GA-sensitive *Rht24* and *ALMT1* genes, responded positively to AMF inoculation in terms of plant growth, grain yield, P uptake, and root colonization at maturity. This enhanced AMF compatibility might be a result of *Rht24*, which encodes *TaGA2ox-A9*, a GA 2-oxidase that hydroxylates active GA into inactive forms, stabilizing DELLA proteins that are known for promoting efficient AMF symbiosis and arbuscule formation (Floss et al., 2013; Tian et al., 2022). To date, no published studies have evaluated AMF colonization or rhizosphere microbial dynamics in *Rht24* genotypes, thereby providing novel evidence that GA-sensitive dwarf cultivars might establish strong AMF associations. The cultivar KS Providence had positive responses to AMF inoculation at maturity in root biomass, root:shoot ratio, plant P uptake, and grain P uptake, despite variable responses in root colonization. This enhanced responsiveness may be attributed to its rye-derived genetic material, as previous studies comparing wheat, triticale, and rye demonstrated that rye has the highest mycorrhizal dependency and responsiveness to AMF inoculation (Pandey et al., 2005).

While modern wheat cultivars are bred for nutrient-abundant environments, wheat landraces are adapted to low-input environments (Scandurra et al., 2024). The landrace Turkey Red was included in this study to serve as the long-term control and consistently produced the highest aboveground and root biomass across all genotypes. The extensive root system of Turkey

Red may be a result of a mechanism for nutrient uptake adapted to low-nutrient environments, favoring broad soil exploration and a strong relationship with the native microbial community (Kelly et al., 2022). In fact, Turkey Red may rely more on its root system and well-established compatibility with native AMF than on added inoculum. Although inoculation initially increased plant growth and P uptake, these benefits did not persist through maturity, as root biomass, root:shoot ratio, and grain P uptake decreased. This pattern suggests that although landraces maintain strong compatibility with native AMF, their responsiveness to additional inoculation may be reduced because their baseline associations and acquisition strategies are already functional and optimized, and early benefits from inoculation are not always sustained through maturity. These findings align with previous work suggesting that root system architecture and genetic background strongly determine the degree of mycorrhizal dependency (Hetrick et al., 1992; Tawaraya, 2003).

Soil enzyme activity is an important indicator of soil biological functioning, as it reflects the metabolic potential of the microbial community and its capacity to mediate key processes that transform soil organic matter and release plant-available nutrients (Mendes et al., 2024; Tabatabai, 1994). Enzymes such as acid phosphatase, β-glucosidase, and arylsulfatase mediate nutrient cycling in the rhizosphere, and their activities often respond to changes in nutrient availability and AMF presence (Qin et al., 2020; Tabatabai, 1994). In parallel, PLFA and NLFA analyses are commonly used to assess microbial community structure and quantify AMF biomass, and are used as indicators of soil health, as they reflect the size and composition of the living microbial biomass and provide a snapshot of community structure (Frostegård et al., 2011; Lehman et al., 2015).

Partially supporting our hypothesis, soil microbial community composition and extracellular enzyme activity were driven primarily by genotype rather than by AMF inoculation or fertilization, indicating that plant genetic background strongly controls rhizosphere functional

processes, potentially via root exudation patterns or root architecture (Iannucci et al., 2021). KS Providence, Turkey Red, and Byrd consistently exhibited high acid phosphatase, arylsulfatase, and β-glucosidase activities, as well as greater abundance of several microbial groups, including total microbial biomass (PLFA) and soil AMF biomass, indicating that these genotypes supported a more biologically active rhizosphere with greater potential for nutrient cycling. In contrast to Campos et al., (2021), who observed reduced rhizosphere acid phosphatase activity following inoculation with *Rhizophagus intraradices* and *Claroideoglomus claroideum* in wheat cultivars, no significant effect of AMF inoculation on acid phosphatase activity was detected in this study, despite clear genotype differences. Genotypes that increase acid phosphatase activity in the rhizosphere enhance the mineralization of organic P pools, thereby improving P availability in soils where inorganic P is limited (Campos et al., 2018; Schmidt & Laskowski, 1961). By stimulating the mineralization of organic P compounds, these genotypes promote more efficient nutrient cycling at the root–soil interface, reducing dependence on external inputs such as P fertilizers (Campos et al., 2018).

KS Providence supported the highest overall microbial biomass (total microbial biomass, gram-positive and gram-negative bacteria, actinomycetes, and saprophytic fungi) and enzyme activity (acid phosphatase, arylsulfatase, and β-glucosidase), suggesting that the 1B.1R rye introgression enhances not only plant–fungus interactions but also broader microbial functions, likely through well-structured root systems and enhanced exudation patterns. For instance, previous studies have reported that the rye chromosome arm 1RS contains a genetic locus associated with increased root biomass in wheat and that rye translocation can increase both root biomass and root:shoot ratio in wheat plants (Ehdaie et al., 2003; Hoffmann, 2008). Jianzhong et al. (2001) reported that the rye chromosomes 1R and 7R carry genes associated with Pi starvation

tolerance and Pi starvation–inducible acid phosphatase expression and reported that 1R-containing plants developed larger root systems, higher root:shoot ratios, and greater acid phosphatase secretion capacity.

Byrd and Turkey Red also presented consistently higher rhizosphere responses, reinforcing the role of *Aegilops tauschii*-derived traits and landrace's extensive root systems in stimulating microbial activity. Wild relatives of wheat, such as *Aegilops tauschii*, and landraces are important sources of genetic diversity that have the potential to enhance wheat resilience and yield (Cavalet-Giorsa et al., 2024; Cheng et al., 2024). These wild-derived root traits and their genetic control offer promising opportunities to enhance belowground resilience and improve wheat performance (Wang et al., 2018). In contrast, modern cultivars without wild or rye-derived contributions generally supported lower microbial activity and biomass. Bob Dole and SY Monument consistently had some of the lowest microbial biomass and enzyme activities among the genotypes evaluated, despite the expectations that these genotypes would show greater rhizosphere stimulation. Since *ALMT1* mediates malate efflux, a labile C source that can stimulate soil microbial activity, we expected higher microbial biomass and extracellular enzyme activity in Bob Dole (Jones et al., 1996). These results suggest that the presence of *ALMT1* alone does not necessarily translate into enhanced rhizosphere microbial stimulation under the conditions of this study, and highlight a potential unintended consequence of domestication and breeding programs that prioritize aboveground traits such as yield, disease resistance, and reduced stature, possibly reducing genetic diversity in belowground traits and limiting beneficial plant–microbe interactions (Reif et al., 2005). These genotypes may have more conservative exudation profiles or root architectures that provide fewer resources for microbial proliferation or might have been bred under high-input conditions where strong mycorrhizal and microbial associations were not

favored. Studies by Hetrick et al. (1992) and Zhu et al. (2001) reported a progressive decline in wheat responsiveness to AMF with more recent cultivar releases, proposing that conventional breeding under high-fertility conditions has inadvertently reduced selection for mycorrhizal responsiveness, likely because the costs of maintaining the symbiosis outweigh its benefits in fertilized cropping systems.

Turkey Red had the highest AMF biomass (NLFA, 16:1ω5) among all genotypes, followed by Byrd, indicating a strong capacity to support AMF communities, likely via extensive roots and compatible exudation that favor AMF. Turkey Red's modest early growth and reduced grain yield responses to the inoculum reinforce the idea that a genotype can be highly mycorrhizal-associated while having limited additional benefit from inoculation when indigenous AMF communities are already active, and root systems are large. Overall, the dominance of genotype effects over fertilization and inoculation effects on rhizosphere metrics suggests that host plant genetic background is a primary driver of rhizosphere functional potential. In this native AMF-rich soil, functional redundancy or compensatory microbial processes may have masked inoculum-driven effects, highlighting the importance of studying AMF interactions in biologically active, unsterilized soils, where plant–microbe interactions are complex and competitive. Genotypes such as KS Providence and Byrd, which support high microbial biomass and enzyme activities, may be especially valuable for low-input or regenerative systems where biological nutrient cycling and soil health are prioritized. Future breeding strategies should combine favorable root architecture, mycorrhizal compatibility, and rhizosphere traits (e.g., exudation patterns that stimulate AMF presence and enzyme activity) to develop cultivars optimized for enhanced nutrient use efficiency, improved soil biological activity, and greater productivity under reduced fertilizer inputs.

Conclusions

This study demonstrated that wheat genotype strongly regulated plant responses to AMF inoculation and P fertilization, with marked differences in plant growth, P uptake, and rhizosphere dynamics. Together, our results provide practical evidence of the importance of considering plant-microbe interactions and rhizosphere traits as selectable characteristics in wheat breeding programs. Agronomic benefits from AMF bioinoculants may be enhanced when matched with wheat genotypes that are both AMF-compatible and not already operating near their maximum P acquisition potential via native symbioses or adapted root traits. The responsiveness of Byrd and KS Providence suggests that incorporating genetic contributions from wild relatives or rye can enhance AMF compatibility, microbial activity, root growth, and grain P acquisition in wheat. Turkey Red's strong baseline AMF associations show the potential of landraces as reservoirs of beneficial root–microbe traits. By contrast, the weaker soil responses of some modern cultivars suggest that decades of selection for aboveground productivity may have reduced the ability of wheat to recruit and sustain beneficial microbial communities. Our study provides novel evidence that wheat genotypes differ not only in their direct responsiveness to AMF inoculation but also in their capacity to shape soil microbial structure and function. We suggest that the root microbiome should be taken into consideration during the plant breeding process, as it is a determinant for several microbial ecosystem services and microbial interactions that are usually overlooked. Integrating these belowground traits into breeding programs could enhance nutrient use efficiency, soil health, and plant resilience, contributing to more sustainable wheat production systems.

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Tables

Table 4.1. Selected soil properties for the soil used in the study.

Phosphorus Mehlich-3 (mg kg $^{-1}$)	pH	Organic Matter (g kg^{-1})	NO_3^- (mg kg^{-1})	NH_4^- (mg kg^{-1})	K (mg kg^{-1})	CEC (cmol$^+$ kg^{-1})	Zn (mg kg^{-1})	Ca (mg kg^{-1})	Mg (mg kg^{-1})	Na (mg kg^{-1})	Fe (mg kg^{-1})	Mn (mg kg^{-1})	Cu (mg kg^{-1})
13	6.6	27	50.5	8.4	269	18.4	0.8	2403	252	12	38	38	0.9

Note: Soil pH – determined by 1:1 soil slurry (Watson & Brown, 1998); P- extracted by Mehlich-3 (Mehlich, 1984); K, Ca, Mg, and Na – extracted by Ammonium Acetate (Warncke & Brown, 1998); Fe, Zn, Cu, and Mn – extracted by DTPA (Whitney, 1998); NO_3^- and NH_4^- – extracted by KCl (Keeney & Nelson, 1982); Organic matter - determined by Loss on ignition (Combs & Nathan, 1998); Cation Exchange Capacity (CEC) – determined by Summation (Warncke & Brown, 1998). Abbreviation: CEC, cation exchange capacity.

Table 4.2. ANOVA *P*-value summary table of the effects of arbuscular mycorrhizal fungal inoculation (A), phosphorus fertilization (F), wheat genotype (G), and their interactions in all response variables.

	A	F	G	A x F	A x G	F x G	A x F x G
Aboveground Biomass at Flowering	**0.007**	**0.003**	**<0.001**	0.182	**0.002**	**0.002**	0.314
Aboveground Biomass at Maturity	**<0.001**	**<0.001**	**<0.001**	0.324	**0.004**	0.056	0.611
Grain Yield	**<0.001**	**0.006**	**<0.001**	0.149	**0.002**	**0.016**	0.290
Root Biomass at Maturity	0.898	0.052	**<0.001**	**0.014**	**<0.001**	**0.031**	**0.032**
Root:Shoot Ratio	0.196	**<0.001**	**<0.001**	**0.004**	**<0.001**	**0.005**	**0.007**
Phosphorus Uptake at Flowering	**<0.001**	**<0.001**	**<0.001**	**0.010**	**<0.001**	0.097	**0.035**
Phosphorus Uptake at Maturity	**0.017**	**<0.001**	**<0.001**	0.872	**0.007**	**0.001**	**0.031**
Grain Phosphorus Uptake	**0.004**	**0.007**	**<0.001**	0.324	**0.013**	0.110	0.638
AMF Hyphal Colonization at Flowering	0.384	**<0.001**	**0.001**	0.614	**0.003**	**0.014**	**0.026**
AMF Arbuscular Colonization at Flowering	0.166	**<0.001**	**<0.001**	0.326	**<0.001**	**<0.001**	**<0.001**
AMF Hyphal Colonization at Maturity	0.982	**0.002**	**<0.001**	0.212	0.778	**<0.001**	**0.023**
AMF Arbuscular Colonization at Maturity	0.726	0.605	**<0.001**	0.487	0.849	**0.001**	0.422
Acid Phosphatase	0.317	0.518	**0.035**	0.187	0.798	0.064	0.274
Arylsulfatase	0.758	0.682	**<0.001**	0.987	0.936	0.935	0.702
Betaglucosidase	0.829	0.766	**<0.001**	0.584	0.939	0.976	0.915
Total Microbial Biomass	0.738	0.932	**<0.001**	0.474	0.767	0.744	0.566
Gram Positive Bacteria	0.730	0.998	**<0.001**	0.637	0.742	0.809	0.617
Gram Negative Bacteria	0.505	0.514	**<0.001**	0.238	0.836	0.513	0.631
Actinomycetes	0.569	0.241	**<0.001**	0.747	0.680	0.553	0.641
Arbuscular Mycorrhizal Fungi	0.894	0.064	**<0.001**	0.837	0.277	0.940	0.468
Saprophytic Fungi	0.958	0.961	**0.042**	0.223	0.860	0.587	0.482

Table 4.3. Summary of selected plant growth metrics from the two-way interaction between phosphorus fertilization and wheat genotypes (F x G) and AMF inoculation and wheat genotypes (A x G), in alphabetical order. Values are mean ± standard error (n=4); Means followed by the same letter were not significantly different at the $P<0.05$ level (Tukey's HSD); Lowercase letters indicate Tukey groupings for AMF × Genotype and uppercase letters for Fertilization × Genotype. No letters present indicate no pairwise difference. Full details of statistical analysis given in Table 4.1.

		Aboveground Biomass at Flowering (g pot^{-1})		Aboveground Biomass at Maturity (g pot^{-1})		Grain Yield at Maturity (g pot^{-1})	
		Absent	Present	Absent	Present	Absent	Present
Bob Dole	AMF Inoculation	1.24 ± 0.37^{f}	1.49 ± 0.20^{de}	2.31 ± 0.88^{e}	3.55 ± 1.05^{cd}	0.73 ± 0.25^{e}	1.28 ± 0.38^{cd}
Byrd		1.32 ± 0.29^{ef}	1.66 ± 0.36^{cd}	3.45 ± 0.97^{cd}	3.71 ± 1.32^{cd}	1.23 ± 0.49^{d}	1.33 ± 0.75^{d}
KanMark		1.76 ± 0.54^{cd}	1.64 ± 0.75^{d}	3.64 ± 0.66^{cd}	3.91 ± 0.86^{bc}	1.49 ± 0.33^{abc}	1.60 ± 0.47^{ab}
KS Providence		1.60 ± 0.35^{cd}	1.75 ± 0.42^{cd}	3.26 ± 0.83^{d}	3.67 ± 0.39^{cd}	1.37 ± 0.28^{bcd}	1.48 ± 0.24^{abc}
SY Monument		1.77 ± 0.18^{c}	1.74 ± 0.35^{cd}	4.28 ± 0.58^{b}	4.38 ± 0.69^{b}	1.58 ± 0.30^{ab}	1.64 ± 0.28^{ab}
Turkey Red		3.73 ± 1.36^{b}	4.73 ± 1.51^{a}	6.15 ± 1.54^{a}	5.94 ± 0.79^{a}	1.77 ± 0.42^{a}	1.52 ± 0.32^{ab}
		Absent	Present	Absent	Present	Absent	Present
Bob Dole	Phosphorus Fertilization	1.20 ± 0.28^{E}	1.53 ± 0.28^{D}	2.26 ± 0.77	3.60 ± 1.06	0.86 ± 0.43^{F}	1.15 ± 0.39^{E}
Byrd		1.45 ± 0.38^{D}	1.53 ± 0.36^{D}	2.66 ± 0.48	4.50 ± 0.73	0.66 ± 0.17^{F}	1.74 ± 0.32^{AB}
KanMark		1.22 ± 0.22^{E}	2.18 ± 0.54^{C}	3.19 ± 0.14	4.36 ± 0.65	1.26 ± 0.21^{DE}	1.84 ± 0.31^{A}
KS Providence		1.35 ± 0.18^{DE}	2.00 ± 0.20^{C}	3.19 ± 0.56	3.74 ± 0.67	1.37 ± 0.28^{CDE}	1.48 ± 0.25^{BCD}
SY Monument		1.56 ± 0.21^{D}	1.95 ± 0.17^{C}	4.04 ± 0.43	4.61 ± 0.67	1.59 ± 0.27^{ABC}	1.63 ± 0.31^{ABC}
Turkey Red		2.98 ± 0.87^{B}	5.47 ± 0.64^{A}	5.36 ± 0.91	6.73 ± 1.06	1.58 ± 0.29^{ABC}	1.71 ± 0.47^{AB}

Table 4.4. Summary of arbuscular colonization at maturity and grain phosphorus uptake from the two-way interaction between phosphorus fertilization and wheat genotypes (F x G) and AMF inoculation and wheat genotypes (A x G), in alphabetical order. Values are mean ± standard error (n=4); Means followed by the same letter were not significantly different at the $P<0.05$ level (Tukey's HSD); Lowercase letters indicate Tukey groupings for AMF × Genotype and uppercase letters for Fertilization × Genotype. No letters present indicate no pairwise difference. Full details of statistical analysis given in Table 4.1.

		Arbuscular Colonization at Maturity (%)		Grain Phosphorus Uptake at Maturity (mg pot^{-1})	
		Absent	Present	Absent	Present
Bob Dole	AMF Inoculation	5.16 ± 1.52	4.68 ± 1.20	4.65 ± 2.27^{e}	7.84 ± 2.79^{bc}
Byrd		42.5 ± 10.3	52.5 ± 5.10	5.93 ± 2.48^{de}	7.69 ± 3.93^{bc}
KanMark		1.74 ± 0.89	3.56 ± 1.51	7.79 ± 1.90^{bc}	7.49 ± 1.92^{bcd}
KS Providence		23.6 ± 7.82	13.4 ± 3.73	6.83 ± 1.35^{cd}	8.89 ± 3.46^{b}
SY Monument		28.1 ± 9.29	25.3 ± 3.19	8.12 ± 1.79^{bc}	8.42 ± 1.88^{bc}
Turkey Red		1.63 ± 0.40	1.70 ± 0.57	11.4 ± 2.66^{a}	8.92 ± 1.79^{b}
		Absent	Present	Absent	Present
Bob Dole	Phosphorus Fertilization	4.50 ± 1.17^{EF}	5.34 ± 1.53 ^{EF}	4.80 ± 2.57	7.69 ± 2.71
Byrd		66.3 ± 3.07^{A}	28.7 ± 5.37^{BC}	4.29 ± 2.06	9.34 ± 2.12
KanMark		3.00 ± 0.92^{F}	2.34 ± 1.57^{F}	6.35 ± 1.01	8.93 ± 1.59
KS Providence		25.1 ± 7.46^{BC}	11.8 ± 3.75^{DE}	7.09 ± 2.47	8.64 ± 2.95
SY Monument		32.6 ± 7.77^{B}	20.8 ± 5.16^{CD}	7.39 ± 1.73	9.15 ± 1.43
Turkey Red		1.85 ± 0.53^{F}	1.5 ± 0.44^{F}	9.91 ± 2.30	10.4 ± 2.90

Figures

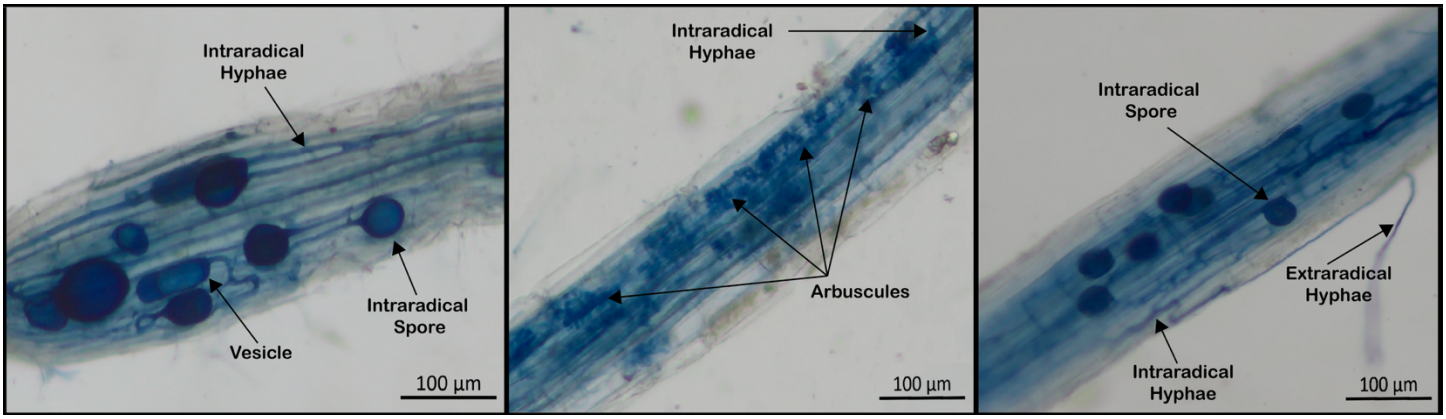


Figure 4.1. Stained roots of wheat (*Triticum aestivum*) genotype Byrd at flowering colonized by the arbuscular mycorrhizal fungus (AMF) *Rhizophagus irregularis* in the inoculated treatment. Arrows indicate characteristic AMF structures, including intraradical hyphae, extraradical hyphae, intraradical spore, vesicle, and arbuscule.

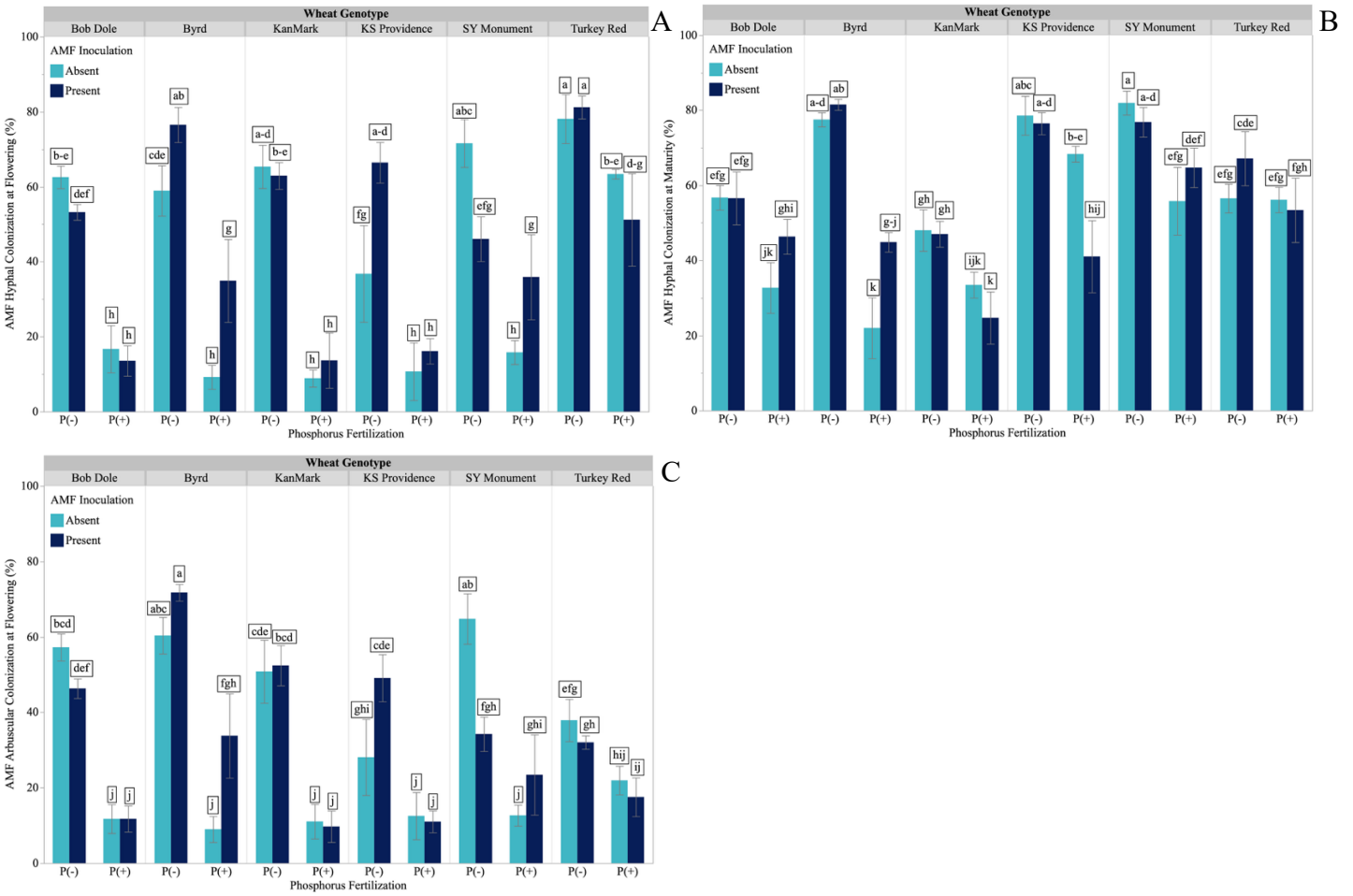


Figure 4.2. AMF hyphal colonization at flowering (A) and maturity (B), and AMF arbuscular colonization at flowering (C) for six wheat (*Triticum aestivum* L.) genotypes with (dark blue bars) and without (light blue bars) arbuscular mycorrhizal fungi (AMF) inoculation under two phosphorus (P) fertilization levels (0 and 50 g P m^{-3}, x-axis). Values are means of four replications. Bars are mean ±standard error. Different letters above the bars indicate significant differences between treatment means by the Tukey test ($P < 0.05$).

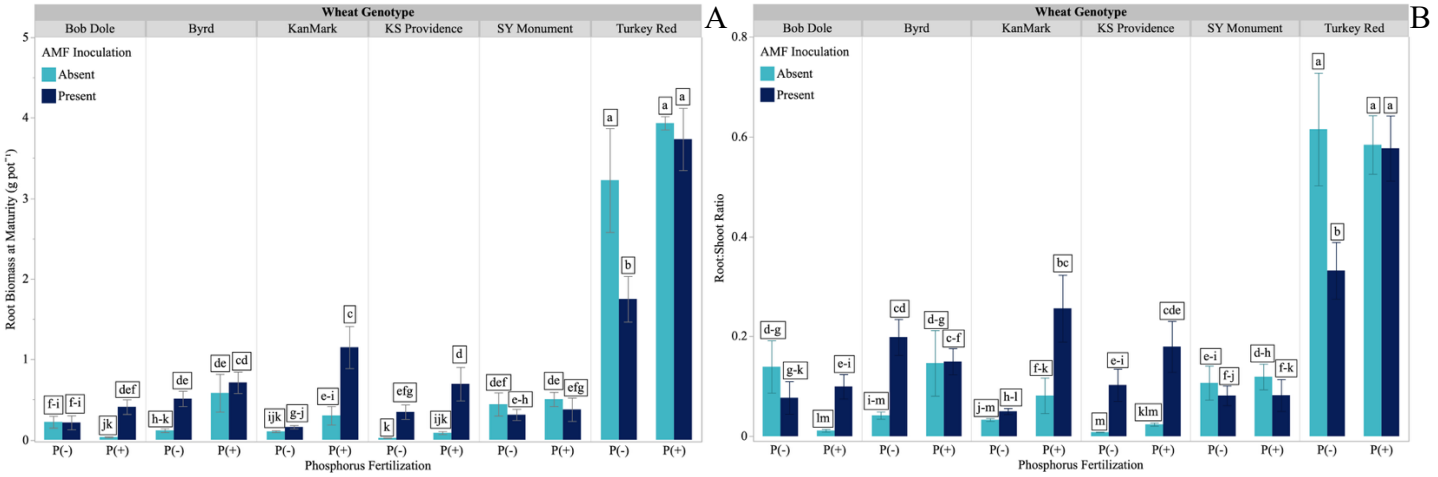


Figure 4.3. Root biomass (A) and root:shoot ratio (B) at maturity for six wheat (*Triticum aestivum* L.) genotypes with (dark blue bars) and without (light blue bars) arbuscular mycorrhizal fungi (AMF) inoculation under two phosphorus (P) fertilization levels (0 and 50 g P m^{-3}, x-axis). Values are means of four replications. Bars are mean ±standard error. Different letters above the bars indicate significant differences between treatment means by the Tukey test ($P < 0.05$).

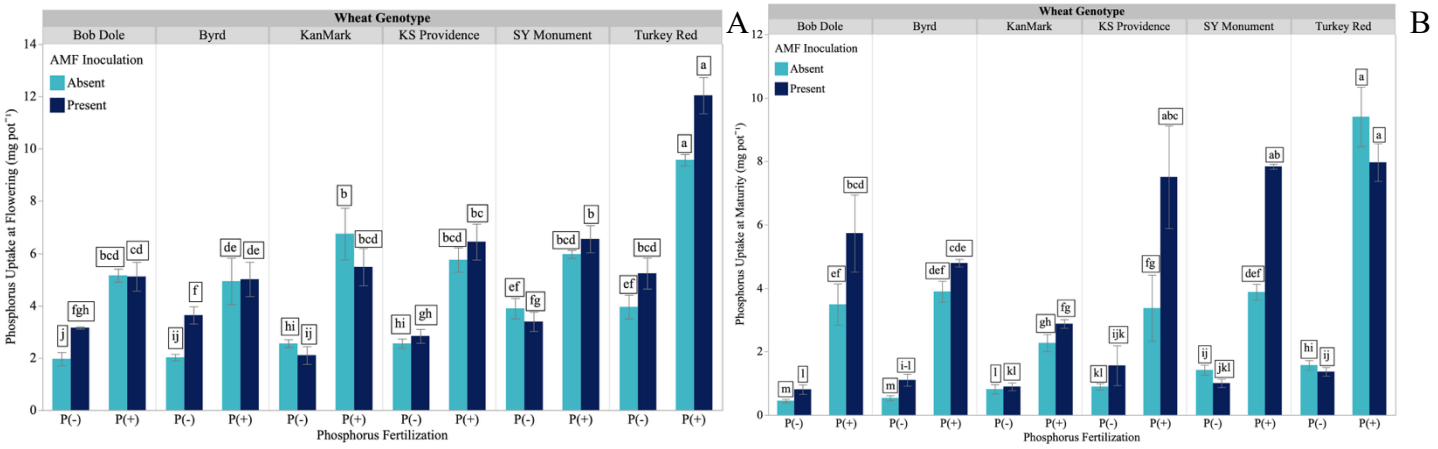


Figure 4.4. Phosphorus uptake at flowering (A) and maturity (B) for six wheat (*Triticum aestivum* L.) genotypes with (dark blue bars) and without (light blue bars) arbuscular mycorrhizal fungi (AMF) inoculation under two phosphorus (P) fertilization levels (0 and 50 g P m^{-3}, x-axis). Values are means of four replications. Bars are mean ±standard error. Different letters above the bars indicate significant differences between treatment means by the Tukey test ($P < 0.05$).

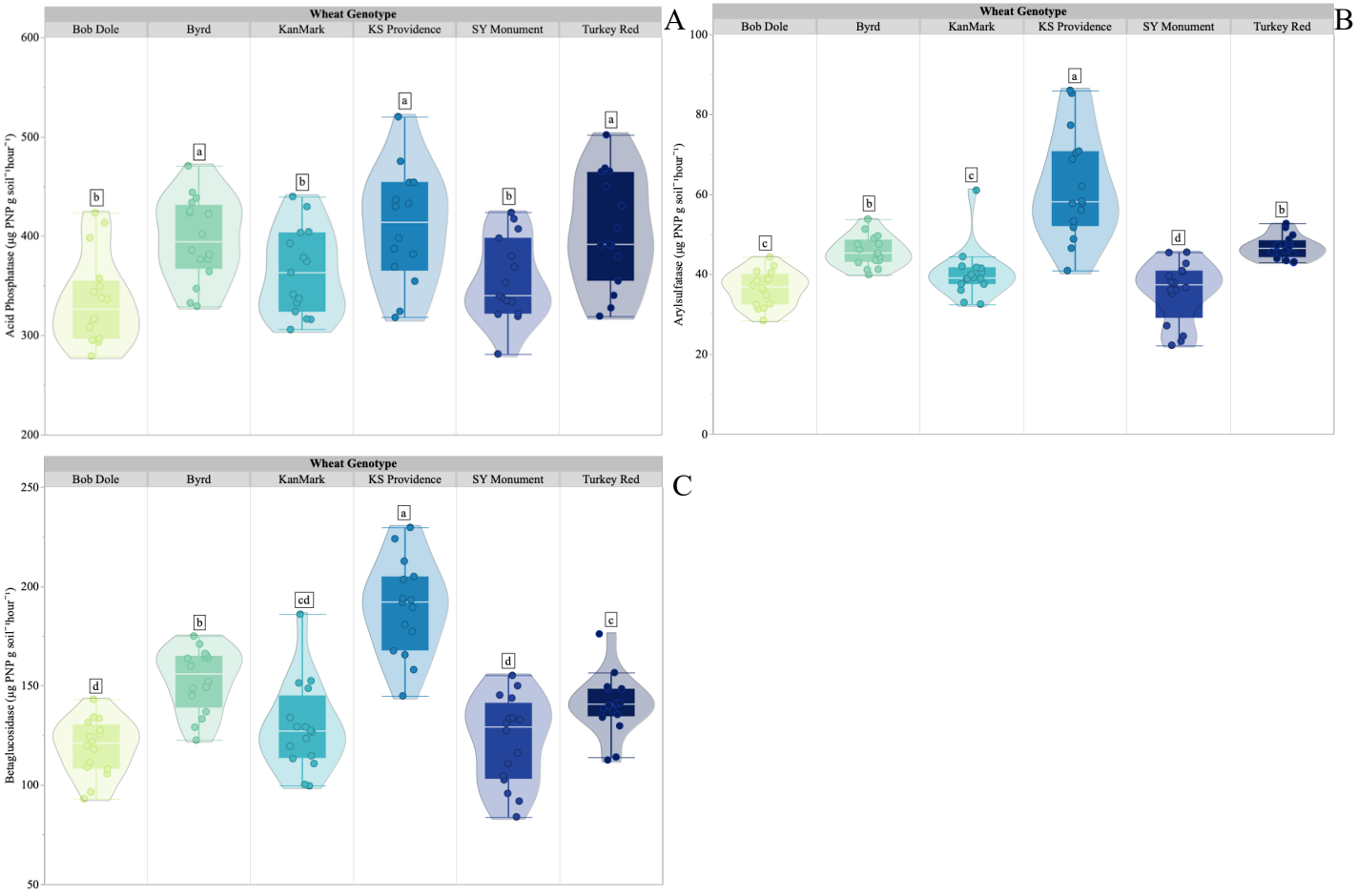


Figure 4.5. Acid phosphatase (A), arylsulfatase (B), and β-glucosidase (C) in soils at maturity for six wheat (*Triticum aestivum* L.) genotypes. Values are means of four replications averaged over both arbuscular mycorrhizal fungi (AMF) inoculation treatments (presence and absence) and two phosphorus (P) fertilization levels (0 and 50 g P m^{-3}). Violin plots illustrate the density distribution of the data, with overlaid box plots representing the median (horizontal line) and interquartile range. Individual points represent raw observations. Different letters above the boxplots represent significant differences between treatment means by the Tukey test ($P < 0.05$).

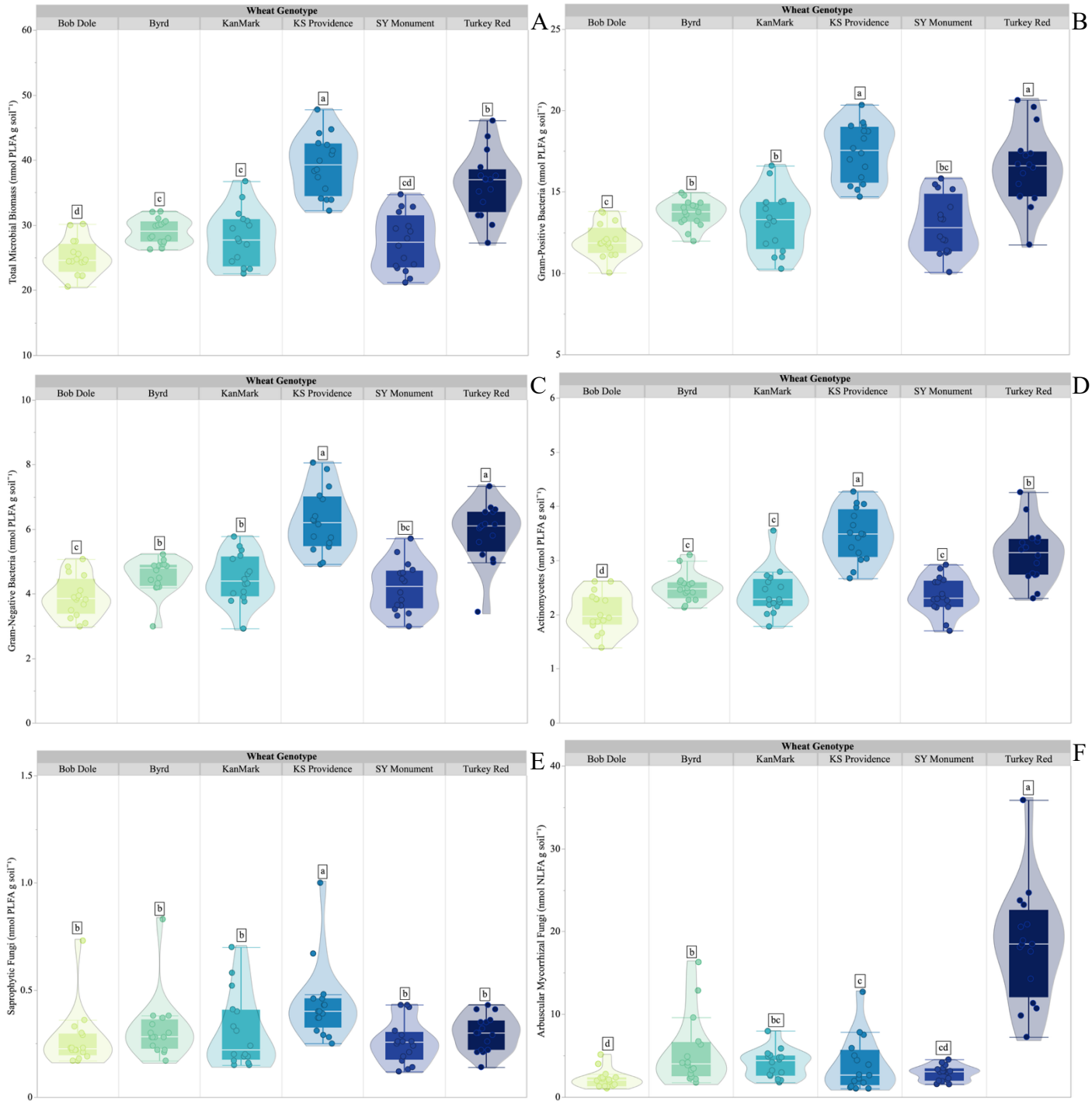


Figure 4.6. Total microbial biomass (A), gram-positive bacteria (B), gram-negative bacteria (C), actinomycetes (D), saprophytic fungi (E), and arbuscular mycorrhizal fungi (F) in soils at maturity for six wheat (*Triticum aestivum* L.) genotypes. Values are means of four replications averaged over both arbuscular mycorrhizal fungi (AMF) inoculation treatments (presence and absence) and two phosphorus (P) fertilization levels (0 and 50 g P m^{-3}). Violin plots illustrate the density distribution of the data, with overlaid box plots representing the median (horizontal line) and interquartile range. Individual points represent raw observations. Different letters above the boxplots represent significant differences between treatment means by the Tukey test ($P < 0.05$).

Chapter 5 - Conclusions and Future Research

The main goal of this dissertation was to evaluate the effects of AMF inoculation and P fertilization on crop performance and soil health under irrigated and dryland field conditions, and to determine how wheat genotype influences AMF inoculation responsiveness under contrasting P levels. By evaluating the effects of AMF inoculation across multiple growing seasons under contrasting soil P levels and environmental conditions, as well as wheat genotype responses under greenhouse conditions, this dissertation provides comprehensive insights into the potential benefits and limitations of AMF bioinoculants in agricultural systems. Overall, our findings demonstrate that the effectiveness of AMF inoculation is highly context-dependent and that understanding the complex interactions between introduced AMF, indigenous microbial communities, soil P, and plant genetic background is essential to developing more effective and reliable bioinoculation strategies.

Chapter 2 evaluated the effects of AMF inoculation and P fertilization on crop productivity, P uptake, and soil health under irrigated corn-soybean rotations in fields with very low initial soil P levels. Across four growing seasons, P fertilization was a stronger and more consistent regulator of plant and soil responses than AMF inoculation, increasing plant biomass, P uptake, grain yield and soil P availability, while progressively reducing AMF root colonization and soil abundance. In contrast, the most consistent benefits of AMF inoculation were associated with improved P uptake, biomass production, apparent P recovery efficiency, and the formation of large water-stable aggregates, while responses in grain yield, soil microbial communities, extracellular enzyme activities, and soil organic carbon stocks were generally absent, limited or inconsistent. Importantly, inoculation benefits were more evident under low P availability and during the first corn years, and they decreased as soil P accumulated over time. Ultimately, our findings provide

practical evidence that the effectiveness of AMF bioinoculants depends strongly on soil P levels and suggest that maintaining an appropriate balance between P fertilization and AMF function is important for maximizing the benefits of mycorrhizal symbiosis. Furthermore, the variability in responses across years and cropping systems reinforces the importance of evaluating bioinoculants over multiple growing seasons under field conditions, rather than relying exclusively on short-term responses or controlled-environment studies.

Chapter 3 investigated the effects of AMF inoculation across a P gradient under dryland continuous corn, at a site where initial soil P availability was near levels considered sufficient for corn production, a common scenario for farmers considering commercial AMF bioinoculants. For the dryland site, AMF inoculation responses varied among years, P rates, and growth stages, with no consistent improvements in plant growth, P uptake, or soil health indicators. Soil P was a stronger and more consistent regulator of AMF root colonization and soil abundance than inoculation, with both decreasing as soil P increased. The greatest agronomic benefits of inoculation occurred in 2023, when increased root colonization, P uptake, and grain yield coincided with the highest precipitation between V6 and R1, a critical period for corn P demand, suggesting that precipitation timing may influence inoculation effectiveness, although this relationship requires further investigation. Inoculation generally had limited effects on most soil biological, chemical, and physical properties, and effects varied by year and depth. Therefore, understanding how management practices, including cover cropping and proper nutrient management, influence indigenous AMF communities may help identify opportunities to support mycorrhizal networks and improve the effectiveness of AMF bioinoculants under dryland conditions.

Chapter 4 investigated how wheat genetic background influences plant responses to AMF inoculation and P fertilization in a greenhouse study using six wheat genotypes with distinct root traits and the potential to influence the soil microbiome. Our findings demonstrated that wheat genotype strongly regulated AMF responsiveness, with significant differences in root colonization, plant growth, P uptake, and rhizosphere microbial dynamics among genotypes. Wheat genotype also strongly influenced rhizosphere microbial communities and extracellular enzyme activities. Some genotypes, particularly Byrd and KS Providence, which contain wild and rye-derived genetic contributions, had greater responsiveness to inoculation, while others such as the landrace Turkey Red, maintained strong associations with AMF regardless of inoculation and supported greater AMF abundance in the rhizosphere. Therefore, our study provided evidence that plant genetic background is an important factor determining the potential benefits of AMF bioinoculants and that differences in root traits and rhizosphere processes may influence the establishment and functioning of mycorrhizal associations, reinforcing the importance of considering AMF compatibility and plant–microbe interactions during the development of new wheat cultivars. Thus, incorporating belowground traits into plant breeding programs could improve nutrient acquisition and soil health while reducing dependence on external inputs.

Importantly, the effectiveness of AMF bioinoculants cannot be fully understood without considering their interactions with indigenous microbial communities. Under field conditions, introduced AMF must compete and interact with microorganisms already adapted to local soil conditions, which can potentially limit their establishment and effectiveness. Their complex interactions are often overlooked in controlled-environment studies, which may help explain why positive responses observed under these conditions are not consistently reproduced in the field, especially when evaluated with sterilized soils or artificial substrates. Additionally, the genotype-

dependent responses observed in Chapter 4 emphasize the importance of plant genetic background and AMF compatibility in determining inoculation outcomes. Therefore, developing more effective bioinoculants requires a better understanding of the interactions that govern the establishment and function of introduced AMF.

The benefits of AMF inoculation are highly dependent on soil P levels, indigenous microbial communities, plant genetic background, and potentially environmental conditions. Furthermore, the contrasting responses between irrigated and dryland conditions suggest that environmental factors, especially water availability, may influence inoculant performance, while established indigenous AMF communities may also limit the additional benefits of introduced fungi. Agricultural management practices such as no-till, cover cropping, diversified crop rotations, and appropriate fertilizer management may help support indigenous AMF communities and create conditions that favor the establishment and effectiveness of introduced AMF. Under favorable conditions, AMF inoculation may provide important benefits, particularly in P-deficient soils and when introduced fungi are compatible with the host plant and local environment. Identifying these conditions is essential for improving the effectiveness and agronomic value of AMF bioinoculants in agricultural systems.

Future Research

Despite the important questions addressed in this dissertation, further research is needed to better understand the factors controlling AMF bioinoculant establishment, persistence, and effectiveness under diverse field conditions. Future research should prioritize long term field experiments across contrasting soil fertility levels, cropping systems, and environmental conditions, as well as understanding the complex interactions between introduced AMF and indigenous microbial communities. To date, little is known about introduced fungi establishment

and persistence in agricultural soils, their long-term ecological consequences, and whether they provide greater benefits to host plants than indigenous AMF communities. Additionally, more research is needed to determine how agricultural management practices can support indigenous AMF communities while maintaining crop productivity without relying on external propagules.

Future studies should also investigate how plant genetic background influences AMF responsiveness on other important crops, such as corn, soybean, and sorghum. Evaluating more crop species and genotypes, particularly under field conditions, could help identify plant traits associated with greater AMF compatibility and nutrient acquisition in other major agricultural crops. Therefore, wild relatives, landraces, and highly mycorrhizal crop genotypes may serve as important genetic resources for breeding cultivars with greater AMF responsiveness and improved rhizosphere microbial function.

Overall, future research should translate these findings into practical guidance on when and where AMF inoculation is likely to be beneficial and how it can complement management practices that support indigenous mycorrhizal communities, improving their reliability and agronomic value under field conditions. Such guidance would align bioinoculant use with the conditions most likely to support its success, improving the reliability and agronomic value of AMF bioinoculants in sustainable agricultural systems.

Appendix A - Chapter 2

Table A.1. Baseline soil physicochemical properties by soil depth (cm) at the experimental fields in Scandia, KS.

	Experimental Field	Phosphorus Mehlich-3 (mg kg $^{-1}$)	pH	Sikora pH	Ca (mg kg^{-1})	Mg (mg kg^{-1})	Na (mg kg^{-1})	K (mg kg^{-1})	Soil Organic Carbon (g C kg^{-1} soil)	Total Soil Nitrogen (mg N kg^{-1} soil)	Sand (%)	Silt (%)	Clay (%)
0-5		4.54	6.54	7.08	1692	308	35	467	15.2	14.2	26	62	13
5-15	Corn-Soybean	2.66	5.92	6.84	1677	305	35	301	10.2	10.1	24	58	18
15-30		1.33	6.19	6.88	2709	519	47	246	10.5	9.47	22	59	20
0-5		7.50	6.08	7.13	1705	303	57	429	18.7	22.1	24	63	13
5-15	Soybean-Corn	5.25	6.30	7.18	1631	281	43	314	12.1	17.6	22	58	20
15-30		2.50	6.00	6.98	2149	389	54	243	12.5	13.4	22	56	23

Note: Soil pH – determined by 1:1 soil slurry (Watson & Brown, 1998); P- extracted by Mehlich-3 (Mehlich, 1984); Ca, Mg, Na, and K – extracted by Ammonium Acetate (Warncke & Brown, 1998); Soil C and N - measured by dry combustion (Bremner & Mulvaney, 1982; Nelson & Sommers, 1983).

Table A.2. Cumulative precipitation and irrigation water inputs (mm) across key corn development stages (VE–V6, V6–R1, and R1–R6) in Scandia, KS (2022–2025).

Corn Development Stages				
Year	**VE-V6**	**V6-R1**	**R1-R6**	**Event**
2022	118	74	138	Precipitation
	0	63.5	159	Irrigation
Total	118	138	297	
All-Season Total			553	
2023	62.5	68.1	262	Precipitation
	0	95.3	191	Irrigation
Total	62.5	163	452	
All-Season Total			678	
2024	66.5	58.9	57.7	Precipitation
	0	159	127	Irrigation
Total	66.5	218	185	
All-Season Total			470	
2025	110	236	87.1	Precipitation
	0	0	191	Irrigation
Total	110	236	278	
All-Season Total			624	

Table A.3. Field operational calendar for corn (*Zea mays* L.), including planting and harvest dates, hybrid selection, and seeding rates across four growing seasons (2022–2025) at Scandia, KS.

Location	Year	Planting and Harvesting Dates	Hybrids	Seeding Rate (seeds ha^{-1})
Scandia	2022	April 26th - September 19th	P1289AM	74,132
	2023	May 17th - October 10th	P1289AM	74,152
	2024	May 29th - October 7th	P1633AM	74,152
	2025	May 5th - September 30th	P1511AM	74,152

Table A.4. Field operational calendar for soybean (*Glycine max*), including planting and harvest dates, cultivar selection, and seeding rates across four growing seasons (2022–2025) at Scandia, KS.

Location	Year	Planting and Harvesting Dates	Cultivar	Seeding Rate (seeds ha^{-1})
Scandia	2022	June 13 - October 11	GH3728X	355,800
	2023	June 16 - October 30	AG33XF2	343,489
	2024	May 15 - October 10	GH3883XF	308,000
	2025	May 6 - October 9	GH3883XF	319,400

Table A.5. Field operational calendar for the cover crop wheat (*Triticum aestivum* L.), including planting and termination dates, cultivar selection, and seeding rates across four growing seasons (2022–2025) at Scandia, KS.

Location	Planting and Termination Dates	Cultivar	Seeding Rate (kg ha^{-1})
Scandia	December 1, 2022- June 7, 2023	KS Western Star	112.1
	November 17, 2023 - May 10, 2024	KS Ahearn	67.3
	November 17, 2024 - May 6, 2025	KS Mako	112.1
	November 14, 2025 - May 4, 2026	KS Mako	112.1

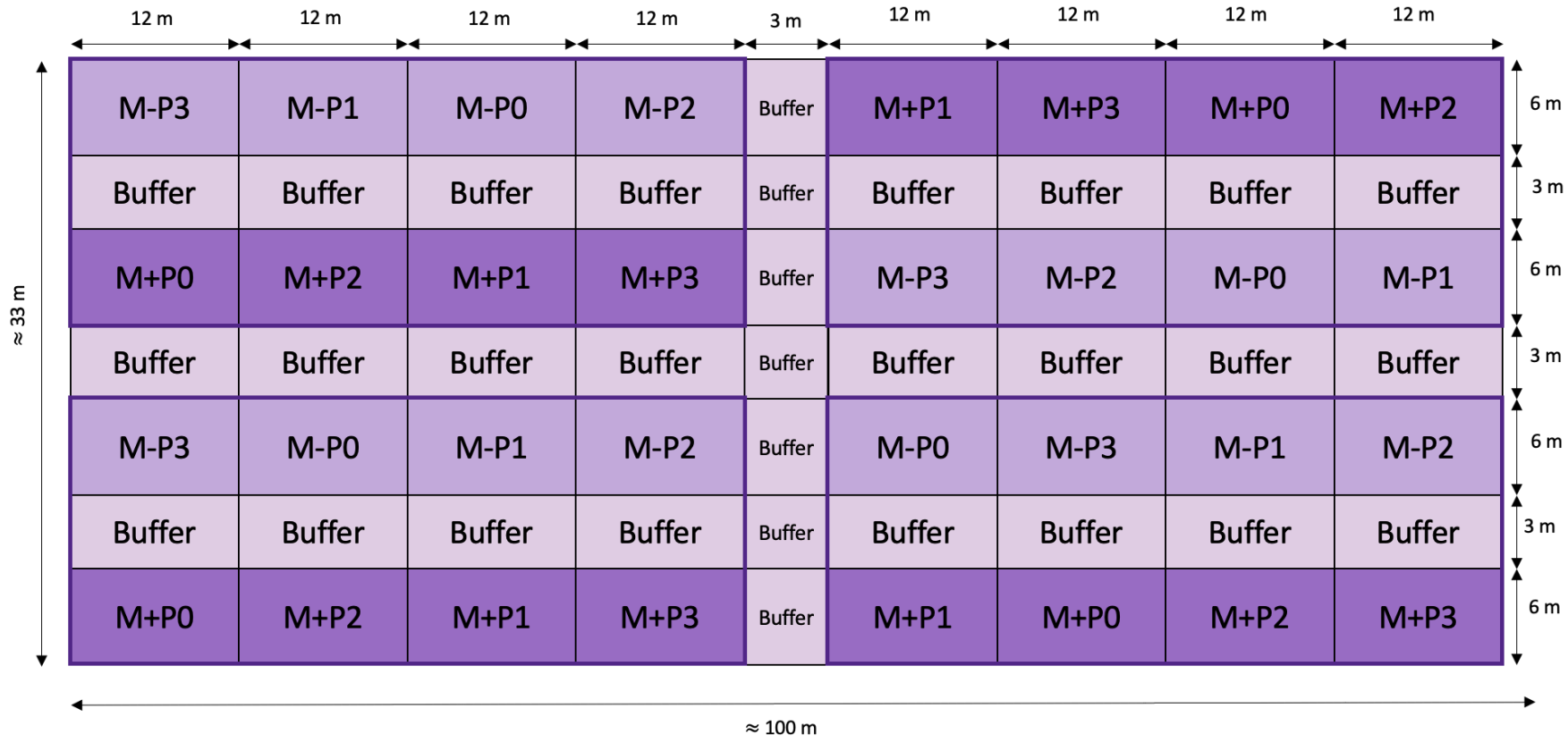


Figure A.1. Experimental layout of the split-plot randomized complete block design (RCBD). Mycorrhizal inoculation was assigned as the main-plot factor, with inoculated (M+) and non-inoculated (M−) treatments, and phosphorus (P) fertilization rates were assigned as subplots: P0 = 0, P1 = 15, P2 = 30, and P3 = 60 kg P ha^{-1}. Buffer areas (3 m) separated plots to minimize potential movement of mycorrhizal inoculum among treatments.

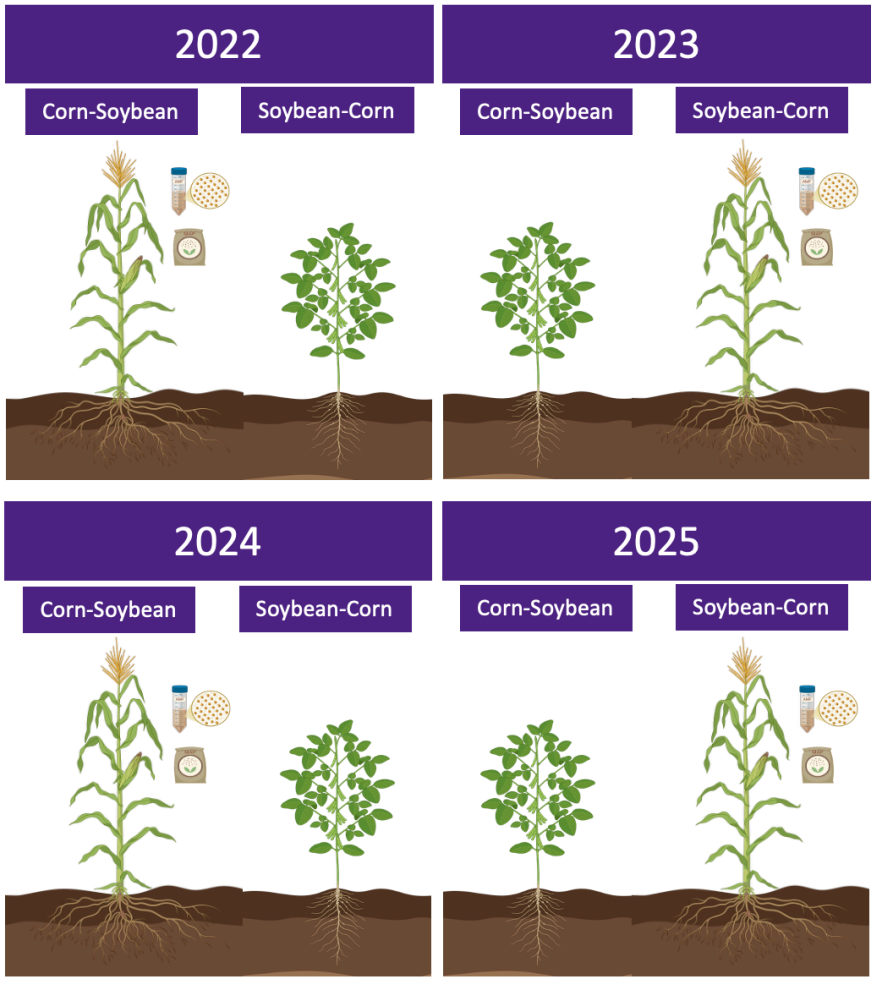


Figure A.2. Schematic representation of the annual crop sequence and treatment application in the Corn–Soybean and Soybean–Corn rotations at Scandia, KS, from 2022 to 2025. Corn and soybean were grown in alternating years in the two cropping systems. Phosphorus (P) fertilization and arbuscular mycorrhizal fungi (AMF) inoculation treatments were applied only during corn-growing years. Consequently, each cropping system received two applications of the P fertilization and AMF inoculation treatments during the four-year period.

Table A.6. ANOVA P-value summary table of the effects of phosphorus fertilization (P), arbuscular mycorrhizal fungi inoculation (F), and their interactions on all plant response variables.

2022			
	P	**F**	**P x F**
Aboveground Biomass at V6	**0.035**	0.373	0.988
Aboveground Biomass at R1	**0.001**	**0.002**	0.078
Aboveground Biomass at R6	**<0.001**	**0.002**	**0.042**
Phosphorus Uptake at V6	**0.070**	**0.029**	0.221
Phosphorus Uptake at R1	**<0.001**	**0.022**	**0.006**
Total Phosphorus Uptake at R6	**0.002**	0.988	0.446
Grain Yield	**0.036**	0.705	0.449
AMF Root Colonization at V6	0.129	0.240	0.615
AMF Root Colonization at R1	0.230	0.852	0.361
AMF Root Colonization at R6	0.307	0.941	0.225
Apparent Phosphorus Recovery Efficiency	0.756	**0.006**	**0.007**
2023			
Aboveground Biomass at V6	**<0.001**	0.325	0.823
Aboveground Biomass at R1	**<0.001**	**0.026**	0.487
Aboveground Biomass at R6	**<0.001**	0.148	**<0.001**
Phosphorus Uptake at V6	**<0.001**	0.425	0.456
Phosphorus Uptake at R1	**<0.001**	0.085	**0.046**
Total Phosphorus Uptake at R6	**<0.001**	0.881	0.833
Grain Yield	**<0.001**	0.721	0.093
AMF Root Colonization at V6	0.566	**0.040**	0.365
AMF Root Colonization at R1	**<0.001**	0.319	0.096
AMF Root Colonization at R6	0.504	0.236	0.220
Apparent Phosphorus Recovery Efficiency	**0.012**	**0.002**	**0.034**
2024			
Aboveground Biomass at V6	0.714	0.670	0.902
Aboveground Biomass at R1	**0.006**	0.489	0.595
Aboveground Biomass at R6	**<0.001**	0.981	0.340
Phosphorus Uptake at V6	**<0.001**	0.989	0.500
Phosphorus Uptake at R1	**<0.001**	0.439	0.212
Total Phosphorus Uptake at R6	**<0.001**	0.334	0.280
Grain Yield	**<0.001**	0.829	0.159
AMF Root Colonization at V6	**0.003**	**0.003**	**0.018**
AMF Root Colonization at R1	**0.021**	0.509	**0.034**
AMF Root Colonization at R6	**<0.001**	0.060	0.147
Apparent Phosphorus Recovery Efficiency	**0.027**	0.557	0.483

2025			
Aboveground Biomass at V6	**<0.001**	**0.016**	0.306
Aboveground Biomass at R1	**<0.001**	0.056	**0.027**
Aboveground Biomass at R6	0.555	0.605	0.885
Phosphorus Uptake at V6	**<0.001**	0.423	0.846
Phosphorus Uptake at R1	**<0.001**	0.822	0.494
Total Phosphorus Uptake at R6	**<0.001**	0.444	0.823
Grain Yield	**<0.001**	0.260	0.940
AMF Root Colonization at V6	**0.004**	**0.002**	**<0.001**
AMF Root Colonization at R1	0.186	0.073	**0.018**
AMF Root Colonization at R6	**0.014**	0.564	0.921
Apparent Phosphorus Recovery Efficiency	**<0.001**	0.670	0.740

Table A.7. Summary of P-values from pairwise contrasts between arbuscular mycorrhizal fungi (AMF)-inoculated and non-inoculated treatments at each phosphorus (P) fertilization rate at the Corn-Soybean rotation across years when significant P × AMF interactions were detected.

Response	Year	Phosphorus Rate (kg P ha^{-1})	P-Value
Phosphorus Uptake at R1	2022	0	0.113
	2022	15	0.208
	2022	30	0.030
	2022	60	0.034
Aboveground Biomass at R6	2022	0	0.009
	2022	15	0.188
	2022	30	0.793
	2022	60	0.729
Phosphorus Uptake at R1	2023	0	0.112
	2023	15	0.498
	2023	30	0.628
	2023	60	0.006
Aboveground Biomass at R6	2023	0	0.164
	2023	15	0.182
	2023	30	0.013
	2023	60	0.000
AMF Root Colonization at V6	2024	0	0.008
	2024	15	0.310
	2024	30	0.472
	2024	60	0.448
AMF Root Colonization at R1	2024	0	0.551
	2024	15	0.230
	2024	30	0.037
	2024	60	0.050
AMF Root Colonization at V6	2025	0	0.006
	2025	15	0.008
	2025	30	0.460
	2025	60	0.028
AMF Root Colonization at R1	2025	0	0.089
	2025	15	0.126
	2025	30	0.018
	2025	60	0.144

Table A.8. ANOVA *P*-value summary table of the effects of phosphorus fertilization (P), arbuscular mycorrhizal fungi inoculation (F), over the years (Y), and their interactions on all response variables of the Corn-Soybean Rotation in Scandia, KS.

0-5 cm							
	P	**F**	**Y**	**P x F**	**P x Y**	**F x Y**	**P x F x Y**
Soil Carbon Stock	0.442	0.560	**<0.001**	**0.008**	0.294	0.478	0.078
Soil Total Nitrogen	0.745	0.798	**<0.001**	**0.016**	0.469	0.998	0.556
Soil pH	**<0.001**	0.549	**<0.001**	0.115	**<0.001**	0.883	0.806
Soil Phosphorus	**<0.001**	0.729	**<0.001**	0.813	**<0.001**	0.555	0.272
Soil Macroaggregates (>2mm)	0.109	0.973	**<0.001**	0.529	**0.006**	0.863	**0.020**
Soil Macroaggregates (2-0.250mm)	0.088	0.076	**<0.001**	0.131	**<0.001**	0.379	**0.018**
Betaglucosidase	**0.012**	0.664	**<0.001**	0.866	0.814	0.146	0.963
Acid Phosphatase	**<0.001**	0.765	**<0.001**	0.685	**0.024**	0.445	0.964
Arylsulfatase	**0.012**	0.780	**<0.001**	0.969	0.961	0.645	0.944
N-acetyl-β-glucosaminidase	0.232	0.672	**<0.001**	0.929	0.983	0.805	0.507
Total Microbial Biomass	0.529	0.844	**0.002**	0.252	0.140	0.187	0.295
Gram-Positive Bacteria	0.479	0.432	**0.010**	0.351	0.620	0.524	0.714
Gram-Negative Bacteria	0.859	0.455	**0.001**	0.274	0.611	0.468	0.332
Actinomycetes	**0.016**	0.149	**<0.001**	0.085	**0.032**	0.508	0.608
Saprophytic Fungi	0.694	0.069	**<0.001**	0.506	0.989	0.362	0.699
Arbuscular Mycorrhizal Fungi	**<0.001**	0.256	**<0.001**	0.517	**<0.001**	0.090	**0.029**
5-15 cm							
	P	**F**	**Y**	**P x F**	**P x Y**	**F x Y**	**P x F x Y**
Soil Carbon Stock	**0.007**	0.202	**<0.001**	0.060	0.285	0.707	0.153
Soil Total Nitrogen	0.071	0.052	**<0.001**	0.323	0.687	0.255	0.461
Soil pH	0.390	0.458	**<0.001**	0.612	0.911	0.137	0.923
Soil Phosphorus	**<0.001**	0.911	**<0.001**	0.894	**0.042**	0.173	0.837
Soil Macroaggregates (>2mm)	**0.002**	0.133	**<0.001**	**<0.001**	0.465	**0.008**	0.427
Soil Macroaggregates (2-0.250mm)	0.724	0.350	**<0.001**	0.792	**0.039**	0.696	0.287

Betaglucosidase	0.565	0.953	**<0.001**	0.314	0.218	0.752	0.056
Acid Phosphatase	0.274	0.120	**<0.001**	0.132	0.596	0.872	0.178
Arylsulfatase	0.688	0.743	**<0.001**	0.583	0.979	0.927	0.795
N-acetyl-β-glucosaminidase	0.459	0.486	**<0.001**	0.529	0.327	0.296	0.755
Total Microbial Biomass	0.762	0.202	**<0.001**	0.102	**0.010**	**0.029**	0.618
Gram-Positive Bacteria	0.991	**0.043**	**<0.001**	0.247	**0.021**	**0.001**	0.801
Gram-Negative Bacteria	0.216	0.067	**<0.001**	0.569	0.430	0.459	0.798
Actinomycetes	0.663	0.230	**<0.001**	0.587	0.539	0.550	0.779
Saprophytic Fungi	0.565	0.347	**<0.001**	0.138	0.810	0.192	0.519
Arbuscular Mycorrhizal Fungi	**<0.001**	0.115	**<0.001**	0.677	**0.038**	0.853	0.159

15-30 cm

	P	**F**	**Y**	**P x F**	**P x Y**	**F x Y**	**P x F x Y**
Soil Carbon Stock	**0.017**	0.167	**0.030**	0.433	**<0.001**	0.721	0.270
Soil Total Nitrogen	0.161	0.074	**<0.001**	0.341	0.107	0.443	0.860
Soil pH	0.479	0.666	**<0.001**	0.839	0.938	0.436	0.889
Soil Phosphorus	**0.026**	0.971	**<0.001**	0.636	**0.008**	0.313	0.235
Soil Macroaggregates (>2mm)	**0.021**	0.193	**<0.001**	0.563	0.731	0.186	0.491
Soil Macroaggregates (2-0.250mm)	0.670	0.225	0.142	0.826	0.122	0.051	0.134
Betaglucosidase	0.396	0.286	**<0.001**	0.371	0.970	0.614	0.225
Acid Phosphatase	0.265	0.406	**<0.001**	0.319	0.721	0.949	0.529
Arylsulfatase	0.295	0.362	**<0.001**	0.625	0.837	0.907	0.539
N-acetyl-β-glucosaminidase	0.514	0.815	**<0.001**	0.772	0.968	0.895	0.813
Total Microbial Biomass	0.660	0.060	**<0.001**	0.445	0.915	0.380	0.544
Gram-Positive Bacteria	0.348	0.117	**<0.001**	0.365	0.702	0.249	0.510
Gram-Negative Bacteria	0.698	0.077	**<0.001**	0.328	0.988	0.774	0.758
Actinomycetes	0.810	0.107	**<0.001**	0.581	0.835	0.526	0.963
Saprophytic Fungi	0.386	0.822	**<0.001**	0.054	0.153	**0.044**	0.559
Arbuscular Mycorrhizal Fungi	**0.016**	0.256	**<0.001**	0.962	**0.001**	0.660	0.444

Table A.9. ANOVA *P*-value summary table of the effects of phosphorus fertilization (P), arbuscular mycorrhizal fungi inoculation (F), over the years (Y), and their interactions on all response variables of the Soybean-Corn Rotation in Scandia, KS.

0-5 cm							
	P	**F**	**Y**	**P x F**	**P x Y**	**F x Y**	**P x F x Y**
Soil Carbon Stock	0.376	0.494	**<0.001**	0.853	0.835	**0.038**	0.087
Soil Total Nitrogen	0.618	0.410	**<0.001**	0.978	0.711	**0.033**	0.363
Soil pH	**<0.001**	0.838	**<0.001**	0.915	**0.004**	0.763	0.184
Soil Phosphorus	**<0.001**	0.583	**<0.001**	0.743	**0.001**	0.932	0.442
Soil Macroaggregates (>2mm)	**0.002**	**<0.001**	**0.009**	0.103	0.456	0.667	0.467
Soil Macroaggregates (2-0.250mm)	**0.019**	0.115	0.576	**0.046**	0.365	0.845	0.379
Betaglucosidase	0.482	0.345	**<0.001**	0.509	0.474	0.918	0.186
Acid Phosphatase	0.058	0.148	**<0.001**	0.587	0.845	0.405	0.404
Arylsulfatase	**0.002**	0.546	**<0.001**	0.565	0.281	**0.034**	0.608
N-acetyl-β-glucosaminidase	0.434	0.877	**<0.001**	0.946	0.480	0.708	0.318
Total Microbial Biomass	0.531	0.921	0.691	0.647	0.792	0.179	0.774
Gram-Positive Bacteria	0.615	0.730	0.375	0.600	0.682	0.099	0.711
Gram-Negative Bacteria	0.423	0.861	0.378	0.621	0.811	0.817	0.851
Actinomycetes	0.900	0.930	0.231	0.829	0.895	0.228	0.796
Saprophytic Fungi	0.066	0.504	**0.002**	0.402	0.547	0.166	0.490
Arbuscular Mycorrhizal Fungi	**<0.001**	0.805	**<0.001**	0.288	**<0.001**	0.293	0.924
5-15 cm							
	P	**F**	**Y**	**P x F**	**P x Y**	**F x Y**	**P x F x Y**
Soil Carbon Stock	0.206	0.264	**<0.001**	0.880	0.091	0.104	0.446
Soil Total Nitrogen	0.059	0.372	**<0.001**	0.862	0.174	0.424	0.511
Soil pH	0.699	0.552	**0.001**	0.098	0.930	0.350	0.532
Soil Phosphorus	**<0.001**	0.259	**<0.001**	0.685	**0.006**	0.244	0.296
Soil Macroaggregates (>2mm)	**<0.001**	0.785	**<0.001**	0.461	**0.044**	0.384	0.565
Soil Macroaggregates (2-0.250mm)	**<0.001**	0.427	**0.010**	0.900	0.950	0.452	0.196

Betaglucosidase	0.905	0.909	**<0.001**	0.521	0.618	0.828	0.552
Acid Phosphatase	0.744	**0.021**	0.247	0.619	0.171	0.128	0.067
Arylsulfatase	0.450	0.615	**<0.001**	0.159	0.095	0.912	0.344
N-acetyl-β-glucosaminidase	0.313	0.970	0.432	0.281	0.182	0.637	0.989
Total Microbial Biomass	0.831	0.906	0.101	0.948	0.079	0.114	0.416
Gram-Positive Bacteria	**0.017**	0.415	0.328	0.584	0.154	0.393	0.544
Gram-Negative Bacteria	0.238	0.309	0.163	0.663	0.067	0.414	0.564
Actinomycetes	0.157	0.415	0.213	0.832	0.086	0.608	0.134
Saprophytic Fungi	0.530	0.708	**<0.001**	0.850	0.062	0.266	0.909
Arbuscular Mycorrhizal Fungi	**<0.001**	0.286	**<0.001**	0.741	**<0.001**	0.847	0.292

15-30 cm

	P	**F**	**Y**	**P x F**	**P x Y**	**F x Y**	**P x F x Y**
Soil Carbon Stock	0.468	0.418	**<0.001**	0.640	**0.028**	0.648	0.155
Soil Total Nitrogen	**0.040**	**0.046**	0.629	0.628	**0.011**	0.159	0.624
Soil pH	0.693	**0.050**	**<0.001**	0.159	0.366	0.254	0.165
Soil Phosphorus	**0.002**	0.122	**<0.001**	0.055	**0.009**	0.622	0.355
Soil Macroaggregates (>2mm)	0.062	0.374	**<0.001**	0.824	0.106	**0.026**	0.977
Soil Macroaggregates (2-0.250mm)	0.518	0.094	0.546	0.817	0.858	0.462	0.477
Betaglucosidase	0.583	**0.012**	**<0.001**	0.524	0.501	**0.006**	0.323
Acid Phosphatase	0.603	**0.030**	**<0.001**	0.339	0.709	0.416	0.820
Arylsulfatase	0.446	0.219	**<0.001**	0.358	0.451	0.158	0.287
N-acetyl-β-glucosaminidase	0.178	**0.022**	**<0.001**	0.749	0.859	0.060	0.971
Total Microbial Biomass	0.715	**0.041**	**<0.001**	0.577	0.278	0.432	0.359
Gram-Positive Bacteria	0.621	0.082	**<0.001**	0.536	0.297	0.983	0.304
Gram-Negative Bacteria	0.373	0.033	**0.005**	0.692	0.355	0.189	0.295
Actinomycetes	0.798	0.096	**<0.001**	0.765	0.335	0.211	0.692
Saprophytic Fungi	0.320	0.114	0.494	0.607	0.175	0.387	0.220
Arbuscular Mycorrhizal Fungi	0.449	0.249	**<0.001**	0.405	**<0.001**	0.346	0.080

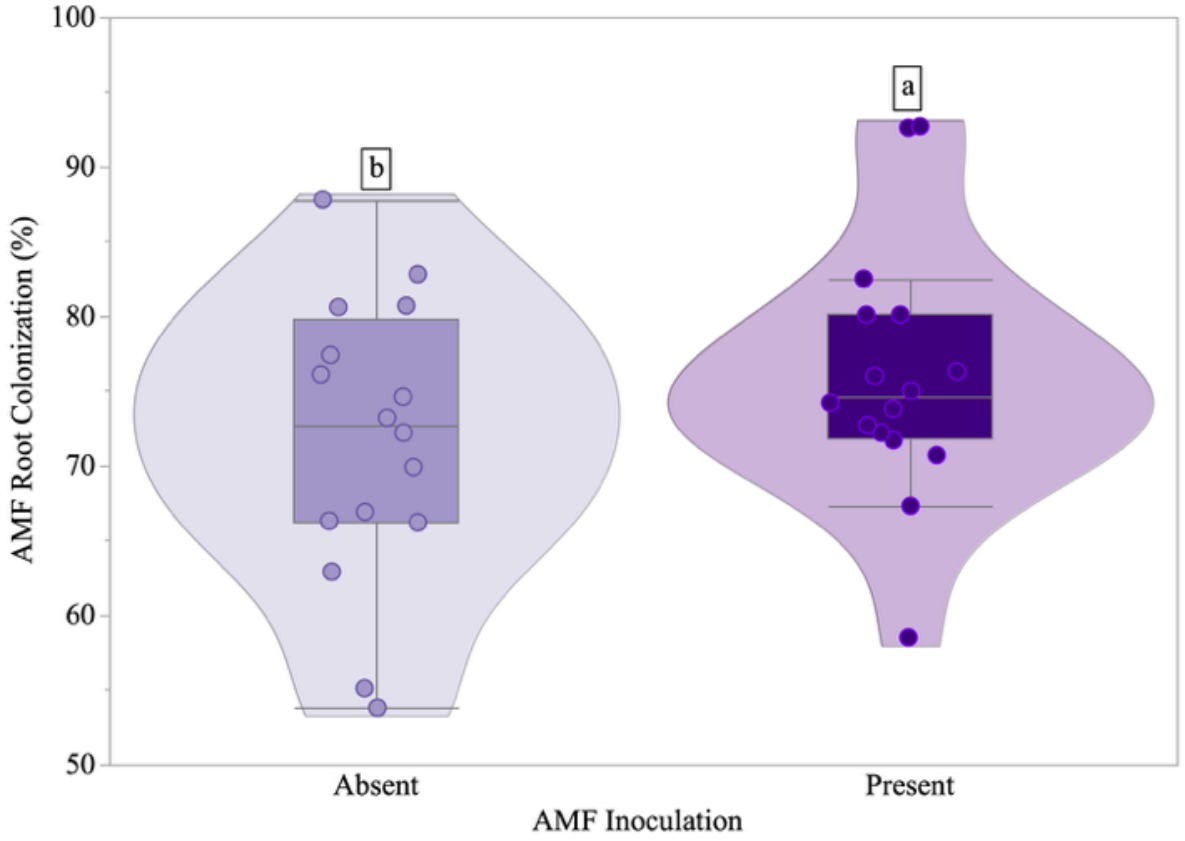


Figure A.3. Effects of arbuscular mycorrhizal fungi (AMF) inoculation on AMF root colonization (%) at V6 in 2023 at the Soybean–Corn rotation. Violin plots illustrate the density distribution of the data, with overlaid box plots representing the median (horizontal line) and interquartile range. Individual points represent raw field observations. Different letters indicate significant differences between AMF inoculation treatments ($P < 0.05$).

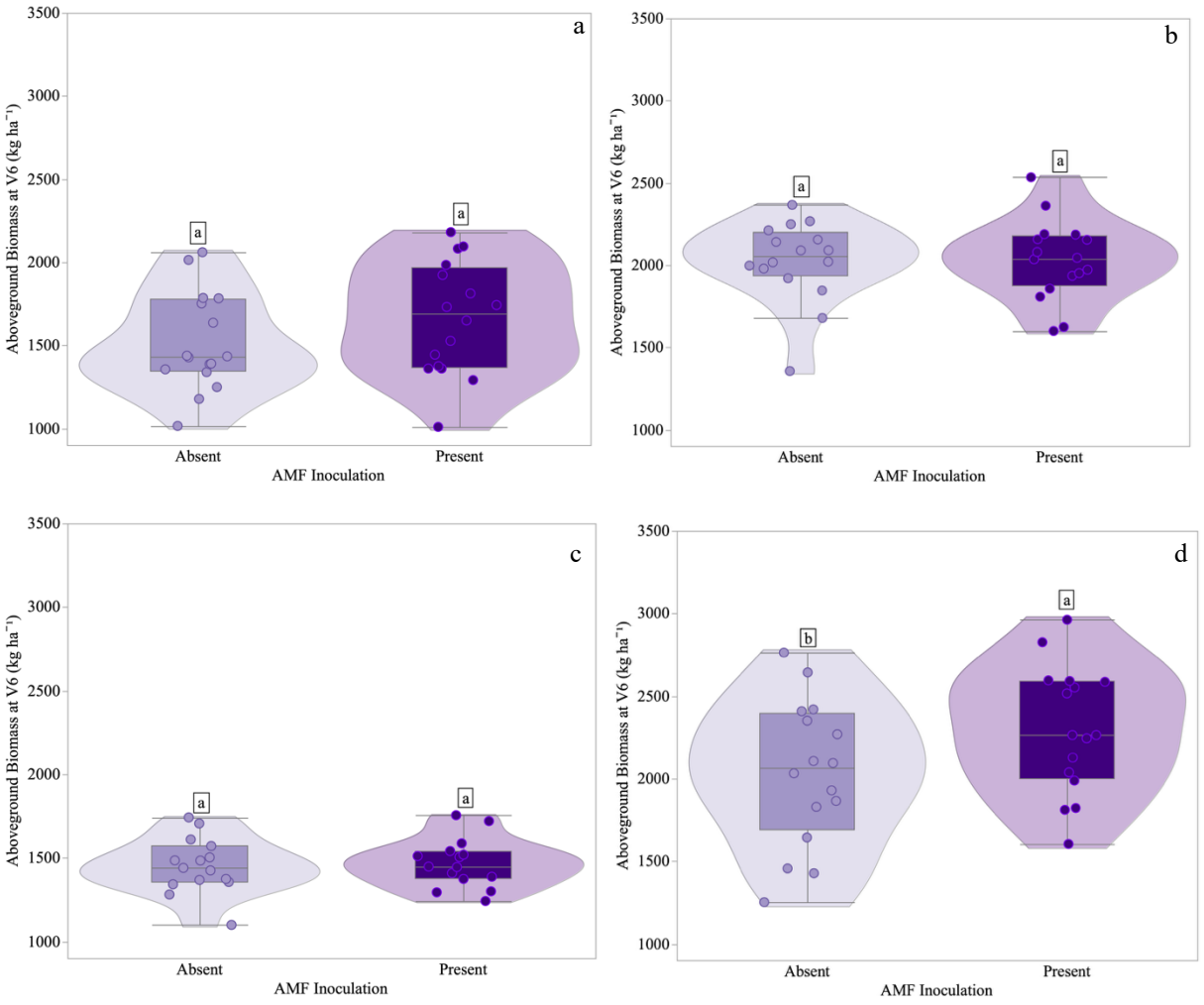


Figure A.4. Effects of arbuscular mycorrhizal fungi (AMF) inoculation on aboveground biomass (kg ha^{-1}) at V6 across years and crop rotations: (a) 2022 and (b) 2024 for the Corn–Soybean rotation, and (c) 2023 and (d) 2025 for the Soybean–Corn rotation. Violin plots illustrate the density distribution of the data, with overlaid box plots representing the median (horizontal line) and interquartile range. Individual points represent raw field observations. Different letters indicate significant differences between AMF inoculation treatments ($P < 0.05$).

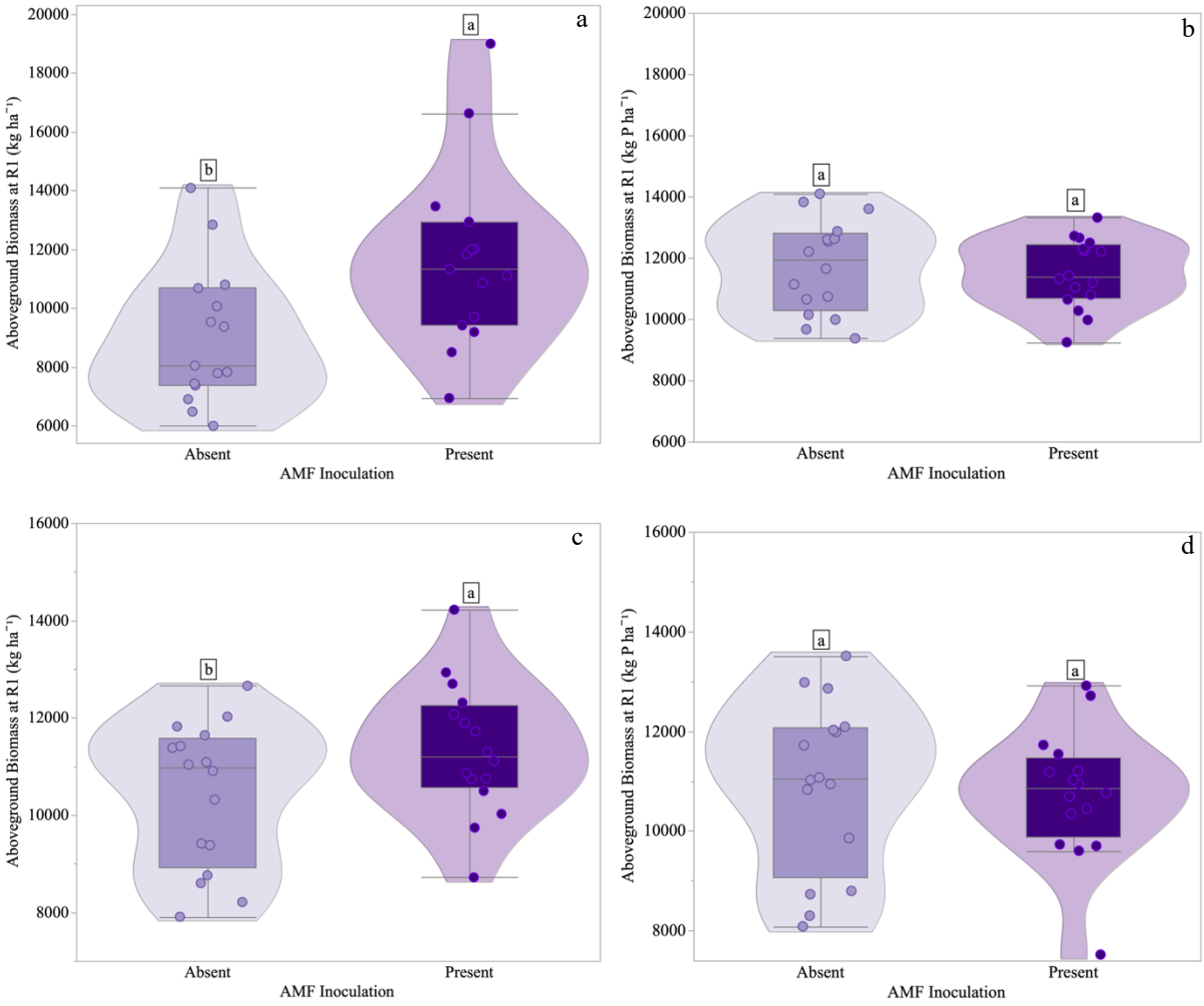


Figure A.5. Effects of arbuscular mycorrhizal fungi (AMF) inoculation on aboveground biomass (kg ha^{-1}) at R1 across years and crop rotations: (a) 2022 and (b) 2024 for the Corn–Soybean rotation, and (c) 2023 and (d) 2025 for the Soybean–Corn rotation. Violin plots illustrate the density distribution of the data, with overlaid box plots representing the median (horizontal line) and interquartile range. Individual points represent raw field observations. Different letters indicate significant differences between AMF inoculation treatments ($P < 0.05$).

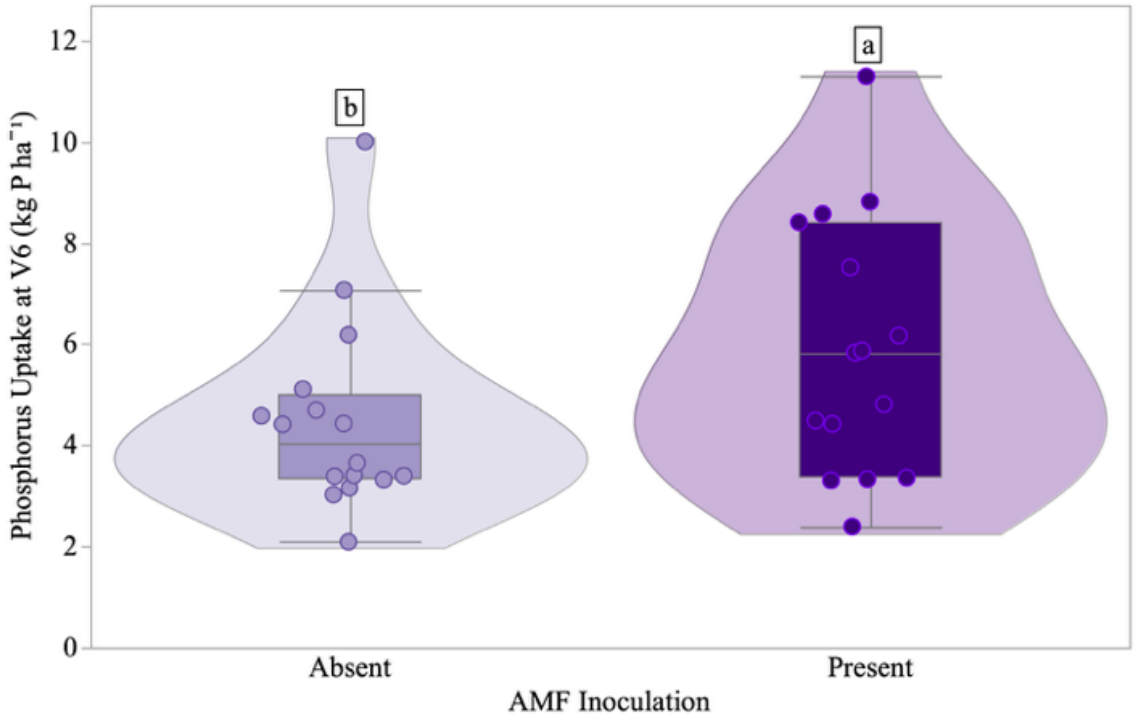


Figure A.6. Effects of phosphorus fertilization and arbuscular mycorrhizal fungi (AMF) inoculation on phosphorus uptake (kg P ha^{-1}) at V6 in 2022 at the Corn–Soybean rotation. Violin plots illustrate the density distribution of the data, with overlaid box plots representing the median (horizontal line) and interquartile range. Individual points represent raw field observations, and different letters indicate a significant difference between AMF inoculation treatments ($P < 0.05$).

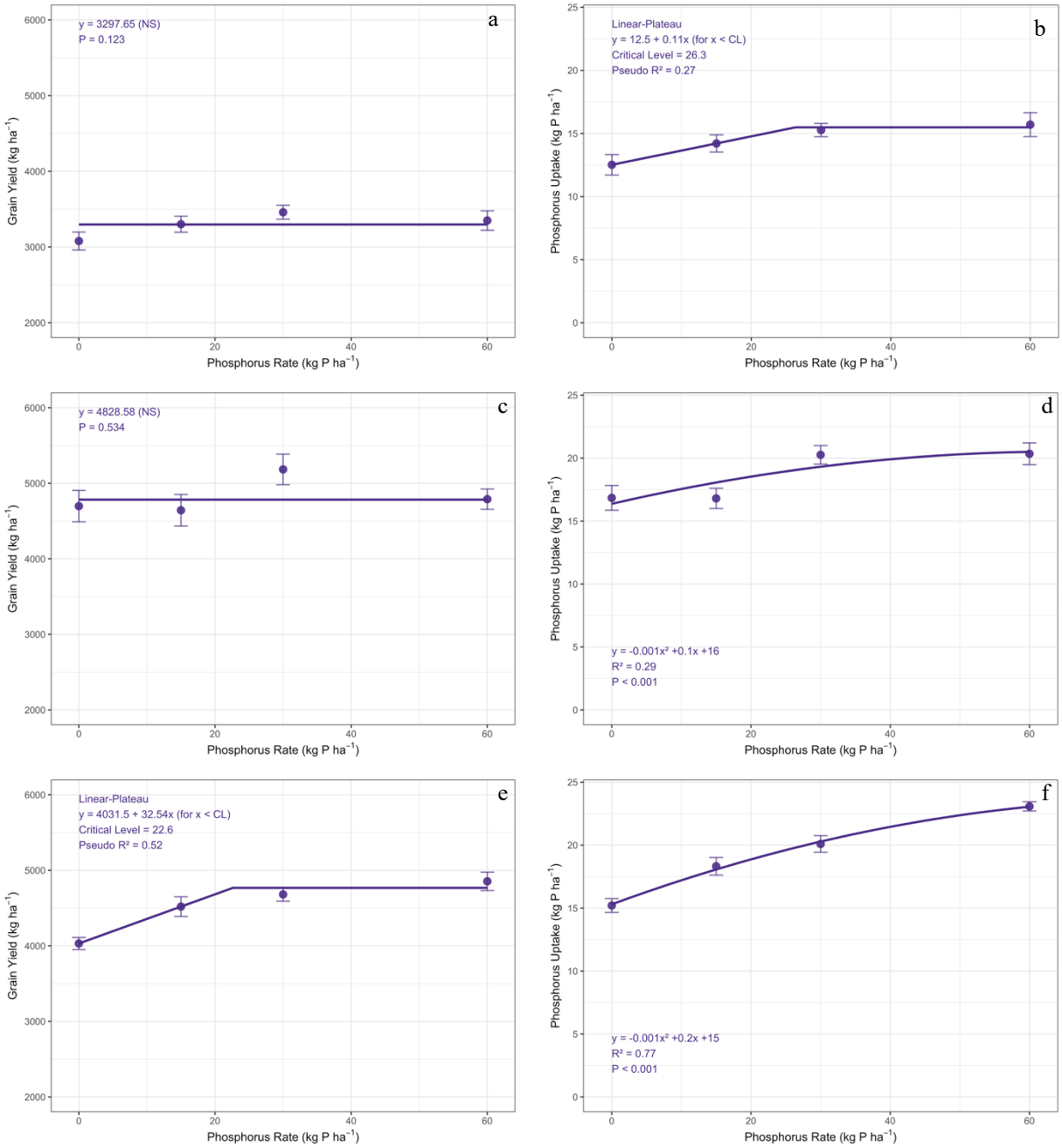


Figure A.7. Soybean grain yield (kg ha^{-1}) and phosphorus (P) uptake (kg P ha^{-1}) in response to P fertilization at Scandia, KS, in (a–b) 2023, (c–d) 2024, and (e–f) 2025. Points represent means ± standard error.

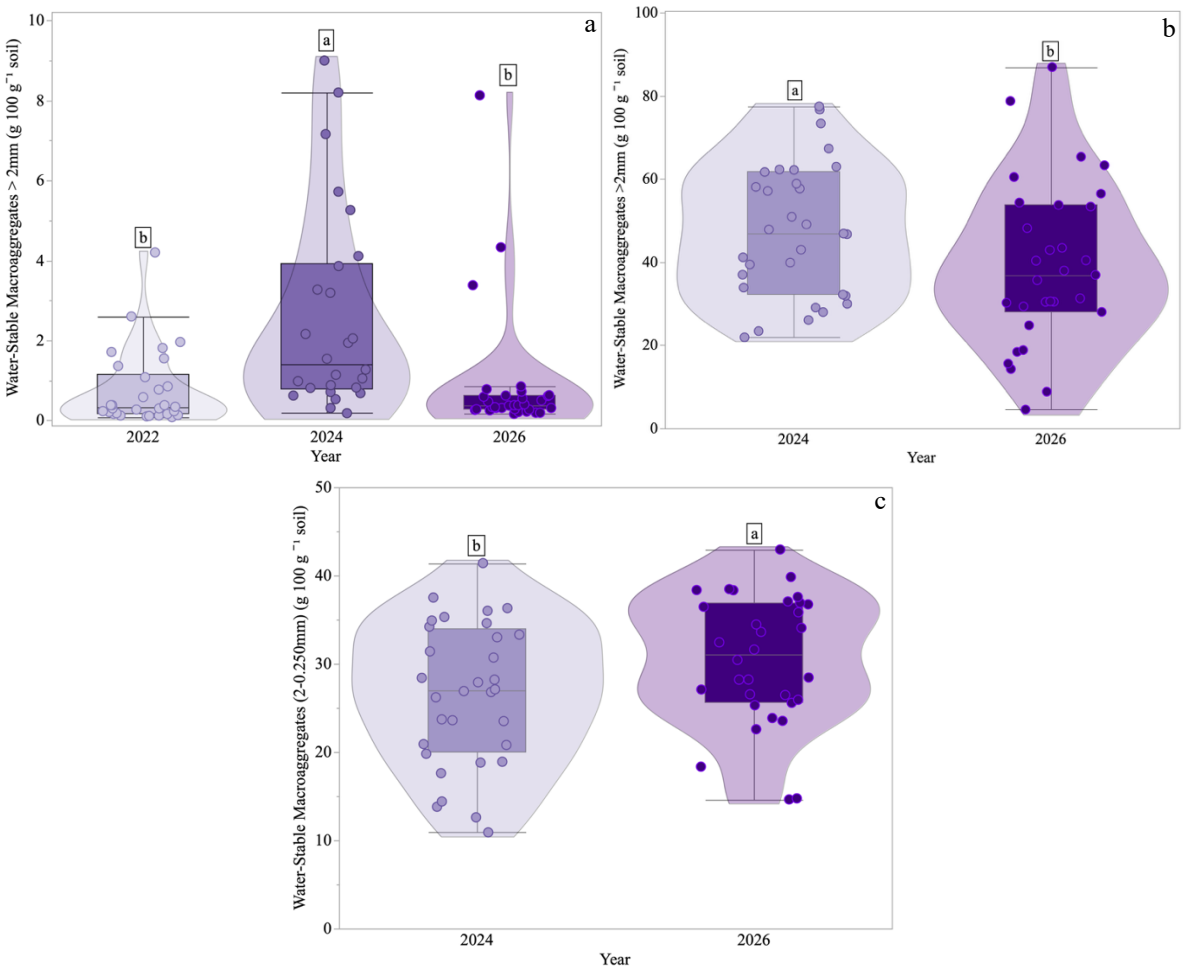


Figure A.8. Water-stable aggregates across sampling years at Scandia, KS: water-stable macroaggregates >2 mm (g 100 g^{-1} soil) in the (a) Corn–Soybean rotation at 15–30 cm and (b) Soybean–Corn rotation at 0–5 cm, and (c) water-stable macroaggregates (0.25–2 mm; g 100 g^{-1} soil) in the Soybean–Corn rotation at 5–15 cm. Violin plots illustrate the density distribution of the data, with overlaid box plots representing the median and interquartile range; individual points represent raw field observations. Different lowercase letters indicate significant differences among years ($P < 0.05$).

Table A.10. Summary of P-values from pairwise contrasts between sampling years at each phosphorus (P) fertilization rate for soil properties with significant P × Year interactions in the Corn-Soybean rotation at Scandia, KS.

Response	Depth (cm)	Phosphorus Rate (kg P ha^{-1})	Year Contrast	P-Value
Soil pH	0-5	0	2022 vs. 2024	<0.0001
	0-5	0	2022 vs. 2026	<0.0001
	0-5	0	2024 vs. 2026	0.219
	0-5	15	2022 vs. 2024	<0.0001
	0-5	15	2022 vs. 2026	<0.0001
	0-5	15	2024 vs. 2026	0.008
	0-5	30	2022 vs. 2024	<0.0001
	0-5	30	2022 vs. 2026	<0.0001
	0-5	30	2024 vs. 2026	0.0026
	0-5	60	2022 vs. 2024	<0.0001
	0-5	60	2022 vs. 2026	0.219
	0-5	60	2024 vs. 2026	<0.0001
Soil Phosphorus	0-5	0	2022 vs. 2024	0.0141
	0-5	0	2022 vs. 2026	0.0015
	0-5	0	2024 vs. 2026	0.4109
	0-5	15	2022 vs. 2024	0.0082
	0-5	15	2022 vs. 2026	<0.0001
	0-5	15	2024 vs. 2026	0.0212
	0-5	30	2022 vs. 2024	0.0048
	0-5	30	2022 vs. 2026	<0.0001
	0-5	30	2024 vs. 2026	0.0003
	0-5	60	2022 vs. 2024	<0.0001
	0-5	60	2022 vs. 2026	<0.0001
	0-5	60	2024 vs. 2026	<0.0001
Acid Phosphatase	0-5	0	2022 vs. 2024	0.0001
	0-5	0	2022 vs. 2026	0.7144
	0-5	0	2024 vs. 2026	<0.0001
	0-5	15	2022 vs. 2024	<0.0001
	0-5	15	2022 vs. 2026	0.9052
	0-5	15	2024 vs. 2026	<0.0001
	0-5	30	2022 vs. 2024	<0.0001
	0-5	30	2022 vs. 2026	0.6344

	0-5	30	2024 vs. 2026	<0.0001
	0-5	60	2022 vs. 2024	0.0001
	0-5	60	2022 vs. 2026	0.0025
	0-5	60	2024 vs. 2026	0.3486
Soil Phosphorus	5-15	0	2022 vs. 2024	0.4101
	5-15	0	2022 vs. 2026	0.0037
	5-15	0	2024 vs. 2026	0.0313
	5-15	15	2022 vs. 2024	0.0167
	5-15	15	2022 vs. 2026	<0.0001
	5-15	15	2024 vs. 2026	0.0495
	5-15	30	2022 vs. 2024	0.0066
	5-15	30	2022 vs. 2026	<0.0001
	5-15	30	2024 vs. 2026	0.0029
	5-15	60	2022 vs. 2024	0.0001
	5-15	60	2022 vs. 2026	<0.0001
	5-15	60	2024 vs. 2026	0.002
Macroaggregates (0.25-2 mm)	5-15	0	2022 vs. 2024	0.0726
	5-15	0	2022 vs. 2026	0.816
	5-15	0	2024 vs. 2026	0.1158
	5-15	15	2022 vs. 2024	0.0318
	5-15	15	2022 vs. 2026	0.0028
	5-15	15	2024 vs. 2026	0.3517
	5-15	30	2022 vs. 2024	0.0197
	5-15	30	2022 vs. 2026	0.0001
	5-15	30	2024 vs. 2026	0.0484
	5-15	60	2022 vs. 2024	0.0021
	5-15	60	2022 vs. 2026	0.0001
	5-15	60	2024 vs. 2026	0.2736
Soil Phosphorus	15-30	0	2022 vs. 2024	0.0077
	15-30	0	2022 vs. 2026	<0.0001
	15-30	0	2024 vs. 2026	<0.0001
	15-30	15	2022 vs. 2024	<0.0001
	15-30	15	2022 vs. 2026	<0.0001
	15-30	15	2024 vs. 2026	<0.0001
	15-30	30	2022 vs. 2024	0.0003
	15-30	30	2022 vs. 2026	<0.0001
	15-30	30	2024 vs. 2026	<0.0001
	15-30	60	2022 vs. 2024	0.0001
	15-30	60	2022 vs. 2026	<0.0001

	15-30	60	2024 vs. 2026	<0.0001
Soil Organic Carbon Stock	15-30	0	2022 vs. 2024	0.7852
	15-30	0	2022 vs. 2026	0.0809
	15-30	0	2024 vs. 2026	0.0348
	15-30	15	2022 vs. 2024	0.4134
	15-30	15	2022 vs. 2026	0.0158
	15-30	15	2024 vs. 2026	0.0017
	15-30	30	2022 vs. 2024	0.1414
	15-30	30	2022 vs. 2026	0.0417
	15-30	30	2024 vs. 2026	0.0008
	15-30	60	2022 vs. 2024	0.7465
	15-30	60	2022 vs. 2026	0.4416
	15-30	60	2024 vs. 2026	0.6542
Actinomycetes	0-5	0	2022 vs. 2024	0.205
	0-5	0	2022 vs. 2026	0.0004
	0-5	0	2024 vs. 2026	0.0102
	0-5	15	2022 vs. 2024	0.0255
	0-5	15	2022 vs. 2026	<0.0001
	0-5	15	2024 vs. 2026	<0.0001
	0-5	30	2022 vs. 2024	0.1085
	0-5	30	2022 vs. 2026	<0.0001
	0-5	30	2024 vs. 2026	<0.0001
	0-5	60	2022 vs. 2024	0.0002
	0-5	60	2022 vs. 2026	<0.0001
	0-5	60	2024 vs. 2026	0.0224
Soil AMF Biomass	5-15	0	2022 vs. 2024	<0.0001
	5-15	0	2022 vs. 2026	<0.0001
	5-15	0	2024 vs. 2026	0.1628
	5-15	15	2022 vs. 2024	<0.0001
	5-15	15	2022 vs. 2026	<0.0001
	5-15	15	2024 vs. 2026	0.4521
	5-15	30	2022 vs. 2024	<0.0001
	5-15	30	2022 vs. 2026	0.0002
	5-15	30	2024 vs. 2026	0.0423
	5-15	60	2022 vs. 2024	0.0004
	5-15	60	2022 vs. 2026	0.0025
	5-15	60	2024 vs. 2026	0.5593
Soil AMF Biomass	15-30	0	2022 vs. 2024	<0.0001
	15-30	0	2022 vs. 2026	<0.0001

	15-30	0	2024 vs. 2026	0.1221
	15-30	15	2022 vs. 2024	0.0005
	15-30	15	2022 vs. 2026	0.0411
	15-30	15	2024 vs. 2026	0.1074
	15-30	30	2022 vs. 2024	0.0005
	15-30	30	2022 vs. 2026	0.1099
	15-30	30	2024 vs. 2026	0.0358
	15-30	60	2022 vs. 2024	0.0054
	15-30	60	2022 vs. 2026	0.9825
	15-30	60	2024 vs. 2026	0.0051

Table A.11. Summary of P-values from pairwise contrasts between sampling years at each phosphorus (P) fertilization rate for soil properties with significant P × Year interactions in the Soybean-Corn rotation at Scandia, KS.

Response	Depth (cm)	Phosphorus Rate (kg P ha^{-1})	Year Contrast	P-Value
Soil Phosphorus	0-5	0	2024 vs. 2026	0.2984
	0-5	15	2024 vs. 2026	0.1163
	0-5	30	2024 vs. 2026	0.0004
	0-5	60	2024 vs. 2026	<0.0001
Soil pH	0-5	0	2024 vs. 2026	<0.0001
	0-5	15	2024 vs. 2026	<0.0001
	0-5	30	2024 vs. 2026	<0.0001
	0-5	60	2024 vs. 2026	<0.0001
Soil Phosphorus	5-15	0	2024 vs. 2026	0.0001
	5-15	15	2024 vs. 2026	<0.0001
	5-15	30	2024 vs. 2026	<0.0001
	5-15	60	2024 vs. 2026	<0.0001
Macroaggregates (>2 mm)	5-15	0	2024 vs. 2026	0.0049
	5-15	15	2024 vs. 2026	0.0001
	5-15	30	2024 vs. 2026	0.0679
	5-15	60	2024 vs. 2026	<0.0001
Soil Phosphorus	15-30	0	2024 vs. 2026	<0.0001
	15-30	15	2024 vs. 2026	<0.0001
	15-30	30	2024 vs. 2026	<0.0001
	15-30	60	2024 vs. 2026	<0.0001
Soil Organic Carbon Stock	15-30	0	2024 vs. 2026	0.2664
	15-30	15	2024 vs. 2026	0.0032
	15-30	30	2024 vs. 2026	0.0005
	15-30	60	2024 vs. 2026	<0.0001
Total Soil Nitrogen	15-30	0	2024 vs. 2026	0.0084
	15-30	15	2024 vs. 2026	0.9699
	15-30	30	2024 vs. 2026	0.7605
	15-30	60	2024 vs. 2026	0.1045
Soil AMF Biomass	0-5	0	2024 vs. 2026	<0.0001
	0-5	15	2024 vs. 2026	0.0004
	0-5	30	2024 vs. 2026	0.0059
	0-5	60	2024 vs. 2026	0.6783

Soil AMF Biomass	5-15	0	2024 vs. 2026	<0.0001
	5-15	15	2024 vs. 2026	0.0728
	5-15	30	2024 vs. 2026	0.0067
	5-15	60	2024 vs. 2026	0.7277
Soil AMF Biomass	15-30	0	2024 vs. 2026	0.0007
	15-30	15	2024 vs. 2026	0.8082
	15-30	30	2024 vs. 2026	0.4801
	15-30	60	2024 vs. 2026	0.0702

Table A.12. Summary of P-values from pairwise contrasts among sampling years within each phosphorus (P) fertilization rate and arbuscular mycorrhizal fungi (AMF) treatment for soil responses in the Corn–Soybean rotation at Scandia, KS, when significant P × AMF × Year interactions were detected.

Response	Depth (cm)	Phosphorus Rate (kg P ha^{-1})	AMF	Year Contrast	P-value
Macroaggregates (>2 mm)	0-5	0	Absent	2022 vs. 2024	0.0197
	0-5	0	Absent	2022 vs. 2026	0.0276
	0-5	0	Absent	2024 vs. 2026	0.888
	0-5	15	Absent	2022 vs. 2024	0.0007
	0-5	15	Absent	2022 vs. 2026	0.5379
	0-5	15	Absent	2024 vs. 2026	0.0037
	0-5	30	Absent	2022 vs. 2024	0.0029
	0-5	30	Absent	2022 vs. 2026	0.3769
	0-5	30	Absent	2024 vs. 2026	0.0287
	0-5	60	Absent	2022 vs. 2024	0.0025
	0-5	60	Absent	2022 vs. 2026	0.2681
	0-5	60	Absent	2024 vs. 2026	0.0425
	0-5	0	Present	2022 vs. 2024	0.0054
	0-5	0	Present	2022 vs. 2026	0.0062
	0-5	0	Present	2024 vs. 2026	0.9554
	0-5	15	Present	2022 vs. 2024	0.0209
	0-5	15	Present	2022 vs. 2026	0.0052
	0-5	15	Present	2024 vs. 2026	0.5607
	0-5	30	Present	2022 vs. 2024	0.0014
	0-5	30	Present	2022 vs. 2026	0.8839
	0-5	30	Present	2024 vs. 2026	0.0009
	0-5	60	Present	2022 vs. 2024	<0.0001
	0-5	60	Present	2022 vs. 2026	0.4472
	0-5	60	Present	2024 vs. 2026	<0.0001
Macroaggregates (0.25–2 mm)	0-5	0	Absent	2022 vs. 2024	0.9951
	0-5	0	Absent	2022 vs. 2026	0.0846
	0-5	0	Absent	2024 vs. 2026	0.1119
	0-5	15	Absent	2022 vs. 2024	0.0028
	0-5	15	Absent	2022 vs. 2026	0.6014
	0-5	15	Absent	2024 vs. 2026	0.0006
	0-5	30	Absent	2022 vs. 2024	0.0108
	0-5	30	Absent	2022 vs. 2026	0.9408
	0-5	30	Absent	2024 vs. 2026	0.0131

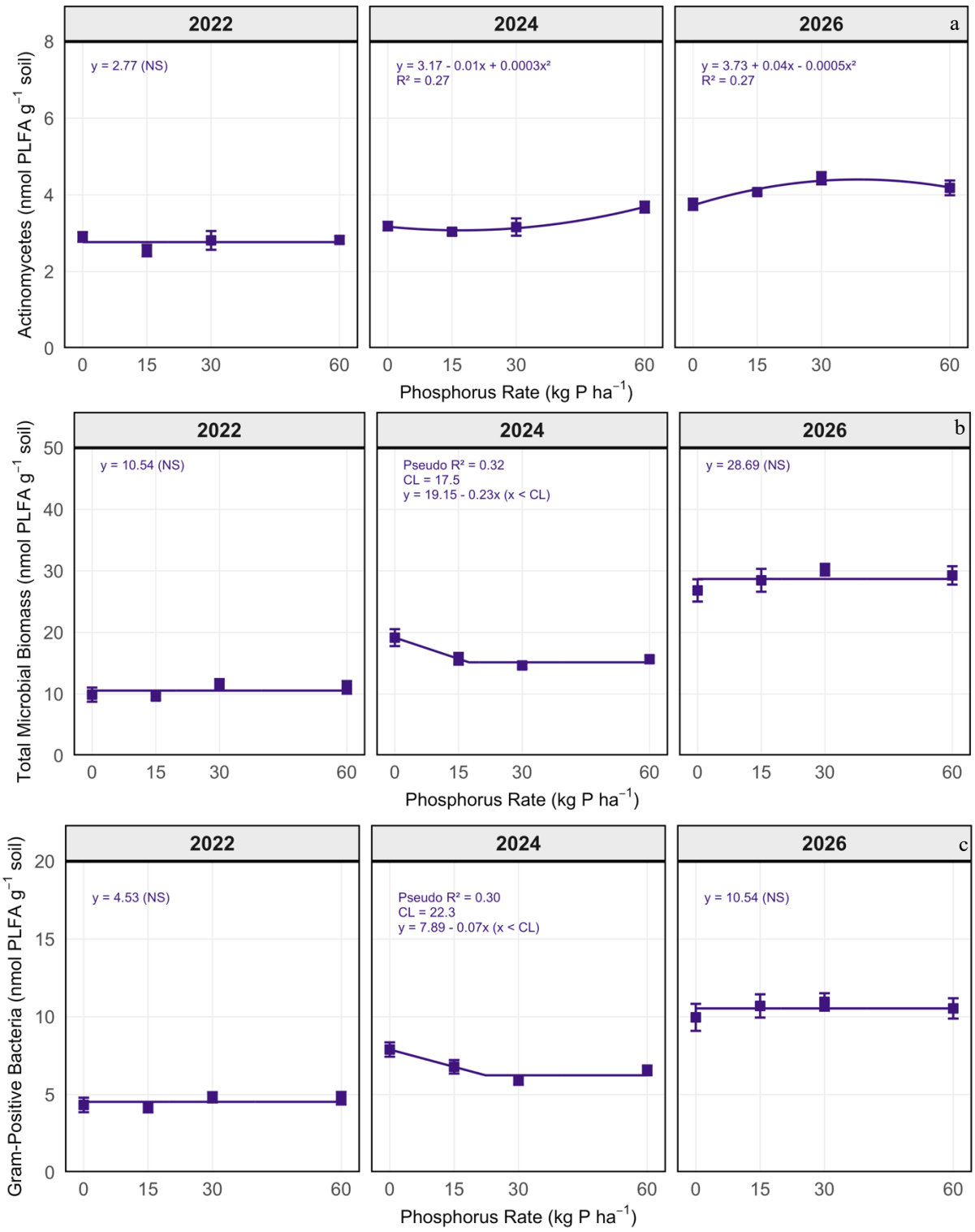
	0-5	60	Absent	2022 vs. 2024	0.7159
	0-5	60	Absent	2022 vs. 2026	0.0485
	0-5	60	Absent	2024 vs. 2026	0.0209
	0-5	0	Present	2022 vs. 2024	0.3266
	0-5	0	Present	2022 vs. 2026	0.4068
	0-5	0	Present	2024 vs. 2026	0.8668
	0-5	15	Present	2022 vs. 2024	0.0246
	0-5	15	Present	2022 vs. 2026	0.01
	0-5	15	Present	2024 vs. 2026	0.5814
	0-5	30	Present	2022 vs. 2024	0.0002
	0-5	30	Present	2022 vs. 2026	0.2854
	0-5	30	Present	2024 vs. 2026	<0.0001
	0-5	60	Present	2022 vs. 2024	0.0087
	0-5	60	Present	2022 vs. 2026	0.5335
	0-5	60	Present	2024 vs. 2026	0.0016
Soil AMF Biomass	0-5	0	Absent	2022 vs. 2024	<0.0001
	0-5	0	Absent	2022 vs. 2026	0.0002
	0-5	0	Absent	2024 vs. 2026	0.4903
	0-5	15	Absent	2022 vs. 2024	0.0016
	0-5	15	Absent	2022 vs. 2026	0.0001
	0-5	15	Absent	2024 vs. 2026	0.4422
	0-5	30	Absent	2022 vs. 2024	0.0079
	0-5	30	Absent	2022 vs. 2026	0.0002
	0-5	30	Absent	2024 vs. 2026	0.2224
	0-5	60	Absent	2022 vs. 2024	0.1645
	0-5	60	Absent	2022 vs. 2026	0.8381
	0-5	60	Absent	2024 vs. 2026	0.1125
	0-5	0	Present	2022 vs. 2024	0.0027
	0-5	0	Present	2022 vs. 2026	<0.0001
	0-5	0	Present	2024 vs. 2026	0.0004
	0-5	15	Present	2022 vs. 2024	0.0047
	0-5	15	Present	2022 vs. 2026	<0.0001
	0-5	15	Present	2024 vs. 2026	0.002
	0-5	30	Present	2022 vs. 2024	0.2523
	0-5	30	Present	2022 vs. 2026	0.2721
	0-5	30	Present	2024 vs. 2026	0.962
	0-5	60	Present	2022 vs. 2024	0.1068
	0-5	60	Present	2022 vs. 2026	0.8795
	0-5	60	Present	2024 vs. 2026	0.1424

Table A.13. Summary of P-values from pairwise contrasts between arbuscular mycorrhizal fungi (AMF)-inoculated and non-inoculated treatments at each phosphorus (P) fertilization rate at the Corn-Soybean rotation across depths and years when significant P × AMF or P × AMF × Year interactions were detected.

Response	Depth (cm)	Year	Phosphorus Rate (kg P ha^{-1})	P-Value
Macroaggregates (>2 mm)	0-5	2022	0	0.942
	0-5	2022	15	0.704
	0-5	2022	30	0.700
	0-5	2022	60	0.748
Macroaggregates (>2 mm)	0-5	2024	0	0.523
	0-5	2024	15	0.126
	0-5	2024	30	0.883
	0-5	2024	60	0.043
Macroaggregates (>2 mm)	0-5	2026	0	0.472
	0-5	2026	15	0.049
	0-5	2026	30	0.177
	0-5	2026	60	0.164
Macroaggregates (>2 mm)	5-15	Across years	0	0.014
	5-15	Across years	15	0.028
	5-15	Across years	30	0.802
	5-15	Across years	60	0.073
Soil AMF Biomass	0-5	2022	0	0.424
	0-5	2022	15	0.780
	0-5	2022	30	0.210
	0-5	2022	60	0.967
Soil AMF Biomass	0-5	2024	0	0.220
	0-5	2024	15	0.507
	0-5	2024	30	0.743
	0-5	2024	60	0.788
Soil AMF Biomass	0-5	2026	0	0.003
	0-5	2026	15	0.077
	0-5	2026	30	0.115
	0-5	2026	60	0.694

Table A.14. Changes in soil microbial community abundance and structure (nmol PLFA g^{-1} soil) across years and soil depths in the Corn–Soybean rotation at Scandia, KS. Values are means ± standard error. Different lowercase letters within each soil depth indicate significant differences among years ($P < 0.05$). Asterisks (*) indicate significant interactions presented as figures.

AMF Inoculation	Year	Total Microbial Biomass	Gram-Positive Bacteria	Gram-Negative Bacteria	Actinomycetes	Saprophytic Fungi
			0-5 cm			
Absent	2022	36.7 c ± 1.06	12.8 c ± 0.47	7.02 b ± 0.25		0.68 c ± 0.05
Present						
Absent	2024	42.5 b ± 1.20	15.4 b ± 0.53	6.27 b ± 0.31	*	1.22 b ± 0.05
Present						
Absent	2026	65.1 a ± 1.58	20.0 a ± 0.40	12.1 a ± 0.38		1.87 a ± 0.11
Present						
			5-15 cm			
Absent	2022	11.0 d ± 0.75	4.66 d ± 0.29	2.26 c ± 0.24	0.97 c ± 0.05	0.06 c ± 0.00
Present		10.2 d ± 0.54	4.41 d ± 0.25			
Absent	2024	16.1 c ± 0.53	6.71 c ± 0.26	2.69 b ± 0.11	1.66 b ± 0.04	0.18 b ± 0.01
Present		16.5 c ± 0.95	6.86 c ± 0.37			
Absent	2026	30.4 a ± 0.92	11.5 a ± 0.38	5.75 a ± 0.33	2.37 a ± 0.07	0.37 a ± 0.01
Present		27.0 b ± 1.12	9.56 b ± 0.47			
			15-30 cm			
Absent	2022	8.77 c ± 0.39	3.52 b ± 0.15	1.37 c ± 0.08	1.00 b ± 0.05	0.02 c ± 0.01
Present						0.02 c ± 0.01
Absent	2024	12.3 b ± 0.56	5.10 a ± 0.23	1.77 b ± 0.09	1.44 a ± 0.07	0.10 b ± 0.01
Present						0.08 b ± 0.01
Absent	2026	15.4 a ± 0.64	5.63 a ± 0.23	2.84 a ± 0.12	1.44 a ± 0.06	0.15 a ± 0.01
Present						0.18 a ± 0.01


2022
2024
2026
a
y = 2.77 (NS)
y = 3.17 - 0.01x + 0.0003x²
R² = 0.27
y = 3.73 + 0.04x - 0.0005x²
R² = 0.27
Actinomycetes (nmol PLFA g⁻¹ soil)
0
2
4
6
8
0
15
30
60
Phosphorus Rate (kg P ha⁻¹)
b
y = 10.54 (NS)
Pseudo R² = 0.32
CL = 17.5
y = 19.15 - 0.23x (x < CL)
y = 28.69 (NS)
Total Microbial Biomass (nmol PLFA g⁻¹ soil)
0
10
20
30
40
50
c
y = 4.53 (NS)
Pseudo R² = 0.30
CL = 22.3
y = 7.89 - 0.07x (x < CL)
y = 10.54 (NS)
Gram-Positive Bacteria (nmol PLFA g⁻¹ soil)
0
5
10
15
20

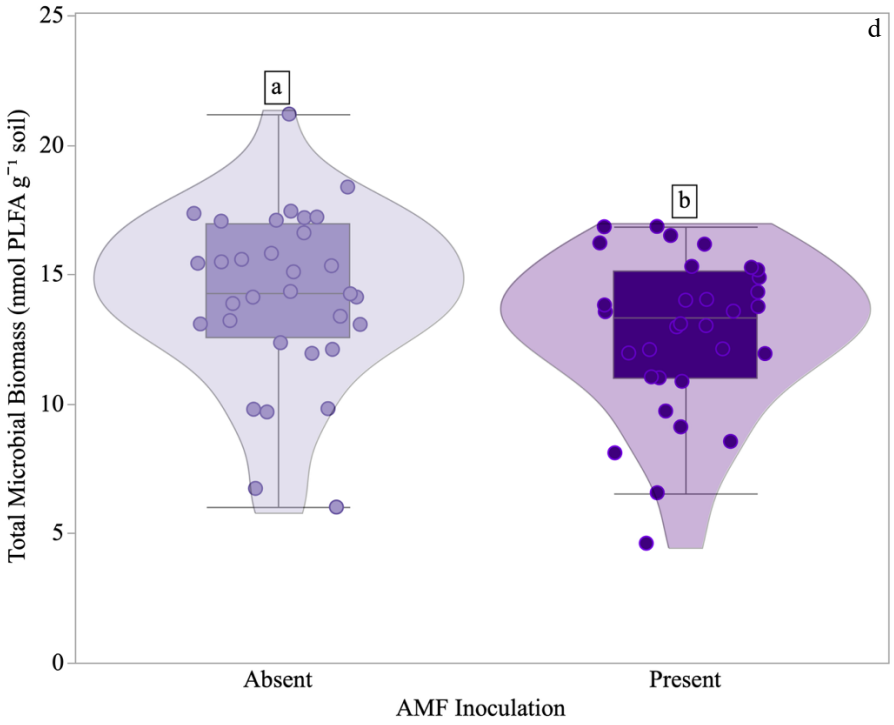


Figure A.9. Effects of phosphorus (P) fertilization across sampling years (2022, 2024, and 2026) on (a) actinomycetes at the 0–5 cm soil depth, and (b) total microbial biomass and (c) Gram-positive bacteria at the 5–15 cm soil depth of the Corn–Soybean rotation at Scandia, KS, and (d) effects of AMF inoculation on total microbial biomass at the 15-30 cm soil depth of the Soybean–Corn rotation. Points represent means with standard error bars. Violin plots illustrate the density distribution of the data, with overlaid box plots representing the median and interquartile range; individual points represent raw field observations. Different lowercase letters indicate significant differences ($P < 0.05$).

Table A.15. Changes in soil microbial community abundance and structure (nmol PLFA g^{-1} soil) across years and soil depths in the Soybean-Corn rotation at Scandia, KS. Values are means ± standard error. Different lowercase letters within each soil depth indicate significant differences among years ($P < 0.05$). Asterisks (*) indicate significant interactions presented as figures.

Year	Total Microbial Biomass	Gram-Positive Bacteria	Gram-Negative Bacteria	Actinomycetes	Saprophytic Fungi
			0-5 cm		
2024	53.0 a ± 1.42	16.9 a ± 0.54	10.2 a ± 0.35	3.70 a ± 0.08	1.61 a ± 0.07
2026	51.8 a ± 3.47	16.9 a ± 1.05	9.59 a ± 0.71	3.45 a ± 0.24	1.35 b ± 0.09
			5-15 cm		
2024	18.9 a ± 0.52	7.62 a ± 0.19	3.92 a ± 0.13	1.90 a ± 0.06	0.22 b ± 0.02
2026	22.1 a ± 1.19	8.41 a ± 0.44	4.54 a ± 0.27	1.78 a ± 0.09	0.33 a ± 0.02
			15-30 cm		
2024	14.8 a ± 0.46	6.00 a ± 0.19	3.01 a ± 0.09	1.82 a ± 0.07	0.14 a ± 0.01
2026	12.1 b ± 0.57	4.77 b ± 0.23	2.56 b ± 0.14	1.10 b ± 0.06	0.16 a ± 0.02

Table A.16. Changes in enzyme activity (µg PNP $soil^{-1}$ h^{-1}) each year, across depths in the Corn-Soybean Rotation at Scandia, KS. Values are means ± standard error. Different lowercase letters within each soil depth indicate significant differences between years ($P < 0.05$). Asterisks (*) indicate significant interactions presented as figures.

Year	Arylsulfatase	β-glucosidase	NAG	Acid Phosphatase
0-5 cm				
2022	75.9 c ± 2.55	192 c ± 6.1	52.7 c ± 2.09	
2024	97.6 b ± 2.28	323 b ± 15.5	73.7 a ± 2.54	*
2026	124 a ± 3.15	420 a ± 9.81	60.6 b ± 1.71	
5-15 cm				
2022	23.9 c ± 1.65	44.3 c ± 2.34	15.8 c ± 0.65	234 c ± 8.25
2024	33.9 b ± 1.21	63.9 b ± 1.94	27.9 a ± 0.74	350 b ± 12.5
2026	42.2 a ± 1.42	84.8 a ± 3.37	24.3 b ± 0.81	382 a ± 9.59
15-30 cm				
2022	30.3 a ± 1.69	48.0 a ± 1.54	30.4 a ± 2.17	242 b ± 8.75
2024	32.4 a ± 1.16	49.0 a ± 1.79	27.2 a ± 1.65	292 a ± 10.9
2026	20.2 b ± 1.07	35.9 b ± 1.06	14.1 b ± 0.79	274 a ± 9.54

Abbreviation: NAG, N-acetyl-β-glucosaminidase

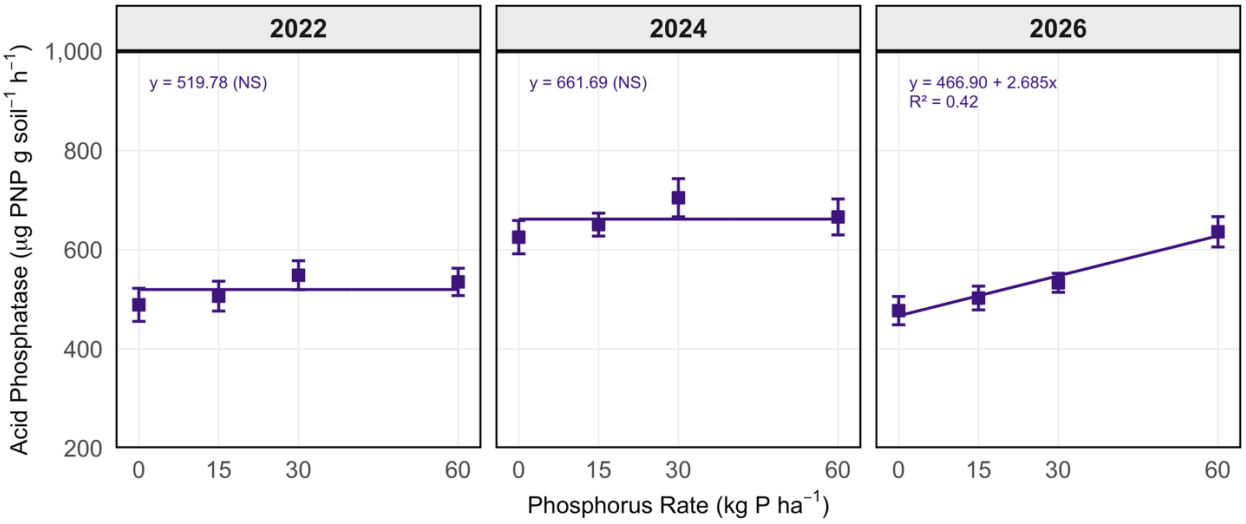


Figure A.10. Response of acid phosphatase activity (µg PNP g^{-1} soil h^{-1}) to phosphorus (P) fertilization across sampling years at the 0–5 cm soil depth in the Corn–Soybean rotation at Scandia, KS. Points represent treatment means with standard error bars.

Table A.17. Changes in enzyme activity (μg PNP $soil^{-1}$ h^{-1}) each year, across depths in the Soybean-Corn Rotation at Scandia, KS. Values are means ± standard error. Different lowercase letters within each soil depth indicate significant differences between years ($P < 0.05$).

AMF Inoculation	Year	Arylsulfatase	β-glucosidase	Acid Phosphatase	NAG
			0-5 cm		
Absent	2024	114 ab ± 5.24	388 a ± 14.4	770 a ± 16.8	71.5 a ± 1.9
Present		110 b ± 6.66			
Absent	2026	131 ab ± 7.26	323 b ± 11.3	595 b ± 14.5	65.1 b ± 2.5
Present		151 a ± 10.0			
			5-15 cm		
Absent	2024	27.1 b ± 1.19	47.6 b ± 2.32	366 ns ± 8.45	22.1 ns ± 0.80
Present					
Absent	2026	47.9 a ± 2.27	60.6 a ± 2.65	382 ns ± 13.2	23.2 ns ± 1.42
Present					
			15-30 cm		
Absent	2024	29.2 a ± 1.41	46.5 a ± 3.22	336 a ± 10.1	22.8 a ± 0.97
Present			36.4 b ± 2.45		
Absent	2026	19.3 b ± 0.93	25.0 c ± 1.57	249 b ± 9.83	12.3 b ± 0.81
Present			24.0 c ± 1.21		

Abbreviation: NAG, N-acetyl-β-glucosaminidase

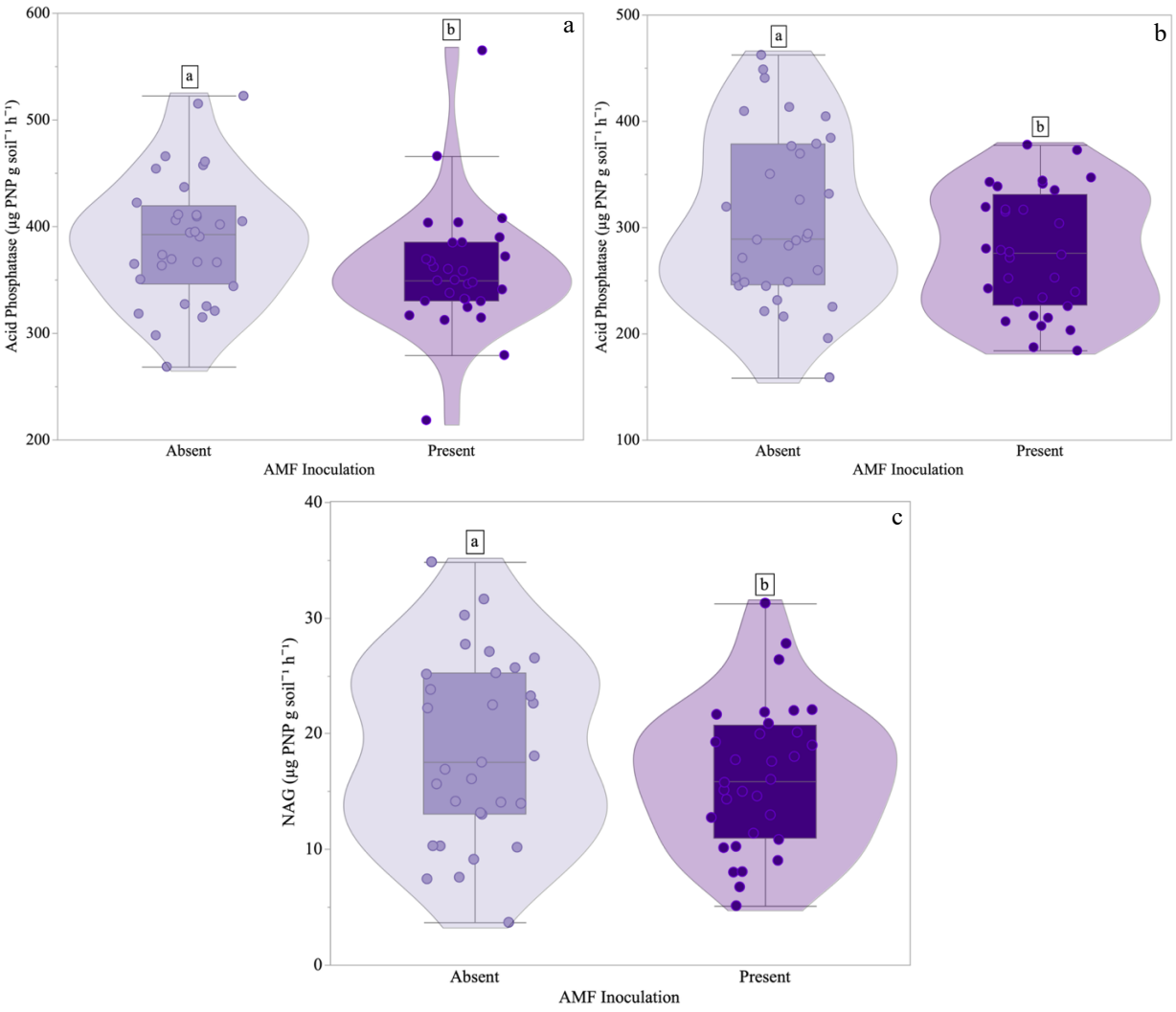


Figure A.11. Effects of arbuscular mycorrhizal fungi (AMF) inoculation on soil enzyme activity in the Soybean–Corn rotation at Scandia, KS: (a) acid phosphatase activity at the 5–15 cm soil depth, (b) acid phosphatase activity at the 15–30 cm soil depth, and (c) N-acetyl-β-D-glucosaminidase (NAG) activity at the 15–30 cm soil depth. Violin plots show the distribution of observations, with box plots representing the median and interquartile range and points representing individual observations. Different lowercase letters indicate significant differences between AMF inoculation treatments ($P < 0.05$).

Table A.18. Changes in total soil nitrogen (Mg N ha^{-1}), soil carbon stock (Mg C ha^{-1}), and soil pH across years and soil depths in the Corn–Soybean rotation at Scandia, KS. Values are means ± standard error. Different lowercase letters within each soil depth indicate significant differences between years ($P < 0.05$); ns indicates no significant difference between years. Asterisks (*) indicate significant interactions presented as figures.

Year	Total Soil Nitrogen	Soil Carbon Stock	Soil pH
		0-5 cm	
2022	0.80 c ± 0.02	11.0 b ± 0.32	
2024	0.88 b ± 0.02	9.66 c ± 0.27	*
2026	1.14 a ± 0.03	13.3 a ± 0.37	
		5-15 cm	
2022	1.35 b ± 0.04	17.2 a ± 0.37	5.92 b ± 0.08
2024	1.55 a ± 0.03	15.8 b ± 0.22	6.30 a ± 0.06
2026	1.46 a ± 0.04	16.7 a ± 0.35	6.17 a ± 0.06
		15-30 cm	
2022	1.89 b ± 0.05		6.23 a ± 0.07
2024	2.10 a ± 0.04	*	6.26 a ± 0.05
2026	1.83 b ± 0.05		5.64 b ± 0.05

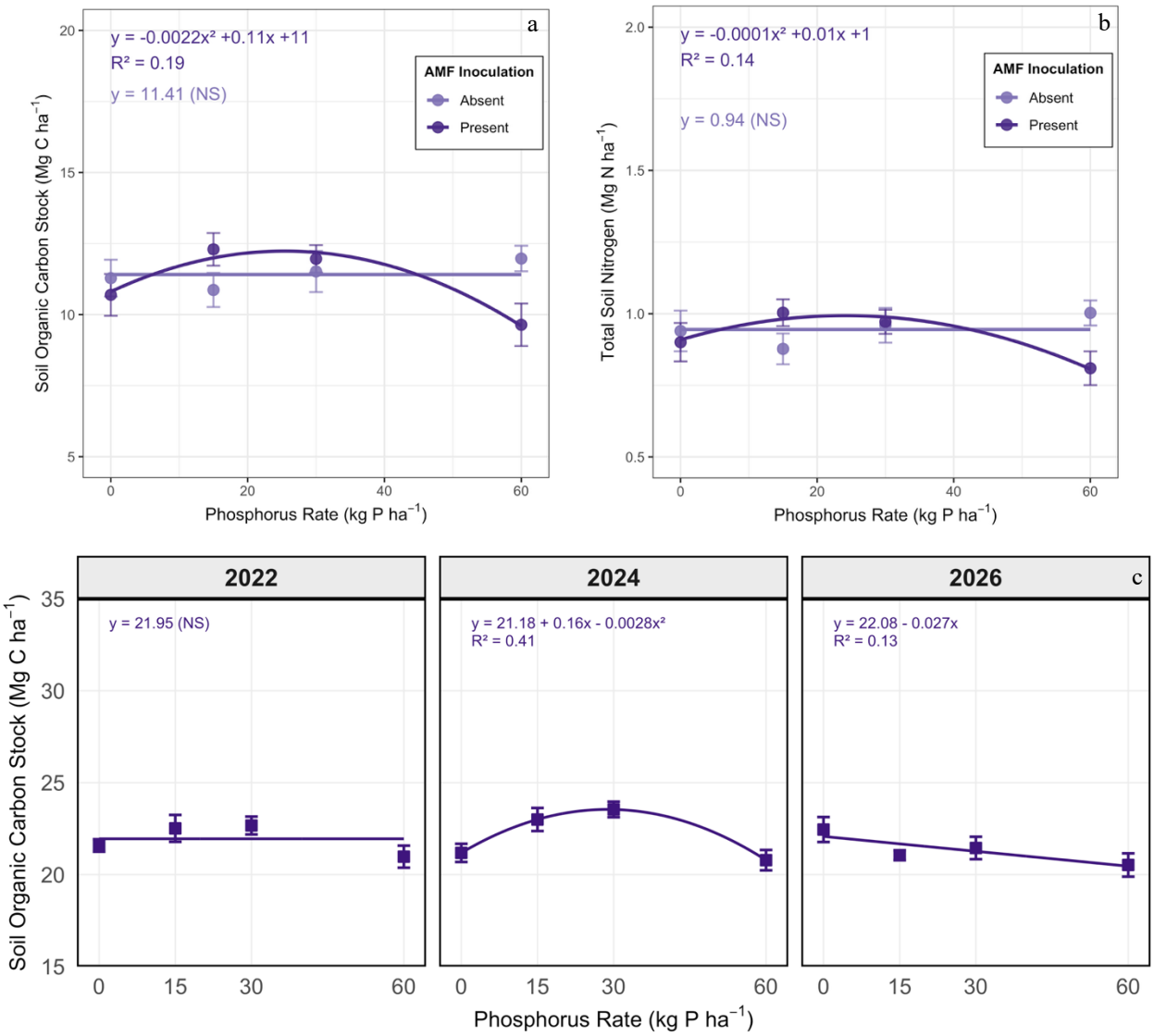


Figure A.12. Effects of phosphorus fertilization and arbuscular mycorrhizal fungi (AMF) inoculation on (a) soil organic carbon stock (Mg C ha⁻¹) and (b) total soil nitrogen (Mg N ha⁻¹) at the 0–5 cm soil depth, and (c) soil organic carbon stock (Mg C ha⁻¹) across sampling years (2022, 2024, and 2026) at the 15-30 cm soil depth in the Corn–Soybean rotation at Scandia, KS. Points represent means with standard error bars.

Table A.19. Changes in total soil nitrogen (Mg N ha^{-1}), soil carbon stock (Mg C ha^{-1}), and soil pH across years and soil depths in the Soybean–Corn rotation at Scandia, KS. Values are means ± standard error. Different lowercase letters within each soil depth indicate significant differences between years ($P < 0.05$). Asterisks (*) indicate significant interactions presented as figures.

Year	Total Soil Nitrogen	Soil Carbon Stock	Soil pH
0-5 cm			
2024	1.07 b ± 0.03	12.2 b ± 0.28	*
2026	1.36 a ± 0.03	15.4 a ± 0.29	
5-15 cm			
2024	1.42 b ± 0.03	16.1 b ± 0.30	6.40 a ± 0.07
2026	1.77 a ± 0.03	17.9 a ± 0.30	6.09 b ± 0.04
15-30 cm			
2024	*	*	6.25 a ± 0.06
2026			5.53 b ± 0.03

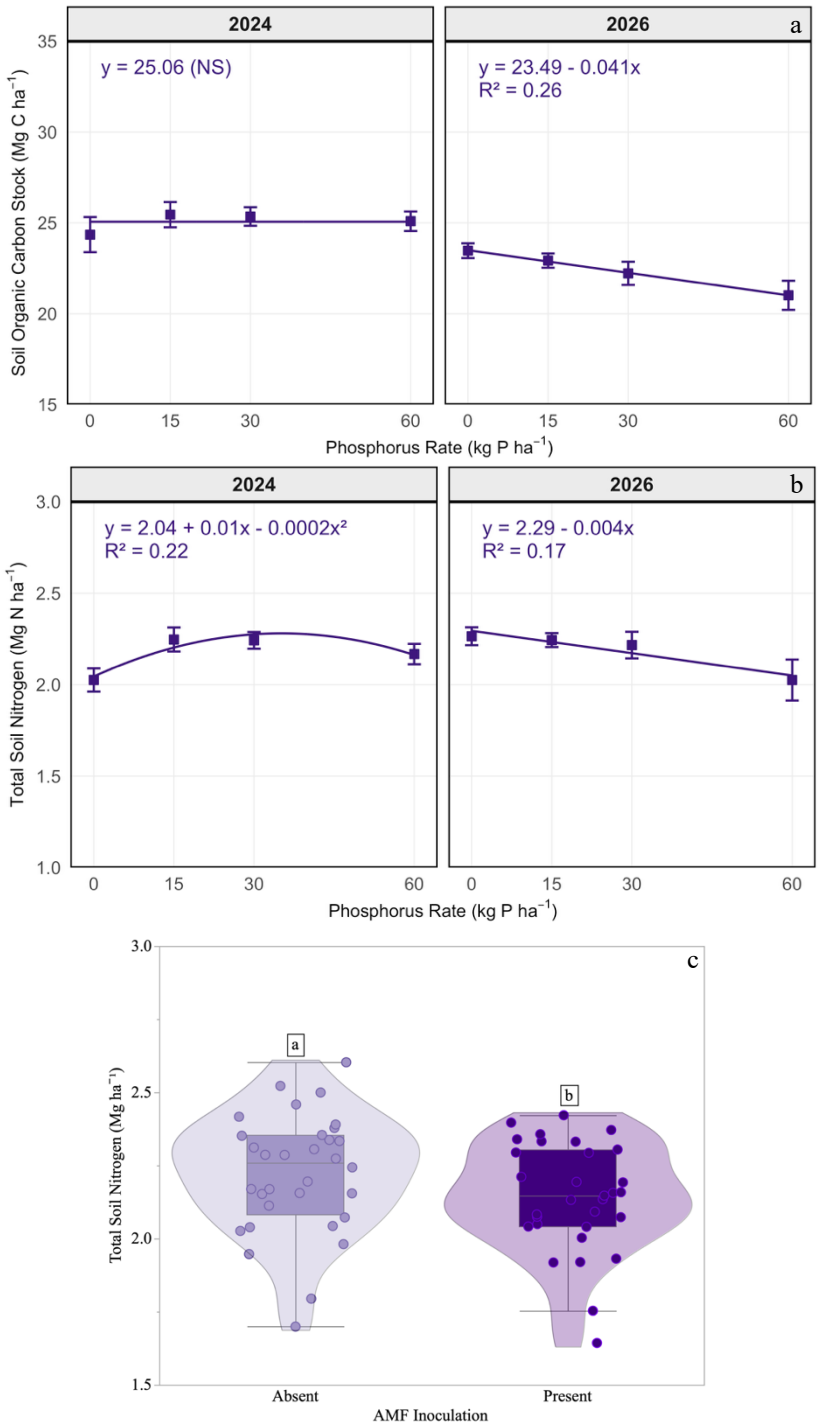


Figure A.13. Effects of phosphorus (P) fertilization on (a) soil carbon stock (Mg C ha^{-1}) and (b) total soil nitrogen (Mg N ha^{-1}) across sampling years (2024 and 2026), and (c) arbuscular mycorrhizal fungi (AMF) inoculation on total soil nitrogen (Mg N ha^{-1}) at the 15–30 cm soil depth in the Soybean–Corn rotation at Scandia, KS. In panels a and b, points represent treatment means with standard error bars. In panel c, violin plots show the distribution of observations, with box plots representing the median and interquartile range and points representing individual observations. Different lowercase letters indicate significant differences between AMF inoculation treatments ($P < 0.05$).

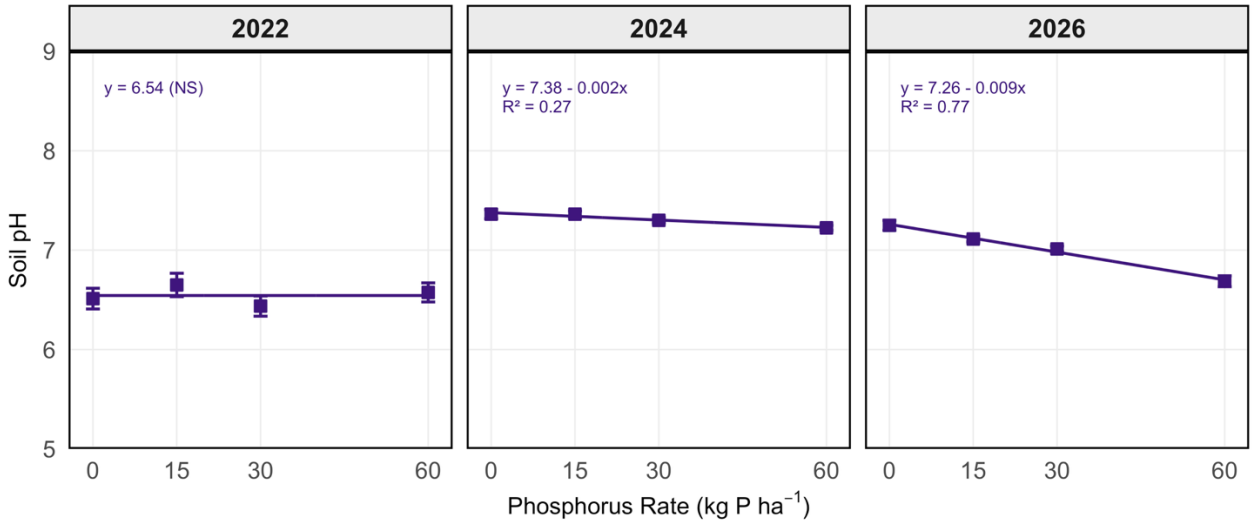


Figure A.14. Soil pH across phosphorus fertilization rates and sampling years at the 0–5 cm soil depth in the Corn–Soybean rotation at Scandia, KS. Points represent treatment means with standard error bars.

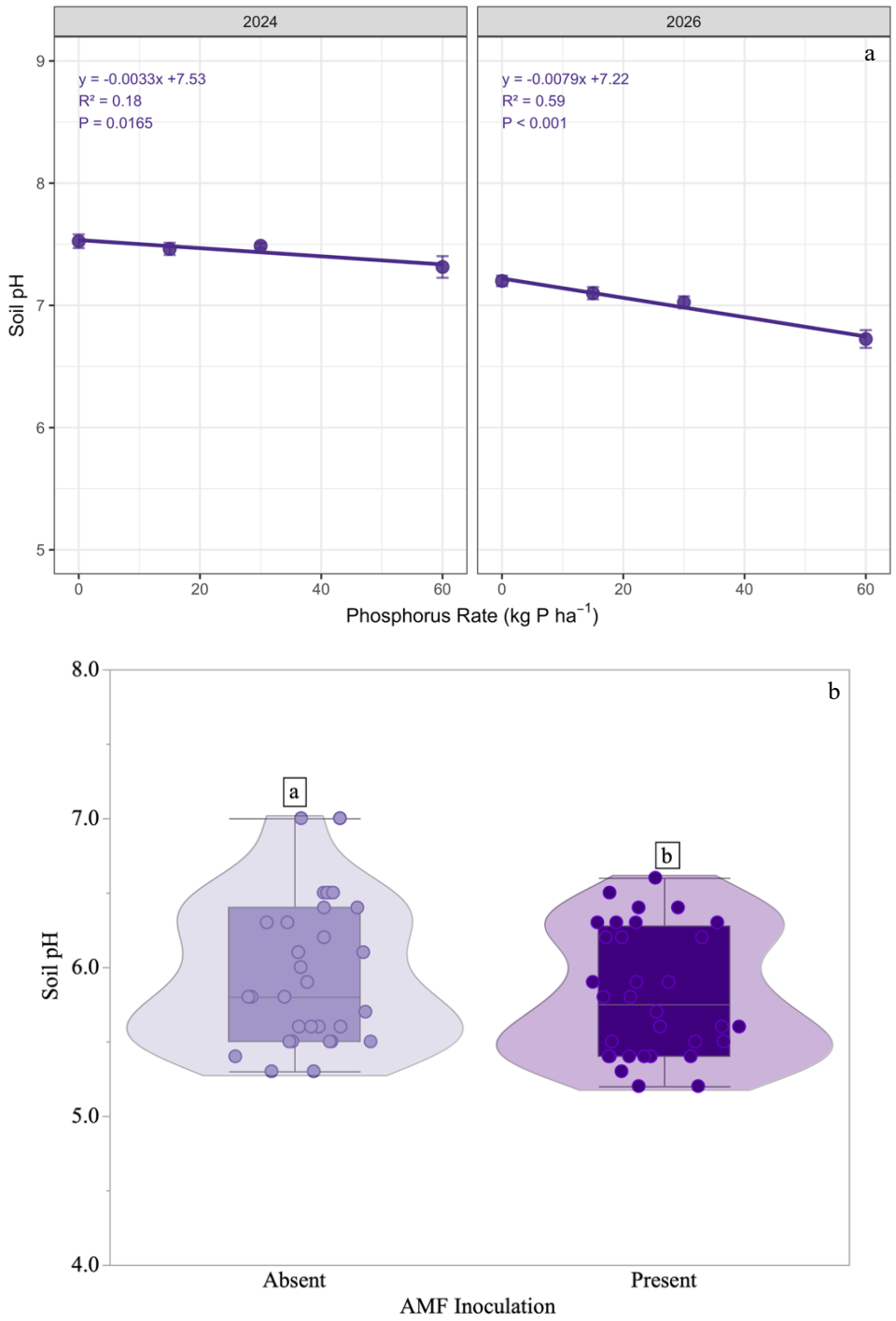


Figure A.15. Soil pH responses in the Soybean–Corn rotation at Scandia, KS. (a) Soil pH across phosphorus fertilization rates and sampling years at the 0–5 cm soil depth. Points represent treatment means with standard error bars. (b) Effects of arbuscular mycorrhizal fungi (AMF) inoculation on soil pH at the 15–30 cm soil depth. Violin plots show the distribution of observations, box plots represent the median and interquartile range, and points represent individual observations. Different lowercase letters indicate significant differences between AMF inoculation treatments ($P < 0.05$).

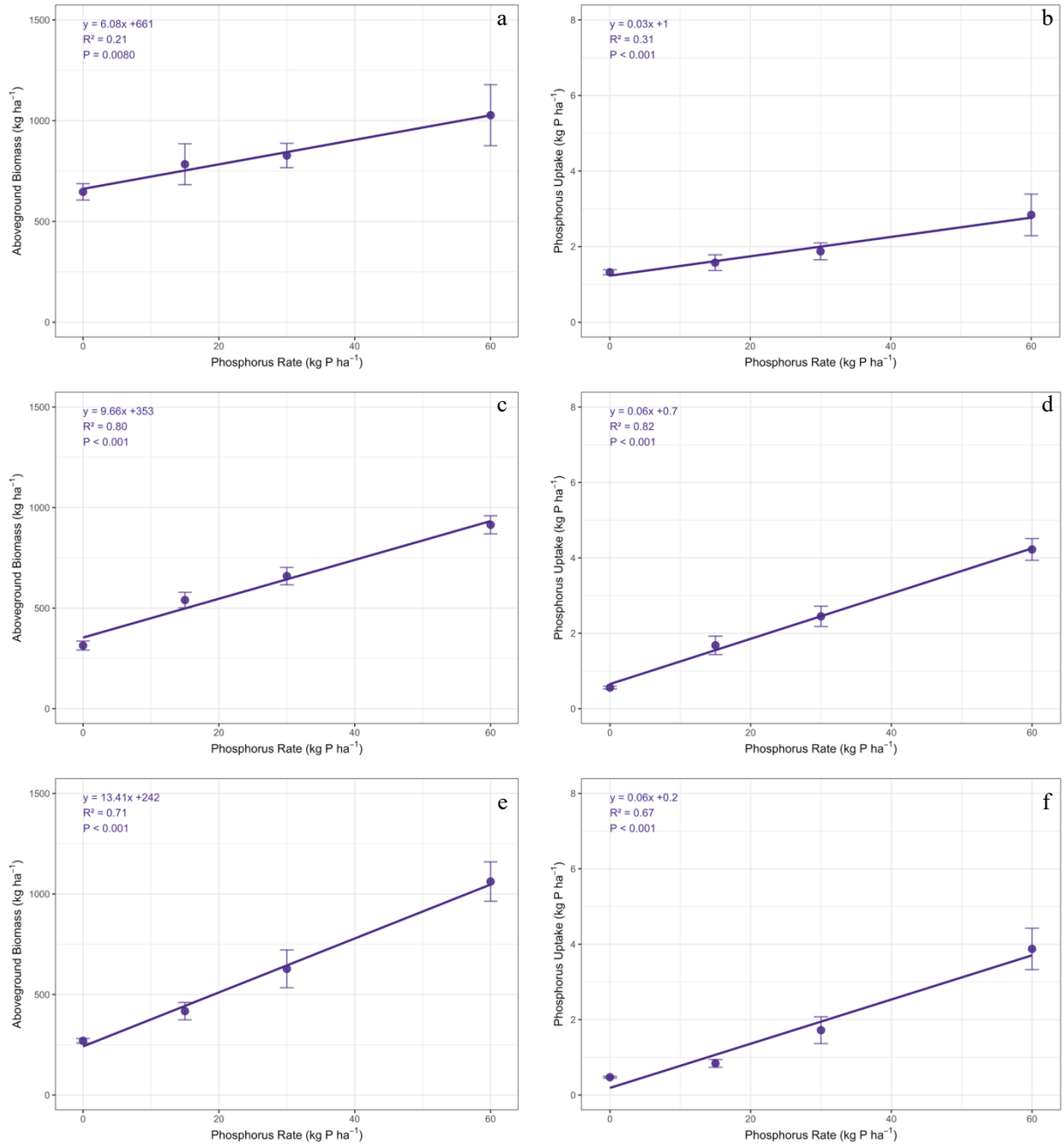


Figure A.16. Main effect of phosphorus fertilization on wheat (cover crop) aboveground biomass and phosphorus uptake (kg P ha^{-1}) at termination across years: (a-b) 2023-2024, (c-d) 2024-2025 (e-f) 2025-2026 for the Corn-Soybean rotation. Points represent means with standard error bars.

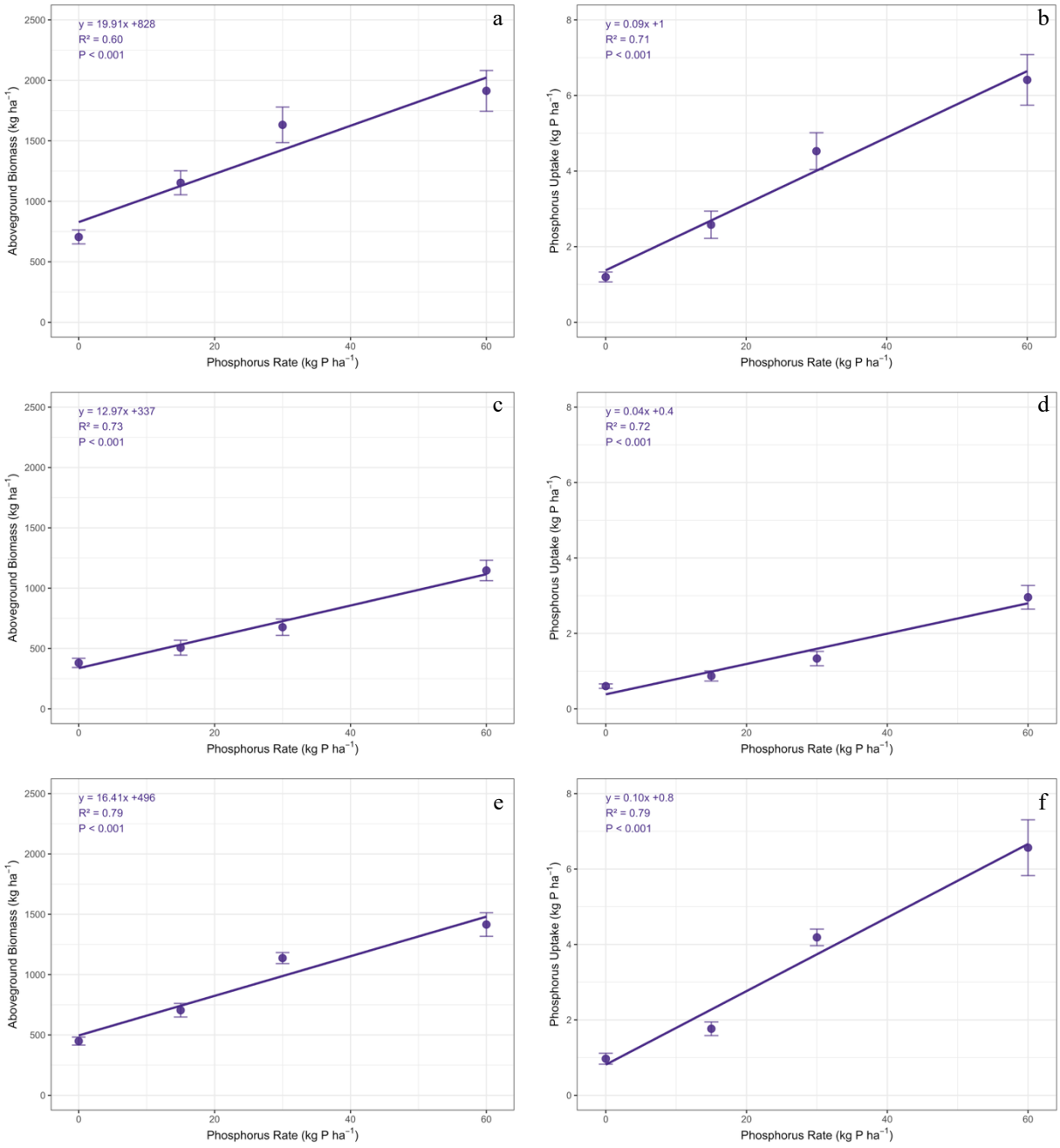


Figure A.17. Main effect of phosphorus fertilization on wheat (cover crop) aboveground biomass and phosphorus uptake (kg P ha^{-1}) at termination across years: (a-b) 2023-2024, (c-d) 2024-2025 (e-f) 2025-2026 for the Soybean-Corn rotation. Points represent means with standard error bars. Solid lines indicate significant ($P < 0.05$) regression responses to phosphorus rates.

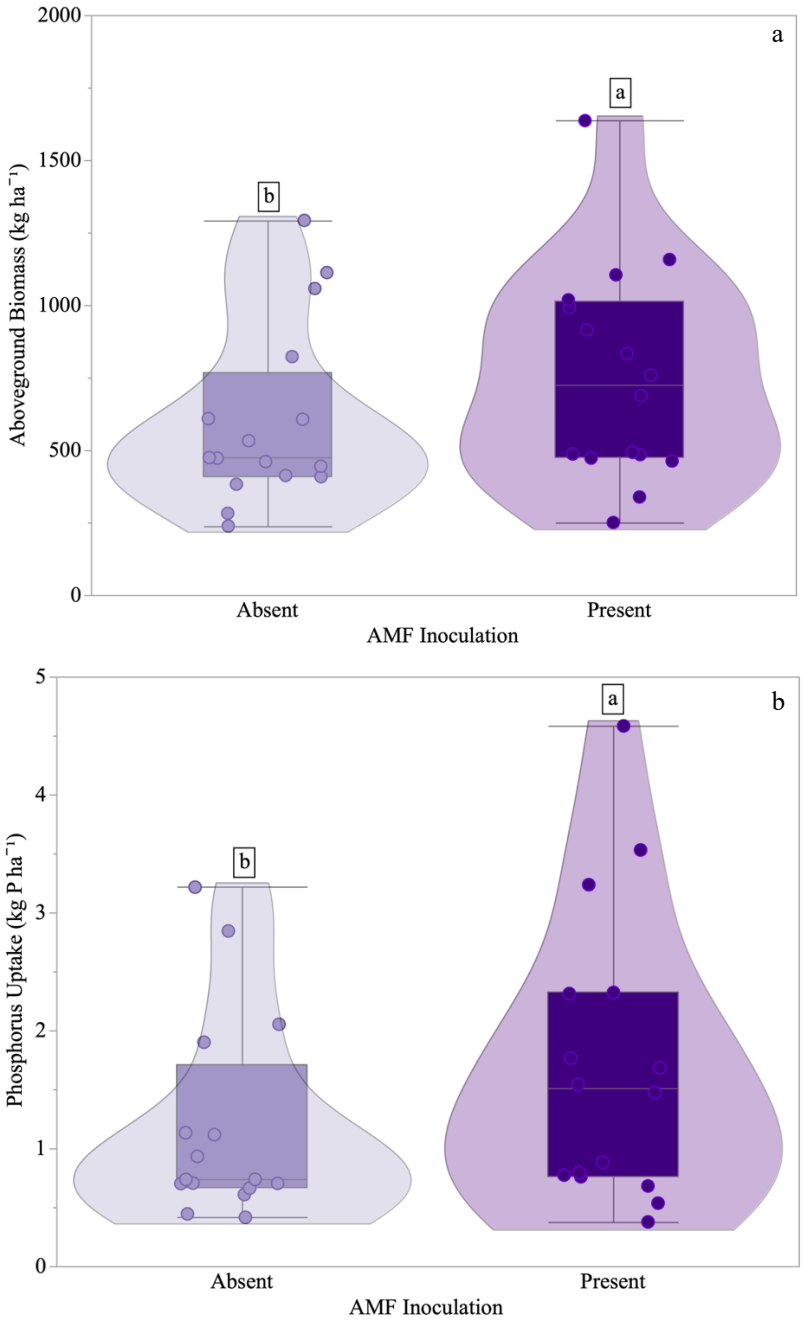


Figure A.18. Main effect of arbuscular mycorrhizal fungi inoculation on wheat (cover crop) aboveground biomass (a) and phosphorus uptake (kg P ha^{-1}) (b) in 2023-2024 for the Soybean-Corn rotation. Violin plots show the distribution of observations, box plots represent the median and interquartile range, and points represent individual observations. Different lowercase letters indicate significant differences between AMF inoculation treatments ($P < 0.05$).

Appendix B - Chapter 3

Table B.1. Baseline soil physicochemical properties by soil depth (cm) at the North Farm in Manhattan, KS.

	Phosphorus Mehlich-3 (mg kg^{-1})	pH	Sikora pH	Ca (mg kg^{-1})	Mg (mg kg^{-1})	Na (mg kg^{-1})	K (mg kg^{-1})	Soil Organic Carbon (g C kg^{-1} soil)	Total Soil Nitrogen (mg N kg^{-1} soil)	Clay (%)	Silt (%)	Sand (%)
0-5	18.8	5.88	6.76	2004	295	23.8	223	15.3	14.3	20	58	22
5-15	7.65	6.17	6.83	2149	292	49.3	134	13.6	12.6	22	56	22
15-30	6.06	6.49	6.89	2256	334	97.9	123	13.7	12.1	23	55	22

Note: Soil pH – determined by 1:1 soil slurry (Watson & Brown, 1998); P- extracted by Mehlich-3 (Mehlich, 1984); Ca, Mg, Na, and K – extracted by Ammonium Acetate (Warncke & Brown, 1998); Soil C and N - measured by dry combustion (Bremner & Mulvaney, 1982; Nelson & Sommers, 1983).

Table B.2. Cumulative precipitation (mm) across key corn development stages (VE–V6, V6–R1, and R1–R6) at the North Farm in Manhattan, KS (2022–2025).

Corn Development Stages				
Year	**VE-V6**	**V6-R1**	**R1-R6**	**Event**
2022	111	70	119	Precipitation
All-Season Total			300	
2023	148	106	94	Precipitation
All-Season Total			348	
2024	151	77	86	Precipitation
All-Season Total			314	
2025	210	51	130	Precipitation
All-Season Total			391	

Table B.3. Field operational calendar for corn (*Zea mays* L.), including planting and harvest dates, hybrid selection, and seeding rates across four growing seasons (2022–2025) at the North Farm in Manhattan, KS.

Location	Year	Planting and Harvesting Dates	Hybrids	Seeding Rate (seeds ha^{-1})
North Farm	2022	June 16, 2022 - October 31, 2022	DKC 64-65	69,200
	2023	April 25, 2023 - September 12, 2023	DKC 64-65	69,200
	2024	May 29, 2024 - October 3, 2024	DKC 64-65	69,200
	2025	May 14, 2025 - September 29, 2025	DKC 64-65	69,200

Table B.4. Field operational calendar for the cover crop wheat (*Triticum aestivum* L.), including planting and termination dates, variety selection, and seeding rates across four growing seasons (2022–2025) at the North Farm in Manhattan, KS.

Location	Planting and Termination Dates	Variety	Seeding Rate (kg ha^{-1})
North Farm	November 18, 2022- April 25, 2023	VNS	134.5
	October 17, 2023 - May 10, 2024	Zenda	134.5
	October 30, 2024 – April 18, 2025	VNS	67.3
	October 20, 2025 - April 24, 2026	KS Mako	100.9

VNS: Variety Not Stated

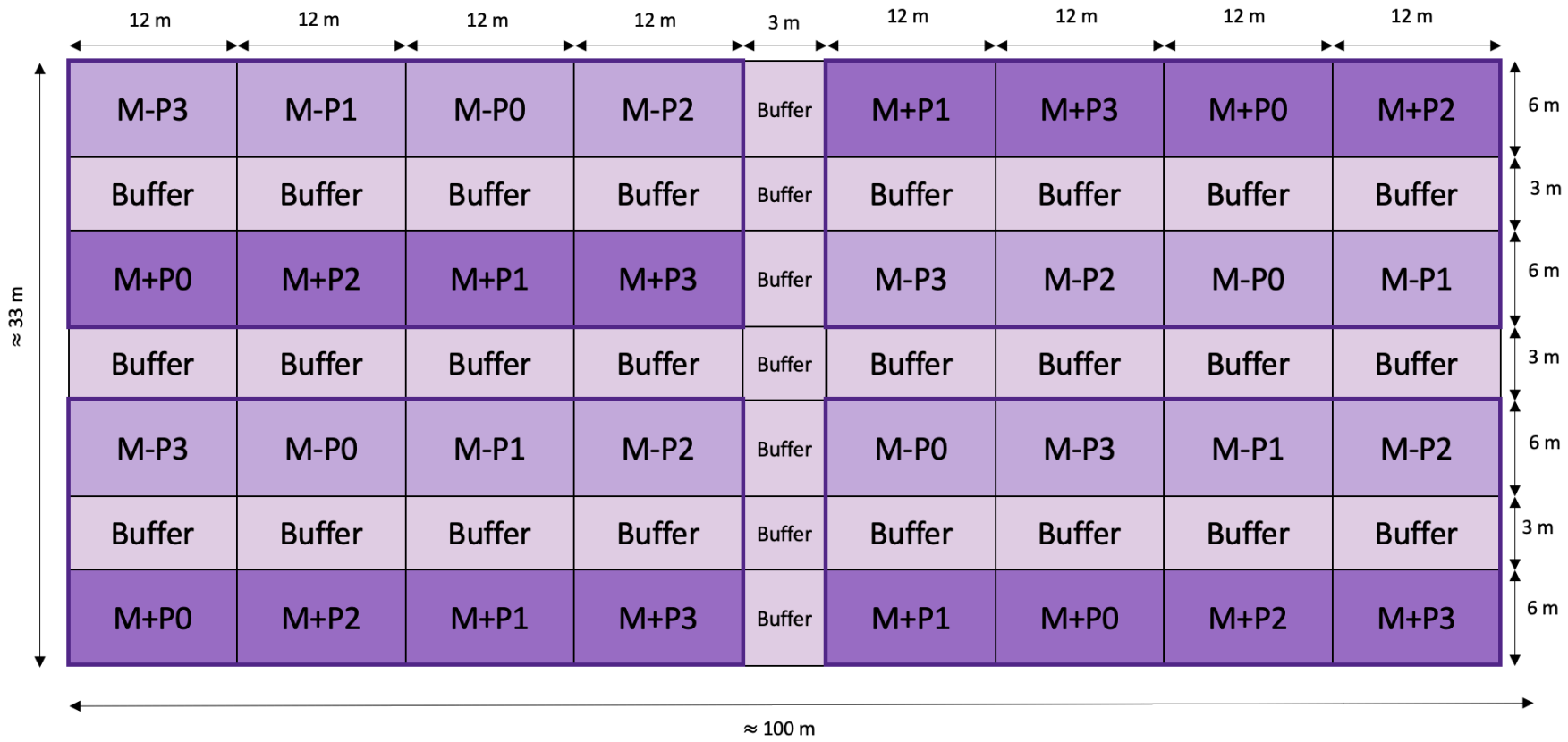


Figure B.1. Experimental layout of the split-plot randomized complete block design (RCBD). Mycorrhizal inoculation was assigned as the main-plot factor, with inoculated (M+) and non-inoculated (M−) treatments, and phosphorus (P) fertilization rates were assigned as subplots: P0 = 0, P1 = 15, P2 = 30, and P3 = 60 kg P ha^{-1}. Buffer areas (3 m) separated plots to minimize potential movement of mycorrhizal inoculum among treatments.

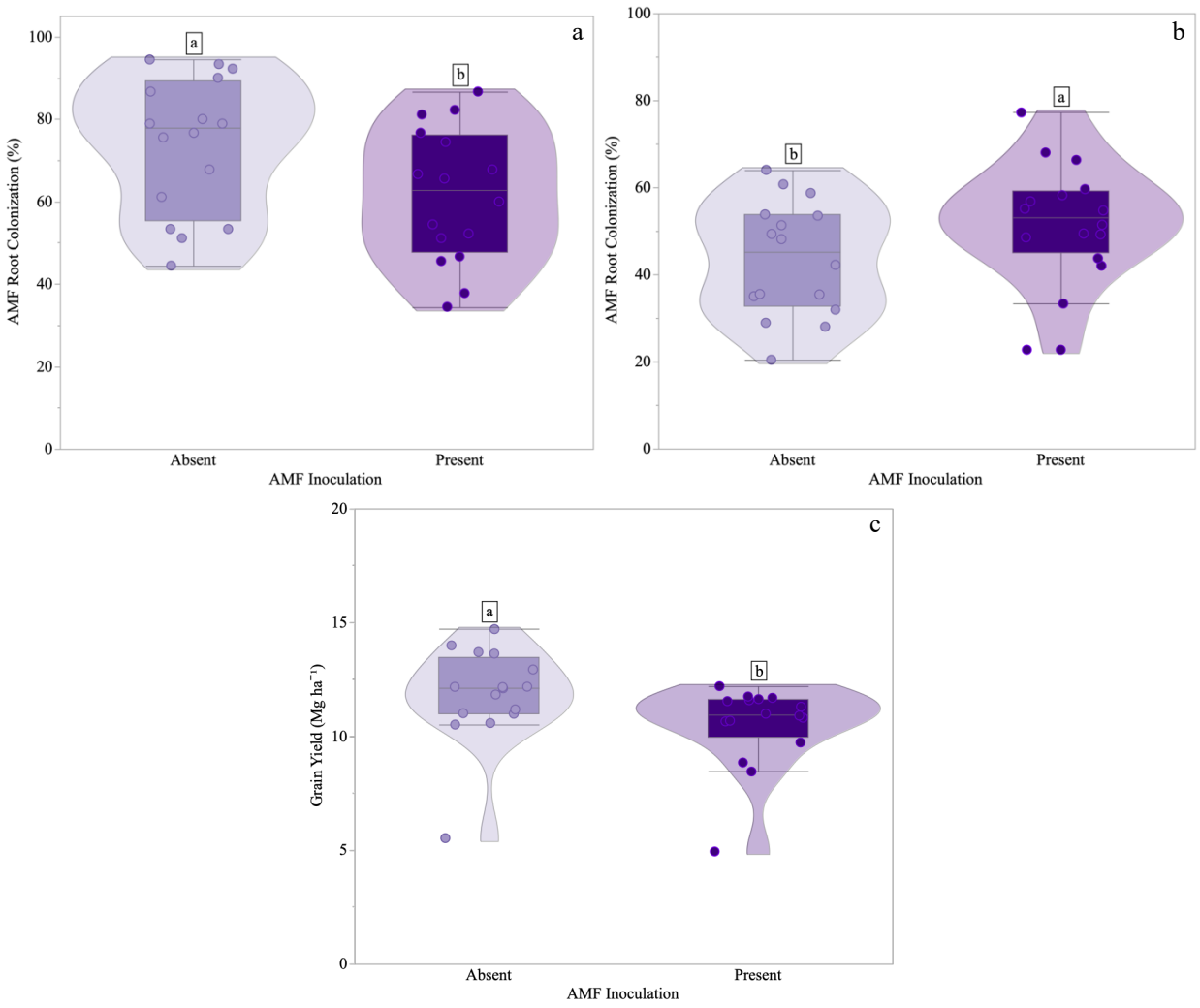


Figure B.2. Main effect of arbuscular mycorrhizal fungi (AMF) inoculation on (a) AMF root colonization at R1 in 2022, (b) AMF root colonization at R6 in 2023, and (c) grain yield in 2022 at the North Farm. Violin plots show the distribution of observations, boxes represent the interquartile range with the median indicated by the horizontal line, and points represent individual observations. Different lowercase letters indicate significant differences between AMF inoculation treatments ($P < 0.05$).

Table B.5. Summary of P-values from pairwise contrasts between arbuscular mycorrhizal fungi (AMF)-inoculated and non-inoculated treatments at each phosphorus (P) fertilization rate across years when significant P × AMF interactions were detected.

Response	Year	Phosphorus Rate (kg P ha^{-1})	P-Value
Biomass at R6	2022	0	0.001
	2022	15	0.499
	2022	30	0.146
	2022	60	0.015
P Uptake at V6	2023	0	0.837
	2023	15	0.435
	2023	30	0.079
	2023	60	0.015
Total P Uptake	2023	0	0.334
	2023	15	0.153
	2023	30	0.400
	2023	60	0.165
Grain Yield	2023	0	0.053
	2023	15	0.317
	2023	30	0.004
	2023	60	0.035
Root Colonization at V6	2023	0	0.792
	2023	15	<0.001
	2023	30	<0.001
	2023	60	<0.001
Biomass at R6	2024	0	0.529
	2024	15	0.007
	2024	30	0.129
	2024	60	0.005
P Uptake at V6	2024	0	0.100
	2024	15	0.288
	2024	30	0.779
	2024	60	0.009
Root Colonization at V6	2024	0	0.189
	2024	15	0.001
	2024	30	0.234
	2024	60	0.007

Table B.6. Summary of P-values from pairwise contrasts between arbuscular mycorrhizal fungi (AMF)-inoculated and non-inoculated treatments at each phosphorus (P) fertilization rate across depths and years when significant P × AMF or P × AMF × Year interactions were detected.

Response	Depth (cm)	Year	Phosphorus Rate (kg P ha^{-1})	P-Value
Soil Phosphorus	0-5	2022	0	0.903
	0-5	2022	15	0.859
	0-5	2022	30	0.769
	0-5	2022	60	0.444
	0-5	2024	0	0.522
	0-5	2024	15	0.608
	0-5	2024	30	0.056
	0-5	2024	60	0.034
	0-5	2026	0	0.034
	0-5	2026	15	0.971
	0-5	2026	30	0.167
	0-5	2026	60	<0.001
Soil pH	0-5	2022	0	0.405
	0-5	2022	15	0.889
	0-5	2022	30	0.486
	0-5	2022	60	0.152
	0-5	2024	0	0.332
	0-5	2024	15	0.134
	0-5	2024	30	0.395
	0-5	2024	60	0.419
	0-5	2026	0	0.675
	0-5	2026	15	0.675
	0-5	2026	30	0.675
	0-5	2026	60	1.000
Acid Phosphatase	5-15	Across Years	0	0.926
	5-15	Across Years	15	0.020
	5-15	Across Years	30	0.121
	5-15	Across Years	60	0.182

Table B.7. Summary of P-values from pairwise contrasts among sampling years within each phosphorus (P) fertilization rate and arbuscular mycorrhizal fungi (AMF) treatment for soil responses when significant P × Year interactions were detected.

Response	Depth (cm)	Phosphorus Rate (kg P ha^{-1})	Year Contrast	P-Value
Gram-Positive Bacteria	0-5	0	2022 vs. 2024	<0.001
	0-5	0	2022 vs. 2026	0.001
	0-5	0	2024 vs. 2026	0.002
	0-5	15	2022 vs. 2024	<0.001
	0-5	15	2022 vs. 2026	0.013
	0-5	15	2024 vs. 2026	0.000
	0-5	30	2022 vs. 2024	<0.001
	0-5	30	2022 vs. 2026	<0.001
	0-5	30	2024 vs. 2026	0.638
	0-5	60	2022 vs. 2024	<0.001
	0-5	60	2022 vs. 2026	0.001
	0-5	60	2024 vs. 2026	0.001
Soil AMF (NLFA)	0-5	0	2022 vs. 2024	0.099
	0-5	0	2022 vs. 2026	0.358
	0-5	0	2024 vs. 2026	0.427
	0-5	15	2022 vs. 2024	<0.001
	0-5	15	2022 vs. 2026	0.462
	0-5	15	2024 vs. 2026	<0.001
	0-5	30	2022 vs. 2024	0.050
	0-5	30	2022 vs. 2026	0.159
	0-5	30	2024 vs. 2026	0.565
	0-5	60	2022 vs. 2024	0.580
	0-5	60	2022 vs. 2026	0.428
	0-5	60	2024 vs. 2026	0.809
Soil Phosphorus	5-15	0	2022 vs. 2024	0.041
	5-15	0	2022 vs. 2026	0.180
	5-15	0	2024 vs. 2026	0.465
	5-15	15	2022 vs. 2024	0.002
	5-15	15	2022 vs. 2026	0.001
	5-15	15	2024 vs. 2026	0.823
	5-15	30	2022 vs. 2024	0.000
	5-15	30	2022 vs. 2026	<0.001
	5-15	30	2024 vs. 2026	0.240

	5-15	60	2022 vs. 2024	<0.001
	5-15	60	2022 vs. 2026	<0.001
	5-15	60	2024 vs. 2026	<0.001
Soil AMF (NLFA)	5-15	0	2022 vs. 2024	<0.001
	5-15	0	2022 vs. 2026	<0.001
	5-15	0	2024 vs. 2026	0.053
	5-15	15	2022 vs. 2024	<0.001
	5-15	15	2022 vs. 2026	<0.001
	5-15	15	2024 vs. 2026	0.022
	5-15	30	2022 vs. 2024	<0.001
	5-15	30	2022 vs. 2026	<0.001
	5-15	30	2024 vs. 2026	0.029
	5-15	60	2022 vs. 2024	<0.001
	5-15	60	2022 vs. 2026	0.003
	5-15	60	2024 vs. 2026	0.032
Soil Phosphorus	15-30	0	2022 vs. 2024	0.037
	15-30	0	2022 vs. 2026	0.001
	15-30	0	2024 vs. 2026	0.251
	15-30	15	2022 vs. 2024	<0.001
	15-30	15	2022 vs. 2026	0.003
	15-30	15	2024 vs. 2026	0.467
	15-30	30	2022 vs. 2024	0.001
	15-30	30	2022 vs. 2026	<0.001
	15-30	30	2024 vs. 2026	0.398
	15-30	60	2022 vs. 2024	0.001
	15-30	60	2022 vs. 2026	<0.001
	15-30	60	2024 vs. 2026	0.001
Soil AMF (NLFA)	15-30	0	2022 vs. 2024	<0.001
	15-30	0	2022 vs. 2026	<0.001
	15-30	0	2024 vs. 2026	<0.001
	15-30	15	2022 vs. 2024	<0.001
	15-30	15	2022 vs. 2026	<0.001
	15-30	15	2024 vs. 2026	<0.001
	15-30	30	2022 vs. 2024	<0.001
	15-30	30	2022 vs. 2026	<0.001
	15-30	30	2024 vs. 2026	0.018
	15-30	60	2022 vs. 2024	<0.001
	15-30	60	2022 vs. 2026	<0.001
	15-30	60	2024 vs. 2026	0.003

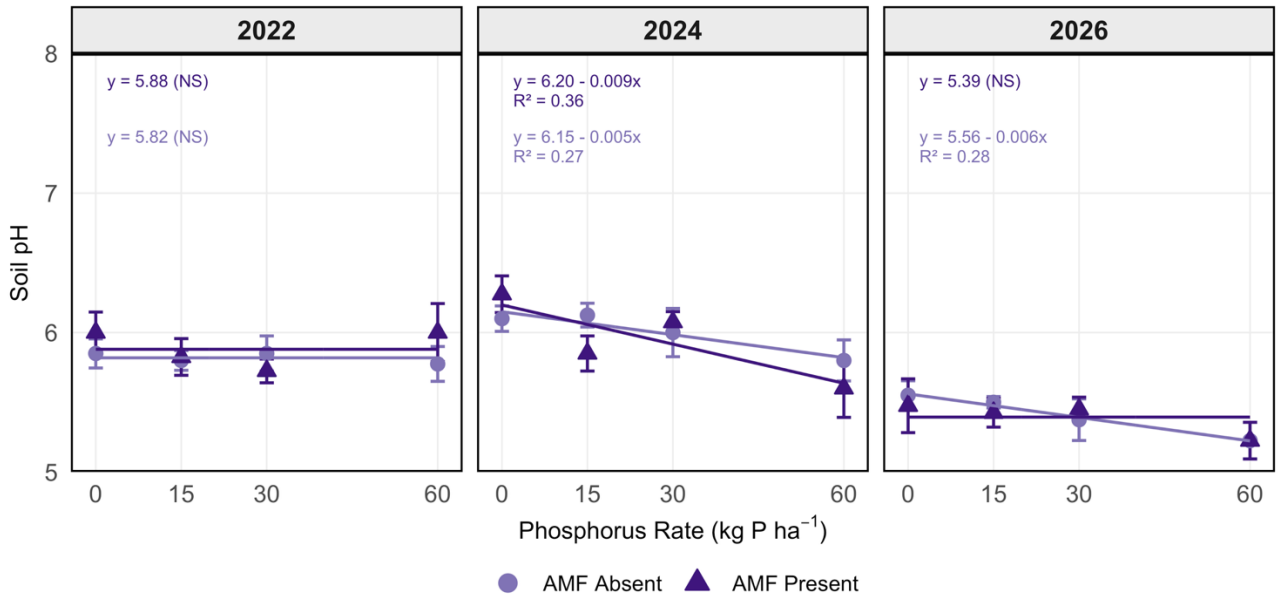


Figure B.3. Effects of phosphorus fertilization and arbuscular mycorrhizal fungi (AMF) inoculation on soil pH across sampling years (2022, 2024, and 2026) at 0–5 cm soil depth. Points represent means with standard error bars. Circles and triangles represent non-inoculated and inoculated treatments, respectively.

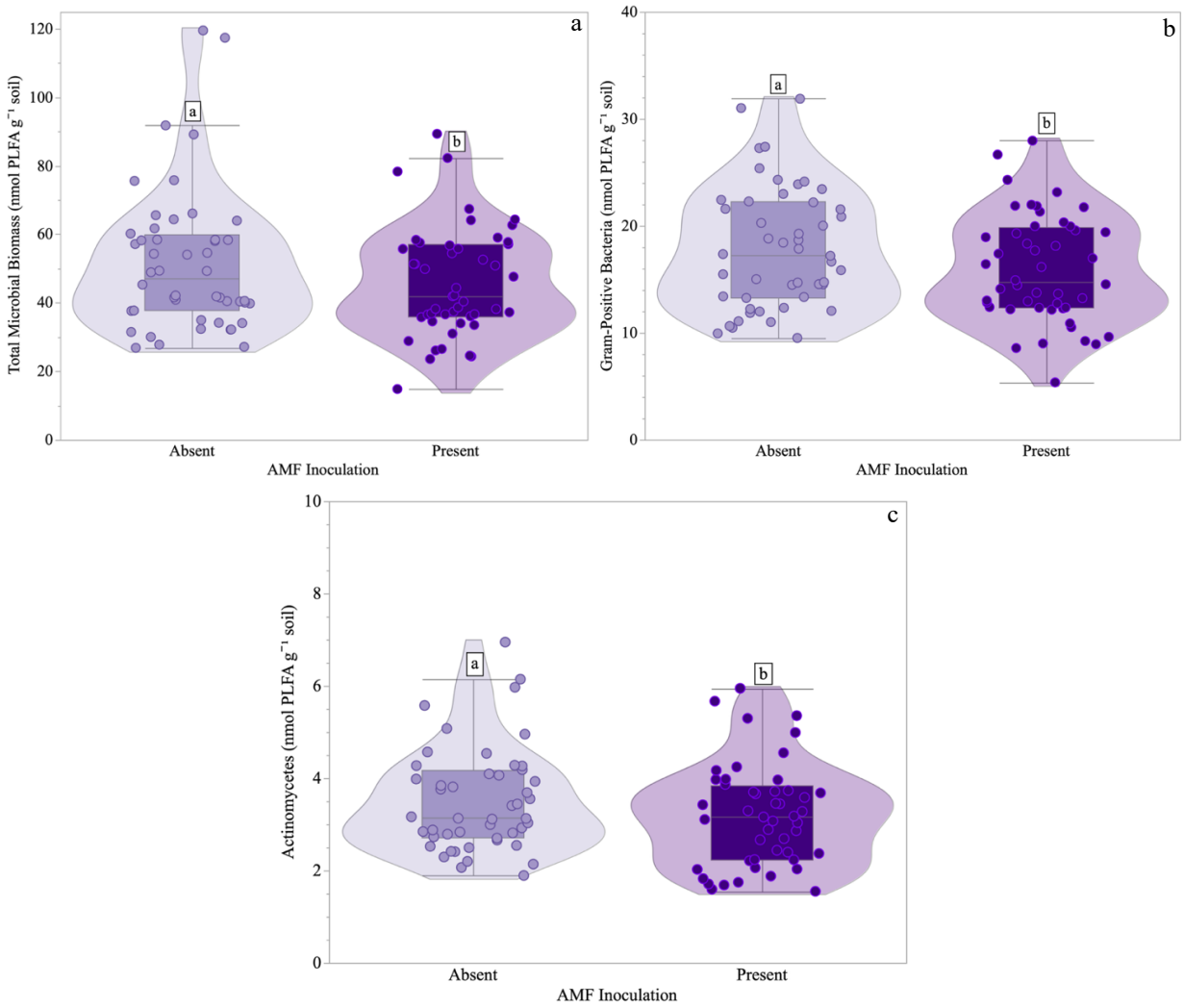


Figure B.4. Effects of arbuscular mycorrhizal fungi (AMF) inoculation on (a) total microbial biomass, (b) Gram-positive bacteria, and (c) actinomycetes at 0–5 cm soil depth. Violin plots show the distribution of observations, box plots represent the median and interquartile range, and points represent individual observations. Different lowercase letters indicate significant differences between AMF inoculation treatments ($P < 0.05$).

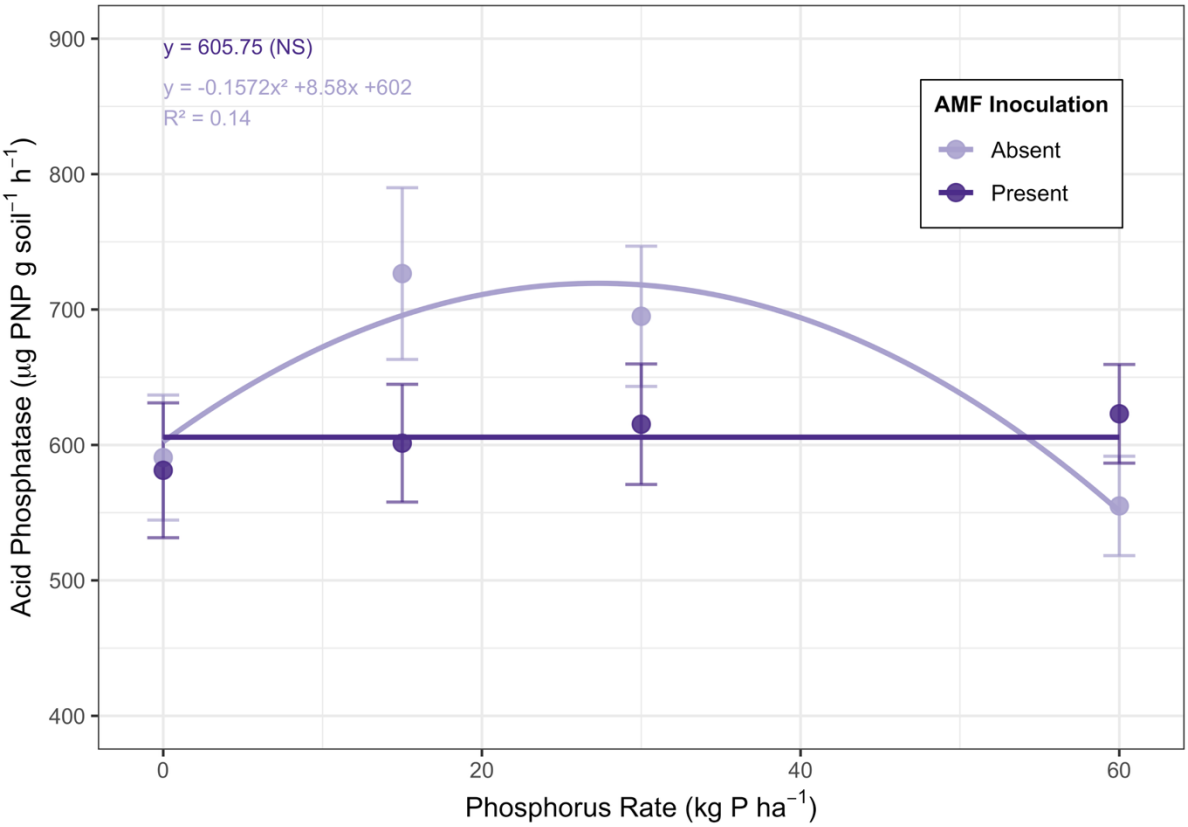


Figure B.5. Effects of phosphorus (P) fertilization and arbuscular mycorrhizal fungi (AMF) inoculation on acid phosphatase activity at 0–5 cm soil depth. Points represent means with standard error bars.

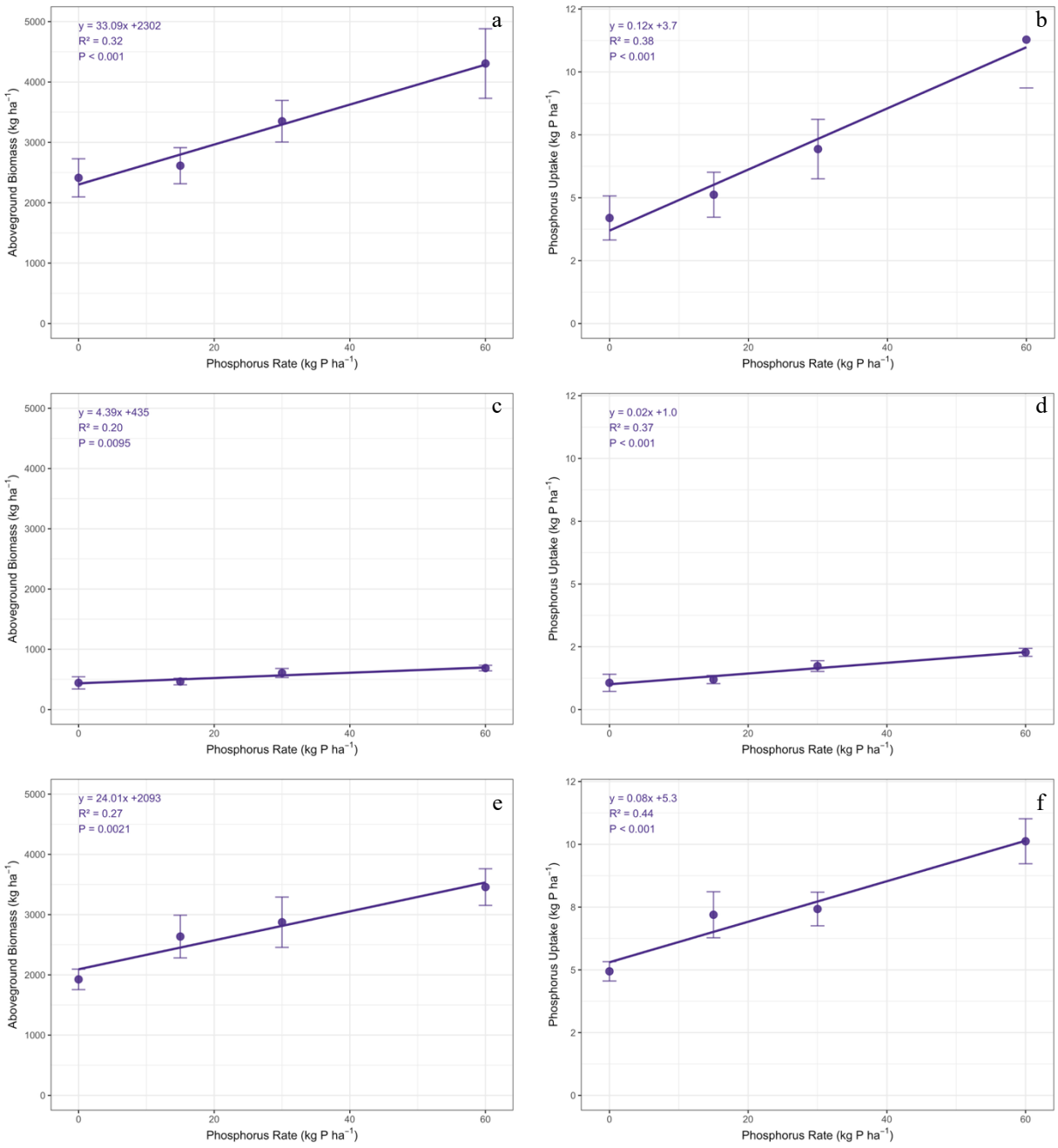


Figure B.6. Main effect of phosphorus fertilization on wheat (cover crop) aboveground biomass and phosphorus uptake (kg P ha^{-1}) at termination across years: (a-b) 2023-2024, (c-d) 2024-2025 (e-f) 2025-2026 for the North Farm field in Manhattan, KS. Points represent means with standard error bars. Solid lines indicate significant ($P < 0.05$) regression responses to phosphorus rates.