

INFLUENCE OF NITROGEN FERTILIZATION AND STRAW RETURNING ON NITROGEN UPTAKE IN UPLAND SOILS: INSIGHTS FROM PHYSICOCHEMICAL PROPERTIES

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Abstract. This study investigated the effects of nitrogen fertilization and straw returning on soil physicochemical properties, enzymatic activities, and soybean yield in upland soils. A field experiment was conducted using three straw-returning levels T0, T1 no straw returning, and T2, combined with four nitrogen application rates N0, N1, N2, and N3. Under T2 + N3, soil total nitrogen reached 1.52 g kg⁻¹, available potassium was 174.3 mg kg⁻¹, and alkaline phosphatase activity measured 323.65 U L⁻¹. With higher straw and nitrogen inputs, urease and phosphatase activities were enhanced, while sucrase activity declined. Agronomic traits improved under T2 + N3, with plant height at 89.67 cm, pods per plant at 73.08, seeds per plant at 171.21, hundred-seed weight at 16.04 g, and yield at 2726.34 kg ha⁻¹. These findings demonstrate that combining higher straw returns with increased nitrogen fertilization effectively enhances soil fertility, enzyme-driven nutrient cycling, and soybean productivity in upland agricultural systems, compared to the low return conventional fertilization regime.

Keywords: *straw returning, nitrogen fertilizer reduction, soil enzyme activity, soybean yield, nutrient management*

Introduction

Soybean (*Glycine max* L.) is a critical global cash crop, playing a vital role in oil production and protein supply, with nitrogen (N) being a key determinant of its growth and yield (Guo et al., 2022). Proper nitrogen fertilization significantly influences soybean growth, development, and yield; however, excessive nitrogen use leads to reduced nitrogen utilization efficiency, increased susceptibility to pests and diseases, and negative impacts on both the yield and quality of soybean crops (Yadav et al., 2017). Since the 1980s, nitrogen fertilizer application rates in China have significantly increased, resulting in resource waste and serious environmental issues, including soil acidification, compaction, reduced fertility, eutrophication of water bodies, and increased greenhouse gas emissions due to nitrogen oxide release (Liu et al., 2022). Thus, optimizing nitrogen fertilizer management to reduce input levels while maintaining crop productivity and environmental sustainability remains a crucial goal in agriculture (Shah and Wu, 2019).

Straw returning, a widely adopted agricultural practice, has emerged as a sustainable approach to enhance soil fertility and structure, increase soil organic matter content, and improve nutrient cycling efficiency (Jin et al., 2020). The incorporation of crop residues into soils enhances microbial activity and enzyme function, positively influencing nutrient availability, including nitrogen, phosphorus, and potassium (Coulbaly et al., 2020). Furthermore, the combined application of nitrogen fertilization and straw returning significantly modifies soil physicochemical properties, microbial communities, and nitrogen cycling processes (Zeng et al., 2025).

This study focuses on evaluating the combined effects of straw biochar and reduced nitrogen fertilization on soil physicochemical properties, nitrogen uptake, and yield and agronomic traits in upland soils. Specifically, the objectives include: (1) assessing the impacts of straw returning and reduced nitrogen fertilizer application on soil nutrient status and quality related physicochemical properties; (2) evaluating the influence of this integrated management practice on soybean agronomic performance and yield. The outcomes aim to provide a theoretical basis for optimizing nitrogen fertilization and biochar application strategies, promoting sustainable soybean production practices.

Materials and methods

Site description and materials

This study was carried out in 2024 in the field trial area of Wuzhan Town, Zhaodong City, Heilongjiang Province (E126°15'53.29", N45°52'3.61"). The region features a cold temperate continental monsoon climate, with pronounced seasonal variations. The frost-free period spans 130–140 days, and the accumulated temperature ($\geq 10^{\circ}\text{C}$) ranges from 2700 to 3000°C, providing suitable conditions for soybean cultivation. The tested soybean variety was “Henong 71,” which has a growth cycle of 126 days from emergence to maturity. This medium maturity variety exhibits an indeterminate pod setting habit and a convergent plant type, with round leaves, purple flowers, and brown pubescence. The seeds are round, with yellow seed coats and yellow hilums, containing 38.74% crude protein and 21.0% crude fat.

The experiment was sown on April 30th using mechanical sowing, with a row spacing of 30 cm and an intra-row spacing of approximately 13 cm. The irrigation strategy relied primarily on natural precipitation, with supplementary irrigation applied as needed during dry spells. During the soybean growing season (May to September), the total recorded precipitation was approximately 430 mm. Notably, significant rainfall events occurred in mid-June (cumulative 62 mm), early July (87 mm), and late August (94 mm). Artificial irrigation was applied twice: 30 mm on May 25th to ensure seedling establishment under early-season dryness, and 35 mm on July 20th to alleviate a short drought period during flowering. The soybean harvest was conducted on September 30th.

Experimental design

This study was carried out in 2024 using a plot experiment method. Each plot had an area of 4.0 m $\times$ 4.0 m. Three straw returning patterns were designed in the experiment, namely no straw returning, half amount straw returning, and full amount, with straw application rates represented by T0 (0 kg·ha⁻¹), T1 (4500 kg·ha⁻¹), and T2 (9000 kg·ha⁻¹), respectively. After the soybean harvest in the previous autumn, when the straw was naturally air-dried, it was cut into segments of about 5 cm in length and then plowed and buried 10–15 cm below the soil surface. Under the three straw returning conditions of T0, T1, and T2, four nitrogen fertilizer application levels (pure nitrogen) were set respectively: 0 kg·ha⁻¹ (N0, with 937.5 kg·ha⁻¹ of phosphorus fertilizer and 187.5 kg·ha⁻¹ of potassium fertilizer), 95 kg·ha⁻¹ (N1), 180 kg·ha⁻¹ (N2), and 225 kg·ha⁻¹ (N3). Each treatment was replicated three times. Other field management measures followed the conventional field production methods.

Soybean samples were collected during the seedling stage, flowering stage, and maturity stages. For each sampling event, five representative plants were randomly selected from the central area of each plot to avoid edge effects. At the seedling stage, the plants were separated into shoots and roots. At the flowering stage, they were separated into leaves, stems, and flowers. At the maturity stage, the samples were separated into leaves, stems, pods, and seeds. The separated samples were oven-dried at 105 °C for 30 min, further dried at 75 °C to a constant weight, weighed, ground, and sieved for nutrient content analysis.

Determination of plant nutrient content

Soil pH was determined in a 2.5:1 water/soil suspension using a pH meter. Soil exchange capacity (EC) was determined by the BaCl₂ compulsive exchange method. CEC was determined via the 1 M ammonium acetate exchange method at pH 7.0. The Walkley-Black titration method was carried out to determine the soil organic matter (SOM) content. The methods of concentrated H₂SO₄ digestion and Kjeldahl were used to determine the total nitrogen content (TN) of the soil samples (Abujabhah et al., 2016). Total phosphorus content (TP) of the soil samples was determined by HClO₄ and H₂SO₄ digestion molybdenum antimony anti-colorimetry (Pan et al., 2016). Total potassium content (TK) was determined by atomic absorption spectrophotometry (Guo et al., 2015). The soil's available nitrogen (AN) was measured using the Alkali-diffusion method (Deng et al., 2016). Determination of the available phosphorus (AP) in soil was measured by using NaHCO₃ extraction- Mo-Sb Anti-colorimetry (Mehlich, 1984). Available potassium content (AK) was extracted with 1 mol·L⁻¹ NH₄OAc, and then determined by flame absorption spectroscopy (Misskire et al., 2019). Ammonium nitrogen in soil was extracted with KCl and the extract was analyzed using a B324 Kjeldahl nitrogen analyzer (Jung et al., 2012). Nitrate nitrogen in soil was extracted with saturated calcium sulfate, and after adding ferrous sulfate zinc to the extract, it was analyzed using a B324 Kjeldahl nitrogen analyzer (Wyszkowska et al., 2022). Alkaline phosphatase was determined by the disodium phenyl phosphate colorimetric method (Cheng et al., 2023). Invertase was determined by the 3,5-dinitrosalicylic acid colorimetric method (Park et al., 2007). Urease was determined by the indophenol colorimetric method (Tavares et al., 2021). At the physiological maturity stage, five representative plants were randomly selected from each plot to evaluate agronomic traits. Plant height was measured from the soil surface to the top of the main stem using a measuring scale. Pods per plant and seeds per plant were counted manually. The total seed weight per plant was recorded by weighing seeds harvested from the sampled plants. Hundred-seed weight was determined by randomly selecting 100 seeds from the bulk sample, and final yield (kg·ha⁻¹) was obtained by harvesting the entire plot area and adjusting grain weight to standard moisture content (Jing et al., 2023).

Statistical analysis

Data were organized using Excel 2010, a software product developed by Microsoft Corporation (USA). One - way analysis of variance (one - way ANOVA) was carried out with SPSS 18.0 software developed by International Business Machines Corporation (IBM, USA). For data with significant differences ($P < 0.05$), the least significant difference (LSD) method was employed for multiple comparisons. Graphs were created using Origin 2021, a software developed by OriginLab Corporation (USA).

Results

Effects of straw returning and nitrogen fertilizer application on soil properties of soybean fields

The experimental soil was generally neutral to slightly alkaline. As shown in *Table 1*, different treatments had no significant effects on the soil pH during the three growth stages of soybean, with soil pH ranging from 6.91 to 7.65. The different treatments significantly affected the soil EC at each soybean growth stage ($P < 0.05$), with soil EC increasing as nitrogen application increased. The soil EC in the T2 + N3 treatments was significantly higher than that in the T0 + N0 treatments, with increases of 62.67%, 61.76%, and 60.31% at the seedling, flowering, and maturity stages, respectively.

Table 1. *Effects of straw returning and nitrogen fertilizer application on soil pH and electrical conductivity (EC) in soybean fields*

Treatments		pH			EC (dS·m ⁻¹)		
		Seedling stage	Flowering stage	Maturity stage	Seedling stage	Flowering stage	Maturity stage
T0	No	7.42 ± 0.08 a	7.17 ± 0.16 a	6.92 ± 0.14 a	0.28 ± 0.01 c	0.26 ± 0.01 c	0.25 ± 0.02 c
	N1	7.54 ± 0.06 a	7.27 ± 0.14 a	7.11 ± 0.09 a	0.38 ± 0.01 c	0.37 ± 0.01 c	0.35 ± 0.01 c
	N2	7.65 ± 0.01 a	6.98 ± 0.03 a	7.06 ± 0.03 a	0.49 ± 0.00 c	0.47 ± 0.01 b	0.44 ± 0.00 b
	N3	7.01 ± 0.03 a	6.94 ± 0.14 a	6.93 ± 0.16 a	0.60 ± 0.01 b	0.57 ± 0.01 b	0.54 ± 0.02 b
T1	No	7.21 ± 0.05 a	7.08 ± 0.10 a	6.98 ± 0.13 a	0.31 ± 0.01 c	0.29 ± 0.01 c	0.27 ± 0.01 c
	N1	7.34 ± 0.02 a	7.32 ± 0.02 a	7.07 ± 0.19 a	0.42 ± 0.00 c	0.40 ± 0.00 c	0.38 ± 0.01 c
	N2	7.32 ± 0.03 a	7.30 ± 0.04 a	7.15 ± 0.12 a	0.54 ± 0.00 c	0.51 ± 0.01 b	0.48 ± 0.01 b
	N3	7.33 ± 0.03 a	6.97 ± 0.06 a	7.19 ± 0.16 a	0.66 ± 0.01 b	0.63 ± 0.01 a	0.59 ± 0.01 a
T2	No	7.32 ± 0.03 a	7.06 ± 0.03 a	7.30 ± 0.01 a	0.34 ± 0.01 c	0.32 ± 0.00 c	0.31 ± 0.01 c
	N1	7.31 ± 0.02 a	7.27 ± 0.09 a	6.93 ± 0.03 a	0.46 ± 0.01 c	0.43 ± 0.01 c	0.41 ± 0.01 c
	N2	7.15 ± 0.05 a	6.91 ± 0.13 a	7.08 ± 0.02 a	0.60 ± 0.01 b	0.55 ± 0.01 b	0.53 ± 0.01 b
	N3	7.23 ± 0.01 a	7.28 ± 0.17 a	6.91 ± 0.06 a	0.75 ± 0.01 a	0.68 ± 0.01 a	0.63 ± 0.00 a

Different small letters in the same column meant significant difference among treatments in the same growth stage for the same season at 0.05 level. The same below

Different treatments had no significant effects on soil CEC across the soybean growth stages (*Table 2*). At the seedling stage, compared with the N0 treatment, CEC values in the N3 treatment increased by 9.63%, 12.93%, and 8.34% in the T0, T2, and T3 treatment groups, respectively. At the flowering stage, CEC values in the N3 treatment increased by 6.14%, 7.52%, and 8.33% in the T0, T2, and T3 groups, respectively, compared with the N0 treatment. At maturity, the N3 treatment increased CEC values by 6.04%, 7.41%, and 8.2 1% in the T0, T2, and T3 groups, respectively, compared with th/e N0 treatment. There were no significant differences in the C/N ratio among treatments during the seedling and flowering stages. At the maturity stage, several treatments showed variation in the C/N ratio, with a statistically significant increase observed in the T1 + N2 treatment compared to T0 + N2 (7.14%, $P < 0.05$), while differences among other treatments were not statistically significant.

Table 2. Effects of straw returning and nitrogen fertilizer application on soil cation exchange capacity (CEC) and C/N

Treatments		CEC (cmol·kg ⁻¹)			C/N		
		Seedling stage	Flowering stage	Maturity stage	Seedling stage	Flowering stage	Maturity stage
T ₀	No	8.16 ± 0.15 a	8.40 ± 0.13 a	8.55 ± 0.06 a	5.80 ± 0.30 a	5.20 ± 0.25 a	4.50 ± 0.20 b
	N1	8.79 ± 0.19 a	8.60 ± 0.12 a	8.75 ± 0.03 a	6.10 ± 0.25 a	5.50 ± 0.20 a	4.80 ± 0.15 ab
	N2	8.91 ± 0.03 a	8.80 ± 0.04 a	8.95 ± 0.13 a	5.75 ± 0.28 a	5.15 ± 0.18 a	4.45 ± 0.18 b
	N3	9.03 ± 0.12 a	8.95 ± 0.05 a	9.10 ± 0.08 a	5.90 ± 0.22 a	5.30 ± 0.22 a	4.60 ± 0.22 ab
T ₁	No	8.21 ± 0.12 a	8.60 ± 0.11 a	8.75 ± 0.12 a	6.05 ± 0.20 a	5.40 ± 0.16 a	4.70 ± 0.14 ab
	N1	8.61 ± 0.15 a	8.85 ± 0.14 a	9.00 ± 0.02 a	5.85 ± 0.32 a	5.25 ± 0.24 a	4.55 ± 0.24 b
	N2	9.36 ± 0.11 a	9.10 ± 0.08 a	9.25 ± 0.13 a	6.20 ± 0.18 a	5.60 ± 0.12 a	4.90 ± 0.12 a
	N3	9.43 ± 0.18 a	9.30 ± 0.04 a	9.45 ± 0.17 a	6.00 ± 0.15 a	5.35 ± 0.15 a	4.65 ± 0.16 ab
T ₂	No	8.90 ± 0.14 a	8.80 ± 0.02 a	8.95 ± 0.18 a	5.95 ± 0.20 a	5.45 ± 0.18 a	4.75 ± 0.18 ab
	N1	9.18 ± 0.15 a	9.10 ± 0.09 a	9.25 ± 0.09 a	6.15 ± 0.22 a	5.55 ± 0.14 a	4.85 ± 0.14 a
	N2	9.48 ± 0.12 a	9.35 ± 0.12 a	9.50 ± 0.10 a	5.88 ± 0.26 a	5.28 ± 0.20 a	4.58 ± 0.20 b
	N3	9.71 ± 0.09 a	9.60 ± 0.02 a	9.75 ± 0.07 a	6.08 ± 0.24 a	5.48 ± 0.16 a	4.78 ± 0.16 ab

Effects of straw returning and nitrogen fertilizer application on soil organic matter in soybean fields

As shown in *Figure 1*, different treatments had a significant impact on the SOM content in soybean fields during various growth stages. Under the T₀ treatment, during the seedling stage, SOM content under the N₃ nitrogen fertilizer level was significantly higher than that under N₀, increasing by 13.35% ($P < 0.05$). Although the SOM content under N₂ appeared higher than that under N₁, only N₃ showed a statistically significant difference compared to N₀. During the flowering stage, SOM content under the N₁ level was significantly higher than under N₀, with an increase of 1.34% ($P < 0.05$). At the maturity stage, the SOM content under N₂ was significantly higher than under N₀, increasing by 8.94% ($P < 0.05$). Under the T₁ treatment, significant increases in SOM content compared to N₀ were observed under the N₂ treatment during both the seedling and flowering stages, with increases of 15.43% and 17.82%, respectively ($P < 0.05$). At maturity, SOM under N₂ was also significantly higher than N₀ by 17.79% ($P < 0.05$). In the T₂ treatment, SOM content at each growth stage increased significantly under the N₂ treatment compared with N₀ ($P < 0.05$). As shown in *Figure 1*, with increasing amounts of straw returned to the field, SOM content in soybean fields tended to increase (T₂ > T₁ > T₀); however, comparisons across nitrogen levels other than N₀ are not supported by statistical significance.

Effect of nitrogen fertilizer on soil nitrogen nutrient content in soybean fields under straw returning

Based on *Figure 2*, the TN content under different straw returning treatments showed significant differences mainly in comparison with the N₀ level. Under the T₂ treatment, TN content at the seedling and maturity stages was significantly higher under N₂ than under N₀, with increases of 33.33% and 22.41%, respectively ($P < 0.05$). For soil nitrogen (NH₄⁺-N, no significant differences were observed among nitrogen levels under the T₀ treatment at the seedling stage. However, at the flowering and maturity

stages, $\text{NH}_4^+\text{-N}$ content under N2 was significantly higher than under N0 by 26.32% and 34.61%, respectively ($P < 0.05$). In the T1 treatment, only at the maturity stage did $\text{NH}_4^+\text{-N}$ content significantly increase under N3 compared to N0 (15.96%, $P < 0.05$). Under the T2 treatment, $\text{NH}_4^+\text{-N}$ content at the flowering stage was significantly increased under N2 and N3 compared to N0, by 27.61% and 19.42%, respectively ($P < 0.05$), and at the maturity stage, N3 led to a 21.93% increase over N0 ($P < 0.05$). For soil $\text{NO}_3^-\text{-N}$, at the flowering stage under T0 and T1 treatments, N2 significantly increased $\text{NO}_3^-\text{-N}$ content compared to N0 by 41.71% and 37.98%, respectively ($P < 0.05$). Under the T2 treatment, $\text{NO}_3^-\text{-N}$ content at the flowering stage was significantly higher under N1 than N0 by 44.84% ($P < 0.05$).

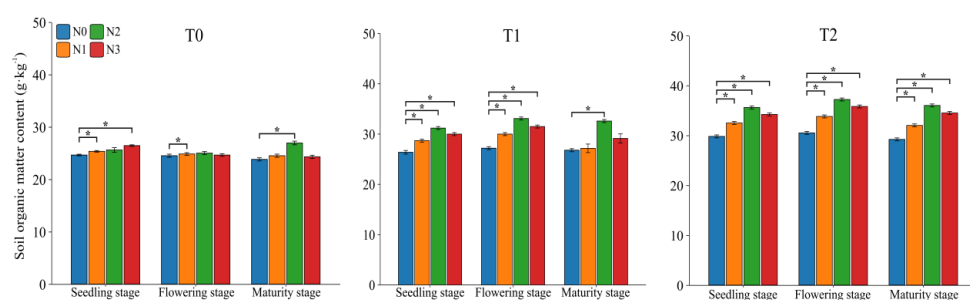


Figure 1. Effects of varying nitrogen fertilizer and straw returning levels on soil organic matter contents in soybean fields. Values labeled with the same lowercase letter were not significantly different (* $P < 0.05$)

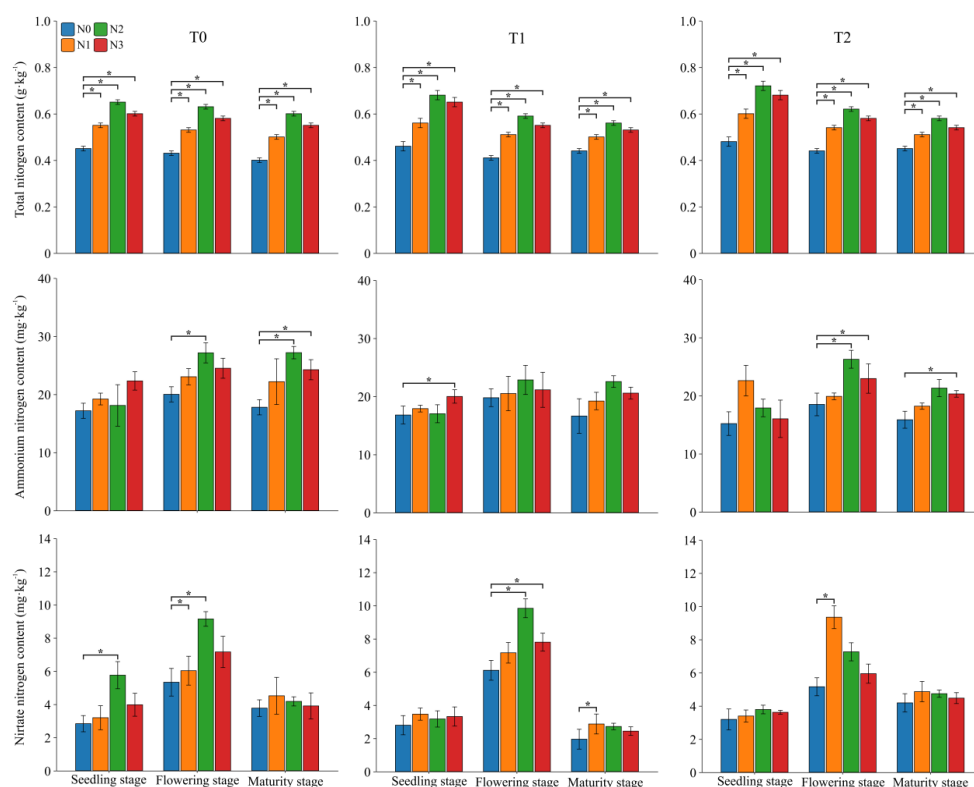


Figure 2. Effects of varying nitrogen fertilizer and straw returning levels on total nitrogen, ammonium nitrogen and nitrate nitrogen contents in soybean fields. Values labeled with the same lowercase letter were not significantly different (* $P < 0.05$)

Effect of nitrogen fertilizer on soil phosphorus nutrient content in soybean fields under straw returning

As shown in *Figure 3*, no significant differences in TP content were observed under T0 and T2 treatments. However, under the T1 treatment at the seedling stage, soil TP content at nitrogen levels N2 and N3 significantly increased by 24.41% and 16.69%, respectively, compared to N0 ($P < 0.05$). In contrast, during the maturity stage, TP content under N1 and N2 significantly decreased by 14.71% and 13.06%, respectively, compared to N0 ($P < 0.05$). For the AP, significant differences were observed among nitrogen treatments when compared to N0. Under the T0 treatment, AP content at N3 significantly increased by 22.56% at the seedling stage and by 20.75% at the flowering stage ($P < 0.05$). At the maturity stage, AP content under N2 was significantly higher than N0 by 21.56% ($P < 0.05$). In the T1 treatment, AP content under N3 significantly increased compared to N0 by 24.41% at the seedling stage and 20.51% at the flowering stage ($P < 0.05$).

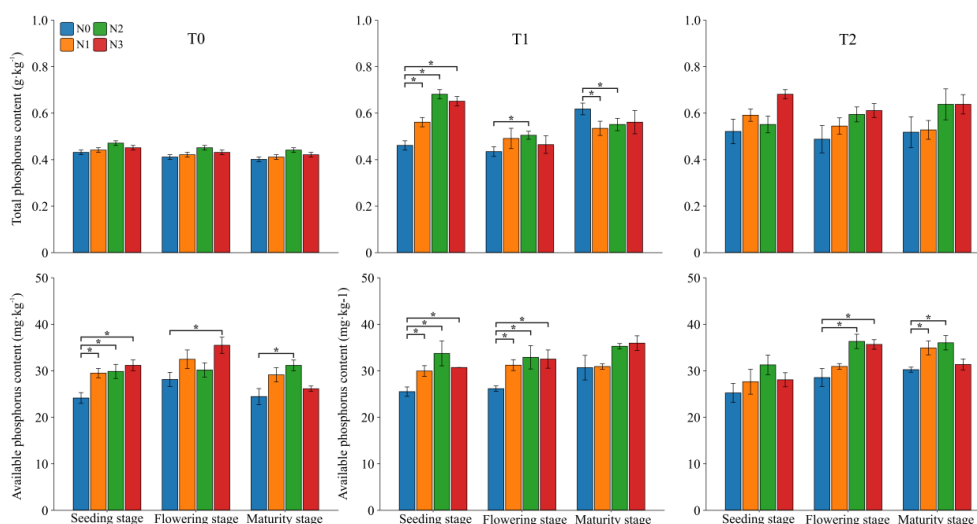


Figure 3. Effects of varying nitrogen fertilizer and straw returning rates on total phosphorus and available phosphorus contents in soybean fields. Values labeled with the same lowercase letter were not significantly different (* $P < 0.05$)

Effect of nitrogen fertilizer on soil potassium nutrient content in soybean fields under straw returning

The effects of nitrogen application rates and straw-returning patterns on the TK content at different soybean growth stages are shown in *Figure 4*. While TK content tended to decrease with increasing nitrogen application rates under T0, T1, and T2 treatments, only comparisons to N0 were supported by the data. Therefore, descriptive ranking among treatments has been removed for clarity. For the AK, significant differences were observed between nitrogen levels and the N0 control. At the flowering stage under the T0 treatment, AK content at N1 was significantly higher than at N0 by 13.39% ($P < 0.05$). At the seedling stage under the T1 treatment, AK content at N1 increased significantly by 14.83% compared to N0 ($P < 0.05$). Under the T2 treatment, AK content significantly increased at N3 compared to N0 by 20.03% at the flowering stage and 13.41% at the maturity stage ($P < 0.05$).

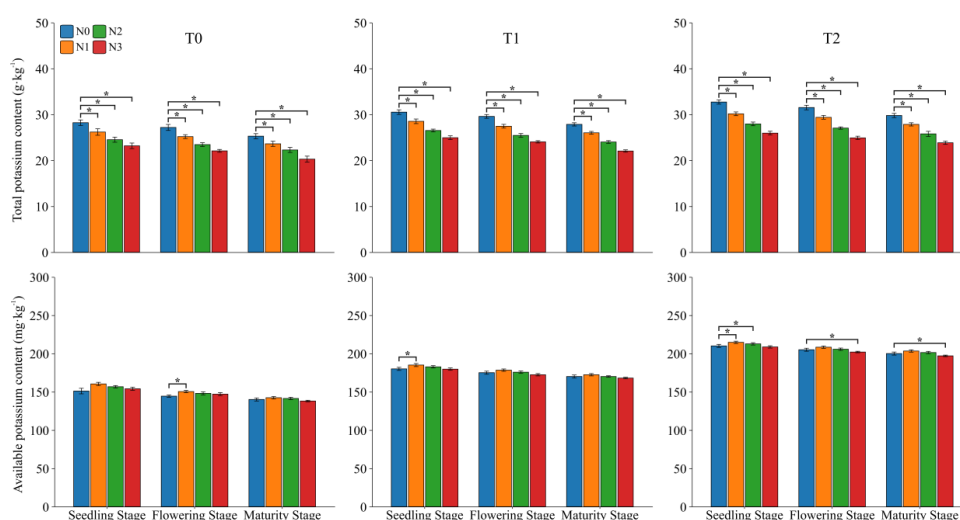


Figure 4. Effects of varying nitrogen fertilizer and straw returning levels on total phosphorus and available phosphorus contents in soybean fields. Values labeled with the same lowercase letter were not significantly different (* $P < 0.05$)

Effect of nitrogen fertilizer on soil enzyme activity in soybean fields under straw returning

As shown in *Figure 5*, under the T0 treatment, soil invertase activity during the seedling stage did not differ significantly among nitrogen levels. During the flowering stage, invertase activity at the N2 and N3 levels significantly increased by 32.58% and 35.13%, respectively, compared with N0 ($P < 0.05$). Under the T1 treatment, invertase activity during the seedling stage significantly increased by 14.94% and 17.91% under N2 and N3, respectively, compared with N0 ($P < 0.05$). At the maturity stage, invertase activity under N2 was significantly higher than under N0 by 17.61% ($P < 0.05$). Under the T2 treatment, nitrogen levels did not significantly affect invertase activity at any growth stage. For alkaline phosphatase activity, under the T0 treatment, activity at the N2 level significantly increased by 33.81%, 29.04%, and 22.53% during the seedling, flowering, and maturity stages, respectively, compared with N0 ($P < 0.05$). Under the T1 treatment, alkaline phosphatase activity under N2 was significantly higher than under N0 by 41.31% at the maturity stage ($P < 0.05$). Under the T2 treatment, alkaline phosphatase activity under N2 was significantly higher than under N0 across all growth stages ($P < 0.05$). For urease activity, under the T0 treatment at the maturity stage, urease activity at the N3 level significantly increased by 16.35% compared with N0 ($P < 0.05$). Under the T1 treatment, urease activity increased progressively with nitrogen application, and at the seedling stage, urease activity at N3 was significantly higher than under N0 ($P < 0.05$).

Effects of nitrogen fertilizer on soybean yield and its components under straw returning

Based on *Table 3*, the different straw-returning patterns and nitrogen application rates significantly influenced soybean yield and yield components. In terms of plant height, significant differences were observed among treatments, with the T2 treatment

showing generally better performance. Under T2, plant height first increased and then decreased with higher nitrogen rates, reaching a maximum of 89.67 cm under N1, which was significantly higher than in T0 and T1 treatments ($P < 0.05$). For pods per plant, the number tended to increase with the amount of straw returned ($T2 > T1 > T0$). While the T2 + N3 treatment showed the highest value (73.08), only two treatment combinations exhibited statistically significant differences compared to T0 at the same nitrogen level ($P < 0.05$), indicating that the observed numerical increases across other treatments were not statistically supported. Therefore, the description has been adjusted to reflect only the significant contrasts. Regarding seeds per plant, an increasing trend was observed with higher nitrogen levels across all straw-returning patterns. The N3 level resulted in the highest seed number within each straw-returning treatment, with T2 + N3 reaching the maximum (171.21), significantly higher than other treatments ($P < 0.05$). Seed weight per plant showed a similar pattern, with T2 + N3 achieving the highest value (26.72 g), also significantly higher than other treatments ($P < 0.05$). Hundred-seed weight showed relatively minor differences among treatments. However, under T2 treatment, higher nitrogen levels slightly increased the hundred-seed weight. The T2 + N3 treatment recorded the highest value (16.04 g), which was significantly higher than those of other treatments ($P < 0.05$). In terms of yield, the T2 treatment consistently outperformed T0 and T1 across nitrogen levels. T2 + N3 achieved the highest yield (2726.34 kg·ha⁻¹), significantly increasing by 3.27% compared to the control (T0 + N0) ($P < 0.05$).

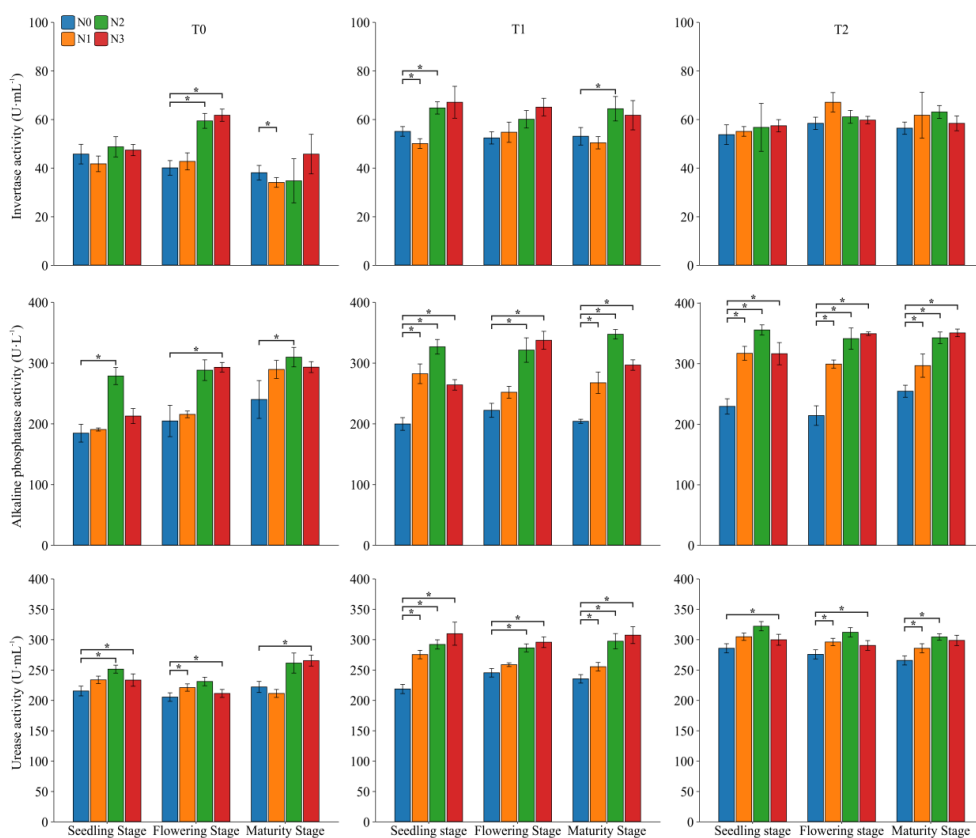


Figure 5. Effect of nitrogen fertilizer on soil invertase, alkaline phosphatase and urease activities in soybean fields under straw returning. Values labeled with the same lowercase letter were not significantly different (* $P < 0.05$)

Table 3. Effects of biochar and nitrogen fertilizer application on soybean yield and components

Treatments		Plant height (cm)	Pods per plant	Seeds per plant	Seed weight per plant (g)	Hundred-seed weight (g)	Yield (kg·ha ⁻¹)
T0	N0	85.04 ± 1.04 b	66.22 ± 0.73 b	153.98 ± 0.86 c	23.29 ± 0.32 a	15.68 ± 0.08 a	2639.91 ± 38.22 a
	N1	83.91 ± 0.58 c	68.13 ± 0.46 a	157.46 ± 1.46 b	22.72 ± 0.19 a	14.99 ± 0.12 a	2641.55 ± 22.74 a
	N2	87.73 ± 0.63 a	68.09 ± 0.69 a	156.97 ± 1.76 b	23.85 ± 0.16 a	15.22 ± 0.18 a	2624.67 ± 24.82 a
	N3	85.68 ± 0.46 b	67.93 ± 0.98 ab	163.34 ± 1.32 a	23.41 ± 0.24 a	15.09 ± 0.23 a	2660.43 ± 33.79 a
T1	N0	87.78 ± 0.51 ab	68.73 ± 0.48 a	160.13 ± 1.43 b	23.86 ± 0.19 c	15.36 ± 0.12 a	2685.64 ± 27.42 b
	N1	87.56 ± 0.52 ab	68.96 ± 1.03 a	160.37 ± 0.82 b	24.59 ± 0.32 b	15.92 ± 0.23 a	2704.45 ± 15.24 a
	N2	86.03 ± 1.19 b	70.06 ± 0.79 a	162.92 ± 1.33 b	24.31 ± 0.25 b	15.96 ± 0.22 a	2742.42 ± 25.96 a
	N3	88.64 ± 1.11 a	70.58 ± 0.76 a	166.38 ± 1.76 a	25.37 ± 0.23 a	15.83 ± 0.13 a	2663.97 ± 27.03 b
T2	N0	88.61 ± 0.81 a	70.56 ± 0.88 b	162.33 ± 2.36 b	24.25 ± 0.32 c	16.09 ± 0.21 a	2677.49 ± 18.26 b
	N1	89.67 ± 1.17 a	72.38 ± 0.99 ab	164.66 ± 1.22 b	24.54 ± 0.23 c	15.99 ± 0.08 a	2699.02 ± 27.14 a
	N2	87.45 ± 0.56 a	71.39 ± 0.63 ab	168.45 ± 1.72 a	25.13 ± 0.36 b	16.08 ± 0.23 a	2695.98 ± 20.37 a
	N3	87.03 ± 0.71 a	72.96 ± 0.39 a	171.21 ± 0.94 a	26.34 ± 0.27 a	16.04 ± 0.19 a	2726.34 ± 26.81 a

Significant differences among treatments are indicated in different lowercase letters ($P < 0.05$)

Discussion

In the current study, nitrogen fertilizer application combined with straw returning significantly influenced the soil's physicochemical properties, demonstrating complex interactions between management practices and soil processes (Akhtar et al., 2023). Soil pH remained largely stable at neutral to slightly alkaline levels throughout the soybean growth stages. However, a slight decrease in pH was observed under the highest nitrogen rate (N3) by the maturity stage, likely due to intensified nitrification that releases hydrogen ions and thus acidifies the soil (Raza et al., 2020). This observation aligns with reports that long-term excessive nitrogen fertilization can acidify soil and reduce fertility, negatively impacting crop yields (Zhu et al., 2023). Soil electrical conductivity (EC) showed significant increases with higher nitrogen input, indicating an accumulation of soluble salts predominantly nitrate (NO_3^-) and ammonium (NH_4^+) ions from the fertilizer (Huang et al., 2017). Elevated concentrations of these ions raise soil salinity, which may adversely affect soybean growth if salinity exceeds threshold levels (Hu et al., 2023). Such findings underscore the importance of optimizing nitrogen rates to avoid soil salinization and related environmental risks (Cuevas et al., 2019).

Although cation exchange capacity (CEC) did not differ significantly among treatments, it exhibited a consistent increasing trend under higher straw return and nitrogen levels (Xiong et al., 2023). This tendency is likely attributable to greater soil organic matter (SOM) accumulation from straw decomposition, which enhances the soil's ability to retain cations and improves overall nutrient-holding capacity (Xin et al., 2024). Similarly, straw incorporation is documented to improve soil structure and nutrient retention through increased organic matter inputs (Huang et al., 2021). The soil carbon-to-nitrogen (C/N) ratio showed minimal variation during early growth stages but increased significantly by the maturity stage in the treatment with moderate straw return and intermediate nitrogen (T1 + N2). This higher C/N ratio suggests enhanced organic carbon sequestration relative to nitrogen mineralization, an indicator of improved soil health and long-term fertility (Sedlář et al., 2023). Such results are consistent with the notion that balanced nutrient cycling is critical for sustainable soil management (Lynch, 2015).

Nitrogen, phosphorus, and potassium nutrient dynamics in the soil were distinctly affected by the straw–nitrogen treatments. Total nitrogen (TN) was notably elevated under the combination of high straw return with intermediate nitrogen (T2 + N2), underscoring how straw-derived organic nitrogen and improved microbial activity contribute to greater nitrogen retention in soil. Meanwhile, changes in soil $\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$ reflected the complex transformation pathways of nitrogen (mineralization, nitrification, immobilization) modulated by straw incorporation (Grzyb et al., 2021). These observations are in line with literature showing that straw returning can significantly alter soil nitrogen cycling dynamics and potentially improve nitrogen use efficiency in agro-ecosystems (Breza and Grandy, 2025). Soil phosphorus behavior was more variable. Total phosphorus (TP) remained relatively stable in the no-straw (T0) and high-straw (T2) treatments, but under moderate straw returning (T1) we observed that higher nitrogen inputs led to a transient increase in TP at the seedling stage (likely due to added fertilizer P and enhanced mineralization). By the maturity stage, however, TP in moderate-N plots had decreased below the level of the N0, presumably because better soybean growth with adequate N resulted in greater P uptake from the soil. In contrast, available phosphorus (AP) was more responsive to nitrogen fertilization: at early growth stages, higher N levels (e.g., N3) significantly increased AP in both T0 and T1 treatments, indicating that sufficient nitrogen can stimulate microbial activity and phosphatase enzymes to release more plant-available P. Even by maturity, moderate nitrogen (N2) treatments often maintained higher AP than the N0 control, highlighting that balanced N supply helps sustain P availability throughout the season. These N–P interactions suggest that judicious nitrogen management is important for maintaining phosphorus availability, supported by evidence that long-term straw return practices enhance soil phosphorus cycling via activated microbial processes (Xu et al., 2025). Potassium (K) dynamics were also influenced by the treatments. Soil total potassium (TK) tended to decline with increasing nitrogen rates, possibly due to intensified crop uptake of K when abundant N stimulates vigorous soybean growth (Zhang et al., 2021). Nevertheless, significantly higher TK and available K (AK) were observed under the high straw return treatment (T2) by the end of the season, reflecting potassium release from decomposing straw residues and microbial mineralization of organic K sources (Li et al., 2021). This straw-mediated K replenishment helps maintain the soil's K balance and ensures sustained availability of this nutrient for crop uptake. Overall, the combined straw returning and nitrogen regimen markedly affected soil N, P, and K status, indicating that integrating organic residue returns with optimized fertilizer N can improve soil nutrient availability while mitigating risks of nutrient depletion or excess.

The observed variations in soil enzyme activities under different treatments indicate that straw returning and nitrogen fertilization jointly influence microbially mediated nutrient cycling. In our experiment, invertase (sucrase) activity showed distinct responses depending on the straw input level and growth stage. Under no straw incorporation (T0), invertase activity increased significantly with higher nitrogen levels (particularly at N2 and N3) during the flowering stage, whereas under high straw return (T2), invertase activity did not change significantly across nitrogen levels at any growth stage. These contrasting patterns suggest that moderate straw inputs together with sufficient nitrogen created a soil environment favorable for sugar-decomposing microorganisms (leading to enhanced invertase activity in T0 with N fertilization), while excessive straw in T2 may have altered microbial community composition or substrate availability, resulting in a dampened invertase response. Consistent with this

interpretation, previous research has noted that optimal organic matter inputs can improve microbial activity and diversity, whereas either insufficient or excessive organic amendments can reduce the efficiency of carbohydrate-degrading microbes (Oren and Hallsworth, 2014). Alkaline phosphatase activity, an enzyme related to phosphorus cycling, was notably heightened by moderate nitrogen addition (N2) under each straw return level. For example, phosphatase activity increased significantly at the N2 rate in T0, T1, and T2 treatments (across various growth stages), indicating that a proper N supply can stimulate microbial populations capable of producing phosphatases by providing the nitrogen needed to support P mineralization processes (Zhu et al., 2018). Moreover, the consistently higher phosphatase activity observed under the high-straw with moderate-N treatment (T2 + N2) suggests that substantial straw inputs by elevating SOM and improving microbial habitat – further boosted the potential for phosphorus mineralization (Tian et al., 2020). These results concur with recent studies showing that balanced nitrogen fertilization combined with straw returning enhances soil phosphatase activity through promoting active microbial communities and steady nutrient availability (Xu et al., 2025).

Urease activity, a key indicator of nitrogen cycling, also responded strongly to the treatments. Under moderate straw return (T1), urease activity rose progressively with higher nitrogen levels, with a significant increase evident as early as the seedling stage at the highest N rate (N3). In contrast, under no straw (T0), a significant increase in urease was only observed at the highest N level, and only at the late growth stage (maturity), implying that without the additional carbon from straw, much more nitrogen input was required to stimulate the microbes that produce urease. Adequate straw incorporation likely provides readily available carbon sources that fuel urease-producing microorganisms, thereby promoting nitrogen mineralization and availability (Xie et al., 2021). On the other hand, extremely high or zero straw inputs might shift the soil C/N balance in ways that inhibit those microbial groups, which could explain the lower-than-expected urease activity under such imbalanced conditions (Gupta et al., 2022). Taken together, our findings indicate that moderate to high nitrogen levels (e.g., N2 and N3) effectively enhanced the activities of invertase, phosphatase, and urease, with straw returning playing a nuanced role in modulating substrate availability and microbial community structure (Liu et al., 2023). Comparisons with other studies confirm that the optimal combination of straw return and nitrogen fertilization can significantly stimulate these key enzyme activities, thereby improving soil nutrient cycling and fertility (Zheng et al., 2023). By contrast, excessive straw additions or imbalanced nitrogen use may offset these benefits by shifting microbial communities toward less efficient functional groups (Martins-Loução et al., 2022). These insights highlight the need for balanced straw–nitrogen management to maximize microbial-mediated nutrient transformations. Future long-term studies should further explore various straw and nitrogen combinations to refine guidelines for sustainable soil fertility and crop production (Kolotylo et al., 2023).

The combined application of nitrogen fertilizer and straw returning also significantly affected soybean yield and yield components. Plant height responded notably to the treatments, with the tallest plants observed under the high straw return regime (T2) at an intermediate nitrogen level (N1). This outcome suggests an interaction where moderate nitrogen availability, coupled with the improved soil organic matter and nutrient cycling from straw incorporation, promotes soybean vegetative growth by enhancing nutrient uptake efficiency and soil structure (Yu et al., 2017). Consistent with this, previous

studies have reported that balanced nitrogen management together with organic amendments can improve crop growth by increasing nutrient availability and fostering beneficial microbial activity in the rhizosphere (Bhunja et al., 2021). We also found a significant increase in pods per plant with greater straw returns. In particular, the high straw treatment (T2) produced more pods per plant than lower straw treatments, reflecting improved reproductive development due to enhanced soil fertility, moisture retention, and nutrient supply from the returned straw (Tang et al., 2023). Under the highest input treatment (T2 + N3), abundant nitrogen availability likely further promoted flower initiation and pod formation, thereby maximizing pod number through better nutrient allocation to reproductive structures (Mathan et al., 2016). Similar findings in other studies indicate that appropriate nitrogen application combined with organic residue amendments can substantially increase crop yield potential by providing sustained nutrient release and favorable soil conditions during the reproductive growth phase (Shah and Wu, 2019).

Seeds per plant and seed weight per plant were also markedly higher under the high straw, high nitrogen treatment (T2 + N3) compared to other treatments. These improvements can be attributed to enhanced nitrogen utilization efficiency facilitated by greater soil nutrient availability and vigorous microbial activity resulting from straw decomposition (Chen et al., 2021). The elevated activities of soil enzymes such as urease and phosphatase in the T2 + N3 treatment likely contributed to more efficient nutrient cycling, supporting higher nutrient uptake by soybean and better seed development (Bello, 2021). This outcome is in agreement with reports that integrating nitrogen fertilization with organic amendments significantly boosts crop productivity by optimizing nutrient bioavailability and promoting efficient nutrient uptake (Oyetunji et al., 2022). Hundred-seed weight showed relatively small differences among treatments, but there was a slight yet significant increase under the T2 + N3 regime, underscoring the benefit of combined high straw and optimal N in enhancing grain filling. This improvement in seed weight could be related to greater photosynthate availability during grain filling, supported by the improved soil fertility and microbial activity under straw return with adequate nitrogen (Gao et al., 2023). Previous studies have similarly noted that proper nitrogen supply coupled with organic amendments can positively influence grain quality and weight by improving photosynthetic efficiency and nutrient availability during critical growth periods (Kubar et al., 2022). Consequently, the T2 + N3 treatment achieved a substantially higher final yield than other treatments, emphasizing the synergistic effects of intensive straw returning and high-rate nitrogen fertilization. This combined approach optimized nutrient availability, enhanced microbial-mediated nutrient cycling, and improved soil structural conditions, all contributing to the highest soybean yield in our study (Han et al., 2025). The pronounced yield gains under T2 + N3 were supported by the elevated soil enzyme activities (particularly alkaline phosphatase and urease) observed in this treatment, which would facilitate greater nutrient transformation and uptake, ultimately translating into superior crop performance (Heidari et al., 2016). These findings are corroborated by other research showing that optimized nitrogen application together with straw incorporation significantly improves yields by positively influencing soil microbial processes and nutrient dynamics (Zhou et al., 2025). Overall, our results highlight the significant interactive effects between nitrogen fertilization and straw returning on soybean growth, yield, and soil quality. The optimal combination in this experiment high straw return with a sufficiently high nitrogen rate consistently produced the best

outcomes, suggesting that this integrated nutrient management strategy can sustainably enhance soil fertility, nutrient use efficiency, and soybean productivity.

Conclusion

This study demonstrated that the combined application of straw returning and nitrogen fertilization significantly influenced soil physicochemical properties, enzyme activities, and soybean agronomic traits in the black soil region of Northeast China. The treatment with 6000 kg·ha⁻¹ straw returning (T2) and 135 kg·ha⁻¹ nitrogen application (N3) notably improved soil total nitrogen (TN) and available potassium (AK), while enhancing enzyme activities such as alkaline phosphatase and urease, thereby creating favorable nutrient conditions for crop growth. Compared to the control (T0 + N0), this integrated strategy significantly increased the number of pods per plant, seeds per plant, seed weight per plant, and 100-seed weight, ultimately achieving the highest yield (2726.34 kg·ha⁻¹). The results further revealed that different combinations of fertilization and straw returning affected yield components differently, with pod number being more responsive to straw incorporation, and seed number and seed weight being more influenced by nitrogen rates. In conclusion, integrating straw returning with appropriate nitrogen fertilization significantly improves soil nutrient status and microbial enzymatic functions, optimizes soybean yield components, and offers a sustainable approach for achieving high efficiency and environmentally friendly agricultural production.

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