Synergistic Effects of Nitrogen Application and Root Interactions on Nitrogen Utilization in Densely Planted Barley-Pea Intercropping Postprint

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Abstract

To address the research gaps in the mechanisms of dense planting in cereal-legume intercropping systems, a barley||pea intercropping system was used as the research subject. Three experimental factors were implemented: nitrogen application [no nitrogen: 0 mg(N) · kg^{-1}(soil); nitrogen application: 100 mg(N) · kg^{-1}(soil)], root separation (no root barrier, root barrier), and density [low density: 15 plants(barley) · pot^{-1}; high density: 25 plants(barley) · pot^{-1}]. Pot experiments were conducted to investigate the effects of nitrogen application and root interaction on nitrogen competition-complementary relationships and utilization efficiency in dense intercropping populations, aiming to provide a regulatory basis for dense planting and efficient nitrogen utilization in cereal-legume intercropping systems. The results showed: 1) Nitrogen application, root interaction, and increased barley density all enhanced nitrogen uptake in barley||pea intercropping populations, with nitrogen application increasing nitrogen uptake by 33.8% compared to no nitrogen, no root barrier increasing nitrogen uptake by 81.1% compared to root barrier, and high density increasing nitrogen uptake by 4.2% compared to low density. Root interaction contributed relatively more to nitrogen uptake under low nitrogen conditions; under no nitrogen and nitrogen application conditions, root interaction increased nitrogen uptake by 92.4% and 11.0%, respectively. Under root interaction conditions, increasing barley planting density significantly increased nitrogen uptake in intercropping populations. 2) Barley was the nitrogen-competitive species, and dense planting significantly increased barley' s nitrogen competition ratio, while nitrogen application weakened barley' s nitrogen competition ratio. The nitrogen competitive advantage of barley relative to pea reached its maximum at the heading stage. 3) Root interaction increased grain nitrogen content in barley and pea by 126.7% and 26.9%, respectively, under nitrogen application conditions, and by 188.5% and

46.5%, respectively, under no nitrogen conditions, and there was a significant interactive effect between nitrogen level and root interaction method on grain nitrogen content in intercropping. 4) High-density barley and root interaction significantly improved nitrogen use efficiency in intercropping populations; under root interaction conditions, increasing barley density increased nitrogen use efficiency in intercropping populations by 59.8%. Barley' s nitrogen competition ratio relative to pea was significantly positively correlated with nitrogen use efficiency in intercropping populations. This study demonstrates that nitrogen application, root interaction, and barley density showed significant interactive effects on nitrogen utilization in barley||pea intercropping. Appropriate nitrogen application and sufficient root interaction are important pathways to support dense intercropping, optimize interspecific nitrogen competition relationships, and ultimately improve population nitrogen uptake and nitrogen use efficiency.

Full Text

Preamble

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Title: Synergistic Effects of Nitrogen Application and Root Interaction on Nitrogen Utilization in Densely Planted Barley-Pea Intercropping System

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Abstract

To address the limited understanding of high-density planting mechanisms in cereal-legume intercropping systems, we conducted a pot experiment using barley-pea intercropping as a model system. The experiment examined three factors, each with two treatment levels: nitrogen application [N0: 0 $mg(N) \cdot kg^{-1}$ soil; N1: 100 $mg(N) \cdot kg^{-1}$ soil], root interaction [no barrier (root interaction) vs. barrier (no root interaction)], and planting density [low density: 15 barley plants per pot; high density: 25 barley plants per pot]. Our objective was to elucidate how nitrogen fertilization and root partitioning affect nitrogen competition, complementary utilization, and use efficiency in densely planted intercropping systems, thereby providing a theoretical basis for optimizing density management and nitrogen utilization in cereal-legume intercropping.

The results demonstrated four key findings. First, nitrogen application, root interaction, and increased barley density all enhanced nitrogen uptake in the barley-pea intercropping system. Specifically, nitrogen application increased total nitrogen uptake by 33.8% compared to the control, root interaction increased uptake by 81.1% compared to the barrier treatment, and high density increased uptake by 4.2% compared to low density. Notably, root interaction contributed more substantially to nitrogen uptake under low-nitrogen conditions, increasing uptake by 92.4% under N0 and 11.0% under N1. Furthermore, increasing barley density under root interaction significantly boosted nitrogen uptake in the intercropping system.

Second, barley was the dominant competitor for nitrogen. High planting density significantly increased barley' s nitrogen competition ratio, while nitrogen application moderately reduced this ratio. The competitive advantage of barley over pea peaked at the heading stage.

Third, root interaction markedly improved grain nitrogen content. Under nitrogen application, root interaction increased barley and pea grain nitrogen content by 126.7% and 26.9%, respectively; without nitrogen, the increases were 188.5% and 46.5%, respectively. A significant interaction existed between nitrogen level and root interaction mode regarding grain nitrogen content.

Fourth, high barley density combined with root interaction significantly improved nitrogen use efficiency (NUE). Under root interaction conditions, increasing barley density enhanced intercropping system NUE by 59.8%. Moreover, barley' s nitrogen competition ratio relative to pea showed a significant positive correlation with NUE in the intercropping system.

In conclusion, nitrogen application, root interaction, and barley density exhibited significant interactive effects on nitrogen utilization in barley-pea intercropping. Appropriate nitrogen application rates and sufficient root interaction are essential for supporting high-density intercropping, optimizing interspecific nitrogen competition relationships, and ultimately enhancing nitrogen uptake and use efficiency.

Keywords: barley-pea intercropping system; root interaction; nitrogen application rate; nitrogen uptake; nitrogen use efficiency; nitrogen competition ratio

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Introduction

Intercropping represents a typical resource-efficient cropping pattern that leverages spatial and nutritional niche complementarity among different crops to achieve efficient utilization of light, water, and nutrients, forming the foundation for high yields. Among various intercropping patterns, cereal-legume intercropping can promote biological nitrogen fixation in legumes and improve nitrogen use efficiency in companion crops, reduce soil mineral nitrogen accumulation, lower farmland nitrogen pollution risks, and benefit agricultural ecosystem protection. Consequently, it is considered an important direction for future sustainable agricultural development.

Research indicates that the high nitrogen use efficiency characteristic of cereal-legume intercropping primarily stems from interspecific nitrogen complementary utilization, while nitrogen application levels and crop density are common measures for regulating interspecific relationships. Studies have shown that nitrogen application can promote nitrogen accumulation in maize-peanut intercropping, though the intercropping advantage decreases with increasing nitrogen rates. Since plants obtain most of their nitrogen from soil through root absorption, enhancing root capacity to absorb and utilize soil nitrogen is crucial for improving crop yield and nitrogen use efficiency. In cereal-legume intercropping systems, physiological and ecological processes regulating nitrogen utilization—such as competitive and complementary nitrogen use, nitrogen inhibition alleviation, and nitrogen transfer—largely depend on root interactions. In practice, planting density is often used to regulate interspecific relationships among component crops in intercropping systems. The effects of intercropping density on competition and compensation depend primarily on resource availability and crop phenological characteristics: competition is relatively weak while compensation effects are strong when resources are abundant, whereas competition intensifies and compensation weakens under resource-limited conditions. With continuous improvement in density-tolerant crop varieties and management techniques, increasing planting density represents a key development direction for further yield and efficiency gains in intercropping systems. Research integrating fertilization, optimization of belowground interactions, and comprehensive density configuration to synergistically improve production efficiency in cereal-legume intercropping can provide reliable theoretical support and practical evidence for enhancing nitrogen utilization advantages and promoting widespread adoption of efficient intercropping patterns.

Among different cereal-legume intercropping systems, barley (*Hordeum vulgare* L.)-pea (*Pisum sativum* L.) intercropping has a symbiotic period exceeding 80% of the total growth duration, providing ample time for interaction and making it an ideal model for studying nitrogen nutrition competition and complementation. Therefore, this study selected barley-pea intercropping as the experimental model and employed root partitioning methodology to distinguish aboveground and belowground interactions, investigating the effects of root interaction and nitrogen application on nitrogen competition and complementary utilization in densely planted intercropping systems. Our aim is to provide theoretical guidance for managing resource competition changes induced by dense planting and improving resource use efficiency in cereal-legume intercropping.

Materials and Methods

1.1 Experimental Design

The pot experiment was conducted during March–July 2012 and March–July 2013 in a solar greenhouse at Gansu Agricultural University, Lanzhou (36°03 N, 103°40 E). Wallerian pots (45 cm height, 30 cm diameter) were used. The test soil was a clay loam collected from Xiajiajing, Qinwangchuan, Yongdeng County, Lanzhou, with a previous barley crop. The soil contained organic matter 12.9 $\mathrm{g \cdot kg^{-1}}$, ammonium nitrogen 2.9 $\mathrm{mg \cdot kg^{-1}}$, nitrate nitrogen 5.0 $\mathrm{mg \cdot kg^{-1}}$, total phosphorus 0.4 $\mathrm{g \cdot kg^{-1}}$, and available phosphorus 0.1 $\mathrm{g \cdot kg^{-1}}$.

After air-drying and sieving through a 2 mm mesh, all fertilizers were applied as basal dressing and mixed thoroughly with the soil before sowing, with 15 kg soil per pot. Phosphorus application rate was 0.1 $\mathrm{g(P_2O_5) \cdot kg^{-1}}$ soil, applied as chemically pure KH_2PO_4. Nitrogen fertilizer was chemically pure urea (46% N). The barley variety was 'Ganpi 4' and the pea variety was 'Longwan 1', both provided by Gansu Academy of Agricultural Sciences.

The experimental design consisted of barley-pea intercropping with three factors, each at two levels: nitrogen application [N0: 0 $\mathrm{mg(N) \cdot kg^{-1}}$ soil; N1: 100 $\mathrm{mg(N) \cdot kg^{-1}}$ soil], root interaction [no barrier (root interaction) vs. barrier (0.025 mm plastic sheet complete partition)], and barley density [D1: 15 plants per pot (low); D2: 25 plants per pot (high)]. Pea density was maintained at 15 plants per pot in all intercropping treatments. Eight treatments were established with three replications each. Both crops occupied half of each pot.

In both experimental years, barley was sown on March 3 and pea on April 1. Barley harvest dates were July 14 (2012) and July 15 (2013), while pea harvest dates were July 2 (2012) and July 1 (2013).

1.2 Measurement Indicators and Methods

Crop nitrogen uptake: Sampling of both barley and pea began 20 days after pea emergence, with four sampling occasions total. The first three samplings were conducted at 20-day intervals (corresponding to barley seedling, tillering, and heading stages), and the fourth sampling occurred at maturity. Three pots per treatment were sampled each time. Samples were oven-dried at 105°C for 30 minutes, then at 80°C to constant weight to determine dry matter weight. Kjeldahl nitrogen determination was used to measure total nitrogen content in barley and pea grains, straw, and whole plants.

Crop nitrogen uptake was calculated as:

$$\text{Nitrogen uptake} = \text{dry matter weight} \times \text{plant nitrogen content} \tag{1}$$

Nitrogen competition ratio of barley relative to pea (NCRBP):

$$\text{NCRBP} = \left(\frac{N_{iB}}{N_{sB}}\right) - \left(\frac{N_{iP}}{N_{sP}}\right) \tag{2}$$

where N_{iB} and N_{iP} represent nitrogen uptake of barley and pea without root barrier, and N_{sB} and N_{sP} represent nitrogen uptake with root barrier. NCRBP > 0 indicates barley has stronger nitrogen competitive ability than pea, while NCRBP < 0 indicates the opposite.

Nitrogen harvest index (NHI):

$$\text{NHI} = \frac{\text{grain nitrogen uptake}}{\text{aboveground nitrogen uptake}} \tag{3}$$

NHI quantifies the degree of dry matter translocation from leaves and stems to grains during late growth stages and reflects nitrogen utilization efficiency.

Nitrogen use efficiency (NUE):

$$\text{NUE} = \frac{\text{N uptake with N application} - \text{N uptake without N application}}{\text{N application rate}} \tag{4}$$

NUE measures the percentage of chemical nitrogen fertilizer absorbed and utilized by the current crop season.

1.3 Data Processing

Data were organized using Microsoft Excel 2013. Statistical and correlation analyses were performed using SPSS 17.0 software. Duncan' s multiple range test was applied to compare treatment means at a significance level of $P < 0.05$.

Results

2.1 Effects of Nitrogen Application, Root Interaction, and Density on Nitrogen Uptake in Intercropped Barley and Pea

As shown in , nitrogen application significantly increased nitrogen uptake in intercropped barley. Under the same density and root interaction mode, nitrogen application increased barley nitrogen uptake by 83.7-175.8% at the seedling stage, 85.4-146.2% at tillering, 63.4-109.6% at heading, and 46.3-88.2% at maturity compared to the no-nitrogen treatment. Root interaction also enhanced barley nitrogen uptake; under the same nitrogen level and density, root barriers reduced barley nitrogen uptake by 16.4-44.9% at seedling, 46.1-60.9% at tillering, 56.1-65.9% at heading, and 82.95-128.14% at maturity. Particularly under no-nitrogen conditions with root interaction, barley nitrogen uptake was 27.8% (2012) and 18.9% (2013) higher than in the nitrogen treatment with root barrier. Increasing density significantly enhanced barley nitrogen uptake; under nitrogen application and root interaction, high density increased total seasonal nitrogen uptake by 21.0% (2012) and 14.0% (2013) compared to low density.

Similar patterns were observed for pea nitrogen uptake. Nitrogen application, root interaction, and increased barley density all promoted pea nitrogen uptake. Under the same density and root interaction, nitrogen application increased pea nitrogen uptake by 4.79–38.42%. Root interaction increased pea nitrogen uptake by 4.4–45.4% at seedling, 37.7–57.0% at tillering, 37.2–52.8% at heading, and 46.76–104.23% at maturity. Under nitrogen application with root interaction, pea nitrogen uptake was 36.3% (2012) and 46.9% (2013) higher than in the nitrogen treatment with root barrier. Under nitrogen application and no barrier, increasing barley density enhanced pea nitrogen uptake by 2.6% (2012) and 9.3% (2013).

For the intercropping system as a whole, nitrogen application and root interaction increased total nitrogen uptake by 20.3–52.4% and 61.16–108.21%, respectively. Root barriers reduced system nitrogen uptake by 48.0% under no nitrogen and 42.2% under nitrogen application, with barley being more affected than pea. This indicates that root interaction is a viable approach to enhance nitrogen uptake in cereal-legume intercropping when soil nitrogen is deficient. Under sufficient nitrogen conditions, increasing barley density improved system nitrogen uptake, raising it by 61.16–81.92% under nitrogen application with no barrier, though density effects were not significant without nitrogen application. This suggests that nitrogen fertilization is essential for density-induced nitrogen uptake increases in intercropping systems.

The effect of nitrogen application on barley nitrogen uptake gradually weakened with advancing growth stages, while the influence of root interaction on both barley and pea strengthened. Root interaction, nitrogen level, and density affected barley more strongly than pea, indicating that these factors primarily regulate system nitrogen uptake through the barley component. Significant interactive effects among all factors were observed for nitrogen uptake at maturity.

2.2 Dynamic Effects of Nitrogen Application, Root Interaction, and Density on Nitrogen Competition Ratio of Barley Relative to Pea

As illustrated in [Figure 1: see original paper], during the seedling stage of barley and pea, the nitrogen competition ratio of barley relative to pea was negative under no-nitrogen conditions. Increasing barley density reduced the pea competition ratio by 86.3%, indicating that pea was the nitrogen-competitive dominant species during the early co-growth period under nitrogen deficiency, and that increased barley density could weaken pea’ s competitive ability.

As growth progressed, barley’ s nitrogen competition ratio increased substantially, making barley the nitrogen-competitive dominant species. By the heading stage, barley’ s competitive advantage peaked. Under no-nitrogen and nitrogen-application conditions, high density increased barley’s competition ratio relative to pea by 29.0% and 47.8%, respectively, compared to low density. Under low and high density, nitrogen application increased the competition ratio by 7.1% and 23.0%, respectively, compared to no nitrogen. This demonstrates that both nitrogen application and increased barley density enhanced barley’ s nitrogen competitive advantage during its growth period.

Comparing the average nitrogen competition ratio across the entire growth period, high density increased barley’ s ratio by 99.7% and 90.10% under no-nitrogen and nitrogen-application conditions, respectively, compared to low density. Under low and high density, nitrogen application increased the ratio by 70.9% and 73.2%, respectively, compared to no nitrogen. These results indicate that increased barley density enhanced barley’ s nitrogen competition ratio, while nitrogen application also strengthened barley’ s competitive advantage.

2.3 Effects of Nitrogen Application, Root Interaction, and Density on Grain Nitrogen Content and Nitrogen Harvest Index of Barley and Pea

2.3.1 Grain Nitrogen Content Under Different Treatments Nitrogen application and root interaction significantly affected grain nitrogen content in the barley-pea intercropping system and its components (Table 2). For barley grain nitrogen content, nitrogen application increased it by 81.8% compared to no nitrogen. Root interaction with pea also improved barley grain nitrogen content, with no-barrier treatments showing 144.8% higher content than barrier treatments. A highly significant interaction existed between nitrogen level and root interaction mode: under nitrogen application, no-barrier treatments showed 126.7% higher content than barrier treatments, while without nitrogen, the increase was 188.5%. A significant interaction also occurred between density and root interaction: under no-barrier conditions, high density increased barley grain nitrogen content by 10.1% compared to low density, whereas under barrier conditions, high density reduced it by 6.3%. This suggests that nitrogen application reduced the positive effect of root interaction on barley grain nitrogen content, while root interaction enhanced the positive density effect.

For pea grain nitrogen content, no-barrier treatments showed 34.5% higher content than barrier treatments, and nitrogen application increased it by 33.3% compared to no nitrogen. Although density and root interaction did not significantly affect pea grain nitrogen content individually, their interaction was significant: under no-barrier conditions, high density reduced pea grain nitrogen content by 8.4% compared to low density, while under barrier conditions, high density increased it by 4.5%. This indicates that barley-pea root interaction had some negative effects on pea grain nitrogen content.

Under nitrogen application and root interaction, intercropping system grain nitrogen content increased by 51.8% and 71.5%, respectively, compared to no nitrogen and barrier treatments. Without nitrogen, root interaction increased system grain nitrogen content by 84.5%, while with nitrogen, the increase was 63.6%. Similar to nitrogen uptake, barley grain nitrogen content was more affected than pea, demonstrating that root interaction is an important pathway for improving grain nitrogen content in cereal-legume intercropping when soil nitrogen is deficient.

2.3.2 Nitrogen Harvest Index Under Different Treatments

Barley and pea nitrogen harvest indices are presented in Table 2. Nitrogen application increased NHI of barley, pea, and the intercropping system by 12.2%, 14.0%, and 13.6%, respectively. Root interaction increased barley NHI by 20.5% but reduced pea and system NHI by 23.3% and 5.1%, respectively. The interaction between density and root interaction significantly affected pea NHI: increasing barley density reduced pea NHI by 11.0% under no-barrier conditions but increased it by 4.0% under barrier conditions. A significant three-way interaction among nitrogen application, barley density, and root interaction affected barley NHI: under root interaction, increasing density improved barley NHI by 3.8% without nitrogen but reduced it by 8.2% with nitrogen application.

These results indicate that nitrogen application increased intercropping system NHI, while root barriers weakened the negative effect of density increase on pea NHI and the positive effect of density on barley NHI. Without nitrogen, root interaction enhanced the positive density effect on barley NHI, whereas with nitrogen, increased density under root interaction reduced the proportion of nitrogen translocated to barley grains.

2.4 Grey Relational Analysis Between Nitrogen Competition Ratio and Grain Nitrogen Content of Intercropped Barley and Pea

To further clarify the relationship between nitrogen competition ratio and grain nitrogen content in the intercropping system, we analyzed the degree of influence of barley’ s nitrogen competition ratio at different growth stages on component crop grain nitrogen content using grey relational analysis. A higher relational degree indicates more similar changing trends and closer relationships.

As shown in , the relational degree between nitrogen competition ratio at different growth stages and intercropping system grain nitrogen content followed the order: heading stage > maturity stage > tillering stage > seedling stage. The nitrogen competition ratios at heading, maturity, and tillering stages showed relatively high relational degrees with grain nitrogen content, indicating these stages significantly influenced component crop grain nitrogen content under different nitrogen and density treatments.

Correlation analysis () revealed that nitrogen competition ratio was positively correlated with barley and system grain nitrogen content. Moderate regulation of barley’s nitrogen competition ratio during the co-growth period could improve intercropping grain nitrogen content, with the heading, maturity, and tillering stages identified as critical management periods for achieving high grain nitrogen content through regulation of interspecific nitrogen competition ratio.

2.5 Effects of Root Interaction and Density on Nitrogen Use Efficiency in Barley-Pea Intercropping

Nitrogen use efficiency (NUE) of the intercropping system is presented in [Figure 2: see original paper]. Density and root interaction mode had highly significant effects on system NUE, with a significant interaction between these factors. Density increase improved system NUE by 33.1%, while root interaction enhanced it by 28.6%. Notably, increasing barley density under root interaction significantly improved system NUE by 59.8%, indicating that root interaction amplified the positive effect of barley density on NUE.

During the barley-pea co-growth period, nitrogen competition ratio showed positive correlations with system NUE: correlation coefficients were 0.592* at seedling stage, 0.797** at tillering stage, 0.764** at heading stage, and 0.324 at maturity. These results suggest that adjusting agronomic practices such as density, nitrogen level, and root interaction to increase nitrogen competition ratio during barley tillering, heading, and seedling stages can effectively improve NUE in intercropping systems.

Discussion

3.1 Effects of Planting Density on Crop Nitrogen Utilization

Planting density ratios in intercropping systems influence both nitrogen use efficiency and competitiveness. Research on maize-soybean intercropping suggests that after soil water and nutrient requirements are met, the intercropping advantage primarily derives from density effects. Studies have shown that appropriate density increases can improve yield and significantly enhance nitrogen use efficiency. In our experiment, the interaction between root interaction and density significantly increased nitrogen uptake and NUE in both barley and pea. However, main effect analysis revealed that density alone did not significantly affect grain nitrogen content, while the interaction between root barrier and density was significant. No-barrier high-density treatments showed significantly higher barley grain nitrogen content than low-density treatments, indicating that density-induced increases in grain nitrogen content in barley-pea intercropping are closely related to root interaction between component crops.

Barley showed greater sensitivity than pea to changes in nitrogen level, density, and root relationship under no-barrier conditions, suggesting that improvements in nitrogen uptake and NUE in barley-pea intercropping result from spatial root overlap and rhizosphere exchange of water and nutrients. Density increase significantly enhanced barley root and canopy biomass, particularly root length density, leading to substantially increased nitrogen absorption. This not only satisfied nitrogen demand for larger aboveground populations but also stimulated biological nitrogen fixation in intercropped pea. Consequently, nitrogen application increased average system nitrogen uptake by 7.5%, with root interaction producing a more significant increase of 11.0%.

Density effects differ between monoculture and intercropping systems. In melon-sunflower intercropping, maximum nitrogen uptake and partial factor productivity occurred at different densities depending on intercropping sowing date. In barley nitrogen absorption studies, maximum uptake and apparent NUE occurred at medium density. Our experiment only tested two barley densities, and under nitrogen application, high density showed higher nitrogen uptake and NUE than low density. However, testing additional density gradients or broader density ranges might reveal different patterns. Therefore, density-induced improvements in nitrogen use efficiency through root interaction likely have limits that require further investigation.

3.2 Relationship Between Root Interaction and Nitrogen Utilization in Intercropping

Intercropped crops compete for space and resources while also exhibiting complementarity through modified microenvironments that increase resource availability. Competition and complementarity coexist, with their relative importance changing during crop development. Previous research shows that interspecific competitive ability, particularly for nitrogen, relates to root growth, distribution, and interaction patterns, with different components having varying nutrient absorption and utilization capacities. Typically, one crop’ s enhanced nutrient absorption occurs at the expense of another, with cereal crops generally having higher root activity than legumes.

In wheat-faba bean intercropping, wheat competition increased symbiotic nitrogen fixation in intercropped faba bean, with the “spared nitrogen” being absorbed by wheat. In our study, barley was the nitrogen-competitive dominant species, consistent with previous findings. Under nitrogen application, root interaction contributed 106.6% and 66.3% to nitrogen uptake in barley and pea, respectively, and 144.7% and 34.5% to grain nitrogen content. Barley’ s strong nitrogen competitiveness resulted in weaker root interaction contributions to pea. Meanwhile, dense planting and nitrogen application significantly increased barley’ s nitrogen competition ratio relative to pea under no-barrier conditions. Although density increased interspecific nitrogen competition, nitrogen application overall showed stronger promotion than competition effects. The barley-pea intercropping system demonstrated intercropping advantages, indicating that root interaction is the primary pathway balancing interspecific nitrogen competition and complementation. Regulating root interactions between cereals and legumes through dense planting can promote soil nitrogen utilization, reduce nitrogen fertilizer dependence, and compensate for nitrogen deficiency, particularly under low soil nitrogen conditions.

3.3 Synergistic Effects of Nitrogen Application, Root Interaction, and Density on Nitrogen Utilization in Intercropping

Research on monoculture wheat indicates that grain yield increases significantly only under optimal nitrogen-density combinations. In monoculture, nitrogen effects on grain yield are closely related to population conditions: moderate density increases can improve grain yield under low nitrogen, while the opposite trend occurs under high nitrogen. Studies combining different densities and nitrogen rates show that neither excessively high nor low densities favor high-yielding populations, while medium-density with high nitrogen or high-density with medium nitrogen can achieve high yields through compensatory effects. Research on winter wheat demonstrates that reducing nitrogen application while increasing density can maintain high grain yield and NUE.

Root interaction makes the synergistic effects of density and nitrogen on nitrogen utilization more complex in intercropping. In our experiment, root interaction increased system nitrogen uptake and grain nitrogen content by 34.3% and 12.6%, respectively, under no-nitrogen conditions. Moreover, density increase contributed 5.9% and 6.2% to barley nitrogen uptake and grain nitrogen content under no-nitrogen conditions with root interaction. This demonstrates that nitrogen nutritional complementation through root interaction compensated for reduced nitrogen uptake and grain content caused by nitrogen omission, while density increase enhanced barley’ s nitrogen absorption and translocation to grains, achieving high grain nitrogen content at low soil nitrogen levels.

Furthermore, through root interaction, barley can alleviate “nitrogen inhibition” in pea and stimulate nodule growth, enhancing pea nitrogen fixation. Increased legume nitrogen fixation in intercropping systems raises nitrogen transfer to cereals, effectively conserving soil nitrogen. In our experiment, root interaction improved pea nitrogen uptake and grain nitrogen content, with greater improvement proportions under no-nitrogen than nitrogen-application treatments. This occurs because when ecosystem resources are limited, interacting species increase niche breadth to maximize marginal returns per unit resource.

Grey relational analysis showed that nitrogen competition ratio at different growth stages influenced barley, pea, and system grain nitrogen content in the order: heading stage > maturity stage > tillering stage > seedling stage. Therefore, production practices should consider regulating root spatial distribution and morphology through planting density, nitrogen level, and nitrogen application timing during different co-growth stages. Utilizing rhizosphere compensatory effects can create complementary nitrogen resource demands between intercropped species, making facilitative interactions exceed competition and maximizing nitrogen absorption and utilization to improve NUE while reducing environmental pollution from nitrogen fertilizer production and application.

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Note: Figure translations are in progress. See original paper for figures.