IMPACTS OF MANAGEMENT PRACTICES AND SOIL PROPERTIES ON FREE-LIVING NITROGEN FIXATION IN SOUTHEASTERN SOUTH DAKOTA

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ABSTRACT

The excessive use of synthetic nitrogen (N) fertilizers in modern agriculture raises economic and environmental concerns. Biological dinitrogen (N_2) fixation offers a sustainable alternative to supply N, with free-living diazotrophs playing a crucial role alongside well-known symbiotic nitrogen-fixing bacteria. Interest in free-living nitrogen fixation (FLNF) has grown due to its potential contribution to sustainable agricultural practices. In recent years, various companies have introduced biofertilizers that could boost FLNF in the Midwestern USA, but many have not considered how soil properties and conservation methods might affect this process. This study examines how crop rotations (2-, 3-, and 4-year systems), tillage practices (conventional vs. long-term no-till), and cover cropping affect potential FLNF in Southeastern South Dakota, by assessing their impacts on key soil properties and how these influence microbial N_2 fixation. Surface soil samples (0-3") were collected at pre-planting, and V5, VT, and R6 corn growth stages in 2024 and 2025 to measure the potential nitrogen fixation rate through $^{15}N_2$ incorporation. Initial findings showed that cover cropping did not significantly impact fixation rates. In 2024, the highest potential fixation was observed in the 2-year corn-soybean rotation, particularly before planting and under conventional tillage, while no-till systems maintained lower but steadier fixation rates throughout the season. Among the soil properties evaluated (potentially oxidizable carbon, cation-exchange capacity, potentially mineralizable nitrogen, exchangeable ammonium-N, nitrate+nitrite-N, and soil pH), soil pH played a mediating role in how tillage is related to FLNF. These results offer valuable insights into how free-living nitrogen fixation operates across different cropping systems and highlight the importance of conservation systems in promoting soil stability.

INTRODUCTION

Synthetic nitrogen (N) fertilization has been contributing to world's food production since the Green Revolution, and it has become a trending topic regarding N use efficiency and N losses. Before the widespread adoption of industrial N, biological dinitrogen (N_2) fixation (BNF) played a crucial role in supplying N to crops. This process remains significant today, especially in regions where soybean production is economically viable without relying on synthetic N inputs such as in the Midwestern USA (Russelle, 2008). Furthermore, N_2 can be fixed by free-living diazotrophs, which can be found in most soils. This topic has had significant interest by researchers since its discovery in the early 20th century until the Green Revolution. With the increasing consumption of synthetic N, research on free-living N fixation began to decline and has been put aside. However, in recent years, there has been a growing interest in this area, reflecting the recognition of its contribution to sustainable agriculture.

Nitrogen fixation that occurs without symbiosis between plants and microbes is known as free-living nitrogen fixation (FLNF). Unlike symbiotic diazotrophs, free-living bacteria lack a stable microenvironment and the resources provided directly by the plant. Under these circumstances, it is important to highlight the significance of soil properties, as these bacteria depend on the broader environment to regulate their activity (Smercina et al., 2019). Key factors that affect free-living N-fixing bacteria include carbon availability, oxygen concentration, soil moisture, temperature, pH, nutrient status, particularly N, phosphorus, molybdenum, and iron. As a result, the rates of N fixation are typically lower than those in symbiotic relationships, with an estimated contribution up to 54 lbs N ac^{-1} yr^{-1} (Orr, 2011). Despite their lesser contribution compared to symbiotic fixers, free-living diazotrophs are widely distributed in various environments. Given proper study and exploration, these organisms have not only the potential to significantly enhance nitrogen fixation rates (Khan et al., 2021) but also act as plant growth-promoting bacteria (Kennedy et al., 2004).

Over the past years, several companies have released biofertilizers with the potential to increase FLNF in the Midwestern USA. However, most of them have overlooked how edaphic properties and conservation practices may influence this process. In South Dakota’s agricultural systems, scientific data on FLNF is limited. Therefore, my research aims to evaluate the impact of different sustainable agricultural practices, such as tillage intensity, crop rotations, and cover crops, on the ability of free-living bacteria to enhance soil nitrogen supply through fixation.

MATERIALS AND METHODS

Site description

This two-year study is being conducted at the South Dakota State University Research Farm near Beresford, South Dakota, during the 2024 and 2025 corn (*Zea mays* L.) growing seasons. The experimental plots are part of a long-term study on tillage and crop rotation. Since 1991, a no-tillage system has been in place, with consistent crop rotation for the past thirteen years and a seven-year history of cover crop planting. All plots are situated on nearly flat areas with slopes under 1%, and the soils belong to the Egan series, characterized as Fine-silty, mixed, superactive, mesic Udic Haplustolls.

Plot layout followed a split-plot arrangement in a randomized complete block design, with four replicates. Crop rotation (2-, 3-, and 4-year systems) was assigned to main plots, tillage (conventional vs. no-till) to subplots, and cover cropping (with vs. without) to sub-subplots.The 2-year crop rotation consisted of corn-soybean (*Glycine max* L. Merr.), the 3-year rotation included corn-soybean-oat (*Avena sativa* L.), and the 4-year rotation had corn-soybean-oat-rye (*Secale cereale* L.). Winter wheat (*Triticum aestivum* L.) was planted in October as the cover crop for both years. Apart from the experimental factors evaluated, all other management practices, including fertilization, were kept uniform across all the sampled plots.

Sampling and data collection

Soil samples were collected from the surface (0-3” depth) throughout both seasons during pre-plant (PP), V5, VT, and R5 corn-growth stages. A composite sample consisting of 4-5 cores was taken from each replication. Part of the sample was kept fresh for potential fixation assessment, while the rest was dried in an oven (50 °C) and ground to <2mm. To measure the potential N_2 fixation by the free-living bacteria, an assay technique involving ^{15}N-labled dinitrogen ($^{15}N_2$) was conducted following the incubation method described by Zhou et al. (2025). The soil parameters analyzed are summarized in Table 1. They included soil pH, determined for a slurry with soil/water ratio of 1:1 (Peters et al., 2015), exchangeable N (NH_4^+-, NO_3^--, and NO_2^--N) by the direct-diffusion method (Khan et al., 2000), potentially mineralizable N (PMN) by the Illinois soil N test-2 (Nunes et al., 2025), permanganate oxidizable carbon (POxC) by Culman et al. (2012), and Bray-1 P with the Ascorbic Acid method (Frank et al., 2015).

Table 1. Summary of soil sampling stages and parameters analyzed.

Sampling stage	Measurements
PP	Soil pH, Mineral Nitrogen, PMN, POxC, Bray-1 P, CEC, Potential N_2 Fixation
V5	Soil pH, Mineral Nitrogen, Potential N_2 Fixation
VT	Soil pH, Mineral Nitrogen, Potential N_2 Fixation
R5	Soil pH, Mineral Nitrogen, Potential N_2 Fixation

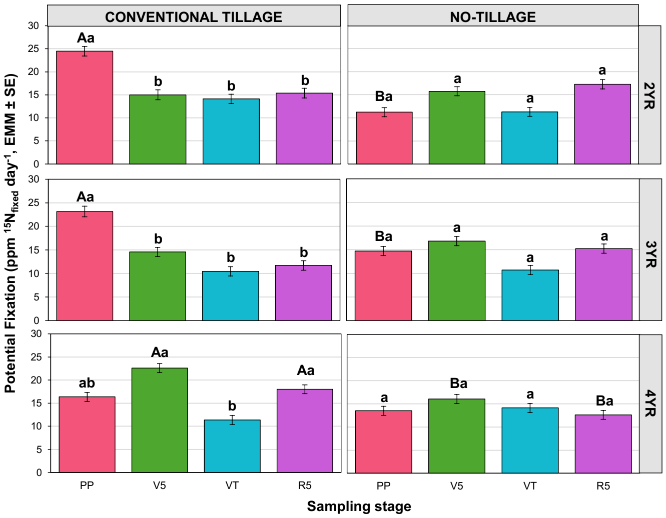
Data analysis was conducted with a linear mixed-effects model (LMM), considering tillage, crop rotation, and sampling stage as fixed factors. Block and their interactions were treated as random effects to account for the split-plot design. The cover crop was excluded from this model because it was not significant in the full model. Soil pH was included as a covariate (mediator) to account for chemical differences across plots. Main effects and interactions were tested using Type III ANOVA with Satterthwaite’s approximation. Tukey’s HSD test was employed for mean comparisons ($\alpha = 0.05$). Significant interactions were further analyzed using estimated marginal means (EMMs).

RESULTS AND DISCUSSION

Across all treatments in 2024, the potential N fixation was heavily influenced by management practices and soil chemical conditions. However, these factors did not act independently; their effects overlapped and interacted, impacting the N fixation by free-living bacteria. The presence of a cover crop did not affect the results ($p>0.05$), likely due to poor establishment and the harsh winter in South Dakota.

Seasonal dynamics under different tillage and rotation systems

Under conventional tillage (CT), higher potential fixation rates were generally observed early in the season, especially before planting. In contrast, under no-tillage, rates were lower but more stable throughout the growing season (Figure 1). Crop rotation influenced potential nitrogen fixation solely under conventional tillage, with the 2-year rotation showing the highest rates before planting, while the 4-year rotation peaked at R5 (Figure 2). In no-till systems, fixation stayed consistent throughout the season, and no effects from rotation were observed. Overall, these results show that tillage and rotation shape the seasonal dynamics of free-living nitrogen fixation, with conservation practices enhancing stability throughout the season.



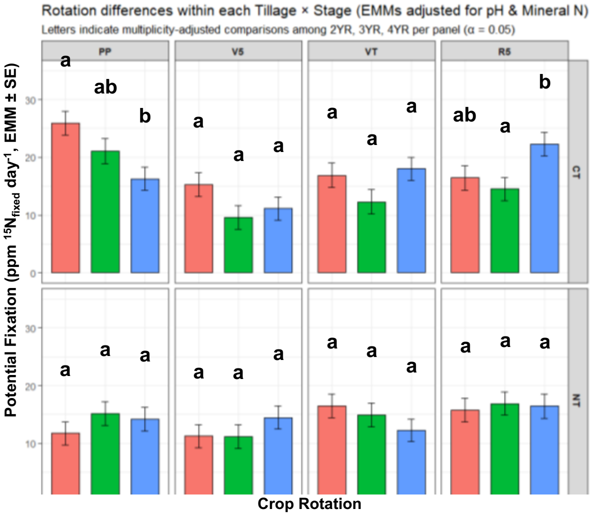


Figure 1. Potential N_2 Fixation throughout the growing season under conventional tillage and no-tillage across the three crop rotations in 2024. Bars represent estimated marginal means ± standard error (EMM ± SE). Different lowercase letters indicate significant differences among sampling stages within each tillage × rotation combination (Tukey's test, α = 0.05). Different uppercase letters indicate differences among tillage systems within each rotation × sampling stage combination (Tukey's test, α = 0.05).

Figure 2. Rotation effects on potential N_2 fixation within each tillage × sampling stage combination in 2024. Bars represent estimated marginal means ± standard error (EMM ± SE). Different lowercase letters indicate significant differences among crop rotations (2-, 3-, and 4-year systems) within each tillage × stage panel (Tukey's test, α = 0.05). CT represents conventional tillage and NT, no-tillage.

Soil pH mediates the effect of tillage on potential N_2 fixation

Among the soil properties evaluated, soil pH played a key role as a mediator, linking tillage to potential fixation rates. Instead of acting independently, tillage interacted with pH (*p*=0.012), influencing how potential fixation responded across the studied pH range (~4.6-6.7). This suggests that tillage modifies the soil chemical environment in ways that affect the sensitivity of free-living nitrogen fixation to soil pH. The distribution of soil pH in categories (Figure 3), ranging from 1 (more acidic) to 5 (closer to neutral), supports this interaction, showing that soils under conventional tillage tended to have higher pH values than those under no-till. Consequently, fixation was more responsive to pH under conventional tillage, while under no-till, the relationship between fixation and pH was weaker, suggesting that this system provides a more buffered environment (Figure 4). In addition, the acidic conditions under no-till also explain the lower overall fixation rates in this system, since many diazotrophs are neutrophiles and are more abundant when soil pH > 6.0 (Martin et al., 1937).

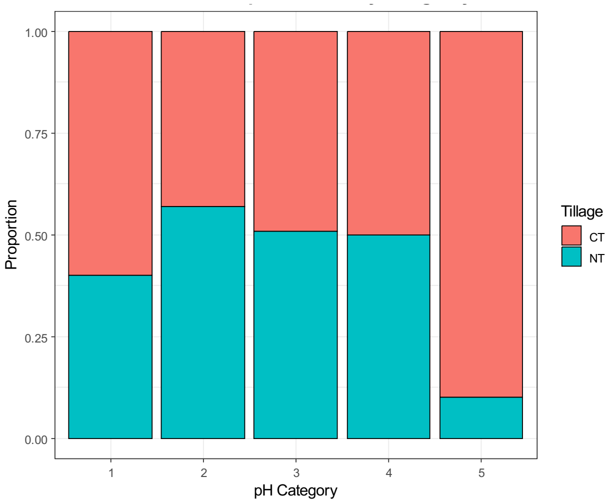


Figure 3. Distribution of soil pH categories under conventional tillage (CT) and no-tillage (NT) systems. Bars represent the proportion of

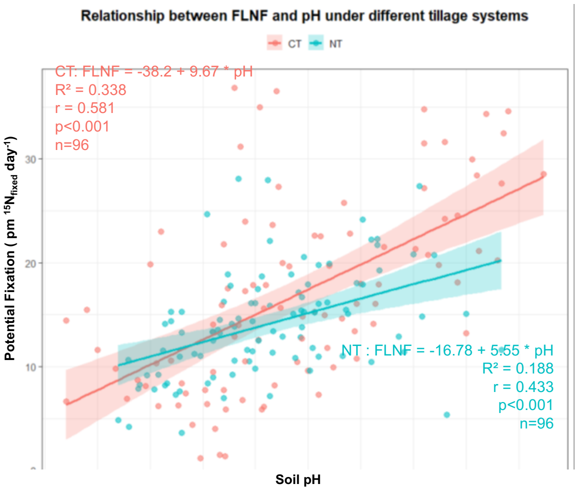


Figure 4. Relationship between soil pH and potential N_2 fixation under conventional tillage (CT) and no-tillage (NT) systems. Each point represents an individual soil sample (n = 96 per tillage system). Regression lines represent linear fits with 95% confidence intervals (shaded areas).

CONCLUSION

Considering how much reliance is placed on synthetic N fertilizer, it is crucial to explore alternatives. However, the use of biological products should take into account the entire agricultural system, and their performance might vary even at a small local scale due to interactions among management factors. This study highlights how agricultural management practices influence the potential free-living N_2 fixation for a specific location in Southeastern South Dakota; nonetheless, it emphasizes the need for proper research into how edaphic properties provide the conditions for these bacteria to thrive.

The results showed that tillage and rotation together shape the seasonal pattern of N_2 fixation by free-living bacteria, with no-tillage systems exhibiting lower but more consistent rates. Among the chemical parameters analyzed (not all results are presented here), soil pH interacted with tillage, indicating that tillage alters the chemical environment and affects the performance of N-fixing bacteria. Overall, this highlights that conservation practices help maintain stable biological N inputs over time, while conventional tillage promotes short-term N fixation during favorable seasonal conditions.

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