

## Advances in the Mechanisms of Efficient Nitrogen Utilization and Agronomic Regulation in Cereal-Legume Intercropping: A Postprint

**Authors:** Chai Qiang, Hu Falong, Chen Guiping

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### Abstract

To ensure food security, chemical fertilizer use is extremely common in agricultural production; however, excessive application often leads to various environmental and ecological problems such as groundwater pollution, intensified greenhouse effects, and reduced biodiversity. The cereal-legume intercropping system, due to differences in the biological characteristics and nitrogen utilization among different crops, can fully leverage the advantages of biological nitrogen fixation through reasonable regulation, thereby reducing chemical fertilizer input and improving production efficiency. It represents a stable, high-yield, efficient, and sustainable cropping system. Within this system, “nitrogen transfer”, mitigation of “nitrogen suppression”, and spatiotemporal differentiation of nitrogen are currently hot research topics, as well as effective pathways to promote nitrogen fixation in legume crops and reduce chemical fertilizer input, enabling efficient nitrogen utilization by both cereal and legume crops. In particular, agronomic measures such as crop variety, nitrogen application regime, spatial arrangement, and planting density are necessary means to regulate interspecific relationships within this system. Rational optimization can effectively promote the competitive and complementary synergistic effects of cereal/legume intercropping, enhance coordinated nitrogen utilization, and thereby tap into the biological potential for efficient nitrogen use in both crops. To this end, based on previous research findings and perspectives on sustainable agricultural development, this paper provides a comprehensive review of the current research status on the main mechanisms of efficient nitrogen utilization in cereal-legume intercropping and related agronomic regulation pathways, both domestically and internationally, aiming to provide strong scientific basis and theoretical support for constructing simple, high-yield, efficient, and nitrogen-saving cereal-legume intercropping models.

## Full Text

### Preamble

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**Research advance in the mechanism and agronomic regulation of high-efficient use of nitrogen in cereal-legume intercropping**

Chai Qiang, Hu Falong, Chen Guiping  
(Gansu Provincial Key Laboratory of Arid Land Crop Science / Faculty of Agronomy, Gansu Agricultural University, Lanzhou 730070, China)

**Abstract:** Chemical fertilizer use is extremely common in agricultural production to ensure food security, but excessive application often causes various environmental and ecological problems, including groundwater pollution, intensified greenhouse effects, and reduced biodiversity. Cereal-legume intercropping systems, due to differences in biological characteristics and nitrogen utilization between crops, can fully exploit the advantages of biological nitrogen fixation through reasonable regulation, thereby reducing chemical fertilizer input and improving production efficiency. This represents a stable, high-yield, efficient, and sustainable cropping system. In these systems, “nitrogen transfer,” alleviation of “nitrogen repression,” and temporal-spatial differentiation of nitrogen are current research hotspots and effective approaches to promote legume nitrogen fixation and reduce fertilizer input, enabling efficient nitrogen utilization by both cereals and legumes. Particularly, agronomic measures such as crop variety selection, nitrogen application regimes, spatial arrangement, and planting density are essential tools for regulating interspecific relationships. Rational optimization can effectively promote the synergistic effects of competition and complementarity in cereal/legume intercropping, enhance coordinated nitrogen utilization, and thus tap the biological potential for efficient nitrogen use in both crops. Therefore, based on previous research findings and perspectives on sustainable agricultural development, this paper comprehensively reviews the current research status on the main mechanisms of efficient nitrogen utilization and related agronomic regulation pathways in cereal-legume intercropping systems, aiming to provide strong scientific basis and theoretical support for constructing simple, high-yield, efficient, and nitrogen-saving intercropping models.

**Keywords:** cereal-legume intercropping; nitrogen transfer; nitrogen repression; interspecific relationship; efficient nitrogen utilization

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**Corresponding author:** CHAI Qiang, E-mail: Chaiq@gsau.edu.cn  
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## 1.2 Nitrogen Transfer in Cereal-Legume Intercropping Systems

Research has shown that when faba bean (*Vicia faba*) is intercropped with barley (*Hordeum vulgare*), the nitrogen fixation amount of faba bean increases significantly [26]. Similarly, for peas (*Pisum sativum*) intercropped with barley, the proportion of biologically fixed nitrogen in total nitrogen accumulation is markedly higher than in monocropped peas [27]. Intercropping maize (*Zea mays*) with lablab bean (*Lablab purpureus*) increased the proportion of atmospheric nitrogen fixed in the system; when the planting ratio of lablab bean to maize reached 75:25, nitrogen fixation reached  $81 \text{ kg} \cdot \text{hm}^{-2}$  per season, compared to only  $49 \text{ kg} \cdot \text{hm}^{-2}$  in monoculture [28]. These studies demonstrate that cereal-legume intercropping promotes biological nitrogen fixation. Using  $^{15}\text{N}$  labeling methods, research has proven that direct nitrogen transfer occurs from legumes to cereals in intercropping systems. Van Kessel et al. [29] used a split-root experiment with  $^{15}\text{N}$ -enriched soil in soybean (*Glycine max*) to demonstrate “nitrogen transfer” from soybean to maize roots. Ledgard [30] attempted new quantitative research on nitrogen transfer through leaf-feeding  $^{15}\text{N}$  methods, finding that 2.2% of nitrogen in white clover (*Trifolium repens*) was directly transferred to perennial ryegrass (*Lolium perenne*). Other reports indicate that 20%-30% of nitrogen uptake by maize intercropped with common bean (*Phaseolus vulgaris*) originated from the bean, accounting for 10%-15% of the bean’s fixed nitrogen [31]. Generally, three pathways are recognized for nitrogen transfer from legumes to non-legumes: (1) direct transfer when intercropped component roots contact each other; (2) indirect transfer through nitrogen residues in soil utilized by non-legume crops later in the season; and (3) indirect transfer through residue nitrogen absorbed by subsequent season crops. However, when crop types, spatial arrangements, or soil baseline characteristics differ in intercropping systems, the effects of nitrogen-efficient utilization through direct or indirect “nitrogen transfer” vary. Therefore, with fixed crop combinations, regulating the spatial layout of main crops and the abundance or deficiency of soil nutrients offers considerable potential for tapping “nitrogen transfer” effects.

## 1.3 Mitigation Effect of Cereal Crops on Legume “Nitrogen Repression”

During the early growth stage of legumes, excessively low soil nitrogen levels create “nitrogen starvation” that reduces nodulation capacity and nitrogen fixation [32], primarily because crop root systems are not yet fully developed and the nodulation and nitrogen fixation processes have not initiated. Applying small amounts of “starter nitrogen” during the seedling stage can eliminate nitrogen deficiency symptoms [33]. During crop growth, nitrogen application leads to decreased nodule numbers and biological nitrogen fixation in legumes [34], a phenomenon known as “nitrogen repression.” Wang et al. [35] found that nitrogen application significantly affected soybean nodule formation, growth, and nitro-

gen fixation capacity; as nitrogen rate increased, nodule dry weight and number initially increased then decreased, while nitrogenase activity and leghemoglobin content showed continuous decline, demonstrating “nitrogen repression.” However, appropriate nitrogen application promotes nodule growth. In soybean monoculture, applying  $25 \text{ kg} \cdot \text{hm}^{-2}$  of “starter nitrogen” and topdressing  $50 \text{ kg} \cdot \text{hm}^{-2}$  at flowering or early seed formation achieved the highest total nitrogen uptake and fixation [33]. Continuous versus non-continuous nitrogen supply affects soybean growth differently; non-continuous supply significantly promotes early seedling growth, while continuous supply benefits post-flowering growth, but sustained nitrogen application inhibits nodule formation and growth, reducing nitrogen fixation efficiency. Therefore, “starter nitrogen,” appropriate nitrogen levels, and application methods are important regulatory factors determining legume nitrogen fixation potential and alleviating “nitrogen repression.”

When intercropped with cereal crops, cereals absorb large amounts of nitrate, maintaining soil mineral nitrogen at low levels and reducing inhibition of legume nitrogen fixation [36]. This effect can be considered as cereal crops mitigating “nitrogen repression” in legumes. Numerous studies have shown that cereal crops can stimulate legume nodulation and nitrogen fixation, possibly because cereals compete for nitrate or ammonium nitrogen in the legume rhizosphere [37]. Additionally, Li et al. [38] designed maize-faba bean intercropping experiments under different nitrogen supply levels in northwestern China to investigate the mitigation effect of maize on faba bean “nitrogen repression.” Results showed that at nitrogen application rates of  $75 \text{ kg} \cdot \text{hm}^{-2}$ ,  $150 \text{ kg} \cdot \text{hm}^{-2}$ ,  $225 \text{ kg} \cdot \text{hm}^{-2}$ , and  $300 \text{ kg} \cdot \text{hm}^{-2}$ , the percentage of nitrogen-induced inhibition of faba bean nodule weight alleviated by maize (Ca%) was 9.7%, -10%, 15.2%, and 10%, respectively, while the percentage of inhibition of nitrogen fixation proportion in total nitrogen uptake (Cis%) alleviated was 20.3%, -0.5%, 17.4%, and 3.9%, respectively. These results fully demonstrate the feasibility of alleviating nitrogen fertilization inhibition on legume nodulation and nitrogen fixation through intercropping with cereals, while providing theoretical basis for selecting reasonable nitrogen application levels in cereal-legume intercropping systems.

#### 1.4 Temporal-Spatial Differentiation Characteristics of Nitrogen Demand in Different Crops

In intercropping systems, different component crops occupy distinct ecological niches due to their genetic and morphological characteristics. Therefore, well-designed intercropping systems can effectively weaken interspecific competition and promote complementary utilization of limited resources. On one hand, different crops in intercropping systems show substantial differences in nutrient sensitivity, with distinct temporal peaks in nutrient uptake, ensuring adequate nutrient supply during each crop’s maximum efficiency period [39]. Additionally, component crops in intercropping systems often have separate sowing and harvest times; early-sown crops have certain advantages in resource utilization and can compete for surplus nutrients from later-sown crops, while later-sown

crops can directly distribute roots in adjacent crop zones to absorb nutrients when they resume growth [8]. Particularly when the same resource becomes limiting, different temporal patterns of resource utilization between two intercropped crops can ensure that nutrient demand does not exceed supply rates during certain periods [40]. On the other hand, different crops have substantially different root biological characteristics, with varying rooting depths and distribution ranges, enabling them to absorb and utilize nutrients from different soil layers, regions, and forms, thereby promoting the formation of rhizosphere nutrient utilization advantages in intercropping [41].

Furthermore, different plant types respond differently to nitrogen forms. Unlike nitrate nitrogen, which inhibits legume nodulation and nitrogen fixation, nitrate generally promotes the growth of cereals such as wheat (*Triticum aestivum*) and maize. Li et al. [20] found that wheat prefers nitrate nitrogen and grows better when nitrate is the sole nitrogen source or constitutes a high proportion of nitrogen fertilizer. Similarly, maize also shows a preference for nitrate nitrogen, which is more pronounced at lower nitrogen rates. However, analyzing chemical nitrogen fertilizer production costs and future development trends, ammonium nitrogen fertilizers will inevitably become mainstream due to advantages in energy savings, making large-scale application of nitrate nitrogen fertilizers relatively unfeasible in crop production. The preference of cereal crops for nitrate nitrogen can only be addressed through efficient nitrification of ammonium nitrogen. In intercropping or relay-cropping systems composed of cereals and legumes, differences in biological characteristics for efficient nitrogen utilization between the two components inevitably create difficulties in precise nitrogen application. Only through scientifically designed nitrogen application systems that simultaneously promote individual crop growth and nitrogen compensatory utilization between crops can economic nitrogen application in intercropping systems be achieved.

## 2 Agronomic Regulation Pathways for Efficient Nitrogen Utilization in Cereal-Legume Intercropping

Related research has demonstrated that the prerequisite for efficient resource utilization in intercropping is scientific regulation of interspecific competition and full exploitation of interspecific complementarity [42-43]. Regulatory factors for efficient nitrogen utilization in intercropping mainly include crop varieties [44], water and fertilizer management [45], crop spatial arrangement, and planting density [46]. Optimizing these regulatory factors can promote the synergistic effects of competition and complementarity in cereal-legume intercropping and improve nitrogen use efficiency.

### 2.1 Selection of Nitrogen-Efficient Varieties

The physiological mechanism of efficient nitrogen utilization in crops is a complex metabolic process that depends not only on crop genotype but also on

physiological metabolism [47]. Clarifying nutrient demand characteristics during vegetative and reproductive growth periods, as well as nutrient recycling metabolism and genetic regulatory mechanisms, are important approaches to improving crop nitrogen efficiency. New breeding objectives should focus on developing novel crop varieties adapted to low nitrogen input. Research shows that crop varieties exhibit obvious genetic diversity in mineral nutrient absorption and utilization, with significant genotypic differences in nitrogen efficiency among different crops such as wheat, maize, soybean, and rice (*Oryza sativa*). Under low nitrogen or nitrogen stress conditions, nitrogen-efficient varieties show the highest plant height, leaf area index, population dry weight, population growth rate, net assimilation rate, and photosynthetic potential at different growth stages [48]. However, existing research on improving nitrogen efficiency through new variety selection has mainly focused on screening nitrogen-efficient varieties and evaluation indicators, mostly under monoculture conditions. Studies on nitrogen absorption and utilization characteristics of different nitrogen-efficient crops at various growth stages in intercropping systems, particularly the interrelationships and internal physiological mechanisms of coordinated nitrogen utilization between crops, remain insufficient. Therefore, combining different nitrogen-efficient crops or varieties to exploit intercropping advantages for physiological nitrogen efficiency has broad research prospects.

## 2.2 Optimization of Nitrogen Application Systems

Nitrogen application level affects the dominant position of intercropping components in the composite population and influences mixed and component yields. Research found that in barley-pea intercropping, barley is the competitive advantage species, with intercropped barley yield and nitrogen absorption similar to monoculture. However, due to competition, individual plant aboveground dry weight and nitrogen accumulation of intercropped peas were significantly lower than in monoculture. Nitrogen application increased barley's relative competitiveness and decreased peas' contribution to mixed yield [4]. Similarly, Corre-Hellou et al. [49] found that barley's rapid root growth during the vegetative stage gave it access to more soil nitrogen than peas in barley-pea intercropping, but this competitive advantage existed only during the vegetative stage and adversely affected final yield only when soil nitrogen supply was low. Under high nitrogen supply, nitrogen sharing between barley and peas was unaffected by the depth of root intermingling. Therefore, nitrogen application systems in cereal-legume intercropping must consider the nitrogen demand characteristics of both crops. Reduced nitrogen application or delayed nitrogen application fully considers differential crop responses to nitrogen, effectively promoting coordinated nitrogen absorption and efficient utilization by both components. Liu et al. [50] found that reduced nitrogen application in maize-soybean relay intercropping significantly increased individual plant nodule number, nodule dry weight, nodule nitrogen fixation potential at soybean R5 stage, and total nitrogen uptake at R8 stage. This reduced nitrogen application also significantly decreased nitrogen residue, loss, and ammonia volatilization while increasing ap-

parent nitrogen recovery efficiency [51]. Delayed nitrogen application not only alleviates early-stage nitrogen repression on legume nitrogen fixation but also promotes post-anthesis dry matter accumulation and translocation to grains in cereals, increases maximum growth rate, and improves grain yield and nitrogen use efficiency [52].

In intercropping systems, moderate competition can improve system-level yield and resource use efficiency, but excessive competition between components inevitably reduces complementarity. Research has proven that nitrogen application rate significantly affects the competitiveness of intercropped cereals and legumes, indicating that optimized nitrogen application can enhance interspecific complementarity. The key challenge lies in quantifying the relationship between crop competitiveness at different growth stages and target yield to formulate refined nitrogen management systems.

### 2.3 Rational Spatial Layout Design

Spatial layout in intercropping mainly refers to the land occupation ratio of different crops, row spacing, and spatial occupancy determined by the length of co-growth periods. Hauggaard-Nielsen et al. [34] designed barley-pea intercropping experiments with three patterns: barley and pea each occupying 100 cm, each occupying 50 cm, and barley occupying 50 cm with pea occupying 100 cm. All three patterns showed significantly higher total nitrogen uptake than monoculture, indicating compensatory effects between the two crops. This compensation mainly resulted from intercropped barley absorbing large amounts of nitrogen, forcing peas to increase dependence on biological nitrogen fixation, though differences among the three patterns were not significant. In maize-pea intercropping, different strip patterns caused variations in total nitrogen uptake and grain yield; compared with the 2:4 pattern, the 3:4 pattern significantly improved nitrogen use efficiency [53]. In maize-soybean intercropping, 4 rows of maize intercropped with 6 rows of soybean achieved the maximum land equivalent ratio and plant nitrogen uptake [54]. Additionally, Davis et al. [55] reported that narrow-row relay intercropping of maize and soybean significantly increased yield, but simultaneous sowing of both crops slightly reduced composite population yield. Appropriately increasing row spacing for simultaneously sown crops could clearly reduce competition, coordinate nitrogen utilization in the composite population, and achieve yield advantages.

In strip intercropping, the adjacent zones between two crops are focal areas for resource competition and complementarity. The traditional spatial structure design principle is “squeeze the middle, empty the sides” —minimizing row spacing of the same crop while maximizing distance between adjacent crop strips within designed density ranges. However, whether this concept is reasonable, particularly in cereal-legume intercropping systems with high nutrient complementarity, and its effects on “nitrogen transfer” and nitrogen repression alleviation lack systematic research. Whether this approach causes intercropping producers to miss opportunities for improving nitrogen use efficiency by

reducing spacing requires further investigation.

## 2.4 Density and Competition Compensation

The effect of planting density on competition and compensation in intercropping mainly depends on resource availability and crop phenological characteristics. Under adequate precipitation and temperature, oat (*Avena sativa*)-pea intercropping produced higher yields at high density, but under drought and high temperature, low density treatments yielded higher. This indicates that when resources are sufficient, intercropped crops experience relatively less competition and greater compensation effects, whereas under resource limitation, competition intensifies and compensation weakens [56]. In oat-pea intercropping, oat is the competitive advantage species, and density can serve as an interspecific competition regulatory factor similar to nitrogen [42]. Hauggaard-Nielsen et al. [46] found that in barley-pea intercropping, barley's earlier emergence gave it certain competitive advantages, but peas showed greater competitiveness after the seedling stage. This competitive advantage reversal mainly depended on the phenological characteristics of both crops. Intercropped barley growth showed significant negative correlation with later-stage density, more pronounced in intercropping populations where peas held competitive advantages. High density could increase the proportion of pea yield, but intercropping did not increase pea dependence on atmospheric nitrogen fixation, and pea land occupation ratio affected intercropping population nitrogen uptake far more than density [57]. In maize-pea intercropping populations, dense planting promoted coordinated utilization of carbon and nitrogen by plants and soil, improving nitrogen use efficiency [58]. In maize-soybean intercropping, dense planting enhanced the alleviation of "nitrogen repression" in intercropped soybean, achieving interspecific promotion and complementary nitrogen utilization [59].

Theoretical research on how density regulation affects nitrogen repression alleviation and promotes efficient nitrogen utilization in cereal-legume intercropping systems remains weak, leaving the biological potential for complementary nitrogen utilization far from fully exploited. Therefore, based on integrated agronomic regulation measures, investigating relationships between different regulatory measures and nitrogen transfer and nitrogen repression alleviation effects will be important for deepening understanding of efficient nitrogen utilization mechanisms and constructing effective regulation technologies.

## 3 Development Status and Existing Problems of Typical Cereal-Legume Intercropping—Maize-Pea Intercropping

Intercropping patterns using soybean and faba bean as legume components have large application areas and long histories in China. However, many researchers consider pea an ideal component crop for organic farming systems [60], particularly in the European Union where pea production accounts for over 80% of grain legumes. Coupled with pea's excellent nitrogen fixation characteristics

and high compatibility with other crops in intercropping, various patterns have formed, including barley-pea [34,46], oat-pea [56], and wheat-pea [61] intercropping. Maize-pea intercropping is a newly emerging pattern in the Hexi Corridor region of Gansu Province, China, with application area exceeding 20,000 hm<sup>2</sup>. Northwest China's inland irrigation areas are rich in light resources, have good soil quality, and thermal conditions sufficient for one but insufficient for two crops, making them suitable for intercropping. However, due to increasingly severe water shortage constraints, traditional patterns such as wheat-maize and wheat-soybean intercropping no longer meet production demands. Since 2005, maize-pea intercropping has rapidly expanded due to its significant water-saving, high-efficiency potential, and capacity for promoting crop-livestock integration.

However, existing production practices manage maize-pea intercropping using nitrogen fertilization and irrigation systems designed for monoculture maize, with spatial layouts and maize density designed by reference to wheat-maize intercropping. This management technology not only ignores nitrogen compensation effects in cereal-legume intercropping but also overlooks potential density and competition effects with different crop combinations and their impacts on nitrogen transfer and nitrogen repression alleviation. Consequently, the advantages of this pattern in improving nitrogen use efficiency, crop yield, and land use efficiency under limited water supply conditions cannot be fully realized. Therefore, researching and designing rational spatial layouts and scientific water-nitrogen management systems is crucial for fully exploiting the biological potential for complementary and efficient nitrogen utilization in maize-pea intercropping.

Coordinating nitrogen supply forms, optimizing soil moisture, temperature, and microbial community structure, and inoculating rhizobia are important measures for promoting legume nodulation and nitrogen fixation. In cereal-legume intercropping systems, significant differences exist in biological characteristics and nitrogen utilization features between crops. Rational regulation and coordination of interspecific relationships can effectively promote competition and complementarity, enhance coordinated nitrogen utilization, and particularly play important roles in promoting nitrogen transfer and alleviating nitrogen repression. Crop variety, water and fertilizer management, spatial layout, and planting density are important agronomic pathways for scientifically regulating interspecific competition and fully exploiting interspecific complementarity. Rational optimization can achieve efficient nitrogen utilization by both cereals and legumes. Reviewing research findings on efficient nitrogen utilization in cereal-legume intercropping reveals abundant results on nitrogen transfer, nitrogen repression alleviation, nitrogen application levels, resource utilization characteristics of intercropping populations, and crop combination and strip pattern design. However, research remains weak on how cereals under different nitrogen application methods and planting densities affect nitrogen repression alleviation in intercropped legumes, and on enhancing cereal promotion of legume nitrogen synergy through spatial layout regulation to improve nitrogen use efficiency. Currently, maize-pea intercropping has been applied on a large scale and is

rapidly expanding in different regions of China, but theoretical and technical research on efficient nitrogen utilization in this system is scarce. Particularly, research on the mechanisms of easily implementable agronomic regulation techniques aimed at increasing biological nitrogen fixation is rarely reported, creating theoretical and technical shortcomings for further efficient development and utilization of this pattern. Therefore, research on high-efficiency production theory and technology focusing on maize-pea intercropping urgently needs further development.

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