

Effects of Different Nitrogen Application Rates and Planting Density on Nitrogen Accumulation and Translocation in Wheat at Anthesis: Post-print

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Abstract

This study used wheat cultivar ‘Zhoumai 22’ as material to investigate nitrogen content and translocation characteristics in various organs with different spatial distributions in the aboveground parts of wheat plants under different nitrogen application rates [0 kg(N) hm², 120 kg(N) hm², 240 kg(N) hm², and 360 kg(N) hm², denoted as N0, N1, N2, and N3] and planting densities (225×10⁴basicseedlings hm², 375×10⁴basicseedlings hm², and 525×10⁴basicseedlings hm², denoted as M1, M2, and M3) at anthesis. Nitrogen translocation, and contribution rate to grain nitrogen in various aboveground vegetative organs were investigated. The results showed that 225×10⁴basic seedlings hm² was relatively suitable for wheat production in the Huang-Huai wheat-maize double cropping region.

Full Text

Effects of Different Nitrogen Application Amounts and Planting Densities on Nitrogen Accumulation and Translocation in Winter Wheat at Anthesis

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Abstract

Using winter wheat cultivar ‘Zhoumai 22’ as experimental material, this study investigated nitrogen contents and translocation characteristics in various organs distributed across different spatial positions of wheat plants under different nitrogen application rates [0 kg(N) · hm⁻², 120 kg(N) · hm⁻², 240 kg(N) · hm⁻²,

and $360 \text{ kg(N)} \cdot \text{hm}^{-2}$, designated as N0, N1, N2, and N3] and planting densities [225×10^4 basic seedlings $\cdot \text{hm}^{-2}$, 375×10^4 basic seedlings $\cdot \text{hm}^{-2}$, and 525×10^4 basic seedlings $\cdot \text{hm}^{-2}$, designated as M1, M2, and M3].

The results showed that nitrogen application amount, planting density, and their interaction had significant effects on nitrogen contents in aboveground organs at both anthesis and maturity stages. Nitrogen content and accumulation in vegetative organs decreased from anthesis to maturity. Individual plant nitrogen accumulation ranged from $7.27\text{--}59.65 \text{ mg} \cdot \text{stem}^{-1}$ at anthesis and $8.48\text{--}60.83 \text{ mg} \cdot \text{stem}^{-1}$ at maturity, with the lowest value observed in the N0M3 treatment and the highest in N3M2. Spatially, nitrogen content, accumulation, post-anthesis translocation, and contribution rate to grain nitrogen all decreased with lower spatial positions in the plant canopy.

Nitrogen content, accumulation, and translocation in vegetative organs increased with nitrogen application rate, with nitrogen translocation rates exceeding 50% in upper and middle canopy organs. The total contribution rate of vegetative organs to grain nitrogen exceeded 67%. Increased nitrogen fertilizer combined with appropriate planting density promoted nitrogen accumulation and translocation in aboveground vegetative organs, particularly in lower canopy organs. Under high nitrogen with medium density (N3M2), nitrogen content and accumulation in the fourth leaf from the top, fourth internode, and remaining leaves and internodes increased, narrowing the differences with upper organs. Population-level nitrogen translocation ranged from $28.56\text{--}549.49 \text{ kg} \cdot \text{hm}^{-2}$ and also increased with nitrogen application, with higher translocation observed in spikes and internodes. Nitrogen application significantly affected grain yield, protein content, and protein yield, while the interaction between nitrogen and density significantly influenced grain protein content and yield, and planting density alone significantly affected protein yield. Based on nitrogen translocation and yield performance, applying nitrogen at $240 \text{ kg} \cdot \text{hm}^{-2}$ with a planting density of 225×10^4 basic seedlings $\cdot \text{hm}^{-2}$ represents a suitable cultivation model for wheat production in the Huanghuai wheat-maize double-cropping region.

Keywords: Wheat; Nitrogen application; Planting density; Nitrogen accumulation; Aboveground organs; Spatial distribution; Yield

Nitrogen fertilizer application and planting density are primary management practices for achieving high wheat yields. As an essential nutrient, nitrogen directly participates in organ development and various physiological and biochemical processes. Previous research has demonstrated that nitrogen assimilation, accumulation, and translocation in wheat vegetative organs are closely related to grain yield and quality, with approximately 20% of grain nitrogen derived from post-anthesis assimilation and 80% translocated from leaves, stems, and glumes after anthesis. Optimizing nitrogen management can promote post-heading dry matter and nitrogen accumulation, facilitate nitrogen translocation to grains,

increase yield and nitrogen use efficiency, and reduce environmental pollution from nitrogen residues. Increasing planting density enhances pre-anthesis nitrogen translocation amount, rate, and contribution to grain nitrogen. In wheat production, nitrogen and density interact, as increasing planting density under appropriate nitrogen reduction conditions facilitates absorption of deep soil nitrogen, reduces soil nitrogen residue, and achieves high yield through improved population photosynthetic performance.

Most current research on wheat nitrogen accumulation under different cultivation practices focuses on the whole plant or individual organs, with limited studies on spatial distribution of nitrogen among organs. Hao et al. investigated effects of nitrogen on vertical distribution of canopy leaf nitrogen in winter wheat under limited irrigation in North China, finding that appropriate nitrogen application ($180\text{--}210\text{ kg}\cdot\text{hm}^{-2}$) increased vertical distribution gradients between leaf layers and promoted nitrogen redistribution within plants. To explore how nitrogen application and planting density affect spatial accumulation and translocation of nitrogen in wheat, this study measured nitrogen contents in organs at different spatial levels at anthesis and maturity under various nitrogen and density treatments, analyzing spatial accumulation and translocation characteristics to provide theoretical guidance for rational fertilization and appropriate planting density to achieve high yield and resource efficiency.

1.1 Experimental Materials and Design

The experiment was conducted during the 2013–2014 growing season at the Xinxiang Comprehensive Experimental Station of the Chinese Academy of Agricultural Sciences (Zhongcao Village, Qiliying Town, Xinxiang County, Henan Province, $35^{\circ}09'\text{N}$, $113^{\circ}45'\text{E}$), using the widely cultivated Huanghuai wheat cultivar ‘Zhoumai 22’. The soil was fluvo-aquic with organic matter content of $15.76\text{ g}\cdot\text{kg}^{-1}$, total nitrogen $0.76\text{ g}\cdot\text{kg}^{-1}$, alkali-hydrolyzable nitrogen $65.74\text{ mg}\cdot\text{kg}^{-1}$, available phosphorus $8.70\text{ mg}\cdot\text{kg}^{-1}$, available potassium $165.86\text{ mg}\cdot\text{kg}^{-1}$, and pH 8.7 in the 0–20 cm layer.

A split-plot design was employed with nitrogen fertilization as the main plot and planting density as the subplot. Nitrogen application rates during the entire growth period were $0\text{ kg(N)}\cdot\text{hm}^{-2}$ (N0), $120\text{ kg(N)}\cdot\text{hm}^{-2}$ (N1), $240\text{ kg(N)}\cdot\text{hm}^{-2}$ (N2), and $360\text{ kg(N)}\cdot\text{hm}^{-2}$ (N3), applied in three splits before sowing, at jointing, and at anthesis in a 5:3.5:1.5 ratio. Planting densities were 225×10^4 basic seedlings $\cdot\text{hm}^{-2}$ (M1), 375×10^4 basic seedlings $\cdot\text{hm}^{-2}$ (M2), and 525×10^4 basic seedlings $\cdot\text{hm}^{-2}$ (M3). Summer maize straw from the previous crop was fully returned to the field. Basal fertilizers of K_2O $90\text{ kg}\cdot\text{hm}^{-2}$ and P_2O_5 $240\text{ kg}\cdot\text{hm}^{-2}$ were applied. Manual furrow sowing was performed on October 11, 2013, with 20 cm row spacing. Each plot measured 19.2 m^2 ($4.8\text{ m}\times 4\text{ m}$) with three replications. Maturity harvest occurred on June 5, 2014.

1.2 Research Methods

At anthesis (April 23) and maturity (June 5), 20 uniform individual stems were selected. Leaves were separated by spatial position into flag leaf, second leaf, third leaf, fourth leaf, and remaining leaves; stems and sheaths into first internode, second internode, third internode, fourth internode, and remaining internodes; and spikes into rachis + glume and grain. Samples were washed, oven-dried to constant weight, and weighed for dry matter mass. Total nitrogen content was determined by semi-micro Kjeldahl digestion after grinding.

1.3 Data Calculation and Analysis

Nitrogen accumulation in samples was calculated based on sample mass and total nitrogen content, and nitrogen translocation amount, translocation rate, and contribution rate to grain nitrogen were calculated for each organ. Data analysis was performed using SPSS 13.0, with multiple comparisons conducted using LSD method. Lowercase letters indicate significant differences at the 0.05 probability level.

2.1 Aboveground Nitrogen Content at Anthesis Under Different Nitrogen and Density Treatments

At anthesis, aboveground organ nitrogen content across different spatial positions ranged from 1.94-52.51 $\text{mg} \cdot \text{g}^{-1}$, with the highest content in the flag leaf, followed by the second and third leaves. Nitrogen content among organs followed the pattern: leaves > rachis + glume > stem + sheath. Spatially, leaf nitrogen content decreased with lower leaf position, showing flag leaf > second leaf > third leaf, with fourth leaf and remaining leaves having lower content. Stem nitrogen content also decreased with lower position, showing first internode > second internode > third internode, with fourth internode and remaining internodes having the lowest content.

Variance analysis indicated that nitrogen rate, planting density, and their interaction had significant effects ($P < 0.05$) on nitrogen content in aboveground organs at anthesis, with nitrogen showing stronger effects. Under fertilization treatments (N1, N2, N3), nitrogen content in all positions was significantly higher than in the N0 treatment ($P < 0.05$). Under M1 density, nitrogen content in rachis + glume, flag leaf, third leaf, fourth leaf, first internode, and second internode showed a “first increase then decrease” trend with increasing nitrogen, peaking at N2. Nitrogen content in second leaf, third internode, fourth internode, and remaining internodes increased with nitrogen rate, with remaining leaves also highest at N3. Under M2 density, remaining leaves and second internode had highest nitrogen content at N3, while other organs peaked at N2. Under M3 density, fourth leaf, remaining leaves, first internode, second internode, and fourth internode had highest nitrogen content at N2, while other organs increased with nitrogen rate and peaked at N3. For lower canopy organs (fourth leaf, remaining leaves, fourth internode, remaining internodes), nitro-

gen content increased more markedly with nitrogen rate, demonstrating that increased nitrogen application effectively enhanced nitrogen absorption in lower leaves and stems, delaying their senescence.

Under N0 treatment, nitrogen content in rachis + glume, leaves (except remaining leaves), and third internode was highest at M1, while remaining leaves, second internode, and remaining internodes peaked at M2. First internode and fourth internode nitrogen content increased with planting density, reaching maximum at M3. Under N1 treatment, fourth internode nitrogen content was highest at M1, while rachis + glume, second leaf, third leaf, remaining leaves, second internode, third internode, and fourth internode peaked at M2. Flag leaf, first internode, and remaining internodes had highest nitrogen content at M3. Under N2 treatment, flag leaf nitrogen content was highest at M1, second internode at M3, and other organs peaked at M2. Under N3 treatment, rachis + glume, flag leaf, second leaf, third leaf, and first internode had highest nitrogen content at M3, while fourth leaf, remaining leaves, second internode, third internode, fourth internode, and remaining leaves were higher at M2. For lower canopy organs (fourth leaf, remaining leaves, fourth internode, remaining internodes) under N2 and N3, nitrogen content was highest at M2, narrowing the gap with upper organs compared to other densities.

2.2 Aboveground Nitrogen Content at Maturity Under Different Nitrogen and Density Treatments

At maturity, aboveground organ nitrogen content ranged from 1.69-28.26 mg · g⁻¹, with grain nitrogen content being the highest. Nitrogen content among organs followed the pattern: grain > leaf > rachis + glume > stem + internode. Spatially, under N0, N1, and N2 treatments, leaf nitrogen content was higher in fourth leaf and remaining leaves, while under N3 treatment, second and third leaves had higher content. Internode nitrogen content was highest in remaining internodes (except under N0M2), with second and third internodes having the lowest content.

Variance analysis showed that nitrogen rate, planting density, and their interaction had significant effects ($P < 0.05$) on nitrogen content in aboveground organs at maturity. For flag leaf and remaining leaves, the effect magnitude was nitrogen > planting density > nitrogen × density interaction. For remaining internodes, the order was nitrogen × density interaction > planting density > nitrogen. For other organs, the pattern was nitrogen > nitrogen × density interaction > planting density. Across nitrogen treatments, aboveground organ nitrogen content increased with nitrogen application rate, reaching higher values under N2 and N3 treatments. Planting density primarily affected nitrogen content in lower canopy remaining leaves and internodes. Under N0 treatment, grain nitrogen content showed no significant differences among densities, while rachis + glume, flag leaf, second leaf, third leaf, first internode, and second internode followed M2 > M1 > M3. Fourth leaf, remaining leaves, third internode, fourth internode, and remaining internodes had highest nitrogen content at

M1. Under N1 treatment, grain, flag leaf, and remaining leaf nitrogen content were significantly higher at M1 and M2 than M3, while rachis + glume, second leaf, third leaf, and fourth leaf peaked at M2, and all internodes were highest at M1. Under N2 treatment, rachis + glume, fourth leaf, first internode, and second internode had highest nitrogen content at M1, while other vegetative organs peaked at M2. Under N3 treatment, grain, rachis + glume, flag leaf, and second leaf had highest nitrogen content at M1, fourth leaf and remaining leaves at M2, and all internodes at M1.

2.3 Aboveground Nitrogen Accumulation at Anthesis and Maturity Under Different Nitrogen and Density Treatments

From anthesis to maturity, total nitrogen accumulation per stem increased while nitrogen accumulation in vegetative organs decreased to varying degrees. At anthesis, individual plant nitrogen accumulation ranged from 7.27–59.63 mg · stem⁻¹, with the lowest in N0M3 and highest in N3M2, followed by N1M2. Under nitrogen treatments, individual plant nitrogen accumulation at anthesis was significantly higher than N0. Organ nitrogen accumulation was higher in rachis + glume and first internode, with leaf and internode accumulation decreasing with lower spatial positions. At maturity, individual plant nitrogen accumulation ranged from 8.48–60.83 mg · stem⁻¹, still lowest in N0M3 and highest in N3M2. Grain had the highest nitrogen accumulation, accounting for 68.30%–76.81% of aboveground nitrogen accumulation, followed by rachis + glume at 3.94%–9.34%. Nitrogen accumulation in internodes from first to fourth decreased with lower positions, while remaining internodes under nitrogen treatments had higher accumulation than upper internodes, second only to the first internode. At maturity, leaf nitrogen accumulation was highest in remaining leaves under N0, N1, and N2 treatments, but lowest in fourth leaf under N3 treatment.

Dividing the plant into upper (flag leaf, first internode, spike), middle (second leaf, second internode, third leaf, third internode), and lower (fourth leaf, fourth internode, remaining leaves, remaining internodes) sections, nitrogen accumulation in all spatial sections under nitrogen treatments was significantly higher than N0. At anthesis, the proportion of nitrogen accumulation in upper, middle, and lower sections was 40%–55%, 30%–40%, and 10%–20%, respectively. Upper and lower section accumulation showed a “first increase then decrease” trend with increasing planting density, peaking at M2. At maturity, the proportions were 80%–90%, 5%–10%, and 5%–10%, respectively. All three sections showed a “first increase then decrease” trend with planting density and an increasing trend with nitrogen rate. The proportion of lower section accumulation was higher at N3 under M1 and M2, but higher at N0 under M3. Middle and upper section proportions were highest at N3 and N2, respectively.

2.4 Aboveground Nitrogen Translocation Under Different Nitrogen and Density Treatments

Nitrogen application rate and planting density regulated nitrogen distribution in plants. From anthesis to maturity, nitrogen translocation in individual aboveground organs decreased with lower spatial positions. Leaves showed higher translocation in flag leaf and second leaf, while internodes had highest translocation in the first internode. Lower canopy organs (remaining leaves, remaining internodes, fourth internode) showed nitrogen input/accumulation under N0 and N1 treatments at some densities, but nitrogen output/translocation under high nitrogen treatments, indicating that nitrogen application significantly promoted nitrogen translocation in lower canopy organs. Organ nitrogen translocation amount increased rapidly then slowly with nitrogen rate, with fertilization treatments (N1, N2, N3) significantly higher than N0 ($P < 0.05$). Across densities, individual organ nitrogen translocation was highest at M1 under N0, N1, and N2, but highest at M2 under high nitrogen (N3).

Nitrogen translocation rates in upper and middle canopy organs exceeded 50%, with nitrogen application promoting organ nitrogen translocation. Lower canopy organ translocation rates increased with nitrogen rate, showing outward translocation of accumulated nitrogen from fourth leaf, fourth internode, and remaining leaves. Planting density affected population-level translocation differently than individual plant translocation. Population aboveground nitrogen translocation ranged from 28.59–549.39 $\text{kg} \cdot \text{hm}^{-2}$. At the population level, translocation was higher in rachis + glume, first internode, and second internode, with internodes > leaves. Population nitrogen translocation increased with nitrogen application. Under N0 and N1, population translocation was significantly higher at M2 than M3 and M1. Under N2 and N3, translocation was significantly lower at M1 than M2 and M3. Among organs, remaining internodes under N0M1, N0M3, N1M1, N1M3, N2M1 and fourth internodes under N0M1 still showed nitrogen accumulation, while other treatments showed nitrogen translocation to other organs.

Organ nitrogen contribution rates to grain ranged from 0.02%–26.10%, with higher rates in rachis + glume and first internode. Total aboveground contribution to grain nitrogen exceeded 67%, highest in N3M2, followed by N1M2, with fertilization treatments higher than N0. Contribution rates decreased with lower spatial positions. Rachis + glume and first internode contribution rates decreased with nitrogen rate, peaking at N1. Leaf contribution rates (except remaining leaves) increased with nitrogen rate, with fertilization treatments (N1, N2, N3) significantly higher than N0 ($P < 0.05$). Remaining internodes and fourth internode contribution rates were higher at N2, while second internode was highest at N0, and other positions peaked at N3. Across densities, contribution rates were highest at M3 under N0, but highest at M2 under fertilization treatments (N1, N2, N3).

2.5 Grain Yield Under Different Nitrogen and Density Treatments

Nitrogen application had significant effects on grain yield ($P < 0.05$), while planting density and nitrogen \times density interaction did not. Grain yield increased with nitrogen rate under N0, N1, and N2 treatments, with slight decrease at N3. Fertilization treatments (N1, N2, N3) increased grain yield by 3.54–5.44 times compared to N0M1, with N2M1 producing the highest yield. Nitrogen rate and nitrogen \times density interaction significantly affected grain protein content, with effect magnitude: nitrogen $>$ nitrogen \times density $>$ planting density. Nitrogen rate, planting density, and their interaction all significantly affected grain protein yield ($P < 0.05$), with effect magnitude: nitrogen $>$ planting density $>$ nitrogen \times density. Grain protein content and protein yield under nitrogen treatments (N3, N2, N1) were significantly higher than N0, increasing with nitrogen rate within N1 and N2 range. At N3, protein content continued increasing while protein yield decreased. Under N0, protein yield showed no significant differences among densities, while under fertilization treatments (N1, N2, N3), protein yield was highest at low planting density (M1).

3 Discussion and Conclusion

Effective nitrogen redistribution in vegetative organs during late growth stages significantly regulates nitrogen nutrition. In this study, nitrogen content in aboveground organs at different canopy levels ranged from 1.94–52.51 $\text{mg} \cdot \text{g}^{-1}$ at anthesis and 1.69–28.26 $\text{mg} \cdot \text{g}^{-1}$ at maturity. Individual plant nitrogen accumulation was 7.27–59.63 $\text{mg} \cdot \text{stem}^{-1}$ at anthesis and 8.45–60.83 $\text{mg} \cdot \text{stem}^{-1}$ at maturity. Nitrogen content and accumulation were highest in leaves at anthesis and in grains at maturity. Nitrogen content and accumulation in vegetative organs decreased from anthesis to maturity, while individual plant and population nitrogen accumulation increased, consistent with previous studies. Spatially, leaf and internode nitrogen content and accumulation decreased with lower canopy positions at anthesis. At maturity, due to nitrogen translocation, spatial distribution differed from anthesis, with internode nitrogen content highest in remaining internodes and leaf nitrogen content higher in remaining leaves and fourth leaf under N0, N1, and N2 treatments, but higher in third and second leaves under N3 treatment.

Hao et al. reported that appropriate nitrogen application (180–210 $\text{kg} \cdot \text{hm}^{-2}$) promoted orderly nitrogen translocation in winter wheat canopy leaves, increasing translocation amount, rate, and contribution to grain. In this study, nitrogen content and accumulation in leaves, stems, and rachis + glume at both anthesis and maturity were significantly higher under fertilization treatments (N1, N2, N3) than N0, increasing with nitrogen rate. Lower canopy leaves (fourth leaf and remaining leaves) and internodes (fourth internode and remaining internodes) as well as grains showed more pronounced increases with nitrogen rate, indicating that increased nitrogen application significantly improved nitrogen content and accumulation in vegetative organs and grains, promoted nitrogen accumulation in lower leaves and internodes, and delayed senescence.

Some studies indicate that high planting density increases population nitrogen accumulation, but post-anthesis nitrogen translocation is more advantageous at lower densities. In this study, planting density primarily affected lower canopy nitrogen content and accumulation, with fourth leaf, remaining leaves, fourth internode, and remaining internodes showing maximum nitrogen accumulation at M2 density, narrowing the gap with upper organs and resulting in more reasonable whole-plant nitrogen distribution.

Coordinated nitrogen accumulation, distribution, and translocation in grains, leaves, stems, and other organs are essential for normal plant growth. High nitrogen translocation amount with appropriate translocation rate in post-anthesis vegetative organs prevents premature leaf senescence due to nitrogen deficiency, while excessive translocation from vegetative tissues can cause leaf premature senescence and reduced photosynthetic capacity, ultimately affecting yield and nitrogen use efficiency. This study showed that nitrogen translocation amount and contribution rate were higher in leaves than internodes, decreasing with lower spatial positions. Vegetative organ translocation amount increased rapidly then slowly with nitrogen rate, with total contribution to grain nitrogen exceeding 67%. Planting density effects on translocation amount and contribution rate varied with nitrogen level: translocation amount was optimal at M1 under N0, N1, and N2, but at M2 under N3; contribution rate was highest at M3 under N0, but at M2 under fertilization treatments (N1, N2, N3). Variance analysis demonstrated that nitrogen rate and planting density jointly regulated nitrogen distribution and translocation, with density effects smaller than nitrogen effects, but their interaction should not be ignored.

This study was conducted in the Huanghuai wheat-maize double-cropping region with previous crop straw return. Both nitrogen application and planting density regulated nitrogen accumulation, translocation, and grain yield traits in aboveground organs at different canopy levels. Nitrogen accumulation and translocation decreased with lower spatial positions. Nitrogen application combined with appropriate planting density promoted nitrogen accumulation in aboveground organs, particularly in lower canopy organs. Based on nitrogen translocation and grain yield performance, the combination of nitrogen application at $240 \text{ kg} \cdot \text{hm}^{-2}$ with planting density of 225×10^4 basic seedlings $\cdot \text{hm}^{-2}$ represents a reasonable cultivation model for this region.

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