DETERMINING THRESHOLD LEVELS OF RESIDUAL SOIL NO_3^- FOR SOYBEAN PRODUCTION

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ABSTRACT

Historically, North Dakota's agricultural system was primarily based on wheat-corn rotations; however, the past twenty years, the state has increased it is soybean planted area in about four times. This new scenario highlights the importance of understanding how residual soil nitrate (NO_3^-) affects soybean nitrogen acquisition, nodulation, and grain yield. Although soybean can obtain nitrogen through biological nitrogen fixation (BNF) and soil mineral nitrogen uptake, elevated residual NO_3^- following corn production may suppress nodulation and reduce the plant's reliance on BNF. This study investigates the relationship between residual soil NO_3^- levels, soybean nodulation, and grain yield across different environments in North Dakota. Seven nitrogen rates (0, 50, 100, 150, 200, 250, and 300 lb N ac^{-1}) were established on the preceding corn crop to generate a range of residual soil NO_3^- levels for the following soybean crop. Soybean response was evaluated by measuring dry nodule mass at R1 and R6 and total grain yield. The results indicate high NO_3^- levels are associated with lower nodulation in R6. However, in one of the sites, greater spring soil NO_3^- was negatively associated with nodule biomass at the R1 growth stage, whereas the other site was associated with a small increase in nodulation with greater NO_3^- rates. Despite negative relationships between NO_3^--N and nodulation observed in some environments, these responses did not consistently translate into lower grain yield. These findings highlight the importance of environmental variability on regulating soybean nodulation and the relationship between residual soil NO_3^- and soybean yield. Understanding these interactions may improve nitrogen management in corn–soybean cropping systems and help clarify when residual NO_3^- can influence soybean dependence on biological nitrogen fixation.

INTRODUCTION

Agriculture remains the backbone of North Dakota's economy, with recent estimates placing the sector's annual gross business volume at $41.3 billion, supporting over 123,000 jobs and $10 billion in labor income (NDSU, 2025). Soybean (Glycine max L. Merr.) play a central role in these figures. Farmers have harvested almost 224 million bushels in 2025, generating $2.16 billion in direct crop value (USDA-NASS, 2026). As the United States stands as the world's second-largest producer, sustaining crop yields as production moves into new regional environments carries weight for both local economies and international trade (USDA-ERS, 2026).

Over the past few decades, North Dakota's agricultural scenario has been through a major transition, marked by a considerable rise in planted soybean fields of acreage (North Dakota Soybean Council, 2017). Soybean production was historically more limited

to the eastern counties while small grains used to dominate the state's major cropland. However, soybean have rapidly moved westward to non-traditional growing regions (NDSU, 2017). The numbers illustrate this shift: planted soybean area has increased from 1.9 million acres in 2000 to 6.85 million in 2026, representing an approximately 261% increase, or a 3.6-fold expansion over 26 years (USDA-NASS, 2026). While the harvested area of wheat declined from 9.43 million acres in 2000 to 5.39 million acres in 2026, soybean acreage expanded substantially, reaching 6.79 million harvested acres in 2026 (USDA-NASS, 2026). As a result, soybeans have surpassed both, wheat and corn (4.75 million acres) in harvested area (USDA-NASS, 2026). This expansion highlights how corn and soybean rotations have increasingly displaced traditional small-grain systems across diverse growing environments in North Dakota.

As soybean production expands across North Dakota, understanding the crop's Nitrogen (N) requirements becomes also increasingly important. Unlike many major field crops, soybean accumulation of N relies heavily on biological N fixation (BNF) to satisfy its high protein demand. (Ciampitti et al., 2018). Once these bacteria infect the root and form functional nodules, they convert atmospheric N into plant-available forms (Döbereiner, 1966). Under controlled conditions, elevated NO_3^- consistently suppresses nodule initiation and functionality (Luan et al., 2025). Beyond affecting nodule formation, NO_3^- can inhibit nitrogenase activity in established soybean nodules, thereby reducing biological nitrogen fixation (Wu and Harper, 1990). NO_3^- can also influence root development and carbon allocation, potentially limiting the resources available to support active nodules, through interconnected physiological and molecular signaling pathways (Li et al., 2023; Guillierme et al., 2025).

Because of the environmental variability, translating controlled laboratory findings to field reality, however, is complicated. Soil NO_3^- concentrations fluctuate during the growing seasons depending on weather and landscapes, to mineral nitrogen turnover, crop uptake, leaching, and denitrification (Santachiara et al., 2019). In field conditions, early-season soil NO_3^- might impact directly symbiotic performance. As early as 1982, Herridge had documented the negative effects of high soil NO_3^- availability on soybean nodulation, reporting delayed nodule development and reduced nitrogen fixation. In a broader evaluation across five environments, planting-time soil NO_3^- ranging from 62 to 248 lb N ha^{-1} have caused wide differences in nodulation rates and the percentage of plant N fixed (Herridge et al., 1990). Observations from the northern Great Plains align with these findings. In a ten years study across the northern U.S., they found out that the direct N applications to soybean decreased both, average nodule number and nodule size (Cigelske et al., 2020; Augustin and Franzen, 2024). However, these studies do not fully address how residual soil NO_3^- from a preceding crop influences soybean nodulation under field conditions.

This gap in knowledge is especially once corn requires substantial mineral N inputs and is highly responsive to N fertilizer, and when applications exceed crop demand, unused NO_3^- accumulates in the soil profile (Zhu and Fox, 2003; Wang et al., 2019; Franzen, 2022). However, residual NO_3^- levels vary across environments and may influence soybean nodulation and nitrogen acquisition differently. This study evaluates the relationships between residual soil NO_3^-, soybean nodulation at the R1 and R6 growth stages, and grain yield across three North Dakota environments, providing insight into

how carryover N from corn production may influence soybean–rhizobium symbiosis and crop productivity under field conditions.

MATERIALS AND METHODS

Three field experiments were set up across the state of North Dakota, USA, utilizing a traditional corn–soybean rotation. This field approach allows tracking N and persistence and assessing how the NO_3^- rates may eventually impact on BNF under realistic agricultural conditions.

During the 2025 crop year, corn N-response trials were established at three North Dakota locations: Dickinson, Mandan, and Valley City. The Mandan site was managed under conventional tillage, whereas the Dickinson and Valley City sites were managed under no-till. To create a gradient of residual soil NO_3^-, we hand-broadcast urea to the corn at seven different rates: 0, 50, 100, 150, 200, 250, and 300 0 lb N ac^{-1} before planting.

To quantify the residual soil NO_3^-, soil samples were collected from each experimental unit in the fall of 2025, right after corn harvest. A second round of samples were taken in the following spring, right before planting soybeans, to measure how much NO_3^- remains after the winter. For every plot, were extracted two soil cores to a depth of 0–2 ft using a soil probe, combining them into a single sample for NO_3^- analysis.

Soybeans were planted in the spring of 2026 at a row spacing of 15 inches. At the Mandan location, CROPLAN 0740 soybean seeds were planted at a seeding rate of 175,000 seeds ac^{-1}, whereas AG03XF2 soybean seeds were planted at Dickinson at a seeding rate of 150,000 seeds ac^{-1}.Dickinson had no previous soybean history and was inoculated twice, whereas Mandan and Valley City had previous soybean history and were not inoculated. Nodule development was evaluated at the R1 and R6 growth stages by sampling three plants per experimental unit at each sampling date. To minimize nodule loss during sampling, one primary plant was carefully excavated along with its root system within a 20 × 20 × 20 inches soil block, while surrounding plants were cut at the soil surface. The excavated root systems were washed to remove soil, and unattached root fragments were discarded. In the laboratory, nodules were individually detached from the roots, and oven-dried at 160°F to constant weight. The dried nodules were then weighed to determine total nodule dry mass per plant. Soybean grain yield was measured at harvest using a small-plot combine harvester. Grain harvested from each experimental unit was collected to determine yield, which was standardized to 15% grain moisture.

Statistical analyses were performed using R and JMP software. Linear regression analyses were conducted to evaluate the relationships between soil NO_3^- concentrations and soybean nodule mass at the R1 and R6 growth stages. Models included soil NO_3^- concentration, environment, and their interaction to measure whether the relationships between soil NO_3^- and nodule mass differed among environments. Relationships

between soil NO_3^- concentrations and soybean yield were also evaluated using linear regression.

RESULTS AND DISCUSSION

The relationship between spring soil NO_3^--N concentrations and soybean nodule dry mass varied between environments and growth stages (Figures 1 and 2).

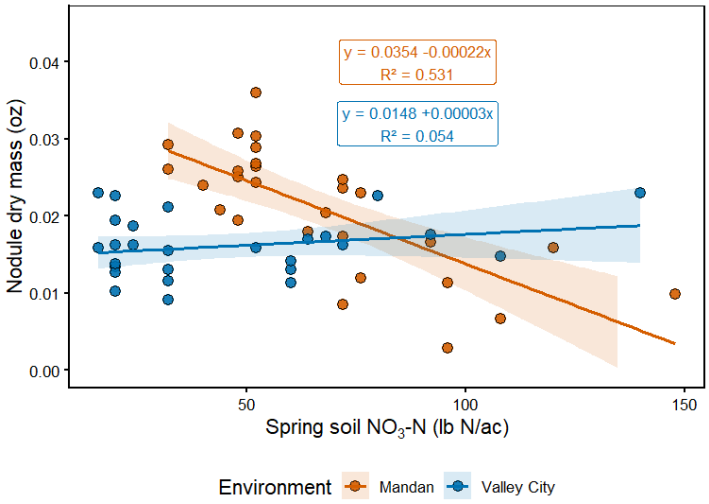


Figure 1: Spring soil NO_3^--N and soybean nodule dry mass at R1.

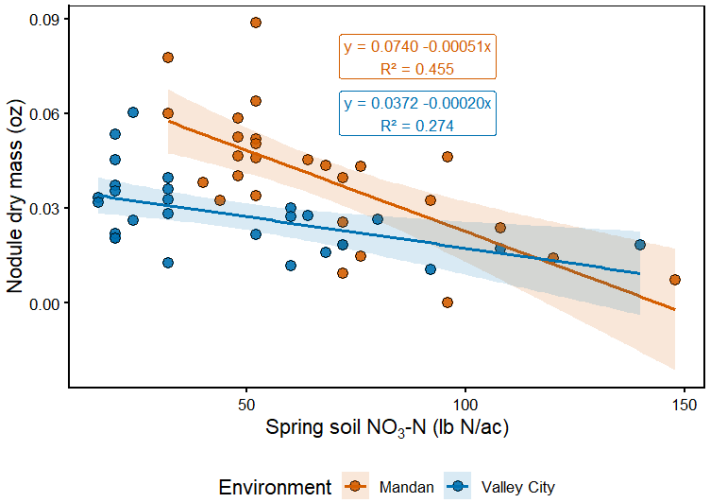


Figure 2: Spring soil NO_3^--N and soybean nodule dry mass at R6.

Spring soil NO_3^--N was negatively associated with soybean nodule dry mass in Mandan at R1 and in both Mandan and Valley City at R6. In contrast, Valley City showed a weak relationship between NO_3^--N concentration and nodule mass at R1. These findings suggest that greater soil NO_3^--N availability was associated with reduced nodulation,

although the magnitude of this relationship varied across environments and growth stages. This pattern is consistent with the potential suppression of nodule development when mineral nitrogen is available, as soybean plants may rely more heavily on soil-derived nitrogen and reduce their investment in biological nitrogen fixation.

Despite the negative relationships between NO_3^--N concentration and nodule mass, soybean yield exhibited positive relationships with spring soil NO_3^--N in Dickinson, Mandan, and Valley City (Figure 3).

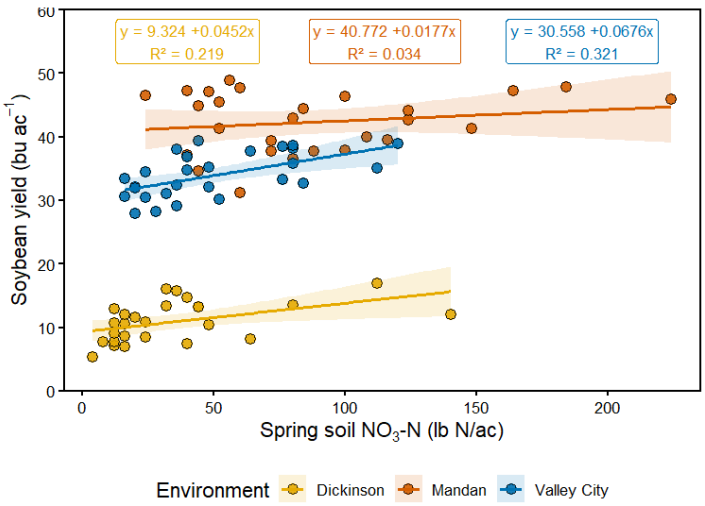


Figure 3: Spring soil NO_3^--N and soybean grain yield.

The relationship was particularly weak in Mandan, suggesting that NO_3^--N concentration alone explained little of the variation in grain yield. These contrasting responses indicate that reduced nodulation did not necessarily correspond to lower yield. Greater soil nitrogen availability may support crop nitrogen uptake and grain production despite reduced nodule development.

CONCLUSION

The results highlight the importance of evaluating soybean nodulation and grain yield separately rather than assessing only the effects of residual soil NO_3^--N and yield. Although higher NO_3^--N concentrations were associated with lower nodule mass in several environments, yield responses were positive or weakly related to NO_3^--N availability. Further research is needed to determine the extent to which soil-derived nitrogen can sustain soybean productivity under conditions associated with reduced nodulation.

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