Biological Factors Affecting Soil Nitrogen Transformation in Shajiang Black Soil Wheat Fields and Their Response to Nitrogen Supply: Postprint

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Abstract

Shajiang black soil is a typical low-to-medium yield soil type in China, and studying the nitrogen transformation processes and mechanisms driven by soil microorganisms can provide a scientific basis for directionally regulating soil nitrogen transformation, improving nitrogen use efficiency, and reducing its negative effects. The experiment was set up with four nitrogen supply rates: 0 kg · hm ^{2}, 120 kg · hm ^{2}, 225 kg · hm ^{2}, and 330 kg · hm ^{2}. Measurements were taken at the winter wheat overwintering, jointing, heading, flowering, grain-filling, and maturity stages to determine the intensity of nitrogen transformation-related microbial activities (ammonification, nitrification, and denitrification) and the activities of soil nitrogen transformation-related enzymes (urease, protease) in wheat rhizosphere soil, as well as soil net nitrogen mineralization rate and changes in soil nitrate nitrogen and ammonium nitrogen contents, thereby investigating the biological factors influencing soil nitrogen transformation in Shajiang black soil wheat fields and their responses to different nitrogen supply rates. The results showed that the active period for soil nitrogen-transforming microorganisms and enzymes was from jointing to grain-filling, after which the intensity of soil ammonification and nitrification, as well as urease and protease activities, decreased; soil net nitrogen mineralization rate was largely consistent with the active period of microbial and enzyme activities related to soil nitrogen transformation, reaching its maximum around the flowering stage. Except for urease activity, which continued to increase with increasing nitrogen supply, the intensity of soil nitrogen-transforming microbial activity and protease activity both increased with nitrogen supply, reaching their maximum under the 225 kg · hm ^{2} treatment; further increasing nitrogen supply to 330 kg · hm ^{2} resulted in varying degrees of decline in microbial activity intensity and enzyme activity. It can be seen that the active period of nitrogen transformation in Shajiang black soil was basically consistent with the peak nitrogen demand period of wheat, which is beneficial for winter wheat growth. However, due to the low intensity of soil nitrification in Shajiang black soil, the soil nitrification capacity is limited, thereby reducing nitrogen availability and increasing the potential risk of soil ammonia volatilization loss. Increasing nitrogen supply within a certain range is beneficial for soil nitrogen transformation, but excessive nitrogen supply (330 kg · hm ^{2}) is not conducive to improving the nitrogen supply capacity of Shajiang black soil.

Full Text

Biological Factors Influencing Nitrogen Transformation in Wheat Fields of Lime Concretion Black Soils and Their Response to Different Nitrogen Supplications

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Abstract: Lime concretion black soil is a typical low-yield field soil in China, characterized by heavy clay structure and poor permeability, which cause imbalances in effective nutrient supply, low soil nutrient supply capacity, and poor production performance. To improve crop yields, chemical fertilizer (especially nitrogen fertilizer) has been excessively applied during production seasons, leading to wastage of agricultural resources and environmental pollution. Soil microbes have always played a predominant role in the processes of soil nitrogen transformation. To provide a scientific basis for directional adjustments to control soil nitrogen transformation processes, improve nitrogen use efficiency, and reduce related negative effects, this study investigated the processes and mechanisms of nitrogen transformation driven by soil microorganisms. A field experiment was carried out from 2012 to 2015 in Xiangcheng, Henan Province, China, using a single factorial design with four nitrogen rates (0 kg · hm ^{2}, 120 kg · hm ^{2}, 225 kg · hm ^{2}, and 330 kg · hm ^{2}). The biochemical action intensity of soil nitrogen transformation microorganisms (ammonification, nitrification, and denitrification), urease activity, protease activity, net nitrogen mineralization rate, and contents of nitrate and ammonium nitrogen in rhizosphere soil were determined at different wheat growth stages to explore the biological factors influencing nitrogen transformation and their response to different nitrogen application rates in wheat fields of lime concretion black soils.

The results showed that the active period of soil nitrogen transformation microorganisms and enzymes was from the jointing stage to the grain-filling stage. After that, ammonification intensity, nitrification intensity, urease activity, and protease activity decreased. Similarly, the soil net nitrogen mineralization rate reached its highest level at the flowering stage. Except for urease activity, which increased with increasing nitrogen application, the intensity of soil nitrogen transformation microorganisms and enzyme activities reached their highest point under the 225 kg · hm ^{2} nitrogen treatment and then decreased with further increases in nitrogen application (330 kg · hm ^{2}). Consistent with dynamic changes in soil nitrogen transformation microbes and enzyme activities, the contents of soil ammonium and nitrate reached their highest points at the heading stage and flowering stage, respectively. Under moderate nitrogen application conditions, soil ammonium content showed an increasing trend, but under excess nitrogen application, there was no significant enhancement in soil nitrate content. It was clear that the active period of soil nitrogen transformation was consistent with the critical period of nitrogen demand for wheat, which was beneficial for winter wheat growth. However, due to low nitrifying bacteria activity, nitrification capacity was limited, which reduced nitrogen availability and increased the potential risk of ammonia volatilization from soil. Increased nitrogen application was beneficial for soil nitrogen transformation, but only within a certain range. Excess nitrogen application (330 kg · hm ^{2}) was not conducive to improving the capacity of soil nitrogen supply or release in lime concretion black soil.

Keywords: Lime concretion black soil; Wheat; Nitrogen transformation; Microorganism; Enzyme; Nitrogen mineralization

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1. Materials and Methods

1.1 Experimental Site and Design

The experiment was conducted during the winter wheat growing seasons from 2012 to 2015 in Molin Town, Xiangcheng City, Henan Province (114.25°E, 33.13°N). The experimental site is located in the transitional zone of the Huang-Huai alluvial plain, with a climate transitioning from subtropical to warm temperate. The soil type is lime concretion black soil, with nutrient contents in the plow layer as follows: organic matter 13.1 g · kg ^{1}, total nitrogen 0.85 g · kg ^{1}, ammonium nitrogen 4.83 mg · kg ^{1}, nitrate nitrogen 4.26 mg · kg ^{1}, available phosphorus 21.52 mg · kg ^{1}, available potassium 86.7 mg · kg ^{1}, and pH 7.26. Dynamic changes in air temperature and soil temperature during the winter wheat growing season are shown in [Figure 1: see original paper].

Four nitrogen application levels were established: N0, N120, N225, and N330, corresponding to pure nitrogen applications of 0 kg · hm ^{2}, 120 kg · hm ^{2}, 225 kg · hm ^{2}, and 330 kg · hm ^{2}, respectively. A randomized block design was used with a plot area of 225 m^{2} and three replications. Before winter wheat sowing, land preparation was performed using a deep loosening-rotary tillage integrated machine combined with straw returning, with a tillage depth of 35 cm. The sowing rate was 150 kg · hm ^{2} with a row spacing of 20 cm. Nitrogen fertilizer used was urea (46% N content), with 50% applied as basal fertilizer during land preparation and the remaining 50% applied as topdressing at the regreening stage (143 days after sowing) combined with irrigation. Phosphorus fertilizer was applied as calcium superphosphate (14% P O content) at 857 kg·hm ^{2}, and potassium fertilizer was applied as potassium chloride (60% K O content) at 200 kg·hm ^{2}, both as basal applications. Other cultivation measures were uniformly implemented according to local high-yield field management practices.

1.2 Sample Collection and Analysis

1.2.1 Soil Sampling and Processing Soil samples were collected at the over-wintering stage (OWS, 69 days after sowing), jointing stage (JS, 156 days after sowing), heading stage (HS, 187 days after sowing), flowering stage (FS, 199 days after sowing), grain-filling stage (GFS, 214 days after sowing), and maturity stage (MS, 229 days after sowing). In each plot, the five-point sampling method was used to excavate root-zone soil samples from 0–20 cm depth. After removing the surface 5 cm of floating soil, rhizosphere soil was collected using the method described by [16]. Soil samples from the five points were mixed to form a composite sample, placed in sterile paper bags, stored in a fresh-keeping box, and transported to the laboratory. Fresh soil samples were passed through a 1 mm sieve. One portion was used for determining soil microbial action intensity, ammonium and nitrate nitrogen contents, and soil water content, while the other portion was air-dried for enzyme activity determination.

1.2.2 Determination of Microbial Action Intensity for Soil Nitrogen Transformation **Ammonification intensity** was determined using the soil incubation method [17]. Soil samples were incubated at 28°C for 10 days, and NH -N content was measured using the Nessler' s reagent method. The increase in NH -N content represented ammonification intensity.

Nitrification intensity was determined using the medium inoculation method [17]. Soil suspensions were incubated at 28°C for 15 days, and NO content was measured using the Griess reagent colorimetric method. The decrease in nitrite nitrogen in the sterilized medium after adding soil suspension represented nitrification intensity.

Denitrification intensity was determined using the phenol disulfonic acid colorimetric method [17]. Samples were placed in a vacuum desiccator, vacuumed, and incubated at 28°C for 2 days. NO -N content was measured using the phenol disulfonic acid colorimetric method, and the decrease in NO -N represented denitrification intensity.

1.2.3 Determination of Rhizosphere Soil Urease and Protease Activities Urease activity was determined using the phenol-sodium colorimetric method [17]. Protease activity was determined using the ninhydrin colorimetric method [17].

1.2.4 Determination of Net Nitrogen Mineralization Net nitrogen mineralization was determined using the in-situ soil incubation method [18]. Two PVC tubes (4 cm diameter, 15 cm length) were inserted 10 cm into the soil in each plot. One tube was brought back to the laboratory for soil analysis, while the other was left in the plot for in-situ incubation with anion exchange resin bags placed at the bottom and top. Fresh soil samples from the PVC tubes before and after incubation were passed through a 1 mm sieve. Ten grams of soil were extracted with 100 mL of 0.01 mol·L ^{1} CaCl , and ammonium and nitrate nitrogen contents were determined using an automatic continuous flow analyzer. Anion exchange resin bags were extracted with 50 mL of 0.01 mol·L ^{1} CaCl solution, and ammonium and nitrate nitrogen contents were also determined. Three replicates were randomly set up in each plot, with incubation starting on the wheat sowing date and sampling every two weeks (with one sampling during the over-wintering period, 100-138 days after sowing, and intensified sampling from regreening to jointing stage).

1.2.5 Determination of Rhizosphere Soil Ammonium and Nitrate Nitrogen Contents Fresh soil samples passed through a sieve were extracted with 0.01 mol·L ^{1} CaCl , and ammonium and nitrate nitrogen contents were determined using an automatic continuous flow analyzer (AA3, SEAL Analytical, Germany).

1.2.6 Data Processing The net nitrogen mineralization rate was calculated as the difference in inorganic nitrogen before and after incubation. The net nitrogen mineralization rate [R, g(N)·g ^{1}·d ^{1}] was calculated using the formula:

$$R = \frac{(Pm_1 + Bm) - Pm_0}{T}$$

where Pm_1 represents the soil inorganic nitrogen (NH -N + NO -N) content after in-situ incubation, Pm_0 represents the initial soil inorganic nitrogen (NH -N + NO -N) content, Bm represents the inorganic nitrogen (NH -N + NO -N) content in the anion exchange resin bag, and T represents the incubation time.

Data processing and graphing were performed using Microsoft Office 2010, and statistical analysis was conducted using SPSS 20.0.

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2. Results

2.1 Dynamics of Microbial Action Intensity for Nitrogen Transformation in Wheat Rhizosphere Soil of Lime Concretion Black Soil and Its Response to Nitrogen Application

As shown in [Figure 2: see original paper]A, ammonification intensity in wheat rhizosphere soil exhibited an inverted "V" shaped pattern during the growth period, with the peak occurring at the flowering stage. Among different nitrogen application rates, ammonification intensity at each growth stage followed the order N225 > N330 > N120 > N0, except at the over-wintering and heading stages. Before the flowering stage, there was no significant difference between N225 and N330, but after flowering, ammonification intensity under N225 was significantly higher ($P < 0.05$) than under other nitrogen rates.

Nitrification intensity showed a similar dynamic pattern to ammonification intensity, increasing initially and then decreasing from the over-wintering stage to the maturity stage, with the highest intensity also at the flowering stage. Before the flowering stage, nitrification intensity increased exponentially ($y = 52.115e^{\cdot 2}$, $R^2 = 0.9625$, where x represents wheat growth stage and y represents nitrification intensity). Among different nitrogen treatments, nitrification intensity increased significantly ($P < 0.05$) at each growth stage as nitrogen application increased from N0 to N225. Particularly at the jointing, heading, and grain-filling stages, differences among N225, N120, and N0 treatments were extremely significant ($P < 0.01$). However, differences between N330 and N225 were mostly not significant (except at the over-wintering stage where N330 was significantly higher than N225, and at the heading stage where N225 was significantly higher than N330).

Denitrification intensity in wheat rhizosphere soil of lime concretion black soil was relatively low, ranging from 54.9 to 74.2 $g \cdot g^{1} \cdot d^{1}$. Two active peaks occurred during the wheat growth period at the jointing stage and maturity stage, with the jointing stage peak being more pronounced. Nitrogen application rate significantly affected denitrification intensity at each growth stage ($P < 0.05$), following the order N225 > N330 > N120 > N0. Similar to nitrification intensity, denitrification intensity increased significantly as nitrogen application increased from N0 to N225, but decreased when nitrogen application increased further from N225 to N330.

2.2 Dynamics of Soil Enzyme Activities in Wheat Rhizosphere Soil of Lime Concretion Black Soil and Their Response to Nitrogen Application

As shown in [Figure 3: see original paper]A, urease activity in wheat rhizosphere soil of lime concretion black soil varied dramatically during the growth period, being lowest at the over-wintering stage, increasing rapidly to the highest level of the entire growth period at the jointing stage, and then gradually decreasing as the growth period progressed.

The effect of nitrogen application on urease activity was significant from the jointing stage to the grain-filling stage, following the order N330 > N225 > N120 > N0 (except at the heading stage where N225 was slightly higher than N330), with significant differences among nitrogen treatments ($P < 0.05$). At the maturity stage, however, differences among the three nitrogen application treatments (excluding N0) were mostly not significant.

Protease activity in wheat rhizosphere soil of lime concretion black soil ranged from 178.7 to 320.9 $g \cdot g^{1} \cdot d^{1}$ during the growth period, with the active peak period from the jointing stage to the flowering stage. After the flowering stage, protease activity decreased rapidly, reaching its lowest level at the maturity stage.

Among different nitrogen application rates, nitrogen treatments significantly affected protease activity at each growth stage (except at the over-wintering stage), following the order N0 < N120 < N225, with significant differences among treatments ($P < 0.05$). However, when nitrogen application increased further from N225 to N330, protease activity at each growth stage decreased significantly.

2.3 Comprehensive Effects of Nitrogen Application on Microbial Action Intensity and Enzyme Activities for Nitrogen Transformation

As shown in , increasing nitrogen application within a certain range (N0–N225) significantly promoted microbial action intensity and enzyme activities for nitrogen transformation. When nitrogen application increased to N330, urease activity continued to increase, nitrification intensity remained basically at the N225 treatment level, while ammonification intensity, denitrification intensity, and protease activity were inhibited to varying degrees.

2.4 Dynamics of Net Nitrogen Mineralization Rate in Wheat Fields of Lime Concretion Black Soil and Its Response to Nitrogen Application

Throughout the winter wheat growing season, the net nitrogen mineralization rate in lime concretion black soil varied significantly, showing two peaks and one trough as the growth period progressed. The first small peak occurred in mid-November (28 days after sowing), with a net nitrogen mineralization rate of 0.86–1.05 $g \cdot g^{1} \cdot d^{1}$. The rate then gradually decreased, reaching its lowest point at the over-wintering stage (70 days after sowing) and remaining stagnant until before the regreening stage (138 days after sowing). It then gradually increased, reaching its highest peak at the heading to flowering stage (182–199 days after sowing), which was 1.7 times that of the pre-winter peak [Figure 4: see original paper].

Among different nitrogen application rates, increasing nitrogen application from N0 to N225 significantly promoted the net nitrogen mineralization rate at most sampling times. However, when nitrogen application increased further to N330, the net nitrogen mineralization rate no longer increased at most sampling times, and its activity showed varying degrees of reduction.

2.5 Dynamics of Ammonium and Nitrate Nitrogen Contents in Lime Concretion Black Soil and Their Response to Nitrogen Application

As shown in , as the wheat growth period progressed, soil NH -N + NO -N, NO -N, and inorganic nitrogen (NH -N + NO -N) contents all showed an initial increase followed by a decrease, but the timing of maximum values differed. Specifically, NH -N content peaked at the heading stage, NO -N content peaked at the flowering stage, and inorganic nitrogen content remained relatively high at both the heading and flowering stages.

With increasing nitrogen application, soil NH -N content at each growth stage mostly increased significantly. The response of NO -N content to nitrogen application was similar to that of NH -N, but there was often no significant difference between N225 and N330 treatments. Inorganic nitrogen (NH -N + NO -N) content showed no difference between N225 and N330 treatments at the over-wintering, jointing, and grain-filling stages, but significant differences among treatments at other stages.

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3. Discussion

3.1 Characteristics of Microbial and Enzyme Action Intensity and Nitrogen Transformation in Wheat Fields of Lime Concretion Black Soil

More than 90% of nitrogen in soil exists in organic form, which must be mineralized into inorganic nitrogen before it can be absorbed and utilized by plants [19-20]. Soil nitrogen mineralization is a microbial-driven biochemical process in which soil nitrogen transformation microorganisms and enzymes play important roles. Specifically, urease, protease, and ammonification convert organic nitrogen into NH , nitrification converts ammonia (or ammonium) in soil into nitrate, and denitrification converts nitrate and other complex nitrogen compounds into N , NO, and N O [5,21]. The results of this experiment showed that ammonification intensity, nitrification intensity, and urease and protease activities in lime concretion black soil wheat fields all showed an initial increase followed by a decrease during the entire wheat growth period, with active peak periods mostly occurring from the jointing stage to the grain-filling stage. The increase in ammonification intensity, urease, and protease activities promoted the conversion of organic nitrogen to NH , increasing soil NH -N content. Since NH -N is the oxidative substrate required for nitrification [5], the increase in ammonification intensity, urease, and protease activities promoted nitrification intensity. From the jointing stage to the flowering stage, soil NH -N and NO -N contents gradually increased under the action of soil nitrogen transformation microorganisms and related enzymes. However, as wheat absorbed nitrogen and soil ammonification intensity, nitrification intensity, urease and protease activities decreased, and denitrification intensity increased, soil NH -N and NO -N contents decreased during the late wheat growth period. This is consistent with the research results of Wang et al. [22] on yellow fluvo-aquic soil. However, in this experiment, urease activity decreased slowly and showed no rebound at the winter wheat maturity stage, which may be related to changes in the physical properties of lime concretion black soil such as water, air, and heat. Due to the heavy texture, poor structure, and low permeability of lime concretion black soil, carbon and energy input from root exudates and root litter that can be utilized by soil microorganisms [23] turned over slowly, while soil nitrogen availability decreased, inhibiting soil microbial and enzyme activities. Compared with the research results of Wang et al. [22] on fluvo-aquic soil, ammonification intensity in wheat rhizosphere soil of lime concretion black soil was greater than that in fluvo-aquic soil after wheat entered the jointing stage, but its nitrification intensity, denitrification intensity, and urease and protease activities were significantly lower than those in fluvo-aquic soil. This is consistent with the research results of Wang et al. [24]. However, the specific amount of nitrogen loss caused by ammonia volatilization and denitrification needs to be further determined through collection and measurement of corresponding gases.

The mineralization process is an important link in soil nitrogen transformation. The results of this experiment showed that the net nitrogen mineralization rate in lime concretion black soil wheat fields fluctuated regularly, with two mineralization peaks occurring in the pre-winter period and the heading-flowering stage, and almost no mineralization occurring during the two-month over-wintering period (70–138 days after sowing). The reasons may include: (1) the influence of soil temperature [25-26]. The fluctuation of net nitrogen mineralization rate was consistent with the change in soil temperature during the winter wheat growth period, with higher temperatures in the pre-winter period and heading-flowering stage, and the lowest temperature during the over-wintering stage. (2) The influence of microbial and enzyme activities. In this experiment, the active periods of ammonifying bacteria, nitrifying bacteria, urease, and protease coincided with the peak periods of net nitrogen mineralization rate.

3.2 Dynamics of Nitrogen Transformation in Wheat Rhizosphere of Lime Concretion Black Soil in Response to Different Nitrogen Application Rates

Sarathchandra et al. [27] believed that fertilization could increase soil microbial quantity and diversity. Other studies have indicated that high nitrogen availability would decrease rhizosphere microbial quantity and activity [28]. Soil microorganisms participate in material transformation in soil and affect soil fertility status, while soil microbial activity is influenced by soil nutrients and soil texture [29]. The results of this experiment showed that compared with no nitrogen application, nitrogen fertilizer application significantly increased soil ammonium and nitrate nitrogen contents. As nitrogen application increased, soil ammonium nitrogen content gradually increased, with this trend being particularly evident from the heading stage to the maturity stage. Nitrate nitrogen content increased with nitrogen application in the range of 0–225 kg · hm ^{2}, but when nitrogen application increased to 330 kg · hm ^{2}, the effect on increasing soil nitrate nitrogen content was no longer significant. This is closely related to the action of soil nitrogen transformation-related microorganisms and enzymes. In this experiment, the increase in urea supply directly provided substrates for urease, thereby stimulating urease activity, which showed a significant enhancement with increasing nitrogen application. Ammonification intensity, nitrification intensity, denitrification intensity, and protease activity were positively correlated with nitrogen application in the range of 0–225 kg · hm ^{2}. However, when nitrogen application increased further to 330 kg · hm ^{2}, all these parameters showed varying degrees of decline. This is mainly because under conditions of high nitrogen availability, rhizosphere microorganisms do not need to obtain nitrogen by decomposing difficult-to-utilize soil organic matter, reducing the secretion of extracellular enzymes for organic matter decomposition. Meanwhile, high nitrogen availability also reduces plant carbon allocation belowground, decreasing rhizosphere microbial quantity and activity. The reduction in rhizosphere microbial biomass, decreased extracellular enzyme secretion, and preferential utilization of root exudates by microorganisms all inhibited soil mineralization [31].

In summary, nitrogen transformation in lime concretion black soil wheat fields is a biochemical process participated in by soil nitrogen transformation microorganisms and is closely related to soil nitrogen supply capacity. The active peak period of nitrogen transformation microorganisms and enzymes in lime concretion black soil and the net nitrogen mineralization rate in wheat fields were consistent with the critical nitrogen demand period for winter wheat, mostly occurring from the jointing stage to the grain-filling stage, which is beneficial for wheat growth. However, in lime concretion black soil, low nitrification intensity is not conducive to further nitrogen transformation, reducing its availability and increasing the potential risk of soil ammonia volatilization loss. Within the nitrogen application range of 0–225 kg · hm ^{2}, increasing nitrogen application enhanced the action intensity of soil nitrogen transformation microorganisms and related enzyme activities, promoted soil nitrogen mineralization, and improved soil nitrogen supply capacity. However, when nitrogen application reached 330 kg · hm ^{2}, soil nitrogen transformation microorganisms and related enzyme activities were mostly inhibited, net nitrogen mineralization rate also decreased to some extent, and the risk of nitrogen loss in soil increased, which is not conducive to efficient nitrogen fertilizer utilization.

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