DISSERTATION

LEGUME INTEGRATION AND NUTRIENT MANAGEMENT IMPACTS ON SOIL HEALTH AND CROPPING SYSTEM PRODUCTIVITY

Submitted by

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ABSTRACT

LEGUME INTEGRATION AND NUTRIENT MANAGEMENT IMPACTS ON SOIL HEALTH AND CROPPING SYSTEM PRODUCTIVITY

Soil degradation threatens food security and livelihoods in sub-Sharan Africa and in smallholder farming systems globally. Thus, proper management of soil is critical for supporting long-term crop production and addressing multiple sustainability challenges in these agricultural systems. Integration of legumes into cropping systems, especially when combined with organic nutrient inputs, holds great promise for supporting soil health. However, legume performance and potential contributions to soil health, likely depend on legume species, management practices and inherent soil properties. The research considered here includes two field experiments in Ethiopia and a greenhouse study in Colorado to investigate the effect of: 1) integration of different legume species and residue retention on nitrogen fixation, soil health and carry-over benefits for subsequent crops, 2) distinct nutrient management strategies on soil health, crop performance and system profitability and 3) residue C input and soil N availability in regulating N fixation and associated microbial communities.

To meet these objectives, I first established a field experiment in Ethiopia with three cropping phases. In phase 1, four legume species: 1) lablab (*Lablab purpureus*), a multipurpose legume, 2) cowpea (*Vigna unguiculata*), 3) common bean (*Phaseolus vulgaris*), and 4) soybean (*Glycine max*) were planted in a randomized complete block design. After legume harvest, each plot was divided into two residue management treatments (residue removal vs. retention), and chickpea (*Cicer arietinum*) was planted uniformly across all plots. Following chickpea harvesting,

maize was planted in all plots. I used a ^{15}N natural abundance approach to quantify the amount of atmospheric N fixed by the four legume species in phase 1. I found that lablab produced the highest biomass and fixed three times more atmospheric N than soybean or common bean. Legume residue retention improved multiple soil health parameters. Chickpea and maize grain yield were highest when lablab residues were retained, and were positively correlated with improved soil health metrics (organic matter pools, aggregate stability, available P). These findings suggest that integrating high-biomass legumes (with high N-fixation capacity) together with residue retention offers great promise for rapidly improving soil health in smallholder farming contexts, with extended benefits to productivity.

For the next study, I established a separate field experiment in Ethiopia to examine the impact of three nutrient management strategies: 1) inorganic fertilizer, 2) manure, and 3) a control, with no nutrient inputs. These were combined with two legume species (common bean and soybean), in a full factorial design with four replicate blocks. Maize was planted in all plots in the following season to understand the residual impacts of the nutrient management and legume treatments on soil health and productivity. I found that manure applications substantially increased grain yield across both legumes relative to the control, while the fertilizer treatment yielded mostly intermediate values. Manure improved key soil health parameters as seen by reduced compaction (bulk density), as well as increased pH, soil organic matter fractions and available P. I also found that manure improved maize yield and net-profit relative to fertilizer and the control. My findings suggest that integrating legumes with manure application can improve soil health and crop yields, which in turn increase profitability for smallholder farmers growing legumes and maize in rotation.

Finally, in a greenhouse experiment in Colorado, I used inputs of crop (wheat, *Triticum aestivum*) residues and nitrogen fertilizer to understand how soil carbon (C) and nitrogen (N)

availability influence N fixation and associated rhizosphere microbial communities in cowpea. Cowpea was grown in pots containing soil amended with: 1) wheat residue alone, with a C:N ratio of 40 (high C:N), 2) wheat residue with urea to achieve a C:N ratio of 25 (medium C:N), 3) wheat residue with urea, for a C:N ratio of 15 (low C:N), 4) urea alone, and 5) no urea or residue inputs (control). Symbiotic N fixation was quantified using a ^{15}N isotope dilution technique, while diazotroph abundance in the rhizosphere soil and cowpea nodules were assessed using *nifH* gene abundance. Full-length 16S rRNA sequencing was also used to profile rhizosphere bacterial communities. Cowpea growth was greatest in the urea and medium C:N treatments. Cowpea grown under the medium C:N fixed the highest amount of N, roughly doubled that observed in the other treatments. Additionally, the medium C:N treatment resulted in the greatest nodulation and the highest percentage of N derived from the atmosphere. In contrast, the low C:N and urea treatments appeared to inhibit nodulation and N fixation. Residue additions increased dissolved organic C and permanganate oxidizable C relative to urea and the control. Bacterial Shannon diversity and *nifH* gene abundance in the rhizosphere were highest in high C:N treatment, and the lowest under urea. Rhizosphere bacterial community structure differed among residue C and N inputs (PERMANOVA, $p = 0.001$), with both medium and high C:N inputs enriching in N-fixing bacteria, *Rhizobium* and *Neorhizobium*. These genera were positively correlated with labile C pools and negatively associated with NO_3^--N. My findings suggest that balancing residue C inputs with small N inputs (medium C:N) creates favorable rhizosphere conditions for symbiotic N fixation in cowpea nodules and the rhizosphere, providing a mechanistic basis for residue management strategies that support soil health and sustainable N management.

The work presented here has important implications for smallholder farming systems. Overall, I found that selecting high-biomass, high N-fixing legumes, retaining residues, and

prioritizing high quality organic nutrient inputs can improve soil health while enhancing crop productivity and profitability. These strategies offer a practical and scalable approach to soil health-centered intensification in resource limited environments.

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DEDICATION

This dissertation is dedicated to Professor Jessica G. Davis, my long-time mentor, who passed away just weeks before my dissertation defense. Thank you, Professor Davis, for letting me join CSU for this PhD journey and for all love that you showed to me and my family. Your loss has left a deep ache in my heart that may last long time. You will always have a special place in my heart.

Professor Davis was named a Legend of Soil and Crop Science Department weeks before her passing.

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CHAPTER 1: INTRODUCTION

The problem we want to solve

Soil provides more than 98% food for humanity, and provides other essential ecosystem services including nutrient cycling, biodiversity conservation, water and climate regulation (Kopittke et al., 2021). Yet, soils across the globe are experiencing rapid degradation, posing a major threat to agricultural sustainability and human wellbeing. Given the widespread nature of soil degradation, it is considered as a "global pandemic" (DeLong et al., 2015) and projected to affect nearly 90% of the world's soils by 2050 (Kraamwinkel et al., 2021), implying that nearly all global ecosystems and populations will be directly impacted, if current trends continue. Soil degradation is manifested as declining soil organic matter (SOM), erosion, compaction, nutrient depletion, acidification, salinization, and a general loss of biological function, which has far-reaching social and economic consequences contributing to reduced productivity and increased food and nutritional insecurity, which is of particular concern in regions dominated by smallholder faming systems (Kopittke et al., 2019). Every year, the cost associated with soil degradation exceeds €50 billion in the European Union (Veerman et al., 2020), over $68 billion in sub-Saharan Africa (SSA)(Mesele et al., 2025), with about $4.3 billion in Ethiopia alone (Wendimu, 2021).

Sub-Saharan Africa represents one of the global hotspots of soil health decline. About 46% of agricultural land is degraded in Africa (Tetteh, 2021). Soil degradation in SSA results from continuous cultivation and monocropping, low fertilizer use, and limited use of organic nutrient inputs, such as residue and manure, among other factors (Chivenge et al., 2011; Mesele et al., 2025). Ethiopia exemplifies these challenges, with 43% of soils affected by acidity, extensive SOM depletion, and persistent nutrient mining and imbalances (Regasa et al., 2024). National nutrient balance assessments estimate annual depletion rates of -23 kg N, -9 kg P, and -7 kg K ha^{-1} $year^{-1}$ from smallholder farms across the country, reflecting continuing nutrient mining driven by low external inputs (van Beek et al., 2016). Continuous monocropping and crop residue removal, commonly practiced by smallholder framers in Ethiopia, further accelerates SOM loss, reduces nutrient cycling, soil biological activity and aggregate formation. Limited use of mineral fertilizer driven by high cost and low accessibility combined with the low application of organic nutrient sources such as manure further worsens nutrient deficiencies on smallholder farms in the country. Consequently, cereal-based smallholder cropping systems across the country exhibit low productivity, contributing to the ongoing challenges of national food security.

In this context, enhancing soil health on smallholder farms has become a priority for improving crop productivity, sustainability and farmers livelihoods in Ethiopia. Soil health refers to the continued capacity of the soil to function as a vital living ecosystem that sustains plants, animals, and humans (Lehmann et al., 2020). Regenerative agricultural management strategies that increase C inputs to the soil and enhance nutrient cycling, particularly through legume integration in the cropping system and the use of organic amendments, may have the potential to reverse soil degradation and support sustainable intensification in smallholder farming systems (Kuyah et al., 2021; Sileshi et al., 2025).

The solution we suggest

Legumes play a crucial role in improving soil health through their symbiotic relationship with the bacterial genus *Rhizobia,* which enables them to fix atmospheric N, the most limited nutrient in most agricultural systems. Legume N fixation can reduce the need for costly synthetic fertilizers and supports soil health, especially important for low-input, smallholder farming systems. Integrating legumes in cereal cropping systems, contribute high-quality residues that can regenerate SOC, improve soil structure, water retention and microbial activity, all of which are critical for enhancing productivity (Qiao et al., 2024; Tripolskaja et al., 2023; van der Pol et al., 2022). Legume residues may also further regulate rhizosphere microbial composition, enhance *Rhizobium* survival in the soil, and improve nodulation and symbiotic N-fixation in legume crops (Bian et al., 2024; Han et al., 2020; Poole et al., 2018). However, the benefit provided by legume may vary with management and environmental factors such as soil pH, N and C availability , water and temperature (Dai et al., 2024; Xie et al., 2021b; Yeremko et al., 2025). In addition, the benefits provided by legumes may vary substantially among species due to differences in N fixation potential, biomass production, residue quality, root depth and rhizosphere interactions (Liu et al., 2019; Palmero et al., 2022). High-biomass legumes may return more N and C to the soil, leading to greater improvements in soil health and productivity, while the low biomass legumes often support greater food production, but may have limited benefits for soil health and subsequent crops (Kehoe et al., 2024; Panagea et al., 2025). Therefore, selecting appropriate legume species and optimizing their management are essential for maximizing their contributions to soil health and crop productivity within the smallholder framer's context in Ethiopia and beyond.

Beyond the type of legume to plant, the residual effects of legumes and residue management on following crops such as maize are also critical to understand in smallholder systems. Because smallholder farmers often remove legume residues, their potential impacts on soil health and crop productivity are often not fully realized (Mungai et al., 2016; Sileshi et al., 2025). To develop integrated and cost-effective nutrient management strategies for smallholder farmers in SSA, we needed to understand how legumes and various nutrient management strategies affect soil health, productivity and smallholder's livelihood.

Along with their direct contributions to the fertility of soil and the productivity of subsequent crops, legumes benefit in smallholder systems are mediated by soil health and nutrient status, which regulate symbiotic interactions in the rhizosphere (Yeremko et al., 2025). In particular, legumes influence rhizosphere microbial community composition, yet the mechanistic pathways by which residue-derived C inputs and soil N availability interact to regulate the rhizosphere microbiome especially diazotrophic communities remain insufficiently understood (Xie et al., 2021a; Zhao et al., 2024). Since symbiotic N fixation is both a plant and microbial regulated process, the balance between microbial C and N demands represents a key control on diazotroph abundance and N fixation, but this regulatory mechanism remains underexplored across many legume systems (Duan et al., 2025; Liang et al., 2023). High soil N availability may suppress legumes nodulation, reduce *Rhizobium* and nifH gene abundance in both soil as well as nodules, and inhibit symbiotic N-fixation, whereas low N availability combined with increased C inputs may stimulate microbial activity, promote proliferation of diazotroph such as *Rhizobium* in the rhizosphere soil and enhance N fixation in legumes. Carbon input in the rhizosphere can reshape diazotrophic communities in the soil and influence N fixation (Xie et al., 2021b). Understanding these microbial regulatory pathways is essential for optimizing legume-based soil fertility strategies.

Given these challenges and knowledge gaps, there is a strong need for a comprehensive, multi-scale investigation that links legume species differences, soil nutrient inputs, microbial processes, and crop productivity across field and greenhouse environments. In Chapter 2, I examined how legumes species vary in their N fixing capacities and how this influences soil health and subsequent crop performance in smallholder systems in Ethiopia. I quantified N fixation capacity of different legume species, (lablab, soybean, common bean and cowpea), using the ^{15}N

natural abundance technique in the field. Then a chickpea crop was grown with split-plot residue retention (vs. residue removal) to assess short-term carryover benefits of legume species. Finally, a maize crop was subsequently planted to evaluate the longer-term carry-over effects of different legumes and residue management. Across these phases, I quantified changes in key soil health indicators, including particulate organic matter (POM), permanganate oxidizable carbon (POXC), soil organic matter (SOM), bulk density, pH, and available phosphorus, while also measuring nodulation and crop yield. This chapter highlights the potential of high biomass legumes and residue retention to improve soil health and productivity of subsequent crops.

Chapter 3 evaluates how distinct nutrient management strategies influence legume performance, soil health and productivity of subsequent crops, as well as overall cropping system profitability. The economic analysis provided insight into the relative affordability and agronomic value of manure and fertilizer for smallholder farmers. This chapter highlights the potential of nutrient inputs to restore degraded soils and support legume-cereal systems in low-input environments, while also providing insights into profitable nutrient management options for smallholders.

In Chapter 4, I investigate the mechanistic pathways underlying residue-driven shifts in the rhizosphere microbiome and biological N fixation. Using a greenhouse experiment, I manipulated residue C inputs and soil N availability to determine their influence on diazotroph abundance, enzyme activity, dissolved organic carbon (DOC), soil N, nifH genes and bacterial community composition using 16S rRNA sequencing. Cowpea served as the test crop, and N fixation was quantified with the ^{15}N isotope dilution technique, enabling precise assessment of changes in symbiotic N fixation. This chapter reveals how residue-derived C inputs and soil N availability, regulate bacterial community assembly, nifH gene abundance, and N-fixation potential, providing mechanistic explanations for the field outcomes observed in Chapters 2 and 3.

Together, the three chapters demonstrate that selecting high-biomass, high N-fixing legumes, retaining their residues, and strategically applying organic nutrient inputs can substantially improve SOM pools, microbial functioning, and crop productivity in smallholder farming systems. By linking field-scale agronomic outcomes with mechanistic insights from greenhouse experiments, the dissertation offers evidence-based strategies for developing soil fertility management strategies that enhance soil health while reducing reliance on costly external inputs and strengthening the resilience of smallholder farming. Ultimately, this work contributes to the broader goals of sustainable intensification and improved food security in Ethiopia and other regions with similar contexts.

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CHAPTER 2: INTEGRATION OF HIGH BIOMASS LEGUMES AND RESIDUE RETENTION IMPROVES SOIL HEALTH AND HAS CLEAR CARRY-OVER BENEFITS FOR PRODUCTIVITY[1]

2.1 Introduction

Legumes are widely considered an essential element for the sustainable intensification of agricultural systems, as they can enhance soil biodiversity, support multiple ecosystem services and improve soil health (Palmero et al., 2022; Shukla et al., 2024). Legumes reduce reliance on synthetic nitrogen (N) fertilizers through their symbiotic relationship with *Rhizobium* bacteria, which fix N from the atmosphere. The benefits of legume N fixation extend beyond the legume crop and can contribute to meeting N needs of succeeding crops through decomposition and mineralization of legume residues and rhizodeposits (Liu et al., 2019). Because of their potential to provide N, legumes integration is particularly important for smallholder farming contexts, where access to nutrient inputs is often limited. Including legumes in cropping systems can also help to mitigate poor soil health by breaking disease cycles and contributing to soil organic matter (SOM) formation (Lal, 2017; van der Pol et al., 2022). However, the amount of N benefit and ability of legumes to help restore soils depends on the legume species planted and other factors, such related to management and environmental conditions (Gou et al., 2023).

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Options for farmers to integrate legumes into their cropping systems are diverse and depend on farmer objectives and knowledge. Soybean (*Glycine max*), common bean (*Phaseolus vulgaris*), chickpea (*Cicer arietinum*) and cowpea (*Vigna unguiculata*) are among the 10 most cultivated legume crops and represent important dietary staples in many regions (FAO, 2025).Options for farmers to integrate legumes into their cropping systems are diverse and depend on farmer objectives and knowledge. Soybean (*Glycine max*), common bean (*Phaseolus vulgaris*), chickpea (*Cicer arietinum*) and cowpea (*Vigna unguiculata*) are among the 10 most cultivated legume crops and represent important dietary staples in many regions (FAO, 2025). In addition to these crops, lablab (*Lablab purpureus*), a multipurpose legume crop used for human consumption, animal feed and soil health management is gaining considerable attention within smallholder farming systems across much of East Africa, including in Ethiopia (Workneh et al., 2024). Despite the many benefits offered by legumes, inconsistent effects and a lack of knowledge on management can limit their adoption. Legume species vary in their quality and quantity of crop residue produced and the potential rotation benefit (e.g., provision of N) for subsequent crops depends greatly on management (Snapp et al., 2019). For smallholder farmers to best leverage and make informed decisions about legume integration in their cropping systems, more information is needed about how different legume species contribute to soil health and benefit subsequent crops.

Legume N fixation is influenced by various environmental factors, including soil N availability (Dai et al., 2024), water, temperature, pH (de Borja Reis et al., 2021), and other factors that affect *Rhizobium* metabolic activity and nodule formation (Porter et al., 2024; Santachiara et al., 2019). The N-fixing potential of annual legumes also varies across species (Liu et al., 2019; Palmero et al., 2022) and is thought to be largely determined by legume productivity (i.e., biomass production; Sanginga et al., 1996). Although some studies on N fixation have been conducted in

East Africa, there is still a paucity of N fixation data and subsequent soil health impacts in the region, including Ethiopia. Studies from Kenya and Ethiopia have compared biological N fixation of legumes and reported considerable variation in their N fixation capacity (Haque & Lupwayi, 2000; Ojiem et al., 2007). For example, lablab was reported to fix higher amounts of N than other legume species in one study (McDonald et al., 2001), whereas Okogun et al. (2005) reported lower N fixation of lablab compared to soybean. Given the uncertainties associated with N-fixation and overall legume performance across different species and contexts, it is difficult to make management recommendations regarding N availability and other soil properties that regulate crop productivity. Thus, more research is needed to understand the potential N benefit of different legumes to subsequent crops, and longer-term soil health impacts within smallholder farming contexts.

Beyond differences in legume performance and N-fixing capacity, management of legume residues can also greatly influence their impact on soil health. Smallholder farmers often remove crop residues from the field for use as livestock feed or fuel (Turmel et al., 2015) or burn them to clear the field for planting (Kumar et al., 2023). Residue removal can negatively impact soil health by reducing SOM, aggregate stability, nutrient availability, and other soil properties (Battaglia et al., 2021; Olayemi et al., 2022). At the same time, retaining residues can improve soil health by feeding soil organisms, contributing to SOM fractions, and recycling nutrients to support soil fertility and overall productivity (Hu et al., 2023; Mugi-Ngenga et al., 2022). This may be especially important for legume residues, that often contain high concentrations of N and thus can contribute greatly to the growth of subsequent crops. Beyond direct impacts on soil health, residue management can also influence nodulation and biological N fixation of subsequent legumes grown in rotation. Previous studies reported that residue retention increased N fixation across a variety of legume species compared to residue removal, but the results are quite variable (Bian et al., 2024; Shah et al., 2003; Xie, Li, et al., 2021). While proper management of crop residues can provide significant long-term economic and environmental benefits (Turmel et al., 2015), there is a need to better understand the effect of residue retention on soil health and subsequent crops in rotation and how these effects vary across different environments and cropping systems.

Based on the great potential of legume integration and the knowledge gaps outlined above, the aims of this study were to: 1) explore the performance and N-fixing capacity of different legume species and their potential to support key soil health parameters and 2) evaluate the effect of legume residue retention (vs. removal) on soil health and subsequent crop yields within a legume-maize rotation . We hypothesized that: 1) legume species with higher biomass production and N-fixing capacity will have greater potential to enhance soil health and improve subsequent crop yields compared to low biomass producing species, and 2) residue retention will further amplify the effect of highly productive species.

2.2 Materials and Methods

2.2.1 Study site

The experiment was conducted at Wolkite University's Plant Science Research Farm, located in Cheha district, Gurage zone, central Ethiopia (8°12'54" N and 37°48'30" E). With an elevation of 1929 m above sea level, the region is characterized by topography of gentle rolling hills. Agriculture in the district is dominated by subsistence farming with enset, teff, maize, wheat, barley, beans, chickpeas, peas, yams, kale and khat as the predominant crops in the area (Nahusenay et al., 2024). The annual average temperature and rainfall are 27 °C and 986 mm yr^{-1}, respectively (based on data from 2005-2015). The area has a bimodal rainfall distribution, with the "Kiremt" (June to September) main rainy season and the "Belg" (March to May) short rainy season. The field site is relatively flat with soils dominantly classified as Typic Haplusters subgroup which are high in cation exchange capacity (between 46 and 66 $cmol_c$ kg^{-1}) (Yitbarek et al., 2018). Soils at the site have a clay texture (11% sand, 21% silt and 68% clay), are moderately acidic (pH = 5.7), have organic matter content of 3.6%, and low total N (0.16 %) and available P (2.69 mg kg^{-1}).

2.2.2 Field experimental design and management

A field experiment was established in May 2022 to evaluate the potential of different legume species to improve soil health and performance of subsequent crops. The field had a history of maize and teff (*Eragrostis tef)* production for at least 9 years prior to experiment implementation. In preparation for the experiment, land was plowed by oxen to a depth of roughly 25 cm and leveled. In the first phase we planted four legume species: 1) lablab (*Lablab purpureus*), 2) cowpea (*Vigna unguiculata*), 3), common bean (*Phaseolus vulgaris*), and 4) soybean (*Glycine max*). Treatments (legume species) were applied to plots (6 m x 3 m) and arranged in a randomized complete block design with four replicate blocks. All plots were separated by a buffer zone of 50 cm between treatments. Legumes were planted with a spacing of 40 cm between rows and 10 cm between plants within rows. Each plot also contained a microplot (1.2 m x 1 m) with kale (*Brassica carinata* A. Braun; variety yellow Dodolla) as a reference plant to evaluate biological N fixation, based on ^{15}N natural abundance. Kale was sown with a spacing of 30 cm between rows and 20 cm within row in the ^{15}N micro-plots. All plots received 46 kg P_2O_5 ha^{-1} (as triple super phosphate) at planting. Based on past experience with legumes at this field site and limited availability of inoculants to smallholder farmers in the region, no *Rhizobium* was applied to legumes planted in the first phase of these study. Both the ^{15}N microplots and main plots were treated similarly and planted and harvested on the same dates.

In November 2022, following the harvest of legumes from the first phase, each plot was prepared and leveled by hand hoeing and then sub-divided into two residue management treatments (residue removal vs. retention) resulting in a split-plot design with a total of 32 plots (3 m x 3 m) across the four blocks for the second phase. The four legume species previously harvested in the first phase, which had been randomly assigned within each block, served as the main plot, and the two residue management treatments were randomly assigned within each of those legume plots. For the residue removal plots, all plants were cut at a height of ~3 cm from the ground and all plant biomass was removed from the field. For the residue retention plots, harvested legume residue from the first phase was equally divided among treatment plots, so that all plots in the same residue retention treatment received uniform inputs of the corresponding legume. Residues were incorporated into the soil to a depth of 20 cm using hand hoeing. The quantities of residue returned to the soil ranged from 1.11 to 4.44 Mg ha^{-1} depending on the legume treatment (Table 2.1). Immediately following residue incorporation, chickpea (*Cicer arietinum;* variety Natoli) was planted across the entire field with a spacing of 30 cm between rows and 10 cm within rows. Within each sub-plot, a new 1.2 m x 1 m ^{15}N micro-plot was established, with kale again planted as a reference crop. Given the greater reliance of chickpea on specialized inoculum, and in accordance with common practices in the region, all plots were inoculated at planting with a commercial inoculant (*Mesorhizobium* strain CP-M41) following the manufacturer's recommendation (Menagesha Biotech Industry P.L.C, Addis Ababa, Ethiopia). All plots received 46 kg P_2O_5 ha $^{-1}$ (as triple super phosphate) at planting. Chickpeas and kale were harvested from each plot at the same time in May 2023.

The third phase was initiated in early June of 2023 after the harvest of chickpeas and subsequent removal or incorporation of chickpea residues in the plots, as designated at the start of

Phase two. The quantity of chickpea residue returned to plots ranged from 1.78 to 2.3 Mg ha^{-1}, again depending on treatment (Table 2.1). Residue was again incorporated into the soil to a depth of 20 cm via hand hoeing. In June 2023, maize (*Zea mays;* variety Limu) was planted in all plots with a spacing of 75 cm between rows and 25 cm within rows to evaluate the residual effect of legume species and their residue return on yield of the succeeding maize crop. The recommended blended NPS fertilizer input for maize in the study area was 19 kg N, 38 kg P_2O_5 and 7 kg S ha^{-1}. To avoid severe yield limitations and allow more sensitive treatment effects, one third of the recommended NPS fertilizer was applied to each plot at planting. Two seeds were planted per hill and thinned to one plant per hill seven days after emergence.

Weed control and cultivation were implemented across all plots within each phase uniformly. Hand hoeing was done to control weeds twice for each phase. Bravo 5 EC (lambda-cyhalothrin 2.5%) was applied at a rate of 400 ml ha^{-1} to control fall armyworm in maize on August 2 and September 27, 2023.

2.2.3 Soil sampling and preparation

Soil samples were collected shortly before maize planting to evaluate treatment effect on soil health and to examine how these effects relate to maize yield. Five sub-samples per plot were collected to a depth of 20 cm using a soil auger and combined into one composite sample per plot for analysis. Two undisturbed core samples were also taken to a depth of 5 cm from each plot to determine bulk density and aggregate stability, as described below.

Key soil health metrics were evaluated using a soil health toolkit designed for smallholder contexts (https://smallholder-sha.org; Nyamasoka-Magonziwa et al., 2020) that relies on low-cost methods adapted from common laboratory procedures and validated to enable research by smallholders and the local research organizations they collaborate with. These tests included pH, permanganate oxidizable carbon (POXC), particulate organic matter (POM), aggregate stability, and bulk density. Available P and total SOM were also measured using standard laboratory procedures (described below).

In the laboratory, the 0-20 cm samples were air-dried in a dust-free environment, passed through a 2 mm sieve after removing visible roots, coarse organic matter and rocks, and stored for subsequent analysis. Soils collected for bulk density and aggregate stability were weighed, and a representative sub-sample (~10 g) was dried at 105°C for determination of bulk density. The remaining field-moist soil was combined to form one composite sample per plot, and soil clods were gently broken along the natural planes of weakness to pass through an 8 mm sieve and then air-dried for determination of aggregate stability.

2.2.4 Physicochemical soil analysis

Aggregate stability was determined by wet-sieving using the method described by Nyamasoka-Magonziwa et al. (2020) and adapted from Elliott (1986). A subsample of 70 g from air-dried, 8 mm sieved soil was placed on top of a 2 mm (2000 µm) sieve in a small basin, and water was added until the soil was submerged. The soil was left for 5 minutes to slake and then wet-sieved by moving the sieve in and out of the water 50 times over a 2-min period. Soil remaining on the 2 mm sieve was rinsed into a pre-weighed aluminum pan using a rinse bottle. The portion that passed through the sieve was transferred to a 250 µm sieve, and the process was repeated to isolate three water-stable aggregate fractions: large macroaggregates (>2000 µm), small macroaggregates (250–2000 µm), and a third fraction comprised of microaggregates along with free silt and clay particles. These fractions were then dried at 105 °C in an oven, and the mass of soil passing through the 250 µm sieve was determined by difference. The aggregate mean weight diameter (MWD) of the aggregate size classes was calculated based on the total initial weight of 70 g of dry soil following equation 1 (van van Bavel 1950).

$$MWD = \sum_{i=1}^{n} (wi \; X \; di) \tag{1}$$

Where *wi* is the total sample weight proportion in the ith aggregate size fraction, and di is average diameter (µm) of ith fraction.

Particulate organic matter was quantified using a density flotation technique with deionized water (Nyamasoka-Magonziwa et al., 2020). A coarse POM fraction (250-2000 µm) was separated from the soil by flotation in water and decanting onto a filter, which was then oven-dried at 60°C and weighed.

Permanganate oxidizable carbon was determined based on labile soil organic C oxidation by potassium permanganate (Nyamasoka-Magonziwa et al., 2020; Weil et al., 2003) and was estimated by measuring absorbance at 525 nm using a Hanna model HI-717 phosphate high range colorimeter (Hanna Instruments Corp., Woonsocket, RI, USA).

Soil pH was measured through a suspension of air-dried soil and water at a ratio of 1:2.5 and determined using a pH meter. SOM content was determined through oxidation using potassium dichromate, following the method of Walkley and Black (1934). Plant-available P in the soil samples was extracted using 0.5 M sodium bicarbonate solution and measured colorimetrically using ammonium molybdate (Olsen, 1954).

2.2.5 Quantifying legume N fixation

At physiological maturity, in both Phase 1 and Phase 2, five legume plants from each treatment plot were harvested and separated into straw and grain. Kale from the microplots was also harvested on the same date along with the legumes. The sample materials were oven dried at 60 °C and ground to pass through a 2 mm sieve. Ground samples were further pulverized to a fine powder in a ball mill and then combined into a composite sample for analysis based on the relative mass of straw and grain. Subsamples (5 ± 0.3 mg) of the finely ground plant materials were weighed out into tin capsules to measure ^{15}N content (atom% ^{15}N), C (% C) and N (% N) on a PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK) at the University of California, Davis Stable Isotope Facility. The percentage of N derived from the atmosphere (% Ndfa) was estimated for the legume crops using the following equations 2 (Shearer & Kohl, 1986; Unkovich et al., 2008):

$$\% \, Ndfa = \frac{\delta^{15}Nref - \delta^{15}Nleg}{\delta^{15}Nref - B\ value} \text{ x } 100 \tag{2}$$

Where δ^{15}Nref is ^{15}N natural abundance of the reference plant (kale), δ^{15}Nleg is ^{15}N natural abundance of legume crops, B value is defined as the δ^{15}N value of the N_2-fixing plant when fully dependent upon N_2 fixation. B value used for lablab, cowpea, soybean, chickpea, and common bean grown on an N free hydroponic culture and fully dependent on biological N fixation were -1.09, -1.61, -1.83, -1.75 and -2.16, respectively(Unkovich et al., 2008).

The total amount of N_2-fixed (kg ha^{-1}) by legume species was estimated using equation 3 and 4.

$$N\ fixed = \frac{\% \, Ndfa\ x\ Sample\ N\ (kg/ha)}{100} \tag{3}$$

Where Sample N is total N content in the shoot and grain of legume species, which was calculated as follows:

$$\text{Sample N content} = \text{Sample N concentration (\%) x Sample dry matter (kg/ha)} \tag{4}$$

2.2.6 Nodule sampling and measurements

Legume nodule sampling was done in both Phase 1 and Phase 2 by excavating the roots of five randomly selected plants from each plot at the mid-flowering stage of the crop. Soil blocks measuring 10 cm in length, 40 cm in width, and 30 cm in depth, containing the root systems, were excavated using a spade and shovel (Yong et al., 2018). The bulk soil was gently removed by hand, and the entire root system was placed in nylon nets with a mesh size of 0.6 mm, soaked in water, and thoroughly washed to eliminate adhering soil, allowing the separation of nodules from the roots. The mean number of nodules per plant was recorded by counting nodules from each of the five plants. The nodule dry weight per plant was determined by removing the nodules from the roots and drying them at 60°C in an oven before weighing.

2.2.7 Grain yield and related parameters

Grain yield, aboveground dry biomass, and harvest index were measured in all three phases. Plant height and branch number were measured during the flowering stages for the second phase. Plant height was measured from ten randomly selected plants from the ground surface to the top of the main stem. The branch and pod numbers per plant were counted based on ten randomly selected plants from each plot at maturity. At harvesting, sub-samples (1 m^2) from each plot were dried at 60°C in an oven to determine aboveground biomass (Mg ha^{-1}). Grain yield was determined by harvesting all plants in each plot. After manual threshing, the grain was weighed, and yield was converted to a per hectare basis. A sub-sample of the grain was utilized for determining grain moisture content, adjusting the actual yield to 10 and 12 % moisture content for pulses and maize, respectively. Harvest index (HI) was calculated as the ratio of grain yield to total aboveground dry biomass.

2.2.8 Statistical analysis

A linear mixed effect model was fitted for all analyses to understand the effects of legume species and residue retention for all plant performance and soil health metrics. For Phase 1, legume species was treated as a fixed effect while blocks were considered as a random effect. For Phase 2 (chickpea) and Phase 3 (maize) legume species, residue management and their interaction were treated as fixed effects. Due to the split plot design, block and legume plot were included as random effects. Data were checked for model assumptions, normality using Q-Q and heteroscedasticity using residual vs fitted plots. Tukey adjusted pairwise comparison was used to compare means when significant differences were detected in ANOVA. Analyses were done in R version 4.3.1 using emmeans, lme4 and lmerTest, packages (R Development Core Team, 2023). All analyses used an α value of 0.05. Simple linear regression was also used to examine correlations between specific soil health parameters and maize grain yield.

2.3 Results

2.3.1 Effect of legume species on biomass production, yield, and N-fixation

The four legume species showed marked differences in aboveground biomass production, grain yield, and harvest index in Phase 1 (Figure 2.1 and Table 2.2). Aboveground biomass yield of lablab was near 6 Mg ha^{-1}, more than double that of soybean and common bean and 82% higher than cowpea. The trend changed for grain yield and harvest index, such that grain yield of common bean was 1.3 Mg ha^{-1}, which was 45 % higher than that of cowpea and soybean and 18 % more than lablab. Harvest index increased in the order of common bean > soybean > cowpea > lablab.

Nodule number, nodule dry weight, % N derived from the atmosphere, and amount of N fixed were also significantly influenced by legume species (Figure 2.1; Table 2.2). The highest nodule number and nodule dry weight were found in lablab. The number of nodules for lablab was 35% and 102% higher than cowpea and soybean, respectively. Though common bean had a similar number of nodules to lablab, these nodules were small and total nodule dry weight for common bean was the lowest among all legume species. Both parameters for cowpea were higher than soybean (Table 2.2). The percentage of N derived from the atmosphere was 92 % for lablab, which was statistically similar to cowpea (Table 2.2). The lowest % N derived from the atmosphere of 48 % was measured in soybean. Amount of N fixed by lablab was 123 kg ha $^{-1}$, 64 % higher than cowpea and roughly three times that of common bean and that of soybean (Figure 2.1C).

2.3.2 Impacts of residue addition and previous legume species on chickpea performance

Both residue management and the previous legume species had significant impacts on chickpea performance and N-fixation metrics (Figure 2.2; Table 2.3). Residue retention from the previous legumes increased chickpea height, pod number per plant, aboveground biomass and grain yield compared to residue removal. However, a significant interaction with legume species for aboveground biomass and grain yield indicates that this effect was not uniform across legume species. For example, residue retention roughly doubled grain yield and increased aboveground biomass of checkpea by at least 30% for common bean, cowpea and lablab, but had only minimal effects for soybean (Figure 2.2; Table 2.3). Plant height and nodule number per plant of chickpea was highest following lablab compared to other treatments. Residue retention consistently increased chickpea nodule number regardless of the previous legume species compared to residue removal (Table 2.3). It also significantly increased chickpea nodule dry biomass, though a significant interaction indicates that the magnitude of the effect varied with previous legume species. Residue retention nearly doubled chickpea nodule dry biomass for lablab, increased by 80 % for cowpea and 50 % for common bean, but only by 20 % for soybean. Additionally, residue retention suppressed % N derived from the atmosphere by chickpea for all previous legume species except soybean. The effect of residue retention on the amount of N fixed by chickpea was inconsistent across the previous legume species; it enhanced amount of N fixed by chickpea for soybean and common bean but suppressed N-fixation for lablab (Figure 2.2B).

2.3.3 Impacts of residue management and previous legume species on soil health and maize yield

Both legume residue retention and the legume species planted in Phase 1 of the study significantly influenced a range of soil properties at the time of maize planting (Table 2.4). Residue retention during the previous phases reduced bulk density by 11% and increased Olsen P by 52% compared to residue removal, across the legume species. Residue retention also increased total SOM, POXC, and POM by 5, 18, and 55%, respectively, compared to the residue removal treatment. When looking at the effect of legume species on soil health, planting lablab in Phase 1 significantly increased SOM compared to common bean and soybean; however, it was on par with cowpea. In addition, we noted that while there were no significant differences in POXC, POM and Olsen P among legume species, soils with lablab tended to have the highest values (Table 2.4). No significant interactions between residue management and legume species were observed for the soil health parameters measured here.

Residue amendment markedly increased maize aboveground biomass and grain yield. However, the significant interaction with legume species indicated that the amount increased was not uniform across legumes. Residue retention almost doubled aboveground biomass and grain yield in plots that previously contained lablab, cowpea and common bean, but had only a moderate effect for the soybean treatment. Residue amendment significantly increased maize HI, from roughly 0.44 in plots without residues to an average of 0.47 in plots where residue was retained

(Table 2.4). Legume species in Phase 1 also had a significant impact on maize HI, such that maize following lablab and cowpea had HI of 0.46 while soybean and common bean registered 0.45 and 0.43, respectively. There was no significant interaction between residue management and legume species on HI.

2.3.4 Ability of the soil health variables to explain variation in maize grain yield

Bivariate correlation between measured soil health predictors and maize grain yield showed significant negative correlation for soil bulk density ($p < 0.01$; $R^2 = 0.22$) and positive correlation for MWD ($p < 0.01$; $R^2 = 0.21$), SOM ($p < 0.001$; $R^2 = 0.39$), POXC ($p < 0.001$; $R^2 = 0.36$), POM ($p < 0.001$; $R^2 = 0.65$), as well as available P ($p < 0.001$; $R^2 = 0.52$) and no correlation for pH ($p < 0.37$; $R^2 = 0.03$; Figure 2.3).

2.4 Discussion

2.4.1 Legume and residue management effects on yield and soil health impacts

The integration of different legume species in Phase 1 of the rotation greatly affected yields and aboveground biomass production of both chickpea and maize. In line with our hypothesis, the high biomass producing lablab fixed the greatest amount of N and resulted in the highest maize and chickpea yields (Tables 3 & 4). The superior performance of lablab in terms of biomass production and N fixation capacity has been previously reported (McDonald et al., 2001). The higher amount of N fixed by lablab likely became available to the subsequent crops through mineralization of nodules and root biomass as well as aboveground biomass (in the residue retention treatment). Legume rhizodeposition, excretion of organic and inorganic N from the living roots, has previously been shown to be an important N source for subsequent crops (Liu et al., 2024; Virk et al., 2022). Release of P and K during mineralization of legume residue (Alamgir et al., 2012; da Silva et al., 2022) might also contribute to increased maize and chickpea yields, as evidenced by the highest available P occurring in the lablab residue retention treatment.

Residue retention improved grain yield, biomass production and HI for both maize and chickpea, compared to residue removal (Tables 2.3 and 2.4), thus supporting our second hypothesis. The doubling of maize grain yield due to residue retention, can likely be explained by improved soil health and fertility, especially increased nutrient availability that is likely associated with the return of high quality (low C:N) legume residues. Previous work has confirmed that legume residue retention can greatly increase cereal grain yield through rapid mineralization of high-quality residues and release of available nutrients to the subsequent crop, as well as improvements to soil physical properties associated with organic matter inputs (Akmal et al., 2015; Uzoh et al., 2019).

In line with our hypotheses, legume residue retention substantially improved soil health indicators compared to residue removal (Table 2.4). Residue retention significantly enhanced SOM and its labile fractions. Higher SOM with legume residue retention is likely attributed to higher inputs of C to the soil at the end of Phase 1, as well as increased productivity and residue return of chickpea in Phase 2 of the study. Higher organic matter input stimulates soil biological activity, which can improve soil aggregation and the physical protection of SOM against decomposition by microbes (Whalen et al., 2024). Additionally, residue retention and the high quality of the legume residues (i.e., low C:N ratio) studied here, may increase the formation of microbially-derived SOM, as nearly 50 % of organic C in agricultural soils is thought to come from microbial residues (Khan et al., 2016). Increase in SOM with legume residue retention compared to residue removal has been previously reported (Li et al., 2019; Tripolskaja et al., 2023). Similar to observed trends for SOM, legume residue retention significantly improved the POXC and POM, which is not entirely surprising since both are important components of SOM. Nyamasoka-Magonziwa et al. (2020) and Hu et al. (2023) reported similar results with legume residue retention. In our study, inclusion of legumes in the cropping system affected SOM; however, the magnitude varied with legume species. SOM accumulated most in plots previously planted to lablab, which may be due to greater rhizodeposition, and especially incorporation of aboveground residues in the residue retention treatments (Virk et al., 2022). An increase in SOM, and improved soil health more broadly, with legume rotation has been reported by others (Shukla et al., 2024; Uzoh et al., 2019).

Bulk density decreased with residue retention, likely due to improved SOM (Topa et al., 2021), and is associated with increased soil porosity, which facilitates root development and water infiltration into the soil. Organic matter additions support soil organisms (bacteria, fungi and soil fauna) that contribute to aggregate formation (Philippot et al., 2024), thus improving porosity and overall soil structure. This idea is supported by a marginally significant increase ($p = 0.08$) in aggregate stability (MWD) with residue retention.

Legume residues decompose easily and enable rapid delivery of plant available nutrients to the soil (da Silva et al., 2022). We suspect that an increase in available P following residue retention is linked to P mineralization from the legume residues, which is critical to improving subsequent crop growth in these P-limited soils. Others have reported that legume residue retention plays a vital role in enhancing available P in the soil by mobilizing soil P during the legume phase and by P addition from residue decomposition (Afkairin et al., 2024; Alamgir et al., 2012). Enhanced cycling of P through microbial biomass might also contribute to increased P availability in the soil with residue retention, especially in acid soils that commonly fix P into plant unavailable forms (Ayaga et al., 2006; Oberson et al., 2001).

The significant positive correlation between soil health parameters (BD, MWD, SOM, POXC and Olsen P) and maize grain yield (Figure 2.3) underpin the direct benefits of improved soil health indices on crop productivity, confirming that improved soil health through legume integration and residue retention is a promising strategy to enhance overall agroecosystem performance and sustainability.

2.4.2 Effect of legume and residue management on chickpea N fixation

The legume species in Phase 1 varied markedly in N fixation and biomass production (Figure 2.1 and Table 2.2) and had a clear influence on the N fixation dynamics of the subsequent chickpea crop. Chickpea showed enhanced nodule formation across legume species when residues were retained (Table 2.3). We suspect that decomposing residues may have increased dissolved organic C, which has been shown to increase the abundance of diazotrophs in the rhizosphere (Bian et al., 2024; Xie, Yu, et al., 2021). Higher nodule number with residue retention has been reported previously for soybean and peanut (El-sherbeny et al., 2023; Herrmann et al., 2014). We note that the residue effect on nodule dry weight largely followed biomass return from aboveground residue for the different species, such that chickpeas grown after lablab with residue retention showed the highest nodule dry biomass, owing to the higher nodule number and nodule size observed from this treatment.

Despite consistent effects of residues on nodule number and biomass, the effects of residue management on % N derived from the atmosphere and total N fixation in chickpea was dependent upon the legume species grown in Phase 1. Residue retention suppressed % N derived from the atmosphere of chickpea for all previous legumes except soybean (Table 2.3). At the same time, residue retention dramatically reduced chickpea N fixation for lablab but increased it for soybean. We suspect that this may be due to higher available N in the soil following lablab, due to the large amount of N in lablab residue inputs. In such a condition, chickpea may rely more on N mineralized from decomposing residues rather than fixing additional N, which incurs an energy cost to the plant. Others have shown that higher N availability can reduce nodule specific activity (Pampana et al., 2017). High mineral N levels in the soil can reduce diazotroph abundance and activity and delay onset of N fixation, hence reducing total N fixed (Liang et al., 2023; van Kessel & Hartley, 2000). Our results contrast with other studies which have reported increased N fixation with residue retention (Rebafka et al., 1993; Xie, Li, et al., 2021). We note, however, that these studies tested the effect of lower quality cereal residues with high C:N ratios that likely immobilized mineral N, thus decreasing availability and stimulating N fixation in the rhizosphere. Similar to our result, Bian et al. (2024) reported that incorporation of legume residue, specifically Chinese milk vetch, suppressed N fixation of the vetch plant in a pot experiment. Unlike other legume species, retention of soybean residue had no effect on % N derived from the atmosphere compared to residue removal in our study, likely due to its lower contribution of available N associated with the low biomass production and lower N fixation measured during Phase 1 (Figure 2.1). The species dependent effect of residue retention on chickpea N fixation highlights the complex interactions between soil N level, residue decomposition rate, and microbial activity, and signals a need for additional research to elucidate these interactions.

The result from the chickpea in Phase 2 presented a nuanced understanding of the carryover effects of previous legume species. In Phase 1, lablab was the legume with highest biomass and N-fixing capacity, which carried over into Phase 2, such that chickpea following lablab yielded the highest. This suggested that residual N and possibly other nutrients from lablab positively influenced chickpea growth. The lower % N derived from the atmosphere in chickpea following lablab with residue retention did not diminish performance of chickpea, indicating that lablab's contribution to soil fertility was large enough to reduce chickpea N demand relative to other treatments. Conversely, soybean had a minimal carryover effect on the subsequent chickpea crop even when residue was retained due to its lower biomass production and N fixation performance during Phase 1.

2.4.3 Tradeoffs and management implications of residue management and legume selection

We observed clear tradeoffs between aboveground biomass and grain yield among legume species in Phase 1 of our study. Understanding this tradeoff is important for optimizing legume selection and management practices to balance the contributions to soil health with crop and forage productivity. Lablab showed the highest aboveground biomass production but lower grain yield than common bean. In contrast, common bean produced the lowest aboveground biomass and the highest grain yield (Figure 2.1 and Table 2.2). Similar findings of higher biomass production of lablab at the expense of grain yield have been previously reported (McDonald et al., 2001; Nord et al., 2020) and suggest that farmer objectives need to be clearly identified and prioritized when selecting legumes to incorporate in their rotations.

Metrics of N fixation further illustrate the tradeoff among legume species (Figure 2.1 and Table 2.2). Lablab demonstrated the highest nodule number and nodule dry weight, and this translated into the highest total N fixation. We note that N fixation ranged from 31 to 123 kg ha^{-1} and was largely associated with the biomass production of each species, implying that both biomass production and N fixation potential (e.g., nodulation) are important criteria to maximize N fixation in cropping systems (Adeleke & Haruna, 2012; Ngwenya et al., 2024). Similar to our result, Haque and Lupwayi (2000), also working in Ethiopia, found the highest N fixation by lablab compared to other legume species and a significant positive linear relationship between amount of N fixed and biomass accumulation among four legume species. We note that the observed 92 % N derived from the atmosphere with lablab is consistent with previously measured values in the tropics (Mugi-Ngenga et al., 2022; Sanginga et al., 1996) and was largely responsible for the high total N fixation by lablab. The maximum N fixation of lablab was also likely associated with its higher nodule number and nodule dry weight (Martins et al., 2022; Nord et al., 2020) as previous work has found increased N fixation with higher nodule dry weight. At the same time, nodule formation alone does not guarantee N fixation (Kiers et al., 2003), as some of the nodules might be unable to produce nitrogenase (Sarr et al., 2016) or less efficient at fixing N (Porter et al., 2024).

Lablab's superior aboveground biomass and N-fixation capacity suggests considerable potential to provide a large quantity of high-quality forage for livestock and to improve soil health in the long term by improving N availability for subsequent crops, SOM pools, and soil structure (Nyamasoka-Magonziwa et al., 2020). However, its low grain yield compared to common bean might not provide immediate economic benefit to farmers, particularly if they prioritize grain yield over soil health. We also note that lablab does not currently have a good market and is not well known in Ethiopia, thus there is lower economic incentive to grow it unless it is used for forage. However, this could change in the future as people become more accustomed to eating the grain and aware of the benefits of lablab residue return. On the other hand, common bean can offer immediate economic return due to its higher grain yield (and price), but its potential contribution to long-term soil health appears quite limited. The moderate biomass, harvest index and N fixation of cowpea, intermediate to lablab and common bean, perhaps offers a compromise in providing grain and/or forage for Ethiopian smallholders, while potentially contributing to soil health (if some residues are retained). Balancing these tradeoffs can enhance sustainability of crop production and contribute to more resilient agricultural systems. Therefore, we suggest that by growing a variety of legume crops, farmers might be able to mitigate the tradeoffs between grain yield and biomass production.

For smallholder farmers, crop residue is an important resource, which is usually in short supply and has many alternative uses, including as a fuel source, livestock feed, soil amendment and for building materials (Tittonell et al., 2015). In Ethiopia, retaining crop residue for soil application presents a significant challenge, as approximately 30% of maize residues are used as fuel, and over half as feed, with only a negligible fraction retained in the fields (Jaleta et al., 2015) Thus, there are clear tradeoffs between retaining residues as a source of C and nutrients vs. removing it for other competing demands (Rodriguez et al., 2017). As supported by our findings, numerous studies have shown residue retention to improve soil health through enhancing SOM and nutrient availability (Gora et al., 2024; Hu et al., 2023). However, it is unclear how long the effect of residue retention persists, and how exactly improved soil health translates into yields in the long-term. In our study, we observed a carry-over effect from Phase 1, such that both chickpea and maize had higher yields with residue retention, especially with lablab. This carry-over effect likely comes from a residual effect of the legume residues incorporated at the end of Phase 1, but also from increased productivity of chickpea in Phase 2. Furthermore, we suspect that increased aboveground biomass production of maize with lablab and residue retention means that more residues would be available to return to the soil at the end of Phase 3, and that the carry-over effect could persist for many subsequent crops in the rotation if managed appropriately. Additional research is needed to help farmers understand how long the benefits of the initial legume inclusion and residue retention could last, and to conduct a careful cost-benefit analysis of residue retention vs. removal over multiple years and crop cycles.

Our findings have important implications for sustainable farm management. The additive benefits of legume species selection and residue management on soil health suggest that these practices can be individually tailored to improve soil health and crop yields. Given the positive correlation observed between enhanced soil health indicators and increased maize yield, it is evident that strategically managing legume residues offers great promise for enhancing sustainable crop productivity. However, using legume residues in sub-Saharan Africa, particularly in Ethiopia, can be challenging because residues are often removed from the field for use as livestock feed and fuel. Therefore, agricultural extension services in this region need to demonstrate the benefits of legume residue on maize yield and soil health. The question of whether smallholder farmers would change the practice of residue removal for feed/fuel and retain the residue in the field to improve soil health and crop production will depend on the relative profitability of both options in both the short term and the long term.

2.5 Conclusion

This study highlights the potential of including legumes with varying biomass production and N-fixing capacities in rotation with maize to rapidly enhance soil health and crop productivity under smallholder farming conditions. Our findings showed that lablab (and to a lesser extent cowpea), with higher biomass production and N fixation capacity, can improve SOM, nutrient availability and soil physical properties. These gains in soil health translated into increased grain yields that extended beyond the following chickpea crop and into the subsequent maize crop, particularly when residues were retained in the field. Residue retention enhanced soil health indicators and grain yields across legume species, with the magnitude of these benefits being greatest for lablab residue and least for soybean. In contrast, the removal of residue reduced soil health and constrained the rotation benefits, underlining the critical role of residue management in sustaining soil fertility and crop productivity.

While our findings come from just one study site, they illustrate the potential of high-biomass legumes, such as lablab, to contribute to addressing fundamental soil health and food insecurity challenges in smallholder cropping systems. However, we acknowledge the competing demands for crop residues for livestock feed and fuel in smallholder systems. Future research should investigate the economic trade-offs of residue management practices and develop strategies to optimize the trade-off depending on smallholder farmer needs and priorities across a range of agroecological contexts. Such efforts would help foster the adoption of sustainable residue management practices and improve long-term resilience of farming systems in Ethiopia and beyond.

CHAPTER 2: TABLES AND FIGURES

Table 2.1 Treatment information (legume varieties, residue input rates, C and N concentrations, planting and harvesting dates) for three phases of a field trial conducted in the Cheha district of Ethiopia between May 2022 and November 2023.

Treatments	Crop	Variety	Planting date (mm/dd/yyyy)	Residue additions (Mg ha^{-1})	Residue C (%)	Residue N (%)	Harvest date (mm/dd/yyyy)
Phase 1							
CB	CB	Awash-Malka	06/30/2022	--	--	--	10/29/2022
CP	CP	Bole		--	--	--	11/23/2022
LL	LL	Gebisa		--	--	--	11/11/2022
SB	SB	Afgat		--	--	--	11/08/2022
Phase 2							
CB +R_{CB}	CH	Natoli	02/28/2023	1.11	41.39	2.04	05/30/2023
CB - R_{CB}				---	--	--	05/25/2023
CP + R_{CP}				2.22	40.46	2.27	06/01/2023
CP - R_{CP}				--	--	--	05/27/2023
LL + R_{LL}				4.44	39.82	3.10	06/01/2023
LL - R_{LL}				--	--	--	05/27/2023
SB + R_{SB}				1.67	42.65	2.62	05/29/2023
SB - R_{SB}				--	--	--	05/25/2023
Phase 3							
CB + R_{CH}	Maize	Limu	06/21/2023	2.00	41.39	1.88	01/03/2024
CB - R_{CH}				--	--	--	01/03/2024
CP + R_{CH}				2.10	41.73	2.22	01/03/2024
CP - R_{CH}				--	--	--	01/03/2024
SB + R_{CH}				1.78	41.17	2.29	01/03/2024
SB - R_{CH}				--	--	--	01/03/2024
LL + R_{CH}				2.30	41.30	2.24	01/04/2024
LL - R_{CH}				--	--	--	01/03/2024

Abbreviations: CB, Common bean; CP, Cowpea; LL, Lablab; SB, Soybean; CH, Chickpea; R, residue.

Table 2.2 Harvest index and different N fixation metrics observed in four legume species (CB, Common bean; CP, Cowpea; LL, Lablab and SB, Soybean) grown in a field trial in the Cheha district of Ethiopia between May 2022 and August 2022. Values are mean ± standard error for treatment combination (n = 4).

Legume species	Harvest index	N fixation metrics		
		Nodule number (plant $^{-1}$)	Nodule dry biomass (g plant $^{-1}$)	N derived from the atmosphere (%)
CB	0.52 ± 0.01^{a}	23.3 ± 0.63^{a}	0.17 ± 0.01^{d}	81.3 ± 1.62^{b}
CP	0.27 ± 0.01^{c}	18.0 ± 0.71^{b}	0.71 ± 0.02^{b}	86.0 ± 1.91^{ab}
LL	0.19 ± 0.02^{d}	24.3 ± 0.72^{a}	1.18 ± 0.01^{a}	92.0 ± 0.84^{a}
SB	0.34 ± 0.01^{b}	12.0 ± 0.51^{c}	0.51 ± 0.07^{c}	48.5 ± 2.91^{c}
p-value	< **0.001**	< **0.001**	< **0.001**	< **0.001**

Note: Means followed by the same letter within a column are not significantly different at $p < 0.05$, according to Tukey adjusted pairwise comparison. *P*-values in bold indicate significant difference at $p < 0.05$.

Table 2.3 Chickpea performance and different N fixation metrics observed in residue management (RM) and legume species (LS) in a field trial in the Cheha district of Ethiopia between February 2023 and June 2023. The legume species tested were: CB, Common bean; CP, Cowpea; LL, Lablab and SB and Soybean. Values are mean ± standard error for treatment combination (n = 4).

		Performance metrics					N fixation metrics		
Legume Species	Residue Management	Plant height (cm)	Branch number (plant^{-1})	Pod number (plant^{-1})	Grain yield (Mg ha^{-1})	Harvest index	Nodule number (plant^{-1})	Nodule dry biomass (g plant^{-1})	N derived from the atmosphere (%)
CB									
	With	50.4±1.7	15.8±1.4	27.8±1.8	1.61±0.17	0.44±0.03	74.2±2.0	3.6±0.15	50.1±4.1
	Without	47.9±2.6	14.1±1.2	18.6±0.9	0.83±0.11	0.35±0.05	61.5±1.1	2.3±0.30	69.4±1.8
CP									
	With	52.9±2.8	16.6±0.7	32.3±2.0	1.76±0.13	0.46±0.01	82.6±2.9	4.3±0.30	43.1±5.9
	Without	48.8±1.6	14.8±0.9	20.6±2.7	0.85±0.13	0.32±0.04	61.2±4.2	2.4±0.50	71.6±3.9
LL									
	With	55.3±2.1	17.7±1.4	35.7±1.9	1.95±0.16	0.46±0.03	83.9±2.5	5.2±0.15	21.3±1.4
	Without	47.5±1.7	15.6±0.8	22.2±2.3	1.05±0.13	0.38±0.04	62.5±5.7	2.7±0.49	56.5±3.5
SB									
	With	49.8±1.0	14.1±1.2	26.0±0.8	1.04±0.24	0.35±0.06	72.3±4.1	2.9±0.29	66.7±4.0
	Without	48.7±1.8	13.7±1.4	18.3±1.4	0.78±0.13	0.31±0.04	59.4±2.5	2.4±0.18	65.9±6.9
p-value									
RM		**0.004**	0.092	**< 0.001**	**< 0.001**	**0.006**	**< 0.001**	**< 0.001**	**< 0.001**
LS		0.661	0.168	**0.021**	**0.005**	0.227	0.120	**0.009**	**0.006**
RM X LS		0.207	0.867	0.417	**0.031**	0.665	0.308	**0.037**	**< 0.001**

Note: The (X) symbol refers to the interaction between treatments. *P*-values in bold indicate significant difference at $p < 0.05$.

Table 2.4 Soil health metrics (BD, bulk density; MWD, mean weight diameter; SOM, soil organic matter; POXC, per manganate organic matter; POM, particulate organic matter; Olsen P, Olsen phosphorus) and maize yield performance evaluated under different residue management (RM) and legume species (LS) treatments. Samples were collected from the 0-20 cm depth before maize planting in a field trial in the Cheha district of Ethiopia between June 2023 and January 2024. The legume species tested were: CB, Common bean; CP, Cowpea; LL, Lablab and SB and Soybean. Values are mean ± standard error for (n = 4).

		Soil health metrics							Maize yield metrics		
Legume Species	Residue Management	BD (g cm^{-3})	MWD (µm)	pH	SOM (%)	POXC (ppm)	POM (%)	Olsen P (ppm)	AGB (Mg ha^{-1})	Yield (Mg ha^{-1})	HI
CB											
	With	1.0±0.03	1193±158	5.7±0.04	3.8±0.04	233±10.6	0.32±0.04	6.0±0.73	4.4±0.27	1.90±0.43	0.47±0.01
	Without	1.1±0.08	1152±61	5.8±0.04	3.6±0.04	208±12.0	0.21±0.01	4.2±0.40	2.8±0.17	0.98±0.09	0.40±0.03
CP											
	With	0.9±0.05	1583±275	5.8±0.06	3.8±0.04	250±9.2	0.35±0.03	7.2±0.66	5.3±0.20	2.73±0.47	0.48±0.01
	Without	1.0±0.07	1266±105	5.8±0.03	3.6±0.02	216±14.4	0.24±0.01	4.5±0.45	2.5±0.05	1.13±0.05	0.44±0.02
LL											
	With	0.9±0.07	1601±138	5.7±0.09	4.0±0.04	285±6.9	0.39±0.01	8.0±1.10	6.2±0.20	3.00±0.15	0.48±0.01
	Without	1.0±0.09	1332±95	5.8±0.03	3.8±0.12	221±8.5	0.22±0.01	4.5±0.63	2.9±0.08	1.32±0.16	0.45±0.01
SB											
	With	1.0±0.04	1219±192	5.7±0.10	3.7±0.05	226±11.4	0.29±0.02	5.2±0.30	3.3±0.12	1.48±0.05	0.46±0.01
	Without	1.1±0.08	1085±178	5.7±0.04	3.5±0.10	197±9.9	0.20±0.02	4.3±0.57	2.3±0.15	1.03±0.09	0.44±0.01
p-value											
RM		**0.015**	0.104	0.177	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	**0.011**	**0.027**
LS		0.596	0.079	0.783	**0.009**	0.054	0.078	0.117	**< 0.001**	**0.002**	**0.047**
RM x LS		0.946	0.753	0.564	0.849	0.062	0.281	0.262	**< 0.001**	**0.041**	0.224

Note: The (X) symbol refers to the interaction between treatments. *P*-values in bold indicate significant effects at $p < 0.05$.
Abbreviations: AGB, aboveground biomass; HI, harvest index.

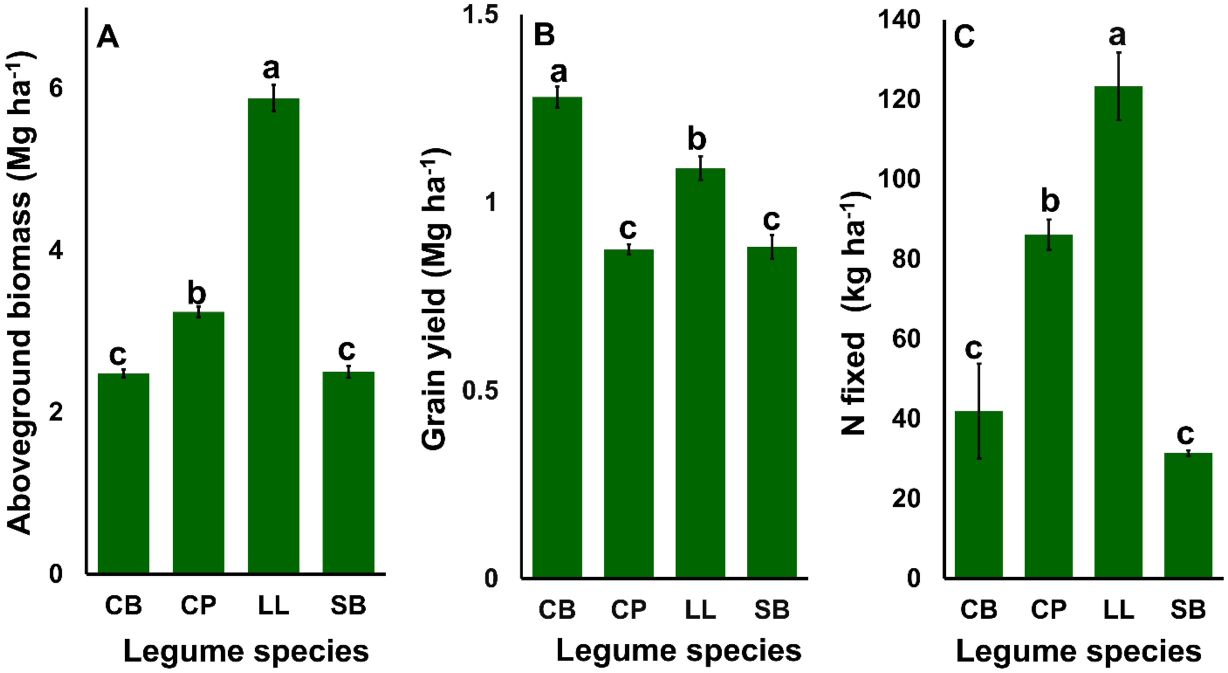

Figure 2.1. Mean values for different plant performance metrics, evaluated at harvest, observed in four legume species (CB, Common bean; CP, Cowpea; LL, Lablab; SB, Soybean) grown in a field trial in the Cheha district of Ethiopia between May and August 2022. Metrics presented are **A** aboveground biomass, **B** grain yield, and **C** total amount of N fixed. Treatments not sharing the same letter differ significantly at $p < 0.05$, according to Tukey adjusted pairwise comparison. Error bars represent standard error of the mean (n = 4).

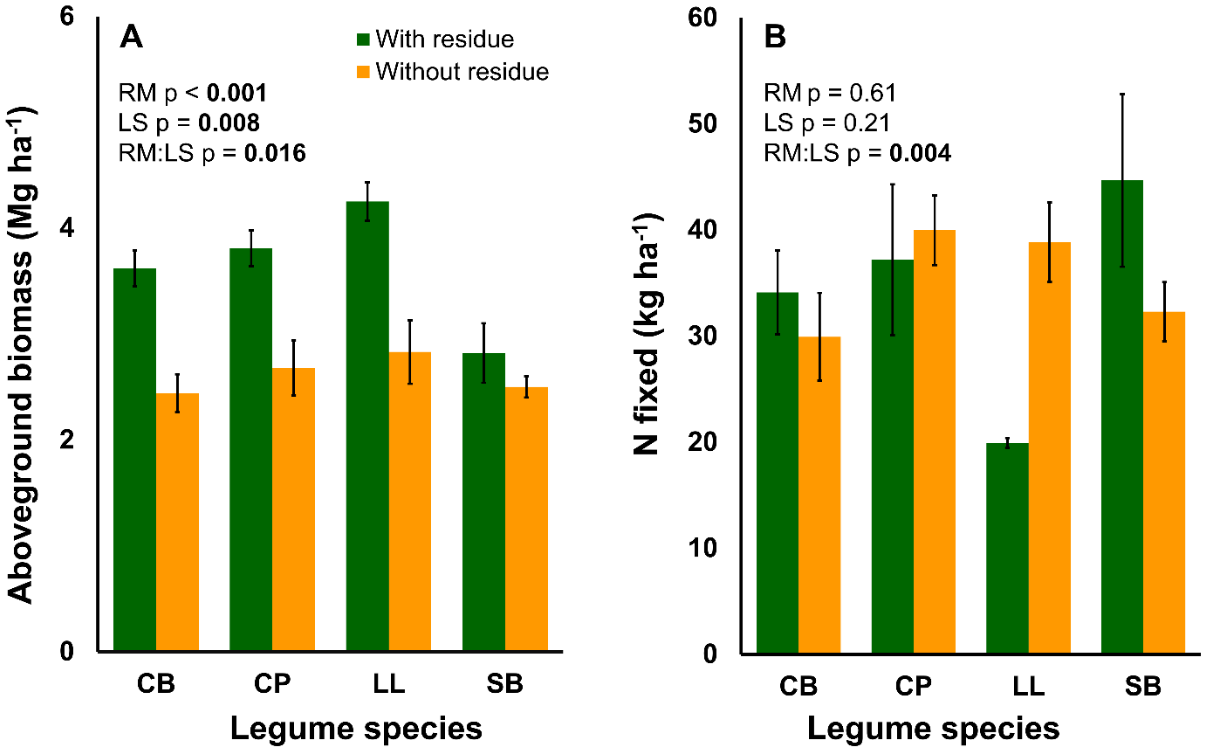

Figure 2.2. Mean **A** aboveground biomass and **B** amount of N fixed from the atmosphere for chickpea, evaluated at harvest, following different residue management (RM) and legume species (LS) treatments (CB: Common bean; CP, Cowpea; LL, Lablab; SB, Soybean) grown in a field trial in the Cheha district of Ethiopia between February 2023 and June 2023. Bold ANOVA *p*-values indicate significant difference at $p < 0.05$. Error bars represent standard error of the mean (n = 4).

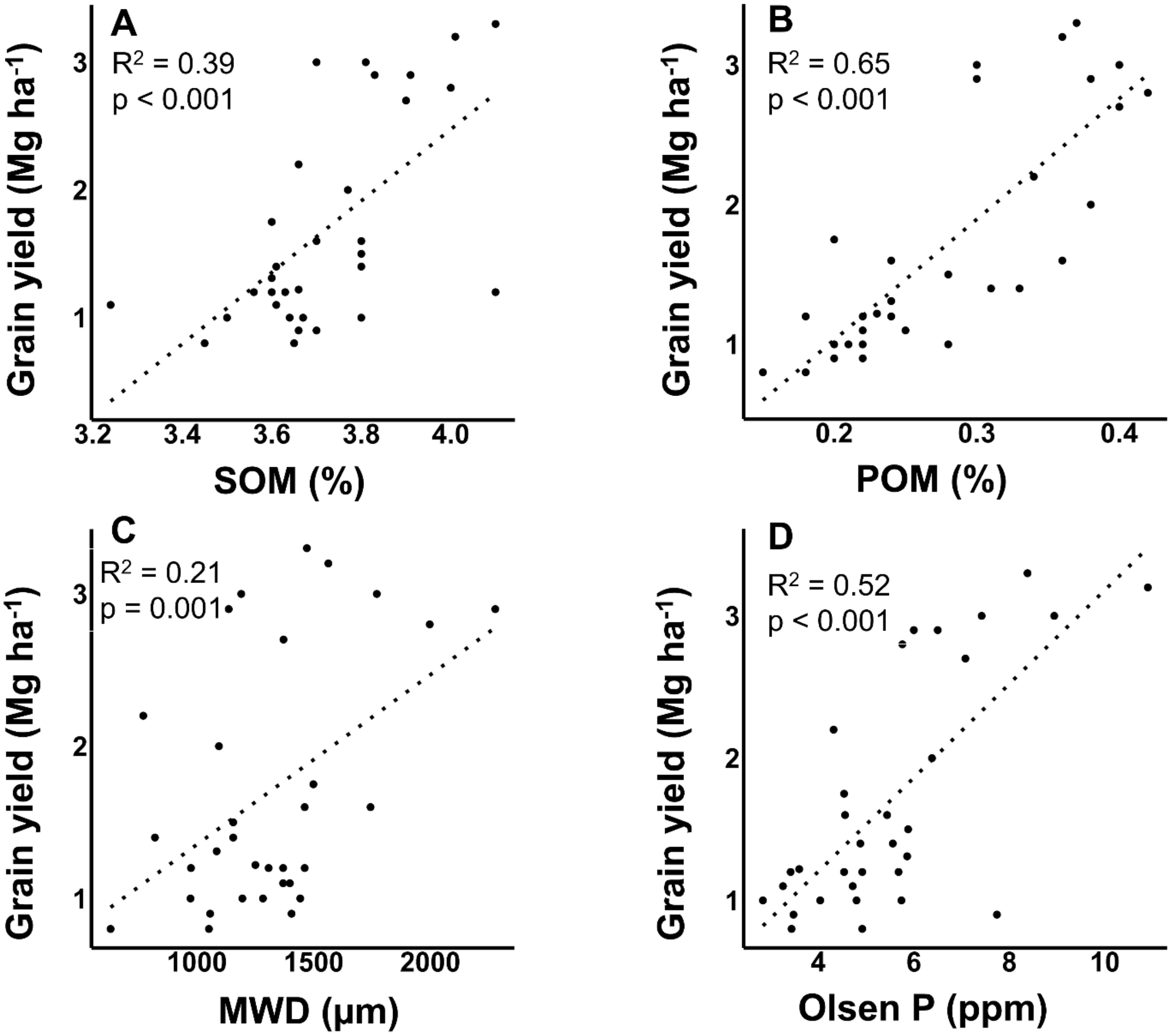

Figure 2.3. Maize grain yield from a field trial in the Cheha district of Ethiopia between June 2023 and January 2024 as explained by soil properties (0-20 cm depth) using simple linear regression with: **A)** total soil organic matter (SOM), **B)** particulate organic matter (POM), **C)** aggregatge mean weight diamter (MWD), **D**) P availability (Olsen P; n = 32).

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CHAPTER 3: ORGANIC NUTRIENT INPUT ENHANCES LEGUME PERFORMANCE AND SUBSEQUENT IMPACT ON MAIZE YIELD AND PROFITABILITY IN A SMALLHOLDER FARMING SYSTEM OF ETHIOPIA[2]

3.1 Introduction

Globally, agriculture faces numerous complex challenges related to climate change, water scarcity, biodiversity loss and widespread soil degradation. These factors threaten the long-term productivity of cropping systems, food security and overall environmental sustainability (Calicioglu et al., 2019; Challinor et al., 2014). Soil health, i.e., the continued capacity of soil to function as a vital living ecosystem that sustains plants, animals and humans, is crucial for addressing sustainability challenges in crop production (Lehmann et al., 2020) and for feeding the growing population, which is projected to reach 9.6 billion by 2050 (Gerland et al., 2014). Healthy soils provide multiple ecosystem services that support food production, biodiversity conservation and climate change mitigation (Rinot et al., 2019), while increasing agricultural profitability and stability (Kane et al., 2021). Despite the importance of maintaining soil health, smallholder farmers in many parts of Africa, including Ethiopia, are facing widespread soil degradation related to depletion of soil organic matter (SOM), acidification, and nutrient deficiency (Sileshi et al., 2025b), which ultimately reduces crop productivity. To address these challenges, it is essential to maintain or restore soil health through more regenerative management techniques that are appropriate to smallholder farming contexts.

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[2] *In revision for* Environmental Research: Food System
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In Ethiopia, smallholder farming is characterized by farm size of < 2 ha, limited access to external input such as fertilizer, commonly managed by family labor, highly dependent on rainfall, and low productivity (Rapsomanikis, 2015; Zerssa et al., 2021).

Diversification of cropping systems has been promoted widely as a means to improve soil health and enhance nutrient cycling (Mihrete and Mihretu, 2025; Nithinkumar et al., 2024). Integration of legumes into cereal-based cropping systems can improve soil health by adding nitrogen (N) through biological N fixation, enhancing soil biological activity, increasing phosphorus (P) availability, contributing biomass that builds SOM, and improving soil structure (Gogoi et al., 2018). These benefits, however, can vary among legume species and the environmental context in which they are grown (Gora et al., 2024). Improvements in cereal productivity following legume cultivation are well documented (Ojiem et al., 2014; Uzoh et al., 2019). However, the effects of different legume species in crop rotation, and interactions with varying soil contexts, on soil health and productivity remain less understood.

Nutrient management is a key element of sustainable agriculture (Sileshi et al., 2025a), especially for smallholder farms where nutrients are highly limiting and inputs are often low (Elrys et al., 2019; Nyamasoka-Magonziwa et al., 2023). Inorganic fertilizers can be an important source of nutrients for improving soil fertility and supporting crop productivity, but are often less accessible to smallholder farmers due to high costs and limited supply (Wolde et al., 2020), leading farmers to apply them below the recommended amounts, if at all. In some regions of sub-Saharan Africa (SSA), cereal crop yields, including maize, have levelled off despite the increased use of inorganic fertilizers, largely due to soil degradation (Kuyah et al., 2021; Sileshi et al., 2025b). Moreover, some suggest that inorganic fertilizers alone often provide only limited profitability for smallholder farmers in SSA, due to high costs and subpar crop response to fertilizer, especially when SOM levels are low (Bonilla-Cedrez et al., 2021; Chivenge et al., 2022). Hence, adopting affordable and accessible soil management practices to mitigate soil health challenges and increase crop productivity is imperative.

Organic nutrients inputs offer a number of benefits to support soil health and long-term productivity. Manure, in particular, has potential to address many soil health related issues including low SOM (Fonte et al., 2009; Howe et al., 2024), reduced soil biological activity (Hurisso et al., 2011), soil compaction (Blanco-Canqui et al., 2015), soil acidity (Dhiman et al., 2019), macro and micronutrient deficiencies (Lupwayi et al., 2000), decline in crop yield and profitability (Kearney et al., 2012; Sileshi et al., 2025a). Manure inputs can also play a key role in increasing various SOM pools, such as particulate organic matter (POM) and permanganate oxidizable C (POXC) (Dungan et al., 2025), and enhancing soil pH buffering and cation exchange capacities (Shi et al., 2019). Beyond the direct benefit of manure for soil, it has also been reported to enhance nutrient uptake, nodule density, N_2 fixation and yield in legumes (Fekadu et al., 2019; Rurangwa et al., 2018). These benefits occur through reduced soil acidity, increased SOM, and improved nutrient availability, driven by decomposition and gradual nutrient release from manure (Otieno et al., 2018). By enhancing legume performance and associated benefits for soil health, manure can also support favorable conditions for subsequent cereal crops. However, how organic nutrient inputs influence legume performance and soil health, and how these effects translate into the productivity of the overall cereal-legume rotation remain less understood in smallholder farming contexts.

Based on the knowledge gaps outline above, we conducted a field experiment under smallholder conditions with the following objectives: i) evaluate how nutrient management strategies influence performance of common bean vs. soybean, ii) quantify impacts of nutrient management and legume species on key soil health indicators and subsequent maize yield, and iii) evaluate the economic benefit of nutrient management strategies for each legume species and maize in smallholder farming systems. We hypothesized that manure application to legumes will increase bean yields and nodulation, enhance key soil health indicators with stronger carryover effect on subsequent maize yield, and improve system profitability compared to inorganic fertilizer.

3.2 Materials and Methods

3.2.1 Study site

The study was conducted on the Plant Science Research Farm of Wolkite University, located in Gurage Zone, Ethiopia (8°12'54" N, 37°48'30" E). This site has an elevation of 1929 m above sea level and a topography dominated by gentle rolling hills. Smallholder farming is common in the region with maize, enset, teff, barley, wheat, beans, peas, chickpeas, kale, and khat being the most common crops in the area (Nahusenay et al., 2024). The mean annual rainfall is 986 mm yr^{-1}, with an average annual temperature of 27 °C (based on data from 2012-2022, Hawassa Metrology Center). The region has a bimodal rainfall distribution, with a short rainy season (Belg) from March to May and the main rainy season (Kiremt) occurring June to September. Soils are classified as Vertisols and are highly deficient in N, P and S (Nahusenay et al., 2024; Yitbarek et al., 2018). The study site soil is moderately acidic (pH = 5.7) with a clay texture (11% sand, 21% silt and 68% clay) and 3.6% SOM.

3.2.2 Field experimental design and management

The field study was established in early May 2022 to evaluate the effect of soil nutrient management strategies on crop performance and soil health in a legume-maize cropping system. Teff (*Eragrostis tef*), a cereal crop native to Ethiopia, and maize were grown in the field for 9 years prior to experiment implementation. At the start of the study, land was plowed by oxen and leveled. In the first phase, three nutrient management strategies [1) inorganic fertilizer, 2) manure, and 3) a control (no nutrient inputs)] were established with two legume species [1) common bean (*Phaseolus vulgaris;* variety Awash-Malka), and 2) soybean (*Glycine max;* variety Afgat)] in a full factorial design. The six treatments were applied to plots (4 m x 4 m) and laid out in a randomized complete block design with four replications. A buffer zone of 50 cm between treatments was used to separate plots. Common beans and soybeans were planted in early July 2022 with a spacing of 10 cm between plants within a row and 40 cm between rows. Aged dairy manure was obtained from Wolkite University dairy farm, and samples were analyzed for nutrient composition at Horticoop Laboratory, Ethiopia (Table 3.1). For the manure treatment, 4.91 Mg ha $^{-1}$ of aged dairy manure (dry weight basis) was uniformly spread on the plots prior to planting (mid-May 2022) and incorporated to a depth of 20 cm using hand hoeing. Manure additions provided a total of 64.8, 27.5 and 12.8 kg ha^{-1} of elemental N, P and S, respectively. For the fertilizer treatment, a recommended and commonly available NPS fertilizer in the region (19% N, 38% P_2O_5 and 7% S) was applied at a rate of 45.8 kg N ha^{-1}, 39.9 kg elemental P ha^{-1}and 16.9 kg S ha^{-1}. Half of the fertilizer was sub-surface banded 5 cm below the seed at planting, and the remaining half was side-dressed 25 days after emergence. Manure and fertilizer (NPS fertilizer) rates were standardized based on equivalent monetary input – i.e., both had the same cost (equivalent to 270.52 US$ ha^{-1}). Manure price was determined based on average local market price in the study area, while cost for fertilizer was obtained from the Cheha District Agricultural Office.

In November 2022, following the harvest of common bean and soybean from the first phase, residue in all plots was retained, averaging 2.5 Mg ha^{-1} for common bean and 2.2 Mg ha^{-1} for soybean, and incorporated into the soil to a 20 cm depth using hand hoeing. The C:N of the common bean and soybean residue was 20.3 and 16.3, respectively. Maize (*Zea mays;* variety Limu) was planted in the same plots on May 24, 2023. Plots were prepared by hand hoeing and maize was planted with a spacing of 25 cm within rows and 75 cm between rows. Two seeds were sown per hill and thinned to a single plant 7 days after emergence. To ensure at least a low level of maize grain production, while also allowing for detection of previous nutrient management differences in the legume phase and associated soil health impacts, we applied 6.3 kg N, 5.5 kg elemental P and 2.3 kg S ha^{-1} as NPS fertilizer, just one third of the recommended rate, according to the local agricultural extension office.

In both phases, all agronomic practices, cultivation and weeding were implemented uniformly across all plots. Hand weeding was carried out two times (3 and 6 weeks after legume emergence) for the first phase and three times (3, 6 and 10 weeks after maize emergence) for the second phase to control weeds in the field. To control fall armyworm in maize, Bravo 5 EC (lambda-cyhalothrin 2.5%) was applied at a rate of 400 mL ha^{-1} on July 28 and September 9, 2023.

3.2.3 Soil sampling and preparation

Soil samples were collected shortly before maize planting (late May 2023) to evaluate the impacts of nutrient management and legume species on key soil health metrics. Five samples (0-20 cm) from each plot were taken following a W pattern using a soil auger (4 cm diameter) and combined into a single composite sample for analysis. Two undisturbed surface samples per plot were also collected using a sharpened metal ring (5 cm diameter) to a depth of 5 cm for determination of aggregate stability and bulk density.

In the laboratory, samples from the top 20 cm were air-dried, sieved through a 2 mm mesh after removing rocks, visible roots, and coarse organic matter and stored for analysis of pH, POM, POXC, total SOM and available P. Soils collected for aggregate stability and bulk density were weighed, and ~10 g representative sub-samples were oven dried at 105 °C to calculate moisture content for bulk density determination. The remaining field-moist soil was combined to form one composite sample per plot, with soil clods gently broken along the natural planes of weakness to pass through an 8 mm mesh sieve and air-dried for evaluation of aggregate stability. Soil analyses were largely conducted following methods outlined by Nyamasoka-Magonziwa et al. (2020), as part of a low-cost, soil health tool kit developed for smallholder farming contexts. The methods were adapted from common laboratory methods and validated, with the goal of providing robust data and more accessible farmer research tools (also see https://smallholder-sha.org).

3.2.4 Soil analysis

Aggregate stability was analyzed by wet-sieving, using a method outlined by Nyamasoka-Magonziwa et al. (2020) and adapted from (Elliott, 1986). A 70 g subsample of air-dried, 8-mm sieved soil was placed on top of a 2 mm sieve in a small basin and submerged by adding water to the basin. After a 5-min slaking period, the soil was wet-sieved by raising and lowering the sieve 50 times over a 2-min period. Soil left on the 2 mm sieve was washed into a pre-weighed aluminum pan using a rinse bottle. The soil that passed through the sieve was then transferred to a 250 µm sieve and sieved again, as described for the 2 mm sieve, to separate the sample into three water-stable aggregate fractions: 1) large macroaggregates (>2000 µm), 2) small macroaggregates (250–2000 µm), and 3) microaggregates with free silt and clay particles (<250 µm). The large and small macroaggregate samples were oven-dried at 105 ºC, and the mass of soil that passed through the 250 µm sieve was determined by difference. The mean weight diameter (MWD) for aggregates in each sample was then calculated based on the initial 70 g of dry soil weight using an equation presented by (van Bavel, 1950):

$$MWD = \sum_{i=1}^{n} (wi\ X\ di)$$

Where *wi* is the proportion of total sample weight in the i^{th} aggregate size fraction, and di is average diameter (µm) of the i^{th} fraction.

A density flotation technique using deionized water was used to determine POM (Nyamasoka-Magonziwa et al., 2020). A coarse POM fraction (250-2000 µm) was separated from 100 g of air-dried soil by flotation in water and decanting onto a filter made of cotton fabric, which was then oven-dried at 60 °C and weighed. Permanganate oxidizable carbon was determined by oxidizing labile soil organic C with potassium permanganate (Weil et al., 2003) and measured by absorbance at 525 nm using a Hanna model HI-717 phosphate high range colorimeter (Hanna Instruments Corp., Woonsocket, RI, USA; Nyamasoka-Magonziwa et al., 2020). Soil pH was determined in a 1:2.5 air-dried soil to water ratio using a pH meter. SOM was measured through potassium dichromate oxidation (Walkley and Black, 1934). Plant-available P in the soil samples was measured using $NaHCO_3$ extraction followed by ascorbic acid colorimetry (Olsen, 1954).

3.2.5 Grain yield and related parameters

At legume flowering, ten randomly selected plants were used to measure plant height and to count pod and branch numbers per plant. At harvest, early-November 2022 for legumes and early-December 2023 for maize, all plants in every plot were harvested, threshed and weighed to determine grain yield. The actual grain yield was adjusted to a moisture content of 10% for legumes and 12% for maize. A sub-sample (1 m^2) from each plot was oven-dried at 60 °C at harvesting to measure aboveground biomass. Harvest index (HI) was calculated as the ratio of grain yield to total aboveground dry biomass.

3.2.6 Nodulation assessment

Root nodules were collected from common bean and soybean in the first phase of the study. At the mid-flowering stage, five randomly chosen plants per plot were excavated, and nodules were collected as described in (Yong et al., 2018). Soil adhering to the roots was carefully removed by hand, and the whole root system was enclosed in a nylon net with a mesh size of 0.6 mm, immersed in water, and gently washed to remove adhering soil, allowing for separation of the nodules from the roots by hand. Nodule number per plant was recorded, and the nodules were oven-dried at 60 ºC for biomass determination.

3.2.7 Cost-benefit analysis of legumes and nutrient management strategies

To understand the relative economic benefit of nutrient management strategies for each legume species and maize, a partial cost-benefit analysis was carried out using an approach similar to (Kearney et al., 2012). Costs for fertilizer and manure inputs were equivalent by design (US$ 270.52 ha^{-1}, based on 1 US$ = 56.65 Ethiopian Birr, on May 13, 2022, and application rate of nutrient sources for beans and maize), while the control only had the small level of fertilizer input applied to maize in the second phase. Labor cost was calculated for fertilizer and manure application by multiplying number of person-days per ha (1 person- day = 8 hours of field work) by the unit wage (US$ 4.47 man-day^{-1}). We excluded planting, weeding, and harvest costs because we did not expect the required person-days associated with these activities to vary much across treatments. Total cost for each plot (on a per ha basis) was thus calculated by summing the cost of nutrient inputs and associated labor, as well as seeds, since these differ between legume species. For each treatment combination, average market value of common bean, soybean, and maize were used to calculate revenues. The market price at harvest was US$ 0.77 kg^{-1} for common bean, US$ 1.36 kg^{-1} for soybean and US$ 0.68 kg^{-1} for maize grain. To calculate the net benefit (i.e., profit;

in US$ per ha), total costs were subtracted from the average market value of beans and maize in each treatment combination.

3.2.8 Statistical analysis

Data was analyzed in RStudio environment (Version 4.3.1) using a linear mixed effect model. Nutrient management strategy, legume species and their interaction were considered as fixed effects, while block was considered as a random effect. Model assumptions were checked using the Shapiro-Wilk test for normality and Levene's test for homogeneity of variance. Means were compared by Tukey-adjusted pairwise comparison. Correlations between maize grain yield and specific soil health parameters were also examined using simple linear regression. Analyses were conducted using lmer4, lmerTest, multcomp and emmeans packages, and an α value of 0.05 was used as a threshold for significance in all analyses.

3.3 Results

3.3.1 Effect of nutrient management strategies on nodulation and performance of legumes

Manure additions increased grain yield across both legume species by 129% relative to the control (figure 3.1), while fertilizer increased yield by 98% on average. Common bean yields were roughly double that of soybean across nutrient management treatments. However, a significant interaction between legume and nutrient management indicated that the beneficial effect of manure (and fertilizer) on yields was much more pronounced for common bean than for soybean (figure 3.1). Aboveground biomass showed similar trends to grain yield, where the manure and fertilizer treatments increased biomass by 78% relative to the control but were not different from each other. Again, a significant interaction suggested that common bean responded more to nutrient inputs (115% increase in biomass) relative to soybean (43% increase). Manure increased HI of common bean by 32% but had no effect on the HI of soybean (Table 3.2). Manure and fertilizer similarly resulted in taller plants and greater pod number than the control, with soybean generally having higher values for both measurements. A slightly higher branch number was observed in the fertilizer treatment compared to manure and control, but a significant interaction suggests that this effect is limited to soybean. Both manure and fertilizer greatly improved nodule number and nodule biomass across legume species with common bean having the highest values and responding most strongly to nutrient input (figure 3.1).

3.3.2 Impact of nutrient management and legumes on soil health and maize yield

Nutrient management strategies and the legume species planted in the first phase of the study significantly affected a range of soil health indicators at the time of maize planting (Table 3.3). Manure applications reduced bulk density by about 20% and increased soil pH by 3.5% compared to the control, with the fertilizer having intermediate values. Manure also increased SOM by 10%, while both manure and fertilizer similarly increased POM, POXC and Olsen P by at least 3, 17 and 23%, respectively, compared to the control. Nutrient management had no effect on MWD, whereas common bean increased MWD by 17% relative to soybean, across nutrient management strategies. Common beans also significantly increased soil pH and Olsen P compared to soybeans (Table 3.3). There were no significant interactions between nutrient management and legume species for any of the measured soil health indicators.

Maize grain yield and aboveground biomass (AGB) were significantly influenced by nutrient management and legume species. Manure (applied to the previous legume crop) significantly increased maize grain yield by 41 and 166% relative to fertilizer and control, respectively. It also improved maize AGB by 21 and 161% compared to fertilizer and control, respectively (Table 3.3). In addition, legume species planted in the previous phase had a significant effect on both maize AGB and grain yield. Common beans increased maize grain yield nearly by 28% (an increase of 1.0 Mg ha^{-1}) and AGB by 20% (1.6 Mg ha^{-1} increase) compared to soybean. No interactive effects of nutrient management and legume species were observed for maize grain yield or AGB.

3.3.3 Variations in maize grain yield as explained by soil health indicators

Correlations between maize grain yield and measured soil health indicators revealed a significant negative correlation for bulk density ($p = 0.001$; $R^2 = 0.38$) and significant positive correlations for pH ($p = 0.008$; $R^2 = 0.28$), SOM ($p < 0.001$; $R^2 = 0.65$), POM ($p < 0.001$; $R^2 = 0.43$), POXC ($p < 0.001$; $R^2 = 0.40$), and Olsen P ($p < 0.001$; $R^2 = 0.51$; figure 3.2).

3.3.4 Economic benefit of nutrient mangment and legume species

The cost-benefit analysis revealed that manure and fertilizer application to common bean or soybean markedly increased yields and thus economic return relative to the control treatment. With the same investment (270.52 US\$ ha^{-1}), manure application in common bean–maize and soybean–maize rotations increased net benefit by 166% (3196.84 US\$ ha^{-1}) and 111% (2032.45 US\$ ha^{-1}) compared to the control, respectively, while fertilizer increased by 117% (2257.53 US\$ ha^{-1}) and 36% (660.84 US\$ ha^{-1}). In both cases, maize accounted for a larger share of the revenue than beans (Table 3.4).

3.4 Discussion

3.4.1 Effect of nutrient management strategies on growth and yield of legumes

Integration of legumes into smallholder cereal-based cropping systems offers great promise for addressing soil degradation and food security challenges, yet management of legumes to best support soil health and long-term productivity is often lacking. We hypothesized that manure would enhance legume performance via improvements to soil health that would, in turn, support N fixation and overall plant growth. In our study, legume performance was generally highest in the manure treatments, and common bean yield was significantly higher in the manure treatment than in both the fertilizer and control treatments (figure 3.1; Table 3.2). While manure applied at the start of the experiment contained more total N than fertilizer, we suspect that only a portion of this N became available during the growing season (Gale et al., 2006), thus overall N availability was likely lower in the manure treatments. At the same time, the manure treatment supplied lower total P and S than was applied with fertilizer; therefore, available P and S amounts over the course of the season were likely quite a bit lower, making it even more impressive that the plots receiving manure did as well or even better than those receiving fertilizer. We suspect that the strong performance of beans in the manure treatment, despite lower levels of available N, P, and S (compared to fertilizer), may be related to multiple factors. First, manure can supply additional nutrients beyond N, P, and S, as it typically contains large amounts of other macro- (K, Ca, and Mg) and micronutrients (e.g., Zn, Fe, and Mn; Davis et al., 2002; Lupwayi et al., 2000). Manure can also influence nutrient availability by modifying soil pH, which may be especially important in highly-weathered, acid soils that are common across much of SSA (Mutai et al., 2025b; Sileshi et al., 2025a). Enhanced legume performance in the manure treatment may also be associated with improved soil physical properties. Increased SOM, resulting from manure, is known to influence soil structure, infiltration and water holding capacity of soils (Liu et al., 2021; Minasny and McBratney, 2018), which can be critical to supporting yields in rainfed systems.

Beyond overall yield impacts, manure application markedly increased nodulation compared to the control (figure 3.1), likely due to its ability to enhance soil health by improving nutrient availability, increasing the labile C pool in the soil, and raising soil pH (Table 3.3), thereby creating favorable conditions for the host plant and rhizobia symbionts. Labile soil C, in particular, has been observed to stimulate symbiotic N fixation by promoting *Rhizobium* bacteria in the rhizosphere and by increasing nitrogenase activity (Bian et al., 2024; Shi et al., 2021). Our findings are consistent with previous studies in Ethiopia and elsewhere, showing that manure can enhance legume nodulation and N fixation by increasing P and C availability and by reducing soil acidity (Fekadu et al., 2019; Ullah et al., 2023; Xie et al., 2021)**.** At the same time, we note that fertilizer also increased nodulation to a similar extent as manure. This is likely associated with increased availability of P and S, which are essential for nodule formation (Li et al., 2021; Scherer and Lange, 1996) and potentially by providing starter N to support early crop growth. Regardless of the type of input, our findings strongly suggest that supplemental nutrients, among other factors, are essential to enhancing growth (and likely N-fixation; Haque and Lupwayi, 2000), and thus optimizing the overall benefits of integrating legumes into smallholder crop rotations.

We note that common bean grain yield and biomass were substantially higher than soybeans across nutrient management strategies and responded more to nutrient inputs than soybean (figure 3.1). This may be due, at least in part, to the observed higher nodulation of common beans, which could have supported greater N fixation. We suspect that this finding may be related to a lack of native and/or effective *Rhizobium* strains for soybean in our experimental field, as we did not inoculate and soybean has been shown to require more specialized *Rhizobium* in its symbiotic association than common bean (Bender et al., 2022). Soybean cultivation in Ethiopia is relatively new, and several studies suggest that soybean-nodulating *Rhizobium* are not yet common in many Ethiopian soils (Aserse et al., 2020, 2012; Muleta et al., 2017). Beyond the presence of compatible *Rhizobia*, we suspect that common bean may be better adapted to the region, and this may also contribute to its relatively higher growth and response to nutrient inputs.

At the same time, we note that our findings are in agreement with others who have shown common bean is more responsive to nutrient supply than that of soybean (Pauletti et al., 2010).

3.4.2 Impact of nutrient management and legume species on soil health and maize yield

Manure applications played a substantial role in improving key soil health indicators compared to the control, with fertilizer resulting in intermediate values (Table 3.3). Notably, manure increased SOM and associated fractions (POM and POXC) in the relatively short time frame considered here (~1 year). Direct C input from the manure and higher legume residue input associated with greater productivity under manure application in the first phase likely explain the significant accumulation of SOM (Howe et al., 2024**.** Our findings are in agreement with a meta-analysis of 42 manure application studies worldwide, which showed an increase in SOC with manure application compared to both fertilizer and unfertilized control treatments, primarily due to the direct manure C input (Maillard and Angers, 2014). Our result is consistent with other studies in East Africa, specifically Kenya, indicating that manure addition improved both POM and POXC (Kapkiyai et al., 1999; Mutai et al., 2025a)**.** We note that inorganic fertilizer also increased these fractions compared to the control (Table 3.3), presumably due to higher above- and belowground biomass input from crop residues (Zhang et al., 2020), but the effect of manure was generally larger for all SOM fractions.

Our findings also indicate that manure increased soil pH relative to the control, while fertilizer had no significant impact (Table 3.3); this effect is likely due to increased soil pH buffering capacity, especially in acidic soil. Cattle manure is an important input of basic cations and buffering capacity in acid soils of Africa (Sileshi et al., 2025b). Similar results on increased soil pH with manure addition to acidic soil have previously been reported (Dhiman et al., 2019; Mutai et al., 2025b).

Manure enhanced Olsen P (available P) by 80% relative to the control. This was likely due to, at least in part, the observed increase in pH, which reduces the potential for P fixation on aluminum and iron oxides (Oladipupo et al., 2020). Additionally, organic inputs are known to support P-availability in acid soils by maintaining P in a more biologically active form (i.e., microbial biomass P) and by physically blocking fixation sites (Oberson et al. 2006). Although the manure treatment supplied less total P than the fertilizer treatment in our study, Olsen P levels under manure were more similar to the fertilized treatment than the control, suggesting that the different mechanisms thereof are quite effective at supporting crop P nutrition. Along with nutrient management impacts, we found the legume species was also important, as we observed greater amounts of available P in the soil following common bean harvest compared to soybean. We suspect that this is due to the release of P following decomposition from the greater residue return of common bean, although it is also possible that the rhizosphere community of common bean solubilized more P than that of soybean (Afkairin et al., 2024; Alamgir et al., 2012).

Manure applications decreased soil bulk density (a measure of soil compaction) compared to the fertilizer and control treatments (Table 3.3), likely by increasing SOM and porosity. Surprisingly, there were no significant effects of manure on aggregate stability, as past research has suggested that organic matter additions (including manure) often enhance aggregation and porosity, which tends to be inversely related to bulk density (Fu et al., 2022). Organic amendments, particularly manure, can lead to relatively rapid reductions in bulk density, especially in soils with initially low organic matter (Agbede, 2025). We note that, common beans improved soil aggregate stability more than soybean, as indicated by the higher MWD (Table 3.3). This might be attributed to the relatively high residue return from common bean, which increased SOM and stimulated biological activity that broadly supports aggregation in soils (Husain and Dijkstra, 2023; Six et al., 2004).

Manure, applied to the legumes at the start of the study greatly increased maize yield and biomass compared to both fertilizer and control (Table 3.3). We suspect that this is, at least in part, attributed to the continued, slow release of nutrients from the manure applied during the legume phase becoming available to the succeeding maize crop due to its slow mineralization rate (Goldan et al., 2023; Hurisso et al., 2024). Others suggest that roughly 20% organic N applied in the form of manure is expected to be available for the succeeding growing season (Eghball, 2000). Consistent with our result, Sileshi et al. (2025b) in a meta-analysis across 14 African countries, observed increased maize yield with manure application compared to no-input control. The significant difference between manure and fertilizer was likely due to the additional macro- and micronutrients supplied by manure beyond those supplied by fertilizer, as well as improvements in soil health. Improved soil properties, beyond just nutrient availability, could also contribute to the increased maize yield in the manure treatment. We note that maize yield was positively correlated with SOM, POM, and pH, and negatively correlated with bulk density (figure 3.2), suggesting that multiple soil properties likely contribute to the overall positive impacts of manure.

Maize grain and biomass yield closely followed the trends in the preceding soybean and common bean, showing a strong rotation benefit from common bean (figure 3.1 and Table 3.3). We suspect this is due to higher nodule formation observed for common bean that likely supported higher N fixation and carryover of N to maize (Wolde *et al., submitted*). Consistent with our findings, Rurangwa et al. (2018) reported higher N uptake and yield in maize planted after common bean compared to soybean in smallholder system in Kenya. The relatively greater amount of common bean residue incorporated after harvest may have provided other non-N associated benefits for example via improvements to soil structure (MWD) and Olsen P, thereby contributing to increased maize yield (figure 3.2; Table 3.3). Improvement in soil pH and physical properties after growing legumes can also contribute to increased yield by promoting root growth and enhancing nutrient foraging capacity of maize (Bossolani et al., 2021; Uzoh et al., 2019).

Overall, manure application, especially when combined with common bean, improved soil health and significantly increased maize yield. We note, these effects may extend well beyond the maize and have significant implications for productivity in the long-term, as has been shown in other studies (Reeve et al., 2012). These findings underscore the importance of soil health improvements through organic inputs and legume integration as a strategy to improve soil health and crop productivity in smallholder farming systems.

3.4.3 Economic implications for smallholder farmers

In Ethiopia, access to fertilizers for smallholder farmers is a major constraint due to high cost and poor distribution systems (Asmamaw et al., 2019; Wolde et al., 2020). As a result, smallholder farmers usually apply little or no external nutrient inputs, contributing to soil degradation and low yields. Our findings showed that nutrient applications can greatly improve legume and subsequent maize yields. While both manure and fertilizer applied to legume-maize rotations significantly increased net benefits compared to the control, manure yielded higher returns than fertilizer, for the same level of investment (Table 3.4). We found that maize following common bean with manure application generated the highest net profit, outperforming maize after common bean with fertilizer, or soybean with either nutrient source. Manure application increased net benefit by 166% for the common beans-maize rotation (from 1928.85 to 5125.69 USD ha^{-1}) and 111% for the soybeans-maize rotation (from 1832.03 to 3864.48 USD ha^{-1}), while fertilizer increased profits by just 117% and 36%, respectively, compared to the control (Table 3.4). The higher profitability with manure application despite the increased labor cost was due to the greater grain yield of both legumes and maize, suggesting considerable potential to improve smallholder incomes. This trend was largely driven by maize, which consistently generated higher revenue than beans, underscoring the importance of considering multiple crops in soil health studies.

The observed soil health and economic advantages of manure in our study, an input locally available especially in mixed crop-livestock farms, aligns with national efforts to promote integrated soil fertility management. Indeed, crop-livestock mixed farming accounts for more than 90% of agriculture in Ethiopia, and the country has the highest number of livestock in Africa (Duguma and Janssens, 2021). However, even where manure is potentially available in sufficient quantities, widespread adoption may be limited due to cultural norms, lack of knowledge, labor, and competing uses of manure (e.g., as a fuel source), especially in areas with limited access to firewood or other energy sources (Lupwayi et al., 2000; Sileshi et al., 2025b). An effective promotion of manure use to improve productivity and soil health could be supported by a holistic set of government policies that address multiple related challenges at once. Such efforts might include improving access to credit for smallholder farmers to enable initial investments, develop labor sharing models for manure application in smallholder communities, subsidizing farmers to access improved manure application technology including tractors at least per farmers village, enhancing access to alternative energy sources (e.g., electric power or biogas) and efficient cooking technologies to reduce manure use as a fuel source, and providing targeted demonstration and extension services, particularly on manure composting and use of small ruminant and poultry manure which are more easily available in smallholder farms, in addition to cattle manure.

3.5 Conclusion

Our study demonstrates the potential role of organic inputs in enhancing legume performance, soil health and subsequent cereal yield. Application of manure or fertilizer is important in supporting legume performance, but our findings indicate that manure has a greater benefit for soil health and carryover effects on subsequent crops and is likely more profitable when considering the whole rotation. These benefits were linked to improvements in soil pH, SOM, and P availability, alongside reduced compaction, which collectively enhance crop yields beyond the initial legume crop. Our findings also suggest that farmer selection of which legume species to plant plays an important role, with common bean performing better than soybean and offering improved support for soil health and subsequent maize productivity. Beyond agronomic and soil health outcomes, our findings revealed significant economic benefits of manure application in legume–maize rotations, with profitability surpassing that of fertilizer, even when accounting for increased labor, thus emphasizing the potential of manure as a locally available and affordable input. Promoting manure use in legume-cereal rotations, while addressing barriers such as competing household uses, labor issues and limited awareness through robust policies and extension education, could offer a practical and accessible pathway towards regenerative nutrient management and long-term sustainability of smallholder agriculture in Ethiopia and similar contexts.

CHAPTER 3: TABLES AND FIGURES

Table 3.1. Nutrient contents and application rate of the aged dairy manure and inorganic fertilizer applied in a field trial in Ethiopia in 2022 and 2023.

Parameters	Nutrient content	
	Manure	NPS fertilizer [ǂ]
Total organic carbon (%)	38.12	---
C:N ratio	28.9	---
Total N (%)	1.32	19
Available N (%)	---	19
Total P (%)	0.56	16.6
Available P (%)	---	16.6
Total K (%)	1.17	---
Total S (%)	0.26	---
Available S (%)	---	7
Total Ca (%)	0.46	---
Total Mg (%)	0.44	---
Total Na (%)	0.14	---
Total Fe (%)	0.20	---
Total B (%)	17.87	---
Total Mn (mg kg^{-1})	0.05	---
Total Cu (mg kg^{-1})	18.99	---
Total Zn (mg kg^{-1})	105.60	---

[ǂ] (Getaneh and Laekemariam, 2021)

Table 3.2. Legume performance metrics observed for different nutrient management strategies (NMS) and legume species (LS) in a field trial in Ethiopia in 2022. Values are mean ± standard error for treatment combination (n=4). P-values in bold denote significant effects at $p < 0.05$, while letters refer to Tukey tests, such that treatments sharing the same letter are not significantly different.

NMS	Legume species	Pod number (plant^{-1})	Branch number (plant^{-1})	Plant height (cm)	Harvest index
Control	Common bean	6.67±0.99	1.7±0.2^{c}	13.9±0.76	0.31±0.01^{b}
	Soybean	11.55±0.92	4.6±0.7^{b}	37.5±2.1	0.23±0.02^{c}
Fertilizer	Common bean	14.15±0.99	3.9±0.5^{b}	24.9±1.1	0.37±0.01^{ab}
	Soybean	20.33±1.8	7.4±0.5^{a}	49.5±2.3	0.20±0.02^{c}
Manure	Common bean	17.93±1.8	4.0±0.3^{b}	29±4.71	0.41±0.02^{a}
	Soybean	19.15±2.05	5.1±0.4^{b}	42.5±2.5	0.23±0.01^{c}
ANOVA	p-value				
NMS		**< 0.001**	**< 0.001**	**< 0.001**	**0.008**
LS		**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**
NMS x LS		0.127	<**0.027**	0.056	**0.009**

Table 3.3. Soil health metrics observed with nutrient management strategy (NMS) and legume species (LS) before maize planting and yield metrics of maize in a field trial in Ethiopia between May 2023 and January 2024. Values are mean ± standard error for treatment combination (n=4). P-values in bold denote significant effects at $p < 0.05$.

		Soil Health Parameters								
NMS	Legume species	BD (g cm^{-3})	MWD	pH	SOM (%)	POM (%)	POXC (mg kg^{-1})	Olsen P (mg kg^{-1})	Maize AGB (Mg ha^{-1})	Maize Yield (Mg ha^{-1})
Control	Common bean	1.10±0.09	654±64	5.76±0.07	3.67±0.01	0.28±0.03	285.3±8.8	3.7±0.1	4.72±0.13	2.25±0.15
	Soybean	1.13±0.10	584±16	5.72±0.12	3.65±0.09	0.27±0.04	280.2±9.4	3.3±0.3	4.48±0.54	2.00±0.32
Fertilizer	Common bean	1.00±0.04	746±72	5.87±0.04	3.85±0.08	0.37±0.01	343.1±14.9	7.3±0.8	10.80±0.46	4.85±0.74
	Soybean	1.03±0.06	673±95	5.75±0.07	3.70±0.10	0.34±0.02	316.8±33.3	6.0±0.4	8.90±0.82	3.18±0.50
Manure	Common bean	0.89±0.03	797±75	6.04±0.10	4.06±0.09	0.41±0.02	364.1±18.3	6.8±0.8	13.33 ±0.5	6.13±0.23
	Soybean	0.91±0.04	618±33	5.82±0.04	4.03±0.06	0.35±0.02	329.0±28.9	5.8±0.3	10.65±0.44	5.18±0.54
ANOVA	p-value									
NMS		**0.017**	0.228	**0.020**	**< 0.001**	**< 0.001**	**0.017**	**< 0.001**	**< 0.001**	**< 0.001**
LS		0.619	**0.037**	**0.019**	0.253	0.084	0.202	**0.009**	**< 0.001**	**0.002**
NMS x LS		0.995	0.571	0.336	0.611	0.412	0.756	0.515	0.079	0.117

BD, bulk density; MWD, Mean weight diameter; SOM, soil organic matter; POM, particulate organic matter; POXC, permanganate organic matter; Olsen P, Olsen phosphorus; AGB, aboveground biomass.

Table 3.4. Cost-benefit analysis for combinations of nutrient management strategies (NMS) and legume species in a field trial in Ethiopia conducted between May 2022 and January 2024. The net benefit (in US\$ ha^{-1}) was calculated by subtracting treatment cost from the average market value of each crop in each treatment combination. IBN; increase in net benefit by nutrient mangement strategy compared to control within legume species.

Treatment combination		Input cost (US\$ ha^{-1})				Grain yield (kg ha^{-})		Market value (US\$ ha^{-1})			Net benefit (US\$ ha^{-1})	IBN (%)
NMS	Legume species	Nutrient input	Legume seed	Labor	Total †	Legume	Maize	Legume	Maize	Total		
Control	Common bean	32.88	27.10	12.04	168.82	740	2250	573.06	1524.60	2097.66	1928.85	0
	Soybean	32.88	38.72	12.04	180.44	485	2000	657.27	1355.20	2012.47	1832.03	0
Fertilizer	Common bean	270.52	27.12	99.16	493.58	1800	4850	1393.92	3286.36	4680.28	4186.38	117
	Soybean	270.52	38.72	99.16	505.2	622	3180	843.61	2154.77	2998.38	2492.87	36
Manure	Common bean	270.52	27.12	238.9	633.32	2073	6130	1605.33	4153.69	5759.02	5125.69	166
	Soybean	270.52	38.72	238.9	644.94	738	5180	999.46	3509.97	4509.43	3864.48	111

† total cost include maize cost for seed (US\$ 96.80 ha^{-1}).

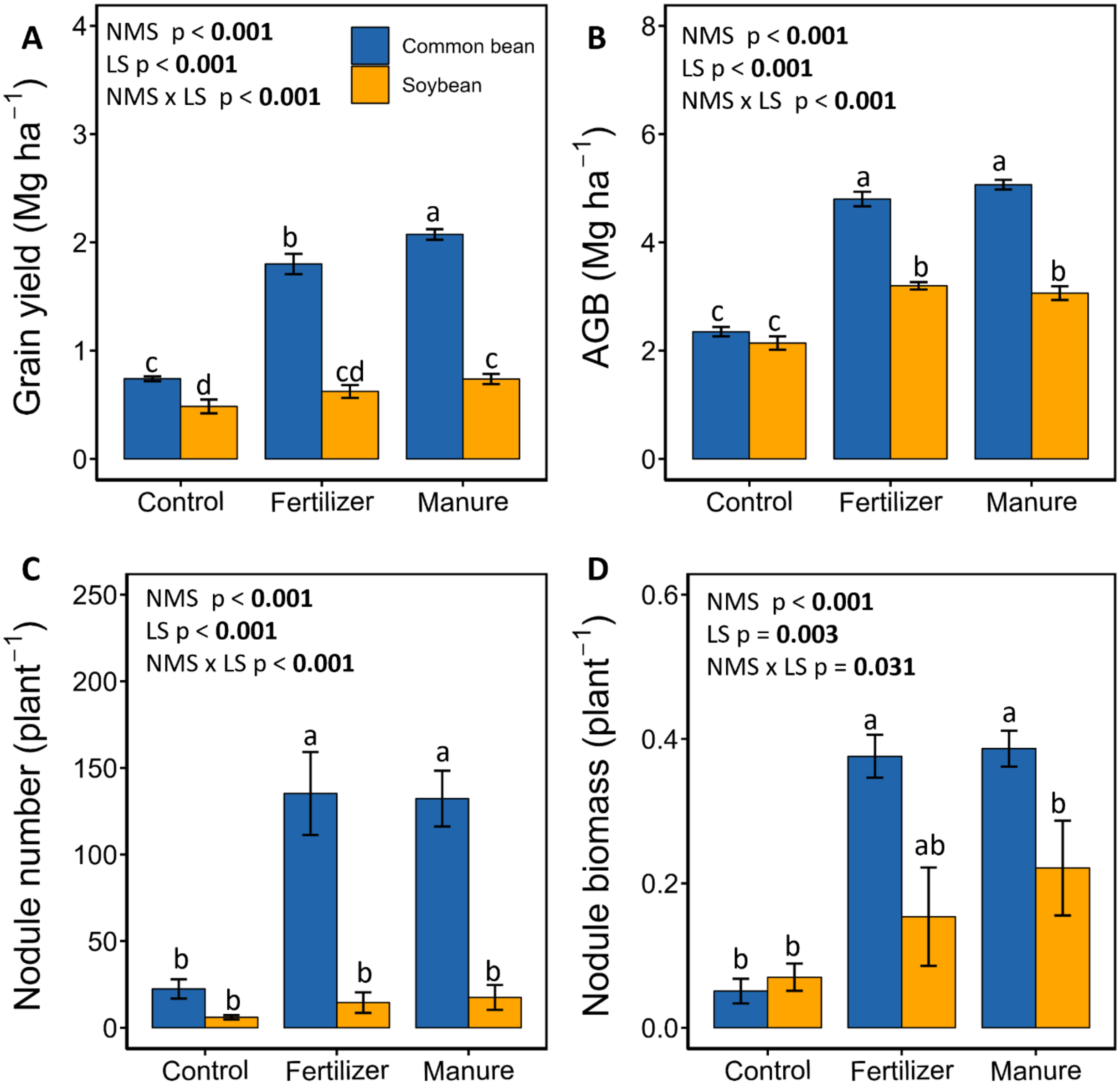

Figure 3.1. Nutrient management strategies (NMS) and legume species (LS) effect on mean A) legume grain yield, B) aboveground legume biomass, C) nodule number and D) nodule dry biomass in a field trial in Ethiopia in 2022. P-values for each factor and their interaction are presented in the top left corner of each panel, with values in bold indicating significance at $p < 0.05$. Error bars represent the standard error of the mean (n = 4), while letters refer to Tukey tests, such that treatments sharing the same letter are not significantly different. AGB, aboveground biomass.

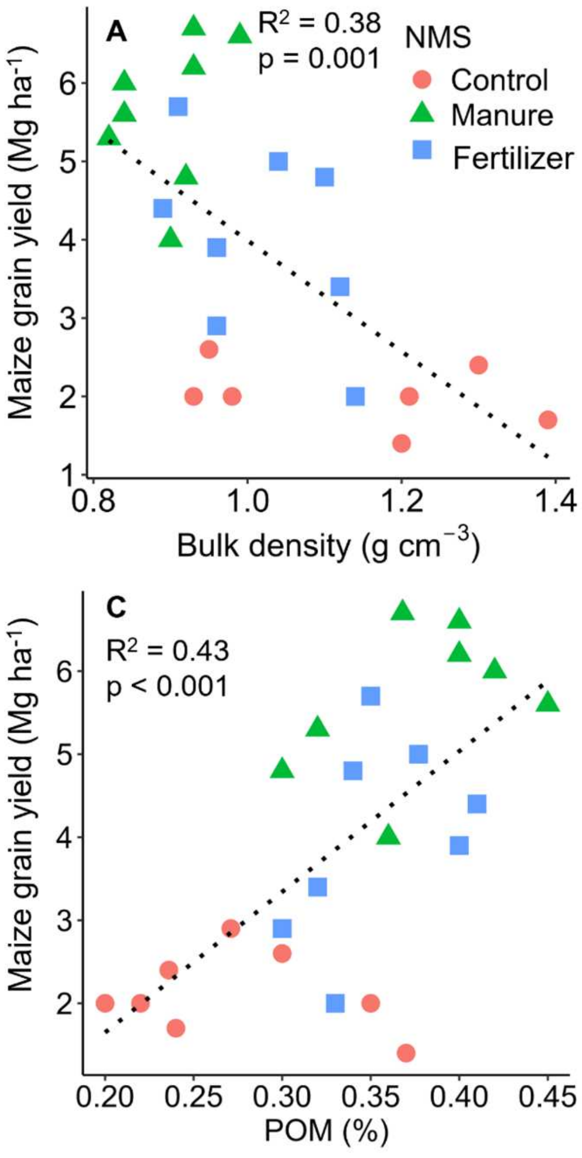

Figure 3.2. Relationship between maize grain yield and key soil health indicators (n = 24): A) Bulk density, B) Soil organic matter (SOM), C) Particulate organic matter (POM) and D) P availability (Olsen P). Soil samples were collected at maize planting (May 2023), while maize yield was collected at harvest (January 2024) in a field trial in Ethiopia with different legume and soil nutrient management strategies.

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CHAPTER 4: RESIDUE C INPUTS INTERACT WITH SOIL N AVAILABILITY TO REGULATE COWPEA N FIXATION AND ASSOCIATED RHIZOSPHERE MICROBIAL COMMUNITIES

4.1 Introduction

The integration of legumes into cereal-based cropping systems plays an important role in addressing multiple sustainability goals. By supplying biologically fixed nitrogen (N) to agroecosystems, legumes can reduce dependence on synthetic N fertilizers and their associated environmental and economic costs (Palmero et al., 2022; Wolde-meskel et al., 2018). Symbiotic N fixation, mediated by legumes and associated *Rhizobium* bacteria, can contribute greatly to soil fertility, cropping system productivity, and long-term soil health (Poole et al., 2018; van der Pol et al., 2022). However, the effectiveness of this symbiosis is sensitive to environmental controls such as soil properties, and management practices that regulate carbon (C) and N dynamics in the rhizosphere (Bian et al., 2024; Moreau et al., 2021).

Crop residue retention promotes soil health by providing a source of organic C inputs that contribute to soil organic matter (SOM), stimulate microbial activity, influence nutrient cycling and other soil properties (Murphy et al., 2016; Siedt et al., 2021). Residue-derived C, particularly more labile fractions such as dissolved organic C (DOC), can enhance microbial growth and promote legume-*Rhizobium* symbiosis (Fang et al., 2025a; Xie et al., 2021b). *Rhizobium* can survive as free-living saprophytes in the rhizosphere before interacting with a legume host to form nodules and a symbiotic relationship. Thus, greater availability of labile C, which serves as an energy source for free-living *Rhizobium*, can enhance their survival in the rhizosphere soil and facilitate nodulation, and ultimately increase symbiotic N fixation (Han et al., 2020; Poole et al., 2018; Xie et al., 2021b). While all residues provide organic C, the quality of this material can vary considerably between residue types. Residue attributes such as C:N ratio can have a strong influence on decomposition rates, microbial N immobilization, and available N in the soil (Breza and Grandy, 2025). Residues with higher C:N ratios generally promote N immobilization and reduce soil N availability, at least in the short-term, while low C:N inputs increase N availability and even can suppress biological N fixation (Hu et al., 2025; Liang et al., 2023). By regulating the relative availability of C and N in soils, residue quality can influence rhizosphere microbial communities, particularly the abundance of diazotrophs, consequently affecting N fixation by legumes and free-living bacteria (Yu et al., 2018).

Following from the effects of residue quality, soil N availability is an important regulator of nodulation and symbiotic N fixation in legumes. For example, high levels of soil inorganic N, particularly NO_3^--N, has been shown to inhibit nodule formation by interfering with nodulation signaling pathway (Danso et al., 2025; Lin et al., 2021), and reducing nodule biomass by limiting photostatic C allocation from the plant host (Alon et al., 2021; Li et al., 2023). Elevated N availability has been shown to reduce N fixation by downregulating N-fixing functional genes as legumes preferentially utilize readily available soil N rather than investing in energetically costly symbioses (Casarin et al., 2025; Dixon and Kahn, 2004). As such, fertilizer N applications have been reported to reduce nodulation and the percentage of N derived from the atmosphere in legumes (Aryal et al., 2024; Favero et al., 2022). However, the effects of C inputs and N availability on N fixation are inconsistent, suggesting that the role of N availability and associated organic matter inputs in regulating N fixation is not yet fully understood.

Rhizosphere microbial communities play a critical role in mediating C and N dynamics in soil. At the same time, residue management and fertilization can reshape the rhizosphere microbiome by selectively enriching or inhibiting taxa such as N-fixing plant symbionts (e.g., *Rhizobia* spp.) and free-living diazotrophs (Bian et al., 2024; Su et al., 2020). Selective enrichment of functionally important taxa such as *Rhizobium* often governs N fixation outcomes in legumes (Badri et al., 2009; Lindström and Mousavi, 2019; Poole et al., 2018). Despite the growing recognition of these mechanisms, few studies, if at any, have simultaneously considered residue C input and soil N availability with direct measurements of symbiotic N fixation, rhizosphere microbial community composition, and N-fixing functional gene dynamics. Consequently, the extent to which residue-derived C inputs and soil N availability independently and interactively regulate symbiotic N fixation in legumes has not been fully elucidated. Addressing this gap is essential for developing residue management strategies that optimize biological N fixation while supporting sustainable N management and soil health.

To address these knowledge gaps, we conducted a pot experiment using cowpea (*Vigna unguiculata*), a promising legume for integration within wheat-based systems of the North American Great Plains region. Wheat residue and inorganic N, common inputs in these systems, were used to manipulate rhizosphere C and N availability. We quantified cowpea symbiotic N fixation using a ^{15}N isotope dilution approach, and examined rhizosphere soil biochemical properties, bacterial community composition, and *nifH* genes abundance. Our objective was to understand how residue C inputs and soil N availability regulate N fixation in cowpea. We hypothesized that: 1) residue-derived C inputs enhance symbiotic N fixation by promoting *Rhizobium* abundance and *nifH* gene enrichment in the rhizosphere, and 2) increasing soil N availability will suppress symbiotic N fixation by reducing the need for biologically fixed-N, and thus diazotrophic abundance and nodulation.

4.2 Materials and methods

4.2.1 Experimental design and treatments

A greenhouse experiment was conducted at the Colorado State University (CSU) Plant Growth Facility. The greenhouse used artificial lighting to maintain a 16 h photoperiod, with temperatures ranging from 18 ºC to 23 ºC. Surface soil (0-30 cm soil) was collected for the pot experiment from a low fertility plot located at the CSU Research, Development, and Education Center (ARDEC) near Fort Collins, Colorado, USA. The soil was a clay loam classified as mixed, superactive, mesic Aridic Haplustalfs (Junaidi et al., 2018). Upon collection, soil was air-dried, passed through an 8 mm sieve and mixed with clean quartz sand in a 1:2 soil:sand ratio (by volume), with approximately 2.4 kg of soil-sand mixture per pot. A sub-sample of the soil-sand mixture was sent off for analysis at a commercial lab (http://www.wardlab.com) and found to have a pH of 7.9, and SOM concentration of 0.9%, with relatively low concentration of available N as well as other macro- and micronutrients.

We examined cowpea (*V. unguiculata*) growth and rhizosphere responses in pots with varying levels C and N input, comprising five input treatments: 1) winter wheat (*Triticum aestivum*) residue addition alone, with a C:N ratio of 40 (high C:N), 2) wheat residue + urea to achieve a C:N ratio of 25 (medium C:N), 3) wheat residue + urea with a C:N of 15 (low C:N), 4) urea alone, with no wheat residues (urea), and 5) a control with no residue or urea. Wheat residue alone (C:N 40) was selected to reflect the common practice in Colorado of farmers leaving wheat residues in the field after harvest. To adjust the C:N ratio, and thus soil N availability, different rates of N in the form of urea were added under the same wheat straw input (4.3 g dry mass pot $^{-1}$, equivalent to ~3 Mg ha^{-1}; Table 4.1). The low and medium C:N treatments were selected to encourage net N mineralization (Paul and Clark, 1989; Trinsoutrot et al., 2000).

Wheat residue was chopped into approximately 1 cm segments and thoroughly mixed with the soil-sand mixture for the treatments containing wheat. Cowpea (California #46 variety) was planted in eight replicate pots (12 x 13.8 x 13 cm, height, top and bottom diameter, respectively) for each treatment in a completely randomized design. Seven seeds were directly planted in each of the cowpea pots in late-November 2024, placed in a greenhouse and were thinned to two seedlings one week after emergence. Urea was ground and applied in a solution according to the treatments (Table 4.1) after thinning, and for all pots a uniform amount (0.368 g pot^{-1}) of triple super phosphate (0 % N, 46 % P_2O_5 and 0 % K_2O) was applied based on the soil test result to avoid P limitation. Soil water content was maintained at approximately 70% field capacity over the course of the experiment. Any leachate following watering was captured on saucers and reapplied to the pots. Pots were re-randomized every two weeks throughout the experiment to avoid potential microclimatic effects in the greenhouse.

4.2.2 Evaluating N-fixation

To estimate the amount of atmospheric N_2 fixed by cowpea, we used a ^{15}N isotope dilution approach, which relies on adding small additions of highly enriched ^{15}N fertilizer (Unkovich et al., 2008) and a non-N fixing reference species. Kale (*Brassica oleracea*) was used as the reference species and planted in three additional pots for each treatment, for a total of 55 pots (40 for cowpea and 15 for kale). One week after emergency of cowpea and kale, in early-December 2024, every pot received 35 mg of 98 atom % ^{15}N NH_4NO_3, equivalent to 12.9 mg N pot$^{-1,}$ to label the plant available N pool in the soil with ^{15}N. The ^{15}N NH_4NO_3 was dissolved in 550 ml distilled water and 10 ml of the ^{15}N solution was applied to the soil surface of each pot in a “W” pattern using a syringe. All pots were then watered to field capacity to help infiltrate the ^{15}N solution and uniformly label the soil-sand mixture. Throughout the growing season, symbiotic N fixation dilutes ^{15}N absorbed into the cowpea tissue relative to kale. The amount of cowpea N fixation is proportional to the amount of dilution.

4.2.3 Plant growth, nodulation, and ^{15}N measurements

Pots were destructively sampled 70 days after planting (when cowpea was at full flowering) to evaluate plant biomass and quantify the amount of N fixed by cowpea. Shoots were harvested by cutting both plants at the soil surface. Rhizosphere soil samples were collected from each cowpea pot. Cowpea and kale root systems were carefully retrieved from the pots and washed gently with tap water. A 0.5 mm mesh was placed underneath the harvested root system to prevent loss of root fragments and nodules. Nodules were separated from the cowpea root by hand and counted to determine the number of nodules per plant. Nodules were then surface sterilized by immersing them in 70 % ethanol, followed by 5 % sodium hypochlorite, and finally rinsed three times in distilled water. The nodule samples were stored at -20°C until DNA extraction. Shoot and root biomass of cowpea and kale were oven-dried at 60 °C and weighed. Immediately prior to harvest, cowpea plant height was recorded by measuring from the soil surface to the tip of the shoot. Chlorophyll levels of young and old leaves were measured a day before harvest by chlorophyll mater (SPAD-502 Konica Minolta; Tokyo, Japan) to provide an idea of plant N limitation.

Roots and shoots were ground separately and each passed through a 2 mm sieve. Ground samples were further milled into a fine powder using mortar and pestle, and root and shoot powder was combined proportionally based on their relative masses. Subsamples of each mixture (5 ± 0.3 mg) were encapsulated in tin capsules to measure ^{15}N (atom % ^{15}N) and N (% N) contents of the biomass on an isotope ratio mass spectrometer, PDZ Europa 20-20 (Sercon Ltd., Cheshire, UK) at the University of California, Davis Stable Isotope Facility. The percentage of N derived from the atmosphere (%Ndfa) due to N_2 fixation for cowpea was calculated using equation 1 (Unkovich et al., 2008).

$$\%\text{Ndfa} = \left[1 - \left(\frac{\text{atom\% 15N excess of CP}}{\text{atom\% 15N excess of RC}}\right)\right] \text{x } 100 \qquad (1)$$

Where CP and RC are N_2 fixing cowpea (CP) and non-N_2 fixing reference crop (RC, kale), respectively.

The amount of N_2-fixed by cowpea was calculated using equation 2:

$$\text{N fixed } \left(\frac{\text{g}}{\text{plant}}\right) = \frac{\%\text{ Ndfa}}{100} \text{ x } \frac{\%\text{ N}}{100} \text{ x biomass (g per plant)} \qquad (2)$$

4.2.4 Rhizosphere soil sampling

Immediately following harvest, cowpea roots were shaken gently to separate and collect the adhered rhizosphere soil. Rhizosphere soil samples used for DNA extraction were stored at -20 ° C while another subsample was stored at -4 ° C for extraction of available N, dissolvable organic carbon (DOC), total dissolved N (TDN) and evaluation of enzyme activity. Bulk soil samples were also collected and air-dried to analyze for permanganate oxidizable C (POXC).

4.2.5 Rhizosphere soil C and N analysis

The rhizosphere soil was evaluated NO_3^--N and NH_4^+-N by extracting with a 2M KCl solution and shaking for 1 hr, and then quantified colorimetrically by Flow Injection Autoanalyzer (Keeney and Nelson, 1982). The sum of NO_3^--N and NH_4^+-N is reported as total mineral N. Dissolved organic C and TDN were analyzed following the methods outlined by Li et al. (2018). Briefly, 8 g of air-dried soil (24 h at room temperature) was placed in 40 ml Milli-Q water (1:5 w/v) were shaken for 8 h at 170 rpm at room temperature, followed by 10 min centrifugation at

2800g. The supernatant was then filtered through syringe tip filter with 0.45 μm cellulose acetate membrane. The DOC and TDN were measured for each sample using a TOC-L Shimadzu analyzer (Shimadzu Corporation, Columbia, MD, USA). The measured DOC and TDN mass were normalized to the rhizosphere soil mass to calculate DOC and TDN, respectively. Active soil organic C was oxidized by potassium permanganate to determine permanganate-oxidizable C (Weil et al., 2003), and absorbance was read at 550 nm on a Cytation 5 microplate reader (BioTek Instruments, Vermont, USA).

4.2.6 Soil enzyme activity

Soil enzyme activity was measured for one N cycling enzyme (NAG, N-acetyl-β-glucosaminidase) and one P cycling enzyme (PHOS, Phosphatase) and four C cycling enzymes: β-glucosidase (BG), α-glucosidase (AG), β-d-cellobiohydrolase (CB) and β-xylosidase (XYL). The substrate, 4-methylumbelliferyl (MUB-gal; dissolved in 2 % of ethanol) was used to measure enzyme activity, producing highly fluorescent MUB cleavage products upon hydrolysis. Rhizosphere soil (1g moist soil) was used to measure enzyme activity in 96-well microplates using fluorometry as outlined by (Bell et al., 2013). Two laboratory replicates (12 replicate wells) were set-up per sample and each standard concentration. Enzyme activities were corrected using a quench control. The microplates were incubated at 25 °C for 4 h in the dark. The fluorescence was read at 365 nm excitation and 450 nm emission wavelength on a Tecan Infinite M Nano Plus microplate reader (Tecan, Mannedorf, Switzerland). The activities were expressed as nmol h^{-1} g^{-1} dry soil

4.2.7 Soil and nodule DNA extraction

To understand how C and N additions influence N-fixing genes (*nifH*) and the bacterial community in rhizosphere soil, and *nifH* gene abundance in nodules, total genome DNA was extracted from 0.25 g of cowpea rhizosphere soil and nodules at harvest using a DNeasy PowerSoil Pro QIAcube DNA extraction kit (Qiagen, Hilden, Germany), according to the manufacturer's instruction. The concentration of DNA (ng/µL) was quantified by Qubit Fluorometer (Thermo Scientific, Illinois, United States). Nodule samples weighing less than 0.25g were directly transferred to the lysis tube for DNA extraction, while extra nodules from those weighing more than 0.25g were used for nodule dry weight measurement. In addition to nodule sterilization during sampling, they were surface sterilized by 70 % ethanol and then 3 % NaClO, and finally rinsed four times in double distilled water before DNA extraction. Once the DNA was extracted, the *nifH* gene was quantified, and 16S rRNA was sequenced.

4.2.8 Quantitative PCR analysis and 16S rRNA amplicon sequencing

The *nifH* gene copy numbers, the most representative genes for the N-fixing bacterial community, were quantified from both the nodule and rhizosphere soil using primer pairs *nifH*-F and *nifH*-R (Rösch et al., 2002). The quantitative polymerase chain reaction (qPCR) was performed in a 20 µL reaction mixture containing 2 µL of DNA template, 10 µL of Maxima SYBR Green/Fluorescein 2X qPCR Master Mix (ThermoFisher, Waltham MA, USA), 2 µL of each primer (10 µM), and 4 µL of nuclease free water, resulting in a final primer concentration of 1 uM. Thermal cycler conditions were as follows: Preincubation at 95°C for 5 min, then 40 cycles of 95°C for 5 sec, 53°C for 15 sec, 72°C for 10 sec. A melt curve was performed to confirm specificity. Concentration of *nifH* genes per gram dry weight were measured against a standard curve using 10 ng/uL ZymoBIOMICS Microbial Community DNA Standard (Zymo Research, Tustin CA, USA) containing $10^{5.537}$ *nifH* copies per uL. The 16S gene from rhizosphere soil was quantified using qPCR targeting the V3-V4 region with primers 341F and 806R as described previously without modifications (Manter et al., 2021).

The full ~1500 bp 16S rRNA gene was sequenced from rhizosphere soil using primers equipped with MinION-specific adapter sequences: 27F-Mn: *TTTCTGTTGGTGCTGATATTGC* AGRGTTYGATYMTGGCTCAG and 1492R-Mn: *ACTTGCCTGTCGCTCTATCTTC* TACCTTGTTACGACTT. The PCR was performed using 4 uL diluted rhizosphere DNA, 14.4 uL water, 20 uL Phusion Hot Start II Master Mix (ThermoFisher, Waltham MA, USA), and 0.8 uL each of 10 uM primer. Thermal cycler conditions were 98°C for 30 sec, then 25 cycles of 98°C for 15 sec, 50°C for 15 sec, 72°C for 60 sec, and final elongation of 72°C for 5 min. The PCR product was bead-cleaned before PCR addition of barcodes (cat# EXP-PBC096, Oxford Nanopore Technologies, Oxford UK) per manufacturer's instructions. PCR products were pooled at equal volumes then bead cleaned. Sequencing adapters were then ligated using Ligation Sequencing Kit V14 (cat# SQK-LSK114, Oxford Nanopore Technologies). The final library diluted to 50 fmol was loaded and sequenced on a MinION Mk1B for 48 hours. Raw sequence reads were basecalled, demultiplexed, and assigned q-scores using dorado v0.9.5 (Oxford Nanopore Technologies). The sequences were then filtered by average q-score (>10) and length (1360-1560 bp) using NanoFilt (De Coster et al., 2018) before classifying with Emu using its default reference database (Curry et al., 2022).

4.2.9 Statistical analysis

Statistical analyses were performed in the Rstudio environment (version 4.5.2). A one-way ANOVA using treatments as a fixed effect was used to evaluate the effect of the five C and N input treatments on cowpea growth, N-fixation metrics, soil chemical properties and enzyme activity. Model assumptions, homoscedasticity and normality of residuals, were checked using Levene's and Shapiro-Wilk tests, respectively. Data was log-transformed as needed to meet these assumptions. Means were compared using ANOVA and pair-wise comparisons using Tukey tests. In all analyses, an α value of 0.05 was used as a threshold for statistical significance.

Alpha diversity of rhizosphere bacterial community was evaluated by calculating observed richness, Shannon, Simpson and Inverse Simpson indices and treatment effect was compared using one-way ANOVA. Additionally, a PERMANOVA, permutational analysis of variance at genus level, with 999 permutations was run to examine the effect of treatments on bacterial community structure in cowpea rhizosphere soil (Bray-Curtis Distance ~ treatments). Bray-Curtis distance, dissimilarity, is a method used to examine differences in community composition by measuring the distance among samples and ordinating them onto axes based on their relationships to reference points (Bray and Curtis, 1957). Post-hoc comparisons were performed using the pairwise Adonis test adjusted by the false discovery rate (FDR) method at an α value of 0.05 to compare bacterial community structure between treatments. The PERMANOVA result was visualized using an ordination, distance-based redundancy analysis. We evaluated the dispersion among treatments in the bacterial community to test the assumption of homogeneity of dispersion required for PERMANOVA. Bray-Curtis matrix was used to calculate Beta diversity dispersion using the betadisper fuction in the vegan package, followed by a permutation test with 999 permutations.

To identify microbial taxa whose abundances differed significantly among treatments, we performed differential abundance analysis using the analysis of compositions of microbiomes with bias correction (ANCOM-BC2) method in the ANCOMBC2 R package (Lin and Peddada, 2024). ANCOM-BC2 applies a log-linear regression framework to bias-corrected microbial abundances, and differential abundance for bacteria genera between treatments and the control was assessed on estimated log-fold change coefficients. The method accounts for the compositional nature of microbiome count data through internal bias correction. Multiple testing was controlled using the Holm-Bonferroni (Holm) family-wise error rate adjustment, with significance assessed at an α level of 0.05. Pseudo-sensitivity analysis (pseudo_sens = TRUE) was enabled to evaluate the robustness of differential abundance results to pseudo-count addition. Spearman correlation was done to test the relationships between relative abundance of differentially abundant genera and rhizosphere soil properties.

4.3 Results

4.3.1 Cowpea performance, nodulation, and N fixation

Cowpea shoot biomass was highest in the urea and medium C:N treatments, more than 40% higher than both high and low C:N treatments, and 123% higher than the control (Table 4.2). Root biomass was highest in the urea treatment and lowest in the high C:N and control treatments. Total biomass and plant height followed a similar trend to shoot biomass, with urea and medium C:N having higher values than the high C:N, low C:N and control treatments. Chlorophyll in young leaves was highest with urea application and lowest in control, while all other treatments showed intermediate values. Significant difference among the treatments was not observed for chlorophyll content in older leaves.

Nodule number, N derived from the atmosphere and amount of N fixed by cowpea were significantly influenced by the different C and N input treatments (Fig. 4.1). The highest nodule count (56 plant^{-1}) was observed in the medium C:N treatment, followed by high C:N (34 plant^{-1}). Application of urea and the low C:N treatments reduced cowpea nodule by 49% compared with the high C:N treatment. The percentage of total plant N derived from the atmosphere (%Ndfa) was highest in the medium C:N treatment (55%), and lowest in the low C:N, urea and control treatments (~30%), with the high C:N treatment having intermediate values (40%). The amount of N fixed by cowpea was 36.4 g plant^{-1} in the medium C:N, which was roughly double that of the other treatments, which fixed 15.1 g plant^{-1}, on average (Fig. 4.1C).

4.3.2 Rhizosphere soil biochemical properties

Rhizosphere soil biochemical properties at harvest varied across the residue and N treatments. Nitrate-N concentration in cowpea rhizosphere showed a decreasing trend with increasing C:N ratio, such that it was highest in the treatment receiving urea alone, intermediate in the treatments with wheat residues, and lowest in the control (Fig. 4.2A). Mineral N (the sum of nitrate and ammonium-N) and dissolved total N followed a similar trend, with the urea showing the highest value and control showing the lowest (Table 4.3). Treatments receiving residue addition (regardless of C:N ratio) enhanced DOC and POXC compared to urea and the control (Fig. 4.2B, C). The pH and NH_4^+-N were not significantly affected by the C:N treatments (Table 4.3). When examining enzyme activity, the NAG (N-cycling) enzyme in the high C:N ratio treatment was nearly double that of the urea and low C:N treatments, and roughly 50% higher than the control (Fig. 4.2D). No significant differences among treatments were observed for the activity of AG, BG, CB, XYL and PHOS enzymes, although the treatments with residue additions generally had higher mean values for the C cycling enzymes (AG, BG, CB, XYL; Table 4.3).

4.3.3 Changes in microbial structure and community composition

A total of 3,443,265 reads of full-length 16S rRNA gene sequence were obtained across all rhizosphere soil samples after Q-score (>10) and length filtering (1360–1560 bp). Sequencing identified 2128 species, 690 genera, 228 families, and 24 phyla across all samples.

Alpha diversity for bacterial communities, as measured by the Shannon index, was significantly influenced by the residue and N input treatments. The Shannon index was highest in the high C:N treatment whereas the lowest values were observed under urea (Table 4.4). Spearman correlation showed that Shannon index was negatively correlated with soil NO_3^--N and mineral N in the cowpea rhizosphere (Fig S4.1). Inverted Simpson index also showed marginally significant differences ($p = 0.057$) between treatments, with the highest value observed in the high C:N and the lowest in the urea treatment. No treatment differences were observed for community richness or the Simpson Index.

The rhizosphere bacterial community structure changed with residue derived C:N ratio ($F = 2.768$, $p = 0.001$) as revealed by distance-based redundancy analysis (db-RDA) which attributed 20.3% of variation to treatment differences (Fig. 4.3A). The first two db-RDA axis explained 45.4% of the variation captured by the principal coordinate analysis (Fig S4.2). Pairwise PERMANOVA shows that the high C:N treatment had a distinct bacterial community structure compared to the low C:N ($p_{adj} = 0.028$), urea ($p_{adj} = 0.001$) and control ($p_{adj} = 0.004$) treatments and was a marginally different community structure than the medium C:N ($p_{adj} = 0.06$). The bacterial community under medium C:N also differed significantly from the urea treatment ($p_{adj} = 0.001$) but showed only marginal difference from the low C:N ($p_{adj} = 0.058$) and control ($p_{adj} = 0.066$) treatments. Similarly, the low C:N treatment exhibited a distinct community structure from urea ($p_{adj} = 0.011$) and was similar to the control ($p_{adj} = 0.282$). The urea and control treatments were also different in the bacterial community structure ($p_{adj} = 0.017$) (Table S4.1). Rhizosphere bacterial dispersion was not significantly different among treatments ($F = 2.22$, $p = 0.07$).

The treatments influenced the rhizosphere soil bacterial composition at the phylum level (Fig. 4.3B). Among the ten most abundant bacterial phyla, Proteobacteria and Bacteroidetes showed the highest relative abundance in the high C:N treatment, whereas Gemmatimonadetes and Acidobacteria had the highest relative abundance in the urea treatment, and Planctomycetes were most abundant in the low C:N treatment (Table S4.2). All other phyla showed similar relative abundances across treatments. The diazotrophic bacterial abundance in the rhizosphere soil, as measured by *nifH* gene copy number, was considerably higher in the high C:N treatment than all other treatments except medium C:N, and lowest in the urea treatment (Table 4.4). Diazotrophic bacteria in the cowpea nodule was more abundant in the medium C:N than all other treatments (Fig. 4.1).

The differential abundance analysis shows a significant differences in rhizosphere soil bacterial abundance between residue-driven C:N treatments and the control (Fig. 4.4). Nine genera (*Rhizobium, Neorhizobium, Agrobacterium, Pseudoxanthomonas, Devosia, Variovorax, Ohtaekwangia, Arenimonas and Peredibacter*) were more enriched in the high C:N treatment compared to the control. *Rhizobium, Neorhizobium* and *Agrobacterium* were also more abundant in the medium C:N treatment relative to control. In contrast, the abundance of five bacteria genera (*Ensifer, Rhizobium, Bdellovibrio, Shinella, and Luteolibacter*) was significantly reduced in the urea treatment compared to the control. There were no differences in bacterial differential abundance between the low C:N and the control in the rhizosphere soil.

4.3.4 Relationships between microbial community and rhizosphere soil properties

Twelve of the thirteen differentially abundant bacteria genera were significantly correlated with at least one of the rhizosphere soil chemical properties (Fig. 4.5). Spearman correlation showed that most bacterial genera were positively correlated with rhizosphere soil DOC, POXC, pH and treatment C:N. In contrast, most of these genera including *Rhizobium* were negatively correlated with rhizosphere soil NO_3^--N, mineral N and dissolved total N.

4.4 Discussion

4.4.1 Effect of C and N input on cowpea performance and N fixation

We observed large differences in growth and N-fixation metrics of cowpea between the different residue and N input treatments, with the best performance observed in the medium C:N and urea treatments (Table 4.2). The higher shoot, root, and total biomass, along with increased plant height and higher chlorophyll content in young leaves under the urea treatment, may be attributed to the higher available N in the rhizosphere, as reflected in soil NO_3^--N and dissolved total N (Fig. 4.2; Table 4.3). In contrast, the improved cowpea biomass in the medium C:N treatment compared to the control, high and low C:N treatments, is likely attributed to enhanced cowpea N nutrition derived from symbiotic N fixation. Improved cowpea N nutrition can enhance photosynthetic CO_2 assimilation, leading to greater biomass yield (Ayalew et al., 2022).

The medium and high C:N treatments increased cowpea nodulation compared to other treatments, likely through enhanced abundance of *nifH* gene and *Rhizobium* in the rhizosphere soil (Table 4.4;Fig. 4.4), which may increase the likelihood of root infection to produce more nodules (Fig. 4.1). This response, together with the positive correlation between *Rhizobium* abundance and DOC, and the negative correlation with NO_3^--N, suggests that increased availability of residue-derived labile C and reduced NO_3^--N level under these treatments promoted nodulation by strengthening *Rhizobium*-cowpea interactions (Fig. 4.2 and 4.5). Decomposing crop residues are a source of labile C in soil and can stimulate the recruitment and activity of beneficial microorganisms including *Rhizobium* in the rhizosphere, thereby potentially mediating root nodulation (Chen et al., 2022; Fang et al., 2025b; Han et al., 2020). Within this high labile C environment, *Rhizobia* can live as free-living saprophytes with an oligotrophic lifestyle prior to symbiosis but exhibit metabolic flexibility in response to increased C availability, whereby labile C enhances *Rhizobia* survival, root colonization, and infection, ultimately promoting nodulation (Poole et al., 2018; Xie et al., 2021b). Consistent with this mechanism, *Rhizobium* abundance was positively correlated with labile C in rhizosphere soil (Fig. 4.5).

We noted that cowpea in the medium C:N treatment produced considerably more nodules than under high C:N, despite similar concentration of NO_3^--N, dissolved total N and C in the rhizosphere at cowpea harvest (Fig 4.1). We suspect that this difference may be attributed to the starter (small) amount of N applied to adjust the straw C:N ratio in the medium C:N treatment. This small N application can enhance nodulation by stimulating early seedling growth and root development, which increases a plant's size and photosynthetic capacity, without significantly impacting available N pools later in the experiment. We suspect that the early pulse of N to support growth was followed by lower levels of N availability, thereby facilitating *Rhizobium* infection, nodule initiation and investment in nodule growth by the plant (Kebede, 2021; Osborne and Riedell, 2006; Sadiq et al., 2023). Similar to our findings, Adegbite et al. (2021) observed a higher nodulation in cowpea when crop residue was applied with N than when residue was applied alone.

The higher concentrations of NO_3^--N and dissolved total N in the urea and low C:N treatments, along with their negative correlation with *Rhizobium* abundance (Fig 4.5), suggests that nodulation was suppressed in these treatments due to elevated N availability. Specifically, increased NO_3^--N in the legume rhizosphere may inhibit the earliest stages of nodulation by disrupting key signaling pathways involved in nodule formation (Danso et al., 2025). Higher NO_3^--N levels can activate specific signaling peptides, and nitrate-responsive transcription factors which suppress the expression of nodulation genes and reduce cytokinin accumulation required for nodule organogenesis (Lin et al., 2021; Moreau et al., 2021). Similarly, Alon et al. (2021) reported that elevated NO_3^--N levels to inhibit nodulation in another legume, *Calicotome villosa*. Consistent with our finding, Liang et al. (2023) observed inhibition of nodulation due to the addition of lower C:N substrate relative to the intermediate C:N treatments in peanut. Thus, our findings are in agreement with past research indicating that high N availability, through the addition of N fertilizer or high quality residues, can inhibit N-fixation through effects on microbial communities and the nodulation process between legumes and their symbiotic *Rhizobia*.

In line with our findings on nodulation, the medium C:N treatment, and to a lesser extent the high C:N treatment, increased the percentage of N derived from the atmosphere (%Ndfa) in cowpea, likely through greater investment in nodules and associated rhizobia, but also by shifting the bacterial community in the cowpea rhizosphere towards diazotrophs (Figs. 4, 5). We note that higher residue-driven C:N treatments (medium and high C:N) resulted in a greater abundance of *Rhizobium* and the *nifH* gene in the cowpea rhizosphere (Fig. 4.4), whereas such enrichment was not evident either at low C:N or urea treatments. The increase in abundance of *Rhizobium* in the rhizosphere soil under higher C:N treatments likely facilitates establishment of a symbiotic relationship with cowpea, via nodule formation and as free-living bacteria, and thus leading to greater overall fixation of atmospheric N_2 (Bian et al., 2024). Consistent with our findings, Xie et al. (2021b) reported that increased dissolved organic C resulting from higher C:N residue inputs enhanced soybean N fixation by enriching *Bradyrhizobium* abundance in the rhizosphere, thereby promoting nodulation. Increases in %Ndfa and N fixation with enhanced C availability due to enrichment of *Mesorhizobium* in Chinese milk vetch has also been reported by Bian et al., (2024).

Along with increased C availability, enhanced N fixation under the medium and high C:N treatments may be related to increased microbial competition for N in the rhizosphere, which fits with the higher observed NAG enzyme activity in these treatments (Fig. 4.2) since microbes often produce N degrading enzymes when N is limiting (Desjardins et al., 2025; Holatko et al., 2023). Others have shown that high C:N organic amendments can promote microbial N immobilization, thus reducing soil NO_3^--N levels, and subsequently enhancing *Rhizobium*- legume symbiosis (Siedt et al., 2021; Wang et al., 2021; Xie et al., 2021b). We note that %Ndfa in cowpea under the low C:N treatment was 87 % lower than the medium C:N, despite having similar levels of labile C (Fig. 4.1), thus suggesting that the suppressive effect of N may be more important than the stimulatory effect of labile C (Zhao et al., 2024). Excess available N in the rhizosphere soil may reduce photosynthetic C allocation to the nodule, limit their function, and may downregulate the transcription of nitrogenase genes including *nifH* gene, ultimately reduce %Ndfa (Dixon and Kahn, 2004; Jing et al., 2025; Khan and Khan, 1981). Further, diazotrophs are known to preferentially utilize inorganic N form the soil when it is available, thereby avoiding the energetically costly process of biological N fixation (Hu et al., 2021).

4.4.2 Rhizosphere bacterial community responses to C and N input

The microbial communities evaluated in the cowpea rhizosphere were greatly impacted by the residue and N treatments. We note that bacterial diversity (Shannon index) was lowest in the urea treatment. A significant negative correlation between Shannon index and N availability metrics, (e.g., NO_3^--N) suggests higher N availability in the rhizosphere as a potential driver of the reduced bacterial diversity (Fig. S4.1). Higher soil N availability may reduce microbial N limitation while simultaneously creating relative C limitation, likely through reduced exudation of labile C from plant root, thereby narrowing niche availability and reducing microbial diversity (Ning et al., 2021; Rappe-George et al., 2017; Wu et al., 2026). Similar declines in bacterial alpha diversity due to N fertilization and associated increases in available N have been reported (Zeng et al., 2016), while others have observed a significant reduction in bacterial (Shannon) diversity following urea application (Staley et al., 2018; Wang et al., 2023). Beyond N availability in the rhizosphere soil, we note that the Shannon index was significantly higher in the low C:N treatment vs. urea, likely due to greater availability of labile C in the low C:N treatment, as evidenced by higher DOC and POXC concentrations (Fig. 4.2).

Beyond effects on alpha diversity, the PERMANOVA and distance-based redundancy analyses showed that residue and N input treatments also impacted soil microbial community structure in the cowpea rhizosphere (Fig. 4.3A). About 20% of the total variation in the community structure was explained by the treatments, suggesting that C and N inputs play a role in shaping the cowpea rhizosphere microbiome. Differences in microbial community structure between the low C:N and the urea treatment (Fig. 4.3A), despite equivalent N input, suggest that C inputs play an important role in shaping the cowpea rhizosphere community. This idea is further corroborated by the observed enrichment of symbiotic N-fixing bacteria (*Rhizobium and Neorhizobium*) and other genera (including *Agrobacterium, Pseudoxanthomonas, Devosia, Variovorax, Ohtaekwangia, Arenimonas and Peredibacter*) in the high C:N compared to the control treatment as well as the positive correlation between measured C fractions (DOC and POXC) and key microbial groups (Figs 4.4 & 4.5). Previous studies have reported that increasing labile C through straw addition can reshape bacterial community in the soil (He et al., 2025; Su et al., 2020). In agreement with our finding, Liang et al. (2023), also observed an increase in relative abundance of N fixing bacteria with increasing C:N ratio of input in the form of glucose and urea.

Diazotrophic abundance (*nifH* gene copy number) in the rhizosphere was greater in the high C:N treatment than the control, which was likely associated with enhanced DOC and POXC amount in the soil (Fig. 4.1). We note that the relative abundance of symbiotic diazotrophs such as *Rhizobium*, were positively correlated with labile C fractions in the rhizosphere (Fig. 4.5). In addition to directly supporting microbial growth, increased labile C may indirectly favor these genera by influencing micronutrient availability including iron in the rhizosphere (Bian et al., 2024). An increase in abundance of symbiotic diazotrophs such as *Bradyrhizobium* in higher C:N input ratio has been reported in peanut and soybean rhizosphere (Liang et al., 2023; Xie et al., 2021b, 2021a) and likely has important implications for N cycling and plant nutrition in these systems.

While increased labile C was associated with greater diazotrophic abundance, we noted that higher N application with residue C input neutralized this stimulatory effect relative to the control, likely because N addition increases microbial relative C limitation (Ning et al., 2021). The lowest diazotrophic abundance was observed in the urea treatment, likely due to high N availability coupled with the low DOC and POXC (Fig. 4.1,4.4). This is further supported by the negative correlations between N-fixing bacteria genera and available N pools (NO_3^- -N, dissolved total N), thus highlighting the potential suppression of beneficial symbiotic diazotrophs such as *Neorhizobium, and Ensifer* unde*r* high N availability (Fig. 4.5). Consistent, with the reduction in diazotrophic abundance in the urea treatment observed here, others have reported an inhibitory effect of N fertilizer on the diazotrophic abundance (e.g., Dietrich et al. 2024). Greater diazotrophic abundance in the wheat rhizosphere was also reported under the combined application of wheat straw and N fertilizer compared with N fertilizer alone, with the highest abundance observed under wheat straw retention rather than removal (Chen et al., 2021).

4.5 Conclusion

Our findings show the potential of C and N inputs to regulate symbiotic N fixation in cowpea and that this appears to be related to impacts on rhizosphere microbial communities and N-fixing functional genes. Residue treatments with medium and high C:N ratios substantially enhanced cowpea nodulation and the percentage of N derived from the atmosphere, coinciding with greater availability of labile C, reduced rhizosphere NO_3^--N, enrichment of *Rhizobium* and related symbiotic taxa, and increased *nifH* gene abundance in the rhizosphere. Moreover, cowpea in the medium C:N treatment fixed the largest amount of N. In contrast, high inorganic N availability, particularly under urea and low C:N treatments, suppressed nodulation and reduced diazotrophic abundance, indicating that excess N availability can override the stimulatory effects of labile C on symbiotic N fixation.

Together, these findings provide mechanistic evidence that residue-derived C inputs combined with limited starter N (medium C:N), can create optimal rhizosphere conditions for symbiotic N fixation by selectively enriching beneficial diazotrophs such as *Rhizobium*, and sustaining N-fixing functional gene abundance. From a soil health perspective, residue C and N inputs, particularly under a balanced C:N ratio, not only supported biological N fixation but also improved microbial functional potential and helped maintained bacterial diversity relative to the urea treatment, key attributes of resilient and biologically active soils. Residue retention strategies that balance C and N inputs can improve sustainable N management by strengthening soil microbial functions, reduce dependence on inorganic N fertilizers, and improve the efficiency of legume-based cropping systems. By linking residue management to microbial community structure, *nifH* gene dynamics, and N fixation, our study advances a process-based understanding of management and soil-microbe-function relationships, central to soil health and agroecosystem sustainability.

CHAPTER 4: TABLES AND FIGURES

Table 4.1. Quantities of wheat residue and urea added to each treatment, along with the target C:N ratios for the five treatments tested in a greenhouse study.

	Description		
Treatments	C:N	Wheat residue (g pot $^{-1}$)	Urea (g pot $^{-1}$)
Control	---	0	0
Urea	0.4	0	0.192
Low C:N	15	4.23	0.192
Medium C:N	25	4.23	0.069
High C:N	40	4.23	0

Table 4.2. Cowpea performance metrics in a greenhouse experiment with varying levels of C and N input. Values represent the means with the standard error (n = 8) reported to the right of each mean. Treatments sharing the same letter within each column are not significantly different at $p < 0.05$, according to Tukey pairwise comparison. Bold ANOVA p-values represent significant value at $p < 0.05$. High C:N, wheat straw with C:N ratio 40; Medium C:N, wheat residue + urea to achieve a C:N ratio of 25; Low C:N, wheat residue + urea with a C:N of 15; Control, no residue or urea added.

Treatments	Shoot dry biomass (g plant $^{-1}$)	Root dry biomass (g plant $^{-1}$)	Total biomass (g plant $^{-1}$)	Plant height (cm)	Young leaves chlorophyll level	Old leaves chlorophyll level
Control	1.3±0.1^{c}	0.30±0.03^{b}	1.64±0.14^{c}	14.9±0.6^{d}	37.1±1.1^{c}	29.1±3.6
Urea	3.0±0.3^{a}	0.46±0.03^{a}	3.46±0.28^{a}	44.8±4.9^{a}	62.7±1.8^{a}	38.2±1.0
Low C:N	2.1±0.1^{b}	0.38±0.03^{ab}	2.43±0.10^{b}	32.2±1.7^{b}	44.1±0.9^{b}	36.1±3.9
Medium C:N	2.9±0.2^{a}	0.39±0.05^{ab}	3.24±0.20^{a}	42.8±2.9^{a}	45.1±1.7^{b}	35.2±2.1
High C:N	2.0±0.1^{b}	0.34±0.02^{b}	2.36±0.15^{b}	25.4±2.8^{c}	44.3±2.1^{b}	37.1±2.5
p-value	**< 0.001**	**0.031**	**< 0.001**	**< 0.001**	**< 0.001**	0.199

Table 4.3. Biochemical properties for rhizosphere soil of cowpea grown in a greenhouse experiment with varying levels of C and N input. Treatments sharing the same letter are not significantly different at $p < 0.05$, according to Tukey pairwise comparison. Bold ANOVA p values represent significant value at $p < 0.05$. Error bars indicated standard error of the mean ($n = 8$). High C:N, wheat straw with C:N ratio 40; Medium C:N, wheat residue + urea to achieve a C:N ratio of 25; Low C:N, wheat residue + urea with a C:N of 15; Control, no residue and urea. DTN, dissolved total N; AG, α-Glucosidase; BG, β-Glucosidase; CB, Cellobiohydrolase; XYL, β-Xylosidase; PHOS, phosphatase.

Treatments	pH	NH_4^+-	Mineral N	DTN	AG	BG	CB	XYL	PHOS
		------------ mg kg^{-1} soil ----------			------------------------ nmol g^{-1} soil h^{-1}------------------------------				
Control	7.2±0.1	0.9±0.1	4.5±0.2^{b}	10.6±0.6^{c}	9.9±1.6	33.8±3.1	8.7±1.8	24.8±4.2	187±1
Urea	7.3±0.1	1.1±0.2	7.1±0.5^{a}	17.6±0.6^{a}	6.5±1.6	31.4±3.9	8.6±2.6	17.4±2.3	160±1
Low CN	7.3±0.1	1.7±0.4	7.0±0.8^{a}	14.2±0.6^{b}	11.4±3.0	35.5±2.1	9.6±1.5	22.0±2.5	157±1
Medium C:N	7.4±0.1	1.5±0.4	6.5±0.7^{a}	13.4±0.7^{b}	11.4±2.6	41.5±6.0	11.9±2.5	29.1±5.3	171±1
High C:N	7.4±0.1	1.5±0.2	6.1±0.3^{a}	13.7±0.6^{b}	11.4±2.8	41.1±4.0	10.6±2.1	25.9±4.8	148±1
p-value	0.196	0.201	**0.005**	**< 0.001**	0.421	0.319	0.814	0.329	0.331

Table 4.4. Response of bacterial alpha diversity and nifH gene abundance in cowpea rhizosphere soil to residue-driven C:N in a greenhouse experiment with varying levels of C and N input. Treatments sharing the same letter are not significantly different at $p < 0.05$, according to Tukey pairwise comparison. Bold ANOVA p-values represent significant values, at $p < 0.05$. Error bars indicate standard error of the mean ($n = 8$). High C:N, wheat straw with C:N ratio 40; Medium C:N, wheat residue + urea to achieve a C:N ratio of 25; Low C:N, wheat residue + urea with a C:N of 15; Control, no residue and urea.

Treatments	Richness	Shannon index	Simpson index	Inverted Simpson	*nifH* gene (x 10^{-8} copies g^{-1} soil)
Control	977±34	5.91±0.05a	0.992	128.2	0.42±0.19b^{c}
Urea	857±25	5.72±0.06b	0.989	99.2	0.25±0.06^{c}
Low C:N	907±35	5.88±0.05a	0.992	132.7	0.5±0.13^{bc}
Medium C:N	913±28	5.85±0.05ab	0.991	124.8	0.6±0.0.08^{ab}
High C:N	948±32	5.95±0.04a	0.993	146.9	2.2±0.0.82^{a}
ANOVA p-value	0.104	**0.035**	0.148	0.057	**0.002**

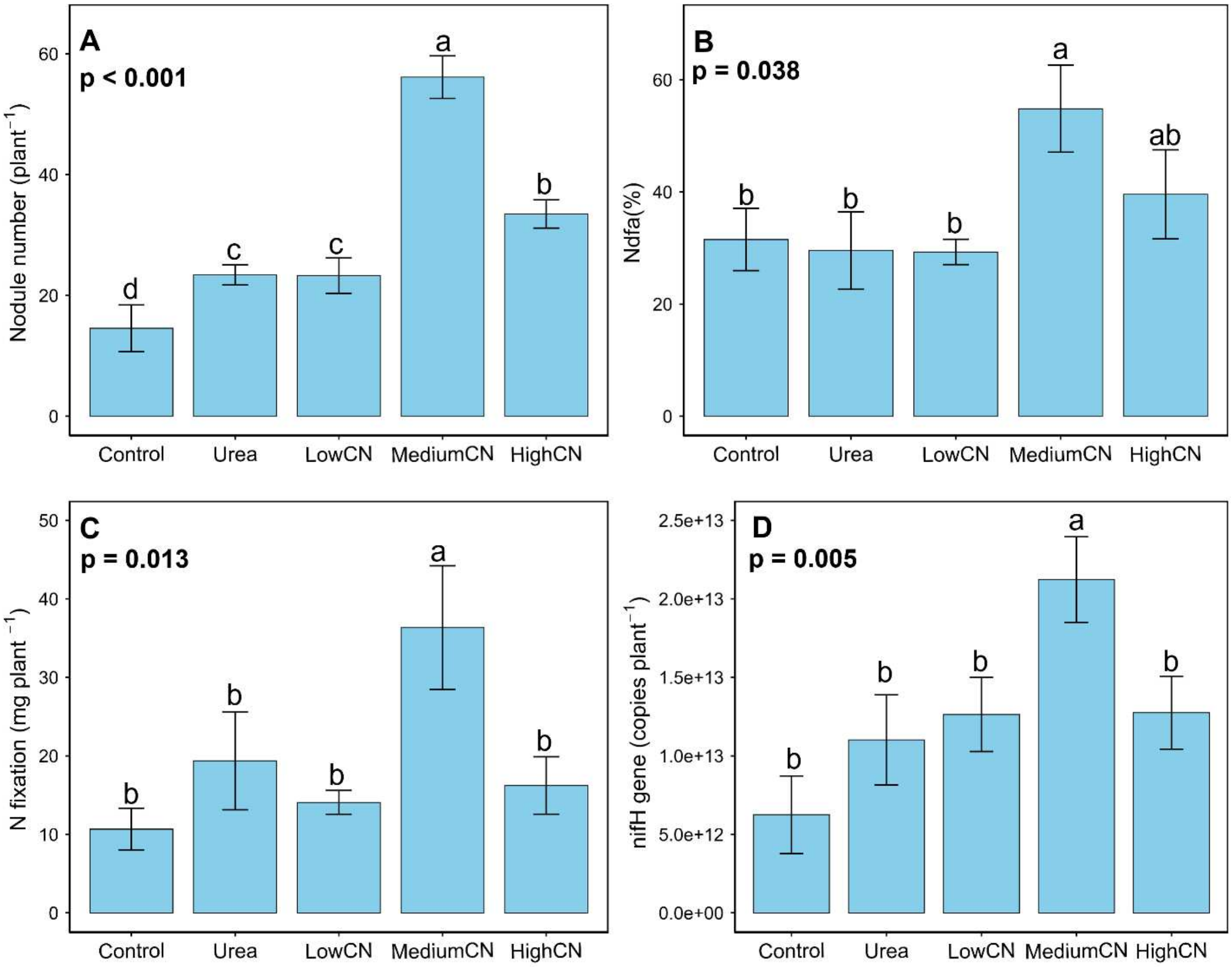

Figure 4.1. Nitrogen fixation metrics (A) nodule number, B) percentage of N derived from the atmosphere, C) total N fixation, and D) absolute *nifH* gene abundance in the nodules of cowpea grown in a greenhouse experiment with varying levels of C and N input. The *nifH* gene plant^{-1} was calculated by multiplying *nifH* gene g^{-1} dry nodule by number of nodule plant^{-}1. Treatments sharing the same letter are not significantly different at $p < 0.05$, according to Tukey pairwise comparison. Bold ANOVA p-values represent significant value at $p < 0.05$. Error bars indicate standard error of the mean ($n = 8$). High C:N, wheat straw with C:N ratio 40; Medium C:N, wheat residue + urea to achieve a C:N ratio of 25; Low C:N, wheat residue + urea with a C:N of 15; Control, no residue or urea.

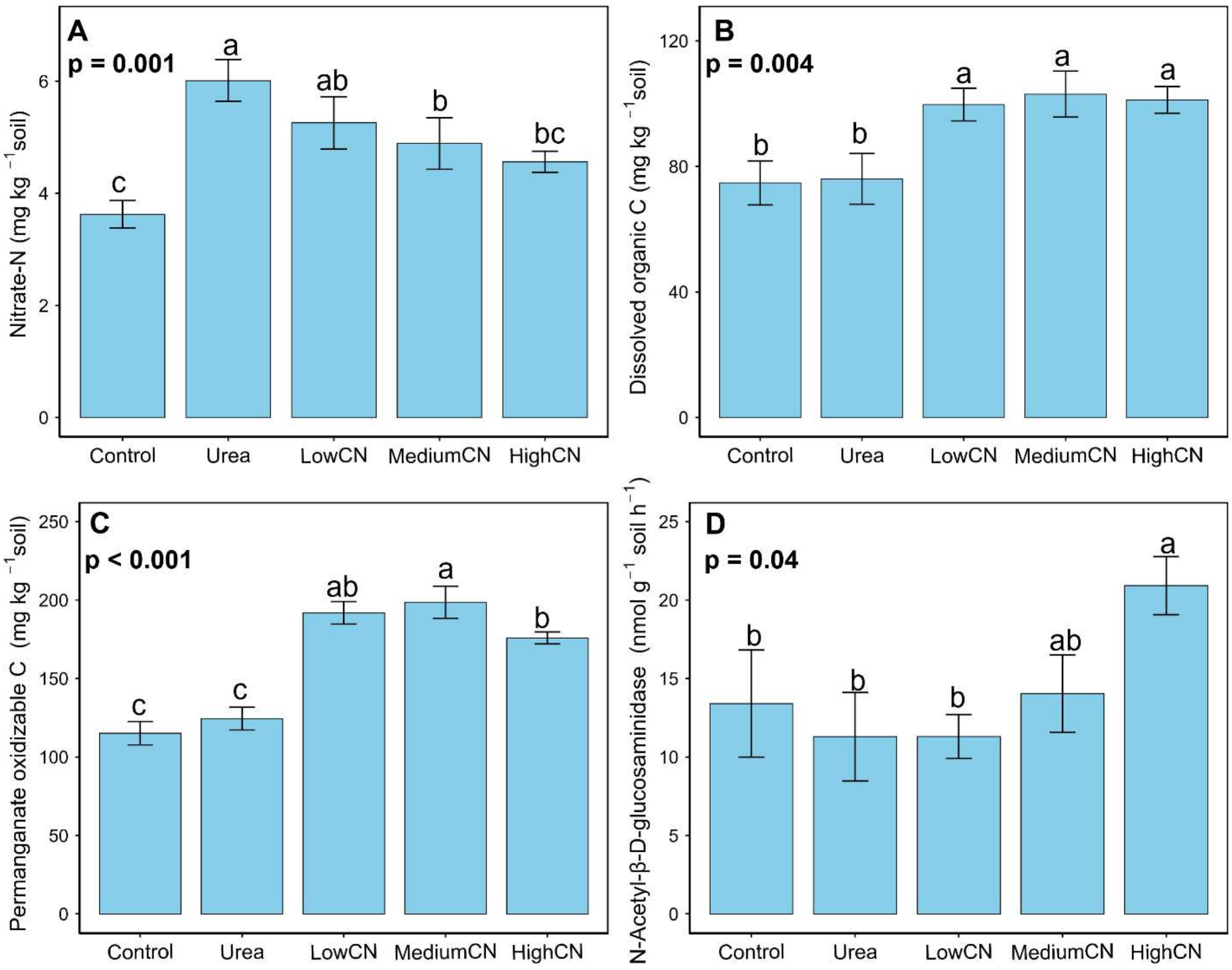

Figure 4.2. Mean soil biochemical properties (A, nitrate-N; B, dissolved organic; C, permanganate oxidizable C; and D, N-acetyl-β-D-glucosaminidase) for rhizosphere soil of cowpea grown in a greenhouse experiment with varying levels of C and N input. Treatments sharing the same letter are not significantly different at $p < 0.05$, according to Tukey pairwise comparison. Bold ANOVA p-value represents significant value at $p < 0.05$. Error bars indicate standard error of the mean (n = 8). High C:N, wheat straw with C:N ratio 40; Medium C:N, wheat residue + urea to achieve a C:N ratio of 25; Low C:N, wheat residue + urea with a C:N of 15; Control, no residue or urea.

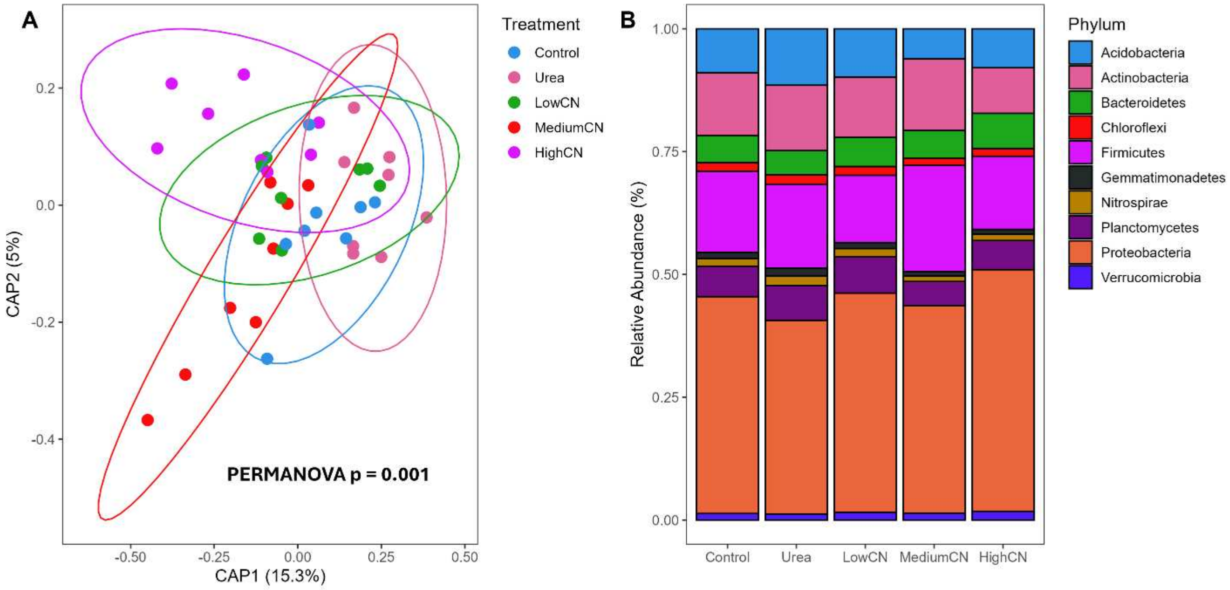

Figure 4.3. A) Distance-based redundancy analysis based on Bray-Curtis dissimilarity showing clustering of bacterial community structure driven by different residue-derived C:N ratios for rhizosphere soil of cowpea grown in a greenhouse experiment with varying levels of C and N input. The colors and ellipses represent different residue-driven C:N ratio treatments: control (blue); urea (pink); lowCN (green); mediumCN (red); highCN (purple). B) Relative abundance of top ten most abundant bacteria phyla in the cowpea rhizosphere varies with residue-driven C:N. HighCN, wheat straw with C:N ratio 40; MediumCN, wheat residue + urea to achieve a C:N ratio of 25; LowCN, wheat residue + urea with a C:N of 15; Control, no residue and urea.

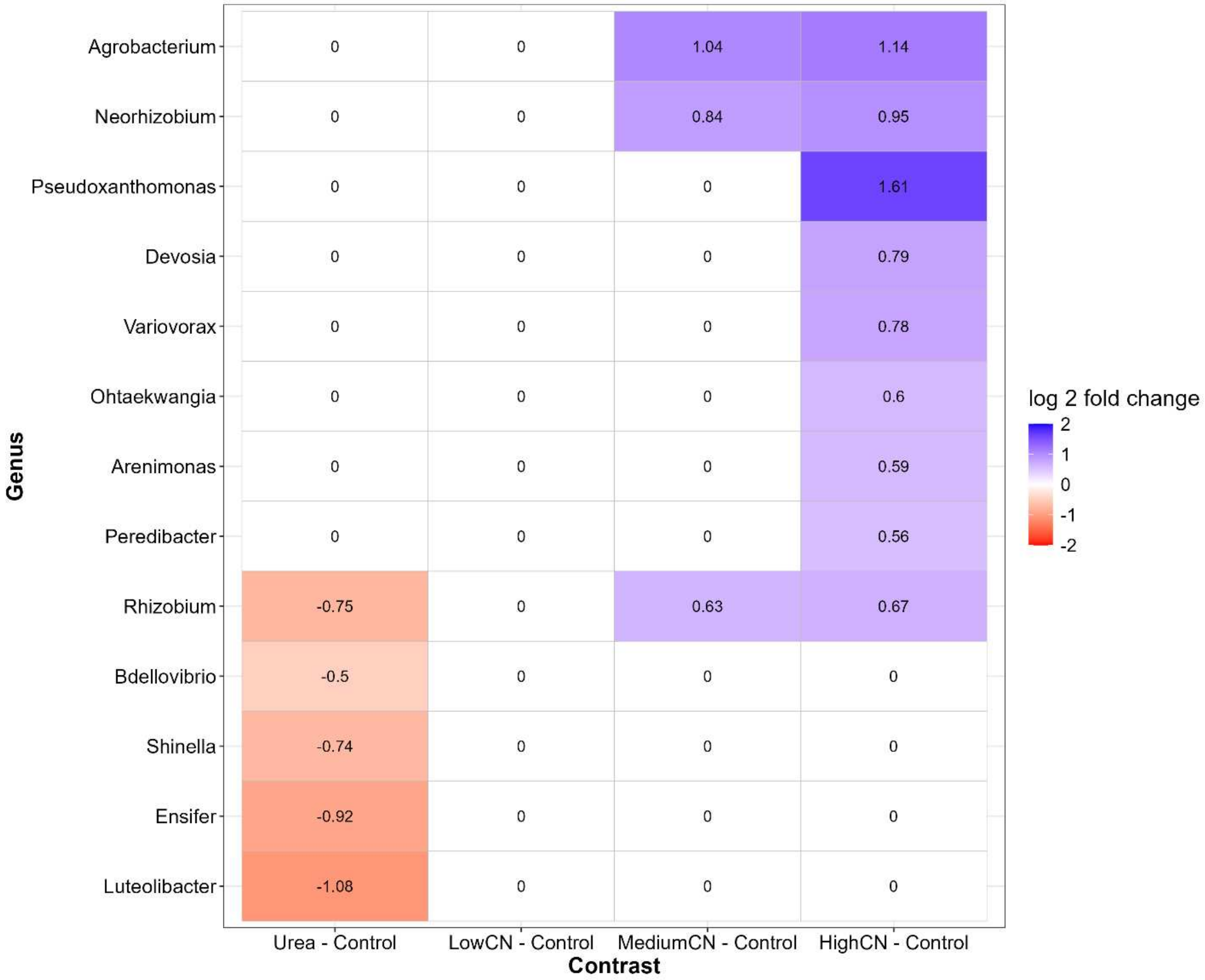

Figure 4.4. Differentially abundant bacteria genera enriched in different plant-driven C:N treatment groups for rhizosphere soil of cowpea grown in a greenhouse experiment with varying levels of C and N input. The column denotes contrast between treatments and control. Rows indicate significant bacteria genera identified by ANCOM-BC2. Numbers in each cell indicate the "Log Fold Change", which is log 2-fold change in abundance between contrasts. Each cell color indicates abundance change: blue refers increase, red signifies reduction and white indicates no difference (no change). Multiple testing correction was done using the Holm-Bonferroni method. HighCN, wheat straw with C:N ratio 40; MediumCN, wheat residue + urea to achieve a C:N ratio of 25; LowCN, wheat residue + urea with a C:N of 15; Control, no residue and urea.

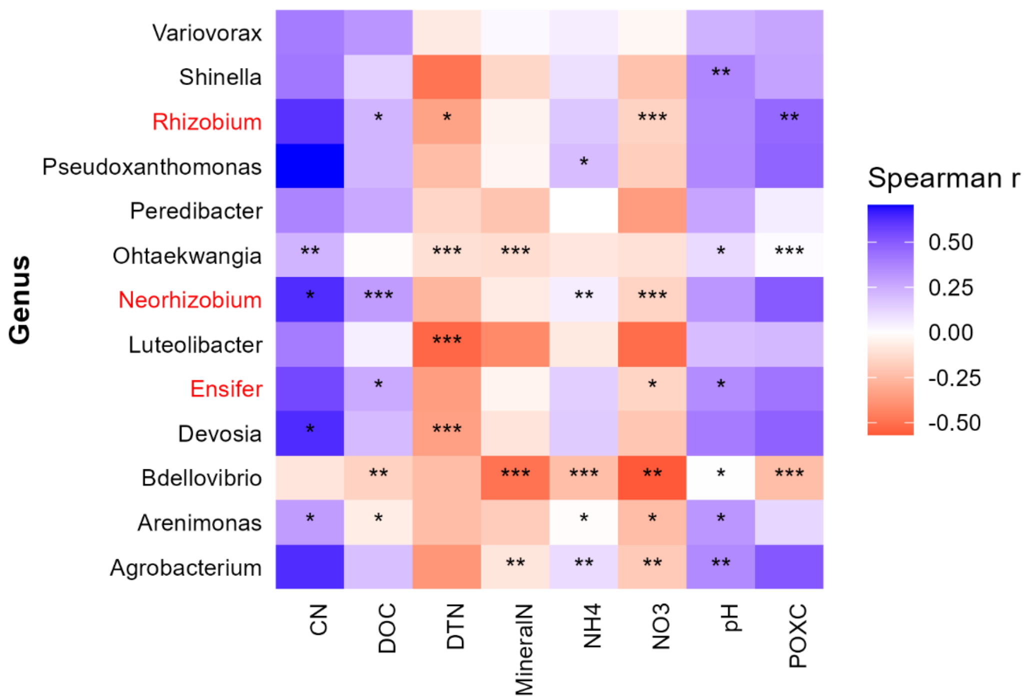

Figure 4.5. Spearman correlation showing association of soil chemical properties and relative abundance of bacteria genera that were differentially abundant among residue-driven C:N ratio treatments in rhizosphere soil of cowpea grown in a greenhouse experiment with varying levels of C and N input. Genera, with their name labeled in red are symbiotic N fixing bacteria. The red cell represents negative correlation, and blue color represents positive correlation. *, ** and *** indicate significant correlation at $p < 0.05$, 0.01 and 0.001. CN, carbon to nitrogen ratio of treatments; DOC, dissolved organic C; DTN, dissolved total N; NH4, ammonium-N; NO3, nitrate-N; pH, rhizosphere soil pH; POXC, permanganate oxidizable C. HighCN wheat straw with C:N ratio 40; MediumCN, wheat residue + urea to achieve a C:N ratio of 25; LowCN, wheat residue + urea with a C:N of 15; Control, no residue and urea.

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CHAPTER 5: CONCLUSION

Soil degradation remains one of the most important challenges to global agriculture and sustainability, particularly across smallholder farming systems in sub-Sharan Africa. Declining soil organic matter (SOM), nutrient depletion, compaction, soil acidity, and loss of biological function continue to constrain crop productivity and threaten food security across the region. In Ethiopia, these challenges are aggravated by continuous cereal monocropping, high cost and limited access to fertilizers, and removal of crop residues. In these contexts, sustainable intensification requires management strategies that simultaneously restore soil health and improve crop productivity, while remaining economically viable for smallholder farmers.

By combining field experiments conducted under smallholder conditions in Ethiopia with a controlled greenhouse study in the United States, this dissertation examine how legume species selection, residue management, and nutrient management interact with legume crops to regulate soil health, soil microbial processes, and crop productivity. Emphasis was placed on understanding the mechanisms through which soil biological function and rhizosphere microbial dynamics mediate symbiotic N-fixation and subsequent crop productivity.

In Chapter 2 I demonstrated that legume species differ substantially in their biomass production and N-fixing capacity, leading to contrasting impacts on soil health and subsequent crop productivity. The high-biomass legumes (lablab) fixed the largest amount of N and returned greater quantities of C and N to the soil compared to lower-biomass grain legumes such as common bean and soybean. This difference translates into measurable improvements in key soil health metrics related to SOM. Our findings show that the benefits of legume integration extended beyond the legume phase. Legume residue retention markedly increased subsequent chickpea and maize yields with the largest yield observed following lablab, highlighting that integration of high biomass legumes within a cropping system can generate strong positive legacy effects on subsequent crops in both the short and medium-term. However, we observed a contrasting response to residue retention in terms of chickpea N fixation, residue retention enhanced amount of N fixation following soybean, while lower N fixation was observed following residue retention with lablab, despite the high chickpea yield. This pattern indicates that when preceded by high biomass legumes, chickpea achieved high productivity while possibly relying more on improved soil N availability rather than symbiotic N fixation, although the reason for reduced N fixation under high residue C input was unclear.

In this same study, residue management emerged as a critical factor in governing the magnitude of legume derived benefits in soil health. Retention of legume residues amplified improvements in SOM pools, nutrient availability (e.g., available P) and reduced soil compaction, whereas residue removal limited improvements in soil health and reduced subsequent crop yields. These results highlight a key trade off in smallholder farming systems, where residues are often removed for livestock feed or fuel. While residue removal may provide short-term household benefits for smallholder farmers, this dissertation demonstrates that retaining legume residues plays a crucial role in sustaining soil health and long-term productivity in smallholder systems.

In Chapter 3, reporting on a separate field study in Ethiopia, we examine how nutrient management strategies affect soil health, productivity and system profitability in legume-maize rotation. Our results show that nutrient inputs in the form of manure or fertilizer consistently enhanced legume productivity relative to the control, where legumes received no nutrient input. Organic nutrient inputs (i.e., manure) had larger benefits for soil health and carryover effects on the subsequent crop. These responses were accompanied by improvements in soil pH, SOM, and reduced compaction, indicating that manure supports soil biological and chemical processes beyond simple nutrient supply. Further, common bean performs better than soybean in terms of soil health support and productivity of the subsequent crop, suggesting that farmers selection of legume species plays an important role. By standardized nutrient inputs, based on equivalent economic cost, this dissertation revealed that manure application significantly enhances profitability in legume-maize rotation system, with economic benefits surpassing that of fertilizer, thus highlighting the potential of manure to restore degraded soils while remaining economically viable for smallholder farmers.

Beyond agronomic outcomes, the dissertation provides mechanistic insight into how residue-derived C inputs and soil N availability regulate symbiotic N fixation. Results from Chapter 4, a greenhouse experiment conducted at CSU, demonstrated that N fixation in legume (cowpea) is controlled by the balance between C and N availability in the rhizosphere. Residue treatments with medium C:N ratios increased labile C availability in the soil while reducing available N, specifically NO_3^--N concentrations, thus creating conditions that favored symbiotic diazotroph abundance (e.g., *Rhizobium*) in the legume rhizosphere, increased nodulation, and markedly enhanced N fixation. In contrast, high inorganic N availability (NO_3^--N) suppressed nodulation and N fixation, even in the presence of residue-derived C in the soil. These findings suggest that legume N fixation is governed by soil biological function and microbial resource balance, highlighting the importance of management practices that regulate C and N availability. By explicitly linking residue management, nutrient availability, microbial community structure, and functional gene abundance to N fixation outcomes, this work advances mechanistic understanding of how nutrient management practices can regulate symbiotic N fixation in legume-based cropping systems.

Overall, the findings of this dissertation have important implications for smallholder farming systems. Selecting high-biomass, high N-fixing legumes and retaining residues, while also prioritizing organic nutrient inputs such as manure can substantially enhance soil health and while improving crop productivity and profitability. These strategies can reduce reliance on costly and often inaccessible synthetic fertilizers (at least in Ethiopia), strengthen internal nutrient cycling, and increase resilience of stallholder farming system to climatic change and economic constraints. As such, they offer a practical and scalable approach to soil health-centered intensification in resource limited environments.

While this dissertation provides robust evidence for the benefits of integrating legumes into cropping systems, and organic nutrient inputs, long-term studies under smallholder conditions are needed to quantify cumulative SOM accrual, system resilience, and environmental tradeoffs. Future work should also expand microbial analyses to include bacterial communities in the nodule, fungal communities in the rhizosphere, and broader soil food web interactions, as well as to evaluate these strategies across diverse agroecological and socioeconomic contexts through participatory on-farm research.

APPENDIX: SUPPLEMENTARY TABLES AND FIGURES

Table S4.1. Pairwise PERMANOVA comparisons of treatment effect on bacterial community based on Bray-Curtis dissimilarity distances for rhizosphere soil of cowpea grown in a greenhouse experiment with varying levels of C and N input. Bold PERMANOVA p value represents significant value at $p < 0.05$for each comparison.

		PERMANOVA		
Treatment pairs for comparison		R^2	p_{adj}-value	Permutation
High C:N	Medium C:N	0.107	0.070	999
	Low C:N	0.142	**0.021**	999
	Urea	0.318	**0.001**	999
	Control	0.175	**0.001**	999
Medium C:N	Low C:N	0.122	0.055	999
	Urea	0.262	**0.001**	999
	Control	0.123	0.052	999
Low C:N	Urea	0.168	**0.011**	999
	Control	0.075	0.286	999
Urea	Control	0.131	**0.017**	999

Table S4.2. Relative abundance of top ten bacteria phyla for rhizosphere soil of cowpea grown in a greenhouse experiment with varying levels of C and N input. Treatments sharing the same letter are not significantly different at $p < 0.05$, according to Tukey pairwise comparison. Bold ANOVA p values represent significant value at $p < 0.05$.

Phylum	High C:N	Medium C:N	Low C:N	Urea	Control	ANOVA p_{adj}-value
Proteobacteria	0.484068^{a}	0.411294^{b}	0.439548^{ab}	0.389194^{b}	0.427035^{ab}	**0.005**
Planctomycetes	0.058326^{ab}	0.048128^{b}	0.073029^{a}	0.069789^{a}	0.059818^{ab}	**0.008**
Gemmatimonadetes	0.009202^{bc}	0.008419^{c}	0.011997^{ab}	0.015101^{a}	0.01155^{bc}	**< 0.001**
Bacteroidetes	0.070521^{a}	0.054909^{ab}	0.058722^{ab}	0.048525^{b}	0.053804^{ab}	**0.033**
Acidobacteria	0.078059^{bc}	0.059226^{c}	0.096763^{ab}	0.113186^{a}	0.086502^{abc}	**0.003**
Verrucomicrobia	0.017323^{a}	0.013404^{a}	0.015399^{a}	0.011971^{a}	0.012689^{a}	0.066
Nitrospirae	0.012905^{a}	0.011139^{a}	0.016103^{a}	0.019414^{a}	0.015334^{a}	0.078
Firmicutes	0.146725^{a}	0.210565^{a}	0.135032^{a}	0.168969^{a}	0.160104^{a}	0.078
Chloroflexi	0.015302^{a}	0.013987^{a}	0.017318^{a}	0.018721^{a}	0.016595^{a}	0.117
Actinobacteria	0.091288^{a}	0.14251^{a}	0.120737^{a}	0.131508^{a}	0.123408^{a}	0.133

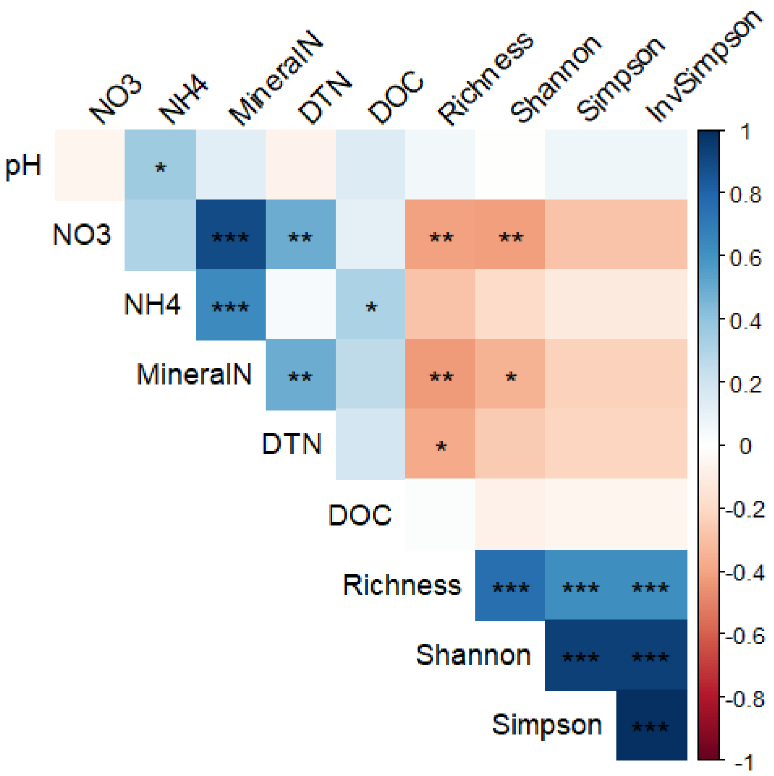

Figure S4.1. Spearman correlation between soil chemical proprties and alpha diversity metrics for rhizosphere soil of cowpea grown in a greenhouse experiment with varying levels of C and N input. Blue and red colors indicate positive and negative correlations, respectively. *, ** and *** refers significant correlation at $p < 0.05$, 0.01 and 0.001. HighCN, wheat straw with C:N ratio 40; MediumCN, wheat residue + urea to achieve a C:N ratio of 25; LowCN, wheat residue + urea with a C:N of 15; Control, no residue and urea.

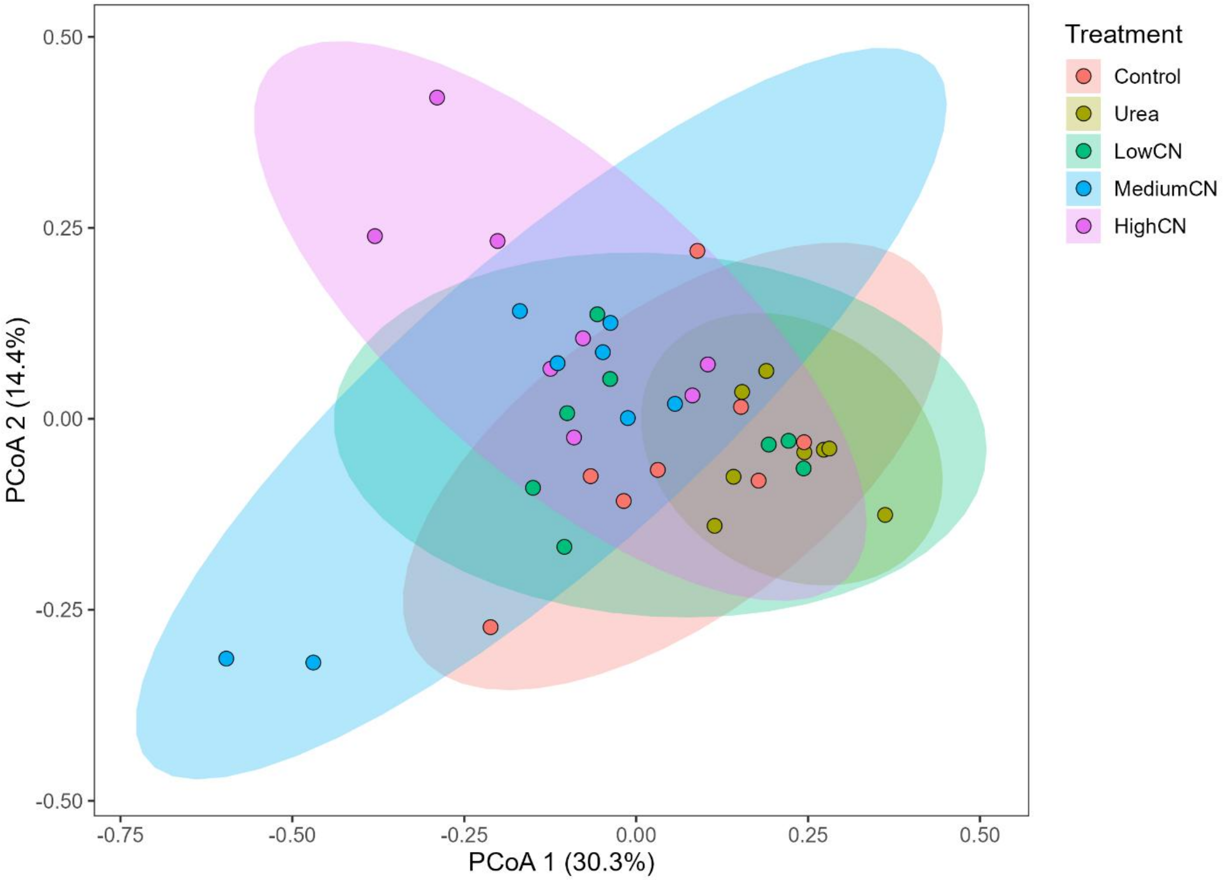

Figure S4.2. Principal coordinate analysis (PCoA) clustering of bacterial community structure for rhizosphere soil of cowpea grown in a greenhouse experiment with varying levels of C and N input. The colors and ellipses represent different plant-driven C:N ratio treatments. HighCN wheat straw with C:N ratio 40; MediumCN, wheat residue + urea to achieve a C:N ratio of 25; LowCN, wheat residue + urea with a C:N of 15; Control, no residue and urea.