

## Postprint: Effect of Cereal-Legume Spacing on Mitigating Nitrogen Suppression in Intercropped Peas

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### Abstract

To address the weak regulatory basis for the synergistic utilization of chemical nitrogen fertilizer and legume nitrogen fixation potential in cereal-legume intercropping, this study, taking the dominant maize/pea intercropping system in the Hexi Corridor region as the research object, investigated the effects of spatial structures with maize-pea intercropping spacings of 15 cm, 30 cm, and 45 cm on the alleviation of nitrogen suppression in intercropped peas, aiming to provide theoretical basis for optimizing spatial structure, alleviating nitrogen suppression, and improving nitrogen use efficiency in cereal-legume intercropping patterns. Results from the two-year study in 2013 and 2014 showed that, compared with monoculture, both effective nodule number and nodule weight of intercropped peas increased significantly, with nodule number increasing by 0-500%, reaching maximum values at a spacing of 30 cm. The nitrogen suppression alleviation effect (Ca) calculated based on nodule number and nodule weight was positive. Under nitrogen application conditions, the nitrogen suppression alleviation effect of the 30 cm spacing treatment between maize and peas was significantly higher than that of the 15 cm and 45 cm spacing treatments, with Ca values calculated from nodule number reaching 78.70% and 161.21% in 2013 and 2014, respectively, indicating that intercropping alleviated nitrogen suppression compared with monoculture. During this period, the nutrient competitive ratio of peas (CR<sub>pm</sub>) was greater than 1, indicating that peas had stronger interspecific competitive ability relative to maize. Cereal-legume intercropping significantly improved nitrogen use efficiency, with the highest value observed in the 30 cm spacing treatment, which on average over the two years of 2013 and 2014 was 21.90% and 21.88% higher than the intercropping patterns with 15 cm and 45 cm spatial structures, respectively. These results demonstrate that optimizing spatial structure can effectively increase nodule number and nodule weight in intercropped peas, enhance nitrogen suppression alleviation

effects, regulate nitrogen uptake and utilization in cereal-legume intercropping systems, and improve nitrogen use efficiency.

## Full Text

### Preamble

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### Effect of Cereal-Legume Spacing in Intercropping System on Alleviating “N Inhibition” in Pea Plants\*

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#### Abstract

To address the weak theoretical basis for synergistic utilization of chemical nitrogen fertilizer and legume nitrogen fixation potential in cereal-legume intercropping systems, we investigated the effects of spatial structure at three intercropping spacings (15 cm, 30 cm, and 45 cm) on alleviating nitrogen inhibition in intercropped pea using the dominant maize/pea intercropping system in the Hexi Corridor region. The aim was to provide a theoretical foundation for optimizing spatial structure, alleviating nitrogen inhibition, and improving nitrogen use efficiency in cereal-legume intercropping patterns. Two-year results (2013-2014) showed that compared with monoculture, intercropping significantly increased effective nodule number and nodule weight in pea, with nodule number increasing by 0-500%. The 30 cm spacing produced the maximum nodule number and weight. The nitrogen inhibition alleviation effect (Ca) calculated from both nodule number and weight was positive. Under nitrogen application, the 30 cm spacing treatment showed significantly higher nitrogen inhibition alleviation effect than the 15 cm and 45 cm spacings. In 2013 and 2014, Ca values calculated from nodule number reached 78.70% and 161.21%, respectively, demonstrating that intercropping alleviated nitrogen inhibition compared with monoculture. During this period, the nutrient competition ratio of pea (CR<sub>pm</sub>) was greater than 1, indicating that pea had stronger interspecific competitive ability than maize. Cereal-legume intercropping significantly improved nitrogen use efficiency, with the 30 cm spacing treatment achieving the highest efficiency. Averaged across the two years, this treatment increased nitrogen use efficiency by 21.90% and 21.88% compared with the 15 cm and 45 cm spacings, respectively. These results demonstrate that optimizing spatial structure can effectively increase nodule number and weight in intercropped pea, enhance nitrogen inhibition alleviation effects, regulate nitrogen uptake and utilization in cereal-legume intercropping systems, and improve nitrogen use efficiency.

**Keywords:** maize/pea intercrop; spatial structure; spacing between intercropped crops; N inhibition; root nodule; nitrogen use efficiency; ratio of nutrient competition

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## Introduction

Cereal/legume intercropping offers numerous advantages including soil protection [1], weed and disease control [2-3], and yield enhancement [4], and is widely practiced in tropical and rain-fed agricultural regions [5-6]. In recent years, as crop production has become increasingly dependent on chemical fertilizers and the associated environmental pollution and sustainability issues have intensified, the capacity of cereal-legume intercropping to improve nutrient use efficiency and reduce fertilizer dependence has attracted considerable research attention [7-8]. This practice is considered an important model for future organic and efficient alternative agriculture. Previous studies have demonstrated that efficient resource utilization in intercropping depends on scientifically regulating interspecific competition [9] and fully exploiting interspecific complementarity. The primary factors regulating competition and complementarity include crop variety [10], nutrient management [11], water regime, and spatial arrangement and density [12-14]. While these findings provide practical guidance for regulating intercropping communities, the theory underlying how optimized crop spatial arrangement, density, and species combinations can enhance nitrogen inhibition alleviation remains weak, leaving the biological potential for efficient nitrogen complementarity in cereal-legume intercropping largely untapped.

During crop growth, nitrogen application reduces nodule number and biological nitrogen fixation in legumes, a phenomenon known as “nitrogen inhibition” [15]. When intercropped with cereals, cereals absorb large amounts of nitrate, maintaining soil mineral nitrogen at a low but appropriate level and thereby reducing inhibition of legume nitrogen fixation [16]. This effect can be considered a “mitigation effect” of cereals on legume nitrogen inhibition. Research indicates that cereals can stimulate nodule formation and nitrogen fixation in legumes, possibly because cereals compete for nitrate or ammonium nitrogen in the legume rhizosphere [17]. Li et al. [18] conducted a maize (*Zea mays*) and faba bean (*Vicia faba*) intercropping experiment in northwestern China under different nitrogen supply levels to investigate the mitigation effect of intercropped maize on nitrogen inhibition in faba bean. Under 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 inhibition alleviation (Ca) was 9.7%, -10%, 15.2%, and 10%, respectively, while the percentage of nitrogen fixation contribution to total nitrogen uptake (Cis) was 20.3%, -0.5%, 17.4%, and 3.9%, respectively. These results demonstrate the feasibility of alleviating nitrogen inhibition effects on legume nodulation and nitrogen fixation through cereal intercropping and provide a theoretical basis for selecting appropriate nitrogen application rates in cereal-legume intercropping systems. Therefore, investigating the relationship between crop

spatial arrangement and nitrogen inhibition alleviation effects, building upon integrated agronomic management practices, will significantly advance our understanding of nitrogen-efficient mechanisms and facilitate the development of effective regulation technologies in cereal-legume intercropping.

The northwest inland irrigation zone is characterized by abundant light resources, good soil quality, and thermal conditions sufficient for one crop but insufficient for two. This region is suitable for intercropping, but traditional wheat (*Triticum aestivum*)/maize and wheat/soybean (*Glycine max*) intercropping patterns are no longer suitable for production practices due to increasing water scarcity. Since 2005, maize/pea intercropping has rapidly expanded due to its significant potential for water saving, high efficiency, and integration of crop and livestock production [19]. However, according to current production practices, maize/pea intercropping follows the fertilization regime of monoculture maize, while spatial arrangement follows the design of wheat/maize intercropping [20]. These management techniques not only ignore the nitrogen compensation effect of cereal-legume intercropping but also overlook potential competition effects between different crop combinations and their influence on nitrogen inhibition alleviation, preventing the full exploitation of this pattern's advantages in improving nitrogen use efficiency, crop yield, and land use efficiency under limited water supply conditions.

Building upon existing theories of nitrogen-efficient utilization in monoculture and cereal-legume intercropping, this study focuses on analyzing the relationship between nitrogen use efficiency and nitrogen inhibition alleviation in maize/pea (*Pisum sativum*) intercropping, clarifying the response of nitrogen inhibition alleviation to spatial arrangement, and identifying the basic mechanisms for improving nitrogen compensation utilization through nitrogen inhibition alleviation effects. This research will provide strong theoretical and practical support for developing simple, high-yield, efficient, and nitrogen-saving cereal-legume intercropping patterns. Therefore, by adjusting the aboveground spatial arrangement in maize/pea intercropping systems, this experiment investigated pea nodulation characteristics under three cereal-legume spacings (15 cm, 30 cm, and 45 cm) and analyzed the effects of different spacings on nitrogen inhibition alleviation in intercropped pea, aiming to provide a theoretical basis for optimizing spatial structure, alleviating nitrogen inhibition, and improving nitrogen use efficiency in cereal-legume intercropping patterns.

### 1.1 Study Area Overview

The study was conducted in 2013 and 2014 at the Oasis Agriculture Research and Teaching Base of Gansu Agricultural University (103°5 E, 37°30 N). The experimental site is located in Huangyang Town, Liangzhou District, Wuwei City, at the eastern end of the Hexi Corridor, in a cold temperate arid climate zone at an altitude of 1,750 m. The frost-free period is approximately 155 days, with multi-year average precipitation of 156 mm, annual evaporation of about 2,400 mm, aridity index of 5.85, and mean annual temperature of 7.2 °C. The

accumulated temperatures above 0 °C and 10 °C are 3,513.4 °C and 2,985.4 °C, respectively. Sunshine duration is 2,945 hours. The region has vast land resources, abundant light, long sunshine hours, and large diurnal temperature differences, making it suitable for multi-cropping systems such as intercropping. During the experimental period, the average daily temperature was 17.85 °C, and effective precipitation was 216.6 mm, higher than the multi-year average. The soil type is irrigation desert soil. Soil nutrient contents in the 0-30 cm layer measured in 2013 are shown in Table 1 .

## 1.2 Experimental Design

The experiment included three planting patterns: monoculture pea (P), monoculture maize (M), and maize/pea intercropping (PM). In the intercropping system, three spacings between maize and pea were established: 15 cm (D1), 30 cm (D2), and 45 cm (D3), with unchanged pea row spacing and adjusted maize row spacing. Two nitrogen application rates were also established for intercropped maize: 0 kg · hm<sup>-2</sup> (N0) and 260 kg · hm<sup>-2</sup> (N1). The experiment comprised 10 treatments, each with three replications. The total nitrogen application rate for monoculture maize and intercropped maize strips was consistent at the local conventional rate of 450 kg · hm<sup>-2</sup>, applied in three splits with a basal:jointing:bell-mouthed stage ratio of 3:2:5. In intercropping, nitrogen fertilizer was applied separately to maize and pea strips according to their land area proportions (11:8). All nitrogen and phosphorus fertilizers for the pea strip were applied as basal fertilizer at once, while topdressing for maize was applied near the maize roots under the plastic film. Fertilization methods were identical for monoculture and intercropping. Urea was used as nitrogen fertilizer and calcium superphosphate as phosphorus fertilizer. Phosphorus fertilizer was applied entirely as basal fertilizer at an N:P ratio of 2:1. Specific fertilization regimes are shown in Table 2 .

Plot size was 45.6 m<sup>2</sup> (8 m length × 5.7 m width), with 0.3 m walkways between plots. Each intercropped plot contained three units: one unit for yield measurement and two units for dry matter sampling at various growth stages. Each unit consisted of three maize rows and four pea rows, with strip widths of 110 cm and 80 cm, respectively. Monoculture maize had 40 cm row spacing and 30 cm plant spacing, while intercropped maize spacing varied by treatment (see Figure 1 [Figure 1: see original paper]). Monoculture maize planting density was 83,000 plants · hm<sup>-2</sup>, while intercropped maize density was 53,000 plants · hm<sup>-2</sup>. Pea row spacing was 20 cm with 20 cm plant spacing, with monoculture pea density at 1.8 million plants · hm<sup>-2</sup> and intercropped pea at 760,000 plants · hm<sup>-2</sup>. Treatments were arranged randomly. The maize strip was fully mulched with plastic film using mechanical application, while the pea strip was not mulched. Other management practices were identical to field production. The plastic film was 0.08 mm thick agricultural film.

The pea cultivar was ‘MZ-1’ (needle-leaf type), planted on April 1 and harvested on July 12. The maize cultivar was ‘Xianyu 335’ , planted on April 25 and

harvested on October 5.

### 1.3.1 Sample Collection and Preparation

- 1) **Plant sample collection and preparation:** Pea and maize sampling was conducted at their respective critical growth stages. Maize sampling began at the seedling stage (15 days after sowing) and continued at 15-day intervals. The first two samplings included 10 plants, while subsequent samplings included 3 plants, totaling eight samples during the growth period. Pea samples were collected at five critical growth stages: branching, bud formation, flowering-podding, grain filling, and maturity, with 20 plants sampled each time. Samples were oven-dried at 105 °C for 2 hours, then at 80 °C to constant weight, weighed as aboveground dry matter, ground with a specialized grinder, passed through a 100-mesh sieve, and bagged for determination of nitrogen content and other indicators.
- 2) **Soil sample collection and preparation:** Before sowing, soil samples from 0-30 cm depth were collected from five randomly selected plots among the 30 plots using an auger. After sowing, 0-80 cm soil samples were collected near plant sampling points using a soil auger at 20 cm intervals during each sampling period. Samples were placed in labeled self-sealing bags, air-dried naturally, sieved through a 100-mesh sieve after moisture evaporation, and bagged for determination of soil nitrate nitrogen, ammonium nitrogen, and total nitrogen.
- 3) **Pea nodule number and weight determination:** In 2013 and 2014, one sampling was conducted 10 days after pea flowering (at the maize bell-mouthed stage, on June 5, 2013 and June 7, 2014). Random sampling was used to select a 0.4 cm wide  $\times$  0.6 cm deep quadrat from the pea strip in each plot. Pea root systems and surrounding soil were carefully excavated with a shovel, plant numbers were recorded, and relatively intact root systems were collected and placed in corresponding sampling bags. Nodule numbers per plot were counted manually on the same day, and nodule dry weight was measured using a JA5003 analytical balance.
- 4) **Plant total nitrogen and soil inorganic nitrogen (nitrate and ammonium) content:** Plant total nitrogen content was determined for whole plants using an Elementar elemental analyzer. Soil inorganic nitrogen content (including nitrate and ammonium nitrogen,  $\text{mg}\cdot\text{kg}^{-1}$ ) was measured using an AA3 continuous flow analyzer produced by Bran+Luebbe GmbH, Germany.
- 5) **Crop yield:** At harvest, a 4-row  $\times$  1.5 m quadrat was sampled for pea yield measurement in each plot, with each plot harvested separately. For maize, 20 plants were sampled from each plot for yield trait measurement, and three 3-row  $\times$  2 m quadrats were harvested for yield determination, with yield calculated by plot.

### 1.3.2 Related Index Calculations

- 1) **Nitrogen inhibition alleviation effect of intercropping (Ca)** [16]: Defined as the change rate of legume nodulation values in intercropping relative to monoculture under the same nitrogen application rate. If  $Ca > 0$ , it indicates that cereal-legume root interactions promote legume nodule growth, increase biological nitrogen fixation, and alleviate the inhibitory effect of nitrogen fertilizer on legume nodulation and nitrogen fixation; conversely, it indicates inhibition.

$$Ca = \frac{Y_i - Y_s}{Y_s} \times 100\% \quad (1)$$

Where  $Y_i$  and  $Y_s$  represent nodulation values of intercropped and monoculture legumes, respectively, under the same nitrogen application level.

- 2) **Nutrient competition ratio (CRpm)** [21]: An indicator measuring crop nutrient absorption capacity. This experiment used the nutrient competition ratio of pea relative to maize to assess competitive ability.

$$CRpm = \frac{U_{ip}/U_{sp} \times F_p}{U_{im}/U_{sm} \times F_m} \quad (2)$$

Where  $U_{im}$  and  $U_{ip}$  are nutrient absorption amounts of intercropped maize and pea, respectively;  $U_{sm}$  and  $U_{sp}$  are nutrient absorption amounts of monoculture maize and pea, respectively; and  $F_m$  and  $F_p$  are the proportions of maize and pea in intercropping. When  $CRpm > 1$ , pea has stronger nutrient competitive ability than maize; when  $CRpm < 1$ , pea has weaker nutrient competitive ability.

- 3) **Nitrogen use efficiency (NUE)**:

$$NUE = \frac{\text{N absorption of N-fertilized crop} - \text{N absorption of non-N-fertilized crop}}{\text{N application rate}} \times 100\% \quad (3)$$

## 1.4 Data Statistics

Experimental data were organized using Microsoft Excel 2007 and analyzed for significance using SPSS 17.0 (LSD test).

### 2.1 Effect of Cereal-Legume Spacing on Nodulation Characteristics of Intercropped Pea

Both spatial structure and nitrogen application significantly affected effective nodule number and nodule weight in pea (Table 3). Compared with monoculture pea, intercropping significantly increased effective nodule number and nodule weight. Under nitrogen application (N1, 135 kg · hm<sup>-2</sup>) in 2013, nodule numbers per plant at 15 cm (D1) and 30 cm (D2) spacings increased by 14.29%

and 71.43% compared with monoculture pea (P), respectively. While nodule weight per plant showed no significant difference between monoculture and intercropped pea, intercropping increased nodule weight by 17.65%, 50.00%, and 11.76% compared with monoculture. In 2014, intercropping increased nodule numbers by 33.33%, 150.00%, and 100.00% at D1, D2, and D3, respectively, and increased nodule weight by 3.85%, 273.08%, and 80.77%, respectively. Under no nitrogen (N0) conditions in 2013, nodule numbers per plant at D1, D2, and D3 increased by 200.00%, 150.00%, and 100.00% compared with monoculture, while nodule weight increased by 168.97%, 68.97%, and 113.79%, respectively. In 2014 under N0, nodule numbers increased by 400.00%, 500.00%, and 350.00% at D1, D2, and D3, respectively, and nodule weight increased by 578.57%, 521.43%, and 292.86%, respectively. Notably, at 30 cm spacing, pea achieved maximum nodule number and weight, significantly higher than monoculture. Nitrogen application significantly increased nodule number and weight in monoculture pea but had no significant effect on intercropped pea, and the interaction between nitrogen application and intercropping was not significant.

## 2.2 Effect of Cereal-Legume Spacing on Nitrogen Inhibition Alleviation in Intercropped Pea

The nitrogen inhibition alleviation effect of intercropping (Ca) was calculated by comparing intercropping with corresponding monoculture under the same nitrogen application. As shown in Table 4, Ca values calculated from both nodule number and weight were positive under both nitrogen application and no-nitrogen treatments, indicating that intercropping alleviated nitrogen inhibition compared with monoculture. Under nitrogen application, the nitrogen inhibition alleviation effect at 30 cm spacing (D2) was significantly higher than at 15 cm (D1) and 45 cm (D3). In 2013, Ca calculated from nodule number reached 78.70, which was 252.60% and 579.03% higher than D1 and D3, respectively. In 2014, Ca at D2 reached 161.21, representing increases of 356.17% and 52.04% compared with D1 and D3, respectively. Under no-nitrogen conditions, Ca at D1 was significantly higher than D2 and D3 in 2013, while no significant differences were observed among spacing treatments in 2014. However, the nitrogen inhibition alleviation effect under no-nitrogen treatment was significantly higher than under nitrogen application, with average Ca values calculated from nodule number being 122.63 and 248.42 higher in 2013 and 2014, respectively. These results demonstrate that cereal-legume intercropping has significant nitrogen inhibition alleviation effects, that appropriate cereal-legume spacing is an important factor for realizing this effect, and that low soil nitrogen conditions can enhance this effect.

## 2.3 Interspecific Competitiveness of Pea Relative to Maize at Different Spacings

As shown in Table 5, nitrogen application significantly increased crop nitrogen absorption, and monoculture crops had significantly higher nitrogen absorption

than intercropped crops. At the pea flowering-podding stage, pea exhibited stronger interspecific competitive ability relative to maize. In 2013, nutrient competition ratios differed significantly between no-nitrogen (N0) and nitrogen application (N1) treatments. Under N0 conditions, all nutrient competition ratios (CR<sub>pm</sub>) were greater than 1, indicating stronger nutrient competitive ability of pea than maize. Under nitrogen application, all CR<sub>pm</sub> values were less than 1, indicating weaker competitive ability of pea than maize, possibly due to higher baseline soil fertility in 2013 that reduced pea's nutrient competitiveness. In 2014, results showed that intercropped pea had stronger interspecific competitive ability relative to maize under both nitrogen treatments, with all CR<sub>pm</sub> values greater than 1. Under N0 conditions, pea's competitiveness relative to maize decreased with increasing spacing, with CR<sub>pm</sub> at D1 (15 cm) and D2 (30 cm) being significantly higher than at D3 (45 cm), showing increases of 33.95% and 28.44%, respectively. No significant differences in CR<sub>pm</sub> among spacings were observed under nitrogen application. These results indicate that 10 days after pea flowering, pea had stronger interspecific competitive ability relative to maize.

#### **2.4 Nitrogen Use Efficiency of Maize/Pea Intercropping at Different Spacings**

Nitrogen application significantly improved nitrogen use efficiency in intercropping systems (Figure 2 [Figure 2: see original paper]). In 2013, nitrogen use efficiency in intercropping systems was significantly higher than in monoculture maize and monoculture pea, with the intercropping community showing 7.7%–45.86% higher efficiency than monoculture maize and 47.37%–99.6% higher than monoculture pea. Among different spacings, nitrogen use efficiency in intercropping communities followed the order D2 > D1 > D3, with D2 being 25.76% and 35.44% higher than D1 and D3, respectively, with significant differences. In 2014, nitrogen use efficiency in intercropping systems was also significantly higher than in both monocultures, with intercropping communities showing 15.16%–34.90% higher efficiency than monoculture maize and 360.79%–439.8% higher than monoculture pea. In the intercropping system, efficiency followed the order D2 > D3 > D1, with D2 being 17.14% and 7.69% higher than D1 and D3, respectively, with significant differences. These results demonstrate that intercropping significantly promotes nitrogen use efficiency, and that the spatial configuration with 30 cm spacing between maize and pea in the 4:3 strip pattern achieves the highest nitrogen use efficiency.

### **Discussion and Conclusion**

In intercropping communities, competition and complementarity between crops enhance yield and nutrient use efficiency through several mechanisms, including promotion of nutrient uptake through root interactions, competition-recovery processes in yield formation, and nutrient mobilization from inefficient to efficient crops [22]. When intercropped with cereals, cereals absorb large amounts

of nitrate, maintaining soil mineral nitrogen at a low but appropriate level and thereby reducing inhibition of legume nitrogen fixation [16]. This study demonstrated that maize/pea intercropping significantly increased nodule number and weight in legumes, with Ca values calculated from both nodule number and weight being greater than 0, indicating that cereal-legume intercropping promotes legume nodulation and significantly alleviates nitrogen inhibition. Nitrogen application significantly affects nodule formation, growth, and nitrogen fixation capacity in legumes. With increasing nitrogen rates, nodule dry weight and number initially increase gradually, while nitrogenase activity and leghemoglobin content continuously decline [23]. Appropriate nitrogen application significantly promotes nodule growth, but insufficient nitrogen supply inhibits nodule growth, and excessive nitrogen supply also inhibits nodule formation [23]. This study found that nitrogen inhibition alleviation effects under no-nitrogen treatment were significantly higher than under nitrogen application, with average Ca values calculated from nodule number being 122.63 and 248.42 higher in 2013 and 2014, respectively. This may be primarily because the nitrogen application rate in this study ( $450 \text{ kg} \cdot \text{hm}^{-2}$ ) represented the local conventional rate, and excessive nitrogen application inhibited nodulation in intercropped pea.

Spatial arrangement in intercropping primarily refers to the land occupation ratio, row spacing, and co-growth period of different crops in the community, which determines spatial occupancy. Hauggaard-Nielsen et al. [24] designed a barley (*Hordeum vulgare*)/pea intercropping experiment and demonstrated that three patterns (barley and pea strips each occupying 100 cm, each occupying 50 cm, and barley strip occupying 50 cm with pea strip occupying 100 cm) had significantly higher total nitrogen uptake than monoculture, indicating compensatory effects between the two crops. This compensation was mainly attributed to increased nitrogen absorption by intercropped barley that forced greater dependence on biological nitrogen fixation in pea, though differences among the three patterns were not significant. This study showed that at 30 cm spacing, legume nodule number and weight were maximized, Ca values were higher than at 15 cm and 45 cm spacings, and pea had stronger interspecific competitive ability relative to maize at the flowering-podding stage (10 days after flowering) with all CRpm values greater than 1. Nitrogen use efficiency in intercropping systems was significantly higher than in monoculture and was highest at 30 cm spacing. These results demonstrate that optimizing spatial structure can effectively improve nodulation status, promote nitrogen inhibition alleviation, and significantly enhance nitrogen use efficiency in cereal-legume intercropping.

In strip intercropping, the adjacent strips of two crops represent the focal area for resource competition and complementarity. The traditional design principle for intercropping spatial structure is “squeeze the middle, space the sides,” which minimizes row spacing within the same crop species while maximizing distance between adjacent strips (spacing). However, this approach may cause intercropping practitioners to miss opportunities to improve nitrogen use efficiency through spacing optimization. The findings of this study will positively contribute to enriching the theory of nitrogen-efficient utilization in cereal-legume

intercropping, increasing the nitrogen fixation contribution of legumes in intercropping communities, and developing nitrogen-saving intercropping production systems.

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

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